Two new species of *Nitella* (Characeae, Charophyceae) from arid-zone claypan wetlands in Australia

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Introduction

Charophytes are macroscopic green algae that grow in wetlands, streams and rivers world-wide. The charophyte flora of Europe is relatively well known (Corillion 1957; Wood 1965; Moore 1986; Krauss 1997; Bruinsma 2010; Romanov and Kipriyanova 2010), but, until recently, few regions of Australia have been well-surveyed for charophytes (*c.f.* van Raam 1995; Garcia 1999; Casanova 2007a, 2007b; Garcia & Chivas 2006). In general, brief surveys for charophytes will reveal only the most common, widespread or perennial species (e.g. *Nitella hyalina* Ag. and *Chara globularis* Thuill.). Collections of short-range endemic species (e.g. *Nitella tumida* Nordst.), annual species (e.g. *Nitella sonderi* A.Braun) cryptic species (e.g. *Nitella verticillata* (Fil. et G.O.Allen ex Fil.) R.D.Wood) or rare species (*Lychnothamnus barbatus* (Meyen.) Leonh.) depends on luck (Casanova *et al*. 2003) or repeated, thorough surveys and culture of seed bank material (e.g. Casanova and Karol 2008). Recently such thorough surveys of the Paroo region wetlands of New South Wales (Porter *et al.* 2007) and wetlands of the Pilbara region of Western Australia (Pilbara Biological Survey, McKenzie *et al.* 2009) have been undertaken.

The genus *Nitella* is characterised by furcate (forked) branchlets, terminal antheridia and a 10-celled coronula on the oogonium (Wood 1965). Charophytes are usually recorded from clear-water habitats (Coops 2002), and are notable for reducing turbidity and maintaining 'clear-water' in water bodies (van Nes *et al.* 2002). Most researchers would not think to look in or try to find charophytes in naturally turbid water bodies. Despite that, charophytes have been shown to cope with very low light levels in the germination phase (de Winton *et al.* 2004), and to grow at depth in clear lakes, at the limits of photosynthetically active radiation (PAR) (Schwarz *et al.* 1996). A large number of water bodies in Australia, including rivers, man-made dams and claypans, are

Abstract

Two new species of *Nitella* Ag. are described. The species are dioecious and grow in temporary arid-zone wetlands called 'claypans'. Both have somewhat inflated, bicelluate fertile dactyls and neither species has mucus associated with the reproductive whorls. *Nitella parooensis* M.T.Casanova & J.L.Porter is characterised by its apparently pluricelluate sterile branchlets with acute, conical end cells, inflated bicellulate dactyls and uniquely twisted oogonia and oospores. *Nitella micklei* M.T.Casanova is characterised by a crisp, inflated appearance, large antheridia and distinctive oospores. The ornamentation on the oospores of both species is essentially reticulate, but for *N. micklei* there are 1–2 thick-walled meshes across the fossa, and in mature oospores of *N. parooensis* the 3–4 meshes have scattered, acute papillae within them.

Key words: Paroo, Pilbara, charophyte, oospore, morphology

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turbid from suspension of inorganic clays (Casanoval *et al.* 2007), rather than algal abundance. This is the first report of charophytes that occur in such turbid habitats.

Materials and methods

Collections of *Nitella* were made from three temporary freshwater wetlands (Woolshed Swamp, Cricket Pitch Claypan and Number 17 Claypan) in Nocoleche Nature Reserve in the arid north-west of New South Wales, within the Paroo-Warrego drainage division. The climate is classified as 'hot desert' under the modified Köppen climate system (Köppen 1918). Annual average rainfall at Wanaaring (29.43º S, 142.01º E; the closest meteorological centre) is 276 mm, with average maximum temperatures reaching 35–45ºC in summer and 16–22ºC in winter (Cunningham *et al.* 1981). Mean annual evaporation rates are high throughout the area (2400–2800 mm/year) so average annual surface run-off in the Paroo drainage division is low (17 mm) and this, combined with low topographic relief, encourages the formation of shallow, temporary, closed depressions (Paijmans *et.al*.1985). On average minor floods in the Paroo occur several times a year, with moderate floods every 2–3 years and major floods about every 5 years (Kingsford & Porter 1999). Riverine connectivity (ability to exchange surface water with rivers) varies considerably among these wetlands and they can fill from river flooding or local rain or both.Woolshed Swamp fills mainly from Paroo River flooding, while the Cricket Pitch and Number 17 claypans fill only from local rainfall.

All collections of *Nitella micklei* were from claypan wetlands in the Paroo region of Western Australia. The region experiences an average of 290 mm of rain annually, most of it falling in the summer via cyclonic activity and thunderstorms. There is high interannual variability in rainfall due to the variability in the paths of cyclones in the region. Claypans occur on the fringes of the Fortescue Marsh and in internally draining areas of the Hammersley Range. Claypans are shallow (<60 cm deep), turbid, temporary wetlands on a red, non-cracking clay substrate (McKenzie *et al.* 2009).

Seed banks and extant plant communities were sampled during a large-scale survey of aquatic plant communities in the Paroo-Warrego and Bulloo River basins from 1988–2004 (Porter 2005), and in the Pilbara Biological Survey between 2002–2004 (McKenzie *et al.* 2009). Seed banks were sampled following the methods of Brock *et al*. (1994) or as a bulk sediment collection. Dried sediment samples were placed in shallow plastic trays, then submerged in tap water or rain water (to 30 cm depth) to stimulate germination.

Plants were either pressed or preserved in 70% alcohol for later examination with the aid of Zeiss microscopes. Oospores were removed from the plants and prepared for scanning electron microscope examination by soaking in a strong detergent solution (Crawford *et al.* 2001) for 20 minutes and removal of the enveloping cells with fine needles followed by three minutes of sonication. Oospores were mounted on stubs using carbon sticky tabs, sputter-coated in gold and examined using an SEM following the methods of Casanova and Karol (2008). Chromosome counts were made on fresh specimens that germinated from the seed bank, or on spirit preserved specimens following the methods of Casanova (1997).

Results

1. ***Nitella parooensis*** M.T.Casanova & J.L.Porter, ***sp. nov.***

Plantae dioeciae, vix heteroclemae, axes graciles, ramuli inaequaliter 0–3-furcati, monopodiales vel quasi monopodiales, 5–7 in quoque verticillo. Ramuli steriles plurcellulares, ramuli fertiles bicellulares. Cellulae terminales conicae et acutae. Ramuli fertiles in verticullis contractis. Oosporae 290–350 µm longae, 250–290 µm latae, tortae. Ornatio reticulata, alveolae 3 vel 4 trans fossam.

***Type*: NEW SOUTH WALES.** Cricket Pitch Claypan, Paroo River region, *J.L. Porter 260* male and female, spirit preserved (r011), (holotype: MEL)

Plants dioecious, scarcely heteroclemous, to 10 cm high, internodes to 3 cm long, *c.* 0.4 mm wide, sterile branchlets to 3 cm long, unevenly 0–3 × furcate, monopodial or nearly so, in whorls of 5–7 at the axis nodes, with an occasional 0 × furcate accessory branchlet at the base of the whorl. Primary branchlet segments to 40 mm long, ranging from 50 to 100% of total branchlet length. Secondary branchlet segments 2 to 3 mm long. Branchlets have the potential to be 3

or more × furcate, but sometimes the development of segments is suppressed and the terminal segments appear to be pluricelluate (up to 5 cells long including the end cell). Dactyls are essentially bicellulate, to 1.5 mm long. Accessory branchlets where present are 0 × furcate (i.e. dactyls). End cells short, conical and acute, the end of the penultimate cell distinctly narrowed so that the base of the end cell is confluent with it. Fertile parts without mucus, whorls on the female plant somewhat contracted, on the male plant in distinct heads. Fertile branchlets 6 in a whorl, 2 × furcate, oogonia from 0.4 to 0.6 mm long, at first and second branchlet furcations, antheridia terminal, to 400 µm in diameter. Oospores 290–350 µm long × 250–290 µm wide, with 4–5 striae of low flanges, dark to chestnut brown. The shape differs from all other species seen so far, rather than being a flattened sphere, oospores of this species appear twisted. Oospore wall ornamentation is coarsely reticulate, with walls of the reticulum *c.* 8 µm wide, 3–4 meshes across the fossa. In mature oospores minute, sparse papillae occur on the walls and cavities of the reticulum. Antheridia to 0.4 mm in diameter. Chromosome number not known. (Fig. 1)

***Additional specimens examined*: NEW SOUTH WALES.** Claypan 17, Nocoleche Nature Reserve, *J.L. Porter 264*, spirit preserved (p650), MEL. Woolshed Swamp, Nocoleche Nature Reserve, *J.L. Porter s.n.*, spirit preserved , MEL.

Distribution and habitat: Claypan wetlands in the Paroo region of north-western New South Wales and south-western Queensland. Small mid- to bright-green plants lacking mucus, with spindly whorls of branchlets terminated with open groups of dactyls; antheridia more prominent than oogonia. Found on heavy clay substrates, often in water so turbid they cannot be located visually (Secchi transparency < 5 cm). Apparently capable of rapid growth and may be short lived.

Associated species: Wetlands fringed by *Eucalyptus ochrophloia* F.Muell., *E. largiflorens* F.Muell., *Eremophila sturtii* R.Br., *Dodonaea angustifolia* L.f. and *Senna* spp., *Babbagia* sp. *Chenopodium* sp. and *Scleroleana* sp with an understorey of *Duma florulenta* (Meisn.) T.M.Schust, *Acacia stenophylla* A.Cunn. ex Benth. and/or *Maireana brevifolia* (R.Br.) Paul G.Wilson. Herbaceous species can include *Eleocharis plana* S.T.Blake, *Marsilea angustifolia* R.Br., *M. drummondii* A.Braun, *Alternanthera denticulata* R.Br., *Cyperus gilesii* Benth., *Aponogeton queenslandicus* H.Bruggen and *Eragrostis australasicus* (Steud.) C.E.Hubb; submerged species include the charophytes *Chara braunii* C.C.Gmelin, *Nitella sonderi* A.Braun and *Nitella cristata* A.Braun.

Etymology: *parooensis* means 'of the Paroo'. This species has not been collected in any other place.

Conservation status: This species has been listed on the New South Wales endangered species list as *Nitella* 'parooensis'.

2. *Nitella micklei* M.T.Casanova, *sp. nov.*

Plantae dioeciae, homeoclemae, ad 10 cm altum, axes crassi, ramuli 1-vel 2-furcati, , 5–7 in quoque verticillo, dactyli inflati, bicellularis. Oosporae 200 µm × 170 µm, ornatio leniter reticulata, alveolae laeves 1 vel 2 trans fossam. Antheridia ad 500 µm diametrum.

Type: **WESTERN AUSTRALIA.** Pilbara Survey Wetland 040, Mulga Downs Outcamp Claypan, 3 km West of Cowra Line Camp Homestead, Mulga Downs Station, 67.5 km ESE of Wittenoom (S 22.3618, E 118.9774) 17.viii.2006, *M.T. Casanova PBS17:R114* (holotype: MEL).

Plants dioecious, homeoclemous, to 90 mm tall, internodes to 35 mm long. Axes stout, to 650 µm in diameter. Sterile branchlets 6 in a whorl, to 18 mm in total, 1(–2) × furcate, primary segments to 6 mm long, 2–3 secondary segments, to 2 mm long, rarely 2–3 tertiary segments, to 2 mm long. Dactyl cells swollen or inflated 1–2 mm long, 0.5–1 mm wide, sometimes narrowing distally to be confluent with the mucronate end cell, 75 µm long × 30–40 µm wide at the base. Fertile branchlets 5–6 in a whorl, to 2 mm long, 1–2 × furcate. Primary segments to 1.5 mm long, 2–3 secondary segments to 1.2 mm long, rarely 2–3 tertiary segments to 0.5 mm long. Fertile dactyls as for sterile, individual segments can be less inflated on male plants. Gametangia on separate plants in contracted whorls. Oogonia to 230 µm long, 190 µm wide, coronula to 18 µm high, 6–7 convolutions. Oospores 200 µm long, 170 µm wide, 6–7 striae of thick ridges, uniting in a small apical crest. Oospore membrane shallowly reticulate with (0–)1–2 smooth meshes across the fossa. Antheridia to 450–470 µm in diameter. Chromosomes n=9. (Fig. 2)

Additional specimens examined: **WESTERN AUSTRALIA.** PSW005 Koodjeepindawarranna Pool 19.viii.2006, *M.T. Casanova PBS40*: *t254* (MEL). PSW005 Koodjeepindawarranna

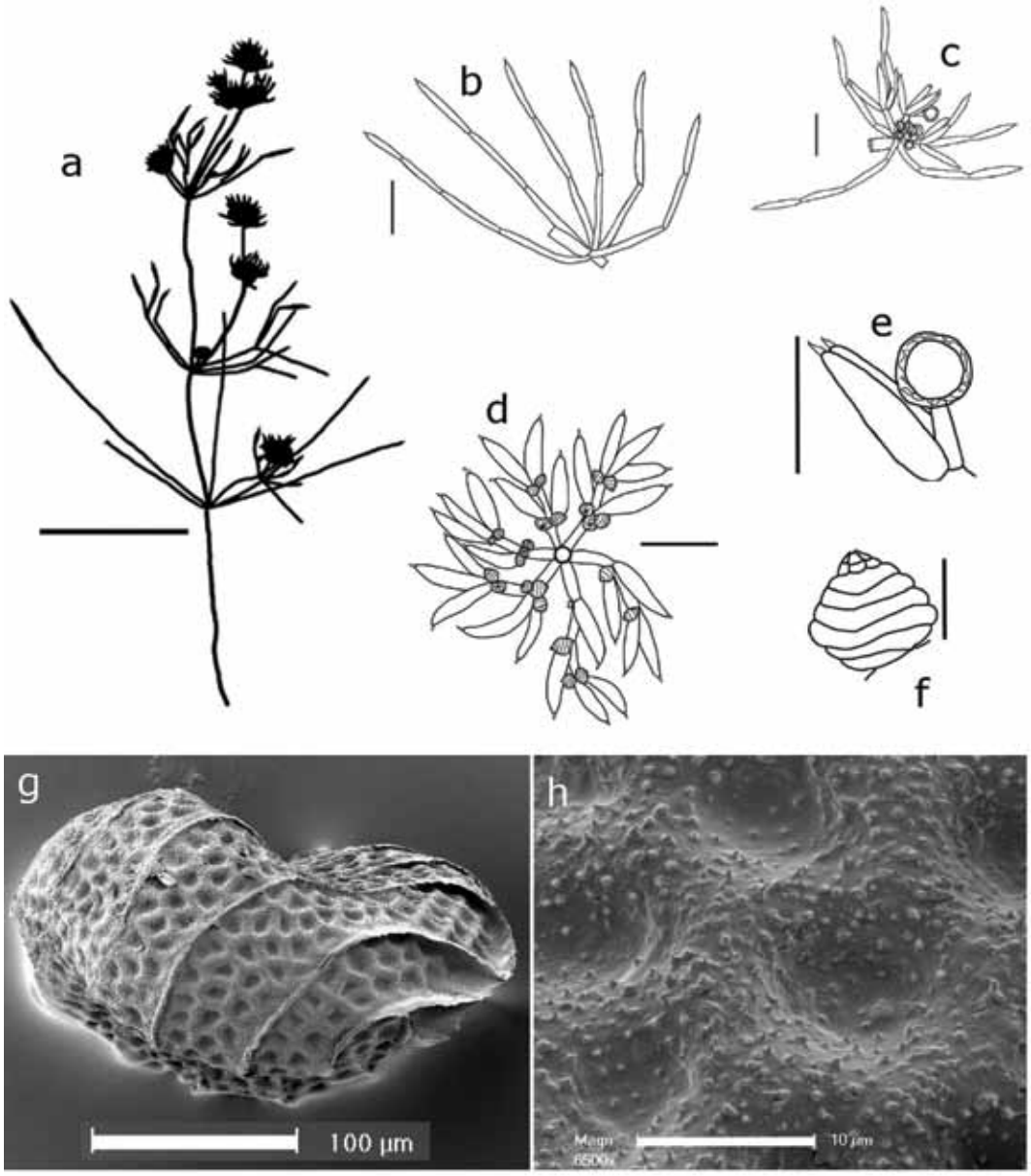

Figure 1. *Nitella parooensis* M.T.Casanova and J.L.Porter. **a.** Whole plant morphology, scale bar = 20 mm; **b.** sterile branchlet whorl showing 4–5 celled branchlets (3–4 cells plus reduced conical end cell), scale bar = 10 mm; **c.** fertile male branchlet node with 0–1 × furcate branchlets, a fertile 'head' and a stalked antheridium, scale bar = 10 mm; **d.** fertile female branchlet node with six 2–3 × furcate branchlets in a whorl, oospores occurring singly, geminate or in clusters at the branchlet nodes, dactyls swollen, scale bar = 1 mm; **e.** fertile male dactyls and stalked antheridium, scale bar = 1 mm; **f.** oogonium with both rows of coronula cells approximately the same size, exhibiting the same sinistral torsion of the oospore, scale bar = 0.2 mm; **g.** whole oospore showing twisted morphology, *c.* six striae with low flanges and reticulate ornamentation, scale bar = 100 µm; **h.** detail of oospore wall showing thick-walled meshes of the reticulum and ornamentation of scattered acute papillae (*c.* 1 µm high), scale bar = 10 µm.

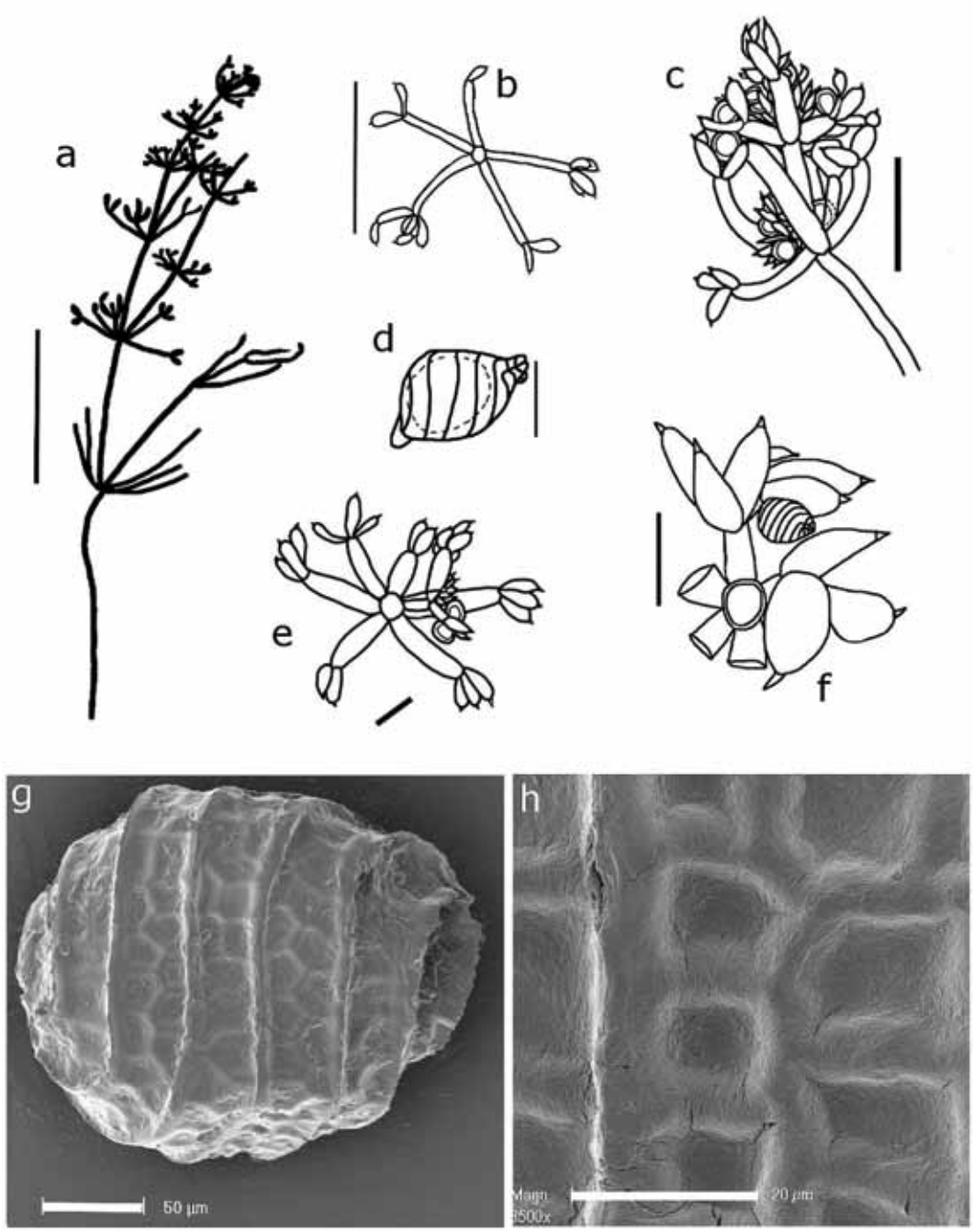

Figure 2. *Nitella micklei* M.T. Casanova. **a.** Whole plant morphology showing whorls of 1 × furcate branchlets, scale bar = 1 cm; **b.** sterile branchlet whorl with five 1–2 × furcate branchlets with inflated dactyls, scale bar = 5 mm; **c.** fertile male shoot with sessile antheridia at base of whorl and first furcations, scale bar = 1 mm; **d.** oogonium, scale bar = 100 µm; **e.** branchlet whorl with six 1 × furcate branchlets and a fertile male side shoot, scale bar = 1 mm; **f.** fertile female branchlets with oogonium at the first furcation and inflated, bicellulate dactyls, scale bar = 100 µm; **g.** whole oospore a flattened sphere 200 µm long and 170 µm wide with *c.* six striae and reticulate ornamentation, scale bar = 50 µm; **h.** detail of oospore wall showing striae of thick ridges and reticulate ornamentation with two thick-walled, shallow meshes across the fossa, scale bar = 20 µm.

Pool 19.viii.2006, *M.T. Casanova PBS44: t257* (MEL). PSW004 Gnalka Gnoona 19.viii.2006, *M.T. Casanova PBS33*: *t256* (MEL). PSW010 Fortescue River at Millstream (Palm Pool) 21.viii.2006, *M.T. Casanova PBS57:t258.* PSW041 Ethel Creek Claypan 1.viii.2004, *D.A. Mickle & M.N. Lyons 3073: t259*.

Distribution and habitat: Shallow turbid claypans in the Pilbara region of Western Australia. Small, turgid, robust plants with short branchlets held perpendicular to the stem. Male plants with large, bright orange antheridia in contracted terminal and axial shoots. The plant is often covered with a layer of fine red clay *in situ*.

Associated species: *Nitella micklei* occurs with *Riella* sp. (bryophyte).

Etymology: Named for David Mickle who, along with Mike Lyons, first collected the species.

Conservation status: Known only from the Pilbara region claypans, locally abundant in shallow water following rain.

Discussion

These two species differ from all other described species of *Nitella* in their oospore and vegetative morphology. The presence of inflated or swollen dactyls is not unique, *N. tumida* in Australia, and several species elsewhere have similar, inflated terminal branchlet segments. However, *N. tumida* has long internodes and branchlets described as 'umbellate' (Nordstedt 1891), and oospores described as 'smooth or with irregular projections' (Wood 1972). Additionally, *N. tumida* occurs in saline habitats (Nordstedt 1891), and is doubtfully recorded from freshwater (García & Chivas 2006). Within subgenus *Tieffallenia* there are a variety of oospore morphologies, but few species in that subgenus exhibit reticulate ornamentation (Leitch *et al*. 1990, Casanova 2009), *cf*. subgenus *Hyella* section *Hyella*, in which almost all species have reticulate oospores (Casanova 2009). Differentiation in oospore morphology has been found to coincide with vegetative morphological differences (John & Moore 1987; Casanova & Karol 2008; Casanova 2009), and with different nucleotide sequences (Sakayama *et al*. 2002; Sakayama *et al*. 2004; Sakayama *et al*. 2005; Sakayama *et al*. 2006). Oospore differences have been used for more than a century to differentiate among charophyte species (Nordstedt 1889; Allen 1896; Williams 1959; Casanova & Karol 2008; Casanova 2009) and only Wood (1965) amalgamated species with different oospore morphologies. On the basis of their distinctive oospores alone these taxa would be distinguished as different species. However, they are also vegetatively distinct from other species of *Nitella*.

The overall shape of the oospore of *N. parooensis* is unique. All other known species of *Nitella* have oospores that are a flattened oval or flattened sphere, so that the definition of the genus (Wood 1965) includes that description. Oospores of *N. parooensis* are essentially a flattened sphere, but the resulting 'disc'-shape is twisted sinistrally. Thus the circumscription of the genus *Nitella* should be amended so that the description of the oospores is 'oospores a flattened sphere or oval *or rarely a flattened sphere sinistrally twisted*'.

Arid zones are not, at first glance, likely habitats for charophytes and other water plants. However, despite the intermittent, unpredictable occurrence of free water for more than a few days at a time, a number of cryptogamic plant and algal species not only occur but also thrive in arid-zone wetlands (Hussain & Khoja 1999, Proctor 1972, Porter 2007, Powling 2009).

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