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TRANSFER OF *PLATYCOLA CIRCULARIS* DONS, 1941, SYMBIONT OF WOOD-BORING ISOPOD *LIMNORIA*, TO *LAGENOPHRYS* (CILIOPHORA, PERITRICHIA)

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ABSTRACT

Dons described several new ciliates on the gribble *Limnoria lignorum* in Norway, including *Platycola circularis* Dons, 1941. My samples of wood borers on Murmansk coast of Barents sea, in White and Black seas and in NW Pacific from Vladivostok to Bering island had only *Lagenophrys* on pleopods. *L. circularis* (Dons, 1941) comb. nov. is redescribed using samples made on Murmansk coast not far from its type locality (Trondheimsfjord). Previous descriptions of this species under 3 different names are analysed.

Key words: Ciliophora, Isopoda, *Lagenophrys*, *Limnoria*, *Platycola*

ПЕРЕНОС ВИДА *PLATYCOLA CIRCULARIS* DONS, 1941, СИМБИОНТА ДРЕВОТОЧЦА *LIMNORIA*, В СОСТАВ *LAGENOPHRYS* (CILIOPHORA, PERITRICHIA)

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РЕЗЮМЕ

Донс описал несколько новых цилиат на древоточце *Limnoria lignorum* в Норвегии, включая *Platycola circularis* Dons, 1941. В моих сборах древоточцев на Мурмане (Баренцево море), в Белом и Черном морях и в Тихом океане от Владивостока до о. Беринга на плеоподах обитает только *Lagenophrys*. *L. circularis* (Dons, 1941) comb. nov. переописан по материалу с Мурмана недалеко от типового местонахождения (Трондхеймсфьорд). Дан анализ прежних описаний вида под тремя видовыми названиями.

Ключевые слова: Ciliophora, Isopoda, *Lagenophrys*, *Limnoria*, *Platycola*

INTRODUCTION

Norwegian zoologist Carl Frederik Lindeman Dons (1882–1949), working in Tromsø and Trondheim, published articles devoted to numerous taxa, from foraminiferans to fishes; the complete checklist of publications of Dons includes 1 book and 137 articles; those devoted to biota of Trondheimsfjord include 96 titles (Sivertsen 1950). Some drawings of ciliates described by Dons are acceptable, for example those of *Metafolliculina nordgardi* Dons, 1924, but most are oversimplified, made without use of oil immersion and with evident mistakes; nevertheless, almost all species, except *Vorticellopsis undulata* Dons, 1918 (primont of a *Zoothamnium* colony?), are identifiable and may be restudied. In one short article (Dons 1941) he described 2 new species *Lobochona limnoriae* and *Platycola circularis* on *Limnoria lignorum* – respectively on setae and on the smooth surface of flat setose pleopods. There were never problems later with the first species and its new genus *Lobochona*; it was overlooked and a synonymous genus *Compsochona* Le Veque, 1947 was established, but later the genus *Lobochona* was accepted and redescribed. Due to bad original drawings, the second species is still ascribed to *Platycola* by Warren (1982), Clamp (1984, 1988, 1991) and all others. Both genera are not related; moreover, they belong to different suborders of sessilid peritrichs, which were recently divided by me into the suborders Vorticellina (with *Platycola*) and Operculariina (with *Lagenophrys*) (Jankowski 2007). In this article I establish the exact place of *P. circularis* inside the subclass Peritrichia.

MATERIALS AND METHODS

Specimens of the wood-boring isopod *Limnoria* (called "gribble" in the USA) were sampled in 1963–1990 on Murmansk coast of the Barents Sea (Biostation in Dalniye Zelentsy, now not existing), in the White sea (Chupa Bay), the Black Sea near Alushta on Southern Crimean coast, and in the NW Pacific on the coasts of Bering Island, Paramushir, Iturup, Kunashir, Putatin Island near Vladivostok, in Nakhodka Bay and in Busse Lagoon (Aniva Bay, southern Sakhalin). Most samples include hundreds of gribbles fixed immediately after their extraction from bored wood to retain symbionts in good cytological state. 5–8% formalin or half–strong Bouin's fluid diluted by sea water were used as fixatives; now in 2013 ciliates and hosts are still in perfect state of conservation. During storage these initial fixators were replaced by new ones made on fresh water, to avoid crystallization of marine salts and polymerization of formalin; bottom sediments in jars with protozoan and metazoan symbionts were carefully retained.

For temporary slides (that may be retained for a year or more, if protected from dust) parts of split fixed hosts were located in a small drop of formalin on a microscope slide; this drop was surrounded by a ring of vaseline oil (= paraffin oil) stained by Sudan Black that gives violet tint to the oil, and a thin coverglass was put on a drop; now the object in formalin is surrounded by a well-visible coloured frame under the coverglass. The same paraffin oil, of course unstained, is used as immersion oil instead of too dense standard cedar oil; it does not solidify and does not require washing from the coverglass and from immersion objective lenses.

A single drawing can be made by free hand or with the aid of a bar or ocular micrometre, but a large series of drawings require exact proportions. An old Zeiss drawing mirror (camera lucida) or a drawing tube proposed later give too small illustrations, their inking is difficult. In the latest years I used an ocular reticle with 16×16 squares, and the pre-fabricated reticle on A4 paper sheets is made by pencil. I store 50 sheets in each set with reticles in distance 0.5 cm between horizontal lines, in range 2 to 5 cm, and 3 additional ones, jointly 10 types of net – sheets are divided into 5 to 14 horizontal lines, with a corresponding number of vertical lines. I followed the recommendations of Langeron (1949) – his valuable book "*Prècis de microscopie*" includes a drawing of an ocular reticle that attracted my attention. Clamp and Kane (2003) used this method for lagenophryids, but without prefabricated sheets of white paper – drawings were made on a translucent paper held over a standard graticule on a white sheet, and with a reticle inside the objective lens.

I find a cell of interest at the lowest magnification (7×8), orient the microscope slide by fingers, examine cell at a medium-power magnification (7 × 20 or 7 × 40) and, if suitable for illustration, change the objective lens for oil immersion (10 × 90). The object is studied for some time, then an eyepiece with a reticle inside (×7 for this object) is used for making a large drawing. From a set of 10 sheet types I select (for this *Lagenophrys*) those with 9 horizontal lines (10 × 7 squares). The main task is to draw as many details as possible in exact proportions; then the eyepiece ×10 without a reticle is returned again, and drawing is continued for a long time. After inking by black Chinese ink, all pencil lines are erased and drawing is ready for scanning. In Figs. 21 and 22, the pencil reticle was erased inside the cells after inking of the object, but was retained and intensified by a black marker outside ciliates; I hope that these drawings explain the method better than the above text, and much recommend it for serial large-sized drawings, showing growth stages and individual variations of protozoans. Measurements of cells and organelles can be made, when needed, on a ready drawing, without an ocular micrometre over the slide; in our case each side of square at 7 × 90 is almost exactly equal to 10 μm.

All drawings of *Lagenophrys* in this article except Fig. 18 are from formol–fixed ciliates; in a balsam layer, shells and cells are clear, not refractile, and their comparison with fixed material becomes difficult or impossible. For staining nuclei of symbiotic ciliates, pleopods and other appendages of split formol–fixed

Limnoria were stained by orcein or Böhmer's haematoxylin, preferably after hydrolysis in 10% HCl at room temperature for 25 min with differentiation by slightly acidified 70% ethanol; objects were dehydrated in dioxan and mounted in a thin layer of Canadian balsam, thus permitting further study under oil immersion.

SYSTEMATICS

Order Sessilida Kahl, 1933

Suborder Operculariina Jankowski, 1980

Family Lagenophryidae Bütschli, 1889

Genus *Lagenophrys* Stein, 1852

Previous descriptions of *Lagenophrys* on *Limnoria*

1. *Platycola circularis* Dons, 1941
(Figs. 1–5)

Dons (1941) published a short article with a description of two new symbionts of *Limnoria lignorum* – *Lobochona limnoriae* and *Platycola circularis* (Figs. 1–5). The accepted publication date is 1941, but on pages of his article we read: "Meddelt 11te november 1940"; "Trykt 9de desember 1940. Aktietrykkeriet i Trondhjem". Some of his previous articles are also frequently cited with a wrong date. Nevertheless, the date 1941, indicated in Nomenclator Zoologicus for *Lobochona* Dons, 1941, and also by Aescht (2001) and Lynn (2008), will be accepted here.

Drawings of *P. circularis* are very bad; only contours of the cell and shell, and nuclei inside cells are shown, nothing more; the shell apex has a simple opening (Figs. 2, 5) or a rigid collar (Figs. 1, 3, 4) – what is correct? Only stained cells in the balsam layer were examined without a high magnification. Figs. 1–12 were redrawn from photocopies of articles of Dons (1922, 1941, 1948). This species is not *Platycola*; the zooid is single, round, the shell has a similar contour, the nucleus is C–like in shape, with an oblique or transversal location. Thus, *P. circularis* in any case must be excluded from *Platycola* and, if not transferred to *Lagenophrys*, must be in a new genus.

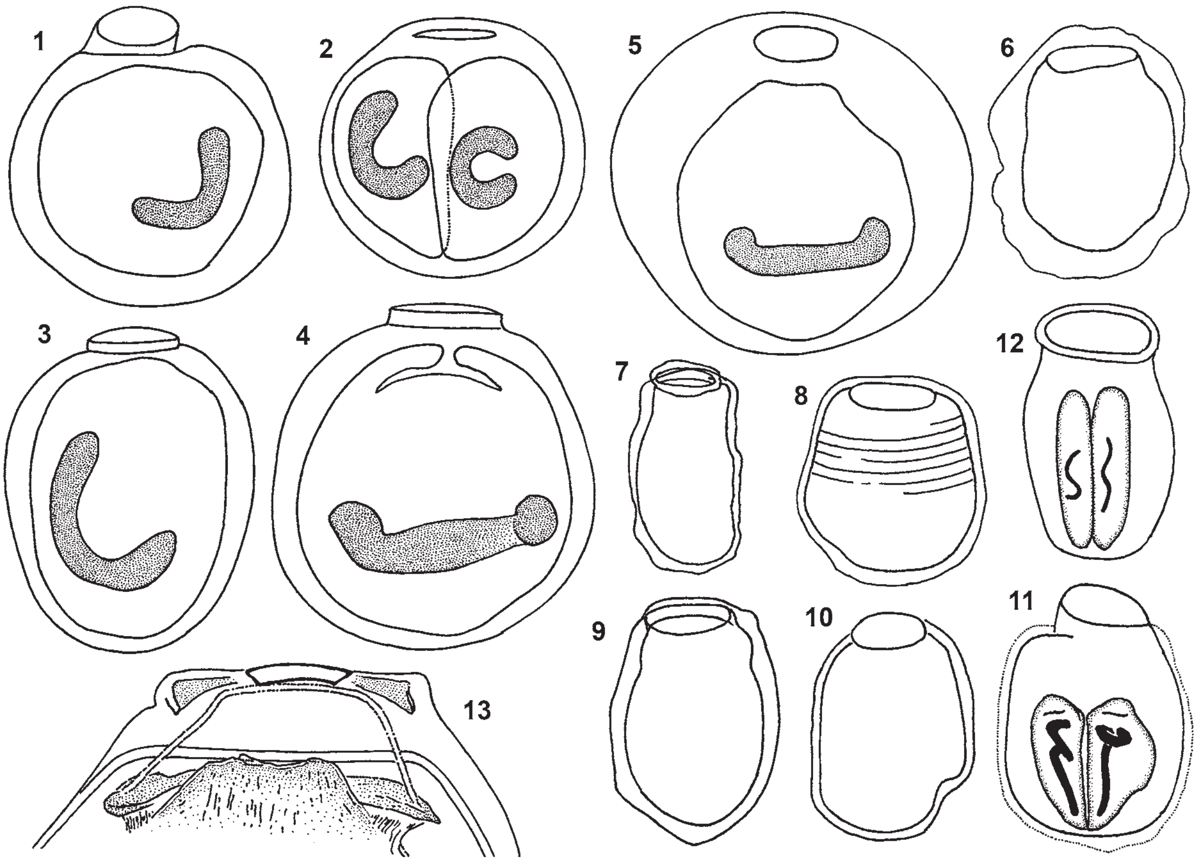
Dons studied symbionts of *Limnoria lignorum* collected in Hjellebotn on the shore of Beitstadfjord, the internal part of the giant, branching Trondheimsfjord (Norway); only fixed material was available for study. Folliculinids *Mirofolliculina limnoriae* (*Freya* in Giard, 1883), *Folliculina gunneri* Dons, 1928 and an atypical vaginicolid *Cothurnia limnoriae* Dons, 1928 were described first (Dons, 1928), the chonotrich *Lobochona limnoriae* and a vaginicolid named *Platycola circularis* – in a later article, respectively on the setae and the smooth surface of "uropods" (pleopods) (Dons, 1941). These 2 species were not found by Dons in other sampling sites in Norway, while folliculinids and cothurnids, noted previously, are widespread; thus Dons supposed that *P. circularis* is brackish-water species, which is absent in the open sea.

The diagnosis of *P. circularis* is short and adds nothing to desperately simple and controversial drawings. Shell colourless, smooth, flat, translucent, usually round; shell mouth located at periphery, without lobes, but sometimes ("jedoch zuweilen") with a very low (short) neck, not more than 5 µm high; cytoplasm structureless; nucleus thick, short (in comparison with *P. donsi*), curved; division equal. Shell diameter 40–65, loricastome width 13–16, nuclear size 30–49 × 5–7 µm. This species differs from other *Platycola* (shown by Kahl 1933, 1935) by a round shell and by a short and relatively thick nucleus. Further text of Dons is devoted to comments on *P. dilatata*, *P. donsi* and *P. striata*, and also includes a discussion of *P. nigra* Wailes, in reality a folliculinid identical with *Lagotia simplex* Dons.

Only 3 type slides of Dons are registered in the Museum of Natural History and Archaeology in Trondheim (Bakken 1999) – *Vaginicola oviformis* Dons, 1948 from the surface of ascidians; *Podocyathus excavatus* Dons, 1938 on hydroids and bryozoans; and a slide with the label "*Folliculina ampulla* and 1 n. sp.", supposedly *Parafolliculina amphora* Dons, 1914 on the shell of the scallop *Pecten islandicus*. But there are also many slides without designation "sp. n." in this Museum and in Trondheim's Biological Station, where type slides of *P. circularis* and fixed host samples must be searched.

2. *Lagenophrys limnoriae* Le Veque, 1947
(Fig. 16)

The unpublished dissertation of Jean Anthony Le Veque (1947), made under guidance of Prof. John Luther Mohr in Los Angeles, is available by aid of University Microfilms, Ann Arbor, Michigan, USA; it is not cited in subsequent literature. The first article of Dons (1928) on limnorian symbionts was



Figs 1–13. Genera *Platycola* and *Lagenophrys*. 1–5 – "*Platycola" circularis* (from Dons 1941); 6–11 – true marine *Platycola* (*P. dilatata*, from Dons 1922), 12 – *P. donsi* (from Dons 1948); 13 – loricastome region of shell of limnorian symbiont (original drawing). Note controversy in loricastome type shown by Dons: rigid collar – 1, 3, 4; slit – 2; wide opening – 5; compare with Fig. 13, made at relief illumination of a fixed zooid under oil immersion. Absence of connection of zooid and loricastome in all drawings of *P. circularis* of Dons is a mistake.

known to both scientists at that time, but the second article (Dons 1941) was overlooked.

Collections were made along Californian coast, within 350 miles; gribbles were identified as *Limnoria lignorum*; main samples were taken in San Pedro and Newport Harbours. The host and its mode of life are shortly described without illustrations, thus the species identity cannot be checked.

Lagenophrys limnoriae, new species of Le Veque, is illustrated by one micrograph, showing cell in a lateral view only (reproduced later by Mohr (1959), with legend "*Lagenophrys* sp."; see Fig. 16). Maximal shell size 60 × 50 µm, size range was not mentioned; macronucleus horse-shoe shaped, 7.5–15 µm wide, with a single adjacent micronucleus. A long description of the contour in the profile view follows; it is omitted here. "The posterior lip is noticeably thicker than the anterior lip and both are bluntly tipped. There are also noticeable thickenings at the tips of the lips. Mouth plates were not demonstrable" (segments of thick margins of lips are meant).

New species was compared with *L. lunatus* and *L. ampulla* of Imamura (1940) (both names are now dubious); but Imamura showed an atypical thin C-like nucleus in *L. lunatus* (now synonymised with *L. eupagurus*), which is revealed during one stage in the cell cycle of any *Lagenophrys*. There are differences in measurements and the dorsal outline of *L. limnoriae* from legs and pleopods; those on pleopods are more round than elliptical, with a mean size of 40 × 36 µm. Whether the two are conspecific or not, remained unclear. By "legs" he must have meant pereopods, but no other author before or later has noted the limnorian *Lagenophrys* in this locus.

Mohr (1950, 1951), who studied limnorian ciliates jointly with Jean Le Veque, Hitoshi Matsudo and

Yuk-Maan Leung, noted *Lagenophrys* sp. on gribbles identified as *L. lignorum* in California and Oregon. In his review of associates of *Limnoria* (Mohr 1959) he mentions 3 limnoriids in California – *L. tripunctata*, *L. quadripunctata* and true *L. lignorum*; only abdominal appendages (pleopods = swimmerets, functioning as gills) are site of adhesion of an undetermined *Lagenophrys* sp., with 15–25 specimens per host; the species is common and widespread here; site specificity (location on the host) is rigid; *Lagenophrys* attach only on the broad surfaces of the pleopods. *Lagenophrys* sp. was mentioned once more by Becker (1968), among other typical limnorian symbionts. The name *L. limnoriae*, given in the unpublished dissertation of Le Veque, is not valid.

3. *Lagenophrys limnoriae* Clamp, 1988
(Figs. 14, 15)

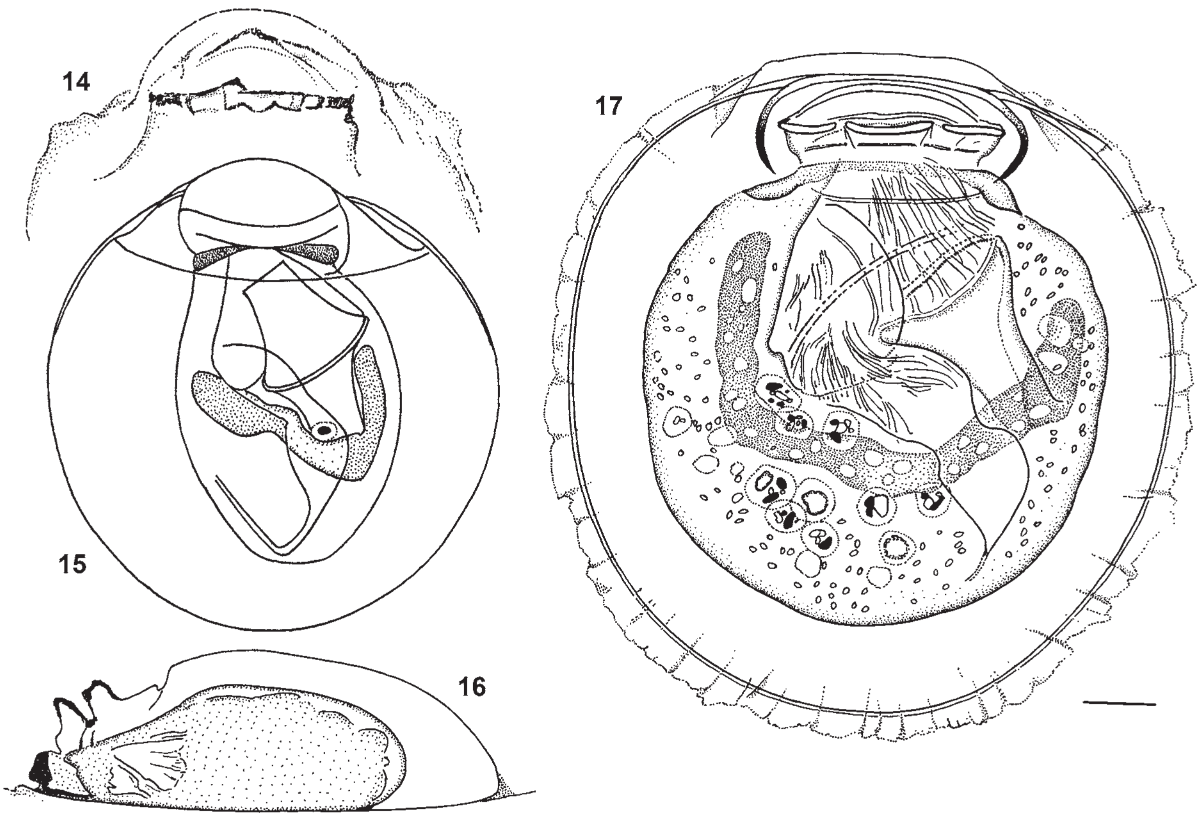
Clamp (1988: 12) lists hosts of *Lagenophrys*, indicating partly copepods, ostracods and cladocerans; five members of the genus occur on marine hosts, and only one of them on marine isopods (*L. cochinensis* is meant). "In addition, an undescribed species of *Lagenophrys* was observed on the marine woodboring isopod *Limnoria* Leach, 1813 by Mohr (1959). I discovered and describe herein a new species of *Lagenophrys* occurring on two species of *Limnoria*. This new species appears to be the one observed by Mohr (1959)" (Clamp 1988). The reference list omits citations of Dons (1941), Le Veque (1947) and Jankowski (1986), which include descriptions and illustrations of *Lagenophrys* on *Limnoria*; Clamp (1984, 1988, 1991) still believes that ciliate of Dons belongs to *Platycola*.

Limnoria tripunctata (type host) was sampled in 2 sites in North Carolina, Atlantic Coast of the USA (type locality); additional host – *L. lignorum* in British Columbia, Canada. Thus both coasts are inhabited by a single species of *Lagenophrys*; any morphological difference between "races" is not mentioned. Pleopods only are inhabited.

The detailed description is supplemented, regretfully, only by a single schematic drawing, reproduced here (Fig. 15), and by a dark SEM micrograph; my Fig. 14 is a redrawing of the loricastome region from this micrograph. According to Clamp (1988: 13), "fixed and stained material was used; living *Lagenophrys* are of little value in taxonomic work because some critical features cannot be seen satisfactorily in the living state". I do not agree with this statement. Fixation denatures proteins, the cytoplasm lacks transparency, the contrast between structures is lost; the buccal cavity, mouth, peristome, cilia, contractile vacuole are much better seen in living cells. The exact structure of loricastome walls is easily seen *in vivo* and *in fixo* under oil immersion, but not in specimens mounted in balsam layer with a high refraction index coinciding with that of a glass slide (1.52). As result, I cannot compare my drawings with 2 illustrations of Clamp (1988). Structures evident in my material are not shown in Clamp's drawing, but something like that may be interpreted as a thickening of the posterior wall in the SEM micrograph; if I understood the text correctly, the margins of loricastome are smooth, but the posterior lip is irregularly folded when applied closely to the opposite lip in a closed loricastome.

The shell size of the Clamp's species is 39–51 (mean 43.2) × 38–46 (42) μm, with a proportion of 0.9–1.17 (1.03); the shell is not exactly spherical, its anterior part is wide, the posteron is more narrow. For a detailed description of the structure of loricastome and some thickenings on its lips, not easy for graphic representation, see Clamp (1988: 13); schematic drawings of the shell, like mine, are absent. A rich material of the type slides of Lagenophryidae, including *L. limnoriae*, was deposited by Clamp in the International Protozoan Type Slide Collection stored in the National Museum of Natural History, Smithsonian Institution, Washington, D.C. (Cole 1994); it includes 2 slides, holotype and paratype, of *L. limnoriae* from the type locality – Topsail Beach, North Carolina, on *Limnoria tripunctata,* and also slides of 24 species of 3 genera of Lagenophryidae deposited prior to Cole's Register, and subsequent types. Smithsonian collection of Crustacea was used by Clamp for study of these symbionts.

This species is regarded valid in all subsequent articles and checklists, e.g. in the checklist of sedentary peritrichs on Crustacea (Fernandez-Leborans and Tato-Porto 2000), that includes also *Platycola circularis* as an independent symbiotic species of peritrichs on limnoriids (thus, a single species is listed under 2 names); *Limnoria lignorum* is only indicated for species of Clamp, *Limnoria* sp. for species of Dons, and both indications are incorrect. Regretfully, *Lagenophrys limnoriae* Clamp is omitted (overlooked) in the checklist of symbiotic ciliates of the Pacific USA, California to Oregon (Landers



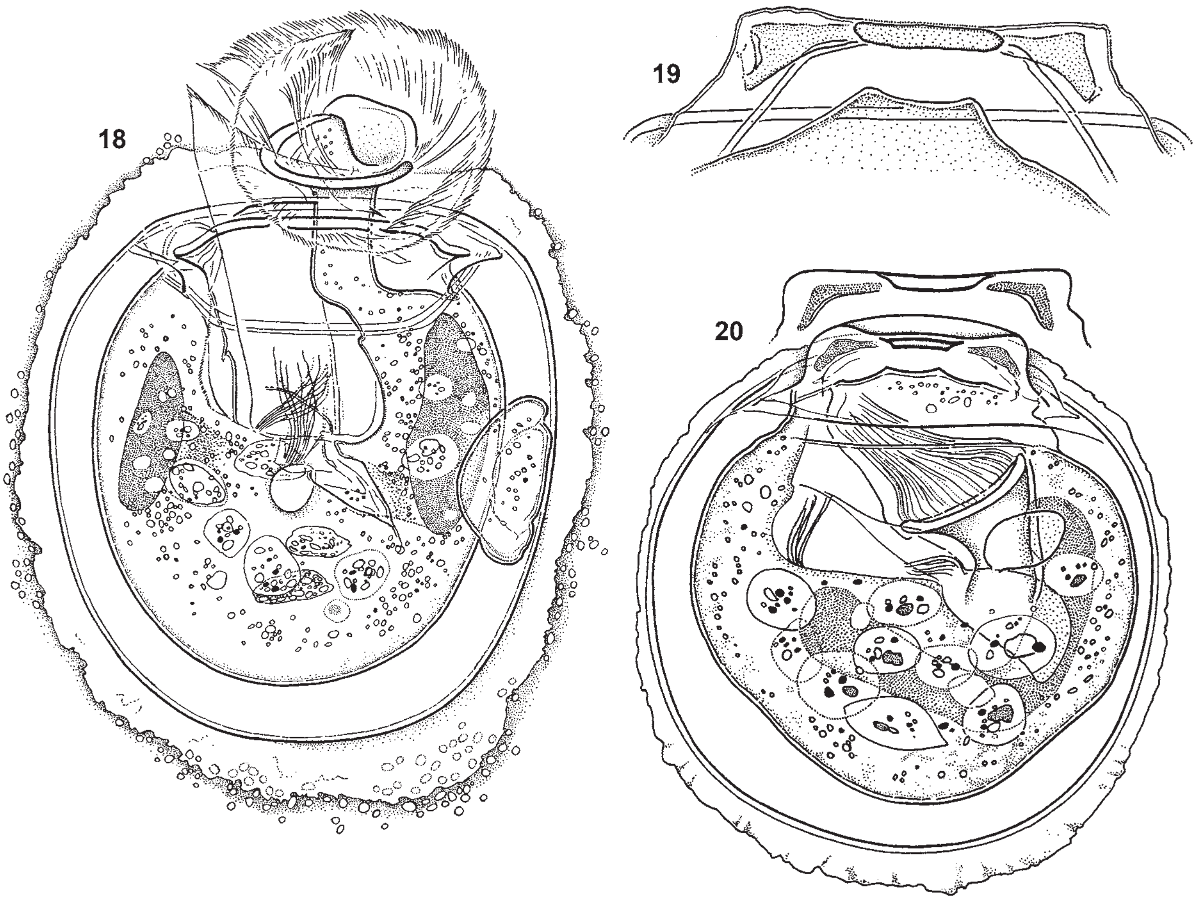
Figs 14–17. Genus *Lagenophrys*. 14, 15 – *Lagenophrys limnoriae* (from Clamp 1988): 14 – redrawing of loricastome region from Clamp's SEM micrograph; 15 – entire shell and zooid; 16 – *Lagenophrys* sp., from one of 3 local species of *Limnoria* in California, USA; host is not named in legend; redrawing of the single micrograph of Mohr (1959), identical with that of Le Veque (1947). 17 – "outgroup" – *Lagenophrys* sp., branchial symbiont of *Pallasea cancellus* in lake Baikal (original). Scale bar 10 µm (only for Fig. 17).

2007), where only first reports of *Lagenophrys* sp. by Mohr (1950, 1951) are listed. The dissertation of Le Veque (1947) and his species *L. limnoriae* Le Veque, and also other symbionts described by this author in the same region are also omitted from references and the checklist of symbionts in a chapter prepared by Landers (2007).

The Internet site of Delgery (accessed in 2009) includes 7 SEM micrographs of symbionts of *Limnoria*; one of these is a photograph of the entire pleopod of *L. quadripunctata* with *Lagenophrys limnoriae* Clamp, from samples made in England; thus 2 American species of symbionts (lagenophryid and chonotrich) were identified instead of 2 European ones of Dons. In a monographic review of Lagenophryidae (Clamp 1991), the article of Dons (1941) is cited, but the species is not included in his checklist of lagenophryids – only *L. limnoriae* Clamp, 1988 is mentioned with its synonym *Circolagenophrys circularis* Jankowski, 1986 (sic), and with a footnote to this name: "Jankowski (1986) incorrectly identified this species as being identical to *Platycola circularis* Dons, 1941. The figure given by Dons (1941) shows that this species does, in fact, belong to the genus *Platycola* and is certainly not a species of *Lagenophrys*".

Redescription of *Lagenophrys circularis* (Dons, 1941) comb. nov.

Type material. The neotype slide was not designated, because the type slide must exist in archives of Carl Dons in Norway. Those who can study the rich scientific archive of Dons, retained in Tromsø and Trondheim, can redescribe species from his slides or fixed samples, or collect new material in the type site – Hjellebotn inside Trondheimsfjord. I store 7 jars with several hundreds of formol-fixed gribbles

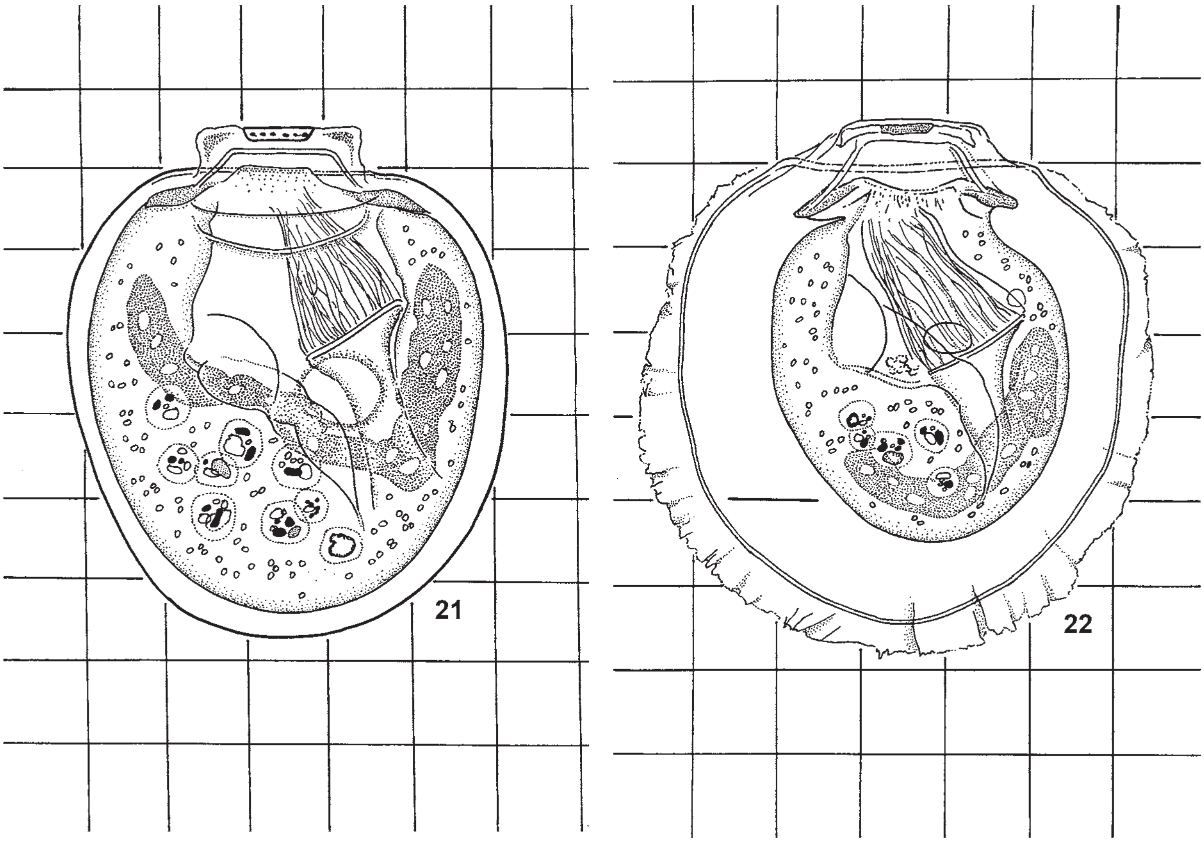


Figs 18–20. *Lagenophrys circularis*. 18, 19 – zooid and closed loricastome; 20 – fixed zooid and loricastome of another specimen; free-hand drawings without scale.

each, and 12 smaller samples, collected in 1963–1990 in Zelenets Bay and in Yarnish Fjord, Murmansk coast of the Barents Sea; appendages with *L. circularis* were stained by orcein and Böhmer's haematoxylin on 26 slides. Fixed isopods and slides are stored in the Laboratory of Protozoology, ZIN, Saint Petersburg, Russia.

Host samples. In rich and perfectly fixed samples of *Limnoria* made in Dalniye Zelentsy ("Far Greenland") Biostation on Murmansk Coast of Barents sea, visited several times over the period of 1963–1990 (abandoned in 1991 and now not existing), all ciliophoran symbionts of local gribbles were studied; peritrichs and folliculinids were illustrated alive, but main research of these two groups and of chonotrichs and suctorians, that are not changed at fixation, was made after return to Saint Petersburg using fixed material. *Lagenophrys* occurs in my samples made in the Barents Sea and in the Pacific from Vladivostok area to Bering island, but in the present article I will describe only Murmansk coast material and will not make any comparison with Pacific forms, to be treated later, specially for comparison only with Norwegian "*Platycola*", discovered by Dons (1941) not far from my sampling site in the entire Global scale. With richly illustrated keys of Menzies (1959), Kussakin (1963) and subsequent sources, the host was identified as widespread *Limnoria lignorum*, not Arctic *L. borealis*.

Morphology. Drawings of *Platycola circularis* from Dons (1941) (redrawn with the aid of a reticle from photocopies) are reproduced on Figs. 1–5; *Lagenophrys* of Murmansk Coast is shown in original Figs. 13, 18–50; a species on branchial plates of amphipods *Pallasea cancellus* from lake Baikal, belonging to the *L. ampulla* group (but with a non-segmented rim of

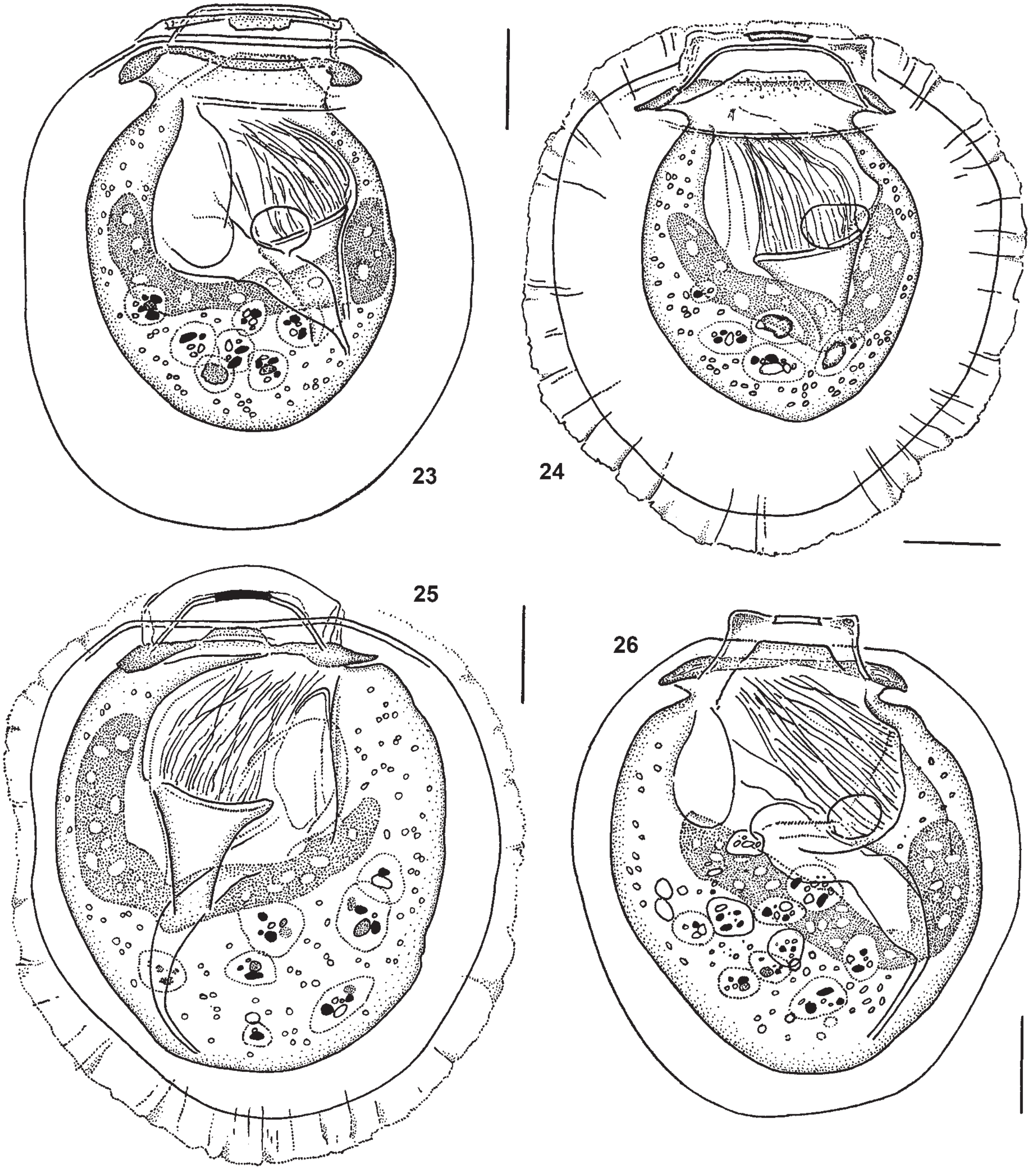


Figs 21–22. *Lagenophrys circularis*. Demonstration of the use of 2 reticles (inside ocular and on paper sheet) for serial large-sized drawings of ciliates, much reduced at printing. Oil immersion, 7 × 90; reticle with16 × 16 squares in ocular ×7; standard A4 sheet of paper is divided by 9 horizontal lines.

the anterior wall, unlike the Clamp's (1991) drawing of typical *L. ampulla*), is shown as a comparative "outgroup" in Fig. 17.

With such illustrative material, showing morphological details and their variability in many specimens, it is enough only to make few comments to my drawings and to give shell dimensions; a single "typical" specimen cannot be selected from my drawings, and only the entire series of illustrations can represent the species. Features of this species common to most lagenophryids are: thin-walled shell; peripheral layer of glue attaching shell to the substratum; thick macronucleus with numerous achromatic nucleoli (stained by acid stains revealing RNA); single micronucleus, small and difficult to reveal, not shown in my drawings; it was seen on orcein-stained slides close to the macronucleus in right-hand angle of that curved nucleus. When specimens are isolated and overturned after splitting of hosts and are seen from the underside (Figs. 25, 36, 40), it seems that only peripheral (marginal) part of flat bottom is used for attachment, and bottom itself, under cells, is not attached. Extended zooid has a narrow cone, spiral ciliary vortex and long undulating membrane (Fig. 18); until now specific differences in the shape of contracted or extended cone were not revealed in any *Lagenophrys*.

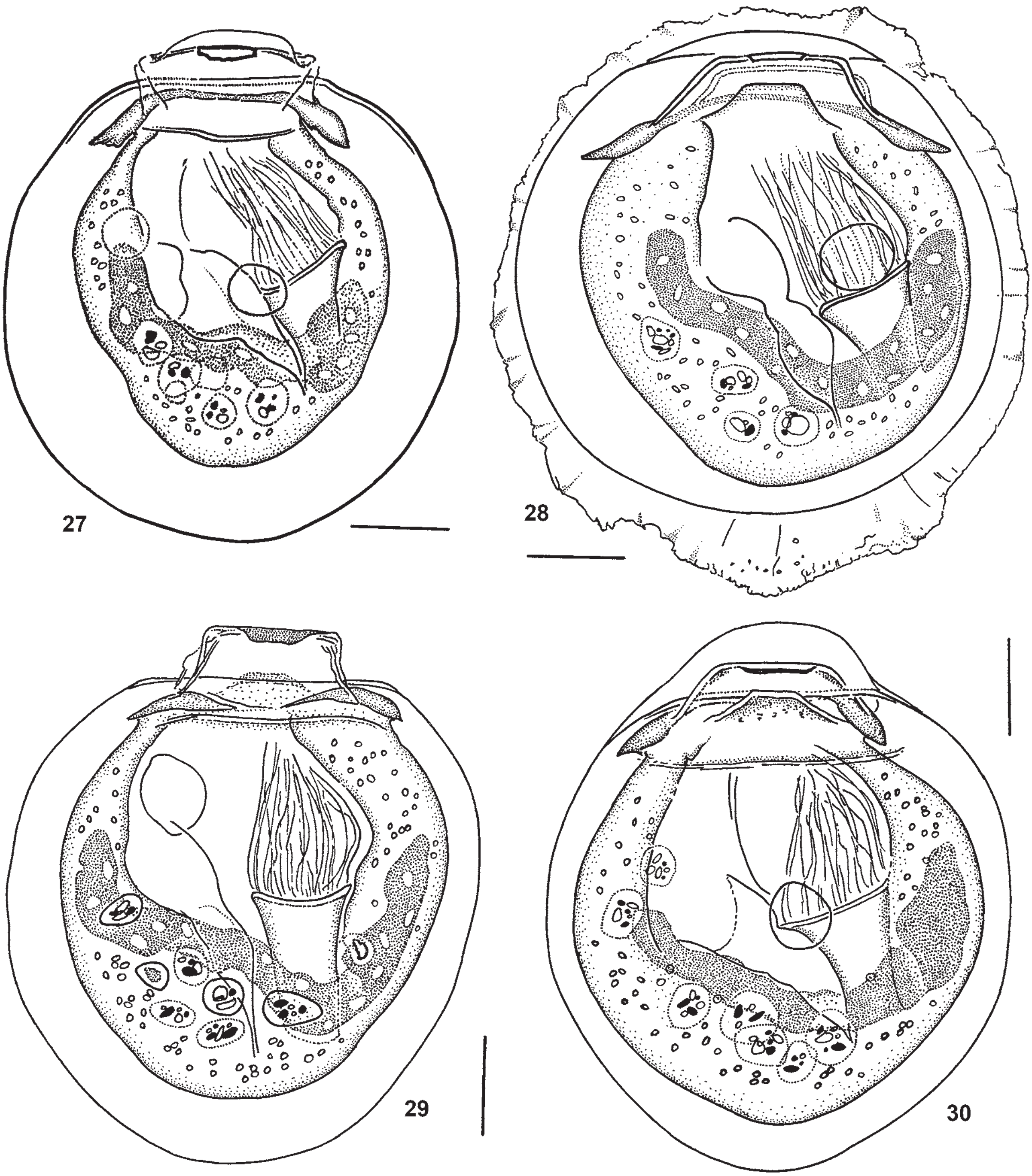
The contractile vacuole without vesicles or radiating channels is located in right-hand body part, in a thin layer of cytoplasm under the dorsal cell surface. The buccal cavity is extensive, with a large mouth opening that leads to a wide cytopharynx with long fibres or thinnest tube (Figs. 25, 26), shown only in some of the drawings. When viewed from the dorsum, in the customary position for the drawings of *Lagenophrys* (Fig. 20), we see first the contractile vacuole, then the peristome, then the macronucleus; the ciliary cone, located below the nucleus, is better



Figs 23–26. *Lagenophrys circularis*, upper view (23, 24, 26) and lower view of separated specimen (25). Scale bar 10 μm.

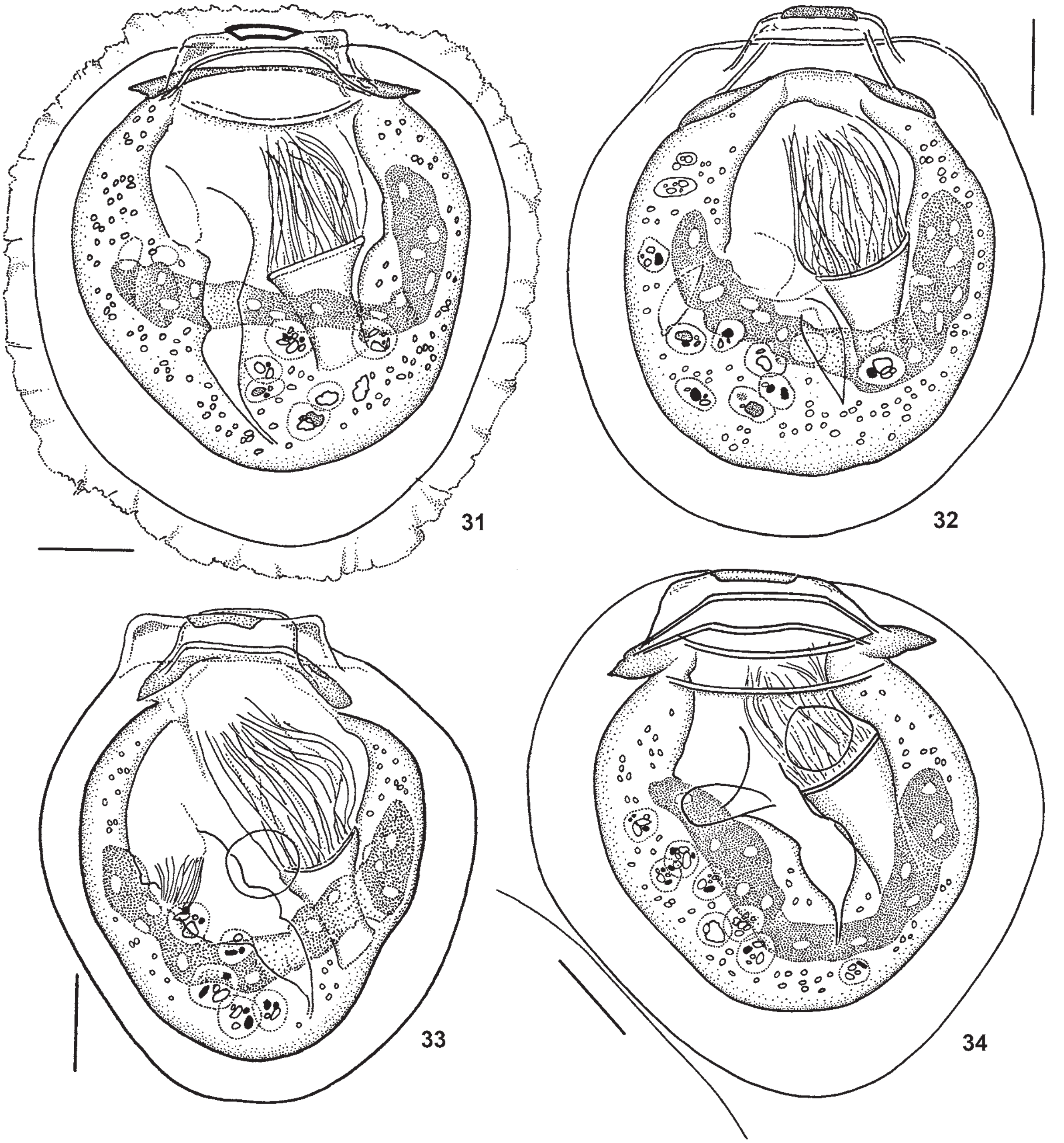
seen in inverted specimens (compare Figs. 25 and 26). Crescentic ribs on the shell surface, evident in the Clamp's SEM micrograph, may be seen in empty shells (Figs. 43, 44). Numerous cuticular furrows, about 40 on the upper cell surface, perhaps corresponding to ancestral cyclonemes of aloricate sessilids, were noted at examination of *Lagenophrys* sp. from *Limnoria magadanensis* in the Kamchatka sample, but after special search were not found in the symbiont of *L. lignorum*.

Shell size of fixed specimens of *L. circularis* (40 measurements with ocular micrometre under oil im-



Figs 27–30. *Lagenophrys circularis*, drawings of fixed specimens in upper view. Scale bar 10 µm.

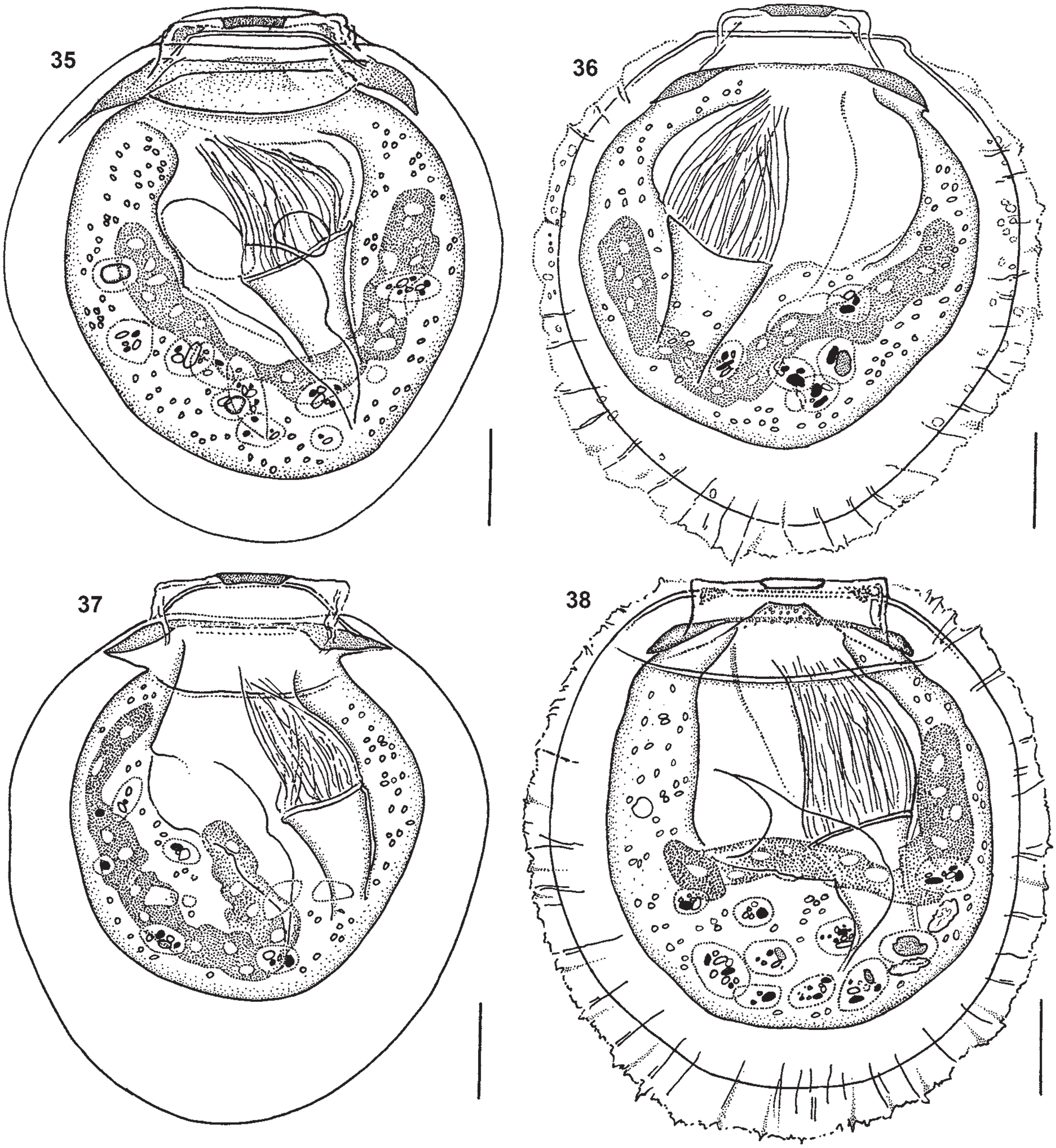
mersion, 10 × 90) 47–57.5 (53) × 45–54.5 (51) µm, loricastome width 17–21 µm; this is one of smallest species of the genus with a very thin shell, and this explains failure of Dons to make exact drawings of stained specimens and their loricastomes in balsam at small magnification; also, study of detached specimens (inverted specimens in Figs. 25, 36, 39, 40, and specimens in the traditional dorsal view in most other illustrations) favours making correct drawings of lagenophryids. Macronuclear size in orcein-stained



Figs 31–34. *Lagenophrys circularis*, drawings of fixed specimens in upper view. Scale bar 10 µm.

cells mounted in balsam 27–36 µm (measured along cell diameter, not along curved nucleus); diameter of micronucleus about 1.6 µm.

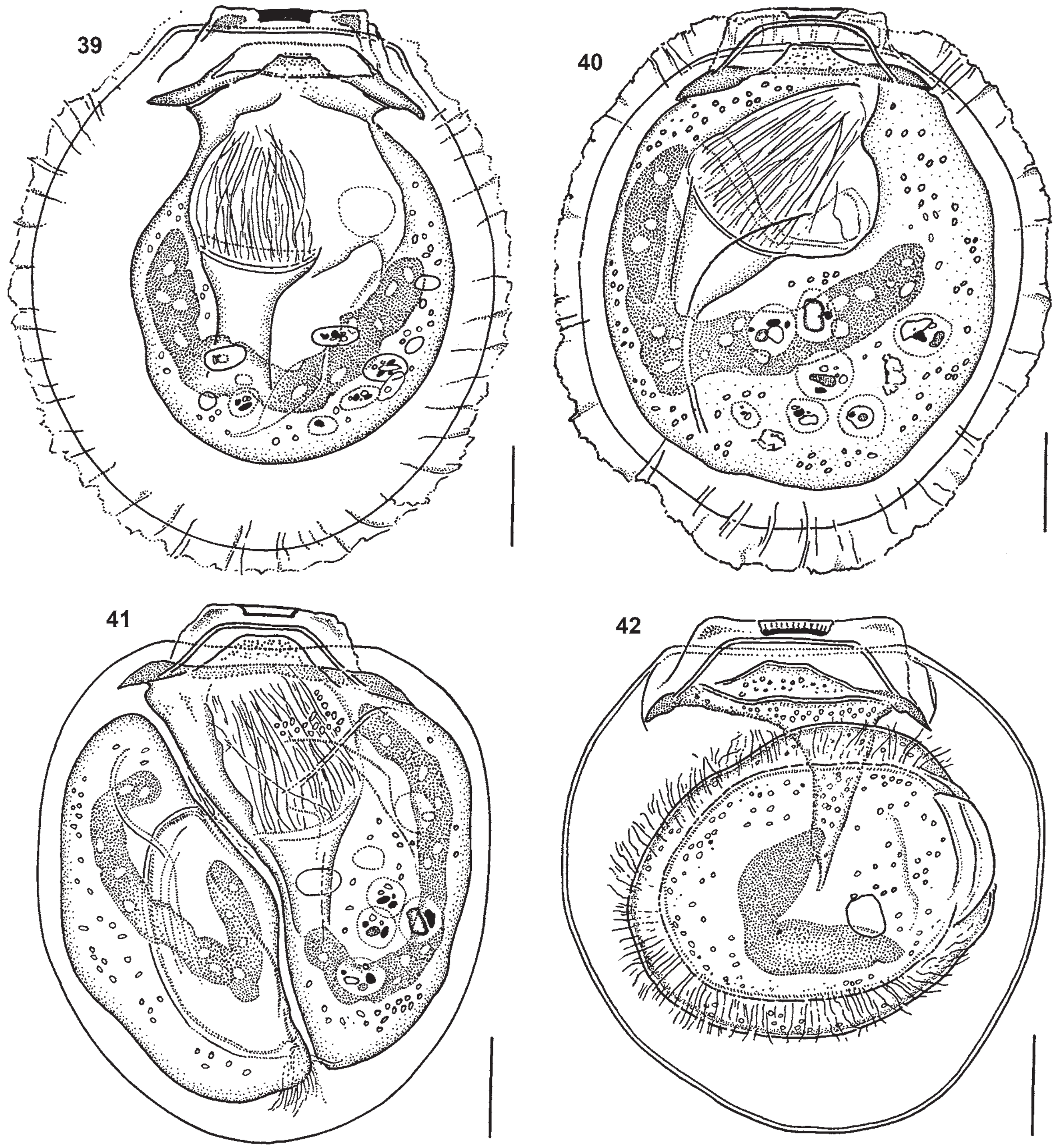
In the entire above description there is nothing special in the structure of this species except for its small size, as compared with other lagenophryids; only loricastome and adjacent area (cell apex, or "anteron") are unique, thus I showed them separately (Figs. 13, 19, 45–50) in addition to those seen on intact specimens (Figs. 21–44). These separate drawings were made as an attempt to understand the structure of the entire complex in fixed specimens, and are a kind of working sketches made by free hand without a scale or reticle. Looking from the dorsal



Figs 35–38. *Lagenophrys circularis*, drawings of fixed specimens in upper (35, 37, 38) and lower view (36). Scale bar 10 µm.

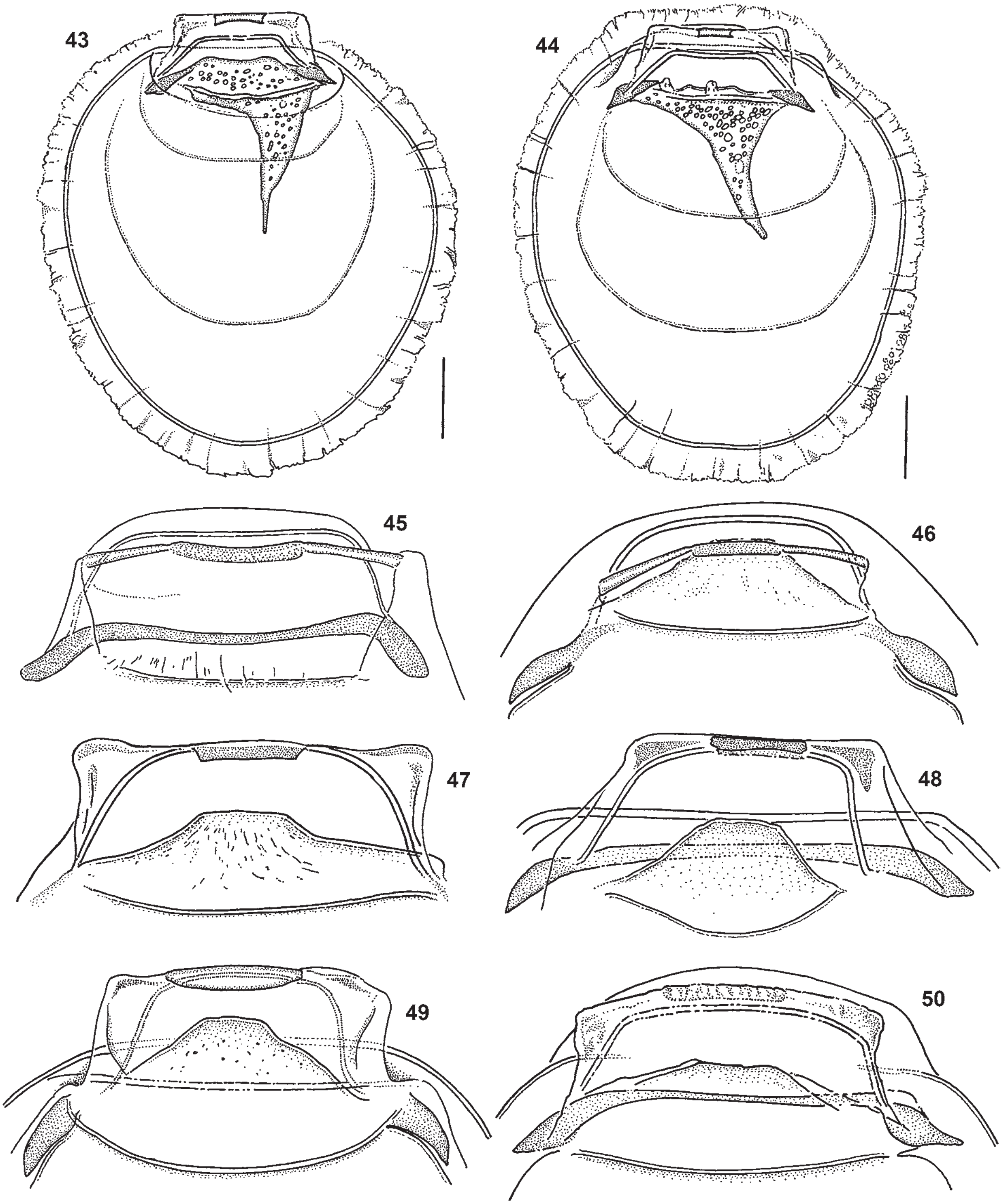
side, we see first the transverse fold of the shell below the entire complex (Figs. 49, 50), then the upper wall of loricastome, called the "posterior wall" by previous authors according to the profile view of the shell (Fig. 16). This wall has a prominent refractile apical thickening and, on both sides of it, less pronounced (thinner) thickenings of a different shape, from unclear (Figs. 47, 49) to very distinct (Figs. 45, 48). Unlike many lagenophryids, e.g. *Lagenophrys eupagurus* Kellicott, 1893, both opercular lips lack indentations and are not segmented; also, refractile hook-like lower ends (crochets) are absent.

Below this wall, we see 2 structures – prominent cytoplasmic cone (Figs. 19, 20, 48, 49; compare with



Figs 39–42. *Lagenophrys circularis*, drawings of fixed specimens in upper (42) and lower view (39–41), on normal intermoult gribbles (39–41) and on moulting host (42). Scale bar 10 µm.

one of the drawings of Dons, Fig. 5) and high arc-like structure, anchored to both ends of a muscle (most evident in Figs. 13, 46, 50). Muscle, or sphincter, is rod-like refractile transversal organelle of unequal width; its enlarged edges, sometimes acute, are easily seen in fixed cells and commonly protrude beyond the cell apex (Figs. 22–24). Such acute edges, protruding to both sides, but not the entire muscle, are known in *L. callinectes* Couch, 1967, symbiont of swimming crabs *Callinectes* in USA. The entire muscle is retained by the cell remnant that is left after transformation of telotroch on moulting hosts (Fig. 42);



Figs 43–50. *Lagenophrys circularis*. 43, 44 – drawings of fixed specimens, with cytoplasmic remnant after transformation of the last zooid into migrant on moulting hosts; note identical concentric ribs on both shells and retention of muscles. Scale bar 10 µm. 45–50 – schematic free-hand drawings of loricastomes of 6 shells.

two empty shells (Figs. 43, 44) are shown specially to prove that such retention is a rule, not exception.

The anterior (= lower) wall of the loricastome is smooth, but with a thick upper margin, and is easily seen in overturned detached cells (Fig. 25). Drawings of the shell of living or fixed specimens is absent in the Clamp's (1988) description of *L. limnoriae*, and comparison of our results is impossible; his dark SEM micrograph shows something similar to apical thickening of the posterior wall of *L. circularis*; his second illustration (Fig. 15) was made from a specimen in balsam, and refractile structures, if present, are not shown. Once again I underline the significance of studying lagenophryids *in vivo* or *in fixo*, not mounted in balsam layer, to make results comparable.

Locus. Preferred site on the host – pleopods, up to 16 shells on a single appendage; small flexible paired branchial plates, devoid of marginal setae, are also inhabited, carrying no more than 5–6 shells; pereopods are inhabited by *Cothurnia limnoriae* and undescribed epistylids only. Branchial plates were not reported before as a locus for this symbiont, only pleopods, covering them; occurrence on pereopods was not documented by Le Veque (1947) by drawings or photographs, but his unpublished thesis attracts attention to possible existence of two species on a single host specimen, like those inhabiting amphipods and shrimps.

Status of *Circolagenophrys* Jankowski, 1986

I have found and studied *Lagenophrys* spp. with a typical round shell on freshwater ostracods, asellote isopods, gammarids (partly in Lake Baikal) and shrimps; only few marine species were found. When the type species was sampled on the freshwater harpacticoid copepod *Canthocamptus*, I was astonished by the difference in the shell shape and structure of this setobiont and typical platybionts, and their loricastomes are not identical also; *Lagenophrys* was split into two different genera, with *Lagenophrys* Stein, 1852, s. str. restricted only to *L. vaginicola* Stein, 1852, and to its probable synonym *L. obovata* Stokes, 1887. All other species except superficially similar Australian setobiont *L. seticola* Kane, 1965 were included in a new genus *Circolagenophrys* (Jankowski 1980, 1982, 1986).

In my review of ciliate genera (Jankowski 2007) I have lowered the rank of *Circolagenophrys* to subgenus, only to avoid further complication of taxonomy of this group with a world-wide distribution; but I have no doubt that *Lagenophrys*, *Circolagenophrys*, *Paralagenophrys*, *Setonophrys* and *Stylohedra* are valid distinct genera, and *Clistolagenophrys* Clamp, 1991, based on mistakes of Swarczewsky (1930), is a synonym of *Circolagenophrys*. Subgenus is useful category in taxonomy, partly in disputable cases – it exists but must not be mentioned. *L. vaginicola* differs from other species not only by setobiosis and pyriform shell, but also by thickening of the posterior part of the shell, well shown in original Stein's drawings, but absent in the drawing of Clamp (1991: 357). This thickening or a kind of double bottom is very distinct in living and fixed specimens (in formalin), but not in those mounted in balsam. Thus not only the shell shape, but also its structure is different in two subgenera.

The type species of *Circolagenophrys* Jankowski, 1980 (now subgenus) is *L. ampulla* Stein, 1852 from freshwater amphipods *Gammarus pulex* and *G. lacustris* in Europe. My drawing of *C. circularis* (Jankowski 1986), reproduced here (Fig. 18), was accompanied in the cited article by the following remark: "We describe below 5 new species of the genus and redescribe a species *Platycola circularis* that in reality belongs to lagenophryans"; and a short text later: "*Circolagen. circularis* comb. n. (= *Platycola circularis* Dons, 1941). Dons (1941) described his new species from "uropods" (pleopods) of wood-boring isopods *Limnoria lignorum* in Norway. The species is common on the same host on Murman and in the White sea, and inhabits pleopods and pereopods. The species is not new, thus we will limit ourselves by reference to the drawing; this is a race from Murman coast limnorians. Dons studied species at small magnification and published a very primitive drawing. On our slides, all features of the species are typical for lagenophryans; mouth walls are differentiated; ventral wall unequal, with mighty central thickening and, on both sides of it, with less distinct folds; dorsal wall without folds and thickenings. Walls of the mouth and shell are very thin, the species is difficult for study. Lagenophryans of such type possibly inhabit all species of limnorians in the entire ocean, and they must be compared with the Scandinavian race".

Review of ciliate genera (Jankowski 2007: 963) includes the diagnosis, hosts and species composition of the subgenus *Circolagenophrys*; the name *L. circularis* was given here without designation "comb. nov.": "Species from Isopoda are – *L. aselli* Plate, 1888;

L. circularis (Dons, 1940 in *Platycola*); *L. platei* Wallengren, 1900; *L. monolistrae* Stammer, 1935; *L. nivalis* Kane, 1969; *L. limnoriae* Clamp, 1988 (synonym or local species on wood-borers). Species *L. cochinensis* Santhakumari et Gopalan, 1980 was described from isopods *Sphaeroma* and tanaids *Apseudes*". This is not the form of transfer permitted by ICZN rules, thus I transfer *Platycola circularis* Dons, 1941 to *Lagenophrys* in this article, as *Lagenophrys* (subgenus *Circolagenophrys*) *circularis* (Dons, 1941) comb. nov.

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