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A new species of *Ophryotrocha* (Annelida: Dorvilleidae) associated with fish farming at Macquarie Harbour, Tasmania, Australia

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Abstract

Ophryotrocha shieldsi, sp. nov. is described from Macquarie Harbour, Tasmania, Australia, where it occurs in high densities beneath the sea cages of fish farms. SCUBA and ROV underwater observations revealed closely spaced mounds of aggregations of the new species. It is closely related to *O. lobifera* Oug, a species reported from fish farms and whale-falls in the North Sea, from which it can be distinguished by its ovate rather than triangular dorsal lateral lobes, palps with small globular rather than longer digitate palpostyles, and additional jaw differences.

Key words: Polychaeta, fish farms, organic enrichment, taxonomy

Introduction

Farming of Atlantic salmon (*Salmo salar* Linnaeus), and ocean trout [*Oncorhynchus mykiss* (Walbaum)] in sea cages has been carried out for more than two decades in Tasmania, Australia. Intensive fish farming produces organic enrichment of the seabed as a result of fish faeces and excess food, leading to an altered benthic fauna, dominated by opportunistic species. Areas of high organic enrichment can develop sediment anoxia and patches of *Beggiatoa*-type mats of sulphur-oxidising filamentous bacteria, typical of deep-sea reducing habitats such as hydrothermal vents and cold seeps (Findlay & Watling 1995).

We are describing below *Ophryotrocha shieldsi*, a new polychaete species of the family Dorvilleidae that occurs in great densities (estimated at up to 100,000 individuals m^{-2}) beneath sea cages at Macquarie Harbour, Tasmania (Fig. 1). Dorvilleids are well known as opportunists, occurring in high densities in organically enriched habitats in shallow water near sewers and pulp mill outfalls (Hilbig 1995) and cage farming of fish (Karakassis et al. 2000), as well as in deep water on whale-falls (Wiklund et al. 2009) and hydrothermal vent and seep sites (Desbruyères et al. 2006).

The systematics of Dorvilleidae is presently unresolved and in need of a revision. The discoveries of hydrothermal vent and whale-fall environments in the last decades have greatly increased the number of known dorvilleid species, particularly of the genus *Ophryotrocha* Claparède & Mecznikov. In an attempt to revise the group, Orensanz (1990) erected, among others, the genus *Palpiphitime* and designated *Ophryotrocha lobifera* Oug the type species, originally described from a strongly organically enriched environment in Norway. The genus was recently redefined with an emphasis on its jaw structure, demonstrating that *Palpiphitime* not only differs from *Ophryotrocha* by the combination of its soft morphological characters but also its jaws (Paxton 2009). The same study described *P. lipovskyae* Paxton from near an Atlantic salmon farm in Hecate Strait, British Columbia, Canada.

Another recent study of dorvilleids from a whale-fall in the north-east Atlantic described three new dorvilleids, one closely related to *P. lipovskyae*, and carried out phylogenetic analyses of available *Ophryotrocha, Iphitime* and *Palpiphitime* species based on the nuclear gene H3 and the mitochondrial genes

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CO1 and 16S (Wiklund et al. 2009). The analysis identified six clades within the *Ophryotrocha* complex with *Iphitime* as sister group to one of the major clades and *Palpiphitime (P. lobifera, P. lipovskyae* and the newly described *O. craigsmithi*, Wiklund et al.) as a major clade with a bootstrap support of 100% (Wiklund et al. 2009: Fig. 5). The authors stated that *Ophryotrocha* was monophyletic in their study only if *Iphitime* and *Palpiphitime* were included. They emphasised the need for a revision of *Ophryotrocha* but opted to include the *Palpiphitime* species within *Ophryotrocha* for the present time. We agree with this decision and view the clade as an informal group of species, namely the *O. lobifera* group, for its oldest known member.

Observations of the life history and feeding behaviour of dorvilleids have only been carried out on laboratory cultures. Their complex maxillary apparatus, consisting of numerous small elements, supported by a pair of mandibles, allows them to graze, similar to the mollusk radula. In laboratory cultures dorvilleids thrive on diets of macerated spinach and/or protozoans, attesting to their omnivorous habits (Åkesson 1967). We are here reporting observations of aggregations of the new species during SCUBA dives and filmed with ROV cameras, providing information on their communal living behaviour.

Material and methods

Study site and collection methods. Macquarie Harbour, situated on the west coast of Tasmania (Fig. 1) is approximately 32 km long by 8 km wide, and 280 km^2 in area. Large areas of shallow waters are present in the northern and north-western areas of the bay, while the depth in much of the central and southern areas exceeds 20 m. Large riverine inputs are received from the Gordon River in the south of the bay and the King River in the north. The river waters, particularly those of the Gordon have high levels of humic substances and organic materials (Carpenter et al. 1991). Due to a pronounced halocline, darker fresh water remains on the surface preventing any light from reaching the underlying marine waters (Cresswell et al. 1989). Since 1883 the King River has carried tailings from the Mount Lyell copper mine near Queenstown into Macquarie Harbour. This has resulted in elevated levels of copper and other heavy metals in the sediments and overlying waters of Macquarie Harbour (Stauber et al. 2000). A narrow entrance (Hells Gates) at the north-western corner of the harbour is the only connection with the open ocean, resulting in a relatively small exchange of marine waters. Specimens were collected while SCUBA diving in 2002 and using a remotely operated vehicle (ROV) in 2009, namely a modified SEABOTIX SE150. An additional Perspex frame was secured to the ROV, providing a lip to scoop up the animals, and allowing for the attachment of a fine mesh bag in which to collect the animals. The modification allowed the bag to remain horizontal whilst moving along the seabed collecting the animals, and to shift into a vertical position whilst being retrieved to the surface, preventing the escape of animals. When retrieved to the surface the fine mesh bag was inverted into a box of seawater (collected with a Niskin bottle from approximately the same depth as that of the animals), and the animals were gently rinsed from the bag into the box. Using a 1 ml plastic pipette, the animals were then placed in 500 ml plastic containers. Some specimens were retained in saltwater and kept cool, attempting to keep them alive for observations and breeding experiments, while specimens intended for taxonomic studies were narcotised using 6% magnesium chloride for 30 minutes, then preserved in 70% ethanol.

Material examined. Specimens were examined with light and scanning electron microscopy (SEM). All drawings were made with the aid of a camera lucida attached to a Zeiss dissecting microscope. SEM preparations were critical-point dried, gold-coated and imaged with a JEOL JSM-6480LA. The type material is deposited in the Australian Museum, Sydney, Australia (AM) and the voucher specimen for the DNA sequences is deposited in the Göteborg Natural History Museum, Göteborg, Sweden (GNM).

Laboratory culture. Some of the specimens collected during SCUBA dives below the fish cages were cultured in the laboratory. The worms were kept in glass bowls in seawater at 18ºC and provided with some sediment. Water was changed once a week, when they were fed with macerated spinach.

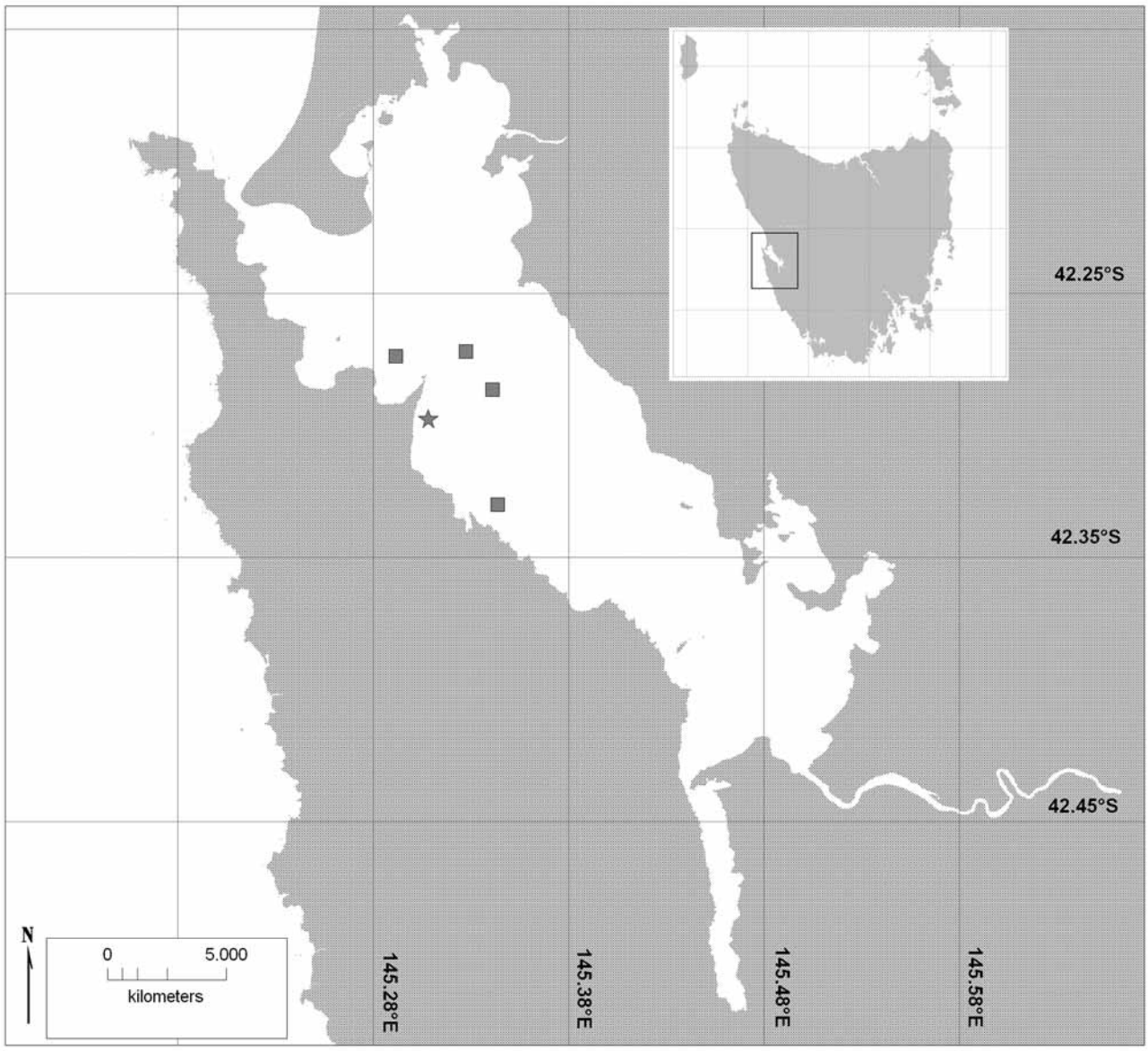

FIGURE 1. Maps of Tasmania (inset) and Macquarie Harbour. Squares and star denote locations of sea cages, with star marking type locality of *Ophryotrocha shieldsi*, sp. nov.

Results

Dorvilleidae Chamberlin

***Ophryotrocha shieldsi*, sp. nov.**
Figs. 1–3

Material examined. Type material: Liberty Point, Macquarie Harbour, Tasmania, 42º18'17.21063"S; 145º19'14.60318"E, beneath sea cages, 20 m, SCUBA diving, February 2002, collectors A. Davey, J. Lane, D. Shields, holotype (AM W.36644), 10 paratypes (AM W.36645); same locality and collectors, 20 m, modified ROV, 4 February 2009, 20 paratypes (AM W.36646). DNA voucher specimen: Same locality and collectors, 4 February 2009 (GNM Polychaeta 13224).

Description. Live specimens up to 16 mm long for 46 chaetigers. Length of holotype 7.3 mm for 43 chaetigers (paratypes 2.5–6.0 mm for 29–36 chaetigers), width of holotype 0.9 mm including dorsal lateral

lobes at chaetiger 10 (paratypes 0.3–0.7 mm). Live specimens with striking colour pattern of salmon pink pigmentation on peristomial rings and lateral segmental lobes, body otherwise whitish. Preserved specimens opaque white. Body long, slender, slightly tapering towards posterior end, dorsal side convex, ventral side slightly concave (Fig. 2A, C). Prostomium (Figs. 2B, 3A) with ciliary ring in front of antennae, continuous across palpophores, additional incomplete band dorsally behind antennae and ventrally in front and behind ciliary ring (Figs. 2C, 3B); peristomial rings and chaetigerous segments encircled by ciliary rings, continuous across lateral dorsal and ventral lobes.

Prostomium about twice as wide as long, bearing pair of dorsolateral cirriform antennae and pair of ventrolateral biarticulate palps. Palps consisting of large palpophore and small globular palpostyle. Pair of oval slanted eyes (Fig. 3A) at centre of posterior edge of prostomium, posteriorly almost touching, anteriorly further apart. Eyes internal, light-reflecting structures, appearing silvery white in live animals under incident light but invisible in preserved specimens. Nuchal organs at level of eyes, two at either side of eyes. Peristomium represented by 2 apodous achaetous rings, similar in length to following chaetigers.

Chaetigers with well developed parapodia and prominent dorsal and ventral lateral lobes. Dorsal lobes (Figs. 2A, 3A, C) ovate, cushion-like, ventral lobes (Figs. 2C, 3B) digitate to triangular; lobes present on all chaetigers but best developed in middle body region. Parapodia (Fig. 2D, 3D) uniramous, long and slender, distally dilating, bearing dorsal and ventral cirrus and acicular lobe; each structure digitate, about as long as median width of parapodium. Parapodia supported by acicula, terminating in acicular lobe and subacicular short simple chaeta or accessory acicula, emerging from ventral chaetal lobe; chaetal lobe in most cases completely retracted (Fig. 2D, E) or expanded to triangular lobe (Fig. 3D). Chaetae long and very thin (Fig. 2E); supra-acicular fascicle with 5-8 simple spatulate chaetae (Fig. 3E), subacicular fascicle with 5-7 heterogomph falcigers (Fig. 3F); upper part of simple chaetae and appendage of falcigers minutely serrated, with blunt tip; shaft of falciger minutely serrated. Pygidium wider than long, with pair of digitate pygidial cirri; anus dorsal (Fig. 3C). Mature males with rosette glands, paired dorsal segmental glandular structures on posterior half of body (Figs. 2F, 3C). Structures consisting of circular clusters of large cells with perforated integument (Fig. 2G) (for discussion of rosette glands see Paxton & Åkesson 2007).

Mandibles strongly sclerotised, black; consisting of two elongate shafts widening to distal cutting plates with slightly curved anterior edge with medial roundish protrusion and 13–16 conical teeth (Fig. 3G). Maxillary apparatus of P- and K-type; maxillae consisting of forceps fused with carrier-like structure and 7 pairs of anterior denticles (D). P-type maxillae occurring in females and immature males, weakly sclerotised with serrated ridges slightly darker (Figs. 2H, 3H). P-forceps with two transverse ridges, each with about 30 alternating larger and smaller teeth and a large fang. Denticles 1–3 similar to ridges of forceps with alternating large and small teeth and fang, D4–7 more delicate, with very finely serrated edge. K-type maxillae only in mature males, darkly sclerotised, almost black (Fig. 3I). K-forceps smooth, distally falcate. Denticles attached by ligament strut to forceps; serration of denticles similar to P-type but with fewer teeth.

Etymology. The new species is named in honour of Derek Shields, who originally observed the aggregations of the new species, and encouraged the second author to study these animals.

Remarks. The new species is the fourth species of the *O. lobifera* group. Like *O. lobifera* it has cushion-like lateral lobes. However, in *O. lobifera* the dorsal lobes are triangular, while in *O. shieldsi* they are ovate. Other differences are: *O. shieldsi* has palps with small globular rather than longer digitate palpostyles, P-maxillae with forceps and denticles serrated by alternating large and small teeth rather than uniform teeth, and mandibles with curved rather than straight anterior edge. *Ophryotrocha lipovskyae* and *O. craigsmithi* differ from both species by possessing lamella-like lateral dorsal lobes. Analysis of DNA sequences of the mitochondrial CO1 and ribosomal 16S genes has demonstrated that *O. shieldsi* is sufficiently genetically isolated from the other three species to warrant specific status (Helena Wiklund, personal communication 2009). Accession numbers for DNA sequences from *O. shieldsi*, published on GenBank: HM181931 (CO1), HM181932 (16S).

Distribution. At present only within Macquarie Harbour, Tasmania, directly underneath salmon cages.

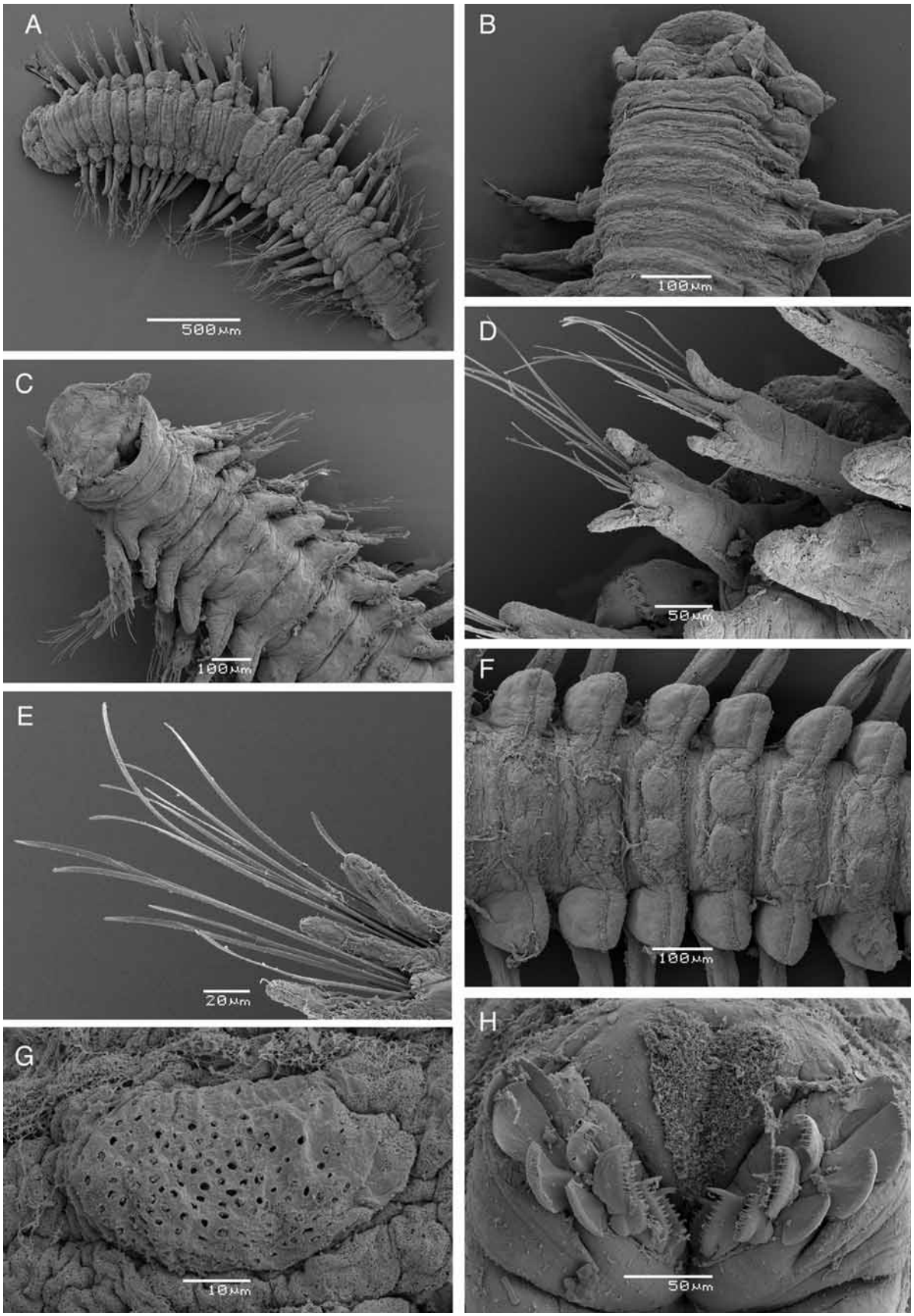

FIGURE 2. *Ophryotrocha shieldsi*, sp. nov. SEM images. A, complete specimen, dorsal view; B, anterior end, dorsal view; C, same, ventral view; D, median parapodia, anteroventral view; E, distal part of parapodium showing chaetae, anterior view; F, posterior body region, showing paired rosette glands, dorsal view; G, enlarged rosette gland; H, P-maxillae, ventral view.

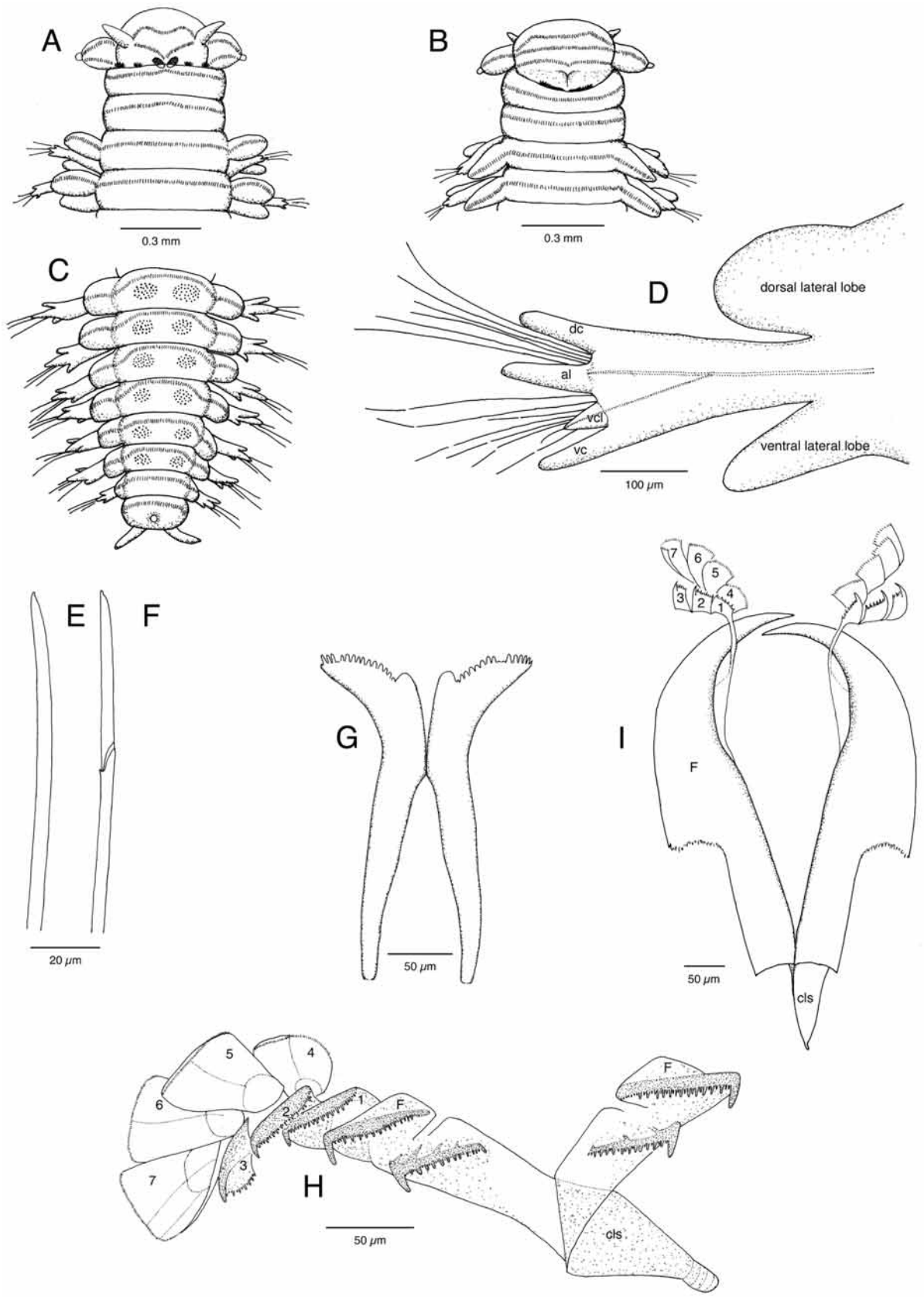

FIGURE 3. *Ophryotrocha shieldsi*, sp. nov. A, anterior end, dorsal view. B, same, ventral view; C, posterior end, dorsal view; D, median parapodium, anterior view; E, simple chaeta; F, heterogomph falciger; G, mandibles, dorsal view; H, P-maxillae, dorsal view; I, K-maxillae, dorsal view; Abbreviations: al, acicular lobe; cls, carrier-like structure; dc, dorsal cirrus; F, forceps; vc, ventral cirrus; vcl, ventral chaetal lobe; numerals refer to anterior denticles.

SCUBA diving observations

The bottom sediment of Macquarie Harbour consists of very fine mud or silt. In most locations where *O. shieldsi* has been located, more than 70% of particles are finer than 63 µm. The presence of *Beggiatoa*-type bacterial mats in Macquarie Harbour has increased since our first observations in 2000. This may be a consequence of increased stocking densities and/or due to the long term build up of organic material. Currently, the mats are quite frequently found under the fish pens.

Video surveys (a monitoring requirement for finfish farms in Tasmania) carried out by Aquenal Pty. Ltd. during September 2000 and January 2001 detected the presence of mass aggregations of dorvilleid polychaetes that were later identified as the new species, directly under finfish sea cages in Macquarie Harbour (Fig. 1). The video footage revealed closely spaced mounds of aggregations up to 30cm high and mostly about 30cm across. The surface of the mounds was densely covered by the fragile tubes, particularly the higher central parts of the mounds. Here the tubes were almost vertically aligned, while on the sloping part the density was lower and the tube arrangement irregular. The mounds of projecting tubes appeared very unstable. Any movement of water brought about by the SCUBA diver or ROV caused the tubes to sway and the mounds to disintegrate. As the lights of the video camera passed over the mounds, the worms were seen to actively retreat into their tubes, and the surface of the mounds appeared like grasses swaying in a breeze.

Laboratory observations

Some of the specimens collected during SCUBA dives below the fish cages were cultured in the laboratory. The worms secreted large amounts of mucus, were covered by thin mucous sheaths or fragile tubes and burrowed into provided sediment and accumulations of spinach and faeces. Some of the tubes were vertically aligned along the walls of the containers. Although they were never thriving, some specimens survived for about a month. Several large females appeared mature, with eggs filling the body cavity. A 38-chaetiger female had eggs from chaetiger 5–6 to 33, while the largest, a 46-chaetiger worm contained eggs from chaetiger 1 to 42. Mature males could be recognised by the presence of the dorsal rosette glands in the posterior half of their body and the large K-jaws. As no spawnings were observed in the mass cultures, a ripe pair was kept separately. When checking on the isolated pair, we happened to witness the spawning process. A large number of eggs (diameter of 150 µm) were on the bottom of the dish, the male was on the eggs, presumably fertilising them, and the female was next to the eggs. Forty-six hours later some of the eggs were observed to move, and a short while later, some were spinning slowly. No further development was observed.

Discussion

Ophryotrocha shieldsi has been observed only under stocked and recently fallowed salmon cages in Macquarie Harbour in depths of 14–45 m. None have yet been detected from control locations (at least 1 km distant from cages). Only two farms are in shallower water (less than 10 m). Although some small patches of bacterial mats have been spotted at one of these farms, *O. shieldsi* appears to be absent. These shallow farms are often stocked with juvenile fish that are on a lighter feeding regime resulting in less accumulating organic waste. Another possible explanation is that light penetration in the shallow farms is better, resulting in higher primary and secondary productivity. Species diversity and abundance is higher in the shallower waters where *O. shieldsi* is presumably outcompeted, while it thrives in the darker, more disturbed and anoxic conditions of the deeper farms. These depths at Macquarie Harbour are characterised by low diversity and abundances compared to sites at similar depth and exposure at Tasmanian estuaries (Edgar et al. 1999). While *O. shieldsi* is the dominant species, the only other invertebrates observed are leptostracans, cirolanid isopods and the occasional polynoid polychaete. Polychaetes are known to graze on filamentous bacterial mats, and *O. lobifera*, the close relative of *O. shieldsi*, was observed to feed off bacterial mats in a whale bone study (Wiklund et al. 2009). Although no identifiable bacterial remains were present in the gut contents of *O.*

shieldsi, it seems possible that the bacteria make up part of their diet and thus the presence of the worms could have an ameliorating effect on the bottom of the fish farms.

Ophryotrocha species secrete large amounts of mucus, are guided by their mucus trails, and are able to burrow into various kinds of substrates (Ockelmann & Åkesson 1990). Tubes range from very fragile mucous envelopes to becoming almost parchmentlike as in *O. cosmetandra* Oug. *Ophryotrocha socialis* Ockelmann & Åkesson produce a common system of mucus-lined tubes with many branches and anastomoses (Ockelmann & Åkesson 1990). However, we are not aware of any reports of the production and habitation of tubes freely suspended from the surface of mounds as observed in the videos of *O. shieldsi*. We do not know what makes up the interior part of the mounds as it was impossible to take any kind of cores. As soon as the SCUBA divers stopped moving they were enveloped in a cloud of silt that allowed no visibility. In several places we saw some worms around un-eaten fish feed pellets and interpreted this as the beginning of a mound. On the basis of our laboratory observations we think it likely that the growing mound is made up of accumulated organic debris mixed with mud and silt and that the worms tunnel through these accumulations creating mostly smallish (about 30 cm diameter) but occasionally very large mounds up to 1 m in diameter. Presumably the age of a mound depends on its organic content and stability of the seabed. *Ophryotrocha* species have a short generation span, thus being able to increase their population very quickly. The only spawning of *O. shieldsi* we observed in the laboratory produced a large number of eggs that developed into free trochophores. This observation is interesting as most of the *Ophryotrocha* species studied have egg masses embedded in a gelatinous matrix, where they undergo direct development (Ockelmann & Åkesson 1990). Freely swimming trochophores have only been observed in *O. maculata* Åkesson and *O.natans* Pfannenstiel.

The surfaces of the mounds are covered by freely projecting tubes containing worms. Due to the fragility of the tubes, we do not think that individual tubes reach far into the mounds. Assuming that the interior of the mounds contain a network of tunnelling worms we would expect that it is here that the worms get their nutrition by eating the organic matter.

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