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Taxonomy, molecular phylogeny and macroevolutionary aspects of sessilid ciliates (Peritrichia: Sessilida), with emphasis on the Epistylididae family

Juiz de Fora
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Dissertação apresentada ao Programa de Pós-Graduação em Biodiversidade e Conservação da Natureza, da Universidade Federal de Juiz de Fora como requisito parcial para a obtenção do título de Mestre em Biodiversidade e Conservação da Natureza.

Orientador: Prof. Dr. Roberto Júnio Pedroso Dias
Coorientadora: Prof. Dra. Mariana Fonseca Rossi

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“Science is the most exciting and sustained enterprise of discovery in the history of our species. It is the great adventure of our time.” – Michael Crichton

RESUMO

Sessilida (Ciliophora, Peritrichia), uma das ordens mais diversas e amplamente distrubuidas dentro os microeucariotos ciliados, é composta por cerca de 600 espécies divididas 14 famílias e mais de 100 gêneros. Os integrantes dessa ordem exibem diversos modos de vida e podem ser encontrados por todo o mundo, em diversos ambientes. Os sessilídeos têm sido investigados desde o início dos estudos com microeucariotos ciliados, porém muitas dúvidas ainda existem quanto a sua morfologia, ecologia e evolução. Visando gerar mais informações para tentar sanar as dúvidas existentes para os indivíduos sesilídeos, principalmente os representantes da família Epistylididae, realizamos uma extensa revisão sobre dois gêneros dessa família, *Apiosoma* e *Rhabdostyla* e, ainda, investigamos a existência de sinal filogenético para o tipo de ambiente e a influência dessa característica ecológica na evolução e diversidade para toda a classe Peritrichia utilizando o marcador 18S-rDNA. Como resultado das revisões, compilamos de uma maneira sistemática e comparativa informações morfológicas para 62 espécies do gênero *Apiosoma* e 45 espécies do gênero *Rhabdostyla,* e de outros gêneros relacionados. Além disso, constatamos a existência de sinal filogenético para o ambiente dentro de Sessilida e ainda, por meio de uma análise de reconstrução do estado ancestral, encontramos resultados que sugerem que os indivíduos dessa subclasse se originaram em ambientes de água doce e somente mais recentemente colonizaram os outros ambientes onde se encontram atualmente.

Palavras-chave: Apiosoma, Rhabdostyla, Sistemática, Reconstrução do estado ancestral.

ABSTRACT

Sessilida (Ciliophora, Peritrichia), one of the most diverse and widely distributed orders within the ciliated microeukaryotes, is composed of about 600 species divided into 14 families and over 100 genera. Members of this order exhibit different ways of life and can be found all over the world, in different environments. Sessilids have been investigated since the beginning of studies with ciliated microeukaryotes, but many doubts still exist regarding their morphology, ecology and evolution. Aiming to generate more information to try to solve the doubts that exist for the sesilid individuals, mainly the representatives of the Epistylididae family, we carried out an extensive review on two genera of this family, *Apiosoma* and *Rhabdostyla*, and also investigated the existence of a phylogenetic signal for the type of environment. and the influence of this ecological feature on evolution and diversity for the entire Peritrichia class using the 18S-rDNA marker. As a result of the reviews, we systematically and comparatively compiled morphological information for 62 species of the genus Apiosoma and 45 species of the genus *Rhabdostyla*, and other related genera. In addition, we found a phylogenetic signal for the environment within Sessilida and, through an ancestral state reconstruction analysis, we found results that suggest that individuals of this subclass originated in freshwater environments and only more recently colonized the other environments where they are currently located.

Key-words: Apiosoma, Rhabdostyla, Systematics, Ancestral state reconstruction.

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General introduction

1. Morphology, Systematics and Evolution of Peritrichia

The subclass Peritrichia Stein, 1859 is one of the largest and most morphologically diverse subclasses of the phylum Ciliophora, containing over 1000 species divided into 19 families and over 120 genera (LYNN, 2008; WU *et al.,* 2020). Individuals in this subclass are very diverse and have a wide geographic distribution (ZHUANG *et al.,* 2018). They are found in freshwater, brackish water, saltwater, and even in soil and bromeliad ponds (FOISSNER *et al.,* 2002; LYNN 2008). Due to their wide dispersion capacity (ZHUANG et al., 2018) and tolerance to high levels of organic pollution (DIAS *et al.*, 2021), peritrichids ciliates are used as bioindicators of environmental quality, both in natural environments, such as rivers, lakes and streams, and artificial, such as sewage treatment plants (DIAS *et al*., 2021; FOISSNER; BERGER, 1996; HENEBRY; RIDGEWAY, 1979; SLEIGH, 1989). These microorganisms have three main ways of life: (1) sessile, when they live attached to the substrate by a peduncle, scapula or lorica; (2) euplanktonic, when they live on a permanent free-swimming stage; and (3) epibiont, when the species ascribed to cyanobacteria, microalgae, invertebrates or aquatic vertebrates.

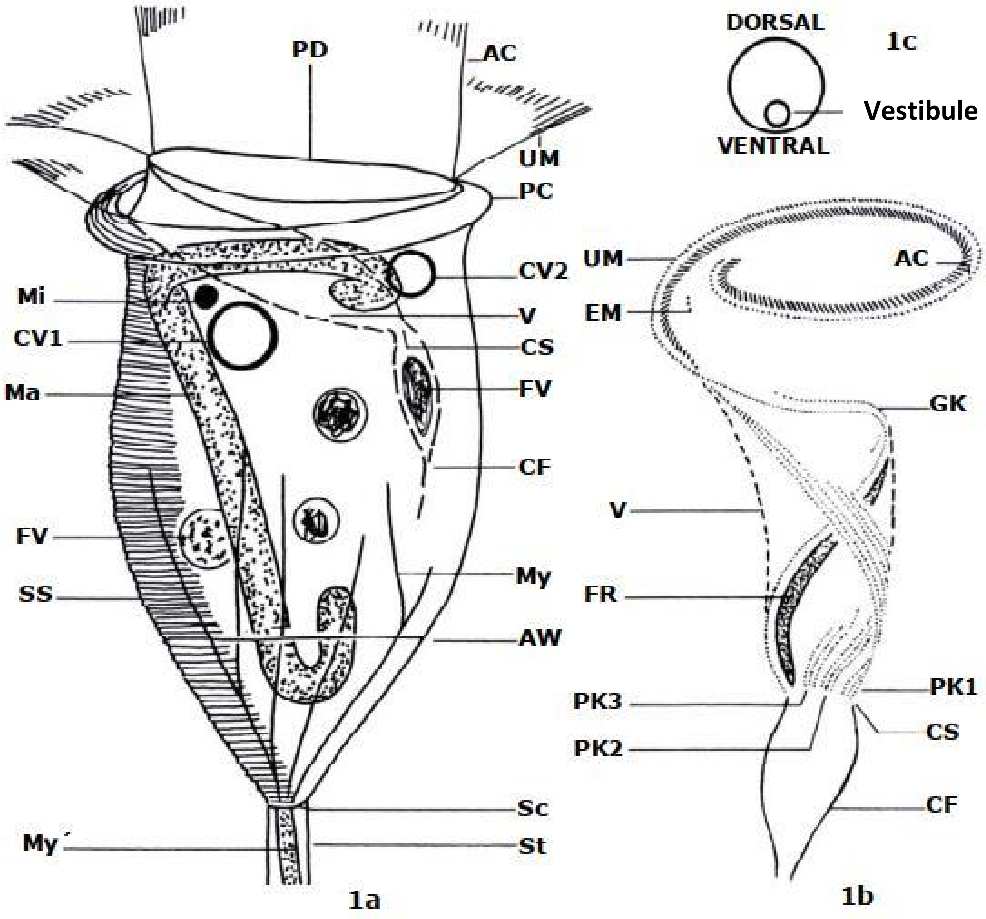
The subclass Peritrichia are usually characterized by presenting (1) zooids, usually in the form of an inverted bell, oval or cylindrical and by a globular free-swimming form containing a prominent aboral ciliary crown; (2) in the aboral region of the zoid, a structure called scopula, which is responsible for the production of the peduncle in sessile forms; (3) short or long peduncles, contractile or non-contractile, branched or not, although some genera have lost this structure; (4) complex myonemal system, containing myonemas in the oral, somatic and peduncle regions (sessile forms), which allows independent movements of the zooid and peduncle; (5) reduced somatic ciliature and developed oral ciliature; (6) adoral spiral ciliature (AC) and undulating membrane (UM) surrounding the peristomial region of the zooid and containing infundibular polykinety (PK's) in its adoral region; (7) free-swimming stage, called telotroch, responsible of dispersion, sexual reproduction and escape under adverse conditions; (8) longitudinal binary fission pattern and buccocinetal stomatogenesis; (CORLISS, 1979; FOISSNER; W., 1999; LYNN, 2008; LYNN; SMALL, 2002)

The main characteristics used in the identification of peritrichids individuals are shown in figures 1-4.

Normally, studies on the systematics of Peritrichia are based on some morphological characters, such as presence and absence of peduncle, presence and absence of lorica, presence and absence of myonemas in the zooid and/or peduncle, solitary or colonial habit, colony shape, pellicular striation pattern, collar and peristomial disc shape and permanence of the aboral ciliary crown in the trophont stage (KAHL 1935, ZHUANG *et al.,* 2018).

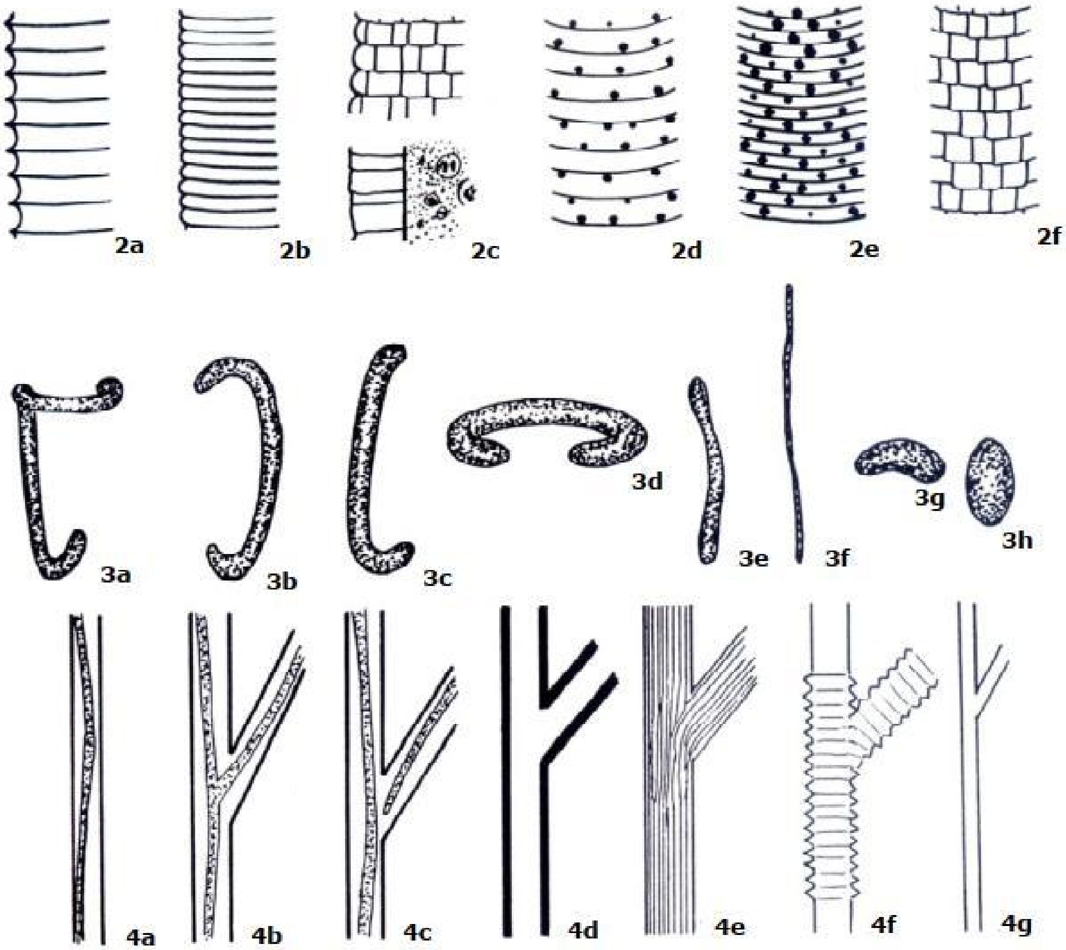
Most of the descriptions of the genera and species ascribed to the subclass Peritrichia were based on *in vivo* observations, which led to the construction of several incomplete and superficial diagnoses (LEITNER; FOISSNER, 1997). In an attempt to standardize the current descriptions of peritrichid and ciliate microeukaryotes in general, WARREN *et al.* (2017) developed a list of requirements for new descriptions of individuals belonging to the phylum Ciliophora. Some of the main recommendations are: (1) the detailed visualization of the cell *in vivo*; (2) the use of silver impregnation techniques; (3) the use of scanning electron microscopy; and (4) DNA extration for molecular analysis. The complete observation proposed by WARREN *et al.* (2020) can help to better establish relationships between families, genera and even species, as some of the techniques reported above shows the species-specific structures that exhibit great variability between species of the same genus, such as oral infraciliature (Fig. 1b) and the pattern of pellicular striations (Fig. 2), which nowadays are essential for the accurate identification of these protists (FOISSNER; BERGER; KOHMANN, 1992; JI *et al.*, 2005; SONG; WARREN; HU, 2009; WARREN *et al.*, 2017).

Figure 1a-c. Schematic drawing of a peritrichids ciliates, showing structures used in the species descriptions. (a) Extended ciliate *in vivo*, (b) details of the oral ciliature, (c) superior view of the ciliate, with detail for the ventral position of the buccal infundibulum. Caption: AC = adoral spiral ciliature; AW = aboral ciliary crown; CF = cytopharynx; CS = cytostome; CV1 = contractile vacuole; CV2 = second contractile vacuole; MS = epistomial membrane; FR = reticular filament; FV = food vacuole; GK = germinal kinetics; Ma = macrokernel; Mi = micronucleus; My = somatic myonema; My' = peduncle myonema or spasm; PC = peristomal collar; PD = peristomal disk; PK1-3 = infundibular or oral polykinesias 1, 2 and 3; Sc = scopula; SS = skin striations (“silverline system”); St = peduncle; UM = undulating membrane; V = vestibule.



Source: Adapted from FOISSNER *et al*. (1999)

Figures 2-4. Different shapes of pellicular streaks (2), macronucleus (3) and peduncles (4) among representatives of the Peritrichia subclass. 2a-d. Types of pellicular streaks seen in live specimens. 2e-f. Types of pellicular streaks seen in silver-impregnated specimens. **3a-h.** Different macronucleus shapes: J-shaped (a), C-shaped (bc), horseshoe-shaped (d), elongated (e), filiform (f), bean-shaped (g) and ellipsoid (H). **4a-g.** Peduncle morphology, with detail for the presence of myonemas and external ornamentation.



Source: Adapted from FOISSNER *et al.* (1999)

Recent molecular studies on the internal phylogeny of Peritrichia have shown a relationship between the families that diverges from the classification based on morphological characters (LI. *et al.,* 2008; SUN *et al.,* 2011; SUN; *et al.,* 2010; UTZ *et al.,* 2010; ZHUANG *et al.,* 2018).

Current works have pointed out some of the main families of Peritrichia, such as Epistylidae and Zoothaminidae, as polyphyletic (SUN *et al.,* 2016; ZHUANG *et al.,* 2018). The relationship between some others families, such as Vorticellidae and Ophrydiidae, has also raised some doubts, given the positioning of their representatives in the constructed phylogenies (SUN *et al.* 2016). However, due to the low representation of the Peritrichia subclass in the molecular databases for the 18S-rDNA gene, only about 6% of species have some kind of molecular representation (GenBank), definitive solutions to the problems presented have not yet been found. This scenario highlights the need to expand molecular and taxonomic studies to better understand the phylogenetic relationships within the Peritrichia subclass.

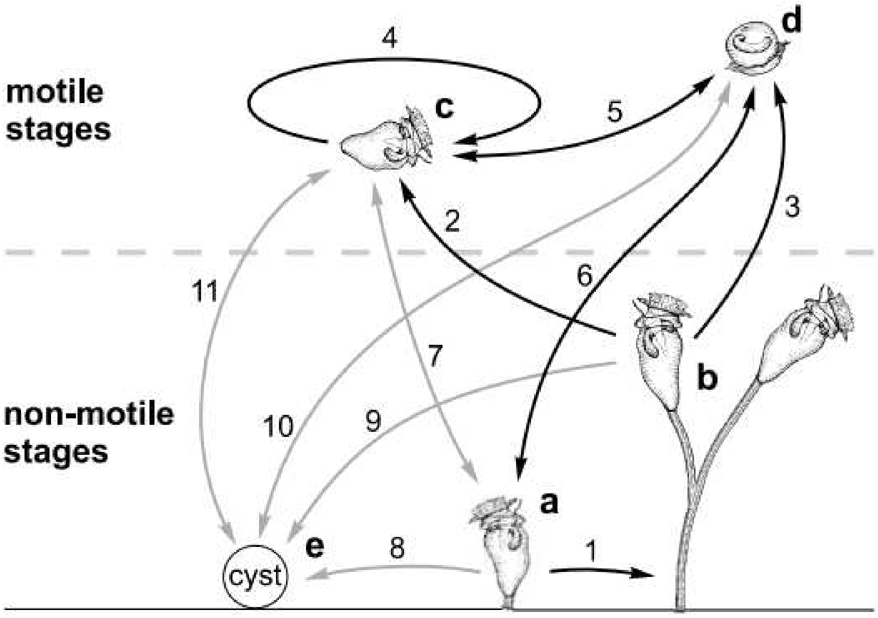
2. Morphology, Systematics and Evolution of representatives of the Sessilida order

Sessilida is the most diverse of the Peritrichia orders, with more than 600 species *(SUN et al.*, 2011; WU *et al.,* 2020) included in 14 families and more than 100 genera. Like the subclass Peritrichia, species of this order can be found in diverse environments such as streams, rivers, lakes, seas, in the soil and in bromeliads (FOISSNER *et al.,* 2010; FOISSNER *et al.,* 2002; LYNN, 2008). They also have different ways of life, being sessile, euplanktonic and epibiont (LYNN, 2008). Among the two orders, Sessilida is one of the most used for water quality assessment work (CABRAL et al., 2018; DIAS et al., 2007, 2021; FOISSNER; BERGER, 1996), given the wide species ranges, due to their great dispersion capability, life cycle features (Fig. 5), the ease of collection (CABRAL et al., 2018; DIAS et al., 2007, 2021; FOISSNER; BERGER, 1996) and a wide variety of ways of life (LYNN, 2008).

Individuals of this order are morphologically well defined and are usually characterized by: (1) body with no cilia, with the exception of those of telotrochs (free-swimming phase) and those belonging to the family Opisthonectidae, which have a telotrochal band; (2) by the oral cilia that continue the spiral ciliature and undulating membrane and form three polykinetics and one haplokinesis; (3) by the peduncle which can be short or long, thin or thick, contractile or non-contractile, branched or unbranched and striated or smooth; (4) the contractile vacuole that empties into the infundibulum; ^{5}the presence of the telotroch in its life cycle (Figure 5); (6) its division plane parallel to the longitudinal axis of the body; and stomatogenesis is buccocinetal (FOISSNER; W., 1999; LYNN, 2008). Other main characteristics of the sessilids are shown in Figure 1-4.

Although the order Sessilida and its 14 families are morphologically well characterized (LYNN, 2008), many diagnoses at the genus and species levels are incipient, often presenting only two or three characteristics (KAHL, 1935). Besides their interfamily relationships have been questioned, given the result of recent molecular analyzes (LI *et al.*, 2016; ZHUANG *et al.*, 2018). Two of the largest families of Sessilida, Epistylididae and Zoothaminiidae appear in various topologies as polyphyletic (ZHUANG *et al.* 2018). Some genera are also polyphyletic, with representatives grouped within clades mostly composed of representatives of different families (ZHUANG *et al.* 2018). Another problem evidenced by recent molecular investigations are unexpected relationships between some families, such as Vorticellidae and Ophrydiidae, the representatives of the family Ophrydiidae appearing as an ingroup of a clade composed soleny by vorticelids (SUN *et al.*, 2016; ZHUANG *et al.*, 2018). Even with these evident discrepancies from what was morphologically expected, few taxonomic changes were made based on molecular results alone (LI *et al.*, 2008). This is a problem, because the molecular representation of the order Sessilida is still precarious. The vast majority of the material available for this group is not identified to species level, which added to a elevate number of clone sequences, majority belonging to the Vorticellidae family (GenBank), make the results demonstrated by the phylogenies not very reliable. This scenario demonstrates the need for new molecular and taxonomic studies on the Sessilida, as with more information about the species and genera, the relations between the families that compose Sessilida can be better understood.

Figure 5. Life cycle diagram of *Epistylis pygmaeum* (Peritrichia, Epistylididae). Life cycle stages: (a) newly fixed zooid (CORLISS, 1979), (b) colony with two zooids, (c) free-swimming zooid, (d) telotrochium (CORLISS, 1979) and (e) cyst. Black arrows indicate well-studied pathways for peritrichous and gray arrows indicate possible pathways that need further studies for further clarification.



Source: Adapted from Gilbert & Schrüder (2003)

3. Morphology, systematics and evolution of Epistylididae

The Epistylididae is one of the largest, most dispersed and most studied families of the Sessilida order (CABRAL *et al.*, 2018; DIAS *et al.*, 2007; SUN *et al.*, 2016; ZHUANG *et al.*, 2018). It comprises more than 300 species, divided into 11 genera (Lynn 2008). It is found in all environments described for the subclass Pertrichia, i.e., streams, rivers, lakes, seas, bromeliads and even soil (FOISSNER *et al.*, 2010; FOISSNER *et al.*, 2002; LYNN, 2008). The individuals included in this family are of great importance for ecological investigations and also have some economic importance, since they are used as bioindicators of environmental quality (CABRAL *et al.*, 2018; DIAS *et al.*, 2021; FOISSNER; BERGER, 1996). Many of them are epibionts of invertebrates and aquatic vertebrates (LYNN, 2008) and some are even considered parasites, as they parasitize fish and some other aquatic vertebrates and can cause damage to their development and even lead to death of fry (BANINA, 1968; LI, M. *et al.*, 2008; MARTINS *et al.*, 2015; SCHMAHL *et al.*, 1989).

This family was described more than 85 years ago by KAHL (1935). On that occasion he characterized the species belonging to this family as species with rigid peduncle, colonial or not, and pointed out the great difficulty in distinguishing speciesfrom this family with another large family of Sessilida, Vorticellidae. Currently, the Epistylididae family is defined as: (1) species with an oval, cylindrical-conical or campanulate shape; (2) contractile trophonts, on non-contractile peduncles that can be difficult to distinguish; (3) present loric, when without peduncle; (4) solitary or colonial; (5) with slightly protruding peristomal apparatus; (6) with the oral ciliature making one to five complete turns around the peristome; (7) with oval, ellipsoid, or band-shaped macronucleus (Lynn 2008).

As for the order, Epistylididae, despite being morphologically well defined, still presents some problems in relation to its more internal groups. There are many species and some genera of this families whose descriptions are uninformative, which makes it difficult to recognize these taxa and raises doubts about the validity of some genera (AESCHT, 2001; BÜTSCHLI, 1889; LYNN, 2008). In addition, the monophyly of this family has been much discussed, since in topologies recovered with several genes (ZHUANG *et al.,* 2018) its representatives have been found in more than one clade (Figure 6-8).

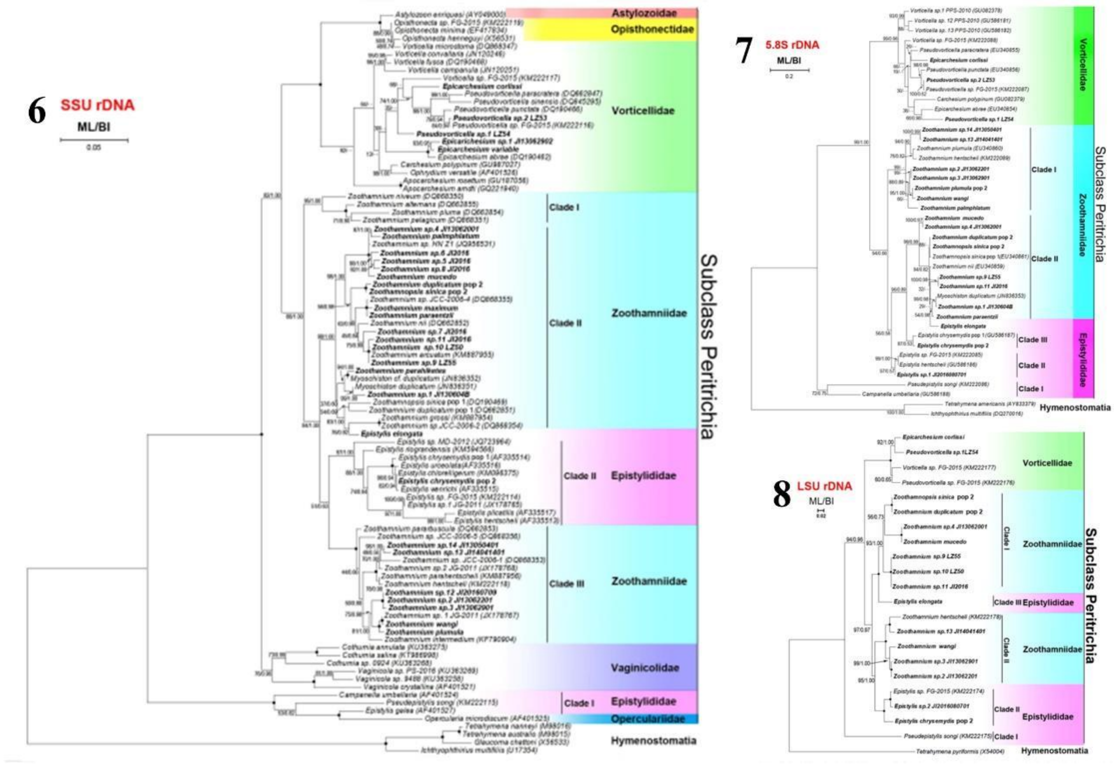
The results of recent molecular analyzes demonstrate that the characters used to classify individuals of the Peritrichia subclass and especially the families that compose it do not represent the evolution and the true relationships between these groups. In order to solve these problems, works that investigate the relationship of other ecological and morphological characteristics with the evolution of the group are necessary. Few studies (SUN *et al.,* 2016, Costa *et al*, 2021; Dias *et al.,* 2021) have already investigated the relationship of other characteristics, such as habitat preference, way of life and saprobic resistance, with the evolution of the group. But the precariousness of much of the morphological and molecular information leave some informational gaps and doubts about the results of these studies and makes it difficult to carry out more studies with this purpose. This scenarios, further demonstrate the importance of excellent taxonomic and molecular surveys, as well as new evolutionary investigations on the group seeking to fill the caps and clear the doubts about the evolutionary history of Peritrichia.

4. Macroevolution in Ciliophora

The relationship between a characteristic, whether morphological or ecological, with the evolution of different animal and plant groups has been the focus of several studies in the past (ROGERS, 2011; COSTA *et al.,* 2021; DIAS *et al.,* 2021). These studies, for a long time, were carried out theoretically, based only on events interpreted through the fossil record (ROGERS, 2011). For several groups of protozoa, such as the phylum Ciliophora, fossil records are scarce, which makes difficult to better understand the evolutionary history of both, morphological and ecological characteristics, of these organisms (ROGERS, 2011). The development of molecular techniques and phylogenetic analysis, bring out the possibility of performing studies on macroevolutionary processes involving ciliates to new insights into evolutionary hypotheses, which were previously based only on morphological data (ROGERS, 2011; SOGIN; SILBERMAN, 1998).

Recently, many works (COSTA *et al.,* 2021; RAJTER; VĎAČNÝ, 2016; STRÜDER-KYPKE *et al.*, 2001; VĎAČNÝ, 2018) have been carried out trying to use macroevolutionary tools to understand how a certain characteristic, whether morphological or ecological, is related with the evolution of the group. More recently, we have the work by COSTA *et al.* (2021), who use macroevolutionary tools to try to understand the relationship between the free-living, mutualistic or parasite way of life of individuals belonging to the phylum Ciliophora with the evolution of the group. In this work, COSTA *et al.* (2021) found a possible relationship with the acquisition of new ways of life with an increase in macroevolutionary rates and in some cases an increase in diversity.

Figure 6-8. Maximum likelihood tree (ML) based on SSU rRNA sequences. The scale bar corresponds to 5 substitutions per 100 nucleotide positions (6). Maximum likelihood (ML) tree based on 5.8S rRNA sequences. The scale bar corresponds to 20 substitutions per 100 nucleotide positions (7). Maximum likelihood tree (ML) based on LSU rRNA sequences. The scale bar corresponds to 2 substitutions per 100 nucleotide positions (8). The first and second values in the nodes represent support values for ML and BI analyses, respectively. Clades with a different topology in the BI tree are indicated by ''- -". The dots at the nodes indicate full support for both algorithms. Blue = Epistylididae; Green = Vorticellidae; Pink = Zoothaminiidae; Yellow = Opisthonectidae; Purple = Vaginicolidae; Dark blue= Operculariidae; Red; Astylozoidae.



Source: Adapted from ZHUANG *et al.*, 2018.

Another ecological feature that has generated interest in phylogenetic investigations is the environment. SUN *et al.* (2016) tries to find within the order Sessilida (Oligohymenophora: Peritrichia) an ecological niche pattern for the environment. The authors classify the environment as freshwater, brackish water, saltwater, and hypersaline, and appear to have found patterns of distribution for this environment in some families. In this work, they also carried out an evolutionary analysis called ancestral state reconstruction to understand the transitions of environments within the constructed phylogeny (SUN *et al.,* 2016), using the Mesquite software (MADDISON; MADDISON, 2007) to infer these transitions. These transitions are not much discussed in this work, apart from that the number of genetic material used is low and contains many replicas of the same group, e.g. representatives of the genus *Vorticella*, these facts added to the little use of the Mesquite software (MADDISON; MADDISON, 2007) give rise to gaps about the relationship between individuals of the Sessilida order and the environment.

Therefore, it is still necessary to carry out further studies, with greater sampling and with more robust software, to try to elucidate the gaps in knowledge about the relationship of Peritrichia and its orders with the environment.

Objectives

The main objectives of this dissertation were carried out a taxonomic review of two genera of Epistylididae family and [2]to investigate the existence of a phylogenetic signal for the environment in the Peritrichia, and also to investigate how these characteristics influence at the evolution of the subclasse.

Specific objectives

- Conduct a systematic review of two genera, *Apiosoma* and *Rhabdostyla*, which are part of the family Epistylididae, presenting checklist, new graphic representations, biometric data, as well as determining valid species;
- Carry out an investigation into the existence of a phylogenetic signal for the environment (freshwater, brackish, marine and soil) in Peritrichia using 18S-rDNA marker sequences and also investigate the influence of these characteristics on the evolution of the subclass by doing a reconstruction of ancestral state.

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Chapter 1:

Brief review of the genus *Apiosoma* Blanchard, 1885 (Ciliophora: Peritrichia: Epistylididae), with notes on geographic distribution and host specificity

Resumo

Os ciliados do gênero *Apiosoma* são importantes ectoparasitos de peixes, porém, há poucas informações sobre distribuição, morfologia, especificidade e potencial patogênico desses microeucariotos unicelulares. A maioria dos estudos sobre esses parasitas está concentrada na antiga URSS, União das Repúblicas Socialistas Soviéticas, com grande parte da literatura no idioma local, o que dificulta a divulgação de informações sobre o gênero. Nos últimos anos, houve uma ampliação dos estudos sobre o grupo, dada a sua importância médico-veterinária, pois em alta densidade podem causar problemas respiratórios, anorexia e dificuldades na natação aos seus hospedeiros. No entanto, a maioria desses estudos não identifica esses ciliados em nível de espécie, o que pode ser um problema, pois a resposta ao tratamento pode variar de acordo com a espécie de ciliado. Para facilitar e incentivar a identificação em nível específico de ciliados do gênero *Apiosoma*, compilamos as principais características morfológicas de 62 espécies dentre as 72 consideradas válidas e fizemos novas ilustrações padronizadas para a maioria dessas espécies. Além disso, analisamos a distribuição dos estudos ao redor do mundo e investigamos a especificidade por hospedeiro e a distribuição das espécies nominais relatadas na literatura recente.

Palavras-chave: Sessilida, Ciliados, Taxonomia, Parasito.

Abstract

The ciliates of the genus *Apiosoma* are important ectoparasites of fish, however, there is little information about the distribution, morphology, specificity and pathogenic potential of these unicellular microeukaryotes. Most studies on these parasites are concentrated in the former USSR, Union of Soviet Socialist Republics, with much of the literature in the local language, which makes it difficult to disseminate information about the genre. In recent years, there has been an expansion of studies on the group, given its medical-veterinary importance, as in high density they can cause respiratory problems, anorexia and difficulties in swimming to their hosts. However, most of these studies do not identify these ciliates at the species level, which can be a problem as the response to treatment may vary by ciliate species. To facilitate and encourage the identification at a specific level of ciliates of the genus *Apiosoma*, we compiled the main morphological characteristics of 62 species out of the 72 considered valid and made new standardized illustrations for most of these species. In addition, we analyzed the distribution of studies around the world and investigated the host specificity and distribution of nominal species reported in recent literature.

Key-words: Sessilida, Ciliates, Taxonomy, Parasite.

Introduction

The Epistylididae family is composed of more than 300 species distributed in 12 genera. It is one of the most diverse and scattered families of the Peritrichia subclass (ZHUANG *et al.*, 2018). The individuals that compose it have different ways of life, which can be free-living, epibionts or parasites (LYNN, 2008). Among the genera with exclusively parasitic representatives, the genus *Apiosoma* BLANCHARD, 1885 stands out.

Apiosoma includes ectoparasite species of aquatic organisms being found mainly on the gills and skin of several fish species, besides other aquatic vertebrates and invertebrates (LI *et al.*, 2008; LOM, 1966). This genus had its first species described under the name of *Spirochona tintinnabulum*, in 1882, by Kent, and nowadays has about 72 valid species (LI *et al.*, 2008). The speceis that compose this genus are solitary and sessile, with the body in a conical shape, and stand out for adhering to their hosts directly through the scopula (LI *et al.*, 2008).

Recent studies on ciliates of the *Apiosoma* genus have been mostly carried out in fishponds with a medical-veterinary focus, as they are ectoparasites of fish, they can cause damage to hosts and generate economic losses. The main approaches of these studies are: new occurrence records/diagnoses (BANINA 1970; JUNTSCHIS *et al.* 1975), morphology (LI *et al.*, 2016; LI *et al.*, 2008), taxonomic review (BANINA, 1968; LI *et al.*, 2008), and control (MEIRA-FILHO *et al.* 2017; SCHMAHL *et al.* 1989). However, due to difficult access or lack of information on the taxonomy and systematics of the group, most of these studies identify these organisms only at the genus level (ALAM *et al.*, 2012), which may prevent more applied research, aimed to evaluate the effect of drugs, due to the difference in response in different species of the same genus (LIU *et al.*, 2017).

Given the medical-veterinary importance and application of knowledge about the genus for the specific identification of individuals, it is necessary to expand taxonomic investigations in representatives of *Apiosoma*. It is a genus with a large number of valid species, which has undergone several important taxonomic changes over the years (BLANCHARD, 1885; BÜTSCHLI, 1889; KENT, 1882), and today it is in one of the families commonly accepted as merophyletic (LI, L. *et al.*, 2008; ZHUANG *et al.*, 2018). Since there are no molecular sequences for representatives of this genus and, therefore, great lack of knowledge about evolution, it is essential to compile the existing morphological and geographic distribution data, in order to increase the accuracy of studies on systematics (integrative taxonomy) and evolution of this group. Therefore, our work aimed to compile data on morphology, geographic distribution and specificity of representatives of the *Apiosoma* genus.

Metodology

An extensive bibliographical survey was carried out on *Apiosoma* species in specialized literature as well in databases such as Scopus and Web of Science, using the keyword “Apiosoma”. Important studies on the morphology and taxonomy of this group, provided by other research groups, were also used (BANINA 1968; FAURÉ-FREMIET 1905; JUNTSCHIS *et al.* 1975; KASHKOVSKY 1975; LOM 1966; SCHEUBEL 1973), in addition to the recent study of LI *et al.* (2008b), where the authors compile a list of valid species. From these works, the main taxonomic information for the group was compiled and standardized, such as body size and shape, fixation apparatus, size, shape and position of the macronucleus, size, shape, and position relative to the macronucleus of the micronucleus. All this information was standardized and organized in a comparative way. All the new illustrations were made based on the original drawings of the species descriptions or the most complete drawing contained in the re-descriptions and revision works. The terminology adopted follows the main taxonomic works of the genus (BANINA, 1968; LOM, 1966; SCHEUBEL, 1973).

For the investigation about geographic distribution and host specificity, we use information taken from 54 articles, acquired from Scopus and Web of Science only. The articles were found using the key-word ‘Apiosoma’, all the articles that registered an encounter of any species of the genus *Apiosoma* was included. That data compiled was then plotted in three maps that were made using the JMP (16.0.0) software. The first map, shows the number of studies with any representative of *Apiosoma* genus from each region of the word. The second map, shows the species richness reported in the works found in the review, where records of *Apiosoma sp.* were counted as a single, regardless of how many were reported and the species not listed in LI *et al.* (2008b) work was treated like valid. The third map, shows the host diversity, in which each host species was counted only once, even if more than one *Apiosoma* species was reported in the same host. On all maps there were records for one or more countries that suffered some kind of territorial division or name change from the time of work to the present day. These records were computed for countries to which the reported region of the record belongs today or for the country resulting from the division that already has a record of *Apiosoma* individuals, the latter case being when the region of record was not properly reported.

Table 1. Comparative table with morphologic characters for 62 valid species of the genus *Apiosoma*.

Species	Dimensions (µm)	Body shape	Fixation apparatus	Macronucleus	Macronucleus position	Macronucleus dimensions (µm)	Micronucleus	Position of the Micronucleus in relation to the Macronucleus	Micronucleus Diameter (µm)	References
Apiosoma beninae	51.75-109.2 x 25.3–32.2	Cupped chalice	Peduncle, long and thin	Conical or oval	In the middle of the body	9.2-19.5 x 5.7–23.8	Rounded and small	Above and slightly to the side	1.7-2.3	Scheubel, 1973
Apiosoma carpelli	20.6-52.2 x 9.0-21.0	Concic, tapered towards the scopula	Scopula, slightly narrower than the body	Oval	-	5.4-11.5 x 2.0-5.4	Polymorphic, Oval, when positioned laterally and club-shaped when positioned anteriorly	Variable, laterally or anteriorly	0.7-5.4 x 0.7-2.2	Banina. 1968
Apiosoma amurensis	32.4-82.8 x 21.6-39.6	Pear-like, sometimes almost spherical, that affiliates towards the scopula	Scopula, narrow with granulations that appear to have fibers attached to the rest of the body	Conical and elongated, with a pointed bottom	-	10.8-21.6 x 6.5-7.2	Oval	To the side	-	Junchis et al.. 1975
Apiosoma lopuchinae	37.0-39.0 x 15.0-17.0	Bell-like when contracted	Scopula, with root-like bulges	Rounded	-	9.4-10.0 x 6.3-8.4	Rounded	Slightly to the side	-	Junchis et al.. 1975
Apiosoma fungiformis	25.2-54.0 x 21.6—36.0	Mushroom-like	Peduncle, short and thick	Ovoid, pointed down	Between the edge of the peduncle and the expanded part of the body	9.0-18.0 x 5.9-14.4	Oblong, sometimes comma-shaped	To the side	-	Junchis et al.. 1975
Apiosoma piscicola	39.6-86.4 x 10.8-36.0	Cup-like	Scopula, a little wider than the preceding body part	Oval or triangular, elongated vertically	In the lower part of the body	5.4-25.2 x 5.4-14.4	Elongated oval or club-shaped	To the side	2.3-6.3 x 1.8-4.3	Banina. 1968; Scheubel, 1973
Apiosoma amoeba	28.8-85 x 14.4-36.0	Conical, tapering sharply toward the scopula	Scopula, which has pseudopod-like projections, with two or three lobes that in vivo show striations	Oval or somewhat triangular, and structured in layers	Lower body, but variable	7.2-21.6 x 7.2-23.4	Polymorphic, spherical, when placed anteriorly, and oval or club-shaped, when found laterally or close to the posterior border of the macronucleus.	Variable, close to the anterior part, laterally or close to the posterior edge.	1.8-5.4 x 1.8-3.2	Banina. 1968, Scheubel, 1973 e Lom, 1966
Apiosoma campanulata	21-68 x 11-28.8	Pear or bell-like	Scopula, small, with variable diameter depending on host	Round or oval	-	5.4-17.9 x 5.4-18.0	Round or oval	Laterally, near the front edge	1.8-3.6 x 1.0-3.6	Banina. 1968 e Scheubel, 1973
Apiosoma shulmani	22.8-46.8 x 13.7-28.8	Amphora-like	-	Rounded	-	5.4-10.1 x 6.1-12.6	Rounded	Laterally or near the back edge	1.8-3.6 x 1.8-2.8	Banina. 1968
Apiosoma leuciscus	34-45 x 25-40	Usually cup-like but can be very variable	-	Oval	In the middle of the body, transverse to the longitudinal axis	-	-	-	-	Scheubel, 1973
Apiosoma olae	36.8-68.8 x 18.9-24.1	High conical cup	Scopula	Rounded	In the middle of the body	17.8-21.0 x 12.6-17.8	Oval rod, large	Always above, at the level of the cell's equatorial girdle	-	Junchis et al.. 1975
Apiosoma megamicronucleata	28.8-50.4 x 14.6-61	Barrel or cup-like	Scopula, wide	Bean-like, big	Across the axis of the body	4.7-13.7 x 7.2-25.0	Big and elongated	In the posterior concavity	2.5-7.2 x 3.6-10.8	Banina. 1968 e Scheubel, 1973
Apiosoma minimicronucleata	16.2-43.2 x 16.3-32.4	Barrel-like	-	/ise elongated, often bean-	-	1.6-9.0 x 7.2-14.4	Transversely elongated but small, barely noticeable	In the posterior concavity	0.42-0.66 x 1.8-2.2	Banina. 1968

Apiosoma apllanatum	22.3-28.7 x 24.6-33.3	Flat	Scopula, very wide	Big and rounded	Takes up large part of the lower body	6,2-7,8 x 17,7-19,6	Rod or oval	Pressed to the macronucleus, inferiorly or superiorly	1.8-3.5 x 2.1-3.6	Shulmna, 1987
Apiosoma lotae	15,7 x 5,2	Bottle-like, small	-	Similar to an equilateral triangle	-	4.2 x 2.1	Rod, very small	To the side	Indistinguivel	Chernyshova, 1976
Apiosoma constricta	36-60 x 27.6-46	Conical, with a deep constriction at the level of the upper edge of the macronucleus	Scopula	Elongated transversely, sometimes bean-shaped	-	11-25.2 x 8.4-14.4	Oval	Below	2.4-4.8 x 1.8-2.4	Kahskovsky, 1975
Apiosoma tenera	33.6-57 x 20-32.4	Barrel-like	Scopula, in the shape of a well-defined saucer	Rounded, sometimes elongated	-	10.8-18 x 10.8-22	Round or oval	On the side or on the top edge	2.4-4.8 x 2.4-3.6	Kahskovsky, 1975
Apiosoma caulata	46.4-73.9 x 31.6-60.6	Pear-like	Peduncle, shorter than the body, with 5.8-11.4 µm in diameter	Round, sometimes cuboidal	In the lower part of the body	13.8-31.2 x 15.0-25.6	Oval	Below	2.8-8.2 x 3.1-9.4	Van as & Viljoen, 1985
Apiosoma curvinucleata	113.7-171.5 x 31.3-50.5	Slim and elongated	Scopula, with 8.8-22.1 µm in diameter	Triangular, elongated	In the lower part of the body, between the ciliary band and the scopula	50.4-70.0 x 15.6-25.9	Not observed	-	-	Van as & Viljoen, 1985
Apiosoma baueri	28.8-57.6 x 19.8-39.6	Barrel-like	-	Rounded	-	9.0-25.2 x 14.4-21.6	Oval	Variable, it can be laterally, close to the posterior edge or even in front	3.6-6.5 x 2.2-3.6	Banina. 1968
Apiosoma mothlapitsis	20.1-37.8 x 11.2-25.8	Wide in the peristomium, tapering towards the scopula	Scopula, 1.5-4.3 µm in diameter	Triangular	-	6.4-13.0 x 10.0- 16.8	Oval	Acentric and above	2.0-6.4 x 2.5-6.7	Van as & Viljoen, 1985
Apiosoma nasalis	27.9-40.0 x 13.8-20.1	Cylindrical, slightly tapered near the scopula	Scopula, with 4.8-8.2 µm in diameter	Oval to cuboidal	-	8.8-15.1 x 10.1- 12.0	Round to oval	Along the bottom	1.8-3.2 x 1.9-3.1	Van as & Viljoen, 1985
Apiosoma oblique	29.2-53.8 x 17.3-34.0	Wide in the peristomium, tapering towards the scopula	Scopula, with 2.63-6.3 µm in diameter	Bean-shaped	-	6.6-14.2 x 5.9-15.2	-	Below	1.1-2.5 x 1.1-3.6	Van as & Viljoen, 1985
Apiosoma phiala	22.5-49.8 x 15.1-34.1	Cylindrical above the ciliary band, tapering slightly towards the scopula	Scopula, with 3.4-9.6 µm in diameter	Triangular	-	9.7-20.5 x 8.8-19.6	Oval	Above	3.9-6.7 x 1.7-4.6	Van as & Viljoen, 1985
Apiosoma micralesti	31.2-54.1 x 19.2-37.4	Wide, which tapers towards the scopula, and when contracted cylindrical	Peduncle, which has a broad base, is almost as long as the body, with 4.2-9.2 µm in diameter.	Rounded	-	11.2-23.2 x 11.8-21.0	Oval	Acentric and below	2.8-7.4 x 2.7-4.5	Van as & Viljoen, 1985
Apiosoma triangularis	52.0-71.0 x 15.0-25.5	Elongated, with an expanded peristomy edge	Scopula, with 4.7-7 µm in diameter	Stick-like	-	12.5-20.5 x 7.0-16.5	Oval to rod	On the top margin	2.0-4.0 x 1.0-2.0	Li & Desser, 1985
Apiosoma micropteri	57 x 24	Urn-like	Scopula, with 6 µm in diameter	Inverted cone, wide	Takes up large part of the lower body	-	Small	On top	-	Surber, 1940
Apiosoma cotti	40-75 x 20-34	Conical	Scopula, small	Truncated conical	In the lower part of the body, extending to the peduncle	-	Spherical	Linked to the macronucleus	-	Scheubel, 1973 e Lom, 1966
Apiosoma pyriformis	38.5-46.9 x 18.0-20.36	Urn or conical-shaped, with a rounded anterior end and an elongated and truncated posterior end	Scopula	Piriform	Pointing to the back	8.9	Spherical	On top	-	Scheubel, 1973 e Lom, 1966
Apiosoma minuta	13-26 x 41395	Trumpet-like	-	Rounded	-	-	Rounded	-	-	Scheubel, 1973 e Lom,
Apiosoma dallii	36-52 x 17-22	Conical, very elongated	-	Conic	-	-	Oval	On the side	-	Scheubel, 1973 e Lom,
Apiosoma robusta	50-75 x 30-45	Conical, almost cylindrical in the adoral part and with a basal part similar to a peduncle	Scopula, small, with the body part just above it, sometimes a little narrower	Spherical, small	Below the pectinal formation zone	-	-	-	-	Scheubel, 1973 e Lom, 1966

Apiosoma poteriformis	55 x 23	Club-like, with constriction in the pectinela formation zone	Scopula, wide	Ovoid to slightly tapered	-	-	-	-	-	Scheubel, 1973 e Lom, 1966
Apiosoma doliaris	28-52 x 15-36	Barrel-like	Scopula, relatively wide	Round to conical	-	-	-	-	-	Scheubel, 1973 e Lom,
Apiosoma basiramosa	20-41 x 44136	Conical, which tapers evenly towards the base	Scopula is supplied with wire-like extensions	Rounded	-	-	Rounded	Above	-	Scheubel, 1973 e Lom, 1966
Apiosoma gobionis	48 x 28	Cylindrical and 'stocky', with the front being the widest part of the body	-	Conical and flat, with a tip towards the scopula	-	22 x 14	-	Sideways to front	-	Scheubel, 1973 e Lom, 1966
Apiosoma phoxini	36-58 x 19-26	Conical, with tapering below the peristomium	Scopula, usually oval	Rounded or Triangular	In the lower part of the body	17 x 12	Spherical to oval	Sideways to front	1,5-3	Scheubel, 1973 e Lom, 1966
Apiosoma piscicola percae	- x -	1–1.4 times smaller than A. piscicola	-	-	-	-	Rounded	Above	-	Chernyshova, 1976
Apiosoma gasterostei	40-70 x 22-34	Conical, sometimes slightly narrowed below the peristomium	Scopula, tapered	Rounded triangular	In the lower part of the body, centrally	-	-	-	-	Scheubel, 1973
Apiosoma tintinnabulum	40-50 x 17-20	Elongated bell-shaped, which tapers towards the scopula	Scopula	Basal rounded conical and in some cases oval, hyaline.	In the lower part of the body, centrally	-	-	-	-	Scheubel, 1973
Apiosoma viridis	32.7-90.8 x 14.3-45.0	Cylindrical above the ciliary band, tapering slightly towards the scopula	Scopula, which is elongated and is attached obliquely to the body	Rounded	In the middle of the body, at the same level as the ciliary band	6.6-14.2 x 5.9-15.2	Not observed	-	-	Van as & Viljoen, 1985
Apiosoma guhli	50-55 x 28-30	Truncated cone	-	Discoidal	Below the pectinal formation zone	-	-	-	-	Scheubel, 1973
Apiosoma filiformis	50 x 25	Short cylinder, slightly wider towards the scopula	Scopula slightly larger than the body, with an adhesive effect reinforced by long finger-shaped appendages that resemble pseudopods.	Discoidal	In the lower part of the body, above the scapula, positioned horizontally	-	-	-	-	Scheubel, 1973
Apiosoma siewingi	40 x 20	Only the adoral part of the body in the shape of a cylinder, from the middle of the body, widens slightly, then very strongly	Scopula, very wide	Flat and rounded	In the lower part of the body, above the scopula	-	-	-	-	Scheubel, 1973
Apiosoma extensa	80-85 x 30-35	Tall and relatively uniform cylinder, which widens only slightly towards the scopula	-	Bean-like or curved disk-shaped	On the upper edge of the basal third of the body	-	-	-	-	Scheubel, 1973
Apiosoma compacta	60-65 x 42	Short cylindrical, slightly narrow below the peristomus, suddenly widening towards the scopula	Scopula, often wavy at the edge	-	-	-	-	-	-	Scheubel, 1973
Apiosoma miniciliata	36-40 x 16-20	Conical, tapering relatively evenly towards the scopula	-	Elongated conical that is usually arched at the top	In the lower part of the body	-	-	-	-	Scheubel, 1973
Apiosoma pseudopiscicola	60 x 16-20	Club-like, with a thicker part of the peduncle	Peduncle	Grande, que ocupa quase um terço do volume do corpo	In the middle of the body	-	-	-	-	Scheubel, 1973
Apiosoma microstyla	50 x 30	Pear or bell-like, highly mobile, with the posterior part thinner like a stalk.	Peduncle, which is short and originates from a very thin scopula	Large, which takes up almost a third of the body's volume	In the middle of the body	-	-	-	-	Scheubel, 1973

Apiosoma lomi	56 x 33	Barrel-like, the aboral half of the body is usually slightly bulbous due to the size of the macronucleus.	Peduncle, very short that ends too broadly towards the substrate.	On the side, it is rounded with a small tail-shaped tip towards the scopula.	In the lower part of the body	-	-	-	-	Scheubel, 1973
Apiosoma maxima	70-100 x 24-60	Cylindrical and tall, which widens towards the scopula	-	Elongated oval	In the lower part of the body	-	-	-	-	Scheubel, 1973
Apiosoma alburni	42 x 29	Compact cylinder	-	Rounded triangle	In the lower part of the body	-	-	-	-	Scheubel, 1973
Apiosoma baninae	29-56 x 14-36	Cup-like, broadly tapered. The aboral end of the body repeatedly widens into the scapula, causing a constriction at this point.	-	Conical, with the tip towards the scopula	In the middle of the body	-	-	-	-	Scheubel, 1973
Apiosoma tretzeli	35-38 x 22-24	Cylindrical, with a narrowing close to the scopula.	Scopula, as wide as the body	Relatively small, conical to ovoid	Upper limit in the pectinella formation zone	-	-	-	-	Scheubel, 1973
Apiosoma matthesi	65-106 x 34-50	Cylindrical and tall, narrow with a small base	Scopula, slightly rounded	Large, usually with clod-shaped inclusions	In the lower part of the body	-	-	-	-	Scheubel, 1973
Apiosoma longiciliaris	33.2-50 x 10.7-20.3	Conical that just below the telotrocal band gradually narrows	-	Oval to conical	-	7-12 x 5-7	Oval to rod	Lateralmente a parte anterior	0.8-1.5 x 1.5-3.3	Mytenev, 1975
Apiosoma baikalensis	65 x 40	-	-	-	-	16 x 20	-	-	4	Scheubel, 1973
Apiosoma gigantea	86-93 x 41-45	-	-	-	-	-	-	-	-	Scheubel, 1973
Apiosoma kessleri	30 x 26	-	-	-	-	-	-	-	-	Scheubel, 1973
Apiosoma mucisani	38.5 x 17.5	-	-	-	-	-	-	-	-	Scheubel, 1973
Apiosoma paracotti	50 x 33	-	-	-	-	-	-	-	-	Scheubel, 1973
Apiosoma uschkani	54 x 18	-	-	-	-	-	-	-	-	Scheubel, 1973

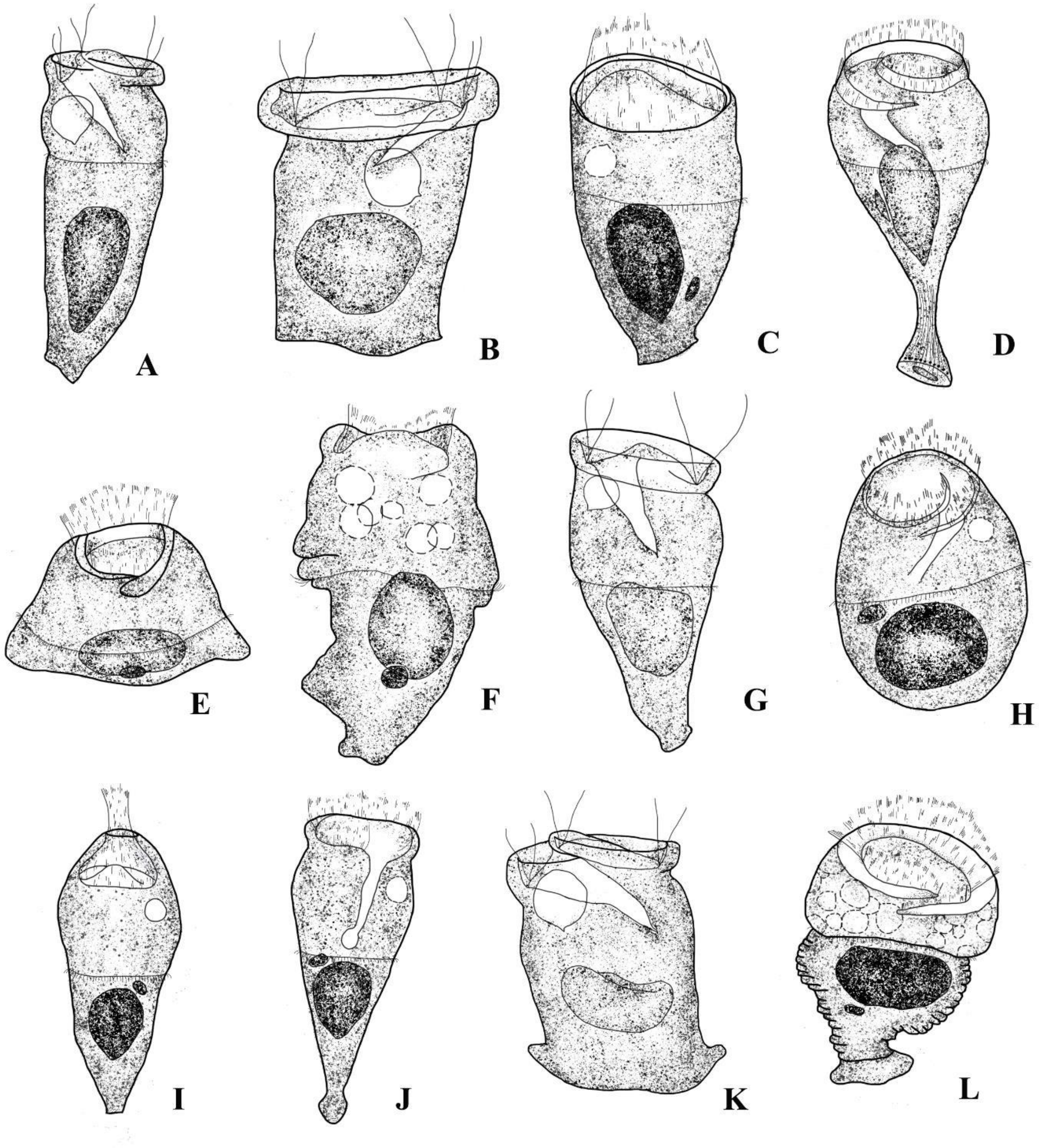
Source: Made by the authors.

Results:

The nine main and most common morphological characters for the *Apiosoma* genus, body size and shape, fixation apparatus, size, shape and position of the macronucleus, size, shape and position relative to the macronucleus of the micronucleus, were compiled comparatively (Table 1) for most of the 62 species recorded here. The new standardized graphical representations (Figures 1-5) were made only for 52 species, due to the lack of associated graphical representations or the presence of incomprehensible graphical representations for the remaining 10 species. A general scheme of individuals of the *Apiosoma* genus was also prepared (Figure 6), to facilitate the identification of the characteristics mentioned in the comparative table.

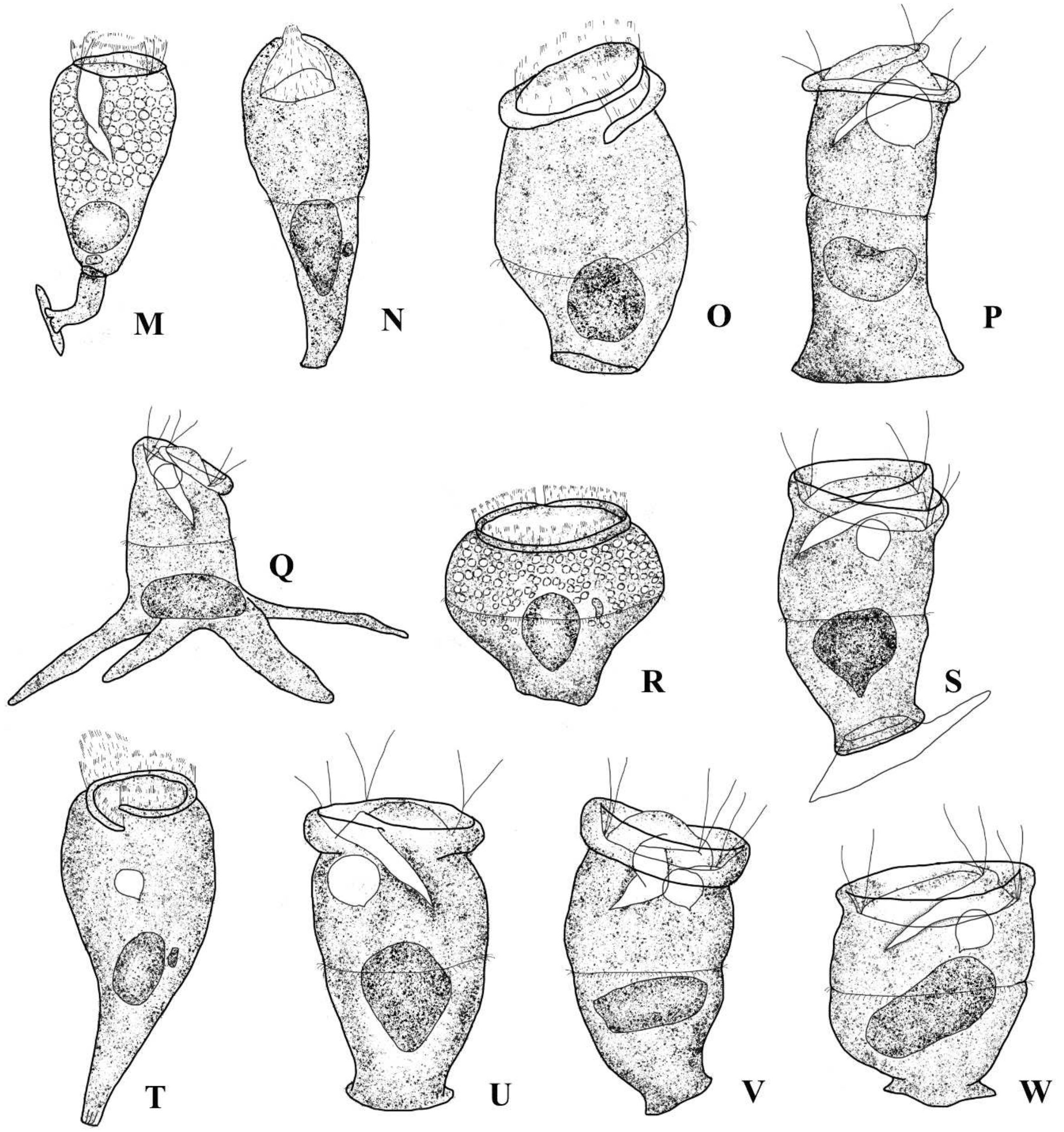
Information on the number of works carried out, number of registered species by region and number of hosts were plotted on maps. In the first map (Figure 7), the number of articles reporting species of *Apiosoma* genus and their respective hosts in each region of the planet were shown, the greatest concentration of these articles was found in the northern region of the globe, with an emphasis on Russia. However, countries like Brazil, The United States and China also have a fair number of articles, which are recent when compared to the ones made in Russia. The second map (Figure 8) demonstrates the richness species around the world. There is a huge concentration of species in Russia, with about 23 species, whereas in the rest of the world the richness is around 2 or less. In the South Africa, Turkey and Serbia, a slightly bigger richness was found. In the third map (Figure 9), which shows the host species richness by country, there is a pattern similar to that found on the first map, where the bigger richness is shown at Russia, however there is a smaller gap in the richness of host between Russia and the other countries, South Africa have only one less species, and countries like Brazil, China and Mexico are not far behind.

Figure 1. Schematic drawing of representatives of the *Apiosoma* genus. A – *Apiosoma matthesi*; B – *Apiosoma alburni*; C – *Apiosoma amoeba*; D – *Apiosoma amurensis*; E – *Apiosoma apllanatum*; F – *Apiosoma baikalensis*; G – *Apiosoma tintinnabulum*; H – *Apiosoma baueri*; I – *Apiosoma campanulata*; J – *Apiosoma carpelli*; K – *Apiosoma compacta*; L – *Apiosoma constricta*.



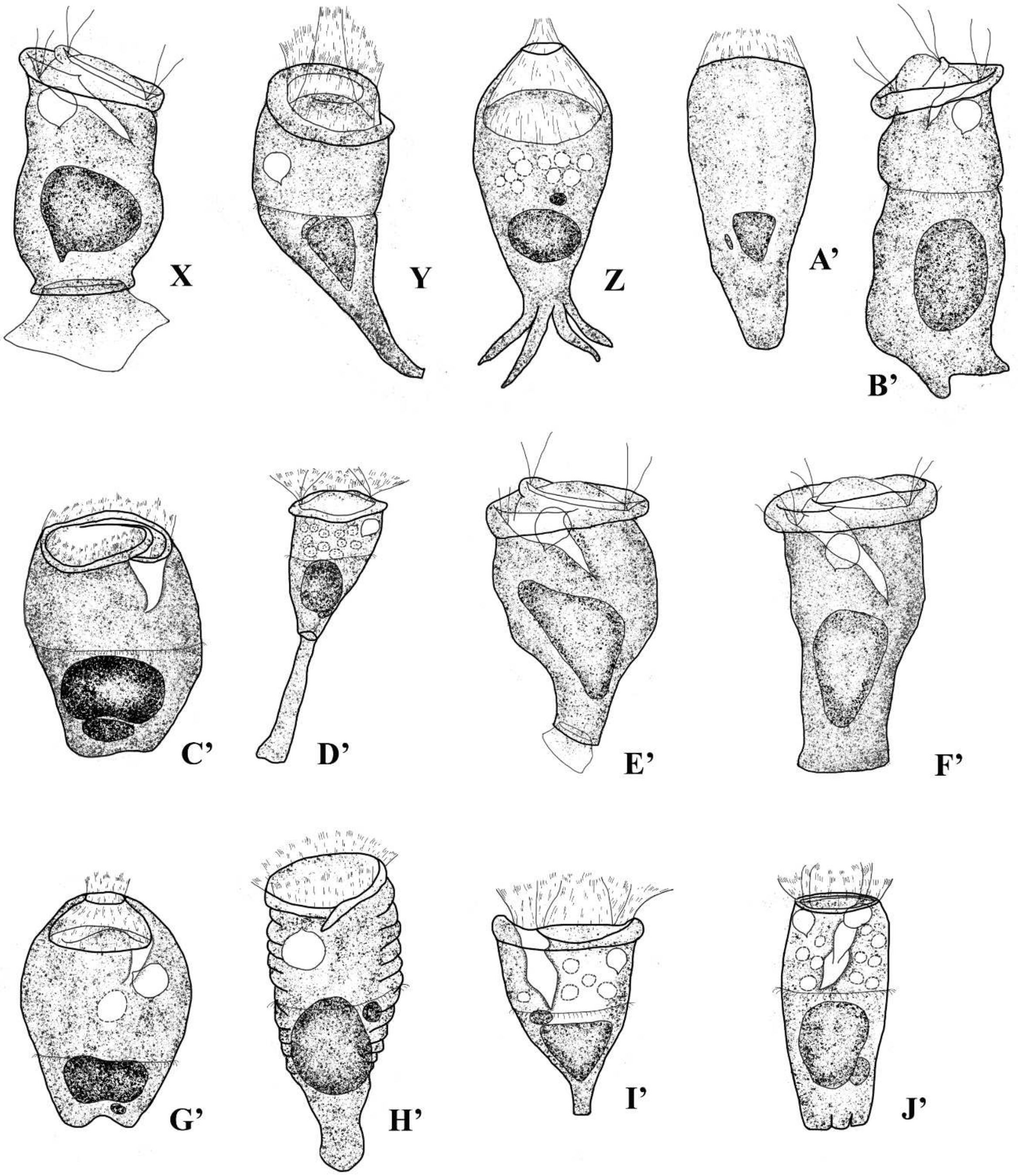
Source: Made by the authors.

Figure 2. Schematic drawing of representatives of the *Apiosoma* genus. M – *Apiosoma curvinucleata*; N – *Apiosoma dallii*; O – *Apiosoma doliaris*; P – *Apiosoma extensa*; Q – *Apiosoma filiformis*; R – *Apiosoma fungiformes;* S- *Apiosoma* gasterostei; T - *Apiosoma gigantea*; U – *Apiosoma gobionis*; V – *Apiosoma guhli*; W – *Apiosoma leuciscus*.



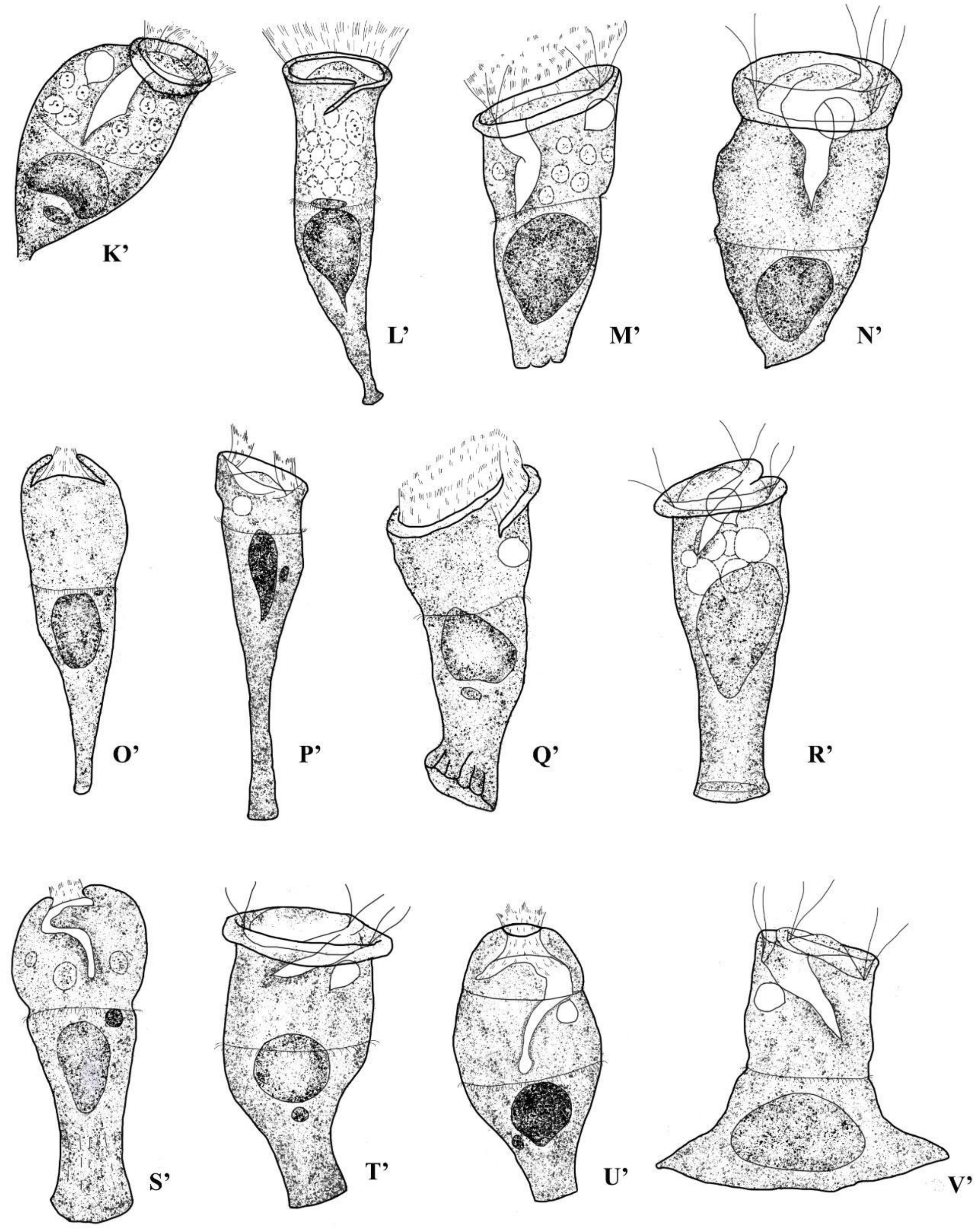
Source: Made by the authors.

Figure 3. Schematic drawing of representatives of the *Apiosoma* genus. X – *Apiosoma lomi*; Y – *Apiosoma longiciliaris*; Z – *Apiosoma lopuchinae*; A' – *Apiosoma lotae*; B' – *Apiosoma máxima*; C' – *Apiosoma megamicronucleata*; D' – *Apiosoma micralesti*; E' – *Apiosoma microstyla;* F' – *Apiosoma miniciliata;* G' – *Apiosoma minimicronucleata*; H' – *Apiosoma minuta;* I' – *Apiosoma mothlapitsis*; J' – *Apiosoma nasalis*.



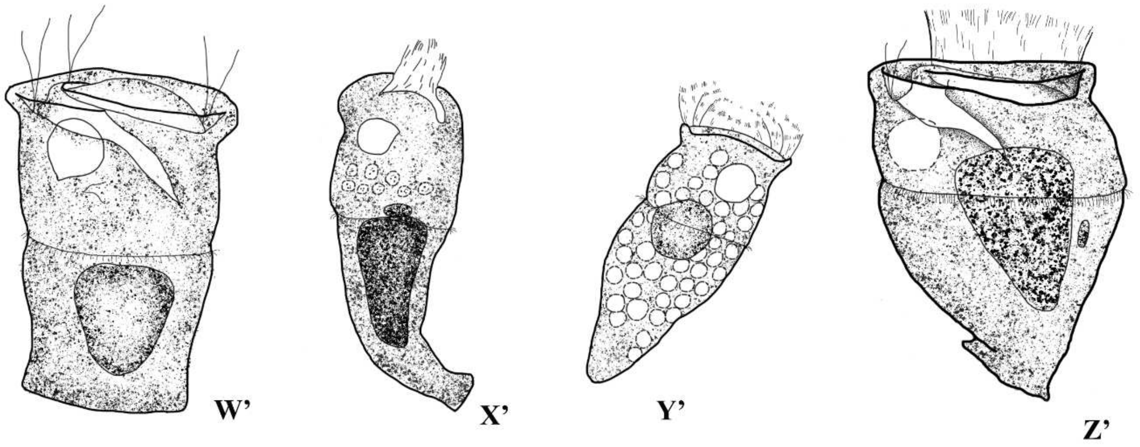
Source: Made by the authors.

Figure 4. Schematic drawing of representatives of the *Apiosoma* genus. K' – *Apiosoma oblique;* L' – *Apiosoma olae;* M' – *Apiosoma phiala*; N' – *Apiosoma phoxini*; O' – *Apiosoma piscícola percae*; P'- *Apiosoma piscícola;* Q' – *Apiosoma poteriformis*; R' – *Apiosoma pseudopiscicola;* S' – *Apiosoma pyriformis*; T' – *Apiosoma robusta*; U' – *Apiosoma shulmani*; V' – *Apiosoma siewingi*.



Source: Made by the authors.

Figure 5. Schematic drawing of representatives of the *Apiosoma* genus. W' – *Apiosoma tretzeli*; X" – *Apiosoma triangularis*, Y' – *Apiosoma viridis*; Z' – *Apiosoma baninae*.



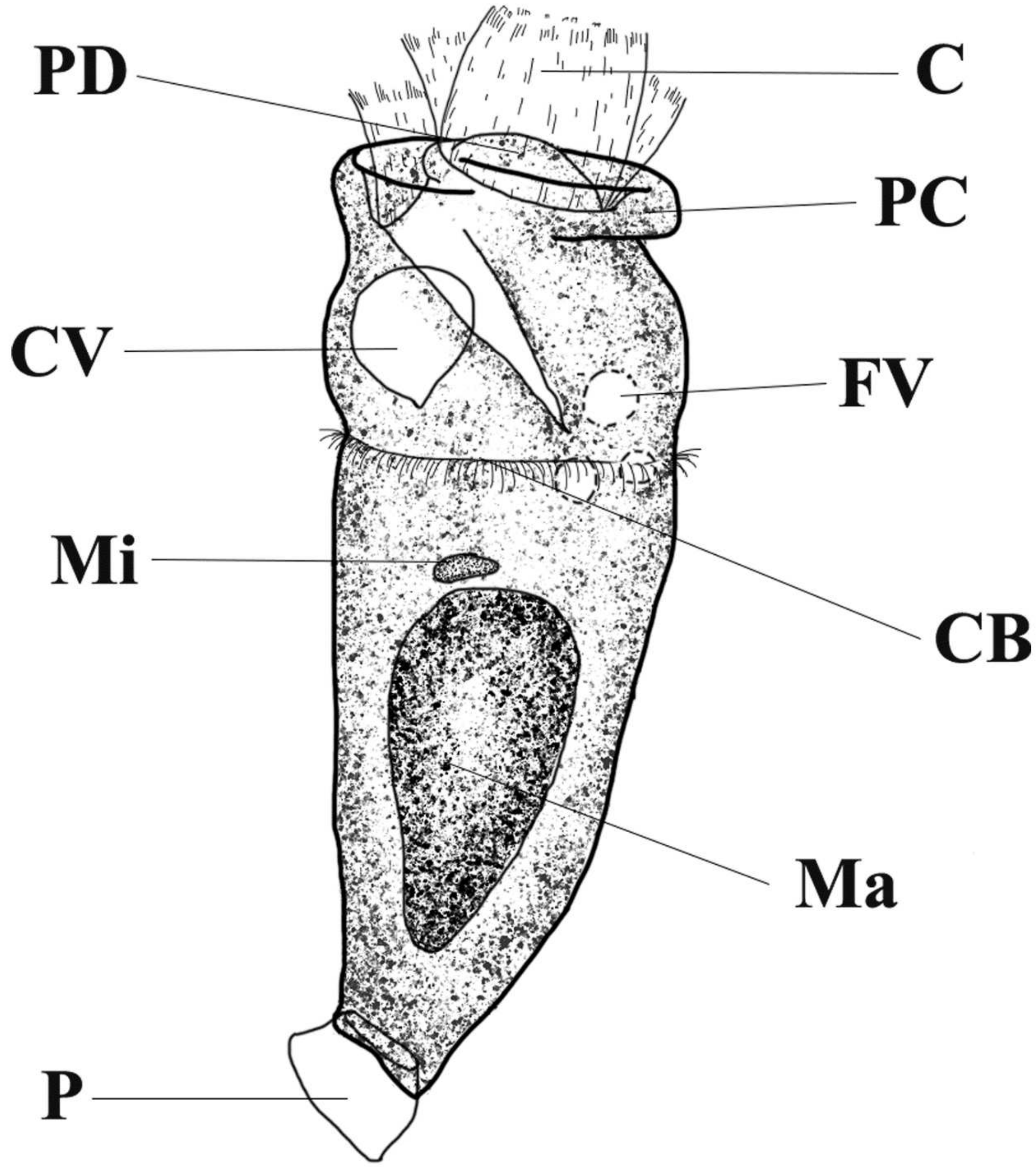
Source: Made by the authors.

Discussion

The first species described for the current *Apiosoma* genus was characterized by KENT (1882) as *Spirochona tintinnabulum*. In 1885, Blanchard created the genus *Apiosoma* to house the fish parasitic species, and does not mention *S. tintinnabulum*. In his review of ciliate taxonomy, BÜTSCHLI (1889) relates the genus described by Blanchard to the genus *Rhabdostyla* Kent, and highlights the inclusion of the species *S. tintinnabulum* in the genus *Spirochona* as inconsistent, thus suggesting the creation of the genus *Glossatella* to group species similar to *S. tintinnabulum* morphologically.

Since Bütschli's proposal was widely accepted, many species described today for the *Apiosoma* genus were considered, at some point, to be of the *Glossatella* genus. The synonymy between *Glossatella* and *Apiosoma* was proposed many years later by LOM (1966) and supported by CORLISS (1979). Currently, all species belonging to the genus *Glossatella* have been recombined to *Apiosoma*.

Figure 6. Generic representation of Apiosoma individuals. C - Ciliature; CB - Ciliary Band; CV - Contractil Vacuole; FV - Food Vacuole; Ma - Macronucleous; Mi - Micronucleous; P - Peduncle; PC - Peristomial Collar; PD - Peristomial Disc.



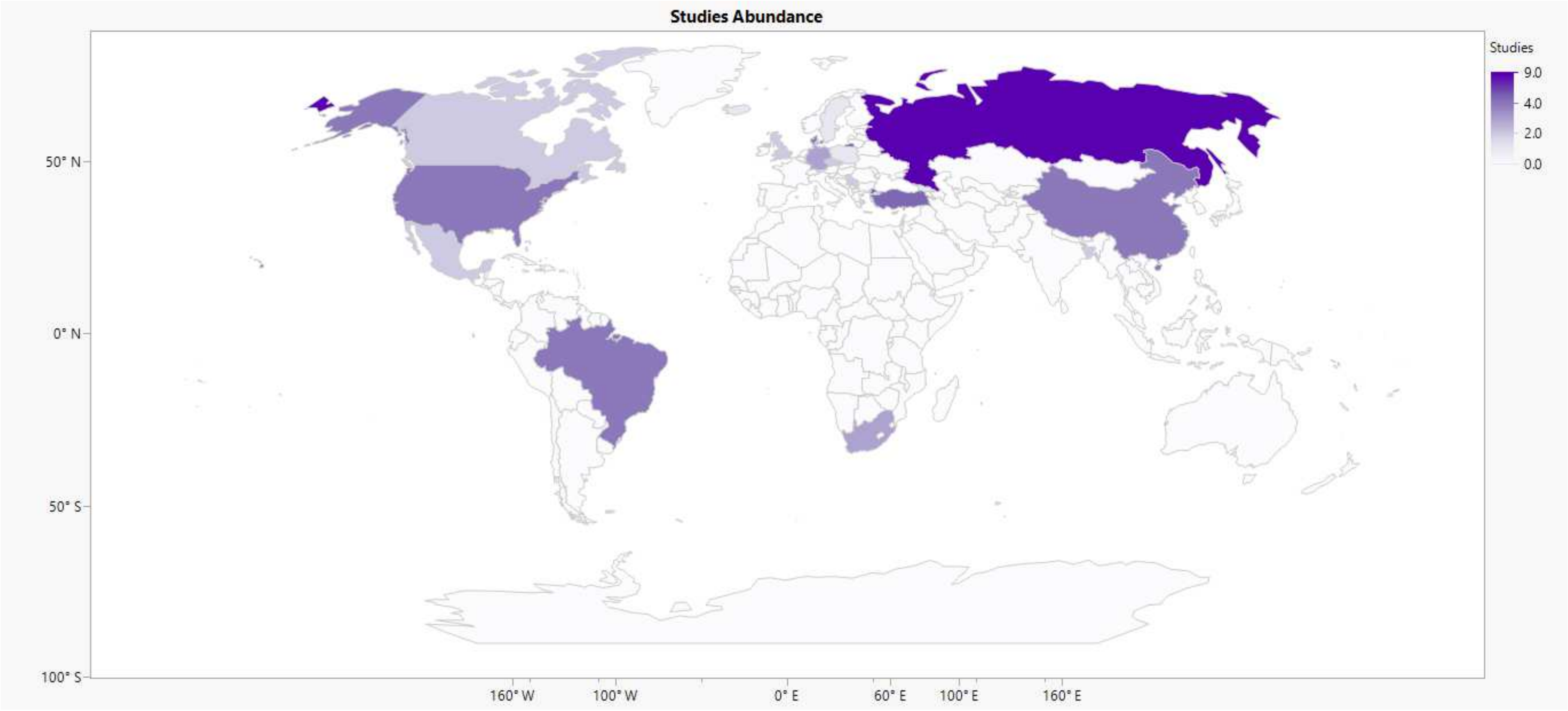
Source: Made by the authors.

According to (LI *et al.*, 2008), there are 72 valid species for the *Apiosoma* genus. In this study, we compiled information for 62 species, given the great difficulty in finding original descriptions of some taxa, as well as the language barrier. Although we have compiled occurrences and records for most species, many studies present a succinct and deficient morphological characterization.

Individuals belonging to this genus are characterized by symbiotic ciliates of aquatic animals, to which they are attached directly by the scopula or by an extremely short peduncle (LOM, 1966). Like other taxa of the Peritrichia subclass, those of the genus *Apiosoma* have considerable intraspecific variability, low parasite specificity and only few morphological characters described (LOM, 1966), which makes it difficult to prepare a complete taxonomic review for the group and, probably, the identification at the species level for these ciliates (ALAM *et al.*, 2012), what is extremely necessary to carry out modern characterizations and re-descriptions of these species.

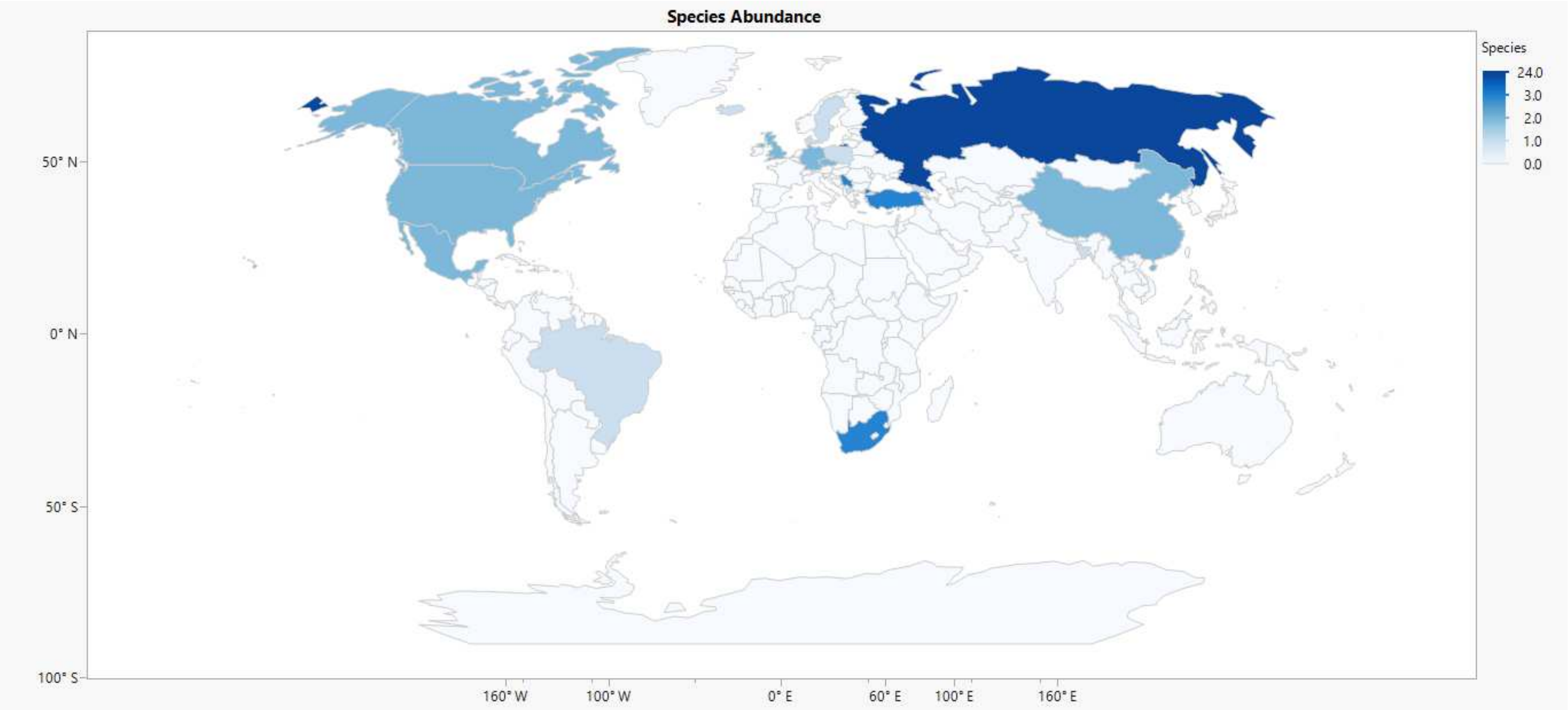
The separation between species of the *Apiosoma* genus is currently based on 9 characteristics, namely: body size and shape, fixation apparatus, size, shape and position of the macronucleus, size, shape and position relative to the macronucleus of the micronucleus. Some of the species can be easily distinguished by their characteristic shape such as *A. filiformis* Scheubel (Figure 2, Q) and *A. fungiformes* Banina & Juchimenko (Figure 2, R). The remaining species may require a more detailed observation to be distinguished. First, they can be divided into two groups, those with an apparent peduncle, such as *A. curvinucleata* Van As & Viljoen (Figure 2, M) and *A. gasterostei* Fauré-Fremiet (Figure 2, S), and those without apparent peduncle (attached directly by the scopula), such as *A. extensa* Scheubel (Figure 2, P) and *A. gobionis* Lom (Figure 2, U). The next step is to look at the shape and position of the macronucleus. They can vary a lot in shape, but tend to be spherical, like *A. olae* Juntschis (Figure 4, L'), oval, like *A. carpelli* Banina (Figure 1, J), triangular, like *A. mothlapitsis* Van As & Viljoen (Figure 3, I'), coniform, such as *A. baninae* Scheubel (Figure 5, A'') or bean-shaped, such as *A. oblique* Van As & Viljoen (Figure 4, K'). As for the position of the macronucleus, they can be found in the anterior part of the body, in the middle of the body or in the posterior part of the body. Next, the micronucleus must be observed, mainly its position in relation to the macronucleus accompanied by its shape, which sometimes can be indistinguishable. The size of the aforementioned structures, body, macronucleus and micronucleus, can also be used to separate the species, but these measurements are quite inconsistent, being influenced, including, in the fixation process (WARREN *et al.*, 2017) and overlap in many species.

Figure 7. Graphical representation of the number of studies with the *Apiosoma* genus representatives recorded across the world.



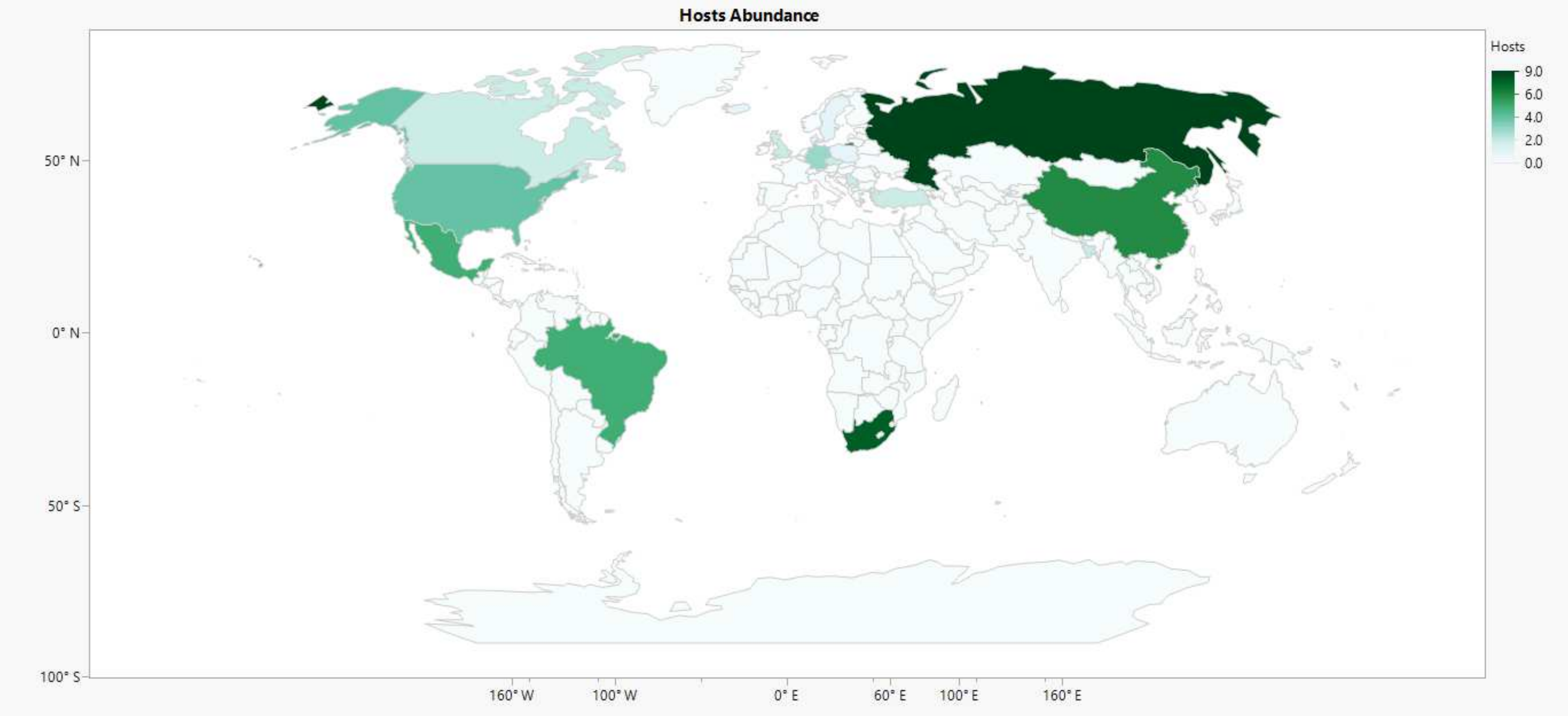
Source: Made by the authors.

Figure 8. Graphical representation of species richness of the genus *Apiosoma* registered across the world.



Source: Made by the authors.

Figure 9. Graphical representation of the number of host species registered for species of the genus *Apiosoma* registered across the world.



Source: Made by the authors.

Observation of properly fixed and stained individuals with techniques such as Heidenhain's method or Ehrlich's hematoxylin (LI *et al.*, 2008), silver impregnation techniques such as Protargol and dry silver, and the use of electron microscopy are also encouraged. Because by allowing visualization of specific characters of taxonomic importance, they represent the gold standard for specific identification of ciliates (WARREN *et al.*, 2017). However, these techniques demand greater investment, time and resources, added to the fact that there is still little material for comparison (LI *et al.*, 2016; MING; SUN; WANG, 2014; MOYSES *et al.*, 2015) turn your use less attractive to non-specialist.

Another characteristic that, historically, was important for *Apiosoma* species was the relationship between its individuals and their hosts. Because, despite not invading the host's cells and not feeding on their tissue, they are treated as parasites. Since, in great abundance in the gills and/or in the body of their hosts, they can cause problems such as breathing and swimming difficulties, respectively (LI *et al.*, 2008; MARTINS; CARDOSO; DE PÁDUA, 2015). In some rarer cases, leading to death, especially in juvenile hosts (SCHMAHL *et al.* 1989). Which can cause economic problems, due to the delay or decrease in the production of fish farms. According to the literature, the species of *Apiosoma* have not host specificity (LOM, 1966), which may be true when analyzing the most recorded species such as *A. piscicola* Blanchard and *A. amoebae* Grenfell, but for species with few records found it may be incorrect. *A. fungiformes*, *A. nasalis* Timofeev, *A. doliare* Timofeev and others are example of that fact, since some were found only one time and some, like *A. constricta* Kashkowskij, were found on the same host species more than one time. Proving the possibility of the existence of a species with host specificity within the genus.

Species of this genus have currently been found in different parts of the world (Figure 8), on a large number of hosts (Figure 9), some of them of great economic importance, such as *Oncorhynchus mykiss* Walbaum, *Cyprinus carpio* Linnaeus and *Oreochromis niloticus* Linnaeus, and also on other organisms' aquatic species such as microcrustaceans and anurans, which was often not reported. However, the geographic distribution of the species is still poorly known. Most of the work with *Apiosoma* individuals was carried out in the region formerly known as the USSR (Figure 7), Union of Soviet Socialist Republics, due to the large concentration of specialists found there (BANINA 1968; GOGEBASHVILI 1980; JUNTSCHIS *et al.* 1975). Until today, the great concentration of knowledge about ciliate biodiversity is in Europe and Asia, and for the *Apiosoma* genus it is no different. There are few works with the group in tropical and subtropical regions (HERRÓZ-ZAMORANO & ALADRO-LUBEL 1996; MOYSES *et al.* 2015b; RELVAS *et al.* 2020; SMIT *et al.* 2017;

VIDAL-MARTÍNEZ *et al.* 2002; VILJOEN & VAN AS 1983), although some of these regions are considered possible strongholds of a great biodiversity of ciliates (FOISSNER; CHAO; KATZ, 2007), a fact that demonstrates that the group, as well as many others, suffer from under sampling due to the lack of specialists and/or investment in these regions, which further reinforces the importance of studies with this group worldwide. This scenario becomes even more alarming, since more recent works usually do not identify the individuals found at a specific level, and some of them are already testing treatments to fight the infestation by the *Apiosoma* species (JENSEN *et al.* 2020; MEIRA-FILHO *et al.* 2017; SCHMAHL *et al.* 1989), which can be a problem because, within Ciliophora, since the different response to a treatment within more restricted groups, such as gender, has already been reported (LIU *et al.*, 2017).

Therefore, despite the work carried out here to facilitate the specific identification of these individuals, several studies addressing the *Apiosoma* species are still needed. Complete alpha taxonomy works, including descriptions and redescriptions with complex morphological analyses, such as silver impregnation techniques and electron microscopy performed by specialists, are necessary for a better separation of the species that make up this genus. This information can make the species-level identification of these individuals by non-specialists even easier and thus generate more reliable records, which will make more applied studies on distribution, evolution, host preference and even the development of drugs or treatments more assertions are carried out. Besides, molecular work is also needed, since there is no molecular record of the group. Phylogenetic analyzes can help to estimate the evolutionary scenario of the group and its relationships with their respective hosts, ways of life and geographic distribution. Furthermore, works with transcriptome analysis can help to better understand the physiology and metabolism of the group, which would enable the development of specific drugs or treatments for these individuals. Finally, histopathological studies are also of great value, as they can provide a better understanding of the influence of these individuals on hosts and the real danger of infestations by this group for animals in culture and free in the wild.

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Supplementary Table 1. List of records of species of the genus *Apiosoma*, their hosts group and species, the country of collection and reference to the work.

Species	**Host**	**Host group**	**Country**	**Year**	**Reference**
Apiosoma sp.	*Oncorhynchus mykiss*	Fish	Denmark	2020	Jessen et al., 2020
Apiosoma piscicola	*Cyprinus carpio*	Fish	Macedonia	2020	Blažeković-Dimovska e Stojanovski, 2020
Apiosoma sp.	*Oreochromis niloticus*	Fish	Brazil	2020	Relvas et al., 2020
Apiosoma sp.	*Ctenopharyngodon idella*	Fish	Brazil	2020	Relvas et al., 2020
Apiosoma sp.	*Astyanax bimaculatus*	Fish	Brazil	2020	Relvas et al., 2020
Apiosoma campanulata	*Gymnocephalus cernuus*	Fish	Russia	2018	Dugarov et al., 2019
Apiosoma sp.	*Mugil liza*	Fish	Brazil	2017	Meira-Filho et al., 2017
Apiosoma sp.	*Oncorhynchus mykiss*	Fish	Turkey	2017	Yandi et al., 2017
Apiosoma piscicola	*Tachysurus fulvidraco*	Fish	China	2016	Li et al., 2016
Apiosoma sp.	*Carassius auratus gibelio*	Fish	China	2016	Cao et al., 2016
Apiosoma sp.	*Hypophthalmichthys molitrix*	Fish	China	2016	Cao et al., 2016
Apiosoma phoxini	*Phoxinus phoxinus*	Fish	Russia	2015	Boutorina & Reznik, 2015
Apiosoma sp.	*Carassius auratus*	Fish	Brazil	2015	Moyses et al., 2015
Apiosoma sp.	*Oreochromis niloticus*	Fish	Brazil	2015	Marengoni et al., 2015
Apiosoma piscicola	*Pelteobagrus fulvidraco*	Fish	China	2014	Li et al., 2014
Apiosoma sp.	*Acipenser oxyrinchus oxyrinchus*	Fish	Poland	2013	Popielarczyk & Kolman, 2013
Apiosoma sp.	*Acipenser oxyrinchus oxyrinchus*	Fish	Canada	2013	Popielarczyk & Kolman, 2013
Apiosoma sp.	*Hypophthalmichthys molitrix*	Fish	Bangladesh	2012	Alam et al., 2012
Apiosoma sp.	*Cirrhinus reba*	Fish	Bangladesh	2012	Mofassahalin et al., 2012
Apiosoma sp.	*Cyprinus carato*	Fish	Germany	2011	Matašin et al., 2011
Apiosoma conicum	*Gymnocephalus cernuus*	Fish	Russia	2010	Odnokurtsev, 2010
Apiosoma amoebae	*Gymnocephalus cernuus*	Fish	Russia	2010	Odnokurtsev, 2010
Apiosoma piscicolum	*Gymnocephalus cernuus*	Fish	Russia	2010	Odnokurtsev, 2010
Apiosoma doliare	*Gymnocephalus cernuus*	Fish	Russia	2010	Odnokurtsev, 2010
Apiosoma baueri	*Gymnocephalus cernuus*	Fish	Russia	2010	Odnokurtsev, 2010
Apiosoma minimicronucleata	*Gymnocephalus cernuus*	Fish	Russia	2010	Odnokurtsev, 2010
Apiosoma piscicolum piscicolum	*Parasalmo mykiss mykiss*	Fish	Russia	2010	Sokolov, 2010
Apiosoma sp.	*Oncorhynchus mykiss*	Fish	Denmark	2009	Jørgensen et al., 2009
Apiosoma piscicolum	*Chondrostoma nasus*	Fish	Czech Republic	2009	Reček et al., 2009
Apiosoma sp.	*Salmo trutta*	Fish	Iceland	2009	Kristmundsson & Richter, 2009
Apiosoma sp.	*Alosa fallax fallax*	Fish	United Kingdom	2008	Nunn et al., 2008
Apiosoma piscicola	*Ctenopharyngodon idella*	Fish	China	2008	Li et al., 2008
Apiosoma piscicola	*Carassius auratus (Var. pengze)*	Fish	China	2008	Li et al., 2008
Apiosoma sp.	*Oncorhynchus mykiss*	Fish	Turkey	2008	Altunay & Yavuzcan Yildiz, 2008
Apiosoma sp.	*Rana aurora aurora*	Amphibian	United States	2007	Nieto et al., 2007
Apiosoma sp.	*Rutilus rutilus*	Fish	Germany	2007	Knopf et al., 2007
Apiosoma piscicolum	*Oncorhynchus mykiss*	Fish	Turkey	2007	Ogut & Akyol, 2007
Apiosoma piscicola var. minor	*Oncorhynchus mykiss*	Fish	Serbia	2006	Nikolić et al., 2006
Apiosoma sp.	*Fundulus heteroclitus*	Fish	United States	2006	Harris & Volgebein, 2006
Apiosoma sp.	*Phoxinus phoxinus*	Fish	Russia	2004	Dorovskikh & Golikova, 2004
Apiosoma sp.	*Tinca tinca*	Fish	Czech Republic	2004	Flajshans et al., 2004
Apiosoma sp.	*Farfantepenaeus aztecus*	Crustacean	Mexico	2002	Vidal-Martínez et al., 2002
Apiosoma sp.	*Farfantepenaeus duorarum*	Crustacean	Mexico	2002	Vidal-Martínez et al., 2002
Apiosoma sp.	*Farfantepenaeus brasiliensis*	Crustacean	Mexico	2002	Vidal-Martínez et al., 2002
Apiosoma sp.	*Farfantepenaeus aztecus*	Crustacean	Mexico	2002	Vidal-Martínez et al., 2002
Apiosoma sp.	*Litopenaeus vannamei*	Crustacean	Mexico	2002	Vidal-Martínez et al., 2002
Apiosoma sp.	*Oncorhynchus mykiss*	Fish	United States	1999	Schisler et al;. 1999
Apiosoma sp.	*Cyprinus carpio*	Fish	Turkey	1999	Ozer & Erdem, 1999
Apiosoma piscicola	*Cyprinus carpio*	Fish	Turkey	1998	Özer & Erdem, 1998
Apiosoma sp.	*Oncorhynchus mykiss*	Fish	Denmark	1997	Buchmann & Bresciani, 1997
Apiosoma piscicola	*Cyprinus carpio rubrofruscus*	Fish	Mexico	1996	Herroz-Zamorano & Aladro Lubel, 1996
Apiosoma robustum	*Perca fluviatilis*	Fish	Serbia	1996	Nikolic & Simonovic, 1996
Apiosoma piscicolum spp. Perci	*Perca fluviatilis*	Fish	Serbia	1996	Nikolic & Simonovic, 1996
Apiosoma sp.	*Oncorhynchus mykiss*	Fish	Denmark	1995	Buchmann et al., 1995
Apiosoma campanulata	*Cottus bairdi*	Fish	United States	1995	Qi & Heckmann, 1995
Apiosoma sp.	*Gasterosteus aculeatus*	Fish	Sweden	1991	Axelsson & Norrgren, 1991
Apiosoma sp.	*Oncorhynchus mykiss*	Fish	South Africa	1991	Bragg, 1991
Apiosoma sp.	*Oncorhynchus mykiss*	Fish	South Africa	1991	Bragg, 1991
Apiosoma amoebae	*Gasterosteus aculeatus*	Fish	Germany	1989	Schmahl et al., 1989
Apiosoma piscicola	*Salvelinus fontinalis*	Fish	Canada	1987	Cone & Odense, 1987
Apiosoma piscicola	*Enteromius (Barbus) paludinosus*	Fish	South Africa	1985	Viljoen and Van As, 1985 in Smit et al., 2017
Apiosoma piscicola	*Enteromius (Barbus) trimaculatus*	Fish	South Africa	1985	Viljoen and Van As, 1985 in Smit et al., 2017
Apiosoma piscicola	*Labeo cylindricus*	Fish	South Africa	1985	Viljoen and Van As, 1985 in Smit et al., 2017
Apiosoma piscicola	*Marcusenius macrolepidotus*	Fish	South Africa	1985	Viljoen and Van As, 1985 in Smit et al., 2017
Apiosoma piscicola	*Micropterus dolomieu*	Fish	South Africa	1985	Viljoen and Van As, 1985 in Smit et al., 2017
Apiosoma piscicola	*Oreochromis mossambicus*	Fish	South Africa	1985	Viljoen and Van As, 1985 in Smit et al., 2017
Apiosoma piscicola	*Pseudocrenilabrus philander*	Fish	South Africa	1985	Viljoen and Van As, 1985 in Smit et al., 2017
Apiosoma piscicola	*Pseudocrenilabrus philander*	Fish	South Africa	1983	Viljoen & Van As, 1983
Apiosoma nasalis	*Pseudocrenilabrus philander*	Fish	South Africa	1983	Viljoen & Van As, 1983

Apiosoma sp.	*Pseudocrenilabrus philander*	Fish	South Africa	1983	Viljoen & Van As, 1983
Apiosoma baninae	*Leuciscus cephalus*	Fish	Georgia	1980	Gogebashvili, 1980
Apiosoma megamicronucleata	*Lota lota*	Fish	Russia	1976	Chernysheva, 1976
Apiosoma amoebae	*Lota lota*	Fish	Russia	1976	Chernysheva, 1976
Apiosoma piscicola	*Lota lota*	Fish	Russia	1976	Chernysheva, 1976
Apiosoma magna	*Lota lota*	Fish	Russia	1976	Chernysheva, 1976
Apiosoma schulmani	*Lota lota*	Fish	Russia	1976	Chernysheva, 1976
Apiosoma phoxini	*Lota lota*	Fish	Russia	1976	Chernysheva, 1976
Apiosoma lotae	*Lota lota*	Fish	Russia	1976	Chernysheva, 1976
Apiosoma megamicronucleata	*Esox lucius*	Fish	Russia	1976	Chernysheva, 1976
Apiosoma campanulata	*Esox lucius*	Fish	Russia	1976	Chernysheva, 1976
Apiosoma amoebae	*Esox lucius*	Fish	Russia	1976	Chernysheva, 1976
Apiosoma piscicola	*Esox lucius*	Fish	Russia	1976	Chernysheva, 1976
Apiosoma magna	*Esox lucius*	Fish	Russia	1976	Chernysheva, 1976
Apiosoma minimicronucleata	*Esox lucius*	Fish	Russia	1976	Chernysheva, 1976
Apiosoma baueri	*Esox lucius*	Fish	Russia	1976	Chernysheva, 1976
Apiosoma esocinum	*Esox lucius*	Fish	Russia	1976	Chernysheva, 1976
Apiosoma olae	*Esox lucius*	Fish	Russia	1976	Chernysheva, 1976
Apiosoma megamicronucleata	*Perca fluviatilis*	Fish	Russia	1976	Chernysheva, 1976
Apiosoma campanulata	*Perca fluviatilis*	Fish	Russia	1976	Chernysheva, 1976
Apiosoma amoebae	*Perca fluviatilis*	Fish	Russia	1976	Chernysheva, 1976
Apiosoma piscicola	*Perca fluviatilis*	Fish	Russia	1976	Chernysheva, 1976
Apiosoma minimicronucleata	*Perca fluviatilis*	Fish	Russia	1976	Chernysheva, 1976
Apiosoma constricta	*Perca fluviatilis*	Fish	Russia	1976	Chernysheva, 1976
Apiosoma constricta	*Perca fluviatilis*	Fish	Russia	1975	Kashkovsky, 1975
Apiosoma tenera	*Cyprinus carpio*	Fish	Russia	1975	Kashkovsky, 1975
Apiosoma longiciliaris	*Phoxinus phoxinus*	Fish	Russia	1975	Mitenev, 1975
Apiosoma olae	*Rutilus rutilus*	Fish	Russia	1975	Juntschis et al., 1975
Apiosoma amurensis	*Phoxinus phoxinus*	Fish	Russia	1975	Juntschis et al., 1975
Apiosoma lopuchinae	*Rutilus rutilus*	Fish	Russia	1975	Juntschis et al., 1975
Apiosoma fungiformis	*Aspius aspius*	Fish	Russia	1975	Juntschis et al., 1975
Apiosoma piscicola	*Gasterosteus aculeatus*	Fish	United Kingdom	1974	Chubb, 1974

Source: Made by the authors.

Chapter 2:

Systematic review of the genera *Rhabdostyla* Kent, 1880 and *Orborhabdostyla* Foissner *et al.* 2010 (Ciliophora, Peritrichia, Epistylididae)

Resumo

O gênero *Rhabdostyla* é um dos mais antigos da família Epistylididae, foi descrito há mais de 120 anos e possui mais de 40 espécies consideradas válidas. Este grupo passou por diversas mudanças sistemáticas ao longo de sua existência e devido à falta de informações taxonômicas, ditas essenciais, para a maioria das espécies que o compõem, algumas dessas mudanças ainda são questionadas. A fim de melhor compreender e validar as espécies e relações identificadas para este gênero, o presente trabalho faz uma breve revisão para o gênero *Rhabdostyla* e seus gêneros relacionados, *Opisthostyla* e *Orborhabdostyla*. Uma análise filogenética de 18S-rDNA também foi realizada para entender a relação entre esses grupos. Como resultado desta revisão, 45 diagnósticos foram compilados e reorganizados, acompanhados de novas representações gráficas para as espécies consideradas válidas para o gênero *Rhabdostyla, Opisthistyla* e *Orborhabdostyla*. As principais mudanças que o gênero *Rhabdostyla* sofreu, como sinonímia com *Opisthostyla* e separação para um novo gênero, *Orborhabdostyla*, foram discutidas à luz de novas informações filogenéticas, mas apesar disso outras informações ainda são necessárias para um veredicto final sobre a validade dessas mudanças.

Palavras-chave: Sessilida, filogenia, epibiontes, 18S-rDNA

Abstract

The genus *Rhabdostyla* is one of the oldest of the Epistylididae family, it was described more than 120 years ago and has more than 40 species considered valid. This group has undergone several systematic changes throughout its existence and due to the lack of taxonomic information, said to be essential, for most of the species that compose it, some of these changes are still questioned. In order to better understand and validate the species and relationships identified for this genus, the present work makes a brief review for the genus Rhabdostyla and its related genera, *Opisthostyla* and *Orborhabdostyla*. A phylogenetic analysis of 18S-rDNA was also performed to understand the relationship between these groups. As a result of this review, 45 diagnoses were compiled and reorganized, accompanied by new graphic representations for the species considered valid for the genus *Rhabdostyla*, *Opisthistyla* and *Orborhabdostyla*. The main changes that the genus *Rhabdostyla* underwent, such as synonymy with *Opisthostyla* and separation to a new genus, *Orborhabdostyla*, were discussed in light of new phylogenetic information, but despite this, other information is still needed for a final verdict on the validity of these changes.

Keywords: Sessilida, phylogeny, epibionts, 18S-rDNA

Introduction

Since their first observation by Leeuwenhoek, ciliates (Ciliophora) have aroused researchers' curiosity about the “microscopic world” (FOKIN, 2004). In more than 250 years of knowledge about Ciliophora, thousands of species have been described and several new technologies have been applied for this purpose (WARREN *et al.*, 2017). With the appearance and development of new tools and technologies, studies on the recognition of ciliate diversity have become increasingly in-depth (FOKIN, 2004; WARREN *et al.*, 2017), which highlights the need for expansion and improvement of older diagnoses of numerous taxa within Ciliophora.

The need for greater depth in species diagnoses has been reported for various classes of ciliates (CEDROLA *et al.*, 2020; LIU. *et al.*, 2017; WARREN *et al.*, 2017), not being different for most of the families that compose the subclass Peritrichia (Ciliophora, Oligohymenophorea). The family Epistylididae is composed of 12 genera and more than 300 species, being the rhabdostylid ciliates (*Rhabdostyla* and *Orborhabdostyla*) an example of this need for more complete redescriptions and a systematic review.

Among the 45 valid rhabdostylid species, only four have morphological data about oral infraciliature available (ÁLVAREZ-CAMPOS *et al.*, 2014; FOISSNER *et al.*, 2010; FOISSNER, Wilhelm, 1979; SONG, 1986), and the vast majority of diagnoses are superficial and incomplete (KAHL, 1935; NENNINGER, 1948), with a clear need for an updated review of the group, as well as the need to carry out redescriptions using appropriate tools. Another problem that plagues rhabdostyloid ciliates is the frequent changes that the group has undergone. The genus *Rhabdostyla*, the main pillar of the group, was synonymous with the genus *Opisthostyla*, because the separation was based only on the presence of a fold at the end of the peduncle (BÜTSCHLI, 1889; AESCHT, 2001; LYNN, 2008) and underwent a change of family (KAHL, 1935), based on a few features, features that today are no longer relevant to Peritrichia's taxonomy. More recently, some species have been removed from the genus *Rhabdostyla* and combined to the genus *Orborhabdostyla* (Foissner et al. 2009), based mainly on the shape of the nucleus and some characteristics that are unknown for most rhabdostylids. These factors show the potential taxonomic inconsistencies in this group and the need for recognition of valid species and a systematic review.

Since the beginning of molecular studies (18S-rDNA) until recent phylogenomic data, the monophyly of the Epistylididae family has been questioned (JIANG *et al.*, 2016; MIAO *et* *al.*, 2004; SUN; CLAMP; XU, 2010; WANG *et al.*, 2017; ZHUANG *et al.*, 2018), however, there are only two studies that presented sequences (18S-rDNA) of rhabdostylid ciliates (*Rhabdostyla*, *Opisthostyla* and *Orborhabdostyla*) (FOISSNER *et al.*, 2010; ZHUANG *et al.*, 2018). These studies suggest that some representatives of the genus *Rhabdostyla* are vorticellids (Vorticellidae), as suggested in a 19th century study (KENT, 1881).

In this scenario of lack of morphological information and the emergence of new molecular information, a review is needed to base studies that may come to investigate these problems. Therefore, we developed a systematic review of the genera Rhabdostyla and Orborhabdostyla, presenting diagnosis, morphometric information, and host data in the case of epibiont species, as well as investigating the phylogenetic position (18S-rDNA) of rhabdostylids using an up-to-date database.

Material and methods:

1. Revision:

Initially, an extensive literature search was carried out, using Google Scholar, Scopus, and Biodiversity Heritage Library databases, to prepare the taxonomic review. The keywords utilizing for the search were ‘Rhabdostyla’, ‘Opisthostyla’ and ‘Orborhabdostyla’. Additionally from the main studies and taxonomic reviews on rhabdostylid ciliates (ÁLVAREZ-CAMPOS *et al.*, 2014; FOISSNER, 1979; KAHL, 1935; KENT, 1881; NENNINGER, 1948; SONG, 1986; STOKES, 1886) the main references of this papers not retrieved during searches in the aforementioned databases were collected. This material was acquired from several research groups and libraries and added to the articles dataset here analyzed. Information on species related to the genera *Rhabdostyla*, *Opisthostyla* and *Orborhabdostyla* was compiled from this database of articles. Broader diagnoses were generated for each species by compiling the morphological information described in different papers. The most complete diagnoses found was complemented with absent information and some information such length was summarized. There are few rhabdostylid species with morphological information generated based on modern techniques. In the remarks section for each species, additional taxonomic information was added, as well as reservations and comments made by the authors/reviewers of the latest diagnoses and the references used to prepare the diagnosis presented and they contribution. The morphological and morphometric data of the species, presented in a comparative way, are available in Table 1. The species drawings were made based on the original drawings of the species descriptions and/or with complementary drawings contained in the re-descriptions. The terminology adopted to designate the morphological structures follows the work of Warren (1986) and Foissner *et al.* (1992). In the synonymies list we added only papers that suggest taxonomic modification, such as synonyms or gender recombination.

2. Phylogenetic analysis:

To investigate the phylogenetic position of rhabdostylid ciliates within the Peritrichia subclass we compiled a wide dataset using 18S-rDNA sequences for sessilids (Oligohymenophora: Peritrichia: Sessilida) listed at the species level, except for the representative of the genus *Opishtistyla* which was identified only to gender level. We collected 109 sequences from the GenBank database in November 2020, which added to *Frontonia leucas* EHRENBERG, 1833 and *Trichodina uniforma* VAN AS & BASSON, 1989, which were used as outgroups, generated a dataset with 111 sequences. The sequences were aligned using the MAFFT software (KATOH; STANDLEY, 2013), with default settings and then visually checked the alignment using the SEAVIEW software (GOUY *et al.,* 2010). After this verification, the GBlocks program (CASTRESANA, 2000) was used to remove the poorly aligned sites and a matrix with 1616 base pairs (bp) was acquired. For phylogenetic inference a Bayesian Inference analysis (IB) was estimated using the Mr. Bayes program (RONQUIST *et al.*, 2012). The analyze used the GTR + GAMMA + I model, which was chosen as the best replacement model by the JModelTest program (POSADA, 2008), implemented by the MEGA-X platform (KUMAR *et al.*, 2018). The topology by Bayesian inference was inferred in two independent Markov Monte Carlo chain runs (MCMC), with 1000000 generations and a burn-in of 25%.

Results:

1. Taxonomic Review:

Class: Oligohymenophorea of Puytorac et al., 1974
Order: Peritrichia Stein, 1859
Family: Epistylididae Kahl, 1933

Genus: *Rhabdostyla* Kent, 1881

Opisthostyla Stokes, 1886b: 565.

[*Rhabdostyla*] (*Rhabdostyla*) Kent, 1881 – Kahl, 1935: 673

[*Rhabdostyla*] (*Opisthostyla*) Stokes, 1886b – Kahl, 1935: 679

Epistylis (*Rhabdostyla*) Kent, 1881 – Stiller, 1971: 64

Diagnosis: Solitary, with a short, non-contractile peduncle, with a generally bell-shaped body and a well-defined peristomial collar.

Type specie: *Epistylis spheroides* (Subsequent designation JANKWOSKI, 2009)

Remarks: initially described by Kent in (1881) as solitary "animalcules", similar to those of the genus *Vorticella*, but with non-contractile peduncle. In a recent taxonomic study, Álvarez-Campos *et al.* (2014) defines the genus as solitary peritrich ciliates with short non-contractile peduncles, with a mainly bell-shaped body and a well-defined peristomial lip. Stokes (1886b) didn't stipulate a type-species to the genus *Opisthostyla*, therefore here we suggest *Opisthostyla annulate* as type species of *Opisthostyla*, this specie was in the original genus description, have a decent diagnosis and attached graphic representation.

Rhabdostyla annulata (Stokes, 1886) nov. comb. – Plate 1, fig. D

Opisthostyla annulata Stokes, 1886(b): 565, fig. 12, 13.

[*Rhabdostyla*] (*Opisthostyla*) *annulate* Stokes,1886 – Kahl, 1935: 679, pl. 129. Fig. 20

Epistylis (*Rhabdostyla*) *annulate* Stokes, 1886 – Stiller, 1971: 70, pl. 36, fig. 6

Diagnosis: Size about 22-35 µm. Conical-bellied body, less than twice long as wide. Slightly changeable in shape, somewhat bumpy or with almost straight side margins. Zooid located obliquely or vertically over the peduncle, which has the same size as the body and a slight curve in the distal portion. Pellicle strongly transversely striated. Revoluted peristomial collar, with ciliary circles. Infundibulum reaches a third of the body's length. Contractile vacuole found on the right margin of the infundibulum in the upper quarter of the body. Macronucleus, thin and horseshoe-shaped, located in the middle of the body, positioned horizontally. Found in freshwater lakes living on algae.

Remarks: morphological information compiled from STOKES (1886), KAHL (1935) and STILLER (1971).

Rhabdostyla arenaria (Cuénot, 1891) - Plate 1, fig. E

[*Rhabdostyla*] (*Rhabdostyla*) *arenaria* Cuénot, 1891 – Kahl, 1935: 678, pl. 129, fig. 25

Diagnosis: Size about 40-56µm. Short peduncle, 10µm in length, reaching the size of the body in some cases. Posterior section of the body interrupted by a transverse groove. Very long infundibulum. Contractile vacuole wide with a very distinct excretory canaliculus. Elongated and band-shaped macronucleus, with small chromatin agglomerations. Found living on the integument of echinoderms.

Remarks: morphological information compiled from CUÉNOT (1891), HENSEN (1911) and KAHL (1935). CUÉNOT (1891) points to the possibility of a spherical micronucleus in the upper part of the body.

Rhabdostyla arenicolae (Fabré-Domergue, 1888) - Plate 1, fig. F

[*Rhabdostyla*] (*Rhabdostyla*) *arenicolae* Fabré-Domergue, 1888 – Kahl, 1935: 677, pl. 129, fig. 30

Diagnosis: Size about 45-60µm. Conical body, wider on the front. Stalk usually measuring half the length of the body. Cylindrical nucleus, with torsion close to the peristomial region. A contractile vacuole in the anterior region. Pellicle transversely striated. Individuals found in the *Arenicola* polychaete gills in marine ecosystems.

Remarks: morphological information compiled from HENSEN (1911) and KAHL (1935).

Rhabdostyla bosminae (Levander, 1907) - Plate 1, fig. G

[*Rhabdostyla*] (*Rhabdostyla*) *bosminae* Lavander, 1907 – Kahl, 1935: 676, pl. 129, fig. 43

Diagnosis: Size about 38-44µm long and 18-20µm wide. Transversely striated pellicle. Stalk measuring about half the size of the body, reaching same size in rare cases. Peristome size about half the width of the body. Horseshoe-shaped nucleus. Usually colonizes the dorsal region of the carapace of cladocerans of the genus *Bosmina*.

Remarks: morphological information according to KAHL (1935).

Rhabdostyla chironomi (Kahl, 1933) - Plate 1, fig. I

[*Rhabdostyla*] (*Rhabdostyla*) *chironomi* Kahl, 1933 – Kahl, 1935: 678, pl. 133, fig. 15

Diagnosis: Size about 60 µm. Very similar in shape to *R. commensalis*, except for the thicker peristomial collar. Peduncle twice as long as wide and with transverse folds. Individuals found on the respiratory tubes of chironomid larvae (Diptera, Chironomidae).

Remarks: morphological information according to KAHL (1935).

Rhabdostyla commensalis (Mobius, 1888) - Plate 1, fig. J

[*Rhabdostyla*] (*Rhabdostyla*) *commensalis* Mobius, 1888 – Kahl, 1935: 677-678, pl. 129, fig. 24

Diagnosis: Size about 62-70 µm. Elongated barrel shaped body. Gently striated pellicle. Peristome narrower than body diameter. Elevated peristomial disc. Very short peduncle, with an enlarged base and no transverse folds. Small, cylindrical macronucleus centrally arranged. Found on the polychaetes, *Capitella capitata* and *Terebellides stromi*.

Remarks: morphological information compiled from articles by KAHL (1935) and PRECHT (1935).

Rhabdostyla congregata (Zacharias, 1903)

[*Rhabdostyla*] (*Rhabdostyla*) *congregata* Zacharias, 1903 – Kahl, 1935: 676

Diagnosis: Size about 38-40 µm long and about 30 µm wide. Body shape like a small *Epistylis*, a little more rounded than other species. Stalk about 8-12 µm long. Smooth film, without streaks. Horseshoe-shaped macronucleus, found in the lower third of the body. One or two contractile vacuoles always present. Specimens found in large numbers, usually more than 25, on rotifers and rarely on crustaceans.

Remarks: morphological information according to ZACHARIAS (1903) and KAHL (1935). ZACHARIAS (1903) he accompanied the encystment of this species. Cyst about 32 and 28 µm wide. The wall of the cysts was very thick, about 2-3 thick, the surface of the cyst was not smooth, it had bumps. Dispersal processes were also reported, as some zooids in the group developed a telotrocal band, detached themselves from the basibiont, and sought new substrates.

Rhabdostyla conipes (Kahl,1935) – Plate 2, fig. K

[*Rhabdostyla*] (*Rhabdostyla*) *conipes* Kahl, 1935: 676, pl. 129, fig 7

Epistylis (*Rhabdostyla*) *conipes* Kahl, 1935 – Stiller, 1971: 64, pl. 34, fig. A

Diagnosis: Size about 40-46µm. Straight and short peduncle, about 6 µm long. Slightly enlarged in the anterior region. Pellicle weakly striated. Wide, almost flat peristomial disc. Short, sausage-shaped macronucleus, curved back at one end and positioned in the middle of the body. Epibiont of cladocerans of the *Daphnia* genus.

Remarks: morphological information diagnosis of articles by KAHL (1935) and STILLER (1971).

Rhabdostyla cyclopis (Kahl, 1935) – Plate 2, fig. L

[*Rhabdostyla*] (*Rhabdostyla*) *cyclopis* Kahl, 1935: 675, pl. 138, fig. 19

Epistylis (*Rhabdostyla*) *cyclopis* Kahl, 1935 – Stiller, 1971: 68, pl. 35, fig. F

Diagnosis: Size about 30µm. Vase-like body. Stalk short and with an expansion at insertion with the body, where the body turns when contracted. Very thick peristomial collar. Convex peristomial disc, with no bulge. Infundibulum reaching a third of the body length. Contractile vacuole found below the peristomial collar. Horseshoe-shaped macronucleus, positioned horizontally on the upper body. Found on species of cladocerans, copepods and ostracodes.

Remarks: morphological information compiled from KAHL (1935) and STILLER (1971).

Rhabdostyla cylindrica (Stiller, 1933) – Plate 2, fig. M

[*Rhabdostyla*] (*Rhabdostyla*) *cylindrica* Stiller, 1933 – Kahl, 1935: 678, pl. 133, fig. 25

Epistylis (*Rhabdostyla*) *cylindrica* Stiller, 1933 – Stiller, 1971: 65, pl. 34, fig. D

Diagnosis: Size about 25-28 µm long and 18-20 µm wid. Robust body with a rounded back. Peduncle measuring one-sixth the height of the body, thick and striated, end ending in an adhesive disk. Pellicle delicately striated. Peristomial collar slightly thickened. Peristomial disc flat, with almost no bulge. Infundibulum narrow reaching a third of the body. Contractile vacuole on the left side of the infundibulum. Macronucleus elongated and horseshoe-shaped, located in the anterior region of the body, tilted horizontally, found less frequently transversely. Several tiny food vacuoles in the cytoplasm. Epibiont of cladocerans of the species *Leptodora kindti*.

Remarks: morphological information compiled from the works of KAHL (1935) and STILLER (1971).

Rhabdostyla dixae (Nenninger, 1948) – Plate 2, fig. N

Epistylis (*Rhabdostyla*) *dixae* Stiller, 1933 – Stiller, 1971: 65, pl. 35, fig. I

Diagnosis: Size about 48.8-55 µm. Jar-like body, strongly protruding in the frontal third. Small stalk, about 10-11 µm to a third of the body size, quite thin. Peristomial collar with two-thirds of the body width, simple, collar-like, notched. Peristomial disc wide and arched, with a pronounced annular groove at the base. Infundibulum reaching about a third of the body's length, which widens to a spherical shape in the lower portion. Contractile vacuole positioned at the lower left end of the infundibulum. Pellicle streaked at regular intervals. The basal part of the body with two lobes, one lobe slightly longer than the other. Macronucleus tape-shaped with folds at the ends, positioned transversely in the anterior part of the body, covering the lower end of the infundibulum, or obliquely reaching the peristomial disc. Found in freshwater environments on insect larvae of the species *Dixa nigra*.

Remarks: morphological information compiled from NENNINGER (1948) and STILLER (1971). According to NENNINGER (1948), the contraction creates a narrow, notched collar and the pellicle striations become more apparent.

Rhabdostyla dubia Foissner 1979 – Plate 2, fig. O

Diagnosis: Size about 30-35µm. Body from a vessel-like form to a funnel-like, when contracted assumes a pear shape with a bifurcated peristomial disc with clear transverse striations on the pellicle. Peduncle measures 2-5 µm, with transverse striations and grooves, usually enlarged at the base. Contractile vacuole found just below the peristomial collar, on the ventral wall of the, wide and quite short, vestibule. Horseshoe-shaped macronucleus, inclined longitudinally. Ciliary of the adoral spiral long and robust. Cytoplasm with large and slightly yellowish granules. Silverline pattern resembles that of *Pseudocarchesium erlangensis*. Found on ostracodes.

Remarks: morphological information based on FOISSNER (1979). He reports for the species cysts with about 30 µm in diameter, ectocysts with numerous mole-shaped elevations. The author also highlights that the position of the contractile vacuole and the form of contraction are the characteristics that distinguish *R. dubia* from other very similar species*, R. ovum* Kent, 1882, *R. cyclopis* Kahl, 1935 and *R. ovum var. culiciidarum* Nenninger, 1948. According to him, it is possible that these 4 species are just an expression of the great variability of a species, especially if we assume that KAHL (1935) and NENNINGER (1948) wrongly drew the contractile vacuole on the dorsal wall of the infundibulum.

Rhabdostyla ephemerae (Kahl, 1935) – Plate 2, fig. P

[*Rhabdostyla*] (*Rhabdostyla*) *ephemerae* Kahl, 1935: 675, pl. 138, fig. 8

Epistylis (*Rhabdostyla*) *ephemerae* Kahl, 1935 – Stiller, 1971: 66, pl. 34, fig. H

Diagnosis: Size about 75 µm. Body variable in shape, but not very asymmetric. Short and thin peduncle. Pellicle with narrow striations. Elongated and sausage-shaped macronucleus, positioned longitudinally, surrounding the infundibulum. Oblique peristomial disc, with distinct protuberance at the margin. Robust peristomial collar. Contractile vacuole positioned in the upper quarter of the body, to the right of the infundibulum. Broad infundibulum. Found in the oral region of Ephemeroptera larvae.

Remarks: morphological information compiled from the works of KAHL (1935) and STILLER (1971).

Rhabdostyla fromentellii (Kent, 1881) – Plate 2, fig. Q

Scyphidia fromentellii Kent, 1881-1882: 659, pl. XXXIII, fig. 53

[*Rhabdostyla*] (*Rhabdostyla*) fromenteli Kent, 1881-1882 – Kahl, 1935: 677, pl. 129, fig. 27

Diagnosis: Size about 80µm. Elongated-claved shape, truncated anteriorly and with the posterior portion without striation when contracted. Peduncle with longitudinal striations. Apparently with 2 or 3 contractile vacuoles. Found in freshwater environments.

Remarks: compiled from the works of KENT (1881) and KAHL (1935). According to KENT (1881) De Fromentel proposed a synonym of this species with *Scyphidia dubia*, which was refuted by Kent due to the existence of a distinct peduncle and three contractile vacuoles.

Rhabdostyla globularis (Stokes, 1889) – Plate 2, fig. R

Opisthostyla globularis Stokes, 1889: 481, pl. X, fig. 8

[*Rhabdostyla*] (*Opishtostyla*) *globularis* Stokes, 1889 – Kahl, 1935: 679, pl. 192, fig. 21

Rhabdostyla globularis Stokes, 1889 – Green, 1974: 459

Diagnosis: Size about 25-36.6 µm. Normally with a subglobus body, but mutable, a little longer than wide. Peduncle slightly longer than body. Pellicle transversely striated. Peristome smaller in diameter than the widest part of the body. Peristomial collar revolute. Peristomial disc not elevated. Found in freshwater environments on algae, *Hydrodictyon utriculatum*, and cladocerans, *Bosmina longirostris* and *Diaphanosoma brachyurum*.

Remarks: morphological information compiled from the works of STOKES (1889), KAHL (1935), NENNINGER (1948) and GREEN (1974).

Rhabdostyla hungarica (Stiller, 1931) – Plate 2, fig. S

[*Rhabdostyla*] (*Rhabdostyla*) *hungarica* Stiller, 1931 – Kahl, 1935: 679, pl. 133, fig. 13

Epistylis (*Rhbadostyla*) *hungarica* Stiller, 1931 – Stiller, 1971: 69, pl. 36, fig. B

Rhbadostyla hungarica Stiller, 1931 – Green, 1974: 459, pl. 17, fig. G

Diagnosis: Size about 35-40µm. Often with a pear-shaped body, but very variable. Plump and short peduncle, 10-15 µm long. Pellicle finely striated. Peristomial collar quite thick. Peristomial disc flat, convex, and sunken, that is, it does not protrude beyond the peristomial collar. Infundibulum reaching a little more than half of the body. Contractile vacuole on the right side of the infundibulum. Anterior macronucleus, horseshoe-shape, inclined transversely. Light gray cytoplasm with coarse granules. Found in a crustacean of the genus *Leptodora*.

Remarks: morphological information compiled from works of morphological information KAHL (1935), STILLER (1971) and GREEN (1974).

Rhabdostyla inclinans (Műller, 1786) – Plate 3, fig. T

Vorticella inclinans Müller, 1786: 309, pl. XLIV, fig. 11

Scyphidia ringens Dujardin, 1841

Gerda inclinans Müller, 1786 – D'udekem, 1864: 51, pl. 1, fig. 6

Scyphidia inclinas Müller, 1786 – Kent, 1882: 659, pl. XXXIV, fig. 2

Rhabdostyla chaeticola Stokes, 1887: 144, pl. VIII, fig. 9

Rhabdostyla inclinans Müller, 1786 – Roux, 1901: 126

Rhabdostyla lumbriculi Penard, 1922: 266, fig. 251

[*Rhabdostyla*] (*Rhabdostyla*) *inclinans* Müller, 1786 – Kahl, 1935: 675, pl. 129, fig. 1 and 2, pl. 138, fig. 6 and 7

Schyphidia lumbriculi Stiller, 1971: 17, pl. 6, fig. E

Rhabdostyla inclinans Müller, 1786 – Foissner et al., 1992: 246, fig. 1-14

Diagnosis: Size about 50-77µm. Cylindrical body twice as long as wide, tapered in the posterior quarter, somewhat ventrally inclined and dorsally convex. Pellicle transversally and finely striated. Cylindrical peduncle, slightly curved with an adhesive disc. Small, arched peristomial disc, slightly prominent. Infundibulum positioned in the middle of the body and reaching the back of the body. Short and not too thick peristomial collar, slightly projected. Contractile vacuole close to the infundibulum, immediately below the peristomial collar. Long, cylindrical micronucleus, positioned longitudinally. Found in association with limnic oligochaetes of the genus *Nais* and *Lumbriculus.*

Remarks: morphological information compiled from the works of MÜLLER (1786), D'UDEKEM (1864), KORSCHELT (1906), PENARD (1922), KAHL (1935), NENNINGER (1948), STILLER (1971) and FOISSNER *et* al. (1992).

Rhabdostyla invaginata Stoke, 1886 – Plate 3, fig. U

Diagnosis: Size about 35µm. Vasiform body, rarely gibbus, twice as wide as long. Pellicle distinctly ringed. Peduncle with a 1/3 to 2/5 of body length. Peristomial collar not detached. Prominent ciliary disc, conical, with two ciliary circles. Expanded peristome, with a flat peristomial disc. Contractile vacuole near the peristomial region. Epibionts of ostracodes, of the genus *Cypris*.

Remarks: diagnosis according to STOKES (1886). According to the same, the contracted body is oval and the peduncle invaginates into the posterior extremity, causing the individual. make real contact with the surface of the object that supports the stalk.

Rhabdostyla libera (Kahl,1933) – Plate 3, fig. V

[*Rhabdostyla*] (*Rhabdostyla*) *libera* Kahl, 1933 – Kahl, 1935: 675, pl. 133, fig. 14

Diagnosis: Size about 60µm. Tapered, extremely similar to that of protists of the genus *Vorticella*. Short and folded peduncle. Delicate longitudinal striations all over the body and transverse striations that can only be seen in the posterior region. Longitudinally inclined macronucleus. Marine individuals and were found on algae.

Remarks: diagnosis according to KAHL (1935).

Rhabdostyla limnophili Kahl, 1935 – Plate 3, fig. W

[*Rhabdostyla*] (*Rhabdostyla*) *limnophili* Kahl, 1935: 678, pl. 138, fig. 29

Diagnosis: Size about 55µm. Oval shaped. Peduncle measuring about two-thirds the size of the body, ringed, sometimes with an unoccupied lateral branch. Elevated peristomial collar, which wraps around the back of the body when contracted. Transversely inclined macronucleus, usually in the middle of the posterior part of the body. Found in fresh water, in large numbers on *Trichoptera* larvae.

Remarks: diagnosis according to KAHL (1935).

Rhabdostyla longipes (Kent, 1880) nov. comb. – Plate 3, fig. X

[*Rhabdostyla*] (*Opisthostyla*) *longipes* Kahl, 1935: 679, pl. 129, fig. 18

Diagnosis: Size about 70 µm. Elongated to bell-shaped body, more attenuated at the posterior end, about twice long as wide when expanded, symmetrically oval in the contracted state. Thin, sinuous peduncle, two or three times longer than the body, centrally enclosing a uniform series of tiny granular particles. Clear cytoplasm, finely grained. Smooth pellicle. Found in freshwater environments.

Remarks: morphological information compiled from the works of KENT (1881) and KAHL (1935). According to KENT (1880), it is very similar to *Vorticella picta*, except for the lack of color in the granulation of the cytoplasm and was found in 'association' with *Vorticella campanula.*

Rhabdostyla mapuche Álvarez-Campos et al., 2014 – Plate 3, fig. Y

Diagnosis: Size about 23-31 µm long and 16-19µm wide. Globular to spherical body. Peduncle short, about 3-6 µm long, and thick, about 4-6 µm wide, with longitudinal sinuation. Pellicle with distinct transverse striations, 36-39 silverlines. Peristomial disc with 20-23µm in diameter and with 9-14 ciliary bands. Macronucleus c-shaped and close to the cell periphery. Round micronucleus, close to the macronucleus. Found on marine polychaetes.

Remarks: diagnoses according to ÁLVAREZ-CAMPOS *et* al. (2014). According to them, this species, when contracted, assumes an oval shape with a deep indentation that contains part of the contracted posterior end, this is because its posterior and anterior folds cover the contracted region.

Rhabdostyla muscorum (Kahl, 1935) – Plate 3, fig. Z

[*Rhabdostyla*] (*Rhabdostyla*) *muscorum* Kahl, 1935: 675, pl. 138, fig. 9, 9a and 9b

Epistylis (*Rhabdostyla*) *muscorum* Kahl, 1935 – Stiller, 1971: 70, pl. 36, fig. I

Diagnosis: Size about 40µm. Elongated vase-like body, with two grooves in the basal part. Thin peduncle, measuring approximately 20 µm to about a third of the body size. Peristomial collar thick. Peristomial disc is flat and low. Infundibulum reaching halfway through the body. Contractile vacuole found on the right side of the infundibulum in the upper third of the body. Macronucleus elongated and positioned longitudinally, has a fold at the top. Found in moss infusions.

Remarks: morphological information compiled from KAHL (1935) and STILLER (1971). According to KAHL (1935), it has a very characteristic contractile form and the furrows in the most basal part of the individuals have been little studied and need more attention.

Rhabdostyla nebulifera (Fromentel, 1874) – Plate 3, fig. B'

Epistylis nebulifera Fromentel, 1874: 239, pl. V, fig. 2, 3

Rhabdostyla nebulifera Kent, 1881-1882, pl. XXXIV, fig. 11, 12

Diagnosis: Size about 125µm. Subglobus body, truncated anteriorly. Short peduncle, with about half the length of the body, which widens the closer to the body ending in a fixation disk. Smooth pellicle. Without projections on the peristomial edge. Found in freshwater environments.

Remarks: diagnosis according to KENT (1881).

Rhabdostyla nereicola Precht 1935 – Plate 3, fig. C'

Diagnosis: Size about 55-62 µm. Shape quite constant, with a thin lower edge. Short peduncle. Pellicle striated. Irregular peristomial collar. Cytoplasm with granules that not reach the end of the body. Macronucleus straight and concave towards the interior of the body. Found on polychaetes, *Nereis dumerili.*

Remarks: diagnosis according to PRECHT (1935).

Rhabdostyla patula Perty,1852 – Plate 3, fig. A’

Scyphidia patula Perty, 1852: 189, pl. II, fig. 7

[*Rhabdostyla*] (*Rhabdostyla*) *patula* Kahl, 1935: 678, pl. 129, fig. 15

Diagnosis: Size about 1/30’”. Peduncle with half the body size. Bell-shaped body. Cytoplasm with a bluish gray coloration.

Remarks: morphological information compiled from the works of PERTY (1852) and KAHL (1935). According to PERTY (1852), this species is similar to *Scyphidia ringens* Müller, 1773. According to KAHL (1935), the original author did not compile enough information, but analyzing the drawing there is no doubt that it is a different species. The 1/30”” size was not changed to the standard measurement (µm), because there was no reference to perform the conversion.

Rhabdostyla porculus (Penard, 1922) – Plate 4, fig. F’

[*Rhabdostyla*] (*Rhabdostyla*) *porculus* Penard, 1922 – Kahl, 1935: 677, pl. 129, fig. 23

Epistylis (*Rhabdostyla*) *porculus* Penard, 1922 – Stiller, 1971: 71, pl. 36, fig. K

Diagnosis: Size about 135-155µm. Broad and cylindrical body. Smooth peduncle, measuring 30 to 35µm, cylindrical shaped with adhesive disc. Milky pellicle with little distinct striations. Wide peristomial disc, almost flat, but protruding from its highest point. Infundibulum reaches half of the body. Very wide contractile vacuole, located at the base of the peristomial collar, to the right of the infundibulum. Broad, relatively little elongated macronucleus, rarely “S” shaped, positioned longitudinally. Species found on debris in small numbers.

Remarks: morphological information compiled from works of PENARD (1922), KAHL (1935) and STILLER (1971).

Rhabdostyla pristinis Righi, 1973 – Plate 4, fig. G’

Diagnosis: Size about 37 µm long and 48 µm wide. Peduncle long, about 1/3 or 1/4 the size of the body, with four or five flexible transverse appendages. Transversely striated pellicle.

Narrow, flat-surfaced peristomial disc with circular peristomial membrane. Infundibulum reaching about half of the body length and constricted in front of the contractile vacuole. Spiral macronucleus. Found on oligochaetes, *Pristinis minuta*.

Remarks: diagnosis according to RIGHI (1973).

Rhabdostyla pusilla (Stokes, 1886a) nov. comb. – Plate 3, fig. D'

Rhabdostyla pussila Stokes, 1886a: 108, pl. I, fig. 17

Opisthostyla pussila Stokes, 1886b: 565

[*Rhabdostyla*] (*Opisthostyla*) *pusilla* Stokes, 1886a – Kahl, 1935: 679, pl. 129, fig. 19

Diagnosis: Size about 22.5-25 µm. Bell or pear-shaped body, thinning posteriorly, less than twice as wide as it is long. Peduncle is slightly longer than the body, the distal end slightly curved. Pellicle cross streaked. Peristome slightly exceeds the width of the center of the body, the peristomial collar is revolute. Found in freshwater ponds over algae of genus *Ceratophyllum*.

Remarks: morphological information compiled from works of Stokes (1886a) and Kahl (1935).

Rhabdostyla ringens (Müller, 1786) – Plate 4, fig. H'

Vorticella ringens Müller, 1786: 309, pl. XLIV, fig. 10

Epistylis ringens Fromentel, 1874: 240, pl. IV, fig. 6

Rhabdostyla ringens Kent, 1881: 666, pl. XXXIV, fig. 1

Diagnosis: Size about 80µm. Conical and elongated body, about twice long as wide. Peduncle short and thin, about a third of the body's length. Pellicle apparently without striations and with a slightly brownish color. Freshwater organisms.

Remarks: morphological information compiled from the works of MÜLLER (1786) and KENT (1881). According to Müller, there is a doubt whether this species is the same as described by Chevalier, as Chevalier's *Vorticella ringes* has no description.

Rhabdostyla similis (Stokes, 1889) nov. comb. – Plate 4, fig. I’

Opisthostyla similis Stokes, 1889: 481, pl. 10, fig. 9

Diagnosis: Size about 22.5 µm. Sub-vasiform body, somewhat mutable in the posterior part. Twice as long as wide, somewhat bumpy, slightly contracted below the peristomial collar. Peduncle slightly longer than the body, distal end slightly curved. Strongly cross-striated pellicle. Posterior region contains two rounded transverse rings, the anterior being the largest, the posterior end often apparently joined to the peduncle by means of a disc-shaped appendix. Peristomial collar revolute. Peristomial disc slightly and obliquely elevated. Found in freshwater lakes, on the roots of floating plants.

Remarks: diagnosis according to STOKES (1889). According to him, the body when contracted is obovate, slightly invaginating the end of the peduncle.

Rhabdostyla scyphidiforme Vavrá, 1961 – Plate 4, fig. J’

Epistylis (*Rhabdostyla*) *scyphidiforme* Vavrá, 1961 – Stiller, 1971: 67, pl. 35, fig. A

Diagnosis: Size about 60-100 µm long and 25-34 µm wide. Peduncle with 64µm to body length, lower end widens into an adhesive disk. Peduncle may be absent, and the body ends are bounded by an irregular circumferential adhesive, as in *Scyphidia*. Peristomial collar slightly thickened and slightly wider than the body. Peristomial disc wide, almost flat, slightly sloping, sometimes with small bumps on top. Pellicle is finely striated. The infundibulum is the size of the first third of the body and is positioned diagonally. Contractile vacuole found below the peristomial collar on the right wall of the infundibulum. Cytoplasm fine-grained and filled with refractive food vacuoles and contains long palate-shaped bacteria. Refractive grains accumulate in the middle of the lower ectoplasmic extremities of the body. Macronucleus curved and at the top of the body. Found on the gills of tadpoles of the species *Rana esculenta.*

Remarks: diagnosis according to STILLER (1971).

Rhabdostyla scyphoides Song, 1986 – Plate 4, fig. K’

Diagnosis: Size about 35-44µm long and 24-27µm wide. Cylindrical body. Peduncle very short, about a third of the body's length, with a widening in the proximal portion. Smooth pellicle. Peristomial disc flat. Cytoplasm with many fine granules. Contractile vacuole in the apical part of the cell. "S"-shaped macronucleus, located longitudinally in the body. The components of the oral ciliary, Polykinety 1 and Polykinety 2 are derived from the Polykinety, Polykinety 3 short, with two rows. Aboral ciliary crown has several rows and a very large scopula.

Remarks: diagnosis according to SONG (1986).

Rhabdostyla sessilis Penard, 1922 – Plate 4, fig. L'

[*Rhabdostyla*] (*Rhabdostyla*) *sessilis* Penard, 1922 – Kahl, 1935: 677, pl. 129, fig. 3

Epistylis (*Rhabdostyla*) *sessilis* Penard, 1922 – Stiller, 1971: 65, pl. 34, fig. B

Diagnosis: Size about 43-45µm. Body elongated, cylindrical or vaguely urceolate, with a more rounded back. Extremely short peduncle, almost invisible, apparently consisting only of the adhesive disk. Pellicle with very thin and indistinct striations. Relatively wide peristomial disc, very low, with an almost flat surface. Large and deep infundibulum, reaching the middle of the body. Very large contractile vacuole, positioned just below the vestibule. Very light macronucleus, longitudinally positioned, sausage-shaped and strongly thickened at its posterior end. Individuals found on *Cyclops* copepods.

Remarks: morphological information compiled from the works of PENARD (1922), KAHL (1935) and STILLER (1971).

Rhabdostyla spheroides (Fromentel, 1874) – Plate 4, fig. M'

Epistylis spheroides Fromentel, 1874: 240, pl. IV, fig. 4

Rhabdostyla sphaeroides Kent, 1881-1882: 666, pl. XXXIII, fig. 10

[*Rhabdostyla*] (*Rhabdostyla*) *sphaeroides* Kahl, 1935: 678, pl. 129, fig. 16

Diagnosis: Size about 60µm. Robust peduncle, measuring about half the size of the body and enlarged proximally. Smooth pellicle. Peristomial disc with convex elevation when expanded, elevation almost forming a sphere. Curved and prolonged infundibulum. Long and robust oral ciliary. Found in freshwater environments.

Remarks: morphological information compiled from the works of de FROMENTEL (1874), KENT (1881) and KAHL (1935).

Rhabdostyla taboadai Álvarez-Campos *et al.,* 2014 – Plate 4, fig. N'

Diagnosis: Size about 30-45 µm long and 15-23 µm wide. Elongated globular body, with a rounded and slightly protruding anterior extremity. Peduncle 14-17 µm long and 7-9 µm wide with a longitudinal twist and adhesive disk 8-12 µm in diameter. Pellicle distinctly transversely striated, with 38-42 silverlines. Peristomial disc is about 26-29 µm in diameter, with 11-18 ciliary bands. Multilobulated macronucleus, usually anterior, close to the cell periphery. Missing micronucleus. Found link to intersegmental furrows, near parapodial bases, of annelid, *Syllis prolifera*.

Remarks: diagnosis according to ÁLVAREZ-CAMPOS *et al.* (2014). According to the authors, when contracted, the posterior and anterior folds cover the contracted part, and this makes it assume an oval shape, with a deep cut containing part of the contracted posterior end.

Rhabdostyla tritora (Nenninger, 1948) nov. comb. – Plate 4, fig. O'

Opisthostyla tritora Nenninger, 1948: 178, pl. 12, fig. a, b

Diagnosis: Size about 180 µm long and 200 µm wide. Body wider than tall, almost spherical. Portion of the body closest to the peduncle is extremely narrow. Peduncle with 1/6 of the body's length. Peristomial disc surrounded by two ciliary chains. Peristomial collar divided into 3 thin layers. Peristomial disc and collar rest on the body like a lid. Infundibulum, which is almost straight, reaches half the length of the body. Contractile vacuole at the end of the infundibulum. Cytoplasm contains coarse grains, except for the tapered end that precedes the peduncle. With several food vacuoles containing dark granulations. Difficult to visualize macronucleus, in the form of a "worm", thick and curved, positioned transversely in the middle of the body. Found in the coastal zone of Regnitz, on annelids, *Helobdella stagnalis*, and algae, *Ceratophyllum*.

Remarks: diagnosis according to NENNINGER (1948).

Rhabdostyla tubificis Nenninger, 1948 – Plate 4, fig. P'

Diagnosis: Size about 60 µm. Vessel-like body, that gradually decreases towards the peduncle. Extremely short peduncle with adhesive disc. Clearly striated pellicle. Peristomial collar moderately thick. Peristomial disc slightly arcuate and bifurcated. Contractile vacuole lies below the peristomial collar. Broad, horseshoe-shaped macronucleus, positioned transversely in the middle of the body, rarely longitudinally. Found over annelids of genus *Tubifex*, only in the head region.

Remarks: diagnosis according to NENNINGER (1948).

Rhabdostyla variabilis (Dons, 1918) – Plate 3, fig. E'

Rhabdostyla variabilis Dons, 1918

Scyphidia variabilis Dons, 1918 – Kahl, 1935: 671, pl. 128, fig. 32

Rhabdostyla variabilis Álvarez-Campos et al., 2014: 276

Diagnosis: Size about 60-90 µm. Peristomial collar approximately with the same width as the largest part of the body. Prominent peristomial disc. Very short peduncle. Kidney-shaped macronucleus. Found on polychaete tentacles.

Remarks: morphological information compiled from the works of KAHL (1935) and ÁLVAREZ-CAMPOS et al (2014). In 1935, KAHL recombines this species to the genus *Scyphidia*, without much justification, and in 2014, ÁLVAREZ-CAMPOS *et al.* (2014) treats this species as belonging to the genus *Rhabdostyla*. Due to these facts, we consider this species as valid for the genus, but a new look must be given considering new information.

Rhabdostyla vernalis Stokes, 1887 – Plate 4, fig. Q'

[*Rhabdostyla*] (*Rhabdostyla*) *vernalis* Stokes, 1887 – Kahl, 1935: 676, pl. 129, fig. 13

Epistylis (*Rhabdostyla*) *vernalis* Stokes, 1887 – Stiller, 1971: 69, pl. 36, fig. A

Diagnosis: Size about 48-50µm long and 20µm wide. Urceolate body, often gibbous, and elongated, twice as long as wide. Peduncle is short, measuring 8-10µm to one-eighth the length of the body. Finely and transversely striated pellicle. Elevated and convex peristomial disc, occasionally developing a central and conspicuous nipple-like projection. With two ciliary circles. Peristomial collar slightly slanted, not revolved. Infundibulum, extends to the middle of the body, prominent vestibular cilia. Contractile vacuole located in the anterior half of the body. Macronucleus hand-like shaped, curved, located in the posterior part of the body. Granular cytoplasm. Found on microcustaceous of the genera of *Cypris* and *Cyclops*.

Remarks: morphological information compiled from STOKES (1887), KAHL (1935) and STILLER (1971). According to STOKES (1887), when contracted, it assumes an oval shape, often waving and the peduncle invaginates in the posterior region of the body.

Rhabdostyla volvocis Nenninger, 1948 – Plate 1, fig. H

Epistylis (*Rhabdostyla*) *volvoci* Nenninger, 1948 – Stiller, 1971: 71, pl. 36, fig. H

Diagnosis: Size about 48.8-60 µm. Funnel-shaped body. Thin pellicle with thickened striations. Peduncle approximately 10 µm in size. Thick, snail-like curved peristomial collar that expands toward the vestibule and is wider than the rest of the body. Vestibule short. Contractile vacuole found just below the peristomial collar. Peristomial disc slightly arched. Macronucleus V-shaped and found in the middle of the body. With some food vacuums and a coarse granulation filling the body. Found on algae, *Volvox globator.*

Remarks: morphological information compiled from NENNINGER (1948) and STILLER (1971). According to NENNINGER (1948), the observation of the nucleus is still absent, it only paid attention to the form for the description.

Genus: *Orborhabdostyla* Foissner *et al.*, 2010

Diagnosis: solitary epistylids, with a discoidal to elliptical macronucleus and a transverse striation pattern (silverlines).

Remarks: A recently created genus, it was added to this revision since most of its members have already been considered of the *Rhabdostyla* genus and because of their close relationship with it.

Type-specie: *Orborhabdostyla bromelicola*

Orborhabdostyla brevipes (Claparède e Lachmann, 1857) – Plate 1, fig. A

Epistylis brevipes Claparède e Lachmann, 1857

Rhabdostyla brevipes Claparède e Lachmann, 1857 – Kent, 1881-1882: 665, pl. XXXIV, fig. 8

[*Rhabdostyla*] (*Rhabdostyla*) *brevipes* Claparède e Lachmann, 1857 – Kahl, 1935: 677: pl. 129, fig. 8

Orborhabdostyla brevipes Claparède e Lachmann, 1857 – Foissner et al., 2010: 293

Diagnosis: Size about 80-90µm. Subcylindrical body, attenuated, about three times longer than wide, similar to *Epistylis plicatilis.* Extremely short peduncle, about one-tenth the size of the body. Provided with an anterior rostrum when contracted. Oval macronucleus centralized in the posterior part of the body. With several transversal grooves in the posterior region when contracted. Found in large numbers on Diptera larvae in freshwater environments.

Remarks: morphological information compiled from the works of KENT (1881) and KAHL (1935).

Orborhabdostyla bromelicola Foissner *et al.*, 2010 – Plate 1, fig. B

Diagnosis: Size about 65 µm long and 15 µm wide in field specimens, while 50 × 15 µm in laboratory cultures. Body narrowly conical. Peduncle usually < 10 µm long and 2 µm wide. Contractile vacuole and cytoproct on dorsal wall of vestibulum slightly posterior of oral bulge. On average 63 silverlines from oral end to anlage of aboral ciliary wreath and 16 silverlines from anlage to scopula. Peniculus 2 shortened posteriorly, peniculus 3 composed of three minute kineties. Freshwater, possibly restricted to tank bromeliads and not colonizing any host.

Remarks: diagnosis according to FOISSNER *et al.* (2010). FOISSNER *et al.* (2010) points that this species is extremely contractile, especially in the posterior third and possibly restricted to bromeliad tanks.

Orborhabdostyla kahli (Nenninger, 1948) – Plate 1, fig. C

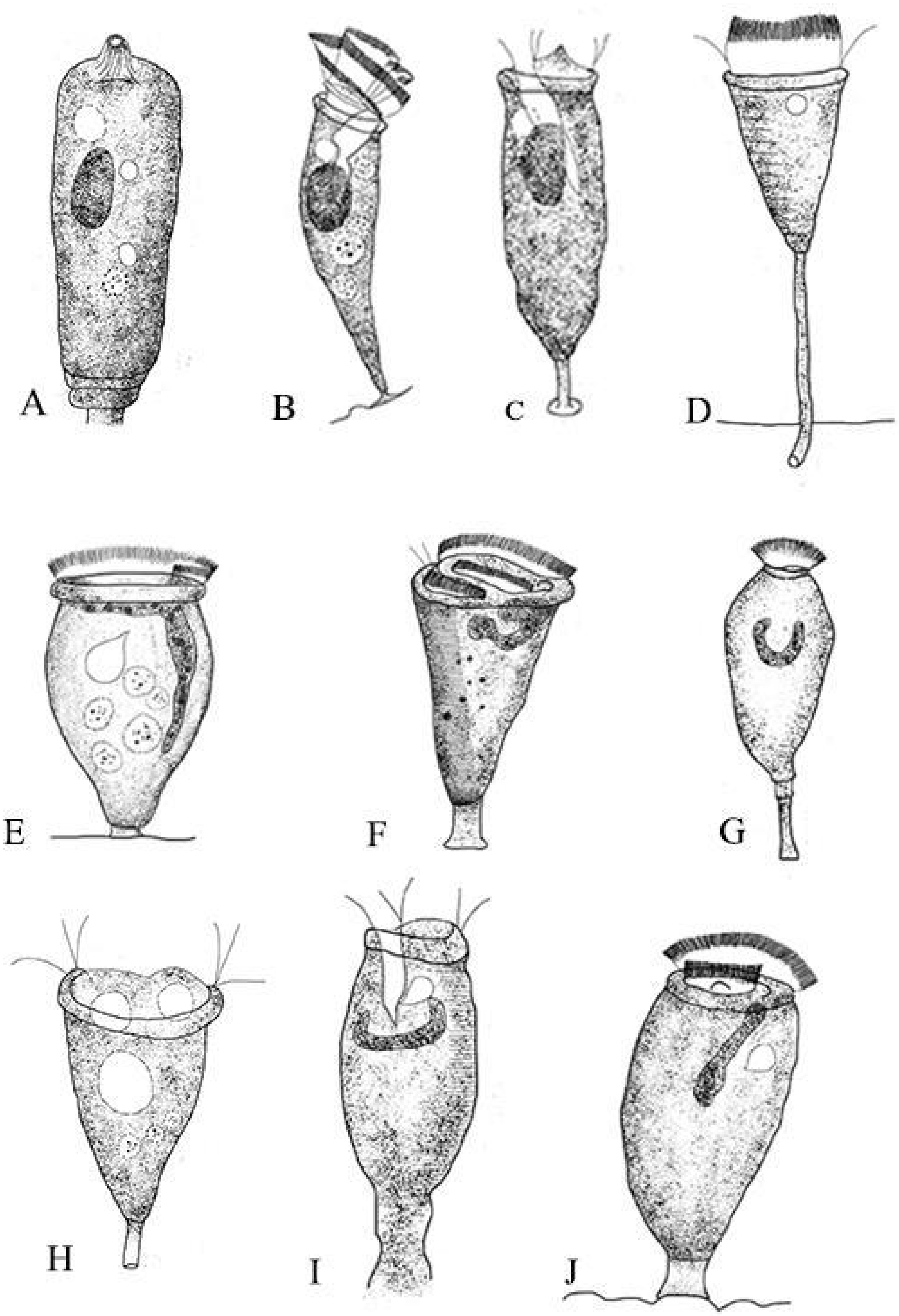
Rhabdostyla kahli Nenninger, 1948

Orborhabdostyla kahli Nenninger, 1948 – Foissner et al., 2010: 293

Diagnosis: Size about 60-70 µm. Peduncle with about 10-14 µm, reaching half its body length. Peristomial collar thick and has a fine fold pattern when contracted. Infundibulum reaches half the length of the body. Contractile vacuole found in the upper fifth of the body to the right of the infundibulum. Oval macronucleus found in the middle of the body on the far right. Found in the final part of the body of the oligochaete *Lumbriculus variegatus.*

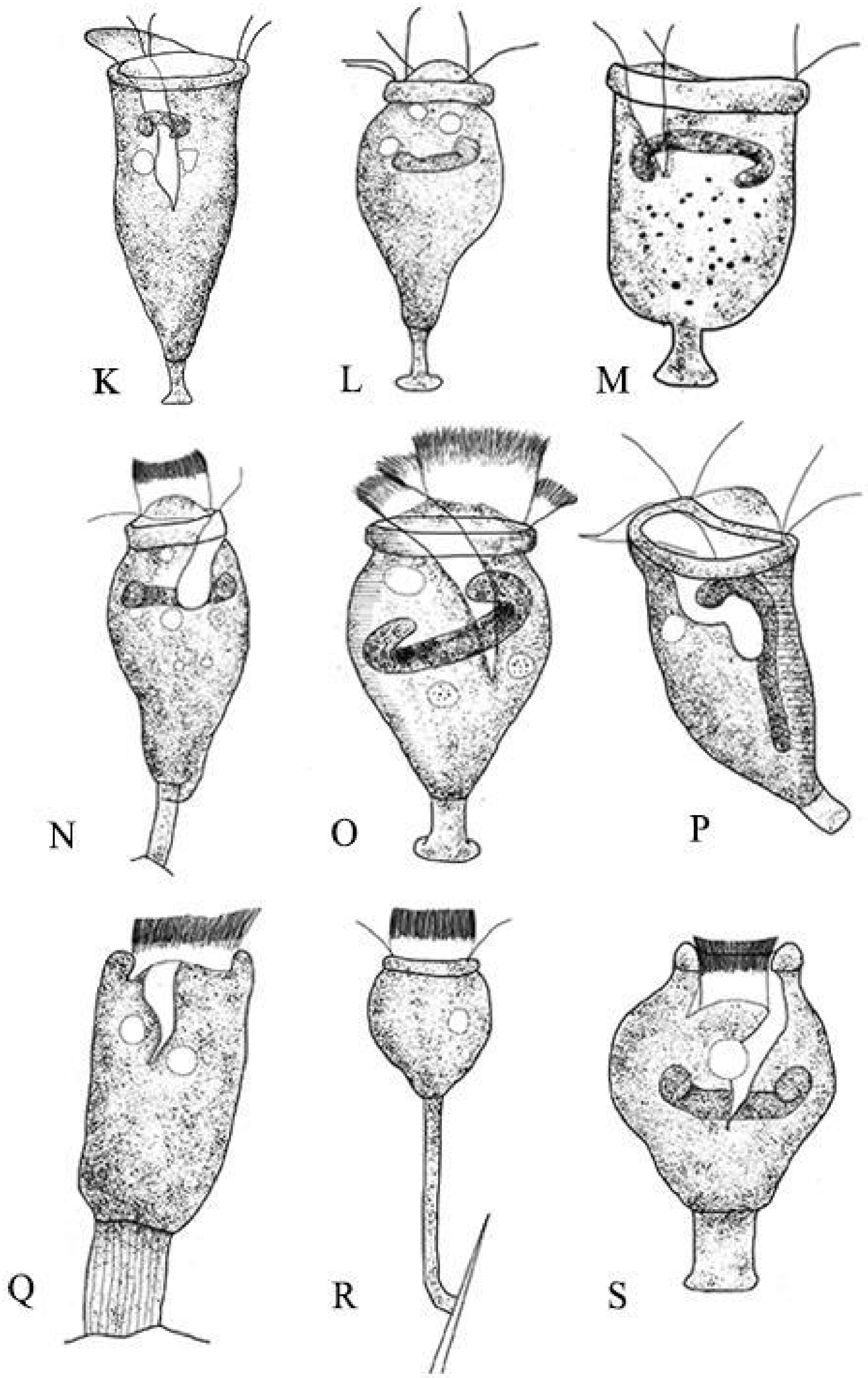
Remarks: diagnosis according to NENNINGER (1948).

Figure 1. Schematic drawing of representatives of the genus *Rhabdostyla* and *Orborhabdostyla*. A: *Orborhabdostyla brevipes*; B: *Orborhabdostyla bromelicola*; C: *Orborhabdostyla kahli*; D: *Rhabdostyla annulate*; E: *Rhabdostyla arenaria*; F: *Rhabdostyla arenicolae*; G: *Rhabdostyla bosminae*; H: *Rhabdostyla volvocis*; I: *Rhabdostyla chironomi*; J: *Rhabdostyla commensalis*.



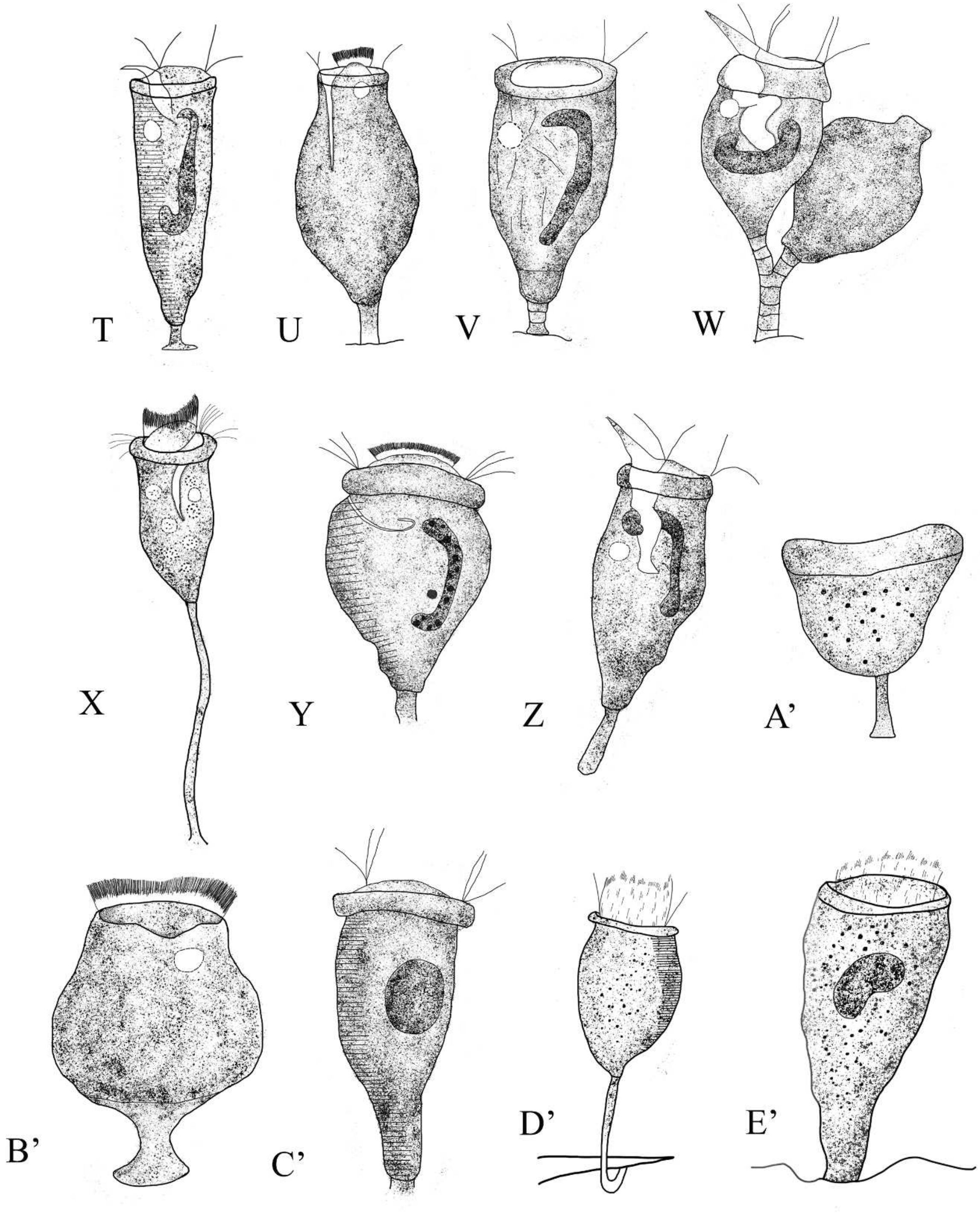
Source: Made by the authors.

Figure 2. Schematic drawing of representatives of the *Rhabdostyla* genus. K: *Rhabdostyla conipes*; L: *Rhabdostyla cyclopis*, M: *Rhabdostyla cylindrica*; N: *Rhabdostyla dixae*; O: *Rhabdostyla dubia*; P: *Rhabdostyla ephemerae*; Q: *Rhabdostyla fromentellii*; R: *Rhabdostyla globularis*; S: *Rhabdostyla hungarica*.



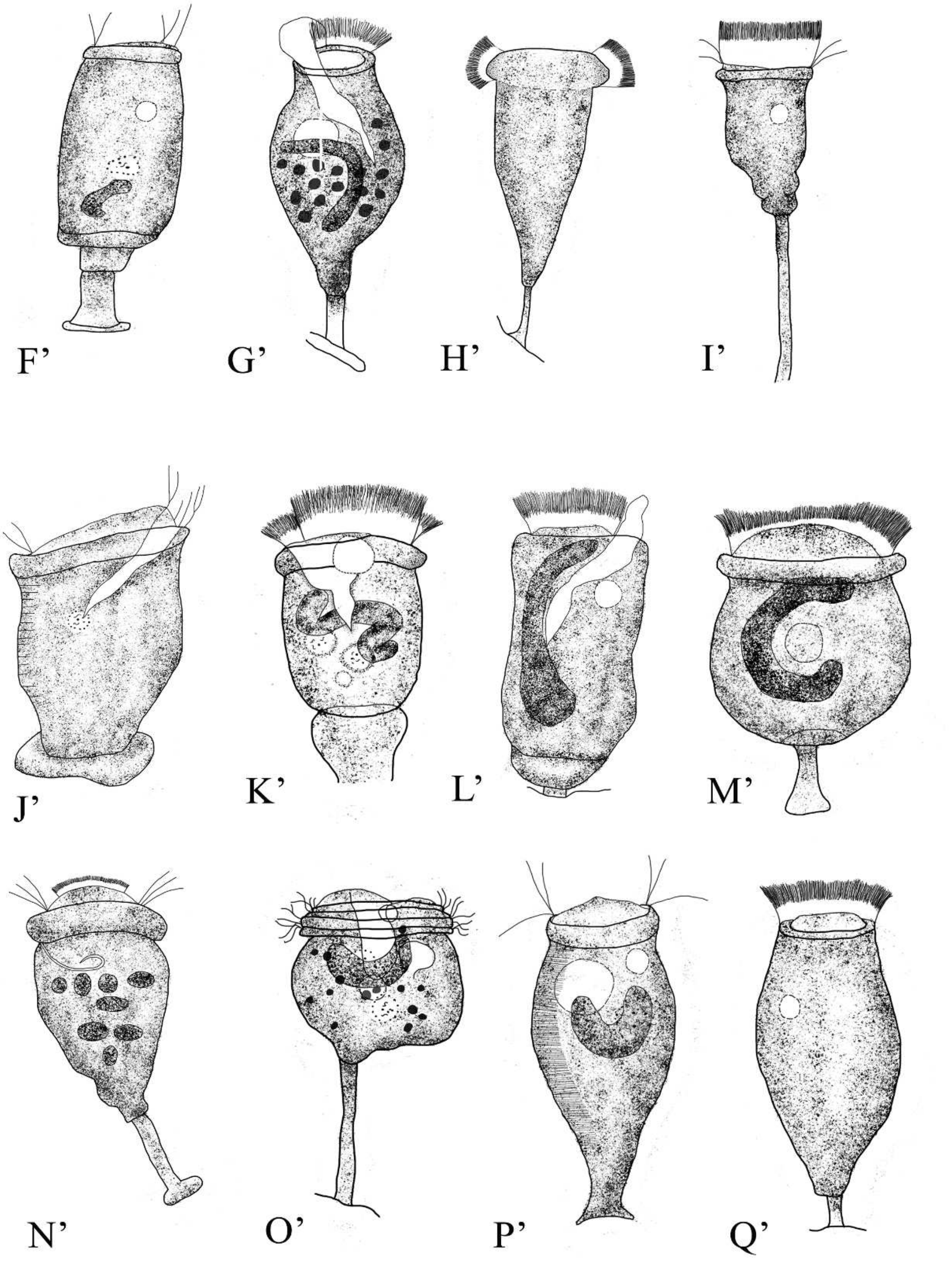
Source: Made by the authors.

Figure 3. Schematic drawing of representatives of the *Rhabdostyla* genus. T: *Rhabdostyla inclinans*; U: *Rhabdostyla invaginata*; V: *Rhabdostyla libera*; W: *Rhabdostyla limnophili*; X: *Rhabdostyla longipes*; Y: *Rhabdostyla mapuche*; Z: *Rhabdostyla muscorum*; A': *Rhabdostyla patula*; B': *Rhabdostyla nebulifera*; C': *Rhabdostyla nereicola*; D': *Rhabdostyla pussila*; E': *Rhabdostyla variabilis*.



Source: Made by the authors.

Figure 4. Schematic drawing of representatives of the *Rhabdostyla* genus. F’: *Rhabdostyla porculus*: G’: *Rhabdostyla pristinis*; H’: *Rhabdostyla rigens*; I’: *Rhabdostyla similis*; J’: *Rhabdostyla scyphidiformis*; K’: *Rhabdostyla scyphoides*; L’: *Rhabdostyla sessilis*; M’: *Rhabdostyla spheroides*; N’: *Rhabdostyla taboadai*; O’: *Rhabdostyla tritora*; P’: *Rhabdostyla tubificis*; Q’: *Rhabdostyla vernalis.*



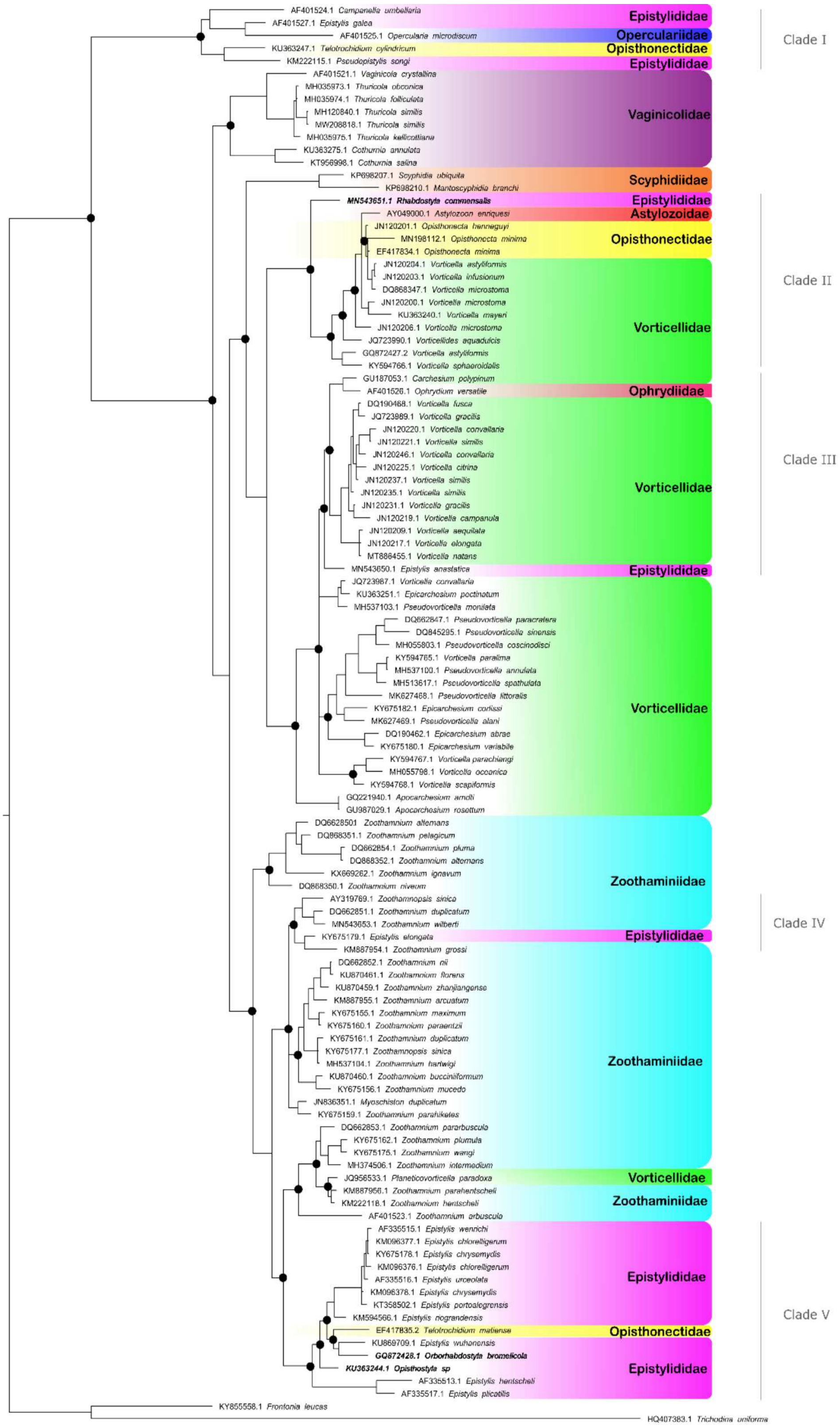
Source: Made by the authors.

2. Molecular phylogeny:

The topologies generated BI analyzes (Figure 1) point to the polyphyly of Epistylididae, Vorticellidae, Opisthonectidae and Zoothaminiidae. Representatives of the family Epistylididae appear in seven positions and in five different clades in the phylogenetic hypothesis retrieved in this study. In clade I, we found three species of the Epistylididae family, *Campanella umbellaria* (AF401524.1), *Epistylis galea* (AF401527.1), *Pseudepistylis songi* (KM222115.1), representatives of the Operculariidae and Opisthonectidae families. In clade II, there are one representative of Epistylididae, *Rhabdostyla commensalis* (MN543651.1), as an external group of a clade that comprise individuals from the Vorticellidae, Astylozoidae and Opisthonectidae families. In clade III, we found only *Epistylis anastatica* (MN543650.1) as representative of the family Epistylididae, it was the sister group of representatives of the families Vorticellidae and Ophrydiidae. In clade IV, we found only *Epistylis elongata* (KY675179.1) as representative of the family Epistylididae, in clade composed only of representatives of the family Zoothaminiidae. Finally, clade V is mostly representatives of the Epistylididae family, except for one representative of the Opisthonectidae family.

A similar scenario is observed with species who are related to the genus *Rhabdostyla*. Rhabdostylids ciliate sequences are grouped at three distinct points on the recovered topology. *Orborhabdostyla bromelicola* (GQ872428.1) is grouped in clade V, among the epistylids representatives, as a sister group of *Epistylis wuhanensis* (KU869709.1). The representative of the genus *Opisthostyla* (KU363244.1) is grouped in clade V as a sister group of the clade that comprises most of the individuals of the genus *Epistylis*. *Rhabdostyla commensalis* (MN543651.1) was grouped in clade II, together with representatives of the Vorticellidae family, specifically as a sister group to the clade comprising individuals from the Astylozoidae, Opisthonectidae and Vorticellidae families.

Figure 5. Bayesian inference (BI) tree based on SSU rRNA sequences. Clades marked with a black dot have more than 70% of support value. All five clades with Epistylididae representatives are demarked by a grey line.



Source: Made by the authors.

Discussion:

The reports and succinct descriptions of rhabdostylid ciliates began in the Age of Discovery and Exploration (CLAMP; LYNN, 2017; FOKIN, 2004) a period that stretched back to the late 1800s, with the taxonomic studies and classifications of KENT (1881), STOKES (1886) and BÜTSCHLI (1889), until the early 1950s, with works by KAHL (1935), NENNINGER (1948) and (LUST, 1950). In the period known as the Infraciliary Era (1950-1970) (FOKIN, 2004; CLAMP & LYNN, 2017) there were some studies with rhabdostylid ciliates (STILLER, 1971), however, descriptions based on information obtained from silver impregnations were only recorded for these ciliates in the 1970s and 1980s (FOISSNER, 1979; SONG, 1986). Thus, in the period of the so-called Era of Ultrastructure (1970-1990), there were no studies on ultrastructure for representatives of the genus *Rhabdostyla*. In the last three decades, although the development of molecular techniques (Era of molecules) has improved the understanding of the evolutionary relationships established between the taxa of the family Epistylididae (MIAO *et al.* 2004; SUN *et al.* 2010; JIANG *et al.* 2016; WANG *et al.* 2017; ZHUANG *et al.* 2018, LU *et al.* 2020), there were few molecular studies involving rhabdostylid ciliates (FOISSNER *et al.*, 2010; LU *et al.*, 2020). In the current so-called Integration Era (CLAMP and LYNN, 2017), studies including rhabdostylid ciliates are increasingly needed, given their enigmatic phylogenetic position in studies with 18S-rDNA, lack of morphological and ultrastructural information, and also its broad relationship with several aquatic metazoans.

The lack of information for ciliates of the genus *Rhabdostyla* also occurs for the other 11 congeners that compose the family Epistylididae that was described in 1935 by KAHL, with the aim of separating individuals, colonial or solitary, with non-contractile peduncle from individuals with contractile peduncle, found in the family Vorticellidae, and individuals without peduncle or with the same almost null, found in the Scyphididae family. The family Epistylididae initially contained 139 species, divided into 5 genera and 6 subgenera. Among the 11 currently valid genera, one is composed of fossil representatives (FOISSNER *et al.*, 2010; LYNN, 2008), and more than 300 species, of which a large part has little taxonomic information, as is the case for rhabdostylids. Of the more than 300 species believed to make up Epistylididae, less than 15% have genetic information deposited and available for analysis, but several works since different genes (such as 18S-rDNA and ITS) until phylogenomics analysis of this material clearly point to merophilia for the representatives of this family (MIAO *et al.* 2004; SUN *et al.* 2010; JIANG *et al.* 2016; WANG *et al.* 2017; ZHUANG *et al.* 2018, LU *et al.* 2020). This shows that possibly the few morphological characters used today to separate the individuals that make up this family do not reflect evolutionary divergence.

Merophilia also occurs in some genera of the family, such as *Rhabdostyla*, which, as well as for the family, can be justified by the several changes made in the history of the genus based on artificial characters. The genus *Rhabdostyla* was initially described by Kent (1881) as representative of the Vorticellidae family, which at the time included solitary or colonial individuals, with or without contractile peduncle and with or without lorica. At the time of the description of the genus Rhabdostyla, there were seven described species, three described by Kent (1881) and four resulting from the recombination of species of the genus *Epistylis*. Kent's (1881) questioning about the proximity of the genera *Rhabdostyla* and *Epistylis* to Vorticella continues to this day in molecular studies (Fig. 1). Stokes (1886) described the genus *Opisthostyla*, closely related to *Rhabdostyla*. Individuals belonging to the genus *Opisthostyla* were characterized by being identical to those of the genus *Rhabdostyla* but with a characteristic body contraction and a fold at the peduncle end, which for the present time would not be enough for this division. In 1889, BÜTSCHLI questions the validity of the genus *Opisthostyla* and suggests the synonymy. BÜTSCHLI (1889) also comments on the possible relationship between *Rhabdostyla* and *Apiosoma*, since, according to him, the only striking difference between the two genera is that *Rhabdostyla* colonizes invertebrate basibionts and *Apiosoma* colonizes vertebrate basibionts. After 46 years and 12 new species described for *Rhabdostyla* and one for *Opisthostyla*, KAHL, in 1935, created the family Epistylididae, inserting the genus *Rhabdostyla* in this new family and describing another 5 new species for this genus. KAHL (1935) accepts part of BÜSTCHLI's (1889) suggestion and appoints *Opisthostyla* as a subgenus of *Rhabdostyla*. The separation between the subgenus was given by the fold at the end of the peduncle, peduncle size and body contraction behavior. Unlike the work of BÜSCHLI (1889), the change proposed by KAHL (1935) is widely accepted. After 36 years only 5 species were described until 1971. STILLER (1971) recombine both of *Rhabdostyla* subgenus as subgenus of *Epistylis*, this recombination was not accepted by the academy, and *Rhabdostyla* still was treated as a valid gender. From 1971 to 2001, next change about the gender *Rhabdostyla*, which 3 species were described (FOISSNER, W., 1979; NENNINGER, 1948; RIGHI, 1973; SONG, 1986). AESCHT (2001) by reviewing valid Ciliophora genera pointed out the full synonymy between *Rhabdostyla* and *Opishtostyla*, which is corroborated a few years later by the review work of LYNN (2008). In recent studies citing rhabdostylids (ÁLVAREZ-CAMPOS *et al.* 2014; CABRAL *et al.* 2010; DIAS *et al.* 2007; FOISSNER *et al.* 2009), only one cites Opisthostyla (ÁLVAREZ-CAMPOS *et al.*, 2014), but emphasizes in recombination. However, we did not find a study in the literature that reviews the combination of species from the old genus *Opisthostyla* to the genus *Rhabdostyla*, as we pointed out in this study. FOISSNER *et al.* (2010) suggest the creation of a new genus for species of the genus *Rhabdostyla* with round or oval macronucleus and transverse-striated silverlines, this genus was named *Orborhabdostyla* and was composed of 3 species, *Orborhabdostyla bromelicola*, *Orborhabdostyla brevipes* and *Orborhabdostyla kahli*. The proposal by FOISSNER *et al*. (2010) was accepted in the scientific community and *Orborhabdostyla* came to be considered a valid genus.

Although the validity of the genus *Orborhabdostyla* needs to be proven using the tools available in the Age of Integration (WARREN *et al*. 2017; LYNN; CLAMP 2017), we do not refute the genus as future molecular data will further elucidate this issue. The main morphological characters used in rhabdostylid diagnosis are: individual size; body shape; peduncle size; shape of the macronucleus and host. Some of these characters are extremely susceptible to environmental influence and do not seem to reflect the evolution of the group (CABRAL *et al*. 2010; ZHUANG *et al*. 2018). Although the drawings of most species show a great difference between them (Figures 2-4), the diagnoses and morphological characters do not show this difference so clearly.

The main characteristics available for valid rhabdostylid species have been compiled (Table 1), this compilation highlight the lack of standardization of descriptions and the few morphological data currently available. An example that highlights the lack of robust information for the separation of rhabdostylids occurs between the species *Rhabdostyla arenaria* and *R. arenicolae*. These species have similar body size (40-56 µm x 45-60 µm), and relative plasticity in body shape, but with very similar feature. Although the size of the peduncle varies among species, it is known that the peduncle varies according to the life cycle/ontogenesis of the different environments that they colonize, therefore, it is not suitable for separation between closely related species. Information on the peristomial apparatus, peristomial collar, peristomial disc, and infundibulum is not detailed for any of the species. There is only information about infundibulum for *R. arenaria*. As for information on the vacuole and macronucleus, there is only information for *R. arenaria* and *R. arenicola*. One of the only information presents for all 2 species is the basibiont host, *R. arenaria* is described as an epibiont of echinoderm individuals, *R. arenicola* is described on annelids of the genus *Arenicola*. As it is a characteristic present for almost all individuals of the genus, and the genus is considered an epibiont, this characteristic is

Table 1. Comparative table with morphologic characters for the 41 species of the genus *Rhabdostyla* and three of the genus *Orborhabdostyla.*

Species	**Length (µm)**	**Width (µm)**	**Body Shape**	**Peduncle**	**Peristomial collar**	**Peristomial disc**	**Infundibulum**	**Contractile Vacuole**	**Pellicle**	**Macronucleus**	**Habitat**	**Host**
Rhabdostyla annulata	22-35	-	Conical-bellied	Same size of the body length, with a slight curve in the distal portion	Revoluted, with two ciliary circles	-	With the third of the body's length	In the right margin of the infundibulum, in the upper quarter of the body	Strongly striated transversely	Thin and horseshoe-shaped	Fresh water	Algae
Rhabdostyla arenaria	40-56	-	-	10 µm long, ringed	-	-	Very long	Wide, with a distinct excretory channel	-	Elongated band-shaped, with small chromatin agglomerations	Marine	Echinoderms
Rhabdostyla arenicolae	45-60	-	Conical, wider on the front	With half of body length	-	-	-	In the anterior region	Transversely striated	Cylindrical , with torsion close to the peristomial region	Marine	*Arenicola sp.*
Rhabdostyla bosminae	38-44	18-20	-	With half of body length, in rare cases same body length	-	Is about half the width of the body	-	-	Transversely striated	Horseshoe shaped	Fresh water	Cladocerans of the genus *Bosmina*
Rhabdostyla chironomi	60	-	Similar to *R. commensalis*	Ringed, twice as short as wide	-	-	-	-	-	-	Fresh water	*Chironomus*
Rhabdostyla commensalis	62-70	-	Elongated barrel	Very short, smooth and with a wide base	-	Elevated	-	-	Gently striated	Small and cylindrical	Marine	Polychaetes
Rhabdostyla congregata	38-40	30	Similar to Epistylis	08-12µm long	-	-	-	One or two	Smooth	Horseshoe shaped	-	Rotifers and Crustacean
Rhabdostyla conipes	40-46	-	Slightly wider in the anterior region	Short ,6 µm long, and straight	-	Wide, almost flat	-	-	Weakly striated	In the shape of a small sausage, curved backwards at one end	Fresh water	*Daphinia*
Rhabdostyla cyclopis	30	-	Vase-like	Short, with a expasion at the insertion of the body	Very thick	Convex, with no bulge	One third of the body length	Below the peristomial collar	-	Horseshoe shaped	-	Cladocerans, copepods and ostrcodes
Rhabdostyla cylindrica	25-28	18-20	Robust, with rounded back	With one-sixth the height of the body, thick and striated, its posterior end ending in an adhesive disk	Slightly thickened	Flat, with almost no bulge	Narrow, reaches a third of the body	On the left side of the infundibulum	Delicately striated	Elongated and horseshoe shape	Fresh water	*Lepdotora kindti*
Rhabdostyla dixae	48.8-55	-	Similar to a jar, strongly protruding in the frontal third	Small, with 10-11 µm to about 1/3 of the body length, and quite thin	With 2/3 of the width of the body, it is simple, similar to a collar, notched	Wide and arched, with a pronounced annular groove at its base.	1/3 of the body length, which widens to a spherical shape in the lower portion	At the lower left end of the infundibulum	Striated at regular intervals	Tape-shaped with folds at the ends	Fresh water	Larvas de *Dixa nigra*
Rhabdostyla dubia	30-35	-	From vassel to funnel	With 2-5 µm, with transverse striations and grooves, usually enlarged at the base	-	-	wide and quite short, reaching the final third of the animal	Positioned just below the peristomial collar, on the ventral wall of the vestibule	Transversely striated	Horseshoe shaped	-	Ostracods
Rhabdostyla ephemerae	75	-	Variable shape, not very asymmetric	Short and thin	Robust	Oblique, with distinct protuberance in the margin	Broad	Positioned in the upper quarter of the body, to the right of the infundibulum	With narrow striations	-	Fresh water	Ephemeroptera
Rhabdostyla fromenteli	80	-	Elongated-claved	With longitudinal striations	-	-	-	Two or three	Smooth	-	Fresh water	-

Rhabdostyla fromenteli	80	-	Elongated-claved	With longitudinal striations	-	-	-	Two or three	Smooth	-	Fresh water	-
Rhabdostyla globularis	25-36.6	-	Subglobe but changeable, a little longer than wide	Slightly larger than body length	Revolute	Not elevated	-	-	Transversely striated	-	Fresh water	*Hydrodictyon utriculatum, Bosmina longirostris* e *Diaphanosoma brachyurum.*
Rhabdostyla hungarica	35-40	-	Usually pear-shaped, but very variable	Short, 10-15 μm long, and plump	Quite thick	Flat, convex and sunken, that is, it does not protrude beyond the peristomial collar	Slightly larger than half of the body length	To the right side of the infundibulum	Finely striated	Horseshoe shaped	-	*Lepdotora*
Rhabdostyla inclinans	50-77	-	Cylindrical, twice as long as wide, tapered in the posterior quarter, somewhat ventrally inclined and dorsally convex	Cylindrical, slightly curved with adhesive disc	Short and not too thick peristomial collar, slightly projected.	Small and arched, slightly prominent	Centrally positioned, reaching the back of the body	Next to the infundibulum, immediately below the peristomial collar	Very thin transversely striated	Long and cylindrical	Fresh water	*Nais* and *Lumbriculus* oligochaetes
Rhabdostyla invarginata	35	-	Vasiform, rarely gibbus, twice as wide as long	1/3 to 2/5 of body length	Not detached, with a prominent ciliary disc, conical, with two ciliary circles	Flat	-	Near the peristomial region	Distinctly ringed	-	-	Ostracods of the genus *Cypris*
Rhabdostyla libera	60	-	Tapered, similar to Vorticella	Short with folds	-	-	-	-	Longitudinal and transverse striated, saw only on the posterior end	Longitudinally inclined	Marine	Algae
Rhabdostyla limnophili	55	-	Oval	With two-thirds the size of the body, ringed, sometimes with an unoccupied lateral branch	Elevated	-	-	-	-	Transversely inclined	Fresh water	*Trichoptera*
Rhabdostyla longipes	70	-	Elongated to bell-shaped	Thin and sinuous, 2 or 3 times larger than body length	-	-	-	-	Smooth	-	Fresh water	-
Rhabdostyla mapuche	22-31	16-19	Globular to spherical	Short, 3-6 μm long, and thick, 4-6 μm wide, with longitudinal sinuation	-	With 20-23 μm in diameter, and with 9-14 ciliary bands.	-	-	Distinctly transversely striated	C-shaped	Marine	Polychaetes
Rhabdostyla muscorum	40	-	Elongated vessel, with two grooves in the basal part	Thin, with 20 μm to about 1/3 of body length	Thick	Flat and low	Reaches half of the body length	On the right side of the infundibulum, in the upper third of the body	-	Elongated, with a fold at the top	Infusion of mosses	-
Rhabdostyla nebulifera	125	-	Subglobus, truncated anteriorly	Short, with 1/2 of body length, with widening in the distal part and with fixation disk	-	-	-	-	Smooth	-	Fresh water	-
Rhabdostyla nereicola	55-62	-	With the thinnest end	Short	Irregular	-	-	-	Transversely striated	Straight and concave towards the interior of the cell	-	*Nereis dumerili*
Rhabdostyla patula	1/30"	-	Bell-shaped	With half of body length	-	-	-	-	-	-	-	-
Rhabdostyla porculus	135-155	-	Broad and cylindrical	Cylindrical, with 30-35 μm and adhesive disc	-	Wide, almost flat, but with projection at its highest point	-	Very wide, at the base of the peristomial collar, to the right of the infundibulum	Milky, with little distinct striations	Broad, relatively little elongated macronucleus, rarely "S" shaped	Animal debris	-
Rhabdostyla pristinis	37	48	-	Long, with 1/3 or 1/4 of the body length, with 4 or 5 transversal appendices	-	Narrow and flat	Reaches half of the body length	One, near the infundibulum	Transversely striated	Spiral	Fresh water	*Pristina minuta*
Rhabdostyla pusilla	22.5-25	-	Bell-shaped or pear-shaped, thinning posteriorly	Slightly larger than the body, with curved distal end	Revolute	-	-	-	Cross-streaked	-	Fresh water	*Ceratophyllum*

Rhabdostyla ringens	80	-	Conical and elongated, with a length about twice of the width	Short, about 1/3 of the body lenght	-	-	-	-	Smooth, with a brownish color	-	Fresh water	-
Rhabdosytla similis	22.5	-	Subvasiform, , somewhat mutable in the posterior part	Slightly longer than the body, its distal end slightly curved	Revolute	Slightly and obliquely elevated	-	-	Strongly cross-striated	-	Fresh water	Floating plant roots
Rhabdostyla scyphidiforme	60-100	25-34	-	With 64μm to body length, the lower end widens into an adhesive disk	Slightly thickened and slightly wider than the body	Wide, almost flat, slightly sloping, sometimes with small bumps on top	Diagonally positioned, with one third of the body length	Below the peristomial collar, to the right of the infundibulum	Finely striated	Curved	Fresh water	Tadpoles, *Rana esculenta*
Rhabdostyla scyphoides	35-44	24-27	Cylindrical body	Very short, about a third of the body's length, with a widening in the proximal portion	-	Flat	-	In the apical part of the cell	Smooth	S shaped	Fresh water	-
Rhabdostyla sessilis	43-45	-	Elongated, cylindrical or vaguely urceolate, with a more roudend back	Extremely short, almost invisible, apparently consisting only of the adhesive disk.	-	Relatively wide, very low, with an almost flat surface	Large and deep, reaching the middle of the body	Very large, positioned just below the vestibulum	With very thin and indistinct striations	Very light, sausage-shaped, strongly thickened at its posterior end	Fresh water	Copepods of the genus *Cyclops*
Rhabdostyla spheroides	60	-	-	Robust, measuring about half the size of the body and enlarged proximally	-	With convex elevation when expanded, elevation almost forming a sphere	Curved and elongated	-	Smooth	-	Fresh water	-
Rhabdostyla taboadai	30-45	15-23	Elongated globular body, with a rounded and slightly protruding anterior extremity.	With 14-17 μm in length and 7-9 μm in width and has a longitudinal twist, with an adhesive disk 8-12 μm in diameter.	-	Approximately 26-29 μm in diameter, with 11-18 ciliary bands	-	-	Distinctly transversely striated	Multilobulated	Fresh water	*Syllis prolifera*
Rhabdostyla tritora	180	200	Almost spherical	With 1/6 of the body length	Divided into 3 thin layers	Surrounded by two ciliary chains	Almost completely straight, reaches halfway up the body	At the end of the infundibulum	-	Worm shaped, thick and curved	Marine	*Helobdella stagnalis e Ceratophyllum*
Rhabdostyla tubificis	60	-	Vessel-like, that gradually decreases towards the peduncle	Extremely short, with adhesive disc	Moderately thick	Slightly arcuate and bifurcated	-	Below the peristomial collar	Clearly striated	Broad and horseshoe-shaped	-	*Tubifex*
Rhabdostyla variabilis	60-90	-	-	Very short	Same width of the largest width of the body	Prominent	-	-	-	Kidney-shaped	-	Polychaetes
Rhabdostyla vernalis	48-50	20	Urceolate body, often gibbous, and elongated, twice as long as it is wide	Short, about 8-10μm at 1/8 of body length	Slightly slanted, not revolting	elevated and convex, occasionally developing a central and conspicuous nipple-like projection.	Reaches the half the body	In the anterior half of the body	Finely transversely striated	In the form of a "hand", curved	Fresh water	*Cypris e Cyclops*
Rhabdostyla volvocis	48.8-49.3	-	Funnel-shaped	Approximately 10 μm	Thick, snail-like that expands towards the entrance of the infundibulum	Slightly arched	Short	Below the peristomial collar	Thin, with thickened striations	V-shaped	-	*Volvox globator*
Orborhabdostyla brevipes	80-90	-	Subcylindrical body, attenuated, about three times longer than wide	Extremely short, 1/10 of body length	-	-	-	-	-	Oval	Fresh water	Dipterous larvae
Orborhabdostyla bromelicola	65	15	Tapered or closely tapered	Usually less than 10 μm long and 2 μm wide	-	Slightly designed, average diameter about 10 μm in vivo, imperceptible to distinctly convex, never umbilised	Reaches half of the body length	In the dorsal wall of the infundibulum	Striated	Usually globular, but may be ellipsoidal	Bromeliads	Without hosts
Orborhabdostyla kahli	60-70	-	-	With 10-14μm to half of the body length	Thick and with a fine fold pattern when contracted	-	Reaches half of the body length	In the upper fifth of the body, to the right of the infundibulum	-	Oval	-	*Lumbriculus variegatus*

Source: Made by the authors.

usually treated as of great taxonomic importance for the group, but as there is no robust data on the specificity of rhabdostylids and their hosts, data of the host are complementary in diagnosis.

The epibiotic relationship is an optional ecological relationship between two organisms, basibiont and the epibiont, during at least one phase of their life cycle (DIAS *et al.,* 2007). As seen in the epibiontial habit of rhabdostylids, some ecological studies were carried out involving ciliates and dipteran hosts (DIAS *et al.* 2007; CABRAL *et al.* 2010; CABRAL *et al*. 2018) and oligochaetes (DIAS *et al.*, 2009). These studies registered a potential correlation between the prevalence of rhabdostylid infestation and the high concentration of organic pollution in lotic systems in Brazil, which raises the potential use of these ciliates as bioindicators. The vast majority of relationships of rhabdostylids individuals occur with annelids (oligochaetes and polychaetes), crustaceans, and insect larvae in specific sites of the basibiont body (ABRAHÃO *et al.*, 2017; CABRAL *et al.*, 2010a; 2018b; CORBI *et al.*, 2016; DIAS *et al.*, 2009, 2010; SMITH, 1986). Many species have been described as being specific to a given host species, such as the species *R. chironomi* and *R. arenicolae*. However, most of the species were reported only in the description, and there are no further records, the scenario on host specificity for these ciliates still needs in-depth studies. This fact, added to the fact that the life cycle of these individuals allows them to colonize several substrates, alive or not, raises the question whether this characteristic is really one of the best to be analyzed in order to build a taxonomy that reflects the evolution of the group. Complete life cycle studies of these individuals, in addition to phylogeny and macroevolution studies analyzing these characteristics, can provide a better understanding of which path to take for the taxonomic classification of rhabdostylid individuals.

The immense lack of morphological, ecological and molecular information is common for most species of the genus *Rhabdostyla* and *Orborhabdostyla*, with only three, out of 45 valid, with more complete morphological information, with analysis of the oral infraciliature and silver impregnation techniques. Due to this limitation of information, the correct identification of the different species and a better discussion about their validity becomes much more difficult, and for this reason we have maintained as valid all the species described for *Rhabdostyla*, *Opisthostyla*, full recombined here to *Rhabdostyla* according to the literature, and *Orborhabdostyla* not recombined to other genera. To solve all these problems, such as the lack of standardization of diagnoses and the lack of description of characters said to be more reliable, such as the number of silverlines and description of the oral infraciliature (WARREN *et al.*, 2017) a great taxonomic effort is needed, with the carrying out of more collections, complete re-descriptions, carried out by specialists, and ecological studies of distribution, life habits, and epibiotic relationships.

Another important piece of information that can help to clarify some doubts about the group, as they have done for other groups within Ciliophora (FOISSNER *et al.*, 2004; HUANG *et al.*, 2018; PETRONI *et al.*, 2002; VĎAČNÝ *et al.*, 2011; ZHANG *et al.*, 2014), are the phylogenetic analyses. Currently, there are only three sequences related to rhabdostyloid individuals, and only two are identified at a specific level, they are: *Orborhabdostyla bromelicola* (GQ872428.1), *Opisthostyla* sp. (KU363244.1) and *Rhabdostyla commensalis* (MN543651.1). Although these sequences have been available for some time, our work is the first to reconstruct the phylogeny of the order Sessilida containing the three rhabdostylid representatives and compare them with other species of Epistylididae available in molecular databases. Our results revive old uncertainties about genders and question some of the relationships cited in the literature. Example of these uncertainties are the relationship between rhabdostylid individuals and the genera *Vorticella* and *Epistylis*, as representatives of *Opisthostyla* and *Orborhabdostyla* were grouped into branches of the family Epistylididae, which despite being clearly polyphyletic (SUN *et al.* 2010; JIANG *et al.* 2016; WANG *et al.* 2017; ZHUANG *et al.* 2018, LU *et al.* 2020) is still considered valid because there are not enough morphological characteristics for a coherent separation. We can clearly observe the polyphilia when analyzing the position of representatives of each genus in the tree. The groups that seem to be most related are *Opisthostyla* and *Orborhabdostyla*. The sequence of *Opisthostyla* sp. was positioned as a sister group of the branch where the sequence of *Orborhabdostyla bromelicola* together with individuals of the genus *Epistylis* and a representative of the family of Opisthonectidae were found. The sequence of *Rhabdostyla commensalis*, appears in a more distant branch, as a sister group of a branch formed by individuals representing the families Astylozoidae, Opisthonectidae and Vorticellidae, and the latter two are also not monophyletic. These results corroborate the hypothesis raised above, that the genus *Rhabdostyla*, and others related to it, are not monophyletic, a fact that can possibly be justified by the turbulent history of the genus and the various changes imposed on them. It is worth mentioning that the different classifications are based on characters that are not divergent from an evolutionary point of view, which calls attention to a generic restructuring based on molecular analysis. However, due to the low number of representatives and the lack of specific epithet for the sequence of the genus *Opishtostyla*, no more abrupt changes will be proposed, we will maintain the conclusions generated by the analysis of the literature and morphology of individuals, that is, the synonymy of *Rhabdostyla* with *Opisthostyla* and the separation of the genus *Orborhabdostyla*. But these results make even more evident the need for morphological recharacterization and molecular characterization of these individuals that make up these genera for a better understanding of these relationships.

Despite the various uncertainties about the rhabdostylid genera, its importance and great diversity is unquestionable. There are 45 valid species so far, with a very wide distribution and a wide range of ecological relationships to be explored. The epibiosis within the group seems to be a very important factor and by studying these genera we can understand a little better how these phenomena work and how the morphology and physiology of the ciliates is altered when they become specialized in this type of interaction. To carry out these studies, improvements in the taxonomy of the group will be necessary, which can be carried out with new collections and re-descriptions of the species using ciliatological techniques, such as silver impregnation, electron microscopy and analysis of the oral infraciliature, as well as molecular characterization, which can help us help to better understand, and may even remedy the polyphilia discovered and pointed out in this work. The general taxonomy of Peritrichia has been widely discussed (MIAO *et al.*, 2004; SUN *et al.,* 2010; ZHUANG *et al.*, 2018). According to the literature, the characters that are used to separate the groups that make up this subclass do not reflect the evolutionary divergence of the group and, of course, the same happens for rhabdostylid individuals. This scenario demonstrates how much a great taxonomic effort is needed to resolve these issues within the group and with this perhaps we can better understand the evolution of these organisms and the relationship of diversity with the taxonomic characters used today. With this information, propose a more correct and complete taxonomy, which really reflects the evolution of the group, which will facilitate the development of several other works, besides possibly raising several other questions to be investigated.

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Chapter 3:

The relation of environment and the subclass Peritrichia: A phylogenetic signal and ancestral state investigation

Resumo:

A subclasse Pertrichia é uma das maiores e mais diversas subclasses de Ciliophora, possui mais de 1000 espécies descritas que podem ser encontradas em diversos ambientes como água doce, água salobra, água salgada e solo. Devido a essa grande diversidade de ambientes que os peritríquios habitam o interesse de investigar a existência de padrões ecológicos e sua relação com a evolução do grupo tem crescido. Poucos trabalhos até o momento realizaram estudos sobre esse tópico, sendo assim muitas lacunas ainda existem quanto a relação do ambiente com a diversidade e evolução de Peritrichia. Com o objetivo de tentar entender melhor a relação do ambiente que os organismos peritriquios habitam com sua evolução, o presente trabalho realiza uma investigação sobre a existência de sinal filogenético para essa característica ecológica e ainda realiza uma análise de reconstrução do estado ancestral para tentar entender ainda melhor como a evolução dessa característica dentro da subclasse. Como resultado foi encontrado forte sinal filogenético para o ambiente e ainda foi constatado que provavelmente os ancestrais da subclasse Peritrchia habitaram ambientes de água doce e que a conquista de novos ambientes aconteceu mais recentemente.

Palavras-chave: Macroevolução, 18Sr-RNA, Ciliados, Mudança de ambiente, História evolutiva.

Abstract:

The Peritrichia subclass is one of the largest and most diverse subclasses of Ciliophora, it has more than 1000 described species that can be found in different environments such as freshwater, brackish water, salt water and soil. Due to this great diversity of environments that peritrichous individuals inhabit, the interest in investigating the existence of ecological patterns and their relationship with the evolution of the group has grown. Few works to date have carried out studies on this topic, so many gaps still exist regarding the relationship between the environment and the diversity and evolution of Peritrichia. With the objective of trying to better understand the relationship between the environment that peritrichids organisms inhabit and their evolution, the present work carries out an investigation into the existence of a phylogenetic signal for this ecological characteristic and also performs an analysis of ancestral state reconstruction to try to understand further better as the evolution of that characteristic within the subclass. As a result, a strong phylogenetic signal was found for the environment and it was also found that probably the ancestors of the subclass Peritrichia inhabited freshwater environments and that the conquest of new environments happened more recently.

Key-words: Macroevolution, 18Sr-RNA, Ciliates, Change of environment, Evolutionary history.

Introduction

The phylum Ciliophora is one of the main groups of eukaryotic microorganisms with about 8000 described species (LYNN, 2008). Among the ciliates, the subclass Peritrichia constitutes a very diverse group, with more than 1200 described species, found all over the world and in a wide variety of environments, such as freshwater, brackish water, salt water, soil, bromeliad ponds and association with several animal groups. (FOISSNER *et al.*, 2010; WANG *et al.*, 2022). The subclass is divided into two orders, Sessilida and Mobilida, both orders very peculiar when compared to the other Ciliophora groups. Sessilids are characterized by having the scapula as a stalk-producing structure that can adhere to various substrates, while mobilids are characterized as ectoparasites that temporarily adhere to the host by means of an adhesive disk (LOM, 1966; LYNN, 2008).

Although the monophyly of the Peritrichia subclass is recognized, the internal phylogeny based on molecular data has many inconsistencies with traditional systematics for the group (CLAMP; WILLIAMS, 2006; LIAO *et al.*, 2018; SUN *et al.*, 2016; WU *et al.*, 2020; ZHUANG *et al.*, 2018). There is still little macroevolutionary information, correlating ecological/environmental parameters and evolution of Peritrichia (COSTA *et al.,* 2021; SUN *et al.*, 2016). Due to the large number of ecosystems and microhabitats that peritrichids inhabit, investigations into these ecological patterns and their relationship to the evolution of the group are a contemporary and fertile topic, that hasn't been covered much yet (SUN *et al.,* 2016).

Sun *et al.* (2016) carried out one of the few studies that investigated the existence of ecological patterns in the evolution of the Peritrichia subclass. The authors detected sudden evolutionary transitions of these ciliates from freshwater to brackish/marine/hypersaline environments using ancestral state reconstruction analysis using Mesquite software (MADDISON; MADDISON, 2007) which is based on the parsimony method. The low sampling for molecular data from representatives of the Peritrichia subclass (WU *et al.*, 2020), the oversampling of few groups (eg, genus *Vorticella*) and the need to use new methodological models for macroevolutionary investigations (e.g. phylogenetic signal) (DIAS *et al.*, 2021) emphasize the need for future evolutionary investigations for the group.

In order to expand the knowledge about the evolutionary dynamics of transitions of the type of environment for representatives of the subclass Peritrichia, the present study carried out an investigation into the existence of a phylogenetic signal for the type of environment in Peritrichia, and also carried out a new analysis of reconstruction of the ancestral state for the characteristic type of environment based on the maximum likelihood phylogenetic reconstruction method and with a broader and updated dataset, containing several new representatives.

Material and methods

1. Phylogenetic analyzes and molecular dating

For molecular investigations on the subclass Peritrichia, we used a dataset composed of short sequences, with more than 1000bp, of 18S-rDNA from ciliates of the class Oligohymenophora compiled from the curated EukRef database (DEL CAMPO *et al.*, 2018) in November 2021. Additional sequences of Oligohymenophora were included in the database due to the presence of primary fossils in this group that were used to build a more robust ultrametric tree for phylogenetic signal analysis and ancestral state reconstruction. Sequences identified at the species level of individuals belonging to the Colpodea, Phyllopharyngea, Plageopylea and Prostomatea classes were used as an outgroup, totaling a dataset with 493 sequences. The dataset was aligned using the MAFFT software (KATOH; STANDLEY, 2013) with the default settings, and, later, the GBLOCKS software (CASTRESANA, 2000) was used to remove the poorly aligned sites, obtaining a matrix with 1551 base pairs (bp). For the inference of the phylogeny, a maximum likelihood analysis (ML) was performed using the RAxML program (STAMATAKIS, 2014). The model used was GTR + GAMMA + I, which was chosen as the best replacement model by the JModelTest program (POSADA, 2008), implemented by the MEGA-X platform (KUMAR *et al.*, 2018). The support of the branches by bootstrap was estimated by the RAxML program, through the autoconvergence criterion, based on 400 pseudo-replicas.

For the estimation of divergence time in Ciliophora, the RelTime software implemented by the MEGA X program (KUMAR *et al.*, 2018) was used, which has shown high performance in large data sets. The fossil species and the secondary time calibration used, as well as their respective minimum and maximum ages, are shown in Table 1. After estimating the divergence time, the Peritrichia clade was extracted using the "extract.clade" function of the ape package (PARADIS; SCHLIEP, 2019) in the R environment (R CORE TEAM, 2021) generating a topology composed of 294 sequences.

Table 1. Fossil records and secondary dates used for node calibration in the Maximum Likelihood (ML) phylogeny of the phylum Ciliophora. The ciliate fossils were recorded in amber and the ages represent the minimum and maximum limits of paleontological dating. For Sessilida the mean of minimum and maximum data was utilized as minimum.

Species	Minimum age (My)	Maximum age (My)	Reference
Paramecium triassicum	93	99	Schönborn et al., 1999 corrected by Schmidt et al., 2001
Sessilida	204	-	Costa et al., 2021
Vorticella sp.	200	-	Bomfleur et al., 2012

Source: Made by the authors.

2. Reconstruction of the ancestral character state

For the analysis of phylogenetic signal and ancestral state reconstruction, the environment classification taken from the cured EukREF database (DEL CAMPO *et al.*, 2018) and standardized in four characters states, that were: (a) Freshwater; (b) Brackish Water; (c) Saltwater; and (d) Soil. For the inference of the environment in which the ancestors of Peritrichia lived, the topology containing only representatives of Peritrichia (n= 247) was used together with the package Phytools v. 1.0-1 (REVELL, 2012) implemented by the R platform (R CORE TEAM, 2021) and has its methodology based on the maximum likelihood method. For this, we use the “ace” function, with discrete data and an “ER” model.

3. Phylogenetic Sign

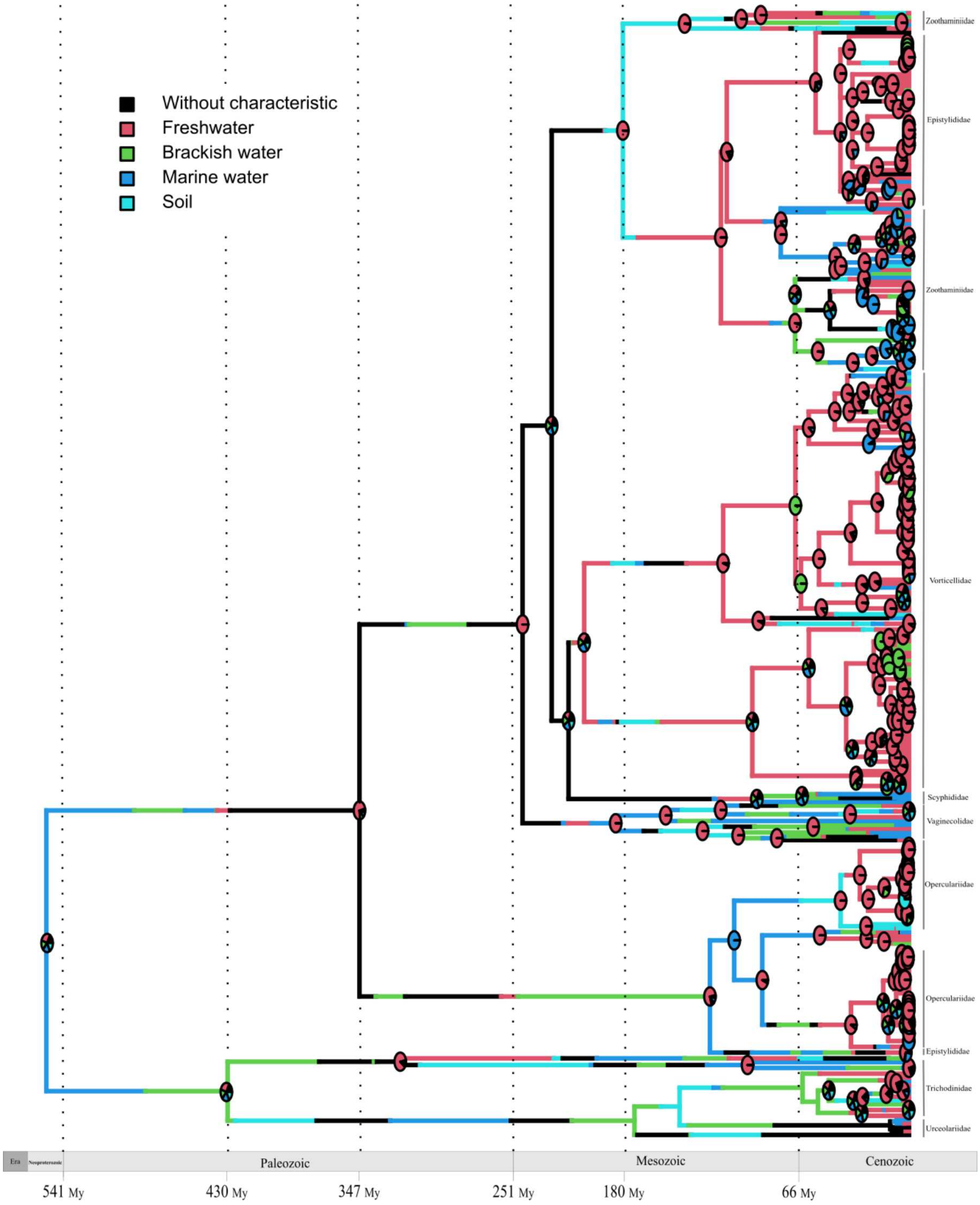
For the calculation of the phylogenetic signal, the topology containing only Peritrichia and the package Phytools v. 1.0-1 (REVELL, 2012) implemented by the R platform (R CORE TEAM, 2021). For this, we use the “phylosig” function with the “lambda” method, which uses the Pagel's index λ (PAGEL, 1999). This index was chosen because it is considered robust and accepts missing characters (MÜNKEMÜLLER *et al.*, 2012), as was the case for some species. Pagel's λ index, which varies from 0 (no signal, ie, randomly distributed characteristics) to 1 (no signal, with distributed characteristics following a phylogenetic model). This index evaluates the variation in branch length throughout the phylogeny, performing a likelihood test between the null hypothesis (absence of signal) and alternative hypothesis (presence of signal), defining the hypothesis with greater likelihood (PAGEL, 1999).

Result

The divergence time estimate for the Peritrichia subclass suggests that its emergence was about 545 Ma ago, in the Cambrian period. The emergence of the two orders of Peritrichia, Mobilida and Sessilida, according to the analysis, took place at 430 Ma and 347 Ma, respectively. At the family level, information was also collected, the largest clade of the large and polyphyletic family Epistylididae probably appeared at about 59 Ma. The largest clade of another large polyphyletic family, Zoothaminiidae, according to the analysis emerged at 72 Ma. The two families that probably appeared the longest are Tricodinidae and Vorticellidae. The estimated ages for the two, respectively, are 173 Ma and 205 Ma, which suggests that in addition to two of the most diverse families of Peritrichia, Trichodinidae and Vorticellidae are also the oldest for their respective orders.

Ancestral state reconstruction analysis suggests that probably the last common ancestor for Peritrichia inhabited freshwater environments (Figure 1). However, according to the results, the transition between different habitats is very common within the subclass and most of these environmental changes were recovered recently. The last common ancestor for the Mobilida order, according to the data available in the dataset used here, seems to have inhabited the freshwater environment (Figure 1). Furthermore, few changes of environment appear to have occurred in the past, as none of the ancestors of the main groups appear to have transitioned to another environment. The conquest of new habitats in Mobilida, such as salt and brackish water, seems to be a recent event (Figure 1), as it is only seen in living groups. Within the Sessilida order, which also has a freshwater inhabitant as its last common ancestor (Figure 1), there are some older environmental transitions, such as the transition to the marine environment of the common ancestor of the family Opercularidae and representatives of the families Epistylididae and Opisthonectidae. Another older transition, now to a brackish water environment, occurred in the last common ancestor of the clade that harbors a large part of the individuals of the Vorticellidae and the representatives of the Ophrydiidae family (Figure 1). However, all of the older transitions were likely to have reverted in subsequent generations and have only recently transitioned back to a greater number of environments.

Figure 1. Dated phylogeny of Maximum Likelihood and ancestral habitat reconstruction for the subclass Peritrichia. The probability of the ancestral characteristic for each clade is represent by the color proportion in each clade sphere. The geological bar shows eras. My, millions of years.



Source: Made by the authors.

Finally, for the phylogenetic signal analysis using the Pagel's λ index and the 247 sequences of peritrichous representatives we found a strong and significant signal ($\lambda = 0.612341$; p-value = 1.59346e-07) for the environmental feature within the Peritrichia subclass, which demonstrates that these environmental characteristics are related to the evolution and diversity of the subclass.

Discussion:

The present study presents the first phylogenetic signal investigation and the first reconstruction study of the ancestral state for the environment using maximum likelihood mothodology for the subclass Peritrichia. Sun *et al.* (2016), did an ancestral state reconstruction using use a parsimony methodology and suggest that the subclass Peritrichia probably originate in a freshwater environment and then diversified and colonized the other habitats where are found today.

The phylogeny presented here differs little from the more recent phylogenies presented for the subclass (SUN *et al.,* 2016; ZHUANG *et al.,* 2018; COSTA *et al.,* 2021), the main difference being the family Opercularidae, which appears as polyphyletic, divided in two clades, which differs from its current position described in the literature, another difference with what is reported in the literature is its relationship with representatives of the families Epistylididae and Opisthonectidae.

The estimate of divergence time found in this work varies from the time estimated for the subclass by the latest works that address the topic (COSTA *et al.,* 2021; JIANG *et al.*, 2019). In the most recent work COSTA *et al.* (2021) estimates the emergence of Peritrichia at 204 Ma (202.2-206 Ma), in the Mesozoic era, which varies from the result found at about 300 Ma less, since the emergence of Peritrichia here was reported for the Cambrian. JIANG *et al.* (2019) estimated the emergence of this subclass around 654 Ma (820-488 Ma), in the Cryogenian. Despite the result reported here being within the range presented in the work by JIANG *et al.* (2019), there is a variation of about 100 Ma less for the date proposed here and the average date propose by JIANG *et al.* (2019). The difference between the results found here and those reported in the literature is probably due to the fossils used and the composition of the datasets. JIANG *et al.* (2019) calibrate their dating with *Triacola ostracodarum* from the early Triassic (252.2-247.2 Ma) (WEITSCHAT; GUHL, 1994) and *Ichthyophthirius multifiliis* (512-461 Ma) (WRIGHT; LYNN, 1997) and their database consisted of 151 orthologs from 34 taxa of the phylum Ciliophora concatenated in a super matrix (JIANG *et al.,* 2019). The work by COSTA *et al.* (2021) uses eleven fossils as calibrations (Table S1) and their dataset was composed of 1142 sequences of the 18S-rDNA gene from different groups of the phylum Ciliophora (COSTA *et al.,* 2021). And this work uses two primary and one secondary calibration (Table 1) and the dataset used here is composed of 493 sequences containing mostly representatives of the Oligohymenophora class but also with some representatives of other classes as outgroups.

To summarize, JIANG *et al.* (2019) focus on the subclass Peritrichia but utilized a smaller dataset and fewer fossils for calibration, this combination results in a wide variation on the possible emerge date for Peritrichia. COSTA *et al.* (2021) used a much larger dataset then the JIANG *et al.* (2019) and more fossils for calibration, which probably is better to select the emerge date for the root of Pertrichia, but since they don't focus on the Peritrichia subclass the representation for this group is much smaller, which may have led to a bias in the result for the internal dates for the subclass. In the present work, we utilized a Peritrichia focus dataset bigger then JIANG's *et al* (2019) and utilized the recovered emergence date for Peritrichia found on COSTA *et al*. (2021) as secondary calibration for the root of the subclass tree.

As well as in the work of Sun *et al.* (2016), the reconstruction of the ancestral state analysis shown possibly the last common ancestor for this subclass, and the common ancestors of the two orders of this subclass, occupied freshwater environments (Figure 1). This result was already expected due to the great diversity of peritrichous species known for this environment (CORLISS, 1979; FOISSNER; BERGER, 1996; FOISSNER.; BERGER, 1992; KAHL, 1935; KENT, 1881; STILLER, 1971), as well as their dominance in the dataset, which may have biased the result. Despite this, transitions to other environments seem to be common, especially at times more recent than 30 Ma, placing most of the subclass environment changes in the Oligocene period.

According to the results of the analysis, the last common ancestors for Mobilida have a high possibility of having inhabited freshwater environments and then transition to marine and brackish environments, most likely happened very recently, the about 12 Ma or less, already in the Miocene. This late transition to other environments may be related to the way of life of these individuals, since most of the individuals that make up this order are ectoparasites of aquatic organisms (LYNN, 2008). Because of this life habit, they would need to parasitize a host that migrated from a freshwater to a saltwater environment. However, the results for this order should be viewed with caution, since its representativeness for this order is smaller then for Sessilida and many of the current groups can be found in saltwater environments, a pattern that can be clearly observed in individuals of the Urceolariidae family.

The Sessilida seems to have conquered new environments, other than freshwater, earlier. According to the result of the ancestral state reconstruction, only some groups within this order conquer new environments only more recently, as is the case for the individuals that make up the Vaginicolidae family. Representatives of this family include loricate individuals, which live as epibionts of plants, animals or on inert substrates (LYNN, 2008), and due to their extremely sessile way of life, they would depend on more factors to conquer new environments, such as colonizing a bashibion that moves between these environments.

Within one of the largest and widely distributed families of Sessilida, Vorticellidae (SUN *et al.*, 2011), the transition to another environment took place much earlier than in any other Peritrichia group. The last common ancestor of one of the clades that make up this family seems to have lived in a brackish water environment, but its descendants soon underwent a reversal and returned to inhabit freshwater environments until more recently (Figure 1). The sister groups Ophridiidae and Vorticellidae for exemple, the last common ancestor the representatives fro theis groups transitioned to a saltwater environment around 25 Ma ago. This characteristic was maintained for one of the representatives of the Ophrydiidae family, but it was reversed for the other and for the representatives of Vorticellidae. This older transition to other environments within the Vorticellidae family may be related to its wide geographic distribuition (SUN *et al.*, 2011), its high resistance to a diversity of environments (DIAS *et al.*, 2021; SLÁDEČEK, 1971; SUN *et al.*, 2011) and the emergence of this family, which is one of the oldest in Sessilida, appearing at least 205 Ma ago.

Another family that apparently transitioned from environment a little earlier was the Zoothaminiidae family. However, this characteristic, as well as for the Vorticellidae family, has been reversed in some groups around 15-20 Ma ago, for fresh and brackish water environments, but most of the family's representatives continue to inhabit the marine environment (Figure 1). This earlier conquest of other environments by the Zoothaminiidae may be related to its wide geographic distribution (FOISSNER & BERGER, 1996; UTZ & COATS, 2008; WU *et al.*, 2020) and to its easy dispersion, due the presence of free-swimming form in your life cicle, and its way of life, since they can be found as epibionts of plants, rotifers, crustaceans, turtles and attached to inanimate objects (LYNN 2008).

Although we can say that the characteristic “type of environment” is related to the diversity and evolution of the subclass Peritrichia, due to the positive and significant result of the phylogenetic signal analysis, an even more explanatory result can be found when more sequences of brackish, marine and soil environments are available. In the dataset studied here, sequences of peritrichous individuals collected from freshwater environments make up 65% of the data, proving a greater focus of studies with peritrichids from the freshwater environment. The second most sampled habitat is saltwater. Samples from this environment account for about 16% of the sequences used here. Next, we have the sequences from brackish water environments, which are equivalent to about 12% of the database. And finally, we have the representatives of the subclass collected from the soil, which are only 3% of the database. This scenario demonstrates very well the lack of knowledge of the true diversity of peritrichous ciliates in these different environments. The focus on studies of ciliates from freshwater environments can be explained by several factors, such as the ease of collection, as several mechanisms of collect have been developed, tested and improved over time (ESTON, 1985; GONG *et al.,* 2005; PRIMC *et al.,* 1967; TAYLOR, 1983), the economic importance, since a large part of the Mobilida order is considered ectoparasites of fish and can bring some problems for the production (MARTINS *et al.*, 2012; MARTINS; GHIRALDELLI, 2008) and the great ecological importance for the subclass, since they can be used as bioindicators for freshwater environments (BICK, 1972; DIAS *et al.*, 2021; FOISSNER; BERGER, 1996).

Despite being the second-best sampled environment, the marine environment and its relationship with Peritrichia diversity are still poorly understood (GIMMLER *et al.*, 2016; SUN *et al.*, 2016). Work with marine ciliates of this subclass happened in small numbers in the past (BASSON *et al.,* 1993; NAGASAWA; WARREN, 1996; SONG *et al.,* 2009) but projects such as the Tara Ocean (GIMMLER *et al.,* 2016) and the interest of large groups, mainly Chinese (JI *et al.*, 2005; JI *et al.,* 2003; SUN *et al.,* 2007; WU *et al.*, 2020; XU; SONG, 2008), have increase our knowledge about the relationship between this environment and the peritrichous that inhabit the marine environment. However, despite the increase in the sampling effort (SONG, 2009), the amount of information and number of available sequences are still reduced. In addition, much of the genetic material deposited for marine individuals comes from metagenomics work (GIMMLER *et al.*, 2016; SCHOENLE *et al.*, 2017) and is therefore not identified, which makes the interpretation of evolutionary analysis results somewhat harder.

The most worrying fact is the representation and knowledge about the other two environments, brackish water and, mainly, soil. Even with the increase in taxonomic studies with peritrichids ciliates coming from the brackish water environment (JIANG *et al.*, 2019; SUN *et al.*, 2015), most of the sequences collected from this environment are still not completely identified (75%), some reach the genus level (29%), but the majority is totally unidentified (45%), which makes it even more difficult to use this material to make evolutionary inferences. This situation is even worse for the representatives of the Peritrichia subclass that inhabit the soil environment. Due to the very low rate of encounter and the very few studies focusing on these organisms (FOISSNER *et al.,* 2002), knowledge about them is extremely precarious, which makes further considerations about this group difficult. For a better understanding of the evolution of Peritrichia and the role of each environment, mainly soil and brackish water, new works focusing on these environments should be carried out. Added to this, new molecular investigations with other genes and with more sequences are necessary to prove the result found here.

Conclusion:

As seen, there is a strong phylogenetic signal for the environment within the Peritrichia subclass, which shows that the environment is probably related to the diversity and evolution of the group. The reconstruction of the ancestral state still demonstrates that possibly the first environment inhabited by these individuals were freshwater and that the conquest of the other environments in which they inhabit today probably happened more recently. However, due to the discrepancy between the representatives of each environment in the dataset analyzed here, a better understanding of the brackish water and soil environments is still necessary. For more robust results and a better understanding of the evolution of the characteristic environment within Pertrichia, more taxonomic and molecular surveys are needed, especially in environments that are still little worked on, such as marine, brackish and especially soil.

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Supplementary Table 1. Fossil records used for node calibration by Costa et al. (2021).

Species	Minimum age (My)	Maximum age (My)	Reference

Bryometopus triquetrus	93	99	Schönborn et al., 1999 corrected by Schmidt et al., 2001
Coleps sp.	220	-	Schmidt et al., 2006
Colpoda sp.	114	-	Martín-González et al., 2008
Cyrtolophosis mucicola	93	99	Schönborn et al., 1999 corrected by Schmidt et al., 2001
Paramecium triassicum	93	99	Schönborn et al., 1999 corrected by Schmidt et al., 2001
Prorodon sp.	114	-	Martín-González et al., 2008
Pseudoplatyophrya nana	92	99	Schönborn et al., 1999 corrected by Schmidt et al., 2001
Tetrahymena rostrata	93	99	Schönborn et al., 1999 corrected by Schmidt et al., 2001
Vorticella sp.	200	-	Bomfleur et al., 2012

My, millions of years

Source: Costa et al. (2021)