Cryptogamie, Bryologie, 2007, 28 (3): 187-195

The taxonomic status of *Platyhypnidium torrenticola* based on ITS sequence data

Olaf WERNER[a], Jairo PATIÑO[b], Juana M. GONZÁLEZ-MANCEBO[b]
& Rosa M. ROS[a]*

[a]Departamento de Biología Vegetal (Botánica), Universidad de Murcia,
Campus de Espinardo, 30100-Murcia, Spain;
E-mail: werner@um.es; rmros@um.es

[b]Departamento de Biología Vegetal (Botánica), Universidad de La Laguna,
C/ Astrofísico Francisco Sánchez s/n,
38071-La Laguna, Tenerife, Islas Canarias, Spain;
E-mail: jpatino@ull.es; glezm@ull.es

(Received 31 October 2006, Accepted 5 February 2007)

Abstract – The taxonomic identity of *Platyhypnidium torrenticola* has been studied using nrITS sequence data. A comparison with the most closely related species, *P. riparioides*, reveals that the nrITS sequences are identical with the majority of the European and African sequences of *P. riparioides*. It is concluded that the morphological characters used to segregate both species are within the variability of *P. riparioides* and that both names correspond to the same species.

Bryophytes / ITS sequence data / Canary Islands / *Platyhypnidium torrenticola* / *Platyhypniudium riparioides* / taxonomy

INTRODUCTION

The moss *Gradsteinia torrenticola* Ochyra, C. Schmidt & Bültmann (Donrichardiaceae), was described from a single locality in Tenerife, the Canary Islands (Ochyra *et al.*, 1998). It is very similar to *Platyhypnidium riparioides* (Hedw.) Dix., with which it grows intermixed, but can be differentiated by the basically bistratose leaf lamina with numerous apparently randomly distributed unistratose patches. In addition, the lamina frequently has longitudinal multistratose ridges and crests arising either as branches or spurs of the costa, or as entirely independent thickenings. The latter are often situated at the margins and form complete or incomplete, prominent and swollen limbidia which may be entire or secondarily spurred. The costa is relatively weak and basically single, with many secondary branches and spurs. It extends up to 5/6 of the leaf length or sometimes vanishes just below the apex (Ochyra *et al.*, 1998). *Platyhypnidium riparioides* has the lamina uniformly unistratose, and the costa is strong and single.

---

[*] Correspondence and reprints: rmros@um.es

One year later, when describing the new species *Platyhypnidium grolleanum* Ochyra & Bednarek-Ochyra from Central Europe (Ochyra & Bednarek-Ochyra, 1999), these authors realized that *Gradsteinia torrenticola* fitted the concept of the genus *Platyhypnidium* M. Fleisch. and shared with other *Platyhypnidium* species the broadly ovate, acute leaves which are sharply and distantly serrulate throughout. Consequently, they transferred the species to this genus as *Platyhypnidium torrenticola* (Ochyra, C. Schmidt & Bültmann) Ochyra & Bednarek-Ochyra and gave the following character combination for this species: the costa that rarely ends more than 3/4 way up the leaf, the leaves imbricate to spreading, the mid-leaf cells 6-11µm wide, and the laminal cells irregularly 1-2 bistratose with frequent 3-4 stratose ridges.

Within the genus *Platyhypnidium*, there is another species that also presents an irregularly pluristratose leaf lamina, *P. mutatum* Ochyra & Vanderp., also described from a single locality in southern Germany (Ochyra & Vanderpoorten, 1999). This species also presents a strong percurrent costa. Although *P. mutatum* and *P. torrenticola* have a similar leaf structure, they can be differentiated morphologically by the leaf form that is lancelolate to oblong-lanceolate in *P. mutatum* and ovate to broadly ovate in *P. torrenticola* (Ochyra & Vanderpoorten, 1999). Chloroplastic *trnL-trnF* and nrITS sequences of *P. mutatum* and *P. riparioides* are identical, except one substitution in the nuclear ribosomal DNA (Stech & Frahm, 1999).

The taxonomic value of the pluristratose lamina in aquatic mosses has been widely discussed, especially in *Platyhypnidium* (Ochyra & Vanderpoorten, 1999), *Gradsteinia* (Stech & Frahm, 2000), and *Palustriella* (Stech & Frahm, 2001; Frahm, 2005).

This paper includes the partial results of a project that deals with the analysis of the main genetic and biogeographical relationships of some Macaronesian endemics, as well as the taxonomical status of the Canary Islands endemic species. It is aimed at achieving DNA sequence information on the moss *Platyhypnidium torrenticola*, considered to be an endemic of the Canary Islands, and comparing it with the broadly distributed *P. riparioides*, in an attempt to clarify its taxonomical status.

MATERIALS AND METHODS

Plant material – A total of seven new accessions were used. One of them represents the species *Platyhypnidium torrenticola* from the only known locality in southwestern Tenerife, and six correspond to *P. riparioides* (Table 1). An exhaustive field sampling of brooks, streams and waterfalls in the Canary Islands was carried out, especially in areas with similar environmental conditions to those of the type locality of *P. torrenticola*. Nevertheless, the rest of specimens collected turned out to be *P. riparioides*. In addition, 12 ITS1 and ITS2 sequences available at GenBank corresponding to samples of *P. riparioides* and eight sequences corresponding to other species of *Platyhypnidium* (including the available sequences of *P. mutatum*) have been included in the study. *Rhynchostegium murale* (Hedw.) Bruch & Schimp. and *R. serrulatum* (Hedw.) A. Jaeger were included due to their close relationship with *Platyhypnidium. Cirriphyllum crassinervium* (Taylor) Loeske & M. Fleisch. was selected as outgroup. The GenBank accession numbers are also included in Table 1.

Table 1. Collection identification and GenBank accession numbers for the taxa included in the molecular analysis. For the sequences obtained from GenBank, only the geograhic origin and the accession numbers are indicated. in cases where only one GenBank accession number is given, the sequences span over ITS1 and ITS2.

Species	*Geographic origin*	*Voucher specimen*	*GenBank accessions ITS1/ITS2*
Cirriphyllum crassinervium (Taylor) Loeske & M. Fleisch.	Unknown		AY848961
Platyhypnidium aquaticum (A. Jaeger) M. Fleisch.	Unknown		AY848963
Platyhypnidium aquaticum	Colombia		DQ200094 DQ200964
Platyhypnidium austrinum (Hook. f. & Wilson) M. Fleisch.	Australia		DQ200095 AY166449
Platyhypnidium hedbergii (P. de la Varde) Ochyra & Sharp	Kenya		DQ336915 DQ200965
Platyhypnidium mutatum Ochyra & Vanderp.	Germany		AF230982 AF230997
Platyhypnidium muelleri (A. Jaeger) M. Fleisch.	Malaysia		DQ200096 DQ200966
Platyhypnidium pringlei (Cardot) Broth.	Mexico		DQ336913
Platyhypnidium riparioides (Hedw.) Dix.	Canary Islands, La Gomera, Barranco de Guada	TFC Bryo 15190, MUB 21738; *J.M. González-Mancebo s.n.*	EF090309 EF090302
Platyhypnidium riparioides	Canary Islands, La Gomera, Barranco de Guada	TFC Bryo 15191, MUB 21737; *J.M. González-Mancebo s.n.*	EF090308 EF090301
Platyhypnidium riparioides	Canary Islands, La Gomera, El Cedro	TFC Bryo 15195, MUB 21710; *J.M. González-Mancebo s.n. & J. Patiño s.n.*	EF090306 EF090299
Platyhypnidium riparioides	Canary Islands, La Gomera, El Cedro	TFC Bryo 15196, MUB 21711; *J.M. González-Mancebo s.n. & J. Patiño s.n.*	EF090307 EF090300
Platyhypnidium riparioides	Canary Islands, Tenerife, Barranco del Riachuelo, Las Cañadas	TFC Bryo 15205, MUB 21715; *J.M. González-Mancebo s.n.*	EF090310 EF090303
Platyhypnidium riparioides	Madeira, entre San Vicente y Porto Moniz	TFC Bryo 15200, MUB 21720; *J.M. González-Mancebo s.n.*	EF090311 EF090304
Platyhypnidium riparioides	Azores		DQ200102 DQ200972
Platyhypnidium riparioides	China 1		DQ200099 DQ200967
Platyhypnidium riparioides	China 2		DQ200097 DQ200963
Platyhypnidium riparioides	Finland		DQ336916 DQ200969

Table 1. Collection identification and GenBank accession numbers for the taxa included in the molecular analysis. For the sequences obtained from GenBank, only the geograhic origin and the accession numbers are indicated. in cases where only one GenBank accession number is given, the sequences span over ITS1 and ITS2. *(suite)*

Species	*Geographic origin*	*Voucher specimen*	*GenBank accessions ITS1/ITS2*
Platyhypnidium riparioides	Georgia		DQ200100 DQ200970
Platyhypnidium riparioides	Germany		AF230981 AF230996
Platyhypnidium riparioides	Kenya		DQ336914
Platyhypnidium riparioides	Madeira		DQ200101 DQ200971
Platyhypnidium riparioides	Portugal		AY848962
Platyhypnidium riparioides	Russia, Altai		DQ333437
Platyhypnidium riparioides	Spain, Cantabria		DQ200098 DQ336917
Platyhypnidium riparioides	USA		DQ336918 DQ200968
Platyhypnidium subrusciforme (Müll. Hal.) M. Fleisch.	Mexico		DQ336919 DQ200974
Platyhypnidium torrenticola (Ochyra, C. Schmidt & Bültmann) Ochyra & Bednarek-Ochyra	Canary Islands, Tenerife, Barranco del Infierno	TFC Bryo 15156, MUB 21734; *J. Patiño s.n.*	EF090305 EF090298
Rhynchostegium murale (Hedw.) Bruch & Schimp.	Unknown		AF230983 AF230998
Rhynchostegium serrulatum (Hedw.) A. Jaeger	USA		AY737451

DNA Extraction – Total DNA was extracted using the NaOH method of Werner *et al.* (2002), in which 5 µl of a crude NaOH extract was diluted with 45 µl of 100 mM Tris – 1 mM EDTA (pH 8.3), stored frozen at – 18°C and used as template for subsequent PCR analysis.

PCR and sequencing – PCR reactions were performed in an Eppendorf Mastercycler using 4 µl of the DNA solution in a 50 µl final volume. The reaction mixture contained the primers 18F and 5.8R for amplification of the ITS1 region, and 5.8F and 25R for amplification of the ITS2 region, at a final concentration of 400 µM, in the presence of 200 µM of each dNTP, 2 mM $MgCl_2$, 2 units Taq polymerase (Oncor Appligene), 1 µl BLOTTO (10% skimmed milk powder and 0.2% NaN_3 in water) and the buffer provided by the enzyme supplier. BLOTTO attenuates PCR inhibition caused by plant compounds (De Boer *et al.*, 1995). The primer sequences are given in Stech and Frahm (1999). Amplification started with 3 min denaturation at 94°C, followed by 35 cycles of 15 s at 94°C, 30 s at 50°C, and 1 min at 72°C, concluding with a final extension step of 7 min at 72°C. Aliquots

(5 µl) of the amplification products were visualized on 6% polyacrylamide gel. Successful amplifications were cleaned with the QIAquick purification kit (Qiagen). The amplification primers were used in the sequencing reactions with the Big Dye sequencing kit and separated on an ABI-Prism 3700 using standard protocols.

Alignment and phylogenetic analysis – Sequences were aligned manually using BioEdit (Hall, 1999). The aligned matrix is available on request. MEGA 3.1 (Kumar *et al.*, 2004) was used to search for the most parsimonious trees. All characters were given equal weight and gaps were excluded from the analysis. The heuristic search used the following settings: close neighbour interchange (CNI) with search level 2; random addition trees = 100 replications. Bootstrap analyses (Felsenstein, 1985) were carried out with 1000 replicates and identical settings as mentioned above.

RESULTS

The obtained ITS1 sequences had a uniform length of 269 bp and the ITS2 sequences of 264 bp. All sequences were identical. Comparing our newly obtained sequences with those available at GenBank, we observed that they were identical with the majority of the European and African sequences. The only exceptions were two sequences deposited in GenBank by Wagner *et al.* (2005), which are separated from the other sequences of *P. riparioides* by three transitions and one transversion in the ITS1 region and one transition and two transversions in the ITS2 region and the sample from the Azores, which showed one transition in the ITS1 region. Furthermore, the ITS1 region of the German sample from Wagner *et al.* (2005) showed a deletion of one bp, a mutation that it shares with *P. mutatum*. As discussed in detail by Stech and Frahm (1999) and Wagner *et al.* (2005), the sequence of *P. mutatum* is almost identical with that of *P. riparioides* used in their analysis. The Georgian sample of *P. riparioides* differed in three transitions and one transversion in the ITS1 region and one transition in the ITS2 region from our new sequences. Contrasting with these results is the situation concerning the Asian and American sequences of *P. riparioides* deposited at GenBank. The MP analyses of these sequences suggest that they are closely related with other species of the genus and separated from the European-African clade. The *Rhynchostegium* Bruch & Schimp. species are nested within the genus *Platyhypnidium.* Figure 1 shows the strict consensus tree of six most parsimonious (MP) trees (RI = 0.901, CI = 0.844, length = 141 steps). The European and African samples of *P. riparioides* are clearly separated into two clades with good bootstrap support. One clade includes the samples used by Wagner *et al.* (2005) and *P. mutatum*, while the other includes specimens from Georgia, Kenya, Macaronesia, the Spanish mainland and Finland.

DISCUSSION

The identity of rapidly evolving DNA sequences like the ITS region are not sufficient to guarantee that two specimens belong to the same species. In rapidly evolving species complexes particularly, speciation may occur faster than

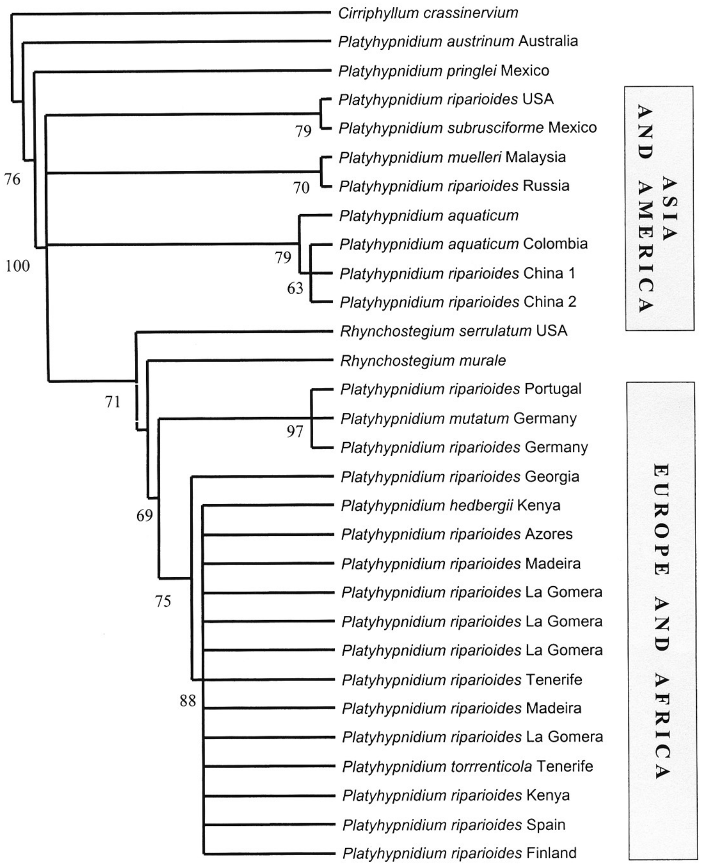

Fig. 1. Strict consensus tree of six most parsimonious trees (RI = 0.901, CI = 0.844) obtained, with bootstrap values given below the branches. *Cirriphyllum crassinervium* was chosen as the outgroup species.

alleles can become fixed within a species. A well known example in the animal kingdom are the cichlid fish of Lake Malawi where the extremely rapidly evolving mtDNA sequences could not be used to clarify the phylogenetic relationships of these species (Albertson *et al.*, 1999). However, in the case studied here, we observe a repeated pattern of variation consisting in the polystratification of the leaf lamina associated with strong waterflow. Some studies have noted that multistratose lamina, mainly in leaf margins are common characteristics in rheophilous bryophytes (Vitt & Glime, 1984). Besides, many aquatic mosses are known to show a high morphological variability depending on growth conditions (Frahm 2005; Hedenäs, 2003; Welch, 1960). On the other hand, there is convincing evidence that, in some cases, species with multistratose longitudinal streaks in the lamina or leaf margins are genetically distinct from similar taxa with simple leaf lamina or margins. This is the case, for example, in the genera *Vittia* Ochyra (Vanderpoorten *et al.*, 2003), *Donrichardsia* H. A. Crum & L. E. Anderson (Vanderpoorten *et al.*, 2002), and also in *Tortula schimperi* M. J. Cano, O. Werner & J. Guerra (Cano *et al.*, 2005).

Even striking morphological differences do not necessarily indicate separation at species level. Similar examples were already analysed shortly after the discovery of the Mendelien laws of heredity. In *Capsella bursa-pastoris* (L.) Medik. (Brassicaceae) L., Shull (1914) described a mutation that changes the typical heartlike fruit to the ovoid form of the variant Heegeri, while in *Lamium album* L. (Lamiaceae) the characteristic zygomorphic flower changes to a peloric morphology caused by the mutation of four genes (Sirks, 1925). However, mutations of this type are not sufficient to recognise these aberrant forms at species level.

In the case here concerned, *Platyhypnidium riparioides* and *P. torrenticola* grow together in the only known location where the latter taxon has been cited, and there are no indications to suggest that the two forms are genetically isolated. With the present state of our knowledge, we therefore conclude that *P. torrenticola* should be synonymized with *P. riparioides.*

It seems that there are still some problems related to the species delimitation within *Platyhypnidium riparioides.* First, the clear separation observed between the two European clades of this species might indicate that there are indeed two species in Europe. Stech and Frahm (1999) did not provide GenBank accession numbers, but gave the complete sequences in their publication. These are identical with those included in the paper by Wagner *et al.* (2005). Furthermore, Stech and Frahm (1999) mentioned that the sample of *P. riparoides* sequenced by them belongs to the var. *atlanticum* (*Rhynchostegium rusciforme* var. *atlanticum* (Brid.) Schimp.). But even if this clade would be given species rank, the rest of the European and African samples forms a monophyletic clade with 75% bootstrap support. On the other hand, the African *P. hedbergii* (P. de la Varde) Ochyra & Sharp has ITS sequences identical with those of our *P. riparioides* samples and therefore its recognition at species level could be questionable. A deeper investigation of these complications is beyond the scope of this paper, but a more detailed study might reveal whether or not the variety *atlanticum* should be given the species rank. Further complications arise because Asian and American ITS sequences of *P. riparioides* deposited at GenBank are even more distant from ours, and are more closely related to Asian and American species of the genus, while the two species of *Rhynchostegium* are nested within the *Platyhypnidium* clade. Since the type specimen is of European origin (Hedwig, 1801), a future re-evaluation of the situation will not affect the name of the typical European specimens. Clearly, a revision based on morphological and molecular data of the whole genus and related taxa is needed. That the genus *Platyhypnidium* is not monophyletic was observed earlier by Huttunen & Ignatov (2004) based on chloroplast and nuclear sequence data. In general terms, the situation is quite similar to that found in another genus of aquatic mosses: *Fontinalis*. In this case, Shaw & Allen (2000) concluded that at least some morphospecies are non-monophyletic and artificial, as defined by convergent leaf forms.

Taxonomy

Platyhypnidium riparioides (Hedw.) Dixon, *Rev. Bryol. Lichénol.* 6: 111. 1934.

= *Platyhypnidium torrenticola* (Ochyra, C. Schmidt & Bültmann) Ochyra & Bednarek-Ochyra, *Haussknechtia Beiheft* 9: 263. 1999. **Basionym:** *Gradsteinia torrenticola* Ochyra, C. Schmidt & Bültmann, *J. Bryol.* 20: 403. 1998. **Type:** [Spain] Canary Islands, S.W. Tenerife, Barranco del Infierno N.E. of Adeje, lat. *ca*

28º17'N, long. 16º42'W; on more or less submerged rocks in waterfall, elev. *ca* 400 m (UTM grid square 28RCS31); 8 April 1993, *H. Bültmann s.n.*, (Holotype: KRAM; Isotypes: B, MSUN, S) **syn. nov.**

Acknowledgements. This paper is part of the projects entitled "Biogeografía y procesos evolutivos en briófitos. Estudios en las Islas Macaronésicas" funded by the Spanish Ministerio de Educación y Ciencia (CGL2005-00028) and "Lista Roja de los Briófitos de las Islas Canarias" funded by the Canary Islands Government (P1042004-028). We are grateful to Ana Losada Lima, Julio Leal Pérez, and the guides of the Barranco del Infierno Special Natural Reserve for their help during the field work. Alain Vanderpoorten is also gratefully acknowledged for his constructive comments on an earlier version of the manuscript.

REFERENCES

ALBERTSON R.C., MARKERT J.A., DANLEY P.D. & KOCHER T.D., 1999 — Phylogeny of a rapidly evolving clade: The cichlid fishes of Lake Malawi, East Africa. *Proceedings of the National academy of sciences of the United States of America* 96: 5107-5110.

CANO M.J., WERNER O. & GUERRA J., 2005 — A morphometric and molecular study in *Tortula subulata* complex (Pottiaceae, Bryophyta). *Botanical journal of the Linnaean society* 149: 333-350.

DE BOER S.H., WARD L.J., LI X. & CHITTARANJAN S., 1995 — Attenuation of PCR inhibition in the presence of plant compounds by addition of BLOTTO. *Nucleic acids research* 23: 2567-2568.

FELSENSTEIN J., 1985 — Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39: 783-791.

FRAHM J.-P., 2005 — *Platyhypnidium mutatum* Ochyra & Vanderpoorten an seinem weltweit einzigem Vorkommen im Schwarzwald. *Bryologische Rundbriefe* 93: 1-2.

HALL T. A., 1999 — BioEdit: a user-friendy biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic acids symposia series* 41: 95-98.

HEDENÄS L., 2003 — *Amblystegiaceae (Musci)*. New York, The New York Botanical Garden Press. [Flora Neotropica. Monograph nº 89].

HEDWIG J., 1801 — *Species Muscorum Frondosorum.*

HUTTUNEN S. & IGNATOV M.S. 2004 — Phylogeny of the Brachytheciaceae (bryophyta) based on morphology and sequence level data. *Cladistics* 20: 151-183.

KUMAR S., TAMURA K. & NEI M., 2004 — MEGA3: Integrated software for Molecular evolutionary genetics and sequence alignment. *Briefings in bioinformatics* 5: 150-163.

OCHYRA R., SCHMIDT C. & BÜLTMANN H., 1998 — *Gradsteinia torrenticola*, a new aquatic moss species from Tenerife. *Journal of bryology* 20: 403-409.

OCHYRA R. & BEDNAREK-OCHYRA H., 1999 — *Platyhypnidium grolleanum* (Musci: Brachytheciaceae), a new species from the Sudetes (Central Europe). *Haussknechtia Beiheft* 9 (Riclef-Grolle Festschrift): 259-264.

OCHYRA R. & VANDERPOORTEN A., 1999 — *Platyhypnidium mutatum*, a mysterious new moss from Germany. *Journal of bryology* 21: 183-189.

SHAW A.J. & ALLEN B. 2000 — Phylogenetic relationships, morphological incongruence, and geographic speciation in the Fontinalaceae (Bryophyta). *Molecular phylogenetics and evolution* 16: 225-237.

Shull M.J., 1914 — Duplicate genes for Capsule form in *Capsella bursa-pastoris. Zeitschrift für induktive Abstammungslehre* 12: 97-149.

Sirks M.J., 1925 — The genotypic character of some aberrant forms of *Lamium. Genetica* 7: 253-272.

STECH M. & FRAHM J.-P., 1999 — The status of *Platyhypnidium mutatum* Ochyra & Vanderpoorten and the systematic value of the Donrichardsiaceae based on molecular data. *Journal of bryology* 21: 191-195.

STECH M. & FRAHM J.-P., 2000 — The systematic position of *Gradsteinia andicola* Ochyra (Donrichardsiaceae, Bryopsida): evidence from nrDNA internal transcribed spacer sequences. *Tropical bryology* 18: 75-85.

STECH M. & FRAHM J.-P., 2001 — *Palustriella pluristratosa* spec. nov. (Amblystegiaceae, Bryopsida), a new moss species with pluristratose lamina from Switzerland. *Botanica Helvetica* 111: 139-150.

VANDERPOORTEN A., HEDENAS L., COX C.J. & SHAW A.J., 2002 — Circumscription, classification, and taxonomy of Amblystegiaceae (Bryopsida) inferred from nuclear and chloroplast DNA sequence data and morphology *Taxon* 51: 115-122.

VANDERPOORTEN A., GOFFINET B., HEDENAS L., COX C.J. & SHAW A.J., 2003 — A taxonomic reassessment of the Vittiaceae (Hypnales, Bryopsida): evidence from phylogenetic analyses of combined chloroplast and nuclear sequence data. *Plant systematics and evolution* 241: 1-12.

VITT D.H. & GLIME J. 1984 — The structural adaptations of aquatic Musci. *Lindbergia* 10: 95-110.

WAGNER D., STECH M. & KÜRSCHNER H., 2005 — The taxonomic status of the neotropical *Platyhypnidium aquaticum* (A. Jaeger) M. Fleisch. (Brachytheciaceae, Bryopsida) based on ITS sequence data. *Nova Hedwigia* 80: 519-526.

WELCH W.H., 1960 — *A monograph of the Fontinalaceae*. The Hague, Netherlands Martinus Nijhoff.

WERNER O., ROS R.M. & GUERRA J., 2002 — Direct amplification and NaOH extraction: two rapid and simple methods for preparing bryophyte DNA for polymerase chain reaction (PCR). *Journal of bryology* 24: 127-131.