Molecular Phylogenetics and Evolution 55 (2010) 631–639

Contents lists available at ScienceDirect

Molecular Phylogenetics and Evolution

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

Molecular phylogenetic relationships and the evolution of the placenta in *Poecilia* (*Micropoecilia*) (Poeciliidae: Cyprinodontiformes)

Robert W. Meredith, Marcelo N. Pires, David N. Reznick, Mark S. Springer *

Department of Biology, University of California, Riverside, CA 92521, USA

ARTICLE INFO

Article history:
Received 12 August 2009
Revised 6 November 2009
Accepted 9 November 2009
Available online 14 November 2009

Keywords:
Cyprinodontiformes
Poeciliidae
Poecilia
Micropoecilia
Acanthophacelus
Placentotrophy

ABSTRACT

Poeciliids are one of the most intensively studied groups within Cyprinodontiformes owing to their use as model organisms for experimental studies on natural and sexual selection, and comparative studies of life-history evolution. Life-history studies have demonstrated multiple origins of placentotrophy and superfetation in poeciliids, including the recent description of placentotrophy in three species of *Poecilia* (*Micropoecilia*): *P. bifurca*, *P. branneri*, and *P. parae*. Here, we use a concatenation of seven nuclear gene segments and two mitochondrial segments to examine relationships within *Micropoecilia* and between this subgenus and other subgenera in *Poecilia* (*Mollienesia*, *Limia*, *Pamphorichthys*, *Acanthophacelus*). The combined molecular data set (8668 bp) was analyzed with maximum parsimony, maximum likelihood, and Bayesian methods. We also employed a relaxed molecular clock method to estimate divergence times within *Poecilia*. All phylogenetic analyses with the combined DNA data set supported the monophyly of *Poecilia* and recovered a basal split between *Poecilia* (*Acanthophacelus*) + *Poecilia* (*Micropoecilia*) and the other three subgenera. Within *Micropoecilia*, *P. bifurca* grouped with *P. branneri*, and these joined *P. parae* to the exclusion of *P. picta*. Ancestral reconstructions based on parsimony and Bayesian methods suggest that placentotrophy evolved once in *Micropoecilia* in the common ancestor of *P. bifurca*, *P. branneri*, and *P. parae*. Divergence time estimates suggest that placentotrophy in *Micropoecilia* evolved in $\leqslant$4 million years.

1. Introduction

The family Poeciliidae (Rosen and Bailey, 1963), equivalent to the subfamily Poeciliinae of Parenti (1981), is a diverse group of freshwater fishes in the order Cyprinodontiformes that comprises ~220 species in 28 genera (Lucinda, 2003; Lucinda and Reis, 2005). The group includes well-known aquarium fishes such as guppies, mollies, and swordtails (Lucinda and Reis, 2005). Poeciliids are endemic to the New World and are widely distributed throughout the Americas and Caribbean. All poeciliids have internal fertilization and all except *Tomeurus* are viviparous (Regan, 1913; Rosen and Gordon, 1953; Rosen and Bailey, 1963). Poeciliids are characterized by the presence of a gonopodium that is formed by modified anal-fin rays 3, 4, and 5 (Lucinda and Reis, 2005). Members of the group are frequently used as model organisms for experimental studies on natural and sexual selection (Endler, 1983; Houde, 1997; Schluter et al., 1998; Hamilton, 2001), and comparative studies of life-history evolution that have demonstrated multiple origins of superfetation and placentotrophy (Grove and Wourms, 1991, 1994; Arias and Reznick, 2000; Reznick et al., 2002, 2007). Most recently, placentotrophy was reported in all populations of *P. parae*, *P. bifurca* and *P. branneri* that were investigated (Pires, 2007; Pires et al., in press). In every case, placentotrophy was documented by a significant increase in embryo dry mass during development, and accompanied by a thickened maternal follicle and enlarged and externalized pericardial membrane in the embryo (Pires et al., in press).

Micropoecilia was proposed as a new genus by Hubbs (1926), who included *M. bifurca*, *M. parae*, *M. picta*, *M. melanozona*, and *M. branneri*, but also indicated that he was not convinced of the distinctness of any of the species and suggested that all may eventually be united with *M. parae*. Rosen and Bailey (1963), in their classic monograph on the family Poeciliidae (=Poeciliinae of Parenti, 1981), described the genus *Poecilia* and its constituent subgenera: *Poecilia*, *Lebistes*, *Pamphorichthys*, and *Limia* (Table 1). Rosen and Bailey (1963) included *Poecilia reticulata* (common guppy), the genus *Micropoecilia* of Hubbs (1926), *Poecilia amazonica*, and *Poecilia scalpridens* in the subgenus *Lebistes*. *Micropoecilia parae*, *M. bifurca*, and *M. melanzona* of Hubbs (1926) were treated as three polymorphs of *Poecilia* (*Lebistes*) *parae* by Rosen and Bailey (1963).

Costa (1991) reallocated *Poecilia* (*Lebistes*) *scalpridens* to the subgenus *Pamphorichthys*. Meyer (1993) reinstated the genus *Micropoecilia* of Hubbs (1926) for *Poecilia parae*, *P. bifurca*, *P. picta*,

* Corresponding author. Fax: +1 909 787 4826.
E-mail address: mark.springer@ucr.edu (M.S. Springer).

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doi:10.1016/j.ympev.2009.11.006

Table 1
Previous classifications of *Poecilia* species relevant to this study.

Hubbs (1924, 1926)	Rosen and Bailey (1963)	Meyer, (1993)	Poeser et al. (2005)	Lucinda and Reis (2005)
Family Poeciliidae	Family Poeciliidae	Family Poeciliidae	Family Poeciliidae	Family Poeciliidae
Subfamily Poeciliinae	Subfamily Poeciliinae	Subfamily Poeciliinae	Subfamily Poeciliinae	Subfamily Poeciliinae
Tribe Poeciliini	Tribe Poeciliini	Tribe Poeciliini	Tribe Poeciliini	Tribe Poeciliini
Genus *Limia*	Genus *Poecilia*	Genus *Poecilia*	Genus *Poecilia*	Genus *Poecilia*
Genus *Micropoecilia*	*P.* (*Poecilia*) *caucana*	*P.* (*Lebistes*) *reticulata*	*P.* (*Acanthophacelus*) *reticulata*	Genus *Limia*
M. bifurca	*P.* (*Poecilia*) *latipunctata*	*P.* (*Pamphorichthys*)	*P.* (*Acanthophacelus*) *wingei*	Genus *Micropoecilia*
M. picta	*P.* (*Lebistes*) reticulata	Genus *Micropoecilia*	*P.* (*Micropoecilia*) *parae*	Genus *Pamphorichthys*
M. branneri	*P.* (*Lebistes*) *parae* [included *Micropoecilia bifurca*]	*M. bifurca*	*P.* (*Micropoecilia*) *branneri*	
M. parae	*P.* (*Lebistes*) *picta*	*M. picta*	*P.* (*Micropoecilia*) *bifurca*	
M. melanzona	*P.* (*Lebistes*) *branneri*	*M. branneri*	*P.* (*Micropoecilia*) *picta*	
Genus *Lebistes*	*P.* (*Pamphorichthys*) minor	*M. parae*	*P.* (*Micropoecilia*) *minima*	
L. reticulatus	*P.* (*Limia*) *dominicensis*			
Genus *Mollienisia*[a]	*P.* (*Limia*) *melanogaster*			
Genus *Allopoecilia*				
A. caucana				
Tribe Pamphoriini				
Genus *Pamphorichthys*				

[a] Hubbs (1924, 1926) used *Mollienisia* rather than *Mollienesia*. Bailey and Miller (1950) subsequently provided justification for using *Mollienesia* instead of *Mollienisia*.

P. amazonica and *P. branneri* based on three derived morphological characters: (1) variegated males and females; most specimens with a relatively well-developed dark humeral spot on the side of the body; (2) males with anal-fin rays 6 and 7 and ventral fins extending to the base of the gonopodium palp; and (3) ray 3 of the gonopodium with 10–16 rose thorn-like spines. Within *Micropoecilia*, Meyer (1993) also proposed synapomorphic characters for *M. bifurca* and *M. picta*, these two plus *M. parae*, and these three plus *M. amazonica*. Meyer (1993) also defined *Poecilia* (*Lebistes*) as a monotypic subgenus that included only *P. reticulata*. Meyer's (1993) *M. amazonica* is of questionable taxonomic value because its type series is composed of a combination of *M. branneri* and *M. parae* individuals (C.A. Figueiredo, pers. comm. to M. Pires).

Subsequent to Meyer (1993), Costa and Sarraf (1997) described *Poecilia* (*Lebistes*) *minima* and hypothesized a close relationship of this taxon to *P. branneri* based on five morphological synapomorphies. Costa and Sarraf (1997) also challenged Meyer's (1993) putative synapomorphies for *Micropoecilia* and argued that (1) the variegated pattern does not occur in both sexes, (2) the humeral spot is not present in all species, and (3) the morphological features of the anal-fin rays and third gonopodial ray are also present in other groups. Given these differences with Meyer (1993), Costa and Sarraf (1997) did not concur with Meyer's (1993) reinstatement of *Micropoecilia* and questioned the monophyly of this genus.

Breden et al. (1999) examined relationships among representatives of the major divisions of the genus *Poecilia* using mitochondrial ND2 sequences. Breden et al. (1999) included two species of *Micropoecilia* (*sensu* Meyer, 1993), *M. picta* and M. *parae*, which grouped together and were the sister taxon to *Poecilia reticulata*. Similarly, Hrbek et al. (2007) and Lucinda and Reis (2005) found support for an association of *P. reticulata* with representative species of *Micropoecilia* based on a multigene concatenation and a matrix of osteological and soft anatomical characters, respectively.

Poeser et al. (2005) described *Poecilia wingei* (the Endler's livebearer, Campoma guppy, or Cumaná guppy), a close relative of *Poecilia* (*Lebistes*) *reticulata*, and placed both species in the subgenus *Acanthophacelus* rather than *Lebistes*. Poeser et al. (2005) argued that apparent autapomorphies of *P. reticulata* were instead synapomorphies of *P. reticulata* and *P. wingei*, and grouped these species in the subgenus *Acanthophacelus*. Poeser et al. (2005) chose *Acanthophacelus* rather than *Lebistes* because the latter name is tied to a molly, not the guppy, and is not available as a subgenus name for *P. reticulata* (Poeser and Isbrücker, 2002). *Acanthophacelus* Eigenmann, 1907 then becomes the first available subgenus name for *P. reticulata* (Poeser and Isbrücker, 2002). Shories et al. (2009) described *Poecilia* (*Acanthophacelus*) *obscura*, which represents a third species in the subgenus *Acanthophacelus*. Poeser et al. (2005) also placed all of Meyer's (1993) *Micropoecilia* species and the newly described *Poecilia* (*Lebistes*) *minima* of Costa and Sarraf (1997) into *Poecilia* (*Micropoecilia*) (see Table 1).

In the present study we use a combined mitochondrial and nuclear DNA data set to examine relationships among species in Poeser et al.'s (2005) subgenus *Micropoecilia* (*bifurca*, *branneri*, *parae*, *picta*). Our study includes all of the placentotrophic *Micropoecilia* species reported by Pires et al. (in press), as well as representatives of most of the other major subdivisions of *Poecilia*. This taxon sampling provides a basis for (1) evaluating the monophyly of *Poecilia* (*Micropoecilia*), (2) determining the number of origins of placentotrophy in *Poecilia* (*Micropoecilia*), and (3) examining relationships among the major lineages within the genus *Poecilia*.

2. Methods and materials

2.1. Taxon sampling

We included all recognized species of *Poecilia* (*Micropoecilia*) except for *P. minima*. We also included two representatives each from the *Poecilia* subgenera *Acanthophacelus*, *Limia*, *Mollienesia*, and *Pamphorichthys*. Two species of *Cnesterodon* were included as outgroups. A previous molecular study that included both mitochondrial and nuclear loci demonstrated that *Cnesterodon* is the closest outgroup to *Poecilia* (Hrbek et al., 2007). All ingroup and outgroup taxa are listed in Supplementary Information Table 1.

2.2. Gene sequences

QUIAGEN DNeasy Tissue extraction kits were used to extract genomic DNA from the skeletal muscle of 95% ethanol-preserved specimens. Two mitochondrial and seven nuclear gene regions were amplified. The first mitochondrial segment included the 3′