



Phylogeny and biogeography of the Malagasy and Australasian rainbowfishes (Teleostei: Melanotaenioidei): Gondwanan vicariance and evolution in freshwater

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Received 16 March 2004; revised 21 June 2004

Available online 25 August 2004

Abstract

Phylogenetic relationships of the Malagasy and Australasian rainbowfishes are investigated using 4394 characters derived from five mitochondrial genes (12S, 16S, tRNA-Valine, ND5, and COI), three nuclear genes (28S, histone H3, and TMO-4c4), and 102 morphological transformations. This study represents the first phylogenetic analysis of the endemic Malagasy family Bedotiidae and includes a nearly complete taxonomic review of all nominal species, as well as numerous undescribed species. Simultaneous analysis of the molecular and morphological datasets results in two equally most parsimonious trees. Results indicate that Bedotiidae (*Bedotia* + *Rheocles*) and *Bedotia* are monophyletic, whereas *Rheocles* is paraphyletic with the inclusion of two recently described species from northeastern Madagascar, *R. vatosoa*, and *R. derhami*. *Rheocles vatosoa* and *R. derhami* are sister taxa, and this clade is recovered as the sister group to *Bedotia*. The remaining species of *Rheocles* are not sexually dimorphic and comprise a clade that is recovered as the sister group to *Bedotia* + (*R. derhami* + *R. vatosoa*), all of which are sexually dichromatic, and sexually dimorphic for pigmentation and fin development. Three geographically distinct clades are recovered within *Bedotia*, one comprising species with distributions ranging from mid- to southeastern Madagascar, another including species restricted to eastern drainages north of the Masoala Peninsula, and a third comprising taxa with distributions extending from the Masoala Peninsula south to the Ivoloina River. The Australian/New Guinean melanotaeniids are monophyletic and are recovered as the sister group to Bedotiidae. The Australasian Telmatherinidae and Pseudomugilidae comprise a clade that is recovered as the sister group to the Melanotaeniidae-Bedotiidae clade. This sister-group relationship between Malagasy bedotiids and a clade restricted to Australia–New Guinea, and the absence of a close relationship between bedotiids and African or Mascarene atheriniforms, is congruent with the break-up of Gondwana, not a scenario reliant on Cenozoic trans-oceanic dispersal. Finally, results of the phylogenetic analysis indicate that Atheriniformes is polyphyletic and further corroborate recent morphological hypotheses, which have recovered Bedotiidae in a derived position within Atherinoidei.

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Keywords: *Bedotia*; *Rheocles*; Bedotiidae; Melanotaeniidae; Systematics; Madagascar; Gondwana

1. Introduction

The atherinoid family Bedotiidae is endemic to Madagascar and comprises two genera, *Bedotia* and *Rheocles*. These small (most species <100.0 mm SL), laterally compressed, and generally colorful fishes are commonly

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referred to as Malagasy rainbows or rainbowfishes. Most bedotiids exhibit varying degrees of sexual dimorphism, which ranges from subtle to striking. *Bedotia* and *Rheocles* occur exclusively in freshwater environments and are distributed in small to medium-sized forested rivers and streams, occasionally in swamps and marshes, spanning nearly the entire eastern slope of Madagascar (a single species of *Rheocles* is recorded from a westward draining basin in the northeast of the island). *Bedotia* (Regan, 1903) comprises six nominal species [*B. madagascariensis* (Regan, 1903), *B. geayi* (Pellegrin, 1907), *B. longianalis* (Pellegrin, 1914b), *B. tricolor* (Pellegrin, 1932), *B. marojejy* (Stiassny and Harrison, 2000), and *B. masoala* (Sparks, 2001)]. Relationships among constituent taxa remain, for the most part, unresolved. Except for coloration and pigmentation, *Bedotia* are morphologically conservative fishes. In a recent checklist of the freshwater fishes of Africa, Maugé (1986) synonymized *B. longianalis* and *B. tricolor* with *B. geayi*, although no justification for this taxonomic rearrangement was given (Sparks, 2001). Based on examination of the primary types for all species of *Bedotia* and comparison of collections made throughout eastern Madagascar, it is apparent that several distinct species of *Bedotia* inhabit the island, many of which are undescribed (Sparks and Stiassny, 2003).

Rheocles (Jordan and Hubbs, 1919) comprises seven valid species [viz., *R. sikorae* (Sauvage, 1891), *R. alaotrensis* (Pellegrin, 1914a), *R. pellegrini* (Nichols and LaMonte, 1931), *R. wrightae* (Stiassny, 1990), *R. lateralis* (Stiassny and Reinthal, 1992), *R. derhami* (Stiassny and Rodriguez, 2001), and *R. vatosoa* (Stiassny et al., 2002)]. *Rheocles* was recently revised by Stiassny (1990), in which a third Malagasy bedotioid genus, *Rheocloides* (Nichols and LaMonte, 1931), was formally synonymized with *Rheocles*. The number of described species has nearly doubled since Stiassny's (1990) revision, and placement of the two most recently described species within the genus is problematic.

Monophyly of *Bedotia*, *Rheocles*, and a clade comprising *Bedotia* and *Rheocles* (= Bedotiidae) has been addressed in several recent studies (Aarn and Ivantsoff, 1997; Dyer and Chernoff, 1996; Stiassny, 1990). This monophyletic assemblage has been given either familial, Bedotiidae (Rosen and Parenti, 1981; Saeed et al., 1994; Stiassny, 1990), or subfamilial rank, Bedotiinae, within Melanotaeniidae (Dyer and Chernoff, 1996). Regardless of rank, neither monophyly nor the composition of Bedotiidae is disputed, yet their placement within the so-called "atherinoid" group of Atherinomorpha has been the subject of considerable debate (Aarn and Ivantsoff, 1997; Dyer and Chernoff, 1996; Stiassny, 1990) (Fig. 1). Rosen and Parenti (1981) concluded that evidence was lacking to support monophyly of atherinoids, an assemblage of six families (Atherinidae, Bedotiidae, Isonidae, Melanotaeniidae, Phallostethidae, and Tel-

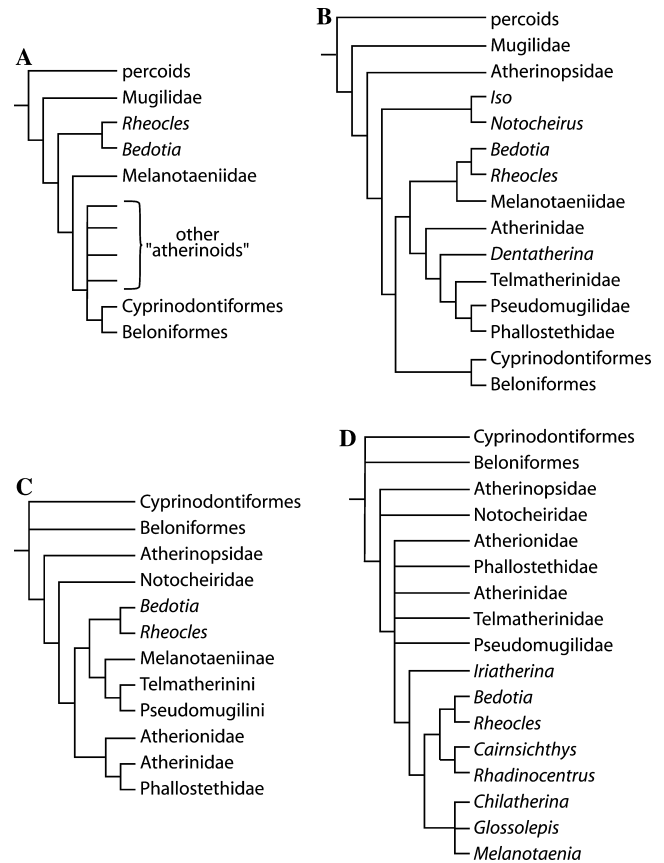


Fig. 1. Hypotheses of inter- and intrafamilial relationships of Bedotiidae and Melanotaeniidae based on morphological evidence according to (A) Stiassny (1990), (B) Saeed et al. (1994), (C) Dyer and Chernoff (1996), and (D) Aarn and Ivantsoff (1997).

matherinidae), which they referred to as Division I of Series Atherinomorpha. Stiassny (1990) and Saeed et al. (1994) failed to provide evidence in support of monophyly of Atherinoidei. A monophyletic Atherinoidei, comparable to that delimited by Rosen and Parenti (1981), however, was recovered by Dyer and Chernoff (1996). Although assigned different ranks by various authors, for clarity and consistency familial-level rank is retained throughout for bedotiids, melanotaeniids, and pseudomugilids (herein including telmatherinids), which we refer to collectively as rainbowfishes.

The similarity between the Malagasy bedotiids and Australian/New Guinean melanotaeniids is striking, and a close relationship has been intimated by numerous authors dating back to the early 20th century (Jordan and Hubbs, 1919). In recent cladistic analyses, bedotiids and melanotaeniids are either recovered as sister taxa (Saeed et al., 1994), bedotiids are the sister taxon to a clade comprising melanotaeniids, pseudomugilids, and telmatherinids (Dyer and Chernoff, 1996), or bedotiids are nested within a paraphyletic melanotaeniid assemblage (Aarn and Ivantsoff, 1997) (Fig. 1). The placement and interrelationships of telmatherinids and pseudomugilids, likewise, varies considerably among analyses.

According to Dyer and Chernoff (1996), pseudomugilids and telmatherinids comprise the sister taxon to melanoetaeniids, and this clade is in turn the sister taxon to bedotiids, whereas the hypotheses presented by Saeed et al. (1994) and Aarn and Ivantsoff (1997) do not unite telmatherinids or pseudomugilids in a clade with bedotiids and melanotaeniids (Fig. 1).

Genotypic and phenotypic characters will be used to test these hypotheses of relationships and to examine the biogeography of Malagasy and Australasian rainbowfishes within a phylogenetic context. Results of recent molecular studies suggest that many of Madagascar's terrestrial vertebrate lineages owe their origin to random dispersal from Africa or Asia, well after the Mesozoic break-up of the Gondwanan supercontinent (Caccone et al., 1999; Jansa et al., 1999; Mausfeld et al., 2000; Nagy et al., 2003; Raxworthy et al., 2002; Yoder and Yang, 2004; Yoder et al., 1996, 2003). The conclusions of these studies reiterate the prevailing opinion offered by paleontologists and many ichthyologists (e.g., Aarn and Ivantsoff, 1997) that Madagascar's extant vertebrates, including freshwater fishes, arrived via oceanic dispersal (Gottfried and Krause, 1998; Krause et al., 1997; Lundberg, 1993; Murray, 2001). Paleontologists have defended dispersal hypotheses by citing a lack of Cretaceous-age fossils for Madagascar's extant assemblages of freshwater fishes (Gottfried and Krause, 1998; Krause et al., 1997; Lundberg, 1993; Murray, 2001) and by pointing to the alleged salinity tolerance of some Malagasy freshwater fishes (Lundberg, 1993; Murray, 2001). Our study will test the sister group relationship of Malagasy and Australasian rainbowfishes recovered by Dyer and Chernoff (1996), a topology consistent with the fragmentation of Gondwana. We will examine the distribution patterns of rainbowfishes throughout their range for congruence with regional vicariance events. For example, it is generally assumed that rainbowfishes have dispersed via the ocean in the Australasian region; we will examine these conjectures in light of phylogenetic and paleogeographic evidence.

We present a phylogenetic hypothesis for rainbowfishes based upon the simultaneous analysis of 4292 molecular characters and the 102 morphological transformations described by Dyer and Chernoff (1996). This analysis includes all nominal bedotiid species, less two members of *Rheocles* (viz., *R. sikorae* and *R. pellegrini*) for which tissue samples could not be obtained. *Rheocles sikorae*, a species from eastern forests that has not been collected in recent years, is presumed to be extinct, and *R. pellegrini*, from northern Madagascar, is known only from the holotype and two (out of three original) remaining paratypes. Representatives from several populations of *Bedotia* hypothesized to represent undescribed species based on detailed morphological comparisons (Loiselle and Stiassny, 2003; Sparks and Stiassny, 2003; Stiassny, pers. comm.) are also included.

The objectives of this study are: (1) to test monophyly of both Bedotiidae and an assemblage comprising the three Australasian rainbowfish families; (2) to determine if a sister-group relationship between the Malagasy bedotiids and Australasian rainbowfishes is supported with the addition of nucleotide characters; (3) to determine if the addition of molecular characters corroborates a derived placement for bedotiids and melanotaeniids within Atheriniformes; (4) to test monophyly of both *Rheocles* and *Bedotia* and resolve intrageneric relationships within these genera; and (5) to determine if the recovered phylogenetic pattern for the Malagasy and Australasian rainbowfishes is congruent with Earth history.

2. Materials and methods

2.1. Taxon sampling

The resulting hypotheses were rooted using the basal acanthomorph *Percopsis*. The putative atherinomorph sister-group, Mugilidae (following Stiassny, 1990), and three non-atheriniform atherinomorphs (*Cololabis*, *Fundulus*, and *Oryzias*) were included to rigorously test the monophyly of the Atheriniformes, rainbowfishes (Melanotaenioidei), and Bedotiidae. Fifty-nine atheriniform taxa were analyzed, including representatives from all atheriniform families. Ten Australasian rainbowfishes, all nominal bedotiid species (except *Rheocles pellegrini* and *R. sikorae*), and 11 undescribed *Bedotia* species were included in the analysis. Finally, both Malagasy non-bedotiid atheriniform genera with members that occur in freshwater (*Atherinomorus* and *Teramulus*) were included.

2.2. Acquisition of nucleotide sequences

Fish tissues were preserved in 70–95% ethanol prior to DNA extraction. Total genomic DNA was extracted from muscle, liver, or fin clips via use of a Qiagen Tissue Extraction Kit (QIAamp or DNeasy Tissue Extraction Kit) following the manufacturer's protocol. PCR was used to amplify fragments from five mitochondrial genes (12S, 16S, tRNA-Val, ND5, and COI) and three nuclear genes (28S, histone H3, and TMO-4c4). Double-stranded amplifications were performed in either 25 or 50 μ L volumes containing 1 \times PCR buffer (Qiagen), 2 mM MgCl₂, 0.2 mM of each dNTP, 0.2–0.5 μ L of each primer (10 μ M), 10–1000 ng of genomic DNA (1–2 μ L), and 1 μ L of *Taq* polymerase (Qiagen), or in a 25 μ L volume containing one Ready-To-Go PCR bead (Amersham Biosciences), 1.25 μ L of each primer (10 μ M) and 2–5 μ L of genomic DNA. PCR primers and profiles for all genes can be found in Smith and Wheeler (2004) or Sparks (2004), except for NADH dehydrogenase

subunit 5 (ND5). To amplify and sequence the ND5 fragment, two primer pairs were designed specifically for use in this study. The primers ND5PercA—L 5'-GGYTGATGATACGGNCGAGCAGA-3' and ND5PercB—H 5'-AGGGCTCAGGCGTTNAGGTG-3' were designed to amplify the ND5 fragment in most included taxa, whereas ND5AthA—L 5'-CTCCACCCTTGACTACCTTCC-3' and ND5AthB—H 5'GGTGAGATGTGTTDAGTGCTTCA-3' were required to amplify a smaller, overlapping segment of ND5 in certain atheriniform taxa that could not be amplified using the initial primer pairs. The double-stranded amplification products were either desalted and concentrated using Qiagen Quick-Spin PCR Purification Columns or an ArrayIt PCR Product Purification Kit (TeleChem International) using a Beckman BIOMEK 2000 laboratory automated pipetting workstation. When multiple bands were amplified, individual fragments were isolated on 1% agarose gels, excised under UV light, and extracted using a Qiagen Gel Extraction Kit. Both strands of the purified PCR fragments were used as templates and directly cycle-sequenced using the original amplification primers and an ABI Prism Dye Terminator Reaction Kit. Nucleotide sequences were analyzed on an ABI 377, ABI 3700, or ABI 3730xl automated DNA sequencer.

All taxa sequenced in the present study, along with collection locality data and GenBank accession numbers, are listed in Table 1. In many cases multiple individuals for each species were sequenced, but only *Bedotia longianalis* showed intraspecific variation. Because of this variation, both specimens were analyzed as separate terminals. Malagasy specimens were obtained from field collections made by the first author or Paul Loiselle (New York Aquarium/WCS). The remaining tissue samples were collected by the second author or obtained from various collectors, researchers, and institutions as documented. For two taxa (*Hypoatherina tsurugae* and *Melanotaenia lacustris*), sequences corresponding to the five mitochondrial genes analyzed were obtained from GenBank (Miya et al., 2003).

2.3. Morphological data

The morphological characters analyzed in this study were taken directly from the study of Dyer and Chernoff (1996), except that we recoded their five weighted characters (77, 80, 81, 82, and 83) to the equivalent number of equally weighted characters (for the POY analysis) and their character 76 in *Rheocles alaotrensis* because this species is not sexually dimorphic (following Stiassny, 1990; Stiassny and Rodriguez, 2001, p. 102; pers. obs.). The aforementioned changes resulted in 102 morphological transformation series. Morphological characters were coded at the generic level in Dyer and Chernoff (1996), and we have retained their coding at the generic

level except in cases where multiple species were sequenced within a genus. In these cases, we have only coded the morphological characters for the species examined by Dyer and Chernoff (1996), with one exception. We did not sequence any *Melanotaenia* species that was examined by Dyer and Chernoff (1996), so we coded the morphological matrix for *M. lacustris*, which McGuigan et al. (2000) recovered in their “clade D” with *M. trilineata*, a species which was examined morphologically by Dyer and Chernoff (1996).

2.4. Phylogenetic analysis

For the phylogenetic analysis, 4394 characters (102 morphological transformations and 4292 aligned nucleotide characters) were simultaneously analyzed under the optimality criterion of parsimony, using equal weights for 63 terminals. Twenty-eight terminals (Table 2) were coded with missing data for the morphological data (primarily numerous bedotiid species), because these terminals were not examined by Dyer and Chernoff (1996). Eleven terminals (*Notocheirus*, *Denatherina*, *Phallostethus*, *Gulaphallus*, *Stenatherina*, *Alepidomus*, *Atherinason*, *Kestratherina*, *Leptatherina*, *Allanetta*, and *Quirichthys*) were coded with missing data for the molecular data because tissue samples for these genera could not be collected. Nine additional DNA fragments were coded as missing. All three nuclear DNA fragments for *Hypoatherina* and *Melanotaenia lacustris* were coded as missing data because their sequences were acquired from Miya et al.'s (2003) study of teleostean mitochondrial genomes, and the ND5 sequences from *Atherinosoma*, *Cairnsichthys*, and *Rhadinocentrus* were coded as missing data in the analysis because these fragments could not be successfully amplified and sequenced.

The parsimony analysis was conducted using direct optimization (Wheeler, 1996) as implemented in the program POY (vers. 3.0, Wheeler, W. C., Gladstein, D., and DeLaet, J., American Museum of Natural History, New York, NY, 2003) and run on the American Museum of Natural History Parallel Computing Cluster. For ribosomal fragments, the sequences were initially broken up according to secondary structure (see Smith and Wheeler, 2004). The analysis began with 200 random addition replicates, followed by TBR branch swapping, tree fusing (Goloboff, 1999), and 200 parsimony ratchet replicates (Nixon, 1999; ratchetpercent 35, ratchetseverity 3). The random replicates from this initial search resulted in 13 unique trees. These 13 trees were submitted to POY for further tree searching using iterative pass (Wheeler, 2003a) and exact. This second analysis began by tree fusing (Goloboff, 1999) the 13 submitted topologies and 20 additional random addition replicates. The resulting trees were submitted to 50 rounds of parsimony ratcheting (ratchetpercent 35, ratchetseverity 3) and a final round of tree fusing and TBR

Table 1
Collection localities and GenBank accession numbers for taxa sequenced for this study

Species	Collection locality	ND5	COI	12S-tVal-16S	16S	Histone H3	TMO-4c4	28S
Bedotiidae								
<i>Bedotia geayi</i>	Mananjary, SE Madagascar	AY444890	AY290799	AY268915	AY266065	AY655547	AY655597	AY655647
<i>Bedotia longianalis</i> 1	Menantany Stream, Eastern Madagascar	AY444880	AY290789	AY268899	AY266055	AY655548	AY655598	AY655648
<i>Bedotia longianalis</i> 2	Mahambo Swamp, Eastern Madagascar	AY444893	AY290802	AY268918	AY266068	AY655549	AY655599	AY655649
<i>Bedotia madagascariensis</i>	Sahameloka, Ivoloina Basin, Eastern Madagascar	AY444885	AY290794	AY268906	AY266060	AY655550	AY655600	AY655650
<i>Bedotia marojejy</i>	Lokofo River, NE Madagascar	AY444883	AY290792	AY268903	AY266058	AY655551	AY655601	AY655651
<i>Bedotia masoala</i>	Masoala Peninsula, NE Madagascar	AY444888	AY290797	AY268913	AY266063	AY655552	AY655602	AY655652
<i>Bedotia tricolor</i>	Karianga, SE Madagascar	AY444891	AY290800	AY268916	AY266066	AY655553	AY655603	AY655653
<i>Rheocles alaotrensis</i>	Perinet Reserve, Eastern Madagascar	AY444895	AY290804	AY268908	AY266070	AY655554	AY655604	AY655654
“ <i>Rheocles</i> ” <i>derhami</i>	Amboabo River, Sofia Drainage, NE Madagascar	AY444896	AY290805	AY268909	AY266071	AY655555	AY655605	AY655655
<i>Rheocles lateralis</i>	Upper Mangoro Drainage, Eastern Madagascar	AY444897	AY290806	AY268910	AY266072	AY655556	AY655606	AY655656
“ <i>Rheocles</i> ” <i>vatosoa</i>	Marovatobe Stream, Lokofo Drainage, NE Madagascar	AY444898	AY290807	AY268902	AY266073	AY655557	AY655607	AY655657
<i>Rheocles wrightae</i>	Mamavo River, Mangoro Basin, Eastern Madagascar	AY444894	AY290803	AY268896	AY266069	AY655558	AY655608	AY655658
<i>Bedotia</i> sp. “antalaha”	Andrakasaka Village, Ankavia Basin, NE Madagascar	AY444882	AY290791	AY268901	AY266057	AY655559	AY655609	AY655659
<i>Bedotia</i> sp. “beforona”	Lazona River, Eastern Madagascar	AY444879	AY290788	AY268898	AY266054	AY655560	AY655610	AY655660
<i>Bedotia</i> sp. “bemarivo”	Bemarivo River, Northeastern Madagascar	AY540119	AY540120	AY540116, AY540117	AY540118	AY655561	AY655611	AY655661
<i>Bedotia</i> sp. “betampona”	Fontsimavo River, Ivoloina Basin, Eastern Madagascar	AY444889	AY290798	AY268914	AY266064	AY655562	AY655612	AY655662
<i>Bedotia</i> sp. “ivoloina”	Ambodiriana, Ivoloina River, Eastern Madagascar	AY444878	AY290787	AY268897	AY266053	AY655563	AY655613	AY655663
<i>Bedotia</i> sp. “mahanara”	Ambavala Village, Mahanara Basin, NE Madagascar	AY444892	AY290801	AY268917	AY266067	AY655564	AY655614	AY655664
<i>Bedotia</i> sp. “makira”	Makira Region, Northeastern Madagascar	AY540124	AY540125	AY540121, AY540122	AY540123	AY655565	AY655615	AY655665
<i>Bedotia</i> sp. “manombo”	Manombo Reserve, SE Madagascar	AY444886	AY290795	AY268911	AY266061	AY655566	AY655616	AY655666
<i>Bedotia</i> sp. “ranomafana”	Ranomafana National Park, SE Madagascar	AY444881	AY290790	AY268900	AY266056	AY655567	AY655617	AY655667
<i>Bedotia</i> sp. “sambava”	Antsahoarana Village, Sambava Basin, NE Madagascar	AY444884	AY290793	AY268904	AY266059	AY655568	AY655618	AY655668
<i>Bedotia</i> sp. “vondrozo”	Vevembe Camp, SE Madagascar	AY444887	AY290796	AY268912	AY266062	AY655569	AY655619	AY655669
Melanotaeniidae								
<i>Cairnsichthys rhombosomoides</i>	Queensland, Australia	Unavailable	AY290816	AY268928	AY266082	AY655574	AY655624	AY655674
<i>Chilatherina bleheri</i>	Aquarium Trade	AY655534	AY655519	AY655504	AY655489	AY655570	AY655620	AY655670
<i>Iriatherina weneri</i>	Aquarium Trade	AY444904	AY290813	AY268905	AY266079	AY655571	AY655621	AY655671
<i>Glossolepis incisus</i>	Aquarium Trade	AY444905	AY290814	AY268924	AY266080	AY655572	AY655622	AY655672
<i>Melanotaenia praecox</i>	Aquarium Trade	AY444906	AY290815	AY268925	AY266081	AY655573	AY655623	AY655673
<i>Rhadinocestrus ornatus</i>	Aquarium Trade	Unavailable	AY655520	AY655505	AY655490	AY655575	AY655625	AY655675
Pseudomugilidae								
<i>Marosatherina ladigesii</i>	Aquarium Trade	AY444899	AY290808	AY268920	AY266074	AY655576	AY655626	AY655676
<i>Pseudomugil gertrudae</i>	Aquarium Trade	AY444907	AY290817	AY268926	AY266083	AY655577	AY655627	AY655677
<i>Pseudomugil signifer</i>	Aquarium Trade	AY655535	AY655521	AY655506	AY655491	AY655578	AY655628	AY655678
Atherinidae								
<i>Atherina hepsetus</i>	Mediterranean Sea	AY444901	AY290810	AY268922	AY266076	AY655579	AY655629	AY655679
<i>Atherinomorus</i> sp.	Karianga, SE Madagascar	AY444903	AY290812	AY268907	AY266078	AY655596	AY655646	AY655696
<i>Atherinomorus lacunosus</i>	Japan	AY655536	AY655522	AY655507	AY655492	AY655580	AY655630	AY655680
<i>Atherinosoma microstoma</i>	Australia	Unavailable	AY655523	AY655508	AY655493	AY655581	AY655631	AY655681
<i>Craterocephalus honoriae</i>	Australia	AY655537	AY655524	AY655509	AY655494	AY655582	AY655632	AY655682
<i>Teramulus waterloti</i>	Bora Reserve, Ankofia Basin, NW Madagascar	AY444902	AY290811	AY268919	AY266077	AY655583	AY655633	AY655683

(continued on next page)

Table 1 (continued)

Species	Collection locality	ND5	COI	12S-tVal-16S	16S	Histone H3	TMO-4c4	28S
<i>Iso rhothophilus</i>	Treachery Beach, New South Wales, Australia	AY444900	AY290809	AY268921	AY266075	AY655584	AY655634	AY655684
<i>Atherinopsidae</i>								
<i>Atherinella panamensis</i>	Panama (Eastern Pacific Ocean)	AY655538	AY655525	AY655510	AY655495	AY655585	AY655635	AY655685
<i>Atherinops affinis</i>	San Diego, California	AY444908	AY290818	AY268927	AY266084	AY655586	AY655636	AY655686
<i>Labidesthes sicculus</i>	Florida	AY655539	AY655526	AY655511	AY655496	AY655587	AY655637	AY655687
<i>Menidia menidia</i>	Shelter Island, NY	AY444909	AY290819	AY268923	AY266085	AY655588	AY655638	AY655688
<i>Phallostethidae</i>								
<i>Neostethus bicornis</i>	Thailand	AY655540	AY655527	AY655512	AY655497	AY655589	AY655639	AY655689
<i>Atherionidae</i>								
<i>Atherion elymus</i>	Japan	AY655541	AY655528	AY655513	AY655498	AY655590	AY655640	AY655690
<i>Outgroups</i>								
<i>Cololabis saira</i>	Off California	AY655542	AY655529	AY655514	AY655499	AY655591	AY655641	AY655691
<i>Fundulus heteroclitus</i>	Shelter Island, NY	AY655543	AY655530	AY655515	AY655500	AY655592	AY655642	AY655692
<i>Mugil cephalus</i>	Panama City, FL	AY655544	AY655531	AY655516	AY655501	AY655593	AY655643	AY655693
<i>Oryzias latipes</i>	Aquarium Trade	AY655545	AY655532	AY655517	AY655502	AY655594	AY655644	AY655694
<i>Percopsis omomistacus</i>	Unknown	AY655546	AY655533	AY655518	AY655503	AY655595	AY655645	AY655695

branch swapping of all shortest trees. The length of the resulting implied alignment (Wheeler, 2003b) was verified in NONA (vers. 3.0, Goloboff, P. A., Fundación e Instituto Miguel Lillo, Tucumán, Argentina, 1998).

To estimate the 'robustness' of the phylogenetic hypotheses recovered, jackknife resampling analyses were performed using NONA (1000 replications, five random addition sequences per replication) and Bremer supports (Bremer, 1988) were calculated using TreeRot (vers. 2, Sorenson, M. D., Boston University, Boston, MA, 1999; twenty random addition sequences per node) in conjunction with PAUP* (vers. 4.0b10, Swofford, D. L., PAUP*: phylogenetic analysis using parsimony and other methods, Sinauer, Sunderland, MA, 2002). Character evolution on the recovered topologies was examined using NONA and WinClada (vers. 0.99, Nixon, K. C., Cornell University, Ithaca, NY, 2000).

2.5. Biogeographic analyses

Alternative biogeographic hypotheses were analyzed and examined using multiple approaches. Taxon-area cladograms for Madagascar's three major clades of freshwater fishes were generated by replacing the names of terminal taxa with their region(s) of occurrence. Congruence between these taxon-area cladograms and a well-corroborated geologic area cladogram was used to select a general area cladogram for the relevant clades of freshwater fishes. In our opinion, congruence between these independently derived hypotheses is the most logical, and least assumption-laden, way to select a general area cladogram (Morrone and Carpenter, 1994). Analyzing taxon-area cladograms is straightforward if there are no widespread taxa, redundant distributions, or missing areas (Morrone and Carpenter, 1994; Nelson and Platnick, 1981), which is effectively the case for aplocheiloid killifishes and cichlids. We examine the aplocheiloid killifish and cichlid taxon-area cladograms for shared components [using protocols outlined by Nelson and Platnick (1981), Rosen (1978)], and discuss these results in light of a well-established geologic cladogram of Gondwanan break-up (i.e., the conventional Gondwanan vicariance model) (Smith et al., 1994; Storey, 1995). Deriving a general area cladogram for all three clades of freshwater fishes (aplocheiloid killifishes, cichlids, and rainbowfishes) simultaneously is less straightforward due to the presence of widespread taxa, redundant distributions, and missing areas. Here we use component analysis (Nelson and Platnick, 1981; Page, 1988) as implemented in COMPONENT (vers. 2, Page, R. D. M., published by the author, 1993) to generate resolved area cladograms (minimize losses option selected). This resulting general area cladogram is interpreted in the context of an independently derived geologic area cladogram of Gondwanan vicariance. Pacific plate boundaries found to be mostly congruent with shorefish

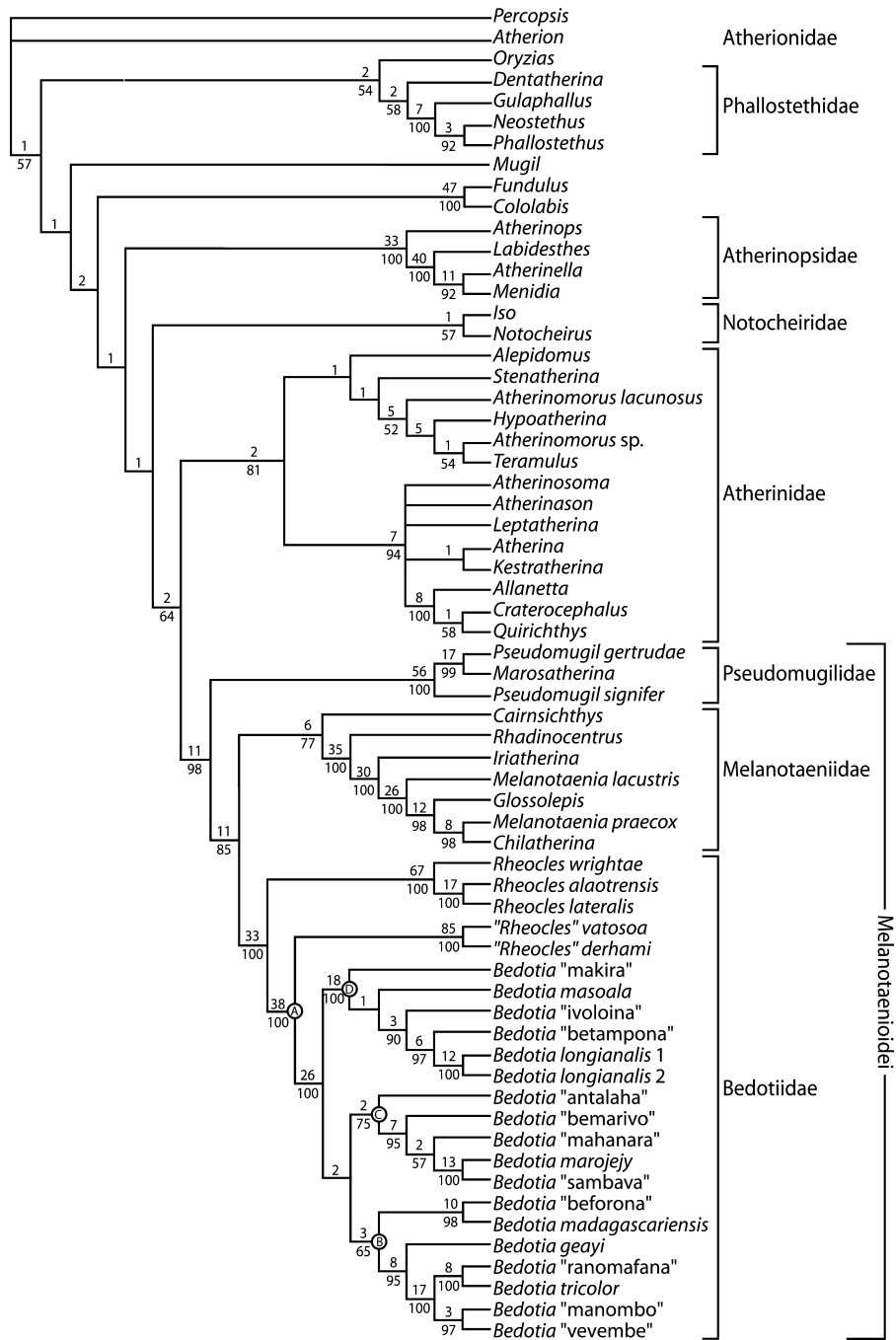


Fig. 2. Strict consensus of both equally most parsimonious trees (TL: 9820 steps, CI: 0.35, RI: 0.56) resulting from a parsimony analysis of 4273 aligned nucleotides and 102 morphological characters. Numbers above branches represent Bremer supports and numbers below branches represent jackknife resampling percentages (>50%) for each resolved node.

telmatherinid included in the analysis (*Marosatherina*) was nested within a paraphyletic Pseudomugilidae. A sister-group relationship between Pseudomugilidae + “Telmatherinidae” and Malagasy (Bedotiidae) + Australasian (Melanotaeniidae) rainbowfishes is recovered and strongly supported.

All traditionally recognized atheriniform families were recovered as monophyletic, except the Pseudomu-

gilidae as noted above. A monophyletic, albeit weakly supported, Atherinidae is recovered as the sister taxon to Melanotaenioidei. The endemic, primarily freshwater, Malagasy *Teramulus* and *Atherinomorus* (*Atherinomorus* sp.) are nested within this atherinid clade (Fig. 2). Notocheiridae and Atherinopsidae are recovered as subsequent sister-groups to the clade composed of Atherinidae + Melanotaenioidei. The Atheriniformes are not

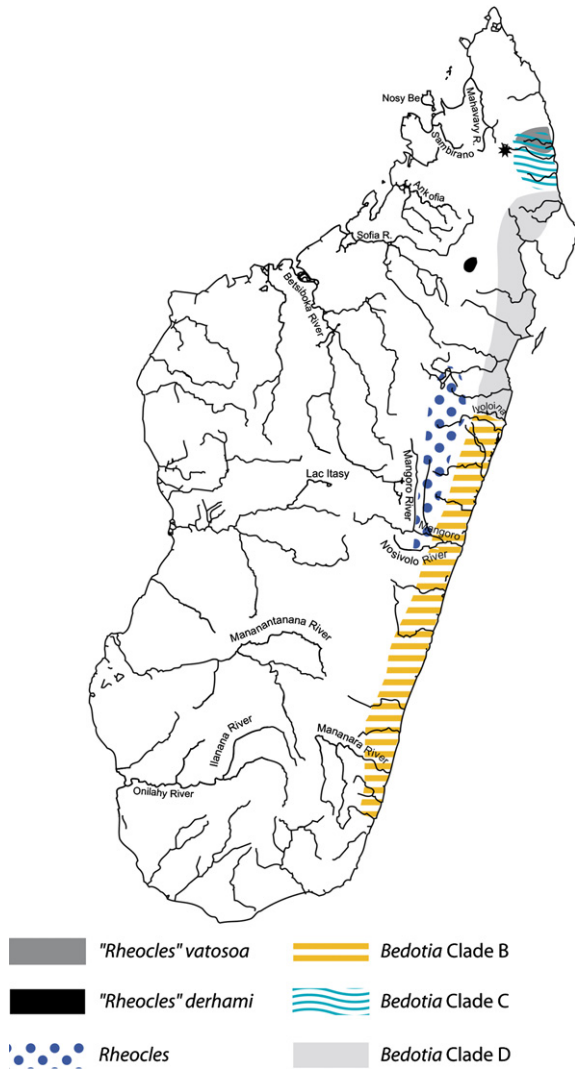


Fig. 3. Map of Madagascar indicating approximate geographic ranges for major bedotiid clades recovered in this study and discussed in text. An asterisk (*) indicates the presumed collection locality for the type series of *Rheocles pellegrini* (i.e., “one day west of Andapa”) according to Stiasny et al. (2002).

recovered as a monophyletic assemblage because the phallostethid and atherionid representatives included were found to be more distantly related to the remainder of the atheriniforms than three of the non-atheriniform outgroups included in the analysis (*Cololabis*, *Fundulus*, and *Mugil*).

In the biogeographic analysis, a general area cladogram is derived using COMPONENT vers. 2.0 for cichlids, aplocheiloid killifishes, and rainbowfishes (Fig. 4). This general area cladogram indicates a closer relationship between Madagascar and India, Australia, and Southeast Asia, than Madagascar to Africa or South America, a pattern congruent with the conventional and well-corroborated geological area cladogram of Gondwanan vicariance (Figs. 4D and E).

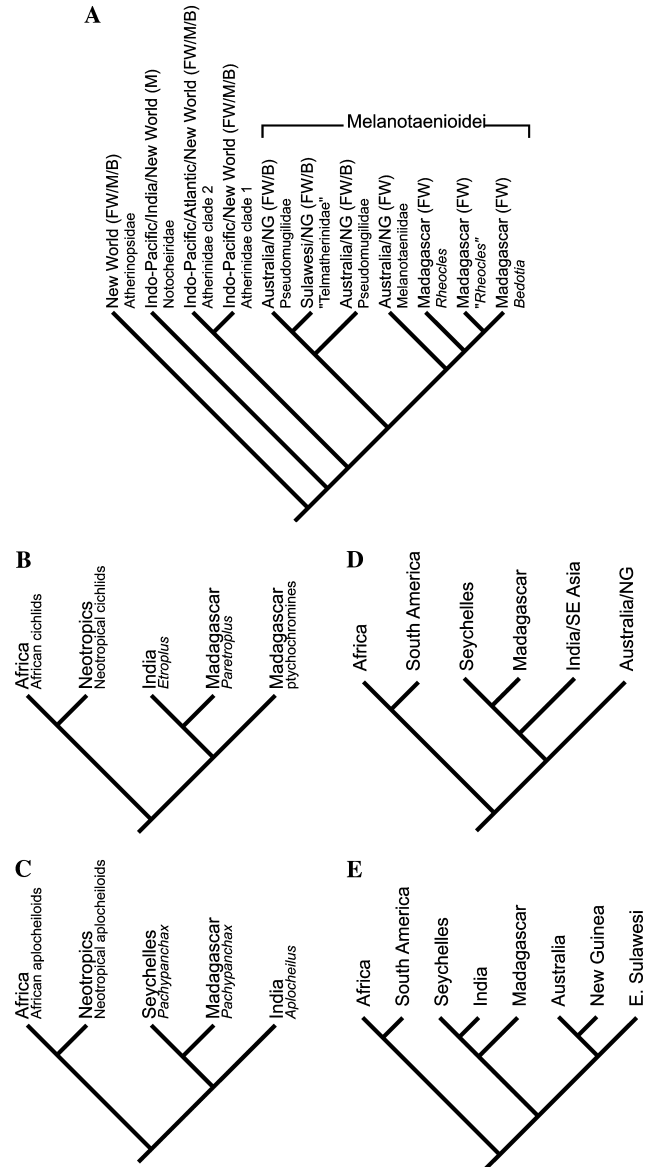


Fig. 4. (A) Taxon-area cladogram of rainbowfish (= Melanotaenioidae) relationships generated from strict consensus phylogeny. Habitat indicated in parentheses following taxon names (B = brackish, FW = freshwater, and M = marine). (B) Taxon-area cladogram of cichlid relationships (after Farias et al., 1999; Sparks, 2004). (C) Taxon-area cladogram of aplocheiloid killifish relationships (after Murphy and Collier, 1997). (D) General area cladogram derived using COMPONENT vers. 2.0 from simultaneous analysis of taxon-area cladograms of cichlids, aplocheiloid killifishes, and rainbowfishes (NG = New Guinea). (E) Geological area cladogram of Gondwanan break up (after Smith et al., 1994; Storey, 1995).

4. Discussion

4.1. Inter- and intrarelationships of rainbowfishes, and their placement within Atheriniformes

The strict consensus topology of the two most parsimonious trees recovered from the simultaneous analysis

of all characters is presented in Fig. 2. Monophyly of the Malagasy rainbowfishes, Bedotiidae, is strongly supported and congruent with hypotheses advanced in prior morphological studies (Aarn and Ivantsoff, 1997; Dyer and Chernoff, 1996; Stiassny, 1990) (Fig. 1). *Bedotia* is monophyletic, whereas *Rheocles* is recovered herein as a paraphyletic assemblage comprising two clades, one (“*Rheocles*”) placed as the sister taxon to *Bedotia* and the other (*Rheocles*) as the sister taxon to “*Rheocles*” plus *Bedotia* (clade A, Fig. 2). Support for monophyly of Bedotiidae, *Bedotia*, and the two recovered clades comprising nominal species of *Rheocles* is compelling, with both high Bremer support (26–85) and jackknife resampling percentages of 100% for all four clades.

In the strict consensus topology, a clade comprising the Australian and New Guinean Melanotaeniidae is recovered as the sister taxon to the Malagasy Bedotiidae (Fig. 2). The single telmatherinid included in the analysis (*Marosatherina*) is nested within a paraphyletic Pseudomugilidae, and we advocate synonymizing the former “Telmatherinidae” with Pseudomugilidae, which has nomenclatural priority. The sister-group relationship between Pseudomugilidae + “Telmatherinidae” and the Malagasy (Bedotiidae) + Australasian (Melanotaeniidae) rainbowfishes is robustly supported, and we accord this clade subordinal rank (= Melanotaenioidei). These results differ slightly from those of Dyer and Chernoff (1996), in which a clade comprising pseudomugilids + telmatherinids was recovered as the sister taxon to Melanotaeniidae. In the topology of Dyer and Cher-

noff (1996), this clade comprising pseudomugilids, telmatherinids, and melanotaeniids was recovered as the sister group to the Malagasy bedotiids. Our revised classification for rainbowfishes (= Melanotaenioidei) is presented in Table 3.

Bedotiids have been considered by many to represent the most primitive or least modified “atherinoids” (Rosen, 1964; Rosen and Parenti, 1981; Stiassny, 1990), whereas the Australasian melanotaeniids have been hypothesized to be more closely related to non-Malagasy atherinoids due to “a more derived condition of the two dorsals” (Rosen and Parenti, 1981, p. 14). To rigorously test the monophyly of Atheriniformes, rainbowfishes (Melanotaenioidei), and Bedotiidae, and as a result, the placement of rainbowfishes within Atheriniformes, numerous atheriniform and non-atheriniform outgroups were included, comprising representatives from all atheriniform families (see Materials and methods). The putative atheriniform sister-group, Mugilidae (following Stiassny, 1990), and three non-atheriniform atheriniforms (*Cololabis*, *Fundulus*, and *Oryzias*) were included, and the resulting hypotheses were rooted using the basal acanthomorph *Percopsis*. Contrary to claims regarding bedotiids as “primitive” atherinoids, and in agreement with the phylogenetic hypothesis advanced by Dyer and Chernoff (1996) based solely on morphological features, our results, based on a combination of morphological and nucleotide characters, also establish a derived position for Bedotiidae (and Melanotaeniidae) within Atheriniformes (Figs. 1 and 2).

4.2. Non-monophyly of *Rheocles*

A clade comprising (*R. wrightae* (*R. alaotrensis*, *R. lateralis*)) is recovered as the sister taxon to the remainder of Bedotiidae. The remaining nominal species of *Rheocles*, *R. derhami* + *R. vatosoa* (“*Rheocles*”), both recently described (Stiassny and Rodriguez, 2001; Stiassny et al., 2002), are monophyletic and recovered as the sister taxon to *Bedotia*. That *Rheocles* is paraphyletic is not particularly surprising. Morphological studies focusing on *Rheocles* have identified only a single (plesiomorphic) morphological feature, “a consolidated lower hypural fan that incorporates a fully fused parhypural” (Stiassny, 1990; Stiassny et al., 2002) to unite members of the group. A similar hypural configuration is present in most melanotaeniids, “telmatherinids,” and pseudomugilids (Allen, 1980; Dyer and Chernoff, 1996; Stiassny, 1990). Additional diagnostic features have been advanced in support of *Rheocles* monophyly (Aarn and Ivantsoff, 1997; Dyer and Chernoff, 1996), although these studies have included only a single representative, *R. alaotrensis*.

The name *Rheocles* appends to the clade composed of *R. alaotrensis*, *R. lateralis*, and *R. wrightae*, and accord-

Table 3

Revised classification of rainbowfishes (Melanotaenioidei) based on results recovered in this study

Series Atherinomorpha
Order Atheriniformes
Suborder Melanotaenioidei new usage
Family Bedotiidae
Genus <i>Bedotia</i>
Genus <i>Rheocles</i> sedis mutabilis
Genus “ <i>Rheocles</i> ” new genus
Family Melanotaeniidae
Genus <i>Cairnsichthys</i>
Genus <i>Iriatherina</i>
Genus <i>Pelangia</i> incertae sedis
Genus <i>Rhadinocentrus</i>
Genus <i>Glossolepis</i>
Genus <i>Melanotaenia</i>
Genus <i>Chilatherina</i>
Family Pseudomugilidae new usage
Genus <i>Kalyptatherina</i> incertae sedis
Genus <i>Kiunga</i> incertae sedis
Genus <i>Marosatherina</i>
Genus <i>Paratherina</i> incertae sedis
Genus <i>Pseudomugil</i>
Genus <i>Scaturiginichthys</i> incertae sedis
Genus <i>Telmatherina</i> incertae sedis
Genus <i>Tominanga</i> incertae sedis

ingly we are describing a new genus to encompass members of “*Rheocles*.” To date, Stiasny (1990) has made the only attempt to resolve relationships within *Rheocles* based on morphological features. Subsequent to the revision of *Rheocles* by Stiasny (1990), the number of included species has nearly doubled (Stiasny and Reinthal, 1992; Stiasny and Rodriguez, 2001; Stiasny et al., 2002). In our reconstruction, the species that were available to Stiasny (1990) comprise a monophyletic assemblage with the addition of *R. lateralis*, described two years subsequent to that study (Stiasny and Reinthal, 1992). *Rheocles sikorae*, type species of the genus, could not be sequenced given that this taxon has not been collected in recent years and is presumed extinct. Stiasny (1990, pp. 21–22) and Stiasny and Reinthal (1992, pp. 5–7) present compelling evidence that *R. sikorae* and *R. wrightae* are sister taxa (including, but not limited to, irregular lateral spotting, a darkly pigmented first dorsal fin, and pigmentation on the genital papilla), and we are confident in placing *R. sikorae* as the sister taxon to *R. wrightae*, within a clade that also includes *R. alaotrensis* and *R. lateralis*.

4.3. Monophyly of *Bedotia*

Although a comprehensive phylogenetic analysis of *Bedotia* has yet to be undertaken based on the comparison of morphological features, monophyly of the genus has been hypothesized in recent studies (Aarn and Ivantsoff, 1997; Dyer and Chernoff, 1996; Stiasny, 1990). A number of diagnostic features have been advanced in support of *Bedotia* monophyly, although in each case these have been based on very limited taxonomic sampling (Aarn and Ivantsoff, 1997; Dyer and Chernoff, 1996; Stiasny, 1990).

Attempts to recover relationships within *Bedotia* have been confounded by a lack of morphological variation, except for coloration and pigmentation pattern. Unfortunately, coloration and pigmentation merited hardly any discussion in the original descriptions of most *Bedotia*, likely due to a loss of color and pigment during preservation and prior to description. Because of this, *Bedotia* is an ideal group to examine using molecular characters. Our results demonstrate that nucleotide characters are quite useful for reconstructing intragenetic relationships within *Bedotia*. Based on these data, all nominal species of *Bedotia* are well differentiated and, thus, appear to be valid [contra Maugé (1986)] (Fig. 2). Moreover, our results identify several populations of *Bedotia* that were originally hypothesized to represent undescribed, distinct species via morphological examination (Loiselle and Stiasny, 2003; Sparks and Stiasny, 2003).

Topotypic material corresponding to all nominal species of *Bedotia* was collected for sequencing (Table 1). Our results support three major, more or less geograph-

ically distinct, clades of *Bedotia*, one comprising species with distributions ranging from mid- to southeastern Madagascar (*B. madagascariensis*, *B. geayi*, and *B. tricolor*, plus four undescribed species; clade B), another including species restricted to eastern drainages north of the Masoala Peninsula (*B. marojejy*, plus four undescribed species; clade C), and a third comprising taxa with distributions extending from the Masoala Peninsula southward to the Ivoloina River (*B. longianalis* and *B. masoala*, plus three undescribed species; clade D) (Figs. 2 and 3). The only distributional overlap reported for any members of these clades occurs in the Ivoloina River basin, where *B. madagascariensis* (clade B) and two undescribed species, *B. sp.* “ivoloina” and *B. sp.* “betampona” (clade D), co-occur, although these species are not syntopic in the drainage (Loiselle, P., pers. comm.).

These three clades of *Bedotia* are not readily separated based on coloration or pigmentation pattern. For example, our phylogeny indicates that features such as lateral spotting, barring or mottling versus the presence of a solid mid-lateral stripe, once considered useful in separating species of *Bedotia* occurring from the Masoala Peninsula northwards from those occurring to the south (Sparks, 2001; Stiasny and Harrison, 2000), are more widely distributed (Fig. 2). Nevertheless, members of the northernmost clade of *Bedotia* (clade C), with the possible exception of *B. sp.* “antalaha,” can be distinguished by the presence of very sparse and irregular lateral blotches. Conversely, members of both clades B (less *B. sp.* “beforona”) and D (less *B. masoala* and *B. sp.* “makira”) can be distinguished by the presence of a prominent, solid mid-lateral stripe, regardless of sex. *Bedotia masoala*, *B. sp.* “makira,” and *B. sp.* “beforona” are, by comparison, characterized by very blotchy, barred, or speckled lateral pigmentation patterns (Sparks, 2001: Figs. 2 and 3).

All *Bedotia* are sexually dimorphic, with males exhibiting larger adult size, enhanced coloration and pigmentation, as well as pronounced development of the unpaired fins. Both species of “*Rheocles*” are also sexually dimorphic (Stiasny and Rodriguez, 2001; Stiasny et al., 2002), exhibiting the same dimorphic attributes observed in *Bedotia*. By comparison, all species of *Rheocles*, the sister taxon to a clade comprising (*Bedotia* + “*Rheocles*”), are not sexually dimorphic (Stiasny, 1990; Stiasny and Rodriguez, 2001, p. 102). It is important to stress, however, that all melanotaeniid genera contain sexually dimorphic species (Allen et al., 2002), and that pseudomugilids and “telmatherinids” are also sexually dimorphic (Kottelat, 1991; Kottelat and Whitten, 1993). Although sexually dimorphic pigmentation and fin development, and sexual dichromatism are plesiomorphic features for *Bedotia* + “*Rheocles*” (clade A), the absence of sexual dimorphism serves to diagnose *Rheocles* within Melanotaeniidae.

4.4. Biogeography

Paleontologists (Gottfried and Krause, 1998; Krause et al., 1997; Murray, 2001) have hypothesized that Madagascar's freshwater fishes owe their origin to Cenozoic trans-oceanic dispersal, well after the Mesozoic break-up of Gondwana. These claims have, for the most part, been advanced due to a lack of Cretaceous-age fossils for Madagascar's extant freshwater fish groups (Patterson, 1993a,b). Such claims, however, are contradicted by the phylogenetic patterns of relationship recovered for the groups of fishes in question in light of a well-corroborated geological area cladogram (Fig. 4). For example, if we examine shared components in their taxon-area cladograms (Nelson and Platnick, 1981; Rosen, 1978), both cichlids (Farias et al., 1999; Sparks, 2004) and aplocheiloid killifishes (Murphy and Collier, 1997) exhibit phylogenetic and biogeographic patterns that are not only congruent with each other, but also with the temporal sequence of Gondwanan fragmentation (compare Figs. 4B, C, and E).

Here we present evidence for a vicariant origin for a third major component of Madagascar's freshwater ichthyofaunal diversity, bedotiid rainbowfishes. Cichlids, aplocheiloid killifishes, and rainbowfishes represent three diverse percomorph orders (Perciformes, Cyprinodontiformes, and Atheriniformes, respectively), and comprise the majority of species (~72%) occurring in Madagascar's freshwaters (Sparks and Stiassny, 2003). These three clades represent the only freshwater fishes with members present in Madagascar that also exhibit a broad Gondwanan distribution, and consequently, that can be used to test a Gondwanan vicariance hypothesis.

The sister-group relationship between the Malagasy Bedotiidae and Australian/New Guinean Melanotaeniidae, with the Australasian pseudomugilids and "telmaterinids" recovered as their subsequent sister group, provides further evidence for a vicariant origin for the major Malagasy freshwater ichthyofaunal assemblages (Figs. 2 and 4A). In light of geographic proximity and a very old rift between Madagascar and Africa (Late Jurassic), a sister-group relationship between Malagasy and African lineages is predicted under a Cenozoic oceanic dispersal scenario. Yet, bedotiids are not the sister group of any insular Malagasy atherinid (i.e., *Teramulus*), nor are they closely related to regional (i.e., Mascarene or African) marine atheriniforms (Fig. 2). Conversely, results of our component analysis (Nelson and Platnick, 1981) including all three Malagasy freshwater fish clades, cichlids, aplocheiloid killifishes, and rainbowfishes, demonstrates that vicariance can simultaneously (= parsimoniously) account for both the presence of sister taxa in Madagascar and Australasia, and the absence of a close affinity of Malagasy lineages with any African form, without invoking transoceanic dis-

persal of freshwater lineages. Our general area cladogram, derived (from three resolved area cladograms; minimal losses nine) using COMPONENT vers. 2.0, indicates a closer relationship between Madagascar and India, Australia, and Southeast Asia, than Madagascar to Africa or South America (Fig. 4D). Madagascar, India, and Australia–New Guinea were separated by thousands of kilometers of open ocean throughout the Cenozoic (Reeves and de Wit, 2000; Smith et al., 1994; Storey, 1995), the time period during which paleontologists conjecture that Madagascar was colonized by members of these fish assemblages, whereas Africa and Madagascar were, by comparison, exceedingly close (separated for ~170 Ma. by at most 430 km across the Mozambique Channel) (Rabinowitz et al., 1983). Lack of a close relationship between Madagascar and Africa, geographically proximal neighbors, and instead an association between the ichthyofaunas of Madagascar and distant Gondwanan landmasses, is predicted under a vicariance model of Gondwanan break up. Our general area cladogram derived using component analysis is largely congruent with the conventional and well-corroborated geological area cladogram of Gondwanan vicariance (Fig. 4E), except for the placement of the Seychelles (but see Torsvik et al., 2000, for an alternative interpretation).

Conversely, if dispersal is invoked to explain these disjunct distributions, one must account for the presence of sister-group pairs on widely separated Gondwanan landmasses, and their concurrent absence from Africa and all intervening Indian Ocean islands of more recent (e.g., volcanic Mascarenes) origin (Sparks and Smith, in press). Further, observations that some Malagasy cichlids exhibit limited tolerance to marine environments (e.g., Lundberg, 1993; Murray, 2001) implies nothing regarding their ability to cross an extended marine barrier. Physiologically, prolonged exposure to seawater presents a formidable barrier for freshwater fishes, a constraint not encountered by terrestrial vertebrates capable of dispersing via rafting or swimming. For example, it has been demonstrated that exposure to seawater results in 100% mortality in less than 12 h for Malagasy cichlids, aplocheiloid killifishes, and bedotiid rainbowfishes (Riseng, 1997).

Although initial rifting between Australia and Antarctica is dated at around 95–90 Ma. (Embleton, 1984; Storey, 1995), with a direct land connection persisting until the Oligocene (ca. 35 Ma.) (Li and Powell, 2001), the timing of separation of India and Antarctica, critical for inferring the latest physical connection between Madagascar and Australia, remains controversial (Hay et al., 1999; Storey, 1995; Storey et al., 1995). Recent paleogeographic evidence suggests that India–Madagascar was connected to Antarctica via the Kerguelen Plateau well into the Late Cretaceous (Hay et al., 1999) (Fig. 5A). Such a connection would have permitted the

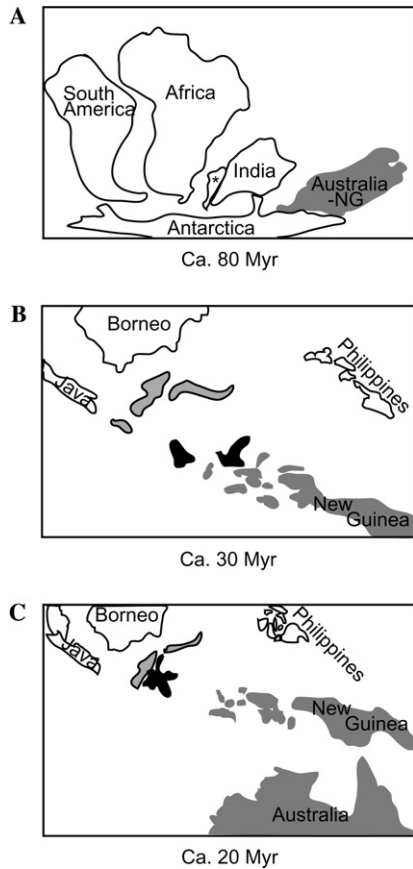


Fig. 5. (A) Late Cretaceous (ca. 80Ma.) plate-tectonic reconstruction of Gondwana after Hay et al. (1999). Madagascar indicated by an asterisk (*). (B) Mid-Eocene (ca. 30Ma.) and (C) Early Miocene (ca. 20Ma.) plate-tectonic reconstructions of Southeast Asia and northwestern Australia (after Hall, 2001; Moss and Wilson, 1998; Wilson and Moss, 1999). Landmasses corresponding to western Sulawesi shaded and outlined in black, those corresponding to eastern Sulawesi solid black.

passage of organisms between India-Madagascar and Australia, exclusive of Africa, presumably as recent as 80Ma. Catfish fossils recovered from the Late Eocene-Early Oligocene of Antarctica (Grande and Eastman, 1986), that are most similar to Recent freshwater lineages, suggest that not only did freshwater corridors via Antarctica exist, but until at least this period the region was not unbearably cold for freshwater fishes that tolerate at most temperate conditions (Nelson, 1994; Scotese et al., 1999; Ng, H. H., pers. comm.). A congruent disjunction between Malagasy and Australasian lineages is also reported for the perciform genus *Mesopristes* (Vari, 1992), which comprises a single endemic Malagasy species, restricted to freshwaters along Madagascar's eastern coast, and four Australasian congeners. A species-level phylogeny for *Mesopristes* is lacking (Vari, 1992), and as a result the genus could not be included in our biogeographic analysis.

Not only can the biogeographic disjunction between the Malagasy Bedotiidae and Australasian Melanotae-

niidae be most parsimoniously explained by vicariance, but the distribution of melanotaenioids in Australasia as well (Figs. 4A, D and E, and 5). It is commonly reported that members of Melanotaeniidae are most likely derived from (regional) marine ancestors (Aarn and Ivantsoff, 1997; Allen et al., 2002; Kottelat, 1991). Our hypothesis of relationships based on the combined analysis of morphological and nucleotide characters, and that of Dyer and Chernoff (1996) based solely on morphology, however, indicate that melanotaenioids are a freshwater radiation (Fig. 4A).

A closer look at Mesozoic through Cenozoic paleogeographic reconstructions of Southeast Asia, as well as the contemporary distributions and habitats of the organisms in question, corroborates this claim. Melanotaeniidae and Pseudomugilidae are found only in Australia and New Guinea, melanotaeniids almost exclusively in freshwater and pseudomugilids in both fresh- and brackish-water habitats (Allen et al., 2002; Saeed et al., 1989). The distribution of these families, ranging primarily from northern Australia to New Guinea, is consistent with a recent (Pleistocene) land connection between these two landmasses (Voris, 2002).

"Telmatherinids" are nested within the pseudomugilids (Fig. 2). Of the nearly 20 species of "telmatherinids" (Fishbase vers. 04, Froese, R., and Pauly, D. eds., published online, <http://www.fishbase.org>, 2003), all but one are confined to freshwater (the single exception tolerates brackish conditions), and except for two species, "telmatherinids" are restricted to eastern Sulawesi (Aarn et al., 1998; Kottelat, 1991; Saeed and Ivantsoff, 1991). Of the two species that occur outside of eastern Sulawesi, one is found in freshwaters of southwestern Sulawesi (Aarn et al., 1998) and the other [recently removed from *Pseudomugil* (Saeed and Ivantsoff, 1991)] in brackish waters on two small islands off the western coast of New Guinea (Saeed and Ivantsoff, 1991). Plate-tectonic reconstructions indicate that eastern Sulawesi is Gondwanan in origin, whereas western Sulawesi is part of Eurasia (Hall, 2001; Moss and Wilson, 1998) (Figs. 5B and C). Until the Mid- to Late Oligocene (ca. 25–30 Ma.), eastern Sulawesi was proximal to northwestern Australia and New Guinea (Hall, 2001; Wilson and Moss, 1999) (Fig. 5B). Western Sulawesi was accreted onto eastern Borneo by the Late Cretaceous, whereas the East Sulawesi Ophiolite remained close to Australia and was not accreted onto Sulawesi until the Late Oligocene or thereafter (see plate tectonic reconstructions in Hall, 1998; Moss and Wilson, 1998; Wilson and Moss, 1999) (Fig. 5C).

Wilson and Moss (1999, p. 329) stress that the East Sulawesi Ophiolite originated as oceanic crust in "a deep marine setting" and would not have "acted as a potential raft for biota" from Australia. Nevertheless, Wilson and Moss (1999, p. 329) also note that "microcontinental fragments accreted onto eastern Sulawesi in the Mio-

cene and Pleistocene may have been emergent as they drifted towards Sulawesi and comprised island hopping/rafting routes for biota of Australian affinity.” Regardless of whether microcontinental fragments acted as ‘rafts,’ the presence of “telmatherinids” in the freshwaters of Sulawesi at most requires relatively short distance marine dispersal from northwestern Australia and New Guinea, possibly via island-hopping. The hypothesis that telmatherinids are descendents of a regional marine ancestor (Aarn and Ivantsoff, 1997; Kottelat, 1991) is simply not corroborated by their current distribution on their phylogenetic placement within a large freshwater radiation (Figs. 2 and 4A; Dyer and Chernoff, 1996; Fig. 5). Further, the fact that “telmatherinids” are absent from Borneo, and have never traversed the extremely narrow Makassar Strait that has separated Borneo from Sulawesi since the Paleogene (Moss and Wilson, 1998), indicates that they do not readily cross even very limited marine barriers. The current distribution of rainbowfishes, which are not widely distributed throughout the Australasian region but very localized, indicates that they are unlikely oceanic dispersers. Specifically, rainbowfishes are only found on landmasses that are, in whole or in part (i.e., Sulawesi), of Gondwanan origin, with only a single species of “telmatherinid” occurring to the west of eastern Sulawesi (i.e., freshwaters of southwestern Sulawesi) (Aarn et al., 1998).

In light of the recovered phylogenetic pattern and paleogeographic evidence, the sister-group relationship between the Malagasy Bedotiidae and Australasian Melanotaeniidae is most parsimoniously explained by the break-up of Gondwana. Given that a direct land connection between Australia and eastern Sulawesi may have never existed, a single, short-distance, dispersal event from northwestern Australia is consistent with the recovered phylogeny and may be required to explain the presence of “telmatherinids” in Sulawesi. Otherwise, as our general area cladogram for these clades of fishes demonstrates, the scenario we outline is entirely congruent with Gondwanan vicariance. The notion that the current distribution of rainbowfishes, and for that matter aplocheiloid killifishes and cichlids, can most parsimoniously be explained by Cenozoic trans-oceanic dispersal simply is not supported by phylogenetic and geologic evidence.

Acknowledgments

Our sincerest thanks to M. Stiassny and P. Loiselle for enthusiastically sharing their unpublished data and vast knowledge of bedotiids with us. We thank K. Smith for reviewing the manuscript and for help with primer design, W. Wheeler and P. Dunlap for kindly providing lab space and equipment, W. Wheeler for providing ac-

cess to the AMNH Parallel Computing Cluster, and to Aarn, R. Hanel, W. Ivantsoff, S. Kimura, K. Lazara, P. Loiselle, N. Lovejoy, R. Mayden, M. McGrouther, R. Schelly, M. Stiassny, P. Unmack, and H. J. Walker for providing tissue samples. Collecting efforts in Madagascar were facilitated by B. Andriamihaja and the MICET (Institute for the Conservation of Tropical Environments, Madagascar) staff, and we are grateful for their support. Fishes were collected under permits obtained from the Direction des Eaux et Foëts and the Association National pour la Gestion des Aires Protégées (ANGAP), Antananarivo, Madagascar, in accordance with the University of Michigan IACUC guidelines. Fieldwork in Madagascar was funded by the American Museum of Natural History, Columbia University, the Wildlife Conservation Society (WCS), an AMNH Lerner-Gray grant for marine research, and by grants from the National Science Foundation (DEB-9300996) and the United States Agency for International Development (University Development Linkage Program, US AID Cooperative Agreement, PCE-5063-A-00-3035-00) to P. Reinthal. Additional funding for W.L.S. was provided by AMNH Lerner-Gray and Columbia University graduate fellowships and the National Science Foundation (DEB-0405246).

References

- Aarn, W., Ivantsoff, W., 1997. Descriptive anatomy of *Cairnsichthys rhombosomoides* and *Iriatherina werneri* (Teleostei: Atheriniformes), and a phylogenetic analysis of Melanotaeniidae. *Ichthyol. Explor. Freshwaters* 8, 107–150.
- Aarn, W., Ivantsoff, W., Kottelat, M., 1998. Phylogenetic analysis of Telmatherinidae (Teleostei: Atherinomorpha), with description of *Marosatherina*, a new genus from Sulawesi. *Ichthyol. Explor. Freshwaters* 9, 311–323.
- Allen, G.R., 1980. A generic classification of the rainbowfishes (Family Melanotaeniidae). *Rec. West Australia Mus.* 8, 449–490.
- Allen, G.R., Midgley, S.H., Allen, M., 2002. Field guide to the freshwater fishes of Australia. Western Australian Museum, Perth, Western Australia.
- Bremer, K., 1988. The limits of amino-acid sequence data in angiosperm phylogenetic reconstruction. *Evolution* 42, 795–803.
- Caccone, A., Amato, G., Gratry, O.C., Behler, J., Powell, J.R., 1999. A molecular phylogeny of four endangered Madagascar tortoises based on MtDNA sequences. *Mol. Phylogenet. Evol.* 12, 1–9.
- Dyer, B.S., Chernoff, B., 1996. Phylogenetic relationships among atheriniform fishes (Teleostei: Atherinomorpha). *Zool. J. Linn. Soc.* 117, 1–69.
- Embleton, B.J.J., 1984. Continental paleomagnetism. In: Veevers, J.J. (ed.), *Phanerozoic Earth History of Australia*. Oxford Geol. Sci. Ser. 2, Clarendon Press, Oxford, pp. 11–16.
- Farias, I.P., Orti, G., Sampaio, I., Schneider, H., Meyer, A., 1999. Mitochondrial DNA phylogeny of the family Cichlidae: monophyly and fast evolution of the Neotropical assemblage. *J. Mol. Evol.* 48, 703–711.
- Farris, J.S., 1989. The retention index and the rescaled consistency index. *Cladistics* 5, 417–419.
- Goloboff, P.A., 1999. Analyzing large data sets in reasonable times: solutions for composite optima. *Cladistics* 15, 415–428.

- Gottfried, M.D., Krause, D.W., 1998. First record of gars (Lepisosteidae, Actinopterygii) on Madagascar: Late Cretaceous remains from the Mahajunga basin. *J. Vert. Paleont.* 18, 275–279.
- Grande, L., Eastman, J.T., 1986. A review of Antarctic ichthyofaunas in the light of new fossil discoveries. *Palaeontology* 29, 133–137.
- Hall, R., 1998. The plate tectonics of Cenozoic SE Asia and the distribution of land and sea. In: Hall, R., Holloway, J.D. (Eds.), *Biogeography and Geological Evolution of SE Asia*. Backhuys Publishers, Leiden, The Netherlands, pp. 99–131.
- Hall, R., 2001. Cenozoic reconstructions of SE Asia and the SW Pacific: changing patterns of land and sea. In: Metcalfe, I., Smith, J.M.B., Morwood, M., Davidson, I.D. (Eds.), *Faunal and Floral Migrations and Evolution in SE Asia-Australasia*. Swets and Zeitlinger Publishers, Lisse, pp. 35–56.
- Hay, W.W., De Conto, R.M., Wold, C.N., Wilson, K.M., Voight, S., Shulz, M., Wold, A.R., Dullo, W.-C., Ronov, A.B., Balukhovskiy, A.N., Söding, E., 1999. Alternative global Cretaceous paleogeography. In: Barrera, E., Johnson, C.C. (Eds.), *Evolution of the Cretaceous Ocean-Climate System*. Geol. Soc. America, Special Paper 332, pp. 1–47.
- Jansa, S.A., Goodman, S.M., Tucker, P.K., 1999. Molecular phylogeny and biogeography of the native rodents of Madagascar (Muridae: Nesomyinae): a test of the single-origin hypothesis. *Cladistics* 15, 253–270.
- Jordan D.S., Hubbs, C.L., 1919. Studies in ichthyology. A monographic review of the family of Atherinidae or silversides. Leland Stanford Jr. Univ. Publ., Univ. Ser. 919 pp. 1–87.
- Kluge, A.G., Farris, J.S., 1969. Quantitative phyletics and the evolution of anurans. *Syst. Zool.* 18, 1–32.
- Kottelat, M., 1991. Sailfin silversides (Pisces: Telmatherinidae) of Lake Matano, Sulawesi, Indonesia, with descriptions of six new species. *Ichthyol. Explor. Freshwaters* 1, 321–344.
- Kottelat, M., Whitten, A.J., 1993. *Freshwater Fishes of Western Indonesia and Sulawesi*. Periplus Editions (HK), Indonesia.
- Krause, D.W., Hartman, J.H., Wells, N.A., 1997. Late Cretaceous vertebrates from Madagascar: implications for biotic change in deep time. In: Goodman, S.M., Patterson, B.D. (Eds.), *Natural Change and Human Impact in Madagascar*. Smithsonian Institution Press, Washington, DC, pp. 3–43.
- Li, Z.X., Powell, C.McA., 2001. An outline of palaeogeographic evolution of the Australasian region since the beginning of the Neoproterozoic. *Earth Sci. Rev.* 53, 237–277.
- Loiselle, P.V., Stiassny, M.L.J., 2003. *Bedotia*. In: Goodman, S.M., Benstead, J.P. (Eds.), *The Natural History of Madagascar*. University Chicago Press, Chicago, pp. 867–868.
- Lundberg, J.G., 1993. African-South American freshwater fish clades and continental drift: problems with a paradigm. In: Goldblatt, P. (Ed.), *Biological Relationships between Africa and South America*. Yale University Press, New Haven, pp. 156–199.
- Maugé, A.L., 1986. Atherinidae. In: Daget, J., Gosse, J.-P., Thys van den Audenaerde, D.F.E. (Eds.), *Checklist of the Freshwater Fishes of Africa*, vol. II. Institut Royal des Sciences Naturelles de Belgique, Bruxelles, Musée Royal de l'Afrique Centrale, Tervuren, Office de la Recherche Scientifique et Technique Outre-Mer, Paris, pp. 277–279.
- Mausfeld, P., Vences, M., Schmitz, A., Veith, M., 2000. First data on the molecular phylogeography of scinid lizards of the genus *Mabuya*. *Mol. Phylogenet. Evol.* 17, 11–14.
- McGuigan, K., Zhu, D., Allen, G.R., Moritz, C., 2000. Phylogenetic relationships and historical biogeography of melanotaeniid fishes in Australia and New Guinea. *Mar. Fresh. Res.* 51, 713–723.
- Miya, M., Takeshima, H., Endo, H., Ishiguro, N., Inoue, J.G., Mukai, T., Satoh, T.P., Yamaguchi, M., Kawaguchi, A., Mabuchi, K., Shirai, S.M., Nishida, M., 2003. Major patterns of higher teleostean phylogenies: a new perspective based on 100 complete mitochondrial DNA sequences. *Mol. Phylogenet. Evol.* 26, 121–138.
- Moss, S.J., Wilson, M.E.J., 1998. Biogeographic implications of the Tertiary paleogeographic evolution of Sulawesi and Borneo. In: Hall, R., Holloway, J.D. (Eds.), *Biogeography and Geological Evolution of SE Asia*. Backhuys Publishers, Leiden, The Netherlands, pp. 133–163.
- Morrone, J.J., Carpenter, J.M., 1994. In search of a method for cladistic biogeography: an empirical comparison of component analysis, Brooks parsimony analysis, and three-area statements. *Cladistics* 10, 99–153.
- Murphy, W.J., Collier, G.E., 1997. A molecular phylogeny for aplocheiloid fishes (Atherinomorpha, Cyprinodontiformes): the role of vicariance and the origins of annualism. *Mol. Biol. Evol.* 14, 790–799.
- Murray, A.M., 2001. The fossil record and biogeography of the Cichlidae (Actinopterygii: Labroidei). *Biol. J. Linn. Soc.* 78, 517–532.
- Nagy, Z.T., Joger, U., Wink, M., Glaw, F., Vences, M., 2003. Multiple colonization of Madagascar and Socotra by colubrid snakes: evidence from nuclear and mitochondrial gene phylogenies. *Proc. R. Soc. Lond. B* 270, 2613–2621.
- Nelson, J.S., 1994. *Fishes of the World*, third ed. Wiley, New York.
- Nelson, G.J., Platnick, N.I., 1981. *Systematics and Biogeography: Cladistics and Vicariance*. Columbia University Press, New York.
- Nichols, J.T., LaMonte, F.R., 1931. *Rheocloides*, a new atherinid fish from Madagascar. *Am. Mus. Novit.* 508, 1–2.
- Nixon, K.C., 1999. The parsimony ratchet, a new method for rapid parsimony analysis. *Cladistics* 15, 407–414.
- Page, R.D.M., 1988. Quantitative cladistic biogeography: constructing and comparing area cladograms. *Syst. Zool.* 37, 254–270.
- Patterson, C., 1993a. An overview of the early fossil record of acanthomorphs. *Bull. Mar. Sci.* 52, 29–59.
- Patterson, C., 1993b. Osteichthys: Teleostei. In: Benton, M.J. (Ed.), *The Fossil Record 2*. Chapman and Hall, London, pp. 621–656.
- Pellegrin, J., 1907. Liste des poissons recueillis à Madagascar par M. F. Geay. Description d'une espèce nouvelle. *Bull. Mus. Natl. Hist. Nat. (Sér. 1)* 13, 201–206.
- Pellegrin, J., 1914a. Sur une Athérine nouvelle des eaux douces de Madagascar. *Bull. Soc. Zool. Fr.* 39, 46–47.
- Pellegrin, J., 1914b. Sur un Athérinidé nouveau de Madagascar appartenant au genre *Bedotia*. *Bull. Soc. Zool. Fr.* 39, 178–180.
- Pellegrin, J., 1932. Athérinidé nouveau de Madagascar appartenant au genre *Bedotia*. *Bull. Soc. Zool. Fr.* 57, 84–86.
- Rabinowitz, P.D., Coffin, M.F., Falvey, D., 1983. The separation of Madagascar and Africa. *Science* 220, 67–69.
- Raxworthy, C.J., Forstner, M.R.J., Nussbaum, R.A., 2002. Chameleon radiation by oceanic dispersal. *Nature* 415, 784–787.
- Reeves, C., de Wit, M., 2000. Making ends meet in Gondwana: retracing the transforms of the Indian Ocean and reconstructing continental shear zones. *Terra Nova* 12, 272–280.
- Regan, C.T., 1903. Descriptions de poissons nouveaux faisant partie de la collection du Musée d'Histoire Naturelle de Genève. *Rev. Suisse Zool.* 11, 413–418.
- Riseng, K.J., 1997. The distribution of fishes and the conservation of aquatic resources in Madagascar. Unpublished M.Sc. thesis. University of Michigan, Ann Arbor.
- Rosen, D.E., 1964. The relationships and taxonomic position of the halfbeaks, killifishes, silversides, and their relatives. *Bull. Am. Mus. Nat. Hist.* 127, 217–268.
- Rosen, D.E., 1978. Vicariant patterns and historical explanation in biogeography. *Syst. Zool.* 27, 159–188.
- Rosen, D.E., Parenti, L.R., 1981. Relationships of *Oryzias*, and the groups of Atherinomorph fishes. *Am. Mus. Novitates* 2719, 1–25.
- Saeed, B., Ivantsoff, W., 1991. *Kalyptatherina*, the first telmatherinid genus known outside of Sulawesi. *Ichthyol. Explor. Freshwaters* 2, 227–238.

- Saeed, B., Ivantsoff, W., Allen, G.R., 1989. Taxonomic revision of the family Pseudomugilidae (Order Atheriniformes). *Aust. J. Mar. Freshw. Res.* 40, 719–787.
- Saeed, B., Ivantsoff, W., Crowley, L.E.L.M., 1994. Systematic relationships of atheriniform families within Division I of the series Atherinomorpha (Acanthopterygii) with relevant historical perspectives. *Voprosi Ikhtiologii* 34, 1–32.
- Sauvage, H.E., 1891. Histoire naturelle des poissons. In: Grandidier, A. (Ed.), *Histoire physique, naturelle et politique de Madagascar*. Vol. 16. Imprimerie Nationale, Paris, pp. 1–543.
- Scotese, C.R., Boucot, A.J., McKerrow, W.S., 1999. Gondwanan palaeogeography and palaeoclimatology. *J. African Earth Sci.* 28, 99–114.
- Smith, A.G., Smith, D.G., Funnell, B.M., 1994. *Atlas of Mesozoic and Cenozoic Coastlines*. Cambridge University Press, Cambridge.
- Smith, W.L., Wheeler, W.C., 2004. Polyphyly of the mail-cheeked fishes (Teleostei: Scorpaeniformes): evidence from mitochondrial and nuclear sequence data. *Mol. Phylogenet. Evol.* 32, 627–646.
- Sparks, J.S., 2001. *Bedotia masoala*, a new species of atherinoid rainbowfish (Teleostei: Bedotiidae) from the Masoala Peninsula, northeastern Madagascar. *Copeia* 2001, 482–489.
- Sparks, J.S., 2004. Molecular phylogeny and biogeography of the Malagasy and South Asian cichlids (Teleostei: Perciformes: Cichlidae). *Mol. Phylogenet. Evol.* 30, 599–614.
- Sparks, J.S., Smith, W.L., in press. Freshwater fishes, dispersal ability, and non-evidence: “Gondwana life rafts” to the rescue. *Syst. Biol.*
- Sparks, J.S., Stiassny, M.L.J., 2003. Introduction to the freshwater fishes. In: Goodman, S.M., Benstead, J.P. (Eds.), *The Natural History of Madagascar*. University Chicago Press, Chicago, pp. 849–863.
- Springer, V.G., 1982. Pacific plate biogeography, with special reference to shorefishes. *Smithsonian Cont. Zool.* 367, 1–182.
- Stiassny, M.L.J., 1990. Notes on the anatomy and relationships of the bedotiid fishes of Madagascar, with a taxonomic review of the genus *Rheocles*. *Am. Mus. Novitates* 2979, 1–33.
- Stiassny, M.L.J., Harrison, I.J., 2000. Notes on a small collection of fishes from the Parc National de Marojejy, northeastern Madagascar, with a description of a new species of the endemic genus *Bedotia* (Atherinomorpha: Bedotiidae). In: Goodman, S.M. (Ed.), *A Floral and Faunal Inventory of the Parc National de Marojejy, Madagascar: With Reference to Elevational Variation*. *Fieldiana Zool.*, new series, 97, 1–286, pp. 143–156.
- Stiassny, M.L.J., Reinthal, P.N., 1992. Description of a new species of *Rheocles* (Atherinomorpha, Bedotiidae) from the Nosivolo Tributary, Mangoro River, Eastern Malagasy Republic. *Am. Mus. Novitates* 3031, 1–8.
- Stiassny, M.L.J., Rodriguez, D.M., 2001. *Rheocles derhami*, a new species of freshwater rainbowfish (Atherinomorpha: Bedotiidae) from the Ambombo River in northeastern Madagascar. *Ichthyol. Explor. Freshwaters* 12, 97–104.
- Stiassny, M.L.J., Rodriguez, D.M., Loisele, P.V., 2002. *Rheocles vatsoa*, a new species of freshwater rainbowfish (Atherinomorpha: Bedotiidae) from the Lokoho River basin in northeastern Madagascar. *Cybius* 26, 71–77.
- Storey, B.C., 1995. The role of mantle plumes in continental breakup: case histories from Gondwanaland. *Nature* 377, 301–308.
- Storey, B.C., Mahoney, J.J., Saunders, A.D., Duncan, R.A., Kelley, S.P., Coffin, M.F., 1995. Timing of hot spot-related volcanism and the breakup of Madagascar and India. *Science* 267, 852–855.
- Torsvik, T.H., Tucker, R.D., Ashwal, L.D., Carter, L.M., Jamtveit, B., Vidyadharan, K.T., Venkataramana, P., 2000. Late Cretaceous India-Madagascar fit and timing of break-up related magmatism. *Terra Nova* 12, 220–224.
- Vari, R.P., 1992. Redescription of *Mesopristes elongatus* (Guichenot, 1866), an endemic Malagasy fish species (Pisces, Terapontidae). *Am. Mus. Novitates* 3039, 1–7.
- Voris, H.K., 2002. Maps of Pleistocene sea levels in Southeast Asia: shorelines, river systems and time durations. *J. Biogeogr.* 27, 1153–1167.
- Wheeler, W.C., 1996. Optimization alignment: the end of multiple sequence alignment in phylogenetics. *Cladistics* 12, 1–9.
- Wheeler, W.C., 2003a. Iterative pass optimization of sequence data. *Cladistics* 19, 254–260.
- Wheeler, W.C., 2003b. Implied alignment: a synapomorphy-based multiple sequence alignment method. *Cladistics* 19, 261–268.
- Wilson, M.E.J., Moss, S.J., 1999. Cenozoic paleogeographic evolution of Sulawesi and Borneo. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* 145, 303–337.
- Yoder, A., Yang, Z., 2004. Divergence dates for Malagasy lemurs estimated from multiple gene loci: geological and evolutionary context. *Mol. Ecol.* 13, 757–773.
- Yoder, A.D., Cartmill, M., Ruvolo, M., Smith, K., Vilgalys, R., 1996. Ancient single origin for Malagasy primates. *Proc. Natl. Acad. Sci. USA* 93, 5122–5126.
- Yoder, A.D., Burns, M., Zehr, S., Delefosse, T., Veron, G., Goodman, S.M., Flynn, J.J., 2003. Single origin of Malagasy Carnivora from an African ancestor. *Nature* 421, 734–737.