Molecular Phylogenetics and Evolution 84 (2015) 158–165

Contents lists available at ScienceDirect

Molecular Phylogenetics and Evolution

journal homepage: www.elsevier.com/locate/ympev

Phylogenetic relationships of *Goneaperca* and the evolution of parental care in darters (Teleostei: Percidae)

Richard C. Harrington [a,*], Thomas J. Near [b]

[a] Department of Earth Sciences, University of Oxford, Oxford, UK
[b] Department of Ecology and Evolutionary Biology and Peabody Museum of Natural History, Yale University, New Haven, CT 06520, USA

ARTICLE INFO

Article history:
Received 8 April 2014
Revised 23 September 2014
Accepted 8 January 2015
Available online 20 January 2015

Keywords:
Species tree
Phylogeny
Gene tree discordance
Etheostoma
Catonotus
Darters

ABSTRACT

Inference of evolutionary relationships among closely related darter species (Teleostei: Percidae) has traditionally proven challenging due to a lack of sufficient numbers of informative morphological characters or reliance on mtDNA sequences. These factors have contributed to longstanding uncertainty of the monophyly of many described taxonomic groups. Although multi-locus data are now available for most darter species, uncertainty has persisted regarding the relationships of some major lineages. Here, we investigate the relationships of darters classified in *Goneaperca*, a clade of 46 species, many of which are characterized by distinct nuptial displays and male-only parental care. Previous phylogenetic analyses of morphological and molecular data have failed to provide strong resolution of relationships among major *Goneaperca* subclades, and especially the monophyly of *Catonotus*. We apply coalescent and phylogenetic analyses to a dataset that includes intraspecific sampling for nearly all species of *Goneaperca* for 13 nuclear genes. Our coalescent species tree analyses resolved a strongly supported sister relationship between *Boleosoma* and a monophyletic *Catonotus*. Ancestor state reconstructions using the posterior distribution of these newly inferred phylogenies support a single origin of male-only parental care in the most recent common ancestor of *Boleosoma* and *Catonotus*.

1. Introduction

Darters, a species-rich clade of freshwater fishes endemic to eastern North America, diversified during the last 34 million years to fill a wide range of ecological niches and life history strategies (Page and Swofford, 1984; Near et al., 2011). Morphological aspects of darter species are generally well described, especially as they relate to alpha taxonomy and species delimitation. These external morphological features have historically been the basis of a classification scheme that includes four genera and up to 18 proposed subgenera in the most species-rich genus, *Etheostoma* (e.g., Page, 1983). However, these data are infrequently used to test hypotheses regarding evolutionary relationships among major darter lineages in a phylogenetic framework. Phylogenetic analyses of darters based solely on morphological data have lacked significant resolution and resulted in low support values for deeper nodes due to insufficient numbers of morphological characters (Shaw, 1996; Ayache and Near, 2009). This lack of support for many proposed taxonomic groups of darters draws into question their utility in evolutionary and ecological studies. With multi-locus genetic data now available for nearly every darter species, our ability to test taxonomic hypotheses at the species level (e.g., Harrington and Near, 2012; Keck and Near, 2013) and among higher taxonomic groupings (Near et al., 2011; Near and Keck, 2013) is rapidly expanding, and molecular systematic studies have provided a set of well-resolved and strongly supported hypotheses of phylogenetic relationships among major lineages of darters (Near et al., 2011).

Of the traditional taxonomic groupings of darter species, one of the most interesting is *Catonotus*, which encompasses 35 species classified into three clades *Oopareia*, *Richiella*, and *Stigmacerca* (Page, 1975; Near et al., 2011). Phylogenetic analyses of both morphological and molecular data either fail to resolve *Catonotus* as a clade or do not provide strong support for its monophyly (Braasch and Mayden, 1985; Porterfield et al., 1999; Sloss et al., 2004; Ayache and Near, 2009; Near et al., 2011). Originally described as a monotypic genus and subsequently synonymized (Agassiz, 1854), *Catonotus* was eventually resurrected as a subgenus (Bailey and Gosline, 1955) and subsequent studies provided diagnoses using external morphological characters (Kuehne and Small, 1971; Braasch and Page, 1979).

Phylogenetic analyses using data matrices containing discretely coded external morphological characters identified three putative synapomorphies for *Catonotus*: a broad and flat genital papilla in

* Corresponding author.
E-mail addresses: richard.harrington@earth.ox.ac.uk (R.C. Harrington), thomas.near@yale.edu (T.J. Near).

http://dx.doi.org/10.1016/j.ympev.2015.01.002
1055-7903/

females, breeding males with a swollen head, and a short first dorsal fin (Braasch and Mayden, 1985; Porterfield et al., 1999). The three subclades traditionally classified in *Catonotus* are resolved within a recently discovered clade, *Goneaperca*, along with *Boleosoma* and *Psychromaster* (Near et al., 2011). Monophyly of *Goneaperca* is strongly supported in analyses of multi-locus DNA sequence datasets, yet the monophyly of *Catonotus*, and support for relationships among subclades of *Goneaperca* varies depending on the loci sampled and methods of phylogenetic inference applied to the nucleotide datasets (Near et al., 2011; Near and Keck, 2013).

Many species of *Goneaperca*, including all members of *Catonotus*, have long been of interest to biologists because they exhibit a suite of complex reproductive behaviors in which males establish nesting territories in benthic cavities, females attach eggs to the upper surface of the cavity in a single layer, and the male guards the nest cavity and eggs until shortly after the eggs hatch (Winn, 1958a,b; Page, 1985). This form of parental care is a derived condition found in approximately 14% of all darter species, whereas most darters simply bury eggs or attach them to substrates but do not provide any form of parental care (Page, 1985). The complex behavior of nest and egg guarding is hypothesized to derive from a two- or three-step transition from either egg scattering or clumping with no care, followed by a transition to egg attaching or clumping with egg and nest guarding (Kelly et al., 2012). The number of reconstructed transitions between modes of parental care depends largely on the monophyly of *Catonotus* and its relationship with other lineages within *Goneaperca*, such as *Boleosoma*, that also contain species which exhibit parental care.

In this study we use an expanded multi-locus dataset sampled from 44 of 46 species of *Goneaperca* to assess the phylogenetic relationships of major lineages within *Goneaperca*, and in particular to test the monophyly of *Catonotus*. This dataset, with intraspecific sampling for thirteen nuclear loci, provides a more robust application of coalescent-based species tree methods than previous analyses, and avoids potential weaknesses associated with concatenation of multi-loci data (e.g., Edwards et al., 2007; Degnan and Rosenberg, 2009). Although a convincing set of morphological characters supporting the monophyly of *Catonotus* has yet to be discovered, our species-tree analyses strongly support monophyly of this long recognized taxonomic group; however, analyses of the concatenated dataset results in *Catonotus* paraphyly. Ancestral state reconstruction of male-only parental care on the posterior distribution of *Goneaperca* species trees results in strong support for a single origin of this trait in the most recent common ancestor of *Boleosoma* and *Catonotus*.

2. Materials and methods

Two to four individuals for each of the 44 sampled species of *Goneaperca* were sequenced for all loci included in this analyses (Table 1). The Qiagen DNeasy Tissue kit was used to isolate DNA from tissue biopsies following the manufacturer's protocol. The following genes were amplified using PCR primers and reaction conditions described in previous studies: *ectodermal neural cortex 1* (*enc1*); *glycotransferase* (*glyt*); *pleiomorphic adenoma protein-like 2* (*plag12*); *hypothetical protein* (*ptr*); *recombination activating protein 1* (*rag1*); *S7 ribosomal protein* (*S7*); *si:dkey-174m14.3* (*sidkey*); *SH3 and PX3 domain-containing 3-like protein* (*SH3PX3*); *t-box brain 1* (*tbr1*); *hypothetical protein* (*ube3A*); *hypothetical protein* (*ube3A-like*); *zic family member protein* (*zic1*); *zinc finger protein 503* (*znf503*) (Chow and Hazama, 1998; Lopez et al., 2004; Li et al., 2007, 2010).

Amplified PCR products were purified using a polyethylene glycol precipitation protocol (Rosenthal et al., 1993). Cleaned PCR products were used as template for Big Dye (Applied Biosystems) cycle sequencing on an ABI 3100 automated sequencer at the Molecular Systematics and Conservation Genetics Laboratory (Department of Ecology and Evolutionary Biology, Yale University, New Haven, CT, USA). Contiguous sequences were created and edited using Sequencher (GeneCodes, Ann Arbor, MI), and aligned manually using Se–Al v. 2.0 (http://tree.bio.ed.ac.uk/software/seal/). The program GARD (available at www.datamonkey.org) was used to test for the presence of recombination at each locus (Kosakovsky Pond and Frost, 2006; Kosakovsky Pond et al., 2006).

Gene trees were estimated from each sampled locus using a partitioned Bayesian strategy executed in the computer program MrBayes 3.1.2 (Ronquist and Huelsenbeck, 2003), and included DNA sequences sampled from *Percina aurantiaca* and *Etheostoma simoterum* as outgroup species. A single data partition was used for each of the nuclear protein-coding genes. Models of molecular evolution were chosen that best fit each of the data partitions using the Akaike information criterion (AIC) as implemented in the computer program MrModeltest version 2.3 (Nylander, 2004) (Table 2). In addition to individual gene trees for each of the nuclear genes, an analysis was run using MrBayes in which all 13 of the nuclear genes were concatenated and each gene was treated as a separate

Table 1
Species, clade membership, and number of individuals.

Species	Clade	Number of individuals
Etheostoma autumnale	*Psychromaster*	3
Etheostoma barbouri	*Oopareia*	3
Etheostoma basilare (Rock Creek)	*Oopareia*	2
Etheostoma basilare (Calf Killer)	*Oopareia*	2
Etheostoma basilare (Cane Creek)	*Oopareia*	2
Etheostoma basilare (Barren Fork)	*Oopareia*	2
Etheostoma basilare (Hickory Creek)	*Oopareia*	2
Etheostoma basilare (Collins River)	*Oopareia*	2
Etheostoma basilare (Mountain Creek)	*Oopareia*	2
Etheostoma cf. virgatum (Buck Creek)	*Oopareia*	3
Etheostoma boschungi	*Psychromaster*	3
Etheostoma brevispinum	*Richiella*	2
Etheostoma chienense	*Stigmacerca*	3
Etheostoma corona	*Stigmacerca*	3
Etheostoma cragini	*Psychromaster*	4
Etheostoma crossopterum	*Stigmacera*	3
Etheostoma derivativum	*Oopareia*	3
Etheostoma flabellare	*Richiella*	3
Etheostoma forbesi	*Stigmacerca*	4
Etheostoma kennicotti	*Richiella*	3
Etheostoma longimanum	*Boleosoma*	3
Etheostoma marmorpinnum	*Richiella*	2
Etheostoma mihileze	*Psychromaster*	3
Etheostoma neopterum	*Stigmacerca*	3
Etheostoma nigrum	*Boleosoma*	3
Etheostoma nigripinne	*Stigmacerca*	4
Etheostoma obeyense	*Oopareia*	3
Etheostoma olivaceum	*Stigmacerca*	3
Etheostoma olmstedi	*Boleosoma*	3
Etheostoma oophylax	*Stigmacerca*	3
Etheostoma cf. oophylax (Clarks River)	*Stigmacerca*	3
Etheostoma pallididorsum	*Psychromaster*	2
Etheostoma percnurum	*Richiella*	2
Etheostoma perlongum	*Boleosoma*	2
Etheostoma podostemone	*Boleosoma*	3
Etheostoma pseudovulatum	*Stigmacerca*	3
Etheostoma punctulatum	*Psychromaster*	3
Etheostoma smithi	*Oopareia*	3
Etheostoma squamiceps	*Stigmacerca*	3
Etheostoma striatulum	*Oopareia*	4
Etheostoma susanae	*Boleosoma*	3
Etheostoma tuscumbia	*Psychromaster*	3
Etheostoma virgatum	*Oopareia*	3
Etheostoma vitreum	*Boleosoma*	3