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The Green Clade grows: A phylogenetic analysis of *Aplastodiscus* (Anura; Hylidae) ☆

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ABSTRACT

Green tree frogs of the genus *Aplastodiscus* occur in the Atlantic Forest and Cerrado biomes of South America. The genus comprises 15 medium-sized species placed in three species groups diagnosed mainly by cloacal morphology. A phylogenetic analysis was conducted to: (1) test the monophyly of these species groups; (2) explore the phylogenetic relationships among putative species; and (3) investigate species boundaries. The dataset included eight mitochondrial and nuclear gene fragments for up to 6642 bp per specimen. The results strongly support the monophyly of *Aplastodiscus* and of the *A. albofrenatus* and *A. perviridis* groups. *Aplastodiscus sibilatus* is the sister taxon of all other species of *Aplastodiscus*, making the *A. albosignatus* Group non-monophyletic as currently defined. At least six unnamed species are recognized for *Aplastodiscus*, increasing the diversity of the genus by 40%. A fourth species group, the *A. sibilatus* Group is recognized. *Aplastodiscus musicus* is transferred from the *A. albofrenatus* Group to the *A. albosignatus* Group, and *A. callipygius* is considered a junior synonym of *A. albosignatus*. Characters related to external cloacal morphology reveal an interesting evolutionary pattern of parallelisms and reversions, suggesting an undocumented level of complexity. We analyze, in light of our phylogenetic results, the evolution of reproductive biology and chromosome morphology in *Aplastodiscus*.

1. Introduction

The hylid tribe Cophomantini includes *Aplastodiscus* Lutz, 1950; *Bokermannohyla* Faivovich, Haddad, Garcia, Frost, Campbell, and Wheeler, 2005; *Hyloscirtus* Peters, 1882; *Hypsiboas* Wagler, 1830; and *Myersiohyla* Faivovich, Haddad, Garcia, Frost, Campbell, and Wheeler, 2005. The largest genus is *Hypsiboas* with 90 species, followed by *Bokermannohyla* and *Hyloscirtus* with 33 species each, *Aplastodiscus* with 15 species, and *Myersiohyla* with six species forming the sister group to the remainder of the tribe (Faivovich et al., 2005, 2013; Frost, 2015).

Aplastodiscus is distributed mainly in the Atlantic Forest of northeastern, southeastern, and southern Brazil, and adjacent Argentina, with one species reaching gallery forests in the Cerrado biome in central-eastern Brazil (Frost, 2015). All but one species are green, with usually colorful eyes with hues of copper, orange, pink, red, violet, and white (Cruz and Peixoto, 1985, 1987; Garcia et al., 2001; Orrico et al., 2006). *Aplastodiscus* formerly was included in the genus *Hyla* Laurenti, 1768; because of the coloration of the frogs, they have traditionally been called "the green species" of *Hyla* of the Brazilian Atlantic Forest.

Aplastodiscus was erected as a monotypic genus by A. Lutz (in Lutz, 1950) to include *A. perviridis*. The genus was considered valid or as a synonym of *Hyla* by different authors (Garcia et al., 2001; Faivovich et al., 2005, for a review). Several authors (Lutz, 1950; Haddad and Sawaya, 2000; Garcia et al., 2001; Hartmann et al., 2004; Haddad et al., 2005) presented grouping evidence for *Aplastodiscus* and some green species formerly included in *Hyla* (*i.e.*, *H. albosignata* and *H. albofrenata* complexes of the *H. albomarginata* Group of Cochran, 1955); this relationship was corroborated by Faivovich et al. (2005) in a comprehensive phylogenetic analysis.

In their review of hylid systematics, Faivovich et al. (2005) included 10 species of the complexes previously included in the former *H. albomarginata* Group in their taxon sampling—*viz.*, the *H. albofrenata* Complex (3 of 6 species included at that time), the *H. albosignata* Complex (4 of the 7 species included at that time),

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and the *H. albomarginata* Complex (3 of the 4 species included at that time). This was the first assessment of the relationships of *Aplastodiscus* and the former *Hyla albofrenata* and *H. albosignata* complexes. Their results showed that the former *Hyla albomarginata* Group was polyphyletic and that the exemplars of the *H. albofrenata* and *H. albosignata* complexes were individually monophyletic, and that *Aplastodiscus* was the sister taxon of the *H. albosignata* Complex.

Faivovich et al. (2005) redefined *Aplastodiscus* to include the former *Hyla albofrenata* and *H. albosignata* complexes, thereby increasing the number of species in the genus from two to fifteen. Furthermore, on the basis of the monophyly of their exemplar species, they recognized each complex as a species group (*A. albofrenatus* and *A. albosignatus* groups), as well as a third group (the *A. perviridis* Group) for the two original species included in *Aplastodiscus*. Based on published information, Faivovich et al. (2005) suggested that the reproductive mode, advertisement call, development of metarcarpal and metatarsal tubercles, and white parietal peritoneum are putative synapomorphies for *Aplastodiscus*. Putative phenotypic synapomorphies for the *A. perviridis* and *A. albosignatus* species groups also were discussed.

Wiens et al. (2005) presented a phylogenetic analysis of Hylidae with a smaller taxon sampling than Faivovich et al. (2005), including only *A. leucopygius* and *A. arildae* (as *H. leucopygia* and *H. albofrenata*, respectively). Their results recovered a large clade composed of the current genera *Aplastodiscus*, *Hypsiboas*, and *Bokermannohyla*, to which they applied collectively the generic name *Boana* Gray, 1825. Aside from *Boana* being a *nomem nudum* (Faivovich et al., 2005), for reasons of priority, these combinations never gained acceptance. Subsequent reanalyses of the DNA sequences produced by Faivovich et al. (2005) yielded a topology for *Aplastodiscus* congruent with that obtained by these authors (Wiens et al., 2005, 2006, 2010: supp. data; Pyron and Wiens, 2011; Pyron, 2014: supp. data).

Currently, three species groups are recognized in *Aplastodiscus*. The *A. perviridis* Group includes two species, *A. perviridis* and *A. cochranae* (Mertens, 1952) that share the lack of webbing between Toes I and II, reduction of webbing among the remaining toes, and a bicolored iris (Garcia et al., 2001). The *A. albofrenatus* Group includes six species: *A. albofrenatus* (Lutz, 1924), *A. arildae* (Cruz and Peixoto, 1987), *A. ehrhardti* (Müller, 1924), *A. eugenioi* (Carvalho-e-Silva and Carvalho-e-Silva, 2005), *A. musicus* (Lutz, 1949), and *A. weygoldti* (Cruz and Peixoto, 1987). The monophyly of this group is supported only by molecular data (Faivovich et al., 2005). The *A. albosignatus* Group includes seven species: *A. albosignatus* (Lutz and Lutz, 1938), *A. cavicola* (Cruz and Peixoto, 1985), *A. callipygius* (Cruz and Peixoto, 1985), *A. flumineus* (Cruz and Peixoto, 1985), *A. ibirapitanga* (Cruz, Pimenta, and Silvano, 2003), *A. leucopygius* (Cruz and Peixoto, 1985), and *A. sibilatus* (Cruz, Pimenta, and Silvano, 2003). Species of this group share elaborated tubercles in the cloacal region (Cruz and Peixoto, 1985; Cruz et al., 2003; Faivovich et al., 2005).

Although the monophyly of the three groups has been tested and corroborated with several species, there are six species that have been unavailable for phylogenetic analyses, and the relationships of these with the groups have been inferred on the basis of putative phenotypic synapomorphies: *Aplastodiscus albofrenatus*, *A. ehrhardti*, *A. flumineus*, *A. musicus*, *A. ibirapitanga*, and *A. sibilatus*.

The taxonomy of *Aplastodiscus* was last reviewed by Cruz and Peixoto (1985, 1987), who described four species related to the former *Hyla albosignata* and three related to the former *Hyla albofrenata* (one of them was considered a junior synonym of the former *H. ehrhardti*; see Faivovich et al., 2002.) Cruz et al. (2003) and Carvalho-e-Silva and Carvalho-e-Silva (2005) described three more species. Thus, the last taxonomic review of the genus was nearly 30 years ago, and several new populations have been discovered since then.

Our goal in this paper is to present a phylogenetic analysis of *Aplastodiscus* to test the monophyly of each species group, as well as to discuss the phylogenetic relationships among all included terminal taxa. On the basis of our results, we discuss the current taxonomy of the genus, putative synapomorphies, and the evolution of cloacal ornamentation, reproductive biology, and chromosome morphology.

2. Materials and methods

2.1. Taxon sampling

We included 48 samples of 14 of the 15 currently recognized species of *Aplastodiscus*. If available, up to five specimens for each species from different localities were included, as well as topotypes for 10 of the species (complete list in Appendix A). *Aplastodiscus musicus* is the only species for which samples were unavailable. This species is known only from its type locality, where it was last collected in 1995 by A. Carvalho-e-Silva, S. Carvalho-e-Silva, and M.T. Rodrigues; despite multiple visits to the type locality, it has never been found again (Berneck, pers. obs.).

Outgroup terminals were selected on the basis of the current phylogenetic hypothesis of Cophomantini (Faivovich et al., 2005) corroborated by several reanalyses (Wiens et al., 2006, 2010: supp. data; Pyron and Wiens, 2011; Faivovich et al., 2013). Thus, we included one member of each species group of *Hypsiboas* (the sister taxon of *Aplastodiscus*), *Bokermannohyla*, *Hyloscirtus*, and *Myersiohyla*; the latter was used to root the trees.

2.2. Character sampling and laboratory protocols

Our analyses included three fragments of mitochondrial genes: the almost complete sequence of ribosomal RNA gene *12S*, the intervening *tRNA-Val*, and partial sequence of *16S*, a fragment including the downstream region of the *rRNA 16S*, the intervening *tRNA-Leu*, the complete sequence of the *NADH dehydrogenase subunit 1 (ND1)*, *a partial sequence of tRNA-Ile*, and a fragment of the gene *Cytochrome c oxidase I* (*COI*).The nuclear gene sequences analyzed include portions of *rhodopsin exon 1* (*RHOD*), *tyrosinase* (*TYR*), *Recombinase-Activation 1* (*RAG-1*), *Tensine 3* (*TNS3*), and *Seventh in Absentia homolog I* (*SIAH*). DNA extraction, amplification, and sequencing methods are those described by Blotto et al. (2013). The *COI* primers are those of Jungfer et al. (2013), the *TNS3* primers are of Smith et al. (2007), and the remaining primers used are those of Faivovich et al. (2013). See Appendix A for voucher data and the GenBank access numbers of sequences employed in this study. We included some sequences produced by Faivovich et al. (2005). For all samples we calculated uncorrected pairwise distance for the *16S* fragment delimited by the primers *16sAR* (Palumbi et al., 1991) and *Wilk2* (Wilkinson et al., 1996) and *COI* using PAUP (Swofford, 2002).

2.3. Phylogenetic analysis

The phylogenetic analysis under direct optimization was performed with POY5.1.1 (Wheeler et al., 2014), using a 1:1:1 weighting scheme (substitutions and insertion/deletion events). Sequences of *12S*, *16S*, *tRNAVal*, and *tRNALeu* were initially delimited in sections of putative homology (Wheeler et al., 2006), and equal-length sequences of protein-coding genes were used as static alignments to accelerate the searches.

Searches were performed using the command "Search." This command implements a driven search, building Wagner trees with random addition sequences (RAS), Tree Bisection and Reconnection

(TBR) branch swapping followed by Ratchet (Nixon, 1999), and Tree Fusing (Goloboff, 1999). The command (Search) stores the shortest trees of each independent run and computes the final fused tree using the pooled trees as a source of topological diversity. The resulting topologies were submitted to a final round of TBR using iterative pass optimization (Wheeler, 2003).

Phylogenetic analyses using POY were executed in parallel using the Museu de Zoologia da Universidade de São Paulo's high-performance computing cluster Ace, which consists of 12 quad-socket AMD Opteron 6376 16-core 2.3-GHz CPU, 16 MB cache, 6.4 GT/s compute nodes (=768 cores total), eight with 128 GB RAM DDR3 1600 MHz (16 × 8 GB), two with 256 GB (16 × 16 GB), and two with 512 GB (32 × 16 GB), and QDR 4X InfiniBand (32 GB/s) networking.

We performed a multiple alignment with MAFFT Version 7 (Katoh and Standley, 2013). For the regions of *12S*, *tRNA-Val*, and the *16S*, *tRNA-Leu*, *ND1* and *tRNA-Ile*, we employed the alignments generated with Q-INS-i strategy (secondary structure of RNA is considered), whereas the alignments for the remaining genes were generated with G-INS-i (global homology considered). For the phylogenetic analysis using parsimony, we used T.N.T Willi Hennig Society Edition (Goloboff et al., 2008). Searches were conducted using the new technology search under Search Level 50, which included sectorial searches, tree drift, and tree fusing (Goloboff, 1999), and requesting the driven search to hit the best length 100 times. Parsimony Jackknife absolute frequencies (Farris et al., 1996) were estimated using new technology, requesting 10 hits with driven searches, each of the 1000 replicates. Trees were edited with FigTree (Rambaut, 2014).

For the Bayesian analysis, models for each partition were chosen with jModelTest v0.1.1 (Posada, 2008). First, second, and third codon positions were treated as separate partitions for each protein-coding gene. The regions of *12S*, *tRNA-Val*, *16S*, *tRNA-Leu*, and *tRNA-Ile* were treated as a single partition for model selection. The Akaike Information Criterion (AIC) was used to select the best fitting model for each gene (Pol, 2004; Posada and Buckley, 2004). Bayesian analyses were performed in MrBayes 3.2 (Ronquist et al., 2012) in the CIPRES web cluster (Miller et al., 2010). Analyses consisted of four runs, each consisting of two replicate Monte-Carlo Markov Chains. Each run used four chains and default settings of priors (Dirichlet for substitution rates and state frequencies, uniform for the gamma shape parameter and proportion of invariable sites, all topologies equally likely *a priori*, and branch lengths unconstrained: exponential). Two analyses running 60 million generations were performed (with a burn-in fraction of 0.20). Stabilization of resulting parameters was evaluated using Tracer (Rambaut et al., 2014).

3. Results

Direct Optimization in POY produced four most parsimonious trees (MPTs) with 11,054 steps (Fig. 1). The static parsimony analysis in T.N.T also yielded in four MPTs with 11,656 steps; Direct Optimization in POY, static parsimony in T.N.T., and Bayesian analysis resulted in similar topologies (Appendix B). The main differences among alternate topologies for each optimality criterion are the internal relationships among terminals of *A. eugenioi* (a polytomy in Direct Optimization but not in static parsimony), and *A. leucopygius* (a polytomy in static parsimony but not in Direct Optimization). For the three analyses, see Fig. 1 and Appendix B.

The monophyly of *Aplastodiscus* is supported with 100% Parsimony Jackknife Frequency (hereafter PJF) (Fig. 1). Six unnamed species were recognized for *Aplastodiscus* based on topology and genetic *p*-distances (Fig. 1). See Table 1 for species and clades distances and Appendix C for pairwise comparisons among all samples; the discussion contains further details and evidence for each unnamed species. A clade formed by *A. sibilatus* and *Aplastodiscus* sp. 1 (aff. *sibilatus*) is the sister taxon of all other species of *Aplastodiscus*, making the *A. albosignatus* Group non-monophyletic as currently recognized. The remaining 13 described species are recovered as a well-supported clade with 95% PJF.

All species of the *A. albofrenatus* Group are monophyletic and recovered with 100% PJF. This clade is the sister taxon of a clade composed of the monophyletic *A. perviridis* Group plus a well-supported clade that includes the remaining six species of the *A. albosignatus* Group, both with 100% PJF support. *Aplastodiscus ehrhardti* is the sister taxon of all other species of the *A. albofrenatus* Group. These are grouped in two clades; one includes *A. arildae* with 100% PJF, and the other has 77% PJF support, and includes *A. weygoldti*, *Aplastodiscus* sp. 2, and *A. albofrenatus* + *A. eugenioi*. The *A. perviridis* Group is monophyletic, with *A. perviridis* being the sister taxon of *A. cochranae* + *Aplastodiscus* sp. 3.

Most species of the *A. albosignatus* Group are monophyletic, with 100% PJF, and with internal relationships having support values of 52–100%. *Aplastodiscus ibirapitanga* and *Aplastodiscus* sp. 4 form the sister taxon of the remaining species in the clade. These are further grouped in two clades. One includes *A. cavicola*, *A. leucopygius*, and *Aplastodiscus* sp. 6. The other clade includes *Aplastodiscus* sp. 5, *A. flumineus*, and a clade including topotypes of *A. albosignatus* and exemplars of *A. callipygius* (including topotypes).

4. Discussion

4.1. Outgroups

As in previous phylogenetic studies, *Hyloscirtus* is the sister taxon of a clade comprising *Hypsiboas*, *Aplastodiscus*, and *Bokermannohyla*, and these genera are monophyletic (Faivovich et al., 2005, 2013; Pyron and Wiens, 2011). The placement of *Hypsiboas* as the sister group of *Aplastodiscus* is corroborated by our analysis. The internal relationships in *Hypsiboas* differ from those recently recovered by Faivovich et al. (2013). However, our study has a much reduced taxon sampling for the study of the relationships within *Hypsiboas*, and thus, does not constitute a valid test of previous phylogenetic hypotheses within this genus.

4.2. Aplastodiscus

Our analysis corroborates the monophyly of the *A. albofrenatus* and *A. perviridis* groups. However, the position of *A. sibilatus* as the sister taxon of all other species of *Aplastodiscus* makes the *A. albosignatus* Group not monophyletic as currently recognized. The relationships recovered among species groups of *Aplastodiscus* (in which the *A. albofrenatus* Group is sister taxon of the *A. albosignatus* + *A. perviridis* groups) corroborate previous results (Faivovich et al., 2005, 2013; Pyron and Wiens, 2011).

Our results indicate the possible existence of at least six unnamed species of *Aplastodiscus*, which represents an increase of 40% of the known diversity of this clade. This inference stems from the analysis of our topological results, vouchers, and current taxonomic knowledge of the group. All these putative new species have been confused in collections, something usual with the admittedly complex taxonomy of *Aplastodiscus*.

4.3. The position of A. sibilatus

In its original description, *Aplastodiscus sibilatus* was associated with *A. albosignatus*, *A. callipygius*, and *A. flumineus* on the basis of the absence of a cloacal flap (Cruz et al., 2003). In the context of our results, this character state is plesiomorphic for *Aplastodiscus*

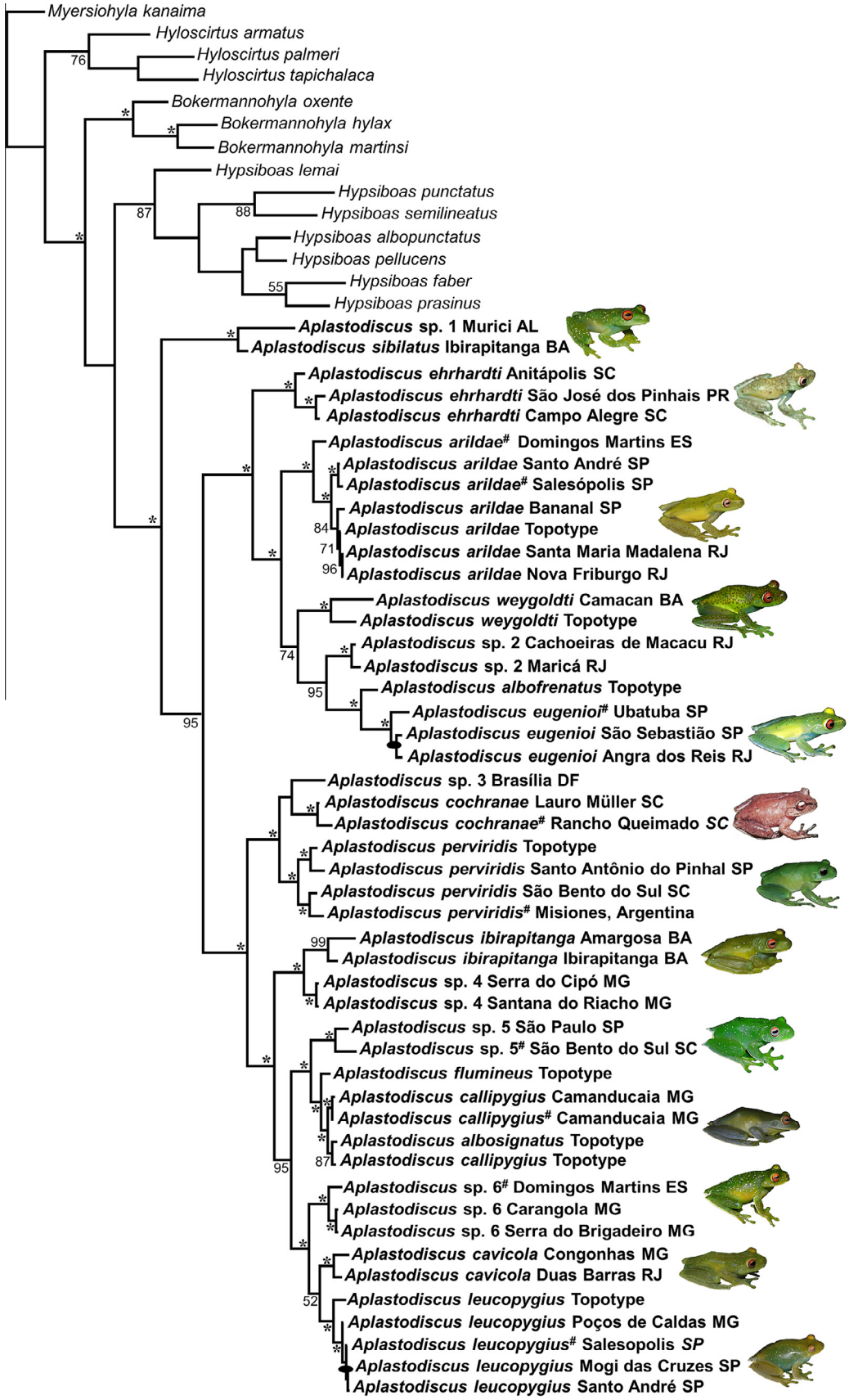

Fig. 1. One of the four most parsimonious trees recovered by direct optimization. Black dots indicate nodes that collapse in strict consensus; an asterisk at nodes indicates 100% Parsimony Jackknife absolute frequency. Samples followed by a # were employed by Faivovich et al. (2005), in some cases using different names, see Appendix A for details and Appendix B for results of the T.N.T. and Bayesian analyses.

Table 1
Ranges of uncorrected pairwise sequence distances (*p*-distances) of the*16s* fragment (below the diagonal) delimited by primers *Ar* and *Wilk2* and *Cytochrome c oxidase subunit 1* (above the diagonal). For individual values of all samples see Appendix C. Values are percentages.

Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1. *A. albofrenatus*	–	19.3	12.1–12.4	19.7	19.9	20.9–21.2	16.4–16.9	7.5–7.8	20.2	19.9	19–19.5	19.9–20.9	22.1	14.1–14.4	22.1	11.9	20.1	19.8–21.8	20.2	18.9
2. *A. albosignatus*	10.45	–	19.0–19.6	3.2	11.8	14.6–14.7	19.8–20.1	19–19.36	5.1	13.8	10.9–11.8	13.5–17.1	21	18.1–19.1	21	18.4	16.1	13.7–15.3	11	11.0
3. *A. arildae*	6.33–6.87	10.83–11.94	–	19.1–20.3	19.5–19.6	17.5–18.8	14.7–15.6	11.7–13.5	19.6–20.8	18.1–19	18–19.2	17.3–20.2	21.0–21.5	12–14.1	21–21.2	11.9–12.9	17.5–17.9	17.4–20	18.8–19	18.4
4. *A. callipygius*	10.8–11.1	0.3–0.7	10.8–12.3	–	12.6	14.6–14.8	19.7–19.7	19.5–19.6	4	13.1	10.4–11.4	13.2–16.5	20.7	18.3–18.6	20.7	19.1	15.8	13.2–14.9	11.2	9.7
5. *A. cavicola*	11.3–11.7	4.77–4.78	11.75–13.4	4.5–4.7	–	12.6	18.1–18.5	20.4–20.7	11.9	14.4	8.3–9.8	13.8–15.5	20.8	19.9–20.2	20.8	18.7	13.8	13–15.3	12.4	9.8
6. *A. cochranae*	11.2–11.4	5.32–5.67	12.72–13.82	5.1–5.6	6.9–7.2	–	19.9–20.4	20.4–20.9	14.8–15.6	16.2–16.5	13.2–15.2	9–10.3	20.3–21.3	17.9–18.9	20.3–21.3	19.5–19.6	9–9.4	14.3–16.3	13.6–13.7	11.8–12.3
7. *A. ehrhardti*	8.4–11.4	11.93–11.98	8.18–10.49	12.1–12.4	12.1–13.4	12–12.7	–	15.4–16.4	19.6–19.9	19.8	18.9–19.9	18.1–20.4	22.5–22.7	16.9–17.4	22.5–22.7	15.6–15.8	18.4–19	18.7–20.8	19.2–19.5	19.3–19.8
8. *A. eugenioi*	2.4–3.3	9.54–10.12	5.70–7.01	9.9–10.4	11.2–11.4	11.1–11.5	7.8–9.5	–	20.6–21.0	19.8–21	18.8–19.9	18.3–20.8	21.9–22.3	12.3–14.7	21.9–22.3	11.4–12.7	19.6–19.9	19.1–21.2	18.8–19.2	18.2–19.5
9. *A. flumineus*	11.71	2.6	11.9–12.8	2.2–2.6	5.4–5.6	5.1–5.5	12.3–12.4	10–10.4	–	13.1	10.2–11.4	13.6–16.8	19.5	18.7–19	19.5	18.5	15.9	12.8–14.4	10.5	10.2
10. *A. ibirapitanga*	11.2–11.7	4.9–5.0	12.1–12.7	4.7–5.2	5.8–6.5	6.7–8.4	12.5–13.5	10.3–11.4	4.4–5.5	–	12.9–13.6	14.5–17.8	20.1	18.2–18.2	20.1	19	16.1	9.13–10.4	14.5	13
11. *A. leucopygius*	11–11.2	3.1–3.3	11.2–12.1	3–3.3	4–5.1	5.8–6.5	12–12.7	10.1–10.6	3.7–3.9	4.1–6.3	–	13–16.4	20.3–20.5	17.5–19.4	20.3–20.5	18.6–19.3	13.3–14.5	12.8–14.7	11.8–13.5	6.2–7.2
12. *A. perviridis*	11.2–11.8	6.2–6.7	12.1–13.6	6.0–6.9	6–7.6	2.1–3	12.2–13.1	11.4–11.8	6.3–7	7.1–8	6–6.4	–	20.1–21.6	17–19.3	20.1–21.6	18.2–19.9	9.2–10.4	13.3–17.3	14.4–16.2	12.5–13.8
13. *A. sibilatus*	10.1–10.8	12.7–13.2	12.8–14.1	12.7–13.6	12.2–13.9	12.9–13.1	11.4–12.3	10–11.8	12.7–13.6	12.4–13.3	13.6–14.3	13.1–14	–	21.9–23.4	–	20.8	21.2	20.3–21.6	21.8	20.9
14. *A. weygoldti*	7.5	11.1	6.7–7.6	11.5–11.5	11.5–12.3	12.6–12.8	9.8–10.2	7–7.2	12.2	11.2–11.6	11.2–11.4	12.4–12.4	11.4	–	21.9–23.4	14.5–15.6	18.4–19.0	17.4–19.6	18.7–19.2	18.4–19.5
15. *A.* sp. 1	10.1	12.7	12.6–12.8	12.7–13.1	13.1–13.3	13.1–13.3	11.4–12.1	10–10.1	12.7	12.1–12.8	13.6–13.8	13.1–14	3.18	11.4	–	20.8	21.2	20.3 – 21.6	21.8	20.9
16. *A.* sp. 2	6.7–6.8	10.4–10.5	6.1–7.4	10.4–10.6	11–11.2	11–11.2	9.1–9.3	6–6.2	11	11.8–12	10.1–10.9	10.9–11	11.4–11.6	6.9–7.1	11.4–11.6%	–	19.6	18.9–20.7	17.9	18.9
17. *A.* sp. 3	12.3	7	13.2–14.1	6.9–7	7.2–8.8	4	12.2–12.7	11.4–12	6.2	7.9–9.5	7.1–7.2	4.6–5.5	14.4	13.5	14.4	11.8	–	15.4–17	15.6	12.9
18. *A.* sp. 4	11.2	5.1	12.7–13.4	4.6–5.1	6.2–6.3	7.6–8	12.7–13.1	11–11.2	5.4	2.6–3.1	5.1–5.7	7.4–8.3	12.9	11.4	12.9	11.2	9	–	12.3–13.5	13–14.2
19. *A.* sp. 5	10.1–11.2	2.6–3.7	10.6–13	2.6–3.8	4.9–6	4.9–5.8	11.4–12	9.4–10.4	2.8–3.3	3.9–5.2	3.2–4.4	5.3–6	12.2–12.5	11.2–11.5	12.2–12.5	9.9–10.4	6–6.3	4.8–5.4	–	11.6
20. *A.* sp. 6	11.2–11.5	3.5–3.7	11.4–12.7	3.5–3.8	3.8–4.7	6.3–6.9	12–12.5	10.6–11	3.53–4.23	4.2–6.1	2.3–3.7	6.5–7.4	13.1–13.3	11.5–11.9	13.1–13.3	10.3–11	6.7–7.4	5.1–5.5	3.3–4.7	–

because the taxonomic distribution of the cloacal flap is restricted to some species of the *A. albosignatus* Group (discussion below); thus, there is no conflict with our finding that *A. sibilatus* is the sister taxon of all other species of *Aplastodiscus*.

Aplastodiscus sibilatus is known from Atlantic Forest patches in southern and central Bahia, and in southwestern Alagoas (Cruz et al., 2003; Lima et al., 2006). Two samples of this clade were included, one from southern Bahia and the other from Alagoas (Appendix A). These two samples have a p-distance of 3.18% for *16S* (Table 1 and Appendix C). A parallel morphological study by Cruz et al. (in prep.) concludes that the voucher from Alagoas belongs to an unnamed species, morphologically distinct from *A. sibilatus*. The *p*-distances are congruent with the specific identity of the population from Alagoas, here referred to as *Aplastodiscus* sp. 1 (Fig. 1).

The topology of *A. sibilatus* and the unnamed species sequence of the recognition of a fourth group of species in *Aplastodisus*, the *A. sibilatus* Group. Some character states, in contrast with other species of *Aplastodiscus* and the outgroups, uniquely characterize *A. sibilatus* and *Aplastodiscus* sp. 1 and are considered putative phenotypic synapomorphies of the *A. sibilatus* Group—*viz.*, subcloacal dermal fold (Cruz et al., 2003; Appendix D) and white gastrointestinal peritoneum (Berneck, pers. obs.). According to Mercês and Juncá (2010), tadpoles of *A. sibilatus* differ from other tadpoles described for *Aplastodiscus*; however, further studies on Cophomantini are needed to corroborate any putative synapomorphies for the *A. sibilatus* Group or other clades of *Aplastodiscus*.

4.4. The Aplastodiscus albofrenatus Group and the putative position of A. musicus

The erection of the *Hyla albofrenata* Complex (now the *A. albofrenatus* Group) was a consequence of the recognition that the name *Aplastodiscus albofrenatus* actually was applied to several different species (*A. arildae*, *A. ehrhardti*, and *A. weygoldti*; Cruz and Peixoto, 1987; Faivovich et al., 2002). Faivovich et al. (2005) reported molecular support for the monophyly of this group. Some authors suggested a red-orange iris as a possible synapomorphy of the group (Carvalho-e-Silva and Carvalho-e-Silva, 2005; Orrico et al., 2006). However, we find that iris color varies among individuals of the same population; at the type locality of *A. arildae*, we recorded two individuals with copper-colored irises, whereas all other individuals had red irises. We have similar observations for *A. calipygius* (of the *A. albosignatus* Group), which is known to have a reddish iris; however, we recorded individuals from Campos do Jordão (State of São Paulo) with white irises. A similar situation was reported by Rivera-Correa and Faivovich (2013) for *Hyloscirtus larinopygion*. Whereas there is notable variation in the pattern and coloration of irises within *Aplastodiscus*, we find it is premature to hypothesize homologies until intraspecific variation and the nature of iris coloration are better known.

The only species of *Aplastodiscus* not available for our study is *A. musicus*, a species that was assigned to the *A. albofrenatus* Group by Cruz and Peixoto (1987) without specific discussion. In her description of *A. musicus*, Lutz (1949) noted some unusual characters, such as an unpigmented nuptial pad in males and a strong peculiar odor of a slimy secretion—characters absent in all species now included in *Aplastodiscus*. A strong odor was reported for *Myersiohyla* by Faivovich et al. (2013), and for *Hyloscirtus* by Rivera-Correa and Faivovich (2013). Lutz (1949) also noted the musical call of *A. musicus*, reminiscent of "an old fashioned glockenspiel," after which the species was named. She described "*a green tree-frog closely akin to* H. albofrenata *and especially to* H. albosignata" and "*though nearer to* H. albosignata, *it is less heavy in build, has a shorter and wider snout and lacks the rows of yellow, milium-like, post-anal glands.*" The contents of the original description and the study of external morphology of six topotypes of *Aplastodiscus musicus* (Appendix E) suggest the association of *A. musicus* with the *A. albosignatus* Group rather than the *A. albofrenatus* Group.

Our results indicate the existence of one new species (*Aplastodiscus* sp. 2) of the *A. albofrenatus* Group. This species is the sister taxon of *A. albofrenatus* + *A. eugenioi*, and was confused with *A. eugenioi* by previous authors (Salles et al., 2012). Although this species is morphologically difficult to diagnose from *A. albofrenatus* and *A. eugenioi*, its specific distinctiveness is supported by a unique advertisement call (Berneck, pers. obs.). Uncorrected *p*-distances of *16S* between *A. albofrenatus* and *A. eugenioi* are 2.4–3.3%. The latter has been distinguished from *A. albofrenatus* by a smaller snout-vent length, larger calcar tubercle, femur and tibia size, iris color, morphology and coloration of the tadpole (Carvalho-e-Silva and Carvalho-e-Silva, 2005), and was considered *A.* aff. *ehrhardti* by Hartmann et al. (2004). Our data suggest that the diagnostic characters of *A. albofrenatus* and *A. eugenioi* require further study.

Only four species of this group were included in previous analyses. The voucher specimen identified as *A. weygoldti* by Faivovich et al. (2005) and subsequent analyses) has been re-identified as *A. arildae* in our analysis. The specimen is from Domingos Martins (State of Espírito Santo). Apparently, both species occur at this locality (Silva-Soares et al., 2011; Silva et al., 2012). Orrico et al. (2006) described the call of *A. weygoldti* from Domingos Martins and compared it with topotypic *A. arildae*, finding them to differ significantly in duration (details in Orrico et al., 2006).

Wiens et al. (2005) included sequences of *12S* and *16S*/*tRNA-Leu*/*ND1* fragment of a voucher specimen identified as *Aplastodiscus albofrenatus* (USNM 208734), collected in 1975 by W.R. Heyer in Boraceia (Salesópolis, São Paulo). So far, only *A. arildae* is known from that locality (Cruz and Peixoto, 1987; Berneck, unpubl.). Without further comments, Wiens et al. (2006) replaced the *12S* fragment with the sequence DQ380346, produced from a voucher identified as *A. albofrenatus* (USNM 303022) from the same locality. The latter sequence is identical to our sequences of *A. arildae* from Boraceia. The position of the terminal USNM 208734 in the analysis of Pyron and Wiens (2011), as the sister taxon of the remaining exemplar species is unusual. Our study of the voucher specimen USNM 208734 indicates that it actually is *A. arildae*. A study of the available *12S* sequences shows striking differences even in conserved regions, when compared with the sequences of other specimens of *A. arildae* and also all other species of the genus. This pattern might indicate problems with sequence quality (Lyra, pers. obs.), but the unavailability of the chromatograms hinders the confirmation of this hypothesis. We did not include the sequences from Wiens et al. (2005, 2006) because they are nearly identical to the samples we have from the same locality (*12S*, Boraceia); moreover, apparently there are sequencing-quality issues.

The *A. albofrenatus* Group is distributed in the Atlantic Forest of Brazil from Bahia (15°25′S; 39°34′W) to Santa Catarina (27°46′S; 49°45′W). The distributions of most members of this group are associated with coastal mountain systems (*e.g.*, Serra do Mar, Serra Bonita), with the notable exception of *A. arildae*, the distribution of which extends westward toward the Serra da Mantiqueira Range and central regions of Minas Gerais (19°56′S; 43°54′W). Putative phenotypic synapomorphies for the *A. albofrenatus* Group are discussed below in "Evolution of external morphology of the cloacal region."

4.5. The Aplastodiscus perviridis Group

Based on observations by Garcia et al. (2001), Faivovich et al. (2005) suggested the following putative synapomorphies for the *A. perviridis* Group—*viz.*, the bicolored iris (the superior third white silver and inferior two thirds reddish copper); absence of an

interdigital membrane between Toes I and II (with instances of homoplasy as identified by Faivovich et al. (2005)); and reduction of webbing between the other toes. In addition, we consider the following features to be putative synapomorphies—the absence of white parietal peritoneum (present in all other species of *Aplastodiscus*) and absence of any cloacal ornamentation as described for the *A. albosignatus*, *A. albofrenatus*, and *A. sibilatus* groups. (In *A. cochranae*, the cloaca is not glandular; only a pigmentary white line is present).

The *A. perviridis* Group includes the nominal species, *Aplastodiscus* sp. 3 a species identified as *A. perviridis* in previous studies (*e.g.*, Bastos et al., 2003; Valdujo et al., 2012), and its sister species, *A. cochranae*. The specific status of *Aplastodiscus* sp. 3 is supported by a smaller snout-vent length and longer advertisement call than those of *A. perviridis*. The uncorrected *p*-distances in *16S* of *Aplastodiscus* sp. 3 are 4.6–5.5% with *A. perviridis* and 4.8% with *A. cochranae* (Table 1). The *A. perviridis* Group is distributed from the states of Minas Gerais and São Paulo, southward to northeastern Argentina and Rio Grande do Sul, and westward to reach the gallery forests in the Cerrado of central-eastern Brazil.

4.6. The Aplastodiscus albosignatus Group

Our results indicate that the *A. albosignatus* Group comprises seven described species (given the exclusion of *A. sibilatus* as discussed above and the inclusion of *A. musicus*), as well as three unnamed species—*viz.*, *Aplastodiscus* sp. 4, *Aplastodiscus* sp. 5, and *Aplastodiscus* sp. 6.

Cruz and Peixoto (1985) distinguished some species of this group by the presence of a white dermal ridge above the cloacal opening (“flap”). Faivovich et al. (2005) suggested that the combination of elaborate tubercles and ornamentation around the cloacal region is a putative morphological synapomorphy of this group. All characters related to the pericloacal region are discussed below in “Evolution of external morphology of the cloacal region.” The presence of a white submandibular dermal fold is also a putative morphological synapomorphy of this group, because it is absent in all species of *Aplastodiscus* from the other three groups and outgroups studied so far.

Abrunhosa et al. (2005) studied nine call parameters of five species of the *Aplastodisus albosignatus* Group and *A. sibilatus* (then in the *A. albosignatus* Group). These authors proposed a phenetic grouping of species based on their observation that the most important parameter is the dominant frequency, which can be found in different harmonics. However, Zina and Haddad (2006b) reported that the dominant frequency varies, depending on social context for *A. leucopygius* and *A. arildae*; thus, it probably does not adequately characterize calls of *Aplastodiscus*.

The topology of the clade including exemplars of *Aplastodiscus albosignatus* and *A. callipygius* indicates that topotypes of the latter are nested within topotypes of the former. The *16S* distances among exemplars of this clade vary from 0.35% to 0.70% (Appendix C). *Aplastodiscus callipygius* and *A. albosignatus* also are morphologically similar. Both have a supratympanic fold, calcar tubercle, and cloacal ornamentation. These species were diagnosed originally by snout shape (rounded in *A. albosignatus* and pointed in *A. callipygius*), size of vocal sac (medium in *A. albosignatus* and large in *A. callipygius*), and size of calcar tubercle (small in *A. albosignatus* and large in *A. callipygius*). However, these differences are not consistent among the many specimens available for study after the description of *A. callipygius*. Abrunhosa et al. (2005) described the calls of *A. albosignatus* and *A. callipygius* as differing somewhat from each other; however, the population on which the call descriptions were based for *A. albosignatus* is, in fact, *Aplastodiscus* sp. 5 (Ribeirão Branco, São Paulo, Brazil). Thus, we consider *Aplastodiscus callipygius* (Cruz and Peixoto, 1985) a junior synonym of *A. albosignatus* (Lutz and Lutz, 1938) based on the position of the topotypes of *A. albosignatus*, nested among the samples of *A. callipygius*, their morphological resemblance (Appendix F), and low genetic distances.

Our results reveal three previously unrecognized species in the *A. albosignatus* Group. *Aplastodiscus* sp. 4, the sister species of *A. ibirapitanga*, differs from that species by having a distinct advertisement call (Berneck, pers. obs.) and smaller size (Pimenta et al., 2014; Cruz et al., 2003). Furthermore, the *16S* distances are 2.66–3.16%. *Aplastodiscus* sp. 5 was previously identified as *A. albosignatus* (*e.g.*, Cruz and Peixoto, 1985; Abrunhosa et al., 2005), but our best hypotheses indicate that it is only distantly related to *A. albosignatus*. Furthermore, it differs in the morphology of the cloacal region. *Aplastodiscus* sp. 6, a species of the clade of *A. leucopygius*, was misidentified initially as *A. leucopygius* or *A. cavicola*. The vouchers are medium-sized and match both species. *Aplastodiscus* sp. 6 is diagnosed by morphology of the pericloacal region, and the *16S* genetic distances are 3.89–4.78% with *A. cavicola* and 2.3–3.72% with *A. leucopygius*. The same clade includes the sample of Faivovich et al. (2005) from Domingos Martins (Brazil, Espírito Santo) identified by these authors as *A. cavicola* owing to proximity to the type locality. Samples of topotypes of *A. cavicola* were not available; therefore, we assigned the terminals belonging to this species on the basis of the vouchers and the holotype.

4.7. Evolution of external morphology of the cloacal region

The presence of calcar tubercles and dermal folds on the limbs (as in most species of *Aplastodiscus*) has been considered as mimetic and disruptive morphology, especially in resting position (Duellman and Trueb, 1986). However, little attention has been given to cloacal ornamentation in anurans and its possible role in disruptive morphology. Several groups of arboreal frogs (*e.g.*, Duellman, 1970; Cisneros-Heredia and McDiarmid, 2007; Faivovich, pers. obs.) have pericloacal ornamentation. Because the variation of pericloacal ornamentation in *Aplastodiscus* is taxonomically informative, it is well described for many species. Information available in the literature allowed us to study the evolution of some characters related to the morphology of the pericloacal region of *Aplastodiscus* and to standardize the terms suggested by different authors (Appendix D).

Our study revealed four hypotheses of character evolution for *Aplastodiscus* and outgroups (Fig. 2). Faivovich et al. (2005) suggested that the presence of ornamentation around the cloaca and elaborated tubercles are putative synapomorphies of this group. We redefined this character with the understanding that the tubercles in the subcloacal region are independent of the pericloacal ornamentation (optimizations in outgroups, character descriptions and figures in Appendix D). The presence of pericloacal ornamentation is a synapomorphy of the *A. albosignatus* Group (present in all species of this group including *A. musicus*, with one instance of homoplasy in *A. albofrenatus* plus *A. eugenioi*). The occurrence of an elliptical area of pericloacal ornamentation around the cloacal opening is a synapomorphy for the clade comprising *A. albosignatus*, *A. flumineus*, and *A.* sp. 5.

Our study indicates a putative morphological synapomorphy of the *A. albofrenatus* Group, the presence of iridophores in the outer cloacal epithelium (with an instance of homoplasy in *A.* sp. 6). The subcloacal dermal fold is a putative synapomorphy for the *A. sibilatus* Group.

The optimization of the characters from pericloacal morphology shows an interesting pattern of parallelisms and reversions, suggesting a level of complexity undocumented thus far (Appendix D). It is unclear why only *Aplastodiscus* among the Cophomantini has this diversity of external cloacal morphology. The morphology described in the *Hyloscirtus bogotensis* and *H. larinopygion* groups

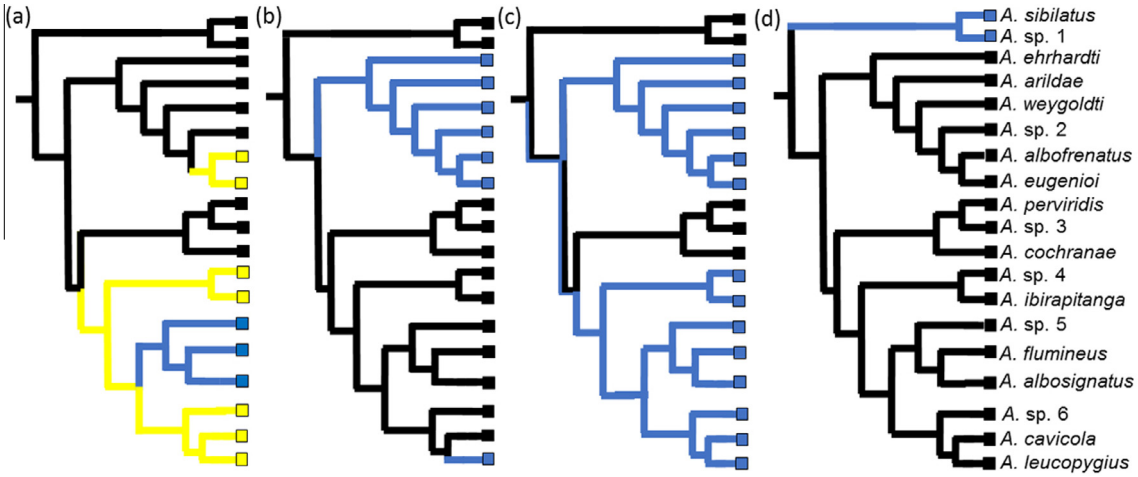

Fig. 2. Optimizations of the four pericloacal characters studied. The characters are (a) Pericloacal ornamentation (yellow: present, restricted to the supracloacal region, blue: present and elliptical around the cloacal opening; black: absent). (b) Iridophores in the outer cloacal epithelium (blue: present; black: absent). (c) Concentration of heterogeneous granules below the cloacal opening (blue: present; black: absent). (d) Subcloacal dermal fold (blue: present; black: absent). See Appendix D for figures of characters and more details. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

possibly differs histologically and anatomically (Faivovich pers. obs.). Members of the *A. perviridis* Group have the same arboreal habits as their congeners; however, cloacal and tarsal ornamentation are absent in all three species, which brings more complexity to the evolution of characters of the cloacal region.

4.8. Reproductive biology

The reproductive biology of *Aplastodiscus* was first reported in detail for *A. leucopygius* (Haddad and Sawaya, 2000), although there were earlier anecdotal observations (e.g., Lutz and Lutz, 1938; Cochran, 1955; Cruz and Peixoto, 1985, 1987). In this species, the male excavates a burrow with an inconspicuous entrance in the mud near, or in, the stream edge. The male then finds a spot to call for females, usually perched on vegetation distant from the ground. When a female approaches a calling male, both exchange a complex sequence of tactile stimuli that includes contact of the hind limbs and snout with hands, snout with snout, and chin on head (Haddad and Sawaya, 2000; Hartmann et al., 2004; Haddad et al., 2005; Carvalho et al., 2006; Zina and Haddad, 2006a,b, 2007), where sexually dimorphic skin glands have recently been described in males (Brunetti et al., 2015). The transit from the calling and meeting point to the burrow can last from 40 min to 9 h (Hartmann et al., 2004; Zina and Haddad, 2007); during this period, the male emits a courtship call (similar to the advertisement call but less intense) while the female follows him to the burrow. Still, after inspection, the female may reject the burrow and leave (Hartmann et al., 2004; Zina and Haddad, 2007; Carvalho et al., 2006). Clutches of *Aplastodiscus* have about 230 unpigmented eggs, which are deposited in a floating layer inside the burrow where they spend the first stages of development (Haddad et al., 2005). After hatching, the exotrophic tadpoles complete metamorphosis in lotic water (Haddad and Prado, 2005).

Haddad et al. (2005) described the reproductive biology of *Aplastodiscus perviridis*. For the *A. albofrenatus* Group, data are available for *A. arildae* (Zina and Haddad, 2006a,b, 2007; Carvalho et al., 2006) and *A. eugenioi* (Hartmann et al., 2004). The descriptions are quite similar to that of *A. leucopygius*. In the *A. albosignatus* Group, data on reproductive biology have been reported for *A. albosignatus* (as *A. callipygius*; Gomes and Peixoto, 1997), *A. leucopygius* (Haddad and Sawaya, 2000; Zina and Haddad, 2006a,b, 2007), and *A. cavicola* (Cruz and Peixoto, 1985). *Aplastodiscus cavicola* commonly calls from inside the burrow (Cruz and Peixoto, 1985). Lutz and Lutz (1938) and Cochran (1955) reported the same behavior for a species identified as *H. albosignata*: it is likely that the species is the one called *A. leucopygius* today. Gomes and Peixoto (1997) and Carvalho et al. (2006) reported male *A. albosignatus* (as *A. callipygius*) and *A. arildae*, respectively, calling from rock crevices. Calling sites are intra- and interspecifically variable (Cruz et al., 2003); on one occasion we found on the same night and along the same stream, a male of *A. ibirapitanga* calling from inside a burrow, whereas others called from shrubs. Males probably call from inside the nest while excavating it and, after the construction is finished, climb the vegetation then used as a calling site (Haddad, pers. obs.). The reproductive biology of *A. sibilatus* is unknown. Cruz et al. (2003) reported that male *A. sibilatus* call from leaf litter, shrubs, and trees, along streams, always above the water level.

Despite occasional observations of males of other species of the *A. albosignatus* Group calling from inside burrows, *A. cavicola* is the only species in which this behavior is common (Cruz and Peixoto, 1985; Cruz et al., 2003; Berneck, pers. obs.). It is unknown if the courtship behavior described for other species of *Aplastodiscus* occurs in this species as well, or if the behavior is modified with respect to the females encountering males directly in the burrow, without the pair moving from an outside meeting point to the nest.

Hylid frogs usually do not call from burrows. Wells (2007) noted that calling from inside burrows, subterranean nests, and rock crevices can interfere with sound transmission, but in some species, the burrow facilitates resonance. In *Aplastodiscus*, only males of the *A. albosignatus* Group call from burrows, and these species have the lowest frequency of advertisement calls of all members of the genus (calls descriptions in Haddad and Sawaya, 2000; Garcia et al., 2001; Hartmann et al., 2004; Abrunhosa et al., 2005; Conte et al., 2005; Haddad et al., 2005;Orrico et al., 2006; Zina and Haddad, 2006b). Lower frequency advertisement calls usually are associated with sound propagation over long distances, and the call of *A. cavicola* has the lowest frequency of all species in the *A. albosignatus* group (Abrunhosa et al., 2005). The taxonomic distribution of calling from burrows may represent a synapomorphy for one of the internal clades of the *A. albosignatus* Group.

4.9. Chromosome evolution in Aplastodiscus

With few exceptions, the diploid number in the subfamily Hylinae is 24 chromosomes (Catroli and Kasahara, 2009; Catroli et al., 2011). So far, among hylids only *Aplastodiscus* has reductions in the diploid chromosome number involving the small-sized pairs (Bogart, 1973; Carvalho et al., 2009a,b; Gruber et al., 2012). Members of the *A. perviridis* Group (*A. cochranae* and *A. perviridis*) have a $2n = 24$ complement, whereas those of the *A. albofrenatus* group have a $2n = 22$ complement (known in *A. albofrenatus*, *A. arildae*, *A. ehrhardti*, and *A. eugenioi*), and those of the *A. albosignatus* Group have a $2n = 20$ or $2n = 18$ complement (*A. albosignatus* with $2n = 20$; *A. leucopygius* with $2n = 18$; Carvalho et al., 2009a,b; Gruber et al., 2012). The karyotype of *A. sibilatus* is unknown.

On the basis of the plesiomorphic $2n = 24$ complement inferred for Hylini (Faivovich et al., 2005), Gruber et al. (2012) hypothesized homeologies for several chromosomes in *Aplastodiscus*, and suggested a plesiomorphic condition of $2n = 24$ for the genus. These authors also suggested two independent events of fusion—one giving rise to large-sized pairs for species with karyotypes of $2n = 20$ and $2n = 18$ (*A. albosignatus* Group) from small chromosomes (pairs 7–10), and another involving fusion of the small Pair 12 chromosomes and the large Pair 3 chromosomes in species of $2n = 22$ (*A. albofrenatus* Group.)

Although our results support the occurrence of independent reductions in chromosome complements in the *Aplastodiscus albofrenatus* and *A. albosignatus* groups, our increased taxon sampling includes many species with unknown karyotypes; thus, there are several ambiguities regarding the nodes at which these chromosome reductions actually occurred in the *A. albosignatus* Group (Fig. 3). It is most parsimonious to infer that the fusion of pairs 12 and 3, leading to a $2n = 22$ karyotype in four species of the *A. albofrenatus* Group (*A. albofrenatus*, *A. arildae*, *A. ehrhardti*, *A. eugenioi*) also occurred in *A. weygoldti* and *Aplastodiscus* sp. 2. The karyotype of *A. sibilatus* is unknown, but putative fusions of pairs 3 and 12 occur neither in the *A. perviridis* and *A. albosignatus* groups, nor

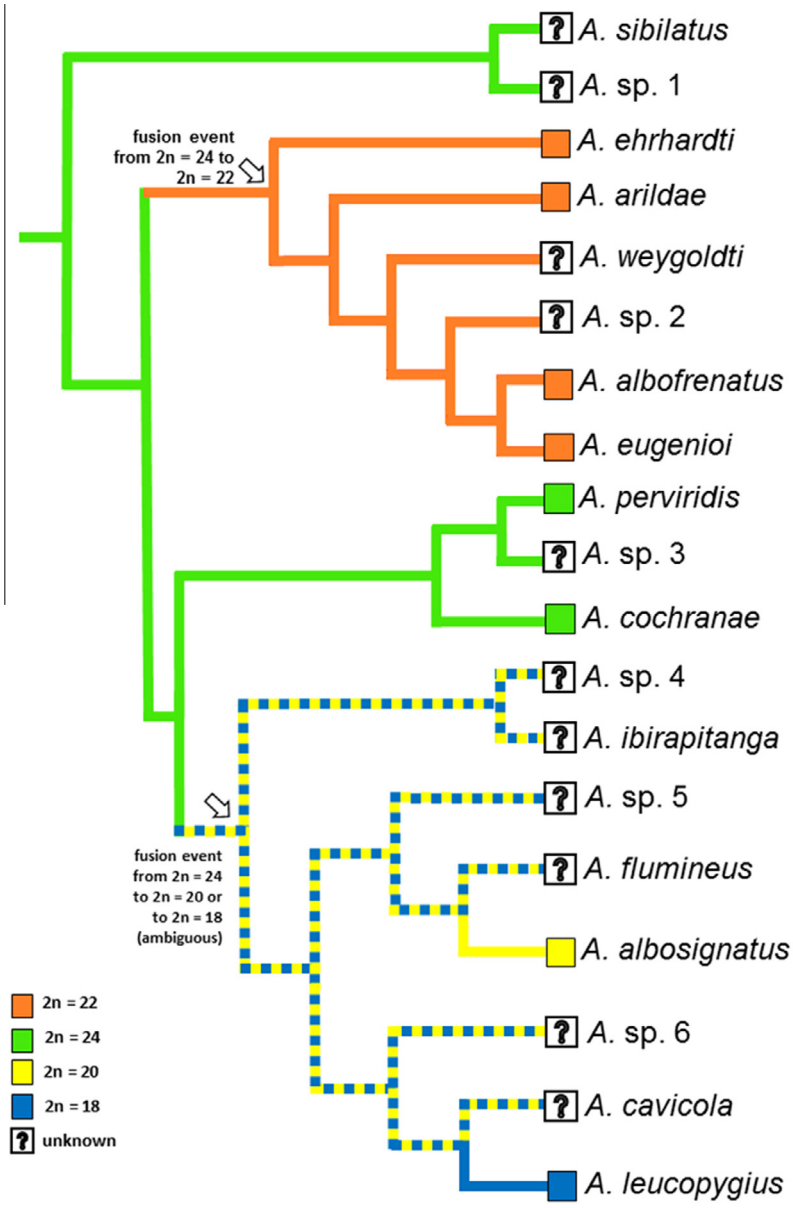

Fig. 3. Optimization of chromosome complement showing reductions in *Aplastodiscus*. Note that the synapomorphies are the events of fusion, which causes the reduction in chromosome number. Data are from Bogart (1973), Carvalho et al. (2009a,b), and Gruber et al. (2012). See text for more details.

in any other known clade of Cophomantini (e.g., Catroli and Kasahara, 2009; Catroli et al., 2011). It is more parsimonious to infer a $2n = 24$ karyotype for *A. sibilatus* and to consider this fusion a synapomorphy of the *A. albofrenatus* Group. (Note that the synapomorphy is the fusion, with the reduction from $2n = 24$ to $2n = 22$ being a consequence of the fusion.)

Transformations in species of the *Aplastodiscus albosignatus* Group leading to the reductions to $2n = 20$ and $2n = 18$ are ambiguous, because karyotypes are known for only three of the nine species included in the group. In the context of our hypothesis, it is most parsimonious to interpret the rearrangement leading to the reduction to $2n = 20$ in *A. albosignatus* to be a synapomorphy at least at the level of the common ancestor of *A. albosignatus*, *A. cavicola*, *A. flumineus*, *A. leucopygius*, *Aplastodiscus* sp. 5, and *Aplastodiscus* sp. 6. Thus far, the additional rearrangement leading to the $2n = 18$ complement has been inferred only in *A. leucopygius*; given that the karyotype is unknown in the closely related *A. cavicola* and *Aplastodiscus* sp. 6, the node at which these rearrangements occurred is ambiguous. When the karyotype of *A. ibirapitanga* is determined, it will be possible to ascertain whether the chromosomal reduction events (*i.e.*, $2n = 24$ to $2n = 20$) are a synapomorphy (or synapomorphies if more than one event can be inferred) of the *A. albosignatus* Group (Fig. 3).

5. Final remarks

Our phylogenetic analyses yielded a stable phylogenetic hypothesis with high support for most nodes. We highlight four major clades of *Aplastodiscus* as named species groups, each of which contains at least one newly revealed species. Our phylogenetic results revise major hypotheses of morphological, behavioral, and chromosomal evolution in *Aplastodiscus*, and identify the data needed for critical testing of these hypotheses.

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Appendices A–F. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ympev.2015.11.014.

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