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Marcos Santos Silva

Redescoberta do material-tipo das espécies de planárias terrestres
(Platyhelminthes) de Schirch e sua revisão taxonômica através de histologia e
microtomografia computadorizada

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(Platyhelminthes) species and their taxonomic revision through histology and
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Dedicatória

Dedicado, genuinamente,
a Maytê e Martin.

O que é o meu ideal senão o Supremo Impossível
Aquele que é, só ele, o meu cuidado e o meu anelo
O que é ele em mim senão o meu desejo de encontrá-lo
E o encontrando, o meu medo de não o reconhecer?

Vinicius de Moraes, *A vida vivida*
(Novos poemas, 1938)

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Em 2016, descobrimos que o material-tipo de 10 das 15 espécies de planárias terrestres (Platyhelminthes, Tricladida, Geoplanidae) brasileiras descritas por Schirch (1929) não estava perdido, mas sim depositado no Museu Nacional do Rio de Janeiro (MNRJ). Schirch somente descreveu a morfologia externa destas espécies. Originalmente no gênero *Geoplana*, as 15 espécies sofreram revisão taxonômica e atualmente são conhecidas como *Issoca rezendei*, *Obama itatiayana*, *Pasipha plana*, *Paraba goettei*, *Pseudogeoplana arpi*, *Ps. bonita*, *Ps. blaseri*, *Ps. bresslaui*, *Ps. cardosi*, *Ps. doerderleini*, *Ps. lumbricoides*, *Ps. obscura*, *Ps. riedeli*, *Ps. theresopolitana* e *Ps. wetzeli*. Ainda, *Geoplana maximiliani* sensu Schirch, 1929 foi renomeada como *Ps. schirchi* Ogren & Kawakatsu, 1990. *Pseudogeoplana* foi proposto para espécies de Geoplaninae cuja morfologia interna se desconhece, especialmente a do aparelho copulador, órgão fundamental para a determinação inequívoca das espécies.

Neste trabalho fizemos uma revisão taxonômica das espécies cujo material-tipo ainda existe, ou seja, *Pasipha plana*, *Pseudogeoplana arpi*, *Ps. bonita*, *Ps. blaseri*, *Ps. bresslaui*, *Ps. doerderleini* e *Ps. wetzeli*. Excluímos do estudo *Obama itatiayana* por não atender aos requisitos de análise adotados (existência de material-tipo maduro e espécie não revisada anteriormente) e *Issoca rezendei* por ser objeto de estudo de outra dissertação. Estudamos o material-tipo e espécimes adicionais coletados recentemente de algumas das espécies. Obtivemos os dados morfológicos a partir de preparações histológicas e, quando viável, a partir de cortes virtuais obtidos por microtomografia computadorizada de raios-X (mCT), técnica que não destrói os espécimes.

Esta abordagem resultou na preservação do corpo inteiro de, pelo menos, um parátipo de cada espécie e do holótipo, por monotipia, de *Ps. bresslaui*. Os espécimes-tipo de algumas espécies são jovens, sem aparelho copulador desenvolvido (*Ps. arpi*, *Ps. doerderleini* e *Ps. wetzeli*); os de outra espécie não pode ser estudado em detalhe por conta da preservação deficiente (*Ps. doerderleini*, Capítulo 1). Nesta revisão taxonômica, propomos a mudança de gênero para duas espécies: *Paraba bresslaui* (Capítulo 1) e *Imbira bonita* (Capítulo 2). Dadas as semelhanças entre *Geoplana goetschi* Riester, 1938 e *I. bonita* (Capítulo 2), também foi estudado o material tipo e espécimes adicionais de *G. goetschi*. O estudo levou à conclusão de que *G. goetschi* é sinônimo júnior de *I. bonita* (Capítulo 2). Por último, também descrevemos uma espécie nova de *Imbira*, simpátrica com *I. marcusii*, e propomos

uma nova diagnose para este gênero (Capítulo 2). A mCT foi uma técnica valiosa para o estudo sistemático de várias espécies de Schirch sem destruir os espécimes-tipo.

Palavras-chave: Morfologia; Taxonomia; Tricladida; Geoplanidae; *Pseudogeoplana*

In 2016, we found that the type-material of 10 of the 15 Brazilian land planarians (Platyhelminthes, Tricladida, Geoplanidae) described by Schirch (1929) were not lost but deposited in the Museu Nacional do Rio de Janeiro (MNRJ). Schirch only described the external morphology of these species. Originally in the genus *Geoplana*, the 15 species underwent taxonomic revision and their current names are *Issoca rezendei*, *Obama itatiayana*, *Pasipha plana*, *Paraba goettei*, *Pseudogeoplana arpi*, *Ps. bonita*, *Ps. blaseri*, *Ps. bresslaui*, *Ps. cardosi*, *Ps. doederleini*, *Ps. lumbricoides*, *Ps. obscura*, *Ps. riedeli*, *Ps. theresopolitana*. Also, *Geoplana maximiliani* sensu Schirch, 1929 was renamed as *Ps. Schirchi* Ogren & Kawakatsu, 1990. *Pseudogeoplana* was proposed for species of Geoplaninae whose internal morphology is unknown, especially that of the copulatory apparatus, organ essential for the unequivocal determination of the species.

In this work we carried out a taxonomic revision of the species having type-material available, namely *Pasipha plana*, *Pseudogeoplana arpi*, *Ps. bonita*, *Ps. blaseri*, *Ps. bresslaui*, *Ps. doederleini* and *Ps. wetzeli*. We excluded from the work *Obama itatiayana* for it does not fulfill the requirements adopted (both existing mature type-material, and species not revised previously) and *Issoca rezendei*, since this species is currently being studied elsewhere. We studied the type-material and additional specimens collected recently, of some of the species. We obtained the morphological data from histological preparations and, when feasible, from virtual sections obtained through X-ray computed microtomography (mCT), a technique that does not destroy the specimens. This approach resulted in the preservation of the entire body of, at least, one paratype of each species, and the holotype, by monotypy, of *Ps. bresslaui*. The type specimens of several species are young, without copulatory apparatus (*Ps. arpi*, *Ps. doederleini* and *Ps. wetzeli*), or are poorly preserved, and hence could not be studied in detail (Chapter 1). As a result of the taxonomic revision, we propose the transfer of two species to other genera: *Paraba bresslaui* (Chapter 1) and *Imbira bonita* (Chapter 2). Given the similarities between *Geoplana goetschi* Riester, 1938 and *I. bonita* (Chapter 2), we also studied the type-material and additional specimens of *G. goetschi*. The study led us to the conclusion that *G. goetschi* is a junior synonym of *I. bonita* (Chapter 2). Finally, we also describe a new species of *Imbira*, sympatric with *I. marcusii*, and propose a new diagnosis for this genus (Chapter 2).

mCT was a valuable technique for the systematic study of several type-specimens of Schirch's species without destroying them.

Key words: Morphology; Taxonomy; Tricladida; Geoplanidae; *Pseudogeoplana*

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Abbreviations used in figures

- (ab) annular-shaped muscle bands
- (c) cilia
- (cg) cerebral ganglion
- (cm) circular muscle
- (cmc) common muscular coat
- (cn) cutaneous nerve net
- (co) common glandular ovovitelline duct
- (cs) creeping sole
- (de) epithelium dorsal
- (dd) decussate diagonal fiber
- (dm) decussate muscle fiber
- (dv) dorso-ventral parenchymal muscle
- (e) eye
- (ef) epithelium of female atrium
- (ej) ejaculatory duct
- (es) esophagus
- (f) fold
- (fa) female atrium
- (g) gonopore
- (gl) glands
- (i) intestine
- (lc) longitudinal cutaneous muscle
- (lm) longitudinal muscle
- (lp) longitudinal parenchymal muscle
- (m) muscle
- (ma) male genital atrium
- (mo) mouth
- (ns) nervous system
- (o) ovary

(ov) ovovitelline duct
(ph) pharyngeal pouch
(pp) penis papilla
(pi) sensory pit
(pv) prostatic vesicle
(sb) subintestinal transverse parenchymal muscle
(sd) sperm duct
(sg) shell glands
(sp) suprainestinal transverse parenchymal muscle
(t) testis
(ve) ventral epithelium
(vi) vitellaria
(vn) ventral nerve plate

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Presentation

Land planarians (Geoplanidae, Tricladida, Platyhelminthes) are free-living flatworms that present diagnostic characters such as a dorsoventrally flattened body, three main intestinal branches, and incomplete digestive system. Land planarians are relatively fragile animals, sensitive to degradation of their environment and limited to humid microhabitats (Kawaguti, 1932; Carbayo et al., 2002; Carbayo et al., 2008). They are predators of a wide range of invertebrates but there is considerable variation in food preference among them (Winsor et al., 2004; Cseh et al., 2017). Some species are specialized in a single prey species; other land planarians are generalist and prey upon snails, earthworms and harvestmen (Silva et al., 2018).

They have been studied for over two centuries by authors including renowned researchers such as Charles Darwin (1844), Fritz Müller (1857), H. N. Moseley (1877), and Ludwig von Graff (1899). The number of terrestrial species known in the world is ca. 822 (Sluys, 1999), of these, approximately 313 belong to Geoplaninae (Carbayo, 2019), a group with Neotropical distribution. Geoplanidae includes also another three subfamilies, namely Bipaliinae, Microplaninae, Rhynchodeminae (including the tribes Rhynchodemini, Caenoplanini, Anzoplanini, Eudoxiatopoplanini, and Pelmatoplaninini).

As with other groups, methods for the taxonomic study of land planarians have evolved accordingly with development of new techniques and equipment. From descriptions of species based upon external aspect only to the current taxonomic works integrating morphological-histological and molecular (ADN) aspects. Some descriptions of land planarians of the 19th and early 20th centuries were exclusively based upon the external aspects of the animals (Darwin, 1844; Schultze & Müller, 1857; Schirch, 1929). Graff (1899) and Riester (1938) described a number of species based in their external aspect only other species were described detailing morphological observations of the internal organs. Currently, descriptions based only on the external appearance would not be welcome by the scientific community for they would generate taxonomic uncertainties. In fact, species whose internal morphology remains unknown, specially that of the copulatory apparatus, are considered in doubtful taxonomic position (Ogren & Kawakatsu, 1990; Carbayo & E. M. Froehlich, 2008). For these situations were proposed the collective genera *Anisorhynchodemus* Kawakatsu, Froehlich, Jones, Ogren & Sasaki, 2003 (Rhynchodemini, Rhynchodeminae), *Australopacifica* Ogren & Kawakatsu, 1991

(Caenoplanini, Rhynchodeminae), *Diversibipalium* Kawakatsu, Ogren, Froehlich & Sasaki, 2002 (Bipaliinae), and *Pseudogeoplana* Ogren & Kawakatsu, 1990 (Geoplaninae).

These genera are nomenclaturally valid, but a non-natural genera, since each one was created, within the respective subfamily with the purpose of grouping the species whose internal morphology - especially the copulatory apparatus- is insufficient to determine and classify them. Species in these collective genera lack a throughout taxonomic revision. An important issue concerns preservation of type-material. There are cases in which type-material went lost even before deposition in a museum, as with Geoplaninae Darwin's and Schirch's species (Schirch, 1929; Almeida et al., 2018). Earlier researchers habitually kept collections in their laboratories where they also housed, eventually, specimens type and non-type specimens. The fate of this material over time is not always known and many cases deposition of type-material was not done.

Anatomical and histological details of the copulatory apparatus are of fundamental value for determining species. These details are traditionally observed in histological sections extended on glass slides with the help of a light microscopy. Even so, some cryptic species have recently been revealed as independent evolutionary lineages with the help of molecular data (e. g., Carbayo et al., 2017).

Details of the internal anatomy of the land planarians such as those related with the cutaneous and parenchymatic muscular systems, the sensory organs, the pharynx and, especially, the copulatory apparatus, are fundamental for the identification of the species and the systematics of the group.

Taxonomy of land planarians and historical Schirch's species

Schirch (1929) described 15 new species from only their external aspect and placed them in *Geoplana* Schultze & Müller, 1857 (Geoplaninae). Four of Schirch's species were revised taxonomically through the examination of additional, mature specimens: *Issoca rezendei*, *Obama itatiayana*, *Paraba goettei* and *Pasipha plana* (Marcus, 1951; Froehlich, 1955a; Carbayo et al., 2013). The remaining eleven species were transferred to the genus *Pseudogeoplana* (Ogren & Kawakatsu, 1990).

Type material of Schirch's species was never reexamined. Moreover, until recently, a consensual thought among the Brazilian researchers was that this type-material were lost. This idea was born in the mind of Ernest Marcus and was transmitted to his followers (E. M. Froehlich, pers. comm.). However, a request by email, done by F. Carbayo to the curator of the Museu Nacional do Rio de Janeiro (MNRJ; Schirch published his paper in the journal of this museum), retrieved an unexpected answer: most of

Schirch's syntypes are deposited in the MNRJ, namely *Issoca rezendei*, *Obama itatiayana*, *Pasipha plana*, *Pseudogeoplana arpi*, *Ps. bonita*, *Ps. blaseri*, *Ps. bresslaui*, *Ps. doerderleini*, *Ps. schirchi* and *Ps. wetzeli*.

Type-material of *Pseudogeoplana cardosi* (Schirch, 1929), *Ps. goettei* (Schirch, 1929) and *Ps. riedlei* (Schirch, 1929) was lost before deposition (Schirch, 1929). From *Ps. lumbricoides* (Schirch, 1929); *Ps. obscura* (Schirch, 1929) and *Ps. theresopolitana* (Schirch, 1929) there is no material in the Museum (Guilherme Muricy, pers. comm.). The remaining species (i.e., *Issoca rezendei*, *Obama itatiayana*, *Pasipha plana*, *Ps. arpi*, *Ps. blaseri*, *Ps. bonita*, *Ps. bresslaui*, *Ps. doerderleini* and *Ps. wetzeli*) there remain still preserved the holotype or some syntypes.

The main objective of this master thesis is to conduct a taxonomic revision of Schirch's species gathering two conditions: type material is still available, and this material includes at least one specimen mature. The holotype by monotypy of *O. itatiayana* is not mature. The internal morphology of this species is known through a the study of additional specimens (Riester, 1938). The internal morphology of *Issoca rezendei* is also known from the study of further, mature specimens (Froehlich, 1955a; Marcus, 1951). Syntypes are mature but were herein not examined for they are being examined elsewhere. In view of the importance of these specimens and of the fact that we master the non destructive technique of microcomputed tomography (mCT), we made an effort to explore this technique over traditional histological procedure whenever possible.

Main results

It is the first time anyone studies the type material of these species after Schirch. The species were studied by means of integrating morphological data obtained with mCT and with traditional histology. One species is described entirely from mCT-derived images, so that the holotype was fully preserved in ethanol. Other five species were redescribed from both histological slides and mCT-derived images combined. Not all specimens were sexually mature. Due to the poor preservation of other specimens neither histological slides not mCT-derived images provided practically any additional information of the internal structures.

Our study is structured in two chapters. Cohesion of the results presented within each chapter motivated us to organize them separated into two chapters. In Chapter 1, we revise the taxonomy of six species (Tab. 1) and reflect on the trade-off between traditional histology and m-CT techniques.

Table 1. Species taxonomically revised in chapter 1, nomenclatural changes proposed and comments on the changes.

Current species name	New name	Comment
<i>Pseudogeoplana bresslaui</i> (Schirch, 1929)	<i>Paraba bresslaui</i> (Schirch, 1929)	Holotype fully mature
<i>Pasipha plana</i> (Schirch, 1929)	-	Syntypes fully mature
<i>Ps. arpi</i> (Schirch, 1929)	-	Syntypes immature
<i>Ps. blaseri</i> (Schirch, 1929)	-	Mislabeled, holotype lost
<i>Ps. doederleini</i> (Schirch, 1929)	-	Syntypes poorly preserved
<i>Ps. wetzeli</i> (Schirch, 1929)	-	Syntypes immature

In Chapter 2, we redescribe in detail *Ps. bonita* (Schirch, 1929). The type specimens are immature but fortunately they present a combination of rare morphological attributes also found in mature specimens collected by us in the type locality. We conclude they are conspecific. Features of this species almost fully match diagnosis of *Imbira* Carbayo et al., 2013. Therefore, we transfer the species to *Imbira*. The external and internal aspects of *Imbira bonita*, including the copulatory apparatus are identical to that of *Geoplana goetschi* Riester, 1938, from the type locality. Therefore, we consider Riester's species a junior synonym. In this chapter, we also describe a new species of *Imbira*, collected in São Paulo/SP, and which presents the same rare morphological attributes found in *I. bonita*. These rare attributes made us to propose a revision of the diagnosis of *Imbira* genus.

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Introduction

Land planarians (Geoplanidae, Tricladida, Platyhelminthes) are free living flatworms with a dorso-ventrally flattened body (Graff, 1899; Carbayo, 2005, Sluys & Riutort, 2018). They live in the soil in humid environments, sheltering under rocks and fallen logs and leaves (Carbayo, 2010; Lemos et al., 2014). They are top predators in their microhabitat and feed on other invertebrates (Froehlich, 1966; Ogren, 1995).

The species number of land planarians known in the world is ca. 920; of these, approximately 180 species are known from Brazil (Almeida et al., 2018, Amaral et al., 2018, Lago-Barcia & Carbayo, 2018, Leal-Zanchet & Marques, 2018). Most of these species were described throughout the 20th century and several of them are in a dubious taxonomic position and need revision (Ogren & Kawakatsu, 1990; Carbayo & E. Froehlich, 2008).

Almost all the land planarians of the Neotropical region belong to the subfamily Geoplaninae, which is a monophyletic group (Carbayo et al., 2013). The subfamily is divided into 24 genera, among them, the collective group *Pseudogeoplana* Ogren & Kawakatsu, 1990. This group houses species whose known morphology is insufficient to determine and classify them. Important taxonomic features of land planarians include the organization of the cutaneous and parenchymal muscular systems, distribution of sensory pits, and the morphology of the pharynx and, mainly, of the copulatory apparatus. This apparatus in species of *Pseudogeoplana* remains unknown because of the immaturity of type specimens or simply they were not histologically sectioned and studied.

Schirch's work

In the year 1929, Paulo F. Schirch published a work on Brazilian land planarians. He described 15 new species, which were originally housed in *Geoplana* (Geoplaninae), namely *Geoplana arpi*, *G. blaseri*, *G. bonita*, *G. bresslaui*, *G. cardosi*, *G. doederleini*, *G. goettei*, *G. itatiayana*, *G. lumbricoides*, *G. obscura*, *G. plana*, *G. rezendei*, *G. riedeli*, *G. theresopolitana* and *G. wetzeli*. All descriptions were exclusively based on external characters such as size, shape and color of the body and distribution of eyes and halos. He did not mention whether type specimens were deposited nor the institution.

In the years following this publication, the knowledge about Geoplaninae was considerably expanded: new species were discovered and described, new genera were proposed, additional diagnostic characters were included and, more recently, molecular data were used to help delimit species. In this process, different authors reviewed four of the 15 species described by Schirch: *G.*

goettei, *G. itatiayana*, *G. plana* and *G. rezendei* (Riester, 1938; Marcus, 1951; Froehlich, 1955a) from conspecific material. Currently these species are classified as *Paraba goettei*, *Obama itatiayana*, *Pasipha plana* and *Issoca rezendei* (Froehlich 1955a; Carbayo et al., 2013). The remaining 11 species, the internal morphology of which is unknown, were transferred to the collective genus *Pseudogeoplana* Ogren & Kawakatsu, 1990, namely *Ps. arpi*, *Ps. blaseri*, *Ps. bonita*, *Ps. bresslaui*, *Ps. cardosi*, *Ps. doederleini*, *Ps. lumbricoides*, *Ps. obscura*, *Ps. riedlei*, *Ps. theresopolitana* and *Ps. wetzeli*.

Non-destructive techniques for morphological studies

Prior to the massive work of Graff (1899), descriptions of land planarians were based on the external morphology. The development and introduction of the histological technique to the study of these organisms facilitated detailed descriptions of internal organs and provided more confident identifications. However, this technique has its inherent limitations and risks to histological processing, such as tissue loss and destruction (Laforsch et. al., 2012; Sombke et. al, 2015).

In recent years, as a non-destructive alternative, techniques such as computerized microtomography (mCT), synchrotron etc. have been used in the taxonomic study of animals (Carbayo et al., 2016; Tessler et al., 2016). But until the work of Carbayo et al. (2016) and Tessler et al. (2016) these techniques had not been successfully applied to describe soft-bodied, i.e., non-mineralized organisms. Carbayo et al. (2016) used this technique, combined with histology, to describe a land planarian species. Tessler et al. (2016) describe leeches. mCT was initially developed and applied to aid medical diagnoses and its usage was expanded to taxonomic and systematic studies of several organisms, such as arthropods, birds and mammals (Metscher, 2009). It is a non-destructive 2D and 3D image reconstruction technique based on the combination of multiple X-ray images of the specimens taken from various angles. The produced images generate virtual reconstructions with a resolution of up to 1 μm that can be observed from an infinite number of angles (Faulwetter et al, 2013). mCT is a well established technique in the study of fossils in paleobiology, even for those fossils that cannot be removed from its casing (Laforsch et al., 2012; Molineux et al., 2007).

Because it is a non-destructive technique, mCT is ideal for the study of museum specimens because it avoids the destruction of the animal and the risks of tissue loss associated with the classical histological technique. During histological processing, serial sections may have suffer distortions and deformations and may render the study unfeasible and even lead to tissue loss throughout processing (Carbayo & Lenihan, 2016).

A limitation of the technique is that, in non-mineralized tissues, it is more difficult to achieve X-ray contrast. A solution to said problem is the use of contrast enhancing agents (Metscher, B. D., 2009). Inorganic iodine and phosphotungstic acid are X-ray contrast enhancers of soft tissues preserved in different solutions. These substances do not cause tissue damage and also do not cause changes at a cellular level (Faulwetter et al, 2013).

On the other hand, mCT also presents its own limitations in planarians taxonomic studies: mCT-based images are composed of different levels of gray and do not reveal acidity/alkalinity of the secretions. Minute structures such as sensory pits, cilia and cell nucleus remained also indiscernible with the equipment used (Carbayo et al., 2016).

Rediscovery of type specimens of Schirch's species

Schirch (1929) stated that the type material of three (namely, *Ps. cardosi*, *Paraba goettei* and *Ps. riedlei*) of the 15 species were lost. After him, Ernest Marcus was the first scientist housed in Brazil to resume studies on this fauna (Carbayo et al., 2009). Marcus believed that types of the species described by Schirch was also lost or their conservation state would be so poor that there won't be point studying it (apud E. M. Froehlich, pers. comm.). However, under our request, Guilherme Muricy, curator of the Invertebrates Department at the Museu Nacional do Rio de Janeiro (MNRJ) checked for this material and found types of the following species: *Pseudogeoplana arpi*, *Ps. blaseri*, *Ps. bonita*, *Ps. bresslaui*, *Ps. doederleini*, *Ps. wetzeli*, *Ps. schirchi*, *Obama itatiayana*, *Pasipha plana* and *Issoca rezendei*.

Herein, we examined the external and internal morphology of the type specimens of six Schirch's species of land planarians, namely *Pseudogeoplana arpi*, *Ps. blaseri*, *Ps. bresslaui*, *Ps. doederleini*, *Ps. wetzeli* and *Pasipha plana*. We made an effort to get the most out mCT equipments to our reach as to avoid, whenever possible destruction of types resulting from histological techniques. Examination of these specimens and additional, non-type material, allowed us to redescribe these species with different levels of detail.

Materials and Methods

All syntypes of the collection of land planarians presumably studied by Schirch (1929) were herein studied. They are housed in the Museu Nacional do Rio de Janeiro (MNRJ). We received these specimens, which include type and non type material. Specimens were mailed to our laboratory wrapped in paper towel moistened with ethanol 80%, each inside a 5-10 ml plastic vial. Upon receipt of the material, we filled the plastic tubes with 80% ethanol. Holotype of *Ps. schirchi* and one of the syntypes of *Ps. doederleini* showed signs of having been accidentally dehydrated (body roughened and rigid) and thus were immersed in Sandison's solution for 5 days for tissue rehydration (Sandison, 1955) before mCT or histological processing. Each syntype was given an additional letter to distinguish it from the remaining type specimens.

We also examined additional specimens of *Pasipha plana*, namely, histological slides of one animal studied by E. M. Froehlich (1955a) and a specimen from FC's wet collection, which were sectioned for this work.

Microcomputed tomography of X-ray (mCT)

The largest syntype or the holotype of each of six Schirch's species was prepared for taxonomic study with mCT (Tab. 1). Specimens selected for mCT were submerged in a solution of 0.3% phosphotungstic acid (PTA) and 3% dimethyl sulfoxide (DMSO) in 95% hydrated ethanol for at least 30 days on a rocky table to facilitate PTA penetration into the body (1 g of iodine diluted in 100 ml ethyl alcohol) (Fernández et al., 2014). Subsequently, specimens were rinsed in 95% ethanol and transferred to a vial with 95% ethanol for mCT data acquisition. Specimens planned to be scanned a week after first scanning, were submerged again into PTA solution prior to scanning. The entire body of each specimen was scanned at low resolution for getting a general aspect of the body and checking if it is mature. Copulatory apparatus of specimens having a body relatively narrow was scanned at higher resolution. Since holotype of *Ps. bresslaui* is mature and its relatively narrow, we made an effort to describe it entirely from mCT dataset.

Two scanners were used for scanning the specimens. General Electric V-Tomex equipment (housed in the Laboratory of Paleontologia e Microtomografia, Museum of Zoologia da Universidade de São Paulo) was used for scanning type material of *Paraba bresslaui*, *Pasipha plana*, *Pseudogeoplana arpi*, *Pseudogeoplana blaseri*, *Pseudogeoplana doederleini* and *Pseudogeoplana wetzeli* specimens under the basic settings ranging from 23-75kV, and 174-340A. The holotype of

Pseudogeoplane bresslaui was scanned with Zeiss Xradia Versa XRM-510 (Laboratório de Caracterização Tecnológica (LCT), Escola Politécnica da Universidade de São Paulo). A total of three final scans of *Paraba bresslaui* specimens were obtained (Tab. 2).

The visualization, edition and manipulation of the volumes, reconstructed microtomography images, was done with the programs myVGL (<https://www.volumegraphics.com/en/products/myvgl.html>) and DataViewer© (<http://bruker-microct.com/products/downloads.htm>) both of free use. 3D images were generated with CTVOX software (<http://www.skyscan.be/next/CTvox64.zip>), also of free use.

Histology

Fixed specimens were examined and photographed with the help of an Olympus SZ61 stereo-microscope and a Leica M80 stereo-microscope with a Leica IC80HD digital camera attached to it. Different regions of the body of the specimens underwent histological processing: dehydration in alcoholic series, dealcoholization in clove oil, inclusion in Paraplast (c), serial sectioning at 5-7 microns in thickness with retracting rotary microtome (Microm HM315 R), rehydration and staining with Mallory's trichrome as modified by Cason (1950). Distribution of eyes was observed in specimens in clove oil. Drawings of the pharynx and copulatory apparatus were performed using a camera lucida coupled with the optical microscope. Nomenclature of body color follows RAL CLASSIC (<https://www.ral-farben.de/content/application-help/all-ral-colours-names/overview-ral-classic-colours.html>). Histological slides were examined and photographed using Olympus BX51 optical microscope and DP72 digital camera attached to it. Whenever possible, all figures are orientated so that anterior extremity of the body is to the left. Descriptions of the species were compared with descriptions of land planarians known in the literature.

Table 2. Representatives (holotype or a syntype) and body regions scanned through X-ray micro-computed tomography. See details of the parameters used in Appendices 1-9.

Scan no.	Species	Body region	Medium voltage (kV)	Current (mA)	Image pixel size (µm)	Scan duration
1	<i>Paraba bresslaui</i>	Entire body	75	290	7.99	0h:59m:15s
2	<i>Paraba bresslaui</i>	Cephalic extremity	23	174	1.62	17h:4m:27s
3	<i>Paraba bresslaui</i>	Copulatory apparatus	70-24	310-175	3.50	6h:51m:27s
4	<i>Pasipha plana</i>	Entire body	55	250	5.92	~50m
5	<i>Pseudogeoplana arpi</i>	Entire body	75	290	17.01	~50m
6	<i>Pseudogeoplana arpi</i>	Pharynx	75	240	2.99	~50m
7	<i>Ps. doederleini</i>	Entire body	75	340	4.24	~50m
8	<i>Ps. wetzeli</i>	Entire body	70	310	4.77	~50m
9	<i>Dolichoplana</i> sp.	Entire body	70	280	11.92	~50m

Taxonomic section

***Paraba bresslaui* (Schirch, 1929), comb. nov.**

Synonymy

Geoplana bresslaui Schirch, 1929 sp. n., p. 31. Type Locality: Teresópolis, Rio de Janeiro, Brazil.

E. M. Froehlich, 1955b, p. 203. [nec *Geoplana bresslaui* Schirch, 1929, in Riester (1938)].

Pseudogeoplana bresslaui: Ogren & Kawakatsu, 1990, p. 114.

Material examined. MNRJ****, **holotype**: Teresópolis, State of Rio de Janeiro, Brazil. P. Schirch *Coll* (unknown year). Preserved in 80% ethanol. Studied through micro-computed tomography.

Distribution. Municipality of Teresópolis, State of Rio de Janeiro, Brazil.

Diagnosis. Species of *Paraba* 25 mm long; dorsal side yellowish green with a thin median black band; long and thin prostatic vesicle, inverted 'U'-shaped; male atrium with 6-8 transverse annular folds; ovovitelline ducts ascend before the gonopore; female atrium lined with an epithelium that protrudes into the male atrium.

External aspect. Fixed holotype is 25 mm long. It is widest at the pharyngeal region, 5 mm. Towards the extremities, the body narrows gently; the anterior extremity is rounded; the posterior, obtuse (Fig. 1A and 1C). Dorsal side is strongly convex; ventral side is flat. Dorsum is ochre brown, with irregular areas of cream color as a result of fading (Fig. 1B). Body margins in anterior 1/6 of the body with the ground color. Ventral side is ochre yellow.

A single row of eyes contour the anterior first millimeter of the body; subsequently, eyes are arranged in a row with 2-4 eyes on each side of the body along 4 mm of body length. Behind, eyes could not be discerned. Sensory pits are simple invaginations located ventro-marginally in a single row from the very anterior body to at least two mm behind (Fig. 1D and 1E). Relative position mouth: body length, 66%. Relative position gonopore: body length, 82%.

Internal morphology. Long paired cerebral ganglia, 2 mm long, commence at 5 mm behind anterior extremity. Ventral nerve plate present. Mouth opening at the end of pharyngeal pouch. Pharynx cylindrical (Fig. 2B and 2C), with dorsal insertion slightly posterior to ventral one. Short esophagus present. Testes rounded, in number of ca. 40, the largest with 200 μ m in diameter. They are dorsally located, and are arranged in a row on each side of the body, each row having up to two testes on a same transverse plane (Fig. 2D). Most anterior testes are located dorsally to the ovaries and 1 mm behind cerebral ganglia, i. e., at a distance from anterior extremity equivalent to 32% of body length; posteriormost testes shortly anterior to pharynx root.

Sperm ducts run posteriorly between the oviducts. Near the prostatic vesicle, the efferent ducts curve vertically to the dorsum to subsequently running down to the posterior region of the prostatic vesicle. Behind the level of the prostatic vesicle, sperm ducts curve forward and medially to communicate with the latero-proximal region of the prostatic vesicle. Prostatic vesicle with an inverted 'U' shape from lateral view, with a short proximal section bifurcated and with folded wall (Fig. 3A); unpaired portion long, with sinuous wall initially, smooth distally. In its distal most course, this vesicle bends posteriorly to level the penis papilla, and subsequently penetrates antero-ventral aspect of the penis bulb. Prostatic vesicle surrounded by a 100 - μ m-thick distinct mass, of presumably muscle and/or glands, and is attached to anterior aspect of the penis bulb. Penis bulb extends 1 mm before the penis papilla.

The prostatic vesicle continues inside the penis bulb as ejaculatory duct. This duct is horizontally positioned, and crosses slightly eccentrically the penis papilla to open at its tip. Penis papilla cylindrical, with the distal portion truncated in lateral view (Fig. 3B and 3C), but conical in dorsal view (Fig. 3B). It fills 1/3 of male atrium length. Male atrium broad, with 6-8 transverse, annular folds. Anteriormost fold is the largest and surrounds the basis of the penis papilla. Length of male atrium twice of that of the female atrium.

Ovaries 230 μ m in diameter and approximately rounded. They are positioned at 8 mm from anterior extremity of the body, and 1 mm behind cerebral ganglia. They are located immediately above the ventral nerve plate (Fig. 3D). Ovovitelline ducts emerge from the dorso-lateral aspect of the ovaries (Fig. 3D), and run posteriorly horizontally until the level of the gonopore. Then they ascend obliquely to the sagittal plane and unite to the common glandular ovovitelline duct above posterior region of female atrium. The ~100 μ m long common glandular ovovitelline duct runs ventrally to join the female

genital canal, the latter being a ~160 μm projection of the postero-dorsal region of the female atrium. Female atrium ca. 1 mm in length and 0.5 mm in height, equivalent of 1/4th of the body height. This female atrium is elongate and is lined with an epithelium with a very high epithelium (~250 μm) which occupies the whole cavity, with the except of an irregular narrow canal (Fig. 4A and 4B). This female lining epithelium presents a cone-shaped protrusion into the male atrium. The gonopore canal is vertical and 500 μm long.

Remarks

The general aspect of the holotype agrees with original description, on shape and shape and size of the body. Body color faded after 90 years of conservation in fluid and unsurprisingly does not fit original description.

The species studied presents all characteristics of the subfamily Geoplaninae, i.e., creeping sole covering most of ventral surface, mouth behind midbody, a well developed cutaneous musculature organized into bundles, part of the parenchymal muscle layers organized in longitudinal fibers, and dorsal testes (Almeida et al., 2018).

Features observed of this species match only one genus of Geoplaninae, namely *Paraba* Carbayo et al., 2013, whose diagnosis reads: "Geoplaninae with small-to-medium-sized body, 6–80 mm in length; body slender, with margins nearly parallel; dorsum and ventral side slightly convex; eyes monolobulated; pharynx cylindrical; prostatic vesicle extrabulbar, generally horizontal; penis papilla protrusible, conical; male atrium not folded; ascending portion of ovovitelline ducts lateral to the gonopore canal or to female atrium, joining each other above female atrium; genital canal dorso-anteriorly flexed, arising from the postero-dorsal region of female atrium; female atrium rounded, clothed with an epithelium with multilayered aspect" (Carbayo et al., 2013). In view of the internal morphology of the species, herein presented for the first time, it is justified to transfer the species to *Paraba*.

This species only stands apart of the diagnostic features of *Paraba* in some details that deserve comment: (a) the dorsum is not slightly but strongly convex, (b) the male atrium is not unfolded, but folded, (c) female atrium is not rounded but elongate, and (d) it is unknown whether the clothing epithelium of the female atrium presents multilayered aspect. These differences probably represent artifacts or intrageneric variation: the two former features (shape of the dorsum and folds of the male

atrium) might have been caused by contraction during fixation or simply the male atrium is folded. An elongate female atrium is also found in other species of *Paraba* (namely *Pa. franciscana*, *Pa. rubidolineata*). We were unable to determine the type of epithelium clothing female atrium. Thus, it should not be excluded the possibility of this epithelium present a multilayered aspect.

Regarding the external aspect, *Ps. bresslaui* differs from any other congeneric species in the color pattern, consisting of a ground color yellowish green, ornated with a median black stripe, and beige pigment spots, often elongate and parallel to main body axis. Any of 14 species of *Paraba* presents dorsal color green yellowish with a median strip black and spots beige stretching longitudinal as in *Ps. bresslaui*.

Regarding the internal aspect there are four species resembling *Ps. bresslaui* in having a U-shaped prostatic vesicle, namely *Pa. cassula* (E. M. Froellich, 1955a), *Pa. goettei* (Schirch, 1929), *Pa. phocaica* (Marcus, 1951) and *Pa. piriana* (Almeida & Carbayo, 2012, in Almeida et al., 2012). However, *Pa. phocaica* is the only species shearing with *Ps. bresslaui* the position of the female atrium from which the female genital canal projects, that is posterior section of the female atrium. Both species also share distinct features: an annular fold around the basis of the penis papilla, a male atrium folded, and the general shape of the prostatic vesicle. Apart from the body color, however, *Ps. bresslaui* distinguishes from *Pa. phocaica* in that the latter species presents a ventral entrance of the prostatic vesicle into the penis bulb, a rounded female atrium, and a lining epithelium of the female atrium not invading the male atrium.

Fig. 1. *Paraba bresslaui* (Schirch, 1929), holotype fixed. **A.** Dorsal view. **B.** Ventral view. **C.** Dorsal view with original label. **D.** Anterior extremity. **E.** Lateral view of a 3D rendering of the μ CT dataset. **F.** Frontal view of a 3D rendering of the μ CT dataset.

Fig. 2. *Paraba bresslaui* (Schirch, 1929), holotype. **A.** Virtual transverse section of pre-pharyngeal region. **B.** Virtual sagittal section of pharynx. **C.** Diagrammatic reconstruction of pharynx. **D.** Virtual horizontal section of testes region.

Fig. 3. *Paraba bresslaui* (Schirch, 1929), holotype. **A.** Virtual section of sagittal of prostatic vesicle. **B.** Virtual section of horizontal of copulatory apparatus. **C.** Virtual section of sagittal of copulatory apparatus. **D.** Virtual horizontal section of ovary region. Asterisk indicates artefact

Fig. 4. *Paraba bresslaui* (Schirch, 1929), holotype. **A.** Virtual sagittal section of copulatory apparatus. **B.** Diagrammatic reconstruction of copulatory apparatus.

***Pasipha plana* (Schirch, 1929)**

Synonymy

Geoplana plana Schirch, 1929 sp. n., p. 33. Type Locality: Teresópolis, Rio de Janeiro, Brazil.

Geoplana plana: Froehlich, 1955a, p. 299.

Pasipha plana: Ogren & Kawakatsu, 1990, p. 51.

Material examined. Type material (Fig. 5D). Four syntypes collected by P. Schirch *Coll* (year unknown) in Teresópolis, State of Rio de Janeiro, Brazil. We received them on loan in 70% ethanol with only a label reading MNRJ 8915. Each syntype was given an additional identification with a letter, A-D. Three dimensional (3D) images and virtual sections of syntype MNRJ 8915A were obtained by microcomputed tomography. Parts of the bodies of syntypes were histologically sectioned as follows. Syntype **MNRJ 8915A**: Transverse sections of posterior extremity on 14 slides; remaining body preserved in 80% ethanol. Syntype **MNRJ 8915B**: Sagittal sections of copulatory apparatus on 156 slides; remaining body preserved in 80% ethanol. Syntype **MNRJ 8915C**: Horizontal sections of anterior extremity on 31 slides; horizontal sections of ovaries on 71 slides; transverse sections of pre-pharyngeal region on 35 slides; sagittal sections of pharynx region on 94 slides; sagittal sections of copulatory apparatus on 124 slides; remaining body preserved in 80% ethanol. Syntype **MNRJ 8915D**: Preserved in 80% ethanol.

Additional material examined: Specimen **MZUSP PL XXXX**, studied by E. M. Froehlich (1955a): Teresópolis, Parque Nacional da Serra dos Órgãos, State of Rio de Janeiro, Brazil (geographic coordinates not available), E. M. Froehlich et al., *Coll* 1953. Transverse sections of pre-pharyngeal region on 2 slides (S761-S762); sagittal sections of pharynx on 1 slide (S763); sagittal sections of copulatory apparatus on 10 slides (S764-S773). **F6484** (MZUSP PL XXXX): Parque Estadual da Pedra Branca, Rio de Janeiro, State of Rio de Janeiro, Brazil, -22.93398, -43.44793, Carbayo et al., *Coll* 9 Dec. 2014. Sagittal sections of copulatory apparatus on 101 slides; remaining body preserved in 80% ethanol.

Distribution. Areas covered with Atlantic forest in the municipalities of Teresópolis and Rio de Janeiro, State of Rio de Janeiro, Brazil.

Diagnosis. Species of *Pasipha* 60-70 mm long; dorsum with black brown dots on an ivory surface; eyes dorsal, occupying a lateral band on each side of the body, 44% of body width; posteriormost testes behind the pharynx; prostatic vesicle inverted U-shaped in lateral view, not bifurcated; ovovitelline ducts emerge from the inner-dorsal side of the ovaries; male atrium folded; length of the male atrium to female atrium ratio, 8:1; female atrium as long as high.

External aspect. Living adult (F6484) 70 mm in length and 10 mm wide. Fixed adults (F6484 and syntype C) 65-71 mm long, 6 mm wide, and 1.7 mm high. Elongated body, with margins approximately parallel; anterior and posterior extremities rounded. Dorsum slightly convex, ventral side flat. The ground color of the dorsum of living animals is ivory, richly ornamentated with black brown dots (Fig. 5A). Towards the body margins, dots tend to be anastomosed into longitudinal striae.

Striae are lacking in a thin medial stripe which is bordered at each side by an irregular line of the same color as the dots. The ventral side is sulfur yellow, with anterior extremity light ivory and the pharyngeal region zinc yellow (Fig. 5C). After 89 years in preservative fluid, the dorsal color of the four syntypes turned into ochre yellow and the dots faded to a terra brown color; their ventral body surface turned into olive yellow (Fig. 5B).

The monolobated eyes surround the anterior extremity of the body. Backwards, they spread onto entire dorsum excepting a thin median line with ~8% of the body width. Towards posterior extremity, they become scarcer (Fig. 6A and 6B). Sensory pits are simple invaginations 25 µm deep, located ventro-marginally in a single row from the very anterior body tip up to at least 57% of body length (syntype C). Relative position mouth: body length, 69%, that of the gonopore, 90% in syntype C (68%, and 91%, in specimen F6484).

Internal morphology. Creeping sole comprising with 96% body width. Rhabditogen cells and glands producing erythrophil granules pierce abundantly dorsal and marginal epidermis; glands producing amorphous orangish-to-reddish secretion pierce marginal epidermis. The ventral epithelium is pierced by glands producing fine erythrophil granules. A glandular margin is absent. The cutaneous musculature comprises three layers, namely a subepithelial circular layer, followed by two diagonal layers with decussate fibers, and then a well developed innermost longitudinal layer. Muscle fibers of the longitudinal layer (120 µm thick dorsally; 88 µm thick ventrally) are arranged into bundles with 30-50 fibers each. Cutaneous musculature thickness relative to body height at the pre-pharyngeal region, 13%. Three parenchymal muscle layers present, all well developed: dorsal layer of decussate diagonal

fibers (60 μm thick), suprainestinal layer of transverse fibers (100 μm thick), and subintestinal layer with transverse fibers (65 μm thick; Fig. 7A-7B). Ventral nerve plate present.

Mouth situated at a distance from root of the pharynx equivalent to 50% of pharyngeal pocket length. Pharynx collar-shaped (Fig. 7C), with dorsal insertion very close to the end of the pharyngeal pocket. Esophagus absent. Outer pharyngeal musculature difficult to be discerned; apparently it consists of a one-fiber-thick subepithelial muscle layer (6 μm thick) of longitudinal fibers followed by an equally thick layer of circular fibers. Inner pharynx musculature consisting of a subepithelial layer of circular fibers (80 μm) in the anterior portion of the pharynx; this circular layer is followed by scattered longitudinal fibers in the posterior portion of the pharynx (syntype C).

Testes are ~300 μm in diameter. They are dorsally located between suprainestinal parenchymal muscle layer and intestine. Anteriormost testes at a distance from anterior extremity of the body equivalent to 29% of body length; posteriormost testes are posterior to the pharynx, near prostatic vesicle, and at a distance equivalent to 85% of body length (syntype C).

Sperm ducts contain sperm in their distal portion; a 60 μm thick circular muscle surround this distal portion. The efferent ducts run between the oviducts, and onto the nervous plate. Laterally the prostatic vesicle, efferent ducts curve forward and medially to communicate with the latero-proximal region of the prostatic vesicle.

Prostatic vesicle extrabulbar, and long and narrow, divided into an anterior half running dorsally, and a posterior half, running ventrally so describing an inverted U course. Initial portion of prostatic vesicle with folded wall, and lined by a 12-30 μm high cuboidal-to-columnar ciliated epithelium which is pierced by abundant glands producing erythrophil (purple) granules. Distal half lined with a 3 μm high epithelium that becomes 10 μm high at its distal section. This distal half pierced by numerous glands producing erythrophil (reddish) granules. Prostatic vesicle surrounded by muscle of fibers variously oriented. This muscle is 200-400 μm thick around proximal portion, and thinner and less dense around distal portion. Entire prostatic vesicle and its musculature are enveloped by a thin coat of muscle fibers, well discerned from that of the common muscle coat.

Prostatic vesicle penetrates ventral aspect of common muscle coat and continues with the ejaculatory duct, which is wide latero-laterally. This duct recurves dorsally before bending abruptly backwards to open into anterior portion of male atrium. Ejaculatory duct lined with a cyanophil, 10-12 μm high, ciliated epithelium, and is surrounded by a circular muscle (10-15 μm thick) of thin fibers (1 μm in diameter) (Fig. 8A).

Male atrium is a 5 mm long cavity (syntype C, 8% of body length), with large folds that occupy its half anterior, especially on its roof, the half posterior is ample and with less folds, it's not ciliated. Male atrium lined with an 8-15 μm high epithelium, the free surface of which is papillar. This epithelium is pierced by glands producing erythrophil (reddish-to-purple) granules; these glands are abundant throughout entire epithelium with the exception of that of lining the sphincter (see below), which is very abundantly pierced by glands producing erythrophil (reddish) granules, especially in its ventral portion (Fig. 9A).

Subepithelial musculature of male atrium consists of a 12 μm thick layer of circular and longitudinal muscle fibers, which sometimes are intermingled, and sometimes arranged in two layers. This muscle is continued with very abundant longitudinal and circular fibers located in the surrounding space delimited by the common muscle coat. A well developed sphincter of circular muscle embraces subterminal portion of the male atrium. Dorsal region of the sphincter is posterior to the ventral (Fig. 8B).

Ovaries ovoid-shaped, 500 μm in maximum length, and situated above the ventral nerve plate (Fig. 7A). They are at a distance from anterior extremity equivalent to 22% of body length (syntype C). Ovovitelline ducts emerge from the inner-dorsal region of the ovaries. Behind the gonopore region, they curve medially, and dorsally and join to form the common glandular ovovitelline duct, behind the female atrium. There are no shell glands discharging their secretion into the ovovitelline ducts. The common glandular ovovitelline duct is 120 μm long and runs dorsally to enter the female genital canal.

This canal (800 μm long in syntype C) is a projection of the postero-dorsal region of the female atrium, and runs backwards and ventrally. Common ovovitelline duct and female genital canal are lined with a ciliated epithelium which is surrounded by a 25 μm thick muscle of thin circular with diagonal fibers (1 μm).

Female atrium short and ample, roughly as long as high. Its posterior wall is projected anteriorly giving to it some square shape. Female atrium lined with a 12-25 μm high epithelium; its ventral and posterior walls presents apical surface with papillar aspect (Fig. 9B). Female atrial epithelium pierced by glands producing erythrophil granules, and surrounded by a muscle layer of longitudinal and circular fibers in some regions, and decussate in other. Male atrium: female atrium ratio, 8:1.

Common muscle coat is constituted by circular and longitudinal muscle fibers interwoven. It is less developed in its posterior region. It envelopes the male and female atria, and the common glandular ovovitelline duct (Fig. 9C and 10).

Remarks

Schirch (1929) described the external appearance of the species only. Froehlich collected one specimen in the type locality and described its external and internal morphology (Froehlich, 1955a). By comparing her specimen with the succinct original description, she understood that they would be conspecific (Froehlich, 1955a). Indeed, she was right: apart from the external aspect, the internal morphology of Froehlich's specimen matches in all aspects that of the syntypes, specially the copulatory apparatus of syntypes A, B, and C, herein described for the first time. This similarity also applies to the specimen F6484, recently collected by us. Thus it is safe to assume that the syntypes, Froehlich's specimen and specimen F6484 are all conspecific.

Currently, *Ps. plana* is placed in *Pasipha* Ogren & Kawakatsu (1990), the diagnosis of which was revised by Carbayo et al. (2013). The genus comprises 24 species (plus 5 species considered *incertae sedis*, see Carbayo et al., 2013). Regarding the external aspect, *Ps. plana* distinguishes from all the congeners excepting *Pasipha hauseri* (Froehlich, 1959) and *Pasipha backesi* (Leal-Zanchet et al., 2012), in that only these species display a dichromatic pattern consisting of dark spots on a clear ground color and a thin median zone devoid of spots. However, they differ in the details, since the dorsum of *Pa. hauseri* is greyish-brown pigment on a light yellow ground on the dorsum. Along the dorsum runs a lighter stripe, ca. 1 mm. broad, due to a thinning of the dark pigment. At the borders of the light stripe the pigment is darker than on the rest of the back, and that of *Pa. backesi* is brown with yellowish median stripe, bordered by dark-brown to black paramedian stripes on the dorsum.

Regarding internal aspect, *Ps. plana* shares only with *Pa. cafusa* (Froehlich, 1956), *Pa. chimbeva* (E. M. Froehlich, 1955a), *Pa. oliveiroi* (Froehlich, 1955b), *Pa. pasipha* (Marcus, 1951), *Pa. penhana* (Riester, 1938), *Pa. pinima* (E. M. Froehlich, 1955a), *Pa. rosea* (E. M. Froehlich, 1955a), *Pa. splendida* (Graff, 1899), *Pa. tapetilla* (Marcus, 1951) and *Pa. velutina* (Riester, 1938) a non-forked prostatic vesicle. However, among these 11 species, only in *Pa. rosea*, *Pa. tapetilla* and *Pa. velutina* the length of the male atrium to the female atrium ratio is $>3:1$, as in *Ps. plana*. In *Ps. plana* this ratio is as high as $8:1$, though. Furthermore, differently from *Ps. plana*, in *Pa. rosea* the prostatic vesicle is not so sinuous as in *Ps. plana*, and the length of the female atrium is twice that of its height, whereas the

female atrium of *Ps. plana* is roughly as long as high; in *Pa. tapetilla*, there is a barrel-shaped and muscular copulatory organ, absent in *Ps. plana*. Finally, differently from *Ps. plana*, in *Pa. velutina* there exist a penis papilla consisting of an annular fold of the proximal region of the male atrium, which otherwise is more spacious and less folded than in *Ps. plana*.

Fig. 5. *Pasipha plana* (Schirch, 1929). **A.** F6484. Specimen alive in dorsal view, anterior extremity to left. **B.** Syntype B fixed in dorsal view, anterior extremity to left. **C.** F6484. Specimen alive in ventral view. **D.** Set of all syntypes.

Fig. 6. *Pasipha plana* (Schirch, 1929), specimen F6484 in clove oil. **A.** Dorsal anterior extremity. **B.** Dorsal midbody extremity.

Fig. 7. *Pasipha plana* (Schirch, 1929). **A.** Specimen F6484. Photomicrograph of a horizontal section of ovaries region. **B.** F6484. Photomicrograph of a transverse section of pre-pharyngeal. **C.** Syntype A. Virtual section sagittal of pharynx.

Fig. 8. *Pasipha plana* (Schirch, 1929). **A.** Specimen F6484. Photomicrograph of distal portion of prostatic vesicle and of ejaculatory duct on a sagittal section. **B.** Syntype A. Virtual section sagittal of copulatory apparatus.

Fig. 9. *Pasipha plana* (Schirch,1929). Specimen F6484. **A.** Photomicrograph of a sagittal section of copulatory apparatus. **B.** Photomicrograph of a sagittal section of copulatory apparatus. **C.** Reconstruction of copulatory apparatus.

Fig. 10. *Pasipha plana* (Schirch, 1929). Syntype A. Virtual section sagittal of copulatory apparatus.

***Pseudogeoplana arpi* (Schirch, 1929)**

Synonymy

Geoplana arpi Schirch, 1929 sp. n., p. 33. Type Locality: Baixo Guandu, Espírito Santo, Brazil

Pseudogeoplana arpi: Ogren & Kawakatsu, 1990, p. 113.

Material examined. Type material. The two syntypes available, collected by P. Schirch in 1917 in Baixo Guandu (former Maylasky), State of Espírito Santo, Brazil. We received them on loan in 70% ethanol with only a label reading MNRJ 8914. Each syntype was given a letter, A-B, for identification. Three dimensional (3D) images and virtual sections of syntype MNRJ 8914A were obtained by microcomputed tomography. Parts of the body of syntype MNRJ 8914B were histologically sectioned as follows. Syntype **MNRJ 8914B**: Transverse sections of anterior extremity on 20 slides; a portion behind anterior extremity, 4.5 mm long, horizontally sectioned on 73 slides; transverse sections of pre-pharynx on 50 slides; sagittal sections of pharynx on 135 slides; remaining body preserved in 80% ethanol.

External aspect. The syntype A fixed is 160 mm long, 10 mm wide, and ~2 mm high (Fig. 11A and 11B); syntype B, 70 mm, ~8 mm, and ~2 mm, respectively. Elongated body, with margins approximately parallel; anterior extremity pointed, posterior rounded, body sides rounded. Dorsum convex, ventral side flat, convex on some portions of the median region most probably as an effect of contraction in the moment of fixation. Ground color of the dorsum clay brown with irregular faded areas visible at the naked eye. Ventral side grey beige (Fig. 11A and 11C).

The monobolated eyes surround anterior extremity of the body and distributed evenly dorsolaterally along all body length (Fig. 12D). Sensory pits are simple invaginations 15 µm deep, located ventro-marginally in a single row that contours anterior extremity and extend posteriorly at least ~4 mm (or 7% of body length). Relative position of mouth: body length, ~55%. Relative position of incipient gonopore: body length, ~70% in syntype B.

Internal morphology. Epithelium ciliated in anterior extremity, dorsally and ventrally. Creeping sole comprising with 92% body width (Fig. 12A). In pre-pharyngeal region, rhabditogen cells and glands producing erythrophil granules pierce abundantly dorsal and marginal epidermis; glands producing amorphous orangish-to-reddish secretion pierce marginal and dorsal epidermis. Ventral

epithelium pierced by glands producing fine erythrophil granules (Fig. 12A and 12B). A glandular margin is absent.

Cutaneous musculature comprises three layers, namely a subepithelial circular layer, followed by a diagonal layer of decussate fibers, and then a well developed innermost longitudinal layer. Muscle fibers of the longitudinal layer (45 μm thick dorsally; 75 μm thick ventrally) arranged into bundles with 13-46 fibers each. Cutaneous musculature thickness relative to body height in the pre-pharyngeal region, 10% (Fig. 12B). No muscle modifications on anterior extremity (Fig. 12A and 12D). Three parenchymal muscle layers present: a dorsal layer (10 μm thick) of decussate fibers, a suprainestinal layer (100 μm thick) with transverse fibers, and a poorly developed subintestinal layer (100 μm thick) of transverse fibers (Fig. 12B). Ventral nerve plate well developed.

Mouth situated at a distance from root of the pharynx equivalent to 32%-47% of pharyngeal pocket length (syntypes A and B, respectively). Pharynx cylindrical, tending to bell-shaped (Fig. 13A and 13B), occupying anterior half of the pharyngeal pocket. Esophagus absent. Free surface of outer pharyngeal epithelium undulate and ciliated, and underlain by a subepithelial longitudinal muscle (8 μm thick), followed by a circular muscle (50 μm thick). Inner pharyngeal musculature consisting of a subepithelial circular muscle (25 μm thick), followed by a longitudinal muscle (5 μm).

Testes poorly developed, rounded, ~100 μm in diameter, and sperm not developed inside them. They are dorsally located among fibers of the suprainestinal transverse parenchymal muscle layer. Anteriormost testes at a distance from anterior extremity of the body equivalent to 26% of body length; posteriormost ones, 36%, i.e., anterior to pharyngeal pocket (Fig. 12C).

Copulatory apparatus very poorly developed (Fig. 13C). It occupies the ventral 2/3th of the body height. Each of the paired sperm ducts opens into the proximal region of the bifurcate prostatic vesicle. This vesicle is intrabulbar, constituted of an unpaired portion running ventro-posteriorly inside a weakly developed penis bulb. Prostatic vesicle opens at the base of a flat, horizontal cavity, 35 μm high and 250 μm wide, delimited by a pair of folds, one dorsal, the other ventral. An ejaculatory duct was not observed. This pair of folds projects from anterior wall of the atrium and has the shape of a horizontal, short and conical penis papilla. This atrium is a 1.5 mm long closed cavity, i.e., not opened to the outside of the body by means of an eventual gonopore canal. The atrium is narrower posteriorly and there is no differentiation between male and female atrium. The atrial epithelium is a mass of tissue, the limit of which is diffuse. Dorsally of atrium, there is a muscular layer of longitudinal fibers

that appear to be part of the atrium, as common muscular coat (Fig. 13D). Quality of histological sections did not allow to describe additional details (Fig. 13C).

Remarks

The species presents all characteristics of the subfamily Geoplaninae, i.e., creeping sole covering most of ventral surface, mouth behind midbody, a well developed cutaneous musculature organized into bundles, part of the parenchymal muscle layers organized in longitudinal fibers, and dorsal testes (Almeida et al., 2018).

Since the syntypes present only poorly developed copulatory organs, there are more than one genera of Geoplaninae into which the species could be included (namely, *Amaga* Ogren & Kawakatsu, 1990, *Bogga* Grau & Sluys, 2012 (Grau et al., 2012), *Gigantea* Ogren & Kawakatsu, 1990, *Imbira* Carbayo et al., 2013, *Obama* Carbayo et al., 2013 and *Pasipha* Ogren & Kawakatsu, 1990). Nonetheless, due to this uncertainty, the species should remain in *Pseudogeoplana*.

Fig. 11. *Pseudogeoplana arpi* (Schirch, 1929). **A.** Fixed syntype A in dorsal view. **B.** Virtual mCT reconstruction of the body of Syntype A. **C.** Dorsal view of syntype B.

Fig. 12. *Pseudogeoplana arpi* (Schirch, 1929), photomicrographs of syntype B. **A.** A transverse section of anterior extremity. **B.** A transverse section of pre-pharyngeal region. **C.** A horizontal section of testis region. **D.** A transverse section of anterior extremity.

Fig. 13. *Pseudogeoplana arpi* (Schirch, 1929). **A.** Syntype A. Virtual sagittal section of pharynx. **B.** Syntype B. Photomicrograph of a sagittal section of pharynx. **C.** Syntype B. Photomicrograph of sagittal section of incipient copulatory apparatus. **D.** Syntype B. Reconstruction of incipient copulatory apparatus.

***Pseudogeoplana doederleini* (Schirch, 1929)**

Synonymy

Geoplana doederleini Schirch, 1929 sp. n., p. 33. Type Locality: Baixo Guandu, Espírito Santo, Brazil.

Pseudogeoplana doederleini: Ogren & Kawakatsu, 1990, p. 153.

Material examined. Type material. Three syntypes collected by P. Schirch *Coll* in 1917 in Baixo Guandu, State of Espírito Santo, Brazil. We received them on loan in 70% ethanol only with a label reading MNRJ 8914. Each syntype was given an additional identification with a letter, A-C. Three dimensional (3D) images and virtual sections of syntype MNRJ 8932A were obtained through microcomputed tomography. Syntype **MNRJ 8932A**: Preserved in 80% ethanol. Whole body of syntype MNRJ 8932B was histologically sectioned as follows. Syntype **MNRJ 8932B**: Transverse sections of anterior extremity on 12 slides; horizontal sections of ovaries on 5 slides; transverse sections of pre-pharynx on 9 slides; sagittal sections of pharynx on 17 slides; sagittal sections of post-pharynx on 17 slides. Syntype **MNRJ 8932C**: preserved in 80% ethanol.

Description. The three specimens were received on loan were rigid and shrunk, with longitudinal cracks (Fig. 14C). The syntype A fixed is 30 mm long, ~3 mm wide (Fig. 14A, 14B), and 1 mm high; syntype B, 26 mm, 2 mm, and ~0.8 mm, respectively. Elongated body, with margins parallel; anterior and posterior extremity rounded, body sides rounded. Dorsum convex, ventral side convex in some regions, concave in other, the latter probably as an effect dehydration. Ground color of the dorsum beige with yellow olive pigment dots, absent in some areas. Ventral side beige. The monolobated eyes surround anterior extremity of the body (Fig. 14D). Posterior to this region, eyes were not observed. Relative position of mouth: body length, 61% (syntype B).

Remarks

As expected, body color is somewhat different from original description, but body size and form and pattern of eyes distribution agree with that description. Histological sections of syntype B are of very poor quality (Fig. 15A and 15B). Pharynx (Fig. 15C) and copulatory apparatus are very

inaccurately outlined and epithelia are indistinct. mCT derived images are also very poor; PTA did not penetrate tissue. By this reason, there is no tissue contrast, hence internal anatomy is not recognizable.

Based in histology of this specimen and on mCT derived images of syntype A, the internal morphology of the species cannot be ascertained. Therefore, species should remain in *Pseudogeoplana*.

Fig. 14. *Pseudogeoplana doederleini* (Schirch, 1929). **A.** Syntype A fixed, in dorsal view. **B.** Virtual mCT reconstruction of the body of syntype A. **C.** The tree syntypes. **D.** Syntype A in oil of clove.

Fig. 15. *Pseudogeoplana doederleini* (Schirch, 1929). Photomicrographs of syntype B. Sections showing poor quality of histological sections. **A.** A transverse section of anterior region. **B.** A horizontal section of anterior half of body. **C.** Sagittal section of pharyngeal region.

***Pseudogeoplana wetzeli* (Schirch, 1929)**

Synonymy

Geoplana wetzeli Schirch, 1929 sp. n., p. 32. Type locality: Baixo Guandu, Espírito Santo, Brazil.

Pseudogeoplana wetzeli: Ogren & Kawakatsu, 1990, p. 160.

Material examined (Fig. 17A). **Type material.** Seven syntypes Baixo Guandu, Espírito Santo, Brazil. P. Schirch *Coll.*, 1917. They were received on loan in 70% ethanol. Parts of the body of syntype **MNRJ 8920 - A** were histologically sectioned: Transverse sections of anterior extremity on 26 slides; horizontal sections of ovaries region on 29 slides; sagittal sections of pharynx region on 33 slides and transverse sections of post-pharyngeal region on 18 slides. Remaining body preserved in 80% ethanol. The following seven syntypes were examined and maintained in 80% ethanol: **MNRJ 8920**, syntypes **B, C, D, E, F, G**.

External aspect. Fixed the largest syntype (syntype A), measured 30 mm long, 3 mm wide, and ~1.2 mm high. Anterior extremity rounded, posterior pointed, body sides rounded. Dorsum convex, ventral side flat. Schirch (1929) illustrated the external aspect of the species (Fig. 16A). In the fixed syntypes the ground color of the dorsum is olive brown with a median band (as wide as 1/3th of body width) of mahogany brown color, that does not reach the extremities of the body (Fig. 16B). Ventral side lemon yellow (Fig. 16C and 16D).

The monolobated eyes are 38 µm in diameter and surround the anterior extremity of the body (Fig. 17B). From the second millimeter behind, the eyes spread onto the dorsum and at 4.5 mm pass to occupy the entire dorsum (syntype A; Fig. 18A and 18D). Sensory pits are simple invaginations 40 µm deep, located ventro-marginally in a single row at least along a body length of ~5 mm (equal to 17% of body length, syntype A; Fig. 18A). Relative position of mouth: body length, ~52% (syntype A). Gonopore not developed in any syntype.

Internal aspect. The creeping sole comprises 72% of body width. Rhabditogen cells and glands producing erythrophil granules pierce dorsal, marginal epidermis and creeping sole. Ventral epithelium is pierced by few glands producing fine xanthophil granules. A glandular margin is absent.

The cutaneous musculature comprises the three layers typical of the subfamily Geoplaninae. A subepithelial circular layer, followed by a diagonal layer with decussate fibers (2 μm thick dorsally; 2.5 μm thick ventrally), and an innermost longitudinal layer. Muscle fibers of the longitudinal layer (20 μm thick dorsally; 25 μm thick ventrally) are arranged into bundles with 6-19 fibers each. Cutaneous musculature thickness relative to body height at the post-pharyngeal region, 4.5%.

Parenchymal musculature. There are a suprainestinal layer of transverse fibers and a subintestinal layer of transverse fibers, both layers interspersed with longitudinal fibers (Fig. 18B). Longitudinal parenchymal muscle fibers were best observed in the region of the ovaries; in the pharyngeal region they are very scarce (Fig. 18C). Suboptimal quality of the horizontal sections did not allow to check whether a third typical layer of Geoplaninae (dorsal layer of decussate fibers) is present. In the cephalic region, cutaneous and parenchymal muscle layers arranged as in the remaining body but less developed. Ventral nerve plate present (Fig. 18B and 18D).

Mouth situated, approximately, in the middle of the pharyngeal pocket. Pharynx cylindrical in shape (Fig. 19A and 19B). Outer pharyngeal musculature consisting of a thin subepithelial longitudinal muscle (5 μm thick), followed by 25- μm -thick circular muscle with longitudinal fibers interspersed (Fig. 19C and 19D). Lining epithelium of pharyngeal lumen sinuous and ciliated, and is underlain by a subepithelial circular muscle (25 μm thick), followed by a longitudinal layer (5 μm). Esophagus absent.

Testes are ~ 40 μm in diameter. They are dorsally located between the suprainestinal parenchymal muscle layer and the intestine, limited dorsolaterally (Fig. 18C). Anteriormost testes are distant from anterior extremity 3 mm, equivalent to 3% of body length; posteriormost testes are distant of anterior extremity 10.4 mm, distance equivalent to $\sim 35\%$ of body length (syntype A). Incipient sperm ducts. Copulatory apparatus not developed.

Ovaries are oval-shaped, 170 μm in maximum length, and situated above the ventral nerve plate. They are located at ~ 6 mm from anterior extremity, equivalent to 20% of body length (syntype A). Ovovitelline ducts emerge from the dorsao-lateral region of ovaries and extend posteriorly only ~ 200 μm . They contain sperm.

Remarks. Diagnostic features of the Geoplaninae are recognized in this species, namely, creeping sole covering most of ventral surface, mouth behind midbody, a well developed cutaneous

musculature organized into bundles, part of the parenchymal muscle layers organized in longitudinal fibers (as they may be present in the subfamily) and dorsal testes (see Carbayo et al., 2013, and Almeida et al., 2018).

It is striking the fact that although the largest specimen presents male and female gonads, even sperm in ovovitelline ducts, the copulatory apparatus is lacking. This lack hinders searching for taxonomic affinities of the species within Geoplaninae.

An uncommon feature within this subfamily, namely, parenchymal longitudinal muscle fibers might shed some light on the genus to which this species belong. These parenchymal fibers are only known from *Geoplana* and *Imbira*. However, *Geoplana* is excluded since in species of this genus eyes in cephalic region are cone-shaped, whereas in *Ps. wetzeli* they are rounded. Regarding longitudinal parenchymal musculature, diagnosis of *Imbira* does not exclude *Ps. wetzeli* but in *Imbira* eyes are only marginally distributed, whereas in *Ps. wetzeli* they are dorsal. In view of the poor morphological knowledge of the species, it should remain in the collective genus *Pseudogeoplana*.

Fig. 16. *Pseudogeoplana wetzeli* (Schirch, 1929). **A.** Original illustration in Schirch (1929). **B.** Syntype A in 80% ethanol in dorsal view. **C.** Syntype A in 80% ethanol in ventral view, on a millimetered paper. **D.** ventral view of specimen A in clove oil.

Fig. 17. *Pseudogeoplana wetzeli* (Schirch, 1929). **A.** Set of all syntypes. **B.** Lateral view of syntype B in clove oil.

Fig. 18. *Pseudogeoplana wetzeli* (Schirch, 1929), photomicrographs of syntype A. **A.** Horizontal section of anterior extremity. **B.** Horizontal section of ovaries region. **C.** Horizontal section of testes region. **D.** Transverse section of anterior extremity.

Fig. 19. *Pseudogeoplanea wetzeli* (Schirch, 1929), syntype A. **A.** Virtual section of pharynx. **B.** Photomicrograph of a sagittal section of pharynx. **C.** Photomicrograph of a tangential section of outer epithelium of pharynx and its muscle on a sagittal plane. **D.** Photomicrograph of a sagittal section of outer epithelium of pharynx and its underlying muscle.

***Pseudogeoplana blaseri* (Schirch, 1929)**

Synonymy

Geoplana blaseri Schirch, 1929

Pseudogeoplana blaseri: Ogren & Kawakatsu, 1990, p. 114.

Remarks

We received on loan only one specimen identified as *Pseudogeoplana blaseri*. This specimen displays an aspect disparately dissimilar from that of the original description of the species. External aspect of *Ps. blaseri* was illustrated by Schirch (1929) (Fig. 20). In Schirch's collection there is no specimen that compares well with original description of *Ps. blaseri*. Most probably, a mislabeling happened before deposition of material or during curatorial actions. Thus, type material of *Ps. blaseri* should be considered lost. In the section "*Dolichoplana* sp." (see below), we describe the specimen labeled as *G. blaseri* to provide evidence of mislabeling.

Fig. 20. Original illustration of *P. blaseri* after Schirch (1929).

Rhynchodeminae Graff, 1896

***Dolichoplana* sp.**

Material examined. MNRJ 8922, specimen: unknown collecting site and date. Misabeled as *G. blaseri*. Three dimensional (3D) images and virtual sections of specimen MNRJ 8922 were obtained through microcomputed tomography. Subsequently, the following body parts of this specimen were histologically processed: Transverse sections of anterior extremity on 38 slides; horizontal sections of a region immediately behind anterior extremity on 31 slides; transverse sections of pre-pharynx on 31 slides; transverse sections of posterior extremity on 18 slides; remaining body preserved in 80% ethanol.

External aspect. Fixed animal with approximately 90 mm long, and 2.5 mm wide. Body elongate, with parallel margins; dorsum convex, ventral side flat, and margins rounded. Anterior and posterior extremities blunt (Fig. 21A and 21B). The dorsum is ochre yellow in color, ornated with terra brown pigment constituting 3-5 longitudinal stripes, namely a median stripe, a pair of lateral stripes and a pair of lateral stripes, these stripes are irregularly faded, mainly in the first 1/4th of the body. Ventral side ochre yellow on a midline, 0.9 mm in width, and terra brown on the body sides (Fig. 21A and 21C).

A pair of eyes present at 4 mm from anterior extremity. Eyes 85 μ m in size (Fig. 22A). Sensory pits are simple invaginations 25-30 μ m deep, located marginally in a single row. They are distributed between the second and fourth millimeters of the body. Mouth at a distance from anterior extremity of the body equivalent to 29% of body length.

Internal morphology. Creeping sole comprising with 35% body width (Fig. 22B). Rhabditogen cells producing erythrophil granules pierce abundantly ventral and marginal epidermis.

The cutaneous musculature comprises three layers, namely a subepithelial circular layer, followed by one diagonal layers with decussate fibers (10 μ m thick), and then a well developed innermost longitudinal layer (70 μ m thick) dorsally, and a subepithelial circular layer, followed by one diagonal layers with decussate fibers (10 μ m thick), and then a well developed innermost longitudinal layer (70 μ m thick) ventrally, the same organization and measured of side dorsal. Muscle fibers of the

longitudinal layer are arranged into bundles with 21-54 fibers each. Cutaneous musculature thickness relative to body height at the pre-pharyngeal region, 18% (Fig. 22A and 22B).

Five parenchymal muscle layers present: a dorsal layer (20 μm thick) of decussate diagonal fibers, a suprainestinal layer (40 μm thick) of transverse fibers, a subintestinal layer (30 μm thick) of transverse fibers, a subneural layer (40 μm thick) of longitudinal fibers, and ventral layer (40 μm thick) of decussate diagonal fibers located to the inside of the ventral cutaneous nerve net (Fig. 22C and 22D).

Central nerve system constituted of a pair of ganglia (Fig. 22A) and a set of nerve fibers with the typical form of a rope ladder (Fig. 22B).

Mouth situated at a distance from root of the pharynx equivalent to 50% of pharyngeal pocket length. Pharynx cylindrical, not folded. Esophagus measures 0.5 mm in length. Pharynx at a distance from anterior extremity of the body equivalent to 29% of body length (Fig. 23). Intestine typical of that of triclads, consisting of an anterior main branch, and two posterior ones, all of them also branched (Fig. 21B). Reproductive organs not developed.

Remarks

It is evident that the morphology of this specimen does not agree with original description of the single type specimen of *Ps. blaseri* (Fig. 21D). Original description reads: "Length: 160 mm. Width: 20 mm. Dorsum almost completely black, in just a few places it shows the ground color. Pharyngeal and gonopore openings are evident on the ventral side, at 95 mm and 110 mm from anterior extremity, respectively. Ventral side irregularly patterned with marble (so distinguishing from *G. rufiventris*)". (Original in Portuguese). Schirch's description and figure of *Ps. blaseri* matches *Obama* (Geoplaninae), but every morphological aspect of the specimen of Schirch's collection labeled with "blaseri" mismatches Schirch's description. In this collection, there is any individual resembling original description of *Ps. blaseri*. Thus, the type material, i. e., the holotype, should be considered lost.

Specimen MNRJ 8922 agrees with the genus *Dolichoplana* (Rhynchodemidae): body shape and size, position of mouth, number of eyes, and thickness of cutaneous longitudinal muscle (Moseley, 1877; Graff, 1899; Hyman, 1940). However, differently from our specimen, the cutaneous muscle consists of only the longitudinal layer dorsally in *Dolichoplana*.

General external aspect resembles that of *Dolichoplana striata* Moseley, 1877 and *D. carvalhoi* Correa, 1947 - the latter being a junior synonym of the former after Froehlich (1967). Unfortunately, the specimen MNRJ 8922 is immature and not further identification could be done.

Fig. 21. *Dolichoplana* sp. Specimen MNRJ 8922. **A.** Specimen fixed in dorsal view. **B.** Volume rendering reconstruction of the body from mCT data. **C.** Fixed specimen.

Fig. 22. *Dolichoplana* sp. Photomicrographs of specimen MNRJ 8922. **A.** A transverse section of cephalic region. **B.** A transverse section of pre-pharynx. **C.** A horizontal section of pre-pharynx region, view dorsal muscles. **D.** A horizontal section of pre-pharynx region.

Fig. 23. *Dolichoplana* sp. Specimen MNRJ 8922. Virtual sagittal section of the pharynx.

On the nomenclatural stability of the species names. As demonstrated above, descriptions of species of land planarians based on features others than those of the copulatory apparatus do not promote nomenclatural stability of their names (Ogren & Kawakatsu, 1990). This is the case of *Ps. arpi*, *Ps. wetzeli* (syntypes not mature) and *Ps. doerderleini* (syntypes poorly preserved). In this work, we also report loss of type material of *Pseudogeoplana blaseri*. These nominal species will probably remain indefinitely in this collective genus.

On the opposite side, detailed descriptions of species, including copulatory apparatus, allow address taxonomic and systematic reflections. This situation is the case of *Pasipha plana* and *Paraba bresslaui*, the internal organs of which are presented here for the first time through the study of type specimens. Indeed, in the latter case, we managed to fully preserve the bodily integrity of the holotype through microcomputed tomography of X-rays.

Trade-offs between preservation and body sectioning. In an earlier paper, Carbayo et al. (2016) discussed the virtues and limitations of mCT explored for the morphological study of these flatworms. Main problems they raised are derived from inoperability of the mCT to differentiate acid from basic secretions, and the small size of structures that are important to the systematic of the group. The acid-basic nature of secretions was not solved in this work since PTA seemingly links to tissue independently from this property. Nevertheless, on the rare occasions this feature of the secretions has been used for determine species, it was one a set of characteristics (e.g., Álvarez-Presas et al., 2015).

Minute structures such as single muscle fibers, sentory pits, cilia and cell nucleus remained also indiscernible. One real limitation of some mCT equipments, such as SkyScan 1173 scanner (used by Carbayo et al., 2016), is that relatively wide objects cannot be scanned at the highest equipment resolution. This is because resolution is object-source of X-rays distance dependent, the higher magnification the shorter distance. Wide objects (e.g., *Obama otavioi* Carbayo et al., 2016 was scanned at 6.04 μm maximal resolution whereas the scan can provide 7 μm resolution) exceed minimum distance from source of X-rays required for scanning at the highest resolution. Zeiss Xradia Versa XRM-510 overcame this problem through a scintillator that converts X-rays to visible light. Subsequently, this light is optically magnified (<https://www.zeiss.com/microscopy/int/products/x-ray-microscopy/zeiss-xradia-510-versa.html>). Highest resolution of this facility reaches 0.7 μm . Thanks to

this, we were able to scan parts of the holotype of *Pa. bresslaui* with high resolution, i.e.,. This allowed us to obtain images with 1.62 μm resolution even though the specimen was bent so doubling its effective width to ~ 10 mm (see Figs. 1E and 1F). Moreover, we succeeded in redescribing this species nearly following current standards from only mCT-derived images, so preserving the integrity of this precious specimen. In exchange for this advantage, use of the facility depends on schedule of facility operators and can cost more than preparation of traditional histological sections.

This is an example of how mCT may be useful for the taxonomic study of soft-bodied organisms.

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Introduction

The subfamily Geoplaninae harbors most of the Neotropical species of land planarians, of which there are 313 species housed in 24 genera (Carbayo, 2019). The taxonomic status of these genera is uneven, with some species of *Imbira* Carbayo et al. (2013) (Geoplaninae) needing a revision. Currently the genus has four species: *Imbira guaiana* (Leal-Zanchet & Carbayo, 2001), type species of the genus; *Imbira marculsi* Carbayo et al., 2013; *Imbira negrita* Negrete & Brusa, 2017 and *Imbira flavonigra* Amaral et al., 2018.

Imbira was proposed to house two species, *Notogynaphallia goetschi* (Riester, 1938, originally in *Geoplana*), from Teresópolis, State of Rio de Janeiro, and *Notogynaphallia guaiana* Leal-Zanchet & Carbayo, 2001, from São Francisco de Paula, in the State of Rio Grande do Sul. Accordingly with the diagnosis of the genus, the two species possess rare morphological attributes such as parenchymatic muscle layers of longitudinal fibers, dorsally and ventrally to the intestine, in addition to the three common parenchymatic muscle layers, and a penis papilla eversible, among main features of the genus.

The type of penis papilla - either eversible or protrusible - in representatives of the genus is controversial. Besides *Geoplana goetschi*, Riester (1938) also described as a specimen with papilla protrusible from Teresópolis and named it *Geoplana fryi* var. *bruna*, even though its copulatory apparatus is much more similar to that of *G. goetschi* than to the organ of *G. fryi* [currently *Obama fryi* (Graff, 1899)]. This similarity led Marcus (1951) to conclusion that *G. fryi* var. *bruna* would be a synonym of *G. goetschi*.

Marcus (1951) studied five specimens from Butantã and Horto Florestal - two localities in the municipality of São Paulo (approximately 350 km away from Teresópolis)-, and other municipalities of the State of São Paulo, namely Santo André (formerly Alto da Serra), Mogi das Cruzes and Mongaguá (formerly Vila Atlântica) and identified them as *Geoplana goetschi*. Among the specimens he sectioned, there was a young individual (see Fig. 191) and made the following observation: “In the young worm, the atrium is partially everted and, therefore, slightly folded. It could, at this stage, speak of penis papilla, constituted, however, only by the contraction of the muscular envelope [...] We marked the orifice (c) of the ejaculatory duct in the worm with 'penis papilla' and in the others, 'without papilla', to show the variability of penis length”.

Later, Ogren & Kawakatsu (1990) transferred *G. goetschi* to *Notogynaphallia* Ogren & Kawakatsu, 1990 since the species, as the diagnosis of the genus, does not present penis papilla.

After examining a number of specimens Zanchet & Carbayo (2001) considered that the species, now *N. goetschi*, does present a permanent penis papilla and stated the need of a taxonomic review.

More recently, Carbayo et al., (2013) inferred a phylogeny of several members of Geoplaninae from molecular data. In that paper, *N. goetschi* and *N. guaiana* have a sister-group relationship. They also found out a rare morphological attribute of the muscular systems in specimens studied by Marcus and the type material of *N. guaiana*, i.e., parenchymatic muscle layers of longitudinal fibers, dorsally and ventrally to the intestine. They did not provide morphological evidences. On the basis of the molecular and morphological congruence, Carbayo et al. (2013) (a) considered considered Marcus's specimens different from *G. goetschi* and proposed they represent a new species they named *Imbira marcus* and (b) transfered *G. goetschi* sensu Marcus (1951) and *N. guaiana* to a new genus, *Imbira*.

Very recently we discovered that part of the type material of the 15 species described by Schirch (1929) have survived until the present (see Introduction of the previous manuscript). Among these specimens, we found representatives of *Pseudogeoplana bonita* (Schirch, 1929). This species, originally placed in *Geoplana*, was transferred to the collective genus *Pseudogeoplana* in view of the insufficient morphological knowledge of its internal organs. Interestingly, this species presents features of *Imbira* as shown in this paper. Based on the type material and additional material herein we (a) redescribe *Pseudogeoplana bonita*, which resulted to be senior synonym of *Geoplana goetschi* Riester and (b) describe a new species of *Imbira*, very alike with *I. marcus* and sympatric with it.

Materials and Methods

We examined the type material of *Ps. bonita* Schirch (1929). Schirch's type material consists of seven syntypes. They are housed in the Museu Nacional do Rio de Janeiro (MNRJ). Syntypes were mailed to our laboratory wrapped in paper towel moistened with ethanol 80%, each inside a 5-10 ml plastic vial. Upon receipt of the material, we filled the plastic tubes with 80% ethanol. Additionally, to a detailed morphological study, we included specimens of *Ps. bonita* collected recently in type locality (Teresópolis/RJ). We searched for these animals on the soil litter and forest trails during the day and at night. We took pictures of them alive, euthanized in boiling water, cut off a small tissue sample, and preserved it in 100% ethanol for DNA extraction. We fixed the remaining body in 10% formalin and, subsequently, transferred it to 80% ethanol. The same protocol was applied to the syntypes of *Imbira* sp. n. The type material of *G. goeschi* was also studied from photomicrographs of histological sections, schematic drawings of the copulatory organs made by one of us (FC) during a short stay in the Senckenberg Museum Frankfurt.

Histology

The syntypes A (MNRJ 8909) and B (MNRJ 8909) of *Ps. bonita* were examined under the stereomicroscope and photographed with the help of an Olympus SZ61 stereo-microscope and a Leica M80 stereo-microscope with a Leica IC80HD digital camera attached to it. Different regions of the body of the syntypes were histologically treated as follows: dehydration in an alcoholic series, dealcoholization in clove oil, inclusion in Paraplast ©, serial sectioning at 5-7 microns in thickness with retracting rotary microtome (Microm HM315 R), rehydration and staining with Mallory's trichrome as modified by Cason (1950). Distribution of eyes was observed in specimens in clove oil. Drawings of the pharynx and copulatory apparatus were performed using a camera lucida coupled with the optical microscope. Nomenclature of body color follows RAL CLASSIC (<https://www.ral-farben.de/content/application-help/all-ral-colours-names/overview-ral-classic-colours.html>). Histological slides were examined and photographed using Olympus BX51 optical microscope and Olympus DP72 digital camera attached to it. Whenever possible, all figures are orientated so that anterior extremity of the body is to the left. The same protocol was applied to the syntypes of *Imbira* sp. n.

Taxonomic section

***Imbira* Carbayo et al., 2013**

***Imbira bonita* (Schirch, 1929), comb. nov.**

Synonymy

Geoplana bonita Schirch, 1929 sp. n., p. 34. Type locality: Teresópolis, Rio de Janeiro, Brazil.

Geoplana bonita: Riester, 1938, p. 78-79. Teresópolis.

Pseudogeoplana bonita: Ogren & Kawakatsu, 1990, p. 55.

Geoplana goetschisp. n. Riester, 1938, p. 20-24. Teresópolis.

Geoplana fryi var. *bruna* Riester, 1938, p. 69-71. Teresópolis.

Material examined. Type material (Fig. 24D). Seven syntypes collected by P. Schirch *Coll* (date unknown) in Teresópolis, State of Rio de Janeiro, Brazil. We received them on loan in 70% ethanol with a label reading MNRJ 8909. Each syntype was given an additional identification with a letter, A-G. Three dimensional (3D) images and virtual sections of syntype MNRJ 8909A were obtained by microcomputed tomography. **MNRJ 8909, syntype - A, C, D, E, F and G:** Body preserved in 80% ethanol. Parts of the body of **MNRJ 8909, syntype - B** were histologically sectioned as follows: Horizontal sections of anterior extremity on 44 slides; horizontal sections of ovaries region on 50 slides; transverse sections of pre-pharyngeal region on 39 slides; sagittal sections of pharynx on 45 slides and sagittal sections of copulatory apparatus on 75 slides (immature). Remaining body preserved in 80% ethanol.

Additional material examined: F2615 (MZUSP PL XXXX): Parque Nacional da Serra dos Órgãos, Teresópolis, State of Rio de Janeiro, Brazil, -22.45642, -42.99514, Carbayo et al., *Coll* 15 July 2008. Body preserved in 80% ethanol. **F4235** (MZUSP PL XXXX): Parque Nacional da Serra dos Órgãos, Teresópolis, State of Rio de Janeiro, Brazil, -22.2705, -43.0005, Carbayo et al., *Coll* 7 December 2010: Sagittal sections of copulatory apparatus on 145 slides. Remaining body preserved in 80% ethanol. **F4236** (MZUSP PL XXXX): *Ibidem*. Transverse sections of anterior extremity on 42

slides, horizontal sections of ovaries region on 49 slides, horizontal sections of post-ovaries region on 56 slides, sagittal sections of pharynx on 111 slides and sagittal sections of copulatory apparatus on 78 slides. Remaining body preserved in 80% ethanol. **F4243** (MZUSP PL XXXX): *Ibidem*. Sagittal sections of copulatory apparatus on 174 slides. Remaining body preserved in 80% ethanol. **F4280** (MZUSP PL XXXX): Parque Nacional da Serra dos Órgãos, Teresópolis, State of Rio de Janeiro, Brazil, -22.2705, -43.0005, Carbayo et al., *Coll* 8 January 2010. Body preserved in 80% ethanol. **F4297** (MZUSP PL XXXX): *Ibidem*. Body preserved in 80% ethanol.

Distribution. Atlantic forest in the municipality of Teresópolis, State of Rio de Janeiro, Brazil.

Diagnosis. Species of *Imbira* with 85 mm in length. Dorsal color with a thin zinc yellow midline separating a pair of black red paramedian stripes, external to which there is an orange yellow lateral stripe. Body margins black red. Dorsal epidermis of anterior extremity ciliated; muscular tube of parenchymal longitudinal fibers wraps intestine from anterior region of the body until near the pharynx; prostatic vesicle divided into a paired anterior portion and an unpaired portion with 40% of the length of the entire prostatic vesicle; ovovitelline ducts emerge from the inner-dorsal region of ovaries; female atrium elongate, 5 times longer than its diameter, and divided into a posterior, ellipse-shaped half that is lined with an epithelium with multilayered aspect, and anterior, folded half that is lined with a columnar epithelium.

External aspect. Living specimens display the dorsum with a thin (3.5 % of body width) zinc yellow midline separating a pair of black red paramedian stripes (each 21% of body width), external to which there is an orange yellow lateral stripe (40% of body width, specimen F4236, Fig. 24A). Body margins black red. Ventral body surface cream-to-beige (Fig. 24B). Adult fixed specimens are 85-110 mm in length. An adult fixed specimen (syntype A) was 85 mm long, 5 mm wide and 3 mm high. Elongated body, with parallel margins; anterior and posterior extremity rounded. Dorsum convex, ventral side flat (specimen F4236). After 89 years in preservation, the dorsal color of the seven syntypes turned into green-beige with two brown-beige paramedian stripes with width of ~1.5 mm each (30% of body width); the remaining body surface turned into lemon yellow (Fig. 24E and 24F). The ventral body surface of the fixed syntypes is green-beige.

Eyes, monolobulated, contour the anterior extremity marginally, and extend until the posterior extremity distributed in an irregular double-to-triple row in the body margins (syntype B).

Besides the creeping sole, dorsal surface of anterior extremity is ciliated (Fig. 25A and 25B). Sensory pits are simple invaginations 22 μm deep, located ventro-marginally in a single row from the anterior body tip of the body backwards to at least 15% of body length (specimen F4236). Relative position of the mouth: body length, ($\sim 68\%$, syntype B; $\sim 55\%$, specimen F4236); relative position of gonopore: body length, ($\sim 69\%$, specimen F4236).

Internal morphology. Creeping sole comprising 90% of body width. Rhabditogen cells pierce abundantly dorsal and marginal epidermis. Two gland types, producing erythrophil granules and amorphous erythrophil secretion, respectively, pierce entire epidermis, the latter type is more abundant in the latero-ventral side of the body; glandular margin absent (Fig. 25C).

Cutaneous musculature comprises three layers, namely a subepithelial circular muscle, followed by a diagonal layer with decussate fibers, and an innermost longitudinal muscle. Muscle fibers of the longitudinal muscle (57 μm thick dorsally; 57 μm thick ventrally) arranged into bundles with ~ 32 fibers each (Fig. 25C). Cutaneous musculature thickness relative to body height at the pre-pharyngeal region, 10%.

Very numerous dorso-ventral parenchymal muscle fibers (Fig. 26A and 26B). Five parenchymal muscle layers present: (a) dorsal layer (160 μm thick) of decussate diagonal fibers, (b) a suprainestinal layer (160 μm thick) of transverse fibers; (c) a 250- μm -thick suprainestinal layer of well defined bundles of longitudinal fibers immediately below the suprainestinal layer of transverse fibers; some fibers of these two layers appear intermingled in horizontal sections (Fig. 25C); (d) a 250- μm -thick layer of longitudinal fibers below the intestine; and (e) a 150- μm -thick layer of subintestinal transverse fibers. Actually, the two longitudinal layers extend laterally to join each other so that they form a tube. Interestingly, these parenchymal longitudinal muscle fibers do occur only along anterior half of the body, that is, from anterior extremity of the body to the pharyngeal region.

Ventral nerve plate ~ 100 μm thick in the pre-pharyngeal region, and 200 μm in the cephalic region (specimen F4236; Fig. 25C and 3A). No definite cerebral ganglia were found.

Mouth situated at a distance from root of the pharynx equivalent to 38% of pharyngeal pocket length. Pharynx bell-shaped. Esophagus ~ 800 μm in length in syntype B and ~ 1800 μm in specimen F4236. Outer pharyngeal musculature consisting of a thin subepithelial longitudinal muscle (5 μm),

followed by a circular muscle (10 μm). Inner pharynx musculature consisting of a subepithelial of circular muscle (40-80 μm , specimen F4236; Fig. 26C) followed by a 10- μm -thick longitudinal muscle.

Testes ~175 μm in diameter. They are dorsally located between the suprainestinal longitudinal parenchymal muscle layer and intestine (Fig. 27A). Anteriormost testes at a distance from anterior extremity of the body equivalent to 22% of body length; posteriormost testes are near the root of the pharynx and at a distance from anterior extremity equivalent to ~55% of body length (syntype B).

Sperm ducts contain sperm in their distal portion and are dilated (specimen F4235). Distally, they bend slightly medially and dorsally to communicate with the ventro-proximal region of the prostatic vesicle.

The extrabulbar prostatic vesicle is horizontal, very long and divided into a bifurcated, proximal portion, ~2500 μm long and 125 μm in diameter, and an unpaired, 2250 μm long and 75- μm in diameter, distal portion (specimen F4236, Fig. 29A-B). Anteriormost portion of prostatic vesicle lateral to pharyngeal pouch. Distally, prostatic vesicle is curved slightly ventrally to enter the penial bulb and communicating with the ejaculatory duct. Epithelium of the prostatic vesicle is ciliated and 25-75 μm high, with intensely pleated wall. It is pierced by gland cells producing erythrophil granules, and is surrounded by a 40- μm -thick muscle of circular and longitudinal fibers. Anterior portion of ejaculatory duct is dilated and with pleated wall; distal portion is canalicular and helicoidal to open into the anteriormost portion of the male atrium. Ejaculatory duct presents a ciliated epithelium and is surrounded by a 10- μm -thick muscle of criss-crossed fibers.

There is no permanent penis papilla but an eversible penis papilla formed by eversion of anterior folds of male atrium, as suggested by the shape of the folds in specimens F4236 and F4235. Male atrium is a long cavity with intensely folded walls. In specimen F4236 (Fig. 29A), the largest folds are the anteriormost. Male atrium lined with a cuboidal-to-columnar, ciliated epithelium that is pierced by cell glands producing erythrophil granules. A 10- μm -thick circular muscle followed by a 10- μm -thick longitudinal muscle surround this epithelium.

Ovaries are ovoid-shaped, 125 μm in longitudinal axis, and situated above the ventral nerve plate. They are at a distance from anterior extremity equivalent to 10% of body length. Ovovitelline ducts emerge from the inner-dorsal region of ovaries. Behind the gonopore region, these ducts curve medially and dorsally, and join to form the common glandular ovovitelline duct above the female atrium (Fig. 27B). Epithelium of the ovovitelline ducts 10 μm high, ciliated and surrounded by a 25- μm -thick decussate muscle. The common ovovitelline duct is ~1000 μm long and runs horizontally

(specimen F4236); shell glands are absent (specimens F4235 and F4236). This duct communicates with the female genital canal, which is a 350- μ m-long projection of the postero-dorsal region of the female atrium. This canal is directed forwards.

Female atrium elongate, 5 times longer than is height. It is divided into two halves (Fig. 28A-B). Posterior half presents the shape of an ellipse and is lined with an epithelium with multilayered aspect that fills almost all the lumen (Fig. 28B). Anterior half is folded and lined with a columnar, ciliated epithelium, the subapical portion of which is erythrophil; the free surface of this epithelium is sinuous. Female atrium receives two types of glands producing erythrophil and xanthophil granules, respectively, the former one much more abundant. A 15- μ m-thick circular muscle followed by a 10 μ m thick longitudinal muscle wrap female atrium. Common muscle coat surrounds male atrium, female atrium and common glandular ovovitelline duct (specimen F4235).

Ecological note

Specimens F4280 and F4297 were found together feeding on a terrestrial leech.

Remarks

Conspicificity of specimens studied. We examined three sets of specimens: type material of *Ps. bonita*, additional specimens collected by us, and type material of *G. goetschi* Riester, 1938. A comparison between Schirch's (1929) textual description of the color pattern of the dorsum and that of our additional material reveals minor differences: median stripe yellowish after Schirch vs. zinc yellow in the additional material; paramedian stripes and body margins dark green vs. black red; lateral stripes orangish vs. orange yellow. However, drawing of the dorsum provided by Schirch (1929) (reproduced here in Fig. 24C) agrees with that of additional material. Furthermore, size and shape of the body of syntypes also match that of additional material. Hence, in this respect, conspecificity of syntypes and additional material cannot be excluded.

Best morphological attributes to test for conspecificity are those of the copulatory organs. Additional specimens are mature but very unfortunately, syntypes are not. However, we found out a further set of rare morphological attributes that sheds light on the conspecificity: syntypes and additional material share (a) a ciliated epidermis on the dorsal side of the anterior extremity; (b) a parenchymal muscular tube of longitudinal fibers extending along anterior half of the body only. To the best of our knowledge, such pattern of distribution of ciliated epidermis is only known in species of

two genera of Geoplaninae, namely *Geobia* (Diesing, 1861) and *Cratera* (Froehlich, 1955a), the first is house of one species and the second is house of nine species, but only two, *C. hina* and *C. joia*, presents ciliated epidermis on the dorsal side. The rarity of this structure reinforces the conspecificity between type material and conspecific-material. A parenchymal musculature of longitudinal fibers is also present in representatives of *Geoplana* either in the form of a layer dorsal to intestine and another layer ventral to the intestine, or as a tube around it. The latter situation is that of *I. bonita*, but this species is unique in that the muscular tube is restricted to the anterior half of the body.

Despite copulatory organs have been used as the ultimate morphological evidence for species identification, the evidences gathered from other body regions support our conclusion of conspecificity between syntypes and additional specimens studied. Moreover, syntypes and additional material were collected in the same locality and we never have found any other species with which *I. bonita* could be confounded.

Taxonomic position of the species. The species studied presents all characteristics of the subfamily Geoplaninae, i.e., creeping sole covering most of ventral surface, mouth behind midbody, a well developed cutaneous musculature organized into bundles, part of the parenchymal muscle layers organized in longitudinal fibers, and dorsal testes (see Almeida et al., 2018). Within this group, only *Geoplana* and *Imbira* include representatives having parenchymal longitudinal musculature. However, *I. bonita* does not fit *Geoplana* since as characteristic of this genus the dorsum is strongly convex; eyes in the anterior region of the body are cone shaped; a prostatic vesicle is absent; the penis papilla is protrusible and very long, extending into female genital atrium; the male atrium is not folded, among other distinctive features.

Our species matches all features of *Imbira* and it should be compared with the remaining species of the genus.

Distinctiveness of I. bonita. This genus houses four species, namely *I. guaiana* (Leal-Zanchet & Carbayo, 2001), *I. marculsi* (Carbayo et al., 2013), *I. negrita* Negrete & Brusa, 2017 and *I. flavonigra* Amaral & Leal-Zanchet, 2018. Regarding the external aspect, *Imbira bonita* differs from *I. guaiana* and *I. negrita* in that these two species display a dark homogeneous dorsum whereas the dorsum of *I. bonita* is striped. A striped pattern is present also in *I. marculsi* and *I. flavonigra*. However, in *I. marculsi* the paramedian stripes are yellow relatively wider and the lateral stripes are yellowish (vs. black red in *I. bonita*) and thinner; and in *I. flavonigra*, the midline is yellow (vs. zinc yellow in *I. bonita*) and

relatively much wider, and this midline is bordered by black stripes (vs. blackred in *I. bonita*) which are relatively much more thinner.

With respect to internal aspect, *I. bonita* presents a combination of features that differs from the remaining species of the genus: the dorsal surface of the anterior extremity is ciliated (vs. non-ciliated in *I. marculsi*; this feature has not been examined in *I. guaiana*, *I. negrita*, and *I. flavonigra*); the parenchymal muscular tube of longitudinal fibers extends along anterior half of the body only (vs. along the entire body length in *I. marculsi*, *I. negrita*, and *I. flavonigra*); 40% of the length of the prostatic vesicle is paired (vs. 25% in *I. guaiana*, 62-100% in *I. marculsi*, 29-70% in *I. negrita* and 10-18% in *I. flavonigra*); the ovovitelline ducts emerge from the inner-dorsal region of ovaries (vs. lateral region in *I. guaiana* and *I. negrita*); and female atrium is five times longer than its diameter (vs. two-and-a-half times in *I. guaiana*, three times in *I. marculsi*, three times in *I. negrita* and two times in *I. flavonigra*), and only posterior half is provided with an epithelium of multilayered aspect (vs. the entire epithelium in the remaining species).

Fig. 24. *Imbira bonita* (Schirch, 1929). **A.** Living specimen F4236 in dorsal view. **B.** Living specimen F2615 in ventral view. **C.** Original illustration by Schirch. **D.** Set of all type-material. **E.** Dorsal view of fixed syntype A on graph paper. **F.** Ventral view of fixed syntype A.

Fig. 25. *Imbira bonita* (Schirch, 1929). Photomicrograph of a transverse section of anterior extremity. **A.** Specimen F4236. **B.** Syntype B. **C.** Specimen F4236.

Fig. 26. *Imbira bonita* (Schirch, 1929). Photomicrographs. **A.** Syntype B. Horizontal section of ventral region. The inset in (A) is shown enlarged. lp, longitudinal parenchymal muscle; dv, dorso-ventral parenchymal muscle. **B.** Syntype B. Horizontal section of dorsal region. The inset in (B) is shown enlarged lp, longitudinal parenchymal muscle; dv, dorso-ventral parenchymal muscle. **C.** Specimen F4236. Sagittal section of pharynx.

Fig. 27. *Imbira bonita* (Schirch, 1929), photomicrographs of specimen F4236. **A.** Horizontal section of testes region. **B.** Horizontal section of ovary region.

Fig. 28. *Imbira bonita* (Schirch, 1929), specimen F4235. Photomicrographs sagittal sections of the copulatory apparatus **A.** Anterior region of female atrium and its columnar, ciliated epithelium (arrow in inset). **B.** Posterior region of female atrium and its epithelium with multilayered aspect (arrow in inset).

Fig. 29. *Imbira bonita* (Schirch, 1929). **A.** Specimen F4236. Photomicrograph of a sagittal section of copulatory apparatus. **B.** Specimen F4235. Reconstruction of copulatory apparatus. * indicates epithelium of female atrium with multilayered aspect.

***Imbira* sp. n.**

Material examined. Type material. **Holotype** (F3717, MZUSP PL XXXX): Parque Estadual da Cantareira, São Paulo, State of São Paulo, Brazil, Carbayo et al., *Coll* 19 April 2009: Transverse sections of anterior extremity on 7 slides; horizontal sections of ovaries on 15 slides; sagittal sections of a portion behind ovaries on 33 slides; transverse sections of pre-pharyngeal region on 16 slides; sagittal sections of pharyngeal region on 28 slides and sagittal sections of copulatory apparatus on 20 slides. Paratypes: **F3503** (MZUSP PL XXXX): Parque Estadual da Cantareira, São Paulo, State of São Paulo, Brazil, Carbayo et al., *Coll* 26 March 2009: Sagittal sections of copulatory apparatus on 21 slides. **F3505** (MZUSP PL XXXX): *Ibidem*. Sagittal sections of pharynx on 27 slides. **F3507** (MZUSP PL XXXX): *Ibidem*. Transverse sections of pre-pharyngeal region on 12 slides; sagittal sections of pharynx on 20 slides. **F3536** (MZUSP PL XXXX): *Ibidem*. Sagittal sections of copulatory apparatus on 56 slides. **F3538** (MZUSP PL XXXX): *Ibidem*. Sagittal sections of copulatory apparatus on 101 slides. **F3539** (MZUSP PL XXXX): *Ibidem*. Sagittal sections of copulatory apparatus on 93 slides. Remaining body of all specimens preserved in 80% ethanol.

Distribution. Areas covered with Atlantic forest in the municipality of São Paulo, State of São Paulo, Brazil.

Diagnosis. *Imbira* of 70-110 mm in length and 10 mm wide. The color of the dorsum between ocher yellow and olive drab; parenchymal muscle tube of longitudinal fibers around intestine from anterior extremity of the body to pre-pharyngeal region; penis papilla protrusible, as long as 3 times its diameter, entirely occupying both male and half of female atrium; ovovitelline ducts emerge from inner-dorsal section of ovaries; female atrium elongate and divided into a keyhole-shaped, posterior half, lined with an epithelium with multilayered aspect, and a dilated anterior half, lined with a columnar epithelium; length of female atrium equal to twice its diameter.

External aspect. A living adult (holotype) measured 70-110 mm in length, 10 mm wide. After fixation, it measured 43 mm long, 8 mm wide, and 1.7 mm high. Elongated body, with margins parallel; anterior extremity pointed, posterior rounded. Dorsum convex, ventral side flat.

Dorsal color olive drab (Fig. 30A) or ocher yellow (Fig. 30B), slightly clearer submarginally, and darker marginally. A thin midline (8% of body width) of sand yellow color may also be present. Ventral side varies between ivory and daffodil yellow color, whitish in the region of pharynx and copulatory apparatus. The color of the body faded after fixation.

Monolobulated eyes contour the anterior extremity (fig. 30C) marginally, extending to the posterior extremity in a row of 2-3 eyes. A minute halo encircles each eye. Sensory pits as simple invaginations, 25 μm deep, located ventro-marginally in a single row that contour anterior extremity of the body (Fig. 30D) and extend posteriorly at least along 3 mm of body length (holotype). Relative position mouth: body length, 60%. Relative position gonopore: body length, 83% (holotype).

Internal morphology. Creeping comprising with 95% body width. Rhabditogen cells pierce dorsal and marginal epidermis. There are additional glands piercing the epidermis; unfortunately suboptimal quality of the staining did not produce details of their affinity for dye. Glandular margin absent. The cutaneous musculature comprises three layers, namely a subepithelial one-fiber-thick circular layer, followed by two diagonal layers with decussate fibers (5 μm thick dorsally, 10 μm thick ventrally), and then a well developed longitudinal layer (90 μm thick dorsally, 70 ventrally) arranged into bundles with 45-54 fibers each. Cutaneous musculature thickness relative to body height at the pre-pharyngeal region, 8% (Fig. 31A-B).

Five parenchymal muscle layers present: (a) dorsal layer (60 μm thick) of decussate diagonal fibers, (b) a suprainestinal layer (150 μm thick) of transverse fibers; (c) a 120- μm -thick suprainestinal layer of well defined bundles of longitudinal fibers immediately below the suprainestinal layer of transverse fibers; some fibers of these two layers appear intermingled in horizontal sections; (d) a 260- μm -thick layer of longitudinal fibers below the intestine; and (e) a 120- μm -thick layer of subintestinal transverse fibers. Actually, the two longitudinal layers extend laterally to join each other so that they form a tube. These parenchymal longitudinal muscle fibers do occur only along anterior half of the body, that is, from anterior extremity of the body to the pharyngeal region (Fig. 32A-B). Ventral nerve plate present.

Mouth situated at a distance from the root of the pharynx equivalent to 32% of pharyngeal pocket length. Pharynx bell-shaped. Esophagus conspicuous, 1250 μm long (holotype). Outer pharyngeal musculature consisting of a subepithelial layer (5 μm) of longitudinal muscle, followed by

a layer (12.5 μm) of circular fibers. Inner pharynx musculature consisting of a subepithelial circular muscle (125 μm) followed by a longitudinal muscle (35 μm ; Fig. 33A).

Testes measure 150 μm in diameter. They are dorsally located at the level of the suprintestinal longitudinal layer; anteriormost testes at a distance from anterior extremity equivalent to 18% of body length; posterior follicles near the pharyngeal root (Fig. 33B). Sperm ducts contain sperm in their distal portion. They are lined with a ciliated, epithelium, 12.5 μm high and receive glands producing erythrophil granules. A 100- μm -thick circular muscle cloths sperm ducts.

The prostatic vesicle is long and horizontal, divided into a bifurcated, proximal portion ~1100 μm long, and an unpaired, 1250 μm long distal portion (holotype, Fig. 34A-B). The proximal portion measures 160 μm in diameter; the distal portion measures 100 μm in diameter. The ciliated epithelium of prostatic vesicle 40-65 μm high; it is pierced by numerous glands producing erythrophil granules and is surrounded by a 170- μm -thick circular muscle in proximal portion that becomes thinner (18 μm thick) distally. Prostatic vesicle penetrates ventro-anterior aspect of the well muscularised penis bulb and continues as an ejaculatory duct through the penis papilla. Ejaculatory duct canalicular, running through anterior 1/3 or 1/2 of penis papilla to open into an irregular intra-penial cavity. This cavity is large and extends along the remaining 1/2 or 1/3 of the penial papilla length (paratype F3539, Fig. 35A-B). Ejaculatory duct is lined with a ciliated epithelium which is pierced by glands producing erythrophil granules. The duct is surrounded by a 13- μm -thick circular muscle.

Penis papilla protrusible, large (3100 μm long in paratype F3717), as long as 3 times its diameter. Ventral insertion of penis papilla posterior to the dorsal one. This papilla occupies entire male atrium and anterior half of the female one. The external surface of the papilla and that of its cavity are folded. Epithelium of papilla 12 μm high, ciliated and receives two types of glands producing erythrophil and xanthophil granules, respectively. This epithelium is underlain by a 10- μm -thick layer of circular muscle fibers followed by a 100- μm -thick layer of longitudinal muscle in the proximal portion (holotype); distally the circular muscle is very thin. Male atrium is a cavity with few and small folds. It is lined with a ciliated, 25 μm high epithelium that receives glands producing erythrophil granules and is surrounded by a 5- μm -thick layer of circular muscle fibers followed by an equally thick longitudinal muscle.

Ovaries oval-shaped, 225 μm in maximum length, and situated at a distance from anterior extremity equivalent to 16% of body length. Ovovitelline ducts emerge from inner-dorsal section of

ovaries (Fig. 35C). Behind the gonopore, they curve dorsally, and join to form an unpaired common ovovitelline duct, dorsally to the female atrium. This unpaired ovovitelline duct is 1 mm long (holotype), and communicates with the female genital duct; this duct is a projection of the postero-dorsal region of female atrium. No shell glands were observed.

The female atrium is elongate, and divided into a posterior, keyhole-shaped half, lined with an epithelium with multilayered aspect, and a dilated anterior half, lined with a columnar epithelium; total length of female atrium equal to twice its diameter. Fine erythrophil granules are discharged into the female atrium (Fig. 35B). A 10- μ m-thick muscle of circular and longitudinal fibers surrounds female atrium. Common muscle coat envelopes male atrium, female atrium and unpaired ovovitelline duct.

Remarks

Distinctiveness of Imbira sp. n. Regarding the external aspect, the new species distinguishes from all species of the genus in that it presents tenuous submarginal stripes (vs. stripes evident). Concerning the internal aspect. *Imbira* sp. n. presents a parenchymal muscle of longitudinal fibers restricted to the pre-pharyngeal region (vs. along the entire body length in *I. marculsi*, *I. negrita*, and *I. flavonigra*); a penis papilla protrusible (vs. eversible penis papilla in *I. guaiana*, *I. marculsi*, *I. negrita*, *I. flavonigra* and *I. bonita*), and occupying both male atrium and half of female atrium (vs. only male atrium, if it exists, in *I. marculsi* and *I. negrita*); ovovitelline ducts emerge from inner-dorsal section of ovaries (vs. lateral region in *I. guaiana* and *I. negrita*); female atrium lined with an epithelium with multilayered aspect only in its posterior half (vs. entire atrium in *I. guaiana*, *I. marculsi*, *I. negrita* and *I. flavonigra*); total length of female atrium equal to twice its height (vs. three times in *I. marculsi*, two-and-a-half times in *I. negrita* and five times in *I. bonita*). Upon this unique combination of morphological attributes, this species should be considered new to science.

Fig. 30. *Imbira* sp. nov. **A.** Living holotype in dorsal view. **B.** Living paratype F3538 in dorsal and ventral view. **C.** Holotype. Photomicrograph of a transverse section of anterior extremity. **D.** Holotype. Photomicrograph of a transverse section of anteriormost extremity.

Fig. 31. *Imbira* sp. nov. Holotype. Photomicrographs of transverse sections. **A.** Anterior extremity. **B.** Pre-pharyngeal region.

Fig. 32. *Imbira* sp. nov. Photomicrographs of holotype. **A.** A horizontal section of dorsal epidermis at the level of ovarian region. **B.** Sagittal section of pre-pharyngeal region.

Fig. 33. *Imbira* sp. nov. Photomicrographs of sagittal sections of holotype. **A.** Pharynx. **B.** Pre-pharyngeal region.

Fig. 34. *Imbira* sp. nov, holotype. **A.** Photomicrograph of a sagittal section of copulatory apparatus. **B.** Reconstruction of copulatory apparatus. * indicates epithelium of female atrium with multilayered aspect

Fig. 35. *Imbira* sp. nov. Photomicrographs. **A.** Paratype F3539. Sagittal section of copulatory apparatus. **B.** Paratype F3539. A sagittal section of copulatory apparatus. **C.** Holotype. A horizontal section of ovarian region. * indicates epithelium of female atrium with multilayered aspect

Discussion

On the diagnosis of the genus. With the exception of the protrusible penis papilla, *Imbira* sp. n. fully matches the diagnosis of Geoplaninae and *Imbira*, even the parenchymal longitudinal muscle. The diagnosis of this genus includes an eversible penis papilla. The copulatory apparatus of three mature specimens studied of *Imbira* sp. n. is provided with a penis papilla, the distal half of which is irregularly folded and housing not an ejaculatory duct, but an also irregular cavity. A similar aspect presents the penis papilla of one of three type specimens of *I. negrita* (see Fig. 5C in Negrete & Brusa, 2017) and one young individual among the three specimens of *I. marcus* (= *Geoplanea goetschi*: Marcus) studied by Marcus (1951, see Fig. 191). Marcus makes the following observation: “In the young worm, the atrium is partially everted and, therefore, slightly folded. It could, at this stage, speak of penis papilla, constituted, however, only by the contraction of the muscular envelope [...] We marked the orifice (c) of the ejaculatory duct in the worm with 'penis papilla' and in the others, 'without papilla', to show the variability of penis length”. No other species of the genus presents a penis papilla. Thus it seems that there is a gradient in the shape of the penis in the genus, ranging from a penis papilla absent (i.e., eversible, as observed in *I. bonita*, *I. flavonigra*, *I. guaiana*), to a penis papilla permanent (i.e., protrusible, as in *I. sp. n.*), being *I. marcus* an intermediate situation, since it presents both forms. Therefore, the diagnosis of the genus should be revised to consider variation in the type of the penis papilla (see below).

Taxonomic actions. With the discovery of variation in the morphology of the species within the genus, it becomes necessary to emend (underlined text) the diagnosis of *Imbira* as follows:

Diagnosis of *Imbira*. Geoplaninae with large-sized body, 90–140 mm in length, body slender, flattened, with margins parallel; eyes monolobulated, marginally arranged along the body; parenchymatic muscle layers of longitudinal fibres, dorsally and ventrally to the intestine, these may be arranged along the body or restricted to half anterior, in addition to the three common parenchymatic muscle layers; prostatic vesicle extrabulbar, long-branched; penis papilla eversible or protrusible; male atrium folded; ascending portion of the ovovitelline ducts lateral to the gonopore canal or to the female atrium and joining each other above female atrium; genital canal dorso-anteriorly flexed, arising from the postero-dorsal region of the female atrium; female atrium rounded, clothed with an epithelium with multilayered aspect.

General discussion

Previous studies have already indicated that features such as cutaneous and parenchymal muscular systems, sensory organs and the anatomy of the pharynx and, especially, the copulatory apparatus are fundamental to land planarian species identification (Riester, 1938; Ogren & Kawakatsu, 1990; Carbayo & E. Froehlich, 2008, Almeida et al., 2018). For this reason the syntypes of *Ps. arpi* and *Ps. wetzeli* (sexually immature) will remain in the collective group *Pseudogeoplana*. This situation also applies to *Ps. doederleini*, the poor tissue preservation of it hampered getting histological not mCT-derived images from it.

We believe that the widespread ADN Sanger sequencing would not produce valuable gene fragment sequences due to the antiquity of the tissues of type specimens. Furthermore, additional conspecific mature specimens should be collected and their ADN sequenced for comparison, a task out of the scope of this work. Nevertheless, as we preserved the tissue of those immature specimens, attempts to sequence ADN are still possible.

On the other hand, species whose type material is mature and well preserved (*Imbira bonita*, *Pasipha plana* and *Paraba bresslaui*) enabled us detailed description through histology and/or mCT. Most iconic case might be *Paraba bresslaui* which we described from mCT-derived images of the holotype without destructing its bodily integrity.

References

Presentation and General discussion references:

Almeida, A. L., Marques, F. P., & Carbayo, F. 2018. 'Endless forms most beautiful': taxonomic revision of the planarian *Geoplana vaginuloides* (Darwin, 1844) and discovery of numerous congeners (Platyhelminthes: Tricladida). *Zoological Journal of the Linnean Society*, 185(1),1-65.

Carbayo, F., Leal-Zanchet, A. M., & Vieira, E. M. 2002. Terrestrial flatworm (Platyhelminthes: Tricladida: Terricola) diversity versus man-induced disturbance in an ombrophilous forest in southern Brazil. *Biodiversity & Conservation*, 11(6), 1091-1104.

Carbayo, F., Pedroni, J., & Froehlich, E. M. 2008. Colonization and extinction of land planarians (Platyhelminthes, Tricladida) in a Brazilian Atlantic Forest regrowth remnant. *Biological invasions*, 10(7), 1131-1134.

Carbayo, F., & Froehlich, E. M. 2008. Estado do conhecimento dos macroturbelários (Platyhelminthes) do Brasil. *Biota Neotropica*, 8(4), 176-197.

Carbayo, F., Álvarez-Presas, M., Olivares, C. T., Marques, F. P., Froehlich, E. M., & Riutort, M. 2013. Molecular phylogeny of Geoplaninae (Platyhelminthes) challenges current classification: proposal of taxonomic actions. *Zoologica Scripta*, 42(5), 508-528.

Carbayo, F., Silva, M. S., Riutort, M., & Álvarez-Presas, M. 2017. Rolling into the deep of the land planarian genus *Choeradoplana* (Tricladida, Continenticola, Geoplanidae) taxonomy. *Organisms Diversity & Evolution*, 18(2), 187-210.

Carbayo, F. 2019. Planárias Terrestres Neotropicais. Uma base de dados sobre os tricládidos. Available at: <<http://planarias.each.usp.br/filtrar/caracteristica>>. Accessed on: Jan 13, 2019.

Cseh, A., Carbayo, F., & Froehlich, E. M. 2017. Observations on food preference of Neotropical land planarians (Platyhelminthes), with emphasis on *Obama anthropophila*, and their phylogenetic diversification. *Zoologia (Curitiba)*, 34(e-12622), 1-8.

Darwin, C. 1844. Brief descriptions of several terrestrial planariae, and of some remarkable marine species, with an account of their habits. *Quarterly Journal of Microscopical Society*, 91(2): 209-214.

Froehlich, C. G. 1955. Sobre morfologia e taxonomia das Geoplanidae. *Boletim da Faculdade de Filosofia, Ciências e Letras, Universidade de São Paulo. Zoologia*, 19(19), 226-234.

Froehlich, E. M. 1955. Sobre espécies brasileiras do gênero *Geoplana*. *Boletim da Faculdade de Filosofia, Ciências e Letras da Universidade de São Paulo, Zoologia*, 19, 298-299.

Kawaguti, S. 1932. On the physiology of land planarians. *Memoirs of Faculty Science Agricultural Taihoku Imperial University*, 7, 15-27, 29-37, 39-55.

Kawakatsu, M., Ogren, R. E., Froehlich, E. M., & Sasaki, G. Y. 2002. Additions and corrections of the previous land planarian indices of the world (Turbellaria, Seriata, Tricladida, Terricola). *Bulletin of the Fuji Women's College Series 2*, 40, 157-177.

Kawakatsu, M., Froehlich, E. M., Jones, H. D., Ogren, R. E., & Sasaki, G. Y. 2003. Miscellaneous papers on Turbellarians. Article II. Additions and corrections of the previous land planarian indices of the world (Turbellaria, Seriata, Tricladida, Terricola). Additions and Corrections, etc. *Bulletin of Fuji Women's University Series 2*, 41, 97-114.

Marcus, E. 1951. Turbellaria brasileiros. *Boletim Faculdade de Filosofia Ciências e Letras, Universidade de São Paulo, Zoologia*, (16) 72-74.

Moseley, H. N. 1877. Notes on the structure of several forms of land planarians, with a description of two new genera and several new species, and a list of all species at present known. *Quarterly Journal of Microscopical Science*, 17, 273-292.

Müller, F. 1857. II. Description of a new species of Earth-worm (*Lumbricus corethrurus*). *Annals and Magazine of Natural History*, 20(115), 13-15.

Ogren, R. E., & Kawakatsu, M. 1990. Index to the species of the family Geoplanidae (Turbellaria, Tricladida, Terricola) Part I: Geoplaninae. *Bulletin of Fuji Women's University Series 1*, 28, 79-166.

Ogren, R. E., & Kawakatsu, M. 1991. Index to the species of the family Geoplanidae (Turbellaria, Tricladida, Terricola): Part II: Caenoplaninae and Pelmatoplaninae. 29, 35-58.

Riester, A. 1938. Beiträge zur geoplaniden-fauna brasiliens. *Abhandlungen Senkenberg Gesellschaft Naturforschung*, (4), 20-24.

Schirch, P. F. 1929. Sobre as planarias terrestres do Brasil. *Boletim do Museu Nacional, Rio de Janeiro*, 5, 27-38.

Schultze, M., & Müller, F. 1857. Beiträge zur Kenntniss der Landplanarien. *Abhandlungen der Senckenberg Naturforschender Gesellschaft für Naturforschung*, (4) 19-38.

Silva, M. S., Willemart, R. H., & Carbayo, F. 2018. Sticky flatworms (Platyhelminthes) kill armored harvestmen (Arachnida, Opiliones) but are not immune to the prey' s weapons. *Journal of Zoology*, 306(2), 88-94.

Sluys, R. 1999. Global diversity of land planarians (Platyhelminthes, Tricladida, Terricola): a new indicator-taxon in biodiversity and conservation studies. *Biodiversity & Conservation*, 8(12), 1663-1681.

von Graff, L. 1899. *Monographie der Turbellarien* (Vol. 2). W. Engelmann.

Winsor, L., Johns, P. M., & Barker, G. M. 2004. Terrestrial planarians (Platyhelminthes: Tricladida: Terricola) predaceous on terrestrial gastropods. *Natural enemies of terrestrial molluscs. Oxfordshire: CAB International*, 227-278.

References chapter 1:

Almeida, A. L., Marques, F. P., & Carbayo, F. 2018. ‘Endless forms most beautiful’: taxonomic revision of the planarian *Geoplana vaginuloides* (Darwin, 1844) and discovery of numerous congeners (Platyhelminthes: Tricladida). *Zoological Journal of the Linnean Society*, 185(1), 1-65.

Almeida, A. L., Kishimoto, R. G., & Carbayo, F. 2012. Two new land planarian species (Platyhelminthes: Tricladida) from the Brazilian Atlantic Forest. *Zoologia*, 29(5), 430-438.

Amaral, S. V., Ribeiro, G. G., Müller, M. J., Valiati, V. H., & Leal-Zanchet, A. 2018. Tracking the diversity of the flatworm genus *Imbira* (Platyhelminthes) in the Atlantic Forest. *Organisms Diversity & Evolution*, 18(1), 87-99.

Álvarez-Presas, M., Amaral, S. V., Carbayo, F., Leal-Zanchet, A. M., & Riutort, M. 2015. Focus on the details: morphological evidence supports new cryptic land flatworm (Platyhelminthes) species revealed with molecules. *Organisms Diversity & Evolution*, 15(2), 379-403.

Carbayo, F. 2005. Procedimentos de campo e laboratório para a caracterização das planárias terrestres neotropicais (Platyhelminthes: Tricladida). *Caderno La Salle 11*, Canoas, 2(1), 131-144.

Carbayo, F., & Froehlich, E. M. 2008. Estado do conhecimento dos macroturbelários (Platyhelminthes) do Brasil. *Biota Neotropica*, 8(4), 177-197.

Carbayo, F., Froehlich, E. M., Leal-Zanchet, A. M., & Amato, S. B. 2009. Turbelários (Platyhelminthes). *Estado da arte e perspectivas para a Zoologia no Brasil*. Paraná: Editora da Universidade Federal do Paraná, 49-64.

Carbayo, F. 2010. A new genus for seven Brazilian land planarian species, split off from *Notogynaphallia* (Platyhelminthes, Tricladida). *Belgian Journal of Zoology*, 140(Suppl.), 91-101.

Carbayo, F., Álvarez-Presas, M., Olivares, C. T., Marques, F. P., Froehlich, E. M., & Riutort, M. 2013. Molecular phylogeny of Geoplaninae (Platyhelminthes) challenges current classification: proposal of taxonomic actions. *Zoologica Scripta*, 42(5), 508-528.

Carbayo, F., Giribet, G. & Francoy, T. M. 2016. Application of non-destructive imaging to describe land planarians: a new species of *Obama* (Platyhelminthes, Tricladida). *Zoologica Scripta*, 45(5), 566-578. doi: 10.1111/zsc.12175.

Carbayo, F. & Lenihan, J. W. 2016. The datasets of micro-computed tomography scans and virtual histological slides of a new land planarian species of *Obama* (Platyhelminthes). *Giga Science*, 5(3), 1-7. doi: 10.1186/s13742-016-0119-4.

Corrêa, D. D. 1947. A primeira *Dolichoplana* (Tricladida terricola) do Brasil. *Boletins da Faculdade de Filosofia, Ciências e Letras, Universidade de São Paulo. Zoologia*, 12(12), 57-81.

Faulwetter, S., Vasiliadou, A., Kouratoras, M., Dailianis, T. & Arvanitidis, C. 2013. Micro-Computed tomography: introducing new dimensions to taxonomy. *ZooKeys*, 263, 1-45.

Froehlich, C. G. 1955a. Sobre morfologia e taxonomia das Geoplanidae. *Boletim da Faculdade de Filosofia, Ciências e Letras, Universidade de São Paulo. Zoologia*, 19(19), 226-234.

Froehlich, C. G. 1955b. Notas sobre geoplanas brasileiras. *Pap. Avul. Dep. Zool.*, 12(7), 198.

Froehlich, C. G. 1956. Tricladida Terricola das regiões de Teresópolis e Ubatuba. *Papéis Avulsos do Departamento de Zoologia*, 12(16), 336-339.

Froehlich, C. G. 1959. On Geoplanides from Brazil. *Boletim da Faculdade de Filosofia, Ciências e Letras, Universidade de São Paulo. Zoologia*, 22(22), 223-225.

Froehlich, C. G. 1966. Notas sobre a ecologia de planárias terrestres. *Progresos en biología del suelo*. 299-301. Montevideo, Uruguay.

Froehlich, C. G. 1967. A contribution to the zoogeography of neotropical land planarians. *Acta Zoologica Lilloana*, 23, 153-162.

Froehlich, E. M. 1955a. Sobre espécies brasileiras do gênero *Geoplana*. *Boletim da Faculdade de Filosofia, Ciências e Letras da Universidade de São Paulo. Série Zoologia, São Paulo*, 19, 298-299, 314.

Froehlich, E. M. 1955b. Chave para a classificação das *Geoplanas* brasileiras. *Pap. Avul. Dpto. Zool*, 8, 203.

Grau, J. H., Sluys, R., Froehlich, E. M., & Carbayo, F. 2012. Reflections on the genus *Amaga* Ogren and Kawakatsu 1990, and description of a new genus of land planarian (Platyhelminthes: Tricladida: Geoplanidae). *Journal of natural history*, 46(25-26), 1529-1546.

Hyman, L. H. 1940. XXXI.—Land planarians from the Palau and Caroline Islands, Micronesia. *Journal of Natural History*, 5(28), 345-362.

Laforsch, C., Imhof, H., Sigl, R., Settles, M., Heb, M. & Wanninger A. 2012. *Applications of computational 3D-modeling in organismal biology*. INTECH Open Access Publisher, 117-141.

Lago-Barcia, D. & Carbayo, F. 2018. Taxonomic revision of four species of the Neotropical land planarian genera *Cratera* and *Geoplana* (Platyhelminthes, Tricladida) with a description of two new species and an emendation of *Cratera*. *Zootaxa*, 4500(4), 517-542.

Leal-Zanchet, A. M., Rossi, I., Seitenfus, A. L. R., & Alvarenga, J. 2012. Two new species of land flatworms and comments on the genus *Pasipha* Ogren & Kawakatsu, 1990 (Platyhelminthes: Continticola). *Zootaxa*, 3583(1), 1-21.

Leal-Zanchet, A. M., & Marques, A. D. 2018. Coming out in a harsh environment: a new genus and species for a land flatworm (Platyhelminthes: Tricladida) occurring in a ferruginous cave from the Brazilian savanna. *PeerJ*, 6, e6007. <https://peerj.com/articles/6007/>

Lemos, V. S., Cauduro, G. P., Valiati, V. H., & Leal-Zanchet, A. M. 2014. Phylogenetic relationships within the flatworm genus *Choeradoplana* Graff (Platyhelminthes: Tricladida) inferred from molecular data with the description of two new sympatric species from Araucaria moist forests. *Invertebrate Systematics*, 28(6), 605-627.

Marcus, E. 1951. Turbellaria brasileiros. *Boletim Faculdade de Filosofia Ciências e Letras, Universidade de São Paulo, Zoologia*, (16) 72-74.

Metscher, B. D. 2009. MicroCT for comparative morphology: simple staining methods allow high-contrast 3D imaging of diverse non-mineralized animal tissues. *BMC physiology*, 9(11), 1-14.

Molineux, A., Scott, R. W., Ketcham, R. A. & Maisano, J. A. 2007. Rudist taxonomy Using X-Ray Computed Tomography. *Palaeontologia Electronica*, 10(3),1-6.

Moseley, H. N. 1877. Notes on the structure of several forms of land planarians, with a description of two new genera and several new species, and a list of all species at present known. *Quarterly Journal of Microscopical Science*, 17, 273-292.

Ogren, R. E., & Kawakatsu, M. 1990. Index to the species of the family Geoplanidae (Turbellaria, Tricladida, Terricola) Part I: Geoplaninae. Bulletin of Fuji Women's University Series 1, 28, 79-166.

Ogren, R. E. 1995. Predation behaviour of land planarians. *Biology of Turbellaria and some Related Flatworms*, 105-111. Springer, Dordrecht.

Riester, A. 1938. Beiträge zur geoplaniden-fauna brasiliens. *Abhandlungen Senkenberg Gesellschaft Naturforschung*, (4), 20-24.

Sandison, 1955

Schirch, P. F. 1929. Sobre as planarias terrestres do Brasil. *Boletim do Museu Nacional*, Rio de Janeiro, 5, 27-38.

Sluys, R., & Riutort, M. 2018. Planarian diversity and phylogeny. *Planarian Regeneration*, 1-56. Humana Press, New York, NY.

Sombke, A., Lipke, E., Michalik, P., Uhl, G. & Harzsch, S. 2015. Potential and Limitation of X-ray Micro-Computed Tomography in Arthropod Neuroanatomy: A Methodological and Comparative Survey. *Journal of Comparative Neurology*, 523(8), 1281-1295.

Tessler, M., Barrio, A., Borda, E., Rood-Goldman, R., Hill, M., & Siddall, M. E. 2016. Description of a soft-bodied invertebrate with microcomputed tomography and revision of the genus *Chtonobdella* (Hirudinea: Haemadipsidae). *Zoologica Scripta*, 45(5), 552-565.

von Graff, L. 1899. *Monographie der Turbellarien* (Vol. 2). W. Engelmann.

References chapter 2:

Almeida, A. L., Marques, F. P., & Carbayo, F. 2018. ‘Endless forms most beautiful’: taxonomic revision of the planarian *Geoplana vaginuloides* (Darwin, 1844) and discovery of numerous congeners (Platyhelminthes: Tricladida). *Zoological Journal of the Linnean Society*, 185(1), 1-65.

Amaral, S. V., Ribeiro, G. G., Müller, M. J., Valiati, V. H., & Leal-Zanchet, A. 2018. Tracking the diversity of the flatworm genus *Imbira* (Platyhelminthes) in the Atlantic Forest. *Organisms Diversity & Evolution*, 18(1), 87-99.

Carbayo, F., Álvarez-Presas, M., Olivares, C. T., Marques, F. P., Froehlich, E. M., & Riutort, M. 2013. Molecular phylogeny of Geoplaninae (Platyhelminthes) challenges current classification: proposal of taxonomic actions. *Zoologica Scripta*, 42(5), 508-528.

Carbayo, F. 2019. Planárias Terrestres Neotropicais. Uma base de dados sobre os tricládidos. Available at: <<http://planarias.each.usp.br/filtrar/caracteristica>>. Accessed on: Jan 13, 2019.

Froehlich, E. M. 1955. Sobre espécies brasileiras do gênero *Geoplana*. *Boletim da Faculdade de Filosofia, Ciências e Letras da Universidade de São Paulo. Série Zoologia, São Paulo*, 19, 298-299, 314.

Leal-Zanchet, A. M., & Carbayo, F. 2001. Two new species of Geoplanidae (Platyhelminthes, Tricladida, Terricola) of south Brazil. *Journal of Zoology*, 253(4), 433-446.

Negrete, L., & Brusa, F. 2017. Increasing diversity of land planarians (Platyhelminthes: Geoplanidae) in the Interior Atlantic Forest with the description of two new species and new records from Argentina. *Zootaxa*, 4362(1), 99-127.

Riester, A. 1938. Beiträge zur geoplaniden-fauna brasiliens. *Abhandlungen Senkenberg Gesellschaft Naturforschung*, (4), 20-24.

von Graff, L. 1899. *Monographie der Turbellarien* (Vol. 2). W. Engelmann.

Marcus, E. 1951. Turbellaria brasileiros. *Boletim Faculdade de Filosofia Ciências e Letras, Universidade de São Paulo, Zoologia*, (16) 72-74

Ogren, R. E., & Kawakatsu, M. 1990. Index to the species of the family Geoplanidae (Turbellaria, Tricladida, Terricola) Part I: Geoplaninae. *Bulletin of Fuji Women's University Series 1*, 28, 79-166.

Schirch, P. F. 1929. Sobre as planarias terrestres do Brasil. *Boletim do Museu Nacional, Rio de Janeiro*, 5, 27-38.

Appendix 1

Parameter for acquisition of mCT images of the anterior extremity of *Paraba bresslaui*

[System]

Scanner=SkyScan1272

Instrument S/N=13C09015

Software Version=1.1.2

Home Directory=C:\SkyScan1272\SkyScan1272

Source Type=HAMAMATSU_L11871_20

Camera Type=XIMEA xiRAY16

Camera Pixel Size (um)=7.4

Camera X/Y Ratio=1.0000

[User]

User Name=microct

Computer Name=LNN107

[Acquisition]

Data Directory=C:\Results\Usuários externos\Fernando - USP\bresslaui-Ant

Filename Prefix=bresslaui-Ant_

Filename Index Length=8

Number Of Files= 3128

Number Of Rows= 3280

Number Of Columns= 4904

Partial Width=OFF

Image crop origin X=0

Image crop origin Y=0

Camera binning=1x1

Image Rotation=-0.12900

Optical Axis (line)= 1742

Camera to Source (mm)=274.90436

Object to Source (mm)=60.33874

Source Voltage (kV)= 23

Source Current (uA)= 174

Image Pixel Size (um)=1.625104

Scaled Image Pixel Size (um)=1.625104

Image Format=TIFF

Depth (bits)=16

Reference Intensity=57000

Exposure (ms)=4015

Rotation Step (deg)=0.060

Use 360 Rotation=NO

Scanning position=40.500 mm

Frame Averaging=ON (3)

Random Movement=ON (30)

Flat Field Correction=ON

FF updating interval=284

Geometrical Correction=ON

Filter=No Filter

Gantry direction=CC

Rotation Direction=CC

Type of Detector Motion=STEP AND SHOOT

Scanning Trajectory=ROUND

Study Date and Time=18 Nov 2015 07h:02m:02s
Scan duration=17h:4m:27s
Maximum vertical TS=5.0
[Reconstruction]
Reconstruction Program=NRecon
Program Version=Version: 1.6.10.2
Program Home Directory=C:\SkyScan1272\SkyScan1272
Reconstruction engine=GPUReconServer
Engine version=Version: 1.6.10
Reconstruction from batch=No
Postalignment=6.00
Reconstruction servers= LNN107
Dataset Origin=SkyScan1272
Dataset Prefix=bresslaui-Ant_
Dataset Directory=C:\Results\Usuários externos\Fernando - USP\bresslaui-Ant
Output Directory=C:\Results\Usuários externos\Fernando - USP\bresslaui-Ant\bresslaui-Ant_Rec
Time and Date=Nov 18, 2015 14:05:22
First Section=114
Last Section=2842
Reconstruction duration per slice (seconds)=6.261634
Total reconstruction time (2729 slices) in seconds=17088.000000
Section to Section Step=1
Sections Count=2729
Result File Type=BMP
Result File Header Length (bytes)=1134
Result Image Width (pixels)=3120
Result Image Height (pixels)=2976
Pixel Size (um)=1.62510
Reconstruction Angular Range (deg)=187.68
Use 180+=OFF
Angular Step (deg)=0.0600
Smoothing=0
Ring Artifact Correction=1
Draw Scales=OFF
Object Bigger than FOV=OFF
Reconstruction from ROI=ON
ROI Top (pixels)=4713
ROI Bottom (pixels)=1734
ROI Left (pixels)=1130
ROI Right (pixels)=4250
ROI reference length=4904
Filter cutoff relative to Nyquist frequency=100
Filter type=0
Filter type description=Hamming (Alpha=0.54)
Undersampling factor=1
Threshold for defect pixel mask (%)=0
Beam Hardening Correction (%)=0
CS Static Rotation (deg)=0.00
Minimum for CS to Image Conversion=0.012471
Maximum for CS to Image Conversion=0.228247
HU Calibration=OFF
BMP LUT=0
Cone-beam Angle Horiz.(deg)=7.556624
Cone-beam Angle Vert.(deg)=5.058237

Appendix 2

Parameter for acquisition of mCT images of the entire body of *Paraba bresslaui*

[System]
Scanner=SkyScan1272
Instrument S/N=13C09015
Software Version=1.1.2
Home Directory=C:\SkyScan1272\SkyScan1272
Source Type=HAMAMATSU_L11871_20
Camera Type=XIMEA xiRAY16
Camera Pixel Size (um)=7.4
Camera X/Y Ratio=1.0000
[User]
User Name=microct
Computer Name=LNN107
[Acquisition]
Data Directory=C:\Results\Usuários externos\Fernando - USP\bresslaui inteiro
Filename Prefix=bresslaui inteiro_~03
Filename Index Length=8
Number Of Files= 317
Number Of Rows= 820
Number Of Columns= 1224
Partial Width=OFF
Image crop origin X=0
Image crop origin Y=0
Camera binning=4x4
Image Rotation=-0.12900
Optical Axis (line)= 433
Camera to Source (mm)=224.90436
Object to Source (mm)=60.75100
Source Voltage (kV)= 25
Source Current (uA)= 180
Image Pixel Size (um)=7.999852
Scaled Image Pixel Size (um)=7.999852
Image Format=TIFF
Depth (bits)=16
Reference Intensity=57000
Exposure (ms)=1706
Rotation Step (deg)=0.600
Use 360 Rotation=NO
Scanning position=40.410 mm
Frame Averaging=ON (4)
Random Movement=ON (30)
Flat Field Correction=ON
FF updating interval=52
Geometrical Correction=ON
Filter=Al 0.25mm
Gantry direction=CC
Rotation Direction=CC
Type of Detector Motion=STEP AND SHOOT
Scanning Trajectory=ROUND
Number of connected scans=4
Current scan number=4
Number of lines to be reconstructed=644
Study Date and Time=17 Nov 2015 13h:23m:19s
Scan duration=0h:59m:15s
Maximum vertical TS=5.0

[Reconstruction]
Reconstruction Program=NRecon
Program Version=Version: 1.6.10.2
Program Home Directory=C:\SkyScan1272\SkyScan1272
Reconstruction engine=GPUReconServer
Engine version=Version: 1.6.10
Reconstruction from batch=No
Postalignment=-1.50
Connected Reconstruction (parts)=4
Sub-scan post alignment [0]=-4.000000
Sub-scan post alignment [1]=-3.000000
Sub-scan post alignment [2]=-2.500000
Sub-scan post alignment [3]=-1.500000
Sub-scan scan length [0]=661
Sub-scan scan length [1]=661
Sub-scan scan length [2]=661
Sub-scan scan length [3]=644
Used extra rotation per scan(deg)= 0.000 0.184 0.381 0.637
Reconstruction servers= LNN107
Dataset Origin=SkyScan1272
Dataset Prefix=bresslaui inteiro_~03
Dataset Directory=C:\Results\Usuários externos\Fernando - USP\bresslaui inteiro
Output Directory=C:\Results\Usuários externos\Fernando - USP\bresslaui inteiro\bresslaui inteiro_Rec
Time and Date=Nov 17, 2015 14:29:18
First Section=69
Last Section=2715
Reconstruction duration per slice (seconds)=0.094254
Total reconstruction time (1984 slices) in seconds=187.000000
Section to Section Step=1
Sections Count=2647
Result File Type=BMP
Result File Header Length (bytes)=1134
Result Image Width (pixels)=720
Result Image Height (pixels)=688
Pixel Size (um)=7.99985
Reconstruction Angular Range (deg)=190.20
Use 180+=OFF
Angular Step (deg)=0.6000
Smoothing=0
Ring Artifact Correction=1
Draw Scales=OFF
Object Bigger than FOV=OFF
Reconstruction from ROI=ON
ROI Top (pixels)=1100
ROI Bottom (pixels)=411
ROI Left (pixels)=279
ROI Right (pixels)=999
ROI reference length=1224
Filter cutoff relative to Nyquist frequency=100
Filter type=0
Filter type description=Hamming (Alpha=0.54)
Undersampling factor=1
Threshold for defect pixel mask (%)=0
Beam Hardening Correction (%)=0
CS Static Rotation (deg)=0.00
Minimum for CS to Image Conversion=0.008289
Maximum for CS to Image Conversion=0.111691
HU Calibration=OFF
BMP LUT=0

Cone-beam Angle Horiz.(deg)=9.214993
Cone-beam Angle Vert.(deg)=6.180784

Appendix 3

Parameter for acquisition of mCT images of the copulatory organ of *Paraba bresslaui*

[System]

Scanner=SkyScan1272

Instrument S/N=13C09015

Software Version=1.1.2

Home Directory=C:\SkyScan1272\SkyScan1272

Source Type=HAMAMATSU_L11871_20

Camera Type=XIMEA xiRAY16

Camera Pixel Size (um)=7.4

Camera X/Y Ratio=1.0000

[User]

User Name=microct

Computer Name=LNN107

[Acquisition]

Data Directory=C:\Results\Usuários externos\Fernando - USP\bresslaui-AC2

Filename Prefix=bresslaui-AC2_

Filename Index Length=8

Number Of Files= 2367

Number Of Rows= 1640

Number Of Columns= 2452

Partial Width=OFF

Image crop origin X=0

Image crop origin Y=0

Camera binning=2x2

Image Rotation=-0.12900

Optical Axis (line)= 866

Camera to Source (mm)=224.90436

Object to Source (mm)=53.15850

Source Voltage (kV)= 24

Source Current (uA)= 175

Image Pixel Size (um)=3.500026

Scaled Image Pixel Size (um)=3.500026

Image Format=TIFF

Depth (bits)=16

Reference Intensity=57000

Exposure (ms)=1897

Rotation Step (deg)=0.080

Use 360 Rotation=NO

Scanning position=28.400 mm

Frame Averaging=ON (3)

Random Movement=ON (30)

Flat Field Correction=ON

FF updating interval=215

Geometrical Correction=ON

Filter=No Filter

Gantry direction=CC

Rotation Direction=CC

Type of Detector Motion=STEP AND SHOOT

Scanning Trajectory=ROUND

Study Date and Time=18 Nov 2015 15h:51m:22s

Scan duration=6h:51m:27s

Maximum vertical TS=5.0

[Reconstruction]
Reconstruction Program=NRecon
Program Version=Version: 1.6.10.2
Program Home Directory=C:\SkyScan1272\SkyScan1272
Reconstruction engine=GPUReconServer
Engine version=Version: 1.6.10
Reconstruction from batch=No
Postalignment=-7.50
Reconstruction servers= LNN107
Dataset Origin=SkyScan1272
Dataset Prefix=bresslaui-AC2_
Dataset Directory=C:\Results\Usuários externos\Fernando - USP\bresslaui-AC2
Output Directory=C:\Results\Usuários externos\Fernando - USP\bresslaui-AC2\bresslaui-AC2_Rec
Time and Date=Nov 18, 2015 16:20:38
First Section=69
Last Section=1576
Reconstruction duration per slice (seconds)=0.426393
Total reconstruction time (1508 slices) in seconds=643.000000
Section to Section Step=1
Sections Count=1508
Result File Type=BMP
Result File Header Length (bytes)=1134
Result Image Width (pixels)=1180
Result Image Height (pixels)=996
Pixel Size (um)=3.50003
Reconstruction Angular Range (deg)=189.36
Use 180+=OFF
Angular Step (deg)=0.0800
Smoothing=0
Ring Artifact Correction=1
Draw Scales=OFF
Object Bigger than FOV=OFF
Reconstruction from ROI=ON
ROI Top (pixels)=2002
ROI Bottom (pixels)=1004
ROI Left (pixels)=821
ROI Right (pixels)=2002
ROI reference length=2452
Filter cutoff relative to Nyquist frequency=100
Filter type=0
Filter type description=Hamming (Alpha=0.54)
Undersampling factor=1
Threshold for defect pixel mask (%)=0
Beam Hardening Correction (%)=0
CS Static Rotation (deg)=0.00
Minimum for CS to Image Conversion=0.002539
Maximum for CS to Image Conversion=0.149284
HU Calibration=OFF
BMP LUT=0
Cone-beam Angle Horiz.(deg)=9.229985
Cone-beam Angle Vert.(deg)=6.180784

Appendix 4

Parameter for acquisition of mCT images of the entire body of *Pasipha plana*

[General]

Version=2.3.0.1032
Version-pca=2
Comment=
LoadDefault=1
SystemName=v|tome|x m

[AutoScO]

Active=1
ImgNr=8
ImageString=312:624:936:1248:1560:1872:2184:2500
Skip=10

[Geometry]

FDD=817.22215760
FOD=49.67906250
Magnification=16.45003179
VoxelSizeX=0.01215803
VoxelSizeY=0.01215803
CalibValue=14.105
cx=344.50000000
cy=1011.50000000
DetectorRot=0.00000000
Tilt=0.00000000
Old_CalibValue=-53.72800000

[CT]

Type=0
NumberImages=2500
StartImg=2501
RotationSector=360.00000000
NoRotation=0
EstimatedTime=0
RemainingTime=2500
ScanTimeCmpl=2500
NrImgDone=2501
NrImgCmplScan=2501
RefDriveEnabled=0
SkipForNewInterval=25
SkipAcc=1
FreeRayFactor=1.00010000
Wnd_L=638
Wnd_T=11
Wnd_R=689
Wnd_B=99
Level=3533.00000000

[VSensor]

EnableTiles=1
Start=0
NumTiles=1
Interval=60
Overlap=9
AdjustImg=1
SingleImgX=700

[Trajectory]

Active=0

[CalibValue]

NumberImages=18

Averaging=2

Skip=3

[FastCT]

Active=0

[Image]

Top=0

Left=662

Bottom=2023

Right=1361

DimX=690

DimY=2024

Rotation=0

MirrorX=0

MirrorY=0

BitPP=16

FreeRay=4462

[ImgProc]

SwBin=1

AddSwBin=0

[Warmup]

Enable=1

Counter=0

MaxTimes=10

TimeTrigOn=0

kV=90

Time=60

[Multiscan]

Active=0

[Multiline]

Installed=0

[CalibImages]

MGainMode=0

MGainPoints=3

Avg=50

Skip=10

EnableAutoAcq=1

MGainVoltage=50:50:50:

MGainCurrent=20:80:260:

GainImg=C:\Program Files\phoenix x-ray\datosx 2 acq\CalibrationImages\G_B1_G400_T500_V1.tif

MGainImg=S:\CT_DATA\Jaime Jardim\Calloplax janeirensis_MZUSP104009

seco\Calloplax

janeirensis_MZUSP104009__bright_55kV_250uA_500ms_1Det.tif

OffsetImg=C:\Program Files\phoenix x-ray\datosx 2 acq\CalibrationImages\offset_B1x1_G400.0%_T500ms.tif

DefPixelImg=C:\Program Files\phoenix x-ray\datosx 2 acq\CalibrationImages\pixmask_B1x1_x2024_y2024.tif

[SectorScan]

Active=0

[DetectorShift]

Enable=1

Mode=0

Amplitude=5

Interval=1

Step=1

[Detector]

InitTimeOut=60000

Name=dxr-250

PixelSizeX=0.20000000

PixelSizeY=0.20000000

NrPixelsX=2024

NrPixelsY=2024

Timing=4

TimingVal=500.092

Avg=3

Skip=1

Binning=0

BitPP=14

CameraGain=3

SatValue=15563

SatPixNrLimit=4096

[Xray]

ComPort=0

Name=xs|300 d

ID=2303

InitTimeout=20000

Voltage=55

Current=250

Mode=0

Filter=Unknown

Collimation=0

WaitTime=1000

WaitForStable=30000

FocDistX=0.00000000

FocDistY=0.00000000

SpinStepkV=10

SpinStepuA=10

Macro=0

RestrictNumSpots=0

PreWarning=0

MinGainCurrent=10

[Cnc]

InitTimeout=8000

CollisionDetection=1

JoyDriveDoorOpen=0

SecPosSample=250.00000000

MinSampleDetPos=300.00000000

EnableKeyboardJoy=0

KeyJoyVelocityFactor=0.25000000

[Axis]

XSample=-0.019688

YSample=279.859375

ZSample=49.679062

RSample=0.000000

XDetector=0.000000

[AcqSrvManager]

RecvPcIp=192.168.10.2

ExePath=c:\Program Files\phoenix x-ray\datosx 2 acq\srv\dtxaSrv.exe

[Net]

Enable=1
IP=192.168.10.2

Appendix 5

Parameter for acquisition of mCT images of the entire body of *Pseudogeoplane arpi*

[General]

Version=2.3.0.1032
Version-pca=2
Comment=
LoadDefault=1
SystemName=v|tome|x m

[AutoScO]

Active=1
ImgNr=8
ImageString=250:500:750:1000:1250:1500:1750:2000
Skip=10

[Geometry]

FDD=817.22215760
FOD=272.56437500
Magnification=2.99827209
VoxelSizeX=0.06670509
VoxelSizeY=0.06670509
CalibValue=-9.758
cx=199.50000000
cy=1011.50000000
DetectorRot=0.00000000
Tilt=0.00000000
Old_CalibValue=0.00000000

[CT]

Type=0
NumberImages=2000
StartImg=2001
RotationSector=360.00000000
NoRotation=0
EstimatedTime=0
RemainingTime=2000
ScanTimeCmpl=2000
NrImgDone=2001
NrImgCmplScan=2001
RefDriveEnabled=0
SkipForNewInterval=25
SkipAcc=1
FreeRayFactor=1.00010000
Wnd_L=2
Wnd_T=11
Wnd_R=75
Wnd_B=125
Level=11126.00000000

[VSensor]

EnableTiles=1
Start=0
NumTiles=1
Interval=60
Overlap=9
AdjustImg=1
SingleImgX=410

[Trajectory]

Active=0

[CalibValue]

NumberImages=18

Averaging=2

Skip=3

[FastCT]

Active=0

[Image]

Top=0

Left=807

Bottom=2023

Right=1216

DimX=400

DimY=2024

Rotation=0

MirrorX=0

MirrorY=0

BitPP=16

FreeRay=12289

[ImgProc]

SwBin=1

AddSwBin=0

[Warmup]

Enable=1

Counter=0

MaxTimes=10

TimeTrigOn=0

kV=80

Time=60

[Multiscan]

Active=0

[Multiline]

Installed=0

[CalibImages]

MGainMode=0

MGainPoints=3

Avg=50

Skip=10

EnableAutoAcq=1

MGainVoltage=70:70:70:

MGainCurrent=30:100:310:

GainImg=C:\Program Files\phoenix x-ray\datosx 2 acq\CalibrationImages\G_B1_G200_T1000_V1.tif

MGainImg=S:\CT_DATA\FernandoCarbayo\Bresslaui AC\Bresslaui AC__bright_70kV_310uA_1000ms_1Det.tif

OffsetImg=C:\Program Files\phoenix x-ray\datosx 2 acq\CalibrationImages\offset_B1x1_G200.0%_T1000ms.tif

DefPixelImg=C:\Program Files\phoenix x-ray\datosx 2 acq\CalibrationImages\pixmask_B1x1_x2024_y2024.tif

[SectorScan]

Active=0

[DetectorShift]

Enable=1

Mode=0

Amplitude=5

Interval=1

Step=1

[Detector]

InitTimeOut=60000

Name=dxr-250

PixelSizeX=0.20000000

PixelSizeY=0.20000000

NrPixelsX=2024

NrPixelsY=2024

Timing=5

TimingVal=1000.082

Avg=1

Skip=0

Binning=0

BitPP=14

CameraGain=2

SatValue=15563

SatPixNrLimit=4096

[Xray]

ComPort=0

Name=xs|300 d

ID=2303

InitTimeout=20000

Voltage=75

Current=290

Mode=0

Filter=Unknown

Collimation=0

WaitTime=1000

WaitForStable=30000

FocDistX=0.00000000

FocDistY=0.00000000

SpinStepkV=10

SpinStepuA=10

Macro=0

RestrictNumSpots=0

PreWarning=0

MinGainCurrent=10

[Cnc]

InitTimeout=8000

CollisionDetection=1

JoyDriveDoorOpen=0

SecPosSample=250.00000000

MinSampleDetPos=300.00000000

EnableKeyboardJoy=0

KeyJoyVelocityFactor=0.25000000

[Axis]

XSample=-0.814687

YSample=236.632125

ZSample=272.564375

RSample=-233.762400

XDetector=0.000000

[AcqSrvManager]

RecvPcIp=

ExePath=c:\Program Files\phoenix x-ray\datosx 2 acq\srv\dtxaSrv.exe

[Net]

Enable=0

IP=

Appendix 6

Parameter for acquisition of mCT images of the pharynx of *Pseudogeoplane arpi*

[General]

Version=2.3.0.1032
Version-pca=2
Comment=
LoadDefault=1
SystemName=v|tome|x m

[AutoScO]

Active=1
ImgNr=8
ImageString=250:500:750:1000:1250:1500:1750:2000
Skip=10

[Geometry]

FDD=817.22215760
FOD=43.21587500
Magnification=18.91023050
VoxelSizeX=0.01057629
VoxelSizeY=0.01057629
CalibValue=-31.904
cx=749.50000000
cy=1011.50000000
DetectorRot=0.00000000
Tilt=0.00000000
Old_CalibValue=0.00000000

[CT]

Type=0
NumberImages=2000
StartImg=2001
RotationSector=360.00000000
NoRotation=0
EstimatedTime=0
RemainingTime=2000
ScanTimeCmpl=2000
NrImgDone=2001
NrImgCmplScan=2001
RefDriveEnabled=0
SkipForNewInterval=25
SkipAcc=1
FreeRayFactor=1.00010000
Wnd_L=18
Wnd_T=1824
Wnd_R=104
Wnd_B=2021
Level=12289.00000000

[VSensor]

EnableTiles=1
Start=0
NumTiles=1
Interval=60
Overlap=9
AdjustImg=1
SingleImgX=1510

[Trajectory]

Active=0

[CalibValue]

NumberImages=18

Averaging=2

Skip=3

[FastCT]

Active=0

[Image]

Top=0

Left=257

Bottom=2023

Right=1766

DimX=1500

DimY=2024

Rotation=0

MirrorX=0

MirrorY=0

BitPP=16

FreeRay=12289

[ImgProc]

SwBin=1

AddSwBin=0

[Warmup]

Enable=1

Counter=0

MaxTimes=10

TimeTrigOn=0

kV=80

Time=60

[Multiscan]

Active=0

[Multiline]

Installed=0

[CalibImages]

MGainMode=0

MGainPoints=3

Avg=50

Skip=10

EnableAutoAcq=1

MGainVoltage=70:70:70:

MGainCurrent=30:100:310:

GainImg=C:\Program Files\phoenix x-ray\datosx 2 acq\CalibrationImages\G_B1_G200_T1000_V1.tif

MGainImg=S:\CT_DATA\FernandoCarbayo\Bresslaui AC\Bresslaui AC__bright_70kV_310uA_1000ms_1Det.tif

OffsetImg=C:\Program Files\phoenix x-ray\datosx 2 acq\CalibrationImages\offset_B1x1_G200.0%_T1000ms.tif

DefPixelImg=C:\Program Files\phoenix x-ray\datosx 2 acq\CalibrationImages\pixmask_B1x1_x2024_y2024.tif

[SectorScan]

Active=0

[DetectorShift]

Enable=1

Mode=0

Amplitude=5

Interval=1

Step=1

[Detector]

InitTimeOut=60000

Name=dxr-250

PixelSizeX=0.20000000

PixelSizeY=0.20000000

NrPixelsX=2024

NrPixelsY=2024

Timing=5

TimingVal=1000.082

Avg=1

Skip=0

Binning=0

BitPP=14

CameraGain=2

SatValue=15563

SatPixNrLimit=4096

[Xray]

ComPort=0

Name=xs|300 d

ID=2303

InitTimeout=20000

Voltage=75

Current=240

Mode=0

Filter=Unknown

Collimation=0

WaitTime=1000

WaitForStable=30000

FocDistX=0.00000000

FocDistY=0.00000000

SpinStepkV=10

SpinStepuA=10

Macro=0

RestrictNumSpots=0

PreWarning=0

MinGainCurrent=10

[Cnc]

InitTimeout=8000

CollisionDetection=1

JoyDriveDoorOpen=0

SecPosSample=250.00000000

MinSampleDetPos=300.00000000

EnableKeyboardJoy=0

KeyJoyVelocityFactor=0.25000000

[Axis]

XSample=-0.544062

YSample=253.753813

ZSample=43.215875

RSample=820.479100

XDetector=0.000000

[AcqSrvManager]

RecvPcIp=

ExePath=c:\Program Files\phoenix x-ray\datosx 2 acq\srv\dtxaSrv.exe

[Net]

Enable=0

IP=

Appendix 7

Parameter for acquisition of mCT images of the entire body of *Pseudogeoplane doederleini*

[General]

Version=2.3.0.1032
Version-pca=2
Comment=
LoadDefault=1
SystemName=v|tome|x m

[AutoScO]

Active=1
ImgNr=8
ImageString=562:1124:1686:2248:2810:3372:3934:4500
Skip=10

[Geometry]

FDD=817.22215760
FOD=57.76625000
Magnification=14.14705226
VoxelSizeX=0.01413722
VoxelSizeY=0.01413722
CalibValue=2.778
cx=149.50000000
cy=1011.50000000
DetectorRot=0.00000000
Tilt=0.00000000
Old_CalibValue=-10.69200000

[CT]

Type=0
NumberImages=4500
StartImg=4501
RotationSector=360.00000000
NoRotation=0
EstimatedTime=0
RemainingTime=4500
ScanTimeCmpl=4500
NrImgDone=4501
NrImgCmplScan=4501
RefDriveEnabled=0
SkipForNewInterval=25
SkipAcc=1
FreeRayFactor=1.00010000
Wnd_L=0
Wnd_T=0
Wnd_R=10
Wnd_B=10
Level=7294.00000000

[VSensor]

EnableTiles=1
Start=0
NumTiles=1
Interval=60
Overlap=9
AdjustImg=1
SingleImgX=310

[Trajectory]

Active=0

[CalibValue]

NumberImages=18

Averaging=2

Skip=3

[FastCT]

Active=0

[Image]

Top=0

Left=857

Bottom=2023

Right=1166

DimX=300

DimY=2024

Rotation=0

MirrorX=0

MirrorY=0

BitPP=16

FreeRay=7294

[ImgProc]

SwBin=1

AddSwBin=0

[Warmup]

Enable=1

Counter=0

MaxTimes=10

TimeTrigOn=0

kV=85

Time=60

[Multiscan]

Active=0

[Multiline]

Installed=0

[CalibImages]

MGainMode=0

MGainPoints=3

Avg=50

Skip=10

EnableAutoAcq=1

MGainVoltage=90:90:90:

MGainCurrent=20:60:200:

GainImg=C:\Program Files\phoenix x-ray\datosx 2 acq\CalibrationImages\G_B1_G200_T500_V1.tif

MGainImg=C:\Program Files\phoenix x-ray\datosx 2 acq\CalibrationImages\G_B1_G400_T500_V1.tif

OffsetImg=C:\Program Files\phoenix x-ray\datosx 2 acq\CalibrationImages\offset_B1x1_G200.0%_T500ms.tif

DefPixelImg=C:\Program Files\phoenix x-ray\datosx 2 acq\CalibrationImages\pixmask_B1x1_x2024_y2024.tif

[SectorScan]

Active=0

[DetectorShift]

Enable=1

Mode=0

Amplitude=5

Interval=1

Step=1

[Detector]

InitTimeOut=60000

Name=dxr-250

PixelSizeX=0.20000000

PixelSizeY=0.20000000

NrPixelsX=2024

NrPixelsY=2024

Timing=4

TimingVal=500.092

Avg=1

Skip=0

Binning=0

BitPP=14

CameraGain=2

SatValue=15563

SatPixNrLimit=4096

[Xray]

ComPort=0

Name=xs|300 d

ID=2303

InitTimeout=20000

Voltage=75

Current=340

Mode=0

Filter=Unknown

Collimation=0

WaitTime=1000

WaitForStable=30000

FocDistX=0.00000000

FocDistY=0.00000000

SpinStepkV=10

SpinStepuA=10

Macro=0

RestrictNumSpots=0

PreWarning=0

MinGainCurrent=10

[Cnc]

InitTimeout=8000

CollisionDetection=1

JoyDriveDoorOpen=0

SecPosSample=250.00000000

MinSampleDetPos=300.00000000

EnableKeyboardJoy=0

KeyJoyVelocityFactor=0.25000000

[Axis]

XSample=-0.100687

YSample=226.507250

ZSample=57.766250

RSample=-110.435250

XDetector=0.000000

[AcqSrvManager]

RecvPcIp=192.168.10.2

ExePath=c:\Program Files\phoenix x-ray\datosx 2 acq\srv\dtxaSrv.exe

[Net]

Enable=0

IP=192.168.10.2

Appendix 8

Parameter for acquisition of mCT images of the entire body of *Pseudogeoplane wetzeli*

[General]

Version=2.3.0.1032
Version-pca=2
Comment=
LoadDefault=1
SystemName=v|tome|x m

[AutoScO]

Active=1
ImgNr=8
ImageString=250:500:750:1000:1250:1500:1750:2000
Skip=10

[Geometry]

FDD=817.22215760
FOD=52.06718750
Magnification=15.69553104
VoxelSizeX=0.01274248
VoxelSizeY=0.01274248
CalibValue=-22.231
cx=299.50000000
cy=1011.50000000
DetectorRot=0.00000000
Tilt=0.00000000
Old_CalibValue=-9.78900000

[CT]

Type=0
NumberImages=2000
StartImg=2001
RotationSector=360.00000000
NoRotation=0
EstimatedTime=0
RemainingTime=2000
ScanTimeCmpl=2000
NrImgDone=2001
NrImgCmplScan=2001
RefDriveEnabled=0
SkipForNewInterval=25
SkipAcc=1
FreeRayFactor=1.00010000
Wnd_L=488
Wnd_T=22
Wnd_R=570
Wnd_B=90
Level=7970.00000000

[VSensor]

EnableTiles=1
Start=0
NumTiles=1
Interval=60
Overlap=9
AdjustImg=1
SingleImgX=610

[Trajectory]

Active=0

[CalibValue]

NumberImages=18

Averaging=2

Skip=3

[FastCT]

Active=0

[Image]

Top=0

Left=707

Bottom=2023

Right=1316

DimX=600

DimY=2024

Rotation=0

MirrorX=0

MirrorY=0

BitPP=16

FreeRay=11352

[ImgProc]

SwBin=1

AddSwBin=0

[Warmup]

Enable=1

Counter=0

MaxTimes=10

TimeTrigOn=0

kV=75

Time=60

[Multiscan]

Active=0

[Multiline]

Installed=0

[CalibImages]

MGainMode=0

MGainPoints=2

Avg=100

Skip=10

EnableAutoAcq=1

MGainVoltage=70:70:70:

MGainCurrent=20:70:220:

GainImg=C:\Program Files\phoenix x-ray\datosx 2 acq\CalibrationImages\G_B1_G200_T1000_V1.tif

MGainImg=

OffsetImg=C:\Program Files\phoenix x-ray\datosx 2 acq\CalibrationImages\offset_B1x1_G200.0%_T1000ms.tif

DefPixelImg=C:\Program Files\phoenix x-ray\datosx 2 acq\CalibrationImages\pixmask_B1x1_x2024_y2024.tif

[SectorScan]

Active=0

[DetectorShift]

Enable=1

Mode=0

Amplitude=5

Interval=1

Step=1

[Detector]

InitTimeOut=60000

Name=dxr-250

PixelSizeX=0.20000000

PixelSizeY=0.20000000

NrPixelsX=2024

NrPixelsY=2024

Timing=5

TimingVal=1000.082

Avg=1

Skip=0

Binning=0

BitPP=14

CameraGain=2

SatValue=15563

SatPixNrLimit=4096

[Xray]

ComPort=0

Name=xs|300 d

ID=2303

InitTimeout=20000

Voltage=70

Current=310

Mode=0

Filter=Unknown

Collimation=0

WaitTime=1000

WaitForStable=30000

FocDistX=0.00000000

FocDistY=0.00000000

SpinStepkV=10

SpinStepuA=10

Macro=0

RestrictNumSpots=0

PreWarning=0

MinGainCurrent=10

[Cnc]

InitTimeout=8000

CollisionDetection=1

JoyDriveDoorOpen=0

SecPosSample=250.00000000

MinSampleDetPos=300.00000000

EnableKeyboardJoy=0

KeyJoyVelocityFactor=0.25000000

[Axis]

XSample=-0.407187

YSample=213.247562

ZSample=52.067187

RSample=624.875950

XDetector=0.000000

[AcqSrvManager]

RecvPcIp=192.168.10.2

ExePath=c:\Program Files\phoenix x-ray\datosx 2 acq\srv\dtxaSrv.exe

[Net]

Enable=0

IP=192.168.10.2

Appendix 9

Parameter for acquisition of mCT images of the entire body of *Dolichoplana* sp.

[General]

Version=2.3.0.1032
Version-pca=2
Comment=
LoadDefault=1
SystemName=v|tome|x m

[AutoScO]

Active=1
ImgNr=8
ImageString=187:374:561:748:935:1122:1309:1500
Skip=10

[Geometry]

FDD=817.22215760
FOD=62.61656250
Magnification=13.05121401
VoxelSizeX=0.01532424
VoxelSizeY=0.01532424
CalibValue=-9.789
cx=494.50000000
cy=1011.50000000
DetectorRot=0.00000000
Tilt=0.00000000
Old_CalibValue=0.00000000

[CT]

Type=0
NumberImages=1500
StartImg=1501
RotationSector=360.00000000
NoRotation=0
EstimatedTime=0
RemainingTime=1500
ScanTimeCmpl=1500
NrImgDone=1501
NrImgCmplScan=1501
RefDriveEnabled=0
SkipForNewInterval=25
SkipAcc=1
FreeRayFactor=1.00010000
Wnd_L=0
Wnd_T=0
Wnd_R=10
Wnd_B=10
Level=11060.00000000

[VSensor]

EnableTiles=1
Start=0
NumTiles=1
Interval=60
Overlap=9
AdjustImg=1
SingleImgX=1000

[Trajectory]

Active=0

[CalibValue]

NumberImages=18

Averaging=2

Skip=3

[FastCT]

Active=0

[Image]

Top=0

Left=512

Bottom=2023

Right=1511

DimX=990

DimY=2024

Rotation=0

MirrorX=0

MirrorY=0

BitPP=16

FreeRay=11060

[ImgProc]

SwBin=1

AddSwBin=0

[Warmup]

Enable=1

Counter=0

MaxTimes=10

TimeTrigOn=0

kV=75

Time=60

[Multiscan]

Active=0

[Multiline]

Installed=0

[CalibImages]

MGainMode=0

MGainPoints=3

Avg=100

Skip=10

EnableAutoAcq=1

MGainVoltage=90:90:90:

MGainCurrent=20:60:180:

GainImg=C:\Program Files\phoenix x-ray\datosx 2 acq\CalibrationImages\G_B1_G200_T1000_V1.tif

MGainImg=

OffsetImg=C:\Program Files\phoenix x-ray\datosx 2 acq\CalibrationImages\offset_B1x1_G200.0%_T1000ms.tif

DefPixelImg=C:\Program Files\phoenix x-ray\datosx 2 acq\CalibrationImages\pixmask_B1x1_x2024_y2024.tif

[SectorScan]

Active=0

[DetectorShift]

Enable=1

Mode=0

Amplitude=5

Interval=1

Step=1

[Detector]

InitTimeOut=60000

Name=dxr-250

PixelSizeX=0.20000000

PixelSizeY=0.20000000

NrPixelsX=2024

NrPixelsY=2024

Timing=5

TimingVal=1000.082

Avg=1

Skip=0

Binning=0

BitPP=14

CameraGain=2

SatValue=15563

SatPixNrLimit=4096

[Xray]

ComPort=0

Name=xs|300 d

ID=2303

InitTimeout=20000

Voltage=70

Current=280

Mode=0

Filter=Unknown

Collimation=0

WaitTime=1000

WaitForStable=30000

FocDistX=0.00000000

FocDistY=0.00000000

SpinStepkV=10

SpinStepuA=10

Macro=0

RestrictNumSpots=0

PreWarning=0

MinGainCurrent=10

[Cnc]

InitTimeout=8000

CollisionDetection=1

JoyDriveDoorOpen=0

SecPosSample=250.00000000

MinSampleDetPos=300.00000000

EnableKeyboardJoy=0

KeyJoyVelocityFactor=0.25000000

[Axis]

XSample=-0.366312

YSample=260.824125

ZSample=62.616562

RSample=778.335000

XDetector=0.000000

[AcqSrvManager]

RecvPcIp=

ExePath=c:\Program Files\phoenix x-ray\datosx 2 acq\srv\dtxaSrv.exe

[Net]

Enable=0

IP=

Appendix 10

Parameter for acquisition of mCT images of the entire body of *Imbira bonita*.

[General]

Version=2.3.0.1032
Version-pca=2
Comment=
LoadDefault=1
SystemName=v|tome|x m

[AutoScO]

Active=1
ImgNr=8
ImageString=562:1124:1686:2248:2810:3372:3934:4500
Skip=10

[Geometry]

FDD=817.22215760
FOD=96.16187500
Magnification=8.49840082
VoxelSizeX=0.02353384
VoxelSizeY=0.02353384
CalibValue=-5.650
cx=149.50000000
cy=1011.50000000
DetectorRot=0.00000000
Tilt=0.00000000
Old_CalibValue=-10.69200000

[CT]

Type=0
NumberImages=4500
StartImg=4501
RotationSector=360.00000000
NoRotation=0
EstimatedTime=0
RemainingTime=4500
ScanTimeCmpl=4500
NrImgDone=4501
NrImgCmplScan=4501
RefDriveEnabled=0
SkipForNewInterval=25
SkipAcc=1
FreeRayFactor=1.00010000
Wnd_L=5
Wnd_T=11
Wnd_R=23
Wnd_B=22
Level=6147.00000000

[VSensor]

EnableTiles=1
Start=0
NumTiles=1
Interval=60
Overlap=9
AdjustImg=1
SingleImgX=310

[Trajectory]

Active=0

[CalibValue]

NumberImages=18

Averaging=2

Skip=3

[FastCT]

Active=0

[Image]

Top=0

Left=857

Bottom=2023

Right=1166

DimX=300

DimY=2024

Rotation=0

MirrorX=0

MirrorY=0

BitPP=16

FreeRay=7294

[ImgProc]

SwBin=1

AddSwBin=0

[Warmup]

Enable=1

Counter=0

MaxTimes=10

TimeTrigOn=0

kV=85

Time=60

[Multiscan]

Active=0

[Multiline]

Installed=0

[CalibImages]

MGainMode=0

MGainPoints=3

Avg=50

Skip=10

EnableAutoAcq=1

MGainVoltage=90:90:90:

MGainCurrent=20:60:200:

GainImg=C:\Program Files\phoenix x-ray\datosx 2 acq\CalibrationImages\G_B1_G200_T500_V1.tif

MGainImg=C:\Program Files\phoenix x-ray\datosx 2 acq\CalibrationImages\G_B1_G400_T500_V1.tif

OffsetImg=C:\Program Files\phoenix x-ray\datosx 2 acq\CalibrationImages\offset_B1x1_G200.0%_T500ms.tif

DefPixelImg=C:\Program Files\phoenix x-ray\datosx 2 acq\CalibrationImages\pixmask_B1x1_x2024_y2024.tif

[SectorScan]

Active=0

[DetectorShift]

Enable=1

Mode=0

Amplitude=5

Interval=1

Step=1

[Detector]

InitTimeOut=60000

Name=dxr-250

PixelSizeX=0.20000000

PixelSizeY=0.20000000

NrPixelsX=2024

NrPixelsY=2024

Timing=4

TimingVal=500.092

Avg=1

Skip=0

Binning=0

BitPP=14

CameraGain=2

SatValue=15563

SatPixNrLimit=4096

[Xray]

ComPort=0

Name=xs|300 d

ID=2303

InitTimeout=20000

Voltage=75

Current=340

Mode=0

Filter=Unknown

Collimation=0

WaitTime=1000

WaitForStable=30000

FocDistX=0.00000000

FocDistY=0.00000000

SpinStepkV=10

SpinStepuA=10

Macro=0

RestrictNumSpots=0

PreWarning=0

MinGainCurrent=10

[Cnc]

InitTimeout=8000

CollisionDetection=1

JoyDriveDoorOpen=0

SecPosSample=250.00000000

MinSampleDetPos=300.00000000

EnableKeyboardJoy=0

KeyJoyVelocityFactor=0.25000000

[Axis]

XSample=-0.275625

YSample=232.849312

ZSample=96.161875

RSample=1943.984600

XDetector=0.000000

[AcqSrvManager]

RecvPcIp=192.168.10.2

ExePath=c:\Program Files\phoenix x-ray\datosx 2 acq\srv\dtxaSrv.exe

[Net]

Enable=0

IP=192.168.10.2