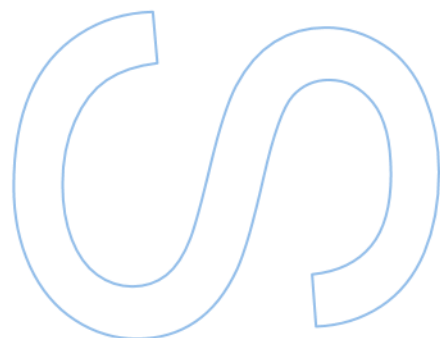
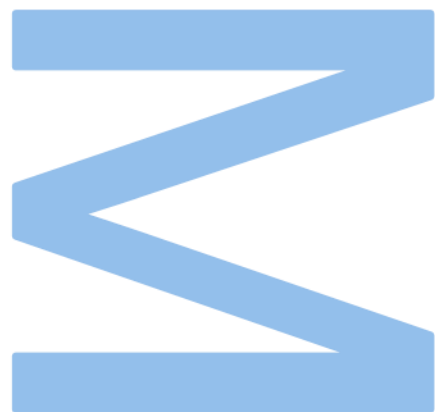


Biometric Characterization and Palp Morphology of the annelid *Hediste diversicolor* (Müller, 1776)

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Master in Biological Aquatic Resources
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Thesis Outline

The research performed for this thesis was shared via submission of abstracts, producing scientific communications in national and international science meetings and manuscripts:

National Scientific Conferences

Almeida, J.C., Lobo-da-Cunha, A., Alves, Â., Antunes, C., Costa, D.A., Rocha, S. (2023). Histochemistry and electron microscopy of the palps from the polychaete *Hediste diversicolor* (Müller, 1776). IJUP, Reitoria da Universidade do Porto, May, 10-12, 2023

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Resumo

O filo Annelida, e particularmente os poliquetas, apresentam uma grande diversidade podendo ser encontrados numa grande variedade de ambientes. Esta tese investiga, em particular, o poliqueta *Hediste diversicolor* (O.F. Müller, 1776), com foco no desenvolvimento de múltiplas estruturas corporais com funções e ultra-estrutura únicas. Esta espécie pode ser encontrada ao longo da costa atlântica da Península Ibérica e é comumente utilizada como isco para a pesca. É também de grande importância ecológica, estando envolvida na manutenção da boa qualidade do sedimento, e pela sua posição na cadeia trófica, reciclando detritos e sendo predada por diferentes animais incluindo peixes e aves. O poliqueta *H. diversicolor* é comumente utilizado como modelo em diferentes áreas científicas, tais como ensaios ambientais e de poluição, testes de drogas e estudos de anatomia, reprodução e desenvolvimento.

Este estudo é composto por dois capítulos principais: O Capítulo I que apresenta uma análise biométrica das várias estruturas corporais de *H. diversicolor*, com o objetivo de identificar as tendências de crescimento associadas ao desenvolvimento de cada novo segmento setígero. Os resultados revelaram o crescimento médio de estruturas específicas, desde o prostómio, peristómio, segmentos setígeros e pigídio por cada novo segmento setígero, e a correlação entre o crescimento de cada uma dessas estruturas e o número de segmentos setígeros, demonstrando os padrões de desenvolvimento da espécie.

O capítulo II objetivou estudar a ultra-estrutura dos palpos utilizando técnicas histoquímicas e microscopia eletrónica de transmissão (TEM). Estes órgãos desempenham um papel muito importante na perceção sensorial, na orientação e na alimentação. Consequentemente, a ultra-estrutura dos palpos apresenta um arranjo celular complexo. Neste sentido, a investigação apresentada neste capítulo fornece novos conhecimentos sobre as diferentes funções dos palpos, como a sensorial e a secretora, e fornece uma base para estudos noutras áreas científicas.

Investigações futuras podem incluir estudos longitudinais, tendo em consideração o fator tempo, para melhor compreender e diferenciar novas fases do ciclo de vida. As metodologias utilizadas no Capítulo II podem ser utilizadas em outros apêndices de *H. diversicolor* ou de outras espécies para comparar a sua anatomia e funções.

Palavras-chave: Recursos aquáticos; Biologia do desenvolvimento; Histoquímica; Microscopia eletrónica de transmissão

Abstract

The phylum Annelida, and particularly the polychaetes, have a great diversity and can be found in a wide variety of environments. This thesis investigates the polychaete *Hediste diversicolor* (O. F. Müller, 1776), with focus on the development of multiple body structures with unique functions and ultrastructure. This species can be found along the Atlantic coast of the Iberian Peninsula and its commonly used as bait for fishing. It is also of great ecological importance, being involved in the maintenance of the good quality of the sediment, and by their position on the trophic chain, recycling detritus and being preyed by different animals including fish and birds. *Hediste diversicolor* is commonly used as a model for studies on different scientific areas, such as environmental and pollution essays, drug testing and anatomy, reproduction and development studies.

This study is composed by two main Chapters: The Chapter I presenting a biometric analysis of the various body structures of *H. diversicolor*, with the aim to identify the growth trends associated with the development of each new chaetiger segment. Results revealed the average growth of specific structures, from the prostomium, peristomium, chaetiger segments, and pygidium per each new chaetiger segments, and the correlation between the growth of each of those structures and the number of chaetiger, demonstrating the developmental patterns of the species.

Chapter II aimed to study the palps ultrastructure using histochemistry techniques and transmission electron microscopy (TEM). These organs play a very important role in sensory perception, orientation and feeding. As a result, the ultrastructure of the palps has a complex cellular arrangement. In that note, the research provided from this chapter gives new insights into the different functions of the palps such as sensory and secretory, and providing a baseline for studies in other scientific areas.

Future research may include longitudinal studies, considering the time factor, to better understand and differentiate new life cycle stages. Methodologies used on Chapter II can be used on other appendages of *H. diversicolor* or other species to compare their anatomy and functions.

Keywords: Aquatic resources; Developmental biology; Histochemistry; Ragworm; Transmission electron microscopy

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List of Abbreviations

CIIMAR/UP	INTERDISCIPLINARY CENTRE OF MARINE AND ENVIRONMENTAL RESEARCH OF THE UNIVERSITY OF PORTO
FCUP	FACULTY OF SCIENCES OF THE UNIVERSITY OF PORTO
ICBAS	SCHOOL OF MEDICINE AND BIOMEDICAL SCIENCES
I3's	INSTITUTE FOR RESEARCH AND INNOVATION IN HEALTH
NEL	NEUROPODIAL SUBACICULAR LIGULE
NOL1	NOTOPODIAL SUPRACICULAR LIGULE
NOL2	NOTOPODIAL SUBACICULAR LIGULE
PAS	PERIODIC ACID-SCHIFF
POL	POST-CHAETAL LOBE
PRL1	NOTOPODIAL PRE-CHAETAL LOBE
PRL2	NEUROPODIAL PRE-CHAETAL LOBE
TEM	TRANSMISSION ELECTRON MICROSCOPY

General Introduction

The phylum Annelida Lamarck, 1802 is one of the richest in terms of number of species, and the polychaetes, or bristle worms, make a large numerical contribution to this taxon (Rouse et al., 2022).

Polychaetes show a remarkable ability to adapt and live in a wide range of environments. They can be found in marine, brackish and freshwater, as well as humid terrestrial environments (Rouse & Giribet, 2016). Some species, notably species that inhabit estuarine/brackish environments (Kristensen, 1984), can tolerate a great range of environmental temperatures, salinity, pressure and oxygen levels, consequently presenting considerable geographical dispersion/ ecological release (Hesselberg, 2003). Polychaetes can be found worldwide, in tropical, temperate and polar aquatic environments, from intertidal and estuary zones to the deep-sea trenches (Brandt et al., 2019).

These organisms play various ecological roles in their ecosystems. Some polychaetes show a borrowing behaviour by moving inside the substrate (Hesselberg, 2003), acting as bioengineers, forming bioturbation that facilitates the oxygenation and nutrient recycling of the substrate and ventilation (Fang et al., 2022). They also actively move matter from the upper layers of the sediment to the deeper layers (Queirós et al., 2013), causing the sediment particles to mix homogeneously as they move the water along their burrows. This activity impacts biogeochemical processes, such as the nitrogen and carbon cycles (Queirós et al., 2013), creating vital conditions for associated benthic communities (Hutchings, 1998).

Polychaetes also have a vital role on the food chain, being prey for other annelids (sometimes exhibiting cannibalistic behaviour), crustaceans, fish and even birds (Kristensen, 1984; Scaps, 2002). In turn, some polychaetes are predatory or omnivorous, being able to feed on algae, fungi, bacteria and other microorganisms, small crustaceans, molluscs and other annelids, as well as on detritus, thus contributing to the recycling of organic matter in their habitat (Fauchald & Jumars, 1979; Dierschke et al., 1999; Jumars et al., 2015).

Recent phylogenetic studies showed that, apart from basal taxa with a lower number of species, the phylum Annelida contains two main clades: Errantia and Sedentaria (Weigert & Bleidorn, 2016; Kuo, 2017). The polychaete body is constituted by several segments, some of which bearing modified structures with diversified functions, such as

antennae, palps, modified cirri or pygidium, and others having structures that aid with locomotion such as parapodia that carry bristles/chaeta also known as chaetiger segments (Viéitez et al., 2004).

According to the current cladistic perspective, Sedentaria is a very large and highly diversified annelid clade, encompassing not only polychaetes with low mobility that spend most of their lives in tubes built of cemented sand grains but also the Clitellata (earthworms and leeches) (Weigert & Bleidorn, 2016; Kuo, 2017; Magalhães et al., 2021). Sedentary polychaetes commonly build a tube where they spend most of their life, leaving for only short periods of time (Viéitez et al., 2004). This is only possible because they have modified body structures, such as antennae, palps and cirri, that make it possible to sustain their necessities without having to leave the tube. These structures are located at distinct body regions, originating what is known as heteronomous segmentation (Viéitez et al., 2004; Rouse & Giribet, 2016).

On the other hand, errant polychaetes carry a pair of bilobated paddle-like structures called parapodia (parapodium as singular noun), that they use to “swim” and move on the sea bottom, displaying high mobility. Errant polychaetes lack a clear differentiation of body regions, presenting homonomous segmentation (Viéitez et al., 2004; Rouse & Giribet, 2016). Usually, the parapodia of errant polychaetes are much more developed than those of sedentary polychaetes, providing them with greater motility, helping them to prey on or escape from other organisms. These polychaetes usually show a more developed cephalization process developing several types of sense organs that help them orientate in the surrounding environment in search for food, to evade predation, and locate potential partners (Purschke, 2005), whereas in sedentary polychaetes these processes are reduced or absent (Viéitez et al., 2004).

One of the most remarkable families of errant polychaetes is Nereididae Blainville, 1818, which members are characterised by powerful jaws and muscular bodies, capable of creating borrows and hunting prey effectively, usually being omnivore, detritivore or carnivore (Costa et al., 2021; Rouse et al., 2022). Nereidids have great ecological diversity and can occupy various niches in marine and estuarine environments, due to their diverse feeding strategies (Fauchald & Jumars, 1979) and reproductive behaviours (Rouse & Pleijel, 2006). These annelids are used in many economic activities, namely as bait in recreational and commercial fishing and as feed in aquaculture due to their high attractiveness to fish and nutritional value (Fidalgo e Costa et al., 2003). The introduction of nereidids in the diet of aquaculture animals can add essential nutrients

and proteins that improve growth rate, reproductive success and health (Jerónimo et al., 2021). Recent studies also show that polychaetes can be used to help mitigate pollution related to aquaculture production as they can feed on the excess nutrients present in the water of aquaculture effluents and integrated aquaculture (Marques et al., 2017; Nederlof et al., 2019; Wang et al., 2019).

Nereidid polychaetes are also commonly used as test subjects in many different studies, including environmental and pollution essays, drug testing and anatomy, reproduction and development studies (Sato, 1999; Levin, 2006; Pires et al., 2016; Silva et al., 2020).

Within the family Nereididae, the genus *Hediste* Malmgren, 1867 commonly known as “ragworms”, is one of the most studied, checking all the boxes on previously mentioned important ecological and economical roles (Scaps, 2002). Members of this genus can be found worldwide in estuarine and intertidal environments (Ashley & Budd, 2020). Various species are included in this genus, displaying differences in their habitat, reproductive strategy, diet and life cycle. The species *Hediste japonica* (Izuka, 1908) is found in northwestern Pacific; *Hediste atoka* Sato & Nakashima, 2003 in the northern Gulf of Mexico; *Hediste diadroma* Sato & Nakashima, 2003 is native to the coasts of northeastern Asia, including Japan, Korea, and eastern Russia (Sato & Nakashima, 2003); *Hediste limnicola* (Johnson, 1903) is found in the northeastern Pacific (Rudy et al., 2013); *Hediste rabatensis* (Mohammad, 1989) occurs in Morocco (Mohammad, 1989), and *Hediste diversicolor* (O. F. Müller, 1776) spreads throughout the European and North African Atlantic coastline and Baltic (Teixeira et al., 2022). It also occurs in the Atlantic coast of North America where it was introduced (Einfeldt et al., 2014). Recently, another two species were identified: *Hediste astae* in the Aegean Sea and *Hediste pontii* in the Adriatic Sea (Teixeira et al., 2022; Read & Fauchald, 2024).

The common ragworm *Hediste diversicolor* (O.F. Müller, 1776) is a common infaunal species in shallow marine and estuarine sandy and muddy sediments. The vast geographical distribution is possible due to the species tolerance to a large variation in abiotic factors, such as temperature, salinity and dissolved oxygen. This species occupies an important position in the trophic chain and its burrowing activity improve sediment reworking and oxygenation (Fidalgo e Costa et al., 1998; Scaps, 2002; Gilbert et al., 2021). A maximum length of 10–11 cm was reported in field studies, and it seems that usually they have no more than 100–110 segments (Clark & Scully, 1964; Abrantes et al., 1999). The common ragworm is an omnivorous and opportunistic species with diverse feeding modes, such as filter-feeding, deposit feeding, scavenging and

predation. Consequently, this polychaete has a wide diet range that includes detritus, bacteria and other microorganisms, macroalgae, salt marshes plants, small crustaceans and other invertebrates (Fidalgo e Costa et al., 2006; Jumars et al., 2015). In turn, it is preyed upon by crustaceans, birds, and fishes (Scaps, 2002). Thus, *H. diversicolor* is commonly exploited as bait for both professional and recreational fishing, having economic value and potential for use as ingredient in aquaculture feeds (Carvalho et al., 2013; Pombo et al., 2020).

Justification and objectives

Although *H. diversicolor* is a species commonly used in fisheries, aquaculture, and other activities of economic interest, there is a need to deepen our knowledge of its development throughout its life cycle. The objective of Chapter I: “Biometric Analysis of various body structures of the polychaeta *Hediste diversicolor* (Müller, 1776)”, is to identify and evaluate distinct developmental trends, attempting to explore the development and growth patterns of various structures with different functionalities. This procedure is the first exploratory characterization of the biometry of multiple structures of *H. diversicolor*, from the prostomium, peristomium, chaetiger segments and pygidium. Relative to this objective, the hypothesis of certain morphological characteristics becoming noticeable in key times associated with the crescent number of chaetiger segments between life cycle stages was put forward.

Besides that, in general, most of the studies carried out on polychaetes are aimed at taxonomy, where the description of species focuses mainly on external morphological characteristics, so ultrastructural studies are less common, and there is a lack of ultrastructural knowledge of the various structures and organs present in these organisms. Therefore, the aim of Chapter II: “Investigating the palps of *Hediste diversicolor* (Annelida; Errantia) through histochemistry and transmission electron microscopy” is to study the histology and histochemistry of sensory appendages in greater detail to help better understand the types of cells associated with these organs. The palps are one of the most important sensory appendages for nereidids, such as *H. diversicolor*, as they are used for orientation, locomotion and feeding. The study of the ultrastructure of the palps therefore adds to our knowledge of the cells that make up this organ, which already has known sensory functions. In addition, these organisms are already a standardized model for toxicological studies. Knowing the ultrastructural organization of the average individual will help to obtain a standard model for

comparison, to evaluate the effects of drugs and other composites or pollutants may have on the ultrastructure, function and histology of their organs.

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Chapter I: Biometric analysis of various body structures of the polychaeta *Hediste diversicolor* (O. F. Müller, 1776)



1.1. Abstract

Hediste diversicolor (O. F. Müller, 1776) is a species of polychaete belonging to the family Nereididae found in estuarine environments along the European Atlantic coast. This species has several appendages with sensory functions along the body, that help the animal navigate its environment. It also has specialized feeding structures in an eversible proboscis, called paragnaths, that are essential for taxonomic identification. The parapodia showcase distinct chaeta arrangements that distinguish them from other species in the same genus. Due to the importance for many economic activities such as fishing and aquaculture, this species is used in and usual protagonist for studies of multiple areas, having a well-documented life cycle. However, knowledge is lacking on the growth and evolution of individual structures and appendages of the body in this species along its life cycle. Thus, the aim of this study is to document the first exploratory characterization of the biometry and anatomy of morphological structures of *H. diversicolor*, using the number of chaetiger segments as an indicator of growth. Results revealed a significant positive correlation between the number of chaetiger segments and most of the morphological structures analysed, evidencing a proportional growth of most of these features, so that their function can be maintained and adapted to the ever-increasing size of the animal. Simple chaetae, a specific type of chaeta only present in genus *Hediste*, were only found in individuals with more than 25 chaetiger segments, possibly indicating a functional change as the worm matures. Simple chaetae tend to be found earlier along the body axis, closer to median chaetigers as the organism matures. The results illustrate how *H. diversicolor* body develops over time, highlighting the distinct developmental processes and evolutionary adaptability that differentiate it from other species within the genus *Hediste*.

Keywords: Developmental biology; Life cycle; Nereididae; Ragworm; Simple chetae

1.2. Introduction

Polychaetes (in ancient Greek = “many chaeta”) are a very diverse group of annelid worms, many of which bearing bristle-like structures called chaetae. They are divided into two main clades: sedentary and errant. Errant polychaetes, which are more mobile due to well-developed parapodia, typically have a more developed cephalisation process with well-defined eyes, palps and antennae, while sedentary polychaetes have a reduced or absent cephalisation process and parapodia (Viéitez et al., 2004). The anterior region of the errant polychaetes comprises the peristomium, which houses the mouth and various sensory structures, and the prostomium, which contains numerous sensory organs and structures that contribute to the characterization of different families (Fig.1).

The family Nereididae Blainville, 1818 is a prominent group of errant polychaetes. Members of this family have a diamond-shaped prostomium with rounded posterior corners that fuses with the peristomium (Fauchald & Rouse, 1997). The prostomium includes two pairs of eyespots arranged in a trapezoid shape, with the posterior pair positioned closer to each other, two antennae, a pair of conical, bi-articulated palps (Fig.1), and small, short ciliated grooves, known as nuchal organs, located posteriorly to the eyespots. The peristomium has usually four pairs of cylindrical tentacular cirri, but this number varies depending on the taxa. Still in the peristomium the mouth has the opening on the ventral side (Fig.1). The eversible muscular proboscis or pharynx, which has chitinous jaws and two distinct rings when everted, is essential for feeding and is

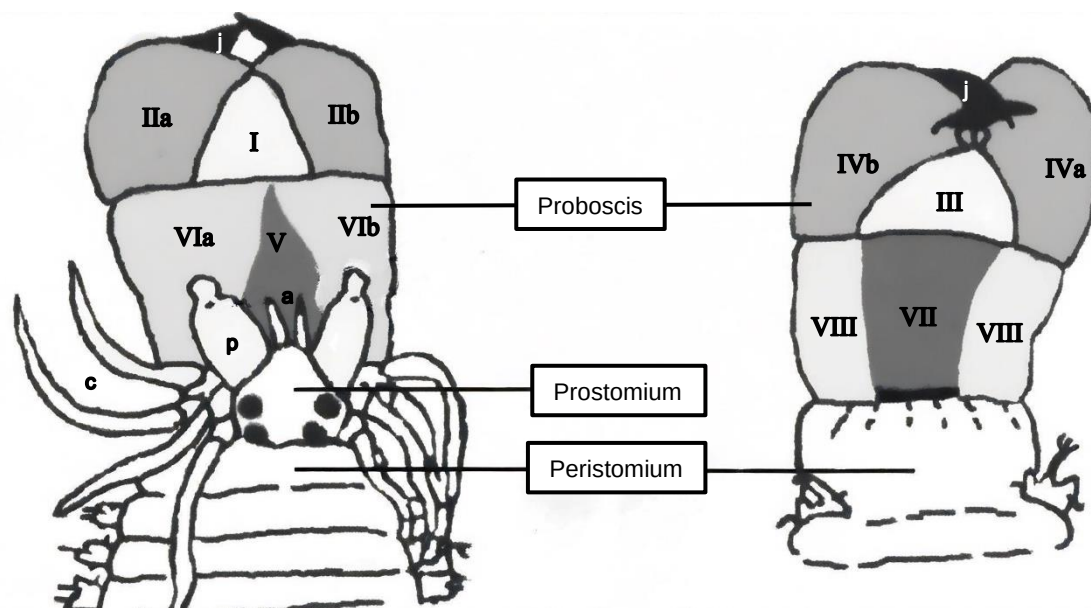


Figure 1. Drawing representing prostomium and peristomium structures of a nereidid polychaete, with the distribution of papillae and paragnaths in the eversible proboscis rings. a, antennae; c, peristomial cirri; j, jaw; p, palp. Source: Adapted from Viéitez, et al. (2004).

used to differentiate taxonomic groups within the family, based on the arrangement of its papillae and/or paragnaths (Fauchald & Rouse, 1997; Rouse & Giribet, 2016). The positions of paragnaths in said rings are extremely important for the taxonomy inside the nereidids. The paragnaths are typically arranged in well-defined areas in both pharyngeal rings (Bakken et al., 2009). A total of eight different areas are distinguishable (usually identified with roman numbers). The number, type and arrangement of the paragnaths in each area are extremely important characters to differentiate genera and species within the family nereididae (Viéitez et al., 2004).

The genus *Hediste* Malmgren, 1867 shares many characteristics with the genera *Nereis* Linnaeus, 1758 and *Neanthes* Kinberg, 1865, to which *H. diversicolor* has been previously assigned, such as the presence of conical paragnaths on both rings of the proboscis, the morphology of parapodia, and the types of chaetae (Viéitez et al., 2004). A distinctive feature of *Hediste* is the presence of a simple chaetae (Fig.2E, J) above the neuropodial acicula on posterior parapodia, along with other types of chaetae such as homogomph spiniger, heterogomph spiniger, and heterogomph falciger.

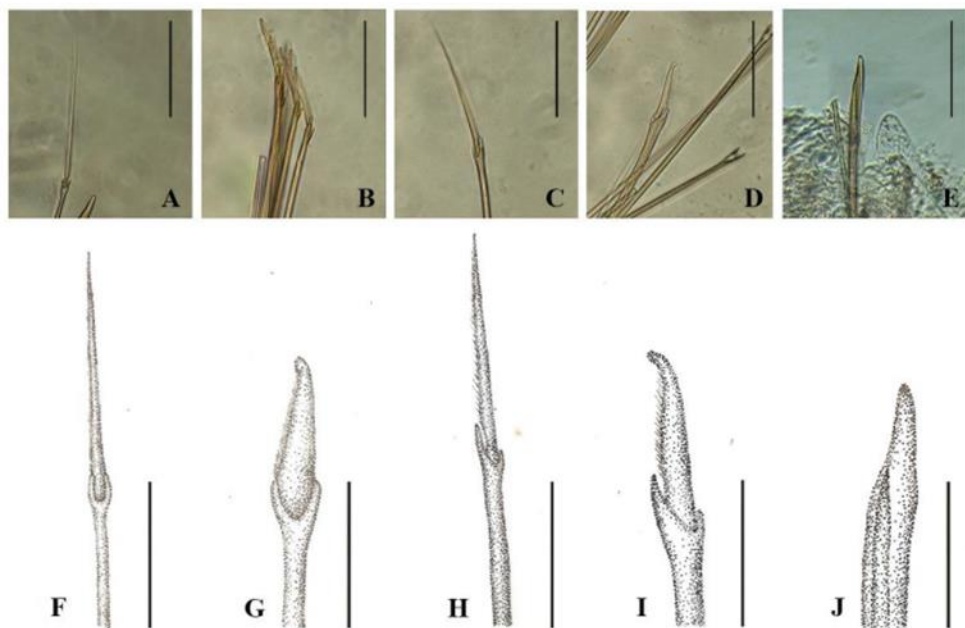


Figure 2. Types of chaetae in nereidid polychaetes. Photos: (A) Homogomph spiniger, (B) Homogomph falciger, (C) Heterogomph spiniger, (D) Heterogomph falciger, (E) Fused "simple" chaetae; Illustrations: (F). Homogomph spiniger, (G) Homogomph falciger, (H) Heterogomph spiniger, (I) Heterogomph falciger, (J) Fused "simple" chaetae. Scales: A–E: 0.1mm, F–J: 0.05mm. Source: unpublished data, Dimitri Costa (2022)

The species *Hediste diversicolor* can be found along the European Atlantic coast in estuarine and brackish areas. Its body can reach up to 10 cm in length and 6 mm in width, and is robust, slightly flattened and tapering towards the posterior end, comprising an average of 90-95 and a maximum of 100–110 chaetiger segments in fully grown

specimens (Clark & Scully, 1964; Abrantes et al., 1999; Viéitez et al., 2004). The prostomium has two short antennae between a pair of palps that are tapered and biarticulated, having an anterior region called palpostyle, wider than long, which is smaller than the posterior region named palpophore. It has two pairs of eyespots set up in a trapezoid shape, being the posterior pair closer together than the anterior one (Fig.1). The peristomium has approximately the same length as the rest of the chaetiger segments. There are four pairs of tentacular cirri formed from the peristomium, with cirrophore wider and shorter than cirrostyle. The posteriormost pair of cirri is wider and longer than the rest. The proboscis has a pair of brown jaws with teeth (serrated). Conical paragnaths can be found on all areas of both proboscis rings, with exception for area V. The chaetiger segments have biramous parapodia with slight differences in shape and length along the body (Fig.3). Each notopodium in the first parapodia is composed of notopodial cirrus and a notopodial ligula, without acicula or chaetae (Fig.3A). From the third chaetiger onwards notopodium has acicula, two notopodial ligules, one above and one below the acicula and a pre-chaetal lobule, with homogomph spiniger chaetae (Fig.2A, F). The neuropodium has acicula and its composed by neuropodial cirrus, a pre-chaetal and a post-chaetal lobule and a neuropodial ligule (Fig.4A). Supra-acicular chaetae are from the homogomph spiniger and heterogomph falciger type (Fig.2D, I), although on the posterior chaetiger segments simple chaetae (Fig.2E, J) can appear. Infra-acicular chaetae are from the heterogomph spiniger and falciger (Fig.2C, D, H, I), although rarely homogomph falciger (Fig.2B, G) can rarely appear. Neuropodium tends

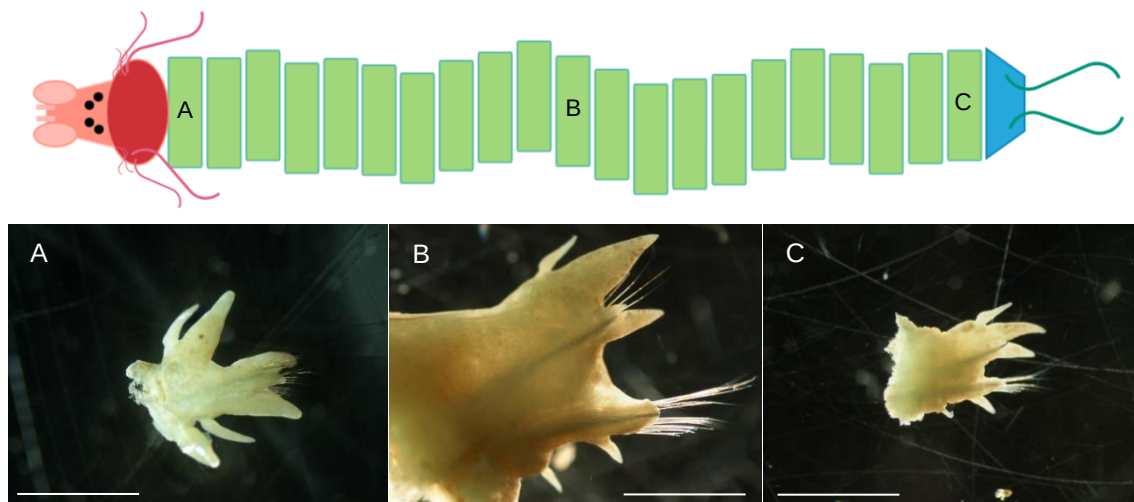


Figure 3. – a) Simplified scheme of *Hediste diversicolor*. Colours refer to different region of the body: Pink - prostomium; Red - peristomium; Green - chaetiger segments; Blue - pygidium; and b) the appearance of different parapodia along the body axis: (A) First anterior parapodium, (B) Median parapodium, (C) Posterior parapodium. Scales: A – C: 1 mm.

to become smaller compared to the notopodium the further posterior the segment (Fig.3) (Viéitez et al., 2004). The last segment is an achaetiger that contains the pygidium, composed by a pair of long anal cirri and the anal orifice (Fig.4B) (Viéitez et al., 2004).

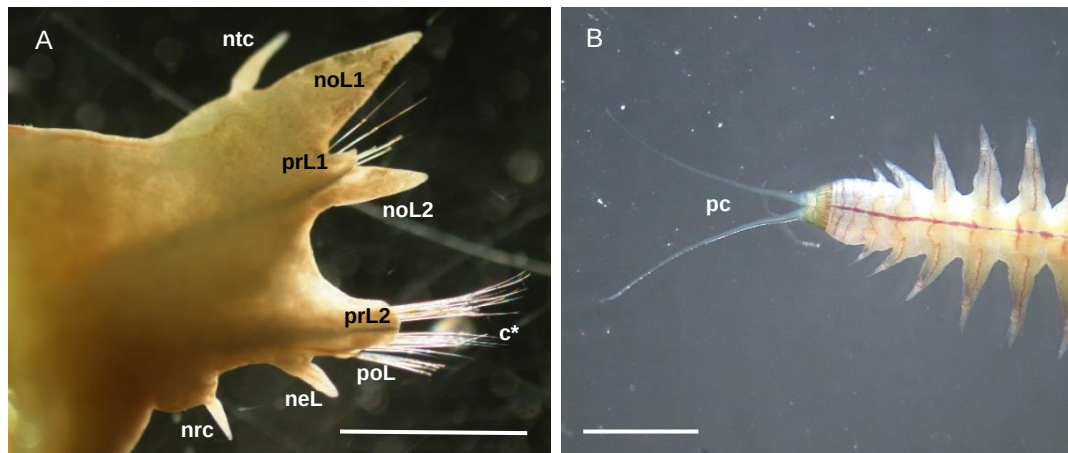


Figure 4. Photographs of *H. diversicolor*, representing: (A) Median parapodium structures: ntc, notopodial cirrus; noL1, notopodial ligule; prL1, pre-chaetal lobe 1; noL2, notopodial ligule 2; prL2, pre-chaetal lobe 2; c*, chaetae; poL, post-chaetal lobe; neL, neuropodial ligule; nrc, neuropodial cirrus; (B) pygidium with pygidial cirrus. pc, pygidial cirri. Scales: A – 1mm; B – 2 mm

This species reproduces only once in a lifetime, with individuals dying shortly after reproduction. They use up to seventy per cent of their total energy in the production of gametes inside the celoma, consequently causing an increase in body mass and a stop to their growth (Fidalgo e Costa, 2003). Sexual maturation is induced by the increase in temperature and the spawning is influenced by the lunar cycle (Dales, 1950). Sexual maturation is reached at an age between one and two years. Both male and female of *H. diversicolor* are indistinguishable before maturation, presenting a reddish-brown colour. After maturation males acquire a light green colour and females a darker green (Rouse & Pleijel, 2006). This difference is explained by the increase of white sperm in the celoma of males (Scaps, 2002).

Unlike other nereidids, *H. diversicolor* lacks an epitoke stage or planktonic larvae stage (swimming stage), having a fully benthonic life cycle. Mature females lay their eggs inside the galleries and mature males lay sperm at the entrance of the galleries. Then females increase their ventilatory activity inside the galleries leading sperm into the eggs and increasing the chance of fertilization (Scaps, 2002). Fertilized eggs develop quickly inside the galleries, still protected by the females until they die. Larvae hatch with 3 chaetiger segments at the trochophore stage and develop (first chaetae appearance; sense organs like eyes, palps, antennae, dorsal and anal cirrus; formation of the jaws; increase in length) while feeding on mucus and bacterial particles inside the gallery. At 10 to 14 days post-hatching, larvae have 6 to 8 chaetiger segments (Bartels-Hardege &

Zeeck, 1990; Wang. et al., 2020) and are ready to leave the parental galleries (Bartels-Hardege & Zeeck, 1990; Scaps, 2002; Hesselberg, 2003). It is at this developmental stage that they start showing burrowing behaviour, carving their own borrows in the substrate (Scaps, 2002).

This annelid is an important part of the trophic chain, serving as prey for various organisms and contributing to the recycling of organic matter in its habitat (Fauchald & Jumars, 1979; Dierschke et al., 1999; Jumars et al., 2015). Additionally, *H. diversicolor* is used as bait in fishing, impacting geographic communities through the dissemination of different populations (Fidalgo e Costa et al., 2003; Font et al., 2018).

While progress has been made in understanding the nervous system and sense organs of mature annelids, studies concerning the development of these structures throughout the life cycle remain limited. Research in this field is crucial for evolutionary development studies and for assessing environmental impacts on organism development (Winchell et al., 2010; Pires et al., 2016).

The present study aims at being the first exploratory characterization of the biometry and anatomy of morphological structures of *H. diversicolor*. By examining these structures across animals at different developmental stages, with different sizes and number of chaetiger segments, we seek to explore and identify morphological changes and potential differences that improve our knowledge on their growth and development.

1.3. Material and methods

1.3.1. Sampling Location and Sites

The research area chosen was the estuary of the river Minho. Located on the northwest coast of the Iberian Peninsula, the Minho river creates a natural border between Portugal and Spain for an 80 km downstream section before flowing into the Atlantic Ocean. The estuary has a maximum width of approximately 2 km next to the mouth. Tidal influence extends approximately 40 km upstream, comprising a total estuarine area of 23 km². The estuary is mesotidal, characterized by a predominantly semidiurnal tidal regime, with a variation between 2 meters at low tides and 4 meters at spring tides, and its maximum depth is 11 meters below the average sea level (Díaz et al., 2020). Sampling sites were selected along the Minho river estuary according to the already-known existence of *H. diversicolor* in the area. Thus, three sites were chosen: Baía de Camarido, Caminha

(41°52'01.2"N 8°51'00.3"W); Pedras Ruivas (41°53'28.4"N 8°49'30.8"W); and Ilha da Boega (41°55'28.2"N 8°46'13.8"W) (Fig.5).



Figure 5. Study area in Minho river, displaying the points selected for sampling. 1 - Baía de Camarido, Caminha (41°52'01.2"N 8°51'00.3"W); 2 – Pedras Ruivas (41°53'28.4"N 8°49'30.8"W); 3 – Ilha da Boega (41°55'28.2"N 8°46'13.8"W). Source: Google Earth. (2022). Google Earth (Version 7.3.6.9326) [Software]. Retrieved December 14, 2022, from <https://earth.google.com>

The sediment at these sites consisted of a mixture of sand and mud with a few large rocks. The exact point for collection of sediment samples was selected upon the identification of bioturbation in the sediment that is typical of this species (Fig.6A, B) (Hesselberg, 2003).



Figure 6. Bioturbation on the sediment: (A) Transversal section of a borrow inside the sediment, (B) *H. diversicolor* exiting the borrow through the surface opening.

1.3.2. Sample Collection

Sediment samples were gathered using shovels and placed in buckets to ease the transportation (Fig.7).



Figure 7. Sampling technique used for sediment collection.

Immediately after arrival at the laboratory, the samples were sorted to protect the organisms from desiccation stress. To ease the collection of the polychaetes, small amounts of sediment were placed in plastic trays where water with matching salinity with the sampling site was added. Annelids were placed in beakers with a premixed water solution to mimic the salinity of the estuary (salinity 22–24 ppm). Specimens with different numbers of segments were selected to ensure that the entire range of chaetiger numbers was represented. These animals were then preserved in 70% alcohol for further analysis. The rest was sent to a purposely build monoculture tank.

1.3.3. Species Identification

A traditional morphological identification was carried out using dichotomous and identification keys from relevant literature to guarantee that all the worms analysed belonged to the target species (e.g. Viéitez et al., 2004). Photographs were taken using a stereo microscope (Leica EZ4W, Germany) to document the essential taxonomic characteristics of *H. diversicolor*.

1.3.4. Monoculture Tank Setup

A monoculture tank with capacity for 200 Litres was set up to hold specimens at different stages of development collected from the estuary. A brackish water solution (salinity 22–24 ppm) was placed in the tank. To keep oxygen at adequate levels and the water clean, the tank was equipped with an aeration pump and a filtration system (Fig.8A). After the sediment from one of the sampling sites had been carefully examined and cleaned of

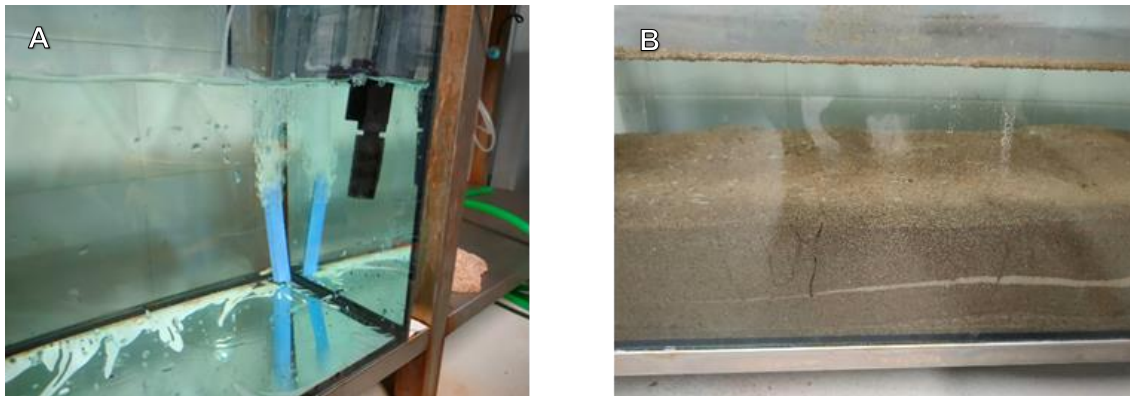


Figure 8. Monoculture tank setup. (A) Tank equipped with aerator and filtration pump filled with a brackish water solution, (B) Tank with sediment and polychaetes. Possible to observe bioturbation formed by the annelids in the sediment.

competing species, it was used as a substrate for the tank (Fig.8B). The polychaetes were fed once a day with SERA® Pond Flakes Nature. The density of polychaetes in the tank was not controlled during the experiment, being possible for the animals to reproduce in the tank. A multiparameter probe was used to monitor water quality and some parameter such as pH, temperature, conductivity, dissolved oxygen, and salinity. Whenever necessary, the salinity was adjusted, adding salt water or adding fresh water conform needed to keep it in a range close to the observed in the Rio Minho estuary.

1.3.5. Biometric and Anatomical Analysis

Biometric analyses were conducted according to the standard descriptive methodology for the family Nereididae (Viéitez et al., 2004) using an optical microscope (Nikon SMZ800, Japan) and stereo microscope (Leica EZ4W, Germany). Measurements were performed using the software ImageJ. The total length of each specimen was measured, and the number of chaetigers counted, as well as the number of paragnaths, and their relative position in the eversible proboscis noted. The maxillae were also measured in width and length and the number of teeth counted. Both sections of the palps (palpophore and palpostyle) were measured for width and length. The width and length of the lateral antennae were measured as well. In the peristomium, the length and width of the cirrophore and cirrostyle was measured for each individual cirrus. A detailed anatomical analysis of parapodia was performed. A set of seven parapodia were chosen according to their relative position, these being the first anterior parapodium; a parapodium in the transition zone between anterior parapodia and median parapodia; a median parapodium located at a distance of the same number of chaetiger segments between the anterior-median transition parapodium and the median-posterior transition parapodium; a median-posterior parapodium; a posterior parapodium; the parapodium immediately preceding the appearance of the simple chaetae; and the first parapodium

with simple chaeta. Parapodia measurements included the width and length of notopodial and neuropodial cirri, notopodial supracicular ligule (noL1), notopodial pre-chaetal lobe (prL1), notopodial subacicular ligule (noL2), post-chaetal lobe (poL), neuropodial pre-chaetal lobe (prL2) and neuropodial subacicular ligule (neL).

The length of the notopodial and neuropodial aciculae were noted when present. The types of chaetae in each parapodial section (notopodial, neuropodial, supra-acicular, and infra-acicular) were noted. If the analysed animals had suffered any trauma prior to the analysis or if the animals had a low number of chaetiger segments, then only the anterior, median and posterior parapodia were sampled. If the animals did not have simple chaeta, these parapodia were not sampled either. Finally, the width and length of pygidium cirri were measured, including the cirri segment lengths. A total of 30 specimens of *H. diversicolor* were biometrically analysed. Due to the size of the different animals, certain characteristics were very difficult to count, such as the number of paragnaths, as these become more markedly coloured during the development of the organism. Other structures such as the parapodia become difficult to differentiate visually when the polychaetes are at an earlier stage of maturation. Some of the animals analysed appeared to have suffered trauma prior to analysis, which is why certain structures were sometimes absent. The number of organisms analysed therefore differs according to the target structure analysed.

1.3.6. Statistics Analysis

The data was organised and analysed using Microsoft Excel™. Length and width measures of each structure covered by this study, as well as other important quantitative data such as the number of paragnaths per area of the proboscis and number of teeth in each maxilla, were analysed by Shapiro-Wilk and Anderson-Darling Normality tests to check for normal distribution, considering a significance value of $p < 0.05$. Homoscedasticity was evaluated by creating a linear regression between the number of chaetiger segments and the measures obtained from each structure in order to obtain fitted values and residuals. With these values a XY scatter plot was created to determinate if the variance was constant. Based on the normal data distribution and homoscedasticity, the parametric linear Pearson correlation test (r) was applied, considering a significance value of $p < 0.05$. All statistic tests were performed using PAST software (v.4.13, Hammer et al., 2001).

1.4. Results

All the data gathered from the quantification and measurement of the *H. diversicolor* structures can be seen in the supplementary material tables (Table S1. – S6.).

1.4.1. Prostomial structures analysis

Data corresponding to the average increase in each measure of the prostomial structures per each chaetiger segment acquired by growth can be observed in the Table 1.

Table 1. Increase in length and width of each studied structure from the prostomium per each new chaetiger segment acquired by growth.

		Increase per each new chaetiger	
		Length (mm)	Width (mm)
Palp	Structure		
	Lateral Antennae	0.0047	0.0017
	Left Palpophore	0.0096	0.009
	Right Palpophore	0.0097	0.0089
	Left Palpostyle	0.0016	0.0014
	Right Palpostyle	0.0026	0.0024

In the prostomium, lateral antennae measurements shown a significant positive correlation (Pearson parametric test correlation: $p \leq 0.5$) between the increase of length and width of the antennae and the number of chaetiger segments (Chart 1.). Regarding the palps, there was also a significant positive correlation ($p \leq 0.5$) between the length and width of these structures in relation to the number of chaetiger segments (Chart 2., 3.).

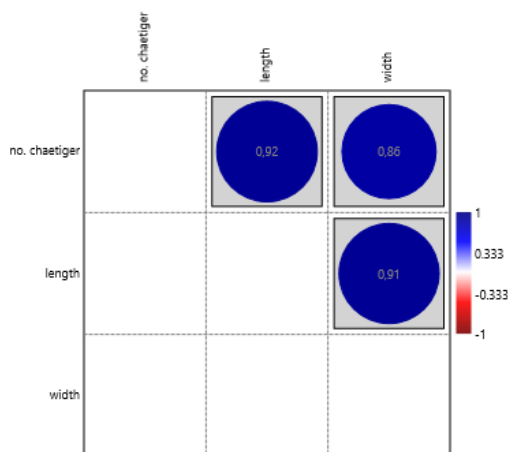


Chart 1. Correlation between the length and width of the lateral antennae and the number of chaetiger segments

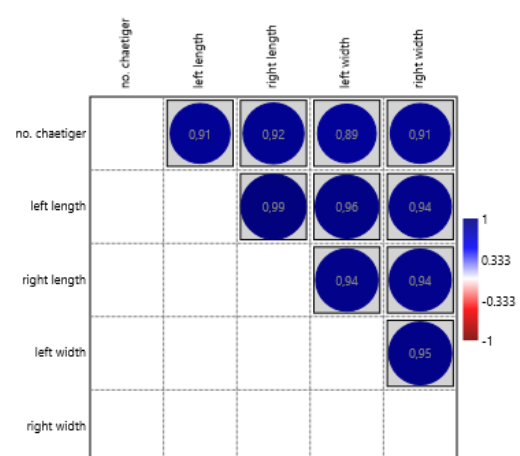


Chart 2. Correlation between the length and width of the right and left palpophore and the number of chaetiger segments

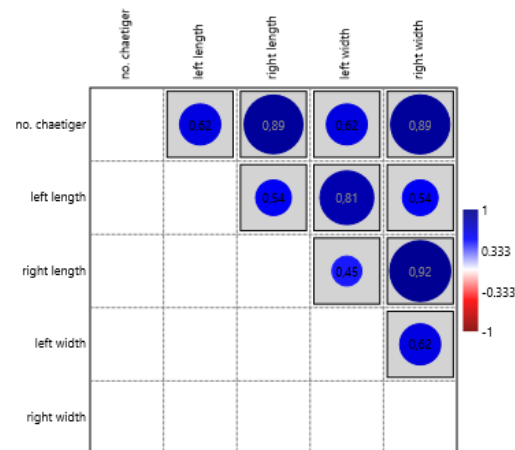


Chart 3. Correlation between the length and width of the right and left palpostyle and the number of chaetiger segments

1.4.2. Feeding structures analysis

On average, for each new chaetiger segment, there was an increase of 1.6967 in the number of paragnaths. A positive correlation was detected between the number of chaetigerous and the number of paragnaths in each section of the proboscis ($p \leq 0.5$). This correlation was significant in all the positions of the proboscis except VIb ($r = 0.32$) (Chart 4.).

Jaw average length increase per new chaetiger segment was 0.0127 mm. A significant positive correlation between the length of each jaw and the number of chaetiger segments was detected. The same was not observed between the number of teeth in each jaw in relation to the chaetiger number where the r value of the correlation was close to zero ($r = -0.03$ for no. of teeth a; $r = -0.10$ for no. of teeth b) (Chart 5.). The number of teeth in each jaw varied between 5 and 9, being the most common amount 8 teeth per jaw.

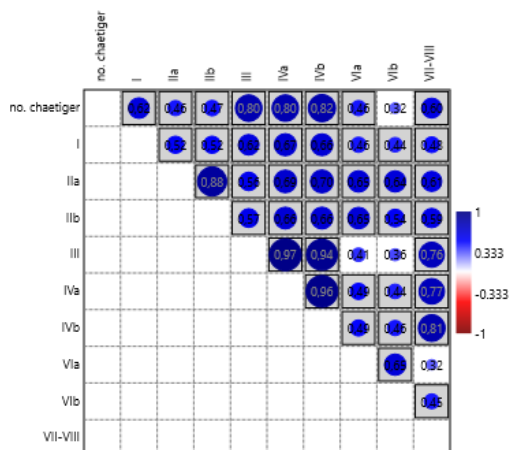


Chart 4. Correlation between the number paragnaths per section of the proboscis with the number of chaetiger

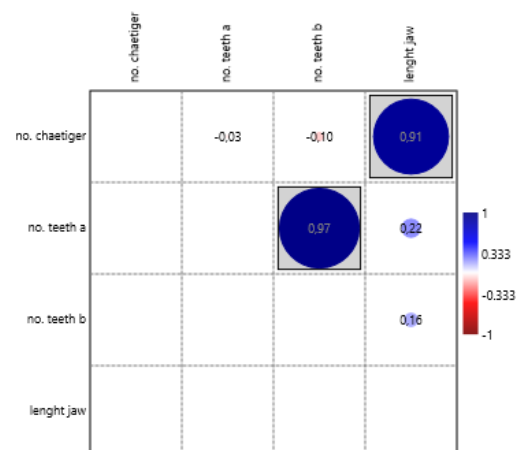


Chart 5. Correlation between the number of teeth from the right and left, and length of the jaw with the number of chaetiger segments

1.4.3. Peristomial structures analysis

Table 2. showcases the average increase of measured peristomial structures for each new formed chaetiger segment.

Table 2. Increase in length and width of each studied structure from the peristomium per each new chaetiger segment

		Increase per each new chaetiger	
	Structure	Length (mm)	Width (mm)
Cirri 1	Left Cirrophore	0.0024	0.0072
	Right Cirrophore	0.0025	0.0071
	Left Cirrostyle	0.0086	0.0018
	Right Cirrostyle	0.0084	0.0017
Cirri 2	Left Cirrophore	0.0027	0.0027
	Right Cirrophore	0.0033	0.0029
	Left Cirrostyle	0.0205	0.0017
	Right Cirrostyle	0.017	0.0018
Cirri 3	Left Cirrophore	0.0021	0.0023
	Right Cirrophore	0.0023	0.0022
	Left Cirrostyle	0.0159	0.0016
	Right Cirrostyle	0.0133	0.0017
Cirri 4	Left Cirrophore	0.0038	0.0027
	Right Cirrophore	0.0042	0.0029
	Left Cirrostyle	0.0292	0.0019
	Right Cirrostyle	0.0272	0.0017

For all the modified peristomial cirri, both the length and width of the cirrophore and cirrostyle had significant positive correlations with the increasing number of chaetiger (Charts 6.–13.).

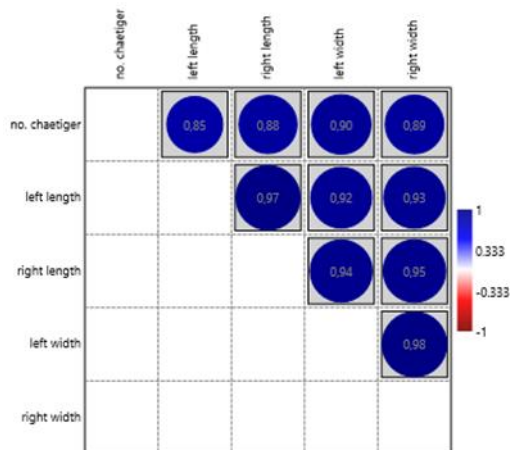


Chart 6. Correlation between the length and width of the first pair of peristomial cirri cirrophore and the number of chaetiger segments

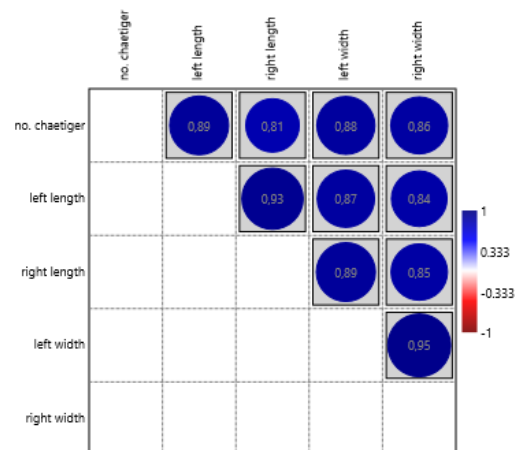


Chart 7. Correlation between the length and width of the first pair of peristomial cirri cirrostyle and the number of chaetiger segments

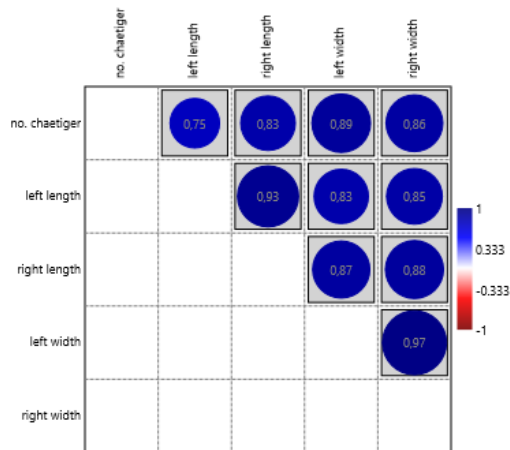


Chart 8. Correlation between the length and width of the second pair of peristomial cirri cirrophore and the number of chaetiger segments

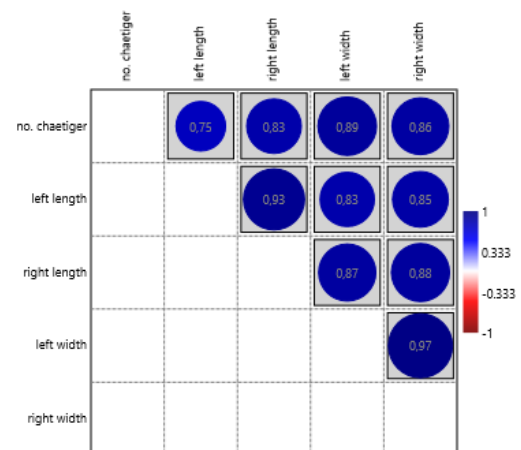


Chart 9. Correlation between the length and width of the second pair of peristomial cirri cirrophore and the number of chaetiger segments

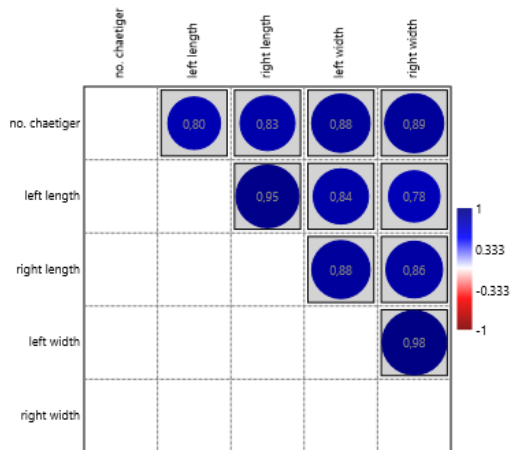


Chart 10. Correlation between the length and width of the third pair of peristomial cirri cirrophore and the number of chaetiger segments

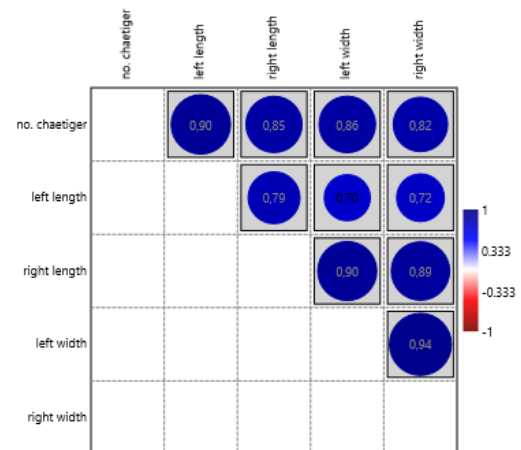


Chart 11. Correlation between the length and width of the third pair of peristomial cirri cirrophore and the number of chaetiger segments

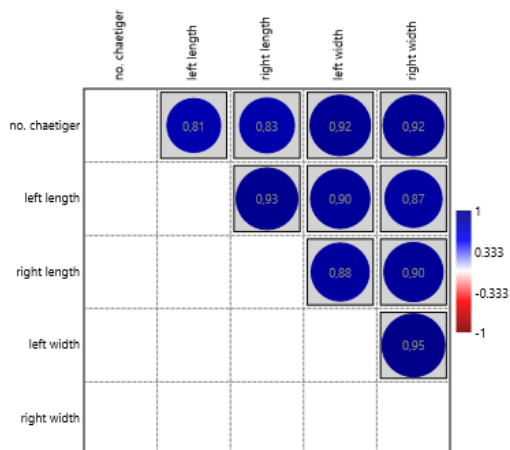


Chart 12. Correlation between the length and width of the fourth pair of peristomial cirri cirrophore and the number of chaetiger segments

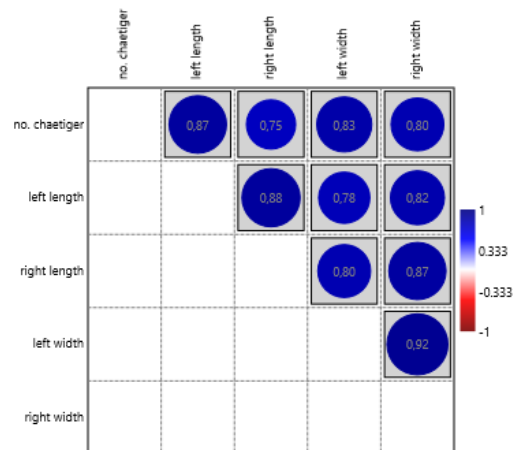


Chart 13. Correlation between the length and width of the fourth pair of peristomial cirri cirrophore and the number of chaetiger segments

1.4.4. Parapodia analysis

The average growth of the studied structures of each parapodia of different body sections for each new chaetiger segment can be seen in Table 3 and 4.

Table 3. Increase in length and width of each studied structure from various parapodium of different body sections per each new chaetiger segment

		Increase per each new chaetiger		
		Structure	Length (mm)	Width (mm)
Anterior	Noto.	Noto. Cirri	0.0054	0.0013
		noL1	0.008	0.0037
	Neuro.	Neuro. Cirri	0.0057	0.0013
		prL2	0.0073	0.0022
		poL	0.0078	0.0021
		neL	0.0074	0.0029
		Acicula	0.0108	-
Transition Anterior-Median	Noto.	Noto. Cirri	0.0034	0.0007
		noL1	0.0205	0.0065
		prL1	0.004	0.0018
		noL2	0.019	0.0032
		Acicula	0.017	-
	Neuro.	Neuro. Cirri	0.0023	0.0009
		prL2	0.0181	0.0033
		poL	0.0197	0.0022
		neL	0.0147	0.002
		Acicula	0.019	-
Before Simple chaetae	Noto.	Noto. Cirri	0.0038	0.0011
		noL1	0.0233	0.0059
		prL1	0.0045	0.0021
		noL2	0.0182	0.003
		Acicula	0.0254	-
	Neuro.	Neuro. Cirri	0.0035	0.0016
		prL2	0.0154	0.0027
		poL	0.015	0.0029
		neL	0.0142	0.0017
		Acicula	0.0235	-

Table 4. Increase in length and width of each studied structure from various parapodium of different body sections per each new chaetiger segment (continued)

		Increase per each new chaetiger		
		Structure	Length (mm)	Width (mm)
First With Simle Chaetae	Noto.	Noto. Cirri	0.004	0.001
		noL1	0.0225	0.0047
		prL1	0.0049	0.0017
		noL2	0.0177	0.0027
		Acicula	0.0255	-
	Neuro.	Neuro. Cirri	0.0036	0.0009
		prL2	0.0154	0.0016
		poL	0.0149	0.0015
		neL	0.0115	0.0017
		Acicula	0.0253	-
Median	Noto.	Noto. Cirri	0.0036	0.0009
		noL1	0.018	0.0053
		prL1	0.0046	0.0016
		noL2	0.0163	0.0026
		Acicula	0.0235	-
	Neuro.	Neuro. Cirri	0.0018	0.0008
		prL2	0.0128	0.0018
		poL	0.0124	0.0023
		neL	0.0089	0.0016
		Acicula	0.0226	-
Transition Median-Posterior	Noto.	Noto. Cirri	0.0017	0.0006
		noL1	0.0128	0.0029
		prL1	0.0006	0.0003
		noL2	0.0103	0.0016
		Acicula	0.0163	-
	Neuro.	Neuro. Cirri	0.0013	0.0008
		prL2	0.008	0.0002
		poL	0.0077	0.0004
		neL	0.0064	0.0007
		Acicula	0.0146	-
Anterior	Noto.	Noto. Cirri	0.0045	0.0009
		noL1	0.0116	0.002
		prL1	0.0015	0.0004
		noL2	0.0106	0.0015
		Acicula	0.0123	-
	Neuro.	Neuro. Cirri	0.0025	0.0005
		prL2	0.0075	0.0008
		poL	0.0076	0.001
		neL	0.0072	0.001
		Acicula	0.0123	-

Results obtained from measuring each structure of the parapodia revealed that there is a positive correlation between the length and width of these structures and the number of chaetiger segments in both the notopodium and the neuropodium (Charts 14.–27.). In the first parapodium of the anterior-median region, a significant positive correlation ($p \leq 0.5$) between the length and width of all the structures analysed and the number of chaetiger segments was detected (Chart 16., 17.). In the notopodium of the anterior-median parapodium, both the width of the cirrus and the width of the prL1 correlated positively but not significantly ($p > 0.5$; $r = 0.41$ for cirrus width; $r = 0.48$ for prL1 width) with the number of chaetigers (Chart 16.). In the neuropodium, analysis of the cirrus revealed that it correlated positively but not significantly ($p > 0.5$; $r = 0.41$ for cirrus length; $r = 0.48$ for cirrus width) with the chaetiger number, while all the other structures analysed showed a significant correlation ($p \leq 0.5$) (Chart 17.). In the parapodium located before the first parapodium with simple chaeta, for the cirri, lobes, ligules and acicula in both the notopodium and the neuropodium a significantly positive correlation ($p \leq 0.5$) with the number of chaetiger segments was obtained (Chart 18.,19.). In the first parapodium with simple chaeta, both the notopodium and the neuropodium had a significantly positive correlation ($p \leq 0.5$) between the dimensions of the structures analysed and the number of chaetiger segments (Chart 20., 21.). In the median parapodium, all the structures of both the notopodium and the neuropodium showed significantly positive correlation ($p \leq 0.5$) to the number of chaetiger segments (Chart 22., 23.), excluding the cirrus length of the neuropodium which showed a non-significant positive correlation ($p > 0.5$; $r = 0.33$) to the number of chaetiger segments (Chart 23.). In the notopodium of the median-posterior parapodium, a significant positive correlation ($p \leq 0.5$) was obtained between all the structures and the number of chaetiger segments, with the exception of the length of the notopodial cirrus and the length and width of prL1 ($p > 0.5$; $r = 0.35$ for the length of the cirrus; $r = 0.09$ for the length of the prL1; $r = 0.18$ for the width of the prL1) (Chart 24.). In the Neuropodium of the median-posterior parapodium, the width of the neuropodial cirrus, the length of the prL2, the poL, the neL and the acicula showed a significant positive correlation ($p \leq 0.5$) to the number of chaetiger segments. The other neuropodial structures showed non-significant positive correlations ($p > 0.5$; $r = 0.49$ for cirrus length; $r = 0.10$ for prL2 width; $r = 0.14$ for poL width; $r = 0.42$ for neL width) to the number of chaetiger segments (Chart 25.). Lastly, in the posterior parapodium, a significant positive correlation ($p \leq 0.5$) was obtained between the dimensions of the notopodium and the neuropodium structures analysed and the number of chaetiger segments (Chart 26., 27.), with the exception of the notopodial prL1, in which a non-significant positive correlation was obtained ($p > 0.5$; $r = 0.34$) (Chart 26.).

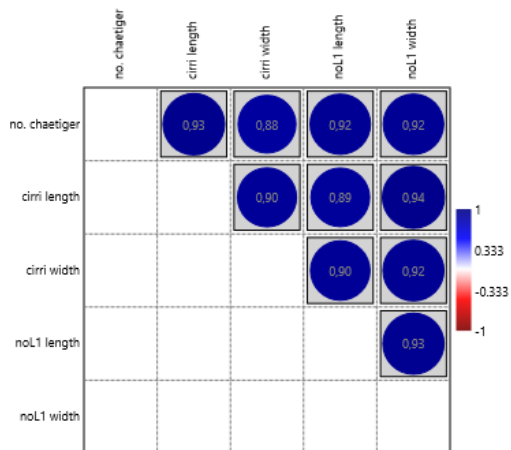


Chart 14. Correlation between multiple structures of the notopodium from anterior parapodium and the number of chaetiger segments

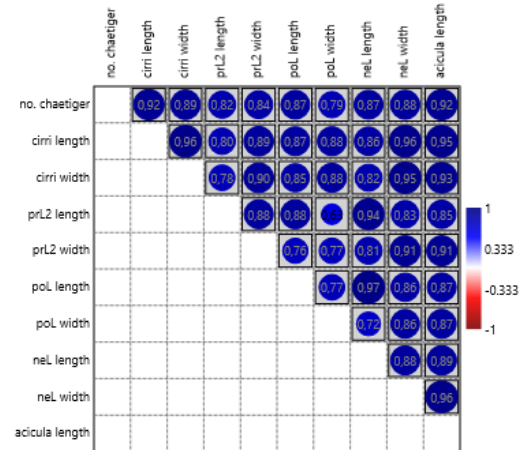


Chart 15. Correlation between multiple structures of the neuropodium from anterior parapodium and the number of chaetiger segments

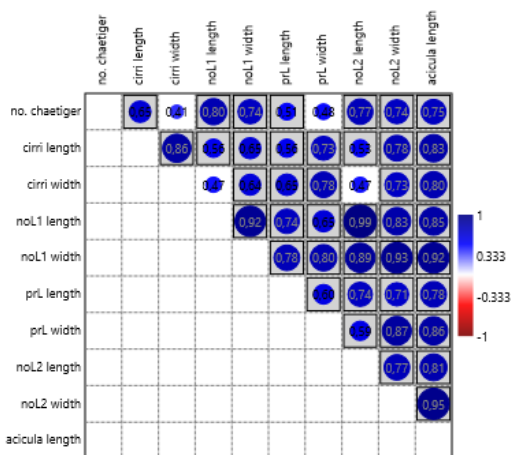


Chart 16. Correlation between multiple structures of the notopodium from transition anterior-median parapodium and the number of chaetiger segments

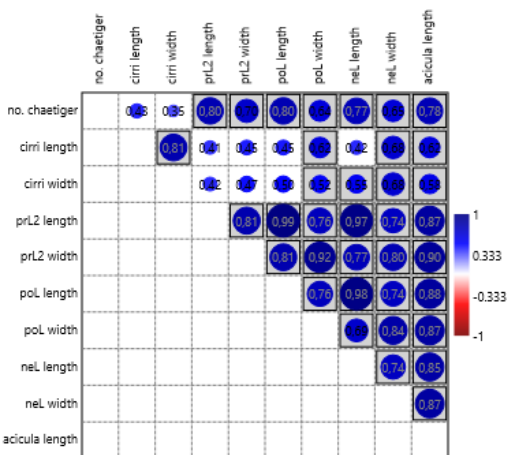


Chart 17. Correlation between multiple structures of the neuropodium from transition anterior-median parapodium and the number of chaetiger segments

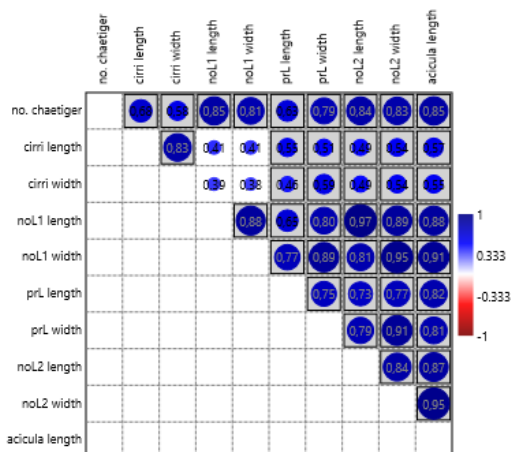


Chart 18. Correlation between multiple structures of the notopodium from the parapodium before the first chaetiger with simple chaeta and the number of chaetiger segments

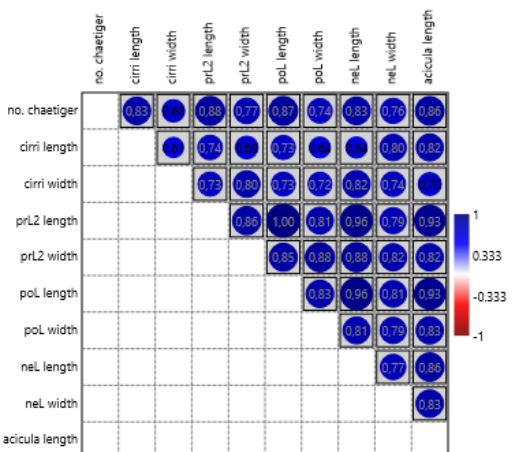


Chart 19. Correlation between multiple structures of the neuropodium from the parapodium before the first chaetiger with simple chaeta and the number of chaetiger segments

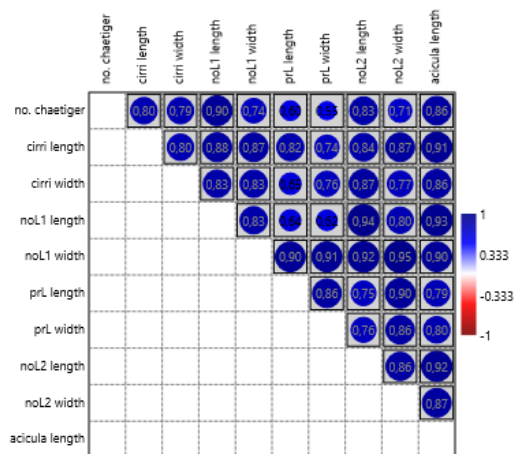


Chart 20. Correlation between multiple structures of the notopodium from the first parapodium with simple chaeta and the number of chaetiger segments

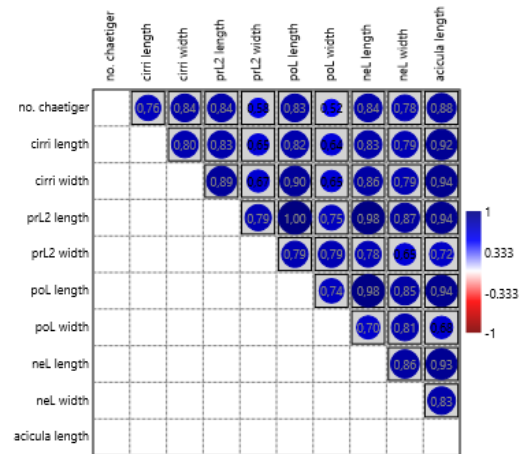


Chart 21. Correlation between multiple structures of the neuropodium from the first parapodium with simple chaeta and the number of chaetiger segments

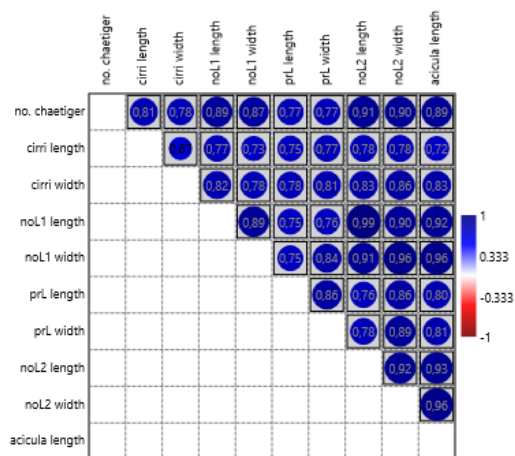


Chart 22. Correlation between multiple structures of the notopodium from median parapodium and the number of chaetiger segments

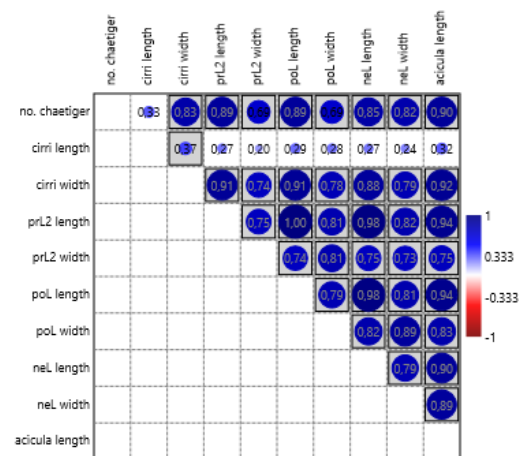


Chart 23. Correlation between multiple structures of the neuropodium from median parapodium and the number of chaetiger segments

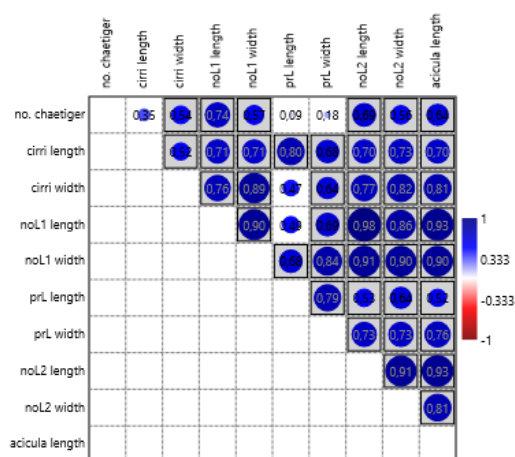


Chart 24. Correlation between multiple structures of the notopodium from transition median-posterior parapodium and the number of chaetiger segments

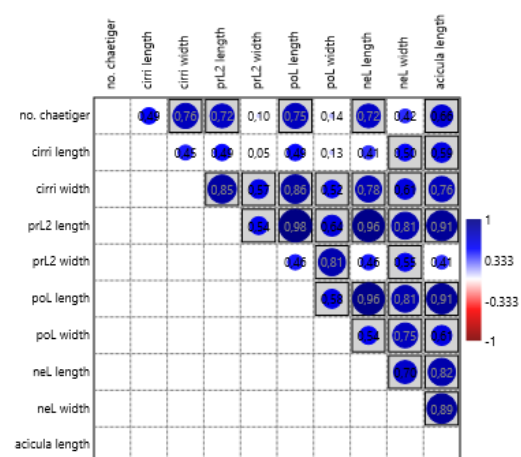


Chart 25. Correlation between multiple structures of the neuropodium from transition median-posterior parapodium and the number of chaetiger segments

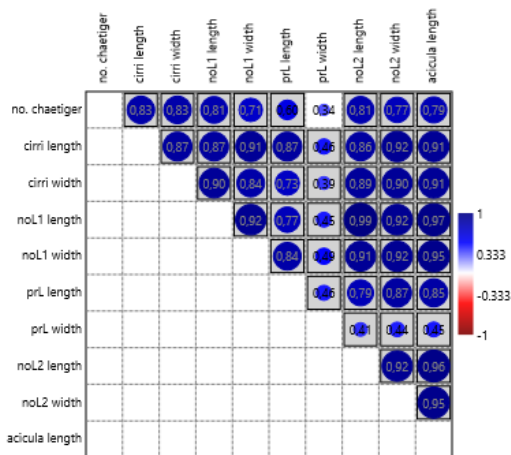


Chart 26. Correlation between multiple structures of the notopodium from posterior parapodium and the number of chaetiger segments

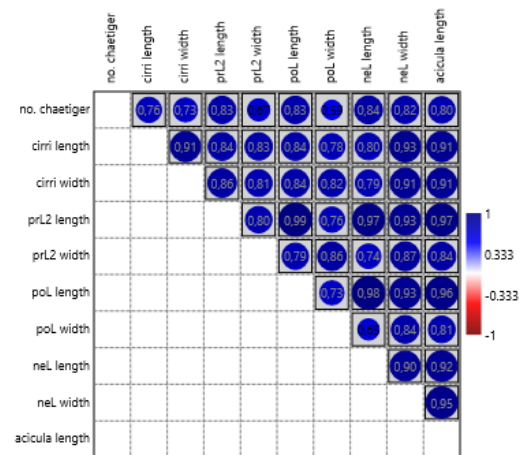


Chart 27. Correlation between multiple structures of the neuropodium from posterior parapodium and the number of chaetiger segments

Regarding the type of chaeta, the notopodium bears only supra-acicular homogomph spiniger type of chaeta. It should be noted that in the first parapodia there is no noto-acicula and, consequently, chaetae are absent in that area. In the neuropodium, there is a greater variety of chaeta types. The supra-acicular chaetae include homogomph spinigers, in higher number, and heterogomph falcigers, in lower number and closest to the acicula. The heterogomph falciger chaetae are replaced by a large fused 'simple' chaetae in the posteriormost chaetigers. In rare cases, homogomph falciger chaetae are found in the neuropodium, where the simple chaetae are usually located. In some instances, it was possible to observe a variation of simple chaeta that appeared to be incomplete or still under formation, that resembled an heterogomph falciger chaeta were the stalk and the blade where partially fused together. Heterogomph spiniger chaetae are found in the infra-acicular region of the neuropodium and heterogomph falciger chaetae are found further away from the acicula. Near the pygidium, the overall number of chaetae per parapodium visually decreases, with bristles sometimes missing from at least one region of both the notopodium and neuropodium.

1.4.5. Relative position of the first parapodium with simple chaeta

Simple chaetae are not observed in individuals with less than 25 chaetiger segments. In individuals with close to 30 chaetiger segments appear in the last third of the body. For organisms with 50 or more chaetigers segments, simple chaetae in formation and typical simple chaetae appear closer to the median segments. Thus, a significant negative

correlation ($p \leq 0.5$) was obtained between the relative position of the first parapodium with simple chaeta and the number of chaetiger segments (Chart 28.).

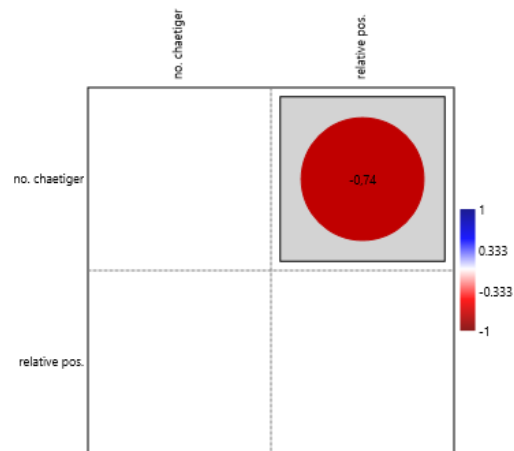


Chart 28. Correlation between the relative positions of the first chaetiger segment with simple chaeta and the number of chaetiger segments

1.4.6. Pygidium analysis

The average increase in length of pygidial cirri and its segments per each new chaetiger segment can be checked in Table 5.

Table 5. Increase in length of the pygidial cirri and cirri segments per each new chaetiger segment

		Increase per each new chaetiger
Structure		Length (mm)
Pygidium	Left Pygidial Cirrus	0.0372
	Right Pygidial Cirrus	0.0346
	Cirri Segment	0.0022

Both right and left pygidial cirri length as well as the length of their sections have a significant positive correlation ($p \leq 0.5$) with the chaetiger number (Chart 29.).

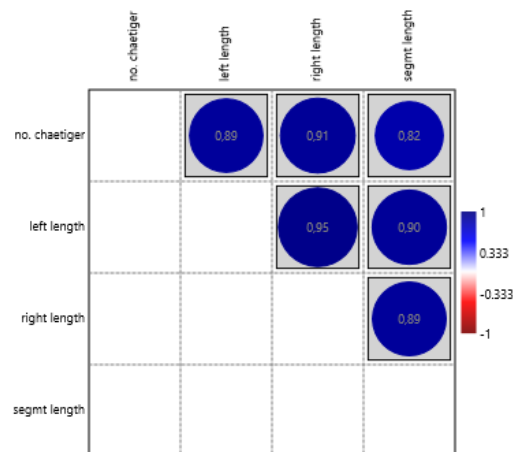


Chart 29. Correlation between the length of the right and left pygidial cirri and their sections and the number of chaetiger segments

1.5. Discussion

From the analysis of correlations between the various target structures with different functions, such as sensory or locomotor, and the number of chaetiger segments, important data was obtained to understand the morphological development of *H. diversicolor*. We can therefore conclude that these structures develop to accompany the maturation of the animal.

1.5.1. Sensory structures

The results indicate a significant positive correlation of the growth of the sensory structures (antennae, palps, peristomial and pygidial cirri), in length and width, with the increase of the number of chaetigerous segments. Those structures are believed to have a chemoreceptor role (Dorsett & Hyde, 1969). The increase in the volume of sensory appendages can be translated into an increase in sensory surfaces, thus improving the reception of sensory stimuli and consequently an increase of sensory capabilities of these organs. This enhancement in perception of the surroundings helps the animal to navigate and interact more effectively with its environment enabling more complex behaviours that may be crucial for the survival and reproduction of the species.

1.5.2. Feeding structures

The paragnaths within the proboscis mainly help to grip the food particles and transfer them to the gut (Maltagliati et al., 2006). The number of paragnaths in most of the sections of the proboscis and the length of the jaws increase with the animal growth. This increase probably positively influences the ability to capture a greater quantity of food, which is crucial since food requirements augment as the animal grows.

Although the length of the jaws correlates positively with the number of chaetiger segments, the same is not true for the number of teeth in each jaw. The left and right jaw had usually the same number of teeth, and the number of teeth seems to be less variable than other characteristics. The number of teeth per jaw in *H. diversicolor*, between 5 and 9, is within the range of other nereidid species such as *Alitta succinea* (Leuckart, 1847) (Hefferan, 1900) and other polychaeta of genus *Hediste* (Sato & Nakashima, 2003). On the other hand, the results obtained for peristomial cirrus showed significant positive correlations between its measurements, including both cirrophore and cirrostyle, and the number of chaetiger segments. Thus, the growth of peristomial cirri is proportional to the growth of the organism.

1.5.3. Locomotor structures

The number of chaetigerous segments and most of the measurements of the parapodial structures were positively correlated, also demonstrating a proportional growth pattern in which both the notopodium and the neuropodium grow as the organism develops new chaetiger segments. This same pattern was also observed in the nereidid species *Laeonereis acuta* (Treadwell, 1923), with the positive correlation between the increase in chaetigers and body size (Omena & Amaral, 2001). As the parapodia are key elements for the movement of these annelids (Hesselberg, 2003), as the size of the animal increases, there is a gradual adaptation of the structures that provide this function. When the worm is younger and smaller, parapodia with smaller dimensions and less developed will be able to perform the basic needs of locomotion and interactions with the sediment for its survival. As the annelid grows, its body gains more volume and mass, requiring its parapodia to become larger and more complex in order to maintain its ability to move, avoid predators and interact with the environment (Hesselberg, 2003; Hesselberg & Vincent, 2006). Some parapodial structures, such as the notopodial cirrus of the anterior-median and median-posterior transitional parapodia, the neuropodial cirrus of the anterior-median, median and posterior median parapodia, the prL1 of the anterior-median, median-posterior and posterior parapodia, and poL and prL2 of the median-posterior transitional parapodia did not show significant correlations. This means that although there is proportional growth of most of the parapodia's structures, some had possible malformations or were influenced by other factors, such as environmental conditions, or mechanical wear/damage, depending on the location of the parapodium where they are found. In the notopodium there is no diversity in the type of chaetae, which are always of the homogomph spiniger type. In the neuropodium, there is diversity between the different parapodia along the body, particularly the transition from

heterogomph falciger to simple chaetae, which appears when an individual of *H. diversicolor* reaches around 30 chaetiger segments, undergoing a possible functional adaptation. For other species of like *Hediste japonica* and other Asian species of *Hediste*, like *H. diadroma*, parapodial development is conditioned by the life cycle phase (Sato & Nakashima, 2003; Kan et al., 2020). In these Asian species, the chaeta transitions occur in different body segments, depending on the stage of development and environmental variables (Sato, 2017), demonstrating greater flexibility in parapodial adaptation than in *H. diversicolor*. This distinction can be interpreted as an adaptation to the more variable habitats that *H. japonica* and *H. diadroma* inhabit, both being catadromous euryhaline nereidids that undergo epitokous metamorphosis (Sato, 2017; Kan et al., 2020), which requires a more adaptable locomotor apparatus. The presence of simple chaetae on the posterior segments of *H. diversicolor* may indicate a functional change as the worm matures, perhaps due to changes in interaction with the sediment or in burrowing behavior as it approaches a mature state.

The presence of simple chaetae on the posterior segments of *H. diversicolor* may indicate a functional change as the worm matures. Statistical analysis on the relative position of the first parapodium with simple chaeta in relation to the number of chaetiger segments revealed a significant negative correlation, indicating that during the annelid development, simple chaetae occur earlier along the body axis. This ontogenetic shift in parapodial chaeta could be linked to the worm's transition from juvenile to adult stages when modifications in burrowing behaviour or substrate interaction become necessary due to their enlarged size. Simple chaetae are thicker and stronger, possibly being used to better anchor their bodies to the borrows as a defence mechanism (Hesselberg & Vincent, 2006), and potentially help in reproductive activities, since in this species fertilisation takes place inside the burrow (Scaps, 2002). This growth tendency is consistent with observations in other *Hediste* species, such as *H. japonica* and *H. atoka*, where the first chaetiger with simple chaetae emergence are similar to the data obtained from *H. diversicolor* (Sato & Nakashima, 2003). For individuals with approximately 100 chaetiger segments, the first parapodia with simple chaeta was discovered to be near the 50th chaetiger segment.

1.5.4. Conclusions

From the results obtained in the correlation analyses between the various structures with different functions, such as sensory, locomotor and feeding, in relation to the number of chaetiger segments, an indicator of growth, important information was discovered about the growth patterns and morphological adaptations of *H. diversicolor*, illustrating how its

body develops throughout growth, and demonstrating the complex developmental processes that shape the anatomy of this species of nereid, differentiating it from other species in the *Hediste* genus. It should be noted that there is a proportional growth trend between most of the structures analysed and the overall growth of the body, and that in the case of some specific structures, they may be subject to different selective pressures or developmental restrictions. Although many developmental patterns, such as the growth of sensory appendages, the feeding apparatus and locomotor structures, are common to all species, there are some specialised adaptations that end up reflecting the unique ecological environment in which each species is inserted, influencing its development. These results show the evolutionary adaptability of the genus *Hediste* and contribute to our understanding of the morphological variability of polychaetes. They can also guide future research into specific characteristics of the *H. diversicolor* that were previously unknown given that animal growth and development tracking of certain key features was lacking.

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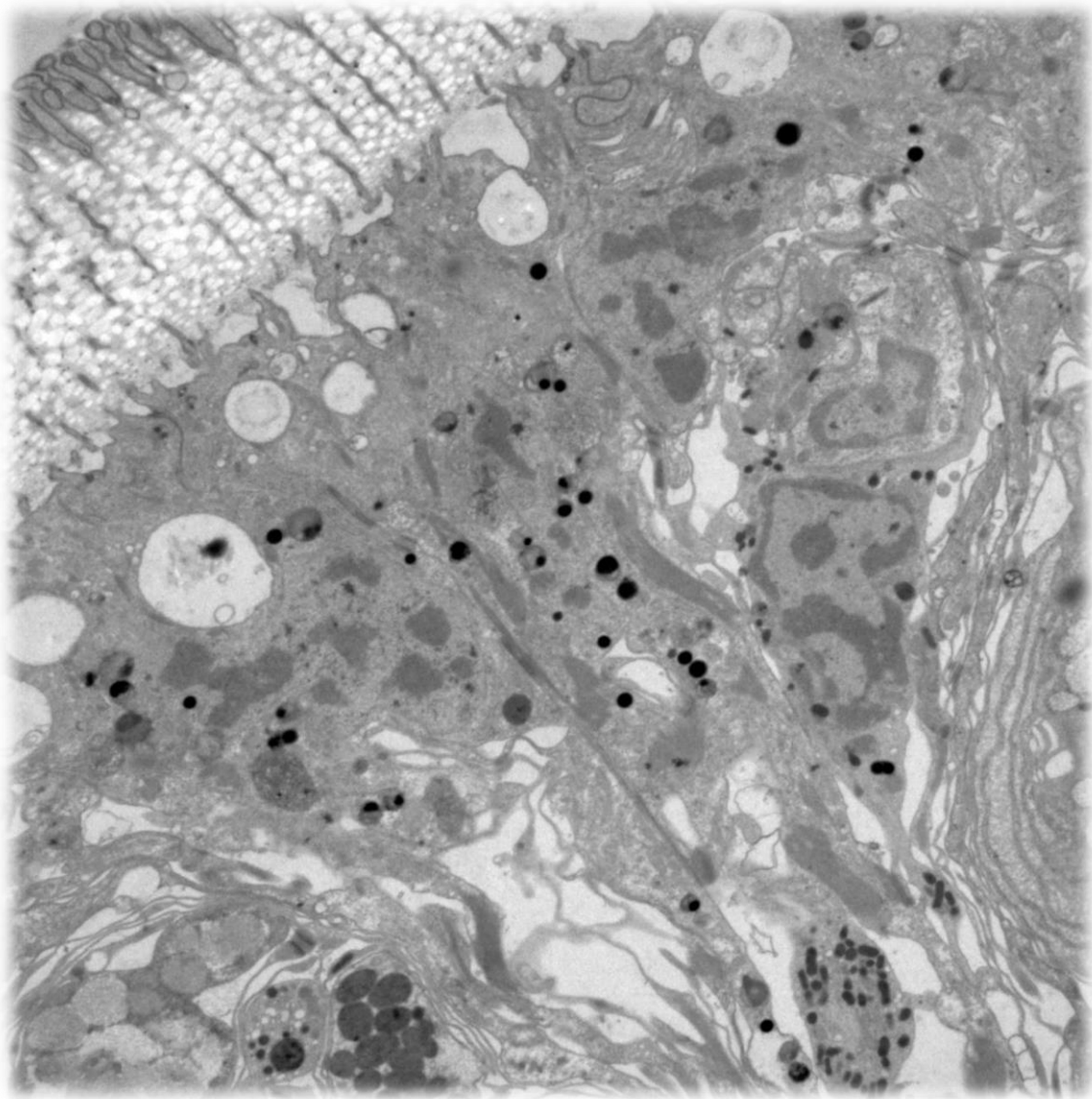
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Chapter II: Investigating the palps of *Hediste diversicolor* (Annelida; Errantia) through histochemistry and transmission electron microscopy



2.1. Abstract

Polychaetes (Phylum Annelida) respond to sensory stimuli through the usage of several organs and appendages, such as palps, which vary in shape and structure depending on their lifestyle. The typical palps of errant polychaetes are tapered appendages divided in two segments. The basal segment, called palpophore, is the wider and longer region that connects to the prostomium. The distal segment is called palpostyle and is the thinner and shorter part of the palp, containing sensory cells. Previous studies on *Hediste diversicolor* palps were focused on these sensory cells. To achieve a more comprehensive view of the histology and ultrastructure of the palps, *H. diversicolor* specimens were collected from the lower estuary of the Minho River and the palps were processed for light (semithin sections) and transmission electron microscopy. The current study revealed details of the cuticle, which is thinner in the palpostyle than in the palpophore. Five types of secretory cells were distinguished mainly based on the characteristics of their secretory vesicles. Two of these types could be classified as protein-secreting cells, and the other three as mucus-secreting cells. Granulocytes and eleocytes were found in the celom cavity of the palps. The latter cells contained lipid droplets and a very large amount of glycogen. In the central region of the palpophore a ring of muscle cells responsible for the retraction of the palpostyle encircled the neuropil formed by numerous axons and glial cells containing bundles of filaments and gliosomes.

Keywords: Nereididae; Ragworm; Cuticle; Sensory organs; Ultrastructure

2.2. Introduction

The common ragworm species *Hediste diversicolor* is among the most abundant species of the macrobenthic community in estuarine environments (Kristensen, 1984; Nithart, 1998; Gillet *et al.*, 2008), including the Minho estuary where the specimens used in this study were collected (Picanço *et al.*, 2014). In polychaetes several sensory organs are located on the head (Fauchald & Rouse, 1997; Purschke, 2005). For this and the other polychaete taxa, sensory organs are crucial for survival in the environment where they live. Due to the large diversity of species, the external morphology and internal anatomy of these appendages varies greatly among polychaetes. The grooved feeding palps, found in Spionida, Sabellida and other sedentary polychaetes, are appendages with a longitudinal ciliated groove for gathering food particles and conveying them to the mouth, but also have sensory functions (Fauchald & Rouse, 1997; Lindsay *et al.*, 2004; Meyer *et al.*, 2021). The elongated prostomial appendages of Protodriliformia errant polychaetes, which have been considered to be palps based on their specific innervation, possess a variety of sensory cells and at least in several species seem not involved in collecting food particles (Purschke, 1993; Wilkens & Purschke, 2009; Lehmacher *et al.*, 2014). In *H. diversicolor* the prostomium contains two pairs of ocelli, a pair of short antennae and a pair of ventral palps, like those found in Nereididae and many other errant polychaetes (Teixeira *et al.*, 2022). These are usually tapered or digitiform and relatively short compared to the grooved palps (Fauchald & Rouse, 1997). The palps are divided in two segments. The basal segment is the palpophore, which is the wider and longer region connected to the prostomium. The palpostyle is the distal segment, which is the thinner and shorter region of the palp. This segment can be retracted into the palpophore or pulled back by muscles attached in a ring forming the appearance of an articulation (Harper, 1979; Fauchald & Rouse, 1997). These appendages are believed to have mainly a chemical sensory function (Dorsett & Hyde, 1969). Among other features, palp morphology is important for species characterization and identification (Santos *et al.*, 2005; Teixeira *et al.*, 2022).

However, few ultrastructural studies concerning these appendages have been carried out previously in the family Nereididae and in the species *H. diversicolor*, and these were mainly focused on sensory cells and nerves (Dorsett & Hyde, 1969; Purschke, 1993; Lindsay *et al.*, 2004; Forest & Lindsay, 2008; Meyer *et al.*, 2021) with little information being available on other cell types. Therefore, the general ultrastructure of *H. diversicolor* palps remain understudied. Thus, this study aimed to improve knowledge on the histology and ultrastructure of palps in *H. diversicolor*.

2.3. Material and Methods

2.3.1. Animal collection

Specimens of the common ragworm *H. diversicolor* were collected from the lower estuary of the Minho River, near Pedras Ruivas (41°53'28.4"N, 8°49'30.8"W), Caminha, Portugal, at low tide in February 2023. The sediment at this location consisted of a mixture of sand and mud, with a few rocks distributed on the surface. Sediment was collected manually around and beneath rocks where borrows and bioturbation typical of *H. diversicolor* were observed. In the laboratory, small quantities of sediment were sequentially observed in a plastic tray containing estuary water (salinity 22–24) to isolate *H. diversicolor* specimens. Adult specimens with more than 90 segments were selected for this study.

2.3.2. Sample processing for light and transmission electron microscopy

The selected specimens were processed according to Beckers *et al.* (2021). Worms were relaxed in a 7.5% solution of MgCl₂ mixed 1:1 with isotonic saltwater. Palps were removed at the base of the palpophore using a scalpel, fixed with a 2.5% glutaraldehyde solution buffered in 0.2M sodium cacodylate with 0.14M NaCl (pH 7.2) at 4 °C for 1h, and rinsed in the same buffer. Post-fixation was carried out with 2% OsO₄ in the same buffer for 2h. Samples were dehydrated in a graded ethanol series, followed by embedding in epoxy resin. For light microscopy observations, semithin sections (2 µm) were either stained with methylene blue-Azure II or processed for histochemical techniques. For transmission electron microscopy (TEM) ultrathin sections were double stained with uranyl acetate and lead citrate. For glycogen and collagen staining, ultrathin sections were treated with 5% tannic acid for 10 min and stained with uranyl acetate for 10 min (Sannes *et al.*, 1978). Ultrastructural observations were preformed using JEOL 100 CXII transmission electron microscope, equipped with a Gatan Orius SC200 camera, operated at 60kV.

2.3.3. Histochemical techniques

Histochemical techniques were applied to semithin sections of the palps. These included the tetrazonium coupling reaction for protein detection, the Periodic Acid Schiff (PAS) reaction for neutral polysaccharides, and Alcian blue stain for acid polysaccharides. For the tetrazonium coupling reaction, the embedding medium was not removed from the semithin sections. These were treated with a freshly prepared 0.2% solution of fast blue salt B in veronal-acetate buffer (pH 9.2) for 15 min, washed with 0.1% HCl followed by water, treated with a saturated solution of β-naphthol in veronal-acetate buffer (pH 9.2), and washed with water (Nöhammer, 1978). For the PAS reaction and Alcian blue

staining, the embedding medium was removed from semithin sections using an alcoholic solution of sodium ethoxide (Lane & Europa, 1965). For the PAS reaction, sections were treated with 1% periodic acid for 20 min, washed with water, and treated with Schiff reagent for 30 min before being washed with water. For the Alcian blue staining, sections were stained with the Alcian blue (pH 1.0 or 2.5) for 30 min and washed with water. All semithin sections were mounted with DPX medium.

2.4. Results

2.4.1. Morphology, histology and histochemistry of the palps

Freshly cut palps exhibit a pale brown hue, with a hint of greenish colour at the base of the palpophore. Under a light microscope, yellowish-brown cells could be observed on both palpophore and palpostyle, being more abundantly along the dorsal side of the palpophore, and in the area between the palpophore and the palpostyle (Fig. 9A). The palps are covered by the cuticle, which is thicker on the palpophore than on the palpostyle (Figs. 9B–D). In semithin sections, the cuticle was weakly stained by PAS reaction and Alcian blue at both pH 1 and 2.5 (Fig. 9E, F), but was not stained by the tetrazonium coupling reaction. Some clusters of ciliated sensory cells were seen along the surface of the palpostyle. These ciliated cells extend to the palp interior where they connect to the neuropil (Fig. 9D).

Diverse cell types occur between the epidermis and the celomic cavity of the palps (Fig. 9B, C). In this region of the palpophore the most abundant were the cells with multivesicular vesicles that were not stained by methylene blue and azure II (Fig. 9C, G). The multivesicular vesicles of these cells were also unstained by the tetrazonium coupling reaction for proteins (Fig. 10A, B), but were stained by Alcian blue at both pH values (Fig. 10C) and very weakly stained by PAS reaction (Fig. 10D). However, the cytoplasm around the multivesicular vesicles was stained by the tetrazonium coupling reaction (Fig. 10A). Other cells contained vesicles that were lightly stained by methylene blue and azure II (Fig. 9G), very weakly stained by the tetrazonium coupling reaction (Fig. 10B) and unstained by Alcian blue (Fig. 10C). The light microscopy technique for protein detection revealed another cell type characterised by very small vesicles strongly stained by this reaction (Fig. 10A). The coelom cavity housed rosette forming small rounded coelomocytes, about 7–9 µm in diameter, and eleocytes with an irregular and variable shape in semithin sections, reaching 20–30 µm in length, containing some osmiophilic lipid droplets (Fig. 9C, E). The cytoplasm of the latter cells was stained by Alcian blue and PAS reaction, except the lipid droplets (Fig. 10C, D). In the central region

of the palpophore, a ring of muscle cells responsible for the retraction of the palpostyle encircled the neuropil (Fig. 9C).

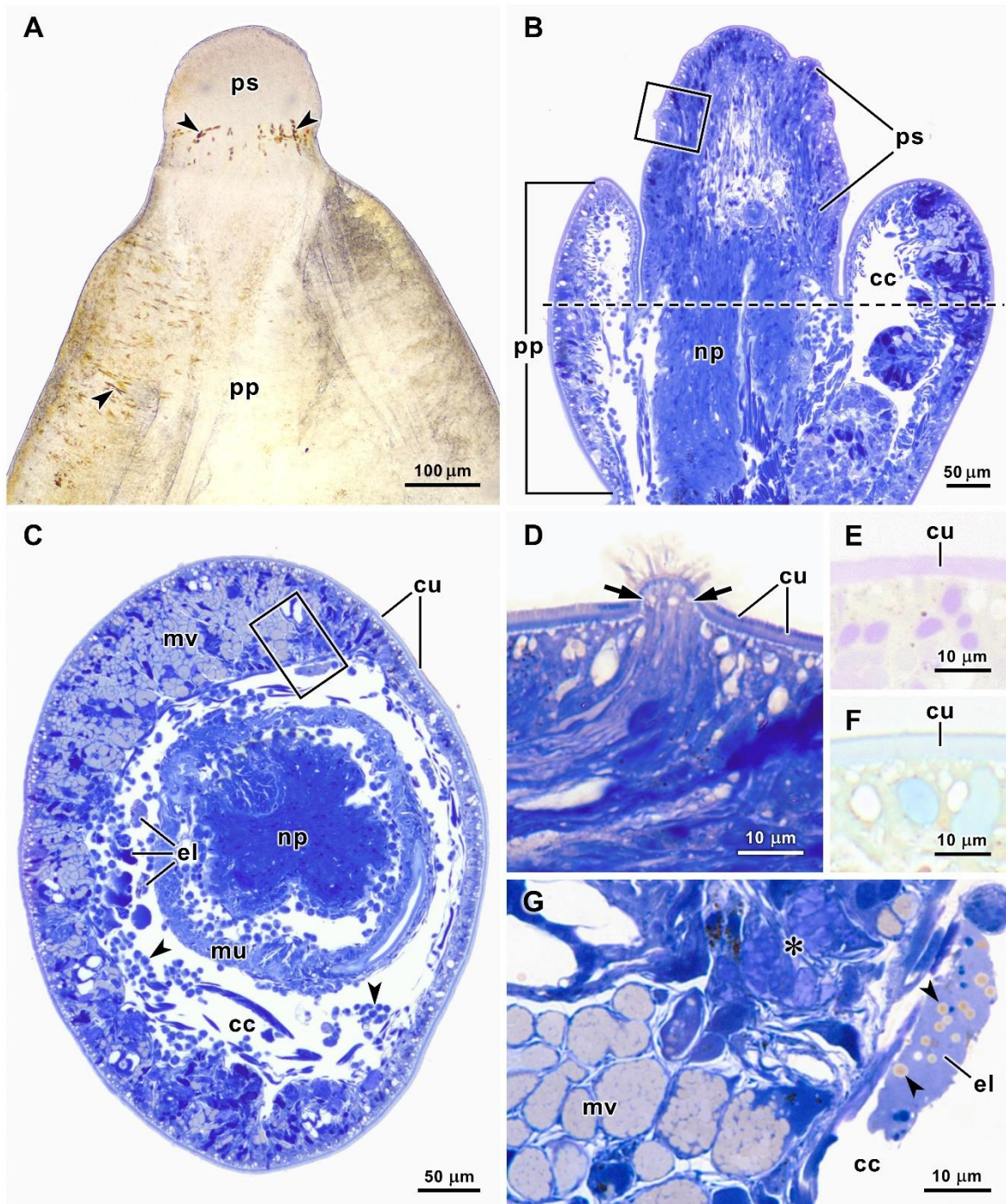


Figure 9. Light microscopy of *Hediste diversicolor* palps, observed fresh. (A) and in semithin sections stained with methylene blue and azure II (B–D, G), PAS reaction (E) and Alcian blue (F). (A) Arrowheads indicate yellowish-brown cells in the palpophore and palpostyle. (B) Palp in longitudinal section. The dashed line corresponds to the plan of section in C, and the box corresponds to the area in D. (C) Transverse section of the palpophore. Cells with unstained multivesicular vesicles (mv) are abundant in this region. Eleocytes (el) and small coelomocytes (arrowheads) are present in the coelom cavity. The box corresponds to the area in G. (D) Cluster of ciliated sensory cells (arrows) of the palpostyle. (E) Cuticle stained by PAS reaction. (F) Cuticle weakly stained by Alcian blue. (G) Palpophore tissues include cells with unstained multivesicular vesicles (mv) and cells with lightly stained vesicles (asterisk). The eleocyte (el) in the coelom cavity (cc) contains several osmiophilic lipid droplets (arrowheads). cc, coelom cavity; cu, cuticle; el, eleocytes; mv, cells with multivesicular vesicles; mu, muscle tissue; np, neuropile; pp, palpophore; ps, palpostyle.

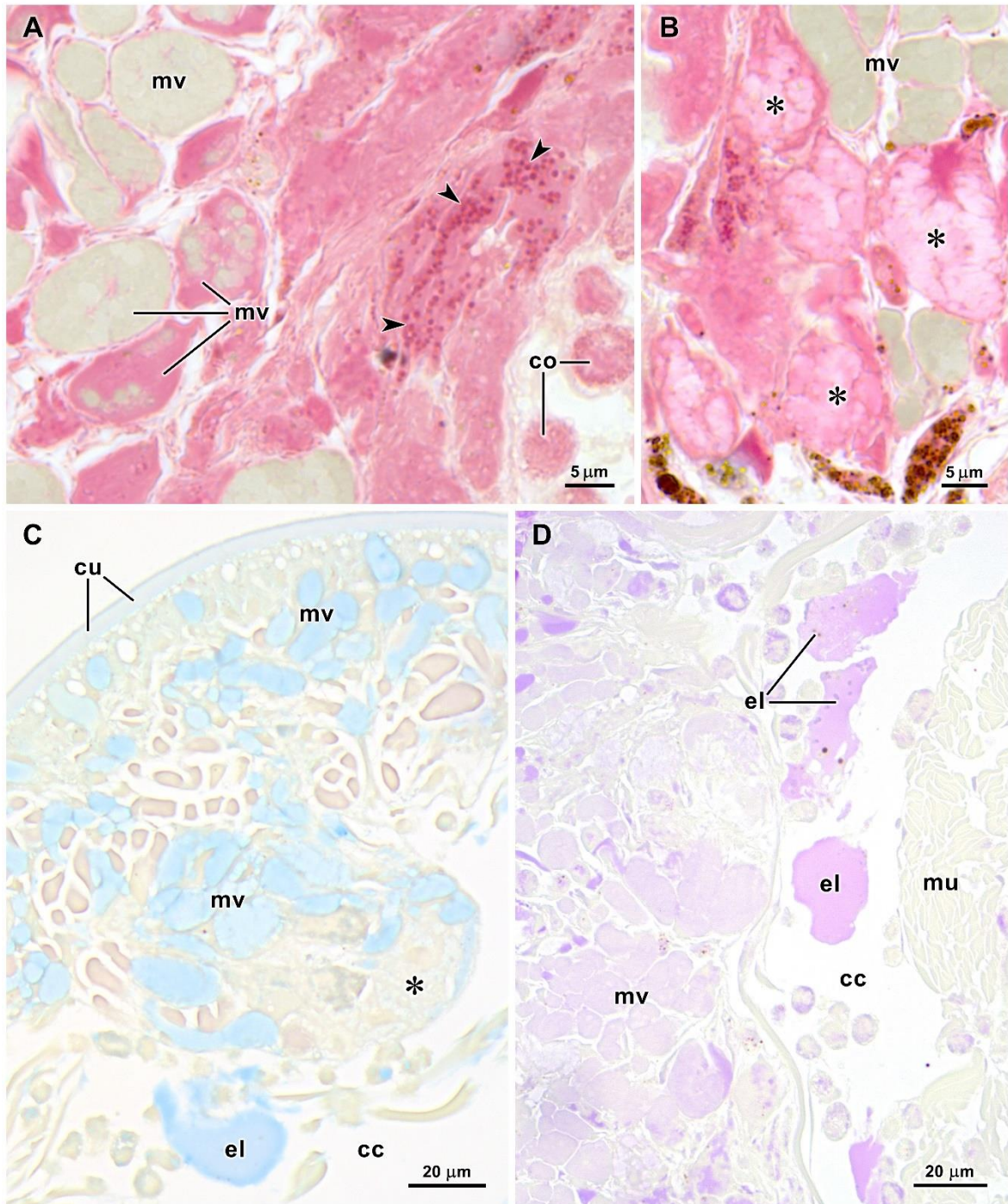


Figure 10. Histochemistry of *Hediste diversicolor* palps in semithin sections. (A) The tetrazonium coupling reaction does not stain the vesicles of cell with multivesicular vesicles (mv), but another cell type contains small vesicles that are strongly stained (arrowheads). The cytoplasm of the different cell types is moderately stained. (B) Cells with vesicles very weakly stained by the tetrazonium coupling reaction (asterisks). (C) Alcian blue stains the cells with multivesicular vesicles (mv) and eleocytes (el). The cuticle (cu) is slightly stained and other cell types are unstained (asterisk). (D) PAS reaction stains the eleocytes (el), but the cells with multivesicular vesicles (mv) are very weakly stained. cc, coelomocytes; cu, cuticle; el, eleocytes; mv, cells with multivesicular vesicles; mu, muscle tissue.

2.4.2. Palp ultrastructure

Ultrastructural analysis put in evidence the difference in cuticle thickness between the palpophore and the palpostyle previously noticed in light microscopy observations. In the palpophore, the cuticle with a total thickness around 4.5 μm comprised a main portion consisting of roughly 26–30 layers of collagen fibres covered by an epicuticle about 0.6–0.8 μm thick with deep pits containing secretion. The collagen fibres were surrounded by a matrix containing electron-dense filaments that also formed a layer with few and thin collagen fibres at the base of the cuticle (Fig. 11A, B). Collagen fibres were absent in the epicuticle that was coated by an electron-dense layer with an approximate thickness of 40 nm. Although electron-lucent in ultrathin section stained with uranyl acetate and lead citrate (Fig. 11A, B), the collagen fibres became strongly stained by uranyl acetate after treatment with tannic acid (Fig. 11C). In each layer the collagen fibres had a parallel orientation, being practically orthogonal in relation to the layer above and below. It was also noticed that the width of collagen fibres was higher in the middle of the cuticle and smaller at the top and basal layers. The thinnest fibres, about 20–25 nm thick, were found in the layers close to the epidermis, whereas the thicker fibres in the middle layers of the cuticle could reached a thickness of 180 nm. Below the epicuticle the thickness of the collagen fibres was reduced to 40–80 nm. The epidermal cells contained large vesicles in the apical region (Fig. 11A, B), in which the tannic acid-uranyl acetate staining revealed the presence of collagen fibres (Fig. 11D). The images also suggested that the fusion of these vesicles with the cell membrane releases collagen fibres into the subcuticular space to be incorporated in the cuticle. Adjacent epidermal cells were connected by zonula adherens, septate junctions, and cell junctions reminiscent of desmosomes. These cells also contained bundles of filaments and formed thin microvilli that crossed the cuticle ending at the bottom of epicuticular pits (Fig. 11B).

In the palpostyle the cuticle presented a similar structure but was thinner, especially over the ciliated sensory cells where the total thickness was about 0.8 μm (Fig. 12A, B), whereas in other parts of the palpostyle the cuticle could reached a thickness of approximately 1.2 μm (Fig. 12C). Digitiform epicuticular projection were observed only around the cilia of sensory cells, and the thin electron-dense coat of the epicuticle also coated the cilia (Fig. 12A, B). At high magnification tubular structures with a width of about 15 nm were observed in the epicuticle, some perpendicularly attached to the epicuticular electron-dense coat (Fig. 12D). In the apical region, the ciliated sensory cells contained several microtubules and small vesicles (Fig. 12A).

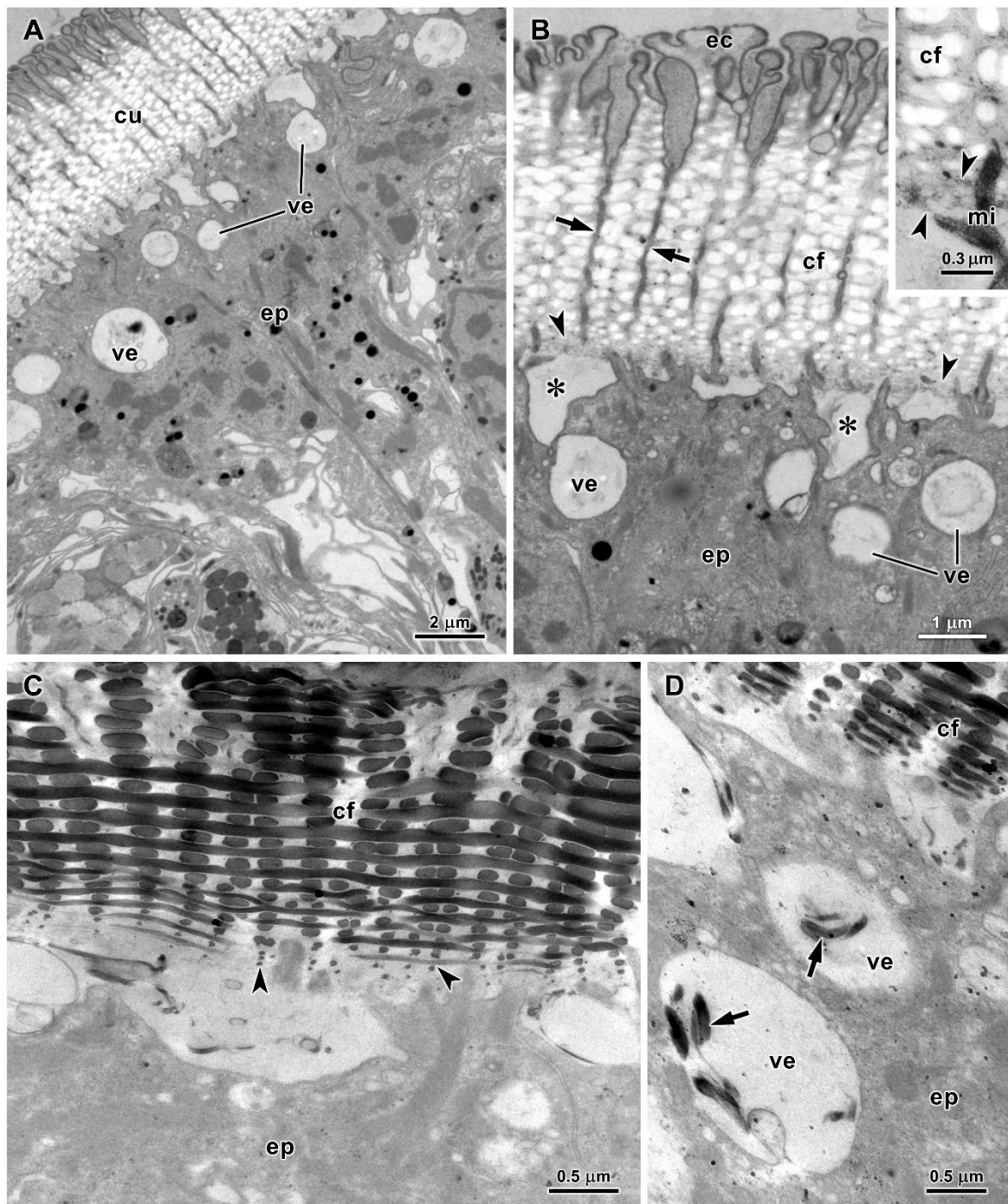


Figure 11. Ultrastructure of the cuticle and epidermis of *Hediste diversicolor* palpophore. (A, B) The cuticle (cu) comprises a main portion with several layers of collagen fibres (cf), the epicuticle (ec) and a basal layer mainly formed by electron-dense filaments (arrowheads). Epidermal cells (ep) possess large apical vesicles (ve) and long microvilli (arrows) that cross the cuticle. A subcuticular space (asterisks) is visible between the cell membrane of epidermal cells and the cuticle. (C, D) Cuticular collagen fibres (cf) are strongly marked by tannic acid-uranyl acetate staining, revealing very thin fibres (arrowheads) close to the epidermis (ep). Stained collagen fibres (arrows) are also visible within apical vesicles (ve) of the epidermal cells. cf, collagen fibres; cu, cuticle; ec, epicuticle; ep, epidermal cells; mi, microvilli; ve, vesicles.

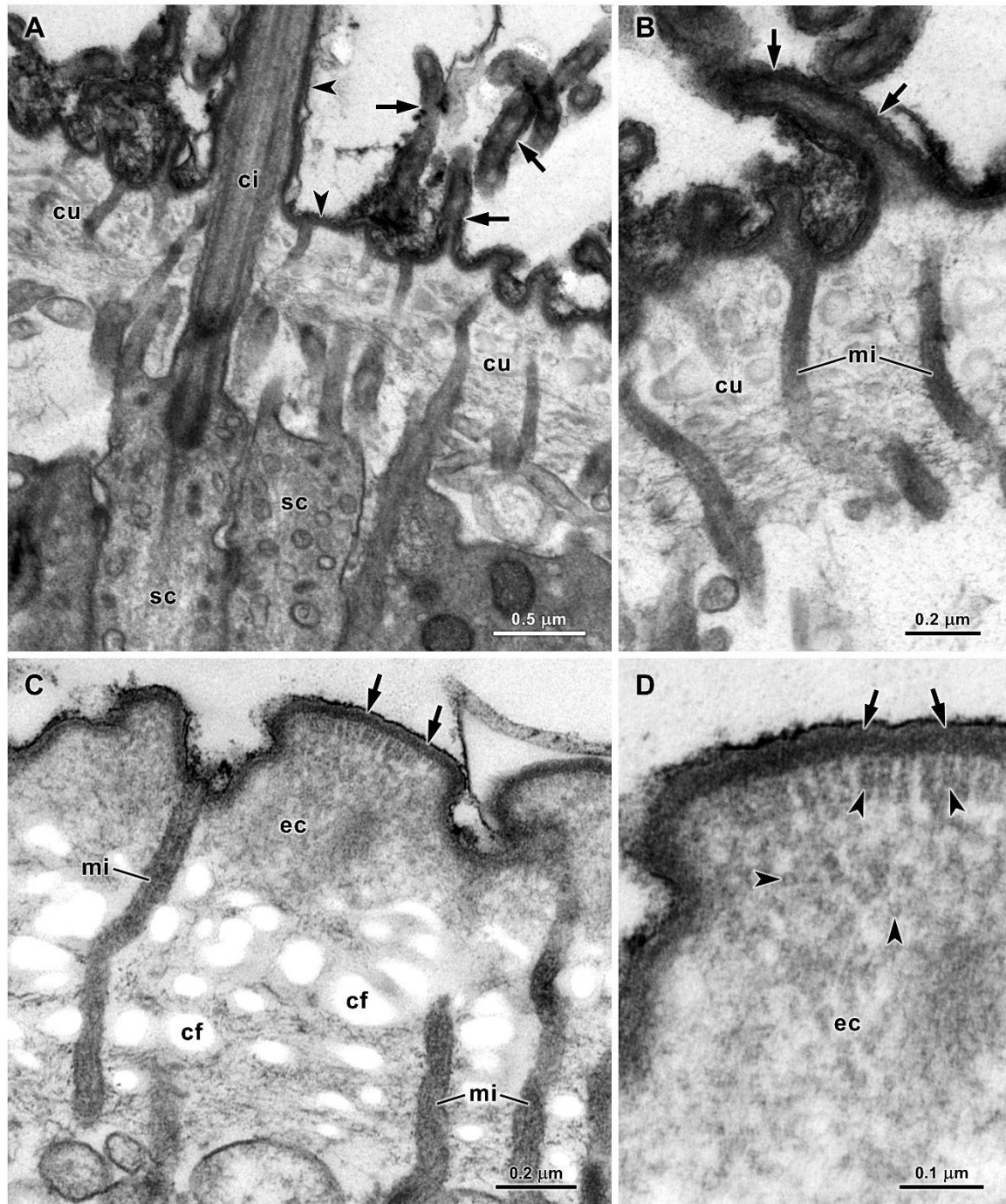


Figure 12. Ultrastructure of cuticle and sensory cells of *Hediste diversicolor* palpostyle. (A, B) The cuticle (cu) above ciliated sensory cells is thinner and displays epicuticular projection (arrows) coated by an electron-dense layer (arrowheads) that also coats the cilia (ci) of sensory cells. Sensory cells contain several vesicles in the apical region. (C, D) Palpostyle cuticle contained only a few layers of collagen fibres (cf) covered by the epicuticle (ec) containing tubular structures (arrowheads), and coated by an electron-dense layer (arrows). cf, collagen fibres; ci, cilium; cu, cuticle; ec, epicuticle; mi, microvilli; sc, sensory cells.

Several types of secretory cells were observed between the epidermis and the celomic cavity, which could be differentiated based on their ultrastructural features. One of these cell types was characterised by the presence of multivesicular vesicles, most of them with a diameter around 1.5–2.5 µm, filled with small vesicles with low electron-density content about 0.1–0.3 µm in diameter. In these cells, the multivesicular vesicles occupied a large portion of the cytoplasm that also contained large amounts of flattened rough endoplasmic reticulum cisternae (Fig. 13A, B). Although the multivesicular vesicles were not stained by the tetrazonium coupling reaction, the portion of these cells stained by this protein detection method for light microscopy (Fig. 10A) should correspond to the cytoplasmic regions with large amounts of rough endoplasmic reticulum cisternae. Another cell type had round vesicles with electron-dense spots. In these cells rough endoplasmic reticulum cisternae with flocculent material were abundant, and some of these cisternae presented dilated portions. The images suggested a gradual condensation of the larger vesicles with flocculent material to form smaller and more electron-dense mature secretory vesicles about 0.6–0.3 µm in diameter (Fig. 13C, D). Some mitochondria could be observed close to the nucleus (Fig. 13C). These cells seem to correspond to the cells with small vesicles strongly stained by the tetrazonium coupling reaction in semithin sections (Fig. 10A).

Other cells contained highly electron-dense elongated secretory vesicles with round or oval cross sections usually about 0.6–0.3 µm in diameter, some of which reached a length of 2.5 µm in ultrathin sections. In these cells several Golgi stacks could be identified, as well as numerous rough endoplasmic reticulum cisternae. Large vesicles with flocculent material found close to the Golgi stacks seemed to be immature secretory vesicles (Fig. 14A, B). Another cell type was characterised by rounded vesicles with fine granular content, with a diameter about 0.9–0.5 µm. These cells also had a large amount of densely packed rough endoplasmic reticulum with a granular content (Fig. 14C, D). These two cell types were not identified in semithin section. The last secretory cell type recognised by transmission electron microscopy enclosed numerous secretory vesicles of variable sizes with median electron-density that showed tendency to fuse with each other. These cells presented less rough endoplasmic reticulum cisternae and were richer in Golgi stacks with flattened cisternae that produced immature secretory vesicles (Fig. 15A, B). This last cell type seems to correspond to the cells with vesicles lightly stained by methylene blue and azure II (Fig. 9G) and weakly stained by the tetrazonium coupling reaction in semithin sections (Fig. 10B).

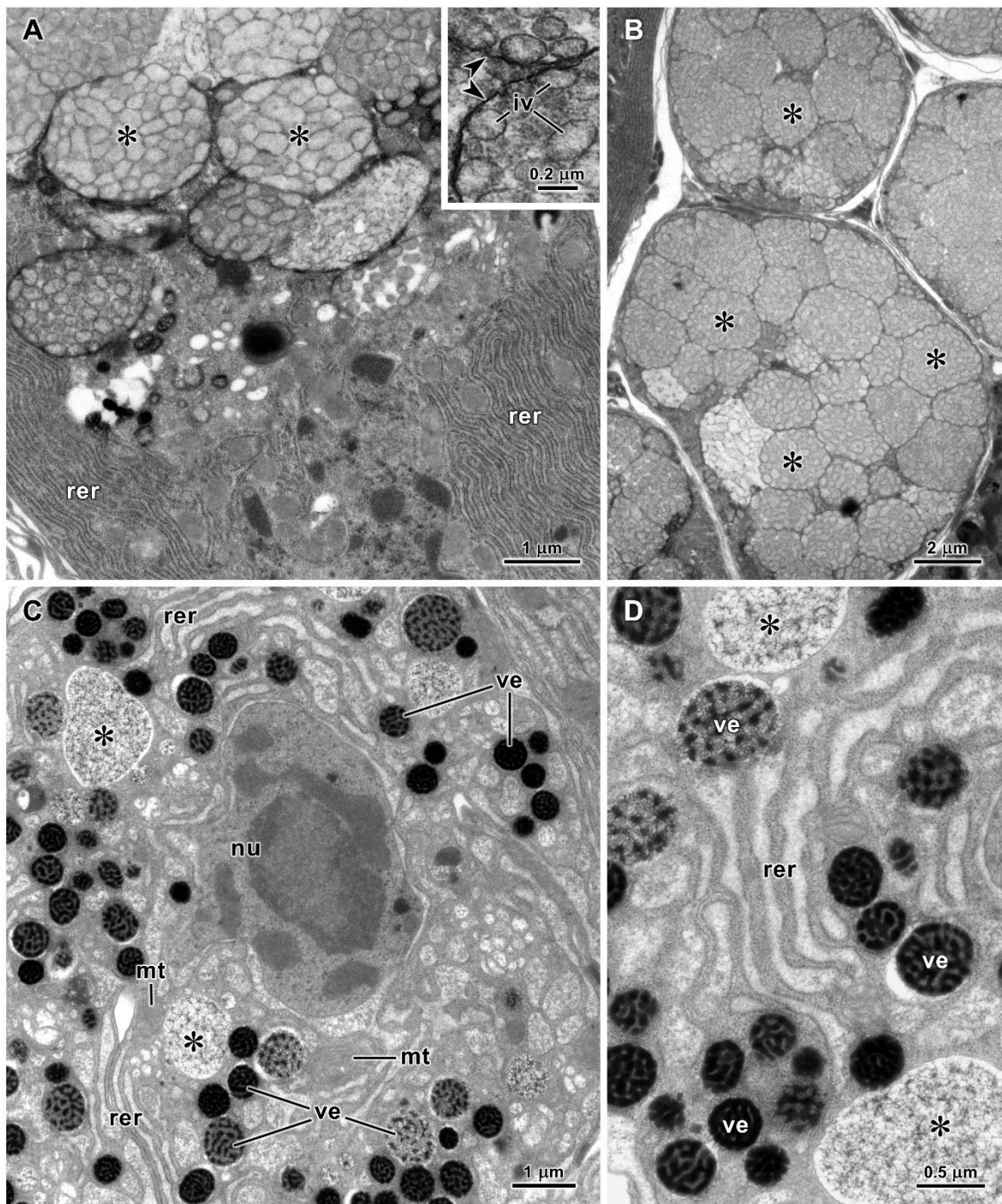


Figure 13. Ultrastructure of secretory cells from the palps of *Hediste diversicolor*. (A, B) Cells with multivesicular vesicles (asterisks) in which the outer vesicle membrane (arrowheads) surrounds a group of inner vesicles (iv). (C, D) Cells with spotted vesicles. Matured secretory vesicles with higher electron-density (ve) seem to result from the gradual condensation of immature vesicles with flocculent material (asterisks). iv, inner vesicles; mt, mitochondria; nu, nucleus; rer, rough endoplasmic reticulum; ve, secretory vesicles.

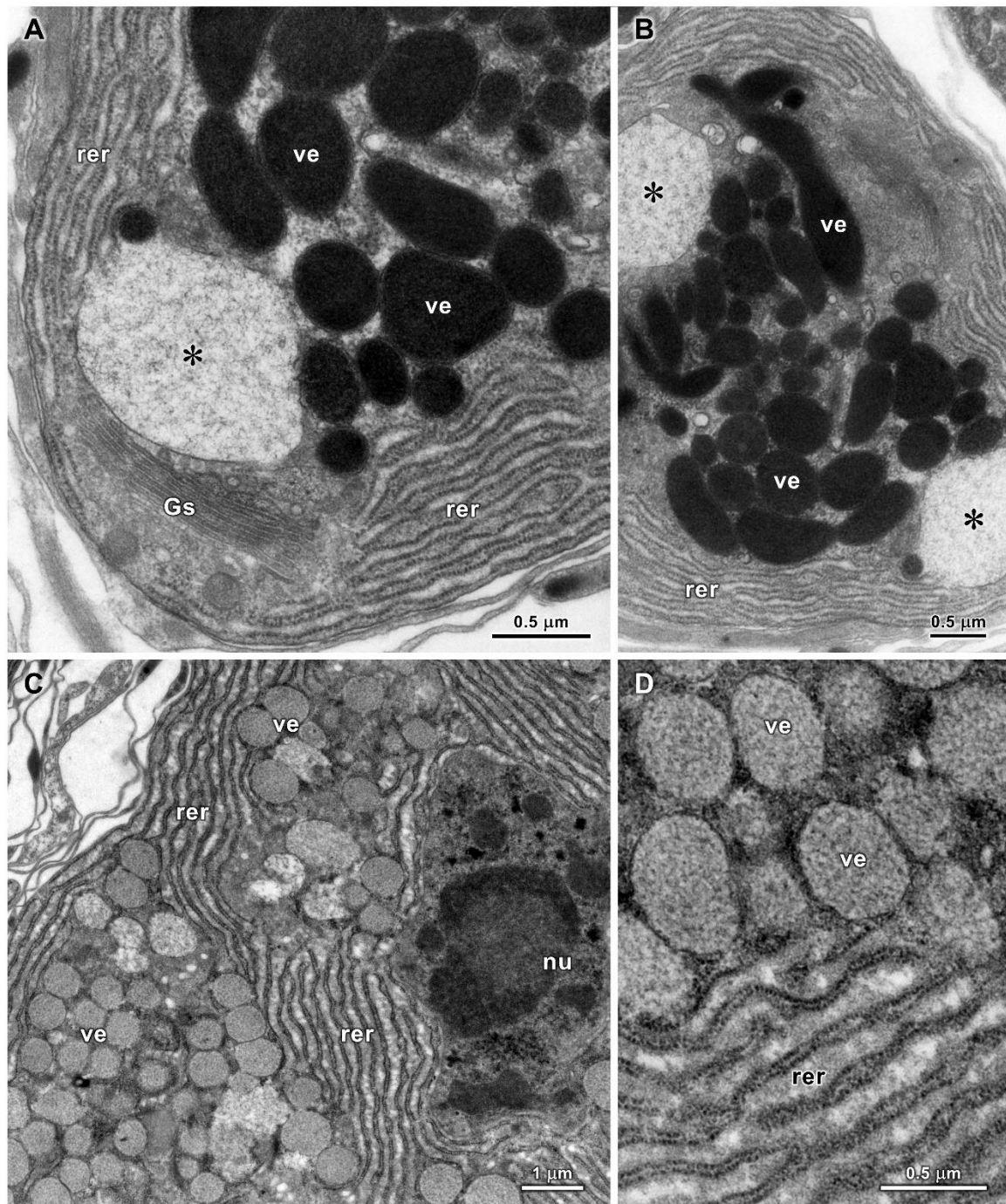


Figure 14. Ultrastructure of secretory cells from the palps of *Hediste diversicolor*. (A, B) In cells with highly electron-dense vesicles (ve) at least some of them are elongated. Immature vesicles (asterisks) can be seen close to Golgi stacks (Gs). (C, D) In another cell type, vesicles have a fine granular content (ve) that is also visible within the rough endoplasmic reticulum cisternae (rer). Gs, Golgi stacks; nu, nucleus; rer, rough endoplasmic reticulum; ve, secretory vesicles.

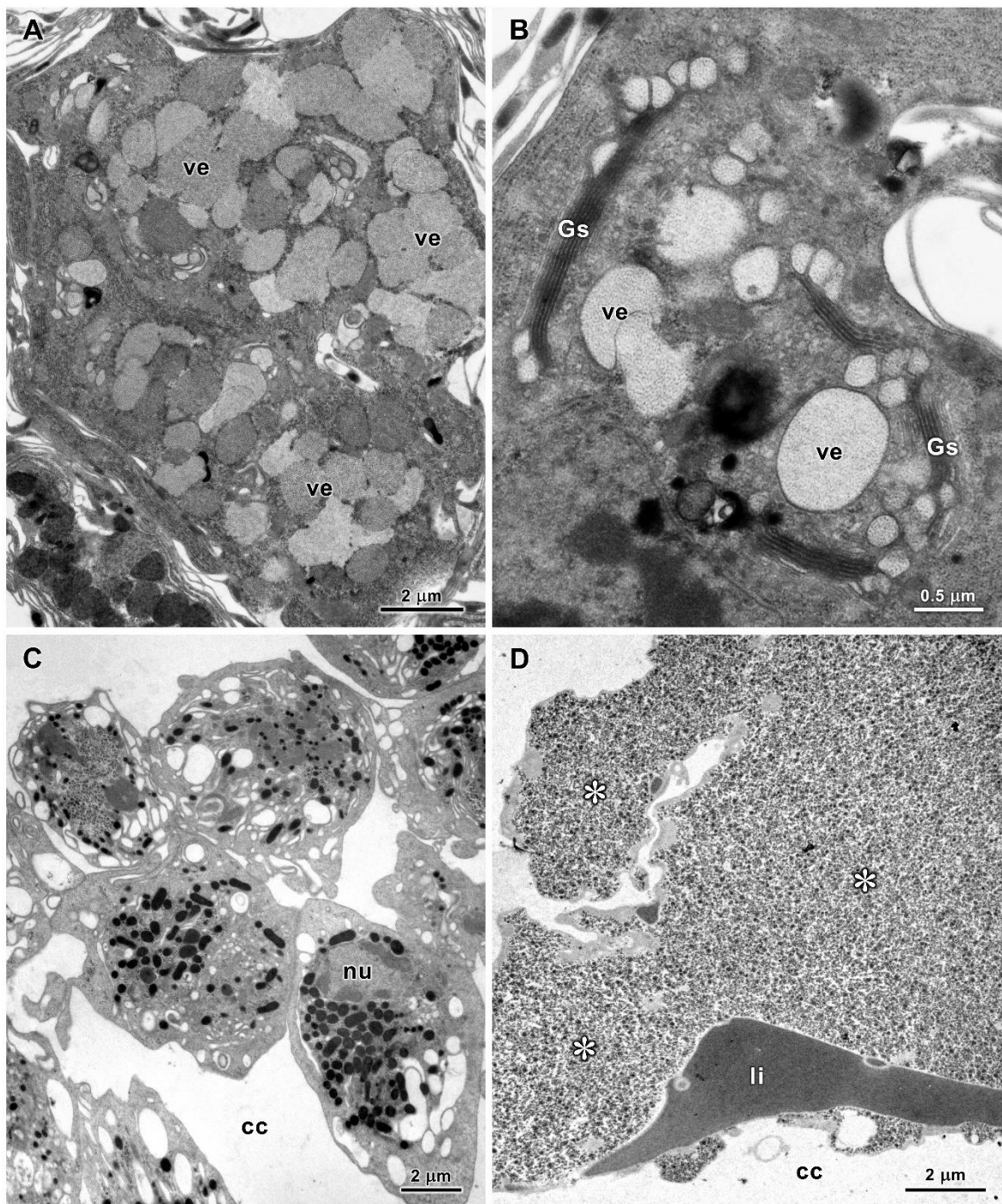


Figure 15. Ultrastructure of secretory cells, coelomocytes and reserve cells from the palps of *Hediste diversicolor*. (A, B) The cells with fusing secretory vesicles (ve) present less rough endoplasmic reticulum cisternae and were richer in Golgi stacks (Gs). (C) Coelomocytes containing electron-dense and electron-lucent vesicles forming a rosette in the celomic cavity (cc). (D) Eleocyte filled with glycogen granules (asterisks) and also with lipid reserves (li); tannic acid-uranyl acetate staining. cc, celomic cavity; li, lipid reserves; Gs, Golgi stacks; nu, nuclei.

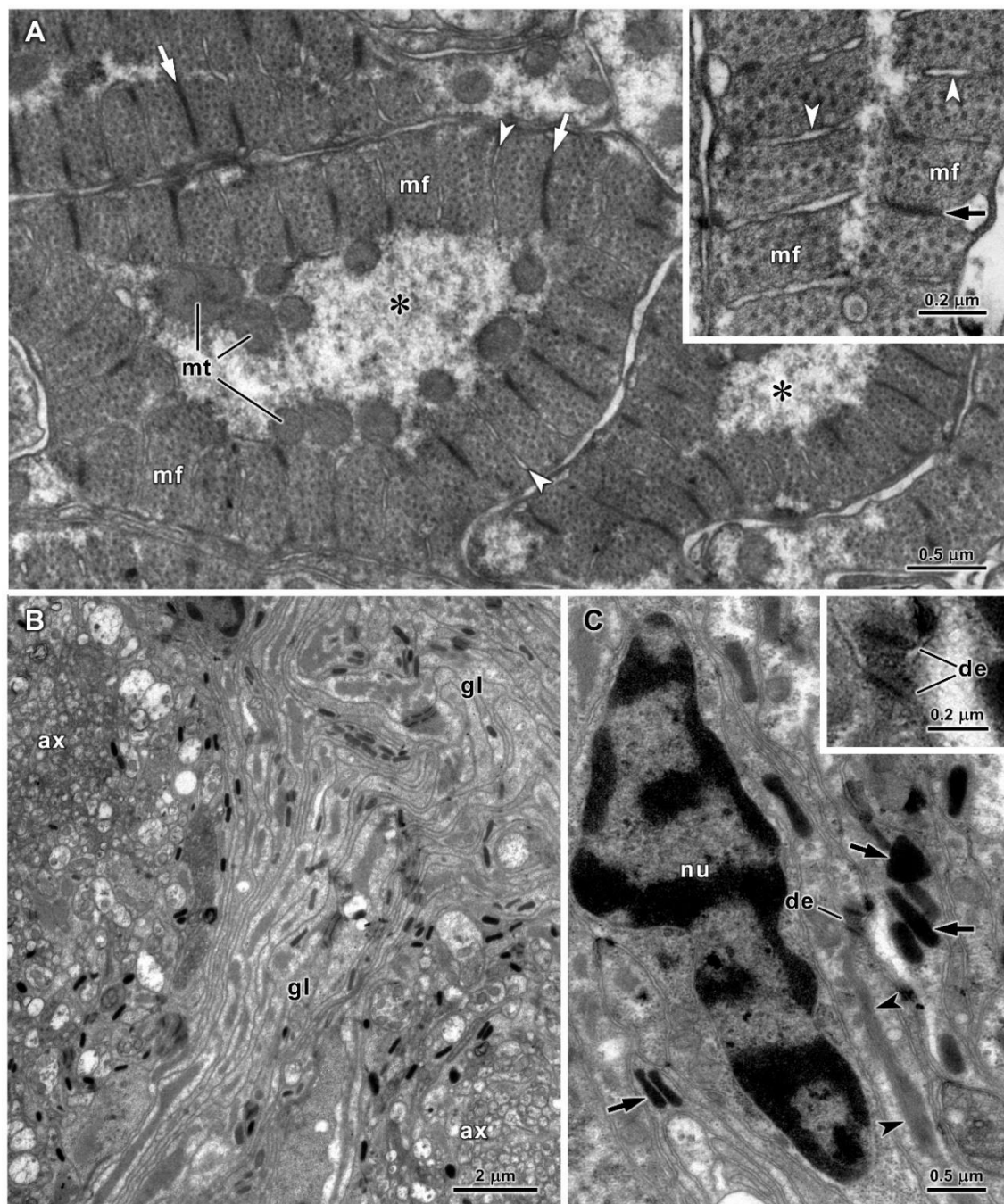


Figure 16. Ultrastructure of muscle fibres and neuropil in the palps of *Hediste diversicolor*. (A) Transverse sections of muscle cells. Electron-dense Z bars (arrows) and sarcoplasmic reticulum tubules (arrowheads) are visible between the myofilaments (mf) at the cell periphery. The central region contains mitochondria (mt) and glycogen deposits (asterisks). (B) A large number of axons (ax) and glia cells (gl) form the neuropil. (C) Glia cells contain bundles of filaments (arrowheads) and gliosomes (arrows) in their fine cellular processes. ax, axons; de, desmosomes; gl, glia cell processes; mf, myofilaments; mt, mitochondria; nu, nucleus.

Two types of coelomocytes could be recognised in the celomic cavity of the palps. The small coelomocytes presented a roughly round or oval shape and formed pseudopodia. The central region of these cells contained the nucleus, a few small mitochondria, rough endoplasmic reticulum cisternae, cisternae with smooth membrane, many small electron-dense and electron-lucent vesicles. A thin peripheral layer of cytoplasm was almost devoid of organelles. The small coelomocytes were frequently seen in rosette aggregates (Fig. 15C). Other coelomocytes, which were much larger and irregular in shape, had their cytoplasm almost entirely filled by glycogen granules. Additionally, some osmiophilic lipidic droplets were also present (Fig. 15D). These large coelomocytes were identified as eleocytes, which were stained by Alcian blue and PAS reaction in semithin sections (Fig. 10C, D). More internally, muscle fibres surrounded the central neuropil of the palps. These cells exhibited arrays of myofilaments at the periphery and the central region contained the nucleus, several mitochondria and glycogen deposits (Fig. 16A). The neuropil consisted of numerous axons with small mitochondria and vesicles, and glia cells with thin cytoplasmic processes (Fig. 16B). Glia cells and their cytoplasmic processes contain several flattened electron-dense gliosomes and bundles of filaments connected to the desmosomes that attach these cells (Fig. 16C).

2.5. Discussion

In annelids the cuticle functions as a strong yet flexible exoskeleton. The thickness and architecture of the annelid cuticle is very variable, with some relationship with species size and lifestyle. A nearly orthogonal grid of collagen fibre layers is the main structural component of the cuticle in many polychaetes. However, the cuticle of very small polychaetes of the interstitial fauna may lack collagen fibres or have them irregularly arranged. Collagen fibres are also absent in the thin cuticle of tubicolous polychaetes of the families Sabellidae and Serpulidae among others (Westheide & Rieger, 1978; Hausen, 2005; Meyer et al., 2021). In the large errant polychaete *Alitta virens* (Sars, 1835) that can reach 30 cm in length (Simonsen et al., 2019), the body cuticle with approximately 40 layers of collagen fibres attains a thickness of 10 µm (Murray et al., 1981). In *H. diversicolor*, which is a smaller errant polychaete, the cuticle has the same structure as in *A. virens*, but with a lower number of collagen fibre layers and less thickness (Pilato & La Rosa, 1992). However, cuticle thickness and number of collagen fibres layers is not the same in all parts of the annelid body. In the anterior segments of *H. diversicolor* the dorsal cuticle has a thickness of 5–6 µm with 33 collagen layers, and the ventral cuticle 6–6.5 µm with 38 collagen layers, but in posterior segments the cuticle thickness is only about 2.5 µm with 17–20 collagen layers (Pilato & La Rosa, 1992). In

the palpopore of *H. diversicolor*, cuticle is thinner than in the anterior segments (approximately 4.5 µm) and has a higher number of collagen fibres layers (26–30) than the 15 layers previously reported in the palps of this species (Dorsett & Hyde, 1969).

In the palps of *H. diversicolor*, collagen fibres are thinner at the basal and top layers of the cuticle and thicker in the central region. This variation in the diameter of collagen fibres was also noticed in the body cuticle of *H. diversicolor* (Pilato & La Rosa, 1992) and in other polychaetes (Murray et al., 1981; Lepescheux, 1988). However, fibre cross section in *H. diversicolor* palpopore and body cuticle (Pilato & La Rosa, 1992) have a lesser diameter than in *A. virens* in which the thicker fibres in the middle layers of the cuticle have a diameter of 250–300 nm (Murray et al., 1981). In annelid cuticle, these fibres are formed by collagen molecules that differ from other collagen types, lacking the banded pattern typical of connective tissue collagens (Murray et al., 1981; Hausen, 2005). In *H. diversicolor* as in other annelids, cuticle staining by Alcian blue and PAS reaction can be mainly attributable to neutral polysaccharide and acid mucopolysaccharide components of the matrix around collagen fibres. Nevertheless, some degree of staining can be due to sugars linked to collagen molecules (Welsch & Storch, 1986; Hausen, 2005). Our observations of *H. diversicolor* palps revealed a marked difference in cuticle thickness between the palpopore and the palpostyle. This difference was mainly due to a substantial reduction in the number of collagen fibre layers in the palpostyle, an aspect that may be relevant to enhance the sensitivity of the palpostyle. In addition, the epicuticular digitiform projections that were only seen around the cilia of sensory cells may contribute to the sensory functions of the palps, or just be involved in the protection of the sensory cilia (Dorsett & Hyde, 1969). A filamentous and fine granular structure was observed in the epicuticle of different annelids (Dorsett & Hyde, 1969; Gustavsson & Erséus, 2000; Hausen, 2005). However, in favourable ultrathin sections of *H. diversicolor* palps, at high magnification, it was possible to see numerous thin tubular structures not previously reported in the epicuticle of annelids.

The use of tannic acid-uranyl acetate staining for visualization of glycogen and collagen by TEM (Afzelius, 1992) was particularly useful to visualize the thinnest collagen fibres at the base of the cuticle and inside the vesicles of epidermal cells, which were invisible in ultrathin sections stained by uranyl acetate and lead citrate. Epidermal cells are regarded as involved in the formation of the cuticle (Bartolomaeus, 1992). In the palps of *H. diversicolor*, the detection of collagen fibres inside large vesicles of epidermal cells indicates that segments of collagen fibres are assembled within epidermal cells prior to being released by exocytosis into the subcuticular space. The epidermal cells of *H.*

diversicolor and other annelids possess numerous microvilli that cross the cuticle and may be involved in the uptake of small organic molecules, such as amino acids, from the interstitial water of the sediments (Westheide & Rieger, 1978). Another feature of annelid epidermal cells are the filament bundles characteristic of cells that need to withstand mechanical forces (Bartolomaeus, 1992; Hausen, 2005).

Several types of secretory cells have been identified in the integument of polychaetes (Gardiner, 1992; Hausen, 2005). Five types of secretory cells could be recognised by TEM in the palps of *H. diversicolor*, which were distinguished mainly by the ultrastructural aspects of the secretory vesicles. The main body of these cells was localised below the epidermis, and their secretion must be released through the cell neck that crosses the epidermis. The most abundant were the cells with multivesicular vesicles, which seem to correspond to the secretory cells of type 5 described by Dorsett & Hyde (1970b), considering the ultrastructural features. However, according to Dorsett & Hyde (1970b) these cells were stained by PAS reaction, but not by Alcian blue. According to our results, the cells with multivesicular vesicles stain with Alcian blue and only weakly with the PAS reaction, indicating that these cells secrete acid mucopolysaccharides. The cells with spotted vesicles were designated as type 1 by Dorsett & Hyde (1970a), and our results clearly show that these are protein-secreting cells. The abundance of flat rough endoplasmic reticulum cisternae and the high electron-density of the secretory vesicles suggest that the cells with these features are also protein-secreting cells, although it was not possible to identify them in semithin sections stained with histochemical reactions. The other two cell types found in the palps of *H. diversicolor* present ultrastructural features of mucus-secreting cells, namely, secretory vesicles with lower electron-density, dilated rough endoplasmic reticulum cisternae and many Golgi stacks. The ones containing dilated rough endoplasmic reticulum cisternae with flocculent material and vesicles with fine granular content that didn't fuse with other vesicles, could correspond to the secretory cells of type 2 described by Dorsett & Hyde (1970a). These cells were not noticed in semithin sections, and according to Dorsett & Hyde (1970a) the secretion of these cells was not stained by PAS reaction neither by Alcian blue. The last cell type, characterised by fusing vesicles with low or median electron-density, doesn't seem to correspond to any of the secretory cells described in the integument of *nereidids* (Dorsett & Hyde, 1970a, 1970b).

Coelomocytes of annelids are involved in defence against infections and parasites (Porchet-Henneré & M'Berri, 1987; Adamowicz, 2005). The smaller coelomocytes found in the celomic cavity of *H. diversicolor* palps can be classified as granulocytes because

they contain numerous membrane-delimited electron-dense granules (Baskin, 1974; Dhainaut, 1984). However, these cells formed pseudopods and were seen in aggregations, which are features more typical of amoebocytes in other annelids (Adamowicz, 2005). The large coelomocytes found in the celomic cavity of *H. diversicolor* palps have features attributed to eleocytes, namely, large size, paucity of cytoplasmic organelles and abundance of glycogen and lipid reserves (Dhainaut, 1984; Adamowicz, 2005). On the other hand, the large acidic vacuole also typical of eleocytes (Baskin, 1974; Dhainaut, 1984; Hoeger *et al.*, 1995) was not observed in the eleocytes found in the palps of *H. diversicolor*. Eleocytes seem to be multifunctional, not only operating as a reserve cell with very large amounts of glycogen and lipids, but also capable of phagocytosis and vitellogenin production in females, naturally presenting different ultrastructural aspects according to the functions in which they are involved (Baskin, 1974; Bonnier *et al.*, 1991).

The neuropile that occupies the central region of the palps comprises numerous axons and supportive glia cells. The nervous system of nereidid polychaetes is rich in glial cells with extensive cytoplasmic processes containing bundles of filaments attached to the numerous desmosomes that link these cells (Baskin, 1971). The electron-dense vesicles of glial cells, known as gliosomes, which are abundant in the neuropil of *H. diversicolor* palps, have important physiological roles in nervous tissue of other animals (Milanese *et al.*, 2009) and certainly will be important also in the nervous system of annelids.

This study offers a comprehensive view of the tissues that constitute the palps of *H. diversicolor*, covering the epidermis and cuticle, secretory cells, coelomocytes, muscle and nerve tissues, providing new information about these sense organs of an errant polychaete species that has great relevance in European estuarine ecosystems.

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General Conclusion and future perspectives

With this work it was possible to obtain the average increase in terms of size or number of all the structures analysed for each new chaetiger segment formed in *H. diversicolor*, as well as to understand how the various structures correlate positively or negatively with the number of chaetiger segments the animal has, creating hypotheses to explain these results. It was also possible to detail from which number of chetiger segments it is possible to observe simple chaeta and their position along the body axis.

Due to the proportional and continuous growth between most of the structures analysed and the increase in the number of chaetiger segments, it was not easy to delimit distinct stages of the life cycle. For this purpose, individuals from the larval stage up to individuals in the mature adult stage would need to be obtained, requiring a more complex and time-consuming procedure. It would also be necessary to place only mature individuals in the tank and analyse water filtrates until the first larval stages appear. Once larvae were detected, adults would need to be removed. Then it would be necessary to collect a specific number of specimens at pre-defined intervals to follow their development. In the case of this study, since one of the criteria for specimen selection was the choice of animals with a uniform distribution in terms of the number of chaetiger segments, there was no such temporal monitoring to record changes at key points that would enable an irrefutable differentiation between different life cycle stages.

In the future it will be possible to follow the evolution of the life cycle of *H. diversicolor* by applying the experimental procedure mentioned above, and combining it with existing data, this time also considering growth in relation to time, for trying to further identify key differences in the development pattern and differentiate distinct life cycle stages.

With regard to the study of the palps using histochemistry and TEM, the results obtained using different staining and contrasting techniques verify, and in some cases correct, the existing literature, as was observed, for example, with regard to the number of collagen filaments in the cuticle of the palpophore. In other cases, it also supplemented with previously unknown information. It was also realised that in addition to their sensory function, palps also have secretory cells that confer to them a secretory function.

In the future, the same techniques used in this study can be applied to study other appendages present in the prostomium, such as the lateral antennae, peristomial structures such as tentacular cirri, multiple structures from the parapodia such as notopodial and neuropodial cirri and even lobes and ligules, or pygidial cirri so that a

comparison can be made between them and possibly better understand their specific functions.

The new data obtained from this experiment can also be used as a baseline for toxicological studies. By using the same methodology, future studies can compare their results with the findings of this work, providing a “before” and “after” exposure scenario of specific substances and compound effects on the ultrastructure of the palps in *H. diversicolor* to assess the impact.

Supplementary Material

Table S1. Biometric and morphological data on different structures of the proboscis and prostomium of *Hediste diversicolor*

Sample Code	Number chatigers	Total Length	Proboscis													Prostomium										
			Paragnaths										Maxillae			length	Palps								Lateral antennae	
			Maxillary ring						Oral ring				Number teeth		palpophore				palpostyle							
			I	II		III	IV		V	VI		VII & VIII	a	b	a		b	a	b	a	b	length	width			
				a	b		a	b		a	b													length	width	length
NATMIP- Apph-0013	88	99,78	3	22	24	59	47	44	0	6	6	40	9	9	1,44	0,91	0,85	0,91	0,90	0,10	0,27	0,11	0,22	0,34	0,18	
PRM_001 8/IX/2022	104	129,86	6	18	17	32	32	33	0	5	4	28	6	6	0,92	0,91	0,90	0,91	0,89	0,41	0,23	0,33	0,23	0,46	0,09	
PRM_002 8/IX/2022	90	74,88	3	17	17	44	34	38	0	4	4	47	7	7	0,99	0,85	0,93	0,82	0,61	0,13	0,22	0,09	0,17	0,48	0,15	
BCC_001 22/IX/2022	107	92,24	2	13	12	32	26	27	0	4	5	31	7	7	1,19	1,12	1,10	1,08	1,10	0,09	0,35	0,11	0,34	0,51	0,22	
RA_001 23/III/2022	40	7,83	1	9	10	9	10	10	0	6	5	16	5	5	0,26	0,22	0,22	0,23	0,21	0,09	0,08	0,09	0,08	0,16	0,05	
Aqua_001 12/X/2022	32	6,42	x	x	x	x	x	x	x	x	x	x	7	7	0,28	0,19	0,16	0,18	0,17	0,09	0,08	0,09	0,08	0,12	0,05	
Aqua_002 12/X/2022	60	18,29	1	9	9	17	14	13	0	3	4	24	7	7	0,64	0,27	0,29	0,28	0,28	0,11	0,17	0,11	0,16	0,25	0,08	
Aqua_003 17/IV/2023	33	6,38	x	x	x	x	x	x	x	x	x	x	7	7	0,29	0,18	0,17	0,18	0,17	0,11	0,07	0,10	0,07	0,17	0,04	
PRM_003 19/IV/2023	92	96,34	7	14	12	50	39	38	0	6	5	29	7	7	1,28	0,64	0,55	0,61	0,51	0,18	0,25	0,18	0,22	0,51	0,15	
Icbas_001 13/IV/2023	76	50,22	2	9	8	19	18	18	0	5	5	13	7	7	1,22	0,54	0,55	0,52	0,79	0,20	0,26	0,12	0,23	0,53	0,21	
Icbas_002 19/V/2023	16	2,85	x	x	x	x	x	x	x	x	x	x	7	8	0,20	0,08	0,10	0,08	0,09	0,03	0,04	0,03	0,04	0,09	0,02	
Icbas_003 19/V/2023	16	2,89	x	x	x	x	x	x	x	x	x	x	8	8	0,23	0,10	0,12	0,10	0,09	0,07	0,06	0,06	0,05	0,09	0,02	
Icbas_004 19/V/2023	25	5,16	0	6	5	2	8	7	0	2	4	8	7	7	0,27	0,15	0,14	0,14	0,13	0,05	0,06	0,05	0,05	0,12	0,04	
Icbas_005 19/V/2023	25	5,90	0	8	9	5	8	7	0	3	3	8	8	8	0,22	0,19	0,16	0,17	0,16	0,05	0,06	0,06	0,06	0,09	0,03	
Icbas_006 19/V/2023	32	7,26	1	7	9	5	10	11	0	1	3	18	7	7	0,23	0,19	0,16	0,20	0,16	0,07	0,07	0,07	0,07	0,12	0,03	
Icbas_007 19/V/2023	35	7,81	1	14	14	8	13	12	0	4	3	18	7	7	0,24	0,21	0,17	0,21	0,20	0,06	0,08	0,06	0,07	0,20	0,05	
Icbas_008 19/V/2023	30	5,85	x	x	x	x	x	x	x	x	x	x	7	7	0,31	0,16	0,16	0,18	0,17	0,08	0,07	0,09	0,06	0,16	0,05	
Icbas_009 03/VI/2023	10	1,54	x	x	x	x	x	x	x	x	x	x	8	8	0,17	0,07	0,08	0,08	0,07	0,06	0,05	0,06	0,05	0,07	0,02	
Aqua_004 20/VI/2023	67	36,43	2	22	16	31	31	26	0	5	5	24	7	7	0,82	0,59	0,45	0,65	0,51	0,16	0,19	0,19	0,17	0,27	0,11	
Icbas_010 19/V/2023	24	4,34	x	x	x	x	x	x	x	x	x	x	7	7	0,36	0,18	0,15	0,19	0,15	0,08	0,08	0,08	0,07	0,13	0,05	
Aqua_005 04/VII/2023	55	16,72	1	15	14	22	22	26	0	5	5	30	7	7	0,45	0,31	0,26	0,37	0,28	0,08	0,10	0,07	0,08	0,23	0,06	
Aqua_006 04/VII/2023	52	19,80	3	20	15	23	23	28	0	5	7	40	7	7	0,64	0,46	0,30	0,43	0,38	0,06	0,10	0,08	0,14	0,28	0,08	
Icbas_011 11/X/2023	74	40,72	2	15	15	18	25	23	0	6	6	30	8	8	1,07	0,66	0,41	0,69	0,44	0,12	0,14	0,13	0,13	0,32	0,12	
Icbas_012 11/X/2023	74	38,90	7	22	23	25	26	28	0	6	7	27	7	7	0,87	0,48	0,42	0,66	0,52	0,10	0,18	0,11	0,19	0,36	0,14	
Icbas_013 11/X/2023	72	37,48	2	20	20	16	20	23	0	8	5	21	8	8	1,06	0,57	0,44	0,61	0,48	0,09	0,16	0,10	0,15	0,36	0,11	
Icbas_015 17/X/2023	48	32,31	4	14	14	22	23	16	0	5	5	33	7	7	0,68	0,72	0,50	0,67	0,51	0,08	0,17	0,15	0,17	0,38	0,12	
Icbas_016 17/X/2023	55	34,63	2	14	18	20	18	15	0	5	5	20	7	7	0,72	0,64	0,70	0,67	0,60	0,12	0,23	0,12	0,17	0,32	0,11	
Icbas_017 17/X/2023	44	22,16	2	21	15	14	19	19	0	5	6	22	7	7	0,67	0,60	0,47	0,56	0,38	0,11	0,14	0,10	0,14	0,27	0,10	
Icbas_021 17/X/2023	43	12,89	x	x	x	x	x	x	x	x	x	x	7	7	0,58	0,34	0,31	0,31	0,30	0,11	0,12	0,10	0,12	0,19	0,07	
Icbas_022 17/X/2023	34	20,04	x	x	x	x	x	x	x	x	x	x	7	7	0,65	0,25	0,38	0,24	0,38	0,12	0,15	0,10	0,16	0,27	0,09	

: Measures in mm

Table S2. Biometric and morphological data on different structures of the peristomium of *Hediste diversicolor*

Sample Code	Number chaetigers	Total Length	Peristomium																																
			Modified Anterior Cirri																																
			Cirri 1								Cirri 2								Cirri 3								Cirri 4								
			Cirrophore				Cirrostyle				Cirrophore				Cirrostyle				Cirrophore				Cirrostyle				Cirrophore				Cirrostyle				
			a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b			
length	width	length	width	length	width	length	width	length	width	length	width	length	width	length	width	length	width	length	width	length	width	length	width	length	width	length	width	length	width	length	width				
'ATMIP- Apph-0013	88	99,78	0,18	0,24	0,20	0,23	0,73	0,23	0,97	0,19	0,24	0,31	0,25	0,32	1,67	0,26	1,44	0,23	0,20	0,23	0,14	0,22	1,14	0,17	1,02	0,19	0,37	0,29	0,29	0,20	1,63	0,25	2,20	0,19	
'RM_001 8/I/X/2022	104	129,86	0,31	0,29	0,29	0,28	0,70	0,19	0,66	0,16	0,29	0,23	0,29	0,23	1,35	0,16	1,50	0,20	0,27	0,21	0,25	0,22	1,14	0,14	1,17	0,18	0,38	0,27	0,38	0,27	2,71	0,17	2,73	0,17	
'RM_002 8/I/X/2022	90	74,88	0,15	0,17	0,16	0,16	0,79	0,17	0,63	0,16	0,21	0,28	0,28	0,25	2,07	0,16	2,05	0,15	0,10	0,22	0,21	0,20	1,47	0,15	1,40	0,13	0,40	0,27	0,30	0,26	2,44	0,23	2,78	0,15	
CC_001 22/I/X/2022	107	92,24	0,17	0,23	0,22	0,22	0,79	0,13	0,48	0,17	0,11	0,25	0,21	0,31	1,57	0,14	1,62	0,10	0,14	0,19	0,15	0,17	1,24	0,13	1,10	0,11	0,15	0,27	0,30	0,28	2,31	0,18	1,57	0,15	
RA_001 23/II/2022	40	7,83	0,07	0,07	0,07	0,07	0,24	0,04	0,25	0,03	0,09	0,09	0,10	0,08	0,54	0,05	0,53	0,06	0,06	0,06	0,06	0,05	0,31	0,04	0,32	0,05	0,11	0,14	0,10	0,14	0,62	0,04	0,79	0,05	
qua_001 12/X/2022	32	6,42	0,06	0,05	0,07	0,07	0,16	0,03	0,14	0,03	0,05	0,05	0,05	0,05	0,37	0,04	0,23	0,06	0,04	0,05	0,06	0,05	0,32	0,03	0,37	0,03	0,09	0,07	0,08	0,06	0,38	0,04	0,59	0,04	
qua_002 12/X/2022	60	18,29	0,05	0,07	0,06	0,07	0,45	0,07	0,44	0,08	0,09	0,08	0,08	0,08	0,56	0,07	0,60	0,06	0,08	0,07	0,08	0,07	1,02	0,07	0,95	0,08	0,10	0,10	0,10	0,11	0,84	0,07	0,81	0,08	
qua_003 17/IV/2023	33	6,38	0,06	0,06	0,06	0,07	0,18	0,03	0,17	0,03	0,08	0,05	0,08	0,05	0,44	0,04	0,40	0,04	0,06	0,05	0,06	0,05	0,22	0,02	0,22	0,02	0,06	0,06	0,05	0,05	0,50	0,05	0,49	0,05	
RM_003 19/IV/2023	92	96,34	0,26	0,21	0,22	0,22	0,69	0,14	0,70	0,11	0,35	0,27	0,33	0,28	2,44	0,13	0,60	0,16	0,28	0,23	0,26	0,18	1,62	0,14	0,63	0,14	0,45	0,30	0,41	0,36	2,85	0,14	1,88	0,15	
bas_001 13/IV/2023	76	50,22	x	x	0,19	0,21	x	x	0,93	0,21	x	x	0,34	0,24	x	x	1,47	0,24	x	x	0,23	0,27	x	x	1,48	0,21	x	x	0,51	0,26	x	x	3,12	0,20	
bas_002 19/V/2023	16	2,85	0,03	0,02	0,03	0,02	0,09	0,02	0,09	0,02	0,03	0,04	0,03	0,04	0,19	0,03	0,19	0,03	0,03	0,03	0,03	0,10	0,03	0,09	0,03	0,03	0,04	0,03	0,04	0,40	0,04	0,36	0,04		
bas_003 19/V/2023	16	2,89	0,03	0,03	0,03	0,03	0,10	0,03	0,11	0,03	0,04	0,04	0,03	0,04	0,22	0,03	0,19	0,03	0,04	0,03	0,03	0,03	0,11	0,03	0,12	0,03	0,04	0,04	0,05	0,04	0,42	0,04	0,38	0,04	
bas_004 19/V/2023	25	5,16	0,03	0,04	0,04	0,04	0,18	0,04	0,15	0,04	0,04	0,05	0,06	0,05	0,30	0,04	0,31	0,04	0,04	0,04	0,03	0,04	0,19	0,03	0,18	0,03	0,07	0,06	0,07	0,06	0,35	0,04	0,56	0,04	
bas_005 19/V/2023	25	5,90	0,04	0,05	0,04	0,05	0,16	0,04	0,19	0,03	0,05	0,07	0,05	0,07	0,34	0,04	0,33	0,04	0,04	0,05	0,05	0,06	0,23	0,04	0,19	0,04	0,05	0,07	0,06	0,07	0,54	0,05	0,64	0,05	
bas_006 19/V/2023	32	7,26	0,04	0,07	0,04	0,06	0,20	0,04	0,21	0,04	0,05	0,07	0,05	0,06	0,29	0,05	0,30	0,04	0,05	0,05	0,05	0,06	0,16	0,03	0,24	0,04	0,06	0,06	0,06	0,07	0,57	0,05	0,70	0,05	
bas_007 19/V/2023	35	7,81	0,07	0,07	0,06	0,07	0,22	0,05	0,24	0,05	0,06	0,08	0,07	0,08	0,39	0,04	0,33	0,05	0,07	0,06	0,07	0,06	0,25	0,03	0,27	0,03	0,08	0,08	0,09	0,07	0,88	0,07	0,66	0,05	
bas_008 19/V/2023	30	5,85	0,05	0,06	0,06	0,06	0,15	0,04	0,23	0,05	0,07	0,05	0,06	0,05	0,33	0,04	0,30	0,04	0,05	0,04	0,06	0,04	0,17	0,03	0,18	0,03	0,09	0,07	0,09	0,07	0,61	0,05	0,49	0,05	
bas_009 03/VII/2023	10	1,54	0,04	0,04	0,02	0,03	0,07	0,03	0,07	0,02	0,03	0,03	x	x	0,18	0,03	x	x	0,02	0,02	0,02	0,03	0,11	0,02	0,08	0,02	0,03	0,04	x	x	0,34	0,03	x	x	
qua_004 20/VI/2023	67	36,43	0,12	0,13	0,13	0,13	0,42	0,12	0,46	0,12	0,22	0,20	0,24	0,16	0,92	0,14	1,01	0,13	0,16	0,16	0,19	0,14	0,47	0,11	0,58	0,10	0,25	0,17	0,28	0,18	1,86	0,13	1,77	0,16	
bas_010 19/V/2023	24	4,34	0,05	0,06	0,05	0,06	0,16	0,04	0,14	0,04	0,05	0,06	0,06	0,05	0,28	0,04	0,32	0,04	0,05	0,04	0,04	0,05	0,15	0,03	0,17	0,03	0,06	0,05	0,08	0,06	0,45	0,05	0,56	0,04	
qua_005 04/VII/2023	55	16,72	0,11	0,10	0,10	0,09	0,28	0,08	0,33	0,10	0,11	0,10	0,10	0,11	0,47	0,07	0,82	0,07	0,09	0,11	0,10	0,08	x	x	0,17	0,03	0,17	0,12	0,17	0,14	1,23	0,09	1,94	0,06	
qua_006 04/VII/2023	52	19,80	0,18	0,15	0,17	0,13	0,58	0,09	0,55	0,08	0,19	0,14	0,20	0,17	1,09	0,09	0,92	0,09	0,12	0,11	0,14	0,12	0,63	0,08	0,57	0,07	0,24	0,17	0,19	0,16	1,51	0,08	1,99	0,10	
bas_011 11/X/2023	74	40,72	0,18	0,15	0,21	0,13	0,83	0,11	0,82	0,10	0,25	0,17	0,27	0,17	1,57	0,13	1,47	0,14	0,14	0,21	0,13	0,20	1,00	0,12	0,99	0,12	0,31	0,17	0,30	0,17	2,48	0,13	2,49	0,10	
bas_012 11/X/2023	74	38,90	0,19	0,13	0,20	0,12	0,68	0,15	0,90	0,13	0,22	0,16	0,22	0,15	1,71	0,15	1,54	0,12	0,20	0,12	0,21	0,16	1,10	0,12	1,00	0,14	0,27	0,16	0,35	0,20	2,42	0,15	2,25	0,14	
bas_013 11/X/2023	72	37,48	0,21	0,20	0,21	0,20	0,80	0,11	0,78	0,09	0,30	0,16	0,31	0,15	1,89	0,12	1,48	0,12	0,19	0,15	0,19	0,15	1,08	0,13	0,97	0,10	0,27	0,16	0,31	0,18	2,77	0,12	3,14	0,14	
bas_015 17/X/2023	48	32,31	0,17	0,17	0,19	0,16	0,53	0,12	0,55	0,13	0,20	0,21	0,15	0,18	1,44	0,14	1,23	0,14	0,26	0,13	0,21	0,13	0,70	0,13	0,72	0,10	0,33	0,18	0,36	0,17	1,65	0,15	2,86	0,17	
bas_016 17/X/2023	55	34,63	0,16	0,17	0,20	0,20	0,61	0,13	0,79	0,15	0,23	0,21	0,31	0,21	1,74	0,16	1,74	0,22	0,22	0,20	0,18	0,20	1,07	0,15	1,04	0,13	0,29	0,20	0,31	0,20	2,69	0,18	2,94	0,18	
bas_017 17/X/2023	44	22,16	0,17	0,12	0,17	0,15	0,67	0,11	0,56	0,08	0,15	0,16	0,20	0,16	0,99	0,09	1,03	0,09	0,14	0,15	0,15	0,12	0,67	0,12	0,45	0,08	0,25	0,16	0,21	0,12	1,28	0,11	0,92	0,10	
bas_021 17/X/2023	43	12,89	0,12	0,10	0,12	0,11	0,29	0,08	0,31	0,08	0,12	0,11	0,13	0,12	0,51	0,08	0,66	0,07	0,10	0,09	0,12	0,10	0,37	0,07	0,40	0,09	0,15	0,16	0,12	0,15	0,95	0,08	0,85	0,08	
bas_022 17/X/2023	34	20,04	0,07	0,13	0,08	0,10	0,26	0,08	0,26	0,08	0,15	0,13	0,16	0,13	1,14	0,10	1,04	0,10	0,12	0,13	0,13	0,12	0,46	0,09	0,56	0,11	0,16	0,13	0,16	0,12	0,17	0,13	1,19	0,12	

 : Measures in mm

Table S3. Biometric and morphological data on different structures of *Hediste diversicolor* from anterior parapodium and the anterior-median transitional parapodium

Sample Code	Number chaetigers	Total Length	Biramous Parapodia																																		
			Anterior (1st parapodium)														Transition anterior-median																				
			Notopodium								Neuropodium						Notopodium								Neuropodium												
			Cirrus		Ligule				Acicula	Cirrus		Ligule				Acicula	Cirrus		Ligule				Acicula	Cirrus		Ligule				Acicula							
length	width	length	width	length	width	length	width	length	length	width	length	width	length	width	length	length	width	length	width	length	width	length	length	width	length	width	length	length	width	length	width	length					
NATMIP- Apph-0013	88	99,78	0,42	0,13	0,62	0,32	Not applicable (1st)				0,47	0,14	0,54	0,19	0,63	0,25	0,53	0,25	0,88	0,39	0,10	1,90	0,62	0,52	0,23	1,80	0,37	1,73	0,34	0,09	1,60	0,35	1,68	0,32	1,20	0,22	1,72
PRM_001 8/IX/2022	104	129,86	0,51	0,09	0,52	0,32	Not applicable (1st)				0,50	0,12	0,29	0,16	0,60	0,26	0,49	0,24	0,98	0,35	0,07	1,51	0,41	0,43	0,23	1,32	0,29	1,36	0,24	0,07	1,43	0,21	1,37	0,21	1,24	0,22	1,36
PRM_002 8/IX/2022	90	74,88	0,46	0,11	0,87	0,38	Not applicable (1st)				0,51	0,13	0,87	0,23	0,67	0,16	0,68	0,30	1,08	0,40	0,11	1,15	0,52	0,48	0,22	1,01	0,32	1,73	0,30	0,08	1,00	0,35	0,99	0,29	0,78	0,21	1,92
BCC_001 22/IX/2022	107	92,24	0,43	0,14	0,97	0,30	Not applicable (1st)				0,46	0,11	1,06	0,24	0,98	0,11	1,03	0,24	0,97	0,47	0,12	1,83	0,52	0,56	0,17	1,93	0,26	1,70	0,25	0,09	1,78	0,29	1,82	0,21	1,53	0,18	1,97
RA_001 23/II/2022	40	7,83	0,15	0,03	0,22	0,12	Not applicable (1st)				0,10	0,04	0,07	0,03	0,10	0,03	0,14	0,06	0,29	Not measured																	
Aqua_001 12/X/2022	32	6,42	0,24	0,04	0,17	0,08	Not applicable (1st)				0,17	0,03	0,13	0,04	0,23	0,04	0,27	0,10	0,20	Not measured																	
Aqua_002 12/X/2022	60	18,29	0,27	0,05	0,34	0,14	Not applicable (1st)				0,18	0,05	0,21	0,05	0,38	0,06	0,30	0,11	0,27	Not measured																	
Aqua_003 17/IV/2023	33	6,38	0,13	0,03	0,14	0,05	Not applicable (1st)				0,10	0,03	0,12	0,04	0,11	0,04	0,14	0,04	0,14	Not measured																	
PRM_003 19/IV/2023	92	96,34	0,57	0,09	0,82	0,31	Not applicable (1st)				0,61	0,12	0,62	0,13	0,94	0,25	0,89	0,29	1,08	0,39	0,09	1,61	0,55	0,67	0,21	1,45	0,30	1,70	0,37	0,07	1,18	0,14	1,27	0,19	0,91	0,20	1,63
Icbas_001 13/IV/2023	76	50,22	0,49	0,15	0,63	0,31	Not applicable (1st)				x	x	0,54	0,23	0,35	0,16	0,53	0,30	0,84	0,58	0,19	1,10	0,48	0,65	0,31	0,97	0,36	1,85	0,53	0,26	1,03	0,24	1,20	0,24	1,13	0,27	1,73
Icbas_002 19/V/2023	16	2,85	0,03	0,01	0,07	0,02	Not applicable (1st)				0,02	0,01	0,05	0,01	0,05	0,02	0,06	0,02	0,08	Not measured																	
Icbas_003 19/V/2023	16	2,89	0,03	0,01	0,10	0,04	Not applicable (1st)				0,03	0,01	0,09	0,02	0,10	0,02	0,07	0,04	0,13	Not measured																	
Icbas_004 19/V/2023	25	5,16	0,09	0,03	0,14	0,03	Not applicable (1st)				0,06	0,02	0,10	0,02	0,10	0,04	0,12	0,03	0,16	Not measured																	
Icbas_005 19/V/2023	25	5,90	0,08	0,03	0,13	0,02	Not applicable (1st)				0,07	0,02	0,09	0,02	0,10	0,03	0,16	0,03	0,14	Not measured																	
Icbas_006 19/V/2023	32	7,26	0,10	0,02	0,14	0,04	Not applicable (1st)				0,06	0,02	0,10	0,03	0,11	0,03	0,14	0,02	0,14	Not measured																	
Icbas_007 19/V/2023	35	7,81	0,12	0,02	0,16	0,04	Not applicable (1st)				0,08	0,02	0,13	0,02	0,16	0,03	0,16	0,04	0,17	Not measured																	
Icbas_008 19/V/2023	30	5,85	0,11	0,02	0,13	0,03	Not applicable (1st)				0,08	0,02	0,08	0,02	0,10	0,03	0,12	0,03	0,16	Not measured																	
Icbas_009 03/VI/2023	10	1,54	0,05	0,01	0,09	0,02	Not applicable (1st)				0,04	0,01	0,07	0,02	0,08	0,01	0,08	0,02	0,12	Not measured																	
Aqua_004 20/VI/2023	67	36,43	0,23	0,06	0,44	0,12	Not applicable (1st)				0,12	0,02	0,40	0,02	0,42	0,04	0,43	0,08	0,42	0,27	0,05	0,69	0,15	0,23	0,07	0,61	0,14	0,65	0,13	0,04	0,46	0,08	0,49	0,07	0,54	0,05	0,66
Icbas_010 19/V/2023	24	4,34	0,07	0,02	0,14	0,02	Not applicable (1st)				0,07	0,02	0,13	0,02	0,11	0,02	0,14	0,03	0,14	Not measured																	
Aqua_005 04/VII/2023	55	16,72	0,25	0,04	0,35	0,08	Not applicable (1st)				0,24	0,05	0,38	0,07	0,29	0,05	0,37	0,08	0,30	0,22	0,04	0,46	0,11	0,41	0,06	0,43	0,11	0,60	0,22	0,05	0,37	0,05	0,37	0,08	0,31	0,06	0,56
Aqua_006 04/VII/2023	52	19,80	0,22	0,08	0,40	0,16	Not applicable (1st)				0,23	0,04	0,44	0,09	0,36	0,09	0,40	0,12	0,50	0,26	0,08	0,73	0,22	0,49	0,11	0,73	0,14	0,90	0,23	0,06	0,73	0,09	0,69	0,10	0,66	0,12	0,89
Icbas_011 11/X/2023	74	40,72	0,25	0,05	0,36	0,16	Not applicable (1st)				0,36	0,07	0,28	0,11	0,18	0,07	0,31	0,14	0,72	0,38	0,09	0,38	0,09	0,21	0,11	0,38	0,16	1,07	0,18	0,04	0,39	0,12	0,32	0,12	0,34	0,16	0,91
Icbas_012 11/X/2023	74	38,90	0,38	0,08	0,41	0,16	Not applicable (1st)				0,29	0,05	0,36	0,13	0,26	0,06	0,31	0,12	0,51	0,31	0,08	0,44	0,24	0,32	0,10	0,48	0,17	0,74	0,27	0,05	0,45	0,13	0,44	0,13	0,39	0,13	0,78
Icbas_013 11/X/2023	72	37,48	0,36	0,08	0,38	0,20	Not applicable (1st)				0,35	0,08	0,33	0,12	0,31	0,08	0,29	0,12	0,51	0,41	0,06	0,61	0,17	0,25	0,10	0,49	0,20	0,92	0,39	0,06	0,48	0,09	0,37	0,17	0,35	0,11	0,94
Icbas_015 17/X/2023	48	32,31	0,29	0,06	0,36	0,18	Not applicable (1st)				0,21	0,07	0,40	0,12	0,31	0,07	0,37	0,15	0,58	0,32	0,08	0,57	0,27	0,19	0,24	0,50	0,19	1,00	0,27	0,05	0,54	0,12	0,43	0,16	0,37	0,14	0,97
Icbas_016 17/X/2023	55	34,63	0,27	0,07	0,40	0,18	Not applicable (1st)				0,30	0,06	0,42	0,14	0,26	0,16	0,32	0,11	0,55	0,38	0,10	0,80	0,23	0,46	0,17	0,78	0,18	1,12	0,17	0,03	0,67	0,16	0,62	0,14	0,58	0,15	0,98
Icbas_017 17/X/2023	44	22,16	0,20	0,06	0,36	0,15	Not applicable (1st)				0,28	0,06	0,36	0,12	0,31	0,08	0,32	0,11	0,48	0,20	0,07	0,68	0,21	0,41	0,12	0,74	0,17	0,87	0,17	0,03	0,64	0,12	0,51	0,15	0,57	0,13	0,93
Icbas_021 17/X/2023	43	12,89	0,13	0,03	0,27	0,06	Not applicable (1st)				0,12	0,03	0,24	0,04	0,18	0,06	0,23	0,06	0,28	0,16	0,04	0,26	0,08	0,14	0,05	0,26	0,07	0,37	0,10	0,03	0,28	0,05	0,28	0,11	0,27	0,07	0,38
Icbas_022 17/X/2023	34	20,04	0,08	0,02	0,25	0,07	Not applicable (1st)				0,08	0,01	0,28	0,08	0,20	0,07	0,27	0,08	0,42	Not measured																	

 : Measures in mm

Table S4. Biometric and morphological data on different structures of *Hediste diversicolor* from the parapodium before the first parapodium with simple chaeta and from the first parapodium with simple chaeta.

Sample Code	Number chaetigers	Total Length	Biramous Parapodia																																					
			Before first simple chaeta parapodium																			First simple chaeta parapodium																		
			Notopodium									Neuropodium										Notopodium									Neuropodium									
			Cirrus		Ligule						Acicula	Cirrus		Ligule						Acicula	Cirrus			Ligule						Acicula	Cirrus		Ligule						Acicula	
length	width	length	width	length	width	length	width	length	width	length	length	width	length	width	length	width	length	width	length	length	width	length	width	length	width	length	width	length	width	length	length	width	length	width	length	width	length	width	length	
ATMP- Apph-0013	88	99,78	0,26	0,10	1,50	0,34	0,27	0,11	1,28	0,24	2,35	0,26	0,07	1,18	0,12	1,19	0,21	0,93	0,10	2,23	0,29	0,10	1,52	0,33	0,28	0,17	1,50	0,16	2,19	0,33	0,09	1,19	0,16	1,21	0,11	0,84	0,08	2,17		
RM_001 8/IX/2022	104	129,86	0,32	0,09	2,56	0,59	0,34	0,26	1,86	0,35	2,18	0,31	0,28	1,49	0,34	1,43	0,34	1,66	0,20	2,05	0,52	0,08	1,96	0,26	0,31	0,09	1,06	0,22	2,36	0,46	0,08	1,13	0,10	1,06	0,12	0,96	0,16	2,31		
RM_002 8/IX/2022	90	74,88	0,40	0,08	1,16	0,51	0,31	0,16	0,66	0,28	1,82	0,21	0,08	0,82	0,27	0,80	0,31	0,79	0,13	1,57	0,39	0,09	1,76	0,36	0,39	0,12	1,50	0,32	1,89	0,24	0,10	1,12	0,17	1,12	0,26	0,81	0,17	1,88		
CC_001 22/IX/2022	107	92,24	0,45	0,11	2,22	0,38	0,35	0,13	1,95	0,21	1,92	0,30	0,08	1,52	0,23	1,39	0,19	1,23	0,11	1,87	0,44	0,12	2,10	0,41	0,29	0,13	1,85	0,22	1,85	0,24	0,08	1,44	0,24	1,44	0,16	1,17	0,14	1,93		
RA_001 23/II/2022	40	7,83	Not measured																			Not measured																		
qua_001 12/X/2022	32	6,42	Not measured																			Not measured																		
qua_002 12/X/2022	60	18,29	Not measured																			Not measured																		
jua_003 17/IV/2023	33	6,38	0,13	0,04	0,26	0,05	0,09	0,04	0,25	0,08	0,46	0,11	0,04	0,22	0,07	0,19	0,06	0,18	0,07	0,44	0,12	0,03	0,28	0,05	0,07	0,03	0,23	0,07	0,45	0,10	0,03	0,21	0,06	0,20	0,06	0,15	0,07	0,42		
RM_003 19/IV/2023	92	96,34	0,49	0,08	1,59	0,43	0,72	0,15	1,56	0,28	2,36	0,31	0,10	1,34	0,25	1,41	0,30	1,23	0,21	2,11	0,47	0,09	1,57	0,49	0,79	0,18	1,58	0,31	2,16	0,31	0,08	1,52	0,13	1,39	0,23	1,11	0,22	2,04		
bas_001 13/IV/2023	76	50,22	0,30	0,12	1,42	0,59	0,63	0,24	1,34	0,27	2,24	0,40	0,12	1,03	0,21	1,00	0,30	0,68	0,20	2,04	0,49	0,11	1,33	0,59	0,75	0,36	1,47	0,39	2,34	0,46	0,08	1,45	0,28	1,42	0,33	1,04	0,19	2,12		
bas_002 19/IV/2023	16	2,85	Not measured																			Not measured																		
bas_003 19/IV/2023	16	2,89	Not measured																			Not measured																		
bas_004 19/IV/2023	25	5,16	Not measured																			Not measured																		
bas_005 19/IV/2023	25	5,90	Not measured																			Not measured																		
bas_006 19/IV/2023	32	7,26	0,12	0,02	0,17	0,04	0,05	0,03	0,19	0,05	0,20	0,08	0,02	0,15	0,02	0,13	0,02	0,12	0,02	0,18	0,11	0,02	0,13	0,03	0,04	0,02	0,15	0,04	0,17	0,06	0,02	0,12	0,03	0,10	0,04	0,09	0,02	0,17		
bas_007 19/IV/2023	35	7,81	0,14	0,03	0,23	0,05	0,05	0,02	0,28	0,05	0,28	0,05	0,02	0,23	0,03	0,23	0,07	0,20	0,02	0,27	0,14	0,03	0,21	0,04	0,06	0,02	0,22	0,06	0,34	0,08	0,02	0,22	0,04	0,23	0,09	0,21	0,03	0,35		
bas_008 19/IV/2023	30	5,85	0,10	0,02	0,10	0,03	0,02	0,02	0,12	0,05	0,17	0,04	0,02	0,11	0,03	0,12	0,06	0,09	0,02	0,17	0,12	0,03	0,13	0,03	0,03	0,02	0,15	0,04	0,15	0,05	0,02	0,14	0,03	0,15	0,05	0,10	0,03	0,16		
bas_009 03/VI/2023	10	1,54	Not measured																			Not measured																		
jua_004 20/VI/2023	67	36,43	0,21	0,05	0,79	0,19	0,29	0,14	0,79	0,12	0,63	0,16	0,04	0,65	0,10	0,71	0,21	0,59	0,11	0,65	0,19	0,05	0,72	0,19	0,22	0,06	0,66	0,15	0,68	0,14	0,04	0,63	0,08	0,61	0,09	0,56	0,11	0,57		
bas_010 19/IV/2023	24	4,34	Not measured																			Not measured																		
jua_005 04/VII/2023	55	16,72	0,23	0,04	0,53	0,12	0,15	0,06	0,54	0,07	0,60	0,13	0,02	0,42	0,05	0,40	0,08	0,36	0,06	0,57	0,23	0,05	0,44	0,09	0,10	0,03	0,47	0,07	0,62	0,11	0,03	0,50	0,07	0,45	0,10	0,38	0,06	0,51		
jua_006 04/VII/2023	52	19,80	0,24	0,05	0,73	0,14	0,26	0,09	0,78	0,13	0,89	0,16	0,04	0,80	0,19	0,75	0,17	0,68	0,10	0,91	0,29	0,03	0,75	0,18	0,37	0,08	0,80	0,14	0,90	0,19	0,03	0,66	0,14	0,59	0,13	0,50	0,09	0,88		
bas_011 11/X/2023	74	40,72	0,38	0,05	0,56	0,22	0,18	0,07	0,41	0,11	1,08	0,23	0,05	0,41	0,08	0,40	0,05	0,26	0,14	1,02	0,24	0,07	0,59	0,17	0,26	0,08	0,58	0,16	1,05	0,18	0,05	0,44	0,12	0,37	0,12	0,33	0,10	1,03		
bas_012 11/X/2023	74	38,90	0,32	0,05	0,50	0,16	0,26	0,08	0,53	0,10	0,88	0,35	0,04	0,45	0,12	0,42	0,09	0,35	0,09	0,85	0,29	0,07	0,66	0,12	0,29	0,09	0,45	0,09	1,02	0,26	0,04	0,44	0,08	0,43	0,08	0,49	0,09	0,97		
bas_013 11/X/2023	72	37,48	0,67	0,22	0,30	0,06	0,24	0,13	0,60	0,15	0,98	0,26	0,05	0,56	0,12	0,54	0,16	0,28	0,08	0,92	0,29	0,04	0,56	0,21	0,28	0,07	0,52	0,15	0,84	0,17	0,04	0,57	0,16	0,49	0,16	0,39	0,09	0,83		
bas_015 17/X/2023	48	32,31	0,33	0,07	0,83	0,21	0,35	0,08	0,72	0,14	1,17	0,16	0,04	0,66	0,13	0,66	0,10	0,54	0,10	1,09	0,34	0,05	0,87	0,27	0,36	0,10	0,81	0,17	1,23	0,19	0,05	0,86	0,20	0,79	0,13	0,80	0,09	1,13		
bas_016 17/X/2023	55	34,63	0,26	0,06	0,69	0,23	0,36	0,11	0,62	0,17	1,26	0,17	0,04	0,54	0,15	0,52	0,22	0,44	0,07	1,18	0,34	0,10	0,78	0,22	0,32	0,11	0,76	0,17	1,18	0,19	0,03	0,62	0,15	0,61	0,21	0,59	0,10	0,97		
bas_017 17/X/2023	44	22,16	0,25	0,06	0,61	0,19	0,30	0,08	0,62	0,13	0,93	0,11	0,02	0,54	0,12	0,54	0,16	0,41	0,10	0,85	0,29	0,06	0,62	0,18	0,26	0,08	0,63	0,14	0,81	0,17	0,04	0,48	0,10	0,42	0,13	0,38	0,10	0,73		
bas_021 17/X/2023	43	12,89	0,16	0,04	0,27	0,08	0,13	0,05	0,29	0,08	0,44	0,08	0,03	0,23	0,08	0,26	0,10	0,18	0,05	0,42	0,18	0,04	0,24	0,08	0,11	0,04	0,26	0,06	0,42	0,09	0,03	0,21	0,08	0,26	0,08	0,18	0,05	0,42		
bas_022 17/X/2023	34	20,04	0,26	0,04	0,56	0,14	0,19	0,05	0,51	0,11	0,79	0,15	0,04	0,38	0,13	0,38	0,09	0,35	0,07	0,72	0,26	0,05	0,51	0,13	0,18	0,06	0,51	0,12	0,73	0,19	0,05	0,45	0,11	0,42	0,12	0,41	0,06	0,77		

Table S5. Biometric and morphological data on different structures of *Hediste diversicolor* from the median parapodium and the median-posterior transitional parapodium.

Sample Code	Number chatgers	Total Length	Biramous Parapodia																																					
			Median																	Transition median-posterior																				
			Notopodium									Neuropodium								Notopodium									Neuropodium											
			Cirrus			Ligule			no12			Aciula	Cirrus			Ligule		no1	Aciula	Cirrus			Ligule			no12	Aciula	Cirrus			Ligule			no1	Aciula					
length	width		length	width	length	width	length	width	length	width	length	width	length	width	length	width	length	width	length	width	length	width	length	width	length	width	length	width	length	width	length	width	length	width	length	width				
NATMP- Apph-0013	88	99,78	0,32	0,10	1,73	0,52	0,22	0,12	1,53	0,23	2,47	0,27	0,10	1,15	0,15	1,14	0,14	0,74	0,12	2,24	0,32	0,11	1,26	0,34	0,19	0,12	1,07	0,15	2,11	0,21	0,07	0,83	0,08	0,83	0,07	0,70	0,09	1,28		
PRM_001 8/X/2022	104	129,86	0,28	0,08	1,31	0,46	0,44	0,12	1,28	0,26	2,16	0,17	0,05	1,00	0,20	0,97	0,20	0,65	0,16	2,07	0,25	0,07	1,25	0,24	0,15	0,08	1,21	0,18	1,47	0,20	0,08	1,00	0,14	0,95	0,20	0,88	0,12	1,52		
PRM_002 8/X/2022	90	74,88	0,36	0,07	1,35	0,62	0,29	0,19	1,27	0,27	1,97	0,19	0,07	1,08	0,22	1,00	0,34	0,77	0,25	1,98	0,43	0,08	1,06	0,24	0,28	0,08	0,95	0,19	1,54	0,19	0,07	0,83	0,12	0,75	0,21	0,53	0,12	1,51		
BCC_001 22/X/2022	107	92,24	0,44	0,10	2,33	0,38	0,39	0,10	2,01	0,23	1,90	0,24	0,09	1,64	0,22	1,55	0,23	1,27	0,12	1,94	0,34	0,12	1,10	0,33	0,25	0,08	1,03	0,24	1,42	0,20	0,09	0,91	0,17	0,87	0,22	0,73	0,10	1,36		
RA_001 23/II/2022	40	7,83	0,18	0,06	0,34	0,13	0,08	0,05	0,43	0,09	0,28	0,12	0,02	0,24	0,06	0,24	0,04	0,24	0,04	0,28	Not measured																			
Aqua_001 12/X/2022	32	6,42	0,11	0,02	0,28	0,06	0,11	0,07	0,28	0,06	0,30	0,10	0,02	0,20	0,06	0,18	0,05	0,18	0,04	0,30	Not measured																			
Aqua_002 12/X/2022	60	18,29	0,49	0,04	0,67	0,17	0,19	0,09	0,66	0,12	0,55	0,19	0,03	0,52	0,08	0,50	0,12	0,40	0,07	0,51	Not measured																			
Aqua_003 17/IV/2023	33	6,38	0,11	0,02	0,31	0,08	0,13	0,07	0,30	0,08	0,35	0,12	0,03	0,24	0,07	0,20	0,06	0,19	0,05	0,36	Not measured																			
PRM_003 19/IV/2023	92	96,34	0,43	0,08	1,35	0,43	0,56	0,15	1,28	0,26	2,13	0,37	0,07	1,11	0,14	1,15	0,22	0,87	0,15	2,07	0,54	0,06	1,24	0,26	0,38	0,10	1,09	0,18	1,61	0,25	0,04	0,85	0,10	0,80	0,15	0,71	0,13	1,56		
Icbas_001 13/IV/2023	76	50,22	0,33	0,16	1,45	0,42	0,58	0,23	1,36	0,27	2,00	0,30	0,09	1,18	0,28	1,08	0,41	0,93	0,17	2,02	0,51	0,11	1,32	0,45	0,61	0,18	1,29	0,26	2,01	0,24	0,08	0,97	0,18	0,93	0,25	0,69	0,18	2,08		
Icbas_002 19/V/2023	16	2,85	0,04	0,01	0,13	0,03	0,03	0,01	0,11	0,02	0,14	0,04	0,01	0,12	0,03	0,10	0,04	0,07	0,02	0,16	Not measured																			
Icbas_003 19/V/2023	16	2,89	0,07	0,02	0,16	0,03	0,02	0,01	0,11	0,02	0,16	0,05	0,02	0,11	0,02	0,10	0,04	0,09	0,02	0,19	Not measured																			
Icbas_004 19/V/2023	25	5,16	0,09	0,03	0,18	0,04	0,05	0,02	0,15	0,06	0,21	0,07	0,02	0,21	0,03	0,19	0,07	0,15	0,06	0,26	Not measured																			
Icbas_005 19/V/2023	25	5,90	0,10	0,03	0,13	0,02	0,04	0,01	0,11	0,03	0,29	0,80	0,02	0,16	0,04	0,17	0,07	0,14	0,03	0,27	Not measured																			
Icbas_006 19/V/2023	32	7,26	0,18	0,05	0,24	0,06	0,09	0,04	0,28	0,06	0,30	0,08	0,02	0,24	0,06	0,25	0,08	0,22	0,04	0,33	Not measured																			
Icbas_007 19/V/2023	35	7,81	0,13	0,04	0,23	0,05	0,08	0,04	0,30	0,07	0,35	0,07	0,02	0,27	0,25	0,25	0,08	0,20	0,03	0,36	Not measured																			
Icbas_008 19/V/2023	30	5,85	0,11	0,02	0,23	0,04	0,05	0,02	0,26	0,04	0,25	0,08	0,02	0,21	0,04	0,21	0,07	0,18	0,03	0,25	Not measured																			
Icbas_009 03/VI/2023	10	1,54	0,08	0,01	0,07	0,02	0,02	0,01	0,10	0,02	0,14	0,07	0,01	0,13	0,03	0,11	0,02	0,12	0,02	0,16	Not measured																			
Aqua_004 20/VI/2023	67	36,43	0,36	0,06	0,72	0,18	0,18	0,14	0,72	0,13	0,63	0,14	0,04	0,59	0,09	0,55	0,09	0,48	0,09	0,61	0,25	0,05	0,62	0,14	0,11	0,07	0,58	0,11	0,54	0,14	0,03	0,51	0,13	0,41	0,12	0,39	0,07	0,57		
Icbas_010 19/V/2023	24	4,34	0,12	0,03	0,18	0,04	0,04	0,02	0,19	0,04	0,21	0,08	0,02	0,15	0,04	0,13	0,07	0,14	0,04	0,22	Not measured																			
Aqua_005 04/VII/2023	55	16,72	0,20	0,04	0,40	0,12	0,15	0,05	0,47	0,09	0,59	0,13	0,02	0,37	0,04	0,33	0,06	0,32	0,06	0,60	0,22	0,05	0,35	0,08	0,13	0,04	0,39	0,07	0,49	0,11	0,02	0,35	0,05	0,41	0,09	0,32	0,06	0,58		
Aqua_006 04/VII/2023	52	19,80	0,31	0,05	0,74	0,22	0,46	0,12	0,75	0,13	0,89	0,18	0,04	0,63	0,12	0,58	0,17	0,52	0,10	0,92	0,31	0,06	0,64	0,21	0,33	0,11	0,63	0,13	0,79	0,16	0,04	0,63	0,12	0,54	0,14	0,47	0,07	0,77		
Icbas_011 11/X/2023	74	40,72	0,30	0,07	0,65	0,19	0,22	0,09	0,60	0,12	1,14	0,21	0,04	0,48	0,11	0,44	0,10	0,28	0,12	1,05	0,21	0,05	0,50	0,13	0,18	0,08	0,55	0,10	0,97	0,32	0,04	0,49	0,05	0,47	0,08	0,36	0,07	0,96		
Icbas_012 11/X/2023	74	38,90	0,27	0,05	0,52	0,13	0,22	0,08	0,52	0,11	0,93	0,31	0,05	0,41	0,09	0,39	0,08	0,23	0,06	0,92	0,29	0,05	0,42	0,09	0,20	0,06	0,44	0,08	0,79	0,15	0,03	0,42	0,06	0,44	0,06	0,33	0,06	0,87		
Icbas_013 11/X/2023	72	37,48	0,20	0,07	0,63	0,20	0,37	0,10	0,62	0,14	0,90	0,39	0,06	0,41	0,17	0,42	0,14	0,36	0,10	0,89	0,27	0,06	0,64	0,18	0,34	0,07	0,56	0,10	0,75	0,21	0,06	0,49	0,15	0,48	0,11	0,34	0,08	0,73		
Icbas_015 17/X/2023	48	32,31	0,33	0,06	0,62	0,24	0,30	0,09	0,53	0,15	1,16	0,17	0,05	0,41	0,11	0,39	0,16	0,38	0,09	1,07	0,34	0,07	0,85	0,20	0,26	0,11	0,81	0,16	1,21	0,17	0,04	0,59	0,16	0,51	0,21	0,48	0,09	1,09		
Icbas_016 17/X/2023	55	34,63	0,34	0,06	0,81	0,19	0,43	0,13	0,66	0,16	1,15	0,19	0,04	0,55	0,15	0,56	0,21	0,47	0,10	1,16	0,38	0,08	0,70	0,18	0,33	0,09	0,74	1,14	1,07	0,21	0,04	0,69	0,13	0,66	0,22	0,50	0,14	1,11		
Icbas_017 17/X/2023	44	22,16	0,21	0,06	0,55	0,19	0,20	0,08	0,48	0,17	0,93	0,17	0,05	0,47	0,15	0,45	0,16	0,37	0,10	0,88	0,27	0,06	0,51	0,15	0,26	0,09	0,65	1,15	0,87	0,15	0,04	0,58	0,16	0,55	0,18	0,49	0,08	0,75		
Icbas_021 17/X/2023	43	12,89	0,18	0,05	0,30	0,08	0,12	0,03	0,31	0,08	0,34	0,11	0,02	0,21	0,08	0,21	0,10	0,21	0,05	0,33	0,19	0,04	0,25	0,06	0,11	0,03	0,26	0,06	0,37	0,11	0,02	0,25	0,08	0,26	0,12	0,22	0,05	0,36		
Icbas_022 17/X/2023	34	20,04	0,27	0,05	0,53	0,15	0,24	0,08	0,47	0,11	0,70	0,14	0,04	0,44	0,14	0,47	0,10	0,42	0,08	0,69	Not measured																			

: Measures in mm

Table S6. Biometric and morphological data on different structures of *Hediste diversicolor* from the posterior parapodium and pygidium.

Sample Code	Number chatigers	Total Length	Biramous Parapodia																		Pygidium			
			Posterior																					
			Notopodium										Neuropodium											
			Cirrus		Ligule						Acicula		Cirrus		Ligule						Acicula		length	
					noL1		prL		noL2						prL2		pot		neL					
length	width	length	width	length	width	length	width	length	width	length	width	length	width	length	width	length	width	length	a	b	Segment length			
NATMIP- Apph-0013	88	99,78	0,43	0,11	1,53	0,29	0,17	0,06	1,40	0,19	1,66	0,27	0,07	0,99	0,10	0,96	0,10	0,88	0,11	1,61	2,26	2,14	0,14	
PRM_001 8/IX/2022	104	129,86	0,43	0,07	0,99	0,15	0,14	0,06	0,97	0,11	0,96	0,18	0,05	0,66	0,08	0,65	0,10	0,66	0,10	0,88	2,66	2,40	0,13	
PRM_002 8/IX/2022	90	74,88	0,56	0,08	0,91	0,25	0,17	0,05	0,74	0,15	0,98	0,25	0,06	0,58	0,10	0,53	0,12	0,46	0,10	0,95	3,87	2,42	0,24	
BCC_001 22/IX/2022	107	92,24	0,34	0,11	0,96	0,09	0,10	0,04	0,87	0,13	0,90	0,14	0,04	0,71	0,07	0,67	0,09	0,65	0,09	0,93	2,53	3,26	0,23	
RA_001 23/II/2022	40	7,83	0,15	0,03	0,21	0,03	0,04	0,03	0,26	0,09	0,15	0,04	0,03	0,17	0,03	0,17	0,02	0,17	0,03	0,16	0,69	0,66	0,05	
Aqua_001 12/X/2022	32	6,42	0,10	0,02	0,15	0,05	0,04	0,04	0,17	0,02	0,17	0,07	0,01	0,17	0,02	0,14	0,01	0,15	0,02	0,16	0,31	0,70	0,11	
Aqua_002 12/X/2022	60	18,29	0,17	0,03	0,32	0,06	0,12	0,05	0,38	0,06	0,33	0,12	0,03	0,34	0,05	0,31	0,06	0,28	0,04	0,36	1,41	1,35	0,13	
Aqua_003 17/IV/2023	33	6,38	0,11	0,02	0,16	0,06	0,04	0,05	0,18	0,02	0,21	0,09	0,02	0,18	0,03	0,16	0,02	0,17	0,03	0,20	0,34	0,33	0,05	
PRM_003 19/IV/2023	92	96,34	0,37	0,07	1,03	0,21	0,11	0,09	0,89	0,15	1,02	0,28	0,04	0,64	0,09	0,78	0,07	0,80	0,11	1,05	3,96	3,95	0,20	
Icbas_001 13/IV/2023	76	50,22	0,57	0,11	1,19	0,29	0,30	0,08	1,09	0,22	1,59	0,39	0,10	0,86	0,14	0,84	0,23	0,68	0,15	1,55	3,62	3,21	0,30	
Icbas_002 19/V/2023	16	2,85	0,04	0,01	0,05	0,02	0,01	0,01	0,04	0,01	0,07	0,02	0,01	0,06	0,01	0,05	0,01	0,04	0,01	0,06	0,30	0,30	0,03	
Icbas_003 19/V/2023	16	2,89	0,02	0,01	0,05	0,01	0,01	0,01	0,04	0,01	0,04	0,01	0,01	0,05	0,02	0,04	0,01	0,03	0,01	0,06	0,31	0,32	0,04	
Icbas_004 19/V/2023	25	5,16	0,09	0,02	0,11	0,04	0,04	0,02	0,09	0,03	0,18	0,07	0,02	0,10	0,02	0,09	0,03	0,11	0,03	0,16	0,44	0,46	0,03	
Icbas_005 19/V/2023	25	5,90	0,06	0,01	0,10	0,02	0,02	0,01	0,08	0,02	0,16	0,05	0,02	0,10	0,02	0,09	0,02	0,08	0,03	0,16	0,54	0,42	0,04	
Icbas_006 19/V/2023	32	7,26	0,15	0,03	0,15	0,05	0,04	0,16	0,03	0,04	0,20	0,08	0,02	0,16	0,03	0,13	0,03	0,10	0,03	0,19	0,62	0,61	0,05	
Icbas_007 19/V/2023	35	7,81	0,11	0,03	0,12	0,03	0,03	0,02	0,15	0,04	0,17	0,05	0,02	0,17	0,03	0,15	0,07	0,14	0,02	0,18	0,75	0,70	0,06	
Icbas_008 19/V/2023	30	5,85	0,09	0,02	0,09	0,02	0,03	0,01	0,10	0,02	0,12	0,02	0,01	0,07	0,01	0,08	0,02	0,06	0,01	0,12	0,51	0,55	0,06	
Icbas_009 03/VI/2023	10	1,54	0,06	0,02	0,11	0,03	0,02	0,01	0,09	0,05	0,13	0,04	0,01	0,10	0,03	0,09	0,02	0,10	0,03	0,11	0,07	0,08	0,04	
Aqua_004 20/VI/2023	67	36,43	0,22	0,06	0,35	0,07	0,05	0,03	0,30	0,07	0,37	0,11	0,03	0,22	0,10	0,24	0,05	0,23	0,05	0,36	1,40	1,39	0,13	
Icbas_010 19/V/2023	24	4,34	0,10	0,03	0,13	0,03	0,04	0,01	0,09	0,03	0,16	0,09	0,02	0,12	0,06	0,11	0,03	0,12	0,03	0,16	0,52	0,53	0,05	
Aqua_005 04/VII/2023	55	16,72	0,17	0,04	0,10	0,03	0,04	0,01	0,15	0,05	0,16	0,05	0,01	0,13	0,03	0,15	0,04	0,14	0,04	0,16	1,03	0,88	0,08	
Aqua_006 04/VII/2023	52	19,80	0,20	0,04	0,28	0,09	0,09	0,04	0,25	0,07	0,35	0,12	0,02	0,26	0,06	0,25	0,07	0,23	0,04	0,42	1,40	0,86	0,10	
Icbas_011 11/X/2023	74	40,72	0,35	0,04	0,44	0,11	0,12	0,04	0,46	0,08	0,65	0,21	0,04	0,35	0,06	0,31	0,08	0,23	0,06	0,64	2,29	2,42	0,15	
Icbas_012 11/X/2023	74	38,90	0,26	0,04	0,30	0,09	0,12	0,03	0,28	0,09	0,53	0,21	0,04	0,29	0,04	0,26	0,06	0,25	0,07	0,54	2,11	1,88	0,21	
Icbas_013 11/X/2023	72	37,48	0,28	0,06	0,31	0,08	0,09	0,04	0,31	0,08	0,69	0,23	0,04	0,32	0,06	0,30	0,04	0,28	0,06	0,71	2,68	2,62	0,22	
Icbas_015 17/X/2023	48	32,31	0,25	0,04	0,20	0,05	0,04	0,02	0,16	0,06	0,35	0,12	0,03	0,16	0,05	0,13	0,04	0,17	0,05	0,36	x	x	x	
Icbas_016 17/X/2023	55	34,63	0,22	0,06	0,21	0,09	0,05	0,02	0,21	0,06	0,33	0,14	0,04	0,15	0,05	0,12	0,07	0,17	0,05	0,33	1,32	1,20	0,16	
Icbas_017 17/X/2023	44	22,16	0,34	0,07	0,50	0,14	0,17	0,07	0,52	0,10	0,70	0,13	0,03	0,36	0,10	0,32	0,14	0,18	0,06	0,71	0,39	0,38	0,06	
Icbas_021 17/X/2023	43	12,89	0,17	0,04	0,28	0,07	0,09	0,03	0,32	0,07	0,42	0,11	0,02	0,27	0,07	0,26	0,10	0,24	0,06	0,40	0,62	0,89	0,09	
Icbas_022 17/X/2023	34	20,04	0,32	0,04	0,45	0,10	0,21	0,05	0,44	0,11	0,58	0,16	0,03	0,33	0,08	0,29	0,08	0,28	0,07	0,50	0,8	0,81	0,10	

Measures in mm