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REVIEW

The widely reported but poorly studied ciliate family Folliculinidae (Protozoa, Ciliophora, Heterotrichea): a revision with notes on its taxonomy, morphology and phylogenetic relationships

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Abstract

Ciliates of the heterotrich family Folliculinidae are widespread in various habitats and are distinguished by their transparent loricae of various shapes, conspicuous peristomial lobes, and dimorphic life cycles. They usually attach firmly to the surface of substrates, feed on bacteria and microalgae, and play a significant role in energy flow and material cycling in the microbial food web. However, little is known regarding their biodiversity and systematics. In this work, we establish the terminology of the family Folliculinidae and select six crucial features for genus recognition. Based on previous studies, we revise the classification of Folliculinidae, supply improved diagnoses for each of the 33 folliculinid genera, and provide a key to their identification. Moreover, phylogenetic analyses based on small subunit ribosomal DNA (SSU rDNA) sequences revealed that the family is monophyletic and comprises two subclades (subclades I II) which can be identified by the flexibility of their peristomial lobes and the sculpturing of their necks. Furthermore, we investigate the evolutionary relationships of folliculinids using the six chosen generic features.

Keywords Ciliates · Evolutionary relationships · Folliculinidae · Heterotrichea · Review

Introduction

Ciliated protozoa are the most complicated and morphologically diverse single-celled eukaryotes. Most are free-living and exist in virtually all aquatic and some terrestrial habitats where they play important roles in the cycling of materials and energy, both within and beyond the microbial web (Liu et al. 2021; Lu et al. 2019; Lynn 2008; Wang et al. 2022; Zhao et al. 2020b, 2021). Moreover, ciliates are ideal biological tools, some of which are used as model organisms in a range of fields, including genetics, regenerative biology, symbiosis, and evolution (Gong et al. 2020; Pan et al. 2020; Wang et al. 2021; Zhao et al. 2020a).

Heterotrich ciliates were among the earliest protozoa to be discovered and observed because of their large body size, conspicuous oral structure, and, in many cases, their bright body color (Campello-Nunes et al. 2018; Chen et al. 2019; Chi et al. 2021; Fernandes et al. 2016; Jin et al. 2021; Lobban et al. 2007; Perrotta et al. 2016; Shazib et al. 2019; Trembley 1745; Wancura et al. 2017). Over the last 300 years, the taxonomic status of the class Heterotrichea has been revised multiple times and, in several recent taxonomic schemes of ciliates, the classes Heterotrichea and

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Special topic: Ciliatology.

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Karyorelictea are classified as members of the subphylum Postciliodesmatophora (Adl et al. 2019; Gao et al. 2016; Lynn 2008; Ma et al. 2021; Small and Lynn 1985). The study of heterotrich ciliates has always been a hot topic in protozoan biology on a global scale with several international ciliate research teams focusing on their species diversity, genetic diversity, and phylogeny (Chen et al. 2017; Chi et al. 2020a, b, 2021, 2022; Fernandes et al. 2016; Hines et al. 2018; Shazib et al. 2014; Wancura et al. 2017).

Currently, the class Heterotrichea contains ten families, that is, Stentoridae, Blepharismidae, Fabreidae, Folliculinidae, Maristentoridae, Gruberiidae, Condylostomatidae, Climacostomidae, Peritromidae, and Spirostomidae. Folliculinidae is the largest of the ten heterotrichous families. Since *Folliculina ampulla* (Müller, 1786) Lamarck, 1816 was collected by O. F. Müller on the coast of Denmark in 1781, about 80 folliculinid species have been discovered and documented (Andrews 1949a; Hadži 1938, 1951; Kahl 1932; Mulisch and Patterson 1987; Mulisch et al. 1993; Ye et al. 2021a, b). Folliculinids live in a bag-shaped lorica from which the group takes its name (Latin foll-, follis, folliculus, meaning shaped like a bag). The posterior end of the body is connected to the lorica via the holdfast organelle, and at the anterior end is a pair of conspicuous peristomial lobes bearing a row of adoral membranelles that spirals into the buccal cavities. In addition, the life cycle of folliculinids is complex and dimorphic, comprising two distinct phases, i.e., the sessile phase (trophont) and the free-swimming phase (swarmer) (Andrews 1949b; Mulisch and Patterson 1987; Ye et al. 2021a, b). The swarmer only exists for a very short time and is difficult to collect directly from the environment. Its morphology is quite different from the trophont, being rod-shaped without a lorica and swims freely in the water. Sometimes, swarmers of folliculinids are mistaken for other taxa/species of heterotrichous ciliates (Wilbert and Song 2005).

Folliculinids are mainly distributed in marine or brackish water and can be found in all parts of the ocean, from brackish wetlands to intertidal zones, shallow to deep waters, and ambient regions to deep-sea hydrothermal vents (Kouris et al. 2007). To date, only four species have been found in freshwater environments. Folliculinids usually live attached to the surface of algae, aquatic plants or invertebrates, and are a crucial component of periphytic ciliate communities (Corliss 1979; Kouris et al. 2010; Lynn 2008). Sometimes, folliculinids will grow in large numbers on the surface of aquatic organisms, inflicting significant damage to their host (Cróquer et al. 2006).

The family Folliculinidae is a speciose group with 80 valid species (Andrews 1953; Deflandre and Deunff 1957; Dioni 1972; Hadži 1951; Jankowski 2009; Mulisch and Patterson 1983; Wilbert and Song 2008; Ye et al. 2021b). However, many species were reported based solely on fixed ecological samples or empty loricae from environmental samples, resulting in many homonyms or synonyms. Due to the limitations in sampling or staining, only nine species have been described in detail with modern taxonomic characterizations (Kouris et al. 2007; Luo et al. 2019; Mulisch and Patterson 1987; Mulisch et al. 1993; Song et al. 2003; Wilbert and Song 2008; Ye et al. 2021a, b). Moreover, only six well-characterized species have SSU rDNA sequences in the GenBank, i.e., less than 10% of the 80 valid species (Hadži 1938, 1951; Kahl 1932; Miao et al. 2009; Mulisch et al. 1993; Song et al. 2003; Ye et al. 2021a, b). The paucity of taxonomically informative morphological features and molecular information on folliculinid species brings many difficulties to the classification and phylogenetic relationships of Folliculinidae.

The current study provides a comprehensive clarification and correction of those errors and confusions in the literature, as well as precise definitions and key for 33 genera. Moreover, a preliminary discussion of the phylogeny of the family Folliculinidae is presented based on accurate morphological characteristics and molecular information.

Terminology

General ciliate terminology is mainly adopted from Lynn (2008) and Song et al. (2009). However, some morphological terminologies of folliculinids are rather specific, as shown in Fig. 1, so we follow Luo et al. (2019), Mulisch et al. (1986) and Ye et al. (2021a, b) who defined the terms used in the description of folliculinid genera/species as follows.

Trophont. The adult, feeding, sessile stage of the life cycle.

Lorica. Chitinous (?) extracellular shell in which the trophont lives and is typically divided into a broadened flask and relatively thinner neck. The morphological features of the lorica are traditionally used for genus and species classification. For example, flask recumbent vs. vertical, flask single-chambered vs. multi-chambered, surface smooth vs. ornamented, neck short vs. long, neck surface smooth vs. sculptured, neck opening simple or with an obvious brim.

Closure device. A special structure on the inner wall of the lorica, usually at the junction of the flask and neck, which is composed of one or more flaps or may be membranous in composition. The presence or absence of this structure is an important generic character.

Basal plate. A transparent, jelly-like structure that attaches the lorica to its substrate.

Peristomial lobe. An anterior structure of the trophont with adoral membranelles distributed along its edge.

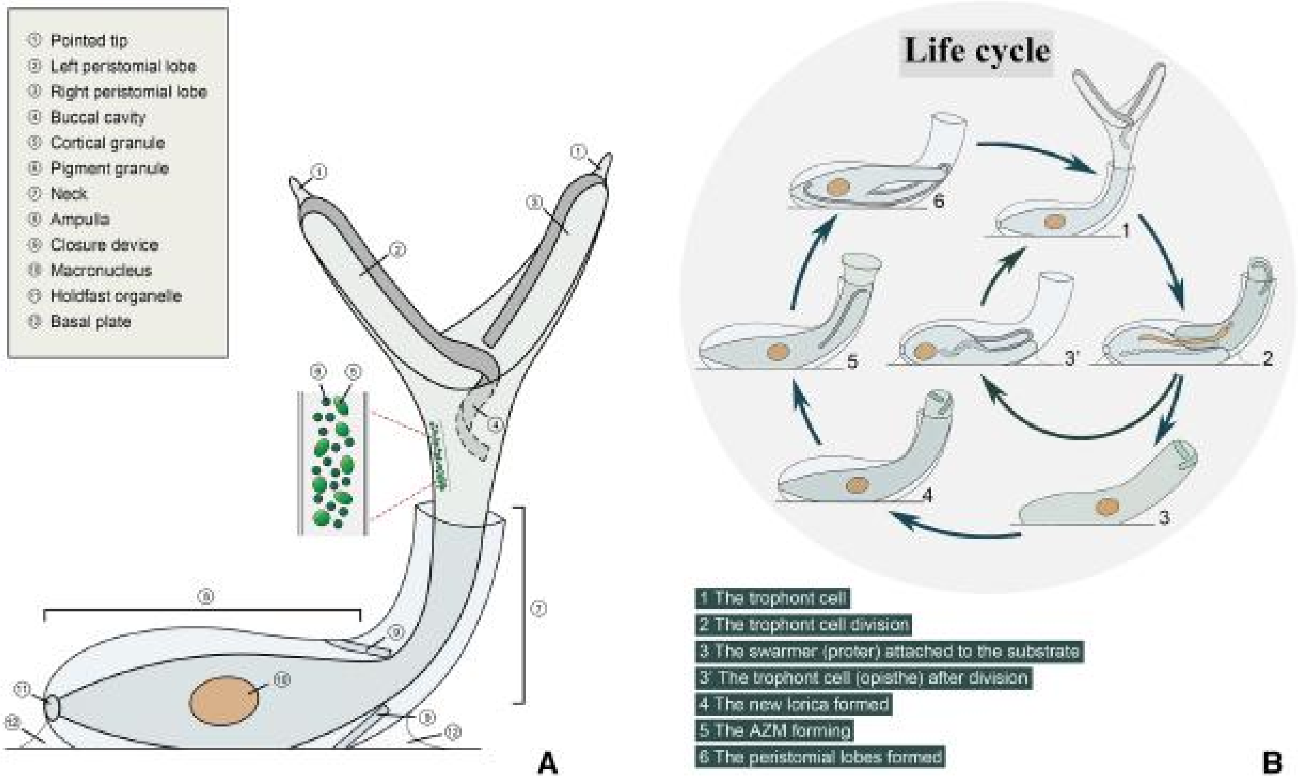


Fig. 1 Terminology and basic characters for generic and specific classification of the Folliculinidae. **A** Lateral view of an extended trophont in vivo. **B** The folliculinid life cycle

The trophont has two peristomial lobes that may be either broad or narrow, equal or unequal in size or shape, flexible or relatively inflexible. Some folliculinid species have a terminal pointed tip at the top of each lobe. Under normal conditions, the peristomial lobes protrude out of the lorica. The beating action of the adoral membranelles creates a water current that carries particulate food such as organic matter, bacteria and microalgae into the oral cavity. The lobes quickly retract into the lorica when the organism is disturbed or otherwise stimulated.

Holdfast organelle. A structure at the posterior end of the trophont by which it attaches to the inner wall of the lorica at the bottom of the flask. The holdfast organelle is fixed to the lorica by one to several bundles of cytoplasmic fibrils. The shape of the holdfast organelle is a character for genus identification.

Swarmer. The naked, free-swimming, and vermiform cell are produced by cell division of the trophont. The swarmer has a membranellar spiral at the apical end, which resembles the adoral zone of membranelles of the trophont. However, the swarmer lacks an oral cavity so it cannot ingest food.

Revision of family Folliculinidae Dons, 1914

Members of the family Folliculinidae have a dimorphic life cycle which is divided into two phases, that is, trophont and swarmer. Trophonts have a sessile lifestyle and usually reside within a transparent lorica. Swarmers develop after cell division and are vermiform and migratory (Corliss 1979; Hu et al. 2019; Lynn 2008; Song et al. 2009; Ye et al. 2021a, b). Although there are morphological differences among folliculinid genera, their basic structures are similar, as summarized in Fig. 2. The only exception to this basic morphological pattern is the case of *Bickella* Wilbert and Song, 2008 which lacks a lorica (Fig. 3A). Unfortunately, molecular data for *Bickella* are lacking so its molecular phylogeny cannot be determined. Once this is resolved, the family Folliculinidae will be phylogenetically well-circumscribed and diagnosed.

The lorica of folliculinids is usually transparent and comprises a flask and a neck. In most genera, the flask is recumbent but in four, namely *Stentofolliculina, Pedifolliculina*, *Pseudofolliculina* and *Pseudoparafolliculina*, the flask stands upright. At the adoral end of the trophont are

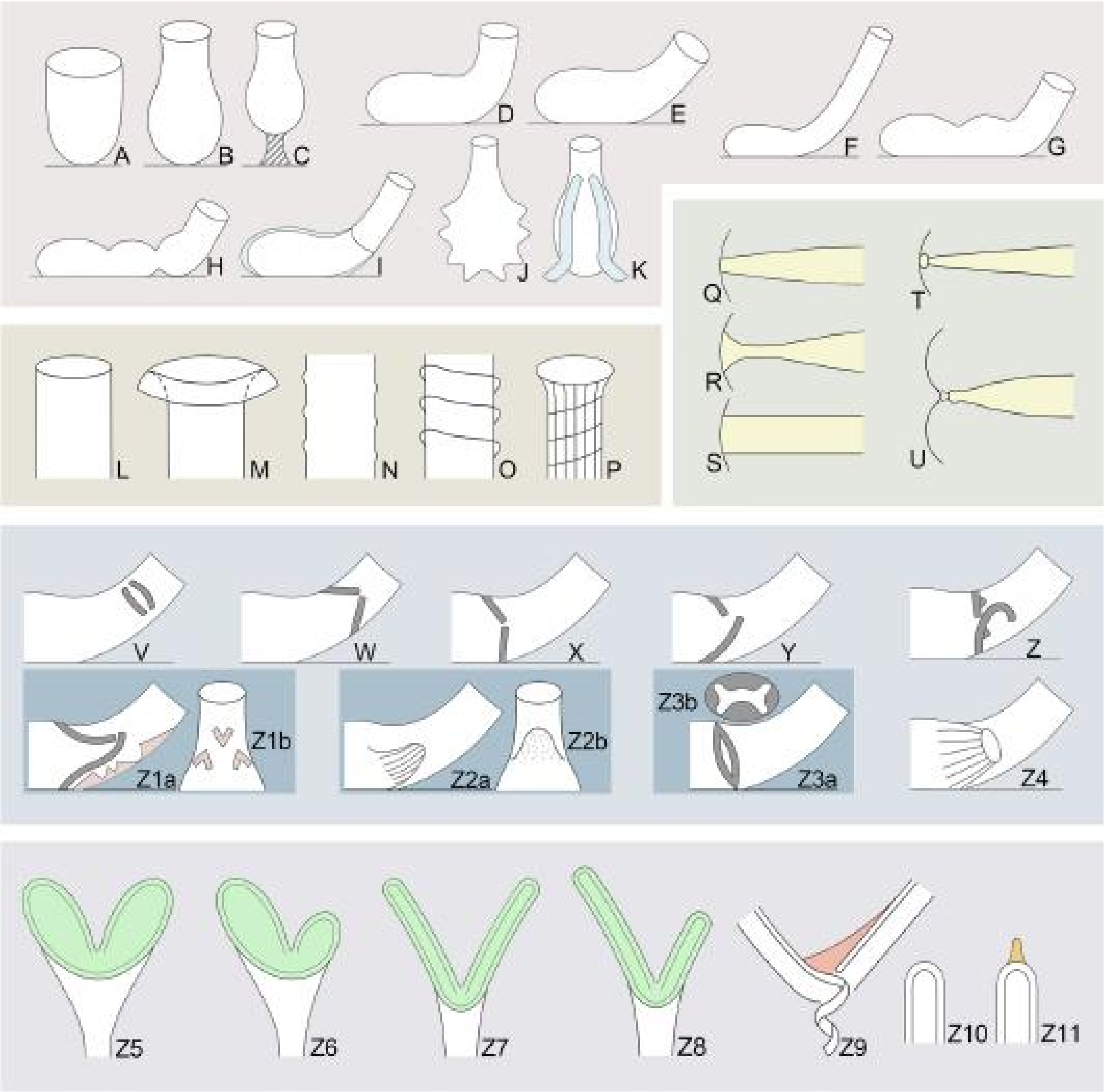


Fig. 2 Summary of five morphological characteristics of Folliculinidae: the macronuclear feature is not shown as it only has two types: single macronucleus or multiple macronuclei. **A**–**K** Different types of lorica. The black and white striped area in **c** indicates the stalk-like basal plate, the blue area in **I** shows the double-layered flask, the blue bands in **K** shows the spines. **L**–**P** Different types of neck. **Q**–**U** Different types of holdfast organelle. **V**–**Z4** Different types of closure devices. The pink areas in **Z1a** and **Z1b** indicate the inner teeth on the lateral and top views. **Z5**–**Z8** Different types of peristomial lobe. **Z9** Showing the left and right peristomial lobes connected in front of the buccal cavity. The pink triangle indicates the pellicular flap in some species. **Z10**, **Z11** Two types of the top of peristomial lobe. The yellow area in **Z11** shows the pointed tip

two conspicuous peristomial lobes that may be equal or unequal in shape and size. The trophont attaches to the inner wall of lorica at the bottom of the flask via a holdfast organelle at its posterior end. When disturbed or otherwise suitably stimulated, the trophont retracts rapidly into the flask. Some species have a closure device that seals the contracted trophont inside the lorica, thus protecting it from predation.

We recognize 33 genera of folliculinids representing a total of 80 valid species. The type genus *Folliculina* was established by Lamarck in 1816. Only four other genera were established in the nineteenth century, that is, *Lagotia* Wright, 1858, *Freia* Claparède & Lachmann, 1858 (objective synonym of *Folliculina* due to these two genera having the same type species, see Aescht 2001), *Ascobius* Henneguy, 1884, and *Pebrilla* Giard, 1888. In the first 30 years of the twentieth century, Dons (1914, 1924, 1927, 1934a) established six new genera, namely *Pseudofolliculina*, *Parafolliculina*, *Semifolliculina* (synonym of *Lagotia*, see the section below on genus diagnoses), *Metafolliculina*, *Mirofolliculina*, and *Echinofolliculina*. Using lorica type as the main generic feature, Kahl (1932) recognized only six genera: *Folliculina*,

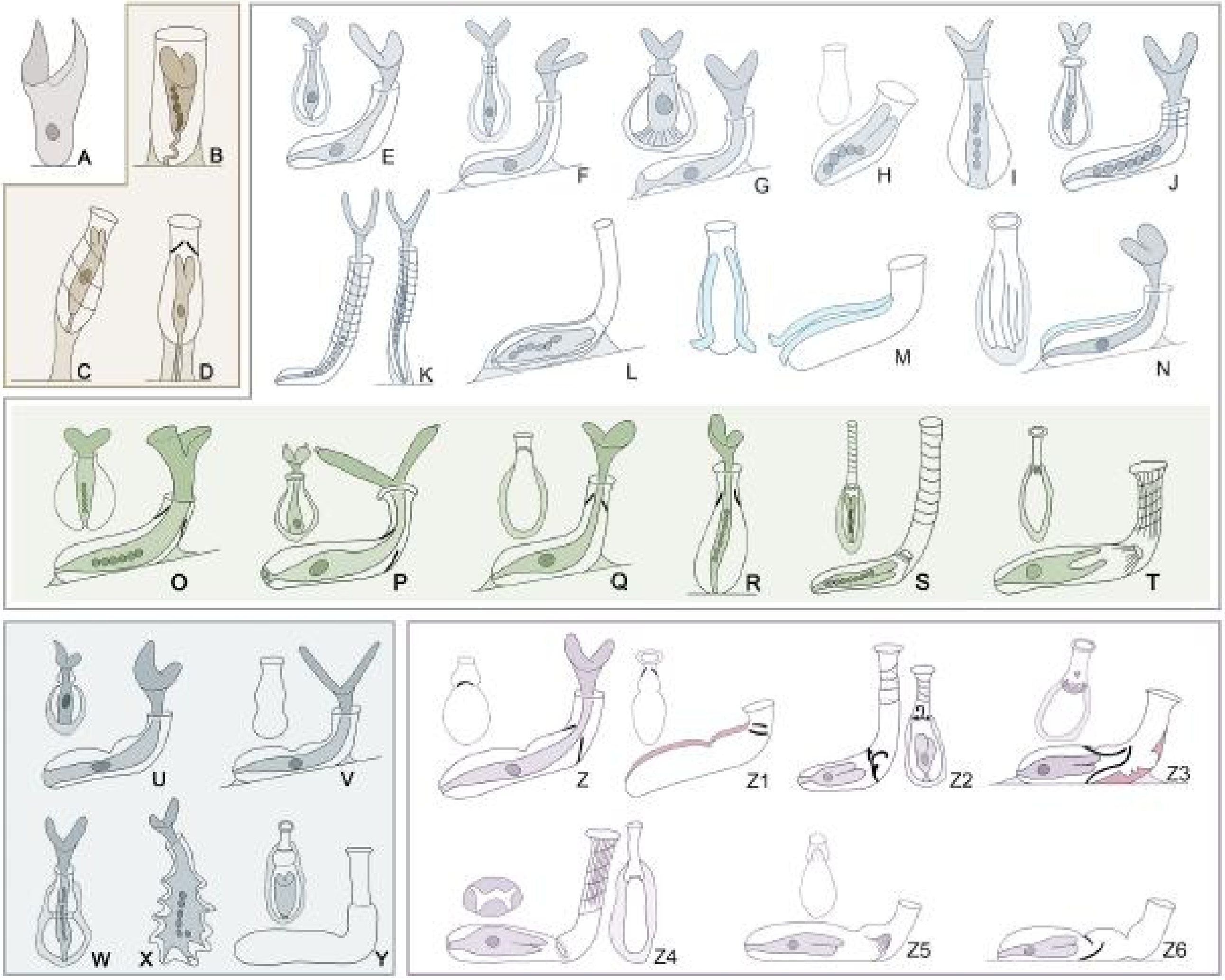


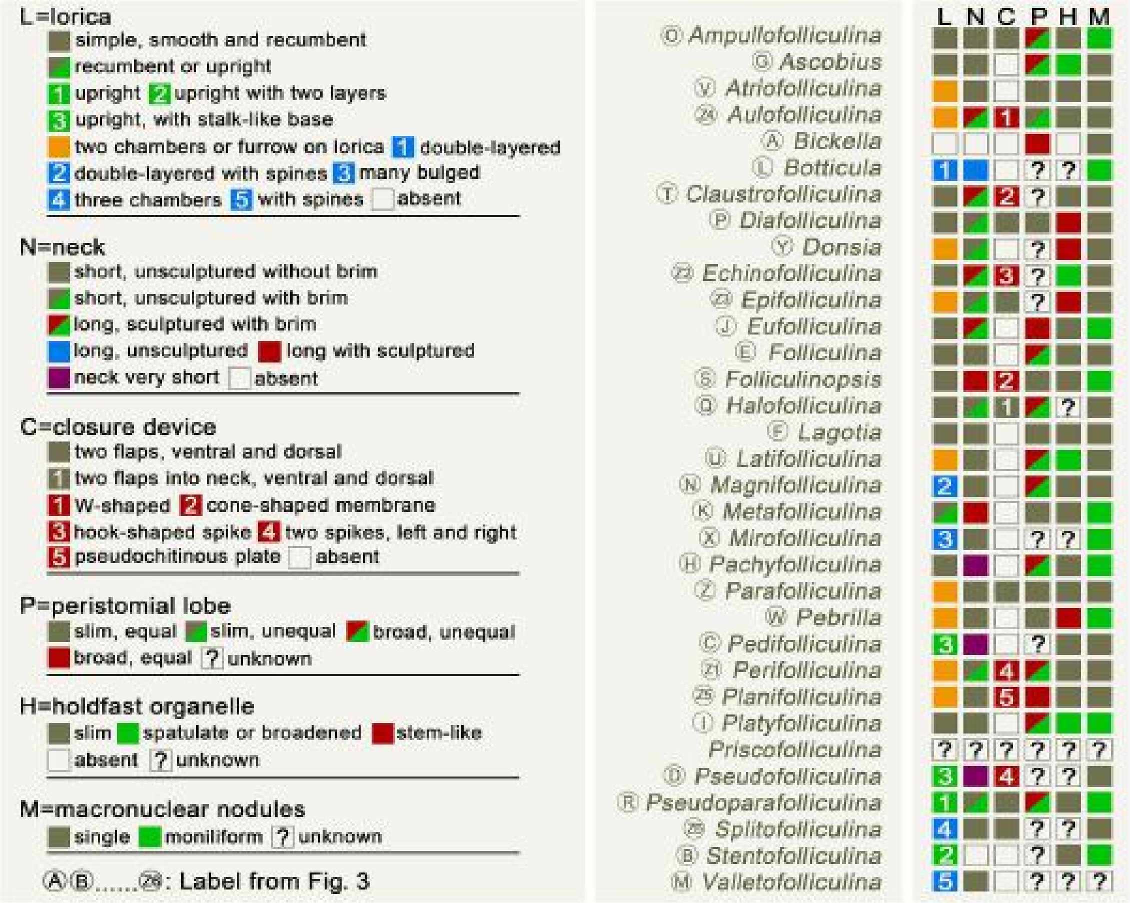
Fig. 3 Schematic illustrations of 32 folliculinid genera based on in vivo observations (fossil genus *Priscofolliculina* is not shown here). **A** The only genus without a lorica. **B–D** Three genera with a vertical flask and a stalk-like basal plate. **E–N** Ten genera that have a single-chambered flask. **O–T** Six genera that have a single-chambered flask and a closure device. **U–Y** Five genera that have a multi-chambered flask and lack a closure device. **Z–Z6** Seven genera that have a multi-chambered flask and a closure device. **A** *Bickella*. **B** *Stentofolliculina*. **C** *Pedifolliculina*. **D** *Pseudofolliculina*. **E** *Folliculina*. **F** *Lagotia*. **G** *Ascobius*. **H** *Pachyfolliculina*. **I** *Platyfolliculina*. **J** *Eufolliculina*. **K** *Metafolliculina*. **L** *Botticula*. **M** *Valletofolliculina*. **N** *Magnifolliculina*. **O** *Ampullofolliculina*. **P** *Diafolliculina*. **Q** *Halofolliculina*. **R** *Pseudoparafolliculina*. **S** *Folliculinopsis*. **T** *Claustrofolliculina*. **U** *Latifolliculina*. **V** *Atriofolliculina*. **W** *Pebrilla*. **X** *Mirofolliculina*. **Y** *Donsia*. **Z** *Parafolliculina*. **Z1** *Perifolliculina*. **Z2** *Echinofolliculina*. **Z3** *Epifolliculina*. **Z4** *Aulofolliculina*. **Z5** *Planifolliculina*. **Z6** *Splitofolliculina*

Metafolliculina, *Pseudofolliculina*, *Mirofolliculina*, *Parafolliculina*, and *Pebrilla*. Fauré-Fremiet (1936) erected a new genus, *Folliculinopsis*, for *Folliculina* species with a moniliform macronucleus, but this was considered as a nomen nudum due to the lack of type species fixation (Aescht 2001; Corliss 1979; Hadži 1951; Lynn 2008). Hadži (1951) resurrected this genus and designated *Folliculinopsis annulata* as the type species. Andrews and Nelson (1942) discovered a new folliculinid and established the genus *Pseudoparafolliculina* for species with an upright flask that attaches directly to the substrate. Hadži discovered nine new folliculinid species from the Adriatic Sea and established 18 new genera, mainly based on lorica shape, the presence or absence of a closure device, the shape of the holdfast organelle, and features of the macronucleus (see Hadži 1951). These 18 nominal genera were: *Alexandrina* (synonym of *Folliculinopsis*, see the section below on genus diagnoses), *Ampullofolliculina*, *Atriofolliculina* (nomen nudum for lack of type species fixation, see Aescht 2001), *Aulofolliculina*, *Claustrofolliculina*, *Diafolliculina*, *Donsia*, *Epifolliculina*, *Eufolliculina*, *Halofolliculina* (nomen nudum for lack of type species fixation, see Aescht 2001), *Latifolliculina*, *Pachyfolliculina*, *Pedifolliculina*, *Perifolliculina*, *Planifolliculinia*, *Platyfolliculina*, *Splitofolliculina* (nomen nudum for lack of type species fixation, see Aescht 2001), and *Stentofolliculina* (see Hadži 1938, 1951). Four other new genera were established in the last half of the twentieth century, namely *Valletofolliculina* Andrews, 1953, *Priscofolliculinia* Deflandre & Deunff, 1957 (fossil genus), *Magnifolliculina* Uhlig, 1964 (nomen nudum for lack of species fixation, see Aescht, 2001), and *Botticula* Dioni, 1972. Wilbert and Song (2008) established a new genus *Bickella*, discovered in Antarctic waters, based on the absence of a lorica. Thus, altogether there are 37 nominal genera of folliculinids, four of which (*Alexandrina*, *Freia*, *Semifolliculina*, and *Tapetofolliculina*) are junior synonyms of other genera.

Based on the data reviewed above and the present investigations, we recognize six features that are critical for genus separation and identification in the family Folliculinidae (Figs. 2, 4): (1) the shape and structure of the flask; (2) the length and sculpturing of the neck; (3) the presence or absence of a closure device; (4) the shape of the peristomial lobes; (5) the type of holdfast organelle; and (6) the shape of the macronucleus and the number of macronuclear nodules.

In most genera, the flask is recumbent and attaches to the substrate via a flattened, transparent basal plate. However, in *Stentofolliculina*, *Pedifolliculina*, and *Pseudofolliculina*, the flask is upright and attaches to the substrate via a stalk-like basal plate (Dons 1914; Hadži 1938). *Pseudoparafolliculina* also has an upright flask but lacks a stalk-like basal plate (Andrews and Nelson 1942). Furthermore, the flask in most genera is single-chambered, whereas in some the flask is divided into two or three chambers by one or two furrows either of the flask or at the junction of the flask and neck (Hadži 1951). In addition, some genera have unique structures of the flask, such as *Valletofolliculina,* which has two ribs with pointed ends, and *Botticula* and *Magnifolliculina,* each of which has a double-layered flask wall (Andrews 1953; Dioni 1972).

The length and sculpturing of the neck are stable characteristics in most folliculinids because the secretion and formation of the lorica occur relatively early during the metamorphosis of trophont from the swarmer. The next steps of the life cycle commence only when this process



L=lorica
🟫 simple, smooth and recumbent
🟫/🟩 recumbent or upright
🟩1 upright 🟩2 upright with two layers
🟩3 upright, with stalk-like base
🟧 two chambers or furrow on lorica 🟦1 double-layered
🟦2 double-layered with spines 🟦3 many bulged
🟦4 three chambers 🟦5 with spines ⬜ absent

N=neck
🟫 short, unsculptured without brim
🟫/🟩 short, unsculptured with brim
🟥/🟩 long, sculptured with brim
🟦 long, unsculptured 🟥 long with sculptured
🟪 neck very short ⬜ absent

C=closure device
🟫 two flaps, ventral and dorsal
🟫1 two flaps into neck, ventral and dorsal
🟥1 W-shaped 🟥2 cone-shaped membrane
🟥3 hook-shaped spike 🟥4 two spikes, left and right
🟥5 pseudochitinous plate ⬜ absent

P=peristomial lobe
🟫 slim, equal 🟫/🟩 slim, unequal 🟥/🟩 broad, unequal
🟥 broad, equal ? unknown

H=holdfast organelle
🟫 slim 🟩 spatulate or broadened 🟥 stem-like
⬜ absent ? unknown

M=macronuclear nodules
🟫 single 🟩 moniliform ? unknown

Ⓐ Ⓑ......㉖: Label from Fig. 3

	L	N	C	P	H	M
Ⓞ *Ampullofolliculina*	🟫	🟫	🟫	🟥/🟩	🟫	🟩
Ⓖ *Ascobius*	🟫	🟫	⬜	🟥/🟩	🟩	🟫
Ⓥ *Atriofolliculina*	🟧	🟫	⬜	🟫	🟫	🟫
㉔ *Aulofolliculina*	🟧	🟥/🟩	🟥1	🟫/🟩	🟫	🟫
Ⓐ *Bickella*	⬜	⬜	⬜	🟥	⬜	🟫
Ⓛ *Botticula*	🟦1	🟦	⬜	?	?	🟩
Ⓣ *Claustrofolliculina*	🟫	🟥/🟩	🟥2	?	🟫	🟫
Ⓟ *Diafolliculina*	🟫	🟫/🟩	🟫	🟫	🟥	🟫
Ⓨ *Donsia*	🟧	🟫/🟩	⬜	?	🟥	🟫
㉓ *Echinofolliculina*	🟫	🟥/🟩	🟥3	?	🟩	🟫
㉒ *Epifolliculina*	🟧	🟫/🟩	🟫	?	🟥	🟫
Ⓙ *Eufolliculina*	🟫	🟥/🟩	⬜	🟥	🟫	🟩
Ⓔ *Folliculina*	🟫	🟫	⬜	🟥/🟩	🟫	🟫
Ⓢ *Folliculinopsis*	🟫	🟥	🟥2	🟫	🟫	🟩
Ⓠ *Halofolliculina*	🟫	🟫/🟩	🟫1	🟥/🟩	?	🟫
Ⓕ *Lagotia*	🟫	🟫	⬜	🟫	🟫	🟫
Ⓤ *Latifolliculina*	🟧	🟫	⬜	🟥/🟩	🟩	🟫
Ⓝ *Magnifolliculina*	🟦2	🟫	⬜	🟥/🟩	🟫	🟫
Ⓚ *Metafolliculina*	🟫/🟩	🟥	⬜	🟫	🟫	🟩
Ⓧ *Mirofolliculina*	🟦3	🟫	⬜	?	?	🟩
Ⓗ *Pachyfolliculina*	🟫	🟪	⬜	🟥/🟩	🟫	🟩
Ⓩ *Parafolliculina*	🟧	🟫	🟫	🟫	🟫	🟫
Ⓦ *Pebrilla*	🟧	🟫	⬜	🟫	🟥	🟩
Ⓒ *Pedifolliculina*	🟩3	🟪	⬜	?	🟫	🟫
㉑ *Perifolliculina*	🟧	🟫/🟩	🟥4	🟥/🟩	🟫	🟫
㉕ *Planifolliculina*	🟧	🟫	🟥5	🟥	🟫	🟫
Ⓘ *Platyfolliculina*	🟫	🟫	⬜	🟥/🟩	🟩	🟩
Priscofolliculina	?	?	?	?	?	?
Ⓓ *Pseudofolliculina*	🟩3	🟪	🟥4	?	?	🟫
Ⓡ *Pseudoparafolliculina*	🟩1	🟫/🟩	🟫	🟥/🟩	🟫	🟩
㉖ *Splitofolliculina*	🟦4	🟫	🟫	?	?	🟫
Ⓑ *Stentofolliculina*	🟩2	⬜	⬜	?	🟫	🟩
Ⓜ *Valletofolliculina*	🟦5	🟫	⬜	?	?	?

Fig. 4 Summary of the character states of the six morphological features (lorica, neck, closure device, peristomial lobe, holdfast organelle and macronuclear nodules) used to characterize the 33 folliculinid genera. The morphology of *Priscofolliculina* is unknown. Illustrations of the other 32 genera are shown in Fig. 3

is entirely completed (see the life cycles of *Ampullofolliculina lageniformis* and *Metafolliculina producta* in Ye et al. 2021a). Occasionally, the neck can be extended in length. For example, the neck of *Metafolliculina nordgardi* can be extended 2–4 times, and the length and number of turns of any spiral ridge(s) vary accordingly (Dons 1924).

The presence or absence and structure of the closure device are considered to be genus-level characters (Andrews 1953; Das 1949; Hadži 1951; Mulisch et al. 1993). For instance, although *Halofolliculina* is very similar to *Lagotia* in most morphological features, the former can be distinguished from the latter by the presence (vs. absence) of a closure device (Hadži 1951). *Folliculinopsis* and *Metafolliculina* are easily confused as they both have a moniliform macronucleus and a long, conspicuously sculptured neck, however, they can be separated by the presence (vs. absence) of a closure device (Fauré-Fremiet 1936; Hadži 1938; Matthews 1968). During a study of folliculinid species, we observed that species without a closure device are more likely to be preyed upon by other ciliates or rotifers than those with a closure device (unpublished). This suggests that the closure device contributes to protecting the trophont and is probably a significant feature in the ecology and evolution of folliculinids.

The shape of the peristomial lobes is very stable and easily observed in living organisms. Historically, however, the shape of the peristomial lobes was not described because the descriptions of most species were based on contracted or shrunken individuals obtained from ecological samples or fixed materials. This feature has been recorded for five genera: *Ampullofolliculina,* which has blunt, unequal lobes; *Diafolliculina,* which has equal lobes that are slender with pointed tips; *Eufolliculina,* which has equal lobes with rounded ends; *Folliculina*, which has unequal lobes with rounded ends; and *Metafolliculina,* which has equal lobes that are long and slender. Mulisch et al. (1993) stressed the importance of this feature and suggested that folliculinids should be divided into two groups according to the fibrous structure of the peristomial lobes. Thus, we suggest using features of the peristomial lobes for genus distinction in future work.

The shape of the holdfast organelle can only be observed in fully extended living trophonts. There are three main types of holdfast organelle: spatula-shaped (Fig. 2R) (as in *Ascobius*), stem-shaped (Fig. 2S) (as in *Latifolliculina*), and slender (Fig. 2Q, T, U) (most other folliculinids). This feature cannot be observed in the contracted individual or if the lorica is empty. Hence, living cells should be carefully observed to provide detailed descriptions of this feature.

The sixth generic feature is the shape of the macronucleus and the number of macronuclear nodules. Fauré-Fremiet (1936) divided *Folliculina* species into two genera based on the presence of a single (*Folliculina*) or multiple (*Folliculinopsis*) macronuclear nodules. The macronuclear apparatus was also considered to be a genus-specific character by other researchers (Das 1949; Hadži 1938, 1951). Thus, we accept macronucleus type as a character for genus distinction. It is noteworthy that the appearance of the macronucleus, e.g., single vs. moniliform, is considered to be a species-level character in other heterotrich families with fewer genera than Folliculinidae, such as Spirostomidae and Stentoridae (Boscaro et al. 2014; Campello-Nunes et al. 2020; Chen et al. 2018, 2019; Chi et al. 2020b, 2021, 2022; Shazib et al. 2019).

The present selection of generic features is based both on previous studies and the experience and judgement of the present authors. It is intended to provide a hypothesis for further investigations, especially those based on molecular phylogeny (see discussion in the section: "Evolutionary significance of morphological features"). Other features that might be significant include the number of somatic kineties, the ultrastructure of peristomial lobe fibers, ontogenetic processes, and cortical and pigment granules. Historically, however, these features were either overlooked or could not be observed due to the lack of appropriate technologies. Therefore, to better review and clarify the historic literature, we selected those features that are available for most genera.

Diagnoses of the 33 nominal genera of the family Folliculinidae

Based mainly on the criteria described above, we redefine all known 33 genera and emend their diagnoses with brief remarks on each genus. Illustrations of selected key characters (Fig. 2) and a visual key of morphological characteristics (Fig. 4) are also provided.

Genus *Ampullofolliculina* Hadži, 1951 (Fig. 3O)

Type species. Ampullofolliculina lageniformis Hadži, 1951

Species assigned. Ampullofolliculina lageniformis Hadži, 1951

Emended diagnosis. Flask single-chambered; neck short and unsculptured; closure device as two flaps, one ventral and one dorsal; peristomial lobes short and dissimilar; holdfast organelle slender; macronucleus moniliform.

Remarks. The genus *Ampullofolliculina* is monotypic and the type species, *A. lageniformis*, was well characterized by Luo et al. (2019) based on a China population. Ye et al. (2021a) described its life cycle for the first time.

Literature. Andrews (1949a), Hadži (1951), Luo et al. (2019) and Ye et al. (2021a).

Genus *Ascobius* Henneguy, 1884 (Fig. 3G)

Type species. Ascobius lentus Henneguy, 1884

Species assigned. Ascobius lentus Henneguy, 1884; *A. claparedi* Hadži, 1951 (syn. *Freia ampulla* sensu Claparède and Lachmann, 1858); *A. faurefremieti* Hadži, 1951 (syn. *Folliculina ampulla* sensu Fauré-Fremiet, 1932); *A. simplex* (Dons, 1917) Hadži, 1951; *A. sileni* Hadži, 1951 (syn. *Folliculina simplex* sensu Silén, 1947)

Emended diagnosis. Flask single-chambered, flat, and recumbent; neck short and unsculptured; closure device absent; peristomial lobes short, broad, and dissimilar; holdfast organelle spatulate or broadened; macronucleus compact and single.

Remarks. The spatulate or broadened holdfast organelle is the main taxonomic character of this genus. The type species is a rare freshwater species that was redescribed in detail by Mulisch et al. (1998). The other four species in this genus await redescription.

Literature. Dons (1917), Hadži (1951), Henneguy (1884), Kahl (1932) and Mulisch et al. (1998).

Genus *Atriofolliculina* Hadži, 1951 [nomen nudum] (Fig. 3V)

Type species. Not designated.

Species assigned. Atriofolliculina andrewsi Hadži, 1951 (syn. *Parafolliculina amphora* sensu Andrews, 1946); *A. hirundo* Hadži, 1951 (syn. *Folliculina amphora* sensu Kent, 1881–1882, *Parafolliculina hirundo* sensu Kahl, 1932); *A. faureana* Hadži, 1951 (syn. *Parafolliculina hirundo* sensu Fauré-Fremiet, 1936)

Emended diagnosis. Flask two-chambered, upright or recumbent with a broad, shallow transverse furrow; neck short and smooth (unsculptured); closure device absent; peristomial lobes long and slender, equal in length; holdfast organelle slender; macronucleus compact and single.

Remarks. Aescht (2001) and Lynn (2008) considered *Atriofolliculina* a nomen nudum for lack of type species fixation. Hadži (1951) erected this genus and transferred into it three *Parafolliculina* species that lack a closure device. The validity of this genus awaits further investigation and the establishment of a type species.

Literature. Andrews (1946), Fauré-Fremiet (1936), Hadži (1951) and Kent (1881–1882).

Genus *Aulofolliculina* Hadži, 1951 (Fig. 3Z4)

Type species. Aulofolliculina labyrinthica Hadži, 1951

Species assigned. Aulofolliculina labyrinthica Hadži, 1951

Emended diagnosis. Flask two-chambered, recumbent; neck long and spirally sculptured, opening with an obvious brim; closure device in the form of a diaphragm with an M-shaped gap; peristomial lobes oblong and slightly disparate; holdfast organelle stem-like; macronucleus single.

Remarks. The closure device is probably the most complex one known to date.

Literature. Hadži (1951).

Genus *Bickella* Wilbert & Song, 2008 (Fig. 3A)

Type species. Bickella antarctica Wilbert & Song, 2008

Species assigned. Bickella antarctica Wilbert & Song, 2008

Emended diagnosis. Lorica absent; trophont free-swimming; peristomial lobes wide and prominent, equal in size and shape; macronucleus globular and single.

Remarks. This genus differs from all other folliculinids by the absence of a lorica and its free-swimming behavior. The original description stated this taxon could be closely related to loricate folliculinids and that the “lorica-free” phenomenon might represent an adaptation to a shortage of food in the Antarctic biotope. Molecular information for this genus is needed to determine its systematic position.

Literature. Wilbert and Song (2008).

Genus *Botticula* Dioni, 1972 (Fig. 3I)

Type species. Botticula ringueleti Dioni, 1972

Species assigned. Botticula ringueleti Dioni, 1972

Emended diagnosis. Flask single-chambered, straight and long, recumbent, double-layered with a thick and fibrous external wall; neck smooth (unsculptured) and long; closure device absent; holdfast organelle slim; macronucleus moniliform.

Remarks. The type species of this monotypic genus is a freshwater form. The original description is the only record of this taxon.

Literature. Dioni (1972).

Genus *Claustrofolliculina* Hadži, 1951 (Fig. 3T)

Type species. Claustrofolliculina clausa Hadži, 1951

Species assigned. Claustrofolliculina clausa Hadži, 1951

Emended diagnosis. Flask single-chambered, tubular and recumbent; neck long and lightly sculptured with longitudinal lines; closure device in the form of a conical membrane; peristomial lobes broad; holdfast organelle slim; macronucleus single.

Remarks. This is the only genus with both a single macronucleus and a closure device in the form of a conical membrane.

Literature. Hadži (1951).

Genus *Diafolliculina* Ye et al., 2021 (Fig. 3P)

Type species. Diafolliculina longilobata Ye et al., 2021

Species assigned. Diafolliculina longilobata Ye et al., 2021; *D. thomseni* (Hadži, 1951) Ye et al., 2021; *D. rotunda* (Hadži, 1951) Ye et al., 2021; *D. similis* (Hadži, 1951) Ye et al., 2021

Emended diagnosis. Flask single-chambered, rounded and recumbent; neck short and unsculptured; closure device as two flaps, one ventral and one dorsal; peristomial lobes may be similar or dissimilar; holdfast organelle spatula-shaped or stout stem-shaped; macronucleus single.

Remarks. Thomsen (1921) described a South American population as *Folliculina boltoni* Kent, 1881. According to this description, the organism has an obvious closure device and a spatula-shaped holdfast organelle, a combination of features not found in any known genus. So Hadži (1951) established a new genus *Diafolliculina* for Thomsen's isolate and renamed it *D. thomseni*. Moreover, Hadži (1951) described two other species, *D. rotunda* and *D. similis*, collected from the Adriatic Sea. However, the type species of *Diafolliculina* was not mentioned by Hadži (1951), so it was nomen nudum. Ye et al. (2021b) collected a new species consistent with the morphological characteristics of *Diafolliculina*. Ye et al. (2021b) reactivated the genus *Diafolliculina* and designated *D. longilobata* as the type species based on the availability of comprehensive morphological information.

Literature. Hadži (1951), Thomsen (1921) and Ye et al. (2021b).

Genus *Donsia* Hadži, 1951 (Fig. 3Y)

Type species. Donsia mirabilis (Dons, 1917) Hadži, 1951

Species assigned. Donsia mirabilis (Dons, 1917) Hadži, 1951

Emended diagnosis. Flask divided into two chambers by a shallow furrow, lower chamber attached to substrate, upper chamber not attached to substrate; neck long and unsculptured, with a furrow at the junction of neck and upper chamber of the flask; closure device absent; holdfast organelle columnar; macronucleus single.

Remarks. Dons (1917) collected a marine folliculinid and described it as *Semifolliculina mirabilis*. This species had a flask with two chambers, both of which were attached to the substrate. Kahl (1932) assigned this species to the genus *Parafolliculina*. Two years later, Dons (1934b) transferred this species to *Lagotia* as it had no closure device. In 1934, Hadži collected from the Adriatic Sea numerous individuals that closely resembled *Lagotia mirabilis* (Dons, 1917) Dons, 1934, however, the upper chamber of the flask was not attached to the substrate. Based on this character, Hadži (1951) erected the genus *Donsia* for this species.

Literature. Dons (1914, 1917, 1934b), Hadži (1951) and Kahl (1932).

Genus *Echinofolliculina* Dons 1934 (Fig. 3Z2)

Type species. Echinofolliculina mortenseni Dons, 1934

Species assigned. Echinofolliculina mortenseni Dons, 1934

Emended diagnosis. Flask single-chambered and elongate, bright to dark green; neck long and spirally sculptured; closure device composed of three pseudochitinous spikes, central one large and fish-hook-shaped, the other two smaller, on each side of the central spike; holdfast organelle with slightly widened end; macronucleus globular and single.

Remarks. Although the original description was relatively simple and lacked an illustration showing the closure device, there is sufficient information in the diagnosis of this genus supplied by Dons (1934a) to confirm its presence: "Zwicchen Wohnraum und Hals befindet sich ein eigentümlicher Schliessapparat, der aus einem Listchen besteht, mit 3 Stacheln versehen ist, von denen der mittlere ziemlich gross, bis 40 µm lang ist, wie eine Fischangel (jedoch ohne Widerhacken) geformt ist und mit gekrümmter Spitze nach aussen und nach oben hin gerichtet ist. An jeder Seite des Zentralstachel befindet sich ein kleiner Nebenstachel" [Translation: "There is a peculiar locking device, consisting of three nails, the middle of which is quite large and fish-hook shaped. On each side of the central one there is a small side-pin"].

Literature. Dons (1934a) and Hadži (1951).

Genus *Epifolliculina* Hadži, 1951 (Fig. 3Z3)

Type species. Epifolliculina diaphana Hadži, 1951

Species assigned. Epifolliculina diaphana Hadži, 1951

Emended diagnosis. Lorica colorless; flask recumbent and two-chambered; neck unsculptured and with a widened opening; closure device as two flaps, one dorsal and one ventral, ventral flap much larger than dorsal one, with one to several inner teeth in front of the ventral flap; macronucleus ovoidal and single.

Remarks. The main characteristics of this genus are the two unequal flaps of the closure device and the presence of one or more teeth in front of the ventral flap.

Literature. Hadži (1951).

Genus *Eufolliculina* Hadži, 1951 (Fig. 3J)

Type species. Eufolliculina moebiusi (Kahl, 1932) Hadži, 1951

Species assigned. Eufolliculina moebiusi (syn. *Eufolliculina ampullacea* Hadži, 1951); *E. latemarginata* (Hadži, 1938) Hadži, 1951; *E. lignicola* (Fauré-Fremiet, 1936)

Hadži, 1951; *E. brunea* Jankowski, 2009; *E. uhligi* Mulisch & Patterson, 1983

Emended diagnosis. Flask single-chambered, recumbent; neck short and either unsculptured or weakly sculptured; closure device absent; peristomial lobes slim and equal in length; holdfast organelle slim; macronucleus moniliform.

Remarks. In 1887, Möbius described a German population of *Folliculina ampulla* with a moniliform macronucleus. Kahl (1932) regarded Möbius' population as a different species and named it *Folliculina moebiusi* because of the macronuclear character. According to the combination of short unsculptured neck and moniliform macronucleus, Hadži (1951) erected the genus *Eufolliculina* for this species which he fixed as the type species, *E. moebiusi*. At the same time, three other species were transferred into *Eufolliculina*, that is, *E. ampullaceal*, *E. latemarginata,* and *E. lignicola* (Hadži 1951). Mulisch and Patterson (1983) provided a review of *Eufolliculina* based on the detailed characteristics of a new species, *E. uhligi*, found in Germany and regarded *E. ampullacea*, *E. latemarginata*, and *E. lignicola* as synonyms of *E. moebiusi.* We agree with this treatment. Recently, Jankowski (2009) described a new *Eufolliculina* species based on ecological samples collected from the Barents Sea and named it *E. brunea*. In a recent study, Ye et al. (2021b) provided a detailed redescription of *E. moebiusi* based on a China population. They deduced that *E. brunea* should be a synonym of *E. moebiusi*.

Literature. Hadži (1951), Jankowski (2009), Mulisch and Patterson (1983), Mulisch et al. (1986) and Ye et al. (2021b).

Genus *Folliculina* Lamarck, 1816 (Fig. 3E)

Type species. Folliculina ampulla (Müller, 1786) Lamarck, 1816

Species assigned. Folliculina ampulla (Müller, 1786) Lamarck, 1816; *F. boltoni* (Kent, 1881) Hadži, 1951; *F. simplex* Dons, 1912

Emended diagnosis. Flask single-chambered and broad; neck short and unsculptured; closure device absent; peristomial lobes broad with rounded ends, unequal in size; holdfast organelle slim; macronucleus single.

Remarks. This is the type genus of the family Folliculinidae. Since its establishment, many species have been assigned to or moved out of this genus. Kahl (1932) carried out a detailed review and described 14 *Folliculina* species. Das (1949) and Hadži (1951) also reviewed this genus and transferred many species to various newly erected genera. Hadži (1951) recognized only two valid species of *Folliculina*, namely *F. ampulla*, the type species, and *F. boltoni*. In the original description, *F. ampulla* has a broad lorica with a short and wide neck, short and rounded peristomial lobes, and multiple macronuclear nodules. Unfortunately, this species has never been re-collected so the macronuclear character cannot be verified. Some researchers even doubted the existence of this species (Das 1949). *Folliculina simplex* has been collected and redescribed several times, including Song et al. (2003) who supplied a detailed redescription based on specimens both in vivo and following protargol staining. Therefore, the emended diagnosis of *Folliculina* in the present work is mainly based on *F. simplex*. Although the diagnostic characters of the type species are controversial, *Folliculina* is the type genus of Folliculinidae, so we agree with Aescht (2001) that this genus should be a nomen conservandum.

Literature. Aescht (2001), Chen et al. (2011), Das (1949), Hadži (1951), Hu et al. (2019), Kahl (1932) and Song et al. (2003).

Genus *Folliculinopsis* Fauré-Fremiet in Hadži, 1951 (Fig. 3S)

Type species. Folliculinopsis annulata Fauré-Fremiet, 1936

Species assigned. Folliculinopsis annulata Fauré-Fremiet, 1936; *F. moebiusi* (Kahl, 1932) Hadži, 1938

Emended diagnosis. Flask single-chambered and recumbent; neck long and sculptured; closure device in the form of conical membrane; peristomial lobes slim with pointed tips; holdfast organelle slim; macronucleus moniliform.

Remarks. Fauré-Fremiet (1936) founded the genus *Folliculinopsis* for species with a moniliform macronucleus, a recumbent flask, and a long neck. He assigned seven species to this genus, including some with a closure device, but no type species was fixed. Therefore, although Fauré-Fremiet's genus is well known, it was regarded as a nomen nudum by Aescht (2001) and Lynn (2008). However, this genus was reactivated by Hadži (1951), who fixed *Folliculinopsis annulata* as the type species and supplied a new genus diagnosis. He also declared the genus *Alexandrina* Hadži, 1938, a junior synonym of *Folliculinopsis*.

Literature. Das (1949), Fauré-Fremiet (1936), Hadži (1938, 1951) and Ji et al. (2004).

Genus *Halofolliculina* Hadži, 1951 [nomen nudum] (Fig. 3Q)

Type species. Not designated

Species assigned. Halofolliculina elegans (Claparède & Lachmann, 1859) Hadži, 1951; *H. annulata* (Andrews, 1944) Hadži, 1951

Emended diagnosis. Flask single-chambered and recumbent; neck narrow; closure device as two flaps that protrude into the neck; peristomial lobes short, broad, with rounded ends, unequal in size; holdfast organelle slim; macronucleus single.

Remarks. The main diagnostic character of this genus is the combination of a single macronucleus, a single-chambered flask, and two closure flaps protruding into the neck. Aescht (2001) and Lynn (2008) regarded this genus as a nomen nudum for the lack of type species fixation. The validity of this genus awaits further investigation and the establishment of a type species.

Literature. Andrews (1944) and Hadži (1951).

Genus *Lagotia* Wright, 1858 (Fig. 3F)

Type species. *Lagotia viridis* Wright, 1858

Species assigned. *Lagotia viridis* Wright, 1858; *L. abyssorum* (Giard, 1883) Hadži, 1951; *L. aculeata* (Claparède & Lachmann, 1858) Hadži, 1951; *L. coerulea* Hadži, 1951; *L. donsi* Hadži, 1951; *L. dinaridica* Primc-Habdija & Matoničkin, 2005; *L. expansa* (Levinson, 1893) Hadži, 1951; *L. faurefremieti* Hadži, 1951; *L. flava* (Silén, 1947) Hadži, 1951; *L. gigantea* (Dons, 1914) Dons, 1934; *L. lutea* (Fauré-Fremiet, 1936) Hadži, 1951; *L. minima* Hadži, 1951; *L. minor* Dons, 1948; *L. obstetrica* (Wright, 1862) Hadži, 1951; *L. similis* (Dons, 1915) Dons, 1934; *L. spirobis* (Dons, 1912) Dons, 1934; *L. stylifer* (Wright, 1862) Hadži, 1951

Emended diagnosis. Flask single-chambered, slightly expanded and recumbent; neck either sculptured or unsculptured; closure device absent; peristomial lobes stiff; holdfast organelle slim; macronucleus single.

Remarks. The genus *Lagotia* was established by Wright (1858), but was treated as a synonym of *Folliculina* in several subsequent studies (Kahl 1932; Kent 1881–1882). Hadži (1951) provided an improved diagnosis based on *Lagotia viridis* Wright, 1858 and transferred into this genus many folliculinids with a single macronucleus. Mulisch et al. (1993) reinvestigated *Lagotia minor* using electron microscopy which revealed that the typical stiffness of the peristomial lobes was due to the arrangement of the nemadesmata of the adoral membranelles. Mulisch et al. (1993) considered this to be a more conservative feature than the morphology of the macronucleus and lorica. Dons (1934b) synonymized *Semifolliculina* Dons, 1914 with *Lagotia* and transferred its two species, *S. gigantea* Dons, 1914, and *S. mirabilis* Don, 1917, into this genus.

Literature. Dons (1914, 1917, 1934b), Hadži (1951), Kahl (1932), Kent (1881–1882), Mulisch et al. (1986, 1993) and Primc-Habdija and Matoničkin (2005).

Genus *Latifolliculina* Hadži, 1951 (Fig. 3U)

Type species. *Latifolliculina incolorea* Hadži, 1951

Species assigned. *Latifolliculina incolorea* Hadži, 1951 (syn. *Parafolliculina amphora* Dons, 1914 sensu Fauré-Fremiet, 1936)

Emended diagnosis. Flask recumbent and two-chambered; neck short and unsculptured; closure device absent; peristomial lobes conspicuously unequal in size; holdfast organelle wide spatula-shaped; macronucleus single.

Remarks. Fauré-Fremiet (1936) collected a folliculinid with two chambers from the Bay of Concarneau and identified it as *Parafolliculina amphora* Dons, 1914. However, Fauré-Fremiet's population lacked a closure device, so Hadži (1951) established the genus *Latifolliculina* for this organism.

Literature. Dons (1914), Hadži (1951) and Fauré-Fremiet (1936).

Genus *Magnifolliculina* Uhlig, 1964 [nomen nudum] (Fig. 3N)

Type species. Not designated

Species assigned. *Magnifolliculina alata* Uhlig, 1964; *M. binalata* Uhlig, 1964

Emended diagnosis. Lorica wall double-layered, outer layer forming single or multiple spines on flask; flask single-chambered and recumbent; neck short and unsculptured; closure device absent; peristomial lobes short and rounded, unequal in size; holdfast organelle slim; macronucleus single.

Remarks. Based on its double-layered lorica, Uhlig (1964) transferred *Parafolliculina roestenis* into this genus. However, *P. roestenis* has a closure device, so Hadži (1951) erected the genus *Perifolliculina* for this species (see *Remarks* in the section on *Perifolliculina*). Aescht (2001) and Lynn (2008) regarded this genus as nomen nudum for the lack of type species fixation. The validity of this genus awaits further investigation and the establishment of a type species.

Literature. Hadži (1951); Uhlig (1964).

Genus *Metafolliculina* Dons, 1924 (Fig. 3K)

Type species. *Metafolliculina nordgardi* Dons, 1924

Species assigned. *Metafolliculina nordgardi* Dons, 1924; *M. andrewsi* Hadži, 1938; *M. ballerina* Mulisch et al. 1986; *M. producta* (Wright, 1859) Dons, 1934 (syn. *M. longicollis* Hadži, 1938); *M. elongata* (Das, 1949) Hadži, 1951

Emended diagnosis. Flask single-chambered, recumbent, or vertical; neck long and sculptured, with prominent spiral or annular ridges; without a closure device; peristomial lobes long and slender, equal in size; holdfast organelle slim; macronucleus moniliform.

Remarks. Dons (1924) erected this genus for *Metafolliculina nordgardi*, which has a vertical lorica, although the original genus diagnosis did not mention this character: "Vorhandensein eines Rosenkranzkernes und andererseits durch einen verhältnismässig kleinen Wohnraum, einen hohen Hals und eine besonders kräftige Spiralleiste karakterisiert" (Dons 1924) [Translation: "macronucleus moniliform, flask small, neck high with particularly strong spiral bar"]. Dons (1934b) reviewed the genus *Metafolliculina* and supplied an emended diagnosis: "mit Rosenkranzkern, Wohnraum schmal, Hals sehr lang, mit kräftiger Spiralverdickung" [Translation: "macronucleus moniliform, flask narrow, neck very long with strong spiral cover"]. He also transferred *Lagotia producta* Wright, 1859 into this genus. Hadži (1951) made a detailed overview of this genus and recognized five valid species. Mulisch et al. (1986) discovered another new species in the United Kingdom and named it *M. ballerina*. Ye et al. (2021a) provided a detailed redescription of *M. producta* based on a Chinese population and described its life cycle for the first time. *Metafolliculina longicollis* was regarded by Ye et al. (2021a) as a junior synonym of *M. producta*.

Literature. Andrews (1952), Das (1949), Dons (1924, 1934b), Hadži (1938, 1951), Mulisch et al. (1986) and Ye et al. (2021a).

Genus *Mirofolliculina* Dons, 1927 (Fig. 3X)

Type species. Mirofolliculina limnoriae (Giard, 1883) Dons, 1927

Species assigned. Mirofolliculina limnoriae (Giard, 1883) Dons, 1927

Emended diagnosis. Flask single-chambered and recumbent, irregular in shape with peripheral part bulged; neck short and unsculptured; closure device absent; main body of trophont wide and with several holdfast organelles; peristomial lobes unequal in size; macronucleus moniliform.

Remarks. The original description of *M. limnoriae*, was very superficial and was based solely on a top view of contracted individuals. It was redescribed by Dons (1927) and although this too was based on contracted individuals, it was sufficient for Dons to establish a new genus for this species.

Literature. Dons (1927), Giard (1883) and Hadži (1951).

Genus *Pachyfolliculina* Hadži, 1951 (Fig. 3H)

Type species. Pachyfolliculina gunneri (Dons, 1927) Hadži, 1951

Species assigned. Pachyfolliculina gunneri (Dons, 1927) Hadži, 1951

Emended diagnosis. Flask single-chambered, short, almost cylindrical, and recumbent; neck very broad, short and unsculptured; closure device absent; peristomial lobes with rounded ends, unequal in size; macronucleus moniliform.

Remarks. Dons (1927) reported a folliculinid which he characterized as follows: "Das Haus ist ziemlich breit, schuhähnlich, mit kurzer, halb nacho ben gerichteter Mündung" [Translation: "Lorica quite wide, foamy, with short and half pointed neck"] and named it *Folliculina gunneri*. Hadži (1951) erected a new genus for this species based solely on this simple description. Therefore, this genus may be a synonym of *Eufolliculina,* however, more data are needed to verify this.

Literature. Dons (1927), Fauré-Fremiet (1936), Hadži (1951) and Silén (1947).

Genus *Parafolliculina* Dons, 1914 (Fig. 3Z)

Type species. Parafolliculina amphora Dons, 1914

Species assigned. Parafolliculina amphora Dons, 1914; *P. americana* Hadži, 1951; *P. glutinata* Hadži, 1951; *P. labiata* Dons, 1942; *P. tristanensis* Dons, 1948; *P. violacea* (Giard, 1888) Dons, 1914

Emended diagnosis. Flask two-chambered; neck may be short or long, unsculptured; closure device comprises two flaps, one dorsal and one slightly larger ventral; peristomial lobes equal in size; holdfast organelle slim; macronucleus single.

Remarks. This genus is characterized by the combination of a two-chambered flask and a closure device comprising two flaps. Kahl (1932) made a short review of this genus and listed four species: *P. amphora*, *P. hirundo*, *P. mirabilis,* and *P. violacea*. Hadži (1951) erected the genus *Donsia* for *P. mirabilis* because this species lacks a closure device and transferred *P. hirundo* to the newly established genus *Atriofolliculina* because it also lacks a closure device and its peristomial lobes are slim and of equal size.

Literature. Andrews (1941, 1942), Dons (1914, 1942), Fauré-Fremiet (1936), Hadži (1951) and Kahl (1932).

Genus *Pebrilla* Giard, 1888 (Fig. 3W)

Type species. Pebrilla paguri Giard, 1888

Species assigned. Pebrilla paguri Giard, 1888

Emended diagnosis. Flask recumbent and two-chambered, with a deep transverse median wrinkle; neck short and unsculptured; closure device absent; peristomial lobes equal in size; holdfast organelle stud-like; macronucleus moniliform.

Remarks. The main features that characterize this genus are the flask with a very conspicuous transverse median wrinkle and the moniliform macronucleus.

Literature. Fauré-Fremiet (1936), Giard (1888) and Hadži (1951).

Genus *Pedifolliculina* Hadži, 1951 (Fig. 3C)

Type species. Pedifolliculina arctica (Dons, 1917) Hadži, 1951

Species assigned. Pedifolliculina arctica (Dons, 1917) Hadži, 1951

Emended diagnosis. Flask single-chambered and upright, spirally sculptured; basal plate long and cylindrical; closure device absent; holdfast organelle slim; macronucleus single.

Remarks. Dons (1912) collected this species from the Norwegian coast and described it as *Folliculina melitta* Laackmann, 1910. Subsequently, he erected the genus *Pseudofolliculina* for this species (Dons 1914). He later noticed several differences between his organism and *P. melitta* sensu Laackmann (1910) and established a new species, *Pseudofolliculina arctica,* for the Norwegian population (Dons 1917). Hadži (1951) listed several morphological differences between *P. arctica* and *P. melitta*, such as the presence (vs. absence) of a stalk-like basal plate and the absence (vs. presence) of a closure device, so he erected the genus *Pedifolliculina* for the former. However, most subsequent researchers did not accept this genus and regarded it as a junior synonym of *Pseudofolliculina*. Unfortunately, *P. arctica* has not been studied using modern methods. In the absence of any new evidence, we agree on the establishment of *Pedifolliculina* by Hadži (1951).

Literature. Dons (1912, 1914, 1917), Hadži (1951) and Laackmann (1910).

Genus *Perifolliculina* Hadži, 1951 (Fig. 3Z1)

Type species. Perifolliculina roestensis (Dons, 1931) Hadži, 1951

Species assigned. Perifolliculina roestensis (Dons, 1931) Hadži, 1951

Emended diagnosis. Flask two-chambered and recumbent; neck short and unsculptured; closure device comprises two flaps (right and left); peristomial lobes slightly unequal in size; macronucleus single.

Remarks. Hadži (1951) erected this genus based only on the rather superficial description supplied by Dons (1931) but noted that the lack of information on the living trophont might question its validity. In our opinion, the structure of the closure device (with one right and one left flap) is sufficient to differentiate this taxon from all other known genera. Therefore, we consider *Perifolliculina* to be a valid genus.

Literature. Dons (1931) and Hadži (1951).

Genus *Planifolliculina* Hadži, 1951 (Fig. 3Z5)

Type species. Planifolliculina cumbens Hadži, 1951

Species assigned. Planifolliculina cumbens Hadži, 1951

Emended diagnosis. Flask recumbent and two-chambered; neck extremely short; closure device in the form of semicircular pseudochitinous plate rising obliquely from the ventral inner wall; holdfast organelle slim; macronucleus single.

Remarks. The main feature that characterizes this genus is the structure of the closure device.

Literature. Hadži (1951).

Genus *Platyfolliculina* Hadži, 1938 (Fig. 3I)

Type species. Platyfolliculina sahrhageana Hadži, 1938

Species assigned. Platyfolliculina sahrhageana Hadži, 1938; *P. paguri* Andrews & Reinhard, 1943

Emended diagnosis. Flask single-chambered and recumbent; neck short and unsculptured; closure device absent; peristomial lobes short and slightly uneven in size; holdfast organelle spatula-shaped; macronucleus moniliform.

Remarks. This genus is characterized by the combination of its moniliform macronucleus and spatula-shaped holdfast organelle.

Literature. Andrews and Reinhard (1943) and Hadži (1938, 1951).

Genus *Priscofolliculina* Deflandre & Deunff, 1957

Type species. Priscofolliculina. pulchra Deflandre & Deunff, 1957

Species assigned. Priscofolliculina pulchra Deflandre & Deunff, 1957; *P. elongata* Deflandre & Deunff, 1957; *P. aegrota* Deflandre & Deunff, 1957; *P. oblonga* Deflandre & Deunff, 1957; *P. lelayi* Deflandre & Deunff, 1957; *P. annuligera* Deflandre & Deunff, 1957

Emended diagnosis. Flask rounded or ovoidal, opening rounded.

Remarks. This genus is known only from fossil specimens. Information is therefore confined to the lorica shape and size.

Literature. Deflandre and Deunff (1957).

Genus *Pseudofolliculina* Dons, 1914 (Fig. 3D)

Type species. Pseudofolliculina melitta (Laackmann, 1910) Dons, 1914

Species assigned. Pseudofolliculina melitta (Laackmann, 1910) Dons, 1914

Emended diagnosis. Flask single-chambered, cylindrical and upright; basal plate columnar and hollow; closure device in the form of two flaps; posterior region of trophont very slim, passes through hollow basal plate and attaches directly to substrate; macronucleus single.

Remarks. Dons (1914) established *Pseudofolliculina* for *Folliculina melitta* sensu Laackmann, 1910, for species having an upright lorica, a stalk-like basal plate and two closure devices. Subsequent researchers widely accepted this genus, but they often misdated the authority, for example, "1915" in Kahl (1932) and Fauré-Fremiet (1936).

Literature. Aescht (2001), Corliss (1979), Dons (1912, 1914), Fauré-Fremiet (1936), Kahl (1932), Laackmann (1910), Lynn (2008) and Silén (1947).

Genus *Pseudoparafolliculina* Andrews & Nelson, 1942 (Fig. 3R)

Type species. Pseudoparafolliculina portitor Andrews & Nelson, 1942

Species assigned. Pseudoparafolliculina portitor (syn. *Tapetofolliculina portitor* Andrews & Nelson in Hadži, 1951)

Emended diagnosis. Flask single-chambered, upright; neck short and unsculptured; basal plate absent; closure device comprises two flaps; peristomial lobes short and of unequal size; holdfast organelle slim; macronucleus moniliform.

Remarks. Hadži (1951) listed the type species as "*Tapetofolliculina portitor* Andrews & Nelson, 1950"and renamed the genus without giving a reason. So *Tapetofolliculina* is an objective synonym of *Pseudoparafolliculina* due to these two genera having the same type species (Aescht 2001).

Literature. Aescht (2001), Andrews and Nelson (1942) and Hadži (1951).

Genus *Splitofolliculina* Hadži, 1951 [nomen nudum] (Fig. 3Z6)

Type species. Not designated

Species assigned. Splitofolliculina adherens, Hadži, 1951; *S. longicollis,* Hadži, 1951

Emended diagnosis. Flask recumbent and three-chambered with a transverse furrow between adjacent chambers; neck short and unsculptured; closure device comprises two flaps (dorsal and ventral); peristomial lobes long and narrow; macronucleus single.

Remarks. Aescht (2001) and Lynn (2008) regarded this genus as nomen nudum for the lack of type species fixation. In Folliculinidae, this is the only genus with a three-chambered flask. The validity of this genus awaits further investigation and the establishment of a type species.

Literature. Hadži (1951).

Genus *Stentofolliculina* Hadži, 1938 (Fig. 3B)

Type species. Stentofolliculina tubicola Hadži, 1938

Species assigned. Stentofolliculina tubicola Hadži, 1938

Emended diagnosis. Lorica single-chambered, upright, and cylindrical, without division into flask and neck; closure device absent; peristomial lobes slightly unequal in size; holdfast organelle slim; macronucleus moniliform.

Remarks. Stentofolliculina has a simple cylindrical lorica that is not divided into a flask and neck. Based on this character, Hadži (1951) considered this to be the most primitive genus in the family Folliculinidae.

Literature. Hadži (1938, 1951).

Genus *Valletofolliculina* Andrews, 1953 (Fig. 3M)

Type species. Valletofolliculina bicornis Andrews, 1953

Species assigned. Valletofolliculina bicornis Andrews, 1953

Emended diagnosis. Flask single-chambered and recumbent, with two prominent hollow horns on the dorsal surface, posterior ends of horns spine-like and protruding beyond posterior margin of flask; neck short and unsculptured; closure device absent; macronucleus single.

Remarks. The type species was collected from sediment at a depth of about 30 min Tomales Bay, California, USA. The original description was based solely on the lorica.

Literature. Andrews (1953).

Key to 33 folliculinid genera

The key is mainly based on the six characters discussed above that can be recognizable without silver staining or electron microscopy. Nonetheless, available infraciliature or ultrastructure should be applied because unknown species, or insufficiently characterized genera, may differ in features recognizable only by their infraciliature or ultrastructure.

1 Lorica absent............................*Bickella* (Fig. 3A)
– Lorica present...2
2 Basal plate solid and stalk-like....................................3
– Basal plate thin and jelly-like......................................5
3 Macronucleus moniliform...*Stentofolliculina* (Fig. 3B)
– Macronucleus as a single nodule................................4
4 Closure device absent...*Pedifolliculina* (Fig. 3C)
– Closure device present...*Pseudofolliculina* (Fig. 3D)
5 Flask single-chambered..6
– Flask with two or more chambers............................21
6 Closure device absent...7
– Closure device present..16
7 Flask with special structure(s), i.e., double-layered wall and/or with spines and/or with spine-like processes..8
– Flask without special structures................................10
8 Flask wall double-layered, without spines..*Botticula* (Fig. 3L)
– Flask wall single-layered, with ridges...........................9
9 Flask with conspicuous horns posterior ends of which extend as spine-like processes...*Valletofolliculina* (Fig. 3M)
– Flask with inconspicuous spine(s)-...*Magnifolliculina* (Fig. 3N)

10 Lorica neck normal size, not heavily sculptured..........11
– Lorica neck very long, heavily sculptured. ....*Metafolliculina* (Fig. 3K)
11 Macronucleus as a single nodule..........12
– Macronucleus moniliform..........14
12 Holdfast organelle not spatula-shaped..........13
– Holdfast organelle spatula-shaped.......... ....*Ascobius* (Fig. 3G)
13 Peristomial lobes flexible.......... ....*Folliculina* (Fig. 3E)
– Peristomial lobes inflexible..........*Lagotia* (Fig. 3F)
14 Holdfast organelle not spatula-shaped. 15
– Holdfast organelle spatula-shaped.......... ....*Platyfolliculina* (Fig. 3I)
15 Lorica almost cylindrical, neck broad and hardly bent- ....*Pachyfolliculina* (Fig. 3H)
– Neck narrower than flask, bent about 30–45° angle. ....*Eufolliculina* (Fig. 3J)
16 Flask upright..........*udoparafolliculina* (Fig. 3R)
– Flask recumbent..........17
17 Closure device in the form of two flaps..........18
– Closure device in the form of conical membrane..........20
18 Macronucleus moniliform. ....*Ampullofolliculina* (Fig. 3O)
– Macronucleus as a single nodule..........19
19 Holdfast organelle spatula-shaped.......... ....*Diafolliculina* (Fig. 3P)
– Holdfast organelle not spatula-shaped.......... ....*Halofolliculina* (Fig. 3Q)
20 Macronucleus moniliform.......... ....*Folliculinopsis* (Fig. 3S)
– Macronucleus as a single nodule. ....*Claustrofolliculina* (Fig. 3T)
21 Closure device absent..........22
– Closure device present..........26
22 Flask single-chambered, irregular in outline, peripheral part bulging..........*Mirofolliculina* (Fig. 3X)
– Flask two-chambered, regular in outline, peripheral part smooth..........23
23 Front chamber of flask not attached to substrate.......... ....*Donsia* (Fig. 3Y)
– Both chambers of flask attached to substrate..........24
24 Macronucleus moniliform.......... ....*Pebrilla* (Fig. 3W)
– Macronucleus as a single nodule..........25
25 Holdfast organelle stem-shaped, as wide as main body..........*Latifolliculina* (Fig. 3U)
– Holdfast organelle slim.......... ....*Atriofolliculina* (Fig. 3V)..
26 Closure device in form of two flaps..........2
7– Closure device as a special structure, i.e., as three spikes, or as a diaphragm with an M-shaped gap or as a semicircular pseudochitinous plate..........30
27 Closure device comprises one right flap and one left flap..........*Perifolliculina* (Fig. 3Z1)
– Closure device comprises one dorsal flap and one ventral flap..........28
28 Flask two-chambered..........29
– Flask three-chambered.......... ....*Splitofolliculina* (Fig. 3Z6)
29 Ventral flap of closure device larger than dorsal flap, with inner teeth..........*Epifolliculina* (Fig. 3Z3)
– Two flaps of closure device almost equal, without inner teeth..........*Parafolliculina* (Fig. 3Z)
30 Neck short, unsculptured.......... ....*Planifolliculina* (Fig. 3Z5)
– Neck long, sculptured..........31
31 Closure device in form of diaphragm with a M-shaped gap..........*Aulofolliculina* (Fig. 3Z4)
– Closure device as three spikes, central one hook-shaped ....*Echinofolliculina* (Fig. 3Z2)

Molecular phylogeny of folliculinids among related heterotrich ciliates

The maximum likelihood (ML) and Bayesian inference (BI) trees based on SSU rDNA data had nearly identical topologies, therefore only the ML tree is shown here with support values from both analyses (Fig. 5). In the molecular phylogenetic tree, all folliculinid sequences clustered together, indicating that the family Folliculinidae is monophyletic. The family Folliculinidae forms a well-supported clade (91% ML, 1.00 BI) that is sister to *Maristentor dinoferus*, the monotypic member of family Maristentoridae.

There are two strongly supported subclades of Folliculinidae, i.e., subclades I and II. Subclade I comprises two lineages: *Metafolliculina* and *Eufolliculina*. In the *Metafolliculina* lineage, *M. producta* clusters with an unidentified *Metafolliculina* species with high support (96% ML, 0.95 BI). In the *Eufolliculina* lineage, *E. uhligi* clusters with *E. moebiusi* with almost full support (98% ML, 1.00 BI). Subclade II includes three lineages: *Folliculina*, *Ampullofolliculina,* and *Diafolliculina*. There is only one sequence in the *Diafolliculina* lineage, i.e., *D. longilobata*, which branches off first with very high support (99% ML, 1.00 BI). The monophyly of each of the other two lineages (*Folliculina* and *Ampullofolliculina*) is fully supported (100% ML, 1.00 BI). *Ampullofolliculina* clusters with two species of *Folliculina* with moderate support (76% ML, 0.78 BI). The two

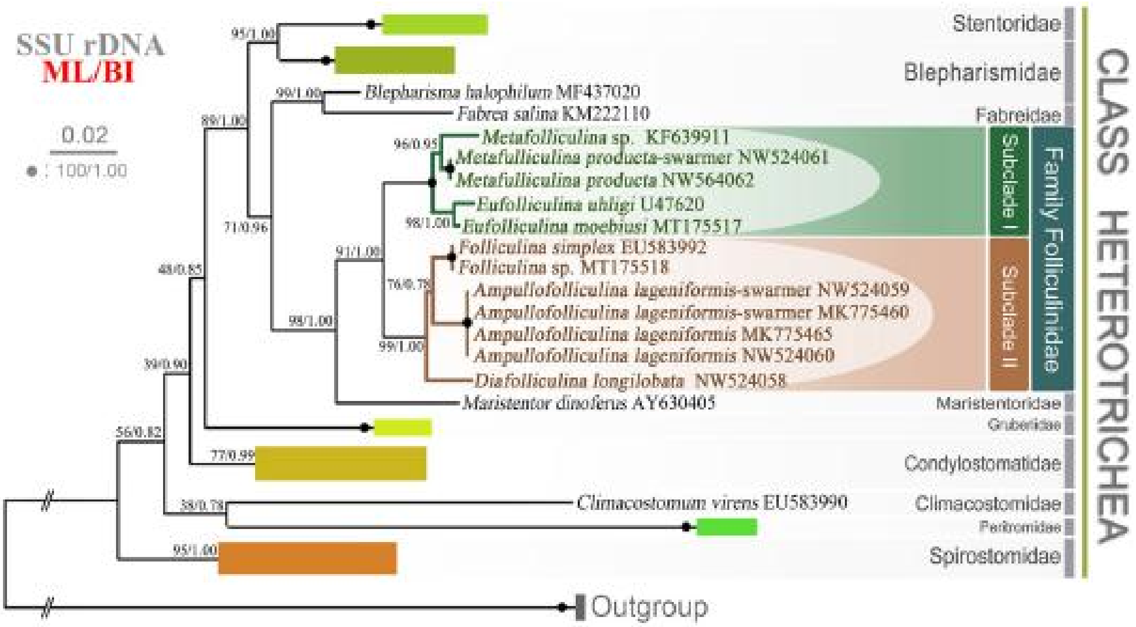


Fig. 5 Maximum likelihood tree inferred from SSU rDNA sequences showing the systematic position of the family Folliculinidae. Numbers near the nodes represent the ML bootstrap values and BI posterior probability values. Asterisks indicate the disagreement between the ML and BI trees, question marks denote sequences the identities which are controversial. Fully supported (100/1.00) branches are marked with solid circles. The bar corresponds to 2 substitutions per 100 nucleotide positions

sequences of *Folliculina simplex* cluster with the other *Folliculina* species with maximal support (100% ML, 1.00 BI).

Discussion on folliculinid phylogeny

In the SSU rDNA tree (Fig. 5), among the ten heterotrich ciliate families, only five (Stentoridae, Blepahrismidae, Fabreidae, Folliculinidae, and Maristentoridae) have very stable relationships. In contrast, the phylogenetic relationships of the other five families (Climacostomidae, Condylostomatidae, Gruberiidae, Peritromidae, and Spirostomidae) are not well resolved, as demonstrated by the low support values at the node of each (Fig. 5) (Campello-Nunes et al. 2020; Chen et al. 2019; Fernandes et al. 2016; Luo et al. 2019; Shazib et al. 2014; Yan et al. 2016).

The family Folliculinidae comprises 33 genera, only five of which have molecular data. These five genera are divided into two highly supported subclades (Figs. 5, 6). Subclade I consists of *Metafolliculina* and *Eufolliculina.* The common characteristics of these two genera are a spirally ridged neck, the absence of a closure device, moniliform macronucleus, slim holdfast organelle, and extremely flexible peristomial lobes that are equal in size and shape and are connected by a pellicular flap located at the junction of the left and right lobes on the ventral side. Moreover, these two genera have identical life cycles (Andrews 1952; Hadži 1951; Mulisch and Patterson 1983, 1987; Ye et al. 2021a, b).

In subclade II, three genera, i.e., *Folliculina, Ampullofulliculina,* and *Diafolliculina*, uniquely share a combination of inflexible peristomial lobes and a smooth lorica neck. Other morphological features do not reflect their phylogenetic affiliation. For example, *Ampullofolliculina* differs from *Folliculina* and *Diafolliculina* in the macronuclear shape (moniliform vs. single ovoidal); *Folliculina* and *Ampullofulliculina* have slim holdfast organelles, whereas in *Diafolliculina* the holdfast organelle is spatula-shaped; and *Ampullofolliculina* and *Diafolliculina* have closure devices, whereas *Folliculina* lacks a closure device (Chen et al. 2011; Hadži 1951; Song et al. 2003; Ye et al. 2021a, b). Among the six morphological characters used here for defining and identifying folliculinid genera, only the flexibility of peristomial lobes and the sculpturing of the neck correspond to the topology of the SSU rDNA tree. These findings support the assertion proposed by Mulisch et al. (1993) that the family Folliculinidae can be divided into two separate evolutionary lines according to the flexibility or stiffness of the peristomial lobes.

Evolutionary significance of morphological features

The family Folliculinidae is a diverse group with 33 genera and 80 valid species. However, most species descriptions lack information on certain taxonomic features as they have not been examined using modern methods. For example,

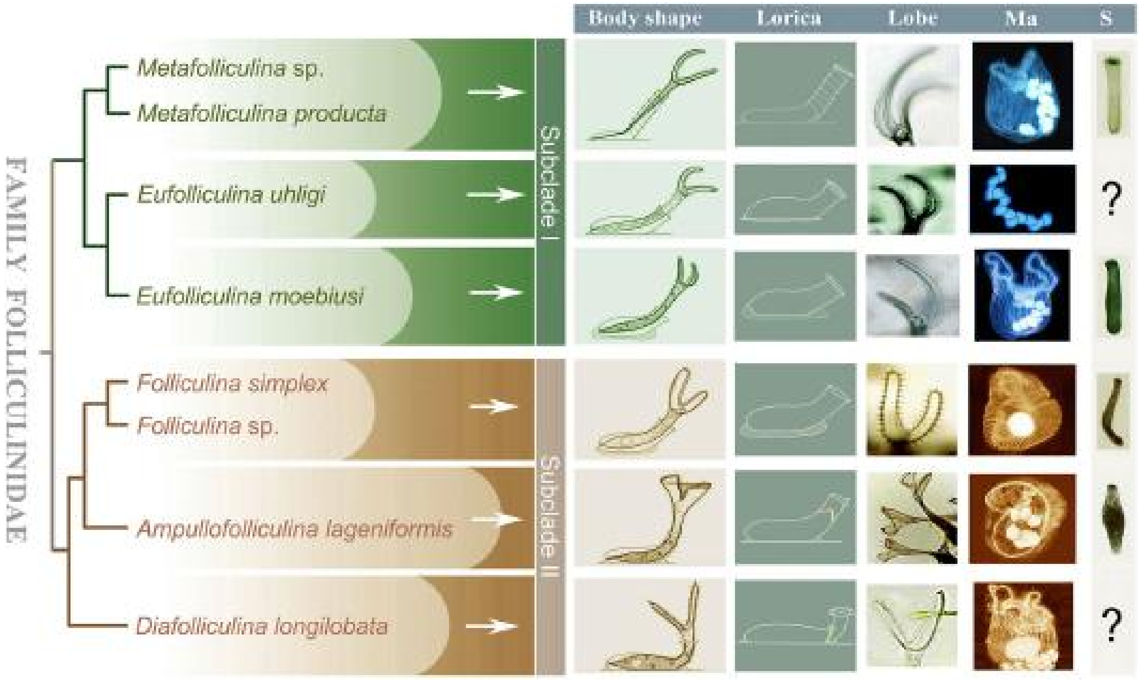


Fig. 6 Maximum likelihood tree inferred from SSU rDNA sequences showing the systematic positions of *Metafolliculina producta, Eufolliculina uhligi, Eufolliculina moebiusi, Folliculina simplex, Ampullofolliculina lageniformis* and *Diafolliculina longilobata*. Question marks denote swarmer not observed. *Ma* macronucleus, *S* swarmer

infraciliature information is available for only nine species and molecular data are available for only six species. The lack of sufficient, accurate information makes it challenging to analyze the evolutionary relationships of this family. In the present work, a simple phylogenetic diagram was constructed to show the possible evolution of Folliculinidae (Fig. 6). The topology of this diagram is mainly consistent with the SSU rDNA tree. We combined six generic characteristics into this evolutionary diagram. Most of these morphological features are not concordant with the phylogenetic diagram. However, only the flexibility of the peristomial lobes and the sculpturing of the lorica neck support the division of the Folliculinidae into two subclades, i.e., subclade I (with flexible peristomial lobes and a sculptured lorica neck) and subclade II (with inflexible peristomial lobes and a smooth lorica neck), with strong support (91% ML; 1.00 BI) in the SSU rDNA tree.

To better analyze evolutionary relationships within the family Folliculinidae, we hypothesize and discuss the roles of the six morphological characteristics listed above that we used to define the 33 known genera. Although folliculinids have other taxonomically informative features, such as cortical granules, somatic kineties, and habitat, they currently cannot be applied to the systematics of folliculinids for a variety of reasons. For example, in the case of cortical granules and somatic kineties, there is a lack of information for a sufficient number of taxa. Concerning habitat, there is a hypothesis based on studies of other organisms that freshwater groups evolved later than marine groups (Subirana and Colom 1987). However, there is no evidence to support this inference in ciliate research. Here, we will only analyze and discuss the six features mentioned above.

The flask, the closure device, and the neck are essential parts of the lorica, and they are critical in safeguarding the trophont. There are many types and structures of the flask: thin or thick/double layers wall, one or more chambers, and special features such as spines. A flask with a thick or double-layered wall (genera *Botticula* and *Magnifolliculina*) makes the lorica stronger and more resistant to external forces. A flask with two chambers (genera *Aulofolliculina*, *Epifolliculina*, *Latifolliculina*, *Parafolliculina*, *Perifolliculina*, and *Planifolliculina*) or three chambers (genus *Splitofolliculina*) also provides better protection for the trophont. Two genera (*Magnifolliculina* and *Valletofolliculina*) have spines on the flask surface. Andrews (1953) speculated that these might provide some protection against the rasping effect of some gastropods, whereas Uhlig (1964) posited that the spines were reinforcing strips to strengthen the stability of the lorica. In any case, the multiple chambers or special structures can better protect the organism.

The closure device is quite effective in safeguarding the trophont. For example, we recently found a folliculinid without a closure device that was attacked and hunted by *Loxophyllum* species. Another folliculinid was invaded and occupied by rotifers to lay eggs (unpublished). Such incidents have never been reported for genera with closure devices, such as *Ampullofolliculin*a and *Diafolliculina* (Andrews 1921, 1949a; Das 1949; Dons 1934b; Fauré-Fremiet 1936; Hu et al. 2019; Ji et al. 2004; Luo et al. 2019). Similarly, there is a strong association between the lorica neck structure and protection. For example, a slender and long neck would be more protective, and sculpturing can strengthen the neck to extend it better. The most typical example is *Metafolliculina producta*, whose long neck is supported by spiral sculptures and can be extended several times (Ye et al. 2021a, b).

In view of the above, we can draw the following preliminary conclusions: complex and strongly constructed loricae (multiple chambers, presence of closure device, long neck) can be more effective in defending the trophont against predation or invasion. Therefore, the evolutionary direction inferred from lorica features should be from single chamber to multiple chambers, from a short and wide opening neck to a long neck, and from the absence of a closure device to the presence of a closure device. The topology of the SSU rDNA tree is concordant with the characteristics of the neck; that is, subclade I, which comprises two genera with a sculptured neck, is separated from subclade II, the members of which have a short, smooth neck. Although the presence or absence of the closure device does not agree with the molecular phylogeny, e.g., *Ampullofolliculina* and *Diafolliculina* (with closure devices) cluster with *Folliculina* (without a closure device), it is likely that the lack of sequence data affected the molecular phylogenetic analyses.

The peristomial lobes are intimately tied to the capacity for feeding. Flexible lobes can be arranged in a variety of shapes to form water currents in diverse directions for better feeding. Furthermore, flexible lobes are more readily contracted, create folds to minimize their size, and can retract quickly into the lorica when simulated. We found that the flexibility of the peristomial lobes closely corresponds to the topology of the SSU rDNA tree, that is, *Metafolliculina* and *Eufolliculina* (with flexible lobes) form subclade I, whereas *Folliculina*, *Ampullofolliculina*, and *Diafolliculina* (with inflexible lobes) form subclade II. These findings support the assertion that the flexibility of the peristomial lobes is a phylogenetically informative character for determining evolutionary relationships within the family Folliculinidae (Mulisch et al. 1993).

The holdfast organelle connects the trophont to the lorica and consists of one to several bundles of cytoplasmic fibrils. Based on the size of the fiber bundle, the holdfast organelle can be divided into three types: spatula-shaped, stem-shaped, and slender-shaped. Furthermore, based on previous experimental results (Ye et al. 2021a, b), there is a direct correlation between the size of the holdfast organelle and the extensibility of the trophont, i.e., the trophont has more extensibility when it has a slender holdfast organelle. Therefore, we speculate that folliculinids with a slender holdfast organelle diverged later than those with a broad holdfast organelle.

In general, macronuclear type is an important taxonomic feature. It also plays a particular role in the evolution of some heterotrichous ciliates. For example, the evolutionary direction predicted by macronucleus shape in *Spirostomum* is consistent with the topology of the SSU rDNA tree, i.e., single macronucleus is the least evolved and moniliform macronucleus is the most developed (Boscaro et al. 2014; Chi et al. 2020a, 2021, 2022). However, in most species of ciliates, the number and shape of macronucleus are not well represented in evolutionary relationships. In the case of folliculinid ciliates, the number and shape of the macronucleus are not concordant with the topology of the SSU rDNA tree, so they do not reflect evolutionary relationships among folliculinids.

Based on the SSU rDNA tree, we propose an evolutionary direction of the morphological characters available in the folliculinids. The transformation of morphological traits is assumed to have changed from a simple lorica (single chamber, without a closure device, short and smooth neck) to a complicated lorica (multiple chambers, with a closure device, long and sculptured neck); from inflexible peristomial lobes to flexible peristomial lobes; and from a broad holdfast organelle to a slender holdfast organelle. Unfortunately, only five (*Metafolliculina*, *Eufolliculina*, *Folliculina*, *Ampullofolliculina*, and *Diafolliculina*) of the 33 known folliculinid genera have both comprehensive morphological and SSU rDNA sequence data. Furthermore, *Lagotia*, *Folliculinopsis,* and *Bickella* only have detailed morphological data obtained by silver-staining or electron microscopy. Therefore, more morphological and molecular data and greater taxon sampling are required to reconstruct an accurate evolutionary scenario of the family Folliculinidae.

Materials and methods

Construction of phylogenetic trees

Sixty-four representative sequences of heterotrichid species with five sequences of karyorelictid species as the outgroup (Supplementary Table S1) were retrieved from the GenBank database and used for phylogenetic analyses. All sequences were aligned with the MAFFT algorithm applying the default parameters provided on the webserver GUIDANCE (Penn et al. 2010a, b). BioEdit v.7.1.3.0 (Hall 1999) was used to trim the ends of alignments. Hypervariable sites were removed using Gblocks v.0.91b with default settings (Castresana 2000; Talavera and Castresana 2007), which resulted in a matrix of 1558 characters.

Maximum likelihood (ML) analysis was performed on GIPRES Science Gateway with RAxML-HPC2 v.8.2.11 on XSEDE (Stamatakis et al. 2008). Bayesian inference (BI) analysis was conducted with MrBayes v.3.2.7 on XSEDE (Ronquist et al. 2012) on the online CIPRES Science Gateway web server, using the GTR + I + G model (selected by MrModelTest v.2.3) (Nylander 2004). The chain length of Markov chain Monte Carlo (MCMC) simulations was 10,000,000 generations with a sampling frequency of every 100th generation. The first 25% of sampled trees were discarded as burn-in. All remaining phylogenetic trees were used to calculate the posterior probabilities using a majority rule consensus. MAGA v.5.0 (Tamura et al. 2011) and TreeView v.1.6.6 (Page 1996) were used to visualize tree topologies. Systematic classification mainly followed Chi et al. (2021), Gao et al. (2016), Hadži (1951), and Shazib et al. (2014).

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Author contributions TY: methodology, visualization, writing original draft, reviewing and editing the manuscript. SC and YX: cultivation. YJ and LL: phylogeny. SA: methodology. MS: writing and editing the manuscript. XC: conceptualization, supervision, writing, reviewing and editing the manuscript. All authors read and approve the final version of the manuscript.

Data availability The data used to support the findings of this study are included within the article.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Animal and human rights statement We declare that all applicable international, national, and/or institutional guidelines for sampling, care, and experimental use of organisms for the study have been followed and all necessary approvals have been obtained.

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