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Genetic relationships among freshwater mussel species from fifteen Amazonian rivers and inferences on the evolution of the Hyriidae (Mollusca: Bivalvia: Unionida)

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ABSTRACT

The current phylogenetic framework for the South American Hyriidae is solely based on morphological data. However, freshwater bivalve morphology is highly variable due to both genetic and environmental factors. The present study used both mitochondrial (COI and 16S) and nuclear (18S-ITS1) sequences in molecular phylogenetic analyses of nine Neotropical species of Hyriidae, collected from 15 South American rivers, and sequences of hyriids from Australia and New Zealand obtained from GenBank. The present molecular findings support traditional taxonomic proposals, based on morphology, for the South American subfamily Hyriinae, currently divided in three tribes: Hyriini, Castaliini and Rhipidodontini. Phylogenetic trees based on COI nucleotide sequences revealed at least four geographical groups of *Castalia ambigua*: northeast Amazon (Piriá, Tocantins and Caeté rivers), central Amazon, including *C. quadrata* (Amazon and Aripuanã rivers), north (Trombetas river), and *C. ambigua* from Peru. Genetic distances suggest that some specimens may be cryptic species. Among the Hyriini, a total evidence data set generated phylogenetic trees indicating that *Paxyodon syrmatophorus* and *Prisodon obliquus* are more closely related, followed by *Triplodon corrugatus*. The molecular clock, based on COI, agreed with the fossil record of Neotropical hyriids. The ancestor of both Australasian and Neotropical Hyriidae is estimated to have lived around 225 million years ago.

1. Introduction

Traditional taxonomic classifications of South American freshwater mussels (for example, d'Orbigny, 1847; Graf, 2000; Lange-de-Morretes, 1949; Ortmann, 1921; Parodiz and Bonetto, 1963; von Ihering, 1890) have been based on morphological characteristics of the shell, soft parts and larval morphology. However, some authors (Alvarenga and Ricci, 1981; Graf, 2000; Hoeh et al., 1998) mention a number of difficulties arising from this approach. Intraspecific morphological variability due to environmental or genetic factors, or parallel evolution, can make it difficult to identify the species (Nagel and Badino, 2001; Roper and Hickey, 1994). Morphologically similar cryptic species may go undetected in analyses based solely on shell characters, as happened, for example, with *Velesunio wilsonii* from Australia (Baker et al., 2003).

The freshwater mussel family Hyriidae is traditionally placed in the subclass Palaeoheterodonta, order Unionida (Nevesskaja,

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2009), superfamily Unionoidea Rafinesque, 1820 (Graf and Cummings, 2006). Hyriidae Swainson, 1840 are found in South America, Australia, New Zealand, New Guinea and the Solomon Islands (Graf and Cummings, 2007; Marshall et al., 2014; McMichael and Hiscock, 1958; Parada and Peredo, 2002; Parodiz and Bonetto, 1963; Walker et al., 2001, 2014). In reviews of the literature, Bogan (2008) reported nine genera and 55 species for Neotropical Hyriidae, whereas Pereira et al. (2013) reported 64 species. The Amazonian species of the subfamily Hyriinae are divided into three tribes: Hyriini (*Triplodon*, *Paxyodon*, *Prisodon*), Castaliini (*Castalia*, *Callonaia*, *Castaliella*), and Rhipidodontini (*Diplodon* and *Rhipidodonta*) (Graf and Cummings, 2006, 2007). The South American freshwater mussel fauna is poorly known in terms of both molecular studies and ecology, and more effort is needed to determine its current phylogenetic and conservation status (Pereira et al., 2013).

Based on molecular sequences of COI and 28S, Whelan et al. (2011) obtained phylogenetic trees of tropical freshwater mussels, including two species of Neotropical Hyriidae (*Castalia ambigua* and *Triplodon corrugatus*) from Peru. Their study showed strong bootstrap values grouping all the Hyriidae species in the trees, however they were unable to provide significant support for inter-family relationships.

Given the large area relative to sampling effort, the Amazonian Hyriidae remain poorly known and the fauna has not yet been completely revised. Simone (2006) left the genera *Triplodon* and *Prisodon* Schumacher, 1817 together as a single genus, *Prisodon*, with *Prisodon corrugatus* and *Prisodon obliquus* (Schumacher, 1817) considered valid, respectively, and *Paxyodon* kept as a distinct genus within the Prisodontini (currently Hyriini). Moreover, Mansur and Pimpão (2008) described *Triplodon chodo*, a new species of Hyriidae from central Amazonia, based on shell descriptions of specimens.

Molecular systematic studies of lower-level taxa of freshwater bivalves have focused on the North American fauna and more studies are needed from other regions (Roe and Hoeh, 2003). Some molecular studies include Hyriidae from Australia and New Zealand (Baker et al., 2003, 2004; Hughes et al., 2004) and South America (Hoeh and Bogan, 2000; Whelan et al., 2011), but further studies, using a molecular approach, are needed to establish robust phylogenetic relationships in this family. In the present study, we analysed 36 specimens of 9 species of Hyriidae collected from 15 rivers in the Amazon region in order to verify the geographic distribution previously described for morphologically identified specimens and the molecular phylogenetic relationships among these species.

2. Material and methods

2.1. Sampling and laboratory methods

We collected Neotropical Hyriidae from the Amazon, Araguaia, Aripuanã, Caeté, Gurubatuba, Irituiá, Laranjal, Piriá, Pará, Tapajós,

Table 1
Specimen identification (species and number), sex, GenBank accession number of COI, 16S and 18S-ITS1 sequences, sampling location (river) and geographic coordinates of sampling locations.

Species and number	Sex	GenBank accession number			Sampling location (river)	Coordinates
		COI	16S	18S-ITS1		
Paxyodon syrmatophorus 1	–	KU888254	–	KU888185	Tocantins	49°25′06.17′W; 2°30′01.28″S
Paxyodon syrmatophorus 2	M	KU888255	KU888215	–	Tocantins	49°25′06.17′W; 2°30′01.28″S
Paxyodon syrmatophorus 3	–	KU888256	KU888216	KU888186	Amazon	51°08′43.81″W; 0°08′05.52″W
Paxyodon syrmatophorus 4	M	KU888257	KU888217	–	Tocantins	49°25′06.17′W; 2°30′01.28″S
Paxyodon syrmatophorus 5	–	KU888258	KU888218	KU888187	Amazon	51°08′43.81″W; 0°08′05.52″W
Paxyodon syrmatophorus 6	–	KU888259	–	–	Pará	48°46′34.86″W; 1°31′40.45″S
Paxyodon syrmatophorus 7	F	KU888260	KU888219	KU888188	Tocantins	49°25′06.17′W; 2°30′01.28″S
Paxyodon syrmatophorus 8	F	KU888261	–	–	Tocantins	49°25′06.17′W; 2°30′01.28″S
Paxyodon syrmatophorus 9	F	KU888262	KU888220	KU888189	Tocantins	49°25′06.17′W; 2°30′01.28″S
Triplodon corrugatus 1	–	KU888245	KU888224	KU888194	Tocantins	49°25′06.17′W; 2°30′01.28″S
Triplodon corrugatus 2	–	KU888246	–	KU888195	Irituia	47°26′01.33″W; 1°46′39.11″S
Triplodon corrugatus 3	F	KU888247	KU888225	KU888196	Iritua	47°26′01.33″W; 1°46′39.11″S
Triplodon corrugatus 4	–	KU888248	KU888226	KU888197	Tapajós	54°43′18.76″W; 2°25′51.08″S
Triplodon corrugatus 5	–	KU888249	KU888227	KU888198	Tapajós	54°43′18.76″W; 2°25′51.08″S
Triplodon corrugatus 6	–	KU888250	KU888228	KU888199	Gurubatuba	54°05′29.75″W; 2°00′51.87″S
Triplodon corrugatus 7	–	KU888251	KU888229	KU888200	Gurubatuba	54°05′29.75″W; 2°00′51.87″S
Triplodon corrugatus 8	F	KU888252	KU888230	KU888201	Piriá	46°27′37.71″W; 1°25′42.765″S
Triplodon corrugatus 9	M	KU888253	KU888231	KU888202	Piriá	46°27′37.71″W; 1°25′42.765″S
Castalia ambigua 1	F	KU888236	KU888207	KU888178	Tocantins	49°25′06.17″W; 2°30′01.28″S
Castalia ambigua 2	F	KU888237	KU888208	KU888179	Tocantins	49°25′06.17″W; 2°30′01.28″S
Castalia ambigua 3	M	KU888238	KU888209	–	Piriá	46°27′37.71″W; 1°25′42.765″S
Castalia ambigua 4	M	KU888239	KU888210	KU888180	Caeté	46°48′54″W; 1°12′32.76″S
Castalia ambigua 5	–	KU888240	KU888211	–	Amazon	56°18′04.83″W; 2°24′00.45″S
Castalia ambigua 6	–	KU888241	KU888212	KU888181	Amazon	56°18′04.83″W; 2°24′00.45″S
Castalia ambigua 7	–	KU888242	KU888213	KU888182	Trombetas	55°52′25.28″W; 1°45′53.28″S
Castalia ambigua 8	–	KU888243	–	–	Trombetas	55°52′25.28″W; 1°45′53.28″S
Castalia quadrata[a]	–	KU888244	KU888214	KU888183	Aripuanã	60°11′39.5″W; 06°01′05.15″S
Castalia stevensi[b]	–	AF231736	–	KU888184	Portuguesa	–
Callonaia duprei 1	–	KU888233	KU888204	KU888175	Araguaia	48°40′22.68″W; 5°22′49.58″S
Callonaia duprei 2	–	KU888234	KU888205	KU888176	Araguaia	48°40′22.68″W; 5°22′49.58″S
Callonaia duprei 3	–	KU888235	KU888206	KU888177	Araguaia	48°40′22.68″W; 5°22′49.58″S
Prisodon obliquus 1	–	–	–	KU888190	Laranjal	49°12′54.71″W; 1°31′40.45″S
Prisodon obliquus 2	–	–	KU888221	KU888192	Trombetas	55°52′25.28″W; 1°45′53.28″S
Prisodon obliquus 3	–	–	KU888222	KU888191	Trombetas	55°52′25.28″W; 1°45′53.28″S
Prisodon obliquus 4	–	–	KU888223	KU888193	Trombetas	55°52′25.28″W; 1°45′53.28″S
Diplodon suavidicus	–	KU888263	KU888232	KU888203	Xingu	52°12′38.47″W; 3°14′28.53″S

[a] Coleção de Moluscos do Instituto Nacional de Pesquisas da Amazônia, INPA # 386.
[b] Illinois Natural History Museum #14939.