ABSTRACT

Aim Any discovery of new biogeographical pattern in landlocked animals may challenge hypotheses that account for the evolution of intercontinental faunas. In this study, we examined the evolutionary origin and historical biogeography of temperate, freshwater perch-like fishes within a molecular phylogenetic framework.

Location Temperate Eastern Eurasia, the Americas and Australia.

Methods Data from five nuclear markers obtained from 15 targeted species and a broad sampling of percomorphs were assembled to investigate their phylogeny and historical biogeography. We (1) applied a fossil-calibrated Bayesian reconstruction to provide a phylogenetic time-scale for the diversification of the taxa, (2) inferred the evolution of habitat preference through a tree-based character reconstruction method, and (3) reconstructed the evolution of range distributions using a dispersal–extinction–cladogenesis model.

Results We recovered a new antitropical freshwater clade, Percichthyoidea, composed of Siniperidae, Percichthyidae (including Percilia but excluding Macquaria colonorum), Elassomatidae and Centrarchidae. Our time-scale analysis indicates that the Percichthyoidea originated around the Cretaceous–Palaeogene transition. Ancestral habitat reconstructions revealed only one marine-to-freshwater transition that preceded the origin of the percichthyoids; the warm-to-temperate transition occurred earlier. Ancestral-area inference indicates a wide-ranging pan-American and Eastern Eurasian distribution of the most recent common ancestor of Percichthyoidea. The initial diversification event occurred between the southern (Percichthyidae) and northern (remaining percichthyoids) clades. A second divergence occurred between Eastern Eurasia (Siniperidae) and North America (Centrarchidae plus Elassomatidae). The trans-Pacific distribution of the Percichthyidae may have preceded the final breakup of south Gondwana.

Main conclusions The present-day distribution of the Percichthyoidea is unique within extant fishes, comprising four trans-Pacific continental blocks across the two hemispheres. Current geological and palaeoenvironmental reconstructions may well explain the longitudinal but not the latitudinal pattern of this freshwater fish clade, as it requires a transequatorial freshwater system connecting temperate regions of South and North America during the Late Cretaceous/Palaeogene. An alternative hypothesis is discussed, in which the ancestor of the Percichthyoidea was not completely adapted to freshwater.

Keywords Antitropical distribution, Centrarchidae, dispersal–extinction–cladogenesis, freshwater fish biogeography, historical biogeography, nuclear markers, Percichthyidae, Percomorpha, vicariance.
INTRODUCTION

Groups of freshwater fishes distributed across oceans are of particular interest to biogeographers because of their limited tolerance to salinity and the assumption of low dispersal capabilities across marine environments (Briggs, 1979). Disjunct intercontinental distributions of related freshwater fishes have been explained both by vicariance (e.g. land fragmentations through tectonics) and dispersal (e.g. land bridges emerging through eustatic sea-level variations). For example, the distributions of several groups of freshwater fishes in South America and Africa are hypothesized to be the direct consequence of the separation of these two continents during the Cretaceous Period (Lundberg, 1993; but see Chen et al., 2013), whereas the distributions of shared freshwater fishes between North America and Eastern Eurasia are better explained by dispersal through the periodically emerged Beringia land bridge during the Mesozoic/Cenozoic (Choudhury & Dick, 1998; Oaks, 2011).

Most transoceanic distributions of freshwater fishes are longitudinal and well documented (Berra, 2007). They mostly occur between South America and Africa [e.g. Cichlidae, Characiformes and Siluriformes; mostly tropical (Murray, 2001; Chen et al., 2013)], North America and Eurasia [paddlefishes (Grande & Bemis, 1991), sturgeons (Choudhury & Dick, 1998) and Catostomidae (Smith, 1992); temperate] or Australia, South America and South Africa [Galaxiidae (Waters & Burridge, 1999); temperate]. Any discovery of a new distribution pattern in groups of freshwater fishes may therefore challenge hypotheses that account for the evolution of intercontinental faunas (Lundberg et al., 2007).

Here, we address the phylogenetic positions and biogeographical patterns of temperate freshwater fishes of the largest extant group of teleost fishes, Percomorpha. It includes about 245 families (Eschmeyer & Fong, 2011; Eschmeyer, 2013) and is deeply nested within the phylogeny of Teleostei (Chen & Mayden, 2010). Most percomorpha families live predominantly in tropical marine environments. Only about 23 families include exclusively or predominantly freshwater species, and only nine of them include exclusively or predominantly temperate freshwater species (Nelson, 2006; Berra, 2007).

![Figure 1 Present-day geographical distribution of the four families of temperate perch-like fishes (Percichthyoidea) examined: Sinipecidae, Centrarchidae, Percichthyidae and Elassomatidae. Distributions follow Berra (2007).](image-url)
new avenues for discussion and research about the biogeographical history of the Percomorpha.

MATERIALS AND METHODS

Taxonomic sampling

We examine five Australian percichthyid species from five different genera, two South American percichthyid species from the genera Percichthus and Percilia, two species of Sini-
percidae from the genera Siniperca and Coropercra, four species of Centrarchidae and two species of Elassomatidae (Ellassoma), as well as a broad sampling of 55 taxa representing the main lineages of the Percomorpha recently identified in molecular analyses (Chen et al., 2003, 2007; Miya et al., 2003; Dettai & Lecointre, 2005; Smith & Craig, 2007; Li et al., 2009; Wainwright et al., 2012). This sampling strategy allows us to: (1) test the monophyly of the four studied families; (2) search for their close relatives; and (3) provide calibration points for fossil-based dating reconstruction. We select two non-percomorph beryciform species, Beryx splen-
dens and Myripristis murdjan, to root the tree. The list of taxa examined in this study is given in Supporting Information (see Appendix S1 in the Supporting Information).

DNA data

DNA sequences were generated from five nuclear genes located on different chromosomes [recombination activation gene 1 (RAG1), rhodopsin (RH), and early growth response protein genes 1, 2B and 3 (EGR1, EGR2B and EGR3); Chen et al., 2008]. Some sequences used in this study were retrieved from GenBank or previously determined in Chen et al. (2013). Protocols for collecting new DNA sequences follow those outlined in Chen et al. (2008). The complete list of primers used in this study is given in Appendix S1. The newly obtained sequences have been deposited in the NCBI GenBank database (see Appendix S1 for accession numbers).

Phylogenetic analysis and divergence time estimation

DNA sequences were edited using CodonCode Aligner 4.0.4 (CodonCode Corporation, Centerville, MA, USA) and Se-Al 2.0a11 (available at http://tree.bio.ed.ac.uk/software/seal/). They were initially aligned with the automatic multiple alignment program MUSCLE (Edgar, 2004) using the online server at http://www.ebi.ac.uk/Tools/msa/muscle/index.html. These were then adjusted manually based on the inferred amino-acid translations. Regions containing large insertion/deletion segments (e.g. tandem repeats in EGR genes), showing high dissimilarity in sequence length, which may result in invalid assertions of homology, were discarded from phylogenetic analyses. The total data matrix included 72 taxa and 5001 nucleotide positions. Maximum-likelihood phylogenetic analyses were conducted on two data matrices and two different partitioning schemes. The first matrix, named ‘123’, includes all first, second and third codon positions (5001 bp). The second matrix, named ‘123 no indel’, excluded all insertion and deletion positions (indels) to minimize the impact of missing or ‘gap’ data (4386 bp), because missing data may mislead phylogenetic reconstruction (Lemmon et al., 2009; Wiens & Morrill, 2011). The two different partitioning schemes were: (1) each codon position (across all genes) was assigned to a partition (three partitions in total); (2) for each of the five individual genes, each codon position was assigned to a partition (15 partitions).

Phylogenetic analyses were performed using the partitioned maximum-likelihood (ML) method as implemented in RAXML-HPC (Stamatakis, 2006), through its graphical interface RAxMLGUI 0.93 (Silvestro & Michalak, 2012). Heuristic searches were conducted under mixed models of sequence evolution, which allows individual model parameters of nucleotide substitutions to be estimated independently for each partition in an analysis. As recommended in the manual of RAXML-HPC (Stamatakis, 2006), a GTR+Γ model (with four discrete rate categories) for each partition was used without incorporating invariant sites. A thorough ML tree search was conducted across 100 distinct runs. The optimal tree was determined by comparison of the likelihood scores among the suboptimal trees obtained per run. To evaluate the robustness of the internal branches of the ML tree, 1000 bootstrap replications (MLBS) were calculated for each data set under the GTR+Γ model.

The partitioned Bayesian method, as implemented in BEAST 1.7.4, incorporating a relaxed molecular clock calibrated with a set of fossils, was used to simultaneously infer phylogenetic relationships and divergence times (Drummond et al., 2012). Given the highly similar ML phylogenetic trees when using previous datasets and data partitioning schemes, we analysed only the smallest dataset (‘123 no indel’), partitioned according to codon positions (three partitions) to save computation time. We chose a GTR+Γ model of sequence evolution for each partition, and unlinked the substitution model, rate-heterogeneity model and base frequencies across partitions.

The fossil record is essential for calibrating molecular-based time estimation, because it offers direct evidence for the presence of a taxon at a defined time in the past – its strict minimum age. Its maximum age needs to be estimated, and this estimation depends on the observer’s perception of the quality of the fossil record: the better the quality of the fossil record, the more precise the estimate of its maximum age is. Our phylogenetic tree was time-calibrated with a series of seven percomorph fossils that provide hard minimum ages and soft maximum age limits through an exponential distribution in which the 95% upper credibility limit was equal to the maximum age of the stratum where the fossil was excavated. †Eolates gracilis is the first fossil assigned to the family Latidae of Lower Eocene age (Ypresian, 48.6–55.8 Ma); we use this fossil to constrain the time to the most recent common ances-
tor (TMRCA) of the clade Centropomus + Lates (Otero, 2004). †Proluvarus necopinatus, is the first fossil assigned to the family
Luvaridae, from the Lower Eocene (Ypresian, 48.6–55.8 Ma); we use this fossil to constrain the TMRCA of the clade Priomusrus + Luvarus (Bannikov & Tyler, 1995; Klanten et al., 2004). †Eophryn e barbutii is the first articulated fossil of the family Antennariidae, from the early Eocene or late Ypresian (47.8–53.0 Ma); it is used to calibrate the TMRCA of the clade Lophius + Anthennarius (Carnevale & Pietsch, 2009). †Archaeotetraodon winterbottomi is the first tetraodontid fossil known from the Oligocene and its age has been estimated at 32–34 Ma; this fossil is used to calibrate the TMRCA of the clade Takifugu + Tetraodon (Carnevale & Tyler, 2010). †Moclaybalistes danekrus is a stem balistoid from the Palaeocene (at 59 Ma); we use this fossil to calibrate the TMRCA of the clade Triacanthodes + Balistes (Santini & Tyler, 2003). The oldest Micropterus fossil is represented by a damaged articular bone from the Toledo Bend site in Texas, dated to the early Miocene of approximately 23.0 Ma; we use this species to calibrate the TMRCA of the clade Micropterus + Lepomis (Albright, 1994). The first fossils of Sparidae are known as early as the Palaeocene (i.e. as early as 65.5 Ma) in Europe and North Africa (Orrell et al., 2002); the first fossils of Moronidae are dated to about 50 Ma (Williams et al., 2012). We therefore used a minimum age of 65.5 Ma to calibrate the TMRCA of the clade ((Morone, Dicentrarchus), Sparus).

Following Santini et al. (2009), we constrained the minimum age of the crown group Beryciformes (Beryx and Myripristis) to 99 Ma (Late Cretaceous) by using the oldest crown group beryciform fossils (such as Hoplopteryx and Trachichthyoides) from the Cenomanian. The maximum age of the root of the tree was constrained to the age of the first acanthomorph otolith fossils (about 125 Ma; i.e. the age of the clade Beryciformes + Acanthomorpha).

Habitat evolution reconstruction

We independently reconstructed the evolution (ancestral condition at each node) of the salinity [i.e. marine, euryhaline (including brackish species) and freshwater] and water temperature preference [i.e. tropical (> 25 °C) and temperate (< 25 °C)] on the BEAST time-calibrated maximum clade credibility tree using the ‘MkI’ model, as implemented in Mesquite 2.72 (Maddison & Maddison, 2009). Salinity and water temperature preferences, along with the distributions of families closely related to the temperate freshwater perches, were compiled from Nelson (2006) and Berra (2007).

Ancestral-range reconstruction

The model-based dispersal-extinction–cladogenesis (DEC) likelihood method of Lagrange (Ree et al., 2005; Ree & Smith, 2008) was used to reconstruct ancestral geographical ranges at tree nodes. The effects of different dispersal rates and area combinations in Lagrange were explored – all rates of dispersal among areas equal to 1, or marine dispersal among areas impossible (rates of 0), and all area combinations included (i.e. 15 combinations possible), or excluding biologically irrelevant combinations, such as Australia plus North America (i.e. 10 combinations possible). We selected the default options of the other parameters; in particular, the total number of ancestral areas was not constrained (up to four).

RESULTS

Phylogenetics

Our first data matrix includes a total of 5001 aligned positions for the exon regions of the five nuclear genes in 72 taxa. The second matrix, in which all indel positions were deleted, includes a total of 4386 positions. These two matrices contained 2485 and 2178 variable sites, respectively, and 1996 and 1790 of those sites, respectively, were parsimony informative. Regardless of the matrix and the partition schemes considered, all analyses yielded mostly identical and strongly supported topologies for recovering the previously resolved main percomorph lineages (i.e. clades F, L and Q, etc.) (Fig. 2; see also Appendix S2). The monophyly of a group composed of the Percichthyoidea (minus Macpauria colonorum), Siniperi dae, Elassomatidae and Centrarchidae is strongly supported (MLBS > 89%). Within this clade (named Percichthyoidae), the last three families form a well-supported monophyletic group (MLBS > 92%); Eurasian Siniperi dae are sister to the North American clade of Elassomatidae + Centrarchidae. The marine Howella and Lateolabrax (Asian sea-perches), both sometimes recognized as members of the Percichthyoidea (Nelson, 2006), were not found to be closely related to the freshwater Australian and South American percichthyids. Instead, Howella and Lateolabrax form a clade related to other marine perches such as Acropomatidae and Symphysanodontidae (Fig. 2). The phylogenetic position of the brackish Australian percichthyid Macpauria colonorum is unexpected, as it is not closely related to the freshwater percichthyids. The South American percichthyid genus Percilia is the sister group of a second South American percichthyid genus, Percichthys, and both are nested within the Australian percichthyids.

The Percichthyoidea and the Cirrhitoidea (Perciformes: Percoidei) form a monophyletic group (MLBS > 96%) that is then the sister group of a clade including the families Kyphosidae, Oplegnathidae, Teraponidae and Kuhlidae (MLBS > 83%) (Fig. 2).

Divergence times

Our Bayesian molecular time-tree calibrated with a set of seven fossils (Fig. 3) provides an age for the origin of the Percichthyoidea of 61.1 Ma [95% credible interval (CI): 47.0–75.9 Ma]. The age of the split leading to the separation of percichthyid taxa from their extant sister-group, Cirrhitoidea, was estimated at 65.9 Ma (CI: 51.2–81.2 Ma). Within the percichthyoid clade, the respective ages of the crown groups are: Percichthyidae, 45.5 Ma (CI: 30.0–61.5 Ma); Siniperidae, 30.8 Ma (CI: 11.5–50.4 Ma); North American Elassomatidae + Centrarchidae clade, 42.8 Ma (CI: 31.4–55.7 Ma).
Evolution of salinity and temperature preference

Our reconstructions of habitat and character evolution (Fig. 4a,b) show that the transition from marine to freshwater environments and the transition from tropical to temperate environments were not synchronized within the ‘centrarchiform’ clade. The marine/freshwater transition probably occurred between the stem origin (divergence with the Cirrhitoida) and crown origin (most recent common ancestor) of the percichthyoid clade, whereas the tropical/temperate transition predated the initial diversification of the ‘centrarchiform’ clade.

Ancestral areas

Our two ancestral-area reconstruction analyses (differing from each other in the constraints applied to the relative dispersal rates and range possibilities) provide similar hypotheses for the distribution of the most recent ancestor of each of the three clades (Centrarchidae, Elassomatidae), ((Centrarchidae, Elassomatidae), Sinipercaidae), and ((Percichthyidae, Percilia), (Nannatherina, Nannoperca)) (Fig. 5). However, the results of the analyses suggest two different hypotheses for the ancestral area of the most recent common ancestor of the percichthyoid clade.
Figure 3 Phylogenetic chronogram of the Teleostei based on a Bayesian relaxed clock approach (using \textit{beast} 1.7.4), using the matrix ‘123 no indel’ partitioned in three, and calibrated with seven fossil-based constraints following exponential distributions (see text for details). The maximum age for the root is constrained to 125 Ma and the minimum age of the crown group (\textit{Beryx splendens} and \textit{Myripristis mordax}) is constrained to 99 Ma. Horizontal time-scale is in million years before present (Ma). Black horizontal bars indicate calibration constraints on the corresponding nodes; grey horizontal bars at nodes are 95% age credibility intervals. Numbers given in parentheses at nodes are the Bayesian posterior probabilities if below 1. Arrowheads indicate the origins of the ‘Centrarchiformes’ and the origin of the Percichthyoidae. Estuarine and marine ‘percichthyids’ are highlighted in bold. Centr., Centrarchidae; Elass., Elassomatidae; Sinip., Siniperidae; Perci., Percichthyidae.
Figure 4 Reconstructions of the evolution of salinity preference (a) and temperature preference (b) within the 'centrarchiform' clade, using likelihood optimization on the Bayesian time-tree topology (Fig. 3). In both reconstructions (a, b), the relative probabilities of each state (sum = 1) are drawn using pie charts at each node. Salinity preference: black, freshwater; grey, euryhaline; white, marine. Temperature preference: black, temperate; white, tropical.

Figure 5 Two ancestral area reconstructions within the temperate freshwater perch-like clade (Percichthyoidea) onto the Bayesian time-tree displayed in Fig. 3, in which all non-percichthyoids have been deleted, using the dispersal–extinction–cladogenesis method as implemented in the software Lagrange (Ree et al., 2005). The most likely ancestral areas are drawn at each node. Each ancestral-area combination is made up of the ranges of its two descendant (daughter) lineages. For example, in the first reconstruction, the inferred ancestral area of the most recent common ancestor of the Percichthyoidea is made of Eurasia, North America plus Australia. The four area units are: North America (N.Am., code colour: yellow); Eastern Eurasia (Eura., green); Australia (Austr., blue); South America (S.Am., red). Vicariance events are indicated with black arrowheads; dispersal events are indicated with stars.
group and of the most recent common ancestor of the Percichthyidae. Notwithstanding the inferred salinity preference evolution (see above), we first consider all intercontinental dispersals to be equiprobable, including transoceanic dispersals (reconstruction 1, Fig. 5). In this reconstruction, the inferred ancestral range of the most recent common ancestor of Percichthyoidea covers North America, Eurasia and Australia. This biogeographical area seems unlikely, because no extant or extinct group of freshwater fishes has such a distribution. When some biogeographically implausible ranges (including the previous range) were excluded and we further imposed limits on dispersals, excluding long-distance transoceanic dispersals following our inferences of the evolution of ecological preference (Fig. 4a), the most recent common ancestor of the Percichthyoidea was distributed in South America, North America and Eastern Eurasia (reconstruction 2, Fig. 5). Each of the two reconstructions (1 and 2, Fig. 5) necessitates one dispersal event between South America and Australia to explain the distribution of the Percichthyidae, but with different timings.

**DISCUSSION**

**A new clade of temperate freshwater fishes**

An exclusive relationship gathering the Sinipercaidae, Elasmommatidae, Centrarchidae and Percichthyidae *sensu* Johnson (1984), minus *Macquaria colonorum*, has never previously been proposed. This clade, named Percichthyoidea, contains only temperate freshwater species and exhibits a unique pattern of distribution among living fishes (Fig. 1). The Percichthyoidea is not closely related to the temperate freshwater perch family Percidae, which belongs to clade X (Fig. 2). Our phylogenetic analyses also corroborate Percichthyoidea as part of the larger clade including Cirrhitoidae, Oplegnathidae, Teraponidae, Kuhlidae and Kyphosidae. The content of this clade is similar to the ‘centrarchiform’ clade hypothesized by Near *et al.* (2012, 2013) (but see Betancur-R. *et al.*, 2013), comprising also the family Enoplosidae, not examined here; the relationships within Percichthyoidea are, however, notably different between these studies.

Using partly overlapping datasets of several nuclear genes, neither Near *et al.* (2012, 2013) nor Betancur-R. *et al.* (2013) recovered a monophyletic Percichthyoidea relative to Cirrhitidae, Cheilodactylidae and Enoplosidae. To assess the reasons for such phylogenetic discordance between our study and these previous studies, we first employed the hypothesis tests for alternative topologies using the likelihood-based approximately unbiased (AU) test (Shimodaira, 2002) based on the most recent and the most complete dataset of Near *et al.* (2013) and on our own dataset. We found that the constrained tree topology in which the monophyly of the Percichthyoidea is enforced is not statistically rejected (AU test, $P_0 = 0.184$) based on the dataset of Near *et al.* (2013), whereas the constrained tree topology of the non-monophyletic Percichthyoidea, *sensu* Near *et al.* (2013), is rejected using our own dataset (AU test, $P_0 = 0.032$). Furthermore, simultaneous analyses combining our five nuclear genes with 14 nuclear genes of Near *et al.* (2012, 2013) and Betancur-R. *et al.* (2013) for individual gene sequences of the common taxa among the studies produced ML trees in which the Percichthyoidea is monophyletic (see Appendix S3). These results together suggest that the nuclear genes used herein provide sufficient phylogenetic signal to resolve the positions of Percichthyidae and the clade (Sinipercaidae, Centrarchidae and Elasmommatidae) relative to Cirrhitidae, Cheilodactylidae and Enoplosidae, and more signal than the markers used by Near *et al.* (2012, 2013) and Betancur-R. *et al.* (2013).

The monophyletic status of each of the families Centrarchidae, Elasmommatidae and Sinipercaidae is well documented (Roberts, 1993; Near *et al.*, 2012), whereas the content of the Percichthyidae remains controversial (Johnson, 1984; Arratia, 2003; Eschmeyer, 2013). Johnson (1984) restricted Percichthyidae to only the South American and Australian temperate freshwater/euryhaline perch-like fishes, based on morphological evidence, thereby excluding all other marine and freshwater taxa that were previously added to this family by Gosline (1966). Our molecular study and other studies (Smith & Craig, 2007; Near *et al.*, 2012, 2013; Betancur-R. *et al.*, 2013) confirm that none of the marine and freshwater taxa excluded by Johnson (1984) is closely related to any freshwater/euryhaline percichthyids. None of Smith & Craig (2007), Near *et al.* (2012, 2013) and Betancur-R. *et al.* (2013), however, found the remaining freshwater/euryhaline Percichthyidae to be monophyletic. Smith & Craig (2007) found *Bostokia porosa* and the brackish-water-adapted *Macquaria novemaculeata* to be only distantly related to the two other Australian percichthyids examined, while Near *et al.* (2012, 2013) and Betancur-R. *et al.* (2013) found *Macquaria novemaculeata* and its sister species *Macquaria colonorum* (both classified in *Percalates* in Johnson, 1984) to be distantly related to other South American and Australian freshwater percichthyids. We also found *Macquaria colonorum* as a separate and distant lineage from the freshwater percichthyids (Fig. 2). The phylogenetic position of the freshwater *Bostokia porosa* warrants further investigation, although Jerry *et al.* (2001) found this species to be related to the genera *Nannoperca* and *Nannatherina*.

**Percichthyoidea exhibits a unique pattern of distribution**

The geographical distribution of the extant percichthyoid clade is unique among freshwater fishes as it encompasses the four main temperate regions surrounding the Pacific Ocean (excluding New Zealand): south-western and south-eastern Australia, southern South America, eastern Eurasia and North America (Fig. 1). The distribution of this group of fishes combines two general biogeographical patterns: the longitudinal trans-Pacific and latitudinal antitropical patterns. An antitropical distribution is a common pattern in temperate marine (e.g. Cheilodactylidae) (Burr ridge, 2002) and anadromous fishes (e.g. lampreys, Osmeridae) (Ilves & Taylor, 2009;
Renaud, 2011), but this pattern has not previously been reported for any strictly freshwater fish lineage.

The noteworthy pattern of the percichthyoid clade relates also to its longitudinal distribution across four continents. Unlike antitropicality, such a distribution pattern is rather common in temperate freshwater fishes (Donoghue & Smith, 2004). For example, sturgeons (Acipenseridae), perches (Percidae), salmonoids and relatives (Salmonidae), and pikes (Esocidae) all have distributions covering most of North America and Eurasia (Berra, 2007). Paddlefishes (Polyodontidae) and suckers (Catostomidae) are known from North America and only Eastern Eurasia, both from fossils and extant species (Smith, 1992; Choudhury & Dick, 1998). The Galaxiidae (mostly freshwater) occurs in South Africa, South America, Australia and New Zealand (Waters et al., 2000; Nelson, 2006).

**Origin of Percichthyoidea: freshwater or marine?**

The first proposed biogeographical scenario for the origin of the Percichthyoidea (‘freshwater origin’) derives from our analyses based on phylogenetics, time estimation, and ancestral habitat and area reconstructions. The phylogenetic analyses support the monophyly of the Percichthyoidea. Our time-estimation analysis provides a relatively robust time-scale for the origin and diversification of the Percichthyoidea at 61.1 Ma (CI: 47.0–75.9 Ma) (Fig. 3). The inferred origin for the percichthyid fishes considerably post-dates the complete separation of Gondwana and Laurasia (dated to about 140 Ma), the separation of Gondwana into West Africa and India–Madagascar block) and East (South America, Antarctica–New Zealand and Australia–New Guinea) (dated to around 100 Ma), and the final separation of New Zealand from Antarctica (dated to around 85 Ma) (Smith et al., 1994). The ancestral-habitat reconstruction supports the hypothesis that the most recent common ancestor of the Percichthyoidea was adapted to temperate freshwater environments. The ancestral-range reconstruction (Fig. 5: reconstruction 2) establishes that the area occupied by the most recent common ancestor of percichthyoids included North America, South America and Eurasia.

Based on these analytical findings, the most recent common ancestor of the Percichthyoidea appears to have been a temperate and freshwater taxon, and was distributed in North America, South America and Eastern Eurasia around the Cretaceous–Palaeogene (K/Pg) transition. This is a challenging scenario, because both current geological and palaeoclimatic reconstructions reject the possibility of a pan-American temperate freshwater habit crossing the equator at that time. Near the K/Pg transition, geological reconstructions show the presence of a proto-Antillean island chain surrounded by shallow seas separating South and North America (reviewed in Hedges, 2006). No primary freshwater fishes – those intolerant to saltwater – are known to have occurred in these islands, and crossing even short marine distances may have proven impossible for restricted freshwater fishes (Sparks & Smith, 2005) (but see below). Interestingly, however, some primary freshwater fishes (e.g. Characiformes and Siluriformes) and some terrestrial vertebrates (e.g. lizards, mammals and dinosaurs) crossed this region around that period and expanded their ranges across the two continents (Cifelli & Eaton, 1987; Nydam, 2002; Hedges, 2006; Newbrey et al., 2009; Chen et al., 2013). The proto-Antillean island arc was not a permanent connection, as shown by the marine faunal similarity between the East Pacific and the West Atlantic (Iturralde-Vinent & MacPhee, 1999), but unidentified episodic island chains between South and North America may explain these biotic exchanges. Such island chain connections have not yet been documented from geological data (White, 1986; Newbrey et al., 2009).

The second difficulty we face in this explanation for the distribution of the ancestor of the Percichthyoidea is that around the K/Pg transition, the global climate, although cooler than during most of the preceding Cretaceous period, may still have been too warm to support a temperate corridor connecting both hemispheres (Scotese et al., 1999). Palaeoclimatology also shows, however, that climatic conditions were unstable during that period, with some episodes of climatic change (Jenkyns, 2003). Biogeography echoes these climatic data, as it shows that, despite an overall warm climate around the K/Pg transition, transequatorial (marine) dispersals of temperate organisms were possible (White, 1986; Burridge, 2002; Waters et al., 2002).

The second scenario for the origin of the Percichthyoidea is more in line with the prevailing geological context at the end of the Cretaceous, but is less compatible with our analytical results. In this marine-origin scenario, the ancestor of the Percichthyoidea was not completely adapted to the freshwater environment. According to this hypothesis, there is no need for a strictly freshwater route between South and North America, and the distribution of the ancestor of the Percichthyoidea would have encompassed the coastal marine areas of South and North America and the region in between. This scenario requires two late complete transitions from marine to freshwater environments in South and North America, along with the extinction of related marine lineages. It is tempting to link these transition events with the late restrictions of epicontinental seas in North and South America (Haq et al., 1987; Harries, 2009) and the extinction events with the increase of sea temperature observed during the Palaeocene and culminating during the Eocene (Zachos et al., 2003; Friedman, 2010). Alternatively, a putative diadromous ancestor may have founded the antitropical groups, followed by the loss of the marine phase of its life history. The loss of marine phases in diadromous taxa is commonly observed across short timescales [e.g. galaxiid fishes becoming non-diadromous in lakes with histories of < 1 Myr old (Waters & Wallis, 2001); see also salmonids (Taylor et al., 1996), stickleback (Ort et al., 1994), etc.]. Thus, such a process could have occurred many times over the several million years of history of the Percichthyoidea.

**Biogeography of the northern clade**

Regardless of the ecological requirements of the ancestral Percichthyoidea, the most recent common ancestor of the
clade (Siniperidae, (Centrarchidae, Elassomatidae)) was probably temperate and freshwater, and occurred in Eurasia and North America during the Palaeocene (54.1 Ma; Fig. 5). At that time, a vicariance event separated the Eurasian Siniperidae lineage from the North American Centrarchidae + Elassomatidae lineage. The distribution pattern of the clade (Siniperidae, (Centrarchidae, Elassomatidae)), covering Eastern Eurasia and North America, has been frequently observed in other groups of freshwater organisms [e.g. paddlefish, catostomids, cyprinid genera Phoxinus (Eurasia) and Chrosomus (North America)]. During most of the Palaeocene, eastern Asia and western North America formed a continuous landmass called Beringia, and considerable evidence exists for biotic interchanges across this landmass from the end of the Cretaceous to the Palaeogene (Sanmartín et al., 2001; Lundberg et al., 2007; Krassilov et al., 2010; Oaks, 2011). Our results indicate that the submergence of the Beringia Bridge at the end of the Palaeocene period may have promoted the allopatric differentiation of the Siniperidae and the clade Elassomatidae + Centrarchidae.

Biogeography of the southern clade

Regarding the southern clade, the Percichthyidae appears to have first experienced a range expansion from South America to Australia between 61 and 45 Ma, at a time where South America and Australia were probably widely connected via Antarctica (Smith et al., 1994). Diversification events in Australia during the Eocene preceded a vicariance event separating the South American percichthyids (Percichthys and Percilia) from their Australian sister clade (Nannoperca and Nannathetina), near the end of Eocene, at about 36 Ma (CI: 21.6–51.4 Ma) (Fig. 3). We cannot reject the hypothesis that the later event was mediated by the complete split of the austral landmass, isolating Antarctica from other continents, that occurred 55–34 Ma (Smith et al., 1994; Lawver & Gahagan, 2003).

The fossil record of Percichthyidae supports the hypothesis that some members of the family were present in South America as early as the Palaeocene. The two oldest percichthyid fossils include †Percichthys lonquimayiensis and †Percichthys sandovali from the Upper Palaeocene (Thanetian, 55.8–58.7 Ma) (Arratia, 1982), but the classification of these two Palaeocene fossils in the genus Percichthys is incongruent with our more recent molecular-based age estimate of extant Percichthyidae. Their phylogenetic positions in relation to the extant percichthyids warrant further investigation and the result should provide significant evidence for the early evolution of the Percichthyidae. The Australian percichthyid fossils are much younger, known from the mid-Eocene, with †Macquaria antiquus, which may be closely related to catadromous Macquaria spp., and from the early Miocene, with †Maccullochella ‘macquariensis’ (see review in Unmack, 2001). Of potential interest is the discovery of two fossil scales in New Zealand freshwater fishes (Central Otago) of Miocene age (about 20 Ma), assigned to the family Percichthyidae (McDowall & Lee, 2005). Because New Zealand separated from Gondwana more than 80 Ma and the freshwater Percichthyidae is younger than 61 Myr, the presence of these two scales may be indicative of marine dispersal capacity in Percichthyidae and this would therefore challenge a strictly freshwater scenario. The evolutionary affinity of these New Zealand fossil taxa is, however, rather elusive (McDowall & Lee, 2005), and they may be related to the catadromous Macquaria spp. lineage. McDowall (2010, p. 334) concluded his chapter on the biogeography of New Zealand freshwater fishes by stating that ‘...the place of this putative New Zealand percichthyid is unlikely to ever be resolved with any clarity unless much more and better fossil material is discovered’.

CONCLUSIONS

We report a new clade of temperate freshwater perch-like fishes, comprising Percichthyidae, Siniperidae, Centrarchidae and Elassomatidae. This clade, Percichthyoidea, has a unique geographical distribution, combining most of the temperate areas surrounding the Pacific Ocean. To explain its antitropical distribution, it is necessary to postulate either the existence of a past pan-American equatorial freshwater and temperate environment never before hypothesized or, alternatively, the existence of a ‘marine’ temperate ancestor and two independent late marine-to-freshwater transitions, in North America and South America.

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**SUPPORTING INFORMATION**

Additional Supporting Information may be found in the online version of this article:

**Appendix 51** Taxa, genes and GenBank accession numbers for nuclear gene sequences of representative species, and a list of primers used in the study.

**Appendix 52** Highlights from maximum likelihood trees of percomorphs obtained from the alternative analyses (see Materials and Methods) depicting the evolutionary relationships of ‘centrarchiform’ fishes.

**Appendix 53** Simultaneous analysis in combining 19 nuclear genes; 15,060 characters and 22 taxa resulting in maximum-likelihood trees, well matched with the monophyly of the Percichthyoidea.

**BIOSKETCH**

Wei-Jen Chen is an associate professor at the Institute of Oceanography, National Taiwan University, and is interested in marine biology and evolutionary biology. The focus of the research team is on biodiversity, biogeography, integrated genomics, natural history, and mechanisms of evolution of ray-finned fishes.

Author contributions: W.-J.C. led the project and conducted the sample and data collection; W.-J.C. and S.L. conceived the ideas, analysed the data, and wrote the paper; L.B.B. and R.L.M. provided samples and contributed to the writing.

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