



A master in disguise? The rediscovery of *Misumena bicolor* Simon, 1875 (Araneae: Thomisidae)

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Abstract

Misumena bicolor Simon, 1875, an enigmatic species known only from the male holotype collected on Corsica (France), has remained elusive since its initial description. In this study, we report new occurrences of *M. bicolor* from Sardinia (Italy) and Bavaria (Germany) based on male material, considerably expanding its known distribution. Employing an integrative taxonomic approach encompassing both morphological and COI (barcode) analyses, we confirm *M. bicolor* as a valid species. As the male of *M. bicolor* apparently has a distinct coloration, we complement the known distributional data with possible sightings of photographic observations from various naturalist portals and hereby provide additional evidence for the establishment of the species on the French mainland and in Germany.

Key words: crab spider, new record, Sardinia, Germany, taxonomy

Introduction

Thomisidae, commonly known as crab spiders, are a very diverse spider family of ambush hunters with more than 170 known genera distributed on all continents except for Antarctica (Benjamin *et al.* 2008). In several thomisid genera the females have the ability of actively changing colors, which may lead to an improved hunting success and/or a reduced predatory pressure (Oxford & Gillespie 1998). Probably one of the most prominent representatives of these species is *Misumena vatia* (Clerck, 1757), named “the chameleon of all spiders” by Platnick (2020). While the physiology, ecology and behavior of *M. vatia* is well known (Fritz & Morse 1985; Gadeau de Kerville 1907; Kevan & Greco 2001; Morse 1982, 1985, 1990, 1994, 2009, 2011; Riou & Christidès 2010), relatively little is known about its taxonomy and that of the other four members of the genus in the Western Palearctic (World Spider Catalog 2024). *Misumena spinifera* (Blackwall, 1862) and *Misumena nigromaculata* Denis, 1963 are Macaronesian endemics with *M. nigromaculata* described from a single female captured in 1940 near Funchal (Madeira) and not described again since (Cardoso *et al.* 2017; Denis 1963; Suárez 2016). *Misumena atrocincta* Costa, 1875 is a doubtful species from Egypt, mentioned in a footnote with very brief description, lacking illustrations as well as information about the sex of the described specimen. Although the species is listed in a recent study (Amal *et al.* 2019), its identification appears highly doubtful in the face of the poor original description. Likewise, very little is known about *Misumena bicolor* Simon, 1875. Since its description by Simon (1875) based on a single male specimen from Corsica, only Pavesi (1880) reported a single specimen collected in Tamerza, Tunisia. Unfortunately, this specimen was lost in 1873 while being sent to E. Simon for consultation. However, Pavesi expressed confidence in his identification,

stating “mi restano però note sufficienti da poterlo riferire a questa specie“ (I have sufficient notes left to refer it to this species).

Thereafter, *M. bicolor* remained forgotten until Lehtinen (2004) re-examined the male holotype deposited at the Muséum National d’Histoire Naturelle in Paris. He came to the conclusion that *M. bicolor* is a valid species and not, as assumed by Simon (1875), possibly only a color variant of *M. vatia*. While he also synonymized another *Misumena* species (*Misumena personata* Simon, 1916) with *M. bicolor*, this was rejected by Breitling *et al.* (2016) and *M. personata* was assigned to *M. vatia*. Breitling *et al.* (2016) also considered *M. bicolor* to be a valid species, but provisionally classified it as *species inquirenda*. As males apparently have a characteristic coloration (black prosoma, unicolored bright opisthosoma) they should be well identifiable in the field.

Here, we present morphological and genetic (COI-barcode) evidence establishing *M. bicolor* as a distinct species. This is supported by the inclusion of recent material collected from Sardinia (Italy) and Bavaria (Germany). To explore other potential locations of *M. bicolor*, plausible photographic records of *Misumena*-specimens fitting the description of *M. bicolor* were collected from popular naturalist networks (iNaturalist 2024; Observation International 2024). Our records and the ones harvested from online resources suggest a distribution of this species ranging from the Western Mediterranean region over southwestern France to Central Europe (Germany).

Material and methods

The Sardinian specimen was collected with a sweep net by the first author during a field trip in June 2022 and stored in 70% Ethanol. The German specimen was collected by hand and preserved in 99% Ethanol.

Morphological examination. Determination was done by using a Zeiss Stemi 305 stereomicroscope and the works of Simon (1875), Lehtinen (2004) and Breitling *et al.* (2016). Nomenclature for structures of the male palp follows Loerbroks (1984). All drawings were made by using drawing attachments on an Olympus SZH stereomicroscope and Zeiss Standard microscope. Habitus images were taken with TouPCam C-MOS Camera, and edited in Adobe photoshop CC. Palps had been temporary fixed in glycerin-gelatin.

Molecular methods. Ethanol-preserved leg tissue from an adult male specimen of *M. bicolor* from Bavaria served as DNA source. For DNA extraction, a silica membrane column of the Blood and Tissue kit by Qiagen (Hilden, Germany) was used, following the manufacturer’s specifications. Polymerase chain reaction targeted 658 bp of the 5’ part of the mitochondrial COI (cytochrome c oxidase subunit 1) gene using primers HCO2198-JJ and LCO1490-JJ (Astrin & Stüben 2008) and the ‘Multiplex PCR Master Mix’ (Qiagen). PCR routine: first cycle set (15 repeats): 35 s denaturation at 94°C, 90 s annealing at 55°C (–1°C per cycle) and 90 s extension at 72°C. Second cycle set (25 repeats): 35 s denaturation at 94°C, 90 s annealing at 45°C, and 90 s extension at 72°C. The PCR product was sent for bidirectional Sanger sequencing to BGI (Hong Kong, China). The remainder of the specimen is stored frozen at LIB Biobank, Museum Koenig, Bonn, and accessible under voucher ID ZFMK-TIS-76343. The corresponding genomic DNA is filed under ZFMK-DNA-FD19583051. Its DNA barcode sequence is accessible on BOLD (Ratnasingham & Hebert 2013) under process ID LIBBB218-23.

Additional DNA sequences of *Misumena vatia*, *M. spinifera*, and *Spiracme striatipes* (L. Koch, 1870) were downloaded from BOLD (Ratnasingham & Hebert 2013) and assembled, inspected and aligned using Geneious vers. R7 (Biomatters, Auckland, New Zealand). As the number of hits on BOLD for *Misumena vatia* was excessive (>500) while genetic variation remained moderate, we randomly selected representatives of the genetically most distinct clusters up to a total number of 20 representatives for *M. vatia*. A Neighbor Joining tree was constructed in Geneious.

To search for further pictures of *Misumena bicolor* the google image search (search word: “Misumena”) was used. In addition the two naturalist networks iNaturalist.org (iNaturalist 2024) and observation.org (Observation International 2024) were searched for images showing *Misumena vatia* males, with filter on European and North African countries and *Misumena bicolor*. The search for *M. vatia* was conducted because it is the species most likely to be confused with *M. bicolor*. The map, showing all known confirmed and unconfirmed records to date, was created with SimpleMappr (Shorthouse 2010).

Abbreviations: **COI**—Cytochrome c oxidase subunit I gene; **Op**—Opisthosoma; **Pr**—Prosoma; **RTA**—retrolateral tibial apophysis; **ZFMK**—Zoological Research Museum Alexander Koenig, Bonn, Germany.

Results

Misumena bicolor Simon, 1875

Figs 1A1–A5, 2A–B, 3, 4A

Misumena bicolor Simon, 1875: 246, pl. 7, f. 2 (holotype male from FRANCE: Corsica [Muséum national d'histoire naturelle, Paris], examined by Lehtinen 2004 and Breitling *et al.* 2016). Lehtinen 2004: 171, f. 86–87; Breitling *et al.* 2016: 70, f. 11.

Other material examined. ITALY: Sardinia: Bolotana, 40.353N, 8.911E, 990 m, 1 male, 12 June 2022, A. Bach leg., sweep netted in a wet meadow near Riu Ilde. **GERMANY: Bavaria:** Deggendorf, Westlicher Stadtgraben 50, 1 male, 2 June 2022, HJ Thorns leg., collected by hand (ZFMK-TIS-76343; BOLD-ID: LIBBB218-23).

Comparative material of *M. vatia*. GERMANY: Hesse: Usingen, close to Eschbacher Klippen, 50.3638N, 8.5381E, 381 a.s.l., 2 males, 2 females, 8 June 2017, leg. S. Lauterbach. **North Rhine-Westphalia:** Bestwig, Ostenberg, old quarry, 51.3505N, 8.4036E, 419 m a.s.l., 7 males, 2 females, leg. S. Lauterbach. **Baden-Württemberg:** Elfmorgenbruch by Karlsruhe, forest clearing, 49.0097N, 8.4547E, 1 male, 23 May 2023, leg. T. Bauer (Coll. T. Bauer).

Diagnosis. Differentiated from other species of the genus by the combination of the orange opisthosoma lacking any longitudinal stripes as well as dark lateral patterns in combination with a less coiled embolus compared to *M. vatia*. In *M. vatia*, the embolus is much more strongly coiled, with the distal part nearly in parallel position to the sides of the cymbium and the tip at a 90° angle (Figs 1 B1, B2, B4)), while in *M. bicolor* the distal part and the tip point, more or less, in the same direction (Figs 1 A1, A2, A5). In addition, the RTA of *M. vatia* bears an additional hump in the basal part (Fig 1 B3), which is missing in *M. bicolor*. The position of the spermophor in the basal part of the embolus differs as well. While the spermophor is positioned more horizontally in *M. vatia*, in *M. bicolor* it enters the embolus in a transversal position.

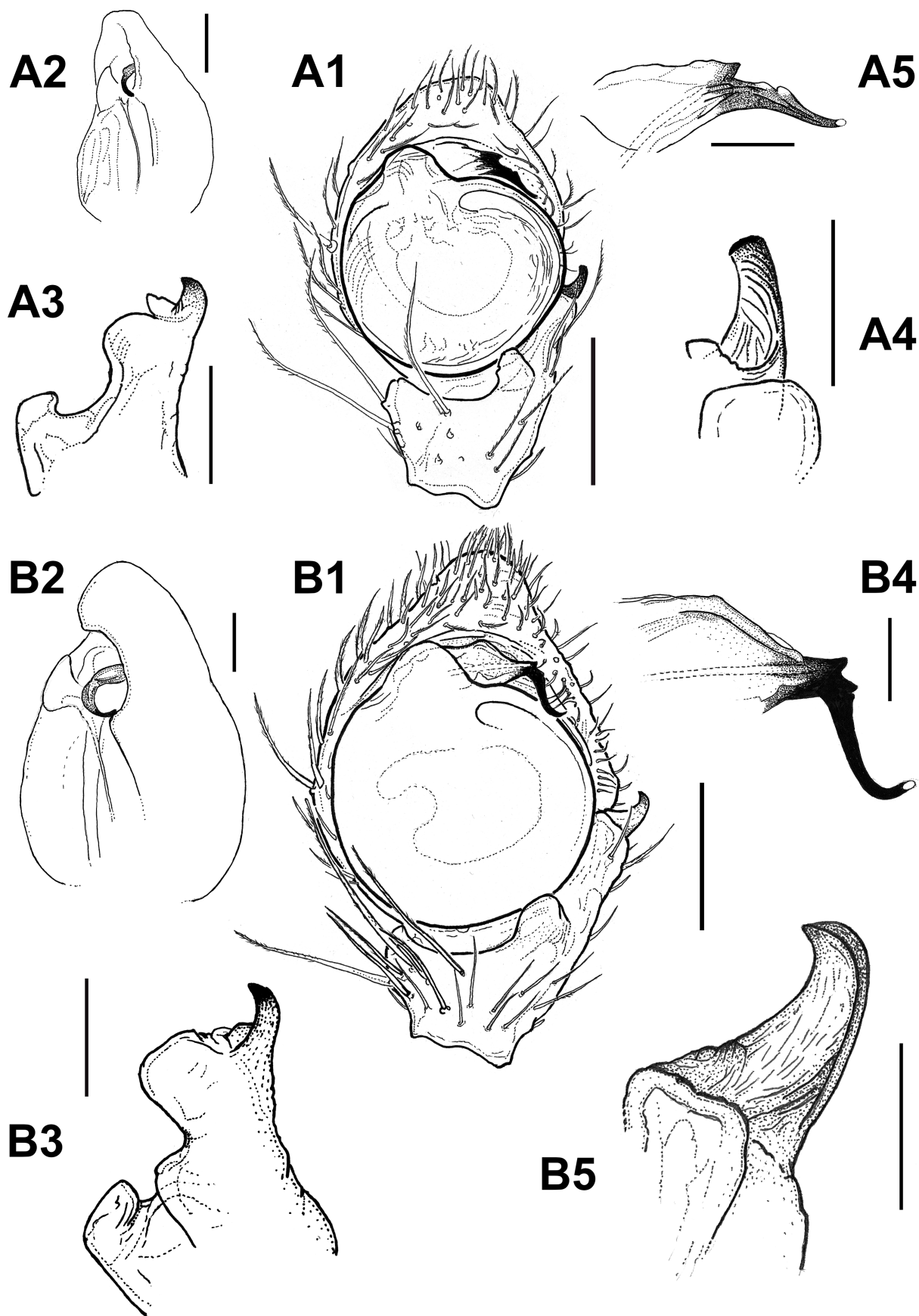
Description. Male (from Sardinia, in ethanol): Pr length 1.3, Pr width 1.35, Op length 1.9, Op width 1.35. The prosoma of *M. bicolor* is black in live specimens, with a lighter area behind the fovea and the eyes. When preserved in ethanol, the prosoma becomes dark reddish-brown. In contrast, the prosoma of *M. vatia* features a light, often yellow, longitudinal stripe. The opisthosoma of *M. bicolor* is a light yellowish tone, covered with conspicuous, short spines, and lacks any longitudinal lines or dark lateral patterns, which sharply contrasts with the opisthosoma of *M. vatia*. The femora of the first pair of legs are prominently dark in color, while the remaining segments display an anterior reddish-brown hue, with a gradual brightening towards the posterior regions Left leg measurements: I. coxa 0.40; trochanter 0.30; femur 2.10; patella 0.70; tibia 1.75; metatarsus 1.50; tarsus 0.50; total length 7.25 II. 0.40; 0.25; 2.05; 0.65; 1.70; 1.45; 0.80; 7.30; III. 0.30; 0.15; 0.90; 0.40; 0.70; 0.55; 0.40; 3.40; IV. 0.35; 0.20; 0.90; 0.40; 0.70; 0.60; 0.40; 3.55.

Female: Unknown.

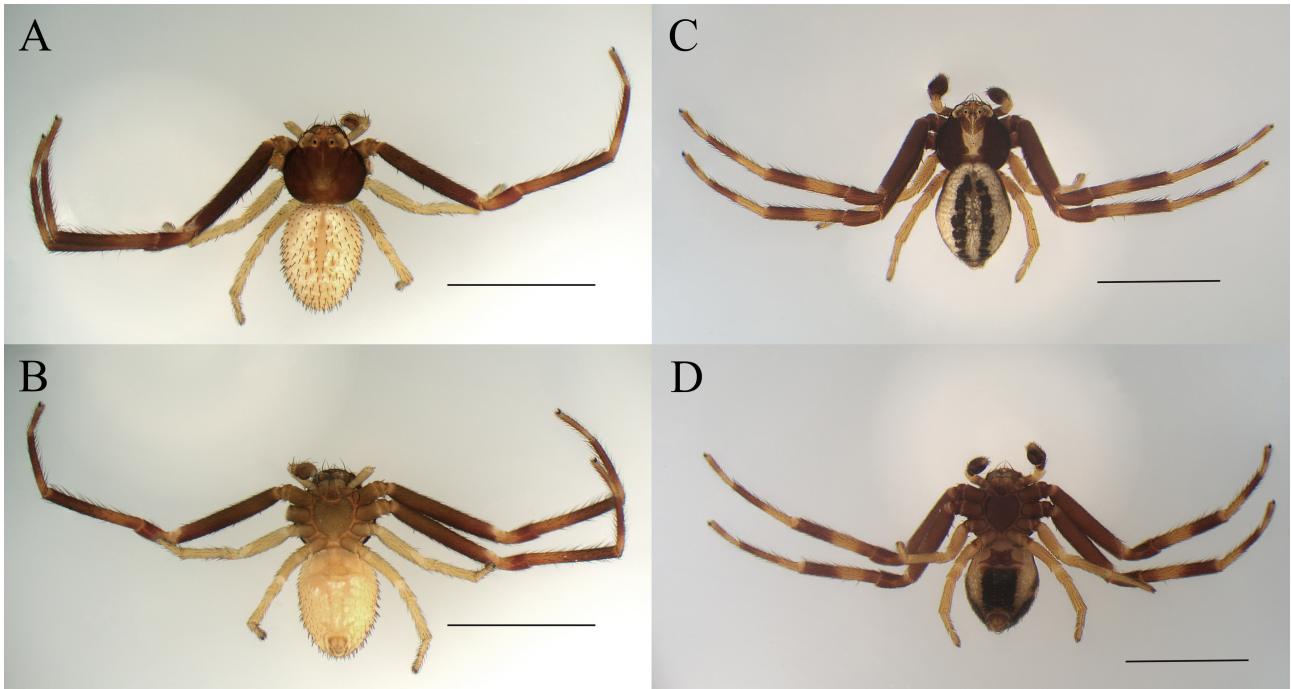
Comment. Our specimens (Figs 2A–B, 3) are consistent with the somatic description provided by Simon (1875). However, concerning the palp, Simon's description merely notes that it is disc-shaped and of a reddish coloration. Similarly, Lehtinen's (2004) description is relatively concise, mentioning only the differences between *M. bicolor* and *M. vatia* with respect to a stronger coiled embolus and details in RTA morphology. However, Breitling *et al.* (2016) highlighted the important difference that the embolus in *M. bicolor* is less coiled than in *M. vatia*, a characteristic which was also observed in both of our specimens (Fig. 1 A5).

Molecular analyses. In the Neighbor Joining tree (Fig. 5), the COI sequence of the *Misumena bicolor* male from Germany clearly falls outside the *M. vatia* cluster. The genetic distances between *M. bicolor* and *M. vatia* range from 6.4 to 8.0% (uncorrected *p*-distance when comparing full sequences of *M. vatia* in the dataset, over a length of 653 bp). Intraspecific distances in *M. vatia* range from 0.0 to 2.3%. This provides strong evidence in favor of allocating the specimens from Germany (and Corsica) to a species distinct from *M. vatia*. A single sequence of a potentially juvenile "*M. vatia*"-specimen from Corsica on BOLD (Process ID LPRCS004-19) is nearly identical to our sequence and represents *M. bicolor* (BIN/Barcode Index Number BOLD:AEC4584) as well.

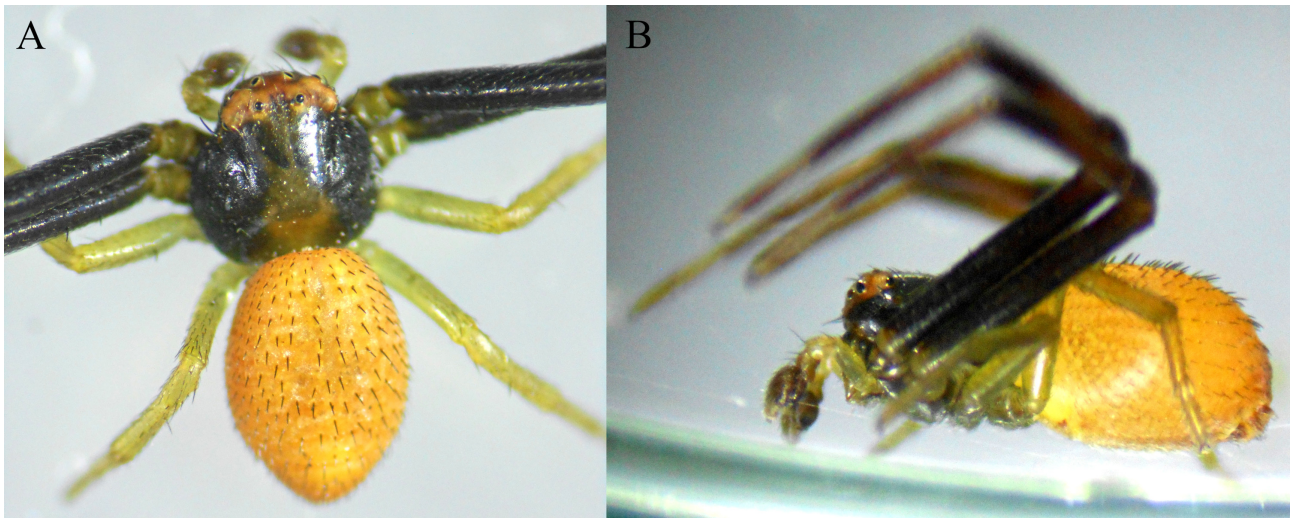
Genetic distance of the *M. bicolor* specimens to the outgroup *Spiracme striatipes* is ca. 12% (same for *M. vatia*) and to *Misumena spinifera* ca. 9%. *Misumena vatia* is separated from *M. spinifera* by around 10%.



FIGURES 1 A1–5, B1–5. A1–A5 *Misumena bicolor*, male from Sardinia; B1–B5 *Misumena vatia*, male from Germany. A1, B1 Palp, ventral, scale: 0.2 mm; A2, B2: Palp without tibia, lateral, scale: 0.1 mm; A3, B3: RTA lateral, scale: 0.1 mm; A4, B4: Apical tip of RTA, scale: 0.05 mm; A5, B5: Embolus ventral, scale: 0.05 mm.

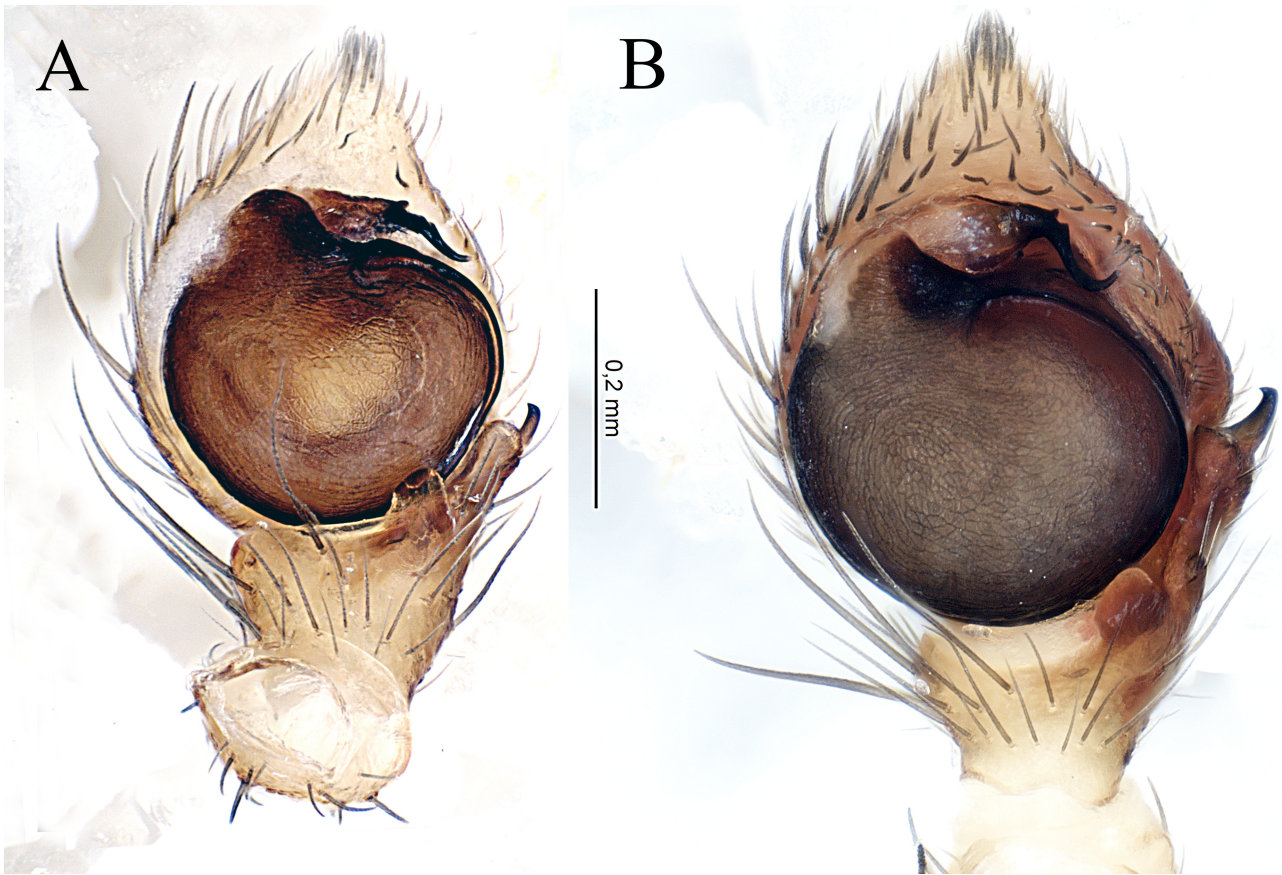


FIGURES 2A–D. Habitus of preserved male specimens of *Misumena bicolor* from Sardinia (Italy) (A, B) and *M. vatia* from Germany (C, D) in dorsal (A, C) and ventral (B, D) view. Scales: 2.00 mm.



FIGURES 3A–B. In vivo habitus of *Misumena bicolor* from Germany in dorsal (A) and lateral (B) view.

Photographic records from naturalist networks. In addition to the two photo-based records from Germany in Breitling *et al.* (2016), fourteen other photo-records from Germany, mainland France, Italy and Austria were identified. Links and coordinates from all sites are given in the supplement table. Photographic evidence of *M. bicolor* in mainland Europe is available from west or north of the Alps. The westernmost photographic record comes from the French commune Astaffort in the department of Aquitaine, while the northernmost locality is in the Solling, a German low mountain range in Lower Saxony (Fig. 6).



FIGURES 4A–B. Images of left male palps in ventral view. A *Misumena bicolor* from Deggendorf, Germany; B *M. vatia* from Karlsruhe, Germany.

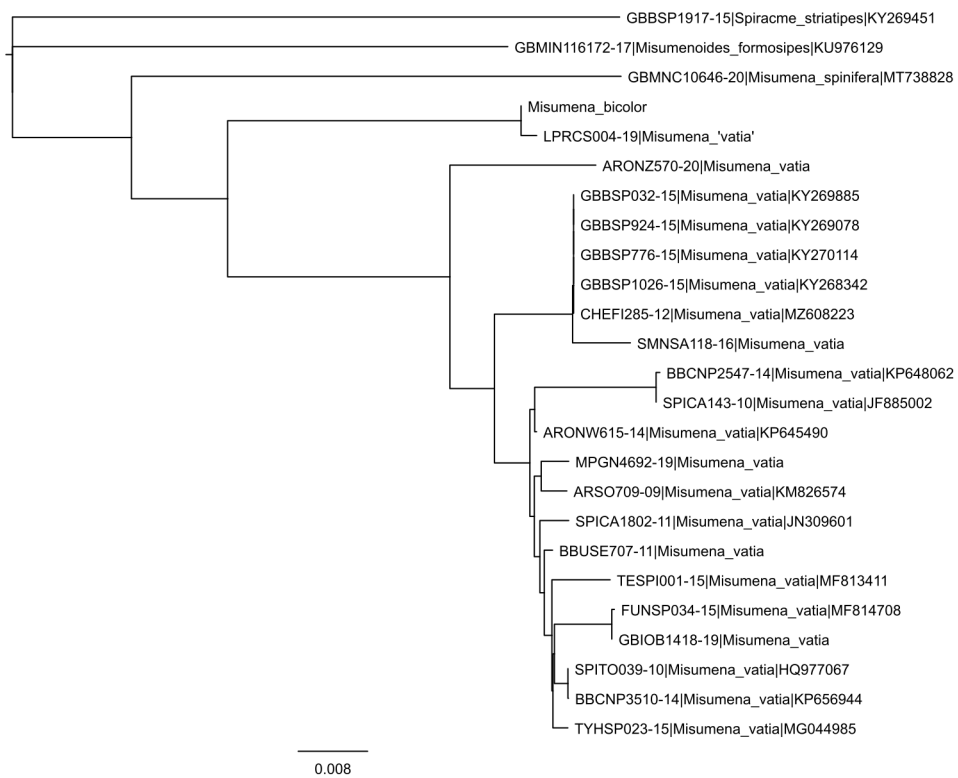


FIGURE 5. Neighbor Joining Tree including the COI-Sequence of *Misumena bicolor* (from Germany; ZFMK-TIS-76343).

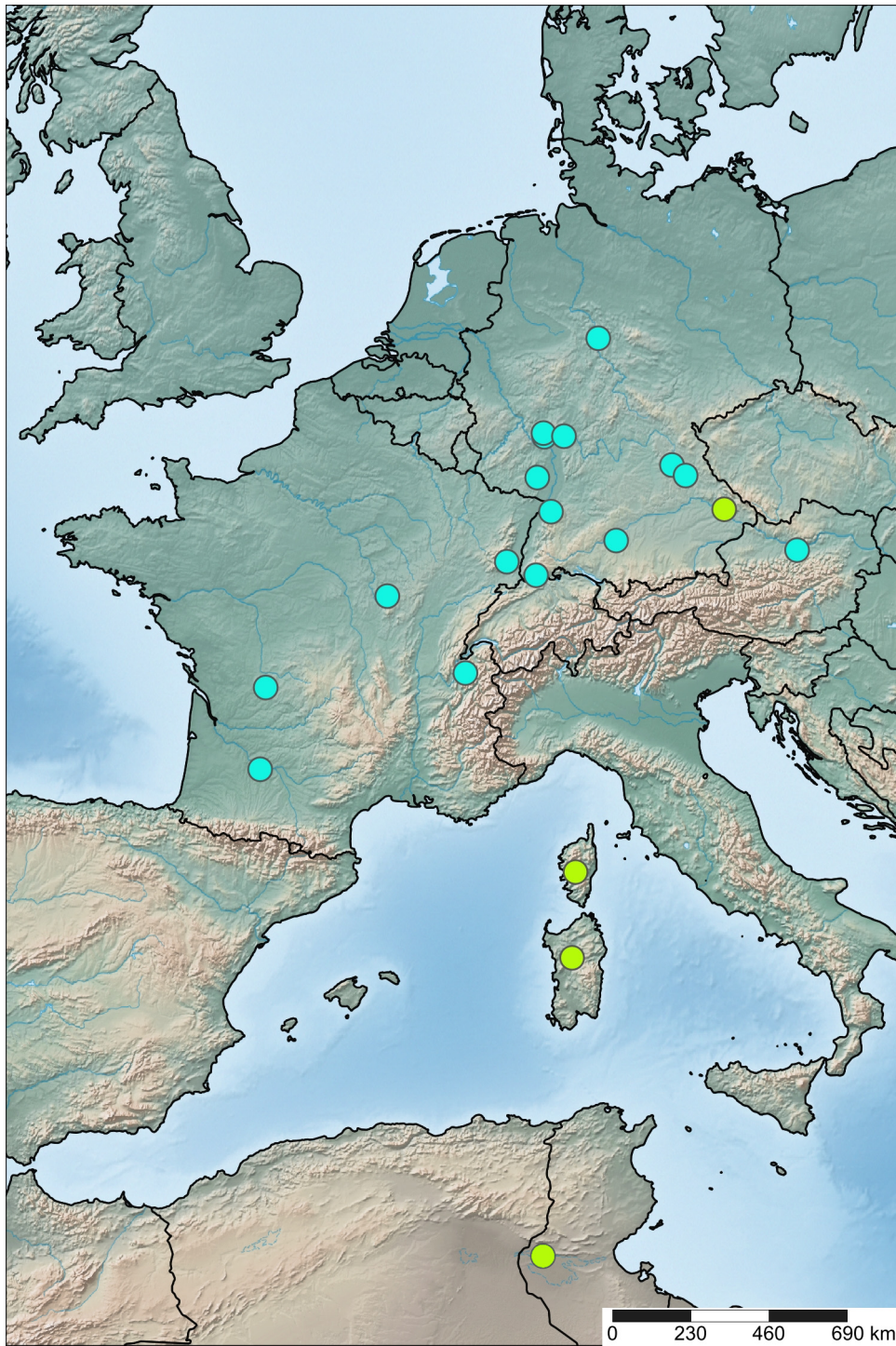


FIGURE 6. Collected (yellow circles) and photographic records (turquoise circles), based on images and literature, of *Misumena bicolor* in the Western Palearctic. The collected specimen from Tunisia is lost (Pavesi 1880).

Discussion

Our study revealed 1) *Misumena bicolor* as a species clearly distinct from *Misumena vatia*, and 2) a probably wide distribution of *M. bicolor* in the Western Mediterranean region and parts of Central Europe. Of particular significance is the confirmation of the species presence in Bavaria, Germany, challenging the prevailing perception of it as a Mediterranean faunal element. The possibly extensive distribution area, coupled with the distinct habitual characteristics of the male, makes it puzzling why this species is absent from over 150 years of arachnological

literature. The detection of cryptic species within supposedly well-known species is not uncommon, especially with new molecular biological approaches (Hebert *et al.* 2004; Muster & Michalik 2020; Trigo *et al.* 2013). Additionally, sometimes species are overlooked due to occurrences in little-investigated microhabitats (e.g., Bauer *et al.* 2022; Lenzini *et al.* 2022), however, it seems unlikely that *M. bicolor* inhabits a special microhabitat like bark or ant nests as judged from its morphology, close relation to *M. vatia* and the known localities.

Nevertheless, the case of *M. bicolor* is special because of the distinctive coloration of the male and the eye-catching behavior, since members of the genus *Misumena* are sit-and-wait predators on flowers and are therefore considered easy to observe (Chien & Morse 1998). The species habitat and microhabitat preferences remains virtually unknown, also Simon (1875) did not mention anything about the habitat in which his specimen was collected. However, the habitat information of our two records do not explain why this species has been so widely overlooked, as the known habitats and localities (see also Breitling *et al.* 2016) do not appear to differ significantly from those of its sister species *M. vatia*. Hence, the reasons why the species has been overlooked for so long remain a mystery. It is likely that the unknown female cannot be reliably distinguished from *M. vatia* females based on somatic or possibly even genital characteristics. The color variability observed in females of *M. vatia*, may have led to a presumed variability in males as well. Roberts (1995), for instance, does not explicitly refute this notion but rather notes that males are “less variable” than females. Further there is no evidence for a greater variability of the male habitus in recent literature (Almquist 2005; Heimer & Nentwig 1991; Kim & Lee 2012; Lehtinen 2004; Locket & Millidge 1951; Mcheidze 1997). All authors described the presence of the dark longitudinal stripes on the white-greenish opisthosoma and no author pointed out a possible variability of the male or even mentioned individuals missing longitudinal stripes. Hence, it is unlikely that any of these authors had access to a specimen of *M. bicolor* that could have been mistaken for a male *M. vatia*. This also suggests the possibility that the species may have expanded northward or northeastwards only very recently. Several authors have demonstrated that many spider species, alien and native, have expanded their distribution rapidly and suddenly, potentially caused by climate change, genetic admixture of formerly isolated lines, genetic accumulation of dispersion traits or other, often unknown environmental factors (Bach *et al.* 2023; Bauer *et al.* 2019; Krehenwinkel *et al.* 2015, 2016; Krehenwinkel & Tautz 2013; Narimanov *et al.* 2022; Parmesan 2006). Given the accumulation of photographic records in Germany and mainland France over the last years, with some of them coming from semi-natural habitats in rural areas (Fig 6.; Breitling *et al.* 2016; iNaturalist 2024) the species is very likely established in these two countries while the status in Austria and Italy needs to be assessed if additional records are made. The known records suggest a wide distribution in both countries, however, at the moment only males are identifiable, so the overall commonness of the species cannot be assessed. It remains unknown whether the species just recently expanded its distribution into more temperate regions. This hypothesis requires additional support through further investigations and more data.

As the females and the overall distribution of *M. bicolor* remain unknown, there is a substantial need for further research. Given the limited number of observations since its description over 150 years ago, *M. bicolor* appears to be a very rare species. However, the male has a distinct coloration and genital morphology and is easily separated from male *M. vatia*. Now, it remains to be seen how long the female of the species can disguise itself from discovery and if it is really morphologically identical to *M. vatia*.

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Supplementary Materials. The following supporting information can be downloaded at the DOI landing page of this paper: [Supplement_Table_1](#).