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A new species of *Cosmocercella* parasite of *Diaglena spatulata* and *Triprion petasatus* (Anura: Hylidae) from Mexico, based on new morphological information for the genus

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Abstract

A new species of *Cosmocercella* as a parasite of the hylid frogs *Diaglena spatulata* and *Triprion petasatus* is herein described. The new species most closely resembles *C. haberi* and *C. anothecae*, both species distributed in Mexico. *Cosmocercella diaglenae* n. sp. differs from these species in the arrangement of the caudal papillae, and mainly in the presence and size of the first pair of papillae situated anteriorly to the anus, but it also differs by the distance between the second and third pairs of plectanes, by the length of the spicules and gubernaculum, by the size and form of the pharynx, as well as the esophageal bulb length-width ratio in females and finally by having larger eggs. In order to find additional taxonomic characteristics to distinguish among species, a morphometrical study was conducted.

Key words: Cosmocercidae, Nematoda, *Diaglena spatulata*, *Triprion petasatus*, Anura, Hylidae, Jalisco, Yucatán

Resumen

Se describe una especie nueva de *Cosmocercella* parásita de los hylidos *Diaglena spatulata* y *Triprion petasatus*. La nueva especie se asemeja a *C. haberi* y *C. anothecae*, ambas especies distribuidas en México. *Cosmocercella diaglenae* n. sp. difiere de estas especies en el arreglo papilar caudal, principalmente en la presencia y tamaño del primer par de papilas situado anteriormente al ano, en la distancia entre el segundo y tercer par de plectanes, en la longitud de las espículas y el gubernaculum, en el tamaño y forma de la faringe, además de la relación entre el largo y ancho del bulbo esofágico en las hembras y en presentar huevos más grandes que los observados en *C. haberi* y en *C. anothecae*. Se realizó un estudio morfométrico con el fin de encontrar más características diagnósticas para distinguir estas especies.

Introduction

Currently, *Cosmocercella* Steiner, 1924 comprises a small group of nematodes parasitic in amphibians and reptiles from the Americas and Asia: *C. haberi* Steiner, 1924 in *H. cinerea* from Alamos Sonora, USA; *C. neveri* Hsü & Hoeppli, 1933 in *Quasipaa spinosa* from Amoy, China; *C. minor* (Freitas & Dobbin, 1961) Baker & Adamson, 1977 in *Phyllomedusa hypochondrialis* from Department Presidente Hayes, Río Negro, Paraguay; *C. anothecae* Baker & Adamson, 1977 in *Anotheca spinosa* from Veracruz, Mexico; *C. uropeltidarum* (Crusz & Ching, 1975) Baker & Crusz, 1980 in *Rhinophis phillipinus* and *R. drummondhayi* from India and Sri Lanka; *C. phyllomedusae* Baker & Vaucher, 1983 in *Phyllomedusa hypochondrialis* from Para-

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guay (type host and locality) and *Ph. palliata*, *Ph. tomopterna* and *Ph. vaillanti* from Peru (Bursey *et al.*, 2001); *C. iwatzukii* Hasegawa, 1989 in *Rhacophorus viridis viridis* from Okinawa, Japan, and *C. phrynomantisi* Moravec, 1990 in *Callulops humicola* (type host), *C. stictogaster*, and *C. wilhelmana* from Mt. Otto, Papua New Guinea. During a survey of the helminth parasites of amphibians in Mexico, an undescribed species of *Cosmocercella* was found in the intestine of *Diaglena spatulata* (Günther) and *Triprion petasatus* (Cope) from Jalisco and Yucatán states, respectively. The new species is described herein and a comparison of caudal papillae and morphometric data among *Cosmocercella* species is presented.

Materials and methods

Specimens of *Diaglena spatulata* and *Triprion petasatus* were collected by hand at localities of Jalisco and Yucatán states, Mexico. Hosts were killed with an overdose of sodium pentobarbital directly to the central nervous system and were examined for internal parasites under a stereoscope. Nematodes found were counted in situ, recorded and fixed in hot (steaming) formalin 4%, cleared in glycerin-ethanol, and mounted on temporary slides with glycerol for microscopical observation. Drawings were made using a drawing tube. Mesurements are provided in millimeters, including range, followed by the average, standard deviation, and a sample size in parentheses when is different from the total number of studied specimens. Hosts bodies were deposited in the Museo de Zoología, Facultad de Ciencias (MZFC), UNAM, Mexico City, Mexico, and parasites were deposited in the Colección Nacional de Helmintos (CNHE), Instituto de Biología, UNAM, Mexico City, and the Harold W. Manter Laboratory of Parasitology (HWML), Lincoln, Nebraska. The following specimens from the United States National Parasite Collection (USNPC), Beltsville, Maryland were examined for comparison: *C. anothecae* 74592 (paratypes); *C. haberi* 74593, 84259, 85904, 85907, 87750, 88959, 88960, 89817, 91714, 92599 (vouchers); *C. iwatsukii* 80368 (paratypes); *C. minor* 85250 (voucher); *C. phyllomedusae* 89547, 89550 and 89554 (vouchers). Current host names used in the present study follow Frost (2008).

Morphological data were tested for normality and homogeneity of variances using Shapiro-Wilks W statistics and Bartlett's test respectively (Zar, 1984). Canonical discriminant-function analyses (Blackith & Reyment, 1971) were used to determine differences between species.

Results

Cosmocercella diaglenae Mata-López, Guillén-Hernández and León-Règagnon, new species
(Figs. 1–3)

Taxonomy: Ascaridida, Cosmocercoidea, Cosmocercidae, *Cosmocercella* Steiner, 1924.

Diagnosis: Nematodes with tapered extremities and lateral narrow wings, commencing from level far anterior to nerve ring and ending at precloacal region in males, and anterior to midtail in females. Cuticle with fine longitudinal striations. Three lips; dorsal lip with two papillae and each sub-ventral lip with one large papilla and one amphid; narrow naked mouth cavity. Esophagus anteriorly with short pharynx, followed by cylindrical portion and terminal esophageal bulb, with valvular apparatus. Nerve ring at midlevel of cylindrical portion of esophagus. Excretory pore ventromedian, anterior to esophageal bulb; excretory vesicle small, surrounded by a large cellular mass.

Description. Male (measurements of 16 specimens): Body length (MBL) 1.537–1.975 (1.683±94.53). Maximum width 0.072–0.090 (77.03± 4.00) (3.8–5.13% of MBL). Pharynx 0.012–0.020 (0.015±0.002) long by 0.012–0.020 (0.016±0.002) (0.74–1.18% of MBL) wide. Ratio of pharynx length to width 0.83–1.33 (1.03±0.15). Esophagus length 0.237–0.267 (0.254±0.009) (12.53–16.42% of MBL) by width 0.020–0.032 (0.022±0.004). Esophageal bulb length 0.052–0.065 (0.059±0.004) (3.04–3.88% of MBL) by width 0.050–

0.060 (0.054±0.003). Esophageal bulb length to width ratio 0.95–1.14 (1.08±0.05). Nerve ring 0.125–0.147 (0.137±0.006) (7.22–8.73% of MBL) and excretory pore 0.222–0.250 (0.238±0.008) (12.41–15.61% of MBL) from cephalic extremity. Single flexed testis distributed posteriorly to the midbody. Spicules subequal, nonalate, distally pointed, bent ventrally; right spicule 0.150–0.302 (0.217±0.039, n=15) (9.33–17.19% of MBL), left spicule 0.192–0.302 (0.231±0.039, n=15) (11.96–18.06% of MBL). Gubernaculum length 0.050–0.065 (0.054±0.004, n=4) by width 0.005. Oblique musculature present in pre–cloacal ventral portion. Anus 1.380–1.800 (1.522±0.089) from cephalic end; tail length 0.140–0.187 (0.161±0.017) (8.28–11.19% of MBL) by width 0.025–0.045 (0.030±0.006). Caudal end bent ventrally. Narrow caudal alae present, ventral, beginning at level of nearest pair of plectanes to anus and reaching the anterior third of the tail. Caudal papillae as follows: 4 pairs of large vesiculated rosette papillae (plectanes), 1 lateral pair of large and wide papillae in the midline between the last pair of plectanes and the anus (named large papillae in this study), between the large papillae and the anus there are 4 pairs of digital-form papillae and another pair in ad-cloacal position, 1 extra pair of digital-form papillae, one on each side of the cloaca, 1 unpaired sessile papilla in posterior lip of the cloaca, 2 ventral and 1 lateral sessile pairs in the midtail, and 2 ventral digital and 1 dorsal sessile pairs at base of spine-like distal portion of tail. Phasmids minute, lateral and anterior to midtail. Distance between cephalic region and first pair of plectanes 0.887–1.207 (0.967±0.070) (54.59–61.14% of MBL); distance to second pair of plectanes 0.987–1.105 (1.078±0.072) (61.09–67.09% of MBL); distance to third pair of plectanes 1.092–1.440 (1.193±0.074) (67.60–73.29% of MBL); distance to fourth pair of plectanes 1.205–1.345 (1.304±0.074) (74.26–79.50% of MBL); distance between cephalic end and large papilla 1.292–1.482 (1.395±0.046, n=12) (83.13–85.36% of MBL).

Holotype: Body length (MBL) 1.662. Maximum width 0.077 (4.66% of MBL). Pharynx 0.017 long (1.05% of MBL) by 0.017 wide. Ratio of pharynx length to width 1.00. Esophagus length 0.265 (15.94% of MBL) by width 0.02. Esophageal bulb length 0.06 (3.61% of MBL) by width 0.055. Ratio esophageal bulb length to width 1.09. Nervous ring 0.142 and excretory pore 0.242 from cephalic extremity (8.57% and 14.59% of MBL, respectively). Right spicule length 0.215 (12.93% of MBL), left spicule length 0.215 (12.93% of MBL). Gubernaculum length 0.055. Anus 1.492 from cephalic end; tail length 0.170 (10.23% of MBL) by width 0.025. Distance between cephalic region and first pair of plectanes 0.917 (55.19% of MBL); distance to second pair of plectanes 1.032 (62.11% of MBL); distance to third pair of plectanes 1.162(69.92% of MBL); distance to fourth pair of plectanes 1.277 (76.84% of MBL); distance between cephalic end and large papilla 1.387 (83.46% of MBL).

Female (measurements of 20 specimens): Body length (FBL) 1.615–2.057 (1.872±0.108). Maximum width 0.085–0.150 (0.13±0.017) (4.63–7.56% of FBL). Pharynx length 0.012–0.020 (0.017±0.002) (0.68–1.12% of FBL) by width 0.015–0.022 (0.018±0.002). Ratio of pharynx length to width 0.75–1.80 (1.12±0.27). Esophagus 0.230–0.312 (0.274±0.026) (12.55–15.98% of FBL) long by 0.022–0.032 (0.024±0.002) wide. Esophageal bulb length 0.057–0.070 (0.064±0.005) (3.00–3.79% of FBL) by width 0.052–0.070 (0.059±0.005). Esophageal bulb length to width ratio 0.92–1.25 (1.09±0.07). Nervous ring at 0.127–0.162 (0.145±0.010, n=19) (7.14–8.53% of FBL) and excretory pore 0.215–0.275 (0.248±0.016) (11.73–14.24% of FBL) from cephalic end. Vulva ventral, with anterior lip protruded and rounded lines in its cuticle, posterior to middle of the body at 0.907–1.305 (1.075±0.080) (53.95–74.47% of BLF) from the cephalic end. Vagina first runs anteriorly, then flexed posteriorly; amphidelphic, anterior uterus directing anteriorly; posterior uterus directing posteriorly, then flexed anteriorly; each uterus with 4–7 large embryoned eggs and 1–2 larvae contained in eggs; anterior ovary flexed posteriorly and ending at vulvar level; posterior ovary ending at vulvar ending. Eggs length 0.085–0.242 (0.149±0.022, n=91) by width 0.050–0.110 (0.085±0.011, n=91). Anus at 1.437–1.820 (1.672±0.095) from cephalic end. Tail conical length 0.165–0.237 (0.199±0.02) (9.15–12.13% of FBL) by width 0.027–0.047 (0.039±0.004).

Allotype: Body length 1.912 (HBL). Maximum width 0.132 (6.93% of MBL). Pharynx long 0.02 (1.05% of MBL) by 0.015 wide. Ratio of pharynx length to width 0.75. Esophagus length 0.295 (15.42% of MBL) by

0.025 width. Esophageal bulb length 0.057 (3.01% of MBL) by width 0.062. Ratio esophageal bulb length to width 0.92. Nervous ring 0.150 and excretory pore 0.252 from cephalic extremity (7.84% and 13.20% of MBL, respectively). Vulva at 1.080 from anterior end (56.47% of HBL). Eggs length 0.012–0.014 by width 0.007–0.01. Anus 1.695 from cephalic end; tail length 0.217 (11.37% of MBL) by 0.032 width.

Taxonomic summary.

Type host: *Diaglena spatulata* (Günther).

Type locality: Tapalcatepec-Jilotlan Road, Jalisco, Mexico (19° 13' 57" N, 102° 51' 52" W, elevation 538 m).

Other records: *Triprion petasatus* (Cope) from Rancho Hobonil (20° 00' 06" N, 89° 02' 30" W, elevation 80 m) (paratypes). *Diaglena spatulata* from Las Palmas-La Estancia Road, Jalisco (20° 49' 08'' N, 104° 58' 21'' W, elevation 538 m) and Talpa-Tomatlan Road, Sierra Cacoma, Jalisco (20° 03' 17'' N, 104° 51' 08'' W, elevation 337 m) (vouchers).

Site of infection: large intestine.

Prevalence and intensity: Eight specimens of *D. spatulata* examined, 6 parasitized (66.6%); 338 nematodes in 6 frogs (56.3). Six specimens examined of *T. petasatus*, 5 infected (83%); 126 worms in 5 frogs (25.2).

Deposited specimens: Holotype: CNHE-6267. Allotype: CNHE-6268. Paratypes: CNHE-6269 (in *D. spatulata* from type locality) and CNHE-6270 (from *T. petasatus*), HWML-48959 (from *D. spatulata*) and HWML-48960 (from *T. petasatus*). Vouchers: CNHE-6271 and CNHE-6272 both in *D. spatulata* from Las Palmas-La Estancia Road and in Talpa-Tomatlan Road, Jalisco, respectively.

Etymology: The new species is named after one of its host genera, *Diaglena*.

Remarks. *Cosmocercella* includes nematode species parasitic in hylids and snakes in the Americas and Asia. In Mexico, two *Cosmocercella* species have been recorded: *C. haberi* and *C. anothecae* from hylids. *Cosmocercella haberi* is widely distributed in North America parasitizing hylid frogs: *Hyla arenicolor*, *Hyla chrysocelis*, *Hyla plicata*, *Hyla versicolor*, *Hyla wrightorum*, *Lithobates pipiens*, *Pachymedusa dacnicolor* and *Pternohyla fodiens* (Baker, 1987; Goldberg *et al.*, 1996; Goldberg *et al.*, 1999; Creel *et al.*, 2000; Goldberg & Bursey, 2002; Goldberg *et al.*, 2003). *Cosmocercella anothecae* has been recorded only in *Anotheca spinosa* from Mexico State, Mexico (Baker & Adamson, 1977).

The distinct characteristic of the genera in Cosmocercidae is the presence of ventral vesiculated papillae in precloacal position (Chabaud, 1978). However, there is a large variation in this feature among *Cosmocercella* species. *Cosmocercella phyllomedusae* possess both vesiculated and rosette papillae (this last kind of papilla is characteristic of species in the genus *Cosmocercoides*) and the papillae present in *C. phrynomantisi* are structured by a ring of minute punctuations surrounding the top of each papillae, which is considered by Moravec (1990) as a kind of inter-stage between rosette and vesiculated papillae; remainder species of *Cosmocercella* possess evident vesiculated papillae.

Cosmocercella neveri and *C. uropeltidarum* possess numerous somatic papillae along the entire body; besides, the first species has alate spicules and smaller and more numerous eggs than other *Cosmocercella* species; the latter species has a wide lateral wing and caudal external appendices. Additionally, *C. iwatzukii* lacks gubernaculum and caudal alae. Specimens of the new species differ from these species by lacking somatic papillae, by having gubernaculum and analate spicules, by showing evident caudal alae, and but having few large eggs in the uterus of females.

Cosmocercella minor, *C. haberi*, *C. anothecae* and *C. diaglenae* possess 4 pairs of precloacal vesiculated papillae and caudal alae. *Cosmocercella minor* is distinguished from them in that it has shorter pedunculate papillae within smaller caudal alae, and the vesiculated papillae show surface swellings of the cuticle. The other three species have large oval structures deeply embedded in the hypodermis (Baker & Vaucher, 1983) (Figure2). Further, *C. minor* shows 20–22 papillae distributed on the tail, whereas the other species have no more than 12 papillae.

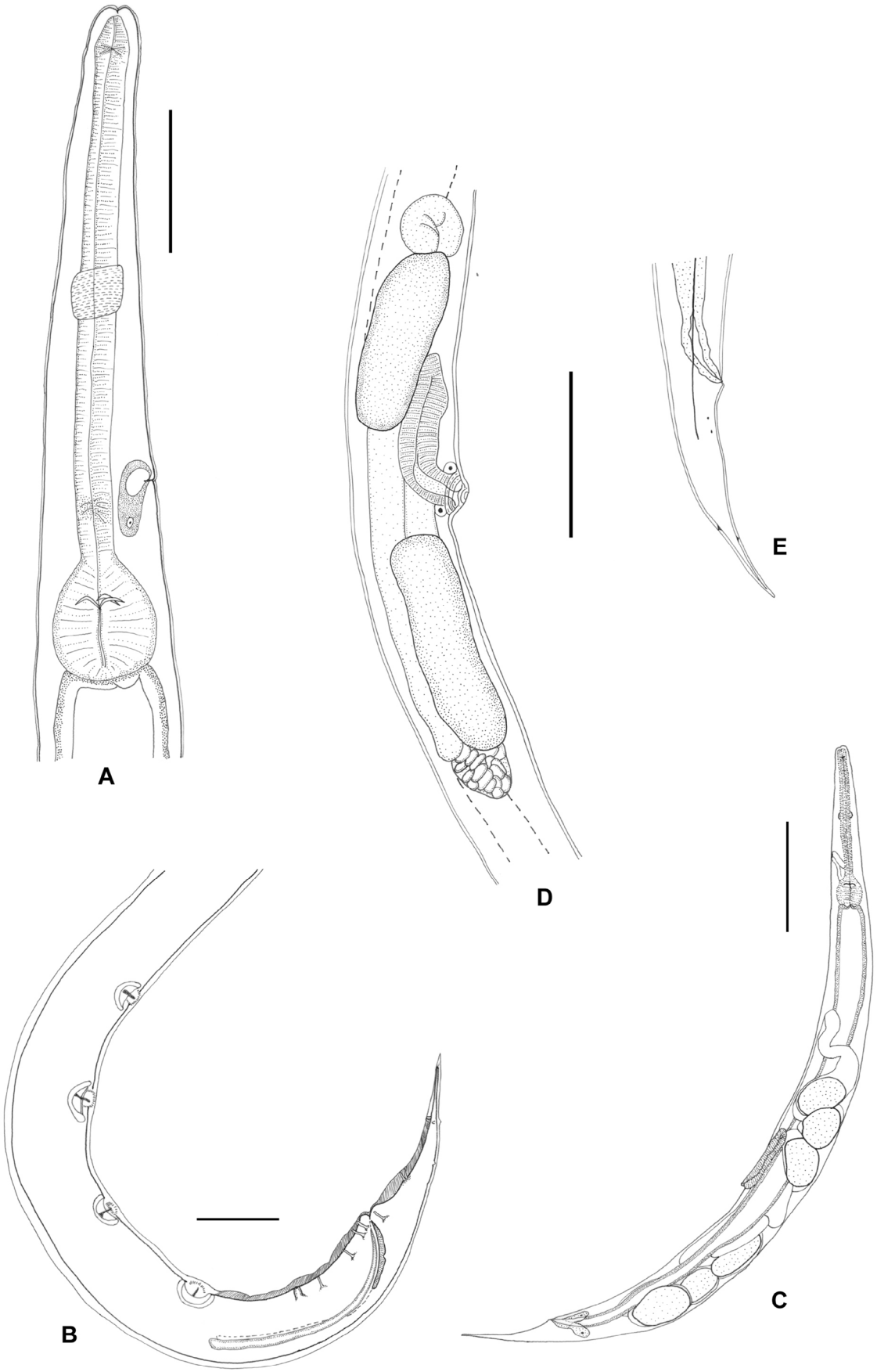

FIGURE 1. *Cosmocercella diaglenae* **A**. Male anterior region. **B**. Caudal male region. **C**. Female lateral view. **D**. Reproductive female system showing vulva, vagina and eggs. **E**. Female tail.

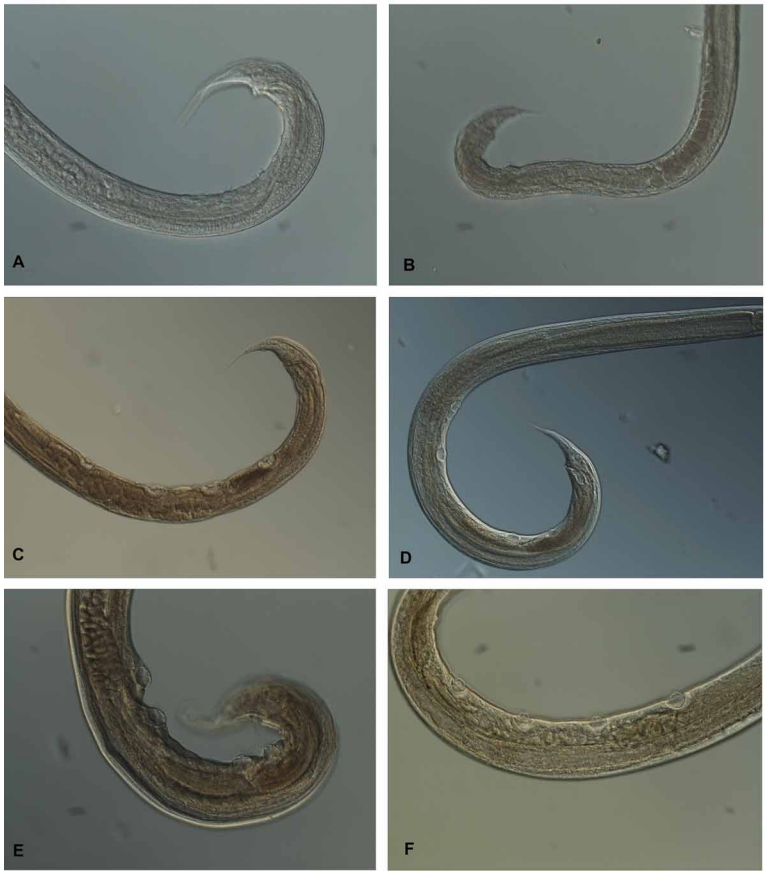

FIGURE 2. Male posterior region. **A**. *Cosmocercella iwatsukii*. **B**. *C. minor.* **C**. *C. anothecae*. **D** and **E**. *C. haberi*. **F**. *C. diaglenae*.

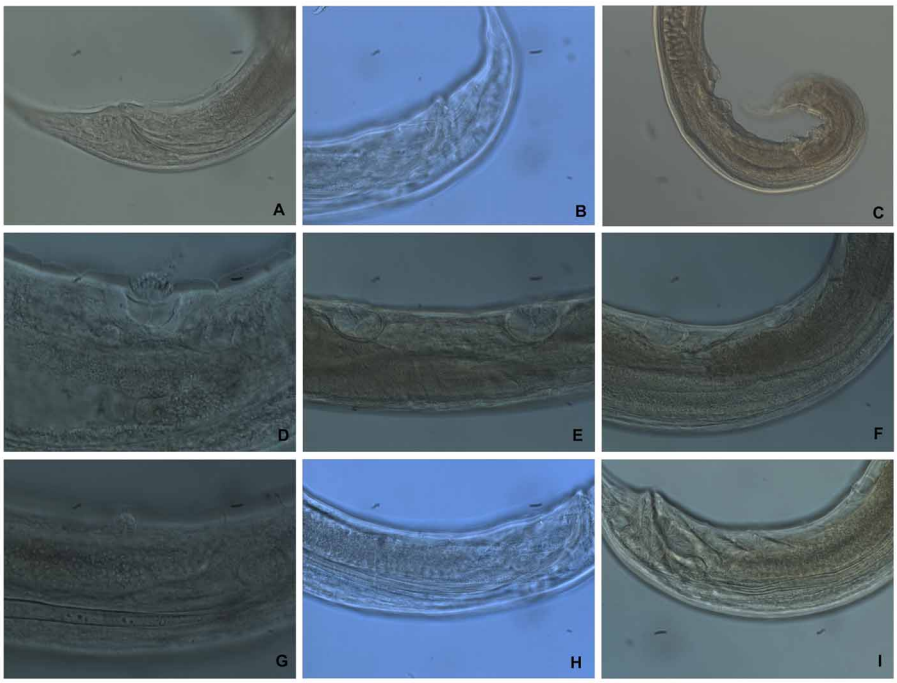

FIGURE 3. Caudal region, Plectanes and large papilla. **A**, **D** and **G**. *C. diaglenae*. **B**, **E** and **H**. *C. anothecae*. **C**, **F** and **I**. *C. haberi*, respectively.

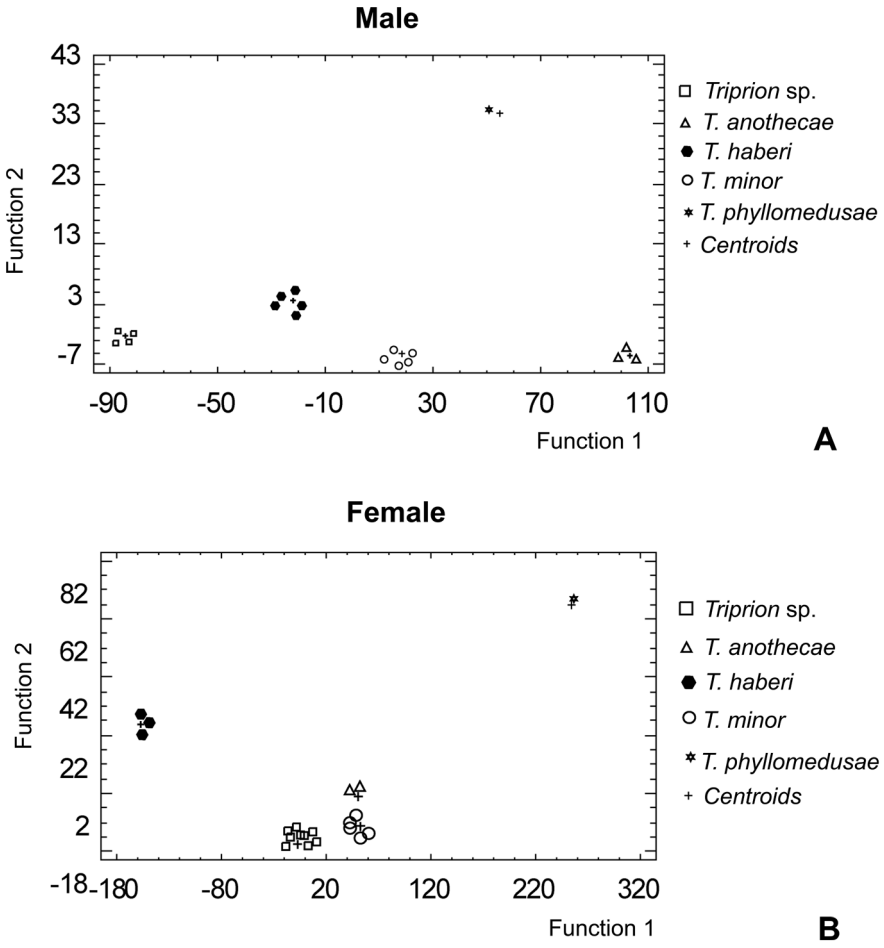

FIGURE 4. Discriminat analyses of the spicule length in males **A** and the esophageal bulb ratio in females **B** in *Cosmocercella* spp.

The new species, *C. diaglenae*, resembles *C. haberi* and *C. anothecae* in the form and number of caudal papillae. It shares the characteristic of possessing 5 precloacal digitiform papillae with *C. haberi*, but the first pair of these, near to proximal pair of plectanes is different. This pair of papillae is larger than that in *C. haberi* (Figure 3D–F). *Cosmocercella anothecae* lacks this kind of papilla, and it has 6 pairs of postcloacal papillae. Other evident difference among these species is the distance between each pair of plectanes. *Cosmocercella haberi* has the second and third pairs of plectanes widely separated one from each other (Figure2, D and E), in this aspect, *C. anothecae* and *C. diaglenae* are similar. These species show the same number of plectanes in a similar position (Figure 2, C and F, respectively), but they are different in the arrangement, number and position of the caudal papillae as we indicated before, and in the size and form of the pharynx, spicules and gubernaculum. *Cosmocercella anothecae* has rounded pharynx instead of the rectangular pharynx in *C. diaglenae* and the spicules and gubernaculum (0.261–362 and 0.059–0.074, respectively) are significantly longer than in *C. diaglenae* (0.150–0.302 and 0.05–0.065, respectively). In contrast, the eggs in *C. diaglenae* are larger (0.085–0.242 x 0.062–0.075) than those in *C. anothecae* (0.148–0.187 x 0.062–0.075).

Twenty different anatomical characters from specimens of *Cosmocercella* spp. were used for the statistical analysis in females and 18 in males. After proved coliniality between variables and in order to conduct a discriminant analysis only 15 and 13 characters were used in females and males, respectively. The classification success of the discriminant analysis indicates a high separation between species (Wilk´s statistic λ= 3.1106; p= 0.000 to males and λ= 1.0150; p= 0.000 to females), all individuals in both sexes (100%) were correctly

classified. Discriminant analysis confirmed differences present in anatomical observations between females and males from the different species (Figure 4). The first eigenvalue is very large in comparison with the second one in both females (93.70% vs 5.69) and males (96.81% vs 2.24), indicating that the first axis captures most of the variance among species. The most relevant anatomical characters to differentiate between species were the esophageal bulb ratio in females and the spicule length in males. The esophageal bulb ratio in females is 1.07–1.08 in *C. haberi*, 0.97–1.03 in *C. anothecae*, while in *C. diaglenae* is 0.92–1.25. As we cited before; the spicule length in these species is 0.261–0.362 in *C. haberi*, 0.406–0.511 in *C. anothecae* and 0.150–0.302 in *C. diaglenae*. This analysis shows that reproductive structures are relevant in the identification of different species in this group of parasites, and spicule length may act as a reproductive isolation factor between them.

Finally, by combining both kinds of information we can distinguish *C. diaglenae* from the other *Cosmocercella* species by the possession of a pair of precloacal large papillae, by having equidistant plectanes, by the length of the caudal alae, that begins at level of most posterior pair of plectanes and reaches the end of the first third of the tail and the length of the spicules in the males, and in the females mainly by the length/width ratio of the esophagus bulb.

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