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Phylogenetic relationships and evolutionary history of the Mesoamerican endemic freshwater fish family Profundulidae (Cyprinodontiformes: Actinopterygii) ☆

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ABSTRACT

Freshwater fishes of Profundulidae, which until now was composed of two subgenera, represent one of the few extant fish families endemic to Mesoamerica. In this study we investigated the phylogenetic relationships and evolutionary history of the eight recognized extant species (from 37 populations) of Profundulidae using three mitochondrial and one nuclear gene markers (~2.9 Kbp). We applied a Bayesian species delimitation method as a first approach to resolving speciation patterns within Profundulidae considering two different scenarios, eight-species and twelve-species models, obtained in a previous phylogenetic analysis. Based on our results, each of the two subgenera was resolved as monophyletic, with a remarkable molecular divergence of 24.5% for mtDNA and 7.8% for nDNA uncorrected *p* distances, and thus we propose that they correspond to separate genera. Moreover, we propose a conservative taxonomic hypothesis with five species within *Profundulus* and three within *Tlaloc*, although both eight-species and twelve-species models were highly supported by the bayesian species delimitation analysis, providing additional evidence of higher taxonomic diversity than currently recognized in this family. According to our divergence time estimates, the family originated during the Upper Oligocene 26 Mya, and *Profundulus* and *Tlaloc* diverged in the Upper Oligocene or Lower Miocene about 20 Mya.

1. Introduction

Profundulidae (Hoedeman and Bronner, 1951), with eight recognized species, represents together with Lacantuniidae (Rodiles-Hernandez et al., 2005), one of the few freshwater fish families endemic to Mesoamerica (Bussing, 1985; Miller, 1966; Myers, 1966). Mesoamerica is regarded as one of the most biologically diverse regions of the world. Its high level of biodiversity has been attributed to its complex geological history and its geographical location, corresponding with a contact zone between the Nearctic and Neotropical biotas. Within Mesoamerica, Profundulidae occurs on both sides of the Isthmus of Tehuantepec, an area that has been recognized as a biodiversity hotspot for several groups of vertebrates (Escalante et al., 2007; Ferrari, 2000; Huidobro et al., 2006; Marshall and Liebherr, 2000; Mulcahy et al., 2006; Paniagua and Morrone, 2009). Currently, most of the species within Profundulidae exhibit a highly restricted distribution, being confined to basin headwaters of southern Mexico, El Salvador, Honduras and Guatemala in both the Pacific and Atlantic versants (Eschmeyer, 2007; Matamoros et al., 2012; Miller, 1955, 2005; Morcillo, 2004), with the exception of *Profundulus punctatus*, which is able to inhabit the montane and piedmont floodplains and coastal plains. This family is mainly distributed within the Grijalva-Usumacinta basin, an area of endemism (western Guatemala and southern Mexico, Fig. 1), which is characterized by exceptional diversity and where 59% endemism is attributed to freshwater fish fauna (Matamoros et al., 2014).

During recent decades, the family has been subjected to several taxonomic changes, where alternative hypotheses proposed by

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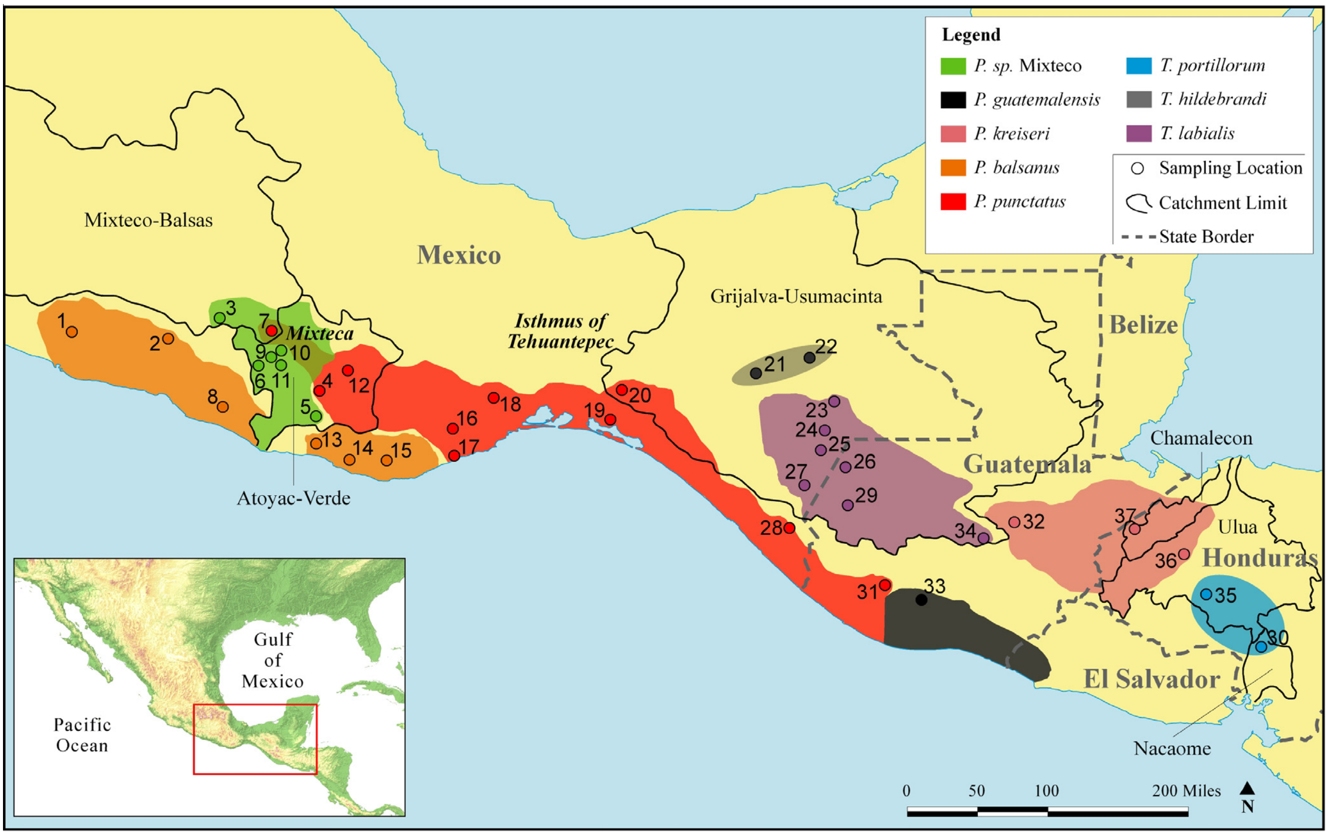

Fig. 1. Sampling localities and species distribution. Map of localities sampled in this study. Numbers correspond to localities shown in Supplementary data, and shaded areas correspond to the 8 species hypothesis proposed for the *Profundulus* and *Tlaloc* subgenera.

morphology and molecular data in some cases have produced contrasting results (Doadrio et al., 1999; Gonzalez-Diaz et al., 2005; Matamoros and Schaefer, 2010; Matamoros et al., 2012; Miller, 1955). These taxonomic changes have arisen in part from difficulties in delimiting species due to a lack of morphological differentiation among some species within the family (i.e. cryptic speciation within (*Profundulus*) *punctatus* species "group", Miller, 1955), at the same time morphological divergence emerges from ecological adaptations in others (i.e. *Profundulus* (*Tlaloc*) *candalarius* and *Profundulus* (*Tlaloc*) *labialis* (Gonzalez-Diaz et al., 2005; Miller, 1955)). Profundulidae represents a feasible model for studying species delimitation, since the family exhibits few species, each with a well-defined distributional range (Fig. 1, Matamoros et al., 2012). According to Miller (2005), the family taxonomy is as follows: one nominal genus, *Profundulus* (Hubbs, 1924) that is divided into two subgenera: *Profundulus* (Hubbs, 1924) comprised of three species, *P.* (*P.*) *guatemalensis*, *P.* (*P.*) *punctatus* and *P.* (*P.*) *oaxacae*, and *Tlaloc* (Alvarez and Carranza, 1951), also including three species, *P.* (*T.*) *hildebrandi*, *P.* (*T.*) *labialis* and *P.* (*T.*) *candalarius*. Recently, two new species were described: *P.* (*P.*) *kreiseri*, which belongs to the *Profundulus* subgenus (Matamoros et al., 2012), and *P.* (*T*). *portillorum*, which belongs to the *Tlaloc* subgenus (Matamoros and Schaefer, 2010). Doadrio et al. (1999) found at least five well-differentiated groups within *P.* (*P.*) *cf. punctatus* based on molecular data (allozymes), which could correspond to a higher number of putative species within the subgenus *Profundulus*. Two of them were considered to be new species not yet described: one was known only from a single spring in the Tehuantepec basin and the second extended mainly through springs in the Mixteca region of Mexico (Fig. 1). On the other hand, within the subgenus *Tlaloc*, the validity of the species *P.* (*T.*) *candalarius* has been questioned because minor and inconsistent morphological differentiation from its sister species *P.* (*T.*) *labialis* (Gonzalez-Diaz et al., 2005) and a lack of resolution between these two species using the mtCytb marker suggest recent divergence between the two species (Matamoros et al., 2012).

The present study, which represents the most extensive geographic survey to date for the Profundulidae using molecular data, has two main goals. The first one is to formulate a robust phylogenetic hypothesis and the second one is to infer the family evolutionary history. In order to achieve these goals, we performed a molecular phylogenetic reconstruction using three mitochondrial genes (ATP8, ATP6 and ND2) and the S7 nuclear gene. In addition, we carried out a Bayesian relaxed molecular clock analysis to assess the time and mode of diversification in the family.

2. Material and methods

2.1. Taxonomic sampling and tissue collection

A total of 44 specimens were collected from 37 localities situated along most of the known geographic distribution for the family, including samples for all currently recognized species in the family (Fig. 1, Table 1, Supplementary data 1.1) (Matamoros et al., 2012; Miller, 2005). These localities included populations on the Pacific slope from the Balsas basin (locations 3 and 7, Fig. 1) Mexico, to the Nacaome basin (location 30, Fig. 1), Honduras, and on the Atlantic slope from the Grijalva-Usumacinta basin (Fig. 1), Mexico, to the Ulua basin, Honduras (locations 35 and 36, Fig. 1).

Specimens were sampled by electrofishing and seining under local authorities permission and released onsite. A small