

## The Systematic Position of *Plagiochila moritziana*, *P. trichostoma*, and *P. deflexa* Based on ITS Sequence Variation of Nuclear Ribosomal DNA, Morphology, and Lipophilic Secondary Metabolites

JOCHEN HEINRICHS, HENK GROTH AND INGO HOLZ

Albrecht-von-Haller-Institut für Pflanzenwissenschaften, Abteilung Systematische Botanik, Universität Göttingen, Untere Karspüle 2, D-37073 Göttingen, Germany

DAVID S. RYCROFT

Department of Chemistry, University of Glasgow, Glasgow G12 8QQ, Scotland, U.K.

CARSTEN RENKER

Institut für Ökologie, Universität Jena, Dornburger Strasse 159, D-07743 Jena, Germany

THOMAS PRÖSCHOLD

Botanisches Institut I, Universität zu Köln, Gyrhofstrasse 15, D-50931 Köln, Germany

**Abstract.** According to phylogenetic analyses of nrDNA ITS1 and ITS2 sequences (including the 5.8S unit) the Neotropical *Plagiochila moritziana*, *P. rutilans* var. *rutilans*, *P. rutilans* var. *standleyi*, *P. trichostoma* (= *P. permista*, syn. nov.), and *P. subtrinitensis* form a monophyletic lineage and are placed in *P. sect. Rutilantes*; all five taxa lack a ca 20 base pair sequence that is present in all the taxa of the other *Plagiochila* sections investigated. The Central American *P. subtrinitensis* is treated as a synonym of the Hawaiian endemic *P. deflexa*. *Plagiochila moritziana* is excluded from *sect. Fuscoluteae* and reduced to a variety of *P. rutilans*; *P. sect. Permistae* is treated as a synonym of *P. sect. Rutilantes*. The sporophytes of *P. trichostoma* and *P. deflexa* are described for the first time. Fresh material of *P. rutilans* var. *moritziana* exhibits a distinct odor of peppermint caused by the presence of several menthane monoterpenoids, principally pulegone. The Central American *P. rutilans* var. *standleyi* is reported from Ecuador, new to South America. Lectotypes are designated for *P. rutilans* var. *moritziana*, *P. subtrinitensis*, and *P. trichostoma*.

*Plagiochila*, with more than 1,600 binomials (Inoue 1989) and an estimated number of 400–450 species worldwide (So & Grolle 2000), is the largest genus of hepatics. Attempts to structure *Plagiochila* (e.g., Carl 1931; Inoue 1958, 1965; Inoue & Schuster 1971; Lindenberg 1839–1844; Schiffner 1901; Spruce 1884–1885) led to the description of more than 80 subgenera, sections, and subsections. Most of these subgeneric units were based solely on gametophytic features such as leaf shape and leaf position, leaf cell pattern, as well as shape and position of the perianths, and comprise only a few taxa each. Recent attempts to subdivide the genus consider both gametophytic and sporophytic features (e.g., Heinrichs & Gradstein 1999; Inoue 1984; Müller et al. 1999), as well as secondary metabolites (Anton et al. 2000; Heinrichs et al. 2000a), and often lead to a reduction of subgeneric units. Although analysis both of gametophytic and of sporophytic material (e.g., Heinrichs & Gradstein 1999; Heinrichs et al. 2000a; Müller et al. 1999) increased the number of taxonomically informative

characters, the number of morphological features is limited. Sporophytes and secondary metabolites of many *Plagiochila* species are still unknown and a satisfactory sectional placement of these species is often impossible.

Carl placed *P. trichostoma* Gottsche and *P. subtrinitensis* Herzog (= *P. deflexa* Mont. & Gottsche, this study) in his *P. sect. Superbae*, alongside species subsequently noted to have a capsule wall epidermis without wall thickenings (Heinrichs et al. 2001b). Sporophytic material of the former two species became available during the course of our study of Neotropical *Plagiochilae*, and the details observed cast doubt on Carl's sectional assignment of both species.

The Neotropical *Plagiochila moritziana* Hampe was collected only a few times in the 19th Century and only gametophytic material is available. Carl (1931) placed the species in his *sect. Caversiae* (= *sect. Fuscoluteae* Carl; Heinrichs & Gradstein 1999), containing species notable for the occurrence of leaf surface waxes (Heinrichs et al. 2000b).

TABLE 1. Geographic origins, voucher numbers, and GenBank accession numbers of the taxa investigated. Vouchers are deposited at GOET, duplicates of those marked with # were or are to be distributed in *Bryophyta Exsiccata Generis Plagiochilae* (BEGP, Heinrichs & Anton 2001). Sequences of species with asterisks were taken from EMBL/GenBank.

| Taxon  | Origin      | Voucher                                  | Accession number |
|--|-------------|--|------------------|
| * <i>Herbertus subdentatus</i> (Steph.) Fulford                              | Bolivia     | <i>Groth s.n.</i>                        | AJ413177         |
| * <i>Plagiochila bifaria</i> (Sw.) Lindenb.                                  | Tenerife    | <i>Drehwald 3922</i>                     | AJ413173         |
| * <i>P. buchtiniana</i> Steph.   | Bolivia     | <i>Groth s.n.</i>                        | AJ413306         |
| <i>P. deflexa</i> Mont. & Gottsche   | Costa Rica  | <i>Heinrichs et al. 4160, BEGP 10</i>    | AJ416083         |
| * <i>P. deflexirama</i> Taylor   | Costa Rica  | <i>Heinrichs et al. 4163, BEGP 14</i>    | AJ413310         |
| * <i>P. diversifolia</i> Lindenb. & Gottsche                                 | Ecuador     | <i>Holz EC-01-17</i>                     | AJ413308         |
| <i>P. fuscolutea</i> Taylor  | Costa Rica  | # <i>Heinrichs et al. 4400, BEGP 148</i> | AJ416086         |
| * <i>P. longispina</i> Lindenb. Gottsche                                     | Costa Rica  | <i>Heinrichs et al. 4148</i>             | AJ413307         |
| * <i>P. punctata</i> (Taylor) Taylor   | Scotland    | <i>Rycroft 01013</i>                     | AJ413174         |
| <i>P. rutilans</i> Lindenb. var. <i>rutilans</i>                             | Bolivia     | <i>Groth 101</i>                         | AJ416081         |
| <i>P. rutilans</i> var. <i>moritziana</i> (Hampe) J. Heinrichs               | Ecuador     | # <i>Holz EC-01-404, BEGP 131</i>        | AJ416080         |
| <i>P. rutilans</i> var. <i>standleyi</i> (Carl) J. Heinrichs & D. S. Rycroft | Ecuador     | # <i>Holz EC-01-499, BEGP 136</i>        | AJ416079         |
| * <i>P. sandei</i> Sande Lac.  | Philippines | <i>Schwarz 5732</i>                      | AJ413176         |
| * <i>P. spinulosa</i> (Dicks.) Dumort.                                       | Scotland    | <i>Rycroft 01012</i>                     | AJ413175         |
| <i>P. superba</i> (Sprengel) Mont. & Nees                                    | Costa Rica  | # <i>Heinrichs et al. 4175, BEGP 22</i>  | AJ416084         |
| <i>P. superba</i>  | Ecuador     | <i>Holz EC-01-65</i>                     | AJ416085         |
| * <i>P. tocarema</i> Gottsche  | Costa Rica  | <i>Heinrichs et al. CR199</i>            | AJ413309         |
| <i>P. trichostoma</i> Gottsche   | Costa Rica  | # <i>Heinrichs et al. 4324, BEGP 56</i>  | AJ416082         |
| * <i>P. virginica</i> A. Evans   | Tenerife    | <i>Rycroft 01068</i>                     | AJ413311         |

Ingo Holz collected fresh material of *P. moritziana* in Ecuador and recognized a distinct odor of peppermint in the species. Similar observations are well known from *Plagiochila rutilans* Lindenb. (Heinrichs et al. 2001a; Huneck et al. 1984; Rycroft & Cole 2001), type species of *P.* sect. *Rutilantes* Carl.

In this paper we analyze the phylogenetic relationships of *P. trichostoma*, *P. deflexa*, and the *Plagiochila rutilans* complex, based on nucleotide sequence variations in the internal transcribed spacer regions of nuclear ribosomal DNA, lipophilic secondary metabolites, and morphological features. The investigation expands a first study of nrDNA ITS sequence variation of members of sect. *Arrectae* Carl, *Cucullatae* Schiffn., *Contiguae* Carl, and *Glaucoscentes* Carl (Heinrichs et al. 2002).

#### MATERIALS AND METHODS

*Morphological studies.*—Using traditional microscopic techniques, we examined herbarium specimens from the herbaria B, BM, C, FH, FLAS, G, INB, JE, LPB, MANCH, M, MO, NY, PC, S, SAAR, U, US, and W and fresh material collected in Bolivia (1997, 2000), Costa Rica (1999, 2000), and Ecuador (2001). S.E.M. study of the leaf surfaces of *P. moritziana* (specimen *Holz EC-01-404*, Ecuador), *P. rutilans* var. *standleyi* (Carl) J. Heinrichs & D.S. Rycroft (specimen *Holz EC-01-499*, Ecuador), *P. deflexa* (specimen *Heinrichs et al. 4160*, Costa Rica), and *P. trichostoma* (*Heinrichs et al. 4323*, Costa Rica) was performed as described in Heinrichs et al. (2000b).

*Phytochemistry.*—The lipophilic secondary metabolites of *P. moritziana* (*Holz EC-01-408*, Ecuador), *P. rutilans*

var. *standleyi* (*Holz EC-01-499*, Ecuador), and *P. trichostoma* (*Heinrichs et al. 4323 4324*, Costa Rica) were determined using GC-MS and/or NMR profiling of deuteriochloroform extracts (Rycroft 1996, 1998; Rycroft & Cole 2001).

*DNA extraction.*—Material to be sequenced (Table 1) was carefully cleaned before drying. Upper parts of shoots were preserved over silica gel prior to extraction of genomic DNA using PUREGENE Genomic DNA Isolation Kit (Gentra Systems).

*PCR amplification, cloning, and sequencing.*—The PCR primers P1 (5'-TGT ACA CAC AAT GCA GCA AAC CAG CG-3') and P2 (5'-CGG GTA ATC TTG CCT GAT CTG AG-3') were used to amplify the internal transcribed spacer (ITS) of the ribosomal DNA that encompasses the 5.8S gene and both the ITS1 and ITS2 regions. Amplification by the polymerase chain reaction (PCR, Saiki et al. 1988) was performed in a total volume of 50  $\mu$ l containing 2U Taq-DNA-polymerase (Promega, Heidelberg, Germany), 5  $\mu$ l Taq polymerase reaction buffer (Promega), 4  $\mu$ l 25 mM MgCl<sub>2</sub>, 4  $\mu$ l dNTP-Mix (2.0 mM each, MBI Fermentas, St. Leon-Rot, Germany), one  $\mu$ l of each of the two primers and one  $\mu$ l of the genomic DNA (100–500 ng). The reactions were performed in 32 cycles under the following conditions: 40 sec. denaturation at 94°C, 30 sec. annealing at 54°C, and 40 sec. elongation at 72°C—using hot-start-PCR with 10 min. of denaturation at 94°C before adding the Taq at 80°C. Finally there were 10 min. of elongation at 72°C.

PCR products were cloned into the pCR4-Topo Vector (Invitrogen) following the TOPO TA Cloning Kit manufacturer's protocol and transformed into TOP10 Chemically Competent *Escherichia coli* DH5 $\alpha$  by the heat shock method (Sambrook et al. 1989). Sequencing was done on an LI-COR DNA Sequencer Long Reader 4200 using the thermo Sequenase fluorescent labelled primer cycle sequencing kit with 7-deaza-dGTP (Amersham Pharmacia Biotech, Little Chalfont, England).

*Phylogenetic analyses.*—Phylogenetic trees were in-

ferred using distance (neighbour joining; NJ), maximum parsimony (MP), and maximum likelihood (ML) criteria using PAUP\* version 4.0b8 (Swofford 1998). An alignment of 19 nrDNA ITS1-, 5.8S-, and ITS2-sequences of *Herbertus subdentatus* and 17 taxa of *Plagiochila* (Table 1) with 828 aligned positions was used as the data set (alignment available from J.H.). To decide on the evolutionary model that fits the data best, the program Modeltest 3.04 (Posada & Crandall 1998) was used that employs two statistics: the likelihood ratio test (LRT) and the Akaike information criterion (AIC, Akaike 1974). Based on the results of the tests, the model selected by the hierarchical LRT was the HKY85 model (Hasegawa et al. 1985) with gamma shape parameter (G) for among site variation calculated from the data set (HKY85 + G). The ML method (with the HKY85 + G model) was used for phylogenetic analyses.

The confidence of branching was assessed using 100 bootstrap resamplings in ML-analysis (using the HKY85 + G model) and 1,000 bootstrap resamplings in distance (neighbour joining method using the HKY85 + G model) and unweighted MP (with 10 addition-sequence replicates) of the data set (Felsenstein 1985).

User defined trees were generated by modifying the tree file of the "best tree" (Fig. 1) using TreeVIEW (version 1.6.2, Page 1996). To compare user defined topologies with the "best tree", the sequence data file was loaded into PAUP\* and used for Kishino-Hasegawa-tests (KH, Kishino & Hasegawa 1989); comparisons [KH tests using bootstrap (1,000 replicates) with full optimization, one-tailed test] were based on ML (model of evolution selected by Modeltest) and MP criteria (Table 2).

## RESULTS AND DISCUSSION

*Morphology* (see also TAXONOMIC TREATMENT).—*Plagiochila rutilans* (type species of *P.* sect. *Rutilantes*), *P. rutilans* var. *standleyi*, *P. moritziana*, *P. deflexa*, and *P. trichostoma* agree in the form of the androecia, perianth shape, and position of the female bracts. Furthermore, *P. rutilans* (Heinrichs et al. 2001a: Figs. 4 and 5), *P. trichostoma* (Fig. 3), and *P. deflexa* (Fig. 5) show a remarkably high degree of similarity in sporophytic features, leading to the hypothesis of a close relationship. Carl (1931) placed *P. deflexa* (as *P. subtrinitensis*) and *P. trichostoma* in sect. *Superbae* Carl (*nom. illeg.*), containing species with a capsule wall epidermis without wall thickenings (Heinrichs et al. 2001b). However, both *P. deflexa* and *P. trichostoma* have epidermal capsule wall thickenings, throwing doubt on Carl's (1931) sectional placement.

Sporophytes of *P. moritziana* are still unknown. Carl placed *P. moritziana* in his sect. *Caversiae*. Heinrichs and Gradstein (1999) treated sect. *Caversiae* as a synonym of sect. *Fuscoluteae* Carl, and Heinrichs et al. (2001b) demonstrated that the *Fuscoluteae* are distinguished by the presence of leaf surface waxes. S.E.M. study of the leaf surface of *P. moritziana* (as well as of *P. rutilans* var. *standleyi*, *P. deflexa*, and *P. trichostoma*) revealed no trace of wax. Thus, assignment to sect. *Caversiae*

(Carl 1931) seems inappropriate and placement within sect. *Rutilantes* probable.

*Phytochemistry*.—The lipophilic compounds (deuteriochloroform extract) of *P. moritziana* are broadly similar to those of *P. rutilans* s. str. The six most abundant compounds are (in parentheses: % w/w extracted from Holz EC-01-408): pulegone (3), terpinolene (0.6),  $\beta$ -phellandrene (0.2), limonene (0.2), bicyclogermacrene (0.1), and menthone (0.1). Peculiaroxide (0.05) and 2-methoxy-6-prenylhydroquinone (0.04) are less abundant than is generally the case in *P. rutilans* s. str. (Heinrichs et al. 2001a; Rycroft & Cole 2001).

Secondary metabolites of *P. rutilans* var. *standleyi* from Costa Rica were reported recently (Heinrichs et al. 2001a; Rycroft & Cole 2001). This taxon has now been found, new to South America, at an elevation of 1,000 m in the Podocarpus National Park near Zamora, Zamora-Chinchipec, Ecuador, and the lipophilic compounds in the deuteriochloroform extract of a 20 mg sample of the gathering have been investigated. The five most abundant compounds are (in parentheses: % w/w extracted from Holz EC-01-499): 3-hydroxy-4'-methoxybibenzyl (6), limonene (2),  $\beta$ -phellandrene (1),  $\alpha$ -terpinene (1), and bicyclogermacrene (0.2). The chemical profile is rather similar to those of the two Costa Rican specimens of var. *standleyi* investigated previously, but the amount of ascaridole observed in the Ecuadorian specimen (0.1 % w/w) is less. Neither peculiaroxide, spathulenol, nor fusicoccadiene was detected.

The three most abundant lipophilic compounds of *P. trichostoma*, based on GC-MS analysis of the deuteriochloroform extract of Heinrichs et al. 4323, are (in parentheses: GC-MS TIC relative integration): 4-hydroxy-3'-methoxybibenzyl (100),  $\beta$ -phellandrene (42; from NMR: 0.3 % w/w extracted from the liverwort), anastreptene (15). Spathulenol was present but peculiaroxide and fusicoccadiene were not detected. The proton NMR spectra of the extracts from Heinrichs et al. 4323 and 4324 were similar, but close-clustered signals at  $\delta$ 2.85 and  $\delta$ 3.78 prevented signal integration and quantification of 4-hydroxy-3'-methoxybibenzyl; these signals suggested the presence of large amounts of other compounds, not observed by GC-MS, containing methoxybenzyl moieties and possibly derived from 4-hydroxy-3'-methoxybibenzyl. A detailed chemical investigation of *P. trichostoma* has been undertaken by Anton (pers. comm.).

The secondary metabolites of *P. deflexa* have been investigated by Anton et al. (1999; chemistry voucher: SAAR, Gradstein & Mues 5670, Costa Rica), when the material had been determined to genus only; several bibenzyl and both cyclic and acyclic bisbibenzyl derivatives were reported.

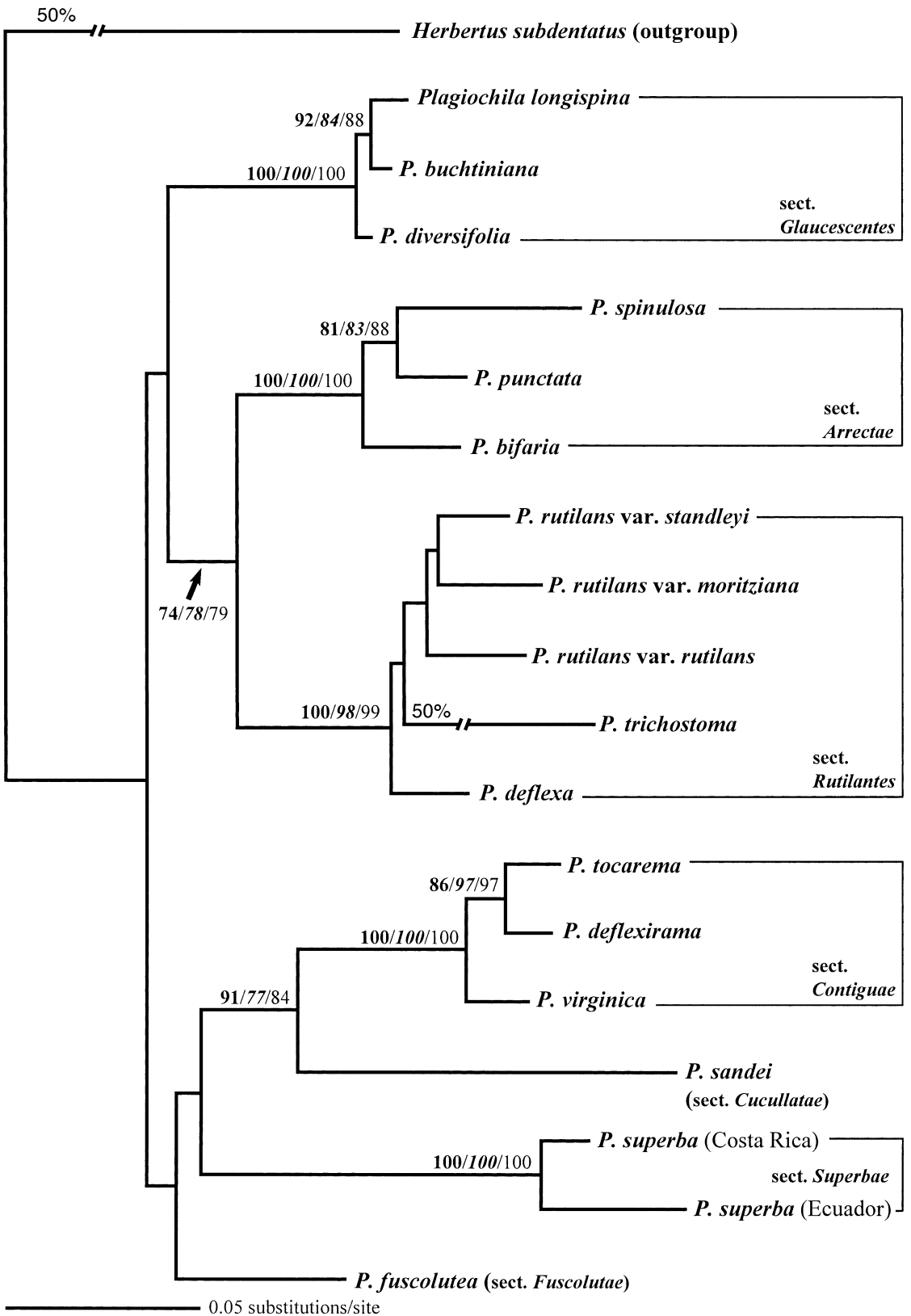


TABLE 2. Comparisons of the maximum likelihood tree in FIGURE 1 with user-defined trees by Kishino-Hasegawa-tests (using bootstrap with 1,000 bootstrap replicates, with full optimization, one-tailed test) using maximum likelihood (ML) and maximum parsimony (MP) methods.

| Tree topology <sup>a</sup> | ML (HKY85+G) <sup>a</sup> |                       | MP                        |           |
|----------------------------|---------------------------|-----------------------|---------------------------|-----------|
|                            | Diff-InL <sup>c</sup>     | <i>p</i> <sup>d</sup> | Diff. length <sup>e</sup> | <i>p</i>  |
| 1. (Best tree; Fig. 1)     | (4520.6) <sup>f</sup>     | —                     | (695) <sup>g</sup>        | —         |
| 2. (Pfuscol; Pmoritz)      | 94.0                      | < 0.001*              | 38                        | < 0.0001* |
| 3. (Pmoritz; Pfuscol)      | 118.0                     | < 0.001*              | 46                        | < 0.0001* |
| 4. (Psuperba; Ptrichos)    | 48.8                      | < 0.001*              | 18                        | 0.0066*   |
| 5. (Ptrichos; Psuperba)    | 52.2                      | < 0.001*              | 17                        | 0.0151*   |
| 6. (Psuperba; Pdeflexa)    | 51.2                      | < 0.001*              | 31                        | < 0.0001* |
| 7. (Pdeflexa; Psuperba)    | 101.9                     | < 0.001*              | 37                        | < 0.0001* |

<sup>a</sup> Maximum likelihood (ML) using the model after Hasegawa et al. (1985) with estimated gamma shape and transition/transversion ratio (HKY85+G).

<sup>b</sup> Tree no. 1 (best tree) is identical with Fig. 1; modifications in use-defined trees no. 2–7 are indicated by following abbreviations:

Tree 2: *Plagiochila fuscolutea* (Pfuscol) as sister to *Plagiochila rutilans* var. *moritziana* (Pmoritz).

Tree 3: *Plagiochila rutilans* var. *moritziana* (Pmoritz) as sister to *Plagiochila fuscolutea* (Pfuscol).

Tree 4: *Plagiochila superba* (both specimen; Psuperba) as sister to *Plagiochila trichostoma* (Ptrichos).

Tree 5: *Plagiochila trichostoma* (Ptrichos) as sister to *Plagiochila superba* (both specimen; Psuperba).

Tree 6: *Plagiochila superba* (both specimen; Psuperba) as sister to *Plagiochila deflexa* (Pdeflexa).

Tree 7: *Plagiochila deflexa* (Pdeflexa) as sister to *Plagiochila superba* (both specimen; Psuperba).

<sup>c</sup> Difference in -log-likelihood between the best tree (Fig. 1) and the user-defined tree.

<sup>d</sup> Probability of getting a more extreme T-value under the null hypothesis of no difference between the two trees (one-tailed test).

<sup>e</sup> Difference in tree length between the best tree (Fig. 1) and the user-defined tree.

<sup>f</sup> -log-likelihood of best tree (Fig. 1).

<sup>g</sup> Length of the optimal tree in the maximum parsimony analysis.

\* User defined tree significantly worse than the best tree at *p* < 0.05.

Although only *P. rutilans* var. *rutilans*, *P. rutilans* var. *standleyi*, and *P. moritziana* exhibit an odor of peppermint, in other respects all five taxa show rather similar chemistry and are characterized by the presence of phenolic compounds (bibenzyl or prenylhydroquinone derivatives) rather than 2,3-secoaromadendranes (plagiophilines). This chemical affinity extends to members of sect. *Arrectae* (Rycroft et al. 1999), where the phenolic class of compounds is represented by bibenzyl, 9,10-dihydrophenanthrene and benzoic acid derivatives.

**Sequence analysis.**—The molecular investigation (Fig. 1) produced a monophyletic lineage for *P. rutilans*, *P. rutilans* var. *standleyi*, *P. moritziana*, *P. deflexa*, and *P. trichostoma*. *Plagiochila rutilans* var. *standleyi* and *P. moritziana* are sister to *P. rutilans* var. *rutilans*. This finding requires reduction of *P. moritziana* to a variety of *P. rutilans* (see below). *Plagiochila trichostoma* and *P. deflexa* are placed at the base of the *Rutilantes* clade. A striking

similarity is found in the ITS1 sequences of *P. deflexa*, *P. trichostoma*, and members of the *P. rutilans* complex. At the end of the ITS1 spacer, all five taxa lack a sequence of about 20 base pairs that is present in all the other members of *Plagiochila* sect. *Arrectae*, *Contiguae*, *Cucullatae*, *Fuscoluteae*, *Glaucescentes*, and *Superbae* investigated. Carl (1931) erroneously treated *P. permista* (type species of *P.* sect. *Permistae*) and *P. trichostoma* as distinct species; based on our results, we combine all five taxa in sect. *Rutilantes* and include sect. *Permistae* in sect. *Rutilantes*.

Placement of *P. trichostoma* and *P. deflexa* in sect. *Superbae* (Carl 1931) as well as *P. rutilans* var. *moritziana* in sect. *Caversiae* (= sect. *Fuscoluteae*) is not supported by the molecular study: *Plagiochila trichostoma*/*P. deflexa* and *P. superba* as well as *P. rutilans* var. *moritziana* and *P. fuscolutea* are placed in different clades.

To evaluate the significance of the phylogenetic

←

FIGURE 1. Molecular phylogeny of *Plagiochila* species based on ITS1-, 5.8S-, and ITS2-nrDNA sequence comparisons using 828 aligned positions. The rooted tree resulted from a maximum likelihood analysis of 19 sequences (including the outgroup sequence), using the HKY85 (HKY85 + G; Hasegawa et al. 1985) model with estimated gamma shape ( $\Gamma = 0.645$ ) and transition/transversion ratio (Ti/Tv = 1.638), calculated as the best model by Modeltest 3.04 (Posada & Crandall 1998); bootstrap percentage values (> 50%) were determined for maximum likelihood (using HKY85 + G; bold), neighbor-joining (HKY85 + G; bold italics) and unweighted maximum parsimony (not bold) methods.

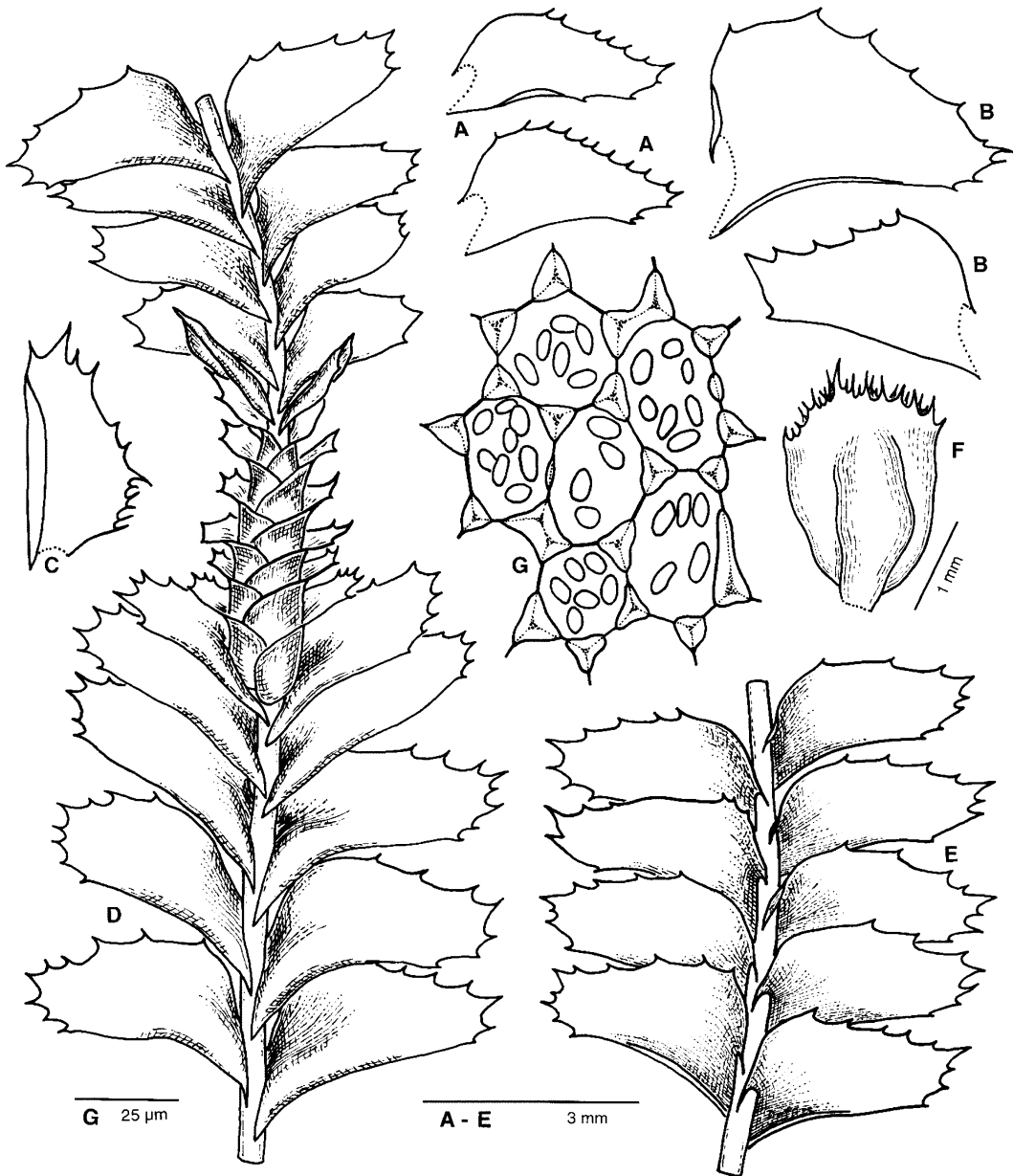


FIGURE 2. *Plagiochila rutilans* var. *moritziana* (Hampe) J. Heinrichs. — A-B. Leaves. — C. Female bract. — D. Part of male shoot with androecium, dorsal view. — E. Part of shoot, ventral view. — F. Perianth, lateral view. — G. Cells from leaf center with oil bodies. [A from lectotype of *P. rutilans* var. *moritziana* (w, Moritz 102), B-G from Holz EC-01-404 (GOET)].

inferences, user-defined trees were created and used for KH tests (Table 2). KH tests clearly reject monophyletic lineages of *P. rutilans* var. *moritziana* and *P. fuscolutea* as well as *P. trichostoma* or *P. deflexa* and *P. superba*.

The molecular investigation places the *Rutilantes* sister to sect. *Arrectae*. The relationships between the *Rutilantes/Arrectae*, *Contiguae/Cucullatae*, *Fuscoluteae*, *Superbae*, and *Glaucescentes* are not resolved by the present molecular study.

#### TAXONOMIC TREATMENT

**PLAGIOCHILA** sect. **RUTILANTES**, Carl, Ann. Bryol. 2 (suppl. 2): 74. 1931. TYPE (art. 22.6 ICBN): *Plagiochila rutilans* Lindenb.

= *Plagiochila* sect. *Permistae* Carl, Ann. Bryol. 2 (suppl. 2): 74. 1931. TYPE (art. 22.6 ICBN): *Plagiochila permista* Spruce (= *P. trichostoma* Gottsche, see below).

Branches lacking or moderate in number, (near-

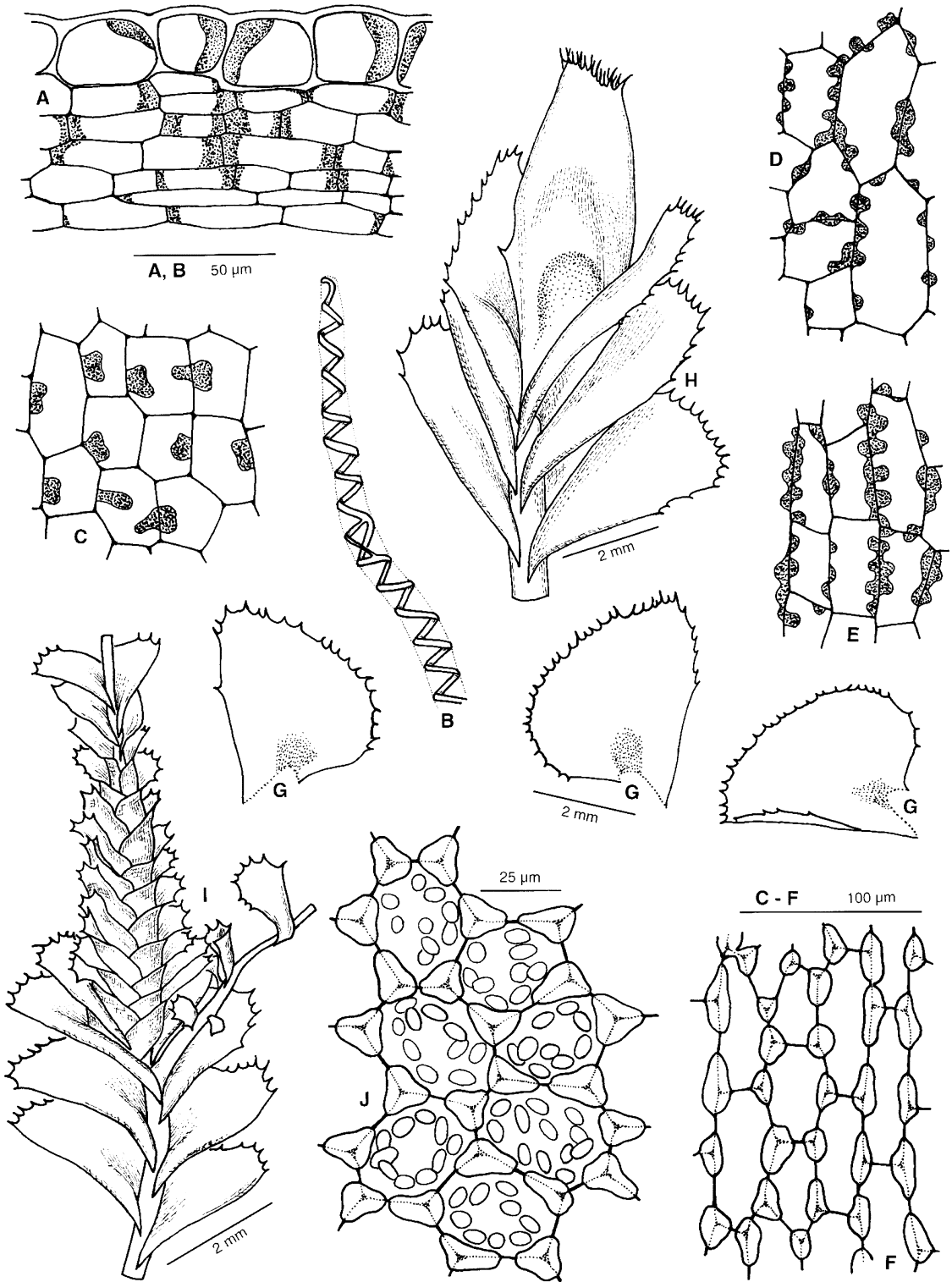


FIGURE 3. *Plagiochila trichostoma* Gottsche. — A. Transverse section of capsule wall. — B. Part of elater. — C. Capsule wall epidermis, surface view. — D. Subepidermal layer of capsule wall, surface view. — E. Innermost layer of capsule wall, surface view. — F. Basal leaf cells. — G. Leaves. — H. Top of female plant with perianth bearing young sporophyte, dorso-lateral view. — I. Part of male plant with androecium, dorsal view. — J. Cells from leaf center with oil bodies. [A–E, J from Heinrichs et al. 4324 (GOET), F–H from lectotype of *P. trichostoma* (G 025342), I from isolectotype of *P. trichostoma* (G)].

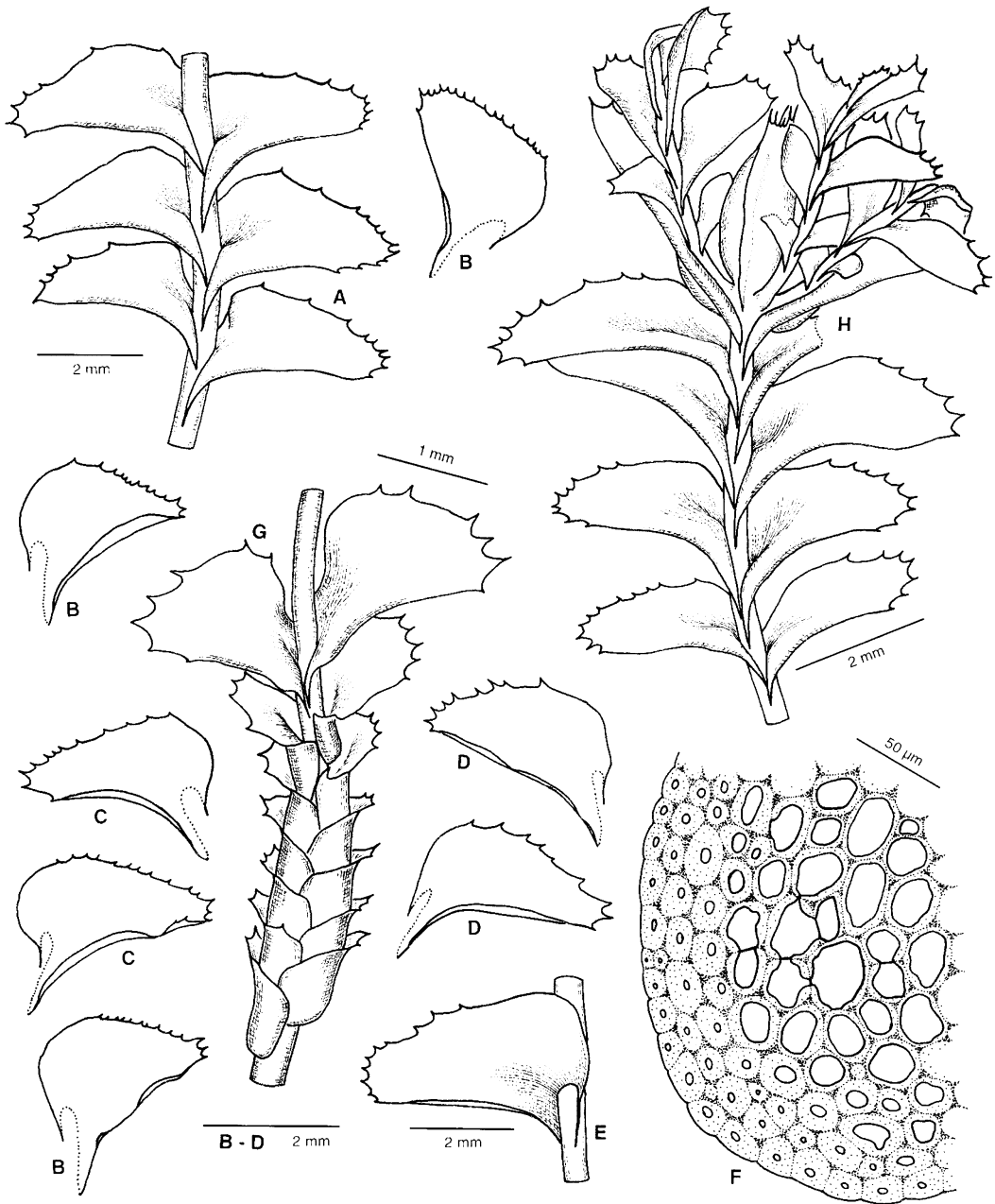


FIGURE 4. *Plagiochila deflexa* Mont. & Gottsche. — A. Part of shoot, dorsal view. — B, C, D. Leaves. — E. Part of stem with leaf, ventral view. — F. Transverse section of stem. — G. Upper part of androecium, dorsal view. — H. Upper part of female plant with perianth, dorsal view. [A, D, F from lectotype of *P. deflexa* (G 001157, Gaudichaud s.n.), B, E from lectotype of *P. subtrinitensis* (JE, Standley 38198), C from Valerio 2 (JE), G from Heinrichs et al. 4171 (GOET), H from Heinrichs et al. 4170 (GOET)].

ly) exclusively of lateral intercalary type. Underleaves vestigial or lacking. Oil bodies colorless, subglobose or ellipsoidal to fusiform, homogeneous to indistinctly coarse segmented. Androecia becoming intercalary, simple, opposite bracts overlapping on dorsal side of shoots. Female bracts  $\pm$  leaflike, covering basal part of perianth or inserted below

perianth. Perianths  $\pm$  elliptical in dorsal view, wingless or dorsal keel with low arch/wing. Capsules subglobose, valves with thickenings in all layers, epidermal cells in surface view with slightly thickened walls and few large, nodulose thickenings; innermost cells in surface view in  $\pm$  irregular pattern, most walls with nodulose to cone-like



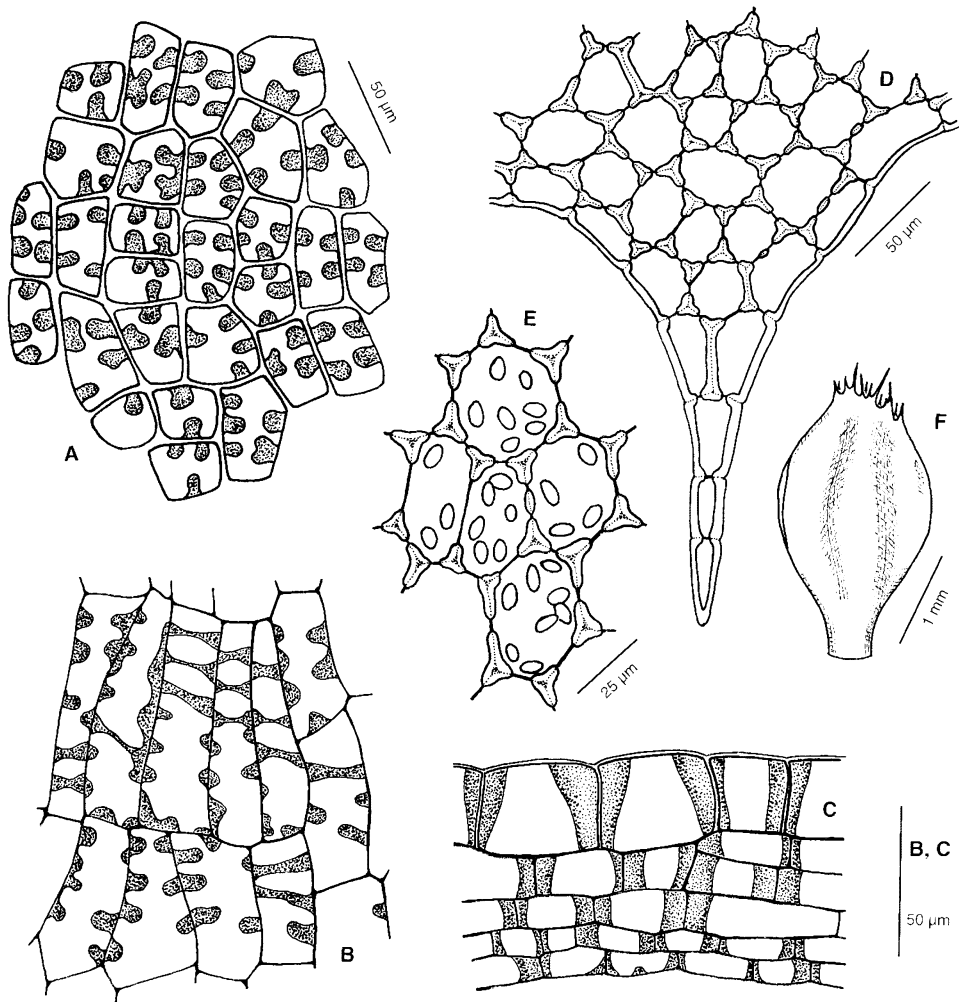


FIGURE 5. *Plagiochila deflexa* Mont. & Gottsche. — A. Cells of capsule wall epidermis, surface view. — B. Innermost capsule wall cells, surface view. — C. Transverse section of capsule wall. — D. Tooth from ventral leaf margin. — E. Cells from leaf center with oil bodies. — F. Perianth, lateral view. [A–C from *Valerio 2* (JE), D from lectotype of *P. deflexa* (G 001157, *Gaudichaud s.n.*), E, F from *Heinrichs et al. 4170* (GOET)].

thickenings often confluent at their base and partially coalesced. Spores 1(–2)-celled. Elaters 1–2(in middle, rarely, 3) spiral.

*Distribution.*—Neotropics, Hawaiian Islands (*P. deflexa*).

***PLAGIOCHILA RUTILANS* Lindenb. var. *MORITZIANA*** (Gottsche & Lindenb. *ex* Hampe) J. Heinrichs, *comb. et stat. nov.*

*Plagiochila moritziana* Gottsche & Lindenb. *ex* Hampe, *Linnaea* 20: 323. 1847. TYPE: VENEZUELA. Colonia Tovar, *Moritz 102* [lectotype, here designated, w hb. Lindenb. 588 (*c. per.*); isolectotypes, BM (2 specimens, *c. per.*), M (*c. per.*), MANCH CC9116 (*c. per. juv.*), S (*c. per.*)].

*Gametophyte.*—Plants with odor of peppermint (fresh material), medium to large in size, (3–)5–

15(–20) cm long and 4–8 mm broad, olive green to brownish green, becoming brownish in herbarium, in diffuse patches, with differentiation into short creeping stoloniform shoots giving rise to ascending or pendent leafy stems. Stems brown, near base ca 380–600 × 320–450 μm, dorsally moderately to widely exposed, ventrally widely exposed to completely covered by leaves, in transverse section ca 19–30 × 16–26 cells across, the cortical cells in (2–)3–4(–6) layers, distinctly thick-walled, ca 14–30 × 10–25 μm, medullary cells thin-walled to slightly thick-walled, in transition zone to cortex sometimes moderately thick-walled, ca (16–)20–30(–35) × (12–)18–26(–32) μm, trigones small, triangular or lacking. Branches lacking to moderate, lateral-intercalary. Creeping stoloniform shoots partly beset with short leaflets, leaves of aerial

shoots wide spreading or weakly bent to ventral side, in dry condition not or weakly inrolled, moderately, occasionally densely imbricate, on top of shoots and on weaker plants often remote, at least on main shoots and stronger branches with distinct ventral shoulder, occasionally weakly ampliate, triangular, elongate-triangular, or triangular-ovate, on weaker branches and shoot tops sometimes narrowly oblong-ovate to oblong, with acute to truncate apex; leaves of main stems ca (2.1–)2.4–3.5(–3.9) × (1.1–)1.3–2.3(–2.6) mm and ca 1.4–1.9(–2.2) times as long as wide, those of weaker branches and shoot tops somewhat smaller and up to 2.4 times as long as wide, ventral margin flat or weakly recurved above leaf base, short to moderately decurrent as an acute strip, basal 2/3 of dorsal margin often recurved, short to long decurrent along dorsal midline of stem, decurrent strip in lower parts often small and acute. Leaves toothed, teeth restricted to apex, upper half of dorsal margin and upper 1/4–3/4 of ventral margin, occasionally complete ventral margin toothed, teeth triangular to elongate triangular, (1–)2–3(–4) cells wide at base and (1–)2–6(–8) cells long, with moderately elongate terminal cell, apical teeth usually larger, sometimes lobe-like and with up to 2 accessory teeth at base, teeth straight, occasionally curved, perpendicular to leaf margin or pointed forwards, occasionally pointed backwards, apical teeth pointing in various directions, ventral margin of stem leaves with (1–)3–10(–12) teeth, apex with (1–)2–4 teeth, dorsal margin with 0–3 teeth. Leaf areolation ± regular, cells in upper leaf half isodiametric to moderately elongate, in center of upper leaf half (28–)30–45(–50) × (18–)20–35(–40) μm and 1.0–1.5(–1.7) times as long as wide, cells towards leaf base more elongate, (35–)40–80(–90) × 25–40 μm and 1.3–3.4 times as long as wide; walls thin except those along leaf margins, trigones (small to) medium sized to large, subnodulose to nodulose, often with rounded corners, subconfluent or confluent on long walls, especially towards leaf base, intermediate thickenings ± frequent on long walls; cuticle smooth. Oil bodies colorless, (5–)7–11(–13) per median leaf cell, 6–10 × 3–4 μm, spheric to ellipsoidal, homogeneous or indistinctly coarse-segmented. Underleaves lacking or vestigial, minute, built by few short cilia or lanceolate. Asexual reproduction by specialized structures not observed. Male plants as large as female plants or somewhat smaller; androecia simple, becoming intercalary, bracts in 5–17 pairs, imbricate, overlapping dorsally, strongly inflated basally, margin of inflated part entire, with thin-walled, partly bulging marginal cells, distal part obliquely spreading, margin with 1–3 blunt to acute, triangular teeth, upper half of bracts composed of cells similar to those of leaves, towards

base with partly weakly bulging cells containing somewhat less chlorophyll than those of leaves and bearing weaker trigones. Antheridia 1–2 per bract, on stalk ca 12–24 cells long, globose to broadly ellipsoid. Gynoecia terminal on main shoots and on branches, 1–2 subgynoecial innovations frequent, often again with perianths, bracts similar to leaves in shape but dorsally and ventrally hardly to moderately decurrent, dorsal margin strongly recurved, often somewhat more densely toothed; perianths elliptic in dorsal view, broadly cylindrical in lateral view, 1.7–2.4 × 1.5–1.9 mm and 1.0–1.3 times as long as wide, dorsal keel often with low, smooth arch, perianth mouth arched, with coarse, elongate triangular teeth.

*Sporophyte*.—Unknown.

*Phytochemistry*.—Lipophilic compounds.—Broadly similar to var. *rutilans*: several menthane monoterpenoids, principally pulegone; peculiaroxide and 2-methoxy-6-prenylhydroquinone present, but less abundant than is generally the case in var. *rutilans* (Heinrichs et al. 2001a; Rycroft & Cole 2001).

*Ecology and distribution*.—*Plagiochila rutilans* var. *moritziana* is known from only a few localities in the northern and central Andes (southwards to Ecuador), and the West Indies. In Ecuador the species grows on trunks in a montane cloud forest at about 2,000 m.

*Specimens examined*.—COLOMBIA. *Goebel s.n.* (BM). ECUADOR. ZAMORA-CHINCHIPE. Estación Científica San Francisco along the highway Loja-Zamora at km 30, Quebrada 2, 1,900–2,100 m, 2001, *Holz EC-01-404, EC-01-408* (GOET). HISPANIOLA (S). VENEZUELA. MÉRIDA. Mérida, *Moritz s.n.* (BM).

#### KEY TO THE VARIETIES OF *P. RUTILANS*

1. Leaves triangular, elongate-triangular, or triangular-ovate ..... *P. rutilans* var. *moritziana*
1. Leaves oblong ..... 2
2. Herbarium specimens greenish brown or brownish after moistening, leaves dorsally hardly to moderately decurrent ..... *P. rutilans* var. *rutilans*
2. Herbarium specimens blackish after moistening, leaves dorsally moderately to long decurrent ..... *P. rutilans* var. *standleyi*

**PLAGIOCHILA TRICHOSTOMA** Gottsche in Triana & Planchon, Ann. Sci. Nat., Bot. (ser. 5)1: 113. 1864. TYPE: COLOMBIA. Bogotá: Tequendama, 2,500 m, 1861, *Lindig 1720b* (lectotype, here designated, G 025342 [c. per., male]; isolectotypes, G [3 specimens, c. per., male], JE [c. per.], M [c. per., male]; holotype [B] destroyed).

= *Plagiochila permista* Spruce, Trans. Proc. Bot. Soc. Edinburgh 15: 481. 1885, *syn. nov.* TYPE: ECUADOR, Tungurahua, Mt. Tunguragua, *Spruce s.n.* (holotype, MANCH CC9200 [c. per.]; isotype, G [ster., scanty, only fragments of 2 leaves]).

*Gametophyte*.—Plants without odor of peppermint (fresh material), medium to large in size, (2.5–)4–12(–16) cm long and (4.5–)5–7(–7.5) mm wide (flattened), with differentiation into creeping stoloniform shoots giving rise to prostrate or ascending leafy stems. Leafy stems dorsally and ventrally flattened, near base ca 480–600 × 420–510 μm, brown, in upper parts often pale brown, well visible between the leaves in dorsal view, ventrally moderately to completely covered by leaves, in transverse section ca 22–28 × 23–30 cells across, the cortical cells in 4–6(–8) layers, very thick-walled, the outer layer sometimes moderately thick-walled only, ca 15–25 × 10–20 μm, medullary cells thin-walled to slightly thick-walled, 20–32(–40) × 17–22(–35) μm, trigones lacking or small, triangular. Branches lacking to moderate, of the lateral-intercalary type. Creeping stoloniform shoots often beset with short leaflets. Leaves of aerial shoots ± moderately secund to ventral side or ± wide spreading and directed transversally to stems, in dry condition weakly to distinctly inrolled, imbricate, on weaker shoots occasionally remote, with distinct ventral shoulder or ampliate, triangular, ovate-triangular or ovate with subtruncate, acute or rounded, occasionally irregularly truncate apex, ca 2.6–3.6 × 2.4–3.4 mm and 0.9–1.3 times as long as wide, ventral margin flat, hardly to moderately decurrent with narrow strip, dorsal margin moderately to strongly recurved, occasionally ± flat, hardly to moderately decurrent along dorsal midline of stem. Leaves toothed, teeth often lacking on basal half of dorsal margin, mature leaves in all with (5–)16–44 teeth. Teeth of dorsal leaf margin forward pointed, others pointing in various directions, straight or curved, elongate-triangular, 1–5 cells wide at base and (1–)2–6(–8) cells long, apical cells elongate, blunt or acute. Leaf areolation ± regular, cells in upper two-thirds of leaf shorter than wide to slightly elongate, cells in center of upper leaf half ca (24–)35–45(–50) × (24–)29–37(–44) μm and (0.8–)0.9–1.4(–1.6) times as long as wide, cells above leaf base moderately to strongly elongate, forming broad vitta, ca (40–)50–80(–92) × 24–34 μm and (1.2–)1.6–3.2(–3.7) times as long as wide; cell walls thin except those along leaf margins, trigones medium sized to large, at leaf base usually large, subnodulose or nodulose, especially towards leaf base, sometimes subconfluent or confluent, especially near leaf margins, forming weak, inconspicuous yellowish marginal border, intermediate thickenings often present on long walls of elongate cells; cuticle smooth. Oil bodies opaque, colorless, ca 7–16 per median leaf cell, 3–6 × 5–9 μm, spheric to ellipsoidal, homogeneous. Underleaves lacking or vestigial, small, variously ciliate or only short cilia present. Asexual reproduction by specialized structures not observed.

Male plants as large as female plants or somewhat smaller. Androecia simple, becoming intercalary; bracts in ca 7–18 pairs, imbricate, opposite bracts overlapping dorsally, strongly inflated basally, inflated part sometimes with some bulging, ± thin-walled marginal cells, distal part obliquely spreading, with 2–7 elongate-triangular, straight or curved teeth, ventral base of bracts usually entire; bracts in upper half composed of cells similar to those of leaves, towards base composed of ± hyaline, elongate, p.p. somewhat bulging cells with small, triangular to subnodulose trigones. Antheridia usually 2 per bract, on long stalk. Gynoeceia terminal on main shoots and on branches, usually with 1–2 innovations (also under sporophytes), female bracts broadly suborbicular to asymmetrically ovate, 3.7–4.5 × 4.1–4.7 mm (flattened) and 0.8–1.0 times as long as wide, dorsal margin usually strongly recurved, teeth present on complete margin except basal third of dorsal margin (occasionally few teeth present near dorsal leaf base), bracts in all with 35–47 elongate triangular to ciliate, straight or curved teeth pointing in various directions, teeth (1–)2–3(–5) cells wide at base and (1–)3–8(–12) cells long, occasionally forked once near base, areolation similar to that of leaves but vitta usually broader. Perianths broadly cylindrical to elliptic in lateral view, cylindrical in dorsal view, usually lengthening during development of sporophyte, lower 2/3–3/4 (1/2 after fertilization) usually covered by bracts, perianths ca 2.7–3.6 mm broad and 4.8–5.5 mm long, after fertilization up to 8 mm long, ca 1.5–2.4 times as long as wide, dorsal keel usually slightly longer than ventral one, wingless or basal half with low, 0–2 toothed wing, perianth mouth truncate or weakly arched upwards, densely beset with slender, elongate triangular to ciliate teeth, teeth 1–2(–4) cells wide at base and (3–)5–12(–14) cells long, larger ones occasionally forked once near base.

*Sporophyte*.—Capsule moderately exserted, long elliptic in lateral view; valves straight, not twisted, brown, ca 3.1–3.4 × 1.1–1.3 mm, ca 2.4–3.0 times as long as wide, epidermal cells in surface view mostly elongate, with few thickenings on most longitudinal and some transverse walls, thickenings large, nodulose to cone-like, often somewhat branched; hypodermal cells in all more elongate and with more thickenings, thickenings smaller than those of epidermal cells, nodulose; innermost cells moderately to strongly elongate and somewhat irregular, with small to medium sized nodulose thickenings both on longitudinal and transverse walls, thickenings discrete, occasionally fused at their base, especially towards margins of valve; valves in transverse section ca 80–95(–100) μm thick, 7–10-stratose, epidermal cells larger than inner ones, ca 18–30 μm thick, inner cells ca 5–12

$\mu\text{m}$  thick. Spores trilete, ca 21–34  $\mu\text{m}$  in diameter, globose to slightly elongate, 1(–2) cellular, sporoderm with  $\pm$  densely spaced bacula and pila. Elaters 1–2 spiral, ca 7–9  $\mu\text{m}$  in diameter, surface  $\pm$  smooth to moderately rough.

**Phytochemistry.**—Lipophilic compounds.—4-hydroxy-3'-methoxybibenzyl most abundant by GC–MS, followed by  $\beta$ -phellandrene and anastreptene. Spathulenol present, but peculiaroxide and fusicocadiene not detected.

**Ecology and distribution.**—*Plagiochila trichostoma* occurs in the northern and central Andes and in mountainous areas of Central America at elevations between 2,000 and 3,700 m. The species is confined to humid, evergreen forests, e.g., Andean cloud forests and Central American oak forests, and to shrubby vegetation of the subparamo. *Plagiochila trichostoma* grows predominantly on soil, rock, rotten logs, and bases of trunks.

**Representative specimens examined.**—COLOMBIA. ANTIOQUIA. Alto del Boquerón, 25 km NE of Medellín, 2,800–3,000 m, 1986, *Churchill et al. 14241* (GOET). NAPO. Santa Bárbara de Sucumbios, valley of Río Clúngual, 2,400 m, 1959, *Harling 4190* (s); RISARALDA. Santa Rosa de Cabal, Hacienda La Sierra, 3,700 m, 1985, *Wolf 2* (v). COSTA RICA. CARTAGO. La Esperanza del Guarco, 2,700 m, 1985, *Kappelle 357* (U); PUNTARENAS. Bocas, Cerro Echandi, 3,200 m, 1983, *Gómez et al. 21812* (U); SAN JOSÉ: San Gerardo de Dota, Río Savegre valley, 2,700 m, 1999, *Heinrichs et al. 4323* (GOET, INB), “Los Robles”, 2,300 m, 1999, *Heinrichs et al. 4324* (GOET, INB). ECUADOR. “S. Franzis.”, river Vascun, *Spruce s.n.* (MANCH CC6525). PERU. AMAZONAS. Chachapoyas, road Chachapoyas-Cajamarca, km 417, 2,800 m, 1982, *Frahm et al. 589, 828* (B).

**PLAGIOCHILA DEFLEXA** Mont. & Gottsche in Montagne, *Ann. Sci. Nat., Bot.* (ser.4) 6: 192, 1856. TYPE: Hawaiian Islands, *Gaudichaud s.n.* [lectotype, designated by Inoue (1976: 427), G 001157 (ster., ex hb. Montagne); isolectotypes, G 001157A (ster.) and G 001164 (ster.), M (ster.)].

= *Plagiochila askenasi* Steph., *Bull. Herb. Boissier* 5: 847. 1897 [= Schinz, *Kenntnis Moosfl. Hawaiiinseln*: 265], *syn. fide* Inoue (1976: 427) and here confirmed. TYPE: Hawaiian Islands, *Baldwin s.n.* (holotype, G 001097 [ster.]).

= *Plagiochila caespitosa* Steph., *Bull. Herb. Boissier* 5: 848. 1897 [= Schinz, *Kenntnis Moosfl. Hawaiiinseln*: 266], *nom. illeg.* [Art. 53.1 ICBN], *non P. caespitosa* Col. 1888, *syn. fide* Inoue (1976: 427) and here confirmed. TYPE: Hawaiian Islands, *Baldwin s.n.* (holotype, G 001150 [ster.]).

= *Plagiochila nahikuna* Steph., *Spec. Hep.* 6: 187. 1921, *syn. fide* Inoue (1976: 427) and here confirmed. TYPE: “Japonia”, Hawaiian Islands. Maui, Nahiku, 1909, *Faurie 278* (holotype, G 001244 [ster.]).

= *Plagiochila rockii* Steph., *Spec. Hep.* 6: 207. 1921, *syn. fide* Inoue (1976: 427). TYPE: Hawaiian Islands, *Rock s.n.* [holotype, G (not available on loan from G in 2000)].

= *Plagiochila subtrinitensis* Herzog, *Hedwigia* 72: 198. 1932, *syn. nov.* TYPE: COSTA RICA. San José, La Palma, ca 1,600 m, 1924, *Standley 38198* [lectotype, here designated, JE (c. spg. juv.); isolectotypes, B 13443 (ster.), FH (c. per.), PC (ster.), US (c. per.)]; San José, near Finca La Cima above Los Lotes N of El Copey, 2,100–2,400 m, 1925, *Standley 42689* [paralectotypes, FH (c. spg. juv.), JE (c. per.), US (c. per.)]; Heredia, Cerros de Zurqui NE San Isidro, 2,000–2,400 m, 1926, *Standley and Valerio 50500* [paralectotypes, B 13441 (c. per.), FH (ster.), JE (ster.), M (ster.), US (ster.)].

**Illustrations.**—Herzog [1932: fig. 2 a–d (as *P. subtrinitensis*)], Stephani [1985: figs. 10510 (as *P. caespitosa*), 10657 (as *P. askenasi*), 10766 and 10767 (as *P. nahikuna*)].

**Gametophyte.**—Plants without odor of peppermint (fresh material), medium to large in size, (3–)5–12(–20) cm long and 5–8 mm broad, olive green to brownish green, becoming brownish in herbarium, in diffuse patches, with differentiation into short creeping stoloniform shoots giving rise to prostrate or ascending leafy stems.

Stems brown, near base ca 550–650  $\times$  370–430  $\mu\text{m}$ , dorsally widely exposed, ventrally widely exposed to completely covered by leaves, in transverse section ca 26–35  $\times$  21–30 cells across, cortical cells in (3–)4–6(–7) layers, distinctly thick-walled, ca 14–25  $\times$  10–25  $\mu\text{m}$ , medullary cells thin-walled to slightly thick-walled, in transition zone to cortex sometimes moderately thick-walled, ca (16–)20–28(–30)  $\times$  (18–)22–35(–41)  $\mu\text{m}$ , trigones small, triangular or lacking. Branches lacking to moderate, predominantly lateral-intercalary, occasionally terminal, of *Frullania*-type. Creeping stoloniform shoots partly beset with short leaflets, leaves of aerial shoots widely spreading or weakly bent to ventral side to horizontally directed, occasionally bent transversely to stems, in dry condition not or weakly inrolled, moderately imbricate to remote, occasionally densely imbricate, at least on main shoots and stronger branches with distinct ventral shoulder or ampliate, triangular, elongate-triangular, triangularly ovate or ovate oblong, on weaker branches and shoot tops sometimes narrowly ovate oblong to oblong, with acute to truncate apex; leaves of main stems ca (2.5–)2.8–3.8(–4.3)  $\times$  2.1–2.9(–3.2) mm and ca 1.1–1.7 times as long as wide, those of weaker branches and shoot tops somewhat smaller and up to 2.2 times as long as wide, ventral margin flat or recurved above leaf base, moderately to very long decurrent as acute strip, dorsal margin often recurved on whole length, (moderately to very) long decurrent along dorsal midline of stem, decurrent strip in lower parts very small and acute, often reaching dorsal base of opposite leaf or dorsal bases partly decurrent along each other. Leaves toothed, teeth restricted to apex, upper half of dorsal margin and upper 3/4 of ventral

margin, occasionally complete ventral margin toothed, teeth triangular to elongate triangular, (1–)2–4(–6) cells wide at base and (1–)3–6(–10) cells long, with moderately elongate terminal cell, apical teeth often larger, sometimes lobe-like, teeth  $\pm$  straight, rightly perpendicular to leaf margin or forward pointed, occasionally pointed backwards, apical teeth pointing in various directions, ventral margin of stem leaves with 4–21(–25) teeth, apex with 2–5(–7) teeth and dorsal margin with 0–4(–7) teeth. Leaf areolation regular to irregular, cells in upper leaf half slightly elongate with scattered distinctly elongate cells and cells isodiametric to shorter than long, cells from center of upper leaf half (28–)30–55(–68)  $\times$  (22–)25–38(–42)  $\mu\text{m}$  and (0.9–)1.1–1.5(–2.2) times as long as wide, cells towards leaf base in all more elongate, building short and inconspicuous vitta above leaf base with cells (45–)50–92(–105)  $\times$  22–38  $\mu\text{m}$  and 1.4–4.1 times as long as wide; cell walls thin except those along leaf margins, trigones ( $\pm$  lacking to) medium sized to large, subnodulose to nodulose, with rounded corners, often confluent, intermediate thickenings  $\pm$  frequent, especially on long walls; cuticle smooth. Oil bodies opaque, colorless, (5–)7–11(–13) per median leaf cell, 4–6  $\times$  5–9  $\mu\text{m}$ , spheric to ellipsoidal, homogeneous or weakly and coarsely botryoidal. Underleaves lacking or vestigial, built by few short cilia. Asexual reproduction by specialized structures not observed. Male plants as large as female plants, androecia becoming intercalary, bracts in 5–17 pairs, imbricate, overlapping dorsally, strongly inflated basally, margin of inflated part with 0–2(–3) triangular, blunt teeth, distal part obliquely spreading, margin with 1–4 blunt to acute, triangular teeth to sinuate, bracts composed of cells similar to those of leaves, trigones well-developed in all cells. Antheridia 1–3 per bract, on stalk ca 14–20 cells long, globose to broadly ellipsoid. Gynoecia terminal on main shoots and on branches, 1(–2) subgynoecial innovations frequent (even below sporophytes), bracts similar to leaves in shape but dorsally and ventrally hardly to moderately decurrent and sometimes slightly longer, often inserted some distance from perianth; perianth  $\pm$  cylindrical to elliptic in dorsal view, elliptic to nearly round in lateral view, 2.7–4.1  $\times$  1.7–2.6 mm and 1.1–1.6 times as long as wide, dorsal keel often with low, smooth arch; perianth mouth truncate to arched, densely beset with ciliate to elongate triangular teeth.

*Sporophyte*.—Capsules short-exserted, subglobose; valves ca 1.4–1.7 mm long and 0.7–0.9 mm broad, not twisted, epidermal cells in surface view broader than long to slightly elongate, walls thick, with 1–2(–3) large, nodulose, occasionally forked, brown thickenings on most long and many short walls, hypodermal cells in surface view more elon-

gate, with small brown thickenings on many walls, basal innermost cells mostly distinctly elongate, median and apical innermost cells shorter, mixed with transversely directed cells, in  $\pm$  irregular pattern, short walls with few, long walls with many nodulose to cone-like thickenings sometimes confluent at their base and partly coalesced; valves in transverse section ca 65–75  $\mu\text{m}$  in diameter, 5–6-stratose with thickenings in all layers, epidermal cells thicker than inner cells, ca 22–30  $\mu\text{m}$  thick. Spores 1-celled, trilete, globose, ca 18–26 in diameter, verrucate to baculate. Elaters smooth, bispiral ca 10–15  $\mu\text{m}$  thick, rarely branched, bispiral, spirals terminating some distance from end of elater on one side or on both sides.

*Representative specimens examined*.—COSTA RICA. ALAJUELA. Parque Nacional Volcán Poás, 2,700 m, 1977, *Griffin & Araya 34* (FLAS); CARTAGO. road Cartago-Cerro de la Muerte, Reserva Foresta Río Macho, 2,500 m, 1993, *Gradstein & Morales 9382* (GOET); GUANACASTE. PN Guanacaste, volcano Cacao, 1,600 m, *Hammel 17647* (MO); HEREDIA. Parque Nacional Volcán Barva, 2,650–2,900 m, 1994, *Gradstein & Mues 5670/9637* (GOET, SAAR, chemistry voucher); LIMON. Chirripó grande, 2,500 m, 1932, *Kupper 1271* (M); SAN JOSÉ. Cordillera Talamanca, San Gerardo de Dota, Río Savegre valley, holiday camp Chacon, trail “Los Gobles”, 2,300 m, 1999, *Heinrichs et al. 4161* (GOET). CUBA. ORIENTE. Sierra Maestra Mts., Pico Joaquín, 1979, *Pócs 9089/B* (= Inoue: *Bryophyta Selecta Exsiccata 568*) (B, C, FLAS, S, U). DOMINICAN REPUBLIC. BARAHONA. Montaña Nueva, near Polo, 1,300 m, 1976, *Judd 1115* (FLAS). HONDURAS. CORTÉZ. Agua Azul, Lake Yojoa, 600 m, 1951, *Morton 7205* (B, US). JAMAICA. Blue Mountain Peak, 1903, *Underwood 1489, 2507, 2582* (GOET, NY). MEXICO. OAXACA. Sierra Juárez, 1962, *Sharp s.n.* (US). NICARAGUA. RIVAS. Isla Ometepe, Volcán Maderas, Balgüe, 1,200–1,260 m, 1984, *Robledo 492* (U), 1,000–1,200 m, 1983, *Nee & Robledo 28112* (U). PANAMA. CHIRIQUÍ. Palo Alto NE of Boquete, 2,000 m, 1979, *Hammel 7517, 7518* (U). U.S.A. HAWAII. Kapauu-Halaula, Hamakua ditch, 1938, *Cranwell et al. 4655, 5403* (NY); KAUAI. Alakai, Kilohuna, 1938, *Cranwell & Skottsberg 5368* (G); MAUI. *Rock s.n.*, *Kryptogamae Exsiccatae 2183* (B, G, M, W); MOLOKAI. Palekuhu Pali, above Kolehole, 1948, *Fagerlind & Skottsberg 7012* (G, NY); OAHU. Koolau Mts., Kahuku Forest Reserve, Laie Trail, 430 m, 1975, *Yoshida 169* (B, NY, U, W).

*Phytochemistry*.—Bibenzyls and bisbibenzyls (in Costa Rican material, Anton et al. 1999).

*Ecology and distribution*.—*Plagiochila deflexa* is widespread in the Hawaiian Islands and Central America, especially Costa Rica (Holz et al. 2001). Furthermore, *P. deflexa* occurs in the West Indies. The species is confined to montane, wet, mossy forests between 500 and 3,000 m. *Plagiochila deflexa* grows predominantly epiphytically, especially on the base and lower portions of trunks. Neotropical specimens are often fertile, bearing androecia or perianths, occasionally sporophytes. In contrast, only sterile plants were known from the Hawaiian Islands (Inoue 1976). In this study, of the 31 Ha-

waiian gatherings examined, only one specimen (*Fagerlind & Skottsberg 7012*) proved not to be sterile, bearing a single male plant.

#### CONCLUDING REMARKS

Traditional concepts (e.g., Carl 1931; Stephani 1902–1905) usually restrict ranges of *Plagiochila* species to one continent/floristic region. Evidence for disjunct ranges of several *Plagiochila* species was presented recently (e.g., Heinrichs et al. 2000a; Inoue 1980; Rycroft et al. 2001). For the first time a disjunct range of an assumed Hawaiian endemic (Inoue 1976), *P. deflexa*, has been detected, being widespread in Central America and the West Indies. This finding leads to a possible occurrence of sect. *Rutilantes* in Asia. The Southeast Asian *P. propinqua* Sande Lac. (Inoue 1984) in particular resembles members of sect. *Rutilantes* in sporophytic features, and perianth shape. Molecular data are not yet available for this species.

The results of the molecular investigation of nrDNA ITS sequences of 17 taxa of *Plagiochila* are in accord with morphological and phytochemical investigations. Thus, ITS-sequencing provides a useful molecular tool for the analysis of this difficult hepatic genus. Our findings demonstrate the need of a broad molecular study to illuminate further the phylogenetic relationships within *Plagiochila*.

#### ACKNOWLEDGMENTS

We express our gratitude to the curators of the herbaria cited for the loan of material, to Monica Moraes and Stephan Beck (La Paz, Bolivia), as well as Maarten Kappelle and Nelson Zamora (Heredia, Costa Rica) for logistic support during fieldwork, and to Nicole Noeske (Göttingen) and Hermann Anton (Saarbrücken) for assistance in the field. The investigation was supported by the Deutsche Forschungsgemeinschaft (grant Gr 1588/1).

#### LITERATURE CITED

- AKAIKE, H. 1974. A new look at the statistical model identification. *IEEE Transactions on Automatic Control* 19: 716–723.
- ANTON, H., R. SCHOENEBOERN & R. MUES. 1999. Bibenzyls and bisbibenzyls from a neotropical *Plagiochila* species. *Phytochemistry* 52: 1639–1645.
- , J. HEINRICHS, R. MUES & S. R. GRADSTEIN. 2000. Chemotaxonomical and morphological characterization of *Plagiochila dusenii* (Hepaticae), most closely related to *P. validissima*. *Journal of the Hattori Botanical Laboratory* 89: 93–112.
- CARL, H. 1931. Die Artypen und die systematische Gliederung der Gattung *Plagiochila* Dum. *Annales Bryologici, supplementum* 2: 1–170.
- FELSENSTEIN, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39: 783–791.
- HASEGAWA, M., H. KISHINO & T. YANO. 1985. Dating the human-ape splitting by a molecular clock of mitochondrial DNA. *Journal of Molecular Evolution* 22: 160–174.
- HEINRICHS, J. & H. ANTON. 2001. *Bryophyta Exsiccata Generis Plagiochilae*, a new Exsiccata distributed by the University of Göttingen Herbarium. *Polish Botanical Journal* 46: 109–111.
- , ———, S. R. GRADSTEIN & R. MUES. 2000a. Systematics of *Plagiochila* sect. *Glaucoscentes* Carl (Hepaticae) from tropical America: a morphological and chemotaxonomical approach. *Plant Systematics and Evolution* 220: 115–138.
- , ———, ———, & I. HOLZ. 2000b. Surface wax, a new taxonomic feature in Plagiochilaceae. *Plant Systematics and Evolution* 225: 225–233.
- & S. R. GRADSTEIN. 1999. On *Plagiochila longiramea* Steph., a poorly known species of Bolivia. *Candollea* 54: 73–81.
- , H. GROTH, S. R. GRADSTEIN, D. S. RYCROFT, W. J. COLE & H. ANTON. 2001a. *Plagiochila rutilans* (Hepaticae): a poorly known species from Tropical America. *THE BRYOLOGIST* 104: 350–361.
- , I. HOLZ & S. R. GRADSTEIN. 2001b. The sporophyte of *Stereochila* (Plagiochilaceae). *Journal of Bryology* 23: 35–41.
- , T. PRÖSCHOLD, C. RENKER, H. GROTH & D. S. RYCROFT. 2002. *Plagiochila virginica* A. Evans rather than *P. dubia* Lindenb. and Gottsche occurs in Macaronesia; placement in sect. *Contiguae* Carl is supported by ITS sequences of nuclear ribosomal DNA. *Plant Systematics and Evolution* 230: 221–230.
- HERZOG, T. 1932. Beiträge zur Kenntnis der Gattung *Plagiochila*. I. Neotropische Arten. *Hedwigia* 72: 195–243.
- HOLZ, I., J. HEINRICHS, A. SCHÄFER-VERWIMP & S. R. GRADSTEIN. 2001. Additions to the hepatic flora of Costa Rica, III. Cryptogamie, *Bryologie* 22: 255–273.
- HUNECK, S., J. D. CONNOLLY, L. J. HARRISON, R. S. I. JOSEPH & T. PÓCS. 1984. 1-(3,4-Dihydroxy-5-methoxyphenyl)-3-methylbut-2-ene from the liverwort *Plagiochila rutilans*. *Phytochemistry* 23: 2396–2397.
- INOUE, H. 1958. The family Plagiochilaceae of Japan and Formosa. II. *Journal of the Hattori Botanical Laboratory* 20: 54–106.
- . 1965. Contributions to the knowledge of the Plagiochilaceae of southeastern Asia. VII. Synopsis of *Plagiochila* Dum. in the Himalayan region. *Bulletin of the National Science Museum (Tokyo)* 8: 375–403.
- . 1976. Notes on the Plagiochilaceae, V. Studies in the genus *Plagiochila* (Dum.) Dum. in the Hawaiian Islands. *Journal of the Hattori Botanical Laboratory* 40: 411–440.
- . 1980. Notes on the Plagiochilaceae, X. *Plagiochila corniculata* (Dum.) Dum. and its allies. *Bulletin of the National Science Museum, Ser. B6*: 115–124.
- . 1984. The Genus *Plagiochila* in Southeast Asia. Academic Scientific Book Inc., Tokyo.
- . 1989. *Plagiochila*, pp. 106–273. In P. Geissler & H. Bischler (eds.), *Naiadea to Pycnoscenus* (Index Hepaticarum vol. 11). Cramer, Berlin, Stuttgart.
- & R. M. SCHUSTER. 1971. A monograph of the New Zealand and Tasmanian Plagiochilaceae. *Journal of the Hattori Botanical Laboratory* 34: 1–225.
- KISHINO, H. & M. HASEGAWA. 1989. Evaluation of the maximum likelihood estimate of the evolutionary tree topologies from DNA sequence data, and the branching order of the Hominoidea. *Journal of Molecular Evolution* 29: 170–179.
- LINDENBERG, J. B. G. 1839–1844. *Monographia Hepaticarum Generis Plagiochilae*. Henry and Cohen, Bonn.

- MÜLLER, J., J. HEINRICHS & S. R. GRADSTEIN. 1999. A revision of *Plagiochila* sect. *Plagiochila* in the Neotropics. *THE BRYOLOGIST* 102: 729–746.
- PAGE, R. D. M. 1996. TREEVIEW: an application to display phylogenetic trees on personal computers. *Computer Applications in the Biosciences* 12: 357–358.
- POSADA, D. & K. A. CRANDALL. 1998. Modeltest: testing the model of DNA substitution. *Bioinformatics* 14: 817–818.
- RYCROFT, D. S. 1996. Fingerprinting of plant extracts using NMR spectroscopy: application to small samples of liverworts. *Chemical Communications*: 2187–2188.
- . 1998. Chemical comparison of liverworts using NMR spectroscopy. *Journal of the Hattori Botanical Laboratory* 84: 105–111.
- , W. J. COLE, N. ASLAM, Y. M. LAMONT & R. GABRIEL. 1999. Killarniensolide, methyl orsellinates and 9,10-dihydrophenanthrenes from the liverwort *Plagiochila killarniensis* from Scotland and the Azores. *Phytochemistry* 50: 1167–1173.
- & ———. 2001. Hydroquinone derivatives and monoterpenoids from the neotropical liverwort *Plagiochila rutilans*. *Phytochemistry* 57: 479–488.
- , J. HEINRICHS, W. J. COLE & H. ANTON. 2001. A phytochemical and morphological study of the liverwort *Plagiochila retrorsa* Gottsche, new to Europe. *Journal of Bryology* 23: 23–34.
- SAIKI, R. K., D. H. GELFAND, S. STOFFEL, S. J. SCHARF, R. HIGUCHI, G. T. HORN, K. B. MULLIS & H. A. EHRLICH. 1988. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science* 239: 487–491.
- SAMBROOK, J., E. FRITSCH & T. MANIATIS. 1989. *Molecular Cloning. A Laboratory Manual* 1(3). Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
- SCHIFFNER, V. 1901. *Expositio plantarum in itinere Suo Indico annis 1893/94 suscepto collectarum, II. Denkschriften der Kaiserlichen Akademie der Wissenschaften, mathematisch-naturwissenschaftliche Classe* 70: 155–218.
- SO, M. L. & R. GROLLE. 2000. Checklist of *Plagiochila* (Hepaticae) in Asia. *Journal of the Hattori Botanical Laboratory* 88: 199–243.
- SPRUCE, R. 1884–1885. *Hepaticae of the Amazon and the Andes of Ecuador and Peru. Transactions and Proceedings of the Botanical Society of Edinburgh* 15: i–xi, 1–589, plates i–xxii.
- STEPHANI, F. 1902–1905. *Plagiochila* Dum. *Species Hepaticarum* 2: 195–595. Geneva.
- . 1985. *Icones Hepaticarum, Microfiche*. Inter Documentation Company bv, Leiden.
- SWOFFORD, D. L. 1998. PAUP\*, phylogenetic analysis using parsimony (\*and other methods), version 4.0b8. Sinauer Associates, Sunderland, MA.

ms. received Oct. 8, 2001; accepted Dec. 19, 2001.