The tubificids (Annelida, Oligochaeta) of Lake Trasimeno and Lake Piediluco in Central Italy, with a study of SEM morphology of some species

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Abstract: Freshwater oligochaetes are studied worldwide because of their relevance as bioindicators in aquatic ecosystems. Nevertheless, species identification is still difficult because it is mainly based on the male reproductive system. The morphology of the chaetae offers an additional set of characters and the fine organization of these elements can help in solving crucial aspects allowing specific attribution. In spite of this, somatic chaetae are poorly investigated under scanning electron microscopy (SEM). A SEM analysis of the chaetae has been carried out on the tubificids Branchiura sowerbyi, Limnodrilus hoffmeisteri, Tubifex tubifex, and Psammoryctides barbatus from Trasimeno and Piediluco lakes (Central Italy). In particular, the chaetal pattern of B. sowerbyi is comparable to that described for specimens from the tropical regions of Asia, and T. tubifex shows the chaetal pattern typical of the nominal species; the palmate chaetae of P. barbatus are characterized by 15–20 fringes, each of them with a distal end thinner and folded backwards. In addition, B. sowerbyi is the dominant species in Lake Trasimeno, whereas it is lacking in Lake Piediluco, where the tubificids are primarily represented by T. tubifex.

Key words: Tubificids, chaetae, scanning electron microscopy

1. Introduction
Tubificids encompass species living mainly in freshwater habitats (Martin et al., 2008; Rota, 2008). In the modern phylogenetic system, all tubificids (former Tubificidae) are considered members of the family Naididae (Erséus et al., 2008), whereas the family Tubificidae is maintained in the evolutionary system as a paraphyletic taxon (Timm, 2012).

Tubificids are ubiquitous organisms in lacustrine ecosystems where, especially in the deep zones, they often represent the largest biomass among the macroinvertebrates, thus constituting an important trophic link with the deposits of detritus. Some tubificids can tolerate, in a different manner, the lack of dissolved oxygen; the typical assemblages of their species, as well as their relative density, have been used for the characterization of lacustrine trophism. Therefore, they are relevant bioindicators of the trophic state in freshwater ecosystems (Brinkhurst, 1971; Brinkhurst and Jamieson, 1971; Milbrink, 1983a, 1994; Svensson et al., 2001; Milbrink et al., 2002). Likewise, tubificids play an important role in the sediment–water exchange processes in a wide range of toxicity and bioaccumulation tests (Egeler et al., 1999; Bouché et al., 2000; Chapman, 2001).

In addition, the relationship between the incidence of chaetae deformities in oligochaete worms and contaminated sediments has been investigated (Milbrink, 1983b; Bouché et al., 1999a).

In recent years, some tubificids have also been identified as intermediate hosts of several Myxozoa that parasitize fish of commercial interest (Wolf and Markiw, 1984; Brinkhurst, 1996; Lom and Dykova, 2006; Marcucci et al., 2009).

In most cases, the identification at the species level of tubificids is based on the morphology and arrangement of the chaetae, in particular of those located dorsally on the preclitellar segments. Sometimes these characteristics are insufficient and it is necessary to examine mature specimens, because only the morphology of the male reproductive system is a valid character (Brinkhurst, 1971). Molecular analyses have shown that, in some cases, sexual characteristics are insufficient for a specific discrimination (Beauchamp et al., 2001; Envall et al., 2012).

For these reasons, investigations of the chaetae at scanning electron microscopy (SEM) levels are an important tool for facilitating better taxonomic identification. At present, studies on the chaetae of aquatic oligochaetes under SEM are scarce (Chapman and Brinkhurst, 1986; Finogenova and Arkhipova, 1994;
Bouché et al., 1999b; Ohtaka and Nishino, 1999; Arslan and Şahin, 2006; Caramelo and Martínez-Ansemil, 2012), and they mainly deal with the morphological changes that these elements undergo in response to both contaminated natural sites (Milbrink, 1983b) and laboratory conditions (Chapman and Brinkhurst, 1987).

The aim of the present study is to evaluate the tubificid assemblage from Trasimeno and Piediluco lakes (Central Italy) and to analyze under SEM the ultrastructure of the chaetae characterizing the members of the examined populations.

2. Materials and methods

2.1. Study area

Lake Trasimeno (43°08′00″N, 12°06′00″E, surface: 124 km², volume: 0.586 km³, maximum depth: 6.3 m) is the largest shallow lake of Italy. It is in a mesotrophic condition, tending to eutrophic (Ludovisi and Gaino, 2010; Di Veroli et al., 2012).

Lake Piediluco (42°32′00″N, 12°45′00″E, surface: 1.7 km², volume: 0.02 km³, maximum depth: 20 m) is a natural lake regulated for hydroelectric purposes. It is in a eutrophic condition (Posati, 2003; Di Veroli et al., 2010). In Lake Trasimeno, the study was seasonally conducted from 2000 to 2006 by means of 16 surveys in 4 stations: st. 1–3, central zone, with an average depth of 4.5 m, and st. 4, littoral zone with an average depth of 1 m (Figure 1a). The central stations (st. 1–3) presented a muddy substrate, whereas the littoral station (st. 4) showed muddy sand with discontinuous submerged vegetation. In Lake Piediluco, 9 seasonal surveys were carried out over the years 2003–2006 at 10 stations (st. 1–10), at a depth varying from 1.25 to 18.5 m (Figure 1b). These stations presented a prevailing muddy substrate.

During the periods of investigation, the surface waters of Lake Trasimeno reached a maximum temperature of about 30 °C, whereas those of Lake Piediluco reached about 25 °C.

Figure 1. Location of the sampling stations: a) Lake Trasimeno (st. 1–4); b) Lake Piediluco (st. 1–10). The arrows in bold indicate the inlet and the outlet of Lake Piediluco.
2.2. Sampling and methods
Tubificids were collected in Trasimeno (st. 1–3) and Piediluco (st. 1–10) lakes by means of a compressed air pump equipped with a base 20 × 20 cm in size, corresponding to an area of 400 cm². In each station, 5 withdrawals were made for a total area of 2000 cm² (Pearson et al., 1973; Drake and Elliot, 1982). The material was removed from the bottom for 60 s, each time collected in a 275-μm mesh net.

In addition, in the central area (st. 1–3) of Lake Trasimeno, the qualitative investigations were carried out by using a small drag net (30-cm opening; 0.5-mm mesh). This qualitative technique was exclusively utilized in the littoral zone (st. 4) of Lake Trasimeno.

In the laboratory, tubificids were cleaned from the sediment, collected with pipettes, and fixed in 70% alcohol. For a preliminary screening, some specimens were rinsed in deionized water, placed in lactic acid for several hours, mounted on a slide with a few drops of liquid Faure, and finally observed under the optical microscope for identification.

For SEM, specimens (10–20 per taxon/lake) were appropriately fixed for 30 min in 3% glutaraldehyde buffered with cacodylate, then repeatedly rinsed in the same buffer. Afterwards, selected material was dehydrated by using ethanol gradients, followed by critical-point drying by means of a CPD 030 Bal-Tec (Bal-Tec Union Ltd., Balzers, Liechtenstein). Specimens were then mounted on stubs with silver conducting paint, sputter-coated with gold-palladium in a sputter-coater (Emitech K550X, Emitech, Ashford, UK), and observed with a Philips XL30 scanning electron microscope (Philips, Eindhoven, the Netherlands) at an accelerating voltage of 18 kV.

Guides used for the identification were Brinkhurst (1971), Campaioli et al. (1994), and Tachet et al. (2010).

3. Results
Tubificids represented the most important component of the benthic macroinvertebrate communities in both lakes. In addition, chironomids almost exclusively constituted the remaining benthic assemblage.

The average density of tubificids in the central area (st. 1–3) of Lake Trasimeno was 165.91 ind. m⁻² (56.38% of the macrobenthos), corresponding to 1095 specimens, represented by Branchiura sowerbyi (69.92%), T. tubifex (13.50%), and undetermined Naididae (4.42%).

In Lake Piediluco, the average density of tubificids was 407.47 ind. m⁻² (54.57% of the macrobenthos), corresponding to 4075 specimens, represented by L. hoffmeisteri (12.16%), T. tubifex (69.92%), P. barbatus (13.50%), and undetermined Naididae (4.42%).

3.1. Morphological observations
The 4 identified tubificid species were analyzed under SEM. No differences in the somatic chaetae of the species living both in Trasimeno and Piediluco lakes were observed.

3.1.1. Branchiura sowerbyi Beddard, 1892 (Figures 2a–2d)
The collected specimens were up to 5 cm in length. Each bundle of the dorsal chaetae, located on the preclitellar region, resulted from thin hair elements (1–4 in number) and numerous bifid chaetae (4–6 in number). These last had an upper tooth smaller than the lower one, with a jagged border in between (Figures 2a–b). The dorsal postclitellar chaetae were similar but lacked the jagged border (Figure 2c). The ventral chaetae were bifid, showing a cut deeper than that of the dorsal chaetae (Figure 2d).

3.1.2. Limnodrilus hoffmeisteri Claparède, 1862 (Figures 2e–2g)
The collected specimens had an elongated and slender body, about 20–35 mm long. On the dorsal pre- and postclitellar regions, long and thin bifid chaetae with a very curved distal end were present. The anterior dorsal bundles were composed of 6–8 chaetae with the upper tooth thinner but longer than the lower one, without a jagged border or intermediate teeth interposed between the teeth (Figures 2e and 2f). The ventral bifid chaetae were shorter than the dorsal ones (Figure 2g).

3.1.3. Tubifex tubifex (Müller, 1774) (Figures 3a–3d)
In the collected specimens, the bundles of the preclitellar dorsal chaetae included long hair chaetae (1–4) and pectinate chaetae (3–5). This last had 3–4 intermediate teeth well separated from each other (Figures 3a and 3b). The hair chaetae were smooth and free of secondary branches (Figures 3a–3c). The characteristic intermediate teeth of the dorsal bifid chaetae were not present in the postclitellar region (Figure 3c). The ventral chaetae have a deep split between the upper and lower teeth (Figure 3d).

3.1.4. Psammoryctides barbatus (Grube, 1861) (Figures 3e–3h)
The collected specimens reached about 4 cm in length. They were easily recognizable owing to the presence of the characteristic preclitellar palmate dorsal chaetae (7–8) associated with very long numerous hair chaetae. The palmate chaetae, consisting of numerous fringes (15–20), had a distal end that was thinner and folded backwards (Figures 3e and 3f). The postclitellar dorsal chaetae were large and markedly bifid (Figure 3g). In the ventral
Figure 2. Chaetae under SEM. Branchiura sowerbyi: a) anterior hair (H) and bifid (B₁) dorsal chaetae; b) detail of a bifid dorsal chaeta (B₁) showing the jagged border; c) postclitellar dorsal chaetae; d) bundles of ventral chaetae. Limnodrilus hoffmeisteri: e) bundles of anterior dorsal chaetae, detailed in f); g) bundles of ventral chaetae.
Figure 3. Chaetae under SEM. *Tubifex tubifex*: a) anterior hair (H) and pectinate (Pe) dorsal chaetae; b) detail of a pectinate chaeta; c) dorsal postclitellar chaetae (H, hair; B₂, bifid dorsal chaeta without intermediate teeth); d) bundles of ventral bifid chaetae. *Psamnoryctides barbatus*: e) bundles of anterior dorsal chaetae (H, hair; Pa, palmate); f) detail of a palmate chaeta showing the fringes, each of them with a distal end thinner and folded backwards; g) dorsal postclitellar chaetae; h) bundles of ventral bifid chaetae, whose detailed apical region is zoomed in the inset.
bundles, the chaetae showed a deep split between the upper and lower teeth (Figure 3h).

4. Discussion

Branchiura sowerbyi is a cosmopolitan species and prefers lentic or slowly flowing warm waters, being originally from the tropical regions of Asia (Timm, 1980; Paunović et al., 2005; Raposeiro et al., 2009). This thermophilous tubificid is considered an invasive species and it is currently found in many countries in Europe (http://www.faunaeur.org).

The specimens were easily recognizable for the presence of gills in the rear of the body. The gills were formed by dorsal and ventral individual filaments on the posterior segments and their rhythmic wave movements create an intense circulation of water. The length, the speed of bending, and the frequency of the undulations of each filament vary in relation with the availability of dissolved oxygen (Ohtaka and Nishino, 1999). These morphological adaptations allow the oligochaete worm to be fairly resistant in environments rich in organic matter.

By comparing our data with those reported in literature, it is evident that the morphology of the dorsal preclitellar chaetae is comparable with that showed by the specimens from China and Sumatra, regions encompassed in the origin area of the species (see Timm, 1980). The ventral bifid chaetae, having a clear cut and well-pronounced inferior tooth, differ from the observations of Ohtaka and Nishino (1999), who reported one-cusped or just bifid ventral chaetae.

This species was not found in Lake Piediluco, probably because of the low water temperature (Di Veroli et al., 2010).

Limnodrilus hoffmeisteri has a wide distribution and it is a useful biological indicator of relevant tolerance to organic pollution. In these conditions, it can reach high population density (Chapman and Brinkhurst, 1984; Lauritsen et al., 1985; Brinkhurst and Gelder, 2001).

The lack of previous ultrastructural data on the chaetae of L. hoffmeisteri, as far as we know, did not allow us to perform a comparative assessment.

Tubifex tubifex represents a species complex (Beauchamp et al., 2001; Crottini et al., 2008), showing a variability of the morphological characters (chaetae and other anatomical characters) (Holmquist, 1983). This species complex has a wide ecological valence, tolerating trophic conditions up to eutrophy, and, in environments with high organic matter content, it may become dominant due to its ability to tolerate low levels of oxygen. It is well known that these species plug into the substrate, building up a tube within which the caudal part, facing outward, swings to facilitate the intake of oxygen (Chapman and Brinkhurst, 1984; Lauritsen et al., 1985; Brinkhurst and Gelder, 2001).

The members of the examined population show the chaetal pattern typical of the nominal species.

Psammoryctides barbatus is a species with broad ecological valence, even though it is less tolerant of polluting factors than the other taxa previously reported in this study. This species is found especially in mesotrophic environments (Ladle and Bird, 1984).

The main outcome is represented by the fine morphology of the palmate chaetae consisting of numerous fringes (15–20), each of them having a distal end thinner and folded backwards.

In conclusion, SEM analysis carried out on the examined tubificids from Trasimeno and Piediluco lakes showed that the chaetal pattern in B. sowerbyi is comparable with that described for the specimens from the original tropical regions of Asia; that in T. tubifex is typical of the nominal species; and the palmate chaetae of P. barbatus consist of 15–20 fringes. In addition, B. sowerbyi was the prevailing taxon in Lake Trasimeno, whereas it was lacking in Lake Piediluco, where the tubificids were primarily represented by T. tubifex.

Acknowledgments

We are very grateful to Francesca Scoccia, PhD, for her graphical support in laying out the figure plates, and to the anonymous referees for their helpful suggestions, which markedly improved the content of the text.

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