UNIVERSITAT DE BARCELONA

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Historia evolutiva y mecanismos de diversificación en arañas mediterráneas de la familia Nemesiidae (Araneae, Mygalomorphae)

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TESIS DOCTORAL

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Memòria presentada per Elisa Mora de Checa, realitzada sota la direcció del Dr. Miquel A. Arnedo en el Departament de Biologa Animal, per a accedir al títol de Doctora per la Universitat de Barcelona.

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A mi madre, mi padre y mi hermano, por dármelo todo en esta vida

A Marc, el millor company de viatge que mai hagués pogut imaginar

Nothing in biology makes sense, **except in the light of evolution**

'The American Biology Teacher', 1973

Theodosius Dobzhansky

Agraïments

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1. Introduction

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1.1 The study of biological systematics

Biological systematics is a discipline that studies the diversity of living and fossil organisms. It basically serves two purposes. First, it deals with the discovery and description of species and, second, it infers the phylogenetic relationships of these species (Wiens, 2007; Wiley & Lieberman, 2011).

One of the main goals of systematics is to understand the evolutionary relationships of taxa, because of that, the development of systematics is closely related to the development of methodologies for taxonomy and phylogenetic inference.

Taxonomy is the science of discovering, describing, naming and classifing extant or extinct organisms (Quicke, 1993). Phylogenetics focuses on understanding the relationships between species, using observable characters from organisms and analyzing them with different methodologies.

Will Henning (Hennig, 1950, 1966) established the theoretical basis of phylogenetic systematics (or cladism). This school states that the only relevant groups for a natural classification are **monophyletic groups** (ie. those that include the ancestor and all its descendants). Since Henning, the classification of organisms has to reflect their evolutionary relationships, which is in turn based on the presence of sinapomorphies, this is shared derived characters inheretid from a commom ancestor.

Ancestor descendant relationships between organisms are represented in the form of a **tree**. This tree is a mathematical structure used to model the evolutionary history of a group of sequences or organisms. This pattern of historical relationships is the **phylogeny** or **evolutionary tree,** which it is possible to be estimated. A tree consists of nodes connected by branches, being the terminal nodes the taxa (specimens or sequences) and the internal nodes representing the hypothetical antecesor. The root of the tree represent the common ancestor of all the sequences or taxa in the tree.

Given a tree, two characters that are identical and this similarity is due to a commom antecesor, represent an instance of **homology**. On the contrary, if they similarity is not due to common ancestry then they are an instance **of homoplasy.** We can distinguish between ancestral and derived homologies. If a character has the same state as the exclusive commom ancestor, then it is the ancestral or **plesiomorphic** state. Otherwise, it is a derived or **apomorphic** state. Unique derived states are autopomorphies, and shared derived states are synapomorhies. Only the synapomorphies are relevant for inferring phylogenetic relationships.

Molecular systematics

Molecular systematics is the use of molecular **genetics** to study the evolution of relationships among individuals and species (Hillis, 1996). Nowaday, the use of molecular data have prevailed over morphological characters because among molecular characters are easier to codify, morphological features can be subtile and require wide experstise.

DNA data come in different flavours: DNA sequences, AFLP's or SNP's, among others. The exponential growth of this discipline in last decades is due to a combination of increased sophistication in molecular biology techniques, and computer advances in hardware and software that allow to model large and complex data sets and to evaluate and test hypothesis.

DNA tools have spurred the development of molecular taxonomy, ie. the use of molecular markers and DNA sequences for description of species or to supplement the classical taxonomy, (Blaxter, 2004; Hebert et al., 2004; Hogg & Hebert, 2004; Smith et al., 2005). This new approach facilitated the discovery of cryptic taxa (Goetze, 2003; Molbo et al., 2003; Feulner et al., 2006).

DNA taxonomy has generated an intense debate (Tautz et al., 2003; Nielsen & Matz, 2006), it proposed replacing the current system of specimen types with molecular information. Although DNA has been a great help to delineate species, it cannot provide the only source of evidence and it is always advisable to combined the DNA data with other sources of information, such as

morphology, behavior, ecology and the geographical distribution of organisms (Balakrishnan, 2005; Knowles & Carstens, 2007).

1.2 Species concept

The ability to recognize species is fundamental to understanding the origin and diversification of the biodiversity. Species are fundamental units of Biology (Darwin, 1859; Dobzhansky, 1937; Mayr, 1942; de Queiroz, 2005; De Queiroz, 2013). Notwithstanding the need for a useful concept and the fact all scientist have a clear idea on what constitutes species, there is a great confusion around their definition. In The Origin of Species Darwin wrote: "*No one definition has as yet satisfied all naturalists; yet every naturalist knows vaguely what he means when he speaks of a species".*

Two hundred years later, the debate continues. In fact, very few topics in biology have raised as much debate as the species concept (Mayr, 1942; Cracraft, 1989; Claridge et al., 1997; Coyne & Orr, 2004; de Queiroz, 2005). Mayden (1997) listed more than 20 different named species concepts. The main problem is that those concepts and their definitions were initially formulated to answer questions from different disciplines, and each one involves at least partially incompatible species concepts (Mayden, 1997; de Queiroz, 1998; Harrison, 1998).

To make matters worse, the species concept has been confused with the issue of species delimitation. This confusion is commonly known as the *species problem*: when both questions were linked. De Queiroz (2005,2007) has proposed a possible solution to the apparent conundrum, by defining the species as a "metapopulation that evolves independently". In this way, he clearly distinguished between the actual definition, and the different lines of evidences used to recognise it. De Queiroz (1998; 2007) suggested that the former species concepts were better interpreted as criteria to delineate species and confirm evolutionary independent status of species. Nowadays, the emerging consensus in the scientific community is that the best approach to delimit species is the use of different sources of evidence and different analytical approaches to extract the most meaningful information (Heethoff et al., 2011).

1.3 Integrative taxonomy

Delimitation of species boundaries is crucial to discover life's biodiversity because it determines whether or not different individual organisms are members of the same entity. Some estimates about global biodiversity calculated that approximately 10 million species remain still undiscovered (Wheeler et al., 2004). The incorrect estimation of biodiversity can led to serious consequences affecting to the general knowledge of nature's patterns and processes and ultimately to conservation efforts (Wiens, 2007).

The delimitation of species boundaries may be particularly challenging when dealing with either morphologically uniform taxa (Stockman & Bond, 2007; Bond & Stockman, 2008; Hendrixson & Bond, 2009; Hamilton et al., 2011, 2014; Hendrixson et al., 2013) or recently evolved taxa (Pons et al., 2006; Shaffer & Thomson, 2007).

Despite the general perception that the major availability of molecular data could be the solution to a general crisis in taxonomy, only a small portion of species delimitation studies provides taxonomic species description. The final decision on what constitutes an actual species will further require the integration of additional sources of evidence (Kekkonen & Hebert, 2014).

The task of describing and classifying organisms is the main goal of the taxonomy. Despite of the technological and methodological advances that have facilitated the generation of a wealth of DNA sequence data and its relevance for addressing taxonomic questions such as the discovery of cryptic lineages and the identification of life stages and parts, some taxonomist have been reluctant to incorporate DNA to their studies (Dayrat, 2005). The gap in communication between the different disciplines involved in delimiting species is an important and overlooked problem in the so-called 'taxonomy crisis'.

To solve this problem, it is suggested that taxonomy becomes integrative, and this is seen as the real challenge for the future of taxonomy. The term 'integrative taxonomy' refers to taxonomy that integrates all available data sources to frame species limits (Yeates et al., 2011). It aims to delimit the units of life's diversity from multiple and complementary perspectives

(phylogeography, comparative morphology, population genetics, ecology, development, behaviour, etc.) (Dayrat et al. 2005,). This integration maximizes the objectivity and robustness of the species delimitation process (Bond & Stockman, 2008; Hendrixson et al., 2013; Edwards & Knowles, 2014). A big change is needed concerning the creation of names in order to achieve this integration and to prevent the over-abundance of both synonyms and names of doubtful application from worsening. Integrative taxonomy gives priority to species delineation over the creation of new species names.

1.4 The importance of maintaining biodiversity

The origin of the term biodiversity is quite recent. In 1980, Thomas Lovejoy introduced the term “biological diversity” to the scientific community and in 1988, E. O. Wilson coined the term biodiversity (Haila & Kouki, 1994). Biologists most often define biodiversity as the "totality of genes, species, and ecosystems of a region" (Larsson, 2001). This definition provided an unified view of the traditional levels of biological variability. In 2003, Anthony K. Campbell defined a fourth level: Molecular Diversity.

At present, one of Earth’s major crises is the accelerating extinction of species due to human activities (Smith et al., 1993; Lawton & May, 1995; Purvis & Hector, 2000; Cardoso et al., 2011). Conservative estimates suggest that 3000 of the 3–100 million extant estimated species are being lost each year, this is eight species per day (Wilson, 2003; González-Oreja, 2008).

The loss of a species implies the loss of functional diversity and the provision of ecosystem services, with consequences to human well-being (Balvanera et al., 2006). For example, the loss of a pollinator may involve the loss of productivity in many crops (Kremen et al., 2002), and the loss of groundwater fauna may cause the disruption of purification and bioremediation processes with the consequent pollution problems (Boulton et al., 2008). To overcome this crisis, it is very important that we protect the places where biodiversity lives. In addition, a poor knowledge of biodiversity can led to wrong decision in defining conservation priorities.

1.5 The Mediterranean region as a hotspot of biodiversity

The Mediterranean region is particularly noted for the diversity of its plants: about 25,000 species are native to the region, and more than half of these are endemic (Myers et al., 2000). This Mediterranean has been recognized as one of the top 25 Global Biodiversity Hotspots (Myers et al., 2000) for conservation priorities. Besides this great richness of plants, a high proportion of Mediterranean animals are unique to the region: 2 out of 3 amphibian species are endemic, 48% of the reptiles, a quarter of mammals, 6% of sharks and rays and 3% of the birds and 14% of dragonflies, as well as half of the crabs and crayfish, (Medail & Quezel, 1997; Blondel et al., 1999).

Figure. 1 Map of the Mediterranean region (adapted from UICN, 2010)

The Mediterranean also hosts 253 species of endemic freshwater fish despite this sea makes up less than 1% of the global ocean surface, an incredibly rich biodiversity for such a small area (Bianchi & Morri, 2000). Furthermore, the Mediterranean's importance for wildlife is not limited to the richness of its resident fauna and flora (Fig.2): millions of migratory birds use Mediterranean wetlands and other habitats as stopover or breeding sites. The Mediterranean basin is expected to be seriously affected by global warming. The Its position of transition between Euro-Siberian, South-template and Saharo-Indic regions gives the region all the characteristics to be severely affected by climatic change.

Figure.2. Mediterranean diversity of fauna and habitats. Copyright Elisa Mora

Geology and climatic shifts are among the main abiotic drivers of species diversification, due to their ability to generate or modify barriers to dispersal (Hewitt, 2004; Esselstyn et al., 2009). The complex geological history and the dramatic climatic changes in the Mediterranean basin played a crucial role in the development of the Mediterranean as a biodiversity hotspot (Myers et al., 2000)

1.6 History of the Mediterranean Basin

The Mediterranean region was formed in the upper Oligocene, about 40 million years ago (Ma), when the Thetis ocean closed due to the convergence of the African plate towards the Eurasian (Blondel et al., 1999). This collision drove the Alpine Orogenic process (Rosenbaum & Lister, 2004) and that extended since the Miocene until today (Fig. 3).

During the Oligocene the Balearic Islands, Corsica, Sardinia, the Calabro-Pretorian massif, the Kabylies and the Beatic-Rift Cordillera microplates formed part of the Hercynian belt. This Hercynian Belt was connected with the eastern part of the Iberian Peninsula and the south of France. As a consequence of the Alpine Orogeny, the Hercynian Belt broke off and the microplates started drifting from the eastern Iberian Peninsula and southern France to their present-day location (Rosenbaum et al., 2002). The displacement of the microplates resulted in the formation of the major basins of the region including Valencia tough, Gulf of Lion, Ligurian Sea, the Alboran Sea and the Tyrrhenian Sea.

The opening started with the formation of the Valencia tough, which traces back to about 25 Ma (Roca et al., 1999). Corsica, Sardinia and Calabria block started rifting counterclockwise direction from the Eurasian plate. This block collided with the western margin of Adria (Apulia) (20-18 Ma) and give rise to the Apenines. The separation of Corsica and Sardinia has been dated between 21-15 Ma, (Speranza et al., 2002; Gattacceca et al., 2007). Corsica and Sardinia subsequently detached from the Apennines in the mid Miocene around 9 Ma and started drifting off towards their present day position (Rosenbaum & Lister, 2004).

The Betic-Rif plate started rifting clockwise from its original location around 23 Ma, and 15 Ma and eventually started fragmenting into the Betic and the Rif blocks, which reached their present day location on both sides of the Strait of Gibraltar by mid Miocene (10 Ma) (Lonergan & White, 1997) . Some authors that suggest that the connection between the southernmost part of the Betic, corresponding to the Gibraltar region of the Iberian Peninsula, with the Rif block persisted until Upper Tortorian/Lower Messinian (~8 – 7.2 Ma), while the water exchange between the Mediterranean and the Atlantic Ocean was facilitated through the Guadalquivir Basin (Braga et al., 2003).

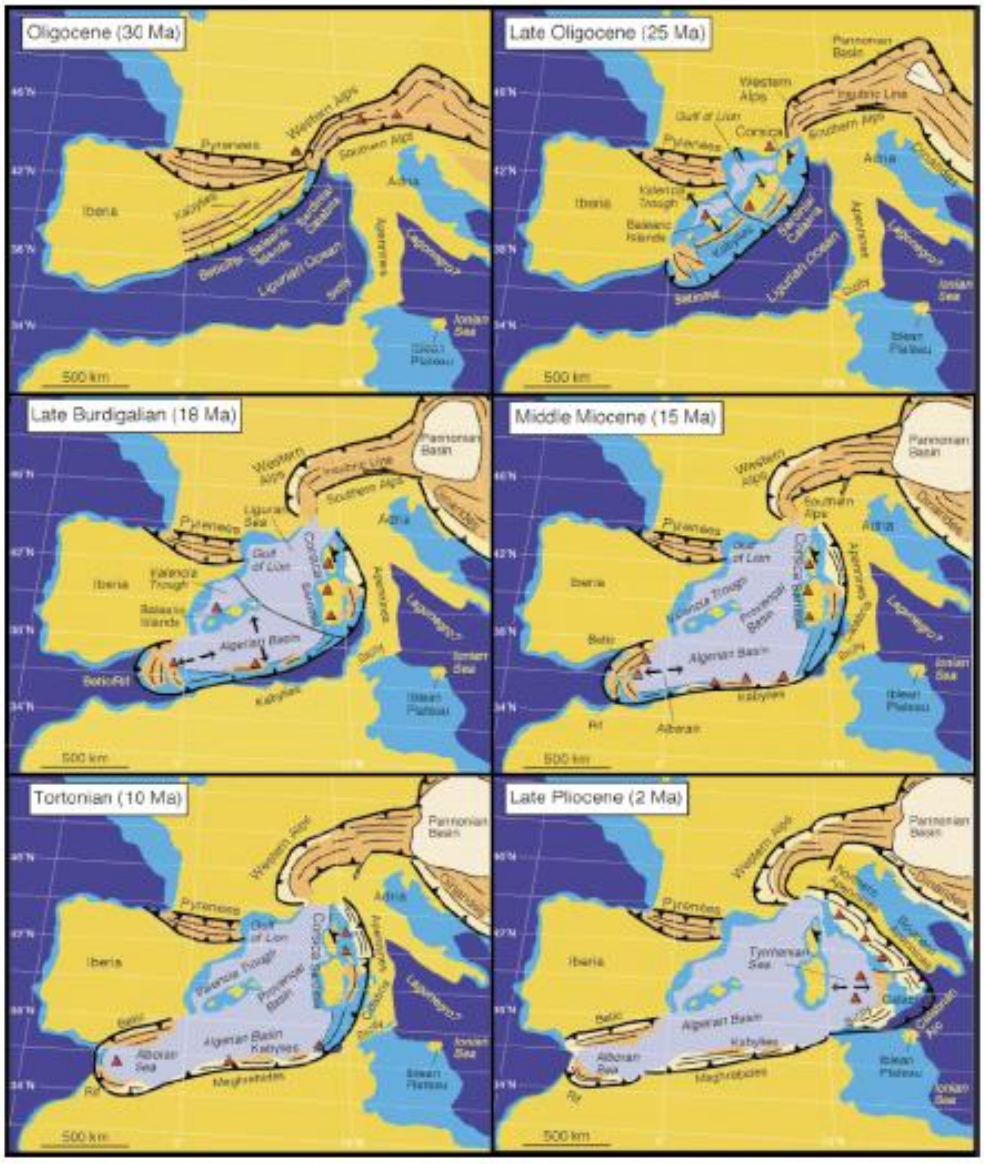


Figure 3. Adapted from Rosembaun et al. 2002

In the Eastern Mediterranean a continuous landmass containing the present day mainland Greece, Crete, the small Aegean islands and parts of Anatolia, started to break up after the formation of the mid-Aegean trench in the Upper Miocene (12–9 Ma), which resulted in the opening of Anatolian sea and the disjunction of Crete about 8 Ma (Creutzburg, 1963; Dermitzakis, 1990).

The Messinian Salinity Crisis (MSC) has been pointed out as one of the main drivers of local diversification. Approximately 5,96 Ma the Strait of Gibraltar closed, isolating the Mediterranean Sea from the Atlantic Ocean with the subsequent almost total desiccation of the Mediterranean Basin (Krijgsman et al., 1999). Emerged landbridges were established among previously isolated

regions, including the Balearic Islands, the Rif, Corsica and Sardinia (Jolivet et al., 2006). The reopening of the Strait of Gibraltar approximately 5.3 Ma restored the water exchange between the Atlantic and Mediterranean, re-establishing effective isolation of northern Morocco and the islands (Krijgsman et al., 1999; Loget & Van Den Driessche, 2005)

In parallel, the Mediterranean region also experimented major climatic changes. At the beginning of the Eocene (~ 55 Ma) the climate was warmer and wetter than today. In the middle Miocene (~15-7 Ma) started a cooling process that led to the establishment of the seasonality as we know today. The subtropical climate progressively changed towards cooler and dryer conditions, culminating on the present-day Mediterranean climate about 3.2 Ma(Suc, 1984; Jiménez-Moreno et al., 2010). This climatic shift was accompanied by the replacement of thermophilous plant communities by typical Mediterranean vegetation.

The Quaternary glacial cycles (2.6-0.1 Ma) are probably the most dramatic climatic shift experimented in the region. Starting 2.58 Ma (Gibbard et al., 2010), glacial oscillations produced major range shifts and population size changes of local organism, which eventually led to either the extinction or the speciation (Hewitt, 1996; Avise et al., 1998) of local biota. The formation of the glacial ice-sheets induced drops of the sea level that restablished land bridges among some islands, for instance Majorca and Minorca.

1.7 Spiders as subject of study

The use of arthropods for biodiversity assessment provides information about diversity patterns and environmental quality at a scale often more relevant than those used with plants and vertebrates (Yen and Butcher, 1997). Moreover, monitoring arthropods may provide early warnings of ecological changes. Because of their higher reproductive rates and shorter generation times arthropods are more sensitive to environmental perturbations than vertebrates, and may reveal the effect of fragmentation on areas where vertebrates are not good indicators (Kremen et al., 1993, 1994).

However, not all arthropods are equally effective as indicators for conservation. Top predators are the most sensitive trophic level to environmental changes. It

has shown that the extinction of top predators may cause important changes in (Estes et al., 2011). Spiders are the fifth most diverse order of animals , with 110 families and more than 45.000 species (World Spider Catalog, 2015).Spider are among the most abundant and conspicuous top predators on Earth.

The infraorder Mygalomorphae comprises American tarantulas, purse-web spiders, funnel-web spiders and trap-door spiders. It s one of the three main lineages recognized within spiders (Platnick & Gertsch, 1976; Bond & Hedin, 2006) (Fig. 4). Mygalomorphs are the sister group of Araneomorphae and are currently classified into 15 families, comprising roughly 2500 species and 300 genera (World Spider catalog, 2015).

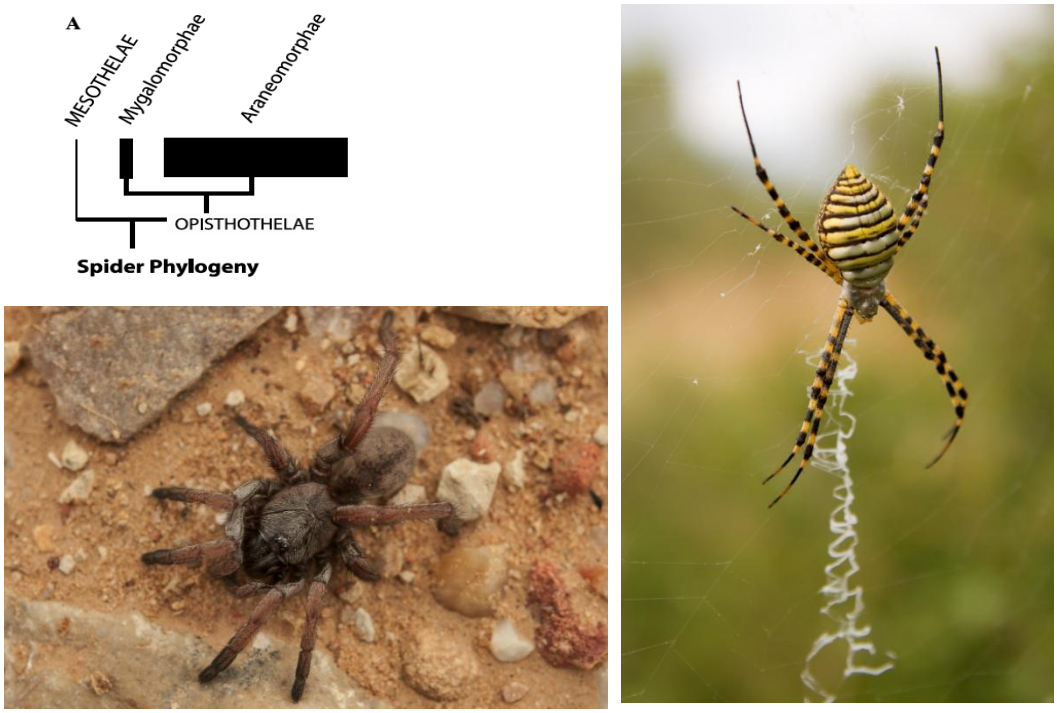


Figure.4. Phylogenetic tree showing the three main linneages of spiders (Bond and Hedin, 2006). Left: mygalomorph spider: *Nemesia*. Right: aranemoporh spider: *Argiope*. Photo credit Elisa Mora.

The mygalomorphs are a cosmopolitan group that inhabits all the continents except Antarctica. The origin of the group has been suggested to be ancient (Marusik, 2011) and the lineage has been commomly described as ‘primitive’ because of the retention of plesiomorphic characters, such as the presence of four book lungs and chelicerae bearing longitudinal fangs with unsynchronized

movement (Raven, 1985). Those spiders have a cryptic lifestyle. They are mostly nocturnal, ground-dwellers that construct burrows lionned with silk, frequelty protected by a closing trapdoor. The burrows can be up to 30cm deep. Females are mostly sedentary and long-lived (almost 20 years in captivity) (Buchli, 1961, 1962, 1965). Unlike females, which live inside the burrow for most of their life, adult males have anual life cycles and disperse after reaching madurity to search for mates. It has been documented that for some species females eat males after mating (Buchli, 1965).

The low vagility, the long-life cycles, the narrow ecological preferences and the restricted distribution make mygalomorphs an excellent model system for biogeographic and evolutionary studies (Hendrixson & Bond, 2005; Hamilton et al., 2011; Opatova et al., 2013; Satler et al., 2013; Opatova & Arnedo, 2014a). Ancient lineages with narrow habitat preferences and poor dispersal habilities are more likely to reflect the fingerprint of geological history (Stock, 1993; Bauzà-Ribot, 2013).

Low vagility has been cited as the main responsible for the high level of local endemism and deep geographic structure usually found in mygalomorphs (Bond et al., 2001; Arnedo & Ferrández, 2007; Decae et al., 2007a; Bond & Stockman, 2008; Satler et al., 2011; Hendrixson et al., 2013).

Unlike in araneomorph spiders, aerial dispersal through ballooning is rare in mygalomorph. Only three nonrelated mygalomorph families have been reported to perform ballooning, namely Atypidae, Ctenizidae and Actinopodidae (Coyle, 1983; Coyle et al., 1985). The presence of mygalomorph such as *Ummidia* (Ctenizidae) in some Caribbean Islands of volcanic origin without previous connection to any landmass (World spider Catalog, 2015), indicates long distance dispersal capability. On the other hand, the deep genetic structuring detected among *Atypus* populations (Atypidae) (Pedersen & Loeschcke, 2001) suggest that airborne dispersal operates at short distances. Recent dispersal studies in *Atypus* suggest that habitat selection, rather than ballooning,is responsible for the agregated colonies found in the field (Řezáč et al., 2007; Deruytter et al., 2012).

Despite their potential, several limitations have hampered ther use of

mygalomorphs as a as biogeographic and evolutionary model. They have a secluded lifestyle and are usually diifcult to observe and collect. Closely related taxa are usually morphologically conserved and diffcult to diagnose (Bond et al., 2006). Additionally, because of their different life-style, males and females are usually not collected together and species descriptions frequently include only one of the sexes.

1.8 The mygalomorph family Nemesiidae

The trap-door family Nemesidae is the most diverse of the six mygalomorph families present in the Mediterranean (Fig.5).

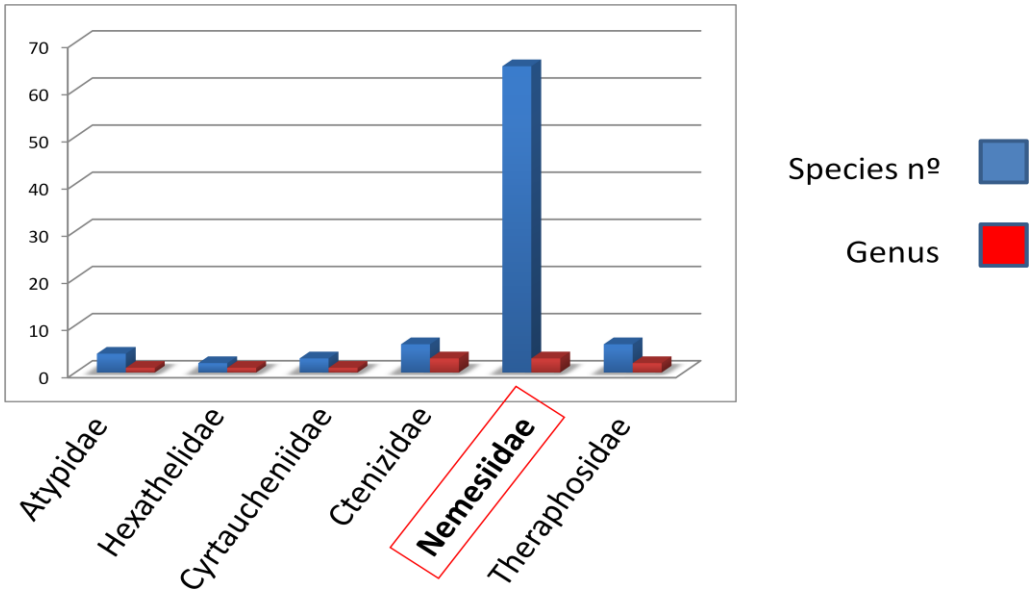


Figure.5. Graphic chart showing diversity in the 6 mediterranean mygalomoprh families

Nemesiidae has a worldwide distribution and ranks second among the largest mygalomorph families (World Spider Catalog, 2015). Four Nemesiidae genera have been recorded in the Mediterranean basin: the genus *Raveniola,*Zonstein 1987 with mostly an Asian distribution that reaches the eastern parts of Anatolia, *Iberesia* Decae & Cardoso 2006*,* from the Iberian Peninsula and the Balearic Islands, *Brachythele,*Pocock 1892 widely spread in the Adriatic and Aegean regions, including Anatolia and Cyprus, and finally *Nemesia,*Audouin 1826 which is distributed throughout the Mediterranean although its type-locality is near Alexandria in Egypt (Fig. 6)

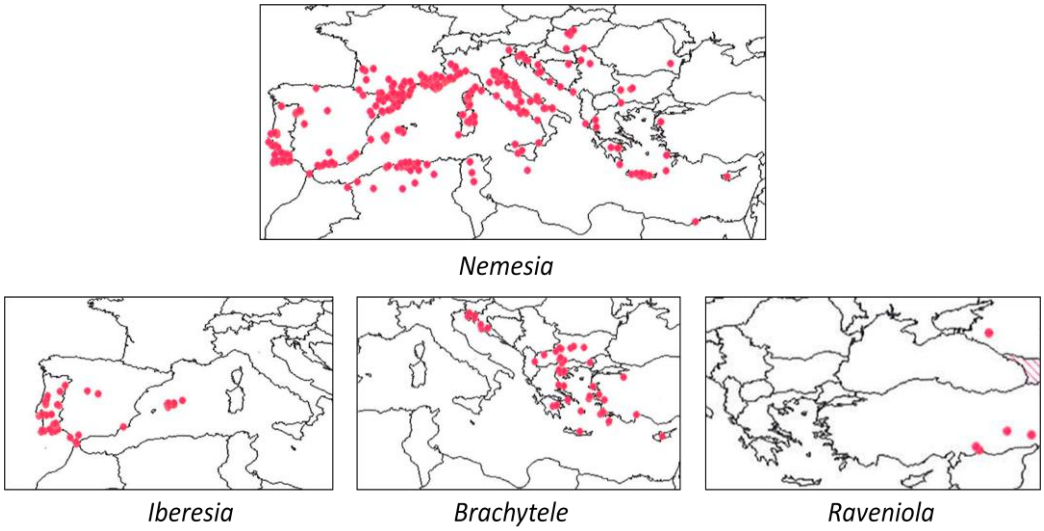


Figure 6. Distribution of the 4 nemesiidae genus (Adapted from Decae, 2010)

1.9 Genus *Nemesia* Audouin, 1826

The genus *Nemesia* comprises spiders from small to large mediterranean trapdoor spiders. They are distinguished from sympatric members of the families Ctenizidae and Cyrtauchenidae by the general brownish color, long legs relative to body size, and recurved fovea (Decae, 2010a) (Fig. 7).

Figure 7. Left: *Nemesia*. Right: *Cyrtauchenius. Photo credit Elisa Mora*

They can be distinguished from sympatric Iberesia by the presence of two spinnerets pairs, whereas Iberesia only has one pair of spinnerets (Decae & Cardoso, 2006). To date, 50 species and 4 subspecies of *Nemesia* have been described (World Spider Catalog, 2015). The species and their distribution are summarized in Fig. 8.

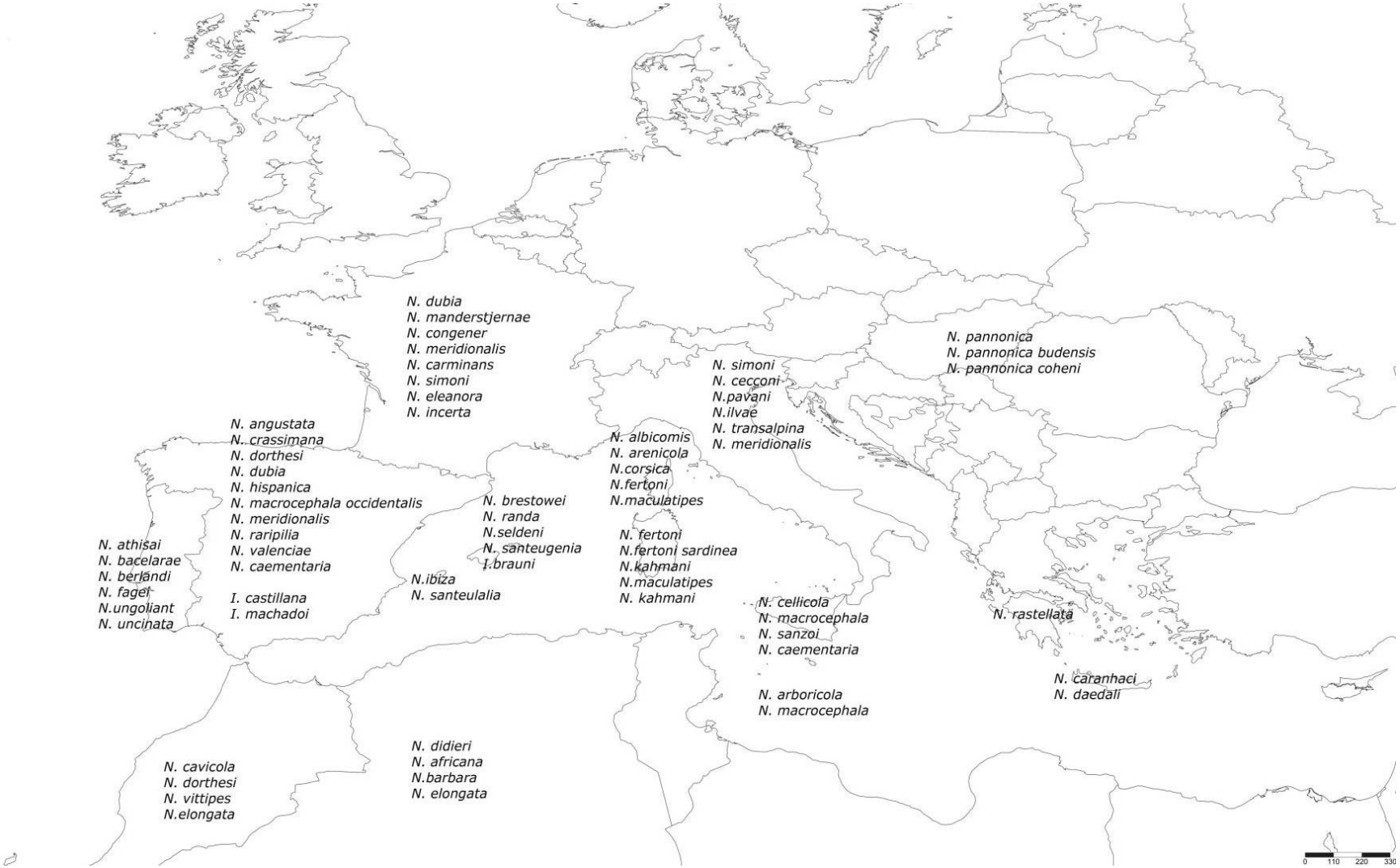


Figure.8 Species of the genus *Nemesia* and *Iberesia* and their distribution along the Mediterranean (World Spider Catalog, 2015)

Like most mygalomorphs, *Nemesia* species are morphologically conservative and hence difficult to tell apart. In arthropods, and spiders in particular taxonomists rely on the morphology of the copulatory organs to distinguish species (Eberhard, 1985). This is due to the fact that sexual characters evolve faster than somatic characters, probably as a result of sexual selection (Eberhard, 1985; Huber, 2003; Hosken & Stockley, 2004; Huber et al., 2005) In *Nemesia*, the variation in the female spermatheca or the male bulb is subtle, and additional characters are needed for species identification (Decae et al., 2007b). Because of the lack of sexual characters, immature stages are usually impossible to identify.

After two hundred years of the first description of *Nemesia,* the taxonomy is still porrly known and understood. There are several reasons that explain the chaotic taxonomic of the genus. **First, old descriptions** were usually inaccurate and ambiguous.Most descriptions date back to the 18th, 19th, and the first half of the 20th centuries, they lack comparative diagnosis, standardised descriptions of structures and, above all, informative illustrations. Second, **lost or misplaced type material**. Eugène Simon was one of the most prolific authors of *Nemesia* species (Simon, 1889, 1892, 1914). Unfortunately the spciemens used in the description were not labelled as type material and they were put together with other specimen considered to belong to the same species but from different localities in the same vials. In some cases it is impossible to know what is the actual type specimen for comparison. Third, **half of the species are known from one of the sexes**. Thus is the result of the sex biased life style. Direct capture by digging burrows usually provides only females, rarely males. Pitfall trapping, on the other hand, mostly captures wandering males.

1.10 The genus *Iberesia* Decae & Cardoso, 2006

The genus *Iberesia* was recently described to accommodate species formerly included in *Nemesia* distinguished by the absence of posterior median spinnerets (PMS) (Decae & Cardoso, 2006). Both the Posterior Lateral Spinnerets (PLS) and the PMS are present in *Nemesia*. *Iberesia* presently includes 3 species from the Iberian Peninsula and the Balearic Islands.

2. Material and Methods

2. Material and methods

2.1 Material

2.1.1 Sampling

The material used in this doctoral thesis was collected in several fieldtrips from 2009 to 2013. Some additional samples were already available from previous collection trips and few others were kindly provided by our collaborators. Mygalomorph spiders were captured by exploring banks and forest grounds, in search for trap-doors, which are usually well concealed. Once the trap-door is identified, a small wooden stick was introduced into the burrow to prevent collapse during excavation. Burrows can be up to 30 cm in depth, and spiders usually run away to the bottom of the burrow when the trap-door is opened. Collecting the specimen require slowly removing the soil from the entrance down to the bottom with the help of a screwdriver and a small shovel. Occasionally males can be found under stones and dead logs, where they usually hide at daylight the mating season. Pitfall traps are ideal for capturing mygalomorph males because they are only active during few days during the year. Pitfall consisted in cylindrical plastic cups of 10 cm in diameter and 15 cm high (Churchill & Arthur, 1999; Ferretti et al., 2012a), placed every 5 m along straight line transects. The cups were covered with funnels made with the same material and from the same diameter. Traps were filled with propilenglycol, because it does not evaporates and preserves DNA for subsequent molecular analyses. One or two legs depending on the specimen were removed and preserved in 100% ethanol at -20º degrees, the voucher was stored in 70% ethanol for morphological studies. Further information on the specimens analysed is included in each chapter.

2.2. Methods

2.2.1 Inferring molecular phylogenies

Molecular systematics can be defined as the use of the information contained in molecular data to reconstruct phylogenetic relationships (Hillis et al., 1996). Because of its ever increasing availability and the easiness to generate and score characters, DNA sequences have become the dominant data in modern

phylogenetic inference. There are three main methodologies to conduct phylogenetic inference: parsimony, maximum likelihood, and Bayesian inference.

2.2.1.1 Parsimony

Parsimony is the algorithmic and computational implementation of cladistic methodology (Farris, 1970; Fitch, 1971; Goloboff, 1998). States that the evolution will choose the easiest pathway understanding this as the minimum number of steps. The most parsimonious tree is the best estimate of phylogeny (Farris, 1970). This tries to maximize congruence among characters while minimizing incongruence (homoplasy) (Farris, 1983).

2.2.1.2 Maximum likelihood

Maximum Likelihood (ML) (Cavalli-Sforza, L. L. & Edwards, 1967; Felsenstein, 1981; Huelsenbeck & Crandall, 1997) is a method that was popularized by Felsenstein in the 80's, but it was computationally impossible to analyze large datasets. In recent years, the developments of methodologies have made this method a widespread choice.

Given an observed data, how can we decide whether our hypothesis is an adequate explanation for the data? One approach to this problem is the concept of likelihood

This method is based in the explanation that makes the observed output the more likely: the probability of observing the data (alignment), with a particular model of sequence evolution, given a phylogenetic tree. The tree that makes the data, the most probable outcome is the maximum likelihood estimate of the phylogeny (Edwards, 1972).

The **likelihood** is the probability of observing the data given a particular model. Different models will make this data more or less plausible. Is very important to distinguish between the probability of getting the observed data from the probability of the model being correct. Likelihood says nothing about the probability of the model itself. It is not the probability that the tree is true, only that the tree gave rise to the data(Page & Holmes, 2000).

The likelihoods for different models can be compared if those models are nested, meaning than one is a special case from another. A model of sequence evolution is necessary for applying this method. This model has two components: the frequency of nucleotides or aminoacids and the rate of change of this states.

Those model make some assumptions: all nucleotide sites change independently; the substitution rate is constant over time; the base composition is at equilibrium, the probabilities of nucleotide substitution don not change with time. Some of these assumptions are not true; in reality states show a wide range of probabilities of rate variation. The most widespread approach is the use of **gamma distribution**; that has a shape parameter **alpha**, which specifies the rate of variation.

Resampling

ML method performs **heuristic searches**, therefore is important to carry out multiple independent replicate in order to search for the optimal tree. For this reason, it requires the calculation of the level of confidence of the evolutionary hypotheses proposed as estimates of statistical support for each node of the tree(Sanderson, 1997). Bootstrap is used in this dissertation for ML analyses and Jackknife is used for Maximum Parsimony analyses.

2.2.1.3 Bayesian Inference

Bayesian Inference (Yang & Rannala, 1996) uses the **posterior probability distribution,** the probability that a tree is correct for the observed data, to construct the phylogenetic tree (Huelsenbeck et al., 2001). Bayes' theorem combines the a priori probability of a phylogeny with the likelihood to produce a posterior probability distribution (PP). This method calculates the posterior probability of phylogenies, by a sampling process that is speed up using **Markov Chain Monte Carlo (MCMC)** algorithm.

This algorithm search randomly in a space of trees in such a way as to settle down into an equilibrium distribution of trees that have the desired distribution (bayesian probability). We need to imagine a space full of trees, the different chains explore this space comparing those trees (generations), not all the trees

are going to be analyzed just the best ones. The chain goes comparing the likelihoods of trees. The output is a distribution of trees. The tree with higher value of PP should be considered the best approximation to the true tree, and the value itself of PP is a statistical support of the evolutionary hypothesis. This has a direct statistical interpretation: the probability that the given tree is true given a model, the premises and the data (Huelsenbeck, 2002).

2.2.2. Gene trees and species trees

The development in recent years of computational resources combined with the big availability of molecular data, allowed the incorporation of big amounts of data. Following the “total evidence” paradigma (Kluge, 1989) that big amount of data may result in robust phylogenies. Furthermore, the inclusion of different genes is basic for a real estimate of the phylogeny of a specie or genus (Pamilo & Nei, 1988).In this context, it is considered that the concatenation of different genes obtained allowed inferring phylogenies more robust that provides more reliable data. The implicit assumption is: when inferring phylogenies individuals and their genes share the same story, meaning that gene tree is congruent with the species tree.

A **species tree** represents the pattern of branching of species lineages via the process of speciation. A **gene tree** represents the pattern of branching of gene copies after they replicate and are passed on to more than one offspring (see Madison, 1997). The relationship between gene-tree and species tree is more complicated than that. At molecular level, each gene family has his own history that not necessary is identical to the history of the organism which contain that gene. So this may result in very incongruent tree topologies (Edwards, 2009).

There are many potential sources of discrepancy between gene trees and species trees. There are differences in patterns of nucleotide evolution, saturation substitution rate and differences in the rate of substitution between sites within the same gene and between genes (Arbogast et al., 2002). These processes influence directly to the length of branches of gene trees, and can lead to incorrect inference when several genes are concatenated (Felsenstein, 1978; Bull et al., 1993; Kolaczkowski & Thornton, 2004; Mossel & Vigoda, 2005; Kubatko & Degnan, 2007). Other causes of incronguence between gene trees

and species trees are proceses as gene duplication, introgression, gene flow and horizontal gene transfer, deep coalescence, or incomplete lineage sorting.

This discrepancy has been known for decades and is especially problematic for closely related species or species with large population sizes. It hasn't been until last decade that development in computation allowed to implement different methodologies to solve this incongruence. Several methods have been applied but the most widespread are method based on **coalescen theory**, known as **multispecies coalescent model** which is base on bayesian inference to reconstruct species tree from different gene trees using an MCMC algorithm to estimate posterior distribution of the species tree(Rannala & Yang, 2003; Heled & Drummond, 2010).

The coalescence (Kingman, 1982; Rosenberg & Nordborg, 2002) is based on the reconstruction of evolutionary history of genetic lineages until to arrive to the most recent common ancestor (MRCA) taking into account the genealogy and the allelic frequencies of the genes (Kingman, 1983). Under this model it is assumed that each gene tree represents the relationship between orthologous genes (derived from a common ancestor) and assumes no events of horizontal gene transfer, hybridization, gene flow and introgression(Heled & Drummond, 2010). Among the programs that implement this model is * **BEAST**, which it is part of Bayesian phylogenetic inference package BEAST (Drummond & Rambaut, 2007; Heled & Drummond, 2010)

2.2.3 Estimation of divergence times

The possibility of dating speciation events is one of the major objectives of evolutionary studies (Lepage et al., 2007) This was possible thanks to the development of the hypothesis of molecular clock.

The **molecular clock hypothesis**, (Zuckerkandl & Pauling, 1965), is one of the most influential concepts in modern evolutionary Biology (Arbogast et al., 2002). This hypothesis proposes that genes evolve at constant rates through time. The molecular clock converts measures of genetic distance between sequences into relative estimates of divergence between two lineages. This hypothesis has revolutionized the way researchers address temporal questions in evolutionary

biology because allowed thorugh calibration of the clock is possible to obtain absolute ages and estimate a timeframe for species diversification.

Rate heterogenity

One of the most fundamental debates concerns the degree to which rates across lineages, are heterogeneous. When lineages do not follow a strict molecular clock, it is necessary to apply other methods that consider these heterogeneity. **Heterogeneity** will almost always confound attempts to accurately estimate evolutionary dates of divergence. The reasons for this heterogeneity are diverse, and include issues such as differences in population sizes, the generation times of the species, or changes in mutation rate due to environmental factors, differences in the DNA repair machinery, and different metabolic rate of the species (Bromham & Penny, 2003; Rutschmann, 2006).One way to solve this problem was the developing of methods that introduced autocorrelated and uncorrelated relaxed clocks that permit variation of evolutionary rates trough time (Sanderson, 1997; Thorne & Kishino, 2002; Drummond et al., 2006; Rannala & Yang, 2007).

One of this methods has been implemented in BEAST (Drummond & Rambaut, 2007) that does not assume autocorrelation of the rate. Non auto-correlated rates means that the rate associated with each branch is drawn from a single underlying parametric distribution such an exponential or log-normal (Heath et al., ; Drummond et al., 2006; Lepage et al., 2007). Nowadays is the widespread option for estimating divergence times. This software uses a Bayesian approach and the MCMA algorithm to estimate posterior distributions of substitution rates and divergence times. Some reasons behind the widely use of Beast is because allows relaxed methods that draw branch lengths from a lognomal or exponential distribution; the topology and rates are estimated simultaneously ,allowing integration of uncertainty into the analyses (confidence intervals of the parameters estimates).

For calibrating the clock to establish an absolute (meaning geological) timescale is necessary to incorporate of one or more calibrations derived from external sources (Kodandaramaiah, 2011).

One possibility is to extrapolate universal rates. For instance, in arthropods one of the most widely used substitution rates is the 2.3% pairwise sequence divergence per million years for the mtDNA (Brower, 1994) Recently, a spider specific mitochondrial substitution rate has also been estimated for the ground-dwelling genus *Parachtes* (Bidegaray-Batista & Arnedo, 2011)(Bidegaray-Batista *et al.* 2011) and in mygalomoprhs one specific nuclear substitution rate for the nuclear gene Elongation Factor 1 – Gamma (Opatova et al., 2013; Opatova & Arnedo, 2014b). The use of a substitution rates, either universal or inferred for closely related groups, is based on the assumption that the substitution rates in the group of interest are the same, which may not be true. For example, an accelerated rate of mitochondrial genes has been reported in mygalomorph spiders(Bond et al., 2001) and scorpions (Gantenbein & Eightley, 2004).Other possibility is to introduce calibration points based on paletontolgical or biogeographic evidence. Those calibration can be fixed ages, such as vicariant biogeographic events biogeographic events such as the emergence of isolation of Balearic Islands during Messinian Salinity Crisis(Lalueza-Fox et al., 2005) (Lalueza-Fox et al. 2005) or the separation of Corsica-Sardinia microplate (Ketmaier & Caccone, 2013). It also can be minimum and maximum constraints, for example fossils provide lower bounds, and the emerge of oceanic islands provide upper bounds.

2.2.4 Species delimitation under the coalescence

As already mentioned above fact, very few topics in biology have raised as much debate as the species concept (Cracraft, 1989; Claridge et al., 1997; de Queiroz, 1998, 2005; Hei, 2001; Coyne & Orr, 2004)., The main problem is that the species concept has been confused with the issue of species delimitation itself. De Queiroz (2005,2007) proposed a solution under the name of the unified *species concept*, where a species is defined as a “metapopulation that evolves independently”; and allows to distinguish between what a species is and the different lines of evidences used to recognise it.

The centrail aim of coalescent-based approaches is to identify independently evolving linages (Fujita et al., 2012). The **General mixed yule coalescent method** (Pons et al., 2006; Fujisawa & Barraclough, 2013) is used for used to

define putative evolutionary lineages. Combines a model of stochastic lineage growth (Yules model) with a coalescence null model to determine the point of transition from species-level to population-level evolutionary processes. The GMYC is implemented in the R package SPLITS (Species Limits by Threshold Statistic, Ezard et al., 2009) and needs an ultrametric tree, that can be inferred with BEAST. Its widely known that the GMYC approach tends to over split the data (Esselstyn et al., 2009; Talavera et al., 2013; Hamilton et al., 2014; Kekkonen & Hebert, 2014) and thus is better interpreted as a first step into the assignment of the individuals into putative species for subsequent analyses (Edwards & Knowles, 2014) The final decision on what constitutes an actual species will further require the integration of different sources of evidence (Kekkonen & Hebert 2014).Nowadays there are sereval approximations and methodologies for DNA-based species delimitation (Carstens et al., 2013; Satler et al., 2013)and plenty of them use the coalescent approach such as: BPP, SpeDeSTEM, Brownie, Bayesian Computation (ABC) and PTP**.**

2.2.5 Integration of different sources of evidence

Integrative taxonomy aims to delimit the units of life's diversity from multiple and complementary perspectives (phylogeography, comparative morphology, population genetics, ecology, development, behaviour, etc.) (Dayrat, 2005; Schlick-Steiner et al., 2010) With that purpose different methodologies were included in this thesis.

2.2.5.1 Geometric morphometrics

Traditional methodologies, as morphometrics, difficult the description of cryptic species in the absence of conspicuous divergence of genitalia ((Bond et al., 2003). **Geometric morphometrics** is a quantitative method used to study morphological variation. This method allows studying the variation of shape by defining it mathematically. This discipline defines the concept of **shape** as all geometric features of a structure except its position, orientation and scale (Klingenberg, 2010). GMM has higher power to detect small differences and also subtle differences that can be biologically relevant, and using methods than can reliably find and report this differences, in a easy, quick, cheap and repeatable way.

The role of the carapace in geometric morphometrics with mygalomorph has been questioned recently (Bond & Beamer, 2006) despite spider carapace is a character that is often scored in higher-level analyses of spider relationships. Major clades within the spider infraorder Mygalomorphae have been at least partially defined on the basis of carapace shape(Park et al., 1988; Goloboff, 1993; Bond, 2004). The shape and elevation of the caput have been used in several subsequent studies further evaluating mygalomorph phylogeny (Goloboff, 1993; Bond et al., 2001; Bond & Beamer, 2006)and caput elevation is a character useful for *Nemesia* description (Mora pers obs).Other approximations as implementing outline have shown that carapece is not very informative for phylogenetic studies (Bond, 2004; Bond & Beamer, 2006)

The most widely used approximation is **landmark** approach. Definition and location must be done according to the morphology of the animals and the biological information that can show different structures related with the life cycle and being informative for detect shape differences.Landmark design is crucial for rigor conclusions.The coordinates of landmarks must be recorded and the two-dimensional x, y Cartesian coordinates digitized using the tpsDig program(Rohlf, 2001). The raw coordinates can be proceesed with a very intuitive packge MORPHOJ (Klingenberg, 2011). Principal component analysis(PCA) is used to examine the variation of multiple variables within a single sample and. CVA is the most widely used method for investigating taxonomic differences (Viscosi & Cardini, 2011).

2.2.5.2.Phenologic studies

Phenologic studies are the study of periodic animal cycles and how this are influenced by seasonality. To present a comprehensive knowledge about the life history of a species in its natural habitat, this activity must be monitored (Aitchison, 1984; Ferretti et al., 2012b). This kind of study are appropriate for mygalomorph for several reasons, first of all, pitfall trapping will allow to capture males, which are otherwise difficult to capture. And second, phenological studies will provide relevant information on the very poorly known life cycle of mygalomorph spiders.

3. Objectives

Objectives

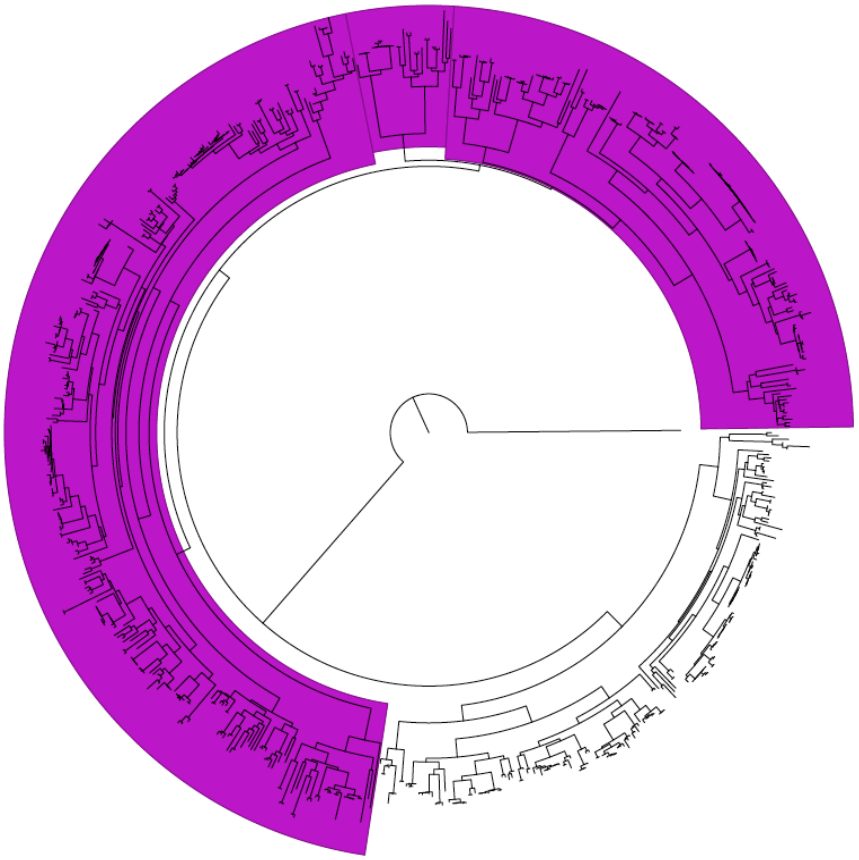
The main aim of this thesis is to study the evolutionary history and drivers of diversification of two genera of trap-door spiders from the Mediterranean region: *Nemesia* and *Iberesia*. To accomplish this, the following objectives were defined:

1) To infer the phylogenetic structure of the genus *Nemesia* and *Iberesia* to confirm their taxonomic status and further test the significance of spinneret morphology for the taxonomy of the group (Chapter 4.1).
2) To infer the phylogenetic relationships of the species rich genus *Nemesia*, estimate a timeframe for its diversification and reconstruct its biogeographic history to elucidate the mechanisms responsible for its high diversity (Chapter 4.1).
3) To infer the phylogenetic relationships, estimate a timeframe of diversification and reconstruct the biogeographic history to test whether the low diversity of *Iberesia* the is the result of a recent origin or, alternatively, its diversity has been overlooked (Chapter 4.2).
4) To infer a dated phylogeny and conduct biogeographic reconstruction to determine their origins and colonization pathways of the Nemesiid species endemic to the Balearic Islands (Chapter 4.3).
5) To use integrative taxonomy to identify and describe species and infer biodiversity patterns of *Nemesia* in Tunisia (Chapter 4.4).
6) To identify the mechanisms of species coexistence in *Nemesia* by integrating taxonomic, molecular, phenological and morphometric information (Chapter 4.5)

4.Results

Chapter 4.1

Systematics and biogeography of the genus *Nemesia* (Araneae, Nemesiidae)



Systematics and biogeography of the Mediterranean trap-door genus *Nemesia* Audouin 1826

1.Introduction

The spider infraorder Mygalomorphae is one of the three main evolutionary lineages recognized within spiders and comprises, among others, the tarantulas, the funnel-web spiders and the trap-door spiders and (Platnick & Gertsch, 1976; Bond & Hedin, 2006). Mygalomorphs are currently classified into 15 families, comprising 2.600 species and 300 genera. Despite being less diverse that their sister group the Araneomorphs, or true spiders, mygalomorph have a cosmopolitan distribution, inhabiting all continents except the Antarctica (World Spider catalog, 2015).

Mygalomorphs have been extensively used to address biogeographic and evolutionary questions, among other factors are remarkable their low vagility, long life cycles, narrow distributions and ecological preferences (Pedersen & Loeschcke, 2001; Ballesteros et al., 2002; Bond, 2004, 2012; Hendrixson & Bond, 2005a, Hendrixson & Bond, 2005b; Bond & Hedin, 2006; Bond & Stockman, 2008; Bailey et al., 2010; Satler et al., 2011, 2013; Hamilton et al., 2011; Opatova et al., 2013; Hendrixson et al., 2013; Opatova & Arnedo, 2014a, 2014b).

The Nemesiidae are the most species rich mygalomorph family in the Mediterranean. To date 53 species and 5 subspecies have been catalogued in two genera: *Nemesia* (50 species and 5 subspecies) and *Iberesia* (3 species) (World Spider Catalog, 2015) (see Table 1 for a list of the nominal species and their reported distribution).

The genus *Nemesia* Audouin 1826 was one of the earliest discovered genera of Mediterranean mygalomorph spiders (Moggridge, 1873, 1874; Latreille, 1799). The spiders of this genus are from small to large sized (body length of adults 9–31 mm). The ground colour is brownish with relatively long legs and recurved fovea, which distinguished them from Mediterranean species of the also mygalomorph family Ctenizidae. *Nemesia* species have a cryptic lifestyle; they are nocturnal sit-and-wait predators that construct silk lined burrows, hidden by

a closing a trapdoor. The females are mostly sedentary and some studies suggest that they have long life cycles, living more than 20 years in captivity (Buchli, 1965). Males have shorter life spans, they leave their burrows in search for females after the adult moult and supposedly mediate gene flow between populations. Low vagility has been cited as the main reason for the high level of local endemism in mygalomorph species and for the deep population structure within species (Bond et al., 2001; Bond & Stockman, 2008; Decae, 2012; Opatova et al., 2013; Opatova & Arnedo, 2014b).The different species usually have a restricted distribution and narrow ecological preferences.

Despite their poor dispersal abilities, *Nemesia* is the only mygalomorph genus distributed throughout the Mediterranean basin and is also present in all main Mediterranean islands. Because of its high diversity and wide distribution across the Mediterranean, *Nemesia* offers unparallel opportunities to test explicit biogeographic hypotheses under the light of the well-known geochronology of the region. Unfortunately, the use of *Nemesia* as a model system for evolutionary research has been hampered by the poorly understood taxonomy of the group. After 200 years of the description of its first species, the real species diversity of the genus cannot be estimated (Decae, 2012). The nemesiids, like other mygalomorph groups, are taxonomically challenging, species are phenotypically conservative, and the few variable characters are frequently polymorphic. The male and, in a lesser degree, the female genitalia and the spinnerets provide the main source of diagnostic characters but in many species have not been adequately studied and illustrated (Decae, 2012) To make things worse, old species often lack designated type material and half of the species are only known from one sex, due to their different life cycle. Additionally, there are numerous species awaiting formal description and many more remain to be discovered (Mora pers. obs.).

There is also little consensus in the supraspecific organization of the genus in terms of the possible existence of sub-generic groups (Decae, 2012). Based on the spination pattern, characters of the scopulaea and the embolus and the burrow structure, Simon proposed three species groups within *Nemesia,* namely *Haplonemesia*, *Nemesia sensu stricto* and *Pronemesia*. Subsequently, (Decae, 2010)based on a quantitative cladistics analyses of 27 morphological

characters proposed two main subgenus within *Nemesia*: *Pronemesia* and *Holonemesia* (including *Nemesia sensu stricto* from Simon). The reduction of the spinning apparatus is the easier diagnostic feature to separate the subgenera of *Nemesia*, as well as the closely related genera *Brachythele* and *Iberesia*.

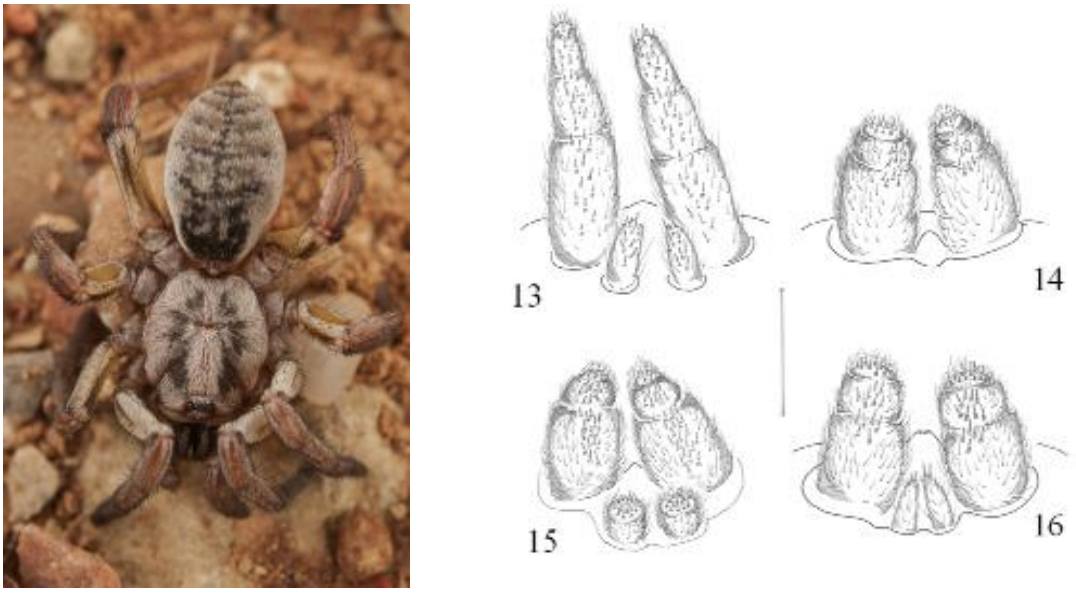


Figure 1. Left: general appareance of *Nemesia*. Right: different degrees of spinneret reduction (adapted from Decae, 2010). Spinnerets 13) *Brachytele*, 14) *Iberesia* 15) *Holonemesia* 16) *Pronemesia*

Pronemesia is distinguished by the conical Posterior Median Spinnerets (PMS) bearing few spigots in the apical part, and the spigots restricted to the apical part of the Posterior Lateral Spinnerets (PLS). *Holonemesia* is distinguished by the thick, knob-shaped PMS spinnerets, and the presence of several spigots distributed along the spinneret both in PMS and PLS. The number and distribution of spinnerets its supposedly related with the burrow construction. The burrow architecture is extremely diverse and may provide additional diagnostic characters at the species level. There is a large diversity of features that characterize the different burrow architectures, including the entrance width, the burrow length, the trap-door shape (e.g. rounded, cogwheel-shaped) and thickness (e.g. waffle-like, cork-like), the amount and distribution of tube-linen silk, the number of galleries (single tube, secondary tubes), the presence

and shape of internal doors, and presence and location of prey remains inside the tube. Some authors have suggested that the architecture of the burrows is related to environmental and biological factors, and that burrows become more complex as the environmental conditions and the intraspecific competition increases (Pickard Cambridge, 1874).

Finally, in a recent study based on the variation in the morphology of both male and female sexual organs (bulbs and spermathecae), Decae (2012) further suggested three male and three female super-specific types with strong geographic structure. The distribution of these genitalic types indicates that the *Nemesia* fauna of the eastern Mediterranean differs markedly from that of the western Mediterranean (Decae, 2012). Unfortunately, this study did not provide any clue to link the genitalic defined types and the formerly proposed subgenera.

All these studies revealed high levels of overlooked subgeneric diversity in this genus, but very few is known about the drivers of diversification that originated this patterns of diversity. In the Mediterranean region. High species richness has been proved that is the result of geology and climatic shifts (Esselstyn et al., 2009). The complex biogeography of the Mediterranean basin may influenced the high diversification of the genus in the region.

From Oligocene to Late Miocene the Mediterranean region suffered extensive changes in the distribution of landmasses. The Mediterranean basin began to form during Oligocene (34-30 Ma) due to a convergence of the African and Eurasian Plates (Krijgsman, 2002; Mansion et al., 2008). At the beginning of the Oligocene (30-25 Ma) the microplates that today are Corsica, Sardinia, Balearic Islands and the area known as the Calabro-Pretorian Massif, the Kabilies and the Betic-Rif area were part of the Iberian Peninsula and the south of France in a region that is known as the Hercynian Belt (Mansion et al., 2008). The broke of the Hercynian Belt involved the subsequent migration of the fragmented plates until they reach their actual positions (Rosenbaum et al., 2002a). At the beginning of this process Corsica, Sardinia and the Calabro-Pretorian Massif started drifting counterclokwise, whereas the Balearic plate and the Kabilies drifted clockwise to the Iberian Peninsula and reached their actual positions.

The closure of the Gibraltar strait at 5.9 Ma drove the dissecation of the Mediterranean basin in a short period of time, known as The Messinian Salinity Crisis (Krijgsman et al., 1999; Jolivet et al., 2006). This process allowed the establishment of land bridges between previously isolated landmasses. The isolation was restablished at 5.3 Ma with the water exchange between the Mediterranean and the Atlantic Ocean (Loget & Van Den Driessche, 2005).

In parallel, the transition from a tropical weather to the seasonality as we known today, and the effects of glacial cycles on Quaternary also contributed the generation of new habitats and connection of previously isolated areas.

The aim of this study is to investigate the origins of the genus *Nemesia* and unravel the drivers of diversification that generated its high diversity in the region. In order to achieve this, we inferred a multi-locus molecular phylogeny that included representatives of the whole known distribution of *Nemesia* to test morphology based supraspecific groups and to trace the evolution of spinneret modifications and burrow architecture. In addition, we identified putative evolutionary lineages and inferred a temporal framework for the diversification of the group.

2.Material and Methods

2.1 Taxonomic sampling

Specimens were collected in 232 localities (Fig. 2) by the authors and collaborators in several collection trips conducted from 2009 to 2013, with special attention paid to the more species rich western Mediterranean region. Further specimens were kindly provided by colleagues. In addition to *Nemesia* specimens, representatives of the closely related genus *Iberesia* were included to test the monophyly of *Nemesia*. One representative of the eastern Mediterranean nemesiid *Brachythele* Ausserer, 1871 and one of the north American genus *Calisoga* Chamberlin, 1973 were included as outgroups. All trees were rooted in the braches joining *Brachythele* with the remaining taxa following (Leavitt et al., 2015). All specimens and localities are summarized in Table 1 (at the end of the chapter).

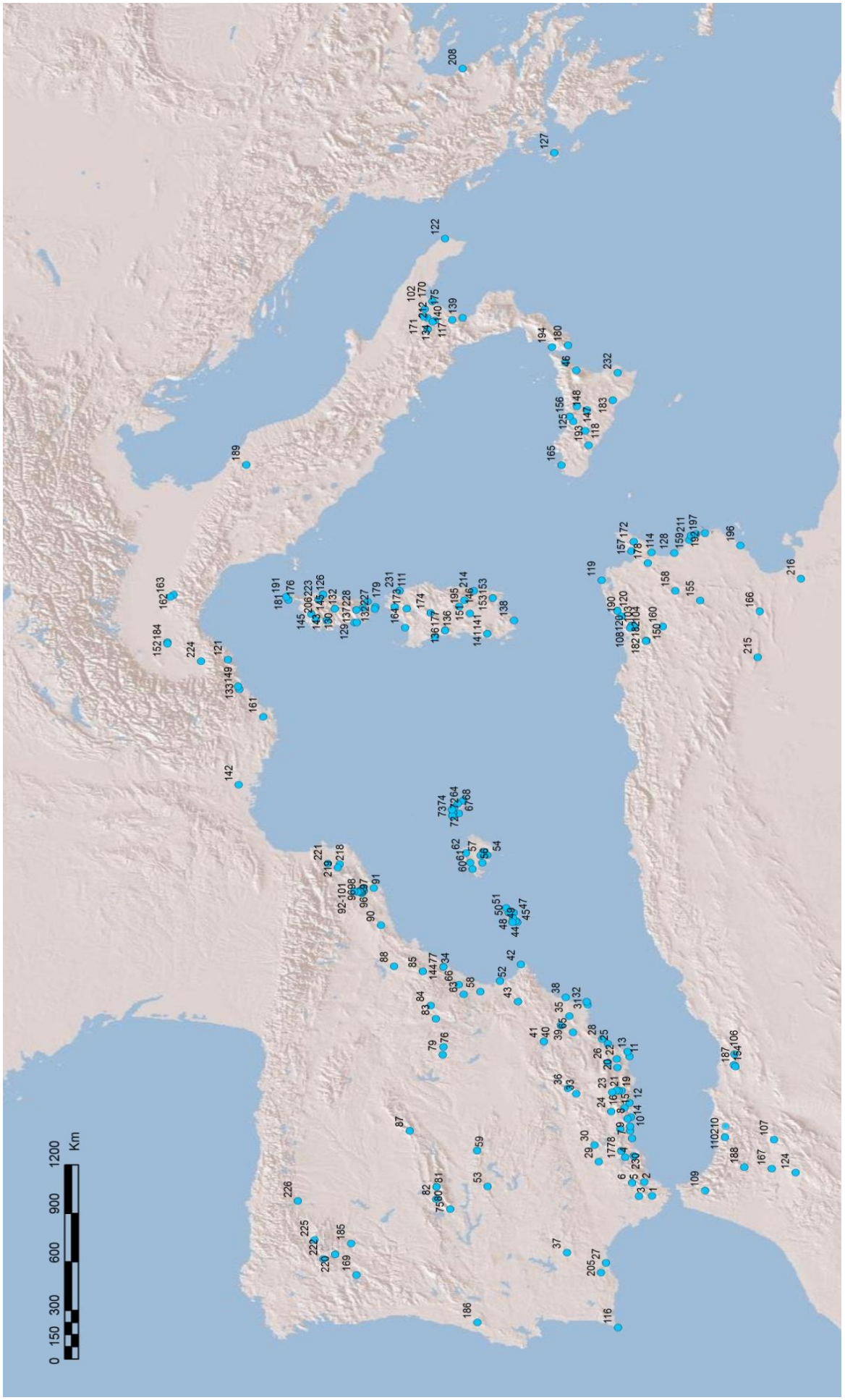


Figure 2. Map of sampling localities included in the present study

2.1 Specimen sorting and identification

All collected specimens were identified to species level when possible with the help of original descriptions and available keys. Specimens were examined with a Leica MZ16A dissection microscope, equipped with a Leica DFC450 digital camera. Female vulvas were removed with the aid of needles, and the muscle tissue was digested with a 35% KOH solution before observation.

One or two legs (depending on the size) were removed and directly preserved in absolute ethanol shortly after specimen collection and stored at -20°C at the freezer facilities of the Department of Animal Biology of the University of Barcelona to ensure DNA preservation. Voucher specimen was preserved in alcohol 70% for morphological studies. All specimen information is summarized in Table 1.

Total Genomic DNA was extracted from legs or pinned abdomens (juveniles) using the DNeasy Tissue Kit (Qiagen) following the manufacturer's protocol. We used a combination of markers most commonly used in mygalomorph systematics for which working primers are available for the focal species, or other spiders Fragments of the following genes were amplified with universal primers: a mitochondrial fragment spanning the 3' half of the 16S rRNA ribosomal subunit (16S), the complete tRNA leu (L1) and the 5' half of the NADH deshydrogenase subunit I (nad1), and fragments of the nuclear genes 28S rRNA (28S), Histone H3 (H3), and Elongation Factor 1 gamma (Ef-1g). Primer sequences and conditions are listed in Table 2. The final volume of each PCR reaction was 25 mL, and used approximately 15 ng of genomic DNA with the following final concentrations of: a unit of Taq DNA polymerase (Biotools), 1.8 mM MgCl2, 0:25 mM of each primer, and 0.5 mM The purified PCR products were sequenced at Macrogen Inc. (Seoul, South Korea). Raw sequences were assembled and edited using Geneious (v. 5.3.7) (http://www.geneious.com, Kearse et al., 2012).

Gene	Primer		Sequence	Annealing Tº	Reference
16S	16SA	F	CGCCTGTTTATCAAAAACAT	48-52º	(Simons et al., 1994)
	Nem_nd1	R	GCC ACT CCT CGA ATT CTT CC		(Mora et al., submitted)
H3	H3aF	F	ATG GCT CGT ACC AAG CAG ACV GC	48º	(Colgan et al., 1998)
	H3aR	R	ATA TCC TTRGGC ATR ATRGTG AC		(Colgan et al., 1998)
28S	28SO	F	GAAACTGCTCAAAGGTAAACGG	50-52º	(Giribet et al., 1999)
	28SB	R	TCGGAAGGAACGAGCTAC		(Giribet et al., 1999)
	28SC	R	GGTTCGATTAGTCTTTCGCC		(Giribet et al., 1999)
Efg	ER1gF78	F	GGCAACAACCAGCTCGTGGA	TD 56 to 46º then 42º	(Ayoub et al., 2007)
	EF1γR1258	R	GTGCTGTTATTATCTTCGCC		(Ayoub et al., 2007)
	Ef-gNkf	F	GTWGGCAACAAYCAGCTSCGKGG	50-52º	(Mora et al., submitted)
	Ef-gNkr	R	TGYTGTCTTCACCAAARAGGC		(Mora et al., submitted)

Table 2.- Primers and annealing temperature (T) used in this study (see text for details). TD: touch down

2.2 Alignment and evolutionary model selection

Ribosomal DNA sequences were aligned using the online version of MAFFT v. 6 (available at http://mafft.cbrc.jp/alignment/server/, (Katoh & Toh, 2008). The protein coding sequences were translated into amino acids to confirm that no stop codons were present. Unlike H3 gene fragments, the nad-1 and Ef-1g sequences showed evidence of indel mutations. Therefore, alignments were build using translated sequences and the MAFFT algorithm as implemented in the program TranslatorX (Abascal et al., 2010) (available at http://www.translatorx.co.uk/).

Gaps were recoded as presence/absence data following the simple method of Simmons & Ochoterena, 2000 as implemented in the program SeqState v1.4.1 (Müller, 2005). This gap treatment facilitates including gap information into the analyses without increasing the weight of overlapping multiple non-homologous gaps (Pons & Vogler, 2006), and are amenable to likelihood, Bayesian and parsimony phylogenetic inference methods. Identical haplotypes were removed from the data matrix for phylogenetic analyses.

Preliminary maximum likelihood analyses (see below for the programs and options implemented) on each gene separately confirmed that there were not conflicting supported clades between the gene trees (results not shown). Concatenation of individual gene matrices was conducted with Geneious (Kearse et al., 2012). Model based analyses were conducted using the best partitioning scheme and corresponding evolutionary models selected by

Bayesian Information Criterion index, as implemented in the program PARTITIONFINDER (Lanfear et al., 2012). Because of the large number of taxa, Bayesian analyses had to be conducted with simpler models to facilitate convergence.

Partition	Model	Partition	Model	Partition	Model
16s-L1	GTR+I+G	16s-L1	GTR+I+G	mitoc	GTR+I+G
*nad1*_2	GTR+I+G	*nad_1*	GTR+I+G		
*nad1*_3	K81uf+G		K81uf+G		
*nad1*_1	HKY+I+G		TVM+G		
28s	GTR+I+G	28s	GTR+I+G	28s	HKY+I+G
H3_2	GTR+I+G	H3	TVMef+I+G	H3	HKY+I+G
H3_3	SYM+G				
H3_1	JC+I				
EF1g_2	K80+G	EF1g	HKY+I+G	EF1g	HKY+I+G
EF1g_3	HKY+I				
EF1g_1	TVMef+G				

Table 3. Partition schemes and corresponding best models used in the present study

2.3 Delimitation of independent lineages

The Generalized Mixed Yule-coalescent (GMYC) method (Pons et al., 2006; Fujisawa & Barraclough, 2013) was used to define coalescent clusters based on the mtDNA information. This method combines a model of stochastic lineage growth (Yules model) with a coalescence null model to determine the point of transition from species-level to population-level evolutionary processes (Fujisawa & Barraclough, 2013).

The GMYC method requires an ultrametric tree, which was inferred with the computer program BEAST v.1.8.1 (Drummond & Rambaut, 2007) defining a single gene partition for the whole mtDNA dataset with the model selected by Partitionfinder. A lognormal relaxed clock prior was selected with the ucld.mean parameter set to 1 (i.e. Relative branch lengths). A constant population size was used as tree prior following Monaghan et al. 2009. Convergence between runs

and correct mixing within each run were visualized with TRACER (Drummond & Rambaut, 2007).

Individual runs were combined using the BEAST accompanying program LOGCOMBINER. The first 10% of the generations of each run was discarded as a burn-in. A consensus chronogram was inferred with the accompanying program TREEANNOTATOR. The GMYC analysis was carried out in the R (R Core Team, 2013,http://www.r-project.org) environment with the help of the SPLITS package (Ezard et al., 2009) using the inferred ultrametric tree. The results of the GMYC were used as guide to sample nuclear genes, such as each GMYC was at least represented by two individuals sequenced for nuclear genes.

2.4 Phylogenetic Inference

Maximum Likelihood (ML) analyses were conducted on RaxMLv.7.2.7 on XSEDE and runned remotely at the CIPRESS computer resources (Miller et al., 2010,www.phylo.org). Gene partitions were assigned a GTR model, while gaps were assigned a binary model. In all cases, models included GAMMA and Invariants. The best tree was obtained form 100 random replicates and clade support assessed with 1000 bootstrap replicates (BS).

Bayesian inference analyses were performed using Mr. Bayes v 3.1.2 (Ronquist & Huelsenbeck, 2003) and were runned at the CIPRESS computer resources (Miller et al., 2010, www.phylo.org). Two independent runs of $5x10^7$ generations, 4 MCMC chains each, were conducted simultaneously, starting from random trees, and saving trees and parameters every 1000 generations. The convergence between the chains was assessed by monitoring the standard deviation of the split frequencies between runs (<0.02), and was confirmed with the help of the software TRACER v. 1.5 (Drummond & Rambaut, 2007).

The parsimony analyses were conducted with the program TNT v1.1 (Goloboff et al., 2008). because the size of the matrices we employed new technology algorithms under equal weights. We combined sectorial searches, with tree drift and tree fusion (50 initial addition sequences, initial level: 10, cycles of drifting: 10), and analyses were conducted until the strict consensus remained stable

five times (with default factor of 75). The search strategy followed the author's recommendations for analysing large difficult datasets (Goloboff, 1999). Heuristic search consisted of 1000 iterations of random addition of taxa followed by TBR branch swapping, holding five trees per iteration and conducting a final round of branch swapping on the held trees. Clade support was assessed via jacknife (Farris et al., 1996) resampling using 1000 replicates with individual heuristic searches consisting of 20 replicates of addition of taxa.

2.5 Estimation of divergence times

A time frame for the diversification of *Nemesia* was inferred in a Bayesian framework as implemented in the software BEAST v.1.8.2 (Drummond & Rambaut, 2007). A reduced matrix with 200 terminals was analysed in order to simplify to speed up computation and facilitate convergence of chains. This matrix included one representative of each GMYC cluster to avoid mixing coalescent and species branch lengths, along with the *Iberesia* specimens and the two outgroups (*Brachytele* and *Calisoga)*.

A simpler, by gene partition scheme was defined to facilitate chain convergence. A strict lognormal uncorrelated molecular clock was assigned to each nuclear gene and a relaxed clock was assigned to the mtDNA genes. A normal distribution with mean and standard deviation 0.0113961, 0.00483085 and 0.00117, 0.0065 was specified for the mtDNA and the Ef-1g ucld.mean parameters, respectively, based on available information in the literature (Bidegaray-Batista et al., 2011; Opatova et al. 2013; Opatova et al 2014).

The rate paramters for the H3 and 28S were estimated during the analyses. We set a maximum age of the tree root to 126 Ma, based on recent phylogenomic and phylogenetic evidence (Bond et al. 2014, Levitt et al. 2015). The Yule Process was selected as a tree prior. We enforced monophily of *Nemesia, Iberesia* and *Nemesia+Iberesia* and the sister group relationship of *Brachythele* to the remaining Nemesiidae (after Levitt et al. 2015). Three independent runs of $5x10^7$ generations were run independently. The processing of the sampled parameter values and trees was done as described above for the GMYC analysis

2.6 Ancestral Area Reconstruction

We used the Bayesian discrete phylogeographic approach (Lemey et al., 2009) as implemented in BEAST v.1.8.2 (Drummond & Rambaut, 2007) to infer the biogeographic history. We defined 12 discrete biogeographic areas corresponding to the main islands in the Western Mediterranean (ie. Ibiza, Majorca, Minorca, Corsica, Sardinia and Sicily) and to the main continental terrains (ie. Iberia, Italy, France and North Africa). Based on the geological history, we distinguished two different areas that corresponded to the present-day Iberian Peninsula, the Iberian Massif and the Betics.

The Betics correspond to a microplate that detached, drifted counterclock-wise and re-joined the rest of the peninsula, rising the Betic cordillera (Rosenbaum et al., 2002a). An eastern-Mediterranean area was defined to score the outgroup (*Brachythele*) and the *Nemesia* from Crete and Greece (defined as Greece in the analises). The discrete phylogeographic analysis was run with the same parameters used in the divergence time analysis, and implementing a strict clock and a symmetric substitution models for the geographic area trait. Three independent MCMC chains were run for 50 million generations. The processing of the sampled parameter values and trees was done as described above.

3. Results

3.1 Species identification

We sampled 232 localities, 224 for *Nemesia* and 13 for *Iberesia*. The two genera coexisted in 5 localities. We analysed 513 specimens, 498 *Nemesia*, 13 *Iberesia* and one *Brachythele* and one *Calisoga* . Morphological identification revealed the presence of 15 nominal species among the studied specimens. All specimens used in the present study, with locality information are summarized in Table1.

3.2 Delimitation of coalescent groups

The GMYC algorithm identified 183 entities (CI: 172-206) (p =1.5 10e-7). The distribution of GMYC clusters is indicated in a Map in Fig. 3, and in boxes in the phylogenetic tree in Fig. 4. Most GMYC clusters corresponded to either single or nearby localities Intestingly several exceptions to this pattern were found. Specially relevant are the localities where more than one distant related GMYC were found (highlited using circles in Fig. 3, and orange boxes in Fig. 4). Furthermore, some GMYC groups were found in distant localities (this exceptions are highlighted in yellow in Fig. 4). Those exceptions are here refeared by other of apparition in the figure.

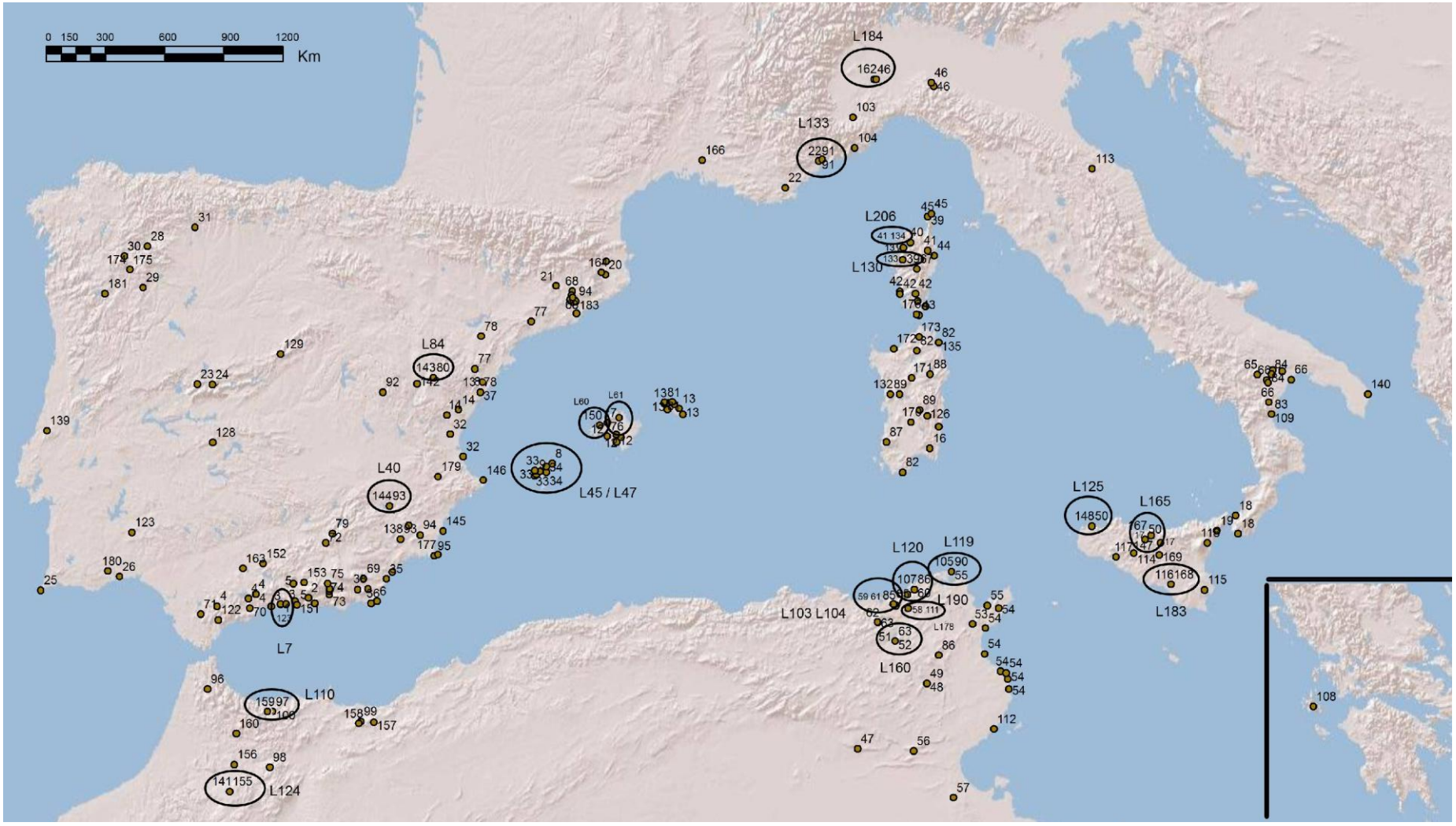
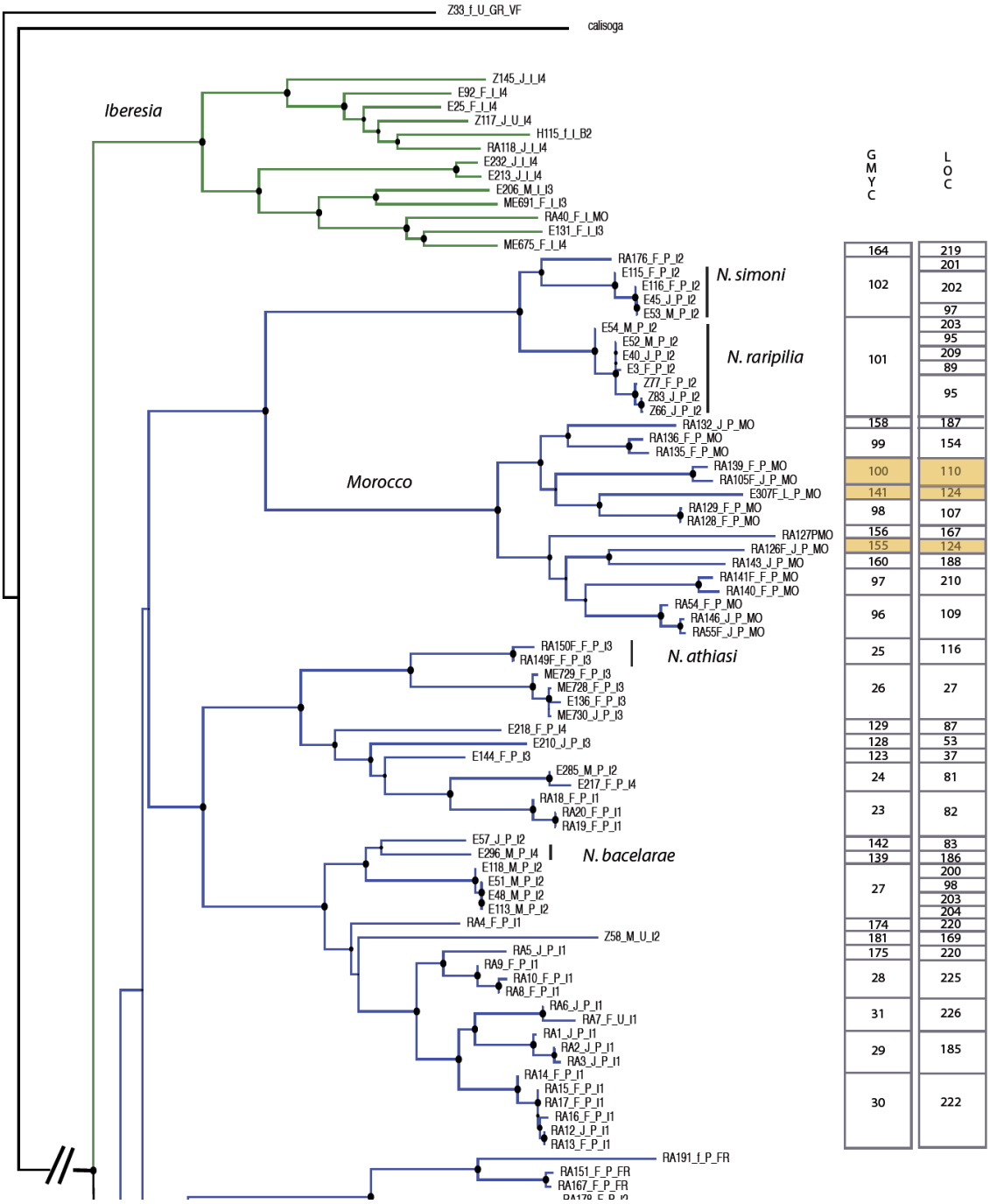


Figure 3. Geographic distribution of GMYC. Circles denote localities including distant related GMYC

Figure 4 Phylogenetic inference for the genus *Nemesia* .ML tree of obtained with Maximum Likelihood with results of GMYC species delimitation method. Clade support for ML is summarized with circle dots indicating suport.Branches' color is according the type of spinnerets, being green for *Iberesia* (no PMS), blue for Pronemesia (cone-shaped PMS, few spigots) and red for Holonemesia (Knob-shapped spinnerets , densely covered by spigots). The first column indicates the GMYC cluster for every specimen. The second row indicates the locality number for every specimen. In orange localities where non-sister GMYC are present, in yellow distant localities included in the same GMYC cluster.



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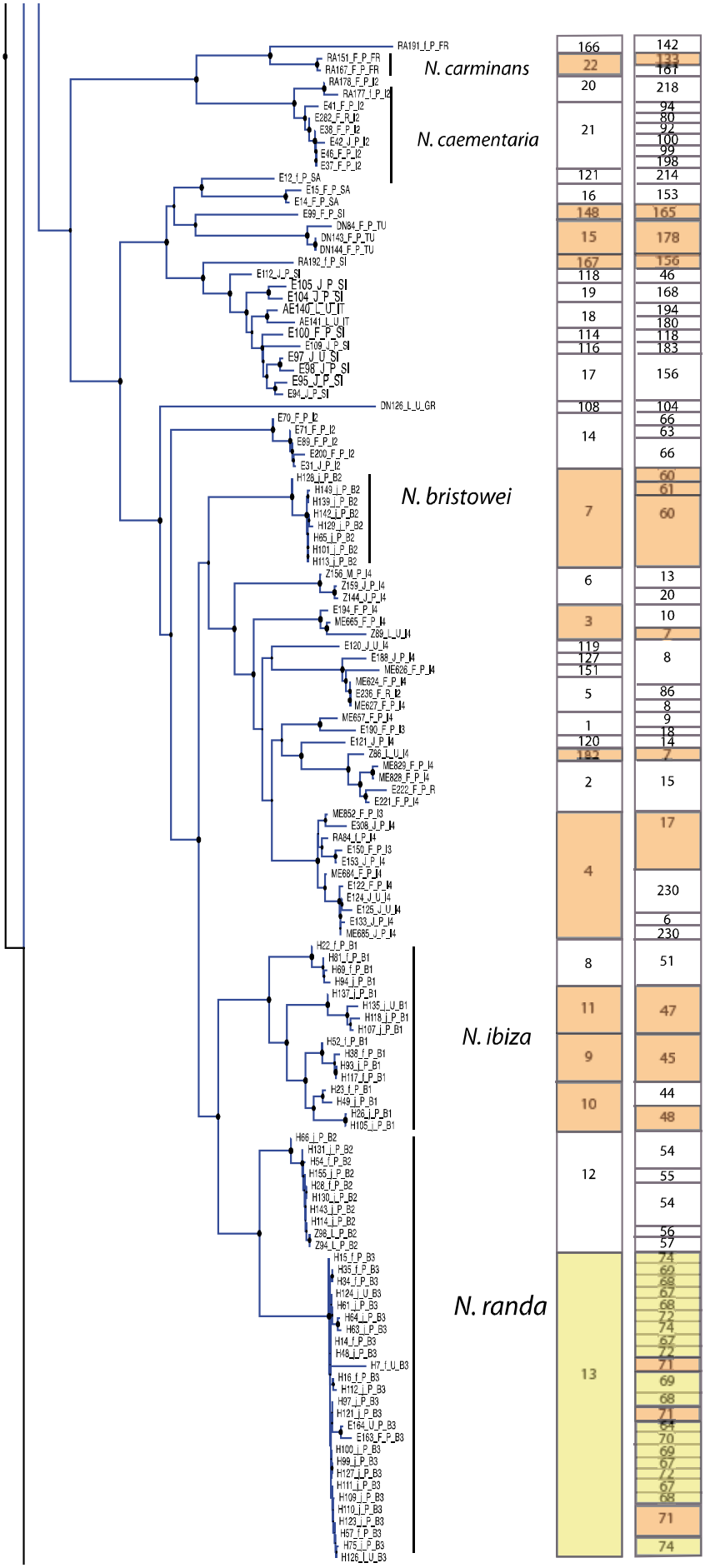

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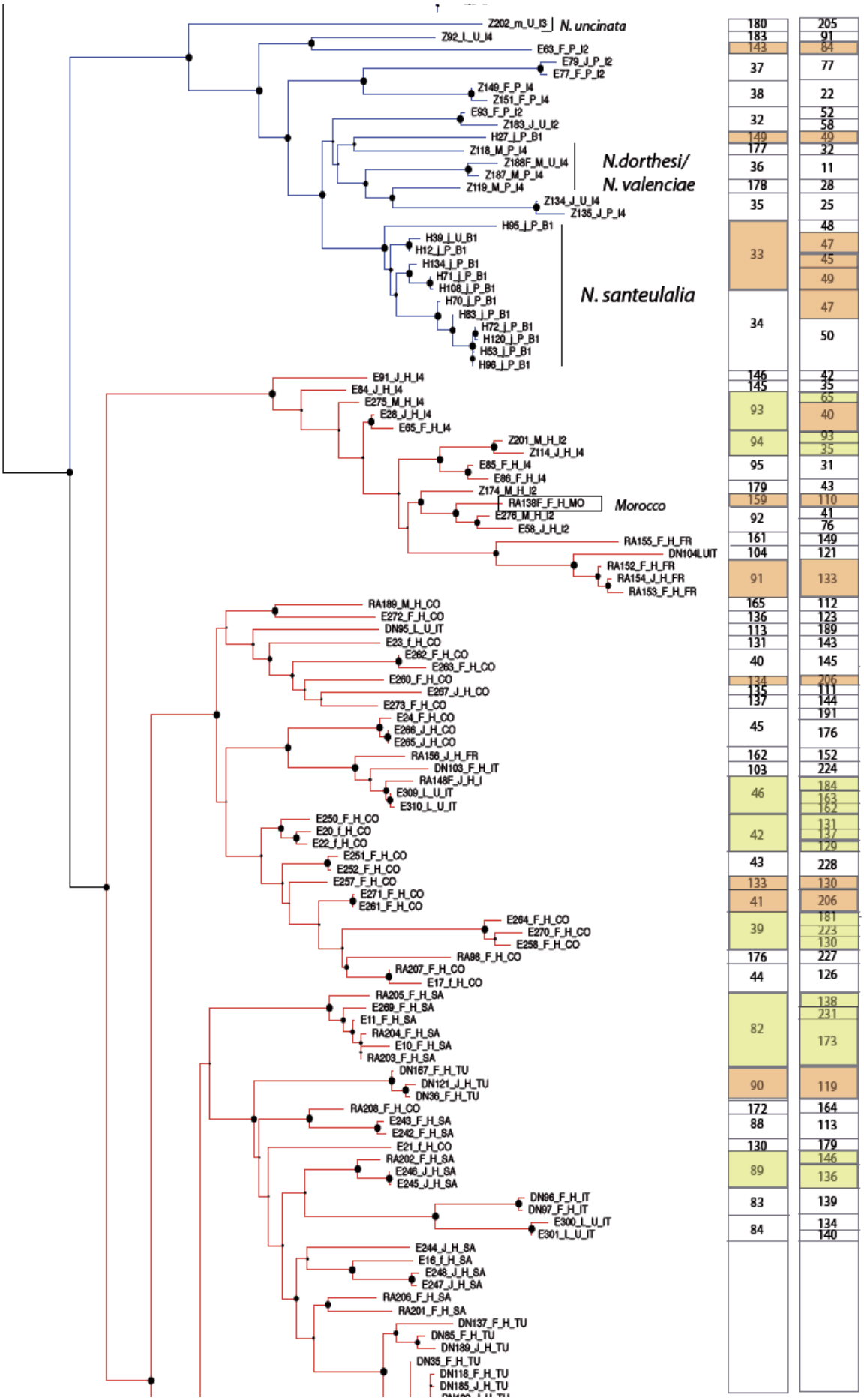
Z202_m_U_I3
N. uncinata
Z92_I_U_I4
E63_F_P_I2
E79_J_P_I2
E77_F_P_I2
Z149_F_P_I4
Z151_F_P_I4
E93_F_P_I2
Z183_J_U_I2
H27_I_P_B1
Z118_M_P_I4
Z188F_M_U_I4
Z187_M_P_I4
Z119_M_P_I4
N.dorthesi/
N. valenciae
Z134_J_U_I4
Z135_J_P_I4
H95_I_P_B1
H39_I_U_B1
H12_I_P_B1
H134_I_P_B1
H71_I_P_B1
H108_I_P_B1
H70_I_P_B1
H83_I_P_B1
H72_I_P_B1
H120_I_P_B1
H53_I_P_B1
H96_I_P_B1
N. santeulalia
E91_J_H_I4
E84_J_H_I4
E275_M_H_I4
E28_J_H_I4
E65_F_H_I4
Z201_M_H_I2
Z114_J_H_I4
E85_F_H_I4
E86_F_H_I4
Z174_M_H_I2
RA138F_F_H_MO
Morocco
E276_M_H_I2
E58_J_H_I2
RA155_F_H_FR
DN104LUIT
RA152_F_H_FR
RA154_J_H_FR
RA153_F_H_FR
RA189_M_H_CO
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E23_f_H_CO
E262_F_H_CO
E263_F_H_CO
E260_F_H_CO
E267_J_H_CO
E273_F_H_CO
E24_F_H_CO
E266_J_H_CO
E265_J_H_CO
RA156_J_H_FR
DN103_F_H_IT
RA148F_J_H_I
E309_I_U_IT
E310_I_U_IT
E250_F_H_CO
E20_f_H_CO
E22_f_H_CO
E251_F_H_CO
E252_F_H_CO
E257_F_H_CO
E271_F_H_CO
E261_F_H_CO
E264_F_H_CO
E270_F_H_CO
E258_F_H_CO
RA98_F_H_CO
RA207_F_H_CO
E17_f_H_CO
RA205_F_H_SA
E269_F_H_SA
E11_F_H_SA
RA204_F_H_SA
E10_F_H_SA
RA203_F_H_SA
DN167_F_H_TU
DN121_J_H_TU
DN36_F_H_TU
RA208_F_H_CO
E243_F_H_SA
E242_F_H_SA
E21_f_H_CO
RA202_F_H_SA
E246_J_H_SA
E245_J_H_SA
DN96_F_H_IT
DN97_F_H_IT
E300_I_U_IT
E301_I_U_IT
E244_J_H_SA
E16_f_H_SA
E248_J_H_SA
E247_J_H_SA
RA206_F_H_SA
RA201_F_H_SA
DN137_F_H_TU
DN85_F_H_TU
DN189_J_H_TU
DN35_F_H_TU
DN118_F_H_TU
DN185_J_H_TU

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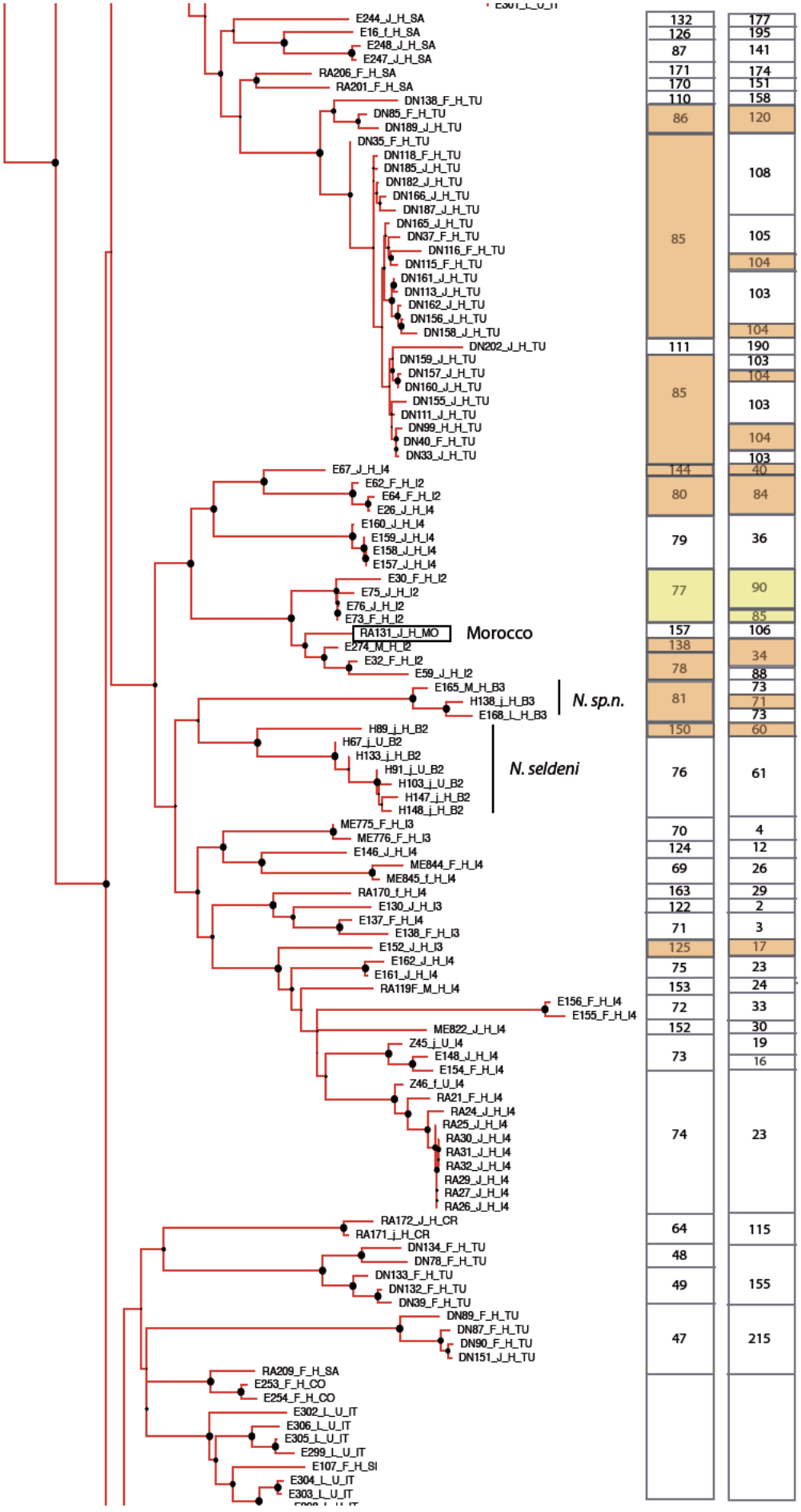
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E73_F_H_I2
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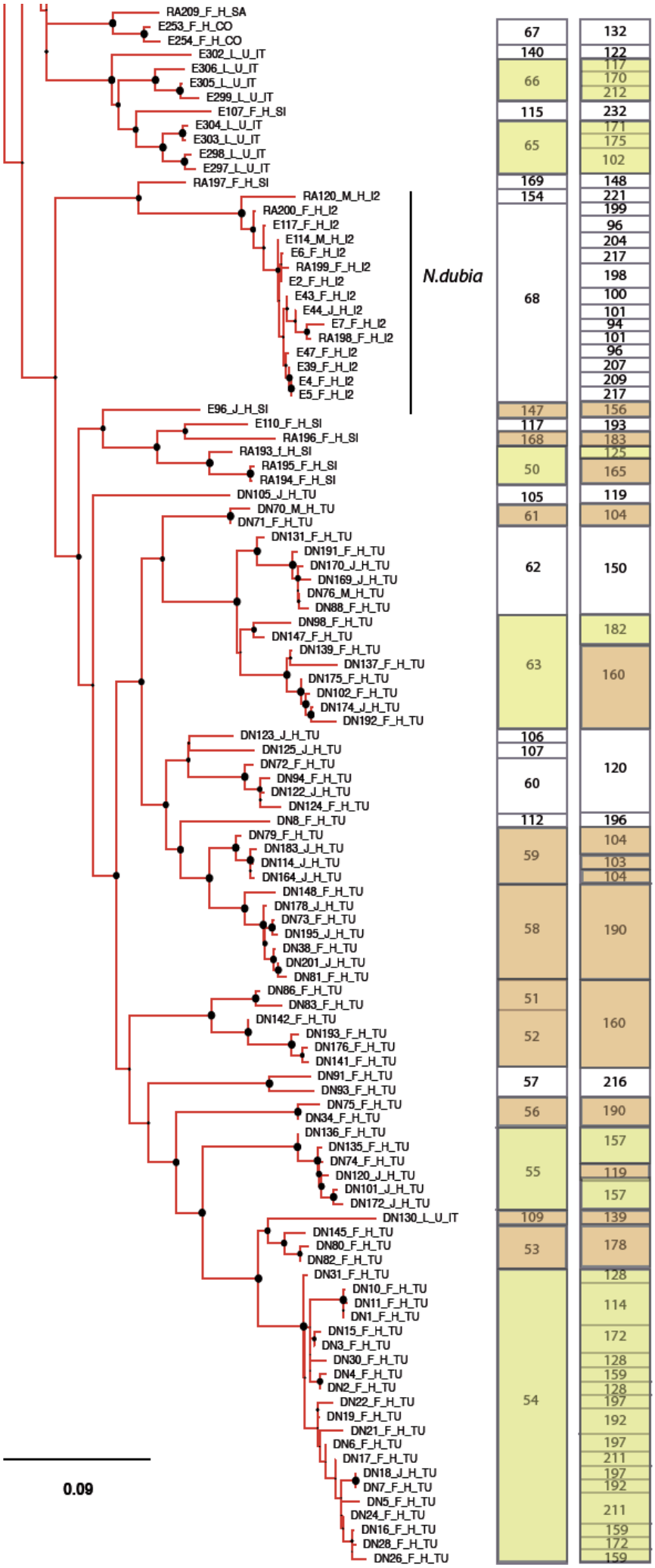
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The first interesting case is locality 110, in Beni-Hadifa, in Morocco , includes two distant related GMYC : G100 (RA105, RA139, both are Pronemesia) and G159 (RA138, which is an Holonemesia). Locality 124, includes two not related GMYC: G141 and G155, both identified as Pronemesia specimens.

In locality 133, Col de Vence, South of France, was found one specimen belonging to GMYC 22 (identified as *N. carminans*, Pronemesia) and other specimens included in GMYC 91 which are Holonemesia (RA152, RA153, RA153) This pattern is repeated: in Luppino ,Sicily referred as locality 165, 2 GMYC are found: G148 and G50 belonging to Pronemesia and Holonemesia respectively. The same pattern is repeated for locality 178, Oued Ez Zid (Tunisia), where GMYC185 includes DN84,DN143, DN144 (the only Pronemesia specimens found in Tunisia) and GMYC 53 including the Holonemesia specimens DN145, DN80, DN82

Same pattern is repeated in several localities along the Balearic Islands, where at least two or three different GMYC's corresponding to already different identified species are found (see chapter 4.3 for a detailed study on Balearic Islands fauna). In Majorca we found this pattern in locality 60 (Can Planiol) where co-occur *N. bristowei* (GMYC 7) and *N. Seldeni* (GMYC 150) , and in locality 61 again *N. bristowei* (GMYC 7) and *N. Seldeni* (GMYC 76).

Malaga, in Locality 7 (South of Spain), includes specimen Z89 (GMYC 3) and specimen Z86 (GMYC 123), despite both are Pronemesia specimens, are not sister groups. Same pattern of coexistence of Holonemesia and Pronemesia is found in Torcal de Antequera , Locality 4 (south of the Iberian Peninsula), were two distant GMYC are found: G4 including a large sample of pronemesia specimens from nearby localities and G125 including only E152 which is an Holonemesia.

Specimens identified as *N. Ibiza* were recovered in 4 GMYC sister groups, corresponding to closer localities, in which ones another distant GMYC were obtained. In locality 47 , Santa Eularia des Riu (Ibiza) three different GMYC groups were found, two of them GMYC 33 and GMYC 34 were sister groups and corresponding specimens were identified as *Nemesia* santeulalia, and GMYC 11 (including H135,H118 and H107) were identifies as *N. Ibiza*. This

pattern is repeated in several localities along the Balearic Islands, where at least two or three different GMYC's corresponding to already different identified species are found (see chapter 4.3 for a detailed study on Balearic Islands fauna). In locality 45, Sa Talaia (Ibiza, Balearic Islands) two distinct GMYC clusters were found corresponding to N. Ibiza (G9) and to N. Santeulalia (G33) In locality 48 two different and distant related gmyc's are found corresponding to N. Ibiza (G10) and *Nemesia* santeulalia (G33).

Different patterns were found where the GMYC appeared in distant localities. An interesting pattern is found in GMYC 13, which includes all specimens identified as *Nemesia randa* from Minorca belonging to distant localities. Only one of the eight localities included more than one GMYC. This was locality 71 including specimens that was not possible to assign no any described specie (see chapter 4.3). Another example of coexistence of Pronemesia and Holonemesia is found in locality 84, namely Cedrillas, North East of Iberian Peninsula, which includes G143 (E63, Pronemesia) and G80 (E62, E64,E26, Holonemesia). Specimens identified as *N. Santeulalia* were clustered in G33 and G34. Specimen H27 which was recognized as *N. Santeulalia* does not cluster with them and appears in G149 (see chapter 4.3 for more detailed information on Balearic Islands).

Regarding to the big Holonemesia clade (Fig. 4, page 3), the first split shows a very interesting pattern of distant localities including two sister gmyc, GMYC 93 is found in Sierra de Espuña (Murcia) and in Sierra de Segura (Loc 40). At the same time, locality 40 includes two distant related GMYC belonging to Holonemesia specimens G93 (E28, E65) and G144 (E67). This cluster appears in a closer locality (65), namely Sierra de Espuña (Murcia). The sister GMYC group, G94 appears in locality 93 (Murcia, south east of the Iberian Peninsula) and Castellar del Vallès (near Barcelona, northeast of the Iberian Peninsula).

The cluster G46 appears in three localities, in Italy, Locality 184 distant from L162-163. Locality 184, in addition, includes other GMYC cluster distantly related G162.

Regarding the Corsican groups two remarkable instances of the same cluster in separated localities are found: GMYC 42 (L131,L137,L129) and GMYC 39

(L181,223,130). Furthermore, there are two localities 130 and 206, that include distant GMYC groups. Locality 130 namely Col da Vizzabona , contains GMYC 133 (E271) and GMYC 39 (E258), both are Holonemesia specimens. The same pattern of two different Holonemesia lineages is shown in the other locality (206) namely Sant Cristophe, includes GMYC 41 (E261,E271)and GMYC 134 (E260).

Interestingly, the fauna of Sardinia shows two instances of GMYC clusters that appeared in distant localities. Those cases are, GMYC 82, formed by specimens belonging to distant localities 138 (E269) , 231 (E11) which is near to 173 (RA204, E10, RA203). This pattern is repeated with the clade including RA202, E246 and E245, where both are included in G89 but were found in localities 146 (Gadoni) and 136 (Cuglieri)

The GMYC 90, is formed by specimens from Tunisia sampled in Locality 119 (Cap Blanc). In this locality 3 different and non sister GMYC clusters were found: GMYC 90 (DN167, DN121, DN36); GMYC 55 (DN120) and GMYC 105 (DN172). This pattern is repeated several times in the Tunisian *Nemesia*. For instance, in locality 120 (Cap Negre) includes four different GMYC clusters, despite this time two of them are sister groups: G60, G85, G106, G107.

GMYC 77 is formed by specimens sampled in distant localities , namely Penya Roja (90) in the North East of the Iberian Peninsula and VallBona (85) in the east coast of the Iberian Peninsula. Different pattern can be found in locality34 (Penya Roja), where distant GMYC G78 and G138 were found.

Regarding the Holonemesian specimens from Balearic Islands, a lineage that was not possible to assign to any known specie was found (E165, E168, H138) in locality 71, as mentioned above, also included G13 in the same locality corresponding to specimens identified as *Nemesia randa*. The lineage including *Nemesia seldeni* specimens was found in two closer localities (L60, G150 and L61, G76), one of them Locality 60 also included specimens identified as *Nemesia bristowei* (G7, Pronemesia)

Relevant instances of GMYC in distant localities are present in the fauna of the South of Italy. Here referred as their appearance order in Fig. 5 (page 5): the GMYC 66 includes specimens from the distant localities L117, L170 and L212.

The same pattern is repeated in the sister group clade, including G65 from localities L171, L175, L102.

A different pattern is found in other Sicilian samples, included in G50 from distant localities 125 (Cerda) and 165 (Luppino). This last, at the same time includes samples belonging to G148 (Pronemesia).

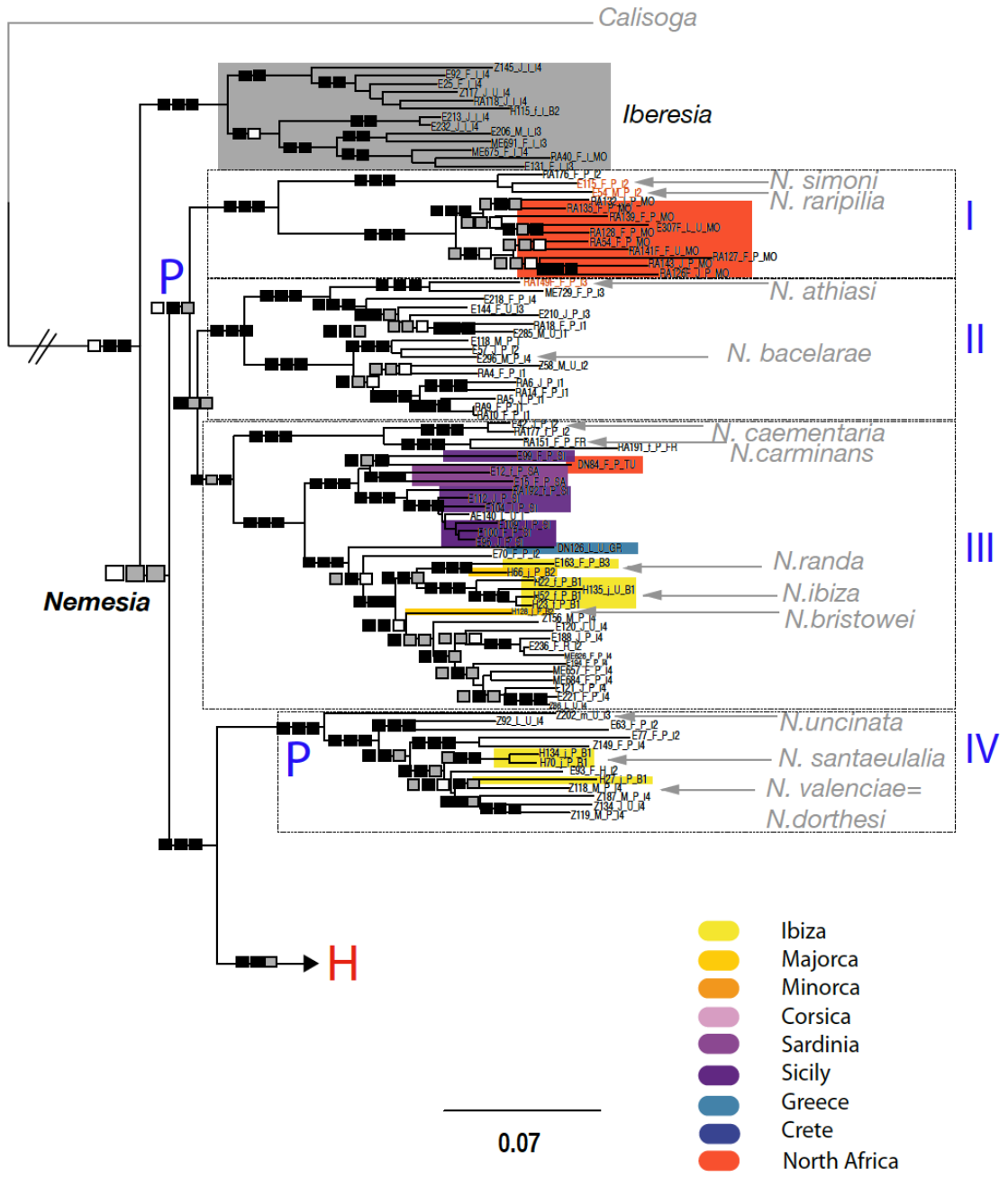
Other interesting case is Locality 165 corresponding to Isnello, Sicily, where we found 3 distant gmyc. This locality contains, G167, together with distant related Pronemesia specimens E97 and E98 (G17) and E96 (G147). A third case of distant related GMYC in Sicily appears in Ponte Olivo (Loc 183) where we found GMYC 116 and GMYC 168.

Despite the fact that some specimens from Tunisa every GMYC correspond to single or nearby localities, there are some remarkable patterns that must be highlighted. Several localities include more than two or even three distant related GMYC. Is the case of Ain Draham 1(L103) that includes G59 and G85; Le Kef (L160) that includes G51, G52 and G63; Aind Draham 2 that contains G59, G61 and G85; and Nezca (L190) that includes G58 and G111. Other relevant pattern show in three instances in Tunisian fauna are three GMYC groups that were found in several distant localities , by order are as follows: G63 in localities 182 and 160 ; G55 in localities 119 and 157; and G54 found in 7 different localities, (detailed study on Tunisian specimens in Chapter 4.5)

3.2 Phylogenetic Inference

Two datasets were used to infer the phylogenetic relationships in the genus *Nemesia*. The first dataset, hereafter referred as the complete dataset, included all specimens (513). The second dataset, hereafter referred as the reduced dataset, included 200 specimens, one for each GMYC cluster identified. Both matrices included 3,339 characters, distributed as follows: 674 characters corresponding to the aligned 16S+L1, 411 to the *nad1*, 800 to the 28S, 328 to the H3, 816 to the Ef-1g and 336 to gaps coded as absence/presence binary characters. The preferred partition corresponded to a by gene for ribosomal and by codon position for protein coding genes scheme (11 partitions, see Table 2).

Results of the ML analyses of the complete data matrix are summarised in Fig.. 4 and results of the ML and MP analyses of the reduced matrix in Fig. 5, only including one specimen by GMYC cluster with identified species and coloured according main Mediterranean islands. The parsimony analysis found 72 trees of 15,718 steps. The ML and parsimony trees were congruent for the most part. BI does not recover the relationship of *Nemesia* and *Iberesia* as sister groups, although internal nodes are not supported.



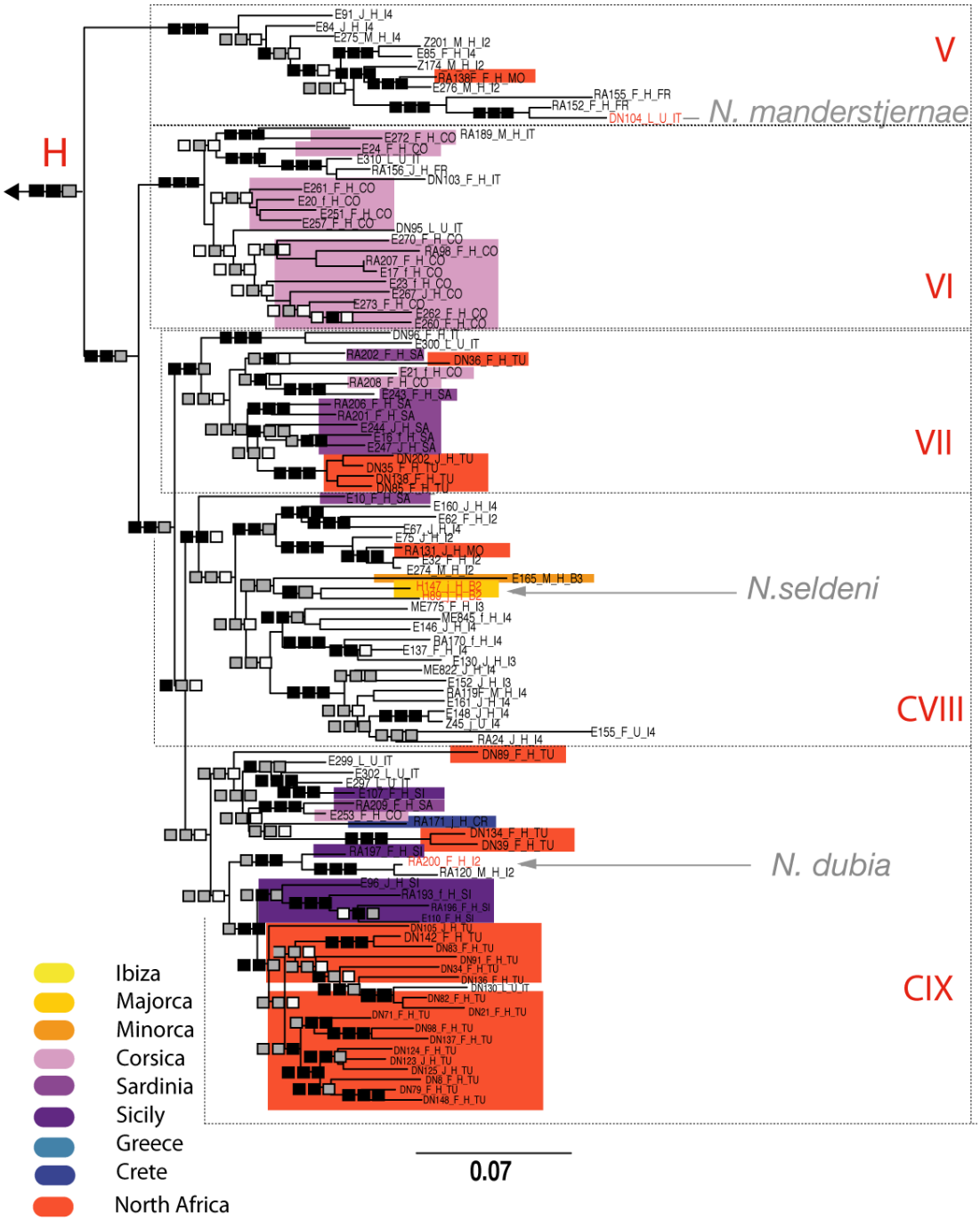


Figure 5. Results of the model based of the reduced concatenated data matrix. Clades are colored according geographic distribution. Boxes on branches indicate suport for the diferent analyses (BI.ML,MP) as follows: black box indicates ML bootstrap, Parsimony Jacckife >75% or Posterior Probability>0.95, grey indicates clade recovered below the formwer thresholds, white box indicates clade not recovered. Color codes are accodring to main islands on the Mediterranean Region.

Analyses of both matrices recovered the reciprocal monophyly of the nemesiids genera *Nemesia* and *Iberesia* albeit with for the *Nemesi*a clade. The big clade including most of Pronemesia is divided into three lineages named Clade I, Clade II, Clade III. Clade I and Clade II are well supported by all methods, despite the relationships of Clade II and III are only supported by BI.

Clade I includes two main lineages. The first branching lineage is the one that includes specimens from Catalonia (North- East of the Iberian Peninsula). Those lineages were identified as *N. rariplia*, E54 and *N. simoni*, E115 (Chapter 4.5) and they are the sister groups of the Moroccan species. All Pronemesia species from Morocco are monophyletic.

Clade II includes specimens from the Iberian Peninsula. At the same time, is itself divided in two clades well supported by all methods. The first branching clade contains specimens distributed all over Iberian massif: RA149 (*Nemesia athiasi*, from South Portugal, Locality 116), ME729 (Huelva. South-West Spain, locality 19), RA18 (Locality 82, Castilla-León, East Spain) and specimens from Central System . The other lineage contains specimens from Catalonia (a new specie, E118, from Sant Llorenç de Munt, described in chapter 4.5) as sister group from specimen E296 is one male from Portugal(Locality 186) identified as *Nemesia bacelarae.*

Clade III includes a big diversification of Pronemesia. This clade is further resolved into three clades. The first lineage is one including samples from France belonging to *N. carminans* (RA151, locality 133,Col de Vence) and samples from Catalonia belonging to *N. caementaria* (E42, from Catalonia, Sant Llorenç de Munt).Those species are supported and confirmed sister species. The second branching lineage is well supported by all methods and includes individuals from Sardinia, Sicilia , Italy and Tunisia, all which supported as monophyletic. Specimens from Sicily (E99, from Luppino locality 165) appear as sister group from the only Pronemesia specimens from Tunisia (DN84, from Oued ez zit, Loc 178), and are closely related with Sardinian Specimens. At the same turn , they are sister groups to a Sicilian lineage, that at the same time is closely related with South Italy specimens AE140 (from Locality 194 , the closest to Sicily).

The third lineage includes one individual from Greece (DN126) in a Balearic-Betic clade. The individuals were identified as *N. randa* (E163) from Minorca , *N. randa* H66 from Majorca , *N. ibiza* (H22,H52,H135,H23) from Ibiza and *N. bristowei* (H128) from Majorca (see chapter 4.3) with their closest groups in the East Coast of the Iberian Peninsula, in the Betic Cordilllera,namely Almeria (Z156, locality 13) and Granada (Locality 8, E120).

Clade IV, includes specimens with Pronemesia-like spinnerets but makes Pronemesia group paraphyletic. Internal relationships are well supported by all methods, includes one specimen from Portugal (Z202, locality 205) identified as *N. uncinata,* closely related with specimens from North-East of the Iberian Peninsula, namely Z92 from Barcelona and E63 from Teruel (Locality 84). Also includes specimens along the East coast of the Iberian peninsula (E77, from locality 77, Castello) related to a clade which includes specimens identified as *N. valenciae* (or *N. dorthesi,* that show same bulb morphology). This specie is the putative sister group of *Nemesia santeulalia*, from Balearic Islands (see chapter 4.3 for a deep study on Balearic Islands).

The big Holonemesia clade is supported by ML and BI, despite the internal relationships are not well resolved. The clade V is the first branching lineage and includes specimens from the East Coast of the Iberian Peninsula. Interestingly specimens Z174 , with Betic distribution (Moixent, Valencian Country Locality, 43) is closely related and highly supported with a clade that includes an specimen from Morocco: RA138 (Bni-hadifa, Locality 110, in the Rift Valley) and E276 (From Castilla la Mancha, South East of Spain, Locality 41). At the same turn, those specimens are sister group of a lineage from South of France and North of Italy. The specimen named as DN104 (Carpassio, Loc 121) has been identified *as Nemesia manderstjernae* (Isaia & Decae, pers. Com.). Those results are congruent with the supposedly distribution of *N. mandertsjernae* which is supposedly distributed in France and in Morocco.

Clade VI is supported, the relationships between lineages are not well resolved, being only supported at the tips. Includes a diversification process in Corsica, and is further resolved into three clades. The first includes two Corsican lineages with sister groups in the North of Italy and South of France. The

second one includes only Corsican lineages. And the third one includes a Corsican lineage that is the sister Group of DN95 a specimen from Central Italy, namely Fossombrone (Locality, 189).

Clade VII is supported by ML and BI, formed by specimens belonging to Sardinia, Corsica, Italy and Tunisia, but without support at deep nodes. Specimens from South Italy appear as sister group of a diversification process between Corsica, Sardinia and Tunisia. Specimens from Sardinia are recovered as sister groups from Tunisia.

The relationship of clade CVIII and C IX as the sister groups is only supported by BI. The Clade VIII can be spitted in four different lineages. The first one into splitting is one lineage from Sardinia. The second split includes specimens from the southernmost of Iberian with Betic distribution and includes specimens from the east-coast of the Iberian peninsula (E32, E274,from Locality 34) which are closely related with high support with a lineage from Morocco (RA131, from Ain Sfa, Locality 106, in the Algerian Border). This pattern is similar to the other Holonemesia from Morocco, suggesting at least three independent process of colonisation occurred. The third split, is a Betic lineage that contains two lineages from Balearic Islands, *N. seldeni* (H147, H89) and the other is a putative new specie from Majorca(E165 see chapter 4.3) as sister group of a Betic diversification process.

Clade IX has no support at internal nodes. Can be further divided into two main lineages. despite tips are well supported. One of them without internal support includes specimens from South Italy (Accentura) as sister groups from Sicily. Furthernore,the relationship of specimen RA171 from Crete is not resolved.

Also includes a lineage from Sicily (RA197) which appears as sister group *of N. dubia* (from Sant Llorenç de Munt, Catalonia, localities 96,97, etc), and one lineage from Sicily as well supported and sister group of a Tunisian diversification. Specimens DN82 and DN21 from Tunisia are recovered as sister groups of DN130, from Locality 139(South Italy).

3.3 Estimation of divergence times

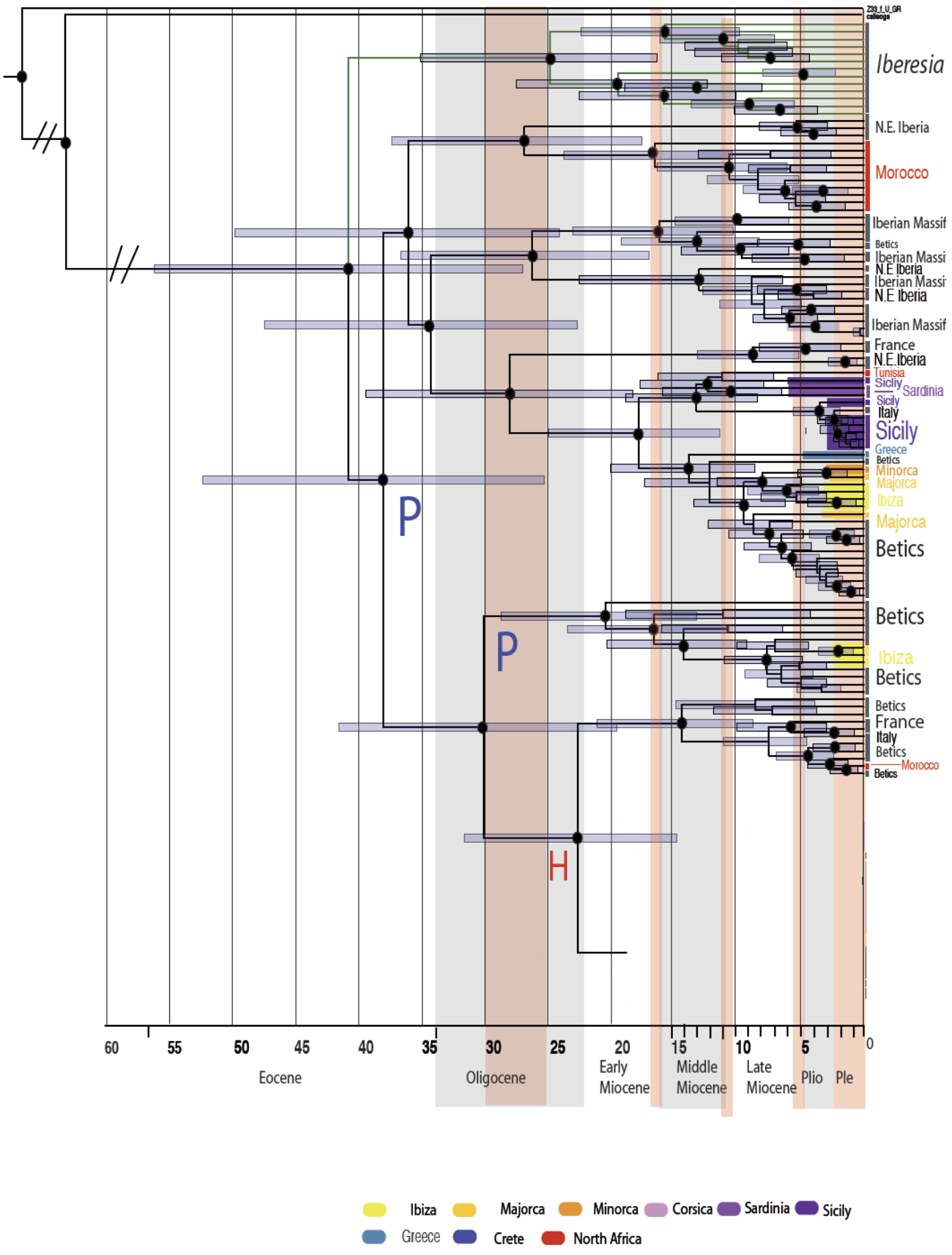
Convergence was confirmed using TRACER v.1.5 and Effective Samples Sizes were above 200, suggesting a good representation of independent samples in the posterior. The chronogram with corresponding confidence intervals obtained is shown in Fig. 6.

Overall, the tree topology and the clade supports were similar to those found in the phylogenetic analyses. Deeper nodes show wider confidence intervals and longer uncertainty and relationship within some Holonemesia spiders are not supported. The most recent common antecessor of *Iberesia*, splitted from *Nemesia* during Eocene at 40.82 Ma (56.18 – 26.99 Ma) which HPD 95% interval may include also Oligocene. The time of the split between the two big *Nemesia* lineages, the Pronemesia and the Holonemesia (this last includes one lineage of Pronemesia) was estimated at 38.08 Ma (52.40-25.30 Ma).

The Most Recent Common ancestor of Pronemesia Lineage was dated at 36.08 Ma (49.83 – 24.09 Ma) and most diversification occurred during. Middle Miocene. The MRCA of the big Holonemesia lineage was dated 30.11 mA (41.34-19.54 Ma) and diversified during Middle Miocene and Pliocene.

The big Pronemesia lineage diversified into 3 lineages. The most recent common antecsor of first the lineage into split was dated at 26.87 Ma (37.38-17.53). Pronemesian Morocco specimens split from their continental sister groups (from North-East Iberia) at 16.55 Ma (23.75 – 10.426 Ma) .The other Pronemesia lineages diverge from a common antecessor that was dated at 34.274 (47.43-22.68). The second lineage was dated at 26.2906 (36.65-16.98 Ma) and includes specimens from the Iberian Peninsula, mainly from the North East and from the Iberian Massif. At the same turn, this lineage can be splitted into two clades. The first includes samples mainly from the Iberian Massif, that diversified during Middle Miocene. The second one includes mainly specimens from North East of Iberian Peninsula, and diversified during Late Miocene but internal relationships are not supported.

The most recent common ancestor of the third Pronemesia lineage was dated at 28.0136 (39.42 -18.2808). At the same time this can be divided into three clades. Samples from South France and North East of Iberian Peninsula are supported as sister groups of a namely Hercynian-Betic diversification. The


Iberesia
N.E. Iberia
Morocco
Iberian Massif
Betics
Iberian Massif
N.E Iberia
Iberian Massif
N.E Iberia
Iberian Massif
France
N.E.Iberia
Tunisia
Sicily
Sardinia
Sicily
Italy
Sicily
Greece
Betics
Minorca
Majorca
Ibiza
Majorca
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Early Miocene
Middle Miocene
Late Miocene
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Ibiza
Majorca
Minorca
Corsica
Sardinia
Sicily
Greece
Crete
North Africa

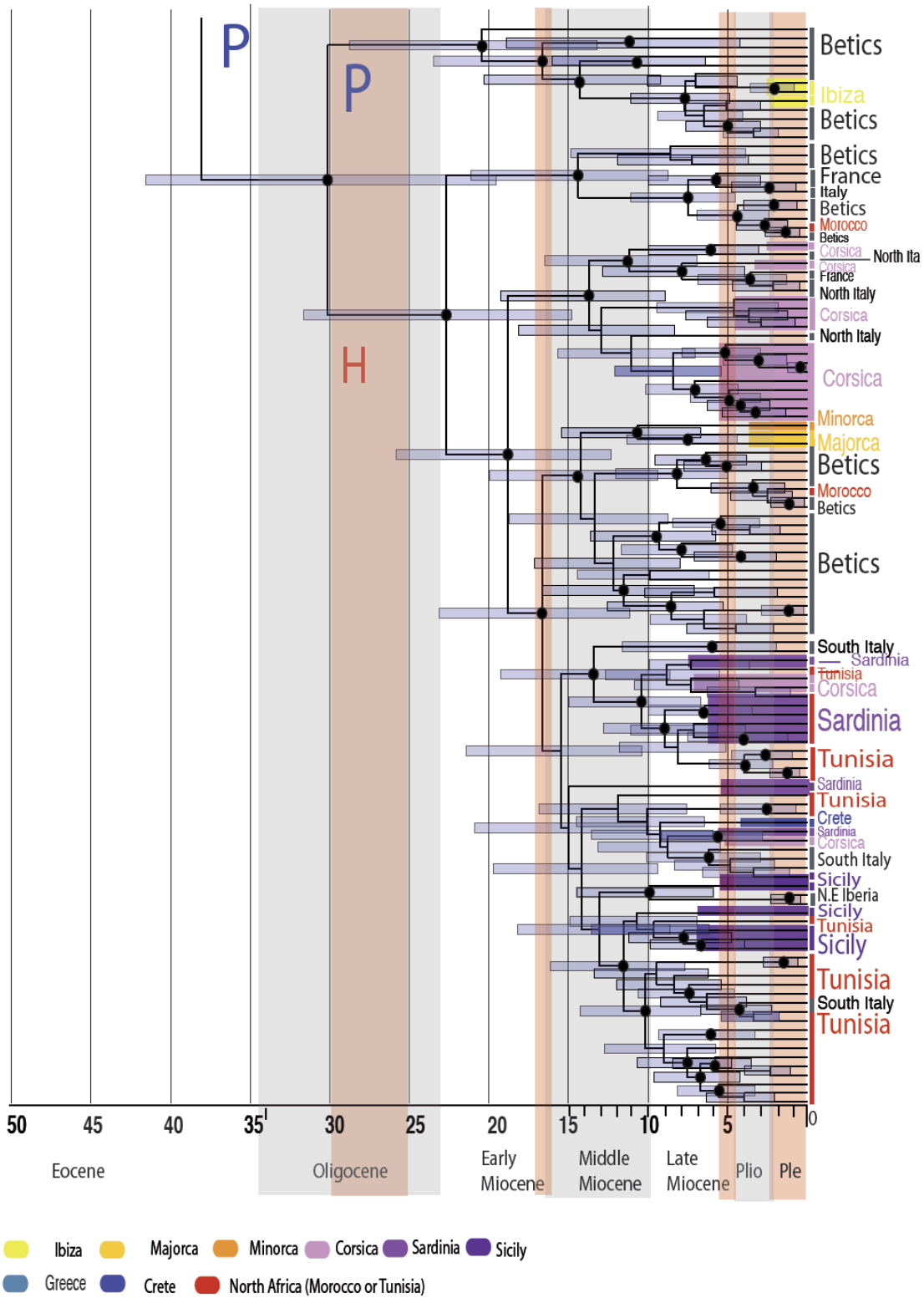

Figure 6. Chronogram inferred with BEAST. Bars on nodes correspond to confidence intervals Dots correspond to >95% support. Clades are colored according biogeographic regions. P correspond to the lineages with pronemesia-like spinnerets, H to the holonemesia-like spinnerets

most recent common ancestor of this two lineages started its diversification at 17.84 Ma (24.96-11.37). The relationship of the Tunisian Pronemesia as sister group of Sicily is not supported, despite this clade supports the closer relationships of Sicilian fauna with southernmost part of Italy. The split from Sicilian fauna is dated at 13.23 (24.96 – 11.37) and the split of the Italian fauna occurred during the Pleistocene and was dated at 2.3 (5.3 – 1.85).

The sister group of this lineage is a Balearic-Betic clade which split was dated at 13.872 (20.07 – 8.64). The split of Balearic specimens from their continental relatives was dated at 9.47 (13.43 – 6.19Ma) for a detailed study on Balearic Islands fauna see chapter 4.3. The Betic clade started their diversification at 8.68 (12.3071-5.609) . The relationship of Majorcan lineage as sister group of Betic specimens is not supported.

The fourth lineage of Pronemesia, is the one that makes this sub-generic division paraphyletic. Holonemesia specimens evolved from this clade. The Most recent common antecessor of this big Holonemesia including one—Pronemesia lineage was dated at 30.11(41.54- 19.54) The specimens with Pronemesia spinnerets splitted at 20.472 (28.74-13.20) and are mainly from the Betics. This lineage diversified during Early and Middle Miocene and includes two lineages from Ibiza that splitted from their continental relatives 7.03 (9.38-4.5)and 5.0734 (7.75-2.9).

The big lineage of Holonemesia splitted from most closer Pronemesia at 22.67 (31.63-14.78)and includes 5 big linneages. The first one into split was dated at 14.32 (21.25 -8.76 Ma). At the same turn can be subdivided into two lineages. The first includes samples from the Betics but their relationship are not supported. The second includes specimens from France as sister group of specimens from the Betics. Their split was dated at 14.32 (11.07 -4.5). Interestingly the colonisation from South France to North Italy was dated at 2.3179 (4.72-0.71Ma). Same clade includes one sample from Morocco (RA138) as sister group a Betic lineage. The split from this Moroccan lineage from their Iberian relatives was dated at 1.34 (2.62-0.44).

The second lineage into split corresponds to a diversification within Corsica. The most recent common ancestor of this lineage was dated at 13.71 (19.22-8.91) and originated three lineages. The only supported lineage can be splitted into two clades, the first one includes specimens from Corsica as sister groups of North Italy, which split was dated at 6.17 (9.98-3.06); and the second includes Corsican samples as sister groups in North Italy dated at 3.59 (6.81-1.2) the relationship with south France is not supported.

The third lineage of Holonemesia is mainly formed by specimens from the Betic area. This lineage includes specimens from Minorca and Majorca as sister groups from a diversification in the Betic area. This lineage was dated at 14.25 Ma(19.93- 9.3) . Those Balearic lineages splitted from their relatives at 10.67 Ma (15.43-6.67). The split between Minorcan and Majorcan lineages was dated a 7.6 (11.32-4.40).

The Betic clade can be divided at the same time into two Betic lineages, that diverged at 13.35 Ma (18.7-8.74), but their relationship is not supported. One of them includes specimens from Morocco. This lineage splitted from the other at 8.18 (12.05-4.98) and the specimen from Morocco splitted from their continental sister groups at 2.51 (4.8-0.91 Ma). The other clade, includes only specimens from the Betic area and most of diversification processes occurred during Late Miocene.

The fourth Holonemesia clade includes specimens from South Italy , Tunisia, Corsica and Sardinia. The most recent common ancestor of this clade was dated 13.42 (19.25-8.6). Specimens from South Italy splitted at 5.9 Ma (11.63 – 11.92) The relationships within Sardinia and Tunisia as sister group of a Corsican lineage is not supported, despite this clades includes a Sardinian lineage closely related with Tunisia.

The fifth clade of Holonemesia is a big lineage that includes specimens from Tunisia, Crete, Sardinia, Corsica, South Italy, Sicily and North East of the Iberian Peninsula but without support. The samples from Corsica and Sardinia confirmed as sister groups which split was dated at 5.62 Ma (9.17-2.78) ; the relationship of North East Iberian Peninsula as sister group of Sicily and dated at 10.054 (14.47 -5.86). The clade includes a lineage showing a major

diversification process in Tunisia, and was dated at 10.303 Ma (14.26-6.07 Ma). This lineage splitted from their Sicilian sister relatives at 11.5565 (16.13-7.6). Sicilian specimens are sister groups of specimens from North East of the Iberian Peninsula, their divergence was dated at 9.91 (14.92-6.92 Ma). This results suggest a closer relationship between samples from Sicily and Tunisia, despite some nodes are not well supported One of these lineages from Tunisia has a closer relative in South Italy, this split was dated at 4.9 Ma.

3.4 Ancestral Area reconstruction

The reconstruction of the ancestral biogeographic distributions are summarised in Fig. 7. Our analyses support the Betic region as the center of origin of *Nemesia*.

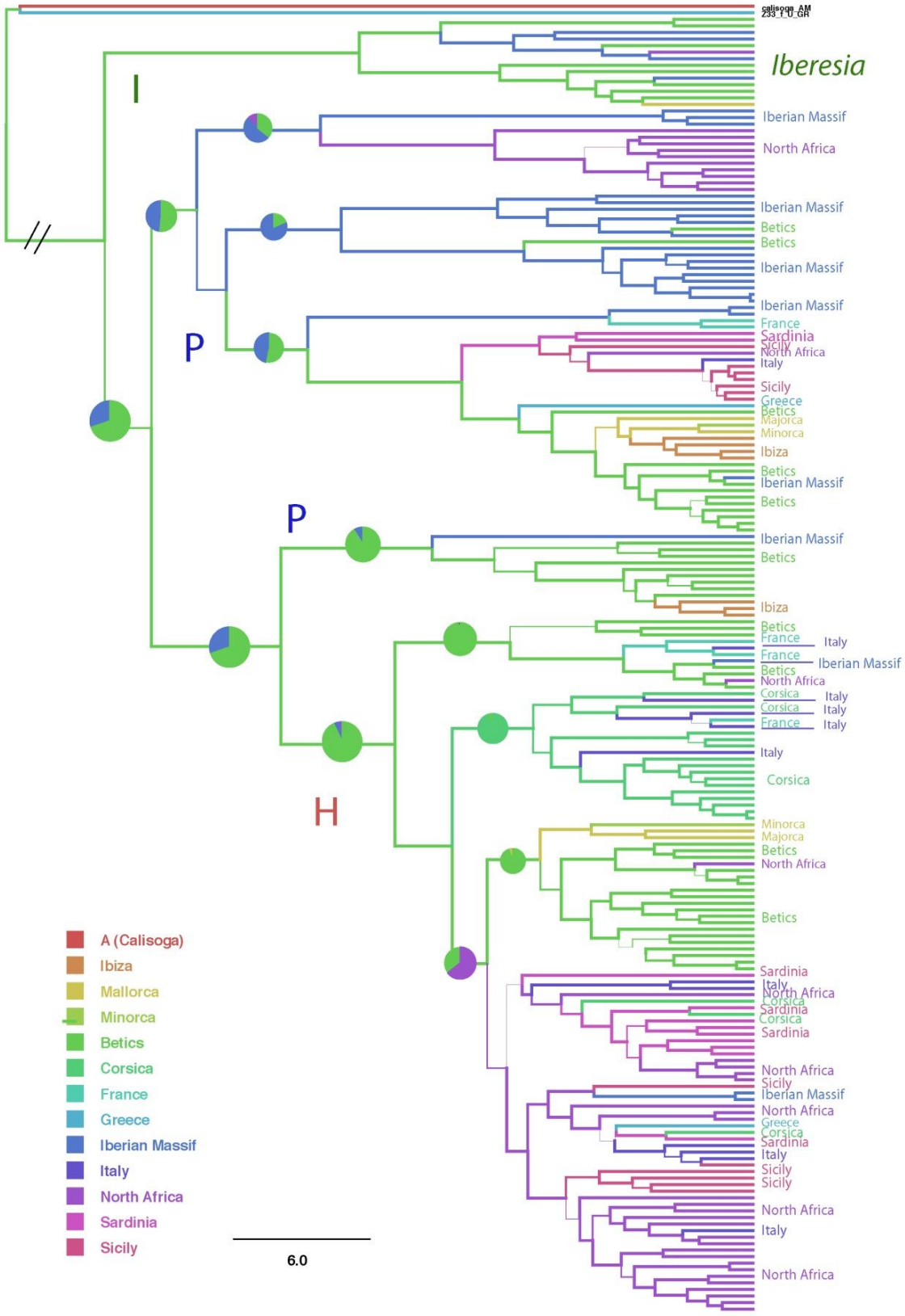

Figure 7. Bayesian-based biogeographic reconstruction of ancestral nodes using the discrete phylogeographic approach (Lemey et al, 2009). Colours correspond to the geographic regions defined in the text (please note that colour codes do not match previous figures). Pies indicate the probability of the different ancestral areas for the relevant nodes.

4.Discusion

This study is the first large-scale molecular phylogeny of the genus *Nemesia*, the most species rich mygalomorph in the Mediterranean. Our results confirm the monophyly of the genus, albeit with low support, and its sister group relationship to *Iberesia* (Decae & Cardoso, 2006; Decae et al., 2007).

The degree of spinneret reduction has been used to define supraspecific groups within *Nemesia* (Decae,2010 ; Simon, 1914) and to define the genus *Iberesia* (Decae & Cardoso, 2006). Mapping the spinneret type onto our phylogenies indicates that the two main types of spinnerets in *Nemesia* evolved once and that the *Holonemesia*-like type evolved from a *Pronemesia*-like ancestor. A closer examination to the spinneret patterns allows a finer split into additional groups. For example, in the clade including samples from Tunisia and south-eastern Iberia, namely Granada and Almeria, Posterior Median Spinnerets (PMS) were extremely reduced (e.g. Z156, E188), and easily confused with the pattern exhibited by *Iberesia*. Interestingly, some of the individuals belonging to these clade have been observed to construct cork-like and cogwheel-like (E188) trap-doors, with few silk coverage, also similar to those reported for *Iberesia*.

Interestingly, all specimens that were observed in the field to build burrows with complex architecture, including turrets, trap-door ornamentations or secondary passages, belong to the clade exhibiting *Holonemesia*–like spinnerets. Therefore it could be concluded that the type of the spinneret apparatus has an effect on the type of nest constructed by the species. Our results also suggest that the burrow architecture may contain phylogenetic signal. All cork-like trap-door and the cog-wheel trap-doors builders collected in different geographic regions belong to the same clades. The systematic value of the burrow architecture, and its use as a species diagnostic character remains to be investigated. Further, more detailed studies will be required to unambiguously relate the specific structures of the burrows with particular clades. Also the degree of intraspecific plasticity in the construction of the burrows and the nest will have to be assessed to confirm its relevance as diagnostic characters for species identification.

Delimitation of independent evolutionary lineages

The GMYC method has been widely used for species delimitation in arthropods (Bidegaray-Batista & Arnedo, 2011; Hendrixson et al., 2013; Planas et al., 2013; Satler et al., 2013; Hamilton et al., 2014; Kekkonen & Hebert, 2014; Opatova & Arnedo, 2014b), although it has been shown to overestimate the number of evolutionary lineages (Satler et al., 2013; Talavera et al., 2013). Oversplitting is particularly significative in mygalomorph spiders due to their poor dispersal capabilities, which results in high levels of population structure (Satler et al., 2013). Here we used GMYC to generate a preliminary hypothesis of lineage delimitation, as an objective first step to recognised independent lineages that can be further validated as species with additional data.

In our study, the GMYC method yielded 183 independent lineages most of which corresponded to either single or nearby localities. These results reflect the existence of strong geographic differentiation among populations, probably as a result of poor dispersal abilities, which suggest oversplitting by GMYC. Interestingly, several instances were detected of distantly related GMYC in the same locality, in several cases corresponding to the co-existence of *Holonemesia* and *Pronemesia* species, which may hint to some kind of ecological segregation.

Biogeographic patterns and diversification drivers in *Nemesia*

Our biogeographic area reconstruction identified the Betic region as the center of origin of *Nemesia*. It also suggests successive independent arrivals to other terrains that once formed part of the Iberian plate. The timing of the diversification of *Nemesia* estimated by the relaxed clock analyses (38.08 Ma, 52.40-25.30 Ma) suggest that the presence of *Nemesia* in other areas was mostly likely driven by the breakup of the Hercynian Belt (Rosenbaum et al., 2002b). Therefore *Nemesia* did not actually disperse to other regions but most likely drifted off with the blocks that later gave rise to the present day major Western Mediterranean islands, north Africa and parts of Italy.

Pronemesia lineages are mainly distributed in the Iberian Peninsula and in Morocco, except for one clade with representatives in Sardinia, Sicily, Greece

and Tunisia. Conversely, Holonemesia is widely distributed in the Islands and Tunisia, and only one *Holonemesia* specimen has been recorded in Morocco. Following estimated times, these asymmetric distribution patterns could be explained by the opening of the Valencia Trough (Fontboté et al., 1990) dated at 25 Ma that constituted an effective biological barrier that hampered the connections between the microplates and the Iberian Peninsula. The Pronemesian Sardinian lineages splitted from their Sicilian relatives around 10 ma, which is in accordance with the beginning of the separation of Sardinia from the Calabro-Pretorian Massif (Rosenbaum & Lister, 2004; Salvo et al., 2010).

The *Holonemesia* lineages evolved from a Pronemesian ancestor and mostly likely originated in the Betics. Our estimates dated the different splits within *Holonemesia* around 18-13 Ma, suggesting that different lineages may correspond to different vicariant events related with the drifting of the Hercynian microplates.

A very interesting patterns emerges within the *Holonemesia* lineages. There is a basal split between a mostly Corsican clade, which also includes some northern Italian and French lineages, and a clade that includes one Iberian clade and one clade mostly formed by Sardinian, Sicilian and Tunisian lineages. This relationship may suggest an ancient separation of Corsican from their closer microplates. The time of the separation of Corsica from Sardinia, dated at 21-15 Ma (Speranza et al., 2002), which matches our time estimates for the origin of the Corsican clade. The close relationships of Corsican species with North of Italy and South of France has also been recovered in a diverse assemblage of arthropods, including cave crickets, wasps or isopods (Ketmaier & Caccone, ; Allegrucci et al., 2005; Dapporto et al., 2007), and may correspond with land connections between these areas either during the drifting period (Meulenkamp & Sissingh, 2003) or the Messinian Salinity Crises (Duggen, S. et al., 2003).

The diversification of *Holonemesia* in the Betics was dated at 14.25 Ma. The Betic-Rif plate that drifted westward and started fragmenting around 15 Ma, reaching their actual position around 10 Ma (Lonergan & White, 1997; Braga et al., 2003). The Betic *Holonemesia* clade includes lineages from the Balearic islands, which splits was dated at 10.67 Ma. This results support the existence

of a Betic-Balearic connection during the Langhian-Serravallian marine regression, dated approximately at 14 Ma (Gibbons & Moreno, 2002), (see chapter 4.3 for a more detailed study of the Balearic species).

The internal relationships of the clade that includes lineages from Sardinia, Sicily, South Italy and Tunisia are poorly supported and inferring biogeographic relationships and its origins seems premature. Corsica and Sardinia are old continental islands and their geological evolution has been reconstructed in good details (Ketmaier & Caccone,2013). The evolutionary history of the fauna of Corsica, Sicily and Sardinia has been influenced by several periods of contact, within islands and with surrounding landmasses in different periods .

Our results suggest closer relationships between Sardinia, Corsica and South Italy indicating a multiple colonisation scenario. Interestingly we didn't found any closer relationship between Sardinia and North Italy, which is surprinsg because Sardinia was connected to the Apennines until the opening of the Tyrrhenian Sea and some land bridges between Corsica, Sardinia and North Italy have been reported around 10 Ma, during Messinian salinity Crisis and during Quaternary (Ketmaier & Caccone, 2013)

Our results suggest the connection of Sicily as part of the Calabro-Pretorian Massif connected with Iberian peninsula as a landmass integrating the Hercynian belt, fact that explain the sister relationships between some North East Iberian with Sicily. Our results suggest closer relationships within Tunisia. Africa and Sicily were in contact by land bridges during Messinian Salinity Crisis. This have already been demonstrated for poor disperser amphibia as *Bufo viridis* (Stöck et al., 2008).

This pattern of multiple colonisation events is repeated for North Africa, with several independent colonisations dated at different periods.. At least three independent colonisations of Morocco occurred. Pronemesian Morocco specimens split from their continental sister groups (North-East Iberia) at 16.8233 Ma (Late Miocene). This pattern has already been reported for other arthropod such as beetles (Faille et al., 2014). The other two independent lineages, are Holonemesia and more recent and splits from Betic fauna were dated during the Pleistocene, during the repeated and lengthy low sea-level

stands in the Pleistocene, a number of submarine banks have emerged as islands, serving as stepping stones from Iberia to Africa (Whittaker, 1998).

Our study lacks sampling in Algeria so the western limits of the Moroccan clade are unknown . An interesting pattern is the discrepancy between the genetic clades and distribution range of north African species, being the Tunisian species the result of several independent colonisations from with closest relatives in Sardinia and Sicily. This pattern was already seen in lacertids (Paulo et al., 2008). Further sampling in Algeria is needed for a deep study of North African fauna.

Our results suggest that causes of speciation within *Nemesia* result in a combination of vicariant events due to the broke of the Hercynian Belt and subsequent rotation and migration of the plates together with dispersal events allowed through land-bridges established between land-masses posterior to the rotation of the microplates.

***Nemesia* taxonomy**

Our study reflects well the poor present day knowledge on *Nemesia* taxonomy. Out of the near 500 specimens studied, we could only identify 15 nominal species. As stated in the introduction, *Nemesia* specimens are difficult to assign to any of the 54 taxon names available due to the ambiguous diagnosis, the poorly illustrated old descriptions, and the lack of information on one of the sexes in half of the species.

The use of a threshold divergence value to distinguish between intra- and interspecific genetic distances has become a common practice as a proxy to assign individuals to species and to discover new species (i.e. DNAbarcoding approaches Hebert et al., 2003a, 2003b). Castalanelli and collaborators (2014) used this approach to identified putative species among a sample of mygalomorphs sampled in the Pilbara region of Australia (most of which were nemesiids). These authors used a 9.5% of uncorrected genetic distance in the mtDNA gene cytochrome oxidase I (the animal barcode) as a cut-off value to delimit candidate species. The application of the 9.5% cut-off value to the species of *Calisoga* found in California, which currently include two nominal

species, resulted in as many as 26 putative species (Leavitt et al., 2015). Unfortunately in the present study we did not sequence the standard barcode gene and hence our results are not directly comparable. The 16S and the nad1 mtDNA genes are known to evolve at different rates than the cox1 in spiders (Bidegaray-Batista & Arnedo, 2011). Applying the 9.5% cut-off value to our data results in a single species, which is highly unlikely given the estimated divergence times and the span of phenotypical and ecological diversity observed. However, the analysis of the distribution of the pair-wise uncorrected mtDNA genetic distances of *Nemesia* with the help of the abdg (Puillandre et al., 2011, website tool http://wwwabi.snv.jussieu.fr/public/abgd/abgdweb.html) and using the same parameter settings implemented in other studies with mygalomorphs (Hamilton et al., 2014), revealed that for prior intraspecific values below 6%, sequences clustered in 138 putative species. For values above, all sequences again collapse in a single cluster. Although we do not claim here that there are as many *Nemesia* species in the Mediterranean, our results suggest that a large part of diversity of the genus remains uncovered and warrants future modern integrative taxonomic revisionary studies to provide a formal recognition of these hidden diversity.

5.Conclusions

The genus *Nemesia* is an excellent model system for evolutionary studies, because of its species richness, high architectural diversity of burrows, interesting biogeographic patterns. In this study we inferred the phylogenetic structure of the genus, its diversification timeframe and biogeographical patterns and shed some light on the large amount of uncover diversity in the group. Our mapping of the spinneret reduction on the phylogenetic trees showed that *Holenemesia* evolved from a *Pronemesia* ancestor. Furthermore we detected a clear relationship between the spinneret structure and burrow architecture suggesting that the degree of complexity of the spinning apparatus may reflect the kind of nest constructed by the species.

Our analyses support the Betic region as the center origin for *Nemesia* and suggest that the opening of the Western Mediterranean Basin may have drove the diversification of the group. Subsequent dispersal may have been accomplished by the emergence of land bridges as a result of sea level changes in the Messinian Salinity Crises and the Quaternary glacial cycles.

Our results confirm the poor taxonomic knowledge on the group and further suggest that a large amount of diversity remains to be described. We propose future species descriptions should be conducted in an integrative framework, combining different sources of evidence, including standardized description, informative images of the sexual organs, ecological information and illustration of burrow architecture and DNA information.

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Table 1. Specimen locality data for the present study. Column S inidcates the spinneret type, GMYC the GMYC cluster and loc_num, the locality number in the map.

label	S	SPEC_CODE	*genus*	sp	LOC_NAME	Province	Country	gmyc	loc_num	El	Long.	Lat.	16s	28s	h3	EFG
AE140_L_U_I	H	CRBAMM002003	*Nemesia*		Scilla - RC - Italia	Italy	Italy	18	194		38,244	15,718	AE140			
AE141_L_U_I	H	CRBAMM002004	*Nemesia*		Pentedattilo - Melito Porto Salvo	Italy	Italy	18	180		37,953	15,762	AE141			
calisoga		calisoga	*calisoga*		obtained from genebank	USA	USA	out	229		USA	USA	calisoga	calisoga	calisoga	Calisoga
DN1_F_H_TU	H	HB4	*Nemesia*		Bouficha	Hammamet	Tunisia	54	114		36,400	10,547	DN1			
DN10_F_H_TU	H	HB3	*Nemesia*		Bouficha	Hammamet	Tunisia	54	114		36,400	10,547	DN10			
DN101_J_H_TU	H	CRBAME001265	*Nemesia*		Kourbous		Tunisia	55	157	74	36,777	10,584	DN101			
DN102_F_H_TU	H	CRBAME001371	*Nemesia*		Le Kef		Tunisia	63	160	556	36,184	8,682	DN102			
DN103_F_H_TU	H	CRBAME001715	*Nemesia*		Vicoforte Mondovi		Italy	103	224		44,393	7,811	DN103		DN103	
DN104_L_U_IT	H	CRBAME001716	*Nemesia*		Carpasio	Imperia	Italy	104	121	326	43,941	7,849	DN104			
DN105_J_H_TU	H	CRBAME001270	*Nemesia*		Cap Blanc		Tunisia	105	119	2	37,332	9,846	DN105	DN105	DN105	DN105
DN11_F_H_TU	H	HB5	*Nemesia*		Bouficha	Hammamet	Tunisia	54	114		36,400	10,547	DN11			
DN111_J_H_TU	H	CRBAME001325	*Nemesia*		Ain Draham 1		Tunisia	85	103	788	36,777	8,707	DN111			
DN113_J_H_TU	H	CRBAME001327	*Nemesia*		Ain Draham 1		Tunisia	85	103	788	36,777	8,707	DN113			
DN114_J_H_TU	H	CRBAME001328	*Nemesia*		Ain Draham 1		Tunisia	59	103	788	36,777	8,707	DN114			DN114
DN115_F_H_TU	H	CRBAME001336	*Nemesia*		Ain Draham 2		Tunisia	85	104	785	36,777	8,703	DN115			
DN116_F_H_TU	H	CRBAME001346	*Nemesia*		Ain Draham 3		Tunisia	85	105	698	36,721	8,677	DN116			
DN118_F_H_TU	H	CRBAME001313	*Nemesia*		Baboucha		Tunisia	85	108	620	36,801	8,642	DN118			
DN120_J_H_TU	H	CRBAME001269	*Nemesia*		Cap Blanc		Tunisia	55	119	2	37,332	9,846	DN120			
DN121_J_H_TU	H	CRBAME001271	*Nemesia*		Cap Blanc		Tunisia	90	119	2	37,332	9,846	DN121			
DN122_J_H_TU	H	CRBAME001289	*Nemesia*		Cap Negre, Road P7 to		Tunisia	60	120		37,040	9,080	DN122			DN122
DN123_J_H_TU	H	CRBAME001284	*Nemesia*		Cap Negre, Road P7 to		Tunisia	106	120		37,040	9,080	DN123	DN123	DN123	DN123
DN124_F_H_TU	H	CRBAME001275	*Nemesia*		Cap Negre, Road P7 to		Tunisia	60	120		37,040	9,080	DN124	DN125	DN124	DN124
DN125_J_H_TU	H	CRBAME001276	*Nemesia*		Cap Negre, Road P7 to		Tunisia	107	120		37,040	9,080	DN125		DN125	DN125
DN126_L_U_GR	H	CRBAME001714	*Nemesia*		Chahiotata. Omala	Kefalonia	Greece	108	127	483	38,203	20,606	DN126			
DN130_L_U_IT	H	CRBAME001719	*Nemesia*		Entre Villapiana i Platici		Italy	109	139	411	39,863	16,454	DN130			
DN131_F_H_TU	H	CRBAME001358	*Nemesia*		Ghardinou to PN El Feija		Tunisia	62	150	559	36,490	8,325	DN131	DN131	DN131	DN131
DN132_F_H_TU	H	CRBAME001394	*Nemesia*		Hbabsa		Tunisia	49	155	444	35,476	9,341	DN132		DN132	DN132
DN133_F_H_TU	H	CRBAME001395	*Nemesia*		Hbabsa		Tunisia	49	155	444	35,476	9,341	DN133	DN133	DN133	DN133
DN134_F_H_TU	H	CRBAME001392	*Nemesia*		Hbabsa		Tunisia	48	155	444	35,476	9,341	DN134	DN134	DN134	DN134
DN135_F_H_TU	H	CRBAME001261	*Nemesia*		Kourbous		Tunisia	55	157	74	36,777	10,584	DN135			
DN136_F_H_TU	H	CRBAME001262	*Nemesia*		Kourbous		Tunisia	55	157	74	36,777	10,584	DN136	DN136	DN136	DN136
DN137_F_H_TU	H	CRBAME001389	*Nemesia*		Le Kef		Tunisia	63	160	556	36,184	8,682	DN137			
DN138_F_H_TU	H	CRBAME001390	*Nemesia*		La grotte de Mina		Tunisia	86	158	900	35,947	9,581	DN138	DN138	DN138	DN138
DN139_F_H_TU	H	CRBAME001375	*Nemesia*		Le Kef		Tunisia	63	160	556	36,184	8,682	DN139	DN139	DN139	DN139
DN141_F_H_TU	H	CRBAME001372	*Nemesia*		Le Kef		Tunisia	52	160	556	36,184	8,682	DN141			
DN142_F_H_TU	H	CRBAME001376	*Nemesia*		Le Kef		Tunisia	52	160	556	36,184	8,682	DN142	DN142	DN142	DN142
DN143_F_P_TU	H	CRBAME001433	*Nemesia*		Oued Ez Zit		Tunisia	15	178	166	36,465	10,279	DN143			DN14
DN144_F_P_TU	H	CRBAME001434	*Nemesia*		Oued Ez Zit		Tunisia	15	178	166	36,465	10,279	DN144			DN143
DN145_F_H_TU	H	CRBAME001430	*Nemesia*		Oued Ez Zit		Tunisia	53	178	166	36,465	10,279	DN145	DN145	DN145	DN145

label	S	SPEC_CODE	*genus*	sp	LOC_NAME	Province	Country	gmyc	loc_num	El	Long.	Lat.	16s	28s	h3	EFG
DN147_F_H_TU	H	CRBAME001368	*Nemesia*		PN El Feidja		Tunisia	63	182	829	36,507	8,321	DN147	DN147	DN147	DN147
DN148_F_H_TU	H	CRBAME001294	*Nemesia*		Nezca to Tabarka, Ain Sebaa		Tunisia	58	190	107	36,960	8,942	DN148	DN148	DN148	DN148
DN15_F_H_TU	H	M5	*Nemesia*		Monastir town	Monastir	Tunisia	54	172		36,729	10,820	DN15		DN15	
DN151_J_H_TU	H	CRBAME001412	*Nemesia*		Thagmerza		Tunisia	47	215	250	34,374	7,911	DN151			
DN155_J_H_TU	H	CRBAME001329	*Nemesia*		Ain Draham 1		Tunisia	85	103	788	36,777	8,707	DN155			
DN156_J_H_TU	H	CRBAME001321	*Nemesia*		Ain Draham 1		Tunisia	85	103	788	36,777	8,707	DN156			
DN157_J_H_TU	H	CRBAME001345	*Nemesia*		Ain Draham 2		Tunisia	85	104	785	36,777	8,703	DN157			
DN158_J_H_TU	H	CRBAME001335	*Nemesia*		Ain Draham 2		Tunisia	85	104	785	36,777	8,703	DN158			
DN159_J_H_TU	H	CRBAME001324	*Nemesia*		Ain Draham 1		Tunisia	85	103	788	36,777	8,707	DN159			
DN16_F_H_TU	H	ML2	*Nemesia*		Lamta	Monastir	Tunisia	54	159		35,683	10,860	DN16			
DN160_J_H_TU	H	CRBAME001322	*Nemesia*		Ain Draham 1		Tunisia	85	103	788	36,777	8,707	DN160			
DN161_J_H_TU	H	CRBAME001323	*Nemesia*		Ain Draham 1		Tunisia	85	103	788	36,777	8,707	DN161			
DN162_J_H_TU	H	CRBAME001326	*Nemesia*		Ain Draham 1		Tunisia	85	103	788	36,777	8,707	DN162			
DN164_J_H_TU	H	CRBAME001343	*Nemesia*		Ain Draham 2		Tunisia	59	104	785	36,777	8,703	DN164		DN164	DN164
DN165_J_H_TU	H	CRBAME001351	*Nemesia*		Ain Draham 3		Tunisia	85	105	698	36,721	8,677	DN165			
DN166_J_H_TU	H	CRBAME001320	*Nemesia*		Baboucha		Tunisia	85	108	620	36,801	8,642	DN166			
DN167_F_H_TU	H	CRBAME001267	*Nemesia*		Cap Blanc		Tunisia	90	119	2	37,332	9,846	DN167	DN167	DN167	DN167
DN169_J_H_TU	H	CRBAME001366	*Nemesia*		Ghardinou to PN El Feija		Tunisia	62	150	559	36,490	8,325	DN169			
DN17_F_H_TU	H	MT10	*Nemesia*		Teboulba	Monastir	Tunisia	54	211		35,653	10,971	DN17			
DN170_J_H_TU	H	CRBAME001359	*Nemesia*		Ghardinou to PN El Feija		Tunisia	62	150	559	36,490	8,325	DN170			
DN172_J_H_TU	H	CRBAME001263	*Nemesia*		Kourbous		Tunisia	55	157	74	36,777	10,584	DN172		DN172	DN172
DN174_J_H_TU	H	CRBAME001370	*Nemesia*		Le Kef		Tunisia	63	160	556	36,184	8,682	DN174			
DN175_F_H_TU	H	CRBAME001374	*Nemesia*		Le Kef		Tunisia	63	160	556	36,184	8,682	DN175		DN175	DN175
DN176_F_H_TU	H	CRBAME001378	*Nemesia*		Le Kef		Tunisia	52	160	556	36,184	8,682	DN176			
DN178_J_H_TU	H	CRBAME001302	*Nemesia*		Nezca to Tabarka, Ain Sebaa		Tunisia	58	190	107	36,960	8,942	DN178			
DN18_J_H_TU	H	MHSM3	*Nemesia*		Sidi Messaoud	Mahdia	Tunisia	54	197		35,555	11,009	DN18			
DN182_J_H_TU	H	CRBAME001316	*Nemesia*		Baboucha		Tunisia	85	108	620	36,801	8,642	DN182			
DN183_J_H_TU	H	CRBAME001341	*Nemesia*		Ain Draham 2		Tunisia	59	104	785	36,777	8,703	DN183			DN183
DN185_J_H_TU	H	CRBAME001314	*Nemesia*		Baboucha		Tunisia	85	108	620	36,801	8,642	DN185			
DN187_J_H_TU	H	CRBAME001319	*Nemesia*		Baboucha		Tunisia	85	108	620	36,801	8,642	DN187			
DN189_J_H_TU	H	CRBAME001291	*Nemesia*		Cap Negre, Road P7 to		Tunisia	86	120		37,040	9,080	DN189		DN189	DN189
DN19_F_H_TU	H	MHS3	*Nemesia*		Salakta	Mahdia	Tunisia	54	192		35,385	11,032	DN19			DN19
DN191_F_H_TU	H	CRBAME001357	*Nemesia*		Ghardinou to PN El Feija		Tunisia	62	150	559	36,490	8,325	DN191		DN191	DN191
DN192_F_H_TU	H	CRBAME001380	*Nemesia*		Le Kef		Tunisia	63	160	556	36,184	8,682	DN192			
DN193_F_H_TU	H	CRBAME001379	*Nemesia*		Le Kef		Tunisia	52	160	556	36,184	8,682	DN193		DN193	DN193
DN195_J_H_TU	H	CRBAME001300	*Nemesia*		Nezca to Tabarka, Ain Sebaa		Tunisia	58	190	107	36,960	8,942	DN195			
DN2_F_H_TU	H	S9	*Nemesia*		Chott Meriem	Sousse	Tunisia	54	128		35,971	10,533	DN2			
DN201_J_H_TU	H	CRBAME001308	*Nemesia*		Nezca to Tabarka, Ain Sebaa		Tunisia	58	190	107	36,960	8,942	DN201		DN201	DN201
DN202_J_H_TU	H	CRBAME001306	*Nemesia*		Nezca to Tabarka, Ain Sebaa		Tunisia	111	190	107	36,960	8,942	DN202		DN202	DN202
DN21_F_H_TU	H	MHS2	*Nemesia*		Salakta	Mahdia	Tunisia	54	192		35,385	11,032	DN21	DN21	DN21	DN21

label	S	SPEC_CODE	*genus*	sp	LOC_NAME	Province	Country	gmyc	loc_num	El	Long.	Lat.	16s	28s	h3	EFG
DN22_F_H_TU	H	MHSM8	*Nemesia*		Sidi Messaoud	Mahdia	Tunisia	54	197		35,555	11,009	DN22			DN22
DN24_F_H_TU	H	MT8	*Nemesia*		Teboulba	Monastir	Tunisia	54	211		35,653	10,971	DN24			
DN26_F_H_TU	H	ML9	*Nemesia*		Lamta	Monastir	Tunisia	54	159		35,683	10,860	DN26			
DN28_F_H_TU	H	M8	*Nemesia*		Monastir town	Monastir	Tunisia	54	172		36,729	10,820	DN28			
DN3_F_H_TU	H	M9	*Nemesia*		Monastir town	Monastir	Tunisia	54	172		36,729	10,820	DN3			
DN30_F_H_TU	H	S8	*Nemesia*		Chott Meriem	Sousse	Tunisia	54	128		35,971	10,533	DN30			
DN31_F_H_TU	H	S5	*Nemesia*		Chott Meriem	Sousse	Tunisia	54	128		35,971	10,533	DN31			DN31
DN33_J_H_TU	H	CRBAME001331	*Nemesia*		Ain Draham 1		Tunisia	85	103	788	36,777	8,707	DN33			DN34
DN34_F_H_TU	H	CRBAME001408	*Nemesia*		Guettar - Bou Oumrane		Tunisia	56	166	341	34,337	9,061	DN34	DN34	DN34	DN35
DN35_F_H_TU	H	CRBAME001311	*Nemesia*		Baboucha		Tunisia	85	108	620	36,801	8,642	DN35	DN35	DN35	DN36
DN36_F_H_TU	H	CRBAME001268	*Nemesia*		Cap Blanc		Tunisia	90	119	2	37,332	9,846	DN36		DN36	
DN37_F_H_TU	H	CRBAME001349	*Nemesia*		Ain Draham 3		Tunisia	85	105	698	36,721	8,677	DN37			
DN38_F_H_TU	H	CRBAME001295	*Nemesia*		Nezca to Tabarka, Ain Sebaa		Tunisia	58	190	107	36,960	8,942	DN38			
DN39_F_H_TU	H	CRBAME001391	*Nemesia*		Hbabsa		Tunisia	49	155	444	35,476	9,341	DN39			
DN4_F_H_TU	H	ML10	*Nemesia*		Lamta	Monastir	Tunisia	54	159		35,683	10,860	DN40			
DN40_F_H_TU	H	CRBAME001338	*Nemesia*		Ain Draham 2		Tunisia	85	104	785	36,777	8,703	DN4			
DN5_F_H_TU	H	MT9	*Nemesia*		Teboulba	Monastir	Tunisia	54	211		35,653	10,971	DN5			
DN6_F_H_TU	H	MHSM5	*Nemesia*		Sidi Messaoud	Mahdia	Tunisia	54	197		35,555	11,009	DN6			
DN7_F_H_TU	H	MHS1	*Nemesia*		Salakta	Mahdia	Tunisia	54	192		35,385	11,032	DN7			
DN70_M_H_TU	H	CRBAME001333	*Nemesia*		Ain Draham 2		Tunisia	61	104	785	36,777	8,703	DN70		DN70	DN70
DN71_F_H_TU	H	CRBAME001334	*Nemesia*		Ain Draham 2		Tunisia	61	104	785	36,777	8,703	DN71	DN71	DN71	DN71
DN72_F_H_TU	H	CRBAME001274	*Nemesia*		Cap Negre, Road P7 to		Tunisia	60	120		37,040	9,080	DN72	DN72	DN72	DN72
DN73_F_H_TU	H	CRBAME001293	*Nemesia*		Nezca to Tabarka, Ain Sebaa		Tunisia	58	190	107	36,960	8,942	DN73			
DN74_F_H_TU	H	CRBAME001260	*Nemesia*		Kourbous		Tunisia	55	157	74	36,777	10,584	DN74			
DN75_F_H_TU	H	CRBAME001404	*Nemesia*		Guettar - Bou Oumrane		Tunisia	56	166	341	34,337	9,061	DN75		DN75	DN75
DN76_M_H_TU	H	CRBAME001354	*Nemesia*		Ghardinou to PN El Feija		Tunisia	62	150	559	36,490	8,325	DN76			
DN78_F_H_TU	H	CRBAME001393	*Nemesia*		Hbabsa		Tunisia	48	155	444	35,476	9,341	DN78		DN78	DN78
DN79_F_H_TU	H	CRBAME001337	*Nemesia*		Ain Draham 2		Tunisia	59	104	785	36,777	8,703	DN79	DN79	DN79	DN79
DN8_F_H_TU	H	SX1	*Nemesia*		Sfax town	Sfax	Tunisia	112	196		34,709	10,722	DN8	DN8	DN8	DN8
DN80_F_H_TU	H	CRBAME001431	*Nemesia*		Oued Ez Zit		Tunisia	53	178	166	36,465	10,279	DN80			
DN81_F_H_TU	H	CRBAME001296	*Nemesia*		Nezca to Tabarka, Ain Sebaa		Tunisia	58	190	107	36,960	8,942	DN81			DN81
DN82_F_H_TU	H	CRBAME001432	*Nemesia*		Oued Ez Zit		Tunisia	53	178	166	36,465	10,279	DN82		DN82	DN82
DN83_F_H_TU	H	CRBAME001388	*Nemesia*		Le Kef		Tunisia	51	160	556	36,184	8,682	DN83		DN83	DN83
DN84_F_P_TU	H	CRBAME001429	*Nemesia*		Oued Ez Zit		Tunisia	15	178	166	36,465	10,279	DN84	DN84	DN84	DN84
DN85_F_H_TU	H	CRBAME001272	*Nemesia*		Cap Negre, Road P7 to		Tunisia	86	120		37,040	9,080	DN85	DN85	DN85	DN85
DN86_F_H_TU	H	CRBAME001373	*Nemesia*		Le Kef		Tunisia	51	160	556	36,184	8,682	DN86	DN86	DN86	DN86
DN87_F_H_TU	H	CRBAME001411	*Nemesia*		Thagmerza		Tunisia	47	215	250	34,374	7,911	DN87		DN87	DN87
DN88_F_H_TU	H	CRBAME001355	*Nemesia*		Ghardinou to PN El Feija		Tunisia	62	150	559	36,490	8,325	DN88			
DN89_F_H_TU	H	CRBAME001410	*Nemesia*		Thagmerza		Tunisia	47	215	250	34,374	7,911	DN89	DN89	DN89	DN89
DN90_F_H_TU	H	CRBAME001409	*Nemesia*		Thagmerza		Tunisia	47	215	250	34,374	7,911	DN90			

label	S	SPEC_CODE	*genus*	sp	LOC_NAME	Province	Country	gmyc	loc_num	El	Long.	Lat.	16s	28s	h3	EFG
DN91_F_H_TU	H	CRBAME001420	*Nemesia*		Tmezret		Tunisia	57	216	446	33,539	9,887	DN91	DN91	DN91	DN91
DN93_F_H_TU	H	CRBAME001428	*Nemesia*		Tmezret		Tunisia	57	216	446	33,539	9,887	DN93		DN93	DN93
DN94_F_H_TU	H	CRBAME001273	*Nemesia*		Cap Negre, Road P7 to		Tunisia	60	120		37,040	9,080	DN94			
DN95_L_U_TU	H	CRBAME001713	*Nemesia*		Rifuggio Ca Fabbri,	Fossombrone	Italy	113	189	761	43,633	12,750	DN95	DN95	DN95	DN95
DN96_F_H_IT	H	CRBAME001717	*Nemesia*		Entre Villapiana i Platici		Italy	83	139	411	39,863	16,454	DN96			
DN97_F_H_IT	H	CRBAME001718	*Nemesia*		Entre Villapiana i Platici		Italy	83	139	411	39,863	16,454	DN97			
DN98_F_H_TU	H	CRBAME001369	*Nemesia*		PN El Feidja		Tunisia	63	182	829	36,507	8,321	DN98		DN98	DN98
DN99_H_H_TU	H	CRBAME001342	*Nemesia*		Ain Draham 2		Tunisia	85	104	785	36,777	8,703	DN99			
E10_F_H_SA	H	CRBAME000315	*Nemesia*		Monte Limbara	Sardinia	Italy	82	173	770	40,858	9,132	E10	E10	E10	
E100_F_P_SI	P	CRBAME000137	*Nemesia*		Cammarata	Sicily	Italy	114	118	901	37,635	13,614	E100			E104
E104_J_P_SI	P	CRBAME000160	*Nemesia*		Mandanicci	Sicily	Italy	19	168	374	37,999	15,329	E104		E104	
E105_J_P_SI	P	CRBAME000161	*Nemesia*		Mandanicci	Sicily	Italy	19	168	374	37,999	15,329	E105			
E107_F_H_SI	H	CRBAME000177	*d*		Cannicattini	Sicily	Italy	115	232	342	37,032	15,073	E107			
E109_J_P_SI	P	CRBAME000185	*Nemesia*		Ponte Olivo	Sicily	Italy	116	183	158	37,121	14,381	E109			
E11_F_H_SA	H	CRBAME000316	*Nemesia*		Monte Limbara	Sardinia	Italy	82	173	770	40,858	9,132	E11			
E110_F_H_SI	H	CRBAME000198	*Nemesia*		Sant anna	Sicily	Italy	117	193	309	37,570	13,244	E110	E110	E110	E110
E112_J_P_SI	P	CRBAME000233	*Nemesia*		Linguaglossa	Sicily	Italy	118	46	792	37,800	15,133	E112	E112	E112	E112
E113_M_P_I2	P	CRBAME000911	*Nemesia*	n.sp.	Agramunt, Pitfall 4	Catalonia	Spain	27	204	553	41,687	2,021	E113			
E114_M_H_I2	H	CRBAME001086	*Nemesia*	dubia	Agramunt, Pitfall 4	Catalonia	Spain	68	204	547	41,687	2,021	E114			
E115_F_P_I2	P	CRBAME001093	*Nemesia*	simoni	Corba sot teixoneres, PNSLLM	Catalonia	Spain	102	201	819	41,687	2,021	E115	E115	E115	E115
E116_F_P_I2	P	CRBAME001094	*Nemesia*	simoni	Sot de teixoneres, PN SLLM	Catalonia	Spain	102	202	819	41,687	2,021	E116			
E117_F_H_I2	H	CRBAME001104	*Nemesia*	dubia	PN STLLM Coll 3 creus	Catalonia	Spain	68	96	873	41,649	1,972	E117	E117	E117	
E118_M_P_I2	P	CRBAME001107	*Nemesia*	n.sp	Agramunt, PN Sant LLorenc Munt	Catalonia	Spain	27	200	547	41,687	2,022	E118		E118	E118
E12_f_P_SA	P	CRBAME000354	*Nemesia*		Tertenia	Sardinia	Italy	121	214	84	39,666	9,578	E12	E12	E12	E12
E120_J_U_I4	U	CRBAME000639	*Nemesia*		Jete	Andalusia	Spain	119	8	95	36,784	-3,672	E120	E120	E120	E120
E121_J_P_I4	P	CRBAME000669	*Nemesia*		Arroba, Ctra de la Cabra	Andalusia	Spain	120	14	1067	36,849	-3,716	E121	E121	E121	E121
E122_F_P_I4	P	CRBAME000673	*Nemesia*		Desfiladero de los gaitanes	Andalusia	Spain	4	230	290	36,892	-4,682	E122			
E124_J_U_I4	U	CRBAME000679	*Nemesia*		Desfiladero de los gaitanes	Andalusia	Spain	4	230	290	36,892	-4,682	E124			
E125_J_U_I4	U	CRBAME000680	*Nemesia*		Desfiladero de los gaitanes	Andalusia	Spain	4	230	290	36,892	-4,682	E125			
E130_J_H_I3	H	CRBAME000710	*nemesia*		Gaucin	Andalusia	Spain	122	2	729	36,536	-5,302	E130	E130	E130	E130
E131_F_I_I3	I	CRBAME000711	*Iberesia*		Grazalema	Andalusia	Spain	OUT	5	832	36,765	-5,323	E131	E131	E131	
E133_J_P_I4	P	CRBAME000724	*Nemesia*		Valle de Abdalajis	Andalusia	Spain	4	6	832	36,765	-5,323	E133			
E136_F_P_I3	P	CRBAME000731	*Nemesia*		Villablanca	Andalusia	Spain	26	27	19	37,249	-7,343	E136			
E137_F_H_I4	H	CRBAME000733	*Nemesia*		Tempul, Finca de Picao	Andalusia	Spain	71	3	112	36,631	-5,663	E137	E137	E137	E137
E138_F_H_I3	H	CRBAME000734	*Nemesia*		Tempul, Finca de Picao	Andalusia	Spain	71	3	112	36,631	-5,663	E138			
E14_F_P_SA	P	CRBAME000363	*Nemesia*		Gola di Rio Cannas	Sardinia	Italy	16	153	364	39,322	9,401	E14			
E144_F_P_I3	P	CRBAME000818	*Nemesia*		Aroche 1	Andalusia	Spain	123	37	250	37,973	-7,081	E144	E144	E144	E144
E146_J_H_I4	H	CRBAME000837	*Nemesia*		Venta del Tarugo	Andalusia	Spain	124	12	1161	36,812	-3,306	E146	E146	E146	
E148_J_H_I4	H	CRBAME000841	*Nemesia*		Sierra Nevada	Andalusia	Spain	73	19	1181	37,015	-3,008	E148			

label	S	SPEC_CODE	*genus*	sp	LOC_NAME	Province	Country	gmyc	loc_num	El	Long.	Lat.	16s	28s	h3	EFG
E15_F_P_SA	P	CRBAME000364	*Nemesia*		Gola di Rio Cannas		Italy	16	153	364	39,322	9,401	E15	E15	E15	
E150_F_P_I4	P	CRBAME000855	*Nemesia*		Torcal de Antequera	Andalusia	Spain	4	17	1013	36,962	-4,519	E150			
E152_J_H_I3	H	CRBAME000859	*Nemesia*		Torcal de Antequera	Andalusia	Spain	125	17	1013	36,962	-4,519	E152	E152	E152	E152
E153_J_P_I4	P	CRBAME000863	*Nemesia*		Torcal de Antequera	Andalusia	Spain	4	17	1013	36,962	-4,519	E153			
E154_F_H_I4	H	CRBAME000864	*Nemesia*		Cherni	Andalusia	Spain	73	16	541	36,960	-3,008	E154			
E155_F_H_I4	H	CRBAME000876	*Nemesia*		Los Rosales	Andalusia	Spain	72	33	826	37,798	-3,084	E155	E155	E155	
E156_F_H_I4	H	CRBAME000877	*Nemesia*		Los Rosales	Andalusia	Spain	72	33	826	37,798	-3,084	E156			
E157_J_H_I4	H	CRBAME000884	*Nemesia*		Sierra de Cazorla	Andalusia	Spain	79	36	1081	37,950	-2,946	E157			
E158_J_H_I4	H	CRBAME000885	*Nemesia*		Sierra de Cazorla	Andalusia	Spain	79	36	1081	37,950	-2,946	E158			
E159_J_H_I4	H	CRBAME000886	*Nemesia*		Sierra de Cazorla	Andalusia	Spain	79	36	1081	37,950	-2,946	E159			
E16_f_H_SA	H	CRBAME000374	*Nemesia*		Seui	Sardinia	Italy	126	195	693	39,833	9,344	E16	E16	E16	
E160_J_H_I4	H	CRBAME000887	*Nemesia*		Sierra de Cazorla	Andalusia	Spain	79	36	1081	37,950	-2,946	E160	E160	E160	
E161_J_H_I4	H	CRBAME000888	*Nemesia*		La Calahurra	Andalusia	Spain	75	23	1723	37,131	-3,041	E161	E161	E161	E161
E162_J_H_I4	H	CRBAME000889	*Nemesia*		La Calahurra	Andalusia	Spain	75	23	1723	37,131	-3,041	E162			
E163_F_P_B3	P	CRBAME001459	*Nemesia*	randa	Trepucó	Minorca	Spain	13	64		39,860	4,297	E163	E163	E163	E163
E164_U_P_B3	P	CRBAME001460	*Nemesia*	randa	Santa Ageda	Minorca	Spain	13	70		40,032	3,990	E164			
E165_M_H_B3	H	CRBAME001461	*Nemesia*	n.sp.	Algaiarens beach 2	Minorca	Spain	81	73		40,050	3,912	E165	E165	E165	E165
E168_L_H_B3	H	CRBAME001876	*Nemesia*	n.sp.	Algaiarens beach 2	Minorca	Spain	81	73		40,050	3,912	E168			
E17_f_H_CO	H	CRBAME000411	*Nemesia*		Cervione, Valle di campoloro	Corsica	France	44	126	338	42,323	9,490	E17	E17	E17	E17
E188_J_P_I4	P	CRBAME000632	*Nemesia*		Jete	Andalusia	Spain	127	8	95	36,784	-3,672	E188			
E190_F_P_I3	P	CRBAME000672	*Nemesia*		Arenas del Rey	Andalusia	Spain	1	18	926	36,983	-3,974	E190			
E194_F_P_I4	P	CRBAME000666	*Nemesia*		Frigiliana	Andalusia	Spain	3	10	379	36,797	-3,904	E194	E194	E194	E194
E2_F_H_I2	H	CRBAME000245	*Nemesia*	dubia	Agramunt, PN SLLM	Catalonia	Spain	68	198	557	41,687	2,021	E2			
E20_f_H_CO	H	CRBAME000483	*Nemesia*		Cupabia, plage	Corsica	France	42	137	0	41,739	8,784	E20	E20	E20	E20
E200_F_P_I4	P	CRBAME000991	*Nemesia*		Embalse Veo	Valencian country	Spain	14	66	347	39,932	-0,338	E200			
E206_M_I_I3	I	CRBAME001734	*Iberesia*	machadoi	Navaconcejo	Extremadura	Spain	OUT	75	375	40,089	-5,985	E206	E206	E206	E206
E21_f_H_CO	H	CRBAME000513	*Nemesia*		Palombaggia, plage	Corsica	France	130	179	3	41,547	9,305	E21	E21	E21	E21
E210_J_P_I3	P	CRBAME001738	*Nemesia*		Berzocama *Loc Leti	Extremadura	Spain	128	53		39,415	-5,418	E210	E210	E210	
E213_J_I_I4	I	CRBAME001745	*Iberesia*		Montes de Toledo	Castilla la Mancha	Spain	OUT	59	884	39,607	-4,512	E213	E213	E213	E213
E217_F_P_I4	P	CRBAME001765A	*Nemesia*		Sierra de Gredos, 4	Castilla la Mancha	Spain	24	81	1130	40,331	-5,421	E217			
E218_F_P_I4	P	CRBAME001767	*Nemesia*		La Granja, Sierra de Guadarrama	Madrid	Spain	129	87	1587	40,808	-4,013	E218		E218	E218
E22_f_H_CO	H	CRBAME000526	*Nemesia*		Ciavari	Corsica	France	42	129	514	41,779	8,780	E22	E22	E22	E22
E221_F_P_I4	P	CRBAME000830	*Nemesia*		Orgiva	Andalusia	Spain	2	15	529	36,902	-3,442	E221			
E222_F_P_R	P	CRBAME000831	*Nemesia*		Orgiva	Andalusia	Spain	2	15	529	36,902	-3,442	E222			
E23_f_H_CO	H	CRBAME000595	*Nemesia*		Foret Aitone	Corsica	France	131	143	996	42,262	8,838	E23	E23	E23	E23
E232_J_I_I4	I	CRBAME001748	*Iberesia*		Montes de Toledo	Castilla la Mancha	Spain	OUT	59	884	39,607	-4,512	E232	E232	E232	E232
E236_F_R_I2	R	CRBAME000623	*Nemesia*		Loc J. Hernandez	Andalusia	Spain	5	86		40,808	4,013	E236			
E24_F_H_CO	H	CRBAME000607	*Nemesia*		Rogliano	Corsica	France	45	191	117	42,958	9,428	E24			

label	S	SPEC_CODE	*genus*	sp	LOC_NAME	Province	Country	gmyc	loc_num	El	Long.	Lat.	16s	28s	h3	EFG
E242_F_H_SA	H	CRBAME000288	*Nemesia*		Bosa	Sardinia	Italy	88	113	278	40,359	8,419	E242			
E243_F_H_SA	H	CRBAME000289	*Nemesia*		Bosa	Sardinia	Italy	88	113	459	40,494	9,406	E243			
E244_J_H_SA	H	CRBAME000307	*Nemesia*		Onani	Sardinia	Italy	132	177	576	40,180	8,585	E244			
E245_J_H_SA	H	CRBAME000334	*Nemesia*		Cuglieri	Sardinia	Italy	89	136	576	40,180	8,585	E245			
E246_J_H_SA	H	CRBAME000335	*Nemesia*		Cuglieri	Sardinia	Italy	89	136	576	40,180	8,585	E246		E246	
E247_J_H_SA	H	CRBAME000340	*Nemesia*		Fiuminimaggiore	Sardinia	Italy	87	141	126	39,421	8,502	E247			
E248_J_H_SA	H	CRBAME000341	*Nemesia*		Fiuminimaggiore	Sardinia	Italy	87	141	126	39,421	8,502	E248			
E25_F_I_I4	I	CRBAME000929	*Iberesia*	sp.	Villalba de la Sierra	Valencian Country	Spain	out	79	978	40,223	-2,100	E25	E25	E25	E25
E250_F_H_CO	H	CRBAME000394	*Nemesia*		Col de Bellevalle	Corsica	France	42	131	457	41,741	9,101	E250			
E251_F_H_CO	H	CRBAME000401	*Nemesia*		Zoza	Corsica	France	43	228	457	41,741	9,101	E251			
E252_F_H_CO	H	CRBAME000402	*Nemesia*		Zoza	Corsica	France	43	228	74	41,414	9,175	E252			
E253_F_H_CO	H	CRBAME000447	*Nemesia*		Col de Parmentile	Corsica	France	67	132	74	41,414	9,175	E253	E253	E253	
E254_F_H_CO	H	CRBAME000448	*Nemesia*		Col de Parmentile	Corsica	France	67	132	1025	42,119	9,132	E254			
E257_F_H_CO	H	CRBAME000488	*Nemesia*		Col da Vizzabona	Corsica	France	133	130	1025	42,119	9,132	E257			
E258_F_H_CO	H	CRBAME000489	*Nemesia*		Col da Vizzabona	Corsica	France	39	130	1025	42,119	9,132	E258			
E26_J_H_I4	H	CRBAME000947	*Nemesia*		Cedrillas	Aragon	Spain	80	84	1359	40,438	0,860	E26			
E260_F_H_CO	H	CRBAME000522	*Nemesia*		St Cristophe	Corsica	France	134	206	813	42,402	9,351	E260	E260	E260	E260
E261_F_H_CO	H	CRBAME000523	*Nemesia*		St Cristophe	Corsica	France	41	206	813	42,402	9,351	E261			
E262_F_H_CO	H	CRBAME000533	*Nemesia*		Foret de Melaha	Corsica	France	40	145	850	42,526	8,993	E262			
E263_F_H_CO	H	CRBAME000534	*Nemesia*		Foret de Melaha	Corsica	France	40	145	850	42,526	8,993	E263			
E264_F_H_CO	H	CRBAME000559	*Nemesia*		Pinzalone	Corsica	France	39	181	103	42,914	9,356	E264			
E265_J_H_CO	H	CRBAME000561	*Nemesia*		Muchieta	Corsica	France	45	176	103	42,914	9,356	E265			
E266_J_H_CO	H	CRBAME000562	*Nemesia*		Muchieta	Corsica	France	45	176	103	42,914	9,356	E266			
E267_J_H_CO	H	CRBAME000565	*Nemesia*		Bocca di Marsolinu	Sardinia	Italy	135	111	0	40,987	9,581	E267	E267	E267	E267
E269_F_H_SA	H	CRBAME000567	*Nemesia*		Cala Sassari	Sardinia	Italy	82	231	693	40,987	9,581	E269			
E270_F_H_CO	H	CRBAME000575	*Nemesia*		Vezzani	Corsica	Italy	39	223	813	42,402	9,351	E270	E270	E270	E270
E271_F_H_CO	H	CRBAME000579	*Nemesia*		St Cristophe	Corsica	France	41	206	813	42,402	9,351	E271			
E272_F_H_CO	H	CRBAME000615	*Nemesia*		Casta	Corsica	France	136	123	547	42,442	8,854	E272	E272	E272	E272
E273_F_H_CO	H	CRBAME000616	*Nemesia*		Foret de Bonifato	Corsica	France	137	144	355	40,213	0,112	E273	E273	E273	
E274_M_H_I2	H	CRBAME001016	*Nemesia*		Torre Domenech	Valencian Country	Spain	138	34	860	37,863	-1,534	E274			
E275_M_H_I4	H	CRBAME001044	*Nemesia*		Sierra Espuna 2	Murcia	Spain	93	65	929	37,863	-1,535	E275	E275	E275	
E276_M_H_I2	H	CRBAME001073	*Nemesia*		La parra de las Vegas	Castilla la Mancha	Spain	92	41	463	38,398	-1,763	E276	E276	E276	
E28_J_H_I4	H	CRBAME000957	*Nemesia*		Cenajo, Sierra de Segura	Castilla la Mancha	Spain	93	40		38,398	-1,763	E28	E28	E28	
E282_F_R_I2	P	CRBAME001181	*Nemesia*	caementaria	Bosc de la Vila, , Gurb	Catalonia	Spain	21	80	1130	40,331	-5,421	E282			
E285_M_P_I1	P	CRBAME001763	*Nemesia*		Sierra de Gredos, 4	Castilla la Mancha	Spain	24	81		40,331	-5,421	E285			
E296_M_P_I3	P	CRBAME001901	*Nemesia*	bacelarae	Private House, portugal		Portugal	139	186		39,602	-8,836	E296	E296	E296	
E297_L_U_IT	U	CRBAME001902	*Nemesia*		Accentura		Italy	65	102		40,490	16,170	E297			

label	S	SPEC_CODE	*genus*	sp	LOC_NAME	Province	Country	gmyc	loc_num	El	Long.	Lat.	16s	28s	h3	EFG
E298_L_U_IT	U	CRBAME001903	*Nemesia*		Accentura		Italy	65	102		40,490	16,170	E298			
E299_L_U_IT	U	CRBAME001904	*Nemesia*		Mater		Italy	66	212	272	40,400	16,360	E299	E299		
E3_F_P_I2	P	CRBAME000251	*Nemesia*	raripilia	Les Rafardes, PNSant Llorenc Munt	Catalonia	Spain	101	89	375	41,721	1,997	E3			
E30_F_H_I2	H	CRBAME001012	*Nemesia*		Penya Roja	Catalonia	Spain	77	90		41,315	1,159	E30			
E300_L_U_IT	U	CRBAME001905	*Nemesia*		Craco		Italy	84	134		40,360	16,390	E300	E300		
E301_L_U_IT	U	CRBAME001906	*Nemesia*		Ferrandina		Italy	84	140		40,495	16,459	E301			
E302_L_U_IT	U	CRBAME001907	*Nemesia*		Casamasella		Italy	140	122		40,180	18,450	E302			
E303_L_U_IT	U	CRBAME001908	*Nemesia*		Montescaglioso		Italy	65	175		40,540	16,680	E303			
E304_L_U_IT	U	CRBAME001909	*Nemesia*		Miglionico		Italy	65	171		40,560	16,490	E304			
E305_L_U_IT	U	CRBAME001910	*Nemesia*		Calendano		Italy	66	117		40,050	16,400	E305			
E306_L_U_IT	U	CRBAME001911	*Nemesia*		Marina di Ginosa		Italy	66	170		40,410	16,870	E306			
E307F_L_P_MO	P	CRBAMM000440	*Nemesia*		Imouzer Kandar		Morocco	141	124	1477	33,644	-5,069	E307F			
E308_J_P_I4	P	CRBAME000856	*Nemesia*		Torcal de Antequera	Andalusia	Spain	4	17	1013	36,962	-4,519	E308			
E309_L_U_IT	U	CRBAME001912	*Nemesia*		Lombardia, Retorbido (Pv)	Lombardia	Italy	46	163		44,899	9,432	E309			
E31_J_P_I2	P	CRBAME000993	*Nemesia*		Embalse Veo	Valencian country	Spain	14	66	347	39,932	-0,338	E31			
E310_L_U_IT	U	CRBAME001913	*Nemesia*		loc pietra , margine bosco		Italy	46	162	850	44,848	9,479	E310			
E32_F_H_I2	H	CRBAME001020	*Nemesia*		Torre Domenech	Valencian Country	Spain	78	34	355	40,213	0,112	E32			
E37_F_P_I2	P	CRBAME000243	*Nemesia*	caementaria	Agramunt, PN SLLM	Catalonia	Spain	21	198	557	41,687	2,021	E37			
E38_F_P_I2	P	CRBAME000250	*Nemesia*	caementaria	Can Torres, P N SLL Munt	Catalonia	Spain	21	92	519	41,613	2,031	E38			
E39_F_H_I2	H	CRBAME000254	*Nemesia*	dubia	Les Rafardes, PNSLLM	Catalonia	Spain	68	207	272	41,721	1,997	E39			
E4_F_H_I2	H	CRBAME000252	*Nemesia*	dubia	Les Rafardes, PNSLLM	Catalonia	Spain	68	209	272	41,721	1,997	E4			
E40_J_P_I2	P	CRBAME000255	*Nemesia*	raripilia	Les Rafardes, PNSLLM	Catalonia	Spain	101	209	272	41,721	1,997	E40			
E41_F_P_I2	P	CRBAME000264	*Nemesia*	caementaria	Les Arenes	Catalonia	Spain	21	94	361	41,640	2,058	E41			
E42_J_P_I2	P	CRBAME000072	*Nemesia*	caementaria	PN STLLM 1	Catalonia	Spain	21	100		41,661	1,992	E42	E42	E42	
E43_F_H_I2	H	CRBAME000077	*Nemesia*	dubia	PN STLLM 2	Catalonia	Spain	68	100	485	41,660	1,996	E43			
E44_J_H_I2	H	CRBAME000240	*Nemesia*	dubia	Marquet Roques, PN SLLM	Catalonia	Spain	68	101	561	41,676	2,019	E44			
E45_J_P_I2	P	CRBAME001097	*Nemesia*	simoni	Sot de teixoneres, PN SLLM	Catalonia	Spain	102	202	819	41,687	2,021	E45			
E46_F_P_I2	P	CRBAME001140	*Nemesia*	caementaria	PN STLLM 1	Catalonia	Spain	21	99		41,661	1,992	E46			
E47_F_H_I2	H	CRBAME001099	*Nemesia*	dubia	PN STLLM Coll 3 creus	Catalonia	Spain	68	96	873	41,649	1,972	E47			
E48_M_P_I2	P	CRBAME000906	*Nemesia*	n.sp.	Agramunt, Pitfall 3	Catalonia	Spain	27	203	551	41,687	2,021	E48			
E5_F_H_I2	H	CRBAME000260	*Nemesia*	dubia	Lligabossa, PN SLLM	Catalonia	Spain	68	217	589	41,783	2,017	E5			
E51_M_P_I2	P	CRBAME000916	*Nemesia*	n.sp.	Coll las tres creus, P4	Catalonia	Spain	27	98	874	41,649	1,972	E51			
E52_M_P_I2	P	CRBAME001152	*Nemesia*	raripilia	Coll las tres creus	Catalonia	Spain	101	95	874	41,648	1,972	E52			
E53_M_P_I2	P	CRBAME001108	*Nemesia*	simoni	Sot de teixoneres	Catalonia	Spain	102	97	873	41,649	1,972	E53			
E54_M_P_I2	P	CRBAME001145	*Nemesia*	raripilia	Les Rafardes, Pitfall 5	Catalonia	Spain	101	213	504	41,721	1,996	E54	E54	E54	E54
E57_J_P_I2	P	CRBAME000924	*Nemesia*		San Blas	Valencian Country	Spain	142	83	1000	40,346	-1,199	E57	E57		
E58_J_H_I2	H	CRBAME000926	*Nemesia*		Embalse de la Toba	Valencian country	Spain	92	76	1165	40,212	-1,900	E58			
E59_J_H_I2	H	CRBAME000930	*Nemesia*		Alcaniz	Aragon	Spain	78	88	336	41,083	0,131	E59			

label	S	SPEC_CODE	*genus*	sp	LOC_NAME	Province	Country	gmyc	loc_num	El	Long.	Lat.	16s	28s	h3	EFG
E6_F_H_I2	H	CRBAME000262	*Nemesia*	dubia	Lligabossa, PN SLLM	Catalonia	Spain	68	217	589	41,783	2,017	E6			
E62_F_H_I2	H	CRBAME000940	*Nemesia*		Cedrillas	Aragon	Spain	80	84	1359	40,438	-0,860	E62	E62	E62	E62
E63_F_P_I2	P	CRBAME000943	*Nemesia*		Cedrillas	Aragon	Spain	143	84	1359	40,438	-0,860	E63	E63	E63	
E64_F_H_I2	H	CRBAME000946	*Nemesia*		Cedrillas	Aragon	Spain	80	84	1359	40,438	-0,860	E64			
E65_F_H_I4	H	CRBAME000948	*Nemesia*		Cenajo, Sierra de Segura	Castilla la Mancha	Spain	93	40		38,398	-1,763	E65	E65	E65	
E67_J_H_I4	H	CRBAME000964	*Nemesia*		Cenajo, Sierra de Segura	Castilla la Mancha	Spain	144	40		38,398	-1,763	E67	E67	E67	E67
E7_F_H_I2	H	CRBAME000268	*Nemesia*	dubia	Les Arenes	Catalonia	Spain	68	94	361	41,640	2,058	E7			
E70_F_P_I2	P	CRBAME000980	*Nemesia*		Embalse Veo	Valencian country	Spain	14	66	347	39,932	-0,338	E70	E70	E70	E70
E71_F_P_I2	P	CRBAME000981	*Nemesia*		Embalse Veo	Valencian country	Spain	14	66	347	39,932	-0,338	E71			
E73_F_H_I2	H	CRBAME001002	*Nemesia*		Vallbona	Valencian Country	Spain	77	85	1110	40,578	-0,001	E73			
E75_J_H_I2	H	CRBAME001014	*Nemesia*		Penya Roja	Catalonia	Spain	77	90	375	41,315	1,159	E75	E75	E75	E75
E76_J_H_I2	H	CRBAME001015	*Nemesia*		Penya Roja	Catalonia	Spain	77	90	375	41,315	1,159	E76			
E77_F_P_I2	P	CRBAME001026	*Nemesia*		Atzeneta	Valencian country	Spain	37	77	355	40,213	0,112	E77	E77	E77	
E79_J_P_I2	P	CRBAME001029	*Nemesia*		Atzeneta	Valencian c.	Spain	37	77	355	40,213	0,112	E79			
E84_J_H_I4	H	CRBAME001046	*Nemesia*		TorreVieja	Valencian Country	Spain	145	38	0	37,993	-0,655	E84	E84	E84	E84
E85_F_H_I4	H	CRBAME001049	*Nemesia*		PRegional Calblanque	Murcia	Spain	95	31	21	37,587	-0,838	E85	E85	E85	
E86_F_H_I4	H	CRBAME001050	*Nemesia*		PRegional Calblanque	Murcia	Spain	95	31	21	37,587	-0,838	E86			
E89_F_P_I2	P	CRBAME001067	*Nemesia*		La Cova Santa	Valencian country	Spain	14	63	555	39,849	-0,575	E89			
E91_J_H_I4	H	CRBAME001077	*Nemesia*		Cova del Rull	Valencian country	Spain	146	42	478	38,811	0,177	E91	E91	E91	
E92_F_I_I4	I	CRBAME001079	*Iberesia*		Cullera. Far	Valencian country	Spain	out	52	0	39,191	-0,243	E92	E92	E92	E92
E93_F_P_I2	P	CRBAME001080	*Nemesia*		Cullera. Far	Valencian country	Spain	32	52	0	39,191	-0,243	E93	E93	E93	E93
E94_J_P_SI	P	CRBAME000081	*Nemesia*		Gangi, road to Nicosia		Italy	17	147	915	37,788	14,228	E94			
E95_J_P_SI	P	CRBAME000085	*Nemesia*		Gangi, road to Nicosia		Italy	17	147	915	37,788	14,228	E95	E95	E95	
E96_J_H_SI	H	CRBAME000097	*Nemesia*		Isnello	Sicily	Italy	147	156	720	37,921	13,968	E96	E96		E96
E97_J_U_SI	U	CRBAME000098	*Nemesia*		Isnello	Sicily	Italy	17	156	720	37,921	13,968	E97			
E98_J_P_SI	P	CRBAME000101	*Nemesia*		Isnello	Sicily	Italy	17	156	720	37,921	13,968	E98			
E99_F_P_SI	P	CRBAME000132	*Nemesia*		Luppino	Sicily	Italy	148	165	288	38,074	12,748	E99	E99	E99	E99
H100_j_P_B3	P	CRBA_AP000039	*Nemesia*	randa	Sant Juan, rd. ME7 Fornells to Mao	Minorca	Spain	13	69	12	40,015	4,123	H100			
H101_j_P_B2	P	CRBA_AP000051	*Nemesia*	bristowei	Can Planiol	Majorca	Spain	7	60	456	39,689	2,578	H101			
H103_j_U_B2	U	CRBA_AP000092	*Nemesia*		rd. Orient to Bunyola; rd. slopes	Majorca	Spain	76	61	545	39,727	2,734	H103			
H105_j_P_B1	P	CRBA_AP000128	*Nemesia*	ibiza	Sa Casilla, road Eivissa-St. Antoni	Ibiza	Spain	10	48	97	38,955	1,342	H105			
H107_j_P_B1	P	CRBA_AP000136	*Nemesia*	ibiza	Santa Eularia des Riu, Coll de Vila	Ibiza	Spain	11	47	172	38,944	1,478	H107			
H108_j_P_B1	P	CRBA_AP000146	*Nemesia*	santeulalia	Sant Antoni, Cala Bassa	Ibiza	Spain	33	49	13	38,966	1,242	H108			
H109_j_P_B3	P	CRBA_AP000007	*Nemesia*	randa	Favaritx	Minorca	Spain	13	68	12	39,953	4,221	H109			
H110_j_P_B3	P	CRBA_AP000017	*Nemesia*	randa	Algaiarens beach, rd to Cala Morell	Minorca	Spain	13	71	109	40,036	3,911	H110			

label	S	SPEC_CODE	*genus*	sp	LOC_NAME	Province	Country	gmyc	loc_num	El	Long.	Lat.	16s	28s	h3	EFG
H111_j_P_B3	P	CRBA_AP000027	*Nemesia*	randa	Cala Mitjana, rd. to Cala Galdana	Minorca	Spain	13	67	11	39,936	3,973	H111			
H112_j_P_B3	P	CRBA_AP000040	*Nemesia*	randa	Sant Juan, rd. ME7 Fornells to Mao	Minorca	Spain	13	69	12	40,015	4,123	H112			
H113_j_P_B2	P	CRBA_AP000052	*Nemesia*	bristowei	Can Planiol	Majorca	Spain	7	60	456	39,689	2,578	H113			
H114_j_P_B2	P	CRBA_AP000065	*Nemesia*	randa	Santuari de Cura	Majorca	Spain	12	54	471	39,422	2,929	H114			
H115_f_I_B2	I	CRBA_AP000073	*Iberesia*		Campanet, Vall de Fangar	Majorca	Spain	out	62	69	39,809	2,979	H115	H115	H115	H115
H117_f_P_B1	P	CRBA_AP000114	*Nemesia*	ibiza	Sa Talaia	Ibiza	Spain	9	45	420	38,907	1,267	H117			
H118_j_P_B1	P	CRBA_AP000137	*Nemesia*	ibiza	Santa Eularia des Riu, Coll de Vila	Ibiza	Spain	11	47	172	38,944	1,478	H118			
H12_j_P_B1	P	CRBA_AP000132	*Nemesia*	santeulalia	Santa Eularia des Riu, Coll de Vila	Ibiza	Spain	33	47	172	38,944	1,478	H12	H12	H12	
H120_j_P_B1	P	CRBA_AP000150	*Nemesia*	santeulalia	Sant Llorenç de Balafir	Ibiza	Spain	34	50	134	39,033	1,472	H120			
H121_j_P_B3	P	CRBA_AP000018	*Nemesia*	randa	Algaiarens beach, rd to Cala Morell	Minorca	Spain	13	71	109	40,036	3,911	H121			
H123_j_P_B3	P	CRBA_AP000020	*Nemesia*	randa	Algaiarens beach, rd to Cala Morell	Minorca	Spain	13	71	109	40,036	3,911	H123			
H124_j_U_B3	U	CRBA_AP000028	*Nemesia*	randa	Cala Mitjana, rd. to Cala Galdana	Minorca	Spain	13	67	11	39,936	3,973	H124			
H126_l_U_B3	U	CRBA_AP000033	*Nemesia*	randa	Cala Torta, rd. to Far de Cavalleria	Minorca	Spain	13	74	29	40,057	4,076	H126			
H127_j_P_B3	P	CRBA_AP000045	*Nemesia*	randa	Santa Agueda	Minorca	Spain	13	72	93	40,038	4,000	H127			
H128_j_P_B2	P	CRBA_AP000053	*Nemesia*	bristowei	Can Planiol	Majorca	Spain	7	60	456	39,689	2,578	H128	H128	H128	H128
H129_j_P_B2	P	CRBA_AP000054	*Nemesia*	bristowei	Can Planiol	Majorca	Spain	7	60	456	39,689	2,578	H129			
H130_j_P_B2	P	CRBA_AP000066	*Nemesia*	randa	Santuari de Cura	Majorca	Spain	12	54	471	39,422	2,929	H130			
H131_j_P_B2	P	CRBA_AP000067	*Nemesia*	randa	Santuari de Cura	Majorca	Spain	12	54	471	39,422	2,929	H131			
H133_j_H_B2	H	CRBA_AP000093	*Nemesia*	seldeni	rd. Orient to Bunyola; rd. slopes	Majorca	Spain	76	61	545	39,727	2,734	H133			
H134_j_P_B1	P	CRBA_AP000119	*Nemesia*	santeulalia	Sa Talaia	Ibiza	Spain	33	45	420	38,907	1,267	H134	H134	H134	H134
H135_j_U_B1	U	CRBA_AP000138	*Nemesia*	ibiza	Santa Eularia des Riu, Coll de Vila	Ibiza	Spain	11	47	172	38,944	1,478	H135	H135		
H137_j_P_B1	P	CRBA_AP000140	*Nemesia*	ibiza	Santa Eularia des Riu, Coll de Vila	Ibiza	Spain	11	47	172	38,944	1,478	H137	H137		
H138_j_H_B3	H	CRBA_AP000021	*Nemesia*	n.sp.n.	Algaiarens beach, rd to Cala Morell	Minorca	Spain	81	71	109	40,036	3,911	H138	H138	H138	H138
H139_j_P_B2	P	CRBA_AP000055	*Nemesia*	bristowei	Can Planiol	Majorca	Spain	7	60	456	39,689	2,578	H139			
H14_f_P_B3	P	CRBA_AP000022	*Nemesia*	randa	Cala Mitjana, rd. to Cala Galdana	Minorca	Spain	13	67	11	39,936	3,973	H14			
H142_j_P_B2	P	CRBA_AP000058	*Nemesia*	bristowei	Can Planiol	Majorca	Spain	7	60	456	39,689	2,578	H142			
H143_j_P_B2	P	CRBA_AP000068	*Nemesia*	randa	Santuari de Cura	Majorca	Spain	12	54	471	39,422	2,929	H143			
H147_j_H_B2	H	CRBA_AP000094	*Nemesia*	seldeni	rd. Orient to Bunyola; rd. slopes	Majorca	Spain	76	61	545	39,727	2,734	H147	H147	H147	H147
H148_j_H_B2	H	CRBA_AP000095	*Nemesia*	seldeni	rd. Orient to Bunyola; rd. slopes	Majorca	Spain	76	61	545	39,727	2,734	H148			
H149_j_P_B2	P	CRBA_AP000096	*Nemesia*	bristowei	rd. Orient to Bunyola; rd. slopes	Majorca	Spain	7	61	545	39,727	2,734	H149			
H15_f_P_B3	P	CRBA_AP000029	*Nemesia*	randa	Cala Torta, rd. to Far de Cavalleria	Minorca	Spain	13	74	29	40,057	4,076	H15			
H155_j_P_B2	P	CRBA_AP000113	*Nemesia*	randa	Ermita de Mont Sio	Majorca	Spain	12	55	214	39,495	3,015	H155			
H16_f_P_B3	P	CRBA_AP000035	*Nemesia*	randa	Sant Juan, rd. ME7 Fornells to Mao	Minorca	Spain	13	69	12	40,015	4,123	H16			
H22_f_P_B1	P	CRBA_AP000121	*Nemesia*	ibiza	Punta des Jonc, Cala Sant Vicenç	Ibiza	Spain	8	51	51	39,079	1,596	H22	H22		H22
H23_f_P_B1	P	CRBA_AP000129	*Nemesia*	ibiza	Cap Blanc	Ibiza	Spain	10	44	160	38,880	1,236	H23	H23		
H26_j_P_B1	P	CRBA_AP000125	*Nemesia*	ibiza	Sa Casilla, deroute C73 road Eivissa-St. Antoni	Ibiza	Spain	10	48	97	38,955	1,342	H26			
H27_j_P_B1	P	CRBA_AP000144	*Nemesia*		Sant Antoni, Cala Bassa	Ibiza	Spain	149	49	13	38,966	1,242	H27	H27	H27	H27
H28_f_P_B2	P	CRBA_AP000060	*Nemesia*	randa	Santuari de Cura	Majorca	Spain	12	54	471	39,422	2,929	H28			
H34_f_P_B3	P	CRBA_AP000009	*Nemesia*	randa	Favaritx	Minorca	Spain	13	68	12	39,953	4,221	H34			

label	S	SPEC_CODE	*genus*	sp	LOC_NAME	Province	Country	gmyc	loc_num	El	Long.	Lat.	16s	28s	h3	EFG
H35_f_P_B3	P	CRBA_AP000036	*Nemesia*	randa	Sant Juan, rd. ME7 Fornells to Mao	Minorca	Spain	13	69	12	40,015	4,123	H35			
H38_f_P_B1	P	CRBA_AP000115	*Nemesia*	ibiza	Sa Talaia	Ibiza	Spain	9	45	420	38,907	1,267	H38			
H39_j_U_B1	U	CRBA_AP000133	*Nemesia*	santeulalia	Santa Eularia des Riu, Coll de Vila	Ibiza	Spain	33	47	172	38,944	1,478	H39			
H48_j_P_B3	P	CRBA_AP000042	*Nemesia*	randa	Santa Agueda	Minorca	Spain	13	72	93	40,038	4,000	H48			
H49_j_P_B1	P	CRBA_AP000130	*Nemesia*	ibiza	Cap Blanc	Ibiza	Spain	10	44	160	38,880	1,236	H49			
H52_f_P_B1	P	CRBA_AP000116	*Nemesia*	ibiza	Sa Talaia	Ibiza	Spain	9	45	420	38,907	1,267	H52	H52		H52
H53_j_P_B1	P	CRBA_AP000147	*Nemesia*	santeulalia	Sant Llorenç de Balafir	Ibiza	Spain	34	50	134	39,033	1,472	H53			
H54_f_P_B2	P	CRBA_AP000062	*Nemesia*	randa	Santuari de Cura	Majorca	Spain	12	54	471	39,422	2,929	H54			
H57_f_P_B3	P	CRBA_AP000012	*Nemesia*	randa	Algaiarens beach, rd to Cala Morell	Minorca	Spain	13	71	109	40,036	3,911	H57			
H61_j_P_B3	P	CRBA_AP000006	*Nemesia*	randa	Favaritx	Minorca	Spain	13	68	12	39,953	4,221	H61			
H63_j_P_B3	P	CRBA_AP000031	*Nemesia*	randa	Cala Torta, rd. to Far de Cavalleria	Minorca	Spain	13	74	29	40,057	4,076	H63			
H64_j_P_B3	P	CRBA_AP000043	*Nemesia*	randa	Santa Agueda	Minorca	Spain	13	72	93	40,038	4,000	H64			
H65_j_P_B2	P	CRBA_AP000049	*Nemesia*	bristowei	Can Planiol	Majorca	Spain	7	60	456	39,689	2,578	H65			
H66_j_P_B2	P	CRBA_AP000063	*Nemesia*	randa	Santuari de Cura	Majorca	Spain	12	54	471	39,422	2,929	H66	H66	H66	H66
H67_j_U_B2	U	CRBA_AP000090	*Nemesia*	seldeni	rd. Orient to Bunyola; rd. slopes	Majorca	Spain	76	61	545	39,727	2,734	H67	H67	H67	H67
H69_f_P_B1	P	CRBA_AP000122	*Nemesia*	ibiza	Punta des Jonc, Cala Sant Vicenç	Ibiza	Spain	8	51	51	39,079	1,596	H69			
H7_f_U_B3	U	CRBA_AP000010	*Nemesia*	randa	Algaiarens beach, rd to Cala Morell	Minorca	Spain	13	71	109	40,036	3,911	H7	H7	H7	H7
H70_j_P_B1	P	CRBA_AP000134	*Nemesia*	santeulalia	Santa Eularia des Riu, Coll de Vila	Ibiza	Spain	34	47	172	38,944	1,478	H70	H70	H70	H70
H71_j_P_B1	P	CRBA_AP000145	*Nemesia*	santeulalia	Sant Antoni, Cala Bassa	Ibiza	Spain	33	49	13	38,966	1,242	H71			
H72_j_P_B1	P	CRBA_AP000148	*Nemesia*	santeulalia	Sant Llorenç de Balafir	Ibiza	Spain	34	50	134	39,033	1,472	H72			
H75_j_P_B3	P	CRBA_AP000034	*Nemesia*	randa	Cala Torta, rd. to Far de Cavalleria	Minorca	Spain	13	74	29	40,057	4,076	H75			
H81_f_P_B1	P	CRBA_AP000123	*Nemesia*	ibiza	Punta des Jonc, Cala Sant Vicenç	Ibiza	Spain	8	51	51	39,079	1,596	H81			
H83_j_P_B1	P	CRBA_AP000135	*Nemesia*	santeulalia	Santa Eularia des Riu, Coll de Vila	Ibiza	Spain	34	47	172	38,944	1,478	H83			
H89_j_H_B2	H	CRBA_AP000050	*Nemesia*	seldeni	Can Planiol	Majorca	Spain	150	60	456	39,689	2,578	H89	H89	H89	H89
H91_j_U_B2	U	CRBA_AP000091	*Nemesia*		rd. Orient to Bunyola; rd. slopes	Majorca	Spain	76	61	545	39,727	2,734	H91			
H93_j_P_B1	P	CRBA_AP000117	*Nemesia*	ibiza	Sa Talaia	Ibiza	Spain	9	45	420	38,907	1,267	H93			
H94_j_P_B1	P	CRBA_AP000124	*Nemesia*	ibiza	Punta des Jonc, Cala Sant Vicenç	Ibiza	Spain	8	51	51	39,079	1,596	H94			
H95_j_P_B1	P	CRBA_AP000127	*Nemesia*	santeulalia	Sa Casilla	Ibiza	Spain	33	48	97	38,955	1,342	H95	H95	H95	H95
H96_j_P_B1	P	CRBA_AP000149	*Nemesia*	santeulalia	Sant Llorenç de Balafir	Ibiza	Spain	34	50	134	39,033	1,472	H96			
H97_j_P_B3	P	CRBA_AP000005	*Nemesia*	randa	Favaritx	Minorca	Spain	13	68	12	39,953	4,221	H97			
H99_j_P_B3	P	CRBA_AP000026	*Nemesia*	randa	Cala Mitjana, rd. to Cala Galdana	Minorca	Spain	13	67	11	39,936	3,973	H99			
ME624_F_P_I4	P	CRBAME000624	*Nemesia*		Jete	Andalusia	Spain	5	8	95	36,784	-3,672	ME624			
ME626_F_P_I4	P	CRBAME000626	*Nemesia*		Jete	Andalusia	Spain	151	8	95	36,784	-3,672	ME626		ME626	
ME627_F_P_I4	P	CRBAME000627	*Nemesia*		Jete	Andalusia	Spain	5	8	95	36,784	-3,672	ME627			
ME657_F_P_I4	P	CRBAME000657	*Nemesia*		Sayalonga	Andalusia	Spain	1	9	332	36,796	-4,016	ME657	ME657	ME657	ME657
ME665_F_P_I4	P	CRBAME000665	*Nemesia*		Frigiliana	Andalusia	Spain	3	10	379	36,797	-3,904	ME665	ME665	ME665	
ME675_F_I_I4	I	CRBAME000675	*Iberesia*		Desfiladero de los gaitanes	Andalusia	Spain	OUT	230	290	36,892	-4,682	ME675	ME675	ME675	ME675
ME684_F_P_I4	P	CRBAME000684	*Nemesia*		Desfiladero de los gaitanes	Andalusia	Spain	4	230	290	36,892	-4,682	ME684	ME684	ME684	ME684
ME685_J_P_I4	P	CRBAME000685	*Nemesia*		Desfiladero de los gaitanes	Andalusia	Spain	4	230	290	36,892	-4,682	ME685			
ME691_F_I_I3	I	CRBAME000691	*Iberesia*		Finca El Peso	Andalusia	Spain	OUT	1	46	36,388	-5,651	ME694	ME691	ME692	ME693

label	S	SPEC_CODE	*genus*	sp	LOC_NAME	Province	Country	gmyc	loc_num	El	Long.	Lat.	16s	28s	h3	EFG
ME728_F_P_I3	P	CRBAME000728	*Nemesia*		Villablanca	Andalusia	Spain	26	27	19	37,249	-7,343	ME728			
ME729_F_P_I3	P	CRBAME000729	*Nemesia*		Villablanca	Andalusia	Spain	26	27	19	37,249	-7,343	ME729	ME729	ME729	ME729
ME730_J_P_I3	P	CRBAME000730	*Nemesia*		Villablanca	Andalusia	Spain	26	27	19	37,249	-7,343	ME730			
ME775_F_H_I3	H	CRBAME000775	*Nemesia*		Cartama Estacion	Andalusia	Spain	70	4	48	36,729	-4,647	ME775	ME775	ME775	ME775
ME776_F_H_I3	H	CRBAME000776	*Nemesia*		Cartama Estacion	Andalusia	Spain	70	4	48	36,729	-4,647	ME776			
ME822_J_H_I4	H	CRBAME000822	*Nemesia*		Mojon Alto	Andalusia	Spain	152	30	748	37,461	-4,371	ME822			
ME828_F_P_I4	P	CRBAME000828	*Nemesia*		Orgiva	Andalusia	Spain	2	15	529	36,902	-3,442	ME828			
ME829_F_P_I4	P	CRBAME000829	*Nemesia*		Orgiva	Andalusia	Spain	2	15	529	36,902	-3,442	ME829			
ME844_F_H_I4	H	CRBAME000844	*Nemesia*		Collado Garcia	Almeria	Spain	69	26	1155	37,216	-2,297	ME844			
ME845_f_H_I4	H	CRBAME000845	*Nemesia*		Collado Garcia	Almeria	Spain	69	26	1155	37,216	-2,297	ME845	ME845	ME845	ME845
ME852_F_P_I4	P	CRBAME000852	*Nemesia*		Torcal de Antequera	Andalusia	Spain	4	17	1013	36,962	-4,519	ME852	ME852	ME852	ME852
RA1_J_P_I1	P	CRBAME000001	*Nemesia*		Portela, rd N103 to Vinhais	Bragança	Portugal	29	185	830	41,836	-6,856	RA1	RA1	RA1	RA1
RA10_F_P_I1	P	CRBAME000010	*Nemesia*		Las Medulas	Galicia	Spain	28	225	713	42,468	-6,761	RA10			
RA105F_J_P_MO	P	CRBAMM000514	*NEMESIA*		Bni-Hadifa		Morocco	100	110	1796	35,005	-4,179	RA105F			
RA118_J_I_I4	I	CRBAMM001069	*Iberesia*		Archena	Murcia	Spain	OUT	39	277	38,085	-1,365	RA118	RA118	RA118	RA118
RA119F_M_H_I4	H	CRBAMM000211	*Nemesia*		Bco. del Espostal, Granada	Andalusia	Spain	153	24		37,150	-3,530	RA119F	RA119F	RA119F	
RA12_J_P_I1	P	CRBAME000012	*Nemesia*		Sobrado	Galicia	Spain	30	222	620	42,314	-7,242	RA12			
RA120_M_H_I2	H	CRBAMM000319	*Nemesia*	dubia	Canyamars	Catalonia	Spain	154	221		42,235	2,711	RA120	RA120	RA120	
RA126F_J_P_MO	P	CRBAMM000439	*Nemesia*		Imouzer Kandar	Ifrane	Morocco	155	124	1477	33,644	-5,069	RA126F			
RA127_F_P_MO	P	CRBAMM000454	*Nemesia*		m–a Djebel Zalach, FŽs	FŽs	Morocco	156	167	816	34,107	-4,969	RA127	RA127	RA127	RA127
RA128_F_P_MO	P	CRBAMM000476	*Nemesia*		Bab-Azhar		Morocco	98	107	1126	34,057	-4,237	RA128		RA128	
RA129_F_P_MO	P	CRBAMM000477	*Nemesia*		Bab-Azhar		Morocco	98	107	1126	34,057	-4,237	RA129			
RA13_F_P_I1	P	CRBAME000013	*Nemesia*		Sobrado	Galicia	Spain	30	222	620	42,314	-7,242	RA13			
RA131_J_H_MO	H	CRBAMM000487	*Nemesia*		Ain-Sfa		Morocco	157	106	567	34,825	-2,087	RA131			
RA132_J_P_MO	P	CRBAMM000494	*Nemesia*		reserva Beni- Snasen, Tafral		Morocco	158	187	888	34,803	-2,397	RA132			
RA135_F_P_MO	P	CRBAMM000497	*Nemesia*		Gorge du Zegzel		Morocco	99	154	441	34,838	-2,358	RA135	RA135	RA135	
RA136_F_P_MO	P	CRBAMM000498	*Nemesia*		Gorge du Zegzel		Morocco	99	154	441	34,838	-2,358	RA136			
RA138F_F_H_MO	H	CRBAMM000512	*Nemesia*		Bni-Hadifa		Morocco	159	110	1796	35,005	-4,179	RA138F			
RA139_F_P_MO	P	CRBAMM000513	*Nemesia*		Bni-Hadifa		Morocco	100	110	1796	35,005	-4,179	RA139			
RA14_F_P_I1	P	CRBAME000014	*Nemesia*		Sobrado	Galicia	Spain	30	222	620	42,314	-7,242	RA14	RA14	RA14	RA14
RA140_F_P_MO	P	CRBAMM000517	*Nemesia*		Taunateel-Kchour, pinus		Morocco	97	210	1796	35,005	-4,179	RA140			
RA141F_F_P_MO	P	CRBAMM000518	*Nemesia*		Taunateel-Kchour, pinus		Morocco	97	210	1796	35,005	-4,179	RA141F	RA141F	RA141F	RA141F
RA143_J_P_MO	P	CRBAMM000545	*Nemesia*		Rhafsai		Morocco	160	188	189	34,632	-4,931	RA143			
RA146_J_P_MO	P	CRBAMM000572	*Nemesia*		Beni - Yder - cherki		Morocco	96	109	331	35,386	-5,522	RA146			
RA148F_J_H_I	H	CRBAMM000586	*Nemesia*		Portacomaro		Italy	46	184		44,950	8,250	RA148F			
RA149F_F_P_I3	P	CRBAMM000719	*Nemesia*	athiasi	Cabo de Sao Vicente	Sagres	Portugal	25	116	49	37,028	-8,969	RA149F	RA149F	RA149F	RA149F
RA15_F_P_I1	P	CRBAME000015	*Nemesia*		Sobrado	Galicia	Spain	30	222	620	42,314	-7,242	RA15			
RA150F_F_P_I3	P	CRBAMM000720	*Nemesia*		Cabo de Sao Vicente	Sagres	Portugal	25	116	49	37,028	-8,969	RA150F			
RA151_F_P_FR	P	CRBAMM000776	*Nemesia*	carminans	Col de Vence	Nice	France	22	133	580	43,747	7,100	RA151			
RA152_F_H_FR	H	CRBAMM000777	*Nemesia*		Col de Vence	Nice	France	91	133	580	43,747	7,100	RA152	RA152	RA152	

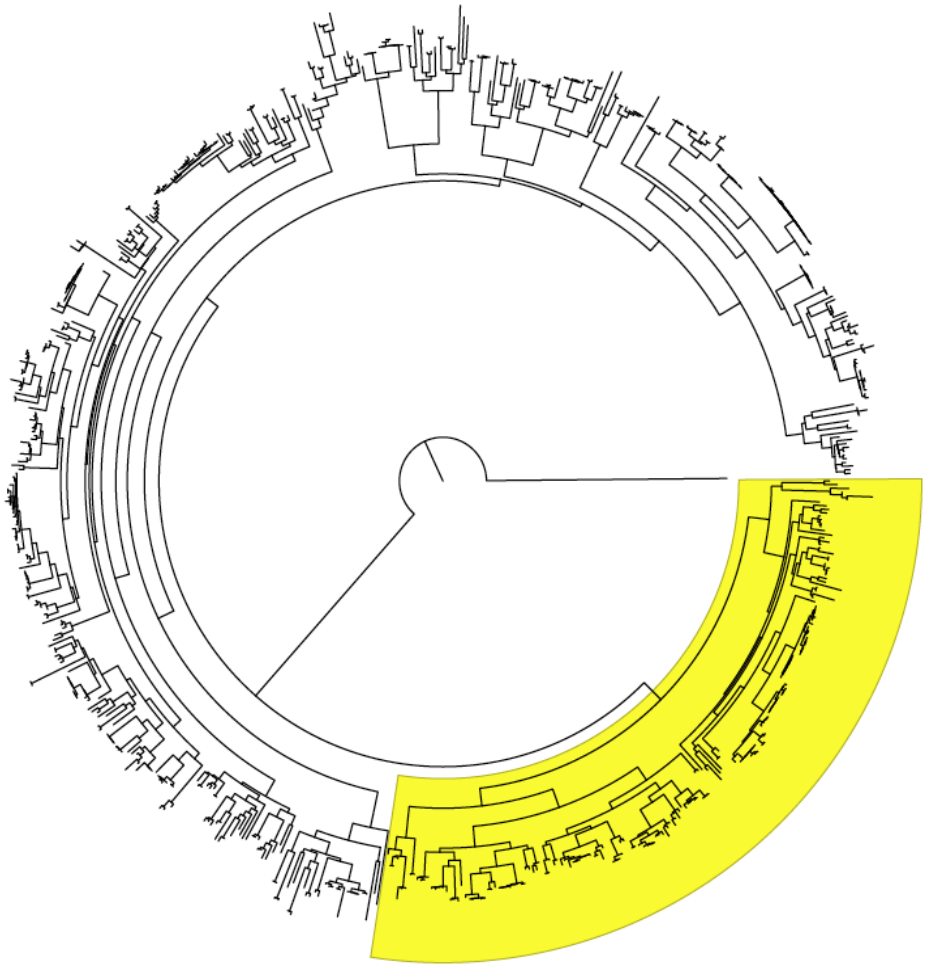
label	S	SPEC_CODE	*genus*	sp	LOC_NAME	Province	Country	gmyc	loc_num	El	Long.	Lat.	16s	28s	h3	EFG
RA153_F_H_FR	H	CRBAMM000778	*Nemesia*		Col de Vence	Nice	France	91	133	580	43,747	7,100	RA153			
RA154_J_H_FR	H	CRBAMM000780	*Nemesia*		Col de Vence	Nice	France	91	133	580	43,747	7,100	RA154			
RA155_F_H_FR	H	CRBAMM000782	*Nemesia*		Gattieres, crtra Tou le Boc		France	161	149	292	43,771	7,175	RA155	RA155	RA155	RA155
RA156_J_H_FR	H	CRBAMM000810	*Nemesia*		Gioia, Portacomaro	Asti	italy	162	152	192	44,952	8,287	RA156			
RA16_F_P_I1	P	CRBAME000016	*Nemesia*		Sobrado	Galicia	Spain	30	222	620	42,314	-7,242	RA16			
RA167_F_P_FR	P	CRBAMM000837	*Nemesia*	carminans	le Mourre, gar de Freinet		France	22	161	325	43,347	6,413	RA167			
RA17_F_P_I1	P	CRBAME000017	*Nemesia*		Sobrado	Galicia	Spain	30	222	620	42,314	-7,242	RA17			
RA170_f_H_I4	H	CRBAMM001014	*Nemesia*		Puente Genil	Andalusia	Spain	163	29	256	37,381	-4,793	RA170	RA170	RA170	RA170
RA171_j_H_CR	H	CRBAME000027	*Nemesia*		Bramiana Lake	Crete	Greece	64	115	69	35,036	25,703	RA171	RA171	RA171	RA171
RA172_J_H_CR	H	CRBAME000028	*Nemesia*		Bramiana Lake	Crete	Greece	64	115	69	35,036	25,703	RA172			
RA176_F_P_I2	P	CRBAME000062	*Nemesia*	simoni	St. Esteve de Llemena	Catalonia	Spain	164	219	294	42,065	2,612	RA176	RA176	RA176	
RA177_f_P_I2	P	CRBAME000066	*Nemesia*	caementaria	Llora	Catalonia	Spain	20	218		42,030	2,701	RA177	RA177	RA177	RA177
RA178_F_P_I2	P	CRBAME000067	*Nemesia*	caementaria	Llora	Catalonia	Spain	20	218		42,030	2,701	RA178			
RA18_F_P_I1	P	CRBAME000018	*Nemesia*	athiasi	El Travieso	Castilla León	Spain	23	82	1879	40,335	-5,732	RA18	RA18	RA18	RA18
RA189_M_H_IT	H	CRBAMM000217	*Nemesia*		Boccu di Barinu	Corsica	France	165	112	754	41,627	9,149	RA189	RA189	RA189	
RA19_F_P_I1	P	CRBAME000019	*Nemesia*	athiasi	El Travieso	Castilla León	Spain	23	82	1879	40,335	-5,732	RA19			
RA191_f_P_FR	P	CRBAMM000844	*Nemesia*	carminans	Fontvielle, PN des Alpilles	Alpes	France	166	142	18	43,759	4,695	RA191	RA191	RA191	RA191
RA192_f_P_SI	P	CRBAME000094	*Nemesia*		Isnello	Sicily	Italy	167	156	720	37,921	13,968	RA192	RA192	RA192	RA192
RA193_f_H_SI	H	CRBAME000106	*Nemesia*		Cerda	Sicily	Italy	50	125	326	37,857	13,846	RA193	RA193	RA193	RA193
RA194_F_H_SI	H	CRBAME000134	*Nemesia*		Luppino	Sicily	Italy	50	165	288	38,074	12,748	RA194			
RA195_F_H_SI	H	CRBAME000129	*Nemesia*		Luppino	Sicily	Italy	50	165	288	38,074	12,748	RA195			
RA196_F_H_SI	H	CRBAME000181	*Nemesia*		Ponte Olivo	Sicily	Italy	168	183	158	37,121	14,381	RA196	RA196	RA196	RA196
RA197_F_H_SI	H	CRBAME000204	*Nemesia*		Garcia, near Villarosa	Sicily	Italy	169	148	401	37,602	14,139	RA197	RA197	RA197	RA197
RA198_F_H_I2	H	CRBAME000237	*Nemesia*	dubia	Marquet Roques, PN SLLM	Catalonia	Spain	68	101	561	41,676	2,019	RA198			
RA199_F_H_I2	H	CRBAME000242	*Nemesia*	dubia	Agramunt, PN SLLM	Catalonia	Spain	68	198	557	41,687	2,021	RA199			
RA2_J_P_I1	P	CRBAME000002	*Nemesia*		Portela	Bragança	Portugal	29	185	830	41,836	-6,856	RA2			
RA20_F_P_I1	P	CRBAME000020	*Nemesia*	athiasi	El Travieso	Castilla León	Spain	23	82	1879	40,335	-5,732	RA20			
RA200_F_H_I2	H	CRBAME000258	*Nemesia*	dubia	Lligabossa, PN SLLM	Catalonia	Spain	68	199	557	41,687	2,021	RA200	RA200	RA200	RA200
RA201_F_H_SA	H	CRBAME000271	*Nemesia*		Giara di Gesturi	Sardinia	Italy	170	151	526	39,738	9,007	RA201	RA201	RA201	RA201
RA202_F_H_SA	H	CRBAME000291	*Nemesia*		Gadoni. quercus forest	Sardinia	Italy	89	146	756	39,926	9,184	RA202	RA202	RA202	RA202
RA203_F_H_SA	H	CRBAME000314	*Nemesia*		Monte Limbara	Sardinia	Italy	82	173	770	40,858	9,132	RA203			
RA204_F_H_SA	H	CRBAME000328	*Nemesia*		Monte Limbara	Sardinia	Italy	82	173	770	40,858	9,132	RA204			
RA205_F_H_SA	H	CRBAME000337	*Nemesia*		Domus de Maria	Sardinia	Italy	82	138	111	38,940	8,834	RA205	RA205	RA205	
RA206_F_H_SA	H	CRBAME000348	*Nemesia*		Monte Rasu	Sardinia	Italy	171	174	896	40,435	9,029	RA206	RA206	RA206	RA206
RA207_F_H_CO	H	CRBAME000410	*Nemesia*		Cervione, Valle di campoloro	Corsica	France	44	126	338	42,323	9,490	RA207			
RA208_F_H_CO	H	CRBAME000428	*Nemesia*		Lu Bagnu	Sardinia	italy	172	164	32	40,891	8,652	RA208	RA208	RA208	RA208
RA209_F_H_SA	H	CRBAME000460	*Nemesia*		Crisciuleddu	Sardegna	Italy	173	135	115	41,073	9,175	RA209	RA209	RA209	RA209
RA21_F_H_I4	H	CRBAMM000046a	*Nemesia*		La Calahurra	Andalusia	Spain	74	23	1723	37,131	-3,041	RA21			
RA24_J_H_I4	H	CRBAMM000046d	*Nemesia*		La Calahurra	Andalusia	Spain	74	23	1723	37,131	-3,041	RA24	RA24	RA24	RA24

label	S	SPEC_CODE	*genus*	sp	LOC_NAME	Province	Country	gmyc	loc_num	El	Long.	Lat.	16s	28s	h3	EFG
RA25_J_H_I4	H	CRBAMM000046e	*Nemesia*		La Calahurra	Andalusia	Spain	74	23	1723	37,131	-3,041	RA25			
RA26_J_H_I4	H	CRBAMM000046f	*Nemesia*		La Calahurra	Andalusia	Spain	74	23	1723	37,131	-3,041	RA26			
RA27_J_H_I4	H	CRBAMM000046g	*Nemesia*		La Calahurra	Andalusia	Spain	74	23	1723	37,131	-3,041	RA27			
RA29_J_H_I4	H	CRBAMM000046i	*Nemesia*		La Calahurra	Andalusia	Spain	74	23	1723	37,131	-3,041	RA29			
RA3_J_P_I1	P	CRBAME000003	*Nemesia*		Portela	Bragança	Portugal	29	185	830	41,836	-6,856	RA3			
RA30_J_H_I4	H	CRBAMM000046j	*Nemesia*		La Calahurra	Andalusia	Spain	74	23	1723	37,131	-3,041	RA30			
RA31_J_H_I4	H	CRBAMM000046k	*Nemesia*		La Calahurra	Andalusia	Spain	74	23	1723	37,131	-3,041	RA31			
RA32_J_H_I4	H	CRBAMM000046l	*Nemesia*		La Calahurra	Andalusia	Spain	74	23	1723	37,131	-3,041	RA32			
RA4_F_P_I1	P	CRBAME000004	*Nemesia*		Prado Cavalo	Galicia	Spain	174	220	790	42,111	-7,128	RA4	RA4	RA4	RA4
RA40_F_I_MO	I	CRBAMM000537	*Iberesia*		Rhafsai		Morocco	OUT	188	189	34,632	-4,931	RA40	RA40	RA40	RA40
RA5_J_P_I1	P	CRBAME000005	*Nemesia*		Prado Cavalo	Galicia	Spain	175	220	790	42,111	-7,128	RA5	RA5	RA5	RA5
RA54_F_P_MO	P	CRBAMM000573	*Nemesia*		Beni - Yder - cherki		Morocco	96	109	331	35,386	-5,522	RA54	RA54	RA54	
RA55F_J_P_MO	P	CRBAMM000575	*Nemesia*		Beni - Yder - cherki		Morocco	96	109	331	35,386	-5,522	RA55F			
RA6_J_P_I1	P	CRBAME000006	*Nemesia*		Embalse Selga	Castilla León	Spain	31	226	1029	42,753	-5,778	RA6	RA6	RA6	
RA7_F_U_I1	U	CRBAME000007	*Nemesia*		Embalse Selga	Castilla León	Spain	31	226	1029	42,753	-5,778	RA7			
RA8_F_P_I1	P	CRBAME000008	*Nemesia*		Las Medulas	Galicia	Spain	28	225	713	42,468	-6,761	RA8			
RA84_f_P_I4	P	CRBAMM001004	*Nemesia*		Torcal de Antequera 2	Andalusia	Spain	4	78	1104	36,961	-4,527	RA84	RA84	RA84	RA84
RA9_F_P_I1	P	CRBAME000009	*Nemesia*		Las Medulas	Galicia	Spain	28	225	713	42,468	-6,761	RA9	RA9	RA9	RA9
RA98_F_H_CO	H	CRBAMM000218	*Nemesia*		Zonza, rd to L«Ospedale	Corsica	France	176	227	938	41,425	9,124	RA98			
Z114_J_H_I4	H	CRBAMM000114	*Nemesia*		El Valle, Alberca	Murcia	Spain	94	35	164	37,930	-1,130	Z114			
Z117_J_U_I4	U	CRBAMM000117	*Iberesia*		Parc regional de Calbalanque,	Murcia	Spain	OUT	32	88	37,608	-0,760	Z117	Z117	Z117	Z117
Z118_M_P_I4	P	CRBAMM000118	*Nemesia*	dorthesi/val.	Parc regional de Calbalanque,	Murcia	Spain	177	32	88	37,608	-0,760	Z118	Z118	Z118	Z118
Z119_M_P_I4	P	CRBAMM000119	*Nemesia*	dorthesi/val.	Sierra de Almagrera	Murcia	Spain	178	28	28	37,322	-1,702	Z119	Z119	Z119	Z119
Z134_J_U_I4	U	CRBAMM000134	*Nemesia*		Vera, pinar en el pueblo	Almeria	Spain	35	25	18	37,213	-1,827	Z134	Z134		Z134
Z135_J_P_I4	P	CRBAMM000135	*Nemesia*		Vera, pinar en el pueblo	Almeria	Spain	35	25	18	37,213	-1,827	Z135		Z135	
Z144_J_P_I4	P	CRBAMM000144	*Nemesia*		Tabernas	Almeria	Spain	6	20	371	37,036	2,418	Z144			
Z145_J_I_I4	I	CRBAMM000145	*Iberesia*		Tabernas	Almeria	Spain	OUT	20	371	37,036	-2,418	Z145	Z145	Z145	Z145
Z149_F_P_I4	P	CRBAMM000149	*Nemesia*		Lucainena de las Torres	Almeria	Spain	38	22	527	37,050	-2,205	Z149	Z149	Z149	Z149
Z151_F_P_I4	P	CRBAMM000151	*Nemesia*		Lucainena de las Torres	Almeria	Spain	38	22	527	37,050	-2,205	Z151			
Z156_M_P_I4	P	CRBAMM000156	*Nemesia*		Punto Polacra, ctra Torre de Lobos	Almeria	Spain	6	13	41	36,848	-2,025	Z156	Z156	Z156	Z156
Z159_J_P_I4	P	CRBAMM000159	*Nemesia*		Punto Polacra, ctra Torre de Lobos	Almeria	Spain	6	13	41	36,848	-2,025	Z159			
Z174_M_H_I2	H	CRBAMM000174	*Nemesia*		Moixent	Valencian C.	Spain	179	43	357	38,869	-0,759	Z174	Z174	Z174	
Z183_J_U_I2	U	CRBAMM000183	*Nemesia*		L' Eliana	Valencian C.	Spain	32	58	95	39,547	-0,510	Z183			
Z187_M_P_I4	P	CRBAMM000187	*Nemesia*	dorthesi/val.	Boca de los Freiles, Cabo de Gata	Almeria	Spain	36	11		36,810	-2,147	Z187	Z187	Z187	Z187
Z188F_M_U_I4	U	CRBAMM000188	*Nemesia*	dorthesi/val.	Boca de los Freiles, Cabo de Gata	Almeria	Spain	36	11		36,810	-2,147	Z188F			
Z201_M_H_I2	H	CRBAMM000201	*Nemesia*		Castell de Vallès	Catalonia	Spain	94	93		41,624	2,086	Z201			
Z202_m_U_I3	U	CRBAMM000202	*Nemesia*	uncinata	Sra. da Graça de Padroes		Portugal	180	205		37,339	-7,580	Z202	Z202	Z202	Z202
Z33_f_U_GR_VF	out	CRBAMM000033	*Brachytele*		Stomio		Greece	out	208		39,867	22,733	Z33	Z33	Z33	Z33
Z45_j_U_I4	U	CRBAMM000045	*Nemesia*		Puerto de ragua	Andalusia	Spain	73	21	1530	37,049	-2,998	Z45	Z45	Z45	Z45
Z46_f_U_I4	U	CRBAMM000046	*Nemesia*		La Calahurra	Andalusia	Spain	74	23	1723	37,131	-3,041	Z46	Z46	Z46	

label	S	SPEC_CODE	*genus*	sp	LOC_NAME	Province	Country	gmyc	loc_num	El	Long.	Lat.	16s	28s	h3	EFG
Z58_M_U_I2	U	CRBAMM000058	*Nemesia*		Margem do sol posto		Portugal	181	169		41,740	-7,643	Z58			
Z66_J_P_I2	P	CRBAMM000066	*Nemesia*	raripilia	Coll las tres creus	Catalonia	Spain	101	95	874	41,648	1,972	Z66			
Z77_F_P_I2	P	CRBAMM000077	*Nemesia*	raripilia	Coll las tres creus	Catalonia	Spain	101	95	874	41,648	1,972	Z77			
Z83_J_P_I2	P	CRBAMM000083	*Nemesia*	raripilia	Coll las tres creus	Catalonia	Spain	101	95	874	41,648	1,972	Z83			
Z86_L_U_I4	U	CRBAMM000086 - AD1	*Nemesia*		Malaga	Andalusia	Spain	182	7		36,765	-4,208	Z86			
Z89_L_U_I4	U	CRBAMM000089 -AD4	*Nemesia*		Malaga	Andalusia	Spain	3	7		36,765	-4,208	Z89			
Z92_L_U_I4	U	CRBAMM000092 - AD7	*Nemesia*		Barcelona	Catalonia	Spain	183	91		41,443	2,100	Z92			
Z94_L_P_B2	P	CRBAMM000094 - AD9	*Nemesia*	randa	nr Castellitx de la Pau	Majorca	Spain	12	57		39,543	2,920	Z94			
Z98_L_P_B2	P	CRBAMM000098 - AD13	*Nemesia*	randa	Half way along the road (Llucmajor /Porreres)	Majorca	Spain	12	56		39,514	2,739	Z98			

Chapter 4.2

Systematics and biogeography of the genus *Iberesia* (Araneae, Nemesiidae)



Systematic and biogeography in a taxonomically challenging group: the study case of *Iberesia*

1.Introduction

Species are fundamental units of Biology (Darwin, 1859; Dobzhansky, 1937; Mayr, 1942; Wiley, 1981; de Queiroz, 2005). Notwithstanding the need for a useful concept and the fact all scientist have a clear idea on what constitutes specie, very few topics in biology have raised as much debate as the species concept (Mayr, 1942; Cracraft, 1989; Claridge et al., 1997; de Queiroz, 1998, 2005; Coyne & Orr, 2004; de Queiroz & Donoghue, 2013). Mayden (1997) listed more than 20 different named species concepts. The main problem is that those concepts and their definitions have been created by different disciplines of biology, and each one involves at least partially incompatible species concepts (Mayden, 1997; de Queiroz, 1998; Harrison, 1998) because they were initially formulated to answer particularly questions from each discipline. For instance, the biological species concept defines a species as "a group of natural populations that are potentially capable of mate and produce fertile offspring, and they are reproductively isolated from other ", this can't be applied to either asexual species, fossils or viruses, and sometimes it is impossible to determine. Those incompatibilities can lead to different conclusions concerning the boundaries and numbers of species.

To make matters worse, the concept of species has been confused with the issue of species delimitation itself. This is commonly known as the *species problem*(Hei, 2001). Hence, species problem involves different questions: on the one hand, the species concept *per se* (an idea of what kind of entity species are), and on the other hand the methodological approach to recognizing species in a particular case. De Queiroz noticed that the main problem was when both questions were linked. Solution was proposed by the same de Queiroz, namely, the unified *species concept* (1998,2005,2007) where a specie is defined as a "metatpopulation that evolves independently"; and allows to distinguish between what a specie is and the different sources of evidences used to recognise it.

De Queiroz (1998; 2007) postulated that all these species definitions from

different disciplines are criteria to delineate species and have a point in common: there are based on demonstrate that species are the result of evolutionary lineages or populations, where lineage refers to a succession of ancestor-descendant. Hence, all definitions are lines of evidence to test the hypothesis of a species and are therefore relevant for species delimitation. Nowadays the emerging consensus in the scientific community is that the best approach is to employ different data types obtained from different sources of evidence and different analytical approaches to extract the most meaningful information.

Species boundaries delimitation may be particularly challenging when dealing with morphologically uniform (Stockman & Bond, 2007; Bond & Stockman, 2008; Hamilton et al., 2011, 2014; Hendrixson et al., 2013) or recently evolved taxa (Pons et al., 2006; Shaffer & Thomson, 2007) and resulting in a misidentification of biodiversity. This can led to serious consequences affecting to general knowledge of nature's patterns and processes and conservation efforts (Wiens, 2007).

Despite the general perception that the major availability of molecular data can be the solution to a general crisis in taxonomy, only a small portion of species delimitation studies provide species description. Although there are some cases where only morphological characters may fail to recognise species, especially with cryptic species were no selective pressures on morphology can hamper a lot of genetic diversity. The final decision on what constitutes an actual species will further require the integration of additional sources of evidence (Kekkonen & Hebert, 2014).

The infraorder Mygalomorphae, comprising tarantulas, purse-web spiders, funnel-web spiders, trap-door spiders, are a very good model to test for species boundaries(Bond, 2004, 2012; Hendrixson & Bond, 2005a, Hendrixson & Bond, 2005b; Bond & Hedin, 2006; Bond & Stockman, 2008; Bailey et al., 2010; Satler et al., 2011, 2013; Hamilton et al., 2011; Opatova et al., 2013; Hendrixson et al., 2013; Opatova & Arnedo, 2014a, 2014b).

Mygalomorph have posed important challenges to taxonomists. Distinguishing species is extremely difficult because most of the closely related taxa are

morphologically homogenous(Hedin & Bond, 2006); most of the descriptions are old and ambiguous, furthermore the diagnostic characters are based only on male reproductive organs, that are available in the field only during a short period of time, whereas direct capture only provides females that in species are not known. In addition, most of the descriptions are made with only one of the two sexes, point that makes extremely difficult a correct identification of samples.

Due to their burrow fidelity, narrow ecological preferences and limited dispersal abilities, mygalomorph populations are typically clustered in isolated aggregations(Bond et al., 2006). As predicted for any kind of species with such lifestyles, species-level investigations of mygalomorphs typically reveal extensive population genetic structure (Starrett & Hedin, 2007; Bond & Stockman, 2008; Opatova et al., 2013; Satler et al., 2013; Opatova & Arnedo, 2014b). Species delimitation is more difficult when empirical systems lack pre-existing divisions (O'Meara, 2010), is the case of mygalomorph species, which poor taxonomy hampered the development of those organisms as system models.

Their conserved morphology combined with the high population structure, make taxonomy studies bases on DNA, but those characteristics leads a high number of clades in mtDNA gene trees, which could result in oversplitting mygalomorph species into dozens of new species (Satler et al., 2013), but also there is the possibility that speciation occurs in absence of morphological or apparent ecological divergence(Bond et al., 2012). Those reasons make them a good candidate to investigate species boundaries.

Our subject of study is the genus *Iberesia* (Decae & Cardoso, 2006), belongs to the familiy Nemesiidae. *Iberesia*, was recently described (2005) only based on morphological information and its supposedly the sister group of the genus *Nemesia*, the most diverse and widespread mygalomorph genus on the Mediterranean region. The genus has a restricted distribution in comparison to her sister group, is endemic to the Iberian Peninsula and Balearic Islands.

The genus was described to receive species from the Iberian Peninsula traditionally included in *Nemesia* Audouin, 1826, but to be distinguished by one

sinapormorphy: the absence of the posterior median spinnerets.

The species of *Iberesia* are in general big sized, robust species with a very aggressive behaviour and construct deep and wide burrow, with a characteristic cork door and covered by a thin layer of silk in the upper half. *Iberesia* can be easily distinguishable from *Nemesia* due to the Supra-Spine in pro-lateral patella III and by having one row of spiky cuspules on the lateral edge of the maxillae (Fig.1).

Fig.1.Up: general appeareance of *Iberesia machadoi.* Photo credit: Elisa Mora.Down: distinguisable characters of *Iberesia*, adapted from Decae & Cardoso, 2006. 1a)Absence of Posterior Median Spinnerets. 1b)Line of spiky cuspules at lateral edge of the maxillae 1c)Supra-spine in prolateral Patella II

The genus includes only three species. Two of them were transferred from *Nemesia : I.brauni* C.L.Koch, 1882 and *I. castillana* Frade & Bacelar, 1931. The third specie *I. machadoi* Decae & Cardoso, 2006 (Fig.1) is the most recently

described and its named in apposition to Antonio de Barros Machado, who was the first author that noticed the reduction of the Posterior Median Spinnerets (PMS). As a matter of fact, Machado thought he was studying specimens of *Nemesia castillana*, but later Decae & Cardoso (2006) found after a deep look at the museum material that the specimens he was studying were a new specie distributed around Portugal, so *I. machadoi* have long been incorrectly identified as *Nemesia hispanica.*Males are distinguishable by embolous with denticles on the tip with the exception of the male of *Iberesia castillana*, which is smooth and slightly bent. Female's spermatheca is bipartite or tripartite (Decae & Cardoso, 2006; Decae et al., 2007).

The main objectives of the present study are: (1) provide for the first time a molecular phylogeny including the whole distribution of *Iberesia* and evaluate the concordance of the results with previous morphological studies; (2) to infer the temporal framework for the diversification of the genus using a multi-locus approach; (3) to explore the interespecific relationships, and the possible presence of unrecognised divergent lineages in *Iberesia.*

2. Materials and Methods

2.1 Taxonomic sampling

Specimens were collected in 82 localities (Fig.2) by the authors and collaborators in several campaigns conducted from 2009 to 2013. Further specimens were kindly provided by colleagues. One hundred and eighty two individuals representing the whole distribution of *Iberesia* were included in the analysis, including specimens from Morocco where the genus was discovered for the first time. We sampled at least 3 individuals per locality, but in some localities individuals were rare. Additionally, 14 representatives of the main evolutionary lineages within the closely related genus *Nemesia* (Mora unpublished data) were included to test the monophyly of *Iberesia*. A representative of the eastern Mediterranean nemesiid *Brachythele* Ausserer, 1871 was included as outgroup to root all trees (Leavitt et al., 2015). All specimens used in the present study with locality information are summarized in Table 1 (at the end of this chapter).

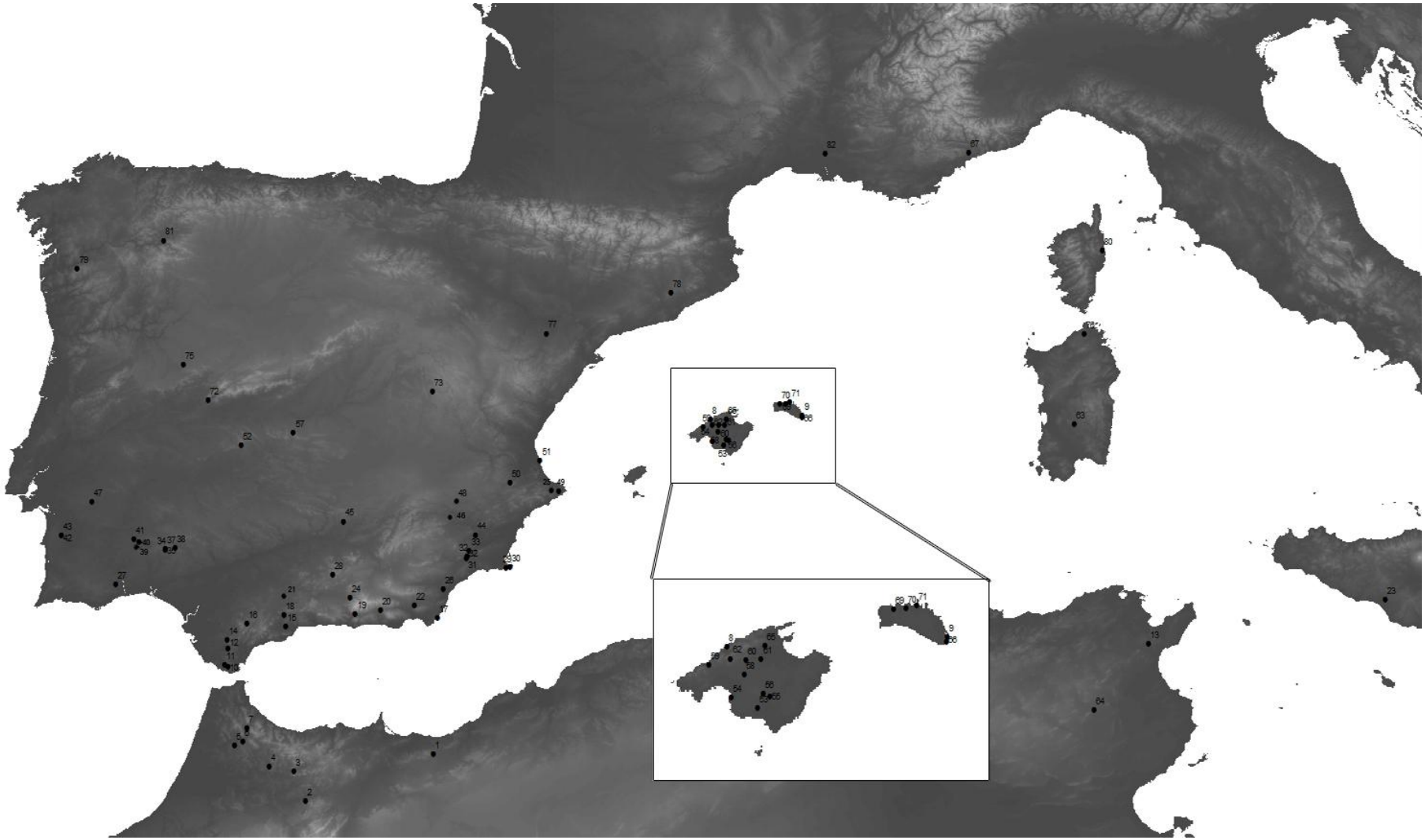


Figure 2. Sampling localities for the present study.

2.2 Specimen sorting and identification

Adult specimens were first sorted out into morphotypes. Only males could be confidently assigned to nominal species. Females were divided into morphotypes according to the general somatic morphology and the shape of the spermatheca. *Iberesia* shows two basic shapes of spermatheca(Decae & Cardoso, 2006)..

The *brauni*-type has two parts (bipartite), a basal, wide conduct and a distal domed receptacle. The *machadoi*-type has three parts (tripartite), since the basal conduct connects by narrow central part to the domed distal receptacle (see Decae and Cardoso, 2006, Figs. 15-16). Among the material examined we found some additional forms. At least one bipartite spermatheca showed an irregular shaped distal receptacle and the tripartite spermatheca could be further divided according to the form of the glandular tissue around the distal receptacle, which could be either covering more or less densely and resulting in different shapes. In addition, some specimens could be split in groups according to the general somatic morphology (carapace pattern, crest zone, eye formation, caput as have been prove to be informative characters for Nemesiidae, see Decae, 2005, Chapter 4.4 and 4.5) Finally, although almost all *Iberesia* specimens collected in the field build circle-shaped trap-doors, some specimens were reported to have drop-shaped trap-doors. Juveniles were associated to female and male morphotypes/species based on the results of the molecular analyses.

Specimens were examined with a Leica MZ16A dissection microscope, equipped with Leica df450 digital camera. Female vulvas were removed with the aid of needles, and the muscle tissue was digested with 35% KOH solution before observation.

One or two legs (depending on the size) were removed and directly preserved in absolute ethanol shortly after specimen collection. The legs were stored at -20°C at the freezer facilities of the Department of Animal Biology of the University of Barcelona to ensure DNA preservation. The rest of the voucher specimen was preserved in alcohol 70% for morphological studies.

2.2. DNA extraction, PCR amplification and sequencing

Total Genomic DNA was extracted from specimen legs or pinned abdomens (juveniles) using the DNeasy Tissue Kit (Qiagen) following the manufacturer's protocol. Fragments of the following genes were amplified with universal primers: a mitochondrial fragment spanning the 3' half of the 16S rRNA ribosomal subunit (16S), the complete tRNA leu (L1) and the 5' half of the NADH deshydrogenase subunit I (nad1), and fragments of the nuclear genes 28S rRNA (28S), Histone H3 (H3), and Elongation Factor 1 gamma (Ef-1g). Primer sequences and conditions are summarized in Chapter 4.1. The purified PCR products were sequenced on both strands were sequenced at Macrogen Inc. (Seoul, South Korea), Raw sequences were assembled and edited using Geneious v. 5.3.7 (Kearse et al., 2012).

2.3 Alignment and evolutionary model selection

Ribosomal DNA sequences were aligned using the online version of MAFFT v. 6 (available at http://mafft.cbrc.jp/alignment/server/, Katoh & Toh, 2008) using the G-INS-i strategy. The protein coding sequences were translated into amino acids to confirm that no stop codons were present. Unlike the nad1 and H3 gene fragments, the Ef-1g sequences showed evidence of indel mutations, and alignments were build using translated sequences and the MAFFT algorithm as implemented in the program TranslatorX (Abascal et al., 2010) (available at(http://www.translatorx.co.uk/).

Gaps were recorded as presence/absence data following the simple method of Simmons & Ochoterena, 2000, as implemented in the program SeqState v1.4.1 (Müller, 2005). This gap treatment facilitates including gap information into the analyses without increasing the weight of overlapping multiple non-homologous gaps (Pons & Vogler, 2006) and are amenable to likelihood, Bayesian and parsimony phylogenetic inference methods.

Model based analyses were conducted on the best partitioning scheme and corresponding evolutionary models selected with the program PARTITIONFINDER (Lanfear et al., 2012) using the Bayesian Information Criterion.

2.4 Delimitation of putative evolutionary lineages

The Generalized Mixed Yule-coalescent (GMYC) method (Pons et al., 2006; Fujisawa & Barraclough, 2013), was used to identify coalescent clusters based on the mtDNA information. The GMYC method combines a model of stochastic lineage growth (Yule model) with a coalescence null model to determine the point of transition from species-level to population-level evolutionary processes (i.e., Branch lengths An ultrametric tree was inferred with the computer program BEAST v.1.8.1 (Drummond & Rambaut, 2007) defining a single gene partition for the whole mtDNA data set with the model selected by Partitionfinder. A lognormal relaxed clock prior was selected with the ucld.mean parameter set to 1 (i.e. relative branch lengths). A constant population size coalescent was used as tree prior following Monaghan et al., (2009). Convergence between runs and correct mixing within each run were visualized with TRACER (Drummond & Rambaut, 2007).

Individual runs were combined using the BEAST(Drummond & Rambaut, 2007) accompanying program LOGCOMBINER. The first 10% of the generations of each run was discarded as a burn-in. A consensus chronogram was inferred with the accompanying program TREEANNOTATOR. The GMYC analysis was carried out in the R (http://www.r-project.org) environment with the help of the SPLITS package (Ezard et al., 2009) using the inferred ultrametric tree. The results of the GMYC were used as guide to sample nuclear genes, such as each GMYC was at least represented by two individuals sequenced for nuclear genes.

2.5 Phylogenetic Inference

Maximum Likelihood (ML) analyses were conducted the software program RAxML and run remotely at the portal CIPRES (Miller et al., 2010, www.phylo.org). .Gene partitions were assigned a GTR model, while gaps were assigned a binary model. In all cases, models included GAMMA and Invariants. The best tree was obtained out of 100 random replicates and clade support was assessed with 1000 bootstrap replicates (BS).

Bayesian inference analyses were conducted with MRBAYES v.3.1.2 (Ronquist

& Huelsenbeck, 2003) and run remotely at the CIPRESS portal (Miller et al., 2010, www.phylo.org). Two with 4 MCMC chains were run simultaneously for run of $2x10^7$ generations, starting from random trees and saving trees and parameters every 1000 generations. Convergence between chains was assessed by monitoring the standard deviation of split frequencies between runs (<0.05). TRACER v. 1.5 (Drummond & Rambaut, 2007) was used to further check chain convergence and correct mixing (ESS value) and to determine the correct number of generations to discard as a burn-in for the analyses (first 10% generations).

Parsimony analyses were conducted using the software TNT v. 1.0 (Goloboff et al., 2008) Each heuristic search consisted of 1000 iterations of Wagner trees constructed with the random addition of taxa and subsequent TBR branch swapping. Clade support was assessed via jackknife resampling (Farris et al., 1996) using 1000 replicates.

2.6 Estimation of divergence times

A time frame for the diversification of *Iberesia* was inferred in a Bayesian framework using the software BEAST v.1.8.1 (Drummond & Rambaut, 2007).
The only fossil tentatively included in Nemesiidae, does not provide any information on the time of origin of the extant genera(Selden, 2002), and hence we relied on biogeographic information and informed priors on the substitution rates. An ongoing study on Mediterranean nemesiids (Chapter 4,3) suggests that *Iberesia* colonized the Balearic Islands during the Messinian Salinity Crisis, which has been dates between 5.9 and 5.3 Ma (Krijgsman et al., 1999). Consequently, we constrained a normal distribution with mean 5.3 and standard deviation 0.1 upon the stem branch of the Balearic *I. brauni* .

A reduced matrix with 67 terminals was analysed in order to simplify to speed up computation and facilitate convergence of chains. This matrix included one sample by GMYC because our main aim was to define coalescence clusters, also included 14 specimens from the genus *Nemesia*. A simpler, by gene partition scheme was defined to facilitate chain convergence. A strict lognormal uncorrelated molecular clock was assigned to each nuclear gene and a relaxed clock was assigned to the mtDNA genes. A normal distribution with mean and

standard deviation 0.0113961, 0.00483085 and 0.00117, 0.0065 was specified for the mtDNA and the Ef-1g ucld.mean parameter, respectively, based on available information in the literature(Opatova et al., 2013; Bidegaray-Batista et al., 2014; Opatova & Arnedo, 2014a, 2014b).. The values for the H3 and 28S were estimated during the analyses. The Yule Process was selected as a tree prior. Three independent runs of $5x10^7$ generations were run independently. Convergence and correct mixing were visualized using the software TRACER (Rambaut et al., 2014). The BEAST accompanying programs LOGCOMBINER and TREEANNOTATOR were used to combine independent runs, following burn-in, and to estimate the consensus chronogram.

3. Results

3.0 Specimens sorting into morphotypes

Only 5 out of the 182 *Iberesia* species examined were males and could be identified to species levels. The 84 female specimens were sorted out into 6 morphotypes based on general morphology, spermatheca shape and trap-door architecture, as follows:

Morphotype I: Bipartite spermatheca, with rounded distal receptacle. Ground body colour orange, with darker pattern on the carapace. Carapace is larger than wide. The crest zone (Decae & Cardoso, 2006) is well delimited by the contrasting darker pattern and the slopes of the caput. Two lighted coloured areas at both sides of the eye group (“cheeks” sensu Isaia & Decae, 2012). The periocular pigmentation (POP) is unbroken. Available field observations indicated that these individuals build drop-shaped trap-doors.

Morphotype II: Bipartite spermatheca, with rounded distal receptacle. Ground body colour dark orange, more intense than morphotype I and darker pattern on the carapace that does not reach the margins. Carapace square-like, as large as wide. Eyes are on steep tubercle and the caput is high. Crest zone and cheeks as morphotype I. POP broken, not including all eyes. Specimens build regular, circle-shaped trap doors.

Morphotype III: Bipartite spermatheca, with distal receptacle of irregular shape. Ground body colour dark orange, showing a diffuse pattern on the carapace.

Carapace slightly larger than wide, the caput is high and wide. No contrasting crest zone. POP broken.

Morphotype IV: Tripartite spermatheca, with fan-shaped distal receptacle. Ground body colour is brown, carapace wider than large. Crest zone not delimited by contrasting colour from the rest of the carapace. Eyes on steep tubercle. Orange “cheeks” at both sides of the eye group that include the slopes of the caput, which is high. POP is broken.

Morphotype V: Tripartite spermatheca, with leaf-toed shaped distal receptacle (this particular shape is named after an analogy to the toe shape of the leaf-toed geckos). Ground body colour is brown. Carapace as large as wide, showing a thin crest zone that tappers through the fovea. The fovea is somewhat angular, wider than in other morphotypes.

Morphotype VI: Tripartite spermatheca, with broccoli-shaped distal receptacle (the shape refers to the apparent granulations that remember the shape of a broccoli). Ground body colour is brown. Carapace larger than wide, delimited on lateral and proximal margins by a purple-brownish line. Caput is high. Eye formation smaller than in other morphotypes.

During the course of the present study we had the opportunity to examine the type material of *Nemesia vittipes* Simon, 1911, stored at the Musee the d'Histoire Naturelle de Paris (MHNP. AR 4462) the original type it’s a juvenile. In the same pot,, there was a male without PMS (MHNP AR.4444), from the same locality Ain Sfa, in the Beni Snassen Mountains. Examination of the male spec revealed that the PMS were missing and hence that this species should be transferred to the genus *Iberesia*, which constitutes the first citation of the genus in North Africa. A formal transfer of *N. vittipes* to the genus *Iberesia* is warranted.

We did sample one juvenile specimen from the very same locality (RA130) of (proposed to be transferred to *Iberesia vittipes*) which cluster with L1 and thus confirm the presence of *Iberesia* in Eastern Morocco.

3.1 Delimitation of coalescent groups

The GMYC algorithm identified 53 entities/clusters ($p=6.5*10^{-9}$), (Table S1, Fig. 1). In most cases, the GMYC groups corresponded to single localities, and each locality included a single GMYC cluster. Five GMYC groups were found in more than one, generally close, (G23 in 5 localities, G22 in 2 and G21 in 6 in the Balearic Islands; and G4 in 4 localities and G7 in 5 in Portugal). Conversely, 5 localities were found to contain more than one GMYC (Fig. 3). Especially relevant are those localities with distantly related GMYC clusters such as locality 11, Facinas Cadis (G35 and G11, G10) and locality 57, Montes de Toledo, Central System (G34 and G27).

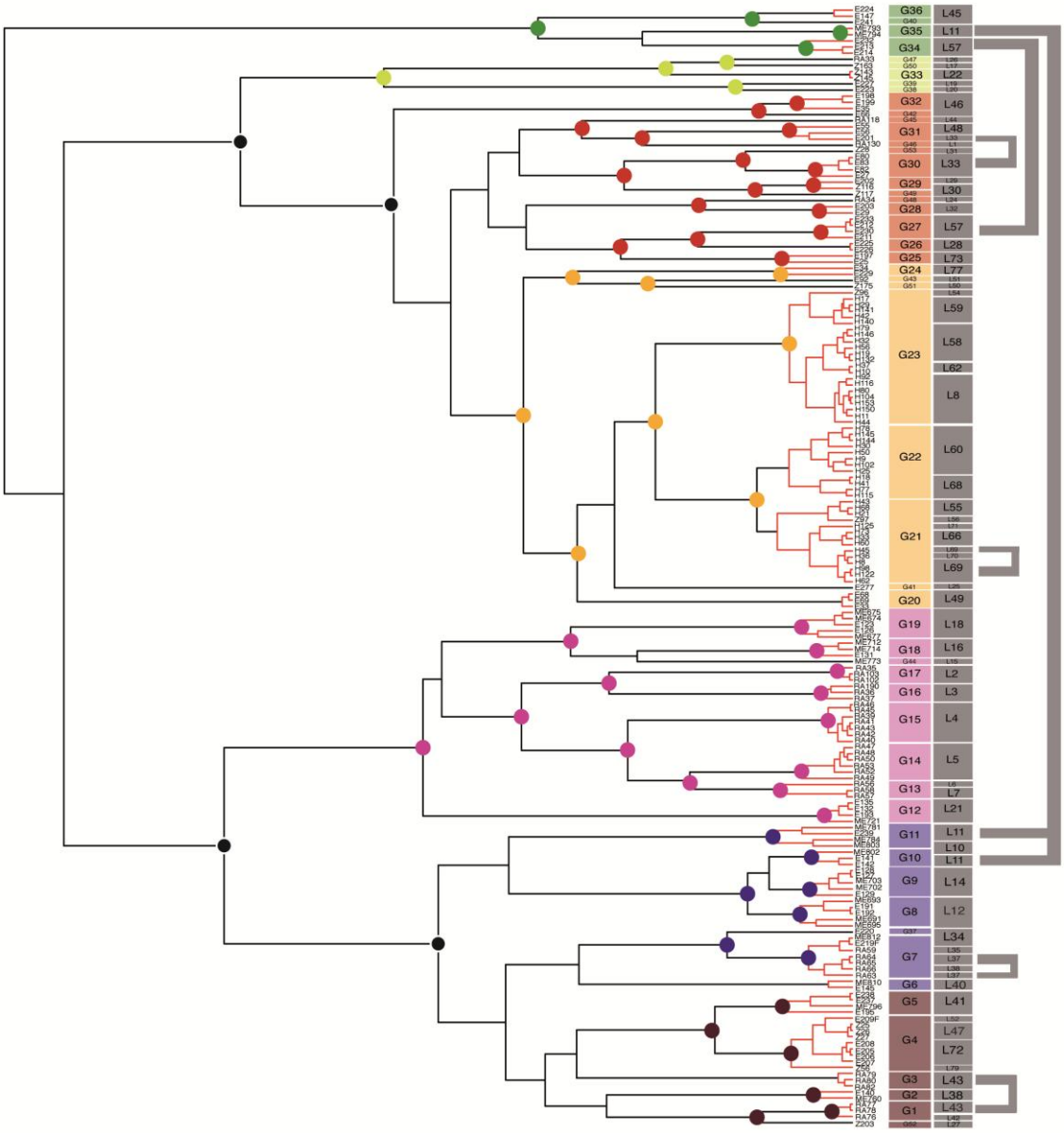


Figure.2. Ultrametric *16s_nad1* BEAST tree. Red branches indicate GMYC independent clusters in the SPLITS analyses, also indicated in first column. Locality column: localities where the respective GMYC cluster was collected. Connected by bars localities where more than GMYC was found, or different GMYC from the same locality.

3.1 Phylogenetic inference

A data matrix of 197 specimens, including 182 *Iberesia* specimens, 14 *Nemesia* specimens and the outgroup *Brachythele* sp. was constructed by concatenating 613 characters corresponding to the 16S+L1, 399 to the *nad1*, 793 to the 28S, 327 to the H3, 814 to the Ef-1g and 136 additional absence/presence coded gaps. Results of the model based (ML, BI) and parsimony analyses of the concatenated data matrix under the preferred partition scheme and evolutionary models are summarised in Fig.3. Partitions implemented in the analyses are shown as follows.

Partition	**Model**	**Partition**	**Model**
16s-L1	GTR+I+G	16s-L1	GTR+I+G
*nad1*_3	HKY+I+G	*nad1*	GTR+I+G
*nad1*_1	GTR+G		
*nad1*_2	GTR+I+G		
28s	TVM+I+G	28s	TVM+I+G
H3_1	GTR+I+G	H3	TVMef+I+G
H3_2	JC+I		
H3_3	TVMef+G		
EF1g_1	F81+G	EF1g	HKY+I+G
EF1g_2	TVMef+G		
EF1g_3	K80+I+G		

Table 3. Partition schemes and corresponding best models used in the present study

All methods recovered the monophyly of *Iberesia*, model based analyses with support (i.e. ML bootstrap>0.75 and PP>0.95), and its sister group relationship to a monophyletic *Nemesia*.

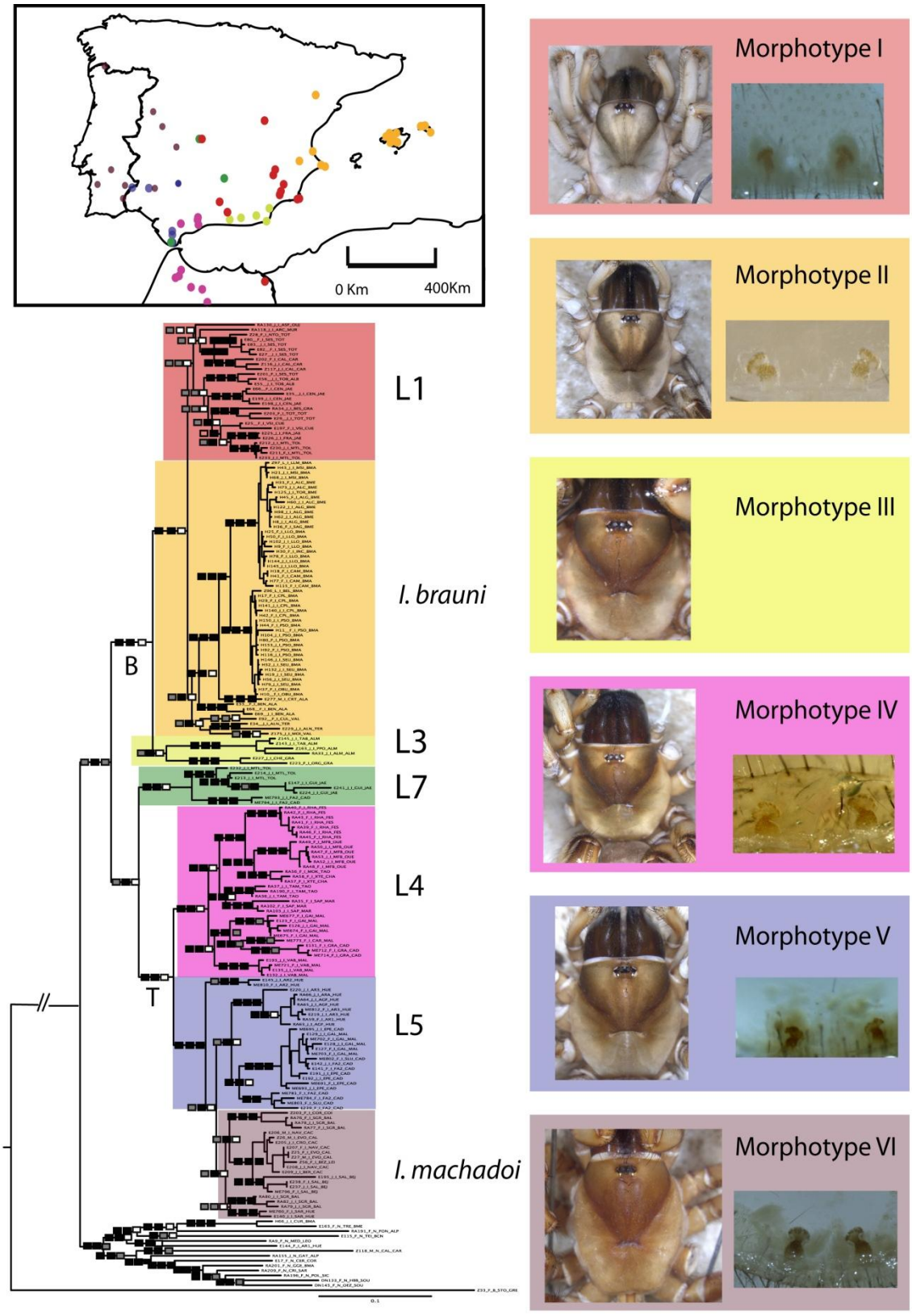


Fig.3 Phylogenetic inference of the genus *Iberesia*. ML tree obtained from concatenated data matrix. Boxes on branches indicate clade support as follows: black rectangles denote ML bootstrap >75% (left), and Bayesian Posterior Probability >0.95, grey rectangles denote clades recovered but with support values below the former thresholds, methods arranged in the same orders as above, and white boxes denote clades not recovered in this particular analyses.

Clades are colored according the morphotypes previously defined with exception of lineage 4 that was not possible to associate to any morphotype.

Iberesia was further split into two main supported clades that correspond to the two basic types of spermatheca described in *Iberesia*: the bipartite, *brauni*-type, and the tripartite, *machadoi*-type, respectively.

The bipartite spermatheca clade (hereafter referred as the BS clade) was further divided into three clades that matched morphotype I, II and III, respectively. Hereafter we refer to this clades as lineage 1 (L1), lineage 3 (L3), and *I. brauni*, since the clade including females identified as morphotype II also included the males identified as *I. brauni*.

The lineage L1, recovered in the model based but not in the parsimony analyses, included specimens from the Iberian Central System (CS) mountain ranges, along with samples from the Betics and specimen RA130 from Morocco, close to the border with Argelia (Ain Sfa).

The *I. brauni* lineage, supported in the BI and recovered in the ML, included the specimens from the Balearic Islands, where the species was originally described and though to be endemic, that form a supported clade in all analyses. The rest of individuals included were found in the eastern Iberian coastal region. One specimen from Alacant (E277) was supported in ML and BI as the sister lineage to the Balearic *I. brauni*.

The sister group relationship of L1 and *I. brauni* was supported in all analyses. The lineage L3, recovered in model based analyses albeit with low support in the ML, was restricted to the south-eastern coastal Betics and included two non-overlapping clades supported in all analyses, namely Almeria and Granada.

The tripartite spermatheca clade (hereafter referred as TS clade) was further divided into three clades, two corresponding to morphotypes IV and V and hereafter referred as lineages L4 and L5, respectively, and one corresponding to morphotype VI but also including the males identified as *I. machadoi* and hereafter referred as the *I. machadoi* lineage.

The *I. machadoi* lineage was recovered in all analyses albeit only supported in BI and was formed by specimens collected in south-Portugal and in the East of Spain including localities from Central System Mountain Range. The lineage L5 was recovered as paraphyletic in all analyses with regards to *I. machadoi*. The specimens from Cadis and Malaga form a supported clade sister to the bulk of specimens from north-western Huelva. Two individuals from Huelva were supported as sister to *I. machadoi* plus the remaining L5 individuals. All analyses supported the clade including L5 and *I. machadoi*.

The lineage L4 was supported in all analyses and further split into two main clades, one including specimens from Morocco and a second one specimens form Cadiz and Malaga. All analyses recovered a clade composed by L4, L5 and *I. machadoi*. An additional clade, which was formed exclusively by juveniles and hence could not be assigned to any of the two major clades defined by the spermatheca type, is hereafter referred as lineage L7.This lineage included three supported clades with disjoint distribution, one from Cadiz, one from Jaen and one from Montes de Toledo (Central System). In Cadiz and Montes de Toledo the L7 lineage overlapped with L5 and L1, respectively. All analyses recovered the sister group relationship of L7 with the TS Clade.

3.3 Estimation of divergence times

The dated chronogram and the corresponding confidence intervals obtained with BEAST is shown in Fig. 4. Overall, the tree topology and the clade supports were very similar to those found in the former phylogenetic analyses, again L5 was recovered as paraphyletic in relation to *I. machadoi*, albeit with low support. Surprisingly, the *I. brauni* clade was recovered paraphyletic as well, because the L1 specimen RA130 cluster within this clade. However, the relationship received no support.

According to our results, *Iberesia* split from *Nemesia* during the Oligocene, approximately 32 Ma (45.6-20.5). The basal split between the two main *Iberesia* lineages, the TS clade plus L7 and the BS lineages, traces back to the Early Miocene, approximately 19.71 Ma (27.34-13.08 Ma). The extant diversity of the BS clade originated during the middle Miocene, approximately 13.54 Ma (18.81-

9.9). The most recent common ancestor of L1 and *I.brauni* was dated at 9.74 Ma (12.25-6.1). The TS clade split from L7 approximately 16.8 Ma (23.52-11.08). The L4 lineage diverged from the remaining TS clade approximately 14.13 Ma (20.33-9.6). The Iberian and Moroccan population of L4 split during the late Tortonian 9.03 Ma (12.82—5.75). The lineage including L5 and *I. machadoi* started diversifying approximately 13.58 Ma (18.69-8.54). Most diversification events within the main lineages (morphotypes) occurred between the Late Miocene and the Pliocene.

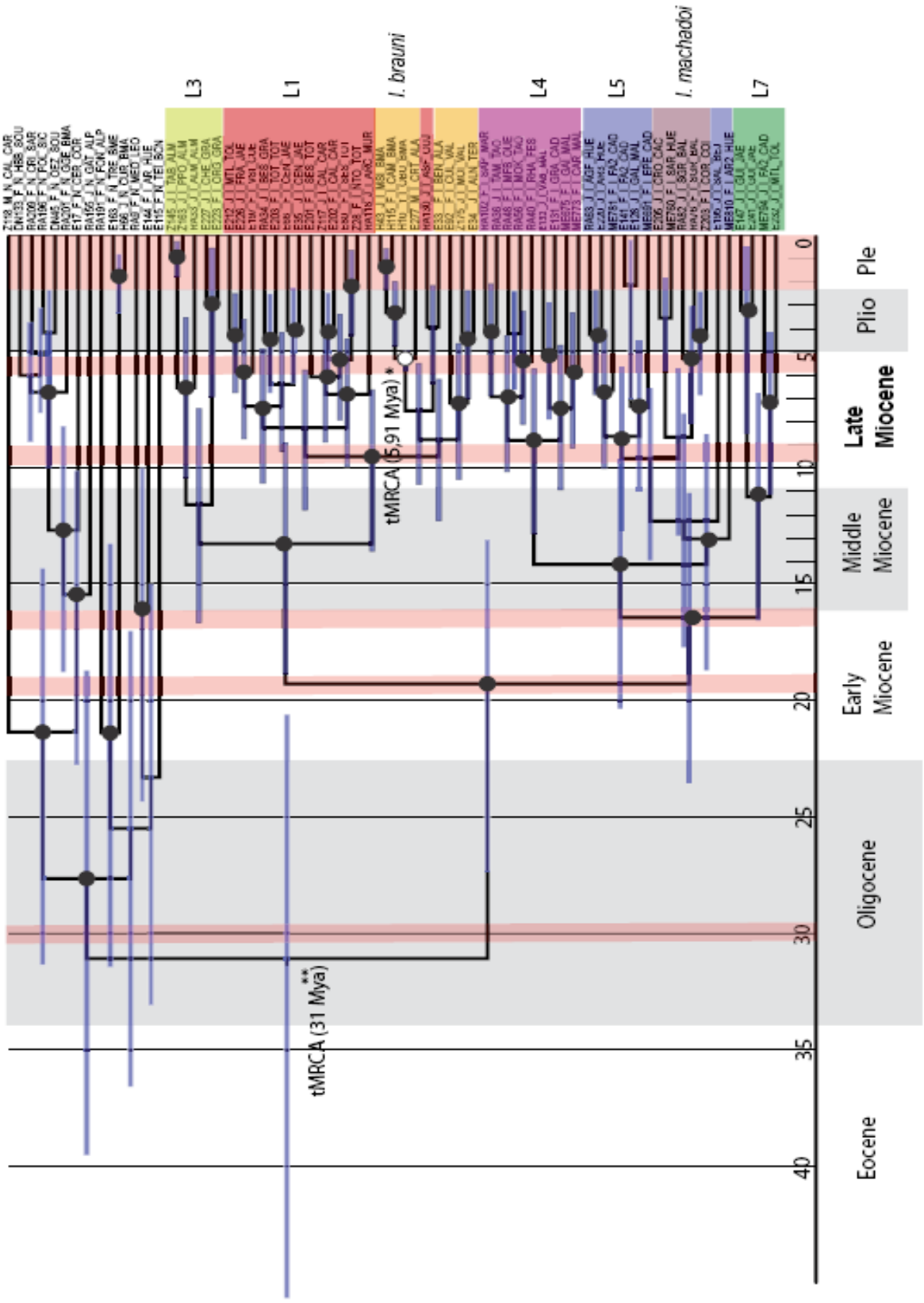


Figure 4. The concatenated approach using BEAST. Dots on nodes denote Bayesian Posterior Probabilities. Node bars indicate 95% HPD confidence intervals of the divergence time. The x-axis is in million years. Terminal colour codes are the same than in the phylogenetic analyses.

4.Discussion

The genus *Iberesia* was proposed to accommodate nemesiid individuals, in some cases belonging to species formerly in *Nemesia*, that lacked posterior median spinnerets (PMS). Given that *Nemesia* shows a whole range of reduction in the PMS, there were good chances that the description of *Iberesia* could have rendered *Nemesia* paraphyletic. This study supports the reciprocal monophyly of *Iberesia* and *Nemesia* and hence provides further corroboration for the validity of *Iberesia* as a distinct genus.

At present *Iberesia* included three species. Two of them were unambiguously sampled in the present study. The identity of the third species, *I. castillana* remains a mystery. This specie is only known from the male type, deposited at the MNHN, with type locality Avila. The female is unknown. In the course of the present study we conducted a thorough sampling in the area around the type locality, without success.

We do have however collected specimens not far from the type locality In particular, the specimen E205 was collected only 16 km form Avila. Unfortunately this is a juvenile. Interestingly it clusters with other specimens identified as belonging to *I. machadoi*. Further collections, especially of males around the original type locality of *I. castillana* would be necessary in order to confirm the validity of the species or propose its synonymy to I*. machadoi*.

Our mapping of identified adult specimens on the ultrametric tree revealed that all morphotypes formed monophyletic groups, with the only exception of morphotype I that was recovered as paraphyletic with regards to morphotype II, and morphotype V than was paraphyletic with regard to morphotype 6. In addition, we identified a deeply divergent lineage formed exclusively by juvenile specimens

The results of the GMYC method yielded 53 independent lineages. This method has been extensively used in arthropods for species delimitation(Bidegaray-Batista & Arnedo, 2011; Hendrixson et al., 2013; Planas et al., 2013a; Kekkonen & Hebert, 2014; Opatova & Arnedo, 2014b). Despite has been demonstrated that tends to overestimate the number of evolutionary lineages

(Satler et al., 2013; Talavera et al., 2015) and its very sensitive to parameters like population size or rate of speciation (Esselstyn et al., 2012). In mygalomorph , has been demonstrated to overestimate the number of putative evolutionary lineages due to their lifestyle, being poor dispersers with high levels of population structure (Satler et al., 2013). The GMYC provided an starting point to reconcile morphological based groupings with genetic relationships and hence provide a preliminary hypothesis of species boundaries to be further validated.

Our results show several deeply divergent lineages indicating that some of them might deserve species status. The examination of female vulva and carapace morphology support the distinct morphology of different lineages.

We did sample one juvenile specimen from the very same locality (RA130) of the type *Nemesia vitipes*, (proposed to be transferred to *Iberesia vittipes*) which cluster with L1 and thus confirm the presence of *Iberesia* in Eastern Morocco.

Origins and biogeographic patterns

Geological events causing vicariance contributed greatly to the present day diversity and distribution of organisms(Sanmartín et al., 2001; McCarthy, 2003; Sanmartín & Ronquist, 2004). However, uncover the processes that occurred at different temporal and spatial frames has proven to be a difficult task, especially in low vagility organisms due to their strong population structure and deep genetic divergences (Bidegaray-Batista, 2012; Opatova et al., 2013; Planas et al., 2013b). The complex geological history of the Mediterranean region and the dramatic climatic changes undergone by the region, have played a key role in generating arthropod diversity, both at the intra and interspecific levels (Blondel et al., 1999)

In this study, we used phylogenetic inference and coalescent methodologies to study phylogenetic relationships and biogeographic patterns of the recently described trap-door genus *Iberesia*. We have reported for the first time the presence of *Iberesia* in Morocco, which according to our result was most likely colonised from the Iberian Peninsula twice independently.

The geographic distribution of the main phylogenetic lineages within *Iberesia* closely resembles those reported for other ground arthropods with poor dispersal abilities such as the trap-door spider genus *Ummidia* (Opatova, submitted), the funnel-web spider *Macrothele* (Arnedo & Ferrández, 2007) or the *Buthus* scorpions (Souse in prep.). Similarities include the basal split between the western and the eastern Iberia, and the existence of different lineages in the Betic region. *Iberesia* is unique among these groups, however, because it colonized the Balearic Islands.

The existence of common biogeographic patterns hints at the commonality of the processes that drove the diversification of these groups. Based on the diversification timeframe inferred in this study, we propose the geological processes undergone by the Betic region lay behind some of the major split event in the group

The Origins of *Iberesia* trace back to the broke of the Hercynian belt , around 32 Ma (Rosenbaum & Lister, 2002), when they started differentiating from *Nemesia*, their sister, group as our results confirm, and generating a new lineage. Based on our estimates the diversification of *Iberesia* take place from Middle Miocene to late Miocene.

The two main lineages, the Bipartite and the Tripartite split around 19,71 Ma, but the 95% HPD includes the interval: 27,34-13.08. this period that matches with the first phase of the broke of the Hercynian belt, and the opening of Valencia Trough at 25 mA(Rosenbaum et al., 2002).

The tripartite clade started its diversification at 16.8 Ma (23.52-11.08) ,during that period, at middle Miocene, the Kabylies block broke and drifted southward in direction to the African margin (Rosembaun et al 2002), that subduction involved that the Betic-Rif landmass comtinued drifting westward and started fragmenting around 15 Ma (Lonergan & White, 1997) until arrive at their nowadays position. The bipartite clade started its diversification at 13.54 Ma (18.81-9.9). Those results match that the split of the two main lineages was medited by the fragmentation of the Betic-Rif, that began 15 Ma, and that their divesrfication into two lineages was mediated by isolation during Tortonian.

Pecisley during tortonian (11.6-7.2 Ma, Braga et al., 2003) the Morocan Rif and the southernmost part of the Iberian peninsula formed a continuos landmass isolated from the Betics (Paulo et al., 2008)..

This is congruent with the distribution of the two main lineages , the tripartite clade would have been isolated from the bipartite clade. which must remained isolated in the Betics. This is congruent with the diversification of L1 and *Iberesia* braun 9.74 Ma (12.25-6.1). Most of speciation process take place in L1 and *Iberesia* brauni occurred during Late Miocene, between 8-6 Ma, which is congruent with the uplift of the Betic cordillera (Braga et al., 2003), that may have had a key role in the isolation of different lineages . During Messinian Salinity Crisis, *Iberesia* colonized the Balearic Islands thanks to land brigdes (Agustí et al., 2006) established during the dissecation of the Mediterranean Sea during Messinian Salinity Crisis(Krijgsman et al., 1999; Meijer & Krijgsman, 2005).

The Iberian and Moroccan population of L4 split during Late Tortonian, which is congruent with the reestablishment of the isolation between Morocco and the most southern part of the Iberian Peninsula..

According our results, the present day distribution of the genus *Iberesia* having the BS lineage has a Betic distribution and the TS lineage has an South -West Iberian distribution matches with the hypothesis of the existence of a Betic Island during Tortonian which was isolated from a Southern-Iberia and Rif Island. This process can be considered together with the uplift of the Betic Cordillera the driver of diversification of the genus.

5.Conclusions

The sinapomrophy that defines *Iberesia* received litlle attention until 2006. This implies that the diversity of these genus have been overlooked during many years.Our results show that one specie previously defined as *Nemesia* must be transferred to the genus *Iberesia*, and some new deeply divergent lineages are found, suggesting a high level of overlooked diversity. Further pitfall sampling is needed because it must be specially focused on the obtention of males to proceed with the descriptions of the new putative species found.

This study show that the geological history of the region played a key role generating the diversity and distribution of the genus *Iberesia*. The broke of the Hercynian belt , and subsequent migration of the Betic-Rift block were the main drivers of diversification within the genus. The isolation of the Betic Island , on the one hand, and the isolation of the Rif with the southernmost part of the Iberian Peninsula, on the other hand, contributed to generate present diversity. Furthermore, the land bidges established during the Messinian Salinity Crisis allowed *Iberesia* to colonize the Balearic Islands. Further species delimitation analyses will be performed in orther to validate the taxonomic status of the new discovered lineages.

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Table 1. Specimens used for the present study

Genus	*Sp.*	SpecimenCode	Gmyc	Morph	Lineage	Label	S	Locality Name	Province	Country	N	El.	Lat.	Lon.	16s	28S	Efg	H3
Iberesia	*sp*	CRBAMM000486	46	I	1	RA130_J_I_ASF_OUJ	J	Ain-Sfa	Oujuda-Angak	Morocco	1	567	34,82454	-2,086	RA130	RA130	RA130	RA130
Iberesia	*sp.*	CRBAMM000212	48	I	1	RA34_J_I_BES_GRA	J	Bco. del Espostal, Granada	GRANADA	Spain	24		37,15	-3,53	RA34	RA34		RA34
Iberesia	*sp.*	CRBAME000848	26	I	1	E225_J_I_FRA_JAE	J	Frailes	JAEN	Spain	28	1075	37,4969	-3,83086	E225	E225	E225	E225
Iberesia	*sp.*	CRBAME000849	26	I	1	E226_J_I_FRA_JAE	J	Frailes	JAEN	Spain	28	1075	37,4969	-3,83086	E226	E226	E226	E226
Iberesia	*sp.*	CRBAME001047	29	I	1	E202_F_I_CAL_CAR	F	P.Regional Calblanque	CARTAGENA	Spain	29	21	37,58681	-0,83789	E202	E202	E202	E202
Iberesia	*sp.*	CRBAMM000116	29	I	1	Z116_J_I_CAL_CAR	J	Parc regional Calbalanque,	CARTAGENA	Spain	30	88	37,6079	-0,75988	Z116	Z116	Z116	Z116
Iberesia	*sp*	CRBAMM000117	49	I	1	Z117_J_I_CAL_CAR	J	Parc regional Calbalanque,	CARTAGENA	Spain	30	88	37,6079	-0,75988	Z117	Z117	Z117	Z117
Iberesia	*sp.*	CRBAMM000028	53	I	1	Z28_F_I_NTO_TOT	F	Totana	TOTANA	Spain	31		37,73	-1,517	Z28	Z28	Z28	
Iberesia	*sp.*	CRBAME001060	28	I	1	E203_F_I_TOT_TOT	F	Totana, near camping	TOTANA	Spain	32	260	37,758	-1,51071	E203	E203	E203	E203
Iberesia	*sp.*	CRBAME001062	28	I	1	E29__J_I_TOT_TOT	J	Totana, near camping	TOTANA	Spain	32	260	37,758	-1,51071	E29	E29	E29	E29
Iberesia	*sp.*	CRBAME001033	31	I	1	E201_F_I_SES_TOT	F	Sierra de Espuña	TOTANA	Spain	33	379	37,85253	-1,47114	E201	E201	E201	E201
Iberesia	*sp.*	CRBAME001041	30	I	1	E27__J_I_SES_TOT	J	Sierra de Espuna	TOTANA	Spain	33	379	37,85253	-1,47114	E27			
Iberesia	*sp.*	CRBAME001032	30	I	1	E80__F_I_SES_TOT	F	Sierra de Espuna	TOTANA	Spain	33	379	37,85253	-1,47114	E80	E80	E80	E80
Iberesia	*sp.*	CRBAME001036	30	I	1	E82__F_I_SES_TOT	F	Sierra de Espuna	TOTANA	Spain	33	379	37,85253	-1,47114	E82			
Iberesia	*sp.*	CRBAME001042	30	I	1	E83__J_I_SES_TOT	J	Sierra de Espuna	TOTANA	Spain	33	379	37,85253	-1,47114	E83	E83	E83	E83
Iberesia	*sp*	CRBAMM001069	45	I	1	RA118_J_I_ARC_MUR	J	Archena	MURCIA	Spain	44	277	38,08524	-1,36514	RA118	RA118	RA118	RA118
Iberesia	*sp.*	CRBAME000954	32	I	1	E198_J_I_CEN_JAE	J	Cenajo, Sierra de Segura	JAEN	Spain	46	463	38,39757	-1,76317	E198			
Iberesia	*sp.*	CRBAME000955	32	I	1	E199_J_I_CEN_JAE	J	Cenajo, Sierra de Segura	JAEN	Spain	46	463	38,39757	-1,76317	E199	E199	E199	E199
Iberesia	*sp.*	CRBAME000962	32	I	1	E35__J_I_CEN_JAE	J	Cenajo, Sierra de Segura	JAEN	Spain	46	463	38,39757	-1,76317	E35	E35	E35	E35
Iberesia	*sp.*	CRBAME000951	42	I	1	E66__F_I_CEN_JAE	F	Cenajo, Sierra de Segura	JAEN	Spain	46	463	38,39757	-1,76317	E66	E66	E66	E66
Iberesia	*sp.*	CRBAME000918	31	I	1	E55__J_I_TOB_ALB	J	Tobarra	ALBACETE	Spain	48	632	38,58025	-1,69101	E55		E55	E55
Iberesia	*sp.*	CRBAME000919	31	I	1	E56__J_I_TOB_ALB	J	Tobarra	ALBACETE	Spain	48	632	38,58025	-1,69101	E56			
Iberesia	*sp*	CRBAME001743	27	I	1	E211_F_I_MTL_TOL	F	Montes de Toledo	TOLEDO	Spain	57	884	39,60709	-4,51189	E211		E211	E211
Iberesia	*sp*	CRBAME001744	27	I	1	E212_J_I_MTL_TOL	J	Montes de Toledo	TOLEDO	Spain	57	884	39,60709	-4,51189	E212	E212	E212	E212
Iberesia	*sp*	CRBAME001746	27	I	1	E230_J_I_MTL_TOL	J	Montes de Toledo	TOLEDO	Spain	57	884	39,60709	-4,51189	E230			
Iberesia	*sp*	CRBAME001750	27	I	1	E233_J_I_MTL_TOL	J	Montes de Toledo	TOLEDO	Spain	57	884	39,60709	-4,51189	E233	E233	E233	E233
Iberesia	*sp.*	CRBAME000928	25	I	1	E197_F_I_VSI_CUE	F	Villalba de la Sierra	CUENCA	Spain	73	978	40,22325	-2,10018	E197	E197	E197	E197

Genus	*Sp.*	SpecimenCode	Gmyc	Morph	Lineage	Label	S	Locality Name	Province	Country	N	El.	Lat.	Lon.	16s	28S	Efg	H3
Iberesia	*sp.*	CRBAME000929	25	I	1	E25__F_I_VSI_CUE	F	Villalba de la Sierra	CUENCA	Spain	73	978	40,22325	-2,10018	E25	E25	E25	E25
Iberesia	*sp.*	CRBAME000968	20	II	*I. brauni*	E33__F_I_BEN_ALA	F	Beniarda, camp oliveres	ALACANT	Spain	49	607	38,68195	0,23138	E33	E33	E33	E33
Iberesia	*sp.*	CRBAME000970	20	II	*I. brauni*	E68__F_I_BEN_ALA	F	Beniarda	ALACANT	Spain	49	607	38,68195	0,23138	E68	E68	E68	E68
Iberesia	*sp.*	CRBAME000977	20	II	*I. brauni*	E69__J_I_BEN_ALA	J	Beniarda	ALACANT	Spain	49	607	38,68195	-0,23138	E69			
Iberesia	*sp.*	CRBAME001074	41	*I. brauni*	*I. brauni*	E277_M_I_CRT_ALA	M	Coll de Rates	ALACANT	Spain	25	420	38,73427	0,07164	E277			
Iberesia	*sp*	CRBAMM000175	51	II	*I. brauni*	Z175_J_I_MOI_VAL	J	Moixent	VALENCIA	Spain	50	357	38,86874	-0,7589	Z175		Z175	Z175
Iberesia	*sp.*	CRBAME001079	43	II	*I. brauni*	E92__F_I_CUL_VAL	F	Cullera. Far	VALENCIA	Spain	51	0	39,19116	-0,24337	E92	E92	E92	E92
Iberesia	*brauni*	CRBAMM000096 - AD11	23	II	*I. brauni*	Z96_L_I_BEL_BMA	L	Bellevista, south east of Arenal	MALLORCA	Spain	54		39,485	2,739	Z96			
Iberesia	*brauni*	CRBA_AP000110	21	II	*I. brauni*	H21__J_I_MSI_BMA	J	Ermita de Mont Sio	MALLORCA	Spain	55	214	39,49533	3,0154	H21			
Iberesia	*brauni*	CRBA_AP000111	21	II	*I. brauni*	H43__J_I_MSI_BMA	J	Ermita de Mont Sio	MALLORCA	Spain	55	214	39,49533	3,0154	H43	h43	H43	h43
Iberesia	*brauni*	CRBA_AP000112	21	II	*I. brauni*	H68__J_I_MSI_BMA	J	Ermita de Mont Sio	MALLORCA	Spain	55	214	39,49533	3,0154	H68			
Iberesia	*brauni*	CRBAMM000097 - AD12	21	II	*I. brauni*	Z97_L_I_LLM_BMA	L	Majorca	MALLORCA	Spain	56		39,51	2,97	Z97			
Iberesia	*brauni*	CRBA_AP000085	23	II	*I. brauni*	H132_J_I_SEU_BMA	J	Santa Eugenia	MALLORCA	Spain	58	121	39,62666	2,83395	H132			
Iberesia	*brauni*	CRBA_AP000086	23	II	*I. brauni*	H146_J_I_SEU_BMA	J	Santa Eugenia	MALLORCA	Spain	58	121	39,62666	2,83395	H146			
Iberesia	*brauni*	CRBA_AP000081	23	II	*I. brauni*	H19__J_I_SEU_BMA	J	Santa Eugenia	MALLORCA	Spain	58	121	39,62666	2,83395	H19	H19	H19	H19
Iberesia	*brauni*	CRBA_AP000082	23	II	*I. brauni*	H32__J_I_SEU_BMA	J	Santa Eugenia	MALLORCA	Spain	58	121	39,62666	2,83395	H32			
Iberesia	*brauni*	CRBA_AP000083	23	II	*I. brauni*	H56__J_I_SEU_BMA	J	Santa Eugenia	MALLORCA	Spain	58	121	39,62666	2,83395	H56			
Iberesia	*brauni*	CRBA_AP000084	23	II	*I. brauni*	H79__J_I_SEU_BMA	J	Santa Eugenia	MALLORCA	Spain	58	121	39,62666	2,83395	H79			
Iberesia	*brauni*	CRBA_AP000056	23	II	*I. brauni*	H140_J_I_CPL_BMA	J	Can Planiol	MALLORCA	Spain	59	456	39,68885	2,57752	H140	H140	H140	H140
Iberesia	*brauni*	CRBA_AP000057	23	II	*I. brauni*	H141_J_I_CPL_BMA	J	Can Planiol	MALLORCA	Spain	59	456	39,68885	2,57752	H141			
Iberesia	*brauni*	CRBA_AP000046	23	II	*I. brauni*	H17__F_I_CPL_BMA	F	Can Planiol	MALLORCA	Spain	59	456	39,68885	2,57752	H17			
Iberesia	*brauni*	CRBA_AP000047	23	II	*I. brauni*	H29__F_I_CPL_BMA	F	Can Planiol	MALLORCA	Spain	59	456	39,68885	2,57752	H29			
Iberesia	*brauni*	CRBA_AP000048	23	II	*I. brauni*	H42__F_I_CPL_BMA	F	Can Planiol	MALLORCA	Spain	59	456	39,68885	2,57752	H42			
Iberesia	*brauni*	CRBA_AP000078	22	II	*I. brauni*	H102_J_I_LLO_BMA	J	Lloseta, rd. to Alaro	MALLORCA	Spain	60	154	39,72051	2,84633	H102			
Iberesia	*brauni*	CRBA_AP000079	22	II	*I. brauni*	H144_J_I_LLO_BMA	J	Lloseta, rd. to Alaro	MALLORCA	Spain	60	154	39,72051	2,84633	H144			
Iberesia	*brauni*	CRBA_AP000080	22	II	*I. brauni*	H145_J_I_LLO_BMA	J	Lloseta, rd. to Alaro	MALLORCA	Spain	60	154	39,72051	2,84633	H145			

Genus	*Sp.*	SpecimenCode	Gmyc	Morph	Lineage	Label	S	Locality Name	Province	Country	N	El.	Lat.	Lon.	16s	28S	Efg	H3
Iberesia	*brauni*	CRBA_AP000075	22	II	*I. brauni*	H25__F_I_LLO_BMA	F	Lloseta, rd. to Alaro	MALLORCA	Spain	60	154	39,72051	2,84633	H25			
Iberesia	*brauni*	CRBA_AP000076	22	II	*I. brauni*	H50__F_I_LLO_BMA	F	Lloseta, rd. to Alaro	MALLORCA	Spain	60	154	39,72051	2,84633	H50			
Iberesia	*brauni*	CRBA_AP000077	22	II	*I. brauni*	H78__F_I_LLO_BMA	F	Lloseta, rd. to Alaro	MALLORCA	Spain	60	154	39,72051	2,84633	H78			
Iberesia	*brauni*	CRBA_AP000074	22	II	*I. brauni*	H9__F_I_LLO_BMA	F	Lloseta, rd. to Alaro	MALLORCA	Spain	60	154	39,72051	2,84633	H9	h9	H9	h9
Iberesia	*brauni*	CRBA_AP000097	22	II	*I. brauni*	H30__F_I_INC_BMA	F	Inca, ermita de St.Magdalena	MALLORCA	Spain	61	190	39,72521	2,95399	H30			h30
Iberesia	*brauni*	CRBA_AP000087	23	II	*I brauni*	H10__f_I_OBU_BMA	F	rd. Orient to Bunyola	MALLORCA	Spain	62	545	39,72712	2,73418	H10		H10	H10
Iberesia	*brauni*	CRBA_AP000088	23	II	*I. brauni*	H37__F_I_OBU_BMA	F	rd. Orient to Bunyola	MALLORCA	Spain	62	545	39,72712	2,73418	H37			
Iberesia	*brauni*	CRBA_AP000103	23	II	*I. brauni*	H104_J_I_PSO_BMA	J	Port de Soller, rd to Fornalutx,	MALLORCA	Spain	8	39	39,80173	2,70703	H104			
Iberesia	*brauni*	CRBA_AP000098	23	II	*I. brauni*	H11__F_I_PSO_BMA	F	Port de Soller, rd to Fornalutx,	MALLORCA	Spain	8	39	39,80173	2,70703	H11	h11		H11
Iberesia	*brauni*	CRBA_AP000104	23	II	*I. brauni*	H116_J_I_PSO_BMA	J	Port de Soller, rd to Fornalutx,	MALLORCA	Spain	8	39	39,80173	2,70703	H116			
Iberesia	*brauni*	CRBA_AP000105	23	II	*I. brauni*	H150_J_I_PSO_BMA	J	Port de Soller, rd to Fornalutx,	MALLORCA	Spain	8	39	39,80173	2,70703	H150			
Iberesia	*brauni*	CRBA_AP000108	23	II	*I. brauni*	H153_J_I_PSO_BMA	J	Port de Soller, rd to Fornalutx,	MALLORCA	Spain	8	39	39,80173	2,70703	H153			
Iberesia	*brauni*	CRBA_AP000100	23	II	*I. brauni*	H44__F_I_PSO_BMA	F	Port de Soller, rd to Fornalutx,	MALLORCA	Spain	8	39	39,80173	2,70703	H44			
Iberesia	*brauni*	CRBA_AP000101	23	II	*I. brauni*	H80__F_I_PSO_BMA	F	Port de Soller, rd to Fornalutx,	MALLORCA	Spain	8	39	39,80173	2,70703	H80			
Iberesia	*brauni*	CRBA_AP000102	23	II	*I. brauni*	H92__F_I_PSO_BMA	F	Port de Soller, rd to Fornalutx,	MALLORCA	Spain	8	39	39,80173	2,70703	H92			
Iberesia	*brauni*	CRBA_AP000073	22	II	*I. brauni*	H115_F_I_CAM_BMA	F	Campanet, Vall de Fangar	MALLORCA	Spain	65	69	39,80901	2,97931	H115	h115	H115	h115
Iberesia	*brauni*	CRBA_AP000069	22	II	*I. brauni*	H18__F_I_CAM_BMA	F	Campanet, Vall de Fangar	MALLORCA	Spain	65	69	39,80901	2,97931	H18			
Iberesia	*brauni*	CRBA_AP000070	22	II	*I. brauni*	H41__F_I_CAM_BMA	F	Campanet, Vall de Fangar	MALLORCA	Spain	65	69	39,80901	2,97931	H41			
Iberesia	*brauni*	CRBA_AP000072	22	II	*I. brauni*	H77__F_I_CAM_BMA	F	Campanet, Vall de Fangar	MALLORCA	Spain	65	69	39,80901	2,97931	H77			
Iberesia	*brauni*	CRBA_AP000003	21	II	*I. brauni*	H33__F_I_ALC_BME	F	Alcalfar, rd. to Sant Lluis	MENORCA	Spain	66	24	39,83172	4,29193	H33			
Iberesia	*brauni*	CRBA_AP000001	21	II	*I. brauni*	H60__J_I_ALC_BME	J	Alcalfar, rd. to Sant Lluis	MENORCA	Spain	66	24	39,83172	4,29193	H60	h60	H60	h60
Iberesia	*brauni*	CRBA_AP000002	21	II	*I. brauni*	H73__J_I_ALC_BME	J	Alcalfar, rd. to Sant Lluis	MENORCA	Spain	66	24	39,83172	4,29193	H73			
Iberesia	*brauni*	CRBA_AP000019	21	II	*I. brauni*	H122_J_I_ALG_BME	J	Algaiarens beach,	MENORCA	Spain	69	109	40,03588	3,91062	H122			
Iberesia	*brauni*	CRBA_AP000011	21	II	*I. brauni*	H45__F_I_ALG_BME	F	Algaiarens beach	MENORCA	Spain	69	109	40,03588	3,91062	H45	H45	H45	h45
Iberesia	*brauni*	CRBA_AP000015	21	II	*I. brauni*	H62__J_I_ALG_BME	J	Algaiarens beach	MENORCA	Spain	69	109	40,03588	3,91062	H62			
Iberesia	*brauni*	CRBA_AP000014	21	II	*I. brauni*	H8__J_I_ALG_BME	J	Algaiarens beach	MENORCA	Spain	69	109	40,03588	3,91062	H8			

Genus	*Sp.*	SpecimenCode	Gmyc	Morph	Lineage	Label	S	Locality Name	Province	Country	N	El.	Lat.	Lon.	16s	28S	Efg	H3
Iberesia	*brauni*	CRBA_AP000016	21	II	*I. brauni*	H98__J_I_ALG_BME	J	Algaiarens beach	MENORCA	Spain	69	109	40,03588	3,91062	H98			
Iberesia	*brauni*	CRBA_AP000041	21	II	*I. brauni*	H36__F_I_SAG_BME	F	Santa Agueda	MENORCA	Spain	70	93	40,0381	4,00035	H36			
Iberesia	*brauni*	CRBA_AP000032	21	II	*I. brauni*	H125_J_I_TOR_BME	J	Cala Torta	MENORCA	Spain	71	29	40,05706	4,0759	H125			
Iberesia	*sp.*	CRBAME000931	24	II	*I. brauni*	E229_J_I_ALN_TER	J	Alcaniz	TERUEL	Spain	77	336	41,08276	-0,13115	E229		E229	E229
Iberesia	*sp.*	CRBAME000935	24	II	*I. brauni*	E34__J_I_ALN_TER	J	Alcaniz	TERUEL	Spain	77	336	41,08276	-0,13115	E34	E34	E34	E34
Iberesia	*sp.*	CRBAMM000163	50	III	3	Z163_J_I_PPO_ALM	F	Punto Polacra Cabo de Gata	ALMERIA	Spain	17	41	36,84788	-2,0249	Z163	Z163	Z163	Z163
Iberesia	*sp.*	CRBAME000834	38	III	3	E223_F_I_ORG_GRA	F	Orgiva	GRANADA	Spain	19	529	36,90213	-3,44176	E223			
Iberesia	*sp.*	CRBAME000873	39	III	3	E227_J_I_CHE_GRA	J	Cherni	GRANADA	Spain	20	541	36,96039	-3,00783	E227	E227	E227	E227
Iberesia		CRBAMM000143	33	III	3	Z143_J_I_TAB_ALM	J	Tabernas	ALMERIA	Spain	22	371	37,0362	-2,41823	Z143	Z143	Z143	Z143
Iberesia	*sp.*	CRBAMM000145	33	III	3	Z145_J_I_TAB_ALM	J	Tabernas	ALMERIA	Spain	22	371	37,0362	-2,41823	Z145	Z145	Z145	Z145
Iberesia	*sp*	CRBAMM000136	47	III	3	RA33_J_I_ALM_ALM	J	Cuevas de Almanzora	ALMERIA	Spain	26	155	37,28217	-1,91695	RA33			RA33
Iberesia	*sp.*	CRBAME000793	35	juv	4	ME793_J_I_FA2_CAD	J	Facinas 2	CADIZ	Spain	11	53	36,14684	-5,69858	ME793			
Iberesia	*sp.*	CRBAME000794	35	juv	4	ME794_J_I_FA2_CAD	J	Facinas 2	CADIZ	Spain	11	53	36,14684	-5,69858	ME794	ME794	ME794	ME794
Iberesia	*sp.*	CRBAME000839	36	juv	4	E147_J_I_GUI_JAE	J	Los Guindos	JAEN	Spain	45	443	38,28559	3,65327	E147	E147	E147	E147
Iberesia	*sp.*	CRBAME000840	36	juv	4	E224_J_I_GUI_JAE	J	Los Guindos	JAEN	Spain	45	443	38,28559	-3,65327	E224			
Iberesia	*sp.*	CRBAME000838	40	juv	4	E241_J_I_GUI_JAE	J	Los Guindos	JAEN	Spain	45	443	38,28559	-3,65327	E241			
Iberesia	*sp*	CRBAME001745	34	juv	4	E213_J_I_MTL_TOL	J	Montes de Toledo	TOLEDO	Spain	57	884	39,60709	-4,51189	E213	E213	E213	E213
Iberesia	*sp*	CRBAME001752	34	juv	4	E214_J_I_MTL_TOL	J	Montes de Toledo	TOLEDO	Spain	57	884	39,60709	-4,51189	E214		E214	E214
Iberesia	*sp*	CRBAME001748	34	juv	4	E232_J_I_MTL_TOL	J	Montes de Toledo	TOLEDO	Spain	57	884	39,60709	-4,51189	E232	E232	E232	E232
Iberesia	*sp*	CRBAMM000472	17	IV	5	RA102_F_I_SAP_MAR	F	Sidi Abdullah	Marrakesh	Morocco	2	483	34,12708	-4,30482	RA102	RA102	RA102	RA102
Iberesia	*sp*	CRBAMM000474	17	IV	5	RA103_J_I_SAP_MAR	J	Sidi Abdullah	Marrakesh	Morocco	2	483	34,12708	-4,30482	RA103			
Iberesia	*sp*	CRBAMM000471	17	IV	5	RA35_F_I_SAP_MAR	F	Sidi Abdullah	Marrakesh	Morocco	2	483	34,12708	-4,30482	RA35	RA35	RA35	RA35
Iberesia	*sp*	CRBAMM000528	16	IV	5	RA190_F_I_TAM_TAO	F	Road to Tahar - Souk	Taounate	Morocco	3	387	34,5612	-4,49946	RA190	RA190		RA190
Iberesia	*sp*	CRBAMM000532	16	IV	5	RA36_J_I_TAM_TAO	J	carretera Tahar - Souk	Taounate	Morocco	3	387	34,5612	-4,49946	RA36	RA36	RA36	RA36
Iberesia	*sp*	CRBAMM000533	16	IV	5	RA37_J_I_TAM_TAO	J	carretera Tahar - Souk	Taounate	Morocco	3	387	34,5612	-4,49946	RA37	RA37	RA37	RA37
Iberesia	*sp*	CRBAMM000536	15	IV	5	RA39_F_I_RHA_FES	F	Rhafsai	FES	Morroco	4	189	34,63186	-4,93112	RA39			
Iberesia	*sp*	CRBAMM000537	15	IV	5	RA40_F_I_RHA_FES	F	Rhafsai	FES	Morroco	4	189	34,63186	-4,93112	RA40	RA40	RA40	RA40

Genus	*Sp.*	SpecimenCode	Gmyc	Morph	Lineage	Label	S	Locality Name	Province	Country	N	El.	Lat.	Lon.	16s	28S	Efg	H3
Iberesia	*sp*	CRBAMM000539	15	IV	5	RA41_F_I_RHA_FES	F	Rhafsai	FES	Morroco	4	189	34,63186	-4,93112	RA41			
Iberesia	*sp*	CRBAMM000540	15	IV	5	RA42_F_I_RHA_FES	F	Rhafsai	FES	Morroco	4	189	34,63186	-4,93112	RA42	RA42	RA42	RA42
Iberesia	*sp*	CRBAMM000541	15	IV	5	RA43_F_I_RHA_FES	F	Rhafsai	FES	Morroco	4	189	34,63186	-4,93112	RA43			
Iberesia	*sp*	CRBAMM000543	15	IV	5	RA45_F_I_RHA_FES	F	Rhafsai,	FES	Morroco	4	189	34,63186	-4,93112	RA45			
Iberesia	*sp*	CRBAMM000544	15	IV	5	RA46_F_I_RHA_FES	F	Rhafsai,	FES	Morroco	4	189	34,63186	-4,93112	RA46			
Iberesia	*sp*	CRBAMM000551	14	IV	5	RA47_F_I_MFB_OUE	F	M.F.B. Bellota	Ouezzanne	Morocco	5		34,94961	-5,52892	RA47			
Iberesia	*sp*	CRBAMM000552	14	IV	5	RA48_F_I_MFB_OUE	F	M.F.B. Bellota	Ouezzanne	Morocco	5		34,94961	-5,52892	RA48	RA48	RA48	RA48
Iberesia	*sp*	CRBAMM000553	14	IV	5	RA49_F_I_MFB_OUE	F	M.F.B. Bellota	Ouezzanne	Morocco	5		34,94961	-5,52892	RA49	RA49	RA49	RA49
Iberesia	*sp*	CRBAMM000554	14	IV	5	RA50_J_I_MFB_OUE	J	M.F.B. Bellota	Ouezzanne	Morocco	5		34,94961	-5,52892	RA50			
Iberesia	*sp*	CRBAMM000556	14	IV	5	RA52_J_I_MFB_OUE	J	M.F.B. Bellota	Ouezzanne	Morocco	5		34,94961	-5,52892	RA52			
Iberesia	*sp*	CRBAMM000557	14	IV	5	RA53_J_I_MFB_OUE	J	M.F.B. Bellota	Ouezzanne	Morocco	5		34,94961	-5,52892	RA53			
Iberesia	*sp*	CRBAMM000577	13	IV	5	RA56_F_I_MOK_TAO	F	Mokrisset, campo de oliveras	Taounate	Morocco	6	343	35,00978	-5,39443	RA56	RA56		RA56
Iberesia	*sp*	CRBAMM000578	13	IV	5	RA57_F_I_XTE_CHA	F	Road Xouen - Tetuan, km 54	chaouen	Morocco	7	280	35,20756	-5,31293	RA57	RA57	RA57	RA57
Iberesia	*sp*	CRBAMM000579	13	IV	5	RA58_F_I_XTE_CHA	F	road Xouen - Tetuan, km 54	chaouen	Morocco	7	280	35,20756	-5,31293	RA58			
Iberesia	*sp.*	CRBAME000773	44	IV	5	ME773_F_I_CAR_MAL	F	Cartama Estacion	MALAGA	Spain	15	48	36,72942	-4,64674	ME773		ME773	
Iberesia	*sp.*	CRBAME000711	18	IV	5	E131_F_I_GRA_CAD	F	Grazalema	CADIZ	Spain	16	832	36,76462	-5,32318	E131	E131		E131
Iberesia	*sp.*	CRBAME000712	18	IV	5	ME712_F_I_GRA_CAD	F	Grazalema	CADIZ	Spain	16	832	36,76462	-5,32318	ME712			
Iberesia	*sp.*	CRBAME000714	18	IV	5	ME714_F_I_GRA_CAD	F	Grazalema	CADIZ	Spain	16	832	36,76462	-5,32318	ME714			
Iberesia	*sp.*	CRBAME000676	19	IV	5	E123_F_I_GAI_MAL	F	Desfiladero de los gaitanes	MALAGA	Spain	18	290	36,89157	-4,68182	E123	E123		E123
Iberesia	*sp.*	CRBAME000681	19	IV	5	E126_J_I_GAI_MAL	J	Desfiladero de los gaitanes	MALAGA	Spain	18	290	36,89157	-4,68182	E126			
Iberesia	*sp.*	CRBAME000674	19	IV	5	ME674_F_I_GAI_MAL	F	Desfiladero de los gaitanes	MALAGA	Spain	18	290	36,89157	-4,68182	ME674			
Iberesia	*sp.*	CRBAME000675	19	IV	5	ME675_F_I_GAI_MAL	F	Desfiladero de los gaitanes	MALAGA	Spain	18	290	36,89157	-4,68182	ME675	ME675	ME675	ME675
Iberesia	*sp.*	CRBAME000677	19	IV	5	ME677_F_I_GAI_MAL	F	Desfiladero de los gaitanes	MALAGA	Spain	18	290	36,89157	-4,68182	ME677	ME677	ME677	ME677
Iberesia	*sp.*	CRBAME000723	12	IV	5	E132_J_I_VAB_MAL	J	Valle de Abdalajis	MALAGA	Spain	21	571	36,97504	4,66431	E132	E132	E132	E132
Iberesia	*sp.*	CRBAME000726	12	IV	5	E135_J_I_VAB_MAL	J	Valle de Abdalajis	MALAGA	Spain	21	571	36,97504	-4,66431	E135			
Iberesia	*sp.*	CRBAME000727	12	IV	5	E193_J_I_VAB_MAL	J	Valle de Abdalajis	MALAGA	Spain	21	571	36,97504	-4,66431	E193	E193	E193	E193
Iberesia	*sp.*	CRBAME000721	12	IV	5	ME721_F_I_VAB_MAL	F	Valle de Abdalajis	MALAGA	Spain	21	571	36,97504	-4,66431	ME721			

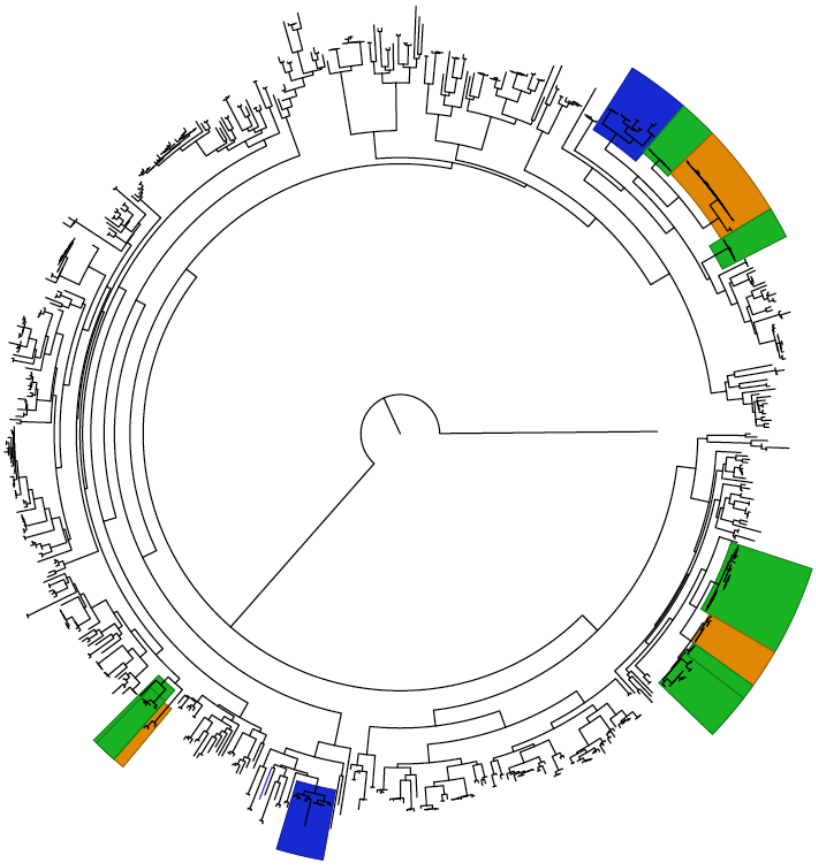
Genus	*Sp.*	SpecimenCode	Gmyc	Morph	Lineage	Label	S	Locality Name	Province	Country	N	El.	Lat.	Lon.	16s	28S	Efg	H3
Iberesia	*sp.*	CRBAME000802	10	V	6	ME802_F_I_SLU_CAD	F	Nuestra Sra de la Luz	CADIZ	Spain	10	145	36,12643	-5,64418	ME802			
Iberesia	*sp.*	CRBAME000803	11	V	6	ME803_F_I_SLU_CAD	F	Nuestra Sra de la Luz	CADIZ	Spain	10	145	36,12643	-5,64418	ME803	ME803	ME803	ME803
Iberesia	*sp.*	CRBAME000783	10	V	6	E141_F_I_FA2_CAD	F	Facinas 2	CADIZ	Spain	11	53	36,14684	-5,69858	E141			
Iberesia	*sp.*	CRBAME000785	10	V	6	E142_J_I_FA2_CAD	J	Facinas 2	CADIZ	Spain	11	53	36,14684	-5,69858	E142			
Iberesia	*sp.*	CRBAME000782	11	V	6	E239_F_I_FA2_CAD	F	Facinas 2	CADIZ	Spain	11	53	36,14684	-5,69858	E239			
Iberesia	*sp.*	CRBAME000781	11	V	6	ME781_F_I_FA2_CAD	F	Facinas 2	CADIZ	Spain	11	53	36,14684	-5,69858	ME781	ME781	ME781	ME781
Iberesia	*sp.*	CRBAME000784	11	V	6	ME784_F_I_FA2_CAD	F	Facinas 2	CADIZ	Spain	11	53	36,14684	-5,69858	ME784	ME784	ME784	ME784
Iberesia	*sp.*	CRBAME000696	8	V	6	E191_J_I_EPE_CAD	J	Finca El Peso	CADIZ	Spain	12	46	36,38845	-5,65146	E191	E191	E191	E191
Iberesia	*sp.*	CRBAME000698	8	V	6	E192_J_I_EPE_CAD	J	Finca El Peso	CADIZ	Spain	12	46	36,38845	-5,65146	E192			
Iberesia	*sp.*	CRBAME000691	8	V	6	ME691_F_I_EPE_CAD	F	Finca El Peso	CADIZ	Spain	12	46	36,38845	-5,65146	ME691	ME691	ME691	ME691
Iberesia	*sp.*	CRBAME000693	8	V	6	ME693_J_I_EPE_CAD	J	Finca El Peso	CADIZ	Spain	12	46	36,38845	-5,65146	ME693	ME693		ME693
Iberesia	*sp.*	CRBAME000695	8	V	6	ME695_J_I_EPE_CAD	J	Finca El Peso	CADIZ	Spain	12	46	36,38845	-5,65146	ME695			
Iberesia	*sp.*	CRBAME000704	9	V	6	E127_F_I_GAL_MAL	F	Puerto de Galiz	MALAGA	Spain	14	386	36,51784	-5,65624	E127			
Iberesia	*sp.*	CRBAME000705	9	V	6	E128_J_I_GAL_MAL	J	Puerto de Galiz	MALAGA	Spain	14	386	36,51784	-5,65624	E128	E128	E128	E128
Iberesia	*sp.*	CRBAME000706	9	V	6	E129_J_I_GAL_MAL	J	Puerto de Galiz	MALAGA	Spain	14	386	36,51784	-5,65624	E129			
Iberesia	*sp.*	CRBAME000702	9	V	6	ME702_F_I_GAL_MAL	F	Puerto de Galiz	MALAGA	Spain	14	386	36,51784	-5,65624	ME702			
Iberesia	*sp.*	CRBAME000703	9	V	6	ME703_F_I_GAL_MAL	F	Puerto de Galiz	MALAGA	Spain	14	386	36,51784	-5,65624	ME703			
Iberesia	*sp.*	CRBAME000814	7	V	6	E219_J_I_AR3_HUE	J	St Anna La Real 3	HUELVA	Spain	34	625	37,86559	-6,72768	E219		E219	E219
Iberesia	*sp.*	CRBAME000817	37	V	6	E220_J_I_AR3_HUE	J	St Anna La Real 3	HUELVA	Spain	34	625	37,86559	-6,72768	E220		E220	E220
Iberesia	*sp.*	CRBAME000812	7	V	6	ME812_F_I_AR3_HUE	F	St Anna La Real 3	HUELVA	Spain	34	625	37,86559	-6,72768	ME812			
Iberesia	*sp*	CRBAMM000602	7	V	6	RA59_F_I_AR1_HUE	F	Sanata Ana la Real	HUELVA	Spain	35		37,8659	-6,72768	RA59			
Iberesia	*SP*	CRBAMM000623	7	V	6	RA63_J_I_AGF_HUE	J	Aguafría	HUELVA	Spain	37	735	37,88387	-6,73797	RA63	RA63	RA63	RA63
Iberesia	*SP*	CRBAMM000624	7	V	6	RA64_J_I_AGF_HUE	J	Aguafría	HUELVA	Spain	37	735	37,88387	-6,73797	RA64			
Iberesia	*SP*	CRBAMM000625	7	V	6	RA65_J_I_AGF_HUE	J	Aguafría	HUELVA	Spain	37	735	37,88387	-6,73797	RA65			
Iberesia	*SP*	CRBAMM000632	7	V	6	RA66_J_I_ARA_HUE	J	Aracena	HUELVA	Spain	38	679	37,88842	-6,56184	RA66			
Iberesia	*sp.*	CRBAME000836	6	V	6	E145_J_I_AR2_HUE	J	Aroche 2	HUELVA	Spain	40	209	37,9743	-7,194	E145			
Iberesia	*sp*	CRBAME000810	6	V	6	ME810_F_I_AR2_HUE	F	Aroche 2	HUELVA	Spain	40	209	37,9743	-7,194	ME810	ME810		ME810

Genus	*Sp.*	SpecimenCode	Gmyc	Morph	Lineage	Label	S	Locality Name	Province	Country	N	El.	Lat.	Lon.	16s	28S	Efg	H3
Iberesia	*machadoi*	CRBAMM000203	52	VI	*I. machadoi*	Z203_F_I_COR_COI	F	A do Corvo, Almodovar	COIMBRA	Portugal	27		37,34946	-7,58626	Z203			Z203
Iberesia	*sp.*	CRBAME000767	2	VI	*I. machadoi*	E140_J_I_SAR_HUE	J	Sierra de Aracena	HUELVA	Spain	38		37,88842	-6,56184	E140			
Iberesia	*sp.*	CRBAME000760	2	VI	*I. machadoi*	ME760_F_I_SAR_HUE	F	Sierra de Aracena	HUELVA	Spain	38		37,88842	-6,56184	ME760	ME760	ME760	ME760
Iberesia	*sp.*	CRBAME000800	5	VI	*I. machadoi*	E195_J_I_SAL_BEJ	J	Sobral de alica	BEJA	Portugal	41	227	38,01664	-7,26973	E195	E195	E195	E195
Iberesia	*sp.*	CRBAME000797	5	VI	*I. machadoi*	E237_J_I_SAL_BEJ	F	Sobral de alica	BEJA	Portugal	41	227	38,01664		E237			
Iberesia	*sp.*	CRBAME000798	5	VI	*I. machadoi*	E238_F_I_SAL_BEJ	F	Sobral de alica	BEJA	Portugal	41	227	38,01664	-7,26973	E238			
Iberesia	*sp.*	CRBAME000796	5	VI	*I. machadoi*	ME796_F_I_SAL_BEJ	F	Sobral de alica	BEJA	Portugal	41	227	38,01664	-7,26973	ME796	ME796	ME796	ME796
Iberesia	*sp*	CRBAMM000744	1	VI	*I. machadoi*	RA76_F_I_SGR_BAL	F	Sierra do Grandola	B. ALENTEJO	Portugal	42	200	38,0809	-8,53047	RA76	RA76	RA76	RA76
Iberesia	*sp*	CRBAMM000745	1	VI	*I. machadoi*	RA77_F_I_SGR_BAL	F	Sierra do Grandola	B. ALENTEJO	Portugal	43	200	38,0809	-8,53047	RA77			
Iberesia	*sp*	CRBAMM000746	1	VI	*I. machadoi*	RA78_J_I_SGR_BAL	J	Sierra do Grandola	B.ALENTEJO	Portugal	43	200	38,0809	-8,53047	RA78	RA78	RA78	
Iberesia	*sp*	CRBAMM000747	3	VI	*I. machadoi*	RA79_J_I_SGR_BAL	J	Sierra do Grandola	B.ALENTEJO	Portugal	43	200	38,0809	-8,53047	RA79			
Iberesia	*sp*	CRBAMM000748	3	VI	*I. machadoi*	RA80_J_I_SGR_BAL	J	Sierra do Grandola	B.ALENTEJO	Portugal	43	200	38,0809	-8,53047	RA80	RA80	RA80	RA80
Iberesia	*sp*	CRBAMM000750	3	VI	*I. machadoi*	RA82_J_I_SGR_BAL	J	Sierra do Grandola	B. ALENTEJO	Portugal	43	200	38,0809	-8,53047	RA82	RA82	RA82	RA82
Iberesia	*machadoi*	CRBAMM000025	4	VI	*I. machadoi*	Z25_F_I_EVO_CAL	F	Evora	C. ALENTEJO	Portugal	47		38,575	-8,006944	Z25			
Iberesia	*machadoi*	CRBAMM000026	4	*I. machadoi*	*I. machadoi*	Z26_M_I_EVO_CAL	M	Evora	C ALENTEJO	Portugal	47		38,575	-8,006944	Z26	Z26	Z26	Z26
Iberesia	*machadoi*	CRBAMM000027	4	*I. machadoi*	*I. machadoi*	Z27_M_I_EVO_CAL	M	Evora	C ALENTEJO	Portugal	47		38,575	-8,006944	Z27			
Iberesia	*sp*	CRBAME001737	4	VI	*I.machadoi*	E209_J_I_BER_CAC	J	Berzocama	CACERES	Spain	52		39,41528	-5,41844	E209	E209	E209	E209
Iberesia	*sp*	CRBAME001735	4	VI	*I. machadoi*	E207_F_I_NAV_CAC	F	Navaconcejo	CACERES	Spain	72	375	40,08857	-5,98488	E207			
Iberesia	*sp*	CRBAME001736	4	VI	*I. machadoi*	E208_J_I_NAV_CAC	J	Navaconcejo	CACERES	Spain	72	375	40,08857	-5,98488	E208			

Genus	*Sp.*	SpecimenCode	Gmyc	Morph	Lineage	Label	S	Locality Name	Province	Country	N	El.	Lat.	Lon.	16s	28S	Efg	H3
Iberesia	*machadoi*	CRBAME001734	4	*I. machadoi*	*I. machadoi*	E206_M_I_NAV_CAC	M	Navaconcejo	CACERES	Spain	72	375	40,08857	-5,98488	E206	E206	E206	E206
Iberesia	*sp*	CRBAME001721	4	VI	*I. machadoi*	E205_J_I_CRO_CAC	J	Ciudad Rodrigo, South	SALAMANCA	Spain	75	783	40,61567	-6,42356	E205	E205	E205	E205
Iberesia		CRBAMM000056	4	*I. machadoi*	*I. machadoi*	Z56_F_I_BEZ_LEI	M	Bezerra	LEIRIA	Portugal	79		42,05	-8,2525	Z56			
Nemesia	*sp.*	CRBAME001395				DN133_F_N_HBB_SOU	F	Hbabsa	SOUSSE	Tunisia	64	444	35,47567	9,34092	DN133	DN133	DN133	DN133
Nemesia	*sp.*	CRBAME001430				DN145_F_N_OEZ_SOU	F	Oued Ez Zit	SOUSSE	Tunisia	13	166	36,46527	10,2791	DN145	DN145	DN145	DN145
Nemesia	*sp*	CRBAME000181				RA196_F_N_POL_SIC	F	Ponte Olivo	SICILY	Italy	23	158	37,12068	14,38138	RA196	RA196	RA196	RA196
Nemesia	*valenciae*	CRBAMM000118				Z118_M_N_CAL_CAR	M	Parc regional Calbalanque,	CARTAGENA	Spain	30	88	37,6079	-0,75988	Z118	Z118	Z118	Z118
Nemesia	*sp.*	CRBAME000818				E144_F_I_AR1_HUE	F	Aroche 1	HUELVA	Spain	39	250	37,97251	-7,081	E144	E144	E144	E144
Nemesia	*randa 98%*	CRBA_AP000063				H66__J_I_CUR_BMA	J	Santuari de Cura, 2 km; on rd slopes	MALLORCA	Spain	53	471	39,42208	2,92913	H66	H66	H66	H66
Nemesia	*sp*	CRBAME000271				RA201_F_N_GGE_BMA	F	Giara di Gesturi	MALLORCA	Italy	63	526	39,73802	9,00701	RA201	RA201	RA201	RA201
Nemesia	*randa*	CRBAME001459				E163_F_N_TRE_BME	F	Trepucó	MENORCA	Spain	9		39,86	4,297	E163	E163	E163	E163
Brachythele	*sp*	CRBAMM000033				Z33_F_B_STO_GRE	F	Stomio	GREECE	Greece	68		39,866667	22,73333	Z33	Z33	Z33	Z33
Nemesia	*sp*	CRBAME000460				RA209_F_N_CRI_SAR	F	Crisciuleddu	SARDEGNA	Italy	76	115	41,07318	9,17543	RA209	RA209	RA209	RA209
Nemesia	*sp.*	CRBAME001093				E115_F_N_TEI_BCN	F	Sot de teixoneres, PN St Llorenc Munt	BARCELONA	Spain	78	819	41,68677	2,02142	E115	E115	E115	E115
Nemesia	*sp.*	CRBAME000411				E17_F_N_CER_COR	F	Cervione, Valle di campoloro	CORSICA	France	80	338	42,32309	9,4898	E17	E17	E17	E17
Nemesia	*sp.*	CRBAME000009				RA9_F_N_MED_LEO	F	Las Medulas	LEON	Spain	81	713	42,4675	-6,7614	RA9	RA9	RA9	RA9
Nemesia	*carminans*	CRBAMM000844				RA191_F_N_FON_ALP	F	Fontvielle, PN des Alpilles	ALPS	France	82	18	43,75919	4,69507	RA191	RA191	RA191	RA191
Nemesia	*sp.*	CRBAMM000782				RA155_J_N_GAT_ALP	J	Gattieres, crtra Tou le Boc	ALPS	France	67	292	43,7711	7,17463	RA155	RA155	RA155	RA155

Chapter 4.3

Rafting spiders or drifting islands? Origins and diversification of the endemic spiders from the Balearic Islands, Western (submitted)



Rafting spiders or drifting islands? Origins and diversification of the endemic trap-door spiders from the Balearic Islands, Western Mediterranean.

1. Introduction

Since the work of Darwin, islands have played a pivotal role in our current understanding of the evolutionary process (MacArthur & Wilson, 1967; Carlquist, 1974) The features such of islands such as isolation, impoverishment, disharmony and replication, have converted islands into the test-tube experiments for evolutionary biologists (Mayr, 1967). According to their geological origin, islands are classified into two primary types. Oceanic islands, located over oceanic plates, originated because of volcanic or tectonic activity and never had contact with the continent (Whittaker, 1998). Because of the lack of a previous, inherited biota and, frequently, a long distance to the continent, oceanic islands are inherently depauperate and are viewed as ideal locations to conduct evolutionary research. The other primary type of island, the fragment or continental island, (Gillespie & Roderick, 2002) was once part of a continental landmasses from which the island later separated through tectonic movements or became isolated because of rising sea levels.

Unlike oceanic islands, continental islands have a previous biological history, shared with their continental counterparts (Gillespie & Roderick, 2002). Whereas the original absence of biota and the long distances to the continent dictate that dispersal is a key driver in the shaping of biological diversity on oceanic islands, the vicariance is traditionally considered the primary explanation for the origins of continental island biota (Wiley, 1988). Moreover, the availability of empty niches promotes high levels of local diversification on oceanic islands (ie. neo-endemism), whereas on continental islands phenomena such as relaxation, which is the loss of species because of se of area effects, and relictualisation, which is the formation of endemics following the extirpation of continental relatives (ie. Paleo-endemism), are dominant (Gillespie & Roderick, 2002).Molecular phylogenies and dating methods provide the essential information to discern the origins of island taxa and hence the ability to assess the relative roles of vicariance versus dispersal and the in situ

diversification events. Notably, the application of molecular tools to investigate the origins of endemic taxa on continental islands has revealed a more predominant role for dispersal than original expectations (Trewick SA, 2000; Carranza & Amat, 2005; Yoder & Nowak, 2006; Gentile et al., 2010; Salvo et al., 2010; Trewick & Gibbs, 2010; Cruaud et al., 2012).The Balearic Islands (Fig. 1) form one of the most isolated archipelagos in the Western Mediterranean (Palmer et al., 1999) and were once part of the Iberian Peninsula (Rosenbaum et al., 2002a; Rosenbaum & Lister, 2004). The islands are the emerged feature of the Balearic promontory, which constitutes an eastern prolongation of the Iberian Betic Mountain range (Fornos et al., 2002). There are four main islands and several islets of the Balearic Islands, with Majorca, the largest island, Minorca, and Cabrera forming the Gymnesian island group and with Ibiza, the closest island to the mainland, and Formentera typically referred to as the Pityusic Islands.

During the Eocene and part of the Oligocene, the Balearic Islands, Corsica, Sardinia, the Calabro-Peloritan massif, the Kabylies and the Betic-Rift Cordillera formed part of the Iberian plate. At the beginning of the Oligocene (approximately 30-25 million years ago, Ma), several blocks separated from their original location at the eastern side of the present-day Iberian Peninsula and southern France, and the resulting microplates started drifting clockwise to the present-day locations (Rosenbaum et al., 2002a; Rosenbaum & Lister, 2004). According to the geological (Martin-Suárez et al., 1993) and fossil evidence (Fontboté et al., 1990; Roca, 1996), part of the Balearic islands remained connected to the Betic belt (the present-day Betic ranges in south-eastern Spain), forming a Betic-Balearic domain. Further fossil evidence suggests that the Balearic terrains were effectively isolated from the Betics by the middle Miocene (approximately (approximately (approximately 16-11.6 Ma) (Gibbons, W. & Moreno, 2002).

The Balearic Islands were reconnected to the continent during the Messinian Salinity Crises, when land bridges emerged as a result of the closing of the Strait of Gibraltar (5.96 Ma) and the subsequent desiccation of the Mediterranean Basin (Jolivet et al., 2006). The reopening of the Strait of

Gibraltar, dated at 5.3 Ma, restored the water exchange between the Atlantic and the Mediterranean and effectively re-established the isolation of the island ecosystems (Loget et al., 2005; Meijer & Krijgsman, 2005). Finally, during the Quaternary glacial cycles, the eustatic sea level changes resulted in the recurrent emergence of land bridges between Majorca and Minorca, but not with Ibiza.

The suborder Mygalomorphae retains some of the characters that are considered primitive among spiders (Raven, 1985), such as two pairs of booklungs and fangs that articulate parallel to the body axis. Although less diverse than their sister taxa, the Araneomorphae or true spiders, the mygalomorphs have a cosmopolitan distribution, with 16 families and more than 2600 species (World Spider Catalog, 2015). Because of the low vagility, long life cycles and narrow distributions and ecological preferences, mygalomorphs have been used extensively to address biogeographic and evolutionary questions (Pedersen & Loeschcke, 2001; Ballesteros et al., 2002; Bond, 2004, 2012; Hendrixson & Bond, 2005a, Hendrixson & Bond, 2005b; Bond & Hedin, 2006; Bond & Stockman, 2008; Bailey et al., 2010; Satler et al., 2011, 2013; Hamilton et al., 2011; Opatova et al., 2013; Hendrixson et al., 2013; Opatova & Arnedo, 2014a, 2014b).

The Nemesiidae is the most species rich mygalomorph family in the Mediterranean, with 67 species in four genera, namely *Nemesia* (55, circum-Mediterranean), *Iberesia* (3, Iberian), *Brachythele* (8, Eastern Mediterranean) and *Raveniola* (1, Turkey) (World Spider Catalog, 2015). These spiders have a cryptic lifestyle—they are sit-and-wait predators that construct silk lined burrows in the gound, hidden by a closable trapdoor. The females have long life cycles and are sedentary. The males have shorter life cycles, leave their burrows in search of females after the adult moult and supposedly mediate the gene flow between populations. The Nemesiidae are the only mygalomorph spiders present in the Balearic Islands. To date, six species of the genus *Nemesia* and one species of the genus *Iberesia,* all endemic to the archipelago, have been recorded. Five species are found on Majorca, namely *Iberesia brauni* (Koch, 1882), *Nemesia bristowei* (Decae, 2005), *N. seldeni* (Decae, 2005), *N. randa*

(Decae, 2005), and *N.santeugenia* (Decae, 2005), and two species are found on Ibiza, *N. santeulalia* (Decae, 2005) and *N. ibiza* (Decae, 2005). To date *Nemesia* species have not been reported on Minorca.

The high levels of diversity and endemism of the nemesiids in the Balearic Islands offer unparalleled opportunities to investigate the relative roles of vicariance versus dispersal to explain the origins of the Balearic biota and to assess the relevance of in situ diversification processes in the shaping of local diversity. Despite their poor dispersal abilities, several mygalomorph spiders have colonised oceanic islands (Park et al., 1988; Raven, 1994; Opatova & Arnedo, 2014b). Ballooning, a type of silk-mediated, airborne dispersal that is characteristic of spiders, was reported in the mygalomorph genera *Ummidia* (Coyle et al., 1985) and *Atypus* (Deruytter et al., 2012) which might explain the long distance, overseas dispersal. Although ballooning has never been reported in the Nemesiidae, the genus *Nemesia* has been recorded on all primary Western Mediterranean islands.

In the present study, we tested whether the nemesiid species in the Balearic Islands were the result of vicariant events related to the break-up of the Iberian plate and the subsequent migration of landmasses caused by tectonic movements (Vicariant hypothesis) or, alternatively, whether the endemic nemesiids were the result of long distance, overseas dispersal events (Dispersal hypothesis). We further tested whether the Balearic species were, in part, the result of local diversification processes or whether the endemics evolved independently from the different continental lineages.

2.Materials and Methods

2.1 Taxonomic sampling

One hundred fifty individuals representing all known nemesiid species on the Balearic Islands, except the rare *N. santeugenia*, and a putative new species from Minorca, were sampled for the present study. Additionally, 59 specimens of non-Balearic nemesiids were included in the study to test the monophyly of the Balearic species and to identify the primary source of colonisation for the islands. An on-going research project aimed at unravelling the genetic and

phylogenetic diversity of the family Nemesiidae within the Mediterranean basin (Mora & Arnedo, in progress) facilitated the informed sampling of the closest relatives to conduct the present analyses. A representative of the closely related genus *Brachythele* Ausserer, 1871 was included as the outgroup to root all trees.

Most specimens were collected in the field by the authors during several campaigns conducted from 2009 to 2012. Additional specimens were kindly provided by colleagues. All specimens used in the present study, with locality information, are summarised in Fig.1.

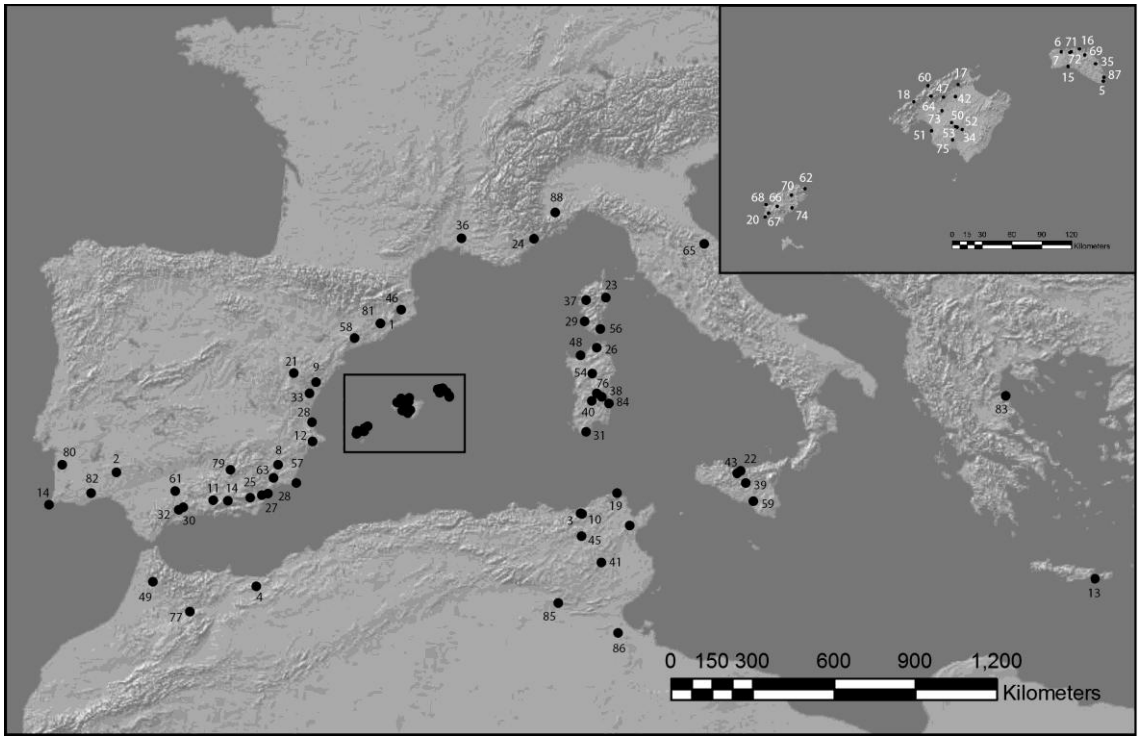


Figure.1. Map with sampling localities included in the present study

One or two legs (depending on the size) were removed and directly preserved in absolute ethanol shortly after specimen collection, and these samples were stored at -20°C in the freezer facilities of the Department of Animal Biology of the University of Barcelona to ensure preservation of the DNA. The voucher specimens were preserved in 70% alcohol for morphological studies.

2.2. DNA extraction, PCR amplification and sequencing

The total genomic DNA was extracted from the legs of the specimens or the pinned abdomens (juveniles) using the DNeasy Tissue Kit (Qiagen), according to the manufacturer's protocol. The fragments of the following genes were amplified with universal primers: a mitochondrial fragment spanning the 3' half of the 16S rRNA ribosomal subunit (16S), the complete tRNA leu (L1), the 5' half of the NADH dehydrogenase subunit I (nad1), and the fragments of the nuclear genes 28S rRNA (28S), Histone H3 (H3), and Elongation Factor 1 gamma (Ef-1 g). The primer sequences and the conditions for gene amplification are listed in Table 1. The PCR reactions were conducted in a final volume of 25 µL using Biotools Pfu DNA Polymerase (Biotools). The PCR products were purified using ExoSAP-IT (USB Corporation) and were cycle-sequenced in both directions using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). The sequence reactions were run on an ABI 3700 automated sequencer at the Scientific and Technical Services of the Universitat de Barcelona (CCiTUB, www.ccit.ub.edu).The raw sequences were assembled and edited using Geneious (v. 5.3.6) (http://www.geneious.com, Kearse et al., 2012).

2.3 Alignment and evolutionary model selection

The ribosomal DNA sequences were aligned using the online version of MAFFT v. 6 (available at http://mafft.cbrc.jp/alignment/server/; Katoh & Toh, 2008), which used the Q-INS-i strategy to account for the RNA secondary structure, with the default settings (gap opening penalty, GOP set to 1.53; offset value set to 0.0). The protein coding sequences were translated into amino acids to confirm that no stop codons were present. Unlike the nad1 and the H3 gene fragments, the Ef-1 g sequences showed evidence of indel mutations. Therefore, the alignments were built using translated sequences and the MAFFT algorithm as implemented in the program TranslatorX (Abascal et al., 2010) (available at http://www.translatorx.co.uk/).

The Gaps were recoded as presence/absence data following the simple method of Simmons & Ochoterena, 2000 as implemented in the program SeqState v1.4.1 (Müller, 2005). This gap treatment facilitated incorporation of the gap information into the analyses without increasing the weight of overlapping

multiple nonhomologous gaps (Pons & Vogler, 2006), and are amenable to likelihood, Bayesian and parsimony phylogenetic inference methods. The identical haplotypes were removed from the data matrix for phylogenetic analyses.

2.4 Phylogenetic Inference

The preliminary maximum likelihood analyses (see below for the programs and options implemented) on each gene separately confirmed that there were not conflicted supported clades between the gene trees (results not shown). The concatenation of the individual gene matrices was conducted with Geneious. Because incongruence among gene trees may exist because of different processes such horizontal gene transfer, gene duplication and incomplete lineage sorting, in this study, we used a concatenation approach based on two considerations: (1) no evident topological incongruences were found among individual gene trees, and (2) our time window of interest (Oligocene/Miocene divergences) minimised the possible effects of coalescent stochastic errors in the sampled markers (Bidegaray-Batista & Arnedo, 2011).

Model based analyses were conducted on the best partitioning scheme and corresponding evolutionary models were selected based on the BIC index, as reported in the program PARTITIONFINDER (Lanfear et al., 2012). The Maximum Likelihood (ML) analyses were conducted with RaxML v 7.4.2 (Stamatakis, 2006) under the graphical interface raxmlGUI (Silvestro & Michalak, 2012). The gene partitions were assigned a GTR model; whereas the gaps were assigned a binary model. In all cases, the models included GAMMA and Invariants. The best tree was obtained from 100 random replicates, and the clade support was assessed with 1000 bootstrap replicates (BS). The Bayesian inference analyses were performed using Mr. Bayes v 3.1.2 (Ronquist & Huelsenbeck, 2003) and were run remotely at the Bioportal computer resources of the University of Oslo (http://www.bioportal.uio.no).

The analyses were first run under the best partition scheme selected (see Results), but results did not become stationary, which was achieved by using the next simpler model (10 partitions, 1st and 2nd codon positions of the protein coding genes in one single partition). Two independent runs of 5x10^{7}

generations, 4 MCMC chains each, were conducted simultaneously, starting from random trees, and saving trees and parameters every 1000 generations. The convergence between the chains was assessed by monitoring the standard deviation of the split frequencies between runs (<0.05), and was confirmed with the help of the software TRACER v. 1.5 (Drummond & Rambaut, 2007). The software TRACER was also used to check for the correct mixing of the chains (ESS value) and to determine the correct number of generations to discard as a burn-in for the analyses (the first 10% of the generations). The parsimony analyses were conducted using the software TNT v1.1 (Goloboff et al., 2008). Each heuristic search consisted of 1000 replicates of random sequence addition, followed by TBR branch swapping, holding five trees per iteration and conducting a final round of branch swapping on the held trees. The clade support was assessed via jack-knife resampling (JS) (Farris et al., 1996), using 1000 replicates with individual heuristic searches composed of 20 replicates with random addition of taxa.

2.5 Delimitation of putative evolutionary lineages

The Generalized Mixed Yule-coalescent (GMYC) method (Pons et al., 2006; Fontaneto et al., 2007) was used to define coalescent clusters based on the mtDNA information. This method combines a model of stochastic lineage growth (Yules model) with a coalescence null model to determine the point of transition from species-level to population-level evolutionary processes. The GMYC method requires an ultrametric tree. The best tree out of 100 replicates was inferred for the combined 16s+L1+nad1 data matrix (mtDNA), under the best partition scheme and the CAT model. The branch lengths were converted to ultrametric using the PATHd8 software (District, 2000; Britton et al., 2007) by arbitrarily setting the root node to 1. The outgroup was removed from the transformed tree. The GMYC analysis was conducted with the R package SPLITS (Species Limits by Threshold Statistic) (Fujisawa & Barraclough, 2013).

2.6 Estimation of divergence times

A time frame for the diversification of the Balearic nemesiids was inferred in a Bayesian framework as implemented in the software BEAST v.1.6.3 (Drummond & Rambaut, 2007). Little is known of the fossil record of the family

Nemesiidae. The only known fossil that tentatively referred to the family Nemesiidae is *Cretamygale chasei*, in amber from the Isle of Wight, dated approximately 121±127 Ma (Selden, 2002). Unfortunately, the lack of available characters of the fossil species precluded inclusion it in any extant nemesiid lineage. Moreover, the family is most likely paraphyletic (Bond, 2012), which would complicate even further the use of the *Cretamygale* as calibration point. Additionally, biogeographic data were not incorporated to avoid circular reasoning. In the absence of both fossil and biogeographic data, the sole source of information from which to infer absolute ages was provided by the substitution rates estimated for close relatives. The substitution rates for Ef-1g were recently estimated for mygalomorphs (Opatova et al., 2013) and the mtDNA substitution rates are available for the haplogyne spider family Dysderidae (Bidegaray-Batista et al., 2014).

A simpler, by gene partition scheme was defined to facilitate chain convergence. A relaxed lognormal uncorrelated molecular clock was assigned to each nuclear gene and to the combined mtDNA genes. A normal distribution with mean values and standard deviations of 0.0113961 and 0.00483085 and 0.00117 and 0.0065 were specified for the mtDNA and the Ef-1g ucld.mean parameters, respectively, which were based on available information in the literature (see above). The values for the H3 and the 28S were estimated during the analyses. The Yule Process was selected as a tree prior. To avoid mixing species and coalescent level branch lengths, single representatives of each coalescent cluster (ie GMYC clusters) were selected for running the analyses, yielding a data matrix of 90 terminals. Six independent runs of $5x10^7$ generations were run independently. The convergence and correct mixing were visualised using the software TRACER. The BEAST accompanying programs LOGCOMBINER and TREEANNOTATOR were used to combine independent runs, following burn-in, and to estimate the consensus chronogram.

2.7 Ancestral Area Reconstruction

A Bayesian discrete phylogeographic approach (Lemey et al., 2009) as implemented in BEAST v.1.7.5 was used to infer biogeographic patterns. The Bayesian Stochastic Search Variable Selection (BSSVS) was used to identify

the rates, i.e. dispersion pathways, that were frequently invoked to explain the diffusion process (Lemey et al., 2009). We defined 12 discrete biogeographic areas corresponding to the main islands in the Western Mediterranean (ie. Ibiza, Majorca, Minorca, Corsica, Sardinia and Sicily) and to the main continental terrains (ie. Iberia, Italy and northern Africa). Based on the geological history, we distinguished two different areas that corresponded to the present-day Iberian Peninsula, the Iberian Massif and the Betics. The Betics area formed a microplate that detached, drifted counterclock-wise and re-joined the rest of the peninsula, lifting the Betic Cordillera (Rosenbaum et al., 2002b). An eastern-Mediterranean area was defined to score the outgroup (*Brachythele*) and the *Nemesia* from Crete. The discrete phylogeographic analyses were run with the same settings for the molecular data that were used in the divergence time analysis, using a strict clock and a symmetric substitution models for the geographic area trait. Three independent MCMC chains were run for 50 million generations. The processing of the sampled parameter values and the trees were conducted as described above for the estimation of the divergence times. The well-supported biogeographic diffusion rates were identified using the Bayes factors test in SPREAD.

Haplotype networks were inferred for the more thoroughly sampled Balearic species, namely *I. brauni, N. randa, N. Ibiza* and *N. santeulalia.* The networks were constructed with the help of the software TCS 1.21 (Clement et al., 2000), which implemented the statistical parsimony approach and the R package PEGAS (Paradis E., 2010). The additional code for building haplotypes by Jimmy O'Donnell (http://jimmyodonnell.wordpress.com/2013/05/20/haplotype-networks-in-r/ accessed 14 January 2014) and for renaming haplotypes by Samuel Brown (available at http://www.r-bloggers.com/haplotype-names-in-r/, 2014) was incorporated. The within and between network uncorrected genetic distances (*p-value*) were calculated with the help of the program MEGA (Tamura et al., 2013).

3. Results

A total of 209 specimens were analysed, in addition to the species *Brachythele* sp., which was used as the outgroup. The concatenated data matrix included

3124 characters that were distributed as follows: 613 characters corresponding to the 16S+L1, 411 to the *nad1*, 800 to the 28S, 327 to the H3, 815 to the Ef-1g and 154 to the absence/presence gaps. The preferred partition corresponded to the "by gene and by codon position for protein coding genes" scheme (11 partitions, see Appendix S2 in Supporting Information). However, this partition scheme resulted in lack of convergence in the Bayesian analyses. Therefore, for the MrBayes analysis the next best partition was implemented, which corresponded to the "by gene, by codon in the mt protein codon genes and by 1+2 vs. 3 codon positions in the nuc protein genes" scheme (Table 2). Furthermore, a simpler (5 partitions, see table 2), "by gene" scheme was implemented in the BEAST analyses, for faster computations.

Partition	**Model**	**Partition**	**Model**	**Partition**	**Model**
16s-L1	GTR+I+G	16s-L1	GTR+I+G	16s-L1	GTR+I+G
*nad1*_1	GTR+I+G	*nad1*_1	GTR+I+G	*nad1*	GTR+I+G
*nad1*_2	K81uf+G	*nad1*_2	K81uf+G		
*nad1*_3	TVM+G	*nad1*_3	TVM+G		
28s	GTR+I+G	28s	GTR+I+G	28s	GTR+I+G
H3_2	SYM+G	H3_1+2	TVMef+I+G	H3	TVMef+I+G
H3_3	SYM+G				
H3_1	JC+I	H3_3	SYM+G		
EF1g_1	K80+G	EF1g_1+2	HKY+I+G	EF1g	TrN+I+G
EF1g_2	HKY+I				
EF1g_3	TVMef+G	EF1g_3	TVMef+G		

Table.2. Partition schemes and corresponding best models used in the present study

3.1 Phylogenetic Inference

The phylogenetic trees obtained under the maximum likelihood (ML), Bayesian inference (BI) and parsimony analyses (MP) are summarised in Figure 2. The parsimony analyses yielded 10,000 trees (overflow) of 8,281 steps; whereas the best likelihood tree reported a *–logL* value of 40288.56. All analyses recovered the reciprocal monophyly of the nemesiid genera *Iberesia* and *Nemesia*, albeit

with low support for the *Nemesia* clade (BS and JS <75, PP<0.95). Similarly, all analyses split the Balearic nemesiids into several independent clades.

The first clade included all the sampled specimens of the species *Iberesia brauni*, a species recovered in all analyses, although only supported under only the ML analysis as the sister group to two other *Iberesia* lineages from southeastern Iberia (E33) and Morocco (RA130). The results showed the paraphyly of the Mallorca specimens with regards to the Minorca specimens.

A second clade, hereafter referred to as the Balearic clade, included the individuals from Majorca, Minorca and Ibiza, which were of at at least three different species, namely *N. bristowei*, *N. ibiza* and *N. randa,* all of which were supported as monophyletic. This second clade was recovered in all the analyses, albeit with low support. Additionally, the Balearic clade was supported in all the analyses as the sister group to the two lineages from the southeastern Iberian Peninsula (unidentified *Nemesia* Z156 and RA84).

The species *N. ibiza* was supported as the sister species to *N. randa*, which was itself divided into two well-supported clades corresponding to the Minorca and the Majorca individuals, respectively. A third clade was supported in all the analyses included the individuals identified as *N. santaeulalia* in addition to four eastern Iberian lineages. Notably, the specimen H27 did not cluster with the remaining specimens from Ibiza, but was more closely related to an individual (Z118) identified as *N. valenciae* from Murcia, in southeastern Iberia.

The fourth clade, recovered in all the analyses, albeit with low support, included two Balearic lineages, *N. seldeni* and a putative new species from Minorca (hereafter referred to as *N.* nsp 'Minorca'); these two Balearic lineages were not sister groups, because the *N.* sp 'Minorca' was recovered as the sister group to three lineages from eastern Iberia, but only the ML provided support, and MP placed the clase as the sister to *N. seldeni* instead.

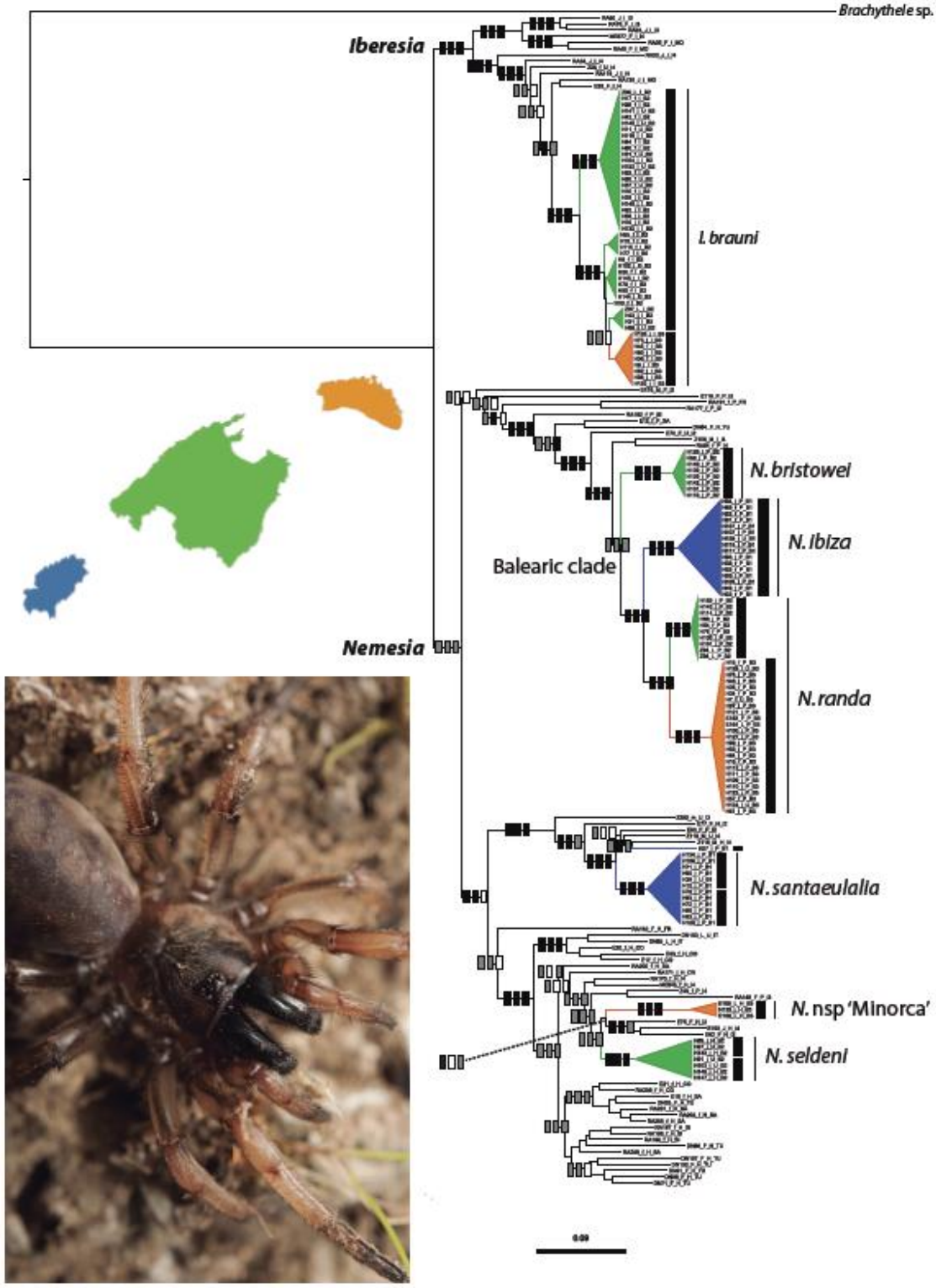


Figure 2. The ML tree obtained from concatenated data matrix. Boxes on branches indicate clade support as follows: black rectangles denote ML bootstrap values >75% (left), Parsimony jackknife values >75% (middle) and Bayesian Posterior Probability values >0.95, grey rectangles denote clades recovered but with support values below the former thresholds, with methods arranged in the same orders as above; and white boxes denote clades not recovered in this particular analysis. Black bars indicate GMYC clusters for Balearic species, all the other individuals represent independent GMYC lineages. Clade color-coded according to islands (see

Map). Picture shows a female of Iberesia brauni from Orient, Majorca (Locality 64).

3.2 Divergence times analyses

The chronogram obtained in the analyses of the divergence times with the corresponding confidence intervals is shown in Fig. 3. The species *I. brauni* split from its continental sister groups approximately 6 Ma (8.4-3.9 Ma), and the time of the most recent common ancestor (MRCA) of the extant diversity was dated at approximately 4 Ma (6-2.5 Ma).

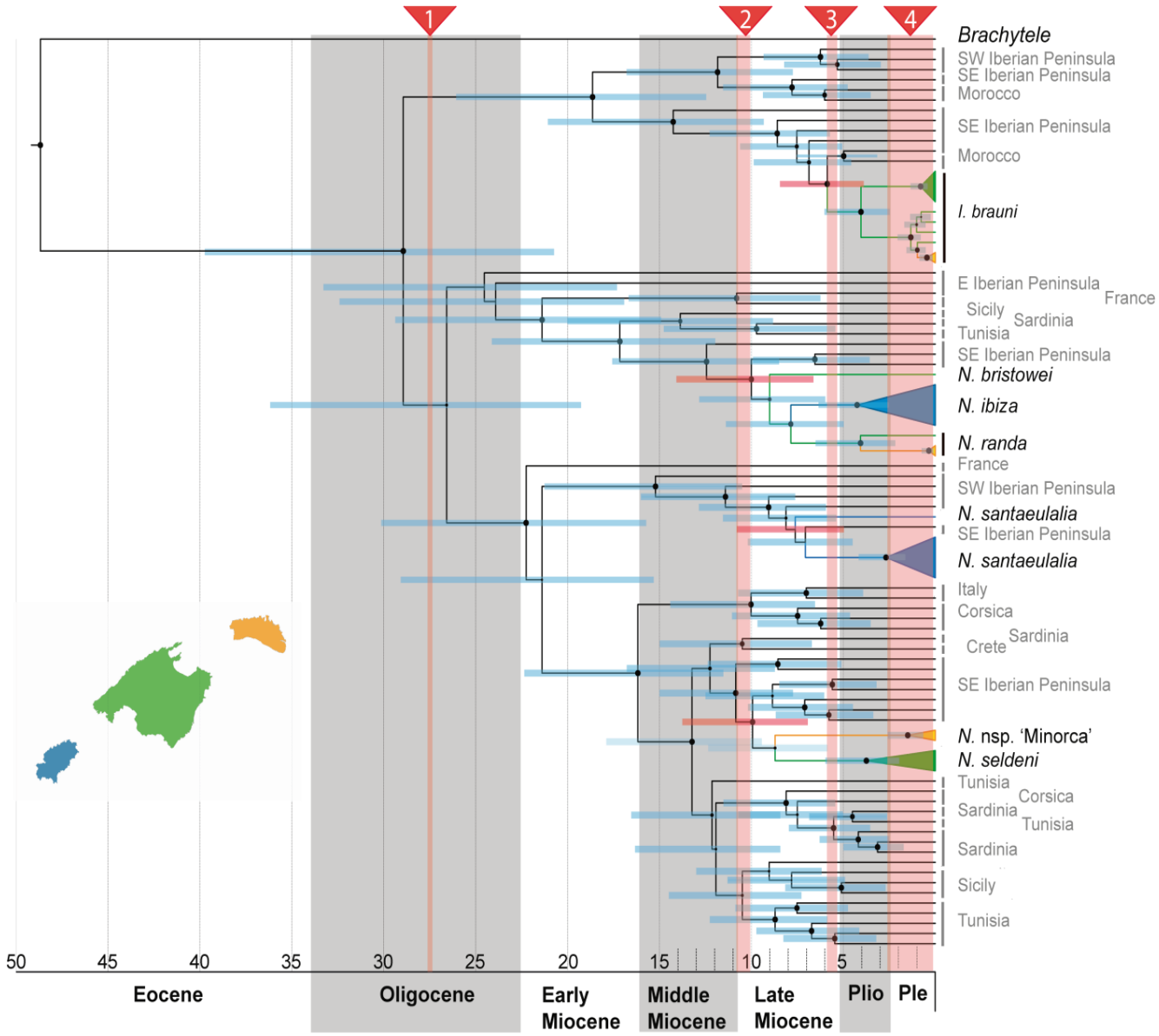


Figure 3. Chronogram inferred with BEAST. Bars on nodes correspond to confidence intervals, and red coloured bars indicate splits of Balearic lineages from their continental sister-taxa. Red lines and orresponding red triangles indicate the timing of relevant geological and climatological events in the region as follows: 1, approximately 25 Ma, the Hercynian block split off the Iberian

Peninsula (Rosenbaum et al., 2002b), 2, the Langhian-Serravallian (middle Miocene) and the Tortonian (late Miocene) (Mein & Adrover, 1982; Quintana & Agustí, 2007), which has been recorded only in Mallorca and Menorca; 3, 5.96 Ma, closing of the Atlanto-Mediterranean marine connection and starting of the Messinian Slainity Crises, with connection restored at 5.3 Ma (Krijgsman et al., 1999; Loget et al., 2005); and 4, the Quaternary glaciations dated between 2.6 and 0.1 Ma (Ehlers & Gibbard, 2008).

The Minorcan population of *I. brauni* split from the Majorcan relatives approximately 1 Ma (1.5-0.5 Ma). The Balearic clade included *N bristowei, N. ibiza and N. randa, which* split from the Iberian relatives approximately 10 Ma (14-6.6 Ma). Intraspecific splits within the archipelago involved the separation of the *N. bristowei* ancestor from the two other species at 9 Ma (12.8– 6 Ma), followed by the split of *N. ibiza* from *N.randa* at 8 Ma (11.4-5 Ma). The Minorcan and Majorcan populations of *N. randa* split approximately 4 Ma (6.5-2 Ma). The MRCA of *N. ibiza* and *N. randa* was dated at 4.2 Ma (2.5-6.3 Ma) and 4.1 Ma (2.2-6.5 Ma), respectively. For the *N. santaeulalia* lineages, the clade split from the continental sister group at 8.1 Ma (5.4-11.5 Ma).

The next split that occurred within the clade included the *N. santaeulalia* individuals and one northeastern Iberian individual, which all fell within the former time span. The time of the MRCA of *N. santaeulalia*, excluding the H27 individuals was dated to 2.7 Ma (1.6-4.1 Ma). Unlike the non-ultrameric, model-based phylogenetic analyses, the BEAST recovered the monophyly of *N. seldeni* and *N.* nsp. 'Minorca', albeit with low support. The split of *N. seldeni* and *N.* nsp. 'Minorca' from their Iberian relatives was dated at 9.9 Ma (7-13.7 Ma).

The split of the two Balearic species was estimated at 8.7 Ma (5.9-12.3 Ma), and the MRCA was dated at 3.7 (2-6 Ma) and 1.5 Ma (0.7-2.5), for *N. seldeni* and *N.* nsp. 'Minorca', respectively.The reconstructions of the ancestral biogeographic distributions are summarised in Fig. 4. The geographic diffusion pathways supported by the Bayes factors (BF>3) were as follow: between Majorca and Minorca, between the Betics and Majorca, Ibiza, the Iberian Massif and northern Africa, between France and the Iberian Massif, between northern Africa and Sardinia and Sicily and between Corsica and Italy and Sardinia. In all cases, the reconstructed area with the highest posterior probability of the MRCA of the Balearic lineages and the continental sister-group was the Betics.

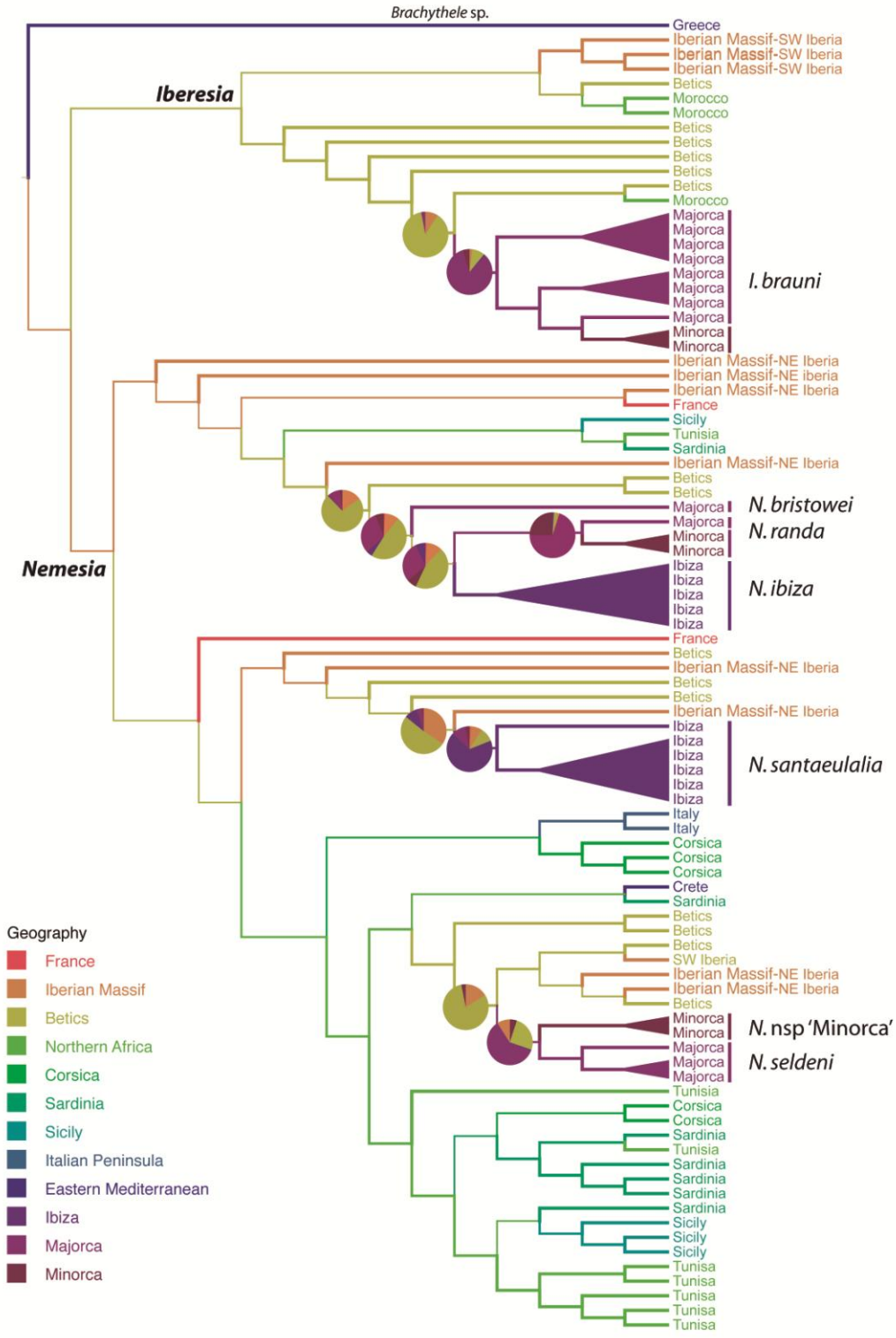


Figure 4. Bayesian-based biogeographic reconstruction of ancestral nodes using the discrete phylogeographic approach (Lemey et al, 2009). Colours correspond to the 12 geographic regions defined in the text (please note that colour codes do not match those in Fig. 2 and 3). Pies indicate the probability of the different ancestral areas for the relevant nodes, when the probability of a particular ancestral state was lower than 0.95.

The haplotype networks that were inferred are shown in Figs. 5, 6 and 7 for *I brauni*, *N. randa*, and *N. santeulalila* and *N. ibiza*, respectively. The *Iberesia brauni* mtDNA haplotypes were resolved as 4 independent parsimony networks (Table 3), with clear geographic structure, namely, the NW Majorca, NE Majorca, S Majorca, and the Minorcan haplotype networks. The recovered networks were more inclusive than but were otherwise congruent with the GYMC results.

	N	H	net	GMYC	π	(st.dev)
I. brauni	52	48(45)	4	6	0.038	(0.0003)
Majorca	42	38	3	5	0.036	(0.0003)
Minorca	10	10	1	1	0.007	(0.00002)
N. randa	49	35(34)	2	2	0.037	(0.0003)
Majorca	13	10	1	1	0.002	(0.000002)
Minorca	36	25	1	1	0.004	(0.00004)
N. ibiza	16	16(16)	5	5	0.05	(0.0007)
N. santaeulalia	13	13(13)	5	5	0.043	(0.0005)

Table 2.- Genetic diversity in mtDNA in selected Balearic nemesiid species. N: number of individuals sampled; H: number of haplotypes, in brackets number of haplotypes when Ns were not considered; net: number of independent haplotype networks identified with TCS; GMYC: number of GMYC clusters identified; and π: nucleotide diversity, with standard deviations in brackets. For *I. brauni* and *N. randa*, values are reported overall and for each island population.

The mean p-value within the networks was 0.008, 0.012, 0.003 and 0.007 for the NW, NE, S Majorca and the Minorca networks, respectively, and the mean internetwork diversity was 0.032. Similarly, the *N. ibiza* haplotypes were resolved into 5 independent networks (Fig. 6), with high geographic structure because each locality was resolved as an independent network, and these networks also matched the GMYC clades that were recovered. The mean p-value within the networks was 0.001, 0.004, 0.004, 0.005 and 0.005, and the mean internetwork diversity was 0.048. Two networks were obtained for *N. randa*, with one corresponding to the Majorcan and one to the Minorcan populations (Fig. 7) which were as also recovered by the GMYC analysis. The Minorcan network showed little geographic structure and had low genetic variability (0.008). The Majorcan network also showed little genetic variability

(0.003) but for the geographic structure, most populations included exclusive haplotypes.

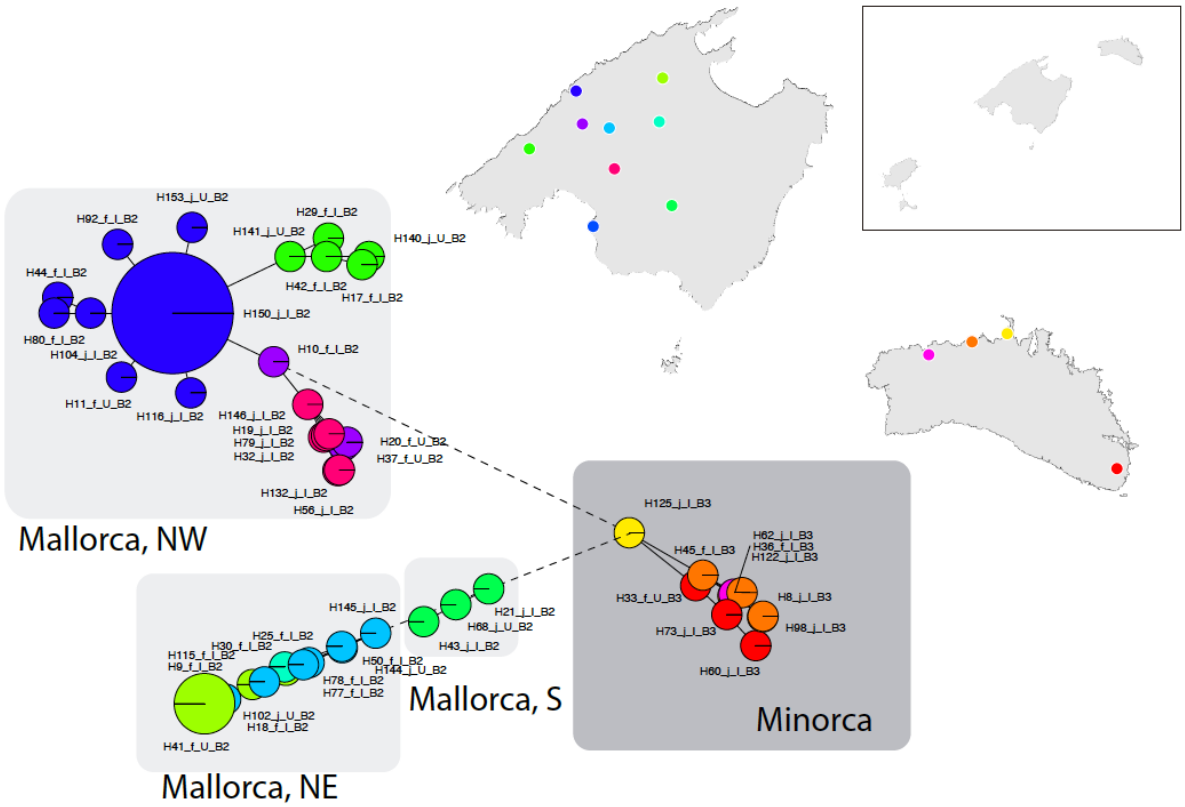


Figure.5. Mitochondrial haplotype networks of I. *brauni.* Pie colour indicates the number of localities where the same haplotype was found,and the size is proportional to the number of individuals with the same haplotypes. Colour codes correspond to the localities shown on the maps. Haplotypes connected by dotted lines denote statistical parsimony probabilities below 0.95. Independent networks are boxed

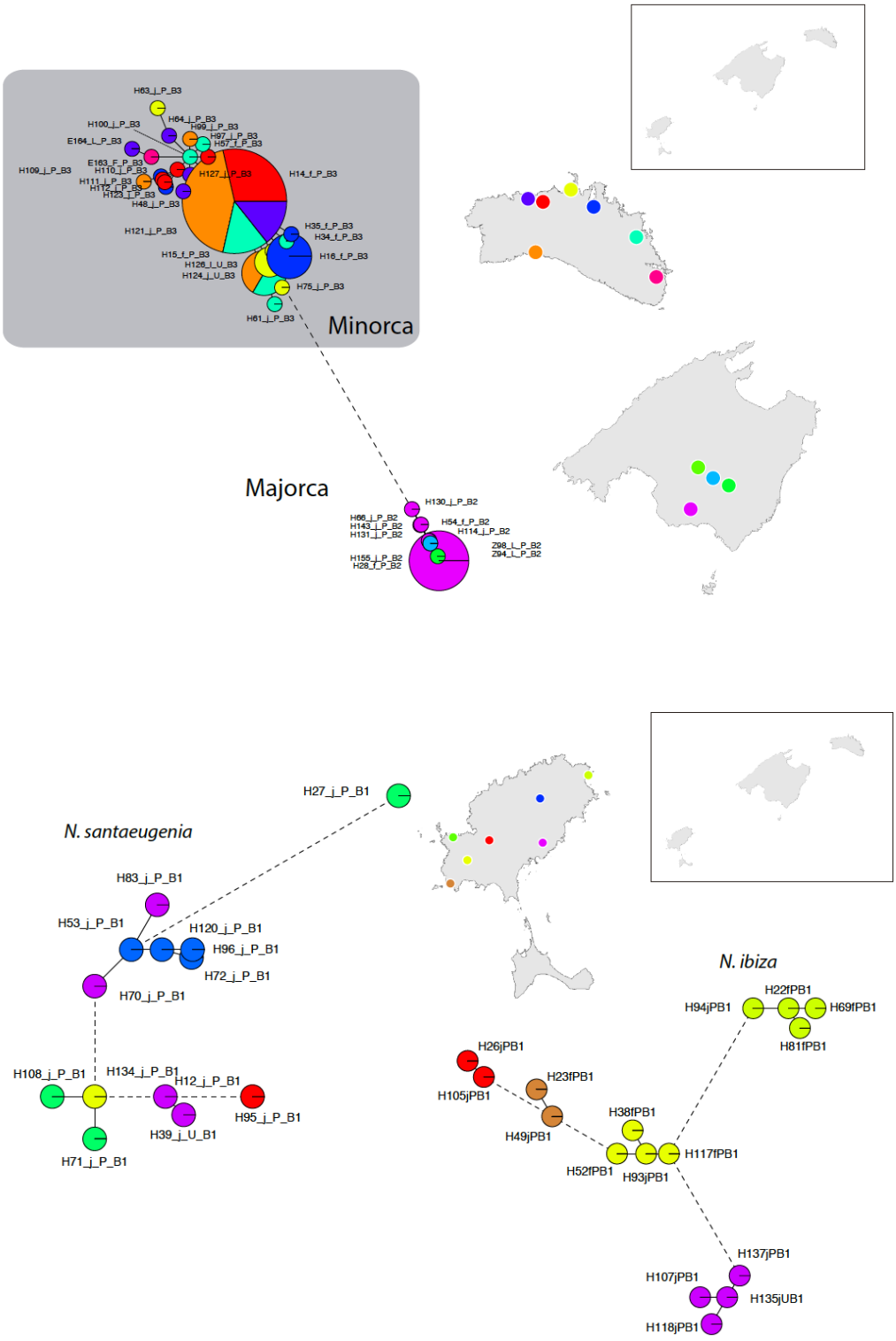


Figure.6 (up) and 7(down) Mitochondrial haplotype networks of *N. santeugenia, N,ibiza* (up) and *N. randa* (down). Pie colour indicates the number of localities where the same haplotype was found,and the size is proportional to the number of individuals with the same haplotypes. Colour codes correspond to the localities shown on the maps. Haplotypes connected by dotted lines denote statistical parsimony probabilities below 0.95. Independent networks are boxed

Unlike the networks of the other species, the *N. randa* networks showed a star-like pattern, with one or a few widespread, frequent haplotypes and many slightly differentiated, exclusive, local haplotypes. The mean internetwork diversity was 0.031. Finally, the *N. santeulalia* haplotypes were resolved into 5 networks (see Appendix S3), which were more inclusive than but were congruent with the GMYC clusters. The networks of *N. santeulalia* showed less geographic structure than the other species; two localities included exclusive haplotypes, whereas the others shared haplotypes from independent networks. Within network genetic diversity was 0.001, 0.007 and 0.01 for the three networks with two or more haplotypes, and the mean internetwork diversity was 0.08 (0.035, when the highly divergent H27 haplotype was excluded).

4. Discussion

Origins of Balearic nemesiids: Vicariance or overseas dispersal?

The Balearic Islands were once part of the Iberian Peninsula. Both geological and paleontological evidence indicated that there were no geographical barriers between the Balearic Islands and the Iberian Peninsula during the Oligocene (Ramos et al., 2001; Gibbons, W., Moreno, 2002). Using molecular dating methods, recent studies revealed that the divergence of some Balearic endemics, including earthworms (Pérez-Losada et al., 2011) and ground spiders (Bidegaray-Batista & Arnedo, 2011) from their Iberian relatives traces back to the late Oligocene-early Miocene (30-25 Ma). Thus, the opening of the Valencia trough constituted an effective biological barrier that hampered the connections of the Balearic terrains with the Iberian plate.

The Balearic microplate and the Betic belt, formed a Betic-Balearic domain, which suffered a WNW progressive thrust sheet stacking during the back arc extension that ended approximately at the time of the middle Miocene (Fontboté et al., 1990). The close faunal similarities between the Balearic Islands and the Betics (Martin-Suárez et al., 1993; Bover et al., 2008; Bailon et al., 2010) provided support for the existence of a Betic-Balearic connection during the Langhian-Serravallian marine regression (middle Miocene, c. 14.2 Ma). Although the limits of the ancestral Balearic Islands remain poorly defined, the fossil evidence, including remains of lagomorphs, rodents and a viper remains

supports an insular nature for the Balearic domains during the middle Miocene (approximately 16-11.6 Ma) (Gibbons & Moreno, 2002).

Our results provided further support for the close affinities between the Balearic and the Betic fauna, by reconstructing the Betics as the most likely ancestral region for the ancestor of the Balearic Nemesiidae. These results suggested that either the Betics served as the source for the overseas colonisation of the Balearic Islands (Dispersal hypothesis) or alternatively than the ancestors of the Balearic species once inhabited the continuous landmass that connected the Balearics and the Betics, which was subsequently severed by the middle Miocene tectonic movements that isolated the Balearics (Vicariant hypothesis).

The time estimates for the split of three of the Balearic lineages, namely the Balearic clade, the *N. santaeulalia* lineage and the *N. seldeni* lineage, from their continental (Betic) counterparts indicated middle to late Miocene divergences (10 Ma, 14-6.6 Ma; 8.1 Ma, 11.5-5.4 Ma, and 9.9 Ma, 13.7-7 Ma, respectively), which were compatible with the vicariant origin hypothesis. Notably, a very similar age was also obtained for the split of the Balearic species of the *Parachtes* ground-dweller spiders and their Betic sister species (9.7 Ma, 16.1-4.1 Ma), which was a group fpor which the diversity was hypothesized to be the result of the opening of the western Mediterranean basin (Bidegaray-Batista & Arnedo, 2011).

The estimated split of *Iberesia brauni* from its continental relatives (6 Ma, 8.4-3.9 Ma), however, was more compatible with a secondary spread into the islands via the land bridges that emerged during the Mediterranean sea level drop following the Messinian Salinity Crises, dated to 5.96-5.3 Ma (Krijgsman et al., 1999; Loget et al., 2005). The arrival to the islands via the terrestrial connections established during the middle-late Pleistocene was also proposed to explain the origin of the extinct endemic caprine genus *Myotragus* (Lalueza-Fox et al., 2005).

The Gymnesian islands showed clear faunistic affinities as a result of the establishment of recurrent land bridges between the two islands following the first episode of insularisation of the archipelago (Palmer et al., 1999). Majorca and Minorca were connected in at least three different periods: during the

Serravallian (13.8-11.6 Ma), as evidenced by the presence of the lagomorph *Gymnesicolagus* aff. *gelaberti* remains (Quintana & Agustí, 2007) during the Messinian Salinity Crises (5.9-5.3 Ma) and during the eustatic sea levels induced by the Quaternary glacial cycles (2.6-0.1 Ma). Notably, the time of the split for each of the three nemesiid species that inhabit Minorca from their Majorcan sister groups suggested that they originated as a result of the marine transgressions that followed each of the former connection events: 8.7 Ma (12.3-5.9 Ma), 4 Ma (6.5-2 Ma) and 1 Ma (1.5-0.5 Ma) for the *N.* n.sp. 'Minorca', *N. randa* and *I. brauni,* respectively. The fossil evidence is abundant for a close faunistic relationship between Majorca and Minorca between the lower and the upper Pleistocene because of the terrestrial connection that resulted from the glacial related eustatic sea level changes (Bover et al., 2008).

The Gymnesian and the Pityusic (ie. Ibiza and Formentera) islands have remained isolated from one another since the end of the Messinian Salinity Crisis (MSC) (Picornell et al., 2004), as was illustrated by the divergence of the two endemic *Podarcis* lizards, *P.lilfordi* (on Mallorca, Minorca and Cabrera) and *P.pityusensis* (on Ibiza and Formentera) (Brown et al. 2008). Although the confidence intervals marginally overlapped, the average time of the split was much older (8 Ma, 11.4-5 Ma) for *N. ibiza* from its Gymnesian relatives. Alternatively, such a split could correspond to an older event; more precisely, the marine transgression recorded in the early Tortonian (11.62-7.25 Ma) (Viseras et al., 2005), which would have isolated the formerly connected islands (Serravallian).

Systematics of Mediterranean Nemesiidae spiders

This study was the first to analyse the phylogenetic relationships within the Mediterranean Nemesiidae. Our results supported the monophyly of the recently described genus *Iberesia* (Decae & Cardoso, 2005; Decae et al., 2007) and its sister group relationship with the genus *Nemesia*, although the *Nemesia* monophyly was poorly supported. The genus *Iberesia* was characterised by the synapomorphic loss of the posterior lateral spinnerets. However, different degrees of spinneret reduction were also described in *Nemesia*, which in combination with the low support retrieved might indicate a paraphyletic status

of the genus. Additionally, the geographically structured differentiation of the genitalia observed within *Nemesia* was also suggestive of independent evolutionary lineages that might deserve generic status (Decae, 2012).

The polyphyletic nature of the Balearic nemesiid assemblage recovered in the present study was previously advanced in the original descriptions of the endemic species (Decae, 2005). Based on the spermathecae morphology, all Balearic species were of the “western type” (E- and F-types sensu Decae, 2012), except for *N. seldeni* and the new Minorcan species, which would be placed with the D-type, a group much more frequent in the Eastern Mediterranean. Our results supported the former suggestion that *N. seldeni* and the new Minorcan species belonged to a clade that also included species from Italy, Tunisia, Crete and the Thyrrenian Islands.

The species *N. randa* and *N. bristowei* were suggested as sister species based on the morphology, behaviour and ecology. The *N. bristowei* constructs a characteristic cogwheel trapdoor that was been documented in species from Sardinia and Tunisia. Although not included in the original description, a similar, although more subtle trapdoor design was also observed in *N. randa* (Arnedo, pers.obs), particularly in the Minorcan populations. Notably, the specimens DN84 from Tunis and E12 from Sardinia, both cogwheel builders, were supported as sister species that fell close to the Balearic clade. Although our results supported the close affinities of *N. bristowei* and *N. randa*, the results also supported the inclusion of *N. ibiza* in the same clade, which was in contradiction with the morphological evidence. The suggestion was that *N. ibiza* was morphologically related to species from France and the Iberian Peninsula that also constructed a similar, cork-like trapdoor, namely, *N. hispanica*, *N. caementaria* and *N. carminans*. Notably, the samples identified as *N. caementaria* (RA177) and *N. carminans* (RA191) were included in a larger clade that also included *N. ibiza*. The distribution of cork and cogwheel trapdoors in our phylogeny suggested a close evolutionary relationship between these two trapdoor types and a complex scenario of multiple independent evolutionary origins of these traits

The morphology also anticipated an independent origin of *N. santaeulalia* and the closest relationships with Iberian and Moroccan species, which was further supported in the present study. Unfortunately, the Majorcan species *N. santaeugenia*, which is morphologically close to *N. santaeulalia*, could not be included here. Finally, as expected, *I. brauni* was the only *Iberesia* representative in the Balearic Islands to colonised the archipelago independently.

5. Conclusions

Our data confirmed the phylogenetic distinctiveness of the six species sampled (all but one) of Balearic nemesiids but also revealed a previously unknown and overlooked diversity. The sampling conducted as part of the present study led to the discovery of a new species in Minorca that was closely related to the Majorcan endemic *N. seldeni*, but with a distinctive morphology (A. Decae pers. obs.). Additionally, deep phylogeographic breaks in the mtDNA variation within some species might indicate overlooked species.

The newly discovered populations of *N. randa* in Minorca, for example, whose divergence from the Majorcan relatives was estimated to trace back to the early Pliocene, constituted a candidate for a new spaces. In the case of *I. brauni*, there was additional deep genetic structure within the Majorcan populations, one of which was more closely related to the Minorcan populations, that deserves further investigation to properly assign species boundaries. Similarly, the highly divergent haplotype identified as *N. santeulalia* could also correspond to a new species. However, additional morphological and ecological evidence will be required to further confirm the species status of these deeply coalescent mitochondrial lineages.

In conclusion, trap-door spiders arrived to the Balearic Islands at least four times independently. The colonisation was most likely accomplished via terrestrial dispersal, using the land connections between the islands and the surrounding landmasses as an advantage to establish through different geological events. Most of the species spread into the Balearic Islands from the nearby Betics region, before the two landmasses separated following the early Tortonian marine transgression. Another species, *I. brauni*, colonised the

Balearic Islands after the emergence of a land bridge during the marine regression that followed the closing of the Gibraltar strait (i.e., Messinian Salinity Crises). The phylogeographic analysis of the genetic variation revealed deep population structure among the more thoroughly sampled species, which suggested the existence of overlooked evolutionary lineages that might deserve species status. Although our analyses confirmed the monophyly of the genus *Iberesia*, the support for *Nemesia* remained low, which together with the high levels of morphological variation observed suggested a paraphyletic status. Further systematic research to assess the phylogenetic limits of *Nemesia* and for the possible subdivision of the genus into additional genera is warranted.

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Table 1. Specimens and corresponding localities for the present study

DNA	Specimen code	Genus	Specie	S	Locality	Region	Island	Country	M	X	Y	16S	28S	H3	EFG	G
H10	CRBA_AP000087	Iberesia	brauni	f	rd. Orient to Bunyola	Majorca	Balearic Islands	Spain	545	39.72712	2.73418	H10_f_I_B2	H10_f_I_B2		H10_f_I_B2	1
H104	CRBA_AP000103	Iberesia	brauni	j	Port de Soller	Majorca	Balearic Islands	Spain	39	39.80173	2.70703	H104_j_I_B2				1
H11	CRBA_AP000098	Iberesia	brauni	f	Port de Soller	Majorca	Balearic Islands	Spain	39	39.80173	2.70703	H11_f_U_B2	H11_f_U_B2	H11_f_U_B2		1
H116	CRBA_AP000104	Iberesia	brauni	j	Port de Soller	Majorca	Balearic Islands	Spain	39	39.80173	2.70703	H116_j_I_B2				1
H132	CRBA_AP000085	Iberesia	brauni	j	Santa Eugenia	Majorca	Balearic Islands	Spain	121	39.62666	2.83395	H132_j_I_B2				1
H140	CRBA_AP000056	Iberesia	brauni	j	Can Planiol	Majorca	Balearic Islands	Spain	456	39.68885	2.57752	H140_j_U_B2	H140_j_U_B2		H140_j_U_B2	1
H141	CRBA_AP000057	Iberesia	brauni	j	Can Planiol	Majorca	Balearic Islands	Spain	456	39.68885	2.57752	H141_j_U_B2				1
H146	CRBA_AP000086	Iberesia	brauni	j	Santa Eugenia	Majorca	Balearic Islands	Spain	121	39.62666	2.83395	H146_j_I_B2				1
H150	CRBA_AP000105	Iberesia	brauni	j	Port de Soller	Majorca	Balearic Islands	Spain	39	39.80173	2.70703	H150_j_I_B2				1
H152	CRBA_AP000107	Iberesia	brauni	j	Port de Soller	Majorca	Balearic Islands	Spain	39	39.80173	2.70703	H152_j_I_B2				1
H153	CRBA_AP000108	Iberesia	brauni	j	Port de Soller	Majorca	Balearic Islands	Spain	39	39.80173	2.70703	H153_j_U_B2				1
H154	CRBA_AP000109	Iberesia	brauni	j	Port de Soller	Majorca	Balearic Islands	Spain	39	39.80173	2.70703	H154_j_U_B2				1
H17	CRBA_AP000046	Iberesia	brauni	f	Can Planiol	Majorca	Balearic Islands	Spain	456	39.68885	2.57752	H17_f_I_B2				1
H19	CRBA_AP000081	Iberesia	brauni	j	Santa Eugenia	Majorca	Balearic Islands	Spain	121	39.62666	2.83395	H19_j_I_B2	H19_j_I_B2		H19_j_I_B2	1
H20	CRBA_AP000089	Iberesia	brauni	f	rd. Orient to Bunyola;	Majorca	Balearic Islands	Spain	545	39.72712	2.73418	H20_f_U_B2				1
H29	CRBA_AP000047	Iberesia	brauni	f	Can Planiol	Majorca	Balearic Islands	Spain	456	39.68885	2.57752	H29_f_I_B2				1
H31	CRBA_AP000099	Iberesia	brauni	f	Port de Soller	Majorca	Balearic Islands	Spain	39	39.80173	2.70703	H31_f_U_B2				1
H32	CRBA_AP000082	Iberesia	brauni	j	Santa Eugenia,	Majorca	Balearic Islands	Spain	121	39.62666	2.83395	H32_j_I_B2				1
H37	CRBA_AP000088	Iberesia	brauni	f	rd. Orient to Bunyola	Majorca	Balearic Islands	Spain	545	39.72712	2.73418	H37_f_U_B2				1
H42	CRBA_AP000048	Iberesia	brauni	f	Can Planiol	Majorca	Balearic Islands	Spain	456	39.68885	2.57752	H42_f_I_B2				1
H44	CRBA_AP000100	Iberesia	brauni	f	Port de Soller	Majorca	Balearic Islands	Spain	39	39.80173	2.70703	H44_f_I_B2				1
H56	CRBA_AP000083	Iberesia	brauni	j	Santa Eugenia,	Majorca	Balearic Islands	Spain	121	39.62666	2.83395	H56_j_I_B2				1
H79	CRBA_AP000084	Iberesia	brauni	j	Santa Eugenia,	Majorca	Balearic Islands	Spain	121	39.62666	2.83395	H79_j_I_B2				1
H80	CRBA_AP000101	Iberesia	brauni	f	Port de Soller	Majorca	Balearic Islands	Spain	39	39.80173	2.70703	H80_f_I_B2				1
H92	CRBA_AP000102	Iberesia	brauni	f	Port de Soller	Majorca	Balearic Islands	Spain	39	39.80173	2.70703	H92_f_I_B2				1
Z96	CRBAMM000096 - AD11	Iberesia	brauni	L	Bellevista	Majorca	Balearic Islands	Spain		39.485	2.739	Z96_L_I_B2				1
H115	CRBA_AP000073	Iberesia	brauni	f	Campanet, Vall de Fangar,	Majorca	Balearic Islands	Spain	69	39.80901	2.97931	H115_f_I_B2	H115_f_I_B2	H115_f_I_B2	H115_f_I_B2	2
H18	CRBA_AP000069	Iberesia	brauni	f	Campanet, Vall de Fangar	Majorca	Balearic Islands	Spain	69	39.80901	2.97931	H18_f_I_B2				2

DNA	Specimen code	Genus	Specie	S	Locality	Region	Island	Country	M	X	Y	16S	28S	H3	EFG	G
H41	CRBA_AP000070	Iberesia	brauni	f	Campanet, Vall de Fangar,	Majorca	Balearic Islands	Spain	69	39.80901	2.97931	H41_f_U_B2				2
H55	CRBA_AP000071	Iberesia	brauni	f	Campanet, Vall de Fangar	Majorca	Balearic Islands	Spain	69	39.80901	2.97931	H55_f_I_B2				2
H77	CRBA_AP000072	Iberesia	brauni	f	Campanet, Vall de Fangar,	Majorca	Balearic Islands	Spain	69	39.80901	2.97931	H77_f_I_B2				2
H102	CRBA_AP000078	Iberesia	brauni	j	Lloseta, rd. to Alaro	Majorca	Balearic Islands	Spain	154	39.72051	2.84633	H102_j_U_B2				3
H144	CRBA_AP000079	Iberesia	brauni	j	Lloseta, rd. to Alaro;	Majorca	Balearic Islands	Spain	154	39.72051	2.84633	H144_j_U_B2				3
H145	CRBA_AP000080	Iberesia	brauni	j	Lloseta, rd. to Alaro;	Majorca	Balearic Islands	Spain	154	39.72051	2.84633	H145_j_I_B2				3
H25	CRBA_AP000075	Iberesia	brauni	f	Lloseta, rd. to Alaro;	Majorca	Balearic Islands	Spain	154	39.72051	2.84633	H25_f_I_B2				3
H50	CRBA_AP000076	Iberesia	brauni	f	Lloseta, rd. to Alaro;	Majorca	Balearic Islands	Spain	154	39.72051	2.84633	H50_f_I_B2				3
H78	CRBA_AP000077	Iberesia	brauni	f	Lloseta, rd. to Alaro;	Majorca	Balearic Islands	Spain	154	39.72051	2.84633	H78_f_I_B2				3
H9	CRBA_AP000074	Iberesia	brauni	f	Lloseta, rd. to Alaro;	Majorca	Balearic Islands	Spain	154	39.72051	2.84633	H9_f_I_B2	H9_f_I_B2	H9_f_I_B2	H9_f_I_B2	3
H21	CRBA_AP000110	Iberesia	brauni	j	Ermita de Mont Sio,	Majorca	Balearic Islands	Spain	214	39.49533	3.0154	H21_j_I_B2				4
H43	CRBA_AP000111	Iberesia	brauni	j	Ermita de Mont Sio,	Majorca	Balearic Islands	Spain	214	39.49533	3.0154	H43_j_I_B2	H43_j_I_B2	H43_j_I_B2	H43_j_I_B2	4
H68	CRBA_AP000112	Iberesia	brauni	j	Ermita de Mont Sio,	Majorca	Balearic Islands	Spain	214	39.49533	3.0154	H68_j_U_B2				4
Z97	CRBAMM000097 - AD12	Iberesia	brauni	L	Llucmajor, Porreres	Majorca	Balearic Islands	Spain		39.51	2.97	Z97_L_I_B2				4
H122	CRBA_AP000019	Iberesia	brauni	j	Algaiarens beach,	Minorca	Balearic Islands	Spain	109	40.03588	3.91062	H122_j_I_B3				5
H125	CRBA_AP000032	Iberesia	brauni	j	Cala Torta,	Minorca	Balearic Islands	Spain	29	40.05706	4.0759	H125_j_I_B3				5
H33	CRBA_AP000003	Iberesia	brauni	f	Alcalfar, rd. to Sant Lluis,	Minorca	Balearic Islands	Spain	24	39.83172	4.29193	H33_f_U_B3				5
H36	CRBA_AP000041	Iberesia	brauni	f	Santa Agueda	Minorca	Balearic Islands	Spain	93	40.0381	4.00035	H36_f_I_B3				5
H45	CRBA_AP000011	Iberesia	brauni	f	Algaiarens beach	Minorca	Balearic Islands	Spain	109	40.03588	3.91062	H45_f_I_B3	H45_f_I_B3	H45_f_I_B3	H45_f_I_B3	5
H60	CRBA_AP000001	Iberesia	brauni	j	Alcalfar,	Minorca	Balearic Islands	Spain	24	39.83172	4.29193	H60_j_I_B3	H60_j_I_B3	H60_j_I_B3	H60_j_I_B3	5
H62	CRBA_AP000015	Iberesia	brauni	j	Algaiarens beach,	Minorca	Balearic Islands	Spain	109	40.03588	3.91062	H62_j_I_B3				5
H73	CRBA_AP000002	Ibereisa	brauni	j	Alcalfar,	Minorca	Balearic Islands	Spain	24	39.83172	4.29193	H73_j_I_B3				5
H8	CRBA_AP000014	Ibereisa	brauni	j	Algaiarens beach	Minorca	Balearic Islands	Spain	109	40.03588	3.91062	H8_j_I_B3				5
H98	CRBA_AP000016	Ibereisa	brauni	j	Algaiarens beach,	Minorca	Balearic Islands	Spain	109	40.03588	3.91062	H98_j_I_B3				5
H101	CRBA_AP000051	Nemesia	bristowei	j	Can Planiol,	Majorca	Balearic Islands	Spain	456	39.68885	2.57752	H101_j_P_B2				6
H113	CRBA_AP000052	Nemesia	bristowei	j	Can Planiol,	Majorca	Balearic Islands	Spain	456	39.68885	2.57752	H113_j_P_B2				6

DNA	Specimen code	Genus	Specie	S	Locality	Region	Island	Country	M	X	Y	16S	28S	H3	EFG	G
H128	CRBA_AP000053	Nemesia	bristowei	j	Can Planiol,	Majorca	Balearic Islands	Spain	456	39.68885	2.57752	H128_j_P_B2	H128_j_P_B2	H128_j_P_B2	H128_j_P_B2	6
H129	CRBA_AP000054	Nemesia	bristowei	j	Can Planiol,	Majorca	Balearic Islands	Spain	456	39.68885	2.57752	H129_j_P_B2				6
H139	CRBA_AP000055	Nemesia	bristowei	j	Can Planiol	Majorca	Balearic Islands	Spain	456	39.68885	2.57752	H139_j_P_B2				6
H142	CRBA_AP000058	Nemesia	bristowei	j	Can Planiol	Majorca	Balearic Islands	Spain	456	39.68885	2.57752	H142_j_P_B2				6
H149	CRBA_AP000096	Nemesia	bristowei	j	rd. Orient to Bunyola;	Majorca	Balearic Islands	Spain	545	39.72712	2.73418	H149_j_P_B2				6
H65	CRBA_AP000049	Nemesia	bristowei	j	Can Planiol,	Majorca	Balearic Islands	Spain	456	39.68885	2.57752	H65_j_P_B2				6
H107	CRBA_AP000136	Nemesia	ibiza	j	Santa Eularia des	Ibiza	Balearic Islands	Spain	172	38.94437	1.47775	H107_j_P_B1				7
H118	CRBA_AP000137	Nemesia	ibiza	j	Santa Eularia des Riu,	Ibiza	Balearic Islands	Spain	172	38.94437	1.47775	H118_j_P_B1				7
H135	CRBA_AP000138	Nemesia	ibiza	j	Santa Eularia des Riu,	Ibiza	Balearic Islands	Spain	172	38.94437	1.47775	H135_j_U_B1		H135_j_U_B1		7
H137	CRBA_AP000140	Nemesia	ibiza	j	Santa Eularia des Riu,	Ibiza	Balearic Islands	Spain	172	38.94437	1.47775	H137_j_P_B1				7
H23	CRBA_AP000129	Nemesia	ibiza	f	Cap Blanc	Ibiza	Balearic Islands	Spain	160	38.87959	1.23648	H23_f_P_B1		H23_f_P_B1		8
H49	CRBA_AP000130	Nemesia	ibiza	j	Cap Blanc,	Ibiza	Balearic Islands	Spain	160	38.87959	1.23648	H49_j_P_B1				8
H117	CRBA_AP000114	Nemesia	ibiza	f	Sa Talaia,	Ibiza	Balearic Islands	Spain	420	38.90655	1.26666	H117_f_P_B1				9
H38	CRBA_AP000115	Nemesia	ibiza	f	Sa Talaia	Ibiza	Balearic Islands	Spain	420	38.90655	1.26666	H38_f_P_B1				9
H52	CRBA_AP000116	Nemesia	ibiza	f	Sa Talaia	Ibiza	Balearic Islands	Spain	420	38.90655	1.26666	H52_f_P_B1		H52_f_P_B1	H52_f_I_B2	9
H93	CRBA_AP000117	Nemesia	ibiza	j	Sa Talaia,	Ibiza	Balearic Islands	Spain	420	38.90655	1.26666	H93_j_P_B1				9
H22	CRBA_AP000121	Nemesia	ibiza	f	Punta des Jonc,	Ibiza	Balearic Islands	Spain	51	39.07929	1.59565	H22_f_P_B1		H22_f_P_B1	H22_f_P_B1	10
H69	CRBA_AP000122	Nemesia	ibiza	f	Punta des Jonc,	Ibiza	Balearic Islands	Spain	51	39.07929	1.59565	H69_f_P_B1				10
H81	CRBA_AP000123	Nemesia	ibiza	f	Punta des Jonc	Ibiza	Balearic Islands	Spain	51	39.07929	1.59565	H81_f_P_B1				10
H94	CRBA_AP000124	Nemesia	ibiza	j	Punta des Jonc,	Ibiza	Balearic Islands	Spain	51	39.07929	1.59565	H94_j_P_B1				10
H114	CRBA_AP000065	Nemesia	randa	j	Santuari de Cura,	Majorca	Balearic Islands	Spain	471	39.42208	2.92913	H114_j_P_B2				11
H130	CRBA_AP000066	Nemesia	randa	j	Santuari de Cura,	Majorca	Balearic Islands	Spain	471	39.42208	2.92913	H130_j_P_B2				11
H131	CRBA_AP000067	Nemesia	randa	j	Santuari de Cura,	Majorca	Balearic Islands	Spain	471	39.42208	2.92913	H131_j_P_B2				11
H143	CRBA_AP000068	Nemesia	randa	j	Santuari de Cura	Majorca	Balearic Islands	Spain	471	39.42208	2.92913	H143_j_P_B2				11
H155	CRBA_AP000113	Nemesia	randa	j	Ermita de Mont Sio	Majorca	Balearic Islands	Spain	214	39.49533	3.0154	H155_j_P_B2				11
H28	CRBA_AP000060	Nemesia	randa	f	Santuari de Cura,	Majorca	Balearic Islands	Spain	471	39.42208	2.92913	H28_f_P_B2				11

DNA	Specimen code	Genus	Specie	S	Locality	Region	Island	Country	M	X	Y	16S	28S	H3	EFG	G
H40	CRBA_AP000061	Nemesia	randa	f	Santuari de Cura,	Majorca	Balearic Islands	Spain	471	39.42208	2.92913	H40_f_P_B2				11
H54	CRBA_AP000062	Nemesia	randa	f	Santuari de Cura	Majorca	Balearic Islands	Spain	471	39.42208	2.92913	H54_f_P_B2				11
H66	CRBA_AP000063	Nemesia	randa	j	Santuari de Cura,	Majorca	Balearic Islands	Spain	471	39.42208	2.92913	H66_j_P_B2	H66_j_P_B2	H66_j_P_B2	H66_j_P_B2	11
H76	CRBA_AP000064	Nemesia	randa	f	Santuari de Cura	Majorca	Balearic Islands	Spain	471	39.42208	2.92913	H76_f_P_B2				11
H90	CRBA_AP000059	Nemesia	randa	j	Santuari de Cura	Majorca	Balearic Islands	Spain	471	39.42208	2.92913	H90_j_U_B2				11
Z94	CRBAMM000094 - AD9	Nemesia	Randa	L	Castellitx de la Pau	Majorca	Balearic Islands	Spain		39.543	2.92	Z94_L_P_B2				11
Z98	CRBAMM000098 - AD13	Nemesia	Randa	L	Llucmajor and Porreres	Majorca	Balearic Islands	Spain		39.514	2.958	Z98_L_P_B2				11
E163	CRBAME001459	Nemesia	nsp.	F	Trepucó	Minorca	Balearic Islands	Spain		39.86	4.297	E163_F_P_B3	E163_F_P_B3	E163_F_P_B3	E163_F_P_B3	12
E164	CRBAME001460	Nemesia	nsp.	L	Santa Ageda	Minorca	Balearic Islands	Spain		40.032	3.99	E164_L_P_B3				12
H100	CRBA_AP000039	Nemesia	randa	j	Sant Juan,	Minorca	Balearic Islands	Spain	12	40.0145	4.12304	H100_j_P_B3				12
H109	CRBA_AP000007	Nemesia	randa	j	Favaritx,	Minorca	Balearic Islands	Spain	12	39.95349	4.22116	H109_j_P_B3				12
H110	CRBA_AP000017	Nemesia	randa	j	Algaiarens beach	Minorca	Balearic Islands	Spain	109	40.03588	3.91062	H110_j_P_B3				12
H111	CRBA_AP000027	Nemesia	randa	j	Cala Mitjana	Minorca	Balearic Islands	Spain	11	39.93589	3.97327	H111_j_P_B3				12
H112	CRBA_AP000040	Nemesia	randa	j	Sant Juan	Minorca	Balearic Islands	Spain	12	40.0145	4.12304	H112_j_P_B3				12
H121	CRBA_AP000018	Nemesia	randa	j	Algaiarens beach	Minorca	Balearic Islands	Spain	109	40.03588	3.91062	H121_j_P_B3				12
H123	CRBA_AP000020	Nemesia	randa	j	Algaiarens beach,;	Minorca	Balearic Islands	Spain	109	40.03588	3.91062	H123_j_P_B3				12
H124	CRBA_AP000028	Nemesia	randa	j	Cala Mitjana	Minorca	Balearic Islands	Spain	11	39.93589	3.97327	H124_j_U_B3				12
H126	CRBA_AP000033	Nemesia	randa	l	Cala Torta,	Minorca	Balearic Islands	Spain	29	40.05706	4.0759	H126_l_U_B3				12
H127	CRBA_AP000045	Nemesia	randa	j	Santa Agueda	Minorca	Balearic Islands	Spain	93	40.0381	4.00035	H127_j_P_B3				12
H13	CRBA_AP000008	Nemesia	randa	f	Favaritx,	Minorca	Balearic Islands	Spain	12	39.95349	4.22116	H13_f_P_B3				12
H14	CRBA_AP000022	Nemesia	randa	f	Cala Mitjana,	Minorca	Balearic Islands	Spain	11	39.93589	3.97327	H14_f_P_B3				12
H15	CRBA_AP000029	Nemesia	randa	f	Cala Torta,	Minorca	Balearic Islands	Spain	29	40.05706	4.0759	H15_f_P_B3				12
H16	CRBA_AP000035	Nemesia	Randa	f	Sant Juan,	Minorca	Balearic Islands	Spain	12	40.0145	4.12304	H16_f_P_B3				12
H34	CRBA_AP000009	Nemesia	randa	f	Favaritx	Minorca	Balearic Islands	Spain	12	39.95349	4.22116	H34_f_P_B3				12
H35	CRBA_AP000036	Nemesia	randa	f	Sant Juan;	Minorca	Balearic Islands	Spain	12	40.0145	4.12304	H35_f_P_B3				12
H46	CRBA_AP000023	Nemesia	randa	f	Cala Mitjana,;	Minorca	Balearic Islands	Spain	11	39.93589	3.97327	H46_f_P_B3				12

DNA	Specimen code	Genus	Specie	S	Locality	Region	Island	Country	M	X	Y	16S	28S	H3	EFG	G
H47	CRBA_AP000030	Nemesia	randa	j	Cala Torta	Minorca	Balearic Islands	Spain	29	40.05706	4.0759	H47_j_P_B3				12
H48	CRBA_AP000042	Nemesia	randa	j	Santa Agueda,	Minorca	Balearic Islands	Spain	93	40.0381	4.00035	H48_j_P_B3				12
H57	CRBA_AP000012	Nemesia	randa	f	Algaiarens beach, slopes	Minorca	Balearic Islands	Spain	109	40.03588	3.91062	H57_f_P_B3				12
H58	CRBA_AP000004	Nemesia	randa	j	Favaritx,	Minorca	Balearic Islands	Spain	12	39.95349	4.22116	H58_j_P_B3				12
H59	CRBA_AP000037	Nemesia	randa	f	Sant Juan,	Minorca	Balearic Islands	Spain	12	40.0145	4.12304	H59_f_P_B3				12
H61	CRBA_AP000006	Nemesia	randa n	j	Favaritx,	Minorca	Balearic Islands	Spain	12	39.95349	4.22116	H61_j_P_B3				12
H63	CRBA_AP000031	Nemesia	randa	j	Cala Torta,	Minorca	Balearic Islands	Spain	29	40.05706	4.0759	H63_j_P_B3				12
H64	CRBA_AP000043	Nemesia	randa	j	Santa Agueda	Minorca	Balearic Islands	Spain	93	40.0381	4.00035	H64_j_P_B3				12
H7	CRBA_AP000010	Nemesia	randa	f	Algaiarens beach	Minorca	Balearic Islands	Spain	109	40.03588	3.91062	H7_f_U_B3	H7_f_U_B3	H7_f_U_B3_2		12
H74	CRBA_AP000024	Nemesia	randa	j	Cala Mitjana,	Minorca	Balearic Islands	Spain	11	39.93589	3.97327	H74_j_P_B3				12
H75	CRBA_AP000034	Nemesia	randa	j	Cala Torta,	Minorca	Balearic Islands	Spain	29	40.05706	4.0759	H75_j_P_B3				12
H85	CRBA_AP000013	Nemesia	randa	j	Algaiarens beach,	Minorca	Balearic Islands	Spain	109	40.03588	3.91062	H85_j_P_B3				12
H86	CRBA_AP000025	Nemesia	randa	j	Cala Mitjana,	Minorca	Balearic Islands	Spain	11	39.93589	3.97327	H86_j_P_B3				12
H87	CRBA_AP000038	Nemesia	randa n	f	Sant Juan,	Minorca	Balearic Islands	Spain	12	40.0145	4.12304	H87_f_P_B3				12
H88	CRBA_AP000044	Nemesia	randa n	j	Santa Agueda,	Minorca	Balearic Islands	Spain	93	40.0381	4.00035	H88_j_P_B3				12
H97	CRBA_AP000005	Nemesia	randa	j	Favaritx	Minorca	Balearic Islands	Spain	12	39.95349	4.22116	H97_j_P_B3				12
H99	CRBA_AP000026	Nemesia	randa	j	Cala Mitjana,	Minorca	Balearic Islands	Spain	11	39.93589	3.97327	H99_j_P_B3				12
H70	CRBA_AP000134	Nemesia	santaeulali	j	Santa Eularia des Riu	Ibiza	Balearic Islands	Spain	172	38.94437	1.47775	H70_j_P_B1	H70_j_P_B1	H70_j_P_B1	H70_j_P_B1	13
H83	CRBA_AP000135	Nemesia	santaeulalia	j	Santa Eularia des Riu,	Ibiza	Balearic Islands	Spain	172	38.94437	1.47775	H83_j_P_B1				13
H120	CRBA_AP000150	Nemesia	santaeulalia i	j	Sant Llorenç de Balafir,.	Ibiza	Balearic Islands	Spain	134	39.03275	1.47228	H120_j_P_B1				14
H53	CRBA_AP000147	Nemesia	santaeulalia i	j	Sant Llorenç de Balafir	Ibiza	Balearic Islands	Spain	134	39.03275	1.47228	H53_j_P_B1				14
H72	CRBA_AP000148	Nemesia	santaeulalia i	j	Sant Llorenç de Balafir,	Ibiza	Balearic Islands	Spain	134	39.03275	1.47228	H72_j_P_B1				14
H96	CRBA_AP000149	Nemesia	santaeulalia i	j	Sant Llorenç de Balafir	Ibiza	Balearic Islands	Spain	134	39.03275	1.47228	H96_j_P_B1				14
H108	CRBA_AP000146	Nemesia	santaeulalia i	j	Sant Antoni, Cala Bassa;	Ibiza	Balearic Islands	Spain	13	38.96596	1.24213	H108_j_P_B1				15
H134	CRBA_AP000119	Nemesia	santaeulalia i	j	Sa Talaia,	Ibiza	Balearic Islands	Spain	420	38.90655	1.26666	H134_j_P_B1	H134_j_P_B1	H134_j_P_B1	H134_j_P_B1	15
H71	CRBA_AP000145	Nemesia	santaeulalia i	j	Sant Antoni,	Ibiza	Balearic Islands	Spain	13	38.96596	1.24213	H71_j_P_B1				15

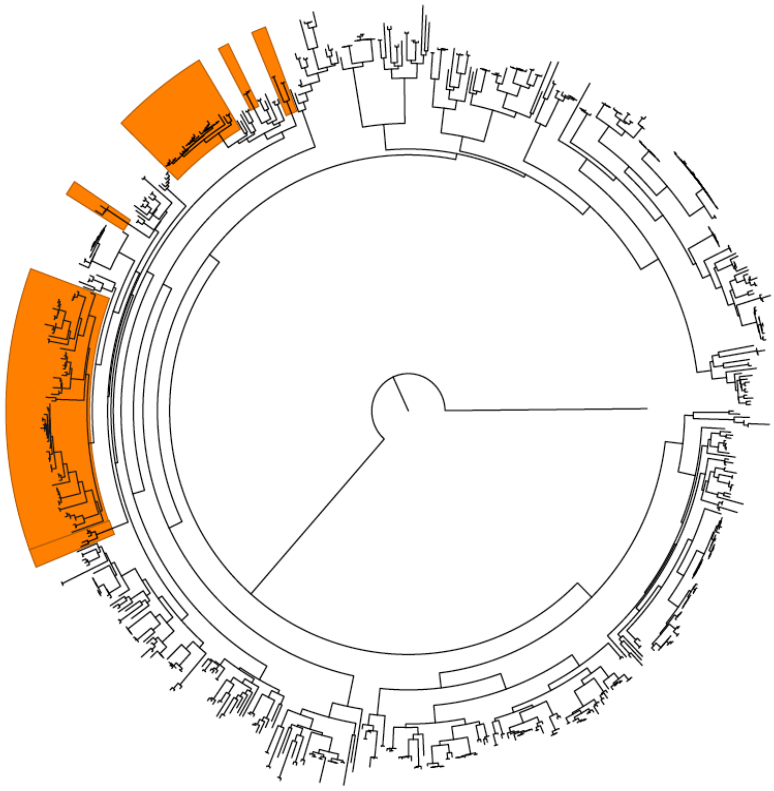
DNA	Specimen code	Genus	Specie	S	Locality	Region	Island	Country	M	X	Y	16S	28S	H3	EFG	G
H12	CRBA_AP000132	Nemesia	santaeulaliai	j	Santa Eularia des Riu	Ibiza	Balearic Islands	Spain	172	38.94437	1.47775	H12_j_P_B1	H12_j_P_B1	H12_j_P_B1	H12_j_P_B1	16
H39	CRBA_AP000133	Nemesia	santaeulaliai	j	Santa Eularia des Riu,	Ibiza	Balearic Islands	Spain	172	38.94437	1.47775	H39_j_U_B1				16
H103	CRBA_AP000092	Nemesia	seldeni	j	rd. Orient to Bunyola;	Majorca	Balearic Islands	Spain	545	39.72712	2.73418	H103_j_U_B2				17
H147	CRBA_AP000094	Nemesia	seldeni	j	rd. Orient to Bunyola;	Majorca	Balearic Islands	Spain	545	39.72712	2.73418	H147_j_H_B2	H147_j_H_B2	H147_j_H_B2	H147_j_H_B2	17
H148	CRBA_AP000095	Nemesia	seldeni .	j	rd. Orient to Bunyola	Majorca	Balearic Islands	Spain	545	39.72712	2.73418	H148_j_H_B2				17
H91	CRBA_AP000091	Nemesia	seldeni .	j	rd. Orient to Bunyola;	Majorca	Balearic Islands	Spain	545	39.72712	2.73418	H91_j_U_B2				17
E23	CRBAME000595	Nemesia	sp..	F	Foret Aitone		Corsica	France	996	42.26166	8.83781	E23_f_H_CO	E23_f_H_CO	E23_f_H_CO	E23_f_H_CO	18
DN103	CRBAME001715	Nemesia		L	Vicoforte Mondovi,			Italia		44.392902	7.81055	DN103_L_U_IT	DN103_L_U_IT			19
DN95	CRBAME001713	Nemesia		L	Rifuggio Ca Fabbri			Italy	761	43.63333	12.75	DN95_L_H_IT	DN95_L_H_IT	DN95_L_H_IT	DN95_L_H_IT	20
RA33	CRBAMM000136	Iberesia	sp	J	Cuevas de Almanzora		Andalusia	Spain	155	37.28217	-1.91695	RA33_J_I_I4	RA33_J_I_I4	RA33_J_I_I4	RA33_J_I_I4	21
ME677	CRBAME000677	Iberesia	sp.	F	Desfiladero de los gaitanes		Andalucia	Spain	290	36.89157	-4.68182	ME677_F_I_I4	ME677_F_I_I4	ME677_F_I_I4	ME677_F_I_I4	22
RA49	CRBAMM000553	Iberesia	sp	F	M.F.B. Bellota			Morocco		34.94961	-5.52892	RA49_F_I_MO	RA49_F_I_MO	RA49_F_I_MO	RA49_F_I_MO	23
RA35	CRBAMM000471	Iberesia	sp	F	Sidi Abdullah,		Al Haouz Province	Morocco	483	34.12708	-4.30482	RA35_F_I_MO	RA35_F_I_MO	RA35_F_I_MO	RA35_F_I_MO	24
RA76	CRBAMM000744	Iberesia	sp	F	Sierra do Grandola,		Alentejo	Portugal	200	38.0809	-8.53047	RA76_F_I_I3	RA76_F_I_I3	RA76_F_I_I3	RA76_F_I_I3	25
RA63	CRBAMM000623	Iberesia	Sp	J	Aguafría		Andalusoa	Spain	735	37.88387	-6.73797	RA63_J_I_I3	RA63_J_I_I3	RA63_J_I_I3	RA63_J_I_I3	26
RA80	CRBAMM000748	Iberesia	sp	J	Sierra do Grandola,		Alentejo	Portugal	200	38.0809	-8.53047	RA80_J_I_I3	RA80_J_I_I3	RA80_J_I_I3	RA80_J_I_I3	27
Z28	CRBAMM000028	Iberesia	sp.	f	,near camping Totana			Spain		37.73	1.517	Z28_f_U_I4	Z28_f_U_I4	Z28_f_U_I4	Z28_f_U_I4	28
E33	CRBAME000968	Iberesia	sp.	F	Beniarda,		Pais Valencia	Spain	607	38.68195	0.23138	E33_F_I_I4	E33_F_I_I4	E33_F_I_I4	E33_F_I_I4	29
H30	CRBA_AP000097	Nemesia	brauni	f	Inca, ermita de St agdalena	Majorca	Balearic Islands	Spain	190	39.72521	2.95399	H30_f_I_B2	H30_f_I_B2			30
RA34	CRBAMM000212	Iberesia	sp.	J	Bco. del Espostal, Granada		Andalucía	Spain		37.15	-3.53	RA34_J_I_I4	RA34_J_I_I4	RA34_J_I_I4		31
RA118	CRBAMM001069	Iberesia	sp	J	Archena,		Murcia	Spain	277	38.08524	-1.36514	RA118_J_I_I4	RA118_J_I_I4	RA118_J_I_I4	RA118_J_I_I4	32
RA130	CRBAMM000486	Iberesia	sp	J	Ain-Sfa			Morocco	567	34.82454	-2.08665	RA130_J_I_MO	RA130_J_I_MO	RA130_J_I_MO		33
RA177	CRBAME000066	Nemesia	sp	f	Llora,		Catalonia	Spain		42.03042	2.70109	RA177_f_P_I2	RA177_f_P_I2	RA177_f_P_I2	RA177_f_P_I2	34
RA191	CRBAMM000844	Nemesia	carminans	F	Fontvielle, PN des Alpilles		Provence-Alpes-Côte d'Azur	France	18	43.75919	4.69507	RA191_f_P_FR	RA191_f_P_FR	RA191_f_P_FR	RA191_f_P_FR	35
E70	CRBAME000980	Nemesia	sp.	F	Embalse Veo		Pais Valencia	Spain	347	39.93237	-0.33758	E70_F_U_I2	E70_F_U_I2	E70_F_U_I2	E70_F_U_I2	36
Z156	CRBAMM000156	Iberesia	sp	M	Punto Polacra,		Andaluc'a	Spain	41	36.84788	-2.0249	Z156_M_I_I4	Z156_M_I_I4	Z156_M_I_I4	Z156_M_I_I4	37

DNA	Specimen code	Genus	Specie	S	Locality	Region	Island	Country	M	X	Y	16S	28S	H3	EFG	G
RA84	CRBAMM001004	Nemesia	sp	F	El Torcal de Antequera,		Andalucía	Spain	1104	36.96065	-4.52693	RA84_f_P_I4	RA84_f_P_I4	RA84_f_P_I4	RA84_f_P_I4	38
H105	CRBA_AP000128	Nemesia	ibiza	j	Sa Casilla	Ibiza	Balearic Islands	Spain	97	38.95495	1.34235	H105_j_P_B1				39
H26	CRBA_AP000125	Nemesia	ibiza	j	Sa Casilla,	Ibiza	Balearic Islands	Spain	97	38.95495	1.34235	H26_j_P_B1				39
E12	CRBAME000354	Nemesia	sp	F	Tertenia		Sardinia	Italy	84	39.66598	9.57818	E12_f_P_SA	E12_f_P_SA	E12_f_P_SA	E12_f_P_SA	40
DN84	CRBAME001429	Nemesia	sp.	F	Oued Ez Zit			Tunisia	166	36.46527	10.2791	DN84_F_H_TU	DN84_F_H_TN	DN84_F_H_TU	DN84_F_H_TU	41
E115	CRBAME001093	Nemesia	sp.	F	Sot teixoneres, PN SLL M		Catalonia	Spain	819	41.68677	2.02142	E115_F_P_I2	E115_F_P_I2	E115_F_P_I2	E115_F_P_I2	42
E118	CRBAME001107	Nemesia	sp.	M	Agramunt, PN SLLM		Catalonia	Spain	547	41.68674	2.02151	E118_M_P_I2	E118_M_P_I2		E118_M_P_I2	43
RA152	CRBAMM000777	Nemesia	Manderstjr.a	F	Col de Vence		Alpes Maritimes	France	580	43.7468	7.10032	RA152_F_H_FR	RA152_F_H_FR	RA152_F_H_FR		44
Z202	CRBAMM000202	Nemesia	uncinata	m	Sra. da Graça de Padroes			Portugal		37.33944	7.57984	Z202_m_U_I3	Z202_m_U_I3	Z202_m_U_I3	Z202_m_U_I3	45
H27	CRBA_AP000144	Nemesia	santaeulalia	j	Sant Antoni, Cala Bassa	Ibiza	Balearic Islands	Spain	13	38.96596	1.24213	H27_j_P_B1	H27_j_P_B1	H27_j_P_B1	H27_j_P_B1	46
Z118	CRBAMM000118	Nemesia	Sp	M	Parc R. de Cabalanque		Murcia	Spain	88	37.6079	-0.75988	Z118_M_H_I4	Z118_M_H_I4	Z118_M_H_I4	Z118_M_H_I4	47
E77	CRBAME001026	Nemesia	sp.	F	Atzeneta		Pais Valencia	Spain	355	40.21255	-0.11158	E77_F_H_I2	E77_F_H_I2	E77_F_H_I2		48
E93	CRBAME001080	Nemesia	sp.	F	Cullera. Far		Pais Valencia	Spain	0	39.19116	-0.24337	E93_F_P_I2	E93_F_P_I2	E93_F_P_I2	E93_F_P_I2	49
H95	CRBA_AP000127	Nemesia	santaeulalia	j	Sa Casilla,	Ibiza	Balearic Islands	Spain	97	38.95495	1.34235	H95_j_P_B1	H95_j_P_B1	H95_j_P_B1		50
Z119	CRBAMM000119	Nemesia	sp	M	Sierra de Almagrera,		Murcia	Spain	28	37.32173	-1.70208	Z119_M_U_I4		Z119_M_U_I4	Z119_M_H_I4	51
E62	CRBAME000940	Nemesia	sp.	F	Cedrillas		Aragon	Spain	1359	40.43811	-0.85964	E62_F_H_I2	E62_F_H_I2	E62_F_H_I2	E62_F_H_I2	52
E75	CRBAME001014	Nemesia	sp.	F	Penya Roja		Catalonia	Spain	375	41.31501	1.15882	E75_F_H_I2	E75_F_H_I2	E75_F_H_I2	E75_F_H_I2	53
E160	CRBAME000887	Nemesia	sp.	J	Sierra de Cazorla		Andalusia	Spain	1081	37.94976	-2.94636	E160_J_H_I4	E160_J_H_I4	E160_J_H_I4		54
ME845	CRBAME000845	Nemesia	sp	F	Collado Garcia		Andalusia	Spain	1155	37.21637	-2.29718	ME845_f_H_I4	ME845_f_H_I4	ME845_f_H_I4	ME845_f_H_I4	55
RA170	CRBAMM001014	Nemesia	sp	F	Puente Genil, crtra A318, olivar		Andalusia	Spain	256	37.38147	-4.79338	RA170_f_H_I4	RA170_f_H_I4	RA170_f_H_I4	RA170_f_H_I4	56
H89	CRBA_AP000050	Nemesia	seldeni	j	Can Planiol,	Majorca	Balearic Islands	Spain	456	39.68885	2.57752	H89_j_H_B2	H89_j_H_B2	H89_j_H_B2		57
H133	CRBA_AP000093	Nemesia	seldeni	j	rd. Orient to Bunyola	Majorca	Balearic Islands	Spain	545	39.72712	2.73418	H133_j_H_B2				58
H67	CRBA_AP000090	Nemesia	seldeni	j	rd. Orient to Bunyola	Majorca	Balearic Islands	Spain	545	39.72712	2.73418	H67_j_U_B2	H67_j_U_B2	H67_j_U_B2	H67_j_U_B2	58
RA149	CRBAMM000719	Nemesia	athiasi	F	Cabo de Sao Vicente, Sagres		Algarve	Potugal	49	37.02757	8.96902	RA149_F_P_I3	RA149_F_P_I3	RA149_F_P_I3	RA149_F_P_I3	59
Z46	CRBAMM000046	Iberesia	sp.	f	La Calahurra, road		Andalusia	Spain	1723	37.13139	-3.04111	Z46_f_P_I4	Z46_f_P_I4	Z46_f_P_I4		60
DN89	CRBAME001410	Nemesia	sp.	F	Thagmerza			Tunisa	250	34.37396	7.91083	DN89_F_H_TU	DN89_F_H_TN	DN89_F_H_TU	DN89_F_H_TU	61

DNA	Specimen code	Genus	Specie	S	Locality	Region	Island	Country	M	X	Y	16S	28S	H3	EFG	G
RA171	CRBAME000027	Nemesia	sp	J	Bramiana Lake		Crete	Greece	69	35.03608	25.70334	RA171_j_H_CR	RA171_j_H_CR	RA171_j_H_CR	RA171_j_H_CR	62
RA209	CRBAME000460	Nemesia	sp	f	Crisciuleddu		Sardegna	Italy	115	41.07318	9.17543	RA209_f_H_SA	RA209_f_H_SA	RA209_f_H_SA	RA209_f_H_SA	63
RA197	CRBAME000204	Nemesia	sp	f	Garcia, near Villarosa		Sicily	Italy	401	37.60246	14.13927	RA197_f_H_SI	RA197_f_H_SI	RA197_f_H_SI	RA197_f_H_SI	64
DN133	CRBAME001395	Nemesia	sp.	F	Hbabsa			Tunisia	444	35.47567	9.34092	DN133_F_H_TU	DN133_F_H_TN	DN133_F_H_TU	DN133_F_H_TU	65
DN86	CRBAME001373	Nemesia	sp.	F	Le Kef			Tunisia	556	36.18403	8.68234	DN86_F_H_TU	DN86_F_H_TU	DN86_F_H_TU	DN86_F_H_TU	66
DN71	CRBAME001334	Nemesia	sp.	F	Ain Draham 2			Tunisia	785	36.77715	8.70288	DN71_F_H_TU	DN71_F_H_TN	DN71_F_H_TN	DN71_F_H_TN	67
DN91	CRBAME001420	Nemesia	sp.	F	Tmezret			Tunisia	446	33.53897	9.88678	DN91_F_H_TU	DN91_F_H_TN	DN91_F_H_TU	DN91_F_H_TU	68
RA192	CRBAME000094	Nemesia	sp	f	Isnello		Sicily	Italy	720	37.92115	13.96773	RA192_f_P_SI	RA192_f_P_SI	RA192_f_P_SI	RA192_f_P_SI	69
RA196	CRBAME000181	Nemesia	sp	f	Ponte Olivo		Sicily	Italy	158	37.12068	14.38138	RA196_f_H_SI	RA196_f_H_SI	RA196_f_H_SI	RA196_f_H_SI	70
RA193	CRBAME000106	Nemesia	sp	f	Cerda		Sicily	Italy	326	37.857	13.84667	RA193_f_H_SI	RA193_f_H_SI	RA193_f_H_SI	RA193_f_H_SI	71
DN167	CRBAME001267	Nemesia	sp.	F	Cap Blanc			Tunisia	2	37.33186	9.84616	DN167_F_H_TU	DN167_F_H_TN	DN167_F_H_TU	DN167_F_H_TU	72
E21	CRBAME000513	Nemesia	sp	F	Palombaggia, plage		Corsica	France	3	41.54668	9.30501	E21_f_H_CO	E21_f_H_CO	E21_f_H_CO	E21_f_H_CO	73
RA208	CRBAME000428	Nemesia	sp	f	Lu Bagnu		Sardinia	italy	32	40.89118	8.65232	RA208_f_H_CO	RA208_f_H_CO	RA208_f_H_CO	RA208_f_H_CO	74
RA202	CRBAME000291	Nemesia	sp	f	Gadoni. quercus forest		Sardinia	Italy	756	39.92644	9.18359	RA202_f_H_SA	RA202_f_H_SA	RA202_f_H_SA	RA202_f_H_SA	75
DN35	CRBAME001311	Nemesia	sp.	F	Baboucha			Tunisia	620	36.80067	8.64215	DN35_F_H_TU	DN35_F_H_TN	DN35_F_H_TU	DN35_F_H_TU	76
E16	CRBAME000374	Nemesia	sp	F	Seui		Sardinia	Italy	693	39.83321	9.34356	E16_f_H_SA	E16_f_H_SA	E16_f_H_SA		77
RA206	CRBAME000348	Nemesia	sp	f	Monte Rasu		Sardinia	Italy	896	40.43473	9.0287	RA206_f_H_SA	RA206_f_H_SA	RA206_f_H_SA	RA206_f_H_SA	78
RA201	CRBAME000271	Nemesia	sp	f	Giara di Gesturi		Sardinia	Italy	526	39.73802	9.00701	RA201_f_H_SA	RA201_f_H_SA	RA201_f_H_SA	RA201_f_H_SA	79
RA205	CRBAME000337	Nemesia	sp	f	Domus de Maria		Sardinia	Italy	111	38.94011	8.83376	RA205_f_H_SA	RA205_f_H_SA	RA205_f_H_SA	RA205_f_H_SA	80
H138	CRBA_AP000021	Nemesia	Sp.	j	Algaiarens beach	Minorca	Balearic Islands	Spain	109	40.03588	3.91062	H138_j_H_B3	H138_j_H_B3	H138_j_H_B3	H138_j_H_B3	81
E165	CRBAME001461	Nemesia	nsp.	L	Algaiarens beach	Minorca	Balearic Islands	Spain		40.05	3.912	E165_L_H_B3	E165_M_H_B3	E165_M_H_B3	E165_M_H_B3	82
E168	CRBAME001876	Nemesia	nsp.	L	Algaiarens beach ground and slopes	Minorca	Balearic Islands	Spain		40.05	3.912	E168_L_H_B3				83
E20	CRBAME000483	Nemesia	Sp	F	Cupabia, plage		Corsica	France	0	41.73851	8.78435	E20_f_H_CO	E20_f_H_CO	E20_f_H_CO	E20_f_H_CO	84
E17	CRBAME000411	Nemesia	sp	F	Cervione, Valle di campoloro		Corsica	France	338	42.32309	9.4898	E17_f_H_CO	E17_f_H_CO	E17_f_H_CO	E17_f_H_CO	85
Z33	CRBAMM000033	Brachythele	sp	f	Stomio		Thessalia	Greece		39.866667	22.733333	Z33_f_U_GR	Z33_f_U_GR	Z33_f_U_GR	Z33_f_U_GR	

Chapter 4.4

Molecular based systematic and biodiversity patterns in taxonomically challenging groups: the Tunisian trap-door spiders of the genus *Nemesia* Audouin, 1826 (Araneae, Nemesiidae)



Please, notice that dissertations do not meet the criteria of publication according to Article 8 of the “International Code of Zoological Nomenclature” (ICZN 1999), and therefore species names used in the following chapter are not considered valid under the current code of zoological nomenclature

Molecular based systematic and biodiversity patterns in taxonomically challenging groups: the Tunisian trap-door spiders of the genus *Nemesia* Audouin, 1826 (Araneae, Nemesiidae)

1.Introduction

Nowadays conservation biology is facing a major challenge: we are witnessing the major mass extinctions recorded on Earth's history, while a large amount of extant species remain undiscovered (Mora et al., 2011). Rapid population declines and extinctions have been reported (Brook et al., 2003; Randhawa et al., 2014), with present-day rates being much faster than in pre-human times (Pimm et al., 1995;) Current estimates of extinction point to approximately 3,000 species being lost each year, this is, eight species per day (Wilson, 2003; Wheeler et al., 2004; González-Oreja, 2008; Cardoso et al., 2011).Estimates on the present day number of species are biased by the methodologies used to delimit them. It has been suggested that studies based only on morphological characters tend to underestimate species diversity (Bickford et al., 2007). This bias is higher in highly diverse and understudied groups, such as the invertebrates, ‘‘the little things that run the world’’ (Evans, 1993; Cardoso et al., 2011; Wilson, 2013)

The incorporation of DNA sequences, along with other sources of information facilitates the task of taxonomists: to delimit and name evolutionary independent lineages. DNA information may reveal the existence of overlooked or cryptic lineages and make the link between different stages and sexes. This term integrative taxonomy has been coined to refer to the integration of multiple sources of evidence, namely morphological, molecular and ecological, to delimit, discover and describe new species (Padial et al., 2010; Schlick-Steiner et al., 2010; Riedel et al., 2013). Integrative taxonomy has been successfully applied to describe diversity in challenging groups with rampant convergent or conservative morphology (Bailey et al., 2010; Siler et al., 2011).

The spider suborder Mygalomorphae comprises the trap-door spiders, the tarantulas, the funnel web spiders and their kin. This suborder retains some of the characters that are considered primitive among spiders (Raven, 1985), such

as two pairs of booklungs and fangs that articulate parallel to the body axis. Despite they are not as diverse as its sister-group, the Araneomorphae or true spiders, they presently comprise more than 2,600 species and more than 300 genera (World Spider Catalog, 2015).

Mygalomorphs usually have cryptic lifestyles. Trap-door spiders, for instance, life in self-dig burrow covered by a trapdoor, usually camouflaged with leaves or other covers that makes them difficult to detect. The females have long life cycles, some studies suggest that females in captivity can live almost 20 years (Buchli, 1965), and usually sedentary. On the contrary, males have short life cycles, they leave their burrows soon after the adult moult in search for females. Males are most likely the main agents for keeping gene flow between close populations. The low vagility has largely been cited as the main reason for the restricted distributions, high level of local endemism and deep population structure reported in mygalomorphs (Bond et al., 2001; Bond & Stockman, 2008; Decae, 2012; Opatova et al., 2013).

Because of their lifestyle, narrow distributions and ecological preferences, mygalomorphs have been extensively used to address biogeographic and evolutionary questions (Pedersen & Loeschcke, 2001; Ballesteros et al., 2002; Bond, 2004, 2012; Hendrixson & Bond, 2005a, Hendrixson & Bond, 2005b; Bond & Hedin, 2006; Bond & Stockman, 2008; Bailey et al., 2010; Satler et al., 2011, 2013; Hamilton et al., 2011; Opatova et al., 2013; Hendrixson et al., 2013; Opatova & Arnedo, 2014a, 2014b). Despite of their potential as model organisms for biodiversity studies (Opatova & Arnedo, 2014b), mygalomorphs systematics remain poorly understood. The cryptic lifestyle and especially, the challenging taxonomy are among the factors that have contributed to the poor knowledge on these animals. Mygalomorph species are highly conserved morphologically. In addition the taxonomy is in very bad shape. Many species descriptions are old and are too vague and poorly illustrated to identify species, type material has been misplaced or lost and, because of their different lifestyle, many species are only known by one sex. Finally, there is a common consensus among researchers that there are a great number of species that remain to be described or even discovered. Therefore the groups offers a great opportunity to apply modern, integrative taxonomy approaches to facilitate

species delimitation, link different stages and sexes and associate identified specimens with independent genetic lineages. One possible solution that has been proposed for taxonomically challenging groups such as mygalomoprhs is the application of “turbo-taxonomy” (Riedel et al., 2013) . This term was created for an approach which included DNA barcoding with short taxonomic descriptions of morphological characters for hyperdiverse parasitic wasps (Butcher et al., 2012). In most cases, laborious traditional descriptions are not necessary to provide informative identification, which can be further supported by the use of DNA information

Nemesia Audouin, 1826 is the most diverse mygalomorph genus in the Mediterranean region, one of Earth’s biodiversity hotspots (Myers et al., 2000). The genus is especially diverse in the Western Mediterranean, but only 9 out of the 54 described species (World Spider Catalog, 2015) has been reported in northern Africa, namely *Nemesia africana* (Koch, C. L. 1838); *Nemesia barbara* (Lucas, 1846); *Nemesia cavicola* (Simon, 1889); *Nemesia didieri* Simon, 1892; *Nemesia elongata* (Simon,1883); *Nemesia dorthesi* Thorell, 1875); *Nemesia maculatipes* Ausserer, 1871; *Nemesia valenciae* Kraus, 1955 and *Nemesia vittipes* Simon, 1911. This species list is in need of revision. For instance *N. valenciae* is most likely a junior synonymy of *N. dorthesi* (Decae pers com; Mora pers. obs) and *Nemesia vittipes* probably belongs to the genus *Iberesia* (see Chapter 4.2). Like other mygalomorphs, *Nemesia* species are difficult to identify because of their overall resemblance and poor taxonomic knowledge. In addition description are frequently only available for one of the sexes. In the case of north African spercies, only 4 out of the 9 species are known from one both sexes.

To date, north African *Nemesia* species are only known from Morocco and Algeria. However, there is evidence of the presence of *Nemesia* also in Tunisia (Decae, 2012)

The aim of this study is to uncover the largely overlooked diversity of *Nemesia* trap-door spiders in Tunisia. We combined morphological, ecological and molecular evidence to delimit and identify species to overcome the still largely incomplete taxonomic knowledge of the group.

2. Material and methods

2.1 Sampling and specimen sorting

A total of 243 specimens, 2 males, 119 females and 122 juveniles, were collected by the authors in two campaigns in May and August 2012. We sampled 25 localities distributed among the main terrestrial habitats across the country (Map in Fig. 1).

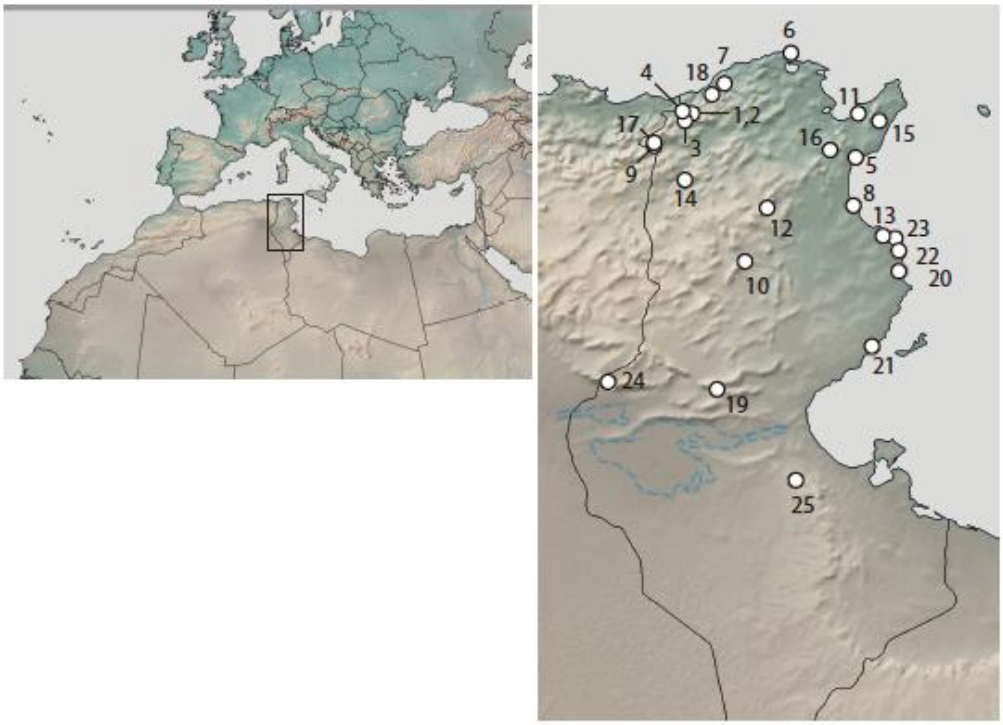


Figure 1. Map of localities sampled in this study.

Specimens were examined with a Leica MZ16A dissection microscope, equipped with a Leica DFC450 digital camera. Female vulvas were removed with the aid of needles, and the muscle tissue was digested with a 35% KOH solution before observation. One or two legs (depending on the size) were removed and directly preserved in absolute ethanol shortly after specimen collection. The legs were stored at -20°C at the freezer facilities of the Department of Animal Biology of the University of Barcelona to ensure DNA preservation. The rest of the voucher specimen was preserved in alcohol 70% for morphological studies. Following sorting of the speciemens into

morphotypes, 124 specimens were selected for further analyses. Specimen information is summarised in Table 1.

2.2 DNA extraction and sequencing

Total Genomic DNA was extracted from legs or pinned abdomens (juveniles) using the DNeasy Tissue Kit (Qiagen) following the manufacturer's protocol. Fragments of the following genes were amplified with universal primers: a mitochondrial fragment spanning the 3' half of the 16S rRNA ribosomal subunit (16S), the complete tRNA leu (L1) and the 5' half of the NADH deshydrogenase subunit I (nad1), and single fragments of the nuclear ribosomal 28S rRNA (28S), and the nuclear protein coding Histone H3 (H3) and Elongation Factor 1 gamma (Ef-1g). Primers sequences and PCR conditions are detailed in Chapter 4.1 and Chapter 4.3. PCR reactions were carried out in a final volume of 25 microL using Biotools Pfu DNA Polymerase (Biotools). The purified PCR products were sequenced at Macrogen Inc. (Seoul, South Korea). Raw sequences were assembled and edited using Geneious (v. 5.3.7) (http://www.geneious.com, Kearse et al., 2012).

DNA sequences were aligned using the online version of MAFFT v. 7 (available at http://mafft.cbrc.jp/alignment/server/, Katoh & Toh, 2008) using the Q-INS-i strategy, which takes in consideration RNA secondary structure, with offset value set to 0.1 as recommended when large gaps are not expected. The protein coding sequences were translated into amino acids to confirm that no stop codons were present. Unlike the nad1 and H3 gene fragments, the Ef-1g sequences showed evidence of indel mutations. Therefore, alignments were built using translated sequences and the MAFFT algorithm as implemented in the program TranslatorX (Abascal et al., 2010) (available at http://www.translatorx.co.uk/).

2.3 Species delimitation based on GMYC method

The Generalized Mixed Yule-coalescent (GMYC) is a single-marker, species delimitation method (Pons et al., 2006; Fujisawa & Barraclough, 2013) that provides an objective way to delimit genetic clusters. GMYC was used to define coalescent clusters based on the mtDNA information. This method combines a

model of stochastic lineage growth (Yules model) with a coalescence null model to determine the point of transition from species-level to population-level evolutionary processes. The GMYC method requires an ultrametric tree.

The computer program BEAST (Drummond & Rambaut, 2007) was used to infer an ultrameric tree for the whole *16s_nad1* data set defining a lognormal relaxed clock, and the partition scheme and models selected by partitionfinder (Lanfear et al. 2012). The ucld.mean parameter was set to 1 to infer relative lengths and and a constant population size coalescent was selected for the tree prior following the rational suggested by Monaghan (2009). Three independent runs of 5×10^7 generations were conducted remotely at the CIPRES computing facility (Miller et al, 2012 www.phylo.org). Convergence between runs and correct mixing within each run were visualized with TRACER (Rambaut et al., 2014). Individual runs were combined using LOGCOMBINER. The first 10% of the generations of each run was discarded as a burn-in. A consensus chronogram was inferred with TREEANNOTATOR.

The GMYC analysis was conducted with the R package SPLITS (Species Limits by Threshold Statistic) (Ezard et al., 2009) with single threshold with different intervals (0,5), (0,10) and (0,20) and multiple thresholds .

2.4 Allele Networks

Haplotype networks in statistical parsimony analyses provide a useful and objective method to delimit individuals into evolutionary significant units (Hart & Sunday, 2007). Allele Networks were reconstructed for the nuclear loci *28s, H3 and EF1-G* using statistical parsimony with 5% connection significance with the software TCS v1.21 (Clement et al., 2000) in search of possible geographic patterns and to detect gene flow between lineages.

2.5 Phylogenetic inference

Maximum likelihood analyses were conducted with RAxML v. 7.2.7 at the CIPRESS computing portal (Miller et al, 2012 www.phylo.org). A GTR plus GAMMA and Invariants model was assigned to each gene partition. The best tree was obtained from 100 random replicates, and the clade support was assessed with 1000 bootstrap replicates (BS).

2.6 Qualitative morphological analysis

We studied the morphological features of all the specimens included in the molecular analyses to asses if the genetic entities recovered displayed phenotypic diagnostic characters. Traditionally, *Nemesia* descriptions were based on colour and spination patterns. However, the spination pattern has been reported to be highly polymorphic within a single species, (Decae, 2005; Decae et al., 2007a). We examined different diagnostic characters that have revealed informative in *Nemesia* taxonomy (Decae 2005, Decae et al., 2007a). Morphological characters examined are listed in List 1 .

2.7 Traditional morphometric analysis

Morphometric measurements were taken on spiders using both a Leica MZ16A dissection microscope, equipped with a Leica DFC450 digital camera. Thirty-four continuos measurements were taken on spiders, measures were taken measuring the pictures with a calliper. A total of 70 females specimens were measured. Measures and their abbreviations are summarized in Fig. 2 and Table 2. GMYC lineages and morphotypes were used as factor levels in the analyses. All analyses were performed in RStudio, integrated development environment for R version 3.1.2 (2014-10-31; http://www.r-project.org/) using package prcomp/princomp and ggplot2 (Wickman, 2009, 2011)

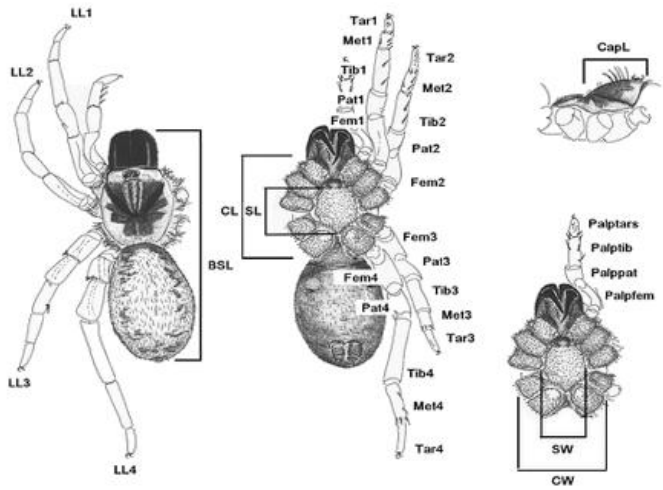


Figure 2. Continuous characters measured for the morphometric analyses (Adapted from Decae, 2005).

Prior of statistical analysis, normality of the data set was tested using the Shapiro-Wilks' W-test (Shapiro & Wilk, 1965) implemented in PAST version 2.03 (Hammer et al., 2001). When non-normally distributed, data were log_{10}-transformed and normalized before statistical analysis in order to meet the assumption of normality more closely and to exclude the biased effect of large measurements in multivariate analysis (D'Elía & Pardiñas, 2004). Principal Components Analysis (PCA) was conducted from the correlation matrix of morphometric measurements in order to compare patterns in shape variability within nemesiids populations. PCA is a data reduction technique used to simplify the sets of multidimensional data in two / three principle reduced dimensions for tracing purposes and for a better visual analysis (Manly, 2004).

PCA with Eigenvalues greater than 1 were retained, and scores for all normalized input data observations of GMYC groups and morphotypes were generated and visualized with prcomp/princomp and ggplot2 functions both implemented in R (Wickham, 2009). A subsequent Ward hierarchical cluster analysis (HCA) was performed with pvclust function implemented in R, Euclidean distance and with multiscale bootstrap (bootstrap= 10000) p values (au: approximately unbiased; bp: bootstrap probability) (Suzuki & Shimodaira, 2006). Normalized Body-shape categories were established by HCA according two cases; one with all normalized dataset and another one with mean normalized morphotypes delimited dataset. Taxonomic identity was compared to the resulting shape classes to assess whether shape classes were evolutionarily linked or not to the phylogeny.

3. Results

3.1. Species delimitation based in GMYC model

The concatenated *16s_nad1* data matrix, including 117 *Nemesia* specimens was analysed using the single-threshold option of the GMYC algorithm, which was shown not to be significantly worse than the multiple-threshold option (p = 0.9226493). The GMYC algorithm identified 29 entities (CI: 25-29) (p = $3.461025*10^{-8}$) (Fig. 3).

In most cases, the GMYC clusters corresponded to single localities (see Fig. 3). Exceptions to this pattern included instances of GMYC clusters found in more than one locality including nearby localities, specially remarkable is the case of G2 which includes 6 localities distributed along the East coast of Tunisia, some separated by approximately 130 km .

Six localities were found to include more than one GMYC, an in 5 of them the GMYC clusters were distantly related (Map in Fig. 3). For example: the closely related GMYC G25, G26 and G13 co-occurred with the distantly related G22 in locality 7, namely Cap Negre. The same pattern was found in locality 2, namely Ain Draham 2 which includes G12 and G14, that aren't closely related and locality Ain Draham 1 and Ain draham 2 were less than 0.5 km apart, that is the reason why they appear as only one point in the map (Locality 1 cointain G5). An interesting pattern is found in Iocality 14, namely, le Kef, where G6 and G7 were found, both closely related but G9, Same pattern of distantly related gmyc is found in Locality 16 that includes G1 and G20.

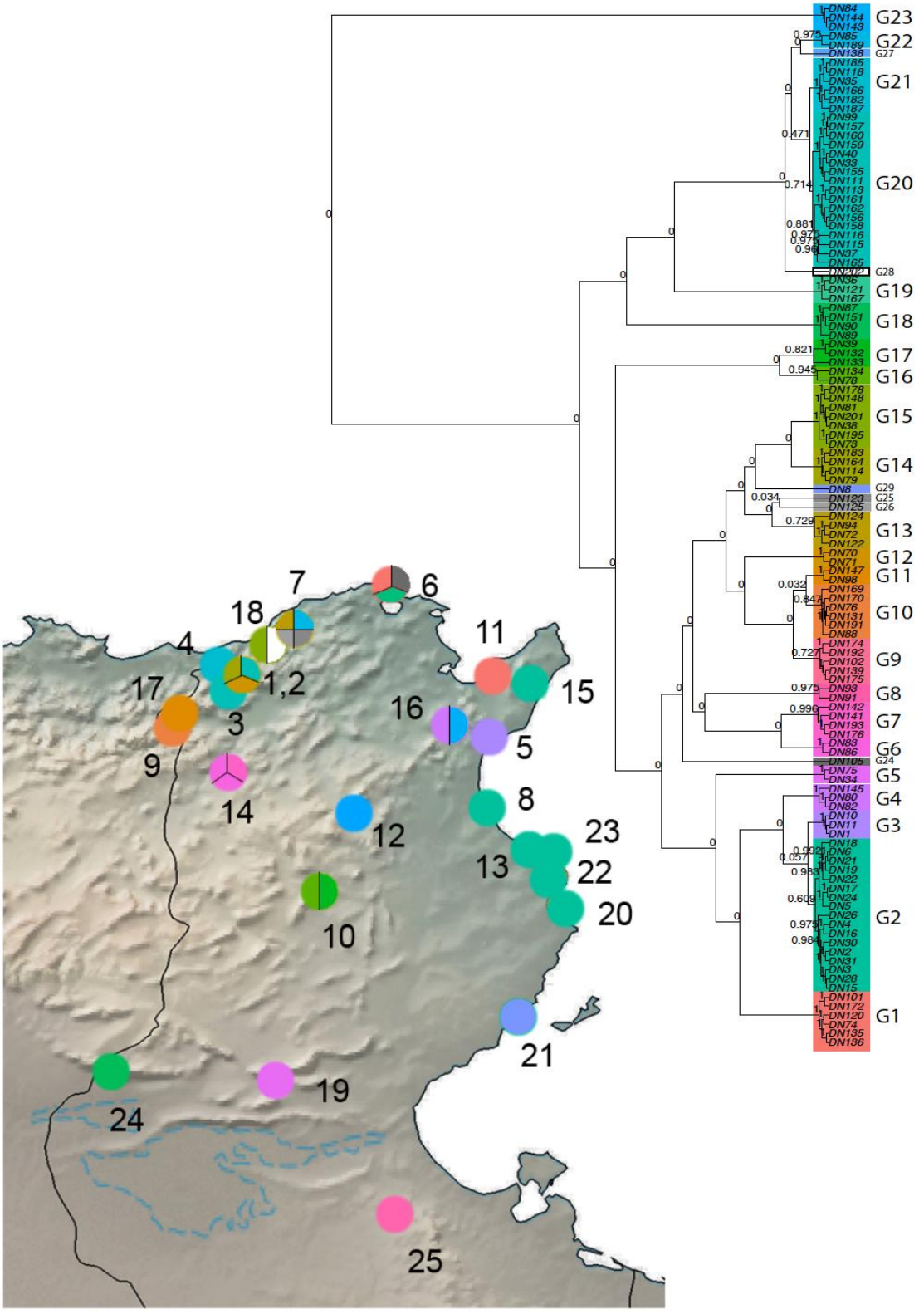


Figure 3. Results of independent GMYC clusters in the SPLITS analyses, labelled as in Table 1. Map showing localities where the respective GMYC cluster was collected. Localities are colored according GMYC clustering

3.3. Nuclear networks

The nuclear allele networks are shown in Fig. 4 (H3) and Fig. 5 (EF1-G). The 28S network had low genetic variability and showed little geographic structure.

The H3 haplotypes were resolved as 15 independent parsimony networks, showing high geographic structure. Some localities resolved as independent networks matched the mtDNA GMYC clades. Also some possible instances of gene flow were detected between GMYC entities. Those cases are highlighetd by discontinued lines. According to our results, several instances for the H3 were found. The H3 allele of specimen DN148 was not related to those found in other specimen belonging to mtDNA G15. Same pattern for the H3 alelle of DN193 not related to mt DNG7, for H3 allele of specimen DN191 which is not related with other specimens found in mtG10 and for H3 allele of DN193 which is not related to those belonging to mt G7.

Similary, two instances of incongruence were found in the EF1-G network: EF1-G allele for specimen DN81 is not related to those found in other specimens belonging to mtDNA G15, that in this case constitutes and independent network. Interestingly EF1-G allele of DN133 is not related to other mtDNA from G7, but its related with the alleles of mtDNA G6 (the sister group).

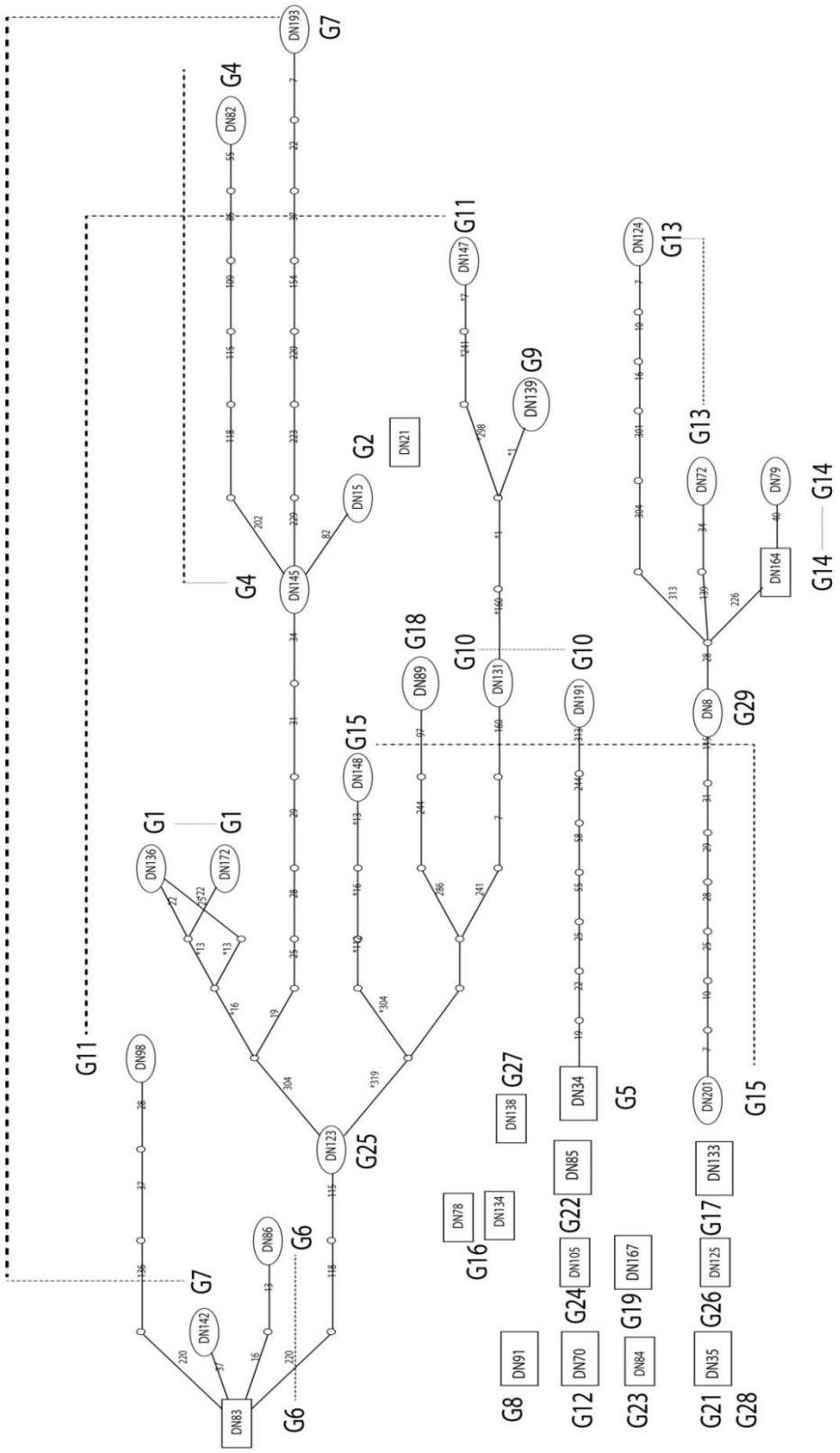


Figure 4. Haplotype parsimony network for H3 where GMYC groups are indicated. Discontinued lines indicate possible instances of gene flow.

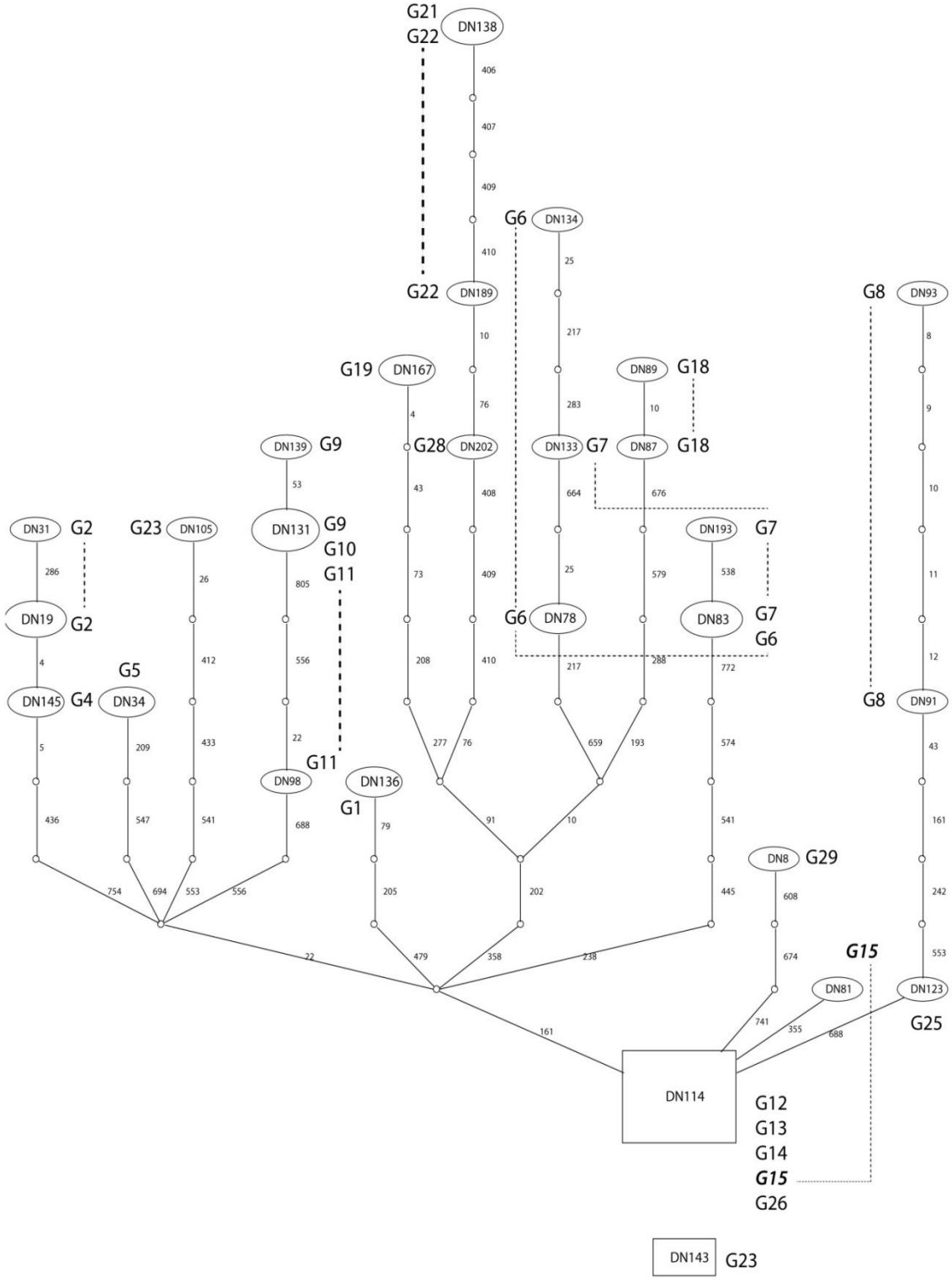


Figure 5. Haplotype network for EF-1-G. GMYC clusteting is indicated. Discontinuos lines show paterrns of gene flow.

3.3. Phylogenetic results

A concatenated matrix for 117 specimens included characters distributed as follows: 611 characters corresponding to the 16S+L1, 392 to the *nad1*, 800 to the 28S, 327 to the H3, 811 to the Ef-1g. Fig. 6 shows the results obtained of the ML analyses. Our results show the evidence of 13 different clusters, that match with the morphotypes recognized in the morphological study.

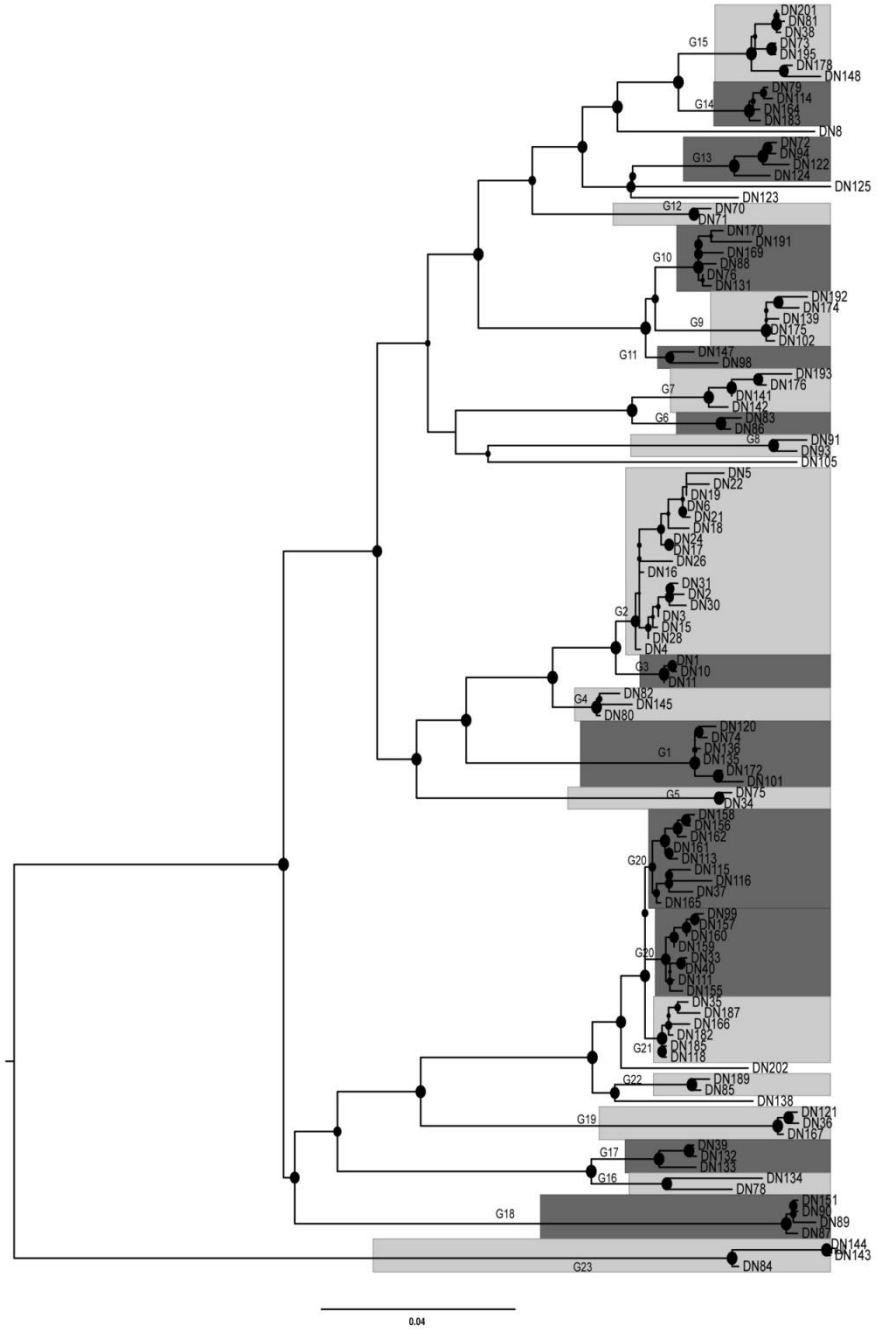


Figure 6. Phylogenetic ML tree Circles denote bootstrap support, clades are coloured according GMYC clustering (indicated at nodes).

3.4. Morphological survey

After examination of morphological diagnosable characters and exhaustive examination of genital features of all sequenced specimens, 13 morphotypes were defined according to the characters previously defined. Mapping identified adult specimens on the ultrametric tree revealed that all morphotypes formed monophyletic groups and included one or more GMYC clusters (Fig 7, 8) :

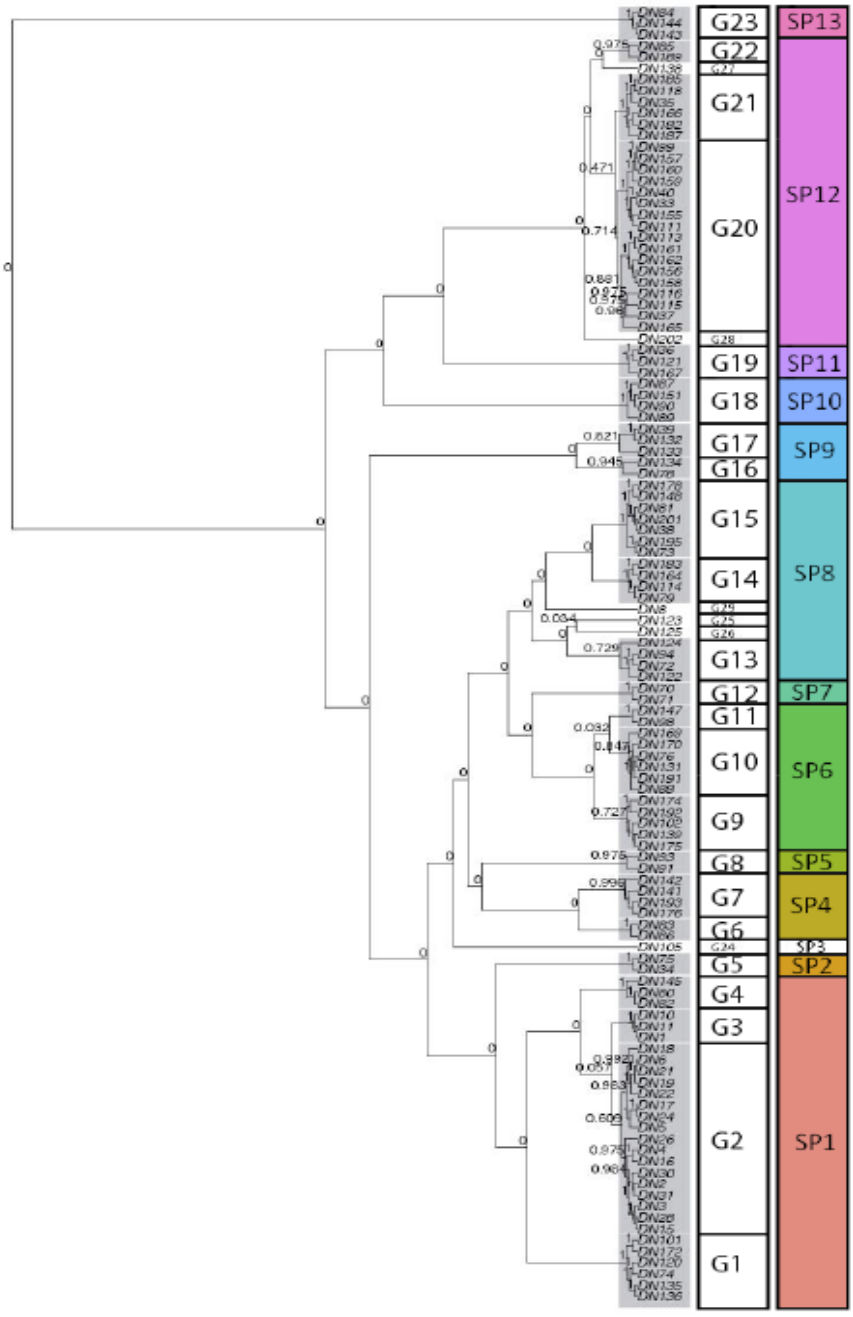


Figure 7. GMYC tree inferred with BEAST. First column indicates GMYC clusters, second column indicates morphotype associated

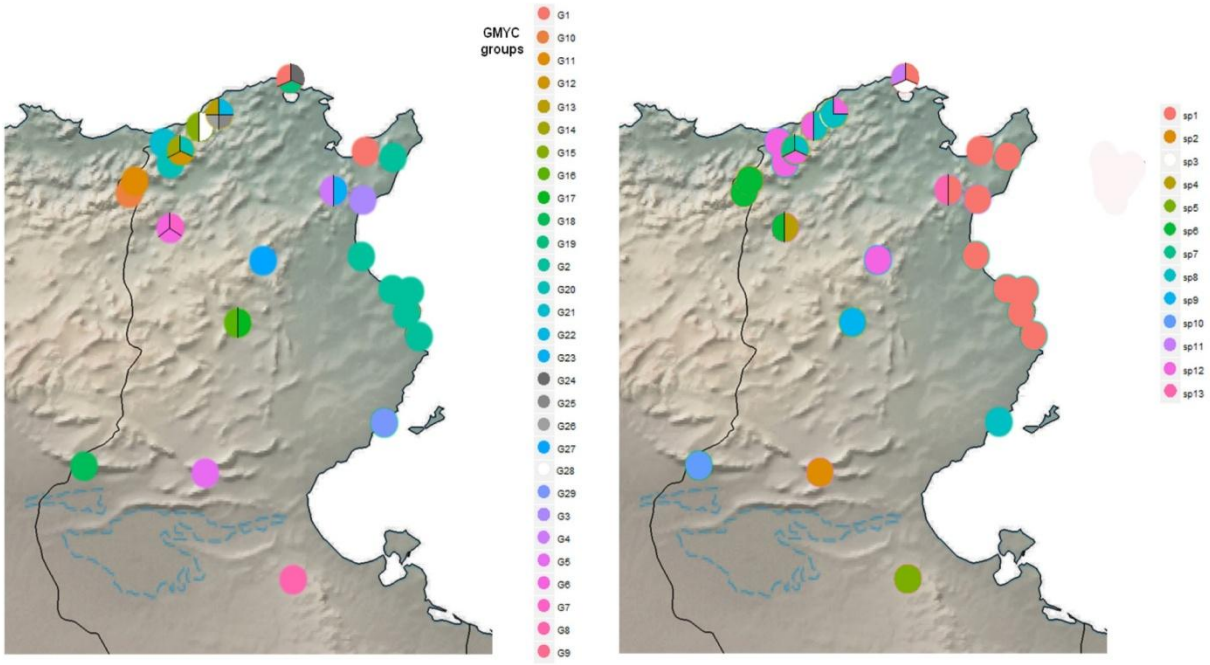


Figure 8. Geographic distribution of GMYC clustering and comparison with geographic distribution of morphotypes.

3.5. Morphometric analysis

The assignment of GMYC entities to putative species was done according to the results obtained from morphological survey (Fig.7). The specimens belonging to GMYC gropus formed only by juveniles were not included on the analyses (GMYC groups G24, G25, G26, G28). Differences in *Nemesia* shape among GMYC groups and delimited morphospecies were analyzed by PCA of size-adjusted measurements. PCA retained the two first principal components with eigenvalues larger than 1. Coefficients of the first principal component, which account 75.3% of all variations, are highly correlated, all of them with negative values (Fig. 9A). PC1 and PC2 both explain 78.29% of the point variability. PCA retained the two first principal components with eigenvalues larger than 1. PC1 and PC2 both explain 78.29% of the point variability (Fig. 9B, Table 3).

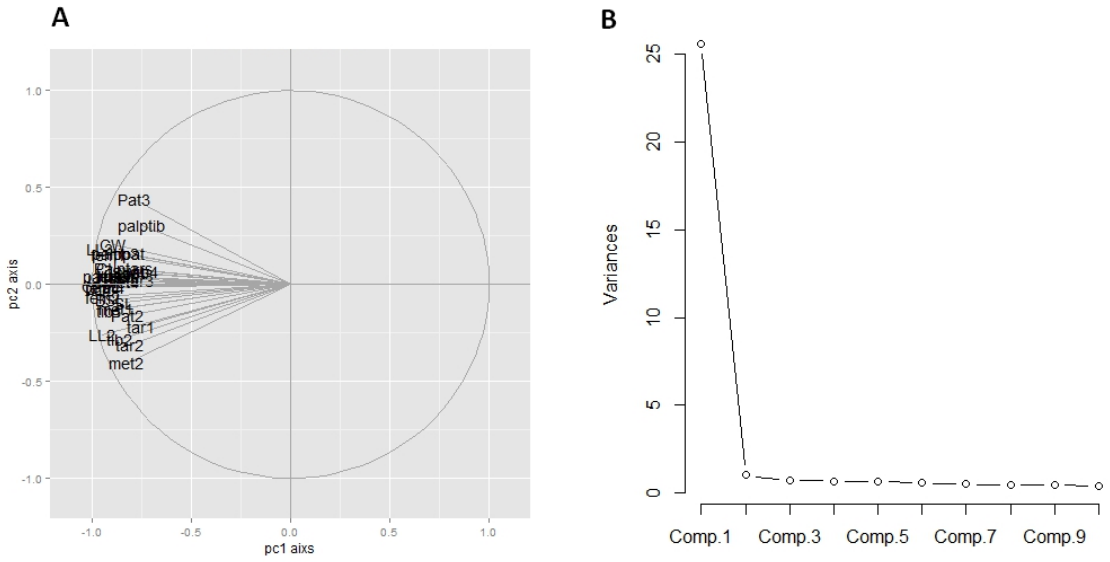


Figure 9: A**:** Circle of correlation of normalized 34 metric characters graph: the direction and length of each arrow shows quality of the correlation between variables and between variables and principal components; **B:** Plot of principal components according their variances.

	PC1	PC 2	PC 3
Eigen values	25.613	1.007	0.673
Standard deviation	5.061	1.003	0.832
Proportion of Variance %	75.3	2.96	2.04
Cumulative Proportion %	75.3	78.29	80.33

Table 3: Table of three first principal components extracted by the PCA on log-transformed morphometric variables.

The scatterplot of the two first principal components among genetic lineages and species delimitations showed that none of the two plots has clear grouping. An overlap of groups is also observed (Fig. 10). PCA failed to separate lineages and morphotypes; no morphological characters of diagnose in tunisian *Nemesia* was found.

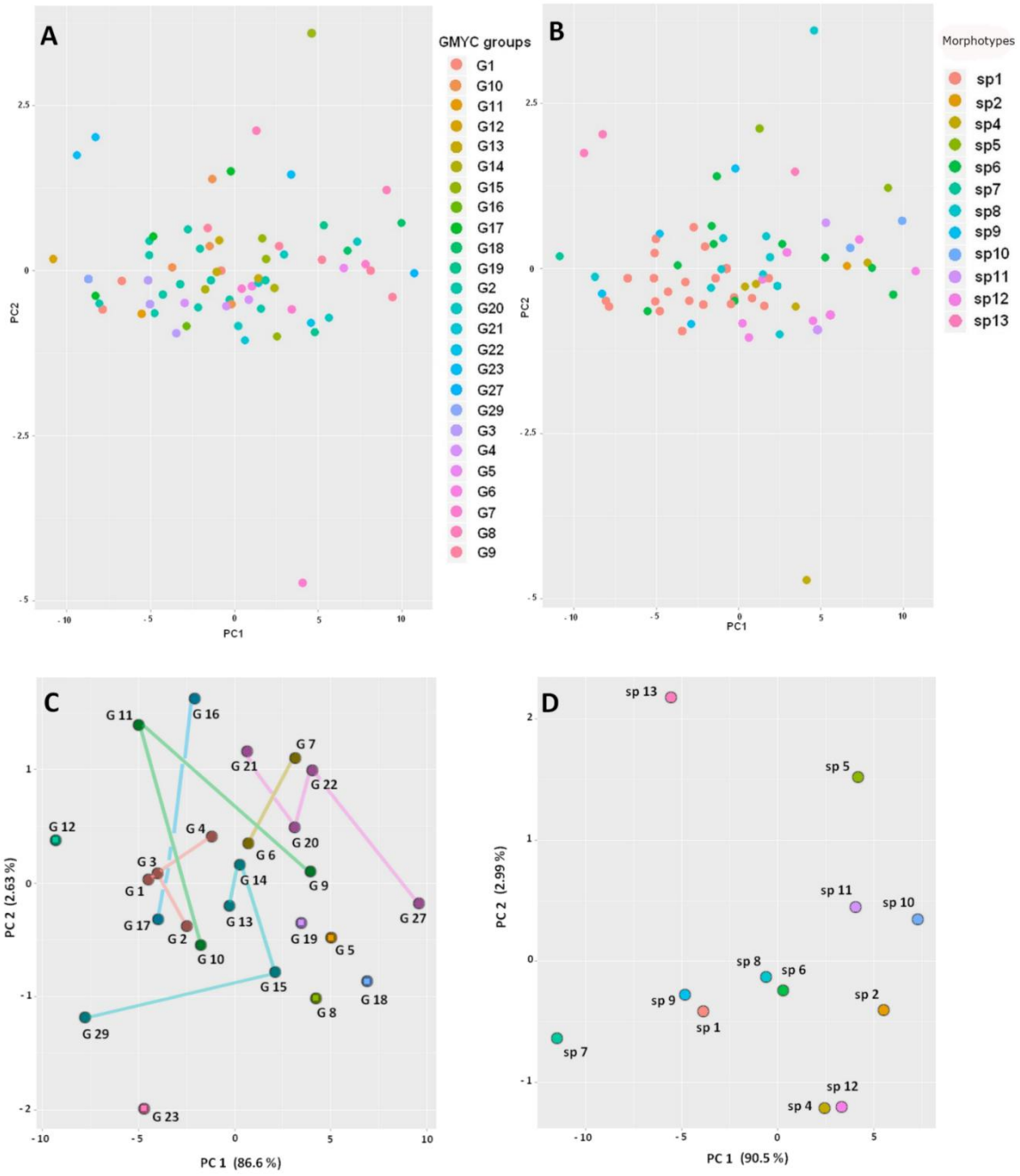


Figure 10: Plot of PCA on log-transformed morphometric variables in PC1/ PC2 space : specimen's genetic lineages (A) , morphotypes (B), means of each GMYC'sgroups (C) and means of delimited morphotypes (D) are used as factor levels

A similar pattern is observed in performed Ward HCA. Among GMYC groups, there is no clear separation (Fig. 11).

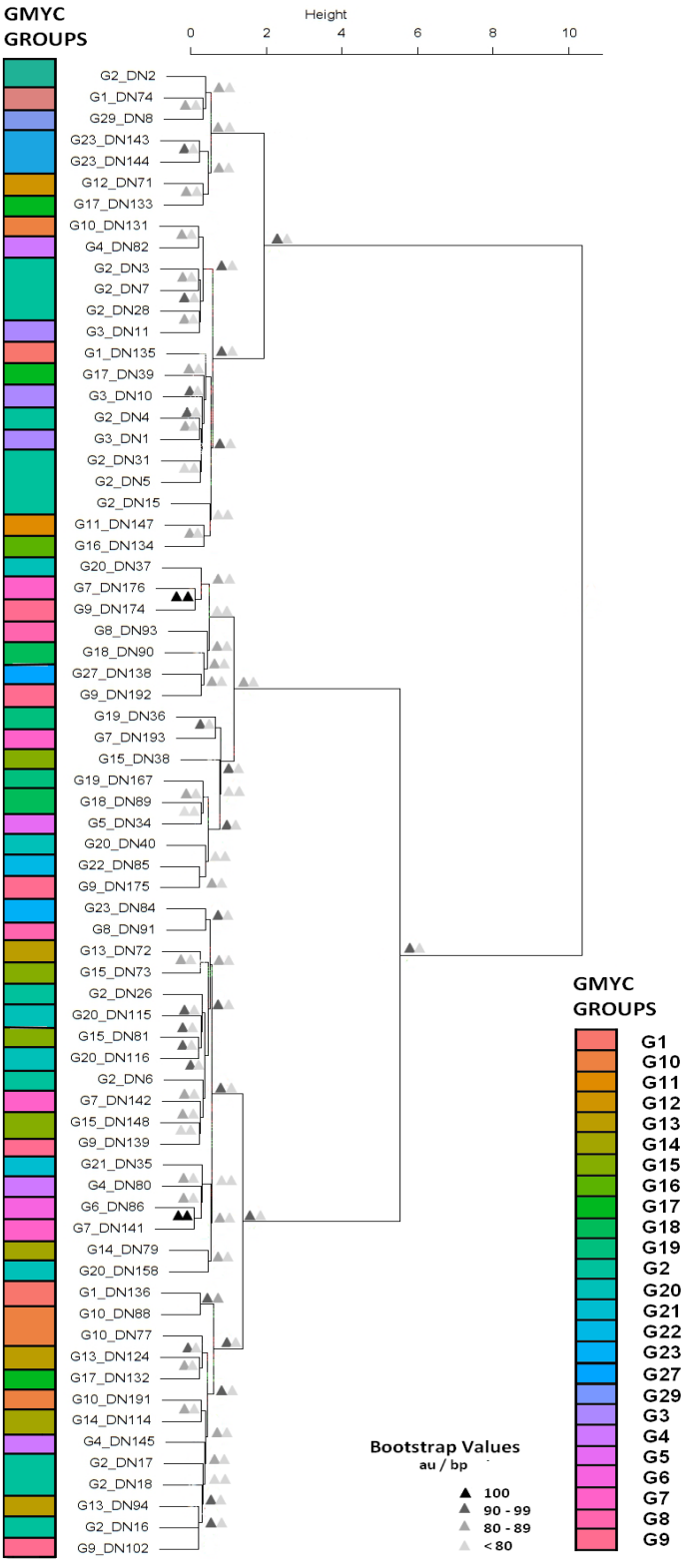


Figure 11. Ward Hierarchical Clustering (HCA) with Bootstrapped p values and Euclidean distance; Values on the edges of the clustering are *p*-values with au/bp values (%).

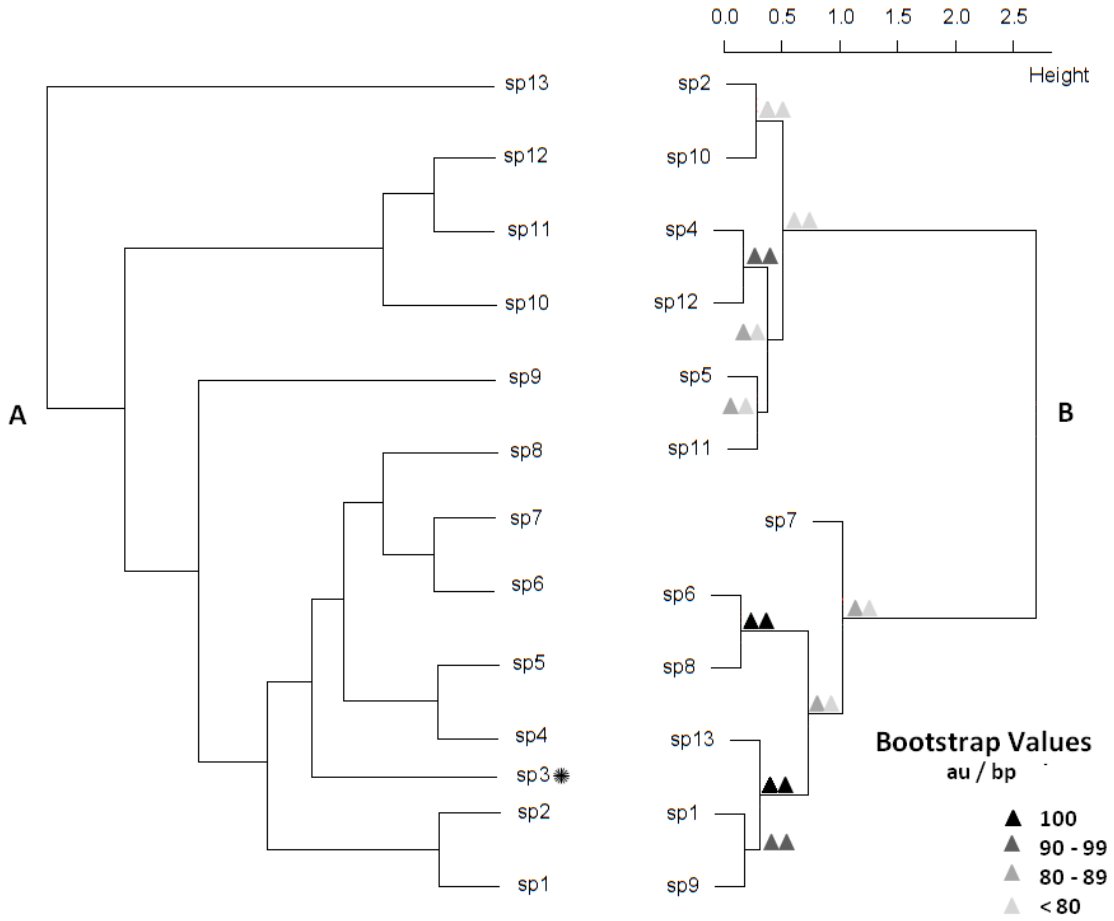


Figure 12.Phylogenetic topology of *Nemesia* species redrawn from GMYC tree(A) compared to Ward HCA dendrogram (B) for corrected mean species measurements and with au/bp values (%).***** sp3 is the only morphotype that lacks adults for the morphometric analysis.

Based on geographical distribution HCA phenogram reveals two major phenotypic patterns (Fig. 12). The first group is composed by *Nemesia* morphotypes sp1, 13, 9, 6, 8 and 7, they are characterized by having the largest total body length BSL (BSL= 22.37 - 16.54 mm) among all specie's size in this study. This group has East, Northeast, North, Northwest and center distribution, specifically along coastline areas.

The second group is composed by *Nemesia* morphs sp2, 10, 4, 12, 5 and 11. Sp12, 4 and 11 has a medium BSL (BSL= 15.30 - 13.54 mm) compared to sp2, 10 and 5 which these latter have smallest total body length (BSL= 13.20 - 12.6 mm). The whole group has a major distribution within inner Tunisia areas along mountain's borders (Fig. 13): the mountain ranges of Khroumerie, Mogods and

Nefza in North-Wester of Tunisia, tunisian ridge in Center-West and the Matmata chain in the South-East of Tunisia

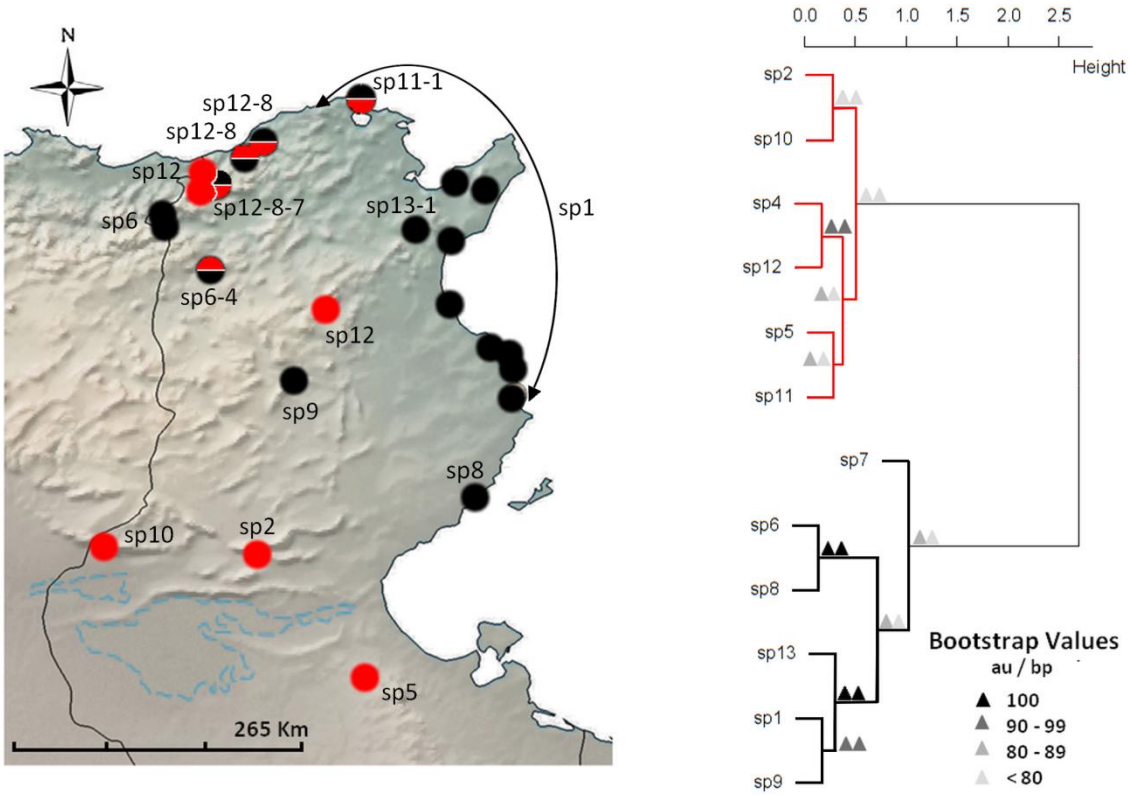


Figure 13. Ward HCA with au/bp values (%) phenogram of corrected mean species measurements correlated to geographic distribution of each species

In order to estimate pattern of species coexistence occurring in a same locality, one-way MANOVA was conducted. Results are shown on Table 4. Like we lack large sample size on each species/localities, results of the analysis were only a global appreciation of species-coexistence. Within the six localities, we carried out analysis only on four of them; “Cap Blanc” and “Road AP7to Nezka, Tabarka” hasn’t all species female correspondent.

Localities	Morphotypes composition	GMYC groups composition	MANOVA intergroup result factors that are significant
	Sp1 →	G1 (juveniles in that locality)	
Cap Blanc	Sp3 →	G24 (juveniles)	No analysis
	Sp11 →	G19 (2 females)	
	Sp8 →	G13 (3 females)	SW (F=153.297 ; p= 0.006)
		G25 (juveniles)	Palptars (F=22.020 ; p=0.043)
Cap Negre		G26 (juveniles)	Fem2 (F = 6.61e+31 ; p=<2e-16)
			Pat3 (F=22.707 ; p=0.041)
	Sp12 →	G22 (1 female)	Fem3 (F=34.787 ; p=0.028)
	Sp7 →	G12 (1 female)	
			Fem1 (F=40.120 ; p=0.024)
Ain draham 2	Sp8 →	G14 (2 females)	Tar2 (F=37.261 ; p=0.026)
			Tib3 (F=20.437 ; p=0.047)
	Sp12 →	G20 (2 females)	
	Sp8 →	G15 (4 females)	
Road AP7 to Nezka, Tabarka			No analysis
	Sp12 →	G28 (juveniles)	
	Sp4 →	G6 (1 female)	
		G7 (4 females)	All factors are not significative between species
le Kef			
	Sp6 →	G9 (5 females)	
	Sp1 →	G4 (3 females)	
Oued Ez Zit			Palppat (F=8.374 ; p=0.044)
	Sp13 →	G23 (3 females)	Tar1 (F=9.823 ; p=0.035)

Table 4: Results of geographic composition on *Nemesia* species in a same locality

Results of AMOVA show that on Cap Negre, “SW”, “Palptars”, “Fem2”, “Pat3” and “Fem3” are significant between the two morphotypes Sp8 and Sp12. While, on Ain draham Sp2, Sp7, Sp8, Sp12 differs by “Fem1”, “Tar2” and “Tib3” lengths. On Oued Ez Zit, Sp1 differs from Sp13 by “Palppat” and “Tar1” lengths. By cons, no significant differences were observed between the two morphotypes Sp4 and Sp6 in “the Kef”.

Significant characters between morphotypes differ on each locality to another, but in general on the total thirty four characters “SW”, “Palptars”, “Palppat”, Fem1,2,3”, “Tar1,2”, “Tib3” and “Pat3” seems to have subtle differences between species groups.

3.6 Taxonomy

Because of the differences in life-style, long-lived sedentary females and short-lived wandering males, direct capture of *Nemesia* specimens in burrows, as conducted in this study, usually results in either female or juvenile specimens. Ten out of the twelve species here described are only known from females

Abbreviation used in text and figures

CRBA: Centre de Recursos de Biodiversitat Animal, **BL**: body length, **CL**: carapace length, **CW:** carapace width, **Ca**: caput length, **AR:** anterior eye-width, **PR:** posterior eye-row width, **EL:** eye-formation length, **Clyp**: clypeus height, **ALE:** diameter anterior lateral eye, **PLE:** diameter posterior lateral eye, **POP:** periocular pigmentation, **PSP:** prolateral spines on patellae III, **PMS:** posterior median spinnerets, **PLS:** posterior lateral spinnerets.

Taxonomy

Family Nemesiidae

Genus *Nemesia* Audouin, 1826

Type Specie: *Nemesia cellicola*, Audouin 1826

Nemesia hipatiae sp. nov., Mora & Dimassi.

Figure 14. *Nemesia hipatiae* n.sp. From left to right: Carapace, dorsal view. Abdomen, dorsal view. Spermatheca.

Holotype. Female CRBAME00M9 (CRBA).

***Paratype*.** 3 Females. Tunisia**:** *Korbous:* Korbous: 36,77661°/10,5843901 iii 2012, leg. M. Arnedo, E. Mora, E. Planas leg. (CRBAME001260-1262).

Type Locality *Monastir:* El Frina, entrance of Monastir town near the sea, (36,728647°N, 10,820411°E)

Diagnosis

Nemesia hipathiae sp.nov. differs from any other *Nemesia* species by its brownish, oval-shaped carapace, and by a distinct dark pubescent pattern consisting in three radii projecting from the fovea on each side reaching the margin of carapace, with dense white pubescence. The spermatheca is straight, thin, corkscrew shaped, twisted once and densely covered with glandular tissue at its basal part. Resembles *Nemesia bellae* sp.nov. by the carapace pattern but differs from this species by the thin orange crest zone, and the shape of the spermatheca, which is tubular, twice twisted and slightly curved in *N. bellae* sp.nov.

Etymology

The species name honours Hypatia of Alexandria (born c. AD 350 – 370; died 415). She was a Greek mathematician, astronomer and philosopher that was murdered because of her scientific convictions. Socrates wrote about her that she was the best philosopher of her times.

Description

Female (holotype). CRBAMEM9. Measurements: BL: 19.46, CL: 9.3, CW: 4.66, AR: 1.19, PR: 1.19, EL:0.62, Cly: 0,26. Carapace twice longer than wide (Fig. 14), brownish, with distinct of dark brown pattern formed by short hairs, radiating from the fovea and extending until the limit of the carapace, contrasting with the lighter background. Caput strongly elevated and clothed with white pubescence. Crest zone extremely narrow compared to other *Nemesia* species, orange and contrasting with the colour of the carapace, bearing a single line of fine bristles. Clypeus is wide (>0.20 mm) , with few hairs. Eye-formation is almost twice longer than wide. Eye AR as long as PR. POP unbroken, PME clearly the smallest. Fovea recurved, with a longitudinal groove.

Chelicerae reddish contrasting with carapace colour. Legs same colour as carapace. Three PSP on patella III. One row of spiky cuspules along the proximal edge of the maxillae. Abdomen (Fig. 14) greyish, with symmetric pattern of purplish lines. PLS bear spigots distributed all over, PMS spigots restricted to the apical part. Spermathecae is wider at the base, corkscrew-like, single twist, covered by dense glandular tissue at proximal part.

Intraspecific variation: CL: 7.54-10.21, CL/CW= 2.01-1.82. Crest zone may vary from a narrow and thin line to a wide orange line.

Natural History

This specie constructs a burrow with two galleries, waffle trap-door, lined with thin silk.

Habitat

Found in banks on roadsides near Olive *tree* plantations.

Distribution.

Found along the North-Eastern coast of Tunisia.

Figure 15. *Nemesia montalcinae* n.sp. From left to right: Dorsal view, Spermatheca.

Holotype. Female CRBAME001408 (CRBA).

Paratype. 1 Female. **Tunisia:** *Gafsa:* 34,33729°N/9,06134° iii 2012, leg. M. Arnedo, E. Mora, E. Planas leg. (CRBAME001404).

Type Locality *Gafsa:* Road between Guettar and Bou Oumrane, 34,33729°N, 9,06134°E; elevation 341m; *Olive* and *Palm* trees

Diagnosis

Nemesia montalcinae differs from other Tunisian species by the small size of adult females, yellowish general appearance, with smooth draws radiating from the fovea due to gray pigmentations that don't reach the edge of the carapace. Shows some black patches near the spinnerets. General appearance might be similar to *N. crowfoodae* but they differ in their spermatheca, that in *N. montalcinae* is straight, tubular and slightly curved before the globular distal receptacle.

Etymology

The name of the specie honours is in apposition of Rita-Levi Montalcini, 1986 Nobel Prize in Physiology or Medicine jointly with Stanley Cohen for the discovery of nerve growth factor (NGF). She died at 103 and her work was one of the most important scientific contributions in the 20th Century.

Description. Female (holotype): CRBAME001408. Measurements (mm): BL: 13.2, CL: 6.35, CW: 3.21, AR:0.76, PR: 0.84, EL: 0.39 , Cly: 0,14. Carapace almost twice longer than wide, yellowish, with an undefined pattern radiating from the fovea by grey pigmentation. Caput is clothed by only white pubescence with a very thin crest zone. Clypeus is narrow. Eye-formation almost twice larger than wide AR/EI= 1.91; PR slightly longer than AR. ALE largest than PLE, POP unbroken, Fovea is recurved and distinctly narrowed. Chelicerae are brown contrasting with carapace. Legs yellowish, with 3 PSP spines on patella III.

The opistosoma shows an irregular pattern of dark symmetric lines at both sides of cardiac mark. Only one row of spiky cuspules is shown along the proximal edge of the maxillae. Few dark patches near to spinnerets. In the PLS the spigots are distributed along the whole spinneret, on the contrary on the PMS where spigots are restricted and densely concentrated into the apical part. The spermathecae is bipartite, long and with globular apical part, the median part of the spermatheca is specially densely covered by glandular tissue.

Natural history

The nest constructed is formed by one single gallery, a very particular trap.door: semi-circular shaped, we define this shape as half-moon shaped.

Habitat

The specimens were found near Olive and Palm trees, in an arid area.

Distribution

We found only specimens in Gafsa Province, in the center of Tunisia.

Nemesia bellae sp. nov., Mora & Dimassi

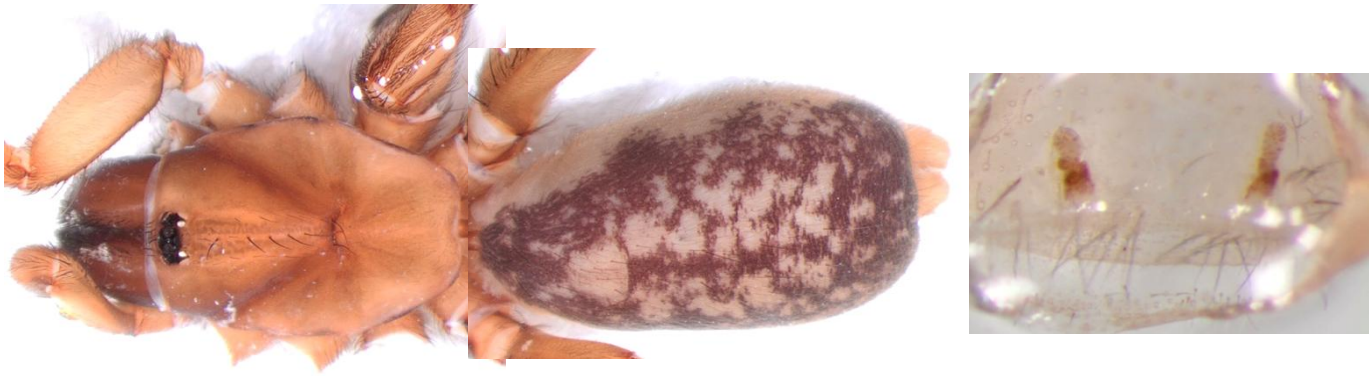


Figure 16. *Nemesia bellae* n.sp. From left to right: Carapace view. Abdomen. Spermatheca.

Holotype. Female CRBAME001372 (CRBA).

Paratype**.** 4 females. **Tunisia:** *Le Kef. Same as Holotype.* iii 2012, leg. M. Arnedo, E. Mora, E. Planas leg. (CRBAME001373, 76, 78-79, 88).

Type Locality *Le Kef:* Pinus forest. 36,18403°N, 8,68234°E

Diagnosis

Nemesia bellae resembles *N. hipatiae* in capace pattern, but differes by dense white pubescence, by the wide crest zone and by the spermatheca with corkscreew shape, globular in distal part, covereded of dense glandular tissue in the twisted zones.

Etymology

The name is in apposition to Jocellyn Bell, is a Northen Irish astrophysicist. She was the first to discover the first radio pulsars, his supervisor was awarded with the Nobel Price while she was excluded (because she was a postgraduate student) despite having been the first to observe and precisely analyse the pulsars. In March 2013 she was elected Pro-Chancellor of the University of Dublin. She was as President of the Royal Society of Edimburg in October 2014

Description. Female (holotype) CRBAME001372. Measurements (mm): BL=16.49; CL=7.73, CW=4.32, Cly=0.19, CL/CW= 1.78, AR= 1.04, PR= 1.07. Carapace almost longer than wide, rhomboid shaped (Fig. 16) orange, without any distinct pattern in the carapace. The edge is delimited by one purplish line. Caput is high and clothed with white pubescence. Crest zone is wide, orange with two well defined lines of darker pigmentation, lighter than carapace, and tappers through the fovea. Three lines of fine crest setae are present. Clypeus is narrow, hairy, short and straight. Eye-formation with AR slightly longer than PR. POP unbroken. Fovea somwheat angular.

Chelicerae are brown, contrasting with the the carapace. Legs brownish, not contrasting with the carapace, with two retrolateral spines on patella III. Abdomen is brown with an irregular pattern of light patches. Show two rows of spiky cuspules along the proximal edge of the maxillae. The PLS are densely covered by spigots, on the contrary on the PMS spigots are restricted to the apical part of the spinneret. The spermathecae (Fig.16) is tripartite, twice twisted, and globular in the apical part. Fully covereded of dense glandular tissue specially in the twisted zones.

Intraspecific variation

Specimens may present one or two rows os spiky cuspules and the spines (PSP) on patella III can vary betwwen 2 or 3.

Natural history

The characteristic kind of nest is also specific for this specie, is a construction of a small tower (from 3 to 5 cm) constructed with pinus leaves, covered by a fine trap-door.

Habitat

Specimens were found on slopes on pinus forest.

Distribution

Specimens were found only on the North Eastern of Tunisia near the city Le Kef.

Nemesia franklina sp. nov., Mora & Dimassi

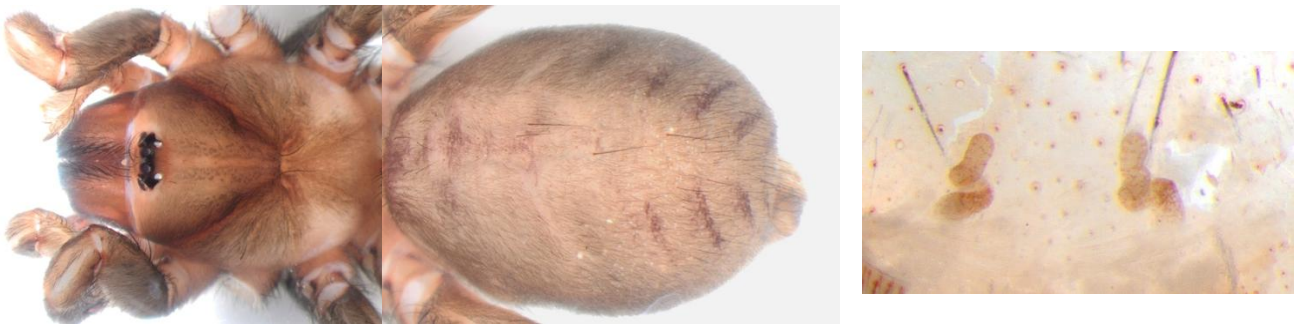


Figure 17. Nemesia franklinae. From left to right: Carapace. Abdomen. Spermatheca.

Holotype. Female CRBAME001420 (CRBA).

Paratype. Female. **Tunisia:** *Tamerzef. Same as Holotype.* iii 2012, leg. M. Arnedo, E. Mora, E. Planas leg. (CRBAME001428).

Type Locality *Tamerze*t. 33.53897 N, 9,886178 E

Diagnosis

This specie differs from other Nemesia by having the carapace densely covered by dense black hairs without any clear pattern, distributed along the whole carapace, radiating from the fovea and including the slopes of the caput (unless the crest zone).

Etymology

The name honours Rosalind Elsie Franklin, which X-Ray diffraction images lead to the discovery of the DNA double Helix. Her data and research was key in determining the structure and formulating Watson's and Crick model. Their collegues wined the Nobel price, and later one member of her research team win the Nobel Prize too. After finishing her work on DNA, with her own research team at Birkbeck College, Franklin led pioneering work on the molecular structures of viruses, including polio virus.

Description. Female (holotype): CRBAME001420. Measurements (mm): spider BL=17.11, CL=8.85, CW =5.2. Clyp=0.2; Ca=3; AR=1.25; PR=1.22; EI=0.6.

Carapace is oval-shaped much longer than wide (CL/CW = 1.70) densely covered by dark hairs radiating from the fovea including the slopes of the caput without showing any clear pattern (Fig. 17). Clypeus is wide (0.20 mm) with few bristles. The caput is high. The crest zone is well delimited by the black pubescence. A thin orange line is delimited at both sides by two lines of pigmentation, that tappers through the fovea. Only has one line of crest setae. Fovea somewhat angular. The Eye posterior row is slightly longer than anterior, AR/PR = 0.97, more than twice as wide as long, AR/EI = 1.86, POP unbroken. Arround the eyes are found lighter “cheeks” (sensu Decae 2012). The chelicerae are dark brown contrasting with the color of the carapace. Legs are brownish , densely covered by black hairs, dorsally light gray with dense pair of lines on the proximal part. Abdomen is yellowish densely covered by short dark hairs with few short and darker long hairs. Shows an irregular pattern of patches violet brownish (color in alcohol).

This specie shows only row of spiky cuspules along the proximal edge of the maxillae. The posterior lateral spinnerets are covered by spigots all over the spinneret, despite in PMS are not abundant, only in the apical part of the segment. The spermathecae (Fig. 17) is tripartite, is twice twisted in median part, with a verydensely covered by glandular tissue. The shape it seems an inverted 4.

Intraspecific variation

The row of spiky cuspules may be absent.

Natural history

This species constructs single galerries with a thin trap-door, semi-circle shaped.

Habitat

This specie was found in Tamerzet, in a desertic area.

Distribution

Northeastern Tunisia

Nemesia joliotcuriae sp. nov, Mora & Dimassi

Figure 18. *Nemesia joliocturiae.* In the upper row, from left to right: :Male: general appareance and Copulatory bulb. Dowm , female; from left to right: Female Carapace. Female Abdomen. Spermatheca.

Holotype*.* Male. CRBAME001354, iii 2012; Leg. M. Arnedo, E. Mora, E. Planas, (CRBA)

Paratypes*.* 1 Female. **Tunisia:** *Jendoua: Ghardinou: PN El Feija* (same as holotype) 36,4899°/ 8,3254303, iii 2012, leg. M. Arnedo, E. Mora, E. Planas (CRBAME001368).3 females. **Tunisia**: Le kef. 36,18403°/8,68234 iii 2012, leg. M. Arnedo, E. Mora, E. Planas coll. (CRBAME001371, 74-75.).

Type Locality: *Ghardinou:* PN El Feija (36,4899°/ 8,3254303)

Diagnosis

Males of *Nemesia joliocturiae* differ from other Nemesia species by having an extremely large and thin embolous which is more than three times larger than the bulb. *Nemesia joliocturiae females* can be distinguished by the dark pattern on the carapace which is totally covered by dark pubescence and dark pigmentations radiating from the fovea, forming irregular lobulations like a star-shaped pattern. The shape of the spermatheca is corkscrew-like, twisted more than twice and densely covered by glandular tissue, ending in a globular shape in the apical part. It might resemble *N. franklinae* due to the pubescence but is easy distinguishable by having thiner and orange crest zone, smaller eye formation and twice twisted spermatheca.

Etymology

This name honours Irène Joliot-Curie (12 September 1897 – 17 March 1956). She was awarded with her husband the Nobel Prize of Chemistry in 1935 for their discovery of artificial radioactivity. She was known for her strong convictions, and together with her husband they decided to stop working on nuclear fission because of the dangers derived form its military use. She was actively involved in actions promoting women's education at Comité nacional de l'Union des Femmes Françaises and also at the World Peace Council.

Description. Male. CRBAME001354. Measurements : BL: 13,242, CL:5.390, CW: 3.069 mm, AR:0.747 , PR:0.734 , EI:0.488 , Cly:0.155. Carapace ovale and orange, with lateral and posterior margin delimited by a line of dark hair (Fig. 18). Carapace shows a dark undefined pattern around fovea due to black pubescence, including the caput but no reaching the edges of the carapace. At the edges pubescene is replaced by a dense cover of white pubescence. Caput is steep and contrasting with carapace colour due to pigmentation and black pubescence in the slopes. Crest zone is a thin, orange and tapers towards the fovea with only one line of few creat seate. Pubescene specially dense and darker from the fovea to the pedicel. Fovea is recurved with a longitudinal groove. Clypeus narrow, with some setae. Eye-group on steep ocular tubercle. POP broken. Eye formation is wide, central and ALE are blue. AR as PR row.

Chelicerae are dark brown , contrasting with the carapace, showing two line of hairs. Legs same colour as carapace but contrasting due to the presence of dense dark hair. Presence of 2 prolateral spines on patella III.

Abdomen yellowish (Fig. 18) showing an irregular pattern of brown patches. There is a big amount of dark and dense pubescence on the cardiac mark zone, which is purplish. No row of spiky cuspules. Spinnerets are of the same color as ventral abdomen, PMS well developed holonemsia-like fully covered with spigots all over the spinneret, PLS basal segment densely covered with spigots along the spinneret specially dense in the apical part of the first segment.

The copulatory bulb of this specie is very easy to differentiate. Embolous of palpal bulb without any longitudinal ribs third times longer than the bulb and is straight , slender and elongated.

Female (paratype): CRBAME001358. Measurements (mm): BL:17.11, CL:8.85, CW: 5.2, Clyp:0.2, AR:1.08; PR:0.1.05; EI=0.58. Carapace oval-shaped much longer than wide (CL/CW = 1.70) (Fig 18) densely covered by dark hairs radiating from the fovea including the slopes of the caput forming an undefined pattern. Clypeus is wide with few bristles. The caput is high. Crest zone is well delimited by black pubescence , is thin and orange and tappers through the fovea. One line of crest setae. Fovea somewhat angular. The Eye posterior row is slightly longer than anterior row, AR/PR = 0.97, more than twice as wide as long, AR/EL = 1.86, POP unbroken. Arround the eyes are found lighter “cheeks” (sensu Decae 2012). The chelicerae are dark brown contrasting with the color of the carapace. The legs are brownish , densely covered by black hairs, dorsally light gray with dense pair of lines on the proximal part.

Abdomen is yellowish densely covered by short dark hairs with few short and darker long hairs. Shows an irregular pattern of violet patches One of spiky cuspules along the proximal edge of the maxillae.

The posterior lateral spinnerets are covered by spigots all over the spinneret, despite in PMS are not abundant, only in the apical part of the segment. The spermathecae is tripartite, is twice twisted in median part, with a very densely

covered by glandular tissue. The shape it seems an inverted 4.

Intraspecific variation.

The row of spiky cuspules may be absent in females.

Habitat.

Specimens found in a Natural park with predominance of *Quercus faginea* and *Quercus suber.*

Natural history

The nest has only one gallery, the trap-door is thin and flat, without any remarkable structures. Its frequent find some plugs inside. Plugs have been cited (Frade & Bacelar, 1932) as protection system against hymenoptera.

Distribution

Found in Northeastern Tunisia.

Figure 19. *Nemesia barresinoussae*. Upper row : male. From left to right::General appareance. Copulatory bulb. Down row: female. From left to right: Carapace. Abdomen. Sperrmatheca.

Holotype*.* 1 Male, CRBAME001333**,** iii 2012 (CRBA).

Paratypes*.* 1 Female. **Tunisia:** *Ain drahaam:* Ain drahaam L2 (same as holotype), 36,77715°/8,70288°, 03 iii 2012, leg. M. Arnedo, E. Mora, E. Planas, (CRBAME001334).

Type Locality: Ain drahaam, forest of *Cork-Oak* trees, Kroumirie mountains. (36,77715°/8,70288°)

Diagnosis

Nemesia barresimoniae is distinguishable by they general brown appareance. May ressamble *Nemesia joliocnturiae,* but differs because carapace is bigger, wider, and shows a defined pattern. Also crest zone is wider in comparision to

N. jolioncturiae which has a thin line. Males differ from *N. jolioncturiae* by having a shorter bulb, and with the tip slightly curved, females differ in their spermatheca from other *Nemesia* by being corkscreew-like, twisted three times and ending in an irregular globular receptacle as wider as the base.

Etymology

The name honours Françoise Barré-Sinoussi (30 July 1947), a French virologist and director of the Regulation of Retroviral Infections Division at Institut Pasteur in France. Her work was fundamental for the identification of the VIH as the cause of AIDS. She was awarded the Nobel Prize in Physiology or Medicine for her discoveries in 2008.

Description.Male (holotype): Measurements (mm): BL: 18,141, CL:6.208 CW: 4.670 mm, AR:0.806, PR:0.948, EL:0.377, Cly:0.278. Carapace is orange longer than wide, CL/CW=1.329 with a dark undefined pattern and densely covered densely covered by white and black pigmentation but not including the caput. Pubescene is specially dense and darker from the fovea to the pedicel. Margin is delimited by a line of dark and dense hair. Caput is steep contrasting with carapace pigmented in the slopes. Crest zone is wide, orange, well delimited by dark pigmentation contrasting with the colour of the caput, and tapers towards the fovea. One line of few crest setae flanked by two lines of few bristles and few white pubescence. Clypeus is wide with few setae. Eye-group on steep ocular tubercle POP is broken not conecing all eyes. AR/PR=0.850, posterior row smaller than anterior row. Eye formation almost twice larger than wide (AR/EL=1.92727). Fovea somewhat angular, and very deep with a longitudinal groove.

Chelicerae are brownish, not contrasting with the carapace, showing two line of hairs. Legs same colour as the carapace. Presence of 2 PSP s on patella III. Abdomen is yellowish in alcohol, showing an irregular pattern of brown patches, dense pubescence on the cardiac mark zone. Only one row of three spiky cuspules. Spinnerets are of the same colour as ventral abdomen, PMS well developed fully covered with spigots all over the spinneret, PLS basal segment densely covered with spigots along the spinneret specially dense in the apical part of the first segment.

The copulatory bulb os this specie is very easy to differentiate . Embolous of palpal bulb without any longitudinal ribs (striae) is two times longer than the bulb and is straight , slender and elongated and slightly curved at the tip.

Female (paratype): Measurements (mm), medium sized spider BL:22.37, CL:11.28, CW :7.16, AR:1.38, PR:1,38, EL:0.67, Cly: 0.29 Carapace is elliptical, longer than wide (CL/CW = 1.50), covered by dense black hairs giving general brownish appereance (Fig. 19), contrasting with the orange background. Shows a characteristic pattern of lobulations (star-shapped) iradiating from the fovea, including whole caput but never reaching margins of carapace. Clypeus is wide, clothed with black hairs and with one light central line. Crest zone is thin, and light orange contrasting with the general coloration of the carapace with one line of crest setae and two lines of fine bristles at both sides. Fovea is recurved and has a deep longitudinal groove. Eye formation is almost twice as wide as long, AR/EI = 2.059 the PR and AR are of the same length. POP unbroken. Chelicerae dark brown contrasting with carapace. Legs are brownish. Abdomen is yellowish, densely covered by fine pubescence with a violet cardiac mark, and some irregular violet paired lines forming a symmetric pattern. Shows one row of spiky cuspules along the proximal edge of the maxillae. The PLS are covered with spigots only in the basal segment, in PMS spigots are distributen all over the spinneret but are not abundant. Spermathecae corkscreew-like, twisted three times and ending in an irregular globular receptacle as wider as the base.

Natural history

This species constructs a single gallery coverede by a thin trap-door

Habitat

Ain Draham is located in Kroumirie mountains, with forest cork oak

Distribution

Northeastern Tunisia

Nemesia maricae sp. nov., Mora & Dimassi

Figure 20. *Nemesia maricae*. From left to right: Carapace. Abdomen, Spermatheca.

Holotype. 1 Female. CRBAME001336, iii 2012 (CRBA)

Paratype. 1 female. **Tunisia:** Sfax; Sfax Town (same as holotype): 34.70881/10.72155, viii 2012, leg Dimassi, (CRBAMESx1); 1 female. **Tunisia:** Ain Sebaa, Road from Nezca to Tabarca: 36.9604/ 8.940213 , iii 2012, leg. M. Arnedo, E. Mora de Checa & E. Planas (CRBAME001294)

Type Locality: **Tunisia:** *Sfax; Sfax Town*: 34.70881/10.72155

Diagnosis

Nemesia maricae differs from all the rest of tunisian *Nemesia* by having black spots in the clypeus. The eyes are steep in an ocular tubercle, despite this is not the only specie in Tunisia showing this carachter. The general apperacence of *Nemesia maricae* is brownish and dark, rather the other specie who have steep eyes is orange and very easy distinguisable. The spermatheca is very characteristic and easily distinguishable from the others being tripartite, thin, and digitiform.

Etymology

The name honours Mileva Maric, a Serbian physicist who was bright and talented and accepted at Zurich EHT at time when women were not admitted. She was honoured in 2005 by the Zurich EHT Eidgenössische Technische Hochschule.

Description.*Female* (holotype) . CRBAME001336. BL: 19.44 ; CL:9.75 CW:5.2 mm; AR:1.06 ; PR:1..12 ; EL:0.57 ; Cly:0.12.. Carapace brownish (Fig. 20) almost twice longer than wide, CL/CW=1.85, posterior and lateral edges delimited by a brown dark line. Carapace shows an undefined pattern of brown zones radiating from fovea over lighter orange background. Is covered with black and white pubescence, including lateral slopes of caput. Caput elevated. Crest zone wide, orange tapering towards the fovea. One line of few crest setae flanked by few bristles. Clypeus narrow, with few setae. Eye-group on steep ocular tubercle. POP connecting all the eyes. PR slightly longer than AR. Eye formation almost twice wide than longer. Fovea smoothly recurved, dropping away from base of caput without a central groove.

Chelicerae are darker than the carapace contrasting with the orange from the crest zone. Legs slightly lighter than carapace. Presence of 2 PSP. Abdomen is grey with complex dorsal-lateral pattern of yellow patches. Only one row of few spiky cuspules. PMS well developed fully covered with spigots all over the spinneret. PLS basal segment densely covered with spigots along the spinneret specially dense in the apical part. The spermatheca is tripartite (Fig. 20), the proximal part is short with thin glandular tissue, middle part is twisted with dense glandular tissue and the distal part is digitiform with thin of glandular tissue.

Intraspecific variation

PSP may vary between 2 or 3. Crest zone may vary between wide below the eyes and decreasing until de caput or being a straight orange line along the caput. Sometimes maculae are present on spinnerets.

Natural History

Most of the species collected constructed a burrow with two galleries and very thin trapdoors.

Habitat

Found in Cork oak forest and in reforested olive tree field.

Distribution

Along the Coast from North, near the Algerian frontier to the center of Tunisia.

Nemesia mcclintockae sp. nov, Mora & Dimassi

Figure 21. *Nemesia mcclintockae*. From left to right: Carapace. Abdomen. Spermatheca.

Holotype. 1f, CRBAME001391, iii 2012. (CRBA)

Paratypes. 2 females. **Tunisia:** Hbabsa, 35.47567/9.34092 (same as holotype), iii 2012, leg. M. Arnedo, E. Mora de Checa & E. Planas, (CRBAME0013927CRBAME001395).

Type Locality: Tunisia: Hbabsa, 35.47567; 9.34092

Diagnosis

Nemesia mcclintockae differs other *Nemesia* by the pattern in the carapace. There is a lobulated black pattern emerging from the fovea radiating along the capace, including the slopes of the caput. This pattern is defined as butterfly-like due to the similarty with the shape of some butterfly wings. The spermatheca is different from the others Tunisian specimens being tubular, thin, straight and bend towars the center. Also is very easy to distinguish by the extremely large PLS.

Etymology

The name honours Barbara Mcclintock, an american cytogeneticist who studied chromosomes and how they change during reproduction. She produced the first genetic map of maize and demonstrated the role of the telomers and centromers in the conservation of genetic information. She was recognized

among the best in the field and elected as a member of the National Academy of Science in 1944. She was awarded with the Nobel Prize in Physiology in 1983 for her discovery of the mobile genetic elements.

Description *Female* (holotype).Measurement. BL: 20.16; CL:9; CW:5.44; AR:0.98; PR:1.02; EL:0.4; Cly:0.12.Carapace yellowish (Fig. 21), longer than wide, CL/CW=1.65 , edges delimited by a brown purplish dark line. Shows a pattern of brown zones radiating from fovea over lighter yellow background strongly covered with black pubescence including slopes of caput. Caput elevated with dark brown slopes. Crest zone wide, orange tapering towards the fovea, darker and contrasting with the yellow pattern form the carapace. In the caput there is one line of few crest setae flanked by two lines of few bristles . Clypeus narrow with few setae. Eye-group on ocular tubercle. POP connecting all the eyes. AR/PR= 0.98, posterior row slightly longer than anterior row, eye formation wider than longer AR/EL=1.225, showing rectangular form. Fovea recurved, dropping away from base of caput without a central groove.

Chelicerae are brown, darker than the carapace contrasting with color from the carapace and the crest zone. Legs are yelowish, slightly lighter than carapace. Presence of 3 PSP.

The abdomen is yellowish (Fig. 21) with brown cardiac mark from the pedicel to almost half abdomen. Two rows of spiky cuspules, one of them near the maxillae with the typical disposition, and the other more than 0.5 cm from the edge of the maxilla with few cuspules. PMS well developed holonemsia-like fully covered with spigots all over the spinneret , the spigots in the apical part specially big. PLS unusually large, surpassing the abdomen, segment densely covered with spigots along the spinneret specially dense in the apical part of the first segment, with some of them differentiate and specially big. The spermatheca is unipartite, thin, double bend and towards the inner ventral part, finished with straight and digitifom shape. The middle part is covered with dense glandular tissue and the distal part is digitiform with thin glandular tissue.

Intraspecific variation.

PSP may vary between 2 or 3. The Chelicerae may have some pattern of orange colored stripes. The number of spiky cupsules may vary but always are distributed in two lines: first row with the typical disposition, the second line, not usually located with few cuspules.

Natural history. Most of the species construct a burrow with two galleries and very thin trapdoors.

Habitat

Found in a *Quercus* forest

Distribution

This specie was only found in one locality, in Hbabsa, located in the center of Tunisia which is an area surrounded by different natural parks, the closer was the Natural park Jebel Mihila.

***Nemesia crowfoodae* sp. nov.,** Mora & Dimassi.

Figure 22 . *Nemesia crowfoodae* From left to right : Carapace. Abdomen. Spermatheca.

Holotype. 1 Female CRBAME001409, iii 2012 (CRBA)

Paratypes. 1 Female. **Tunisia:** Thagmerza, 34.37396/7.91083, iii 2012; leg. M. Arnedo, E. Mora de Checa & E. Planas (CRBAME001410).

Type Locality: Thagmerza, 34.37396/7.91083

Diagnosis

Nemesia crowfoodae is easily distinguishable from the other *Nemesia* species by the small size and the general yellowish appereance. Also by the lack of any pattern, pubescence or pigmentaton in the carapace. Is distinguishable by flat caput and by the small “cheeks” at boths sides of eye formation.

Etymology

The name honours Dorothy Crowfoot (1910–1994), a biochemist that conducted research on protein chrystallography. Her work advanced the technique of X-ray crystallography used to determine the 3D structure of molecules. She became the third woman awarded with Nobel Prime after Marie Curie and Irene Joliot-Curie.

Description. Femal***e*** (holotype). Measurements: BL: 11.23, CL:5.53, CW:3.29 mm, AR:0.81, PR:0.86, EL:0.41, Cly:0.13. Carapace is yellow (Fig. 22), more than 1,5 times longer than wide, with absence of any pigmentation or pubescence, showing dark zone that delimits the slopes of the caput. Caput is flat. Crest zone wide, from the same colour as carapace , from the posterior lateral eyes, tapering towards the fovea. The crest zone shows a darker orange thin line contrasting with the yellow pattern form the carapace. One line

of crest setae and few setae in the narrow clypeus. Eye-group on ocular tubercle. POP not covering including posterior eyes. Eye posterior row slightly longer than anterior row AR/PR= 0.97. Eye formation twice wider than longer AR/EL= 2. Lighter spots next to the eyes, contrasting with the back POP. Fovea angular with longitudinal grove.

Chelicerae light orange, slightly darker than the carapace but contrasting slightly with the the crest zone. Legs yellowish, same colour than carapace, only contrasting in prolateral view being lighter. Presence of 2 PSP. Palp slighty darker than anterior legs. Abdomen grey yellowish with white cardiac mark from the pedicel to almost half abdomen. Pedicel light brown . Absence of spiky cuspules. Spinnerets with PMS well developed with spigots only in the apical part. PLS large, surpassing the abdomen, distal part covered with few spigots, specially dense in the apical part of the first segment. The spermatheca (Fig. 22) is wide in the proximal part and densely covered with glandular tissue, tapering and connecting to a digitform distal receptacle.

Intraspecific variation

PSP may vary between 1 or 2. Chellcerae can be slightly darker than the carapace.

Natural history

Most of the specimens collected in the field build a very particular nest, in sandy soil making a tower with a very particular trap-door, covered by very fine silk

Habitat

Specimens were found in palm tree forest.

Distribution

Found only in the south west of Tunisia in Tagmerza, very near to the Algerian frontier.

Nemesia noetherae sp. nov., Mora & Dimassi.

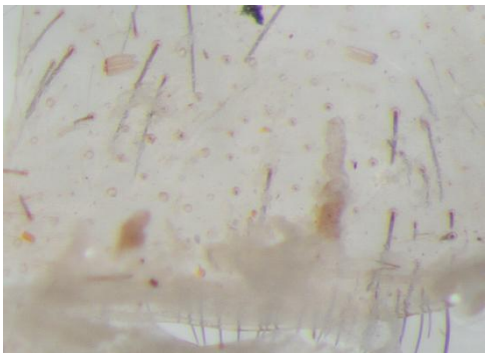


Figure 23. *Nemesia noetherae* .From left to right: General appearance. Spermatheca

Holotype. 1 Female. CRBAME001268, iii 2012 (CRBA).

Paratypes. 1 juv. **Tunisia:** Cap blanc, 37.33186/ 984616 (same as holotype), iii 2012; leg. M. Arnedo, E. Mora de Checa & E. Planas. (CRBAME001271).

Type locality: Cap blanc, North East Coast, 37.33186/ 984616

Diagnosis

Nemesia noetherae is easily distinguishable from the other *Nemesia* species by the small size and the general orange appearance. Also to the lack of pattern and pubescence make this specie very easy to recognise at first sight. Differs from *Nemesia hipatiae* by its yellowish and has no pigmentation in the carapace and no spiky cuspules, rather than *hipatiae* has 2 rows of spiky cuspules, dark pigmentation around the caput, an higher number of spines in prolateral patella III .Tthe shape of the spermatheca is thin, twisted and with non dense glandular tissue its makes no possible confusion .

Etymology

The name honours Amalie Emmy Noether, a celebrated mathematician mostly recognised by her contributions to theoric physics and abstract algebra. Her work on differential invariants in the calculus of variations, called Noether's theorem has been referred as "one of the most important mathematical

theorems ever tested among those who guide the development of modern physics".

Description. *Female* (holotype). Measurements. Bl=12.66, CL:6.56, CW:4, AR:0.909, PR:0.9, Cly:0.16. Carapace orange (Fig. 23), longer than wide; with a characteristic pattern of black pigmentation over the orange light colour radiating from fovea (hourglass-like). Caput flat, pigmented on the slopes, delimiting a orange wide crest zone. Crest zone contrasting with the colour of the carapace, tapering towards the fovea. One line of few crest setae. The clypeus is extremely narrow. Eye-group is on steep ocular tubercle. POP unbroken. Eye PR as AR. Eye formation twice wider than longer, showing rectangular form. Fovea is recurved with longitudinal grove.

Chelicerae are brownish and orange, contrasting with the carapace with some stripes orange in dorsal view. Legs are yellowish, of the same colour than carapace, only contrasting in prolateral view being lighter. Presence of 4 PSP.. Abdomen is full of bristles, grey , with white cardiac mark. Shows two rows of spiky cuspules. Spinnerets same color as ventral abdomen, PMS well developed with few spigots only in the apical part. PLS large, surpassing the abdomen, distal segment is covered with few spigots. The spermatheca is tripartite, wide and doubled bended: corkscrew-like. The proximal part is densely covered with glandular tissue.

Intraspecific variation

PSP may vary between 2 or 4. Chelicerae can show different orange patterns.

Natural History

Most of the species collected in the field build a nest with one gallery and thin trapdoor.

Distribution

From the north west of Tunisia, near the coast.

Habitat

Specimens were found in *Quercus* forest

Nemesia germainae sp. nov., Mora & Dimassi.

Figure 24. From left to right: Carapace. Abdomen. Spermatheca. .

Holotype*.* 1 Female. CRBAME001311, iii 2012 (CRBA).

Paratypes*.* 1 Female. **Tunisia:** *:* Ain Draham 36.77715/ 8.70288, iii 2012; leg. M. Arnedo, E. Mora de Checa & E. Planas (CRBAME001338). 1 Female. La Grotte de Mina; 35,94722/ 9.58096, iii 2012 , leg. M. Arnedo, E. Mora de Checa & E. Planas (CRBAME001390). 1 Female. Cap Negre 37.03957/9.08034, iii 2012, leg. M. Arnedo, E. Mora de Checa & E. Planas (CRBAME001272).

Type locality: Tunisia; Baboucha: Baboucha. 36-80067/8.64215

Diagnosis

Nemesia germanie is easily distinguishable from the other *Nemesia* species by the the presence of maculae in the legs and in the spinnerets. The carapace has a lobulated dark pattern irradiating from fovea contrasting with the orange carapace. This pattern is present in the slopes on the caput still present pigmentation at both sides of the crest zone, contrasting with it. The spermatheca is bend and twisted showing a very high dense glandular tissue in the proximal part.

Etymology

The name honours Marie Sophie Germain (april 1776 – June 1831) a French matematician, physicist and philosopher. As a woman, Germain was barred from attending the École Polytechnique. She was self-taught, masquerading as a man to enter mathematical study places. In his research and studies, he

autographed as "Mr. Leblanc," to hide her identity. Germain began sending her work to Joseph Louis Lagrange, already a faculty member who became her mentor. One of her main contributions was the Sophie Germain prime number. Because of her condition, she could not develop an academic career and worked independently during her whole life.

Description . Female (holotype). Measurements. BL=16.53, CL:6.56, CW:4, AR:1.0, PR: 1.06, EL=0,54, Cly:0.22. Carapace is brownish (Fig. 24), twice longer than wide, CL/CW=1.93; with a dark pattern of 4 lobulations, over the orange colour radiating from fovea. Dark pattern due to the dark pubescence and black pigmentation. Edge and the lateral margins delimited by a dark purplish line. Caput is flat and showing dark pigmentation at both sides of the crest zone. The clypeus is wide with few bristles. Eye-group steep on ocular tubercle. POP not including the posterior row. Eye PR slightly AR. Eye formation twice wider than longer. Fovea is recurved with alongitudinal groove .

Chelicerae contrast with the carapace with some orange stripes in dorsal view.. Presence of 3 PSP. Abdomen full of bristles, with no defined pattern. Only shows one row of three spiky cuspules. PMS well developed holonemsia-like with spigots distributed all over the spinneret. PLS fully covered with spigots all over the spinneret. The spermatheca is tripartite, same wide proximal than distal part, and double bended.

Intraspecific variation

PSP may vary between 1 to 3 . The Chelicerae can show different orange patterns and being darker or showing the same colour of the carapace.

Natural history

Most of the species collected in the field build a nest with one two galleries and thin trapdoor.

Habitat

Specimens were found in Quercus forests.

Distribution

Distributed along the north of the country following the coast line.

Nemesia meitneraesp. nov., Mora & Dimassi

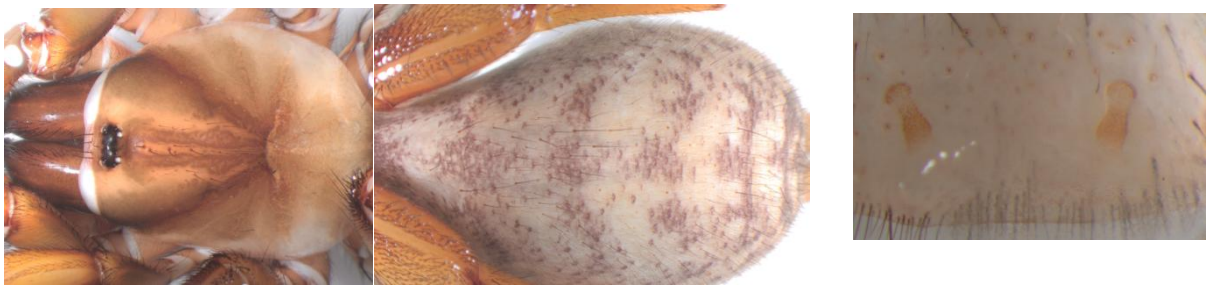


Figure 25. From left to right: Carapace. Abdoomen. Spermatheca.

Holotype; 1 Female. CRBAME001434, iii 2012 (CRBA)

Paratypes*.* 2 Females. **Tunisia:** Hammamet**:** Oued Ez Zid, 36.46527/10.2791; iii 2012; leg. M. Arnedo, E. Mora de Checa & E. Planas (CRBAME001429/CRBAME001434)

Type locality: **Tunisia***:* Oued Ez Zid, 36.46527/10.2791

Diagnosis

Nemesia meitnerae is easily distinguishable from the other Tunisian species by being big sized *Nemesia* showing strong reduction of the PMS. The spermatheca is bipartite, similar to species western Mediterranean, but differs by POP unbroken, the absence of pubescence on carapace and by grey pigmentation in carapace.

Etymology

The name honours Lise Meitner, an Austrian physicist who investigated radioactivity and nuclear fission. She introduced the concept of nuclear fission was part of the team who discovered it. Otto Hahn, her colleague, received the Nobel Price for the discovery, but she was omitted.

Description. *Female* (holotype) . Measurements (mm): BL=23, CL:11.69, CW:7.44, AR:1.28, PR: 1.27, EL=0.63 Cly:0.26. Big -sized spider. Carapace , yellowish (Fig. 25), is 1.5 times fold longer than wide, CL/CW=1.5. Shows with

a grey pigmentation pattern radiating from fovea. Absence of of any kind of pubescence in the carapace. Caput is somehow elevated and grey contrasing the with the orange crest zone. Crest zone is partculary wide, tapering to the fovea with two dark lines of the same color of the carapace. Clypeus iand darker tha the carapace with few bristles. Eye-group on ocular tubercle. POP , small than regular. Surrounding and connecting all the eyes but not including the posteror row. Eye AR slightly longer than PR. Eye formation more than twice wider than longer AR/EL= 236, showing rectangular form.

Chelicerae brown with some orange stripes in dorsal view. Legs yellow, with presence of 3 prolateral spines on patella III.. Abdomen full of bristles, with a brown and grey undeterminend pattern. One row of seven cuspules. , PMS very reduced , with spigots only in the distal part. PLS not surpassing the abdomen only showin spigots in the distal part.

The spermatheca in *Nemesia meitnerae* is bipartite, showing thickening in the distal part, similar to other spermatheca of specimens from Balearic slands already defined as mushroom-like. The proximal part is wide and densely covered with glandular tissue tapering to the distal part .

Intraspecific variation.

PSP may vary between 1 to 3

Natural History

The trapdoor of the nest is cork-like and shows a cog-wheel form and the nest is covered by very few amount of silk .

Habitat

Those spiders were found in a *Quercus* forest,

Distribution

Found at the noth of Tunisia, in a margin slope on a open area.

All new described species and their distributions are summarized in Fig. 26.

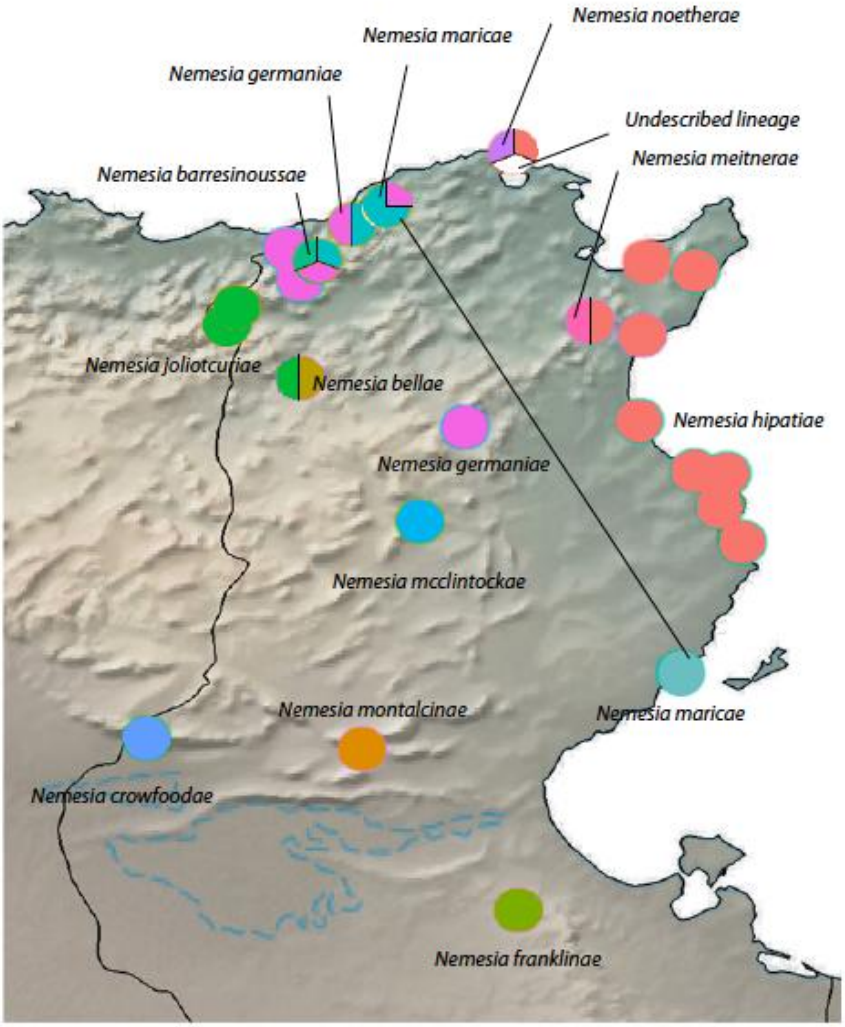


Figure 26. Species distribution of the new described species in this study along Tunisia

4.Discusion

Mygalomorph spiders are a challenging group for species delimitation. Previous studies find the extensive population structure and the lack of distinctiveness of somatic characters the reason behind the high diversity uncovered (Bond, 2004b; Hendrixson & Bond, 2005b; Hamilton et al., 2011; Opatova & Arnedo, 2014b).

The lack of up to date taxonomic information, and the unavailability of access to type material in most cases hamper the species identification leading to a big amount of uncovered diversity. Our results show how the inclusion of moleculars phylogenetics and coalescent-based methods for species delimitation revealed high levels of overlooked diversity in morphotipically uniform phenotypes. No information was kwon previous to this study about the nemesian fauna of Tunisia.

The GMYC method has been largely used in arthropods for species delimitation (Bidegaray-Batista & Arnedo, 2011; Planas et al., 2013; Opatova & Arnedo, 2014b) Furthermore, has been demonstrated that tends to overestimate the number of evolutionary lineages (Satler et al., 2013; Talavera et al., 2013). In this study GMYC identified 29 GMYC clusters that were associated to 12 morphotypes (13 if we count the lineage formed by juveniles). These pattern has already been reported for mygalomoprh, because due to their lifestyle, are poor dispers with high levels of population structure (Satler et al.,2013). This is the reason why GMYC was used as an objective preliminar delimitation approach.

Nuclear networks were applied to study possible patterns of gene flow. Although results do not show clear evidence. Only 3 particular cases in H3 AND two cases in EF-1G. Most shared alleles can be explained by ancestral polymorphism, a larger sample and more variable nuclear markers would be required to assess the actual level of genetic isolation.

We grouped the morpholohical variation in morphoespecies (sensu Dayrat 2005). The definition of morphoespcies, as hypothesis that should be tested via different approaches with different kind of data (molecular, ecogogical,

morphological) , because definition of species only based on morphology has some limits (Dayrat, 2005).

The definition and interpretation of different characters and states has always a subjective component (Padial et al., 2010) , the combination of morphological survey and phylogenetic data is fundamental for succeeding in species delimitation. The examination of female genitalia revealed small variation in closely related taxa. Its is important to remark that the preparation of female genitalia was problematic due to the weakly spermatheca and that stalks do not remain in a fixed position, furthermore we are dealing with 3-dimensional structures that can change dramatically if they are not well oriented. So we used valuable qualitative diagnostic characters that have been proved as useful before (Decae, , 2005; Decae & Cardoso, 2005; Decae et al., 2007b) and apply a morphometric study with quantitive characters.

The approach discussed here is the power of metric characters on different *Nemesia* spiders to perform a rigorous species delimitation. All morphometric analysis of *Nemesia* using metric-based approach failed to delineate discrete groups and clusters that correspond neither to GMYC lineages nor to delimited species. Based on morphometric data, an overlap occurs in *Nemesia* groups. Furthermore clustering provided by morphometric analysis is not recovering monophyletic groups. HCA dendogram recovers two main groups which are not recovered in phylogenetic analyses.

The only characters measured that were able to detect subtle differences within morphotypes were measures mainly from leg I,II,III. This might be suggesting that subtle differences within species can be detected by differences in their legs which are directly involved in burrow construction, that has been found very diverse and almost specific in Tunisan samples. This results show that methods with high power to detect subtle differences need to be incorporated to the species delimitation process within *Nemesia*.

How many species inhabit Tunisia?

The nemesiidae fauna of North Africa is poorly known. To date only 9 species have been described, from Morocco and Algeria. Most descriptions date back to

the 18th and 19th, are broad and poorly illustrated, and often lack diagnostic characters to identify species unambiguously. The possibility that this species should be assigned to other *Nemesia* species from the North of Africa was discarded despite there was no possibility to examine type material. Taking into account that most of them were described by Simon (Simon, 1889, 1892, 1911, 1914), with the inconvenient that he didn't label the type specimen in most cases, therefore is not possible to identify the type in most of his descriptions.

An extensive bibliographic search was performed in search of some diagnosable characters to be recognized in our sampling including morphological characters, distribution and burrow architectue. Was impossible to establish any relationship between ancient descriptions and our observations. Its remarkable that North African descriptions don't include any references or cites to the fauna of Tunisia. Only there is one recent study (Bosmans, 2003) where 4 species are cited, but according to our findings, no one of this described species can be related. Taking into account all this evidences we considered that all species found were new for science and deserve taxonomic status.

5.Conclusions

In this study we identified a high level of overlooked diversity in Tunisia. Our work strongly supports the importance of combining difference lines of evidence for species delimitation. The application of molecular tools combined with traditional morphological survey to the study of *Nemesia* highlighted the need for integrative approaches to identify all extant diversity. Mersitic measures were unable to detect subtle differences between species, that were detected using qualitative characters. Our results allowed the description of 12 from 13 new species. The high diversity and evolutionary significance of burrow architectures suggests the relevance of this feature to explain the high species diversity of the genus.

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Labels	Specimen	Genus	Sp	sex	Spin	Locality Name	Country	LN	Elevation	LocLatitude	LocLongitude	16S-ND1	DNA_code	28S	Efg	H3	G
DN1_F_H_TU	HB4	Nemesia	sp.	F	H	Bouficha	Tunisia	5		36,400242	10,547136	DN1	DN1				21
DN10_F_H_TU	HB3	Nemesia	sp.	F	H	Bouficha	Tunisia	5		36,400242	10,547136	DN10	DN10				21
DN101_J_H_TU	CRBAME001265	Nemesia	sp.	J	H	Kourbous	Tunisia	11	74	36,77661	10,58439	DN101	DN101				19
DN102_F_H_TU	CRBAME001371	Nemesia	sp.	F	H	Le Kef	Tunisia	14	556	36,18403	8,68234	DN102	DN102				9
DN105_J_H_TU	CRBAME001270	Nemesia	sp.	J	H	Cap Blanc	Tunisia	6	2	37,33186	9,84616	DN105	DN105	DN105	DN105	DN105	23
DN11_F_H_TU	HB5	Nemesia	sp.	F		Bouficha	Tunisia	5		36,400242	10,547136	DN11	DN11				21
DN111_J_H_TU	CRBAME001325	Nemesia	sp.	J	H	Ain Draham 1	Tunisia	1	788	36,77677	8,70712	DN111	DN111				5
DN113_J_H_TU	CRBAME001328	Nemesia	sp.	J	H	Ain Draham 1	Tunisia	1	788	36,77677	8,70712	DN113	DN113				5
DN114_F_H_TU	CRBAME001336	Nemesia	sp.	F	H	Ain Draham 2	Tunisia	2	785	36,77715	8,70288	DN114	DN114	pcr p	DN114		13
DN115_F_H_TU	CRBAME001346	Nemesia	sp.	F	H	Ain Draham 3, going out from	Tunisia	3	698	36,72058	8,67731	DN115	DN115	pcr p	pcr p		5
DN116_F_H_TU	CRBAME001347	Nemesia	sp.	F	H	Ain Draham 3, going out from	Tunisia	3	698	36,72058	8,67731	DN116	DN116				5
DN118_F_H_TU	CRBAME001313	Nemesia	sp.	J	H	Baboucha	Tunisia	4	620	36,80067	8,64215	DN118	DN118				5
DN120_J_H_TU	CRBAME001269	Nemesia	sp.	J	H	Cap Blanc	Tunisia	6	2	37,33186	9,84616	DN120	DN120				19
DN121_J_H_TU	CRBAME001271	Nemesia	sp.	J	H	Cap Blanc	Tunisia	6	2	37,33186	9,84616	DN121	DN121				3
DN122_J_H_TU	CRBAME001289	Nemesia	sp.	J	H	Cap Negre, Road P7 to	Tunisia	7		37,03957	9,08034	DN122	DN122		DN122		12
DN123_J_H_TU	CRBAME001284	Nemesia	sp.	J	H	Cap Negre, Road P7 to	Tunisia	7		37,03957	9,08034	DN123	DN123	DN123	DN123	DN123	25
DN124_F_H_TU	CRBAME001275	Nemesia	sp.	F	H	Cap Negre, Road P7 to	Tunisia	7		37,03957	9,08034	DN124	DN124		DN124	DN124	12
DN125_J_H_TU	CRBAME001276	Nemesia	sp.	J	H	Cap Negre, Road P7 to	Tunisia	7		37,03957	9,08034	DN125	DN125	DN125	DN125	DN125	24
DN131_F_H_TU	CRBAME001358	Nemesia	sp.	F	H	Ghardinou to PN El Feija	Tunisia	9	559	36,4899	8,32543	DN131	DN131	DN131	DN131	DN131	11
DN132_F_H_TU	CRBAME001394	Nemesia	sp.	F	H	Hbabsa	Tunisia	10	444	35,47567	9,34092	DN132	DN132		DN132	DN132	7
DN133_F_H_TU	CRBAME001395	Nemesia	sp.	F	H	Hbabsa	Tunisia	10	444	35,47567	9,34092	DN133	DN133	DN133	DN133	DN133	7
DN134_F_H_TU	CRBAME001392	Nemesia	sp.	F	H	Hbabsa	Tunisia	10	444	35,47567	9,34092	DN134	DN134	DN134	DN134	DN134	6
DN135_F_H_TU	CRBAME001261	Nemesia	sp.	F	H	Kourbous	Tunisia	11	74	36,77661	10,58439	DN135	DN135				19
DN136_F_H_TU	CRBAME001262	Nemesia	sp.	F	H	Kourbous	Tunisia	11	74	36,77661	10,58439	DN136	DN136	DN136	DN136	DN136	19
DN138_F_H_TU	CRBAME001390	Nemesia	sp.	F	H	La grotte de Mina	Tunisia	12	900	35,94722	9,58096	DN138	DN138	DN138	DN138	DN138	22
DN139_F_H_TU	CRBAME001375	Nemesia	sp.	F	H	Le Kef	Tunisia	14	556	36,18403	8,68234	DN139	DN139	DN139	DN139	DN139	9
DN141_F_H_TU	CRBAME001372	Nemesia	sp.	F	H	Le Kef	Tunisia	14	556	36,18403	8,68234	DN141	DN141				17
DN142_F_H_TU	CRBAME001376	Nemesia	sp.	F	H	Le Kef	Tunisia	14	556	36,18403	8,68234	DN142	DN142	DN142	DN142	DN142	17
DN143_F_P_TU	CRBAME001433	Nemesia	sp.	F	P	Oued Ez Zit	Tunisia	16	166	36,46527	10,2791	DN143	DN143		DN143		1
DN144_F_P_TU	CRBAME001434	Nemesia	sp.	F	P	Oued Ez Zit	Tunisia	16	166	36,46527	10,2791	DN144	DN144		DN144	DN144	1

Labels	Specimen	Genus	Sp	sex	Spin	Locality Name	Country	LN	Elevation	LocLatitude	LocLongitude	16S-ND1	DNA_code	28S	Efg	H3	G
DN145_F_H_TU	CRBAME001430	Nemesia	sp.	F	H	Oued Ez Zit	Tunisia	16	166	36,46527	10,2791	DN145	DN145	DN145	DN145	DN145	20
DN147_F_H_TU	CRBAME001368	Nemesia	sp.	F	H	PN El Feidja	Tunisia	17	829	36,50709	8,3208	DN147	DN147	DN147	DN147	DN147	10
DN148_F_H_TU	CRBAME001294	Nemesia	sp.	F	H	Road AP7 from Nezca to Tabarka, Ain Sebaa	Tunisia	18	107	36,9604	8,94213	DN148	DN148	DN148	DN148	DN148	14
DN15_F_H_TU	M5	Nemesia	sp.	F	H	Monastir town	Tunisia	15		36,728647	10,820411	DN15	DN15				21
DN151_J_H_TU	CRBAME001412	Nemesia	sp.	J	H	Thagmerza	Tunisia	24	250	34,37396	7,91083	DN151	DN151				2
DN155_J_H_TU	CRBAME001329	Nemesia	sp.	J	H	Ain Draham 1	Tunisia	1	788	36,77677	8,70712	DN155	DN155				5
DN156_J_H_TU	CRBAME001321	Nemesia	sp.	J	H	Ain Draham 1	Tunisia	1	788	36,77677	8,70712	DN156	DN156				5
DN157_J_H_TU	CRBAME001345	Nemesia	sp.	J	H	Ain Draham 2	Tunisia	2	785	36,77715	8,70288	DN157	DN157				5
DN158_J_H_TU	CRBAME001335	Nemesia	sp.	F	H	Ain Draham 2	Tunisia	2	785	36,77715	8,70288	DN158	DN158				5
DN159_J_H_TU	CRBAME001324	Nemesia	sp.	J	H	Ain Draham 1	Tunisia	1	788	36,77677	8,70712	DN159	DN159				5
DN16_F_H_TU	ML2	Nemesia	sp.	F	H	Lamta	Tunisia	13		35,683025	10,859511	DN16	DN16				21
DN160_J_H_TU	CRBAME001322	Nemesia	sp.	J	H	Ain Draham 1	Tunisia	1	788	36,77677	8,70712	DN160	DN160				5
DN161_J_H_TU	CRBAME001323	Nemesia	sp.	J	H	Ain Draham 1	Tunisia	1	788	36,77677	8,70712	DN161	DN161				5
DN162_J_H_TU	CRBAME001326	Nemesia	sp.	J	H	Ain Draham 1	Tunisia	1	788	36,77677	8,70712	DN162	DN162				5
DN164_J_H_TU	CRBAME001343	Nemesia	sp.	J	H	Ain Draham 2	Tunisia	2	785	36,77715	8,70288	DN164	DN164		DN164	DN164	13
DN165_J_H_TU	CRBAME001351	Nemesia	sp.	J	H	Ain Draham 3, going out from	Tunisia	3	698	36,72058	8,67731	DN165	DN165				5
DN166_J_H_TU	CRBAME001320	Nemesia	sp.	J	H	Baboucha	Tunisia	4	620	36,80067	8,64215	DN166	DN166				5
DN167_F_H_TU	CRBAME001267	Nemesia	sp.	F	H	Cap Blanc	Tunisia	6	2	37,33186	9,84616	DN167	DN167	DN167	DN167	DN167	3
DN169_J_H_TU	CRBAME001366	Nemesia	sp.	J	H	Ghardinou to PN El Feija	Tunisia	9	559	36,4899	8,32543	DN169	DN169				11
DN17_F_H_TU	MT10	Nemesia	sp.	F	H	Teboulba	Tunisia	23		35,653394	10,971478	DN17	DN17				21
DN170_J_H_TU	CRBAME001359	Nemesia	sp.	J	H	Ghardinou to PN El Feija	Tunisia	9	559	36,4899	8,32543	DN170	DN170				11
DN172_J_H_TU	CRBAME001263	Nemesia	sp.	J	ABD DEF	Kourbous	Tunisia	11	74	36,77661	10,58439	DN172	DN172		DN172	DN172	19
DN174_J_H_TU	CRBAME001370	Nemesia	sp.	F	H	Le Kef	Tunisia	14	556	36,18403	8,68234	DN174	DN174				9
DN175_F_H_TU	CRBAME001374	Nemesia	sp.	F	H	Le Kef	Tunisia	14	556	36,18403	8,68234	DN175	DN175		DN175	DN175	9
DN176_F_H_TU	CRBAME001378	Nemesia	sp.	F	H	Le Kef	Tunisia	14	556	36,18403	8,68234	DN176	DN176				17
DN178_J_H_TU	CRBAME001302	Nemesia	sp.	J	H	Road AP7 from Nezca to Tabarka,	Tunisia	18	107	36,9604	8,94213	DN178	DN178				14
DN18_J_H_TU	MHSM3	Nemesia	sp.	F	H	Sidi Messaoud	Tunisia	22		35,554522	11,008853	DN18	DN18				21
DN182_J_H_TU	CRBAME001316	Nemesia	sp.	J	H	Baboucha	Tunisia	4	620	36,80067	8,64215	DN182	DN182				5
DN183_J_H_TU	CRBAME001341	Nemesia	sp.	J	H	Ain Draham 2	Tunisia	2	785	36,77715	8,70288	DN183	DN183		DN183		13

Labels	Specimen	Genus	Sp	sex	Spin	Locality Name	Country	LN	Elevation	LocLatitude	LocLongitude	16S-ND1	DNA_code	28S	Efg	H3	G
DN185_J_H_TU	CRBAME001314	Nemesia	sp.	J	H	Baboucha	Tunisia	4	620	36,80067	8,64215	DN185	DN185				5
DN187_J_H_TU	CRBAME001319	Nemesia	sp.	JJ	H	Baboucha	Tunisia	4	620	36,80067	8,64215	DN187	DN187				5
DN189_J_H_TU	CRBAME001291	Nemesia	sp.	J	H	Cap Negre, Road P7 to	Tunisia	7		37,03957	9,08034	DN189	DN189		DN189	DN189	4
DN19_F_H_TU	MHS3	Nemesia	sp.	F	H	Salakta	Tunisia	20		35,385331	11,031575	DN19	DN19		DN19		21
DN191_F_H_TU	CRBAME001357	Nemesia	sp.	F	H	Ghardinou to PN El Feija	Tunisia	9	559	36,4899	8,32543	DN191	DN191		DN191	DN191	11
DN192_F_H_TU	CRBAME001380	Nemesia	sp.	F	H	Le Kef	Tunisia	14	556	36,18403	8,68234	DN192	DN192				9
DN193_F_H_TU	CRBAME001379	Nemesia	sp.	F	H	Le Kef	Tunisia	14	556	36,18403	8,68234	DN193	DN193		DN193	DN193	17
DN195_J_H_TU	CRBAME001300	Nemesia	sp.	J	H	Road AP7 from Nezca to Tabarka,	Tunisia	18	107	36,9604	8,94213	DN195	DN195				14
DN2_F_H_TU	S9	Nemesia	sp.	F	H	Chott Meriem	Tunisia	8		35,970928	10,532675	DN2	DN2				21
DN201_J_H_TU	CRBAME001308	Nemesia	sp.	J	H	Road AP7 from Nezca to Tabarka,	Tunisia	18	107	36,9604	8,94213	DN201	DN201		DN201	DN201	14
DN202_J_H_TU	CRBAME001306	Nemesia	sp.	J	H	Road AP7 from Nezca to Tabarka,	Tunisia	18	107	36,9604	8,94213	DN202	DN202	pcr p	DN202	DN202	5
DN21_F_H_TU	MHS2	Nemesia	sp.	F	H	Salakta	Tunisia	20		35,385331	11,031575	DN21	DN21	DN21	DN21	DN21	21
DN22_F_H_TU	MHSM8	Nemesia	sp.	F	H	Sidi Messaoud	Tunisia	22		35,554522	11,008853	DN22	DN22		DN22		21
DN24_F_H_TU	MT8	Nemesia	sp.	F	H	Teboulba	Tunisia	23		35,653394	10,971478	DN24	DN24				21
DN26_F_H_TU	ML9	Nemesia	sp.	F	H	Lamta	Tunisia	13		35,683025	10,859511	DN26	DN26				21
DN28_F_H_TU	M8	Nemesia	sp.	F	H	Monastir town	Tunisia	15		36,728647	10,820411	DN28	DN28				21
DN3_F_H_TU	M9	Nemesia	sp.	F	H	Monastir town	Tunisia	15		36,728647	10,820411	DN3	DN3				21
DN30_F_H_TU	S8	Nemesia	sp.	F	H	Chott Meriem	Tunisia	8		35,970928	10,532675	DN30	DN30				21
DN31_F_H_TU	S5	Nemesia	sp.	F	H	Chott Meriem	Tunisia	8		35,970928	10,532675	DN31	DN31		DN31		21
DN33_J_H_TU	CRBAME001331	Nemesia	sp.	J	H	Ain Draham 1	Tunisia	1	788	36,77677	8,70712	DN33	DN33				5
DN34_F_H_TU	CRBAME001408	Nemesia	sp.	F	H	Road Between Guettar and Bou Oumrane	Tunisia	19	341	34,33729	9,06134	DN34	DN34	DN34	DN34	DN34	18
DN35_F_H_TU	CRBAME001311	Nemesia	sp.	F	H	Baboucha	Tunisia	4	620	36,80067	8,64215	DN35	DN35	DN35	DN35	DN35	5
DN36_F_H_TU	CRBAME001268	Nemesia	sp.	F	H	Cap Blanc	Tunisia	6	2	37,33186	9,84616	DN36	DN36		DN36	DN36	3
DN37_F_H_TU	CRBAME001349	Nemesia	sp.	F	H	Ain Draham 3, going out from	Tunisia	3	698	36,72058	8,67731	DN37	DN37				5
DN38_F_H_TU	CRBAME001295	Nemesia	sp.	F	H	Road AP7 from Nezca to Tabarka,	Tunisia	18	107	36,9604	8,94213	DN38	DN38				14
DN39_F_H_TU	CRBAME001391	Nemesia	sp.	F	H	Hbabsa	Tunisia	10	444	35,47567	9,34092	DN39	DN39				7
DN4_F_H_TU	ML10	Nemesia	sp.		H	Lamta	Tunisia	13		35,683025	10,859511	DN4	DN4				21
DN40_F_H_TU	CRBAME001338	Nemesia	sp.	F	H	Ain Draham 2	Tunisia	2	785	36,77715	8,70288	DN40	DN40				5
DN5_F_H_TU	MT9	Nemesia	sp.	F	H	Teboulba	Tunisia	23		35,653394	10,971478	DN5	DN5				21
DN6_F_H_TU	MHSM5	Nemesia	sp.	F	H	Sidi Messaoud	Tunisia	22		35,554522	11,008853	DN6	DN6				21

Labels	Specimen	Genus	Sp	sex	Spin	Locality Name	Country	LN	Elevation	LocLatitude	LocLongitude	16S-ND1	DNA_code	28S	Efg	H3	G
DN7_F_H_TU	MHS1	Nemesia	sp.	F	H	Salakta	Tunisia	20		35,385331	11,031575	DN7	DN7				21
DN70_M_H_TU	CRBAME001333	Nemesia	sp.	M	H	Ain Draham 2	Tunisia	2	785	36,77715	8,70288	DN70	DN70		DN70	DN70	8
DN71_F_H_TU	CRBAME001334	Nemesia	sp.	F	H	Ain Draham 2	Tunisia	2	785	36,77715	8,70288	DN71	DN71	DN71		DN71	8
DN72_F_H_TU	CRBAME001274	Nemesia	sp.	F	H	Cap Negre, Road P7 to	Tunisia	7		37,03957	9,08034	DN72	DN72	DN72	DN72	DN72	12
DN73_F_H_TU	CRBAME001293	Nemesia	sp.	F	H	Road AP7 from Nezca to Tabarka,	Tunisia	18	107	36,9604	8,94213	DN73	DN73				14
DN74_F_H_TU	CRBAME001260	Nemesia	sp.	F	H	Kourbous	Tunisia	11	74	36,77661	10,58439	DN74	DN74				19
DN75_F_H_TU	CRBAME001404	Nemesia	sp.	J	H	Between Guettar and Bou Oumrane	Tunisia	19	341	34,33729	9,06134	DN75	DN75		DN75	DN75	18
DN76_M_H_TU	CRBAME001354	Nemesia	sp.	M	H	Ghardinou to PN El Feija	Tunisia	9	559	36,4899	8,32543	DN76	DN76				11
DN77_F_H_TU	CRBAME001356	Nemesia	sp.	F	H	Ghardinou to PN El Feija	Tunisia	9	559	36,4899	8,32543	DN77	DN77				11
DN78_F_H_TU	CRBAME001393	Nemesia	sp.	J	H	Hbabsa	Tunisia	10	444	35,47567	9,34092	DN78	DN78		DN78	DN78	6
DN79_F_H_TU	CRBAME001337	Nemesia	sp.	F	H	Ain Draham 2	Tunisia	2	785	36,77715	8,70288	DN79	DN79	DN79	DN79	DN79	13
DN8_F_H_TU	SX1	Nemesia	sp.	F	H	Sfax town	Tunisia	21		34,708817	10,72155	DN8	DN8	DN8	DN8	DN8	26
DN80_F_H_TU	CRBAME001431	Nemesia	sp.	F	H	Oued Ez Zit	Tunisia	16	166	36,46527	10,2791	DN80	DN80				20
DN81_F_H_TU	CRBAME001296	Nemesia	sp.	F	H	Road AP7 from Nezca to Tabarka,	Tunisia	18	107	36,9604	8,94213	DN81	DN81		DN81		14
DN82_F_H_TU	CRBAME001432	Nemesia	sp.	F	H	Oued Ez Zit	Tunisia	16	166	36,46527	10,2791	DN82	DN82		DN82	DN82	20
DN83_F_H_TU	CRBAME001388	Nemesia	sp.	J	H	Le Kef	Tunisia	14	556	36,18403	8,68234	DN83	DN83		DN83	DN83	16
DN84_F_P_TU	CRBAME001429	Nemesia	sp.	F	P	Oued Ez Zit	Tunisia	16	166	36,46527	10,2791	DN84	DN84	DN84	DN84	DN84	1
DN85_F_H_TU	CRBAME001272	Nemesia	sp.	F	H	Cap Negre, Road P7 to	Tunisia	7		37,03957	9,08034	DN85	DN85	DN85	DN85	DN85	4
DN86_F_H_TU	CRBAME001373	Nemesia	sp.	F	H	Le Kef	Tunisia	14	556	36,18403	8,68234	DN86	DN86	DN86	DN86	DN86	16
DN87_F_H_TU	CRBAME001411	Nemesia	sp.	J	H	Thagmerza	Tunisia	24	250	34,37396	7,91083	DN87	DN87		DN87	DN87	2
DN88_F_H_TU	CRBAME001355	Nemesia	sp.	F	H	Ghardinou to PN El Feija	Tunisia	9	559	36,4899	8,32543	DN88	DN88				11
DN89_F_H_TU	CRBAME001410	Nemesia	sp.	F	H	Thagmerza	Tunisia	24	250	34,37396	7,91083	DN89	DN89	DN89	DN89	DN89	2
DN90_F_H_TU	CRBAME001409	Nemesia	sp.	F	H	Thagmerza	Tunisia	24	250	34,37396	7,91083	DN90	DN90				2
DN91_F_H_TU	CRBAME001420	Nemesia	sp.	F	H	Tmezret	Tunisia	25	446	33,53897	9,88678	DN91	DN91	DN91	DN91	DN91	15
DN93_F_H_TU	CRBAME001428	Nemesia	sp.	F	H	Tmezret	Tunisia	25	446	33,53897	9,88678	DN93	DN93		DN93	DN93	15
DN94_F_H_TU	CRBAME001273	Nemesia	sp.	F	H	Cap Negre, Road P7 to	Tunisia	7		37,03957	9,08034	DN94	DN94				12
DN98_F_H_TU	CRBAME001369	Nemesia	sp.	J	H	PN El Feidja	Tunisia	17	829	36,50709	8,3208	DN98	DN98		DN98	DN98	10
DN99_H_H_TU	CRBAME001342	Nemesia	sp.	J	H	Ain Draham 2	Tunisia	2	785	36,77715	8,70288	DN99	DN99				5

List 1. Qualitative characters, based on Decae 2005

1) Crest zone: is the colour pattern showed by the integument of the caput;

2) Crest Setae: the number of rows of hairs /bristles on the crest of the caput (varies consistently between most species);

3) Pubescence: the presence/absence and the colour of hair on the carapace, chelicerae and leg segments may be of important diagnostic value in Nemesiidae at the species level;

4) POP: is the pattern of the black pigmentation around the eyes, it can be broken if its not including all eyes

5) Fovea: the shape, and the presence/or absence of a longitudinal groove

6) Cuspules: the number, pattern and shape of the maxillary cuspules may vary between species –

7) Spinneret morphology: is a very important important diagnostic character in *Nemesia.* First of all because in absence of molecular information help to relate conspecific males and females. Also spinneret are closely related with nest construction.

PMS: reduced in *Nemesia* and absent in *Iberesia* (Decae & Cardoso 2005), the pattern of spigots however, are of important diagnostic value at the species level in *Nemesia*.

PLS, the pattern of spigots on this segment are of diagnostic value. The distrubution of spigots along the spinnerets its closely realted with the morphology of the nest which is from diagnosic value in most species (Deacae, 2010, Mora pers comm)

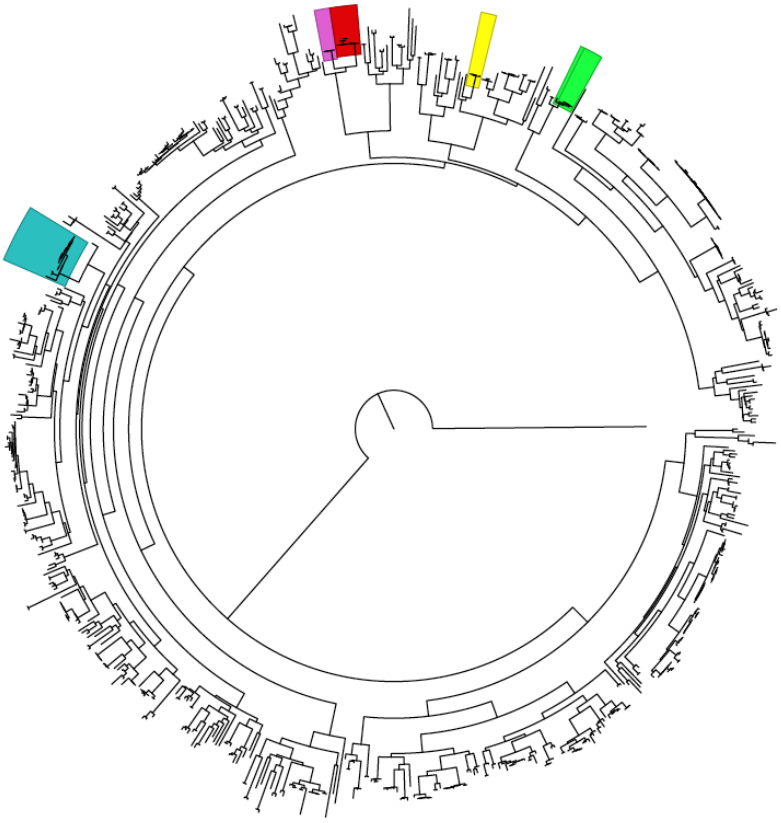
8) Maculae, the presence of dark pigmented patches (maculae) on the external leg segments and/or on the external basal segment of the PLS

Table 2. List of the 34 morphometric characters and their abbreviations.

N°	Characters	Abbreviations
1	Body Spider length	BSL
2	Carapace length	CL
3	Carapace width	CW
4	Caput length	CapL
5	Sternum length	SL
6	Sternum width	SW
7	Tarsus palp length	Palptars
8	Tibia palp length	Palptib
9	Ptella palp length	Palppat
10	Femur palp length	Palpfem
11	Tarse length on leg 1	Tar1
12	Metatarse length on leg 1	Met1
13	Tibia length on leg 1	Tib 1
14	Patella length on leg 1	Pat1
15	Femur length on leg 1	Fem1
16	Tarse length on leg 2	Tar2
17	Metatarse length on leg 2	Met2
18	Tibia length on leg 2	Tib 2
19	Patella length on leg 2	Pat2
20	Femur length on leg 2	Fem2
21	Tarse length on leg 3	Tar3
22	Metatarse length on leg 3	Met3
23	Tibia length on leg 3	Tib 3
24	Patella length on leg 3	Pat3
25	Femur length on leg 3	Fem3
26	Tarse length on leg 4	Tar4
27	Metatarse length on leg 4	Met4
28	Tibia length on leg 4	Tib4
29	Patella length on leg 4	Pat4
30	Femur length on leg 4	Fem4
31	Total leg length 1	LL1
32	Total leg length 2	LL2
33	Total leg length 3	LL3
34	Total leg length 4	LL4

Chapter 4.5

Alongside but separate: High local diversity and the mechanisms underlying species co-existence in Nemesia trap-door spiders (Mygalomorphae, Nemesiidae)



Alongside but separate: High local diversity and the mechanisms underlying species co-existence in *Nemesia* trap-door spiders (Mygalomorphae, Nemesiidae)

1. Introduction

Understanding the mechanisms behind the coexistence of closely related species is one of the main goals of ecological studies and it is a key factor for understand how biodiversity is generated and maintained (Hutchinson & MacArthur, 1959; Tilman & Pacala, 1993; Brown, 1995; Chesson, 2000a; Gaston, 2009) Several mechanisms have been put forward to explain the maintenance of species coexistence (Chesson, 2000b; Hubbell, 2001; Emerson & Gillespie, 2008; Mayfield & Levine, 2010). Theories based on niche differentiation were popular during the first half of the 20th century. Under this paradigm, coexisting species although apparently very similar, would differ even in very subtle ways, for instance in their responses to environmental shifts or in their specific role in the community (Begon et al., 1990),. Niche differentiation may be accomplished by temporal partitioning, this is species avoiding competition by using similar resources but at different times,, or by spatial partitioning; this is species avoid competition because they live in slightly different habitats. Scale plays a major role in ecological segregation because partitioning may occur at very different spatial and temporal levels. Frequently, morphological differentiation may hint to the use of different resources by coexisting close relatives (Pyke et al., 2012).

There are, however, exceptions to the general rule and some coexisting species do not show obvious evidences of niche partitioning, challenging the explanatory power of the Niche theorem (Kelly et al., 2008). There has been documented, for example, that co-occurring hispine beetles use the same resources and show no evidence of niche differentiation, hence contradiction the competitive exclusion principle (Strong, 1982).

Hubbell's Unified Neutral Theory of Biodiversity and Biogeography (UNTB) (Hubbell, 2001) attempted to address some of the limitation of the Niche theorem'. During last years this theory has generated a substantial debate because of its rejection of some of the fundamental concepts of traditional

ecological research and niche theory (Chesson, 1990). UNTB proposes that species are ecologically equivalent (Bell, 2000; Hubbell, 2001; Leibold & McPeek, 2006), and states that coexistence is mediated by similarities within the species. When competitive abilities are conserved, closely related species will be more prone to coexist than distant ones (Mayfield & Levine, 2010).. Provided that coexistence is mediated by neutral dynamics, then the single most important species-specific trait to explain coexistence is dispersal ability (Hubbell, 2001; Gravel et al., 2006; Michalko & Pekár, 2015). Hubbell himself acknowledged that ecological communities are undoubtedly governed by both niche-assembly and dispersal-assembly rules, along with ecological drift. The question then arises: what is the relative contribution of niche segregation and species dispersal to local community assembly. Recent studies have pointed out that the co-occurrence of ecologically similar or equivalent species is not incompatible with the niche theory, because niche relationships may sometimes favour the coexistence of similar species (Leibold & McPeek, 2006). More efforts are required toward a better integration of the predictions of the two theories.

Spiders as a model system

Spiders are a good model system for studying species coexistence. They are among the most abundant, diverse and ubiquitous predators in most terrestrial ecosystems There are numerous cases in the literature that seems to suggest niche partitioning in co-occurring species (Michalko & Pekár, 2015), including different foraging strategies (Olive, 1980) body sizes (Richardson & Hanks, 2009) microhabitat use (Harwood et al., 2003), or phenologies (Herberstein & Elgar, 1994).

Mygalomorphae are one of the three main lineages of spiders (Stock, 1993; Hedin & Bond, 2006), and include, among others, the American tarantulas, the trap-door spiders and the fennel web spiders. They are usually large, long lived and sedentary ground-dwellers. Despite not being are not as diverse as their sister group, the araneomorphs, they comprise more than 2600 species in approximately 300 genera (World Spider Catalog, 2015) . The genus *Nemesia* is the most diverse mygalomorph genus in the Mediterranean region, where 54

species and 4 subspecies are currently recognized (World Spider Catalog, 2015). These spiders have a cryptic lifestyle, they are usually found in self-digged burrows protected by a trapdoor. The trapdoor adopt a diverse array of shapes and is usually camouflaged with the surrounding areas, which difficult finding them in the field. The females have long life cycles, some studies have reported that females can live in captivity for almost 20 years (Buchli, 1961, 1962, 1965), and are mostly sedentary. Conversely, males have shorter life cycles, after the adult moult they leave their burrows in search for females. Gene low between populations is hence mostly mediated by males. Species usually have restricted distributions and narrow ecological preferences.

The high level of local endemisms is most likely the result of their low vagility, as revealed by the deep population structure found in numerous molecular studies (Bond et al., 2001; Arnedo & Ferrández, 2007; Bond & Stockman, 2008; Decae, 2012; Opatova & Arnedo, 2014). *Nemesia* species are excellent candidates for investigating the mechanisms that allow species coexistence: they are phenotypically conserved but are commonly found co-occurring in the same locality. Unfortunately, *Nemesia* taxonomy remain poorly understood. Old descriptions are usually ambiguous and do not provide good diagnostic characters, many species are know from single individuals and one of the sexes and a large part of its diversity remains to be catalogued (Decae, 2012).

Here we aim to identify the mechanisms underlying the coexistence of closely related *Nemesia* species in the Sant LLorenç de Munt i la Serra de l'Òbac Natural Park (Catalonia, north-eastern Iberian Peninsula), where five *Nemesia* species have been recorded living in close proximity(Parera, 1984). We use an integrative approach to delimit species boundaries and facilitate identification (Dayrat, 2005; Hebert & Gregory, 2005; Padial et al., 2010) to circumvent the inherent problems of a poor background taxonomy and phenotypic uniformity. Once the species are properly delimited, we can then investigate temporal and spatial separation of the species, as predicted by the niche theory suggests or, in absence of evidence of species segregation, species coexistence in *Nemesia* is an example in favour of the neutral dynamic theory.

2. Material and Methods

2.1 Study Area

The Natural Park of Sant Llorenç de Munt and la Serra de L'Òbac, is located in Catalonia at the north-east of the Iberian Peninsula (N 41.5973, E 2.0259). The park covers 13 ha at the confluence of two Mountain ranges separated by a stream that form part of the Catalan Pre-littoral mountain range. The climate of the region is typically Mediterranean with irregular and intense rainy periods mostly in winter, but also in autumn and spring. The summer is normally dry with high temperatures. The main precipitations varies significantly among different zones, ranging from above 800 mm in La Mola (the highest peak) to approximately 603 mm in the lower parts.

The predominant vegetal community is the evergreen oak *Quercus ilex* forest, with high predominance of *Buxus sempervirens*, This forest is restricted to the highest areas (> 800 m) due to human activity, and is enriched with species typical from humid places. The lower lands of the massif are mostly covered by the Aleppo pine tree (*Pinus halepensis*), with some groves of hazel (*Corylus*) and *Quercus petraea* oaks. About, .4.600 ha from the total extension were burned in 2003. As a results of the fires, large extensions with predominance of *Cistus*, *Rosmarinus* and *Erica* are present.

2.2 Sampling for DNA taxonomy and phenology

A first sampling campaign was performed along the Natural Park in search for the colonies of *Nemesia* .Specimens were collected by direct capture and later pitfall trapping were placed by the authors and collaborators in several campaigns conducted from 2011 to 2012.Pitfall traps are ideal sampling method for capturing mygalomorph males because they are only active during few days during the year. Pitfall traps consisted in cylindrical plastic cups of 10 cm in diameter and 15 cm high (Churchill & Arthur, 1999; Ferretti et al., 2012), placed every 5 m along 20 m straight line transects. The cups were covered with funnels made with the same material and from the same diameter. Traps were filled with propilengycol, because it does not evaporates and preserves DNA for subsequent molecular analyses. The proyplenglycol was changed every

sampling day. The traps were covered with flat stones to prevent flooding, elevated about two centimetres from the ground to avoid capturing microvertebrates but not interfering with the spider sampling.

Five localities were selected to maximize the representation of the main plant communities in the Park. As those spiders live permanently in burrows, we expected them to be especially sensitive to the microclimatic regime, which is in turn significantly influenced by plants; along with soil parameters and habitat stability (Řezáč et al., 2007). The localities included three different kinds of forests: *Quercus ilex* (el Coll de les tres creus), *Pinus halepensis* (Les Refardes) and *Q. petraea* (Sot de Teixoneres) and two localities that burnt in the 2003 fire (Agramunt and Lligabossa). A map the localities in shown in Fig. 1. One pitfall transect was sampled for each locality. The samplings period began in May 2011 and ended in February 2013. The pitfalls were revised monthly except for the raining period (autumn). Males predominantly disperse after the first rains, and hence sampling was done weekly or bimonthly during the rainy season to record the peak of male activity. Localities are summarized in Table 1.

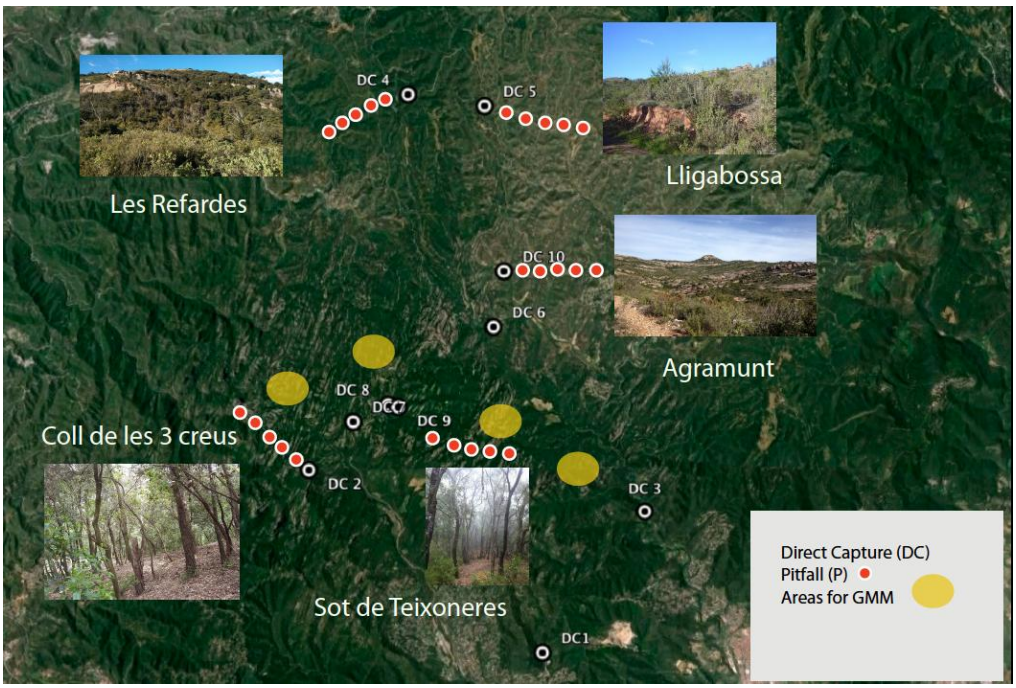


Fig. 1. Map of sampling localities for the present study. Direct capture sampling is indicated with black and white dots (DC). From these 10 localities, pitfall trapping was located in 5 of them which are indicated with

red dots. Areas where samples for GMM were collected are highlighted in yellow. Due to the fine scale of pitfall samples, red points on map are only a visual representation.

2.3 Specimen sorting and identification

All collected specimens were sorted into morphotypes and subsequently identified to species level with the help of original descriptions and available keys. Specimens were examined with a Leica MZ16A dissection microscope, equipped with a Leica DFC450 digital camera. Female vulvas were removed with the aid of needles, and the muscle tissue was digested with a 35% KOH solution before observation. One or two legs (depending on the size) were removed and directly preserved in absolute ethanol shortly after specimen collection. The legs were stored at -20°C at the freezer facilities of the Department of Animal Biology of the University of Barcelona for subsequent DNA analyses. The rest of the voucher specimen was preserved in alcohol 70% for morphological studies.

A former inventorying in the Park conducted in a *Quercus ilex* forest (our *Quercus ilex* forest was located in the same area) have recorded the presence of 5 *Nemesia* species, namely *Nemesia caementaria* (Latreille, 1799), *N. dubia* (Pickard Cambridge, 1874), *N.. manderstjernae* (Koch, 1838)*, N. raripila S*imon, 1914 *and N.* cf. *maculatipes* (Ausserer, 1875)(Perera, 1986). Unfortunately, we were not able to locate the original material used in the identifications. However, we considered some of the records doubtful because they are far from the known distribution of some of the species, The southernmost record of *N. manderstjernae* is southern France and the species has never been recorded south of the Pyrenees (Blasco, 1984; Medail & Quezel, 1996)while specimens belonging to the *maculatipes* group have been so far only reported from Sardinia and Sicily (Decae, 2012).

2.4 Species delimitation and identification

DNA sequence information was generated for a subset of individuals including all stages and representing all sampled areas. Total Genomic DNA was extracted from specimen legs' or pinned abdomens (juveniles) using the DNeasy Tissue Kit (Qiagen) following the manufacturer's protocol. Fragments of the following genes were amplified with universal primers: a mitochondrial

fragment spanning the 3' half of the 16S rRNA ribosomal subunit (16S), the complete tRNA leu (L1) and the 5' half of the NADH deshydrogenase subunit I (nad1), a fragment of the nuclear genes 28S rRNA (28S), Histone H3 (H3) and Elongation Factor 1 gamma (Ef-1g). The primers and PCR conditions for each gene are indicated in Chapter 4.3. Amplicons were sequenced in both directions by **Macrogen Inc**. (Seoul, Korea). Raw sequences were assembled and edited using Geneious v. 5.3.7(Kearse et al., 2012).

The ribosomal gene sequences were aligned using the online version of MAFFT v. 6 (available at http://mafft.cbrc.jp/alignment/server/, (Katoh & Toh, 2008) using the Q-INS-i strategy, which takes in consideration RNA secondary structure, with default settings (gap opening penalty, GOP set to 1.53; offset value set to 0.0). The protein coding sequences were translated into amino acids to confirm that no stop codons were present

Pairwise, and average within and between **u**ncorrected genetic distances were estimated with MEGA v6 (Tamura et al., 2013). Graphic representation of distances and divergence thresholds to delimit sequence clusters were investigated with the web version of ABGD (Puillandre et al., 2011). Haplotype networks were constructed using the TCS method (Clement et al., 2000) as implemented in the program Popart (available at http: //popart.otago.ac.nz.)**.**

Maximum Likelihood (ML) analyses were performed by raxmlGUI version 1.5 defining unlinked GTR-CAT models by gene and, in the case of protein coding gene, codon partition. The best ML tree and the bootstrap support were inferred simultaneously using the –f a algorithm and the autoMRE option (Stamatakis, 2006).

2.5 Geometric morphometrics

Geometric morphometrics (GMM) is a quantitative method used to study morphological variation. This method allows studying the variation of shape by defining it mathematically. The concept of shape is defined as all geometric features of a structure except its position, orientation and scale (Klingenberg, 2010). GMM has higher power to detect small and also subtle differences that

can be biologically relevant, and using methods than can reliably find and report this differences.

2.5.1 Specific design and sampling

Due to the high number of samples required for GMM analysis, here we focused in studying morphological differences in the two morphs originally identified as belonging to *N. raripilia* in two different forest: *Q. ilex* and *Q. petraea..*Each forest type had a replicate separated at least by one kilometer of distance within the park. Three colonies were sampled for each replicate, collecting 15 adult females per colony. If the number of females in a colony was less than 15, we sampled additional colonies until reaching the right number of females. We focused on females because they are more sedentary and have longer life spans and hence are the stage/sex most exposed to evolutionary and environmental pressures. Bidimensional images were digitalized of each specimen from dorsal and lateral views of the carapace as shown in Fig. 4. All legs were removed to facilitate imaging. Pictures in tiff format were taken using a LEICA MZ16A equipped with a Nikon DXM1200 and a high resolution LEICA DFC 450.

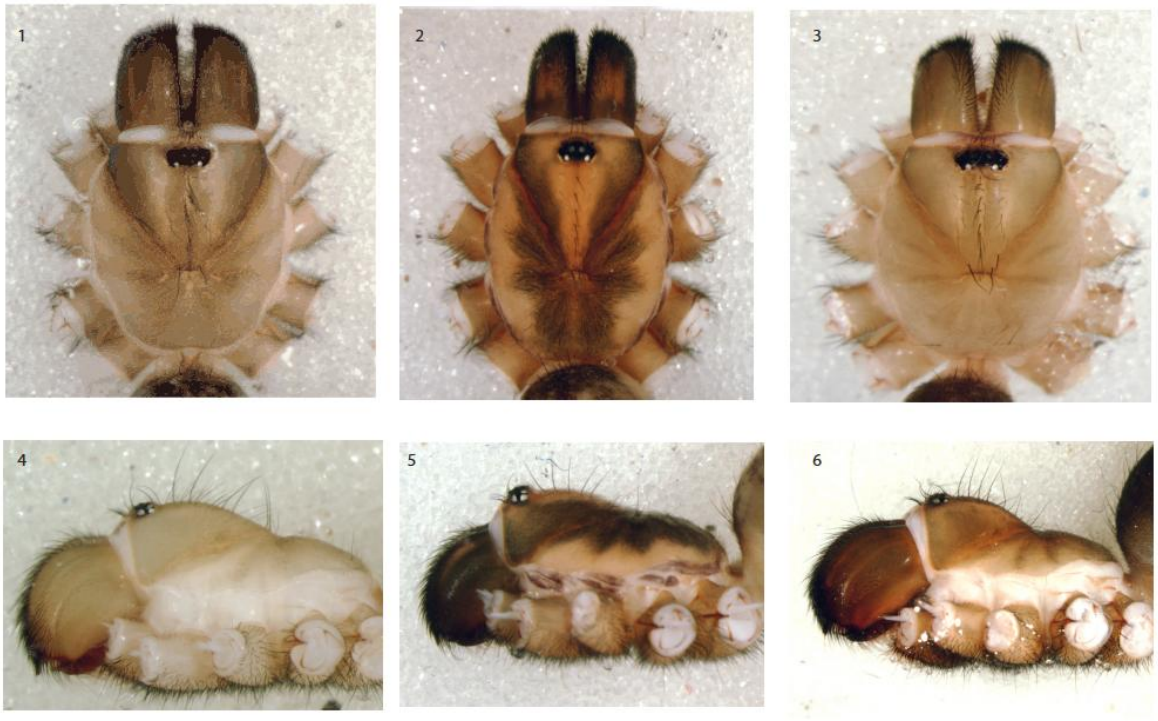


Figure 2. Images of carapace dorsal (1,2,3) and lateral (4,5,6) views. (1,4) *Nemesia raripilia*, morph 1 (Q.ilex); (2,5) *Nemesia dubia*; (3,6) *Nemesia raripilia*, morph 2 (*Q. petraea*)

The carapace is a character that is often scored in higher-level analyses of spider relationships. Major clades within the spider infraorder Mygalomorphae have been at least partially defined on the basis of carapace shape (Raven, 1985; Goloboff, 1993; Bond et al., 2001). The shape and elevation of the caput have been used in several subsequent studies further evaluating mygalomorph phylogeny (Goloboff, 1993; Bond et al., 2001; Bond & Beamer, 2006) and caput elevation seems to be useful character in *Nemesia* diagnosis (Mora pers obs). The utility of the geometric morphometrics if the carapace for mygalomorph taxonomy, however, has been recently out into question.(Bond & Beamer, 2006)

We used a landmark approximation. The definition and location of landmarks was based on the morphology and the putative biological relevance of shape differences. Landmarks were located precisely on each specimen under study and corresponded in a one-to-one from specimen to specimen. Twenty-three landmarks were defined from the dorsal view and 8 from the lateral view. Landmark definitions are shown in Fig. 5.

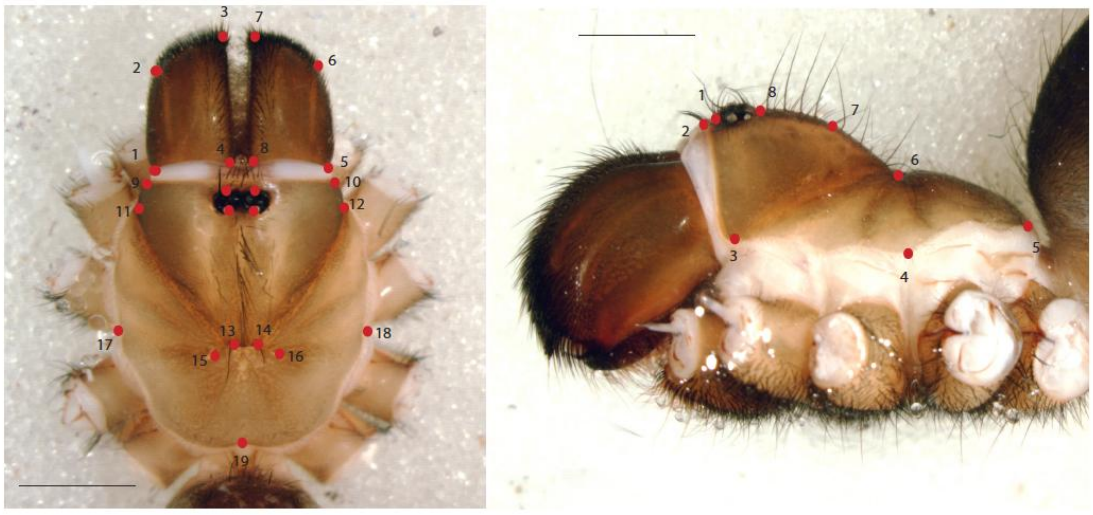


Figure 3. Landmark positions defined in the present study.

Those landmarks are defined as follows.

Dorsal View

1) On Left chelicerae; Insertion of the basal part (external side) of chelicerae into the carapace
2) On the left chelicerae, external part; landmark at the beginning of the curvature
3) On the left chelicerae, inner part : landmark at the top of the structure

4) On Left chelicerae; Insertion of the basal part (internal side)on the carapace
5) On right chelicerae; Insertion of the basal part (external side) of chelicerae into the carapace
6) On the right chelicerae, external part; landmark at the beginning of the curvature
7) On the right chelicerae, inner part : landmark at the top of the structure
8) On right chelicerae; Insertion of the basal part (internal side)on the carapace
9) Left marginal end of the carapace
10) Right marginal end of the carapace
11) Left part of the carapace, beginning of the line that delimits the caput
12) Right part of the carapace, beginning of the line that delimits the caput.
13) Left , junction when the caput tapering towards the fovea
14) Right, junction of junction when the caput tapering towards the fovea
15) Left end of fovea
16) Right end of fovea
17) Point when the left second leg insert on the carapace
18) Point hen the right second leg inserts on the carapace
19) Middle point on the inferior edge of the Carapace (insertion of inner musculature)
20) In the anterior eye row from, inner extreme for the left eye
21) In the anterior eye row from, inner extreme for the right eye
22) In the posterior eye row from the left side inner extreme for the biggest left eye
23) In the posterior eye row, fro the right side, inner extreme for the biggest right eye

Lateral view

1) Clypeus delimitation, landmark in front of the eyes, in straight line from the median eyes
2) Carapace margin edge delimiting the proximal edge of the carapace
3) Carapace low limit, where begins the line that delimits the caput
4) Curvature defining musculature insertion, between 2nd and 3rd leg.
5) Posterior margin carapace edge
6) Point delimiting the fovea (thoracic height)
7) Point delimiting the eye steep tubercle
8) Highest point of the curvature between 7 and 8

The coordinates of the landmarks were recorded and the two-dimensional x, y Cartesian coordinates digitized using the tpsDig program (Rohlf, 2001). The raw coordinates were analysed in the MORPHOJ package (Klingenberg, 2011) . Alignment was performed using Procrustes fit (Procrustes superimposition) (Dyrden & Mardia, 1998), whereby variation due to position, orientation and size was removed from the data, leaving only shape variation for further analysis. An allometry regression was performed to account for the long life span of female *Nemesia*. Procrustes coordinates were subjected to a principal component analysis (PCA) and a canonical variate analysis (CVA). Principal component analysis (PCA) was used to examine the variation of multiple variables within a single sample. It is often also used for a first exploratory analysis of a larger data set composed of several samples. We performed a Canonical variate analyses rather than discriminant analyses because we were comparing three groups. CVA is the most widely used method for investigating taxonomic differences (Viscosi & Cardini, 2011).

2.9 Meristic measurements

Carapace width (Hutchinson, 1959) was measured for all the males captured with the pitfall trapping survey, considering carapace width is a measure of overall spider size (Hagstrum, 1971) and being the measure with less percentage of error. Analysis of repeatability was performed with R (R Core Team, 2013)to discard differences due to error measurement using the package rptRP(Nakagawa & Schielzeth, 2010). For calculating the coefficient a subsample was measured three times in different weeks. After using RCCP the coefficient was R=0,94 which means no significant error in the measurements. Differences in carapace width, as measure of body size, were measured to detect differences between species.

2.10 Phenology

Phenology charts were made in R environment (R Core Team, 2013). The meteorological data used in this study was obtained from the nearest meteorological stations to the study area located at Rellinars (located at 3 km to the park), and kindly provided by Meteocat service from Generalitat de Catalunya. Due to the altitudinal differences, temperatures were corrected using the R package (Deepayan, 2008).

3. Results

3.1 Species delimitation and identification

We collected a total of 538 individuals, 241 males, 204 females and 93 juveniles. Morphological identification revealed the presence of 3 nominal species among the studied specimens, namely *Nemesia dubia* Pickard Cambridge, 1874, , 1874*, Nemesia caementaria* Latreille, 1799 *and Nemesia raripila* Simon 1914*.* We further recognised some morphological differences between the *N. raripilia* from *Q. ilex* forests at those from *N. raripilia* populations in *Q. pretraea* forests and hence two different morphs were considered. Finally, a fifth morph apparently corresponding to an undescribed species was identified. Thirty-five individuals including all stages and representing all sampled areas were sequenced for the 16snad1 genes (554 aligned characters 16S+L1 and 359 *nad1*), resulting in 10 different haplotypes. Analyses of the

pairwise p-values distances with ABGD reveal three mean distance categories, a group with pairwise distances below 0.1%, a group with distances above 18% and a group with distances between 9 and 10%. Any intraspecific cut-off above 0.1% yielded 5 main clusters of sequences. Fig. 2 shows the phylogenetic tree inferred from the 16Snad1, were the 5 main cluster are made evident. The five clusters match exactly the 5 species/morph initially recognised in the morphological study. We further sequenced one individual from each cluster for the nuclear genes 28S (749 aligned characters), H3 (327) and EF1g (768). TCS network if each gene is shown in Fig. 3. All clusters shown exclusive nuclear sequences whit deep genetic divergences among them. The closest sequences were those of the two *N. raripilia* morphs, that in the 28S only shown 1 mutation difference.

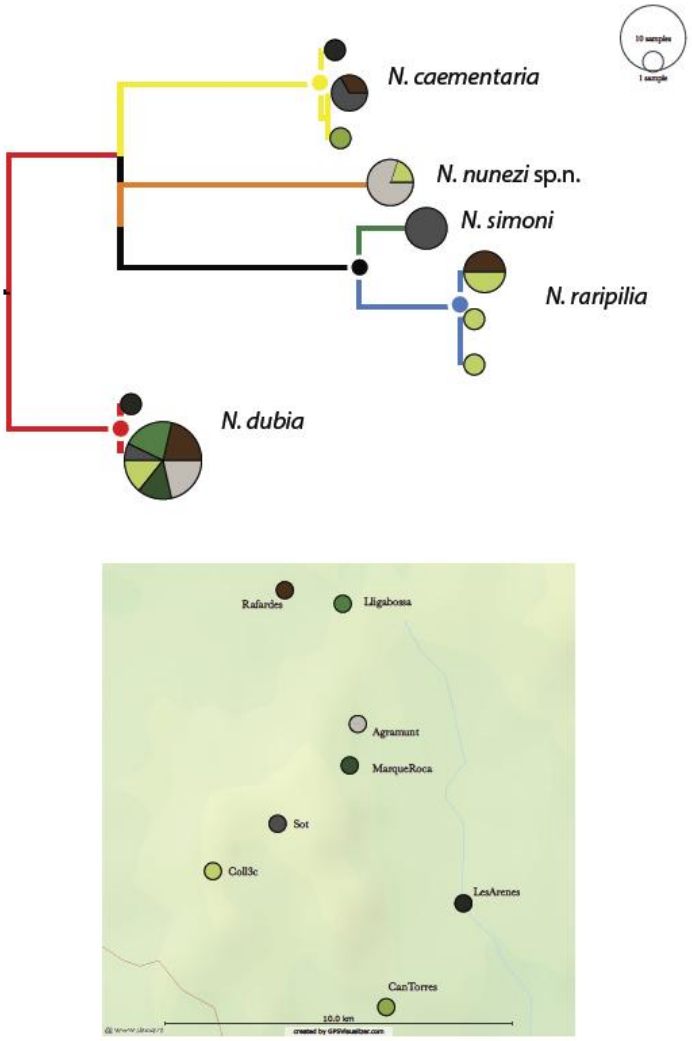


Figure 4. Phylogenetic tree inferred from 16_nad1 matrix. The area of pie plots is proportional to the number of individuals found for each allele. Pie portions represent the proportion of individuals carrying the allele from the same geographic area, as defined in map

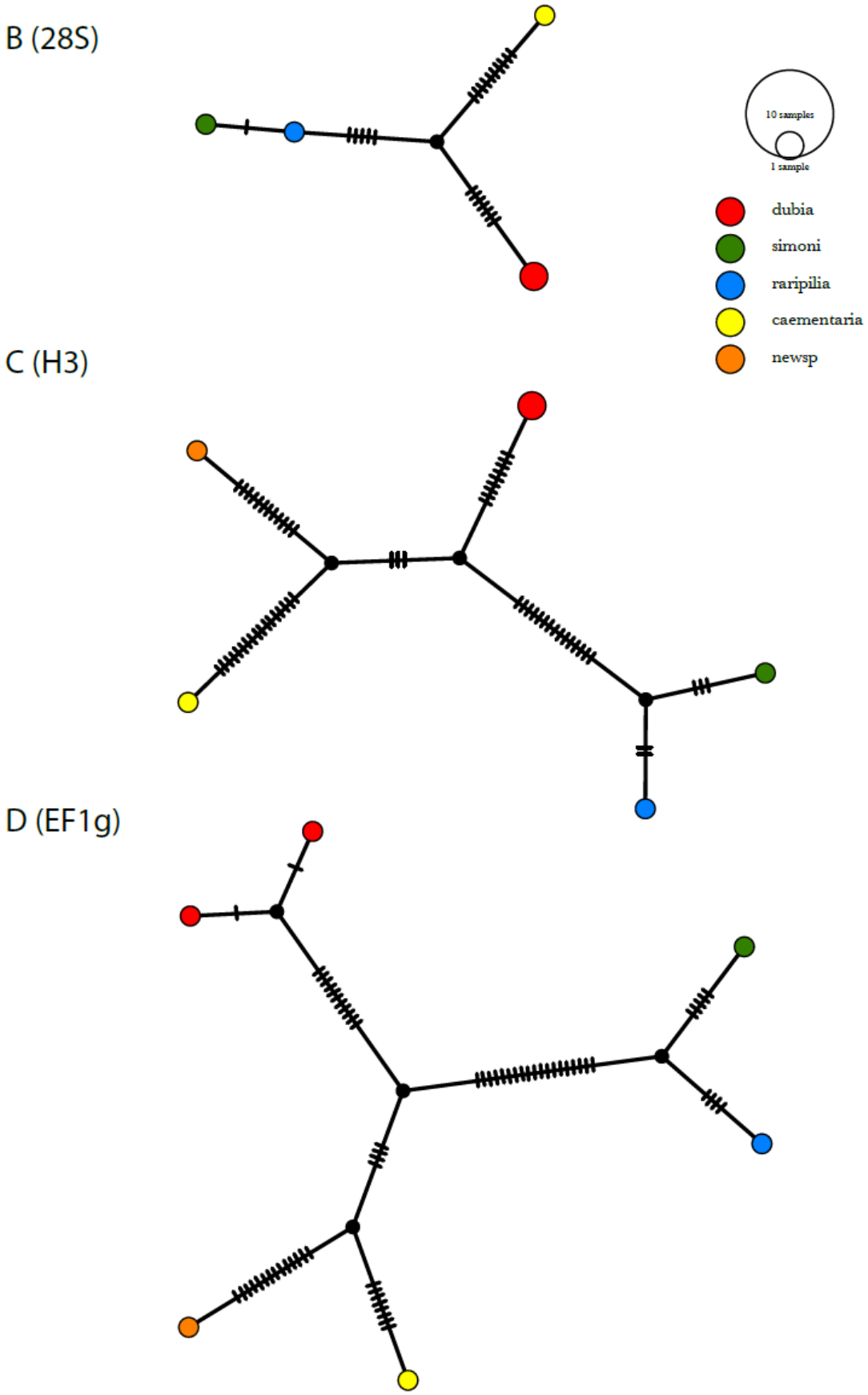


Figure.5. Statistical parsimony networks for different nuclear genes (28S, H3, EF1g).The area its proportional to the number of individuals found for each allel Colors are according to the species. Small lines indicate missing or exctinct alleles.

3.2 Geometric Morphometrics

Due to the high number of samples required for GMM analysis, here we focused in studying morphological differences in the two morphs originally identified as belonging to *N. raripilia* in two different forest types. Population 1 included colonies of *N. raripilia* in *Q. ilex* forest, where also populations of *N. ubia* were present. Population 2 included colonies of *N. raripilia* in *Q. pretraea*. Here we refer as colonies a group if individuals living in close proximity (less that 10 m^2). Geometric morphometric analyses were performed to find further morphological evidence to separate the two morphs identified by subtle spermatheca differences and supported by genetic divergences.

A total of 207 specimens were collected in 25 localities; including males, females and juveniles. After removing males and juveniles, 163 left were digitalized for analysis (Information is summarised in Table 1), 70 females of *N. raripilia* "Q. ilex", 78 females of *N. raripilia pop. "Q. petraea".* In addition, 15 females of the morphologycally divergent *N. dubia* were included as a control.

According to the mygalomorph lyfe-cycle, an allometry regresion was performed. It allowed removal of the within-species allometric variation. P-values were significative for all habitats and species. After size correction MorphoJ gives a size-corrected output. Two views of the spiders were analyzed: dorsal view and lateral view for the Carapace. For Carapace Dorsal View, the first three principal components accumulated 82,737% of variance (Pc1=61,582%, pc2=16,317%, pc3=4,837%). All p-values were significative. Shape changes are of every component are shown in Fig. 6.

The PC1 shows the displacements vectors corresponding to the shape changes on the carapace of *Nemesia raripilia* population 1 being smaller than *the specimens belonging to the population 2* , PC2 corresponds with the shape of *Nemesia raripilia population 2* showing a carapace wider and more robust and PC3 corresponds with the shape of *Nemesia dubia* being oval, smaller and carapace twice larger than wider. The differences between PC1 and PC2 allow to distinguish thanks to the two linneages.

The Principals components for carapace lateral view show the same pattern.

Three component acumulate 79,516% of variance (Pc1=41,650%, pc2=30,67%0, pc3=7,837%). All p-values were significative. Again, the shape changes are shown in the wireframe graph corresponding to the shape changes from different species analised. In this case second principal component shows a higher value, almost as big as the first (Fig. 6).

The differences for lateral view of the carapace clearly show the differences between the species in relation to caput height and carapace lenght. Being *Nemesia raripilia pop 2* the one with highest caput, *Nemesia raripilia* population 1 with caput of intermediate height and *Nemesia dubia* being completely flat Fig. 6.

Its important to consider that PCA doesn't take into account any structure on data, they are usually an exploratory analices. We performed a Canonical Variate analisys because is the most widely used method for investiganting taxonomic differences (Viscosi & Cardini, 2011).

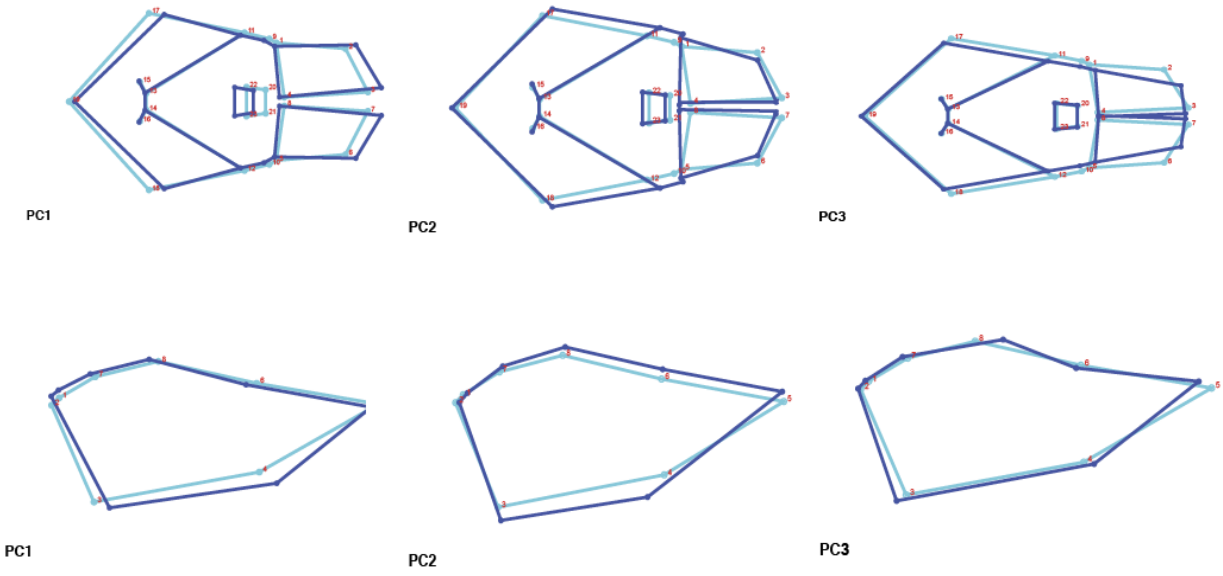


Figure. 6. Wireframe graphs showing variation in the three PCA's that accumulated for all variance. First row in dorsal view of the carapace. Second row on dorsal view. First individual *N. rariplia* from *Q. ilex forest*, second morph for *Q. petraea* forest, third_*Nemesia dubia.*

CVA detected 2 significal canonical variates axes for the dataset of all

specimens, meaning that species differed significantly from the others along the axis. All p-values were significative. For dorsal view of the carapace, the two target species variate along the CV2 (CV1:73,441; CV2:26,559). Visualizing the CVA dispersion graphic we can see that CV1 shows variation along the length of the carapace. *N. raripilia* morph 1 and 2 both show a carapace almost as long as wide, on the contrary the caracape of *N. dubia* is almost twice long than wide. So the CV1 summarizes the variation along the length of the carapace. CV2 resumes the variation in the carapace width, being the morph 2 much wider and robust than morph 1. For lateral view of the carapace variation our target species was along the CV1 (63,247; cv2: 36,753). Visualizing the CVA dispersion graphic we can see that CV1 shows clearly the shape variation produced by differences in the caput height, which allows to distinguish the specimens belonging to the cluster 2 from the two other species. CV2 summarizes the variability also from the length of the carapace. This results are consistent with the genetic differences observed within the two populations and are shown in Fig.7.

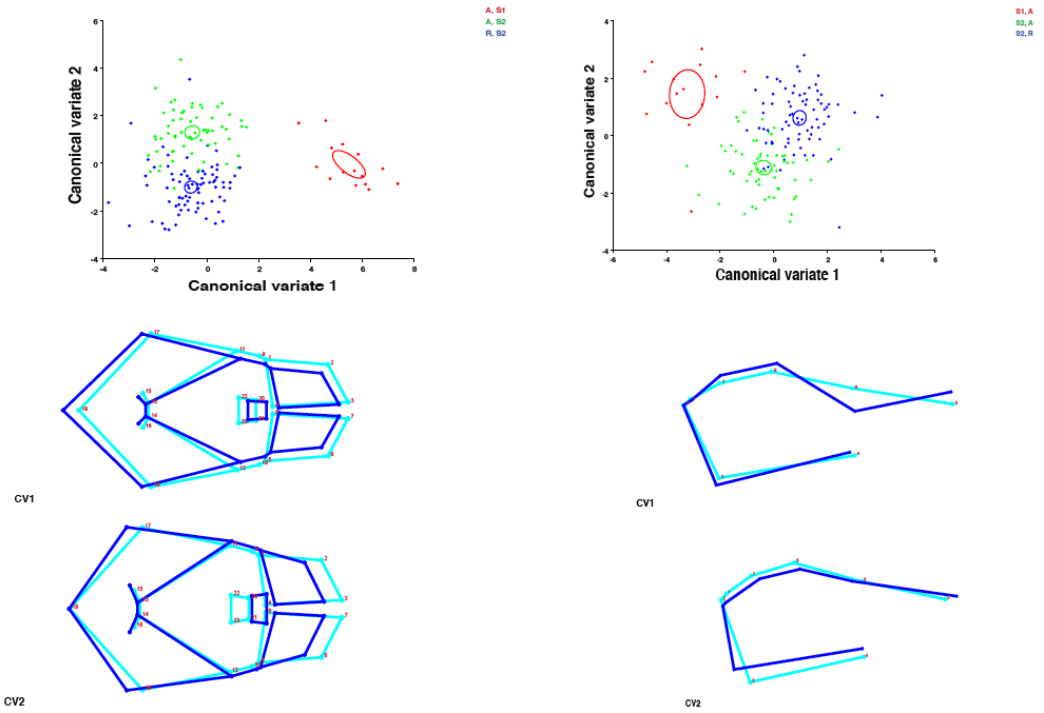


Figure. 7. PC scores for the CVA analysis for carapace dorsal and carapace lateral view (from left to right). Points are colored to different morphs: red for *Nemesia dubia,* green for *Nemesia raripilia* , populations of morph 1 *(Q. ilex* forest) and in blue for second morph in the *Q. pretraea* forest. Under the axis are shown wireframe graphs for each canonical variate.

3.5 Carapace measurements

Males from different lineages are show difference in size. In the case of *Nemesia caementaria* only one male was found. The new specie of *Nemesia* was the smallest showing an average carapace width of only 0,33 mm Again there are considerable differences within the two populations of raripiliaThe measurements of average of carapace width from different specimens are: *Nemesia caementaria* = 0,38 mm (only one specimen found); *Nemesia dubia*=0.38 mm; *Nemesia sp.n*.=0,332 ; *Nemesia raripilia* morph 1= 0,39; *Nemesia rariplia* morph 2 (new sp.n.)rou=0,45

3.6 Phenology

A total of 249 spider specimens were collected in pitfalls during the period May 2011 to February 2013, including 233 males, 11 juveniles and 5 females. The monitoring of male dispersal is shown in Fig. 8. *Nemesia rariplia* was the most abundant specie, 116 males, 4 juveniles and 1 female were caught in the pitfalls. The second was *Nemesia* sp.n. *(nunezi)* with 68 males, 2 juveniles and 3 females. The third most abundant was *N. simoni* 36 males and 4 juveniles. Only 13 males of *N. dubia* were collected and *N. caementaria* was rarest species, only two specimens were, collected one male and one female.

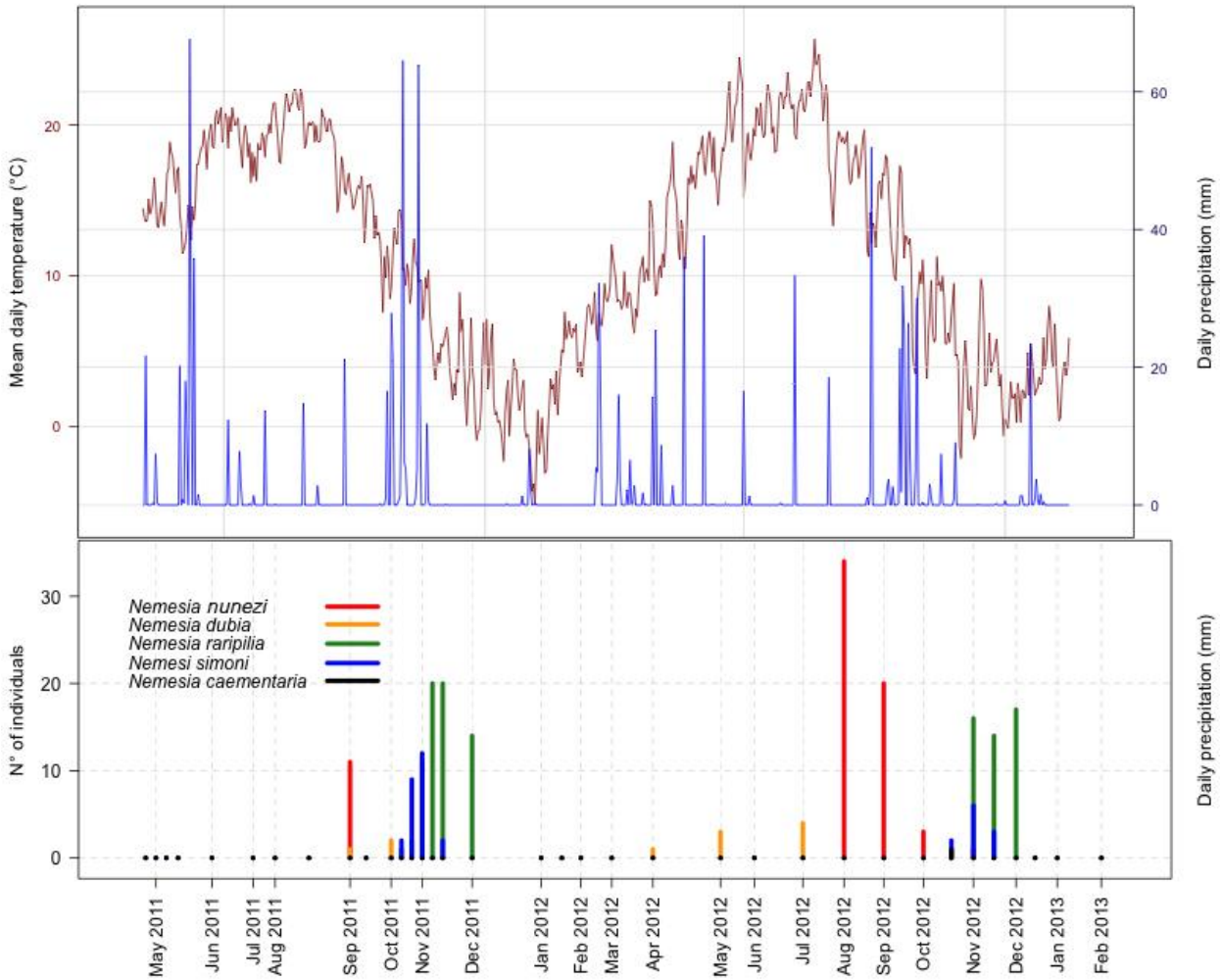


Figure 8 Phenologic chart showing in the upper part Mean Daily Temperature and Daily precipitation (mm). In the lower part the number of individuals collected with pitfall monitirong from May 2001 to February 2003.

The 5 localities used in the study showed differences in their species composition, the number of males found was distributed as follows:

	Agramunt	Coll 3 Creus	Lligabossa	Refardes	Teixoneres
cae	0	0	1	0	0
dub	4	1	5	3	0
new	24	3	28	13	0
rar	0	97	0	19	0
sim	0	0	0	0	36

Nemesia sp.n. (nunezi, morph 2) and *Nemesia dubia* are the two species showing wide distribution and have appeared in 4 of the 5 localities (both were absent at Sot de Teixoneres). *Nemesia caementaria* appears only Lligabossa (in the localities were found females but only one male was caught during the study,). Males of *Nemesia raripilia* were caught in Coll de les 3 Creus and les Refardes. The most restricted preferences are shown by the morph identified as *Nemesia simoni* wich appears only in Sot de Teixoneres.

The phenologic chart shows clearly that the period of male dispersal is different for every lineage. During 2011 not all species were caught in the pitfalls, that's the reason why one more locality was added in spring of 2012.

Dispersal activity

Our results show that different species have different dispersal periods, whith different peaks coinciding with times of higher rainfall periods in the Mediterranean region (spring and autumn).

Nemesia dubia was the specie showing the largest dispersal period. During the first year of monitoring, males of this specie were caught from September to middle October. During the second year, specimens were caught from middle April to middle November, showing a dispersal period of 6 months.. In both years *Nemesia dubia* shows a double peak of dispersal. Males of *Nemesia sp. (nunezi)* were active from end of August until middle October in 2011, and in 2012 the active period was registered from end of May to middle October. Juveniles were found two months before male dispersal.

Nemesia raripilia showed a two months dispersal period. From middle October to middle December. Juveniles and females were found before the peak of male dispersal. *Nemesia simoni* was active only for a month approximately. Near the end to October to end of November in 2011, and was during November in 2012. Juveniles of this specie were found during all the activity period. *Nemesia caementaria* is the specie showing the shorter period of 15 days, being found only once in the beginning of November. One female was caught in July.

3.6 Taxonomy: species revalidation and description of a new specie

Mitocondrial and nuclear information, GMYC species delimitation, GMM results, carapace average width and different phonologic behaviour confirm the morphology and the distinctiveness of those 5 lineages at species that is also confirmed in genitalic morphology. GMM analyses confirmed that slightly differences in shape are correlated with genetic differences and habitat preferences. The specimens previously refered as *Nemesia raripilia* Morph 2 were found only in one locality with dispersal period and slightly differences in their morphology but noticeable differences in female spermatheca..The combination of these different lines of evidence and a extensive bibliographic reaserch allowed us to identified *Nemesia raripilia* population 2 as *Nemesia simoni.*

One clade in genetic cluster anaylisis was not possible to relate to any other nemesian specie known and the combination of different methodologies allows us to describe it as a new specie. Due to the lack of information regarding to genus *Nemesia* and the ancient and ambiguous descriptions, not allowing easy identification. The integration of different methodologies provide enough lines of evidences to make the redescription of the 4 species found following a modern description and the description of a new specie.

Abreviations

CRBA: Centre de Recursos de Biodiversitat Animal, BL: Total Body Length, CW: Carapace Width, CL: Carapace Length; Ca: Caput Length, Cly: Clipeus Width, Th: Thoracic height, EL:,Eye Longitude; ALE: Anterior Lateral eyes, PLE:Posterior Lateral Eyes, PR: Eye Posterior Row, AR= Eye Anterior Row.

Nemesia caementaria, (Latreille, 1799)

Figure.9. *Nemesia caementaria*. First row left to right: Female. Male of *Nemesia caementaria*. Second row:Spermatheca. Copulatory Bulb.

Type material –examined, Musee d' Histoire Naturelle de Paris (MHNP)

Diagnosis

Nemesia caementaria resembles *N. carminans* by the bifid tip of the embolous, but they differ by the distal tips of the embolous, which are curved in *N. carminans* (Fig. 9). The spermatheca shape is characteristic (Fig. 9). Differs from other species by the shape of the tripartite spermatheca with a wide and square receptacle (twice wider than large) that shows an enlarged tubular part that ends in a globular distal receptacle not densely covered by glandular tissue. Dark pattern in carapace allows to distinguish both males and females from other species. Is distinguishable from Iberesia by the two small posterior median spinnrets.

Description.Male CRBAME001815.(Fig. 9). Measurements (mm). BL: 8,85, CW: 3.22, CL:4.164, Ca: 2.553, Th:1.22, EL: 0.438, ALE:0.176, PLE:0.215, PR:

0.805, AR: 0.547. Carapace rounded, slightly longer than wide, CL/CW = 1.29, generally glabrous, yellowish. Pubescence only at the distal part of the caput. Shows a pattern of 4 dark suffused lobulations from the fovea until the margins of the carapace. Caput is elevated and darker than thorax, even darker around the eyes. Few setae on crest of caput and on clypeus(narrow).Eyes steep on ocular tubercle. Eye-group almost as wide as long, AR/EI = 1.24 PR longer than AR, AR/PR = 0.67, ALE shorter than PLE, ALE/PLE = 0.818. Ocular tubercle higher in front, lower behind. POP is broken difficult to appreciate due to the dark coloration of caput distal part. Crest zone with 3 orange lines contrasting with dark caput. The central line is more intense than the two lateral, which are diffused. Fovea deep, curved.

Chelicerae brown but contrasting with the color yellowish carapace.Legs lighter yellow than carapace, with dense pubescence on femora. Abdomen pubescent, mostly around the pedicel with irregular pattern of purple-brownish spots, contrasting with yellow background coloration. Ventral general aspect yellowish colored. Few, reduced spiky cuspules near prolateral, proximal margin. The PLS with few spigots covering the most distal part, small spigots grouped around one central large spigot. The PMS are reduced with few spigots in the distal part. Male bulb with short (half distance than bulb) and curved embolus, with bifid tip. Tibial spur wide at proximal margin, inwardly curved. (revisar). Ventral clasper field on metatarsus I. The ventral clasper is mostly straight, slightly curved inwards at the tip, and thin compared to other species

Female CRBAME001449. (Fig.9) Measurements (mm). BL: 15.098, CW: 4.663, CL:5.82,; Ca:3.81, Cly: 0.25, Th=1.871mm; Ch= 3.146 mm; EI= 0.527 mm; ALE=0.229 mm; PLE=0.202 mm;PR=0.970 mm; AR= 1,012 mm. Large, yellowish. Carapace slightly longer than wide, CL/CW = 1.24, glabrous, yellowish, contrasting with the dark suffused and lobulated pattern of the darker central zones and lighter lateral zones on thorax. The caput is elevated and darker than thorax, even darker around the eyes. Females have two diffused orange spots at both sides of the eye group. Few setae in a row on the crest zone and clypeus. Crest zone with 3 orange lines contrasting with dark caput tapering towards the fovea. The lines show brown diffused spots along them. Fovea is deep and angular. Two lines of fine bristles in the crest zone. Clypeus

is wide (>0.20 mm) . Eye-group almost twice longer than wide, AR/El = 1.92 ; AR almost as longer than PR, AR/PR = 1.04, ALE longer than PLE, ALE/PLE =1.13. Eye formation on steep eye tubercle. POP broken, not including all eyes.

Chelicerae brown, contrasting with the yellowish thorax. The internal side of the chelicerea orange. Row of black bristles at the internal side. Legs yellow, lighter than carapace, with dense pubescence on femora. Abdomen clothed with black hairs, specially on the pedicel, with irregular pattern of pourple-brownish spots contrasting with pale coloration of the whole abdomen. Ventral side yellowish. One row of spiky cuspules near prolateral proximal margin.The spermatheca is enlarged, bipartite without straight conducts. The PLS have spigots covering the distal part, specially on the distal end. Apical spigots grouped around one large central spigot. The PMS are very reduced, with few spigots on the distal end.

Natural history

Its often found in dry places, in slopes exposed to the sun. The burrow is very characteristic by showing a Cork door, which is closed during summer due to aestivation and is frequent to find juveniles from different periods inside (Mora personal observation).

Habitat

Can be found in open areas, in slopes of areas exposed to the sun.

Distribution

This specie is distributed from the South of France to the North-East of Iberian Peninsula

Remarks

Sometimes can be confusion because Simon assigned by mistake the male of *N. carminans* to *N.caementaria*

***Nemesia simoni*,** O. Pickard-Cambridge, 1874

Figure. 10. Nemesia simoni. First row from left to right: Male of N. simoni. Female of N. simoni Second row: Copulatory bulb. Tarsal spur . Spermatheca

Type material, not examined, British Museum of Natural History (BMNH)

Diagnosis

Males of *Nemesia simoni* differs from other *Nemesia* by the bulb ending in a fish.hook tip and shows small ornaments. Despite are very similar to *Nemesia raripilia,* (the bulb is almost identical) males and females differ by some violet spots delimiting eye formation. Females differ from other species by some angulations in the caput slopes and in the shape of the spermatheca. The spermatheca is bipartite, and curved, wide at the base and densely covered by glandular tissue, curves and narrows until a globular distal part which is not densely covered by glandular tissue.

Description. Male.CRBAME001573 (Fig.10). BL: 12.056, CW: 3.954, CL:4.937, Ca: 2.953, Cly: 0.178, Th: 0.834mm, Ch: 1.920, EL: 0.412, ALE:0.2, PLE:0.169, AR:0,773, PR: 0.852. Carapace is oval, orange, longer than wide

CL/CW=1.248, contrasting with the darker pubescence that covers the whole spider. Carapace has a irregular dark patter, irradiating from fovea. Middle distal contour of the carapace is covered by dense hairs. Caput is dark specially the area surrounding the eyes, very high in comparison with other nemesian spiders (Pickard Cambridge, 1874), slopes are steep, fully covered with dark pubescence at both sides of the crest zone. Caput width tapers considerably towards the fovea. Shows a fline of crest and two lines of fine bristles. Crest zone is delimited by black pigmentation, slightly lighter, towards a deep, recurved fovea with a longitudinal groove. Clypeus is narrow with few setae. Eyes on ocular tubercle steep in front sloping behind. Eye-group almost twice as wide as long, AR/El = 1.876 PR longer than AR, AR/PR = 0.907, ALE slitghly larger than PLE. POP unbroken connecting all eyes. Four spots violet-brownish, delimit the margins of Anterior Lateral Eyes and Posterior Laterial Eyes in some kind of “eyebrown” pattern.

Chelicerae are brown and contrasting with the orange baclground of the thorax. Legs are densely covered by hairs specially in the femorae, contributing to the dark coloration of the spider. Abdomen pubescent densely covered, specially at the pedicel with irregular pattern of spots violet-brownish and yellow along the whole abdomen. Present a group of two lines of spiky cuspules near the proximal edge on the maxillae. The PLS show spigots covering the apical part until middle spinneret. The PMS show spigots only in the apical part of distal segment. Embolous is enlarged at proximal part and tapperings trough distal part, ending in a fish-hook tip. This shows two small denticles in the convex part. The appearance in prolateral wiew is completely different. The piriform cymbium tappers and curves until a large and thin bifid tip ornamented with two denticles and the tips are from different length. Clasper, inwardly curved. Ventral clasper field with a short line of stiff hairs.

Female.CRBAME001094. BL: 19,24, CW: 4,976, CL:6,888, Ca: 4,195 , Cly: 0.335 , Th:1,946 , Ch: 3,315 ,EL: 0.549 , ALE:0.341, PLE:0.217,AR: 1,189 , PR:1,222. Carapace larger than wider CL/CW=1,38. with an irregular pigmentation pattern, follows three longitudinal grooves irradiating from fovea. The pattern of color delimits these grooves and the edges of the caput making it steeper slope until the edge of the carapace. Specially darker at the contact

zone with the pedicel. Caput is particulary elevated in this specie; dark at the proximal edge of the carapace and at slopes of caput. Crest zone delimited by lighter areas at both sides and shows two brown lines and a very thin orange line tappering trough the fovea. One line of crest setae is present and two lines of fine bristles. Few setae are present when caput joins the fovea. Clypeus wide in relation to other *Nemesia* species (0.335) with a considerable number of setae. Eyes on ocular tubercle steep, eye-group twice as wide as long. PR slightly longer than AR, AR/PR = 0.97, ALE larger than PLE, ALE/PLE= 1.57. Eyes darker in comparison to other nemesian species. POP unbroken connecting all eyes. Four spots violet-brownish delimit the margins of ALE and PLE in some kind of “eyebrows” pattern.

Chelicerae contrasting with the orange coloration of the thorax. with pa wide line of Black hairs along the chelicerae. Legs are orange, densely covered by. Abdomen pubescent densely covered, specially in the pedicel with irregular pattern of spots violet-brownish and yellow patches.Two lines of spiky cuspules near the proximal edge on the maxillae. The PLS show spigots covering the apical part until middle spinneret. The PMS show spigots only in the apical part of distal segment. Spermatheca is bipartite. Basal part is wide and densely covered with glandular tissue, then middle parts twisted and tappers , the distal presents globular shape occasionally covered with glandular tissue.

Natural history

The burrow is single, quite similar to *raripilia,* but in the case of *simoni* as is reported from O. P.Cambridge that the nest is extremely large and turns and very deep in comparison with the nest of *raripilia*.

Habitat

This specie was found in *Q. petraea* forest, which is rare in Mediterranean areas. Characteristic from the high Mountain Systems such as Pyrenees.

Distribution

South of France, along the Pyrenees and Nort-East of Iberian Peninsula.

Nemesia raripilia, Simon 1914

Figure 11. First row from left to right: Male of *N.rariplia*. Female of *N. raripilia* Second row: Copulatory bulb. Spermatheca

Diagnosis

Males of *Nemesia raripilia* are diagnosable by their bulb with a piriform cymbium which tappers and curves until a large and thin tip ornamented with two denticle, this bulb is easily confused with the bulb of *N. simoni*, but differ by being smaller in size and not showing any spotted pattern around the eyes. Females are distinguishable by their spermatheca which is bipartite, tube shaped no twisted and densely covered at the base with glandular tissue, and globular shaped at distal part. Both show a pattern of longitudinal grooves in the carapace irradinting from fovea, that allows to distinguish them from other nemesian species.

Description. Male CRBAME001209. (Fig.11). Measurements (mm). BL:10.125,

CW: 3.130, CL:4.738, Ca: 2.893, Cly: 0.198, Th:1.27 , Ch: 2.0107, EL: 0.416 , ALE: 0.238, PLE:0.165, AR: 0.802, PR: 0.808. Carapace is orange, elipitical, longer than wider CL/CW=1.513. (Fig, 11.).Shows an irregular dark pattern, pigmentation pattern spreads from the fovea to the proximal margin of the carapace, (hourglass-like). Contour is covered by groups of dense black hairs. Fovea with a deep longitudinal groove. Caput is elevated, delimited by black pigmentation. Crest zone is delimited by black pigmentation. Caput width tapers considerably towards the fovea. A few line of crest setae is present and two lines of fine bristles.Clypeus is narrow, covered with setae. Eyes on ocular tubercle. Eye-group twice wide as long, AR/El =1,95. Eyes from AR only slightly shorter than PR, AR/PR = 0.98, ALE larger than PLE (ALE/PLE=1.44). POP unbroken connecting all eyes. Anterior Lateral Eyes and central eyes have a blue coloration.

Chelicerae orange, with three pubescent brown lines contrasting with the coloration of the thorax. The legs are orange,densely covered by black hairs. Abdomen pubescent, particulary dense hairs the pedicel zone showing an . irregular pattern of spots. Shows a group of two lines of spiky cuspules near the proximal edge on the maxillae. The PLS have spigots distributed along the spinneret. The PMS show spigots only in the distal segment.

The embolous of the bulb is enlarged at proximal part and tapers through distal part, ending in a fish-hook tip. This shows two small denticles in the convex part. Appareance in prolateral view is different, the piriform cymbium tappers and curves until a large and thin tip ornamented with two denticles. .Clasper, inwardly curved. Ventral clasper field with a short line of stiff hairs.

Female.CRBAME01598 (Fig.11); BL= 14,487 mm ; CW= 4,624 ;CL=6,179 mm; Ca= 3,771; Cly: 0,309 mm; Th=1,383 mm; Ch= 2,745 mm; EI= 0.511 mm; ALE=0.237 mm; PLE=0.171 mm; AR= 1,050 mm; PR=0,889. General appearance is dark. Carapace is brownish, longer than wider CL/CW=1.33 with an irregular dark pigmentation pattern, irradiating from fovea (.same as males), follows three longitudinal grooves irradiating from fovea along the carapace. Caput is specially dark at slopes. Crest zone showing two brown lines delimiting an orange one, tapering towards a deep, somewhat angular fovea

with a longitudinal groove. A line of crest setae is present and two lines of fine bristles. Few setae are present when caput joins the fovea. Clypeus is wide, with few setae. Eyes on steep tubercle. Eye-group three times longer than wide,. AR slightly longer than PR, AR/PR = 1.181, ALE is larger than PLE (ALE/PLE= 1.38). ALE eyes are darker in comparison to other nemesian species. POP is connecting all eyes. Females show too a very characteristic coloration pattern around the eyes.

Chelicerae contrasting with the orange coloration of the thorax, in the inner part a wide line of Black hairs along the chelicerae. The legs are orange, but densely covered by hairs specially in the femora, contributing to the dark coloration of the spider.

Abdomen pubescent densely covered, specially in the pedicel with irregular pattern of spots violet-brownish and yellow along the whole abdomen. Ventral general aspect is yellowish . Show a group of two lines of spiky cuspules near the proximal edge on the maxillae. The PLS have spigots covering the whole spinneret. The PMS show spigots only in the distal segment The spermatheca is bipartite, and tubular. Wide at the proximal part densely covered by glandular tissue, ending in a globular part.

Natural History

The bulb of this specie is one single gallery, but filled with high amount of silk.

Habitat

This specie was found in *Quercus ilex* and *Pinus* forests.

Distribution

Nort-east of the Iberian Peninsula and south France.

Nemesia dubia, O Pickard-Cambridge ,1874

Figure 12.. First row from left to right: Male of N.dubia, Female of N. dubia.. Second row:Tarsal spur. Copulatory bulb .Spermatheca

Type material –examined, Musee d' Histoire Naturelle de Paris (MHNP)

Diagnosis

Nemesia dubia is one of the most feasibly species of *Nemesia* recognizable due to the very characteristic pattern in the carapace, in females. The corkscrew spermatheca and the large and slightly curved bulb in the tip of the embolous allow recognition with no confusion. The burrow is branched and sometimes one of the trapdoors is closed by the spider. O-P. Cambridge notes that there can ve a slightly obstruction from one of the entrances that can be easily form away, this matches with our field observations.

Description. Male. CRBAME001808. BL: 11.55 mm, CW: 3.247, CL:5,161,; Ca: 3.016, Cly: 0.125, Th:1.281, Ch: 1.435, EL: 0.472, ALE:0.239, PLE:0.174 , AR: 0.715, PR:0.803. Carapace oval (Fig.12), longer than wide, CL/CW = 1,58, orange contrasting with a characteristic lobulated pattern of dark pigmentation irradiating from fovea along the thorax (hourglass-like) that don't reach the marginal edge. Proximal margin is fully covered by dense black pigmentation. Edge of the carapace delimited by line of hairs along the whole margin. Caput is flat , black pattern delimits a wide and orange crest zone tappering towards fovea. One line with few setae is present and two lines of few bristles. Fovea is deep and curved with a longitudinal groove. Clypeus narrow with few setae. Eye formation on steep eye tubercle, much longer than wider, AR/El = 1.51 ; AR slightly shorter than PR, AR/PR = 0.890 ALE much bigger than PLE, ALE/PLE = 1.37. POP including all eyes.

Chelicerae brown, contrasting with the orange thorax, showing some orange patches and a thick lines of black pubescence at the inner part . Legs yellow, with dense pubescence on femora. Abdomen pale yellow clothed with black pubescence, cardiac mark violet brownish, high density of pubescence in the pedicel. Ventral general aspect is yellowish . Shows two line of thick spiky cuspules near prolateral proximal margin.

The PLS are bipartite with spigots along the whole spinneret. The PMS are thick and knob-shaped fully covered with spigots. The bulb (Fig. 12) in ventral view is large in comparison with the cymbium, thin and slighty curved at the tip. The clasper (Fig. 12) is curved inwards and the metatarsus I shows a dense comb.

Female. CRBAME00101. Measurements (mm). BL:9.810, CW: 2.457, CL:3,759, Ca: 2.191, Cly: 0.2, Th:0.8, Ch: 1.69, EL: 0.391, ALE:0.200, PLE:0.163, AR: 0.593, PR:0.5093. Carapace oval, longer than wide, CL/CW = 1.52, general appearance yellowish-orange contrasting with a characteristic pattern of dark lobulations and dense pubescence irradiating from fovea including lateral slopes of the caput. This pattern don't reach the marginal edge of the carapace..Carapace margin is delimited by a violet line.

Caput is flat (but litlle higher in female). Black pubescence delimits a wide an orange crest zone tapering towards fovea. One line with few setae is present

and two lines of few bristles. Caput slopes are dark contrasting with some irregular orange and diffused patters at both sides of eye formation. Crest zone is wide and orange surrounded by two thin lighter lines contrasting with dark caput, tapering along the caput to the fovea. Fovea is deep and curved. Clypeus= 0.17 is narrow with few setae.

Eye formation longer than wider, AR/El = 1,51 AR sligltly longer than PR, AR/PR = 1.16; ALE larger than PLE, ALE/PLE = 1.22. Eye formation is on steep eye tubercle. POP including all eyes.. Chelicerae brown, contrasting with the yellowish thorax (orange in alcohol) showing some orange patches and a thick line of black pubescence. Legs lighter yellow than carapace, with dense pubescence on femora.

Abdomen is pale yellow clothed with black pubescence, cardiac mark violet brownish with high density of pubescence in the pedicel.

Ventral general aspect is yellowish. Shows two line of thick spiky cuspules near prolateral proximal margin. The PLS are bipartite with spigots along the whole spinneret. The PMS are thick and knob-shaped fully covered with spigots.

The spermatheca (Fig. 12) is tripartite, and twice twisted, also defined as corkscrew shaped (Isaia & Decae, 2012) densely covered by glandular tissue in the proximal part.

Natural history The burrow is branched and sometimes one of the trapdoors is closed by the spider. O-P.Cambridge notes that there can ve a slightly obstruction from one of the entrances that can be easily form away, this matches with our field observations.

***Nemesia nunezi* sp.nov,** Mora 2015

Figure 13.. First row from left to right: Male of *N. Nunezi* . Female of *N. Nunezi*. Second row, from left to right: Tarsal spur. Copulatory bulb) Spermatheca

Holotype. Male. CRBAME001501 . (CRBA)

Paratype 1 Female. Catalonia, Barcelona, PN Sant Llorenç de Munt i la Serra de l'Òbac, Agramunt, same as holotype. CRBAME001505, leg. E. Mora, R. Garcia(CRBAME001505),

Type locality: Catalonia, Barcelona, PN Sant Llorenç de Munt i la Serra de l'Òbac, Agramunt, (41.689/2.02)

Diagnosis

Nemesia nunezi males resemble *Nemesia dubia*, is distinguiable by thin spinnerets not densely covered by spigots and the embolous of copulatory bulb is shorter in relation to *Nemesia dubia,* and has a curved tip..Resembles *to N. bacalarae* from Portugal but is distinguishable by the absence of teeth in the

embolus. The clasper of tarsal I is short and thin in comparison to other nemesian species and the tip is curved. Female spermatheca are similar to N. bacelare, also “mushroom”-like (sensu Deace, 2005) but differ in the tubular part that connects with distal receptacle.

Etymology

The name is in appostion to Marc Nuñez which help during the whole development of this study was invaluable. Without his help this specie will remain unkown.

Description.Male. Holotype CRBAME001051. Measurements (mm). BL: 10.117, CW: 3,09, CL: 4,209, Ca: 2.588, Cly: 0.159, Th:1,114, Ch:1.82, EL: 0.399, ALE:0.230, PLE=:0.143, AR: 0.774, PR:0,757. Carapace is orange, oval (Fig. 13), larger than wider, CL/CW = 1,36. with a characteristic “star” pattern of dark pigmentation irradiating from fovea along the thorax).This pattern reach almost the marginal edge of the carapace. The margin of the carapace is delimited by a purplish line along the whole edge wich is also densely covered by dense pubescence along the margin.

Caput is flat, slopes delimited by pigmentation, with a wide an orange crest zone tapering towards fovea with three lines of crest setae. Fovea is deep and somewhat angularClypeu is narrow with few setae. Eye formation is on steep eye tubercle, almost as twice wide as long, AR/El = 1.99 AR almost as larger as PR, AR/PR = 1.02 ALE much bigger than PLE, ALE/PLE = 1,608. POP is broken, not including all eyes.

Chelicerae are orange, contrasting with the coloration of the thorax showing two a thick lines of black pubescence. Legs lighter orange than carapace, with dense pubescence on femora. Abdomen pale yellow clothed with dense black pubescence, cardiac mark violet brownish, high density of pubescence in the pedicel. Abdomen shows a symmetric pattern of violet patches.

Ventral general aspect is yellowish. The PLS are bipartite with spigots only in the distal part of the spinneret. The PMS are reduced and conical fully covered with spigots only in the apical part. The embolous in ventral view us short in comparision to the cymbyum. The tip of the embolus is thin (Fig. 13), tapering

through the tip and curved like a fishhook. The clasper (Fig.13)is short, thin and twisted. Small fine bristles are present on metatarsus I not exceeding the longitude of the claspers.

Paratype: CRBAME001505. Female. Measurements (mm). BL: 12.765, CW: 3.142, CL:4.644, Ca: 2.958, Cly: 0.17, Th:1.906, Ch: 1.361, EL: 0.442, ALE:0.267, PLE:0.204, AR: 0.546, PR:0,859. Carapace oval, longer than wide, CL/CW = 1,478, orange, contrasting with a characteristic " star" pattern of dark pigmentation irradiating from fovea along the thorax extended to the margins of the carapace. The pigmentation is darker in the lateral slopes of the caput. Carapace margin delimited by a brown line at proximal edge of the carapace. Caput is elevated, shows a wide orange crest zone tappering towards fovea. Fovea is deep and curved. One line with few setae is present and two lines of few bristles. Caput slopes are dark contrasting with some irregular orange and very thin diffused orange coloration around the eyes. Some setae are present at the junction of the caput with the fovea. Clypeus= 0.17 is narrow with few setae.

Eye formation is almost as twice longer than wider, AR/El = 0.1914; AR is not longer than PR, AR/PR = 1.16 ALE is bigger than PLE, ALE/PLE = 1,30. Eye formation on steep eye tubercle. POP broken, not including all eyes.

Chelicerae dark brown, contrasting with the orange thorax showing a thick line of black pubescence. Legs lighter orange than carapace, with dense pubescence on femora.

Abdomen pale yellow clothed with black pubescence, cardiac mark violet brownish , high density of pubescence in the pedicel. Abdomen shows a symmetric pattern of violet patches. Ventral general aspect is yellowish. Shows two lines of thick spiky cuspules near prolateral proximal margin. The PLS are bipartite with spigots along the whole spinneret. The PMS are thick and knob-shapped fully covered with spigots. The spermatheca is bipartite, the shappe have been defined befote as mushroom like

4.Discusion

Species delimitation analysis

Mygalomorph spiders, specially *Nemesia*, being the most divers genus in the Mediterranean region, is a real challenge for evolutionary biologists. The ability to fingerprint the evolutionary processes which allowed a high level of diversification is underestimated due to the difficulty to delimit species boundaries. The high genetic structure due to their lack of dispersal and the morphological conservatism is a real nightmare when evolutionary biologists try to decipher the drivers of diversification in this group(Bond et al., 2003; Opatova et al., 2013; Opatova & Arnedo, 2014). Despite there is a common need about a definition of what a specie is, this is still a major debate in the scientific community. There is a commom consense that species are populations or metapopulations that evolve independently, but dealing with poor dispersers with phenotipically conserved, establishing a threshold of what a specie is requires a high level of confidence in the methodology used.

Molecular results show the existence of five independent evolutionary lineages, and therefore are confirmed using differences sources of evidence. Despite the incorporation of genetic data in systematics has been a key factor for discovery hidden diversity, employing different lines of evidence like geometric morphometrics is very important because they are better for detecting subtle differences in overall changes in shape. If this study had included only traditional observations, two cryptic and sister species *(N. simoni* and *N. raripilia*) wouldn't have been recognised due to their similarities. The incorporation of methodologies such as geometric morphometrics rather than traditional morphometrics provide a usefull tool for taxonomist and systematitians for species delimitation and description.

Phenological studies can play an important role in integrative taxonomy studies, especially because very few is known about mygalomorph spiders and its life cycle. Only some literature from middle sixties' from is known. The gap of information of mygalomorph life cycle can be an impediment for landscape genetic studies due to the lack of information of basic life cycle traits. Our results show a clear stationaly that coincides with the two rainy periods of the

Mediterranean region: spring and autumn. But this result is not surprising because it is commonly know that mygalomorph spiders are humidity-dependant due to the existence of the 4 book-lungs which expose the inner body directly to the environment (Foelix, 1996). During the first period of monitoring, not all the species were found. The addition of an extra locality shows the importance of the sampling effort to increase the probability of detecting the species (Engelbrecht, 2013). Otherwise the male of *N. caementaria* wouldn't have been found. Also *Nemesia caementaria* is the only nemesian specie whereby is reported that female eats male after the mating, this may be a reason why less only one male could be found. Also there are some references that noticed that the reproduction of *Nemesia Nemesia caementaria* is biannual, and that different generations of juveniles can be found in the same nest. This fact is confirmed by our fieldwork where some nests of *N. caementaria* appeared different juveniles form two different sizes (Mora pers obs.).

Different species were active at different periods along the year with variyng durations. This was already been demosntradet for antriodaedids in North Africa (Coyle et al., 1985) .Even more, not all species were found in the same habitats, this can be an indicator as habitat preference (Engelbrecht, 2013). Discrete periods of activity, or phenology, of adult male mygalomorphs have been demonstrated for for antrodiaetids in North America (Coyle et al., 1985) . Recent studies in *Atypus affinis*, a Mediterranean widespread mygalomorph, showed that is the habitat use and not the limitations of dispersal the phenomena behind the aggregated population structure shown by ths mygalomorph (Řezáč et al., 2007; Deruytter et al., 2012). Despite *Atypus* can make ballooning, this have been not reported to *Nemesia* yet. Molecular ongoing studies point that is the limited dispersal hability behind the high population structure in *Nemesia* being most of the species local endemics.

Different species were found in different kind of habitats. There is a particular pattern in this results. The most widespread species, which males have the activity period in a season with high temperatures, also are the ones living in more dried habitats and exposed to sun radiations. *Nemesia dubia* is a clear example. Sampling by direct capture methods, *Nemesia dubia* is easily found in

slopes exposed to the sun light and it becames difficult to find as deeper as go we into the forest. On the contrary, *N. raripilia* and *N. simoni* have a very restricted dispersal coinciding with the raniy period and the lower temperatures . *N. simoni* is only found in the *Q. petraea* forest. The distribution of Q. petraea forest is not commom in such Mediterranean zone. Due to climatic phenomena it is stablished a micro climate typical from the Pyrenees (Fusalba, 1986; Fusalba,1995). This is the reason behind presence of *Q. petraea* and therefore it may explain the presence of *N. simoni*, wich distribution is typical from the Pyrynees.

The species show in the Natural Park clearly show habitat preferences but deeper studies on meteorological conditions, vegetal coverage and soil characteristics are needed. Different species showed different dispersal peaks, with maximum of activity that don't overlap, this is a clear indicator that the different species of *Nemesia*, in fact, use spatial separation for avoiding competition. We also detected microhabitat preferences, meaning that differences species have clear habitat preferences. This pattern has already been found. Recent publications demonstrated that in the genus *Attypus* ,who has been documented that disperses through ballooning(Deruytter et al., 2012), the habitat selection is the reason behind the aggregated colonies(Řezáč et al., 2007; Michalko & Pekár, 2015).

5. Conclusions

Integrative taxonomy plays a key role for dealing with spiders from the genus *Nemesia*. Integrating different lines of evidence makes easier the species delimitation process in those challenging organisms. In this particular case, the integrative taxonomy its crucial at the point of defining species boundaries. In this particular study different species of *Nemesia* were found coexistin. Coexistence is maintained due to mechanisms of spatial and temporal separation such as habitat selection and differences in dispersal period respectively.

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Table 1. Specimens sequenced, and localities. Sampling localities are classified according to the specific pat of the study DC (direct capture), GMM localities sampled for the geometric morphometric study, P indicates localities where pitfall trapping monitoring was conducted.

DNA	Labels	Specimen code	Genus	sp.	Sex	Sampling	Vegetation	Loc_name	Alt	Lat	Long	16Snad1	28S	EFG	H3
E118	E118_M_P_I2	CRBAME001107	Nemesia	nw	M	DC	Pinus & bushes, post-fire	Agramunt	547	41.68674	2.02151	E118	E118	E118	E118
E2	E2_F_H_I2	CRBAME000245	Nemesia	dubia	F	DC	Pinus & bushes, post-fire	Agramunt	557	41.68669	2.02141	E2			
RA199	RA199_F_H_I2	CRBAME000242	Nemesia	sp	F	DC	Pinus & bushes, post-fire	Agramunt	557	41.68669	2.02141	RA199			
			Nemesia	new	M	DC	Pinus & bushes, post-fire	Agramunt (Pitfall 1)	551	41.68693	2.02118				
			Nemesia	new	M	P	Pinus & bushes, post-fire	Agramunt (Pitfall 2)	551	41.6892	2.02122				
E48	E48_M_P_I2	CRBAME000906	Nemesia	new	M	P	Pinus & bushes, post-fire	: Agramunt (Pitfall 3)	551	41.68693	2.02118	E48			
E113	E113_M_P_I2	CRBAME000911	Nemesia	new	M	P	Pinus & bushes, post-fire	Agramunt (Pitfall 4)	553	41.68696	2.02141	E113			
E114	E114_M_H_I2	CRBAME001086	Nemesia	dub	M	P	Pinus & bushes, post-fire	Agramunt (Pitfall 4)	553	41.68696	2.02141	E114			
E49	E49_M_P_I2	CRBAME000910	Nemesia	new	M	P	Pinus & bushes, post-fire	Agramunt (Pitfall 4)	553	41.68696	2.02141	E49			
			Nemesia	dub	M	P	Pinus & bushes, post-fire	Agramunt (Pitfall 5)	547	41.68705	2.02094				
E38	E38_F_P_I2	CRBAME000250	Nemesia	caementaria	F	DC	slope forest quercus ilex, pinus	Can Torres	519	41.61313	2.03147	E38			
Z66	Z66_J_P_I2	CRBAMM000066	Nemesia	sp.	1 juv	DC	q ilex forest, with buxus sempervirens	Coll de les Tres Creus (Loc1)	874	41.64839	1.97175	Z66			
Z77	Z77_F_P_I2	CRBAMM000077	Nemesia	raripilia	1 f	DC	q ilex forest, with buxus sempervirens	Coll de les Tres Creus (Loc1)	874	41.64839	1.97175	Z77			
Z83	Z83_J_H_I2	CRBAMM000083	Nemesia	sp.	1 juv	DC	q ilex forest, with buxus sempervirens	Coll de les Tres Creus (Loc1)	874	41.64839	1.97175				
E117	E117_F_H_I2	CRBAME001104	Nemesia	dubia	F	DC	q ilex forest, with buxus sempervirens	Coll de les Tres Creus (Loc2)	873	41.64872	1.972	E117	E117	E117	E117
E47	E47_F_H_I2	CRBAME001099	Nemesia	dubia	F	DC	q ilex forest, with buxus sempervirens	Coll de les Tres Creus (Loc2)	873	41.64872	1.972	E47			
E52	E52_M_P_I2	CRBAME001152	Nemesia	raripilia	M	DC	q ilex forest, with buxus sempervirens	Coll de les Tres Creus (Loc2)	873	41.64872	1.972	E52			
			Nemesia	raripilia	J	P	q ilex forest, with buxus sempervirens	Coll de les Tres Creus (Pitfall 1)	870	41.64872	1.972				
			Nemesia	raripilia	M	P	q ilex forest, with buxus sempervirens	Coll de les Tres Creus (Pitfall 2)	873	41.64892	1.97182				
	E286_M_P_I2	CRBAME001864	Nemesia	raripilia	M	P	q ilex forest, with buxus sempervirens	Coll de les Tres Creus (Pitfall 3)	874	41.649	1.97176				
			Nemesia	new	M	P	q ilex forest, with buxus sempervirens	Coll de les Tres Creus (Pitfall 3)	874	41.649	1.97176				
E51	E51_M_P_I2	CRBAME000916	Nemesia	new	M	P	q ilex forest, with buxus sempervirens	Coll de les Tres Creus (Pitfall 4)	873	41.64911	1.97173	E51			
			Nemesia	new	M	P	q ilex forest, with buxus sempervirens	Coll de les Tres Creus (Pitfall 5)	873	41.64919	1.97163				
			Nemesia	sp.	J	GMM	q ilex forest, with buxus sempervirens	Coll de les Tres Creus A1 L10	865	41.64946	1.97157				
			Nemesia	sp.	F	GMM	q ilex forest, with buxus sempervirens	Coll de les Tres Creus A1 L10	847	41.64948	1.97292				
			Nemesia	dubia	F	GMM	q ilex forest, with buxus sempervirens	Coll de les Tres Creus A1 L2	875	41.64887	1.97147				
			Nemesia	sp.	F	GMM	q ilex forest, with buxus sempervirens	Coll de les Tres Creus A1 L3	874	41.64869	1.9718				
			Nemesia	sp.	F	GMM	q ilex forest, with buxus sempervirens	Coll de les Tres Creus A1 L3	874	41.64869	1.9718				
			Nemesia	sp.	J	GMM	q ilex forest, with buxus sempervirens	Coll de les Tres Creus A1 L4	870	41.64865	1.97197				
			Nemesia	sp.	F	GMM	q ilex forest, with buxus sempervirens	Coll de les Tres Creus A1 L5	871	41.64878	1.97167				
			Nemesia	sp.	F	GMM	q ilex forest, with buxus sempervirens	Coll de les Tres Creus A1 L6	871	41.64883	1.91175				
			Nemesia	sp.	F	GMM	q ilex forest, with buxus sempervirens	Coll de les Tres Creus A1 L7	872	41.64888	1.9717				

DNA	Labels	Specimen code	Genus	sp.	Sex	Sampling	Vegetation	Loc_name	Alt	Lat	Long	16Snad1	28S	EFG	H3
			Nemesia	sp.	F	GMM	q ilex forest, with buxus sempervirens	Coll de les Tres Creus A1 L7	872	41.64888	1.9717				
			Nemesia	sp.	F	GMM	q ilex forest, with buxus sempervirens	Coll de les Tres Creus A1 L8	871	41.64867	1.97195				
			Nemesia	sp.	F	GMM	q ilex forest, with buxus sempervirens	Coll de les Tres Creus A1 L8	871	41.64867	1.97195				
			Nemesia	sp.	J	GMM	q ilex forest, with buxus sempervirens	PN Sant Llorenç del Munt:	870	41.64885	1.9718				
			Nemesia	sp.	F	GMM	q ilex forest, with buxus sempervirens	Coll de les Tres Creus A1L1	875	41.64865	1.97169				
E41		CRBAME000264	Nemesia	sp.	F	DC	grass and pinus , near river	Les Arenes	361	41.64024	2.05818	E41			
E7	**E7_F_H_I2**	CRBAME000268	Nemesia	dubia	F	DC	grass and pinus , near river	Les Arenes	361	41.64024	2.05818	E7			
			Nemesia	new	M	P	pinus halepensis near small river	Les Rafardes (Pitfall 1)	553	41.7208	1.99685				
E50	E50_M_H_I2	CRBAME000915	Nemesia	dub	M	P	pinus halepensis near small river	Les Rafardes (Pitfall 3)	513	41.72094	1.99679	E50			
			Nemesia	raripilia	M	P	pinus halepensis near small river	Les Rafardes (Pitfall 3)	513	41.72088	1.9967				
			Nemesia	raripilia	M	P	pinus halepensis near small river	Les Rafardes (Pitfall 4)	465	41.721	1.9965				
E54	E54_M_P_I2	CRBAME001145	Nemesia	raripilia	M	P	pinus halepensis near small river	Les Rafardes (Pitfall 5)	504	41.7212	1.99637	E54	E54	E54	E54
E3	E3_F_P_I2	CRBAME000251	Nemesia	sp.	F	DC	pinus halepensis near small river	Les Rafardes Loc 1	272	41.72094	1.99679	E3			
E37	E37_F_P_I2	CRBAME000243	Nemesia	caementaria	F	DC	pinus halepensis near small river	Les Rafardes Loc 2	272	41.72094	1.99679	E37			
E39	E39_F_H_I2	CRBAME000254	Nemesia	dubia	F	DC	pinus halepensis near small river	Les Rafardes Loc 3	272	41.72094	1.99679	E39			
E4	E4_F_H_I2	CRBAME000252	Nemesia	dubia	F	DC	pinus halepensis near small river	Les Rafardes Loc 4	272	41.72094	1.99679	E4			
E40	E40_J_H_I2	CRBAME000255	Nemesia	sp.	J	DC	pinus halepensis near small river	Les Rafardes Loc 5	272	41.72094	1.99679	E40			
E5	E5_F_H_I2	CRBAME000260	Nemesia	sp	F	DC	bush (hyparrenia hyrta), post-fire	Lligabossa	557	41.7177	2.0164	E5			
E6	**E6_F_H_I2**	CRBAME000262	Nemesia	dubia	F	DC	bush (hyparrenia hyrta), post-fire	Lligabossa	557	41.7177	2.0164	E6			
RA200	RA200_F_H_I2	CRBAME000258	Nemesia	sp	F	DC	bush (hyparrenia hyrta), post-fire	Lligabossa	557	41.7177	2.0164	RA200	RA200	RA200	RA200
			Nemesia	new	M	P	bush (hyparrenia hyrta), post-fire	Lligabossa (Pitfall 1)	706	41.71852	2.01654				
			Nemesia	new	M	P	bush (hyparrenia hyrta), post-fire	Lligabossa (Pitfall 2)	642	41.71859	2.01658				
			Nemesia	new	M	P	bush (hyparrenia hyrta), post-fire	Lligabossa (Pitfall 4)	638	41.71865	2.01659				
			Nemesia	new	M	P	bush (hyparrenia hyrta), post-fire	Lligabossa (Pitfall 5)	638	41.71878	2.01659				
E44	E44_J_H_I2	CRBAME000240	Nemesia	dubia	J	DC	paisatge de ribera, humit, vall d'horta	Marquet Roques	561	41.676	2.01882	E44			
RA198	RA198_F_H_I2	CRBAME000237	Nemesia	dubia	f	DC	paisatge de ribera, humit, vall d'horta	Marquet Roques	561	41.676	2.01882	RA198			
			Nemesia	sp.	J	GMM	Quercus ilex open forest	Alzinar L1	795	41.65842	1.98298				
			Nemesia	sp.	F	GMM	Quercus ilex open forest	Alzinar L2	795	41.65834	1.98228				
			Nemesia	sp.	F	GMM	Quercus ilex open forest	Alzinar L3	795	41.65834	1.98228				
			Nemesia	sp.	F	GMM	Quercus ilex open forest	Alzinar L3 C1	7801	41.65797	1.98264				
			Nemesia	sp.	F	GMM	Quercus ilex open forest	Alzinar L3 C2	791	41.65797	1.98256				
			Nemesia	sp.	F	DC	q. ilex mixed with pinus,	slope in a north-oriented Main rd		41.65783	1.9831				
E42	E42_J_P_I2	CRBAME000072	Nemesia	caementaria	j	DC	Slope, open area, mainly bushes	slope on the margin of Road near to Sot Teixoneres		41.66109	1.99198	E42	E42	E42	E42

DNA	Labels	Specimen code	Genus	sp.	Sex	Sampling	Vegetation	Loc_name	Alt	Lat	Long	16Snad1	28S	EFG	H3
E43	E43_F_H_I2	CRBAME000077	Nemesia	dubia	F	DC	Slope, open area, mainly bushes	slope on the margin of Road near to Sot Teixoneres		41.66109	1.99198	E43			
			Nemesia	sp.	F	DC	Slope, open area, mainly bushes	slope on the margin of Road near to Sot Teixoneres		41.66109	1.99198				
E53	E53_M_P_I2	CRBAME001108	Nemesia	simoni	M	P	quercus petraea forest	Sot Teixoneres (Pitfall 1)	799	41.66065	1.99457	E53			
			Nemesia	simoni	M	P	quercus petraea forest	Sot Teixoneres (Pitfall 2)	801	41.66066	1.99489				
			Nemesia	simoni	M	P	quercus petraea forest	Sot Teixoneres (Pitfall 3)	803	41.66066	1.99489				
			Nemesia	sp.	M	P	quercus petraea forest	Sot Teixoneres (Pitfall 4)	806	41.66044	1.99495				
			Nemesia	simoni	J	P	Quercus petraea forest	Sot Teixoneres (Pitfall 5)	806	41.66034	1.99503				
E115	E115_F_P_I2	CRBAME001093	Nemesia	sp.	F	DC	quercus petraea forest	Sot Teixoneres 2	813	41.66013	1.9949	E115	E115	E115	E115
E116	E116_F_P_I2	CRBAME001094	Nemesia	sp.	F	DC	quercus petraea forest	Sot Teixoneres 3	813	41.66013	1.9949	E116			
E45	E45_J_P_I2	CRBAME001097	Nemesia	sp	J	DC	quercus petraea forest	Sot Teixoneres 4	813	41.66013	1.9949	E45			
			Nemesia	sp.	J	GMM	q petraea mixed with q ilex	Sot Teixoneres 2 L1 C1	907	41.66216	2.00397				
			Nemesia	sp.	J	GMM	q petraea mixed with q ilex	Sot Teixoneres 2 L1 C3	931	41.66232	2.00391				
			Nemesia	sp.	F	GMM	q petraea mixed with q ilex	Sot Teixoneres 2 R2 L2	904	41.6624	2.00323				
			Nemesia	sp.	F	GMM	q petraea mixed with q ilex	Sot Teixoneres 2 R2 L3	900	41.66207	2.00287				
E46	E46_F_P_I2	CRBAME001140	Nemesia	caementaria	F	direct_capture	Quercus petraea forest	Sot Teixoneres 3	485	41.660429	1.995547	E46			
			Nemesia	sp.	F	DC	Quercus petraea forest	Sot Teixoneres R1 L1 C1	796	41.66072	1.99466				
			Nemesia	sp.	F	GMM	Quercus petraea forest	Sot Teixoneres R1 L1 C2	787	41.66061	1.99462				
			Nemesia	sp.	F	GMM	Quercus petraea forest	Sot Teixoneres R1 L2	847	41.66037	1.99496				
			Nemesia	sp.	F	GMM	Quercus petraea forest	Sot Teixoneres R1 L3 C2	813	41.66079	1.99601				
			Nemesia	sp.	J	GMM	Quercus petraea forest	Sot Teixoneres R1 L3 C4	821	41.66075	1.9961				

5. General Discussion

General discussion

The results of the present study represent a major contribution towards our understanding of the systematics of the western Mediterranean mygalomorph genera *Nemesia* and *Iberesia* and the evolutionary processes responsible for their diversification.

Because of their cryptic lifestyle and conservative morphology, mygalomorphs have posed important challenges to taxonomists and their potential use as model organisms for biodiversity research has not been fully developed. Before the present study, knowledge on Mediterranean nemesiids was restricted to their taxonomy and distribution, and some sporadic accounts on their behavior and natural history. Molecular studies had not been conducted on the group and little was known about their evolutionary history.

Here we used an integrative approach that combined molecular systematics using multiple locus, traditional taxonomy, geometric morphometrics and phenological studies to decipher the origins, the drivers of diversification and the mechanisms of coexistence of Mediteranean nemesiids. We reconciled our results with former taxonomic studies to establish the foundations of the future research on the ecology and evolution of the group.

Specific results have been discussed in detail in each chapter. In this section, I will summarize some of the main findings, highlight and discuss some of the general patterns and discuss future prospects on mygalomorph spider research. In the first chapter, we unravel the origins of the genus *Nemesia* and investigate the drivers of diversification that generated its high species richness. We provided the first molecular phylogeny of the Mediterranean nemesiids, which recovered the reciprocal monophyly of *Nemesia* and *Iberesia* (Decae & cardoso, 2005, Decae 2007).

The degree in spinneret reduction had been used to define supra-specific groups in Mediterranean nemesiids (Decae, 2010). The lost of the PLS is the main synapomorphy that defines *Iberesia* (Decae and Cardoso, 2006). Mapping spinneret morphology on our preferred tree revealed that the Holonemesia type was monophyletic and evolved from a *Pronemesia*-like ancestor. Furthermore,

a more detailed examination of the different spinneret morphology suggested that spinneret types can be subdivided further in additional groups. Interestingly, we detected a relationship between spinneret morphology and burrow architecture: the *Holonemesia* type is found in species that construct more complex burrows and trap-doors, while Pronemesia like species construct simplier burrows similar to those in *Iberesia*. This observation indicates that the reduction on the spinning apparatus directly influence the architecture of the burrows.

We found support for the origin of *Nemesia* in the Betic Cordillera. *Nemesia* split from *Iberesia*, its siter taxa, approximately 40 Ma (56.2–27 Ma), which predates the opening of the Western Mediterranean Basin (Rosembaun et al 2002). Subsequent divergences roughly match the geochronology of fragmentation of the Hercynian Belt, dated at 30-25 Ma.

We propose that the diversification of *Nemesia* and its present day presence in the micropates that once formed part of Iberia was driven by the vicariant events following the fragmentation of the Hercynian belt and the subsequent drift of their main blocks.

The complex pattern of multiple independent lineages on the major islands and some of the former Iberian terrains suggest that the ancestor of the lineages were already present in the Hercynian belt or that they dispersed from other regions taking advantage of the the land bridges emerged during subsequent sea level oscillations, first during the Messinian Salinity Crises then with the Pleistocene.Unfortunately, internal relationships were not always well supported, and hence more effort will be required to provide a fully, well supported phylogeny to study biogeographic patterns. In this regard, we have been working in developing novel nuclear markers using next generation sequencing approaches specifically designed for *Nemesia*.

The GMYC method has been largely used to delimit evolutionary lineages in arthropods (Bidegaray-Batista & Arnedo, 2011; Planas et al., 2013; Hendrixson et al 2013, Hamilton et al, 2014; Opatova & Arnedo, 2014) but has been demonstrated to over split the number of lineages (Satler et al., 2013; Talavera et al., 2013). This behavior is specially pervasive in mygalomorphs due to their

poor dispersal abilities. The GMYC method delineated 183 coalescent clusters in *Nemesia*. In most cases, clusters corresponded to single or nearby localities, in agreement with the deep population structure usually recovered in mygalomorph taxa. In several cases, different GMYC clusters were recovered from the same locality, which may suggest coexistence of different evolutionary lineages. Interestingly, in many cases coexistence involved *Holonemesia* type with *Pronemesia* type lineages.

Our study exemplifies the chaotic state of *Nemesia* taxonomy. Only 15 out of the 54 available species names could be identified among the approximately 500 specimens examined. The application of genetic divergence threshold values proposed for other mygalomorph taxa suggest that there is a great amount of overlooked diversity in *Nemesia* that remains to be described. Our results highlight the need for a through integrative taxonomic revision of the genus.

In Chapter 4.2, we investifate deeper the systematics and evolution of the genus *Iberesia*. In contrast to *Nemesia,* the genus includes only three species and has narrow distribution, restricted to the south half of the Iberian Peninsula and the Balearic Islands. Here we have reported for the first time its presence in Morocco. Our results confirmed the monophyly of *Iberesia* (Decae and Cardoso, 2006) and, albeit with low support, its relationship as sister group of *Nemesia*. We further identified 7 well supported lineages, two of which correspond to two of the nominal species (*I. brauni* and *I machadoi*, respectively*)* and 4 additional linegaes shown morphological diagnostic characters that may warrant their description as new species. We were not able to establish if any of the new lienages could actually corrrspond to the nominal species *I. castillana*. As already observed in *Nemesia* and other mygalomorphs groups, the GMYC method overestimated the number of independent lineages (53 clusters). GMYC clusters most likely reflect the deep populations structure of the group, since in most cases clusters corresponded to single or nearby localities. Further efforts are required to formally describe the new evolutionary lineages identified in our study.

The diversification of *Iberesia* also seems to have been closely linked to the fragmentation of the Hercynian Belt (Rosembaun et al, 2002). *Iberesia* split from *Nemesia* approximately 32 Ma (45.16 - 20.5) and most lineages diversified from the middle to late Miocene. The geographic distribution of the main lineages within *Iberesia* closely resembles those reported for other arthropods with poor dispersal abilities such as the trap-door spider genus *Ummidia* (Opatova, submitted), or the *Buthus* scorpions (Souse pers. Comm.). The lineage start diversifying approximately 20 Ma, most probably on the Betic Cordillera. Most of the basal divergences closely matched the isolation of the Betic-Rif region and the subsequent opening of marine passages isolating the Rif area and the southernmost part of Betics during Tortonian (Braga et al. 2013). The subsequent uplift of the Betic Cordillera during the Upper Messinian further contribute to the isolation of some lineages. The land bidges emerged during the Messinian Salinity Crisis further facilitated *Iberesia* to reach the Balearic Islands.

In Chapter 4.3, we investigated how *Nemesia* and *Iberesia* managed to colonized the most isolated archipelago in the Mediterranean region, the Balearic Islands. Our results revealed that the Balearic species were the result of vicariant events following isolation of the Balearic Islands from the Betic region in the Early Tortonian. In the case of *Iberesia*, the colonization of the islands was most likely accomplished by terrestrial dispersal following the establishment of emerged land bridges with the continent during the Messinian Salinity Crisis (Krijsman et al 1999). Our study reported for the first time the presence of populations of *I. brauni* and *N. randa* in Minorca, which are most the result of terrestrial dispersal through land bridges emerged during the Quaternary glacial cycles, and discovered a new species on Minorca.

In Chapter 4.4, we examined the largely unknown nemesiid fauna of Tunisia. Although the presence of *Nemesia* in Tunisia have been documented in forrner studies, no species had been formally listed for the country. Despite of its size, Tunisia spans a diverse array of climatic zones and a high diversity of habitats. Moreover, the region is of great biogeographic interesting because of its geological connection to Sicily, southern Italy and the Kabylie. We conducted a

systematic sampling of the country in search of nemesiid specimens. The use of molecular markers combined with traditional morphometrics, and the morphological study of quantitative characters revealed high levels of genetic and phenotypic diversity, as well as burrow architectures, pointing towards the existence of overlooked species. The geographic distribution of GMYC clusters revealed similar patterns to those observed in other regions, including the deep population structure, most GMYC cluster corresponded to single or nearby localities, and the coexistence of not far related lineages.

An integrative taxonomic approach identified 13 independent lineages, one of which only included immature individuals. Twelve lineages were here formally described as new species. The possibility that any of the new species could actually correspond to the species known form other regions in northern Africa was discarded after consulting the literature. Interestingly, the morphometric study using meristic characters was unable to detect differences within species that were detected using qualitative characters (Decae 2005.). On the other hand, diagnostic burrow architectures were documented for some of the new species, which confirms the taxonomic relevance and of these characters. The high diversity and evolutionary significance of burrow architectures in *Nemesia* hints to the relevance of this feature to explain the high species diversity of the genus. This study confirmed the importance of including different sources of evidence for a rigorous delineation of species in this genus.

In Chapter 4.5, we deciphered the mechanisms underlying species coexistence in *Nemesia*. We monitored *Nemesia* populations of several species co-occurring in the Sant Llorenç del Munt and Serra de l'Obac natural park. The distribution and phenological data was combined with molecular and geometric morphometric tools to establish the patterns of species diversity and coexistence. Our results demonstrated that *Nemesia* spiders show strong habitat selection, similar to what has been found in other European mygalomorph such as *Atypus* (Rezac et al.,2007). The phenological data revealed a temporal segregation in male activity, and hence the mating season, across species: each specie had its own, non overlapping time of dispersal. Geometric morphometrics revealed subtle differences in species living in different habitats that had been previously overlooked using traditional

taxonomy. Our survey further resulted in the discovery of a new species, which was formally described, along with the redescription of the four species already known in the park.

All in all, our study emphasize the need for the use of integrative approaches to fulfil the full taxonomic revision of the Mediterranean nemesiids and the use of standardized, well-illustrated descriptions to facilitate species diagnostic. Future systematic work in this group will also have to consider the burrow architecture as a relevant evolutionary and diagnostic character and will have to consider the possibility of further split *Nemesia* in different genera, based among other characters in spinneret morphology. Additional, more informative molecular characters will have to be incorporated to recover fully, well-supported relationships. The use of genomic NGS derived RADseq or target enrichment approaches provide promising alternatives to sanger sequencing of household genes.

6. Conclusions

Conclusions

1) Phylogenetic analyses of the genus *Nemesia* and *Iberesia* support their reciprocal monophyly and sister group relationship.

1) The phylogenetic relevance of the morphology of the spinning apparatus is confirmed. The *Holonemesia* type appeared once and evolved from a *Pronemesia* type ancestor. The *Pronemesia* type is mainly distributed along Iberian Peninsula and Morocco, while *Holonemesia* is widespread along the Western Mediterranean Islands, Italy, France and Tunsia.

2) The paraphyletic status of some of the spinneret morphologies along with the discovery of additional types of spinnerets provide support for further splitting *Nemesia* into additional genera.

3) Molecular species delimitation approaches revealed a large amount of overlooked diversity within *Nemesia*, which awaits formal description.

4) Divergence times analyses and biogeographic reconstruction suggest that *Nemesia* diversifications was largely driven by vicariant events as a result of the opening of the Western Mediterranean basins. The present day distribution was further shaped by the dispersal through land bridges emerged during the Messinian Salinity Crises and the Quaternary glaciations.

5) Molecular phylogenetic analysis and morphological data revealed the existence of 7 evolutionary lineages in *Iberesia* that may deserve species status. Only two of the lineages corresponded nominal species, suggesting that diversity of the genus has been largely overlooked.

6) In spite of the overlooked diversity, *Iberesia* is by far less species rich than *Nemesia*. The simple and highly conserved burrow architecture, probably as a result of the reduction of the spinneret apparatus, in *Iberesia* and the rare cases of species coexistence hints at burrow diversity and ecological segregation as key factors to explain the remarkable diversity of *Nemesia*.

7) Time estimation and biogeographic reconstruction suggest that *Iberesia* diversification was most likely driven by the establishment of marine passages across the Betic region and the subsequent mounting building phase that followed the progressive collision of the Betic plate with the Iberian massif.

8) The *Nemesia* species from the Balearic Islands are the result of vicariant events following the isolation of the Balearics from the the Betics during the early Tortonian. *Iberesia*, on the other hand, dispersed to the Balearic island using the land bridges emerged during the Mesianin Salinity Crises.

9) The presence of the Majorcan species *I. brauni* and *N. randa* is here reported for the first time in Minorca. The Minorcan populations were most likely established following dispersal through the land connections emerged during the Quternary glaciations. A new species of *Nemesia* was also discovered in Minorca.

10) A systematic sampling combined with an integrative taxonomic approach revealed high levels of overlooked diversity in Tunisia. The geographic patterns of genetic diversity confirm deep population structure in *Nemesia* and also high levels of species coexistence, usually related to different burrow architecture.

11) Although the presence of *Nemesia* in Tunisa had been previously reported, none species had been formally documented. Here we formally described 12 new species of *Nemesia* for Tunisa.

12) Fine scale monitoring of *Nemesia* populations in the Sant Llorenç del Munt i Serra de l'Obac natural park, revealed the occurrence of 5 *Nemesia* species with contrasting patterns of habitat preference and spatial distribution.

13) Geometric morphometrics revealed previously overlooked phenotypic differences in closely related species leaving in different habitat types.

14) Comparative phenology analyses revealed the existence of spatial and

temporal segregation in the peak of adult male dispersal, this is in the mating season, between species.

15) A new species of *Nemesia* was described for the Sant Llorenç del Munt i Serra de l'Obac natural park, and four additional species inhabiting the park were redescribed.

7.Summary in catalan

1. Introducció

1.1 L'estudi de la sistemàtica biològica

La sistemàtica biològica és una disciplina científica que s'encarrega de l'estudi de la diversitat en els organismes vius i fòssils. D'una banda s'encarrega del descobriment i descripció de les espècies, d'altra banda, determina les relacions filogenètiques d'aquestes espècies (Wiens, 2007; Wiley & Lieberman, 2011). Un dels principals objectius de la sistemàtica és entendre quines són les relacions evolutives dels taxons, per això, el desenvolupament d'aquesta disciplina està estretament relacionada amb el desenvolupament de la taxonomia i la inferència filogenètica.

La taxonomia és la ciència que s'encarrega de descobrir, descriure, anomenar i classificar els organismes existents o extints (Quicke, 1993). La filogènia se centra en la comprensió de les relacions entre les espècies, mitjançant l'ús de caràcters observables en els organismes d'organismes i analitzant-los emprant diferents metodologies.

Will Henning (Hennig, 1950, 1966) va establir les bases teòriques de la sistemàtica filogenètica (o cladisme). Aquesta escola afirma que els únics grups rellevants per a una classificació natural són grups monofilètics (és a dir. aquells que inclouen l'avantpassat i tots els seus descendents). Des de llavors la classificació dels organismes ha de reflectir les seves relacions evolutives, que, al seu torn, es basen en la presència de sinapomorfies (caràcters derivats compartits heretats d'un ancestre comú).

Aquestes relacions entre els organismes poden representar en forma d'arbre. Aquest arbre és una estructura matemàtica que s'utilitza per modelar la història evolutiva d'un grup de seqüències o organismes. El patró de les relacions històriques és la filogènia o arbre evolutiu. Un arbre consta de nodes connectats per branques. els nodes terminals o tàxons (espècimens o seqüències) i els nodes interns que representen l'ancestre hipotètic. L'arrel de l'arbre representa l'ancestre comú de totes les seqüències o tàxons en l'arbre.
Donat un arbre concret, dos caràcters que són idèntics i aquesta similitud és a causa d'un antecessor comú, representa un una instància d'homologia. Per

contra, si la similitud no és causa de l'ancestre comú, aleshores es tracta de homoplasia. Podem distingir entre homologies ancestrals i derivades. Si un caràcter té el mateix estat que l'ancestre comú, llavors aquest és l'estat ancestral o plesiomòrfic. En cas contrari, és un estat derivat o apomòrfic.
Estats derivats únics són autopomorfies, i els estats derivats compartits són sinapomorfies Els estats dels caràcters derivats únics són autopomorfies, i els estats derivats compartits són sinapomofies. Només les sinapomorfies són rellevants per inferir relacions filogenètiques.

Sistemàtica molecular

La sistemàtica molecular consisteix en l'ús de la genètica molecular per estudiar l'evolució de les relacions entre els individus i espècies (Hillis, 1996). En l'actualitat, l'ús de dades moleculars preval sobre l'ús dels caràcters morfològics perquè, entre d'altres, les dades moleculars són molt més fàcils de codificar, mentres que les característiques morfològiques poden ser subtils i normalment requereixen un cert grau d'experiència.

Dades d'ADN vénen en diferents sabors: seqüències d'ADN, de AFLP o SNP, entre d'altres. El creixement exponencial d'aquesta disciplina a les últimes dècades es deu a una combinació d'una major sofisticació en tècniques de biologia molecular, i els avenços informàtics en maquinari i programari que permeten modelar els conjunts de dades grans i complexos, i avaluar i la hipòtesi de prova.

L'ús d'eines moleculars han estimulat el desenvolupament de la taxonomia molecular, és a dir. l'ús de marcadors moleculars i les seqüències d'ADN emprades per a la descripció de les espècies o per complementar la taxonomia clàssica (Blaxter, 2004 ;. Hebert et al, 2004; Hogg & Hebert, 2004; Smith et al., 2005). Aquest nou enfoc facilita el descobriment de tàxons críptics (Goetze, 2003 ;. Molbo et al, 2003 ;. Feulner et al, 2006). Malgrat els avantatges, la taxonomia d'ADN ha generat un intens debat (Tautz et al., 2003; Nielsen & Matz, 2006) degut a què es va proposar substituir l'actual sistema de mostres amb tipus per la informació molecular. Cal destacar que malgrat el ADN ha estat una gran ajuda per a delimitar les espècies, no pot ser l'única font d'evidència emprada durant aquest procés i sempre és aconsellable combinar

les dades d'ADN amb altres fonts d'informació, com ara la morfologia, el comportament, l'ecologia i la distribució geogràfica dels organismes (Balakrishnan 2005; Knowles & Carstens, 2007).

1.2 El concepte d'espècie

La capacitat de reconèixer les espècies és fonamental per entendre l'origen i la diversificació de la biodiversitat. L' espècie és la unitat fonamental de la Biologia (Darwin, 1859; Dobzhansky, 1937; Mayr, 1942; de Queiroz, 2005; De Queiroz, 2013). Malgrat que tot científic té una idea clara sobre el què considera una espècie, hi ha una gran confusió al voltant de la seva definició. De fet Darwin en L'origen de les espècies va escriure: "*Cap definició té encara satisfets tots els naturalistes; però, cada naturalista sap vagament a què es refereix quan parla d'una espècie "*.

Dos-cents anys després, el debat continua. De fet, molt pocs temes en biologia han generat un debat tan ampli com el concepte d'espècie (Mayr, 1942; Cracraft., 1989; Claridge et al, 1997; Coyne & Orr, 2004; de Queiroz, 2005). Mayden (1997) va redactar una llista amb més de 20 conceptes d'espècie diferents. El principal problema és que aquests conceptes i les seves definicions van ser formulats per respondre a les preguntes de diferents disciplines, i cadascun d'ells implica almenys conceptes parcialment incompatibles (Mayden, 1997; de Queiroz, 1998; Harrison, 1998). De fet un fet que agreuja aquesta situació ha estat que s'ha confós el concepte d'espècie en sí amb el procés de delimitació d'espècies. Aquesta confusió es coneix com el "species problem': quan s'han vinculat ambdues preguntes.

De Queiroz (2005,2007) ha proposat una possible solució, mitjançant la definició de l'espècie com una "metapoblació que evoluciona de forma independent". D'aquesta manera, es fa una distinció entre la definició real, i les diferents línies d'evidències que s'utilitzen per a reconèixer les espècies. De Queiroz (1998; 2007) va suggerir que els conceptes antics d'espècies estaven millor interpretats com a criteris per delimitar-les i confirmar-les com a unitats evolutives independents. Avui en dia, el consens en la comunitat científica és que el millor enfocament per delimitar les espècies és l'ús de diferents fonts

d'evidència conjuntament amb mètodes analítics diferents per extreure la informació més significativa (Heethoff et al., 2011).

1.3 Taxonomia Integrativa

La delimitació dels límits de les espècies és crucial per a descobrir la biodiversitat de la vida, ja que determina si ens trobem davant d'organismes individuals que són membres d'una mateixa entitat. Algunes estimes sobre la biodiversitat global calculen que aproximadament encara queden uns10 milions d'espècies per descobrir (Wheeler et al., 2004). Una mesura incorrecta de de la biodiversitat existent pot conduït a conseqüències greus que afecten al coneixement general dels patrons i processos de la natura i en última instància als esforços en biologia de la conservació (Wiens, 2007).

La delimitació d' espècies pot ser particularment difícil quan es tracta de tàxons que són morfològicament uniformes (Stockman & Bond, 2007; Bond &i Stockman, 2008; Hendrixson & Bond, 2009; Hamilton et al, 2011, 2014; Hendrixson et al, 2013) .Malgrat que existeix la percepció general de que la major disponibilitat de dades moleculars pot ser una solució a la crisi general en taxonomia, només una petita part dels estudis de delimitació d'espècies proporcionen descripcions. La decisió final sobre el que constitueix una espècie requereix la integració de les fonts d'evidència (Kekkonen & Hebert, 2014).
Tot i que els avenços tecnològics i metodològics han facilitat la generació d'una gran quantitat de dades de seqüències d'ADN i s'ha demostrat la seva rellevància per abordar qüestions taxonòmiques: com per exemple el descobriment de llinatges críptics, encara hi ha alguns taxònoms reticents a incorporar ADN als seus estudis (Dayrat, 2005). Aquesta falta de comunicació entre les diferents disciplines involucrades en la delimitació de les espècies és un problema important i passat per alt en l'anomenada "crisi de la taxonomia. Per a resoldre'l, es suggereix que la taxonomia es converteixi en quelcom integrador, i és precisament aquest punt el què suposa un veritable repte per al futur de la taxonomia. El terme 'taxonomia integradora "es refereix a la taxonomia que integra totes les fonts de dades disponibles per a emmarcar límits espècies (Yeates et al., 2011). El seu objectiu és delimitar les unitats de la diversitat de la vida a partir múltiples perspectives que alhora són

(filogeografia, morfologia comparativa, genètica de poblacions, l'ecologia, el desenvolupament, comportament, etc.) (Dayrat et al. 2005,). Aquesta integració maximitza l'objectivitat del procés de delimitació d'espècies (Bond & Stockman, 2008 ;. Hendrixson et al, 2013; Edwards & Knowles, 2014). Es necessita un gran canvi en relació amb la creació de noms per tal d'aconseguir aquesta integració i evitar l'abundància de sinònims i noms d'aplicació dubtosa. Precisament la taxonomia integrativa dóna prioritat a la delimitació de les espècies sobre la creació de nous noms de les espècies.

1.2 La importància de mantenir la biodiversitat

L'origen del terme biodiversitat és bastant recent. L'any1980, Thomas Lovejoy va introduir el terme "diversitat biològica" per a la comunitat científica i el 1988, EO Wilson va fer servir el terme biodiversitat (Haila & Kouki, 1994).

Els biòlegs defineixen la biodiversitat com "la totalitat de gens, espècies i ecosistemes d'una regió" (Larsson, 2001). Aquesta definició proporciona una visió unificada dels nivells tradicionals de la variabilitat biològica. El 2003, Anthony K. Campbell defineix un quart nivell: Diversitat Molecular.

En l'actualitat, una de les grans crisis del planeta és l'extinció accelerada d'espècies a causa de les activitats humanes (Smith et al., 1993; Lawton i maig de 1995; Purvis i Hector., 2000; Cardoso et al, 2011).Les estimacions més conservadores indiquen dades alarmants: es perden al voltant d'unes 3.000 espècies cada any, és a dir vuit espècies cada dia (Wilson, 2003; González-Orella, 2008). La pèrdua d'una espècie implica la pèrdua de la diversitat funcional i per tant, la pèrdua de la prestació de serveis dels ecosistemes, amb conseqüències per al benestar humà (Balvanera et al., 2006). Per exemple, la pèrdua d'un pol·linitzador pot implicar la pèrdua de la productivitat en molts cultius (Kremen et al., 2002), i la pèrdua de fauna d'aigua subterrània pot causar la interrupció dels processos de purificació i de bioremediació amb els conseqüents problemes de contaminació (Boulton et al , 2008).Resulta un factor clau, doncs, la protecció de les zones on es troba aquesta biodiversitat

1.4 La regió de la Mediterrània com un hotspot de biodiversitat.

La regió mediterrània és particularment coneguda per la diversitat de les seves plantes: prop de 25.000 espècies són natives de la regió, i més de la meitat d'elles són endèmiques (Myers et al., 2000). La regió Mediterrània ha estat reconeguda com un dels 25 principals Global Biodiversity Hotspots (Myers et al., 2000) a tenir en compte per a les prioritats de conservació. A més d'aquesta gran riquesa de plantes, una alta proporció d'animals mediterranis són exclusius de la regió: 2 de cada 3 espècies d'amfibis són endèmics, el 48% dels rèptils, una quarta part dels mamífers, el 6% dels taurons i ratlles, i 3% de la aus i el 14% de les libèl·lules, així com la meitat dels crancs (Medail & Quézel., 1997; Blondel et al, 1999).

La Mediterrània també alberga 253 espècies de peixos d'aigua dolça endèmics malgrat aquest mar representa menys de l'1% de la superfície oceànica mundial, un area increïblement rica en relació a la mida que té (Bianchi i Morri, 2000). D'altra banda, la importància de la Mediterrània per a la vida silvestre no es limita a la riquesa de la seva fauna i flora , sinó que també milions d'aus migratòries utilitzen els aiguamolls mediterranis i altres hàbitats com llocs de parada o de reproducció

La història geològica complexa i els canvis climàtics dramàtics a la conca del Mediterrani van jugar un paper crucial en el desenvolupament de la Mediterrània com un punt calent de biodiversitat (Myers et al., 2000). La geologia i els canvis climàtics són alguns dels principals factors abiòtics que promouen la diversificació d'espècies, degut a la seva capacitat per generar o modificar les barreres a la dispersió (Hewitt, 2004 ;. Esselstyn et al, 2009).

1.5 Història de la conca mediterrània

La regió mediterrània es va formar al Oligocè superior, fa uns 40 milions d'anys (Ma), quan l'oceà Tetis es va tancar a causa de la convergència de la placa Africana cap a la Eurasiàtica (Blondel et al., 1999). Aquesta col·lisió va generar el procés conegut com la orogènia Alpina (Rosenbaum & Lister, 2004) des del Miocè fins avui (fig. 3).

Durant l'Oligocè les Illes Balears, Còrsega, Sardenya, el massís Calabro-Pretorià, les Kabilies i la zona de la serralada Betico-formaven part del cinturó Hercinià. Aquest cinturó estava connectat amb la part oriental de la Península Ibèrica i el sud de França. Com a conseqüència de l'orogènia Alpina, el Cinturó Hercínico es va trencar i les microplaques van desplaçar-se a la deriva des de l'est de la península Ibèrica i el sud de França fins arribar a la seva ubicació actual (Rosenbaum et al., 2002). El desplaçament de les microplaques va donar lloc a la formació de les principals conques de la regió, incloent el Golf de València , Golf de Lleó, Mar de Ligúria, el Mar d'Alborán i el Mar Tirrè.

L'obertura es va iniciar amb la formació del Golf de València , que es remunta a al voltant de 25 Ma (Roca et al., 1999). Còrsega, Sardenya i el bloc Calabro-Pretorià van escindir-se i es van desplaçar a la deriva, a l'esquerra de la placa euroasiàtica. Aquest bloc va xocar amb el marge occidental d'Adria (Pulla) (20-18 Ma) i donar lloc als Apenins. La separació de Còrsega i Sardenya s'ha datat entre 21-15 milions d'anys, (Speranza et al, 2002 ;. Gattacceca et al., 2007). Còrsega i Sardenya posteriorment es van separar dels Apenins a mitjans del Miocè, i al voltant de 9 Ma i van començar la deriva cap a la seva posició actual (Rosenbaum & Lister, 2004).(veure fig.3 a la introducció)

La placa Betica-Rifenya va començar a moure's en el sentit les agulles del rellotge des de la seva ubicació original al voltant de 23 Ma i 15 Ma. Finalment, va començar a fragmentar-se en el que són actualment la zona Bètica i els blocs del Rif (del Marroc). Aquesta placa va arribar a la seva ubicació , actual (a banda i banda de l'Estret de Gibraltar) a mitjans del Miocè (10 Ma) (Lonergan & White, 1997). Alguns autors suggereixen que la connexió entre la part més meridional de la Bètica, que correspon a la regió de Gibraltar de la Península Ibèrica, amb el bloc de Rif va persistir fins a l'Alt Tortonià / Baix Messinià (~ 8-7,2 Ma), mentre que l'intercanvi d'aigua entre el Mediterrani i l'Oceà Atlàntic va ser possible a través de la conca del Guadalquivir (Braga et al., 2003).

A la Mediterrània oriental una massa de terra contínua que conté el que avui en dia és Grècia continental, Creta, les petites illes de l'Egeu i parts d'Anatòlia, va començar a fragmentar-se en el Miocè Superior (12-9 Ma), i va donar lloc a

l'obertura de mar d'Anatòlia i la disjunció de Creta, estimada a uns 8 Ma (Creutzburg, 1963; Dermitzakis, 1990).

La Crisi de Salinitat del Messinià (MSC) ha estat considerada com un dels principals impulsors de la diversificació local. Aproximadament fa uns 5,96 Ma, l'Estret de Gibraltar es va tancar, aïllant al mar Mediterrani de l'Oceà Atlàntic, amb la conseqüent dessecació gairebé total de la conca de la Mediterrània (Krijgsman et al., 1999). Aleshores van sorgir ponts terrestres que van establir connexions entre les regions anteriorment aïllades, incloent les Illes Balears, el Rif, Còrsega i Sardenya (Jolivet et al., 2006). La reobertura de l'Estret de Gibraltar, aproximadament fa uns 5,3 Ma va restaurar l'intercanvi d'aigua entre l'Atlàntic i el Mediterrani amb el conseqüent restabliment de l'aïllament del nord del Marroc i les illes (Krijgsman et al., 1999; Loget & Van Den Driessche, 2005)

Paral·lelament, la regió mediterrània també va experimentar grans canvis climàtics. A principis de l'Eocè (~ 55 Ma) el clima era més càlid i humit que l'actual. Al Miocè mitjà (~ 15-7 Ma) es va iniciar un procés de refredament que va conduir a la creació de l'estacionalitat de la regió tal i com la coneixem avui en dia. El clima subtropical va canviar progressivament cap a condicions més fredes i seques, fins a assolir el clima mediterrani d'avui dia al voltant de 3,2 Ma (Suc, 1984; Jiménez-Moreno et al., 2010). Aquest canvi climàtic va ser acompanyat per una substitució de les comunitats de plantes termòfiles per vegetació típica mediterrània.

Els cicles glacial quaternari (2,6-0,1 Ma) també han jugat un paper clau i són probablement el canvi climàtic més dramàtic experimentat a la regió. A partir de 2,58 Ma (Gibbard et al, 2010), les oscil·lacions glacials van produir canvis de mida de la població d'organismes locals, que van com portar l'extinció o la especiació (Hewitt, 1996 ;. Aviseu et al, 1998). La formació de les capes de gel glacial va comportar la disminució del nivell del mar amb el conseqüent establiment ponts de terra entre algunes illes, per exemple, Mallorca i Menorca induïts.

1.5 Les aranyes com a subjecte d'estudi

L'ús d'artròpodes per a l'avaluació de la biodiversitat ofereix informació sobre patrons de diversitat i de qualitat del medi ambient a una escala més rellevant que els utilitzats amb plantes i vertebrats (Yen i Butcher, 1997). D'altra banda, els artròpodes poden proporcionar alertes primerenques dels canvis ecològics. Això es deu a causa de les seves altes taxes de reproducció i temps de generació molt curts i per tant els fa més sensibles a les pertorbacions ambientals que no pas els vertebrats. D'aquesta manera poden mostrar l'efecte de la fragmentació en zones en què els vertebrats no són bons indicadors per a tal efecte (Kremen et al., 1993, 1994).

No obstant això, no tots els artròpodes són igualment eficaços com a indicadors per a la conservació. Els depredadors són el nivell tròfic més sensible als canvis ambientals. Les aranyes es troben entre els més abundants i conspícuus depredadors a la Terra, són el cinquè ordre més divers d'animals, amb 110 famílies i més de 45.000 espècies (World Spider Catalog, 2015). L'Infraordre Mygalomorphae comprèn les taràntules americanes, aranyes d'embut i aranyes de trampeta. És un dels tres llinatges principals reconeguts dins de les aranyes (Platnick & Gertsch, 1976; Bond & Hedin, 2006) (fig. 4 introducció). Són el grup germà dels araneomorfs i es classifiquen actualment en 15 famílies, que comprenen aproximadament 2.500 espècies i 300 gèneres (World Spider Catalog, 2015).

Els migalomorfs són un grup cosmopolita, es poden trobar en tots els continents excepte a l'Antàrtida. L'origen del grup s'ha suggerit com antic (Marusik, 2011) i el grup s'ha descrit com a "primitiu" a causa de la retenció dels caràcters plesiomòrfics com els quatre pulmons en llibre i els quelícers amb ullals longitudinals amb moviment no sincronitzat (Raven, 1985). Aquestes aranyes tenen un estil de vida críptica. Són majoritàriament nocturnes, construeixen uns caus enterrats a terra revestits amb seda i protegits per una trampa que tanca el niu. Els caus poden tenir fins a 30 cm de profunditat. Les femelles són majoritàriament sedentàries i, de llarga vida (gairebé 20 anys en captivitat) (Buchli, 1961, 1962, 1965). A diferència de les femelles, que viuen dins del cau per a la major part de la seva vida, els mascles adults tenen cicles

de vida anuals, i es dispersen després d'assolir la maduresa sexual per a l'aparellament. S'ha documentat que per a algunes espècies femelles mengen els mascles després de l'aparellament (Buchli, 1965).

La baixa capacitat de dispersió, els cicles vitals llargs, les preferències ecològiques concretes i la distribució restringida fan dels migalomorfs un excel·lent model per a estudis biogeogràfics i evolutius (Hendrixson & Bond, 2005 ;. Hamilton et al, 2011; Opatova et al, 2013 .; . Satler et al, 2013; Opatova & Arnedo, 2014A).

A diferència dels araneomorfs, la dispersió aèria mitjançant balooning és rara en migalomorfs. Només tres famílies que a més no estan emparentades el presenten: Atypidae, Ctenizidae i Actinopodidae (Coyle, 1983; Coyle et al., 1985). La presència de migalomorfs com *Ummidia* (Ctenizidae) en algunes illes del Carib d'origen volcànic i sense connexió prèvia a qualsevol massa de terra (World Spider Catalog, 2015), indica la capacitat de dispersió a llarga distància. D'altra banda, l'estructuració genètica profunda detectada entre les poblacions *Atypus* (Atypidae) (Pedersen & Loeschcke, 2001) suggereixen que la dispersió aèria funciona a només distàncies curtes. Estudis de dispersió recents en *Atypus* suggereixen que la selecció d'hàbitat, en lloc de la dispersió aèria, és la responsable de les colònies agregades que es troben en el camp (Řezáč et al, 2007; Deruytter et al, 2012).

Malgrat el seu potencial, els migalomorfs no han estat utilitzats com a organisme model per a estudis biogeogràfics i evolutius. Tenen un estil de vida criptic i en general són difícils d'observar i recol·lectar. A més a més, els taxons estretament relacionats són generalment morfològicament conservats i difícils de diferenciar (Bond et al., 2006). Si això no fós suficient, a causa del seu dimorfisme sexual i el seus diferents cicles de vida, mascles i femelles no solen recol·lectar-se junts amb freqüència moltes descripcions d'espècies es fan només amb un dels sexes.

1.7 La família Nemesiidae

La família Nemesidae és la més diversa de les sis famílies d'aranyes migalomorfes presents a la regió Mediterrània (Fig.5 de la

introducció).Nemesiidae té una distribució mundial i és la segona familia més diversa d'aranyes migalomorfes (World Spider Catalog, 2015) Quatre gèneres de Nemesiidae es troben a la conca mediterrània: el gènere *Raveniola*, Zonstein 1987 amb distribució asiàtica que arriba fins a la part oriental d'Anatòlia; *Iberesia* Decae& Cardoso de 2006, de la Península Ibèrica i les Illes Balears; *Brachythele* Pocock 1892, àmpliament distribuïda en les regions de l'Adriàtic i de l'Egeu, incloent Anatolia i Xipre, i, finalment, *Nemesia* Audouin 1826 que es troba àmpliament distribuïda a tota la Mediterrània encara que la seva localitat tipus és prop d'Alexandria a Egipte (fig. 6 , Introducció)

1.8 Gènere *Nemesia* Audouin, 1826

El gènere Nemesia comprèn aranyes de trampeta de mida de petites a grans. Es distingeixen de possibles aranyes simpàtriques de les famílies Ctenizidae i Cyrtauchenidae pel color marró en general, per les seves llargues potes en relació amb la mida del cos, i per fòvea recorbada (Decae, 2010) (fig. 7 , introducció).

Es poden distingir de les *Iberesia* en simpatria per la presència de dos parells de fileres, mentres que *Iberesia* només té un parell de fileres (Decae & Cardoso, 2006). Fins a la data, 50 espècies i 4 subespècies de *Nemesia* s'han descrit (World Spider Catalog, 2015). Les espècies i la seva distribució es resumeixen en la Fig. 8(Introducció).

Com és el cas de la majoria de migalomorfs, les espècies de *Nemesia* són morfològicament conservades i, per tant, difícils de diferenciar. En els artròpodes en general, i aranyes en particular, els taxònoms depenen de la morfologia dels òrgans copuladors per distingir espècies (Eberhard, 1985). Això és degut molt probablement a que els caràcters sexuals evolucionen més ràpidament que els caràcters somàtics, probablement com a resultat de la selecció sexual (Eberhard, 1985; Huber, 2003; Hosken & Stockley, 2004; Huber et al, 2005).

En *Nemesia,* la variació en la espermateca de les femelles o el bulb dels mascles és subtil, i es necessiten caràcters addicionals per a la identificació d'espècies (Decae et al., 2007b). Després de dos-cents anys de la primera

descripció de Nemesia, la taxonomia segueix sent desconeguda. Hi ha diverses raons que expliquen la caòtica taxonòmica del gènere. En primer lloc, les descripcions antigues solien ser descripcions imprecises i ambigües. Moltes es remunten als segles 18 , 19, i la primera meitat del segles 20, no tenen un diagnòstic comparatiu, les descripcions no estan estandarditzades i, sobretot,no les acompanyen il·lustracions informatives. En segon lloc,el material tipus en molts casos o bé s'ha perdut o és molt difícil de trobar. Eugène Simon, que va ser un dels autors més prolífics d'espècies de Nemesia (Simon, 1889, 1892, 1914) desafortunadament no va etiquetar els espècimens utilitzats en la descripció com a material tipus i es van posar al costat d'altres mostres considerades de pertànyer a la mateixa espècie però de diferents localitats en els mateixos vials. Per tant, en alguns casos és impossible saber el quin és el tipus real per a la comparació. En tercer lloc, la meitat de les espècies són conegudes només per un dels sexes com a resultat del seu estil de vida. Aquestes diferències generen un esbiaix en el mostreig degut a què la captura directa de nius proporciona només les femelles, els mascles rarament. I les trampes de captura, d'altra banda, només proporcionen mascles errants.

1.9 El gènere *Iberesia*

El gènere Iberesia va ser descrit recentment per donar cabuda a les espècies anteriorment inclosa en *Nemesia* que es distingeixen per l'absència de fileres mitjanes posteriors (Posterior Median Spinnerets) (Decae & Cardoso, 2006). Tant les fileres laterals (Posterior Lateral Spinnerets) com les mitjanes (PMS són presents en *Nemesia*. *Iberesia* actualment inclou 3 espècies de la Península Ibèrica i les Illes Balears.

Objectius

L'objectiu principal d'aquesta tesi és estudiar la història evolutiva i els mecanismes de diversificació dels dos gèneres d'aranyes de trampeta de la regió mediterrània: *Nemesia* i *Iberesia*. Per aconseguir-ho, es van definir els següents objectius:

1) Inferir l'estructura filogenètica del gènere *Nemesia* i *Iberesia,* confirmar el seu estat taxonòmic i més provar la significació de la morfologia de les fileres en la taxonomia del grup (Capítol 4.1).

2) Inferir les relacions filogenètiques del altament divers gènere *Nemesia*, estimar un termini per a la seva diversificació i reconstruir la seva història biogeogràfica per esbrinar quins han estat els mecanismes responsables de la seva alta diversitat (Capítol 4.1).

3) Inferir les relacions filogenètiques, establir un termini de diversificació i reconstruir la història biogeogràfica per provar si la baixa diversitat de Iberesia el és el resultat d'un origen recent o bé, si per contra, la seva diversitat ha estat infraestimada (Capítol 4.2).

4) Inferir una una datació i dur a terme la reconstrucció biogeogràfica per determinar els orígens i vies de colonització de les espècies de nemesiids endèmiques de les Illes Balears (Capítol 4.3).

5) Utilitzar la taxonomia integradora per identificar i descriure les espècies i inferir els patrons de biodiversitat de *Nemesia* a Tunísia (Capítol 4.4).

6) Identificar els mecanismes de coexistència de les espècies en *Nemesia* mitjançant la integració de la informació taxonòmica, molecular, fenològic i morfomètrics (capítol 4.5)

Resultats i discusió general

Els resultats d'aquest estudi representen una important contribució a la nostra comprensió de la sistemàtica dels gèneres d'aranyes migalomorfes de la regió mediterrània *Nemesia* i *Iberesia,* així com dels els processos evolutius responsables de la seva diversificació.

Degut al seu estil de vida críptica i la seva morfologia conservada, els migalomorfs han plantejat importants reptes als taxònoms i el seu ús potencial com a organismes model per a la investigació de la biodiversitat no s'ha desenvolupat completament. Abans d'aquest estudi, el coneixement sobre nemesiids mediterranis era restringit a la seva taxonomia i distribució, i alguns estudis esporàdics existien sobre el seu comportament i història natural. No

s'havien realitzat estudis moleculars en el grup i se sabia poc sobre la seva història evolutiva.

Aquí hem utilitzat un enfocament integrador que combina la sistemàtica molecular utilitzant múltiples locus, taxonomia tradicional, morfometria geomètrica i estudis fenològics per a desxifrar els orígens, les causes de la diversificació i els mecanismes de coexistència de nemesiids mediterranis.

En el primer capítol, investiguem els orígens del gènere *Nemesia* i així com les causes de diversificació que ha generat aquesta elevada riquesa d'espècies. Proporcionem la primera filogènia molecular dels nemesiids de la Mediterrània, que recuperar la monofilia recíproca de *Nemesia* i *Iberesia* (Decae& Cardoso, 2005, Decae 2007).

El grau de reducció de les fileres s'havia utilitzat prèviament per definir grups supra-específics en nemesiids mediterranis (Decae, 2010). La pèrdua dels PLS és la sinapomorfía principal que defineix *Iberesia* (Decae & Cardoso, 2007). El mapeig de la morfologia de les fileres al nostre arbre filogenètic va revelar que el *Holonemesia* era monofilètic i havia evolucionat a partir d'un ancestre *Pronemesia*. D'altra banda, un examen més detallat de la morfologia de les diferents fileres suggereix que els diferents tipus de filera podrien subdividir-se en grups addicionals. Curiosament, s'ha detectat una relació entre la morfologia de la filera i l'arquitectura del niu: el tipus Holonemesia es troba en les espècies que construeixen caus i trampetes més complexoes, mentre que les espècies de Pronemesia construeixen caus molt més senzills similars als de *Iberesia*. Aquesta observació indica que la reducció en l'aparell de filat influeixen directament en l'arquitectura de les caus.

La serralada Bètica és suportada com el probable origen del gènere *Nemesia*. *Nemesia* es separa d'*Iberesia* anteriorment a l'obertura de la conca de la Mediterrània Occidental (Rosembaun et al 2002). Divergències posteriors coincideixen amb la geocronologia de la fragmentació de la cinturó Hercinià, datat en 30-25 Ma. Proposem que la diversificació de Nemesia i la seva presència actual les regions que van formar part d'Iberia va ser impulsat pels esdeveniments vicariants següents a la fragmentació de la cinturó Hercinià i el posterior desplaçament dels seus principals blocs. El complex patró de

múltiples llinatges independents en les principals illes i alguns dels antics terrenys ibèrics suggereix que l'ancestre ja era presents al cinturó Hercinià o bé que van dispersar a altres regions aprofitant els ponts de terra que van sorgir degut a les oscil·lacions d'aquest, primer durant les crisis de salinitat Messinià i després durant el Pleistocè.

Les relacions internes no sempre són suportades, i es requerirà, per tant, un major esforç per proporcionar una filogènia totalment ben recolzada per a poder estudiar els patrons biogeogràfics. En aquest sentit, hem estat treballant en el desenvolupament de nous marcadors nuclears utilitzant mètodes de seqüenciació de pròxima generació dissenyat específicament per Nemesia.

El mètode GMYC s'ha utilitzat en gran mesura per delimitar llinatges evolutius en artròpodes (Bidegaray-Batista i Arnedo, 2011; Planas et al, 2013; Hendrixson et al 2013, Hamilton et al, 2014; Opatova i Arnedo, 2014), però s'ha demostrat tendeix a sobreestimar el nombre de llinatges (Satler et al, 2013; .. Talavera et al, 2013). Aquest fenòmen és especialment característic en mygalomorphs a causa de les seves pobres habilitats de dispersió. El mètode GMYC va obtenir 183 grups de coalescència a *Nemesia*. En la majoria dels casos, les agrupacions corresponien a localitats individuals o properes, en concordància amb l'elevada estructura poblacional. En diversos casos, els diferents grups GMYC (no estretament relacionats) van ser recuperats de la mateixa localitat, el que pot suggerir coexistència de diferents llinatges evolutius. Curiosament, en molts casos la coexistència tenia lloc entre llinatges de Holonemesia i Pronemesia.

El nostre estudi exemplifica l'estat caòtic de la taxonomia del gènere *Nemesia*. Només 15 espècies de les 54 existents, van poder ser identificades entre els aproximadament 500 espècimens estudiats. Els nostres resultats posen de manifest la necessitat d'una revisió taxonòmica d'integració a través del gènere.

En el capítol 4.2, investiguem la sistemàtica i evolució del gènere *Iberesia*. En contrast amb *Nemesia*, el gènere inclou només tres espècies i té una distribució limitada a la mitjana al sud de la Península Ibèrica i les Illes Balears. Aquí hem trobat per primera vegada la seva presència al Marroc. Els nostres resultats van confirmar la monofilia de *Iberesia* (Decae i Cardoso, 2006) i, encara que

amb baix suport, la seva relació com a grup germà de *Nemesia*. Es van identificar més de 7 llinatges ben suportats, dos dels quals corresponen a dues de les espècies nominals (*I. brauni* i *I. machadoi*, respectivament) i 4 llinatges addicionals, que van mostrar caràcters diagnòstics morfològics que poden justificar la seva descripció com a nova espècie. No va ser possible establir si algun dels nous llinatges podria correspondre a *I. castillana*. Com ja s'ha observat en *Nemesia* , el mètode GMYC va sobreestimar el nombre de llinatges independents (53 grups). Aquests clusters molt probablement reflecteixen l'elevada estructura poblacional del grup, ja que en la majoria dels casos les agrupacions corresponien a localitats individuals o properes. Calen més esforços per descriure formalment els nous llinatges evolutius identificats en el nostre estudi.

La diversificació d' *Iberesia* també sembla estar estretament vinculada a la fragmentació deL cinturó Hercinà (Rosembaun et al, 2002). Iberesia va separar de Nemesia aproximadament 32 milions d'anys, la majoria dels llinatges diversificaren a mitjans o finals del Miocè. La distribució geogràfica dels principals llinatges dins *Iberesia* s'assembla molt a la d'altres artròpodes amb poques habilitats de dispersió com aranyes del gènere *Ummidia* (Opatova, enviat), o els escorpins Buthus (Souse.Pers. Com.). El llinatge comença la seva diversificació aproximadament fa 20 milions d'anys, molt probablement a la Serralada Bètica. La majoria dels resultats obtinguts concorden amb l'aïllament de la regió betico-rifenya i amb la posterior obertura de passatges marins que va aïllar la zona del Rif i la part meridional de les Serralades Bètiques durant el Tortonià (Braga et al. 2013). L'aixecament posterior de la Serralada Bètica durant el Messinià superior va contribuïr encara més a l'aïllament d'alguns llinatges. Els ponts de terra que es van formar durant la Crisi de Salinitat del Messinià van facilitar que *Iberesia* arribés a les Illes Balears.

En el capítol 4.3, es va investigar com *Nemesia* i *Iberesia* van aconseguir colonitzar l'arxipèlag més aïllat a la regió de la Mediterrània, les Illes Balears. Els nostres resultats mostren que les espècies Balears són el resultat dels esdeveniments vicariants que van comportar l' aïllament de les Illes Balears de la regió Bètica durant el Tortonià. En el cas de *Iberesia*, la colonització de les illes va ser molt probablement degut a dispersió terrestre després de

l'establiment de ponts de terra formats durant la Crisi de Salinitat del Messinià(Krijsman et al 1999). El nostre estudi cita per primera vegada la presència de poblacions d'*I. brauni* i *N. randa* a Menorca, que són el resultat de la dispersió terrestre a través de ponts de terra durant els cicles glacials del Quaternari, i hem descobert una nova espècie a Menorca.

En el capítol 4.4, es va analitzar la fauna de nemesiids, desconeguts en gran mesura de Tunísia. Encara que la presència de *Nemesia* a Tunísia s'han documentat en estudis, cap espècie s'havia inclòs formalment. Malgrat la seva mida petita, Tunísia compta amb una gran varietat de zones climàtiques i una alta diversitat d'hàbitats. A més, la regió és de gran interès biogeogràfic a causa de la seva connexió geològica amb Sicília, el sud d'Itàlia i de Cabília. Es va realitzar un mostreig sistemàtic del país a la recerca d'espècimens. L'ús de marcadors moleculars combinats amb tècniques de morfometria tradicionals, i l'estudi morfològic dels caràcters quantitatius revelen alts nivells de diversitat genètica i fenotípica, així com arquitectures cau, que apunta cap a l'existència d'espècies d'alt. La distribució geogràfica dels grups GMYC va revelar patrons similars als observats en altres regions, incloent l'estructura profunda de la població, el patró d'agrupació principal va correspondre a localitats individuals o properes, i la coexistència dels llinatges no molt relacionats. L' enfoc integrador va permetre identificar 13 llinatges independents, un dels quals només incloïen individus immadurs. Dotze llinatges van ser descrits aquí formalment com a noves espècies. La possibilitat que alguna d'aquestes noves espècies podria en realitat correspondre a les espècies conegudes es va descartar després de consultar la literatura. Curiosament, l'estudi morfomètric utilitzant caràcters merístics no va poder detectar diferències dins de les espècies que es van detectar utilitzant caràcters qualitatius (Decae 2005). D'altra banda, les arquitectures del niu es van documentar per a algunes de les noves espècies, el que confirma la importància taxonòmica d'aquest caràcter. L'elevada diversitat i la importància evolutiva de la arquitectura del niu en *Nemesia* insinua a la rellevància d'aquesta característica per a explicar l'alta diversitat d'espècies del gènere. Aquest estudi confirma la importància d'incloure diferents fonts d'evidència per a una delimitació rigurosa de les espècies d'aquest gènere.

En el capítol 4.5, desxifrem els mecanismes subjacents a la convivència entre espècie de *Nemesia*. Es van Monitoritzar poblacions *Nemesia* de diverses espècies co-ocurrents al parc natural de Sant Llorenç del Munt i la Serra de l'Òbac Serra de. La distribució i les dades fenològiques es va combinar amb eines moleculars i de morfometria geomètrica per establir els patrons de diversitat d'espècies i la convivència. Els nostres resultats demostren que *Nemesia* mostren una forta selecció d'hàbitat, similar al que s'ha trobat en altres mygalomorph Europea com *Atypus* (Řezáč, Řezáčová, & Pekár, 2007). Les dades fenològiques revelen una segregació temporal en el pic de dispersió dels mascles que no se superposa. La morfometria geomètrica va revelar diferències subtils en les espècies que viuen en diferents hàbitats que havien estat prèviament confoses mitjançant l'ús de la taxonomia tradicional. El nostre estudi va resultar en el descobriment d'una nova espècie, descrita formalment, juntament amb la redescripció de les quatre espècies ja conegudes al parc.

El nostre estudi posen l'accent en la necessitat de la integració de diferents línies d'evidència per complir amb la revisió taxonòmica completa dels nemesiids Mediterrani i l'ús de descripcions estandarditzades, ben il·lustrades per a facilitar la identificació. Treballs sistemàtics futurs en aquest grup també haurien de considerar l'arquitectura del niu com un de diagnòstic corresponent i hauràn de tenir en compte la la possibilitat d'una major divisió *Nemesia* en diferents gèneres, basada entre altres en les morfologies de la fileres. Més informació moleculars addicional és necessària per a obtenir relacions filogènetiques ben suportades.

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