

## Threatened or threatening? Evidence for independent introductions of *Macrothele calpeiana* (Walckenaer, 1805) (Araneae: Hexathelidae) and first observation of reproduction outside its natural distribution range

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### Summary

Despite being endemic to the south of the Iberian Peninsula, *Macrothele calpeiana* has been repeatedly reported far from its native range (e.g. northern Italy, Switzerland, Belgium, Holland). Here, we confirm, using DNA barcode data, that the specimens collected in northern Catalonia, Majorca, and northern Italy are indeed recent introductions. Additionally, our data suggest that they originated from different source populations, and corroborate former suggestions that the recent expansion of *Macrothele* may be linked to the commercial transport of olive trees. Former species distribution modelling studies have revealed that the exotic localities reported are far from the ecological optimum of the species and hence that introduced specimens have slim chances of survival. However, a recent finding of several individuals of different sex and stages in a coastal town in northern Catalonia (northeastern Iberian Peninsula) suggests that *M. calpeiana* is able to reproduce outside its native range and that microclimate conditions may allow establishment of accidentally introduced populations.

### Introduction

The funnel-web spider *Macrothele calpeiana* (Walckenaer, 1805) is the largest spider in Europe (Márquez-Rodríguez 2014), and the only one protected under European law. The species is presently listed under annex IV of the EU Habitats directive (92/43/EEC, Annex IV) as a species of community interest in need of strict protection. Together with *Macrothele cretica* (Kulczyński, 1903), another species of conservation concern listed in the IUCN as data deficient, they are the only representatives of the spider family Hexathelidae in Europe. The two species have both evolutionary and biogeographic relevance. A recent study has revealed that their closest relatives live in South-East Asia and China, and that the ancestors of the two species arrived to Europe independently (Opatova & Arnedo 2014). The two European *Macrothele* are narrow endemics, *M. cretica* is confined to a few localities on the island of Crete (Snazell & Allison 1989) and *M. calpeiana* is mostly restricted to the southwestern Iberian Peninsula (Jiménez-Valverde & Lobo 2006).

*M. calpeiana* was originally thought to be closely linked to the highly threatened cork-oak forests (Snazell 1986) and its inclusion in the Bern convention was most likely motivated by its supposed role as bioindicator of this type of forest. However, subsequent collections demonstrated that the species could also be found in other type of habitats,

including human-transformed habitats such as pine reforested areas, olive plantations, and even rubbish dumps (van Helsdingen & Decae 1992). The fact that the species is not an obligate dweller of cork-oak forests, and may even benefit from a light human intervention, has led some authors to question its conservation status and the need for protection (van Helsdingen 1993). However, although the species may have a wider distribution and ecological range than originally proposed, phylogeographic studies have revealed that *M. calpeiana* populations are highly structured and that some deeply divergent mitochondrial lineages, that may trace back to the early Pliocene, have extremely restricted distributions (Arnedo & Ferrandez 2007).

In recent years, *M. calpeiana* has increasingly been reported from localities distant from its native range, both in the Iberian Peninsula and other European regions, including southern France (Siaud & Raphaël 2013), northern Italy (Pantini & Isaia 2008), the Netherlands, Belgium, and Switzerland (Jiménez-Valverde *et al.* 2011). Because of their dispersion and isolation, these new localities suggest a human-mediated dispersal, probably associated with the commercial export of olive trees (Ferrández 2010; Pantini & Isaia 2008).

The potential of *M. calpeiana* to become an invasive species has been investigated by means of Species Distribution Modelling (SDM) approaches (Jimenez-Valverde, Decae & Arnedo 2011). This study suggested that locations other than southern Iberia and northern Morocco were not environmentally suitable for the species, and concluded that the persistence of the species in such places seemed improbable. However, the study also cautioned that rapid adaptation to local conditions could facilitate on the future the spread of *M. calpeiana* across Europe.

Here, we report for the first time a case of successful reproduction of introduced *Macrothele* individuals in the northern Iberian Peninsula, where SDM models had not anticipated climatic suitability. In addition, we recovered DNA barcode information from four specimens introduced to Italy, the northern Iberian Peninsula, and the Balearic Islands, and use phylogenetic and phylogeographic methods to demonstrate that they can be traced back to different populations in the native area of the species.

### Material and methods

The existence of a colony of *M. calpeiana* in a private estate at Begur, Girona (Catalonia, Spain) was brought to the attention of the first author after the examination of a sample submitted for identification to the Universitat de Girona in summer 2014. Subsequent visits to the locality revealed the existence of a persistent colony of *M. calpeiana*.

Four specimens of *M. calpeiana* collected in localities supposedly out of the native range of the species were available for molecular analyses. One female collected in a garden centre in Girona (Catalonia, Spain), one female captured in a private house in Inca, Majorca (Balearic Islands, Spain), and two individuals captured in a garden centre and olive plantation in Villa d'Almè, Bergamo (Lombardia, Italy) (Fig. 1). In addition, we included one

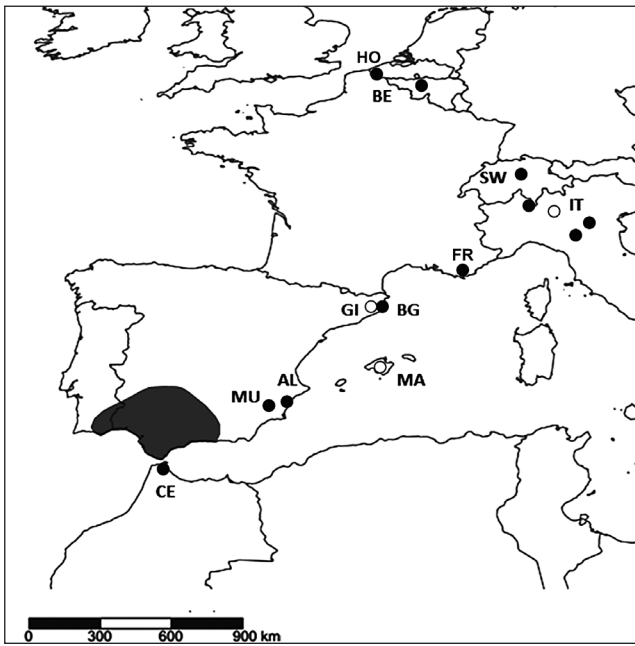


Fig. 1: Distribution map of *M. calpeiana*. Natural range in black. Localities where *M. calpeiana* has been reported out of its suspected range are indicated with filled circles (modified from Jimenez-Valverde, Decae & Arnedo 2011). Open circles denote localities of specimens used for molecular analysis. AL = Almeria, Spain; BE = Belgium; BG = Begur, Girona, Spain; CE = Ceuta, Spain; FR = France; GI = Girona, Spain; HO = Holland, IT = Italy; MA = Majorca, Spain, MU = Murcia, Spain; SW = Switzerland.

| Species               | Accession No. | Locality                                      |
|-----------------------|---------------|---|
| <i>M. amamiensis</i>  | KJ686396      | Japan: Setouchi                               |
| <i>M. calpeiana</i>   | KX538932      | Iberian P.: Viver de Plajts (GI)              |
| <i>M. calpeiana</i>   | KX538934      | Italy: Villa d'Alme (BE)                      |
| <i>M. calpeiana</i>   | KX538933      | Italy: Villa d'Alme (BE)                      |
| <i>M. calpeiana</i>   | KX538935      | Balearic Is.: Majorca, Inca                   |
| <i>M. calpeiana</i>   | EF127271      | Iberian P.: Calera de León (BA)               |
| <i>M. calpeiana</i>   | EF127253      | Iberian P.: Mojón de la Vibora (CA)           |
| <i>M. calpeiana</i>   | EF127254      | Iberian P.: Arroyo Jautor, N Los Barrios (CA) |
| <i>M. calpeiana</i>   | EF127255      | Iberian P.: S Paterna de la Ribera (CA)       |
| <i>M. calpeiana</i>   | EF127257      | Iberian P.: Los Romerales (CA)                |
| <i>M. calpeiana</i>   | EF127264      | Iberian P.: Paterna de la Rivera (CA)         |
| <i>M. calpeiana</i>   | EF127266      | Iberian P.: Paterna de la Rivera (CA)         |
| <i>M. calpeiana</i>   | EF127261      | Iberian P.: Puente Genil (CO)                 |
| <i>M. calpeiana</i>   | EF127256      | Iberian P.: P. Natural de Sierra Huétor (GR)  |
| <i>M. calpeiana</i>   | EF127263      | Iberian P.: Fuente de la Teja, Huétor (GR)    |
| <i>M. calpeiana</i>   | EF127267      | Iberian P.: Embalse de Quéntar (GR)           |
| <i>M. calpeiana</i>   | EF127258      | Iberian P.: A493, nr Niebla crossing (HU)     |
| <i>M. calpeiana</i>   | EF127262      | Iberian P.: Valdehervasio (HU)                |
| <i>M. calpeiana</i>   | EF127265      | Iberian P.: Molares (HU)                      |
| <i>M. calpeiana</i>   | EF127269      | Iberian P.: Santa Ana la Real (HU)            |
| <i>M. calpeiana</i>   | EF127259      | Iberian P.: Peña del Aguila, Mancha Real (JA) |
| <i>M. calpeiana</i>   | EF127260      | Iberian P.: Casas de Guadalmedina (MA)        |
| <i>M. calpeiana</i>   | EF127268      | Iberian P.: El Torcal de Antequera (MA)       |
| <i>M. calpeiana</i>   | EF127270      | Iberian P.: Archena, Las Arboledas (MU)       |
| <i>M. cretica</i>     | KX538936      | Crete: Topolia                                |
| <i>M. gigas</i>       | KX538937      | Japan: Iriomote                               |
| <i>M. gigas</i>       | KJ686390      | Taiwan: Kenting                               |
| <i>M. holsti</i>      | KJ686388      | Taiwan: Jiaoxi                                |
| <i>M. taiwanensis</i> | KJ686428      | Taiwan: Dashuashang                           |
| <i>M. yaginumai</i>   | KJ686400      | Japan: Iriomote                               |

Table 1: Information on the specimens used in the present study.

specimen of *M. cretica* and one of *M. gigas* from Taiwan in our analysis.

No specimens were sacrificed for the present study. Two to three segments of the third leg were removed for DNA extraction. The genomic DNA extractions, conditions of PCR amplifications, and sequencing followed protocols detailed by Arnedo & Ferrández (2007). The DNA barcode fragment of the mitochondrial cytochrome c oxidase subunit I (coi) was amplified using the primer pairs C1-J-1490 (Folmer *et al.* 1994) with C1-N-2191 (Simon *et al.* 1994) or, alternatively, C1-N-2198 (Folmer *et al.* 1994).

Homologous sequences of *M. calpeiana* individuals from several localities, as well as sequences from other available *Macrothele* species available in Genbank, were downloaded and combined with the new sequences in a single matrix. Alignment was trivial since no indel events were detected.

A Maximum Likelihood (ML) phylogenetic analysis was conducted with the help of the program RAxML 8.2.0 (Stamatakis 2014) with the aid of the graphical interface RAXML-GUI 1.3 (Silvestro & Michalak 2012). We assigned independent GTR+CAT models to each codon position, and conducted 1000 bootstrap replicates to assess support and the best tree was inferred out of 100 of the former replicates.

An haplotype network was constructed for *M. calpeiana* sequences using the TCS method (Templeton, Crandall & Sing 1992) as implemented in the programme Popart (available at <http://popart.otago.ac.nz>).

## Results

### *First observation of reproductive success in introduced individuals*

In the summer of 2014, a specimen of a large black spider was found dead in a pool of a private estate at Begur (Catalonia, Spain), and sent to the University of Girona, where it was identified by the first author as a male *M. calpeiana*. The owners of the property reported the presence of large spider webs with funnel-like retreats at the same property. On 29th August 2014, the first author visited the property, found the webs, and confirmed that they belonged to *M. calpeiana*. The presence of an adult female with spiderlings were observed in the retreat of one of the webs, in holes eroded in limestone that formed part of a decorative stone circle around an olive tree (Fig. 2). The tree itself had been transported there nine to ten years ago and there were no other similar trees nearby. Two additional adult specimens were found following examination of the remaining stones around the tree. On 22th November 2014, the property was visited again and a new web was located on the other side of the same tree. The web seemed abandoned but, after turning upside down the stone protecting the funnel retreat, one male, one female, and two juveniles were found. An additional young individual was found on the older web already observed three months before.

As described in the molecular section, another specimen was found in a garden centre in Girona, approximately 30 km east of Begur, further inland.



Fig. 2: Photographs of the private estate where male, female, and immature individuals of *M. calpeiana* were found in Begur, Girona, Spain. **A** limestone rocks encircling an ornamental olive tree where males, females, and immatures were found in successive visits in silk retreats within the holes and crevices of the stones; **B** *M. calpeiana* spiderlings hiding in the silk funnel retreat.

### Molecular analysis

Sequences obtained in the present study were submitted to Genbank under accession numbers KX538932–37 (Table 1). A matrix of 30 *Macrothele* terminals and 472 bp of the *coi* gene was assembled by combining the new sequences obtained here with those downloaded from Genbank (see Table 1). The best ML was rooted in the branch joining the Japanese species *M. yaginumai* to the remaining species as recovered in Opatova & Arnedo (2014) (Fig. 3). All samples of *M. calpeiana*, including the new sequences from allegedly introduced individuals, form a supported clade. All other *Macrothele* species analysed are highly divergent from *M. calpeiana*. The other European species, *M. cretica*, is not closely related to *M. calpeiana*, although support for basal relationships is low.

A reduced matrix including only fully overlapping *M. calpeiana* sequences (23 sequences, 429 bp) was used to infer an haplotype network (Fig. 4). The individual from Majorca (RA258) was found to share the same haplotype as an individual from Jaen (EF127259) belonging to the haplogroup IV in Arnedo & Ferrández (2007), while the individuals from Girona and Italy shared the same haplotype, which was also found in haplogroup II from Cadis.

### Discussion

Molecular data confirmed the identification of the *M. calpeiana* specimens recently collected outside its natural range, and further suggested that the specimens are the result of recent introductions as they all shared identical

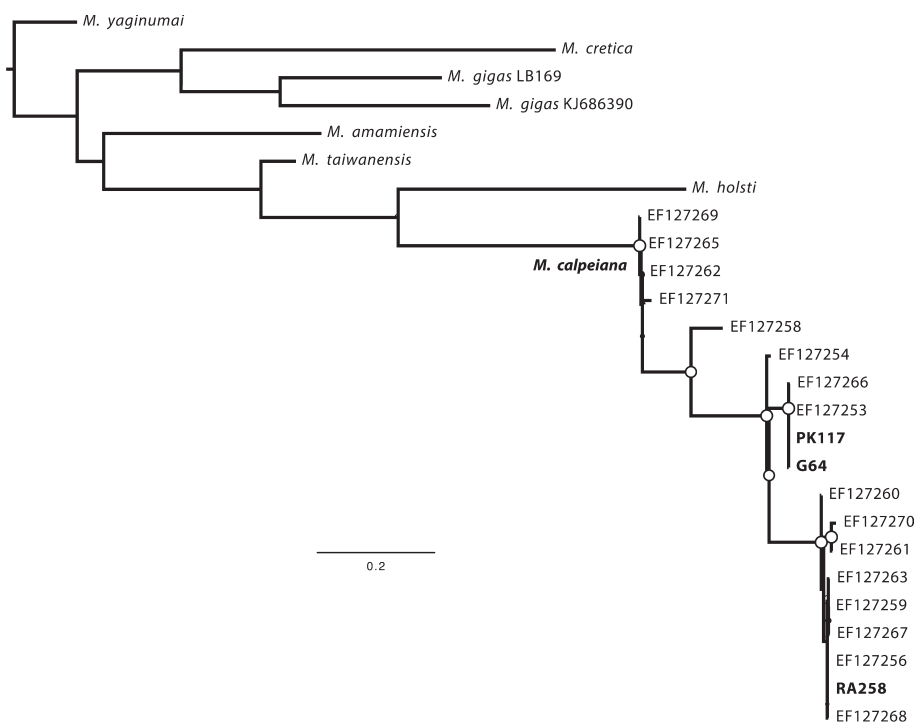


Fig. 3: Best Maximum Likelihood tree. White circles on nodes denote Bootstrap support above 80%. Accession numbers in terminals as in Table 1. Scale bar indicates number of nucleotide substitutions per site



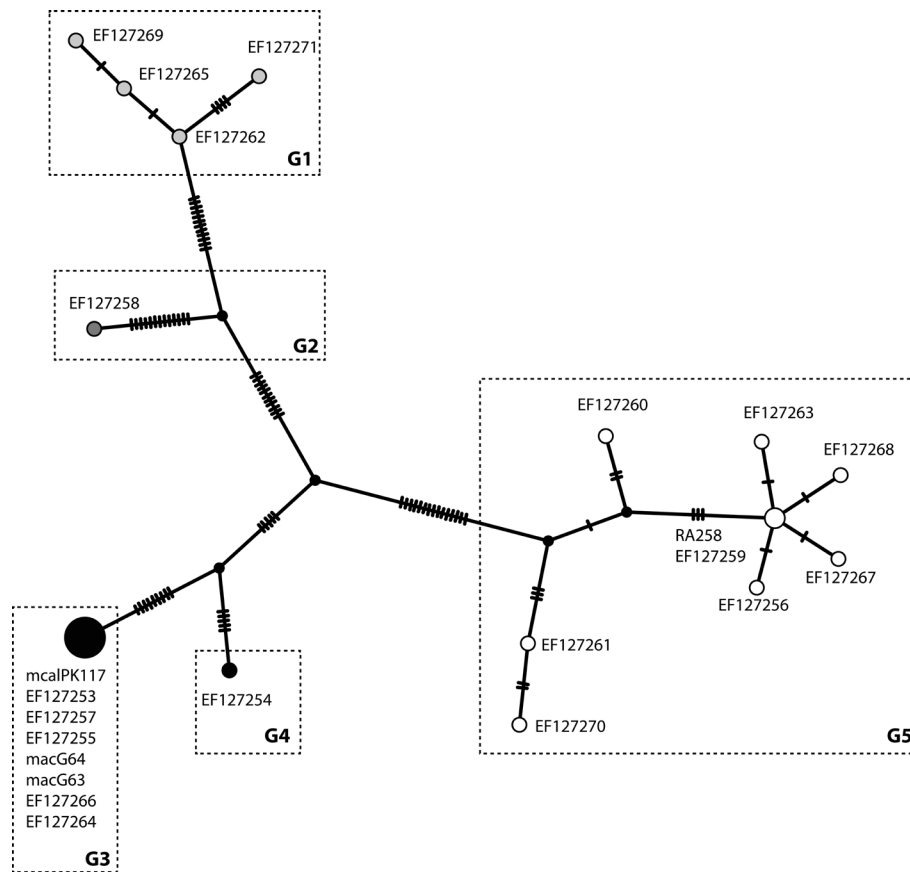


Fig. 4: Statistical parsimony haplotype network of coi. Haplotypes grouped and labelled after Arnedo & Ferrández (2007).

haplotypes to those found in native populations. Interestingly, our results point out to at least two different sources of invasion. The specimens from Girona and Italy share haplotypes with individuals collected in Cadis, and the specimen from Majorca with Jaen populations. Incidentally, the Jaen population itself has been suggested to be the result of either human introduction or recent natural expansion, since the species was only recently discovered in this province (Ferrández *et al.* 1998; Ruiz-Luque 2001); most populations are found in secondary-growth pine forests (Pedrajas Pulido & Sanz del Pozo 2010), and molecular data reveals close affinities with populations further south (Málaga, Granada) (Arnedo & Ferrández 2007).

The recent finding of *M. calpeiana* in localities far from its known distribution range has been related to the commercial export of olive trees from southern Spain (Ferrández 2010; Pantini & Isaia 2008). A *Macrothele* specimen recently captured in southern France (Cuges-les-Pins) was collected from the hollow of a centenary olive tree, in this case brought from Alicante (Siaud & Raphaël 2013). All the specimens analysed here were collected in garden centres and private gardens close to ornamental olive trees.

The identification of more than one source population among only three samples analysed suggest that the introduction of *Macrothele*, and probably other species associated with gardening, is not an exception but a general trend. For example, 18 species of terrestrial planarians are known to have been introduced in Europe (Álvarez-Presas *et al.* 2014), most likely as a result of the transport of live garden plants with soil (Justine *et al.* 2014). The evidence for the recurrent introduction of exotic fauna calls for

the implementation of additional measures to control the commercial transport of live plants at regional and national levels.

To date, all introduced specimens of *M. calpeiana* have been found in human settlements, hence there is no evidence that the spider has spread into natural habitats. Invasive predators may have pernicious effects on local diversity, not only on potential prey but also on native predators (Snyder & Evans 2006). Several cases of invasive spiders have been reported in the literature (Houser, Ginsberg & Jakob 2014), but their impact on native fauna, either as a result of competition or direct predation, is idiosyncratic. *M. calpeiana* is a large, generalist predator (Gallon 1994) and its introduction could potentially have a significant impact on local ecosystems. Although cases of envenomation have been reported for its Taiwanese relatives (Hung & Wang 2004), there is no record so far that European species may pose a threat to human health.

The chances for *M. calpeiana* to invade natural areas will depend on the existence of adequate environmental conditions, the ability to disperse from human settlements to natural areas, and a viable number of adult individuals. An earlier study suggested that most localities where *M. calpeiana* had been newly reported fell outside the predicted distribution range of the species based on climatic variables. However, we report here for the first time the existence of a likely reproductive population in Begur, an area not predicted by climatic models (Jimenez-Valverde, Decae & Arnedo 2011). Because of its close proximity to the Mediterranean Sea, the lower minimum and higher maximum temperatures in Begur are more moderate than those in

nearby inland areas such as Girona, where the species has also been found in a garden centre. Particular microclimatic conditions may explain why climatic models fail to predict the presence of species at lower geographic scales.

## Conclusion

We provide evidence that *M. calpeiana* has been introduced in different places in Europe and the Iberian Peninsula from different source populations in its native range, most likely as a result of the commercial transport of olive trees for agriculture and gardening. Moreover, we report the existence of a reproductive population that may have been present in northern Catalonia for a decade, which confirms that the species may actually be able to invade regions not predicted by broad scale climatic models. Our results support the recurrence of *Macrothele* introductions, and call for the implementation of measures to control the transport of olives and probably other ornamental trees.

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## Bibliography

- ÁLVAREZ-PRESAS, M., MATEOS, E., TUDÓ, À., JONES, H., & RIUTORT, M. 2014: Diversity of introduced terrestrial flatworms in the Iberian Peninsula: a cautionary tale. *PeerJ* **2**: e430.
- ARNEDO, M. A. & FERRANDEZ, M. A. 2007: Mitochondrial markers reveal deep population subdivision in the European protected spider *Macrothele calpeiana* (Walckenaer, 1805) (Araneae, Hexathelidae). *Conservation Genetics* **8**: 1147–1162.
- FERRÁNDEZ, M. A. 2010. *Macrothele calpeiana*. In R. Hidalgo (ed.), *Bases ecológicas preliminares para la conservación de las especies de interés comunitario en España: invertebrados*. Madrid: Ministerio de Agricultura, Alimentación y Medio Ambiente Secretaría General Técnica, Centro de Publicaciones: 1–60.
- FERRÁNDEZ, M. A., FERRÁNDEZ DE CESPEDES, H. & PERUCHO, A. 1998: *Macrothele calpeiana*, la araña negra de los alcornocales. *Quercus* **146**: 14–18.
- FOLMER, O., BLACK, M., HOEH, W., LUTZ, R. & VRIJENHOEK, R. 1994: DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology* **3**: 294–299.
- GALLON, R.C. 1994: Observations on *Macrothele calpeiana* (Walckenaer, 1805) in southern Iberia. *Journal of the British Tarantula Society* **1**: 1–12.
- HELSDINGEN, P. J. VAN 1993: Can *Macrothele calpeiana* (Walckenaer) (Araneae, Hexathelidae) be used as a bio-indicator? *Bulletin de la Société Neuchâtoise des Sciences Naturelles* **116**: 253–258.
- HELSDINGEN, P. J. VAN & DECAE, A. 1992: Ecology, distribution and vulnerability of *Macrothele calpeiana* (Walckenaer) (Araneae, Hexathelidae). *Tijdschrift voor Entomologie* **135**: 169–178.
- HOUSER, J. D., GINSBERG, H. & JAKOB, E. M. 2014: Competition between introduced and native spiders (Araneae: Linyphiidae). *Biological Invasions* **16**: 2479–2488.
- HUNG, S.-W. & WANG, T.-L. 2004: Arachnid envenomation in Taiwan. *Annals of Disaster Medicine* **3**: S12–S17.
- JIMENEZ-VALVERDE, A., DECAE, A. E. & ARNEDO, M. A. 2011: Environmental suitability of new reported localities of the funnelweb spider *Macrothele calpeiana*: an assessment using potential distribution modelling with presence-only techniques. *Journal of Biogeography* **38**: 1213–1223.
- JIMÉNEZ-VALVERDE, A. & LOBO, J. M. 2006: Distribution determinants of endangered Iberian spider *Macrothele calpeiana* (Araneae, Hexathelidae). *Environmental Entomology* **35**: 1491–1499.
- JUSTINE, J.-L., WINSOR, L., GEY, D., GROS, P. & THÉVENOT, J. 2014: The invasive New Guinea flatworm *Platydemus manokwari* in France, the first record for Europe: time for action is now. *PeerJ* **2**: e297-22.
- KULCZYŃSKI, W. 1903: Araneorum et opilionum species in insula Creta a comite Dre Carolo Attems collectae. *Bulletin International de l'Académie des Sciences de Cracovie* **1903**: 32–58.
- MÁRQUEZ-RODRÍGUEZ, J. 2014: Primera cita de *Macrothele calpeiana* (Walckenaer, 1805) (Araneae: Hexathelidae) en la campiña de Sevilla. *Nova Acta Scientiarum Compostelanae Biologia* **21**: 5–6.
- OPATOVA, V. & ARNEDO, M. A. 2014: From Gondwana to Europe: inferring the origins of Mediterranean *Macrothele* spiders (Araneae: Hexathelidae) and the limits of the family Hexathelidae. *Invertebrate Systematics* **28**: 361–374.
- PANTINI, P. & ISAIA, M. 2008: New records for the Italian spider fauna (Arachnida, Araneae). *Arthropoda Selecta* **17**: 133–144.
- PEDRAJAS PULIDO, L. & SANZ DEL POZO, B. 2010: Nuevas citas de *Macrothele calpeiana* (Walckenaer, 1805) en la provincia de Jaén (España). *Revista Ibérica de Aracnología* **18**: 107–109.
- RUIZ-LUQUE, F. J. 2001: Nuevos datos de *Macrothele calpeiana* (Walckenaer, 1805) para Jaén (España). *Revista Ibérica de Aracnología* **4**: 34.
- SIAUD, P. & RAPHAËL, B. 2013: Première observation en Provence d'une mygale andalouse: *Macrothele calpeiana* (Walckenaer, 1805). *Mésogée. Bulletin du Muséum d'Histoire Naturelle de Marseille* **69**: 5–11.
- SILVESTRO, D. & MICHALAK, I. 2012: raxmlGUI: a graphical front-end for RAXML. *Organisms Diversity & Evolution* **12**: 335–337.
- SIMON, C., FRATI, F., BECKENBACH, A., CRESPI, B., LIU, H. & FLOOK, P. 1994: Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. *Annals of the Entomological Society of America* **87**: 651–701.
- SNAZELL, R. G. 1986: The spider genus *Macrothele* Ausserer in Spain (Araneae, Dipluridae). *Bulletin of the British Arachnological Society* **17**: 80–83.
- SNAZELL, R. G. & ALLISON, R. 1989: The genus *Macrothele* Ausserer (Araneae; Hexathelidae) in Europe. *Bulletin of the British Arachnological Society* **8**: 65–72.
- SNYDER, W. E. & EVANS, E. W. 2006: Ecological effects of invasive arthropod generalist predators. *Annual Review of Ecology, Evolution and Systematics* **37**: 95–122.
- STAMATAKIS, A. 2014: RAXML Version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* **30**: 1312–1213.
- TEMPLETON, A. R., CRANDALL, K. A. & SING, C. F. 1992: A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping and DNA sequence data. III. Cladogram estimation. *Genetics* **132**: 619–633.
- WALCKENAER, C. A. 1805: *Tableau des aranéides ou caractères essentiels des tribus, genres, familles et races que renferme le genre Aranea de Linné, avec la désignation des espèces comprises dans chacune de ces divisions*. Paris: Dentu.