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Molecular Phylogenetics and Biogeography of Galaxiid Fishes (Osteichthyes: Galaxiidae): Dispersal, Vicariance, and the Position of *Lepidogalaxias salamandroides*

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Abstract.—The galaxiid fishes exhibit a gondwanan distribution. We use mitochondrial DNA sequences to test conflicting vicariant and dispersal biogeographic hypotheses regarding the Southern Hemisphere range of this freshwater group. Although phylogenetic resolution of cytochrome *b* and 16S rRNA sequences is largely limited to more recent divergences, our data indicate that the radiation can be interpreted as several relatively recent dispersal events superimposed on an ancient gondwanan radiation. Genetic relationships contradict the findings of recent morphological analyses of galaxioid fishes. In particular, we examine several hypotheses regarding phylogenetic placement of the enigmatic *Lepidogalaxias*. Although most workers consider *Lepidogalaxias* to be an unusual scaled member of the Southern Hemisphere galaxioids, it has also been suggested that this species is related to the Northern Hemisphere esocoids. Our data strongly suggest that this species is not a galaxiid, and the alternative hypothesized esocoid relationship cannot be rejected. The species-rich genus *Galaxias* is shown to be polyphyletic and the generic taxonomy of the Galaxiinae is reassessed in the light of phylogenetic relationships. Juvenile saltwater-tolerance is phylogenetically distributed throughout the Galaxiinae, and the loss of this migratory phase may be a major cause of speciation. [Biogeography; diadromy; evolution; Gondwana; molecular clock; mtDNA; phylogeny; secondary structure.]

The galaxiid fishes are a major element of the relatively depauperate Southern Hemisphere freshwater fish fauna. As with several components of the southern temperate biota (e.g., podocarps, southern beeches, ratite birds), the galaxiids exhibit a gondwanan distribution (Fig. 1), with present representation on all gondwanan continents except Antarctica and India. The family Galaxiidae is dominated by the species-rich tribe Galaxiini, which comprises ~50 species across six genera: *Galaxias* (at least 35 species; widespread), *Paragalaxias* (4 species; Tasmania), *Neochanna* (5 species; New Zealand, Tasmania, and Australia), *Galaxiella* (3 species; Australia and Tasmania), *Brachygalaxias* (1 species; Chile), and *Nesogalaxias* (1 species; New Caledonia). Galaxiini lack both an adipose fin and scales.

GALAXIID TAXONOMY

The currently accepted taxonomy of the galaxioid fishes is outlined in Table 1. The exclusively Southern Hemisphere superfamily Galaxioidea includes the families Retropinnidae (six species across three genera; Australia and New Zealand) and Galaxiidae. The revision by Johnson and Patterson (1996), based on morphology, divided the family Galaxiidae into two subfamilies: Lepidogalaxiinae and Galaxiinae. The Lepidogalaxiinae comprises just two species: the Western Australian *Lepidogalaxias salamandroides* and the Tasmanian *Lovettia sealii*. The Galaxiinae consist of the tribes Galaxiini (see above) and Aplochitonini. The tribe Aplochitonini contains a single genus, *Aplochiton*, of stout, moderate-sized fishes that have an adipose fin and a forward dorsal fin (McDowall, 1971). *Aplochiton* is represented by two species on the Chilean coast of South America and in the Falkland Islands. Despite the recent study (Johnson and Patterson, 1996), phylogenetic and taxonomic relationships within the Galaxiidae, and the deeper relationships of the superfamily Galaxioidea, remain controversial. In particular, considerable disagreement surrounds the placement of the salamander fish *Lepidogalaxias salamandroides*, a species notable for its unusual life history (see Berra and Pusey, 1997).

Placement of Lepidogalaxias

Various hypotheses concerning the placement of *Lepidogalaxias* are illustrated in Figure 2. As Williams (1987) noted, although "all investigators have noted similarities between *Lepidogalaxias* and the esocoids

TABLE 1. Current classification of the Galaxioidea, after Johnson and Patterson (1996).

Superfamily	Family	Subfamily	Tribe	Genus	No. of species
Galaxioidea	Retropinnidae	–	–	*Prototroctes*	2
		–	–	*Retropinna*	3
		–	–	*Stokellia*	1
	Galaxiidae	Lepidogalaxiinae	–	*Lepidogalaxias*	1
			–	*Lovettia*	1
		Galaxiinae	Aplochitonini	*Aplochiton*	2
			Galaxiini	*Brachygalaxias*	1
				Galaxias	35
				Galaxiella	3
				Neochanna	5
				Nesogalaxias	1
				Paragalaxias	4

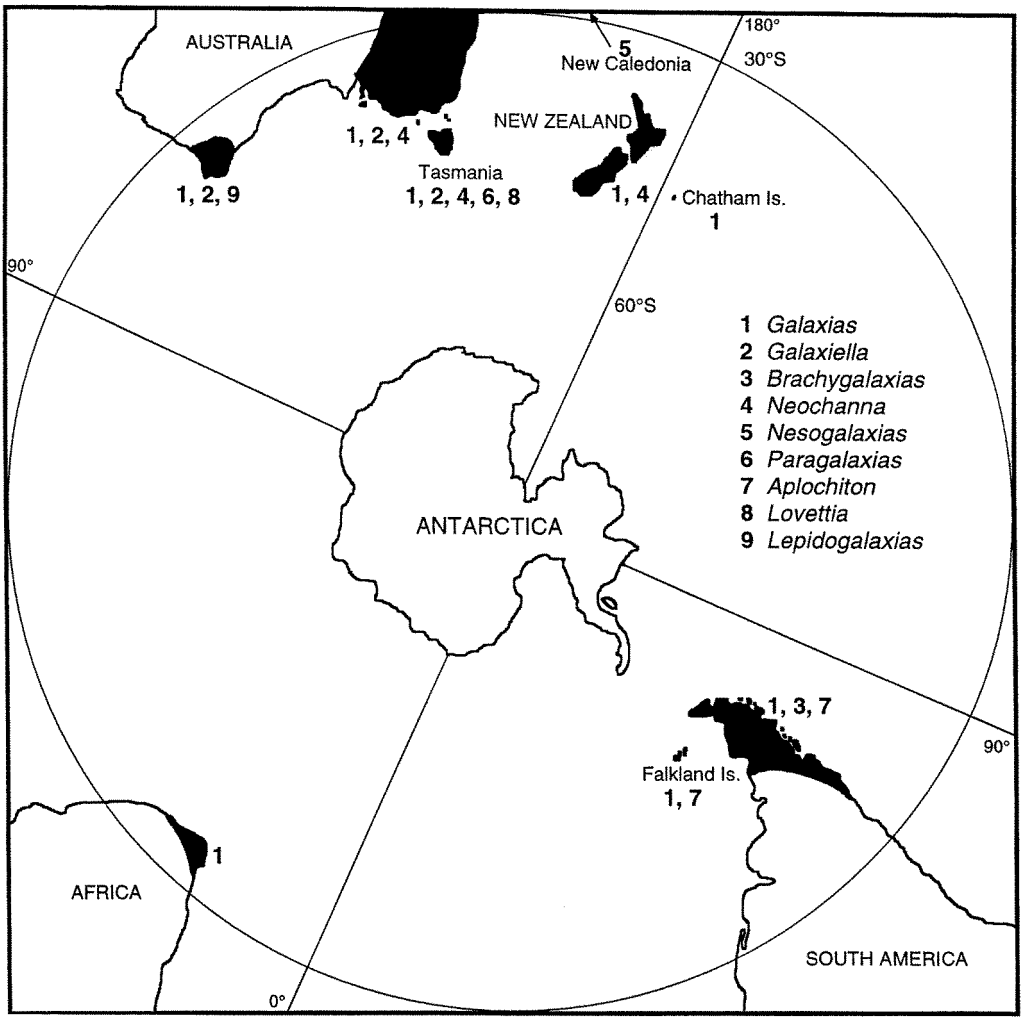

FIGURE 1. Map of Southern Hemisphere with shaded areas representing the geographic range of the galaxiid fishes. Numbers refer to the distribution of galaxiid genera.

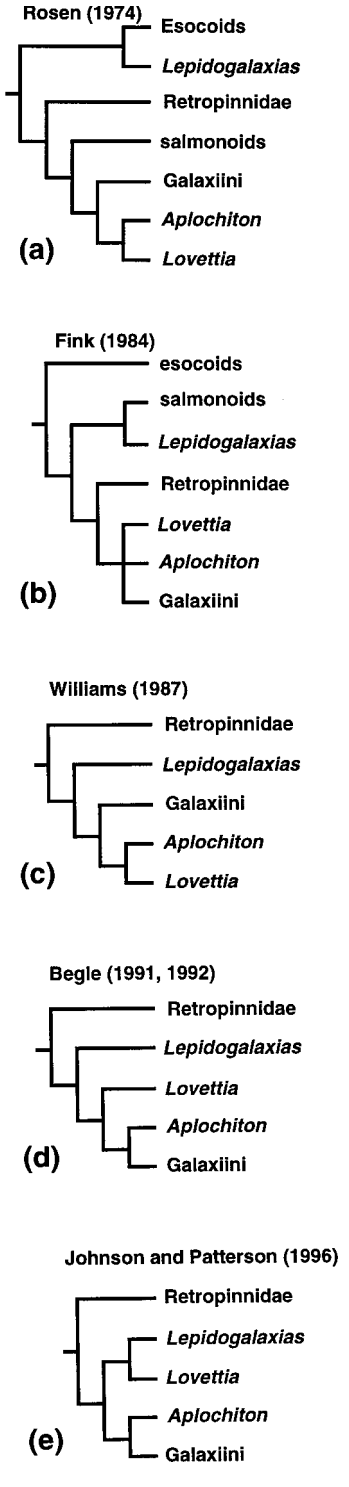

FIGURE 2. Various recent hypotheses regarding the evolutionary relationships of galaxioid fishes, with particular emphasis on the position of *Lepidogalaxias*. Trees have been redrawn and, in some cases, taxa that are not included in the current study (e.g., salangids, argentinids, osmerids) have been removed for the sake of clarity.

(especially the Umbridae) . . . only Rosen proposed that these similarities are indicative of phylogenetic relationship". Most other workers have argued that *Lepidogalaxias* is part of the Southern Hemisphere galaxioid assemblage. *Lepidogalaxias* was originally described as an unusual scaled member of the Galaxiini (Mees, 1961). Frankenberg (1969) examined the osteology of this species and suggested that it is not a galaxiine but the sole member of a closely related superfamily. Rosen (1974) concluded that *Lepidogalaxias* (Australia) belongs with the otherwise Northern Hemisphere esocoids, a suggestion that presented major biogeographic difficulties. This hypothesis was questioned by Fink and Weitzman (1982), who left the genus unplaced. Fink (1984) argued that although *Lepidogalaxias* has many similarities with galaxiids, it is related to neither the galaxiids nor the esocoids; rather, he considered *Lepidogalaxias* to be the sister group of neoteleosts. Williams (1987, 1996) concluded that *Lepidogalaxias* is the sister of Galaxiini + [*Aplochiton, Lovettia*]. Begle (1991) conducted a phylogenetic analysis of the osmeroid fishes and agreed that *Lepidogalaxias* is a galaxioid. Johnson and Patterson (1996) and Patterson and Johnson (1997) were highly critical of Begle's data, suggesting that the majority of his characters were incorrectly coded. However, they concurred that *Lepidogalaxias* is a galaxioid and placed it as the sister group of *Lovettia* (Table 1).

Generic Taxonomy of the Galaxiini

There is phylogenetic and taxonomic disagreement concerning the relationships and status of various galaxiine genera. McDowall (1969) described the generic arrangement of the Galaxiini as poorly defined and "in a state of flux". This was reflected in the recent transfer of the Australian mudfish from the large and widespread genus *Galaxias* to the more specialized and largely New Zealand genus *Neochanna* (McDowall, 1997), a change supported by molecular data (Waters and White, 1997). Controversy also surrounds the generic status of geographic outliers, such as species endemic to New Caledonia and South Africa. The New Caledonian species, originally described as *Galaxias neocaledonicus* (Weber and De Beaufort, 1913), was later placed in a separate genus *Nesogalaxias* (Whitley, 1935), a decision ultimately supported by McDowall (1968). Scott

(1936) placed the South African Cape galaxias as a monotypic subgenus within *Galaxias*. Stokell (1945) rejected this and subsequently placed it in the otherwise Tasmanian genus *Paragalaxias*. Scott (1966) promoted the subgenus *Agalaxias* to a full genus. McDowall (1969, 1973a) rejected all the above views, regarding *G. zebratus* as a "very ordinary" member of *Galaxias*.

The generic status of a group of small, striped Tasmanian and Australian galaxiines, placed in *Galaxiella* by McDowall (1978a; see also Berra and Allen, 1989), has been controversial. Stokell (1954), Whitley (1960), Scott (1966), and Frankenberg (1969) all accepted an expanded *Brachygalaxias* that included these Australian species as well as the South American *B. bullocki*. In contrast, McDowall (1970) chose to avoid the supposedly "considerable geographic problems" associated with the occurrence of congeneric nonmigratory species on separate continents. He argued that similarities between these taxa represented convergent evolution rather than any close phylogenetic relationship. Subsequently, McDowall (1973b) limited the genus *Brachygalaxias* to *B. bullocki*.

Southern Temperate Distribution—Dispersal or Vicariance?

Several galaxiine species have a juvenile marine "whitebait" phase that may stay at sea for as long as 6 months. Such species are termed diadromous or migratory and in general are widely distributed. For example, the range of diadromous *Galaxias maculatus* encompasses Australia, New Zealand, and South America; migratory *G. brevipinnis* occur on both sides of the Tasman Sea. Three additional migratory species are found throughout New Zealand. Conversely, freshwater-limited (nonmigratory) species tend to have restricted distributions.

McDowall (1970, 1978b) considered the marine larval phase to be a primitive feature of the galaxiines, with derived species being the result of landlocking. He hypothesised that the galaxiines achieved their current distribution by means of oceanic dispersal. As evidence, McDowall cited the wide geographic range of the migratory *G. maculatus*, noting the fact that juveniles of this species have been collected as for as 700 km from continental land. Recent molecular studies seem to support the importance of marine dispersal in *G. maculatus* (Berra et al., 1996; Waters and Burridge, 1999; Waters et al., 2000). McDowall argued that Australia, with 20 species, represents the "centre of origin" for the galaxiines, and that other areas were sequentially colonized with the aid of the circumpolar current, representing a "chain of dispersal".

Plate tectonics provide an alternative explanation for the austral distribution of galaxiines. Vicariance theorists (Croizat et al., 1974; Rosen, 1974, 1978; Craw, 1979) supported an ancient gondwanan origin in place of the dispersal hypothesis. Rosen (1974, 1978) disputed McDowall's claim that the morphological similarity of isolated populations of *G. maculatus* was due to recent dispersal. Instead, he argued that a slow rate of evolution in this species has prevented substantial differentiation since continental separation. Craw (1979) pointed to the similarity of Miocene fossil galaxiines and extant species as evidence of such phenotypic stability. Croizat et al. (1974) rejected the Darwinian "centre of origin" theory, arguing that dispersal theory relies on events that are not testable. These authors suggested that the only strong evidence for dispersal was seen in taxa with distribution patterns that could not be explained by vicariance. Instead, vicariance theorists claim that galaxiid distribution can be entirely explained by geological events. McDowall (1990) maintained that dispersal has played a major role in galaxiine biogeography but conceded that the fragmentation of Gondwana may have also influenced galaxiine distribution.

DNA Sequences: Testing Biogeographical Theories

Molecular techniques provide a powerful means of resolving long-standing biogeographic debates (Avise, 1994). Under a vicariance scenario and in the absence of dispersal, the galaxiid fauna of each continent might be expected to be monophyletic. This hypothesis assumes that current galaxiine diversity has arisen entirely from phylogenetic separations subsequent to the fragmentation of Gondwana. If diversification occurred before continental separation, then, under a vicariance model, the phylogenetic relationships (and associated divergence times

derived from molecular clock calibrations) within clades should still be consistent with the established order and timing of continental separations.

Until recently, dispersal hypotheses remained difficult to test and were viewed by some as nonscientific (e.g., Ball, 1975; Michaux, 1991). Nevertheless, phylogenetic analysis of DNA sequence data and the use of molecular clocks to estimate timing of genetic divergences can provide tests of dispersal hypotheses. First, clock calibrations that provide divergence estimates substantially smaller than those proposed by vicariant events suggest recent dispersal rather than ancient vicariance. Vicariance theorists also assume that common distributional patterns result from shared vicariance events. Under a vicariance model, taxa with parallel distributions would be expected to exhibit similar amounts of genetic divergence. Conversely, dispersal may be inferred if common distribution patterns are not reflected by comparable amounts of molecular divergence (assuming clocklike molecular evolution).

Several studies based on isoenzymes have examined the relationships of various galaxiine species, generally concentrating on Tasmanian or New Zealand members of *Galaxias* (e.g., Mitchell and Scott, 1979; Barker, 1987; Allibone and Wallis, 1993; White and Ovenden, unpubl.). Some of these studies provide support for species groups that are generally accepted on the basis of morphology (e.g., *G. truttaceus, G. auratus, G. tanycephalus; G. brevipinnis, G. johnstoni, G. fontanus*). In contrast, they provide little resolution above the genus level.

In the current study we use phylogenetic analysis of mitochondrial DNA (mtDNA) sequences to test the monophyly of galaxiine genera and to shed light on poorly understood relationships among the Galaxiini. We also aim to discriminate among various hypotheses regarding the phylogenetic placement of the enigmatic *Lepidogalaxias*. Finally, we use phylogenetic analysis and molecular clock calibrations to help resolve the biogeographic (dispersal/vicariance) controversy surrounding the southern temperate distribution of the galaxiines.

Materials and Methods

Tissue samples were obtained from 29 fish (27 species), representing the Southern Hemisphere range of the group (Fig. 1). Previous phylogeographic studies revealed much mtDNA sequence variation in *G. zebratus* (Waters and Cambray, 1997) and *G. maculatus* (Waters and Burridge, 1999), so representatives of two divergent populations were included for both of these species (see Appendix). The 27 species sampled include representatives of all six galaxiine genera and additional galaxioids (*Aplochiton, Lovettia, Retropinna*, and *Lepidogalaxias*). Sequences from nine outgroup taxa (seven genera; see below) were obtained from GenBank. Specimens were collected with hand nets or electrofishing apparatus. Tissue was stored frozen or placed in 70–90% ethanol.

Total DNA was extracted by either the Chelex method outlined in Waters and Cambray (1997) or the CTAB protocol adapted from Saghai-Maroof et al. (1984; see Grewe et al., 1993). Purified DNA from *G. postvectis* was provided by Lucette Dijkstra (NIWA; Christchurch, New Zealand). The 5′ region of the cytochrome *b* gene was amplified with primer H15149 (5′-CCCTCAGAATGATATTTGTCCTCA-3′; Kocher et al., 1989) and either L14841 (5′-CCATCCAACATCTCAGCATGATGAA A-3′; Kocher et al., 1989) or L14724 (5′-CGAAGCTTGATGAAAAACCATCGTTG-3′; Pääbo, 1990). Then ~550 bp of the mitochondrial 16S rRNA gene was amplified by using the universal primers 16Sar (5′-CGCCTGTTTATCAAAAACAT-3′) and 16Sbr (5′-CCGGTCTGAACTCAGATC ACGT-3′; (Palumbi et al., 1991). Double-stranded polymerase chain reaction (PCR) amplifications and were carried out as in Waters and Cambray (1997). Amplified DNA was purified either directly with the Wizard PCR Prep System (Promega) or after gel purification with the Bresa-Clean (Bresatec) method.

Most purified PCR products were sequenced with Sequenase ver. 2.0 (U. S. Biochemical), separated by polyacrylamide gel electrophoresis, and visualized by autoradiography. Alternatively, some PCR products were sequenced with the ABI Prism Dye-Terminator kit (Perkin-Elmer) and, after precipitation with ethanol, analyzed with an ABI Prism 377 autosequencer. Sequencing reactions were performed in both directions to allow verification of character states.

Initial alignments of the 16S rRNA sequences were performed with CLUSTAL W

(Thompson et al., 1994). Alignments were then adjusted by eye on the basis of 16S rRNA secondary structure (De Rijk et al., 1998). Phylogenetic analyses of galaxioids were conducted by including sequences from Northern Hemisphere salmonids (*Salmo, Salvelinus*, and *Oncorhynchus*) and esocoids (*Esox, Novumbra, Dallia*, and *Umbra*) as outgroups.

Phylogenetic congruence of cytochrome *b* and 16S rRNA data sets was tested by the partition homogeneity test of Farris et al. (1994) with PAUP 4.0b2 (Swofford, 1999). Three hundred partition replicates were analyzed by maximum parsimony (MP), using the heuristic option and 10 replicates of random addition for each replicate. Pairwise sequence divergences were calculated by using the Kimura (1980) two-parameter model of sequence evolution. Phylogenetic trees were constructed with MP and maximum likelihood (ML) methods implemented in PAUP 4.0b2. In MP analyses, optimal trees were recovered with the global heuristic option and using 20 replicates of random sequence addition. Phylogenetic confidence in the MP topology was estimated by bootstrapping (Felsenstein, 1985a), analyzing 1,000 replicate data sets with the full heuristic option. Decay indices (Bremer, 1990) were determined by MP analysis with the constraints option of PAUP 4.0b2.

For ML analyses we used likelihood-ratio tests (Felsenstein, 1981) to justify the choice of a nucleotide substitution model (Table 2). First, we tested the model of equal base frequencies and equal substitution rates (Jukes and Cantor, 1969). Second, we tested the two-parameter model of Hasegawa et al. (1985), which incorporates both unequal base frequencies and a transition (TI) bias. Third, we tested the general time reversible (GTR) model (Yang, 1994), which treats all substitutions as independent events. Finally, we implemented the GTR model with a Γ distribution correction, which allows for among-site variation (Gu et al., 1995). Likelihood-ratio tests indicated that the latter model (GTR + Γ) significantly improved likelihood (Table 2). This model of molecular evolution was used for subsequent ML analyses. Heuristic ML searches were performed with a starting tree obtained from MP analysis. Bootstrap estimates for ML were obtained from 100 replicates by using the "fast" stepwise-addition option.

The TI bias was estimated with ML and an associated transversion (TV) weighting scheme (TV:TI = 2:1) was implemented for MP, using a stepmatrix. Indels in 16S rRNA sequences were treated as a fifth base for MP, with a weighting of 2:1 relative to TIs. Indel weights were implemented using a stepmatrix. Character weights of 2:1 and 4:1 were applied to first, and second-codon positions relative to third positions, respectively, for cytochrome *b*. A weight of 2:1 was applied to stem regions relative to loop regions of 16S rRNA, according to the secondary structure of De Rijk et al. (1998). Various alternative character/substitution weighting and inclusion schemes (e.g., unweighted; TIs excluded; gaps missing) were examined, and the resulting topologies were evaluated with MP and ML (Table 3).

Different MP topologies were compared by the nonparametric two-tailed Wilcoxon signed-ranks test (Templeton, 1983; Felsenstein, 1985b) performed by PAUP 4.0b2. Significantly different ML topologies were identified by the two-tailed test of Kishino and Hasegawa (1989) implemented in PAUP 4.0d65. Tests for clocklike behavior of cytochrome *b*, 16S rRNA, and combined data were performed with the two-cluster test (linearized tree method) of Takezaki et al. (1995) by using programs distributed by the authors. Branch lengths were also calculated under the assumption of clocklike evolution for the estimation of lineage divergence times, by using the Kimura (1980) two-parameter model of sequence evolution.

TABLE 2. ML scores for a constrained MP tree topology, calculated under a variety of nucleotide substitution models. Models that significantly increase likelihood were identified with likelihood-ratio tests (see Materials and Methods). Degrees of freedom were determined by the difference in the number of parameters associated with the various models.

Substitution model	ln likelihood	Parameters	Comparison	*P*
Jukes–Cantor (JC)	−11124.61	0	–	–
Hasegawa–Kishino–Yano (HKY)	−10592.19	1	HKY vs. JC	<0.001
General time reversible (GTR)	−10479.40	5	GTR vs. HKY	<0.001
GTR + Γ	−8838.08	6	GTR + Γ vs. GTR	<0.001

TABLE 3. MP tree lengths and likelihood scores for tree topologies derived from MP under a variety of weighting strategies. The various topologies were evaluated against optimal weighted MP and ML topologies by using Templeton (1983; MP) and Kishino–Hasegawa (1989; ML) tests. Probability values from these tests are given in separate columns: Templeton (MP) and K-H (ML). The extra steps (MP) and extra ln likelihood (ML) forced by the alternative topologies are indicated. Optimal tree scores are shown in bold.

Weighting strategy	MP tree length	Extra MP	Templeton (MP)	In likelihood	Extra ML	K-H (ML)
MP wtd, all data	**3137**	–	–	−8845.43	14.65	0.2935
ML all data	3164	27	0.2619	**−8830.03**	–	–
MP unwtd, gaps excluded	3160	23	0.8355	−8854.17	24.14	0.1898
MP unwtd, gaps included	3172	35	0.6022	−8854.92	24.89	0.1586
MP wtd, Tls + gaps excluded	3181	44	0.1316	−8850.92	20.20	0.1840
MP wtd, Tls excluded	3157	20	0.5183	−8843.13	13.10	0.2675
MP wtd, 3rd codon Tls excluded	3142	5	0.7728	−8839.34	9.31	0.4948
MP wtd, 3rd codons excluded	3148	9	0.9297	−8843.96	13.94	0.2926

wtd, weighted; unwtd, unweighted; TI, transition.

Because of the lack of galaxiid fossil data, we chose to attempt clock calibrations using published rates of evolution for mitochondrial genes.

RESULTS

Sequence Variation: Cytochrome b

No variation in length was detected among galaxioid cytochrome *b* sequences. Of the 303 nucleotide positions sampled, 158 (52%) were invariant. Of the 145 variable sites, 101 (70%) were third-codon positions (representing every third position sampled), 31 (21%) were first positions, and 13 (9%) were second positions. The majority (65%) of first-position substitutions were silent changes at codons encoding leucine.

Within the Galaxiidae, K2P sequence divergences ranged from 2.1% (*Galaxias truttaceus–G. auratus*) to 39.9% (*G. maculatus–Lepidogalaxias*). Relatively few pairwise comparisons revealed <10% sequence divergence. The very large intraspecific divergences within *G. maculatus* (20.4%; see Waters and Burridge, 1999) and *G. zebratus* (14.0%; see Waters and Cambray, 1997) are more typical of interspecific and even intergeneric comparisons. For example, 7.8% divergence was detected within *Paragalaxias*, whereas mean divergences of 17.2% and 17.4% were detected within *Neochanna* and *Galaxiella*, respectively. The range of cytochrome *b* divergence detected among members of *Galaxias* was 2.1–32.0%. The smallest intergeneric divergence was 9.0%, observed between the New Caledonian *Nesogalaxias neocaledonicus* and Tasmanian *G. brevipinnis*.

Sequence Variation: 16S rRNA

Because the alignment of some 16S rRNA sequences is uncertain at deep levels, we deemed it necessary to exclude 31 characters (27 from loop regions, 4 from stem regions) from species other than galaxiines. Of the 525 aligned nucleotide positions sampled, 163 (31%) were variable. The maximum divergence within the Galaxiidae was 25.2% between *Lepidogalaxias* and *Galaxias truttaceus*. Large intraspecific divergences (~5%) detected within both *G. maculatus* and *G. zebratus* were again similar to values for mean intrageneric comparisons (5.5% *Neochanna*; 6.3% *Galaxiella*; 7.6% *Galaxias*). Divergence among members of the genus *Galaxias* ranged from 1.0% (*G. truttaceus* and *G. auratus*) to 13.9% (*G. zebratus* and *G. maculatus*). Relatively few pairwise comparisons revealed < 5% sequence divergence; *Nesogalaxias* 16S rRNA, however, was separated from that of *G. brevipinnis* by only 3.6% divergence.

We used a general model for the secondary structure of large subunit rRNA available from a Universiteit Antwerpen database (De Rijk et al., 1998) to aid alignment. Note there are some substantial differences between this model (Fig. 3a) and that used in analysis of gymnotiform (Alves-Gomes et al., 1995) and characiform (Ort´ et al., 1996; Ort´, 1997; Ort´ and Meyer, 1997) fish. In those papers, helices G8 to G14 (encompassing variable regions *l* to *n*) are improperly paired or absent, resulting in a large unpaired loop (*l* to *m*). Other regions of the molecule (E24 to G7; G15 to G18) correspond closely at both the primary sequence and secondary structural level with the other model (encompassing variable

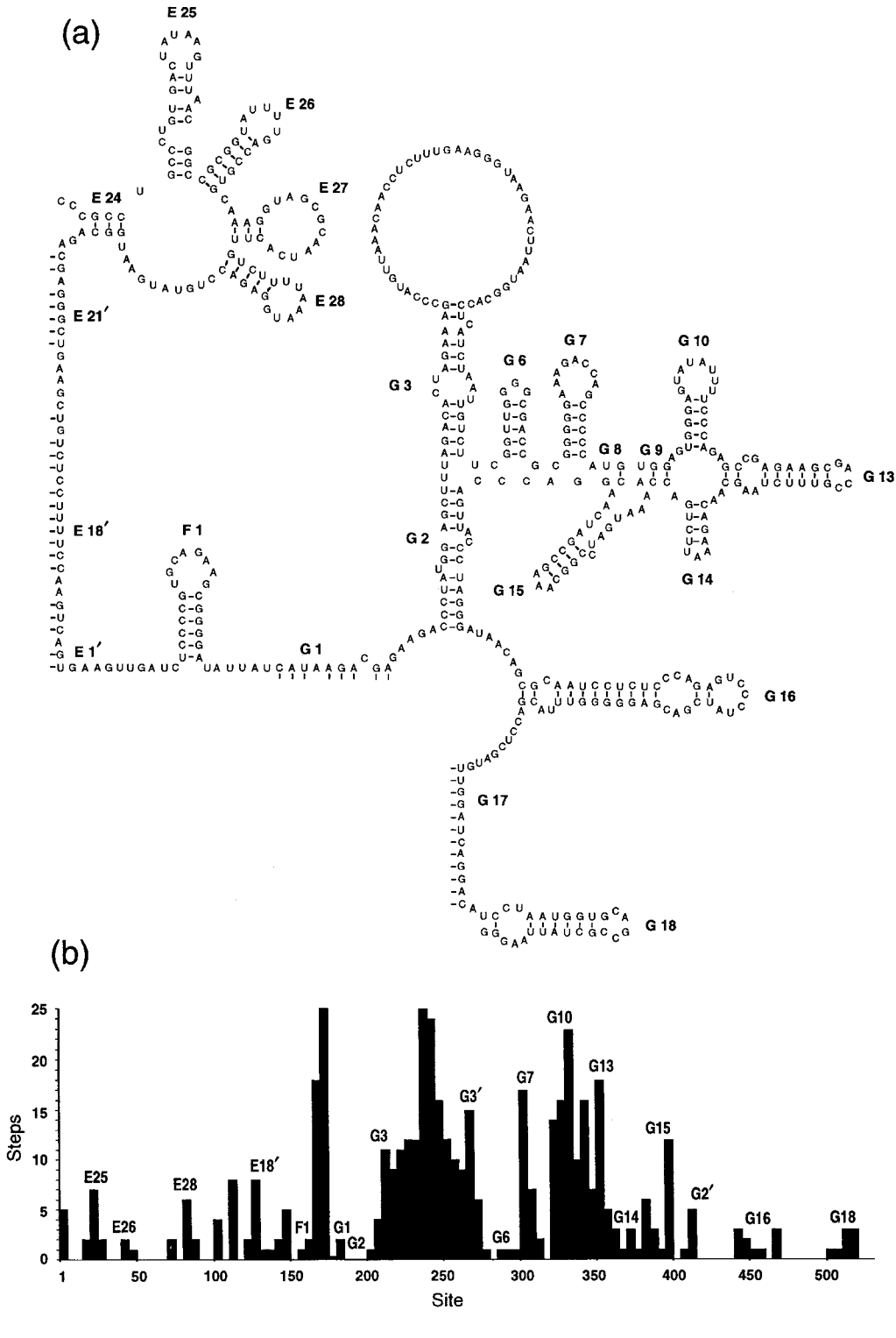

FIGURE 3. (a) Secondary structure for *Galaxias brevipinnis* 16S rRNA based on the large-subunit RNA model and helix notation of De Rijk et al. (1998). Only bonding given in the global model is shown; there could well be some additional bonds. (b) Sliding window analysis (width = 5 bp) presenting the number of evolutionary steps when mapped onto a 27-taxon galaxiine ML tree. Helix numbers map the graph to the secondary structure model above.

regions a–k; and p–q). We also identify an extension to G3 including another 5 bp within variable region *j* of the authors above. The sliding window analysis (Fig. 3b) identifies four other variable regions: between F1 and G1, G7, between G9 and G13, and G15. A more detailed analysis of molecular evolution of 16S in relation to secondary structure will be presented elsewhere.

Phylogenetic Analyses

The partition homogeneity test indicated phylogenetic congruence between cytochrome *b* and 16S rRNA data sets ($P = 0.410$), justifying their combination in phylogenetic analyses. Phylogenetic relationships recovered by MP (3137 steps; Fig. 4) and ML (ln likelihood −8830.64; Fig. 5) methods from combined data are shown with associated bootstrap estimates. Alternative character and substitution weighting schemes failed to change tree topologies significantly ($p > 0.10$; Table 3). Both MP and ML topologies suggest that *Lepidogalaxias* is better placed not in the Galaxiidae but rather as a sister to the rest of the galaxioids (bootstrap 80%; MP). The proposed sister relationship between *Lepidogalaxias* and *Lovettia* added 44 steps to MP tree length and was statistically rejected by Kishino–Hasegawa tests (Table 4). The forced inclusion of *Lepidogalaxias* in the Galaxiidae added 28 steps to MP tree length. *Lepidogalaxias* aside, the monophyly of the galaxiids was strongly reinforced. Specifically, *Aplochiton*, *Lovettia*, and the Galaxiinae represented a monophyletic group (Bremer support = 28 steps). The monophyly of the salmonids was well supported, as was the monophyly of the esocoid genus *Umbra*. The combined monophyly of

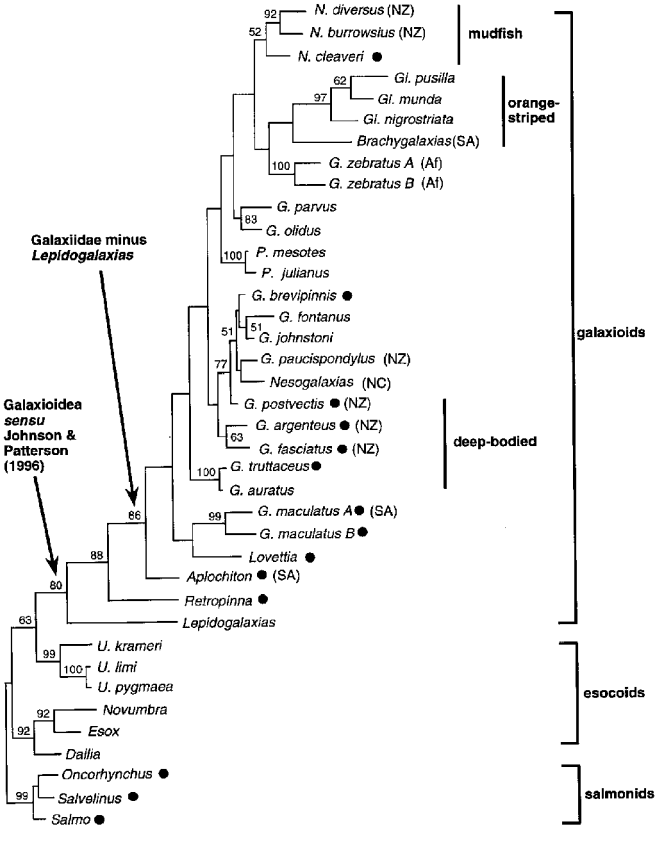

FIGURE 4. MP tree of galaxioid relationships based on combined cytochrome *b* and 16S rRNA sequences. Bootstrap estimates are derived from 1,000 replicates (values <50% are not shown). Species marked with closed circles are migratory; the remaining species are freshwater-limited. Galaxioid taxa originate from Tasmania/Australia unless otherwise stated. Abbreviations: G. = *Galaxias*; Gl. = *Galaxiella*; N. = *Neochanna*; P. = *Paragalaxias*; NZ = New Zealand; NC = New Caledonia; SA = South America/Falkland Islands; Af = South Africa.

TABLE 4. The number of extra steps, and percentage of MP tree length (3137), forced under the constraints of various evolutionary hypotheses. Alternative hypotheses proposed by previous studies are illustrated in Figure 2. The monophyly of deep-bodied species was proposed by McDowall (1970, 1990), and the combined monophyly of *Aplochiton* and *Lovettia* was supported by Williams (1987, 1996). Constrained topologies were evaluated against the optimal MP topology with Templeton (1983; MP) and Kishino–Hasegawa (1989; ML) tests. Associated probabilities are given and significantly ($P < 0.05$) worse topologies are indicated by asterisks.

			P	
Hypothetical grouping	No. of extra steps	% of tree length	Templeton (MP)	K-H (ML)
Lepidogalaxias–Lovettia	44	1.4	0.0826	0.0483**
Lepidogalaxias–Galaxiidae	28	0.9	0.5630	0.1285
Lepidogalaxias–esocoid	15	0.5	0.4984	0.1912
Esocoid monophyly	9	0.3	0.8488	0.3343
Aplochiton–Lovettia	13	0.4	0.1335	0.1091
Galaxiini monophyly	8	0.3	0.1012	0.1603
Galaxias monophyly	68	2.2	0.0001**	0.0000**
Deep-bodied monophyly	12	0.4	0.6548	0.0589
Rosen (1974)	71	2.3	0.0043**	0.0007**
Fink (1984)	44	1.4	0.0814	0.0712
Williams (1987)	55	1.8	0.0142**	0.0069**
Begle (1991, 1992)	47	1.5	0.0018**	0.0136**
Johnson and Patterson (1996)	52	1.7	0.0120**	0.0032**

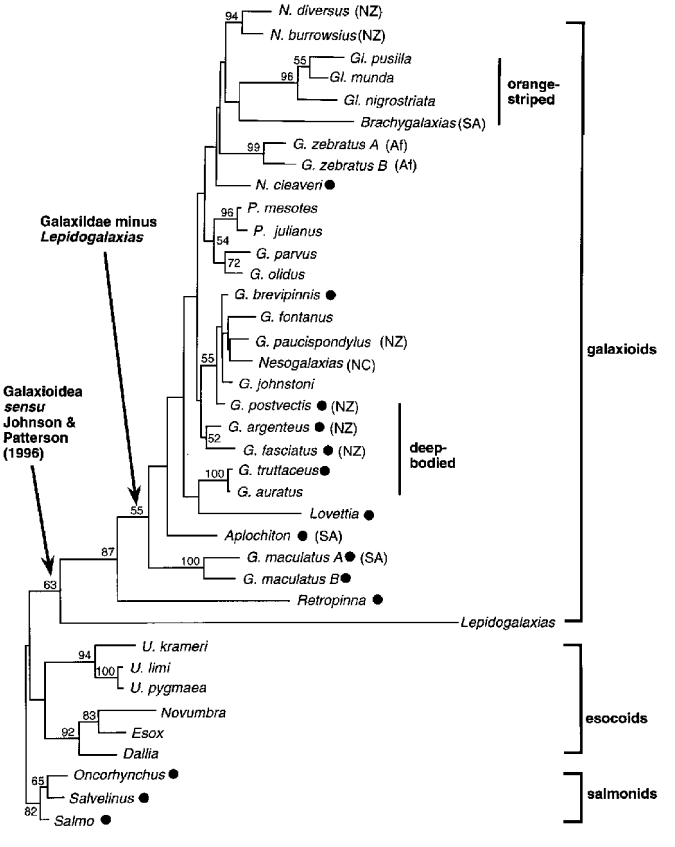

FIGURE 5. ML tree of galaxioid relationships based on combined cytochrome *b* and 16S rRNA sequences. Bootstrap estimates are based on 100 replicates (values <50% are not shown). Species marked with closed circles are migratory, while the rest are freshwater-limited. Galaxioid taxa originate from Tasmania/Australia unless otherwise stated. Abbreviations: G. = *Galaxias*; Gl. = *Galaxiella*; N. = *Neochanna*; P. = *Paragalaxias*; NZ = New Zealand; NC = New Caledonia; SA = South America/ Falkland Islands; Af = South Africa.

the esocoids was supported by the ML topology (bootstrap 40%) but not MP (bootstrap 33%). The controversial placement of *Lepidogalaxias* as sister to *Umbra* was not rejected, receiving 20% and 19% bootstrap support from MP and ML analyses, respectively.

Phylogenetic resolution among galaxiines was generally limited to recent relationships; interior nodes received little support (Figs. 4, 5). Analyses indicated that species possessing a marine larval stage do not represent a monophyletic group. Rather, migratory ability was distributed widely throughout the MP and ML trees, and represented in a number of clades. Both topologies reinforced the morphological similarity of some galaxiine species, with strong support for monophyletic *Paragalaxias* and *Galaxiella* groups, and for the close relationship of *Galaxias truttaceus* and *G. auratus*. The combined monophyly of Tasmanian and New Zealand mudfishes (*Neochanna*) was supported by the MP topology (Fig. 4) with an associated bootstrap value of 52%. The conspecific status of geographic isolates of both *G. zebratus* and *G. maculatus* was reinforced with high bootstrap values.

Substantial evidence supported the polyphyly of the widespread genus *Galaxias* (Table 4). First, the New Caledonian *Nesogalaxias* were clustered within a group of Australian and New Zealand members of *Galaxias* (Figs. 4, 5). Second, *G. parvus* and *G. olidus* formed a monophyletic group (83% bootstrap; MP) that may be sister to *Paragalaxias* (54% bootstrap; ML). Third, the positions of *G. zebratus* and *G. maculatus* appeared to conflict with generic taxonomy, but the relationships of both lineages remained unresolved, probably reflecting an ancient divergence. The enforced monophyly of *Galaxias* markedly increased MP tree length, requiring the addition of 68 steps (Table 4).

Two-cluster tests (Takezaki et al., 1995) performed for rate constancy in Galaxiini + *Aplochiton* + *Lovettia* cytochrome *b* sequences yielded ($\chi^2_{[25]} = 36.3$ ($P > 0.05$), failing to reject clocklike evolution. Similar tests rejected clocklike evolution for 16S rRNA ($\chi^2_{[25]} = 69.9$; $P < 0.05$) and combined ($\chi^2_{[25]} = 60.0$; $P < 0.05$) sequences. More specifically, rate constancy was statistically rejected for branches at three nodes for 16S rRNA and at six nodes for combined data. Molecular clock calibrations were restricted to clades for which clocklike evolution was supported.

Discussion

Phylogenetics of the Galaxioidea

Phylogenetic analyses of the Galaxioidea provide strong support for the monophyly of the Galaxiidae as defined by Johnson and Patterson (1996)—with the notable exception of *Lepidogalaxias*. More specifically, our analyses contradict the current placement of *Lepidogalaxias* as sister to *Lovettia* in the Lepidogalaxiinae (Fig. 2e). The alternative placement of *Lepidogalaxias* as sister to the rest of the Galaxioidea receives substantial bootstrap support in MP and ML analyses—in conflict with the conclusions of Fink (1984), Williams (1987), Begle (1991), and Johnson and Patterson (1996) (Fig. 2). Of the many investigators to have documented the similarities of *Lepidogalaxias* and *Umbra*, only Rosen (1974) suggested a close phylogenetic relationship between these taxa. Our analyses failed to reject this element of Rosen's hypothesis (Table 4). However, the overall phylogeny proposed by Rosen (1974; see Fig. 2a) was statistically rejected (Table 4), largely because of his proposed sister relationship for galaxiids and salmonids. In keeping with the results of Lopez et al. (2000), MP analyses suggest that the esocoids may represent a paraphyletic group. Specifically, although *Novumbra, Esox*, and *Dallia* form a well-supported clade, *Umbra* is placed as sister to the galaxioids. In contrast, the ML topology is consistent with current taxonomy, supporting esocoid monophyly.

Relationships Within the Galaxiidae

The mtDNA sequence data support the combined monophyly of *Aplochiton, Lovettia*, and the Galaxiini. In this regard the data are consistent with the conclusions of a clear majority of morphological studies (e.g., Rosen, 1974; Fink, 1984; Williams, 1987; Begle, 1991; but *not* Johnson and Patterson, 1996) (Fig. 2). However, the relationships among the three groups are still not clear. No strong molecular evidence has yet been produced that Galaxiini represents a monophyletic group. The MP topology places *Aplochiton* as sister to *Lovettia* and the Galaxiini, but this placement receives

very low bootstrap support, as do alternative topologies. Williams' (1987, 1996) proposed sister relationship for *Aplochiton* and *Lovettia* (Fig. 2c) is not rejected.

Monophyly of Galaxiine Genera

The mtDNA data agree, at least partially, with the current generic taxonomy of the Galaxiini in the sense that three of the six genera are supported as monophyletic. Specifically, species of *Paragalaxias* and *Galaxiella* are respectively grouped with high bootstrap support (>95%), and the monophyly of Neochanna receives 52% bootstrap support from MP. The probable monophyly of the two monotypic genera (*Brachygalaxias* and *Nesogalaxias*) remains untested by our data.

In contrast, the genetic data indicate that the widespread and speciose genus *Galaxias* is *not* monophyletic. Specifically, enforced monophyly significantly worsens MP and ML topologies (Table 4). The polyphyly of *Galaxias* is further indicated by the inclusion (in as many as 77% of bootstraps) of *Nesogalaxias* in a clade that otherwise contains *Galaxias* species from Tasmania and New Zealand. In addition, ML suggests a sister relationship for *Paragalaxias* and *G. parvus–G. olidus*. The generic placements of the widespread *G. maculatus* and the South African *G. zebratus* are also questionable, given that both are highly divergent, to the point of saturation, from all other members of the tribe. As is generally the case within the Galaxiini, deep phylogenetic relationships remain uncertain, making the generic status of divergent species a matter of debate.

Phylogenetic Resolution Within the Galaxiini

The lack of phylogenetic resolution at deep levels within the Galaxiidae may be attributed to several factors, including saturation effects, rate heterogeneity, and rapid cladogenesis. Perhaps the most probable explanation involves distantly related galaxiine sequences becoming saturated with changes. The fact that some cytochrome *b* sequences (e.g., *G. zebratus, G. maculatus*) differ from others by >30% suggests that they may have reached saturation. Graybeal (1993) noted that because amino acid replacements are rare, 25% divergence represents the approximate saturation level for cytochrome *b*.

Biogeography—Tectonics, Dispersal, and Genetics

Africa began drifting away from Gondwana 135 million years ago (MYA), with substantial distance between southern Africa and Antarctica by 120 MYA. However, even at 100 MYA, freshwater interchange between Africa and South America was still possible. Africa was entirely separated from Gondwana by 85 MYA (C. Burrett, pers. comm.). New Zealand separated from Gondwana ~80 MYA (Weissel et al., 1977; Kamp, 1986) and has been separated from Australia by at least 1,200 km (and as much as 2,000 km) for the last 60 MY (Cooper and Milliner, 1993). Similarly, New Caledonia separated from the northern part of the Lord Howe Rise in the late Cretaceous between 74 and 64 MYA with the opening of the New Caledonia Basin (Yan and Kroenke, 1993). However, some sea mounts along the Norfolk Ridge could have emerged during Miocene periods of low sea level. Australia and southern South America remained more or less connected by Antarctica until 40 MYA (Veevers et al., 1991). However, ocean temperatures remained warm until 30 MYA, when continental separation was great enough to allow an unimpeded circumpolar current (Veevers, 1991).

New Zealand's freshwater habitats were limited by historical marine transgressions. For example, in the mid-Oligocene, at least 80% of New Zealand's present land area was probably submerged Cooper and Milliner, 1993). Pole (1994) suggested that this event may have eliminated remnant gondwanan biota. He argued that the present flora of New Zealand can be largely, if not totally, attributed to long-distance dispersal rather than vicariance. Even the podocarps and southern beeches, which are usually interpreted as gondwanan, may be present in New Zealand as a result of dispersal (Hill and Jordan, 1993; Pole, 1994).

Molecular studies of southern biota increasingly indicate that vicariance and dispersal are far from being the incompatible alternatives sometimes portrayed (Croizat et al., 1974; Rosen, 1978; Craw, 1979). For instance, Van Tuinen et al. (1998) proposed an evolutionary scenario for the ratite birds that combines aspects of both dispersal and vicariance theory. Phylogenetic relationships among *Nothofagus* species (Linder and Crisp,

1995) conflict with the accepted pattern of continental vicariance, suggesting that recent transoceanic dispersal has occurred in some lineages of this gondwanan group. Molecular clock calibrations similarly indicate that phylogenetic separation of Australian and African baobabs occurred well after the breakup of Gondwana (Baum et al., 1998).

mtDNA data have similarly increased our understanding of fish biogeography. Sequence data suggest that the major lineages of characiform fishes arose before the separation of Africa and South America, indicating a vicariant origin for their current distribution (Ort´ and Meyer, 1997). Gondwanan origins are also supported for extant lineages of cichlids (Farias et al., 1999) and aplocheiloid fishes (Murphy and Collier, 1997). In contrast, shallow mtDNA divergence among Chilean, South African and Australian populations of *Sardinops* suggests a recent history of dispersal rather than ancient vicariance for these taxa (Bowen and Grant, 1997). Although the mtDNA divergences among regional haplotypes of *Galaxias maculatus* are far deeper than those in *Sardinops*, they still seem to support a more recent phylogenetic separation than predicted by models based on plate tectonics (Waters and Burridge, 1999). More importantly, the sister relationship of Australian and New Zealand haplotypes conflicts with the accepted pattern of continental fragmentation.

Molecular Clock Calibrations—Galaxiine Evolution and Biogeography

Molecular clock calibrations were used to estimate the times of well-supported phylogenetic divergences within the Galaxiinae (Table 5). The estimation of divergence times was generally restricted to terminal rather than interior clades, because predominantly the former received substantial bootstrap support. The separation of *Brachygalaxias* and *Galaxiella* was also dated, despite relatively low bootstrap support, because of substantial morphological evidence indicating that these taxa form a clade. These rough divergence timings should be interpreted with caution, for they are based on calibrations derived from other fishes, a strategy necessitated by a lack of galaxiid fossil data. Nevertheless, the use of a wide range of calibrated rates should allow us to discriminate between ancient (vicariant) and recent (dispersal) hypotheses of galaxiid biogeography.

Clock calibrations postdate continental fragmentation (Table 5), and thus favor marine dispersal rather than vicariance for the wide distributions of *Neochanna* (see also Waters and White, 1997) and *Galaxias maculatus* (see Waters and Burridge, 1999). All estimated divergence times were within the last 30 million years, ranging from 0.8–4.3 MYA for *G. truttaceus–G. auratus* to 8.6–30.0 MYA between *Brachygalaxias* and *Galaxiella*. The 24.0% divergence between *Brachygalaxias* and *Galaxiella* cytochrome *b* suggests that these sequences are approaching or have reached saturation, probably confounding clock calibrations for these taxa.

Deep-Bodied Galaxiines

Landlocking and subsequent speciation have been hypothesized as major factors in the diversification of galaxiine fishes. Ovenden et al. (1993) stated that nonmigratory deep-bodied galaxiines endemic to central Tasmanian lakes, such as *G. auratus*, were probably derived from landlocked populations of the migratory *G. truttaceus*. Similar speciation may be under way in more

TABLE 5. Divergence times (MY = million years) for selected phylogenetic splits within the Galaxiini as based on molecular clock calibrations for mitochondrial protein-coding genes (Ort´ et al., 1994; Taylor and Dodson, 1994; McKay et al., 1996), fish rRNA (Alves-Gomes, 1999) and salmonid mtDNA (Martin and Palumbi, 1993). Nodes that failed two-cluster tests (rate constancy; Takezaki et al., 1995) are excluded from calibrations.

Phylogenetic split	Cyt *b* %	0.8–2.6%/MY	16S%	0.23%/MY	mtDNA %	0.5–0.9%/MY
G. zebratus (Olifants–Noetsie)	13.6	14.9–17.0	5.3	23.0	8.0	8.9–16.0
G. maculatus (Tasmania–Falklands)	21.5	7.7–26.9	4.0	17.4	9.6	10.7–19.2
G. truttaceus–G. auratus	2.2	0.8–2.8	1.0	4.3	1.4	1.6–2.8
Nesogalaxias–G. brevipinnis	9.0	3.2–11.3	2.9	12.6	6.2	6.9–12.4
G. olidus–G. parvus	15.3	5.5–19.1	2.3	10.0	6.8	7.6–13.6
P. julianus–P. mesotes	7.7	2.8–9.6	–	–	2.6	2.9–5.2
N. diversus–N. burrowsius	13.2	4.7–16.5	4.9	21.3	–	–
N. cleaveri–(*N. burrowsius, N. diversus*)	17.6	6.3–22.0	–	–	9.5	10.6–19.0
Galaxiella–Brachygalaxias	24.0	8.6–30.0	–	–	–	–

recently landlocked *G. truttaceus* (Ovenden and White, 1990). The lakes containing *G. auratus* are probably <100,000 years old (M. Banks, pers. comm.). However, the estimated timing of the *G. truttaceus–G. auratus* divergence (0.8–4.3 MYA) predates the hypothesized lake formation. We hypothesize that *G. auratus* evolved before the formation of Lake Crescent. Alternatively, the discrepancy in timing may reflect the presence of genetic diversity within *G. truttaceus* before the bottleneck and stochastic lineage-sorting associated with landlocking.

McDowall (1990) has suggested that the migratory deep-bodied galaxiines endemic to New Zealand (*G. fasciatus, G. argenteus*, and *G. postvectis*) share a close relationship with Australian *G. truttaceus* (and *G. auratus*), possibly reflecting successive episodes of trans-Tasman dispersal and speciation. Our current study suggests that only *G. fasciatus* and *G. argenteus* comprise a clade. However, enforced monophyly of all five deep-bodied species requires only 12 extra steps, not enough for rejection. Nevertheless, we think it likely that these taxa represent two or more colonizations by migratory Australian ancestors. If trans-Tasman marine dispersal is highly sporadic, as indicated by *G. maculatus* mtDNA sequences (Waters and Burridge, 1999; Waters et al., 2000), speciation may have rapidly followed colonization.

New Caledonian Galaxiine

Under vicariance theory, the New Caledonian galaxiine is an ancient lineage that has occupied New Caledonia since its separation from Gondwana ~70 MYA. However, McDowall (1968) suggested that this species colonized New Caledonia by marine dispersal during a cool period of the Pliocene or Pleistocene, with the subsequent warming confining the species to upland lakes. The mtDNA data suggest that *N. neocaledonicus* and migratory *G. brevipinnis* diverged from a common ancestor 3–13 MYA. Unless land–island links between New Zealand and New Caledonia were present as recently as the late Miocene/Pliocene, marine dispersal is the most probable explanation for the origin of *N. neocaledonicus*.

McDowall noted that *N. neocaledonicus* is morphologically similar to some members of the genus *Galaxias*. His decision to elevate the species to generic level was based on the absence of pleural ribs posterior to the pelvic girdle. The present study, however, indicates that *Nesogalaxias* is a member of a clade comprising *G. brevipinnis* and other closely related (mostly nonmigratory) species of *Galaxias*; thus we recommend that the New Caledonian species be restored to *Galaxias*.

Paragalaxias

Paragalaxias mesotes and *P. julianus* are estimated to have diverged 3–10 MYA. This supports the suggestion of McDowall and Fulton (1978) that *Paragalaxias* predates the Pleistocene (>1.65 MYA). Both species occur in the Tasmanian central plateau, with *P. julianus* occurring in south-flowing drainages, whereas *P. mesotes* is restricted to a north-flowing drainage. The distribution of *Paragalaxias* mirrors the distribution of the endemic syncarid crustacean *Paranaspides* (Fulton, 1982). If, as suggested (McDowall and Fulton, 1978), this shared distribution pattern was caused by river capture, then the timing of the *Paragalaxias mesotes–P. julianus* divergence may represent an approximate date for the hypothesized change in drainage pattern.

McDowall and Fulton (1978) noted that "the primitive stock from which *Paragalaxias* is derived is not yet evident". They suggested that this genus could be derived from *Aplochiton* or *Lovettia* by the loss of the adipose fin or, more likely, derived from a galaxiine by the forward movement of the dorsal fin. The findings of the present study indicate that the latter hypothesis is correct. Specifically, mtDNA sequences suggest that *Paragalaxias* is closely related to *G. olidus–G. parvus*.

No previous study has suggested that *G. parvus* and *G. olidus* share a close phyletic relationship. These species were paired by phenetic analyses of morphometric and meristic data (Johnson et al., 1983) but this unexpected result was interpreted as reflecting convergence rather than a phylogenetic relationship. However, the findings of the present study suggest that the latter is true. Biogeographical implications may have prevented serious consideration of such a phylogenetic relationship by past workers. Tasmanian *G. parvus* and Victorian *G. olidus* apparently diverged from a common ancestor 5–20 MYA.

Coupled with their apparent morphological similarity (Johnson et al., 1983), the genetic similarity of *G. olidus* and *G. parvus* leaves little doubt that they represent a clade. The mtDNA phylogeny suggests that they could be allocated to *Paragalaxias*. Alternatively, *G. olidus* and *G. parvus* may belong in a new separate genus. Until a thorough morphological examination is complete, the generic status of these species should remain unchanged.

Brachygalaxias–Galaxiella

The mtDNA phylogeny provides only tentative support for the common ancestry of the South American genus *Brachygalaxias* and the Australian genus *Galaxiella*. Originally, the Australian and South American species were all included in *Brachygalaxias*. McDowall (1973b), however, limited the genus to contain only the South American species because of its extended premaxillary alveolar process. Nevertheless, the orange stripe, posterior dorsal fin, large eyes, and reduced supraneural series common to both genera are unique within the Galaxiidae. McDowall (1973c, 1978a) noted the obvious similarities between *Galaxiella* and *Brachygalaxias* but claimed that there was little to indicate a phylogenetic relationship. In other words, he considered the similarities to reflect convergence rather than common ancestry. Given the strong morphological similarities and the mtDNA support (albeit weak) for their monophyly, we recommend that the Australian species be restored to *Brachygalaxias*, with *Galaxiella* used for subgeneric ranking if necessary.

Assuming that the grouping of *Brachygalaxias* and *Galaxiella* reflects their true phylogeny, the presence of this clade in both Australia and South America could reflect either dispersal or vicariance. Both *Brachygalaxias* and *Galaxiella* contain only freshwater-limited taxa. Given the lack of a migratory phase, there is probably no reason to invoke oceanic dispersal for this clade. In contrast, because Australia and South America an thought to have been virtually connected by Antarctica until 40 MYA vicariance seems to be the most plausible explanation for the distribution of *Brachygalaxias* and *Galaxiella*. The molecular data do not necessarily conflict with this hypothesis, suggesting that the phylogenetic split occurred ≤ 30 MYA, possibly much earlier if saturation of cytochrome *b* is taken into account.

Conclusions

We conclude that *Lepidogalaxias* is not a galaxiid but rather an ancient lineage that may have diverged well before the breakup of Gondwana. In contrast, the monophyly of the remaining eight galaxiid genera (*Lovettia* + *Aplochiton* + Galaxiini) is well-supported. *Lepidogalaxias* can be interpreted as sister to other Galaxioidea, although a position as sister of *Umbra* (Rosen, 1974) cannot be rejected. This contradicts the hypotheses of Johnson and Patterson (1996) and others.

Our data generally support the hypothesis that migratory ability is a primitive feature of the galaxiine fishes. Specifically, a marine phase is often present in basal members of clades (e.g., *Galaxias postvectis* and *N. cleaveri*) but absent from more-derived species within such clades. In some instances (e.g., *G. brevipinnis* and related species) a single migratory species may have given rise to several landlocked species through the repeated loss of diadromy (Allibone et al., 1996).

Galaxiine radiations fall into three general biogeographical categories. The first includes clades of nonmigratory species limited to a single landmass, which probably attained their current distribution as a result of changing drainage patterns through river capture or sea-level fluctuation. Such clades include *Galaxiella, Paragalaxias*, and divergent populations of *Galaxias zebratus*. The second group of clades include migratory species and are represented on multiple continents, but exhibit divergences that postdate continental separation. Such groups include *Neochanna*, the *G. brevipinnis* clade, and populations of *G. maculatus* and are best explained by oceanic dispersal. Within such groups, loss of migratory ability has apparently given rise to species such as *G. fontanus* and *G. johnstoni* in Tasmania and the New Zealand members of *Neochanna*. Indeed, the loss of a marine juvenile phase may be an important mechanism of speciation in galaxiine fish. Finally, one nonmigratory clade is represented on more than one landmass and may be best explained by continental separation. Specifically, *Galaxiella* and *Brachygalaxias* are likely to represent an ancient gondwanan

radiation. The divergence of South African *Galaxias zebratus* is clearly ancient and may also date back to the rifting of Gondwana.

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APPENDIX. LOCATION AND GENBANK ACCESSION DETAILS FOR FISH AND ASSOCIATED MTDNA SEQUENCES

Species	Origin	Locality	GenBank accession no.	
			Cytochrome *b*	16S rRNA
Neochanna burrowsius	New Zealand	Canterbury	AF022098	AF022106
Neochanna cleaveri	Tasmania	Allens Ck.	AF022096	AF022104
Neochanna diversus	New Zealand	Hamilton	AF022097	AF022105
Galaxiella munda	Western Australia	Pemberton	AF112305	AF112329
Galaxiella nigrostriata	Western Australia	Chesapeake Rd.	AF112306	AF112324
Galaxiella pusilla	Tasmania	Forester Lodge	AF112304	AF112325
Brachygalaxias bullocki	Chile	Talca	AF112307	AF112328
Paragalaxias mesotes	Tasmania	Arthurs L.	AF112308	AF112331

APPENDIX. Continued.

Species	Origin	Locality	GenBank accession no.	
			Cytochrome *b*	16S rRNA
Paragalaxias julianus	Tasmania	Carters L.	AF112309	AF112332
Nesogalaxias neocaledonicus	New Caledonia	Len Huit	AF112315	AF112323
Galaxias argenteus	New Zealand	unknown	AF112317	AF112334
Galaxias auratus	Tasmania	LCrescent	AF112319	AF112339
Galaxias brevipinnis	Tasmania	Snug A.	AF022094	AF022102
Galaxias fasciatus	New Zealand	unknown	AF112318	AF112333
Galaxias fontanus	Tasmania	Swan R.	AF112313	AF112338
Galaxias johnstoni	Tasmania	Clarence Lagoon	AF112316	AF112337
Galaxias maculatus A	Falkland Islands	Saunders Island	AF049466	AF007034
Galaxias maculatus B	Tasmania	Sandy Bay R.	AF007023	AF022101
Galaxias olidus	Victoria	Glenelg R.	AF112311	AF112336
Galaxias parvus	Tasmania	48 Ck.	AF112310	AF112335
Galaxias paucispondylus	New Zealand	Wilberforce R.	AF112314	AF112330
Galaxias postvectis	New Zealand	unknown	AF112312	AF112343
Galaxias truttaceus	Tasmania	Allens Ck.	AF022092	AF022100
Galaxias zebratus A	South Africa	Noetsie R.	U66611	AF112326
Galaxias zebratus B	South Africa	Olifants R.	U66609	AF112327
Aplochiton zebra	East Falkland	Deep Arroja R.	AF022099	AF022107
Lovettia sealii	Tasmania	Tamar R.	AF112320	AF112341
Retropinna tasmanica	Tasmania	Derwent R.	AF112321	AF112342
Lepidogalaxias salamandroides	Western Australia	Chesapeake Rd.	AF112322	AF112340
Esox lucius	North America	unknown	AF060439	AF060446
Dallia pectoralis	North America	unknown	AF060440	AF060448
Novumbra hubbsi	North America	unknown	AF060438	AF060447
Umbra krameri	North America	unknown	AF060437	AF060444
Umbra limi	North America	unknown	AF060436	AF060443
Umbra pygmae	North America	unknown	AF060435	AF060442
Oncorhynchus mykiss	North America	unknown	L29771	L29771
Salmo salar	North America	unknown	U12146	U12143
Salvelinus leucomaenis	Japan	unknown	AF060441	AF060445