



## NEW ACANTHODRILID EARTHWORMS OF GENERA *PROTOZAPOTECIA* AND *TRIGASTER* FROM CUBA (ANNELIDA, CRASSICLITELLATA)

### NUEVAS LOMBRICES DE TIERRA ACANTHODRILIDAS DE LOS GÉNEROS *PROTOZAPOTECIA* Y *TRIGASTER* DE CUBA (ANNELIDA, CRASSICLITELLATA)

CARLOS FRAGOSO,<sup>1,\*</sup> AND CARLOS RODRÍGUEZ<sup>2</sup>

<sup>1</sup> Lab. de Invertebrados del Suelo. Red de Biodiversidad y Sistemática. Instituto de Ecología, A.C. Carretera Antigua a Coatepec No. 351, El Haya, Xalapa, 91070, Veracruz, México. <carlos.fragoso@inecol.mx>

<sup>2</sup> Mayía Rodríguez No. 61, entre la Cret y Luis Estévez. Santo Suárez, La Habana, 10500, Cuba

\* Autor de correspondencia: Carlos Fragoso <carlos.fragoso@inecol.mx>

Recibido: 27/05/2016; aceptado: 21/02/2017

Editor responsable: Pedro Reyes Castillo

**Fragoso, C., & Rodríguez, C.** (2017) New acanthodrilid earthworms of genera *Protozapotecia* and *Trigaster* from Cuba (Annelida, Crassicolitellata). *Acta Zoológica Mexicana* (n. s.), 33(1), 67-75.

**ABSTRACT.** Four new Cuban species of the family Acanthodrilidae are described from west (*Protozapotecia angelesae* sp.nov.), center (*Protozapotecia centralis* sp.nov., *Protozapotecia cubensis* sp.nov.) and east (*Trigaster setarmata* sp.nov.) regions. The new species are closely related to northeast and east Mexico (*Protozapotecia* and *Zapatadrilus*) and Puerto Rico and Virgin Islands earthworms (*Trigaster*). Whereas new *Protozapotecia* species possess two gizzards and holonephridia, *Trigaster setarmata* sp.nov. has three gizzards and meronephridia. *Protozapotecia angelesae* sp.nov. is separated from all Mexican species of the genus by the absence of typhlosole and penial setae and by presence of free lamellae in the esophagus of **11** and **12**. The presence of penial setae and typhlosole relates the other two new *Protozapotecia* species to Mexican worms; from all of them, *Protozapotecia cubensis* sp.nov. is separated by a dorsal pore in 4/5, free calciferous lamellae in **13** and a lateral spermathecal diverticulum, whereas *Protozapotecia cubensis* sp.nov. is differentiated by last hearts in **13** and calciferous lamellae in segments **13**, **14** and **15**. *Trigaster setarmata* sp.nov. is separated from the four recognized species of the genus by the presence of penial setae and racemose prostatic glands. Clarification of the origin of Cuban acanthodrilids (dispersion or vicariant events) will depend on future sampling and on the elaboration of robust molecular phylogenies.

**Keywords:** Acanthodrilidae, Caribbean, Cuba, earthworms.

## INTRODUCTION

The majority of native earthworms of Cuba (84%) belong to family Acanthodrilidae, subfamilies Benhamiinae (*Eutrigaster*) and Acanthodrilinae (*Diplostroma*, *Exxus*, *Protozapotecia* and *Zapotecia*). Whereas these species

**Fragoso, C. y Rodríguez, C.** (2017) Nuevas lombrices de tierra acanthodrilidas de los géneros *Protozapotecia* y *Trigaster* de Cuba (Annelida, Crassicolitellata). *Acta Zoológica Mexicana* (n. s.), 33(1), 67-75.

**RESUMEN.** Se describen cuatro nuevas especies de la familia Acanthodrilidae del oeste (*Protozapotecia angelesae* sp.nov.), centro (*Protozapotecia centralis* sp.nov., *Protozapotecia cubensis* sp.nov.) y este de Cuba (*Trigaster setarmata* sp.nov.). Las nuevas especies están cercanamente relacionadas con la fauna del noreste y este de México (géneros *Protozapotecia* y *Zapatadrilus*) y de Puerto Rico e islas Vírgenes (*Trigaster*). Las nuevas especies de *Protozapotecia* se caracterizan por tener dos mollejas y holonefridios, mientras que la especie nueva de *Trigaster* presenta tres mollejas y meronefridios. *Protozapotecia angelesae* sp.nov. se separa de las especies mexicanas del género por carecer de tiflosol y quetas peneales y por presentar láminas esofágicas libres en los segmentos **11** y **12**. Por la presencia de quetas peneales y tiflosol, las otras dos especies nuevas de *Protozapotecia* se acercan más a la fauna mexicana; mientras que *Protozapotecia centralis* sp.nov. se separa de todas ellas por el poro dorsal anterior en 4/5, las láminas esofágicas libres en el segmento **13** y el divertículo espermathecal lateral sésil, *Protozapotecia cubensis* sp.nov. se diferencia por los últimos corazones en el segmento **13** y por las láminas esofágicas libres en los segmentos **13**, **14** y **15**. *Trigaster setarmata* sp.nov. se separa de las cuatro especies conocidas del género por presentar quetas peneales y próstatas racemosas. Clarificar el origen de la fauna de acanthodrilidos cubanos (dispersión o vicarianza) dependerá de futuras colectas y de contar con filogenias moleculares robustas.

**Palabras clave:** Acanthodrilidae, Caribe, Cuba, lombrices de tierra.

are related to central and northeast Mexican high altitude earthworms (Fragoso & Rojas 2016), South American affinities are scarce, being represented only by two species of the family Rhinodrilidae (Rodríguez *et al.* 2007).

In the more recent checklist of the earthworms of this country, Rodríguez *et al.* (2007) recorded a total of 47



species, 20 exotics and 23 endemic, eight of these quoted as new non-described species. Preliminary descriptions of some of these species were made by Rodríguez (1999), as part of his Doctoral dissertation. However, and in accordance with article 9.12 of the International Code of Zoological Nomenclature (ICZN 1999), descriptions provided in thesis are considered unpublished. Accordingly, in this paper we are publishing these descriptions and making them widely available.

### MATERIALS AND METHODS

Sampling was performed during years 1984, 1994 and 1995 in west and center Cuba. All worms were handsorted by qualitative and quantitative (soil monoliths) manual extraction methods; each worm was fixed in an ethanol-formalin mixture (1:1) and preserved in formalin (4%). For each species (in brackets), individuals were grouped according to the following convention: juveniles (without any sexual character), non clitellate adults (adults with external sexual characters) and clitellate adults. In each description segments are indicated in bold Arabic numbers. All material is deposited at the collection of the Soil Fauna Laboratory, Biology Faculty, Havana University (FBUH). Definition of included families, subfamilies and genera are according to Csuzdi (2010), James & Davidson (2012) and Fragoso & Rojas (2016).

### RESULTS

Family Acanthodrilidae

Subfamily Acanthodrilinae

Genus *Protozapotecia* James, 1993

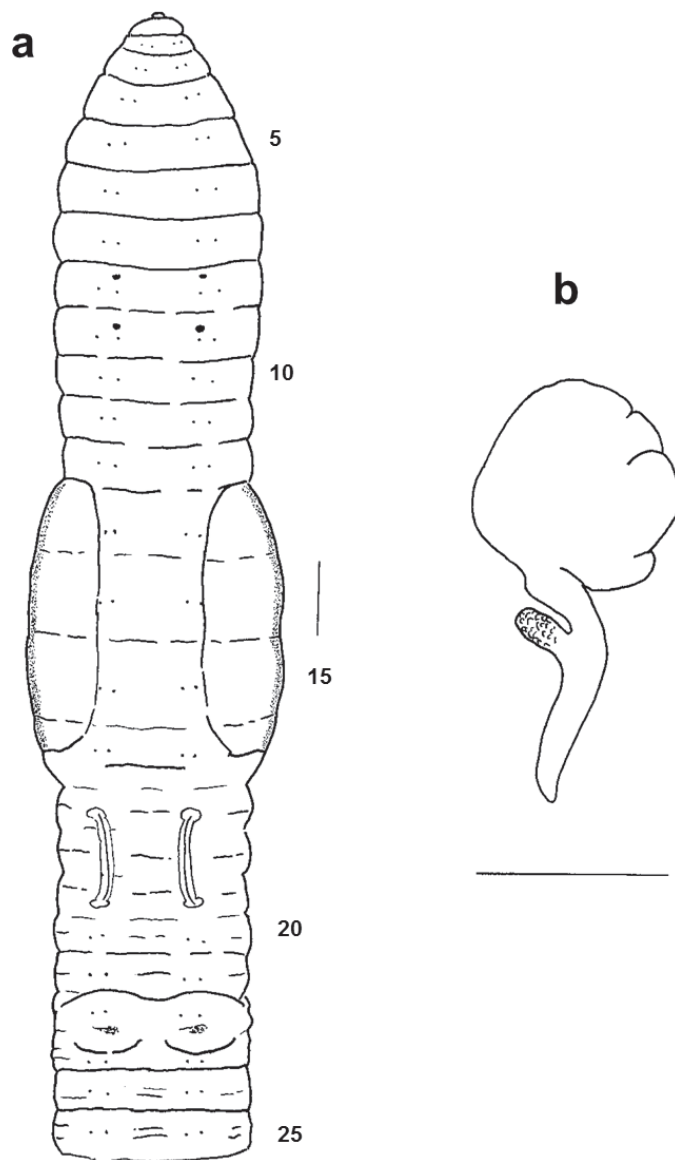
***Protozapotecia angelesae* Rodríguez and Fragoso sp.nov.**

(Fig. 1a, b)

**Material.** Province of La Habana, municipality of Boyeros, Capdevila, Campus of the Institute of Ecology and Systematics, within the soil of a secondary semi-deciduous forest. 23° 02' 6.51" N, 82° 22' 44.99" W, 76 m.a.s.l. Ma. de los Angeles Martínez, coll., October 1994. Holotype: Clitellate adult FBUH 306, Paratype: Juvenile, FBUH 307.

#### Description

**External:** Length 40-67 mm, postclitellar diameter 1.5-2.4 mm. Number of segments 212-215. Cylindrical body in cross section throughout. Pigment absent; after preser-



**Figure 1.** *Protozapotecia angelesae* sp.nov. (Holotype FBUH 306). a) External ventral view of anterior region (scale 0.9 mm); b) Dorsal view of right spermatheca from segment 9. Scale 1 mm.

vation in formalin it acquired a yellowish colour. Prostomium open epilobic, bearing a great number of minute papillae. Post-clitellar segments with secondary or tertiary secondary furrows. Setae lumbricine, closely paired throughout, *cd* lateral, in regular longitudinal rows; setal formula (*aa:ab:bc:cd*) **10**: 3.3:1:3:1, **30**: 5:1:9:1. Without penial and genital setae. First dorsal pore 8/9. Nephridiopores at *d*. Clitellum yellow-orange in **13-16, 17** only dorsally, saddle-shaped without reaching ventral setae and covering setae *cd* (Fig. 1a). Spermathecal pores paired in



**8** and **9**, in front of setae *a* (Figure 1a). Male and female pores inconspicuous. Prostatic pores at the end of nearly straight seminal grooves at *AB* level in **17** and **19** (Fig. 1a). Genital markings represented by a ventral pair of papillae in segments **22-23** (Fig. 1a).

**Internal:** Septum 5/6 membranous, 6/7-9/10 gradually increasing thickness and then decreasing. Alimentary canal with two discrete gizzards in **5** and **6**, each one divided from the other by a deep furrow. Esophagus apparently with mesially free calciferous lamellae in **11** and **12**. Intestinal origin in **14**. Typhlosole and caeca were not seen.

Dorsal vessel single throughout, ventral vessel present, subneural vessel absent. Lateroesophageal hearts in **9-12**, lateral hearts joined to dorsal and ventral vessels in **6-8**, supraesophageal vessel in **8-12**.

Excretory system holoic, avesculate and stomate.

Male sexual system holandric, iridescent testes and funnels free in **10** and **11**; a pair of seminal vesicles in **11** and **12**, with a fine and spongy texture. Tubular prostates in **17** and **19** extending backwards until **23**, the distal portion plicated; the thick, muscular and plicated ducts are covering two segments. Penial setal follicles and penial setae absent. Fan-shaped ovaries in **13**. Paired spermathecae in **8** and **9**; the voluminous and ovoidal ampulla joins a duct as long as the ampulla diameter. Lateral and near to the ampulla base there is a short digit-form diverticulum with coagulated sperma (Fig. 1b), which is also present in the basal portion of the ampulla.

**Etymology.** The name of the species is dedicated to our colleague and good friend Dr. Ma. de los Angeles Martínez.

**Systematics.** The bigiceriate and holoic genus *Diplocardia*, distributed from USA to northeast Mexico, is characterized by two nearly fused gizzards in segments **5** and **6**, tubular prostates open in segments **18** and **20** or backwards, intestine starting in or after segment **17** and a membranous nature of septum 5/6 (Fragoso & Durán 2014, Fragoso & Rojas 2016). Conversely, the three Mexican described species of the genus *Protozapotecia* possess tubular prostates in segments **17** and **19**, intestine beginning in segment **14** or **15** and the two gizzards of **5** and **6** separated by a muscular septum 5/6 (James 1993). By possessing prostatic pores in **17** and **19**, an intestine beginning in a more anterior segment (**14**) and the two gizzards separated by a membranous septum and a clear furrow, the new species is closer to *Protozapotecia* than to *Diplocardia*, even though the latter genus shares the condition of a membranous septum 5/6. By the absence of typhlosole and penial setae and by the presence of

free lamellae in the esophagus of **11** and **12**, *P. angelesae* sp.nov. is clearly separated from the three Mexican species of *Protozapotecia*, *P. aquilonalis* James, 1993, *P. australis* James, 1993 and *P. koebeli* (Eisen, 1900).

***Protozapotecia centralis* Rodríguez and Fragoso sp.nov.**

(Figs. 2a, b, c)

**Material.** Province of Cienfuegos, Municipality of Palmira, close to the agroindustrial Center “Espartaco”, within soil of a secondary semi-deciduous tropical forest. 22° 16' 27" N, 80° 20' 43.17" W, 67 m.a.s.l. Carlos Rodríguez, coll., May 1994. Holotype: Clitellate adult FBUH 155, Paratypes: Two clitellates, one pre-clitellate and two juveniles, FBUH 156-159.

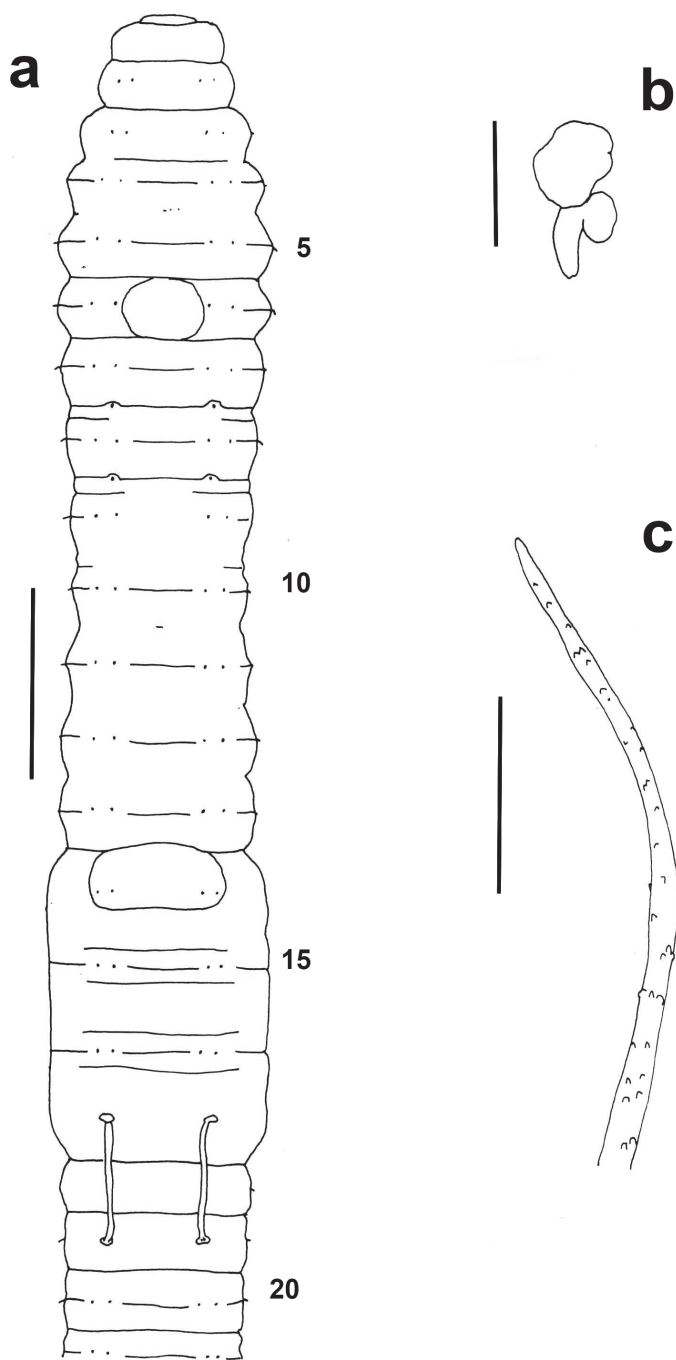
**Description**

**External.** Length 28-70 mm (holotype 48 mm), postclitellar diameter at segment **30** 1-2 mm (holotype 1 mm). Number of segments 53-202 (holotype 193). Body cylindrical in cross section. Pigment absent; color after preservation in formalin, clear grey to pinky greyish. Prostomium closed epilobic, covering 1/2-1/3 of peristomium. Secondary annulation characterized by a single furrow, more evident in pre-clitellar segments. Eight setae per segment in lumbricine arrangement, closely paired throughout, visible from segment **2** in regular longitudinal rows; setal formula (*aa: ab:bc:cd*) **10**: 2.5:1:2:1.8, **30**: 3.8:1:2.5:1.6. Penial setae present, genital setae absent. First dorsal pore in 4/5. Nephridiopores at *c*, conspicuous in the clitellum. Clitellum yellow-ochre, saddle shaped, covering segments **14-17**, and with the intersegments barely visible (Figure 2a). Spermathecal pores paired in **8** and **9**, small and in front of *a* or *ab* (Fig. 2a). Male and female pores not visible. Prostatic pores in **17** and **19**, located on straight seminal grooves at *AB* level (Fig. 2a). Genital markings variable, characterized by single midventral papillae in **6** (only in the holotype), **14** (3 ind.) (Figure 2a), **16** and part of **17** (1 ind.) or in **20** (1 ind.).

**Internal.** Septum 5/6 partially muscular, 6/7-8/9 gradually increasing in thickness until 9/10 which is the thickest; from then septa are gradually more membranous. Two discrete gizzards in **5** and **6**, preceded by a proventriculus in **4**. Esophagus with intramural mesially free calciferous lamellae in **13**. Intestinal origin in **14**. Laminar typhlosole starting in **17**. Caecae not seen.

Lateroesophageal hearts in **10-12**, lateral hearts joined to dorsal and ventral vessels in **6-9**, supraesophageal vessel in **10-12**.

Excretory system holoic, avesculate and stomate.



**Figure 2.** *Protozapotecia centralis* sp. nov. a) External ventral view of anterior region (Holotype FBUH 155) (scale 1 mm); b) Dorsal view of left spermatheca from segment 9 (scale 0.5 mm); c) Distal part of penial seta from segment 17 (scale 50 μm).

Holandric, testes and large iridescent funnels in 10 and 11 with abundant coagulum; a pair of multi-lobed seminal vesicles in 11 and 12. Paired tubular prostates in 17 and 19, the former limited to its segment, and the posterior

ones reaching part of segment 20. Penial setal follicles muscular and anterior to prostatic pores. Penial setae almost straight (0.59-0.63 mm length, 4-9 μ width), with a slight curvature in the distal portion; ornamentation characterized by several and irregular thorns (Fig. 2c). Fan-shaped ovaries in 13, with eggs in rows. Large paired spermathecae in 8 and 9, with the ovoidal ampulla joined to a thick duct, slightly shorter than ampulla diameter; the almost sessile and ellipsoidal diverticulum projects from the ental and lateral portion of duct (Fig. 2b).

**Etymology.** The name of the species indicates that it was found in the central region of Cuba.

**Remarks.** The new species belong to the group of Mexican *Protozapotecia* species with penial setae, typhlosole and seminal vesicles in 11 and 12. It differs from all of them by the more anterior dorsal pore (4/5 vs 6/7-9/10) and the free calciferous lamellae of segment 13 (absent). It also differs from *P. aquilonalis* and *P. australis* by the beginning of typhlosole (in 17 vs. 19 in the Mexican species) and the shape of the spermathecal diverticulum (lateral, sessile vs. reniform perpendicular to ampulla axis); from *P. koebeli* and *P. aquilonalis* it is also separated by the lack of genital setae. From *P. angelesae* sp.nov. is separated by the presence of penial setae and typhlosole.

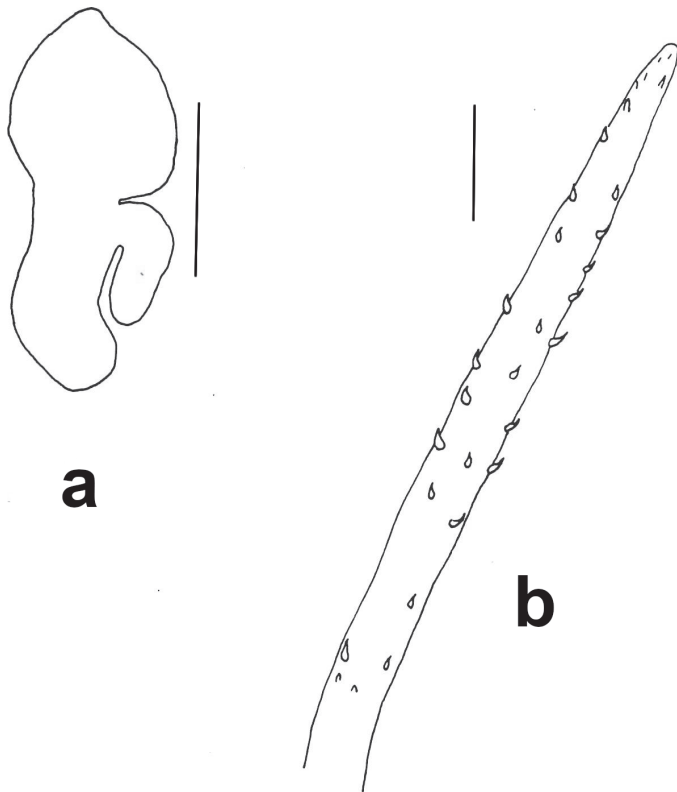
***Protozapotecia cubensis* Rodríguez and Fragoso sp.nov.**

(Fig. 3a, b)

**Material.** Province of Cienfuegos, Municipality of Palmira, close to the agroindustrial Center “Espartaco”, within soil of a secondary semi-deciduous tropical forest. 22° 16' 27''N, 80° 20' 43.17''W, 67 m.a.s.l. Carlos Rodríguez, coll., May 1994. Holotype: Non-clitellate adult FBUH 146, Paratypes: 8 Juvenile (6 fragmented), FBUH 147-154.

**Description**

**External.** Length 95-130 mm (holotype 105 mm), postclitellar diameter at segment 30 2.5 mm (holotype). Number of segments 218-242 (holotype 245). Body cylindrical in cross section throughout. Pigment absent; color after preservation in formalin clear grey to pinky greyish. Prostomium closed epilobic, covering 1/3 of peristomium. Two or three secondary furrows from segment 5 or 6. Eight lumbricine setae per segment, closely paired throughout, visible from segment 2 in regular longitudinal rows, *a* and *b* conspicuous and large; setal formula (*aa:ab:bc:cd*) 10: 2.2:1:1.8:1.3, 30: 1.9:1:1.7:1.4. Penial setae present, genital setae absent. First dorsal pore in 4/5. Nephridiopores at *c*. Clitellum not yet developed; genital marks not seen



**Figure 3.** *Protozapotecia cubensis* sp. nov. (Holotype FBUH 146). a) Dorsal view of right spermatheca from segment 9 (scale 0.5 mm); b) Distal part of penial seta from segment 17 (scale 30  $\mu$ m).

(absent or not developed?). Spermathecal pores paired in 7/8 and 8/9, in *a*. Male and female pores inconspicuous. Prostatic pores at the end of straight seminal grooves in 17 and 19 each pore with two associated (*a* and *b*) penial setae.

**Internal.** Septum 5/6 membranous, 6/7-10/11 muscular, 11/12 less muscular. Two clearly delimited gizzards in 5 and 6, the anterior preceded by a proventriculus in 4. Esophagus with mesially free calciferous lamellae in segments 13, 14 and anterior region of 15. Intestinal origin in 17. Laminar typhlosole visible from 20/21, occupying 1/2-1/3 of intestinal lumen. Intestinal caeca absent.

Supraesophageal vessel in 9-13. Latero-esophageal hearts in 10-13, lateral hearts joined to dorsal and ventral vessels in 6-9.

Excretory system holoic, avesciculate and stomate.

Holandric, incipient funnels in 10 and 11; Acinous seminal vesicles in 11 and 12; not fully developed, they cover the dorsal part of the esophagus. Two pairs of tubular prostates in 17 and 19, small and coiled. Penial setae within muscular setal follicles, measuring 0.5 mm in

length and 17-19  $\mu$  width (Holotype); ornamentation limited to the distal region, characterized by several irregular thorns (Fig. 3b). Ovaries not seen. Paired spermathecae in 8 and 9; ovoidal heart-shaped ampulla and duct of equal length; a sessile digit-form diverticulum, ectally oriented, projects from the ental part of the duct (Figure 3a).

**Etymology.** The name of the species refers to the country where it was found.

**Remarks.** *Protozapotecia cubensis* sp.nov. and *P. centralis* sp.nov. are unique in the genus by the anterior location of dorsal pores (4/5); from the latter species it is separated by the beginning of intestine (17 in *P. cubensis* vs 14 in *P. centralis*) and typhlosole (20/21 vs 17). The new species shares some morphological features that relate it with both *Diplocardia* and *Protozapotecia*. With the latter it shares the position of prostatic pores in 17 and 19 and seminal vesicles in 11 and 12, whereas with (some) *Diplocardia* it shares the last hearts in 13, calciferous lamellae in segments 13-15 and beginning of intestine and typhlosole in 17 and 20/21, respectively (Fragoso & Rojas 2016). However the first dorsal pore in 4/5, only shared with *P. centralis* sp.nov. and never recorded for any *Diplocardia* or any other *Protozapotecia* species suggest a common ancestor for both species. This common ancestor should have had seminal vesicles in segments 11 and 12, a character shared by all *Protozapotecia* species.

A recent molecular analysis (COI gene) of some holoic Mexican species of *Protozapotecia* and *Diplocardia* (two gizzards) and *Zapotecia* (three gizzards) (Cervantes *et al.* 2014), revealed that *Protozapotecia* and *Zapotecia* cluster together, clearly separated from *Diplocardia*. Based on this phylogenetic analysis, these authors suggested that the trigiceriate *Zapotecia* and the bigiceriate *Protozapotecia* correspond to the same lineage; by priority the name of this lineage should be *Zapotecia*.

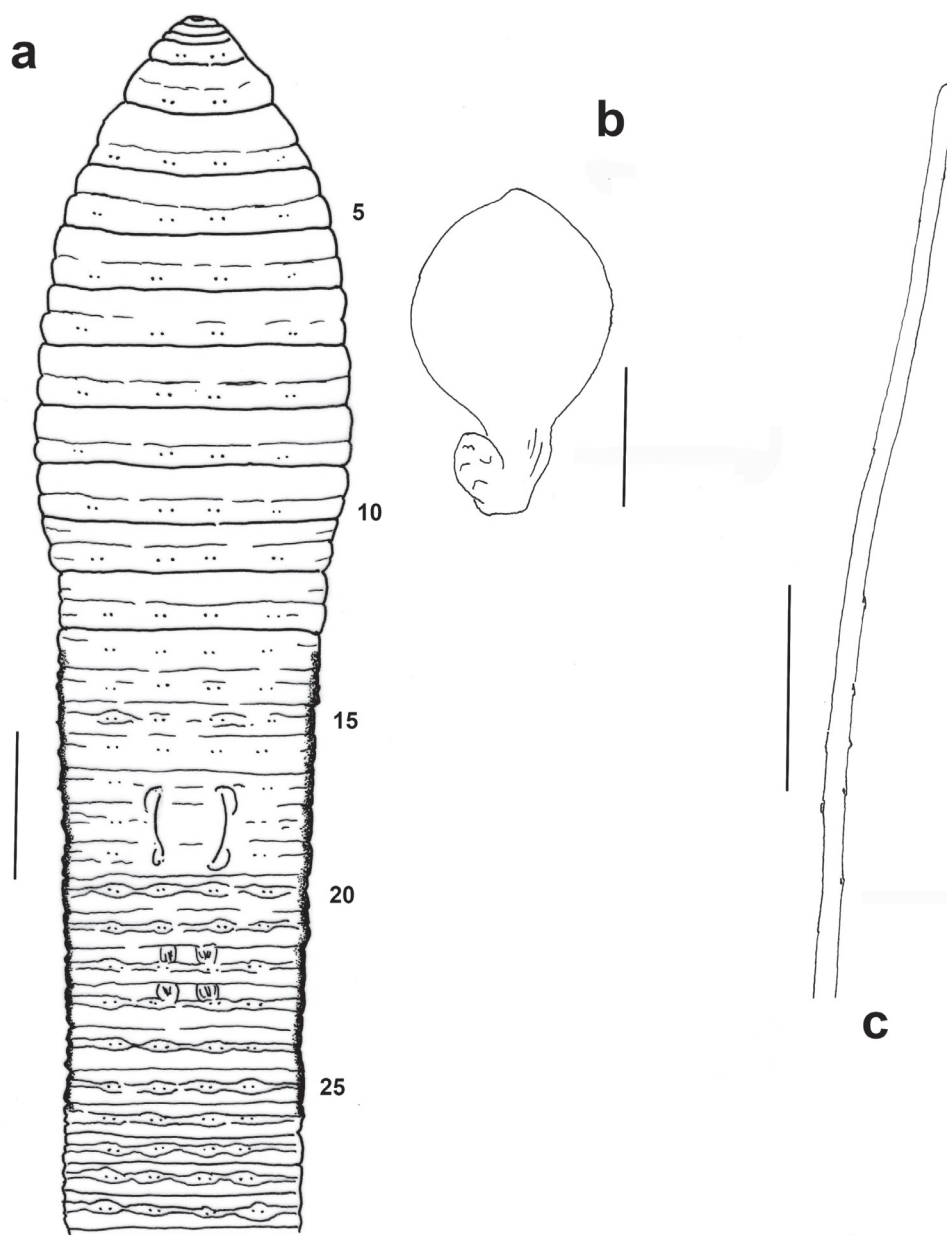
However, and in spite of the high support values of the Bayesian inference analysis, Cervantes *et al.* (2014) recommended further analysis with more genes to confirm the observed topology. In the meantime we prefer to maintain both genera separated, hoping that future molecular analysis with more genes and including the Cuban species will definitively set the relationships of Mexican and Caribbean multigiceriate acanthodriline earthworms.

Genus *Trigaster* Benham, 1886

***Trigaster setarmata* Rodríguez and Fragoso sp.nov.**

(Fig. 4a, b, c)

**Material.** Province of Camaguey, Municipality of Sierra de Cubitas, within soil of a semideciduous microphile for-



**Figure 4.** *Trigaster setarmata* sp. nov. (Holotype FBUH 003). a) External ventral view of anterior region (scale 4 mm); b) Dorsal view of right spermatheca from segment 9 (scale 1 mm); c) Distal part of penial seta from segment 17 (scale 100  $\mu$ m).

est from “Sierra de Cubitas”. 21° 40’ 27.1” N, 77° 46’ 1.8” W, 84 m. a.s.l. L. F. de Armas, coll. June 1984. Holotype: Clitellate adult FBUH 003; Paratype: one juvenile FBUH 004.

**Description**

**External.** Length 305 mm, postclitellar diameter 6.8 mm. Number of segments 584. Body cylindrical in cross section, throughout. Pigment absent, color after preservation



in formalin greyish. Prostomium prolobous. Secondary furrows mainly in faint clitellum and post-clitellum regions. Setae in lumbricine arrangement, minute, closely paired throughout, and in regular longitudinal rows; setal formula (*aa:ab:bc:cd*) **10**: 1.6:1:3.8:1.3, **30**: 5:1:9:1. Penial setae present. First dorsal pore in 11/12. Nephridiopores inconspicuous. Clitellum slightly developed in  $\frac{1}{2}$  **13- $\frac{1}{2}$  26**; yellowish. Spermathecal, female and male pores inconspicuous. Prostatic pores in **17** and **19**, at the end of mesially curved seminal grooves at *ab* (Figure 4a). Barely developed, ventral paired genital marks in **13** in *AB* line, and in **22** and **23**, in *A* (Fig. 4a).

**Internal.** Septa 5/6-11/12 muscular, funnel shaped and displaced backwards, 12/13 and 13/14 slightly muscular. Alimentary canal with three gizzards in **5**, **6** and **7**; anterior part of gizzard of segment **5** apparently forming a proventriculus, but a septum was not seen. Intestinal origin in **20**. Typhlosole and caeca lacking.

Dorsal vessel single throughout, supraesophageal visible only in **10-13**. Lateral hearts in **10-11**, lateroesophageal in **12-13**, last two pairs voluminous; ventral vessel lacking in **5**.

Excretory system meroic; a pair of dense fringes of micronephridia on segments **3** and **4**; in **30** there are 32 to 36 avesculate and astomate nephridia per segment.

Male sexual system metandric, characterized by scarcely developed free testes and male funnels in **11**; a pair of small, granular and digit-shaped seminal vesicles in **12**. Two pairs of incipient racemose prostatic glands in **17** and **19**, with slender and short ducts. Slightly curved penial setae present in segments **17** and **19**; they are thin, 1.7-2.3 mm by 0.01 mm, with some dispersed sub-apical notches (Fig. 4c). Penial setal follicles, just anterior to ducts. Fan shaped ovaries in **13**. Paired spermathecae in segments **8** and **9**, projecting from posterior face of septa 7/8 and 8/9, near mid-ventral line and just in front to setae *ab*. Ampulla nearly spheroidal, with a duct shorter than the ampulla diameter and measuring  $\frac{1}{3}$  of the spermatheca length. Located near the ectal portion of the duct there is a small, spheroidal diverticulum with some incipient chambers (Fig. 4b).

**Etymology.** The species name refers to the presence of penial setae.

**Systematics.** James (1991) divided the multigiceriate and meroic genus *Trigaster* in four lineages. In the new arrangement, *Trigaster* was restricted to metandric species with one seminal vesicle in **12**, tubular prostates in **17** and **19**, three gizzards and eight setae per segment; the genus so defined was restricted to Virgin Islands and Puerto

Rico. The new species shows several morphological characters that relate it to *Trigaster*: three gizzards, metandry, absence of ventral vessel in some portion of the body (in **5** in this species) and spermathecae in **8** and **9**. From all the species of the genus (*T. lankesteri*, *T. calwoodi*, *T. intermedia* from Virgin Islands and *T. longissimus* from Puerto Rico), *T. setarmata* sp.nov is separated by the presence of penial setae and racemose prostatic glands. Accordingly the definition of the genus should be modified to include tubular or racemose prostates and with or without penial setae.

Blakemore (2007) suggested that this species should be placed in the highly debated family Exxidae, because it is assumed that racemose prostates in an acanthodrilid arrangement constitute an evolutionary novelty that appeared only once (in the ancestor of Exxidae). Considering that tubular and racemose prostates are also present in bigiceriate genera of Cuba (*Exxus*) and North México (*Zapataadrilus*, Cervantes *et al.* 2016), we prefer to maintain *Trigaster* in Acanthodrilidae because current evidence supports (e.g. molecular phylogenies) that the number of gizzards is more conservative than the type of prostates.

## DISCUSSION

The four species described in this paper share the common character of multiple gizzards, two gizzards in the species of *Protozapotecia* and three in *Trigaster setarmata* sp.nov. Within Acanthodrilidae, the multiple gizzard condition is observed in two main lineages: subfamily Benhamiinae (tribe Benhamiini) and the so called diplocardins of south USA, northeast and central Mexico and Caribbean islands (Fragoso & Rojas, 2016). Whereas in the first case the majority of species present two gizzards (there are some genera with a single gizzard) in combination with kidney-shaped calciferous glands in the region of segments **14-19** (Csuzdi 2010), in the diplocardins there are two or three gizzards but no extramural calciferous glands occur. All Benhamiinae species are meroic; in diplocardins, on the other hand, both meroic and holoic species occur.

The most recent molecular studies (Buckley *et al.* 2011 and James & Davidson 2012) indicate that Benhamiinae represents a phylogenetic independent lineage; in the case of the diplocardine worms (for the entire list of species see the recent paper of Fragoso & Rojas 2016) all the evidence points out that they constitute also an independent lineage. A future morphological and molecular revision should be made in order to confirm the relationship of all

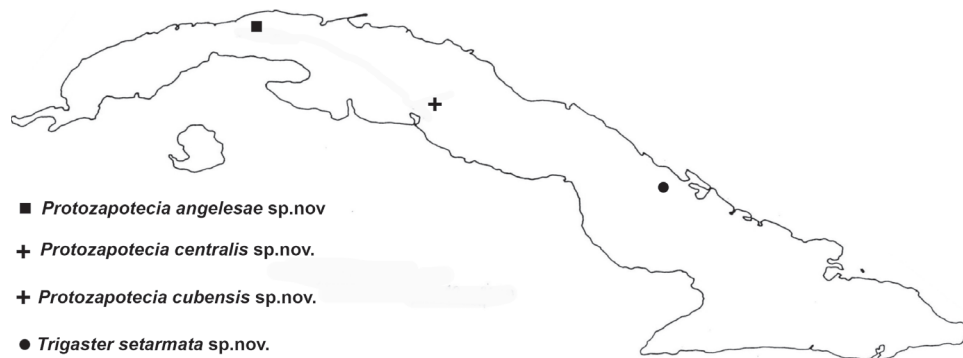


Figure 5. Geographical distribution in the island of Cuba of the new described taxa.

these diplocardine multigiceriate worms; if a single origin is demonstrated, then, and as suggested by Blakemore (2005) and James & Davidson (2012), all these worms should be grouped in the resurrected subfamily Diplocardinae (in the sense of Eisen 1900).

All the species described in this study were found in west (*Protozapotecia angelesae* sp.nov.), central (*Protozapotecia centralis* sp.nov., *Protozapotecia cubensis* sp.nov.) and east Cuba (*Trigaster setarmata* sp.nov.) (Figure 5). Before being tempted to speculate on their biogeographical origin (dispersal vs vicariance), what we can state without any doubt is that the new *Protozapotecia* species are more closely related to the North American *Diplocardia* and north Mexican *Protozapotecia* than to any other acanthodriline worm of southern Mexico or elsewhere. In the case of *Trigaster setarmata* sp.nov. it should be clarified why this genus occurs in Cuba, Puerto Rico and Virgin Islands, without any record in Jamaica and Hispaniola. When and how these worms arrived to Cuba? The answer to this question should wait until robust molecular phylogenies of the entire native Cuban earthworms clarify their relationship with continental genera and species.

**ACKNOWLEDGEMENTS.** We are greatly indebted to Dra. María de los Angeles and Dr. Luis F. de Armas, for providing us with very interesting earthworm material, that turned out to be new species. C. Fragoso acknowledges A. Angeles for its help in the scanning of some figures. C. Rodríguez acknowledges the support of CYTED PIP XII.3 and Mexican CONACYT for the support to carry out several research visits at the Institute of Ecology, A.C., Xalapa. Finally we acknowledge two anonymous referees, whose comments greatly improved the manuscript.

## LITERATURE CITED

- Blakemore, R. J.** 2005. Whither Octochaetidae? – its family status reviewed. pp. 63–84. *In*: A. A. Pop, & V. Pop (Eds.). *Advances in Earthworm Taxonomy. II*. Cluj University Press, Romania.
- Blakemore, R. J.** 2007. Checklist and phylogeny of Exxidae Blakemore, 2000 (Oligochaeta). *European Journal of Soil Biology*, 43, s9–s11.
- Buckley, T. R., James, S., Allwood, J., Bartlett, S., Howitt, R., & Prada, D.** 2011. Phylogenetic analysis of New Zealand earthworms (Oligochaeta: Megascolecidae) reveals ancient clades and cryptic taxonomic diversity. *Molecular Phylogenetics and Evolution*, 58, 85–96.
- Cervantes, G., Fragoso, C., James, S. W. & Espinosa de los Monteros, A.** 2014. Assessing the evolution of morphological characters in Mexican acanthodriline earthworms with multiple gizzards (Oligochaeta: Acanthodrilidae). pp. 17–25. *In*: T. Pavlíček, P. Cardet, M. T. Almeida, C. Pascoal & F. Cássio (Eds.). *Advances in Earthworm Taxonomy VI (Annelida: Oligochaeta)*. Kasperek Verlag, Heidelberg, Germany.
- Cervantes, G., Fragoso, C., Espinosa de los Monteros, A., Sánchez-Ramos, G., Lara-Villalón, M., Yañez-Pacheco, M.J., Lázaro-Castellanos, J.O. & James, S. W.** 2016. New species of the earthworm genus *Zapatadrilus* (Clitellata, Acanthodrilidae) from northern Mexico. *Zootaxa*, 4189 (2), 311–326.
- Csuzdi, C.** 2010. *A monograph of the Paleotropical Benhamiinae earthworms*. Hungarian Natural History Museum & Hungarian Academy of Sciences, Budapest, Hungary, 348 pp.
- Eisen, G.** 1900. Researches in the American Oligochaeta, with special reference to those of the Pacific coast and adjacent islands. *Proceedings of the California Academy of Sciences*, 2, 85–276.
- Fragoso, C. & Durán, S. H.** 2014. On the identity of an earthworm species of the genus *Diplocardia* found in central west Mexico. pp. 26–31. *In*: T. Pavlíček, P. Cardet, M. T. Almeida, C. Pascoal & F. Cássio (Eds.). *Advances in Earthworm Taxonomy VI (Annelida: Oligochaeta)*. Kasperek Verlag, Heidelberg, Germany.
- Fragoso, C. & Rojas, P.** 2016. *Lavellodrilus notosetosus* sp.nov. (An-





- nelida, Crassicitellata): a new Mexican earthworm with remarkable characters, revealed by a preliminary revision of subfamily Acanthodrilinae. *Zootaxa*, 4154 (2), 101-138.
- ICZN** 1999. *International Code of Zoological Nomenclature*. Fourth Edition. The International Trust for Zoological Nomenclature, London, UK. 306 pp.
- James, S.W.** 1991. New species of earthworms from Puerto Rico, with a redefinition of the earthworm genus *Trigaster* (Oligochaeta: Megascolecidae). *Transactions of the American Microscopical Society*, 110, 337-353.
- James, S.** 1993. New acanthodrilina earthworms from Mexico (Oligochaeta: Megascolecidae). *Acta Zoológica Mexicana (n.s.)*, 60, 1-21.
- James, S. & Davidson, S.** 2012. Molecular phylogeny of earthworms (Annelida: Crassicitellata) based on 28S, 18S and 16S gene sequences. *Invertebrate Systematics*, 26, 213-229.
- Rodríguez, C.** 1999. Lombrices de tierra (Oligochaeta: Haplotaxida y Moniligastrida) de Cuba. Tesis doctoral no publicada. Universidad de La Habana. Facultad de Biología. Departamento de Biología Animal y Humana. Cuba.
- Rodríguez, C., Borges, S., Martínez, M., Fragoso, C., James, S. & González, G.** 2007. Biodiversidad y ecología de las lombrices de tierra en las islas caribeñas. pp. 79-98. In: G. G. Brown & C. Fragoso (Eds.). *Minhocas na América Latina: Biodiversidade e Ecologia EMBRAPA Soja*. Brasil.