Microscopic anatomy of aquatic oligochaetes (Annelida, Clitellata): a zoological perspective

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The taxonomic identification of oligochaetes (clitellate annelids) mainly relies on internal organs due to the simplicity of their external anatomy. This fact, added to the small size of most of the aquatic oligochaetes (“microdriles”) has made the microscope an indispensable tool of study. In this paper we summarize the contribution of different microscopical techniques - optical microscopy, transmission electron microscopy (TEM), scanning electron microscopy (SEM) and confocal laser scanning microscopy (CLSM)- to the growing knowledge on aquatic oligochaetes from a zoological perspective. The use of the optical microscope has been essential for taxonomical studies, ever since the first descriptions of aquatic oligochaetes made by Müller (1773) to the currently known ca.1700 species. During the last four decades, electron microscopy has generated new scenarios. Thus, TEM contributed to subcellular and physiological studies, mainly on sense organs, muscles and spermatozoa. In this context, it is worth noting that the ultrastructure of spermatozoa provided interesting topics for discussion on the phylogeny of the Clitellata. Studies with SEM, mainly focusing on chaetae, sense organs and mating structures, provided interesting insights on the field of the functional anatomy of worms. The number of papers published based on observations with CLSM on aquatic oligochaetes is reduced and these are mostly devoted to the structure of nervous system and its regeneration after fragmentation, together with embryonic development of the musculature.

Keywords Annelida; aquatic oligochaetes; optical microscopy; TEM; SEM; CLSM

1. Introduction

Oligochaetes are hermaphroditic clitellate annelids, without suckers and provided with chaetae and spacious coelom. External anatomy of these segmented worms is apparently simple. Hence, the clitellum -a thickened glandular region in the body wall which produces the cocoon-, and the chaetae -usually four bundles protruding from the body wall of each mesosomial segment-, generally represent the only two distinctive elements of their external anatomy. As a consequence, the identification of oligochaetes mainly relies on internal organs such as: genitalia, digestive tract, pharyngeal glands, nephridia and a few other structures (Fig. 1).

Oligochaetes are widely distributed and diversified in terrestrial and aquatic habitats, including the marine environment. About one-third of the almost 5000 currently known species of oligochaetes are aquatic. Aquatic oligochaetes are generally small-sized annelids (“microdriles”), ranging from 1 mm to a few centimeters in length and 50 to 500 µm in diameter. In fact, most of the about 1700 aquatic species known to date belong to one of the 13 microdrile families, which are truly aquatic with the only exception of the primarily terrestrial family Enchytraeidae. Only about 60 species of “megadriles” have been found in aquatic habitats [1].

The aim of this paper is to summarize the growing knowledge on aquatic oligochaetes from a zoological perspective, using different microscopic techniques: optical microscopy, transmission electron microscopy (TEM), scanning electron microscopy (SEM) and confocal laser scanning microscopy (CLSM).

2. Historical background

The small size of aquatic oligochaetes has made the microscope an indispensable tool ever since the first observations and descriptions of species were produced. The diagnosis of the first species described were superficial and essentially based upon the external anatomy of some naidines (Stylaria lacustris (Linnaeus, 1767), Nais elinguis Müller, 1773, Dero digitata (Müller, 1773), Dero (Aulophorus) furcatus (Müller, 1773), Ophidonais serpentina (Müller, 1774), Nais barbata Müller, 1774), tubificines (Tubifex tubifex (Müller, 1774)), lumbriculids (Lumbriculus variegatus (Müller, 1774)) and enchytraeids (Lumbricillus lineatus (Müller, 1774)).

The pace at which new species of aquatic oligochaetes were described using the optical microscope was slow over a century. Thus, in the early seventies of the 19th century, hardly fifty species belonging to twenty genera were known. Thereafter, the description of new species of aquatic oligochaetes increased exponentially to reach the ca. 1700 currently known [1]. In the last third of the 19th century, the great development of the knowledge of the diversity of aquatic oligochaetes was accompanied with some anatomical studies of extraordinary accuracy (e.g. [2]). (Fig. 2).
At the beginning of the 20th century, Michaelsen [3] established a complete and elaborate classification that can be regarded as the stem of all subsequent ones. This classification, intended to discriminate among families, selected a set of characters that would remain in use in the future [4-7]. The location and number of different components of the genital apparatus, with special attention to the number of testes and ovaries, and the relative position of spermathecae, genital pores and sperm funnels were the main characters in which these classifications were based. Details of almost all of the anatomical structures observable with optical microscopy were used for species diagnosis: somatic chaetae, presence, number and type of prostates, development of atria and vasa deferentia, presence and shape of structures for sperm transfer (genital chaetae, pseudopenes, penes, cuticular sheaths), shape of spermathecae, presence of espermatozeugmata, presence and type of coelomocytes, shape of nephridia, etc. (Figs 3-12).

In the eighties and early 90’s, the studies both on aquatic oligochaetes and other zoological taxa aimed to organize the diversity of groups according to a natural classification system. Hence, a growing body of literature appeared from the perspective of Cladistics (e.g. [8-10]). However, discrepancies among authors when considering the supposed homology of many of the characters used as indicators of relationship arouse, and often derived in highly contradictory conclusions [11]. As a consequence, phylogenetic studies based on anatomical characters declined in favor of molecular-based phylogeny. Nevertheless, given the importance of the anatomical knowledge to generate interesting hypothesis in the field of phylogeny, some authors keep on devoting to fine anatomy studies, even attempting to elucidate the embryonic origin of some structures [12-14].

In spite of the microscope being in decline as a tool to study the diversity of aquatic oligochaetes, new genera and even higher-level taxa are still being described nowadays [15-20].

The earliest studies performed with transmission electron microscopy (TEM) on aquatic oligochaetes date from the 50’s and mainly focused on the description of embryonic subcellular structures (e.g. [21-24]). Jamieson’s revision [25] on the ultrastructure of the oligochaete showed that 20% of the more than 300 papers published to date using the TEM were devoted to the study of aquatic oligochaetes. These studies were generally patchy and focused on a few structures: epidermis, clitellum, sensory organs, coelomocytes, chloragogen cells, muscles, sperm (e.g. [26-32]). Most of these studies have a clear physiological orientation whereas only some of them aim for a zoological perspective, incorporating new elements of discussion in the field of systematics (e.g. [31, 33]).

In the recent decades, TEM observations have been particularly focused on the muscle wall and the ultrastructure of sperm. Papers on muscles were merely descriptive [34-36], and only some of them established comparisons among taxa, under a taxonomic or phylogenetic orientation [37-39]. Most studies of sperm ultrastructure have a clear phylogenetic orientation, providing interesting topics for discussion within the Clitellata [40-47]. (Figs 13, 14).

The first studies of aquatic oligochaetes using scanning electron microscope (SEM) date from the early 70’s [48]. Papers carried out since then to the end of the century were very scarce and focused almost exclusively on external sensory organs [49-51] and somatic chaetae [52-58]. The few observations on sense organs complemented the initial knowledge derived from the use of TEM, and the observations of somatic chaetae were generally intended to complement the diagnosis of some taxa, although in some cases [52, 55, 56] were used as a tool for tracking changes in environmental conditions. Yáñez et al. [59] and Caramelo and Martínez-Ansemil [60] published two extensive studies on the typology and distribution patterns of ciliated sense receptors in the main families of microdriles. In addition to
In the last third of the 19th century, the great development in the knowledge of the diversity of aquatic oligochaetes was accompanied with anatomic studies of extraordinary accuracy: two images from Vejdovský, 1884 [2]. A. Anterior region of Chaetogaster diaphanus (Grüthuisen) (Plate V, fig. 1). B. Nephridium of Psammoryctides barbatus. (Grube) (Plate. IX, fig. 1).

These studies and some publications that show images of somatic chaetae with SEM, in 2001, Cuadrado and Martínez-Ansemil [61] published the first work on attachment and sperm transfer. Recently, Caramelo and Martínez-Ansemil [62] provided new insights into sensory organs, chaetae, mating systems, as well as about some other structures not previously observed such as coelomic pores and genital marks (Figs 15-18).

The use of confocal microscopy techniques (CLSM) and/or immunohistochemistry for the study of microdriles started in the late 90's with the observations of the central nervous system of some species (e.g. [63, 64]). Molnár et al. [65, 66] addressed the study of peripheral nervous system of one species of tubificine. In addition, Bergter and Paululat [67] studied the embryonic development of the musculature, and Yoshida-Noro et al. [68] and Müller [69] performed observations on the developing nervous system during regeneration after a process of fragmentation on different species. (Figs 14-19).

3. Conclusions

Optical microscopy is an indispensable tool to ascertain the biodiversity of aquatic oligochaetes, knowledge that is experiencing an exponential growing. In spite of the increasing number of molecular-oriented studies, some new anatomic structures and many new taxa are still being described with optical microscopy, thus providing important contributions in the field of taxonomy and phylogeny of the clitellates. Many questions are still to be solved, especially those regarded to functional anatomy (mating systems, sensory organs, etc). In this context, the use of electron and confocal microscopy is contributing important results that are expected to be increased in the next future.

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Optical microscopy is an indispensable tool to ascertain the diversity in aquatic oligochaetes (microdriles). Many anatomical structures are used for their identification. **Fig. 3.** Cernosvitoviella atrata (Bretschner), anterior body region. **Fig. 4.** Henlea perpusilla Friend, anterior segments. **Fig. 5.** Lumbricillus rivalis (Levinsen), central nervous system. **Fig. 6.** Stylodrilus glandulosus Giani & Martinez-Ansemil, transversal section segment X. **Fig. 7.** Tubifex tubifex (Müller), male apparatus. **Fig. 8.** Limnodrilus udekemianus Claparède, cuticular penis sheath. **Fig. 9.** Psammaryctides barbatus (Grube), dorsal chaetae. **Fig. 10.** Amphichaeta sannio Kallstenius, anterior body region. **Fig. 11.** L. rivalis, chaetal bundle. **Fig. 12.** Stylodrilus heringianus Claparède, chaetal bundles. (at) atrium; (b) brain; (c) coelomocytes; (cc) circumpharyngeal connectives; (co) coelom; (d) diatoms inside the gut; (g) gut; (m) mouth; (pb) penial bulb; (pe) penis; (pg) prostate gland; (ph) pharynx; (phg) pharyngeal glands; (php) pharyngeal pad; (pr) prostomium; (rmp) retractor muscles of the pharynx; (sv) seminal vesicle; (vd) vas deferens; (vnc) ventral nerve cord.
Figs 13-19 Electron microscopy and CLSM contribute with new insights to the zoological knowledge of aquatic oligochaetes. Fig. 13. Phylogeny of some Oligochaeta, using spermatozoal ultrastructure (phylogram for the sperm of 11 species), from Jamieson et al. 1987 [45]. Fig. 14. Prostomial pit of Bothriocentrotus vejdovskyanum Stolc (TEM). Fig. 15. Genital chaeta of Potamothrix hammoniensis (Michaelsen) (SEM). Fig. 16. Coelomic pore of Protuberodrilus tourenqui Giani & Martinez-Ansemil (SEM). Fig. 17. Ciliate sense receptor of Eiseniella tetraedra (Savigny) (SEM). Fig. 18. Genital region of Peristodrilus montanus (Hrabé) (ventral view). Fig 19. Ventral nerve cord and nephridium of B. vejdovskyanum. (ab) anchorage bridge; (ci) cilium; (cu) cuticle; (ep) epicuticular projections; (m) muscle; (mv) microvilli; (n) nephridium; (pc) penial chaetae; (vnc) ventral nerve cord; (za) zonula adhaerens.
References


