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Morphologic and Genetic Variability in the Marine Planktonic Ciliate *Laboea strobila* Lohmann, 1908 (Ciliophora, Oligotrichia), with Notes on its Ontogenesis

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Abstract

Laboea strobila Lohmann, 1908 is a conspicuous oligotrich ciliate in the marine plankton. In order to compare different populations, the morphology of specimens from the Mediterranean Sea, North Sea, and Irish Sea was investigated using live observation, protargol impregnation, and scanning electron microscopy. Furthermore, the PCR-amplified products of the SSrRNA gene from a monoclonal culture of *L. strobila* from the Mediterranean Sea were sequenced and aligned with sequences of other oligotrichs, including a population of *L. strobila* from the Atlantic coast of the USA. Finally, the data from the ecological literature were summarized and the cultivation methods were described. The SSrRNA gene sequences of the two distantly located *L. strobila* populations from the North Atlantic are identical. Likewise, the morphometrics of most populations so far investigated after protargol impregnation (i.e. from the North Atlantic) do not show obvious differences. In all computed phylogenetic trees, *L. strobila* groups with *Strombidium* species, forming a monophyletic taxon corresponding to the subclass Oligotrichia. These results are corroborated by the ontogenetic comparison. Since no type species was fixed for *Laboea* Lohmann, 1908, *L. strobila* was designated in the present paper.

Keywords

Intraspecific variability; live observation; phylogeny; protargol impregnation; scanning electron microscopy; SSrRNA; Strombidiidae; taxonomy

WITHOUT fixing a type, Lohmann (1908) established the genus *Laboea* for *L. conica*, *L. strobila*, and *L. globosa* and distinguished it from *Strombidium* Claparède and Lachmann, 1859 by the presence of cortical platelets. Wulff (1919), Busch (1921), and Kahl (1932), however, considered *Laboea* as synonym of *Strombidium* because the latter was also found to have cortical platelets. Later, Fauré-Fremiet (1970) emended the diagnosis of *Laboea* by including the spiralled cell shape and thus obviously excluded *L. conica* and *L. globosa* from the genus. Unfortunately, he also did not definitely designate *L. strobila* as type. The screw-like appearance was subsequently regarded as the unique feature of the monotypic genus. Accordingly, specimens with such a cell shape were always and easily identified as *L. strobila*.

The species seems to be well-known because it was investigated in vivo, after preservation and protargol impregnation, and with epifluorescence microscopy (e.g. Fauré-Fremiet 1924; Laval-Peuto and Rassoulzadegan 1988; Montagnes et al. 1988; Wulff 1919). Furthermore, its respiration, photosynthesis, and carbon metabolism were studied (e.g. Jonsson 1987; McManus and Fuhrman 1986; Putt 1990b; Stoecker and Michaels 1991; Stoecker, Michaels, and Davis 1987; Stoecker et al. 1988). Several other investigations focussed on its temporal and spatial distribution (e.g. James and Hall 1995; McManus and Fuhrman 1986; Milani et al. 1991; Modigh 2001; Putt 1990a; Rassoulzadegan 1977; Sanders 1995; Stoecker, Taniguchi, and Michaels 1989; Tempelman and Agatha 1997). Nevertheless, there are still large gaps in our knowledge about *L. strobila* with respect to detailed live observations and investigations of the ultrastructure and ontogenesis. Furthermore, it is unclear whether *L. strobila* is truly cosmopolitan or is in fact a species complex, especially, as *L. strobila* might have occasionally been confused with *Tontonia grandis* Suzuki and Han, 2000, which has a similar screw-like appearance. Thus, the morphologic and genetic variability were investigated using distantly located populations.

MATERIALS AND METHODS

Collection and cultivation

Laboea strobila was isolated by A. Beran from a plankton sample taken in February 2002 in the Gulf of Trieste (Northern Adriatic; ~45°42'N 13°42'E). The ciliates were transferred from the sample into sterile culture medium A (Table 1) and washed twice to minimize the initial contamination by other organisms. Clonal cultures were established in sterile, HCl-washed glass bowls (Arcoroc, France) containing 5 ml of medium enriched with *Isochrysis galbana* and an unidentified cryptophyte as food; the bowls were covered by a Petri dish. The cultivation temperature was kept close to that of the sample (10 °C vs. 7 °C). Weekly, ~100 ciliates were transferred from the growing clonal cultures into bowls (pre-treatment, see above) with 20 ml of medium and food algae. The cultures were checked microscopically at intervals for eventual growth of bacteria and or degradation of ciliates and food. No attempt was, however, made to keep the cultures bacteria-free because the cryptophytes needed bacteria. Parallel cultures were maintained at 15.5 °C. Food algae were sustained as batch cultures in 100 ml borosilicate Erlenmeyer flasks with 50 ml medium B (Table 1) at 15.5 °C. All organisms were cultured at a 10 h dark to 14 h light cycle with an irradiance of ~10 $\mu\text{E m}^{-2} \text{s}^{-1}$ for *L. strobila* and the cryptophyte and ~50 $\mu\text{E m}^{-2} \text{s}^{-1}$ for *I. galbana*.

The other populations of *L. strobila* were collected by the senior author in the harbour of List, Island of Sylt (German North Sea coast; ~55°01'N 08°27'E) and in the Irish Sea near the Port Erin Marine Laboratory, Isle of Man (~54°05'N 04°45'W).

Morphological investigations

The behaviour was studied in a Petri dish under a dissecting microscope. The morphology was investigated under a compound microscope equipped with a high-power oil immersion objective as well as bright-field and interference contrast optics. Protargol impregnation followed the protocol of Song and Wilbert (1995). For scanning electron microscopy, cells were fixed for 30 min in a modified Parducz solution made by 6 parts of 2% OsO_4 (w/v) in sea water and one part of saturated aqueous HgCl_2 (Valbonesi and Luporini 1990); further steps followed Foissner (1991).

Counts and measurements on protargol-impregnated cells were performed at 1,000 $\times$; in vivo measurements were made at 4–1,200 $\times$. Cell sizes measured in vivo (5–6 individuals) provide an estimate of shrinkage due to fixation and staining.

Illustrations of live specimens summarize information and are based on mean measurements, while those of protargol-impregnated specimens were made with a camera lucida (for details, see Foissner et al. 2002). Note that the cells in micrographs were often compressed or shrunken, especially, in the scanning electron micrographs.

Slide deposition

Montagnes et al. (1988) deposited neotype material of *L. strobila* in the Smithsonian Institution, Washington, D.C., USA. Voucher slides from the Italian and Irish Sea population have been deposited in the Biology Centre of the Museum of Upper Austria (LI) in A-4040 Linz (Austria), with relevant cells marked.

Terminology

The area, in which the anterior membranelles insert, is named collar region. It merges anteriorly into a thin apical protrusion. The direction of kinety twining is determined according to Montagnes and Taylor (1994). The part of the girdle kinety extending horizontally from the ventral membranelar zone to the middle of dorsal side in *L. strobila* is named left portion, while the remaining, sinistrally spiralled part is called right portion.

DNA extraction and amplification

DNA of the Trieste population was extracted from the ethanol-fixed cells, following the modified Chelex extraction described by Strüder-Kypke and Lynn (2003). Ten to twenty microliters of the supernatant were used in the subsequent PCR reactions.

The PCR amplification was performed in a Perkin-Elmer GeneAmp 2400 thermocycler (PE Applied Biosystems, Mississauga, ON, Canada), using the internal forward primer 300F (5'-AGGGTTCGATTCCGGAG-3'; Elwood, Olsen, and Sogin 1985) and the reverse primer C (5'-TTGGTCCGTGTTTCAAGACG-3'; Jerome and Lynn 1996). PCR products were purified with the GeneClean Kit (Qbiogen, Carlsbad, CA, USA) and sequenced in both directions in an ABI Prism 377 Automated DNA Sequencer (Applied Biosystems Inc., Foster City, CA, USA), using dye terminator and Taq FS with two forward and two reverse internal SSrRNA primers (Elwood, Olsen, and Sogin 1985) and the amplification primers.

Sequence availability and phylogenetic analyses

The nucleotide sequences used are available from the GenBank/EMBL databases and have the following accession numbers: *Codonellopsis americana* AY 143571 (Strüder-Kypke and Lynn 2003), *Eutintinnus pectinis* AY 143570 (Strüder-Kypke and Lynn 2003), *Eutintinnus* sp. (strain ENB99) AY 143569 (Strüder-Kypke and Lynn 2003), *Favella panamensis* AY 143572 (Strüder-Kypke and Lynn 2003), *Gonostomum strenuum* AJ310493 (Bernhard et al. 2001), *Halteria grandinella* AF194410 (Shin et al. 2000), *Laboea strobila* AF399151 (Snoeyenbos-West et al. 2002), *Laurentiella strenua* AJ310487 (Bernhard et al. 2001), *Metacylis angulata* AY 143568 (Strüder-Kypke and Lynn 2003), *Metacylis* sp. (strain MNB99) AY 143567 (Strüder-Kypke and Lynn 2003), *Novistrombidium testaceum* AJ488910 (Modeo et al. 2003), *Onychodromus quadricornutus* X53485 (Schlegel, Elwood, and Sogin 1991), *Oxytricha granulifera* X53486 (Schlegel, Elwood, and Sogin 1991), *Rhabdonella hebe* AY 143566 (Strüder-Kypke and Lynn 2003), *Steinia sphagnicola* AJ310494 (Bernhard et al. 2001), *Strobilidium caudatum* AY 143573 (Strüder-Kypke and Lynn 2003), *Strobilidium* sp. AF399123, AF399124 (Snoeyenbos-West et al. 2002), *Strombidinopsis* sp. AF399132 (Snoeyenbos-West et al. 2002), *Strombidium inclinatum* AJ488911 (Modeo et al. 2003), *Strombidium purpureum* U97112 (Hirt et al., unpubl. data), *Strombidium* sp. (strain SBB99-1) AY 143565 (Strüder-Kypke and Lynn 2003), *Strombidium* sp. (strain SNB99-2) AY 143564 (Strüder-Kypke and Lynn 2003),

Strombidium sp. (clone Stromb00ssu_4) AF399116 (Snoeyenbos-West et al. 2002), *Tetmemena pustulata* (as *Stylonychia pustulata*) X03947, M 14600 (Elwood, Olsen, and Sogin 1985). *Tintinnidium mucicola* AY 143563 (Strüder-Kypke and Lynn 2003), *Tintinnopsis dadayi* AY 143562 (Strüder-Kypke and Lynn 2003), *Tintinnopsis fimbriata* AY 143560 (Strüder-Kypke and Lynn 2003), *Tintinnopsis tocatinensis* AY 143561 (Strüder-Kypke and Lynn 2003), *Uroleptus pisces* AF164131 (Prescott et al., unpubl. data).

The *Laboea strobila* sequence was added to the existing DCSE (Dedicated Comparative Sequence Editor; De Rijk and De Wachter 1993) database and automatically aligned to the already existing *L. strobila* sequence. Considering secondary structural features of the SSrRNA molecule, we further refined the alignment. MrBayes ver. 2.01, a phylogenetic program, employing the Bayesian Inference (Huelsenbeck and Ronquist, in press), determining the maximum posterior probability of a phylogeny out of 500,000 trees, and approximating it with the Markov Chain Monte Carlo (MCMC), was used to infer a phylogenetic tree from our data set. Genetic distances were computed with TreePuzzle 5.0 (Schmidt et al. 2002), employing the Tamura-Nei algorithm and assuming rate heterogeneity with gamma-distribution. Quartet puzzling with 10,000 puzzling steps was used to construct a maximum likelihood tree with support values for the internal branches and maximum likelihood branch lengths. A maximum parsimony analysis was performed with PAUP* ver. 4.0b10 (Swofford 2002) and the data were bootstrap resampled 1,000 times.

RESULTS

Family Strombidiidae Fauré-Fremiet, 1970

Genus *Laboea* Lohmann, 1908

- 1908 *Laboea*—Lohmann, Wiss. Meeresunters., Abt. Kiel, 10:298.
- 1910 *Conocylis* g. n.—Meunier, Camp. Arctique 1907:146 (pro parte).
- 1919 *Chonocylis*—Wulff, Wiss. Meeresunters., Abt. Helgoland, 13:112 (misspelling).
- 1919 *Concycles*—Wulff, Wiss. Meeresunters., Abt. Helgoland, 13:112 (misspelling).
- 1919 *Strombidium* Claparède & Lachmann—Wulff, Wiss. Meeresunters., Abt. Helgoland, 13:112 (pro parte).
- 1921 *Strombidium*—Busch. Arch. Protistenk., 42:365 (pro parte).
- 1929 *Laboea* Lohmann 1908—Hamburger and Buddenbrock, Nordisches Plankton:79.
- 1932 *Strombidium*—Kahl, Tierwelt Dtl., 25:500 (pro parte; first reviser).
- 1932 *Loboea*—Kahl, Tierwelt Dtl., 25:496 (misspelling).
- 1970 *Laboea* Lohmann, 1908—Fauré-Fremiet, Protistologica, 5 (year 1969):349.
- 1979 *Laboea* Lohmann, 1908—Corliss, The Ciliated Protozoa:302.
- 1985 *Strombidium* Claparède and Lachmann, 1858—Maeda and Carey, Bull. Ocean Res. Inst., Univ. Tokyo, 19:5 (pro parte; second revisers).
- 1985 *Laboea* Lohmann, 1908—Small and Lynn, Illustrated Guide to the Protozoa, 1st ed.: 419.
- 1994 *Laboea*—Laval-Peuto, Grain, and Deroux, Traité de Zoologie:171.

1999 *Laboea* Lohmann, 1908—Song, Zhu, and Chen, Yellow Sea, 5:82.

2002 *Laboea* Lohmann, 1908—Lynn and Small, Illustrated Guide to the Protozoa, 2nd ed.: 467.

Synonymy—The synonymization of *Woodania* Leegaard, 1915 with *Laboea*, as supposed by Song (1999) and Song, Zhu, and Chen (1999), is not justified, as the former has no spiralled girdle kinety. Since the original diagnosis mentioned only the cortical platelets as distinguishing feature, several authors synonymized the genus with *Strombidium* (see above). Fauré-Fremiet (1970) emended the diagnosis by including the screw-like appearance. Although Alekperov and Mamajeva (1992) and Maeda and Carey (1985) regarded this distinguishing feature as insufficient for the separation of *Strombidium* and *Laboea*, we follow Fauré-Fremiet (1970), but improve the diagnosis by adding the number and direction of the spiral whorls performed by the girdle kinety.

Improved diagnosis—Strombidiidae with anterior and ventral membranellar zone and girdle kinety performing more than one sinistral turn around cell.

Remarks—For *Laboea* Lohmann, 1908 no type species has been fixed, not even by Aescht (2001) who first recognized this lack. Since the genus has been established before 1930 and satisfies the articles 8, 11, and 12 (ICZN 1999), its name is available with Lohmann as author (article 50.1; ICZN 1999). Although all originally included nominal species have been eliminated by Fauré-Fremiet (1970), except for *L. strobila*, this is not automatically the type (article 69.4; ICZN 1999). Accordingly, *Laboea strobila* Lohmann, 1908 is now designated as type.

Type species—*Laboea strobila* Lohmann, 1908.

Laboea strobila Lohmann, 1908

1908 *Laboea strobila* n. sp.—Lohmann, Wiss. Meeresunters., Abt. Kiel., 10:299.

1910 *Conocylis helix* sp. n.—Meunier, Camp. Arctique 1907:147 (pro parte; Plate 7 Fig. 28, Plate 10 Fig. 32, 33).

1915 *Laboea strobila* Lohmann—Leegaard, Nytt Mag. Naturvid., 53:9 (pro parte; Fig. 1, 2e).

1919 *Chonocylis helix*—Wulff, Wiss. Meeresunters., Abt. Helgoland, 13:112 (misspelling).

1919 *Strombidium strobilum* (Lohmann) Wulff—Wulff, Wiss. Meeresunters., Abt. Helgoland, 13:114.

1924 *Laboea strobila* Lohmann—Fauré-Fremiet, Bull. biol. Fr. Belg., Suppl. 6:77.

1929 *Laboea strobila* Lohm.—Hamburger and Buddenbrock, Nordisches Plankton:80.

1930 *Strombidium strobilum*—Busch, Abh. Mus. Nat. Heimatk. Magdeburg, 6:86.

1932 *Strombidium (Laboea) strobilus* (Lohmann, 1908)—Kahl, Tierwelt Dtl., 25:499 (first reviser).

1933 *Strombidium strobilus* (Lohmann 1908)—Kahl, Tierwelt N.- und Ostsee: 101.

1943 *Strombidium (Laboea) strobilus* (Lohmann)—Wailes. Canadian Pacific Fauna:12.

1970 *Conocyclis helix*—Fauré-Fremiet, Protistologica, 5 (year 1969): 349 (misspelling).

1985 *Strombidium strobilum* (Lohmann, 1908) Wulff, 1919—Maeda and Carey, Bull. Ocean Res. Inst., Univ. Tokyo, 19:39 (second revisers).

1988 *Laboea strobila* Lohmann, 1908—Montagnes et al., J. Protozool., 35:195 (redescription after protargol impregnation).

1993 *Laboea strobila* Lohmann, 1908—Lynn and Gilron, J. mar. biol. Ass. U.K., 73:64.

1994 *Laboea strobila*—Laval-Peuto, Grain, and Deroux, Traité de Zoologie:171.

1999 *Laboea strobila* Lohmann, 1908—Petz, South Atlantic Zooplankton 1:298.

2001 *Laboea strobila* Lohmann, 1908—Agatha, European Register of Marine Species:43.

Improved diagnosis (on own and appropriate literature data)—Size in vivo 50–150 × 25–75 µm, usually 95–130 × 50–70 µm, after protargol impregnation usually 80–110 × 40–60 µm; obconical, with screw-like appearance. 43–68 macronuclear nodules on average. Extrusomes acicular and 17–24 × 0.5–0.7 µm in size. Girdle kinety usually performs 4.5–5 whorls, dikinetidal. Membranellar zones distinctly separate, anterior zone composed of 11–18 membranelles on average, ventral zone of 16–28. Obligate mixotrophic.

Description of specimens from Trieste—Size in vivo 110–145 × 55–75 µm, usually ~120 × 65 µm; after protargol impregnation 93–121 × 49–63 µm, usually 102 × 56 µm (Table 2). Cell with screw-like appearance due to sinistral spiral of girdle kinety in pointed obconical posterior portion, occupying ~70% of cell length, collar region conical and truncate, with ~4 µm high apical protrusion; circular in cross-section (Fig. 1a, b, 2a, b). Macronuclear nodules numerous and rather variable in number, scattered, usually broadly ellipsoidal, with few globular nucleoli ~2 µm across (Fig. 1c). Up to eight 1–2 µm-sized, faintly impregnated globules, possibly micronuclei. Neither a contractile vacuole nor a cytopyge recognized. Extrusomes insert in shallow bulge above girdle kinety, but absent in last half whorl (possibly the ventral kinety), usually form distinct clusters each with two longitudinal rows of up to five alternating organelles, extend obliquely into cytoplasm overlapping those of previous whorl. Resting extrusomes acicular, decrease in size from 24 × 0.7 µm in anterior whorls to 17 × 0.5 µm in posterior, often well-impregnated, but then distinctly deformed (Fig. 1e, h, 3a–c, e, f). Individual extrusomes in irregularly shaped compartments and surrounded by curved tubules, composed of a lumen encircled by several concentric sheets and a hexagonal electron-dense layer (Fig. 4h, i). Ejected extrusomes filiform, i.e. up to 400 µm long and ~1 µm wide, slightly narrowed in anterior portion, only found when the cell has burst. Argyrophilic structures (probably fibres) in bulge above girdle kinety, except for last half whorl (possibly the ventral kinety) and occasionally the first 1–6 dikinetids; in darkly-impregnated cells, ~5 µm long and inverted L-shaped with a short structure at the left end of the horizontal anterior portion, extending obliquely-posteriorly underneath the next fibre on the left (Fig. 1g, 4d). Cortex fragile, cell thus easily bursts when contacts slide, water surface or cover glass; with rhomboidal to polygonal cortical platelets, forming stripe underneath girdle kinety (Fig. 1d, 2h, i, 3i). Cell surface distinctly distended in preserved specimens, where it forms conspicuous blisters between whorls of girdle kinety (Fig. 1b, 4e). Cells usually dark at low magnification because filled with fat globules, 1–6 µm across, and sequestered plastids; cytoplasm colourless. Swims moderately fast in spirals by rotation about main cell axis, interrupted by zigzag movements; escapes by rapid backwards swimming, but never jumps (Fig. 1k).

Somatic cilia $\sim 1\ \mu\text{m}$ long and rod-shaped, arranged in sinistrally spiralled row interpreted as girdle kinety due to its ontogenetic behaviour; no clear separation of last half whorl of ciliary row recognizable, although its lack of extrusomes and fibres as well as its sharp curvature might indicate a ventral kinety nature (see below; Fig. 1b, c, 3d, g–i). Girdle kinety usually performs 4.5 whorls: commences $\sim 29\%$ back from anterior cell end left of buccal vertex and extends horizontally across dorsal to right side, where it starts its steady spiral, usually terminating at posterior end of dorsal side. Girdle kinetids equidistant, with common cilium at each left basal body, while the right basal body occasionally forms a minute bulge (Fig. 3f); kinetids in dorsal portion of first whorl frequently closely spaced, as recognizable in protargol preparations and scanning electron micrographs (Fig. 3e), possibly comprising a single ciliated basal body.

Oral apparatus occupies anterior end of cell (Fig. 1a, j, 2d–g). Anterior membranelles form C-shaped, ventrally widely open zone, with cilia decreasing in length from $\sim 45\ \mu\text{m}$ in mid-portion to $\sim 1.5\ \mu\text{m}$ at both ends of membranelles; polykinetids $\sim 6\ \mu\text{m}$ apart, slightly curved, composed of three rows of basal bodies $\sim 19\ \mu\text{m}$ long. System of argyrophilic structures associated with anterior membranelar zone: (i) a C-shaped, ventrally open, probably fibrillar structure and (ii) a granule row subjacent to each polykinetid abutting posteriorly on C-shaped structure (Fig. 1i). Ventral membranelar zone longitudinally orientated, distinctly separate from anterior zone, i.e. extends dorsolaterally in deep peristome, terminating $\sim 39\%$ back from anterior cell end in oral cavity; outline elliptical due to gradually shortened polykinetids at both ends of zone. Ventral polykinetids more or less perpendicular to zone's main axis and composed of three rows of basal bodies, except for proximal-most ones, which are almost parallel to zone's axis and consist of only two rows. Ventral membranelles equilateral triangular because length of cilia up to $25\ \mu\text{m}$ in mid-portion and decreasing to $\sim 1\ \mu\text{m}$ at both ends of membranelles. In swimming cells, membranelles perpendicular to main cell axis; otherwise, directed anteriorly. Paroral membrane extends on inner wall of buccal lip, accompanied by a row of argyrophilic granules, covered with perilemma or composed of bare basal bodies as no cilia, but a ridge, are recognizable in scanning electron micrographs (Fig. 1j, 2e–g, 4a–c). Pharyngeal fibres commence probably at left row of argyrophilic granules (basal bodies?) and extend parallel to each other rightwards and slightly posteriorly.

Ontogenesis—Although late dividers were rare in the protargol-impregnated material from Trieste, the stages found permit the reconstruction of the main ontogenetic processes (Fig. 4d–g, 5).

A neoformation organelle is not recognizable in morphostatic specimens. First signs of ontogenesis are probably the closely spaced, ciliated kinetids (monokinetids?) in the first dorsal kinety whorl. Stomatogenesis commences with the apokinetal development of a longitudinally orientated cuneate field of basal bodies in a shallow depression underneath the beginning of the girdle kinety. While the oral primordium elongates posteriorly into a subsurface tube, membranelles composed of two rows of basal bodies immediately differentiate from anterior to posterior (Fig. 4d, f, 5a). The paroral membrane probably originates *denovo*. After the final number of membranelles has been formed, the oral primordium orientates inversely to the parental oral structures in the middle cell portion (Fig. 4e, 5b). Simultaneously, the membranelles become ciliated. When the new oral apparatus evaginates left laterally, the left portion of the girdle kinety curves posteriorly below the oral primordium, as indicated by the strong curvature of this kinety portion in middle dividers (Fig. 5c). Subsequently, the girdle kinety splits left laterally and the left product generates the opisthe's girdle kinety, as inferred from late dividers, where the proter's girdle kinety commences only mid-dorsally and extends in a sinistral spiral to the posterior cell end, while the opisthe's kinety already performs ~ 1.5 sinistral turns (Fig. 5d).

In the posterior cell portion, neither a further split of the ciliary row nor a short kinety fragment are recognizable, which would indicate the presence of a ventral kinety (see ontogenetic comparison). One live and one protargol-impregnated late divider show that the new oral apparatus has apparently migrated from the proter's left side to its right (Fig. 4g, 5d).

During ontogenesis, the parental oral ciliature does not reveal any signs of reorganisation.

SSrRNA gene sequence/primary structure—The PCR product of the partially amplified SSrRNA (small subunit ribosomal RNA) gene of *Laboea strobila* is 1373 nucleotides in length and the sequence is accessible under the GenBank accession number AY302563. Its sequence is identical to the sequence of *L. strobila* (clone Lstr00ssu_1) collected from Long Island Sound, Connecticut, USA (Snoeyenbos-West et al. 2002).

Phylogenetic analyses—Three different phylogenetic analyses were performed: Bayesian Inference (BI), Maximum Likelihood (ML), and Maximum Parsimony (MP). In all analyses, the stichotrich ciliates were used as outgroup and several strombidiid and choreotrich species were included to determine the phylogenetic position of *L. strobila*. In all computed phylogenetic trees, *L. strobila* groups with the *Strombidium* species, forming a monophyletic taxon, corresponding to the subclass Oligotrichia. This topology is strongly supported with 100% posterior probability for BI (Fig. 6), 90% support value for ML, and 88% bootstrap support for MP (data not shown).

The BI analysis depicts *Laboea* as adelphotaxon to *Strombidium*, branching basally within the Oligotrichia (97% and 100% posterior probability; Fig. 6), ML and MP analyses do not resolve the topology within the group (data not shown).

The genetic distance within the class Oligotrichia is high, as computed by TreePuzzle ver. 5.0, assuming rate heterogeneity with gamma-distribution ($\alpha = 0.18$) for nucleotide substitution. The maximum genetic distance between *Strombidium* and *Laboea* is $d = 0.18$ (*L. strobila* and *Strombidium* sp., strain SBB99–1). However, the largest distance between two *Strombidium* species is $d = 0.13$ (*Strombidium* sp., strain SBB99–1 and *Strombidium* sp., clone Stromb00ssu-4).

DISCUSSION

Comparison with related genera

A girdle kinety performing several sinistral turns around cell characterizes only *L. strobila* and *Tontonia grandis* Suzuki and Han, 2000. The latter has a ciliated, highly contractile tail typical for *Tontonia* (for further distinguishing features, see 'Comparison with similar species'), while *L. strobila* was never observed to form a tail neither in raw material nor cultures or experiments (this study; Jonsson 1987; McManus and Fuhrman 1986; Putt 1990b; Stoecker et al. 1988). *Spirostrombidium* species also have a spiralled girdle kinety, but the spiral performs only up to two dextral turns with the posterior portion parallel to the ventral kinety (SA., pers. observ.; Lei, Xu, and Song 1999; Montagnes and Taylor 1994; Petz, Song, and Wilbert 1995).

The extrusome structures of *Strombidium* (Fauré-Fremiet and Ganier 1970; Modeo et al. 2001) and *Novistrombidium* (= *Strombidium* type S1, Modeo et al. 2001) are similar to that of *L. strobila*, except for the lack of the irregular compartments, which might be caused by different preparation methods.

Ontogenetic comparison

Our knowledge of oligotrich ontogenesis is more or less restricted to the investigations of three *Strombidium* species (Agatha 2003; Petz 1994; Song and Wang 1996) and some observations on *Novistrombidium* (Agatha 2003) and *Tontonia grandis* (SA., unpubl. data). Similar to these strombidiids, the parental oral ciliature of *L. strobila* does not reveal any signs of reorganisation. In contrast to the freshwater genera *Limnostrombidium* Krainer, 1995 and *Pelagostrombidium* Krainer, 1991, a neoformation organelle (permanent tube in which the oral primordium develops) has not been observed in the other oligotrich genera, including *Laboea*. According to the studies mentioned above and the present results, the divisional behaviour of the girdle kinety apparently depends on its position relative to the origin of the oral primordium: the left portion of the girdle kinety is above or at the level of the oral primordium in early dividers of *Strombidium* and *Laboea* and migrates posteriorly below the new oral apparatus in middle dividers (Fig. 7a–c, g–i; Agatha 2003; Petz 1994; Song and Wang 1996), whereas this kinety portion is already underneath the oral primordium and does not migrate distinctly during ontogenesis in *Novistrombidium* (Fig. 7d–f; Agatha 2003; Anigstein 1913). Due to the same ontogenetic behaviour, the spiralled kinety of *L. strobila* is probably homologous to the girdle kinety of *Strombidium* and *Novistrombidium*. Since *Laboea* and *Strombidium* apparently recapitulate the course of the girdle kinety posterior to the oral primordium in *Novistrombidium*, they are assumed to be more derived with the left kinety portion above the oral primordium as a synapomorphy. Although *Novistrombidium* and *Strombidium* generate their somatic ciliature like *L. strobila* by intrakinetal proliferation of basal bodies and a simple split of the kineties, distinctly closer spaced kinetids as the first sign of ontogenesis have not been observed in these two genera. Furthermore, the oral primordium occupies the posterior cell portion in middle dividers of *Strombidium*, but it is in the middle portion in *Laboea* possibly because the posterior cell portion is occupied by the proter's spiralled girdle kinety.

The daughter's ventral kinety is the posterior divisional product of the old kinety and migrates only slightly to obtain its final position in *Strombidium* and *Novistrombidium* (Fig. 7a–f; Agatha 2003; Petz 1994; Song and Wang 1996). Since the last half whorl of the ciliary row in *L. strobila* differs from the anterior portion in the lack of extrusomes and associated fibres, it might represent the ventral kinety. In the available divisional stages of *L. strobila*, however, neither a split within the last half kinety whorl nor the migration of a kinety fragment from the proter's posterior to the end of the growing girdle kinety of the opisthe is recognizable. Thus, investigations of later dividers may definitely answer the question, whether or not a ventral kinety is present.

Phylogenetic analyses

The species has always been grouped together with strombidiid ciliates, using morphological features (Fauré-Fremiet 1924; Kahl 1932; Montagnes et al. 1988). Our data of the SSrRNA gene sequences of different *Strombidium* species and *L. strobila* support this relationship through phylogenetic analyses, as it was shown in previous studies (Modeo et al. 2003; Snoeyenbos-West et al. 2002). Only a few *Strombidium* species, one *Novistrombidium* species, and *L. strobila* as representatives of the subclass Oligotrichia have been sequenced so far; it is thus difficult to resolve the branching pattern within the Oligotrichia due to the large genetic distances and this unequal sampling.

Comparison with similar species

The screw-like appearance of *Laboea strobila* was thought to be unique, until Suzuki and Han (2000) described *Tontonia grandis* from the East China Sea based on protargol-impregnated material. This species possesses also a screw-like cell shape and multiple macronuclei. The tail, the main distinguishing feature, is often hardly recognizable due to its

contraction or loss caused by fixation or handling. However, there are further features distinguishing *L. strobila* from *T. grandis*: the number of kinety whorls (3.5–5 vs. 3.0–3.5); the number of ventral membranelles (12–24 vs. 24–38; SA., unpubl. data; Suzuki and Han 2000); and the extrusome shape (acicular vs. curved acicular; SA., unpubl. data). Furthermore, the specimens of *T. grandis* are larger than *L. strobila* from the same sample ($146\text{--}189 \times 71\text{--}88 \mu\text{m}$ vs. $64\text{--}108 \times 35\text{--}61 \mu\text{m}$ after protargol impregnation; SA., unpubl. data), they lack the backwards escape movement, swim very slowly, and the cell surface is usually covered by overlapping cortical platelets. These differences and the fact that tail formation was never observed in *L. strobila* indicate that *L. strobila* and *T. grandis* are distinct species, although they are difficult to distinguish, especially after fixation.

Morphological comparison of populations

Most literature data are based on preserved or protargol-impregnated material and usually provide only the mean or range of cell length. These values fall into the size range of the populations described above and mentioned in Table 3. Bernard and Rassoulzadegan (1994), however, found only $30 \mu\text{m}$ long cells, while specimens with more than $130 \mu\text{m}$ length were recorded by several other authors (e.g. Dolan and Marrasé 1996; Jonsson 1987; Ota and Taniguchi 2003). The cells found in the Antarctic region of the Atlantic by Mamaeva (1989) probably represent a different species as they measured $300 \mu\text{m}$, which is even larger than *Tontonia grandis* (see above). The number of macronuclear nodules shows a rather high variability within and occasionally between populations (Table 3); Hofker (1931) recorded extremely few, i.e. only 28 nodules. The mean number of anterior membranelles is 13–15, except for the populations from the Arctic Sea (probably 11; Alekperov and Mamajeva 1992) and the Island of Sylt (18). The Arctic specimens are also exceptional, regarding the great length of the collar region and the high number of ventral membranelles (27–30 but 23, as estimated from the figure in Alekperov and Mamajeva 1992). Although the latter falls into the range of *T. grandis*, a confusion of both species is unlikely because the illustration shows a specimen with at least four kinety whorls (Alekperov and Mamajeva 1992). Further studies are required to verify conspecificity.

The presence of a ventral kinety is controversially discussed. Indeed, the last half whorl of the girdle kinety differs from the anterior portion in some respects, i.e. the absence of extrusomes and associated fibres. Since such a lack characterizes the ventral kinety of strombidiids, except for *Strombidium lingulum* (Montagnes and Humphrey 1998), this kinety portion was regarded as ventral kinety by Montagnes et al. (1988). The authors also observed a gap, separating both parts, and a different kinetid structure in the girdle (ciliated monokinetids) and ventral (bare dikinetids) kinety. Different kinetid structures in the girdle and ventral kinety are, however, unlikely, assuming an ancestor with dikinetidal somatic kineties, as in the hypotrichs and stichotrichs. Moreover, the reinvestigation of the type material kindly provided by the Smithsonian Institution (Catalogue No. 39705) showed exclusively ellipsoidal, clockwise inclined, more or less equidistantly located granules (probably dikinetids) with a cilium arising from the left portion, while a distinct and constantly occurring gap was not found. Instead, the apparent lack of extrusomes and fibres in the last half kinety whorl may be due to the short distance to the posterior cell end. Additionally, the limited ontogenetic data do not indicate a ventral kinety, whose absence is not an extraordinary feature in strombidiids (Alekperov and Asadullayeva 1997; Krainer 1991; Lynn and Gilron 1993; Lynn, Montagnes, and Small 1988). As pointed out in the ‘Ontogenetic comparison’, however, the investigation of later divisional stages might answer the question, whether or not *L. strobila* has a ventral kinety.

Cilia of the paroral membrane are neither recognizable in the scanning electron micrographs nor in the protargol preparations from the type and other populations. Thus, Montagnes et al.

(1988) obviously studied specimens, in which the perilemma did not cover the membrane or interpreted the fibres, originating from the argyrophilic granule row as cilia.

There are some populations deviating from the thoroughly studied ones mainly by cell shape (Alekperov and Mamajeva 1992; Leegaard 1915; Small and Lynn 1985) and size (see above); they thus possibly represent different species. The other populations mentioned above do not show conspicuous differences in the morphometrics; however, most records are not substantiated by illustrations and/or detailed data. Accordingly, reliable information is only available for specimens from the Atlantic coast of North America (Montagnes et al. 1988), the North Sea, Irish Sea, and Mediterranean Sea (this study), but probably lacks for the Pacific Ocean, Indian Ocean, and the South Atlantic. Therefore, there is only evidence by morphometric data and gene sequence analyses for the conspecificity of the North Atlantic populations.

Genetic comparison of populations

The two *Laboea strobila* populations collected from different locations in the Atlantic Ocean, i.e. at the coast of Connecticut (USA) and the Mediterranean Sea, are identical in their SSrRNA gene sequence (this paper). Likewise, the internally transcribed spacer regions of these populations and the one from the Isle of Man are identical (Katz, L., pers. commun.).

Occurrence and ecology

Laboea strobila was registered in the North Atlantic (e.g. Margalef 1973; Møller and Nielsen 2000; Montagnes et al. 1988) and connected regions, such as the North Sea (e.g. Van Goor 1923), the Baltic Sea (e.g. Lohmann 1908), and the Mediterranean Sea (e.g. Ibanez and Rassoulzadegan 1977). Moreover, it was found in the Arctic Sea (e.g. Sime-Ngando et al. 1997; Wulff 1919) and the Pacific Ocean (e.g. Chang 1990; Fonda Umani and Monti 1991; Hasle 1960; Wailes 1943). In the Antarctic part of the Atlantic, extraordinarily large cells occurred, whose identity is thus doubtful (see above; Mamaeva 1989). There is only a single record from the Pacific Ocean substantiated by an illustration and morphometrics (Alekperov and Mamajeva 1992). These specimens from the Barents Sea, however, are exceptional in several respects and therefore possibly represent a different species (see above). Records from the Indian Ocean and the South Atlantic are probably lacking. The wide range of temperatures (0–30 °C) and salinities (2–38‰) tolerated by the species explains, at least partially, its wide spatial and seasonal distribution, covering spring, summer and autumn, and occasionally even wintertime (Levinsen, Nielsen, and Hansen 2000; Martin and Montagnes 1993). *Laboea strobila* is obligate mixotrophic, i.e. sequesters chloroplasts from different algal taxa for photosynthesis, but requires particular food for continued growth (e.g. Stoecker et al. 1988). Due to its large size, the species contributes occasionally significantly to the biomass of mixotrophic ciliates or oligotrichs (e.g. Stoecker, Taniguchi, and Michaels 1989), while its numerical contribution is usually negligible (e.g. Modigh 2001).

Since it cannot be excluded that *Tontonia grandis* was often confused with *L. strobila*, there is some uncertainty in assigning these literature data without illustrations and/or morphometrics to *L. strobila*.

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Fig. 1a–k.

Laboea strobila, specimens from the Gulf of Trieste (a–e, g–k) and the Isle of Man (f) from life (a, d, h, k) and after protargol impregnation (b, c, e–g, i, j). **a.** A representative specimen showing the spiralled stripe of extrusomes accompanying the girdle kinety, except for the last half whorl. **b, c.** Ventral and dorsal views of same specimen. The girdle kinety performs ~4.5 turns around the cell, terminating dorsally near the posterior end (arrowhead). Although extrusomes and fibrillar structures are lacking in the last half kinety whorl, there is no change in kinetid structure and distance recognizable neither in protargol preparations nor in scanning electron micrographs (Fig. 3d, g–i). The macronuclear nodules are scattered throughout the cell. **d.** Pattern of cortical platelets. **e, h.** The resting extrusomes are deformed in protargol preparations (e), while acicular and $\sim 24 \times 0.7 \mu\text{m}$ in vivo (h). **f.** Pattern formed by extrusome attachment sites and the horizontal portion of an associated fibre. **g.** Dikinetidal girdle kinety with associated fibres. Arrowheads denote fibre portions extending underneath the following structure. **i.** Subjacent each anterior membranelle a row of argyrophilic granules extends. Their distal ends are connected by a C-shaped, ventrally open fibrillar structure. **j.** Anterior ventral cell portion. The paroral membrane probably comprises bare basal bodies as cilia are neither recognizable in protargol preparations nor in scanning electron micrographs (Fig. 2f, g). It is often accompanied by an additional longitudinal row of granules (arrowhead). **k.** Swimming trace. AM, anterior polykinetids; DC, distended cell surface; E, extrusomes/extrusome attachment sites; F, probably fibres; GK, girdle kinety; GR, granule rows; MA, macronuclear nodules; OF, oral fibres; PM, probably paroral membrane; VM, ventral polykinetids. Scale bars = $40 \mu\text{m}$ (a–c), $10 \mu\text{m}$ (h), $20 \mu\text{m}$ (j).

Fig. 2a-i.

Laboea strobila, specimens from the Gulf of Trieste (a, c-i) and the Isle of Man (b) in the scanning electron microscope (a, b, d-h) and from life (c, i). **a.** Oblique ventral view. The ~1 μ m long somatic cilia are arranged in a sinistrally spiralled girdle kinety underneath a stripe of extrusome attachment sites. **b.** Left lateral view showing the screw-like appearance of the cell due to the sinistrally spiralled girdle kinety. **c.** Live specimen. The dark inclusions are food vacuoles and sequestered plastids. **d, f.** Anterior ventral cell portions. Note the short cilia at the proximal and distal ends of each anterior membranelle (arrowheads). **e.** Top view showing the ventrally widely open zone of anterior membranelles and the separate ventral membranelar zone extending into the oral cavity. **g.** Oral cavity. Protargol preparations show the basal bodies of the paroral membrane. Instead of cilia, the SEM, however, reveals a ridge (arrowhead). **h, i.** Details of cell surface. Rhomboidal to polygonal cortical platelets cause the reticulation recognizable in scanning electron micrographs and compressed live cells. AM, anterior membranelles; AP, apical protrusion; BL, buccal lip; E, just exploding extrusomes; GK, girdle kinety; VM, ventral membranelles. Scale bars = 20 μ m (a, b, d-f, i). 40 μ m (c), 10 μ m (g, h).

Fig. 3a-i.

Laboea strobila, specimens from the Gulf of Trieste (a–g, i) and the Isle of Man (h) from life (a–c) and in the scanning electron microscope (d–i). **a.** Posterior cell portion of live specimen. The closely spaced extrusomes form a sinistrally spiralled stripe parallel to the girdle kinety. The cell is filled with fat droplets and sequestered plastids (asterisks). **b, c.** The resting extrusomes are acicular and up to $24 \times 0.7 \mu\text{m}$ long. **d, g–i.** Lateral (d, g, h) and polar (i) views of posterior cell portion. The somatic cilia are arranged in a kinety, which commences underneath the buccal vertex and usually performs 4.5 sinistral spirals, terminating with an arc near the posterior end of dorsal side. The last kinety whorl is not accompanied by extrusomes and argyrophilic structures (probably fibres). A distinct gap, separating this kinety portion from the remaining ciliary row, is not recognizable. Its kinetids have the same distance like those of the remaining portion and protargol-impregnated cells do not reveal any change in the kinetid structure. Thus, a ventral kinety is not evident. **e, f.** Details of girdle kinety and stripe of extrusome attachment sites. The girdle kinety is composed of dikinetids each with a $\sim 1 \mu\text{m}$ long cilium at the left basal body and a ciliary stub, forming a bulge, at the right (f; arrowheads); occasionally, the basal bodies and cilia are more closely spaced in the dorsal portion of the first kinety whorl, possibly in specimens commencing division (e). E, extrusomes/extrusome attachment sites; GK, girdle kinety. Scale bars = $20 \mu\text{m}$ (a–d, g–i), $2 \mu\text{m}$ (e, f.)

Fig. 4a-i.

Laboea strobila, morphostatic specimens (a-c, h, i) and dividers (d-g) from the Gulf of Trieste after protargol impregnation (a-f) and from life (g), and cells from Sweden in the transmission electron microscope (h, i; unpubl. micrographs kindly provided by P. Jonsson).

a-c. Buccal vertices with posterior portion of ventral membranellar zone. **d, f.** The oral primordium originates ventrally between the first and second kinety whorl left of midline. The formation of the new membranelles commences immediately. **e.** Dorsal view of a middle divider. Arrowhead denotes distended cell surface. **g.** Late divider. Arrowhead marks opisthe. **h.** Cross section of extrusomes. The extrusome structure is similar to that in other strombidiids, viz., a central lumen is surrounded by several concentric sheets and an electron-dense layer. Between the irregular compartment and the hexagonal electron-dense layer curved tubules extend (arrows). **i.** Oblique cross-section of anterior cell portion showing proximal ventral membranelles and arrangement of extrusomes. CP, cortical platelets; E, extrusomes; EL, electron-dense layer; F, probably fibres; FV, food vacuoles; GK, girdle kinety; GR, granule rows; L, extrusome lumen; OAM, opisthe's anterior polykinetids; OF, oral fibres; OP, oral primordium; OPM, opisthe's paroral membrane; OVM, opisthe's ventral polykinetids; P, plastids; PAM, proter's anterior polykinetids; SC, somatic cilium; VM, ventral polykinetids. Scale bars = 10 μm (a-d, f, i), 40 μm (e, g), 1 μm (h).

Fig. 5a–d.

Laboea strobila, dividers from the Gulf of Trieste after protargol impregnation. **a.** Ventral view of an early divider. The oral primordium develops posterior to the left kinety portion, i.e. between the first and second kinety whorl on ventral side. The first membranelles immediately originate. **b.** Dorsal view of a middle divider. The formation of the membranelles has finished. The oral primordium shows an inverted orientation to the proter's oral apparatus with the ventral membranelles and the paroral membrane (arrow) located dorsally. The minute breaks within the girdle kinety are likely preparation artifacts because not recognized in other middle dividers. Each macronuclear nodule shows a replication band. **c.** Ventral view of a late middle divider. The new oral apparatus evaginates between the first and second kinety whorl. The left girdle kinety portion is curved to surround the new membranellar zone posteriorly (arrow). Later, it separates from the right girdle kinety portion and forms the opisthe's girdle kinety. In contrast to most hypotrichs and stichotrichs, the macronuclear nodules do not fuse before division but each elongates and divides alone, as indicated by the division furrows. **d.** Dorsal view of a late divider. The opisthe grows out, which apparently causes a shift of the new oral apparatus from the proter's left to the right half of dorsal side. The girdle kinety of the proter commences mid-dorsally and extends sinistrally spiralled to the posterior cell end, while the opisthe's girdle kinety already performs 1.5 turns. The structures (asterisk) at the centre of dorsal side are possibly newly forming extrusomes. Most macronuclear nodules have divided. DC, distended cell surface; GK, girdle kinety; OGK, opisthe's girdle kinety; OP, oral primordium; PGK, proter's girdle kinety; PM, paroral membrane; R, ridge. Scale bar = 40 μm .

Fig. 6. Phylogenetic tree for *Laboea strobila* computed with MrBayes ver. 2.01, employing the Bayesian Inference (Huelsenbeck and Ronquist, in press). The numbers are posterior probability values out of 32,300 trees.

Fig. 7a-i.

Schematized illustrations of the main ontogenetic events in three strombidiid genera. **a-c.** In *Strombidium*, the oral primordium develops underneath the girdle kinety (a). Later, the left portion of the girdle kinety curves along the posterior margin of the new, evaginating oral apparatus (b) and splits left laterally (c; based on Agatha 2003; Petz 1994; Song and Wang 1996). **d-f.** In *Novistrombidium*, the oral primordium originates above the left portion of the girdle kinety, which later surrounds the new, evaginating oral apparatus posteriorly and splits left laterally (e, f; based on Agatha 2003; Anigstein 1913). **g-i.** The ontogenesis of *Laboea strobila* is similar to that of *Strombidium* in the origin of the oral primordium underneath the left portion of the girdle kinety (g), which later curves posteriorly underneath the new, evaginating oral apparatus (h) and splits left laterally (i). The arrowhead marks the anterior end of the proter's girdle kinety. Since *Strombidium* and *Laboea* recapitulate the ontogenetic behaviour of the girdle kinety in *Novistrombidium*, they are probably more derived. The oral primordium is represented by a shaded circle, the opisthe's girdle kinety by a stippled line, and its ventral kinety by a dashed line. GK, girdle kinety; OP, oral primordium; VK, ventral kinety.

Table 1

Growth media for ciliates and phytoplankton based on Medium L1 (Guillard 1995). All stock solutions are filtered through sterile 0.2 μm syringe filters. The vitamin stock is stored frozen in small portions, while nutrient and trace element stocks are stored at 4 °C. Two different trace element working stocks are prepared: one with Na_2EDTA , the other with citric acid as chelating agent. Ferric citrate is added directly to the chelator in the working stock. The remaining trace elements are added as 10 ml primary stock to one litre of the respective working stocks. Primary and working stocks in the table are given as litres, but it is recommended to prepare aliquots of 100 ml, in order to use fresh solutions. All media are prepared and stored in sterile Teflon bottles. Seawater for preparation of media is adjusted with ultra pure Milli-Q® water to a salinity of 32 PSU and filtered by means of a peristaltic pump with silicone tubing using a filtration ramp made of Teflon with 0.22 μm Durapore membrane (Millipore GVWP 142 50). Medium A contains 0.2 ml trace element working stocks and 1 ml vitamin stock per litre; 0.1 ml Na_2EDTA working stock is added to one litre of seawater before autoclaving, while 0.1 ml citric acid working stock and 1 ml vitamin stock are added afterwards. Medium B contains 1 ml Na_2EDTA working stock (0.5 ml added before and 0.5 ml after autoclaving), 1 ml nutrient stock, and 1 ml vitamin stock (both added after autoclaving) per litre. Both media are filtered 24 h after autoclaving by means of a peristaltic pump with silicone tubing with sterile in-line filters (0.22 μm Durapore membrane, Millipore GVWP 04750) to remove precipitates.

Chemicals	Medium A		Medium B		Trace Elements		Nutrients working stock [g litre ⁻¹]	Vitamins working stock [g litre ⁻¹]
	final concentration		working stock [g litre ⁻¹]	primary stocks [g litre ⁻¹]	primary stocks [g litre ⁻¹]	primary stocks [g litre ⁻¹]	primary stocks [g litre ⁻¹]	
	[Mol litre ⁻¹]	[Mol litre ⁻¹]						
a) Trace elements								
Na ₂ EDTA·2H ₂ O	1 × 10 ⁻⁶	1 × 10 ⁻⁵	3.72					
Citric acid	2 × 10 ⁻⁶	–	3.85					
Fe-(III)-citrate	1 × 10 ⁻⁶	5 × 10 ⁻⁶	1.31					
MnCl ₂ ·4H ₂ O	2 × 10 ⁻⁷	1 × 10 ⁻⁶			19.79			
ZnSO ₄ ·7H ₂ O	2 × 10 ⁻⁸	1 × 10 ⁻⁷	plus		2.88			
Na ₂ MoO ₄ ·2H ₂ O	2 × 10 ⁻⁸	1 × 10 ⁻⁸	10 ml		2.42			
CoCl ₂ ·6H ₂ O	1 × 10 ⁻⁸	5 × 10 ⁻⁸	of each		1.19			
CuSO ₄ ·5H ₂ O	2 × 10 ⁻⁹	1 × 10 ⁻⁸	primary		0.25			
H ₂ SeO ₃	2 × 10 ⁻⁹	1 × 10 ⁻⁸	stock		0.13			
NaVO ₃	2 × 10 ⁻⁹	1 × 10 ⁻⁸	liter ⁻¹		0.12			
NiSO ₄ ·6H ₂ O	2 × 10 ⁻⁹	1 × 10 ⁻⁸			0.26			

Chemicals	Trace Elements		Nutrients working stock [g litre ⁻¹]	Vitamins working stock [g litre ⁻¹]
	Medium A	Medium B		
	<u>final concentration</u>	working stock [g litre ⁻¹]		
	[Mol litre ⁻¹]	[Mol litre ⁻¹]		
b) Macronutrients				
NaNO ₂	–	8.83×10^{-4}	75.0	
NaH ₂ PO ₄ ·H ₂ O	–	3.63×10^{-5}	5.0	
c) Vitamins				
Thiamine·HCl	3×10^{-7}	3×10^{-7}		0.1
Biotin	2.1×10^{-9}	2×10^{-9}		0.5
Vitamin B ₁₂	3.7×10^{-10}	1×10^{-10}		0.5

Morphometric data on three populations (Pop) of *Laboea strobila* from Trieste (T), the Island of Sylt (S), and the Isle of Man (I).

Table 2

Characteristics ^a	Pop	$\bar{x}$	M	SD	SE	CV	Min	Max	n
Cell length ^b	T	102.1	100.0	7.9	1.8	7.7	93.0	121.0	19
	S	81.1	83.0	8.4	1.8	10.4	64.0	94.0	23
	I	89.1	88.0	12.7	3.3	14.2	64.0	108.0	15
Cell width ^b	T	55.7	56.0	4.4	7.8	49.0	63.0	1.9	19
	S	43.7	43.5	2.9	0.6	6.5	39.0	49.0	23
	I	50.9	51.0	6.4	1.7	12.6	35.0	61.0	15
Cell length : width	T	1.8	1.9	0.1	0.0	4.9	1.6	1.9	19
	S	1.9	1.9	0.1	0.0	7.8	1.6	2.1	23
	I	1.8	1.8	0.2	0.0	10.0	1.4	2.0	15
Anterior cell end to girdle kinety, distance	T	30.3	31.0	3.8	0.9	12.6	20.0	35.0	19
	I	21.5	21.0	3.3	0.9	15.4	14.0	26.0	15
Anterior cell end to buccal vertex, distance	T	39.8	40.0	3.4	0.8	8.4	33.0	49.0	19
	I	37.4	38.0	2.5	0.7	6.8	31.0	41.0	15
Anterior membranelles, width ^c	T	18.9	19.0	0.9	0.2	5.0	18.0	21.0	19
Macronuclear nodules, length	T	4.9	5.0	0.7	0.2	14.5	4.0	6.0	19
	S	3.2	3.0	0.6	0.1	20.2	2.0	4.0	26
Macronuclear nodules, width	I	4.5	4.0	0.8	0.2	18.4	3.0	6.0	15
	T	3.5	3.0	0.6	0.1	17.3	3.0	5.0	19
	S	3.2	3.0	0.6	0.1	20.2	2.0	4.0	26
Macronuclear nodules, number	I	3.6	4.0	0.6	0.2	17.6	3.0	5.0	15
	T	58.7	59.0	10.4	2.4	17.7	39.0	83.0	19
Anterior membranelles, number ^d	S	43.0	43.0	6.6	1.4	15.4	32.0	58.0	23
	I	48.3	47.0	10.0	3.5	20.8	33.0	61.0	8
Anterior membranelles, number ^d	T	14.5	14.0	0.8	0.2	5.8	13.0	16.0	19
	S	17.5	17.5	1.4	0.6	7.9	16.0	19.0	6

Characteristics ^a	Pop	$\bar{x}$	M	SD	SE	CV	Min	Max	n
Ventral membranelles, number ^d	I	14.0	14.0	–	–	–	13.0	15.0	3
	T	16.4	16.0	0.9	0.2	5.5	15.0	18.0	19
	S	17.4	16.5	2.8	0.9	16.1	15.0	24.0	10
Girdle kinety, number of whorls	I	18.1	18.0	0.9	0.3	5.1	17.0	20.0	9
	T	4.4	4.5	0.2	0.0	3.5	4.0	4.5	19
	S	4.8	5.0	0.4	0.1	8.4	4.0	5.0	26
Girdle kinety, number of kinetids per 10 μm	I	4.4	4.5	0.3	0.1	6.4	3.5	4.5	15
	T	10.6	11.0	0.7	0.2	6.4	10.0	12.0	19
	I	10.0	10.0	1.3	0.3	12.5	9.0	13.0	15

^aData are based on protargol-impregnated, mounted, and randomly selected specimens from clonal cultures (Trieste population) or raw material (Sylt and Isle of Man populations). Measurements in μm . CV, coefficient of variation in %; M, median; Max, maximum; Min, minimum; n, number of individuals investigated; SD, standard deviation; SE, standard error of arithmetic mean; $\bar{x}$, arithmetic mean.

^bMeasured without distended cell surface.

^cMeasured as cord of organelle.

^dCounted in properly orientated morphostatic specimens or middle dividers.

Table 3

Main morphometrics of eight populations of *Laboea strobila*.

Characteristics ^a	Woods Hole ^b			Aretic Sea ^c	Trieste ^d	Syllt ^d	Isle of Man ^d
Cell length	60–100 (81) ^e	68–101 (82) ^e	59–101 (86) ^e	63–106 (93) ^e	100–120	93–121 (102) ^f	64–94 (81) ^f 64–108 (89) ^f
Cell width	20–50 (39)	34–50 (42)	34–50 (44)	38–56 (47)	?	49–63 (56) ^f	39–49 (44) ^f 35–61 (51) ^f
Macronuclear nodules, number		44–93 (61) ^g			60–75	39–83 (59)	32–58 (43) 33–61 (48)
Girdle kinety, number of whorls		4–5 (5) ^g			?	4–4.5 (4.4)	4–5 (5) 3.5–4.5 (4.5)
Girdle kinety, structure		dikinetids with one cilium ^g			?	dinetids with one cilium	
Ventral kinety	— ^g	— ^g	— ^g	— ^g	?	—	—
Anterior membranelles, number	12–18 (14)	12–15 (14)	12–15 (13)	12–14 (14)	10–12	13–16 (15)	16–19 (18) 13–15
Ventral membranelles, number	12–20 (16)	15–22 (18)	15–22 (19)	12–20 (18)	27–30	15–18 (16)	15–24 (17) 17–20 (18)

^aData are based on protargol-impregnated material; mean values in parenthesis. Measurements in μm.

^b21–34 specimens investigated; Montagnes et al. (1988).

^cMore than 15 specimens investigated; Alekperov & Mamajeva (1992).

^dUp to 26 specimens investigated; this paper.

^eApical protrusion excluded.

^fDistended cell surface excluded.

^gReinvestigation of type slides; 19 specimens investigated.