

Public DNA barcoding data resolve the status of the genus *Arboricaria* (Araneae: Gnaphosidae)

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Abstract. An analysis of public DNA barcoding data confirms that the extraction of *Arboricaria* Bosmans, 2000 from the genus *Micaria* Westring, 1851 would require the division of *Micaria* into at least five (and probably more) individual genera, to restore the monophyly of *Micaria* sensu stricto. Such an excessive splitting of a homogenous and well-defined genus would be neither desirable nor practical, and consequently *Arboricaria* should be considered a subjective junior synonym of *Micaria*, as suggested earlier (**syn. conf.**).

Keywords: Araneae, DNA barcoding, cladistics, phylogenetic systematics, paraphyly, spider.

Zusammenfassung. Öffentliche DNA-Barcode-Daten klären den Status der Gattung *Arboricaria*. Eine Analyse von öffentlich zugänglichen DNA-Barcode-Daten bestätigt, dass die Herauslösung von *Arboricaria* Bosmans, 2000 aus der Gattung *Micaria* Westring, 1851 eine Aufteilung von *Micaria* in mindestens fünf Einzelgattungen erfordern würde, um die Monophylie der Restgattung *Micaria* im engeren Sinne wiederherzustellen. Diese exzessive Aufteilung einer homogenen und klar definierten Gattung wäre weder wünschenswert noch praktikabel, und folglich sollte *Arboricaria*, wie bereits früher vorgeschlagen, als subjektives jüngeres Synonym von *Micaria* behandelt werden (**syn. conf.**).

The genus *Arboricaria* was established by Bosmans (in Bosmans & Blick 2000) for the three species of the *subopaca* group of *Micaria* defined by Wunderlich (1980) and two newly described species. No explicit justification was provided for the decision to place this particular species group, but not others, in its own genus, and the status of the new genus has been controversial from the beginning. Platnick, as organizer of the World Spider Catalog and previous reviser of the Nearctic members of *Micaria* (Platnick & Shadab 1988), rejected the genus, “as [Bosmans] provided no evidence whatever that these taxa [included in *Arboricaria*] constitute the sister group of all other *Micaria*, or that the remaining *Micaria* do not constitute a paraphyletic group from which a relatively autapomorphic subgroup has been artificially extracted” (Platnick 2014), thus effectively synonymizing *Arboricaria* with *Micaria*. In this assessment he was followed by Wunderlich (2017), an earlier reviser of the Palearctic *Micaria* species (Wunderlich 1980), who formalized the synonymy and concluded that *Arboricaria* should be considered as a “species-group of *Micaria* or as a subgenus”. In contrast, Mikhailov (2016) argued with reference to the International Code of Zoological Nomenclature that “there are no formal grounds to reject the validity of *Arboricaria*”, and consequently the genus is considered as valid in the latest version of the World Spider Catalog (WSC 2017).

However, just like Bosmans, Mikhailov failed to provide unambiguous synapomorphies for the remaining 100 or so species of *Micaria*, so that Platnick’s concerns about the potential paraphyly of *Micaria* sensu stricto remain unresolved. The formalistic argument based on the regulations of the ICZN is obviously insufficient. As Minelli & Kraus (1999) as president and former president of the International Commission on Zoological Nomenclature explain in their Preface to the Fourth Edition of the Code “[t]he conventional Linnaean hierarchy [embodied in the Code] will not be able to survive alone: it will have to coexist with the ideas and terminology of phylogenetic (cladistic) systematics”, stating explicitly that the traditional nomenclature can be perceived as “too permis-

sive, in so far as it may be equally applied to paraphyletic as to monophyletic groups.” This is exactly the issue at hand: while *Arboricaria* is quite likely to be a monophyletic group, the resulting truncated *Micaria* could equally likely be paraphyletic. As has been elaborated extensively, following the historical debate between Ernst Mayr (1974) and Willi Hennig (1975), paraphyletic taxa are non-monophyletic, differ only in subtle ways from polyphyletic ones (Platnick 1977), and have arguably no useful place in a phylogenetic taxonomy and nomenclature.

The recent availability of large amounts of DNA barcoding data for spiders (e.g., Astrin et al. 2016, Blagoev et al. 2013, 2016) now offers a unique opportunity to resolve this issue: a sufficiently large number of *Micaria* species, as well as sequences for an undisputed member of *Arboricaria*, *A. subopaca* (Westring, 1861), have been made publicly available for phylogenetic analysis to answer Platnick’s key question: is *Arboricaria* the sister group of all other *Micaria*, or does its extraction leave *Micaria* sensu stricto as a paraphyletic group?

Material and methods

The results presented below are based entirely on the use of public datasets, analysed using freely available tools with easy and intuitive user interfaces, not requiring programming skills. While the correct use and interpretation of the output of these tools depends on some understanding of sequence alignments and molecular phylogeny, the type of analysis presented here should be widely accessible to practicing spider taxonomists in general.

All public DNA barcode sequences (based on the mitochondrial cytochrome c oxidase I gene, COI) for *Micaria* and *Arboricaria* species represented by Barcode Index Numbers (Ratnasingham & Hebert 2013) in the BOLD database (Ratnasingham & Hebert 2007) as of 21 February 2017 were downloaded in FASTA format, together with a random selection of single sequences for a diverse range of other gnaphosid species to be used as an outgroup (including representatives of *Callilepis*, *Cesonia*, *Drassyllus*, *Gnaphosa*, *Haplodrassus*, *Herpnyllus*, *Nodocion*, *Nomisia*, *Orodassus*, *Parasyrisca*, *Scotophaeus*, *Sergiolus*, *Sosticus* and *Zelotes*). Sequences were managed in BioEdit v7.2.5 (Hall 1999), which was also used for exploratory sequence alignment using ClustalW 1.4 (Larkin et al.

2007) and initial phylogenetic analysis using the neighbor joining and parsimony algorithms implemented in PHYLIP 3.5c (Felsenstein 1989). Some particularly short or redundant (identical) barcodes were removed from the analysis, to minimize the computational effort. The resulting dataset contained barcodes for 144 specimens of 12 *Micaria* species, including representatives from a wide range of morphologically defined species groups. The final phylogenetic analysis was performed using phylogeny.fr (Dereeper et al. 2008), with twelve different workflows, using sequence alignment by MUSCLE version 3.8.31 (Edgar 2004) or ClustalW 2.1, curation using Gblocks 0.91b (Castresana 2000) or by removing positions with gaps, and phylogenetic inference using the Maximum Likelihood approach implemented in PhyML 3.1 (Guindon & Gascuel 2003), the Neighbor Joining method of BioNJ 3.66 (Gascuel 1997) or the Maximum Parsimony algorithm of TNT 1.1 (Goloboff et al. 2008), using default settings. Bootstrap support was estimated for each of the tree building methods in combination with MUSCLE alignment and gap removal. The nucleotide substitution model for the maximum-likelihood analysis was the very general default Generalised Time-Reversible (GTR) model, with Gamma shape parameter 0.725. Phylogenetic trees were visualized and explored in iTOL v3 (Letunic & Bork 2016). All the conclusions discussed below are independent of the exact choice of sequences, alignment method and tree inference algorithm.

No attempt was made to optimize the parameters of any of the methods or to optimize the alignments by manual editing. Also, the choice of tree building methods was dictated by a desire to cover a wide range of conceptually diverse methods (including the neighbour-joining approach, which is not strictly a phylogenetic inference method), rather than trying to use a few theoretically preferred inference approaches. Such an intentionally diversified strategy would be suboptimal in the context of a comprehensive phylogenetic analysis, where maximal resolution and careful assessment of the support of each node in the tree is the aim. It is, however, a suitable approach in the present analysis, which has a more focused ambition, namely to test if *any* of the methods tried would allow us to reject Platnick's hypothesis that *Arboricaria* is nested within a paraphyletic *Micaria* sensu stricto.

Results and discussion

The two different alignment methods resulted in identical alignments, and results were independent of the treatment of gaps in the alignments. Overall, relationships among the *Micaria* species were very similar in all three tree building approaches. A summary of the preferred majority-rule consensus tree resulting from the phylogenetic analysis is shown in Fig. 1 (the full trees for all methods are included in the electronic supplementary files, including sequence accession numbers, branch lengths and bootstrap support information). While this tree, based exclusively on mitochondrial COI data for a limited sample of species, should not be considered as a strongly supported and reliable phylogeny of *Micaria* in general, it allows a clear answer to Platnick's questions: while *Micaria* sensu lato is a consistently recovered monophyletic group, *Arboricaria subopaca*, as the representative member of *Arboricaria* (i.e., Wunderlich's *subopaca* group), is never recovered as sister to the remaining *Micaria* species, and *Micaria* sensu stricto would be paraphyletic. More specifically, in all analyses that provided sufficient phylogenetic resolution *A. subopaca* was found to be more closely related to, e.g., *M. aenea*, *M. longipes*, *M. alpina* and the species of the *pulicaria* species group than to the members of the *dives* or *scenica* groups. More diverse sequence data would be required to resolve the exact relationships: bootstrap support for the exact placement of *A. subopaca* is low, and different methods place it closer to either *M. aenea* (as suggested already by Wunderlich (1980)) or to *M. alpina/longipes*, and the entire clade containing these four species is nested within the *pulicaria* group in some of the analyses. The *pulicaria* group according to Wunderlich (1980) includes the type species of *Micaria* (*M. fulgens*). Obviously, no conclusion is possible regarding the monophyly of *Arboricaria*, as only one species is represented in the analysis, but this monophyly has not been contentious in earlier discussions of the status of the genus (Platnick 2001, Wunderlich 2017) and is irrelevant for the question at hand.

Confidence in the phylogenetic results is provided not only by the stability of these findings towards the choice of analytical methodology, but also by the fact that all individual species represented by more than one specimen are robustly monophyletic (with bootstrap support between 66 and 100 %). A single exception is the closely related species pair

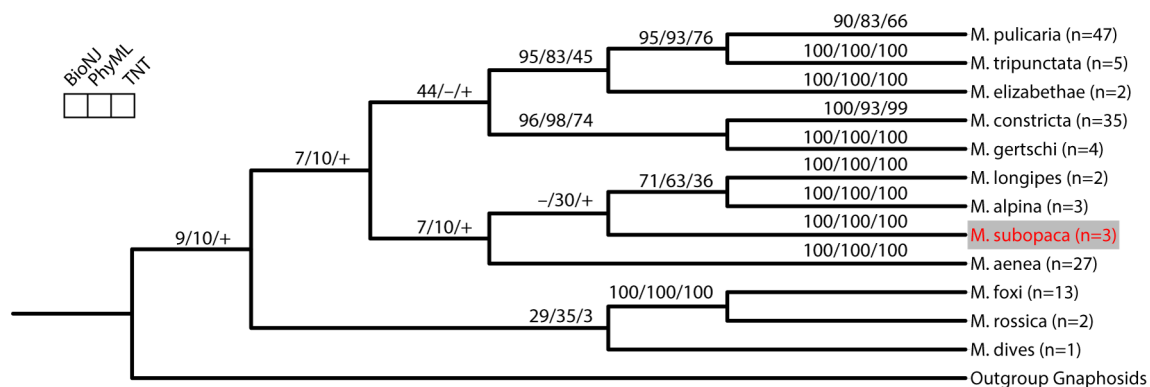


Fig. 1: Preferred phylogeny of barcoded *Micaria* species, based on a majority-rule consensus of analyses in phylogeny.fr, using three different phylogenetic inference algorithms (BioNJ, PhyML and TNT). The bootstrap support for each clade in each of the analyses is indicated above the branches (–: clade not recovered in this analysis, +: clade not consistently resolved in this analysis). A set of gnaphosid species from 14 genera was used as outgroup to root the tree. The number of sequences (n) included in the analysis is indicated for each species.

M. foxi/*M. rossica*, which is not distinguished by the barcode sequences. In this case, the two Alaskan *M. rossica* specimens may be misidentified (G. Blagoev pers. comm.), and two Russian specimens of *M. rossica* added to the database after the conclusion of this study are clearly distinct, but still sister to *M. foxi*. Most importantly, the species groups identified earlier, based on morphological analyses (Wunderlich 1980) and in the first morphology-based phylogenetic analysis of the genus (Platnick & Shadab 1988), are consistently recovered in the majority of the phylogenetic analyses whenever the necessary barcode sequences are available. This concerns the *pulicaria* group (represented by *M. pulicaria*, *M. elizabethae*, *M. gertschi*, *M. constricta* and *M. tripunctata*; only *M. aenea* seems to be misplaced in this group, and Wunderlich (1980) had already indicated a possible closer relationship to the *subopaca* group, as recovered here), as well as the *scenica* group (represented by *M. foxi* and *M. rossica*). Other consistently recovered clades, such as the one joining *M. constricta* and *M. gertschi* (bootstrap support 74 to 96%), and the one joining *M. longipes* and *M. alpina* (bootstrap support 36 to 71 %), indicate the value of DNA barcoding in highlighting potential relationships that are not immediately obvious morphologically.

Restoring the monophyly of *Micaria* with regard to *Arboricaria* as currently defined would require splitting the genus into at least five individual genera (for an extended *pulicaria* group [*Micaria* s. str.], an extended *dives* group [*Micariolepis*], the *subopaca* group [*Arboricaria*], and new genera for the *longipes* group and for *M. aenea*), and possibly more, as several species groups are not yet represented in the DNA dataset, nor in earlier morphological analyses. Given the notable morphological homogeneity of the genus *Micaria* s. lat., as well as its distinctive morphological and ecological synapomorphies pointed out by Wunderlich (2017) – e.g., squamose and iridescent hairs, diurnal life style and ant-mimicry – such an excessive splitting of the genus would be undesirable, turning a clearly differentiated genus into a complex of poorly resolved genera that would be very challenging to diagnose reliably.

One could, of course, argue that the results are weakened by the absence of the type species of *Arboricaria*, i.e. *A. cyrnea*, in the barcode dataset. However, as *Arboricaria* was explicitly established for “the species from the former *M. subopaca*-group” (Bosmans & Blick 2000), even if *A. cyrnea* would turn out to be the sister species of all the *Micaria* species considered here, the resulting drastic re-definition and relimitation of *Arboricaria* would seriously undermine its taxonomic usefulness.

It is noteworthy that the molecular phylogeny places *M. dives* close to the root of *Micaria*, compatible with *M. dives* (plus the *scenica* group) being the sister to all other *Micaria* species. *M. dives* could therefore with some justification be placed in its own genus *Micariolepis* Simon, 1879, as had been suggested by Simon (1878, sub *Chrysothrix*, preoccupied) and followed by numerous later authors (e.g., Reimoser 1937, Bucher 1962, Brændegård 1966, Miller 1971). But even then, the sequence analyses indicate that maintaining monophyly of *Micaria* would either require establishing an additional new genus for the *scenica* group or the extension of *Micariolepis* to include the *scenica* group at the cost of losing morphological diagnosability. Moreover, the morphological gap separating *Micariolepis* and the analysed representatives of the *scenica* group from the rest of *Micaria* is at best very narrow

and the unambiguous diagnosis of *Micariolepis* so challenging (Wunderlich 1980) that such a formal separation would be hardly informative and is better avoided.

In conclusion, the molecular barcoding data fully vindicate the suspicions raised by Platnick (2001) and support Wunderlich’s (2017) decision to formally treat *Arboricaria* as a subjective junior synonym of *Micaria* (**syn. conf.**).

The decision to perform analyses using non-optimized default parameters and to combine results from a diverse set of methods into a single consensus tree should alleviate concerns regarding the possibility of fine-tuning or cherry picking the results in favour of the preferred outcome. But it also means that there is considerable room for improvement should there be interest in a more comprehensive phylogenetic analysis of *Micaria* and gnaphosids in general: ideally, such a study would include an even wider range of species, additional genes (including nuclear ones), and carefully optimized alignments and parameters, while being restricted to the most appropriate phylogenetic inference methods, including Bayesian approaches, which because of computational constraints were not included in the present study.

The case of *Arboricaria* illustrates the value of barcoding information beyond its primary purpose of documenting biodiversity and assisting species identification and discovery (Hebert et al. 2003). While the molecular data in isolation will not be able to replace traditional, integrative taxonomy (Will et al. 2005, Ebach & de Carvalho 2010), they can provide highly valuable complementary information to resolve long-standing taxonomic problems in arachnology (Padial & de la Riva 2007). A systematic analysis of the publicly available data will certainly reveal numerous analogous cases in other spider taxa in the near future, and as the availability of data increases similar studies should soon become part of taxonomic routine in arachnology.

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- Supplementary File 1:** Supplement1.txt, phylogenetic trees in Newick format
- Supplementary File 2:** Supplement2.txt, consensus trees of 100 bootstrap replicates in Newick format
- Supplementary File 3:** Supplement3.pdf, phylogenetic trees in pdf format
- Supplementary File 4:** Supplement4.pdf, consensus trees of 100 bootstrap replicates in pdf format