ORIGINAL ARTICLE

Cryptic diversity in ant-mimic *Micaria* spiders (Araneae, Gnaphosidae) and a tribute to early naturalists

Christoph Muster 🆔 | **Peter Michalik** 🆔

Zoologisches Institut und Museum, Universität Greifswald, Greifswald, Germany

Correspondence
Christoph Muster, Zoologisches Institut und Museum, Universität Greifswald, Greifswald, Germany.
Email: cm@christoph-muster.de

Abstract

Spiders of the genus *Micaria* are ground-living mimics of ants. Species delineation in these spiders is challenging, mainly because of exceptional high levels of intraspecific variation masking species boundaries. As implied by preliminary DNA barcode data from Central Europe, the Holarctic and very widely distributed glossy ant-spider *M. pulicaria* shows cryptic diversity. Here, we disentangle the hidden diversity by means of an integrative taxonomy approach, using mitochondrial DNA, morphometrics, traditional genitalic characters and ecology. Our data suggest the clear delineation of two distinct species, which supports the conception of 19th century taxonomists. These early naturalists distinguished *M. pulicaria* and a second closely related species based on morphology and natural history, which were synonymized in subsequent taxonomic studies. Therefore, we re-circumscribe *M. pulicaria* and revalidate the long forgotten *M. micans*. These two *Micaria* species co-occur sympatrically in vast areas of the western Palearctic, while the Nearctic region is populated by *M. pulicaria* alone. Male genitalic traits are more dissimilar in the area of sympatry than in allopatry, suggesting a decisive role of reproductive character displacement in species diversification. Our study emphasizes the value of the early taxonomic literature in integrative taxonomic studies, as it may contain crucial information on natural history that are not regularly recorded by modern taxonomists.

KEYWORDS
ground spiders, Holarctic distribution, integrative taxonomy, morphological crypsis, myrmecomorphy, species delimitation

1 | INTRODUCTION

Species are the fundamental units in most biological disciplines, including evolution, genetics, ecology, paleontology, behavioural and developmental biology, systematics and conservation (Claridge, Dawah, & Wilson, 1997; de Queiroz, 2005; Sites & Marshall, 2003). To that effect, species are frequently used as the currency in comprehensive comparisons, for example analyses of macroecological and biodiversity patterns. The "species concept" issue (see below) is particularly relevant in the current situation of unprecedented rates of biodiversity loss and species extinctions that puts humanity at risk (IPBES, 2019).

Most biologists acknowledge that species represent a unique level of self-organizing entities in nature. However,

the recognition and delineation of species are controversial, depending on theoretical, technical and analytical potentialities. Zachos (2016) compiled 32 different "species concepts," whose application to the natural world strongly effects the resultant biodiversity estimates. Until the early 20th century, species discrimination was almost entirely based on morphological distinctiveness. According to Shull (1923) species "may be defined as easily recognized kinds of organisms, and … their recognition should rest on simple gross observation such as any intelligent person can make with the aid only, let us say, a good hand-lens". The taxonomists in the early era of this "morphological species concept" spent much time collecting and observing specimens in the field. Thus, information on natural history, such as behaviour, habitat affinity or interspecific interactions, certainly influenced their perception of species entities. The emergence of the "biological species concept" (Dobzhansky, 1937; Mayr, 1942) strongly influenced not only the debate of what a species is, but also had an impact on taxonomy since the 1940s. The focus on reproductive isolation allowed for large degrees of intraspecific morphological variation. Consequently, a period of prevailing species lumping arose in taxonomy. For example, Sierwald (1987) synonymized in one paper not less than 35 nominal species of pisaurid spiders with one, *Nilus curtus* O. Pickard-Cambridge (sub *Thalassius spinosissimus*). With the advent of new methods and data sources in taxonomy and systematics in the late 20th century, a variety of new species concepts has been proposed, with a focus on species differentiation below the level of morphological distinctiveness (see summary in Zachos, 2016). A certain consensus has been reached through advancement of the unified species concept by de Queiroz (2005, 2007). In general, it seems of importance to distinguish between ontological ("what is a species") and operational ("how species are delimited") level. As pointed out by de Queiroz (2007), several species concepts refer to different biological properties (e.g. morphological and biological species concept, see also above). Thus, those "concepts" are rather operational and are combined in the framework of an integrative taxonomy (Padial, Miralles, Riva, & Vences, 2010).

Here, we present a textbook example that illustrates the impact of changing "species concepts" on taxonomic inference over time. During reinvestigation of species boundaries in closely related ant-mimic *Micaria* species, we found it particularly interesting that the perception of historical authors gets closer to our conclusions (based on principles of integrative taxonomy), than the view of authors of modern revisions. *Micaria* are small, ant-mimicking ground spiders (Gnaphosidae) with an iridescent abdomen. Their spider-ant association is of the type myrmecomorphy (Batesian mimicry): the ant-like appearance is a strategy to reduce attacks from hunting predators, but the mimics probably do not prey on ants (Cushing, 2012; Platnick & Shadab, 1988). The genus comprises 107 species with a primarily Holarctic distribution (WSC, 2019). The Palearctic (Mikhailov, 1988; Wunderlich, 1980) as well as the Nearctic species (Platnick & Shadab, 1988) have been thoroughly revised in the last decades.

Taxonomists of the 19th century separately described several closely related *Micaria* species, namely *pulicaria, nitens, formosa, micans* and *similis* (Koch, 1839; Blackwall, 1861; Westring, 1861; Koch, 1866; Ohlert, 1867; Menge, 1872, 1873; Bösenberg, 1902). Although these authors already recognized the close relationship between *M. pulicaria* and their pertaining species, they used diagnostic characters inconsistently and were apparently puzzled with their morphological separation ("Es geht aus allem wol hervor, dasz die feinern microscopischen merkmale bei untersscheidung der arten wol nicht zu entbehren sind" [It becomes clear that the finer microscopic characteristics are indispensable in differentiation of these species] Menge, 1873:328). Reimoser (1937) listed all the aforementioned names (except for *similis*) in synonymy of *Micaria pulicaria* (Sundevall), and this view has been adopted in the later revisions (Platnick & Shadab, 1988; Wunderlich, 1980) with reference to the extraordinary high degree of intraspecific variation in size, proportions, bristle configuration, colouration and genitalic structures. To date, *M. pulicaria* has been synonymized with a total of eight nominal species (see supplement taxonomy, and WSC, 2019). Interestingly, however, data from the German Barcode of Life campaign (GBOL, https://www.bolgermany.de/) revealed that COI sequences of *M. pulicaria* fall into two distinct clusters with a p-distance of ca. 6% (Astrin et al., 2016), further suggesting that the species deserves a thorough taxonomic reconsideration.

Here, we used an integrative approach to untangle the cryptic diversity of *M. pulicaria*. Our assessment reveals two sibling species, which occur sympatrically in large parts of the western Palearctic and have been mistaken for high levels of putative random variation in somatic and genitalic characters. Furthermore, we (a) confirm a high level of intraspecific variation in the female genitalia, which is rare in spiders; (b) found a pattern of exaggerated divergence of male genitalic traits in sympatry as compared to allopatry, which may be indicative of character displacement (Pfennig & Pfennig, 2009); and (c) confirmed an instance of circum-Holarctic distribution in spiders, which is remarkable as only <1% of the Holarctic spider species show such a wide distribution (Marusik & Koponen, 2005).

2 │ MATERIALS AND METHODS

2.1 │ Material examined

We examined 347 lots with a total of 737 specimens of *M. pulicaria* s.l. from 20 countries of Europe, Northern Asia and

North America (Table S1). The material is stored in the following collections and institutions:

BIOUG – Centre for Biodiversity Genomics, University of Guelph, Ontario, Canada

Coll. S. Danflous – Collection Samuel Danflous, Conservatoire d'Espaces Naturels de Midi-Pyrénées, Mauressac, France

Coll. S. Déjean – Collection Sylvain Déjean, Conservatoire d'Espaces Naturels de Midi-Pyrénées, Ferrières-sur-Ariège, France

Coll. C. Muster – Collection Christoph Muster, Putbus, Germany

Coll. S. Otto – Collection Stefan Otto, Leipzig, Germany

Coll. S. Pekár – Collection Stanislav Pekár, Masaryk University, Brno, Czech Republic

Coll. A. Trotta – Collection Alessio Trotta, Finale Ligure, Italy

FMNH – Field Museum of Natural History, Chicago, USA

MCZ – Museum of Comparative Zoology, Cambridge, Massachusetts, USA

MMUE – Manchester Museum, University of Manchester, UK

NHMUK – The Natural History Museum, London

NHRS – Swedish Museum of Natural History, Stockholm, Sweden

NMB – Naturhistorisches Museum Basel, Switzerland

OUMNH – University Museum of Natural History, Oxford, UK

PSU – Perm State University, Perm, Russia

SMNK – Staatliches Museum für Naturkunde, Karlsruhe, Germany

SZM – Siberian Zoological Museum, Institute of Animal Systematics and Ecology, Novosibirsk, Russia

UAM – University of Alaska Museum, Fairbanks, Alaska, USA

ZFMK – Zoologisches Forschungsmuseum "Alexander Koenig", Bonn, Germany

ZIMG – Zoological Institute and Museum, University of Greifswald, Germany

ZMMU – Zoological Museum, Moscow State University, Moscow, Russia

ZSM – Zoologische Staatssammlung, München, Germany

2.2 | Molecular data

A total of 71 COI barcode sequences of *Micaria pulicaria* were downloaded from the BOLD public data portal by 3 June 2019 (http://www.boldsystems.org; Ratnasingham & Hebert, 2007). Two sequences shorter than 500 bp were excluded from the analysis to avoid genetic distance artefacts.

We received permission from the corresponding project managers to use further 14 BOLD sequences that were nonpublic at that date. Additional seven COI sequences were newly generated at ZFMK using the same primers and laboratory protocols as described in Astrin et al. (2016). The newly generated and the previously unpublished sequences are available in the public data set, "DS-MICPUL Micaria pulicaria barcode additions" (dx.doi.org/10.5883/DS-MICPUL) on BOLD. One COI sequence of each species, *Micaria tripunctata* and *M. elizabethae*, were downloaded from the BOLD public data portal and were used as outgroups, because these were the closest relatives according to Platnick and Shadab (1988). The final data set comprised 92 sequences (Table S2 with BOLD and GenBank accession numbers).

Sequence alignment was performed in MEGA X v. 10.0.5 (Kumar, Stecher, Li, Knyaz, & Tamura, 2018) using MUSCLE (Edgar, 2004) with the default settings. The resulting alignment (Appendix S3) was trimmed to length of 653 bp corresponding to Astrin et al. (2016). The shortest included sequence was composed of 540 bp.

Maximum likelihood (ML) analysis was performed with IQ TREE 1.6.11 (Nguyen, Schmidt, Haeseler, & Minh, 2015). We used the implemented ModelFinder (Kalyaanamoorthy, Minh, Wong, Haeseler, & Jermiin, 2017) to find the best-fit substitution model for partitioned analysis by codon position. The selected models were HKY (7.3298) + F (0.2079, 0.1758, 0.2301, 0.3860) + I (0.8719) for partition 1 (1st and 2nd positions) and TN (28.3748, 55.9415) + F (0.3363, 0.0161, 0.1093, 0.5381) for partition 2 (3rd position). Branch support was estimated by using the ultrafast bootstrap approximation with 1,000 replicates (Hoang, Chernomor, Haeseler, Minh, & Vinh, 2018). The consensus ML tree was annotated with iTOL v4 at https://itol.embl.de/ (Letunic & Bork, 2019).

2.3 | Morphology

Representative specimens from the resulting OTU clusters were investigated for discrete differences in somatic and genital morphology. Once diagnostic characters for the OTUs were established and/or allopatric distributions observed, museum specimens were included in the analyses. Twenty-five males and 25 females from each OTU, and the type specimens of *M. gentilis, M. montana* and *M. perfecta* were measured. We took morphometric measurements of nine somatic characters in both sexes, plus eight measurements of the male palpal organ and five measurements of the female epigyne (Appendix S2, Table S3). Measurements were taken using a ZEISS Stemi 2000 stereomicroscope equipped with an ocular micrometre. All measurements are given in millimetres.

For the analysis of morphometric data, we applied the multivariate statistical framework developed by Baur and Leuenberger (2011). Shape PCA (principal component

analysis) was used in order to disentangle the effects of size and shape in the multivariate morphospace. Once the final species hypotheses have been generated, we used the LDA ratio extractor to determine those morphometric ratios that allow the best discrimination among the species. The analyses were performed in R 3.5.2 (R Core Team, 2018).

2.4 | Climatic data

We retrieved mean annual temperatures (1970–2000) from 240 collecting sites of examined specimens using the Senckenberg dataportal (http://dataportal-senckenberg.de/dataExtractTool/). Data were extracted from WorldClim (https://www.worldclim.org/, Hijmans, Cameron, Parra, Jones, & Jarvis, 2005) at a spatial resolution of <1 km^2.

2.5 | Species delimitation

Species boundaries were determined within the formalized framework of integrative taxonomy (Dayrat, 2005; Schlick-Steiner et al., 2010; Will, Mishler, & Wheeler, 2005) by integrating information from molecular, morphological, ecological and distribution data. Integrative taxonomy acknowledges the inherent failure rate of any single source of evidence, and disagreement among them is considered helpful in understanding the evolution of the study system.

Our primary species hypothesis is based on the major, well supported clades in the ML phylogeny, henceforth numbered as operational taxonomic units (OTU; Figure 1). For molecular based species delineation, popular distance-based and tree-based methods were used. For distance-based analysis, the Barcode Index Number (BIN) clustering algorithm was employed as implemented in BOLD 4 (http://v4.boldsystems.org). BIN clusters are supposed to show high concordance with species limits (Ratnasingham & Hebert, 2013). The multi-rate Poisson Tree Processes model (mPTP) was applied as a tree-based molecular species delineation method. The method uses number of substitutions in a given tree to model speciation by assuming that the mean number of substitutions per site between species is higher than the number of intraspecific substitutions, and it accounts for different levels of intraspecific genetic diversity (Kapli et al., 2017). In contrast to the Generalized Mixed Yule Coalescent model (GMYC, Pons et al., 2006), this method does not require time-calibrated ultrametric input trees, whose calculation is notoriously error-prone. mPTP species were inferred from the rooted ML tree using the web-based interface (http://mptp.h-its.org).

Morphological characters with potential value to discriminate species were identified by careful comparison of specimens of the OTU clusters. Morphometric data were analysed

separately for each sex by pairwise linear discriminant analyses (LDA) among OTUs. LDA was performed with stepwise selection of characters in the software package SPSS Statistics v21 (IBM, USA). Species were considered as morphologically distinct when more than 95% of the specimens were correctly classified in cross-validated LDA.

To test for differences in thermal niche, the mean annual temperatures at the collecting sites of the OTUs were statistically compared. Locations were allocated to OTUs either by COI barcodes or morphology of the specimens, or by geography (in areas of nonoverlapping clade distributions).

3 | RESULTS

3.1 | Molecular analyses

The ML consensus tree (Figure 1) reveals three major clusters within *M. pulicaria*, henceforth referred to as OTU-1, -2 and -3, that were further tested in species delimitation analysis. Genetic distances among the OTUs were similar (mean K2P distance OTU 1–2: 5.5%, OTU 1–3: 4.8%, OTU 2–3: 5.3%). OTU-1 shows a shallow genetic structure (mean K2P distance within clade 0.6%) and is distributed in the western Palearctic east to Tajikistan. OTU-2 shows a pronounced genetic structure, the mean K2P distance within the clade was 2.7%, and it occurs in the temporal and boreal regions of the Palearctic, as well as in north-western parts of the Nearctic (British Columbia, Alberta). OTU-3 is widespread in temperate and boreal biomes of the Nearctic, from the Pacific to the Atlantic Ocean. Genetic diversity within the clade was low (mean K2P distance 1%).

In the molecular delineation analyses, specimens were consistently assigned to seven entities. The composition of BIN clusters and mPTP species was identical. OTU-1 corresponds to mPTP-1, while OTU-2 was divided into four mPTP-taxa, of which mPTP-2 was restricted to Europe, mPTP-3 recorded from Europe and the Far East, and mPTP-4 and -5 were found in western Canada. OTU-3 was divided into two mPTP entities of wide Nearctic distribution.

3.2 | Morphological characters

Our study confirmed extraordinary high levels of morphological variation within the scrutinized *Micaria* specimens. For example, the size and shape of the receptacula of the female genitalia are highly variable (Figure 2). The variation in the aforementioned morphological feature as well as several others is continuous and seemingly not correlated with phylogenetic relationships of the specimens. Without a priori hypotheses derived from independent evidence, the task to infer species boundaries given such variability appears futile.
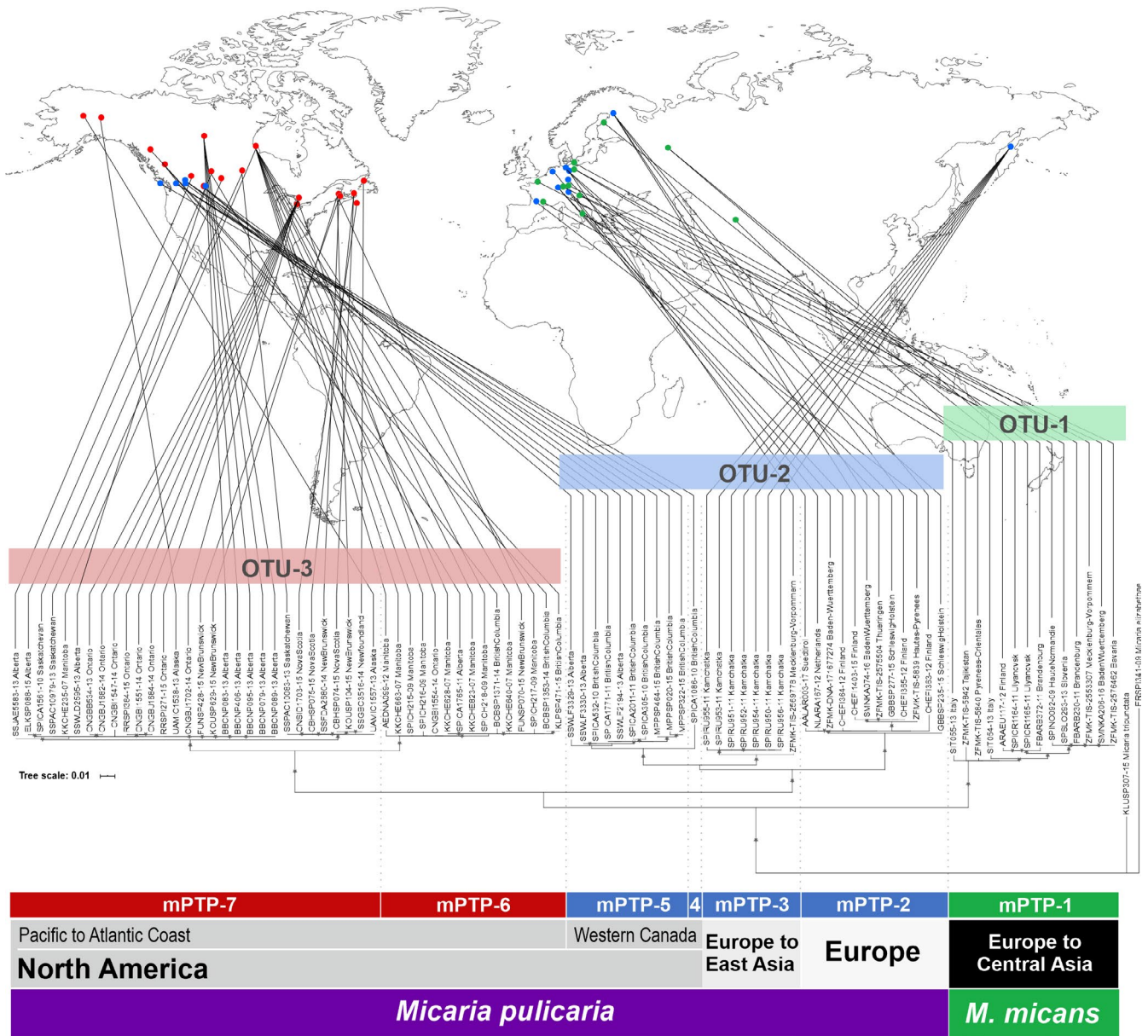
FIGURE 1 Consensus tree (log likelihood −1861.4513) from maximum likelihood analysis of the COI gene fragment. Stars indicate nodes with >75% support from 1,000 replicates of ultrafast bootstrapping. Operational taxonomic units (OTU) are indicated as primary species hypotheses, species delineation as derived from mPTP analysis (BIN clusters are identical), geographic distribution of the clades, and final species delimitation inferred from integrative taxonomy [Colour figure can be viewed at wileyonlinelibrary.com]

However, based on the grouping hypothesis from the ML analysis, we discovered that certain combinations of features clearly distinguish the specimens of OTU-1 from all other specimens. To our surprise, these features include a trait that is easily recognizable in both sexes and even in old museum material—a dark longitudinal striation at the dorsal face of the femora III and IV (sometimes also recognizable at the tibiae of these legs), which is caused by stripe-like arrangement of dark hairs and dark pigmentation of the cuticula (Figure 3). Noteworthy, this character has been already described by Wunderlich (1980), but was considered as highly variable and taxonomically uninformative. Additionally, all specimens

with striped femora show a number of associated diagnostic characters in genitalic traits. In males, the retrolateral margin of the tegulum shows a distinct notch (while being almost straight in OTU-2 and -3), the embolus is slender (as opposed to stouter) and the terminal curve of the sperm duct is situated in the basal half of the tegulum (in distal half in the remaining OTUs) (Figure 4). In females, the epigyne is as long as wide (wider than long in the remaining specimens), the anterior transversal fold is M-shape curved (as opposed to straight to weakly curved), and the copulatory ducts run parallel in part (evenly curved in the other material; Figure 2). For more details, see Appendix S1.
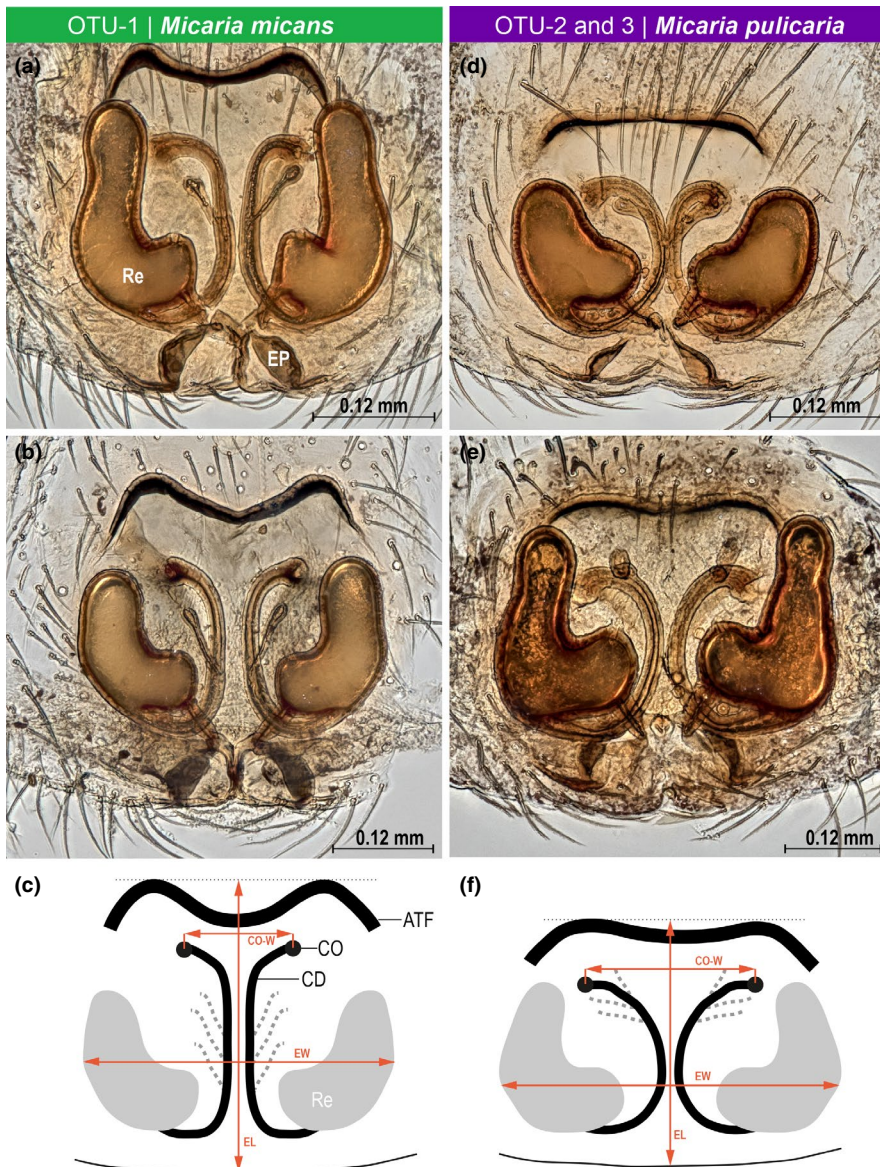
FIGURE 2 Variation in the structure of the female genital organs. (a) ID200 from Jena; (b) ID207 from Köllitsch; (d) ID188 from Mangfallgebirge; (e) ID193 from Süderleda (see Table S1 for sample IDs). The schematic drawings (c, f) illustrate the main characters and indicate the measured distances for the morphometric analysis. The dotted grey lines indicate the position of the accessory bulbs in different specimens. ATF, anterior transverse fold; CD, copulatory ducts; CO, copulatory openings; CO-W, width between copulatory openings; EL, epigyne length; EP, epignyal pocket; EW, epigyne width; Re, receptaculum [Colour figure can be viewed at wileyonlinelibrary.com]

3.3 | Morphometrics

LDA allowed 100% discrimination between males of OTU-1 and OTU-2. For the other comparisons (OTU 1–3, OTU 2–3), only 94.2% of the specimens were correctly classified in cross-validated analyses. Figure 5a shows that males of OTU-1 and OTU-2, which co-occur sympatrically in vast areas of the Palearctic, are completely separated in the shape space of the first two principal components, which account for 59% of the total variance. OTU-3 with its Nearctic distribution, which is largely allopatric to OTU-1 and OTU-2, presents an intermediate position in morphology. The examined type specimens of *M. gentilis* and *M. perfecta*, both from North America, are positioned in the central area of the morphospace of OTU-3.

A different pattern was observed in females (Figure 5b). Specific difference was evident between females of OTU-1

and OTU-3, with 96.2% correctly classified specimens in cross-validated LDA. The rates were below the significance threshold for OTU 1–2 (94%) and OTU 2–3 (86.8%). Again, the examined type specimens of *M. gentilis* and *M. montana*, both from North America, are positioned in the central area of the morphospace of OTU-3 with its Nearctic distribution.

3.4 | Thermal niche

The mean annual temperature was significantly higher at collection sites of OTU-1 (8.9°C) than at localities of OTU-2 (4.9°C) (Wilcoxon test, $W = 6,859$, $p < .001$) and the North-American OTU-3 clade (3.5°C) ($W = 4,232$, $p < .001$), but it was not different between OTU-2 and OTU-3 ($W = 3,137$, $p = .087$) (Figure 6).
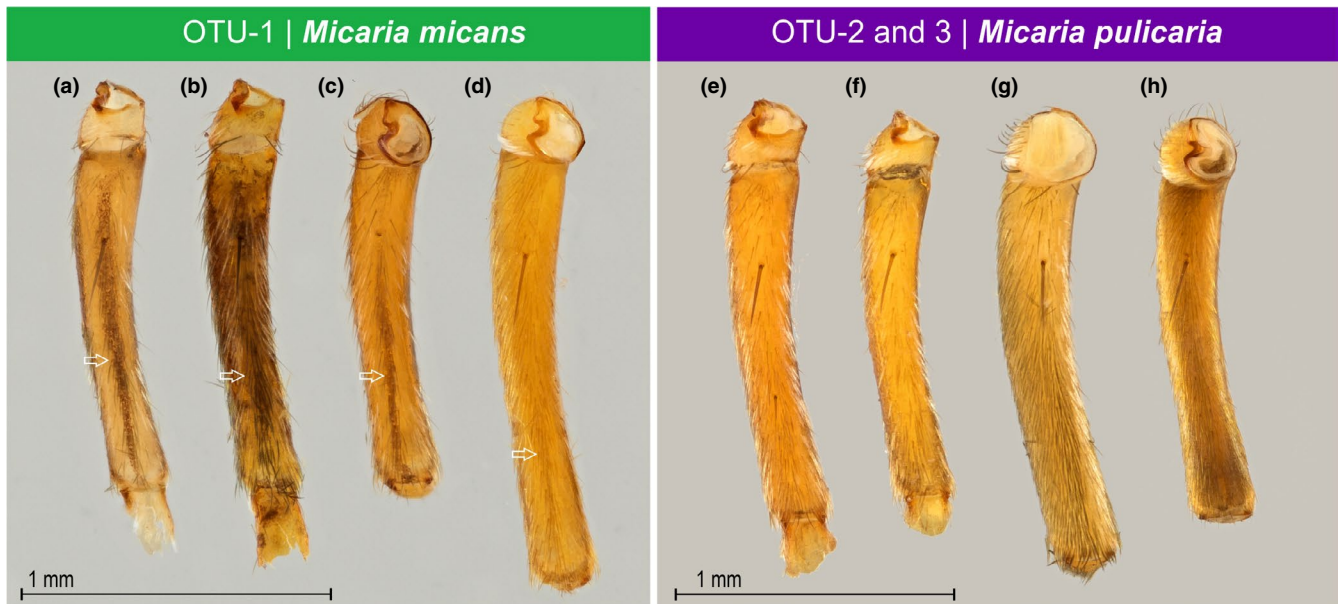
FIGURE 3 Leg colouration (femur IV in dorsal view) in specimens from OTU-1 (*Micaria micans*) and OTU-2 + OTU-3 (*M. pulicaria*). The arrows point to the diagnostic dorsal striation at femora IV in *M. micans*. (a) ID207 from Köllitsch; (b) ID214 from Ahlden; (c) ID175 from Kent; (d) ID181 from Kara-Alma/Kyrgyzstan; (e) ID189 from Allgäu Alps; (f) ID70 from Schmilka; (g) ID176 from Kent; (h) ID117 from Khevsureti/Georgia (see Table S1 for sample IDs) [Colour figure can be viewed at wileyonlinelibrary.com]

3.5 | Species delimitation

The species status of OTU-1 was supported by all lines of evidence, except for male morphometrics as compared to OTU-3, and female morphometrics in comparison with OTU-2 (Table 1). OTU-2 and OTU-3 were recognized as distinct species by the molecular delineation methods, but neither taxonomic characters, morphometrics, nor ecology supported this view. In conclusion, we can distinguish two species in the *M. pulicaria* complex. *Micaria micans* (Blackwell, 1858) (OTU-1) is distributed in the temperate regions of the western Palearctic east to central Asia. This species prefers lowland habitats, the highest record comes from 1,400 m.a.s.l. in the Caucasus. *Micaria pulicaria* (Sundevall, 1831) (OTU-2 + OTU-3) is widely distributed in the temperate and boreal biomes of the Holarctic region. This species occurs up to the alpine zone, with highest records in the European mountains in 2,200 m and up to 4,000 m in North America. *Micaria gentilis, M. montana* and *M. perfecta* remain in synonymy of *M. pulicaria*.

The mean K2P divergence in the COI gene between *M. micans* and *M. pulicaria* was 5.1%, it was 0.6% within *M. micans* and 3.4% within *M. pulicaria*. The two species are readily distinguishable by colouration of the hind legs and genitalic characters (see 3.2 and full taxonomic account in Appendix S1). Furthermore, the results of the LDA ratio extractor revealed that the two species are almost perfectly separable by taking four morphological measurements (Figure S2-1). In males, the best discriminating ratio was *length of femur IV* divided by *d* (distance from terminal curve of sperm

duct to distal edge of bulbus; Figure S2-1; standard distance *Dij* = 3.99). More than 87% of the discriminating power was due to shape components as compared to size (δ = 0.128). The next discriminating body ratio being as little correlated as possible with ratio 1 was *prosoma width at position of the posterior eye row* divided by *b* (distance between basal and terminal curve of sperm duct). In females, the best discriminating ratio was *length of femur IV* divided by *length of epigyne* (Figure S2-1); standard distance *Dij* = 3.72). More than 88% of the discriminating power was due to shape components as compared to size (δ = 0.115). The second best discriminating body ratio being as little correlated as possible with ratio 1 was *total length* divided by *width between the copulatory openings*.

4 | DISCUSSION

4.1 | How many species in *Micaria pulicaria*?

Taxonomists faced problems in species delimitation of the *M. pulicaria* species complex for almost 200 years. Using an in-depth integrative taxonomy approach, we propose the species status for two out of eight names that are currently in synonymy of *M. pulicaria* (WSC, 2019). We applied data from morphology, mitochondrial DNA and ecology (Table 1), and species were accepted only if they differed in all three disciplines. This is based on rationales provided by Schlick-Steiner et al. (2010), who demonstrated that three disciplines
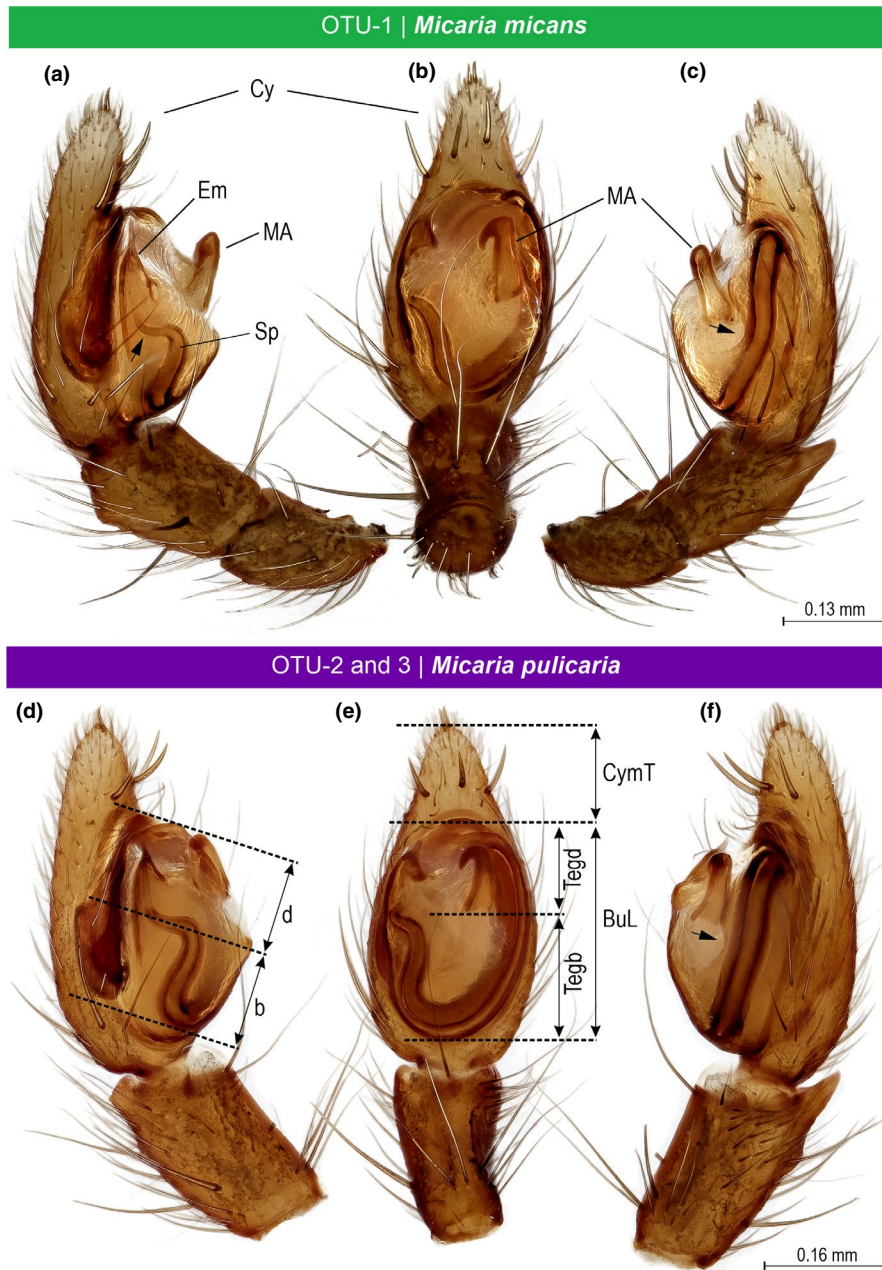
OTU-1 | *Micaria micans*

(a) (b) (c)

OTU-2 and 3 | *Micaria pulicaria*

(d) (e) (f)

0.13 mm

0.16 mm

FIGURE 4 Male palp of *Micaria micans* (a–c; ID200 from Jena) and *M. pulicaria* (d–f; ID189 from Allgäu Alps) in prolateral (a, d), ventral (b, e) and retrolateral view (c, f). The arrow in (a) points to the terminal curve of the sperm duct; arrows in (c, f) point to the notch at the retrolateral margin of tegulum, which is distinct in *M. micans*. BuL, bulbus length; Cy, cymbium; CymT, cymbium tip; Em, embolus; MA, median apophysis; Sp, sperm duct; see Appendix S2 for further measurements [Colour figure can be viewed at wileyonlinelibrary.com]

are required to lower the average error rate in species delimitation below 5%. This may be considered a conservative approach. Nowadays, many species are described primarily based on molecular delineation (Atherton & Jondelius, 2018; Zhang et al., 2018), and Barcode Index Numbers (BINs) are frequently used as a species proxy (Hebert et al., 2016). In our study, reliance exclusively on molecular delineation methods would result in splitting *M. pulicaria* into seven species, of which only two were morphologically distinguishable. We do not consider morphological differentiation essential for species distinction, as morphological crypsis among species is not uncommon (Bickford et al., 2007). However, we assume that morphological variation reflects genome-wide divergence better than single gene trees. This effect is shown in the lower failure rate of morphology as compared to

mitochondrial DNA and nuclear DNA in a literature survey on species delimitation (Schlick-Steiner et al., 2010). More importantly, molecular delimitation methods detect lineages, which must not necessarily correspond to species. It is well known that popular delineation methods that were designed for single-locus molecular data, such as GMYC, PTP and BIN, tend to overestimate species diversity (Carstens, Pelletier, Reid, & Satler, 2013; Hawlitschek, Scherz, Ruthensteiner, Crottini, & Glaw, 2018; Luo, Ling, Ho, & Zhu, 2018; Miralles & Vences, 2013). In a recent simulation study, Sukumaran and Knowles (2017) have convincingly shown that even the multispecies coalescent model, which is increasingly used with genomic data, consistently overestimated the number of true species due to misidentification of population structure for species entities. Since overinflation

FIGURE 5 Scatter plot of the PCA analysis of morphological measurements in shape space. (a) Males; (b) Females. Colours of OTUs correspond to Figure 1 [Colour figure can be viewed at wileyonlinelibrary. com]
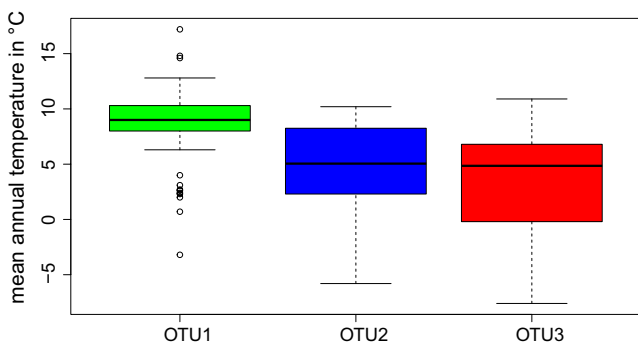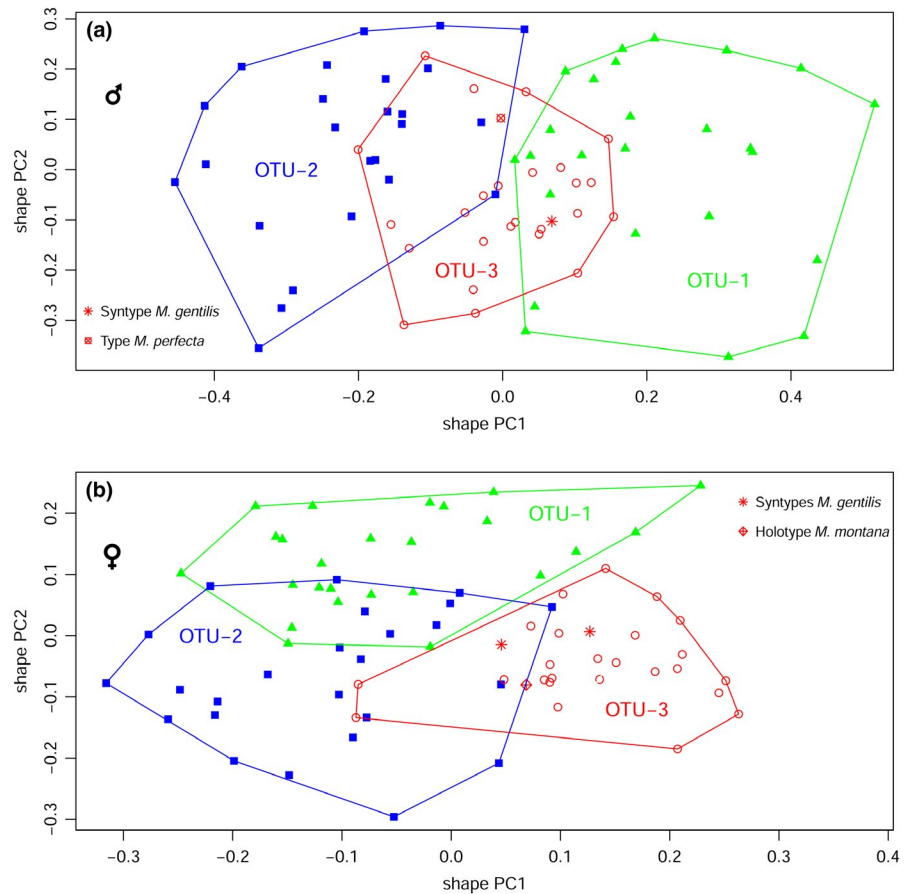

FIGURE 6 Box plot of mean annual temperatures at collection sites of examined specimens of OTU 1 to OTU-3. Boxes indicate interquartile range (IQR: between upper [Q3] and lower [Q1] quartile). Black bars designate medians, whiskers indicate values within 1.5× IQR beneath Q1 or 1.5× above Q3. Circles depict outliers (above or below 1.5× IQR). Colours of OTUs correspond to Figure 1 [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 1 Summary of species delineation results among OTUs in the *Micaria pulicaria* species complex in the framework of integrative taxonomy

	OTU 1–2	OTU 1–3	OTU 2–3
BIN	✓	✓	✓
mPTP	✓	✓	✓
Male morphometrics	✓	×	×
Female morphometrics	×	✓	×
Male genitalia	✓	✓	×
Female genitalia	✓	✓	×
Somatic morphology	✓	✓	×
Thermal niche	✓	✓	×

of species numbers may have serious consequences, for example in global biodiversity estimates or for conservation strategies (Larsen, Miller, Rhodes, & Wiens, 2017; Robuchon et al., 2019) the integration of genetic and nongenetic sources of data (morphological, ecological and ethological information) is generally recommended. In our *Micaria* example, OTU-2 populates vast areas of the Holarctic region with its complex Pleistocene climatic history. Therefore, lineage sorting is not

expected to be complete and we predict a profound genetic structure that should not be mistaken for putative species unless supported by other lines of evidence.

While we are confident that the relatively deep divergence within Palearctic *M. pulicaria* (OTU-2) is attributable to phylogeographic structure instead of speciation, the situation is more challenging with respect to species status of OTU-3. This clade is exclusively Nearctic and overlaps with OTU-2 only in a small area near the Pacific coast. The oldest available name for North-American specimens is *Micaria montana* Emerton, 1890 with the type locality Mt. Washington

situated in New Hampshire. Since only OTU-3 occurs in this region, this would be the valid name for the widespread North-American clade. The morphometric analysis (Figure 5a, b) confirms that the holotype of *M. montana* as well as type material of *M. gentilis* Banks, 1896 and *M. perfecta* Banks, 1896 correspond to OTU-3. The crucial question is whether OTU-3 should be considered conspecific with OTU-2 or not. Our data suggest diagnostic substitutions for each clade in the COI gene. However, we do not know whether these characters would remain specific if the sampling was more comprehensive than in our study (Bergsten et al., 2012). On the other hand, we did not detect significant differences in morphological characters nor in ecology between OTU-2 and OTU-3. For the sake of taxonomic stability, we therefore advocate to keep *M. montana* (and also *M. gentilis* and *M. perfecta*) in the synonymy of *M. pulicaria,* as proposed by Hackman (1954) and Platnick and Shadab (1988) until contradicting evidence is provided. Phylogenomic data may once decide upon this matter.

4.2 | The legacy of early naturalists

An appealing aspect of this study is the concordance of our species delimitation results with the views of 19th century arachnologists, and the discrepancy with later revisions. We acknowledge that in the era of the phenotypic species concept many varieties were described that may correspond to DNA delineated species only by accident. However, the consistency in the parallel treatment of *M. pulicaria* and a second closely related species in almost all regional monographs on European spiders from Koch (1839) to Bösenberg (1902) let us assume that those authors had good reasons to distinguish these taxa. Most arachnologists of that time worked in regular professions; they were forest rangers, customs officers, merchants, physicians or teachers who spent a lot of time observing and collecting spiders in their natural habitats. They knew the local communities around their residence towns Regensburg (C.L. Koch), Llanrwst in north Wales (Blackwall), Göteborg (Westring), Nuremberg (L. Koch), Königsberg/Kaliningrad (Ohlert), Danzig/Gdansk (Menge) or Pforzheim (Bösenberg) very well. In all these areas, both *Micaria* species occur sympatrically and distinctive traits could have been perceived by the careful observer. Specific characters may include colouration of living specimens, foraging and reproductive behaviour, activity patterns, preference for certain microstructures or association with different ant species. Also differences in habitat selection probably did not escape the attention of these early arachnologists. For example, Menge (1872, 1873) found *M. micans* (sub *M. pulicaria*) "only at sunny spots," while he reported *M. pulicaria* (sub *M. nitens*) "from the foot of pines." From label information of the examined material (Table S1), we can confirm that

M. micans prefers dry and warm open habitats (grassland, fields, gardens and forest edges), while European *M. pulicaria* is associated with habitats of higher humidity (forests and bogs). Furthermore, only *M. pulicaria* occurs above the timberline. On the small scale, a mosaic pattern of mutually exclusive distributions depending on habitat quality is the rule: we recorded only few instances of syntopic occurrence.

Despite their intuitive perception of two similar *Micaria* species coexisting in their areas, early arachnologists failed to identify reliable diagnostic characters. Some of the descriptions do not deal with diagnostic issues at all, others proposed distinctive features that did not hold up thorough investigation, for example differences in spination of the femora (Koch, 1866) or differences in leg tarsus claws, shape of the iridescent scales and spinneret morphology (Menge, 1873). But it was only Bösenberg (1902) who recognized the diagnostic value of the colouration variants at femora III and IV.

In the era of integrative taxonomy, morphological and natural history data still prove crucial to validate species hypotheses derived from DNA data. The forgotten knowledge of early naturalists may provide useful information on various aspects, especially with regard to the natural history of the study organisms; they may even stimulate own observations. In any case, new species should not be described without considering all old descriptions and synonymies, as exercised, for example by Kovblyuk and Nadolny (2008) in their revision of Crimean *Micaria* species.

4.3 | The evolution of morphological crypsis in *Micaria pulicaria*

The spider fauna of central Europe is well known. In such faunas, the detection of hidden diversity is a rare phenomenon. Moreover, *M. pulicaria* is a common species. According to the Atlas of the European Arachnids (https://atlas.arages.de/), the species is placed 59 out of 1,050 in descending order of grid frequencies of spider species in Germany. Thus, it is rather surprising that two widespread species have been mistaken for such a long time. If one accepts the definition of cryptic species as two or more distinct species that were earlier classified as one (Bickford et al., 2007), then *M. micans* and *pulicaria* are cryptic species. Processes underlying the evolution of morphological crypsis have gained vast attention in recent years (Struck et al., 2018; Wagner et al., 2018). Which factors could have driven crypsis in *Micaria* spiders?

The long-lasting taxonomic confusion of the two *Micaria* species certainly relates to the high levels of intraspecific variation as compared to low interspecific dissimilarity. On the one hand, the organization of both, the male palp and the female epigyne features, is simple as compared to other spiders, and few diagnostically useful characters exist. On the

other hand, we found exaggerated variation within species—particularly in vulva structures—that is almost unrivalled in spiders (but see Crews, 2009). Spiders generally show no to very little intraspecific variation in genitalic characters (Kraus, 2002). However, spider genitalia are usually distinct even among closely related species and thus reliable indicators of species limits (Huber, Rheims, & Brescovit, 2005). The reasons for higher intraspecific variation in spider genitalia in some taxonomic groups than in others are still not clear (Eberhard & Huber, 2010). We hypothesize that the high levels of standing variation within species as well as overall morphological stasis among the two *Micaria* species could be triggered by their myrmecomorphy. As frequency and distribution of model species changes, the mimics have to adapt, thus intraspecific polymorphism is an important characteristic of Batesian mimicry (Joron & Mallet, 1998). On the other hand, morphological conversion in the speciation process is constrained by selection towards maintenance of mimetic similarity with the model species. Hence, myrmecophily is listed as one source for the evolution of cryptic species in Bickford et al. (2007).

Presumably, the exceptional high levels of variation within the two *Micaria* species also promoted character displacement in male genitalic traits. Reproductive character displacement is the selective process by which reproductive traits diverge in order to minimize the risks of hybridization; it results in a geographic pattern in which species are more dissimilar where they occur together than in allopatry (Pfennig & Pfennig, 2009). Exactly such a pattern has been observed in *M. micans/pulicaria* (Figure 5a): Palearctic *M. pulicaria* (OTU-2) and *M. micans* (OTU-1), which occur sympatrically in vast areas of Eurasia, are separated in morphospace, while the Nearctic OTU-3 takes an intermediate position. Although our morphometric analysis includes nongenitalic traits, the characters of the male palp contribute by far the highest loadings to shape PC1. Moreover, species may also diverge in traits that are not directly involved in reproduction owing to correlated evolution with those traits actually targeted by character displacement (Pfennig & Pfennig, 2005). At the moment, we can only speculate whether character displacement in *M. micans/pulicaria* results from postspeciation divergence, for example reinforcement of specific differences that evolved in allopatry, for example through range contraction during Pleistocene glaciations, or if character displacement itself initiated speciation. During reproductive character displacement, female preferences on male traits may become so divergent that females in sympatry fail to recognize allopatric males as acceptable mates (or vice versa), ultimately resulting in reproductive isolation (Hoskin, Higgie, McDonald, & Moritz, 2005). Effective character displacement would also explain the absence of signals for introgression between *M. pulicaria* and *micans*.

Introgression through occasional hybridization needs certainly to be considered in morphologically and ecologically similar congeners that occur in sympatry. Recent genomic studies have shown that it may be more prevalent in spiders than previously assumed (Ivanov, Lee, & Mutanen, 2018; Leduc-Robert & Maddison, 2018). However, we observed no single case of disagreement between morphology and mtDNA (i.e. no signs of mitonuclear discordance) in the studied *Micaria* species.

ORCID

Christoph Muster 🔾 https://orcid.org/0000-0001-7639-2547
Peter Michalik 🔾 https://orcid.org/0000-0003-2459-9153

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