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# Charophytes of the Pilbara region of Western Australia

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#### Introduction

Family Characeae has five recognised genera in Australia, three of which (*Lamprothamnium*, *Chara* and *Nitella*) occur in the Pilbara region of Western Australia. All the Characeae are currently being revised and as a result of that revision, and studies into previously poorly collected areas like the Pilbara, a number of new species are being found and described. The two diverse genera *Chara* and *Nitella* are divided up into subgenera and sections, containing several species each. However, even these higher level classifications are undergoing revision as a result of genetic analysis (Mc Court et al. 2000; Karol et al. 2000). No subdivisions of the genus *Lamprothamnium* have been made, but in this report species of *Chara* and *Nitella* are listed under their sections.

The most uniformly useful taxonomic characters in the family are found on the oospores (sexually produced propagules). Oospores have a desiccation-resistant multi-layered wall with distinctive spiral ornamentation. The features are best viewed and analysed using scanning electron microscopy. The most valuable features for taxonomic assessment include

- The presence of a gyrogonite (calcareous covering of the oospore)
- The size and shape of the gyrogonite and number of spiral circumvolutions
- The size and shape of the oospore and number of spiral circumvolutions
- The ornamentation on the fossa wall of the oospores

Additional useful taxonomic characters in the genus Lamprothamnium are

- Sexual state: monoecious (with both male and female organs on the same plant) or dioecious (separate male and female plants)
- The character of the coronula (crown of cells present at the top of the female reproductive organ)
- Length and shape of stipulodes, bract-cells, bracteoles and branchlet cells

Additional useful taxonomic characters in the genus Chara are

- Sexual state
- The character of the coronula
- Cortication on the axis in relation to branchlet number (1x, 2x, 3x)
- Cortication on the branchlets
- Number of stipulodes in relation to branchlet number (1x, 2x, etc)
- Number of tiers of stipulodes (1 or 2)
- Length and shape of stipulodes, bract-cells, bracteoles, branchlets and spine cells

Additional useful taxononomic characters in the genus Nitella are

- Sexual state
- The character of the coronula
- Number of branchlet whorls at the nodes
- Number of branchlet furcations
- Number of cells in the terminal branchlet segments
- Presence or absence of contracted fertile branchlet whorls (heads or spikes)
- Relative lengths of branchlet segments

On the basis of these characters the following 27 taxa of charophytes have been determined.

#### Lamprothamnium

The genus Lamprothamnium was thought to consist of three species: L. papulosum with a world-wide distribution excepting North America, but centred on Europe, L. succinctum from Africa and southern Asia and L. hansenii from the Baltic Sea (Wood 1965). These taxa were originally included in Chara, but were separated on the basis of developmental and morphological criteria (Groves 1916). The variation within Australian collections was recognised as being large (Wood 1972), and originally L. succinctum var. australicum and L. macropogon were described (Ophel 1947). However, Wood (1965) lumped all Australian taxa into the European L. papulosum albeit with two varieties and several forms recognised and named (var. toletanum and var. papulosum with forms papulosum, carrissoi, pouzolsii, aragonense and macropogon). In his later consideration of the Australian material alone, he listed four new forms for Australia (forms compactum, australicum, capitatum and succinctoideum; Wood 1972). Current taxonomic treatments reinstate Australian L. macropogon as a separate species (van Raam 1995; García 1999; Meiers et al., 1999). and recognise the presence of L. succinctum in Australian coastal lagoons (Garcia et al, 2000). Recent collections in the Paroo region of New South Wales resulted in the delineation of L. heraldii, the only dioecious representative of the genus (Garcia and Casanova 2003). The discovery of this dioecious species in Australia led to the hypothesis that Lamprothamnium had an Australian or Gondwanan origin (García and Casanova 2003). Further work by Garcia and Chivas (2004) revealed that L. succinctum was more widespread, there is a fossil species (L. williamsii) from the Lake Eyre basin, and a possible new monoecious species from the south of Western Australia (Garcia 2004). The collections from the Pilbara region contain specimens of three species of Lamprothamnium, one of them similar to L. succinctum in the paucity of accessory structures, another dioecious taxon that has significantly different oospores but can be compared with both L. heraldii and L. williamsii, and a third, completely new species with a number of distinctive features including a stellate coronula. This coronula structure is completely different from all previously described charophytes, not just species of Lamprothamnium. The Pilbara species give greater support to the idea that the Australian continent was a centre of origin for this genus.

#### Lamprothamnium sp. aff. succinctum (A. Braun in Asch.) R.D. Wood.

Lamprothamnium succinctum has been recorded from New South Wales coastal lagoons with fluctuating salinity, Africa, India, Mauritius and New Caledonia (Wood 1965. García et al. 2000). It is distinctively different from the common Australian Lamprothamnium macropogon (A. Braun) Ophel. The Pilara material appears to take the reduction in stipulodes and bract cells that characterises the taxon succinctum to an extreme. All appendages except the branchlets and gametangia are very reduced, making the specimens appear similar to Chara australis. There are no stipulodes internal to the branchlet whorls, and the gametangia are basal and infrequently sejoined on the branchlets. This material will be maintained in culture until breeding experiments can be undertaken with L. succinctum and L. macropogon. Plant thallus entirely without cortication (Fig. 5). A few down-ward pointing stipulodes present. Few, short bract-cells, no internal stipulodes (Fig. 7). Oospores enclosed in a calcareous lime-shell (gyrogonite Fig. 1). Oospores 550 µm long, 280 um wide, 11 striae (Fig. 2). Striae consisting of a low ridge with an undulating margin. Ornamentation of low circular grains, 0.3 to 0.8 µm wide, one to two grainwidths apart (Fig. 3). Basal cell 80 to 100 µm in diameter (Fig. 4). Example p657 Mundabullananga Pool, seed bank culture. MTC 27/10/04. Chromosomes n=28 (Fig. 6).





#### Lamprothamnium sp. aff. williamsii García and Chivas

The fossil taxon, L. williamsii was described on the basis of gyrogonites found in Holocene sediments associated with Lake Eyre (García and Chivas 2004). No extant material has been found. The gyrogonites of the Pilbara material are similar to one extreme of the gyrogonites included in the delineation of L. williamsii, and it is likely that the fossil taxon actually consists of more than one species. The Pilbara species is a dioecious taxon, characterised by large gyrogonites with 7-8 circumvolutions (Fig. 9), oogonia on stalks and poorly developed stipulodes and bract cells. The oospores are c. 500 µm long, almost rectangular in side view and with 7-8 striae (Fig. 8). The oospore wall is occasionally without the characteristic granular ornamentation typical of Lamprothamnium (Fig. 10 cf. Fig. 3). The only other dioecious Lamprothamnium previously described (Lamprothamnium heraldii García and Casanova) is from the Paroo region of western NSW and Queensland. L. heraldii has well-developed stipulodes and bract cells and oospores with 8-10 striae and granulate walls. Its gyrogonites have convex calcification, with 8-10 circumvolutions. Its oogonia are rarely on stalks (A. García personal communication). The discovery of this, second, dioecious species of Lamprothamnium supports the contention that Australia is the centre of origin of this genus (García and Casanova 2003), and that monoecy has evolved from dioecy in this group (Proctor 1980). Representative specimen p635 MN Lyons & DA Mickle 3079, PSW043 T1 4/6/04.







#### Lamprothamnium sp. nov.

A unique apparently dioecious *Lamprothamnium* with elongate branchlet cells and no stipulodes or bract-cells. The branchlets are tipped with a tiny two-celled mucro (Fig. 12), the end-cell of which is frequently deciduous. No male material has been seen. The oogonia are rounded with a stellate coronula (Fig. 11). The gyrogonite (only one seen) is uncalcified at the top and has concave convolutions (Fig 13). The oospore (one inside the gyrogonite) has not yet been examined. Spherical bulbils are present at the base of the plant. Other species of *Lamprothamnium* have a two-celled end-segment, although not as mucronate as in these specimens. All other charophytes have rounded, or at most, 'crown-like' coronula cells at the apex of the oogoinum or female reproductive organ (see Fig. 7 for typical a *Lamprothamnium* coronula). A stellate coronula consisting of elongated, pointed cells perpendicular to the top of the oogonium is unique to this species. Representative specimens *p666* MN Lyons & DA Mickle 3080 PSW043 T2 3/6/04 (1) and MN Lyons & DA Mickle 3078 PSW043 T2 3/6/04.





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#### Chara section Charopsis

Section Charopsis of Chara consisted of C. corallina (distributed through Asia, Australia and the Pacific), C. socotrensis (restricted to India, Burma, Indonesia and Socotra Island) and C. braunii (world-wide) according to Wood (1965). Recent genetic analysis indicates that C. braunii is not closely related to the other taxa (Meiers et al 1999), and that C. corallina consists of more than one species (Meiers et al 1999). In Woods revision of Australian Characeae (Wood 1972), he amalgamated endemic dioecious C. australis with monoecious C. corallina, but segregated distinct variants to varieties corallina and nobilis with the recognition that var. corallina was consistently dioecious in Australia (in contrast to the rest of its range through Asia), and that var. nobilis could be separated into four forms: nobilis, stuartiana. simplicissima and inflata (Wood 1972). Genetic analysis indicates that C. stuartiana and C. australis can be recognised at species level (Meiers et al 1999), and that C. inflata is in a different genus (Ken Karol personal communication), which is supported by analysis of the gyrogonite morphology (García and Karol 2004). Chara simplicissima was originally described as Tolypellopsis simplicissima Filarszky, and later as Protochara australis (Womersley and Ophel 1947). Breeding experiments between C. australis and a New South Wales specimen identified as Protochara australis resulted in renaming this entity C. australis subsp. estipulodica (Macdonald and Hotchkiss 1956). It is likely that C. simplicissma will be recognised at species level (Garcia 2004). The Pilbara collections contain specimens of C. simplicissima and C. australis as well as an undescribed taxon with similar morphology but distinctly different oospores.

#### Chara australis R. Brown

*Chara australis* is a robust, elongate species with naked axes and branchlets, few and reduced stipulodes, bract-cells and bracteoles, bright orange oogonia (Fig. 19) and antheridia on separate plants (Figs 17, 18) with those organs occurring inside the base of the branchlet whorls and in twos and threes (and occasionally fours) on the branchlets. The oospores are smooth and oval when viewed from the side, with 4 to 6 low striae on their surfaces (Fig 15). The impression of the basal cell on the oospore is about 60% of the diameter of the oospore when seen from above (Fig. 16). The branchlets are occasionally inflated, and although they usually have only one terminal branchlet cell, can occasionally develop two (through the development of a terminal branchlet node bract-cell. Chromosomes n=14. Representative specimen p639 (PSW3057). This species occurs throughout Australia. Figures 17 to 19 are from New South Wales specimens, Figures 14 to 16 are from specimen p639 from the Pilbara region.











#### Chara simplicissima (Filarszky) García

This species is very similar to *Chara australis*, but has in-curved, inflated branchlets, no stipulodes, bract-cells or bracteoles, and the gametangia are not clustered inside the base of the whorls. The oospores are smaller and rectangular or 'dumb-bell shaped' in side view, and the basal cell diamter is 80% of the diameter of the oospore when seen from above. This species is restricted to Western Australia.

Representative specimen: p673 MN Lyons & DA Mickle 3070A, PSW038 21/5/04 ADJ.



#### Chara sp aff. australis (pachygyra)

This species is similar in morphology to *C. simplicissima* and *C. australis*. It differs in its smaller size, the greater development of the accessory cells (stipulodes, bractcells etc) and in the morphology of the oospores, which have thickened and raised striae (pachygyra). This morphology has not been described for the genus *Chara*, although there are examples in *Nitella*. Similar corticate and ecorticate entities with pachygyra oospores have been found in the Paroo region of New South Wales, but are not yet described. Representative specimens: *p664* MN Lyons & DA Mickle 3065, PSW035A, *p665* MN Lyons & DA Mickle 3077, PSW041 1/6/04, *p672* MN Lyons & DA Mickle 3070B, PSW038 21/5/04 ADJ.



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#### Chara section Agardhia

Section Agardhia consists of charophytes with corticated axes and naked branchlets, with branchlets tipped with a cluster of bract-cells. Wood included some taxa with some cortication on the axes, but genetic analysis indicates that those species are not closely related to the rest of Agardhia. For Australian Agardhia Wood recognised two dioecious taxa (*C. leptopitys* and *C. ecklonii*) and one broad taxon consisting of both monoecious and dioecious entities (*C. fibrosa*) (Wood 1972). This section is closely related to section Braunia (differing in the character of the branchlet tips), which was erected by Wood to contain *C. baueri* (vars *baueri* and *muelleri*), but is likely to be revised to also contain *C. braunii* which is currently in section Charopsis (Ken Karol personal communication). Neither *C. braunii* nor *C. bauerii* were collected from the Pilbara, but should be considered when examining material in Agardhia because of the morphological similarity of the species.

*Chara leptopitys* was thought to consist of one dioecious taxon, endemic to Australia, but distributed widely. The unifying characteristics are the presence of basal gametangia, a corticated axis and naked branchlets (Wood 1972). *Chara ecklonii* was the product of amalgamating endemic Tasmanian *C. mollusca* and African *C. ecklonii* (Wood 1965), to which was added *C. ecklonii* var. *albaniensis* on the basis of a single incomplete specimen collected in Albany, WA (Wood 1972). Wood (1965; 1972) followed the work of Zaneveld (1940) fairly closely in his treatment of *C. fibrosa*, amalgamating *C. gymnopitys* and *C. fibrosa*, and relegating *C. myriophylla* and *C. hookeri* to varietal status. Other taxa (*C. flaccida*, *C. benthamii*, *C. acanthopitys*, *C. dichopitys*, *C. drumondii*, *C. arnhemensis*, *C. microphylla*, *C. preissii*, *C. submollusca*, and *C. subtilis*) were either subsumed into *C. fibrosa* var. *fibrosa* form *fibrosa* or recognised as forms.

It has not been possible to designate definitive names for most of this group from the Pilbara. However *C. fibrosa* subspecies *benthamii* (black oospores, bicellulate), *C. sp aff flaccida* (monoecious, brown oospores) and *C. sp aff fibrosa* are tentative names for Pilbara taxa. There are three other dioecious taxa with distinctive vegetative morphology and oospores, which are designated here as *C. sp. aff. preissii* 1 (brown oospores) and *C. sp aff. preissii* 2 (pachygyra oospores), and *C. ?albaniensis*.

#### Chara fibrosa subspecies benthamii A. Braun

Monoecious species with corticated axes and naked branchlets. This taxon keys out to *C. fibrosa* var. *fibrosa* in Wood (1972), but was previously described as *C. fibrosa* subsp. *benthamii* (Zaneveld 1940). It is likely that this taxon is a separate species within section Agardhia. Distinguishing characteristics are the two-celled terminal segment on the branchlets, the size and shape of the oospore and the distribution of the taxon in the tropics. The type collection was from Hong Kong. The Pilbara specimens were not mature, so oospores cannot be illustrated. Representative specimen p633. PSW022 T2 M.N. Lyons and D.A. Mickle 3052.

#### Chara sp aff preissii 1

*Chara preissii* was described on the basis of specimens collected in Western Australia (York) by Preiss in 1838. Specimens were dioecious with smooth black oospores with 8-9 striae, large antheridia and verticillate bract cells. Pilbara material is more robust with twice the number of stipulodes as branchlets, fewer, smaller bract cells, large antheridia singly at the nodes, and golden-brown oospores with 7 flanged striae (Figs 29, 30) and a small, sunken basal cell impression (Figs 33, 34). The texture of the oospore wall varies from relatively smooth (Fig. 31) to minutely granulate (Fig, 32).

Representative specimens *p642* M.N. & S.D. Lyons 3041, PSW025 T1; *p685* M.N. & S.D. Lyons 3003, PSW003, *p636* M.N. & S.D. Lyons 3002, PSW003.



#### Chara sp. aff. preissii 2

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This taxon is similar to *C. preissii* in that it is also dioecious, however there are only 6 branchlets in a whorl, the corticated axis is triplostichous and the oospores are very similar to those of *C.* sp aff. *australis*. The oospores are less rectangular in side view than those of *C.* sp aff. *australis* (Figs 35, 38), with a smaller basal cell impression (Fig 37). This taxon also occurs in the Paroo region of New South Wales, where it was collected for the first time by Brian Timms in about 1988. It is as yet undescribed.

Representative specimens: *p675* MN Lyons & SL Lyons 3064, PSW004 T1 4/9/03; *p669* MN Lyons & SD Lyons 3019, PSW017 T2 14/9/03.



#### Chara sp aff fibrosa

*Chara fibrosa* is currently under revision, and contains at least four, and probably six good species in Australia. It was amalgamated with the tropical SE Asian species *C. gymnopitys*, as well a number of other taxa. Until the group is revised it is difficult to allocate names. The Pilbara material has markedly different oospores from the approximately 150 other specimens I have examined from Australian herbaria (Casanova 1997). The oospores are ovate, have 8 to 9 striae (Figs 39, 41) with a roughened to unevenly vertucate oospore wall (Figs 40, 42). The impression of the basal cell is small (Fig. 43). Representative specimens *p668* MN Lyons & SD Lyons 3019, PSW017 T2 14/9/03; *p684* MN Lyons and DA Mickle 5 PSW045 T1 5/6/04.



#### Chara sp aff flaccida

*Chara flaccida* A. Braun was reduced to a subspecies of *C. fibrosa* by Zaneveld (1940) on the basis of its variable morphology. The one consistent feature that kept it as a subspecies was that the oospores were 'golden-brown' (Zaneveld 1940). These specimens are relatively consistent with the description of *C. flaccida*, having a 2x corticated axis and naked branchlets, one row of stipulodes of 2x stipulodes with bright orange antheridia and oogonia at all branchlet nodes, right to the terminal branchlet nodes, and golden-brown oospores. There is some variation in the vegetative morphology of these specimens, but the oospores are all sufficiently similar to be the same taxon. Oospores are 480 to 520  $\mu$ m long, with 7 to 8 striae (Figs 44, 46). The oospore wall is roughly vertucae (Fig. 45) and the basal cell impression small (Fig. 47). Example *p646* MN Lyons & DA Mickle 3060 PSW032 T1 17/5/04; *P684* MN Lyons & DA Mickle 5 PSW045 T1 5/6/04.









#### **Chara** ?albaniensis

Chara ecklonii var. albaniensis was described on the basis of a single, male specimen collected at Albany WA by Blow (Wood 1972). Wood's amalgamation of African C. ecklonii and Australian C. mollusca could well be in error, given the disjunct distrubution, and the fact that most of what he recognised as varieties will be eventually reinstated as species (he retained the taxon of mollusca as a variety of C. ecklonii). The description of var. albaniensis indicates that it was 1x corticate, with 2x stipulodes, with inflated stipulodes, spine cells and bract cells. Oospores were not present on the type specimen so they are not described. The Pilbara material is also dioecious, with inflated stipulodes etc, and brown oospores. The oospores are 450  $\mu$ m long with 7 striae, a minutely vertucate oospore wall and a rounded basal cell impression c. 50  $\mu$ m in diameter. SEM images are not currently available for this taxon. If this is the same taxon, these are the only specimens to be collected since its type collection. Example, p652 MN Lyons & SD Lyons 3002, PSW003 T1 5/9/03.

#### Chara section Chara

Section *Chara* in genus *Chara* is the section to which the greatest diversity of Northern Hemisphere taxa belongs. Australian specimens are, however, much less diverse. Two taxa were described on the basis of Australian specimens: *C. contraria* var. *behriana* and *C. contraria* var. *australis*. Wood (1965) amalgamated *C. contraria* and *C. vulgaris* on the basis of morphological similarity and *C. vulgaris* had priority. In his treatment of Australian charophytes he therefore referred the Australian taxa to *C. vulgaris* var. *vulgaris* and *C. vulgaris* var. *gymnophylla* (Wood 1972). The European workers never subscribed to Woods revision (e.g. Corillion 1956), and the two taxa have always been easily distinguished on the basis of the arrangement of spine cells on the corticated axis. Given the difficulty of working out the range of variation in the original concept of *C. contraria* since it was not retained as even a separate form in Woods (1965) taxonomy, the early Australian names are currently retained for these taxa. On that basis, Pilbara specimens can be referred to *C. contraria* var. *australis* on the basis of the completely ecorticate branchlets, occasional occurrence of two rows of stipulodes and 2x corticated axes.

#### Chara contraria var. australis Muell.

(= *Chara vulgaris* var. *vulgaris* L. em R.D. Wood). An endemic Australian variety of *C. contraria* distinguished on the basis of vegetative and sexual characteristics (Fig. 48). This taxon was previously thought to be restricted to South Australia (Wood 1972), so records of it in the Pilbara region extend its distribution across the arid zone and into the wet-dry tropics. Axis diplostichous (Fig. 49), monoecious, partly corticate branchlets (Fig. 50). Stipulodes are usually in one row, but with occasional development of a second row of stipulodes below. Oospores are oval in side-view (Figs 54, 55), covered by a calcareous gyrogonite (Figs 52, 53), with a rounded basal cell impression (Figs 56, 57) and a rough surface (Figs 58, 59). Example *p659* Palm Spings at Cave Creek, seed bank culture MTC 27/10/04, *p643* MN and SD Lyons 3013 PSW009 T1 21/9/03. Chromosome count n=28 (Fig.51).







#### Chara section Grovesia

This section and subsection consist of widespread *C. globularis* and restricted *C. setosa* in Australia (Wood 1972). *C. globularis* consists of a complex of interrelated entities with similar morphology, that occurs world-wide (Proctor 1971). Wood (1972) found that there were three varieties in Australia, var. *globularis*, var. *leptosperma* and var. *virgata* none of which were endemic. The Australian taxa fit quite well into these categories, but more recent genetic and morphological research has shown that at least *C. globularis* and *C. virgata* are separate species (IRGC newsletter 2004). Variety *leptosperma* was distinguished on the basis of its yellow oospores. Although Wood thought this was merely a growth condition, it is probably one of a suite of differences between these taxa. Some of the Pilbara material fits the description of *C. globularis* var. *globularis*, but there is other material that does not fit well into any previously described species or vareity. It has distinct differences in its vegetative morphology, sexual structures, oospores and gyrogonites, and has 14 chromosomes, a number not recorded for a dioecious charophyte before. It is referred to as *C. sp aff globularis* 

#### Chara globularis var. globularis

A widespread and relatively uniform taxon in Australia. A large degree of variation occurs in this taxon in the Northern Hemisphere, and several other species have been described there, including dioecious ones. The vegetative plant is characterised by a dark green, narrow thallus with a strong foetid or 'garlic' smell. The axis is triplostichous with few, globular spine cells and stipulodes (Fig. 60), the branchlets are also corticated, generally with the last two segments ecorticate (Fig. 61). Bract cells are usually globular, with bracteloles present when nodes are fertile. The oogonium and antheridia occur singly, conjoined at the lowest two to three branchlet nodes. The oospore is elongate and has up to 12 striae (Fig. 62), and there is often a weakly developed gyrogonite. The base of the oospore is frequently extended into a 'basal cage' and 'claws' (Fig. 63).

Representative example MN Lyons and SD Lyons 3022, PSW0017 14/9/03. Figures 60 to 64 below are from a NSW example of this taxon which has 42 chromosomes (Fig. 64).





#### Chara sp. aff. globuaris Thuillier em. R.D.Wood.

This species keys out to be *C. globularis*, but has small but significant differences. Overall the entire thallus has a large amount of red pigmentation (Fig. 65), unlike the dark green of typical *C. globularis*. The gyrogonites, oogonia and oospores are much broader than in typical *C. globularis*, the coronula is globular, rather than appressed and the spine cells, while usually globular, are larger, more frequent and more conspicuous (Fig. 66). Chromosome counts on fresh antheridia reveal that this species has 14 chromosomes (Fig. 67), a number that has not been recorded for *C. globularis* before (Wood 1972). It is also an unusual number for a monoecious species. Monoecious species of *Chara* usually have multiples of 14 chromosomes (e.g. 28, 42 Proctor 1971). The oospores are much more oval than *C. globularis* (Fig 68), with no extension of the striae into a 'basal cage' (Fig 69) and a roughened surface (Fig 70). Representative specimen: *p658* MTC Hammersley Gorge 7 (1) seed bank culture, 24/10/04.



#### **Chara section Willdenowia**

Australian charophytes in this section consist only of *Chara zeylandica*. *C. zeylandica* is widespread through tropical regions, but reaches its greatest diversity in Central America (V. Proctor personal communication). The first specimen in this group was collected in Lake Titicaca, but the type specimen comes from Sri Lanka (Ceylon) (V. Proctor personal communication). Woods examination of the specimens indicated only the type variety and form in Australia (Wood 1972). The Pilbara specimens fit into the morphological variation outlined in Wood (1972), with some specimens displaying the elongate basal segment referred to (Wood 1972 p 21). These have been keyed out in Wood 1965 and are referred to as *C. zeylandica* var. *zeylandica* (short basal cell) and form *humboltiana* (long basal cell). The relative value of this character is not known.

#### Chara zeylandica var. zeylandica form zeylandica

Triplostichous, corticate branchlets, stipulodes in two rows, two per branchlet. Differs from C. *zeylandica* var *zeylandica* form *humboltiana* on the basis of 8 branchlet cells with a short naked basal branchlet cell. Gyrogonites broad (Figs 71, 76) with a small basal cell impression (Fig 74). Oospores oval with 11-12 striae (Fig. 72) and a concave basal cell impression (Fig. 74). Ornamentation apparently ridged and consisting of a roughened granular surface (Fig 73). In general a larger, more robust plant. Representative specimen: *P648* MN Lyons and SD Lyons 3015 PSW041 T2 1/6/04 2.



#### Chara zeylandica var. zeylandica form humboltiana

Triplostichous, corticate branchlets, stipulodes in two rows, two per branchlet. Differs from C. *zeylandica* var *zeylandica* form *zeylandica* on the basis of 6 to 7 branchlet cells with a long naked basal branchlet cell, and with 8 branchlets in a whorl. Gyrogonites smaller than form *zeylandica* (Figs 78, 79), oospores c. 650  $\mu$ m long with 11 striae (Figs 77, 82). Basal cell impression concave (Fig. 81), and ornamentation regularly granulate (Figs 80, 83). In general a smaller, finer plant than form *zeylandica*.

Example: P649 M.N.Lyons and D.A.Mickle, 3076 PSW041 T2 1/6/04 2.



#### Nitella section Hyella

Woods (1965) concept of Hyella in Nitella consisted of the pluricellulate species: dioecious N. myriotricha, N. gelatinifera (and all its varieties) and N. tasmanica and monoecious N. leptostachys, and N. leonhardii. Wood (1972) amalgamated N. leonhardii and N. leptostachys, but these two taxa are distinct based on morphology, oospores and gene sequences (K. Karol personal communication). Wood also amalgamated N. gelatinifera and N. tasmanica, with the name tasmanica having priority. He kept the taxa as separate subspecies, gelatinifera and tasmanica, with N. tasmanica subspecies gelatinifera having five varieties (gelatinifera, afoliata, microcephala, cryptostachys and beaugleholei). Variety gelatinifera has five forms (gelatinifera, elongata, compacta, zonata and tenuis) and var. microcephala has four (forms microcephala, cladostachya, podostachya and simplex). van Raam (1995) revised the Tasmanian members of the group, and reinstated the species N. gelatinifera and N. tasmanica but kept the varieties of N. gelatinifera. On the basis of this and until further revision, the pluricellulate Nitella from the Pilbara region can be distinguished as N. gelatinifera.

#### Nitella gelatinifera (R.D. Wood) R.D. Wood

Nitella gelatinifera is the name given by Wood to the illegitimately named N. gelatinosa, since the name gelatinosa had been used before. Wood's first reference to this taxon was as a subspecies of N. tasmanica (Wood 1965) but in his monograph he listed it as a 'microspecies' (Wood 1965). The original taxon was described for a specimen collected by Preiss in the Canning River in Perth. The Pilbara specimens fit the description of that taxon quite well. Oospores are 190  $\mu$ m long and wide with 6 striae (Figs 84, 86). The ornamentation on the fossa wall consists of low ridges runnning perpendicular to the striae, continuing up the 15 to 20  $\mu$ m high flanges as small lumps (Fig. 85). The ornamentation can be obscure or reduced in immature oospores.(Fig. 87). Representative specimen p640 MN Lyons and DA Mickle 3056, PSW027 T2 27/5/04; p638 MN Lyons & DA Mickle 3044, PSW027 T2 27/5/04.



#### Nitella sections Tieffallenia and Gioallenia

These sections of Nitella are characterised by species with bicellulate dactyls and homeoclemous whorls (i.e. nodes with a single whorl of branchlets present). Wood (1965) differentiated between Tieffallenia and Gioallenia on the basis of the shape of the dactyl end-cell. However, genetic analysis (Karol et al. 2000) indicates that this is not a good character for separation of sections, and that the broader group contains other taxa, placed in other sections by Wood (1965; 1972) (N. partita in Muelleria, N. penicillata in DeCandollea, N. tumida in Vogania). The majority of Australian Nitellas are homeoclemous, bicellulate and dioecious. Clearly defined Australian taxa include N. partita (Georgina R., Paroo R. Middle Is.), N. ignescens (NSW), N. confusa (Tas), N. haagenii (Tas), N. monopodiata (Tas) and N. gloeostachys (Tas). Less well defined taxa in this group are N. furcata (Wood amalgamated N. furcata and N. orientalis, good taxa in their own rights), N. sonderi (in which the type specimen differs from the generally accepted species concept and which occurs in eastern Australia), N. pseudoflabellata (which was made so broad that it contains approximately 19 varieties and forms in Australia and Asia), N. subtilissima (which vegetatively agrees with Wood's concept of N. furcata, but is not related to that taxon, occurring in WA, Vic, NSW), N. penicillata (which has a confused nomenclatural history and occurs in Vic. and Tas.), and N. aemula, N. robertsonii and N. remota which have not been re-collected since the type collections. Other dioecious taxa in this group not recorded for Australia are N. globulifera (=Wood's N. gracillis subspecies gracillis var. leptosoma form globulifera (India)), N. annandalei (= Wood's N. gracillis subspecies gracillis var. leptosoma form annandalei (India)), N. flagelliformis (=Wood's N. furcata subspecies flagelliformis (India)), N. dixonii (= Wood's N. tenuissima subspecies ornithipoda form dixonii (Portugal)). Comparison of the oospores and vegetative morphology rule out all the previously noted Australian taxa, and monoecious taxa that occur in south-east and southern Asia (Zaneveld 1940, Pal 1931, Pal 1940, Imahori 1954). It is possible that the Pilbara specimens represent new taxa, which are here designated N. sp aff tenuissima, N. sp aff. orientalis, N. sp 1(shallow-reticulum), N. sp 2 (pin-dots), N. sp 3 (aff. parooensis) and N. sp 4 (rippled).

#### Nitella sp. 1 (shallow-reticulum)

Bicellulate homeoclemous 2-3x furcate, no heads. Antheridia singly or in twos at first, second and third furcations. Dioecious. Golden yellow oospores c. 250  $\mu$ m long, 250  $\mu$ m wide with 6 striae (Fig 88). Low flanges on the striae and the fossa wall rippled into a shallow, irregular reticulum (Fig. 89). Example *p647* MNLyons and DAMickle 3054, PSW027 29/5/04.



### Nitella sp. 2 (pin-dots)

Bicellulate dioecious, no heads, no mucus, homeoclemous,  $2 \times furcate$ , oogonia and at first furcations. Very long primary branchlet cells, dactyls of varying lengths. Oospores c. 150 µm broad (Fig. 93) 275 µm long and 275 µm wide, with 7 striae (Fig. 90), with weak flanges 10 to 15 µm high (Fig. 91), and ornamentation consisting of minute papillae 0.2 to 0.3 µm high (Fig. 92). Representative specimens *p645* MNLyons & DA Mickle 3066 PSW036 T1 1 22/5/04.



#### Nitella sp. 3 (aff parooensis)

John Porter from New South Wales National Parks and Wildlife recently collected a new, dioecious *Nitella* with inflated dactyls from the Paroo region of New South Wales. That species has similar morphology and oospores as this one, although the oospores are occasionally more ornamented than these. Delineation of this taxon will be with reference to the Paroo specimens. The Pilbara specimens are dioecious, bicellulate, except that the sterile branchlets appear simple, and all dactyls are inflated. Gametangia clustered into a head, occasional furcations, oospores dark brown when mature. Six branchlets in a whorl, gametangia in 2s and 3s at fertile branchlet nodes, 2x furcate. Oospores are c. 190 µm in diameter and 200 µm long (Fig. 94), and 80 to 100 µm broad (Fig. 96) with 5 to 6 striae. The fossa is coarsely, shallowly reticulate, with one to two reticulae across the fossa (Fig. 95). The striae are rarely extended into thick flanges near the top of the oospore (Fig. 94). Representative specimen: p676 MN Lyons & DA Mickle 3072, PSW040 T2 27/5/04.



#### Nitella sp cf tenuissima

Nitella tenuissima is largely a monoecious taxon, with the exception of a poorly described entity recorded from Portugal (*N. dixonii*) (Wood 1965). Other members of *N. tenuissima* occur India and Japan, but these are not dioecious. The overall description of the Pilbara material approximates *N. tenuissima*, but it is dioecious. Specimens bicellulate, dioecious with very fine axes, branchlets and dactyls, with no real heads, no mucus. Some specimens are slightly calcified, branchlets are 1 x furcate, long dactyls, 6 branchlets in a whorl. The oospores are very small, approximately 140 to 200  $\mu$ m long, 150  $\mu$ m wide (Figs 97, 100) and 90  $\mu$ m broad (Fig. 99). There are 3 to 4 striae with weak flanges 10 to 20  $\mu$ m and the fossa wall is ornamented with small lumps (Fig. 101) or shallow ripples aligned perpendicular to the striae (Fig. 98). Representative specimen *p644* MN Lyons & DA Mickle 3008 A PSW007 T1 23/9/03.



#### Nitella sp. 4 (rippled)

This Pilbara *Nitella* is bicellulate, dioecious, homeoclemous, with brown oospores. The oospores are c.200  $\mu$ m long and wide (Figs 102, 104) with relatively thick flanges on the striae (Fig. 103). The ornamentation on the oospore wall consists of low verrucae 0.8.to 1  $\mu$ m in diameter, sometimes united into lumpy ridges (Figs 105, 106). Representative specimen *p670* MN Lyons and SD Lyons 3040, PSW025 T1 10/9/04.



#### Nitella sp aff. orientalis

*Nitella orientalis* was described on the basis of Japanese material and is monoecious. Wood retained this species as a subspecies of *N. furcata*. However, every time I have determined a non-fertile specimen as *N. furcata* genetic analysis has shown that it is identical to *N. subtilissima*. *N. subtilissima* is a dioecious taxon, initially described on the basis of specimens collected in the Swan River in Perth, it has the fertile branchlets contracted into long, non-mucus covered spikes. This taxon, being dioecious, is not *N. orientalis* or *N. furcata*, nor is it *N. subtilissima*. This taxon is homeoclemous, dioecious and bicelluate, somewhat brachydactylous with no heads. Gametangia occur at second and third furcations. The oospores are 220 µm long and 200 µm wide with c. 6 striae (Figs 107, 110). The ornamentation of short, 0.5 µm high papillae (Fig 108), runs up the delicate flanges on the striae (Fig. 109), gradually petering out (Figs 111, 112). Representative specimens *p641* MN Lyons and DA Mickle 3057, PSW027 T2 29/5/04; *p671* MN Lyons and DA Mickle 3070A PSW038 21/5/04 ADJ.



#### Nitella section DeCandollea

This section contains bicellulate *Nitellas* with two or more whorls of branchlets at the nodes (heteroclemy). *Nitella hyalina* is the well recognised representative of this section with a world-wide distribution. It is monoecious and most variable in South-East Asia. According to Woods revision (1972) Australia has two other dioecious species in the section; *N. congesta* and *N. lhotzkyi* (= *N. heterophylla*, *N. conglobata* and *N. biformis*). Wood also placed *N. penicillata* in this section, but recent genetic analyses indicates it is more closely related to members of section Tieffallenia (K. Karol personal communication). The Pilbara region has two monoecious taxa that key out to *N. hyalina* and one dioecious taxon keys out to *N. lhotzkyi*. The dioecious taxon does not have the distinctive oospore illustrated for the type material by Kützing, and it is likely that the Pilbara specimens are a different taxon. The designations used here are *N. hyalina*, *N. sp aff lhotzkyi* (pom-pom).

#### Nitella hyalina

Plants to 10 cm high, branchlets in more than one whorl at the node. Primary branchlets furcate, gametangia conjoined at second and third branchlet fucations. Ultimate branchlet segments consisting of two cells, the end cell short, conical and acute. Oospores laterally compressed without lime-shells (gyrogonites), 230  $\mu$ m long, 180  $\mu$ m wide (Fig. 114) with large flanges, to 50  $\mu$ m long (Fig. 113). Flanges extended into an apical 'crest' and basal 'cage'. Oospore ornamentation spongy, continuing up the flanges (Fig. 113). Representative specimen: *p637* M.N. Lyons & SD Lyons 3000, PSW001 T1 5/9/03.



#### Nitella sp aff hyalina (Bannister River)

Nitella hyalina is a relatively uniform taxon, and genetic analysis reveals that taxa from around the world have identical rbcL sequences, except for one specimen collected from Bannister River in the south-east of Western Australia. Further collections of that taxon have been made from the Pilbara. This species differs in its longer dactyls, and large number of accessory branchlets, both inside and outside the whorls. The overall morphology is robust. Oospores are 230 to 240  $\mu$ m long and 200 to 210  $\mu$ m wide (Figs 115, 116) with 7 to 8 striae with short flanges (Fig. 117), sometimes extended at the top and bottom of the oospore. The ornamentation is composed of anastomosing fibres, making up a spongy appearance (Figs 117, 118). Representative specimen: p682 MN Lyons & SD Lyons 3021, PSW015 T2 17/9/03.



#### Nitella sp aff lhotzkyi (pom-pom)

Nitella lhotzkyi was described by Braun in 1843 on the basis of Preiss' Western Australian collections. Subsequent similar specimens were allocated to sub-taxa, var. heterophylla, var. conglobata and N. biformis. Wood (1972) amalgamated all these and the species name lhotzkyi had priority. The type material have distinctive oospores with ornamenation consisting of low verrucae joined by low walls, producing a moniliform or 'string-of-beads' appearance on the fossa wall. The Pilbara specimens do not have oospores with this morphology, so it is likely they belong to another taxon, perhaps one of the ones that Wood subsumed into lhotxkyi. Final designation awaits further analysis.

Pilbara specimens are dioecious, bicellulate and heteroclemous, with a dense whorl of secondary branchlets at the base of the whorl. The primary branchlet whorl consists of branchlets with a long primary branchlet cell, and short subsequent branchlet cells, producing the appearance of 'pom-poms' at the end of the branchlets. Oospores are c. 120  $\mu$ m broad (Fig. 122), 300  $\mu$ m long, 280  $\mu$ m wide with 6 to7 striae with large flanges to 50  $\mu$ m long (Figs 119, 121). The underlying structure of the oospore wall is fibrous (Figs 123, 124), and in mature oospores is covered with a relatively smooth and unornamented layer (120). Representative specimen: *p679, p674, p677, MN* Lyons & SD Lyons 3046, PSW004 T1 4/9/03.



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**RMB L620** Westmere Vic 3351 10 December 2004

Department of Conservation and Land Management WA Wildlife Research Center P.O Box 51 Wanneroo WA 6946 Mr. M. N. Lyons Mr D.A. Mickle

Dear Mike.

Re: task 4 of our contract: 'A list of charophyte species for the Pilbara region of Western Australia'. This task entailed production of a report combining species that were collected and preserved in the field, and species that germinated from the seed bank.

Fourteen taxa of Chara, three of Lamprothamnium and ten of Nitella were present in the collections. The distribution of these 27 species within the sites is tabulated (Table 1), and provided as an Excel spreadsheet on compact disk. A description of each taxon with illustrations of the thallus and oospores (where available) is also provided (Charophytes of the Pilbara region of WA).

Seed banks are experiencing a new flush of germination of charophytes from the seed bank, so I will keep the seed bank study going until next winter, and update the species list and species determinations until that time. I anticipate receiving more soil and specimens from you in the near future (early autumn is likely to be the ideal time for inundating seed bank samples) with an extension of this contract.

Thank you for payments received to date, please find enclosed invoice for task 4: list of charophyte species from the Pilbara.

Balance of contract Extra seed bank material Extra specimens Total

1000 600 (extra 3 samples x \$200/sample) 120 (extra 6 specimens x \$20/specimen) \$1720 + GST

Yours truly,

Mehelle Casanora

Michelle Casanova

No. species	Nitella sp 4 (rippled)	Nitella sp 3 (aff. parc	Nitella sp 2 (pin-dots	Nitella sp 1 (shallow	Nitella sp cf tenuissi	Nitella sp aff Ihotzky	Nitella sp aff hyalina	Nitella hyalina	Nitella sp aff orienta	Nitella gelatinosa	Chara contraria var.	Chara zeylandica va form humboltiana	form zeylandica	Chara zevlandica va	Chara sp aff globula	Chara globularis	Chara sp aff preissil	Chara sp aff preissi	Chara ?albaniensis	Chara sp aff fibrosa	Chara fibrosa subsp benthamii	Chara sp. aff. flaccid	Chara sp aff austral	Chara australis	Chara simplicissima	Lamprothamnium sp	Lamprothamnium sp	Lamprothamnium sp succinctum	species		
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