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Protoraphis atlantica sp. nov., a New Marine Epizoic Diatom

by

Robert A. Gibson*

With 6 plates

Abstract: A new species of *Protoraphis* is described. *Protoraphis atlantica* sp. nov. differs from the type, *P. hustedtiana,* in having only a straight apical axis. The ultrastructure of this new species is described and compared with that of *Pseudohimantidium pacificum* Hustedt & Krasske. As in *P. pacificum* this new species was found epizoic by means of mucilaginous stalks on marine planktonic copepods. Also, as in *P. pacificum,* its occurrence was found to be linked to the mating behavior of the copepod hosts.

Introduction

Simonsen (1970) established the family Protoraphidaceae to include the genera *Pseudohimantidium* and *Protoraphis.* The principle characters of the family are a sinuous axial area and a row of large pores at the valve ends. Simonsen speculated that all members of the family live attached by gelatinous stalks.

Russell & Norris (1971) described the large pores in *Pseudohimantidium pacificum* as "unequal raphes". However, Hasle (1973) observed a row of internal labiate processes at the valve ends of this diatom. Gibson (1978b) found that these processes interrupted a narrow external groove; the entire structure was termed a labiate groove. Gibson also found that the labiate groove (or pore row) was not associated with stalk production in *P. pacificum.* Similar studies of *Protoraphis hustedtiana* Simonsen have not been made, and the pore row structure in this diatom is unknown.

*Harbor Branch Foundation, RR 1, Box 196, Ft. Pierce, FL 33450, U.S.A.

The stalk extruded by *P. pacificum* is attached to body segments and appendages of several host copepods (Russell & Norris 1971; Gibson 1978a). Russell and Norris also observed that diatom infestation was linked to copepod mating behavior. The ecology and life history of *P. hustedtiana* are unknown, possibly because of its rather scant distribution, i.e., the Arabian Sea in the Indian Ocean (Simonsen 1970, 1974).

Epizoic diatoms were found attached to individuals of several copepod species collected from coastal waters of Florida, U.S.A. These waters are part of the Florida Current. Among these diatoms a form was collected which was similar to *P. hustedtiana* but varied in a number of key characters. It is the intention of this paper to describe this diatom, its morphology and ecology in relation to other members of the Protoraphidaceae.

Materials and Methods

Surface plankton collections were made from 25 August 1977 to 17 April 1978, 1-5 km E of the Ft. Pierce Inlet, Florida, in Florida Current waters of the northwestern Atlantic Ocean (ca. 27° 29'N, 80° 15'W). Infested copepod individuals were collected, sorted and preserved as described by Gibson (1978a).

Diatom frustules were cleaned using the dichromate technique (Patrick in Patrick and Reimer 1966). Salts were removed by diluting the acid-diatom suspension 10✕ with distilled water, filtering this suspension onto a 3.0 µm screen with approximately 0.5 *l* of distilled water followed by 1.0 *l* of boiling distilled water. Diatom valves cleaned with this treatment were dried onto coverglasses and either mounted onto microscope slides with Hyrax and sealed with fingernail polish or mounted onto aluminum stubs and sputter coated with gold-palladium.

Individuals of the copepod *Labidocera scottii* Giesbrecht with attached diatoms were dehydrated, critical point dried, mounted onto aluminum stubs and coated with gold-palladium.
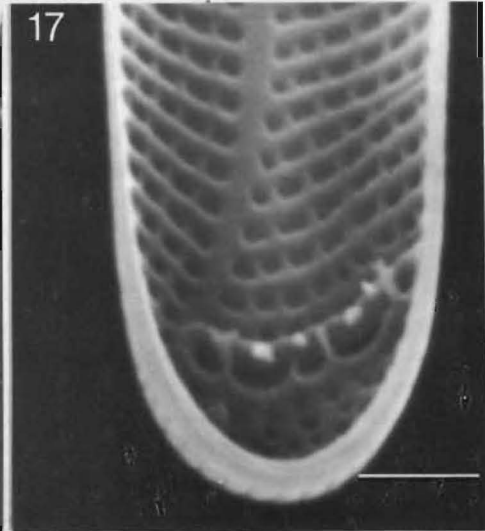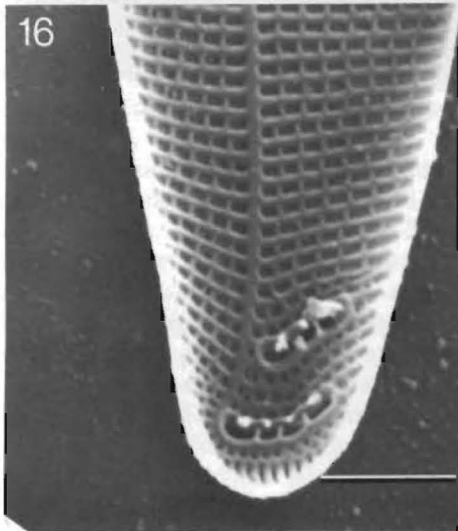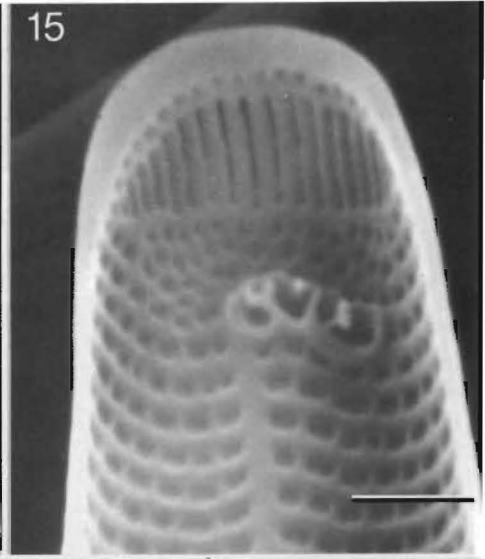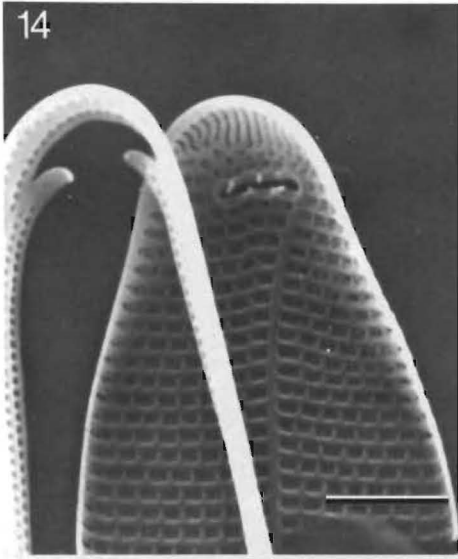
Other formalin-preserved copepods infested with diatoms were stained with periodic acid-Schiff's reagent (Humason 1972) or treated with a 20% HF solution to determine stalk composition. These specimens
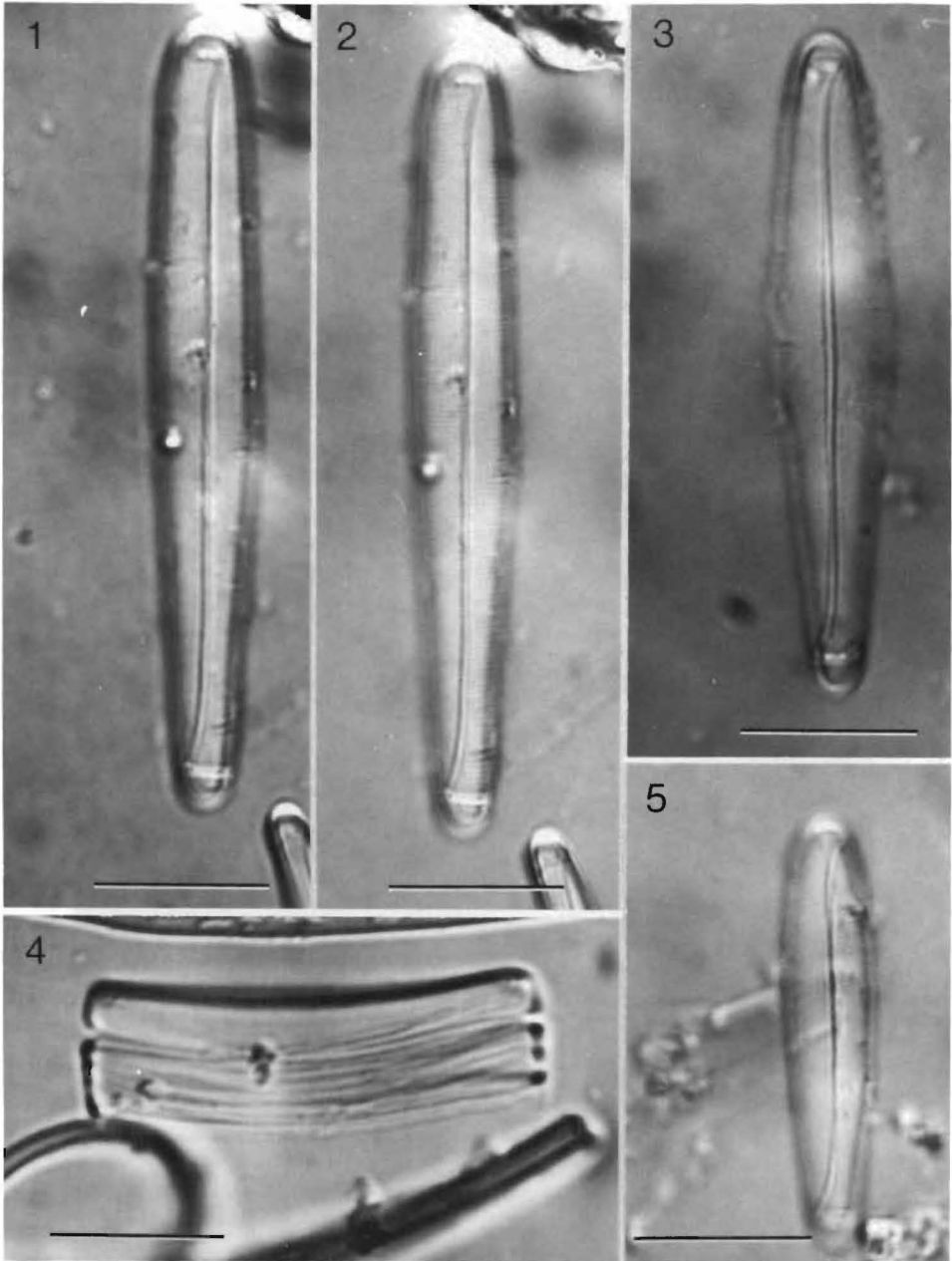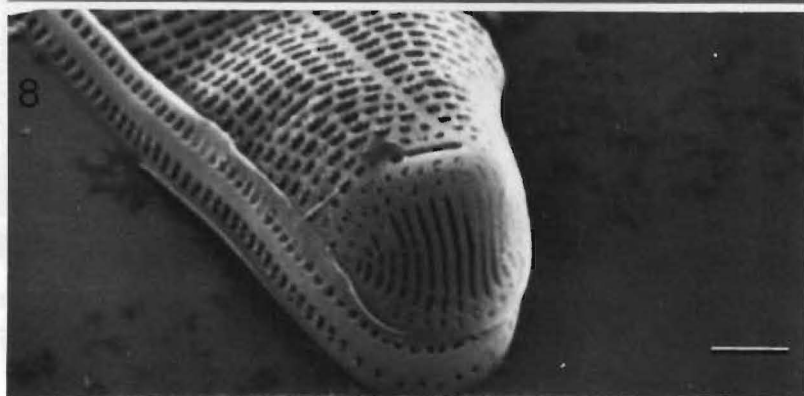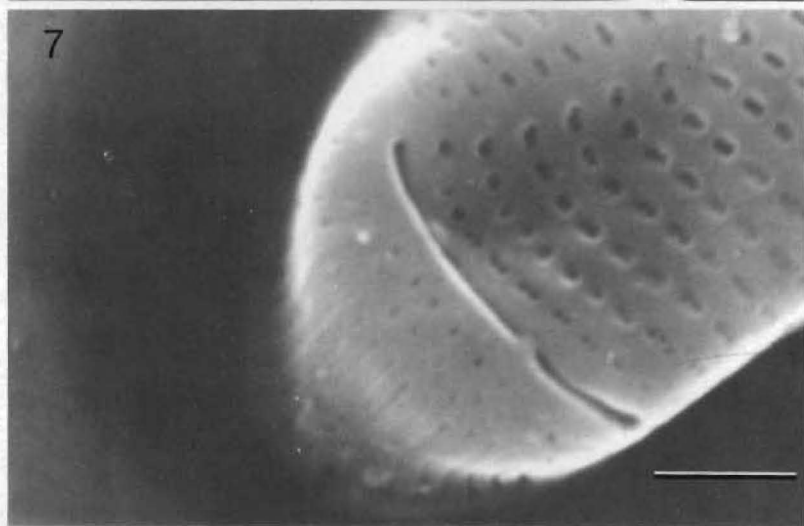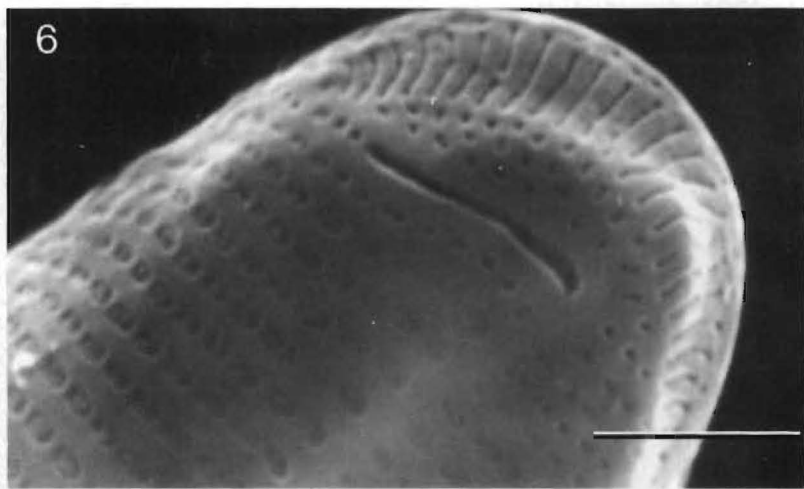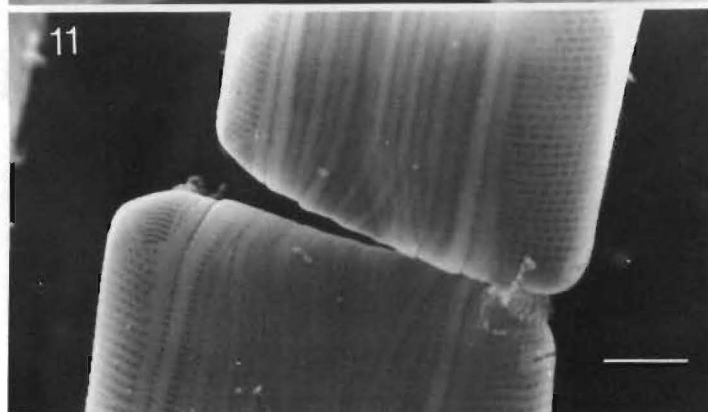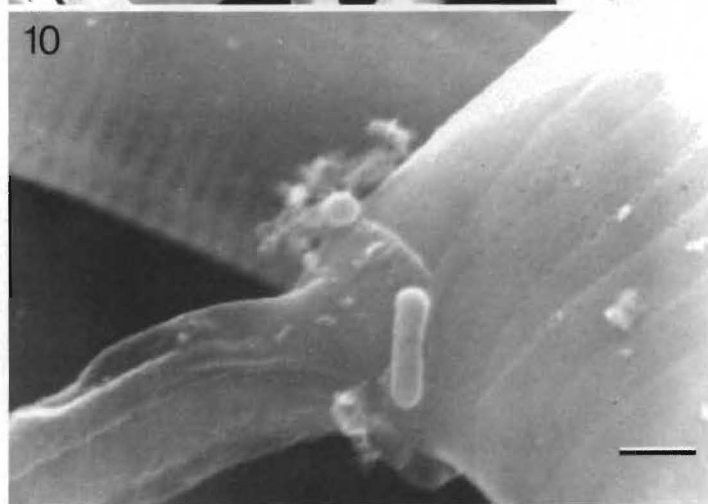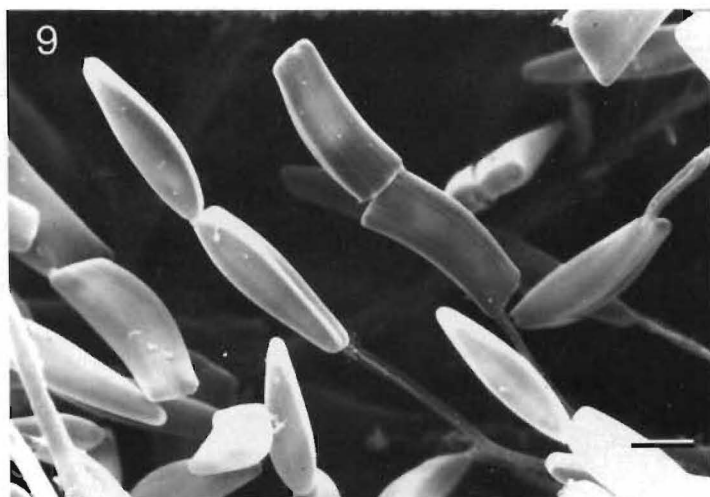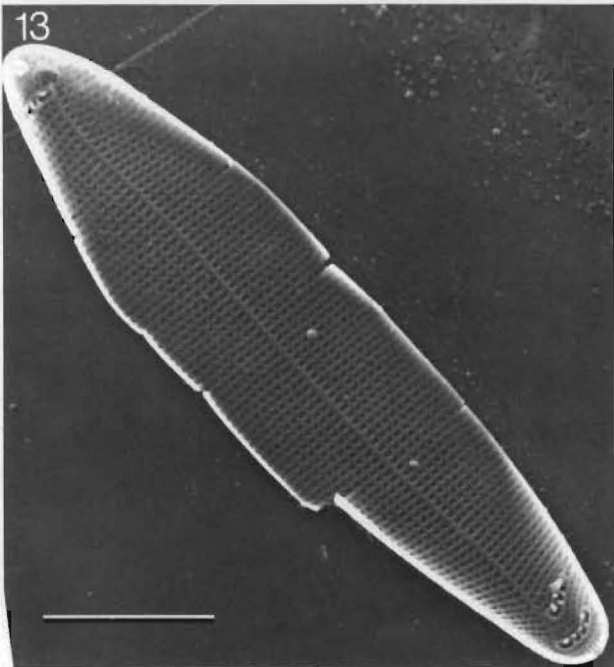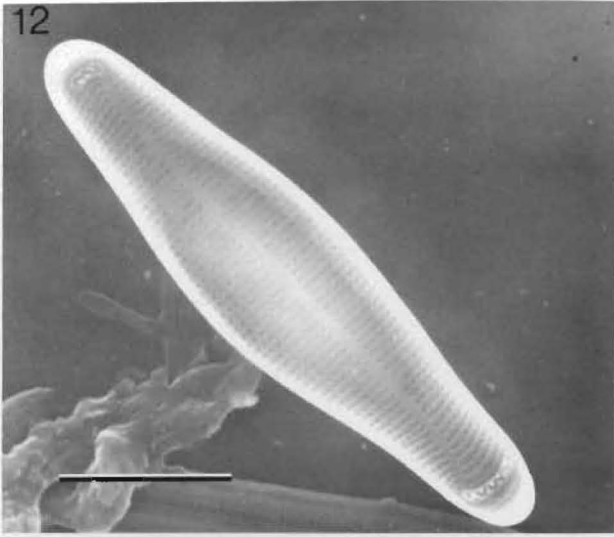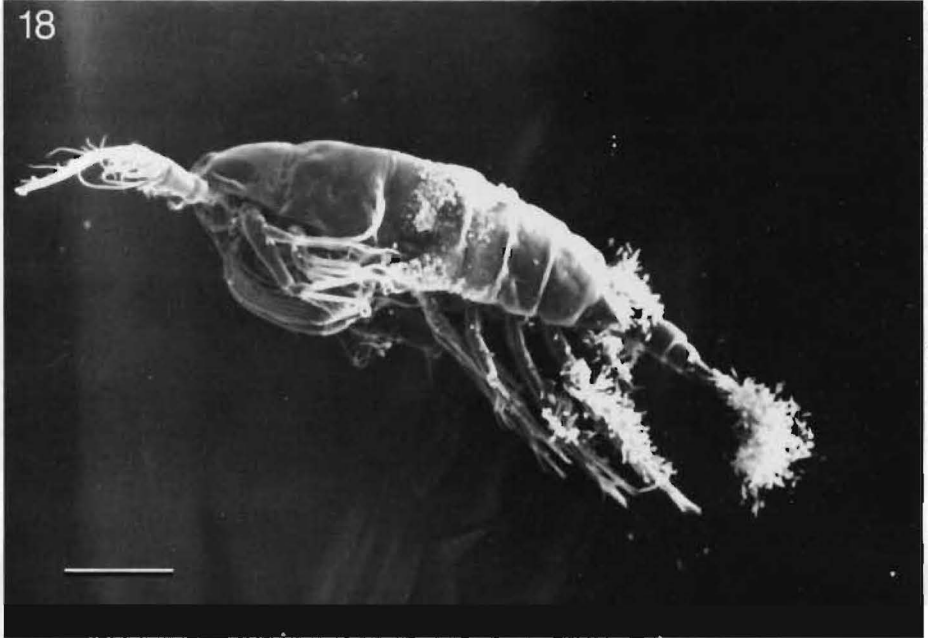
were dehydrated and mounted with Permount as permanent slides. Permount and Hyrax preparations were observed with a Zeiss Universal microscope equipped with phase-contrast and differential interference contrast optics.

Cleaned diatom valves and diatoms attached to copepod individuals which had been mounted onto stubs were scanned with a Zeiss Novascan 30 scanning electron microscope at an accelerating voltage of 15 kv.

Diatom infestation on *Labidocera aestiva* Wheeler was determined by sorting 27 copepod individuals as to sex and counting the diatom cells attached to each body segment or appendage.

Observations

Protoraphis atlantica sp. nov.

Frustulum in aspectu cincturae rectangulare ad leviter cuneiforme, frustula aliqua cum axi apicali leviter inflexo. Longitudo axis pervalvaris 7-10 μm. Chromatophora magna duo, apicaliter disposita in extremo alterutro cellulae. Frustula affixa singulatim aut binatim ad substratum hospitis pedicellis mucilaginibus extrusis unde areis apicalibus limbis poris ad polis pedibus ubi binatim, cellulae affixae ad polo capitulis. Partes cincturae 15-20; utrumque perforatae serie singula punctarum, 55-60 in 10 μm. Valva clavata-lanceolata, apicibus rotundatis. Longitudo maxima transapicallis in valvis cum ambitu distincte clavato est circiter 1/3 longitudinis apicalis infra polo capitulo. Ambitus graditim angustus, fere concavescens super polo pedi. Longitudo axis apicalis 18-50 μm. Longitudo axis transapicalis 4-11 μm. Area axialis perangusta et recta, tantum ad extremis valvae in directiones oppositas flexa. Striae transapicales subtiliter punctatae, parallelae, 33-40 in 10 μm. Areae apicales limbi pororum compositae pororis apicalibus connexis sulcis; series pororum circiter 60 in 10 μm. Area axialis desinens in apicibus valvae in sulco externo disposito parallele axi transapicali. Sulcus interne interruptus a fascia silicea plus minusve plicata. Sulci versus polis pedis longiores quam ad polis capitulis. Epizoica in copepodis marinis planctonicis in aquis marinis litoralibus Maris Atlantici boreo-occidentalis, longitudo 27° 29'N, latitudo 80° 15'W.

Holotypus: Praeparatio no. A.-G.C.-53761, Diatom Herbarium, Academy of Natural Sciences of Philadelphia. Isotypi: Praeparatio no. Zu 1/33, Collectio Hustedt, Institut für Meeresforschung, et in collectione auctoris.

Frustule in girdle view rectangular to slightly wedge-shaped, some frustules with apical axis slightly inflexed, bent or bowed. Length of pervalvar axis 7-10 μm. Two large chromatophores arranged apically in either end of the cell. Frustules attached singly or in pairs to their

host substrate by mucilaginous stalks extruded from apical mantle pore fields at the foot poles; when in pairs, cells attached at their head poles. Girdle elements 15-20 per frustule; each element perforated by a single row of puncta, 55-60 in 10 μm. Valve clavate-lanceolate, symmetrical to the apical axis, asymmetrical to the transapical axis, with rounded apices. Greatest transapical length in those valves with a distinctly clavate outline is about 1/3 of the apical length below the head pole. The outline then gradually narrows, becoming nearly concave above the foot pole. Length of apical axis 18-50 μm. Length of transapical axis 4-11 μm. Axial area very narrow and straight, flexed in opposite directions only at the valve apices. Transapical striae finely punctate, parallel throughout the valve, 33-40 in 10 μm. Apical mantle pore fields composed of pores apically connected by grooves; pore rows about 60 in 10 μm. Axial area ending at the valve apices in an external groove arranged parallel to the transapical axis. Groove interrupted internally by a more or less plicate siliceous band. Grooves longer at the foot pole than at the head pole. Epizoic on marine planktonic copepods in neritic waters of the western North Atlantic Ocean.

Type locality: 5 km east of the Fort Pierce Inlet, Florida, Florida Current, western North Atlantic Ocean, longitude 27° 29'N, latitude 80° 15'W.

Holotype material: Slide number A.-G.C.-53761 Diatom Herbarium, Academy of Natural Sciences of Philadelphia. Isotype material: Slide number Zu 1/33 Hustedt Collection, Institut für Meeresforschung and in the author's collection.

Light microscope. The outline of *Protoraphis atlantica* valves is asymmetrical about the transapical axis, lanceolate, varying from slightly clavate to distinctly clavate (Figs 1-3, 5). In girdle view (Fig. 4) the frustules are curved with concave and convex valves, many appearing wedge-shaped. Internal septa were not observed in any of the specimens.

Valves are without raphes, the axial area lying in a median position throughout its extent except at the poles (Figs 1-3, 5). The straight axial area is flexed in opposite directions at either pole thereby appearing sigmoid. As shown in Figure 2 the axial area is connected at both apices to what appears to be a straight, transapically parallel row of large pores. The large pore row is usually longer at the foot pole than the head pole. Transapical striae are straight, finely punc-

tate and radiate from the axial area conforming to its contours at the cell ends. The puncta also form more or less straight longitudinal striae which are less apparent than the transapical striae. Cells have two equal parietal chromatophores, apically oriented and positioned near the middle of the cell.

The stalk attached at the cell end is unbranched, uniform in diameter and structureless throughout its length. Stalk material was heavily stained by periodic acid Schiff's reagent as were some of the cell contents. Treatment with hydrofluoric acid appeared to have no effect on the stalk material. As many as four cells were observed attached end to end, their apices abutting, with the basal cell attached to the substrate by the stalk.

Electron microscope. External valve surface: Transapical striae are formed of ellipsoidal to rectangular puncta and interrupted only by the axial area. The puncta abruptly become smaller and circular at the end of the axial area where they surround a straight, narrow, transapically parallel groove or slit positioned at the end of the flexed axial area (Figs 6, 7). The groove at the head pole (Fig. 6) was usually shorter than that at the foot pole (Fig. 7). A series of apical lamellar pores is positioned on the mantle at either valve end (Fig. 8).

As shown in Figures 9 and 10 stalk material was extruded at cell apices on one valve. Cell to cell attachment is by means of a short stalk or pad extruded from the lamellar slit field at the other cell end on the opposite valve (Fig. 11).

Internal valve surface: As shown in Figure 13 puncta appear to penetrate the basal siliceous layer such that the area between the transapical striae as well as the axial area appears as siliceous ribs. The external groove is interrupted by a more or less plicate internal siliceous band (Figs 12, 14-17). The structure of this band was inconsistent from valve to valve. Portions of the band also appeared to project into the cell interior as shown in Figure 15. A few valves possessed two siliceous bands at one valve end (Fig. 13). One band was in its normal position at the end of the axial area and another, usually shorter band, was further way from the end of the axial area (Fig. 16). This situation was rare in the valves examined and was considered abnormal. The lamellar pore series at the valve ends appeared similar to that observed externally.

Table 1. Copepods collected from Florida Current waters epizoic with *Protoraphis atlantica.*

order	family	genus	species	collection date
Calanoida	Pontellidae	Labidocera	aestiva Wheeler	Aug. 25, Aug. 29, Sept. 12, Sept. 15, Sept. 23, 1977, Jan. 17, 1978
		Labidocera	scottii Giesbrecht	Sept. 12, 1977
		Pontellopsis	brevis Giesbrecht	Oct. 27-Oct. 5, 1977
		Pontellopsis	villosa G. Brady	Oct. 27-Oct. 5, 1977
		Pontella	meadii Wheeler	Apr. 10, 1978 Apr. 17, 1978
		Anomalocera	ornata Sutcliffe	Apr. 10, 1978
	Candaciidae	Candacia	pachydactyla (Dana)	Jan. 17, 1978
		Candacia	curta (Dana)	Jan. 17, 1978
		Paracandacia	simplex (Giesbrecht)	Jan. 17, 1978
Harpacticoida	Miraciidae	Miracia	efferata Dana	Jan. 17, 1978
	Longipediidae	Longipedia	helgolandica Klie	Apr. 17, 1978

Ecology. Table I gives a list of copepod species infested with *Protoraphis atlantica* from the Florida Current. The diatom cells were found on posterior body appendages and segments of nine calanoid species representing two copepod families. The diatoms were found on all body segments of the species representing two harpacticoid families.

Infestation by *P. atlantica* on four *Labidocera aestiva* males was equally distributed on the 4th periopod, the dorsal and ventral surfaces of the genital segment and the caudal furcae. In contrast, two-thirds of the infestation of 23 *L. aestiva* females occurred on the caudal furcae and the rest primarily on the 4th pair of swimming legs and the dorsal surface of the genital segment. An example of this diatom infestation on the copepod is shown in Figure 18.

Discussion

Protoraphis atlantica has characteristics which conform to that of the family Protoraphidaceae described by Simonsen (1970). Prior to this time the family was composed of two unispecific genera, *Pseudohimantidium* and *Protoraphis.* In girdle view cells of these species are straight, linear or club-shaped. The valves are linear or sickle-shaped, sometimes club-shaped, with punctate transapical striae. The valves have no raphe, and the axial area lies in a more or less median

position recurved in opposite directions only at the apices. The axial area is connected to a row of large pores at either valve end. The genera were distinguished by curvature of the apical axis. The new species, *Protoraphis atlantica,* also has these characters of the family but does not fit either key character given by Simonsen (1970) for the two genera. It has neither three straight isopolar axes, nor are the valves sickle-shaped. Cells of *Pr. atlantica* have a straight isopolar to heteropolar apical axis, a clavate transapical axis and a bent pervalvar axis.

Symmetry about the apical axis then seems to be a more consistent differentiating character between *Protoraphis* and *Pseudohimantidium* than transapical symmetry. For that reason I have placed this diatom in *Protoraphis* and propose the following generic key and emended description of *Protoraphis:*

 I. *Protoraphis,* with a straight apical axis.

 II. *Pseudohimantidium,* apical axis curved so that the frustule is sickle-shaped.

Emended descriptions of *Protoraphis* Simonsen: Genus with the characteristics of the family, moreover with a straight apical axis. Symmetry: cells isopolar or heteropolar, diagonally symmetrical to the apical axis because of the "S"-shaped, bent axial area. Apical and transapical planes straight; valvar planes straight or bent.

 I. Cells with three straight isopolar axes, *Pr. hustedtiana.*

 II. Cells with a straight heteropolar to isopolar apical axis, clavate transapical axis and a bent or bowed pervalvar axis, *Pr. atlantica.*

Protoraphis atlantica and *Pr. hustedtiana* both have an axial area flexed in opposite directions at the apices, and connected to transapically parallel pore rows. However, in *Pr. atlantica* one pore row is usually longer than the other, the longer row usually on the foot pole. This heteromorphic character at the valve ends is also found in *Ps. pacificum* and *Licmophora flabellata* (Greville) Agardh. However, *Pr. atlantica* valves are not sickle-shaped as in *Ps. pacificum,* nor do they have internal septa as does *L. flabellata.*

The pore rows in *Pr. atlantica* and *Ps. pacificum* are partially interrupted external grooves. The pore row in *Ps. pacificum* consists

of a row of internal labiate processes which interrupt the external groove (Hasle 1973), termed a labiate groove by Gibson (1978b). The internal groove in *Pr. atlantica* is not interrupted by labiate processes but rather by a somewhat variable siliceous band. This band may in fact be the lips of a labiate process row. The ultrastructure of the pore rows in *Pr. hustedtiana* is unknown but would presumably either have a labiate groove or a groove similar to that in *Pr. atlantica*.

Cellular attachment in *Pr. atlantica* neither resembles the branched stalked system in *Ps. pacificum* (Russell & Norris 1971; Gibson 1978b) or *L. flabellata* (Hendey 1964). *Protoraphis atlantica* is attached by an unbranched, nonsiliceous mucopolysaccharide stalk extruded through an apical mantle slit field on one valve. Stalk extrusion in *Ps. pacificum* was shown by Gibson (1978b) to occur through similar apical pore fields but on both valves of the cell. The function of the external groove in *Pr. atlantica* is unknown but, as Gibson (1978b) had shown for *Ps. pacificum*, has no relation to stalk extrusion. Cellular attachment and stalk morphology in *Pr. hustedtiana* are unknown.

Protoraphis atlantica infestation occurs on specific body segments and appendages of the copepod *L. aestiva*, especially so on female individuals. This infestation specificity tends to discount chance encounter as a mode of infestation. Specificity by *Ps. pacificum* on *Corycaeus affinis* McMurrich was described by Russell & Norris (1970). They attributed the diatom occurrence to copepod copulatory positions. Prior to copulation in *L. aestiva* the male geniculate antennae and the fifth swimming legs were in contact with the female caudal setae and furcae, abdominal segment and genital segment. During and subsequent to copulation the male's fifth pair of swimming legs and possibly the last thoracic segment were in contact with the female abdominal segment and genital segment (Blades & Youngbluth, unpublished observations). The animals were observed to assume the precopulatory positions for as long as 30 minutes whereas copulation occurred within one minute. Thus it appears that *Pr. atlantica* infestation on *L. aestiva* is also linked to copepod mating behavior.

Similar infestation by *Pr. atlantica* on the posterior body segments and appendages was observed in all of the calanoid copepod host species. However, diatom infestation on the harpacticoid species was considerably less specific, i.e. cells occurring equally on nearly all body segments and appendages. Haq (1972) has described mating

behavior in the harpacticoid copepod, *Euterpina acutifrons* Dana. The copulatory positions in this copepod are relatively complex and involve nearly every body segment and appendage to some extent. Also the entire process can extend for as long as 10 hours. Similar copulatory behavior in *Miracia efferata* and *Longipedia helgolandica* could also explain the observed diatom infestation.

The rather scant information on this diatom species, indeed with the entire family Protoraphidaceae, suggests that the diatoms occur epizoic on species of two marine copepod orders and that the infestation is transferred via the mating behavior of the hosts. One might then easily hypothesize diatom interference with host mating which would imply important copepod population adjustments. However, observations by Blades (personal communication) of successful copulation by heavily infested (>1000 diatom cells $\cdot$ copepod^{-1}) *L. aestiva* individuals indicates that diatom infestation may not interfere with host mating behavior. This information seems to indicate a commensal type of two-species population interaction, however, several other possibilities might exist.

It is unknown whether copepod swimming speed is impaired by diatom attachment. This might reduce the copepod's ability to escape predation. The diatom cells could also derive some ecological benefit by their attachment to a moving host continually replenishing their nutrient supply. Perhaps more importantly, it is unknown whether predators on this diatom-copepod association make use of the additional energy and nutrient supply afforded by the attached diatoms.

This diatom as well as the other species of the Protoraphidaceae have evidently been overlooked by planktologists, and it would appear that these diatoms may be more common than previously thought. Several specimens of *Pontellopsis brevis* collected in 1977 by Dr. A. Fleminger from surface waters of the Caribbean Sea were also infested with *Pr. atlantica*. Dr. Fleminger (letter of 27 September 1978) states that it is not uncommon to find epizoic diatoms on pontellids and other near-surface neritic copepods. Therefore, although the occurrence of *Pr. atlantica* is presently reported from the western North Atlantic Ocean, the distribution of the species is probably similar to that of *Ps. pacificum.*

Acknowledgements

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Explanation of Plates

Plate 1

Figs 1-5. *Protoraphis atlantica*, LM. Scale $= 10\,\mu$m. Fig. 1. Whole valve with focus on grooves at the valve ends. — Fig. 2. Same valve as in Fig. 1 except focus on axial area and valve surface. — Fig. 3. Whole valve, focus on grooves at the valve ends. — Fig. 4. Girdle view showing girdle bands and concave valve surface. — Fig. 5. Whole valve, focus on axial area and valve surface.

Plate 2

Figs 6-8. *Protoraphis atlantica*, SEM. Scale $= 1\,\mu$m. Fig. 6. Outside view of groove on head pole. — Fig. 7. Outside view of groove on foot pole. — Fig. 8. Outside view of apical lamellar pore field on valve mantle.

Plate 3

Figs 9-11. *Protoraphis atlantica*, SEM, critical point dried preparations. Fig. 9. View of stalk and cellular attachment. Scale $= 10\,\mu$m. — Fig. 10. Stalk extrusion at location of apical lamellar pore field. Scale $= 1\,\mu$m. — Fig. 11. Detail of mucilage pad extrusion from apical lamellar pore fields. Scale $= 2\,\mu$m.

Plate 4

Figs 12, 13. *Protoraphis atlantica*, SEM. Scale $= 5\,\mu$m. Fig. 12. Internal view of whole valve showing sinuous bands interrupting grooves at valve ends. — Fig. 13. Internal view of whole valve (split along margin) showing double grooves at foot pole.

Plate 5

Figs 14-17. *Protoraphis atlantica*, SEM. Scale $= 2\,\mu$m except where otherwise noted. Fig. 14. Internal view of head pole with groove; a portion of the girdle band also shown. — Fig. 15. Internal view of head pole with groove and apical lamellar pore field; note projections from groove margin. Scale $= 1\,\mu$m. — Fig. 16. Internal view of foot pole with double grooves. — Fig. 17. Internal view of foot pole with single groove. Scale $= 1\,\mu$m.

Plate 6

Fig. 18. SEM, critical point dried preparation of *Labidocera scottii* with cells of *Protoraphis atlantica* attached to thoracic segments 1-5, swimming legs 1-5, genital segment and caudal furcae. Scale = 400 μm.