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## Three new species of *Hemienchytraeus* (Enchytraeidae, Oligochaeta) from Amazonian forest soil

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### Abstract

The species diversity of tropical enchytraeids is still largely unexplored, although knowledge of them is a *desideratum* regarding the potential of species-related data as biological soil quality indicators. Here we describe three new species of the mainly terrestrial enchytraeid oligochaete genus *Hemienchytraeus* from a primary rain forest plot at the Brazilian Agroforestry Research Facility close to Manaus, Brazil (EMBRAPA-CPAA: Empresa Brasileira de Pesquisa Agropecuária, Centro de Pesquisa Agroflorestal na Amazônia Ocidental). *Hemienchytraeus siljae*, *H. patricii*, and *H. tanjae* spp. nov. were discovered in a study of soil fauna and litter decomposition in the framework of a project series that was aimed at developing methods for sustainable land use in Amazonian rain forests (SHIFT ENV 52: Studies on Human Impact on Floodplains and Forests in the Tropics). The specimens were investigated alive and as stained whole mounts. The detailed descriptions cover almost the entire light-microscopical anatomy of the animals, following results achieved with other enchytraeid genera where it has been demonstrated that taxonomical difficulties in Enchytraeidae can be overcome by more detailed descriptions. The three new species differ among each other in at least 16 different traits. Most notable are: (1) the size difference of chaetae in terminal and preclitellar segments; (2) the branching pattern of the oesophageal appendage; (3) the number and location of secondary pharyngeal gland lobes; (4) the number and location of preclitellar nephridia; and (5) the distribution pattern of clitellar gland cells, especially on the ventral side. About half of the differentiating characters are non-sexual, which increases the possibility of identifying juvenile specimens to the species level—an important point for ecological studies. Most similar to *H. siljae* and *H. patricii* is *H. stephensoni* and most similar to *H. tanjae* is *H. bifurcatus*. The comparison of these species is made difficult by the poor original descriptions of *H. bifurcatus* and *H. stephensoni* and by an apparent overestimation of their intraspecific morphological variability in following accounts. *Hemienchytraeus tanjae* is distinguished from *H. bifurcatus* in coelomocyte size and texture, in the shape of the brain and in the absence of nephridial terminal vesicles. Furthermore, *H. bifurcatus* is considered as *species inquirenda*, due to the lack of important details in the original description and to the loss of type material. A literature-based distinction of *H. siljae* and *H. patricii* from *H. stephensoni* remains inconclusive; type specimens of *H. stephensoni* (syntype specimens of the nominal species *Enchytraeus cavicola*, *E. myrmecophilus* and *E. rangoonensis*), however, differ clearly from *H. siljae* and *H. patricii* by a ventrally fully developed clitellum and by strongly thickened preclitellar septa, among other characters.

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## Introduction

Enchytraeid oligochaete worms are a common component of the soil fauna, not only in temperate, boreal and arctic regions, but also in the tropics and subtropics. However, the diversity of enchytraeid species living at lower latitudes is still largely unexplored (Römcke 2003). For example, only three enchytraeid species (out of some 600 worldwide) are known so far from the entire Amazonas catchment area. In temperate regions, the species composition of enchytraeids gives useful information on the biological state of the soil (Graefe and Schmelz 1999; Didden 2002; Ruf et al. 2003); a similar indicator function can be assumed for enchytraeids in warmer climates. Knowledge of tropical and subtropical enchytraeid species is therefore highly desirable not only from a biodiversity perspective but also as a tool in environmental assessments, ranging from ecotoxicology over land-use effects to conservational aspects (Jänsch et al. 2005).

*Hemienchytraeus* is among the frequently cited genera in the very few faunistic or ecological studies that have been carried out on enchytraeids in these regions (e.g. Righi 1974; Christoffersen 1979; Nakamura 1984; Dash 1995; Healy 1996). *Hemienchytraeus* worms are between 3 and 15 mm long. The genus was erected by Černosvitov (1934) for species that possess: (1) always two chaetae per bundle; (2) a dorsal, tubular, bifurcating oesophageal appendage that inserts with an unpaired root behind the pharyngeal pad; (3) no further appendages or diverticula on the intestine; (4) nephridia with large anteseptale; and (5) blind-ending spermathecae devoid of diverticula—among other characters. The genus has been subdivided into the two subgenera *Hemienchytraeus* and *Cotinchytraeus* (Righi 1974); this distinction, however, is not beyond doubt, as it is based on not more than the presence or absence of secondary branches on the oesophageal appendage. We will not use the subgeneric level in the following.

Eighteen species of *Hemienchytraeus* are described worldwide, among them eight from South America. In this paper, we describe three more species of *Hemienchytraeus* from that continent, named *H. siljae*, *H. patricii*, and *H. tanjae*. They were found in soil samples taken from a primary rain forest plot at the Brazilian Agroforestry Research Facility close to Manaus, Brazil (EMBRAPA-CPAA: Empresa Brasileira de Pesquisa Agropecuária, Centro de Pesquisa Agroflorestal na Amazônia Ocidental). A series of studies has been carried out at that site with the aim of developing methods for sustainable land use in Amazonian rain forests (SHIFT: Studies on Human Impact on Floodplains and Forests in the Tropics) (Höfer et al. 2001). One of these studies (ENV 52) focused on soil fauna and litter decomposition, and several new oligochaete species were discovered: Enchytraeidae, Naididae, and one species each of Tubificidae and Glossoscolecidae. Naidid and tubificid species are described in Collado and Schmelz (2000a, 2000b, 2001, 2002), the new glossoscolecid species is described in Zicsi et al. (2001). The enchytraeid fauna at that site is dominated by *Hemienchytraeus* with regard to both species richness and abundance of specimens (Römcke and Meller 1999). *Hemienchytraeus patricii* and *H. tanjae* spp. nov. were the most abundant *Hemienchytraeus* species in the soil samples. Other species of *Hemienchytraeus* found in the soil samples will be described when more material is available. Further enchytraeid species, most of them new, belong to *Enchytraeus*, *Guaranidrilus*, *Achaeta*, and to other, probably new, genera, to be described elsewhere.

## Material and methods

The study area of the SHIFT project ENV 52 is located at km 29 on the road Manaus–Itacoatiara close to the agroforestral research station EMBRAPA-CPAA in the state of Amazonas, Brazil (Collado and Schmelz 2000a, Figure 1; Höfer et al. 2000, Figure 1). The geographical coordinates are 02°53'47"S and 59°59'45"W. The area is flat without elevations, its altitude is 50–100 m above sea level. Mean annual precipitation is 2400 mm. The area is divided into three study plots, a silvicultural plantation, a secondary forest and a primary forest adjacent to the experimental plantation. The sites from which the species are described are all located in the primary forest. It is a typical “terra firme” site, i.e. never flooded, and comprises a mixed vegetation of hundreds of different plant species. None of the approximately 40 tree species can be regarded as being dominant. The soil is a sandy acid clay (xanthic Ferrasol according to FAO typology) with 60% clay, 25% sand, and 15% silt. Other parameters of the top soil are: average pH 4.0, organic matter content 6.7–7.7%, nitrogen 0.27–0.31%, mean soil moisture 22%. Temperatures in the litter layer usually range from 25.6 to 27.7°C (min. 23.7°C, max. 34.7°C), and in the top soil from 25.7 to 26.1°C (min. 23.7°C, max. 27.7°C). Average air humidity in the litter layer is 97%. The primary forest plot of the SHIFT ENV 52 study area is the type locality of the three new species described here.

Samples (both litter and mineral soil) were taken every 3 months from July 1997 to March 1999, resulting in eight sampling dates. The type specimens are from three samples taken separately in December 1997, March 1998, and December 2000. Enchytraeids were extracted from the litter fraction using modified versions of the wet funnel technique of O'Connor (1955). The mineral soil fraction was practically without enchytraeids. Animals were investigated alive and as stained whole mounts, using a light-microscope with interference contrast (Nomarski) optics. Specimens were fixed in 70°C hot Bouin's fluid, stained with Paracarmin, passed through a graded ethanol/xylol series, and mounted whole between two coverslips in Malinol, a synthetic substitute of Canada balsam. Some specimens were anaesthetized with ethanol prior to fixation.

The new *Hemienchytraeus* species are named *H. siljae*, *H. patricii*, and *H. tanjae*, honouring Silja, Patrick and Tanja Römbke, who helped with the sampling. The descriptions combine observations on living and preserved specimens. Dimensions taken from living and preserved animals are marked by “viv” and “fix”, respectively. Fixation usually causes size reductions in all organs except the chaetae. Terminology and mode of description follow Schmelz (2003). Types are deposited at the Instituto Nacional de Pesquisas da Amazônia (INPA), Manaus, Brazil, and at the Hamburgisches Zoologisches Museum und Institut (ZIM), Germany.

Additionally, the following type material, kindly sent for loan from the Invertebrates II Section of the Natural History Museum, London, was reinvestigated, in order to clearly distinguish *H. siljae* and *H. patricii* from *H. stephensoni*: *Enchytraeus cavicola*, BMNH 1933.2.23.3.301–309, eight specimens on slides (four as whole mounts, two in longitudinal sections, one in oblique sections, one in transverse sections); *Enchytraeus myrmecophilus*, BMNH 1949.3.1.968, four specimens in sections on slides (three longitudinal, one transverse); *Enchytraeus rangoonensis*, BMNH 1933.2.23.321–323, five specimens on slides (two as whole mounts, three in longitudinal sections). All specimens are syntypes.

The following abbreviations are used in the figures: IX, X, etc., ninth, tenth segment etc.; 7/8, 8/9, etc., intersegmental region at VII/VIII, VIII/IX, etc.; ad, ectal dilatation of spermathecal ampulla; af, afferent fascicle of pharyngeal glands; br, brain; bs, bursal slit=secondary male pore; ct, connecting tube of spermathecal ampulla; dl, dorsal lobe of

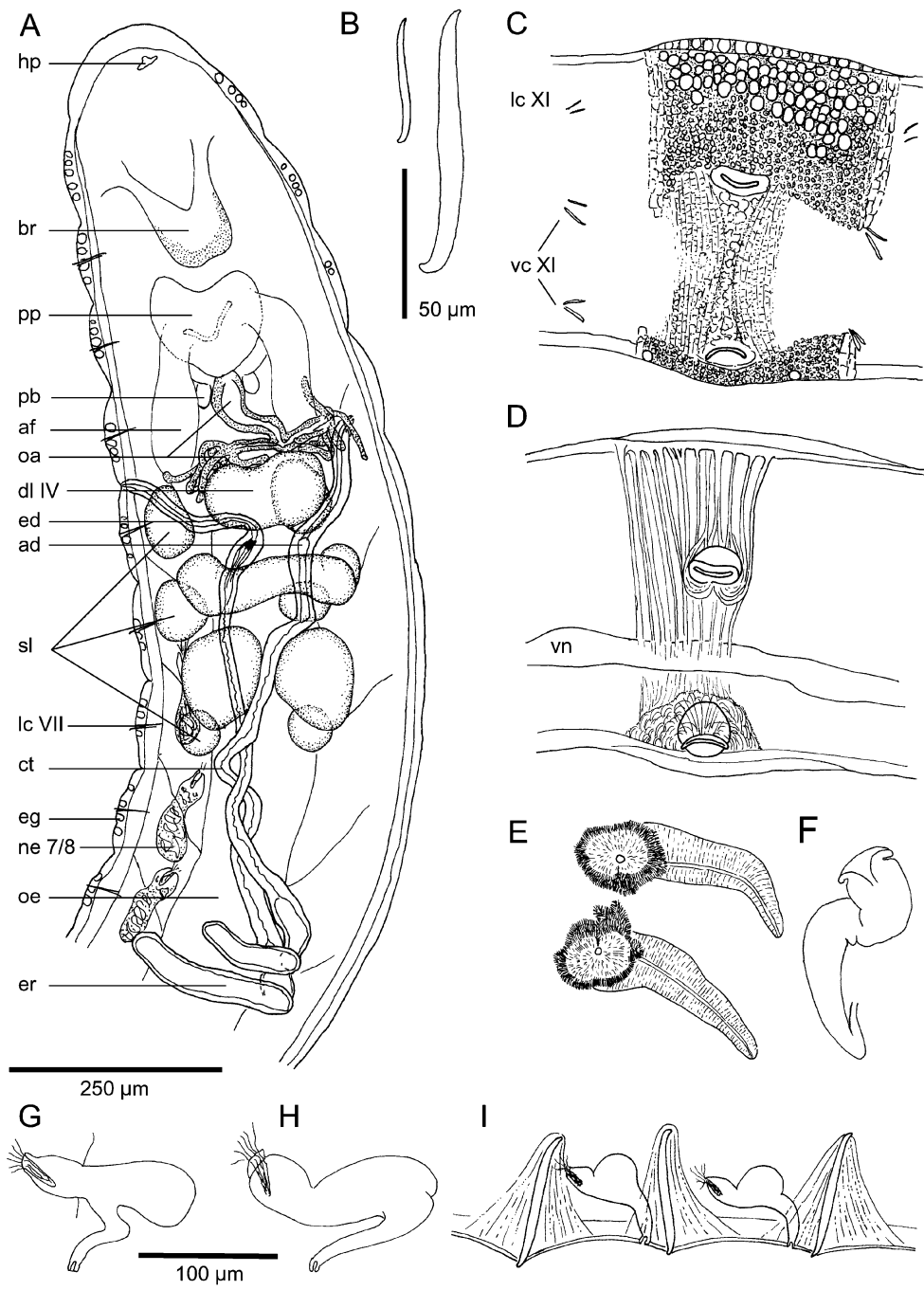


Figure 1. *Hemienchytraeus siljæ* sp. nov. (A) Anterior body region of a mature specimen, dorsal view; lateral chaetae to the right omitted; (B) lateral chaeta in IV (left), ventral chaeta, 42nd segment (right); (C) clitellum and bursal slits, ventro-lateral view; (D) male copulatory organ with bursal slit, glandular body and copulatory muscles; (E) sperm funnels with sperm nuclei attached to flared collar, sperm flagella not drawn; (F) sperm funnel, submature specimen; (G) nephridium in IX; (H) nephridium in XV; (I) nephridia in hindmost segments, ventral chaetae and chaetal muscles, lateral view. All figures from whole-mounted specimens: (C–E) drawn from same specimen. Scale bars: 250 µm (A, C–F); 50 µm (B); 100 µm (G–I).

pharyngeal gland; ed, spermathecal ectal duct; eg, epidermal gland cell; ep, spermathecal ectal pore; er, ental reservoir of spermathecal ampulla; et, layer of epidermis and transverse (=ring) muscles; gb, glandular body of male copulatory organ; hp, head pore; lc, lateral chaetae; lm, longitudinal body wall musculature; ne, nephridium; oa, oesophageal appendage; oe, oesophagus; oo, mature, yolky oocyte; ov, ovarium; pb, post-pharyngeal bulb; pl, primary ventral lobe of pharyngeal gland; pp, pharyngeal pad; sf, sperm funnel; sl, secondary ventral lobes of pharyngeal glands; te, testis; vc, ventral chaetae; vd, vas deferens; vn, ventral nerve cord.

## Descriptions

### *Hemienchytraeus siljae* sp. nov.

(Figure 1)

#### *Type material*

Holotype: INPA 121, fully mature specimen, stained and whole-mounted. Paratypes: INPA 122, one specimen, submature, with severed posterior end; ZIM OL 14281, one specimen, fully mature. All types from a sample collected in December 1997.

#### *Further material investigated*

Three mature specimens, examined alive, not conserved.

#### *Description*

Length 10–12 mm (viv) or 8–11 mm (fix), diameter (fix) ca 0.3 mm, up to 0.35 mm in XII. Segment number 43–45. Two chaetae per bundle throughout, present laterally in XII also in mature specimens. Chaetae (Figure 1B, I) slightly sigmoid, without nodulus, pointed distally, blunt proximally; ventral chaetae distinctly larger (longer and thicker) than lateral chaetae; chaetae in posterior segments much larger than chaetae in anterior segments; lateral preclitellar chaetae 37–47 µm long, 3–4 µm thick, increasing in posterior segments from 40 × 3 µm (XIII) up to 85 × 7 µm in terminal segments; ventral preclitellar chaetae 40–65 µm long and 4–4.7 µm thick; gradually increasing in posterior segments up to 90 × 9 µm in terminal segments. Head pore (Figure 1A) on prostomium in form of a small transverse slit. Epidermal gland cells (Figure 1A): in preclitellar segments, four to five segmental rows of transversely elongate, quasi-rectangular cells; most cells situated laterally, few cells mid-dorsally and mid-ventrally. First segment and part of prostomium densely covered with cells; in postclitellar segments one to three rows per segment, with fewer cells than in preclitellar segments.

Clitellum (Figure 1C) in XII–1/2 XIII, posterior end of clitellum immediately before chaetae of XIII dorsally and at level of chaetae of XIII latero-ventrally; clitellum present on all sides except in posterior half of mid-ventral part; cellular arrangement in indistinct rows to reticulate; mid-ventrally (anterior half) in dense rows, irregularly warty between bursal slits. On dorsal body half, hyalocytes and granulocytes present, hyalocytes (diameter 10–19 µm, height 16 µm, fix) larger than granulocytes (diameter 5–8 µm), the latter

inconspicuous, occupying interspaces between hyalocytes. Ventro-laterally only granulo-cytes, here clitellum thinner.

Body wall 15–25 µm thick (fix), cuticle 1–1.5 µm thick (fix), always distinct at  $\times 250$  magnification, longitudinal muscle layer well developed, 10–20 µm thick. Preclitellar septa not thickened. Brain (Figure 1A) about as long as wide, or shorter (measured dimensions:  $66 \times 66$  µm and  $70 \times 82$  µm, fix), deeply incised anteriorly, slightly indented posteriorly, sides parallel. Post-pharyngeal bulbs (Figure 1A, pb) conspicuous. Ventral nerve cord perikarya concentrated in segmental ganglia from V on, i.e. no perikarya in the region of the septa. Oesophageal appendage (Figure 1A) with long unpaired root, reaching pharyngeal glands of IV, short primary branches and about four to five elongate secondary branches on each side. Large proximal chamber present. Primary branches thinner than unpaired trunk and thicker than secondary branches. Pharyngeal glands (Figure 1A) widely connected dorsally in IV, with narrow dorsal connection or separate in V, glands dorsally separate in VI. Three pairs of secondary ventral lobes, in V, VI, and VII, smallest in VII. Afferent fascicles (Figure 1A, af) comparatively thick. Chloragocytes from V. Dorsal vessel from XIV to XV. Inflated ventral gut epithelium from XXIII to XXX, extending over six or seven segments.

Nephridia (Figure 1A, G–I). Preclitellar segments: four pairs, in 6/7–9/10, anteseptale globular, with minute and numerous brownish granules at periphery; funnel orientated obliquely ventrad, with small and narrow anterior projection; postseptale twice as long as anteseptale (total lengths measured 85–116 µm, fix), adseptal to medial origin of efferent duct. Postclitellar segments: first pair at 14/15; number of nephridia reduced in mid-body region, slightly larger than preclitellar nephridia (length ca 140 µm, fix); nephridia in terminal segments more numerous, small, about half as long (ca 50–55 µm, fix) as preclitellar nephridia; postseptale compressed, bulged dorsad, rise of efferent duct terminal. Coelomocytes flat and broadly oval, with central nucleus and finely granular matrix (viv), 15–30 µm long (fix), large cells  $1.5 \times$  as long as wide, small cells with circular outline.

Seminal vesicle very small or absent; a small, dense, conspicuous aggregation of developing sperm present around the sperm funnel collars. Sperm funnel (Figure 1E, F) about as long as body diameter,  $4\text{--}6 \times$  as long as wide (viv); funnel body cone-shaped, gradually and regularly tapering distad (e.g. from 65 to 20 µm, fix), broadly oval in cross-section, not circular; collar flared, wider than funnel body in mature specimens (up to  $1.5 \times$  as wide), outline wavy, irregular, like a brim, conspicuous by dense masses of attached spermatozoa. Spermatozoa at least 125 µm long (viv), head lengths not measured. Vasa deferentia in XII, in loose or tight irregular coils, diameter tapering from 13 proximally to 8 µm distally, entering male copulatory organ dorsally. Male copulatory organs with strongly developed musculature. Male glandular body globular, diameter ca 55 µm (fix). Bursa laterally flattened, short, extending halfway dorsad into glandular body; bursal slit (Figure 1C, D) longitudinal, slightly bent, tips curved outward. Musculature strongly developed (Figure 1D), surrounding glandular body and obscuring its outline in living specimens; muscle system complicated, extending over entire segment of XII; dense aggregations of strands anteriorly, posteriorly, laterally and dorso-laterally of glandular bulb; orientation of strands mainly from dorso-lateral to mid-ventral; some strands connecting glandular body and body wall, some strands connecting dorso-lateral and mid-ventral side of body wall in a regular pattern. In two specimens, aggregations of hyaline bodies of varying size and with smooth outline interspersed between the muscular strand concentrations. No accessory copulatory glands present.

Spermathecae (Figure 1A) not attached to oesophagus, extending into VI, VII or VIII, each consisting of ectal duct and ampulla, the latter subdivided into ectal dilatation, connecting tube and ental reservoir. No ectal gland, epidermis thickened in a small circular field (diameter twice the ectal duct diameter) around ectal pore; thickening projecting inside. Ectal pore 3 µm wide (fix), distalmost stretch of ectal duct canal 4–5 µm wide (fix), lined with cuticle for about 25 µm; further proximally, cuticle light-microscopically not distinguishable; canal often inconspicuous here; ectal duct about one segment length long; ectal dilatation of ampulla 16–30 µm wide (fix), ca twice as wide as ectal duct; connecting tube about as wide as ectal duct; ental reservoir thin-walled, usually bent dorsad, of varying length (one or two segments) and width (35–80 µm). Sperm present in ectal dilatation and ental reservoir; in ectal dilatation, sperm arranged side-by-side in a wisp with nuclei orientated distad and flagella extending straight into proximal tube; in ental reservoir, spermatozoa arranged in a dense and irregular coil. One mature egg at a time.

#### Remarks

One paratype specimen has slightly reduced pharyngeal glands in VI.

### *Hemienchytraeus patricii* sp. nov. (Figure 2)

#### Type material

Holotype: INPA 123, mature specimen, coll. December 1997. Paratypes: INPA 124, 13 specimens, 11 mature, two submature, coll. December 1997. ZIM OL 14282, 13 specimens, 11 mature, two submature, coll. December 1997 (eight specimens), March 1998 (five specimens). All types stained and whole-mounted.

#### Further material investigated

Three mature specimens, ZIM OL 14283, stained whole mounts, with abnormal pharyngeal glands, one of them infected by parasites.

#### Description

Slim and active worms, body transparent, due to thin body wall and sparse coelomocytes. Body length ca 7–8 mm (viv) or 5–7 mm (fix); diameter 0.2–0.23(–0.25) mm (viv, fix). Segment number (28)–31–34–(35). Two chaetae per bundle throughout, present laterally in XII also in mature specimens. Chaetae sigmoid without nodulus, pointed distally, blunt proximally, proximal bend stronger than distal curve. Chaetae in preclitellar bundles 25–35 µm long, smallest in II, gradually increasing in length posteriad, diameter ca 3 µm; lateral and ventral chaetae of equal size. Chaetae in postclitellar bundles gradually increasing in size towards rear end, size increase much more pronounced in ventral than in lateral bundles. In terminal segments ventral chaetae 70–80 µm long and 7–8 µm thick, lateral chaetae ca 50 µm long and 5 µm thick. Head pore (Figure 2A, B) on prostomium in form of a small transverse slit. Epidermal gland cells numerous in first four segments including prostomium; cells in up to seven rows per segment, cells transversely elongate except at chaetal level; here cells about as long as wide, larger than in adjacent rows;



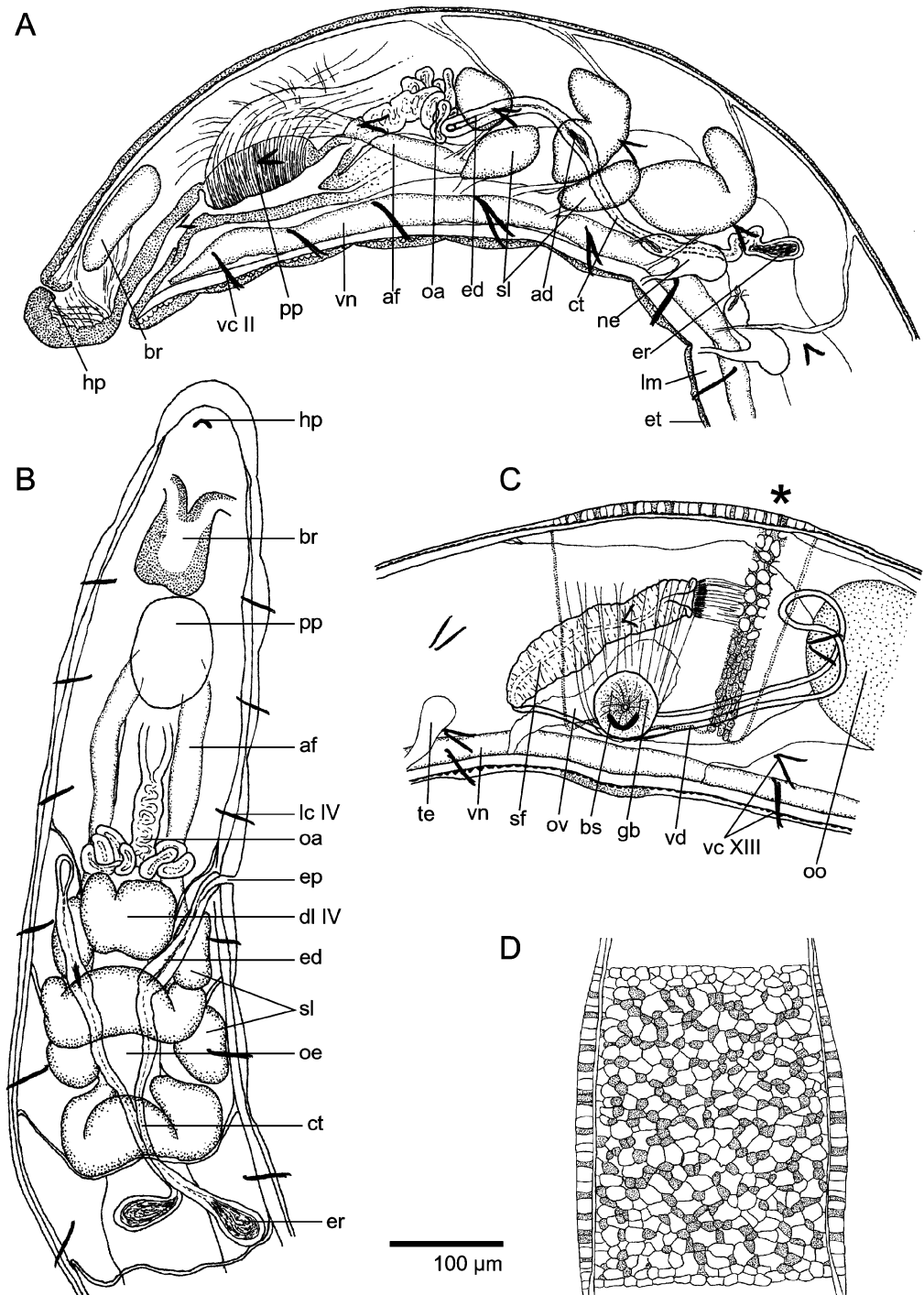


Figure 2. *Hemienchytraeus patricii* sp. nov. (A) Anterior body region, lateral view; (B) anterior body region, dorsal view; (C) segments XI–XIII, clitellar region, ventro-lateral view; double-dotted line: borders of clitellum; field below asterisk: detail of clitellum, showing gland cell distribution; (D) clitellum, dorsal view. (A–C) From whole-mounted specimens; (D) from live photograph. Scale bar: 100 µm.

posteriorly fewer cells, quasi-rectangular. No cells mid-ventrally in II–IV; from V on quasi-rectangular square cells present.

Clitellum (Figure 2C, D) short, covering little more than one segment length: anterior margin behind septum 11/12, immediately before male copulatory organ, posterior margin at some distance before chaetae of XIII. Clitellum saddle-shaped, not developed mid-ventrally. Ventral clitellar margins (i.e. the width of the cell-free ventral area) marked by the bursal slits. Cellular arrangement in two distinct patterns. (1) In dorsal half, down to the longitudinal level of lateral chaetae, hyalocytes and granulocytes present. Hyalocytes  $2\text{--}3 \times$  as large (diameter) as granulocytes, but not more numerous than the latter; cellular arrangement reticulate, no transverse orientation. Cells about as high as wide, higher than wide when fully developed ( $12\text{--}18\text{ }\mu\text{m}$ , fix). (2) Ventro-laterally, in a field between the longitudinal level of lateral chaetae to that of bursal slits, only granulocytes present, cellular arrangement in transverse rows, cells less elevated than in dorsal body half. Each bursal slit surrounded by a field with stronger staining nuclei; this area not marked in living specimens.

Body wall usually  $6\text{--}12\text{ }\mu\text{m}$  thick (viv, fix), cuticle thin, often invisible. Body wall thicker laterally and ventrally in anterior seven segments and ventrally in terminal segments with enlarged chaetae, here up to  $25\text{--}30\text{ }\mu\text{m}$  thick (fix). Preclitellar septa not thickened. Brain (Figure 2A, B) about as long as wide or longer ( $55\text{--}77\text{ }\mu\text{m} \times 48\text{--}64\text{ }\mu\text{m}$ , fix), incised anteriorly, sides parallel or slightly converging anteriad, posteriorly slightly indented or truncate. Post-pharyngeal bulbs inconspicuous. Ventral nerve cord perikarya concentrated in segmental ganglia from V on, i.e. no perikarya in the region of the septa. Oesophageal appendage (Figure 2A, B): unpaired root with large proximal chamber; following section longer than proximal chamber, with thick, meandering canal; first bifurcation into primary branches immediately anteriorly of dorsal pharyngeal glands of IV; primary branches shorter than unpaired root; numerous secondary branches of different length and thickness, difficult to distinguish, often compacted into a thickened cauliflower-like mass. Pharyngeal glands (Figure 2A, B) in IV–VI, with single unpaired dorsal lobe in each segment, and primary ventral lobes in V and VI; small secondary ventral lobes present in V and VI. Dorsal lobe large in IV and V, smaller in VI, connection between dorsal lobe and ventral lobes wide in V, narrow in VI. Primary ventral lobes in VI distinctly projecting anteriad, ventral lobes in V not or only slightly projecting anteriad, compared to dorsal lobe. Chloragocytes flat, beginning in V, absent in XI and XII. Dorsal blood vessel originating in XIII–XIV. Gut widening gradually. Inflated ventral gut epithelium in  $1/2\text{ XX--}1/2\text{ XXV}$ , extending over two to four, usually three, segments. Cells filled with vesicles apically, of same colour as intestinal content.

Nephridia (Figure 2A). Preclitellar segments: three pairs, from 6/7 to 8/9; anteseptale globular, ca half as long as postseptale (total length ca  $95\text{ }\mu\text{m}$ , fix), adseptale to subterminal rise of efferent duct; terminal vesicle inconspicuous or absent. Postclitellar segments: first nephridium at 14/15, mid-body region with very few nephridia, elongate, terminal rise of efferent duct; in terminal segments nephridia shorter (ca two-thirds as long as preclitellar nephridia), with subterminal rise of efferent duct. Coelomocytes sparse but readily visible and present throughout the body; cells not in aggregations, most of coelom free. Cells flat, ellipsoid, about twice as long as wide, with very fine, regular, pale granulation (viv); granules much smaller than chloragocyte vesicles. Central nucleus visible; cell periphery often framed by a distinct hyaline margin.

No seminal vesicle, stages of developing sperm free in XI, scattered, not numerous, not compacted, not coloured. Mature spermatozoa as dense brush on top of sperm funnel

collars, spermatozoa short, exact length not measured. Sperm funnels (Figure 2C)  $6-8 \times$  as long as wide, as long as or longer than body diameter (viv), shorter in fixed material; funnel body not wider than collar, usually tapering distad, but often with constrictions or areas with equal diameter; distal end attached to ventral part of septum 11/12. Vas deferens (Figure 2C) of medium length, confined to XII, in large loose coils, or in numerous regular narrow coils of equal diameter. Male copulatory organ (Figure 2C) with bursa, glandular bulb and surrounding musculature. Bursa and gland compact, roughly spherical, diameter ca  $50 \mu\text{m}$ , e.g.  $55 \mu\text{m}$  long,  $50 \mu\text{m}$  wide,  $40 \mu\text{m}$  high (fix). Vas deferens piercing glandular bulb centrally; surrounding gland cells finely granulated centrally, in aster- or modiolus-like arrangement around cuticularized terminus of vas deferens, distinct in living specimens. Bursal slit mainly longitudinal, staple-shaped, bursa not deep, often with central fold in lateral wall. No accessory copulatory glands present.

Spermatheca (Figure 1A, B) free, not attached to oesophagus, extending posteriorly into VII or VIII, consisting of ectal duct and ampulla, the latter subdivided into ectal dilatation, connecting tube and ental reservoir. No ectal gland, but epidermis thickened in a circular field around ectal pore, here occasionally foreign matter adhering to body surface. Ectal pore in intersegmental furrow 4/5, in lateral line slightly ventrally of lateral chaetae; pore ca  $3 \mu\text{m}$  wide in fixed specimens, cuticular lining of ectal duct canal visible for ca  $15 \mu\text{m}$ ; later on cuticle absent or very thin. Ectal duct about one segment length long; ampullar ectal dilatation (Figure 2A, B: ed) ca twice as wide as ectal duct, thick-walled, spermatozoa in longitudinal and roughly parallel arrangement, orientated distally, entirely filling out the narrow lumen of the dilatation; connecting tube narrower than ectal duct, widening into thin-walled ental reservoir filled with sperm in irregular arrangement. In living specimens, canal visible throughout, from ectal pore to ental reservoir; in fixed specimens, canal only visible in most distal, cuticularized, stretch of ectal duct. One mature egg at a time.

#### Remarks

Three specimens of *H. patricii* have not been included in the type series because of malformed or reduced pharyngeal glands. In one of these specimens the glands are infected by microcystid cysts.

#### *Hemienchytraeus tanjae* sp. nov.

(Figure 3)

#### Type material

Holotype: INPA 125, stained and whole-mounted specimen, fully mature, coll. December 2000. Paratypes: INPA 126, four specimens, coll. December 1997 (four specimens); ZIM OL 14284, four specimens, coll. December 1997 (one specimen), December 2000 (three specimens). All paratypes stained and whole mounted, fully mature.

#### Description

Length ca  $5 \text{ mm}$  (viv) or  $4-5 \text{ mm}$  (fix), diameter  $0.125 \text{ mm}$  (viv) or ca  $0.12 \text{ mm}$ , up to  $0.15 \text{ mm}$  in XII/XIII, down to  $0.1 \text{ mm}$  in terminal segments (fix). Segment number 31–34. Segments following clitellum  $2-3 \times$  as long as wide when stretched, segments shortening

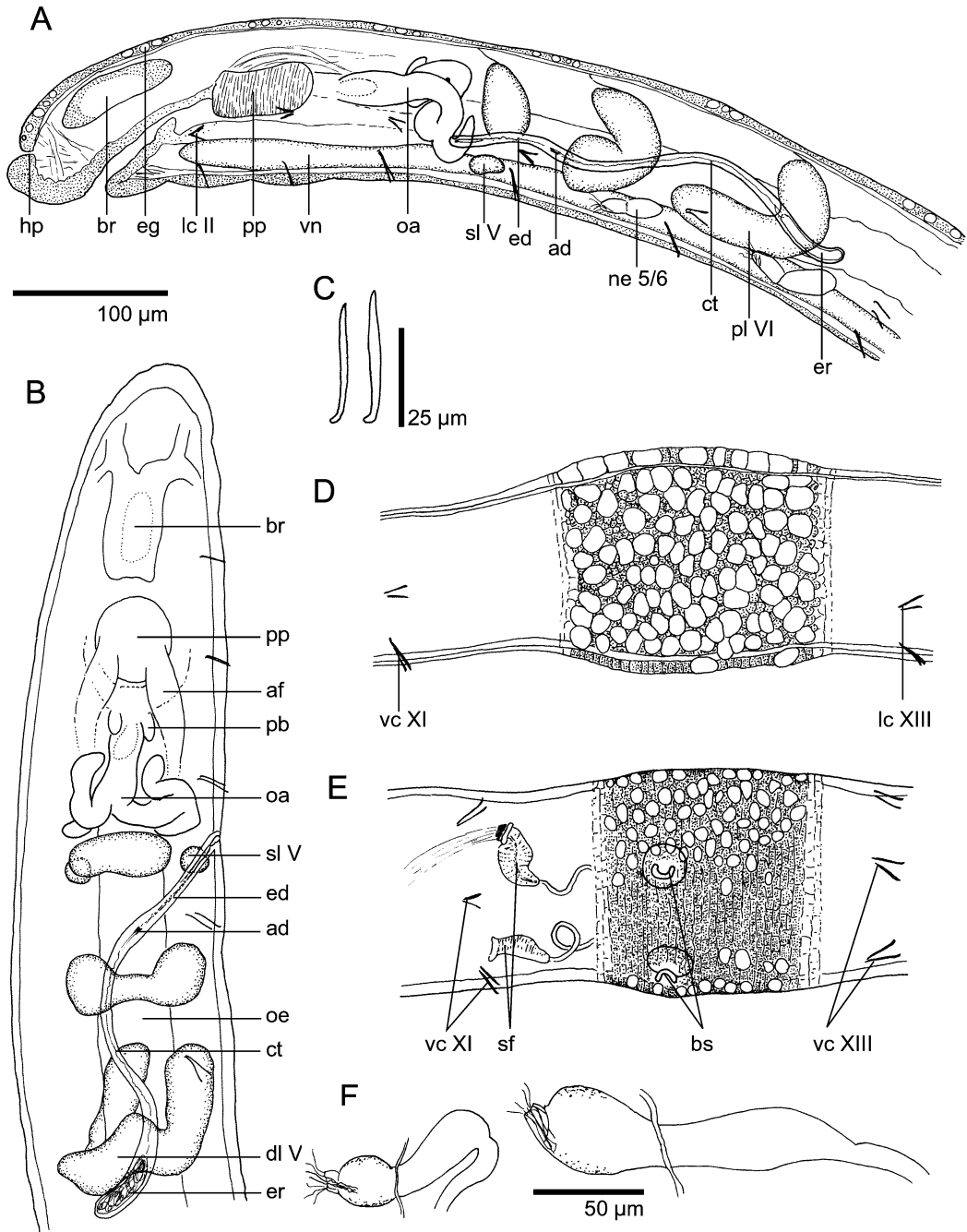


Figure 3. *Hemienchytraeus tanjae* sp. nov. (A) Anterior body region, lateral view; (B) anterior body region, dorsal view; lateral chaetae and spermatheca to the left not shown; (C) chaetae in preclitellar (left) and in terminal (right) segment; (D) clitellum, dorsal view; (E) clitellum and male reproductive system, ventral view. Note size difference of hyalocytes between (D) and (E): (E) is drawn from slightly less mature specimen (egg not fully developed); (F) nephridia in preclitellar (left) and terminal (right) segments. All figures from stained whole mounts. Scale bars: 100 µm (A, B, D, E); 25 µm (C); 50 µm (F).

towards posterior end. Two chaetae per bundle throughout, present laterally in XII also in mature specimens. Chaetae without nodulus, pointed distally, blunt proximally, only faintly sigmoid, distal curve rather slight, often inconspicuous (Figure 3C). Chaetae only slightly enlarged in posterior segments; ventrals 28 µm long and 2.5 µm thick in anterior segments, 33 µm long and 3 µm thick in terminal segments. Head pore (Figure 3A) on prostomium in form of a small transverse slit. Epidermal gland cells in three to four rows in preclitellar segments, more cells on dorsal half than on ventral half; in posterior segments one row per segment at chaetal level.

Clitellum (Figure 3D, E) girdle-shaped, i.e. fully developed ventrally, and short, i.e. extending over little more than one segment length, ending at some distance before chaetae of XIII; cellular arrangement reticulate or in indefinite rows on all sides except ventrally; hyalocytes about twice as wide (diameter ca 20 µm, *viv*) as granulocytes (diameter ca 12 µm, *viv*); difference more marked in fixed specimens. Hyalocytes largest in specimens with fully developed egg (Figure 3D). Ventrally, in a field as wide as the distance between bursal slits, only granulocytes, arranged in dense transverse rows, clitellum less elevated here.

Body wall usually 4–5 µm thick, up to 10 µm ventrally in preclitellar segments (*fix*), cuticle thin (<1 µm), preclitellar septa not thickened. Brain (Figure 3A, B) ca twice as long as wide (e.g. 65 µm × 35 µm, *fix*), deeply incised anteriorly, slightly indented posteriorly, sides parallel or slightly converging anteriorly. Post-pharyngeal bulbs conspicuous (Figure 3B). Ventral nerve cord perikarya concentrated in segmental ganglia from V on, i.e. no perikarya in the region of the septa. Oesophageal appendage (Figure 3A, B): unpaired root with moderately large proximal chamber; root and primary branches of about equal length and equal canal width. Two secondary branches on each side, short, with narrow canal lumen. Diameter of appendage decreasing from unpaired root over primary to secondary branches. Pharyngeal glands (Figure 3A, B) all united dorsally in IV, V and VI, connection widest in IV, narrow in VI; primary ventral lobes in V and VI, secondary ventral lobes in V, small, attached to septum 4/5, apparently absent in one specimen. Efferent fascicles (Figure 3B) not conspicuously enlarged. Chloragocytes as a thin layer from V, filled with light brown vesicles (same colour as gut contents). Dorsal blood vessel from XIV. Inflated ventral gut epithelium inconspicuous, from 1/2 XIX–XXI, only slightly higher than epithelium in adjacent anterior and posterior segments, higher than dorsal epithelium in same segments, recognizable mainly by granular texture and less intensive staining in fixed specimens.

Nephridia (Figure 3A, F) in preclitellar segments: four pairs, from 5/6 to 8/9; anteseptale globular, with minute and numerous brownish granules at periphery; funnel orientated obliquely ventrad, with small and narrow anterior projection; postseptale elongate, ca twice (1.7–2.5 ×) as long and about as high as anteseptale; length of nephridia 50–85 µm (*fix*), mostly subterminal rise of efferent duct (also medial and terminal); terminal vesicle small, inconspicuous. Postclitellar segments: first pair at 14/15, nephridia reduced in number in following segments, often unpaired, many segments without nephridia; nephridia in posterior body half larger than in preclitellar segments, longer (up to 140 µm, *viv*) than body diameter, shape as in preclitellar segments, with mostly terminal rise of efferent duct. Coelomocytes small, length ca 15 µm (*viv*, *fix*: 12–16 µm), longer than wide; colour pale, margin wavy, texture with blurred vesicles (*viv*); cells not numerous, often aggregations postero-dorsally in V, VI and VII and in terminal segments.

Seminal vesicle absent. Few sperm on top of sperm funnel collar, spermatozoa longer than funnel (ca 70 µm, *fix*), heads short. Sperm funnel (Figure 3E) small, ca twice as long

as wide, less than half as long as body diameter (e.g.  $53 \times 22 \mu\text{m}$ , viv,  $50 \times 19 \mu\text{m}$ , fix), funnel body as wide as or narrower than collar, tapering distad. Vas deferens in XII, usually coiled in small, irregular, consecutive loops ventro-laterally; diameter  $3 \mu\text{m}$  (fix). Male copulatory organ (Figure 3E): glandular body globular, diameter  $24\text{--}28 \mu\text{m}$  (fix), bursal slit longitudinal (viv), staple-shaped or broadly U-shaped in fixed specimens, shorter than diameter of glandular body. Accessory copulatory glands absent.

Spermatheca (Figure 3A, B) inconspicuous, often difficult to see (viv, fix), extending into VI or VII, consisting of ectal duct and ampulla, the latter subdivided into ectal dilatation, connecting tube and ental reservoir. Ectal dilatation not seen in all specimens, here ectal duct continuously merging into connecting tube. Ectal duct ca one segment length long, canal and wall epithelia of about the same diameter; ectal pore  $2\text{--}3 \mu\text{m}$  wide (fix), up to  $4 \mu\text{m}$  in living specimens, canal lined with cuticle distally, cuticle thinning out proximad. Ectal dilatation of ampulla  $10\text{--}12 \mu\text{m}$  wide (fix), connecting tube with inconspicuous canal, ental reservoir thin-walled,  $13\text{--}15 \mu\text{m}$  wide (fix). Sperm present in ectal dilatation and ental reservoir; in ectal dilatation, sperm arranged side-by-side in a wisp with nuclei orientated distad and flagella extending straight into proximal tube; in ental reservoir, spermatozoa arranged in a dense and irregular coil. One mature egg at a time, extending over two to three segments when fully developed.

### Remarks

The secondary pharyngeal gland lobes are quite small, and apparently absent (i.e. not seen) in one of the paratype specimens. Other characters (especially details of oesophageal appendages, nephridia, and clitellum) are without exception. Two further specimens found at the type locality, one submature, one juvenile, agree with the diagnosis of *H. tanjae* in the pattern of pharyngeal glands (one pair of secondary lobes in V) and in the distribution of preclitellar nephridia (four pairs, from 5/6 to 8/9). However, the secondary pharyngeal gland lobes are large and conspicuous, terminal chaetae are almost twice as large as preclitellar chaetae and the submature specimen is larger (body diameter  $0.225 \text{ mm}$ , fix) than mature specimens of *H. tanjae*. In view of the clearcut differences it would seem unjustified to include these specimens into *H. tanjae*. Their identity must remain uncertain until more and mature material has been found.

## Discussion

### Species comparison

*Hemienchytraeus siljae*, *H. patricii*, and *H. tanjae* are clearly separable from each other; Table I lists conspicuous differentiating characters. Likewise, the majority of the 18 presently described *Hemienchytraeus* species differ in several characters from each of the three new species. This is demonstrated in Table II, where only those differentiating traits are listed that apply to *H. siljae*, *H. patricii*, and *H. tanjae* alike; actually, the number of differences is higher. Only two species require a detailed discussion, *H. bifurcatus* Nielsen and Christensen, 1959 for its similarity with *H. tanjae*, and *H. stephensoni* (Cognetti, 1927) for its similarity with *H. siljae* and *H. patricii*.

*Hemienchytraeus stephensoni* and *H. bifurcatus* are the only species in the genus with several records after the original description. Both appear to be cosmopolitan. *Hemienchytraeus bifurcatus*, originally described from Denmark (Nielsen and Christensen 1959), has been recorded from Poland (Makulec 1983), France (Healy 1980), India (Dash

Table I. Characters distinguishing *H. siljae*, *H. patricii*, and *H. tanjae*.

	<i>H. siljae</i>	<i>H. patricii</i>	<i>H. tanjae</i>
Body dimensions (fix)	ca 10 × 0.3 mm	ca 6 × 0.2 mm	5 × 0.12 mm
Segment number	43–45	Mostly 31–34	31–34
Chaetae in hindmost segments	Almost twice as large as largest preclitellar chaetae	More than twice as large as largest preclitellar chaetae	Only slightly larger (1.2 ×) than preclitellar chaetae
Brain	As long as wide	Slightly longer than wide	Twice as long as wide
Oesophageal appendage: no. secondary branches	4–5 each	4–5 each	2 each
Secondary pharyngeal gland lobes	3 pairs, in V, VI, VII	2 pairs, in V, VI	1 pair, in V, or absent
Ventrally inflated gut epithelium	XXIII–XXX, 6–7 segments	1/2 XX–1/2 XXV, 2–4 segments	Inconspicuous, 1/2 XIX–XXII
Preclitellar nephridia	4 pairs, in 6/7–9/10	3 pairs, in 6/7–8/9	4 pairs, in 5/6–8/9
Nephridia in terminal segments	Very small, 1/2 as long as preclitellar nephridia	Small, 2/3 as long as preclitellar nephridia	Large, 5/3 as long as preclitellar nephridia
Clitellum			
Length	1 1/2 segments	1 segment	1 segment
Mid-ventrally	Present anteriorly, absent posteriorly	Absent	Present throughout
Sperm funnel			
Length	As long as body diameter	Longer than body diameter	Shorter than 1/2 body diameter
Length: width	4–6:1	6–8:1	2:1
Shape	Cone	Cone/cylindrical	Cylindrical
Collar	Wider than funnel body, flared	About as wide as funnel body, not flared	About as wide as funnel body, not flared
Male copulatory muscles	Strongly developed	Medium	Weakly developed

1983, p 121), Japan (Nakamura 1984) and Florida (Healy 1996); the latter two papers include morphological notes. *Hemienchytraeus stephensoni* consists actually of three nominal species: (1) *Enchytraeus cavicola* Stephenson, 1924 from India (renamed *E. stephensoni* by Cognetti (1927) because *cavicola* was preoccupied), (2) *E. myrmecophilus* Černosvitov, 1930, from Argentina, and (3) *E. rangoonensis* Stephenson, 1931 from Burma. Černosvitov (1934), recognizing their similarity and at the same time their differences from other previously described enchytraeid species, erected a new genus for them and united them into a single species in the same paper, as *Hemienchytraeus stephensoni*. Further records of the species are from Lake Titicaca (Černosvitov 1939), the isle of Madeira (Bell 1962), Brazil (Christoffersen 1979) including Amazonia (Righi 1981), Japan (Nakamura 1984), Ecuador (Dózsa-Farkas 1989), Florida (Healy 1989, 1996), and China (Xie et al. 1999). All papers mentioned provide more or less detailed morphological notes, those of Bell (1962), Righi (1981) and Healy (1989) excepted.

Curiously, *H. bifurcatus* and *H. stephensoni* are also the only *Hemienchytraeus* species in the genus that have been considered as morphologically highly variable [see Černosvitov (1934, 1939) and Healy (1996) for *H. stephensoni* and Healy (1996) for *H. bifurcatus*]. In fact, the range of variation of the species has expanded with almost each redescription and includes characters that in our material are intraspecifically constant and species-distinguishing. There is more variation in *H. stephensoni* than in *H. bifurcatus*, which coincides with the fact that the former has been redescribed more often. Christoffersen (1979) even distinguished four different morphological variants in Brazilian specimens. If

Table II. Comparison of *Hemienchytraeus* species with *H. siljae*, *H. patricii*, and *H. tanjae*.

	Characters differing from <i>H. siljae</i> , <i>H. patricii</i> , and <i>H. tanjae</i> , respectively
<i>H. africanus</i> Černosvitov, 1935 [Kenya/Uganda]	(1) Body length up to 17 mm (2) More than 50 segments (3) Nephridial postseptale bilobed, i.e. with a deep dorsal kerb (4) Ental reservoir of spermathecal ampulla very large and thick (Characters derived from Michaelsen 1914; Černosvitov 1938)
<i>H. brachytheus</i> Xie, Wang and Liang, 1999 [China]	(1) Oesophageal appendage with two short secondary branches on each side, branching again into 3–4 tertiary branches (2) Five preclitellar pairs of nephridia, from 6/7 to 10/11 (3) Spermathecae short, not extending beyond segment V
<i>H. brasiliensis</i> (Cognetti, 1900) [Brazil]	(1) 27 segments (2) Male copulatory organ without glandular bulb (Very poorly described species <i>incertae sedis</i> : description based on only one specimen, probably not mature: clitellum and eggs not dealt with; genus-diagnostic character of <i>Hemienchytraeus</i> (oesophageal appendage) not dealt with; description fits several other genera as well)
<i>H. cipoensis</i> Righi, 1973 [Brazil]	(1) Oesophageal appendage without secondary branches (2) Clitellar gland cells arranged in regular transverse rows throughout (3) Male copulatory organ absent
<i>H. csuzdii</i> Dózsa-Farkas, 1989 [Ecuador]	(1) Body dimensions (length 2.5–4 mm, 24–28 segments) (2) Spermatheca short, not extending beyond segment V (3) Clitellar gland cells arranged in ca 30 transverse rows
<i>H. guineanus</i> Omodeo, 1958 [Guinea]	(1) More than 50 segments (2) Vas deferens extending beyond clitellum, as far backward as segment XV (3) Male copulatory organ with minute glandular body (4) Sperm in ental reservoir of spermathecal ampulla arranged in many discrete, regularly oval packages
<i>H. inversus</i> Omodeo, 1958 [Guinea]	(1) First pair of nephridia at 4/5 (2) Spermathecal ectal duct canal dilated, sperm-containing (The peculiarity that gave the name, the backward orientation of the sperm funnels, is not a taxonomic character; a reversal of orientation is frequently observed in living enchytraeids)
<i>H. khallikotosus</i> Dash and Thambi, 1978 [India]	(1) Oesophageal appendage without secondary branches (2) Pharyngeal glands separate dorsally in IV, V and VI [The “many-layered” clitellum (Dash and Thambi 1978, p 131) is doubtful]
<i>H. loksai</i> Dózsa-Farkas, 1989 [Ecuador]	(1) Oesophageal appendage with secondary and tertiary branches (comp. <i>H. brachytheus</i> ) (2) Clitellum arranged in transverse rows (3) Vas deferens extending backwards into XIV–XVIII
<i>H. makusi</i> Righi, 1988 [Venezuela]	(1) Body length 15–17 mm (2) Segment number >50 (3) Seminal vesicle very large, extending over 3–5 segments (4) Ental reservoir of spermathecal ampulla half as thick as body diameter (5) Sperm in ental reservoir of ampulla arranged in discrete, digitiform packages
<i>H. mauriliae</i> Righi, 1981 [Ecuador]	(1) Septa 4/5–9/10 strongly thickened (2) Five pairs of preclitellar nephridia (3) Nephridial anteseptale slightly longer than postseptale
<i>H. planisetosus</i> Xie, Wang and Liang, 1999 [China]	(1) Anterior chaetae with flattened and widened distal tips (2) Five pairs of preclitellar nephridia (The coelomocytes are said to be spindle-shaped, but this may refer to cells as seen in oblique position.)



Table II. (Continued.)

	Characters differing from <i>H. siljae</i> , <i>H. patricii</i> , and <i>H. tanjae</i> , respectively
<i>H. rixae</i> Righi, 1974 [Brazil]	(1) Oesophageal appendage without secondary branches (2) Clitellar gland cells in regular rows (3) Male copulatory organ conical
<i>H. shirensis</i> Bell, 1954 [Kenya]	(1) More than 50 segments (2) Body wall very thick, 1/4 of worm diameter (3) Clitellum extending over 2 segment lengths, from 1/2 XI–1/2 XIII
<i>H. solimoensis</i> Righi, 1978 [Brazil]	(1) Coelomocytes very small (diameter 9 µm in fixed specimens) (2) Spermatheca small, confined to segment V
<i>H. theae</i> Prabhuo, 1960 [India]	(1) Oesophageal appendage without secondary branches (2) Collar of sperm funnel narrow, less than half as wide as funnel body

Species are listed in alphabetical order. The country of origin (type locality) is added in square brackets. The differences listed in the second column are derived from the original descriptions unless stated otherwise; they apply to all three new species alike. Taxonomic comments are added when necessary. All nominal species of *Hemienchytraeus* are listed, except *H. bifurcatus* and *H. stephensoni* with its junior synonyms. They are dealt with separately in the text.

we followed this trend, *H. siljae* and *H. patricii* would rank as just two more variants of the polymorphic *H. stephensoni*, and *H. tanjae* would be lumped into *H. bifurcatus*. However, Christoffersen (1979) himself, and also Dózsa-Farkas (1989), already pointed to the possibility that *H. stephensoni*, as presently conceived, is a group of species rather than a single polymorphic species; the same may apply to *H. bifurcatus*. Regarding the character distribution in our material, it seems that the morphological variability of at least *H. stephensoni* has been overestimated in the previous literature.

An evidently needed complete taxonomic revision of *H. stephensoni* and *H. bifurcatus* would go beyond the scope of this paper. It is not necessary here, either, our only objective being the demonstration that *H. siljae* and *H. patricii* are not junior synonyms of *H. stephensoni* and that *H. tanjae* is not a junior synonym of *H. bifurcatus*. This is achieved by naming taxonomic differences derived from the original descriptions and name-bearing types. Such differences, if present, confirm the validity of the three new species irrespective of the taxonomic status of *H. stephensoni* or *H. bifurcatus*. All accounts of the species since the original descriptions will be disregarded in this context. This includes *H. stephensoni* as conceived by Černosvitov in his revisionary paper (Černosvitov 1934). Instead, we turn to the original descriptions and types of *Enchytraeus cavicola/stephensoni*, *E. myrmecophilus*, and *E. rangoonensis*. Again, we neither confirm nor reject the synonymies established by Černosvitov (1934).

*Hemienchytraeus bifurcatus* and *H. tanjae* differ in (1) size and (2) texture of the coelomocytes; they are described for *H. bifurcatus* as being “...slightly smaller than the length of the setae; they contain small, refractile granules” (Nielsen and Christensen 1959, p 45, italics added). In *H. tanjae*, the largest coelomocytes are distinctly smaller than the chaetae (15 µm versus 28–33 µm), and the texture is without refractile granules. Both species differ also in (3) the shape of the brain, being about as wide as long in *H. bifurcatus* (Nielsen and Christensen 1959, p 140, Figure 27), and in (4) the nephridia; they possess large terminal vesicles in *H. bifurcatus* (Nielsen and Christensen 1959, p 139; Figures 24, 25). The latter two characters are not dealt with in the text of the description. All four characters are species-constant in our material; the brain is twice as long as wide even in strongly contracted specimens of *H. tanjae*. [Presence or absence of nephridial terminal vesicles is indeed a good taxonomic character and not a transient adaptation to local

edaphic factors or an effect of extraction conditions, as one might possibly assume. In a forthcoming paper, we will describe another new *Hemienchytraeus* species from South America with constantly large nephridial terminal vesicles. This character is species-constant also in *Achaeta* (U. Graefe, personal communication)]. Two further differences between *H. tanjae* and *H. bifurcatus* can be extracted from the original description of the latter, although they are not fully reliable: (5) *Hemienchytraeus bifurcatus* is twice as long (10 mm) as *H. tanjae*; however, body dimensions in Nielsen and Christensen (1959) are often exaggerated (Schmelz 2003, p 24). (6) The clitellum is “strongly elevated” (Nielsen and Christensen 1959, p 45), but no precise dimensions are given.

The apparent similarity of *H. tanjae* and *H. bifurcatus* is probably due to the poor and imprecise original description of the latter. Unknown, for example, are the following characters: (1) size of terminal chaetae; (2) clitellum ventrally; (3) number and distribution of secondary pharyngeal gland lobes; (4) number and distribution of preclitellar nephridia; (5) relative size of nephridia in terminal segments; (6) shape and size of the male copulatory organs. Each of these characters is, from our experience, crucial for a correct identification of *Hemienchytraeus* species; with respect to the lack of information regarding these characters, *H. bifurcatus* should rather be considered as *species inquirenda*. Type material of *H. bifurcatus* is lost (B. Christensen, personal communication). The type locality—a moist meadow in the grounds of the Mols Laboratory, Denmark—was resampled twice by one of us (R. M. Schmelz, in November 1998 and June 2000), but the species was not found.

A literature comparison of *H. stephensoni* with *H. siljae* and *H. patricii* is even more difficult. All original descriptions (i.e. those of *E. cavicola/stephensoni*, *E. myrmecophilus*, and *E. rangoonensis*) lack many details that we consider as taxonomically important. *Hemienchytraeus patricii* is actually indistinguishable from *E. cavicola/stephensoni*, and *H. siljae* differs only in body size and segment number. Fortunately, type material of all three nominal species (*E. cavicola/stephensoni*, *E. myrmecophilus*, and *E. rangoonensis*) is extant and available. A complete description of this material together with a critical discussion of the taxonomic history of *H. stephensoni* and its degree of morphological variation will be provided elsewhere. Here we only highlight some characters that the name-bearing types of all three nominal species have in common, in order to facilitate the taxonomic discussion.

The investigated syntype specimens of *E. cavicola/stephensoni*, *E. myrmecophilus*, and *E. rangoonensis* share the following characters: (1) clitellum girdle-shaped, ventrally complete; (2) clitellum strongly developed, cells 2–4  $\times$  as high as wide (30–45  $\mu$ m high), more granulocytes than hyalocytes; (3) two pairs of secondary ventral pharyngeal gland lobes, in V and VI; (4) four pairs of preclitellar nephridia, from 6/7–9/10; (5) several preclitellar septa strongly thickened. These characters are not dealt with in the original descriptions, except characters (2) and (5) in the account of *E. myrmecophilus*. A further common character can be derived from the original descriptions (Stephenson 1924; Černosvitov 1930; Stephenson 1931): (6) body length below 1 cm, segment number below 40. These six characters are sufficient to establish *H. siljae* and *H. patricii* as species of their own: *H. siljae* differs in characters (1), (2), (3), (5), and (6); *H. patricii* differs in characters (1), (2), (4), and (5).

### Concluding remarks

The descriptions of *H. siljae*, *H. patricii*, and *H. tanjae* have been carried out to the greatest possible detail, and they combine observations on living and preserved material. It has been demonstrated in other enchytraeid genera, especially in *Fridericia*, that intraspecific

character variability had been overestimated in previous studies (Rota 1995; Schmelz 2003). Higher intraspecific constancy of characters allows *and* necessitates the elaboration of species descriptions in much finer detail; furthermore, the combined investigation of living and preserved material has proved to be advantageous if not necessary for unequivocal species delineations (Rota and Healy 1999; Schmelz 2003). These findings established in *Fridericia* are, from our experience with the Amazonian material, directly applicable to *Hemienchytraeus*. Character deviations that occur in single specimens must nonetheless be assessed carefully in order to distinguish between intraspecific variants and interspecific differences. For example, deviations in the shape of the pharyngeal glands were observed in some specimens of all three species; their peculiarity suggested some sort of malformation, partly induced by parasites. In two specimens initially identified as *H. tanjae*, however, a deviation in the size of the secondary pharyngeal gland lobes coincided with two other character differences (large size differences between anterior and posterior chaetae and a generally larger body), suggesting a different species rather than intraspecific variation.

The three new species described here are distinguishable from each other in at least 16 different traits. Most notable are: (1) size difference of chaetae in terminal and preclitellar segments; (2) branching pattern of the oesophageal appendage; (3) number and location of secondary pharyngeal gland lobes; (4) number and location of preclitellar nephridia; (5) distribution pattern of clitellar gland cells, especially on the ventral side. It should be noted that the first four characters (and seven of the 16 characters listed in Table I) acquire their species-specific idiosyncrasy already in juvenile specimens, hatchlings perhaps excepted. This is good news for ecological studies. Using these non-sexual characters, and provided that the species composition of a given site is more or less known, it should be possible to identify correctly a large number of juveniles to species level, in addition to the sexually mature specimens, which usually make up only a small percentage of all individuals.

On the other hand, only one of the above-mentioned characters (no. 2) has been dealt with accurately in the majority of previous species descriptions; all other characters were often considered as intraspecifically variable, or they were not dealt with at all, especially in the older literature. Consequently, a much lower number of characters was available here for comparisons with previously described species. The differences are slight in the case of *H. tanjae* and *H. bifurcatus*. However, we question the validity of the latter, because type material that could help to complement the poorly detailed original account is lost. Regarding *H. siljae* and *H. patricii*, the reinvestigation of the types of *H. stephensoni* was necessary in order to establish species-separating differences. The situation encountered here with *H. bifurcatus* and *H. stephensoni* is not uncommon in Enchytraeidae taxonomy, where the still largely unrevised older literature is almost as great an obstacle for an accurate species identification as is the animals' difficult anatomy itself. We hope that this contribution, together with forthcoming revisions, will facilitate more extensive taxonomic research in the tropics on this important group of soil animals.

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