Journal of Nematology 41(1):64–83. 2009.

A Partial Revision of the Marine Nematode Genus *Elzalia* (Monhysterida: Xyalidae) with New Characters and Descriptions of Two New Species from Khung Kraben Bay, East Thailand

W. Duane Hope,[1] Chittima Aryuthaka[2]

Abstract: Elzalia bipectinella[1] *n. sp.* and *E. tuberculata*[2] *n. sp.* from Thailand are described and the males of three species from the Gulf of Mexico, *E. federici* Castillo-Fernandez and Lambshead (1990), *E. kimae* Castillo-Fernandez and Lambshead (1990), and *E. poli* Castillo-Fernandez and Lambshead (1990) are re-described from type and topotype specimens using light and scanning electron microscopy. New morphological characters are described that include ornamentations at the distal end of the spicula and features of the gubernaculum including the *manus* with digits, *pontis* with sensory receptor and accessory process, and the *condylus* with either conical or foliate projections. Also, ejaculatory and rectal glands are described for the first time for *Elzalia*. The relevance of the new characters to the taxonomy of *Elzalia* is discussed. A key to identification of males is provided. A generalized description of females is given, although characters are lacking by which females of each species may be identified.

Key words: Elzalia bipectinella, *Elzalia tuberculata*, *Elzalia federici*, *Elzalia kimae*, *Elzalia poli*, morphology, spicula, gubernaculum, ejaculatory glands, rectal glands.

Ecological investigations of meiofauna have been conducted in the mangrove areas of Ranong, south Thailand (Nozawa *et al.*, 1983); in seagrass beds in Ban Thung Nang Dam, Phang Nga Province and in Ban Tha Lens, Krabi Province along the Andaman Sea Coast (Sommerfield *et al.*, 2002); in intertidal flats in southern Thailand (Swennen and Witte, 1989), in Khung Kraben Bay, Gulf of Thailand (Aryuthaka, 1991); and in aquaculture habitats, including shrimp culture ponds in the Khung Kraben area (Aryuthaka, 1994) and green mussel (*Perna viridis* (I.)) cultures. Although nematodes were the dominant form of meiofauna in all of these investigations, there are only a few previous taxonomic investigations of marine nematodes from Thailand. Jensen (1989) described three new species of the family Aponchiidae. Kito and Aryuthatka (1998) described two new species of the family Monhysteridae and one new species from Xyalidae. They also described a new species of Siphonolaimidae from mangrove communities on the western coast of the Gulf of Thailand (Kito and Aryuthaka, 2006). In this study we describe two new species of the genus *Elzalia* (Xyalidae) collected by the junior author while conducting ecological investigations in Khung Kraben Bay.

New characters were found while studying the new species of *Elzalia*, and inconsistencies were discovered between our observations of the gubernaculum and interpretations reported in the literature. In order to provide as near as possible a comparable understanding of all species of the genus, we have included in this study a redescription of three of the seven nominal species of *Elzalia*, namely *E. federici* Castillo-Fernandez and Lambshead, 1990, *E. kimae* Castillo-Fernandez and Lambshead, 1990, and *E. poli* Castillo-Fernandez and Lambshead, 1990, based upon a study of the original type specimens and new topotype material. Type specimens were not available for *E. floresi* Gerlach, 1957, *E. mediterranea* Vitiello, 1971, *E. gerlachi* Zhang and Zhang, 2006 and *E. striatitenuis* Zhang and Zhang, 2006. The genus is redefined, and a key to the identification of males is provided.

Materials and Methods

Specimens of the two new species described here were collected by the junior author during an investigation of benthic meiofauna in Khung Kraben Bay, Chanthaburi, East Thailand between March, 1989 and April, 1990. The Bay is situated at latitude 12°34′ to 12°36′N and longitude 101°53′ to 100°35′E. The Bay is fringed by mangrove swamps except on the northwest side where the bay opens seaward through a narrow inlet. Specimens were collected using a 25 cm × 25 cm Eckman grab from stations 1-7.

The sediment was sandy silt at almost all stations with little seasonal change. Organic content ranged from 10 to 45 mg DW.gsed - 1 with the higher content at stations 1-3. The depth in the Bay ranges from 1 to a maximum of 4.5 m at station 3. Salinity varied in the dry season (Jan. – April) from 24.5 ± 1.7 – 30.9 ± 0.5 and in the wet season (Aug. – Nov.) from 20.0 ± 4.3 – 25.3 ± 2.0 psu. Details of the study area have been reported by Aryuthaka (1991).

The nematodes were preserved in 10 % formalin in seawater and mounted in anhydrous glycerine with coverslip supports for light microscopy. Specimens of

Received for publication June 4, 2009.

[1]Department of Invertebrate Zoology, National Museum of Natural History, Smithsonian Institution, Washington, D. C. 20560 USA.

[2]Department of Marine Science, Faculty of Fisheries, Kasetsart University, Thailand.

Acknowledgments. We thank Dr. Castillo-Fernandez for providing topotype specimens and Abbie Yorkoff and Ashleigh Smythe for their critical reviews of the manuscript.

This paper was edited by Kris Lambert.

[1] Specific epithet is a combination of the Latin prefix bi-, for two, and the Latin suffix pectinella, masculine gender, meaning comb, referring to the two, small comb-like ornamentations at the distal end of each spiculum.

[2] The specific epithet denotes the minute tubercles situated subterminally near the distal end of each spiculum.

the new species selected for scanning electron microscopy (SEM) were washed in distilled water, dehydrated with ethanol by vapor diffusion (Hope, 1982), and critical point dried with a Denton Critical Point Dryer. Specimens were pre-coated with carbon, coated with gold/palladium, and examined with a Hitachi S-570 and a Leica 440.

Type specimens of *E. federici*, *E. kimae* and *E. poli* borrowed from the British Museum (Natural History) were remounted in instances where faulty seals had resulted in the specimens being inadequately immersed in glycerin[3]. Before remounting the holotypes, each was placed head down in a small cylindrical cavity drilled in an aluminum stub. This well was filled with glycerine and the specimens positioned so that the posterior end of the specimen projected beyond the cavity and glycerine. This preparation made it possible to examine the tips of the spicula with a scanning electron microscope without coating the specimens or in any way compromising their quality for future examination with light microscopy. Although resolution was not good, this procedure enabled us to confirm for the original type material the existence of new characters observed in the topotypes. Although the spicula of *E. poli* were observed in this fashion, the distal end of the gubernaculum was not projected beyond the cloacal aperture, and details of it were observed with light microscopy only (Fig. 10B).

Our investigations revealed that *E. poli* paratype specimen Ep3 (BM(NH)1989.1.3) is a specimen of *E. federici* and Ep4 (BM(NH) 1989.1.7) is not a member of the genus *Elzalia*, but possibly a member of the genus *Paramonhystera*. In the case of Ep3, it is likely that the distorted condition of the gubernaculum accounts for the misidentification. Our re-identification of Ep3 is based upon the lengths of the spicula relative to body length.

Our redescriptions of *E. federici*, *E. kimae* and *E. poli* are based on our own measurements of the original type specimens and topotype specimens provided by Castillo-Fernandez. We have not included body widths and ratios of the original types because of the flattened condition of those specimens, and where other data is not obtainable it is indicated by [ND]. Morphometric and meristic data for the holotype specimens of all five species are in brackets. The length of the gubernaculum is measured on the arc from the distal surface of the *manus* to the proximal end of the sheath on the ventral or anterior surface of the spiculum.

Morphometric data were obtained from camera lucida drawings and from electron micrographs using a Summagraphics digitizer. Statistical calculations were made using "Nemes", a program written by Dr. Yoshihisa Shirayama to run in conjunction with a Summagraphics digitizer. The ranges for morphometric and meristic data are followed by the mean and one standard deviation in parentheses.

Descriptions of all five species of *Elzalia* and the key were prepared with DELTA (Dallwitz et al., 1993). Type specimens of *Elzalia bipectinella* n. sp. and *E. tuberculata* n. sp. and topotype specimen of *E. federici*, *E. kimae*, and *E. poli* are deposited in the National Museum of Natural History, Smithsonian Institution, Washington, D. C.

Morphology

Copulatory apparatus of male Elzalia: Examination of male specimens of our two new species led to the conclusion that previous interpretations (Castillo-Fernandez and Lambshead, 1990) of the gubernacula of *E. federici* Fernandez and Lambshead, 1990, *E. kimae* Fernandez and Lambshead, 1990, and *E. poli* Fernandez and Lambshead, 1990 were inaccurate, presumably because those interpretations were based upon flattened and distorted preparations. This was confirmed by examination of type and topotype specimens of each of the three species in question. Examinations with light and scanning electron microscopy of spicula and gubernacula protruded from the cloacal aperture of males of the new species from Thailand and topotype specimens of the species described by Castillo-Fernandez and Lambshead (1990) have provided new characters and character states for the spicula and an improved understanding of the structure of the gubernacula. Character states of the spicula are species specific with taxonomic and phylogenetic implications. Unfortunately, there are no type specimens of *E. floresi* or *E. mediterranea*, and type specimens of *E. gerlachi* Zhang and Zhang, 2006 and *E. striatitenuis* Zhang and Zhang, 2006, are not available, so details of the male copulatory apparatus of those species remain unknown. New details of the male copulatory apparatus from specimens at hand are as follows:

Spicula: The spicula in males of *Elzalia* are elongate and acicular. The shaft is cylindrical whereas the distal tip is either cylindrical or slightly spatulate with the edges rolled ventrad. Spicula that are cylindrical throughout their length are provided with various arrangements of terminal or subterminal conical tubercles (Figs. 2B, 4B, and 8B), whereas those that are distally spatulate have serrated edges (Figs. 6A and 10A). Examination at high resolution with the SEM failed to disclose any evidence of sensory or glandular pores at the tips of the tubercles or serrations. Each spiculum is enclosed within a sheath of protractor muscle whose insertion is on the slightly cephalated proximal end of the spiculum.

Gubernacula: The distal end of the gubernaculum is comprised of right and left halves joined as a single functional unit. Both halves are exposed to the same degree when protruded from the cloaca. Each half is comprised of a broad, anterior, hand-like structure, here termed the *manus*, with four antero-dorsally directed digits (Figs. 2A, 4A, 8B and 10B). The most lateral digit

[3]The paratype of E. kimae, Ek2, was damaged in the process of making new slide mounts. A topotype specimen has been deposited at the British Museum in its place.

on each side is the largest, with digits becoming progressively smaller toward the saggital plane. Spines may project laterally from the lateral surface of each *manus* or from the lateral surface of the first digit. One or more spines may also extend posteriorly from the posterior edge of each *manus* nearest the sagittal plane.

Posteriorly each *manus* is continuous with a narrow bridge, the *pontis* (Figs. 2A, 8B). The latter always bears a ventrally-directed, cylindrical process that is hollow and assumed to have a chemosensory function (Figs. 2A and C, 4A, 6C, and 8B). There is an accessory process lateral to each sensory receptor that is cylindrical in *E. bipectinalla* (Fig. 2C), but conical in all other species included in this investigation (Figs. 4A, 6B and C, and 8B).

The posterior end of each *pontis* is continuous with the *condylus*, which bears either foliate ridges, as in the case of those specimens from Thailand (Fig. 2A, B and C and Fig. 4A), or conical processes as in those from the Gulf of Mexico Figs. 6B and C, 8B and 10B). When foliate ridges are present they are collectively dispersed fan-like across lateral, posterior and mesal surfaces of the *condylus*. A single, cuticular apophysis extends dorsally from the right and left halves of the *condylus*, to which are attached the right and left protractor muscles of the gubernaculum respectively and perhaps also, in part, the protractor muscles of the right and left spicula. A cuticular sleeve also projects dorsally from the right and left sides of the gubernaculum, which ensheath distal portions of the right and left spicula respectively.

The structures identified by Castillo-Fernandez and Lambshead (1990) as the sheaths of the spicules and the apophysis of the gubernaculum are in accord with our interpretations. Because of the complexity of the main body of the gubernaculum and their distorted condition, other structures designated by them cannot be easily related to the terms used in the present investigation, except it is likely that the structures designated as "DP" probably corresponds to the *manus*, and "P" and "leaf-like" projections are the conical projections of the *condylus*.

Ejaculatory and rectal glands: We have observed for the first time for this genus the presence of what are here provisionally interpreted to be an ejaculatory gland and a smaller rectal gland on each side of the male body. There are two to eight cells in each ejaculatory gland. They may be of one type only (Figs. 3C, 5C and 9C), or with a proximal series of smaller cells followed by two additional, longer, oblong anterior cells (Fig. 1B, 2E and 7C). Each cell in these glands has an attenuated neck extending to an ampulla in the region of the cloaca (Fig. 1C, 3C, 5C, 7C, and 9C), but the precise location of the efferent openings of these glands is unknown. The rectal gland is either slender (Figs. 1B and 9C) and consists of two tandem swellings (Fig. 3C), or is bifurcated (Figs. 5C, 7C). It is not known if it is comprised of one or, especially in the case of the two tandem swellings or bifurcated glands, of two cells. In any case, this gland also appears to terminate in the vicinity of the cloaca. Fixation was not sufficient to resolve detailed structure of rectal gland cells.

Systematics

XYALIDAE Chitwood, 1951
XYALINAE Chitwood, 1951
ELZALIA Gerlach, 1957
Elzalia Gerlach, 1957: 454.

Synonymy: Megalolaimus Timm, 1961: 40 op Hope and Murphy, 1972: 39.

Type species: Elzalia floresi Gerlach, 1957: 454.

Synonymy: Megalolaimus speculifer Timm, 1961: 40 (Doubtful species described from one female.)

Emended generic diagnosis: Xyalidae. Body fusiform, distinctly tapered in region of esophagus; tail conical, with short, cylindrical terminus. Cuticle with fine transverse striae and longitudinal interstrial lines (Fig. 2D). Six papilliform, inner labial sensilla; six outer labial and four paralabial[4] setiform sensilla joined in single circle, sometimes appearing segmented. Amphid circular and nearly as wide as corresponding body diameter. Subventral, precloacal rows of sensilla present, each subventral sensilla approximately same length as sublateral somatic sensilla. Head truncate with six, thin, overlapping labial flaps covering oral aperture. Buccal cavity spacious, goblet-shaped or cylindrical, and unarmed. Buccal walls distinctly cuticularized with two, closely-spaced transverse seams near level of paralabial sensilla. Posterior end of buccal capsule enveloped by anterior end of esophagus. Esophagus clavate; marginal tubes absent. Ventral gland (renette) absent. Spicula aciculate and ensheathed in protractor muscle. Distal end of spicula cylindrical or spatulate; if cylindrical then with terminal, comb-like ridges or clusters of subterminal tubercles; if spatulate then distal margins serrate. Distal end of gubernaculum complex with digitate *manus*; *pontis* with cylindrical sensory receptor and cylindrical or conical process accessory to sensory receptor; and c*ondylus* with posterior conical or foliate projections. Gubernaculum with proximal sleeves closely adhered to shaft of spicula. Males diorchic with anterior testis on left and posterior on right of gut. Ejaculatory gland with variable numbers of one or two kinds of cells; cells open into ampulla near cloaca; efferent aperture of each ampulla apparently situated in wall of cloaca near cloacal vent. Rectal glands present and opening into or near cloaca. Ventromedian supplements slit-like with raised, lateral, crescent-shaped margins (Fig. 2D). Females monodelphic. Tail anteriorly conical and posteriorly cylindrical with three elongate, terminal sensilla. Caudal glands with separate, subterminal openings.

[4]The term paralabial sensilla is here used in reference to the third circle of four sensilla, whether joined with or separate from the outer labial sensilla, and the term cephalic sensilla is inclusive of inner and outer labial and paralabial sensilla. See Hope, 2007.

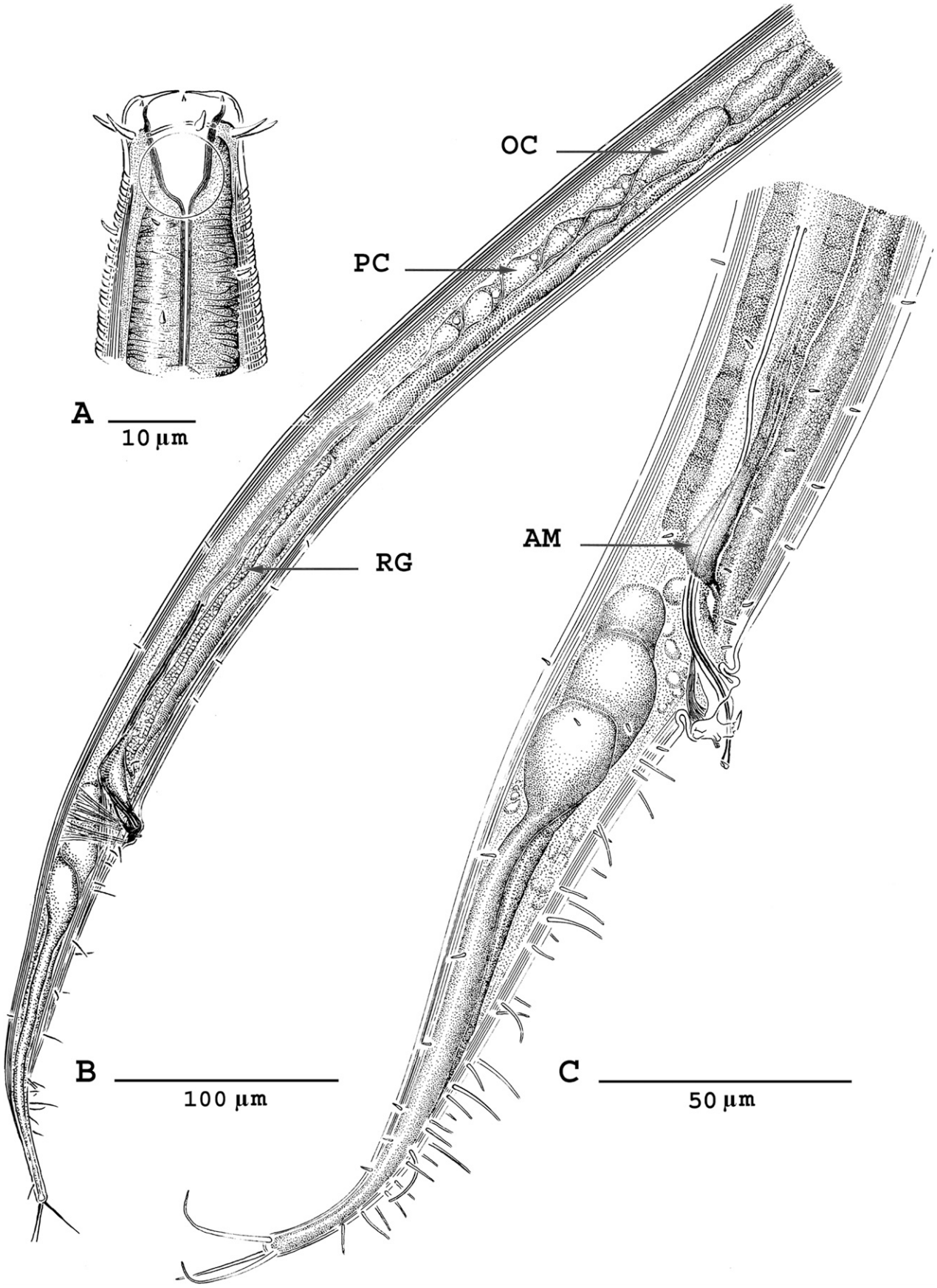

Fig. 1. *Elzalia. bipectinella* n. sp. Holotype, USNM No. 171268. A. Head. B. Posterior body region with rectal gland (RG) and oblong (OC) and pyriform (PC) cells of the ejaculatory gland. C. Tail with ampulla (AM) of the ejaculatory gland.

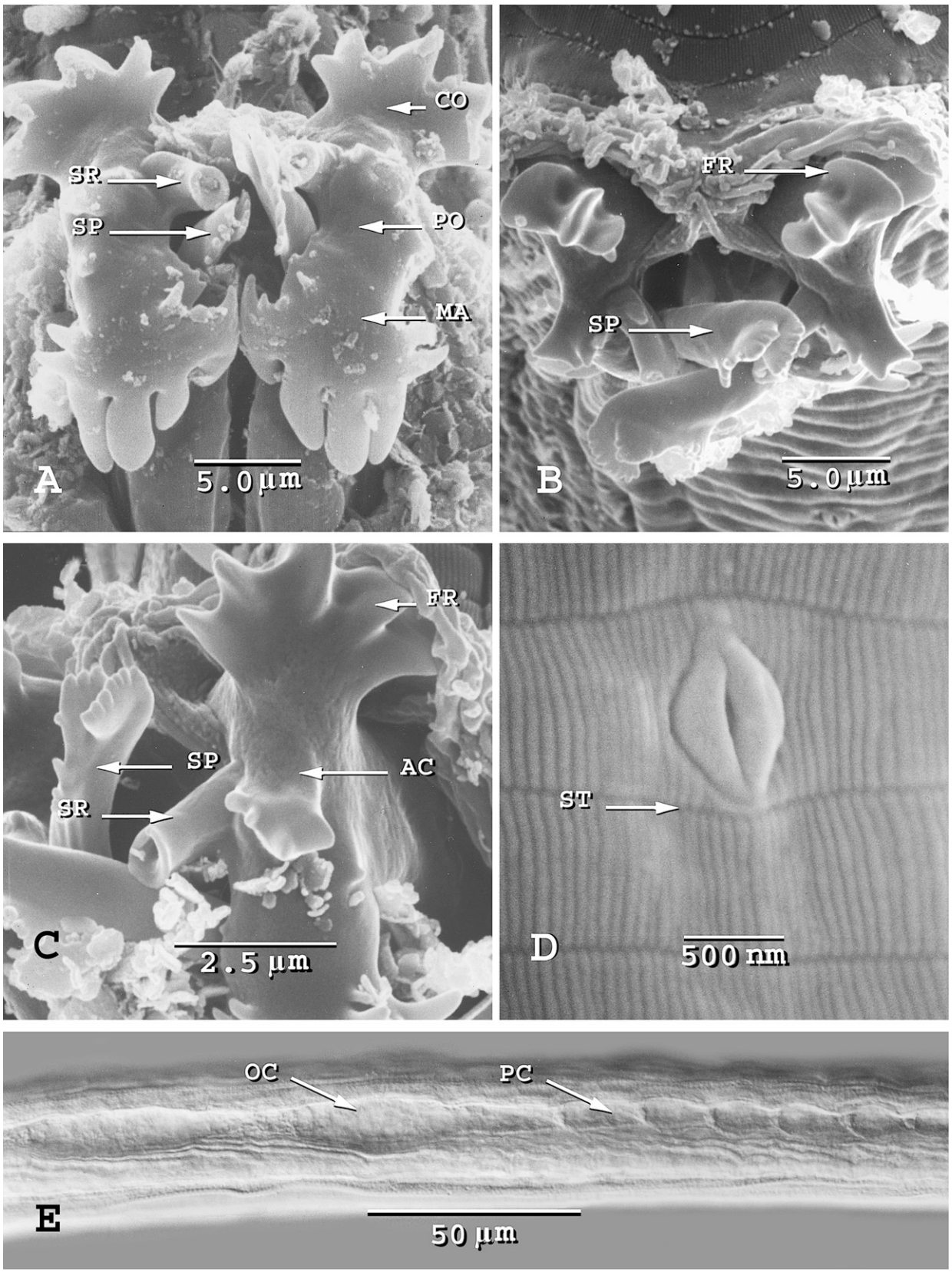

FIG. 2. *E. bipectinella* n. sp. A. Spicule (SP) and *manus* (MA), *pontis* (PO), *condylus* (CO) and sensory receptor (SR) of gubernaculum of USNM No. 171278. B. Spicula (SP) and foliate ridges (FR) on *condylus* of USNM No. 171279. C. Spicule (SP), sensory receptor (SR), accessory process (AC) and foliate ridges (FR) on the gubernaculum of USNM No. 171279. D. Ventromedian supplement and transverse (ST) and longitudinal striae, USNM No. 171280. E. Photomicrograph of two oblong (OC), distal cells and shorter, pyriform (PC) cells of the ejaculatory gland.

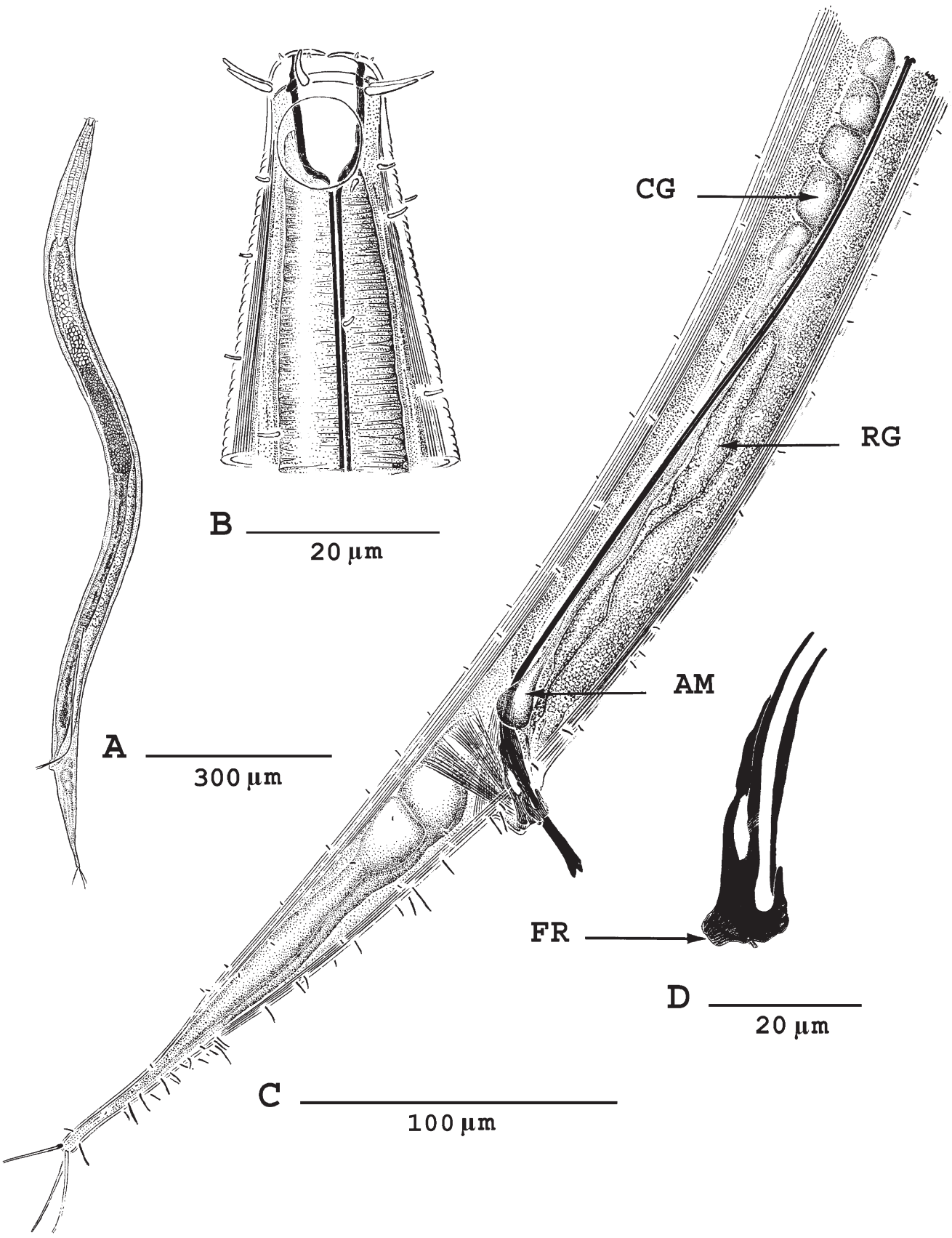

FIG. 3. *Elzalia tuberculata* n. sp. Holotype, USNM No. 171284. A. Habitus. B. Head. C. Posterior body region with rectal gland (RG) and cells (CG) and ampulla (AM) of the ejaculatory gland. D. Gubernaculum with foliate ridge (FR) on the *condylus*.

Nominal species of *Elzalia*

E. bipectinella n. sp.
E. federici Castillo-Fernandez and Lambshead, 1990: 68
E. floresi Gerlach, 1957: 454
E. gerlachi Zhang and Zhang, 2006
E. kimae Castillo-Fernandez and Lambshead, 1990: 68
E. mediterranea Vitiello, 1970: 656
E. poli Castillo-Fernandez and Lambshead, 1990: 66
E. striatitenuis, Zhang and Zhang, 2006
E. tenuis (Allgén, 1960) Gerlach, 1963: 650
Syn: *Filipjeviella tenuis* Allgén, 1960: 498
E. tuberculata n. sp.

E. tenuis was inadequately described from a single female and has not been redescribed since. It is here regarded as *species dubium.*

Artificial Key to Males of Elzalia

1(0). Spicula length greater than 200 µm 2
Spicula less than 200 µm 3
2(1). Posterior projections on gubernaculum foliate; spicula with subterminal cluster of numerous tubercles . . . . . *tuberculata* n. sp.
Posterior projections on gubernaculum conical; spicula with serrated margin and single, acute, distal process . . . . . *poli*
3(1). Body length less than 1200 µm 5
Body length greater than 1200 µm 4
4(3). Posterior projections on gubernaculum foliate . . . . . *bipectinella* n. sp.
Posterior projections on gubernaculum conical . . . . . *gerlachi*
5(3). Body length more than 700 µm 6
Body length less than 650 µm . . . . . *mediterranea* and *striatitenuis*[5]
6(5). Spiculum less than 150 µm 7
Spiculum more than 150 µm . . . . . *floresi*
7(6). Spicula length between 50 and 80 µm, 6-7% of body length; spicula with subterminal sigmoid bend and small terminal tubercules; ejaculatory gland 34-40% of body length with 2 oblong and 4-7 pyriform cells . . . . . *kimae*
Spicula length between 90 and 130 µm, 8-12% of body length; spicula distally spatulate with marginal serrations; ejaculatory gland 16-18% of body length with 2-3 gland cells of one type . . . . . *federici*

Elzalia bipectinella n. sp.
(Figs. 1 A - C, 2A - E)

Description of [holotype] and12 male paratypes: De Man's Ratios: a = 25 - 36 [26] (30 ± 3.4); b = 5.8 – 6.7 [6.2] (6.3 ± 0.3); c = 7.1 – 8.2 [7.3] (7.6 ± 0.4). L = 1,348 – 1,374 [1,330] (1,333 ± 53) µm.

Body fusiform, mostly tapered anteriorly from base of esophagus and in tail region. Head diameter 12 – 14 [13] (13 ± 3.4) µm; body diameter at level of amphid 12 – 14 [14] (13 ± 3.4) µm, at level of nerve ring 29 – 39 [36] (34 ± 2.8) µm, at base of esophagus 33 – 42 [42] (39 ± 3.0) µm, at midbody 36 – 50 [50] (44 ± 4.9) µm, and at level of cloacal vent 28 – 41 [32] (32 ± 3.7) µm.

Cephalic sensilla 6 + 10 (Fig. 1A). Inner labial sensilla papilliform. Outer labial sensilla 5 – 7 [7] (6.4 ± 0.9) µm long and 54 % of head diameter; paralabial sensilla 4 – 5 [5] (4.6 ± 0.4) µm or 29 – 42 [38] (35 ± 4.4) % of head diameter. Distance from oral surface to anterior margin of amphids 4 – 6 [5] (4.6 ± 0.7) µm. Length of amphid 8 – 12 [11] (10 ± 1.4) µm and width 9 – 11 [10] (9.6 ± 0.7) µm, or 61 - 81 [71] (71 ± 6.4) % of corresponding body diameter.

Cervical sensilla 2 - 4 (2.5 ± 0.6) and somatic sensilla 3 - 4 (3.4 ± 0.3) µm long. Periodicity of transverse striae 1.6 µm in midbody region. Interstrial, longitudinal lines present at least in region of body bearing ventromedian supplements and on tail; periodicity of longitudinal lines 63 nm (Fig. 2D).

Buccal capsule goblet-shaped (Fig. 1A), 10 – 13 [13] (12 ± 0.9) µm; maximum width 3 – 8 [8] (6 ± 1.9) µm at level of buccal seam; 49 – 71 [60] (61 ± 6.7) % of buccal length overlapped by amphid. Clavate esophagus 202 – 222 [213] (209 ± 6.5) µm long. Distance to nerve ring from oral surface 92 – 107 [107] (102 ± 4.2) µm, or 41 – 51 [50] (49 ± 2.8) % of esophagus length.

Tail length 162 – 193 [181] (175 ± 9.5) µm, or 4 – 6 [5.7] (5.5 ± 0.5) cloacal body diameters long; cylindrical portion 19 – 31 [22] (25 ± 4.1) µm, or 11 – 18 [12] (14 ± 2.6) % of tail length. Subventral rows of sensilla present (Figs. 1B and C), and more abundant and longer near posterior end of tail. Three terminal sensilla present 13 – 23 (18 ± 3.4) µm long.

Subventral, precloacal rows of sensilla present, each sensillum approximately same length as sublateral somatic sensilla. Number of ventromedian supplements 5[6]; length of each supplement equal to interstrial distance (Fig. 2D), with 30 - 31 (30 ± 1.0; n = 4) striae between supplements. Distance from cloacal vent to first (furthest posterior) ventromedian supplement 22 µm; from first ventromedian supplement to second 25 µm; from second to third 26 µm; from third to fourth 24 µm; and from fourth to fifth 20 µm.

Anterior testes 190 - 392 [300] (313 ± 58) and posterior testes 86 - 221 [192] (175 ± 42) µm long; length of posterior testes 25 - 94 [64] (59 ± 21) % of anterior testes.

Ejaculatory glands with two distal oblong cells and variable number of shorter, proximal, pyriform cells. Ejaculatory gland on right side of body (Fig. 1B) with 5 – 8 [7] (6.3 ± 1.0) and gland on left with 6 – 8 [8] (6.7 ± 0.7) serially arranged cells of two types; length of each

[5] These two species cannot be separated reliably on the basis of the criteria given by Zhang and Zhang (2006).

[6] The ventromedian supplements are not reliably detectable from longitudinal optical sections of wholemount preparations for light microscopy. This count is from a single specimen examined with scanning electron microscopy.

anterior oblong cell 44 – 63 [61] (61 ± 10) on right side and 42 – 73 [52] (61 ± 10) μm on left; remaining cells oval to pyriform, 22 – 29 [23] (26 ± 2.6) μm long on right side and 17 - 28 [28] (25 ± 2.6) μm on left. Neck from each oblong and pyriform cell of right and left glands joined duct-like with right and left ampullae situated lateral to cloaca (Fig. 1C). Distance from cloaca to anterior end of ejaculatory gland on right side 426 – 485 [474] (463 ± 23) μm, or 33 – 36 [36] (35 ± 1.1) % of total body length, and 382 – 598 [494] (486 ± 69) μm on left side, or 30 - 42 [38] (36 ± 4.0) % of body length.

Elongate rectal gland (Fig. 1B) ventral to, and parallel with, duct of ejaculatory gland; cytoplasm granular. Distance from cloaca to anterior end of rectal gland 112 – 199 [198] (173 ± 26) and 163 – 205 [205] (179 ± 15) μm on right and left sides respectively, or 12 -15 [15] (13 ± 1.1) % of total body length on both sides.

Length of right and left spicula 109 – 139 [118] (121 ± 10) and 104 – 142 [115] (121 ± 12) μm respectively; lengths of right and left spicula combined 8 – 11 [9] (9 ± 1.0) % of body length and 3.2 – 4.4 [3.7] (4.0 ± 0.4) cloacal body diameters. Spicula arched ventrad near cloaca; shaft of spicula flexible with or without shallow, sigmoid bends (Fig. 1C). Proximal tip of spicula slightly enlarged; distal tip of each spiculum with two parallel, comb-like ridges (Figs. 2A - C).

Length of gubernaculum 32 – 36 [34] (34 ± 1.3) μm, or 0.8 – 1.14 [1.1] (1.07 ± .11) cloacal body diameters. *Manus* with four, dorsally-directed, digits; base of first (lateral) digit with two or three laterally directed spines (Fig. 2A), and rim of *manus* at posterior edge of fourth (medial) digit with two to four spines directed posteroventrally toward protruded spicula (Fig. 2A). Each *pontis* with sensory receptor (Figs. 2A and 2C) and with cylindrical, blunt, ventrad accessory process bearing small tubercles (Fig. 2C). Sensory receptor cylindrical, truncate and with terminal aperture (Figs. 2A and C). Posterior, fist-like *condylus* with four or five foliate ridges; first (lateral) ridge largest with each ridge toward medial plane progressively smaller (Figs. 2A - C).

Diagnosis: The total length of the body, including the tail, is 1,348 to 1,374 μm. The ejaculatory gland consists of a tandem series of five to six pyriform cells and two anterior, oblong cells. The combined length of right and left ejaculatory glands, measured from the anterior end of each gland to the level of the cloaca, ranges from 382 to 598 μm, or 30 to 42 % of the body length. The combined average length of right and left spicula is 104 to 142 μm, or 8 to 11 % of body length and 62 to 83 % of the tail length. The distal tip of each spiculum has two, terminal, parallel, ridges with comb-like serrations. Each half of the *condylus* has four or five posterior, foliate ridges. The lateral is largest with gradual transition in size to the medial and smallest ridge. The accessory process of the sensory receptor is peg-like bearing small protuberances.

Material examined: Male holotype, USNM No. 171268; male paratypes, USNM Nos. 171269 - 171280.

Type locality: Khung Kraben Bay, situated beyond the mangrove swamp in the coastal area of Chanthaburi, east Thailand; 12° 35′ N; 101° 55′ E; collected March 8, 1989.

Type habitat: Muddy sand with dense growths of seagrasses (*Enhalus acoroides*, *Halodule pinifolia* and *Halophila* sp.) and seaweeds (*Acetabularia sp.* and *Sargassum sp.*). Mean depth 0.8 - 4.5 m.

Elzalia tuberculata n. sp.
(Figs. 3A - D and 4A and B)

Description of [holotype] and 7 male paratypes: De Man's Ratios: a = 22 – 32 [23] (28 ± 4); b = 5.7 – 6.5 [6.4] (6.2 ± 0.3); c = 6.6 – 7.7 [7.0] (7.4 ± 0.4). L = 1,164 – 1,478 [1,287] (1,341 ± 96) μm.

Body fusiform, mostly tapered anteriorly from base of esophagus and in tail region (Fig. 3A). Head diameter 11 – 14 [12] (12 ± 1) μm; at level of nerve ring 35 – 40 [35] (37 ± 2) μm; at base of esophagus 40 – 52 [49] (45 ± 4) μm, at midbody 41 – 58 [55] (49 ± 6) μm, and at level of cloacal vent 28 – 41 [41] (34 ± 5) μm.

Cephalic sensilla 6 + 10. Inner labial sensilla papilliform. Outer labial sensilla 4 – 6 [6] (5 ± 1) μm long, or 29 – 55 [50] (40 ± 9) % of head diameter and paralabial sensilla 4 – 6 [4] (5.6 ± 1) μm, or 33 – 63 [33] (50 ± 10) % of head diameter. Distance from oral surface to anterior margin of amphid 4 – 6 [6] (5.6 ± 0.6) μm; length of amphid 10 – 12 [10] (10.5 ± 1) μm; width 9 – 11 [10] (10 ± 1) μm or 62 – 85 [77] (75 ± 6) % of corresponding neck diameter.

Cervical sensilla situated in subdorsal, sublateral and subventral longitudinal rows; cervical sensilla and somatic sensilla 2 - 4 [3] (2.5 ± 0.6) μm long. Periodicity of transverse striae 1.3 μm. Longitudinal striae present at least in region of body bearing ventromedian supplements and on tail.

Buccal capsule goblet-shaped (Fig. 3B); length 11 – 14 [14] (13 ± 1) μm; maximum width 4 - 7 [6] (6 ± 1) μm at level of buccal seam; 45 - 64 [57] (56 ± 6) % of buccal length overlapped by amphid. Length of esophagus 182 – 238 [203] (216 ± 18) μm; distance from oral surface to nerve ring 83 - 120 [98] (105 ± 12) μm, or 46 – 51 [48] (49 ± 2) % of esophagus length.

Tail length 174 – 194 [183] (182 ± 8) μm, or 4.4 – 6.3 [4.4] (5.5 ± 1) cloacal body diameters long; cylindrical portion 25 – 35 [35] (30 ± 4.5) μm long, or 13 – 21 [19] (17 ± 3) % of tail length. Tail with subventral rows of prominent sensilla, more abundant at posterior end of tail (Fig. 3C); length of caudal sensilla 4 - 9 (6.4 ± 1.5) μm. Terminal sensilla 20 – 26 [26] (23 ± 2) μm long.

Subventral, precloacal rows of sensilla present, each subventral sensillum approximately same length as sublateral somatic sensilla. Number of ventromedian supplements 5[7]; each supplement slit-like with raised,

[7] Observed in SEM preparation of specimens TR4 (n = 1).

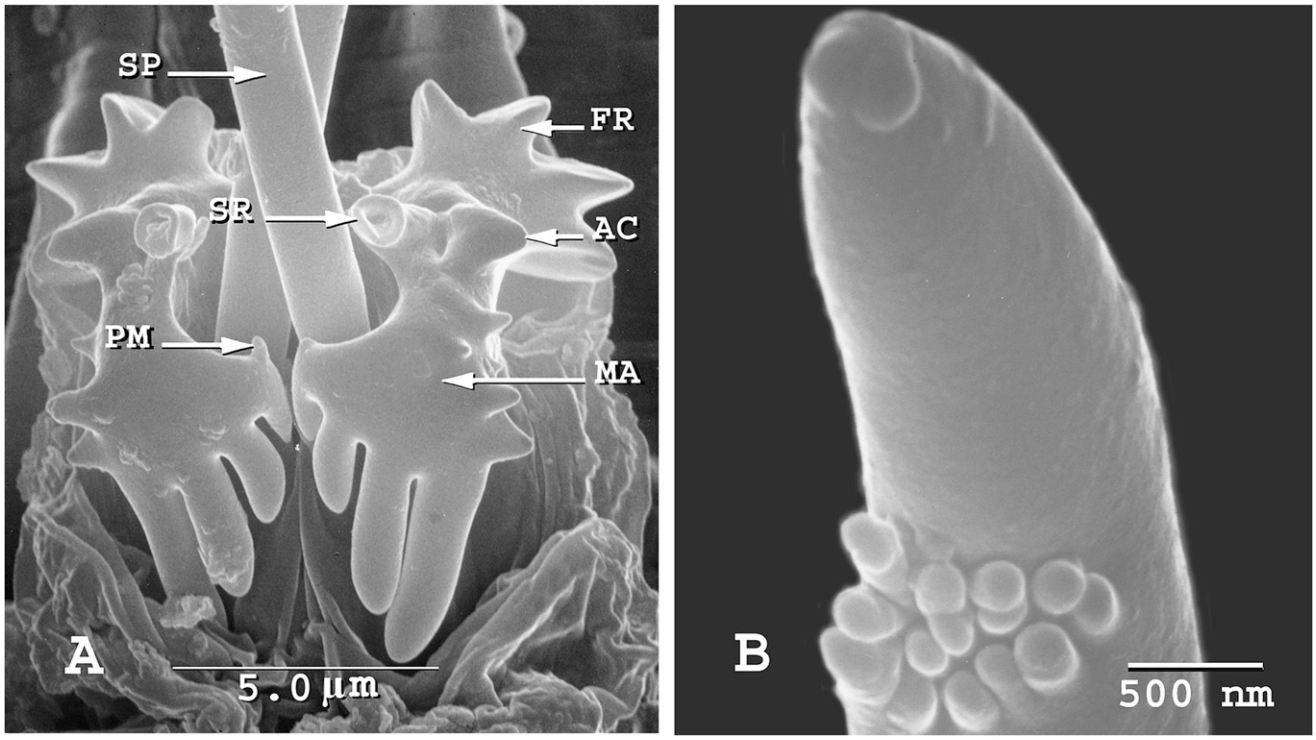

FIG. 4. *E. tuberculata* n. sp. A. *Manus* (MA), shaft of spiculum (SP), posterior process of the *manus* (PM), sensory receptor (SR), accessory process (AC) and foliate ridges (FR) at distal end of the gubernaculum of USNM No. 171291. B. Cluster of tubercles at distal end of the spiculum of USNM No. 171291.

lateral, crescent-shaped margins; length of each supplement equal to interstrial distance, with 17 - 37 (32 ± 3) striae between supplements. Distance from cloacal vent to first (furthest posterior) ventromedian supplement 16 µm; from first to second 17 µm; from second to third 31 µm; from third to fourth 28 µm; and from fourth to fifth 33 µm.

Anterior testes 271 – 448 [335] (353 ± 57) and posterior testes 115 - 246 [163] (173 ± 57) µm long; length of posterior testes 36 - 66 [49] (48 ± 11) % of anterior testes.

Ejaculatory gland on right side with 5 [5] (5 ± 0) and on left side with 4 [4] (4 ± 0) serially arranged cells of one type only. Cytoplasm of each cell body hyaline with anteriorly displaced nucleus (Fig. 3C). Length of cells in right ejaculatory gland 12 - 27 [14, 16, 13, 19, 20] (20 ± 5) and in left gland 16 - 35 [20 - 27 (24 ± 3)] (23 ± 4.4) µm. Distance from cloaca to anterior end of ejaculatory gland on right 265 - 322 [243] (291± 36) µm, or 19 – 24 [19] (21 ± 2.6) % of body length; on left 221 - 312 [253] (270 ± 36) µm, or 16 - 23 [20] (20 ± 2.6) % of body length.

Rectal gland cytoplasm with spherical granules. Distance from cloaca to anterior end of rectal gland on right side 139 – 197 [162] (162 ± 23) µm, or 11 - 14 [13] (12 ± 1.2) % of body length; on left side 142 - 198 [172] (165 ± 22) µm, or 11 – 14 [13] (12 ± 1.3) % of body length.

Right spicula 262 - 299 [299] (275 ± 12) µm long; left spicula 252 - 277 [277] (264 ± 10) µm long. Right and left spicula combined 19 - 23 [22] (20 ± 2) % of body length, or 6.7 – 9.3 [7.0] (8.0 ± 1.0) cloacal body diameters. Spicula arched ventrad near cloaca; shaft of spicula flexible with elongate bend (Fig. 3C). Proximal tip of spicula slightly thickened; distal tip of each spiculum obtusely conical with cluster of 7 - 14 [14] (10 ± 4) tubercles, 1.7 - 2.6 [1.7] (2.2 ± 0.4) µm from distal tip of spicula (Fig. 4B).

Gubernaculum 33 - 43 [43] (38 ± 3) µm long, or 1.0 – 1.4 (1.1 ± 0.2) cloacal body (Fig. 3D) diameters. *Manus* with four, dorsally-directed, digits; lateral surface of *manus* and anterior end of *pontis* with three or four laterally directed spines; rim of *manus* at posterior edge of fourth (medial) digit with single, small spine directed posteroventrally toward spicular shaft (Fig. 4A). *Pontis* with sensory receptor cylindrical, truncate sensory receptor with terminal opening (Fig. 4A), and with conical accessory process. *Condylus* with three to five foliate ridges; first (lateral) ridge slightly larger than inner (medial) ridge (Fig. 4A).

Diagnosis: The total length of the body is 1,164 to 1478 µm. The ejaculatory gland is comprised of a series of four to five cells of one kind, larger anterior oblong cells being absent. The combined length of right and left ejaculatory glands measured from the anterior end of each gland to the level of the cloaca ranges from 221 to 322 µm, or 16 to 24 % of the body length. The combined lengths of right and left spicula range from 252 to 299 µm, or 19 to 23 % of the body length and 140 to 160 % of the tail length. The distal end of each

spiculum has a single cluster of subterminal tubercles. The *condylus* bears a variable number of foliate ridges.

Material examined: Male holotype, USNM No. 171284; male paratypes, USNM Nos. 171285 - 171291.

Type locality: Khung Kraben Bay, situated beyond the mangrove swamp in the coastal area of Chanthaburi, east Thailand; 12° 35′ N; 101° 55′ E; collected March 8, 1989.

Type habitat: Muddy sand with dense growths of seagrasses (*Enhalus acoroides, Halodule pinifolia* and *Halophila sp.*) and seaweeds (*Acetabularia sp.* and *Sargassum sp.*). Mean depth 0.8 – 4.5 m.

Females of *E. bipectinella* n. sp. and *E. tuberculata* n. sp.

Material examined: Females, USNM Nos. 1128809 – 112828.

Locality: Khung Kraben Bay, situated beyond the mangrove swamp in the coastal area of Chanthaburi, east Thailand; 12° 35′ N; 101° 55′ E; collected March 8, 1989.

Type habitat: Muddy sand with dense growths of seagrasses (*Enhalus acoroides, Halodule pinifolia* and *Halophila sp.*) and seaweeds (*Acetabularia sp.* and *Sargassum sp.*). Mean depth XX m.

Description of Females: De Man's Ratios: a = 23.3 - 30.1 (26.5 ± 2.4); b = 5.8 - 6.7 (6.2 ± 0.3); c = 7.6 - 8.0 (7.8 ± 0.1); V = 47 - 51 (49 ± 1)%.

General morphology similar to males. Reproductive system monodelphic, prodelphic, on left side of gut; anterior end of ovary often near base of esophagus, and anterior region of ovary frequently with sigmoid bend. One or two well-developed ova present in uterus.

Posterior seminal receptacle continuous with uterus and possibly derived from posterior branch of gonoduct. Seminal receptacle comprised of narrow, thick-walled, proximal region and distal, saccate region, both situated on left side of gut. Lumen of saccate region usually filled with sperm. Large, granular, ventromedian gland present on anterior and posterior sides of vagina; gland openings apparently near orifice of vulva.

Elzalia federici Castillo-Fernandez and Lambshead, 1990
(Figs. 5A – D and 6A - C)

Description of the holotype, 6 paratypes, 5 topotypes and Ep3: De Man's Ratios: a = 24.3 - 27.8 (26 ± 1); b = 4.5 - 5.4 (5.1 ± 0.3); c = 6.8 – 8.3 (7.4 ± 0.3). L = 745 - 1,064 [960] (945 ± 86) μm.

Body fusiform, mostly tapered anteriorly from base of esophagus and in tail region (Fig. 5A). Head diameter 10 - 11 (10.7 ± 0.4) μm; body diameter at level of amphid 10 - 13 (11 ± 1.3) μm; at level of nerve ring 26 - 30 (29 ± 1.5) μm; at base of esophagus 30 - 36 [45] (34 ± 2.4) μm; at midbody 29 - 38 (35 ± 3.6); and at level of cloacal vent 22 - 28 (25 ± 2.2) μm.

Cephalic sensilla 6 + 10. Inner labial sensilla papilliform. Distance from oral surface to outer labial and paralabial sensilla 2 - 4 [2] (3 ± 0.8) μm; outer labial sensilla 5 [5] (5 ± 0) μm long; paralabial sensilla 3 - 4 [3] (3.4 ± 0.5) μm, or 30 - 40 [27] (35 ± 4.3) % of head diameter. Distance from oral surface to anterior margin of amphids 3 - 5 [5] (4 ± 0.6) μm. Length of amphid 9 - 11 [12] (10 ± 0.8) μm and width 8 - 10 [10] (9 ± 1.0), or 73 - 85 (78 ± 5.3) % of corresponding body diameter.

Cervical sensilla situated in subdorsal, sublateral and subventral longitudinal rows. Periodicity of transverse striae 0.9 - 1.2 [1.1] (1.1 ± 0.1) μm in midbody region.

Buccal capsule nearly cylindrical 13 – 15 [14] (13.5 ± 0.8) μm long (Fig. 5B); maximum width 5 - 6 [5] (5.2 ± 0.4) μm at level of buccal seam; 51 - 76 [65] (68 ± 9.5) % of buccal capsule length overlapped by amphid. Esophagus 164 - 189 [184] (180 ± 9.2) μm long. Distance to nerve ring from oral surface 78 - 98 [93] (90 ± 7) μm, or 48 - 52 [51] (50 ± 1) % of esophagus length.

Tail length 104 - 130 [113] (117 ± 8.5) μm, or 3.3 - 5.2 (3.8 ± 0.6) cloacal body diameters long; cylindrical portion 16 - 21 [16] (18 ± 2.8) μm, or 14 - 16 [14] (15 ± 1) % of tail length. Subventral rows of sensilla present on tail (Fig. 5C); sensilla most abundant and longer near posterior end of tail; sensilla length 2 - 10 (6 ± 2.2) μm. Terminal sensilla 11 - 15 [15] (13 ± 2.8) μm long. Number of ventromedian supplements unknown.

Anterior testes 184 - 252 [ND] (219 ± 34) and posterior testes 81 - 132 [ND] (102 ± 27) μm long; length of posterior testes 43 - 52 [ND] (46 ± 5) % of anterior testes.

Ejaculatory gland on right side with 3 (0) and gland on left with 2 - 3 [2] (2.3 ± 0.5) serially arranged cells of one kind (Fig. 5C). Distance from cloaca to anterior end of gland on right side 161 - 170 [ND] (165 ± 5) μm, or 17 - 18 [ND] (17.6 ± 0.6) % of body length; on left side 151 - 173 [153] (160 ± 10) μm, or 16 - 18 [16] (17 ± 0.8) % of body length.

Rectal gland (Fig. 5C) situated ventral to duct of ejaculatory gland and bifurcated (two celled?); cytoplasm granular. Distance from cloaca to anterior end of rectal gland 98 - 113 [ND] (107 ± 8) μm on right side, or 11 - 13 [ND] (12 ± 1) % of total body length, and 62 - 132 [62] (98 ± 24) μm on left side, or 7 – 13 [7] (11 ± 2.5) % of body length.

Length of right and left spicula 91 - 117 [105] (109 ± 9.1) and 105 - 125 [106] (113 ± 9.0) μm respectively. Combined right and left spicula lengths 10 - 16 [11] (12 ± 2) % of body length, or 82 – 106 [92] (97 ± 7.9) % of tail length. Spicula arched ventrad near cloaca; shaft of spicula apparently flexible with or without shallow, sigmoid bends (Fig. 5C). Distally each spiculum spatulate with each edge bearing rounded serrations (Figs. 6A - C).

Gubernaculum (Fig. 5D) 20 - 28 [27] (25 ± 2.5) μm long, or 0.7 - 1.1 [0.9] (.89 ± 0.15) cloacal body diameters. Each *manus* with four (observed in paratype with light microscopy), dorsally-directed, digits; rim of *manus* at posterior edge of fourth digit with single, large conical projection directed posteriorly toward shafts of spicula (Fig. 6C). Right and left sides of *pontis*

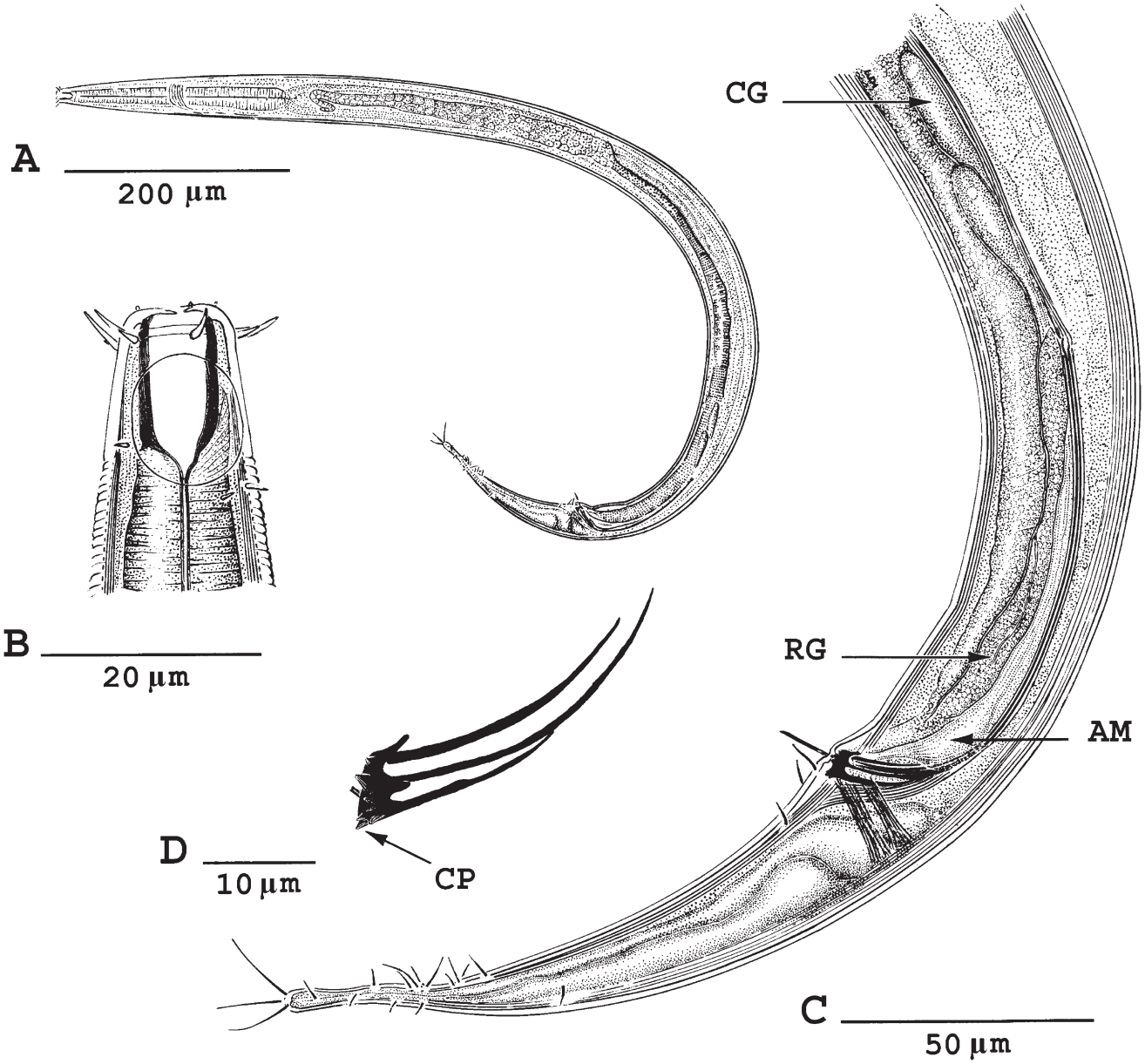

FIG. 5. *Elzalia federici*. Topotype, USNM No. 171304. A. Habitus. B. Head. C. Posterior body region with bifurcate rectal gland (RG) and cells (CG) and ampulla (AM) of the ejaculatory gland. D. Gubernaculum with conical projections (CP) on the *condylus*.

each with a cylindrical, truncated, sensory receptor with terminal aperture (Fig. 6C); accessory process of sensory receptor conical (Fig. 6B and C). Each *condylus* with one or two conical protuberances directed laterally and one or more directed posteriorly (Fig. 6C).

Diagnosis: The body length is 745 - 1,064 [960] (945 ± 86) µm. Each ejaculatory gland is comprised of 2 or 3 elongate cells of one type, the larger oblong cells present in *E. bipectinella* being absent. The combined length of right and left ejaculatory glands ranges from 151 - 173 µm, or 16 - 18 [16] (17 ± 0.8) % of the body length. The lengths of right and left spicula range from 91 to 117 µm, or 10 to 16 % of the body length and 82 to 106 % of the tail length. The distal region of each spiculum is trough-like, the edges of the trough provided with rounded serrations. The posterior and lateral projections of the *condylus* and accessory process of the sensory receptor are conical. Overall the distal end of the gubernaculum is not as large as it is in the other species studied here.

Material examined: Male holotype, Ef1, BM(NH)1989.1.6.; EP3, BM(NH)1989.1.3, which was originally misidentified as a paratype of *Elzalia poli*; paratypes Ef2, BM(NH) 1989.1.1, Ef3, BM(NH) 1989.1.4, Ef4, BM(NH).1.5, Ef5, BM(NH).1.8, Ef6, BM(NH).1.3, Ef7, BM(NH).1.9; topotypes, USNM Nos. 171301 - 171305.

Type locality: Near oil drilling platforms in the Cayo Arcas region, Campeche Sound, Gulf of Mexico; Station 8, 19°25′ longitude N and 91°50′ latitude W; depth 42 m; collected 1986.

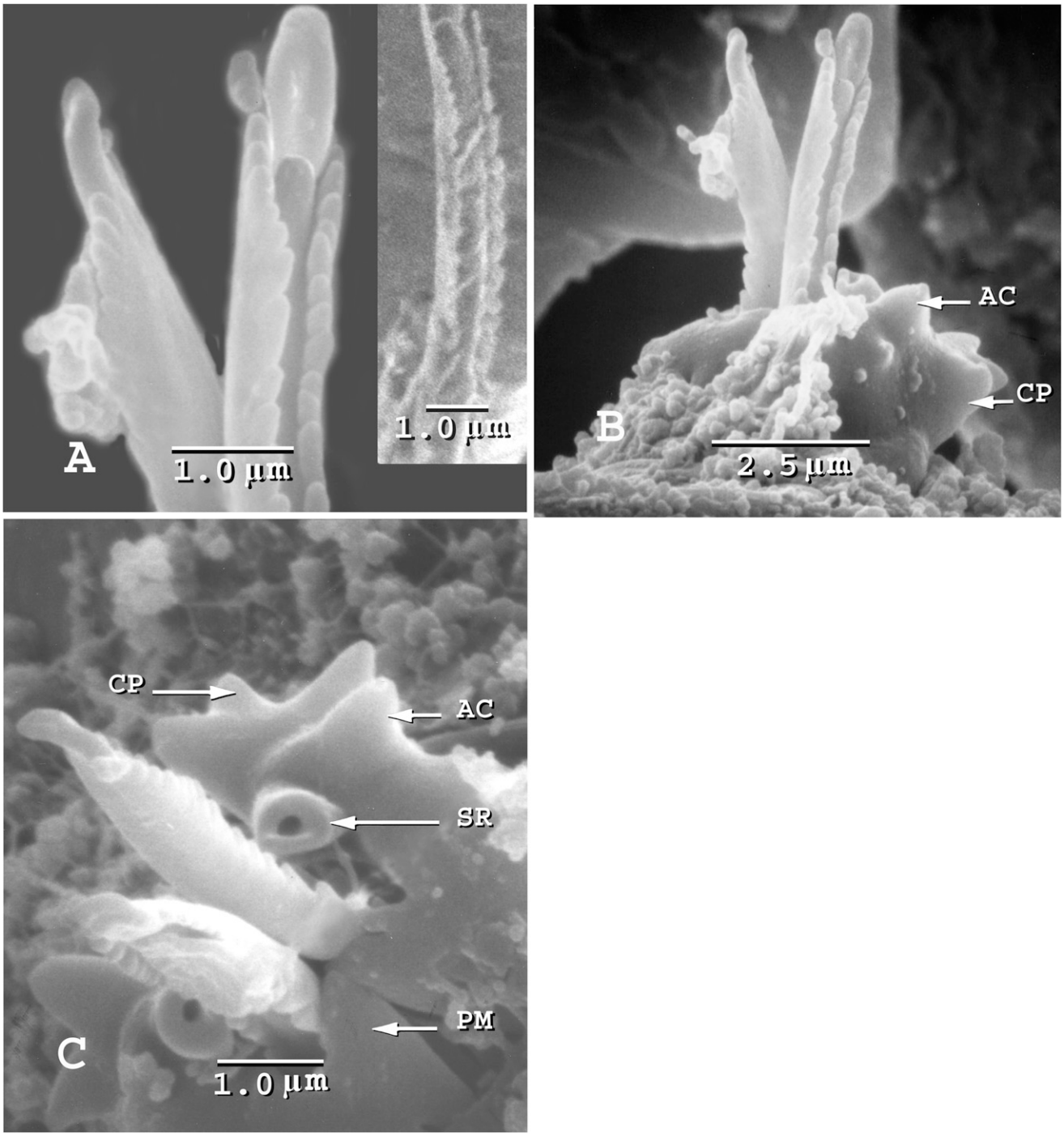

FIG. 6. *E. federici*. Topotype, USNM No. 171305. A. Distal tips of spicula with serrate margins. Inset: Distal tip of spiculum of holotype BM No. Ef 1. BM(NH) 1989.1.6. B. Spicula and gubernaculum with accessory process (AC) and conical projections (CP) on the *condylus*. C. Spicula and gubernaculum with sensory receptor (SR), accessory process (AC) and conical projections (CP) on the *condylus*, and posteriorly directed process (PM) of the *manus*.

Habitat: Grey, jelly-like clay sediment.

Elzalia kimae Castillo-Fernandez and Lambshead, 1990
(Figs. 7A – D and 8A - B)

Description of [holotype] and 5 male topotypes: De Man's Ratios: a = 27 - 32 (29 ± 2); b = 4.8 - 5.5 [5.8] (5.4 ± 0.3); c = 7.2 - 9.1 [8.7] (8.4 ± 0.7). L = 994 - 1042 [984] (1013 ± 21) µm.

Body fusiform, mostly tapered anteriorly from base of esophagus and in tail region (Fig. 7A). Head diameter 12 - 13 [11] (12.7 ± 0.6) µm; body diameter at level of amphid 13 - 15 [11] (14 ± 1) µm, at level of nerve ring 27 - 31 [ND] (30 ± 2) µm, at base of

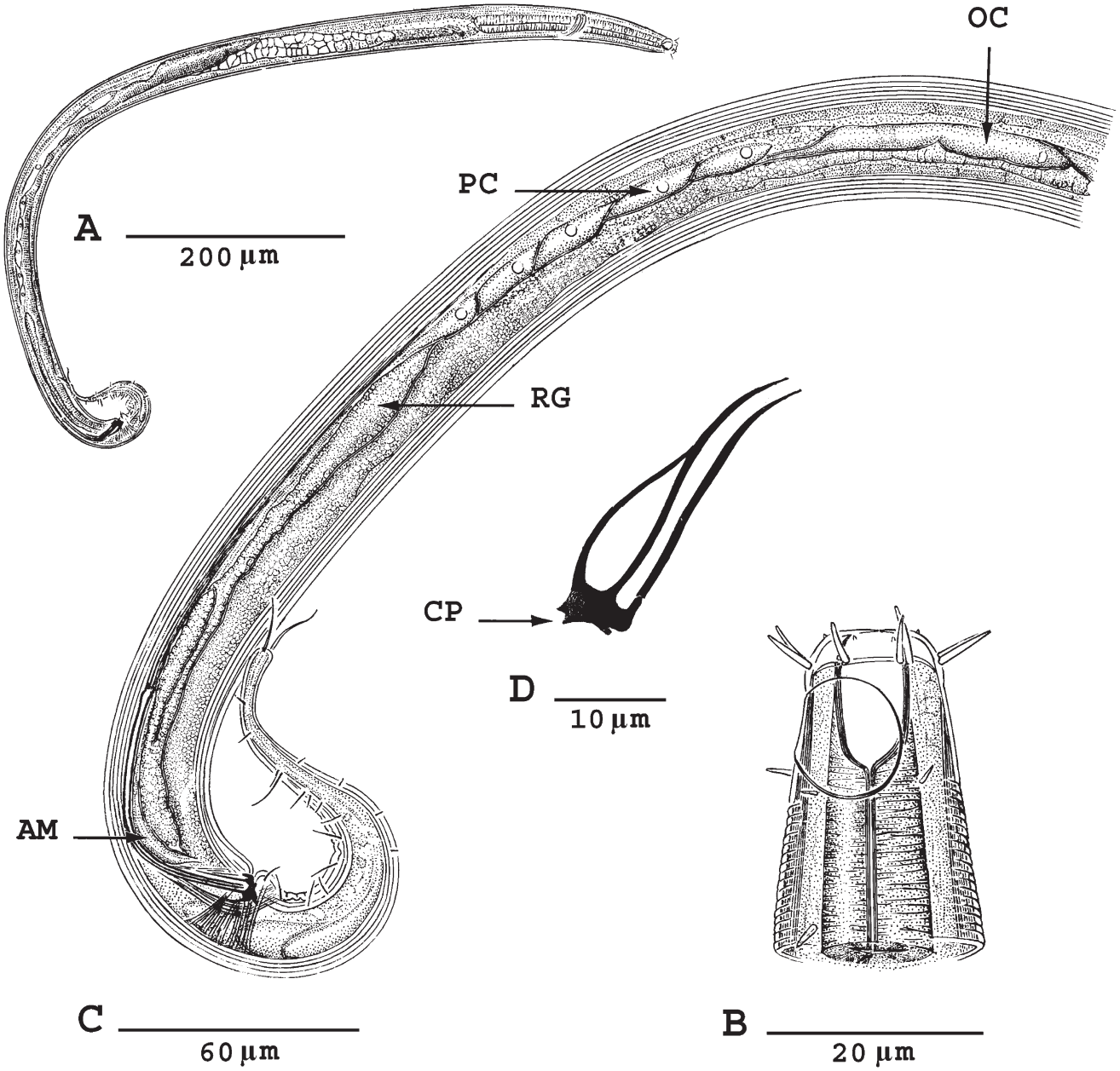

FIG. 7. *Elzalia kimae*. Topotype, USNM No. 171296. A. Habitus. B. Head. C. Posterior body region with bifurcate rectal gland (RG) and ampulla (AM) and oblong (OC) and pyriform (PC) cells of the ejaculatory gland. D. Gubernaculum with conical projections (CP) on the *condylus*.

esophagus 31 - 36 [ND] (34 ± 2) µm, at midbody 31 - 36 [ND] (35 ± 2) µm, and at level of cloacal vent 21 - 25 [ND] (23 ± 2) µm.

Cephalic sensilla 6 + 10. Inner labial sensilla papilliform. Distance from oral surface to outer labial and paralabial setiform sensilla 2 - 3 [2] (2.4 ± 0.5); outer labial sensilla 6 - 7 [6] (6 ± 0.6) µm, and paralabial sensilla 4 - 5 [5] (4.7 ± 0.6) µm long µm, or 31 - 42 [45] (38 ± 5.2) % of head diameter. Distance from oral surface to anterior margin of amphids 3 -5 [ND] (4.4 ± 1.0) µm. Length of amphid 11 - 12 [ND] (11.2 ± 0.4) µm and width 9 - 10 [ND] (9.8 ± 0.5) µm, or 62 - 81 [ND] (72 ± 9) % of corresponding body diameter. Periodicity of transverse striae 1.0 - 1.3 [ND] (1.2 ± 0.1) µm in mid body region.

Buccal capsule cylindrical to goblet-shaped (Fig. 7B), 11 - 13 [11] (12 ± 1.0) long and 6 - 7 [6] (6.5 ± 0.5) µm wide at level of buccal seam; 58 - 71 [65] (63 ± 5) % of stoma length overlapped by amphid. Length of esophagus 181 - 206 [169] (190 ± 10) µm. Distance oral surface to nerve ring 90 - 103 [82] (95 ± 5.0) µm, or 48 - 51 [49] (50 ± 1.0) % of esophagus length.

Tail length 114 - 139 [113] (120 ± 10) µm, or 4.7 - 6.3 (5.3 ± 0.7) cloacal body diameters long; cylindrical portion 20 - 28 [27] (23 ± 3.3) % of tail length. Subventral rows of caudal sensilla present (Fig. 7C); sensilla more

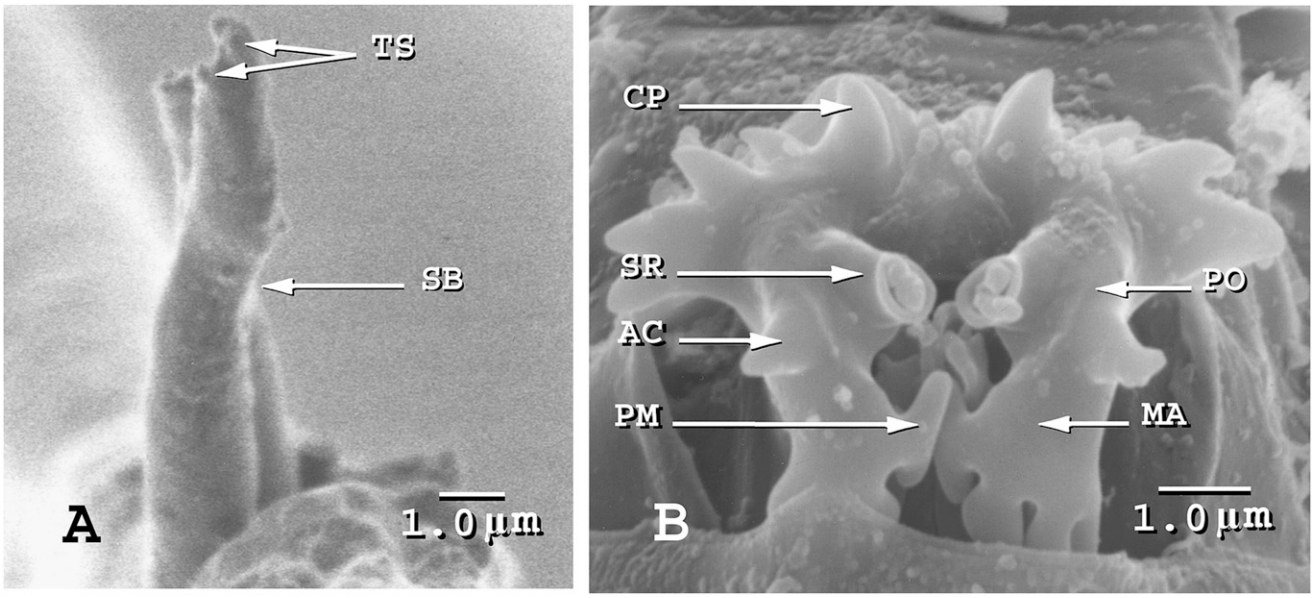

FIG. 8. *E. kimae*. A. Distal tip of a spiculum with slight subterminal bend (SB) and subterminal and terminal tubercles (TS) of holotype, BM No. Ek 1. BM(NH) 1989.1.4. B. Gubernaculum with conical projection (CP) on the *condylus*, sensory receptor (SR) and accessory process (AC) of the *pontis* (PO), and posterior process (PM) of the *manus* (MA) of topotype USNM No. 171297.

abundant and longer near mid and anterior regions of tail; length of caudal sensilla 4 - 13 [ND] (7 ± 2.7) µm. Terminal sensilla 9 - 17 [ND] (13 ± 3.0) µm long.

Anterior testes 158 - 195 [158] (175 ± 19) and posterior testes 71 - 101 [71] (91 ± 17) µm long; length of posterior testes 45 - 59 [45] (52 ± 7.0) % of anterior testes. Anterior testis straight or flexed; posterior testis straight.

Ejaculatory gland comprised of serially arranged cells of two types. Length of gland on right side 342 - 418 [ND] (390 ± 29) µm, or 34 - 40 [ND] (38 ± 3) % of total body length, and 370 - 408 [ND] (393 ± 15) µm on left side, or 37 - 40 [ND] (39 ± 1) % of body length. Two anterior-most cells oblong, 39 - 73 [ND] (50 ± 13) µm long on right side and 44 – 69 [ND] (57 ± 10) on left; remaining cells smaller, oval to pyriform, each with anteriorly displaced nucleus. Number of pyriform cells on right and left sides identical 4 - 7 [ND] (5 ± 1) (Fig. 7C); length of pyriform cells on right and left sides 20 - 25 [ND] (22 ± 2) and 16 - 23 [ND] (19 ± 3) respectively.

Rectal gland bifurcated (two-cells?) (Fig. 7C) with granular cytoplasm. Distance from cloaca to anterior end of rectal gland 108 - 173 [ND] (154 ± 27) on right side, or 11 - 17 [ND] (15 ± 3) % of total body length, and 152 - 171 [ND] (161 ± 8) µm, or 15 - 16 [ND] (16 ± 0.5) % of total body length on left side; cell body length on right and left 31 - 49 [ND] (40 ± 7) and 36 - 57 [ND] (47 ± 9.0) µm respectively.

Length of right and left spicula 60 – 72 [65] (67 ± 5) and 59 - 78 [69] (67 ± 7.0) µm respectively; combined right and left spicula lengths 6 - 7 [7] (6.8 ± 0.5) % of body length, or 45 - 60 [59] (56 ± 6) % of tail length. Spicula arched ventrad near cloaca (Fig. 7C); spicula distally set off by shallow sigmoid bend and with one subterminal tubercle and two small terminal tubercles (Fig. 8A).

Gubernaculum 29 – 37 [31] (33 ± 3) µm long, or 1.3 - 1.6 [ND] (1.4 ± 0.1) cloacal body diameters. Each *manus* with four digits (Fig. 8B); first (lateral) digit of *manus* longest with each progressively shorter toward fourth, nearest sagittal plane; base of first digit with or without a single lateral spine, and fourth medial digit with single, posteriorly directed, cylindrical process with bluntly rounded terminus (Fig. 8B). Sensory receptor cylindrical, truncate and with terminal opening; accessory process of sensory receptor conical (Fig. 8B). Each *condylus* with three to five conical, protuberances directed laterally and posteriorly and/or ventrally (Fig. 8B).

Diagnosis: The body length is 984-1042 µm. The ejaculatory gland is comprised of a series of pyriform to lenticular cells, with terminal oblong cells present. The combined length of right and left ejaculatory glands ranges from 342 to 418 µm, or 34 to 40 % of the body length. The spicula of *E. kimae* (59 - 78 µm) are the shortest, relative to body length (6 to 8 % of the body length), among the species studied here. The sensory receptor is cylindrical, truncate and with terminal aperture. The accessory process of the sensory receptor is conical. The processes on the posterior margin of the *condylus* of the gubernaculum are conical.

Material examined: Male holotype, Ek1, BM(NH)1989. 1.4; male topotypes, USNM Nos. 171295 - 171298.

Localities: Type locality near oil drilling platforms in the Cayo Arcas region,Campeche Sound, Gulf of Mexico; Station 6, 20° 00′ longitude N and 91° 30′ latitude W; depth 31 m; collected 1986.

Habitat: Grey, jelly-like clay sediment.

Elzalia poli Castillo-Fernandez and Lambshead, 1990
(Figs. 9A – D and 10A - B)

Description of [holotype] and 3 male topotypes: De Man's Ratios: a = 23 – 28 (25 ± 2.2); b = 5.2 - 5.8 [5.8] (5.5 ± 0.3); c = 7.4 - 7.7 [7.4] (7.5 ± 0.2). L = 1,067 – 1,269 [1,269] (1,167 ± 77) µm.

Body fusiform, mostly tapered anteriorly from base of esophagus and in tail region (Fig. 9A). Head diameter 12 - 13 (12.3 ± 0.7) µm; body diameter at level of nerve ring 31 - 38 (35 ± 4) µm, at base of esophagus 36 - 44 [44] (40 ± 6) µm, at midbody 41 - 50 (45 ± 5) µm, and at level of cloacal vent 27 - 33 (31 ± 4) µm.

Cephalic sensilla 6 + 10. Inner labial sensilla papilliform. Outer labial sensilla 4 - 6 [6] (5 ± 1) µm long,

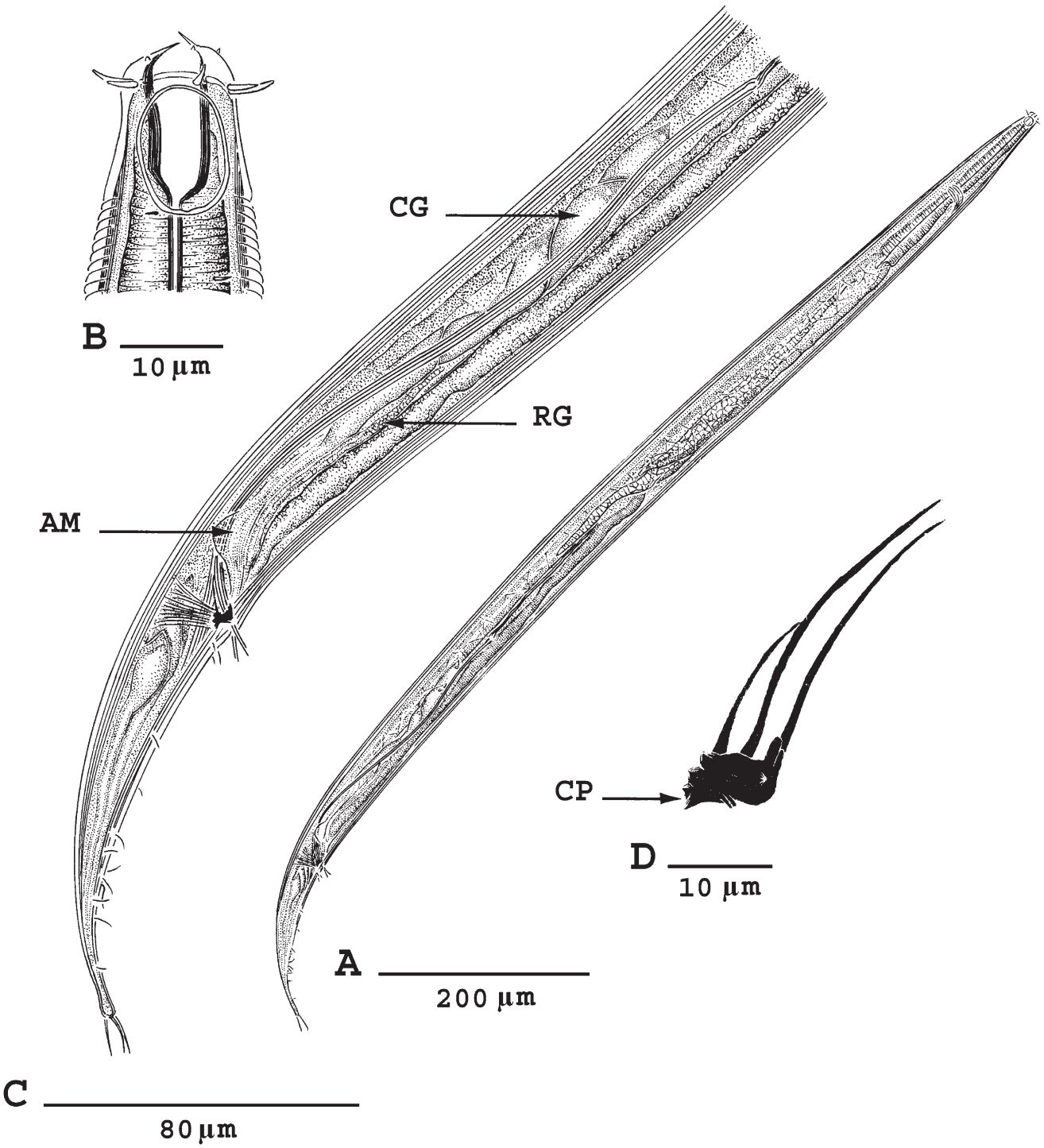

Fig. 9. *Elzalia poli*. Topotype, USNM No. 1128833. A. Habitus. B. Head. C. Posterior body region with rectal gland (RG) and cells (CG) and ampulla (AM) of the ejaculatory gland. D. Gubernaculum with conical projections (CP) on the *condylus*.

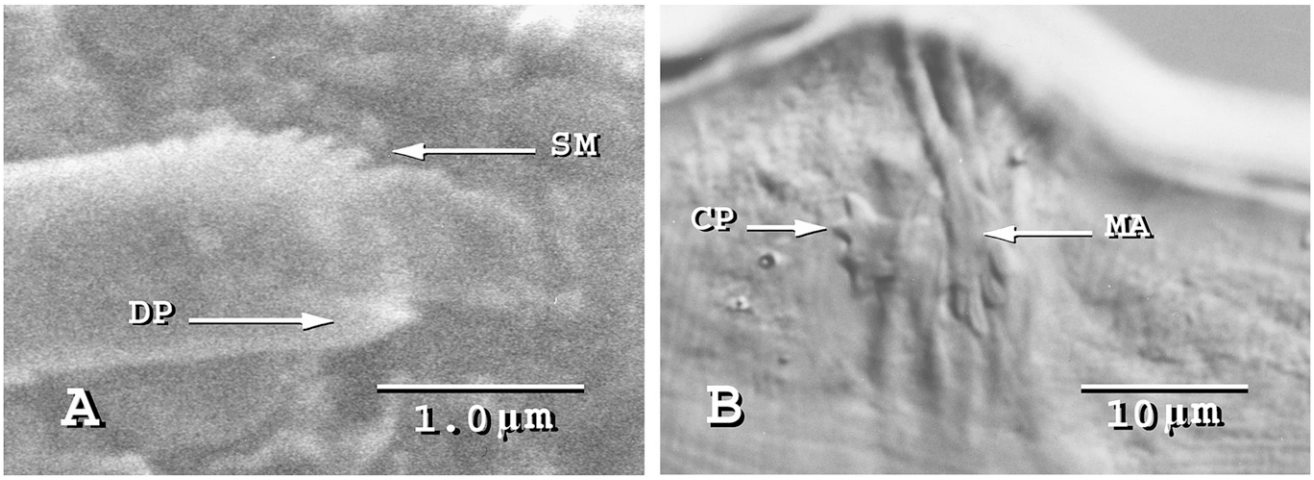

FIG. 10. *E. poli*. Holotype, BM No. Ep 1. BM(NH) 1989.1.2. A. Distal tip of spiculum with serrated margin (SM) and opposite edge with single, acute, distal process (DP). B. Photomicrograph of gubernaculum on original slide mount showing distorted positions of the *pontis* with its conical projections (CP) and the *manus* (MA) with four digits.

33 - 50 (40 ± 9) % of head diameter, and paralabial sensilla 3 - 5 (4 ± 1) µm, or 25 - 33 (30 ± 4) % of head diameter. Distance from oral surface to anterior margin of amphids 3 - 4 [3] (3.3 ± 0.6) µm. Amphid length 12 [12] (0) µm; width 9 – 10 [10] (9.7 ± 0.6) µm, or 67 - 83 [78] (76 ± 8) % of corresponding body diameter. Periodicity of transverse striae 1.1 - 1.4 (1.3 ± 0.2) µm in mid body region.

Buccal capsule cylindrical (Fig. 9B); length of 14 - 15 [14] (14.3 ± 0.6) µm; width at level of buccal seam 6 [6] (0); 69 - 83 [79] (77 ± 7) % of stoma length overlapped by amphid. Esophagus 199 - 216 [202] (206 ± 9) µm long. Distance to nerve ring from oral surface 99 - 106 (103 ± 4) µm, or 49 - 52 [52] (50 ± 2) % of esophagus length.

Tail anteriorly conical and posteriorly cylindrical (Fig. 9C); tail length 147 - 171 [171] (160 ± 10) µm, or 4.2 - 5.4 (4.4 ± 0.9) cloacal body diameters long; cylindrical portion 26 - 31 [ND] (28 ± 3) µm, or 18 - 21 [ND] (19 ± 2) % of tail length. Subventral rows of caudal sensilla present (Fig. 9C); sensilla more abundant and longer in posterior third of tail; length of caudal sensilla 4 - 12 [ND] (7 ± 4) µm. Terminal sensilla 13 - 15 [ND] (14 ± 1) µm long.

Anterior testes 235 - 280 [ND] (259 ± 23) and posterior testes 117 - 142 [ND] (130 ± 13) µm long; length of posterior testes 45 - 60 [ND] (51 ± 8) % of anterior testes.

Ejaculatory glands comprised of one cell type (Fig. 9C). Gland on right side of body with three or four serially arranged cells and two gland cells on left. Distance from cloaca to anterior end on right side 231 [ND] (n = 1) µm, or 20 % of total body length, and 204 [ND] µm on left side, or 17 [ND] % of body length.

Distance from cloaca to anterior end of rectal gland 120 and 110 µm on right and left sides respectively, or 10 and 6 - 9 [ND] (7 ± 2) % of total body length.

Spicula thin, elongate and ensheathed in protractor muscle (Fig. 9C); length of right and left spicula 271 - 330 [ND] (298 ± 20); right and left spicula pooled 24 - 30 [ND] (26 ± 2.6) % of body length, or 1.8 - 2.0 [ND] (2.0 ± 0.1) tail lengths. Proximal tip of spicula slightly enlarged; distally each spiculum with marginal series of acute serrations on one side and opposite side with single, acute, distal process (Fig. 10A).

Gubernaculum 33 - 35 [35] (34.5 ± 1) µm long, or 1.0 - 1.3 [ND] (1.1 ± 0.2) cloacal body diameters. Each *manus* with four, dorsally-directed, digits; first (lateral) digit of *manus* longest with each progressively shorter toward fourth, nearest sagittal plane (Fig. 10B). Each half of condylus with four or five conical projections (Fig. 10B).[8]

Diagnosis: The body length is 1,067 – 1,269 µm. The ejaculatory gland is comprised of a series of three or four cells of one size only, the larger oblong cells being absent. The combined length of right and left ejaculatory glands, measured from the anterior end of each to the level of the cloaca, ranges from 204 to 231 µm, or 17 to 20 % of the body length. The combined lengths of right and left spicula range from 271 - 330 µm, or 24 – 30 % of the body length and 1.8 to 2.0 tail lengths. There are acutely-pointed serrations on one margin of the distal ends of the spicula, and a single, acute distal process on the other margin. The posterior and lateral projections of the *condylus* are conical.

Material examined: Male holotype, E.p.1, BM(NH)1989.1.2; male paratypes Ep2 BM(NH)1989.1.3 and Ep3 BM(NH) 1989.1.3 and male topotypes, USNM Nos. 1128833 – 1128835.

Localities: Type locality near oil drilling platforms in the Cayo Arcas region, Campeche Sound, Gulf of Mexico; Station 4, 20° 11′ longitude N and 91° 53′ latitude W; depth 42 m; collected 1986.

[8] The distal tip of the gubernaculum was not protruded beyond the cloacal aperture in any of the original type and topotype male specimens of *E. poli*. Observations of the gubernaculum in this species are limited to the original slide preparation of the holotype (Fig. 10B).

Habitat: Grey, jelly-like clay sediment.

Discussion

The new morphological features described here offer improved means by which species of this genus may be identified one from another.

The taxonomic value of these character states of the spicula are somewhat diminished by the fact that they are not observable without use of an SEM and then only in cases where tips of the spicula protrude beyond the cloacal aperture. These limitations aside, the distal ornamentations are new, important means by which the species included in this study may be distinguished one from another, and they may eventually be important in phylogenetic investigations. The spicula of *E. federici* and *E. poli* have serrations along at least one margin of the somewhat flattened distal end of the spicula, which suggests they may be closely related. Possibly the distal tubercles on the spicula of *E. tuberculata* and *E. kimae* are variant homologs of the serrations. The same may be true of what appear to be diminutive tubercles along the crest of the combs on the spicula of *E. bipectinella*. Should they be homologs, determination of which character state is ancestral and the lineages by which the variations have evolved require further investigation. Tubercles and/or serrations are not known to exist among other members of Xyalidae.

More accessible diagnostic features of the spicula are their absolute lengths and their lengths relative to body lengths. When spicule length is plotted against body length for the five species included in this study, it became apparent that spicule length was closely proportional to body length, and data clusters for each species are separate and distinct from others (Fig. 11). It was of interest then to plot mean spicule length against mean body length for the other four nominal species of *Elzalia*. In so doing, the datum for *E. striatitenuis* fell near that of *E. mediterranea*. The diagnostic critera given by Zhang and Zhang (2006) are insufficient to differentiate these two species. However, Vitiello (1971) states that the anterior rim of the amphid of *E. mediterannea* is situated at the same level as the posterior end of the buccal capsule, which is in contrast to all other known species in which the anterior rim is at the level of the outer labial and paralabial sensilla. This may be a criterion by which *E. mediterranea* can be sparated from *E. striatitenuis*, but in the case of the latter species Zhang and Zhang (2006) were unable to observe the amphid. We prefer not to synonymize these two species because of the large distances separating their known geographic distributions and because there is insufficient information concerning their detailed morphology.

Body and spicule length are also close for *E. tuberculata* and *E. poli*, and those species might be considered conspecific were it not for the differences we have shown in the structure of the spicula and gubernacula.

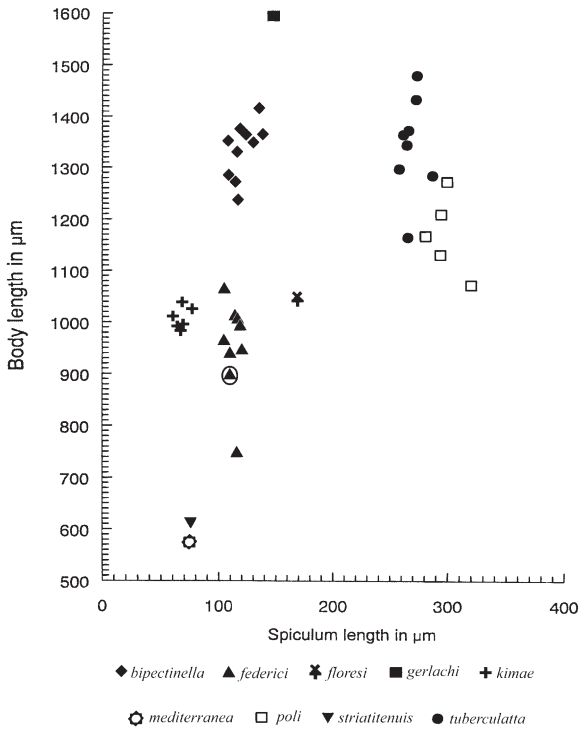

Fig. 11. The combined average of right and left spiculum lengths plotted against body length for individual specimens of *E. bipectinella*, *E. federici*, *E. kimae*, *E. poli*, and *E. tuberculata*. Population means from the literature are plotted for *E. floresi*, *E. gerlachi*, *E. mediterranea*, and *E. striatitenuis*. Ep 3. BM(NH) 1989.1.3 is circled.

Castillo-Fernandez and Lambshead (1990) were of the opinion that the range of spicula lengths of *E. federici* overlaps that of *E. poli*, but employed features of the gubernaculum to discriminate between these two species. Because of distortions of the gubernaculum in their wholemount preparations, their identifications of specimens in the type series of their species are suspect. Consequently, we plotted the spicule length against body length for all individuals of both species, including topotypes, using our own measurements without regard to how each specimen had been identified by Castillo-Fernandez and Lambshead (1990), and we excluded Ep4 (BM(NH) 1989.1.7), which is not a species of *Elzalia*. In so doing, the data plotted for these two species became separate and distinct from one another, and *E. poli* paratype Ep3 (BM(NH)1989.1.3), which is circled in figure 11, fell within the cluster of values for *E. federici*. Therefore, we concluded that their "E. poli" paratype specimen Ep3 is a specimen of *E. federici*.

The character states of the gubernaculum are also of taxonomic value, especially when the protruded distal end of gubernacula may be observed with an SEM, but they have value as well in light microscope observations of whole mount specimens. For example, it is apparent in light microscopic observations (as well as with the SEM) that the *condylus* of the gubernaculum in both

species from Thailand have foliate posterior projections (Figs. 2B, 3D, and 4A), whereas they are conical in all species from the Gulf of Mexico (Figs. 6C, 8B, 9D and 10B). In SEM observations a process adjacent to the sensory receptor in *E. bipectinella* is columnar, blunt and bears minute projections (Fig. 2C). The process adjacent to the sensory receptors in *E. tuberculata*, *E. federici* and *E. kimae* are thorn-like (Figs 4A, 5D, 6B and C, 7D, 8B, and 9D). The form of this process is not known for *E. poli*.

Although this is the first report of ejaculatory glands in the genus *Elzalia*, their presence has been documented for diverse taxa of nematodes. As noted by Riemann (1967) in his review of literature, ejaculatory glands have been reported in members of Dorylaimida and Mononchida (Coomans and Loof, 1986), members of Mononchida (Coomans and Lima 1965), and in several instances (Murphy and Canaris, 1964; Hopper, 1966; Riemann, 1967; Vincx and Coomans, 1983; and Nicholas and Stewart, 1997) among species belonging to the same order as *Elzalia*, namely Monhysterida. In all instances, including those among the Dorylaimida and Mononchida, ejaculatory glands are situated on both sides of the posterior intestine and are comprised of a few to several tandomly arranged cells, each with a long duct that proceeds posteriorly, as is the case in *Elzalia*. The gland ducts open into the cloaca in *Anatonchus amiciae* Coomans and Lima, 1967 (Coomans and Lima, 1967), in species of *Amphimonhystera* and *Echinotheristus cimbricus* Von Thun and Riemann, 1967 (Riemann, 1967), and in *Daptonema williamsi* Vincx and Coomans, 1983 (Vincx and Coomans, 1983). They open into the vas deferens in *Actinca costata* Schneider, 1935 and other dorylaims (Coomans and Loof, 1986) and into the ejaculatory duct in *Gonionchus australis* Nicholas and Stewart, 1997 (Nicholas and Stewart, 1997). By contrast, Murphy and Canaris (1964) were of the opinion that glands of a similar nature in *Theristus pratti* Murphy and Canaris, 1964 opened, not by way of the cloaca or vas deferens, but by way of a "lateral piece" flanking each side of the gubernaculum. They interpreted these to be sclerotized copulatory gland openings. What are doubtless homologs of the lateral pieces was observed by Riemann (1967) in the case of *Amphimonhystera anechma* (Southern, 1914) and by Hope and Zhang (1995) in their description of *Hopperia hexadentata* Hope and Zhang, 1995, but it was the opinion of the latter authors that these are sensory in function rather than gland openings. We assume that in *Elzalia* the gland cell ducts open indirectly into either the vas deferens or the cloaca by way of the ampullae, one of which is situated on each the right and left sides of the juncture of vas deferens and cloaca. We have seen no evidence that the efferent openings of ejaculatory glands are through the cylindrical process on the *pontis*, which we believe to have a chemosensory function.

The ejaculatory gland cells in *Elzalia* have either one kind of relatively small cell, or they may consist of two larger, oblong, anterior cells and a series of smaller posterior cells. Only one species from Thailand, namely *E. bipectinella* (Fig. 9B), and one from the Gulf of Mexico, *E. kimae*, (Fig. 7C) have both kinds of gland cells, whereas the other three species included in our study have only one type of cell. When the larger oblong cells are present, they are paired, and glands with the additional oblong cells are longer than those without them. The ejaculatory glands are slightly longer on the left side in those species from Thailand, whereas they are longer on the right side in those from the Gulf of Mexico. Although the lengths of the glands differ among all species in this study, their lengths are closely correlated with body length. For example, the two species that have oblong cells in addition to the pyriform cells, namely *E. bipectinella* and *E. kimae*, have gland lengths from 474 - 494 and 342 - 418, respectively, and accordingly the percent of the body lengths they occupy are 36 - 38% and 34 - 40%. Likewise, for *E. tuberculata*, *E. federici*, and *E. poli*, the gland lengths are 243 - 255, 151 - 173, and 204 - 231 μm, respectively. Whereas the ranges of these values differ, the ranges as a percent of the body length, namely 19 - 20, 16 - 18, and 17 - 20%, are much closer with some overlap. As to be expected, these percent values differ significantly from the percent of body length occupied by those with two cell types.

Ejaculatory glands may be more common among nematodes than is known at present, in part perhaps because they may be difficult to observe unless well-preserved. Their phylogenetic importance is unknown at present.

Rectal glands have been reported less frequently than ejaculatory glands, and more often for members of Dorylaimida and Mononchida. In *A. amiciae* and *Laimydorus* spp. (Dorylaimida) each consists of three cells situated posteriorly from the spicula with their ducts terminating on the posterior end of the cloaca (Coomans and Lima, 1965). In *A. costata* (Mononchida) there are also three cells in each rectal gland, but each gland is situated anteriorly from the spicula with ducts first extended posteriorly past the spicula and then turned anteriorly between spicula to become attached to the posterior end of the cloaca (Coomans and Loof, 1986). It is not clear in this case if there is one gland on each side of the body, or only one gland on either side. Coomans and Loof (1986) note that the gland cells produce refractive granules that fill the ducts to the extent that the granulation may obscure the spicula. The rectal gland cells in *Elzalia*, one on each side of the intestine, also lie anteriorly from the spicula. Although it was not possible to trace the ducts posteriorly to an exact point of attachment, they are similar to *A. costata* in that the cells and ducts were filled with opaque and light refractive granules. It is of interest to note that

whereas Vincx and Coomans (1983) do not mention the presence of rectal glands in *D. williamsi*, they state that second to the last of five cells in each ejaculatory gland always has a dark granular appearance, which contrasts with the bright appearance of the other four cells. Given the similarity of its content with the rectal-gland-cells in *A. costata* and in *Elzalia*, the question comes to mind as to whether or not that dark granular cell in *D. williamsi* is actually a rectal gland cell.

Our observations concerning the number of testes and their position relative to the gut differ in part from what is reported in the literature. Our observations that the males of *Elzalia* are diorchic are in agreement with Castillo-Fernandez and Lambshead (1990). However, they state that whereas the position of the anterior testis relative to the gut among members of *E. kimae* could not be determined, they state that the anterior testes of *E. federici* and *E. poli* are situated on the right side of the gut. If this were true, it would differ in that respect from the two new species described here, which have the anterior testis consistently on the left of the gut. Whereas we found that the condition of all type specimens of *E. poli* and the paratype specimens of *E. federici* were too poor to determine the position of the anterior testis, it was possible to do so in the case of the holotype of *E. federici* and the topotypes of *E. federici* and *E. poli*. In all of these specimens the anterior testis is on the left side of the gut, and the posterior is on the right. Because this conforms to the position of the anterior testes in *E. bipectinella* n. sp. and *E. tuberculata* n. sp., we assume that Castillo-Fernandez and Lambshead (1990) were in error and that the anterior and posterior testes are on the left and right sides of the gut respectively in all species of *Elzalia*. Zhang and Zhang (2006) also state that the anterior testis in *E. gerlachi* is on the left, but they claim that there is only the one testis. We suspect the latter is an erroneous observation. They do not give information about testes in *E. striatitenuis*.

Although we have introduced new characters for the systematics of *Elzalia*, too little is know about the four species not included in this study to begin to formulate hypotheses concerning phylogenetic relationships within the genus.

Abbreviations

AC – accessory process of gubernaculum
AM – ampulla of ejaculatory gland
CB – comb-like bifurcations
CG – cells of ejaculatory gland
CO – *condylus*
CP – conical process of *condylus*
DP – dentiform process of spiculum
FR – foliate ridge of *condylus*
MA - *manus*
OC – oblong cell of ejaculatory gland
PC – pyriform cell of ejaculatory gland
PM - posterior projection on *manus*
PO - *pontis*
RG – rectal gland
SB – spicule bend
SM – serrated margin of spicule
SP – spiculum
SR – sensory receptor of gubernaculum
SS – shaft of spiculum
ST – transverse striation
TS – tubercles of spiculum

Literature Cited

Allgén, C. 1960. Antarktische moistens neue freilebende marine nematoden aus dem Graham-Land. Zoologischer Anzeiger 164:474–499.

Aryuthaka, C. 1991. Meiofauna community in Khung Kraben Bay, Chanthaburi, east Thailand. Thai Marine Fisheries Research Bulletin 2:47–57.

Aryuthaka, C. 1994. Preliminary report on the meiofauna community in shrimp culture ponds in the Khung Kraben Bay area, Chanthaburi, eastern Thailand. Pp. 41–52. *in* A. Snidvongs, W. Utoomprukporn, and M. Hungspreugs, eds. Proceedings of the NRCT – JSPS Joint Seminar on Marine Science, December 2 – 3, 1993, Songkhla, Thailand. Department of Marine Science, Chulalongkorn University, Bangkok.

Castillo-Fernandez, D., and Lambshead, P. J. D. 1990. Revision of the Genus *Elzalia* Gerlach, 1957 (Nematoda: Xyalidae) including Three New Species from the Gulf of Mexico, with a Discussion of the Sibling Species Problem. Bulletin of the British Museum (Natural History). Zoology 56:63–71.

Coomans, A., and Lima, M. B. 1965. Description of *Anatonchus amiciae* n. sp. (Nematoda: Mononchidae) with Observations on it Juvenile Stages and Anatomy. Nematologica 11:413–431.

Coomans, A., and Loof, P. A. A. 1986. Observations on the glands of the male reproductive system in dorylaims and it phylogenetic importance. Revue Nématology 9(3):261–265.

Dallwitz, M. J., Paine, T. A., and Zurcher, E. J. 1993. DELTA User's Guide. A General System for Processing Taxonomic Descriptions. 4th ed. Canberra, Australia: CSIRO Division of Entomology, 136 pp.

Gerlach, S. A. 1957. Die Neamtodenfauna des Sandstrandes an der Küste von Mittelbrasilien. Mitteilungen aus dem Zoologischen Museum in Berlin 35(2):411–459plus 4 tables.

Gerlach, S. A. 1963. Über freilebende meeresnematoden: Revision der Linhomoeidae. Zoologische Jahrbücher. Abteilung für Systematic. Ökologie und Geographie der Tiere 90:599–658.

Hope, W. D. 1982. Structure of Head and Stoma in the Marine Nematode Genus *Deonstostoma* (Enoplida: Leptosomatidae). Smithsonian Contributions to Zoology 353:1–22.

Hope, W. D. 2007. Nematoda. Pp. 234–266. *in* J. T. Carlton, ed. The Light and Smith Manual: Intertidal Invertebrates from Central California and Oregon. Berkely: University of California Press.

Hope, W. D., and Murphy, D. G. 1972. A Taxonomic Hierarchy and Checklist of the Genera and Higher Taxa of Marine Nematodes. Smithsonian Contributions to Zoology 137:1–101.

Hope, W. D., and Zhi-nan, Z.hang. 1995. New nematodes from the Yellow Sea, *Hopperia hexadentata* n. sp. and *Cervonema deltensis* n. sp. (Chromadorida: Comesomatidae), with observations on morphology and systematics. Invertebrate Zoology 114(2):119–138.

Jensen, P. 1989. Revision of Aponchidae Gerlach, 1963 (Nematoda: Monhysterida), Epibiotic Nematodes on Shells of Intertidal Epibenthic Invertebrates, with Description of New Species. Phuket Marine Biological Center. Research Bulletin 50:1–24.

Kito, K., and Aryuthaka, C. 1998. Free-living marine nematodes of shrimp culture ponds in Thailand. I. New species of the genera *Diplolaimella* and *Thalassomonhystera* (Monhysteridae) and *Theristus* (Xyalidae). Hydrobiologia 379:123–133.

Kito, K., and Aryuthaka, C. 2006. New mouthless nematode of the genus *Parastomonema* Kito, 1989 (Nematoda: Siphonolaimidae) from a mangrove forest on the Coast of Thailand, and erection of the new subfamily Astomonematinae within the Siphonolaimidae. Zootaxa 1177:39–49.

Murphy, D. G., and Canaris, A. G. 1964. *Theristus pratti* n. sp., a Marine Nematode from Kenya. Proceedings of the Helminthological Society of Washington 31(2):203–208.

Nicholas, W. L., and Stewart, A. C. 1997. Ultrastructure of *Goniochus australis* (Xyalidae, Nemtoda). Journal of Nematology 29(2):133–143.

Nozawa, K., Yoshikawa, N., Shokita, S., and Limsakul, S. 1983. Mangrove meiofauna in Thailand. Mangrove Ecology in Thailand. Bulletin of Thai-Japanese Cooperative Research Project on Mangrove Productivity and Development, pp. 63–72.

Riemann, F. 1967. Die Gattung *Amphimonhystera* Allgén, 1929. (Nematoda: Monhysteridae). Veröffentlichungen des Instituts für Meeresforschung in Bremerhaven X:217–225.

Sommerfield, P. J., Supaporn, Y., and Aryuthaka, C. 2000. The effects of green mussel *Perna viridis* (I.) (Mollusca: Mytilidae) culture on nematode community structure in the Gulf of Thailand. The Raffles Bulletin of Zoology 48:263–272.

Sommerfield, P. J., Yodnarasri, S., and Aryuthaka, C. 2002. Relationships between seagrass biodiversity and infaunal communities: implications for studies of biodiversity effects. Marine Ecology Series 237:97–109.

Swennen, C., and Witte, J. 1989. Meiofauna in two intertidal flats in southern Thailand. Wallaceana 55 & 56:12–13.

Timm, R. W. 1961. The Marine Nematodes of the Bay of Bengal. Proceedings of the Pakistan Academy of Science 1(1):25–88.

Vincx, M., and Coomans, A. 1983. *Daptonema williamsi* n.sp. (Nematoda, Xyalidae) from the Solomon Islands. Zoologica Scripta 12(4):237–244.

Vitiello, P. 1971. Nématodes Libres Marins des Vases Profondes du Golf du Lion III. Monhysterida, Araeolaimida, Desmodorida. Téthys 2(3):647–690.

Zhang, Y., and Zhang, Z. N. 2006. Two new species of the genus *Elzalia* (Nematoda: Monhysterida: Xyalidae) from the Yellow Sea, China. Journal of the Marine Biological Association, United Kingdom 86:1047–1056.