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New acoels (Acoela, Acoelomorpha) from North Carolina

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Abstract

From a survey of interstitial acoel flatworms at Lockwoods Folly Inlet, Oak Island, North Carolina, the new species Anaperus singularis sp. nov. (Anaperidae), Diopisthoporus lofolitis sp. nov. (Diopisthoporidae), Conaperta earnhardti sp. nov. (Convolutidae), Kuma flava sp. nov. (Haploposthiidae), Pseudohaplogonaria cerasina sp. nov. (Haploposthiidae), and Proporus carolinensis sp. nov. are described. Seven previously described acoel species and one nemertodermatid species were also collected from Lockwoods Folly, these were: Diopisthoporus gymnopharyngeus, Endocincta punctata, Neochildia fusca, Parahaploposthia thiophilus, Paratomella rubra, Polychoerus caudatus, Pseudaphanostoma smithrii, and Flagellophora apelti. A previously described species, Anaperus trifurcatus (Anaperidae) is transferred to the genus Amphiscolops (Convolutidae).

Key words: Acoelomorpha, meiofauna, turbellarians, Anaperus, Diopisthoporus, Conaperta, Pseudohaplogonaria, Kuma, Proporus

Introduction

Acoels from North Carolina have been used in many important investigations of flatworm ultrastructure (e.g., Tyler 1973, Crezée 1975, Crezée & Tyler 1976, Doe 1981, Smith & Tyler 1985, Rieger et al. 1991), and eight of the 40 acoel species known from the Atlantic coast of North America were originally described from North Carolina (see Hooge & Tyler 2003), including four species of the genus *Solenofilomorpha* (see Crezée 1975). There are many more acoels in North Carolina that have yet to be described, and we found that the shallow subtidal of Lockwoods Folly Inlet, Oak Island, North Carolina is especially abundant with acoel flatworms. We herein describe six previously unknown species, and report range extensions for two additional species.

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Specimens were extracted from sediment using magnesium-chloride anesthetization (Sterrer 1971). Live animals in squeeze preparations were viewed by light microscopy, sketched and photographed (e.g., see Fegley et al. 1984).

For histological study, specimens were relaxed in isotonic magnesium chloride, fixed in phosphate–buffered 2.5% (v/v) glutaraldehyde or warm Bouin's fixative, washed in phosphate buffer (Millonig's, 0.1 M), fixed in phosphate-buffered 1% (v/v) osmium tetroxide, dehydrated in acetone, and embedded in EMBed/Araldite epoxy resin. Dehydration was quickened by microwave radiation (Samsung oven, two 7-sec irradiations at 650 W separated by a 20-sec interim, with specimen-vial on ice and with water ballast of two filled 300-ml beakers; Giberson & Demaree 1995). Serial thick sections of 1.5 μ m were made according to Smith & Tyler (1984) and stained in toluidine blue.

Body-wall musculature of worms was revealed through F-actin staining of whole mounts with fluorescently labeled phalloidin (Alexa 488; Molecular probes, Eugene, OR) according to Hooge (2001).

Type material has been deposited in the American Museum of Natural History (AMNH), New York, New York, USA.

Results

Family Anaperidae Dörjes, 1968 Genus Anaperus Graff, 1911 Anaperus singularis sp. nov. (Figs. 1–3)

Type Material. Syntypes. AMNH PLATY 1642 and AMNH PLATY 1643, two sets of 1.5-µm-thick serial sagittal sections of epoxy-embedded specimens stained with toluidine blue, collected October 2002. Paratype. AMNH PLATY 1644, epoxy-embedded whole mount.

Type Locality. Oak Island, NC, from shallow subtidal medium grained sand at the Lockwoods Folly Inlet (33° 55' 03"N, 78° 13' 58"W).

Other Material Examined. Living specimens in squeeze preparations; set of 1.5- μ m-thick serial sagittal sections of epoxy-embedded specimen; one whole mount for fluorescence imaging of musculature.

FIGURE 1. Anaperus singularis **sp. nov.**; reconstructions to show arrangement of organs. A. Dorsal reconstruction of whole specimen with paired ovaries and unpaired eggs. B. Dorsal reconstruction of whole specimen with paired ovaries and paired eggs. C. Sagittal reconstruction of whole organism. D. Sagittal reconstruction of reproductive structures. a, adenodactyls; bn, bursal nozzle; cop, male copulatory apparatus; e, egg; fg, frontal gland; gl, gland cell secretions; m, mouth; mg, mucoid gland; mgp, male gonopore; mw, muscular wall surrounding penis; pmg, mucoid glands of male gonopore; sb, seminal bursa; sp, sperm; st, statocyst; t, testes; v, vacuole.



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Etymology. Species name comes from the Latin *singularis*, alone, and refers to the single bursal nozzle in this species.

Synonyms. Anaperus sp.: Smith 1981, Smith & Tyler 1985.

Description. Mature specimens ~1100 μ m long and ~200 μ m wide (Figs. 1, 2A). Body somewhat flattened. Anterior and posterior ends rounded.

Epidermis completely ciliated. By transmitted light epidermis reddish-brown in color due to pigment granules. Reddish-orange rod-shaped gland secretions present in long tracts underneath ventral epidermis (Fig. 2C). Scattered mucoid glands present in epithe-lium (Fig. 2B, 3A).



FIGURE 2. Anaperus singularis **sp. nov.**; photomicrographs of living specimen. A. Wholemount. B. Focus on epidermal cells and mucoid glands. C. Gland secretions in body wall. D. Dorsal view of posterior. a, adenodactyls; bn, bursal nozzle; cop, male copulatory apparatus; e, egg; mg, mucoid glands; sb, seminal bursa; sp, sperm.



FIGURE 3. *Anaperus singularis* **sp. nov.**; photomicrographs. A. Sagittal histological section. B. Sagittal histological section of posterior. C. Whole-mount stained with Alexa-488-labeled phalloidin and viewed with confocal microscopy. Projection of musculature associated with male copulatory organ. a, adenodactyl; bn, bursal nozzle; cop, male copulatory apparatus; e, egg; mg, mucoid glands; p, penis; sb, seminal bursa; sp, sperm.

Body-wall musculature with circular muscles that encircle the body along entire length of animal; straight longitudinal muscles present between frontal organ and anterior $\overline{442}$

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Frontal organ well developed; cell bodies of frontal glands positioned $\sim 200 \,\mu m$ behind frontal pore in fixed specimens (Fig. 1A–C).

Mouth opening on ventral surface, middle of body. Several examined specimens had conspicuous vacuole in immediate vicinity of mouth (Fig. 1C).

Ovaries paired, ventral. Egg strands paired or unpaired; extend from position anterior of mouth posteriorly to bursal nozzle (Figs.1, 3A).

Testes paired, lateral to eggs, follicular; separate from ovary. Testes extend from position near level of mouth posteriorly to level of bursal nozzle (Fig. 1).

Female gonopore absent. Unwalled seminal bursa leads to curved bursal nozzle ~40 μ m long (Figs. 1, 2D, 3A).

Male gonopore ventral at posterior end of body. Pore surrounded by mucoid glands that stain pink in toluidine blue. Male copulatory organ composed of a loose arrangement of muscles surrounding a small muscular penis. Copulatory organ surrounded by strands of darkly staining glandular secretions (Figs. 1C, 2D, 3B). Several actin-rich stimulatory organs known as adenodactyls also present (Figs. 1, 2D, 3B, C). Two adenodactyls present anterior to copulatory organ; extend through the ventral epidermis anterior to the male gonopore. Large adenodactyl positioned immediately behind copulatory organ; opens into male antrum (Figs. 1, 3B, C). Additional adenodactyls, flanking lateral sides of male copulatory organ present in some specimens (data not shown).

Remarks. This species is placed in the family Anaperidae due to the presence of needle-like adenodactyls. The presence of a bursal nozzle along with an unwalled seminal bursa is characteristic of members of the genus *Anaperus*. Like other anaperids, the adenodactyls of *Anaperus singularis* are closely associated with gland cells, and our phalloidin stained specimen reveals that the adenodactyls in this species also contain a considerable amount of actin. Besides having a unique arrangement of adenodactyls, *A. singularis* is distinguished from all other known species in the genus *Anaperus* in having only a single bursal nozzle; other known species have two or more nozzles.

Family Diopisthoporidae Westblad, 1940 Genus *Diopisthoporus* Westblad, 1940 *Diopisthoporus lofolitis* sp. nov. (Figs. 4–5)

Type Material: Holotype. AMNH PLATY 1645, set of 1.5-µm-thick serial frontal sections of male and female mature epoxy-embedded specimen stained with toluidine blue, collected October 2002. Paratype. AMNH PLATY 1646, set of 1.5-µm thick serial frontal sections of female mature, epoxy-embedded specimen stained with toluidine blue.

Type Locality. Oak Island, NC, from shallow subtidal medium grained sand inside Lockwoods Folly Inlet (33° 54' 53"N, 78° 14' 06"W).

Other Material Examined. Living specimens in squeeze preparations; one whole mount for fluorescence imaging of musculature.

Etymology. Species name is a combination of the words Lockwoods Folley Inlet, the type locality of this species.

Synonyms. Diopisthoporus cf. longitubus: Smith 1981, Smith & Tyler 1985.



FIGURE 4. *Diopisthoporus lofolitis* **sp. nov.**; sagittal reconstruction of whole organism to show arrangement of organs. e, egg; fg, frontal gland; gc, gland cells; fsv, false seminal vesicle; m, mouth; ma, male antrum; mgp, male gonopore; ph, pharynx; st, statocyst; sv, seminal vesicle; t, testes.

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Description. Male mature specimen ~300 long and ~80 μ m wide; male immature specimens ~410 μ m long (Figs. 4, 5). Body cylindrical. Anterior end rounded, posterior end blunt.

Epidermis without coloration by transmitted light. Epidermis completely ciliated. Rhabdoid and mucoid glands absent.

Body-wall musculature a simple gridwork of outer circular muscles and inner longitudinal muscles. A few longitudinal fibers angle diagonally at mid-body to cross over other straight diagonal muscles, then resume their longitudinal orientation (Fig. 5C).

Frontal organ well developed; cell bodies of frontal glands positioned \sim 70 µm behind frontal pore (Figs. 4, 5C).



FIGURE 5. *Diopisthoporus lofolitis* **sp. nov.**; photomicrographs. A. Dorsal view of whole animal. B. Frontal histological section of whole animal. C. Lateral view of whole-mount stained with Alexa-488-labeled phalloidin and viewed with confocal microscopy. e, egg; fg, frontal gland; fsv, false seminal vesicle; ma, male antrum; ph, pharynx; sp, sperm; st, statocyst; sv, seminal vesicle. Mouth opening terminal at posterior end of body, ventral side; opens to large ciliated pharynx lined with circular and longitudinal muscle fibers (Figs. 4, 5).

Ovary unpaired, dorsal. In male mature specimen ovary extends from statocyst posteriorly to anterior end of pharynx (Fig. 4).

Female gonopore, seminal bursa, and female accessories absent.

Testis unpaired, ventral, compact; separate from ovary. Testes extend from level of statocyst posteriorly to level of seminal vesicle. Sperm from testes extends from ventral to dorsal side to enter proximal end of seminal vesicle (Fig. 4).

Male gonopore terminal at posterior end of body; dorsal side. Gonopore leads to unciliated male antrum surrounded by mucoid gland cells that stain pink in toluidine blue. Proximal end of antrum opens to walled seminal vesicle (Figs. 4, 5B).

Remarks. The genus *Diopisthoporus* contains four other known species, including the filiform *D. gymnopharyngeus*, which was also present in our collections from Lockwoods Folly Inlet. *D. lofolitis* is similar in general appearance to *D. psammophilus* Dörjes, 1968, and *D. longitubus* Westblad, 1940, in having a large, terminally opening, ciliated pharynx. However, a male copulatory organ has not been found in *D. psammophilus*, and although *D. longitubus* does have a terminally-opening male gonopore on its dorsal side, it is without mucoid gland cells surrounding the male antrum. Additionally, the testis of *D. longitubus* is positioned dorsally and the eggs ventrally—the reverse of what occurs in *D. lofolitis*.

Family Convolutidae Graff, 1905 Genus *Conaperta* Antonius, 1968 *Conaperta earnhardti* sp. nov. (Figs. 6–8)

Type Material: Holotype. AMNH PLATY 1647, set of 1.5-µm-thick serial sagittal sections of epoxy-embedded specimen stained with toluidine blue, collected October 2002. Paratypes. AMNH PLATY 1648, set of 1.5-µm-thick serial sagittal sections; AMNH PLATY 1649, epoxy-embedded whole mount.

Type Locality. Oak Island, NC, from shallow subtidal medium grained sand inside Lockwoods Folly Inlet (33° 54' 53"N, 78° 14' 06"W).

Other Material Examined. Living specimens in squeeze preparations; four sets of 1.5-µm-thick serial sagittal sections of epoxy-embeded specimens; whole mounts for fluorescence imaging of musculature (eight specimens).

Etymology. Species named in memory of race-car driver, and North Carolina native, Dale "The Intimidator" Earnhardt.

Description. Examined specimens 350 to 650 µm long and ~125 µm wide (Figs.6A, B, 7A, 8A). Body cylindrical. Anterior and posterior ends rounded.

Epidermis completely ciliated. Body without coloration in transmitted light, but gut contents green in color. Many mucoid glands present; arranged in distinct rows.

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FIGURE 6. *Conaperta earnhardti* **sp. nov.**; reconstructions to show arrangement of organs. A. Dorsal reconstruction of whole specimen. B. Sagittal reconstruction of whole organism. C. Sagittal reconstruction of reproductive structures. bn, bursal nozzle; cop, male copulatory apparatus; cv, chordoid vacuole; e, egg; fg, frontal gland; gp, gonopore; gr, granules; m, mouth; mg, mucoid gland; p, penis; sb, seminal bursa; sp, sperm; sph, muscular sphincter surrounding vagina; st, statocyst; sv, seminal vesicle; t, testes; v, vagina.

Body-wall musculature with circular muscles that encircle the body along entire length of animal; straight longitudinal muscles present between frontal organ and anterior edge of mouth; longitudinal muscles with a longitudinal orientation anteriorly that bend medially to cross diagonally over the body (longitudinal-cross-over fibers present in dorsal and ventral body walls; longitudinal muscles in the anterior half of body that wrap around the posterior rim of mouth (U-shaped muscles) present in ventral body wall (Fig. 8A).

Frontal organ well developed; cell bodies of frontal glands positioned ~100 μ m behind frontal pore (Fig. 6B).

Mouth opening on ventral surface, middle of body. Digestive central syncytium extends from frontal glands posteriorly to level of male copulatory apparatus.

Ovaries paired, ventral; extend from frontal glands posteriorly to bursal nozzle (Fig. 6B).

Testes paired, lateral to eggs, compact; separate from ovary. Testes extend from level of statocyst posteriorly to level of bursal nozzle.

Common genital pore opens posteriorly to male copulatory apparatus and anteriorly to vagina (Figs. 6, 7B, C).

Vagina positioned somewhat to the right side of male copulatory organ, rather than directly anteriorly. Vagina filled glandular secrections in the form of tear drop-shaped clusters of granules. Vagina surrounded by thick muscular sphincter (Figs. 6, 7, 8B). Walled seminal bursa leads to well-developed bursal nozzle (Figs. 6, 7B, C).

Gonopore opens directly to well-developed penis (Figs. 6C, 7C). Slightly curved penis with outer longitudinal and inner circular muscle fibers that surround lumen filled with spherical clusters of granular gland secretions (Figs. 6A, 7B, C, 8C). Proximal end of penis somewhat bulbous. Penis invaginated into muscular seminal vesicle filled with sperm that surrounds proximal end of penis.

Remarks. The unusual vagina in *Conaperta earnhardti* distinguishes it from other known species of the genus. The distinctive granular secretions in the lumen of the vagina, and the sizable muscular sphincter surrounding the vagina are diagnostic characters for this species. *Conaperta krana* and *C. thela* also have vaginal sphincters (although much less well-developed that that of *C. earnhardti*), but these species have differently constructed penes, and have eye-spots and zooxanthellae—features absent in *C. earnhardti*.







FIGURE 7. *Conaperta earnhardti* **sp. nov.**; photomicrographs. A. Ventral view of living specimen. B. Sagittal view of reproductive organs in living specimen. C. Sagittal histological section. bn, bursal nozzle; e, egg; p, penis; sb, seminal bursa; sph, muscular sphincter surrounding vagina; sv, seminal vesicle.





FIGURE 8. Conaperta earnhardti **sp. nov.**; whole-mount stained with Alexa-488-labeled phalloidin and viewed with confocal microscopy. A. Projection of ventral body-wall musculature. For explanation of muscle patterns see Hooge (2001). B. Projection of musculature associated with reproductive organs. C. Projection showing penis musculature inside of seminal vesicle. cm, circular muscles; cop, male copulatory aparatus; lpm, longitudinal penis muscle; m, mouth; p, penis; sph, muscular sphincter surrounding vagina; u, U-shaped muscles; vcm, ventral crossover muscles.

Family Haploposthiidae Westblad, 1948 Genus *Kuma* Marcus, 1950 *Kuma flava* sp. nov. (Figs. 9–10)

Type Material: Holotype. AMNH PLATY 1650, set of 1.5-µm-thick serial frontal sections of epoxy-embedded specimen stained with toluidine blue, collected October 2002. Paratypes. AMNH PLATY 1651, set of 1.5-µm-thick serial sagittal sections; AMNH PLATY 1652, epoxy-embedded whole mount.

Type Locality. Oak Island, NC, from shallow subtidal coarse to medium grained sand inside Lockwoods Folly Inlet (33° 54' 53"N, 78° 14' 06"W).

Other Material Examined. Living specimens in squeeze preparations; set of 1.5µm-thick serial frontal sections of epoxy-embeded specimen.

Etymology. Species name comes from the Latin *flavus*, gold-colored, and refers to the species body color.

Synonyms. Kuma sp.: Smith 1981, Smith & Tyler 1985.



FIGURE 9. *Kuma flava* **sp. nov.**; reconstructions to show arrangement of organs. A. Dorsal reconstruction of whole organism. B. Frontal reconstruction of reproductive structures. cs, digestive central syncytium; e, egg; fg, frontal gland; m, mouth; ma, male antrum; mg, mucoid glands; n, nucleus; rh, rhabdoid gland; sp, sperm; st, statocyst; sv, seminal vesicle; t, testes.

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Description. Mature specimens ~510 μ m long and ~100 μ m wide (Figs. 9A, 10A). Body cylindrical. Anterior and posterior ends rounded; posterior more blunt. Body color gold by transmitted light. Epidermis completely ciliated. Many large rhabdoid glands present; mostly concentrated at anterior end (Fig. 9A).

Frontal organ well developed; cell bodies of frontal glands positioned ~80 μ m behind frontal pore (Fig. 9A).

Mouth opening on ventral surface, middle of body.

Ovary unpaired, ventral; extends ~150 µm posteriorly from level of mouth (Fig. 9A).

Testes paired, lateral to ovary, compact; separate from ovary. Testes extend from level of mouth posteriorly to seminal vesicle.

Female gonopore, seminal bursa, and female accessories all absent.

Male gonopore terminal at posterior end of body; gonopore closer to ventral side. Proximal opening of long (~60 μ m) ciliated antrum surrounded by ring of large mucoid glands whose tips open into antrum (Figs. 9, 10B). Antrum body-wall musculature composed of circular and longitudinal muscles; antrum musculature surrounded by darkly staining nuclei-perhaps insunk nuclei of antrum epithelium. Large unwalled seminal vesicle positioned at proximal end of male antrum (Figs. 9B, 10B).



FIGURE 10. *Kuma flava* sp. nov.; photomicrographs of living specimen. A. Whole-mount. B. Dorsal view of posterior. e, egg; ma, male antrum; mg, mucoid glands; sv, seminal vesicle.

Remarks. The taxa *Haploposthia* and *Kuma* are closely related genera with only minor differences between them. The germinal center for eggs and sperm are separate in *Kuma*, but combined in *Haploposthia*. Although the diagnosis provided by Faubel (1976) for the genus *Kuma* includes the character "uncolored", this does not seem to preclude our species from inclusion in the genus, especially since two other species of *Kuma*, *K*.

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zootaxa 442 *monogonophora* (Westblad, 1946) and *K. viridis* (An der Lan, 1936) have green coloration. Among species of *Kuma*, *K. flava* is similar to *K. albiventer* (Marcus, 1954), *K. monogonophora*, and *K. viridis*, which all have conspicuous gland cells ringing the proximal end of the male antrum. However, *K. albiventer* has a ventrally opening male gonopore, *K. monogonophora* has several stimulatory needles that open into the male antrum, and *K. viridis* has a female gonopore, all features not shared with *K. flava*.

Family Haploposthiidae Westblad, 1948 Genus *Pseudohaplogonaria* Dörjes, 1968 *Pseudohaplogonaria cerasina* sp. nov. (Figs. 11–12)

Type Material: Syntypes. AMNH PLATY 1653 and AMNH PLATY 1654, two sets of 1.5-µm-thick serial oblique longitudinal sections of epoxy-embedded specimens stained with toluidine blue, collected October 2002.

Type Locality. Oak Island, NC, from shallow subtidal coarse to medium grained sand inside Lockwoods Folly Inlet (33° 54' 53"N, 78° 14' 06"W).

Other Material Examined. Living specimens in squeeze preparations; one whole mount for fluorescence imaging of musculature.

Etymology. Species name is from the Latin *cerasinus*, cherry-colored, and refers to the bright red rhabdoids in this species.

Description. Mature specimens approximately $650 \ \mu m$ long and $100 \ \mu m$ wide (Figs. 11, 12A). Body cylindrical. Anterior and posterior ends rounded. Uncolored body by transmitted light.

Epidermis completely ciliated. Many bright red as well as uncolored rhabdoids present in body wall; mostly concentrated on ventral side (Fig. 11, 12C).

Musculature with circular muscles that encircle the body along entire length of animal; straight longitudinal muscles present between frontal organ and anterior edge of mouth; longitudinal muscles with a longitudinal orientation anteriorly but then bend medially to cross diagonally over the body (longitudinal-cross-over fibers) present in both dorsal and ventral body wall; anterior end with ventral diagonal muscles positioned between outer circular and inner longitudinal muscles (data not shown).

Frontal organ well developed; cell bodies of frontal glands positioned ~250 μ m behind frontal pore (Fig. 11A, B).

Mouth opening on ventral surface, middle of body. Digestive central syncytium extends nearly entire length of body.

Ovary unpaired, ventral; extends from mouth posteriorly to bursal nozzle (Fig. 11B).

Testes paired, dorsal, compact; separate from ovary. Testes extend anteriorly to position \sim 220 µm behind anterior tip and posteriorly to male copulatory organ.

Female gonopore and vagina absent. Seminal bursa leads to robust bursal nozzle \sim 30 µm in length (Figs. 11B, C, 12B, D).



FIGURE 11. *Pseudohaplogonaria cerasina* **sp. nov.**; reconstructions to show arrangement of organs. A. Dorsal reconstruction of whole specimen. B. Sagittal reconstruction of whole organism. C. Sagittal reconstruction of reproductive structures. bn, bursal nozzle; cop, male copulatory apparatus; cs, digestive central syncytium; cv, chordoid vacuole; e, egg; fg, frontal gland; m, mouth; mgp, male gonopore; p, penis; rh, rhabdoid gland; sb, seminal bursa; sp, sperm; st, statocyst; t, testes.

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photomicrographs. A. Dorsal view of living specimen. B. Dorsal view of posterior of living specimen. C. Rhabdoid glands. D. Sagittal view of reproductive organs in living specimen. bn, bursal nozzle; cv, chordoid vacuole; p, penis; sb, seminal bursa; sp, sperm.

Male gonopore ventral, at posterior end; leads to non-muscular penis composed of spongy tissue with large nuclei (Figs. 11C, 12B, D). Large masses of sperm present at lateral sides of penis; true seminal vesicle absent.

Remarks. Among species in the family Haploposthiidae, members of the genus Pseudohaplogonaria are united in having a seminal bursa with a sclerotized bursal nozzle, and a weakly developed or absent seminal vesicle. P. cerasina is most similar to P. minima (Ehlers & Dörjes, 1979), which also has paired testes, an unpaired ovary, conspicuous rhabdoids, and a bursal nozzle. *P. minima*, however, is more stout-bodied than *P. cerasina*, has a smaller male copulatory organ, and a common gonopore that opens to the seminal bursa via a short vagina.

Family Proporidae Graff, 1882 Genus *Proporus* Schmidt, 1848 *Proporus carolinensis* sp. nov. (Figs. 13–15)

Type Material: Syntypes. AMNH PLATY 1655 and AMNH PLATY 1656, two sets of 1.5-µm-thick serial sagittal sections of epoxy-embedded specimens stained with toluidine blue, collected October 2002. Paratype. AMNH PLATY 1657, epoxy-embedded whole mount.

Type Locality. Oak Island, NC, from shallow subtidal medium grained sand at the Lockwoods Folly Inlet (33° 55' 03"N, 78° 13' 58"W).

Other Material Examined. Living specimens in squeeze preparations; one whole mount for fluorescence imaging of musculature.

Etymology. Species name refers to North Carolina, the state from which the type material was collected.

Description. Mature specimens 650 to 800 μ m long and ~150 μ m wide (Figs. 13, 14A, 15A). Specimens fixed for histological sectioning contracted considerably.

Body cylindrical. Anterior end rounded, posterior end tapers to blunt point.

Epidermis completely ciliated. Two types of rhabdoid glands present in epithelium; one large and cylindrically shaped, the other smaller with an irregular shape (Fig. 13, 14B).

Body-wall musculature a simple gridwork of outer circular muscles and inner longitudinal muscles (Fig. 15C).

Frontal organ well developed; cell bodies of frontal glands positioned ~150 μ m behind frontal pore in fixed specimens (Fig. 13).

Mouth opening on ventral surface, anterior half of body; usually posterior to statocyst (Fig 13, 14A, 15A), in one fixed specimen mouth opening extended anterior to level of statocyst (Fig. 15A). Mouth opens to ciliated pharynx with well-developed circular and longitudinal muscle fibers (Fig. 13, 15A, C, D). Digestive central syncytium extends from frontal glands to posterior end of body.

Ovary unpaired, ventral; extends from level of pharynx posterior to middle of body. (Fig. 13). Eggs not present in all examined specimens (Fig. 15A).

Testes paired, dorsal, follicular; separate from ovary. Testes extend posteriorly from position behind frontal glands to seminal vesicle (Figs. 13, 15A, B).

Female gonopore absent. Male gonopore terminal at posterior end opens to ciliated, tubular male antrum (Figs. 13, 15B). Wall of antrum with circular and longitudinal muscles, lined with nuclei, but without obvious glands. Proximal end of antrum opens to seminal vesicle with sperm arranged in parallel with longitudinal body axis (Figs. 14A, 15B).

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FIGURE 13. *Proporus carolinensis* **sp. nov.**; sagittal reconstruction to show arrangement of organs. cs, digestive central syncytium; e, egg; fg, frontal gland; m, mouth; ma, male antrum; ph, pharynx; rh, rhabdoid gland; st, statocyst; sv, seminal vesicle; t, testes.

Remarks. *Proporus carolinensis* appears to be most similar to *P. lonchitus* and *P. minimus*. These three species have pharynges that open posterior to the level of the statocyst; this is unlike the condition found in the other three known species of *Proporus* in which the mouth is located anterior to the statocyst. In addition, *P. carolinensis* and *P. lonchitus* are the only known species in the genus to have paired testes. In contrast, *P. carolinensis* lacks the gut musculature found in *P. minimus*, and the glands surrounding the pharynx of *P. lonchitus*. Most importantly, *P. carolinensis* does not have glands opening into the male antrum, as is the case in all other known species in the genus.

Proporus carolinensis lacks accessory muscles that fan out from the mouth opening as found in another species of the Proporidae, *Proporus bermudensis* Hooge & Tyler, 2001, and other pharynx-bearing turbellarians such as *Macrostomum hystricinum* (see Rieger et al. 1994).





FIGURE 14. *Proporus carolinensis* **sp. nov.**; photomicrographs of living specimen. A. Ventral view of of whole animal. B. Rhabdoid glands.

New Distributional Records

In addition to the newly described species, we also collected six species of acoelomorphs from Lockwoods Folly already known to occur in North Carolina; these were: *Diopisthoporus gymnopharyngeus* Smith & Tyler, 1985, *Endocincta punctata* Crezée, 1975, *Flagellophora apelti* Faubel & Dörjes, 1978, *Neochildia fusca* Bush, 1975, *Parahaploposthia thiophilus* Fegley et al., 1984, and *Paratomella rubra* Rieger & Ott, 1971. Also found in our collections were two species originally described from the northwest Atlantic and previously unrecorded from North Carolina: *Polychoerus caudatus* Mark, 1892, and *Pseudaphanostoma smithrii* Hooge & Tyler, 2003.





FIGURE 15. *Proporus carolinensis* **sp. nov.**; photomicrographs. A, B. Sagittal histological sections. C, D. whole-mount stained with Alexa-488-labeled phalloidin and viewed with confocal microscopy. C. Focus on ventro-lateral body-wall musculature and mouth. D. Focus on musculature of pharynx. ma, male antrum; sv, seminal vesicle; t, testes.

Our specimens of *P. caudatus* were 1.3 to 2.4 mm long and ~600 μ m wide, without obvious body coloration in transmitted light, and always with three caudal cirri and two bursal nozzles. In the original description from Woods Hole, Massachusetts by Mark (1892), specimens of *P. caudatus* were 3 to 4 mm long, 1.5 to 2 mm, with 1 to 5 caudal

cirri, and typically possessing 40 to 50 bursal nozzles, but sometimes having as few as 8 to 10. Although our North Carolina specimens were smaller and had far fewer bursal nozzles, the morphology of their male copulatory organs were indistinguishable from those described from Massachusetts. Uncolored specimens, presumably similar to our specimens, were also collected by Bob Smith from Sewage Beach, Virginia Key, Florida in October 1964 (Louise Bush, personal correspondence).





FIGURE 16. Photomicrographs of living specimens collected from Lockwoods Folly Inlet, North Carolina. A. *Polychoerus caudatus*. B. *Flagellophora apelti*.

Reassignment of Anaperus trifurcatus Beltagi, 1983 to the genus Amphiscolops

Anaperus trifurcatus was described by Beltagi (1983), who collected the worm from brown algae taken from the upper intertidal zone in the Red Sea. This species was placed in the genus Anaperus despite lacking the stimulatory organs (adenodactyls) that are diagnostic for the family (Dörjes 1968). A. trifurcatus has a trilobed posterior end, zooxanthellae, frontal glands, a statocyst, eight bursal nozzles, and a single male gonopore and is better suited for placement in the genus Amphiscolops Graff, 1904 (see discussion in Winsor 1990). While one other known species of Amphiscolops, A. bermudensis Hyman, 1939, also has a trilobed posterior end, A. trifurcatus is apparently unique in lacking eyes and a female gonopore, and in its possession of ciliated "sensory" pits on both sides of the male gonopore. We hereby transfer this species to the genus Amphiscolops.

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