

## ***Eufolliculina brunea* sp. nov. (Ciliophora, Heterotrichida), a symbiont of wood-boring *Limnoria* isopods**

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Two well-known and one new species of folliculinids, obligate symbionts of the gribble (*Limnoria* Leach, 1814) are illustrated, and the new species is described in detail. Its placement in *Eufolliculina* Hadzi, 1951 is temporary because of the doubtful taxonomic status of this genus.

**Key words:** Ciliophora, Heterotrichida, *Eufolliculina*, new species

### **INTRODUCTION**

Two species of folliculinids associated with *Limnoria* Leach, 1814 are very widespread with their hosts – *Pachyfolliculina gunneri* (Dons, 1927) in massive dark-brown shells on the ventral side of the thorax (Figs 5–7) and *Mirofolliculina limnoriae* (Giard, 1883) with folded blue-green shells on the pleotelson (Figs 4, 8–10). There is evidence of the presence of a third form, that differs morphologically from the both mentioned species and has the restricted host specificity and distribution.

Le Veque (1947) described *M. limnoriae* in the text, but his single photograph shows this third form, not a true *M. limnoriae*; the host gribble was identified as *L. lignorum* (Rathke, 1799) in samples along the Californian coast. Mohr (1959) corrected these mistakes, and the hosts were identified as *L. lignorum*, *L. tripunctata* Menzies, 1951 and *L. quadripunctata* Holthuis, 1949. In the Mohr's (1959) short review of protozoan symbionts of these gribbles some photographs of a true *M. limnoriae* and of the third symbiont were given. The last one was regarded as “form of *M. limnoriae* in temperate zone”, although they occurred in the same samples of bored wood. Brunel (1963) also recognized the existence of two different folliculinids on the dorsum of gribbles in

Atlantic coastal waters of Canada, but again the aberrant species (here on *L. japonica* Richardson, 1909, common in region of sampling in spite of its name) was considered as a local (geographic) form of *M. limnoriae*; this statement cannot be proved. This third “form” has never been described and named, and its generic placement within the family Folliculinidae Dons, 1914 (with some 30 generic names, including supposed synonyms) is unknown.

In the present article, this third symbiont is recognized as a valid new species, not a “local form” of *M. limnoriae*, and its generic placement is discussed below (see Discussion). Formally it belongs to *Eufolliculina* Hadzi, 1951, one of most difficult genera in protozoan taxonomy.

### **TAXONOMIC PART**

#### **Order HETEROTRICHIDA Stein, 1859**

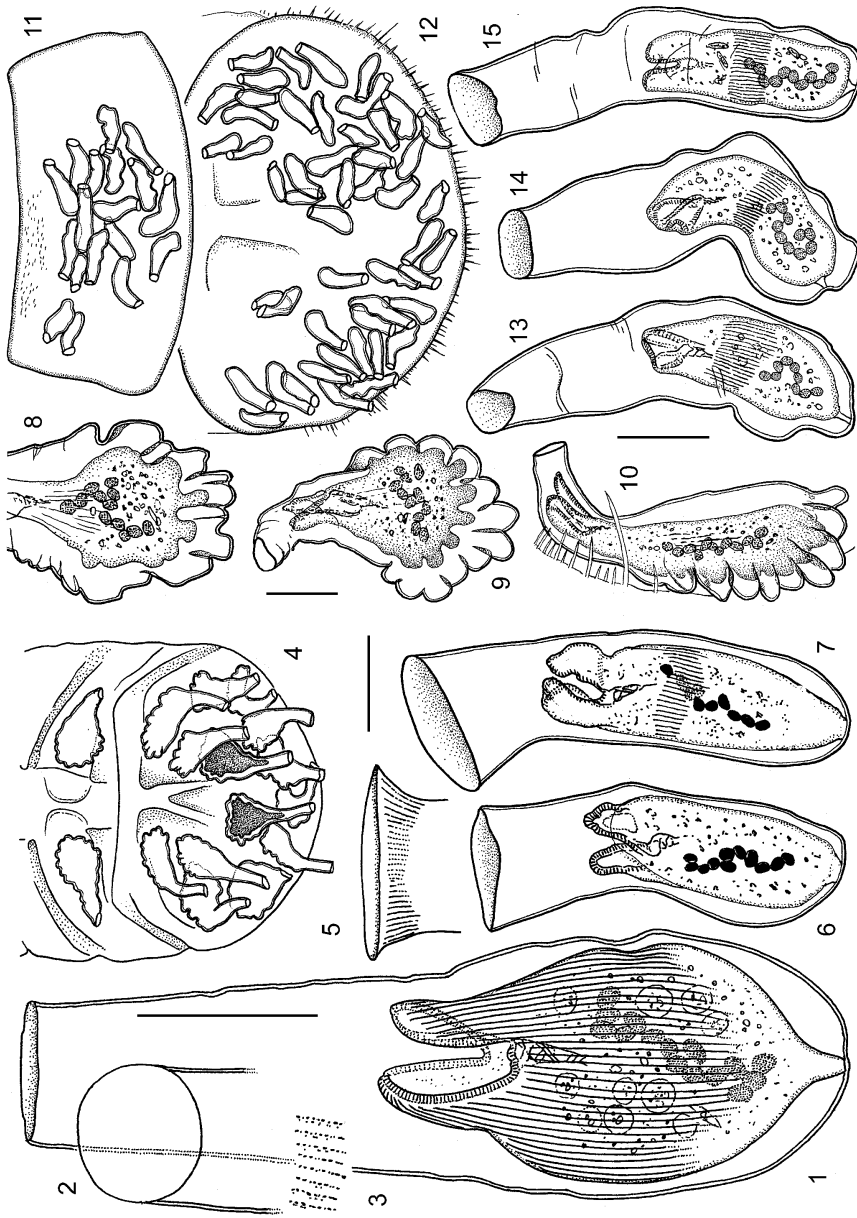
#### **Family FOLLICULINIDAE Dons, 1914**

#### **Genus *Eufolliculina* Hadzi, 1951**

#### ***Eufolliculina brunea* sp. nov.**

(Figs 1–3, 11–15)

*Hapantotype* (holotype). A slide, 1968-71, include fragments of *Limnoria borealis* Kussakin, 1963, from Kola Bay, Barents sea, from samples



**Figs 1–15.** Folliculinidae on wood-boring isopods (gribbles) *Limnoria borealis* (1–3, 11–15) and *Limnoria lignorum* (4–10): 1–3, *Eufolliculina brunea* sp. nov., a contracted zooid in the shell (1), a round shell mouth (2), granules along the somatic kineties (3); 4, 8–10, *Mirofolliculina limnoriae* on a pleotelson; 5–7, *Pachyfolliculina gunneri* on ventral side of the thorax; 11–15, *Eufolliculina brunea* sp. nov. on the dorsal side of the somite and the pleotelson. Orcein staining. Scale bars: 50  $\mu$ m.

dated 1932, with numerous specimens of *Eufolliculina brunea* on the pleotelson and the adjacent somites; deposited in the Laboratory of Protozoology, Zoological Institute, Academy of Sciences, Saint Petersburg, Russia.

*Additional material.* A slide 1968–72; same as 1968–71.

Formol-fixed fragments were refixed in Bouin's fluid, hydrolyzed in 10% HCl at room temperature during 25 minutes and then stained with orcein, for intense staining of macronuclear chains. After dehydration in dioxan, fragments were covered by cover-glasses with thin layer of Canadian balsam on underside, thus permitting oil immersion study. Brown colour of shells is still perfectly retained (and also in two other symbionts treated in a similar way) and fine details of the zooids and the shell are well visible.

*Description.* Shell size 202–234 × 54–71 μm (20 measurements at oil immersion, 10 × 90); shell mouth width 32–41 μm; shell brown-colored, narrow, elongated, with straight or slightly wavy contour, sometimes curved, almost entirely attached to substratum. Zooid chamber (ampulla) gradually pass into wide long neck, usually without definite separation from ampullar region occupied by contracted zooid; neck raised above telson, when viewed in lateral view, and less distinct in upper view; neck mouth round, neck rim not campanulate. Any additional structures of shell, common in folliculinids, are absent – careful examination at oil immersion does not reveals double walls, closig system, atrium or lids at neck base.

So, this species belongs to the group of simple folliculines. Also, the neck is always free from any external rib pattern – concentric ribs or spirals – common in *E. uhligi* Mulisch & Patterson, 1983.

Only fixed material was available for the study; contracted animals within shells are large, with a distinct attachment cone at the cell base (Figs 1, 13–15), with relatively short peristomal wings; the ciliature is in about 50 longitudinal rows, with pigment grains along the kineties (fig. 3); the

macronucleus is moniliform (chain-like), with 12–18 nodes. The macronuclear type is similar in all three symbionts of *Limnoria* (*Eufolliculina*, *Pachyfolliculina* Hadzi, 1951 and *Mirofolliculina* Dons, 1927), and all these species do not have any internal complications of their simple shells. The two latter atypical genera may be independent derivatives of a simple *Eufolliculina*.

Like *Mirofolliculina*, this species inhabits the upper part of pleotelson plates of *Limnoria*, but occurs only in the complete absence of *M. limnoriae* (compare Figs 4 and 12) on different hosts. When ciliates are numerous, they adhere also to the dorsal part of somites, but never to the ventral surface of the thorax commonly occupied by species of *Pachyfolliculina*.

*Hosts and Distribution.* Hosts of *Pachyfolliculina* and *Mirofolliculina* are *Limnoria lignorum* (Rathke, 1799) Leach, 1814, *L. tripunctata* Menzies, 1951, *L. quadri-punctata* Holthuis, 1949 and some other unidentified species; *Eufolliculina* is restricted at present to *L. borealis* Kussakin, 1963 and *L. magadanensis* Jesakova, 1961; fixed samples of these two species were kindly given to me for identification of their symbionts by the late Oleg G. Kussakin. Brunel (1963) noted *Eufolliculina* on *L. japonica* Richardson, 1909. Infested specimens of *L. borealis* were collected in Kola Bay, Barents Sea (depth 188–220 m) and in the Vladivostok area, Sea of Japan (92–112 m). *Limnoria magadanensis* was fixed in Avacha Bay, Kamchatka; the depth was not indicated on the label.

Regretfully, *Eufolliculina* has always been absent from my personal samples. In 1963–1990 I fixed *Limnoria* in Dalniye Zelentsy (Murmansk Coast), Chupa Bay (White Sea), off Bering Island, Iturup, Kunashir, Sakhalin (Busse Lagoon in Aniva Bay), Putatin Island near Vladivostok, south Crimean shore near Alushta. Most samples contain many hundreds of gribbles, fixed one by one immediately after isolation from bored wood to retain protozoan morphology. Both pierces and floating wood

were examined for the presence of gribbles. In spite of such rich samplings, only *Pachyfolliculina* and *Mirofolliculina* were found. Absence of deep-water gribbles in my samples may explain the absence of *Eufolliculina* on littoral crustaceans – possibly I have not sampled its hosts. This underlines significance of studying museum material of crustaceans for the presence of ciliophoran symbionts; such material should be available for symbiologists, not only for specialists on Crustacea.

## DISCUSSION

The family Folliculinidae includes six groups, some of these are regarded as sub-families (Hadzi, 1951), are not recognized here because the molecular analysis was scanty and the monophyly of the groups was not proved. Nevertheless, these groups are useful for preliminary sorting of species and identification at generic level.

In the groups 1, 2 and 3, represented by genera *Lagotia* Strethill Wright, 1857, *Parafolliculina* Dons, 1913, and *Halofolliculina* Hadzi, 1951, all genera possess a simple spherical macronucleus. In the groups 4 and 5 (examples – *Mirofolliculina* Dons, 1927 and *Folliculinopsis* Faure-Fremiet, 1935) the nucleus is moniliform (chain-like). The group 6 has the both types of the nucleus. The genus *Pebrilla* Giard, 1888, occurring only on pagurid anomurans, has a moniliform nucleus and an extensive atrial chamber. The shell neck is wide in other genera having a round nucleus (*Atriofolliculina* Hadzi, 1951, *Latifolliculina* Hadzi, 1951 and *Donsia* Hadzi, 1936).

There is an internal septa in *Folliculinopsis* Faure-Fremiet, 1935, *Ampullofolliculina* Hadzi, 1951, *Botticula* Dioni, 1972 and *Pseudoparafolliculina* Andrews & Nelson, 1942 (= *Tapetofolliculina* Andrews & Nelson in Hadzi, 1951) of the group 5 with a chain-like nucleus.

The group 4 remains, with the genera *Stentofolliculina* Hadzi, 1938, *Eufolliculina* Hadzi, 1951, *Metafolliculina* Dons,

1925, *Mirofolliculina* Dons, 1927, *Pachyfolliculina* Hadzi, 1951 and *Platyfolliculina* Hadzi, 1938, with a moniliform nucleus and a simple shell. *Stentofolliculina* and *Metafolliculina* have a vaginicolid shell (like in the cothurnid genus *Vaginicola* Lamarck, 1816) – a straight wide tube without an ampulla. I suppose that species of *Metafolliculina* which possess the ampulla must be excluded from this genus. *Platyfolliculina* has a wide flat ampulla and a narrow neck. The difference of the shell shape in *Eufolliculina*, *Pachyfolliculina* and *Mirofolliculina* is evident from Figs 1–15.

The new species from *Limnoria* corresponds to the diagnosis of *Eufolliculina* Hadzi, 1951, given by Ringuet (1953): the ampulla not flattened, with a short neck lacking any sculpture, without atrium or closing apparatus, with a small aboral pedicle and a moniliform macronucleus. But this diagnosis is restricted only to the type species indicated by Ringuet – *E. ampulacea* Hadzi, 1951, because other species have a ribbed neck. The name *E. ampulacea* was not based on any personal material of Hadzi (1951) but was given to one of the drawings of "*Folliculina ampulla* (Müller, 1786)" in a paper by Laackmann (1910). This is a simple schematic drawing made without oil immersion. It is certainly simplified and, to my opinion, is incorrect. The specimens of this type species has never been found and the species will not most probably be re-described. It has not been even mentioned in any faunistic lists since a century. The proposal of a new genus for the doubtful drawing was inappropriate by both Hadzi (1951) and Ringuet (1953).

Mulisch & Patterson (1983) discussed the situation, but unfortunately have not reproduced the old drawings from Mobius (1887) and Laackmann (1910), thus their proposals are not convincing. They designate *E. moebiusi* (Kahl, 1932) as type species of *Eufolliculina*, and proposed to restrict the name *E. ampulacea* (changed by them to *E. ampullacea*) for folliculines with the dark green shell, near 415 µm long; *E. moebiusi* is

yellow to brown and smaller (200–250  $\mu\text{m}$ ). By priority of Ringuélet's (1953) designation, *E. ampulacea* remains the type species. However, this name is not linked with any reliable description or at least one good drawing. The new species *E. brunea* differs from *E. uhligi* in having a smooth (not ribbed) neck, and a brown shell, and from *E. moebiusi* by thin walls of the shell. *Eufolliculina ampulacea* as defined by Mulisch and Patterson (1983) has a dark green shell and a straight neck in contrast to the new species *E. brunea* with a brown shell and an oblique neck.

Thus, *Eufolliculina* belongs to ciliate genera in which specimens of their type species are unknown and cannot be identified with certainty due to the fact that their original images are of bad quality. Besides *Eufolliculina*, such examples are *Vorticellopsis* Dons, 1918 (type species *V. undulata* Dons, 1918), *Trichopodiella* Corliss, 1960 (= *Trichopus* Claparede & Lachmann, 1859) (type species *Trichopus dysteria* Claparede & Lachmann, 1859), *Bertolinella* Caprano, 1941 (type species *B. intestinalis* Caprano, 1941), *Tricholeptus* Fromentel, 1875 (type species *T. aculeatus* Fromentel, 1875), *Euploia* Lohmann, 1920 (type species *E. pelagica* Lohmann, 1920) and many other genera, including universally accepted genera of peritrichs and suctorians with the worldwide distribution such as *Scyphidia* Dujardin, 1841 (type species *S. rugosa* Dujardin, 1841), *Rhabdostyla* Kent, 1881 (type species *Epistylis spheroides* Fromentel, 1874), *Sphaerophrya* Claparede & Lachmann, 1859 (type species *S. pusilla* Claparede & Lachmann, 1859). Their further retention is doubtful (Jankowski, 2007). In this situation it seems appropriate to consider the new species – a small brown folliculinid from *Limnoria* – as a member of the genus *Eufolliculina* until the situation with this problematic genus is cleared.

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