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Is sexual selection driving diversification of the bioluminescent ponyfishes (Teleostei: Leiognathidae)?

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Abstract

Sexual selection may facilitate genetic isolation among populations and result in increased rates of diversification. As a mechanism driving diversification, sexual selection has been invoked and upheld in numerous empirical studies across disparate taxa, including birds, plants and spiders. In this study, we investigate the potential impact of sexual selection on the tempo and mode of ponyfish evolution. Ponyfishes (Leiognathidae) are bioluminescent marine fishes that exhibit sexually dimorphic features of their unique light-organ system (LOS). Although sexual selection is widely considered to be the driving force behind ponyfish speciation, this hypothesis has never been formally tested. Given that some leiognathid species have a sexually dimorphic LOS, whereas others do not, this family provides an excellent system within which to study the potential role of sexual selection in diversification and morphological differentiation. In this study, we estimate the phylogenetic relationships and divergence times for Leiognathidae, investigate the tempo and mode of ponyfish diversification, and explore morphological shape disparity among leiognathid clades. We recover strong support for a monophyletic Leiognathidae and estimate that all major ponyfish lineages evolved during the Paleogene. Our studies of ponyfish diversification demonstrate that there is no conclusive evidence that sexually dimorphic clades are significantly more species rich than nonsexually dimorphic lineages and that evidence is lacking to support any significant diversification rate increases within ponyfishes. Further, we detected a lineage-through-time signal indicating that ponyfishes have continuously diversified through time, which is in contrast to many recent diversification studies that identify lineage-through-time patterns that support mechanisms of density-dependent speciation. Additionally, there is no evidence of sexual selection hindering morphological diversity, as sexually dimorphic taxa are shown to be more disparate in overall shape morphology than nonsexually dimorphic taxa. Our results suggest that if sexual selection is occurring in ponyfish evolution, it is likely acting only as a genetic isolating mechanism that has allowed ponyfishes to continuously diversify over time, with no overall impact on increases in diversification rate or morphological disparity.

Keywords: bioluminescence, disparity, diversification, leiognathids, sexual selection

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Introduction

Sexual selection may influence the tempo and mode of evolution by facilitating genetic isolation among populations (Andersson 1994). In this study, we investigate whether sexual selection caused increased rates of diversification in a family of bioluminescent fishes that utilize photic displays during courtship behaviour. Sexual selection has been conjectured to be the driving force behind speciation in leiognathids (ponyfishes) because of their highly variable and strongly sexually-dimorphic luminescent system, but this hypothesis has remained untested until now. In theory, if sexual selection is occurring within Leiognathidae, it may lead to accelerated rates of diversification and produce reproductive isolation independent of environmental factors (Panhuis *et al.* 2001). There have been many comparative studies of the potential effects of sexual selection on speciation in various taxa that corroborate these theoretical signals (e.g. Mitra *et al.* 1996; Møller & Cuervo 1998; Hodges & Arnold 1995; Masta & Maddison 2002).

Leiognathids are a common and widespread family of shallow-water Indo-Pacific marine fishes that are diagnosed by the presence of a unique light-organ system (LOS). The circumesophageal light organ houses symbiotic bioluminescent bacteria (*Photobacterium*), which produce light that the host fish co-opts for predator avoidance via ventral counter-illumination, distress displays, and for courtship signalling (Harvey 1921; Hastings 1971; McFall-Ngai & Dunlap 1984; Woodland *et al.* 2002; Sasaki *et al.* 2003). The LOS is comprised of a multilobed circumesophageal light organ, a silvery, guanine-lined gas bladder, and translucent regions of the gas bladder, head and/or trunk that are frequently species-specific in morphology (Sparks *et al.* 2005; Chakrabarty & Sparks 2007; Chakrabarty *et al.* 2011). Bacterial luminescence is transmitted from the light organ into the reflective, guanine-lined gas bladder via a chromatophore studded light organ 'window', which along with muscular shutters on the light organ and corresponding chromatophore studded translucent head and flank regions, controls the emission of light into the environment (McFall-Ngai & Dunlap 1983; Sparks *et al.* 2005). The orientation, shape and pigmentation of the translucent external patches differ among ponyfish clades, and it can be inferred that the intensity, flashing pattern, and possibly wavelength of emitted light varies interspecifically based on morphological differences associated with the LOS.

In addition to species-specific LOS variation, most leiognathid species are sexually dimorphic with regard to light organ volume and shape (McFall-Ngai & Dunlap 1984; Sparks *et al.* 2005). In ponyfishes with sexually dimorphic LOSs, males not only have larger light organs (by volume) than similarly sized conspecific females, but typically exhibit characteristic translucent patches on their flank, gular, buccal, nuchal and/or opercular regions (Sparks *et al.* 2005; Chakrabarty & Sparks 2007). These sexually dimorphic LOS traits allow males to both utilize and emit bacterially generated luminescence in unique ways not available to females, which lack these anatomical specializations. Given that these male-specific LOS traits would appear to make males more conspicuous to predators, it has been hypothesized that the LOS is a target of sexual selection and that these selective pressures could lead to genetic isolation and taxonomic diversification (Sparks *et al.* 2005).

Sexual selection has previously been hypothesized to be an important isolating factor in the diversification of ponyfishes (Sparks *et al.* 2005) given that (i) leiognathids are externally conservative in morphology and are often found in mixed confamilial species assemblages whose members lack conspicuous external sexually dimorphic features (exclusive of translucent patches) and physiognomy (McFall-Ngai & Dunlap 1983; Sparks *et al.* 2005), (ii) leiognathids are, in general, found in great abundance throughout their range with few obvious geographical isolating barriers and (iii) ponyfishes have pelagic larvae that are theoretically capable of dispersing over great distances via ocean currents (Trnski & Leis 2000). The LOS therefore represents the only character complex of sexually dimorphic anatomical traits (apart from anatomical differences related to sex itself) known in the family. Herein, we investigate whether rates of diversification and morphological differentiation in body shape are influenced by potential sexual-selective pressures acting on the LOS.

The main objectives of this work are to: (i) reconstruct a comprehensive phylogeny for Leiognathidae, (ii) estimate divergence times for all major ponyfish lineages, (iii) examine the tempo and mode of ponyfish diversification and (iv) investigate morphological disparity in both body plan and the LOS. We analyse a greatly expanded taxonomic sampling of ponyfishes relative to previous phylogenetic studies (Ikejima *et al.* 2004; Sparks & Dunlap 2004; Sparks *et al.* 2005) to resolve the relationships of ponyfishes in a Bayesian framework, while simultaneously estimating lineage divergence times via the inclusion of leiognathid and other acanthomorph fossils. Results of this analysis are then used as a framework for addressing questions regarding ponyfish diversification rates and morphological disparity that we outline below.

To examine whether sexual selection acting on the LOS has affected the tempo and mode of ponyfish diversification, we address three major questions. Is there greater taxonomic richness in sexually dimorphic

clades vs. the nondimorphic lineages? Are increased rates of diversification associated with sexually dimorphic clades? Are lineage accumulation patterns consistent with density-dependent speciation or continuous diversification through time? If sexual selection acting on the LOS influences diversification within Leiognathidae, we might expect there to be greater taxonomic richness in sexually dimorphic clades vs. the non-sexually dimorphic lineages, *Aurigequula* and *Leiognathus* lineages, because of higher rates of diversification in sexually dimorphic lineages. Significant shifts in the rates of diversification in specific ponyfish lineages may also indicate that sexual selection is impacting ponyfish evolution, such as an increase in diversification rates in sexually dimorphic clades, or conversely, a rate slowdown in the nondimorphic lineages. A common lineage accumulation pattern has been observed across many taxonomic groups (e.g. birds, lizards, fishes; Harmon *et al.* 2003; Ruber & Zardoya 2005; Phillimore & Price 2008; McPeek 2008; Rabosky & Lovette 2008) whereby lineages accumulate rapidly early on in the evolutionary history of a clade, followed by a marked decrease in lineage diversification over time. This pattern is often associated with density-dependent speciation, where lineages diversify rapidly until niche spaces are filled, at which point diversification slows down (Seehausen 2007). If sexual selection on the LOS is playing a prominent role in the diversification of ponyfishes, a pattern of continuous diversification through time might be expected. Sexual selection acting on the LOS is a potential isolating mechanism that would promote continuous diversification through time regardless of habitat or niche constraints and other environmental factors.

To explore whether sexual selection acting on the LOS affects morphological disparity (quantitatively measured variance in morphology) within ponyfishes, we investigate the following question: Are sexually dimorphic ponyfish species less morphologically disparate than nonsexually dimorphic taxa with respect to overall body shape? Panhuis *et al.* (2001) suggested that one of the conclusive signatures indicating that sexual selection is influencing the evolutionary history of a group is whether closely related species that are sexually dimorphic exhibit little variation in morphological traits other than those related to the potential mating signal (i.e. morphology of the LOS). Therefore, if the sexually dimorphic LOS is the target of sexual selection, then disparity among other morphological features within ponyfishes, such as body shape, should be muted. Sexual communication via a sexually dimorphic LOS may inhibit or perturb body shape disparity within a clade because visual cues important for female choice are potentially focused on photic (LOS) signals rather than other physical traits (e.g. pigmentation pattern, body shape). In contrast, the nonsexually dimorphic LOS lineages may exhibit greater morphological disparity in external body form because of chance, given that allopatric speciation processes would include isolating mechanisms other than those related to sexual selection.

Materials and methods

Data acquisition

To provide a robust test of leiognathid monophyly and examine interrelationships of the family, 21 outgroup taxa were included in the analysis (Table 1). Taxonomic sampling includes groups historically hypothesized to be closely related to ponyfishes, including Carangidae, Gerreidae, Sparidae and Chaetodontidae (Günther 1862; Starks 1911; Regan 1913; Weber & de Beaufort 1931; James 1975; Jones 1985; Springer & Orrell 2004; Sparks *et al.* 2005; Smith & Wheeler 2006; Thacker 2009; Chakrabarty *et al.* 2011). Ingroup sampling includes a taxonomically comprehensive, species-level sampling of leiognathids (38 of 45 valid species and six putatively new species from recent collecting expeditions in Indonesia, Madagascar, Malaysia, Singapore, Sri Lanka, Taiwan and Thailand).

Nucleotide characters were sampled from seven mitochondrial (16S, COI, ND4, ND5, tRNA-His, tRNA-Ser, and tRNA-Leu) and two nuclear genes (28S, histone H3). Markers were selected to complement our previous work (Sparks *et al.* 2005). All ND4, tRNA-His, tRNA-Ser, tRNA-Leu, as well as some of the ND5 sequences used in this analysis, were obtained from previous studies (Table 1). Fish tissues were preserved in 95% ethanol and/or stored frozen prior to DNA extraction. Total genomic DNA was extracted from dorsal flank muscle or fin clips using a Qiagen Tissue Extraction Kit following the manufacturer's protocol. The polymerase chain reaction (PCR) was used to amplify the target gene sequences.

Double-stranded amplifications were performed in a 25-μL volume containing one Ready-To-Go PCR bead (Amersham Biosciences), 1.25 μL of each primer and 2–5 μL of genomic DNA. Amplification profiles and primer sequences for all genes can be found in Smith & Wheeler (2004), Sparks & Dunlap (2004), and Sparks & Smith (2004). The novel double-stranded amplification products analysed in this study were desalted and concentrated using AMPure (Agencourt Biosciences Corporation). Both strands of the purified PCR fragments were used as templates and directly cycle sequenced using the original amplification primers and an ABI Prism Big Dye Terminator Reaction Kit (version 1.1). The sequencing reactions were cleaned and desalted using cleanSEQ (Agencourt Biosciences Corporation).

Table 1 Taxa sampled for the phylogenetic analysis with corresponding GenBank accession numbers. Collection localities are provided for ponyfish taxa. Asterisks denote taxa that include ND4 and tRNAs with ND5 fragment

Species	Country	16S	COI	ND5+	H3	28S
Beryx splendens		DQ027918	DQ027987	NC003188	DQ028088	DQ028177
Anoplogaster cornuta		JF965513	JF965514	NC004391	JF965507	JF965510
Hoplostethus mediterraneus		AY538968	EU869820		AY539177	AY539073
Calamus penna		AY662700	AY662747	DQ028061	AY662876	DQ028192
Enoplosus armatus		DQ532873	NC013181	NC013181	DQ533370	DQ533030
Gasterosteus aculeatus		DQ027919	DQ027988	NC003174	DQ028089	DQ028178
Morone saxatilis		AY538941	AY662754	NC014353	AY539255	AY539150
Scomber scombrus		DQ027929	DQ027999	NC006398	DQ028101	DQ028191
Xiphias gladius		DQ532983	NC012677	NC012677	DQ533480	DQ533143
Trachinotus ovatus		DQ027921	DQ027991	DQ028050	DQ028091	DQ028180
Gerres equulus		AY541668	AY541643	DQ028053	DQ028098	DQ028187
Capros aper		DQ532846	NC010958	NC010958	DQ533343	DQ533001
Monodactylus argenteus		DQ532912	NC010958	NC009858	DQ533409	DQ533071
Microcanthus strigatus		DQ532910	NC013182	NC013182	DQ533407	DQ533069
Zanclus cornutus		DQ532984	NC009852	NC009852	DQ533481	DQ533144
Luvarus imperialis		DQ532902	NC009851	NC009851	DQ533399	DQ533061
Mola mola		DQ532911	NC005836	NC005836	DQ533408	DQ533070
Triacanthodes anomalus		DQ532973	NC004391	NC009861	DQ533470	DQ533133
Triodon macropterus		DQ532975	NC009859	NC009859	DQ533472	DQ533135
Chaetodon auripes		NC009870	NC009870	NC009870	JF965508	JF965511
Heniochus diphreutes		NC009871	NC009871	NC009871	JF965509	JF965512
Equulites (*Photoplagios*) *absconditus*	Madagascar	DQ027964	DQ028034	Unavailable	DQ028150	DQ028241
Equulites antongil	Madagascar	DQ027963	DQ028033	Unavailable	DQ028148	DQ028239
Equulites (*Photoplagios*) *elongatus*	Japan	AY541652	AY541627	AB100016*	DQ028149	DQ028240
Equulites (*Photoplagios*) *laterofenestra*	Malaysia	EU366342	Unavailable	Unavailable	Unavailable	Unavailable
Equulites (*Photoplagios*) *leuciscus*	Philippines	AY541657	AY541632	Unavailable	DQ028152	DQ028243
Equulites lineolatus	Sri Lanka	DQ027966	DQ028036	Unavailable	DQ028153	DQ028244
Equulites n. sp. Malaysia	Malaysia	HQ993124	HQ993152	Unavailable	HQ993180	Unavailable
Equulites (*Photoplagios*) *rivulatus*	Japan	AY541661	AY541636	AB100019*	DQ028154	DQ028245
Equulites stercorarius	Thailand	HQ993125	HQ993153	AB100021*	HQ993181	HQ993205
Eubleekeria jonesi	Thailand	HQ993116	HQ993144	Unavailable	HQ993172	HQ993197
Eubleekeria splendens	Taiwan	HQ993117	HQ993145	AB100020*	HQ993173	HQ993198
Gazza achlamys	Philippines	AY541648	AY541623	AB100025*	DQ028107	DQ028198
Gazza cf. rhombea	Taiwan	HQ993114	HQ993142	Unavailable	HQ993170	HQ993195
Gazza dentex	Thailand	Unavailable	Unavailable	AB100026*	Unavailable	Unavailable
Gazza minuta	Philippines	AY541649	AY541624	AB100027*	DQ028109	DQ028200
Gazza squamiventralis	Madagascar	DQ027938	DQ028008	Unavailable	DQ028112	DQ028203
Karalla daura	Sri Lanka	DQ027955	DQ028025	Unavailable	DQ028133	DQ028224
Karalla dussumieri	Sri Lanka	DQ027959	DQ028029	DQ028074	DQ028137	DQ028228
Leiognathus equulus	Taiwan	DQ027948	DQ028018	AB100017*	DQ028124	DQ028215
Aurigequula fasciatus	Madagascar	DQ027951	DQ028021	Unavailable	DQ028127	DQ028218
Aurigequula longispina	Singapore	DQ027944	DQ028014	Unavailable	DQ028118	DQ028209
Leiognathus n. sp. Fiji	Fiji	DQ027940	DQ028010	DQ028067	DQ028114	DQ028205
Aurigequula n. sp. Madagascar	Madagascar	DQ027942	DQ028012	DQ028069	DQ028116	DQ028207
Aurigequula n. sp. Singapore	Singapore	HQ993118	HQ993146	Unavailable	HQ993174	HQ993199
Leiognathus robustus	Singapore	DQ027953	DQ028023	Unavailable	DQ028129	DQ028220
Aurigequula striatus	Sri Lanka	DQ027945	DQ028015	Unavailable	DQ028119	DQ028210
Nuchequula blochii	Thailand & Malaysia	EU741826	Unavailable	AB100018*	Unavailable	Unavailable
Nuchequula cf. flavaxilla	Philippines	AY541655	AY541630	DQ028071	DQ028131	DQ028222
Nuchequula cf. gerreoides	Sri Lanka	DQ027957	DQ028027	DQ028072	DQ028135	DQ028226
Nuchequula decora	Australia	DQ027956	DQ028026	AB100015*	DQ028134	DQ028225
Nuchequula longicornus	Thailand	HQ993121	HQ993149	Unavailable	HQ993177	HQ993202
Nuchequula mannusella	Taiwan	HQ993119	HQ993147	Unavailable	HQ993175	HQ993200
Nuchequula nuchalis	Japan	AY541658	AY541633	AB100028*	DQ028139	DQ028230
Photopectoralis aureus	Philippines	AY541650	AY541625	Unavailable	DQ028145	DQ028236

Table 1 (*Continued*)

Species	Country	16S	COI	ND5+	H3	28S
Photopectoralis bindus	Philippines	AY541651	AY541626	Unavailable	DQ028146	DQ028237
Photopectoralis cf. aureus	Thailand	HQ993128	HQ993156	Unavailable	HQ993184	HQ993208
Photopectoralis n. sp. East China Sea	Taiwan	DQ027962	DQ028032	Unavailable	DQ028144	DQ028235
Photopectoralis panayensis	Philippines	AY541659	AY541634	Unavailable	DQ028147	DQ028238
Secutor cf. hanedai	Taiwan & Thailand	Unavailable	Unavailable	AB100022*	Unavailable	Unavailable
Secutor indicius	Taiwan	HQ993133	HQ993161	AB100023*	HQ993189	HQ993212
Secutor insidiator	Taiwan	DQ027971	DQ028041	Unavailable	DQ028161	DQ028252
Secutor megalolepis	Philippines	AY541666	AY541641	AB100024*	DQ028163	DQ028254
Secutor n. sp. Madagascar	Madagascar	DQ027967	DQ028037	Unavailable	DQ028156	DQ028247
Secutor ruconius	Sri Lanka	DQ027973	DQ028043	Unavailable	DQ028164	DQ028255

The sequencing reactions were electrophoresed on an ABI 3730 or 3730xl automated DNA sequencer. Contigs were built in Sequencher version 4.1 (Gene Codes) using DNA sequences from the complementary heavy and light strands. Sequences were edited in Sequencher and Bioedit (Hall 1999). All novel sequences are deposited in GenBank (Table 1).

Phylogeny reconstruction and divergence time estimation

Sequences were aligned with MAFFT (Katoh *et al.* 2002) using default parameters. All alignments were visually inspected, confirmed and manually concatenated by the authors. Topology reconstruction and relative divergence times were estimated simultaneously in BEAST v1.6.2 (Drummond & Rambaut 2007) using a template from BEAUTI v1.6.2, with results visualized in TRACER v.1.4 (Drummond & Rambaut 2007). Each gene was assigned a separate GTR + I + G model, which was recommend by MRMODELTEST v2.0 (Nylander 2004) using the Akaike information criterion (AIC). Mean substitution rates were not fixed, with substitution rates estimated under a relaxed uncorrelated lognormal clock that allows for independent rates to vary on different branches within the topology (Drummond *et al.* 2006). Under this model, there is no *a priori* correlation between any rates in the tree. Four separate analyses were performed with 20 million generations each, with a burn-in of 2 million generations for each analysis. Parameters and trees were sampled every 1000 iterations for a total of 80 000 trees, 72 000 post-burnin. The program TRACER v 1.41 (Rambaut & Drummond 2007) was used to inspect the effective sample size (ESS) of all parameters in each analysis and verify parameter stationarity. All parameters appeared to converge on a stationary distribution and possessed ESS's >200, suggesting that all analyses satisfactorily sampled the posterior distributions of each parameter. A 50% maximum clade credibility (mean heights) tree was generated from the posterior tree distribution and served as a framework for diversification analyses.

Fossil calibrations. All fossil calibrations were assigned a lognormal prior, with hard minimum ages of clades set *a priori*. The minimum dates were assigned based on the oldest known fossil of each clade discussed below.

Anoplogaster + *Hoplostethus* (C1): The node representing the most recent common ancestor (MRCA) of this beryciform clade was given a minimum age of 94 Ma (million years ago), based on the fossil taxa †*Hoploteryx lewesiensis* and †*Hoplopteryx simus*, known from Middle–Upper Cenomanian deposits (Patterson 1993). A conservative soft upper bound was set to 150 Ma, the age of the oldest known fossil euteleost †*Leptolepides sprattiformis* (Arratia 1997, 1999). The lognormal prior was given an offset of 94 Ma, with a standard deviation of 1.0 and a mean of 2.07.

Tetraodontiformes (C2): The fossil taxon †*Triodon antiquus*, known from lower-middle Ypresian deposits (Tyler & Patterson 1991), was used to assign a minimum age of 55 Ma for the MRCA of Tetraodontiformes. A conservative soft upper bound of 94 Ma was used, with the lognormal prior given an offset of 55 Ma, with a standard deviation of 1.0 and a mean of 1.7.

Chaetodontidae (C3): A minimum age of 30 Ma was assigned based on the oldest known fossil representative of the family, Chaetodontidae cf. *Chaetodon*, from Rupelian deposits (Blum 1988; Micklich *et al.* 2009). A soft upper bound of 94 Ma was set on the lognormal prior (offset of 30, standard deviation of 1.0, mean of 2.2).

Gazza (C4): Fossil leiognathids are rare and dubious (Matt Friedman, personal communication), and those that can be unambiguously identified as leiognathids are extremely difficult to place within the family. In our analyses, we included a calibration for the minimum age of the MRCA for the genus *Gazza* (12 Ma; mid-Miocene).

This age is based on the unambiguous leiognathid fossil, '†*Leiognathus*' *tottori* (Yabumoto & Uyeno 1994), whose generic placement within the family can be resolved based on the presence of canine teeth on the premaxilla, a feature diagnostic of *Gazza*. A soft upper bound of 94 Ma was placed on the lognormal prior (offset of 12, standard deviation of 1.0, mean of 2.45).

Diversification rate variation

The resulting maximum clade credibility tree from BEAST was trimmed to exclude all non-leiognathid taxa. Additionally, this tree was pruned further for use in the various diversification analyses described below. The first topology (T1) included a representative of all 44 putative ponyfish species included in this study. The second topology (T2) included only one representative for each monophyletic genus as a terminal for use in combined taxonomic and phylogenetic analyses that included information regarding the known species diversity for each genus assigned to its respective terminal.

Exceptional taxonomic richness. We used the methodology of Magallon & Sanderson (2001, eqns 8–11) as implemented in the R platform package LASER (Rabosky 2006a) to test whether any ponyfish lineages exhibit statistically significantly high or low diversification rates. This method calculates a 95% confidence interval (CI) of the potential expected number of species within a clade given a net diversification rate (*r*), a relative extinction rate (*eps*) and clade age. A plot of CI ranges was generated for a net diversification rate calculated from an estimator of *r* implemented in LASER that incorporates both taxonomic and phylogenetic data (Rabosky *et al.* 2007; eqns 2.1–2.3). The generic-level topology (T2) was used for the phylogenetic data, with the terminal for each lineage (i.e. genus) assigned its corresponding number of known species richness, and the estimated *r* was 0.067. Ranges for the CI values were calculated for two separate *eps* values that represent the extremes of possible relative extinction rates (*eps* = 0, 0.99). Clade age for each ponyfish lineage was then plotted against the number of known species in that lineage within the context of the 95% CIs that were generated. Ponyfish clade ages were based on the mean clade ages estimated from the BEAST analysis. If the known species diversity for a lineage given its age lies outside either the upper or lower CI bounds of expected taxonomic richness, then that clade is subject to statistically significantly high or low diversification.

Diversification patterns. We compared rate-constant and rate-variable models under a maximum likelihood approach as described by Rabosky (2006b), which measures the fit of each model using AIC in LASER. These tests were conducted on the pruned topology that incorporates a representative of each ponyfish species (T1). Rate-constant models included Yule (pure birth) and birth-death models, whereas rate-variable models included a two-rate Yule variant and both lognormal and exponential density-dependent speciation models (Rabosky 2006a). The fit of the best rate-constant model is compared to the best rate-variable model to determine which model best represents the data as given by Rabosky's (2006a) equation:

$$\Delta AIC_{RC} = AIC_{RC} - AIC_{RV}$$

ΔAIC_{RC} is positive when a rate-variable model fits the data best and is negative when a rate-constant model fits best. To reduce the possibility of a Type 1 error (incorrectly reject a true null hypothesis), we calculated the 95th percentile of ΔAIC_{RC} scores (corresponding to $\alpha = 0.05$) from 1000 simulated phylogenies under the null hypothesis that rates are constant, as recommended by Rabosky (2006b), using a pure birth model. The observed ΔAIC_{RC} score from our empirical tree (T1) was then compared to our simulated distribution of ΔAIC_{RC} scores to determine the statistical significance of our observed ΔAIC_{RC}. Simulated trees started with a taxonomic size that reflects the total known (= formally described and newly discovered species that await formal description) species diversity of ponyfishes (58 species). Incomplete taxon sampling was then taken into account by randomly pruning taxa from the simulated phylogeny to include the same number of taxa in our empirical study. Additionally, a lineage through time plot was generated in LASER by plotting log-lineages through time given our species level chronogram (T1).

Shifts in diversification rate. Models of diversification rate shifts were calculated using MEDUSA (Alfaro *et al.* 2009) in R, and implemented the Ape (Paradis *et al.* 2004) and Geiger (Harmon *et al.* 2008) libraries. The MEDUSA analysis estimates rates of speciation and extinction on a chronogram with taxonomic information. The pruned-to-genera topology (T2) with accompanying taxonomic information was utilized for this analysis. The maximum likelihood MEDUSA method begins by estimating birth and death values and an AIC score for a model with no shifts in diversification and a single birth and death value across the tree. The method then fits models of increasing complexity by incorporating a branch where rates of diversification change, with an additional birth and death value calcu-

lated for the clade where the shift point occurred. If the new model has an AIC score that is lower than the previous model by a AIC cut-off value determined by the researcher (4 is a common threshold for AIC significance and is recommended as a starting point by Alfaro *et al.* 2009), then the model incorporating a rate shift is retained. This step-wise procedure continues adding additional shift points throughout the tree until the AIC threshold criterion is no longer met. At this point, a backwards elimination procedure begins that individually removes shift points and reevaluates the models. After both a forward and a downward step, a single model is chosen as the most likely.

Morphological disparity

Disparity is a measure of the variance of the body shapes within a group and is used to approximate the magnitude of overall morphological diversity not related to internal features. To measure disparity among leiognathid clades, 312 adult specimens representing 34 species from all ponyfish genera were chosen to best represent all major lineages on the recovered phylogeny. Whenever possible, a minimum of 10 individuals were chosen per taxon to account for intraspecific variation. Only unbent specimens were analysed to minimize the amount of variation due to artefacts that might affect measurements of shape. Further, only adult specimens were used to avoid introducing allometric growth effects on measurements of variation. Digital images were taken from the left side of all specimens, with landmarks (discrete points on anatomical structures that could be located on every specimen, i.e. putatively homologous points) selected to best represent the external shape around the body (Fig. 1). The program, TPSDIG2 (Rohlf 2006), was used to digitize the landmarks on images of the left side of the body for each individual.

Morphological disparity was measured to approximate overall morphological diversity. Disparity, following Foote (1993), can be represented as $(D) = \sum (d_i^2)/(N - 1)$, where d_i^2 is the squared Procrustes distance between the mean shape of a species and the mean shape over all species in the sample (i.e. the grand mean shape of a group), divided by the number of species (N) minus one; this number is then summed over all the species in the sample. Information unrelated to shape, and therefore not important to the analysis of external morphological disparity, was removed. This information, including size, orientation, and position, was removed from the configuration of landmarks by rescaling, rotation and translation. Removal of these features is effected by fixing specimens at a centroid size of one and superimposing them using generalized least

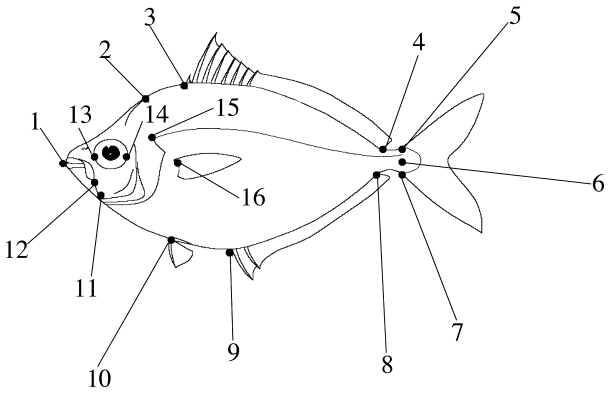

Fig. 1 Landmarks used for disparity analysis: (1) rostral tip of premaxilla, (2) posterior end of nuchal spine, (3) anterior insertion of dorsal fin, (4) posterior insertion of dorsal fin, (5) dorsal insertion of caudal fin, (6) midpoint of caudal border of hypural plate, (7) ventral insertion of caudal fin, (8) posterior insertion of anal fin, (9) anterior insertion of anal fin, (10) dorsal base of pelvic fin, (11) ventral margin of lower jaw articulation, (12) posterior margin of maxilla, (13) anterior margin through midline of eye, (14) posterior margin through midline of eye, (15) dorsal margin of opercle, (16) dorsal base of pectoral fin. Base figure modified from Nelson (2006).

squared Procrustes superimposition. In the optimal superimposition, the distance minimized is the Procrustes distance, calculated as the square root of the summed squared distances between homologous landmarks (Rohlf & Slice 1990; Goodall 1991).

Calculations of disparity and Procrustes superimposition were carried out in DisparityBox6 (Sheets 2007a). This program measures the disparity and generates 95% CIs using a bootstrap method. In this procedure, the original data for a species are resampled (at the specimen level) with replacement to determine the range of possible disparity values. If the 95% CIs of the disparity measurements of two taxa do not overlap, they are significantly different ($P \leq 0.05$). Further tests of statistical significance ($P \leq 0.001$) were also conducted. Statistical significance for differences of morphological disparity was determined by completing a bootstrap test of the range of variation in the disparities, where specimens were resampled with replacement (Efron & Tibshirani 1993). The bootstrap test, carried out in PairDisparity6 (Sheets 2007b), permutes the departure from the within group mean (i.e. the multivariate measures of difference from the means). One thousand bootstrap iterations were completed for each pairwise comparison. In multigroup comparisons, a Bonferonni adjustment was included in the test of significance.

To permit comparison to relevant disparity results from the literature, a slight modification to the results of Chakrabarty (2005) was required, given that there is

an unclear relationship between the number of landmarks and disparity. To make the results of Chakrabarty's (2005) Rift Lake cichlid disparity analysis comparable to Zelditch *et al.*'s (2003) piranha disparity analysis and this study, the number of landmarks had to be reduced from 18 to 16 in the results of Chakrabarty (2005). Therefore, landmarks five and seven were removed from the analysis of Chakrabarty (2005), because they caused the least amount of information loss (change in disparity value) from the total analysis.

Specimens used in comparative morphological analyses are deposited at the following institutions: American Museum of Natural History, New York (AMNH); Australian Museum, Sydney (AMS); Natural History Museum, London (BMNH); California Academy of Sciences, San Francisco (CAS); Faculty of Fisheries, Fisheries Research Laboratory, Mie University, Japan (FRLM); Natural History Museum of Los Angeles County (LACM); Museum National d'Histoire Naturelle, Paris (MNHN); Scripps Institution of Oceanography, Marine Vertebrates Collection, La Jolla (SIO); University of Michigan, Museum of Zoology, Ann Arbor (UMMZ); National Museum of Natural History, Smithsonian Institution, Washington, DC (USNM).

Results

Phylogeny reconstruction and divergence time estimation

The maximum clade credibility tree with 95% higher posterior densities (HPD) is shown in Fig. 2. The HPD correspond to the 95% interval of age ranges sampled for each node in the posterior distribution. Posterior probabilities and HPD ranges for nodes (Fig. 2) are listed in Table 2. A Gerreidae+Chaedodontidae clade was recovered as the sister group of ponyfishes as shown in Fig. 2, albeit with weak support. Monophyly of the family Leiognathidae was strongly supported with a mean clade age of 82 Ma (95% HPD 55–110), indicating that ponyfishes diverged during the Late Cretaceous and that all major lineages were established prior to the end of the Paleogene (Fig. 2).

Nonsexually dimorphic ponyfish species were recovered as a grade leading to a monophyletic Gazzinae (Fig. 2), a pattern previously recovered by Sparks *et al.* (2005). Gazzinae comprises all sexually dimorphic ponyfish species. *Aurigequula* + *Leiognathus* have also been recovered as monophyletic and referred to as the subfamily Leiognathinae (Chakrabarty *et al.* 2011). In the current study, the genus *Aurigequula* was recovered as the sister group to a *Leiognathus* + Gazzinae clade. Within Gazzinae, four strongly supported and morphologically distinct (with regard to internal and external features of the LOS) lineages of Paleogene age were recovered including the tribes Equulitini, Nuchequulini, Eubleekerini and Gazzini (diagnosed in Chakrabarty *et al.* 2011). Gazzini was recovered as the sister group to Eubleekerini, and Equulitini was recovered as the sister group to Nuchequulini, both with strong support (Fig. 2).

Diversification rate variation

The ultrametric tree (Fig. 2) was pruned to include each unique ponyfish species. For diversification studies that utilize taxonomic information, the ultrametric tree was further pruned to include only a single representative of each genus (Fig. 3). Species richness numbers that correspond with currently recognized ponyfish generic-level diversity were matched to each terminal (Fig. 3).

Exceptional taxonomic richness. The plot of 95% CIs for expected species richness of a clade over time is shown in Fig. 4. Confidence intervals were calculated under a relative diversification rate (r) of 0.067 estimated from the combined taxonomic and phylogenetic tree (Fig. 3) and two relative rates of extinction (eps = 0, 0.99). The expected taxonomic richness of five lineages, including Leiognathidae, *Secutor*, *Nuchequula*, *Photopectoralis* and *Equulites*, fall outside the CIs when considering the HPD range of estimated divergence ages, and these lineages are significantly species rich given a moderate to high rate of relative extinction depending on a specific divergence time (see Fig. 4 for divergence time ranges). The subfamily Gazzinae, comprising taxa sexually dimorphic for features of their LOSs, had a statistically significant species richness over time under both a low (0) and a high rate of relative extinction (0.99) for the mean age of divergence of the clade. When the range of estimated divergence ages is considered, this clade exhibits statistically significant species richness, regardless of the relative rate of extinction depending on the specific date of divergence (see Fig. 4).

Diversification patterns. Rate-constant and rate-variable models of diversification were compared on the pruned-to-species topology (T1) of ponyfish relationships (Fig. 2). Results of the likelihood-based model fitting approach are shown in Table 3. The best fitting rate-constant model selected was a pure birth model (AIC_{RC} = 129.54), whereas the best fitting rate-variable model was a Yule model with two diversification rates (AIC_{RV} = 122.39). The rate-variable model fits the data set better than a rate-constant model, and the difference in AIC scores (ΔAIC_{RC} = 7.15) was shown to be statistically significant based on the results of our simulated ΔAIC_{RC} distributions (ΔAIC_{RC} of 7.15 has a P = 0.006).

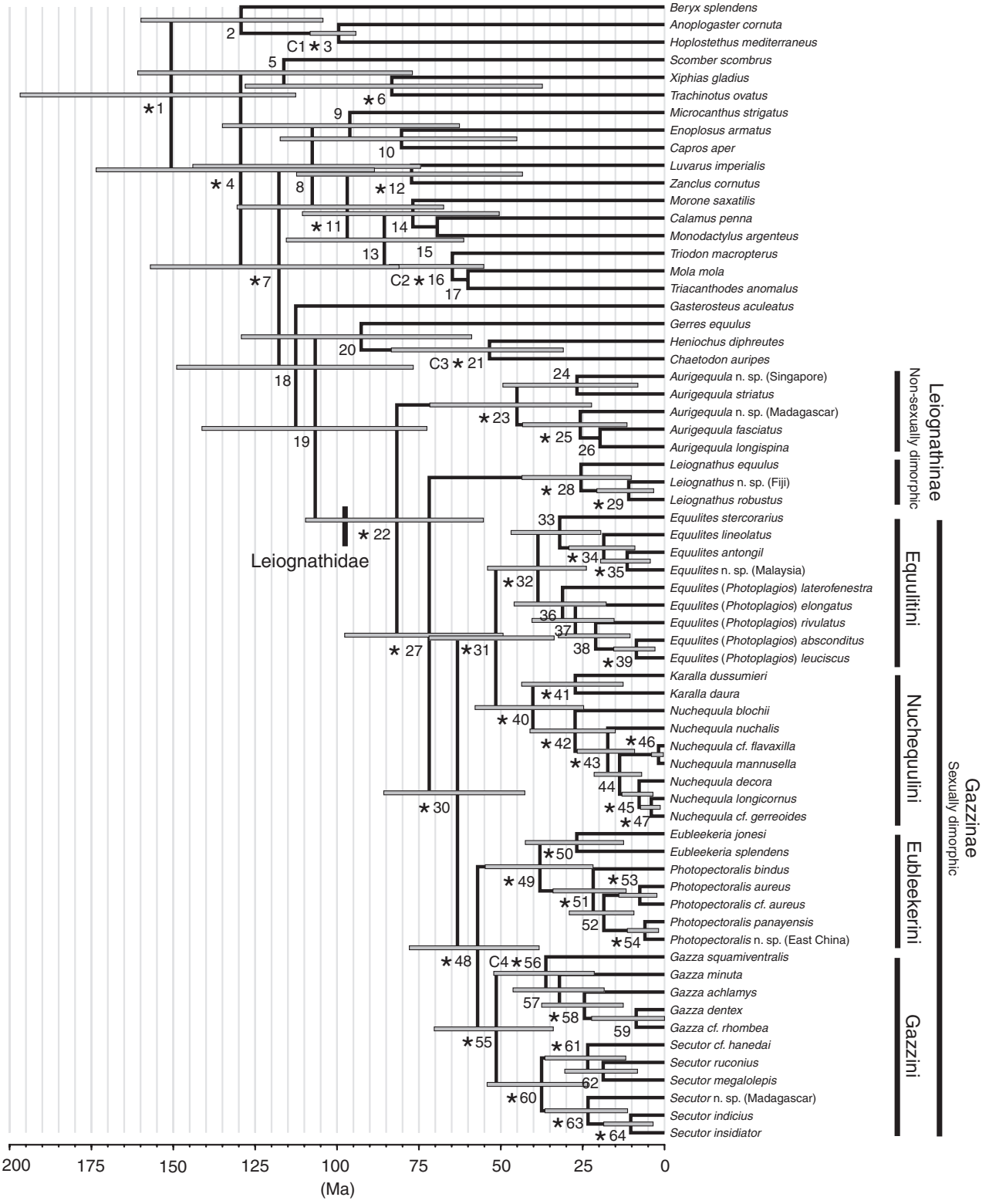

Fig. 2 Maximum clade credibility phylogeny of ponyfishes with divergence time estimations. Horizontal gray bars denote 95% higher posterior densities (HPD). Numbers at nodes refer to clades in Table 1, which includes information regarding mean clade age, 95% HPD and posterior probabilities of nodes. Nodes with an asterisk were recovered with a posterior probability >95%. Taxa labelled with an asterisk were pruned from diversification analyses that included only one terminal representing each species. Scale in millions of years (Ma).

Table 2 Divergence times of ponyfishes as shown in Fig. 3. Clades marked with C# were constrained to a minimum age

Clade/node	Posterior probability	Mean age (Ma)	95% higher posterior densities age
1	1.00	151	113–197
2 Beryciformes	0.84	129	104–160
3 Family (C1)	0.99	99	94–108
4	1.00	129	89–174
5	0.54	116	77–161
6	1.00	83	37–128
7	0.99	118	81–157
8	0.73	108	75–144
9	0.72	96	63–135
10	0.57	80	45–118
11	0.96	97	67–130
12	0.99	77	43–112
13	0.89	86	61–116
14	0.76	77	51–111
15	0.45	70	NA
16 Tetraodontiformes (C2)	0.99	65	55–84
17	0.50	60	NA
18	0.74	113	77–150
19	0.70	107	73–141
20	0.71	93	59–129
21 Chaetodontidae (C3)	1.00	54	31–83
22 Leiognathidae	1.00	82	55–110
23 *Aurigequula*	0.99	45	22–72
24	0.62	27	8–49
25	0.99	26	11–43
26	0.46	20	NA
27	1.00	72	49–98
28 *Leiognathus*	1.00	26	10–44
29	1.00	11	3–21
30 Gazzinae	1.00	63	43–86
31	1.00	52	34–72
32 Tribe Equulitini, *Equulites*	1.00	39	24–54
33	0.90	32	19–47
34	1.00	19	9–29
35	1.00	11	4–20
36 *Equulites* (*Photoplagios*)	0.63	31	18–46
37	0.82	27	15–40
38	0.83	21	11–32
39	1.00	9	3–16
40 Tribe Nuchequulini	1.00	40	25–58
41 *Karalla*	0.98	27	13–44
42 *Nuchequula*	1.00	27	15–41
43	1.00	17	9–27
44	1.00	14	7–22
45	0.90	2	0.5–4
46	1.00	8	4–13
47	1.00	4	1–7
48	1.00	57	38–78
49 Tribe Eubleekerini	1.00	38	22–55
50 *Eubleekeria*	0.99	27	13–43
51 *Photopectoralis*	1.00	22	12–34
52	0.51	19	9–29
53	1.00	8	2–14
54	1.00	6	2–11
55 Tribe Gazzini	0.99	51	34–70
56 *Gazza* (C4)	1.00	36	22–52
57	0.54	32	18–46
58	0.95	25	13–38
59	0.82	9	0.01–22
60 *Secutor*	1.00	38	24–54
61	1.00	24	12–36
62	0.63	19	8–31
63	1.00	23	11–37
64	0.99	10	4–19

The lineage through time plot (Fig. 5) indicates that ponyfish lineages have accumulated continuously through time, with no apparent slowdown in diversification. A pattern of lineage accumulation associated with density-dependent speciation (solid curved line) is shown in Fig. 5, which is in marked contrast to the constant-through-time lineage accumulation pattern (dashed line) observed for ponyfishes (black circles).

Shifts in diversification rate. The maximum likelihood step-wise AIC model test using MEDUSA indicates that there is no strong evidence for a diversification rate shift (either speed up or slow down) within ponyfishes when analysed on the phylogeny that incorporates taxonomic information (Fig. 3). The MEDUSA analysis identified a two-parameter single birth and death model as the best fitting model of ponyfish evolution (AIC = 105.5). The best fitting model chosen that incorporated a rate shift indicated an increase in diversification rate for the sexually dimorphic clade Gazzinae; however, this rate shift model was significantly worse (AIC = 108.19) than the best fit rate-constant (two parameter single birth and death) model. Also the best rate shift model did not satisfy the constraints of the AIC cut-off score of 4 necessary for choosing a rate-variable model over the rate-constant model. Further, no rate-variable models were chosen over the rate-constant model when the AIC cut-off score was relaxed to 1, 2 or 3.

Morphological disparity

The subfamily Gazzinae, the clade including all sexually dimorphic ponyfishes with regard to internal and external features of the LOS, is significantly ($P < 0.01$) more disparate than the combined members of *Aurigequula* and *Leiognathus* (i.e. Leiognathinae), which include all nonsexually dimorphic ponyfish species with

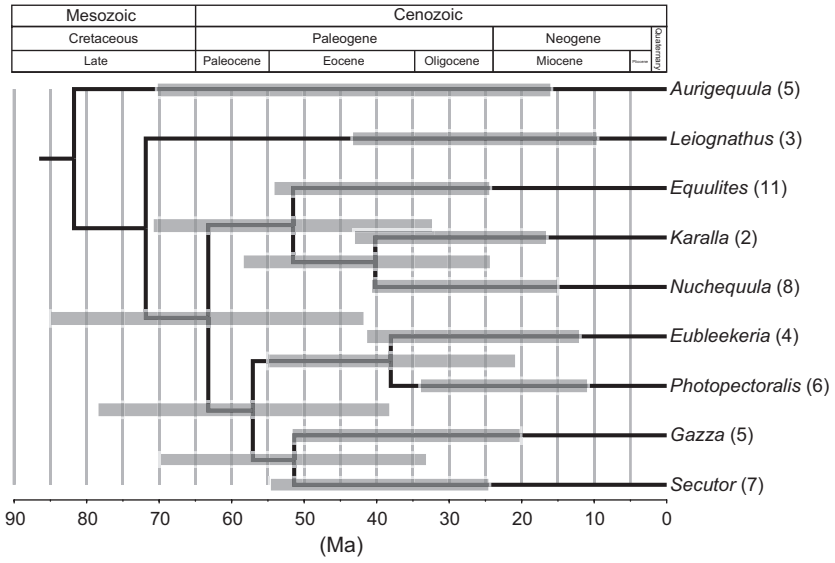

Fig. 3 Phylogeny of ponyfishes pruned to include only a single representative of a strongly supported monophyletic lineage (e.g. a genus) with combined taxonomic richness information. Numbers at terminals represent the known number of species within each lineage. Tree pruned from maximum clade credibility tree presented in Figure 2. Gray horizontal bars indicate 95% HPD.

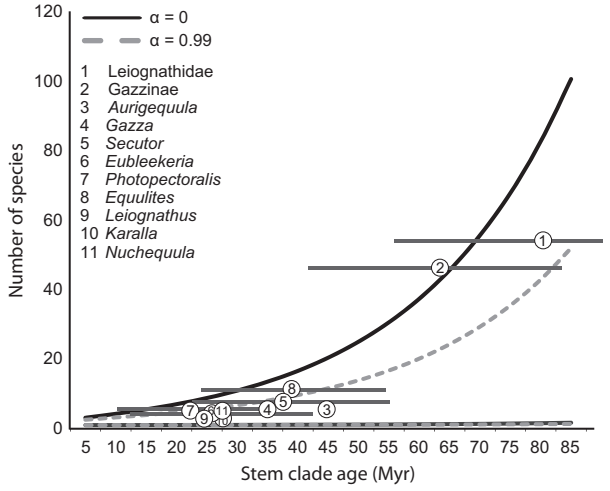

Fig. 4 Plot of clade age vs. extant species diversity of ponyfishes. Solid and dashed curves indicate the 95% confidence intervals regarding expected species richness through time, given an estimated net diversification rate (0.067) and a relative rate of extinction (0, 0.99). (Note: EPS values denoted with an alpha.) Circles represent the estimated mean clade age for each ponyfish lineage, with horizontal bars indicating the age range of the 95% higher posterior densities for lineages where the range extends outside a set of confidence intervals.

regard to LOS structure (Fig. 6). At the tribal level, the externally strongly sexually dimorphic tribe Equulitini is significantly more disparate than its nonexternally dimorphic sister group Nuchequulini. At the generic level, the externally sexually dimorphic *Photopectoralis* is significantly more disparate than its nonexternally dimorphic sister group *Eubleekeria*.

To gauge the level of morphological diversity of ponyfishes relative to other teleostean taxa, we compared our disparity analysis results to analogous studies involving cichlids and piranhas. Ponyfishes had a total morphological disparity of 0.006, which is significantly less than that of Rift Lake cichlids (Chakrabarty 2005) and considerably higher than that of adult piranhas (Zelditch *et al.* 2003).

Discussion

Ponyfish divergence times and evolutionary relationships

Our analyses indicate that Leiognathidae initially diverged during the Late Cretaceous and that all of the extant genera were established during the Paleogene. The topology recovered in this analysis represents a significant advance over previous attempts to generate a taxonomically comprehensive family level phylogeny (e.g. Sparks *et al.* 2005), with 38 of 45 described species, as well as several putative new species included. Many taxonomic changes have been affected since the publication of that earlier phylogeny, primarily on the basis of apomorphic morphological features of the LOS (Chakrabarty & Sparks 2007, 2008; Sparks & Chakrabarty 2007; Kimura *et al.* 2008a,b; Chakrabarty *et al.* 2009, 2010a,b, 2011). The vast majority of the taxonomic changes proposed in recent papers are congruent with the phylogenetic pattern recovered in this work.

Table 3 Likelihood-based model-fit test for rate-constant and rate-variable models on ponyfish pruned to species tree (T1, Fig. 2). Model names as written and implemented in LASER (Rabosky 2006a) are indicated in parentheses. The best rate-constant and rate-variable models are shown in boldface type

Model	Rate category	Akaike information criterion (AIC)
Yule (Pure birth)	**Constant**	**129.54**
Birth-death (bd)	Constant	131.54
Yule with 2 rates (Yule-2-rate)	**Variable**	**122.39**
Density-dependent logistic (DDL)	Variable	122.41
Density-dependent exponential (DDX)	Variable	127.70
	ΔAIC ($AIC_{RC} - AIC_{RV}$)	7.15
	P-value (based on simulated ΔAIC)	**0.006**

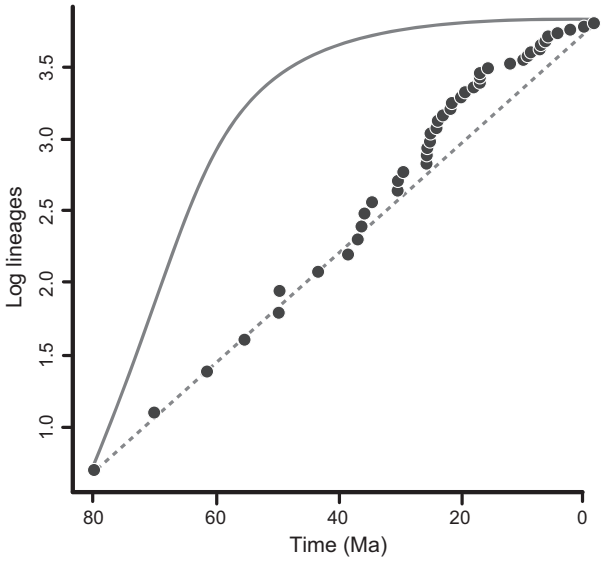

Fig. 5 Log lineage through time plot for ponyfishes (black circles). Curved solid line represents a commonly observed pattern associated with density-dependent speciation, whereas dashed line represents a pattern of continuous diversification through time.

Tempo and mode of ponyfish diversification

Overall, taxonomic richness is greater in sexually dimorphic ponyfish lineages than in those lineages lacking sexually dimorphic LOSs (Fig. 4); however, this result is dependent on combinations of potential divergence times and relative rates of extinction. The subfamily Gazzinae was shown to have greater than expected species richness under the assumption of high relative rates of extinction. Gazzinae may also exhibit significantly greater species richness under low rates of relative extinction depending on the range of estimated divergence ages recovered for this clade (Fig. 4 and Table 2). Specifically, species richness is significant regardless of extinction rate if Gazzinae is younger than 64 Ma. If Gazzinae is older than 64 Ma, then exceptional species richness significance depends on the rate of extinction being moderate to high. In contrast, the nonsexually dimorphic genera *Aurigequula* and *Leiognathus* were shown not to have greater than expected species richness given estimated clade age under any rate of relative extinction and across the range of estimated divergence times. These results indicate that diversification rates in Leiognathidae are higher in sexually dimorphic than nondimorphic taxa, with four additional genera (*Gazza*, *Photopectoralis*, *Nuchequula*, and *Equulites*) exhibiting greater than expected species richness given estimated age ranges and relative rates of extinction. Our taxonomic richness analysis suggests that sexually dimorphic lineages exhibit greater species richness over time; however, we caution that for most genera such a result is dependent on combinations of potential divergence times and extinction rates. In contrast, Rabosky *et al.* (2007) observed an unambiguous greater than expected species richness given time and extinction rates in arid-adapted lineages of sphenomorphine skinks, suggesting that diversification rates in those lineages were elevated in relation to nonarid-adapted lineages. A potential connection between sexual selection acting on the LOS and increased species richness is ambiguous in the sexually dimorphic ponyfishes because their greater than expected species richness is so highly dependent on combinations of potential divergence times and rates of extinction. A connection between sexual selection and increased rates of diversification in sexually dimorphic ponyfishes would have stronger support if the species richness was greater than expected given time in sexually dimorphic lineages regardless of divergence time and rates or extinction.

Likewise, we do not recover compelling evidence for any significant shifts in diversification rate within ponyfishes. The MEDUSA analysis did not recover a rate shift for any specific lineage with any statistical significance. This suggests that neither evolution of the LOS nor speciation as a result of sexual selection caused increases in diversification rates within ponyfishes. Furthermore, our results show that the evolutionary history of ponyfishes is not punctuated by any robust increase or decrease in diversification rate. Notably the sexually dimorphic clade Gazzinae did show exceptional

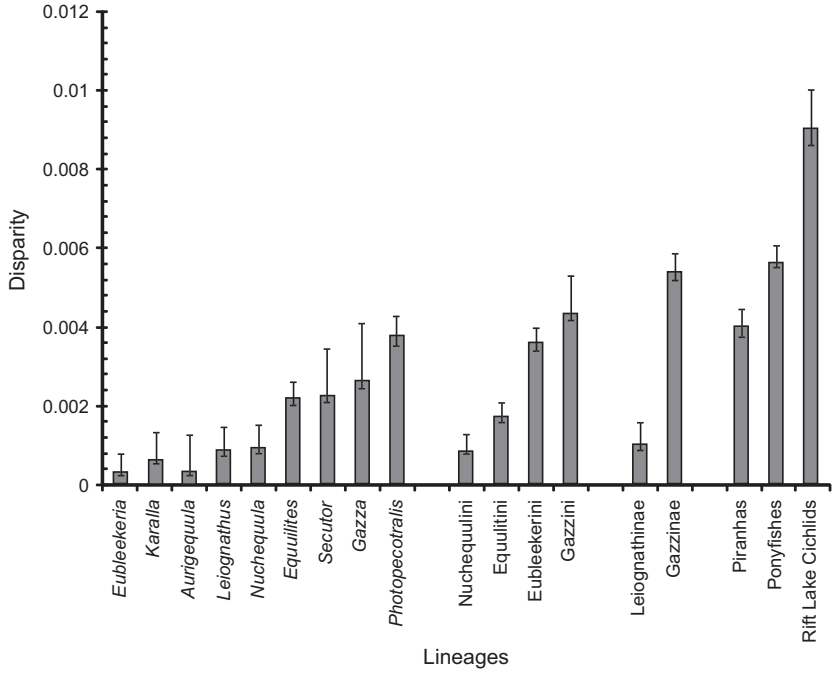

Fig. 6 Results of the morphological disparity analysis showing the comparison of ponyfish genera (*Eubleekeria* to *Photopectoralis* on left of plot), tribes (Aurigequulini to Gazzini, centre of plot), nondimorphic and sexually dimorphic lineages (Leiognathinae and Gazzinae, center right of plot), and a comparison of ponyfishes to other fish clades for which comparable data are available. Error bars reflect 95% confidence intervals. Nonoverlap between confidence intervals among groups reflects significant differences in morphological disparity at the level $P > 0.05$.

taxonomic richness independent of extinction rate, and it also was recovered in one model as having a significant rate increase relative to the nonsexually dimorphic ponyfish lineages. However, these results were recovered under a rate-variable model, which did not provide a better fit than a rate-constant model. Therefore, we cannot conclude that Gazzinae has undergone a significant increase in diversification rate.

Potential reproductive isolating mechanisms for marine species with pelagic larvae are difficult to demonstrate (Taylor & Hellberg 2005). In the case of the sexually dimorphic ponyfishes, anatomical changes in the LOS and correlated changes in female preference are potential candidates for reproductive isolation. Sexual selection as an isolating mechanism could account for continuous diversification through time, regardless of niche space or habitat size and other environmental factors. The recovered pattern of lineage accumulation through time for ponyfishes indicates that leiognathids have continued to diversify throughout their evolutionary history (Fig. 5). This is in stark contrast to the commonly observed lineage accumulation curve associated with density-dependent speciation, a pattern recovered in many recent diversification studies of various taxonomic groups (e.g. Price 2008; Rabosky & Lovette 2008), including other marine fish lineages (Ruber & Zardoya 2005). The lack of a decrease in lineage accumulation over time suggests that ponyfishes are capable of continuous diversification notwithstanding intrinsic factors that may limit genetic isolation in marine fauna (e.g. pelagic larvae, ocean currents, homogeneous environments, lack of potential barriers to dispersal, broad distributions) and that sexual selection acting on the LOS is a potential mechanism of speciation that has contributed to their continual diversification through time.

Morphological disparity in ponyfishes

Our results indicate that Gazzinae, which comprises all ponyfish species with a sexually dimorphic LOS, is both significantly more disparate in body shape and has a higher disparity rate than the nonsexually dimorphic members of Leiognathidae, *Aurigequula* and *Leiognathus* (Leiognathinae, Fig. 6). Further, leiognathid clades that are sexually dimorphic for external features of the LOS (e.g. translucent buccal, gular, opercular, or flank patches in males) are significantly more disparate than their nonexternally sexually dimorphic sister clades. These results suggest that mechanisms other than sexual selection are influencing the external morphological diversity of leiognathids. The high degree of morphological disparity within Gazzini, for example, may

suggest that ecology and habitat are important factors influencing morphological diversification within this clade, as exemplified by the evolution of two very different dietary and feeding mechanisms, piscivory (*Gazza*) and surface feeding (*Secutor*), in members of Gazzini (Sparks *et al.* 2005; Chakrabarty *et al.* 2009). When compared to similar disparity studies of cichlids (Chakrabarty 2005), ponyfishes exhibited low overall external morphological disparity in regard to body shape (Fig. 6). Further, as discussed earlier, our disparity results within Leiognathidae do not support the hypothesis that sexual selection is driving diversification within ponyfishes.

Conclusions and future directions

Overall, our study indicates that there is no conclusive evidence that sexual selection mechanisms have influenced any significant increases or decreases in the rates of diversification in this group. As discussed earlier, for lineages that are sexually dimorphic for the LOS, no unambiguous correlation was recovered suggesting greater than expected species richness given time, nor were any significant increases in diversification rate detected. This is in contrast to studies that have documented greater species richness in lineages exhibiting sexual selection mechanisms, such as taxa with promiscuous mating systems in birds (Mitra *et al.*, 1996), and floral nectar spurs in plant groups (Hodges & Arnold 1995). However, it is important to note that these studies tested for greater than expected species richness without incorporating temporal information regarding the age of their respective lineages. Accounting for time is necessary to accurately distinguish whether a significant greater species richness result is not simply an artefact of the age of the lineage, but rather the result of an elevated diversification rate.

The results from our disparity analysis are also in conflict with the data we would expect to observe if sexual selection is a driving factor in ponyfish diversification, as sexually dimorphic taxa are found to be more morphologically disparate with respect to body plan than nondimorphic taxa. This suggests that other factors relating to natural selection (e.g. ecology, feeding mechanisms and habitat) must figure significantly in driving morphological diversification within this clade.

The pattern of continuous lineage diversification through time recovered for Leiognathidae in this study suggests that ponyfishes have continued to diversify throughout their evolutionary history without any detectable slow downs in diversification rate. Sexual selection may potentially explain this uncommon diversification pattern. Sexual selection could be acting on the LOS to facilitate genetic isolating mechanisms that would allow for continual diversification in the presence of otherwise limiting factors. These factors limiting isolation include the fact that ponyfishes have pelagic larvae and are often found across a wide homogenous marine range in mixed species assemblages. Therefore, despite the fact that our results indicate that sexual selection is not the driving force behind ponyfish diversification, we cannot rule out the possibility that sexual selection may function in this system to provide genetic isolation that supplements other mechanisms of diversification (e.g. allopatry; see Ritchie 2007).

Ultimately, applying the tests performed here to other sexually dimorphic clades of bioluminescent fishes will provide for further examination of the roles of sexual selection and bioluminescence in clade diversification. Although sexually dimorphic luminescent systems are well documented in a number of diverse marine fish clades (e.g. Stomiiformes, Lophiiformes and Myctophiformes) and are hypothesized to occur in many others (Herring 2007), the impact of sexual selection on diversification rates in these clades remains entirely unexplored.

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Data accessibility

Concatenated and aligned data used in generating the phylogeny is deposited at Dryad: doi:10.5061/dryad.8987.