

Zoelucasa sablensis n. gen. et n. sp. (Cercozoa, Incertae Sedis), a New Scale-covered Flagellate from Marine Sandy Shores

Kenneth H. NICHOLLS

Summary. *Zoelucasa sablensis* n. gen et n. sp. is a small heterotrophic flagellate housed within a pyriform lorica of relatively large imbricate, circular siliceous scales. It was found in near-shore benthic sand/seawater samples of both the Pacific and Atlantic Oceans (west and east coasts of Canada; salinity = 32–33 ppt). The median length and width of the lorica was 18 and 11 μm , respectively ($n = 29$). This taxon lacks chloroplasts and swims with a slow zig-zag motion controlled by a short (5–7 μm long), anteriorly-directed flagellum and a longer trailing flagellum, 15–20 μm in length. Its classification within the phylum Cercozoa (most likely, Class Imbricatea) is tentative, as there are no known morphological homologues (discoidal, overlapping siliceous plate-scales forming a test or lorica enclosing a heterotrophic flagellate). Further study of cultured and wild material, including a search for other possible non-flagellate (e.g. amoeboid?) life history stages, TEM examination of cell sections, and rDNA sequencing will most certainly provide more opportunities for a justifiable classification, possibly including a new Order.

Key words: Imbricatea (Silicofilosea), sand-dwelling protist, psammon, silica scales, flagellate.

INTRODUCTION

Sand-welling marine protists include taxa representing a wide variety of forms and phylogenetic lines. Among the best known are dinoflagellates (Faust 1995, Hoppenrath 2000), testate amoebae (Golemansky 1994, Golemansky and Todorov 2006) and various non-dinophyte flagellates (Larsen and Patterson 1990, Okamoto *et al.* 2009, Chantangsi and Leander 2010). An unusual flagellate housed in a lorica or test constructed of large, circular imbricate scales was discovered during examination of near-shore beach sand/seawater

samples collected from both the east and west coasts of Canada. Owing to its apparently unique morphology, it is described here as a new monospecific genus and has been tentatively assigned to the class Imbricatea of the phylum Cercozoa based on its integument of silica scales, the apparent absence of a chloroplast, its single anteriorly directed short flagellum and longer trailing flagellum.

MATERIAL AND METHODS

Collections of nearshore sand mixed with seawater at low tide were made on the North Beach of Sable Island, Nova Scotia (Atlantic Ocean; N43°56', W59°58') in August of 2007 and from the south end of Vancouver near Esquimalt (Pacific Ocean; N48°25.635', W123°27.628' in July of 2008 and at another Pacific Ocean location

Address for correspondence: Kenneth H. Nicholls, S-15 Concession 1, RR # 1, Sunderland, Ontario, Canada L0C 1H0; E-mail: khnicholls@interhop.net

about 800 km NW of the Esquimalt location in December of 2008 in Skidegate Inlet (Haida Gwaii) near Gillat Island (N53°14.46', W131°53.88'). Sampling and laboratory methods for these locations were described in Nicholls (2009a, b). Salinity was measured with a refractometer (model FG-211, Sun Instruments Corp., Torrance CA) with automatic temperature compensation, when the samples arrived at my Ontario laboratory a few days after collection.

Confirmation of likely siliceous nature of the scales of this taxa was determined from specimens that had been isolated, washed with distilled water and allowed to dry on a No. 1 cover slip. This preparation was inverted on a standard glass slide and anchored at the corners with Canada Balsam. The specimen was located with a low-power (16×) objective and then observed with a high-power (100×) phase-contrast objective while concentrated sulphuric acid (H₂SO₄; f.w. = 98.08) was introduced as a droplets at the edges of the cover slip. This procedure was repeated in a separate preparation using concentrated nitric acid (HNO₃; f.w. = 63.01). Enough acid was added to totally envelop and permeate the previously dried specimen. The scaled loricae were observed at regular intervals over periods of several hours.

A search for a chloroplast was conducted on several single *Zoelucasa* cells isolated with a micro-pipette and allowed to slowly compress during evaporation of the aqueous preparation under a #1 cover slip while being examined with 100× oil immersion DIC and phase contrast LM systems. In addition, cells were examined for chlorophyll fluorescence using a Nikon Optiphot 2 fluorescence microscope with a 60× oil immersion objective lens. Isolated cells of *Zoelucasa* were air dried on a coverglass along with single cells of small pennate and centric diatoms isolated from the same Sable Island preserved sample; these diatoms were selected for their small, but easily seen chloroplasts (normal white light illumination) and served as controls on the 2-year period of storage of these cells in formalin and on the cell drying step included in the procedure outlined above, on the understanding that aldehyde-based fixatives and air drying might influence chlorophyll fluorescence (Hallier and Park 1969, Shuvalov and Heber 2003). Incident illumination was from a mercury burner providing all wavelengths in the UV/Visible spectrum, and specimens were observed after a long pass filter was used to block emitted wavelengths below 600 nm.

RESULTS

Zoelucasa sablensis n. gen. et n. sp.

Zoelucasa n. gen.

Diagnosis: Cells lacking chloroplasts; cells with one long trailing flagellum and one shorter flagellum directed antero-laterally. Cells within a lorica formed from a layer of siliceous scales of a single morphological form. Type species: *Zoelucasa sablensis*.

Zoelucasa sablensis n. sp. (Fig. 1a–r)

Diagnosis: Cells about 10 µm in diameter, housed within a radially symmetric pyriform lorica, about 1.5–2 × the cell diameter in length (smaller in dried

specimens). Lorica comprised of 10–15 large, overlapping, circular to slightly elliptical scales, 3–6 µm in diameter, with no surface ornamentation and a convex distal surface. Trailing long flagellum, about 1–1.5 × lorica length; length of the shorter flagellum, usually less than the cell diameter. Both flagella emerge from a parallel position in an anterior flagellar pocket.

Etymology: The genus name is in honour of Zoe Lucas, a Sable Island biologist, ecologist and educator with an impressive knowledge of Sable Island natural history and a life-long mission to understand and protect its flora and fauna. The species name is a direct reference to the type locality, Sable Island, Nova Scotia, Canada.

Type specimen: Fig. 1c

Type locality: Benthic near-shore sand, north shore, Sable Island (Atlantic Ocean, Nova Scotia; N43°56', W59°58'); Salinity = 33 ppt; collected 20 August, 2007.

Material from the type locality: Retained by the author (aqueous dilute formalin solution) as preserved sample # V-2081.

The pyriform shape of the lorica (Fig. 1a, b, c, d) was invariable in this species. The lorica was circular in optical cross-section through the broad basal region through which the lorica widths were measured. Lorica lengths and widths ranged from 14–22 µm and 9–13 µm, respectively (original aqueous samples), with median length and width of 18 and 11 µm, and with coefficients of variation on length and width measurements of 9.8 and 8.2%, respectively (n = 29). Cells contained a centrally or posteriorly located nucleus (Fig. 1a, f, g). Cell shape was spherical when occupying the basal region of the lorica, but was elongate-pyriform in actively swimming specimens.

Flagella emerged in parallel from a well-defined anterior pocket in the cell (Fig. 1g, j). Swimming form was a slow zig-zag pattern, directed alternately about 40° right and left of the forward direction of travel (Fig. 1k). Forward movement was at a rate of about 10–20 µm s⁻¹.

The most distinctive feature of this taxon is the lorica of large, mainly circular, imbricate scales (Fig. 1b) that appeared in SEM images to have smooth unpatterned external surfaces (Fig. 1c, d). The slight concavity of their shape (distal surface is convex) was visible in phase contrast (Fig. 1g, h) and DIC (Fig. 1e) images. From TEM images, however, the scales appeared to be comprised of distal and proximal layers enclosing an internal space occupied by a reticulated meshwork of electron dense material (Fig. 1n–r). The distal and prox-

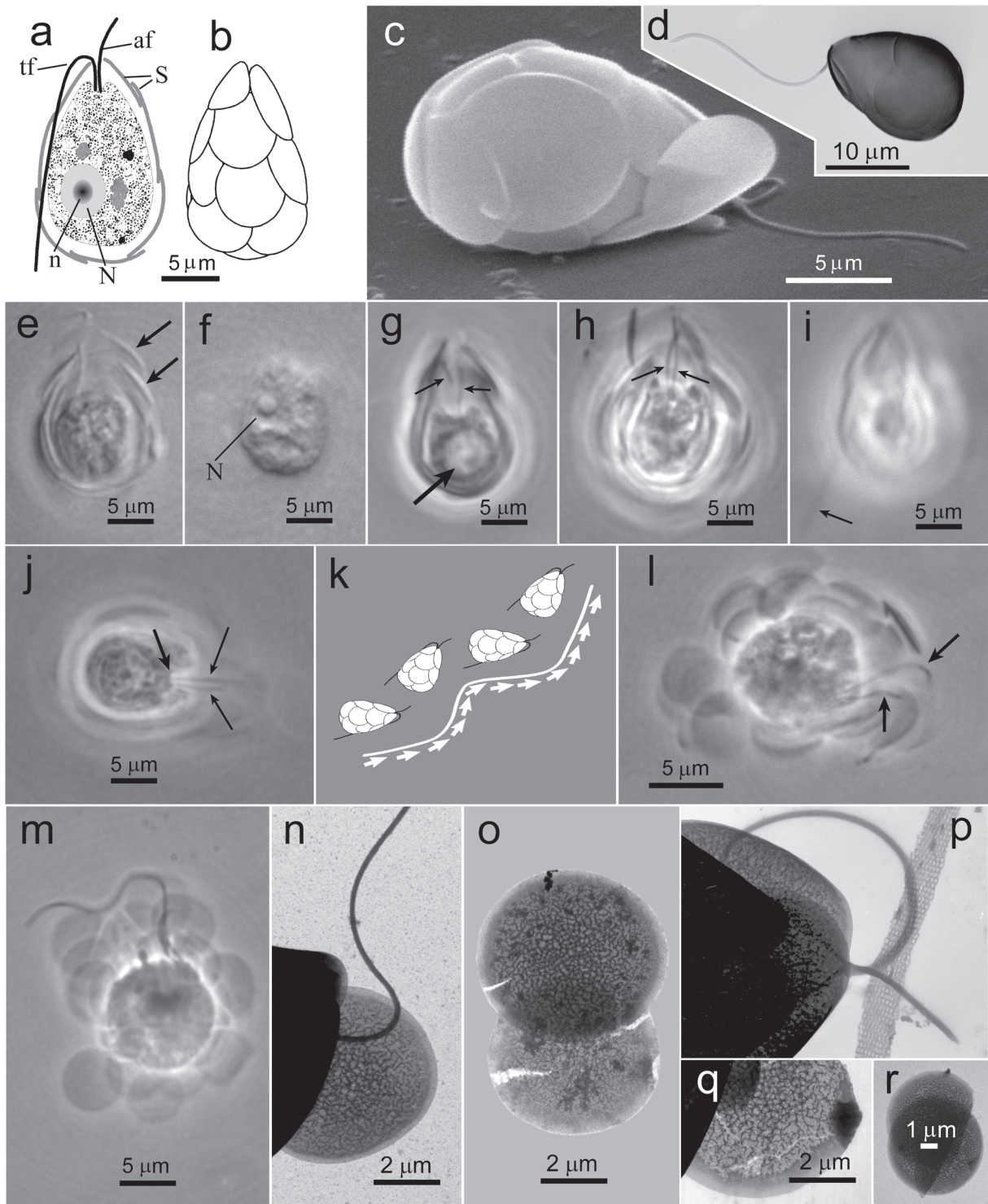


Fig. 1. *Zoelucasa sablensis*. **a** – diagrammatic representation of cellular components, where: af – anterior flagellum, tf – trailing flagellum, S – scales, N – nucleus, n – nucleolus; **b** – external arrangement of scales; **c**, **d** – SEM images of whole, dried cells showing covering of scales and flagella; **e** – DIC image of cell showing optical section of the scale layer (arrows); **f** – nucleus (N); **g**, **h** – nucleus (large arrow) and parallel flagellar insertion in anterior pocket (small arrows) of cells that have retracted to a posterior position within their loricae; **i** – trailing flagellum in a “swimming” cell; **j** – anterior pocket (larger arrow) and parallel emergence of two flagella (small arrows); **k** – diagrammatic representation of the zig-zag pattern of cell “swimming” movement; **l**, **m** – compressed cells showing the flagella (arrows) and disassociated scales around the cells (note the fractures of some scales in **m**); **n–r** – TEM images of scales.

imal layers of the scales are fused around the periphery revealing a marginal unpatterned zone 0.15–0.42 μm wide. This double plate structure with its internal porous support probably serves to provide rigidity given the thinness (and undoubtedly light weight) of the outer surfaces of the scale. Still, the scales were easily fractured, often cracking upon drying (Fig. 1m, o). The scales are very likely siliceous in composition; there was no apparent decomposition after several hours soaking in either sulphuric acid or nitric acid.

While chloroplasts of the control diatom specimens fluoresced bright red and were clearly visible (thus indicating that a 2-year period of storage in formalin did not preclude chlorophyll fluorescence), only a weak pink colour was visible in some of the *Zoelucasa* cells. Owing to its diffuse, and faint nature, this fluorescence was attributed to the likely presence of chlorophyllous particles ingested by the living cells prior to fixation in formalin, rather than to the possible presence of inherent chloroplasts of host origin.

DISCUSSION

The year following the 2007 discovery in the Atlantic Ocean samples, I also found many living specimens in beach sand samples Canada's west coast (Pacific Ocean; see methods) at similar salinity (31–32 ppt). This organism would therefore appear to perhaps have a global marine distribution, at least in the northern hemisphere.

The scale-covered lorica of *Z. sablensis* superficially resembles that of some *Epipyxis* (Chrysophyceae) or *Sphenoderia* (Rhizaria, Euglyphida). The overlapping scales comprising the loricae of *Epipyxis* species are comprised of pectin (not silica) and the cell has chloroplasts and a typical chrysophycean flagellar structure with right-angled insertion of the long and short flagella; as well, the longer flagellum has mastigonemes, and is not trailing (Nicholls and Wujek 2002). *Sphenoderia* (e.g., *S. fissirostris* Penard, 1890), although with large imbricate siliceous scales superficially resembling those of *Zoelucasa sablensis*, is flattened anteriorly (bi-laterally symmetric) and has pseudopodia; no flagellate stage is known for *Sphenoderia* (Meisterfeld 2002).

Although the contemporary concept of the cercozoan class Imbricatea (Howe *et al.* 2011) does include two orders with several genera possessing endogenously produced silica scales (the Thaumatomonadida and the

Euglyphida), there are no known genera in either order that has the combination of large plate-like, imbricate scales enclosing a biflagellate cell of the sort described here for *Z. sablensis*. At the present time, there appears to be no order of protists that can reasonably accommodate *Zoelucasa* given its scaled covered cell and its flagellar characteristics. Further study of cultured and wild representatives of this taxon, including a search for other possible non-flagellate (e.g., amoeboid?) life history stages, TEM examination of cell sections, and rDNA sequencing will most certainly provide more opportunities for a justifiable classification, possibly including a new order and a new family.

Acknowledgements. I acknowledge the assistance of Zoe Lucas (for Sable Is. sampling), David Evans (for southern Vancouver Island sampling) and Doug Bures (for Haida Gwaii sampling). Karen Rethoret, Biology Department, York University, Toronto, provided technical assistance with the SEM, TEM and fluorescence microscope.

REFERENCES

- Chantangsi C., Leander B. S. (2010) An SSU rDNA barcoding approach to the diversity of marine interstitial cercozoans, including descriptions of four novel genera and nine novel species. *Int. J. Sys. Evol. Micr.* **60**: 1962–1977
- Faust M. A. (1995) Observation of sand-dwelling toxic dinoflagellates (Dinophyceae) from widely differing sites, including two new species. *J. Phycol.* **31**: 996–1003
- Golemansky V. (1994) On some ecological preferences of marine interstitial testate amoebas. *Arch. Protistenkd.* **144**: 424–432
- Golemansky V., Todorov M. T. (2006) New data to the shell structure and the biometry of the marine interstitial testate amoebae (Rhizopoda: Testaceafilosia). *Acta Protozool.* **45**: 301–312
- Hallier U. W., Park R. B. (1969) Photosynthetic light reactions in chemically fixed *Anacystis nidulans*, *Chlorella pyrenoidosa*, and *Porphyridium cruentum*. *Plant Physiol.* **44**: 535–539
- Hoppenrath M. (2000) Morphology and taxonomy of *Sinophysia* (Dinophyceae, Dinophysiales) including two new marine sand-dwelling species from the North German Wadden Sea. *Eur. J. Phycol.* **35**: 153–162
- Howe A. T., Bass D., Scoble J. M., Lewis R., Vickerman K., Arndt H., Cavalier-Smith T. (2011) Novel cultured protists identify deep-branching environmental DNA of Cercozoa: new genera *Tremula*, *Micrometopion*, *Minimassisteria*, *Nudifila*, *Peregrinia*. *Protist* **162**: 332–372
- Larsen J., Patterson D. J. (1990) Some flagellates (Protista) from tropical marine sediments. *J. Nat. Hist.* **24**: 801–937
- Meisterfeld R. (2002, but dated 2000 in error) Testate amoebae with filopodia. In: *An Illustrated Guide to the Protozoa*, Second Edition, Vol. II, (Eds. J. J. Lee, G. F. Leedale, P. Bradbury). Society of Protozoologists, Lawrence, Kansas, 1054–1084
- Nicholls K. H. (2009a) Six new marine species of the genus *Paulinella* (Rhizopoda: Filosea, or Rhizaria: Cercozoa). *J. Mar. Biol. Assoc. UK* **89**: 1415–1425
- Nicholls K. H. (2009b) A multivariate statistical evaluation of the “*acolla*-complex” of *Corythionella* species, including a descrip-

- tion of *C. darwini* n. sp. (Rhizopoda: Filosea, or Rhizaria: Cercozoa). *Eur. J. Protistol.* **45**: 183–192
- Nicholls K. H., Wujek D. E. (2002) Chrysophyceae. In: *Freshwater Algae of North America: Classification and Ecology*, (Eds. J. D. Wehr, R. G. Sheath). Academic Press, San Diego, 471–509
- Okamoto N., Chantangsi C., Horák A., Leander B. S., Keeling P. J. (2009) Molecular phylogeny and description of the novel katablepharid *Roombia truncata* gen. et sp. nov., and establishment of the Hacrobia Taxon nov. *PLoS ONE* **4**: e7080. doi:10.1371/journal.pone.0007080
- Shuvalov V. A., Heber H. (2003) Photochemical reactions in dehydrated photosynthetic organisms, leaves, chloroplasts and photosystem II particles: reversible reduction of pheophytin and chlorophyll and oxidation of β -carotene. *Chem. Physics* **294**: 227–237

Received on 13th December, 2011; revised on 14th March, 2012; accepted on 25th March, 2012