

# Two ascidicolous copepods, *Haplostomides otagoensis* n. sp. and *Botryllophilus* cf. *banyulensis* Brément, living in compound ascidians from Otago Harbor, New Zealand

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# Abstract

This paper includes a description of *Haplostomides otagoensis* n. sp. and a new record from New Zealand of *Botryllophilus* cf. *banyulensis* Brément. These copepods, belonging to the family Ascidicolidae (according to Illg & Dudley, 1980), live in the ascidians *Aplidium thomsoni* and *A. benhami*, respectively. The female of the new species of *Haplostomides* is easily distinguished from seven congeners by these characters: labrum with a median cleft; mandible hook-shaped, with 3 setae; maxilla forming a rounded protrusion, with 2 setae, from a broad basal portion that has 1 seta; caudal ramus with 2 unequal marginal setae and 2 unequal terminal spines; copulatory pore within an oblong area surrounded by a sclerotized ridge. A single female of *B. cf. banyulensis* was found during examination of many colonies of its ascidian host.

# Introduction

The coastal rocks at the Portobello Peninsula Point (on the Otago Peninsula in Otago Harbor), where the Marine Laboratory of the University of Otago is located, support a particularly rich ascidian fauna (Brewin, 1964). During my stay at the Marine Laboratory in 1996, it was possible to examine many kinds of colonial ascidians, such as *Aplidium*, *Didemnum* and *Distaplia*, from this area. Among them, however, only two species of *Aplidum* have been recognized as hosts for copepods; one for the new species of *Haplostomides*, the other for *Botryllophilus* cf. *banyulensis* Brément.

Seven species (females) of the genus *Haplostomides* have been described from various coastal waters of the Northern Hemisphere: *H. hibernicus* (T. & A. Scott, 1895), from the eastern North Atlantic Ocean (Valentia Island, Ireland); *H. scotti* Chatton & Harant, 1924, from the English Channel (Pempoul, France); *H. brementi* Chatton & Harant, 1924, from the Mediterranean Sea (Argelès-sur-Mer, France); *H. amarouci* (Blake, 1929), from the western North Atlantic Ocean (Mount Desert region, U.S.A.); *H. bellus* Ooishi & Illg, 1977, and *H. luteolus* Ooishi & Illg, 1977, from the eastern North Pacific Ocean (Whiffin Spit, Vancouver Island, Canada); and *H. hawaiiensis* Ooishi, 1994, from the central North Pacific Ocean (Honolulu, U.S.A.).

Four of these copepods have also been found in other localities of the Northern Hemisphere: (1) *H. hibernicus*, from the English Channel (Roscoff, France; Chatton & Brément, 1910) and the eastern North Atlantic Ocean (Strangford Lough, Northern Ireland; Gotto, 1952); (2) *H. scotti*, from Strangford Lough (Gotto, 1952); (3) *H. amarouci*, from the western North Atlantic Ocean (Woods Hole region, U.S.A.; Dudley & Illg, 1991); and (4) *H. luteolus*, from the western North Pacific Ocean (Akkeshi Bay and Zaga Island, Japan; Ooishi, 1991). The new species of *Haplostomides* from New Zealand is the first species belonging to this genus from the South Pacific Ocean, as well as from the Southern Hemisphere.

The specimen of *B*. cf. *banyulensis* reported from New Zealand closely resembles *B*. *banyulensis* as described by Brément (1909) from the Mediterranean Sea (Port Vandre). A difference from the Mediterranean species will be discussed in this paper.

There are several records of the genus *Botryllophilus* from the Southern Hemisphere: *B. africanus* Schellenberg, 1922, from the South Atlantic Ocean (Angola); *Botryllophilus* sp. Brément, 1909, from the South Pacific Ocean (Strait of Magellan, Chile); *Botryllophilus* sp. Schellenberg, 1922, from the Indian Ocean (Plettenberg, South Africa). Illg & Dudley (1980), however, designated them as indeterminable species of the genus. Therefore, *B.* cf. *banyulensis* from New Zealand is the first record available for comparison with 14 recognized species of this genus described from the Northern Hemisphere.

## Materials and methods

Sampling of copepods (females) from the ascidian hosts was undertaken at Portobello Marine Laboratory (45° 50' S, 170° 46' E), University of Otago, New Zealand, during January 1996. Rounded, light orange colonies (usually 2–3 cm in diameter) of *Aplidium thomsoni*, the host of the new species of *Haplostom-ides*, were collected on the internodes of the alga *Homosira banksii* (Turn), just below high water level, at Portobello Peninsula Point. Brilliant orange, irregularly shaped colonies of *Aplidium benhami*, the host of *Botryllophilus* cf. *banyulensis*, were obtained on the undersurface of rocks at a lower water level at the same locality. Only a single specimen of *Botryllophilus* was collected during the examination of the ascidian colonies (more than 1 kg wet weight).

Macrophotographs were taken of living specimens of the new species of *Haplostomides*. Copepods fixed in 95% ethanol and stored in 70% ethanol were used for scanning electron micrographs (SEM) and all other studies. Specimens were measured, dissected and drawn with the aid of a camera lucida while in lactic acid. Photomicrographs were also based on specimens in lactic acid. In the formula for the armature of exopods of legs 1–4 (P<sub>1–4</sub>), the total number of spines (Roman numerals) is noted first and connected by a dash with the number of setae (Arabic numerals). The total number (T) of these elements in each leg is indicated within parentheses.

The specimen of *Botryllophilus* from New Zealand has been examined by making macrophotographs and drawings in the same way. In this paper, however, only the general aspect of body and certain legs of



Figure 1. Haplostomides otagoensis, n. sp., female, macrophotograph of living specimen, dorsal.

the specimen (preserved) are noted, and no figures or photographs are used.

#### Results

*Haplostomides otagoensis* n. sp. (Subfamily Haplostomatinae Chatton & Harant, 1924)

*Type material*: Holotype  $\varphi$  (MNZ-CR.9553), collected from *Aplidium thomsoni* Brewin, 20 January 1996, Portobello Peninsula Point, Otago Harbor, New Zealand, and paratypes 5  $\varphi \varphi$  (MNZ-CR.9554), collected from the same host and locality, 21 January 1996, deposited in the Museum of New Zealand, Wellington, New Zealand.

*Female*: In living specimens, body (Fig. 1) vermiform, white, opaque; eye small and reddish orange, located internally near anterior margin of cephalosome; gut large and orange or yellowish orange; eggs in body and embryos in egg sacs lavender or mauve. Egg sacs cylindrical, usually longer than body, and eggs (embryos) arranged multiserially.

Body (Fig. 2a–c) 1.56 mm long in preserved specimen. Range of length, based on 7 specimens, from 1.5 to 1.75 mm. Proportional lengths 1:5.4:1 for cephalo-



*Figure 2. Haplostomides otagoensis*, n. sp., female. (a) body form, dorsal; (b) same specimen, left, lateral; (c) same specimen, ventral; (d) cephalosome, ventral; (e) cephalosome, left, lateral. A1 = antennule, A2 = antenna, MD = mandible, MX1 = maxillule, MX2 = maxilla, MXP = maxilliped, S = large mammiform sensillum.

some, metasome, and urosome. Integument (Figs 2d and 5a,b) of body and appendages ornamented with large and small sensilla; pores also present. Large sensillum (S) distinctive, consisting of stout setule-like element protruded from middle of somewhat concave apex of large mammiform projection. Arrangement of large sensilla apparently bilaterally symmetrical (Figs 2a, d and 3a).

Cephalosome (Figs 2a, d, e and 5a) wider than long, with appendages through maxillipeds. Dorsal cephalic sclerite (Fig. 3a) subtriangular, wider than long, including unsclerotized portion posterocentrally. Rostrum (Figs 2d and 3b) subtriangular, approximately as long as wide; lateral and posterior margins sclerotized. Sclerotized lateral margin on each side widened toward apical margin of cephalosome and ornamented with transverse row of 7 spinules (8 or 9, in some specimens) anteriorly. Unsclerotized central portion of rostrum with more than 5 small sensilla.

Metasome (Fig. 2a–c) cylindrical, consisting of 5 segments. First to third segments distinctly divided by indentations, but fourth and fifth segments fused and forming fourth metasomal section without indentation. However, boundary between last 2 segments recognizable by small sclerite middorsally and also midventrally. First segment slightly shorter than those of second to fourth segments, and shortest fifth segment about one-third as long as fourth segment. Greatest width 0.43 mm in third segment. Posterolateral corners of short fifth segment (or fourth metasomal section) protruded into small conical protrusions, with setae representing fifth legs.

Urosome (Fig. 2a–c) gradually tapered posteriorly and divided into 4 segments; first one corresponding to genital double somite containing genitalia, this followed by 2 abdominal segments and anal segment with caudal rami terminally. First 3 segments also recognized by arrangement of transverse muscle strands (Fig. 4e).

Antennule (Figs 2d and 3c) lobate, unsegmented. Proximal four-fifths massive and gradually tapered. Remaining distal portion distinctly narrowed; its apical two-thirds narrowed further. Armature consisting of 18 simple setae; 5 large stout setae on massive portion and 13 setae (5 small stout, 7 slender, 1 short curved) on narrowed portion. Anterior surface with many small and few large sensilla.

Antenna (Figs 2d and 3d) consisting of 3 segments, with proportional lengths 1:2:2 from first to third segments. Narrowed third segment with 4 graduated conical spines; 3 (midway, almost subterminal, subterminal) on medial margin, and 1 terminal. Smallest proximal spine about half as large as largest terminal spine.

Labrum (Figs 3e and 6a) with median cleft on distal margin, without armature.

Mandible (Figs 2d and 3f) unsegmented, hookshaped, but 3 portions recognizable by sclerotizations or slight constrictions between portions. Proportional lengths 3:2:1 for proximal, middle and distal portions. Proximal portion more than twice as wide as distal portion. Proximal and middle portions directed medially but distal portion directed posteriorly. Armature on distal portion consisting of 2 small subterminal setae on anterior surface and 1 large stout seta at apex. All setae simple.

Maxillule (Figs 2d and 3g) consisting of unsegmented structure, but distal half divided into smaller inner lobe and larger outer lobe. Inner lobe with 4 simple stout setae along rounded apical margin. Outer lobe with 4 similar setae along lateral margin and 1 seta apically.

Maxilla (Figs 2d and 3h, i) unsegmented, forming rounded distal protrusion from transversely broad basal portion. Mediodistal margin of rounded distal protrusion with 2 simple setae (longer anterior, shorter posterior). Medial margin of basal portion with 1 much longer simple seta. All setae directed medially.

Maxilliped (Figs 2d and 3j) consisting of large protopod and endopod with curved terminal claw. Basis with 2 simple setae (anterior, posterior) on medial margin. Claw directed medially and resembling subchela.

Legs 1-4 (Figs 3k, 1 and 4a, b) alike in shape. Basis represented by oval sclerite surrounding basal portion of endopod and exopod fused; lateral seta not visible. Endopod represented by subconical protrusion from fused basal portion on basis and ornamented with large (1 or 2) and small (4-6) sensilla on anterior surface. Exopod narrow, longer than endopod, and slightly shorter than width of sclerotized basis. Lateral margin of exopod sclerotized and articulated on basis. Armature consisting of 2-4 graduated curved spines and 1 seta proximally to spines, arranged along distal one-third of lateral sclerotized margin. In legs 1-4, terminal (largest) and subterminal spines close together at base and minutely serrated; margin of segment close to these spines also serrated. Formula for armature on exopods of legs 1-4:



*Figure 3. Haplostomides otagoensis*, n. sp., female. (a) cephalosome, dorsal; (b) rostrum, ventral; (c) antennule, left, anterior; (d) antenna, left, anterior; (e) labrum, anterior; (f) mandible, left, anterior; (g) maxillule, left, anterior; (h) maxilla, left, posterior; (i) maxilla, left, upper view; (j) maxilliped, left, posterior; (k) leg 1, left, anterior; leg 2, left, anterior.



*Figure 4. Haplostomides otagoensis*, n. sp., female. (a) leg 3, left, anterior; (b) leg 4, left, anterior; (c) posterior metasome with fifth legs and urosome with genitalia and caudal rami, ventral; (d) leg 5, right, dorsal; (e) urosome, arrow indicating copulatory pore, left, lateral; (f) genital region with gonopores and sensilla, dorsal; (g) apparatus at right gonopore, arrow indicating 2 massive sclerites on internal row of armature, dorsal; (h) sclerotized ridge and copulatory organs (copulatory pore, long copulatory duct, seminal receptacle and receptacle ducts), ventral; (i) anal segment with left caudal ramus, dorsal; (j) left caudal ramus, lateral.



*Figure 5. Haplostomides otagoensis* n. sp., female, scanning electron micrographs. (a) cephalosome, ventral, scale bar =  $100 \ \mu$ m; (b) large mammiform sensillum, viewed upper surface, scale bar =  $1 \ \mu$ m. A1 = antennule, MXP = maxilliped, R = rostrum, S = large mammiform sensillum on antennule.

	Exopod	(T)
<b>P</b> <sub>1</sub>	IV–1	(5)
$P_2$	III–1	(4)
$P_3$	II–1	(3)
<b>P</b> <sub>4</sub>	II–1	(3)

Leg 5 (Fig. 4c, d) represented by 4 simple short setae on small conical protrusion; 1 slender seta proximally on dorsal side, and 3 setae (1 stout, 2 slender) around apical margin.

In genital area (Fig. 4f), dorsal cuticle between gonopores sclerotized, with 2 sensilla posteriorly. Apparatus at gonopore (Figs 4g and 6b, c) consisting of 2 rows (external, internal) of armature elements on medial margin of cuticular flap covering gonopore. External row with 2 closely spaced unequal spines (larger blunt distal spine unarticulated; smaller proximal spine articulated) and 1 small conical protrusion more proximally. Internal row with 5 elements; 3 conical spines distally and 2 blunt massive sclerites (arrows) proximally.

Midventral genital area with copulatory organs (Figs 4c, e, h and 6d). Single copulatory pore (CP) connecting with seminal receptacle (SR) through

markedly long copulatory duct (CD), and receptacle ducts (RD) diverging from both sides of seminal receptacle toward genital antra. Copulatory pore within oblong cuticle area surrounded by sclerotized ridge; many small sensilla present posterior to ridge.

Anal segment (Fig. 4c, e, i) wider than long, and ornamented with more than 10 small and 2 or 3 pairs of large sensilla.

Caudal ramus (Fig. 4i, j) conical, sclerotized. Armature consisting of 2 unequal simple setae (midway on lateral margin, subterminal on mediodorsal margin) and 2 unequal terminal spines (1 ventral, 1 dorsal). Lateral seta twice as long as subterminal seta. Ventroterminal spine conical and one-third as long as dorsoterminal spine, this serrated at truncated apical margin.

### Male: Unknown.

*Etymology*: The species is named after Otago Harbor where the new *Haplostomides* species, the first to be described from New Zealand, was found.

*Botryllophilus* cf. *banyulensis* Brément (Subfamily Botryllophilinae Sars, 1921)



*Figure 6. Haplostomides otagoensis* n. sp., female, photomicrographs (scale of each organ referred to that of corresponding drawing). (a) labrum, anterior; (b) apparatus at right gonopore, showing 1 long blunt distal spine and 1 short conical proximal spine on external row of armature, dorsal; (c) same specimen, arrows indicating 2 massive proximal sclerites on internal row of armature, dorsal; (d) copulatory organs, showing internal relationship between organs (integument with sclerotized ridge turned inside out toward posterior urosome), ventral. CP = copulatory pore, CD = copulatory duct, RD = receptacle duct, SR = seminal receptacle.

*Material examined:* Single specimen, living in *Aplidium benhami* Brewin, collected on 20 January 1996, from Portobello Peninsula Point, Otago Harbor, New Zealand; the dissected specimen deposited in the author's collections.

*Female*: Body (excluding caudal spines) 1.48 mm long in preserved specimen, and divided into cephalosome, metasome, and urosome. Ratio of length of prosome to that of urosome 1.8:1. Unsegmented metasome expanded dorsally, and cylindrical urosome 5-segmented, representing morphotype A (see Ooishi & IIIg, 1988). Legs 1–4 biramous and right and left legs asymmetrical. Leg 5 uniramous, with 3 short setae (1 proximal, others almost subterminal and subterminal) dorsally and 1 long seta terminally, representing morphotype A.

## Discussion

### Haplostomides otagoensis

The female of *H. otagoensis* adequately fits this genus by having three pairs of mouthparts with setal armature. It differs from females of *Haplostoma*, which lacks one to three pairs, and *Haplosuccus*, which has three pairs but lacks setal or spinal armature. All three genera belong to the subfamily Haplostomatinae (Ooishi & Illg, 1977; Ooishi, 1994).

The main characteristic features of the female of *H. otagoensis* are as follows: (1) distal margin of labrum with medial cleft (other species examined lack this

cleft); (2) hook-shaped mandible with 3 setae distally (this shape not observed in any other species, although *H. scotti* and *H. hawaiiensis* have similar armature); (3) maxilla forming a rounded distal protrusion from a broad basal portion (a simple protrusion in all other species); (4) caudal ramus with 2 unequal terminal spines (1 terminal spine in all other species); (5) copulatory pore within an oblong area surrounded by a sclerotized ridge (this ridge not observed in any other species examined).

An additional characteristic feature of *H. otagoensis* is found in the gonoporal apparatus, which includes two blunt massive sclerites in addition to three conical spines (5 elements consisting of only conical spines in other species examined). The ornamentation of the cuticle, which includes large mammiform sensilla, has been studied by SEM for the first time in this genus. Further studies are required to establish the exact distribution of the sensilla and other ornamentation and also for the function of these structures.

A key to eight species (including the new species) of the genus *Haplostomides* is presented here. It is a revision of a similar key made for seven species by Ooishi (1994).

Key to species (females) of Haplostomides

2a.	Mandible with 1 seta, maxillule with 4 setae, and maxilla with 3 setae $\dots H$ .
2b.	Mandible with 3 setae, maxillule with 7 setae, and maxilla with 2 setae <i>H. hawaiiensis</i> Ooishi, 1994
3a.	Mandible consisting of unarticulated structure, with armature terminally 4
3b.	Mandible consisting of 2 portions, each with armature5
4a.	Maxilla with 2 setae; mandible with 2 or 3 setae
4b.	Maxilla with 3 setae; mandible with 3 setae
5a.	Maxilla with 3 elements of armature7
5b.	Maxilla with 4 setae <i>H. luteolus</i> Ooishi & Illg, 1977
6a.	Mandible with 2 setae <i>H. brementi</i> Chatton & Harant, 1924
6b.	Mandible with 3 setae <i>H. scotti</i> Chatton & Harant, 1924
7a.	Maxillule with 9 setae <i>H. amarouci</i> (Blake, 1929)
7h	Maxillule with 10 setae <i>H</i> hellus

.....H. bellus Ooishi & Illg, 1977

### Botryllophilus cf. banyulensis

The body length (1.48 mm) of the female of *B*. cf. banyulensis is comparable to that (1.5 mm) of B. banyulensis from the Mediterranean Sea, as studied by Brément (1909: 72). The ratio (1.8:1) of the length of the prosome to that of the urosome is also almost the same as that (2:1) in the Mediterranean species.

The formula indicated by total numbers of the armature elements on the right and left exopods of leg 1 of the specimen from New Zealand has been confirmed as six (spines, right) and five (setae, left). This formula exactly corresponds to that of B. banyulensis presented by Brément (1909: 73, Fig. 9). In B. banyulensis, the spinal armature (numbers 1-6, counting from outer to inner) on the right exopod of leg 1 consists of three elongated spines (numbers 1, 5, 6) and three short spines (numbers 2–4); the longest spine (number 5) is more than twice as long as the ramus. The arrangement and morphology of these spines seem to be the most distinctive features of B. banyulensis. The specimen from New Zealand has these characters.

The total number (8) of the armature elements on the endopod of right leg 1 of the specimen from New Zealand is the same as that of B. banyulensis from the Mediterranean. However, there is a disagreement with respect to the composition of the elements. In B. banyulensis from the Mediterranean, according to Brément's description and illustration of right leg 1, the armature of the endopod consists of setae only, whereas in the specimen from New Zealand it includes one elongated spiniform element in addition to seven setae. Because of this difference, the specimen from New Zealand has been designated, for the present, as B. cf. banyulensis. Further discussion of this problem will be presented in a future study (manuscript in preparation) of species of Botryllophilus from the Mediterranean Sea (Napoli).

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#### References

- Blake, C. H., 1929. Crustacea. New Crustacea from the Mount Desert region. Biol. Surv. Mount Desert Reg. part 3: 1-34.
- Brément, E., 1909. Contribution à l'étude des copépodes ascidicoles du Golfe du Lion. Arch. Zool. exp. gén. ser. 5, 1 (Notes Rev. 5): 61-89
- Brewin, B. L., 1946. Ascidians in the vicinity of the Portobello Marine Biological Station, Otago Harbour. Trans. Proc. r. Soc. N. Z. 76: 87-131.
- Chatton, E. & E. Brément, 1910. Sur trois ascidicoles du genre Aplostoma Canu: Aplostoma magellanica n. sp., A. hibernica (T. & A. Scott), A. sacculus n. sp. Bull. Soc. zool. Fr. 35: 80-92.
- Chatton, E. & H. Harant, 1924. Notes sur les copépodes ascidicoles. XVII.-Le nouveau genre Haplostomides et deux espèces

nouvelles de ce genre: Haplostomides scotti et Haplostomides brementi. Bull. Soc. zool. Fr. 49: 406-412.

- Dudley, P. L. & P. L. Illg, 1991. Marine flora and fauna of the eastern United States. Copepoda, Cyclopoida: Archinotodelphyidae, Notodelphyidae, and Ascidicolidae. NOAA Tech. Rep. NMFS 96: 1–40.
- Gotto, R. V., 1952. Copepods new to British waters. Nature 170: 674.
- Illg, P. L. & P. L. Dudley, 1980. The family Ascidicolidae and its subfamilies (Copepoda, Cyclopoida), with descriptions of new species. Mém. Mus. natn. Hist. nat., Paris, A (Zool.) 117: 1–192.
- Ooishi, S., 1991. North Pacific copepods (Cyclopoida: Ascidicolidae) associated mostly with compound ascidians. Bull. Plankton Soc. Japan. spec. vol.(1991): 49–68.
- Ooishi, S., 1994. Haplostomides hawaiiensis, new species (Copepoda: Cyclopoida: Ascidicolidae), associated with the ascidian

Polyclinum constellatum at Honolulu, Hawaii. Hydrobiologia 292/293: 89–96.

- Ooishi, S. & P. L. Illg, 1977. Haplostominae (Copepoda, Cyclopoida) associated with compound ascidians from the San Juan Archipelago and vicinity. Spec. Publ. Seto mar. Biol. Lab. 5: 1–154, pl. 1.
- Ooishi, S. & P. L. Illg, 1988. Two morpho-types of *Botryllophilus* (Cyclopoida, Ascidicolidae). Hydrobiologia 167/168: 561–566.
- Schellenberg, A., 1922. Neue Notodelphyiden des Berliner und Hamburger Museums mit einer Übersicht der ascidienbewohnenden Gattungen und Arten. Mitt. zool. Mus. Berlin 10: 219-274; 277–298.
- Scott, T. & A. Scott, 1895. XLVII. On some new and rare British Copepoda. Ann. Mag. nat. Hist. 16: 353-362, pl. 15–17.