

***Protoglossus graveolens*, a new hemichordate (Hemichordata:
Enteropneusta: Harrimanidae) from the northwest Atlantic**

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Abstract.—A new hemichordate species, *Protoglossus graveolens*, is described from Maine, U.S.A. *Protoglossus graveolens* is the second member of its genus and the only North American protoglossid. *Protoglossus graveolens* occurs intertidally, usually with another enteropneust, *Saccoglossus bromophenolosus* King et al., 1994. The new species is assigned to the genus *Protoglossus* based on the following criteria: 1) proboscis musculature; 2) proboscis coelom; 3) proboscis septa; 4) a basal sheath at the posterior end of the proboscis stalk; 5) inclination of the collar; 6) periahaemal spaces in the collar; 7) lack of esophageal pores. *Protoglossus graveolens* is differentiated from *Protoglossus koehleri* (Caullery & Mesnil, 1900) on the basis of size, coloration, proboscis and branchial skeleton morphologies, collar dimensions and internal organization of the collar.

Three harrimaniid species have been reported from coastal Maine. Two of these were considered a single species but have recently been differentiated into *Saccoglossus bromophenolosus* King et al., 1994 and *S. kowalevskii* Agassiz, 1873. *Saccoglossus bromophenolosus* occurs in colder waters, ranging from southern Maine northward at least to Nova Scotia (Prefontaine & Brunel 1962, Bromley 1979, King et al. 1994); populations are also known from Oregon and Washington (Bullock 1975, Kozloff 1987). In contrast, *S. kowalevskii* occurs from Georgia to southern Maine (Colwin & Colwin 1953, 1962; Elder 1973, Jaffe 1983, Fox & Ruppert 1985, Woodin et al. 1987, King et al. 1994). The third species reported from Maine, *Stereobalanus canadensis* (Spengel, 1893) occurs subtidally, and has been reported from Nova Scotia (Spengel 1893, 1901), Frenchman's Bay, Maine (Reinhard 1942), the Gulf of St. Lawrence (Prefontaine & Brunel 1962), the west coast of Scotland (Burdon-Jones & McIntyre 1960), San Diego, California (Bullock & Rao unpubl.

observations cited in Burdon-Jones & McIntyre 1960) and the Norwegian Sea (Romero-Wetzel 1989). We describe here a fourth and new harrimaniid from three adjacent sites in the Damariscotta River estuary, Maine, U.S.A. (43°56'N, 69°34'W).

The new species is presumptively assigned to the genus *Protoglossus* (van der Horst, 1927). This genus is referred to by different names throughout the literature. Placement of the first protoglossid species, *P. koehleri*, was uncertain with proposals for the genus *Balanoglossus* Delle Chiaje, 1829 and a new genus *Balanocephalus* (Harmer, 1899) (Caullery & Mesnil 1900). The latter genus was renamed *Protobalanus* (Caullery & Mesnil, 1904) and placed in a new family, Protobalanidae (Caullery & Mesnil 1904). *Protobalanus* was renamed *Protoglossus* (van der Horst, 1927) and subsequently placed in the family Harrimaniidae (Burdon-Jones 1956). Although Benito (1982) places *Protoglossus* in a separate family, Protoglossidae, the character overlap between *Saccoglossus* Shmukewitsch, 1892 and *Protoglossus* (Table

1), and the intermediate position of *P. graveolens* between these genera substantiates its placement in the family Harrimaniidae.

Materials and Methods

Specimens were collected from three tidal mudflats, Lowes Cove and Clarks Cove (43°56'N, 69°34'W) and Mears Cove (43°58'N, 69°34'W), in the Damariscotta River estuary, Maine (salinities range from 30–35 ppt; average range of diurnal tides about 3 m). The location of *P. graveolens* was approximated by inspection of the sediment surface for distinctive fecal coils that were easily distinguished from those of the co-occurring species *S. bromophenolosus* by size and coloration. Fecal coils of *P. graveolens* were generally 1–2 mm diameter and dark, while those of *S. bromophenolosus* were usually less than 1 mm diameter and lighter in color. *Protoglossus graveolens* was extracted from the sediment after overturning large portions of mud and exposing individuals in their burrows. Intact animals were seldom obtained due to their fragility, and frequent extension to >40 cm depth within the sediment. Several 1 m² plots were also excavated in order to quantify *P. graveolens* density. Morphological observations, measurements of body dimensions and gill pore counts were obtained from 48 freshly collected individuals following relaxation in 5% magnesium chloride. Four individuals were anaesthetized in 7.5% magnesium chloride and their proboscis skeletons examined after dissection and immersion in 4% sodium borate (Thomas 1968). Internal characters were examined using 7–13 µm thick sections through the proboscis, collar and trunk regions of three adult individuals. The specimens were relaxed in 5% magnesium chloride, fixed in Bouin's for 24–48 hours, dehydrated through several ethanol washes and embedded in paraffin (Humason 1979, K. Eckelbarger & S. Sampson, pers. comm.). Sections were obtained by the use

of a manual microtome (AO Spencer Model 815 Rotary Microtome, American Optical Company) and stained with Gomori's solution (Humason 1979; K. Eckelbarger, pers. comm.). Specimens for analysis of bromophenols were placed in a small dish of clean seawater, and incubated overnight at ambient field temperature to facilitate complete discharge of sediment from the gut. After anaesthesia with 7.5% magnesium chloride, specimens were separated into proboscis, collar and trunk regions, then immediately placed in 1.8 ml screw-cap vials containing 250 µl hexane; the vials were subsequently sealed using teflon-faced neoprene septa. Tissues were extracted for 12–24 hours before 0.7–1.0 µl of the hexane was injected into a Varian 3400 gas chromatograph fitted with a flame ionization detector as described previously (King 1986, 1988). Bromophenol identification was confirmed by gas chromatography-mass spectroscopy according to King et al. (1994).

Harrimaniidae

Type genus.—*Protoglossus* (van der Horst, 1927)

Family diagnosis.—Well-developed proboscis muscles. Elongate proboscis skeleton crura divide buccal diverticulum in collar. Perihæmal spaces may exist in collar but peribuccal cavities not present. Tongue bars hang free in gill slit, varying lengths of skeletal rods in tongue bars, no synapticules. No lateral trunk septa. No hepatic sacculations or caeca. Large oocytes, ranging 200 µm to >1 mm in diameter.

Remarks.—The lack of a number of characters exclude *P. graveolens* from the Ptychoderidae: genital wing formation through a dorsolateral septum (Fig. 1); peribuccal coelomic cavities in the collar (Fig. 2); synapticules in the branchial apparatus; lateral septa and hepatic sacculations in the trunk (Fig. 3) (van der Horst 1930, 1932; Hyman 1959, Woodwick & Sensenbaugh 1985, Hayward & Ryland 1990). Similarly,

Table 1.—Comparison of commonly used internal and external characters of *Saccoglossus* and *Protoglossus* species; bold print indicates characters which *Protoglossus graveolens* shares with members of the two genera (compiled from: van der Horst 1930; Kirk 1938; Brambell & Goodhart 1941; Burdon-Jones 1951, 1956; Thomas 1956; Burdon-Jones & Patil 1960; Thomas 1968; Hadfield 1975; King et al. 1994). *S. silcatus* Spengel, 1893 was excluded due to the lack of information.

Character	<i>Protoglossus graveolens</i> new species	<i>Protoglossus Koehleri</i> (Cautley & Mesnil, 1900)	<i>Saccoglossus bromopharatosus</i> King et al., 1994	<i>Saccoglossus kovatevskii</i> Agassiz, 1873	<i>Saccoglossus atagoensis</i> (Benham, 1899)	<i>Saccoglossus pygmaeus</i> Hinricks & Jacobi, 1938	<i>Saccoglossus ruber</i> Tattersall, 1905	<i>Saccoglossus apantesis</i> Thomas, 1956
Proboscis groove	Deep dorsal in posterior 1/2	Deep dorsal in posterior 1/3	Shallow dorsal groove	Shallow dorsal groove	Deep dorsal groove	Absent	Dorsal groove in posterior 2/3	Slight dorsal groove
Proboscis skeleton	Crura extend to posterior collar	Crura extend to posterior collar	6-7 concentric rings	4-5 concentric rings	Crura extend to posterior collar	Crura extend half into collar	Crura extend half into collar	Crura extend half into collar
Proboscis muscle	Not concentric, radial sectors	Not concentric, radial sectors	Small	Small	3-4 concentric rings	4-6 concentric rings	9-11 concentric rings	9-11 concentric rings
Proboscis coelom	Large	Large	Small	Small	Small	Small	Small	Small
Proboscis complex	Pericard large, stomach capped	Pericard large, stomach capped	Pericard large, stomach capped	Pericard large, stomach capped	Glomerulus does not cap stomach	Glomerulus does not cap stomach	Glomerulus does not cap stomach	Glomerulus caps stomach
Proboscis septa	Dorsal replaced by pericardium	Dorsal replaced by pericardium	Dorsal replaced by pericardium	Dorsal replaced by pericardium	Ventral short	Ventral short	Ventral short	Ventral short
Basal sheath	1, dorso-left	1, dorso-left	1, dorso-left	1, dorso-left	1, left side	1, left side	1, dorso-left	1, dorso-left
Collar dimensions	Present	Present	Present	Present	Not reported	Not reported	Not reported	Not reported
Collar inclination	Broader than long	As long as broad	As long as broad	Longer than broad	Broader than long	Broader than long	Longer than broad	Longer than broad
Collar operculum	Dorso-ventrally at posterior end	Dorso-ventrally at posterior end	Dorso-ventrally at posterior end	Dorso-ventrally at posterior end	Ventra-dorsally at anterior end	Ventra-dorsally at anterior end	Ventra-dorsally at anterior end	Ventra-dorsally at anterior end
Collar groove	Present, fused ventrally	Present, fused ventrally	Present, fused ventrally	Present, fused ventrally	Present	Present	Absent	Present
Collar musculature	Present	Present	Present	Present	Weak	Absent	Present	Present
Perihaemal cavities	Weak	Weak	Weak	Weak	Fused anteriorly	Fused in posterior third	Fused posterior to crura extension	Fused posterior to crura extension
Collar mesenteries	Extend through 2/3 of collar	Extend only to tips of crura	Extend only to tips of crura	Extend only to tips of crura	Both complete	Both complete	Both complete	Both complete
# of gill pores	Both complete >100	Both complete 14-30 pairs	Both complete 60-100 pairs	Both incomplete ~100 pairs	Both absent 10 to 15 pairs	Both complete 9 to 22 pairs	Both complete 60-95 pairs	Both complete 30-45 pairs
Branchial skeleton	Septa > tongues	Septa > tongues	Septa > tongues	Septa > tongues	Septa > tongues	Septa > tongues	Septa > tongues	Septa > tongues
Esophageal pores	Absent	Absent	4-6 pairs	4-6 pairs	1 pair	1 pair	8-12 pairs	6-8 pairs
Gonads start at	Few mm from collar	Halfway down branchial region	Few mm from collar	1 mm behind collar	4th gill pore	None in branchial	Mid-branchial	Mid-branchial
Oocyte size	380 µm diameter	430 µm diameter	~250 µm diameter	~250 µm diameter	400 by 300 µm	400 by 300 µm	310 by 285 µm	310 by 285 µm

Table 1.—Extended.

Character	<i>Saccoglossus autakoensis</i> Thomas, 1968	<i>Saccoglossus horsti</i> Brambell & Goodhart, 1941	<i>Saccoglossus pusillus</i> (van der Horst, 1930)	<i>Saccoglossus gurneyi</i> Robinson, 1927	<i>Saccoglossus bournei</i> Menon, 1904	<i>Saccoglossus inhacensis</i> Kapelus, 1936	<i>Saccoglossus mierschlowskii</i> Wagner, 1885	<i>Saccoglossus carabicus</i> van der Horst, 1924
Proboscis groove		Dorsal and ventral grooves						
Proboscis skeleton	Crura extend half into collar	Crura extend $\frac{3}{4}$ – $\frac{1}{2}$ into collar	Crura extend to posterior collar	Crura embrace buccal cavity	Endplate with dorsal spine	Endplate extends into proboscis spine	Endplate with dorsal spine	Endplate extends into proboscis spine
Proboscis muscle	9–10 concentric rings	>9 concentric rings	6–7 concentric rings	Not concentric	Not concentric	Not concentric	7–10 concentric rings	Not concentric
Proboscis coelom	Small	Small, 1/10 of proboscis diam	Medium					
Proboscis complex	Glomerulus caps stomochord	Pertcard large , no capping	Glomerulus only ventral at tip	Pericardium small	Glomerulus caps stomochord	Pericardium small	Glomerulus caps stomochord	Extends dorso-anteriorly
Proboscis septa	Ventral short	Ventral short	Ventral short	Extend past stomochord	Ventral short	Ventral short	Ventral short	Ventral extends to tip of stomochord
Proboscis septa	1, dorso-left	1, left side	1, left side	1, mid-dorsal	Not reported	Not reported	Not reported	1, mid-dorsal
Basal sheath	Not reported	Not reported	Not reported	Broader than long	Broader than long	Broader than long	As long as broad	Not reported
Collar dimensions	Longer than broad	Longer than broad	Longer than broad					Longer than broad
Collar inclination	Ventra-dorsally at anterior end					Ventra-dorsally at anterior end		
Collar operculum	Present	Present but not pronounced	Absent					
Collar groove		Absent						
Collar musculature								Well-developed circular muscles
Perhaemal cavities	Extend into stalk	Extend to stalk, fused at crurae	Fused in proboscis stalk					Well-developed
Collar mesenteries	Both incomplete	Dorsal absent at anterior $\frac{1}{3}$	Dorsal incomplete at anterior end	Ventral absent at anterior end	Ventral absent at anterior end	Dorsal absent, ventral complete	Ventral incomplete anteriorly	Do not extend to stalk, not fused
# of gill pores	12–25 pairs	100 to 140 pairs	60 pairs	40 to 60 pairs	>62 pairs	>82 pairs	50 pairs	Both complete
Branchial skeleton	Tongues > septa	Tongues > septa	1 pair	Absent?	Collar	4 pairs	Septa > tongues	>50 pairs
Esophageal pores	2–8 pairs	4–8 pairs	12–17th gill pore	Collar	Collar	4th gill pore	7 pairs	
Gonads start at	Collar	1 mm from collar					Mid-branchial	
Oocyte size		230 by 170 μ m						4th gill pore



Fig. 1. *Protoglossus graveolens*, new species. Photograph of individual missing posterior portion of trunk, magnified 3 \times . a: proboscis, b: proboscis groove, c: collar, d: branchial region of trunk, e: gill pores, f: mid-dorsal ridge separating rows of gill pores, g: hepatic region of trunk.

lack of a proboscis appendix, esophageal pores and circular muscles in the trunk (Fig. 3) exclude *P. graveolens* from the Spengelidae (Hyman 1959, Woodwick & Sensenbaugh 1985). The absence of both peribuccal spaces in the collar and synapticules in the branchial apparatus further differentiate *P. graveolens* from three of the Spengelid genera, *Spengelia*, *Schizocardium* and *Willeyia*. Well-developed proboscis muscles (Fig. 4), division of the buccal diverticulum in the collar by the proboscis crura (Fig. 2), large oocytes (Figs. 3 & 5), and the lack of peribuccal spaces in the collar (Fig. 2), synapticulae in the branchial apparatus, lateral septa and hepatic caeca in the trunk (Fig. 3) are fundamental characters of the family Harrimaniidae (Hyman 1959, Woodwick & Sensenbaugh 1985). Since *P. graveolens* shares all of these attributes, its placement within the family Harrimaniidae is indicated.

Protoglossus (van der Horst, 1927)

Type species—*Protoglossus koehleri* (Caullery & Mesnil, 1900).

Genus diagnosis.—Proboscis short with deep dorsal groove along posterior $\frac{1}{3}$ to $\frac{1}{2}$. Proboscis musculature non-concentric but arranged in sectors that project into proboscis coelom along radial fissures. Proboscis coelom third to half of proboscis diameter. Large pericardium replaces dorsal proboscis septum at anterior end, glomerulus caps anterior end of short straight stomochord which extends through $\frac{1}{4}$ to $\frac{1}{3}$ of proboscis. Basal sheath covers posterior half of proboscis stalk. Proboscis skeleton crura extend to posterior end of collar and embrace and divide buccal diverticulum. Collar inclined dorso-ventrally towards the posterior, well-defined operculum at posterior end of collar fused along ventral edge. Collar mesenteries complete, perihæmal spaces ex-

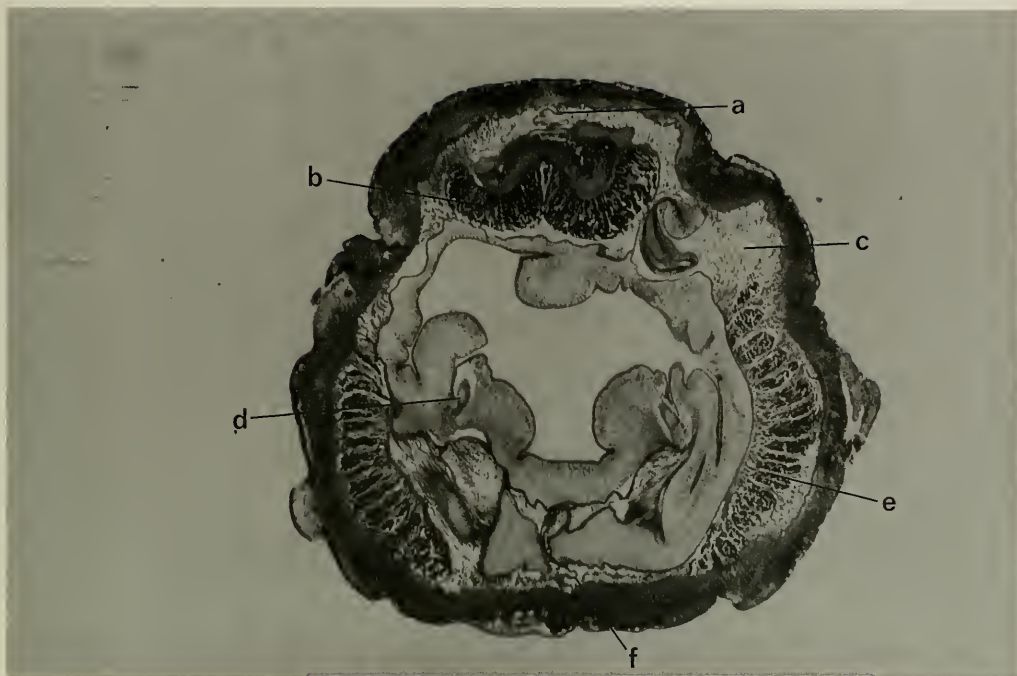


Fig. 2. *Protoglossus graveolens*, new species. Collar, transverse section through posterior end, magnified 18 \times . a: dorsal septum, b: perihæmal cavities, c: coelom, d: proboscis skeleton crura, e: muscles of the proboscis skeleton, f: ventral septum.

tend anteriorly only through $\frac{2}{3}$ of collar. No esophageal pores.

Remarks.—Within the family Harrimaniidae, *P. graveolens* is readily excluded from the genera *Stereobalanus* (Spengel, 1901), *Harrimania* Ritter, 1900, and *Xenopleura* Gilchrist, 1925. Dorsolateral placement of the gonads (Fig. 3), two parallel rows of 60–143 gill pores running along a dorsal ridge in the branchial region and a comparatively higher length to width ratio of the trunk (Fig. 1) readily exclude *P. graveolens* from *Stereobalanus*. In *S. canadensis* the gonads are situated within short dorsal and ventral folds immediately behind the collar while the gill pores, which number far fewer than in *Protoglossus*, are fused to common slits that are hidden between the genital folds (Spengel 1893, Reinhard 1942, Hyman 1959, Burdon-Jones & McIntyre 1960).

Both members of the genus *Harrimania*, *H. kupfferi* (von Willemoes-Suhm, 1871)

and *H. maculosa* Ritter, 1900, are characterized by a lower length to width ratio, two proboscis pores, radiating muscle plates and a very short bilateral coelom in the proboscis, a four-lobed branchial region, and gonads ventral and dorsal to the gill pores (Ritter 1900, Hayward & Ryland 1990). *P. graveolens* shares none of these features.

Although the description for *Xenopleura vivipara* Gilchrist, 1925 is incomplete, several key differences exclude *P. graveolens* from *Xenopleura*. *Xenopleura* is characterized by fusion of the posterior end of the collar to the branchial region, the presence of a differentiated buccal roof in the collar, medullary folds and internal hepatic caeca in the trunk, and the absence of an operculum (Gilchrist, 1925). These characters are not shared by *P. graveolens*.

Protoglossus graveolens shares external and internal morphological characteristics with both of the remaining harrimaniid genera, *Saccoglossus* Shimkewitsch, 1892 and

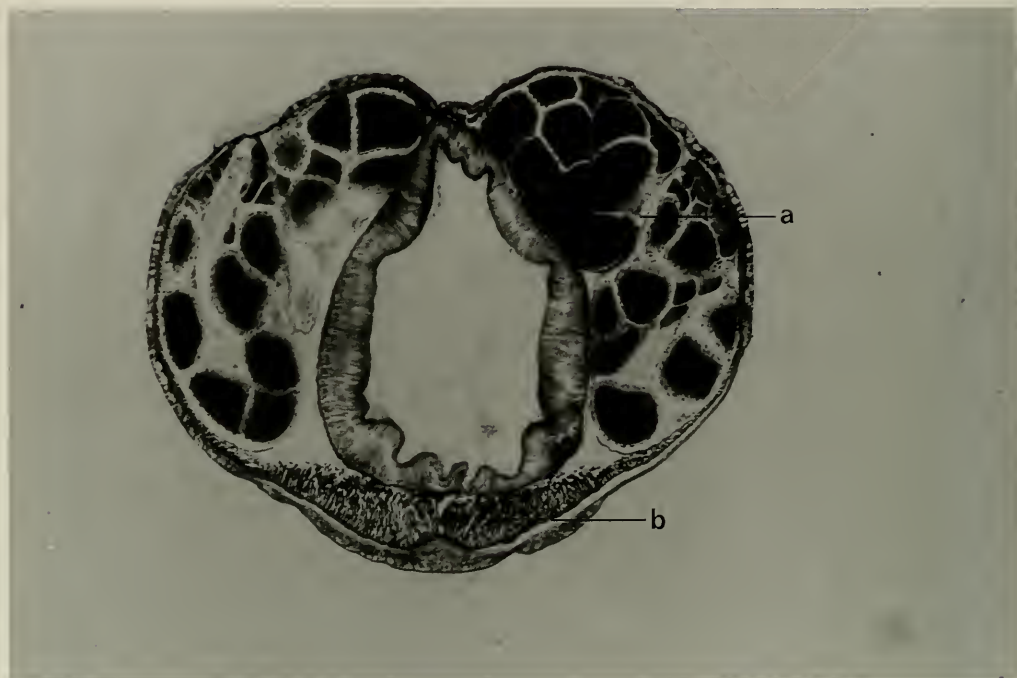


Fig. 3. *Protoglossus graveolens*, new species. Trunk hepatic region, transverse section, magnified 25 \times . a: dorsolaterally placed oocyte bundles, b: ventral longitudinal muscles.

Protoglossus (van der Horst, 1927). This is consistent with the results of a comparison of the commonly used characteristics of harrimaniid species which reveals a substantial overlap between the protoglossids and saccoglossids (Table 1) suggesting a close association between these genera. The anatomical organization of the *P. graveolens* proboscis stalk and collar (Figs. 2 & 6), and the morphology of its branchial skeleton (Fig. 7) are similar to the saccoglossids; the internal characters of the *P. graveolens* proboscis (Figs. 4 & 8), the extension of the perihæmal cavities in the collar (Fig. 2) and external features such as the basal sheath, inclination of the collar (Fig. 9) and lack of esophageal pores are more similar to *P. koehlerii*.

The deep dorsal groove of the *P. graveolens* proboscis (Figs. 1 & 9) and the thickening of the adjacent dorsal nerve layer (Fig. 8) are characters shared with several saccoglossid species (van der Horst

1930, Brambell & Cole 1939, Brambell & Goodhart 1941, Thomas 1956, Burdon-Jones & Patil 1960, Thomas 1968) as well as *P. koehlerii* (Burdon-Jones 1956) (Table 1). Other features shared between these genera include: the number and location of proboscis pores, a large pericardium (Fig. 8), the capping of the stomochord by the glomerulus (Fig. 4), the collar dimensions, the presence of a posterior collar operculum and collar groove (Fig. 9), the collar musculature and mesenteries (Fig. 2), the extension of the proboscis skeleton crurae through the collar (Fig. 2), the number of branchial gill pores, the relative proportions of tongues and septa in the branchial skeleton (Fig. 7), the starting location of gonads along the trunk and the size of oocytes (Table 1). These various characters appear unreliable as diagnostic tools both for differentiating between the two genera and within the genus *Saccoglossus*.

Burdon-Jones & Patil (1960) have pre-



Fig. 4. *Protoglossus graveolens*, new species. Proboscis, transverse section, magnified 16X. a: proboscis coelom, b: glomerulus, c: longitudinal proboscis musculature separated by radial fissures, d: anterior-most portion of stomochord.

viously questioned the taxonomic value of the posterior collar groove for saccoglossids. Inspection of Table 1 indicates that another feature commonly associated with *Saccoglossus*, the concentric arrangement of the proboscis musculature, is also an unreliable diagnostic character; five saccoglossid species lack an obvious concentric musculature. Similarly, collar mesenteries may be unreliable taxonomic indices for the genus *Protoglossus*. Complete collar mesenteries and full separation of the coelom in the collar, as observed in *P. graveolens*, have been suggested as a primitive feature characteristic of *Protoglossus* (Burdon-Jones 1956, Hyman 1959). However, complete mesenteries are also reported for *S. caraibicus* van der Horst, 1924, *S. pygmaeus* Hinrichs & Jacobi, 1938 (Brambell & Goodhart 1941) and *S. ruber* Tattersall, 1905 (Burdon-Jones & Patil 1960). Although the dorsal mesentery is absent in the anterior third of the collar in *S. horsti*, the

collar coeloms are reported as being completely separated in this saccoglossid as well (Brambell & Goodhart 1941). These similarities perhaps suggest a closer phylogenetic relationship between *Protoglossus* and *Saccoglossus*, than between either of these genera and the confamilial *Stereobalanus*.

However, in spite of the similarities between *Protoglossus* and *Saccoglossus*, these genera are clearly distinct when all characters are viewed collectively. This is especially apparent for the new species reported here. When all characters are considered, it is well-differentiated from *Saccoglossus*, but closely associated with *P. koehleri* (Table 1). Three external features are diagnostic. First, *P. graveolens* is characterized by a proboscis stalk sheath, a feature thus far reported only for *P. koehleri* (Burdon-Jones 1956). Second, lack of esophageal pores is shared with *P. koehleri*, but not the saccoglossids. With only one ex-



Fig. 5. *Protoglossus graveolens*, new species. Oocytes packaged in bundles of 13–29, as observed through body wall along hepatic region of the trunk, magnified 18 \times .

ception, all saccoglossids possess esophageal pores, the number varying between 1–12 pairs for different species. Esophageal pores are doubted only for *S. gurneyi* (Thomas 1956). Third, the dorso-ventral inclination of the collar (Fig. 9) is not reported for any saccoglossid, but is a characteristic of *P. koehleri*. Internally, the morphology of the *P. graveolens* proboscis is unlike that of saccoglossids. The organization of the longitudinal muscles in sectors along radial fissures (Fig. 4), the wide proboscis coelom (Fig. 4 & 8) and the large pericardium that replaces the dorsal proboscis mesentery anteriorly (Fig. 8) are characteristics of the new species shared with *P. koehleri*. In contrast, the longitudinal proboscis muscles of *Saccoglossus* are typically organized either in concentric circles or form a continuous mass, the coelom is usually small, and extension of the pericardium to replace the dorsal mesentery has not been reported. The morphology of periaemal cavities

within and through the collar is also diagnostic. *P. koehleri* has no anterior extensions of the periaemal cavities past the crura of the proboscis skeleton (Burdon-Jones 1956). Except for *S. caraibicus*, periaemal spaces extend into or through the proboscis stalk of all described saccoglossids. The periaemal spaces in *P. graveolens* extend past the crura but disappear $\frac{2}{3}$ of the way through the collar (Fig. 2).

Protoglossus graveolens,
new species
Figs. 1–11

Species diagnosis.—Body large, average adult length 24 cm, largest specimen 47 cm. Collar broader than long (0.2–0.35 cm long, 0.3–0.45 cm diameter). Proboscis coloration cream-white; collar cream-white to orange-brown; branchial region translucent yellow to light orange-brown; hepatic region brown; intestinal region pale yellow,

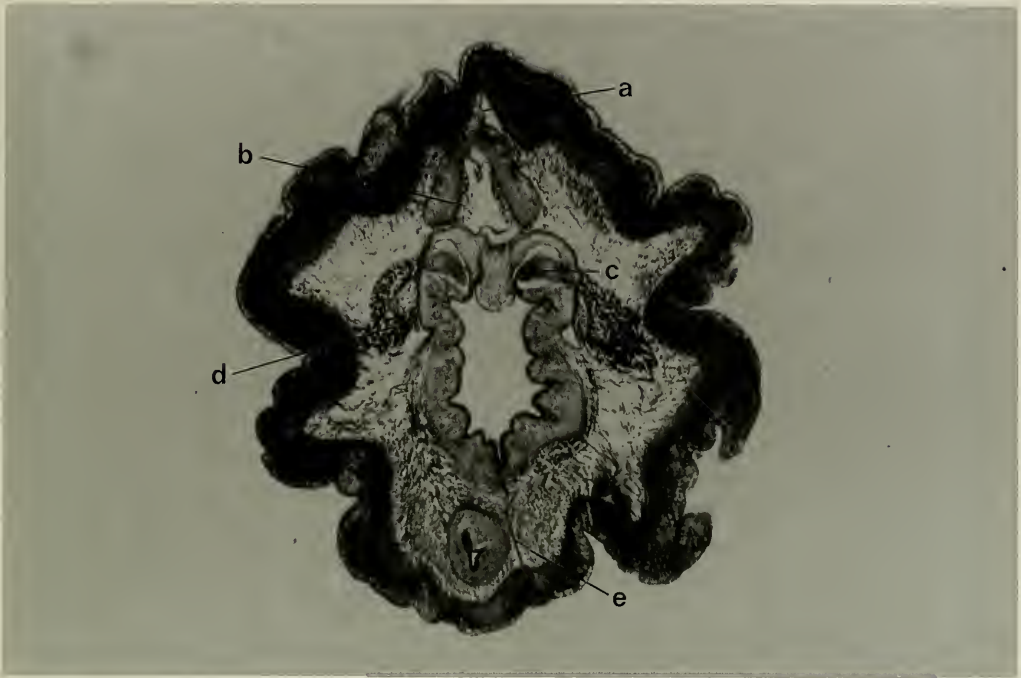


Fig. 6. *Protoglossus graveolens*, new species. Collar, transverse section mid-way through length, magnified 22X. a: dorsal septum, b: rudimentary nature of perihæmal cavities at this point along collar, c: proboscis skeleton crura, d: muscles of the proboscis skeleton, e: ventral septum.

fades posteriorly. Sixty to 142 gill pores, posterior-most 20–30 microscopic. Proboscis groove extends through posterior half of proboscis. Proboscis coelom large ($\frac{1}{3}$ to $\frac{1}{2}$ of proboscis diameter). Stomochord extends through $\frac{1}{3}$ of proboscis. Proboscis skeleton curvature approximately 90 degrees between keel and horns. Perihæmal spaces



Fig. 7. *Protoglossus graveolens*, new species. Branchial skeleton, magnified 46X.

extend through $\frac{2}{3}$ of collar, beyond posterior limit of proboscis skeleton crura. Collar coeloms occupied by muscles and interstitial tissue. Branchial skeleton made up of elongate septa and tongues, septa longer than tongues.

Remarks.—*Protoglossus graveolens* is easily differentiated externally from *P. koehleri* by its size and coloration. The average total length for *P. graveolens* is 24 cm while the largest specimen recorded for *P. koehleri* was only 7.5 cm (Burdon-Jones 1956). The number of gill pores is also proportionately greater in *P. graveolens*, ranging between 60–142 depending on size, while the number reported for the latter species varies between 14–30 (Burdon-Jones 1956). The coloration also differs dramatically between the two species. In *P. graveolens*, the proboscis is cream-white, the collar cream-white to orange brown, the branchial region translucent yellow to light orange brown and the intestinal region pale

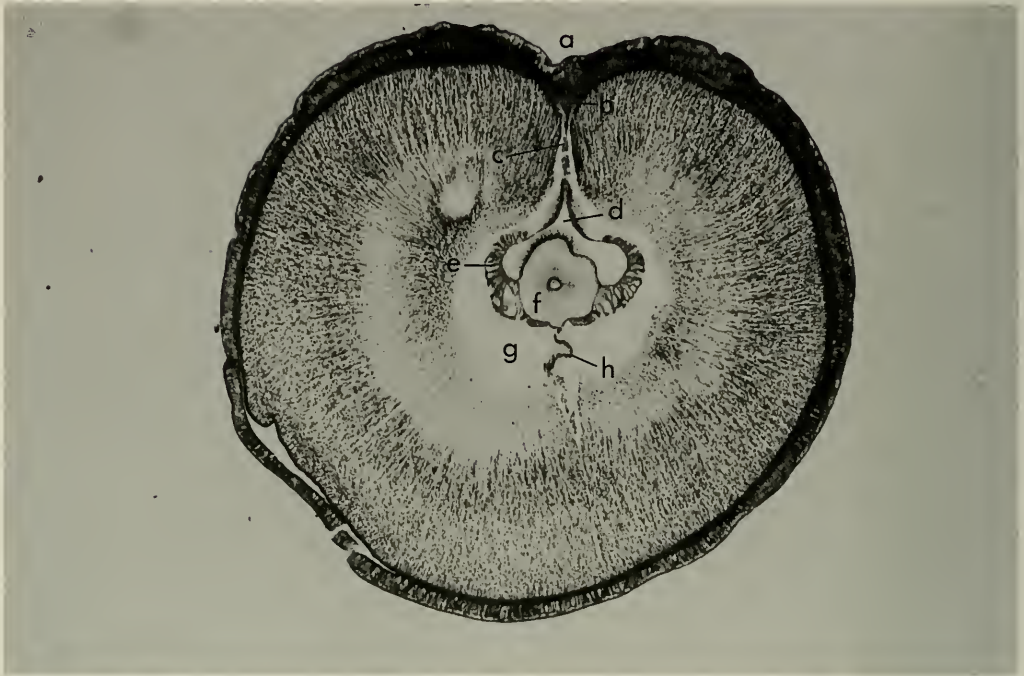


Fig. 8. *Protoglossus graveolens*, new species. Proboscis, posterior end, transverse section through proboscis complex, magnified 16 \times . a: dorsal groove, b: dorsal nerve layer thickening, c: dorsal septum, d: pericardium, e: glomerulus, f: stomochord, g: proboscis coelom, h: ventral septum.

yellow. In *P. koehleri* the coloration is golden yellow or honey for the proboscis and collar, with additional dark green spots that are lacking in *P. graveolens*, pale bluish green or translucent fawn for the branchial region, and amber fading to grey or cream posteriorly for the intestinal region (Burdon-Jones 1956).

The internal organization of the collar and the morphology of the proboscis and branchial skeleton also distinguish *P. graveolens* from its congener. Perihaemal cavities in *P. graveolens* extend more than halfway into the collar (Figs. 2 & 6) while in *P. koehleri* they reach only the posterior tips of the proboscis skeleton crura. The collar coelom, which is described as very distinct in *P. koehleri* (Burdon-Jones 1956), is filled with muscle and connective tissue in *P. graveolens* (Fig. 2). The proboscis skeleton of *P. graveolens* shows a greater curvature in the crura, reaching nearly 90 degrees. The branchial skeleton of *P. ko-*

ehleri is unusual among the harrimaniids because the tongue bars are short or non-existent (Burdon-Jones 1956, Hyman 1959). On the other hand, the branchial skeleton of *P. graveolens* has well-developed tongue bars, much like those of the saccoglossids (Fig. 7).

Etymology.—The species name *graveolens* (L. 'strong smelling, noisome') describes the characteristic strong bromoform odor that emanates from the animal, associated sediment and any other surface with which it has come in contact.

Type material.—Holotype, female carrying eggs, total length 22.5 cm (USNM 173686); paratypes, 1 male, total length 20 cm (USNM 173687), 3 females, total length 23–47 cm (USNM 173688–90), 1 juvenile, total length 2.9 cm (USNM 173691) from Lowes Cove, Maine (43°56'N, 69°34'W), collected by C. Giray, 14 July 1994.

Description.—Holotype: female with eggs, total length 22.5 cm (15 cm or greater

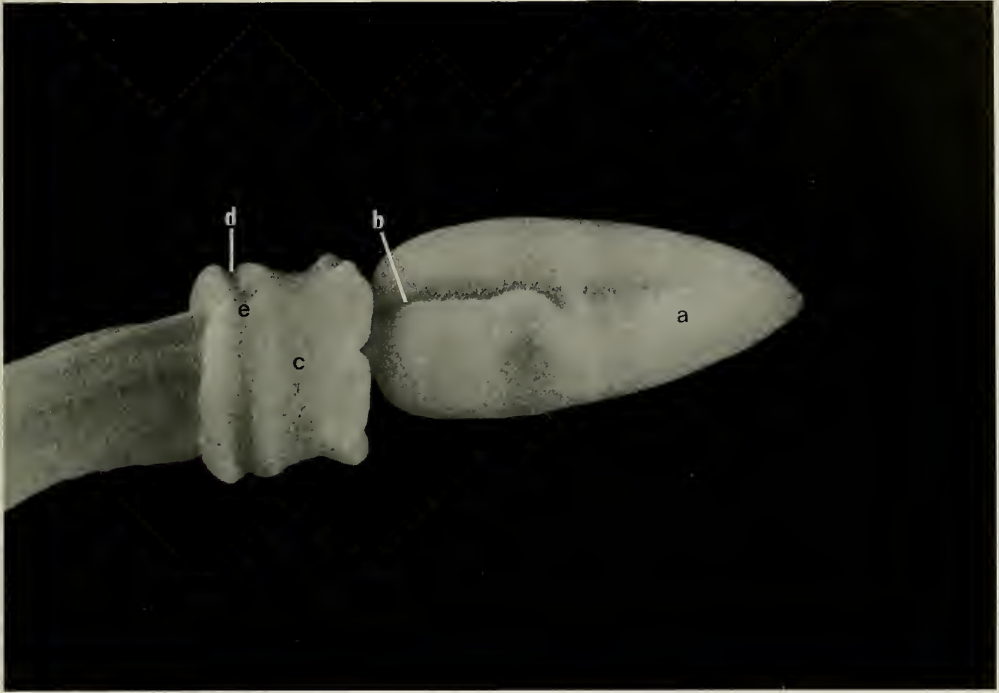


Fig. 9. *Protoglossus graveolens*, new species. Photograph of anterior end, close-up view, magnified 9.5 \times . a: proboscis, b: proboscis groove, c: collar, d: collar groove, e: collar operculum.

portion of trunk intestinal region lost during collection as deduced from remains recovered through further excavation).

Proboscis short and broad, tapered toward anterior tip, cream-white, 0.75 cm long, 0.45 cm wide at base. Deep, distinctive, dorsal groove extends from base of proboscis to anterior end, becoming shallower towards tip (Figs. 1 & 9); only noticeable as basal cleft post-fixation. Basal sheath covers posterior half of proboscis stalk. Single proboscis pore located just anterior of basal sheath dorsolaterally on left side of proboscis stalk. Proboscis skeleton slender, keel and horns of equal length, stalk wing not present. End plate, visible through proboscis stalk, fans out laterally, forming skewed pair of funnels. Curvature between keel and horns approximately 90 degrees, concretions not observed.

Collar 0.3 cm long by 0.45 cm in diameter. Anterior-most region of collar projects to cover most of proboscis stalk while pos-

terior-most region, or operculum, covers first pair of gill pores (Fig. 9).

Trunk consists of three easily distinguished regions. Branchial region (Fig. 1), anterior-most and housing gill pores, 2.8 cm long, 0.35 cm diameter; greatest diameter coincides with gill pore 6 or 7; translucent yellow; surface texture smooth; pronounced mid-dorsal ridge that becomes shallower toward posterior branchial region. Gill pores, dorsal, 100 pairs, arranged in two rows parallel to mid-dorsal ridge. Anterior gill pores slit-like (200 μm maximum width); about 25–30 pair of posterior-most pores rounded and microscopic (10–20 μm maximum width, presumably gill pores in early phases of development), occupy last 0.2–0.3 cm of branchial region (Fig. 10). Hepatic, second trunk region, easily distinguished by wrinkled surface texture (Fig. 1). Occurs immediately posterior to branchial region, 13 cm long, diameter decreases from 0.35 cm at anterior end to 0.2 cm at posterior end.

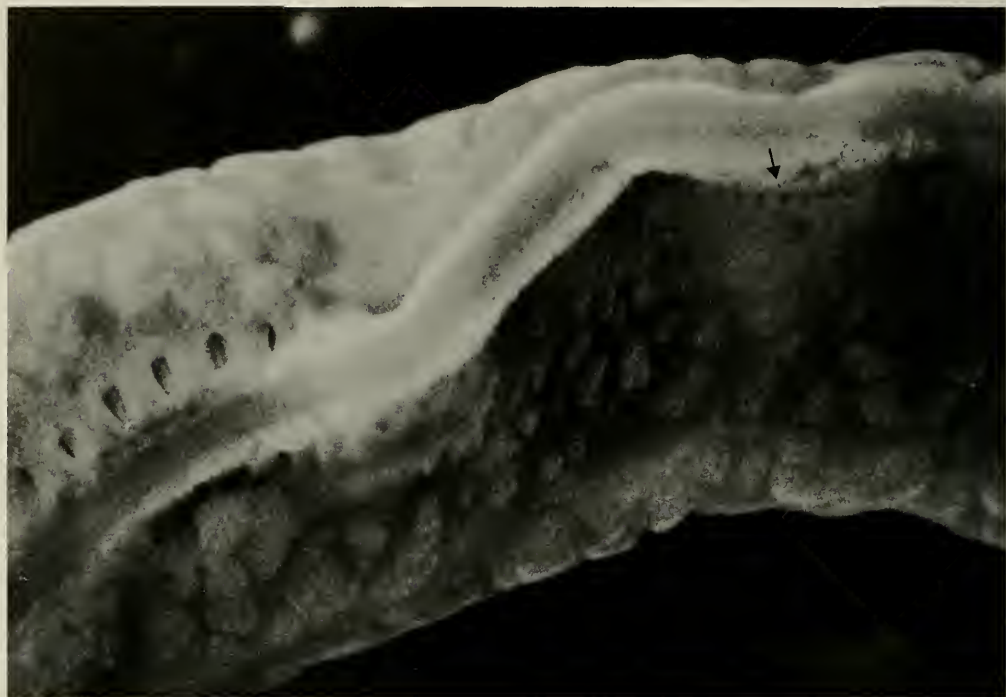


Fig. 10. *Protoglossus graveolens*, new species. Trunk branchial region, posterior end, magnified 30 \times to show the transition from the comparatively larger gill pores which cover most of the branchial to the posterior 25–30 smaller pores (arrow); dorsal groove also becomes significantly raised in this region.

Anterior 3 cm of hepatic region yellow, remaining length brown. Hepatic sacculations absent. Transition into third region, intestinal, marked by change in trunk coloration from brown to pale yellow. Intestinal region extends remaining length of animal, coloration fading rapidly towards posterior end. Total length of intestinal region uncertain but at least 20 cm, only 5.5 cm recovered intact. Diameter, 0.2 cm throughout most length, tapers off to 0.1 cm towards posterior end.

Gonads, dorsolateral, visible through body wall (Figs. 3 & 5), and occur in first two trunk regions; begin 1.8 cm behind collar, extend through branchial region and overlap proximal 6 cm of hepatic region; total extent along trunk, 7 cm. Body wall of gonadal region inflated to 0.35–0.4 cm diameter. Oocytes, pink, 320–380 μm diameter, in clusters of 13–29 (Fig. 5).

Variations.—Four adult paratypes, one

male, three females; share similar features with holotype but greater in length by 1–1.5 cm in branchial region and 7–15 cm in total trunk; total length of most complete specimen, a female, 47 cm; longest intact intestinal region collected, 21 cm. Gill pores, 125–143 pairs, number increasing proportionately with size of individual. Proboscis, collar and branchial region of two paratypes exhibit enhanced orange shade in coloration. Male gonads, creamy white; sperm carried in irregularly shaped packets (approx. 800 μm in diameter), packets usually shed if ripe individual is disturbed. The sperm morphology of *P. graveolens* (Fig. 11) resembles that described for several saccoglossid species (Burdon-Jones 1952, Hyman 1959, Franzen et al. 1985). The head of the spermatozoa measure 3.2 μm in length and 2.2 μm in diameter at the widest part. The acrosome, nucleus and mid-piece measure 1.1, 1.0 and 1.1 μm , respectively,

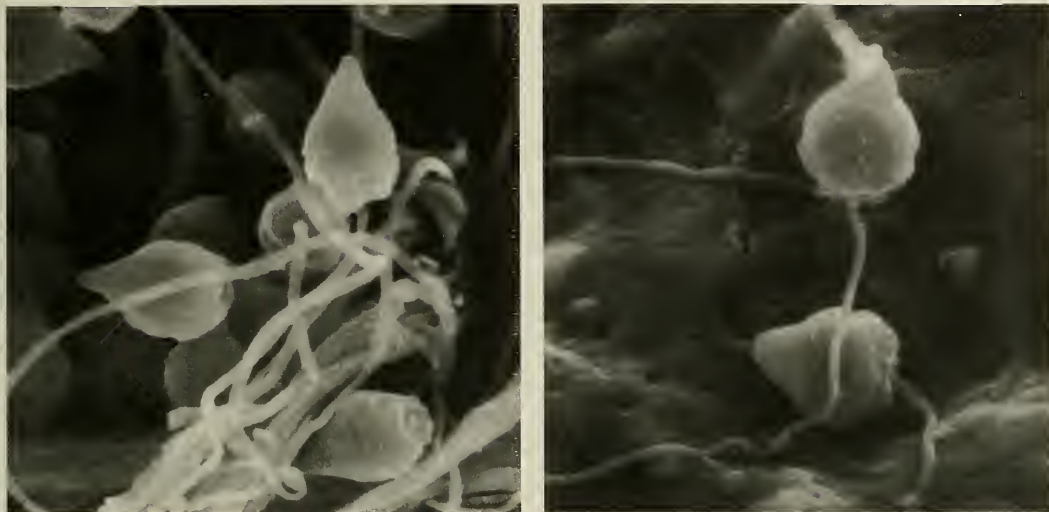


Fig. 11. Spermatozoa (SEM), magnified 10,000 \times . Left: *Protoglossus graveolens*, new species; total length of acrosome, nucleus and middle-piece, 3.2 μm ; maximum width, 2.2 μm ; length of tail 55.5 μm . Right: *Saccoglossus bromophenolus*; total length of acrosome, nucleus and middle-piece, 3.2 μm ; maximum width, 2.5 μm ; length of tail, 56 μm .

while the tail has a length of 55.5 μm (measurements are average values from 12 spermatozoa). In contrast to spermatozoa of *S. bromophenolus* (Fig. 11), the head of *P. graveolens* spermatozoa is narrower and pointed at the tip of the acrosome (Fig. 11). Female gonad coloration varies from pink to olive-gray depending on stage of development. Extent of gonads along trunk also varies with development; in fully mature individuals gonads occur up to 14 cm total length along trunk. Although specimens with developing gonads were collected as early as January, spawning was not observed until late August-early September. Fifth paratype, juvenile; total length 2.9 cm; proboscis 0.35 \times 0.2 cm, collar 0.15 \times 0.2 cm length and diameter, respectively; branchial region, 1 cm long; gill pores, 63 pairs; posterior trunk length, 1.4 cm; coloration, cream-white throughout.

Haloorganic content.—2,4-dibromophenol (DBP) was detected in hexane extracts of *P. graveolens*, with average concentrations from 7 $\mu\text{mol/g}$ fresh weight in the post-branchial trunk to 15 $\mu\text{mol/g}$ fresh weight in the proboscis and 20 $\mu\text{mol/g}$ fresh

weight in the collar and branchial region. A second compound, 4-bromophenol (BP), was also detected but at much smaller levels and mainly in the posterior trunk; average concentration 0.08 $\mu\text{mol/g}$ fresh weight. DBP occurs in *S. bromophenolus*, but differs considerably in concentration and distribution from that in *P. graveolens*. In the latter, the highest DBP concentrations occur in the collar, surpassing the corresponding concentration in *S. bromophenolus* by nearly two orders of magnitude. In *S. bromophenolus*, the highest DBP concentrations occur in the proboscis, at about double the concentrations detected for *P. graveolens*. DBP concentrations are 5–6 times greater in the branchial region of *P. graveolens* than in *S. bromophenolus* while nearly equal levels occur in the posterior trunk regions of the two species. Bromoindoles, which occur in several other hemichordates (Higa et al. 1980), including *S. bromophenolus* (King et al. 1994), were not detected in *P. graveolens*. Minor amounts of BP were detected in hexane extracts of both *P. graveolens* and *S. bromo-*

phenolosus with somewhat higher concentrations in the former species.

Distribution.—*Protoglossus graveolens* occurs intertidally, spanning from 15 to over 40 cm depth through the sediment. Its vertical position varies with sediment particle size and water content, extending to at least 50 cm in softer muds. *P. graveolens* is unusual among the harrimanids in its ability to burrow deep. *S. bromophenolosus* and *S. canadensis* in comparable substrate are found at less than 20 cm maximum sediment depth. *Protoglossus graveolens* was also collected from a shallow subtidal area. Populations of *P. graveolens* are distributed patchily within individual mudflats; densities in the Lowes and Clarks Cove sites range from 1–9 individuals m⁻². *Saccoglossus bromophenolosus* co-occurs with *P. graveolens* at all sampling sites, usually at greater densities. Burrows of *P. graveolens* are always deeper but not as branched as those of *S. bromophenolosus*. In addition, the burrows of *P. graveolens* lack the thick red iron oxyhydroxide coating typical of those of *S. bromophenolosus*. *P. graveolens* has been collected from only three adjacent sites in the Damariscotta River estuary. Therefore, its range along the eastern North American coastline is uncertain.

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Literature Cited

- Agassiz, A. 1873. The history of balanoglossus and tornaria.—Memoirs of the American Academy of Arts and Science 9:421–429.
- Benito, J. 1982. Hemichordata. Pp. 819–821 in S. P. Parker, ed., Synopsis and classification of living organisms, McGraw-Hill Inc., 1232 pp.
- Brambell, F. W. R., & H. A. Cole. 1939. *Saccoglossus cambrensis*, sp. n., an enteropneust occurring in Wales.—Proceedings of the Zoological Society of London B109:211–236.
- , & C. B. Goodhart. 1941. *Saccoglossus horsti* sp. n., an enteropneust occurring in the Solent.—Journal of the Marine Biological Association of the United Kingdom 25:283–301.
- Bromley, J. E. C. 1979. A preliminary checklist of marine fauna of Minas Basin and Minas Channel.—Proceedings of the Nova Scotia Institute of Science 29:517–541.
- Bullock, T. H. 1975. Phylum Hemichordata. Pp. 618–619 in R. I. Smith & J. T. Carlton, eds., Light's manual: intertidal invertebrates of the Central California Coast, University of California Press, Berkeley, 716 pp.
- Burdon-Jones, C. 1951. Observations on the spawning behavior of *Saccoglossus horsti* Brambell and Goodhart, and of other Enteropneusta.—Journal of the Marine Biological Association of the United Kingdom 29:625–638.
- . 1952. Development and biology of the larva of *Saccoglossus horsti*.—Philosophical Transactions of the Royal Society of London B236: 553–590.
- . 1956. Observations on the enteropneust, *Protoglossus koehleri* (Caullery & Mesnil).—Proceedings of the Zoological Society of London 127:35–58.
- , & A. D. McIntyre. 1960. *Stereobalanus*, a genus new to the old world.—Nature 186:491–492.
- , & A. M. Patil. 1960. A revision of the genus *Saccoglossus* (Enteropneusta) in British waters.—Proceedings of the Zoological Society of London 134:635–645.
- Caullery, M., & F. Mesnil. 1900. Sur une nouvelle espece de *Balanoglossus* (*B. koehleri*) habitant les cotes de la Manche. Pp. 256–259 in G. Masson, ed., Comptes Rendus Hebdomadaires des seances et memoires de la Societe de Biologie, Vol. 52.
- , & ———. 1904. Contribution a l'etude des Enteropneustes. *Protobalanus* (n. g.) *koehleri* Caull. et Mesn.—Zoologische Jahrbücher 20: 227–256.
- Colwin, A. L., & L. H. Colwin. 1953. The normal embryology of *Saccoglossus kowalevskii*.—Journal of Morphology 92:401–432.

- Colwin, L. H., & A. L. Colwin. 1962. Induction of spawning in *Saccoglossus kowalevskii* (Enteropneusta) at Woods Hole.—*Biological Bulletin* 123:493.
- Elder, H. Y. 1973. Distribution and functions of elastic fibers in the invertebrates.—*Biological Bulletin* 144:43–63.
- Fox, R. S., & E. E. Ruppert. 1985. Shallow-water marine benthic macroinvertebrates of South Carolina, University of South Carolina Press, Columbia, S.C., 329 pp.
- Franzen, A., K. H. Woodwick, & T. Sensenbaugh. 1985. Spermiogenesis and ultrastructure of spermatozoa in *Saxipendium coronatum* (Hemichordata, Enteropneusta), with consideration of their relation to reproduction and dispersal.—*Zoomorphology* 105:302–307.
- Gilchrist, J. D. F. 1925. *Xenopleura vivipara*, g. et sp. n. (Enteropneusta).—*Quarterly Journal of Microscopical Science* 69:555–570.
- Hadfield, M. G. 1975. Hemichordata. Pp. 185–240 in A. C. Giese & J. S. Pearse, eds., *Reproduction of marine invertebrates*, Academic Press, New York, 712 pp.
- Hayward, P. J., & J. S. Ryland. 1990. Hemichordata and Urochordata. Pp. 872–876 in *The marine fauna of the British Isles and North-West Europe*, Vol. 2 Mollusks to Chordates. Clarendon Press, Oxford., 388 pp.
- Higa, T., T. Fujiyama, & P. J. Scheuer. 1980. Halogenated phenol and indole constituents of acorn worms.—*Comparative Biochemistry and Physiology* 65B:525–530.
- Humason, G. L. 1979. *Animal Tissue Techniques*. W. H. Freeman & Company, San Francisco, 641 pp.
- Hyman, L. H. 1959. The enterocoelous coelomates—Phylum Hemichordata. Pp. 72–207 in *The Invertebrates: smaller coelomate groups* (Chaetognatha, Hemichordata, Pogonophora, Phoronida, Ectoprocta, Brachiopoda, Sipunculida, the coelomate Bilateria), Vol. 5. McGraw-Hill Book Co. Inc., New York, 783 pp.
- Jaffe, L. A. 1983. Fertilization potentials from eggs of the marine worms *Chaetopterus* and *Saccoglossus*. Pp. 211–218 in *The physiology of excitable cells*. Alan R. Liss Inc., New York, New York, 508 pp.
- King, G. M. 1986. Inhibition of microbial activity in marine sediments by a bromophenol from a hemichordate.—*Nature* 323:257–259.
- . 1988. Dehalogenation in marine sediments containing natural sources of halophenols.—*Applied and Environmental Microbiology* 54:3079–3085.
- , C. Giray, & I. Kornfield. 1994. A new hemichordate, *Saccoglossus bromophenolosus* (Hemichordata:Enteropneusta:Harrimaniidae), from North America.—*Proceedings of the Biological Society of Washington* 107:383–390.
- Kirk, H. 1938. Breeding habits and early development of *Dolichoglossus otagoensis*.—*Transactions of the Proceedings of the Royal Society of New Zealand* 68:49–50.
- Kozloff, E. N. 1987. *Marine invertebrates of the Pacific Northwest*. University of Washington Press, Seattle, Washington, 511 pp.
- Prefontaine, G., & P. Brunel. 1962. Liste d'invertébrés marins recueillis dans l'estuaire du Saint-Laurent de 1929 a 1934.—*Le Naturaliste Canadien* 54:239–263.
- Reinhard, E. G. 1942. *Stereobalanus canadensis* (Spengel), a little-known enteropneustan from the coast of Maine.—*Journal of the Washington Academy of Sciences* 32:309–311.
- Ritter, W. E. 1900. Papers from the Harriman Alaska expedition. II. *Harrimania maculosa*, a new genus and species of Enteropneusta from Alaska, with special regard to the character of its notochord.—*Proceedings of the Washington Academy of Sciences* 2:111–132.
- Romero-Wetzel, M. B. 1989. Branched burrow-systems of the enteropneust *Stereobalanus canadensis* (Spengel) in deep-sea sediments of the Voring-Plateau, Norwegian Sea.—*Sarsia* 74:85–89.
- Spengel, J. W. 1893. Die enteropneusten des Golfes von Neapel.—*Fauna und Flora des Golfes von Neapel*, monograph 18. Pp. 297–308, plate 17.
- . 1901. Die benennung der enteropneustengattungen.—*Zoologische Jahrbücher, Abt. Syst.* 15:209–218.
- Thomas, I. M. 1956. *Saccoglossus apantesis*, a new species of enteropneust from South Australia.—*Transactions of the Royal Society of South Australia* 79:167–176.
- . 1968. Two species of *Saccoglossus* (Enteropneusta) from South Australia.—*Transactions of the Royal Society of South Australia* 92:73–84.
- van der Horst, C. J. 1930. Observations on some Enteropneusta. (Papers from Dr Th. Mortensen's Pacific Expedition, 1914–16).—*Videnskabelige Meddelelsen Fra Dansk naturhistorisk Forening Kjøbenhavn* 87:135–200.
- . 1932. Enteropneusta. Vol. 3 Part 2 in W. Kuenthal & T. Krumbach, eds., *Handbuch der Zoologie*.
- Woodin, S. A., M. D. Walla, & D. E. Lincoln. 1987. Occurrence of brominated compounds in soft-bottom benthic organisms.—*Journal of Experimental Marine Biology and Ecology* 107:209–217.
- Woodwick, K. H., & T. Sensenbaugh. 1985. *Saxipendium coronatum*, new genus, new species (hemichordata: Enteropneusta): the unusual spaghetti worms of the Galapagos rift hydrothermal vents.—*Proceedings of the Biological Society of Washington* 98:351–365.