



## Morphology and taxonomic distribution of a newly discovered feature, postero-lateral glands, in pelagic nemerteans

Pamela Roe<sup>1</sup> & Jon L. Norenburg<sup>2</sup>

<sup>1</sup>Department of Biological Sciences, California State University Stanislaus, 801 W. Monte Vista Ave., Turlock, CA 95382, U.S.A.

E-mail: pam@science.csustau.edu

<sup>2</sup>Department of Systematic Biology, National Museum of Natural History, Smithsonian Institution, Washington, DC 20560-0163, U.S.A.

**Key words:** pelagic nemertean, epidermal glands, morphology, taxonomy

### Abstract

A variety of pelagic nemerteans from our collections off California and Hawaii between 1992 and 1997 have a pair of epidermal structures, usually visible on the intact specimens, located on the ventro-lateral margins near the caudal end of the body. The only previous reports of similar structures from pelagic or any other nemerteans are for two species of the genus *Plotonemertes*. Histological serial sections of at least one specimen from each of about 16 morpho-species demonstrate that these are specialized glandular regions of the epidermis, which we broadly term postero-lateral glands. The objects of this study are to describe these glands at the level of light microscopy and to consider their systematic implications and possible functions. Most of the glands consist of two more or less spatially segregated types of secretory cells. One type is usually at the anterior end of the gland and resembles typical mucous goblet cells. The other type usually is the more abundant, and resembles a nemertean serous cell, with secretion that probably is relatively proteinaceous. The glands of one of the *Plotonemertes* specimens have two additional types of secretory cells that are relatively abundant. This study reports on postero-lateral glands from 30 specimens: three specimens of *Plotonemertes* in the family Protipelagonemertidae, 18 in at least three genera of the family Pelagonemertidae, six in the monotypic family Balaenanemertidae, and three of *Proarmaueria* in the family Armaueriidae. The glands are relatively large, with large quantities of secretory vesicles, indicating that they must be of considerable importance to the animals. However, neither structure nor location offer self-evident clues to function of these glands. We also report on different forms of regional specialization in the epidermis of *Crassonemertes* and *Nectonemertes*, both of which lack postero-lateral glands.

### Introduction

Pelagic nemertean specimens we collected between September 1992 and July 1997 from various sites off California and Hawaii were often retrieved in excellent condition, providing unparalleled opportunity for morphological studies. On several of these freshly collected specimens we noticed a pair of reflective, whitish epidermal patches on the ventro-lateral margins near the caudal end of the body. Histological examination showed these structures to be glandular. Subsequent careful examination of other specimens in our collection yielded a diverse assortment of pelagic nemerteans that have these glands, not all of

which were easy to observe unaided or even with a stereomicroscope.

The epidermis of polystyliferous pelagic nemerteans has received little attention, because it is missing in most specimens collected prior to 1992. Coe (1926) described the epidermis of pelagic nemerteans as being thin and delicate, but bearing the same general arrangement of ciliated and glandular cells as occurs in the epidermis of littoral nemerteans. He described epidermal ciliated cells as being somewhat less numerous in pelagic nemerteans, and glandular cells as being of two kinds, those filled with a granular secretion and those filled with a clear mucus secretion. He also remarked that there are no other integument-

ary glands, and that pelagic nemerteans apparently secrete much less mucus than do littoral species. Kato & Tanaka (1938) described the epidermis of *Pelagoneurtes moseleyi* Bürger, 1895, as consisting of ciliated columnar cells and two kinds of glandular cells, corresponding to those of Coe (1926). Eosinophilous glandular cells were described as being especially abundant in the ventral epidermis of the caudal fin (Kato & Tanaka, 1938). Presumed integumentary sensory organs, sometimes called bulb-shaped organs, consisting of a conical group of very slender cells with elongated basal nuclei, were known from most species that had sufficient epidermis. These occur irregularly over the whole body of *P. moseleyi*, as well as many other pelagic nemertean species (Brinkmann, 1917a; Coe, 1926; Kato & Tanaka, 1938).

Norenburg (1985) described the epidermal glandular cells found in members of all four orders of benthic nemerteans as belonging to three types, mucous, serous and bacillary. The mucous cell contains a large vesicle of primarily mucopolysaccharide content, which is relatively labile in aqueous fixatives and staining media. This probably accounts for poor staining when the mucous cell is in the epidermis, as it is in most nemerteans other than heteronemerteans; the secretion is primarily basophilic and cyanophilous (Norenburg, 1985). The serous cell forms a goblet of secretory material that is relatively stable in aqueous and alcoholic fixatives and has staining reactions suggestive of a high proportion of proteinaceous material; usually it is acidophilic and azanophilous. The secretory mass is initially an accumulation of finely granular material that may condense to form large, homogeneous or irregular, opaque bodies or granules, which may be secreted before they coalesce (Norenburg, 1985). Bacillary cells comprise a diverse grouping. Staining reactions are more variable, but also point to relatively high proteinaceous content; their secretory material is in the form of discrete, often paracrystalline, membrane-bound vesicles that are usually of uniform size and shape within a cell type, from 0.2 to 0.6  $\mu\text{m}$  in diameter or length, and spaced uniformly in the cytoplasmic matrix (Norenburg, 1985). Several types are often found within a species, and their ultrastructural morphology shows wide variation across species. Although one or more bacillary cells may be distributed throughout the epidermis, they are associated spatially and temporally with specialized activities, such as adhesion and reproduction (Norenburg, 1985).

The epidermis of benthic hoplonemerteans is dominated by the mucous goblet cell, followed in abundance by the serous cell. The scant previous evidence and our own observations suggest the same for pelagic hoplonemerteans (Brinkmann, 1917a; Coe, 1926; unpubl. obs.). A remarkably specialized pair of glandular epidermal structures was reported by Brinkmann (1917a,b) from a male specimen of a pelagic hoplonemertean, *Plotonemertes adhaerens* Brinkman, 1917. These structures are located on the ventral surface near the posterior end of the body and were described as semicircular folds of the integument, leading posteriorly beneath the surface into a blind glandular pouch (Coe, 1926). Brinkmann (1917a,b) suggested that the glands might aid the male in adhering to the female during spawning. Coe (1936) studied 27 specimens, including females, of *P. adhaerens*, as well as a female of a new species, which he described as *Plotonemertes aurantiaca* Coe, 1936, in which the glands are widely separated. Coe (1936) described the organs as being comprised of deep convolutions of surface epithelium extending beneath the basement membrane, occurring in both sexes, but being more highly developed in males. He reported that the organs receive large branches from the adjacent lateral nerve cords and concluded from this that they might be sensory as well as glandular (Coe, 1936).

We know of few other references to epidermal specialization, other than the bulb-shaped organs, in pelagic nemerteans. Bürger (1909) noted specialized glandular epithelium surrounding the male gonopores of *Balaenanemertes chuni* Bürger, 1909, now known from several additional species (see Norenburg and Roe, 1998). Brinkmann (1917a) mentioned a pair of ventro-lateral oval structures lying along the caudal end of *Balaenanemertes lobata* (Joubin, 1906) that he considered sensory. Indeed, his text (1917a: 131) and his figure (1917a: plate 16, Fig. 12) describe a ciliated epithelium distinguished by a lack of glandular cells. Korotkevich (1955) described a pair of 'band-shaped' organs in several armaueriids. These are distinguished by a unique elaboration of the lateral the nerve cords and bear no resemblance to the glands we will discuss (Maslakova, pers. comm.; unpubl. obs.).

The objectives of this study are to describe, with light microscopy, the general properties of the glands we call postero-lateral glands in specimens collected by us. These properties include the shape, size, thickness, types of glandular cells, and general location of the glands. Our material is not of sufficient quality for detailed cytological, histochemical or ul-

trastructural studies. We consider the implications of these glands for biology and evolutionary diversification in pelagic nemerteans. For that reason, we also include preliminary observations on regional specializations of epidermis found in *Crassonemertes* and *Nectonemertes*.

## Materials and methods

Specimens used in this study were collected from four sites during eight cruises off central California and one cruise off Hawaii between September 1992 and July 1997, on oceanographic cruises of the R.V. Point Sur and the R.V. New Horizon directed by Dr. James Childress or personnel from Monterey Bay Aquarium. The four sites off California were within an area from Point Lobos in the north (latitude  $36^{\circ} 36.7' N$ ) to the California/Mexico border in the south (latitude  $32^{\circ} 27.30' N$ ), and ranged from about 47 to over 160 km from shore (between longitudes  $117^{\circ} 57.12' W$  and  $123^{\circ} 08.85' W$ ). The cruise off Hawaii was approximately 13 km west of Waianae, Oahu (between latitudes  $21^{\circ} 14.66' N$  and  $21^{\circ} 63.0' N$  and longitudes  $158^{\circ} 10.48' W$  and  $158^{\circ} 73' W$ ), April–May, 1993 (Roe & Norenburg, 1999). All specimens, except two, were collected using a modified Tucker Trawl (MTT) (Childress et al., 1978). The MTT has a  $10\text{-m}^2$  opening, 6-mm mesh net, a 30-l thermally insulated closing cod end, and was towed at speeds between 1 and 1.5 kt (Roe & Norenburg, 1999). Specimens 263 and 274 were collected using a Multiple Opening/Closing Net and Environmental Sensing System (MOCNESS-1D) (Wiebe et al., 1985), with  $1\text{-m}^2$  opening and 0.505-mm mesh net, towed at about 1.94 kt (Roe & Norenburg, 1999). Specimens collected off Hawaii are designated by HI before specimen numbers; specimens designated by numbers only were collected off California.

Specimen length and width to nearest mm, and sex, when obvious, were recorded either on shipboard or postfixation. Specimens were relaxed in  $MgCl_2$  isotonic with local seawater and were fixed in 8–10% buffered seawater, Hollande's cupri-picric-formal-acetic fluid or Streck Tissue Fixative (Streck Laboratories, Omaha, NE). Specimens were cleared in benzyl benzoate and photographed prior to embedding for light microscopy. Specimens were embedded in paraffin ( $56^{\circ}C$  m.p.), sectioned at  $8\ \mu m$ , and stained with Heidenhain's 'azan' (Galigher & Kozloff, 1971) or a trichrome method adapted from Mallory, Gomori

and Gurr-McConaill (Crandall, National Museum of Natural History, Washington, D.C., pers. comm.).

We observed a total of 30 serially sectioned specimens, including 24 intact specimens in which postero-lateral glands were visible, plus an additional three intact specimens that were similar to some of the 24 specimens but were lacking visible glands, and three specimens of *Proarmaneria* in which glands were not visible to the unaided eye. Material sectioned specifically for this study included at least one specimen from each of 10 morpho-species in which the glands were visible in intact specimens. An additional 11 previously sectioned specimens were also studied, for a total of 14–17 morpho-species. Sectioned material included specimens of all morpho-species in which glands were visible in intact specimens. Also studied were sections of *Crassonemertes* and *Nectonemertes*. After additional taxonomic work, all specimens will be deposited as vouchers in the collections of the National Museum of Natural History, Washington, D.C.

Characteristics of the dorsal blood vessel were sufficient to classify specimens to family level. Genus designation was determined by comparison of specimen characteristics to literature descriptions, but sometimes by a best approximation (taxon name followed by '?').

Position of the postero-lateral glands was recorded on photographs of whole specimens. Glands were easily recognized in serial sections by concentrations of red glandular secretion. Greatest width and height were measured with an ocular micrometer and averaged from sections; ratio of average maximum width of both glands to average corresponding section widths was calculated (Table 1). Lengths of glandular areas were determined by multiplying the number of sections by  $8\ \mu m$ . Unless a gland on one side of the body was damaged or missing, average length was recorded. We were unable to differentiate the anterior, mucoid portion of the gland of specimen #36 sections because of poor staining. Specimen lengths were determined prior to embedding. Thus, gland-to-specimen-length ratios are affected by dehydration. To estimate gland-to-specimen-length ratios for #338 and #303, lengths of specimen and gland were both measured from a 35-mm slide of each specimen.

## Results

### *Specimen classification and sex*

In 24 of the 30 serially sectioned specimens, the dorsal blood vessel enters the rhynchocoel, and ends blindly a short distance posteriorly, still within the rhynchocoel; this is characteristic of the Pelago- and Balaenanemertidae. Within family Pelagonemertidae, seven of our specimens (#68, 88, 416, 489, 720, 857, and 858), all females, belong to two or three species in genus *Cuneonemertes*; and two others (#334, female and 338, male) possibly belong to genus *Cuneonemertes* as well. Three specimens (#27, male; 87, male or hermaphroditic in male phase; and 263, male) are probably in genus *Probalaenanemertes*. Two specimens (HI 42, probably immature male; and 529, female) belong to separate species in genus *Pelagonemertes*. Four specimens (#93, female; HI 11, male; 198, female; and HI 13, male), each representing a different species, appear to be members of family Pelagonemertidae; but we have not determined their generic placement. Six specimens, all male, belong to the genus *Balaenanemertes*, family Balaenanemertidae (#HI 24, 36, 274, 726, 753 and 1002). Three specimens (146, male; 303, female; 737, male) belong to *Plotonemertes*, family Protopelagonemertidae. The two males belong to different *Plotonemertes* species; the female is probably in the same species as male 737. Three specimens (USNM 174040-174042) are identified as *Proarmaueria cf. pellucida*, in the family Armaueriidae. Our specimens in this species appear to be sequential hermaphrodites, showing either well-developed ovaries with a few small, poorly developed testes or well-developed testes with one or two small ovaries. Two of the specimens are in the female-dominant phase and one is in the male-dominant phase.

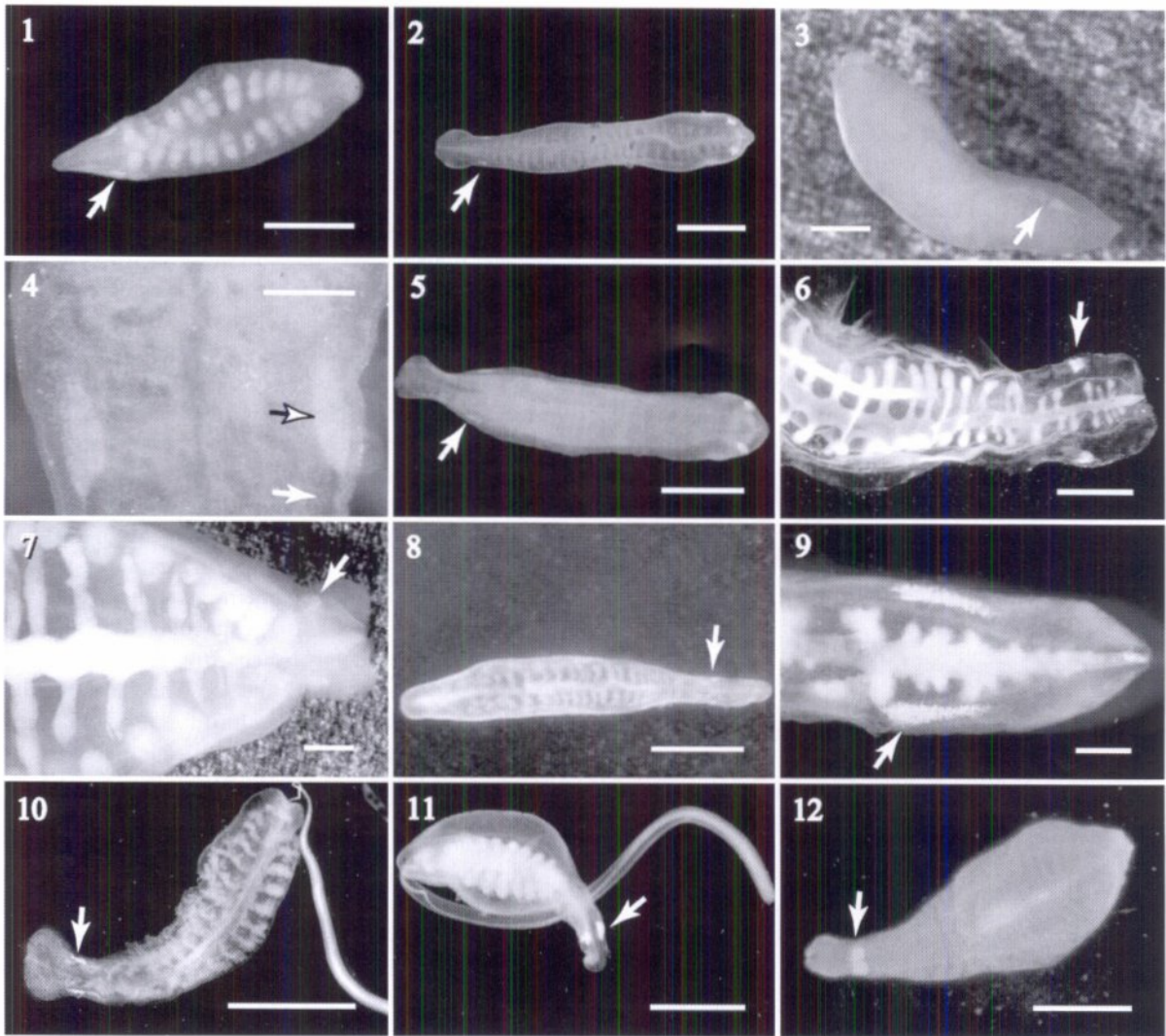
### *Gland description, Pelagonemertidae and Balaenanemertidae*

On whole specimens of pelago- and balaenanemertids, postero-lateral glands usually appear to the naked eye as reflective, whitish, triangular or oval patches distinct from adjoining epidermis, and range from barely visible to conspicuous (Figs 1–11). Glands differ in appearance between genera, and occasionally between species within genera. The two most common forms are long, narrow, backward-pointing triangles (Fig. 1), as in *Cuneonemertes*, or shorter, narrow triangles, resembling ribbons, as in *Balaenanemertes*

(Fig. 2). Variations of the narrow triangle are seen in HI 11 (Fig. 9) and HI 13 (Fig. 11). Most variations are different combinations of the two regions of the gland. Glands of some intact specimens (*Probalaenanemertes*? 27; *Probalaenanemertes*? 87, Fig. 4; and #93, Fig. 8) have the appearance of ice cream cones topped with vanilla ice cream, with the anterior part whitish and oval, and the narrow 'cone' a yellow to brown triangle. In others (#263, Fig. 5; 198, Fig. 10; 338; Fig. 3; and HI 42, Fig. 6), both parts are whitish. The glands of *Pelagonemertes* 529 are small, lumpy circles, near the front of the tail fin (Fig. 7), rather than elongate triangles as in all of our other specimens in these two families.

The postero-lateral glands are approximately ventro-laterad or laterad to the lateral nerve cord on each side of the body. On average, they are at about 80% of the body length from the tip of the head (Table 1) and in front of the caudal fin when a fin is present. The two specimens of *Pelagonemertes*, (HI 42, Fig. 6 and 529, Fig. 7) differ from all other pelago- and balaenanemertids; the glands are at about 90% from the cephalic tip and located on the tail fins, which places the glands relatively distant from the lateral nerve cords. Glands on average comprise about 82% of body length in pelagonemertids (Table 1). Again, the two *Pelagonemertes* specimens differ from other pelagonemertids; their glands comprise less than half of the average percent of body length (Table 1). Glands of the balaenanemertid specimens also are a much smaller percent of body length (average = 4.2%, excluding specimen #36) than most of the pelagonemertids (Table 1).

The glands are easily recognized in sections (Figs 13–38) as strongly differentiated regions of epidermis, usually with an abundance of strongly stained red or fuschia granules. Glands of pelagonemertids and balaenanemertids in sections are approximately rectangular, ranging from nearly square (Figs 23–24, *Cuneonemertes* 858) to extremely long and short (*Pelagonemertes* HI 42, Figs 28–29). Height of the glandular epidermis is about the same as adjacent epidermis, ranging from about 30 to 88  $\mu\text{m}$  in all pelago- and balaenanemertids except in HI 13, where it appears to be considerably thicker (Figs 37 and 38, and Table 1). A mixture of ciliated and mostly mucous glandular cells border the glands. The epidermis of the glands in pelago- and balaenanemertids is further differentiated into an anterior region containing primarily mucoid cells with cyanophilous secretion that stains more strongly than other epidermal mu-



Figures 1–12. Intact specimens. Arrows point to postero-lateral glands. Scale bar lengths in parentheses. (1) *Cuneonemertes* sp. female #416 (5 mm). (2) *Balaenanemertes* sp. male #36 (5 mm). (3) *Cuneonemertes?* sp. male #338, arrow between two parts of postero-lateral gland (1 mm). (4) *Probalaenanemertes?* sp. male #87, black arrow at white part of gland, white arrow at tan cone-shaped gland region, right postero-lateral gland (500  $\mu$ m). (5) *Probalaenanemertes?* sp. male #263 (3 mm). (6) *Pelagonemertes* sp. immature male? #HI 42 (2.5 mm). (7) *Pelagonemertes* sp. female #529 (2.5 mm). (8) Pelagonemertidae sp. female #93 (3 mm). (9) Pelagonemertidae sp. male #HI 11 (500  $\mu$ m). (10) Pelagonemertidae sp. female #198 (5 mm). (11) Pelagonemertid male #HI 13 (3 mm). (12) *Plotonemertes* sp. male #737 (5 mm).

cous goblets (Figs 13–14, 16, 20, 21, 26, 30, 31, 33, 35), and a posterior region of azanophilous (red-staining) serous cells (Figs 13, 17–19, 22, 23, 25, 27–30, 32, 34, 36–38). This pattern probably accounts for the differentiation observed in the glands of some intact specimens. The anterior mucoid cell region is usually the shorter of the two glandular regions; comprising about 43% of the total gland in *Cuneonemertes* specimens; 45% in *Probalaenanemertes?* specimens; 25% in *Pelagonemertes* specimen HI 42 and

63.5% in *Pelagonemertes* 529; 69% in specimen 93 (in which the mucoid glandular region is especially well-developed, Figs 33 and 35); 35% in HI 11; 33% in 198; and 50% in HI 13. In two specimens/species of *Balaenanemertes*, the mucoid region varied from 62% (#24) to 17.5% (#1002); poor staining of sections prevented identification of the anterior gland region in specimen #36. Branches from the lateral nerve cords extend to, or at least toward, the postero-lateral glands in sections of a few specimens, but these seemed no

Table 1. Genus, specimen number, distance of postero-lateral gland from anterior end of specimen, percent of total body length and body width comprised of glands, and gland height ( $\mu\text{m}$ ) of specimens in families Pelagonemertidae and Balaenanemertidae, and genus *Plotonemertes*.

Genus Specimen #	Distance (%)	% Body Length	% Body Width	Height. ( $\mu\text{m}$ )
<i>Cuneonemertes</i>				
68	79	8.4	12.2	74.3
88	NA	4.2	16.8	79.2
416	79	8.5	18.2	29.7
489	85	8.2	23.4	29.7
720	NA	10.4	15.6	49.5
857	74	11.3	21	54.5
858	89	8.2	16.4	79
<i>Cuneonemertes?</i>				
338	77	10	NA	NA
<i>Pelagonemertes</i>				
HI 42	87	4.0	28.2	39.6
529	92	2.6	1.9	32.2
Genus Unassigned				
93	82	10.6	25.8	54.5
198	84	7.7	18.8	74.3
HI 11	80	11	28.8	59.4
HI 13	80	10	57.6	79.2
<i>Probalaenanemertes?</i>				
27	NA	8.7	33.2	49.5
87	62	8.1	10	39.6
263	76	NA	NA	NA
<i>Balaenanemertes</i>				
HI 24	81	4.3	36.7	40
36	83	2*	25.7	40
726	81	NA	NA	NA
1002	80	4.1	28.3	88
<i>Plotonemertes</i>				
146	86	3.3	82.9	254.1
303	85	3.1	4.2	158.4
737	75	5.8	81.2	222.8

Specimen numbers preceded by HI are from Hawaii; others are from California. NA, data not available. Glands were not visible on specimens *Cuneonemertes?*, #334 and *Balaenanemertes* #274 and 753. See Materials and methods for measurement methods.

\*Partial measurement.

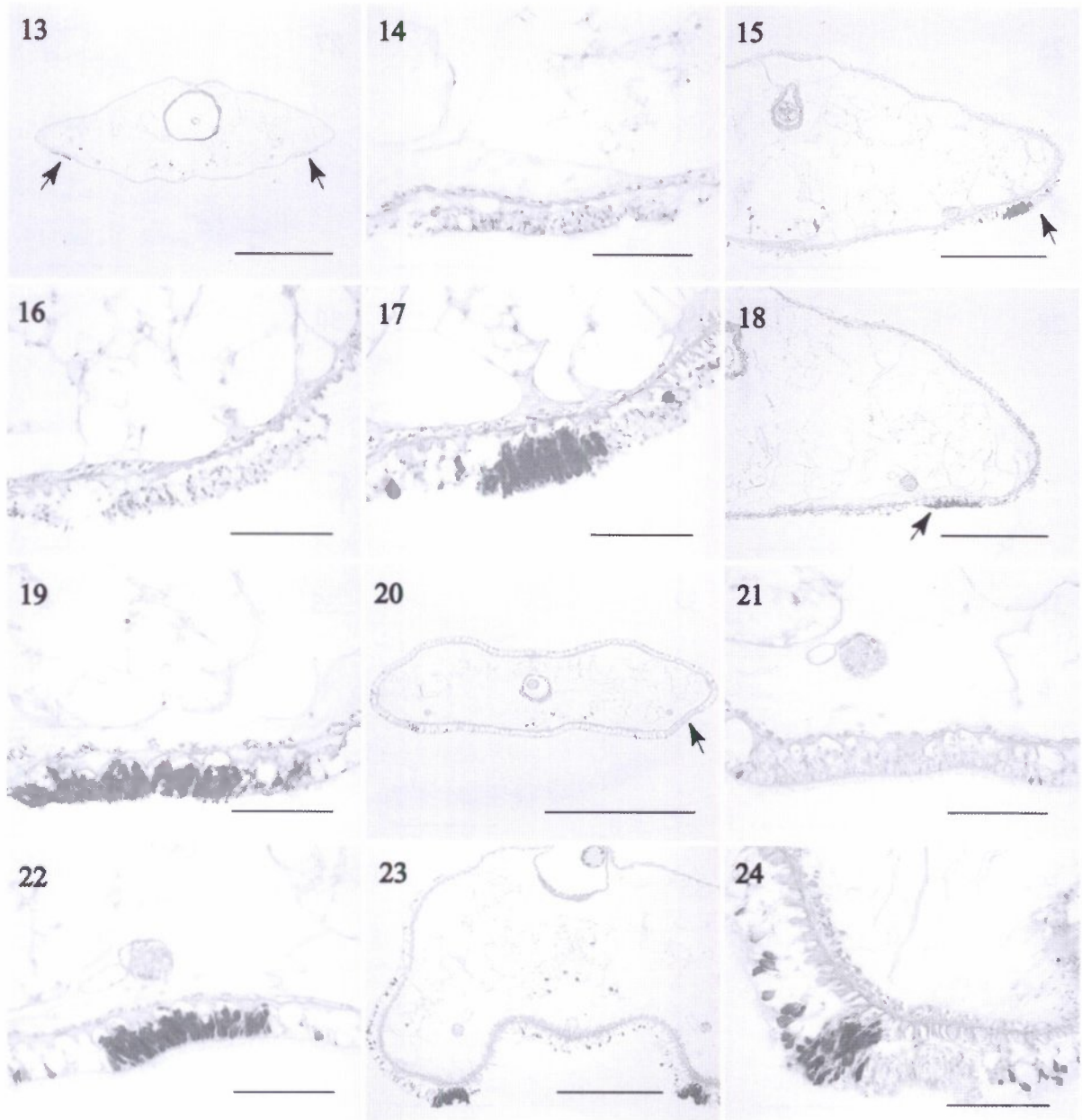
more conspicuous than the branches going to other parts of the body wall. Bulb-shaped sensory organs were not found in any of the glands, but they usually were present in the general body epidermis when this was present.

#### Gland description, *Plotonemertes*, Family *Protopelagonemertidae*

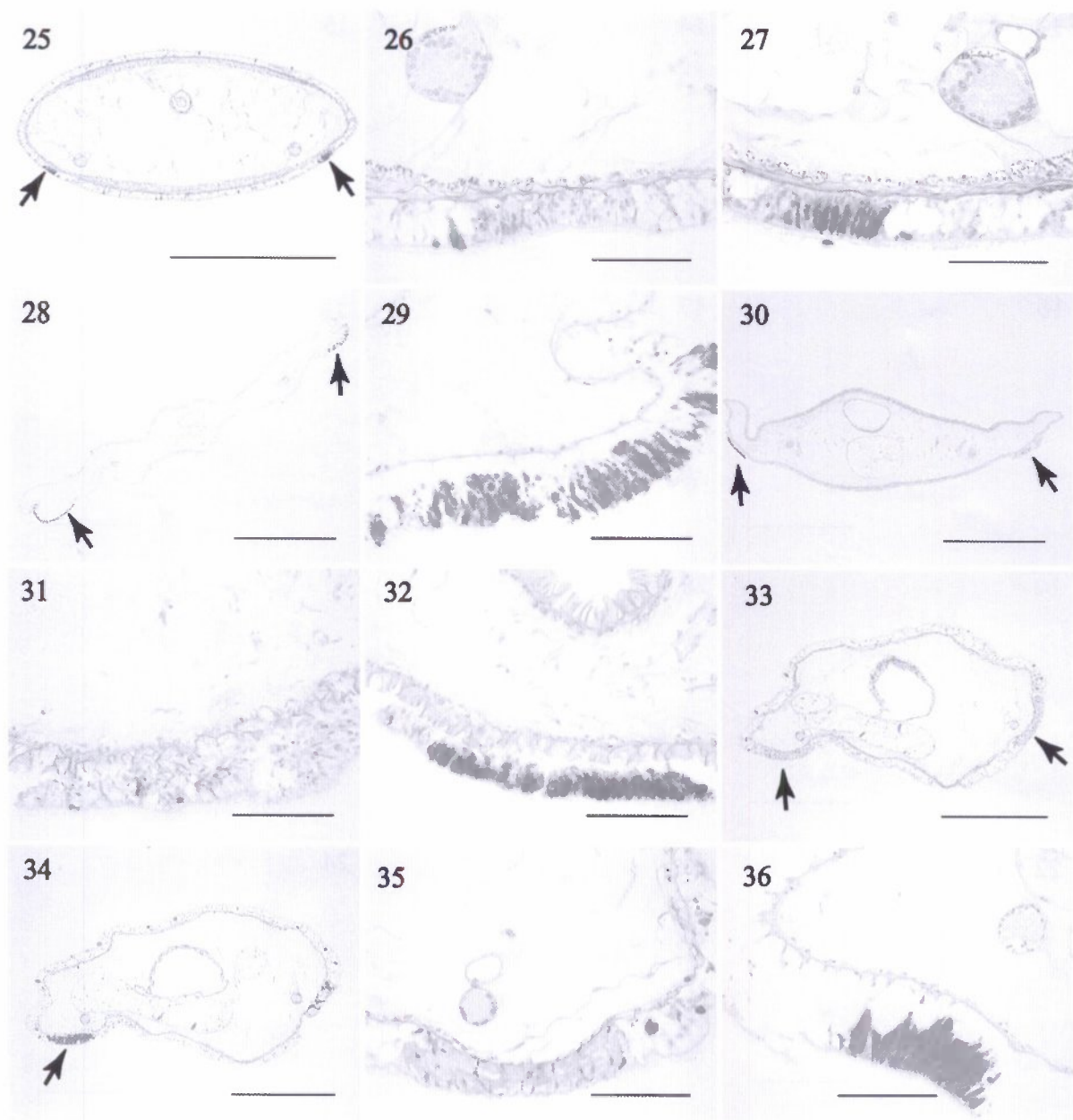
Specimens #146 and #737 are both males, belonging to separate species in genus *Plotonemertes*. Specimen #303 is female, probably of the same species as specimen #737. Glands in the males are clearly visible to the naked eye, much more prominent than in the female. In males, they are bulging circles appearing to meet mid-ventrally at the front of the narrow caudal region (#737, Fig. 12 and Table 1). The glands of the female appear as small, inconspicuous squares at the front of the narrow caudal region and do not meet mid-ventrally (Table 1). Sections reveal that the glandular epidermis of all three specimens is convoluted (Figs 39–45) and conspicuously thicker than the general body epidermis (Table 1), with much of the gland projecting deep into the ventral body wall. In #146, non-folded but similarly glandular ventral epithelium connects the two folded parts throughout the length of the gland (Figs 39 and 40). In specimen #737, the two parts are connected only by a narrow, posterior band of ventral epidermis (Figs 42 and 45). The gland pouches lie directly under and very close to the lateral nerve cords (Figs 39 and 42). As in our pelago- and balaenanemertid specimens, branches from the lateral nerve cords extend to or toward the glands but seem no more conspicuous than nerve branches going to other parts of the body wall. The anterior mucoid glandular region is not well-differentiated, but strongly blue-stained mucoid cells are mostly restricted to the anterior of the gland and mixed with red-stained cells (Figs 39 and 40). The main population of glandular cells in both males (and presumably in the female, the sections of which did not stain well) consists of granular serous cells (Fig. 41). In specimen #737, which has the most elaborate glands, in addition to mucoid and serous cells, there is a large population of what appears to be blue-stained bacillary cells in the central part of the gland, with granules about 2–2.5  $\mu\text{m}$  in diameter (Fig. 43). In addition, there is a large population of slender cells, concentrated in the central region of the gland, containing widely spaced bacillary granules, about 1  $\mu\text{m}$  diameter, with strong orange-G staining affinity (Fig. 44). As in other groups we studied, bulb-shaped sensory organs are present in the general body epidermis but not in the glands.

#### Gland description, *Proarmaueria cf. pellucida*, Family *Armaueriidae*

The postero-lateral glands of *Proarmaueria cf. pel-*

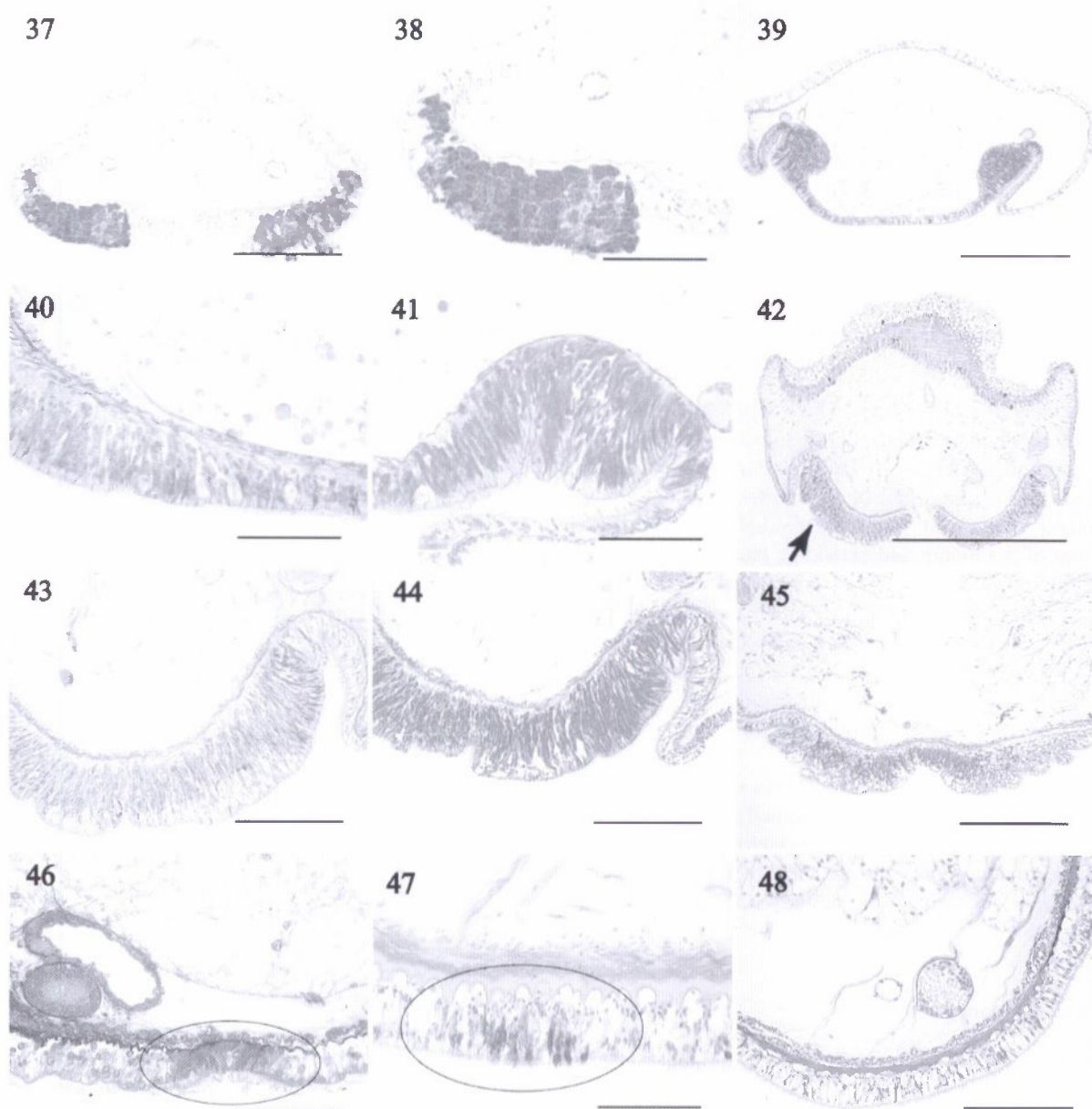


Figures 13–24. Transverse sections showing postero-lateral glands, 8  $\mu\text{m}$  thickness. Original magnification and scale bar lengths in parentheses. (13) *Cuneonemertes* sp. #416, left arrow points to serous cells of posterior glandular region (4 $\times$ , 1 mm). (14) *Cuneonemertes* sp. #416, right, side mucoid cells in anterior region of gland (40 $\times$ , 100  $\mu\text{m}$ ). (15) *Cuneonemertes* sp. #857, right side, beginning of serous cell portion of gland with some mucoid cells still present at far right (10 $\times$ , 400  $\mu\text{m}$ ). (16) *Cuneonemertes* sp. #857, right side, mucoid cells of anterior region of gland (40 $\times$ , 100  $\mu\text{m}$ ). (17) *Cuneonemertes* sp. #857, right side, serous cells in posterior region of gland (40 $\times$ , 100  $\mu\text{m}$ ). (18) *Cuneonemertes* sp. #489, right side, serous cells in posterior region of gland (10 $\times$ , 400  $\mu\text{m}$ ). (19) *Cuneonemertes* sp. #489, right side, serous cells, higher magnification (40 $\times$ , 100  $\mu\text{m}$ ). (20) *Cuneonemertes* sp. #720, mucoid cells in anterior region of glands (4 $\times$ , 1 mm). (21) *Cuneonemertes* sp. #720, right side, mucoid cells (40 $\times$ , 100  $\mu\text{m}$ ). (22) *Cuneonemertes* sp. #720, right side, serous cells (40 $\times$ , 100  $\mu\text{m}$ ). (23) *Cuneonemertes* sp. #858, serous cell posterior region of glands (10 $\times$ , 400  $\mu\text{m}$ ). (24) *Cuneonemertes* sp. #858, right side, mucoid cells to right, serous cells to left (40 $\times$ , 100  $\mu\text{m}$ ).



Figures 25–36. Transverse sections showing postero-lateral glands, 8  $\mu\text{m}$  thickness. Original magnification and scale bar lengths in parentheses. (25) *Probalaenanemertes?* sp. #87, serous cells in posterior region of glands on both sides of section (4 $\times$ , 1 mm). (26) *Probalaenanemertes?* sp. #87, right side, mucoid cells (40 $\times$ , 100  $\mu\text{m}$ ). (27) *Probalaenanemertes?* sp. #87, left side, serous cells (40 $\times$ , 100  $\mu\text{m}$ ). (28) *Pelagonemertes* sp. #HI 42, long, thin posterior serous cell regions of glands on tail fin, both sides of body (4 $\times$ , 1 mm). (29) *Pelagonemertes* sp. #HI 42, right side, serous cells (40 $\times$ , 100  $\mu\text{m}$ ). (30) *Pelagonemertes* sp. #529, mucoid cell anterior region of gland on right, serous cell posterior region of gland on left (4 $\times$ , 1 mm). (31) *Pelagonemertes* sp. #529, mucoid cells (40 $\times$ , 100  $\mu\text{m}$ ). (32) *Pelagonemertes* sp. #529, serous cells (40 $\times$ , 100  $\mu\text{m}$ ). (33) Pelagonemertid #93, mucoid anterior regions of glands, both sides, especially well developed on this specimen (10 $\times$ , 400  $\mu\text{m}$ ). (34) Pelagonemertid #93, serous cell region (10 $\times$ , 400  $\mu\text{m}$ ). (35) Pelagonemertid #93, right side, mucoid cells (40 $\times$ , 100  $\mu\text{m}$ ). (36) Pelagonemertid #198, left side, serous cells (40 $\times$ , 100  $\mu\text{m}$ ).





Figures 37–48. Transverse sections showing postero-lateral glands, 8  $\mu\text{m}$  thickness. Original magnification and scale bar lengths in parentheses. (37) Pelagonemertid #HI 13, thick serous cell region of gland on both sides of section (10 $\times$ , 400  $\mu\text{m}$ ). (38) Pelagonemertid #HI 13, serous cell region, higher magnification (40 $\times$ , 100  $\mu\text{m}$ ). (39) *Plotonemertes* sp. #146, glands bulge inward; epithelium is somewhat convoluted; glandular, non-convoluted epithelium connects the lateral parts (10 $\times$ , 400  $\mu\text{m}$ ). (40) *Plotonemertes* sp. #146, left side, ventral connector, serous cells with mucoid cells interspersed (40 $\times$ , 100  $\mu\text{m}$ ). (41) *Plotonemertes* sp. #146, right side, inward bulging part of gland with adjacent epithelium, mainly serous cells (40 $\times$ , 100  $\mu\text{m}$ ). (42) *Plotonemertes* sp. #737, glands do not meet at midline except at their back end (4 $\times$ , 1 mm). (43) *Plotonemertes* sp. #737, right side, mucoid bacillary cells (20 $\times$ , 200  $\mu\text{m}$ ). (44) *Plotonemertes* sp. #737, right side, serous cells plus a few cells with bacillary granules having strong orange-G staining properties (20 $\times$ , 200  $\mu\text{m}$ ). (45) *Plotonemertes* sp. #737, midventral meeting of glands at their back end (10 $\times$ , 400  $\mu\text{m}$ ). (46) *Proarmaueria* cf. *pellucida* (USNM 174040), left side, serous cells in small glandular area (enclosed by oval line) medial to lateral nerve cord (40 $\times$ , 100  $\mu\text{m}$ ). (47) *Crassonemertes* cf. *robusta* (USNM 174054), left side, glandular cells (enclosed by oval line) of anterior lateral body edge (40 $\times$ , 100  $\mu\text{m}$ ). (48) *Nectonemertes* cf. *mirabilis* (USNM 174034), right side, glandular lateral epidermis differs from mucus-dominated dorsal and ventral epidermis (20 $\times$ , 200  $\mu\text{m}$ ).

*lucida* were first recognized by Svetlana Maslakova, who will be providing a more detailed account in a review of the family. It is sufficient here to point out that the glands are located medially to the lateral nerve cords, but in approximately the same posterior position as postero-lateral glands (Fig. 46). Although quality of staining is poor, the gland appears to consist entirely of a relatively narrow ribbon of strongly stained azanophilous serous cells without mucoid cells. The gland was not visible to the unaided eye.

#### *Regional specializations of epidermis, Crassonemertes and Nectonemertes*

The specimen of *Crassonemertes* (USNM 174054) (Family Planktonemertidae) examined has a very well-preserved epidermis. It lacks postero-lateral glands, but it does have a pair of well-differentiated, ventro-lateral ribbons of glandular epithelium meeting in front of the mouth and extending backward for about 2 mm. The dominant cell type is an azanophilous granular serous cell, among which mucoid cells are distributed that stain more strongly than those of adjacent epidermis (Fig. 47). All of the cell types are represented in the rest of the epidermis, but it is dominated mostly by empty-appearing mucoid goblet cells.

The specimen of *Nectonemertes* (USNM 412601) (Family Nectonemertidae) examined shows strong differentiation of lateral epidermis along much of the trunk region, concentrating a higher proportion of azanophilous serous and bacillary cells in this region (Fig. 48).

#### Discussion and conclusions

To date, we have found postero-lateral glands visible to the unaided eye only in members of the families Pelagonemertidae, Balaenanemertidae and the genus *Plotonemertes* in family Protopelagonemertidae. The glands in specimens belonging to families Pelagonemertidae and Balaenanemertidae generally appear to the unaided eye as lines, triangles, or ovals. Some of these, when viewed with a stereomicroscope, are shown to be more complex; for instance, yellowish-brown, smooth-edged, narrow, triangle behind an oval, spindle-shaped, or linear white region. Some differences in overall shape of the glands within specimens or between specimens may be due to damage, especially in cases where much or all of the adjoining epidermis is missing. Other differences in gland structure

may be species- or genus-specific and taxonomically useful. For example, members of *Probalaenanemertes* and *Balaenanemertes* are strikingly similar except for the lack of externally-evident tentacles in *Probalaenanemertes* and differences in overall appearance of their postero-lateral glands. Differences in general appearance of glands in specimen #338, initially regarded as a *Cuneonemertes* by us, and otherwise similar *Cuneonemertes* specimens, indicate that #338 may not belong to that genus. The glands of two species of *Pelagonemertes*, HI 42 and #529, are very different from each other as compared to other pelagonemertids. Differences in the glands of males of the two species of *Plotonemertes* (specimens #146, #737) are evidently species-specific.

In cross section, glands of members of Pelagonemertidae and Balaenanemertidae appear fairly uniform, extending laterad from a position under or external to the lateral nerve cords. Additionally, the mucoid and serous cells are segregated into anterior and posterior regions respectively.

Pelagonemertidae and Balaenanemertidae share, uniquely among polystyliferous pelagic nemerteans, a dorsal blood vessel that ends blindly in the rhynchocoel a short distance after its entry point into the rhynchocoel. The primary characteristic separating members of these two families is a pair of tentacles near the brain, in both sexes, in the Balaenanemertidae. The similarities we found in the postero-lateral glands of members of these two families support an inference of close relationship between them, and support Korotkevich's (1955) synonymy of the two groups.

*Plotonemertes*, in contrast to members of families Pelagonemertidae and Balaenanemertidae, has enormously developed glands, covering much of the ventral body surface near the caudal end in males. In males, the two glands are connected ventrally by glandular, non-folded epithelium, apparently in species-specific fashion. The glands in our female specimen of *Plotonemertes* are far smaller than those of the males, as was found also by Coe (1936), making the glands of the female more similar to those of the pelagonemertids and balaenanemertids. However, the glandular epithelium of *Plotonemertes*, females and males, differs from postero-lateral glands in other genera in that it is convoluted, much thicker (two to three times) than adjacent epidermis, and the mucoid and serous cell types are not spatially segregated. The ventral body wall in both males and females of *Plotonemertes*, again unlike that of our other specimens, is recessed as

two deep inward-projecting folds of the entire body wall, as was noted by Brinkmann (1917a) and Coe (1936).

Postero-lateral glandular specializations not visible to the unaided eye occur in *Proarmaueria* cf. *pelucida*, family Armaueriidae. These glands resemble those of the Pelago- and Balaenanemertidae more closely than those of *Plotonemertes*, but they lack mucoid cells and they are located completely medial of the lateral nerve cords.

The sex-related dimorphism of glands in *Plotonemertes* found by Coe (1936) and by us might reasonably be expected to reflect differences in functional importance of the glands to each sex. We found glands in both male and female specimens throughout the genera we studied. However, with the exception of *Plotonemertes*, we did not have adequate sectioned material of both male and female specimens for any one species or genus to provide additional insight on sexual dimorphism.

Neither the structure of these glands nor their location on the body offer self-evident clues to function. From the fact that they exist one can reasonably infer that the postero-lateral glands are of considerable importance to these animals. In fact, they may be a novel adaptive feature of pelagic nemerteans. They are large relative to specimen size, about 8% of total body length and 23% (pelago- and balaenanemertids) to 82% (males of *Plotonemertes*) of body width in the narrowed caudal region where the glands are located. The glands produce large numbers of presumed secretory vesicles. Proteinaceous secretions are among the most costly to make, and such concentrations are rare among benthic hoplonemerteans (unpubl. obs.). This cost might be especially important in the deep sea, where these nemerteans live, because the deep sea is thought to be more nutrient-poor than shallow/surface regions (Nybakken, 1997).

Brinkmann (1917a,b) suggested that the glands of *Plotonemertes* might aid the male in adhering to the female during spawning. Coe (1936) suggested that the glands of *Plotonemertes* might be sensory as well as secretory, because they receive large branches from the adjacent lateral nerve cords. We observed no such nerve elaboration. The secretions from these glands could be toxic, as in other hoplonemerteans (e.g., Kem, 1985); bioluminescent, or convey pheromones or other chemical messengers. Bioluminescence has evolved in nearly every group of marine organisms (Haddock & Case, 1999), reaching its most complex development in mesopelagic animals (Nybakken,

1997). S. Haddock and P. Roe were unsuccessful in causing a living female specimen of *Cuneonemertes* cf. *nigra* (Coe, 1945) to produce light from her postero-lateral glands (unpubl. obs.). Additionally, S. Haddock (pers. comm.) does not think the cytological structure of the postero-lateral glands, at the level of light microscopy, resembles the structure of light producing areas of animals he has studied. Thus, pelagic nemerteans continue to comprise one of the few groups of mesopelagic animals in which bioluminescence has not been documented (S. Haddock, pers. comm.).

Secretion of the postero-lateral glands might convey substances that function as chemical messengers (e.g., pheromones, repellents) but no evidence for this is available in our histological preparations. Species-specific messengers could be extremely useful, as most mesopelagic nemertean species appear to be exceedingly thinly dispersed (Roe & Norenburg, 1999). Neither pheromones nor pheromone-like substances have been recognized from any nemertean group to date, although synchronized reproductive behavior of several species might suggest pheromonal recognition (e.g., swimming by *Cerebratulus* spp. at time of spawning).

The sexual dimorphism seen in *Plotonemertes* points suggestively to a sex-related function of postero-lateral glands. However, that could reflect specialization subsequent to some other function. The glands in the other species of this study are poorly positioned to function in tandem for the purpose of adhering to a partner. Perhaps these glands deploy strings of sticky mucus to entangle small prey or discharge noxious mucoids to deter predators. A prominent attribute of postero-lateral organs is that they usually are the last of the epidermis to be lost, which suggests that these structures are more firmly bonded to the underlying connective tissue. That would be a desirable property if the glands were used in adhesion of any sort.

#### Acknowledgements

James Childress and Monterey Bay Aquarium personnel (especially Karen Light, John O'Sullivan and Gilbert Van Dykhuizen) respectively, generously provided both authors opportunities to participate in research cruises during which these nemerteans were collected. Karen Light provided several of the specimens in this study from other cruises. Barbara Littman

and Yolanda Villacampa serially sectioned and stained most of the specimens. This study was supported in part by NSF grant OEC 91-1551 to James Childress, NSF PEET grant DEB 9712463 to Diana Lipscomb and Jon Norenburg, and by a Smithsonian Institution Short Term Visitor Grant to P. Roe.

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