Phylogenetic Placement of Retropinnid Fishes: Data Set Incongruence Can Be Reduced by Using Asymmetric Character State Transformation Costs

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Abstract.—We used mitochondrial DNA sequences to determine the phylogenetic placement of southern smelts (Retropinnidae), a group of diadromous fishes endemic to New Zealand and Australia. Our genetic data strongly support a sister group relationship between retropinnids and northern hemisphere smelts (Osmeridae), a relationship that seems consistent with the similar appearance and life history strategies of these two groups. Our analysis indicates that Retropinnidae and Osmeridae together represent the sister group to the southern hemisphere galaxiid fishes (Galaxiidae). However, this finding conflicts with several recent osteological analyses, which supported a sister relationship for Retropinnidae and Galaxiidae, giving a monophyletic southern hemisphere assemblage (Galaxioidea). We review cases of incongruence and discuss factors that might explain significant disagreement between molecular and morphological data matrices. We suggest that repeated evolutionary simplification may have undermined the accuracy of morphological hypotheses of osmeroid relationships. Although equally weighted parsimony analysis of morphological data rejects the molecular hypothesis (Osmeridae + Retropinnidae), implementation of a range of weighting schemes suggests that incongruence is nonsignificant under asymmetric character transformation models. We propose that a simple “equal transformation cost” parsimony analysis may be biologically unrealistic, especially when reductive homoplasy is widespread; as is increasingly being accepted, complex character states are more readily lost than gained. Therefore, we recommend that morphological systematists routinely implement a range of character transformation models to assess the sensitivity of their phylogenetic reconstructions. We discuss the antitropical biogeography of osmeroid fishes in the context of vicariance and transequatorial dispersal. [16S; Australia; character transformation; congruence; cyt b; galaxiid; New Zealand; osmerid; retropinnid; smelt.]

The osmeroid fishes (suborder Osmeroidei; Johnson and Patterson, 1996; Table 1) are a major element of the world’s temperate freshwater fish fauna. In the northern hemisphere, Osmeroidei are represented by the single family Osmeridae (northern smelts), comprising 11 genera with about 30 species. The discovery of a fossilized smelt from the Paleocene (around 60 million years ago [Ma]; Wilson and Williams, 1991) in North America confirmed that osmerids are an ancient component of the northern ichthyofauna. In the southern hemisphere, Osmeroidei are represented by the Galaxioidea, which comprise approximately 60 species in two families: Galaxiidae (galaxiids) and Retropinnidae (southern smelt). Galaxioids are thought to represent an ancient component of the southern hemisphere fauna, with fossil evidence indicating their presence in New Zealand certainly as early as 20 Ma (McDowall and Pole, 1997) and in South Africa around 70 Ma (Anderson, 1998). The gondwanan distribution of galaxioids, and the broader antitropical distribution of osmeroid fishes, have generated considerable biogeographic controversy (McDowall, 1990). Some workers explain the group’s biogeography in terms of oceanic dispersal (e.g., McDowall, 1978; Taylor and Dodson, 1994; Berra et al., 1996); others consider that it reflects plate tectonics (e.g., Croizat et al., 1974; Rosen, 1978). Recent DNA sequence analyses provide evidence that both vicariance and dispersal have influenced osmeroid distribution (Waters et al., 2000a, b).

OSMEROID INTERRELATIONSHIPS

The Retropinnidae sensu Johnson and Patterson (1996) comprises three genera: Retropinna, Prototroctes, and Stokellia. These fishes are characterized by an adipose fin, cycloid scales, the absence of a lateral line, and a cucumber odor, among other traits. The genus Retropinna (southern smelt) contains three small species: R. tasmanica (Tasmania;
Table 1. Current phylogenetic classification of the Osmeroidei, after Johnson and Patterson (1996).

<table>
<thead>
<tr>
<th>Superfamily</th>
<th>Family</th>
<th>Subfamily</th>
<th>Tribe</th>
<th>Genus</th>
<th>No. of species</th>
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<td></td>
<td></td>
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</tr>
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<tr>
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<td></td>
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<td>Salanx</td>
<td>4</td>
</tr>
<tr>
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<td></td>
<td></td>
<td></td>
<td>Neosalanx</td>
<td>4</td>
</tr>
<tr>
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<td></td>
<td></td>
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<td>Osmerus</td>
<td>3</td>
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<tr>
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<td></td>
<td></td>
<td></td>
<td>Allosmerus</td>
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<td>Spirinchus</td>
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<td>Thaleichthys</td>
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<td>—</td>
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<tr>
<td></td>
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<td>—</td>
<td>—</td>
<td>Stokellia</td>
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<tr>
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<td>Lovetta</td>
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<td>Paragalaxias</td>
<td>4</td>
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anadromous), *R. semoni* (Australia; euryhaline), and *R. retropinna* (New Zealand; anadromous). The genus *Prototroctes* (southern graying) consists of the relatively large amphidromous fishes *P. maraena* (Australia) and *P. oxyrynchus* (New Zealand, now extinct; McDowall, 2000). Stokell's smelt, *Stokellia anisodon* (New Zealand; anadromous) is the sole representative of the third retropinnid genus (McDowall, 2000). Although McDowall (1969, 2000) and Nelson (1994) place *Prototroctes* in a separate family Prototroctidae, they do not dispute the monophyly of Retropinnidae sensu Johnson and Patterson (1996). Rosen (1974) hypothesized a paraphyletic origin for the galaxioids, with retropinnids sister to osmerids. However, most systematists have subsequently supported the monophyly of the galaxioid assemblage (e.g., Fink, 1984; Begle, 1991; Johnson and Patterson, 1996; Williams, 1996). In contrast, morphologists have had considerable difficulty resolving the relationships of osmerid genera. Indeed, Johnson and Patterson (1996:303) went so far as to suggest that “osmerids are unique in the disparity of opinion on their interrelationships.” For instance, the placement of the Asian subfamily Salangini (icefishes; sensu Johnson and Patterson, 1996) is highly controversial, reflecting the paedomorphic features of this group. Suggested placements include the following:

1. Greenwood et al. (1966) included the salangids in the Galaxioidea. However, (2) Weitzman (1967) suggested that, rather than being close to either the galaxioids or osmerids, the salangids may represent a separate group. (3) McDowall (1969:816) argued that salangids do not constitute part of the galaxiid radiation, considering them to be a “very specialized offshoot of salmonoids.” (4) Fink (1984) supported their placement in the Osmeroidei but was undecided as to whether the salangids formed part of the northern osmerid or southern galaxioid assemblages. (5) Williams (1987, 1996) hypothesized that salangids belong in the northern osmerid radiation. In contrast, (6) Begle (1991) supported placement of the salangids in the Galaxioidea. However, (7) Johnson and Patterson (1996) and Patterson and Johnson (1997) strongly criticized Begle’s data matrix and instead assigned the salangids as the sister group of the osmerid genus *Mallotus*. Johnson and Patterson (1996) recognized that even their extensive morphological analysis of osmerid fishes had probably failed to provide a final solution for osmerid relationships.

**Molecular Analysis—Methods**

Representative samples of Osmeridae (five genera) and Retropinnidae (five species; three genera) were collected, anaesthetized,
and placed in ethanol. Protocols for DNA extraction, PCR amplification, and DNA sequencing are as described in Waters et al. (2000b). In several cases only one individual per species was sequenced, but all sequences were compared against reference euteleost sequences to confirm homology. Moreover, sequencing of various osmerid and retropinnid taxa was performed independently in New Zealand and Japanese laboratories to guard against contamination. The 5′ region of the cytochrome \(b\) gene was amplified with primer H15149 (5′-CCCTCAGATGATTTGTTCCTCA-3′; Kocher et al., 1989) and either L14841 (5′-CCATCCACATCTCAGCATGATGAA-3′; Kocher et al., 1989) or L14724 (5′-CG AAGCTTGATGAAAACCATCGTTG-3′; Pääbo, 1990). Approximately 550 base pairs (bp) of the mitochondrial 16S rRNA gene was amplified by using the universal primers 16Sar (5′-CGCCTGTTTATCAAAAACAT-3′) and 16Sbr (5′-CCGGTCTGAACTCAGATCACGT-3′; Palumbi et al., 1991). The mitochondrial 16S rRNA and cytochrome \(b\) sequences of representatives of the Galaxiidae (10 species; seven genera) were taken from Waters et al. (2000b). Sequences of Lepidogalaxias, Salmo, and Esox were included as outgroups (see Waters et al., 2000b). Data were submitted to TreeBASE (study accession S697; matrix M1111; http://treebase.org); GenBank accession details for sequences are listed in Table 2.

Evidence is growing that secondary structure analysis presents the most accurate method of ribosomal sequence alignment (Hickson et al., 1996, 2000; Morrison and Ellis, 1997; Page, 2000). Some paired regions of 16S rRNA contain highly conserved structures and motifs, which aid alignment (Buckley et al., 2000), whereas some variable loop regions are prone to indels, making their alignment questionable (Hickson et al., 1996). Initial alignments of our 16S rRNA data were performed by eye and then were adjusted on the basis of secondary structure (De Rijk et al., 1998; see Fig. 1). The full 16S alignment was 529 bp long. However, 29 loop region characters had to be excluded (18 from the loop between G3 and G3′, 9 between G10 and G10′, 2 between G15 and G15′). Across the remaining 500 bp, gap lengths never exceeded 1 bp.

Chi-square tests for homogeneity of nucleotide frequencies across ingroup taxa performed on phylogenetically informative sites by using PAUP 4.0b4 (Swofford, 1999) revealed significant heterogeneity of base composition across taxa for both 16S rRNA (\(P = 0.0020\)) and cytochrome \(b\) (\(P = 0.0001\)). However, nucleotide bias appeared to have little effect on the molecular phylogeny: A minimum evolution analysis (LogDet + I) revealed that secondary structure analysis presents the most accurate method of ribosomal sequence alignment (Hickson et al., 1996, 2000; Morrison and Ellis, 1997; Page, 2000).

### Table 2

GenBank accession details for retropinnid, osmerid, galaxiid, and outgroup mtDNA sequences.

<table>
<thead>
<tr>
<th>Family</th>
<th>Species</th>
<th>Origin</th>
<th>16S rRNA Accession</th>
<th>Cytochrome (b) Accession</th>
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removing invariant sites in proportion to constant sites yielded a topology similar to the maximum likelihood (ML) tree (see below).

Phylogenetic congruence of mitochondrial cytochrome \( b \) (402 bp) and 16S rRNA (500 bp) data sets was tested by the partition homogeneity test (incongruence length difference, ILD; Farris et al., 1994) using PAUP. Maximum parsimony (MP) analysis of 1,000 replicates failed to reject phylogenetic congruence between cytochrome \( b \) and 16S rRNA data sets (\( P = 0.621 \)), apparently justifying their combination in subsequent phylogenetic analyses. However, results of the ILD test should be treated with caution (Dolphin et al., 2000; Yoder et al., 2001; Dowton and Austin, 2002).

Phylogenetic trees based on combined sequence data were constructed with the ML method. Likelihood ratio tests (Felsenstein, 1981) indicated that a parameter-rich
model of nucleotide evolution significantly improved likelihood (data not shown). Subsequently, ML was performed under a general time reversible model of sequence evolution (GTR; Yang, 1994), as follows—A–C: 6.34; A–G: 22.40; A–T: 8.98; C–G: 1.95; C–T: 39.39—with corrections for invariable sites (I; 0.4410) and among-site rate variation (Γ; Gu et al., 1995; 0.5041) estimated with PAUP using a constrained MP topology. Confidence in the ML topology was assessed with 500 bootstrap replicates using the “fast” stepwise-addition option for heuristic searches. Alternative ML topologies were compared statistically by using the Shimodaira–Hasegawa (1999) test implemented in PAUP.

Optimal MP trees were recovered with the global heuristic option of PAUP, using 20 replicates of random sequence addition. Phylogenetic confidence in the MP topology was estimated by bootstrapping (Felsenstein, 1985) with 1,000 replicate data sets analyzed with the “full heuristic” option. A transversion (TV) to transition (TI) weighting of 2:1 was implemented, and gaps in 16S rRNA sequences were treated as a fifth base for MP, with a weighting of 2 relative to Ts. Several alternative transformation weighting schemes were also implemented to examine their effect on MP topology.

**Molecular Analysis—Phylogenetic Relationships**

ML and weighted MP analyses of the combined sequence data yielded substantial bootstrap support for the monophyly of Johnson and Patterson’s (1996) Retropinnidae (78–86%; Retropinna + Prototroctes + Stokellia) and for their Osmeridae (75–97%; Fig. 2). In keeping with the findings of Waters et al. (2000b), our analyses provided substantial bootstrap support (60–88%) for the monophyly of the Galaxiidae sensu Johnson and Patterson (1996)—with the notable exception of Lepidogalaxias. Furthermore, the galaxioid relationship (Galaxiidae + Retropinnidae) proposed by most morphologists was not supported. Rather, both MP and ML strongly supported (99%; Fig. 2) a sister relationship between southern and northern smelts (Retropinnidae + Osmeridae). These results remained robust under a variety of MP weighting strategies (not shown).

Conflicting hypotheses of retropinnid affinities were assessed by using ML. Specifically, the ML tree (supporting Retropinnidae + Osmeridae) was compared with an alternative topology that was identical except for the proposed sister relationship of Galaxiidae and Retropinnidae. The latter topology (galaxioid monophyly) was statistically rejected by the Shimodaira–Hasegawa (1999) test (P < 0.001).

Within Retropinnidae, support was strong for the paraphyly of the genus Retropinna. The Australian taxa (R. semoni and R. tasmanica) were strongly monophyletic (92%; Fig. 2), whereas the sole New Zealand representative was placed sister to Stokellia (90–100%). Stokellia is probably a local derivative from a Retropinna stock that has undergone rapid morphological evolution. We suggest that genus Stokellia could be submerged into Retropinna. This decision depends on the importance attributed to phylogenetic relationships (see Avise, 2000) over more traditional Linnean considerations.

**Molecules Versus Morphology**

The key finding of our genetic analysis is that the southern smelts (Retropinnidae) and northern smelts (Osmeridae) represent a monophyletic group. Initially, this conclusion does not seem surprising, given that retropinnids “closely resemble the osmerids in both appearance and life history strategy” (McDowall, 1988:59). McDowall (1969) was undecided as to retropinnid affinities, but later noted that Retropinnidae “have a close and perhaps common ancestry in the northern hemisphere smelts” (McDowall, 1990:346). However, this phylogenetic placement strongly contradicts galaxioid monophyly as generally supported by recent morphological studies (e.g., Fink, 1984; Howes and Sanford, 1987; Williams, 1987, 1996; Begle, 1991; Nelson, 1994; Johnson and Patterson, 1996). Of recent workers, only Rosen (1974) supported the combined monophyly of southern and northern hemisphere smelts.

Incongruence between molecular and morphological data sets is not an uncommon phenomenon (Patterson et al., 1993; Paterson et al., 1995). For instance, recent studies have noted molecular and morphological incongruence in crocodilians (Poe, 1996), elephants (Thomas et al., 2000), bovids
Figure 2. ML tree of osmerid relationships based on combined cytochrome b and 16S rRNA sequences (ln likelihood—6326.8111). Bootstrap estimates above nodes are derived from ML analysis of 500 replicate data sets using “quick” stepwise-addition. Bootstrap estimates below nodes are from weighted MP analysis of 1,000 replicates.
(Gatesy and Arctander, 2000), pelicaniforms (Hedges and Sibley, 1994), diving ducks (McCracken et al., 1999), teals (Kennedy and Spencer, 2000), shags and cormorants (Kennedy et al., 2000), iguanids (Wiens and Hollingsworth, 2000), and pickerel weeds (Graham et al., 1998). Incongruence may have several causes. In some instances, apparent incongruence may merely reflect a lack of phylogenetic resolution in either or both data sets (e.g., Graham et al., 1998; Thomas et al., 2000; Kennedy et al., 2000) and turn out to be statistically nonsignificant. Incongruence may also stem from long branch attraction, a potential pitfall of parsimony analysis that can be detected with other phylogenetic reconstruction methods (Felsenstein, 1978). In other cases, phylogenetic conflict might reflect natural selection and associated functional convergence of morphological characters (McCracken et al., 1999) or the nonindependence of characters. The incongruence between morphological and genetic interpretations of osmeroid relationships requires an explanation. Hedges and Sibley (1994) argued that, in such cases of incongruence, morphological evidence should be reevaluated. Therefore, we focused on the work of Johnson and Patterson (1996) as the most comprehensive and explicit analysis of osmeroid morphology available.

**Morphological Support for Galaxioidea**

At face value, the cladograms of osmeroid relationships proposed by Johnson and Patterson (1996:302) provide compelling support for the monophyly of the southern hemisphere galaxiid assemblage (Galaxiidae + Retropinnidae). Specifically, more than 20 characters, including at least 12 “uncontradicted synapomorphies,” support Galaxioidea. However, closer inspection of these characters reveals a preponderance of reductive features (i.e., involving the loss of an ancestral state; Begle, 1991), such as the absence of the dermthmoid (character no. 1 from Johnson and Patterson, 1996), supramaxilla (no. 30), maxillary teeth (no. 29), supraorbital and antorbital (no. 34), accessory neural arch (no. 52), and caudal median cartilages (no. 73). Numerous additional reductive characters unite Galaxiidae + Retropinnidae, including many that are homoplastic with salangids. Such characters include the absence of the mesocoracoid (no. 81), ossified epineurals (no. 57), third uroneural (no. 72), caudal scutes (no. 74), urodermal (no. 76), internal limb of posttemporal (no. 78), sensory canal in posttemporal and supracleithrum (nos. 99, 100).

**Problems with the Morphological Phylogeny?**

In our view, several potential problems might confound the morphological analysis presented by Johnson and Patterson (1996). First, some osmeroid fishes exhibit few novel morphological characters from which to infer relationships. This is particularly the case with respect to taxa such as *Lovettia* and the salangids, which have highly reduced osteology (Frankenberg, 1969; McDowall, 1969). Johnson and Patterson (1996) suggested that a lack of morphological novelty tended to obscure the phylogenetic relationships of osmeroid fishes.

Second, if repeated paedomorphosis (see Bemis, 1984; Wake, 1991; Hufford, 1996) has produced parallel effects on numerous osteological characters, these characters “might overwhelm those supporting the true phylogeny” and lead us to an inaccurate estimate of the true phylogeny (Swofford and Maddison, 1992:189). In particular, closely related characters (e.g., uncinate processes on adjacent epibranchials [nos. 41–43]; Johnson and Patterson, 1996) might lack independence in such situations.

Third, a priori considerations may cloud character choice. For instance, Johnson and Patterson (1996:305) say that in “searching for the sister group of *Lepidogalaxias*, we tried hard to place it within Galaxiidae, for example, as related to the diminutive *Galaxiella*... with which it is sympatric.” Similar biogeographic subjectivity might be extended to any problematic taxa. Because both Galaxiidae and Retropinnidae are restricted to the southern hemisphere, it might be tempting to seek out (and thereby favor) characters that support their combined monophyly.

Fourth, Johnson and Patterson (1996) clearly had difficulty assigning evolutionary polarities to some characters. The ancestral states for many of their characters (e.g., nos. 46, 52, 55, 59, 62, 63, 100, 103, and 111) seem debatable. For instance, the interpretations of a marine life history as plesiomorphic (no. 111-0) and diadromy as derived (no. 111-1)
seem suspect, given the widespread occurrence of diadromy in galaxioids, osmeroids, and salmonoids. The problem of polarity may have been compounded by their use of a hypothetical outgroup (see Appendix 1) in combination with a generalized salmonoid. Barriel and Tassy (1998) recommended the use of multiple “real” taxa rather than a subjective outgroup.

Fifth, and perhaps most importantly here, the evolutionary implications of equal transformation cost in parsimony analysis is arguably biologically unrealistic in many cases. As with the vast majority of morphological phylogenies, the analysis of Johnson and Patterson (1996) relies on the simple assumption that all evolutionary changes are equally likely. In interpreting the evolution of a complex character, such a model would favor (for example) three parallel gains, rather than four parallel losses, because the former hypothesis requires fewer steps. In the case of osmeroids, despite the general absence of these character states from Galaxioidea, one *Retropinna* has a toothed maxilla (no. 29-0), *Aplochiton* has an accessory neural arch (no. 52-0), and Galaxiidae have both a supraorbital and an antorbital (no. 34-0). Johnson and Patterson (1996:306) interpret such states as reversals, apparent evidence of unlikely evolutionary processes, such as the first “well-attested instance of reacquisition of maxillary teeth.”

Bateman (1996:116) recognized “a threshold of ‘two steps forward, one step back’ character change beyond which the evolutionary pattern is no longer parsimonious.” It is widely accepted that complex characters are more readily lost than gained (e.g., Gould, 1970; Templeton, 1983; Kennedy et al., 1996; Omland, 1997). As McShea (1996:221) points out, “deletions ought to be more probable on average than additions, for no other reason than it is easier to destroy than to create.” Clear examples of parallel deletions include repeated limb loss in tetrapods (Lande, 1977), the evolution of flightlessness in birds (e.g., Trewick, 1996, 1997) and insects (e.g., Emerson and Wallis, 1995), multiple losses of dichromatism in dabbling ducks (and other birds; Omland, 1997), and the evolution of blindness and pigment-loss in cave-dwelling fauna (“use it or lose it”; Holt, 2000). Similar reductive parallelism is also a characteristic of galaxiid fishes, which repeatedly lose their marine life history phase (McDowall, 1990; Waters et al., 2000b; Waters and Wallis, 2001).

Some morphological characters may evolve in a virtually irreversible manner (Camin and Sokal, 1965). For example, one might suggest that the loss of pelvic fins in some members of the galaxiid genus *Neochanna* is final. Any small chance of a reversal is thought to diminish with the passage of time, as the underlying “developmental trajectory” deteriorates (McShea, 1996:217). Similarly, under Dollo’s Law (Gould, 1970), the gain of a complex character state (forward change) will occur only once, whereas losses (reversals) may occur repeatedly and with relative ease (Dollo parsimony; Farris, 1977; Maddison and Maddison, 1993). The parallel evolution of specific complex novelties is thought to be “possible in principle, but exceedingly improbable in practice” (McShea, 1996:208). Thus it seems wiser to code such transformations as unlikely rather than impossible.

If one accepts that some evolutionary transformations are more likely than others (McShea, 1996), the default assumption of equal costs “may be simply misleading” (Swofford and Maddison, 1992). Moreover, uncertainty as to the magnitude of a transformation bias “does not imply that a 1:1 weighting is more objective or otherwise safer than a 1.5:1 or a 2:1 weighting” (Swofford and Maddison, 1992:217; see also Ree and Donoghue, 1998). Indeed, molecular phylogeneticists routinely assign higher costs to less likely transformations (e.g., non-synonymous vs. synonymous substitutions; TVs versus TJs; Maddison and Maddison, 1993). In molecular studies, too, a range of character transformation weighting schemes are commonly implemented to assess the sensitivity of associated phylogenetic reconstructions (e.g., Waters et al., 2000b). There is no reason, in principle, why such practices should not be extended to morphological phylogenetics.

**Morphological Reanalysis**

**Revised Morphological Matrix**

We created an amended version of the matrix of Johnson and Patterson (1996) (Appendix 1; TreeBASE accession M1112). First, we corrected a coding error for *Lepidogalaxias*, which Johnson and Patterson (1996)
incorrectly coded as lacking vomerine teeth (no. 4-1; actually, it has two strong teeth). Second, we made several corrections regarding the identification and coding of multistate taxa. Johnson and Patterson (1996) incorrectly coded Galaxiidae as polymorphic for nos. 111-0 and 111-1 (entirely marine or diadromous; McDowall, 1990) when they are actually polymorphic for states 111-1 and 111-2 (diadromous or entirely freshwater). Retropinna and salmonoids also exhibit this polymorphism. Additional errors concerning polymorphisms for Retropinnidae (no. 29 0-1) and Galaxiidae (nos. 3 0-1; 34 0-3; 54 0-1; 91 0-1) were corrected.

Furthermore, we reversed the polarity of five characters. We suggest that the presence of epipleurals (no. 59; present in 13 osmeroid genera and widely in euteleosts), nuptial tubercles (no. 103) and cucumber odor (no. 110; in argentinids, osmerids, and retropinnids) might each represent an ancestral state, and their losses therefore might be derived. In each of these characters, parallel losses may have led to the disjunct distributions of these features in euteleosts. Similarly, the fusion of anterior neural arches and centra (no. 55; Lepidogalaxias, Aplochiton, Lovettia, umbriids; Wilson and Veilleux, 1982) and the presence of laminar keels on posterior spines (no. 62; see Rosen, 1974) could be interpreted as ancestral conditions.

**Partition Analysis**

To examine the nature of morphological support for Retropinnidae + Galaxiidae, we partitioned morphological characters according to skeletal region (Table 3; after Johnson and Patterson, 1996). We found no strong evidence that any single suite of morphological characters was solely (or even largely) responsible for the incongruence (Table 3). For example, the alternative topologies remained significantly incongruent when characters associated with the jaws, braincase, gill arches, and axial skeleton were alternately excluded (Table 3). These results should be treated with caution, given problems with the Kishino–Hasegawa (1989) test (see below). Nevertheless, little evidence suggests that the incongruence reflects correlated character evolution (functional convergence; McCracken et al., 1999). Our focus therefore shifted to character state transformation costs and their effect on congruence.

**Character Weighting**

We reanalyzed the morphological data (amended from Johnson and Patterson, 1996) under various character transformation models. An initial morphological analysis was performed by PAUP with all characters unordered, multistate taxa treated as polymorphic, and all transformations assigned equal weight. Our analysis yielded a tree topology identical to that reported in Johnson and Patterson (1996), but 13 steps longer (272 steps; Fig. 3). Bootstrap analysis provided strong support for Retropinnidae + Galaxiidae (96%; Fig. 3).

The MP tree was compared to an alternative topology that differed only with

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**Table 3.** Reanalysis of the data matrix revised from Johnson and Patterson (1996) with characters partitioned according to morphological category (e.g., jaw; braincase; gill arches; axial skeleton). Categories of morphological characters are excluded to examine their effect on the phylogenetic placement of Retropinnidae. The topology of Johnson and Patterson (their Fig. 19) was statistically compared with an alternative topology (identical except sister relationship of Retropinnidae + Osmeridae) by Kishino–Hasegawa (1989) tests. Significance levels associated with probability values are indicated by asterisks.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>272</td>
<td>288 (+16)</td>
<td>0.0003***</td>
</tr>
<tr>
<td>Jaw (7)</td>
<td>262</td>
<td>275 (+13)</td>
<td>0.0013**</td>
</tr>
<tr>
<td>Suspensorium (6)</td>
<td>249</td>
<td>265 (+16)</td>
<td>0.0001***</td>
</tr>
<tr>
<td>Braincase (20)</td>
<td>231</td>
<td>244 (+13)</td>
<td>0.0013**</td>
</tr>
<tr>
<td>Gill arches (13)</td>
<td>241</td>
<td>255 (+14)</td>
<td>0.0007***</td>
</tr>
<tr>
<td>Sensory canal (4)</td>
<td>261</td>
<td>275 (+14)</td>
<td>0.0008***</td>
</tr>
<tr>
<td>Pectoral (9)</td>
<td>250</td>
<td>265 (+15)</td>
<td>0.0002***</td>
</tr>
<tr>
<td>Pelvic (5)</td>
<td>263</td>
<td>280 (+17)</td>
<td>0.0001***</td>
</tr>
<tr>
<td>Median fins (5)</td>
<td>262</td>
<td>278 (+16)</td>
<td>0.0002***</td>
</tr>
<tr>
<td>Axial skeleton (25)</td>
<td>214</td>
<td>225 (+11)</td>
<td>0.0039**</td>
</tr>
<tr>
<td>Reproductive (5)</td>
<td>260</td>
<td>276 (+16)</td>
<td>0.0002***</td>
</tr>
</tbody>
</table>

*P < 0.05; **P < 0.01; ***P < 0.001.
Figure 3. MP analysis of osmeroid relationships based on morphological data amended from Johnson and Patterson (1996). Bootstrap estimates (1,000 replicates) above nodes are derived from equally weighted parsimony, whereas values below nodes are from asymmetric character weighting.

Respect to placement of Retropinnidae as sister to Osmeridae. Under equal weighting, the alternative tree required 16 extra steps (Retropinnidae + Osmeridae; 288 steps) and was rejected by the Kishino–Hasegawa (1989) test ($P = 0.0003$). The Shimodaira–Hasegawa (1999) test would represent a more appropriate method for topology evaluation here, because the competing trees were determined a posteriori (see Goldman et al., 2000). However, the Shimodaira–Hasegawa test is at present unavailable for MP analysis and hence is inapplicable to morphological data (D. Swofford, pers. comm.). Future morphological studies could be conducted using likelihood, now that a model of
evolution for morphology has recently become available (Lewis, 2001). The results of our Kishino–Hasegawa tests should be treated with caution.

Each of the 112 morphological characters discussed by Johnson and Patterson (1996) was classified into one of five categories (see Appendix 2): (1) binary reductive—derived state equals loss of ancestral feature (43 characters); (2) multistate reductive—several derived states, one of which equals loss of ancestral feature (19 characters); (3) binary nonreductive—single derived state not involving loss of ancestral feature (24 characters); (4) multistate nonreductive—several derived states not involving loss of ancestral feature (11 characters); (5) undetermined (15 characters; e.g., regarding no. 38, is an incised opercular margin a reductive or a nonreductive change?).

Binary reductive characters (category 1) were analyzed under a moderated form of Camin–Sokal (1965); irreversible parsimony, applying transformation costs of 2:1, 3:1, and 4:1 to reversals (1 to 0) relative to forward changes (0 to 1). Similar reversal costs (2:1, 3:1, 4:1) were applied for multistate reductive characters (category 2). For instance, a 3:1 weighting was applied to character 1 (derm-methmoid; 0 = median; 1 = paired; 2 = absent) according to the following stepmatrix:

<table>
<thead>
<tr>
<th>To:</th>
<th>0</th>
<th>1</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>From:</td>
<td>0</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

Binary nonreductive characters (category 3) were analyzed under a moderated form of Dollo parsimony, with cost ratios of 2:1, 3:1, and 4:1 for forward changes versus reversals. Multistate nonreductive characters (category 4) and undetermined characters (category 5) were treated as unordered in all analyses.

Under the equal transformation cost model, the retropinnid–galaxiid relationship of Johnson and Patterson (1996) was significantly better than the retropinnid–osmerid alternative (16 extra steps; $P = 0.0003$; Table 4). However, when reversals for reductive characters (categories 1, 2) were assigned costs of 3 or 4, the difference between the topologies was less marked (11 extra steps; $P = 0.02$; Table 4). When both reversals (categories 1, 2) and forward changes (category 3) were assigned costs of 4, the retropinnid–galaxiid relationship of Johnson and Patterson (1996) was not significantly better than the retropinnid–osmerid alternative (5 extra steps; $P = 0.25$; Table 4). Likewise, this weighting scheme reduced bootstrap support for the retropinnid–galaxiid relationship from 99% (equal weighting) to 87% (Fig. 3).

With regard to retropinnid relationships, we have shown that the results of Johnson and Patterson’s (1996) equally weighted morphological study are significantly incongruent with our molecular findings. However, the incongruence becomes non–significant when asymmetric transformation weights are applied to morphological characters.

**Morphological Evolution—Conclusions**

Bateman (1996:116) suggested (and we agree) that “repeated simplification is arguably the class of evolutionary changes

<table>
<thead>
<tr>
<th></th>
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<th></th>
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</thead>
<tbody>
<tr>
<td>Unweighted</td>
<td>272</td>
<td>288 (+16)</td>
<td>0.0003***</td>
<td>0.0003***</td>
</tr>
<tr>
<td>Reductive 2:1</td>
<td>284</td>
<td>297 (+13)</td>
<td>0.0041**</td>
<td>0.0046**</td>
</tr>
<tr>
<td>Reductive 3:1</td>
<td>288</td>
<td>299 (+11)</td>
<td>0.0157*</td>
<td>0.0164*</td>
</tr>
<tr>
<td>Reductive 4:1</td>
<td>288</td>
<td>299 (+11)</td>
<td>0.0157*</td>
<td>0.0164*</td>
</tr>
<tr>
<td>Reductive &amp; nonreductive 2:1</td>
<td>318</td>
<td>329 (+11)</td>
<td>0.0157*</td>
<td>0.0164*</td>
</tr>
<tr>
<td>Reductive &amp; nonreductive 3:1</td>
<td>346</td>
<td>354 (+8)</td>
<td>0.0450*</td>
<td>0.0455*</td>
</tr>
<tr>
<td>Reductive &amp; nonreductive 4:1</td>
<td>360</td>
<td>365 (+5)</td>
<td>0.2531</td>
<td>0.2513</td>
</tr>
</tbody>
</table>

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. 

---

The table above provides a summary of the parsimony analysis of a morphological data matrix modified from Johnson and Patterson (1996). Reductive characters were weighted so as to favor multiple losses over reversals, whereas nonreductive characters were weighted to favor reversals over parallel gains. For each weighting strategy, the topology of Johnson and Patterson (their Fig. 19) was statistically compared with an alternative topology (identical except sister relationship of Retropinnidae + Osmeridae) by using Kishino–Hasegawa (1989) and Templeton (1983) tests. Significance levels associated with probability values are indicated by asterisks.
most likely to undermine the accuracy of a morphological cladogram.” We are not suggesting that asymmetric weighting of evolutionary transformations represents a cure-all for morphologically based phylogenetic analyses. Nevertheless, we believe the default assumption of equal weighting is often unrealistic, especially in cases where reductive characters dominate the matrix, and when a priori evidence suggests that homoplasy is widespread (e.g., multiple losses of diadromy; McDowall, 1988). In the case of retropropinnids, the implementation of asymmetric weighting substantially decreases the incongruence between morphological and molecular data. We recommend that morphological systematists routinely implement a range of character transformation models to help assess the sensitivity of their phylogenetic reconstructions. All too often, such studies present only an optimal tree based on equal transformation costs, along with a few associated measures of homoplasy. In addition to asymmetric weighting, we suggest that morphological analyses might benefit from the types of statistical methods that are routinely applied to similar molecular studies (e.g., statistical evaluation of alternative hypotheses).

BIogeographic Implications

The Evolution of an Antitropical Distribution

The Osmeroidea are restricted to temperate regions of the southern hemisphere (Retropropinnidae, Galaxiidae) and the northern hemisphere (Osmeridae). This biogeographic pattern might be termed “antitropical” (Hubbs, 1952). The biogeographic implications of the [galaxiid, [retropropinnid, osmerid]] relationship might seem more challenging than the more conventional [osmerid, [retropropinnid, galaxiid]] relationship, because the former group requires two transequatorial events (if a northern origin for retropropinnids + osmerids is assumed) instead of the single one for the latter. The description of a fossil osmerid (Speirsaeigma) from the Paleocene (60 Ma) of North America (Wilson and Williams, 1991) suggests an ancient origin for northern smelts. In addition, the presence of high taxonomic (and genetic) diversity in Osmeridae (11 genera; ~50 species), compared with the relatively low diversity in Retropropinnidae (3 genera; 6 species) might favor a northern origin. On the other hand, the fact that the sister group of retropropinnids + osmerids (Galaxiidae) is restricted to the southern hemisphere supports a southern origin.

Under a vicariance scenario, one might envision that antitropical taxa diverged as a result of plate tectonics (Croizat et al., 1974; Rosen, 1974, 1978). Such an explanation would necessarily imply ancient divergence, because Laurasia and Gondwana split in the Mesozoic. Alternatively, a dispersalist interpretation might involve transequatorial movement during cooler periods such as Pleistocene glaciations (Lindberg, 1991). A third scenario might involve a combination of dispersal and vicariance mechanisms: a global geographic range (dispersal) disrupted by climate change (vicariance). For instance, some antitropical distributions may have formed as a result of a Miocene increase in tropical temperatures (White, 1986, 1989). Indeed, vicariance and dispersal processes are not mutually exclusive (McDowall, 1978; Waters et al., 2000a,b).

Diadromy provides a clear mechanism for marine dispersal in retropropinnid and osmerid fishes (McDowall, 1988). Similarly, juvenile-mediated dispersal appears to be an important biogeographic factor for galaxiid fishes (McDowall, 1990; Waters et al., 2000a,b). Dispersal across the equator may seem highly unlikely for temperate-limited retropropinnids. However, the literature describes numerous examples of such dispersal. The otherwise temperate-limited Galaxiidae are represented in the tropics (Nesogalaxias; New Caledonia), probably as a result of Miocene or Pliocene marine dispersal (McDowall, 1968; Waters et al., 2000b). Some marine fish groups provide evidence of multiple transequatorial dispersal events (e.g., anchovies: Grant and Bowen, 1998; hakes: Grant and Leslie, 2001), possibly driven by oscillations in ocean temperatures. In a review of antitropicality in marine fishes (focusing within species or among closely related taxa), Burridge (2002) noted molecular support for Pleistocene transequatorial dispersal in seven antitropical taxa, with several other taxa yielding divergences suggestive of Miocene and Pliocene dispersal.

Cytochrome b divergences between the southern hemisphere retropropinnids and the northern hemisphere osmerids (28.9–30.6%;
Kimura [1980] 2 parameter) suggest a minimum divergence estimate of around 10–40 Ma, based on fish protein-coding gene calibrations of 0.8–2.6%/Ma (Ort et al., 1994; Taylor and Dodson, 1994; McKay et al., 1996). The slower-evolving 16S rRNA gene gives osmerid–retropinnid 16S divergences ranging from 7.6% to 12.0%. A crude molecular clock calibration for fish 16S (0.23%/Ma; Alves-Gomes, 1999) suggests that the associated phylogenetic separation occurred around 30–50 Ma. These estimates yield a time frame that is intermediate between the extreme biogeographic scenarios of ancient vicariance (Pangaea break-up, 180 Ma) and recent dispersal (Pleistocene, <1.64 Ma). Despite the ancient divergence estimate (as much as 50 Ma), marine dispersal is the favored biogeographic explanation for the antitropical distribution of smelts. The ancient osmerid record from Canada (60 Ma; Wilson and Williams, 1991) suggests a northern origin for osmerids, with retropinnids reflecting north–south dispersal. However, we stress the tentative nature of our divergence estimates and the associated biogeographic conclusions.

Trans-Tasman Dispersal

Marine dispersal seems to be a key factor in the biogeography of retropinnid fishes in the Australasian region. Retropinnids exhibit trans-Tasman 16S divergences (Australia vs. New Zealand; 4.1–6.2%) more or less equivalent to those observed for some galaxiid fishes (Neocharina, 5.1–6.7%; Waters et al., 2000a; Galaxias maculatus, 4.4%; Waters and Burridge, 1999). In contrast, Galaxias brevipinnis exhibits substantially less trans-Tasman divergence (1.2%; J. M. W., unpubl. data). The variable values for divergence probably reflect the stochastic nature of long distance dispersal (Waters et al., 2000a). A molecular clock calibration for fish 16S (see above) suggests that these divergence values are consistent with trans-Tasman dispersal in the mid- to late Tertiary. The alternative biogeographic hypothesis of vicariance (continental fragmentation at 80 Ma; Cooper and Millener, 1993) is rejected for these fishes. Indeed, dispersal is becoming increasingly recognized as an important biogeographic process in this region (Pole, 1994; Trewick, 2000; Waters et al., 2000a; Wright et al., 2000).

ACKNOWLEDGMENTS

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REFERENCES

DOWTON, M., AND A. D. AUSTIN. 2002. Increased congruence does not necessarily indicate increased


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### Appendix 1. Morphological data matrix modified from Johnson and Patterson (1996). Our changes are indicated in bold (see text).

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Data</th>
<th>Data</th>
<th>Data</th>
<th>Data</th>
</tr>
</thead>
<tbody>
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<td>Outgroup</td>
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<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Salmonid</td>
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<tr>
<td>Hypomesus</td>
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<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
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<td>Thaleichthys</td>
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<tr>
<td>Retropinna</td>
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<tr>
<td>Prototroctes</td>
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<td>Lovettia</td>
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<td>0.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>

Polymorphic taxa: $b = 0/1; c = 1/2; d = 0/1/2/3$. 

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**APPENDIX 2. CATEGORIZATION OF CHARACTERS FROM JOHNSON AND PATTERSON’S (1996) AMENDED MORPHOLOGICAL DATA MATRIX**

1. Binary reductive ($n = 43$)
   - 4–6
   - 8
   - 11
   - 12
   - 17
   - 29
   - 30
   - 33
   - 35
   - 41
   - 42
   - 44
   - 47
   - 48
   - 51
   - 52
   - 55
   - 57–59
   - 62
   - 64
   - 67
   - 70
   - 74
   - 76
   - 78
   - 81
   - 86
   - 87
   - 89
   - 90
   - 93
   - 94
   - 96
   - 98
   - 101–103
   - 107
   - 110

2. Multistate reductive ($n = 19$)
   - 1–3
   - 13
   - 23
   - 34
   - 39
   - 40
   - 49
   - 50
   - 56
   - 61
   - 68
   - 73
   - 77
   - 79
   - 80
   - 97
   - 111

3. Binary nonreductive ($n = 24$)
   - 7
   - 9
   - 14
   - 20
   - 27
   - 32
   - 33
   - 43
   - 54
   - 63
   - 66
   - 69
   - 71
   - 82
   - 85
   - 88
   - 91
   - 95
   - 104
   - 106
   - 108
   - 109

4. Multistate nonreductive ($n = 11$)
   - 15
   - 16
   - 18
   - 26
   - 37
   - 45
   - 46
   - 60
   - 75
   - 105
   - 112

5. Undetermined ($n = 15$)
   - 10
   - 19
   - 25
   - 53
   - 92
   - 21
   - 22
   - 24
   - 99
   - 100
   - 32
   - 38
   - 72
   - 83
   - 84