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Evolution and biogeography of marine angelfishes (Pisces: Pomacanthidae)

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Abstract

Phylogenetic relationships among angelfishes (Pomacanthidae) and their putative sister taxon, the butterflyfishes (Chaetodontidae), were examined using 12S and 16S mitochondrial DNA sequences. ML and MP trees were highly congruent with good basal resolution. Monophyly of the two families was supported, although a clade comprising the Chaetodontidae and one of the outgroups, the Scatophagidae, formed the sister clade to the Pomacanthidae. All genera and subgenera within the Pomacanthidae were examined. The relationships among the 24 representative species were consistent with traditional generic boundaries, with the exception of the genus *Centropyge*, but differed from previous phylogenies. Estimated ages of divergence based on trans-isthmian pairs were compared with independent fossil evidence. Trans-isthmian estimates were highly conservative, while fossil-calibrated estimates were most consistent with available evidence. Fossil calibrated estimates suggest that the family has been impacted by both the Terminal Tethyan Event and the closure of the Isthmus of Panama. Within the family, ecological diversity and species-level diversification are restricted primarily to a single pygmy angelfish clade with an origin near the Oligocene–Miocene boundary.

Keywords: Pomacanthidae; Chaetodontidae; Biogeography; Molecular clock; Fossil validation; Trans-isthmian pairs

1. Introduction

With global distribution patterns and a history spanning over 50 million years, coral reef fishes represent one of the most interesting groups for studying evolution and biogeography in the marine realm. Of all families, the angelfishes (f. Pomacanthidae) are among the most promising subjects. The family is of a relatively small size (88 species), yet it represents one of the most conspicuous components in extant coral reef fish assemblages, with representatives in all tropical seas (Allen et al., 1998; Debelius et al., 2003). Within the family there is a diverse range of ecological traits including striking variation in body size, colour patterns, reproductive systems, and diets, which range from herbivory to planktivory. The family therefore, offers an ideal model for examining the impact of marine biogeographic events on the evolution of a widespread fish group and on the evolution of a wide array of distinct ecological traits.

Unlike many reef fish families, however, the evolutionary history of the angelfishes is poorly understood. Their fossil record is restricted to otoliths (Patterson, 1993), with the inevitable limitations that this imposes (Bellwood and Wainwright, 2002). Although numerous representatives of the higher squamipinnes (*sensu* Tyler et al., 1989) are represented in the Eocene (50 Ma) deposits of Monte Bolca, including the Scatophagidae, Ephippidae, Siganidae, Zanclidae, and Acanthuridae (Bellwood, 1996), the Pomacanthidae is conspicuously absent (cf. Bannikov, in press). This is particularly striking given the suggestion that the pomacanthids and chaetodontids together may represent the sister group to a clade (the Acanthuroidei) containing all of the remaining families in the higher squamipinnes (Tyler et al., 1989). The evolutionary history of the Pomacanthidae based on cladistic analyses is likewise relatively unclear, although the morphological observations of

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Shen and Liu (1978) and recent allozyme data of Chung and Woo (1998) have provided clear hypotheses of the relationships among genera (Fig. 1). The monophyly of the family is widely assumed, although the angelfishes have for a long time been regarded as close relatives of the butterflyfishes (Chaetodontidae). Indeed, the pomacanthids were placed within the Chaetodontidae until as recently as the 1970s (Burgess, 1974; Nelson, 1994).

The aims of this study therefore, are three fold. First, we aim to evaluate the monophyly of the Pomacanthidae. To do this, we use the molecular evidence from both the 12S and 16S regions of the mitochondrial genome to examine the relationships of representative species from all pomacanthid genera, along with species from the three major lineages within the Chaetodontidae (the putative sister taxon). Additional outgroups include a basal member of the Acanthuroidei (Scatophagus) and the Microcanthidae. The latter family has been linked with both the Chaetodontidae (Kuiter, 2002) and Pomacanthidae (Gosline, 1985), and was used as the outgroup for both by Mok and Shen (1983). A more distant outgroup with a similar body shape is also included (the Kyphosidae). The use of multiple outgroups enables us to evaluate the monophyly of each family and the putative pomacanthid–chaetodontid clade.

Second, we investigate the relationships among pomacanthid genera. We use at least two representatives from each genus (and include all subgenera). This enables us to examine relationships among major lineages within the family and to explore the origins of evolutionary novelty. Finally, we use the phylogeny and estimated ages of divergence (based on a molecular clock calibrated using trans-isthmian species pairs) to examine the evolutionary and biogeographic history of the component lineages within the family. These estimated ages are independently evaluated by direct comparison with the fossil record.

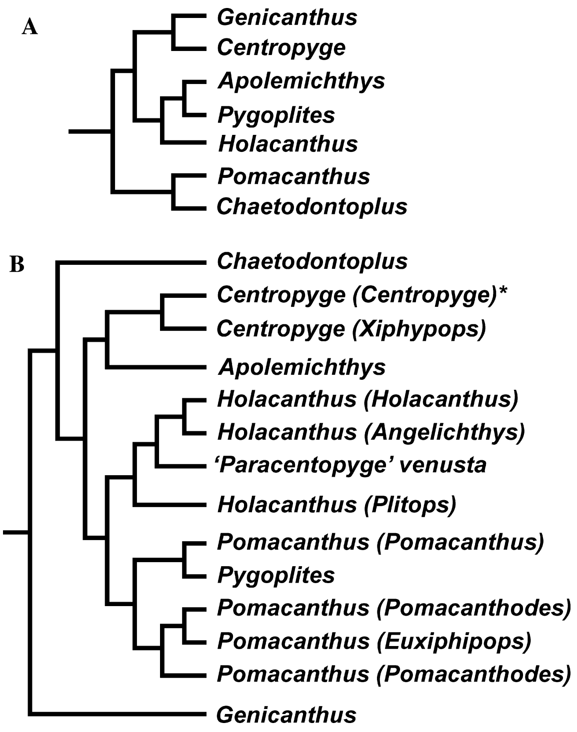

Fig. 1. Phylogenetic hypotheses of relationships among genera in the Pomacanthidae based on (A) morphological data (after Allen, 1981; Shen and Liu, 1978) and (B) allozyme data (after Chung and Woo, 1998).

2. Materials and methods

2.1. Species and outgroup selection

A total of 24 pomacanthid species were examined, with two individuals of each species (in most cases) and at least two species from each of the eight genera (except the monotypic *Pygoplites*). This included representatives from all subgenera (Table 1). The species names, collection locations and GenBank accession numbers are given in Table 2. Taxonomic categories follow Allen et al. (1998) and Debelius et al. (2003). As the putative sister taxon to the Pomacanthidae, the Chaetodontidae was represented by five species, with a representative from each of the three major lineages, including the basal lineage *Amphichaetodon* (following Ferry-Graham et al., 2001).

2.2. Tissue samples, amplification, and sequence preparation

All tissues were collected from freshly euthanized or recently collected specimens. Fishes were held in an ice–water slurry until dissected. All tissue samples were of muscles from the dorsal flank, preserved in 70% ethanol and stored at −12 °C. Specimens were collected using

Table 1
Generic and subgeneric groupings within the Pomacanthidae following Allen et al. (1998)

Genus	Subgenus	Species examined herein
Apolemichthys	—	*A. trimaculatus*, *A. xanthurus*
Centropyge	*Centropyge*	*C. bicolor*, *C. flavissimus*
Centropyge	*Xiphypops*	*C. aurantonota*, *C. loriculus*, *C. potteri*
Chaetodontoplus	—	*C. duboulayi*, *C. mesoleucus*
Genicanthus	—	*G. lamarck*, *G. melanospilos*
Holacanthus	*Angelichthys*	*H. bermudensis*, *H. ciliaris*
Holacanthus	*Holacanthus*	*H. tricolor*
Holacanthus	*Plitops*	*H. passer*
Paracentropyge	—	*P. multifasciata*, *P. venusta*
Pomacanthus	*Euxiphipops*	*P. sexstriatus*
Pomacanthus	*Pomacanthodes*	*P. asfur*, *P. zonipectus*, *P. semicirculatus*
Pomacanthus	*Pomacanthus*	*P. arcuatus*, *P. paru*
Pygoplites	—	*P. diacanthus*

Table 2
Species identification and collection locations of material examined and for which sequences were generated in this study

Genus	Species	Location	GenBank #12S[a]/16S
Ingroup species			
Amphichaetodon	*howensis*	Lord Howe Is.	AY530830/AY530860
Apolemichthys	*trimaculatus*	Philippines[b]	[a]AF108548/AY530843
Apolemichthys	*xanthurus*	Sri Lanka[b]	[a]AF108549/AY530842
Centropyge	*aurantonota*	Caribbean[b]	AY547300/AY530851
Centropyge	*bicolor*	Lizard Isl., GBR	[a]AF108551/AY530845
Centropyge	*flavissimus*	Moorea	[a]AF108556/AY530841
Centropyge	*loriculus*	Moorea	[a]AF108557/AY530856
Centropyge	*potteri*	Hawaii[b]	AY530832/AY530863
Chaetodontoplus	*duboulayi*	Mackay, GBR	[a]AF108566/AY530846
Chaetodontoplus	*mesoleucus*	Philippines[b]	AY530823/AY530847
Genicanthus	*lamarck*	Indonesia[b]	[a]AF108569/AY530849
Genicanthus	*melanospilos*	Indonesia[b]	[a]AF108570/AY530850
Holacanthus	*bermudensis*	Florida[b]	AY530835/AY530867
Holacanthus	*ciliaris*	Belize[b]	[a]AF055593/AY530861
Holacanthus	*passer*	Sea of Cortez[b]	AY530828/AY530857
Holacanthus	*tricolor*	Brazil[b]	AY530833/AY530864
Paracentropyge	*multifasciata*	Indonesia[b]	AY530825/AY530853
Paracentropyge	*venusta*	Philippines[b]	AY530831/AY530862
Pomacanthus	*asfur*	Red Sea[b]	AY530826/AY530854
Pomacanthus	*paru*	Belize[b]	AY530824/AY530852
Pomacanthus	*semicirculatus*	Indonesia[b]	[a]AF108574/AY530844
Pomacanthus	*sexstriatus*	Lizard Isl., GBR	[a]AF108575/AY530858
Pomacanthus	*arcuatus*	Caribbean[b]	AY530836/AY530868
Pomacanthus	*zonipectus*	Costa Rica[b]	AY530840/AY530874
Pygoplites	*diacanthus*	Yonge R., GBR	[a]AF108577/AY530873
Outgroup species			
Microcanthidae			
Atypichthys	*latus*	Lord Howe Is.	AY530829/AY530859
Chaetodontidae			
Chaetodon	*ornatissimus*	Moorea	AY530834/AY530866
Chaetodon	*trifascialis*	Hick's Reef, GBR	AY530827/AY530855
Chaetodon	*kleinii*	Yonge R., GBR	[a]AF108516/AY530865
Forcipiger	*flavissimus*	Pohnpei	[a]AF108540/AY530848
Kyphosidae			
Kyphosus	*cinerascens*	Rib Reef, GBR	AY530839/AY530872
Kyphosus	*vaigiensis*	Rib Reef, GBR	AY530838/AY530871
Scatophagidae			
Scatophagus	*argus*	Indonesia[b]	AY530837/AY530869
Selenotoca	*multifasciata*	Unknown[b]	[a]AF055599/AY530870

[a] Indicates existing 12S sequences which were obtained from GenBank, otherwise sequences were all generated in this study.

[b] Denotes species from the aquarium trade.

nets or spears on the Great Barrier Reef, in French Polynesia, Micronesia, Indonesia, and Papua-New Guinea. This material was supplemented by specimens obtained directly from commercial aquarium collectors, or wholesalers who were able to provide detailed collection information. The specimens have been retained for associated morphological, biomechanical and kinematic analyses (Konow and Bellwood, in preparation).

We extracted DNA using standard salt-chloroform procedures (Sambrook et al., 1989), which was then amplified for all species using universal fish 16S rRNA primers LR-J-12887 5′-CCG GTC TGA ACT CAG ATC ACG T-3′ and LR-N-13398 5′-CGC CTG TTT ACC AAA AAC AT-3′ (Simon et al., 1994), using touchdown PCR in three phases of 5, 5, and 20 cycles per annealing temperature (51–49–47 °C) in the presence of 3.5 mM $MgCl_2$. The 12S region was amplified using universal fish 12S primers L1091 5′-AAA AAG CTT CAA ACT GGG ATT AGA TAC CCC ACT AT-3′ and H1478 5′-TGA CTG CAG AGG GTG ACG GGC GGT GTG T-3′ (Simon et al., 1994), from all species (with the exception of a few species for which 12S sequences were available in Genbank; Table 2), using 30 cycles at an annealing temperature of 50 °C, in the presence of 3.5 mM $MgCl_2$. PCR products were purified by isopropanol precipitation and sequenced directly in both directions on an ABI377 automated sequencer using the amplification primers and dye terminator chemistry (ABI) following manufacturer's instructions. Sequences were aligned by eye using ESEE (Cabot, 1997). Gaps were introduced to maintain alignment between the 24 ingroup and 11 outgroup taxa.

2.3. Phylogenetic analyses

Aligned sequences for 16S and 12S (578 and 364 bp), respectively, were analyzed separately and concatenated in PAUP* 4.0b10 (Swofford, 1998) for 34 species, 24 of which were in the Pomacanthidae. We follow Cunningham (1997), Yoder et al. (2001), and Darlu and Lecointre (2002) who show that data sets can, and in many cases should, be combined even if incongruent length differences are identified in partitioned data. Further, Glazko and Nei (2003, and references therein) showed that when estimating times of divergence from sequence data, it is better to combine data because variances and covariances of the estimates are reduced. In our data, phylogenetic analyses were therefore performed on the combined 12S–16S sequences. When examined independently the 12S sequences were largely uninformative. The optimal substitution model for the combined data was identified using likelihood approaches implemented in Modeltest (Possada and Crandall, 1998). The following specifications were used for analysing the 942 bp of combined sequences: maximum likelihood (ML) analysis, substitution model GTR + G + I, with gamma shape parameter, $G = 0.5217$, proportion of invariable sites, $I = 0.328$ nucleotide frequencies $A = 0.254$, $C = 0.227$, $G = 0.234$, $T = 0.285$, 100 bootstrap replicates. Trees were rooted with the kyphosid outgroup species *Kyphosus cinerascens* and *Kyphosus vaigiensis.*

The ML tree topology was confirmed with Bayesian analysis using the program Mr Bayes version 3.0B4 (Huelsenbeck and Ronquist, 2001) with a maximum likelihood model employing six substitution types, with base frequencies estimated from the data, and rate variation across sites modelled using a γ distribution, as given above. The Markov chain Monte Carlo search was run with four chains for 1,000,000 generations, with trees sampled every 100 generations. The first 10,000 trees were discarded as "burn-in," after which the likelihood scores had stabilized.

Maximum parsimony (MP) heuristic analyses with 1000 bootstrap pseudo-replicates were also performed with random addition of taxa and the ACCTRAN option in effect. To test whether Allen's (1981) morphological phylogeny (Fig. 1A) was significantly different to the molecular phylogeny generated in this study, an additional exhaustive MP analysis was performed, after consensus sequences were generated for each of the seven pomacanthid genera studied by Allen (1981). The molecular tree topology was forced to be identical to the morphological tree topology using MacClade 4.03 (Maddison and Maddison, 2001), after which a Kishino–Hassegawa test was performed in PAUP* to determine if the two MP trees were significantly different (Swofford, 1998).

2.4. Molecular clock considerations and biogeography

Evolutionary and biogeographic analyses were restricted to the pomacanthid clade. Four methods of age estimation were employed: direct calculations, a Langley Fitch enforced molecular clock (LF), and penalized likelihood (PL) and non-parametric rate smoothing approaches (NPRS). For each approach two independent calibration methods were employed: geminate pairs across the Isthmus of Panama and ages based directly on the fossil record. These two independent approaches provided a means of testing the utility of the various calibration approaches and the four methods of age estimation. For fossil calibrated ages, the estimated age of the geminate species pairs should be at least 3.1 Ma. Any younger and the calibration and/or methodology should be rejected. For Isthmus calibrated ages the basal node should be between 50 and 65 Ma, estimates that lie outside this range are of questionable value (Bellwood and Wainwright, 2002).

To provide a robust molecular clock calibration based on the formation of the Isthmus of Panama, three potential trans-isthmian pairs were examined: *Centropyge aurantonota*, *Centropyge loriculus/potteri*; *Pomacanthus zonipectus*, *Pomacanthus paru/arcuatus*, and *Holacanthus passer*, *Holacanthus bermudensis/ciliaris*. Of these three only the latter contained a geminate species pair (it also exhibited the least divergence of the three putative pairs). The *H. passer–bermudensis* pair was therefore used to calibrate the molecular clock, taking a conservative estimate of 3.1 Ma as the date of final closure of the Isthmus of Panama (Coates and Obando, 1996). Direct fossil calibration utilized two dates: 65 Ma, marking the transition between Mesozoic and Cenozoic marine fish faunas, and the probable earliest origins of many reef fish lineages (Bellwood and Wainwright, 2002), and 50 Ma, marking the earliest known fossil evidence of most reef fish families, including the Scatophagidae, one of the outgroups in the present study (Bellwood, 1996). When using the fossil based calibration, trees were constrained at the basal node (the age of divergence between the Pomacanthidae and its sister clade) at either 65 or 50 Ma.

The ages of individual nodes were estimated independently using the four methods. First, ages were directly calculated from the branch lengths of the ML phylogram (without enforcing a clock). For the Isthmus of Panama calibration the rate of change was based on the *H. passer–bermudensis* split with a divergence rate of 0.32% per Ma. Maximum ages were calculated by taking the oldest estimated age (i.e., the longest branch length) at each successive node, cumulatively. Minimum ages were based on the youngest age (shortest branch length) at each successive node cumulatively. Fossil calibration used the cumulative maximum and minimum branch lengths at the basal node to calibrate divergence rates. This yielded rates of 0.15–0.25% per Ma for a 50 Ma calibration (0.12–0.19% per Ma for 65 Ma).

Second, the ages of nodes were estimated by using the Langley Fitch method (LF), which enforces a molecular clock (Langley and Fitch, 1974), implemented in r8s (Sanderson, 2003). In these analyses a χ^2 distribution was used to determine if the two trees, obtained with or without enforcing a molecular clock, were significantly different (df = number of taxa −2). Third, a penalized likelihood (PL) approach was used, as per Sanderson (2002), again implemented in r8s (Sanderson, 2003). This is a semi-parametric rate-smoothing approach, which allows changes in the rates of molecular evolution, but attempts to minimize rate changes between ancestral and descendant branches on the tree (mean rate = 0.15% change per Ma; ± 0.06% SD). The truncated Newton (TN) algorithm was specified for estimating divergence times, in both LF and PL approaches, because this algorithm is recommended as the best and fastest option for use with LF or PL approaches (Sanderson, 2003). This algorithm tolerates age constraints and uses gradients for improved convergence guarantees. Finally, ages were estimated using a NPRS technique. As the TN algorithm is not yet implemented for the NPRS approach, the recommended Powell algorithm was used. Both divergence time algorithms (TN and Powell) require that at least one internal node be fixed or constrained. This we did for the fossil calibrated ages. Where this is not possible, as in the isthmus-based calibrations, it is recommended that the root is fixed with an arbitrary age and then all calculated rates and times of divergence scaled relative to this specified age. As the geminate species pair is at a terminal node, we assigned an arbitrary age of 100 Ma to the root node (this maintains the independence of the fossil evidence which can then be used to test the utility of the various approaches).

Biogeographic distribution data was taken from published descriptions (Allen et al., 1998) and existing databases (Connolly et al., 2003; Hughes et al., 2002). Ecological data were taken from published descriptions (Allen, 1981; Allen et al., 1998; Aburto-Oropeza et al., 2000; Debelius et al., 2003; Howe, 1993; Hourigan et al., 1989).

3. Results

3.1. Phylogenetic analyses

A strong phylogenetic signal was obtained from the combined data, with 313 of the 942 characters (or 33%) being parsimony informative. The transition:transversion (ti:tv) ratio was 2.1:1. All methods of phylogenetic analysis (Bayesian, ML, and MP) produced consensus trees of similar topology with strong bootstrap support for all genus level and most inter-generic nodes, particularly from Bayesian analyses (Fig. 2). Consensus trees were based on 990,000 (Bayesian), 122 (ML), and 4 (MP) trees that were equally likely for each method of analysis as identified from Kishino–Hasegawa tests, $p > 0.105$ (ML) and shortest trees (MP tree length of 1402).

All trees strongly support the monophyly of the Pomacanthidae. The Chaetodontidae also appears to be monophyletic, although the Scatophagidae sits as its sister group. The Chaetodontidae–Scatophagidae lineage is the sister-group to a monophyletic Pomacanthidae.

The combined data set also supports the monophyly of most pomacanthid genera. The nine clades (Fig. 2) strongly reflect existing taxonomic boundaries: *Apolemichthys* (Clade 2), *Genicanthus* (Clade 3), *Paracentropyge* (Clade 5), *Holacanthus* (Clade 6), *Chaetodontoplus* (Clade 7), *Pomacanthus* (Clades 8 and 9). *Pygoplites* is monotypic, but its basal position within a *Holacanthus* lineage (Clade 6) is not inconsistent with the monophyly of the two genera. Furthermore, the weak support for the monophyly of *Holacanthus* (with just 81% support in Bayesian analyses) means that this genus can only be tentatively accepted at this time. The only notable exception to the general support for existing taxonomic groups is in *Centropyge* where two distinct clades are evident. Interestingly, they both conform to conventional divisions reflecting the two *Centropyge* subgenera: *Centropyge* (Clade 1) and *Xiphypops* (Clade 4).

The relationships among genera are relatively well resolved, with four major lineages. In all analyses the *Pomacanthus* species form a distinct, basal, clade with the species dividing into two branches (Clades 8 and 9; Fig 2). One comprises only east Tethys (Indian Ocean and west-central Pacific) species (Clade 8), the other branch only west Tethys species (East Pacific, Caribbean, and tropical Atlantic; Clade 9).

A second major clade consistently contains two smaller clades, one comprising *Holacanthus* and *Pygoplites* (Clade 6), the other containing all remaining taxa except *Chaetodontoplus*. The latter 'pygmy angelfish' clade (containing Clades 1–5) includes both of the *Centropyge* clades (*Centropyge* and *Xiphypops*), *Apolemichthys*, *Genicanthus*, and *Paracentropyge*. All individual genus-level clades, and the major clades are well supported with bootstrapped ML, MP and Bayesian support (Fig. 2). The main exception to this pattern is in the basal relationships among the 'pygmy angelfish' Clades (1–5), which are not well resolved by either ML or MP analyses. Thus the degree of separation between the two *Centropyge* Clades (1 and 4) remains equivocal. A monophyletic *Centropyge* cannot be rejected, however, our data provide no support for such an association.

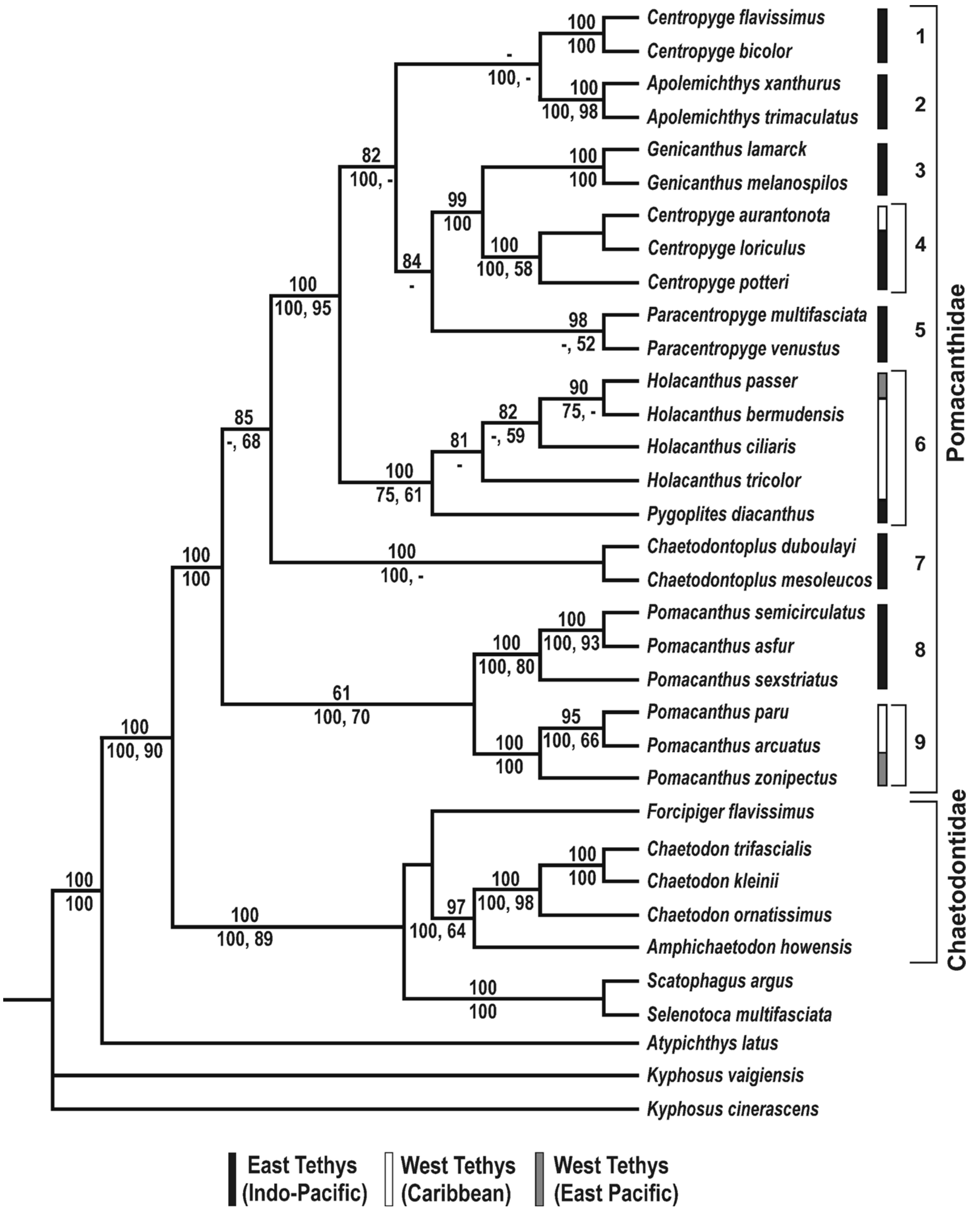

Fig. 2. Preferred maximum likelihood consensus tree of the combined 12S and 16S data sets for the ingroup and all outgroup taxa (rooted using the kyphosids), showing support from Bayesian analyses (above branches) and bootstrap support (below branches) for MP and ML trees. If MP = ML support, only one value is given below the line. — indicates no support >50% was obtained with the particular method of analysis. Biogeographic provinces of species are indicated to the right (see key). Genus-level clades are numbered (1–9) and family membership is indicated.

The genus *Chaetodontoplus* invariably forms a discrete clade and is well supported. However, it is the least stable lineage in the tree being either basal to the whole family or, most frequently, a sister taxon to the combined *Holacanthus* and 'pygmy angelfish' clades.

A single molecular MP tree of length 438 was obtained for the seven pomacanthid genera studied by Allen (1981) (not shown) and this tree was tested against an MP tree constrained to have the topology generated from Allen's morphological analysis (1979) (Fig. 1), which has a length of 447. The tree topologies were found to be significantly different ($p < 0.05$), indicating that the morphological and molecular topologies are not congruent.

The relative ages of the main clades within the Pomacanthidae can be seen in Fig. 3, while details of the variation in age estimates for major clades are summarized in Table 3. The LF method yielded a χ^2 value of

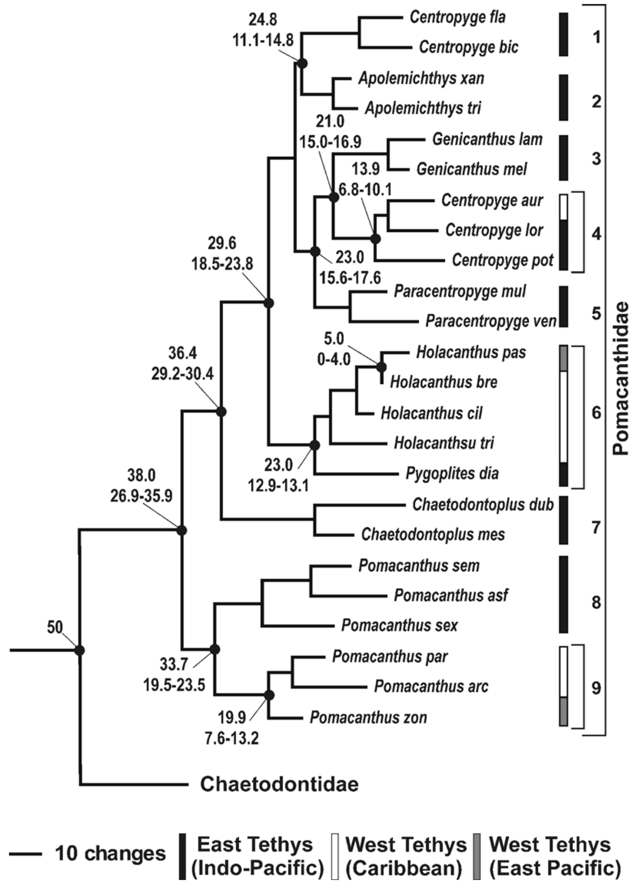

Fig. 3. Preferred maximum likelihood tree of the combined data set (12S and 16S) showing estimated ages in Ma calculated using, penalized likelihood estimates (above) and direct maximum and minimum estimates (below) based on cumulative branch lengths. Both sets of estimates are calibrated using fossil evidence of a 50 Ma diversification at the basal node. The biogeographic distributions of the component taxa are indicated by bars (following Fig. 2). Additional age estimates are provided in Table 3.

90.32 (df = 25), indicating that the molecular clock had to be rejected. However, the ages obtained from this method are given in Table 3 for comparative purposes. The ages obtained by the PL method, produced a χ^2 value of 2.73 (df = 25), indicating that this approach represents an appropriate method for calculating ages of divergence of clades in the Pomacanthidae. When only the geminate species pair divergence age was fixed, in the absence of an arbitrary fixed age for the root node, calculated divergence ages of the deeper nodes were an order of magnitude too large.

When examining the two calibration methods and four approaches a clear pattern arises: regardless of which of the four methods are used, the estimated age of the basal node (Pomacanthidae–Chaetodontidae [inc. Scatophagidae]) was invariably outside the 50–65 Ma window indicated based on fossil evidence. The LF estimate was far too old (91.7 Ma), and the remaining methods all gave estimates that were too young (the

Table 3
Estimated ages (in Ma) of major nodes within the Pomacanthidae using four methods: direct estimates (based on maximum and minimum branch lengths of the phylogram), a Langley Fitch enforced molecular clock (LF), penalized likelihood rate smoothing estimate (PL) and non-parametric rate smoothing analyses (NPRS)

	Direct estimates						LF			PL			NPRS		
	IOP 3.1 Ma		Fossil 65 Ma		Fossil 50 Ma		IOP 3.1 Ma	Fossil 65 Ma	Fossil 50 Ma	IOP 3.1 Ma	Fossil 65 Ma	Fossil 50 Ma	IOP 3.1 Ma	Fossil 65 Ma	Fossil 50 Ma
	min	max	min	max	min	max									
Root node															
Pomacanthidae–Chaetodontidae	23.5	38.5	65.0	65.0	50.0	50.0	91.7	65.0	50.0	22.9	65.0	50.0	34.6	65.0	50.0
Internal nodes															
Pomacanthus-sister clade	12.7	27.7	35.0	46.7	26.9	35.9	48.7	36.8	28.6	17.9	46.7	38.0	25.0	47.0	36.1
Chaetodontoplus-sister clade	13.8	23.4	38.0	39.5	29.2	30.4	50.3	34.6	26.9	17.2	44.4	36.4	23.8	44.7	34.4
Pomacanthus subclades	9.2	18.1	25.4	30.6	19.5	23.5	41.9	28.8	22.4	16.2	40.6	33.7	22.0	41.3	31.8
Holacanthus-pygmy angelfishes	8.7	18.4	24.1	31.0	18.5	23.8	35.2	25.0	19.6	13.8	34.7	29.6	18.6	34.9	26.9
Terminal nodes															
Holacanthus–Pygoplites	6.1	10.1	16.8	17.0	12.9	13.1	23.6	16.7	13.1	11.1	26.3	23.0	14.2	26.7	20.5
P. zonipectus–paru/arcuatus	3.6	10.2	9.9	17.2	7.6	13.2	19.5	13.6	10.6	9.8	22.1	19.9	12.1	22.7	17.5
C. aurantonota–loriculus/potteri	4.7	5.2	8.8	13.1	6.8	10.1	14.7	11.5	8.2	5.0	14.7	13.9	7.3	13.5	11.6
H. bermudensis–passer	0.0	3.1	0.0	4.0	0.0	4.0	3.1	2.2	1.7	3.1	5.9	5.0	3.1	5.8	4.5

For each method three different calibrations were considered: (A) the Isthmus of Panama (IOP) as marked by separation of trans-isthmian geminate species pairs at 3.1 Ma, (B) fixing the basal node at 65 Ma, marking the K/T boundary before which no extant perciform reef fish families have been recorded in the fossil record, and (C) fixing the basal node at 50 Ma marking the first fossil record of the sister taxon to the ingroup.

38.5 Ma based on direct maximum ages was the closest to expected values).

Of the fossil calibrated ages, LF was again at variance with other techniques, yielding ages for the geminate species pair younger than the acceptable minimum age of 3.1 Ma. However, the remaining estimates (direct, PL, NPRS) were all broadly similar, with the age of the *Holacanthus bermudensis-passer* pair ranging from 4.0 (with 50 Ma calibration and a directly estimated maximum ages) to 5.9 (with the PL method and 65 Ma calibration; the 0 for the direct minimum estimates are not included); all are acceptable as they are older than the 3.1 minimum age for division by the isthmus. Variation in estimated ages among methods was limited. The LF method and the molecular clock was rejected and the results reflect this status. As expected, the 65 Ma fossil calibrations gave proportionately older estimates than the 50 Ma. Overall, direct estimates gave slightly younger ages than either PL or NPRS methods.

4. Discussion

4.1. Pomacanthid relationships

The Pomacanthidae is clearly a monophyletic group. This is consistent with traditional taxonomy. The monophyly of the Chaetodontidae likewise agrees with previous classifications. However, the presence of one of the outgroups, the Scatophagidae, as the sister group to the Chaetodontidae is unexpected. This relationship contrasts markedly with both traditional taxonomic classifications (Nelson, 1994) and phylogenetic hypotheses (Ferry-Graham et al., 2001; Tang et al., 1999; Tyler et al., 1989). The Scatophagidae is usually recognised as a basal family within the Acanthuroidei (Tyler et al., 1989). If supported by additional evidence, the placement of the Scatophagidae as the sister clade to the Chaetodontidae would require a fundamental reevaluation of relationships within the squamipinnes. Nevertheless, the Chaetodontidae and Scatophagidae together do appear to be the sister-clade to the Pomacanthidae.

Apart from its association with the Scatophagidae, the other feature of the chaetodontid clade that contrasts markedly with most previous phylogenies (Ferry-Graham et al., 2001, Smith et al., 2003) is in the location of *Amphichaetodon*; it is usually considered to be the most basal chaetodontid lineage (with just two species in one genus). However, our data do agree with morphology-based phylogenies in the placement of *Forcipiger* and *Chaetodon* in separate clades. Until the relationships of all families and genera in the squamipinnes are resolved any inferences must remain tentative. However, our results clearly demonstrate the utility of 12S and 16S regions in resolving relationships among these families and for separating genera within families.

Within the Pomacanthidae, the combined 12S and 16S data support the monophyly of all component genera with the exception of *Centropyge*, which comprises two discrete clades (matching the traditional subgenera *C. Centropyge* [Clade 1] and *C. Xiphypops* [Clade 4]). These results largely agree with traditional taxonomic boundaries of Allen et al. (1998) and Debelius et al. (2003) (only *Centropyge* and the subgeneric divisions within *Holacanthus* and *Pomacanthus* are not supported). In *Holacanthus*, internal support for the branches is insufficient to evaluate subgeneric groupings. However, the close relationship between *H. passer* and *H. bermudensis* suggest that the current division between *H.* (*Angelichthys*) (= *H. africanus, H. bermudensis*, and *H. ciliaris*) and *H.* (*Plitops*) (= *H. clarionensis, H. limbaughi* and *H. passer*) is unwarranted. The only subgenus within *Holacanthus* to obtain support, albeit weak, is *H.* (*Holacanthus*) (= *H. tricolor*). However, as a basal monotypic subgenus, recognition of *H.* (*Holacanthus*) with subgeneric ranking seems unnecessary. From the data currently available it appears that the most parsimonious taxonomic structure would be to reject all subgeneric groupings within *Holacathus*. Indeed, the status of *Pygoplites* and *Holacanthus* as separate genera is equivocal; *Pygoplites* may simply be a basal species within *Holacanthus*, and even this basal position is not well supported.

Within *Pomacanthus* the existing subgeneric groupings are likewise only partially supported. The monophyly of *Pomacanthus paru* and *P. arcuatus* as a clade is consistent with the traditional subgeneric grouping *P.* (*Pomacanthus*), as these are the only species in the subgenus. However, these two species together with *P. zonipectus* form a well-supported Clade–Clade 9. *P. zonipectus* is the type species for *P.* (*Pomacanthodes*). As *P.* (*Pomacanthus*) has priority this entire subgeneric clade is therefore *P.* (*Pomacanthus*); *P.* (*Pomacanthodes*) is no longer a valid subgenus. The sub-generic status of the remaining seven species, in what was *P.* (*Pomacanthodes*), and the three in *P.* (*Euxiphipops*) is equivocal. These may represent sister clades within Clade 8, however, a more comprehensive analysis of species-level relationships within *Pomacanthus* is clearly needed to resolve this issue. Regardless of the outcome, none of these groups can be termed *P.* (*Pomacanthodes*).

Although our data support most traditional generic groupings, our phylogeny differs markedly from previous phylogenies based on either morphology (Allen, 1981; Shen and Liu, 1978) or biochemistry Chung and Woo (1998), in terms of both the composition of genera and their interrelationships (cf. Fig 1). In the most recent evaluation, using allozyme data, Chung and Woo (1998) identify a unified (if phenetic) *Centropyge* and markedly different *Holacanthus–Pomacanthus* associations (they find *Paracentropyge* to be nested within

Holacanthus, and *Holacanthus* and *Pomacanthus* to be sister taxa). Contrary to Chung and Woo (1998) and Pyle and Randall (1993), our data support the genus *Paracentropyge*, which although morphologically and ecologically distinct, has been associated with *Centropyge* and *Holacanthus* by previous authors. In our analyses the greatest instability was in the placement of *Chaetodontoplus* (in ML analyses it was often basal to the whole Pomacanthidae or associated with *Pomacanthus*) and genera within the pygmy angelfish clade (Clades 1–5). Although the latter clade received reasonable support (especially from parsimony analyses) the topology within the clade is poorly resolved.

Chung and Woo (1998) characterized *P. zonipectus* as "biochemically distinct from con-subgeners" in *Pomacanthus* (*Pomacanthodes*), for which it is the type species. In the present analysis, *P. zonipectus* sits as the basal taxon of the *Pomacanthus* (*Pomacanthus*) clade (Clade 9; with *P. arcuatus* and *P. paru*). This association is further supported by a shared juvenile colour pattern (black and yellow narrow vertical stripes) in contrast to the blue and white stripes seen in juveniles of the two other *Pomacanthus* subgenera (Allen et al., 1998; Debelius et al., 2003). As the type genus for *Pomacanthus* (*Pomacanthodes*), the position of *P. zonipectus* calls for a reallocation of species among subgenera.

The confused literature in recent years (reviewed by Chung and Woo, 1998) largely reflects the limited range of morphological characters, disagreement over generic or subgeneric rankings, and a lack of comprehensive cladistic evaluations. While we find significant differences between our phylogeny and that of Shen and Liu (1978) and Allen (1981) it is reassuring to see that the differences lay mainly in terms of relationships. There were relatively few taxonomic changes indicated. A taxonomic revision of the family is outside the scope of the current study (only 2–5 species per genus were examined), however, it is clear that the family is in need of a taxonomic re-evaluation. The possible reinstatement of *Xiphypops* at the full generic rank (following Smith, 1955) stands as a clear priority. Nevertheless, the strong support of the molecular evidence for existing generic groups offers promise for a robust and stable classification. Furthermore, the clear division along taxonomic lines strongly suggests that the remaining species are highly likely to lie within the clades identified and that the patterns described herein are unlikely to be changed as a result of more extensive sampling.

4.2. The origins of ecological novelty

The pattern of ecological diversification within the family is well delineated, with each of the four main clades having a distinctive suite of ecological characteristics (Fig. 4). The *Holacanthus–Pygoplites* (Clade 6) and *Chaetodontoplus* (Clade 7) clades are both composed of oblong shaped fishes, while the *Pomacanthus* clade has both oblong (east Tethys) and rounded (west Tethys) forms (Clades 8 and 9, respectively). In all three Clades (6, 7, and 8 + 9) the fishes are invariably large (25–55 cm total length), generalist omnivores (sponges, algae and attached invertebrates make up the majority of the diet), with a social/reproductive system based primarily on solitary individuals or relatively stable pair bonds (Allen et al., 1998; Debelius et al., 2003; although harems have been described in some *Holacanthus* species Moyer et al., 1983).

All of the large angelfish clades (Clades 6–9) have a distinct juvenile stage. In *Chaetodontoplus* the juveniles are dark with a single pale vertical bar behind the head; in the remaining species the juveniles all have a distinctive pattern characterized by narrow pale vertical stripes (Debelius et al., 2003). In *Pygoplites* the striping is retained in the adult (heterochronic paedomorphosis). This suite of characters in the basal clades is in marked contrast to the 'pygmy angelfish' clade (Clades 1–5 in Fig. 4), which contains a range of evolutionary novelties. In this latter clade, the maximum body size is often small (max 35 cm, most <20 cm, many <10 cm) and although invariably oblong shaped, there is a wide diversity of reproductive and trophic modes. It is only within this clade that we have clear examples of haremic social structures (*Centropyge*, *Apolemichthys*, and *Genicanthus*), protogynous hermaphroditism (*Centropyge*, *Genicanthus*) and complete sexual dichromatism (*Genicanthus*) (Allen et al., 1998; Debelius et al., 2003).

All taxa within the Pomacanthidae possess a unique intra-mandibular articulation that permits closure of the jaws while protruded (Konow and Bellwood, in preparation), a feature that is structurally and functionally distinct from similar linkages in other teleost fishes (cf. Bellwood, 1994; Purcell and Bellwood, 1993). The ecological implications of this specialization are yet to be fully quantified, but appear to be most significant in the larger body-sized clades (*Pomacanthus*, *Holacanthus*) where it combines with a tearing force generated by the jaw adductor muscles to facilitate predation on structurally resilient, and partially hidden prey, such as sponges, ascidians and tunicates (cf. Allen et al., 1998; Konow and Bellwood, in preparation). However, this is a basal trait within the family; most departures are again seen in the 'pygmy angelfish' clade. Here, the pattern of jaw protrusion differs significantly, with one of the two *Centropyge* subgenera (Fig. 3), *C. Xiphypops*, exhibiting a marked antero-ventral, or subterminal, trajectory of jaw protrusion, contrasting with the typical anteriorly directed protrusion as seen in *C. Centropyge*, and most other taxa. Other significant trophic novelties within this clade include planktivory and herbivory. Planktivory is largely restricted to *Genicanthus* where all species are planktivores. Elsewhere, it has only been recorded occasionally in *Holacanthus passer*, as coprophagy,

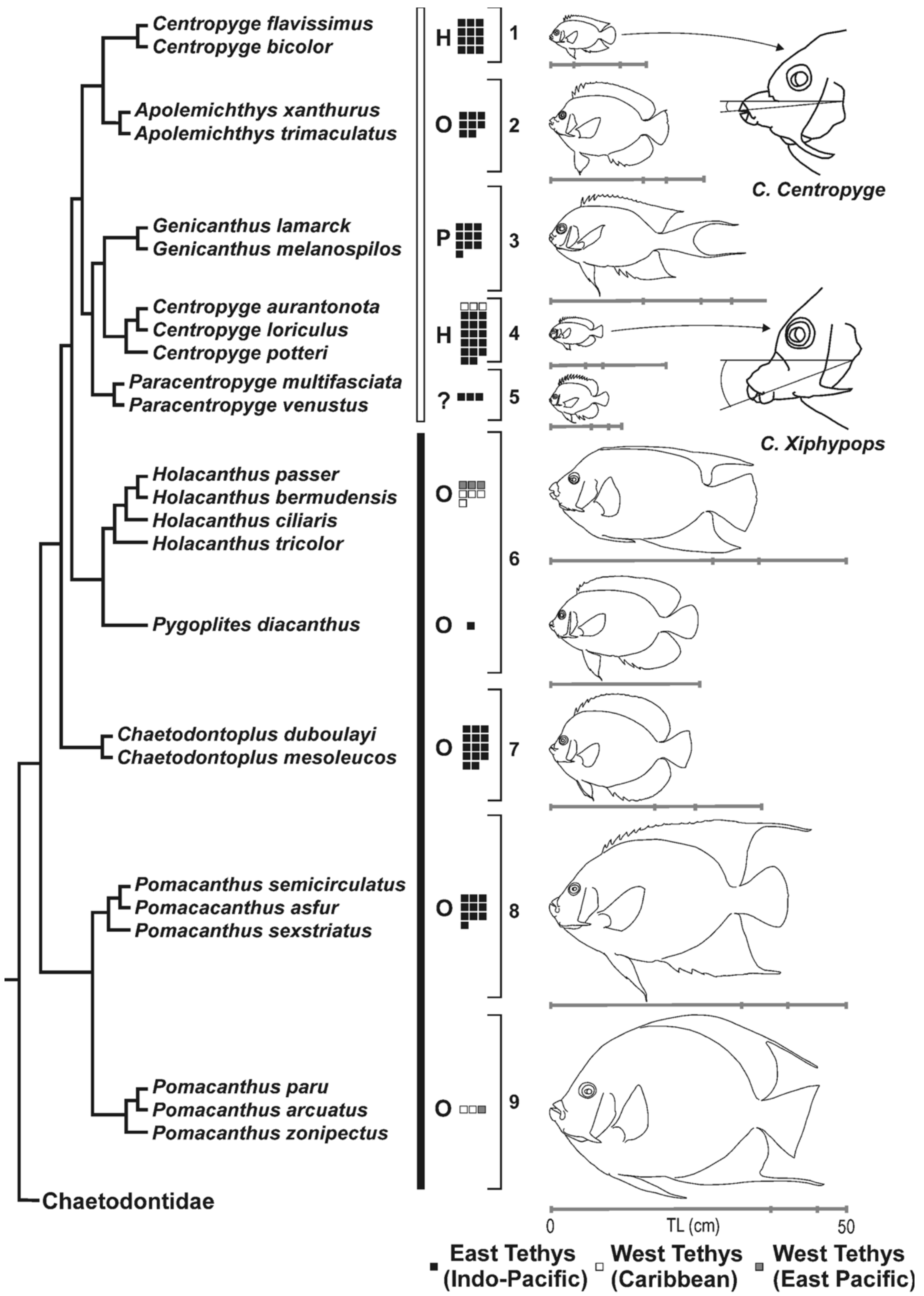

Fig. 4. The evolution of ecological novelty in the Pomacanthidae. Biological traits are mapped on the cladogram with clear evidence of a transition in the sizes of individuals and in the complexity of dietary modes. Most changes are restricted largely to the 'pygmy angelfish' clade (Clades 1–5). The vertical bar indicates the presence (solid) or absence (open) of distinctive juvenile colour patterns. The vertically arranged letters indicate the dominant feeding mode within each clade: H, herbivorous; O, omnivorous; P, planktivorous; ? — unknown. In the block matrices, each square represents one species (with shading denoting biogeographic distributions). The fish figures are all drawn to the same scale, representing the mean size within each genus or subgenus. The horizontal bars indicates the minimum, mean and maximum lengths in each clade. The head silhouettes show the two major jaw morphologies in protruded positions (based on video analyses) illustrating the novel sub-terminal protrusion in *Centropyge* (*Xiphypops*).

(Aburto-Oropeza et al., 2000) and in *Pomacanthus rhomboides* (Debelius et al., 2003). Herbivory is restricted to members of the genus *Centropyge* (*Centropyge* and *Xiphypops*) (Allen, 1981).

The origin of the 'pygmy angelfish' clade with their small body size appears to have laid the foundation for extensive radiation within the family. Today, this clade contains 53 species, 60% of all species in the family, with the two smallest bodied clades (both *Centropyge* at present) containing 32 species (36%). The extent of morphological divergence (disparity) is not known (it is currently under investigation) but the extensive speciation within the small-bodied clades offers support for the suggestions that divergence and diversification may be distinct processes, with divergence predating diversification (Streelman et al., 2002), and that small body size may be a significant factor underpinning diversification in reef fishes (Munday and Jones, 1998).

Colour patterns differ markedly among the clades, particularly considering the striped juvenile phase. A distinct juvenile colouration with narrow pale vertical lines is present in two clades (*Pomacanthus*, *Holacanthus*). The tree cannot resolve whether this represents a basal trait (lost in the pygmy clade and *Chaetodontoplus*) or independent origins. However, it is noteworthy that its presence is correlated in both clades with large adult sizes. It has been suggested that the distinct striped juvenile phase is a mechanism to avoid antagonistic interactions (Fricke, 1980), particularly when the adult size greatly exceeds that of the juvenile. Alternatively, it may represent camouflage or disruptive colouration; juvenile angelfishes in the striped phase are invariably closely associated with cover, often in crevices or caves in shallow water. This pattern is lost in larger juveniles and adults as they move off over the reef. In *Pygoplites* the cave-dwelling habit is retained throughout life and the striped pattern is likewise retained. This retention appears to be an example of heterochronic paedomorphosis.

4.3. Notes on molecular clock calculations

Although we have no fossil pomacanthids, fossil and extant members of the higher squamipinnes have been intensively studied in recent years using morphological features and cladistic methodologies (e.g., Bannikov and Tyler, 1995; Tyler et al., 1989). The well-documented presence of the Scatophagidae in Monte Bolca (Tyler and Sorbini, 1999) means that the age of the pomacanthid–chaetodontid/scatophagid division must be at least 50 Ma. However, the almost complete absence of perciform fish fossils prior to the K/T boundary at 65 Ma would suggest that the oldest dates of origin for the major perciform lineages (including the pomacanthids) should be no more than 65 Ma (Bellwood and Wainwright, 2002). The fossil record, therefore, places the origins of the Pomacanthidae between 65 and 50 Ma. The estimated age of the basal pomacanthid–chaetodontid node therefore, should lie within this range (50–65 Ma).

Of three putative trans-isthmian pairs, only one geminate species pair was identified. The other potential pairs all had basal associations within clades. Nevertheless, this was by far the most promising pair. The *Holacanthus passer–bermudensis* pair had the fewest base changes of all three alternatives. Furthermore, both species (and the derived *Holacanthus* clade as a whole) are characterized by a close affinity with shallow coastal reef and non-reef substrata. As such, they are likely to be among the last species to have been impacted by the raising of the Isthmus of Panama. They therefore, present a relatively good basis for estimating divergence times within the family, and should provide conservative, i.e., minimum, ages for diversification among lineages (cf. Knowlton and Weigt, 1998). A single calibration time was used for the *Holacanthus* pair of 3.1 Ma, resulting in an estimated rate of base pair changes of 0.32% per Ma.

We provide estimated ages from an enforced molecular clock (LF) alongside PL, NPRS and direct estimates of maximum and minimum ages for each clade, to provide a clear indication of the potential range of errors associated with the methods used in calculating age estimates. If we ignore the LF results, with its spurious age estimates, the estimated ages of the basal pomacanthid–chaetodontid node using the three remaining methods (direct, PL, and NPRS) all fail to meet the range of ages required by fossil evidence. Using the Isthmus of Panama calibration, the estimated age of the basal node is invariably at least 11 Ma too young. The 3.1 Ma isthmus calibration appears to be far too conservative and underestimates ages by approximately 20–50% (the latter based on PL estimates).

The corollary is that if fossil ages are used to calibrate age estimates, all three valid methods (LF is rejected) yield dates for the youngest geminate species pair that is considerably older than 3.1 Ma (range 4.0–5.9); some reaching almost twice the minimum value of 3.1 Ma. Such early divisions are not inconsistent with the available evidence (discussed below). For more detailed analyses of ages within the tree therefore, the fossil based estimates were selected, rather than IOP calibrated ages, as they provide the only values that are consistent with both calibration methods and independent geological and molecular evidence.

Our divergence rates of 0.32% base pair changes per Ma (based on the IOP calibration), 0.15–0.25% (based on max/min direct estimates using a 50 Ma calibration) and 0.15% (mean value from the 50 Ma calibrated PL method) vary from parity to more than twice the rate of 0.14% previously used for coral reef fishes (e.g., Bernardi et al., 2000; Streelman et al., 2002; [as per Ritchie et al., 1996]). However, our rate is based on ts + tv, whereas the Ritchie et al. (1996) rate is tv only, so direct comparisons may be inappropriate. Hanel et al. (2002) obtained a rate of 0.35% for ts + tv based on a fossil calibrated clock (0.14% for tv only). Unfortunately, the calibration of Hanel et al. (2002) is based on fossils of questionable value. All are based on old descriptions. The fossils have not been placed in genera based on synapomorphies, rather, overall similarity in shape and form. Examined by one of us (DRB) as part of a review of labrid fishes (Bellwood and Schultz, 1991; Schultz and Bellwood, in preparation), one example, *Labrus agassizi*, could not even be placed with confidence in the Labridae. The disparity between our results and those of

Hanel et al. (2002) may be partially a result of the limitations of their fossil evidence.

Overall, by using two independent calibrations we were able to cross-validate our estimates. Ages calibrated based on what is arguably one of the most intensively studied vicariance events in marine biogeography, the closure of the Isthmus of Panama (Jackson et al., 1996), provided a direct means of calculating divergence rates within the study group (rather than using rates derived from other taxa). Nevertheless, the results were highly conservative. A direct evaluation of the reef fish fossil record, and the presence of well-characterized fossil specimens, however, provided a rigorous, independent, evaluation of our age estimates and yielded a robust estimate of ages within the family.

There has for a long time been a problem with conflicting estimates of the age of divergence among clades when comparing molecular evidence with the fossil record. Fossil dates are invariably too young, while molecular dates are often exceptionally old (Bellwood and Wainwright, 2002; Benton and Ayala, 2003). Interestingly, our estimated ages, based on either a well-established biogeographic event or direct evaluation of recently classified fossils provides a rare consensus, with fossils providing the oldest age estimates, bringing these two conflicting sets of ages together for reef fishes. The high degree of complementarity bodes well for future analyses, and offers some degree of confidence in subsequent biogeographic interpretations.

4.4. Historical biogeography of the Pomacanthidae

The phylogram (Fig. 3) provides an overview of the evolutionary history of the family in relation to major biogeographic events. The estimated age of the family, at 50 Ma is a minimum based on fossil ages of the sister clade, which incorporates Scatophagidae (the independent isthmus estimate merely asserts that the age of the basal node is at least 22.9 Ma; Table 3). On this basis, the timing of the basal pomacanthid divisions is consistent with the lack of pomacanthid specimens in Monte Bolca. The Eocene (50 Ma) deposits of Monte Bolca in northern Italy contain fossils of numerous extant reef fish families (Bellwood, 1996) including many families in the higher squamipinnes (Acanthuridae, Siganidae, Ephippidae, Scatophagidae, and Zanclidae). The Pomacanthidae and Chaetodontidae are conspicuously absent. The division of the Pomacanthidae from its sister clade at 50 Ma suggest that pomacanthid and chaetodontid specimens may eventually be recovered from the deposits. The recent discovery of true wrasses (Labridae) and damselfishes (Pomacentridae) suggests that these groups may have been relatively rare, however, their eventual discovery was consistent with phylogenetic expectations (Bellwood, 1996; Bellwood and Sorbini, 1996). In contrast, the lack of parrotfishes (Scaridae) and blennies (Blenniidae) appears to reflect the post-Eocene origins of these lineages (Stepien et al., 1997; Streelman et al., 2002).

Of the main divisions within the Pomacanthidae, two nodes appear to be associated with the Terminal Tethyan Event (TTE), *Holacanthus–Pygoplites* and a division within *Pomacanthus*. In each case, the association is marked by both the location of the present geographic ranges (Fig. 5) and the timing of the divisions. The ages in the following sections follow Fig. 3 (using PL and direct estimates based on a 50 Ma fossil calibration). The east-west division is marked by species occurring either side of the Red Sea land bridge, the spatial location of the TTE. The final closure of the land bridge occurred between 12 and 18 Ma (Bellwood and Wainwright, 2002), and effectively divided the tropical oceans into two: a western province comprising the east Pacific, Caribbean and tropical Atlantic (the west Tethys, WT) and an eastern province comprising the Indian Ocean and the west-central Pacific (the east Tethys, ET). In *Pomacanthus*, the basal node separates the two major clades, with the three (and only) WT forms (Clade 9) separated from the three ET representatives (Clade 8) at 33.7 Ma (19.5–23.5 Ma). In *Holacanthus–Pygoplites* a division between *Holacanthus* (all with WT distributions; 4 of 7 extant species were examined) and *Pygoplites* (ET distribution) occurred at approximately 23.0 Ma (12.9–13.1 Ma).

Both ages predate the TTE. The presence of such early divisions predating geological events is not uncommon (Bellwood and Wainwright, 2002). This may reflect either a number of discrete vicariance events in this region, or a prolonged period during which a gradual restriction of the seaways sequentially separated populations, with the timing of divisions reflecting the ability of populations to maintain genetic connectivity through a narrowing seaway. It must be noted in this context, that the TTE marks the final physical closure of the seaway and that ecological and genetic barriers were probably in place much earlier (as noted for the Isthmus of Panama).

In addition, there are a number of divisions that appear to be associated with the other major biogeographic division within tropical oceans, the rising of the Isthmus of Panama (IOP). This is seen in the *Centropyge* (*Xiphypops*) clade at the *C. aurantonota-loriculus*/*potteri* node (*C. aur-lor-pot* in Fig. 3) dated to 13.9 Ma (9.4–12.7 Ma) and in the *Pomacanthus* clade with the *P. zonipectus-paru*/*arcuatus* node (*P. zon-par*/*arc*) at 19.9 Ma (7.6–13.2 Ma). Again, both of the divisions considerably predate the final closure at 3.1 Ma (Coate and Obando, 1996), although this pattern is corroborated by the IOP calibrated age estimates (Table 3). While the situation for *Centropyge* is tentative because of limited sampling, the complete representation of the WT *Pomacanthus* species support these older dates.

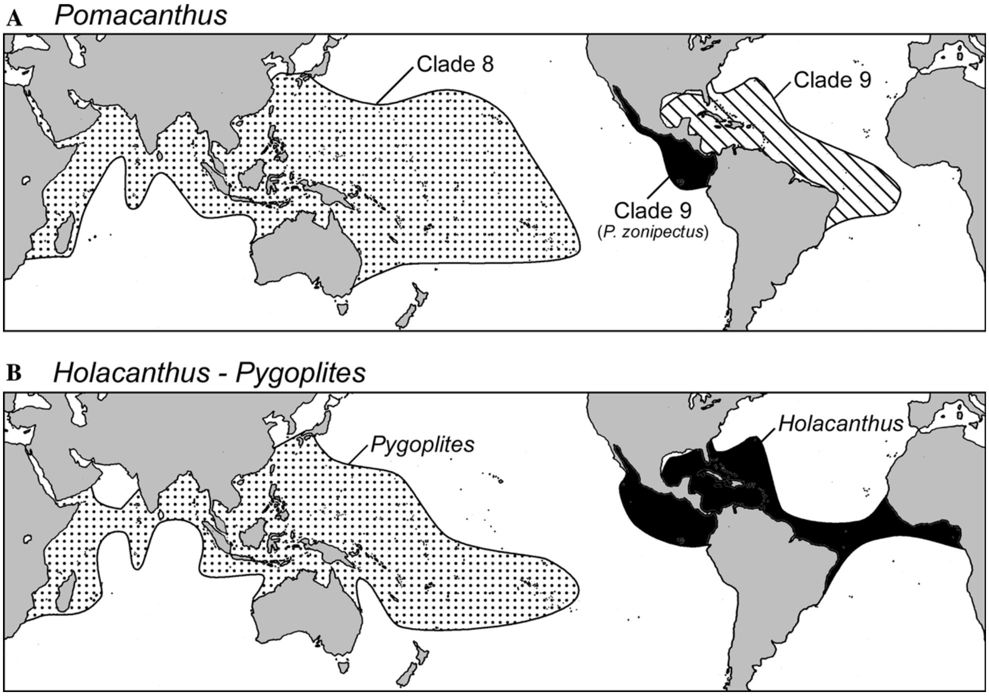

Fig. 5. Biogeographic distribution patterns of the two clades in which vicariance may be associated with the early stages of the Terminal Tethyan Event (the final closure of the Red Sea land bridge at approximately 12–18 Ma). (A) The two *Pomacanthus* clades (diverging between 20 and 34 Ma). (B) The *Holacanthus–Pygoplites* division (approximately 19–30 Ma). The full range of age estimates is given in Table 3.

These early divergence dates are not unusual, with a number of previous studies providing evidence of diversification prior to the final closure of the IOP (Knowlton and Weigt, 1998; Lessios et al., 1995). Nevertheless, the estimated ages of the species examined herein emphasise the potential for diversification of reef fish lineages in the region to predate closure of the IOP by a considerable period of time (>10 Ma).

In terms of longevity, it is interesting to note how successful the Pomacanthidae has been in the west Tethys, retaining representatives in all three major clades. The species in two genera, *Holacanthus* and *Pomacanthus* appear to belong to old lineages that have survived in the WT since before the TTE. This survival in the face of extensive loss in other fish taxa (Bellwood, 1997, Bellwood and Wainwright, 2002) may be associated with their strong non-reef associations. Almost all WT species are well represented in non-reef habitats and there is frequently a strong association between juveniles and mangrove, coastal or shallow-water seagrass habitats (Humann, 1994). It is likely that the west Tethys pomacanthids were one of the main groups to successfully utilize these areas as refuges during low sea stands (cf. Bellwood, 1997). This ability (or at the least an ability to utilize a broad range of habitats) would represent a significant advantage in the face of loss of reef habitat and shallow waters during the Plio-Pleistocene (Bellwood and Wainwright, 2002). Such non-reef links appear to be particularly important features of Atlantic WT reef fish faunas (Bellwood, 1997; Nagelkerken et al., 2002).

The lack of *Chaetodontoplus* in the west Tethys is striking. As one of the older clades it may be expected to be widespread. However, today it is restricted to the Indo-Australian Archipelago (IAA). This distribution may be relictual, although it may equally just reflect limited dispersal. It is interesting to note in this context that the current IAA, while a centre of biodiversity (Bellwood and Hughes, 2001; Hughes et al., 2002), may not have been a significant centre at the time of origin of the *Chaetodontoplus* clade. At this time (36.4 Ma), the IAA had little reef structure (Wilson and Rosen, 1998), and the centre of diversity was probably closer to the central Tethys (the location of the paleo-mid-domain; cf. Connolly et al., 2003).

Finally, one must consider the status of *C. aurantonota*. There are only three *Centropyge* species (all *Xiphypops*) in the west Tethys (WT) (*argi*, *aurantonota*, and *resplendens*). The fourth species in the '*argi*' complex (based on colour patterns) is *C. acanthops*, which is widespread along the east coast of Africa. The number of WT taxa is small compared to the 29 *Centropyge* (17 *Xiphypops*) in the east Tethys. The age of the division between *aurantonota* and the WT species (13.9 Ma; 6.8–10.1 Ma) is equivocal. With the date offering two conflicting possibilities. If the oldest age is the most accurate, then this may be another example of a TTE vicariance event. Alternatively, if the younger dates are supported, this suggests that the division was after the TTE but before the closure of the IOP. Initially, one may hypothesise that the WT *Centropyge* lineage entered the WT after the closure of the TTE but before the IOP. The question is how? The most common explanation for these patterns is that the species dispersed across the east Pacific Barrier. This has been suggested based on phylogenetic evidence for two other reef fish genera, *Scarus* (Bellwood, 1994) and *Bodianus* (Gomon, 1997), and is consistent with patterns described in *Thalassoma* by Bernardi et al. (2004). However, the location of the fourth '*argi*' species, along the coast of east Africa, and the almost identical colour patterns between the African *C. acanthops* and *C. resplendens* from Ascension Island, in the southern Atlantic, and the absence of the '*argi*' complex in the central or east Pacific, strongly suggest that they represent the traces of a post-IOP invasion of the Atlantic from the Indian Ocean via the Horn of Africa. The presence of chaetodontids around the horn (Allen et al., 1998) lends credence to this suggestion by demonstrating the physiological capacity of closely related taxa to survive conditions in this region. Complete sampling of the remaining *Centropyge* should help resolve this dilemma.

Overall, the evolutionary history of the Pomacanthidae appears to share many of the features displayed by other reef fish families. The tree topology and fossil evidence suggest that the family arose over 50 Ma ago in the Eocene or, possibly, Palaeocene. This estimate receives independent support from geologically-calibrated evidence, indicating that the family arose at least 22.9 Ma ago. There is evidence of diversification of major lineages in the late Eocene, Oligocene, and early Miocene, with all genera in place by the mid-Miocene. As with many other groups the pomacanthids appear to have been strongly influenced by the major divisions in the tropics, including the Terminal Tethyan Event and the closure of the Isthmus of Panama. Ecological diversification within the family is largely restricted to a single relatively derived clade (the 'pygmy angelfish' clade), which exhibits distinct changes in body size, social and reproductive patterns, colour patterns and trophic status. These changes also appear to have been associated with extensive speciation within this clade.

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