



Fish collection of the Universidade Federal de Rondônia: its importance to the knowledge of Amazonian fish diversity

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ABSTRACT. Biological collections preserve the biodiversity of a nation. The fish collection of the Universidade Federal de Rondônia, recently established, contains about 41% of freshwater fish species known to occur in Brazil, and 24% of South America. The biological material is distributed into 1,067 species, 21,963 lots and 149,192 specimens, gathered during five years of work. From this collection, 99% of the lots have georeferenced location, and 94% of the species were sampled in the Madeira River basin, the largest tributary in flow, drainage area and sediment discharge of the Amazonas River basin. Among the 1,008 species collected in the Madeira River basin, 80% were reviewed by Brazilian, North American and French experts, and represents the highest richness among tributaries of the Amazonas River and other rivers worldwide. Fish collection from the Madeira River basin deposited in the ichthyological collection of the Universidade Federal de Rondônia accounts for about 75% of the lots and specimens of fish from that basin available for study in collections worldwide.

Keywords: Brazil, freshwater fish, Madeira River basin, high species richness.

A coleção ictiológica da Universidade Federal de Rondônia: sua importância para o conhecimento da diversidade de peixes da Amazônia

RESUMO. Coleções biológicas preservam a biodiversidade de uma nação. A coleção ictiológica da Universidade Federal de Rondônia, recentemente estabelecida, contém cerca de 41% das espécies de peixes de água doce conhecidas para o Brasil e 24% da América do Sul. O material biológico encontra-se distribuído em 1.067 espécies, 21.963 lotes e 149.192 exemplares, organizados durante 5 anos de trabalho. Desse acervo, 99% dos lotes possuem localidade georreferenciada e 94% das espécies foram coletadas na bacia do rio Madeira, o maior afluente em vazão, área de drenagem e descarga de sedimentos da bacia Amazônica. Do total de 1.008 espécies coletadas na bacia do rio Madeira, 80% foram revisadas por especialistas brasileiros, norte-americanos e franceses, e constitui a maior riqueza de espécies conhecida para um afluente do rio Amazonas e entre outros rios do mundo. O acervo de peixes da bacia do rio Madeira depositado na Coleção de peixes da Universidade Federal de Rondônia representa cerca de 75% dos lotes e de exemplares de peixes dessa bacia disponíveis para estudos em coleções ictiológicas do mundo.

Palavras-chave: Brasil, peixes de água doce, bacia do rio Madeira, elevada riqueza de espécies.

Introduction

According to the Statute of Museums enacted in 2009 (Brazilian Law n. 11904), museums are:

[...] nonprofit institutions that preserve, investigate, communicate, interpret and exhibit, for purposes of preservation, study, research, education, contemplation and tourism, sets and collections of historical, artistic, scientific and technical value or of any other cultural nature, open to the public, at the service of society and its development (BRASIL, 2009).

Within this context and holding all these numerous possibilities, museums of natural history, including biological collections, have been active primarily in the storage, preservation and classification of collections of specimens, playing a major role in representing the biological diversity (ZAHER; YOUNG, 2003; SUAREZ; TSUTSUI, 2004). The documentation of biodiversity is considered highly relevant, since collections also permit to demonstrate environmental changes over time through comparisons of the existing fauna to that of former times (PONDER et al., 2001;

BAIRD, 2010). Thus, the absence of voucher specimens of flora and fauna in biological collections may lead to a potentially irreversible loss of knowledge.

Regional biological collections are of unquestionable importance as regards to knowledge of biological diversity. If, on the one hand, national museums hold a broader biological representation of a nation or continent, regional collections commonly maintain more representative and detailed collections of current and past biological diversity of a particular region. Information available on biological collections can be essential for decisions relating to biodiversity conservation by the government (PONDER et al., 2001). Defining priority areas or species for conservation and recovery are examples of measures likely to be taken in line with a review of the information contained in a collection. Furthermore, predictions of impacts of human activities, such as the construction of hydroelectric plants, deforestations, and climatic change can be made from sets of information obtained from scientific collections.

This work discusses the importance of the fish collection of the Universidade Federal de Rondônia (UNIR) in the context of actions for the preservation of natural resources of the Amazon, as a source of material and information for assessment of megadiverse natural systems, and as a tool for regional development.

Material and methods

The ichthyological collection of UNIR (Figure 1), acronym UFRO-I, built by Santo Antônio Energia, as a compensation for the grant of use of hydropower of the Madeira River, has an area of 301 m², black walls with thermal insulation, special floor to support the weight of the vials containing the preserved lots, smoke detection system, fire door, and fire-fighting and cooling system. The lots are taxonomically arranged according to the classification of Reis et al. (2003), with the genera and species displayed in alphabetical order. Most of the deposited material is stored in 70° GL ethanol, and some cleared and stained specimens are maintained in 90% glycerol. Large specimens are kept in 50–200 L plastic drums. The vast majority, however, consists of specimens of small to medium size, kept in 30–5000 mL glass bottles.

Material cataloging is performed using the *Specify 6* software, which was designed to catalog and manage collections. Information used herein was obtained from the Fish Collection database in December 2013. Curatorial area is provided with

five computers connected to a server for simultaneous cataloging of different lots. Labels are printed with indelible ink (*DuraBrite Epson*[®]), which makes the text resistant to discoloration even when the label is immersed in liquid. The paper used for the manufacture of labels (*Resistall Paper*[®]) has cotton fibers and is treated for longer durability and high humidity resistance.



Figure 1. External view of the building of the Coleções Zoológicas e Laboratório Integrados (A) and internal view of the fish collection of the Universidade Federal de Rondônia (B).

The fish collection maintains a collection of biological tissues stored in two freezers. Most tissues taken from muscles, barbels or fins come from vouchers fish specimens deposited in the collection. The material is individualized in 2 mL *ependorf* tubes and systematically arranged within boxes. Ethyl alcohol (100% PA) is the most used product for the conservation of tissue samples.

Species deposited in the collection were identified primarily with the support of specialized literature. However, taxonomic reviews for most families were done by taxonomist researchers, either by sending material to researchers or by visits to the collection.

To ensure the quality of the material deposited in the UFRO-I, we developed a protocol that allows the preservation and conservation of small and medium-sized fish without deformations in the body and maintains optimal concentration of alcohol in the lot. This activity is performed simultaneously with the taxonomic identification of specimens. The protocol included the following steps:

1-Removal of formaldehyde: after two or three days of fixation in 10% formalin, removal of fish samples from the solution, placing them in plastic trays with water for about 30 minutes for large specimens, or 15 min. for small specimens.

Thereafter, exchange of the water at least three times;

2-Sample screening: removal of the water from the tray and grouping the individuals according to species. Moisture of this material is constantly monitored;

3-Identification: after sorting with the naked eye, species identification is performed. For each set of specimens of the same species, a label on tracing paper containing the information required for the cataloging is done;

4-Packaging: in this phase, specimens of the same species are anatomically positioned in straight inside a rigid and perforated plastic bag, in which the label is also inserted. Subsequently, fish are transferred to a glass bottle with 75° GL alcohol, so as to ensure that even after the introduction of the biological material the concentration of alcohol is not less than 70° GL;

5-Rest: fish remain in alcohol for five or more days, until the occurrence of stiffening and establishment of the straight anatomical position of specimens;

6-Cataloging: the alcohol used during the resting phase is replaced with a new solution having 70° GL concentration. The lot data are then inserted into a database, which generates a cataloging number so that each lot is institutionalized.

From the moment that the material receives a catalog number, it becomes available for various activities, including loans, exchanges or donations to other institutions.

The acronyms of museums or institutions that have loaned specimens follow Reis et al. (2003) for INPA, MZUSP, MCP, MPEG, MNRJ and UFPB except for NUP (Coleção Ictiológica do Núcleo de Pesquisas em Limnologia, Ictiologia e Aquicultura da Universidade Estadual de Maringá), MZUEL (Museu de Zoologia da Universidade Estadual de Londrina), DZSJRP (Departamento de Zoologia e Botânica da Universidade Estadual Paulista), LIRP (Laboratório de Ictiologia de Ribeirão Preto da Universidade de São Paulo), UCF (University of Central Florida), UFAM (Universidade Federal do Amazonas) and UFRS (Universidade Federal do Rio Grande do Sul).

To evaluate the importance of the UNIR Fish Collection, the number of lots and specimens deposited in that institution were compared to the total of lots and samples collected to date in the Madeira River basin and maintained in 19 of the major fish collections in the world. The data for this comparison were obtained by consulting the database repositories on the Internet (SpeciesLink and Neodat), as well as through direct consultation with the curators of the collections (data kindly provided by Dr. Roberto E. Reis).

Results

Fish specimens deposited in the UFRO-I consists of 149,192 individuals belonging to 1,067 species distributed in 21,963 lots. Among the cataloged species, 1,008 (94%) originated from the Madeira River basin in the Brazilian territory, the others originated from the rivers Purus, Tapajós and Paranapanema.

All lots from the Madeira River basin deposited in the UFRO-I are originated from different locations in the states of Mato Grosso, Rondônia and Amazonas (Figure 2). They came specifically from the rivers Guaporé, Mamoré, Abunã, Machado, Jaciparaná Marmelos, Manicoré, Aripuanã and from the main channel of the Madeira River. There are records of lots from lakes (Cuniã, Puruzinho and Sampaio), streams (igarapés) and small rivers that flow directly into the Madeira River (Araras, São Lourenço, Mutumparaná, Karipunas, Caracol, Jatuarana I, Jatuarana II, Bate-Estacas, Belmont, Candeias and Preto), and also from the rapids of Santo Antônio, Teotônio and Jirau, currently submerged by the Santo Antônio and Jirau Hydroelectric Power Plant reservoirs (UHEs).

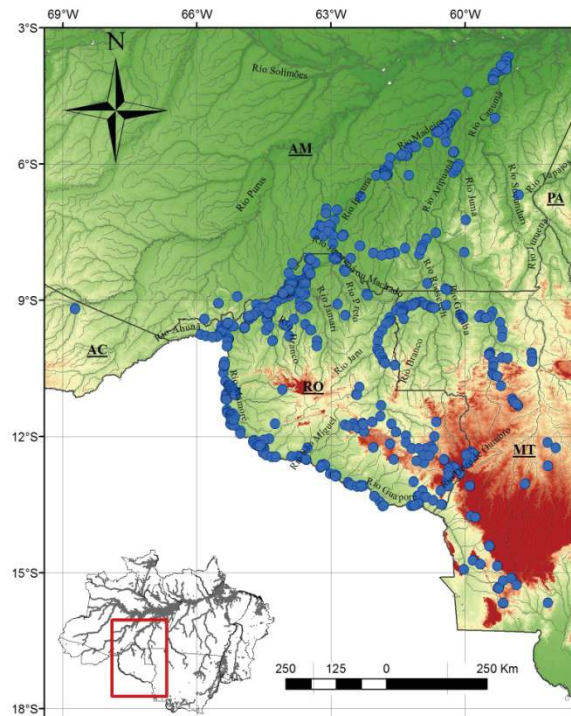


Figure 2. Fish deposited in UFRO-I with the main localities of collection in the Madeira River basin in Brazilian territory, including the States of Mato Grosso, Rondônia and Amazonas. Each dot on the map (blue) may represent more than one collecting event.

Most lots are represented by few specimens: approximately 50% (10,817) have only one

specimen; 14% (3,094) have two; and 7.5% (1,656) have three specimens. Lots with four or more specimens represent less than 26.9% of the material cataloged. Among the cataloged lots, the order Characiformes represents about 51% (10,972 lots); Siluriformes, 28% (6,048); Perciformes, 10% (2,232); Gymnotiformes, 7% (1,564) and the other orders together add up to 3% (651). Among the most representative families, Characidae represents 30% (6,386 lots), Loricariidae 10% (2,074), Cichlidae 9% (2,009), Pimelodidae 5% (991) and Serrasalminidae 4% (861).

The two species with the largest number of lots are piabas or lambaris (Characidae): *Hemigrammus* cf. *bellottii* (Steindachner, 1882) with 217 lots and *Moenkhausia collettii* (Steindachner, 1882) with 204 lots. Traíra (Erythrinidae) *Hoplias malabaricus* (Bloch, 1794) has 194 lots, acará (Cichlidae) *Apistogramma resticulosa* Kullander, 1980 has 174 lots and acará bicudo (Cichlidae) *Satanoperca jurupari* (Heckel, 1840) 173 lots.

The total of 1,067 species included 42 from the Juruena River, Tapajós River basin; ten from the Ipixuna River, Purus River basin, and seven species from the Paranapanema River. Among the species deposited, approximately 801 (80%) were reviewed by expert taxonomists. Of the 21,963 lots cataloged, only a small fraction (7.5%) was collected before 2008. The oldest specimen in the collection is a specimen of *Hypostomus* cf. *plecostomus* (Linnaeus, 1758) (UFRO-I 6258), dated April 11st, 1997 and collected in the Ouro Preto River, a tributary of the Pacaás Novos River, Guaporé River drainage.

The fish collection has 1,242 tissue samples from 385 species. Characiformes represents 45% (562 samples), Siluriformes, 34% (425), Perciformes, 9% (116) and Gymnotiformes, 8% (95). The most representative families in number of samples are Characidae with 14% (173 samples) and Loricariidae 13% (153 samples). Following these, Cichlidae (103), Serrasalminidae (103) and Pimelodidae (98) represent about 8% each.

The protocol used for fixation and conservation of cataloged fish allows small and medium-sized specimens to remain indefinitely in a straight position. This protocol is recommended for all species, especially those having a compressed and elongated body, such as Gymnotiformes, Engraulidae, Acestrorhynchidae, Ctenoluciidae and Cynodontidae, as well as for species that have a long, cylindrical body, such as Trichomycteridae, Heptapteridae, Lebiasinidae, Parodontidae and some Crenuchidae.

Since 2009, 89 formal loans to 15 institutions, involving a total of 4,300 specimens were made. Fish collections of INPA, MZUSP and NUP concentrated 56.7% of total loans and 74.6% of the total loaned specimens. Part of this material can be donated, retained or bartered for fish identification, and also as a strategy to maintain replicates of part of the UFRO-I collection safely in other institutions.

All fish lots and specimens deposited in the UFRO-I collection were compared to the total lots of fish from the Madeira River basin deposited in the 19 other surveyed collections. The total number of fish lots from the Madeira River available in the 20 collections (including UFRO-I) was 29,716, representing 9.7% of the Brazilian fish lots in these collections (306,189 lots). Also, the total number of specimens of this basin in these lots was 196,663, representing 6.3% of the total specimens of fish from Brazil in 20 collections (3,110,615 specimens). These numbers indicate UFRO-I collection contains almost three times more specimens from the Madeira River basin than all other collections together, i.e., about 75% of the lots and specimens of this basin in the Brazilian territory are available for study at that institution.

Discussion

The history of exploitation of natural resources in the Rondônia State is strongly related to the extraction of rubber and the construction of the Madeira-Mamoré Railway during the last century (FERNANDES; GUIMARÃES, 2002). However, most recently, the environmental landscape of Rondônia was marked by a very high population growth associated with the construction of the BR 364 Highway. Consequently, logging, mining, agriculture and cattle ranching resulted in massive deforestation in the Rondônia State (PEDLOWSKI et al., 2005).

Biological records from that time in the form of voucher material deposited in collections in the state have never been performed. For a long time, the knowledge of the fish fauna of Rondônia was limited solely to the work of Fowler (1913). The most recent studies in the Madeira River basin were conducted in the 1990's in smaller portions of the basin (SANTOS, 1995; RAPP PY-DANIEL et al., 2007; CAMARGO; GIARRIZO, 2007; PEDROZA et al., 2012), but material resulting from these studies was not deposited in the collections of institutions in the Rondônia State. With the implementation of the Ichthyofauna Conservation Program and the establishment of the fish collection at UNIR, the material started to remain in the state,

and new works related to the basin started to be developed (TORRENTE-VILARA et al., 2011; SOUSA; BIRINDELLI, 2011; OHARA, 2012; MARINHO; OHARA, 2013; QUEIROZ et al., 2013a; QUEIROZ et al., 2013b; OHARA; LIMA, 2015). Concurrently, many undergraduate students of UNIR and other state institutions can use this collection to carry out their thesis and dissertation projects, and researchers began to visit and examine the material from the collection, and various loans, donations and exchanges occurred among institutions. The whole process resulted in an accurate recognition of a large number of fish species in the basin.

The remarkable richness of the Madeira River deposited in the UFRO-I collection reflects one of the greatest peculiarities of the Amazon: a high biological diversity and low levels of endemic species. In fact, among the rivers known for having the largest number of species in the world, most are concentrated in the Amazon basin (Table 1).

Table 1. Number of fish species from the Madeira River basin deposited at UFRO-I compared to other large tropical rivers of the world.

Drainage	Richness	Reference
Rio Madeira	1,008	This study
Rio Orinoco	658	Maldonado-Ocampo et al. (2008)
Rio Branco	584	Ferreira et al. (2007)
Rio Congo	560	Roberts (1972)
Rio Tocantins	520	Lima and Caires (2011)
Rio Xingu	467	Camargo et al. (2004)
Rio Negro	450	Goulding et al. (1988)
Rio Yangtze	361	Fu et al. (2003)

The Madeira River, when compared to the largest rivers of the world, has greater richness of fish (1,008) than the Nile [129 species (LÉVÊQUE et al., 1991)], Yangtze [361 species (FU et al., 2003)] and Mississippi [250 species (ROBERTS, 1972)] together. Rivers with higher discharge, such as the Congo [560 species (ROBERTS, 1972)], Ganges-Brahmaputra [143 species (SARKAR et al., 2012)] and Yangtze [361 species (FU et al., 2003)], do not exceed the species richness known for the Madeira River in the Brazilian territory. The number of species currently known for the studied portions of the Madeira River demonstrably recorded in the UNIR fish collection outnumbers the known species richness of Europe, Russia and Oceania together (Table 2). A compilation of information on the occurrence of fish species in the Madeira River basin in the Bolivian and Peruvian territories suggest that the Madeira River basin has at least 1,300 species (our pers. obs.). This number represents approximately 50% of the species currently known in the Amazon River basin (LÉVÊQUE et al., 2008;

ALBERT et al., 2011). This study exemplifies, through the fish species deposited in the UFRO-I collection, the importance of a regional collection as a source of relevant information for the conservation of the Amazonian biodiversity.

Table 2. Number of freshwater fish species surveyed in the Brazilian stretch of the Madeira River, and freshwater fish species richness known by continents or country (LÉVÊQUE et al., 2008).

Continents	Richness
Rio Madeira	1,008
South America	4,035
Asia	3,553
Africa	2,945
North America	1,411
Europe	330
Oceania	260
Russia	206

One of the largest and most renowned fish collections in the Amazon is the Instituto Nacional de Pesquisas da Amazônia (INPA), Manaus, Amazonas State, with 40,000 lots and about 1,200 species (RAPP PY-DANIEL, pers. com). The collection of the Museu Paraense Emílio Goeldi (MPEG), with 28,000 lots and about 250 thousand specimens (WOSIACKI, pers. com.) is the second largest fish collection in the Amazon region. The high biodiversity of the Amazon stands out as one of the main characteristics of the region, thus the low number of scientific collections located in the Amazon is worrisome. Likewise, the limited financial resources for hiring specialized professionals and maintaining collections are unacceptable in face of the accelerated environmental changes of the region.

Although being a regional and still emerging collection, the importance of UFRO-I represents 41% of the species known in Brazil and listed in Buckup et al. (2007), and 24% of the entire freshwater fish diversity described for Central and South America reported by Reis et al. (2003). Over 80% of the species deposited in the collection were reviewed by North American, Brazilian and French experts, from 2009-2012, which allowed the preparation of the book "Peixes do rio Madeira" (QUEIROZ et al., 2013a). This initiative also resulted in high accuracy in the identification of the collection and recognition of 80 potentially new species, coming mainly from tributaries in the periphery of the Madeira River basin (and under severe threat by human impacts). In this context, the fish collection of UNIR is likely one of the collections in the country with the highest accuracy in taxonomic identification of the deposited lots. Also noteworthy is the number of species with tissue samples preserved and available for molecular studies.

An evaluation of the collection sites of the lots deposited at UFRO-I (Figure 2) indicates a broad sampling coverage in the Madeira River basin, whose outermost points are more than 1,500 km far from each other. Currently, among all Brazilian collections, UFRO-I has the largest representativeness of cataloged fish lots from this river basin and there are still about 15,000 preserved lots to be cataloged. Thus, it is possible that the Madeira River, together with the Tocantins River, is one of the best-sampled tributary of the Amazon basin, due to the large number of hydroelectric projects and environmental studies (AGOSTINHO et al., 2009).

Lots with one or two specimens predominate in the collection and represent 64% of all cataloged lots. The dominance of Characiformes and Siluriformes in freshwaters of the Neotropical region (LOWE-MCCONELL, 1999) is represented among the lots of the collection. Within these two main orders, Characidae and Loricariidae are the most diverse families. In addition, among all fish families in the world, Characidae and Loricariidae represent the fourth and fifth families with the highest number of described species, respectively (NELSON, 2006).

One of the most important aspects in collections consists of the preparation and preservation of the biological material so that it does not degrade and remains viable for study for hundreds of years. The protocol used at UFRO-I increases the quality of preservation by maintaining the prescribed concentration of alcohol and allowing the specimens to remain in proper anatomical position. The position of the fish can decisively affect biometrics for purposes of taxonomic and ecological studies. The adequate position facilitates visualization and counting of external morphological structures; allows a better utilization of the internal space of the bottle where the lot is stored; and prevents certain structures from breaking with rough handling. Curatorial activities commonly involve cataloging the newly collected fish. Nevertheless, newly collected fish retain a lot of water in the tissues. When immersed in 70° GL alcohol, the loss of water by natural dehydration of the specimens results in dilution of the solution. This fact, besides the natural evaporation of alcohol, can dramatically decrease quality and shelf life of the material.

Conclusion

UFRO-I is the largest and most complete collection of fish from the Madeira River basin. Unfortunately, in many regional development

projects being conducted in the Amazon, most of the biological material collected is improperly identified, and sometimes specimens are discarded. The UFRO-I collection demonstrates the importance of professional involvement and the quality of environmental studies for the appropriate knowledge of the biological diversity of the Amazon. Thus, the maintenance of this collection is a pivotal measure for the development of research, education/training, and an essential attitude towards the knowledge and conservation of this valuable natural heritage of the country.

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