

First record of *Macrothele calpeiana* (Araneae: Macrothelidae) in Austria

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Abstract. *Macrothele calpeiana* (Walckenaer, 1805) (Macrothelidae) is reported for the first time in Austria (Untermeierhof in Lower Austria). Identification to species level was performed by molecular methods (DNA barcoding).

Keywords: introduced species, molecular species identification

Zusammenfassung. Erstnachweis von *Macrothele calpeiana* (Araneae: Macrothelidae) für Österreich. Die Andalusische Trichternetzspinne *Macrothele calpeiana* (Walckenaer, 1805) (Macrothelidae) wurde das erste Mal für Österreich in Untermeierhof in Niederösterreich nachgewiesen. Die Bestimmung auf Artniveau erfolgte mittels molekularer Methoden (DNA Barcoding).

The species *Macrothele calpeiana* (Walckenaer, 1805) (Macrothelidae) is one of the largest European spiders and the only one listed in the Bern Convention (1979, Appendix II) as well as the Habitats Directive (92/43/EEC, Annex IV). In addition, this species is included in the national Red Book of Invertebrates of Spain (Verdú et al. 2011). Its natural distribution is described as covering southern Spain (Blasco & Ferrández 1986), Portugal (Jiménez-Valverde et al. 2007) and northern Africa (Benhacene et al. 2023). Some doubts, however, exist regarding occurrence of *M. calpeiana* in Africa (Jiménez-Valverde 2009).

In the last centuries several records of *M. calpeiana* have been reported for different European countries: Belgium (Jiménez-Valverde et al. 2011), Italy (Pantini & Isaia 2008, Pantini et al. 2016), Holland (Van Keer 2010), Germany (Bauer & Wendt 2022), England (Sherwood 2022), France (Siaud & Raphaël 2013) as well as Switzerland (Hänggi & Stäubli 2012). As location sites were mostly close to garden centers many reports appeared to be related to non-native ornamental trees and other garden goods. Although most findings probably represent isolated specimens, reproduction outside of the natural habitat has been proven at least in one location in Spain (Bellvert & Arnedo 2016).

Herein we describe the identification of a *Macrothele calpeiana* specimen found in Lower Austria, providing the first record of this species for Austria.

Material and methods

Morphological identification was carried out using the online determination key of Nentwig et al. (2025). Photographic documentation of the specimen was carried out by Thomas Schwaha using a Hirox RH-2000 microscope (Hirox Europe, Limonest, France).

Genomic DNA (gDNA) was extracted from one leg of the specimen using the QIAamp DNA Micro Kit (QIAGEN, Hilden, Germany) following the manufacturer's instructions. To amplify the standard barcoding region of the mitochon-

drially encoded cytochrome c oxidase subunit I gene (COI), we used the standard primer set LCO-1490 and HCO-2198 (Folmer et al. 1994) and applied the following thermocycler settings: Initial denaturation at 95°C for 3 minutes followed by 38 cycles of 95°C for 15 seconds, 50°C for 15 seconds and 72°C for 15 seconds, and a final elongation step at 72°C for 5 minutes.

The purified PCR product was then sent to Microsynth Austria GmbH (<https://www.microsynth.com/home-ch.html>) for Sanger sequencing (forward and reverse). Sequence plausibility was checked by performing a BLAST search against the NCBI nucleotide database (<https://www.ncbi.nlm.nih.gov/nucleotide>) as well as analyzing the chromatogram using the software Geneious Prime (Version 2024.0.7; Dotmatrix, Boston, USA). The same software was used to conduct all subsequent bioinformatical analyses. The COI sequence obtained in this study was aligned with 24 reference sequences from NCBI by applying multiple sequence alignment according to MUSCLE 5.1 by Edgar 2022 (Algorithm: PPP). Details on all sequences used for this analysis are given in Supplementary Tbl. 1 (NCBI taxonomy ID, sampling locality, authorship). The alignment was further used to calculate a Maximum Likelihood (ML) tree by applying RAxML 8.2.11 (Stamatakis 2014) using default Geneious Prime settings for the GTR GAMMA nucleotide model. Support values were estimated based on 1.000 bootstrap pseudo-replicates. In addition, we calculated a ML tree applying PhyML 3.3.20180621 (Guindon et al. 2010) which is shown in Supplementary Fig. 1. This was done by using default Geneious Prime settings for the GTR model and support values were estimated based on 100 bootstrap pseudo-replicates. For both ML trees the COI sequence of *Allothelae australis* (GenBank: KY01772.1) was chosen as the outgroup.

The described specimen is stored in the collection of the Natural History Museum of Vienna (Reference: NHMW-ZOO-AR-30390) and the COI sequence was uploaded on the NCBI GenBank (Accession number: PX488387).

Results and discussion

On the 15. Jun. 2025 one specimen belonging to the spider family Macrothelidae was found dead in a pool in Untermeierhof in Lower Austria, 48.03994°N, 16.08514°E about 440 m a.s.l. (Fig. 1).

The body length of the specimen was 28 mm, the prosoma showed a length of 11 mm and a width of 9 mm (Fig. 2). Based on four main morphological criteria we could determine that the specimen found belongs to the genus *Macrothele* within

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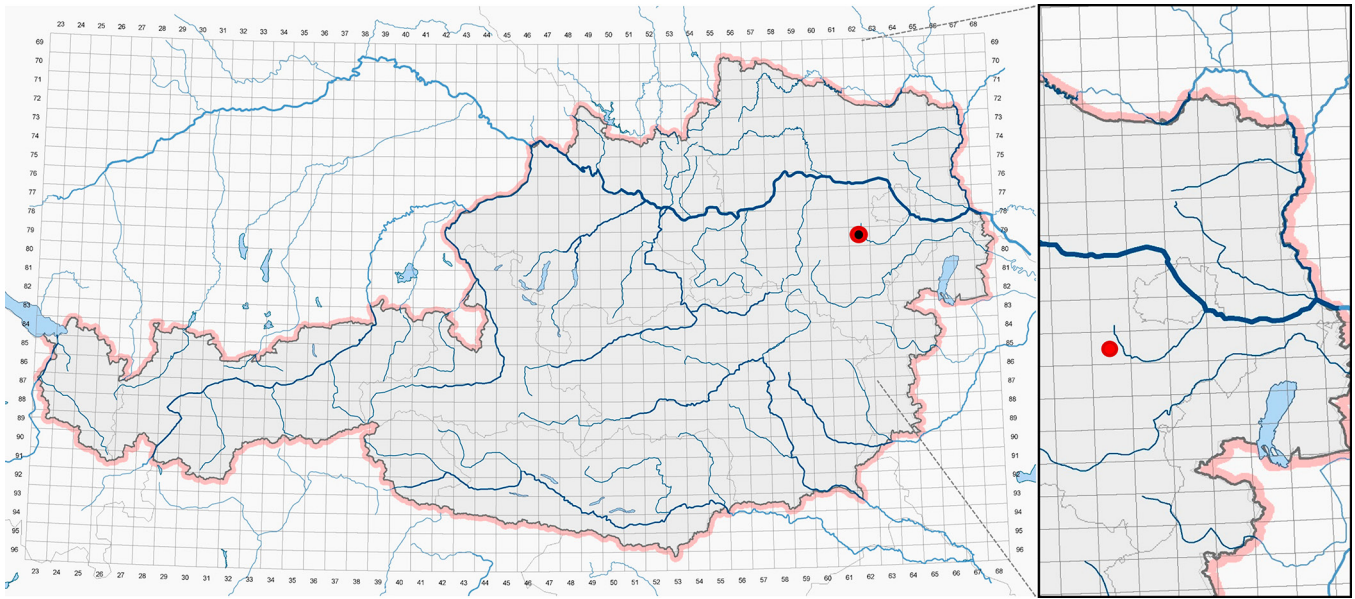


Fig. 1: Geographical map of Austria showing the location where the animal was found, 48.03994°N, 16.08514°E. Untermeierhof in Lower Austria is marked by the red dot

Macrothelidae. Firstly, the chelicerae were orientated in an orthognath position. Secondly, only four spinnerets were present with the posterior lateral ones being very long. Finally, scopulae on anterior tarsi and metatarsi were absent.

No male palpal structures were formed which indicates that the specimen represents either a subadult or female individual. However, all attempts to prepare the epigyne failed as the animal had completely dried out and fully hardened at the time of collection. A scientifically sound determination based on morphological criteria was therefore only possible down to genus level.

We further applied DNA barcoding using COI sequencing data to facilitate molecular species identification. The most significant matches on NCBI showed high similarity (98.0% to 99.8% nucleotide identity) to several *Macrothele calpeiana* COI sequences. In more detail, the sequence of the individual we found in Austria was almost identical to those from sam-

ples collected in the Spanish provinces of Murcia and Andalusia. Other sequences assigned to *M. calpeiana* showed noticeably lower similarity values (approx. 92.0%). This pattern can be explained by high population fragmentation and subdivision which is known to result in deep genetic divergence across populations of the Iberian Peninsula (Arnedo & Fernández 2007). To further support this, we calculated two different Maximum likelihood trees including 24 different COI sequences published on NCBI as well as the COI sequence obtained from the animal found in Austria. The resulting ML trees are displayed in Fig. 3 and Supplementary Fig. 1. Concerning the relationships between groups outside of *Macrothele*, the data set used could not accurately resolve the same relationships as shown in Opatova et al. 2020 (note that both ML trees represent the same topology although some nodes show low bootstrap support values). These discrepancies are expected since COI is not meant to resolve deep phylogenetic relationships (V. Opatová, personal communication). However, it shows that the COI sequence of the specimen found in Austria clearly clusters together with other samples from the Iberian Peninsula described as *Macrothele calpeiana*. Together with the morphological description, the molecular analysis of the COI locus could therefore reliably identify the found specimen as *Macrothele calpeiana*.

How the individual described herein came to Austria is unknown. The import of live garden plants in soil, like for instance ornamental trees, is known to be a source for the introduction of alien species (Bellvert and Arnedo 2016). Two such examples are reported for *M. calpeiana* in Germany (Bauer & Wendt 2022). In the present case, no plants or soil imported from the spider's original distribution area have been recently placed in the garden where the animal was found. However, it is not clear whether this applies to surrounding gardens as well. Furthermore, no spider web could be found at, or in proximity to, the location of collection.

Therefore, we cannot answer the question whether the species was recently introduced to Austria or is already established here. As the Austrian climatic conditions differ notably



Fig. 2: *Macrothele calpeiana* found in a pool in Untermeierhof in Lower Austria. The distal part of the posterior spinnerets has become broken off. Scale: 10 mm, photo: Thomas Schwaha

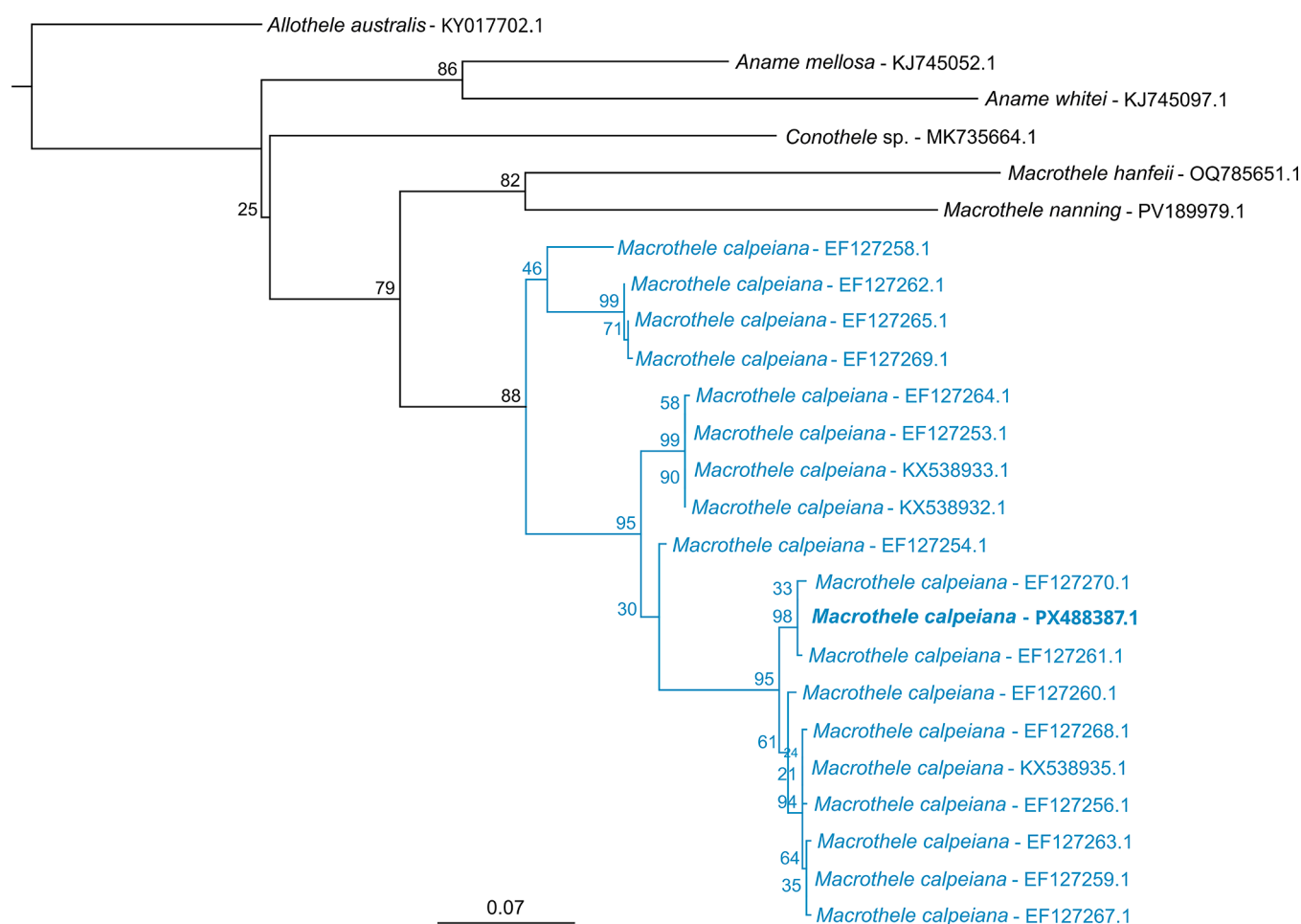


Fig. 3: RaxML Maximum likelihood tree based on COI data, using *Allothele australis* (GenBank: KY017702.1) as the outgroup. Each node is labelled with the corresponding bootstrap support value. The sequence of the *Macrothele calpeiana* specimen found in Lower Austria (GenBank: PX488387) is highlighted in bold. Accession numbers for all samples are included

from those in the original habitat of *Macrothele*, the establishment of this species in Austria under natural circumstances is doubtful. This especially refers to extended winter periods with sub-zero temperatures and is also mentioned for other Central European locations by Jiménez-Valverde et al. (2011).

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Conflicts of Interest. The authors have no conflicts of interest to declare that are relevant to the content of this article.

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