A New Cryptic Species of South American Freshwater Pufferfish of the Genus *Colomesus* (Tetraodontidae), Based on Both Morphology and DNA Data

Cesar R. L. Amaral, Paulo M. Brito, Dayse A. Silva, Elizeu F. Carvalho

**Introduction**

The Tetraodontidae are an Acantomorpha fish family with circumglobal distribution composed of 189 species grouped in 19 genera, occurring in seas, estuaries, and rivers between the tropical and temperate regions. Of these, the genus *Colomesus* is confined to South America, with what have been up to now considered only two species. *C. asellus* is spread over the entire Amazon, Tocantins-Araguaia drainages, and coastal environments from the Amazon mouth to Venezuela, and is the only freshwater puffers on that continent. *C. psittacus* is found in coastal marine and brackish water environments from Cuba to the northern coast of South America as far south as to Sergipe in Brazil. In the present contribution we used morphological data along with molecular systematics techniques to investigate the phylogeny and phylogeography of the freshwater pufferfishes of the genus *Colomesus*. The molecular part is based on a cytochrome C oxidase subunit I dataset constructed from both previously published and newly determined sequences, obtained from specimens collected from three distinct localities in South America. Our results from both molecular and morphological approaches enable us to identify and describe a new *Colomesus* species from the Tocantins River. We also discuss aspects of the historical biogeography and phylogeography of the South American freshwater pufferfishes, suggesting that it could be more recent than previously expected.

**Abstract**

The Tetraodontidae are an Acantomorpha fish family with circumglobal distribution composed of 189 species in 19 genera, occurring in seas, estuaries, and rivers between the tropical and temperate regions. Of these, the genus *Colomesus* is confined to South America, with what have been up to now considered only two species. *C. asellus* is spread over the entire Amazon, Tocantins-Araguaia drainages, and coastal environments from the Amazon mouth to Venezuela, and is the only freshwater puffers on that continent. *C. psittacus* is found in coastal marine and brackish water environments from Cuba to the northern coast of South America as far south as to Sergipe in Brazil. In the present contribution we used morphological data along with molecular systematics techniques to investigate the phylogeny and phylogeography of the freshwater pufferfishes of the genus *Colomesus*. The molecular part is based on a cytochrome C oxidase subunit I dataset constructed from both previously published and newly determined sequences, obtained from specimens collected from three distinct localities in South America. Our results from both molecular and morphological approaches enable us to identify and describe a new *Colomesus* species from the Tocantins River. We also discuss aspects of the historical biogeography and phylogeography of the South American freshwater pufferfishes, suggesting that it could be more recent than previously expected.
species from the Upper Tocantins drainage based on both morphological and molecular data.

Methods

Specimens of *Colomesus asellus* were collected from three distinct populations with about 2200 km of mean distance separating them. The collection localities were Ilha do Mosqueiro, Belém, Brazil; Upper Tocantins River - Porto Nacional, Tocantins, Brazil; and Nanay River - Iquitos, Peru (Figure 1).

Ethics Statement

No statement from an ethics committee was necessary, and the manuscript did not involve any endangered or protect species. All samples were extracted from dead specimens collected with appropriate permissions under authorization number 22512 issued by SISBIO/Instituto Chico Mendes de Conservação da Biodiversidade. We used the ice-slurry method for killing following [33] as they are tropical warm water species and the collected specimens are all smaller than 5 cm SL. All specimens were preserved in alcohol. The reported localities do not include protected areas.

Nomenclatural Acts

The electronic version of this document does not represent a published work according to the International Code of Zoological Nomenclature (ICZN), and hence the nomenclatural acts contained in the electronic version are not available under that Code from the electronic edition. Therefore, a separate edition of
### Table 1. Taxonomic sampling and accession numbers.

| Taxon                        | Accession No. | Accession No. |
|------------------------------|---------------|---------------|
| **Família Triodontidae**     |               |               |
| Triodon macropterus          | JQ841396      | JQ840304      |
| **Família Diodontidae**      |               |               |
| Diodon holocanthus           | AP009177      | GU440524      |
| Chilomycterus reticulatus    | AP009188      | KC959927*     |
| **Família Tetraodontidae**   |               |               |
| Lagocephalus laevigatus      | AP011934      | GU225665      |
| Lagocephalus inermis         | FJ434549      | GU225664      |
| **Lagocephalus lunaris**     |               |               |
| **Lagocephalus lagocephalus**|               |               |
| **Lagocephalus guentheri**   |               |               |
| **Lagocephalus Wheeleri**    |               |               |
| **Lagocephalus spadiceus**   |               |               |
| **Takifugu ocellatus**       |               |               |
| **Takifugu poecilorotus**    |               |               |
| **Takifugu snyderi**         |               |               |
| **Takifugu oblongus**        |               |               |
| **Takifugu pardalis**        |               |               |
| **Takifugu niphobles**       |               |               |
| **Takifugu porphyreus**      |               |               |
| **Tetraodon biocellatus**    |               |               |
| **Tetraodon nigroviridis**   |               |               |
| **Sphoeroides pachygaster**  |               |               |
| **Sphoeroides greeleyi**     |               |               |
| **Sphoeroides nephelus**     |               |               |
| **Sphoeroides spengleri**    |               |               |

(*) Sequences newly determined in this study.

doi:10.1371/journal.pone.0074397.t001
Figure 2. Neighbor-Joining tree based on the barcode region of the COI. The numbers near the branches represent bootstrap probabilities higher than 50%.
doi:10.1371/journal.pone.0074397.g002
A New Colomesus (Tetraodontidae) from Brazil
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In addition, this published work and the nomenclatural acts it contains have been registered in ZooBank, the proposed online registration system for the ICZN. The ZooBank LSIDs (Life Science Identifiers) can be resolved and the associated information viewed through any standard web browser by appending the LSID to the prefix “http://zoobank.org/”. The LSID for this

Figure 3. Maximum-likelihood phylogeny based on the barcode region of the COI marker. The numbers near the branches represent the bootstrap probabilities.
doi:10.1371/journal.pone.0074397.g003

Figure 4. Distribution of K2P distances (%) for COI: A) within species; B) within genera; C, normalized distribution of K2P distance (%) within species. The analyses included the following taxa: Tetraodon nigroviridis, Tetraodon biocellatus, Sphoeroides testudineus, Lagocephalus laevigatus, Colomesus asellus, Colomesus psittacus, and the freshwater Colomesus from the Tocantins drainage.
doi:10.1371/journal.pone.0074397.g004

Figure 5. Neighbor-Joining phylogeny of the freshwater Colomesus and haplotype determination.
doi:10.1371/journal.pone.0074397.g005
Morphological Analyses

Specimens from all three localities were cleared and stained following the methodology of [34].

Molecular Analyses

The molecular systematic analyses used newly determined sequences obtained from the mitochondrial barcode marker COI as well as previously published sequences obtained from the NCBI and BOLD databases.

For the newly determined sequences, a fragment of epaxial musculature was submitted to the standard protocol for DNA extraction and purification from the Qiagen QIAamp DNA FFPE Tissue kit. The fragments were amplified and sequenced using the primers VF2_t1 and FishR2_t1 [35–37]. All primers were appended with M13 tails on sequencing reactions. The PCR profile consisted of 2 min at 95°C, 35 cycles of 30 sec at 94°C, 40 sec at 52°C, and 1 min at 72°C, with a final extension step for 10 min at 72°C. Sequencing reactions were performed with the use of the BigDye® Terminator v.3.1 Cycle Sequencing kit (Applied Biosystems, Inc.), with 25 cycles of 10 sec at 95°C, 5 sec at 50°C and 4 min at 60°C. Sequencing products were processed in an ABI 3500 capillary system (Applied Biosystems, Inc.). The chromatograms were checked and aligned using the BioEdit 7.053 [38] software with its built-in ClustalW routine [39]. The alignment was visually inspected for accuracy and to minimize missing data. All the newly determined sequences are available at the BOLD database (http://www.barcodinglife.com) under the project acronym PUFER. The GenBank accession numbers for all newly determined and previously published sequences used in the present manuscript are summarized in Table 1. The dataset consisted of a 651 bp COI matrix, and we used the MEGA 5.06 software [40] to determine the TN93+G+I as the most appropriate model of sequence evolution based on the Akaike criterion (AIC) [41].

The neighbor-joining (NJ) and maximum-likelihood (ML) trees that encompass the genera Triodon, Diodon, Chilomycterus, Lagocephalus, Tetraodon, Takifugu, Sphoeroides, and Colomesus were constructed using the MEGA 5.06 software [40]. The neighbor-joining sequence divergences were calculated based on the Kimura Two Parameter (K2P) distance model [42] on BOLD workbench and MEGA 5.06 software [40]. The haplotype determination was carried with the use of the server FaBox (http://birc.au.dk/software/fabox/).

Results and Discussion

The neighbor-joining (NJ) and maximum-likelihood (ML) result trees are presented in Figures 2 and 3, respectively. The genus Colomesus was recovered as monophyletic inside the group formed by the sampled Sphoeroides species, in except for Sphoeroides pachygaster. Lagocephalus was recovered in a basal phylogenetic position in relation to Sphoeroides and Colomesus, therefore corroborating recent results such as those presented by [43–45].

Colomesus was recovered deep inside the group formed by the remaining Sphoeroides species, therefore suggesting Sphoeroides as paraphyletic, with S. pachygaster being recovered as basal in relation

| Table 2. Morphometric and meristic data of the type series of Colomesus tocantinensis nov. sp. |
| Register | SL | HL | PR | DR | AR | CR | IOL |
|----------|----|----|----|----|----|----|-----|
| PNT.403  | 30.84 | 10.75 | 15 | 10 | 9 | 11 | 5.8 |
| PNT.404  | 34.9 | 11.83 | 15 | 10 | 9 | 11 | 5.9 |
| PNT.405* | 29.62 | 10.37 | 16 | 10 | 9 | 11 | 5.47 |
| PNT.395  | 30.35 | 11.25 | 15 | 10 | 9 | 11 | 6.35 |
| PNT.396  | 29.28 | 11.1 | 16 | 9 | 9 | 11 | 6.78 |
| PNT.397  | 29.59 | 10.79 | 16 | 10 | 9 | 11 | 5.48 |
| PNT.398  | 29.46 | 10.6 | 15 | 10 | 9 | 11 | 5.77 |
| PNT.399  | 30.66 | 11.99 | 16 | 10 | 9 | 11 | 5.55 |
| PNT.400  | 32.92 | 11.75 | 15 | 10 | 9 | 11 | 6.03 |
| PNT.401  | 27.02 | 9.61 | 16 | 10 | 9 | 11 | 5.12 |

SL, standard length; HL, head length; PR, pectoral fin rays; DR, dorsal fin rays; AR, anal fin rays; CR, caudal fin rays; IOL, interorbital length.

(*)Holotype.

Figure 6. External morphology of the genus Colomesus. A) Colomesus tocantinensis nov. sp. – Tocantins (holotype PNT.ERJ.405 highlighted in white); B) Colomesus asellus – Iquitos; C) Colomesus asellus – Belém.
doi:10.1371/journal.pone.0074397.g006
to all the remaining *Sphoeroides* species in all the analyses. Additionally, *Colomesus* was also recovered as the sister-taxa of the group formed by the species *Sphoeroides nephelus*, *S. tyleri*, and *S. greeleyi* in the NJ result, although it was recovered as the sister-taxa of *S. greeleyi* in the ML results.

In the same way, *Colomesus* is clearly distinguishable from the group formed by all the *Sphoeroides* species mainly by the banded
pigmentation pattern present in all the Colomesus species; the presence of two lateral lines, with the ventral line running the full length of the caudal peduncle; and the absence of an upraised horizontal ridge of skin ventrolaterally along the caudal peduncle. The color pattern was used by [46], along with pectoral fin ray counts, the presence of a dark bar underside of caudal peduncle, and the presence of dermal flaps across the chin, to distinguish between what at that time were considered to be the only two species of the genus, the marine/estuarine C. psittacus, and the freshwater C. asellus. The presence of a dark bar on the underside of the caudal peduncle is a prominent feature for specimens of C. asellus from Iquitos, but this bar is present or not in specimens from both Belém and Tocantins. Dermal flaps were observed in all specimens from both Iquitos and Belém, but such flaps were not observed in any of the examined specimens from the Tocantins drainage.

DNA Barcode and Deep Sequence Divergence

COI amplicons were obtained from all the specimens included in the analyses. The obtained sequences clearly identified both previous accepted Colomesus species (C. asellus and C. psittacus), therefore being in accordance with the previous morphological diagnose presented by [46].

The K2P divergence distances between congeneric species ranged from 5.557% to 12.394% with a mean distance of 8.546%, while the uncorrected K2P distance ranged from 0 to 4.472% within species. The mean K2P distance within the analyzed populations was 0.657% and the mean normalized distance within species is 1.079% (Figure 4).

Deep sequence divergence was observed regarding the freshwater Colomesus from the Tocantins drainage (Figure 5). The mean sequence divergence of the specimens from both Belém and Iquitos was estimated at 1.079%, while the Tocantins distances ranged from 1.955% to 3.063%, with a mean distance of 2.166%. The observed sequence divergence values together with the congruence observed from both molecular and morphological phylogenetic approaches used here suggest the existence of an overlooked species within the genus Colomesus.

A New Colomesus species from the Tocantins River, Brazil

Systematics. Tetraodontiformes sensu Tyler, 1980 [47] Tetraodontidae sensu Santini & Tyler, 2003 [45] Colomesus Gill, 1885 [2] Colomesus tocantinensis nov. sp. urn:lsid:zoobank.org:act:9B8ACCB5-FF55-4514-901B-6366FB6EA307

Derivation of name. The specific epithet tocantinensis refers to the type locality, Porto Nacional, State of Tocantins, Brazil.
Figure 9. Right and top photographs from cleared-and-stained specimens of: A–B) Colomesus tocantinensis nov. sp. – Tocantins (PNT.UERJ.398); C–D) Colomesus asellus – Iquitos (PNT.UERJ.470); E–F) Colomesus asellus – Belem (PNT.UERJ.386).
doi:10.1371/journal.pone.0074397.g009

Figure 10. Isolated opercles from: A) Colomesus tocantinensis nov. sp. – Tocantins (PNT.UERJ.398); B) Colomesus asellus – Iquitos (PNT.UERJ.470); C) Colomesus asellus – Belem (PNT.UERJ.386); D) Colomesus psittacus – Belem (PNT.UERJ.387). Scale bar equals 1 mm.
doi:10.1371/journal.pone.0074397.g010
Holotype. PNT.UERJ.405 (Figure 6).
Paratypes. PNT.UERJ.396, PNT.UERJ.397, PNT.UERJ.398, PNT.UERJ.399, PNT.UERJ.400, PNT.UERJ.401, PNT.UERJ.402, PNT.UERJ.403, PNT.UERJ.404.
Type-locality. The specimens are from the Tocantins River near Porto Nacional, State of Tocantins, Brazil.
Diagnosis. Colomesus species diagnosed by six to seven basal pterygiophores and nine rays in the anal fin (contra ten to eleven in both C. asellus and C. psittacus); ten basal pterygiophores and rays in the dorsal fin (contra eleven for both C. asellus and C. psittacus); the absence of dermal flaps across the chin (contra its presence uniquely in C. asellus); a caudal peduncle with eight vertebrae; and an opercle with a posterior ventral border subdivided in a ventral and a posterior region, the herein called “inverted V” shape (contra the triangular opercle exhibited by both C. asellus and C. psittacus).

Description. The holotype (PNT.UERJ.405) is 29.62 mm SL (Figure 6), with 10.37 mm HL; the entire type-series ranges from 27.02 mm to 34.9 mm SL. The meristic and morphometric data of the type series is presented in Table 2. The extent of the dorsal and ventral lateral lines is similar to those found in C. asellus. The prickles extend along the dorsal, lateral, and ventral surfaces of the body, from the level of the eye to the origin of the dorsal fin.

The color pattern of Colomesus tocantinensis nov. sp. is essentially the same as that of Colomesus asellus, with five transverse dark bars across the dorsal region of the body. A dark blotch on the underside of the caudal peduncle, which is a state used by [46] to diagnose Colomesus asellus, is present or absent, being vestigial to unobservable or absent in several specimens. The interspaces between the dark bars are light yellow, with gradually decreasing pigmentation and becoming white in the ventral region (Figure 6). However, the light yellow to pale pattern presented by C. tocantinensis nov. sp. clearly contrasts with the gold-yellow pattern present in specimens from Iquitos and Belém.

The nasal sac is higher than that presented in the specimens of C. asellus. Two large lateral and anteromedial nostrils are present. They are similar to those found on C. psittacus, rather than the two small nostrils exhibited by C. asellus. The anterior surface of the nasal sac is smooth while the posterior surface of it is folded as in C. psittacus, exhibiting a “T-shaped” ridge with a relatively small dorsal flap. This flap seems much smaller than the one found on C. asellus, although more flexible when compared to C. psittacus.

The presence of dermal flaps across the chin is another character used by [46] to distinguish C. asellus from C. psittacus. No dermal flaps could be seen in the examined specimens from the Tocantins River, although they are always present in examined specimens from Iquitos and Belém.

The skull is partially similar to those found in Colomesus asellus described and figured by [46], although the frontals exhibit a wide posterior border and prominently participate in the orbital margin (Figures 7–9). The prefrontals are triangular and articulate medially with the ethmoid, which posteriorly articulates with the frontals and anteriorly with the palatines (Figure 8). The supraoccipital is roughly triangular and well developed, with an elongate posterior process which covers the first vertebrae (Figure 8). The sphenotics articulate posteriorly with the pterotics (Figures 7 and 8) articulates posteriorly with the slender supracleithrum and medially with the epipotics, which articulate medially with the supraoccipital (Figure 9).

In lateral view, the skull is characterized by the wide preopercle with about 110 degrees between both horizontal and vertical rami (Figure 7), with the preopercular canal running along its anterior border, and by the opercle which is divided in two distinct regions, having ventral and posterior wings, the herein called “inverted V” shape, distinct from the condition found in all other examined specimens of Colomesus (Figure 10). The subopercle is sturdy, with two small dorsal processes.

The parasphenoid is elongate and does not exhibit any developed dorsal flange (Figures 7 and 9). The hyomandibula is roughly triangular and has a slender ventral region; its wide head articulates dorsally with the sphenotics, and its upper posterior edge with the anterior end of the opercle (Figure 7).

Figure 11. Isolated branchial apparatus from: A) Colomesus tocantinensis nov. sp. – Tocantins (PNT.UERJ.404); B) Colomesus asellus – Iquitos (PNT.UERJ.470); C) Colomesus asellus – Belém (PNT.UERJ.386). Scale bar equals 2.5 mm. doi:10.1371/journal.pone.0074397.g011
The palatine is wide and somewhat triangular, with a robust anterior process for the maxilla (Figure 7). The maxilla is robust, with an anterodorsal region articulating with the premaxilla and a posterior expanded region, medially concave for muscle insertion. The ectopterygoid articulates dorsally with the palatine and ventrally with the anterodorsal border of the quadrate. The metapterygoid is wide and composes almost the entire ventral orbital region (Figure 7). It articulates anteriorly with the mesopterygoid (Figure 7), and with the posterior end of the large and triangular quadrate (Figure 7). The quadrate exhibits a well-developed posteroventral spine articulating posteriorly with the slender symplectic (Figure 7), and anteriorly with the articular. The articular is “L” shaped and articulates anteriorly with the robust dentary and ventrally with the small angular (Figure 7).

Five branchiostegal rays (Figure 7) are present and the branchial apparatus is strikingly similar in all the examined specimens (Figure 11).

The pectoral girdle is robust and formed by a wide cleithrum, somewhat triangular and posteriorly expanded, articulating dorsally with the slender supracleithrum. The supracleithrum articulates ventrally with the two postcleithra; a slender dorsal postcleithrum, followed by the posteriorly expanded ventral postcleithrum (Figure 7). There are four radials and sixteen pectoral fin rays (Figure 7).

The axial skeleton has 19 vertebrae. The dorsal fin originates between vertebrae 7–8 and has ten basal pterygiophores and ten fin rays (Figures 12–14). The anal fin is located beneath the 9th vertebra and has six basal pterygiophores and nine fin rays.

The caudal skeleton (Figure 12) has a wide ural centrum formed by the preural centrum 1, the ural centrum, the ventral hypural plate, and the postero-dorsal expansion which articulates anteriorly with the almost triangular epural, and posteriorly with the dorsal hypural plate (Figure 12). Eleven caudal fin rays, five dorsal and six ventral, are present in all of the specimens, both the uppermost and the two lowermost rays are unbranched.

**Phylogeography of the South American Freshwater Pufferfishes**

Although the influence of marine incursions after the Miocene is still under debate, the Caribbean (or Miocene) marine incursion, via the Llanos Basin (Colombia-Venezuela), is well accepted based on both geological and paleontological evidence, suggesting that these incursions may have isolated marine taxa within the western South America freshwater environments [48–52]. This might be the case for the freshwater tetraodontids. As pointed by [53], this scenario predicts that the distribution of the marine sister groups of marine lineages should be related with the Caribbean or western Atlantic, the age of freshwater taxa should be coincident with marine incursions, and the biogeographic congruence should be observed among multiple unrelated taxa, conditions only partially filled by the genus *Colomesus*.

The timing of divergence between the brackish/marine *C. psittacus* and the freshwater *C. asellus* was recently discussed [45], based on a multiple loci approach including both nuclear and mitochondrial markers. The authors dated the split between 2.5-7My, therefore postulating the Miocene marine incursions usually
used to explain the presence of several marine groups within the western Amazon. In this sense, as observed by [45], the colonization carried by the tetraodontids in South America could be presumably related to the Pliocene global climate oscillations. Additionally, the basal split of the *Colomesus* from Tocantins and from Iquitos/Belem agrees with the general area cladogram of neotropical fishes presented by [54] in which the Xingu/Tocantins-Araguaia group was recovered in a basal position in relation to the groups from the lowlands of Western and Eastern Amazon.

It was recently proposed [55], based on the distribution of characiforms, that recent marine incursions would have isolated fish populations in upland terrains or refuges, where lineage divergence is maximized, followed by dispersal episodes back to the lowlands. The “museum hypothesis” predicts that lowlands exhibit a higher number of species, but lower levels of endemism, than highlands, and that the upland refuges would represent areas of high endemism.

Looking on the molecular phylogeny of the serrasalmids *Pygocentrus* and *Serrasalmus*, [56] proposed a phylogenetic test which predicts that basal lineages in a phylogeny of widespread fishes would occur in highland areas, and lowland lineages would have originated only during the last 5 Ma. Additionally, [57] studying the genetics of *Symphysodon* cichlids, indicated the effects that marine incursions would have in population structure, stating that populations in upland terrains or refuges would exhibit reduced genetic variation, while populations in lowlands would represent multiple upland sources, therefore exhibiting a high level of genetic variation, and that populations in lowlands would show a demographic pattern of expansion.

Our results recovered the Upper Tocantins lineages in a basal position in relation to all the remaining specimens, with the sequences being collapsed in uniquely two haplotypes (Figure 5), the first one (h1), represented by eight sequences, and the second haplotype (h2), represented by a unique sequence. This suggests low genetic variation, at least among the studied sampling, and a history initially related with the eastern Amazon, followed by a subsequently expansion to the western South America.

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Figure 13. Left view photographs from cleared-and-stained specimens of: A) *Colomesus tocantinensis* nov. sp. (PNT.UERJ.403); B) *Colomesus asellus* – Iquitos (PNT.UERJ.470); C) *Colomesus asellus* – Belém (PNT.UERJ.386). Scale bar equals 5 mm.

doi:10.1371/journal.pone.0074397.g013

Figure 14. High-definition x-ray images of: A–D, *Colomesus psittacus* USNM.393077; E–G, *Colomesus asellus* USNM.191569. Scale bar equals 10 mm.

doi:10.1371/journal.pone.0074397.g014
The Tocantins-Araguaia Ichthyofauna

The Tocantins-Araguaia drainage is the fourth largest Brazilian drainage, draining part of the northern end of the Brazilian shield directly to the eastern end of the Amazon Basin. It exhibits a recent geomorphological history, within a still tectonically active sedimentary basin with recent subsidence episodes, which are related with the high load of sediments observed within the basin, leading the development of the Bananal Plain, in the lower part of the phylogeny of the taxa of Colomesus, Sphoeroides, and Leporinus.

The timing of divergence between the marine/brackish species Colomesus tocutamensis and the freshwater group formed by C. aestivalis and C. tocutamensis, as recovered by [45], postdates the Miocene marine incursions usually used to explain the presence of tetraodontids within the Amazon freshwater environments. Therefore, it suggests that the freshwater colonization in South America, at least for the tetraodontids, could be more recent than previously expected. Additionally, together with the observed distribution of haplotypes, our results suggest that the history of tetraodontids into the Amazonian freshwater environments could be presumably related to the Pliocene global climate oscillations and its effects inside the eastern Amazon and subsequently to the western South America.

Finally, our results reinforce the Upper Tocantins drainage as an area of high endemism within the Tocantins-Araguaia drainage, although the composite nature of the entire drainage is unquestionable.

Acknowledgments

We would like to thank Dr. James C. Tyler (Smithsonian Institution, Washington) for his support and helpful comments on the manuscript. We are also grateful to Dr. Francesco Santini (Università degli Studi di Torino), Dr. Dorothée Huchon (Tel Aviv University), and an anonymous reviewer for the valuable suggestions during the review of the manuscript. Dr. Leonor Gusmão and Dr. Antonio Amorim (Universidade do Porto) for the comments during the initial discussion of the results, Yuri Modesto (Universidade do Estado do Rio de Janeiro) for the specimens from the Tocantins drainage, Lúcio Paulo Machado and Diogo de Mayrink (Universidade do Estado do Rio de Janeiro) for the specimens from Iquitos; Ms. Sandra Karedon (Smithsonian Institution, Washington) for the x-rays of tetraodontids, Dr. Richard Pyle (Hawaii Biological Survey) for the LSID numbers, and Kleyton M. C. Severiano and Anna Carolina Chaves (Universidade do Estado do Rio de Janeiro) for the technical assistance.

Author Contributions

Conceived and designed the experiments: CRLA PMB DAS EFC. Performed the experiments: CRLA PMB DAS EFC. Analyzed the data: CRLA PMB DAS EFC. Contributed reagents/materials/analysis tools: CRLA PMB DAS EFC. Wrote the paper: CRLA.

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