

# ***Coronostoma claireae* n. sp. (Nematoda: Rhabditida: Oxyuridomorpha: Coronostomatidae) from the Indigenous Milliped *Narceus gordanus* (Chamberlain, 1943) (Diplopoda: Spirobolida) in Ocala National Forest, Florida**

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**Abstract:** Twenty-four individuals of *Narceus gordanus* (Diplopoda: Spirobolidae) were collected in Ocala National Forest, FL, between November 2013 and July 2014. Each specimen was dissected to extract the intestine, which was removed and examined for parasitic nematodes. *Coronostoma claireae* n. sp. was collected from the hindgut and midgut of 10 specimens, and its morphology was examined with brightfield, differential interference contrast, phase contrast, and scanning electron microscopy. This species is separated from other *Coronostoma* spp. by the following characteristics: body length less than 3 mm; head sense organs pit-like; first annule long, extending past middle of corpus, width similar to that of second annule; basal bulb pyriform; eggs larger than 60 × 50 μm. This species is the first North American record for the genus *Coronostoma*, which is removed from Thelastomatoidea: Thelastomatidae and reassigned to Coronostomatidae on the basis of presumed apomorphies. A key is provided for known *Coronostoma* spp. The superfamily Coronostomatoidea is re-established for Coronostomatidae and Traklosiidae.

**Key words:** host-parasitic relationship, key, morphology, predator, nematophagy, scanning electron microscopy (SEM), Spirobolida, taxonomy, Thelastomatoidea, United States.

Nematodes that parasitize diplopod intestines are Rhabditida, primarily in the infraorders Rhigonematomorpha and Oxyuridomorpha. The latter infraorder consists of intestinal parasites of both vertebrates and invertebrates. The invertebrate parasites typically are placed in the superfamily Thelastomatoidea, which are well represented in milliped intestines. Both infraorders are most often reported from tropical and subtropical regions (Hunt, 1998; Carreno et al., 2013). Their phylogenetic position has been well established within clade III of Nematoda, but they are not considered to be monophyletic (Adamson, 1989; Adamson, 1994; Blaxter et al., 1998; Nadler et al., 2007). Most nematodes that inhabit the gut of diplopods feed on bacteria (Jex et al., 2005); however, species of *Coronostoma* Rao, 1958 are believed to be nematophagous (van Waerebeke, 1986; van Waerebeke and Adamson, 1986; Jex et al., 2005).

*Coronostoma* previously has been observed exclusively in spirostreptidan millipeds from Brazil (Kloss, 1961), Madagascar (van Waerebeke, 1986; van Waerebeke and Adamson, 1986), Burkina Faso (Van Waerebeke and Adamson, 1986), India (Rao, 1958), and from an Australian cockroach (Jex et al., 2005).

Between November 2013 and July 2014, we collected 24 specimens of *Narceus gordanus* (Chamberlin, 1943) (Diplopoda: Spirobolida) from Ocala National Forest, Florida. The intestines of 10 individuals contained a new species of *Coronostoma*, which we name *C. claireae*.

This new species is the first record of *Coronostoma* in North America and the first report of a *Coronostoma* sp. from a non-spirostreptidan milliped.

## MATERIALS AND METHODS

Specimens of *N. gordanus* ( $n = 24$ ) were collected between December 2013 and July 2014 in Ocala National Forest, Marion County, FL (29.210833 N, -81.770556 W, elevation 30.4 m). Sex and morphometric data (weight, length, width) were recorded for each milliped. The specimen was then decapitated with a razor blade and the telson was severed. The intestinal tract was removed with the aid of fine-tipped forceps and placed in distilled water, then dissected. Nematodes were separately collected and processed by sequential dissection from the three intestinal regions (Crawford et al., 1983): (i) hindgut – rectum to ileum, (ii) midgut – pyloric region to posterior section of foregut, and (iii) foregut. The intestine was dissected from posterior to anterior and examined for nematodes, gregarines, trichomycetes, acanthocephalans, and ciliated protists with the aid of a Zeiss Stemi 2000 stereo microscope. Collected nematodes were sorted to morphotaxa from each intestinal section, counted, and identified to stage (adult females, adult males, and juveniles). Voucher milliped and nematode specimens are deposited in the Entomology and Plant Pathology Department, University of Tennessee, Knoxville, TN.

Most nematodes were prepared for permanent preservation in glycerin, while others were preserved for molecular analyses or for scanning electron microscopy (SEM). For permanent preservation, nematodes were placed into distilled water, fixed with 4% hot formalin, processed to glycerin by means of a rapid method (Seinhorst, 1959), and mounted in anhydrous glycerin on glass slides. All images were made from glycerin-mounted specimens. Most images were produced with a 14-megapixel Q-camera on an Olympus

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TABLE 1. Morphometrics of female *Coronostoma claireae* n. sp.

Character	Holotype female	Paratype females (n = 13)	
		Mean	Range
Measurements ( $\mu\text{m}$ )			
Length	2,359	2,234	1,672–2,674
Maximum width	192	182.6	129–277
First annule width	87.0	83.5	71–97
Second annule width	13.9	10.3	7–14
Body annule width	13.6	13.3	9–17
Esophagus length	203	194	177–213
Corpus length	103	100	90–112
Bulb length	94.8	96.1	87–104
Distance of excretory pore to anterior end of head	444	375	322–417
Distance of vulva to anterior end of head	1,190	1,030	797–1,250
Distance of anus to anterior end of head	1,823	1,617	1,178–2,057
Egg length	61.6	70.3	59–80
Egg width	49.7	48.2	44–50
Tail length	593	625	499–892
Ratios			
a	12.3	12.8	9.4–20.8
b	11.6	11.6	9.2–15.1
c	4.0	3.6	3.0–4.1
V (%)	50.4	46.6	40–51
V'	0.65	0.64	0.59–0.68

BX-63 DIC microscope system; Figs. 5D and E were obtained with a 17-megapixel DP-73 camera on an Olympus BX-53 phase-contrast microscope. Terminology for stomal structures follows De Ley et al. (1995). Terminology for arcade-like cells and coelomocytes follows Peregrine (1973), Tahseen (2009), and Weinstein (2006). Measurements given in Table 1 were made from glycerin-preserved specimens.

For scanning electron microscopy, formalin-fixed nematodes were washed in distilled water for 20 minutes then dehydrated in a 30- $\mu\text{m}$  microporous specimen capsule (Electron Microscopy Services, Hatfield, PA) using a graded series consisting of 25%, 50%, 75%, 95%, and 100% ethanol, each for 20 minutes. Afterwards, a 1:1 mixture of 100% ethanol and reagent grade hexamethyldisilazane (HMDS) was used in lieu of a critical point dryer. The HMDS series consisted of 25%, 75%, 100%, and a second 100% HMDS dehydration, each for 20 minutes. Nematodes were placed on carbon tape affixed to aluminum stubs and sputter-coated with gold for 10 seconds at 20  $\mu\text{A}$  in a SPI-Module Sputter Coater (West Chester, PA). Specimens were viewed with a Hitachi TM 3030 electron microscope.

## RESULTS

### Systematics

Coronostomatoidea (Kloss, 1961) Poinar, 1977

Coronostomatidae Kloss, 1961

### Emended description

Obligate inhabitants of the intestine of arthropods. Cuticle without spines. Oral aperture surrounded by 12 equal lobes and an inner ring (*corona radiata*) of numerous

setiform or plate-like projections; four inconspicuous to elongated cephalic papillae; amphid aperture near the tip of a projecting conical horn-like structure. Stoma without conspicuous teeth, but subventral stegostomal sectors transverse, plate-like, multi-denticulate. First annule three or more times wider than other annules. Esophagus massively muscular, composed of a procorpus and basal bulb with or without a short, broad isthmus; grinding valves absent. Secretory-excretory system X-shaped (oxyuroid type) with prominent excretory cell and canals. Reproductive system amphidelphic, vulva transverse and in middle of body, vagina directed anteriorly; each gonad with multiple flexures; zero, one or both spermathecae with sperm, usually posterior gonad with sperm, anterior gonad often lacking sperm. Tail long, tapering, pointed. Egg surface smooth or with small blebs, without filaments.

Male shorter and more slender than females, esophageal isthmus longer. Anterior end without elaborate *corona radiata* but with amphidial horns. Genital cone projecting with basal and subapical pairs of papilliform supplements; tail with pair of supplements at midpoint. Spicules, gubernaculum and bursa absent. Sperms broadly to narrowly oval.

*Sole genus:* *Coronostoma* Rao, 1958

*Synonym:* *Laticorpus* van Waerebeke, 1969 (van Waerebeke, 1986)

*Type species:* *C. singhi* Rao, 1958

### Other species

*Coronostoma australiae* Jex, Schneider, Rose and Cribb, 2005

*C. bulbicorpus* Kloss, 1961

*C. claireae* n. sp.

*C. dentata* van Waerebeke and Adamson, 1986

*C. diplopodicola* (Dollfus, 1964) van Waerebeke, 1986

*C. gautuni* van Waerebeke and Adamson, 1986

*Hosts*: Diplopoda of the orders Spirostreptida, Spirobolida and Polydesmida.

*Coronostoma* was established by Rao (1958) for a new species of nematode (*C. singhi*) inhabiting the intestine of a spirostreptid millipede from Andhra Pradesh, India. He placed this taxon in Aoruridae on the basis of the female esophageal shape and tail morphology of the male. Kloss (1961) erected Coronostomatidae for *Coronostoma*, with a very short diagnosis: female procorpus

bulbiform, muscular; female esophageal bulb well-developed but without grinding valves; male lacking spicules, gubernaculum, and preanal cup; eggs smooth.

*Coronostoma claireae* Phillips & Bernard, n. sp.  
(Table 1; Figs. 1–5)

*Description*

*Female* (n = 13): Measurements are listed in Table 1.

*Type locality and habitat*: Ocala National Forest, FL, 29.210833 N, -81.770556 W, elevation 30.4 m, sand

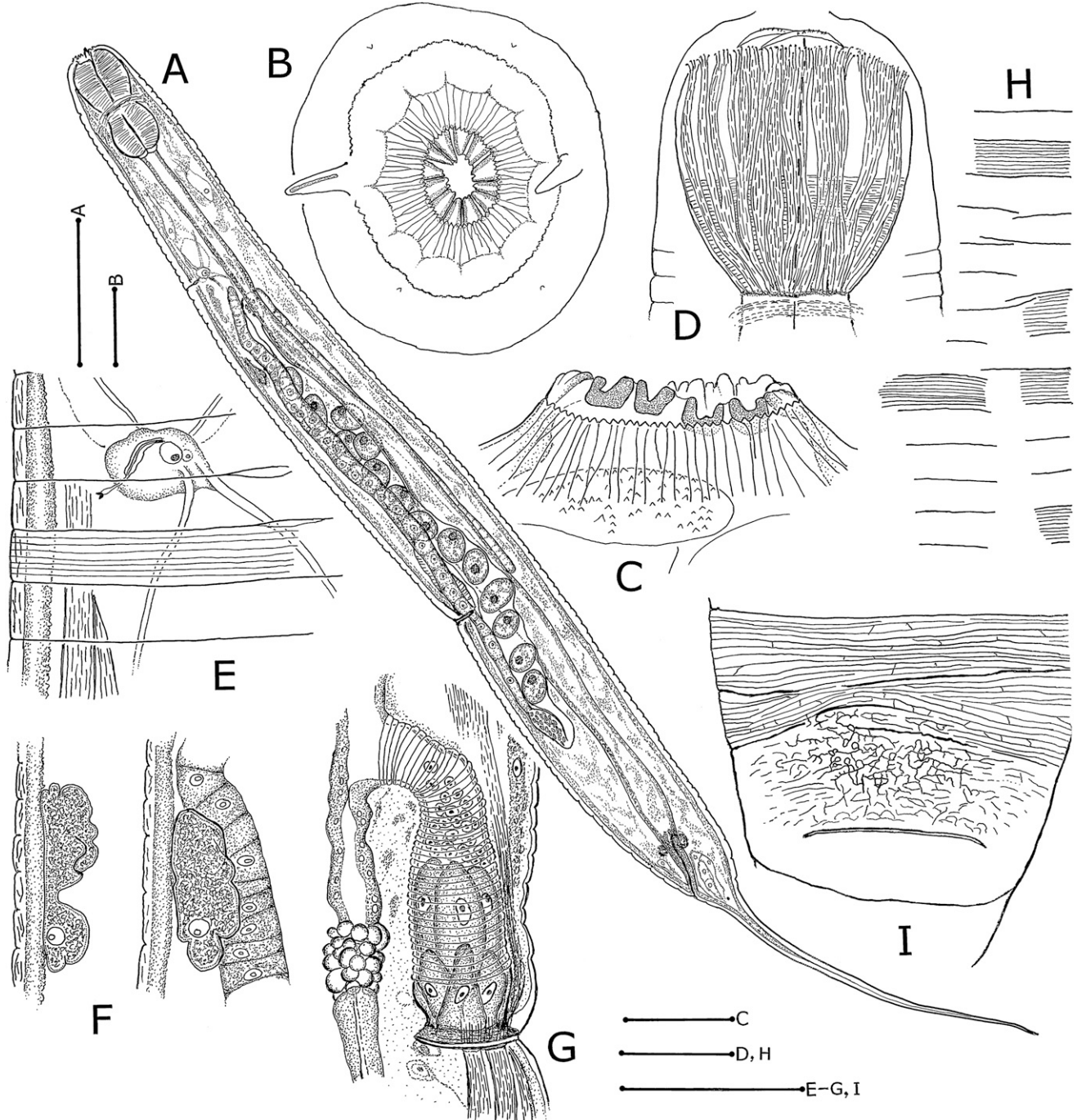


FIG. 1. *Coronostoma claireae* n. sp. A) Female. B) En-face view. C) *Corona radiata*. D) Longitudinal muscles surrounding procorpus, procorpal radial muscles partially drawn. E) Secretory-excretory system complex. F) Giant coelomocytes near anterior ovary. G) Base of reproductive system of young female. H) Lateral field posterior to basal bulb. I) Cuticular region around vulva, ventral view. Scales: A, 250  $\mu$ m; B, C, 10  $\mu$ m; D–I, 50  $\mu$ m.

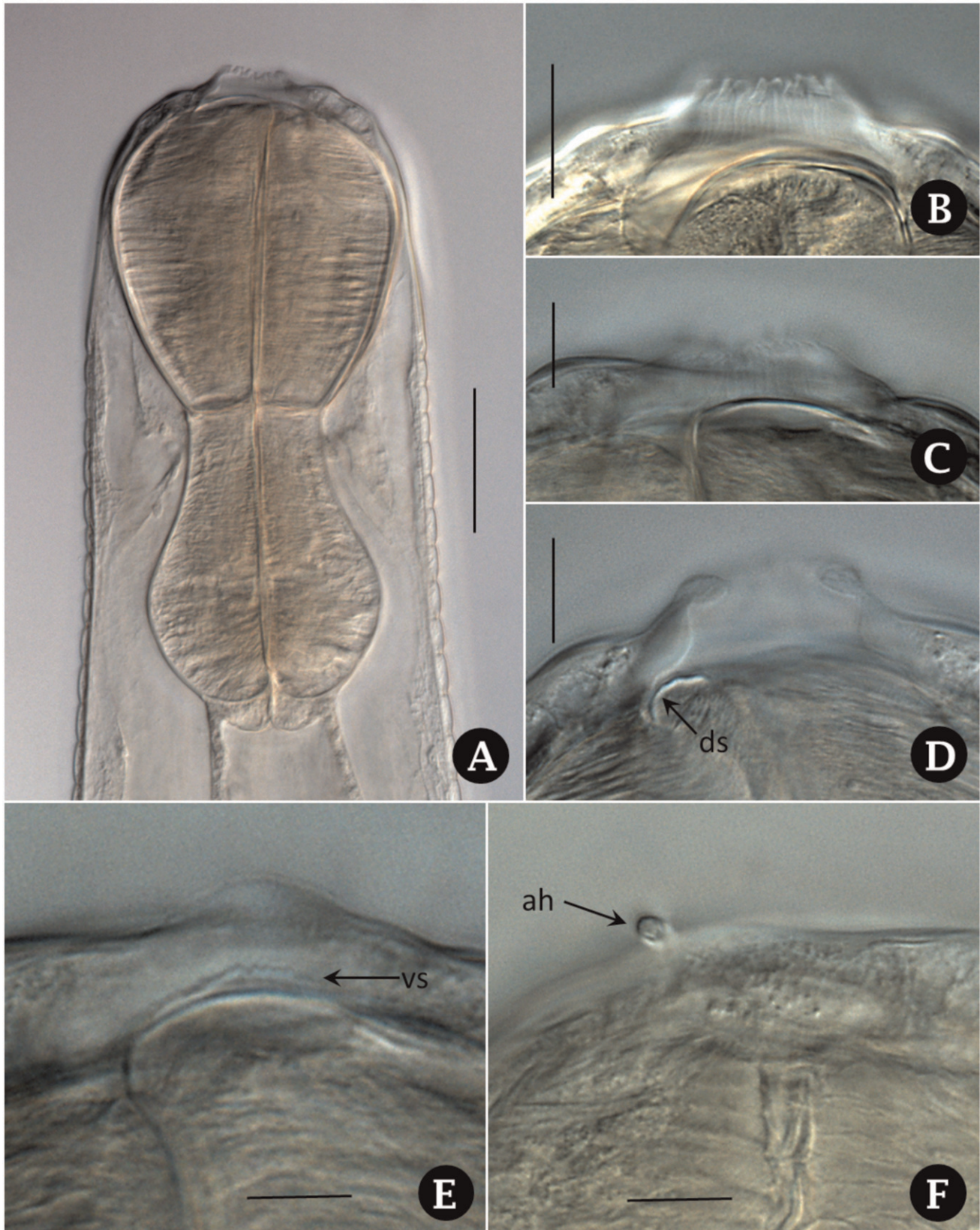


FIG. 2. *Coronostoma claireae* n. sp. A) Anterior region. B) *Corona radiata* and associated ridges. C) Serrated oral margin. D) Anterior region of stoma with profile of dorsal stegostomal plate (ds). E) Subventral stegostomal plate (vs) showing denticles in profile. F) Subventral stegostomal plate, oblique view, with numerous denticles; ah: amphidial horn. Scales: A, 50 μm; B, 20 μm; C–F, 10 μm.

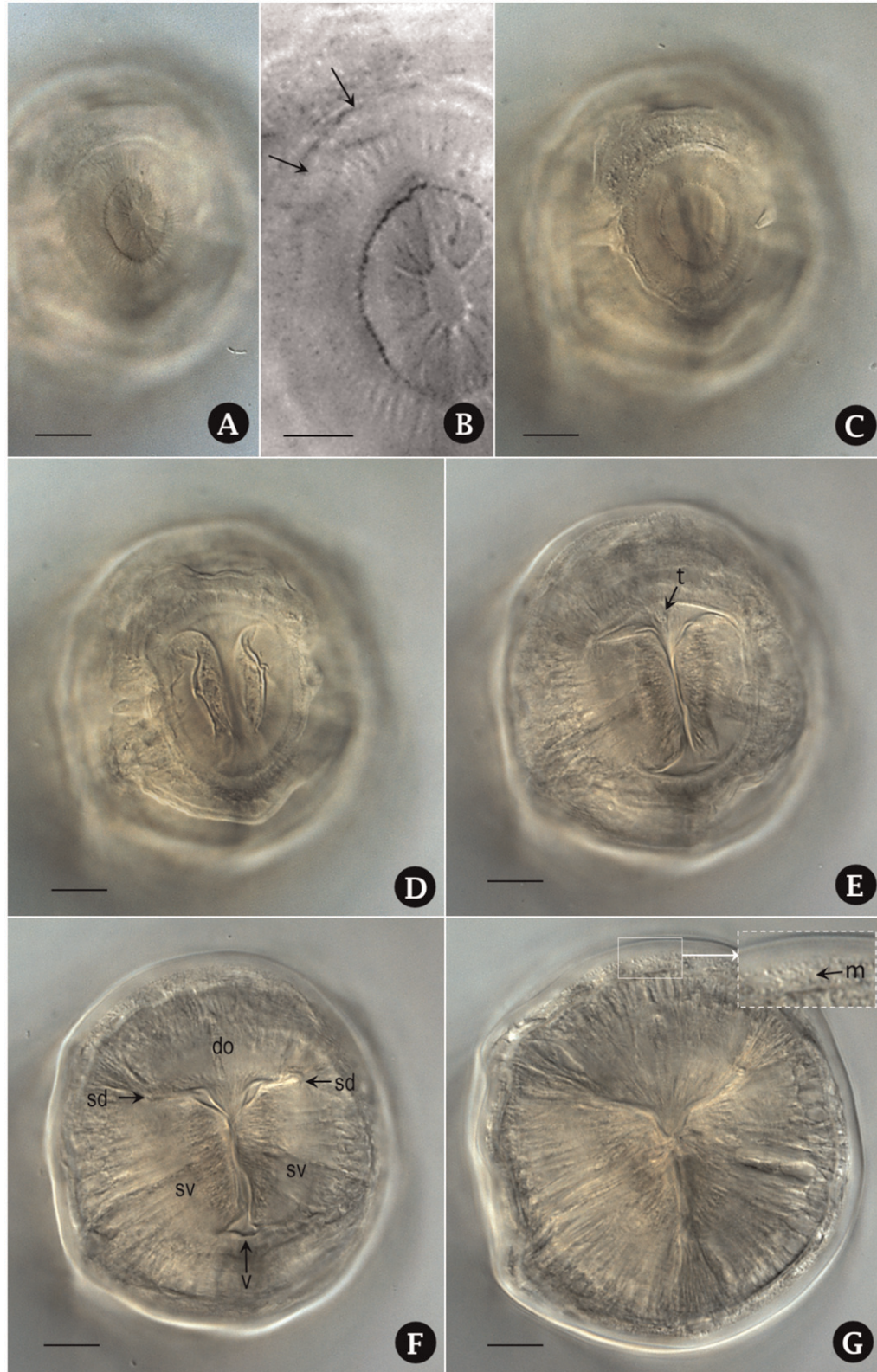


FIG. 3. *Coronostoma claireae* n. sp., optical cross-sections through first 23 μm from anterior end. All images oriented dorsal side up. A) *En-face* view of anterior end. B) Close-up of *en-face* view showing serrated oral margin, fine ridging on oral disc, and weakly lobed edge of oral disc (two of 12 lobes indicated by arrows). C) Cross-section 4 μm below anterior end, interior serrated oral lining and amphidial horns visible. D) Cross-section 6 μm below anterior surface, showing denticulated subventral stegostomal plates. E) Cross-section 12 μm below surface, with small dorsal sector and large subventral sectors; dorsal sector with small tooth (t). F) Cross-section 16 μm below anterior surface, ventral arm (v) of esophageal lumen much longer than subdorsal (sd) arms; dorsal muscular sector (do) much smaller than subventral muscular sectors (sv). G) Cross-section 23 μm below anterior surface, esophageal muscle sectors approximately equal in size. Inset: longitudinal muscle fibers (m) around periphery of esophagus.

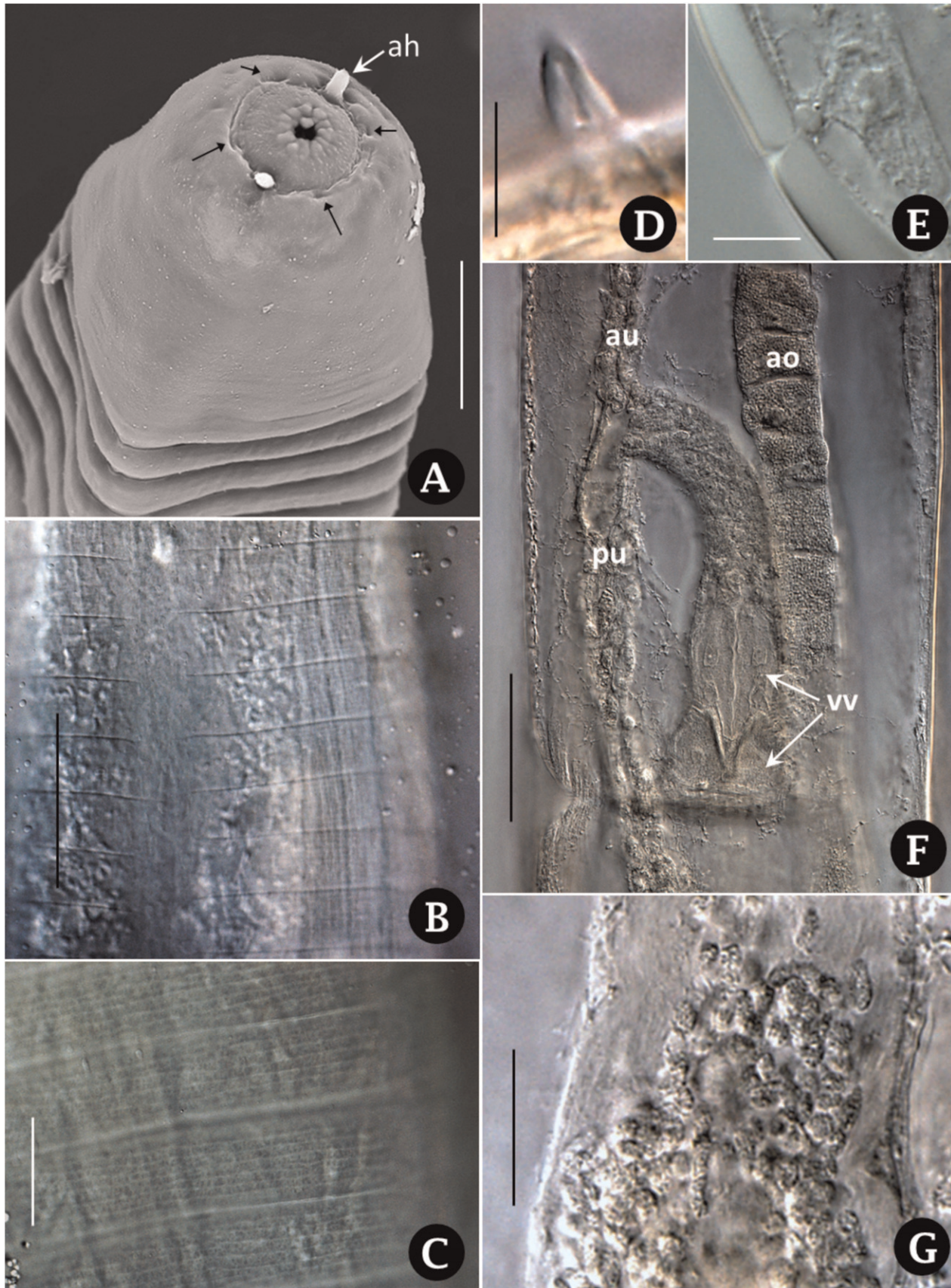


FIG. 4. *Coronostoma claireae* n. sp. A) Scanning electron micrograph of anterior end; ah: amphidial horn. Small arrows indicate pore locations. B) Lateral field of interrupted annules. C) Annules posterior to vulva with fine transverse lines. D) Amphidial horn with aperture. E) Phasmid pore and associated subculticular body. F) Basal region of reproductive system, ventral view; vv: cells of *vagina vera*; au: anterior uterus; ao: reflexed anterior ovary; pu: posterior uterus. G) Portion of posterior spermatheca with sperm. Scales: A, B, F, 50  $\mu$ m; C, G, 20  $\mu$ m; D, E, 10  $\mu$ m.

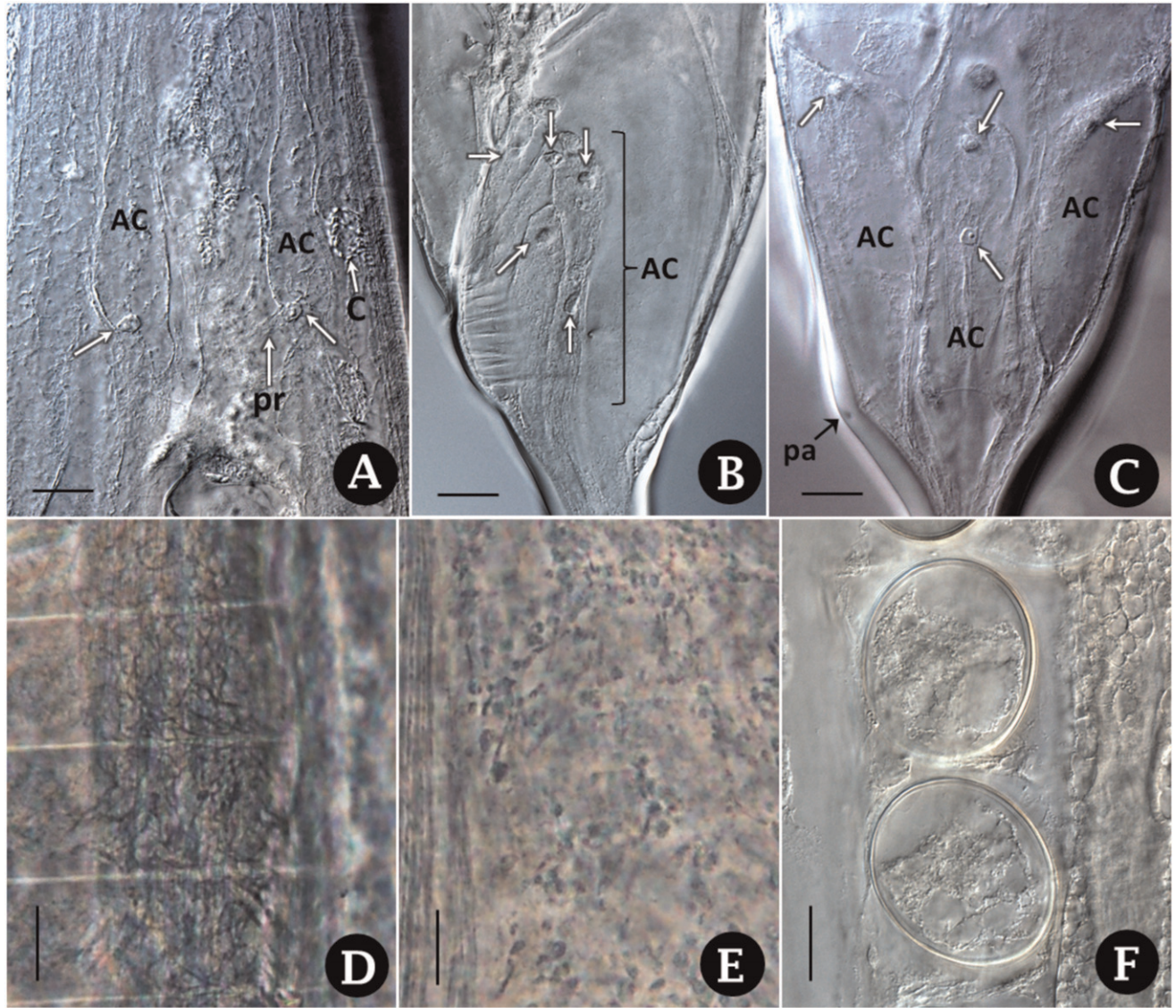


FIG. 5. *Coronostoma claireae* n. sp., internal, cuticular and subcuticular features. A) Arcade-like cells and multi-vesiculate coelomocyte between basal bulb and gonad region. B) Posterior arcade-like cells associated with anus and tail, lateral view. C) Posterior arcade-like cells associated with anus and tail, ventral view. D) Net-like structures of presumed medial zone of cuticle, 2  $\mu\text{m}$  below surface. E) Non-muscular dorsal region with numerous irregular bodies, 4  $\mu\text{m}$  below surface. F) Eggs. AC: arcade-like cell; C: multivesiculate coelomocyte; pa: phasmid aperture; pr: process extending from AC toward intestine. Arrows without labels indicate relevant nuclei. Scales: A–C, F, 20  $\mu\text{m}$ ; D, E, 10  $\mu\text{m}$ .

pine scrub ecoregion. Dissected from the intestine of *N. gordanus* (Spirobolida: Spirobolidae).

*Type designation and deposition:* Holotype female (Accession number T-695t) and two paratypes (Accession numbers T6771p–T6772p) deposited in the USDA Nematode Collection, Beltsville, MD. Seven paratypes deposited in the nematode collection in the Entomology & Plant Pathology Department, University of Tennessee, Knoxville, TN.

*Description of females:* Body cylindrical, stout, head end rounded, tail abruptly narrowing behind anus tapering to a long filiform tip (Fig. 1A). First head annule very large (71–97  $\mu\text{m}$ ) reaching to three-fourths of the procorpus (Figs. 1D; 2A; 4A); remaining body annules 9–17  $\mu\text{m}$  wide, each annule with 7–10

fine transverse lines (Figs. 1E,H; 4C); annule size and line number becoming less regular near anus, cuticle anterior to anus with pattern of short lines, forks and whorls (Fig. 1I). Body without lateral alae; lateral field indicated by annule breaks or anastomoses beginning posterior to esophagus (Figs. 1H; 4B). In cross-section, cuticle with short longitudinal lines in each annule (Fig. 1E), lines net-like in tangential view (Fig. 5E). Body musculature in long bands of >200  $\mu\text{m}$ , diagonal muscles not evident. Numerous minute cell-like hypodermal bodies just below cuticle in dorsal and ventral views (Fig. 5E).

Head end with two protuberant amphidal horns, amphidal apertures longitudinal, slit-like (Figs. 1B; 2F; 3C; 4A,D). Oral opening surrounded by cheilostom

forming a *corona radiata* of 12 entire or bifurcate plates, extending from finely serrated rim (Figs. 1B,C; 2B–D; 3A–C; 4A). Between *corona radiata* and amphidial horns, lip region divided into 12 indistinct sectors (Figs. 1B; 3B). Four minute cephalic pits on anterior end of large head annule (Figs. 1B; 4A). Stoma very shallow, cheilostom and gymnostom fused (Fig. 2D). Subventral stegostomal sectors basally with flattened, enlarged, obliquely oriented plates, covered on anterior surface with numerous minute denticles (Figs. 1C; 2E,F; 3D); dorsal metastegostomal plate smaller (Fig. 1D), with one tooth (Fig. 3E).

All parts of esophagus muscular. Procorpus swollen, slightly larger than pyriform basal bulb, isthmus present but short, grinding valve absent, corpus cardiacum prominent (Fig. 2A). Procorpus with longitudinal bands of muscle overlaying transverse muscles (Fig. 1D) anchored at isthmus and almost at level of stegostomal plates (Fig. 3G). Ventral arm of esophageal lumen much wider than subdorsal arms in anterior part of procorpus (Fig. 3F), arms of equal width more posteriorly (Fig. 3G). Nerve ring encircling esophagus at isthmus. Secretory-excretory system distinct, X-shaped, excretory cell and nucleus large, excretory canal and pore minute, generally just anterior to flexure of anterior gonad (Fig. 1E). Intestinal epithelium composed of discrete polygonal cells. Large arcade-like cells present in pseudocoelom posterior to basal bulb and surrounding anus (Figs. 5A–C); coelomocytes present but not fully catalogued; multilobed giant coelomocyte associated with anterior portion of gonad (Fig. 1F), multivesiculate coelomocyte near anterior arcade-like cells (Fig. 5A).

Vulva at about two-thirds of the head-to-anus length. Vulva transverse, anterior lip not extending as flap over posterior lip. Vagina long, directed anteriorly, with a *vagina vera* composed of two groups of four large cells each (Figs. 1G; 4F). In young females anterior gonad on right side of body, posterior gonad on left side; in older females gonads longer and not strictly confined to one side or the other due to flexures. In mature females each gonad reflexed at least once; spermatheca when present occurring in reflexed region. Presence of distinct spermatheca with sperm variable; of 11 females, three without distinct spermatheca with sperm, five with filled posterior spermatheca, three with both spermathecas filled. Sperms narrow-oval, variable in shape, presumed amoeboid (Fig. 4G). Eggs broadly oval, outer shell usually smooth (Fig. 5F), occasionally eggs with blebs on shell.

Phasmid aperture a minute pore posterior to anus, usually with associated small, spherical, subcuticular chamber (Figs. 4E; 5C).

Males not known.

*Differential diagnosis:* With the inclusion of the new species, there are now seven described species of *Coronostoma*. Discrimination of these species in earlier papers was partly reliant on doubtful characters such as

position of the nerve ring, length of the gonads, and number of ovarian flexures. Other characters that may be valid will need to be re-evaluated by examination of type material or new specimens. For instance, Rao (1958) and van Waerebeke and Adamson (1986) depicted *C. singhi*, *C. gautuni*, and *C. dentata*, respectively, as having four prominent head papillae in addition to the amphidial horns, whereas *C. claireae* n. sp. has minute pits. In the *en-face* view of *C. australiae*, Jex et al. (2005) placed the amphidial horns and cephalic sense organs within the lip region; this interpretation may have been due to the SEM image of a severely collapsed specimen. The characteristics used in the key are those that are obvious from the relevant illustrations or are measurements that have enough separation to be useful.

#### Key to species of *Coronostoma* Rao, 1958

1. Esophagus with short to moderate isthmus, basal bulb pyriform..... 2
- 1' Esophagus without discernible isthmus, basal bulb subspherical..... 6
2. First annule (mega-annule) reaching two thirds or more of corpus..... 3
- 2' First annule reaches only to middle of corpus.....  
..... *C. gautuni*
3. Diameter of first annule swollen, wider than succeeding annules ..... *C. dentata*
- 3' Diameter of first annule width similar to succeeding annules ..... 4
4. Body length at least 4.5 mm ..... *C. singhi*
- 4' Body length less than 3 mm ..... 5
5. Tail length less than 675  $\mu\text{m}$ , eggs larger than  $60 \times 50 \mu\text{m}$ ; head sense organs as minute pits..... *C. claireae* n. sp.
- 5' Tail length greater than 700  $\mu\text{m}$ , egg size less than  $56 \times 41 \mu\text{m}$ ; head sense organs as protuberant papillae ..... *C. diplopodicola*
6. Basal bulb larger and wider than procorpus.....  
..... *C. bulbicorpus*
- 6' Basal bulb and procorpus of equal size.....  
..... *C. australiae*

#### DISCUSSION

The 24 *N. gordanus* dissected for this study contained 40–1,750 oxyuridomorph and rhigonematomorph nematodes per millipede in the hind and midgut. Only 10 of these millipeds contained *C. claireae*, with a maximum of nine *C. claireae* in a millipede that contained 1,389 total nematodes. Previous reports of *Coronostoma* spp. similarly list one or a few individuals per millipede. Van Waerebeke (1986) observed partly digested nematodes in the intestine of several *C. diplopodicola*, suggesting that this nematode was predacious. During the current study we also found fragmentary remains of a small nematode (stoma, cuticular pieces) in the intestine of a female *C. claireae* n. sp. Therefore, this genus appears to consist of specialized predators of other



nematodes inhabiting the millipede's intestine. However, it seems unlikely that all stages are nematode-predacious. The eggs are of typical nematode size and hatched juveniles would be too small to ingest other nematodes. Rather, small juvenile *Coronostoma* may subsist first on bacteria in the millipede intestine, then switch to predation in the later stages. Similar diet-switching is known in the predacious terrestrial free-living order Mononchida (Yeates, 1987).

How *Coronostoma* spp. actually ingest other nematodes is not completely clear from the stomal analysis of *C. claireae* n. sp. or from shorter descriptions in other papers. The presence of large subventral, multidenticulate stegostomal plates in *C. claireae* n. sp. is an original feature not present in any other terrestrial predacious nematode genus. Another unique feature is the presence of strong longitudinal muscle bands around the periphery of the procorpus. These muscles are anchored to the esophageal isthmus and to the body wall near the level of the stoma. Thus, they occupy the approximate position of stylet protractor muscles except they extend posteriorly to the isthmus. We hypothesize that contraction of both the transverse and longitudinal procorpus muscles pulls the prey into the stoma while shortening the procorpus, then relaxation of the longitudinal muscles lengthens the procorpus (pulling the prey in farther) and closes the stegostomal plates against the prey to hold it with the denticles. Repetition of the process along with muscular contractions of the entire esophagus could assist in further ingestion.

*Coronostoma* spp. may be more diverse than their very similar morphologies suggest. The esophagus of *C. claireae* n. sp. at the stomal base is bilaterally symmetrical due to its much-enlarged subventral sectors, assuming a triradiate appearance more posteriorly. The stomal region sketch of *C. diplopodicola* (van Waerebeke, 1986) illustrates one small and two large denticulate regions, which suggests an architecture similar to that of *C. claireae* n. sp. On the other hand, *en-face* figures of *C. dentata*, *C. gautuni* (van Waerebeke and Adamson, 1986) and *C. australiae* (Jex et al., 2005) show three symmetrical lobes.

Specimens of *C. claireae* were not examined exhaustively for arcade cells and coelomocytes, but very large, conspicuous, nucleated sac-like cells were observed posterior to the esophagus and in the anal-tail region. These cells resemble arcade cells, narrowing and extending anteriorly, but unlike arcade cells were posterior to the esophagus. These arcade-like cells sometimes had distinct protuberances extending into the pseudocoel. In several specimens a vesiculate coelomocyte attached to the hypodermis was observed next to an arcade-like cell. Typical arcade cells are found in the esophageal region (Peregrine, 1973; Altun and Hall, 2009). A cluster of arcade-like cells also occurred around the anal region, and appeared to

have extensions into the tail, superficially resembling the spinneret organ found in Plectida. However, these extensions did not lead to any pore. A single multi-lobed, giant coelomocyte (Peregrine, 1973) up to 50  $\mu\text{m}$  long was found in the vicinity of the anterior gonad. This particular coelomocyte and its location are known in many nematodes (Peregrine, 1973).

Adamson (1989) provided a list of synapomorphies defining Oxyurida (= Oxyuridomorpha): "...single rather than paired spicule, reduced number of caudal papillae in male, absence of externolateral cephalic papillae, prominent X-shaped excretory system with vesiculate terminal duct, conical spermatozoa, a life cycle involving two moults in ovo with no extraintestinal phase in the host, and haplodiploid reproduction" (p. 176). In addition, the eight-celled *vagina vera* seems to be a distinctive feature of female oxyuridomorphs (Chitwood and Chitwood, 1933, 1950). The infra-order is commonly divided into two superfamilies: Oxyuroidea (vertebrate parasites) and Thelastomatoidea (invertebrate parasites). Current classification of Thelastomatoidea generally follows Adamson (1989) and Adamson and van Waerebeke (1992), who recognized five families in Thelastomatoidea, with *Coronostoma* placed in Thelastomatidae.

Coronostomatidae and Robertiidae Travassos and Kloss, 1961 were segregated from Thelastomatidae and placed in a new superfamily, Coronostomatoidea (Poinar, 1977). A separate diagnosis of this superfamily was not presented but it was separated in the accompanying key from other Oxyurida by the lack of a valve in the basal bulb. Adamson and van Waerebeke (1992) placed Coronostomatidae in synonymy with Thelastomatidae without explanation, but they did suggest that Thelastomatidae was paraphyletic and lacked unifying synapomorphies. Shah et al. (2012) also included *Coronostoma* in their key to genera of Thelastomatidae. Jex et al. (2005) avoided assigning thelastomatoid species to families, and Carreno (2014) did not refer to *Coronostoma* in his taxonomic review of Thelastomatoidea.

Coronostomatidae differs from Thelastomatidae *s. str.*, and indeed from Thelastomatoidea, in several important characters. The amphid apertures are carried near the tips of horn-like protrusions, while thelastomatids have pore-like amphids directly on the lips. This horn-like amphid structure appears to be unique among Nematoda. Coronostomatids have 12 equal, shallow lobes surrounding the oral aperture; thelastomatid nematodes typically have eight such lobes. The described males of *Coronostoma* lack a spicule, whereas some male Thelastomatidae possess a spicule. Finally, the coronostomatid esophagus is strongly muscled and consists of enlarged procorpus and basal bulb, the latter with little or no isthmus and without grinding valves. In contrast, thelastomatids have a long, slender procorpus and a basal bulb equipped with grinding valves. Coronostomatidae is differentiated from the

other thelastomatoid families (Adamson and van Waerebeke, 1992; Shah et al., 2012) by the above characters as well as the midbody location of the vulva (anterior to esophageal base in Protrelloididae); lack of cervical spines (present in Hystrignathidae); and lack of egg filaments (present in Pseudonymidae and Travassosinematidae).

Validity of a separate superfamily Coronostomatoidea is supported morphologically by the 12-lobed lip region, unique amphidial horns and the massively muscled esophagus that lacks the grinding valve present in Thelastomatoidea. Coronostomatoidea and Thelastomatoidea, however, are sister taxa on the basis of the morphology of the male tail. We did not collect any male *Coronostoma*, but males are known for *C. dentata*, *C. gautuni* and *C. singhi* (Rao, 1958; van Waerebeke and Adamson, 1986). Males of these species are much reduced in size relative to the female and have an arrangement of cloacal and tail papillae similar to that of many thelastomatoid males.

Poinar (1977) included the poorly known beetle and millipede-parasitic family Robertiidae Travassos and Kloss, 1960 (genera *Robertia* Travassos and Kloss, 1961 and *Triumphalisnema* Kloss, 1962; see Bernard and Phillips (2015) for chronology problems with Robertiidae and *Robertia*) in Coronostomatoidea on the basis of an apparently similar esophagus. The family and type genus are now Traklosiidae and *Traklosia* due to homonymy with a fossil synapsid (Bernard and Phillips, 2015). Traklosiidae generally fits the superfamily only on the basis of the muscular esophagus lacking a grinding valve, although an *en-face* SEM of *Triumphalisnema biabulaundatum* Hunt, Sutherland, and Machon, 1989 shows 11 or 12 irregular lobes surrounding the oral opening (Hunt, 1989). *Coronostoma* spp. typically have 12 lobes in the lip region (van Waerebeke and Adamson, 1986; this paper) but Jex et al. (2005) illustrated the head end of *C. australiae* with 11 lobes. The position of Traklosiidae cannot be more precisely determined without additional specimens and analysis, and so the family is retained in Coronostomatoidea.

Most *Coronostoma* spp. have been found in millipeds, with only one species from a cockroach. Millipeds are among the oldest terrestrial arthropods, with molecular clock results and paleobiogeographic reconstructions converging at an origin date of about 524 mya (Pisani, 2009; Shelley and Golovatch, 2011). As detritivores, millipeds likely would have been among the first arthropods ingesting bacterivorous nematodes or nematode eggs (Adamson, 1994). In the near-neutral fermentative intestine of millipeds (and somewhat later, cockroaches), such nematodes would have been preadapted for feeding on the rich bacterial flora that inhabited the gut. In this environment morphological adaptations for feeding directly on a host would not be necessary. Present-day invertebrate-inhabiting oxyuridomorphs

can differ spectacularly in external head morphology (e.g., *Travassosinema* Rao, 1958, see Spiridonov and Cribb, 2012), but in general, morphology and internal anatomy are quite uniform for this large taxon of some 850 species (Adamson, 1994; Spiridonov and Cribb, 2012). Functionally, all oxyuridomorphs of invertebrates are bacterivorous kleptoparasites rather than true parasites, with the exception of the predator *Coronostoma*, which likely represents a relict group of early predacious nematodes within Oxyuridomorpha. The scattered distribution of *Coronostoma* spp. and their evident infrequency and rarity in hosts suggests an early taxon, strengthening the argument that the genus belongs in a separate superfamily coordinate with Thelastomatoidea.

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