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A taxonomic reassessment of the Vittiaceae (Hypnales, Bryopsida): evidence from phylogenetic analyses of combined chloroplast and nuclear sequence data

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Abstract. The Vittiaceae are a small family of aquatic mosses that are defined based on gametophytic traits whose interpretation has led to conflicting taxonomic arrangements. Phylogenetic analyses of two cpDNA regions, *trn*L-*trn*F and *atp*B-*rbc*L, indicate that *Vittia* is nested within the Amblystegiaceae s. str., suggesting that the family Vittiaceae should not be recognized. *Platylomella lescurii* appears nested within the Thuidiaceae/Leskeaceae. This suggests that the series of character states shared by *Vittia* and *Platylomella*, including a differentiated leaf border, short laminal cells, stiff stems, and a thick costa, are convergent features that arose independently in unrelated lineages of aquatic Hypnales. Within the Amblystegiaceae, phylogenetic analyses of the two cpDNA regions combined with ITS sequence data show that *Hypnobartlettia*, *Vittia elimbata spec. nov.*, *V. pachyloma*, and *V. salina*, despite their strong morphological similarity to aquatic *Amblystegium* species, form a clade that is sister to the *Drepanocladus*/*Pseudocalliergon* complex. This combined clade is unresolved at a polytomy that includes *Amblystegium serpens* and a clade including all the other *Amblystegium* species. The occurrence of *A. serpens* outside the strongly supported clade including other *Amblystegium* species suggests that *A. serpens* may be better accommodated in a distinct genus. *Amblystegium serpens* is the type species of *Amblystegium* and thus retains the name. The other species are accommodated in their own genus, *Hygroamblystegium*, including *H. fluviatile*, *H. humile* comb. nov., *H. noterophyllum*, *H. tenax*, and *H. varium*.

Key words: *Vittia*, *Platylomella*, *Hygroamblystegium*, Amblystegiaceae, convergent evolution, aquatic mosses, ITS, *atp*B-*rbc*L spacer, *trn*F-*trn*L region.

Unrelated plant species growing in similar habitats may often exhibit a similar appearance, a process known as convergent evolution. In fast flowing waters, plant species often have a suite of convergent morphological characters, including extensive attachment to their substrates, increased tensile strength, increased flexibility to dissipate energy through elasticity, and/or a streamline shape (Suren et al. 2000). Mosses, which are common in running waters, are rarely flexible but increase strength with a suite of morphological characters such as stiff wiry stems, small thick-walled leaf cells, a strong costa, and often pluri-stratose leaf borders or laminae (Vitt and Glime 1984).

Due to an assumed greater exposure of the moss gametophyte to selective environmental pressures, much of the suprageneric classification of mosses has traditionally been based on sporophytic characters. However, the assumption that gametophytic characters are more labile than sporophytic ones has been challenged (e.g. Buck 1991; Buck et al. 2000; Hedenäs 1999, 2001, 2002). As a consequence, a number of gametophytic traits were given primacy at various taxonomic levels in recent moss classifications. For example, within the Hypnales, the most diverse of the two orders of pleurocarpous mosses, the thickness of leaf lamina has been interpreted as an important taxonomic character (Ochyra 1986a) to such an extent that new species [e.g. *Palustriella pluristratosa* Stech and Frahm (Stech and Frahm 2001b)], genera [e.g. *Donrichardsia* (Grout) H.A. Crum & L.E. Anderson (Crum and Anderson 1979), *Gradsteinia* Ochyra (Ochyra 1990, Ochyra et al. 1998), *Ochyraea* Vana (Vana 1986), *Richardsiopsis* Ochyra (Ochyra 1986b)] and, in some cases, families [e.g. the Donrichardsiaceae (Ochyra 1985) and the Vittiaceae (Ochyra 1987a)], have been erected to accommodate peculiar pleurocarpous aquatic mosses with variously multistratose leaf laminae. Recent analyses of morphological (Hedenäs 1995, 1997, 1998; Ochyra and Bednarek-Ochyra 1999; Ochyra and Vanderpoorten 1999) and molecular data (Stech and Frahm 1999, 2001a; Stech et al. 1999, Vanderpoorten et al. 2001, 2002a) suggest, conversely, that these taxa are often extreme expressions nested within larger genera, including *Platyhypnidium* Fleisch., *Drepanocladus* (Müll. Hal.) Roth, *Hygrohypnum* Lindb., and *Amblystegium* Schimp. Hence, the taxonomic position of many aquatic mosses has been an area of controversy. For example, the peculiar gametophytic features of one such aquatic moss, *Vittia pachyloma* (Mont.) Ochyra (Vittiaceae), including multistratose leaf laminae, short laminal cells, a strong, percurrent to excurrent costa, and a leaf margin composed of many layers of linear, thick-walled cells (Ochyra 1987a), led to a large number of alternative taxonomic placements. Montagne (1838) first interpreted the taxon, with hesitation, as a member of *Gymnostomum* Nees and Hornsch. (Pottiaceae), indicating its similarity, especially the possession of a thickened leaf border, with *Cinclidotus* P. Beauv. Subsequently, Hooker and Wilson (1844) transferred the taxon into *Sciaromium* (Mitt.) Mitt. within the Amblystegiaceae. This treatment was followed in subsequent classification systems (Vitt 1984) until Ochyra (1987a) erected the monospecific genus *Vittia* and family Vittiaceae to accommodate the taxon. This taxonomic position, however, remained debatable, and was discussed by Hedenäs (1995) who interpreted the taxon as a specialized *Amblystegium* with a few autapomorphies presumably connected with the adaptation to growth in running water. Most recently, Buck and Goffinet (2000) and Ochyra and Matteri (2001) retained *Vittia* as a distinct genus within the Amblystegiaceae. Other taxa with at least partly polystratose leaf laminae and a well-defined leaf border, such as *Platylomella* A. L. Andrews, were also included in the family (Buck and Goffinet 2000).

In this paper, we use variation in sequences from chloroplast and nuclear loci to clarify the phylogenetic relationships of *Vittia pachyloma* and the recently described species, *V. salina* Hedenäs and Munoz (Hedenäs and Munoz 2002). The analysis also included other aquatic Hypnales to determine whether their morphological similarities reflect common ancestry or convergent responses to growth in running waters.

Material and methods

Taxon sampling. Two sampling strategies were employed, the first to confirm the placement of *Vittia* within the Amblystegiaceae and to assess familial relationships of morphologically similar taxa. The second dataset was constructed to assess sister group relationships within the Amblystegiaceae. *Vittia* and related taxa included six accessions of *V. pachyloma* from different Chilean provinces; two accessions of *V. salina*; one accession of a puzzling aquatic moss from Bolivia previously interpreted as an extreme *Amblystegium* expression; and three accessions of *Platylomella lescurii* (Sull.) A. L. Andrews, a rheophilous moss endemic to the Appalachian mountains (Table 1). The inclusion of these taxa within the Amblystegiaceae was tested by sampling members of the Amblystegiaceae s. str., plus representative members of Hypnalean families whose potential relationships with the Amblystegiaceae have been suggested by morphological (Hedenäs 1995, 1998; Ochyra and Vanderpoorten 1999) and molecular data (Vanderpoorten et al. 2002b) (dataset 1, appendix 1). These Hypnalean families include the Anomodontaceae (*Anomodon* Hook. and Tayl.), Thuidiaceae (*Thuidium* Bruch and Schimp, *Haplocladium* Müll. Hal., *Abietinella* Müll. Hal., *Helodium* Warnst.), Hypnaceae (*Hypnum* Hedw., *Caribaeohypnum* Ando and Higuchi, *Ptilium* De Not.), Rhytidiaceae (*Rhytidium* (Sull.) Kindb), Brachytheciaceae (*Platyhypnidium*), and Leskeaceae (*Leskea* Hedw.). *Neckera pennata* Hedw. and *N. douglasii* Hook., two members of the suborder Neckerineae (Brotherus 1925, De Luna et al. 2000), were used as outgroups.

Generic relationships within the Amblystegiaceae were investigated using a taxon sampling including members of all the genera currently included within the family sensu stricto (dataset 2, appendix 1). The tree was rooted with *Palustriella* and *Cratoneuron*, which are sister to the other members of the family (Vanderpoorten et al. 2002b).

Phylogenetic analyses. Familial relationships within the Hypnales were investigated using the two cpDNA regions *trn*L-*trn*F and *atp*B-*rbc*L that were sequenced according to the protocols described in Vanderpoorten et al. (2002b), and which could be unambiguously aligned across the Hypnales (dataset 1). The cpDNA dataset was analyzed under maximum likelihood after selection of the substitution model best fitting the data. The model was selected on the basis of the likelihood of a neighbor-joining tree under 56 different nested models as implemented by Modeltest 3.04 (Posada and Crandall 1998). The model parameters were subsequently fixed in heuristic searches with 300 random addition replicates with TBR branch swapping. Support for clades was assessed using bootstrap analyses which were conducted with 100 replicates, using simple taxon addition, and saving no more than 20,000 trees per replicate.

Within the Amblystegiaceae, the sample of closely related species allowed the use of the internal transcribed spacers (ITS) of the 18S-26S nrDNA, in combination with the two cpDNA regions. The decision to combine the partitions was made after comparing both the topologies and the support for the branches as assessed by nonparametric bootstrapping. If a taxon was resolved as part of two distinct monophyletic clades supported each by bootstrap proportions of 70% or higher in bootstrap analyses of individual partitions, these partitions would be considered incongruent (Mason-Gamer and Kellog 1996). In such cases, taxa causing the incongruence were removed from the analysis. The ML analyses were conducted as described above.

Results

The substitution model maximizing the likelihood of the neighbor-joining tree derived from the first cpDNA dataset ($-\ln L = 5080.6074$) was a general time-reversible model (Rodriguez et al. 1990). The rate heterogeneity among sites was assumed to follow a gamma distribution with a shape parameter of 0.8564. The model was implemented with the following settings: rate matrix R (A-C) = 1.0958; R(A-G) = 2.4858; R(A-T) = 0.3164; R(C-G) = 2.2292; R(C-T) = 2.4858; R(G-T) = 0.3164; base frequencies A = 0.4074; C = 0.1012; G = 0.1252; T = 0.3662; proportion of invariable sites = 0.3762. Heuristic searches under ML resulted in a unique most likely tree ($-\ln L = 4573.2927$) (Fig. 1). Taxa with multistratose laminae, including *Platylomella, Vittia, Palustriella, Platyhypnidium,* and *Donrichardsia,* were scattered throughout the phylogeny. The genus *Vittia* was nested within the Amblystegiaceae s. str., supported with a bootstrap value of 90%. *Platylomella lescurii* was not included within the family and its relationships with the Leskeaceae/Thuidiaceae complex was supported at 77%. *Donrichardsia* formed a clade with *Platyhypnidium* and *Hygrohypnum montanum* that was supported at 84%.

Separate analysis of cpDNA and ITS sequence datasets within the Amblystegiaceae

Table 1. Voucher information and GenBank accession numbers of the taxa for which the ITS (nrDNA repeat), *atp*B-*rbc*L intergenic spacer, and *trn*L-*trn*F region have been obtained and combined with a previous dataset for a phylogenetic reassessment of the Vittiaceae. BG and AV represent the private herbaria of Bernard Goffinet and Alain Vanderpoorten, respectively

Taxon	Locality	Voucher	GenBank accession number (ITS/*atp*B-*rbc*L/*trn*L-*trn*F)	Habitat
Drepanocladus longifolius (Mitt.) Broth. ex Par.	Chile, Prov. of Cordillera	Goffinet 5603 (DUKE, BG, AV)	AY062888/AY062884/ AY062890	Mountain stream
Hypnobartlettia fontana Ochyra	New Zealand, South Island, Nelson District	Frahm X12-1 (BONN, AV, BG)	AY242368/AY242359/ AY242376	Limestone dwell, submerged in water
Vittia elimbata Hedenäs, Vanderpoorten & Goffinet	Bolivia, La Paz	Lewis 87816 (MO, DUKE, S, NY)	AF464999/AF464976/ AF465017	In running water at 3810 m
Vittia pachyloma (Mont.) Ochyra	Chile, Prov. of Cordillera	Goffinet 5605 (DUKE, BG)	AY062886/AY062883/ AY062889	Mountain stream
	Chile, Prov. of Bio-Bio	Goffinet 5582 (DUKE, BG)	AY242365/AY242356/ AY242384	Mountain stream
	Chile, Prov. La Araucania	Goffinet 5534 (DUKE, BG)	AY242366/AY242357/ AY242383	Mountain stream
	Chile, Magallanes	Goffinet 6679 (BG)	AY242367/AY242358/ AY242375	Mountain stream
	Chile, Prov. of Cordillera	Goffinet 5610 (DUKE, BG)	AY062887/AY062885/ AY062891	Mountain stream
Vittia salina Hedenäs & Munoz	Argentina, Catamarca	Biasuso et al. 99-60 (S)	AY242369/AY242360/ AY242377	Rocks and sandy banks of salt water rivulet at 3700 m
	Argentina, Catamarca	Biasuso et al. 99-56a (S)	AY242370/AY242361/ AY242378	Fast running salt water stream at 3000 m
Platylomella lescurii (Sull.) Andr.	USA, North Carolina	Vanderpoorten s.n. (AV, BG)	AY242371/AY242362/ AY242379	Fast-flowing mountain stream
	USA, South Carolina	Vanderpoorten s.n. (AV, BG)	AY242372/AY242363/ AY242380	Fast-flowing mountain stream
	USA, North Carolina	Vanderpoorten 4889 (AV, BG)	AY242373/AY242364/ AY242381	Fast-flowing mountain stream

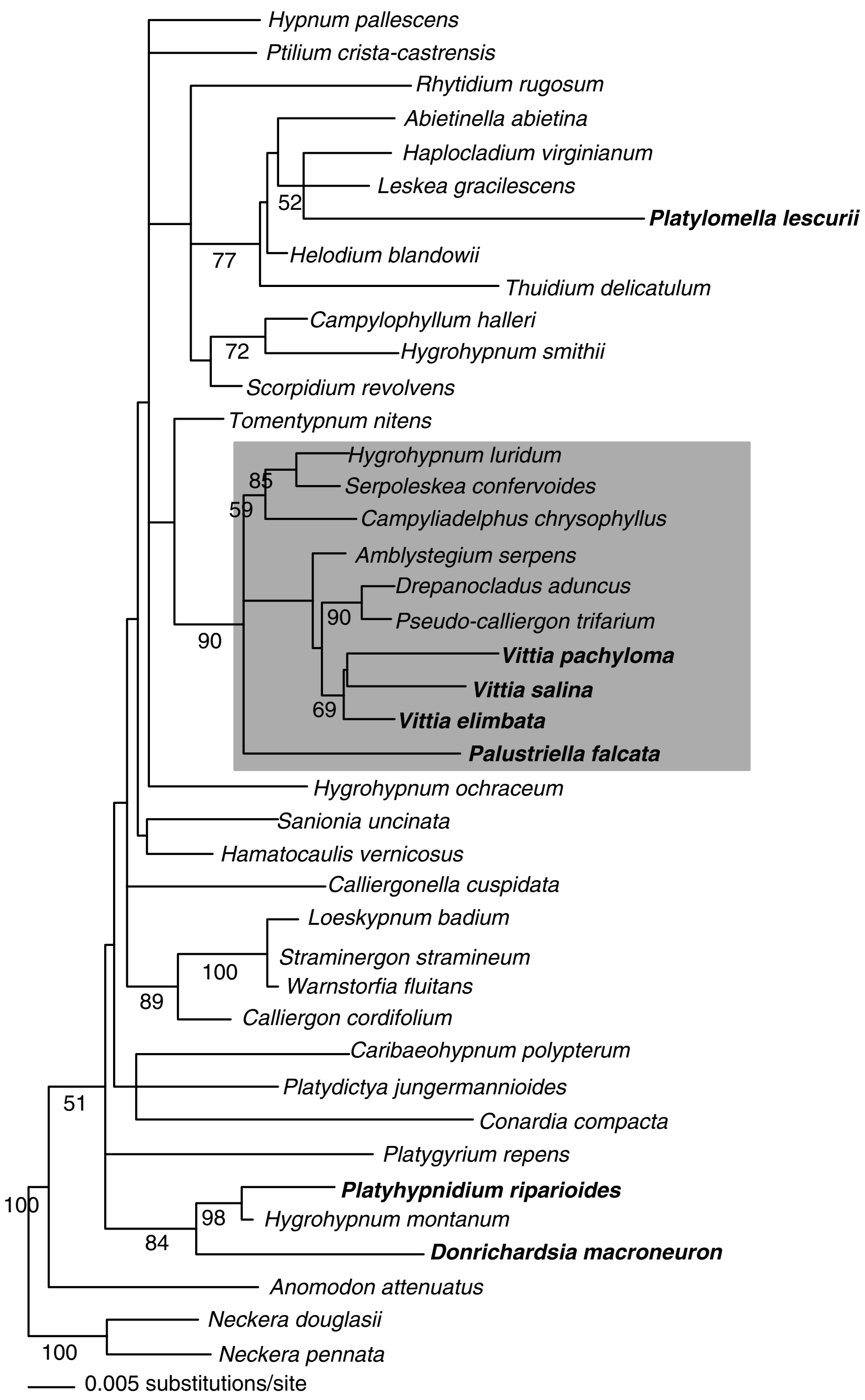

Fig. 1. Phylogram of the unique most likely tree from a maximum likelihood analysis of *trn*L-*trn*F and *atp*B-*rbc*L sequence data for a taxon sampling including the Amblystegiaceae (highlighted in gray) and related Hypnalean families (dataset 1). Branches of maximum length = 0 were collapsed. Bootstrap values higher than 50% are indicated below the branches. Taxa in boldface have (or can have in the case of *Palustriella* and *Platyhypnidium*) multistratose leaf laminae

(dataset 2) gave no evidence of incongruence between relationships implied by cpDNA vs. nrDNA. The datasets were consequently combined. The substitution model maximizing the likelihood of the neighbor-joining tree derived from the combined data set ($-\ln L = 4130.4741$) was a general time-reversible model (Rodriguez et al. 1990) with the rate heterogeneity among sites following a gamma distribution (shape parameter = 0.1645). The model was implemented with the following settings: rate matrix R (A-C) = 1.0529; R(A-G) = 1.6299; R(A-T) = −3636; R(C-G) = 1.0725;-1.0725; R(C-T) = 1.6299; R(G-T) = 1.0000; base frequencies A = 0.3104; C = 0.1892; G = 0.1935; T = 0.3070; proportion of invariable sites = 0. Heuristic searches under ML resulted in a unique most likely tree ($-\ln L =$ 4129.1662) (Fig. 2). *Vittia* constituted with *Hypnobartlettia* a clade that was sister to the *Drepanocladus*/*Pseudo-calliergon* complex (Fig. 2). This combined clade was unresolved at a polytomy that included *Amblystegium serpens* (Hedw.) Schimp.and a clade, supported at 100%, including all the other *Amblystegium* species. Within the *Vittia* clade, accessions of *Vittia pachyloma* formed a clade supported at 77%. The two accessions of *V. salina* composed a monophyletic group with 76% support. *Vittia elimbata*, described as a new species here (Fig. 3, Table 2), appeared to be sister to *V. salina*. *Vittia elimbata* shares with all these taxa, except *V. salina*, a partly bistratose upper leaf lamina but differs from all these species in having unistratose leaf margins. Furthermore, *V. elimbata* differs from *V. pachyloma* in having much narrower pseudoparaphyllia. Based on leaf morphology and anatomy, *V. elimbata* is similar to *Hypnobartlettia fontana*, but the latter has paraphyllia, its lamina is bistratose almost throughout rather than in the upper portion only, and its pseudoparaphyllia are broader.

Discussion

Phylogenetic relationships and circumscription of *Vittia*. The results presented here strongly support recent interpretations based on morphology (Hedenäs 1995, Buck and Goffinet 2000, Ochyra and Matteri 2001) that *Vittia* is a member of the Amblystegiaceae. Together with other families erected to accommodate other pleurocarpous mosses with multistratose leaf laminae such as the Hypnobartlettiaceae and the Donrichardsiaceae (Hedenäs 1995; Stech and Frahm 1999, 2000, 2001a; Stech et al. 1999), the Vittiaceae should be abandoned.

Within the Amblystegiaceae, *Vittia* forms with *Hypnobartlettia* a clade that is sister to the *Drepanocladus*/*Pseudo-calliergon* complex. This combined clade is unresolved at a polytomy that included *Amblystegium serpens* and a clade including all the other *Amblystegium* species. Although the apparent non-monophyly of the genus *Amblystegium* may reflect insufficient phylogenetic signal in our datasets, the occurrence of *A. serpens* outside the strongly supported clade including other *Amblystegium* species suggests that *A. serpens* may be better accommodated in a distinct genus. *Amblystegium serpens* is the type species of *Amblystegium* and thus retains the name, probably together with *A. subtile* (Hedw.) Schimp. (not included in this study). The other species [*A. fluviatile* (Hedw.) Schimp., *A. humile* (P. Beauv.) Crundw., *A. tenax* (Hedw.) C.E.O. Jensen, *A. varium* (Hedw.) Lindb., and probably *A. noterophyllum* (Sull.) Holz. (not included in this study)] should be accommodated in *Hygroamblystegium* Hedw., a genus that we previously hesitated to recognize based on phylogenetic analyses of a smaller dataset (Vanderpoorten et al. 2002a). *Amblystegium tenax, A. fluviatile,* and *A. noterophyllum,* were already tentatively included by some authors within *Hygroamblystegium* on the basis of a larger size, stronger and longer costa, and a tendency to occur in aquatic habitats (e.g. Buck 1998). Our results support the inclusion of *A. varium* within *Hygroamblystegium* (Buck 1998) and we see no reason to recognize the genus *Orthotheciella* Ochyra for this species (Ochyra 1998, Ochyra and Matteri 2001). Hence, *Hypnum* sect. *Orthotheciella* Müll. Hal. [Forschungsreise Gazelle, 4 (Bot.): 36. 1889] and *Orthotheciella* (Müll. Hal.) Ochyra [The Moss Flora of King George Island Antarctica: 224. 1998] are synomized with *Hygroamblystegium*. *Amblystegium humile*, conversely, has been either included within *Amblystegium* (Crundwell 1981), or even in *Leptodictyum* because of populations sharing with the latter a distinctly complanate habit (Ochyra 1981). The new combination, *Hygroamblystegium humile* (P. Beauv.)

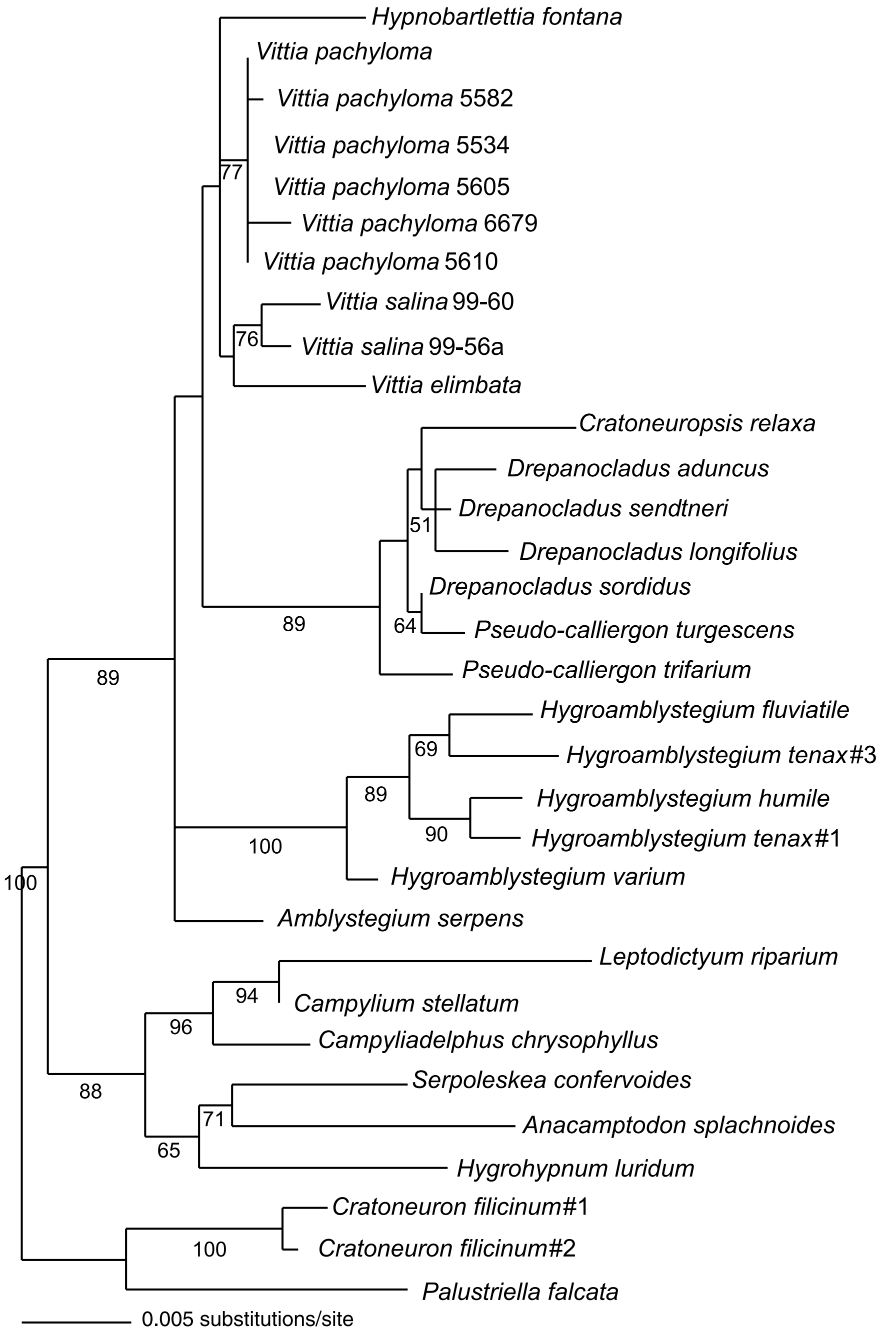

Fig. 2. Phylogram of the unique most likely tree from a maximum likelihood analysis of *trn*L-*trn*F, *atp*B-*rbc*L and ITS sequence data for the Amblystegiaceae (dataset 2). Branches of maximum length = 0 were collapsed. Bootstrap values higher than 50% are indicated below the branches

Vanderpoorten, Hedenäs & Goffinet, comb. nov. (Basionym: *Hypnum humile* P. Beauv., Prodrome des Cinquième et Sixième Familles de l'Aethéogamie 65. 1805), is therefore introduced. As circumscribed here, *Hygroamblystegium* differs morphologically from both *Amblystegium* s. str. and *Leptodictyum* by the occasional presence of foliose paraphyllia.

Morphologically, *Hygroamblystegium* is similar to the *Hypnobartlettia*/*Vittia* complex. Both complexes share a series of characters assumed to correlate with the occurrence in fast-flowing water (Vitt and Glime 1984), such as a thick costa and stiff stems. *Vittia elimbata* especially resembles an extreme expression of *Hygroamblystegium* in its general appearance

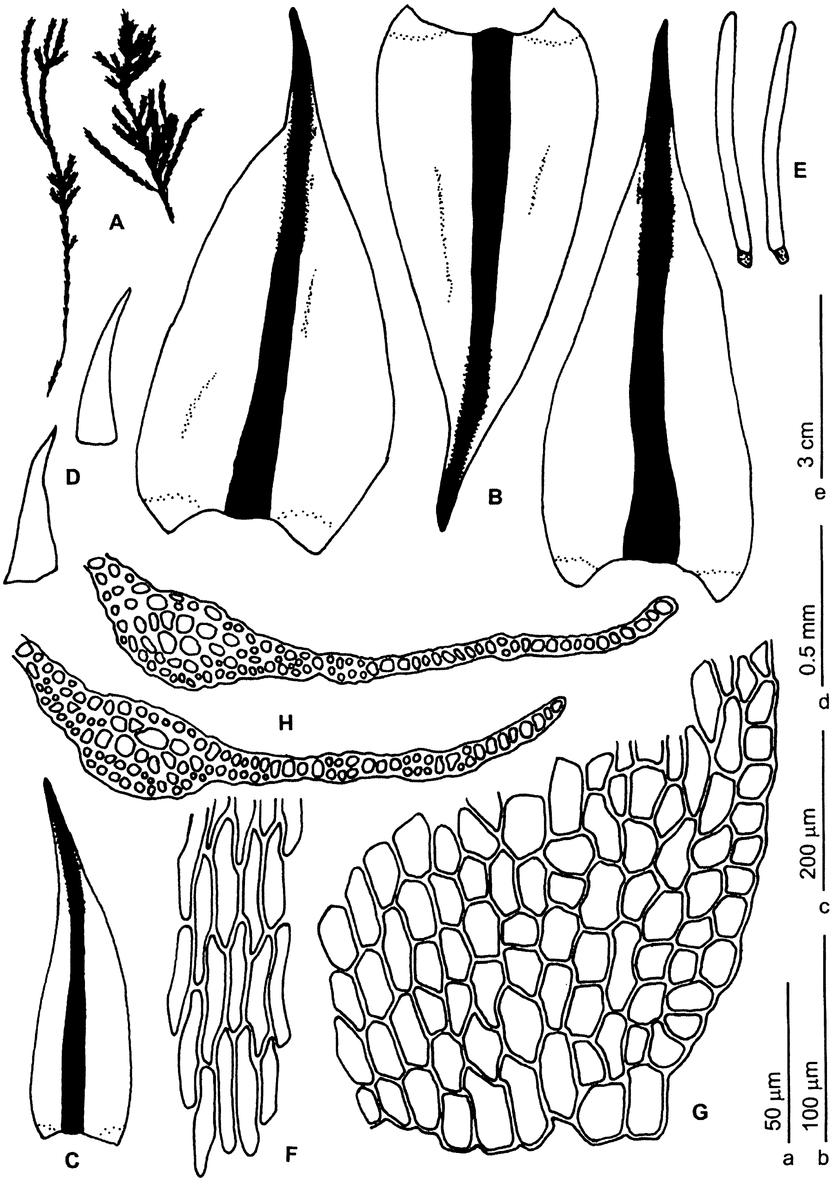

Fig. 3. *Vittia elimbata* spec. nov. **A** Habit; **B** Stem leaves; **C** Branch leaf; **D** Pseudoparaphyllia; **E** Axillary hairs; **F** Median cells of stem leaf lamina; **G** Alar cells of stem leaf; **H** Transverse sections of stem leaf, 65–75% way up leaf. Scales: a: F, G; b: E, H; c: D; d: B, C; e: A

and microscopical features, including the lack of a defined leaf border and undifferentiated alar cells. The taxa of the *Vittia*/*Hypnobartlettia* complex differ from *Hygroamblystegium* in having a variously multistratose leaf lamina and thickened leaf borders. These features, however, are not strongly expressed in all the species of the complex. In *Vittia pachyloma*, the leaf margins are (3–)4–8 stratose, and the lamina varies from unistratose to partly or entirely 2–4-stratose, whereas in both *V. salina* and *V. elimbata* the bistratose portions of the lamina are less strongly developed. *Vittia salina* has a partly or entirely bistratose leaf border sometimes becoming 3(-4)-stratose near the base but has an otherwise unistratose lamina. *Vittia elimbata* has small bistratose portions in its upper lamina, especially close to the costa. The same kinds of peculiar gametophytic features are encountered in the Haitian *Limbella bartlettii* (H. A. Crum & Steere) W. R. Buck but are combined in a different way. *Limbella bartlettii* possesses a 2(-3)-stratose leaf margin and a leaf lamina that is bistratose

Table 2. Description of *Vittia elimbata* Hedenäs, Vanderpoorten & Goffinet, sp. nov.

Diagnosis: *V. salinae* affinis, sed limbidio absenti et lamina folii superne ex parte bistratosa.

Type. Bolivia. Dept. La Paz, Inquisivi, Laguna Ventanani, along the Arco Pongo trail between Japo-Cargadero, SW slope of Cerro Ventanani c. 3 km N of Japo and 28 km NE of Inquisivi, 16°45′ S, 66°56′ W, 3810 m a.s.l., in running water on face of small waterfall, 6 August 1987, M. Lewis 87816 (DUKE, holotype; isotypes in MO, NY, S).

Plants medium-sized, yellow-green. Stem stiff, irregularly or pinnately branched in one plane, with a central strand, and a cortex of 2(-3) layers of small and incrassate cells; pseudoparaphyllia triangular or narrowly so; paraphyllia absent; axillary hairs with 1-celled upper part, 8.5–10.5 μm wide, hyaline when young, basal 1–2 cells quadrate or rectangular, brown; rhizoids not seen, but rhizoid initials present on stem just below leaf costa insertion. Stem leaves 1.6–1.7 mm long, 0.6–0.8 mm wide, erect-patent to almost patent, straight or slightly homomallous, rounded-triangular or ovate-triangular, gradually narrowed to acuminate or sometimes blunt apex, slightly concave; margin finely and obtusely denticulate; costa single, percurrent or shortly excurrent, 105.0–136.5 μm wide at base, diffusely delimited above, 4–5-stratose below, dorsal layers of smaller cells than ventral layers, in upper leaf biconvex, (4-)5-stratose; median laminal cells 21.0–61.0 × 6.5–10.0(-10.5) μm, slightly incrassate, eporose, in upper part near costa bistratose, close to leaf apex frequently bistratose in larger portions; alar cells rectangular, shortly rectangular or especially in upper part of group often quadrate, not or sometimes in basalmost portion slightly widened, forming a relatively large, rather indistinct, transverse or triangular group that extends from margin 1/2–2/3 of distance to costa, shortly decurrent. Branch leaves smaller and narrower than stem leaves, proximal branch leaves ovate to orbicular, with acute or rounded apiculate apex. [Sexual branches and sporophyte unknown].

in streaks. This species most likely also belongs to *Vittia*, but the lack of recent collections precluded the inclusion of the species in the present molecular study.

Morphological evolution of *Vittia*. A multistratose leaf lamina is a synapomorphy for the *Vittia*/*Hypnobartlettia* complex, which shares this peculiar leaf anatomy with many unrelated aquatic Hypnales such as *Donrichardsia macroneuron*, *Platyhypnidium torrenticola* (Ochyra, C. Schmidt & Bültmann) Ochyra & Bednarek-Ochyra, *P. mutatum* Ochyra & Vanderpoorten, and *Ochyraea tatrensis* Vana. Thus, polystratification of leaf lamina appears as a convergent feature associated with strong water flow that independently arose in a number of unrelated aquatic Hypnalean taxa.

The results thus support previous interpretations that morphological features associated with physical reinforcement of leaves are homoplastic and unstable (Vitt and Glime 1984). However, the present analyses do not support the interpretation that all the members of the families formerly defined by such features, including the Donrichardsiaceae, Hypnobartlettiaceae, and Vittiaceae, are extreme forms of Amblystegiaceae (Crosby et al. 1999, Buck and Goffinet 2000). In fact, among the aquatic pleurocarpous mosses with multistratose leaf laminae, molecular evidence only confirms the placement of *Hypnobartlettia*, *Vittia*, and *Gradsteinia andicola* within the Amblystegiaceae. *Richardsiopsis lacustris* (Herz. & Rich.) Ochyra, a rare endemic moss occurring in Brazil, Chile, Ecuador, and Peru, is synonym to *Drepanocladus perplicatus* (Dus.) Roth and also belongs to the family (Hedenäs 1997). Conversely, the data presented here show that *Platylomella lescurii*, which has traditionally been interpreted as a specialized rheophilous *Amblystegium* solely differing by a differentiated leaf border (Crum and Anderson 1981), is related to the Thuidiaceae/Leskeaceae. Similarly, *Ochyraea tatrensis* was shown to be closely related to *Hygrohypnum smithii* (Stech and Frahm 2001a), a taxon that formed with *Campylophyllum* a clade sister to the Leskeaceae/

Thuidiaceae and the Hypnaceae in the present analyses. *Limbella tricostata* (Sull.) Müll. Hal. ex E.B. Bartram was shown to be unrelated to the Amblystegiaceae and transferred to the Thamnobryaceae (Ochyra 1987b), a hypothesis supported by the results of a phylogenetic survey of the pleurocarps based on *rbc*L sequence data (Arikawa and Higuchi 1999).

These results parallel previous analyses of morphological evolution in the Hypnales, suggesting species associations that were not expected from analyses of morphological variation (Vanderpoorten et al. 2002b). Phylogenetic analyses of pleurocarpous mosses including molecular evidence indicate that convergent evolution and reversals in morphological characters have been common, and underline that in many cases molecular data are necessary to resolve relationships at the familial level.

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Appendix 1. Taxon sampling and Genbank accession numbers (*trn*L-*trn*F, *atp*B-*rbc*L, ITS) from Vanderpoorten et al. (2002b)

Abietinella abietina (Hedw.) Fleisch., AY009850, AF322308, AY009802-*Amblystegium fluviatile* (Hedw.) B, S. & G., AY009822, AF322324, AF168154-*A. humile* (P. Beauv.) Crundw., AY009823, AF322359, AF168165- *A. serpens* (Hedw.) B., S. & G., AY009827, AF322326, AF168152- *A. tenax* (Hedw.) C. Jens. #I, AY980024, AF322360, AF168164- *A. tenax* (Hedw.) C. Jens. #III, AY009821, AF322327, AF168157- *A. varium* (Hedw.) Lindb., AY009825, AF322328, AF168159- *Anacamptodon splachnoides* (Brid.) Brid., AY009816, AF322336, AY009810- *Anomodon attenuatus* (Hedw.) Hueb., AY009851, AF322309, AF168133- *Calliergon cordifolium* (Hedw.) Kindb, AY009836, AF322341, AF168146- *Calliergonella cuspidata* (Hedw.) Loeske, AY009859, AF322310, AF168145- *Campyliadelphus chrysophyllus* (Brid.) Kanda, AY009831, AF322355, AF168150- *Campylium stellatum* (Hedw.) C. Jens., AY009832, AF322354, AF168151- *Campylophyllum halleri* (Sw. ex Hedw.) Fleisch., AY009853, AF322311, AF168134- *Caribaeohypnum polypterum* (Mitt.) Ando & Higuchi, AY009846, AF322353, AY009799- *Conardia compacta* (Muell. Hal.) Robins., AY009865, AF322312, AY009806- *Cratoneuron filicinum* (Hedw.) Spruce, AY009817, AF322332, AY009812- *C. filicinum#2* (Hedw.) Spruce, AY009826, AF322362, AF168155- *Cratoneuropsis relaxa* (Hook. & Wils.) Fleisch., AF098525, AF322337, AF152388-AF152391- *Donrichardsia macroneuron* (Grout) Crum & Anderson, AY009848, AF322323, AF167350- *Drepanocladus aduncus* (Hedw.) Warnst., AY009828, AF322331, AF180949- *D. sendtneri* (Schimp. ex Muell. Hal.) Warnst., AY009818, AF322338, AY009811- *D. sordidus* (Muell. Hal.) Hedenäs, AY009868, AF322333, AY009792- *Hamatocaulis vernicosus* (Mitt.) Hedenäs, AY009819, AF322343, AF315073- *Haplocladium virginianum* (Brid.) Broth., AF161133, AF322305, AF168160- *Helodium blandowii* (Web. & Mohr) Warnst., AY009852, AF322313, AY009803- *Hygrohypnum luridum* (Hedw.) Jenn., AY009862, AF322339, AF168137- *H. montanum* (Lindb.) Broth., AY009863, AF322320, AY009804- *H. ochraceum* (Wils.) Loeske, AY009861, AF322345, AF168138- *H. smithii* (Sw.) Broth., AY009856, AF322306, AF168139- *Hypnum pallescens* (Hedw.) P. Beauv., AY009844, AF322314, AY009796- *Leptodictyum riparium* (Schimp.) Warnst., AY009830, AF322325, AF168163- *Leskea gracilescens* Hedw., AF161135, AF322356, AF176277- *Neckera douglasii* Hook., AF315070, AF322358, AY009808- *N. pennata* Hedw., AF315072, AF322357, AY009809- *Palustriella falcata* (Brid.) Hedenäs, AY009829, AF322330, AF168158- *Platydictya jungermannioides* (Brid.) Crum, AY009857, AF322307, AF168162- *Platygyrium repens* (Brid.) Schimp., AF161131, AF322317, AY009798- *Platyhypnidium riparioides* (Hedw.) Dix., AF260908, AF230981-AF230996-*Pseudo-calliergon trifarium* (Web. & Mohr) Loeske, AY009835, AF322329, AY009793- *Pseudo-calliergon turgescens* (Jens.) Loeske, AY009843, AF322335, AY009794- *Ptilium crista-castrensis* (Hedw.) De Not., AY009847, AF322316, AY009800- *Rhytidium rugosum* (Hedw.)

Appendix 1 (Continued)

Kindb., AY009849, AF322318, AY009801- *Sanionia uncinata* (Hedw.) Loeske, AY009860, AF322321, AF168148- *Serpoleskea confervoides* (Brid.) Kartt. AY009858, AF322334, AF168142- *Straminergon stramineum* (Kindb.) Hedenäs, AY009833, AF322349, AF168143- *Thuidium delicatulum* (Hedw.) B., S. & G., AF161132, AF322322, AF176278- *Tomentypnum nitens* (Hedw.) Loeske, AY009854, AF322352, AF168161- *Warnstorfia fluitans* (Hedw.) Warnst., AY009838, AF322350, AF168149.

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