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The systematic position of *Plagiochila* (*Jamesoniella*) *dependula* (Jungermanniopsida) inferred from nrITS sequences and morphology, with notes on its differentiation from *Plagiochila fuscolutea*

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SUMMARY

Based on morphology and phylogenetical analyses of nrITS sequences, the Andean endemic *Plagiochila* (*Jamesoniella*) *dependula* is placed in *Plagiochila* sect. *Fuscoluteae*. Perianths, oil bodies and sporophytes are described for the first time. Morphologically, *P. dependula* is characterized by entire, dorsally mostly shortly decurrent, laterally appressed, reniform leaves which are covered by irregular papillae and membranous wax platelets, papillose oil bodies, intercalary androecia with opposite bracts that overlap dorsally, a plagiochilid perianth with an entire mouth, a 250 μm thick capsule wall with thickenings in all layers, large 1–4 celled spores, and bispiral elaters. Morphotypes of *Plagiochila fuscolutea* with laterally appressed leaves differ from *P. dependula* by an at least moderately decurrent, broad dorsal leaf base, a ± smooth upper leaf surface, and by its often toothed leaf apices. *Plagiochila wolframii* is placed in the synonymy of *P. fuscolutea.* Maximum likelihood and parsimony analyses of 29 *Plagiochila* ITS sequences resolve *P. dependula* in a well-supported clade with several other representatives of *P.* sect. *Fuscoluteae*. Branch lengths within sect. *Fuscoluteae* point to a sudden diversification. Accessions of *P. heterophylla* s.str. from Great Britain (formerly known as *P. atlantica*) and Costa Rica form an unsupported monophyletic lineage.

KEYWORDS: Jungermanniopsida, Plagiochilaceae, *Plagiochila*, *Jamesoniella*, wax, phylogeny, nrITS.

INTRODUCTION

Sterile *Plagiochila* (Dumort.) Dumort. phenotypes with laterally appressed, entire or few-toothed leaves and species of *Jamesoniella* Steph. or *Syzygiella* Spruce have been confused on several occasions. Examples include *Syzygiella anomala* (Lindenb. & Gottsche) Steph., which was originally described as *Plagiochila* (Gottsche, Lindenberg & Nees, 1844–1847), *Jamesoniella trigonifolia* Steph./*Syzygiella trigonifolia* (Steph.) Herzog which was treated as a synonym of *Plagiochila fuscolutea* Taylor by Grolle (1971) but removed to *Syzygiella anomala* by Heinrichs (2002), and *Plagiochila carringtonii* (Balfour) Grolle which was placed in *Jamesoniella* and several other genera until its true identity was eventually discovered (see Grolle, 1964; Renker *et al.*, 2002).

Taylor (1846) described *Plagiochila dependula* Taylor, based on a single sterile specimen from the Central Andes. The species was subsequently transferred to *Jamesoniella* by Stephani (1895). Grolle (1971) accepted the position in *Jamesoniella* with some misgivings. In doing so he pointed out that only the discovery of perianths would allow a doubt-free classification. On the basis of the exclusively lateral-intercalary branches of *Plagiochila*/*Jamesoniella dependula*, Inoue (1989), however, argued for a placement in *Plagiochila.* During fieldwork by ASV, JHei, SL-Y, SY and RW in Northern Ecuador in August 2004, copious material with sporophytes was collected, allowing for a

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complete morphological description and accompanying molecular investigation.

MATERIALS AND METHODS

Morphology

The investigation is based on specimens deposited in the herbaria B, FH, G, GOET, JE, QCA, U and W. Specimens were examined using conventional microscopic techniques.

Scanning electron microscopy

Air-dried herbarium specimens of *Plagiochila/Jamesoniella dependula* were examined by SEM using a ZEISS DMS 960 after fixing on double-sided adhesive tape and sputter coating with gold. In order to determine whether or not leaf surface wax was present, leaves and stem fragments from the same plants were examined by SEM following washing of the shoots in chloroform 2–3 min prior to coating. Classification of surface waxes follows Barthlott *et al.* (1998).

DNA extraction, PCR amplification and sequencing

Upper parts of a few shoots of herbarium specimens were isolated (Table 1). DNA was extracted with Invisorb Spin Plant Mini Kit (Invitek, Berlin, Germany). PCR amplification followed the protocol of Heinrichs *et al.* (2004a). Sequencing was carried out on an ABI 3100 capillary sequencer using the BigDye^{TM} Terminator Cycle Sequencing v2.0 kit (PE Biosystems).

Taxon sampling and phylogenetic analyses

The *Plagiochila/Jamesoniella dependula* nrITS sequence and a new sequence of *P. heterophylla* from Costa Rica were compared with GenBank sequences using the BLASTN program and integrated into a large alignment of Plagiochilaceae ITS sequences. In all cases sequences of *Plagiochila* 'clade A' *sensu* Groth & Heinrichs (2003) were identified as most similar to the new sequences (data not shown). Based on these results, representatives of the related *Plagiochila* sects. *Adiantoideae* Lindenb., *Fuscoluteae* Carl and *Hylacoetes* Carl were sampled. *Plagiochila* (sect. *Cucullatae*) *sandei* Sande Lac. of

Table 1. *Geographic origins, voucher numbers, GenBank/EMBL accession numbers and character matrix of the investigated taxa. All the specimens are in GOET. The coded characters are: 1, cell walls of capsule epidermis thin (0), with thickenings (1); 2, cells of innermost layer of capsule wall with ladder-like thickenings (0), with a mixture of ladder-like and nodulose thickenings (1); 3, male bracts dorsally overlapping (0), not overlapping (1); 4, surface wax present (0), lacking (1); 5, verrucosane-type diterpenes always present (0), lacking or rarely present as minor compound (1).*

Taxon	Origin	Voucher	Accession number	Matrix (characters 1 2 3 4 5)
Plagiochila adiantoides Lindenb.	Costa Rica	Heinrichs *et al.* 4314	AJ422027	1 1 0 1 0
P. aerea Taylor	Costa Rica	Heinrichs *et al.* 4321	AJ422027	1 0 0 0 1
P. amicta Steph.	Costa Rica	Heinrichs *et al.* 4178	AJ422022	0 0 1 1 1
P. boryana Steph.	Bolivia	Anton *et al.* Bol2	AJ866752	0 0 1 1 1
P. boryana	Bolivia	Heinrichs *et al.* 4182	AJ866753	0 0 1 1 1
P. boryana	Uganda	Pócs 97142	AY275161	1 1 0 1 1
P. cristata (Sw.) Lindenb.	Costa Rica	Heinrichs *et al.* 4192	AJ422015	0 0 0 1 1
P. cucullifolia Jack & Steph. var. *cucullifolia*	Costa Rica	Heinrichs *et al.* 4402	AJ422012	0 0 0 1 1
P. cucullifolia. var. *cucullifolia*	Ecuador	Schmidt-Lebuhn 384	AJ620671	0 0 0 1 1
P. cucullifolia. var. *anomala* Heinrichs & Gradst.	Ecuador	Holz EC-01-558	AY330711	0 0 0 1 1
P. dependula Taylor	Ecuador	Heinrichs *et al.* 4420	AJ966736	1 0 0 0 ?
P. dimorpha var. *ecuadorica* (Inoue) Heinrichs	Costa Rica	Holz CR-00-499	AJ422013	0 0 ? 1 1
P. ensiformis Taylor	Ecuador	Sauer & Gradstein E115	AY275163	0 0 1 1 1
P. flabelliflora Steph.	Brazil	Schäfer-Verwimp 10669	AF394462	0 0 1 1 1
P. fuscolutea Taylor	Costa Rica	Heinrichs *et al.* 4400	AJ416086	1 0 0 0 1
P. grandicrista Steph.	Costa Rica	Heinrichs *et al.* 4183	AJ422030	? ? ? 1 0
P. heterophylla Lehm. var. *heterophylla*	Costa Rica	Heinrichs *et al.* 4487	AJ966737	1 0 0 0 1
P. heterophylla var. *heterophylla*	Great Britain	Rycroft 97006	AY275165	1 0 0 0 1
P. longiramea Steph.	Bolivia	Heinrichs *et al.* 4071	AF539463	1 0 0 0 1
P. macrostachya Lindenb.	Ecuador	Holz EC01-80	AY422016	0 0 1 1 1
P. patriciae Heinrichs & H.Anton	Costa Rica	Holz CR-00-176	AY275170	0 0 1 1 1
P. sandei Sande Lac.	Indonesia	Gradstein 9970	AJ414634	0 0 1 1 1
P. sandei	Philippines	Schwarz 5732	AJ413176	0 0 1 1 1
P. superba (Spreng.) Mont. & Nees var. *superba*	Costa Rica	Heinrichs *et al.* 4175	AJ416084	0 0 1 1 1
P. superba var. *superba*	Ecuador	Holz EC-01-65	AJ416085	0 0 1 1 1
P. tabinensis Steph.	Costa Rica	Heinrichs *et al.* 4156	AJ422026	? ? 0 0 1
P. turgida Herzog	Ecuador	Holz EC-01-70	AJ422024	0 0 1 1 1
P. vincentina Lindenb.	Costa Rica	Heinrichs *et al.* 4331	AY275175	0 0 1 1 1
P. vincentina	Ecuador	Andersson 3505	AJ422025	0 0 1 1 1

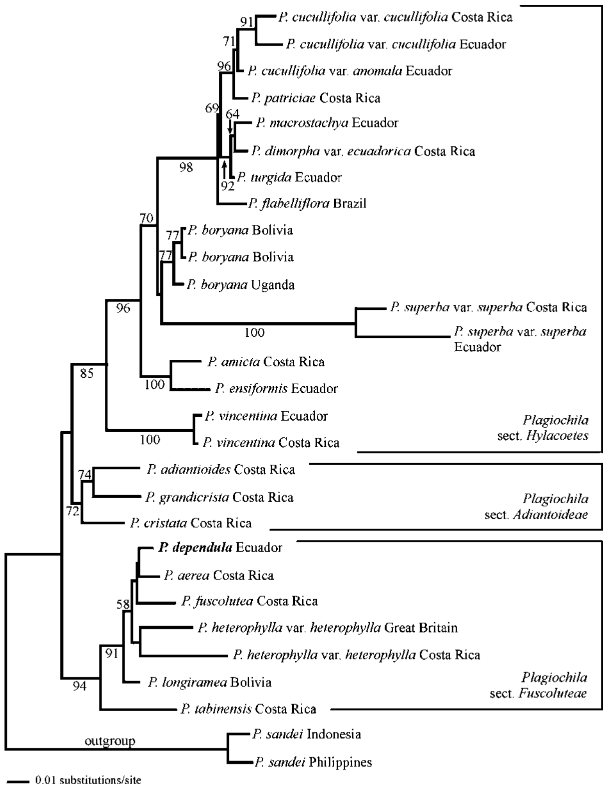

Figure 1. Molecular phylogeny of *Plagiochila* 'clade A' (Groth & Heinrichs, 2003). The unique most likely tree (–ln=3606.8437) resulted from a maximum likelihood analysis, using the TrN model with estimated gamma shape (G=0.3505). Bootstrap support (>50%) is indicated at the branches.

Plagiochila 'clade B' (Groth & Heinrichs, 2003) was chosen as the outgroup.

Twenty-seven ITS1-, 5.8S- and ITS2-sequences from Heinrichs (2002), Heinrichs *et al.* (2002a, b, 2003, 2005a), Renker *et al.* (2002), Groth *et al.* (2003) and the two new sequences (Table 1) were aligned manually in BioEdit version 5.0.9 (Hall, 1999), resulting in an alignment including 740 putatively homologous sites (alignment available from JHei).

Phylogenetic trees were inferred using maximum likelihood (ML) and maximum parsimony (MP) criteria as implemented in PAUP* version 4.0b10 (Swofford, 2003).

ML analyses To decide on the nucleotide substitution model with the smallest number of parameters that best fits the data, the program Modeltest 3.06 (Posada & Crandall, 1998) was used. Both the hierarchical likelihood ratio test and the Akaike information criterion (Akaike, 1974) selected the TrN model (Tamura & Nei, 1993) with gamma shape parameter (G) for among site variation calculated from the data set (TrN+G). An ML analysis (with the TrN+G model) was implemented as an heuristic search with 100 random-addition sequence replicates. The confidence of branching was assessed using 250 bootstrap resamplings in the ML analysis (Felsenstein, 1985).

MP analysis MP analyses of the molecular matrix and a matrix augmented with four morphological characters and one chemical character (Table 1), derived from Anton (2001) and Heinrichs (2002), were performed with the following options implemented: heuristic search mode with 10000 random-addition-sequence replicates, tree bisection–reconnection branch swapping (TBR), MULTrees option on, and collapse zero-length branches off. Morphological and chemical characters were assigned to all accessions of a species; characters known to have a high content of homoplasy at sectional level (e.g. leaf shape and dentation; Heinrichs, 2002) were not scored. All characters were treated as equally weighted and unordered. Gaps were coded as unknown characters. Clade support was estimated from 1000 bootstrap replicates using heuristic searches with TBR branch swapping.

RESULTS

Molecular investigation

ML analysis The ML analysis produced a single tree (Fig. 1) in which the 27 ingroup sequences are placed in three robust sectional clades. *Plagiochila* sect. *Adiantoideae* is sister to *P.* sect. *Hylacoetes*, albeit without support. *Plagiochila dependula* is located within the well-supported sect. *Fuscoluteae* which are otherwise represented by *P. aerea* Taylor, *P. fuscolutea* Taylor, *P. heterophylla* Lehm. var. *heterophylla*, *P. longiramea* Steph. and *P. tabinensis* Steph. *Plagiochila tabinensis* is placed sister to the remainder of *Fuscoluteae* in a well-supported sister relationship. Other relationships within *Fuscoluteae* lack bootstrap support or are weakly supported. *Plagiochila dependula* is placed sister to *P. aerea* albeit without support. Accessions of *P. heterophylla* from the British Isles and Costa Rica form an unsupported monophyletic lineage. Basal branches within *Fuscoluteae* are short.

MP analysis Among the 740 characters investigated (molecular dataset), 167 were parsimony informative, 91 autapomorphic and 482 constant. The heuristic search recovered six equally most parsimonious trees (not shown) with a length of 487 steps, a consistency index (CI) of 0.67, a CI excluding uninformative characters of 0.58, a retention index (RI) of 0.79, and a rescaled CI of 0.52. The strict consensus of these trees (Fig. 2A) is largely congruent with the ML tree (Fig. 1), albeit without good support for sects. *Adiantoideae* and *Hylacoetes*. Addition of the morphological–chemical matrix (all characters parsimony informative) results in three equally most parsimonious trees (not shown) with a length of 493 steps, a CI of 0.67, a CI excluding uninformative characters of 0.59, a RI of 0.79, and a rescaled CI of 0.53. Contrary to the ML topology and the MP tree in Fig. 2A, the strict consensus of these trees (Fig. 2B) shows a well-supported sister relationship of the robust sects. *Adiantoideae* and *Fuscoluteae*.

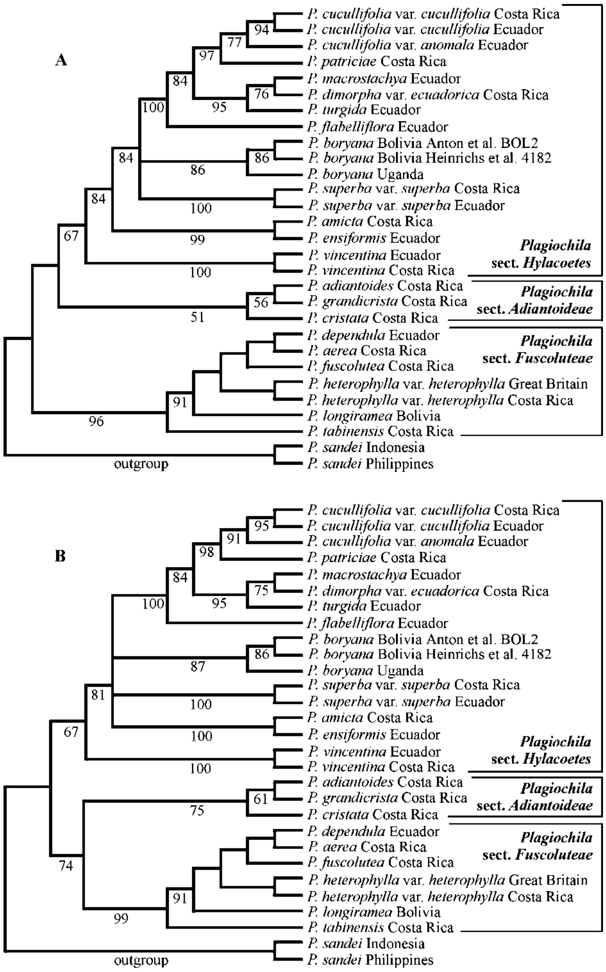

Figure 2. Rooted strict consensus of (A) six and (B) three equally parsimonious trees recovered during 10 000 random taxon addition heuristic searches of the molecular dataset (A) and the combined molecular–morphological–chemical dataset (B). Bootstrap support (>50%) is indicated above the branches.

Morphology

SEM investigation of leaves of Ecuadorian and Colombian *P. dependula* specimens demonstrates the presence of surface wax in the form of membranous platelets which can be removed by chloroform. Wax was only present in low concentrations and at best detectable in basal parts of young leaves. All leaves were densely covered by irregular, round to ellipsoidal or angular papillae which were still present after treatment with chloroform for 3 min.

The newly detected plagiochilid perianths and sporophytes allow for a complete description as follows (Figs 3, 4, 5A).

Plagiochila dependula Taylor, London J. Bot. 5: 265. 1846. *Jamesoniella dependula* (Taylor) Steph., Hedwigia 34: 47. 1895. Type: Ecuador ('Peru') 'East side of Cordilleras', *Jameson 125* (holotype, FH [ster.]).

= *Plagiochila pendula* Steph. Spec. Hep. 6: 196. 1921, syn. fide Inoue (1989), here confirmed. Type: Ecuador, Quito, *Jameson s.n.* (holotype, G 026376 [ster.]).

Gametophyte. Plants medium to large, (3-)6-22(-26) cm long and *ca* 1–1.5 mm wide (dorsal view), yellowish brown to olivaceous green, basal parts often brownish, forming mats, with short creeping stoloniform shoots that give rise to leafy aerial stems. *Leafy stems* dorsiventrally flattened, near base *ca* $420-650\times320-500$ μm, blackish brown to brown, in upper parts often pale or greenish brown, completely concealed by leaves, in cross section *ca* $18-27\times16-25$ cells across, the cortical cells in 3-5(-6) layers, thick-walled, $16-32\times12-24$ μm, medullary cells thin-walled to moderately thick-walled, $18-45\times16-40$ μm, trigone-like thickenings small, triangular or lacking. Branches lacking or rare, of the lateral-intercalary type. Creeping stoloniform shoots often beset with short leaflets. *Leaves of aerial shoots* laterally appressed, only margins weakly inrolled when dry, imbricate, strongly ampliate, reniform, with broadly rounded apex, *ca* 2.4–6.0 (from ventral to dorsal margin) × 1.5–3.3 mm and 1.5–2.3 times as long as wide, margins flat, entire, ventral margin weakly to moderately decurrent, dorsal margin short to moderately, occasionally long decurrent, basal part of decurrent strip narrow, only 1–2 cells thick.

Leaf areolation regular to irregular, cells in upper and lateral portions of leaf broader than long to elongate, *ca* $28-60(-75)\times24-45$ μm and 0.7–2.2 times as long as wide, cells above leaf base moderately to strongly elongate, forming a broad, inconspicuous vitta, *ca* $45-85\times28-42$ μm and 1.4–2.9 times as long as wide; cell walls thin except those along leaf walls, trigones large, nodulose, occasionally confluent, intermediate thickenings occasionally present on long walls; cuticle with irregular, small, round to ellipsoidal or angular papillae, cuticle, at least of young leaves, also covered by membranous wax platelets (SEM). *Oil bodies* colourless, (4-)5-8(-10) per median leaf cell, *ca* $5-12\times3-6$ μm, broadly ellipsoid, sometimes globose, formed of many protruding oil-globules. *Underleaves* lacking or vestigial, *ca* 0.1–0.2 mm long and 0.2–0.3 mm broad, consisting of numerous, densely spaced, short cilia often terminated by slime papillae. Asexual reproduction by specialized structures not observed.

Male plants as large as female plants or somewhat smaller. *Androecia* terminal and sparsely *Frullania*-type branched or simple, becoming intercalary on main stems and on branches, bracts closely imbricate, the basal ones sometimes moderately imbricate, entire, opposite bracts overlapping dorsally, strongly inflated basally, distal part obliquely spreading, bracts composed of cells similar to those of the leaves but cells near base often not or only slightly chlorophyllose. *Antheridia* 1–2 per bract, on a short stalk, broadly ellipsoidal, *ca* $200-330\times250-380$ μm.

Gynoecia terminal on main shoots and on branches, with 0–2 innovations, female bracts broadly and asymmetrically ovate to reniform, with rounded apex, dorsally and ventrally hardly or shortly decurrent, slightly larger than subtending leaves, entire, areolation similar to that of leaves

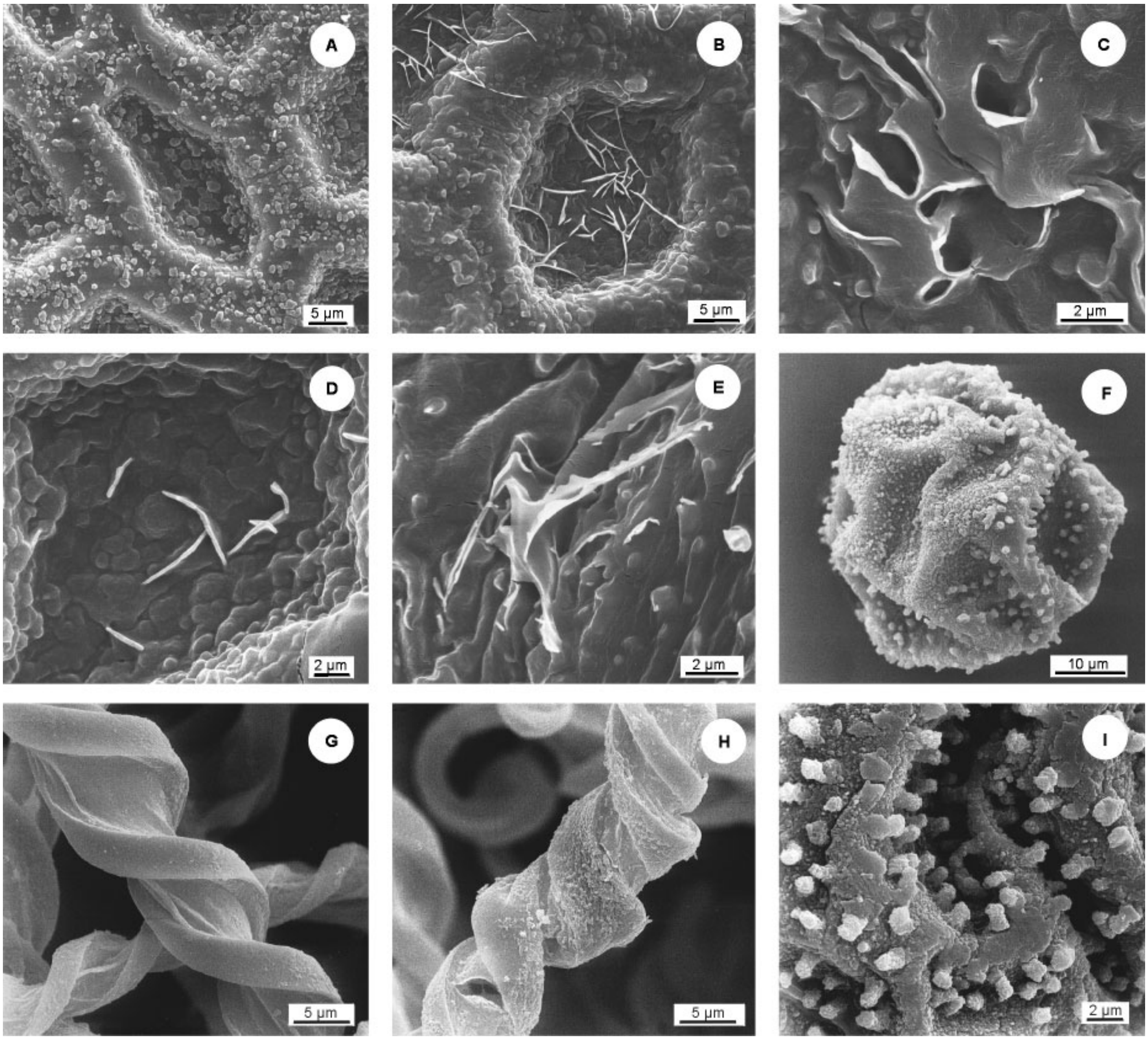

Figure 3. SEM micrographs of *Plagiochila dependula*: A–E, leaf surface (A with papillae, B–E with papillae and membraneous wax platelets); F, spore; G, H, parts of elaters; I, detail of sporoderm (all from *Heinrichs et al. 4420* (GOET)).

but vitta zone often larger. *Perianths* broadly cylindrical in lateral view, ±elliptical in dorsal view, lower half covered by bracts, perianths *ca* 5–7 mm long and 3–6 mm broad, *ca* 1.2–1.7 times as long as wide, both keels of ±similar length or dorsal keel slightly longer, keels unwinged. Perianth mouth truncate or weakly arched upwards, entire.

Sporophyte. Capsule moderately exerted, ±globose; valves straight, not twisted, brown, *ca* 1.8–2.8 mm long and 1.3–1.9 mm broad, *ca* 1.4–1.9 times as long as wide; epidermal cells in surface view broader than long to elongate, with 1-3(-5) thickenings on most walls, thickenings nodulose, occasionally cone-like or weakly branched, opposite thickenings of long walls or of long and short walls sometimes coalesced; hypodermal cells generally larger and with more thickenings, thickenings small, brown, ±nodulose, often confluent at their base, median innermost cells in surface view mostly strongly elongate, mixed with transversely arranged cells, in indistinctly alternating parallel pattern, apical innermost cells shorter, with more transversely arranged cells, innermost cells with ladder-like thickenings, thickenings partly confluent at base giving a fenestrate impression; valves in cross-section *ca* 180–260 μm thick, 9–13-stratose, epidermal cells as large as or slightly larger than inner ones, *ca* 25–35 μm thick, inner cells *ca* 12–30 μm thick. *Spores ca* 30–60 μm in diameter, ±globose, 1–4 cellular, trilete, sporoderm with granular basal surface and ±densely spaced bacula and pila. Elaters bispiral, in the middle often trispiral, 9–16 μm in diameter, ±smooth or covered with low papillae.

Representative specimens examined:

COLOMBIA. META: Páramo de Sumapaz, 4170–4200 m, 1973, *Cleef 8067 & 8167* (U); RISARALDA: Municipio de Santa Rosa, Finca La Sierra, 3730 m, 1980, *Aguirre & Gradstein 1342* (U). ECUADOR. NAPO: Páramo de Quilindaña, Laguna Yurac Cocha, 4150 m, 1979, *Kieft et al. 195 A* (U); PICHINCHA: Papallacta Pass, 3800–4000 m, 2004, *Heinrichs et al. 4419, 4420, 4468* (GOET, QCA,

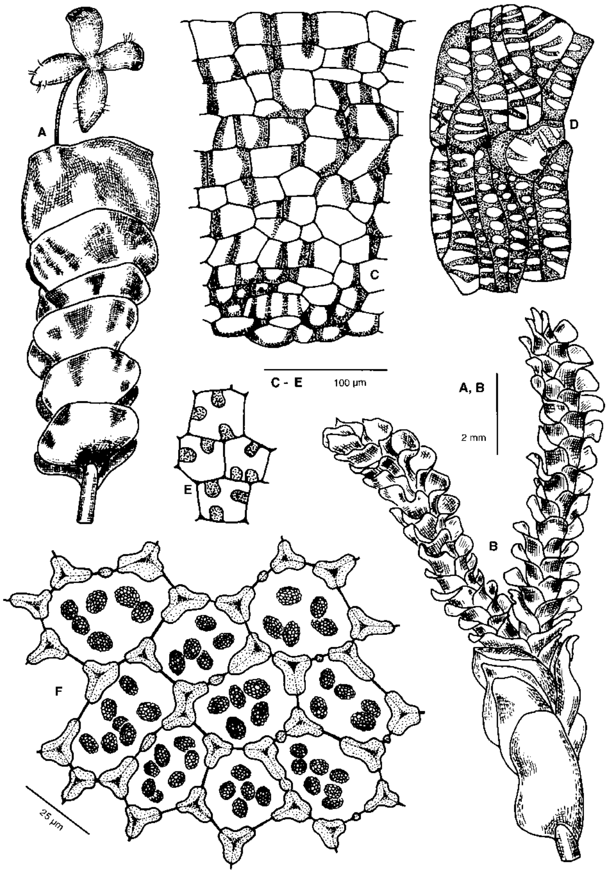

Figure 4. *Plagiochila* (sect. *Fuscoluteae*) *dependula* Taylor: A, top of shoot with sporophyte, lateral view; B, top of shoot with androecium, dorsal-lateral view; C, cross-section of capsule wall; D, median part of innermost layer of capsule wall, surface view; E, epidermal layer of capsule wall, surface view; F, leaf cells with oil bodies (all from *Heinrichs et al. 4420* (GOET))

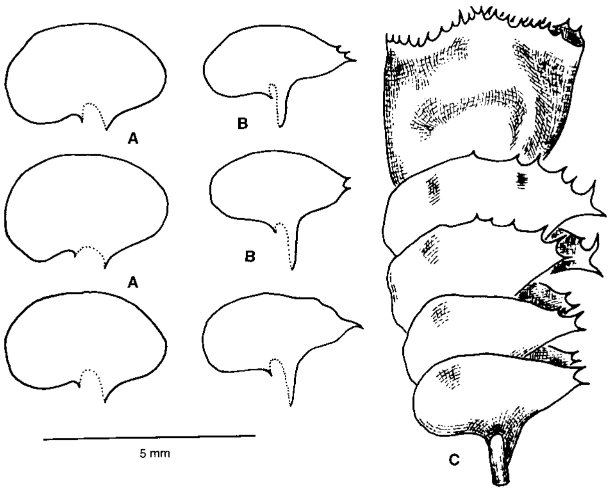

Figure 5. *Plagiochila dependula* Taylor (A) and *P. fuscolutea* (B, C): A, B, leaves; C, top of shoot with perianth (A from *Heinrichs et al. 4420* (GOET), B from isotype of *P. wolframii* (B), C from *Kürschner et al. 95-343* (Peru, Amazonas, B)).

distributed in Bryophyta Exsiccata Generis Plagiochilae fasc.VI). BOLIVIA. LA PAZ: Unduavi, *Pearce s.n.* (G).

Plagiochila dependula occurs in upper cloud forests and páramo vegetation of the northern and central Andes at altitudes between 3500 and 4200 m where it grows on soil and on leaning trunks. The species is able to form large, deep cushions. At the Papallacta Pass (Napo-Pichincha border, Ecuador) *Plagiochila dependula* is very common in open *Polylepis* forests above 3700 m, often growing intermingled with *Plagiochila ensiformis* Taylor and *P. ovata* Lindenb. & Gottsche.

Plagiochila dependula is morphologically close to forms of *Plagiochila fuscolutea* Taylor with strongly ampliate leaves (Fig. 5). Differences between these species are summarized in Table 2.

Plagiochila wolframii Inoue has the laterally appressed leaves of *P. dependula*; however, the leaves are often toothed, dorsally at least moderately decurrent, and in upper parts ±smooth. The taxon is interpreted here as an extreme phenotype of *P. fuscolutea*, leading to the following new synonymy:

Plagiochila fuscolutea Taylor, London J. Bot. 5: 263. 1846. Type: Ecuador ('Peru'), *Jameson s.n.* (holotype, FH [c.per.]).

Syn. nov. *Plagiochila wolframii* Inoue; in Inoue H, ed. *Studies on Cryptogams in Southern Peru*: 99. 1987. Type: PERU. AMAZONAS: Chachapoyas, road Cajamarca-Chachapoyas, 3200 m, 1982, *Frahm et al. 1138* (isotype, B [c.per.]).

Species of *Jamesoniella* are usually much smaller than typical *P. dependula*, and have ventral-intercalary branches (e.g. Grolle, 1971; Gradstein, Churchill & Salazar-Allen, 2001).

DISCUSSION

The plagiochilid perianths described here allow the generic placement of *Plagiochila/Jamesoniella dependula* to be decided with conviction. Verification of surface wax

Table 2. *Differentiation of* Plagiochila dependula *and* P. fuscolutea.

	Plagiochila dependula	*Plagiochila fuscolutea*
Leaves	Laterally appressed	Spreading, rarely laterally appressed
Dorsal leaf base	Short decurrent, if long deccurent the decurrent strip only 1–2 cells thick	Moderately to long decurrent, decurrent cell strip broad
Leaf apex	Broadly rounded, entire	Rounded to subtruncate or acute, often with some teeth
Upper leaf surface	Papillose (papillae and wax)	±Smooth (wax only)
Perianth mouth	Entire	Entire or toothed

strongly supports the placement in *Plagiochila* sect. *Fuscoluteae* as was also seen in the molecular investigation. All members of the *Fuscoluteae* identified so far have surface wax which is otherwise unknown in *Plagiochila* (Heinrichs *et al.*, 2000, 2001; Heinrichs & Rycroft, 2001; Heinrichs, 2002). The extremely thick capsule walls and the large, pluricellular spores of *P. dependula* strongly resemble those of the morphologically similar *Plagiochila fuscolutea* and *P. longiramea* of sect. *Fuscoluteae*. The vast majority of *Plagiochila* species have capsule walls that are only 50–120 μm thick (e.g. Inoue, 1984; Heinrichs, 2002). Similar thick capsule walls have also been reported from *Plagiochila cucullifolia* Jack & Steph. of sect. *Hylacoetes*; however, species of *Hylacoetes* lack wall thickenings in the epidermal layer of the capsule (Heinrichs, 2002).

Within *Plagiochila* sect. *Fuscoluteae*, *P. dependula* stands out by the papillose leaf surface which was originally recognized by Grolle (1971). Only a few species of *Plagiochila* have papillae on their leaves, e.g. *P. papillifolia* Steph. (Heinrichs *et al.*, 2002c) and *P. stricta* of sect. *Arrectae* (Rycroft *et al.*, 2002), *P. boryana* Steph. of sect. *Hylacoetes* (Heinrichs, 2002), as well as *P. pleurata* (Hook. f. & Taylor) Gottsche et al. and *P. fruticella* (Hook. f. & Taylor) Gottsche et al. of sect. *Fruticellae* Carl (Inoue & Schuster, 1971). However, all these species have round to ellipsoidal papillae, whereas the papillae of *P. dependula* are irregular and partly angular, and resemble those found within the Lejeuneaceae (Reiner-Drehwald & Goda, 2000; Reiner-Drehwald, 2005) rather than those of Plagiochilaceae. Interestingly, androecia of the population of *P. dependula* collected on the Papallacta pass, Ecuador, are often terminal and sparsely *Frullania*-type branched. Within the *Plagiochila* 'clade A' *sensu* Groth & Heinrichs (2003), this type of androecium is more typical of representatives of the *Hylacoetes* than for *Adiantoideae* or *Fuscoluteae* species which usually produce simple, intercalary androecia (Heinrichs, 2002). However, Grolle (1971) described the androecia of *P. dependula* as intercalary, indicating that both types occur within this species.

With the exception of two species (*P. boryana* and *P. heterophylla*; Heinrichs, 2002; Heinrichs *et al.*, 2005a), representatives of *Plagiochila* clade A (Groth & Heinrichs, 2003) are restricted to the Neotropics. *Plagiochila* (sect. *Fuscoluteae*) *heterophylla* occurs also in Atlantic Europe (British Isles, northwestern France) where it was known as *P. atlantica* F.Rose (Jones & Rose, 1975). Molecular data (Figs 1, 2) support the synonymy of these taxa as proposed by Heinrichs (2002) based on morphological comparisons. Resolution within the *Fuscoluteae* is poor. Basal branches within the *Fuscoluteae* are short (Fig. 1), indicating a sudden diversification within this group. The genetic similarity is also reflected in morphology: all the *Fuscoluteae* are characterized by the occurrence of surface wax, papillose oil bodies, at least weakly trabeculate leaf cell pattern, dorsally overlapping male bracts, a capsule wall with thickenings in all layers, etc. Species discrimination results from differences in leaf shape and dentation, shape and size of underleaves, and presence and shape of paraphyllia (Heinrichs, 2002). However, nearly all extreme morphotypes are linked by intermediates, making it impossible to identify every collection without doubt. The *Fuscoluteae* are chemically also rather uniform. The *Fuscoluteae* investigated so far (*P. aerea*, *P. fuscolutea*, *P. heterophylla*, *P. longiramea*, *P. paraphyllina* and *P. tabinensis*; Asakawa & Inoue, 1987; Rycroft, 1998, 2003; Rycroft & Cole, 1998; Anton, 2001; Heinrichs & Rycroft, 2001; Heinrichs *et al.*, 2001) are characterized by the presence of numerous flavone-C-glycosides, plagiochiline-type secoaromadendranes and fusicoccanes (Anton, 2001). Only *P. tabinensis* is chemically somewhat isolated from other *Fuscoluteae* by the presence of plagiochiline M and related plagiochilines; the other *Fuscoluteae* investigated so far, produce plagiochiline A or C (Anton, 2001; Heinrichs *et al.*, 2001). The chemical differences are also reflected in the molecular topologies. *Plagiochila tabinensis* is placed sister to the remainder of the *Fuscoluteae* with good support.

The morphologically similar *Plagiochila fuscolutea*, *P. dependula, P. longiramea* and *P. paraphyllina* Herzog colonize the same habitat; open, humid cloud forests and (with the exception of *P. longiramea*) páramo vegetation. Among these four, *P. fuscolutea* is the most variable and widespread species (Heinrichs, 2002). Lack of stable morphological characters and diffuse distribution of extreme morphotypes currently prevents us from infraspecific subdivision of *P. fuscolutea*. *Plagiochila wolframii*, proposed here as a new synonym of *P. fuscolutea* is clearly different from many phenotypes of *P. fuscolutea* and stands out by the laterally appressed, strongly ampliate leaves. However, similar leaves occur in forms of *P. fuscolutea* that were described as *P. jelskii* Loitlesb. and *P. scopulosa* Steph. (Heinrichs, 2002). The type material of *P. scopulosa* (G!) is especially close to that of *P. wolframii*. However, the *P. scopulosa* type comprises plants with spreading as well as with laterally appressed leaves, throwing doubt on the usability of this character for *Fuscoluteae* taxonomy. Judging from the available material, *Plagiochila dependula* can be well separated from *P. fuscolutea* forms with laterally appressed leaves by the entire, reniform, dorsally short decurrent papillose leaves. Hence, species rank is maintained for the taxon.

In the nrITS trees, *Plagiochila aerea* with rectangular, spinosely toothed leaves cannot be separated from *P. dependula* and *P. fuscolutea* with ampliate, entire or sparsely triangularly toothed leaves, indicating that leaf shape and dentition may alter rapidly in *Plagiochila.* Similar observations exist for *P.* sect. *Hylacoetes* (Heinrichs *et al.*, 2003) and *P.* sect. *Arrectae* Carl (Heinrichs *et al.*, 2004b). As is the case with the latter groups, the *Fuscoluteae* also have their centre of diversity in moist neotropical forests above 2000 m. It has already been suggested that late Miocene uplift of the Andes (Gregory-Wodzicki, 2000) and the resulting more humid climate may be responsible for the diversification of these montane species groups of *Plagiochila* (Heinrichs *et al.*, 2003, 2005b).

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TAXONOMIC ADDITIONS AND CHANGES: *Plagiochila fuscolutea* Taylor (syn. *Plagiochila wolframii* Inoue).

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