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

DOI:
10.5431/aramit5405

Document Version
Final published version

Link to publication record in Manchester Research Explorer

Citation for published version (APA):
Breitling, R. (2017). Public DNA barcoding data resolve the status of the genus Arboricaria (Araneae: Gnaphosidae). Arachnologische Mitteilungen, 54, 24-27. https://doi.org/10.5431/aramit5405

Published in:
Arachnologische Mitteilungen

Citing this paper
Please note that where the full-text provided on Manchester Research Explorer is the Author Accepted Manuscript or Proof version this may differ from the final Published version. If citing, it is advised that you check and use the publisher's definitive version.

General rights
Copyright and moral rights for the publications made accessible in the Research Explorer are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

Takedown policy
If you believe that this document breaches copyright please refer to the University of Manchester's Takedown Procedures [http://man.ac.uk/04Y6Bo] or contact uml.scholarlycommunications@manchester.ac.uk providing relevant details, so we can investigate your claim.
Public DNA barcoding data resolve the status of the genus *Arboricaria* (Araneae: Gnaphosidae)

Rainer Breitling

**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, paraphyle, spider.

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 autopomorphic 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 Palaearctic *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 permissive, 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*, *Herpyllus*, *Nadocion*, *Nomisia*, *Orodrasus*, *Parasyrisca*, *Scotophaeus*, *Sergielus*, *Sosticus* and *Zeletes*). 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. 1999).
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, curtail 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 three 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.](image-url)
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. aenca 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 dice group [Micariolepis], the subopaca group [Arboricaria], and new genera for the longipes group and for M. aenca), 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. dice close to the root of Micaria, compatible with M. dice (plus the scenica group) being the sister to all other Micaria species. M. dice 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 (Padih & 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.

Acknowledgements
Gergin Blagoev, Theo Blick, Robert Bosmans, Norman Platnick, and Jörg Wunderlich provided helpful comments on an earlier version of the manuscript. I also thank Jonas Astrin and an anonymous referee for their constructive suggestions that helped improving the analysis.

References
Astrand J, Höfer H, Spelda J, Holstein J, Bayer S, Hendrich L, Huber BA, Kielhorn KH, Krammer HJ, Lemke M, Monje JC, Morinière J, Rulik B, Petersen M, Janssen H & Muster C 2016 Towards a DNA barcode reference database for spiders and harvestmen of Germany. – PLoS ONE 11 (e0162624): 1-24 – doi: 10.1371/journal.pone.0162624
Blagoev GA, DeWaard JR, Ratnasingham S, DeWaard SL, Lu LQ, Robertson J, Telfer AC & Hebert PDN 2016 Untangling taxonomy: a DNA barcode reference library for Canadian spiders. – Molecular Ecology Resources 16: 325-341 – doi: 10.1111/1755-0998.12444
Blagoev GA, Nikolova NI, Sobel CN, Hebert PDN & Adamowicz SJ 2013 Spiders (Araneae) of Churchill, Manitoba: DNA barcodes and morphology reveal high species diversity and new Canadian records. – BMC Ecology 13 (44): 1-17 – doi: 10.1186/1472-6785-13-44
Bosmans R & Blick T 2000 Contribution to the knowledge of the genus Micaria in the West-palaearctic region, with description of the new genus Arboricaria and three new species (Araneae Gnaphosidae). – Memorie della Società Entomologica Italiana, Genova 78: 443-476
Brændegård J 1966 Edderkopper eller spindlere I.– Danmarks Fauna 72: 1-224
Buchar J 1962 Beiträge zur Arachnofauna von Böhmen I. – Acta Universitatis Carolinae Biologicae Caroliniae 1962: 1-7
Castesana J 2000 Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. – Molecular Biology and Evolution 17: 540-552 – doi: 10.1093/molbev/seh043
Dereeper A, Guignon V, Blanc G, Audic S, Buffet S, Chevenet F, DuFayard JF, Guindon S, Lefort V, Lescor M, Claverie JM & Gascuel O 2008 PHYLIp - Phylogeny Inference Package (Version 3.2). – Cladistics 5: 164-166 – doi: 10.1111/j.1096-0031.1989.tb00562.x
Gascuel O 1997 BIONJ: an improved version of the NJ algorithm based on a simple model of sequence data. – Molecular Biology and Evolution 14: 685-695 – doi: 10.1093/oxfordjournals.moleve.a025808
Goloboff PA, Farris JS & Nixon KC 2008 TNT, a free program for phylogenetic analysis. – Cladistics 24: 747-786 – doi: 10.1111/j.1096-0031.2008.00217.x
Guindon S & Gascuel O 2003 A simple, fast and accurate algorithm to estimate large phylogenies by maximum likelihood. – Systematic Biology 52: 696-704 – doi: 10.1080/10635150390235520
Hall TA 1999 BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. – Nucleic Acids Symposium Series 41: 95-98
Hebert PD, Cywinska A, Ball SL & deWaard JR 2003 Biological identifications through DNA barcodes. – Proceedings of the Royal Society B, Biological Sciences 270: 313-321 – doi: 10.1098/rspb.2002.2218
Hennig W 1975 Cladistic Analysis or Cladistic Classification? A reply to Ernst Mayr. – Systematic Zoology 24: 244-256 – doi: 10.2307/2412765
Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Miller EM, Smith TF, Wilm A, Deleage D, Thury-Hermant D, Declerck P, Hoon K, Lopez R, Redaschi N, Thompson JD, Gouy M, Gilson E, Izquierdo R, Lefort V, Clement M & Audic S 2007 Clustal W and Clustal X version 2.0. – Bioinformatics 2007 23: 2947-2948 – doi: 10.1093/bioinformatics/btm404
Letunic I & Bork P 2016 Interactive tree of life (iTOl) v3: an online tool for the display and annotation of phylogenetic and other trees. – Nucleic Acids Research 44: W242-W245 – doi: 10.1093/nar/gkw290
Mayr E 1974 Cladistic Analysis or Cladistic Classification? – Zeitschrift für zoologische Systematik und Evolutionsforschung 12: 94-128 – doi: 10.1111/j.1439-0469.1974.tb00160.x
Mikhailov KG 2016 On the spider genus Arboricaria with the description of a new species (Araneae, Gnaphosidae). – ZooKeys 558: 153-169 – doi: 10.3897/zookeys.558.6521
Miller F 1971 Pavouci-Araneida. – Klíč zvířeny ČSSR 4: 51-306
Minelli A & Kraus O 1999 Preface to the Fourth Edition. In: International Commission on Zoological Nomenclature. (1999) International Code of Zoological Nomenclature Fourth Edition. The International Trust for Zoological Nomenclature, London. 306 pp.
Padial JM & de la Riva I 2007 Integrative taxonomists should use and produce DNA barcodes. – Zootaxa 1586: 67-68
Platnick NI 1977 Paraphyletic and polyphyletic groups. – Systematic Zoology 26: 195-200 – doi: 10.2307/2412841
Platnick NI 2001 The World Spider Catalog, version 2.0. American Museum of Natural History, New York. – Internet: http://www.nsm.nmbe.ch/resources/archive/catalog_2.0/INTRO1.html
Platnick NI 2014 The World Spider Catalog, version 15. American Museum of Natural History, New York. – Internet: http://www.nsm.nmbe.ch/resources/archive/catalog_15.0/index.html – doi: 10.5531/db.iz.0001
Platnick NI & Shadab MU 1988 A revision of the American spiders of the genus Micaria (Araneae, Gnaphosidae). – American Museum Novitates 2916: 1-64
Ratnasingham S & Hebert PDN 2007 BOLD: The Barcode of Life Data system (www.barcodinglife.org). – Molecular Ecology Notes 7: 355-364 – doi: 10.1111/j.1471-8286.2007.01678.x
Ratnasingham S & Hebert PDN 2013 A DNA-based registry for all animal species: The Barcode Index Number (BIN) system. – PLoS ONE 8 (e66213): 1-16 – doi: 10.1371/journal.pone.0066213
Reimoser E 1937 Spinennetiere oder Arachnoidea. 16. Familie: Gnaphosidae oder Plattbauchspinnen. – Die Tierwelt Deutschlands 33: 1-41
Simon E 1878 Les arachnides de France. Tome IV. Roret, Paris. 334 pp.
Simon E 1879 [Replacement names for Gaetulia and Chrysotrich]. – Annales de la Société Entomologique de France, Bulletin Entomologique (5) 9: CLX-CLXI
Will KW, Mishler BD & Wheeler QD 2005 The perils of DNA barcoding and the need for integrative taxonomy. – Systematic Biology 54: 844-851 – doi: 10.1080/10635150500354878
WSC 2017 World Spider Catalog. Version 18.0. Natural History Museum Bern. – Internet: http://wsc.nmbe.ch (22 March 2017)
Wunderlich J 1980 Revision der europäischen Arten der Gattung Micaria Westring 1851, mit Anmerkungen zu den übrigen palaarktischen Arten (Arachnida: Araneida: Gnaphosidae). – Zoologische Beiträge (N.F.) 25: 233-341
Wunderlich J 2017 Descriptions, notes and synonyms of some mainly Mediterranean and Macaronesian spiders (Araneae) of various families. – Beiträge zur Araneologie 10: 298-326

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