A little learning is a dangerous thing – on the usefulness of barcode data for genus-level taxonomy

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Over the last 40 years, the analysis of molecular genetic markers has become a generally accepted and largely uncontroversial component of the taxonomist’s tool kit. In an early review of the field, one of its pioneers, John C. Avise, stated that the only valid reasons not to employ molecular genetic markers are the considerable training required and the high monetary costs involved (Avise 1994:15). The wide availability of easy-to-use online tools for molecular phylogenetic analysis, the predominance of (DNA) sequence-based markers, which are particularly straightforward to analyse, and the gradual acceptance of the foundations of molecular genetics as part of a rounded general education have largely addressed the first impediment. Rapidly accumulating, freely available public datasets of molecular marker information are now promising to overcome the second barrier, bringing affordable molecular marker information within easy reach of any taxonomists with an internet connection. In particular, sequences of the mitochondrial cytochrome C oxidase I gene (COI), collected in vast numbers as molecular “barcodes” for rapid species identification and biodiversity assessments (Hebert et al. 2003; Ratnasingham & Hebert 2007), could be an unprecedented resource in this respect.

In a recent publication in this journal (Breitling 2019a), I presented evidence that publicly available DNA barcode sequences can form a highly valuable source of complementary information to supplement morphological data and help resolve controversial taxonomic issues at the genus level. I illustrated the approach with a number of examples across spider biodiversity, thus focusing on a group for which the available barcode data are particularly abundant. Many other authors are using barcode data as part of an integrative taxonomic approach above the species level, in a wide variety of organisms (e.g., Thacker et al. 2018 for genera of gobies, Srisuka et al. 2019 for species groups of black flies, and Huang et al. 2019 for genera of leeches). While the use of barcode data (and other mitochondrial sequences) for taxonomic purposes has been repeatedly challenged (e.g., Ballard & Whitlock 2004, DeSalle et al. 2005, and Ebach & Holdrege 2005), there seems to be an emerging consensus that these sequences are not inherently misleading and can make an important contribution when used in combination with other sources of evidence (see, e.g., the balanced review of the debate by Rubinoff & Holland 2005, which already pre-empts many of the arguments discussed here).

An approach that combines barcode data with a diverse range of other sources of evidence, from morphology to zoogeography, would thus appear to be largely non-controversial. It was, therefore, rather surprising that
in response to my article, the team of the World Spider Catalog (WSC) has implemented a new policy stating that “COI barcoding [is] not useful on genus level” and that the resulting taxonomic proposals will normally not be adopted in the catalogue (Anonymous 2019). Of course, one could easily dismiss this new policy with a reference to Rubinoff & Holland (2005), considering it merely an example for the WSC’s preference for morphological over molecular data (this is, e.g., also evident in the catalogue’s splitting of the spider family Psilodercidae from Ochyroceratidae and the even more disruptive merging of Prodidomidae and Gnaphosidae, both strongly contradicted by comprehensive molecular evidence). However, it is quite possible that the WSC team’s concerns continue to be shared by others, especially classically trained taxonomists without extensive experience in molecular genetics and phylogeny, who could be the main beneficiaries of the accumulating wealth of publicly available barcode data. It would therefore seem of interest to have a closer look at the arguments provided to support the new WSC policy. Such a careful discussion can hopefully avoid future confusion about the scope and applicability of barcode-based taxonomy at the genus level and the prudent use of this valuable information. The following text addresses each of the WSC’s stated reasons for considering COI barcoding as not useful at the genus level in their original order and wording.

1. “Due to its maternal inheritance as a mitochondrial gene, strong differences between resulting gene tree and real phylogeny have been found (e.g., Edwards & Bensch (2009) Molecular Ecology 18: 2930-2933, doi: 10.1111/j.1365-294X.2009.04270.x)”

This seems to be a rather peculiar argument: the maternal inheritance of COI as a mitochondrial gene is usually considered a helpful feature making it particularly valuable for phylogenetic inference. Mitochondrial genes are not affected by recombination, and show a much higher substitution rate than many nuclear genes, and thus higher signal at shallow branches of the phylogenetic tree. Also, differences between gene and species trees are the normal consequence of stochastic lineage sorting and to be expected at the early stages of speciation – this is the case for all genes, no matter whether they are inherited maternally, paternally, or from both parents. But, importantly, organisms are usually haploid and homoplasmic for their mitochondrial genes, and due to their maternal inheritance the effective population size for these genes is further halved compared to that of nuclear genes, leading to a four times faster coalescence and thus a much lower probability of discrepancies between gene and species trees as a result of incomplete lineage sorting. Perhaps the WSC team intended to imply that despite its maternal inheritance, a single mitochondrial gene phylogeny might not always agree with the actual species tree (Sánchez-Garcia & Castresana 2012). However, this problem of deep coalescence as a result of stochastic effects typically affects estimates of recent speciation history; it is not relevant at the genus level: if two species share COI haplotypes as a result of incomplete lineage sorting, they obviously belong in the same genus (even closely related pairs of sibling species, such as Araniella cucurbitina/A. opisthographa or Metellina segmentata/M. mengei, are separated by a barcode gap of more than 5%, corresponding to several dozen fixed nucleotide differences). Curiously, the cited reference (Edwards & Bensch 2009) does not even discuss the maternal inheritance of COI barcodes or its consequences at all. Moreover, it only discusses examples of phylogenetic inference applied to populations within a single species or superspecies, and is therefore not informative when trying to establish the value of barcodes for genus-level taxonomy. Clearly, Edwards & Bensch (2009) make an important contribution to the discussion of barcode-based phylogeography, but their work does not support the claim made by the WSC team.

2. “Widely spread endosymbionts such as Wolbachia lead to introgression of mtDNA from other species and falsify the results (e.g., Klopfstein et al. (2016) Zoological Journal of the Linnean Society 177: 541-557, doi: 10.1111/zoj.12380).”

Mitochondrial introgression, whether driven by maternally inherited endosymbionts or simply the result of genetic drift and / or selection, requires successful fertile hybridization as its essential precondition (Toews & Brelsford 2012). It can lead to severe underestimates of the genetic divergence between subspecies, semispecies or closely related species pairs, whenever pre- and postzygotic reproductive barriers are insufficiently developed to prevent fertile hybridization (Hurst & Jiggins 2005). There is no evidence whatsoever that hybridization in spiders occurs across genus boundaries, and the possibility that such an inter-genus mating would result in fertile offspring seems remote, given the major divergence in genitalia and / or behaviour even in closely related species pairs (a single reported inter-genus mating in spiders later turned out to be based on a misidentification; Schmidt 1981, Wunderlich 1992). As a result, the examples of
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genus-level taxonomy discussed in Breitling (2019a) will not be negatively affected by introgression artefacts. Quite the contrary is the case: if two species originally placed in different genera could be shown to share similar barcodes as a result of introgression, this observation would provide very strong support for placing them in a single genus, either by synonymy or by transfer. The cited reference (Klopfstein et al. 2016) discusses barcode introgression between some species within a single genus of wasps, and does not comment on the application of barcodes for genus-level taxonomy. This is another example where the cited reference certainly makes a valid contribution to the general debate on barcode analysis, but does not support the argument of the WSC team regarding the use of barcode data at the genus level.

3. “COI on genus level is usually strongly saturated, making phylogenetic trees unreliable (long branch attraction). Intensive sampling and adding as many taxa as possible may help to counter this a bit (e.g., Quicke et al. (2012) Molecular Ecology Resources 12: 676-685, doi: 10.1111/j.1755-0998.2012.03143.x).” It is not quite clear what is meant by this statement, and what might be the underlying evidence. It is correct that COI sequences are diverging rapidly; that is the reason why they can be used to distinguish even closely related species. This rapid divergence, and the fact that the barcodes are only relatively short, means that, at some point, multiple mutations will occur at the same site, or identical changes will appear independently in separate lineages, obscuring the phylogenetic signal. This makes it impossible to use COI data for the confident inference of deep branches in a phylogeny (e.g., at the level of tribes or subfamilies). In those cases, a larger amount of sequences, including more slowly evolving ones, will be required. However, at the genus level, there is no evidence of a major confounding impact of mutational saturation. This is shown, e.g., by the accurate assignment of barcodes to their morphologically defined genera (for spiders see Coddington et al. 2016), as well as the generally excellent congruence between comprehensive barcode-based sequence trees and morphology-based genus (and even family!) assignments (e.g., Astrin et al. 2016: Supplementary figures S1 and S2). In those example trees, misplaced specimens or species are usually very notable by their appearance in entirely unrelated groups; the case that a species is by coincidence misplaced within a closely related genus is much less likely. Long-branch attraction is a serious problem in phylogenetic inference (Bergsten 2005), but its relevance in the present context is unclear – it is certainly not the main reason why phylogenetic trees based on mutationally saturated sequences are unreliable. In any case, as the WSC team acknowledges, long-branch attraction artefacts can be minimized by denser taxon sampling; the COI barcode dataset has certainly the densest taxon coverage of all molecular marker data presently available. Incidentally, the dense taxon sampling will also increase the probability that type species (or closely related, confidently placed species) are represented in the dataset; an important advantage for making taxonomic assessments at the genus level, as shown in Breitling (2019a). The cited reference, once more, disagrees with the WSC team and explicitly advocates the usefulness of barcode data for the correct placement of difficult taxa at the genus (and even tribe or subfamily) level (Quicke et al. 2012:680).

4. “Databases such as BOLD or the NCBI databases contain also species that are not correctly identified. Relying exclusively on such databases and/or including low numbers of specimen may result in error-prone and misleading phylogenies.” It is certainly true that these databases contain some barcodes for specimens that are not correctly identified. However, there is no indication that a substantial fraction of the barcode data are based on misidentified specimens. Moreover, the BOLD barcode database (Ratnasingham & Hebert 2007) provides a large amount of useful metadata that allow validating relevant identifications, including detailed georeferencing, photos of voucher specimens, and the identity of the identifying expert. These metadata are much easier to access for validation purposes than the information for most non-barcoded museum specimens. Also, in many cases, several (often many) specimens have been independently identified and barcoded; the correspondence of the resulting barcode sequences adds further internal evidence for the correct identification. This is, e.g., the case for all examples discussed in Breitling (2019a). In addition, misidentifications will typically involve closely related species pairs within the same genus, at least for the well-known Holarctic spider fauna (often the same species pairs that are suspected of exchanging mitochondrial genomes through introgressive hybridization). Their effect on genus-level phylogenies will thus be minimal.

5. “A further good argumentation can be found here: https://waynemaddison.wordpress.com/2018/11/22/please-dont-use-co1-barcodes-alone-for-spider-phylgeny/”
This blog post makes a very strong case against using COI barcodes in isolation to decide phylogenetic relationships at higher taxonomic levels (tribes or subfamilies). As discussed above, this is a very important warning: for many families, the relationships above the genus level are poorly defined or have never been systematically explored using morphological evidence. There might be some temptation to fill this void using barcode data – as a result of saturation at this level of divergence, this would be a hopeless enterprise, with or without supporting morphological data. The rapid mutation rate of COI, which makes these barcode sequences so useful for species identification and phylogenetic inference at the genus level, makes them generally inappropriate for deeper phylogenetic resolution. The phylogenetic tree used to illustrate the argument in the blog post supports this assessment: various tribes and subfamilies are widely scattered across the tree, as would be expected when the phylogenetic signal becomes obscured by saturation, but the two genera that are represented by more than one species (Portia and Omoedus) are recovered correctly as monophyletic groups. The evidence presented by Maddison certainly does not contradict the conclusion that barcode data can be highly informative at the right levels of phylogeny and do allow successful generic assignment in the majority of cases (Zink & Barrowclough 2008, Coddington et al. 2016).

So, should we be afraid of using COI barcodes to support genus-level taxonomy? It appears that the arguments advanced against this application are based on a misunderstanding and misrepresentation of the well-known limitations of barcode information. Introgression and incomplete lineage sorting sometimes make it difficult to correctly distinguish closely related species pairs at the barcode level; possible examples among spiders are Alopecosa cuneata / A. pulverulenta, Tibellus oblongus / T. maritimus, Enoplognatha ovata / E. latimana or Xysticus audax / X. cristatus (Astrin et al. 2016), and similar cases are known for most other taxonomic groups. Furthermore, as a result of rapid saturation, barcodes are usually unsuitable for resolving the deeper branches of spider phylogeny, where even large multi-gene datasets can fail to produce stable and consistent results (e.g., compare the subfamily relationships for Theraphosidae proposed in Lüddecke et al. 2018 and in Turner et al. 2017). At the genus level, in contrast, barcode information can be very informative, and every practicing taxonomist should be excited about the possibility of making use of this information to complement her morphological assessments (Figure 1).

But wouldn’t it be better to use a multitude of molecular markers? Perhaps even entire genome sequences? There can be no doubt that this would be essential for resolving deep branches in the phylogeny, and it would also be highly advisable when designing a study to collect new genetic data to resolve the details of the phylogenetic relationships within and between genera. The major reason for using COI as a single
molecular marker is that these barcode data are already available freely and in large quantities for an enormous range of species. Neglecting this information, merely because having more data would be even better, would seem foolish. The barcode data, in contrast to phylogenomic datasets, are available to anyone with access to the internet, as are the tools to analyse them on a standard desktop computer, and we should make use of this opportunity to challenge and refine our taxonomic hypotheses at the genus level.

Where the arachnologists of the WSC team are correct is the conclusion that barcode data at the genus level should always be interpreted within the existing framework of morphology-based taxonomy. Such an integrative analysis will easily identify when the COI results turn out to be uninformative, e.g., as a result of saturation at higher levels of phylogeny or the misidentification of critically important specimens. However, where barcode data strongly agree with previous morphological assessments, as in the cases discussed in Breitling (2019a), it would appear negligent and unscientific to ignore the barcode information. A few selected examples, again focusing on spiders as an illustrative case, will show the kind of insights that would be lost if barcode information were to be uncritically discarded. For instance, when Wunderlich (1984) proposed the synonymy of Acantholycosa with Pardosa, he did so specifically because of the close relationship of Acantholycosa species with the nigra group of Pardosa. Based on morphological data alone, this decision was not convincing enough, as the suggested supporting synapomorphies could also be interpreted as homoplastic. As a result of the remaining uncertainty, Buchar & Thaler (1993), and subsequently the WSC, rejected the synonymy for reasons of convenient communication (“aus Gründen der Verständigung”), although they did not refute the proposed relationship especially with Pardosa nigra. When the barcode data recover exactly the same relationship (Breitling 2019a), and place Acantholycosa well within the genus Pardosa, as a member of the extended nigra group, this cannot be dismissed as the result of mere coincidence. Instead, this observation provides strong support for Wunderlich’s earlier taxonomic decision.

To get a better impression of the minute probability of observing these relationships by chance, a simple computation might be useful. In the Breitling (2019a) dataset we have barcodes for about 300 species of wolf spiders, including about 100 species of Pardosa (10 of which are part of the nigra group in the broad sense) and 2 species of Acantholycosa. Three hundred species can be arranged in $3.4 \times 10^{700}$ unrooted trees (Felsenstein 1978). In $5.1 \times 10^{53}$ of these possible trees, the 100 Pardosa species will form a monophyletic group, while in $3.2 \times 10^{528}$ trees, 100 Pardosa plus 2 Acantholycosa will be grouped together. The 102 species of Pardosa plus Acantholycosa can be arranged in $1.3 \times 10^{499}$ different ways. Of these, $1.1 \times 10^{164}$ will show a monophyletic grouping of the two Acantholycosa species and the 10 members of the nigra group.

Thus, out of all the possible random trees, only 1 in $1.1 \times 10^{572}$ trees will show Pardosa plus Acantholycosa as monophyletic among lycosids, and among these only 1 out of $1.2 \times 10^{35}$ will include Acantholycosa forming a (monophyletic) clade with the members of the nigra group. For comparison, the observable universe is estimated to contain less than $10^{82}$ atoms, has a volume of $4 \times 10^{98}$ femtoliters, and has existed for about $4.35 \times 10^{23}$ microseconds. Thus, hitting on such a result in random agreement with the morphological evidence would be infinitely unlikely. This calculation should also call attention to the fact that traditional measures of branch support (such as bootstrap values or posterior probabilities) need to be interpreted with caution when the results are placed in the context of well-defined alternative phylogenetic hypotheses, instead of relying on sequence data alone. In this situation, the barcode trees are used for hypothesis testing, not for hypothesis discovery, and the interpretation of the statistical support values has to be adjusted accordingly (typically using more relaxed significance thresholds).

In the actual barcoding dataset, the situation is obviously more complex than in the preceding example calculations: e.g., Pardosa is consistently recovered as paraphyletic with respect to Acantholycosa, but also with another “pardosine” genus, Wadicosa, represented by two barcoded species, in agreement with the larger molecular dataset of Piacentini & Ramírez (2019), which additionally indicates Draposa as another member of Pardosa s. lat. Also, one of the Pardosa barcodes is consistently placed very far from the rest of the genus (and is possibly based on a low-quality sequence or misidentified specimen). Moreover, the exact composition of the nigra group in the broad sense is not entirely clear, based on the morphological evidence. But even taking all of these uncertainties into account, the probability of a random placement of Acantholycosa in the nigra-group within a monophyletic Pardosa s. lat. remains negligible.
The same argument applies to the treatment of *Centromerita* and *Tallusia* as synonyms of *Centromerus*, the placement of “*Aphileta*” *microtarsa* in the genus *Eulaira*, and the transfer of “*Leptyphantes*” *nodifer* to the genus containing its close relative *Anguliphantes angulipalpis* (Breitling 2019a). In each of these cases, previous morphological analyses had already convincingly made this case, and it would require an almost mystical believe in coincidence to reject the barcode data, which fully support these placements, as uninformative. None of these proposals can be reasonably construed as being “based mainly on COI” as theWSC asserts, but in each case the barcode data are merely the last piece of evidence that decisively tips the balance in favour of an already well-supported hypothesis.

Incidentally, the barcode data also strongly refute a supplementary argument the WSC provides for not accepting the synonymies proposed by Breitling (2019a, 2019b): the catalogue entries now state that the genera *Pardosa*, *Centromerus* and *Clubiona* are “polyphyletic” and their further splitting is to be expected. The barcode data show very consistently and with strong support that at least the large Holarctic core of each of these genera is clearly monophyletic (or paraphyletic, if the proposed synonymies of *Acantholycosa*, *Tallusia*, *Centromerita* and *Porrhoclubiona* are not accepted). In some cases, extralimital species may require their own genera in the future (e.g., some of the Australian members of the *Clubiona*; Breitling 2019b), but that does not affect the conclusion that the core of each of these hyperdiverse genera is a quite recently diversified monophyletic group. The catalogue unfortunately does not provide a literature reference for its statement to the contrary. While, for practical reasons, a subdivision into species-groups or subgenera could be desirable (and has been proposed many times), the wanton disruption of these well-established taxa into multiple independent genera would amount to taxonomic vandalism.

Even in the absence of previous morphological analyses, the barcode data can suggest compelling new hypotheses that can then be tested against complementary data from other sources. For instance, the barcode-based placement of “*Cryptachaea*” *riparia* in *Parasteatoda* is strongly supported by the similarities in the male and female genitalia, which share supposed synapomorphies with *Parasteatoda* (e.g., a “large and distinct epigynal depression, without a posterior lobe, and a large subtegulum and relatively small tegulum on the male palp”; Breitling 2019a, Yoshida 2008). The species also does not have the median apophysis fused to the embolus, as would be expected for a genuine member of *Cryptachaea* (Yoshida 2008). Zoogeographic considerations also provide a strong argument in support of the revised placement: *Parasteatoda* has several undisputed native members in Europe (*P. simulans* and *P. tabulata*), and many more in the eastern Palaearctic, while *Cryptachaea* is predominantly an American genus (with more than 80 American species), represented in the Palaearctic only by two cosmopolitan adventive species (*C. blattea* and *C. veruculata*), which are closely related to the Australian synanthropic *C. gigantipes* and quite possibly belong to a separate genus. Three further *Cryptachaea* species reported from the Oriental region (*C. ogatai*, *C. projectivulva* and *C. uncina*) are probably also all misplaced in this genus: *C. ogatai* appears to be very similar in morphology and behaviour to *P. riparia*, its alveolus appears to extend beyond the cymbium (Yoshida 2016:fig. 37 and 38), and the median apophysis seems to form a separate sclerite (Yoshida 2016: fig. 39); the species is therefore here transferred to *Parasteatoda* (as *Parasteatoda ogatai comb. nov.*). *C. projectivulva* is known only from the female sex and can currently not be placed with confidence; the original description by Yoshida (2001) mentions that the species resembles *Parasteatoda culicivora*. *C. uncina* has no similarity to the type species of *Cryptachaea* and lacks a key diagnostic character of this genus as defined by Yoshida (2008), as its median apophysis is not fused to the embolus base (Gao & Li 2014: fig. 27a, 29A); its correct placement remains uncertain. None of the earlier authors who proposed the alternative placement of *Parasteatoda riparia* in *Cryptachaea* provided an explicit argument to support this decision.

The barcode data can also call attention to questionable taxonomic decisions that have not attracted sufficient attention previously. E.g., the analysis in Breitling (2019a) clearly shows that the recent extensive splitting of *Leptyphantes* s. lat., which has never been supported by a comprehensive cladistic argument, has led to the establishment of a number of strongly supported new genera (e.g., *Tenuiphantes*), but has in other cases resulted in considerable oversplitting into numerous small and poorly diagnosed genera, which make the correct assignment of new species exceedingly difficult (van Helsdingen 2009). The barcode-supported proposal to remedy the situation by synonymising *Anguliphantes*, *Impropohantes*, *Piniphantes* and *Mansuphantes* with *Oryphantes* is incidentally also supported by a large molecular dataset including not only
mitochondrial sequences (COI and 16S), but also several nuclear markers (18S, 28S, H3; Wang et al. 2015), which also finds *Anguliphantes* and *Oryphantes* to be mutually polyphyletic with strong bootstrap support.

A similar case is presented by the results for the *Savignia* genus group of Linyphiidae. This group was defined by Millidge (1977) based on characters of the male pedipalps, as containing the genera *Savignia*, *Diplocephalus*, *Erigonella*, *Araeoncus*, *Dicymbium*, *Saloca*, *Glyphesis*, *Alioranus*, and *Diastanillus*. He suggested, expanding on an earlier proposal by Holm (in litt. in Millidge 1977), that the palpal conformation of these species is so similar that it would be advantageous to group all species in a single large genus (*Savignia* having priority). Frick et al. (2010) reanalysed the group using cladistic methods and a much larger set of morphological characters. They propose that *Alioranus* and *Saloca* need to be removed from the *Savignia* group to render it monophyletic (they did not include *Diastanillus* in the analysis, due to a lack of material). They also find that *Erigonella* and *Diplocephalus* are both non-monophyletic, confirming the suspicions of Millidge and Holm regarding the validity of genus boundaries in this group.

The barcode data presented in Breitling (2019a) include sequence information for all genera of Millidge’s *Savignia* genus group, except *Alioranus* and *Diastanillus*. Interestingly, the resulting preferred barcode tree recovers a monophyletic *Savignia* group, including *Saloca* (Figure 2). This seems to confirm the suggestion by Frick et al. (2010) that the removal of *Saloca* is the result of neglecting relevant characters (such as the presence of a round supratergal apophysis), which *Saloca* shares with the other members of the *Savignia* group. The barcode data also confirm that *Erigonella* and *Diplocephalus* are not monophyletic, and extend the problem to *Araeoncus* and *Dicymbium*, which are both not recovered as monophyletic in the larger set of species covered by the barcode data. The internal details of the *Savignia* group, however, do not correspond to the subdivisions suggested by Millidge (1977) or Frick et al. (2010), which in turn are mutually incompatible in almost all respects. In the barcode data, *Minyriolus* is identified as a new member of the group, close to *Glyphesis*. The two genera are also morphologically very similar (several species of *Glyphesis*, including the type species, were initially placed in *Minyriolus* by Simon, before he separated *Glyphesis* as a new genus). Also, *Diplocephalus cristatus* is not found as a particularly close relative of *Savignia frontata*, in contrast to the cladistic analysis of Frick et al. (2010); instead, the barcode data consistently find *Diplocephalus barbiger* as sister to *S. frontata*. *D. barbiger* is one of only two species currently in *Diplocephalus* that had been placed in *Savignia* multiple times by earlier authors. The striking commonality of all three studies — pedipalp morphological (Millidge), cladistic morphological (Frick et al.), and barcode-based (Breitling) — is their suggestion that current morphological genus definitions in this group are flawed and do not reflect the true relationships within the group. Their mutual lack of any degree of agreement further emphasises that a workable subdivision of the group into monophyletic and morphologically definable units is not going to be easily achieved. For the time being, a formal synonymy of a large part of the genera involved with *Savignia* s. lat. should probably wait until barcode data for *Alioranus* and *Diastanillus*, as well as for more recently proposed members of the *Savignia* group, become available.
Also, it would be important to examine genera placed by Millidge (1977) in the Savignia group in the broader sense, including Dactylopistes, Aulacocyba, Janetschekia and Thaumatoncus. That such a synonymy will ultimately be unavoidable seems to be obvious, even though the barcode data indicate a few consistent subdivisions within the group.

A final example of a controversial genus separation usefully illuminated by barcode data is the separation of Pirata and Piratula, two very similar genera (both morphologically and ecologically), for which no convincing synapomorphies have been proposed to support their mutual monophyly. The barcode data show clearly that Pirata + Piratula is a monophyletic group – and this assessment is supported by several other molecular markers (e.g., in Piacentini & Ramírez 2019 and Murphy et al. 2006) –, but that the supposed members of the two genera are widely scattered across several deeply separated clades within this group. This complete scrambling of the genetic markers seems impossible to explain if these were indeed well-separated genera, unless we postulate a particular form of molecular orthogenesis. While it would be extremely interesting to obtain additional molecular information for a wider sample of members of Pirata s. lat., for the time being a closer look at the characters used by Omelko et al. (2011) to support the separation suggests that the morphological evidence is so weak that it would be hard to justify the considerable amount of ad hoc hypotheses that would be required to maintain the two genera as separate clades in the light of the molecular data: according to Omelko et al. (2011), the only consistent morphological difference appears to be the presence of a prolateral spine on tibia I in females of Piratula, while all other differences (e.g., differences in total size, in the arrangement of the eyes, in sternum pattern and coloration, and the presence or absence of teeth on the upper arm of the tegular apophysis) apply only to some of the species, and are limited to character systems that are notorious for an extreme degree of homoplasy. The proposed synonymy of Piratula and Pirata (in Breitling 2019a), which had previously already been suggested by Dondale & Redner (1981), seems therefore to be entirely justified by the combined evidence.

All of these cases also illustrate the major advantages of barcode data compared to many morphology-based or molecular cladistic analyses: especially Holarctic groups are already very intensely sampled, often with multiple specimens from several widespread populations per species, and with great taxon density, facilitating the selection of suitable outgroups for each analysis. As the WSC team acknowledges (Anonymous 2019), these are important preconditions for high-quality phylogenetic studies – and they are now much easier to meet using the huge public collections of COI sequences. For example, the taxon coverage and sampling intensity of the analysis presented in Breitling (2019a) far exceeds that in earlier complementary studies.

In conclusion, while barcoding data are not a panacea to solve all taxonomic problems, their free availability and broad coverage of animal biodiversity make them a promising complementary source of evidence, which in combination with morphological information can be used successfully to resolve a number of long-standing taxonomic controversies with high confidence, while at the same time challenging established working practices in a stimulating and constructive way. Dismissing this kind of data as generally unreliable is not justified by the available scientific evidence.

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