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Short communication

The phylogenetic position of the Black-collared Bulbul *Neolestes torquatus*

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The Black-collared Bulbul *Neolestes torquatus* is a resident African passerine occupying the lightly wooded savannah of central Africa. It occurs from Gabon to central Angola, Congo and extends northwest to the D. R. Congo (Keith 2000). Its range almost entirely falls within the Western and Southern Congolian Forest Savanna Mosaic as delimited in WWF's Terrestrial Ecoregions for Africa (Burgess *et al.* 2004). Its taxonomic affinities have been a matter of contention for more than a century (Dowsett *et al.* 1999). *Neolestes* has a striking plumage, with a grey forecrown and nape, a black mask across the eye curving around the white throat to connect with the wide black collar across the breast, hence its vernacular name. In general shape and plumage pattern, it strongly resembles malaconotid bush-shrikes (Malanonotidae) and was originally described as an aberrant Malaconotid by Cabanis (1875). Although Gadow (1883) remarked that *Neolestes* 'does not appear to be a Bush-Shrike, but to be allied to the Bulbuls or Pycnonotidae', an association with the Malaconotidae or an enlarged Laniidae held for more than half a century (e.g. Reichenow 1902–1903, Sharpe 1903, Sclater 1930). Chapin (1921) was the first to observe and study the species in the field during his expeditions to the Congo. In both behaviour and morphology *Neolestes* was considered by him to be placed among the bulbuls (Pycnonotidae). Although an affinity with bulbuls has been the prevailing opinion ever since (White 1962, Hall & Moreau 1970, Wolters 1982, Sibley & Monroe 1990, Keith 2000), a link to the bush-shrikes has never been completely discounted (Bannerman 1953). More recently, Olson (1989) examined a fluid-preserved specimen of *Neolestes*. He suggested that the species be placed *incertae sedis* near the helmet-shrikes (Prionopidae), due to *Prionops* and *Neolestes* sharing tufts of stiff, plush, chestnut feathers behind the ear.

In a reappraisal of its phylogenetic position, Dowsett *et al.* (1999) re-evaluated the morphological and behavioural evidence for an association of *Neolestes* with bulbuls, bush-shrikes or helmet-shrikes, together with molecular data. Although some morphological characters were equivocal in placing *Neolestes* among the bulbuls, they clearly indicated that any association with bush- or helmet-shrikes was unwarranted. In particular, *Neolestes* shares with bulbuls similarities in syrinx, carpometacarpus, humerus and various skull structures, juvenile plumage, nest structure, eggs, clutch size, diet and song (Dowsett *et al.* 1999). The molecular data were limited to a short cytochrome-*b* sequence from a dozen oscine passerines. Despite the limited sampling, *Neolestes* clustered basally in a monophyletic clade with the other two bulbuls included in that dataset and far away from *Laniarius* and *Prionops*. Weighing both morphological and molecular evidence, Dowsett *et al.* (1999) suggested that *Neolestes* should be included among the bulbuls. They also predicted that it would probably represent a basal clade, or even the sister taxon of the remaining bulbuls.

Two recent papers (Moyle & Marks 2006, Johansson *et al.* 2007) conducted phylogenetic analyses of bulbul relationships using molecular data. Whereas Moyle and Marks (2006) studied the relationships at the family level, Johansson *et al.* (2007) focused their attention on the Afrotropical radiation. Neither of these studies included *Neolestes*, and thus its placement within the Pycnonotidae remains unanswered. In the present paper we use nuclear and mitochondrial sequences to clarify the phylogenetic position of *Neolestes torquatus*.

METHODS

Based on the results of Moyle and Marks (2006) and Johansson *et al.* (2007), 46 bulbul taxa were selected, encompassing all major lineages previously recovered within the family. The outgroup taxa include eight species belonging to the Sylvioidea clade that have been shown to represent the closest taxa to the Pycnonotidae (Alström *et al.* 2006, Johansson *et al.* 2008). The included taxa, sample accession numbers, and GenBank accession numbers of all sequences are listed in Table 1. The nomenclature follows Dickinson (2003) except for the use of *Alophoixus* for the Asian species formerly included in *Criniger* (Pasquet *et al.* 2001, Moyle & Marks 2006).

We chose six loci, the mitochondrial NADH dehydrogenase subunit II (ND2) and subunit III (ND3), and four nuclear introns: intron 5 of β-fibrinogen (β-Fib5), intron 7 of β-fibrinogen (β-Fib7), intron 2 of myoglobin and introns 6–7 of ornithine decarboxylase (ODC); most

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Table 1. Samples and sequences included in this study, with museum accession numbers and collection localities: [ti] indicates a blood/tissue sample without voucher. Sequences published previously are listed together with their GenBank accession numbers and references.

Taxon	Accession number	ND2	ND3	β-Fib5	β-Fib7	Myoglobin	ODC	Locality
Acrocephalus arundinaceus	NRM 986607	GQ242092	GQ242126	GQ242047	GQ242070	DQ008530 [5]	GQ242151	Sweden
Alophoixus pallidus	NRM 20046822 [ti]	GQ242078	GQ242112	EF626743 [4][a]	GQ242055	DQ008559 [5][a]	EF625332 [4][a]	Vietnam
Andropadus ansorgei		DQ402195 [1]	DQ402256 [1]	EF626708 [4]	DQ402313 [1]	EF625246 [4]	EF625297 [4]	
Andropadus curvirostris		DQ402198 [1]	DQ402259 [1]	EF626706 [4]	DQ402359 [1]	EF625247 [4]	EF625295 [4]	
Andropadus gracilirostris	NRM 86447	GQ242082	GQ242116	EF626705 [4]	GQ242060	EF625249 [4]	EF625294 [4]	Ghana
Andropadus gracilis		DQ402196 [1]	DQ402257 [1]	EF626709 [4]	DQ402357 [1]	EF625245 [4]	EF625298 [4]	
Andropadus importunus	NRM 86388	GQ242081	GQ242115	EF626713 [4]	GQ242059	EF625252 [4]	EF625302 [4]	South Africa
Andropadus latirostris	NRM 20046809 [ti]	GQ242089	GQ242124	EF626710 [4][a]	GQ242068	DQ008560 [5][a]	EF625299 [4][a]	Uganda
Andropadus masukuensis	NRM 86250	GQ242085	GQ242120	EF626698 [4]	GQ242064	EF625238 [4]	EF625287 [4]	Congo
Andropadus tephrolaemus	NRM 20086239 [ti]	GQ242086	GQ242121	GQ242044	GQ242065	GQ242109	GQ242147	Nigeria
Andropadus virens		DQ402197 [1]	DQ402258 [1]	EF626711 [4]	DQ402358 [1]	EF625250 [4]	EF625300 [4]	
Apalis thoracica	ZMUC O5368 [ti]	GQ242094	GQ242128	GQ242049	GQ242072	DQ008548 [5]	GQ242153	Tanzania
Baeopogon clamans		DQ402203 [1]	DQ402264 [1]	EF626716 [4]	–	EF625256 [4]	EF625305 [4]	
Baeopogon indicator		DQ402204 [1]	DQ402265 [1]	EF626717 [4]	DQ402317 [1]	EF625255 [4]	EF625306 [4]	
Bleda canicapillus	NRM 86188	DQ402201 [1]	DQ402262 [1]	GQ242043	DQ402315 [1]	GQ242108	GQ242146	Ghana
Bleda syndactylus	NRM 86179	AY136592 [8]	GQ242119	EF626738 [4]	GQ242063	EF625276 [4]	EF625327 [4]	Ghana
Calyptocichla serina		DQ402199 [1]	DQ402260 [1]	EF626715 [4]	DQ402314 [1]	EF625254 [4]	EF625304 [4]	
Chlorocichla flavicollis		DQ402205 [1]	DQ402266 [1]	EF626721 [4]	DQ402318 [1]	EF625259 [4]	EF625310 [4]	
Chlorocichla flaviventris	ZMUC 117578 [ti]	GQ242087	GQ242122	EF626719 [4][a]	GQ242066	AY228290 [6][a]	GQ242148	Kenya
Criniger barbatus		DQ402208 [1]	DQ402269 [1]	EF626740 [4]	–	EF625278 [4]	EF625329 [4]	
Criniger ndussumensis		DQ402209 [1]	DQ402270 [1]	EF626741 [4]	DQ402324 [1]	EF625279 [4]	EF625330 [4]	
Hemixos flavala	NRM 86032	DQ402224 [1]	DQ402285 [1]	GQ242038	DQ402327 [1]	GQ242104	GQ242141	Malaysia
Hippolais icterina	NRM 20056193	GQ242091	GQ242125	GQ242046	GQ242069	DQ008531 [5]	GQ242150	Sweden
Hirundo rustica	NRM 976238	GQ242090	DQ402247 [1]	GQ242045	DQ402309 [1]	AY064258 [7][a]	GQ242149	Sweden
Hypsipetes leucocephalus	NRM 20047104 [ti]	GQ242080	GQ242114	GQ242040	GQ242058	GQ242105	GQ242143	Vietnam
Iole olivacea	NRM 86056	DQ402220 [1]	DQ402281 [1]	GQ242036	DQ402354 [1]	GQ242102	GQ242139	Malaysia
Ixonotus guttatus	NRM 86201	GQ242088	GQ242123	EF626718 [4]	GQ242067	EF625257 [4]	EF625307 [4]	Congo
Ixos mcclellandii	NRM 20046796 [ti]	GQ242079	GQ242113	GQ242039	GQ242056	DQ008558 [5][a]	GQ242142	Thailand
Ixos philippinus	ZMUC 117590 [ti]	DQ402226 [1]	DQ402287 [1]	EF626742 [4]	GQ242057	EF625280 [4]	EF625331 [4]	Philippines
Locustella fluviatilis	NRM 20016513 [ti]	GQ242093	GQ242127	GQ242048	GQ242071	DQ008527 [5]	GQ242152	Bulgaria
Microscelis amaurotis	NRM 85997	DQ402222 [1]	DQ402283 [1]	GQ242037	DQ402325 [1]	GQ242103	GQ242140	Japan
Neolestes torquatus	NRM 71084	GQ242083	GQ242117	GQ242041	GQ242061	GQ242106	GQ242144	Congo
Orthotomus sutorius	NRM 20046853 [ti]	GQ242095	GQ242129	GQ242050	GQ242073	DQ008542 [5]	GQ242154	Vietnam
Phyllastrephus albigularis		DQ402210 [1]	DQ402271 [1]	EF625272 [4]	DQ402330 [1]	EF625323 [4]	EF626734 [4]	
Phyllastrephus debilis		DQ402213 [1]	DQ402274 [1]	EF626737 [4]	DQ402335 [1]	EF625275 [4]	EF625326 [4]	
Phyllastrephus fischeri		DQ402216 [1]	DQ402277 [1]	EF626730 [4]	DQ402339 [1]	EF625266 [4]	EF625319 [4]	
Phyllastrephus flavostriatus		DQ402214 [1]	DQ402275 [1]	EF626736 [4]	DQ402336 [1]	EF625274 [4]	EF625325 [4]	
Phyllastrephus hypochloris		DQ402215 [1]	DQ402276 [1]	EF626727 [4]	DQ402337 [1]	EF625265 [4]	EF625316 [4]	
Phyllastrephus icterinus		DQ402211 [1]	DQ402272 [1]	EF626731 [4]	DQ402331 [1]	EF625269 [4]	EF625320 [4]	
Phyllastrephus placidus		DQ402212 [1]	DQ402273 [1]	EF626729 [4]	DQ402332 [1]	EF625268 [4]	EF625318 [4]	
Phyllastrephus poensis	NRM 20086270 [ti]	GQ242084	GQ242118	GQ242042	GQ242062	GQ242107	GQ242145	Nigeria
Phyllastrephus terrestris		DQ402217 [1]	DQ402278 [1]	EF626724 [4]	–	EF625262 [4]	EF625313 [4]	

Table 1. (Continued)

Taxon	Accession number	ND2	ND3	β-Fib5	β-Fib7	Myoglobin	ODC	Locality
Phyllastrephus xavieri		DQ402219 [1]	DQ402280 [1]	EF626733 [4]	DQ402340 [1]	EF625271 [4]	EF625322 [4]	
Phylloscopus collybita	NRM 20036909	DQ125988 [2]	GQ242130	GQ242051	GQ242074	DQ125966 [2]	GQ242155	Sweden
Pycnonotus atriceps	NRM 86511	DQ402231 [1]	DQ402292 [1]	GQ242029	DQ402341 [1]	GQ242096	GQ242132	Thailand
Pycnonotus barbatus		DQ402232 [1]	DQ402293 [1]	EF626746 [4]	DQ402342 [1]	EF625284 [4]	EF625335 [4]	
Pycnonotus eutilotus	NRM 86490	DQ402236 [1]	DQ402297 [1]	GQ242030	DQ402346 [1]	GQ242097	GQ242133	Malaysia
Pycnonotus finlaysoni	NRM 20046850 [ti]	GQ242076	GQ242110	GQ242033	GQ242053	GQ242100	GQ242136	Vietnam
Pycnonotus jocosus	NRM 20046820 [ti]	GQ242077	GQ242111	GQ242034	GQ242054	DQ008557 [5][a]	GQ242137	Vietnam
Pycnonotus melanicterus	NRM 20046769 [ti]	DQ402243 [1]	DQ402304 [1]	GQ242032	DQ402353 [1]	GQ242099	GQ242135	Vietnam
Spizixos semitorques	NRM 20086548 [ti]	DQ402244 [1]	DQ402305 [1]	GQ242031	DQ402356 [1]	GQ242098	GQ242134	Vietnam
Thescelocichla leucopleura		DQ402200 [1]	DQ402261 [1]	EF626722 [4]	DQ402312 [1]	EF625260 [4]	EF625311 [4]	
Tricholestes criniger	NRM 86086	DQ402223 [1]	DQ402284 [1]	GQ242035	DQ402326 [1]	GQ242101	GQ242138	Malaysia
Urosphena squameiceps	NRM 20056750 [ti]	AY382399 [3]	GQ242131	GQ242052	GQ242075	DQ008563 [5]	GQ242156	Vietnam

Museum acronyms: NRM, Swedish Museum of Natural History; ZMUC, Zoological Museum, University of Copenhagen.
References: [1] Moyle and Marks (2006); [2] Fuchs *et al.* (2006); [3] Drovetski *et al.* (2004); [4] Johansson *et al.* (2007); [5] Alström *et al.* (2006); [6] Ericson and Johansson (2003); [7] Ericson *et al.* (2002).
[a]Published sequence obtained from the same sample used in this study.

sequences had been published by Moyle and Marks (2006) and Johansson *et al.* (2007), and we sequenced the few missing ones.

The fresh tissue samples were extracted using the Qiagen DNA Mini Kit (Qiagen, Valencia, CA, USA) following the manufacturer's protocol. The DNA of *Neolestes* was extracted from the toe-pad of a study skin, following the procedure described in Zuccon (2005) and Irestedt *et al.* (2006).

For fresh tissue samples, standard primers and procedures were used to amplify all genes with the exception of β-Fib7 (ND2: Sorenson *et al.* 1999, ND3: Chesser 1999, myoglobin: Irestedt *et al.* 2002, ODC: Allen & Omland 2003, β-Fib5: Fuchs *et al.* 2004). For β-Fib7 the new primers bFib7-PycnoIntF (5′-TCTGTAATA-TAGGCTAACAGATCA-3′) and bFib7-PycnoIntR (5′-GAACTGTAAGTAACCATAGTTATC-3′) were designed using the published sequences as a guide. These primers amplify a region of 182–263 bp near the 5′ end of the intron. The amplification profile was: initial denaturation for 5 min at 95 °C, followed by 35–40 cycles of denaturation for 30 s at 95 °C, annealing for 30 s at 50 °C, extension for 45 s at 72 °C, with a final extension for 5 min at 72 °C. *Neolestes* was amplified in a series of short, overlapping fragments of 200–300 bp, using a large set of specific primers. The primer sequences and the amplification conditions are provided in the Supporting Information.

PCR products were cleaned using the QIAquick PCR Purification Kit (Qiagen) and run on an ABI Prism 3100 automated DNA sequencer (Perkin-Elmer Applied Biosystems, Waltham, MA, USA). The DNA sequences were aligned with MegAlign™ (DNAStar, Madison, WI, USA) and, for the intron alignments only, adjusted manually by eye.

The concatenated dataset and each gene partition were analysed under Bayesian inference and the maximum likelihood optimality criteria. We used MrBayes 3.1 (Ronquist & Huelsenbeck 2003) for the Bayesian analyses. A mixed-model approach was implemented to account for the potential differences in evolutionary model parameters between the six data partitions corresponding to the six genes. The most appropriate nucleotide substitution models for each gene partition were determined with MrModelTest (Nylander 2004) using the AIC criterion in conjunction with PAUP* (Swofford 2003). A GTR + Γ+ I substitution model was considered optimal for the mitochondrial genes, an HKY + Γ model for myoglobin and the GTR + Γ model for the three remaining introns. We assumed uniform interval priors for the parameters, except for base frequencies, which were assigned a Dirichlet prior (Huelsenbeck & Ronquist 2001). Two independent runs of four incrementally heated Metropolis-coupled MCMC chains were run for 5 million generations, sampling every 1000 generations, yielding 5000 trees. We examined the likelihood plots

from each run to ensure that the chain had reached stationarity and we discarded the first 1000 trees as the burn-in.

Maximum likelihood searches of the partitioned dataset were conducted with RAxML v. 7.0.3 (Stamatakis 2006) using a GTR + Γ + I model and random starting tree, with α-shape parameters, GTR-rates and empirical base frequencies estimated and optimized for each partition. Nodal support was estimated using 100 bootstrap pseudoreplicates.

Individual gene partitions were also analysed under the Bayesian inference and maximum likelihood optimality criterion using the same analytical parameters indicated above, with the exception of using the appropriate nucleotide substitution models for each gene. The ND2 gene accounts for almost half of the parsimony-informative characters (see Supporting Information). To evaluate a possible bias in the phylogenetic signal caused by ND2, we also analysed a concatenated dataset under the same conditions indicated above, but with ND2 omitted.

RESULTS

Sequences were obtained for almost all taxa. The β-Fib7 gene is missing for only three species, *Phyllastrephus terrestris*, *Baeopogon clamans* and *Criniger barbatus*. The sequence alignment was straightforward, due to the limited number of indels in the four introns. The six genes were concatenated in a single dataset of 3820 bp. Table S2 presents a summary of the molecular properties of each gene.

Bayesian inference and maximum likelihood recovered an identical, well-resolved and well-supported topology (Fig. 1). In the Bayesian tree, only four nodes in the ingroup received a posterior probability below the 0.95 threshold, and only three ingroup nodes in the maximum-likelihood topology were below the 75% threshold. The Pycnonotidae radiation is composed of two major clades, an exclusively Afrotropical clade and a primarily Asian clade, which includes the Afrotropical *Pycnonotus barbatus*. Within the Afrotropical clade, *Neolestes torquatus* forms an isolated, deep lineage, just above the clade containing *Andropadus importunus*, *Calyptocichla serina* and *Andropadus gracilirostris*, and sister to the remaining Afrotropical radiation. Similar topologies are supported by the analysis of the ND2 gene alone and by the combined dataset without ND2, whereas the individual introns and ND3 provide poorly resolved trees with few nodes receiving significant support. However, none of the supported nodes in individual gene trees conflicts with the topology obtained from the combined dataset (Fig. 1).

An examination of the intron indels provided further support for the placement of *Neolestes*. All taxa in the Afrotropical clade share one synapomorphic insertion of 7 bp in β-Fib5 and one synapomorphic deletion of 1 bp in ODC. *Neolestes* also shares one synapomorphic insertion of 3 bp in β-Fib7 with all Afrotropical taxa, with the exclusion of the three basal species in the *Calyptocichla* clade (Fig. 1). Several other nodes are supported by inferred synapomorphic indels (see Supporting Information Fig. S1).

DISCUSSION

The topology in Figure 1 is broadly congruent with published phylogenies (Moyle & Marks 2006, Johansson *et al.* 2007), but some differences exist. Although our dataset has fewer taxa, it sampled twice as many characters. As a consequence we have obtained a better resolved tree, with higher posterior probability values that improve our understanding of relationships within the Pycnonotidae. The ND2 gene provides 46% of all parsimony-informative characters. However, the topologies obtained with and without ND2 are very similar, suggesting that all gene partitions share the same phylogenetic signal.

Our results confirm that *Neolestes* is indeed a bulbul, in agreement with the morphological and ecological data (Dowsett *et al.* 1999). However, contrary to previous suggestions, *Neolestes* is not just a basal branch of, or the sister group to, the bulbuls; instead, it is embedded within the African radiation. The plumage divergence from the other members of the family remains remarkable, considering how conservative the Afrotropical bulbuls are in shape and plumage patterns. The plesiomorphy in plumage characters is so evident that only molecular data were able to identify the polyphyly in the genera *Andropadus* and *Chlorocichla*, whose members actually belong to four and two distinct clades, respectively (Johansson *et al.* 2007).

The quite strongly curved and broad-based bill and distinctive colour pattern in *Neolestes* is more like members of the genus *Pycnonotus* (Asian radiation) than like a greenbul (African radiation), although *Andropadus importunus* has a similar bill shape. The very aberrant colour pattern in *Neolestes* relative to other members of the African greenbul radiation could be seen as an adaptation for visual communication in more open habitats than in other greenbul species, which tend to be associated with forest or closed broadleaved woodland.

Fry *et al.* (2000) state that the 'presence of an ossified nasal margin is one of the best characters defining the Pyconotidae [*sic*]'. However, the presence or absence of the nasal ossification does not seem to be a fully reliable character in diagnosing this family. According to Dowsett *et al.* (1999), the ossification is present in all bulbul genera, but it is less developed in *Andropadus milanjensis* and *Andropadus nigriceps*, missing in some but not all individuals of *Andropadus curvirostris* and *A. gracilirostris*, and missing altogether in *A. gracilis*,

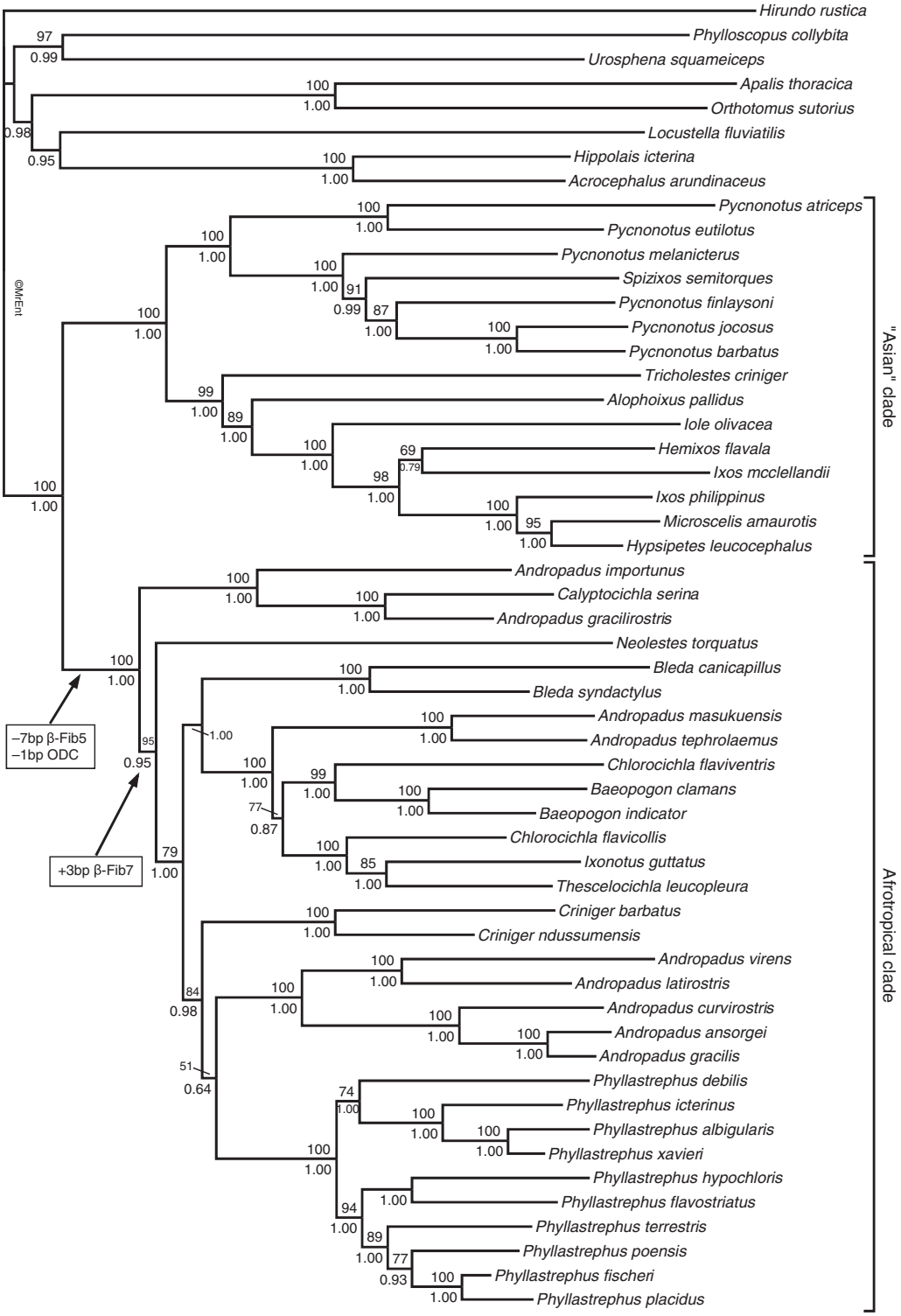

Figure 1. The majority rule consensus tree obtained from the mixed-model Bayesian analysis of the concatenated dataset. Posterior probability values are indicated below the node. Bootstrap support values obtained from the maximum-likelihood analysis are indicated above the node. The inferred synapomorphic indels for two basal nodes in the Afrotropical clade further support the placement of *Neolestes torquatus* within this clade.

A. importunus and *N. torquatus*. That in two species the ossification is present in some individuals but missing in others casts doubts on the value and reliability of this character. However, the two species of *Andropadus* without ossification belong to the basal clade within the African radiation, just below *Neolestes*. We did not have access to a skull of *Calyptocichla*, but it would be relevant to assess its condition and re-evaluate the pattern of the gain and loss of ossification of the nasal margin.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Figure S1. Mapping onto the Baysian topology (see Fig. 1) of inferred synapomorphic insertions and deletions in the four introns. A plus indicates an inferred insertion, a minus an inferred deletion.

Figures S2–S7. Trees recovered from the analysis of the individual genes. S2: β-Fibrinogen intron 5; S3: β-Fibrinogen intron 7; S4: Myoglobin intron 2; S5: Ornithine decarboxylase introns 6–7; S6: NADH dehydrogenase II; S7: NADH dehydrogenase III. A: Bayesian inference (posterior probability indicated at the node); B: maximum likelihood (bootstrap support indicated at the node).

Figure S8. Trees recovered from the analysis of the concatenated dataset without ND2. A: Bayesian inference (posterior probability indicated at the node); B: maximum likelihood (bootstrap support indicated at the node).

Table S1. Primer pairs used in the amplification and sequencing of the longer genes of *Neolestes torquatus* (β-fibrinogen intron 5, myoglobin intron 2, ornithine decarboxylase introns 6–7 and NADH dehydrogenase II). For all combinations, the amplification profile was: initial denaturation 5′ at 95°C, 40 cycles of denaturation 40″ at 95°C, annealing 40″ at the temperature indicated for each primer pair, extension 60″ at 72°C, with a final extension of 5′ at 72°C. ND3 and β-Fib7 were short enough for direct amplification using standard primers (see main text). [1]: Fuchs *et al.* 2004; [2]: Irestedt *et al.* 2002; [3]: Allen & Omland 2003 (see the main text for the full references).

Table S2. Sequence characteristics of the six genes analysed.

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Supporting Information

The phylogenetic position of the Black-collared Bulbul *Neolestes torquatus*

Dario Zuccon & Per G. P. Ericson

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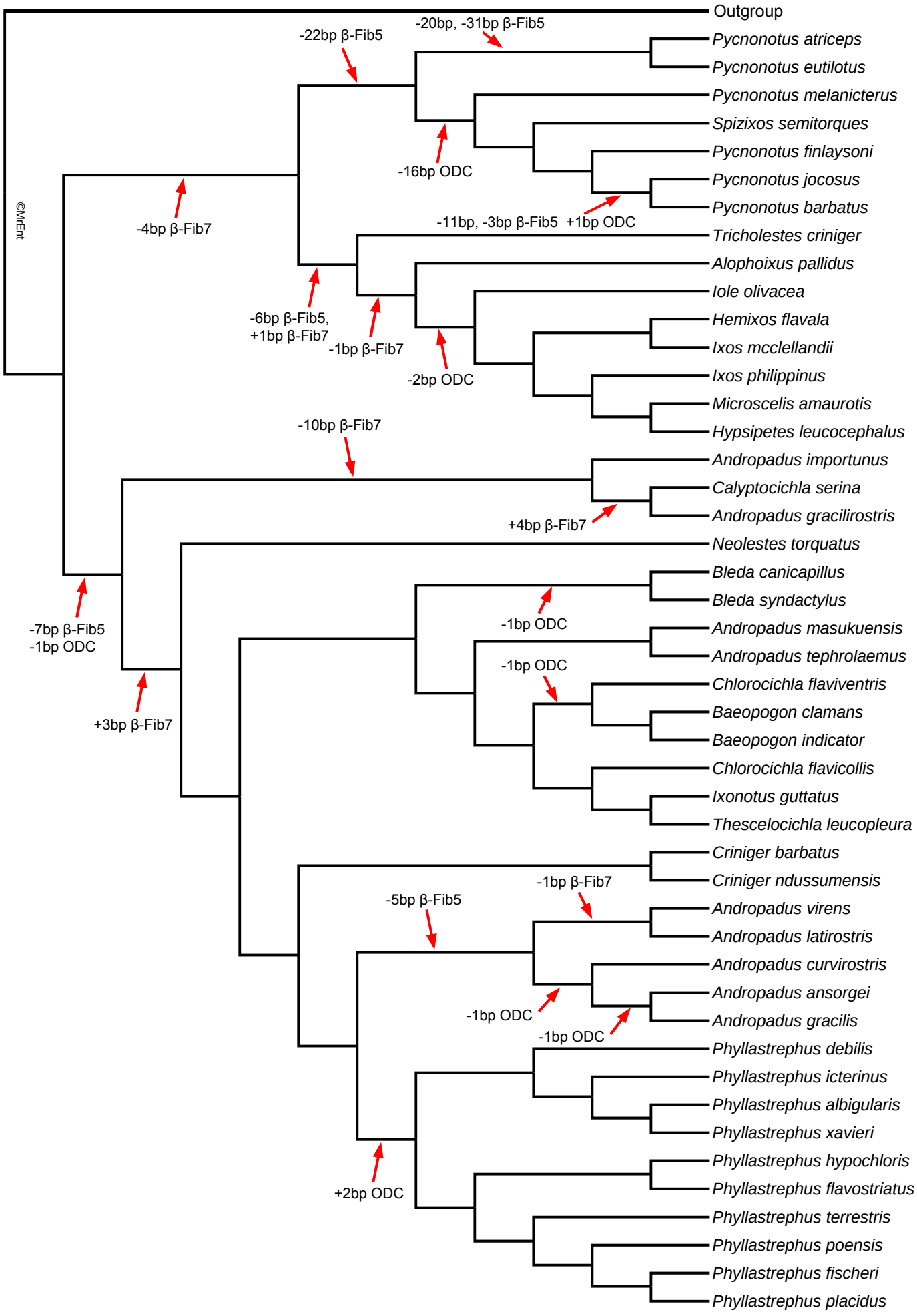
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Spizixos semitorques
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Pycnonotus jocosus
Pycnonotus barbatus
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-11bp, -3bp β-Fib5
+1bp ODC
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Alophoixus pallidus
Iole olivacea
-6bp β-Fib5,
+1bp β-Fib7
-1bp β-Fib7
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Ixos mcclellandii
-2bp ODC
Ixos philippinus
Microscelis amaurotis
Hypsipetes leucocephalus
-10bp β-Fib7
Andropadus importunus
Calyptocichla serina
Andropadus gracilirostris
+4bp β-Fib7
Neolestes torquatus
Bleda canicapillus
Bleda syndactylus
-7bp β-Fib5
-1bp ODC
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-1bp ODC
Chlorocichla flaviventris
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Baeopogon clamans
Baeopogon indicator
Chlorocichla flavicollis
Ixonotus guttatus
Thescelocichla leucopleura
Criniger barbatus
-1bp β-Fib7
Criniger ndussumensis
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Andropadus latirostris
Andropadus curvirostris
Andropadus ansorgei
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Andropadus gracilis
-1bp ODC
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Phyllastrephus icterinus
Phyllastrephus albigularis
Phyllastrephus xavieri
Phyllastrephus hypochloris
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Phyllastrephus flavostriatus
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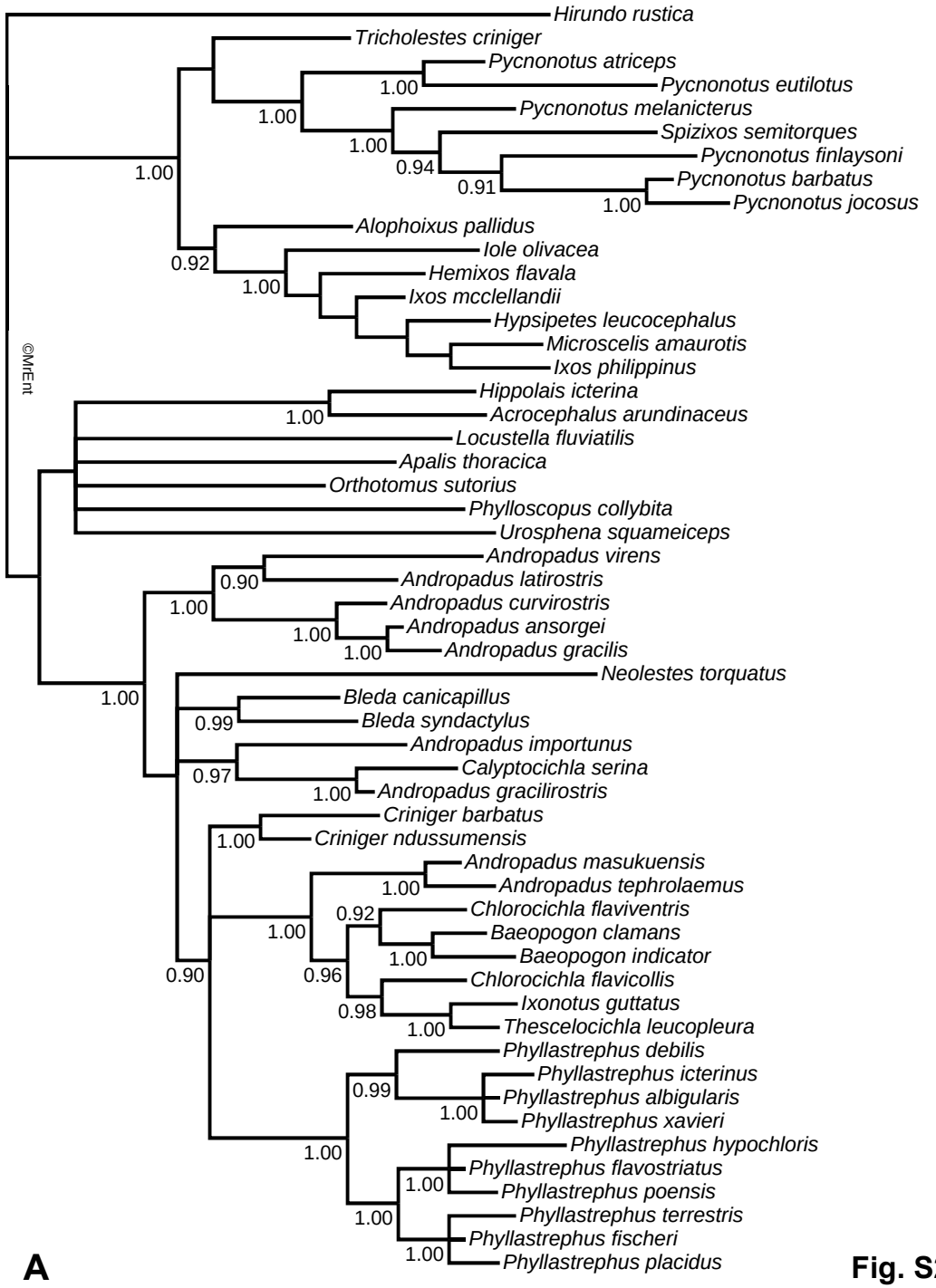
Fig. S1.



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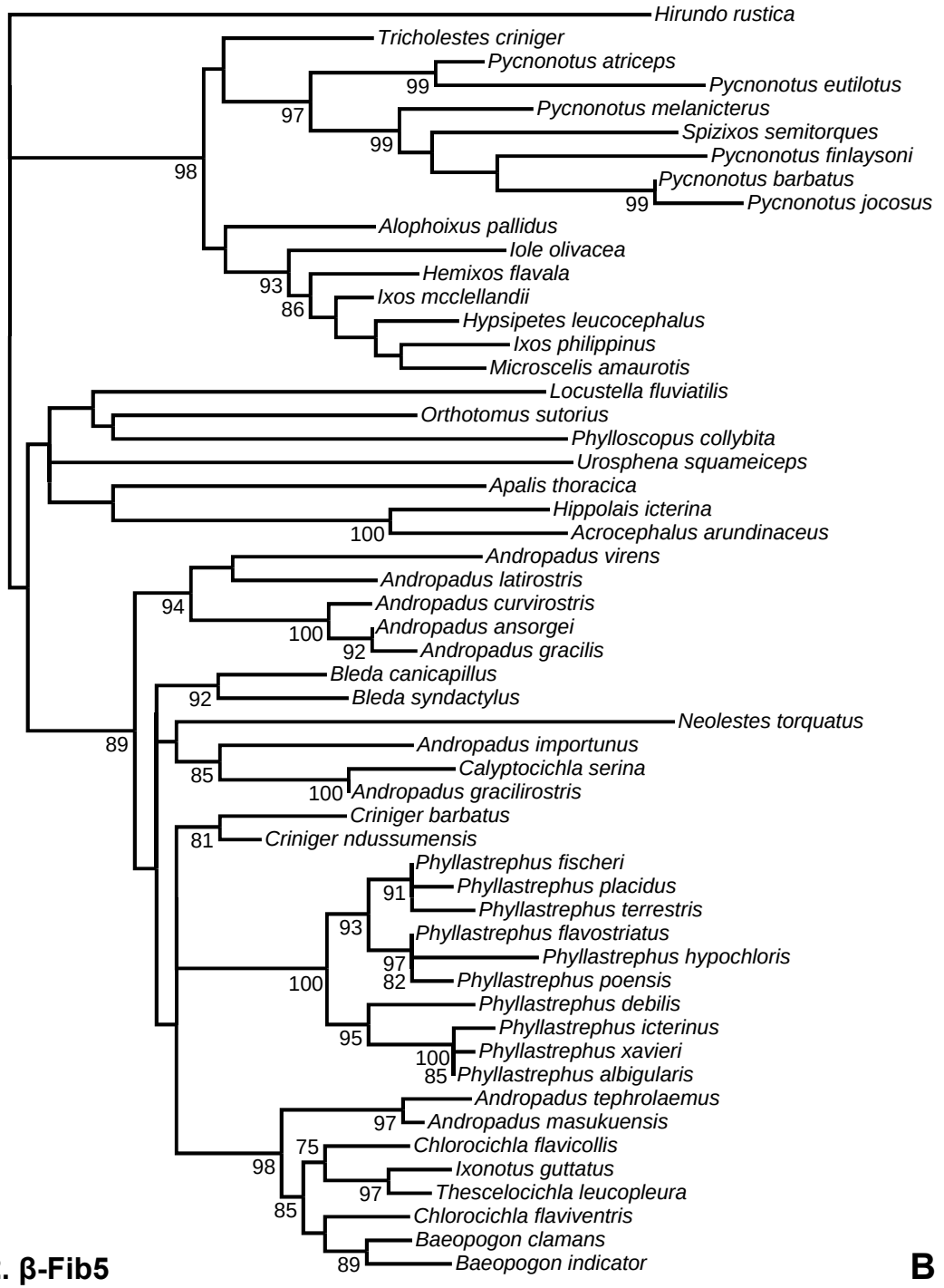

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Fig. S2. β-Fib5

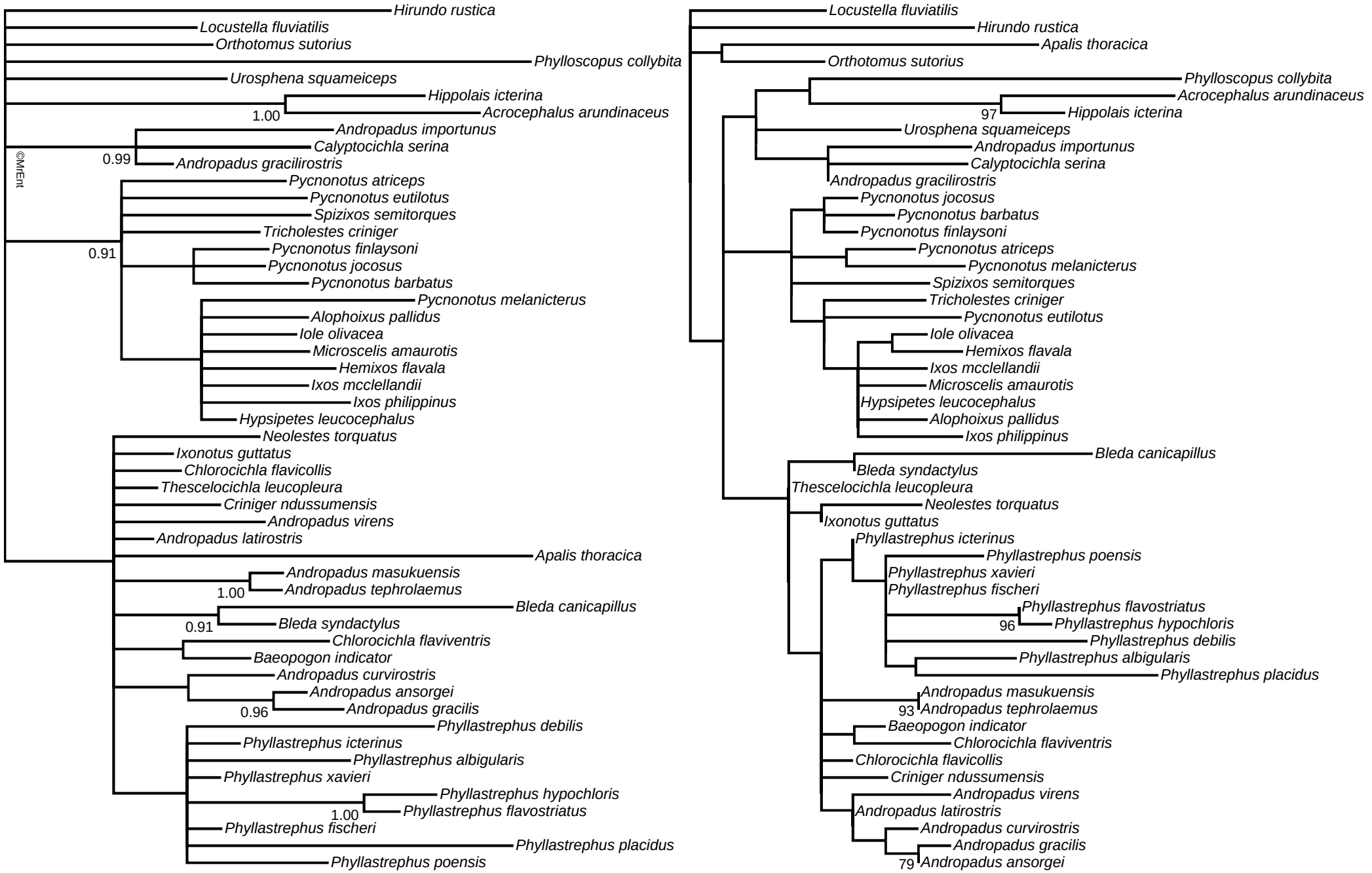

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Fig. S3. β-Fib7

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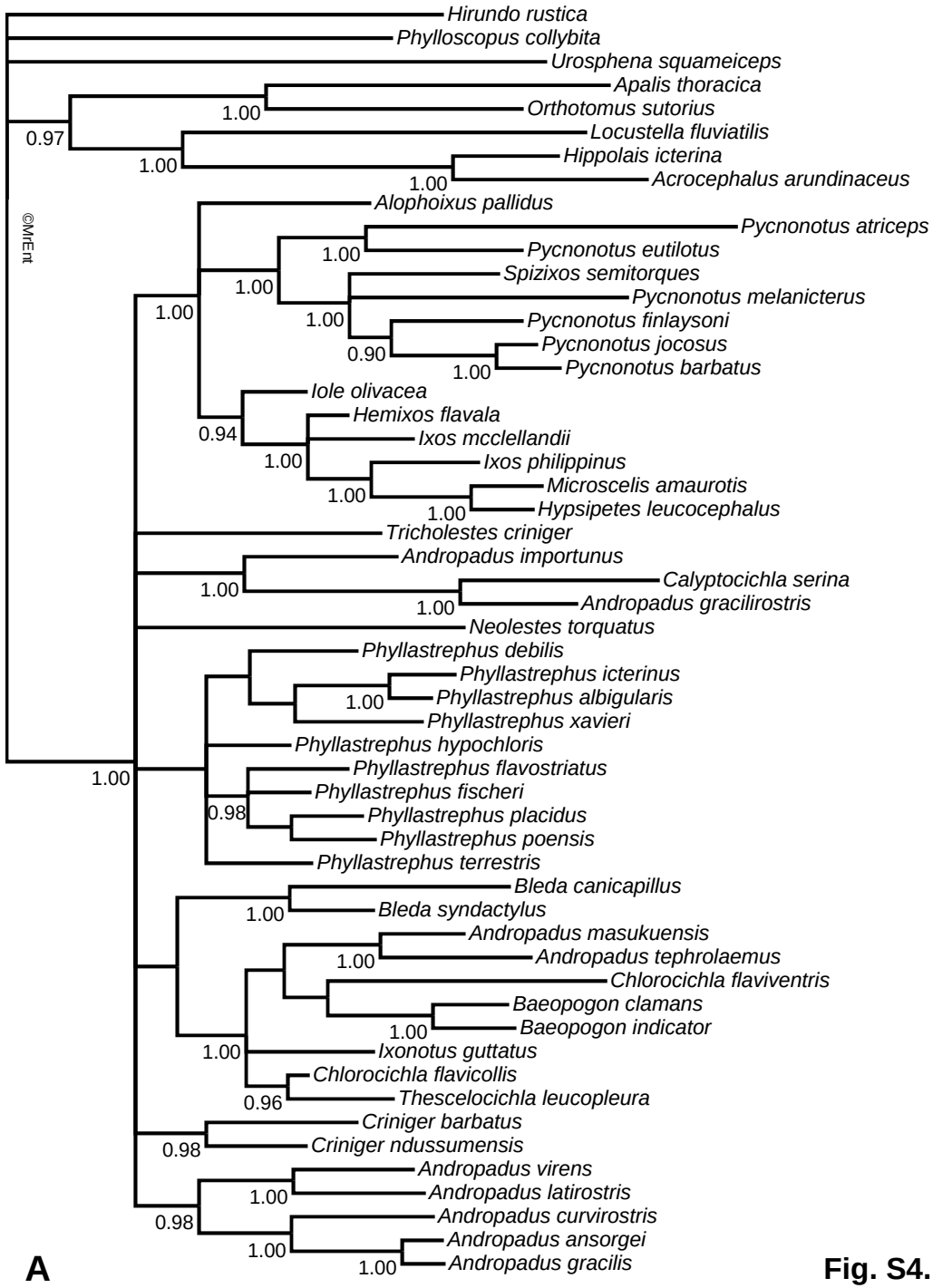
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Alophoixus pallidus
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Baeopogon indicator
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Thescelocichla leucopleura
Criniger barbatus
Criniger ndussumensis
Andropadus virens
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Andropadus curvirostris
Andropadus ansorgei
Andropadus gracilis
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A
Fig. S4. Myoglobin
B

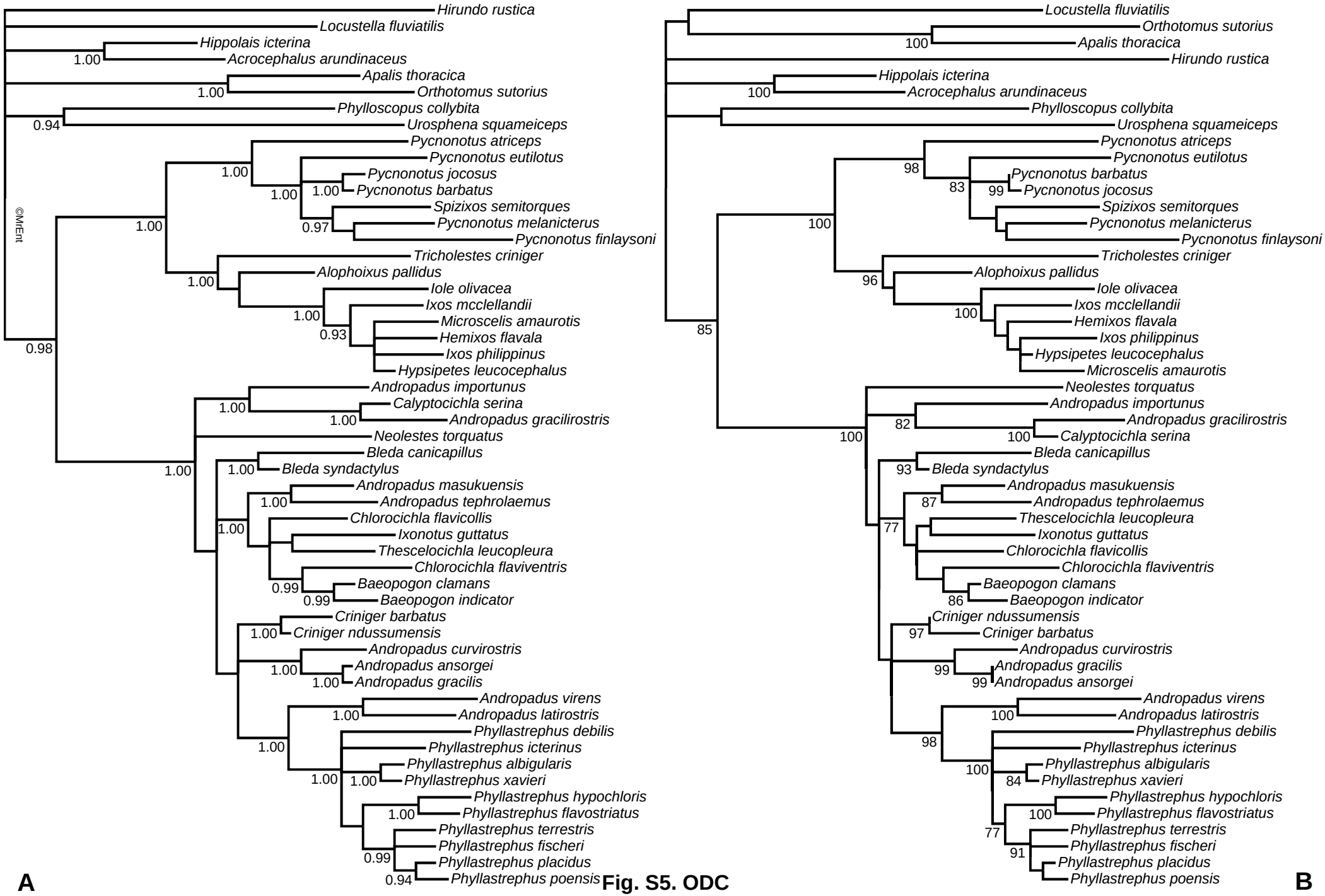
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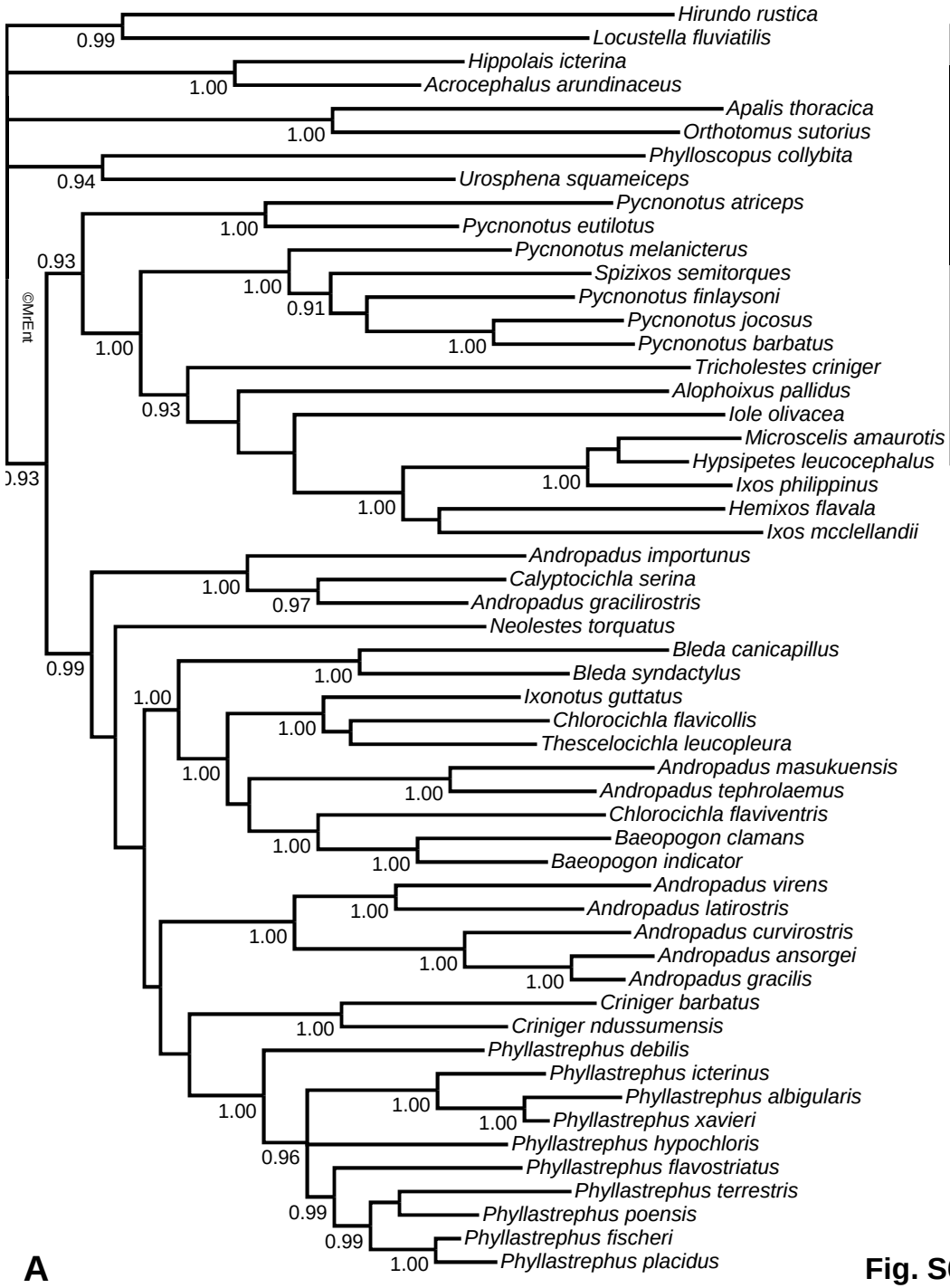
Fig. S5. ODC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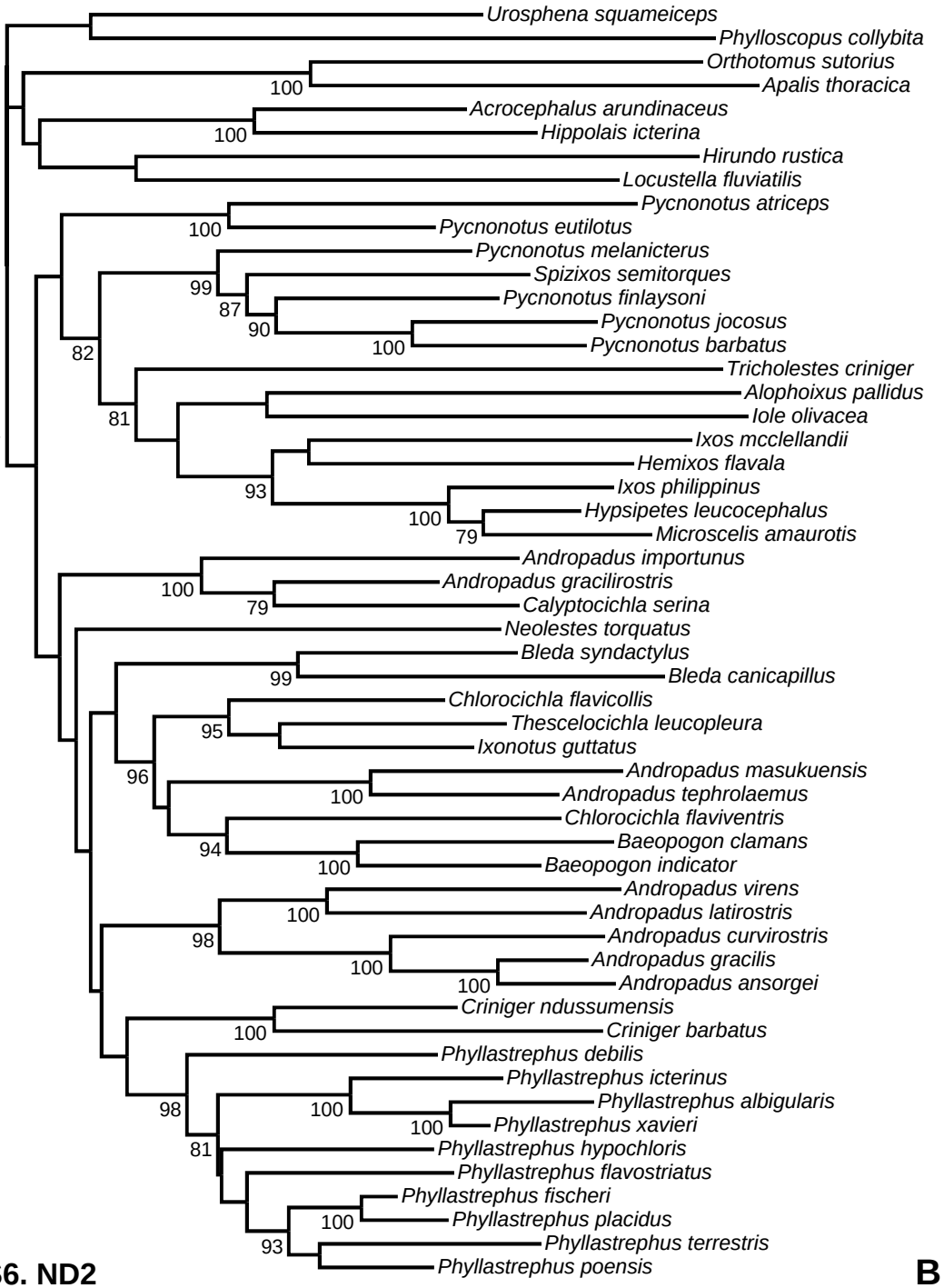
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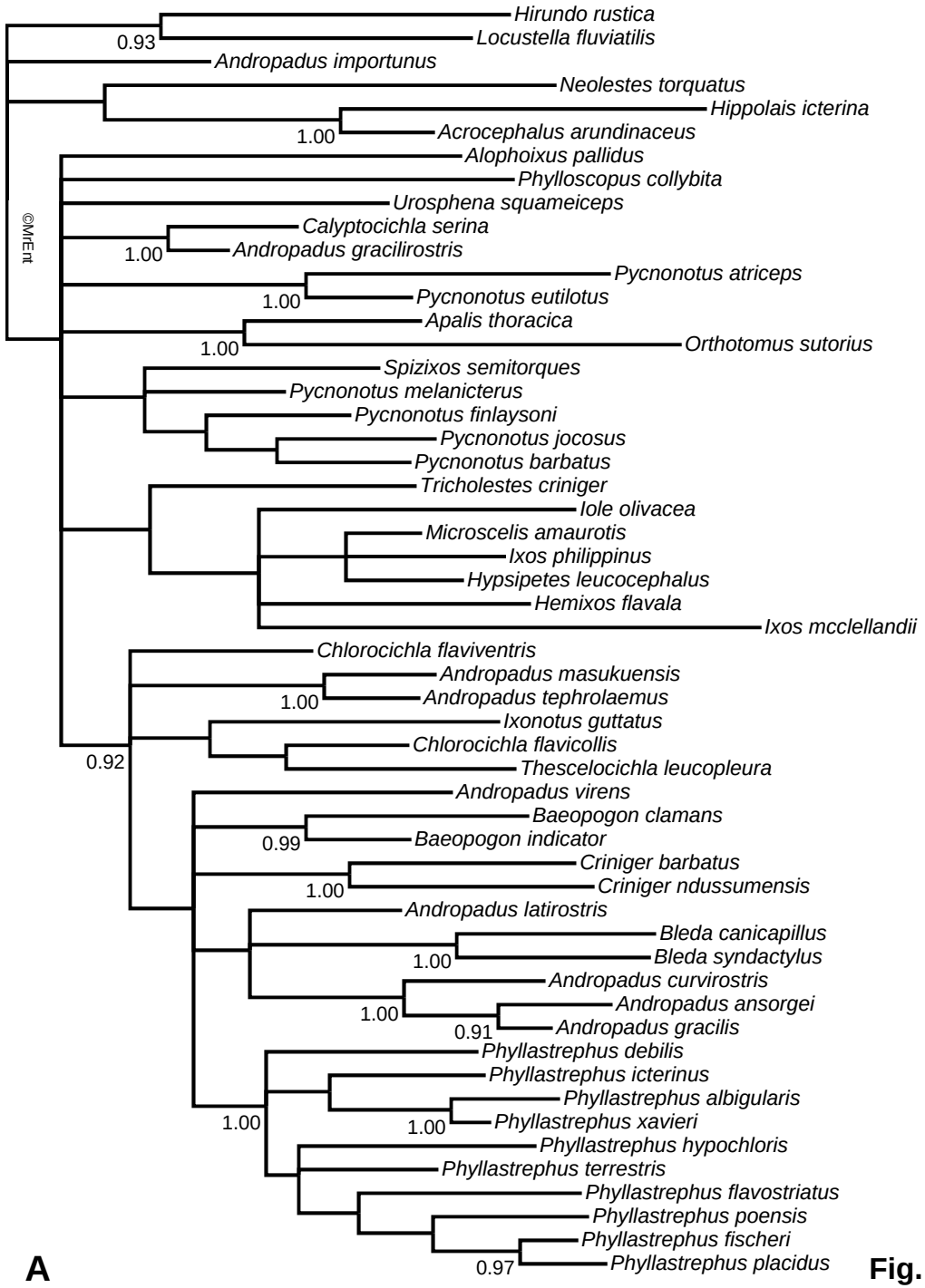
B

Fig. S6. ND2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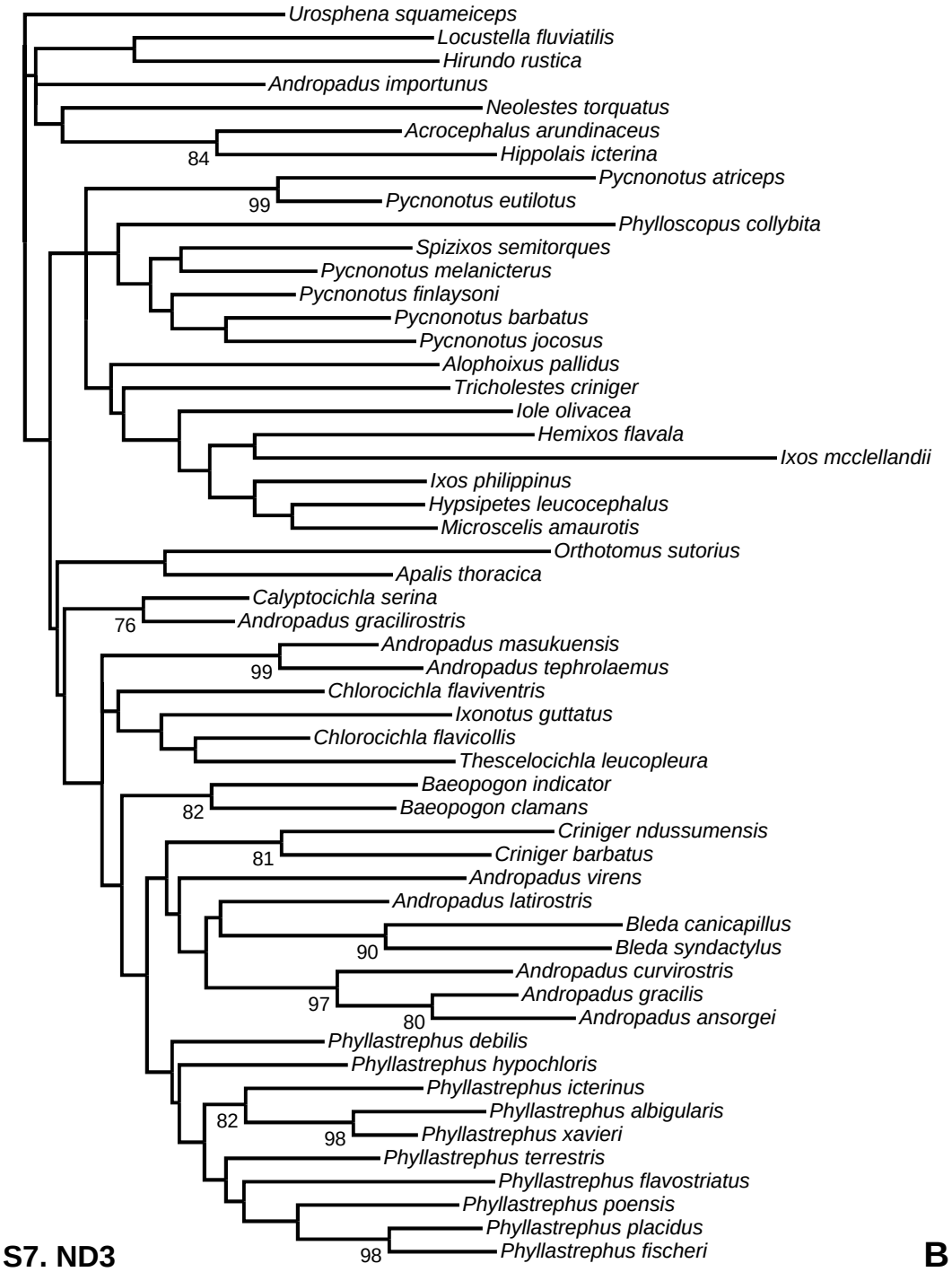
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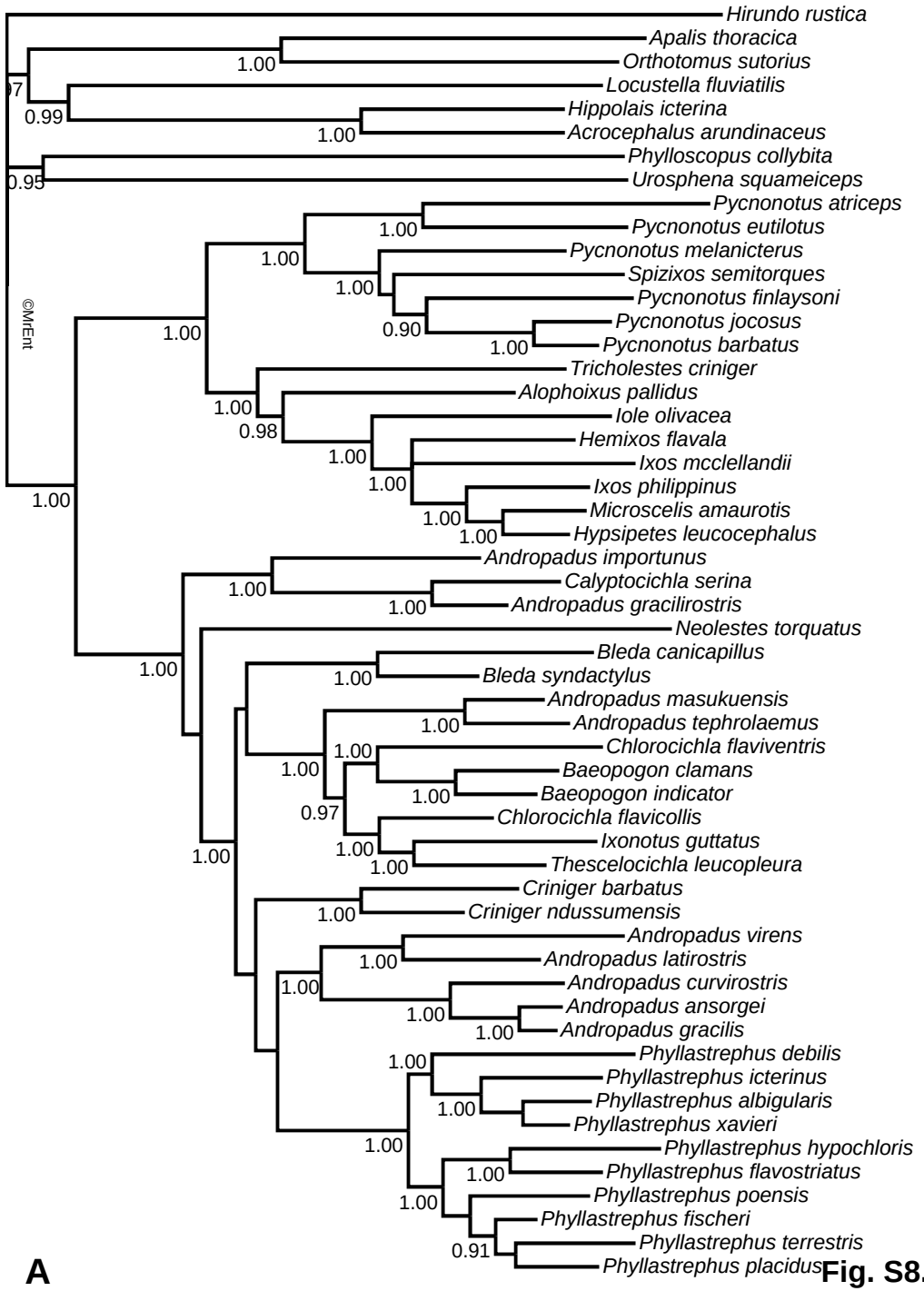
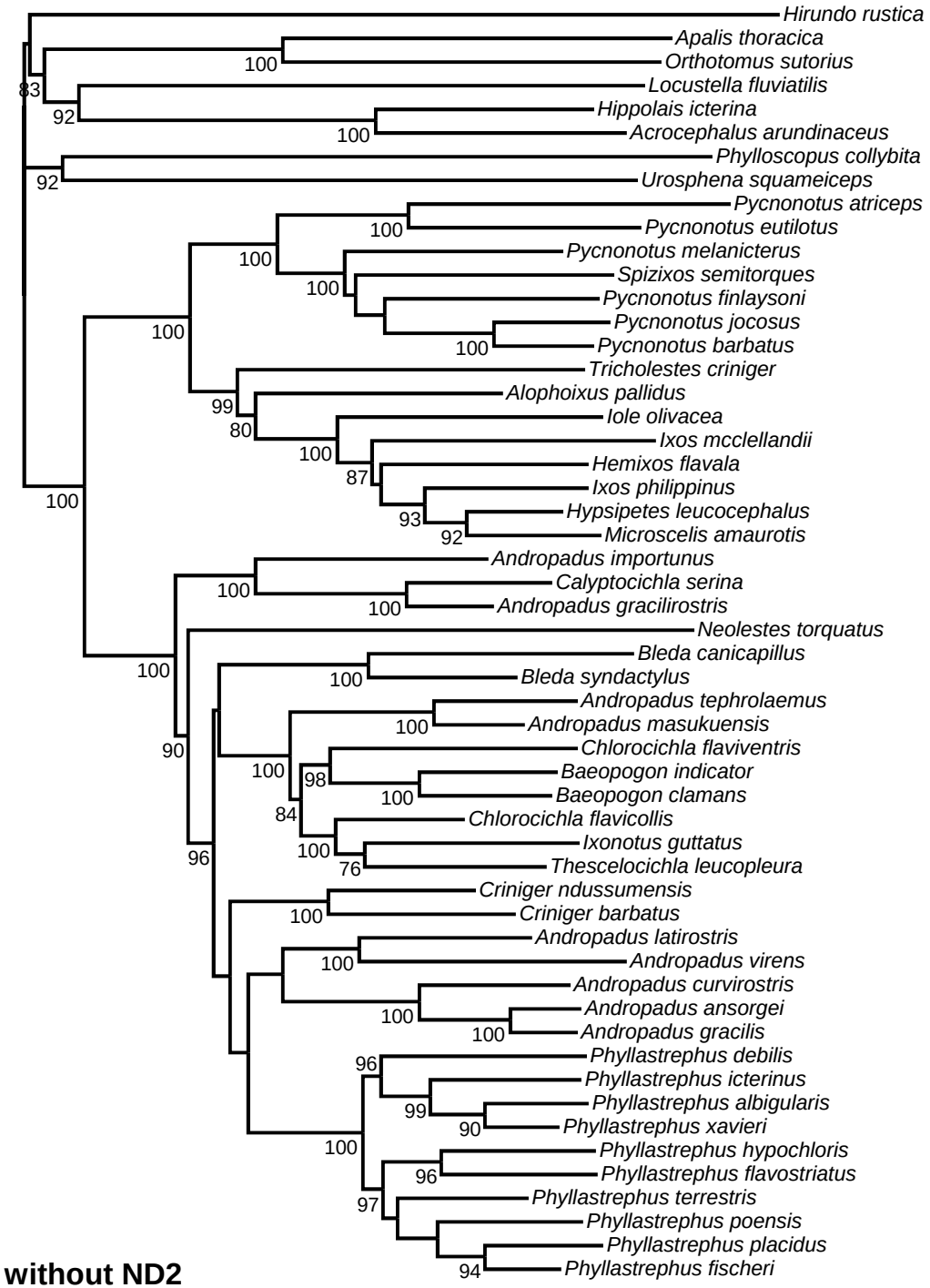
Bleda canicapillus
90
Bleda syndactylus
Andropadus curvirostris
97
Andropadus gracilis
80
Andropadus ansorgei
Phyllastrephus debilis
Phyllastrephus hypochloris
Phyllastrephus icterinus
82
Phyllastrephus albigularis
98
Phyllastrephus xavieri
Phyllastrephus terrestris
Phyllastrephus flavostriatus
Phyllastrephus poensis
Phyllastrephus placidus
98
Phyllastrephus fischeri

B

Fig. S7. ND3





A

Fig. S8. without ND2

B

Table S1. Primer pairs used for the amplification and sequencing of the longer genes of *Neolestes torquatus* (β-fibrinogen intron 5, myoglobin intron 2, ornithine decarboxylase introns 6-7 and NADH dehydrogenase II). For all combinations, the amplification profile was: initial denaturation 5' at 95°C, 40 cycles of denaturation 40" at 95°C, annealing 40" at the temperature indicated for each primer pair, extension 60" at 72°C, with a final extension of 5' at 72°C. ND3 and β-Fib7 were short enough for direct amplification using standard primers (see main text). [1]: Fuchs *et al.* 2004; [2]: Irestedt *et al.* 2002; [3]: Allen & Omland 2003 (see the main text for the full references).

Forward		**Reverse**		**Annealing Temp.**
Fib5 [1]	5’-CGCCATACAGAGTATACTGTGACAT-3’	bFib5-R325	5’-AAGGATGrCCCTGGTCTTTTsTC-3’	56
bFib5-F292	5’-TGGCTCATTTCTTACCCTGCTCCA-3’	Fib6 [1]	5’-GCCATCCTGGCGATTCTGAA-3’	56
Myo2 [2]	5’-GCCACCAAGCACAAGATCCC-3’	Myo-R180	5’-TGAGCCTTTTCTGTGCCTCCTGCT-3’	59
MyoF138	5’-CAGTGAGCTCTCCCTCAAGTCCA-3’	MyoR340	5’-TCCCTTGTGTCCGGTCACTGACA-3’	59
MyoF291	5’-AGCCTATGCATGCCTGGGAAACTG-3’	MyoR546	5’-GGAGCCTGGGCTAGGCAGAAGCA-3’	59
MyoF493	5’-GGTCTACTCAAGGTCATGAAGCA-3’	Myo3F [2]	5’-AAGTCATTATCAAGGTCCTTGCTGAA-3’	61
OD6 [3]	5’-GACTCCAAAGCAGTTTGTCGTCTCAGTGT-3’	ODC-R255	5’-TGTACATCCACTTCCAACGTGGAA-3’	57
ODC-F202	5’-ACTAATTTGCCAAATAGCAAGTGATA-3’	ODC-R506	5’-ATTGAGCTRCCARTTTTAGTGCAT-3’	56
ODC-F469	5’-AGCTTACTTTGACCAGCTTGGCAA-3’	OD8R [3]	5’-ATTGGTGGTGGCTTCCCTGGCTCTGAAGA-3’	58
ND2-ExtF	5’-AGCTATCGGGCCCATACCCCGAA-3’	ND2-R387	5’-TGGTGGGAATTTTATGGCTGTGGA-3’	58
ND2-F308	5’-AAAACTAGGACTAGCCCCCTTCCA-3’	ND2-R571	5’-TTTGGGGCTGTAGACTATGACGA-3’	58
ND2-F476	5’-CTCTGCAGCCCTAGGCGGAT-3’	ND2-R768	5’-AGTCATTTGGGGAGGAAGCCTGT-3’	58
ND2-F701	5’-GAGCAAAAACCCCAGCTTTAAGCGC-3’	ND2-R873	5’-TGCGCAGTATGCAAGTCGGAGGTA-3’	58
ND2-F828	5’-GTAGCAACAATCATCGCCCTCCT-3’	ND2-ExtR	5’-TTGAAGGCCTTCGGTTTAGGTGA-3’	56

Table S2. Sequence characteristics of the six genes analyzed.

Locus	**βFib5**	**βFib7**	**Myoglobin**	**ODC**	**ND2**	**ND3**
Alignment length	611	296	734	787	1041	351
Number of variable bases (%)	198 (32.4%)	98 (33.1%)	291 (39.6%)	337 (42.8%)	644 (61.9%)	199 (56.7%)
Number of parsimony informative bases (%)	150 (24.5%)	41 (39.6%)	133 (18.1%)	162 (20.6%)	577 (55.4%)	181 (51.6%)
% A nucleotides (range)	29.1 (27.6-30.1)	33.4 (25-37.2)	28.0 (27.3-29.9)	28.0 (27.0-30.0)	30.4 (27.7-33.4)	28.4 (24.9-32.2)
% C (range)	17.1 (16.1-18.0)	18.5 (12.9-25)	22.4 (21.2-23.2)	16.8 (15.4-17.9)	34.5 (31.7-37.0)	33.2 (30.5-36.2)
% G (range)	21.4 (20.0-22.6)	16.8 (9.57-25.0)	23.0 (22.0-23.7)	20.4 (19.5-21.4)	11.1 (8.83-12.8)	13.6 (11.7-16.2)
% T (range)	32.3 (31.5-34.2)	31.3 (25.0-38.0)	26.6 (25.7-27.9)	34.8 (33.5-36.5)	24.0 (21.8-26.0)	24.8 (22.8-27.4)
χ^2 (d.f. = 159)	12.25 (P = 1.00)	90.37 (P = 0.99)	10.60 (P = 1.00)	18.88 (P = 1.00)	131.84 (P = 0.94)	56.50 (P = 1.00)
Selected substitution model	GTR+Γ	GTR+Γ	HKY+Γ	GTR+Γ	GTR+Γ+I	GTR+Γ+I