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GENEALOGY IN MICROSPORA (CHLOROPHYCEAE)

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SUMMARY

The genealogical relationships in the green algal genus *Microspora* (Chlorophyceae) are determined by phylogenetic analysis based on 15 ecological, morphological and reproductive characters. It is found that the traditional defining characters like cell shape and distinctness of the H-shaped pieces (composing the cell wall) play a major role in rooting of the cladograms and that species – forming a prominent rope-like growth habit in rapid streams – are closely related. It is suggested that the pH value of the habitat proves to give a more substantial phylogenetic signal than the type of water movement.

Key words: Chlorophyceae, *Microspora*, cladistics, phylogeny.

INTRODUCTION

The cosmopolitan green algal genus *Microspora* Thur. consists of at least 9 species (Lokhorst, 1999) and is characterised by uniseriate simple filaments which are built up of cells with bipartite walls composed of overlapping segments (H-shaped in optical lateral view). A specific property of this wall construction is especially seen during zoospore release when both cell wall pieces become separated. This unique cell wall construction is also found in the genus *Tribonema* Derbès & Solier which represents a prominent member of the Xanthophyceae (Tribophyceae) (Ettl, 1978). Based on similarities in flagellar apparatus structures, it is recently suggested that *Microspora* is evolutionary closely related to taxa (e.g., *Bracteacoccus* Tereg, *Dictyochloris* Vischer) which were formerly classified in the traditional Chlorococcales (Lokhorst & Star, 1999).

The genus *Microspora* has a wide ecological distribution pattern in freshwater varying from acidic stagnant waters, usually without developing an extensive plant carpet, to alkaline, rapid streams, often with formation of extensive (bottom-covering) rope-like plant mats of many decimetres long (Simons et al., 1999).

In the last century two thorough systematic studies were carried out in *Microspora*, by Wichmann (1937) and Lokhorst (1999). In the latter study, it is found that species delimitation in *Microspora* remains primarily based on traditional features like cell dimensions, cell shape, and the distinctness of cell wall components (the H-shaped pieces). The number of zoospores per cell and the ability to form an apical cap in filamentous germinating stages prove to be of additional diagnostic significance.

The availability of a relatively great number of diagnostic features encouraged us to obtain a better understanding of the phylogenetic interrelationships within *Microspora*.

Table 1. Ecological, morphological and reproductive features selected for phylogenetic analysis in the genus *Microspora*.

Distribution pattern in the field

1. Type of water movement
   1 = clear preference for rapid streams or turbulent waters
   2 = widespread occurrence in moving as well as stagnant waters
   3 = clear preference for stagnant waters
2. pH value
   1 = mainly in non-acidic habitats
   2 = in acidic as well as alkaline habitats
   3 = mainly in acidic habitats

Morphology

3. Macroscopic growth habit in the field
   1 = also forming skein-like strands of many decimetres long
   2 = not producing skein-like strands of many decimetres long
4. Filament shape
   1 = primarily straight
   2 = primarily curled and tortuous
5. Cell shape
   1 = basically cylindrical
   2 = cylindrical as well as doliform
   3 = basically doliform
6. Cell length/width ratio
   1 = often almost equal giving rise to filaments seemingly built up of square cells
   2 = usually not equal throughout the filament
7. Cell wall texture
   1 = looking firm (also when thin), cell wall visible
   2 = looking soft, cell wall hardly visible
8. Cell wall shape
   1 = primarily homogeneous (with camouflaged H-shaped pieces)
   2 = primarily heterogeneous (with distinct H-shaped pieces)
9. Transverse cell wall covering
   1 = clothed with yellowish ring
   2 = without yellowish ring
10. Apical cell covering in juveniles
    1 = with hyaline cap
    2 = without hyaline cap
11. Shape of basal cell in juveniles
    1 = (soon) rhizoidal
    2 = unmodified or slightly differentiated, not typically rhizoidal
12. Chloroplast
    1 = reticulate, simple
    2 = reticulate, at full development compound by formation of extra projections

Reproduction

13. Number of zoospores
    1 = up to 2 per zoosporangial cell
    2 = also more than 2 per zoosporangial cell
14. Shape of zoosporangial cell at ripening of zoospores
    1 = not considerably swollen and without huge production of mucilage
    2 = considerably swollen, with huge production of mucilage
15. Formation of akinetes and sexual reproduction
    1 = infrequent formation of akinetes in absence of sexual reproduction
    2 = abundant formation of akinetes in presence of sexual reproduction

MATERIAL AND METHODS

For phylogenetic analyses, potentially informative characters were extracted from a recent study on *Microspora* (Lokhorst, 1999), listed in Table 1. Two ecological characters (characters 1 and 2) were included in the selection, because some species have a distinct habitat preference. For example, *M. pachyderma* occurs only in acidic, predominantly stagnant waters. In addition, the growth habit of an alga may be correlated with the alga's occurrence in the field. For example, *M. floccosa* forms prominent long algal strands in rapid streams but a less remarkable homogeneous algal coat around submerged (organic) substratum in (acidic) stagnant waters.

The 15 selected characters (ecological, morphological, and reproductive) were translated into multistate codes, and, subsequently, entered into the data matrix (Table 2). Within characters, states were defined so as to leave as little overlap as possible. Two types of polymorphism were recognised. Species may show gradual overlap between two defined character states of a particular character, because they express a character state with a broad amplitude. This was found for the ecological characters (characters 1 and 2) and the morphological character of cell shape (character 5). These characters were coded as multistate, represented by three character states, whereby the state with the broad amplitude was consequently separately coded as state 2. Polymorphism may also occur as a condition, whereby species may show two distinct, discontinuous character states of a particular character (one of the two character states is usually rare). These characters are coded as polymorphic with states '1&2'. Note, however, that for *M. floccosa* in Table 2 the state of the multistate morphological character of cell shape (character 5) is coded as 1&2. Both cylindrical and doliform cell shapes occur in this species, but the doliform cell shape is rare and presumably aberrant (Lokhorst, 1999).

The green alga *Binuclearia tectorum* (Kütz.) Beger – in several aspects resembling *Microspora* (see Lukavský, 1970) – was chosen as outgroup. According to literature (Lukavský, 1970), this species produces one zoospores per cell, but it may also form four zoospores per cell (pers. obs.). Hence, the character state of character 13 is coded for this alga as 2 (Table 1).

Table 2. Data matrix of the character states of *Microspora* species added with those of the outgroup species *Binuclearia tectorum*.

Characters / Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
M. quadrata	2	2	2	1&2	1	1	1	1&2	2	2	1	1	1	1&2	1
M. tumidula	2	2	2	1	2	2	1	2	2	1	2	1	1	1	1
M. lauterborni	2	3	2	2	3	1	1	2	2	1	2	1	1	1	1
M. tenuiderma	3	2	2	1	2	2	2	1	2	1	2	1	2	1	1
M. pachyderma	3	3	2	1	3	2	1	2	2	1	1	1	1	1	1
M. stagnorum	2	1	1	1	1	2	1	1	1	2	2	1	1	1	1
M. floccosa	2	2	1	1	1&2	2	1	1&2	1&2	1	2	1	2	2	2
M. wittrockii	2	2	2	1&2	1	2	1	1	2	2	1	1	2	2	1
M. amoena	1	2	1	1	1	2	1	2	2	1	1	2	2	1&2	1
B. tectorum	3	3	2	1	2	2	1	2	2	1	2	?	2	1	?

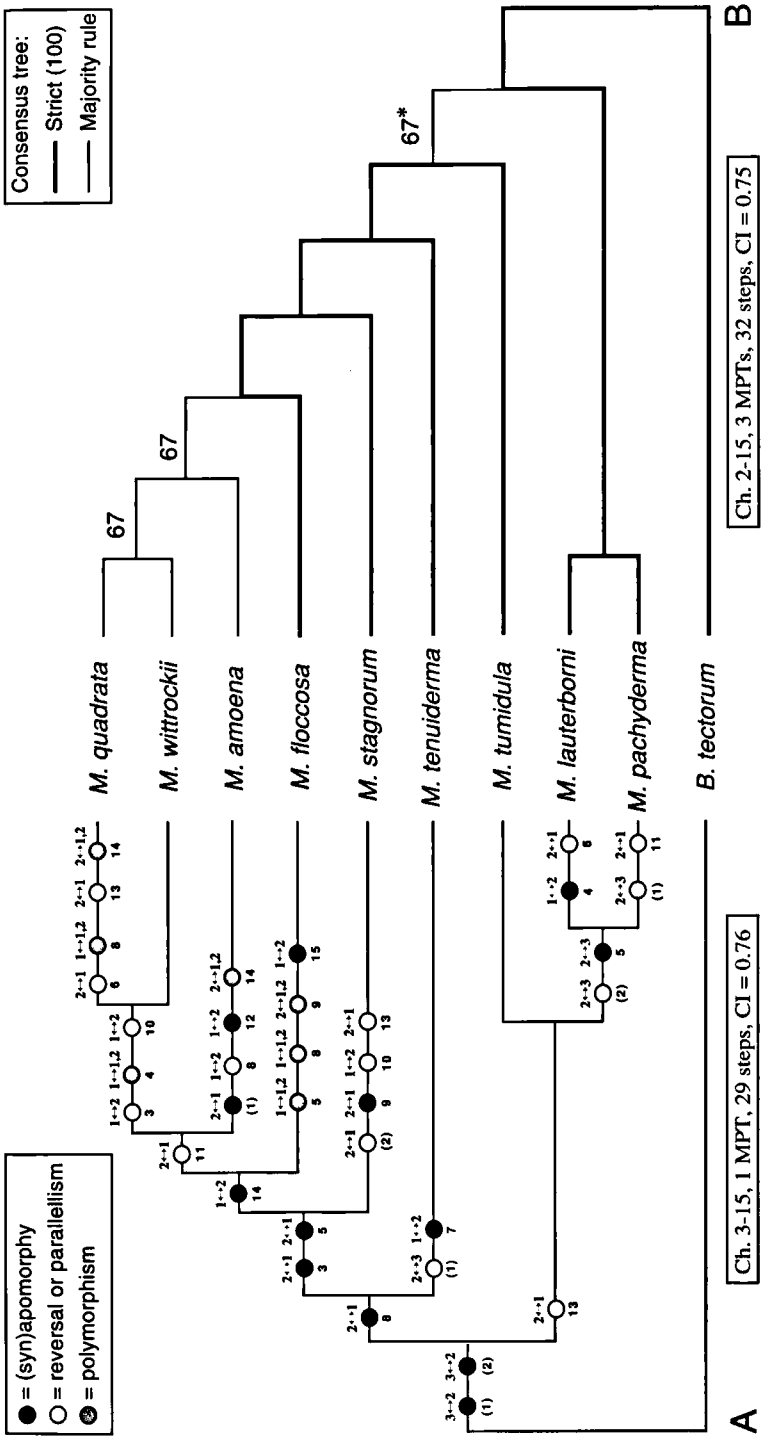

Fig. 1. Phylogenies of *Microspora*. Tree A shows the distribution of the character state changes (numbers above the circles) of the characters (number below the circles). Tree B represents a consensus tree. Characters 1 and 2 (not included in the analysis, see text) are placed between parentheses on tree A. Abbreviations: Ch. = the number of characters involved in the analysis; MPT = the resulting number of most parsimonious trees; CI = consistency index. * = branch in tree B, which is supported in strict consensus tree of two MPTs resulting from analyses without the outgroup (see text).

Unfortunately, the lack of an adequate number of comparative light microscopical features precluded the selection of traditionally chlorococcalean algae (e.g. *Bracteacoccus, Dictyochloris*) – having a flagellar apparatus structure comparable to that of the filamentous *Microspora* (Lokhorst & Star, 1999) – as outgroup species.

The PAUP software packages version 3.1.1 and 4.0b (Swofford, 1993, 1998) were used for the phylogenetic analyses. Cladograms were obtained by computing a set of most parsimonious trees (MPTs) using the exhaustive search option. Characters and character states were treated a priori as unordered and unweighted. Character state changes were evaluated using MacClade version 3.0.8 (Maddison & Maddison, 1992). The informative value of the ecological characters were evaluated by comparing analyses, which were run with and without ecological data and with and without the outgroup.

RESULTS AND DISCUSSION

When using all characters or when the ecological character 2 is omitted, the phylogenetic analyses including the outgroup result in numerous most parsimonious trees with an unresolved consensus tree (not shown). When the ecological character 1 is eliminated from the analysis, it results in three most parsimonious trees (consensus trees Fig. 1B). When both ecological characters are eliminated, the analysis results in a single most parsimonious tree (Fig. 1A).

The results of all four analyses without the outgroup include two MPTs, of which the strict consensus tree is similar to the strict consensus tree of Fig. 1B (but slightly better resolved). The results of the analyses without the outgroup are independent from the exclusion of one or both ecological characters.

Two phylogenies of *Microspora* are depicted in Fig. 1. The left-hand cladogram (A) represents the most parsimonious tree resulting from the cladistic analysis of the characters 3–15 including the outgroup (Table 1). Although the ecological characters 1 and 2 were eliminated from the analysis, they are plotted a posteriori between brackets on tree A in Fig. 1. The right-hand cladogram (B) represents the consensus tree of three MPTs resulting from an analysis with the outgroup and with only one ecological character (character 2) included.

The most striking outcome in the trees of Fig. 1 is the clade *M. pachyderma – M. lauterborni*. In Fig. 1A, this clade is obtained by the analysis of morphological and reproductive data, but it is also well supported by a low pH value (state 3 of character 2; Fig. 1B, ecological data plotted in Fig. 1A).

The traditional specific characters of cell shape and cell wall shape (e.g. used in Heering, 1914; Wichmann, 1937), respectively characters 5 and 8, play a significant role in the phylogeny of *Microspora*. In both trees of Fig. 1, the distinct H-shaped pieces (character 8, state 2) are plesiomorphic for *M. pachyderma, M. lauterborni* and *M. tumidula*. For the remaining species, camouflaged H-shaped pieces (character 8, state 1) is synapomorphic. The distinct H-shaped pieces in *M. amoena* and the polymorphic condition of *M. floccosa* and *M. quadrata* is regarded here as a reversal.

In both trees of Fig. 1, the 'cylindrical as well as doliform' cell shape (character 5, state 2) is a plesiomorphic condition. The doliform cell shape is a synapomorphy for the clade *M. pachyderma – M. lauterborni*. The cylindrical cell shape is a synapomorphy for the clade *M. stagnorum, M. floccosa, M. amoena, M. wittrockii, M. quadrata*.

The peculiar polymorphism of *M. floccosa* for this character is treated as an autapomorphy.

It is further remarkable that all species forming an impressive rope-like plant mass in rapid streams (*M. amoena*, *M. floccosa* and *M. stagnorum*; character 3, state 1) take a congenial position in both trees of Fig. 1. The less massive growing habit of *M. wittrockii* and *M. quadrata* is a reversal.

The pH value of the habitat (character 2) issues a more substantial phylogenetic signal for *Microspora* than the type of water movement (character 1). From an ecological point of view, this may well be explained by our field observations. Water flow in *Microspora*-habitats seems to be easily affected by external conditions like (temporarily severe!) rainfall resulting in drastically varying water movements and water levels. This actually may imply that water movement as an ecological factor in *Microspora*-habitats cannot simply be defined in terms of current velocity, but that the frequency and amplitude of flow changes have to be taken into account as well. The pH value of *Microspora*-habitats seems to be less sensitive to changes in external conditions due to the buffering capacity of dissolved organic and anorganic compounds (or colloid soil particles).

It is earlier assumed that a low(er) pH value promotes the distinctness of the H-shaped pieces in the cell wall of *Microspora* (Lokhorst, 1999). For example, field material of the acidic species *M. lauterborni* and *M. pachyderma* consistently shows this specific cell wall configuration. However, a low pH value is not the only issue which promotes the distinctness of the H-shaped pieces. Especially the two latter species require special growth conditions for this configuration. This is shown when they are exposed to culture conditions, which results in an immediate growth stop and subsequent ailing of filaments (*M. lauterborni*) or in poor growth of filaments which are (non-typically!) characterised by a thin, homogeneous cell wall (*M. pachyderma*). Even when humic acids are added to the media (Lokhorst, 1999), the total spectrum of required specific external conditions cannot be imitated in cultures.

Despite the assumed phylogenetic significance attributed to the pH, the usefulness of ecological data in general in a phylogenetic analysis of the genus *Microspora* can be questioned, because some freshwater habitats, in which representatives of this genus occur, deserve the predicate of being unstable, caused by periodic rainfall, changing temperatures, pollution pressure, etc.

ADDITIONAL NOTES

Tree A is slightly better resolved than tree B (Fig. 1). In both analyses, the phylogenetic signal is strong as judged by the single, respectively three, most parsimonious trees that resulted from each analysis and by the significant skewed g1 values ($P<0.01$) obtained from a comparison with random matrices (Källersjö et al., 1992; Hillis & Huelsenbeck, 1992). The bootstrap values tend to be low in all analyses (1000 replicates, <50%, not shown). However, the application of this method on small (morphological) data matrices is doubtful because the amount of characters on each branch is limited (≤ 3), which makes these data matrices very sensitive to bootstrap resampling (Sanderson, 1989).

Substantial homoplasy (CI ≤ 0.5) was found in the characters 1, 3, 6, 10, 11 and 13 (Fig. 2). Three characters (characters 7, 12, 15) are in terms of parsimony non-informative (autapomorphous) and their exclusion from the analysis can therefore be considered. However, it is chosen to maintain these autapomorphies, because they firstly increase the heuristic value of the datamatrix, meaning that: a) in the future the impact of newly discovered (and incorporated) character states can immediately be tested by the establishment of new synapomorphies; b) new species can easily be added by which existing phylogenies can be evaluated at once; c) the data matrix is more open for a critical (re)interpretation by other scientists; d) the data matrix is more suitable for a priori outgroup analysis. Secondly, it more directly links the phylogeny with the taxonomy, including that: a) the data matrix is also usable as a synoptic key; b) the taxa are positively defined; c) an impression of the morphological distinction is given by the amount of character changes (anagenese) on the terminal branches.

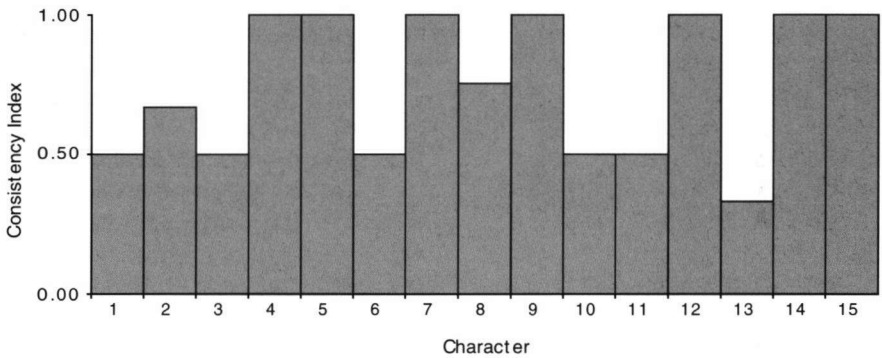

Fig. 2. Amount of homoplasy of each character found in the left-hand (Fig. 1A) one MPT.

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