AUSTRALIAN MUSEUM SCIENTIFIC PUBLICATIONS

Heep, T., J. Rohozinski, A. Simpson and David J. Patterson, 1998. *Stentor amethystinus* (Protista: Ciliophora: Heterotrichida), a common protozoan member of fresh-water plankton in Australia. *Records of the Australian Museum* 50(2): 211–216. [7 October 1998].

doi:10.3853/j.0067-1975.50.1998.1280

ISSN 0067-1975

Published by the Australian Museum, Sydney

Records of the Australian Museum (1998) Vol. 50: 211–216. ISSN 0067-1975

Stentor amethystinus (Protista: Ciliophora: Heterotrichida), A Common Protozoan Member of Fresh-water Plankton in Australia

THOMAS HEEP,[1] JAN ROHOZINSKI,[2] ALASTAIR SIMPSON[3] & DAVID J. PATTERSON[3]

[1] Lehrstuhl für experimentelle Morphologie, Zoologisches Institut, Weyertal 119, Universität zu Köln, 50923 Köln, Germany

[2] Research School Biological Sciences, Australian National University, PO Box 475, Canberra City ACT 2601, Australia

[3] School of Biological Sciences A08, University of Sydney NSW 2006, Australia

Address correspondence to David J. Patterson
djp@bio.usyd.edu.au

ABSTRACT. Large numbers of a brown to violet ciliate can often be seen in freshwater lakes and billabongs in Australia. Uninterpreted records by light microscopy and electron microscopy are provided. The ciliates are identified as *Stentor amethystinus* Leidy, 1880. Despite the abundance of this species, this is a new record for Australia.

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Stentor Oken, 1815 (Oken, 1815) is a widespread and familiar genus of heterotrich ciliates (protozoa). Cells are typically trumpet-shaped and often distinctly coloured; different species being green, black, blue, pink or brown (Foissner & Wölfl, 1994; Kahl, 1932; Tartar, 1961).

A brown species of *Stentor* has been reported as occurring in very large numbers in fresh-water bodies in New South Wales, the Australian Capital Territory and Victoria (Laybourn-Parry *et al.*, 1997). It occurs throughout the year but blooms, which can discolour the water, occur at various times from mid-spring to late autumn. Up to 4200 individuals per litre have been counted, and the organism is argued to make a significant contribution to primary production (Laybourn-Parry *et al.*, 1997). It has also probably been reported as "cf. *Climacostomum*" (Walker & Hillman, 1977).

The aim of this study was to establish the identity of this ciliate. The genus *Stentor* has been reviewed most recently by Foissner & Wölfl (1994) who recognised 19 species. Species distinctions are normally made on the basis of cell shape and size, presence/absence of a mucilagenous sheath, macronuclear form, the number of micronuclei and their location, numbers of kineties and membranelles, pigmentation, presence/absence of symbiotic algae. One study (Nilsson, 1986) has added ultrastructural characteristics to assist in distinguishing species. Brown species

have been observed from America (Leidy, 1880), India (Murthy & Bai, 1974), Africa (Nilsson, 1986), South America (Foissner & Wölfl, 1994; Laybourn-Parry *et al.*, 1997), New Zealand (cited in Laybourn-Parry *et al.*, 1997) and from Europe (Foissner *et al.*, 1992; Schneider, 1992). The brown colour results from the combination of symbiotic green algae and numerous dark plum-coloured pigment granules.

The first description of *Stentor* in Australia was of *S. polymorphus* (Shephard, 1891), Shephard found large numbers in pools in the area of Sandringham, Victoria. Other records of *S. polymorphus* in Australia are provided by Schewiakoff (1893) and Gillies (1915). Other species reported from Australia are: *S. igneus* (Shephard, 1904), *S. roeselii* (Stickland & Stickland, 1895), and several unnamed species (Fielder, 1893; Ingram *et al.*, 1997; Laybourn-Parry *et al.*, 1997; Stickland, 1923).

Materials and methods

Stentor was collected in 1994 from Corin Dam and Lake Burley Griffin, both in the Australian Capital Territory close to Canberra, Googong Dam in New South Wales, in 1996, from Lake Tuggeranong (ACT, Canberra) and from a pond near Camden, south-west of Sydney.

Water samples containing *Stentor* were kept at 5 to 14°C in plastic bottles. Attempts to establish long term cultures of the organism have been unsuccessful. Exposure to temperatures above 23° results in a rapid decrease in the number of individuals. Stocks were maintained for up to three months at 18°C under continuous light.

Live ciliates were used for morphological studies. For some detailed studies, a small drop of 0.01M EGTA and 0.1M MgCl2 was added on a slide to stop movement and prevent contraction. Movement and morphology were documented by video records and black and white photography using a *Leica DM* microscope equipped with a dedicated flash system (Patterson, 1982) The sizes of cells were measured using an ocular micrometer.

To obtain typical cell profiles, groups of about 100 cells were left undisturbed on a dissection microscope for 12 or more hours in the dark.

For electron microscopy, cells were fixed simultaneously with ice cold 2.5% glutaraldehyde and 0.2% osmium tetroxide in 50 mM cacodylate buffer. Cells were washed free of fixative in buffer, collected, and dehydrated through a series of ethanols before embedding in Araldite. Fixed material was sectioned with a diamond knife using a *Reichert Ultracut S* ultramicrotome. Sections were collected on *Pioloform* coated slot grids, carbon coated, and stained with lead citrate and uranyl acetate prior examination using a *Zeiss 902* electron microscope.

Results

The species observed is a brown-coloured ciliate with symbiotic algae (Fig. 1a). It is most usually encountered as a short conical swimming cell (Fig. 1b). Cells from the different collecting sites had similar sizes—with lengths varying from 150–220 μm (mean 188 μm, n = 34) and with widths from 106–150 μm at the peristomial region (mean 128 μm, n = 34). When sedentary, cells either lie on their sides on the substrate or attach to vertical surfaces by their posterior ends when they adopt a slightly more elongate body form. The attachment to the substrate is very slight and swimming is resumed at the slightest disturbance. When the animal is incubated at 25°C in the dark overnight the body becomes more trumpet-shaped and the peristomial bulge is withdrawn slightly. The increase in length is achieved principally a slight extension of the posterior end. Under these conditions on average, only 9% of the cells were attached, 7% were rounded swimming cells and 83% were conical swimming cells. No elongate trumpet forms—typical of most other species of *Stentor*—were observed at any time.

The peristomial region typically adopts a domed appearance, and is surrounded by an adoral zone of about 190 (from 168 to 204, mean 191) membranelles (Figs. 1a,b,c). This adoral zone of membranelles (AZM) makes two clockwise turns leading into the buccal cavity with the cytostome located about the quarter of the way into the cell. There are approximately 29 kineties(24 to 31) in the peristomial region. There are approximately 90–100 longitudinal kineties (50–158) on the cell body. The basal bodies give rise to kinetodesmal (*km*) fibres (Fig. 1d), and there are typically about 5–7 overlapping *km* fibres running alongside the kineties (Figs. 1d, e).

The cells were not able to contract completely, although local contractions were observed. Electron-microscopy confirms the existence of a myonemal system—arranged in the form of a perforated layer—the perforations being penetrated by membrane bound lacunae (Fig. 1f).

There is a single spherical to oval macronucleus measuring on average 44 μm × 38 μm (range: 37.5–53 μm × 30–47 μm) (Fig. 1a). The macronucleus is located approximately 2/3 of the way down the cell. The nucleus is typically surrounded by the pigment granules, and these also cluster around the numerous (10 or more) micronuclei which lie close to the surface of the macronucleus (Fig. 1g).

A single large contractile vacuole is located near the middle of the cell and to the left of the buccal cavity. The interior of the cell contains a large number of symbiotic green algae (Fig. 1a) which are about 4 μm in diameter. Under illuminated conditions these are mostly found near the cell surface and aligned along the kineties. There are a large number of small dark pigment granules (<1 μm in diameter), mostly occurring in 4–5 rows between kineties, in the vicinity of the AZM, and also throughout the cytoplasm. (Fig. 1c). These are a dark burgundy colour, and together with the symbiotic green algae give the animal its dark brown, plum or black colour.

When swimming, *Stentor* describes a spiral path with a clockwise rotation when viewed from the front. The axis of rotation can be described by a line drawn from the posterior tip through the middle of the dorsal surface opposite the buccal cavity.

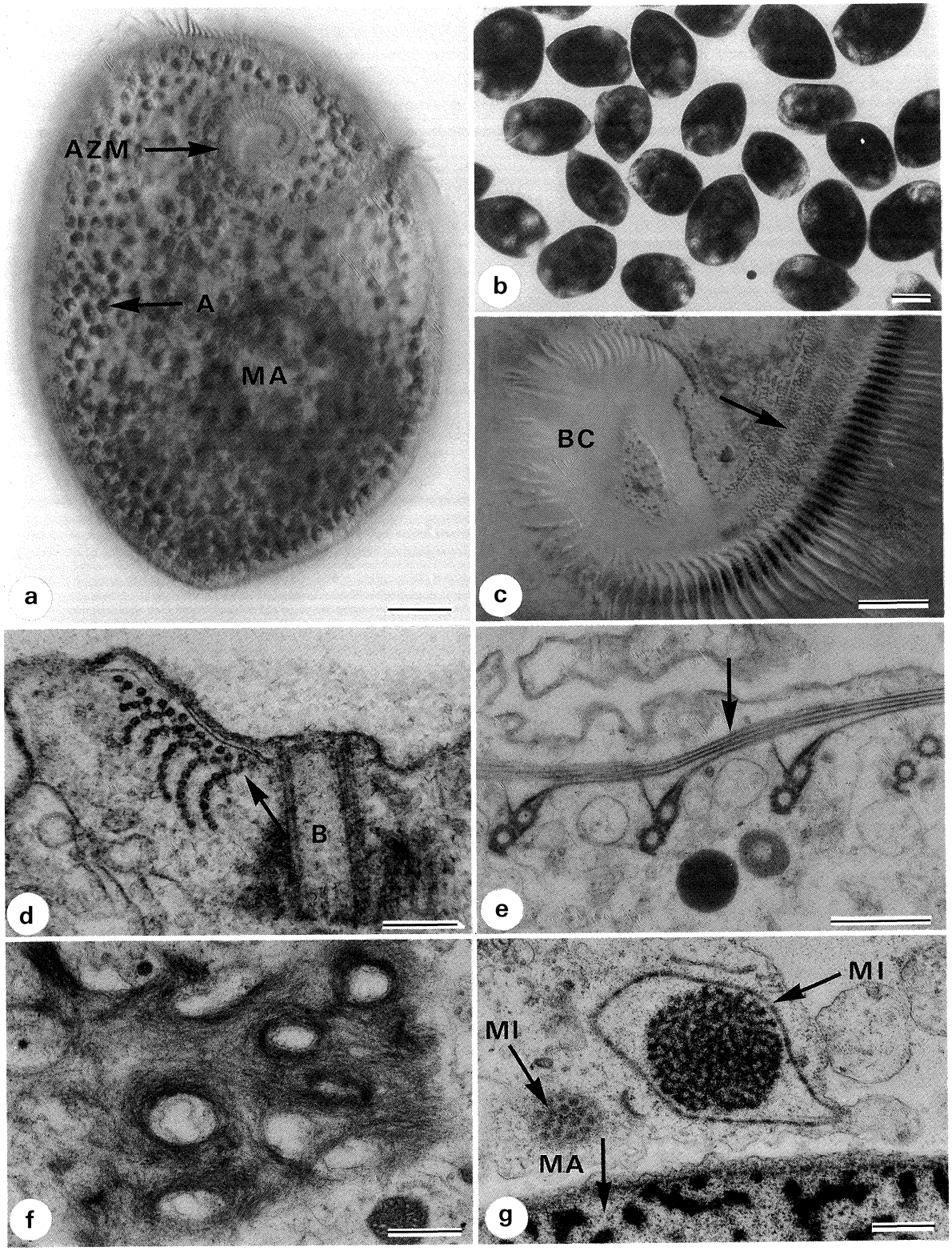

Figure 1. *Stentor amethystinus*. **a**, Ventral view of living cell showing adoral zone of membranelles (AZM), macronucleus (MA) and symbiotic green algae (A), DIC, scale bar 20 µm. **b**, Typical conical forms of living cells, bright-field, scale bar 80 µm. **c**, AZM leading in the buccal cavity (BC), pigment granules (arrow), DIC, scale bar 8 µm. **d**, Electron-micrograph of basal body (B) with associated microtubules of the kinetodesmal fibres in cross section (arrow), scale bar 0.2 µm. **e**, Electron-micrograph showing kintodesmal fibres (arrow) in longitudinal section arising from the basal bodies, scale bar 1 µm. **f**, Electron-micrograph of myonemal system, scale bar 1 µm. **g**, Electron-micrograph of the border of macronucleus(MA) and two mircronuclei (MI). The nucleus to the left has been sectioned through the nuclear envelope and shows the nuclear pores, while that on the right has been sectioned through its centre, scale bar 0.5 µm.

Discussion

The genus *Stentor* is characterised as a genus of contractile and trumpet-shaped heterotrich ciliates, with longitudinal kineties, posterior holdfast, an anterior adoral zone of membranelles (AZM) following a clockwise spiral when seen from above, and no undulating membrane (Kahl, 1932; Tartar 1961). The cells observed by us have these features and are assignable to the genus *Stentor*.

The only members of the genus which have a brown colour are: *S. amethystinus* Leidy 1880 (in Anonymous, 1880); *S. andreseni* Nilsson, 1986; *S. fuliginosus* Forbes, 1891; *S. igneus* Ehrenberg, 1838; *S. niger* (Müller, 1773) Ehrenberg, 1831; and *S. tartari* Murthy & Bai, 1974. *Stentor andreseni* has been synonymized with *S. tartari* (Foissner & Wölfl, 1994).

Stentor tartari is a binucleate species (Murthy & Bai, 1974; Nilsson, 1986). The Australian isolates can be distinguished from this species on the basis of the nuclei alone. Nilsson (1986) used ultrastructural characteristics to add additional characters which distinguish *S. tartari* and *S. amethystinus*. Given the uncertainty over the identity of *S. amethystinus* (see below), we also obtained ultrastructural characteristics. As in Nilsson's *S. amethystinus* and unlike *S. tartari*, the micronuclei do not occur in macronuclear depressions, but unlike the report of Nilsson, a close association between micronuclei and pigment granules was not observed in our ultrastructural studies. We also note that, in contrast to Nilsson's written statement, the myonemal layer of the Australian isolate is perforated—although Nilsson's micrographs suggest that the myonemal layer in her isolates may have discontinuities. We regard the differences as minor, and regard our observations to be of the same species as those of Nilsson on the taxon referred to as *S. amethystinus*, and that this can be distinguished from *S. tartari* on nuclear morphology.

Stentor niger can be distinguished from *S. amethystinus* and the organisms observed by us because it lacks symbiotic algae (Foissner & Wölfl, 1994). Like *S. amethystinus* it has a single macronucleus and the cell tends to be conical in shape. Although generally larger than the cells observed by us, the size range of 250–300 μm does overlap with that reported for *S. amethystinus* (Table 1). As some ciliates with symbiotic algae may loose them, it is possible that *S. niger* is conspecific variety of *S. amethystinus*.

Stentor igneus has a single macronucleus, but with a length of about 100 μm, an ability to adopt the elongate body form, and the absence of symbiotic algae, it can be distinguished from *S. amethystinus* and the organisms observed by us. One isolate has, however, been reported with algae (Foissner & Wölfl, 1994).

Stentor fuliginosus was inadequately described as a variety of *S. igneus* from America, has only recently been given a clear identity (Foissner & Wölfl, 1994). It can be found as a short conical form or as an elongate form. It is relatively small—Foissner & Wölfl suggest that it is usually under 150 μm—but extended cells may be as long as 300 μm. These authors do reflect on possible synonymy with *S. amethystinus* but note that the micronuclei of *S. fuliginosus* are not surrounded by pigment granules. We note that *S. fuliginosus* has a smaller macronucleus and more adoral membranelles than the organisms observed by us. We do not therefore regard our observations to be of *S. fuliginosus sensu* Foissner & Wölfl.

Comparison is therefore best made with the species referred to as *S. amethystinus*. The original description of *S. amethystinus* was by Leidy and reported anonymously to the Academy of Natural Sciences of Philadephia (Anonymous, 1880). It is an incomplete account, lacks illustrations, but referred to a ciliate that measured up to 820 μm long and was encountered readily in an extended form. A number of workers have since referred to and added new observations about this species (Table 1). Of these, only Dragesco (1970) has made new observations on an isolate that adopts the extended trumpet shape. All other records are of an organism that only adopts a rounded or short conical form (Nilsson, 1986; Foissner *et al.*, 1992; Foissner & Wölfl, 1994; Schneider, 1991). The Australian isolate broadly agrees in shape, overlaps in size range, has similar macronuclei and micronuclei (where they have been recorded) with these records from Europe. The Australian isolates do not achieve the same maximum size, have more kineties, and differs subtly in myonemal organization and proximity of pigment granules to the micronuclei.

Dragesco's African isolate does not achieve the same maximal size as the American or European isolates. Sometimes it has more than one macronucleus (see Nilsson, 1986). It may occasionally lack symbiotic algae (see also comments to *S. niger* above). Dragesco & Dragesco-Kernéis (1986) comment that they do not think that the African isolate is the same species as the European isolate. The Australian isolates differ from the African isolate in being smaller, and not being capable of adopting the extended form.

It would appear then that there are subtle differences among different geographical isolates of *S. amethystinus* and these are of comparable magnitude as between *S. amethystinus* and some other brown species of *Stentor*. Because of differences in maximum size and the capacity to adopt a trumpet-shape, we do not believe that the isolates observed by Leidy are identical to those observed by Nilsson (1986), Foissner *et al.* (1992), Foissner & Wölfl (1994) and Schneider (1991), nor to those observed by Dragesco or by us. Indeed, we believe that Leidy's observations were of organisms which more closely correspond with *S. fuliginosus* because of its larger size and occurrence of an extended form. *Stentor fuliginosus* was originally reported from North America. If we were to regard *S. fuliginosus* to be a junior synonym of *S. amethystinus*, it would require that the European isolates be given a new name. Given the inadequacy of the original descriptions of both *S. amethystinus* and *S. fuliginosus* and given that we are unable to assess if the subtly different isolates represent incomplete documentation of a continuum of form or if they represent stable discontinuities (species), we believe that no benefits would accrue from this course of action, and there would be disadvantages of nomenclatural instability. Given that at least one clear

Table 1. Summary of reported characteristics of *Stentor amethystinus*. Dash = not specified; AZM = adoral zone of membranelles.

	Leidy in Anonymous 1880	**Kahl, 1932**	**Tartar, 1961**	**Dragesco, 1970**	**Foissner, 1980**	**Nilsson, 1986**	**Schneider, 1992**	**Foissner *et al.*, 1992**	**Foissner & Wölfl, 1994**	**Australian isolate**
Source	USA	—	—	Cameroon	Austria	Austria	Europe	—	—	Australia
Length (µm)	180–840 (long trumpet)	300–400	—	250–350	135–500	about 300	150–600	135–500	—	150–220
Kineties	—	—	—	90–100	60	80	—	90–110	90–120	80–100
Kineties in oral-region	—	—	—	—	25	—	—	20–25	20–25	28–32
Colour	lilac, amethystine	brown, amethystine	blue-violet	blue-violet	—	brownish	blue-violet, red-violet	dark-violet	violet to purple-red	dark plum, brownish
Shape	conical to extended	—	pyriform, conical	extended conical	spherical	short trumpet	conical	—	—	mostly conical
Extended form observed	yes	—	doesn't stretch	yes	no	no	no	—	—	no
Nucleus diameter (µm)	spherical, 30	—	oval	rounded, 23–30	rounded, 20–25	spherical	rounded, 25–30	rounded, 20–30	—	spherical-oval
Membranelles	—	—	—	160 in AZM	150	150	—	200–300 in AZM	200–300	190–200 in AZM
Zoochlorellae	(abundance of chlorophyll)	yes	yes	—	4–6 µm	4 µm	6 µm	4–7 µm	—	4 µm
Micronuclei	—	—	—	—	—	6 micronuclei close to macro-nucleus	—	—	—	at least 10 micro-nuclei, close to macronucleus

contemporary concept of *S. amethystinus* has emerged (Foissner & Wölfl, 1994) we believe that we will be better served by regarding the species observed by us as being the same as the European isolates and as *S. amethystinus*. We expect that future exploration of this taxon may lead to the concept of this species becoming broader to confidently include all geographic varieties and *S. fuliginosus*, or narrowed such that the geographical varieties are given specific status.

ACKNOWLEDGMENTS. We acknowledge the financial support of Australian Biological Resources Study and the Australian Research Council and input from W. Foissner and, less I rush, an un-named referee.

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Manuscript submitted 15 December 1997; revised 1 May 1998; accepted: 10 June 1998.

Assoc. Ed.: G.D.F. Wilson.