



Nomenclatural stability does not justify recognition of paraphyletic taxa: A response to Scherz et al. (2016)



Pedro L.V. Peloso^{a,b,*}, Christopher J. Raxworthy^b, Ward C. Wheeler^c, Darrel R. Frost^b

^a Museu Paraense Emílio Goeldi, Coordenação de Zoologia, Avenida Perimetral, 1901, Terra Firme, CEP 66077-530 Belém, Pará, Brazil¹

^b Division of Vertebrate Zoology (Herpetology), American Museum of Natural History, Central Park West at 79th Street, 10024 New York, NY, USA

^c Division of Invertebrate Zoology, American Museum of Natural History, Central Park West at 79th Street, 10024 New York, NY, USA

ARTICLE INFO

Article history:

Received 8 July 2016

Revised 16 March 2017

Accepted 18 March 2017

Available online 20 March 2017

Keywords:

Amphibia

Cophylinae

Madagascar

Microhylidae

Phylogeny

Taxon-Naming Criteria (TNC)

ABSTRACT

Peloso et al. (2015: PELOSO) published a comprehensive phylogenetic study of the frog family Microhylidae, which resulted in the discovery that several taxa were not monophyletic. To remedy this, a series of nomenclatural changes were proposed (several generic synonymies and two new subfamilies named). A recent study published in this journal by Scherz et al. (2016: SCHERZ), provided a novel phylogeny for the Malagasy subfamily Cophylinae. SCHERZ dispute the analyses and taxonomic conclusions of PELOSO. Their study is, however, based on *substantial* reduction of data from the PELOSO study, limited addition of new data, and different analytical methods. In spite of the fact that their own results are consistent with the taxonomy of PELOSO, SCHERZ reject that conservative taxonomy and suggest the revalidation of *Platypelis* (from the synonymy of *Cophyla*), the revalidation of *Stumpffia* (from the synonymies of *Rhombophryne*), and the creation of at least two new genera (only one named therein). In doing so, SCHERZ accept the recognition of likely paraphyletic taxa, with *Stumpffia* paraphyletic in their parsimony analysis. Herein, we provide a response to several points raised in SCHERZ: (1) we discuss issues with their interpretation (and selective use) of available phylogenetic and phenotypic evidence; (2) and provide a new phylogenetic analysis of all the data in PELOSO and SCHERZ combined. In the new analysis *Stumpffia* is paraphyletic with respect to *Rhombophryne*, whereas *Cophyla* and *Platypelis* are both monophyletic and sister taxa. We provide a case for the use of the taxonomy suggested in PELOSO.

© 2017 Elsevier Inc. All rights reserved.

1. Introduction

Microhylidae represent approximately 8.8% of the global frog diversity and are found in almost every tropical landmass on earth. Despite dense sampling and repeated attempts to infer phylogenetic relationships among members of the family (e.g., de Sá et al., 2012; Peloso et al., 2015; van der Meijden et al., 2007), relationships, particularly among the nominal subfamilies, are largely unstable, and several genera and subfamilies are still suspected to be para- or polyphyletic.

Peloso et al. (2015: hereafter PELOSO) performed a sensitivity analyses (sensu Wheeler, 1995) on a variety of combinations of taxa (up to 142 taxa) and genomic data (up to 73 loci) to infer the phylogeny of microhylids. Based on their phylogenetic results, PELOSO reviewed the classification of Microhylidae suggesting several taxonomic updates to generic classification, and also

naming two new subfamilies in the process. After PELOSO, Microhylidae was considered to be composed of 13 subfamilies.

1.1. The generic content in Cophylinae

Cophylinae is endemic to Madagascar and is composed of 72 named species (third largest microhylid subfamily), plus an apparent high number of unnamed taxa (Köhler et al., 2010; Perl et al., 2014; Scherz et al., 2016; Wollenberg et al., 2008). The phylogeny of Cophylinae has received considerable attention compared to most other subfamilies (Andreone et al., 2005; Blommers-Schlösser and Blanc, 1993; Scherz et al., 2016; Wollenberg et al., 2008); hence, multiple alternate hypotheses of relationships have been suggested. PELOSO sampled 32 cophylinae taxa in their analyses, including six of the seven genera recognized at the time (excepting *Madecassophryne*, for which tissue samples are unavailable). The results in PELOSO corroborated previous suspicions that some cophylinae genera are not monophyletic: *Platypelis* (paraphyletic with respect to *Cophyla*), *Stumpffia*, and *Rhombophryne* (with respect to each other). To remedy this, PELOSO suggested,

* Corresponding author at: Museu Paraense Emílio Goeldi, Coordenação de Zoologia, Avenida Perimetral, 1901, Terra Firme, CEP 66077-530 Belém, Pará, Brazil.

E-mail address: pedropeloso@gmail.com (P.L.V. Peloso).

¹ Present Address.

among other things: (1) *Platypelis* Boulenger, 1882 should be treated as a synonym of *Cophyla* Boettger, 1880; and (2) *Stumpffia* Boettger, 1881 should be treated as a synonym of *Rhombophryne* Boettger, 1880.

1.2. Scherz et al. (2016)

A recent study published in this journal, by Scherz et al. (2016; hereafter SCHERZ), disputed the data, results and taxonomic conclusions of PELOSO. The principal taxonomic actions of SCHERZ were to: (1) reject the synonymy of *Platypelis* with *Cophyla*, (2) reject the synonymy of *Stumpffia* with *Rhombophryne*, and (3) create a new genus (*Anilany*).

In support of their taxonomic decisions, SCHERZ provided novel phylogenetic analyses (with many added analytical assumptions) of Cophylinae, which also purported to constitute “a re-analysis of the cophylina members of the PELOSO dataset”. However, when doing so, SCHERZ inexplicably excluded a large fraction of the PELOSO dataset. 75% of the taxa and 97% of the genetic data from PELOSO were completely discarded without much discussion or justification. Despite the availability of up to 73 loci from the PELOSO study, data for 71 loci were discarded. Only data from the mitochondrial genes *16S ribosomal RNA (16S)* and *Cytochrome Oxidase Subunit I (COI)* generated by PELOSO were included by SCHERZ. A substantial amount of data available for outgroup taxa were also ignored—SCHERZ deleted all data for non-Malagasy microhylids.

SCHERZ employed two methods for phylogenetic inference: Bayesian inference (via MrBayes) and parsimony (via TNT)—their implementations based on a two-step procedure (multiple sequence alignment + phylogenetic inference: i.e., similarity-alignment). Nevertheless, SCHERZ largely ignored the results from their own parsimony analysis (which do not support their preferred taxonomy) in favor of the Bayesian topology (which marginally supports their preferred taxonomy).² Furthermore, SCHERZ never discussed the fact that their discovery operations (similarity-alignment) are based on conspicuously different theoretical foundations than that of PELOSO (direct optimization: Sankoff, 1975; Wheeler, 1996—tree-alignment). Several authors have discussed the issue of assessing DNA sequence homology, whereas many of them agree that multiple-sequence alignment is best performed with explicit reference to the phylogeny (Felsenstein, 1988, p. 525; Sankoff, 1975; Sankoff and Cedergren, 1983; Wheeler, 1996).

SCHERZ’s arguments for the rejection of the taxonomic review of PELOSO are largely based on: (1) sample misidentifications in the dataset of PELOSO; (2) the claim that the changes are unnecessary to attain a monophyletic classification; and (3) that SCHERZ’s classification promotes taxonomic stability. We discuss these topics below.

2. A response to SCHERZ et al. (2016)

SCHERZ “re-analysis” of PELOSO, and the conclusions drawn from it, are questionable. In this section, we address several logical, analytical, and theoretical issues in SCHERZ’s study.

2.1. Sample misidentifications and their impact in the proposed taxonomy

SCHERZ provided a series of corrections and updates to identifications of samples used in PELOSO. The authors claim that these identification mistakes “caused erroneous genus-level changes

within the Cophylinae”, but fail to recognize that most of these changes were not a result of misidentifications. Rather, the changes proposed by PELOSO stem from the fact that paraphyletic taxa have been historically recognized in the subfamily.

SCHERZ thoroughly reviewed the identification of the genetic samples of cophylina taxa used in PELOSO. This was accomplished by direct comparisons with new (published with SCHERZ) and legacy (GenBank) sequences. The potential sources of the identification errors were discussed in SCHERZ (Supplementary Material). SCHERZ’s co-author Miguel Vences (MV), through the Technische Universität Braunschweig, supplied many (almost 30%) of the mislabeled samples used in PELOSO. Some of these mislabeled samples included tissues supposedly taken from type specimens of species collected and described by MV (and colleagues). A sample labeled *Rhombophryne matavy* D’Cruze, Köhler, Vences, and Glaw, 2010 in PELOSO, turned out to represent a tissue of the holotype of *Plethodontohyla fonetana* Glaw, Köhler, Bora, Rabibisoa, Ramilijaona, and Vences, 2007, whereas a sample labeled as one of the paratypes of *Rhombophryne mangabensis* Glaw, Köhler, and Vences, 2010, was re-identified as an unnamed species of *Stumpffia*. This, however, does not exempt PELOSO from the responsibility of actually incorporating these samples into their work. Clearly, however, future workers should beware of identifications of frog tissue samples provided by the Technische Universität Braunschweig (including type specimens).

Regardless of the source of the misidentifications, SCHERZ’s statement that these mistakes are the main source of erroneous changes to the taxonomy is misleading. Contrary to their claim, the evidence reported by PELOSO was not the sole argument for the taxonomic changes proposed. Fig. 1 shows the cophylina section of the optimal tree from PELOSO with updated and corrected sample IDs (as corrected in SCHERZ)—this reevaluation shows that even with updated sample identifications (assuming the identifications provided in SCHERZ are 100% correct), the taxonomy proposed in PELOSO is monophyletic, whereas the one suggested by SCHERZ is still not—i.e., after sample identification corrections *Cophyla* is still nested within *Platypelis* (rendering the latter paraphyletic), and *Stumpffia* is nested within *Rhombophryne* (rendering the latter paraphyletic).

Finally, we emphasize that there is abundant phylogenetic evidence supporting the taxonomy advocated in PELOSO—this comes not only from the dataset and analyses in PELOSO itself (Fig. 1), but also from previously published papers, many by the authors in SCHERZ (e.g., Perl et al., 2014; Pyron and Wiens, 2011; Rakotoarison et al., 2015; Scantlebury, 2013; Wollenberg et al., 2008). The sample identification errors are, therefore, insufficient to reject the taxonomy proposed in PELOSO.

2.2. To name or not to name?

SCHERZ argued that their taxonomy is formalized based on the *Taxon-Naming Criteria* (TNC) proposed by Vences et al. (2013). However, the criteria were applied inconsistently. When advocating for the TNCs, Vences et al. (2013) established a series of considerations and priorities to be addressed when suggesting name changes in a given group’s classification.

Vences et al. (2013) suggested that economy of change should be “a main priority for biological classification”. Vences et al. (2013) further considered that monophyly, clade stability, and phenotypic diagnoses should also receive high priority in taxonomic decisions. SCHERZ allegedly follow these priorities, but favor a taxonomy that (i) created a monotypic genus (*Anilany*) of unstable relationships (possibly rendering *Stumpffia* paraphyletic, even according to their own parsimony analyses), and (ii) will likely require further generic changes in the near future—at least two additional new genera are already necessary (one for *Stumpffia tri-*

² Their Bayesian topology does support the monophyly of *Cophyla*, *Platypelis*, *Rhombophryne*, and *Stumpffia*, albeit with very low posterior probability.

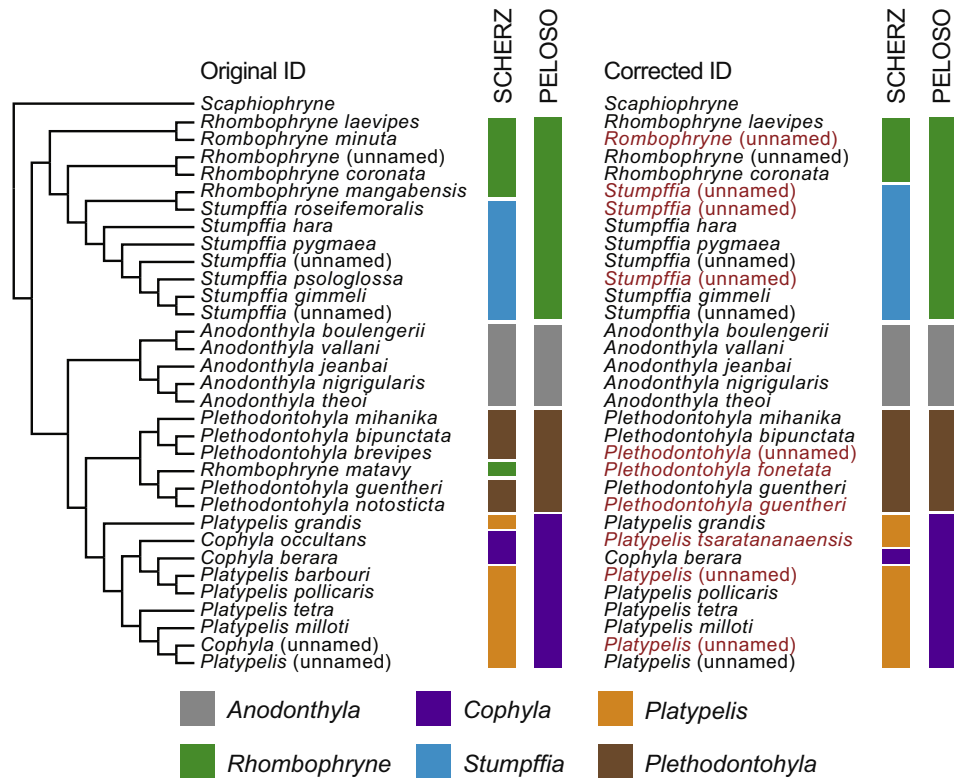


Fig. 1. Cophylinae section of the phylogenetic tree of PELOSO with the original identifications (left) and updated identification provided by SCHERZ (right: corrected identification labeled in red). Bars after taxon names refer to generic names advocated in PELOSO and SCHERZ. Note that even after sample re-identifications, the taxonomy proposed by SCHERZ would still be non-monophyletic according to this topology. Tree based on 73 loci and direct optimization parsimony. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

dactyla, and another to two unnamed species previously identified, respectively, as *Stumpffia* spCa15 and *Stumpffia* spCa16 on the basis of molecular data). Moreover, we argue that following the taxonomy suggested in SCHERZ actually does not entirely satisfy any of the priority TNCs proposed in Vences et al. (2013).

Monophyly—There is mounting evidence that *Stumpffia* and *Rhombophryne* are not monophyletic (Peloso et al., 2015; Perl et al., 2014; Scherz et al., 2016). PELOSO found that *Rhombophryne* is paraphyletic with respect to *Stumpffia*. SCHERZ, even after they named a new genus to rescue *Stumpffia* from paraphyly, and recovered *S. tridactyla* as not being part of that genus on their parsimony analysis. Our own analysis (see below) also failed to recover monophyly of *Stumpffia*—*S. tridactyla* is the sister taxon of *Anilany* (but with very low support: Fig. 2).

The monophyly of *Platypelis* is also not well established. Both PELOSO and Rakotoarison et al. (2015) provided evidence that *Platypelis* may be paraphyletic with respect to *Cophyla*. Our analysis supports the monophyly of both genera with acceptable support values (Fig. 3).

Stability—An evaluation of recent phylogenetic analyses of Cophylinae clearly shows that monophyly and relationships among some cophylinae genera are largely unstable with respect to dataset and method of analysis (Table 1). SCHERZ claim they are confident that their taxonomy will entail fewer future rearrangements (p. 380), but they did not provide a solution to the potential non-monophyly of *Stumpffia* due to the ambiguous position of *S. tridactyla*. Moreover, they did not address the problem that the clade formed by *Rhombophryne* spCa07 and *Stumpffia* Ca34 (both unnamed) is the sister clade of *Stumpffia* in their Bayesian analysis, but deeply nested in *Rhombophryne* in our parsimony analysis (also nested within *Rhombophryne* in their analysis, see Figs. 2 and 4). The taxonomy of PELOSO avoids both of these problems.

Diagnosable taxa—The consensus among authors working on Malagasy fauna is that the phenotypic diagnosis of most cophylinae genera is extremely difficult, if at all possible. Even SCHERZ admit to that: “our revised classification is not free of ambiguities in diagnosing genera by morphology alone” (p. 380).

Therefore, it is relatively easy to argue that SCHERZ are not strictly following the TNCs, but rejecting *ad hoc* most of the provisions set forth by the criteria. In fact, and rather ironically, SCHERZ agree that the taxonomy proposed in PELOSO would also fit the TNCs and, in many cases, would be an even better fit for them: “It is true, however, that the two clades [*Cophyla* + *Platypelis*] together form a monophyletic group (satisfying the Stability of Monophyly TNC) and might together become more easily diagnosable in external morphology (which would satisfy the Diagnosability TNC)” (SCHERZ: p. 377, taxon names in brackets were added herein).

3. Re-analysis of SCHERZ's data reveals several analytical issues

3.1. NMDS: Biased coding forces separation between *Stumpffia*, *Rhombophryne*, and *Anilany*

The coding of morphological characters used by SCHERZ in their NMDS is inadequate to provide a meaningful morphological diagnosis of these genera. The characters were, in their own words, “chosen ad-hoc to maximize diagnostic power to distinguish the two taxa”. We recoded some of the morphological characters used for the NMDS using a more objective coding method (Supplementary Data 1), and we also included *Anilany* in the matrix. The new analysis provided more robust results (e.g., smaller stress values despite a larger matrix) but also a less clear separation between *Rhombophryne* and *Stumpffia*. The two genera are still segregated

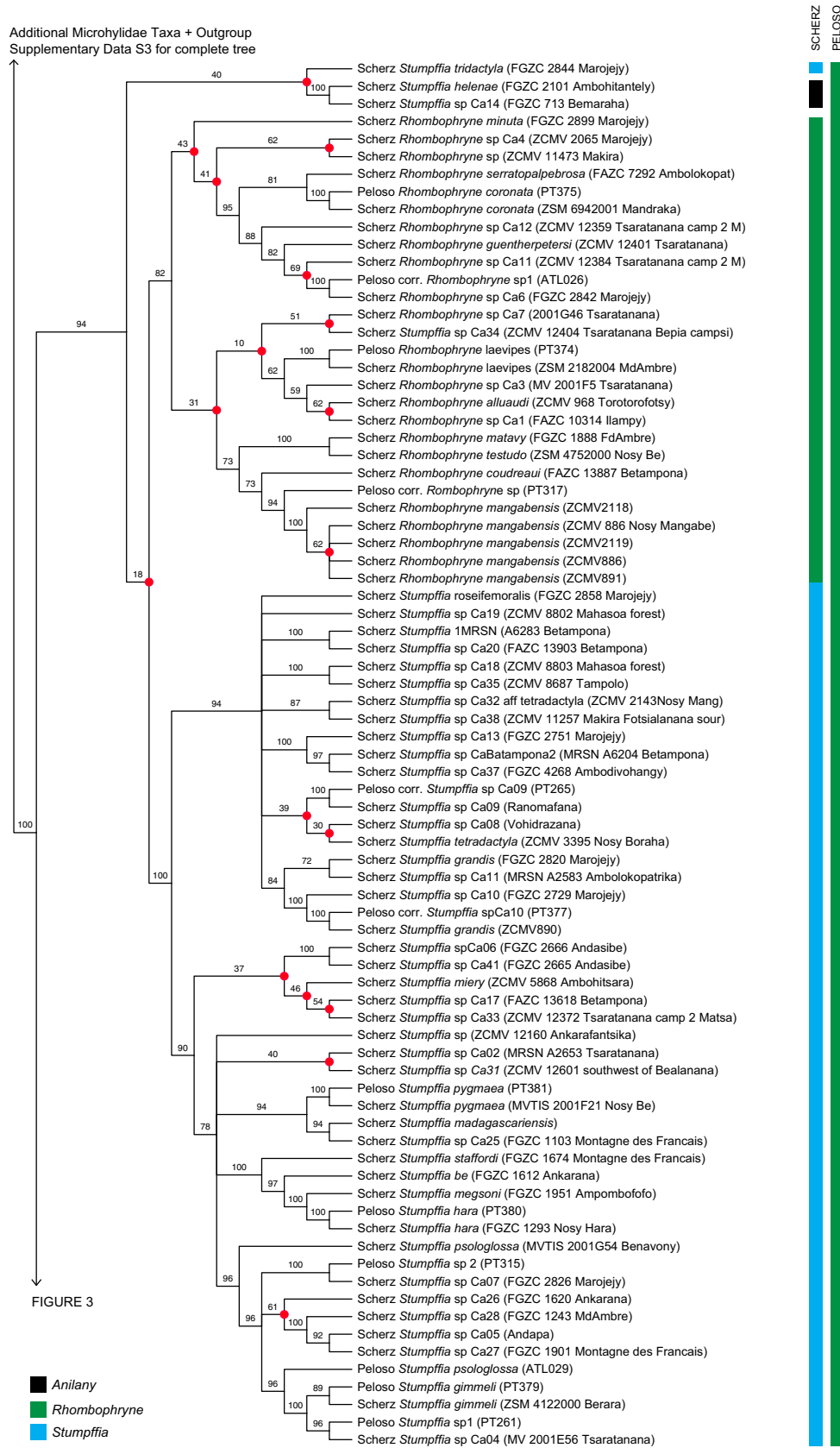


Fig. 2. Strict consensus (part) of five equally most parsimonious trees of the combined data of PELOSO and SCHERZ. Numbers on branches are Jackknife values (nodes with JK < 70 are marked with a red circle). The complete tree is available as [Supplementary Data S3](#). Corr. stands for samples from PELOSO reidentified according to SCHERZ. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1

Clade instability reflecting on the taxonomy of Cophylinae. TNT4 = Parsimony with gaps as missing data; TNT5 = Parsimony with gaps as a fifth state; POY = Direct Optimization parsimony; ML = Maximum Likelihood; UN = unresolved polytomy; NT = not tested, only a single terminal included; Asterisk (*) = Monophyletic only after unnamed species are disregarded. P&W = Pyron and Wiens (2011). RDSA = de Sá et al. (2012). PERL = Perl et al. (2014). Monophyly verified using the taxonomy proposed in SCHERZ.

Clade arrangement	P&W	DESA	PERL	PELOSO				SCHERZ		Present study
	ML	ML	ML	TNT4	TNT5	ML	POY	TNT ^a	ML	TNT5
<i>Cophyla</i> monophyletic	Yes	NT	Yes	UN	NT	NT	NT	Yes	Yes	Yes
<i>Platypelis</i> monophyletic	Yes	NT	No	UN	No	Yes	No	Yes	Yes	Yes
<i>Rhombophryne</i> monophyletic	Yes	Yes	No	No	UN	Yes	No	No	Yes*	Yes*
<i>Stumpffia</i> monophyletic	No*	Yes	No	Yes	Yes	Yes	No	No	Yes*	No
<i>Cophyla</i> + <i>Platypelis</i> monophyletic	No	NT	No	UN	Yes	Yes	Yes	Yes	Yes	Yes
<i>Rhombophryne</i> + <i>Stumpffia</i> monophyletic	No	Yes	No	Yes	Yes	Yes	Yes	No	No	No

^a It is unclear whether SCHERZ's parsimony analysis optimized gaps as missing data or as a fifth nucleotide state (the latter was assumed because it is TNT's default) or as missing data.

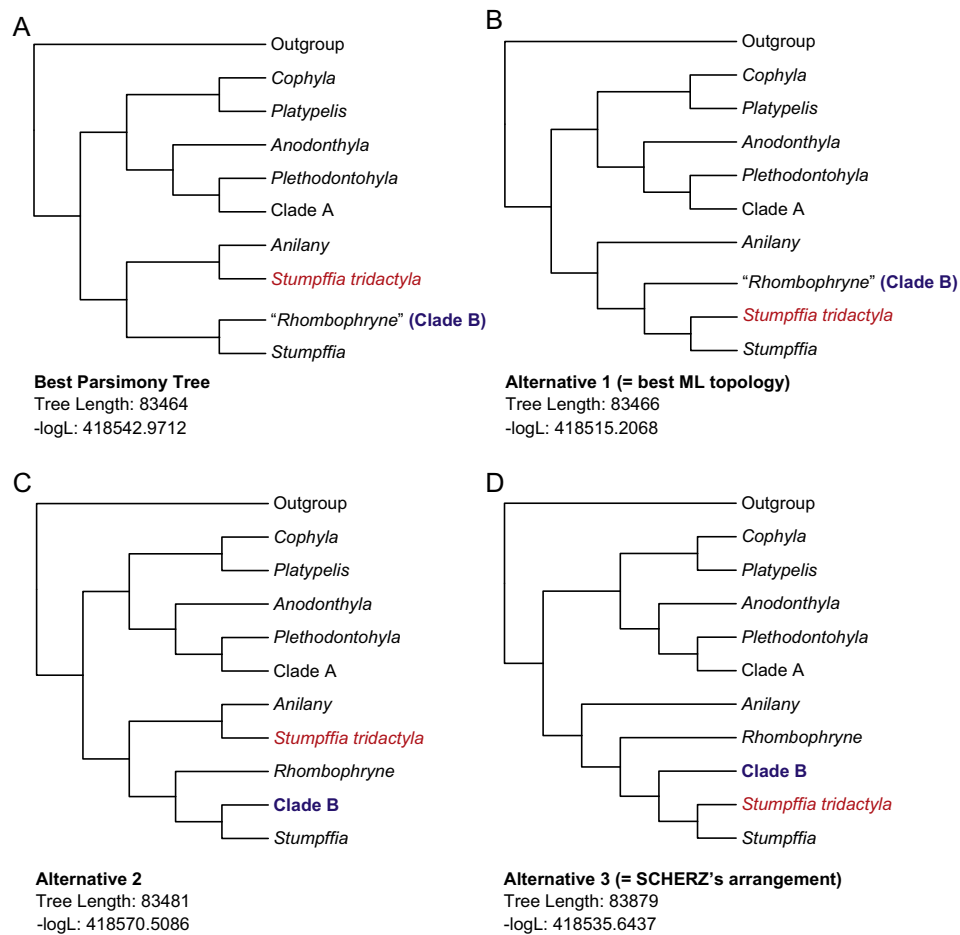


Fig. 4. Alternative placements of a few selected taxa with their respective tree scores for parsimony and Maximum Likelihood—Clade A = (*Stumpffia* spCa15 + *Stumpffia* spCa16). Clade B = (*Rhombophryne* spCa07 + *Stumpffia* spCa34). (A) Preferred arrangement, based on the results from the parsimony optimization—Clade B is nested in *Rhombophryne*. (B) Alternative arrangement 1: *Stumpffia tridactyla* constrained as the sister taxon of all other *Stumpffia*—Clade B is nested in *Rhombophryne*. (C) Alternative arrangement 2: Clade B is constrained as the sister taxon of all other *Stumpffia*. (D) Alternative arrangement 3: Both Clade B and *S. tridactyla* have their positions constrained—this is the preferred topology in SCHERZ.

by the analysis, although with significant overlap. The inclusion of *Anilany* in the NMDS provided no separation whatsoever between *Anilany* and *Stumpffia*.

The recoded matrix and results of the analyses are given as [Supplementary Data \(Supplementary Data S1\)](#).

3.2. A re-analysis of PELOSO's and SCHERZ's phylogenetic data

SCHERZ deleted the vast majority of the data available from PELOSO (75% of the taxa and 97% of the genetic data were deleted), including virtually all representatives from microhylid subfamilies

other than Malagasy taxa. SCHERZ included a single non-Malagasy taxon, *Kaloula pulchra* (Microhylinae), which was used to root the tree. This approach is problematic for two reasons: (1) it implies the monophyly of Malagasy microhylids, which is not well established; and (2) it downplays the role of taxon sampling in phylogenetic inference—the latter issue has been widely discussed in the literature (see Kirchnerberger et al., 2014; Wilberg, 2015 for recent examples), including in PELOSO.

To remedy the aforementioned issues, we re-analyzed the phylogenetic dataset of SCHERZ in combination with the totality of the data from PELOSO.

3.2.1. Phylogenetic analysis of the combined data from PELOSO and SCHERZ

Sequence data from both studies were obtained from Data-Dryad (SCHERZ, doi: 10.5061/dryad.1b2k5; PELOSO, doi: 10.5061/dryad.8112f). Overlapping loci (*16S* and *COI*) were combined into single alignments, whereas redundant taxa (i.e., those present in both datasets) were manually removed. All loci were individually realigned with the MAFFT version 7.222 (Katoh et al., 2002) plugin in Geneious version R9 (Kearse et al., 2012) using the default parameters. All loci were then concatenated into a single dataset in Sequence Matrix (Vaidya et al., 2011). The combined dataset contained 292 taxa and 36,458 characters (Supplementary Data S2).

For comparability with the analysis presented in SCHERZ, we used a static-alignment approach to phylogenetic inference of the combined dataset. Parsimony analysis was performed in TNT version 1.1 (Goloboff et al., 2008) with the *xmult* command, which implements a variety of search algorithms—Random Addition Sequences (RAS), Tree Bisection and Reconnection branch swapping (TBR), Tree Fusing (Goloboff, 1999), Sectorial Searches (Goloboff, 1999), and Tree Drifting (Goloboff, 1999). Search was allowed to run until the best solution was hit 1000 times (command *hits 1000*). Nodal support was assessed using Jackknife (Farris et al., 1996), estimated with 1000 replicates with five search iterations using *xmult* per replicate, and a removal rate of e^{-1} (=0.36) following the suggestion of Farris et al. (1996) that this is the value most congruent with bootstrapping. For both tree search and Jackknife analyses, gaps were treated as a fifth nucleotide state (default).

To test the influence in tree scores of differential placement of selected taxa, we manipulated the strict consensus topology by hand in Mesquite 3.0 (Maddison and Maddison, 2016). Trees were then read into PAUP 4.1 (Swofford, 2002) where we calculated the parsimony and maximum likelihood (ML) scores for the alternative topologies. ML scores were calculated assuming a single concatenated partition and a GTR+G model. Manipulations were restricted to the positions of *Stumpffia tridactyla* and the clade composed of ('*Rhombophryne*' spCa07 + '*Stumpffia*' spCa34).

4. Results and discussion

4.1. Phylogenetic analysis

The TNT analysis of the combined data from PELOSO and SCHERZ recovered 72 equally most parsimonious trees of 83,100 unordered, equally weighted, transformations. The strict consensus of these trees requires 83,464 transformations (see Figs. 2 and 3 for the Cophylinae section of the strict consensus trees; full tree given in Supplementary Data S3). The result supports the monophyly of Microhylidae and of most subfamilies with two important exceptions. Scaphiophryinae is polyphyletic: *Scaphiophryne* is the sister taxon of a monophyletic Cophylinae, whereas *Paradoxophyla* is the sister taxon of Kalophryinae. The phylogenetic position of *Paradoxophyla* is uncertain, with different analyses reporting different conclusions regarding whether it is the sister taxon of *Scaphiophryne*. On the basis of larval morphology, Haas (2003) did not recover a monophyletic Scaphiophryinae, whereas most analyses based on DNA sequence data support monophyly of the subfamily. *Hoplophryne* (Hoplophryinae) is nested within Gastrophryinae but with marginal support. We refrain from commenting on these issues at the moment and focus on cophylinae systematics.

Cophylinae is weakly supported as the sister taxon of Scaphiophryinae, but it is well supported as a monophyletic group. *Anodonthyla*, *Cophyla*, *Platypelis*, and *Plethodontohyla* are unambiguously corroborated as monophyletic groups. *Cophyla* and *Platypelis* are sister taxa. In PELOSO, *C. berara* was nested inside *Platypelis*, whereas here we found this species within a monophyletic *Cophyla* (in accordance to SCHERZ; see Supplementary Data S3).

The unambiguous monophyly of *Stumpffia* is rejected, as *S. tridactyla* is recovered as the sister taxon of SCHERZ's *Anilany* (*A. heleanae* + an unnamed species tentatively assigned to this genus by SCHERZ) (Fig. 4A). It is noteworthy that this relationship received very low support (JK: 40), which attests to the unstable relationship of this taxon to the nuances of different optimality criteria (see also Bayesian versus Parsimony inference in SCHERZ) for choosing among trees. To force the monophyly of *Stumpffia* (*S. tridactyla* as the sister taxon of all other *Stumpffia*) a single branch rearrangement is necessary, and the resulting tree requires 83,466 (two transformations longer than the strict consensus) (Fig. 4B).

Rhombophryne is monophyletic although an unnamed species previously recognized as *Stumpffia* by two separate phylogenetic analyses (ZCMV 12,404: *Stumpffia* spCa34 in Perl et al., 2014 and SCHERZ; *Stumpffia* sp39 in Klages et al., 2013) is nested inside the genus. These two species (*Rhombophryne Rhombophryne* spCa07 + *Stumpffia* spCa34) are collectively called Clade B (Fig. 4). SCHERZ's parsimony analysis did not recover Clade B. On the other hand, SCHERZ's Bayesian analysis recovered Clade B as the sister taxon of all *Stumpffia* (except for *S. tridactyla*, which in turn is the sister species of this clade + all remaining *Stumpffia*). To force SCHERZ's arrangement onto our favored tree, two rearrangements are needed, and the resulting tree is 83,879 transformations (415 steps longer than the optimal topology) (Fig. 4D).

Two additional samples previously identified as unnamed *Stumpffia* by the phylogenetic analysis of Perl et al. (2014) were not found to be members of the *Stumpffia* clade. *Stumpffia* spCa15 + *Stumpffia* spCa16, instead, form the sister clade of *Plethodontohyla* with modest support (JK: 56; Fig. 4). This was already reported in SCHERZ (Bayesian analysis) and we corroborate their findings—a new generic name might be needed whenever these two unnamed species are described.

Two additional samples previously identified as unnamed *Stumpffia* by the phylogenetic analysis of Perl et al. (2014) were not found to be members of the *Stumpffia* clade. *Stumpffia* spCa15 + *Stumpffia* spCa16, instead, form the sister clade of *Plethodontohyla* with modest support (JK: 56; Fig. 4). This was already reported in SCHERZ (Bayesian analysis) and we corroborate their findings—a new generic name might be needed whenever these two unnamed species are described.

4.2. Optimal taxonomic conclusions

Clearly, *Stumpffia* is not assuredly monophyletic with respect to both *Rhombophryne* and *Anilany*. Although there are conspicuous topological differences, this result is in accordance to the phylogenetic analysis of PELOSO, and with the parsimony analysis of SCHERZ. Furthermore, the phenotypic divergence between these three taxa is considerably more subtle than that reported in SCHERZ (Supplementary Data S1).

Cophyla and *Platypelis* are monophyletic, sister taxa; therefore, the taxonomic conundrum involving both genera persists. Considering our topology alone, the decision to maintain both genera as valid or as a single taxonomic unit (for which *Platypelis* has priority) seems subjective and a matter of opinion. However, given previous evidence that the relationships between these two genera are highly unstable (PELOSO, SCHERZ, but also Pyron and Wiens, 2011; Rakotoarison et al., 2015), and the fact that there are no known morphological synapomorphies that would support either genus as monophyletic (Glaw and Vences, 1994, 2007; Rakotoarison et al., 2015; but see SCHERZ), a single name should suffice to recognize the diversity in the group.

In summary, we find a compelling case remains for the generic recognition of the well-supported monophyletic *Platypelis* + *Cophyla* (which thus considers *Platypelis* as a junior synonym of *Cophyla*) and *Stumpffia* + *Rhombophryne* + *Anilany* (which thus considers *Anilany* and *Stumpffia* as junior synonyms of *Rhombophryne*). This taxonomy (discussed in Appendix A) also avoids the signifi-

cant practical problem of trying to diagnose probable paraphyletic genera from each other using dubious morphological characters.

Acknowledgements

We thank the editors and one of two anonymous reviewers for critically reviewing the manuscript. This work was supported by the National Science Foundation (NSF-DEB 1311442 to P.L.V.P and D.R.F.) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (grant numbers 400252/2014-7 and 313680/2014-0 to P.L.V.P).

Appendix A

The future of generic taxonomy of Cophylinae: few monophyletic genera, or several likely non-monophyletic ones?

A1. *Cophyla* + *Platypelis*

These two genera are almost indistinguishable on the basis of external morphology (Glaw and Vences, 1994, 2007; Rakotoarison et al., 2015; Vences et al., 2005) and there are very few osteological characters that would unambiguously support such a distinction. Scherz et al. (2016) mention only the condition of the vomer as a potentially diagnostic character. SCHERZ further suggests that the presence of clavicles in *Platypelis* (versus absence in *Cophyla*) is diagnostic, but clavicles are present at least in *C. puel-larum* (Rakotoarison et al., 2015). Furthermore, both the vomers and clavicles are known to vary widely in microhylids (Carvalho, 1954; Zweifel, 1972), and their conditions are not well surveyed across the diversity of cophylines (conditions unknown in many *Platypelis*). Therefore, their use as diagnostic characters should be employed with extreme caution, and be viewed with skepticism.

Even if we consider that phenotypic diagnosability is not a necessity for recognition of taxa (genotypic synapomorphies should suffice), monophyly definitely is. Collectively, *Cophyla* + *Platypelis* are easily recognizable as a diagnosable monophyletic group. Separately, the monophyly of both genera cannot be assumed with such certainty, as current evidence is ambiguous. SCHERZ state that their own paper “and a recent revision (Rakotoarison et al., 2015) have shown that species of *Cophyla* and *Platypelis* are monophyletic sister groups”. Our interpretation of evidence presented by Rakotoarison et al. (2015) is, however, different. Rakotoarison et al. (2015) performed two separate analyses on their dataset (with different amounts of evidence included in each of them) and did not recover a monophyletic *Platypelis* in one of them. They state that “because the first data set did not support the reciprocal monophyly of *Platypelis* vs. *Cophyla*, we assembled a more comprehensive data set of four nuclear and four mitochondrial genes for a reduced set of taxa” Rakotoarison et al. (2015: p 64). Here, we question their interpretation of what a “more comprehensive dataset” is. The first analysis included more taxa (and more terminals per taxon) than the second, whereas the second included more genes. Why the second is considered more comprehensive is unclear, as no logical justification is provided to select one proposal over the other. PELOSO also did not recover a monophyletic *Platypelis*—the sole species of *Cophyla* included in their analysis is nested within *Platypelis*. SCHERZ, with increased taxon sampling did find both genera to be monophyletic.

Our own analysis of the combined data from PELOSO and SCHERZ does support the monophyly of *Cophyla* and *Platypelis* (Fig. 2). Recognition of both genera as valid or as synonyms is, in this case, a matter of opinion—ours is that all species from this cluster should be considered members of a single genus: *Cophyla*.

A2. *Anilany* + *Rhombophryne* + *Stumpffia*

SCHERZ claim the taxonomic decision in PELOSO to synonymize *Rhombophryne* and *Stumpffia* was done without proper evidence, and thus resurrect *Stumpffia* from the synonymy with *Rhombophryne*. However, in addition to phylogenetic evidence for the non-monophyly of *Rhombophryne* with respect to *Stumpffia* provided in PELOSO (Fig. 1), there is additional published evidence to suggest that *Rhombophryne* and *Stumpffia* are not monophyletic (Perl et al., 2014; Pyron and Wiens, 2011; Scantlebury, 2013; Wollenberg et al., 2008). To be fair, Wollenberg et al. (2008) had previously suggested the recognition of a distinct genus for *Stumpffia helenae* and the unnamed taxon from Bemahara (*Stumpffia* sp. “8” in Wollenberg et al., 2008)—the new genus was, however, not officially named until SCHERZ, almost a decade later (named *Anilany* therein). Furthermore, the parsimony analysis in SCHERZ recovered a paraphyletic *Stumpffia* with, curiously, their newly erected genus *Anilany* nested within it.

To support their claim, SCHERZ highlight the differences among the type species of *Rhombophryne* and *Stumpffia* as justification for the supposedly easy morphological diagnosability of both genera. There is no argument here that the type species of each genus (respectively *R. testudo* and *S. psologlossa*) are in fact very different from each other. Our concern is when you start to add the variation within the group. For some members of *Stumpffia* and *Rhombophryne*, it is impossible to place them in one genus or the other based on morphology alone (therefore the large number of misidentified specimens in museum collections and of sequences in GenBank). “Barcode” identification performed by SCHERZ’s co-authors have previously identified several unnamed species of *Stumpffia*, some of which were recovered deeply nested within *Rhombophryne* by SCHERZ and in our analysis (Fig. 2).

Our reanalysis of the combined PELOSO and SCHERZ data does not unambiguously support the monophyly of *Stumpffia*. We therefore support the claim in PELOSO that *Stumpffia* should be considered a junior synonym of *Rhombophryne*. Furthermore, given our phylogenetic results, we also consider that *Anilany* should be considered a synonym of *Rhombophryne*.

This taxonomy solves the long-standing problem of *Stumpffia* paraphyly (Perl et al., 2014; PELOSO, SCHERZ; Pyron and Wiens, 2011; Scantlebury, 2013; Wollenberg et al., 2008) and would obviously be more useful and avoid practical problems of generic assignment based on phenotype (see also Supplementary Data S1). The alternative to this taxonomy would be to name another monotypic genus to accommodate *Stumpffia tridactyla*—this is the preferred action by SCHERZ.

Appendix B. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.ympev.2017.03.016>.

References

- Andreone, F., Vences, M., Vieites, D.R., Glaw, F., Meyer, A., 2005. Recurrent ecological adaptations revealed through a molecular analysis of the secretive cophylinae frogs of Madagascar. *Mol. Phylogenet. Evol.* 34, 315–322.
- Blommers-Schlösser, R.M.A., Blanc, M., 1993. Amphibiens (deuxième partie). *Faune de Madagascar* 75, 385–530.
- Carvalho, A.L., 1954. A preliminary synopsis of the genera of American microhylid frogs. *Occas. Pap. Mus. Zool. Univ. Mich.* 555, 1–19.
- de Sá, R.O., Streicher, J.W., Sekonyela, R., Forlani, M.C., Loader, S.P., Greenbaum, E., Richards, S., Haddad, C.F.B., 2012. Molecular phylogeny of microhylid frogs (Anura: Microhylidae) with emphasis on relationships among New World genera. *BMC Evol. Biol.* 12, 1–21.
- Farris, J.S., Albert, V.A., Källersjö, M., Lipscomb, D., Kluge, A.G., 1996. Parsimony jackknifing outperforms neighbor-joining. *Cladistics* 12, 99–124.

- Felsenstein, J., 1988. Phylogenies from molecular sequences: inference and reliability. *Annu. Rev. Genet.*, 22.
- Glaw, F., Vences, M., 1994. A Field Guide to the Amphibians and Reptiles of Madagascar. Vences & Glaw Verlags GbR, Köln, Germany.
- Glaw, F., Vences, M., 2007. A Field Guide to the Amphibians and Reptiles of Madagascar. Vences & Glaw Verlags GbR, Köln, Germany.
- Goloboff, P.A., 1999. Analysing large datasets in reasonable times: solutions for composite optima. *Cladistics* 15, 415–428.
- Goloboff, P.A., Farris, J.S., Nixon, K.C., 2008. TNT, a free program for phylogenetic analysis. *Cladistics* 24, 774–786.
- Haas, A., 2003. Phylogeny of frogs as inferred from primarily larval characters (Amphibia: Anura). *Cladistics* 19, 23–89.
- Katoh, K., Misawa, K., Kuma, K., Miyata, T., 2002. MAFFT: a novel method for rapid multiple sequence alignment based on Fast Fourier transform. *Nucleic Acids Res.* 30, 3059–3066.
- Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Mentjies, P., Drummond, A., 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* 28, 1647–1649.
- Kirchberger, P.C., Sefc, K.M., Sturmbauer, C., Koblmüller, S., 2014. Outgroup effects on root position and tree topology in the AFLP phylogeny of a rapidly radiating lineage of cichlid fish. *Mol. Phylogenet. Evol.* 70, 57–62.
- Klages, J., Glaw, F., Köhler, J., Müller, J., Hipsley, C.A., Vences, M., 2013. Molecular, morphological and osteological differentiation of a new species of microhylid frog of the genus *Stumpffia* from northwestern Madagascar. *Zootaxa* 3117, 280–300.
- Köhler, J., Vences, M., D'Cruze, N., Glaw, F., 2010. Giant dwarfs: discovery of a radiation of large-bodied 'stump-toed frogs' from karstic cave environments of northern Madagascar. *J. Zool.* 282, 21–38.
- Maddison, W.P., Maddison, D.R., 2016. Mesquite: A Modular System for Evolutionary Analysis. Version 3.11. Available at: <<http://mesquiteproject.org>>.
- Peloso, P.L.V., Frost, D.R., Richards, S.J., Rodrigues, M.T., Donnellan, S., Matsui, M., Raxworthy, C.J., Biju, S.D., Lemmon, E.M., Lemmon, A., Wheeler, W.C., 2015. The impact of Anchored Phylogenomics and taxon sampling on phylogenetic inference in narrowmouthed frogs (Anura, Microhylidae). *Cladistics* 32, 113–140.
- Perl, R.G.B., Nagy, Z.T., Sonet, G., Glaw, F., Wollenberg, K.C., Vences, M., 2014. DNA barcoding Madagascar's amphibian fauna. *Amphibia-Reptilia* 35, 197–206.
- Pyron, R.A., Wiens, J.J., 2011. A large-scale phylogeny of Amphibia including over 2800 species, and a revised classification of extant frogs, salamanders, and caecilians. *Mol. Phylogenet. Evol.* 61, 543–583.
- Rakotoarison, A., Crottini, A., Müller, J., Rödel, M.O., Glaw, F., Vences, M., 2015. Revision and phylogeny of narrow-mouthed treefrogs (*Cophyla*) from northern Madagascar: integration of molecular, osteological, and bioacoustic data reveals three new species. *Zootaxa* 3937, 61–89.
- Sankoff, D., 1975. Minimal mutation trees of sequences. *SIAM J. App. Math.* 28, 35–42.
- Sankoff, D., Cedergren, R.J., 1983. Simultaneous comparison of three or more sequences related by a tree. In: Sankoff, D., Kruskal, J.B. (Eds.), *Time Warps, String Edits, and Macromolecules: The Theory and Practise of Sequence Comparison*. Addison-Wesley, Reading, MA, pp. 253–264.
- Scantlebury, D.P., 2013. Diversification rates have declined in the Malagasy herpetofauna. *Proc. R. Soc. B* 280. <http://dx.doi.org/10.1098/rspb.2013.1109>.
- Scherz, M.D., Vences, M., Rakotoarison, A., Andreone, F., Köhler, J., Glaw, F., Crottini, A., 2016. Reconciling molecular phylogeny, morphological 1 divergence and classification of Madagascan narrow-mouthed frogs (Amphibia: Microhylidae). *Mol. Phylogenet. Evol.* 100, 372–381.
- Swofford, D.L., 2002. *Phylogenetic Analysis Using Parsimony (* and Other Methods)*. Version 4. Sinauer Associates, Sunderland, MA.
- Vaidya, G., Lohman, D.J., Meier, R., 2011. SequenceMatrix: concatenation software for the fast assembly of multi-gene datasets with character set and codon information. *Cladistics* 27, 171–180.
- van der Meijden, A., Vences, M., Hoegg, S., Boistel, R., Channing, A., Meyer, A., 2007. Nuclear gene phylogeny of narrow-mouthed toads (Family: Microhylidae) and a discussion of competing hypotheses concerning their biogeographical origins. *Mol. Phylogenet. Evol.* 44, 1017–1030.
- Vences, M., Andreone, F., Glaw, F., 2005. A new microhylid frog of the genus *Cophyla* from a transitional forest in northwestern Madagascar. *Afr. Zool.* 40, 143–149.
- Vences, M., Guayasamin, J.M., Miralles, A., De la Riva, I., 2013. To name or not to name: criteria to promote economy of change in Linnaean classification schemes. *Zootaxa* 3636, 201–244.
- Wheeler, W.C., 1995. Sequence alignment, parameter sensitivity, and the phylogenetic analysis of molecular data. *Syst. Biol.* 44, 321–331.
- Wheeler, W.C., 1996. Optimization alignment: the end of multiple sequence alignment in phylogenetics? *Cladistics* 12, 1–9.
- Wilberg, E.W., 2015. What's in an Outgroup? The impact of outgroup choice on the phylogenetic position of *Thalattosuchia* (Crocodylomorpha) and the origin of Crocodyliformes. *Syst. Biol.* 64, 621–637.
- Wollenberg, K.C., Vieites, D.R., van der Meijden, A., Glaw, F., Cannatella, D.C., Vences, M., 2008. Patterns of endemism and species richness in Malagasy cophyline frogs support a key role of mountainous areas for speciation. *Evolution* 62, 1890–1907.
- Zweifel, R.G., 1972. Results of the Archbold Expeditions No. 97. A revision of the frogs of the subfamily Asterophryinae family Microhylidae. *Bull. Am. Mus. Nat. Hist.* 148, 413–546.