

Strain survey on three continents confirms the polyphyly of the genus *Pediastrum* (Hydrodictyaceae, Chlorophyceae)

Mrutyunjay JENA^{1,2,3}, Christina BOCK^{1,4}, Chhandashree BEHERA^{1,2}, Siba P. ADHIKARY⁵
& Lothar KRIENITZ^{1*}

¹Leibniz–Institute of Freshwater Ecology and Inland Fisheries, Alte Fischerhütte2, D–16775 Stechlin–Neuglobsow, Germany; *e–mail: krie@igb–berlin.de, tel.: 033082 69926, fax: 033082 69917

²Department of Botany, Utkal University, Bhubaneswar–756014, Odisha, India

³Department of Botany, Berhampur University, Berhampur–760007, Odisha, India

⁴University of Essen, Faculty of Biology, D–45141 Essen, Germany

⁵Department of Biotechnology, Visva–Bharati Santiniketan–731235, West Bengal, India

Abstract: Approximately 100 new strains of the *Pediastrum*–phenotype were isolated from inland waters of India, eastern and southwestern Africa, and Germany and were studied by light and scanning electron microscopy. The 18S rRNA and ITS genes of 28 strains were sequenced and compared with 25 sequences of Hydrodictyaceae from the GenBank database. Phylogenetic analyses confirmed the polyphyly of *Pediastrum*. The new species *Sorastrum pediastriforme* was described. This species resembled morphologically *Pediastrum*, however, the phylogenetic analyses revealed its affiliation within the genus *Sorastrum*. Furthermore, the following new combinations: *Monactinus sturmi*, *Pseudopediastrum alternans*, *Pp. brevicorne*, *Pp. integrum*, and *Pp. pearsonii* were established.

Key words: CBC, ITS, *Lacunastrum*, *Monactinus*, morphology, *Pediastrum*, phylogeny, *Pseudopediastrum*, *Sorastrum*, SSU, *Stauridium*

INTRODUCTION

The family Hydrodictyaceae (Chlorophyceae) is from a morphological point of view, one of the most diverse natural lineages of coccoid green algae and comprises microscopic colonies of *Pediastrum* MEYEN, *Euastropsis* LAGERH. and *Sorastrum* KÜTZ. as well as macroscopic colonies of *Hydrodictyon* ROTH. Inspired by members of the genus *Pediastrum*, systematists have established more than 350 taxa (PARRA 1979). A flood of observations and revisions were published about *Pediastrum* and summarized by KOMÁREK & FOTT (1983). The most recent review reduced the number of *Pediastrum*–species to 24 (KOMÁREK & JANKOVSKÁ 2001). The other genera included smaller numbers of species: one of *Euastropsis*, seven of *Sorastrum*, and five of *Hydrodictyon* (according to KOMÁREK & FOTT 1983).

The first molecular phylogenetic studies including only two members of Hydrodictyaceae showed the close relationship of *Hydrodictyon reticulatum* (L.) LAGERH. and *Pediastrum duplex* MEYEN (WILCOX et al. 1992; LEWIS 1997) and their placement within Sphaeropleales (BUCHHEIM et al. 2001). An expanded phylogenetic analysis of 26S rRNA, 5.8S + ITS2 genes using sequences of seven

strains of *Hydrodictyon*, 29 strains of *Pediastrum* and three strains of *Sorastrum* revealed a pattern of colony–form evolution within the family from two–dimensionality to three–dimensionality (McMANUS & LEWIS 2005). Another molecular phylogenetic study of 28 hydrodictyacean strains resulted in taxonomic revisions (BUCHHEIM et al. 2005). It was shown that the genus *Pediastrum* is of polyphyletic origin. Therefore, beside *Pediastrum* the genera *Monactinus* (TURPIN) E. HEGEWALD, *Stauridium* (PRINTZ) E. HEGEWALD, *Pseudopediastrum* E. HEGEWALD and *Parapediastrum* E. HEGEWALD were delineated. Nevertheless, the number of strains analysed was very low, for example the genera *Monactinus* (formerly *Pediastrum simplex*) and *Parapediastrum* (*P. biradiatum*) were described from a single strain (BUCHHEIM et al. 2005). Members of the unicellular genus *Tetraedron* KÜTZ. were linked phylogenetically to the Hydrodictyaceae (BUCHHEIM et al. 2005; McMANUS & LEWIS 2005) however, the relative position was not clear from these analyses.

The morphospecies *P. duplex* was shown within different phylogenetic lineages of Hydrodictyaceae (McMANUS & LEWIS 2005). Therefore, one of these lineages was established as new genus *Lacunastrum* H. McMANUS (McMANUS et al. 2011). More detailed phylogenetic analyses based on 26S rRNA and *rbcl*

genes on *P. duplex* and its comparison to other members of Hydrodictyaceae were provided by Mc MANUS & LEWIS (2011).

We have isolated ca. 100 strains of Hydrodictyaceae from three different continents – Africa, Asia and Europe – and studied them morphologically. From this collection, we subjected 28 strains to molecular phylogenetic analyses by sequencing the nuclear 18S rRNA gene and ITS genes and the analyses confirmed the polyphyly of the genus *Pediastrum*. For species delineation, we applied the CBC concept (COLEMAN 2003). In the frame of this study we described the new species *Sorastrum pediastriforme* JENA, C. BOCK, BEHERA et KRIENITZ, and established five new combinations in the genera *Pseudopediastrum* and *Monactinus*.

MATERIAL AND METHODS

Algal cultures. About 100 unialgal strains of *Pediastrum*-phenotype were isolated from field samples of inland waters of India, Israel, Kenya, Namibia, and Germany by microcapillaries and grown in a modified Waris solution (McFADDEN & MELKONIAN 1986). All strains were maintained at the algal strain collection of the Leibniz-Institute of Freshwater Ecology and Inland Fisheries (IGB) at Stechlin, Germany, and grown in suspensions at 15 °C under a 14:10 h light–dark regime. The strain of the newly described *Sorastrum pediastriforme* and the strains representing the five new combinations were deposited at the Culture Collection of Algae at the University of Göttingen (SAG, Germany).

Microscopy. The algae were examined using a Nikon Eclipse E600 light microscope (LM) with differential interference contrast. Photomicrographs (Figs 3–6) were taken with a Nikon Digital Camera DS-Fi1, and Nikon software NIS-Elements D (Nikon Corporation, Tokyo, Japan). For scanning electron microscopy (SEM) the colonies of cultured material were fixed in 2.5% glutaraldehyde in culture medium for 30 min at 5 °C, washed three times in 3 mM Hepes buffer (pH 7.0), and postfixed in 1% osmium tetroxide in a 1:1 mixture of 2% OsO₄ and 3 mM Hepes for 1 h. The fixed cells were then washed three times in Hepes buffer and dehydrated in a graded series of ethanol dilutions. Following dehydration, the material was dried at the critical point using aBAL-TEC CPD 030 critical point dryer (Balzers, Schalkmühle, Germany). Dried material was mounted on aluminium stubs, coated with gold/palladium by a Polaron CA 508 sputter-coater (Fisons Instruments, VG Microtech, Uckfield, UK) and examined under using the field-emission SEM Hitachi S-4500 (Hitachi Corporation, Tokyo, Japan) at 5 kV, 10 µA.

DNA Isolation, PCR and Sequencing. A total of 28 strains were selected for molecular phylogenetic analyses. Algal cells were mechanically disrupted in the presence of glass beads (~ 0.5 mm in diameter, Carl Roth GmbH + Co. KG, Karlsruhe, Germany) using the Retsch MM 2000 (Retsch, Hahn, Germany) bead disruptor. Total Genomic DNA was extracted according to KRIENITZ et al. (2012). The 18S rRNA and ITS–2 rRNA genes were amplified and sequenced as described by BOCK et al. (2011). The newly sequenced strains

were compared with 24 sequences of Hydrodictyaceae and one (outgroup) *Scenedesmus*–sequence from the GenBank database (National Center for Biotechnology Information [NCBI] <http://www.ncbi.nlm.nih.gov/>). The origin and accession numbers of all these 53 strains are listed in Table 1.

Phylogenetic analyses. Based on a concatenated data set (18S rRNA and ITS–2 rRNA) of 47 sequences with 1872 aligned base positions (excluding intron) phylogenetic analyses were performed. The Sequence alignment was done manually according to their secondary structure (COLEMAN 2003) using the SequentiX Alignment Editor (HEPPERLE 2004). The ITS–1 rRNA was not unambiguously alignable and was, therefore, excluded from the data set. In total, 28 strains that include representatives of the genera *Pediastrum*, *Pseudopediastrum*, *Parapediastrum*, *Monactinus* and *Sorastrum* were chosen from the GenBank database (National Center for Biotechnology Information [NCBI] <http://www.ncbi.nlm.nih.gov/>). The selection of additional sequences were done based on the publications of BUCHHEIM et al. (2005) and McMANUS & LEWIS (2005, 2011). *Scenedesmus obliquus* UTEX 393 was selected as outgroup. The phylogenetic tree in Figure 1 was inferred from a concatenated dataset using Treefinder (JOBBA 2008). The applied models were chosen as proposed by Treefinder: For 18S rRNA (1640 bases) the Tamura Nei model with Gamma distribution (TN:G:5), and for ITS–2 the transitional model with Gamma distribution (J2:G:5) were chosen.

To confirm the tree topology, bootstrap analyses were calculated by distance (neighbor-joining; NJ; 1000 replicates), parsimony (MP; 1000 replicates) using PAUP*, version 4.0b10 (SWOFFORD 2002) and maximum likelihood (ML; 1000 replicates; settings as described above) criteria. Bayesian inference (Bayesian posterior probabilities [BPP]; using the covarion model; 1 million generations) were calculated using MrBayes version 3.1 (HUELSENBECK & RONQUIST 2001). The Bayesian analyses were carried out until the average standard deviations of split frequencies between two runs was lower than 0.01. The first 25% of the calculated trees was discarded as burn-in. A 50% majority-rule consensus tree was calculated for posterior probabilities (PP).

Secondary structures analysis. Secondary structures of ITS–2 were constructed with the help of mfold (ZUCKER 2003). The program 4SALE (SEIBEL et al. 2006, 2008; SCHULTZ & WOLF 2009) was used for comparison of ITS–2 among the strains to locate compensatory base changes (CBCs) including Hemi-CBCs in the aligned ITS–2 sequences. The overview graphic was performed with PseudoViewer (BYUN & HAN 2006). In order to find molecular criteria to discriminate the species, these secondary structures were checked for Compensatory Base Changes (CBCs) including Hemi-CBCs using 4SALE (SEIBEL et al. 2006, 2008).

RESULTS

The concatenated set of 18S rRNA and ITS–2 sequences of 47 strains was included in the analyses to assess the phylogeny of Hydrodictyaceae. The 28 newly sequenced strains were placed in seven

subclades of the phylogenetic tree (Fig. 1). Generally, the family of Hydrodictyaceae formed a monophyletic group well supported in the statistical analyses, only the ML analyses resulted in a lower bootstrap value of 67. Members of the genus *Pediastrum* sensu lato, which were in the focus of this study, were grouped into different lineages. According to suggestions of BUCHHEIM et al. (2005) these lineages were regarded as genera and designated as *Pediastrum*, *Pseudopediastrum*, *Monactinus*, *Stauridium* and *Parapediastrum*. Two strains belonged to the recently erected genus *Lacunastrum* (McManus et al. 2011). *Hydrodictyon* was closely related to *Pediastrum*, and *Sorastrum* is placed on the base of the tree as sister to all other hydrodictyacean subclades. The bootstrap support of different genera was high, only the *Pseudopediastrum* clade earned low support. The position of *Tetraedron* remained ambiguous.

The strain MJ 2008/46 (SAG 2392) from a dam near Nyahururu in Kenya clustered in the *Sorastrum* clade. Members of the genus *Sorastrum* normally, were characterized by three-dimensional colonies. However, the MJ 2008/46 (SAG 2392) strain showed flat coenobia (Figs 2a–d) resembling morphospecies of the genus *Pediastrum*, in particular those of *P. duplex*. Only few cells displayed a three-dimensional (tetrahedral) shape (Fig. 2d). This material was described as new species *Sorastrum pediastriforme* (see taxonomic conclusions).

Members of the genus *Monactinus* were found in India, Kenya and Germany. Whereas the strain from Germany exhibited the typical morphology of *M. simplex* var. *simplex* with large irregular holes within the coenobium (Figs 3a–b), the four tropical strains resembled *M. simplex* var. *sturmii* with stocky cells and smaller holes (Figs 3c–d).

Six new strains clustered in the *Pseudopediastrum* clade. These strains showed characteristic differences (Figs 4a–d, 5a–d). Whereas the strains MJ 2008/33 and MJ 2008/35 from Lake Dollgow in Germany fully met the characteristics of *Pseudopediastrum boryanum* (TURP.) MENEGH. (not shown), the others exhibited features of other taxa. Strain MJ 2008/83 from the river Cuando in Namibia possesses long conical lobes and deep incisions, resembling with *Pseudopediastrum alternans* NYG. (Figs 4a–b). Strain MJ 2008/28 from Lake Dollgow in Germany had short lobes with shallow incisions and resembled *Pp. boryanum* var. *brevicorne* A. BRAUN (Figs 4c, d). Strain MJ 2008/86 from a pond in Namibia with short rounded apices and very shallow incisions resembled *Pseudopediastrum integrum* NÄGELI (Figs 5a, b). Strain KR 2006/01 from Lake Naivasha, Kenya, similar in morphology but different in molecular criteria, resembled *Pp. integrum* var. *pearsonii* (G.S. WEST) FRITSCH (Figs 5c, d) known from tropical regions. Because of the clustering of these strains in the same clade, they all were considered as species of

the genus *Pseudopediastrum*. For the essential new combinations see taxonomic conclusions.

Members of the former morphospecies *Pediastrum duplex* clustered in two different clades of the tree. Six of these strains remained in *P. duplex*, whereas two other strains were determined as *Lacunastrum gracillimum* (Figs 6a–d). These strains had relatively long processes and the ultrastructure of the cell wall was rippled, smooth, but not net-shaped and granular. These results confirmed the findings of McMANUS et al. (2011). Five new strains of *Stauridium tetras* (EHRENB.) E. HEGEWALD from Kenya and India and *S. privum* (PRINTZ) E. HEGEWALD from Kenya and Germany clustered in the clade of *Stauridium*. One strain of *Parapediastrum biradiatum* (MEYEN) E. HEGEWALD from Germany clustered together with a strain from Sweden in the *Parapediastrum* clade. These results confirmed the findings of BUCHHEIM et al. (2005).

To test the results of the topology of the phylogenetic tree, the secondary structure of the ITS-2 was studied, with the main goal to find CBCs and hemi-CBCs associated with individual taxa. The secondary structure of the taxa studied exhibited the common pattern with four helices, in which the helix I was branched, shown here using *Sorastrum pediastriforme* (Fig. 7) as exemplar. To establish an overview, we made a pairwise comparison of the CBCs of the conserved regions of all helices between the species which were discovered to be the closest relatives to each other according to the tree. *Sorastrum pediastriforme* differs from *S. spinulosum* (UTEX 37) by 2 CBCs in Helix I (not shown). *Monactinus simplex* and *M. sturmii* differed by 2 CBCs and 5 or 6 hemi-CBCs in helix III (Table 2). The species of *Pseudopediastrum* differed in the following numbers of CBC (Table 3): *Pp. boryanum* differed from *Pp. kawraiskyi* by 2 CBCs in helix III. *Pp. alternans* differed from *Pp. brevicorne* only by hemi-CBCs, 3 in helix I and 1 in helix III. *Pp. integrum* differed from *Pp. kawraiskyi* by 2 CBCs in helix III. *Pp. pearsonii* differed from *Pp. integrum* by 1 CBC in helix I and 1 CBC in helix III (not shown).

DISCUSSION

Our study confirmed the polyphyletic origin of members of the genus *Pediastrum* which was already shown by BUCHHEIM et al. (2005) and McMANUS & LEWIS (2005). Furthermore, we confirmed the generic conception of Hydrodictyaceae and studied new strains from all genera of the relationship of *Pediastrum* sensu lato (*Parapediastrum*, *Pseudopediastrum*, *Lacunastrum*, *Monactinus*, and *Stauridium*) as well as *Sorastrum*. Generally, the differentiation between the genera of Hydrodictyaceae is validated by both, morphological and molecular criteria. However, an interesting example was uncovered contrasting with this general

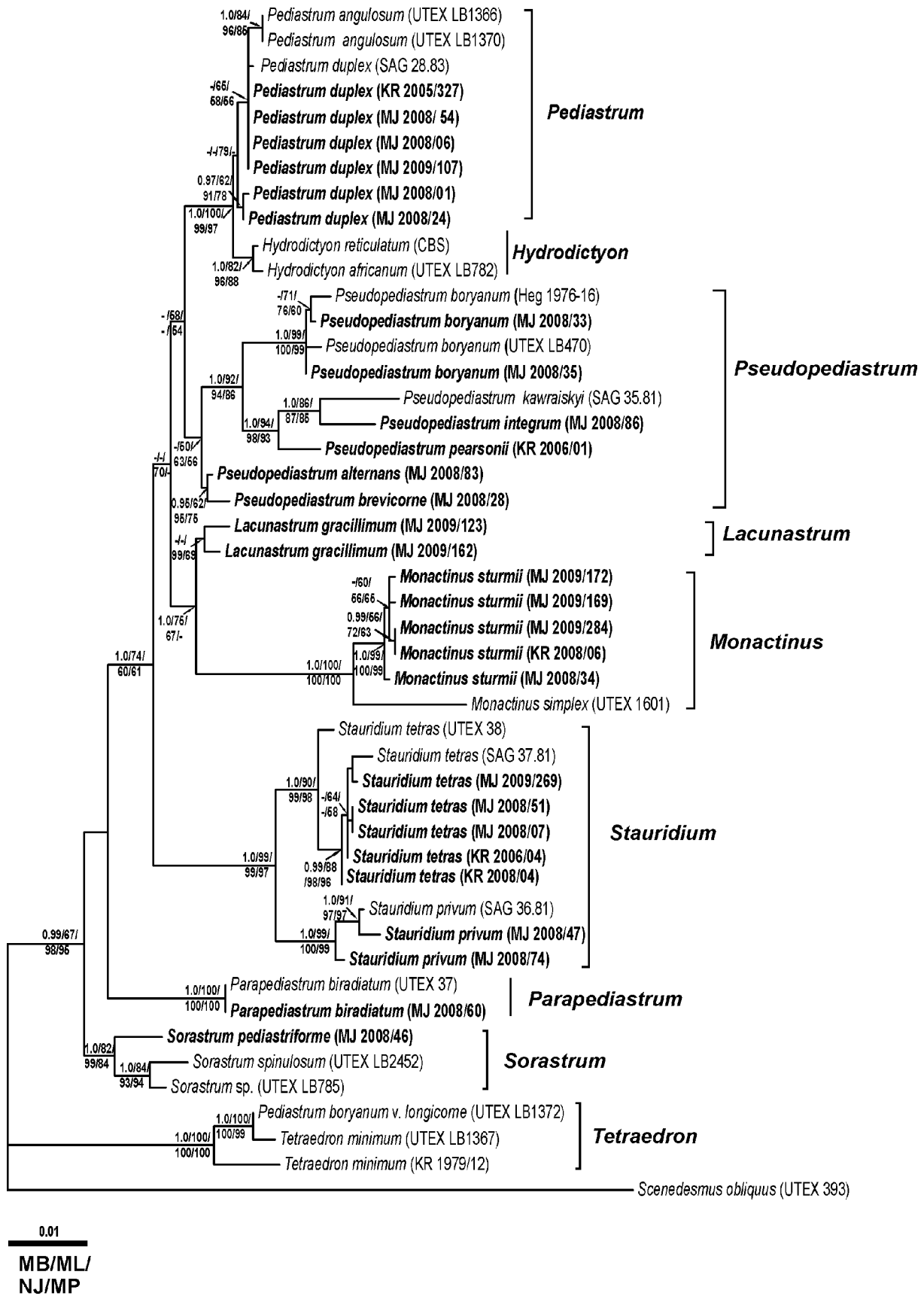


Fig. 1. Phylogeny according to the concatenated SSU and ITS-2 rRNA gene data set of the studied Hydrodictyceae. Values at the nodes indicate statistical support estimated by four methods – Bayesian support (MB BPP), maximum likelihood (ML), maximum parsimony (MP) and neighbour joining (NJ). Hyphens indicate support below 50% for ML, MP, NJ and below 0.95 for MB BPP.

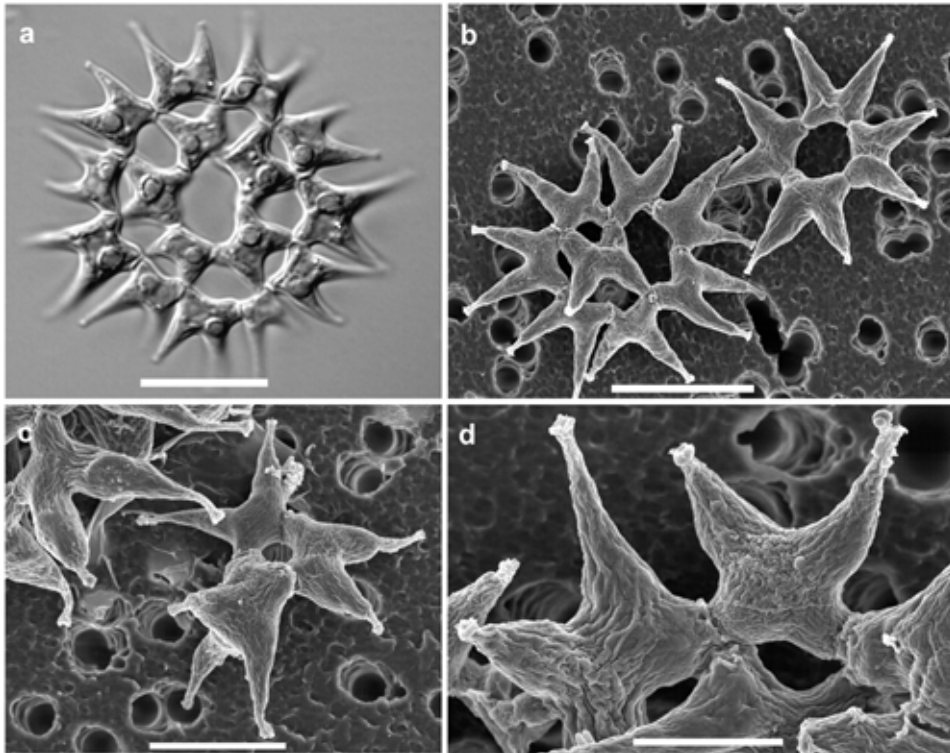


Fig. 2. Micrographs of *Sorastrum pedastriforme* (MJ 2008/46) in culture: (a) LM; (b–d) SEM. The arrow in (d) indicates a tetrahedric cell. Scale bar 2 μm (a); 14 μm (b); 6 μm (c), 10 μm (d).

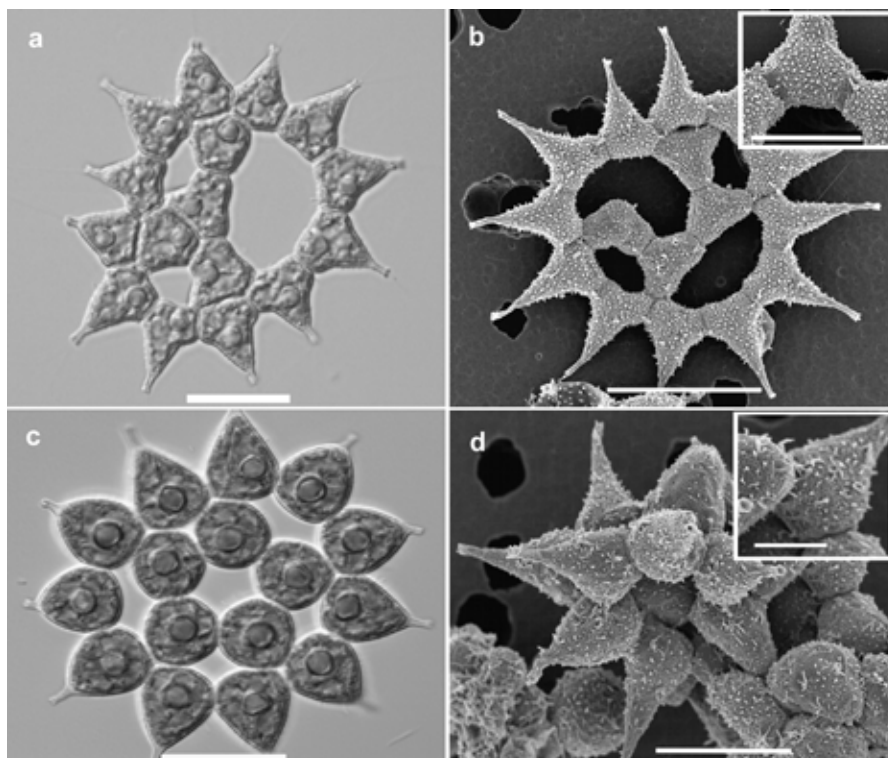


Fig. 3. Micrographs of *Monactinus* in culture: (a, b) *M. simplex* (MJ 2008/34); (c, d) *M. sturmii* (MJ 2009/172). Scale bar 10 μm (a); 20 μm (b); 15 μm (c, d).

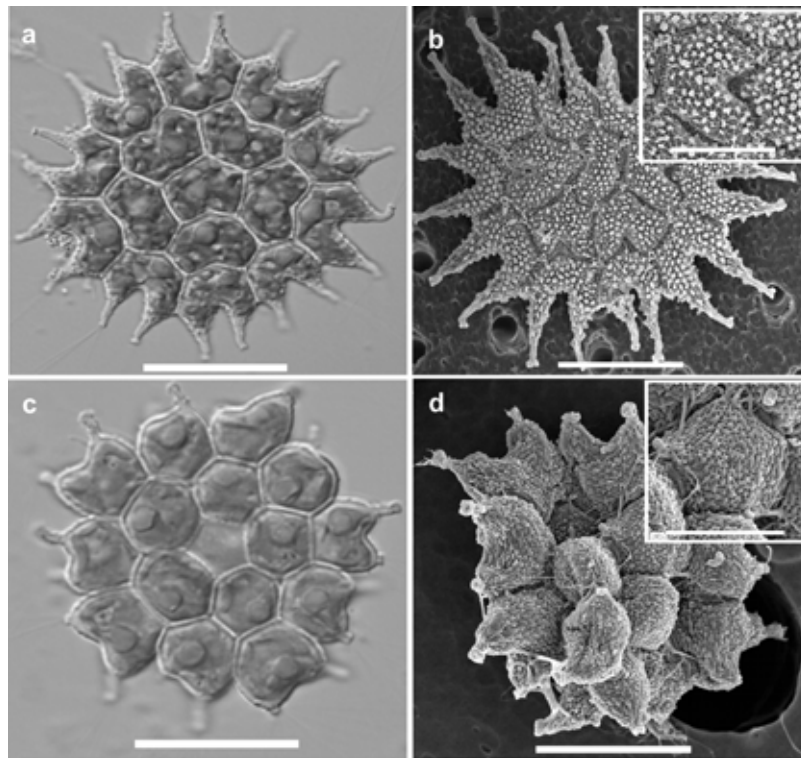


Fig. 4. Micrographs of different *Pseudopediastrum* strains: (a, b) *Pp. alternans* (MJ 2008/83); (c, d) *Pp. brevicorne* (MJ 2008/28). Scale bar 15 μm (a); 12 μm (b); 10 μm (c, d).

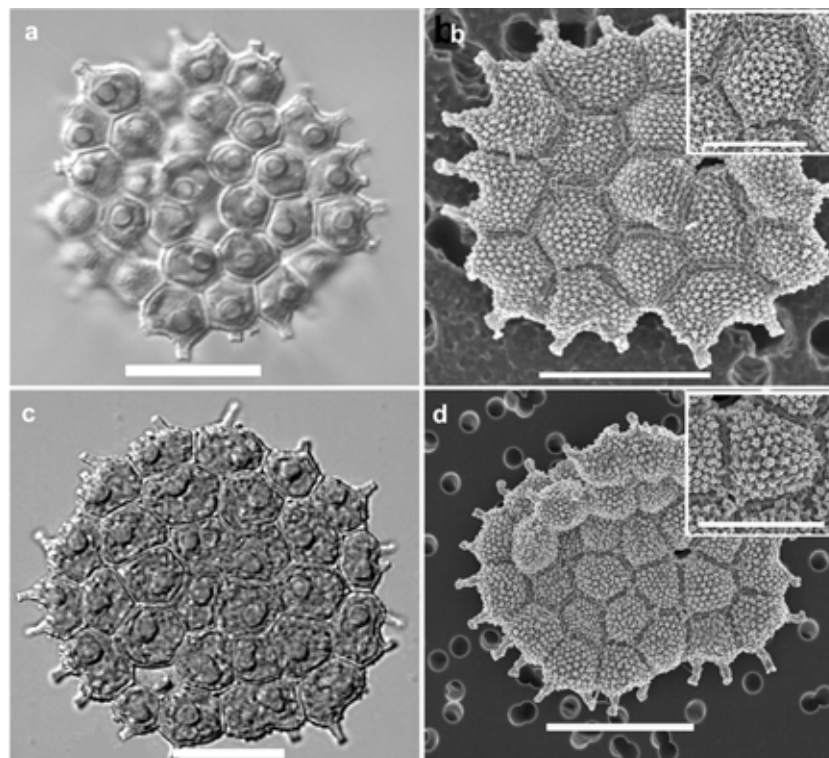


Fig. 5. Micrographs of different *Pseudopediastrum* strains: (a, b) *Pp. integrum* (MJ 2008/86); (c, d) *P. pearsonii* (KR 2006/01). Scale bar 12 μm (a); 20 μm (b); 10 μm (c); 12 μm (d).

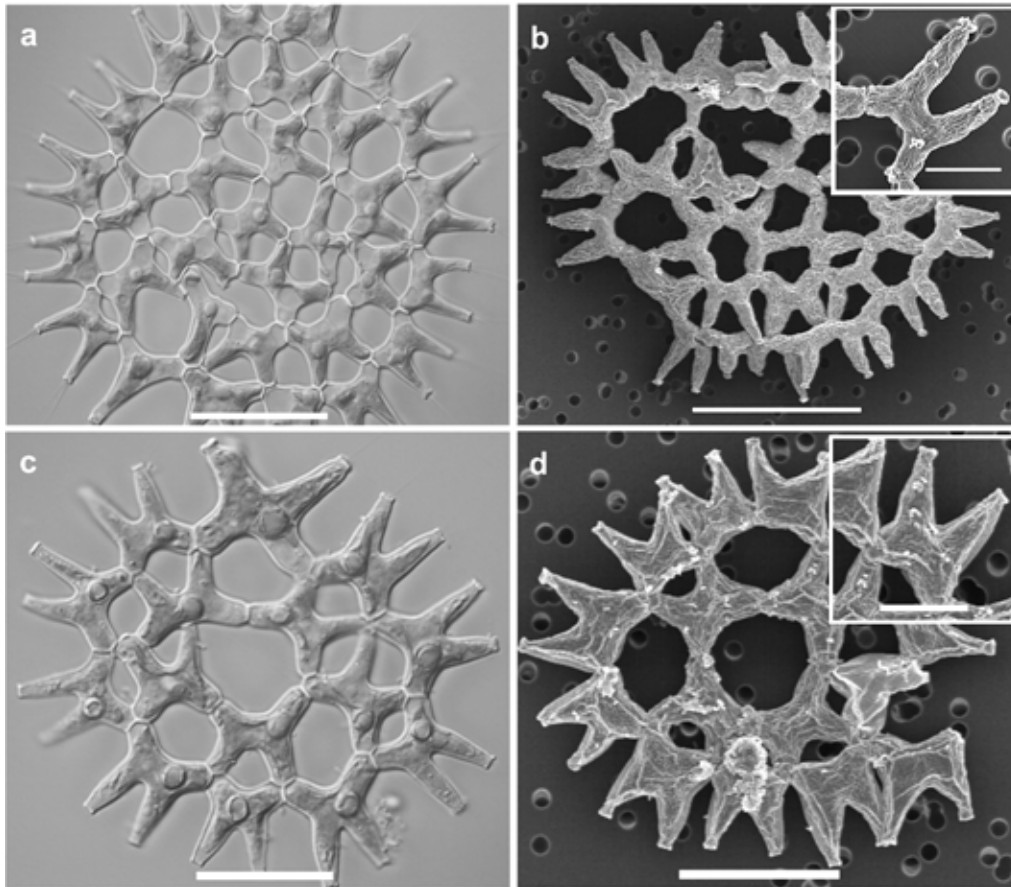


Fig. 6. Micrographs of *Lacunastrum gracillimum* strains: (a, b) MJ 2009/123; (c, d) MJ 2009/162. Scale bar 25 μm (a); 36 μm (b); 20 μm (c); 22 μm (d).

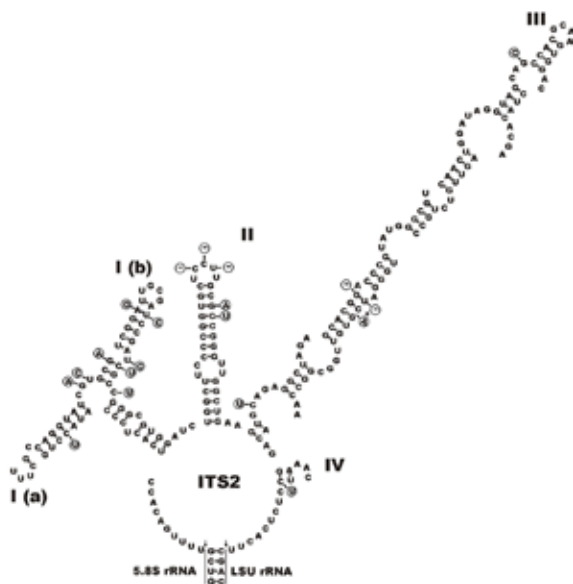


Fig. 7. Secondary structure of the ITS-2 of *Sorastrum pediatriforme* strain SAG 2392. *Sorastrum spinulosum* strain UTEX LB2452 as core structure, intraspecific base changes between strain SAG 2392 and UTEX LB2452 are marked in circles.

view. The new species *Sorastrum pediatriforme* which morphologically resembles *Pediastrum* but molecular phylogenetic analyses reveals its alliance with *Sorastrum*.

Studying two strains of *Lacunastrum gracillimum* from India, we confirmed the polyphyly of the *Pediastrum duplex* phenotype revealed by McMANUS et al. (2005) and the delineation of the genus *Lacunastrum* by McMANUS et al. (2011). It was shown that phylogenetic findings sharpen the eyes for subsequent careful morphometric evaluation of diagnostic features to discriminate the two very similar phenotypes of *P. duplex* and *L. gracillimum* (McMANUS et al. 2011).

In the present state we are still working to clarify the interplay between morphology and phylogeny in Hydrodictyaceae with reference to the other two main clades of planktonic Chlorophyceae, the Scenedesmeaceae and Selenastraceae. The interpretation of the state widely depends on exemplarily treated groups. In Scenedesmeaceae an extraordinary level of morphological variability must be reconciled to a scarcity of molecular peculiarities such as compensatory base changes (VANORMELINGEN et al. 2007). On the other side, the relatively uniform spindle-shaped phenotype of *Acutodesmus* represents

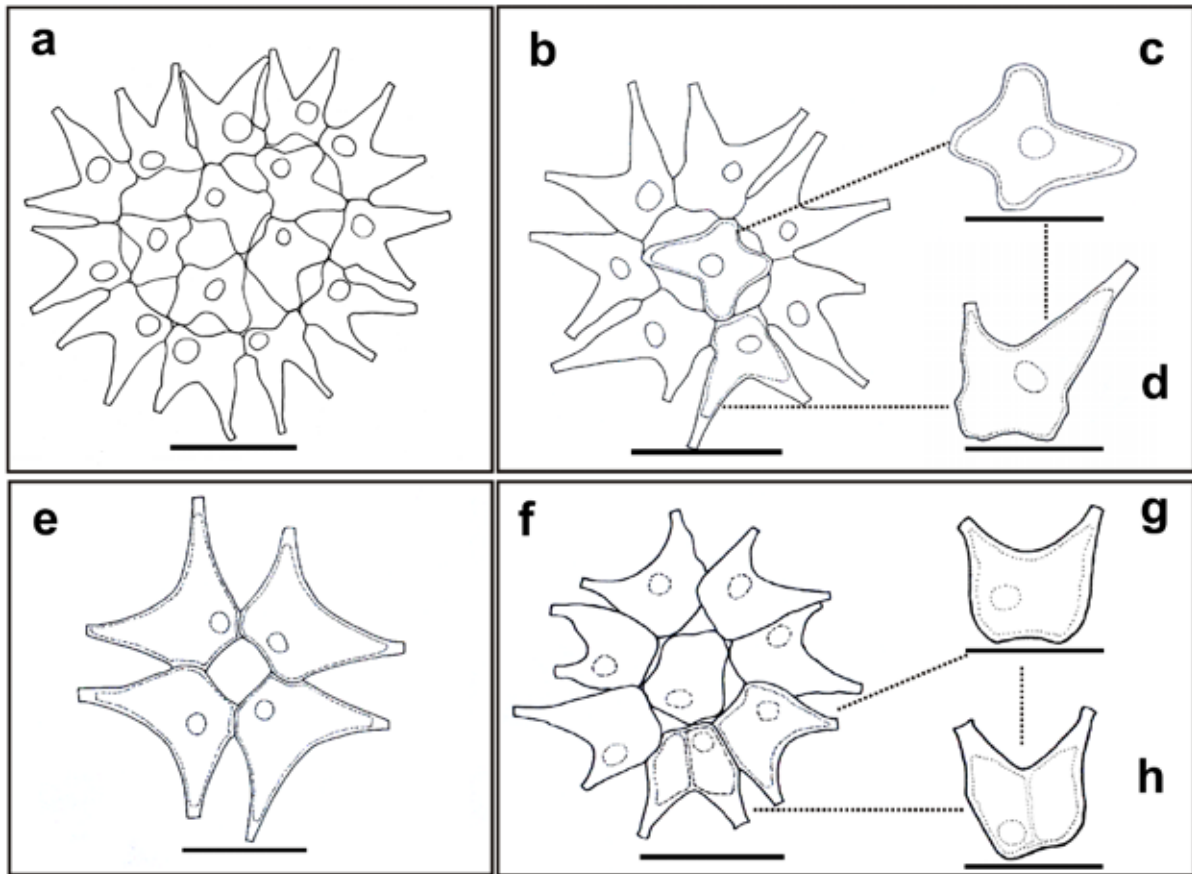


Fig. 8. Drawings of *Sorastrum pediastriforme* (MJ 2008/46). Scale bar 10µm (a, b, e, f); 6 µm (c, d, g, h). (a) iconotype.

different phylogenetic clades (HEGEWALD & WOLF 2003; LEWIS & FLECHTNER 2004; HEGEWALD et al. 2013). In Selenastraceae similar phenotypes such as the needle-shaped or crescent-shaped morphology hide a multitude of phylotypes (FAWLEY et al. 2007; KRIENITZ et al. 2011). In the Hydrodictyceae members of the genus *Pseudopediastrum* are characterized both by high morphological diversity and phylogenetic divergence resulting in four new combinations in this study. Regardless of the molecular background that has revealed overwhelming phenotypic diversity of the Hydrodictyceae, it seems unlikely that more genus-level diversity will be uncovered. It is suspected that the complicated *Pediastrum*-like phenotype of most members of this family has evolved infrequently, at best, and independently than easier constructed phenotypes such as spheres which undergo convergent evolution (POTTER et al. 1997; KRIENITZ & BOCK 2012).

Our survey of *Pediastrum* and related genera from three continents did not provide arguments for endemism of this group, because most of the taxa were found in different geographic regions. Even species formerly known only from tropical or subtropical regions like *Monactinus* (*Pediastrum*) *simplex* extended their distribution area into the temperate zone (COESEL & KRIENITZ 2008). The species *P. pearsonii* known from tropics (KOMÁREK & JANKOVSKÁ 2001) we

found only in Kenya. Probably, this is the exception that proves the rule of cosmopolitan distribution.

Taxonomic conclusions

Sorastrum pediastriforme JENA et C. BOCK sp. nov. (Figs 2a–d, 9a–h)

Descriptio: *Coenobia* plus minusve *planae* multicellularis, 4–8–16 cellulae continens. Cellulae externae per duos lobis formae, rare trilobates. Lobis complanes, elongates cum apicibus coronatis. Cellulae interiores quadricornutae per foramen inter cellulae. Chloroplastus unicus, parietale, massivus, cum pyrenoido. Propagatio zoosporis.

A speciebus ceteris generis ordine nucleotidorum in 18S and ITS rRNA differt.

Coenobia more or less flat, multicellular, containing 4–8–16 cells. Outer cells two-lobed, seldom trilobated. Lobae elongated with crown-like apices. Inner cells four-lobed with holes between the cells. One parietal, massive chloroplast per cell with a pyrenoid. Propagation by zoospores. The species differs from other species by the order of the nucleotides in SSU and ITS rRNA gene sequences.

Holotype: an air-dried sample of the authentic strain SAG 2392 (MJ 2008/46) deposited at the Botanical Museum at Berlin-Dahlem, Germany under the

designation B40 0040819.

Iconotype: our Fig. 9a

Authentic strain: SAG 2392 deposited at the Culture Collection of Algae at the University of Göttingen (SAG, Germany).

Type locality: Kenya, dam near Nyahururu

Etymology: The species epithet was selected because of the morphological similarity of the colonies to species of the genus *Pediastrum*.

***Monactinus sturmii* (Reinsch) JENA et C. BOCK comb. nov.**

Basionym: *Pediastrum sturmii* Reinsch 1867. Die Algenflora des mittleren Theiles von Franken, p. 90, pl. VII, fig. 1a–d.

Synonym: *Pediastrum simplex* var. *sturmii* (Reinsch) Wolle 1887.

Epitype (designated here): An air-dried as well as a formaldehyde-fixed sample of the strain MJ 2009/172, deposited at the Botanical Museum at Berlin–Dahlem under the designation B40 0040814.

Authentic strain: SAG NA2013.007 (MJ 2009/172) deposited at the Culture Collection of Algae at the University of Göttingen (SAG, Germany).

***Pseudopediastrum alternans* (Nygaard) JENA et C. BOCK comb. nov.**

Basionym: *Pediastrum alternans* Nygaard 1949. Kongl. Dansk Vid. Selskab., Biol. Skr. København, p. 42, fig. 16.

Epitype (designated here): An air-dried sample of the strain MJ 2008/83 deposited at the Botanical Museum at Berlin–Dahlem under the designation B40 0040815.

Authentic strain: SAG NA2013.008 (MJ 2008/83) deposited at the Culture Collection of Algae at the University of Göttingen (SAG, Germany).

***Pseudopediastrum brevicorne* (A. BRAUN) JENA et C. BOCK comb. et stat. nov.**

Basionym: *Pediastrum boryanum* var. *brevicorne* A. Braun 1855. Alg. unicell., p. 86, fig. IIB.

Epitype (designated here): An air-dried sample of the strain MJ 2008/28 deposited at the Botanical Museum at Berlin–Dahlem under the designation B40 0040816.

Authentic strain: SAG NA2013.009 (MJ 2008/28) deposited at the Culture Collection of Algae at the University of Göttingen (SAG, Germany).

***Pseudopediastrum integrum* (NÄGELI) JENA et C. BOCK comb. nov.**

Basionym: *Pediastrum integrum* Nägeli 1849. Gattungen einzelliger Algen, p. 97, pl. V: B, fig. 4.

Epitype (designated here): An air-dried sample of the strain MJ 2008/86 deposited at the Botanical Museum at Berlin–Dahlem under the designation B40 0040817.

Authentic strain: SAG NA2013.010 (MJ 2008/86) deposited at the Culture Collection of Algae at the

University of Göttingen (SAG, Germany).

***Pseudopediastrum pearsonii* (G.S. WEST) JENA et C. BOCK comb. nov.**

Basionym: *Pediastrum pearsonii* G.S. West 1912. Ann. S. Afr. Mus. 9, p. 79, figs. 30–32.

Synonym: *Pediastrum integrum* var. *pearsonii* (G.S. West) Fritsch in Fritsch et Stephens 1921.

Epitype (designated here): An air-dried sample of the strain KR 2006/01 deposited at the Botanical Museum at Berlin–Dahlem under the designation B40 0040818.

Authentic strain: SAG NA2013.011 (KR 2006/01) deposited at the Culture Collection of Algae at the University of Göttingen (SAG, Germany).

ACKNOWLEDGEMENTS

This work was supported in part by a fellowship grant of the German Academic Exchange Service for Mrutyunjay Jena. We thank Reingard Rossberg for joint work at the SEM and Monika Papke for technical assistance.

REFERENCES

- BOCK, C.; PRÖSCHOLD, T. & KRIENITZ, L. (2011): Updating the genus *Dictyosphaerium* and description of *Mucidosphaerium* gen. nov. (Trebouxiophyceae) based on morphological and molecular data. – J. Phycol. 47: 638–652.
- BRAUN, A. (1855): Algae unicellularium genera nova vel minus cognita I–III. – 111 pp., W. Engelmann, Leipzig.
- BUCHHEIM, M.; BUCHHEIM, J.; CARLSON, T.; BRABAND, A.; HEPERLE, D.; KRIENITZ, L.; WOLF, M. & HEGEWALD, E. (2005): Phylogeny of the Hydrodictyaceae (Chlorophyceae): Inferences from rDNA data. – J. Phycol. 41: 1039–1054.
- BUCHHEIM, M.A.; MICHALOPULOS, E.A. & BUCHHEIM, J.A. (2001): Phylogeny of the Chlorophyceae with special reference to the Sphaeropleales: A study of 18S and 26S rDNA data. – J. Phycol. 37: 819–835.
- BYUN, Y. & HAN, K. (2006): PseudoViewer: web application and web service for visualizing RNA pseudoknots and secondary structures. – Nucl. Acids Res. 34: W416–W422.
- COESEL, P.F.M. & KRIENITZ, L. (2008): Diversity and geographic distribution of desmids and other coccoid green algae. – Biodiversity & Conservation 17: 381–392.
- COLEMAN, A.W. (2003): ITS2 is a double-edged tool for eukaryote evolutionary comparisons. – Trends Genet. 19: 370–375.
- FAWLEY, M.W.; DEAN, M.L.; DIMMER, S.K. & FAWLEY, K.P. (2005): Evaluating the morphospecies concept in the Selenastraceae (Chlorophyceae, Chlorophyta). – J. Phycol. 42: 142–154.
- GEMEINHOLZER, B.; DROEGE, G.; ZETSCHKE, H.; KNEBELSBERGER, T.; RAUPACH, M.; BORSCH, T.; KLENK, H.–P.; HASZPRUNAR, G. & WAEGELE, J.W. (2009): DNA Bank Network Webportal.
- HEGEWALD, E. & WOLF, M. (2003): Phylogenetic relationships of *Scenedesmus* and *Acutodesmus* (Chlorophyta, Chlorophyceae) as inferred from 18S rDNA and ITS–2 sequence comparisons. – Plant Syst. Evol. 241: 185–191.
- HEGEWALD, E.; BOCK, C. & KRIENITZ, L. (2013): *Pectinodesmus* and the new genera *Verrucodesmus* and *Chodatodesmus* (Chlorophyta, Chlorophyceae). – Fottea 13: 149–164.
- HEPERLE, D. SequentiX Alignment Editor. (2004): Distributed by Author.
- HUELSENBECK, J.P. (2002): Testing a covariotide model of DNA substitution. – Mol. Biol. Evol. 19: 698–707.
- HUELSENBECK, J. P. & RONQUIST, F. (2001): MRBAYES: Bayesian inference of phylogenetic trees. – Bioinformatics 17: 754–755.
- KOMÁREK, J. & FOTT, B. (1983): Chlorophyceae (Grünalgen)

Table 1. List of strains used in this study.

Strain ^{a)}	Species	Origin	Accession number ^{b)}	Reference
UTEX LB782	<i>Hydrodictyon africanum</i>	South Africa, Cape Flats	AY663031	Buchheim et al. 2005
CBS	<i>Hydrodictyon reticulatum</i>	USA, Indiana	AY779861 AY779858 AY779862	Buchheim et al. 2005
MJ 2009/123	<i>Lacunastrum gracillimum</i>	India, Orissa, pond at Athagarh	HM021304	this study
MJ 2009/162	<i>Lacunastrum gracillimum</i>	India, Orissa, pond at Chadneswar	HM021305	this study
UTEX 1601	<i>Monactinus simplex</i>	unknown	AY780664 AY780655	Buchheim et al. 2005
KR 2008/06	<i>Monactinus sturmii</i>	India, Kerala, pond at Kochi	HM021323	this study
MJ 2008/34	<i>Monactinus sturmii</i>	Germany, Lake Dollgow	HM021323	this study
MJ 2009/169	<i>Monactinus sturmii</i>	India, Orissa, pond at Chadneswar	HM021322	this study
MJ 2009/172 <u>SAGNA2013.007</u>	<i>Monactinus sturmii</i>	India, Orissa, River Mohanadi	HM021321	this study
MJ 2009/284	<i>Monactinus sturmii</i>	Kenya, Lake Baringo	HM021325	this study
UTEX 37	<i>Parapediastrium biradiatum</i>	Sweden	AY663034 AY779865	Buchheim et al. 2005
MJ 2008/60	<i>Parapediastrium biradiatum</i>	Germany, Lake Schwanter See	HM021320	this study
UTEX LB1366	<i>Pediastrum angulosum</i>	Czech Republic, Podbansko pool, High Tatra Mountains	AY663032 AY779863	Buchheim et al. 2005
UTEX LB1370	<i>Pediastrum angulosum</i>	Czechoslovakia, Doksy, Machovo jezero pool	AY663033 AY779864	Buchheim et al. 2005
LN020INC	<i>Pediastrum duplex</i>	USA, North Carolina, Lake Norman	AY577744	McManus & Lewis 2005
SAG 28.83	<i>Pediastrum duplex</i>	Germany, Pfälzer Wald, forest pond	AY780662 AY780653	Buchheim et al. 2005
SAG 84.80	<i>Pediastrum duplex</i>	Germany, Schöhsee, Plön	AY577748	McManus & Lewis 2005
SF0202NY	<i>Pediastrum duplex</i>	USA, New York, Stockpot Flats	AY577757	McManus & Lewis 2005
KR 2005/327	<i>Pediastrum duplex</i>	Israel, Lake Kinneret	HM021298	this study

Table 1 Cont.

MJ 2008/01	<i>Pediastrum duplex</i>	Germany, Lake Dagow	HM021300	this study
MJ 2008/06	<i>Pediastrum duplex</i>	Kenya, dam near Nyahururu	HM021301	this study
MJ 2008/24	<i>Pediastrum duplex</i>	Germany, Lake Geron	HM021303	this study
MJ 2008/54	<i>Pediastrum duplex</i>	Kenya, dam near Nyahururu	HM021299	this study
MJ 2009/107	<i>Pediastrum duplex</i>	India, Orissa, pond at Bhubaneswar	HM021302	this study
RL0201FR	<i>Pediastrum</i> sp.	France, Reilhac Lake	AY577749	McManus & Lewis 2005
<u>MJ 2008/83</u> <u>SAG NA2013.008</u>	<i>Pseudopediastrum alternans</i>	Namibia, River Cuando near Kongola	HM021311	this study
OL0301MN	<i>Pseudopediastrum boryanum</i>	USA, Minnesota, Otter Lake	AY577759	McManus & Lewis 2005
EL0203CT	<i>Pseudopediastrum boryanum</i>	USA, Storrs, Eagleville Lake Connecticut	AY577742	McManus & Lewis 2005
UTEX LB1372	<i>Pseudopediastrum boryanum</i> v. <i>longicorne</i>	Czech Republic, pond Brehyne, Doksy	AY663036 AY779867	Buchheim et al. 2005
<u>MJ 2008/28</u> <u>SAG</u> <u>NA2013.009</u>	<i>Pseudopediastrum brevicorne</i>	Germany, Lake Dollgow	HM021310	this study
Heg 1976-16	<i>Pseudopediastrum boryanum</i>	unknown	AY780661 AY780652	Buchheim et al. 2005
UTEX LB470	<i>Pseudopediastrum boryanum</i>	UK, Malham Tam, Yorkshire	AY663035 AY779866	Buchheim et al. 2005
MJ 2008/33	<i>Pseudopediastrum boryanum</i>	Germany, Lake Dollgow	HM021306	this study
MJ 2008/35	<i>Pseudopediastrum boryanum</i>	Germany, Lake Dollgow	HM021307	this study
<u>MJ 2008/86</u> <u>SAG NA2013.010</u>	<i>Pseudopediastrum integrum</i>	Namibia, pond at Mariental	HM021309	this study
SAG 35.81	<i>Pseudopediastrum kawraiskyi</i>	Germany, Steinhuder Meer	AY780663 AY780654	Buchheim et al. 2005
<u>KR 2006/1</u> <u>SAG NA2013.011</u>	<i>Pseudopediastrum pearsonii</i>	Kenya, Lake Naivasha	HM021308	this study
SAG 13.94	<i>Sorastrum americanum</i>	USA, Minnesota, Lake Itasca State Park	AY780657	Buchheim et al. 2005
<u>MJ 2008/46</u> <u>SAG 2392</u>	<i>Sorastrum pediastriforme</i>	Kenya, dam near Nyahururu	HM021312	this study

Table 1 Cont.

UTEX LB2452	<i>Sorastrum spinulosum</i>	Finland, pond	AY663041 AY779872	Buchheim et al. 2005
UTEX LB785	<i>Sorastrum</i> sp.	USA, Bloomington, Indiana	AY663040 AY779871	Buchheim et al. 2005
SAG 36.81	<i>Stauridium primum</i>	Finland, Kuusjärvi, Saukkolahti near Jyväskylä	AY780666 AY780656	Buchheim et al. 2005
MJ 2008/47	<i>Stauridium primum</i>	Kenya, dam near Nyahururu	HM021319	this study
MJ 2008/74	<i>Stauridium primum</i>	Germany, Lake Stechlin	HM021318	this study
SAG 37.81	<i>Stauridium tetras</i>	unknown	AY779891 AY780656	Buchheim et al. 2005
UTEX 38	<i>Stauridium tetras</i>	Czechoslovakia	AY779892 AY779869	Buchheim et al. 2005
KR 2006/04	<i>Stauridium tetras</i>	Kenya, Lake Naivasha	HM021317	this study
KR 2008/04	<i>Stauridium tetras</i>	India, Kerala, pond at Kochi	HM021316	this study
MJ 2008/07	<i>Stauridium tetras</i>	Kenya, dam near Nyahururu	HM021315	this study
MJ 2008/51	<i>Stauridium tetras</i>	Kenya, dam near Nyahururu	HM021314	this study
MJ 2009/269	<i>Stauridium tetras</i>	Kenya, Thika, ricefield	HM021313	this study
UTEX 1367	<i>Tetraedron minimum</i>	Slovakia. Rezabinec pond, Razice	AY663042 AY779875	Buchheim et al. 2005
KR 1979/12	<i>Tetraedron minimum</i>	Germany, pond at Schortewitz	AY780670 AY780659	Buchheim et al. 2005

^{a)} Boldface + underlined: holo- or epitypes; ^{b)} Accession numbers in bold: sequenced in the frame of this study

Table 2. Number of CBCs and Hemi-CBCs (in brackets) in conserved regions of ITS-2 of *Monactinus*.

Taxa compared	Helix 1	Helix II	Helix IIIa	Helix IV
UTEX 1601 vs MJ 2008/34	0 (2)	0 (0)	2 (5)	0 (0)
UTEX 1601 vs KR 2008/06	0 (2)	0 (0)	2 (6)	0 (0)
UTEX 1601 vs MJ 2009/284	0 (2)	0 (0)	2 (6)	0 (0)
UTEX 1601 vs MJ 2009/169	0 (3)	0 (0)	2 (6)	0 (0)
UTEX 1601 vs MJ 2009/172	0 (2)	0 (0)	2 (6)	0 (0)

Table 3. Number of CBCs and Hemi-CBCs (in brackets) in conserved regions of ITS-2 of *Pseudopediastrum*.

Taxa compared	Helix 1	Helix II	Helix III	Helix IV
<i>P. boryanum</i> MJ 2008/33 vs <i>P. boryanum</i> Hg 1976-16	0 (0)	0 (0)	0 (0)	0 (0)
MJ 2008/33 vs <i>P. boryanum</i> var. <i>cornutum</i> (UTEX 470)	0 (2)	0 (0)	0 (0)	0 (0)
MJ 2008/33 vs <i>P. kawraiskyi</i> 35.81	0 (1)	0 (1)	2 (0)	0 (0)
MJ 2008/33 vs MJ 2008/35	0 (0)	0 (0)	0 (0)	0 (1)
MJ 2008/33 vs MJ 2008/86	0 (1)	0 (0)	0 (3)	0 (0)
MJ 2008/33 vs KR 2006/01	1 (1)	0 (1)	1 (2)	0 (0)
MJ 2008/33 vs MJ 2008/83	1 (2)	0 (1)	0 (3)	0 (1)
MJ 2008/33 vs MJ 2008/28	1 (0)	0 (1)	0 (4)	0 (1)
<i>P. alternans</i> MJ 2008/83 vs <i>P. boryanum</i> Hg 1976-16	1 (2)	0 (1)	0 (3)	0 (1)
MJ 2008/83 vs <i>P. boryanum</i> var. <i>cornutum</i> (UTEX 470)	0 (4)	0 (1)	0 (3)	0 (1)
MJ 2008/83 vs <i>P. kawraiskyi</i> 35.81	0 (1)	0 (2)	1 (2)	0 (0)
MJ 2008/83 vs MJ 2008/28	0 (3)	0 (0)	0 (1)	0 (0)
<i>P. brevicorne</i> MJ 2008/28 vs <i>P. boryanum</i> Hg 1976-16	1 (0)	0 (1)	0 (4)	0 (1)
MJ 2008/28 vs <i>P. boryanum</i> var. <i>cornutum</i> UTEX 470	1 (2)	0 (1)	0 (4)	0 (1)
MJ 2008/28 vs <i>P. kawraiskyi</i> 35.81	0 (4)	0 (2)	2 (1)	0 (0)
<i>P. integrum</i> MJ 2008/86 vs <i>P. boryanum</i> Hg 1976-16	1 (1)	0 (0)	0 (3)	0 (0)
MJ 2008/86 vs <i>P. boryanum</i> var. <i>cornutum</i> UTEX 470	0 (3)	0 (0)	0 (3)	0 (0)
MJ 2008/86 vs <i>P. kawraiskyi</i> 35.81	0 (0)	0 (1)	2 (1)	0 (0)
MJ 2008/86 vs KR 2006/01	1 (0)	0 (1)	1 (1)	0 (0)
MJ 2008/86 vs MJ 2008/83	1 (1)	0 (1)	0 (2)	0 (0)
MJ 2008/86 vs MJ 2008/28	0 (4)	0 (1)	0 (4)	0 (0)

Table 3 Cont.

<i>P. pearsonii</i> KR 2006/01 vs <i>P. boryanum</i> Hg 1976-16	1(1)	0(1)	1(2)	0(0)
KR 2006/01 vs <i>P. boryanum</i> var. <i>cornutum</i> UTEX 470	1(3)	0(1)	1(2)	0(0)
KR 2006/01 vs <i>P. kawraiskyi</i> 35.81	1(0)	0(2)	2(0)	0(0)
KR 2006/01 vs MJ 2008/83	1(1)	0(1)	1(1)	0(0)
KR 2006/01 vs MJ 2008/28	1(2)	0(0)	1(1)	0(0)

- Ordnung: Chlorococcales. – In: HUBER–PESTALOZZI, G. (ed.): Das Phytoplankton des Süßwassers 7. Teil, 1. Hälfte. – 1044 pp., E. Schweizerbart'sche Verlagsbuchhandlung (Nägele u. Obermiller), Stuttgart.
- KOMÁREK, J. & JANKOVSKÁ, V. (2001): Review of the green algal genus *Pediastrum*; implication for pollenanalytical research. – *Bibl. Phycol.* 108: 1–127.
- KRIENITZ, L. & BOCK, C. (2012): Present state of the systematics of planktonic coccoid green algae of inland waters. – *Hydrobiologia* 698: 295–326.
- KRIENITZ, L.; BOCK, C.; NOZAKI, H. & WOLF, M. (2011): SSU rRNA gene phylogeny of morphospecies affiliated to the bioassay alga „*Selenastrum capricornutum*“ recovered the polyphyletic origin of crescent-shaped Chlorophyta. – *J. Phycol.* 47: 880–893.
- KRIENITZ, L.; BOCK, C.; KOTUT, K. & PRÖSCHOLD, T. (2012): Genotypic diversity of *Dictyosphaerium*–morphospecies (Chlorellaceae, Trebouxiophyceae) in African inland waters, including the description of four new genera. – *Fottea* 12: 231–253.
- LEWIS, L.A. (1997): Diversity and phylogenetic placement of *Bracteacoccus* Tereg (Chlorophyceae, Chlorophyta) based on ribosomal RNA gene sequence data. – *J. Phycol.* 33: 279–285.
- LEWIS, L.A. & FLECHTNER, V.R. (2004): Cryptic species of *Scenedesmus* (Chlorophyta) from desert soil communities of western North America. – *J. Phycol.* 40: 1127–1137.
- McFADDEN, G.I. & MELKONIAN, M. (1986): Use of Hepes buffer for microalgal culture media and fixation for electron microscopy. – *Phycologia* 25: 551–557.
- McMANUS, H.A. & LEWIS, L.A. (2005): Molecular phylogenetics, morphological variation and colony–form evolution in the family Hydrodictyaceae (Sphaeropleales, Chlorophyta). – *Phycologia* 44: 582–595.
- McMANUS, H.A. & LEWIS, L.A. (2011): Molecular phylogenetic relationships in the freshwater family Hydrodictyaceae (Sphaeropleales, Chlorophyceae), with an emphasis on *Pediastrum duplex*. – *J. Phycol.* 47: 152–163.
- McMANUS, H.A.; LEWIS, L.A. & SCHULTZ, E.T. (2011): Distinguishing multiple lineages of *Pediastrum duplex* with morphometrics and a proposal for *Lacunastrum* gen. nov. – *J. Phycol.* 47: 123–130.
- NÄGELI, C. (1849): *Gattungen einzelliger Algen*. – 139 pp., Friedrich Schulthess, Zürich, Switzerland.
- NYGAARD, G. (1949): Hydrobiological studies on some Danish ponds and lakes. – *Kongl. Dansk Vid. Selskab., Biol. Skr. København* 7:1–293.
- PARRA, C.O. (1979): Revision der Gattung *Pediastrum* Meyen (Chlorophyta). – *Bibl. Phycol.* 48: 1–83.
- POTTER, D.; LAJEUNESSE, T.C.; SAUNDERS, G.W. & ANDERSON, R.A. (1997): Convergent evolution masks extensive biodiversity among marine coccoid picoplankton. – *Biodiversity & Conservation* 6: 99–107.
- REINSCH, P.F. (1867): Die Algenflora des mittleren Theiles von Franken. – *Abh. Naturforsch. Ges. Nürnberg* 1866: 1–238.
- SCHULTZ, J. & WOLF, M. (2009): ITS2 sequence–structure analysis in phylogenetics: A how–to manual for molecular systematics. – *Mol. Phyl. Evol.* 52: 250–252.
- SEIBEL, P. N.; DANDEKAR, T.; MÜLLER, T. & WOLF, M. (2008): Synchronous visual analysis and editing of RNA sequence and secondary structure alignments using 4SALE. – *BMC Res. Notes* 91: 1–7.
- SEIBEL, P. N.; MÜLLER, T.; DANDEKAR, T.; SCHULTZ, J. & WOLF, M. (2006): 4SALE – A tool for synchronous RNA sequence and secondary structure alignment and editing. – *BMC Bioinf.* 7: 498.
- SWOFFORD, D.L. (2002): Phylogenetic analysis using parsimony (* and other methods), PAUP version 4.0b 10. 2003. Massachusetts, Sinauer Associates, Sunderland.
- VANORMELINGEN, P.; HEGEWALD, E.; BRABAND, A.; KITSCHKE, M.; FRIEDL, T.; SABBE, K. & VYVERMAN, W. (2007): The systematics of a small spineless *Desmodesmus* species, *D. costato–granulatus* (Sphaeropleales, Chlorophyceae), based on ITS2 rDNA sequence analyses and cell wall morphology. – *J. Phycol.* 43: 378–396.
- WEST, G.S. (1912): Percy Sladen memorial expedition in South–West Africa, 1908–1911. Report No. 14. Fresh–water algae. – *Ann. S. Afr. Mus.* 9: 61–90.
- WILCOX, L.W.; LEWIS, L.A.; FUERST, P.A. & FLOYD, G.L. (1992): Assessing the relationships of autosporic and zoosporic chlorococcalean green algae with 18S rDNA sequence data. – *J. Phycol.* 28: 381–406.
- ZUKER, M. (2003): Mfold web server for nucleic acid folding and hybridization prediction. – *Nucl. Acids Res.* 31: 3406–3415.

© Czech Phycological Society (2014)

Received September 13, 2013

Accepted December 18, 2013