Pedonais crassifaucis n.gen., n.sp. (Naididae) and Bothrioneurum righii n.sp. (Tubificidae), two new tropical soil-dwelling species of "aquatic" oligochaetes (Clitellata, Annelida) from Central Amazonia

by

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Abstract

Two new species of so-called aquatic Oligochaeta are described from soil and litter samples originating from terra firme sites of the Amazonian primary rain forest. *Bothrioneurum righii* n.sp. (Tubificidae) is characterized by posterior dorsal chaetae with very large distal teeth and much reduced proximal teeth. The new genus *Pedonais* (Naididae) resembles *Allonais* but differs from it in the absence of hair chaetae, in the similarity of dorsal and ventral chaetae, and in the presence of well-developed pharyngeal glands. *Pedonais crassifcaucis* n.sp. reproduces asexually by fragmentation and subsequent regeneration, and the same is assumed for *B. righii*. Sexually mature specimens were not found. Both species exhibit traits that can be conceived as adaptations of aquatic oligochaetes for a successful colonization of terrestrial habitats.

Resumo

Duas novas espécies terrestres dos chamados oligoquetos aquáticos foram descritas em amostras de solo e detritos provenientes de sítios amostrais de "terra firme" da Floresta Amazônica Primária. A espécie *Bothrioneurum righii* n.sp. (Tubificidae) caracteriza-se por cerdas posteriores dorsais com dentes distais muito largos e dentes proximais bastante reduzidos. O novo gênero *Pedonais* (Naididae) aproxima-se de *Allonais* mas difere do mesmo nos seguintes pontos: a ausência de cerdas capilares, a semelhança entre as cerdas dorsais e ventrais, e a presença de glandulas faríngeas fortemente desenvolvidas. *Pedonais crassifaucis* n.sp. reproduz-se assexuadamente por fragmentação e subseqüente regeneração. O mesmo pode ser inferido para *Bothrioneurum righii*. Não foram encontradas espécimens sexualmente amadurecidas.

Ambas as espécies exibem características que podem ser compreendidas como adaptações dos oligoquetos aquáticos à colonização de habitats terrestres.

Introduction

In Central Amazonia (Manaus, Brazil), soil biological studies are currently being carried out in the context of a project aiming at developing methods for sustainable land use in Amazonian rain forests (SHIFT: Studies on Human Impact on Floodplains and Forests in the Tropics) (RÖMBKE & MELLER 1999; RÖMBKE et al. 1999a). These investigations include an inventory of the taxonomic diversity of the soil fauna present at the experimental sites. A part of the study area is a primary rain forest plot at the Brazilian Agroforestry Research Facility (EMBRAPA-CPAA: Empresa Brazileira de Pesquisa Agropecuária, Centro de Pesquisa Agroflorestal na Amazônia Ocidental) near Manaus, Brazil. The sites are "terra firme", i.e. truly terrestrial and not part of the floodplain. A few megadrile and about a dozen new enchytraeid species were found here (RÖMBKE & MELLER 1999; RÖMBKE et al. 1999b; SCHMELZ, pers. obs.), but also an exciting diversity of oligochaetes belonging to taxa generally considered as aquatic. The bulk of species belong to *Pristina* (as redefined by COLLADO & SCHMELZ 2000), but also a new *Bothrioneurum* species and another naidid species of uncertain generic affiliation were discovered. These two species are described in this paper.

Bothrioneurum righii n.sp. (Tubificidae) differs from other species of the genus in the particular pattern of dorsal chaetae. Most species of *Bothrioneurum* are described from tropical environments, only *B. vejdovskyanum* is regarded as cosmopolitan. However, *B. righii* is the first species in the genus that is a true soil-dweller. For the second species, we established the new genus *Pedonais*, because its character combination does not fit any of the existing genera.

The taxonomic investigation was enhanced by the possibility of studying the animals alive, additionally to the classical investigation of stained whole mounts. Of *P. crassifaucis*, flourishing cultures could be established and maintained over a period of several months, allowing observations on burrowing, feeding, and regeneration.

Material and methods

A detailed description of the study area and the methods of investigation are given in COLLADO & SCHMELZ (2000). The species were found in samples of soil and litter from the terra firme primary forest plot (SHIFT ENV 52), located on the agroforestry research station EMBRAPA-CPAA, state of Amazonas, Brazil, 02°53'47''S and 59°59'45''W. This plot is the type locality of the two new species described here.

Specimens were extracted alive using the hot/wet funnel technique established for enchytraeids by O'CONNOR (1955) and investigated with a dissecting microscope (x10 - x40) and a compound microscope (x100 - x1000). Specimens were examined alive and as preparations (stained whole mounts, squash preparations). All microscopical observations, live observations included, were documented photographically. Figures were drawn from photographs of live specimens and from fixed and whole-mounted animals, the latter using a drawing tube. Some of the determined specimens of *Pedonais* were cultured at room temperature on a mixture of 0,5% Agar-Agar and mineral soil from the habitat in polystrol Petri dishes. The cultures survived and reproduced for more than a year.

Type and other reference material is deposited at the Instituto Nacional de Pesquisas da Amazônia

(INPA), Manaus, Brazil and at the Zoologisches Museum und Zoologisches Institut, Universität Hamburg (ZMUH), Germany. Reference specimens are stained and whole mounted or ethanol-preserved. The following descriptions of the new species combine observations on live and preserved specimens.

Taxonomic part

Pedonais n.gen.

Type species: *Pedonais crassifaucis* n.sp.

Prostomium small, pharynx large, pharyngeal glands well-developed, body wall thick, anal branchial fossa or gills absent, reproduction by architomy, five segments regenerated anteriorly, dorsal chaetae from VI or VII on, dorsal and ventral chaetae bifid crotchets, much alike, no hairs, no needles, no eyes, coelomocytes present, no swimming.

Derivatio nominis: *Pedonais* (from the Greek pedon = soil): "the soil-dwelling *Nais*".

Pedonais crassifaucis n.sp. (Figs. 1a, 2a-j, 3a-b)

Type locality: Manaus, Brazil (see above).

Holotype: INPA 117. Paratypes: INPA 118a-e, 5 specimens; ZMUH OL 14157, 3 specimens. All type specimens whole mounted. Other material examined: 8 whole mounted specimens, 20 specimens fixed in 70% ethanol; 18 specimens observed alive.

Derivatio nominis: *crassifaucis*: with fat gullet, or with very large pharynx.

Colour of live specimens (top light) brownish-reddish with whitish to ivory-coloured fields or spots. No swimming. Individual worms considerably varying in length (1.5-8.5 mm, fixed specimens) and segment number (41-101), due to mode of reproduction by fragmentation; observed minimum fragment length 12 segments; maximum length in live specimens ca. 10 mm. Body diameter 230-270 µm at VI, 180-310 µm at XII.

Dorsal chaetae (Fig. 1a, Fig. 2a-d) generally from VI or VII, one stout bifid crotchet per bundle, 53-76 µm long, 3-5 µm thick, nodulus slightly distal, upper tooth thinner and shorter than lower. No hairs, no needles. Dorsal chaetae often missing in some segments, one specimen without dorsal chaetae until XX. Ventral chaetae (Fig. 2e-j) all bifid crotchets, with nodulus slightly distal, 3/5 from the distal end, 56-73 µm long; in II-V 5-15 chaetae per bundle, usually 8-11, teeth equally thick and equally long (1.8-2.5 µm), occasionally upper teeth slightly longer. From VI on 5-15 per bundle, usually 6-10, numbers decreasing posteriad, down to 5-3 in some specimens; posteriad upper teeth increasingly shorter and thinner; lower teeth increasingly thicker. Abrupt change in thickness of lower tooth from V to VI in most but not all specimens (comp. Figs. 2f and 2g). The thickness of chaetae varies between specimens (comp. Figs. 2c and 2d), but the dorsals are always thicker than the ventrals.

Prostomium (Fig. 1a, 3a) rounded, distinct but small, often inconspicuous, wider (90-130 µm at base) than long (40-82 µm). Mouth shifted anteriorly. No eyes. External segmentation present from VI/VII. Body wall thick, longitudinal muscles well-developed (Fig. 3b), ring muscles conspicuous. No epidermal glands cells observed. Brain (Fig. 1a, 3a) in segments I-II, with a median incision anteriorly and a median and two lateral incisions posteriorly, resulting in four posterior lobes, all rounded, the inner projecting farther backwards than the outer. Pharynx large, with dorsally and laterally thickened walls (pharyngeal pad), occupying three segments (III-V) and nearly filling entire body cavity; pharyngeal pad widest anteriorly, slightly tapering posteriorly (dorsal view, fixed specimens). Pharyngeal glands well-developed, in IV, V and parts of VI, surrounding pharyngeal pad dorsally and laterally, also surrounding oesophagus, partly attached to septa of IV/V and V/VI, to inner body wall, partly free; multi-lobed, not arranged in discrete portions, entire glanduar mass adhering to pharyngeal pad (Fig. 1a). Chloragocytes from segment

VI, brownish-reddish, forming a continuous, dense layer from the beginning, giving the main body colour. Intestine widening in X (Fig. 1a), endothel thickened, but no stomachal dilatation with specialized cells present. Coelomocytes (Fig. 3b) abundant, globular, granulated, with refractile corpuscules, forming whitish to ivory-coloured spots or fields in the body (top light), dark through transmittent light, 7.5-15.6 μm in diameter. Blood vessels: dorsal vessel to the left posteriorly, bifurcating in front of brain (Fig. 3a) into circumoral connectives that rejoin in II to form the ventral vessel; one pair of commissural vessels per segment branching off the dorsal vessel in II-VIII, those in II-V with consecutive branchings before joining ventral blood vessel, commissurals unbranched in VI-VIII. Nephridia present from VI/VII; anteseptale consisting of ciliated funnel; postseptale consisting of a bulbus adjacent to septum and a consecutive long and coiled canal, arranged in adjacent loops; opening anterior to ventral chaetal bundles. Anus medially or shifted dorsally, with stiff, non-moving cirri on its outer surface. Hindgut of posteriormost segments strongly ciliated, dilated, funnel- (live specimens) or spindle-shaped (live and preserved specimens). No branchial fossa, no gills.

No sexual organs observed. No fission zones observed. Reproduction by fragmentation and subsequent regeneration of anterior and posterior ends (architomy). Five segments regenerated anteriorly. In fresh fragments, dorsal chaetae of anteriormost segment sometimes fallen out, hence the commencement of dorsals in VII in some specimens.

Taxonomic remarks

We established a new genus for this species because its character combination does not fit any of the existing genera. The architomic mode of reproduction and the lack of dorsal chaetae in some anterior segments aligns it to *Allonais* SPERBER, 1948 and to *Dero* OKEN, 1815 subgenus *Allodero* SPERBER, 1948 in the Naididae. Species of both taxa are mainly found in the tropics.

A comparison with *Dero* (*Allodero*) is difficult, because this subgenus comprises a heterogeneous group of species, some of them free living, some of them parasitic or commensalic in amphibians. Parasitic (or commensalic) species are: *D. (A.) lutzi* (MICHAELSEN, 1926), D. (*A.*) *bauchiensis* (STEPHENSON, 1930), D. (*A.*) *hylae* (GOODCHILD, 1951) and *D. (A.) floridana* HARMAN, 1971; free living species are *D. (A.) malayana* (STEPHENSON, 1931), and *D. (A.) prosetosa* LIANG, 1964. (Not mentioned here are species of doubtful or controversial generic affiliation, see BRINKHURST & JAMIESON 1971: 363) In *D. (A.) lutzi*, both a free living and a parasitic life form is described, and they differ much from one another morphologically. It has been assumed that free living forms must exist in the other parasitic species as well (STEPHENSON 1930a; HARMAN & LAWLER 1975), but HARMAN's statement (1971), that SPERBER (1948) erected *Allodero* for parasitic or symbiontic species within the genus *Dero*, is incorrect because *D. (A.) malayana* is only known as free living. Parasitism is therefore not a diagnostic feature of the subgenus.

The morphological diagnosis of *Dero* (*Allodero*) is not clear, and only the uniform shape of the ventral chaetae is without exception. Other traits are not ascertained for all species or differently developed: there are hairs and needles dorsally, but parasitic forms are often without dorsal chaetae at all. Dorsal chaetae start in IV, except for *D. (A.) prosetosa* where they start in III. Anal respiratory fossae or gills are present, but often rudimentary or absent in parasitic specimens. Reproduction is by fragmentation, but budding zones were observed in *D. (A.) hylae* (HARMAN 1973). BRINKHURST & JAMIESON (1971: 361) view *Allodero* as "little more than an assemblage of parasitic and free living forms". In many species important characters are not known, such as the presence of pharyngeal glands, the position of nephridia, type of coelomocytes, chloragocytes, etc. Nothing is known about the sexual organs of the *Allodero* species, because all species are described from immature specimens.

A number of conspicuous characters of *P. crassifaucis* are shared by some species in *Dero* (*Allodero*): remarkably large pharyngeal glands are found in *D. (A.) malayana*; anal fossae or branchia are lacking in, *D. (A.) bauchiensis*, *D. (A.) lutzi* (parasitic form) and *D. (A.) floridana*; dorsal crotchets are found in *D. (A.) hylae* and *D. (A.) malayana*; a well developed layer of chloragogen tissue along the gut is described

226

for *D. (A.) lutzi* (free living form) and *D. (A.) hylae*; abundant coelomocytes are characteristic of *D. (A.) floridana* and *D. (A.) hylae*. Five segments are regenerated anteriorly in *D. (A.) bauchiensis*.

On the other hand, *P. crassifaucis* has unique characters that separate it from all species so far explicitly or tentatively (for the latter see BRINKHURST & JAMIESON 1971: 363) assigned to *Allodero*: (1) Dorsal chaetae begin in VI or VII, (2) hair chaetae are entirely absent, (3) dorsal and ventral chaetae are much alike, (4) dorsals are stouter than ventrals, (5) branchial fossae are absent. Traits (2) and (5) are distinguishing characters in that they apply to non-parasitic, free living individuals here. (Absence of hairs is also known for parasitic forms of *D. (A.) lutzi* and *D. (A.) hylae*, and the branchial fossa is rudimentary in the commensalic *D. (A.) floridana*.) If the new species were to be included in *Allodero*, all five mentioned characters would even further enlarge the subgeneric morphological diagnosis, ample and imprecise as it already is.

A comparison with *Allonais* is more promising. *Pedonais crassifaucis* agrees with the generic diagnosis (SPERBER 1948) in that dorsal chaetae begin in VI or VII and in the architomic way of asexual reproduction. Further characters in common are: no stomachal dilatation, branched commissural blood vessels in II-V, simple vessels in following segments, coelomocytes present, ventral chaetae of II-V slightly different from those of the following segments. There are more similarities with single species in the genus: in *A. pectinata* (STEPHENSON, 1910), the circumoral connective blood vessels reunite ventrally also in segment II, and the anteriormost nephridium is at VI/VII (AIYER 1929). In *A. paraguayensis aequatorialis* (CERNOSVITOV, 1938), the prostomium is short and the pharynx is very large, extending down to septum 6/7. A large pharynx, extending from II-V, is also described for *Allonais rayalaseemensis* NAIDU, 1963 (NAIDU & SRIVASTAVA 1980). The brain is incised anteriorly and posteriorly in *A. pectinata* (AIYER 1929) and in *A. lairdi* NAIDU, 1965. These characters were not included in the generic diagnosis by SPERBER (1948: 200).

There are three characters in the generic diagnosis of *Allonais* which *Pedonais crassifaucis* does not fit: (1) The dorsal chaetal bundles consist of hairs and characteristically shaped needles; (2) the needles are much different in shape from the ventral bifid crotchets, and (3) septal glands are absent. In *P. crassifaucis*, hairs are absent, ventral and dorsal chaetae are much alike (Fig. 2), and there are large pharyngeal glands. The third character may at first sight not appear as a difference at all, as the absence of *septal* glands in *Allonais* does not necessarily imply the absence of *pharyngeal* glands. The distinction between these two types of glands, however (and even a further type, the 'oesophageal glands', to complicate the picture), is most probably artificial. Following SPERBER (1948: 14ff.), pharyngeal glands are near to and closely associated with the pharynx, whereas septal glands are located more posteriorly, attached to the septa, arranged in paired packages, and without apparent connection to the alimentary tract. At least in enchytraeids, however, the septal gland cells are morphologically and functionally connected with the pharynx (UDE 1977). Live observations on such glands in Naididae (e.g. *Pristina* sp.) suggest the same (SCHMELZ, pers. obs.). Any kind of gland mass around and adjacently posterior to the pharynx of naidids is now uniformly termed 'pharyngeal' (ERSÉUS & GRIMM 1998) or 'pharyngo-oesophageal' (COLLADO & SCHMELZ 2000); these structures certainly deserve ultrastructural and histochemical reinvestigation.

Definite statements about the presence or absence of glands associated with the pharynx are rare for *Allonais* species. Only MEHRA (1920) describes small groups of ca. 30 µm long pyriform cells on top of the pharynx of *Nais pectinata* var. *inaequalis* STEPHENSON, 1911 (now *Allonais inaequalis*). In CERNOSVITOV's (1938) sketch of *A. paraguayensis aequatorialis*, the pharynx is apparently naked, without glandular mass covering its outer surface. On the other hand, the well-developed glands in *P. crassifaucis* are at least in parts attached to the septa, and may also be taken as 'septal' glands, especially in fixed specimens, where the glands appear to be widely attached to septum VI/VII (comp. Fig. 1a). Thus, at the very least, pharyngeal glands of the new species are much more developed and projecting much further posteriad than in *Allonais* species, and in this way it serves as distinguishing character.

To summarize, *P. crassifaucis* exhibits a number of striking similarities with *Allonais*, but the differences (no hair chaetae, dorsal and ventral chaetae similar, pharyngeal glands well-developed) would entail an enlargement of the morphological boundaries of that genus, if the new species were to be included.

227

It may appear premature to establish a new genus based on these few characters, especially when the sexual organs are not known. In Tubificidae, most of the larger genera comprise species with and without hair chaetae. In the Naididae, however, chaetal characters are more significant on the generic level and species with and species without hairs are usually assigned to different genera. (The only exception is *Piguetiella* SPERBER, 1939, where *P. michiganensis* HILTUNEN, 1967 is hairless and *P. blanci* (PI-GUET, 1906) may or may not possess single hairs in some bundles; both species, however, are probably synonymous (BRINKHURST 1986), and the variability in the presence/absence of hairs is intraspecific rather than interspecific). Furthermore, the morphological relationship betwen *Pedonais* and *Allonais* closely corresponds to that between *Uncinais* LEVINSEN, 1884 and *Nais* MÜLLER, 1773. The former is separated from the latter by the absence of hair chaetae and by the similarity of dorsal and ventral chaetae (SPERBER 1948); all other characters, sexual organs included, are alike.

P. crassifaucis bears some superficial resemblance to *Ophidonais serpentina* (MÜLLER, 1773) which also has no hairs, one single stout chaeta per dorsal bundle (often missing), a blunt prostomium, and which is also unable to swim. There are many differences, however, the most striking being (in *O. serpentina*) the paratomic mode of reproduction, the presence of eyes, a stomachal dilatation, and sensory papillae on the body wall, and the difference in shape between dorsal and ventral chaetae.

In discussing the identity and systematic position of *Pedonais*, we deliberately refrained from arguing in phylogenetic terms, because the taxa in question, especially *Dero* (*Allodero*) and *Allonais*, are too insufficiently known to allow justifiable statements on their possible monophyly. Nevertheless, the morphological differences between *Pedonais* and *Allonais* may be interpreted in evolutionary terms, as adaptations of an aquatic oligochaete for living in a terrestrial habitat. *Allonais* species are freshwater dwellers, whereas *P. crassifaucis* lives in forest litter and mineral soil; active burrowing was observed in our laboratory cultures. Loss of long hair chaetae, thickening of dorsal chaetae, loss of ability to swim, and reinforcement of body wall musculature match the requirements for a burrowing way of life. (Enlarged dorsal chaetae, however, are also found in the aquatic *Allonais arieli* GLUZMAN, 1990.) Theoretically, the reverse is possible as well, namely that the aquatic species have evolved out of terrestrial forms; but this would question the general conception of Naididae as a principally aquatic family. As was mentioned above, the phylogenetic relationships between *Allonais* and *Pedonais* cannot be resolved here.

Bothrioneurum righii n.sp. (Figs. 1b,c, 4a-i)

Holotype: INPA 115, whole mounted specimen. Paratypes: INPA 116 a-e, 4 whole mounted specimens.
Other material examined: 12 fixed specimens, 2 of them observed alive prior to fixation.
Derivatio nominis: named in honour of the late Dr. Gilberto Righi, outstanding Brazilian oligochaetologist.

Worms yellowish-brown when alive, active, swimming in water with longitudinal body contractions. Length about 4-9 mm in fixed specimens, diameter 220-300 μm. Segment number (37) - 45-50. A growth zone forming new segments observed at the posterior end of several worms. Prostomium (Fig. 1b) short, conical, wider than long (e.g. 65 μm long, 150 μm wide at base), with a large, ciliated, longitudinal dorsal groove or "sensory pit", proximally surrounded by about a dozen subspherical cells projecting into prostomial lumen.

Dorsal chaetae (Fig. 4a-e) from segment II, all bifid crotchets with nodulus slightly distal, 1-4 per bundle, usually 3; upper tooth longer than lower, size difference increasing posteriad with upper tooth increasing and lower tooth decreasing in size: length ratios of upper to lower tooth 2:1 in III, 3:1 from IX-XIV on (variable among specimens) and up to 5-6:1 in posterior segments; here lower tooth stump-like, shorter than wide, and upper tooth strongly curved. Chaetae 75-90 μm long, 3 μm thick, only in II smaller, 50-60 μm long, 1.5 μm thick. Ventral chaetae (Fig. 4f-i) also bifid crotchets, nodulus slightly distal, in II-IX/X usually 4 chaetae per bundle, 80-90 μm long, slightly shorter than in posterior segments; posteriorly mostly 3, sometimes 2 chaetae per bundle. Upper tooth in anterior segments slightly longer than lower tooth, gradually decreasing in length posteriad, to become slightly shorter than lower in poster-

iormost segments. All chaetal bundles with conspicuous 'chaetal follicle' cells at its base, projecting into the coelomic cavity, consisting of 2 cells each; cells finely granular, darker than coelomocytes.

Brain (Fig. 1b) in segment I, broader than long, broadly concave anteriorly and deeply incised posteriorly, with four posterior lobes; inner lobes larger than lateral lobes, projecting farther posteriad. Pharynx occupying segments II-III. Two pairs of pharyngeal glands in IV and V (Fig. 1b), conspicuous, well-separated from one another, but with somewhat irregular wavy outline. Anteriormost chloragocytes (Fig. 1b) in IV, continuous layer of chloragocytes from VI. Coelomocytes (Fig. 1c) abundant, ellipsoid, 22-28 μm long, pale, with longitudinal streaks and a few refractile yellowish granula concentrated in the center but otherwise irregularly distributed. Coelomocytes and chloragocytes clearly distinct. Blood reddish. Commissural blood vessels in II-VIII; in II-VI not pulsating, nearly as thick as dorsal vessel; in VII and VIII thicker, prominent and strongly pulsating ('intestinal hearts'). Commissurals apparently simple, but some branching pattern observed in II. Intestinal vascular network present. Anterior nephridia in X, XII, XIII and/or XIV, all unpaired.

Only immature specimens observed. No signs of reproduction by fragmentation found (incomplete, specimens, regenerating anterior or posterior ends).

Taxonomic remarks

Although no mature specimens were found, the generic placement of the new species is beyond doubt due to the dorsal sensory pit on the prostomium, which is typical of *Bothrioneurum* and not found outside the genus. Besides, other characters found in this species are characteristic of (though not exclusively found in) *Bothrioneurum*: presence of coelomocytes, presence and location of large pulsating lateral vessels, so-called "intestinal hearts" (STEPHENSON 1930b: 145, Fig. 71), only bifid crotchets in all bundles, no hair chaetae, enlarged chaetal follicle cells.

B. righii is the first species in the genus described to possess posterior dorsal chaetae with very long upper teeth and stump-like, rudimentary, lower teeth. It is especially this feature that justifies the recognition of *B. righii* as a new species. Only *B. schiemeri* TIMM, 1997 has dorsal chaetae with upper teeth longer than the lower in all bundles. In the other known species of *Bothrioneurum*, this pattern is found only in anterior segments; posteriorly upper and lower teeth are always of about equal length. The species-level taxonomy of *Bothrioneurum* is unsettled and the number of valid species is uncertain. For example, BRINKHURST & JAMIESON (1971) list five species: *B. vejdovskyanum* STOLC, 1886, *B. iris* BEDDARD, 1901, *B. americanum* BEDDARD, 1894, *B. brauni* DU BOIS-REYMOND MARCUS, 1949, and *B. pyrrhum* MARCUS, 1942, but they are inclined to believe that there is only one variable species in the genus. The dorsal chaetal pattern of *B. righii*, however, differs from all of them and the taxonomic status of the new species is hence unaffected by this problem.

Bothrioneurum righii differs from *B. schiemeri* mainly in that the lower teeth of the dorsal chaetae decrease considerably in size posteriorly whereas the upper teeth gradually increase in size, becoming 5-6 times longer than the lower one (Fig. 4a-e). In *B. schiemeri* all locomotory chaetae are alike, ventrals and dorsals, with upper teeth 2-3 times longer than the lower. The pattern of the ventral chaetae of the new species also differs from that of *B. schiemeri*: the upper teeth are longer in anterior segments, and the teeth become equal in size posteriorly (Fig. 4f-i). This pattern is more similar to those of the other species of the genus. Besides, *B. schiemeri* is also larger than *B. righii*, longer (up to 22 mm), wider (0.4-0.5 mm), with more segments (100-110) and more chaetae per bundle (4-8 in anterior bundles). Furthermore, it differs from the new species in the shape of the coelomocytes (roundish in *B. schiemeri*, vs. ellipsoid in *B. righii*), in the distribution of pharyngeal gland cells (diffusely surrounding oesophagus down to VII, vs. in discrete packages, confined to IV and V), and in the chaetal follicle cells (inconspicuous, not always observed, vs. always distinct). These characters have not yet been used for species separation in *Bothrioneurum*; they are rarely or insufficiently described in other species, perhaps because usually ethanol-preserved material is investigated.

A stronger focus on characters other than chaetae and male reproductive apparatus might help to solve

the species-level taxonomic problems in *Bothrioneurum*. For example, shape and texture of the coelomocytes may be species-specific and diagnostic: in *B. righii* (Fig. 1c) they are oval and with a conspicuous patterns of stripes and yellowish refractile granula. AIYER (1925) says that the coelomocytes in *B. iris* are oval, flattened and faintly granular, TIMM (1997) states that the coelomocytes in *B. schiemeri* are round (globular?) and granulate. This character is certainly best examined in live specimens.

The enlarged upper tooth of posterior dorsal chaetae is a unique trait in the genus and may be interpreted as an adaptation to the terrestrial environment. Many terrestrial enchytraeids have enlarged posterior chaetae, especially some tropical species (e.g. RIGHI 1974). Other traits interpretable as adaptations, however, are not found. The animals are even able to swim. *B. righii* was constantly found in litter during a litter bag colonization experiment, but always in low numbers (GUSTAVSSON, pers. com.). The bags were exposed in the primary and secondary forest plot. It cannot be excluded that the specimens of *B. righii* found in the soil are just washed out from ponds higher up in the trees.

Discussion

P. crassifaucis reproduces by architomy, but the mode of reproduction of *B. righii* is unknown. It is striking, though, that among all so-called aquatic oligochaetes found at the EMBRAPA terrestrial site, the only tubificid species belongs to a genus where asexual reproduction occurs. All other species of 'aquatic' oligochaetes are naidids, reproducing by paratomy, see COLLADO & SCHMELZ (2000), or by architomy, see *P. crassifaucis*. A presumed mode of reproduction of *B. righii* by architomy would allow the generalization that the colonization of terrestrial sites of aquatic oligochaetes is only possible for groups that are able to reproduce asexually, either because transient droughts must be compensated for by a rapid build-up of large populations, or because the conditions for sexual reproduction are unfavourable in the soil. It may well prove that sexual stages are found higher up in the trees, where tree holes or Bromeliaceae provide a habitat more akin to the original freshwater environment. Both species, however, exhibit traits conceivable as adaptations to the terrestrial environment, *P. crassifaucis* much more evidently than *B. righii*.

The knowledge of the sexual organs of both species remains a desideratum, from a systematic and from a phylogenetic point of view. Fortunately, the sampling site is not a remote spot once visited on an expedition but a well-run experimental area where repeated sampling is possible; so we are confident to complement the description of both species in the near future.

Acknowledgments

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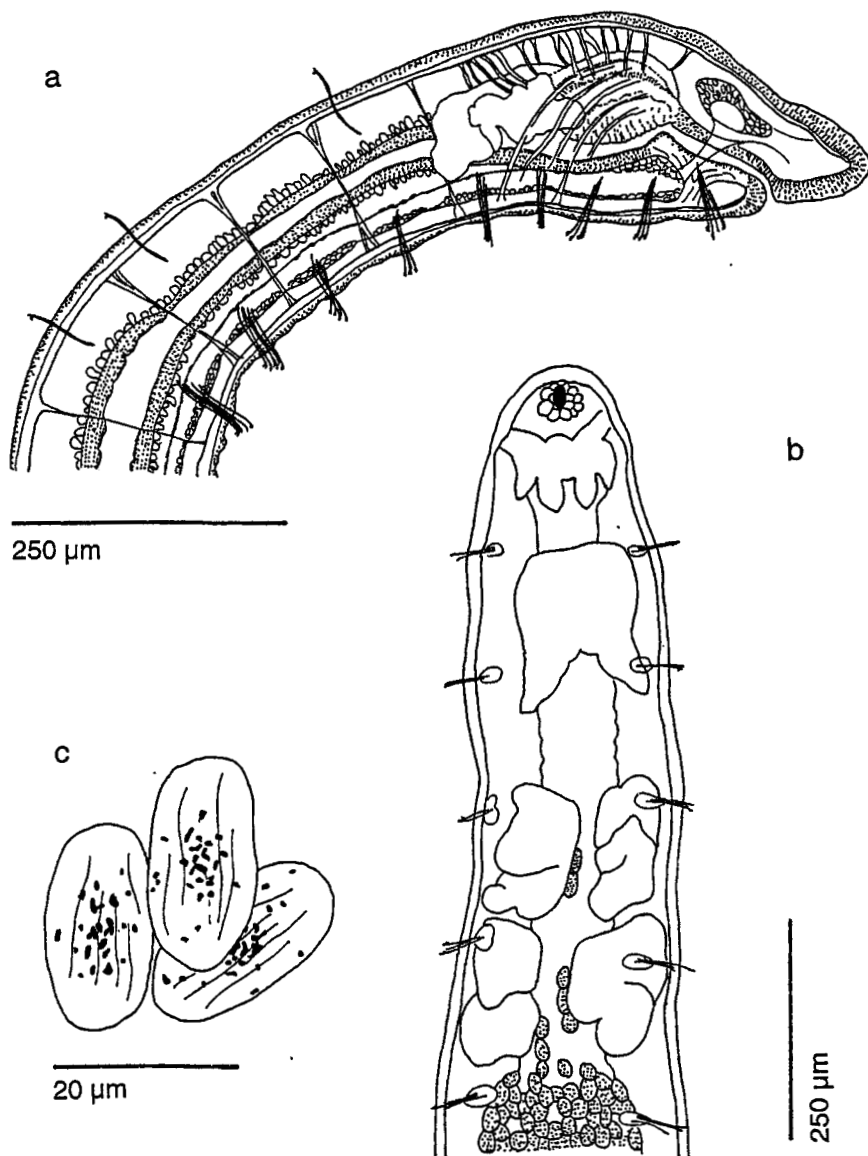
Fig. 1:

Pedonais crassifaucis n.gen., n.sp. and *Bothrioneurum righii* n.sp.

a: *P. crassifaucis*, paratype, anterior end, lateral view; dorsal chaetae missing in VIII in this specimen; note the strong muscular investment of the pharyngeal pad and the pharyngeal glands (in IV-VI) adhering to the oesophagus and the posterior part of the pharyngeal pad; **b**: *B. righii*, anterior end, dorsal view; note the quadrilobed brain, the chatael follicle cells and the two pairs of pharyngeal gland packages in IV and V, respectively; **c**: *B. righii*, coelomocytes. Figure 1a drawn from stained whole mount, 1b,c drawn from photographs of live specimens.
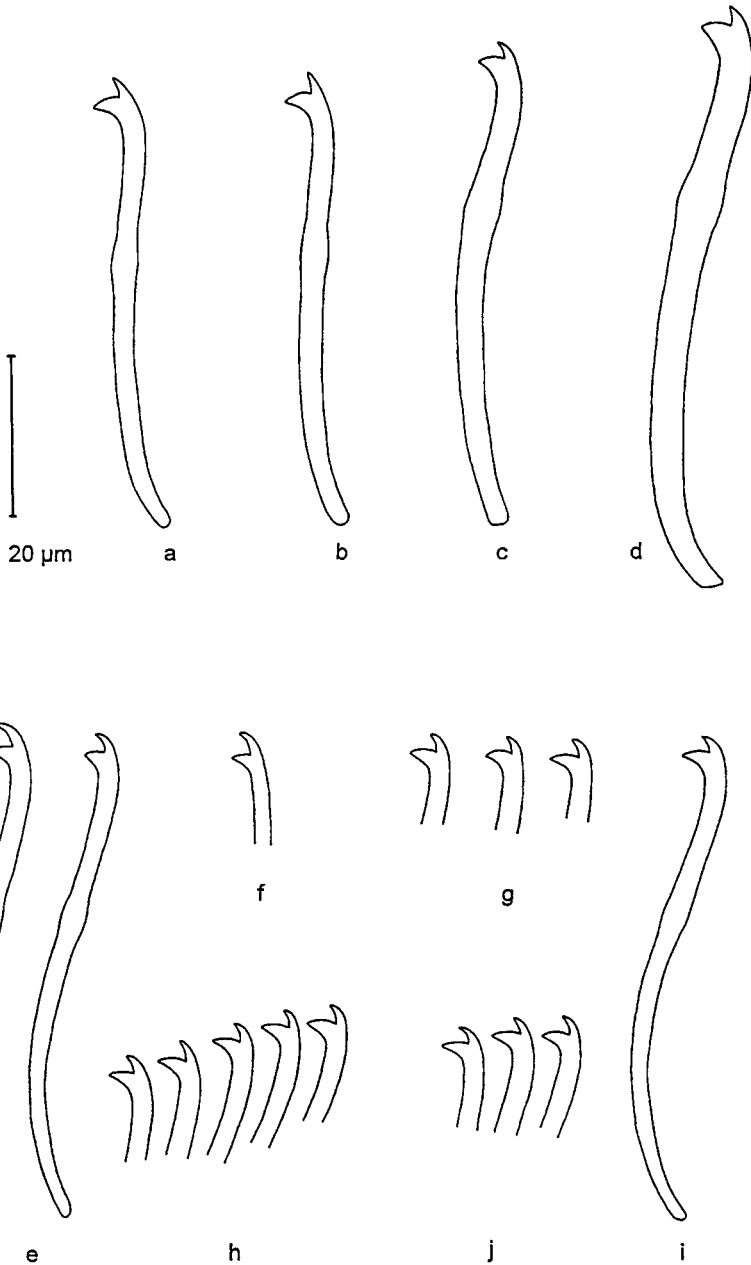
Fig. 2:
Pedonais crassifaucis n.sp.
a - b: posterior dorsal chaeta; c: median dorsal chaeta; d: dorsal chaeta of VII in larger specimen;
e: ventral chaetae of IV; f: ventral chaeta of V; g: ventral chaetae of VIII; h: ventral chaetae of XVIII;
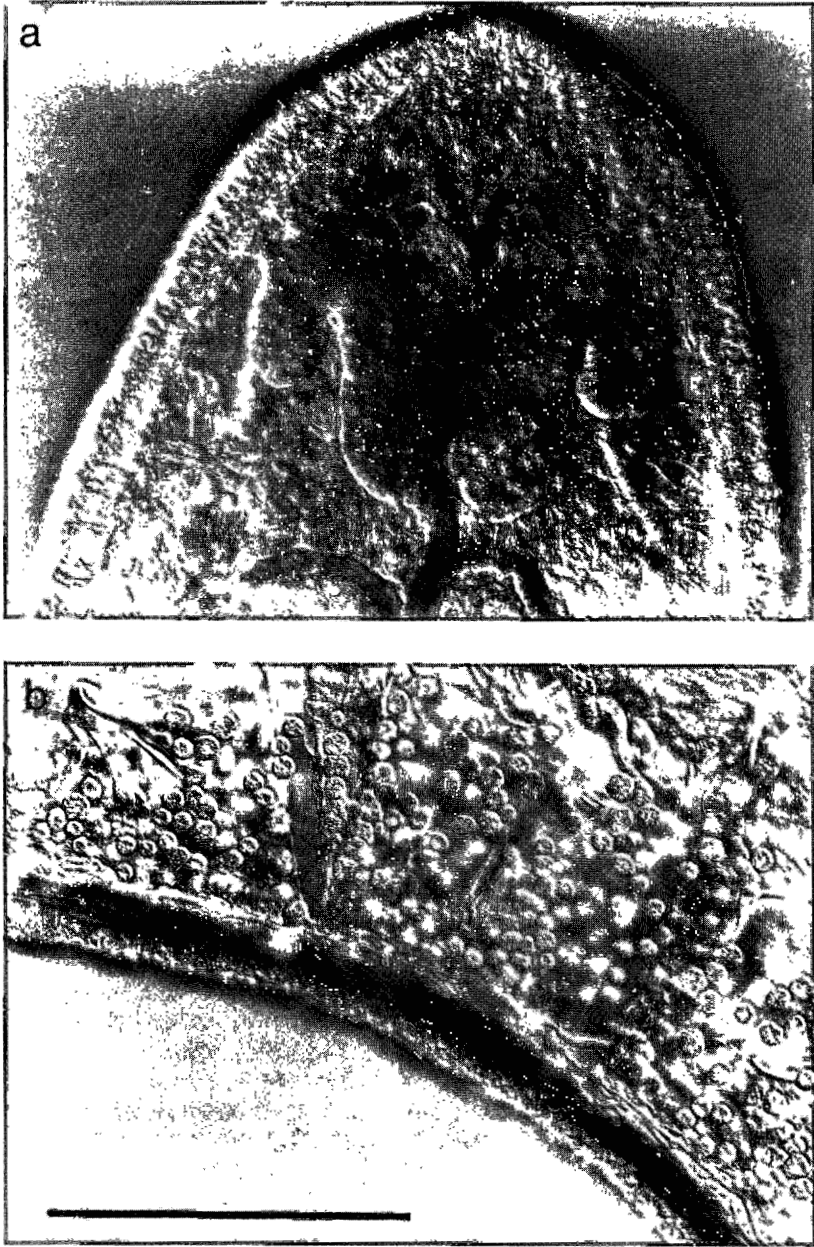i: ventral chaeta of XXXII; j: ventral chaetae in terminal segment.

Fig. 3:
Pedonais crassifaucis n.sp., photographs of live specimens;
a: prostomium and first segment with brain and blood vessels, dorsal view; **b**: mid-body region with globular refractile coelomocytes and well-developed body wall musculature; bar = 100 μm.
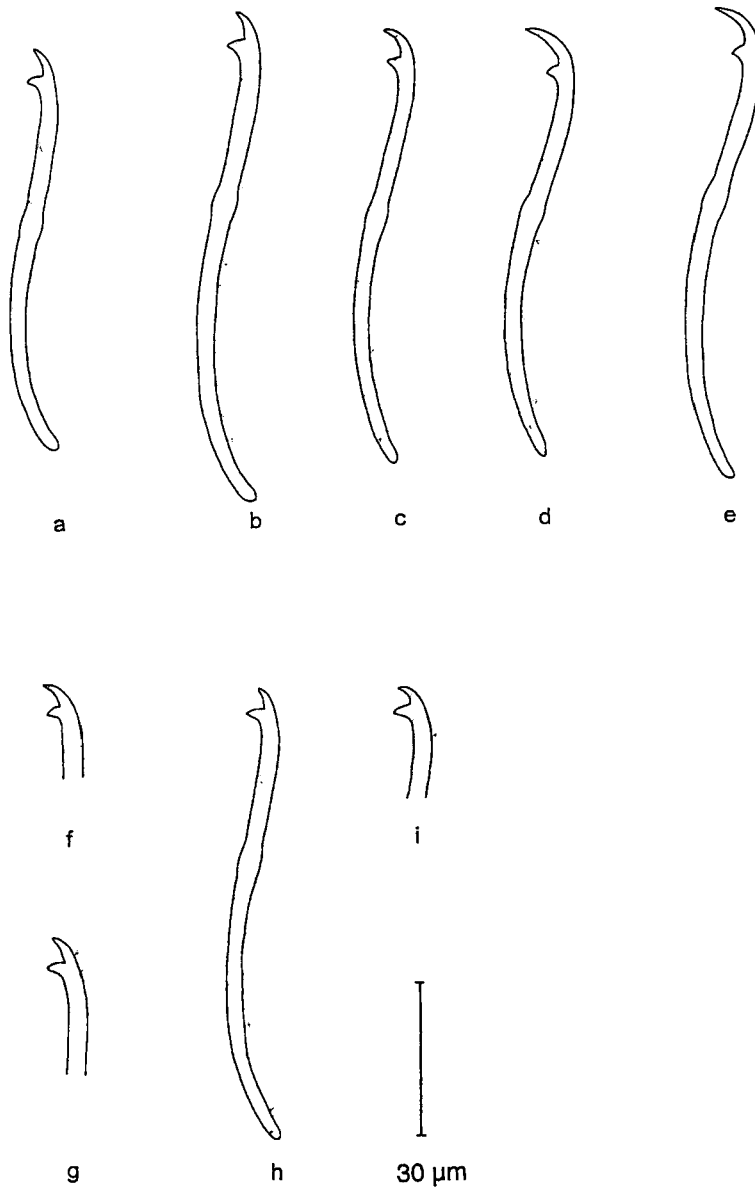
Fig. 4:
Bothrioneurum righii n.sp.
a: dorsal chaeta of III; b: dorsal chaeta of IX (larger specimen); c: dorsal chaeta of XIII; d: dorsal chaeta of XXI; e: dorsal chaeta of XLVII; f: ventral chaeta of III; g: ventral chaeta of IX; h: ventral chaeta of XXXV; i: ventral chaeta of LXVIII.