

## PHYLOGENETIC REVISION OF THE GENUS *PELTIGERA* (LICHEN-FORMING ASCOMYCOTA) BASED ON MORPHOLOGICAL, CHEMICAL, AND LARGE SUBUNIT NUCLEAR RIBOSOMAL DNA DATA

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*Peltigera* (Peltigerineae, lichenized Ascomycota) is one of the most widespread lichen genera incorporating bi- and trimembered associations involving fungi, green algae (cf. *Coccomyxa*), and cyanobacteria (cf. *Nostoc*). A wide range of morphological and chemical (secondary compounds) variation at both the intra- and inter-specific levels is present in this genus. Compared to many other genera of macrolichens, its taxonomy, including chemotaxonomy, still remains poorly understood. Existing infrageneric classifications of *Peltigera* are almost exclusively based on photobiont composition of the thallus. These classifications assumed that bi- and trimembered taxa were distinct monophyletic entities. The genus *Peltigera* has never been the focus of a comprehensive phylogenetic study. The most recent and widely accepted subdivision of the genus into seven groups is based mainly on morphological and chemical characters. Relationships among species of *Peltigera* are investigated here using chemical, morphological, and large subunit nuclear ribosomal DNA (LSU nrDNA) data. We test the monophyly of these seven morpho-chemical *Peltigera* groups and propose a classification based on a phylogenetic approach. Data sets of 42 chemical characters (terpenoids), 31 morphological characters, and 1135 LSU nrDNA characters for 96 samples representing 38 *Peltigera* species, eight undescribed putative *Peltigera* species, and nine species from seven potentially closely related genera from Peltigerineae were subjected to maximum parsimony analyses. Morphological, chemical, and molecular analyses were carried out independently and on a combined data set. Monophyly of *Peltigera*, including *Hydrothyria*, was confirmed. The genus *Hydrothyria* is transferred to *Peltigera* and a new combination *Peltigera hydrothyria* Miadlikowska & Lutzoni is proposed. Eight monophyletic sections within the genus *Peltigera*, with high bootstrap support, are circumscribed: sections *Peltigera*, *Polydactylon* Miadlikowska & Lutzoni, *Chloropeltigera* Gyeln., *Peltidea* (Ach.) Vain., *Horizontales* Miadlikowska & Lutzoni, *Retifoveatae* Miadlikowska & Lutzoni, *Phlebia* Wallr., and *Hydrothyriae* Miadlikowska & Lutzoni. Unequivocal morphological and chemical synapomorphies for all sections except section *Peltidea* are recognized and presented. A key for identification of the sections is provided. In addition, a key based on four main terpenoids for determination of the chemotypes and species within section *Polydactylon* is included. Five terpenoids (50–54) identified on thin-layer chromatography plates for *P. elisabethae* and *P. horizontalis* chemotype I are added to the list of substances found in *Peltigera*. Five chemotypes, mainly from Poland and Norway, are reported from *Peltigera* thalli for the first time: *P. malacea* chemotype V, *P. leucophlebia* chemotype II, *P. hymenina* chemotypes II and III, and *P. collina* chemotype IV. Three main types of vein structure in *Peltigera* were recognized based on SEM studies.

**Keywords:** infrageneric classification, large subunit nrDNA, lichen-forming Ascomycota, morphology, *Peltigera*, Peltigerineae, phylogeny, systematics, terpenoids.

### Introduction

The lichen-forming genus *Peltigera* occupies a central position in the family Peltigeraceae. This family of lichenized Ascomycota is classified within the suborder Peltigerineae of the order Lecanorales (Eriksson and Winka 1998). *Peltigera* includes terricolous and muscicolous foliose macrolichens that are common and widespread on most continents. The genus is well defined by the absence of a lower cortex and the presence of a dense arachnoid-tomentose pilema that usually bears anastomosing pale or dark veins with numerous solitary or

confluent rhizines. Symbiotic entities within this genus are represented by two different types of associations: (1) bimembered symbioses involving a cyanobacterium (cf. *Nostoc*) and a fungus and (2) trimembered symbioses involving a green alga (cf. *Coccomyxa*) as the main photobiont, cyanobacteria (cf. *Nostoc*) located in external cephalodia on the upper or lower surface of the thallus, and a fungus. Cephalodia of some trimembered *Peltigera* species can develop into bimembered thalli called cyanomorphs.

Members of the genus *Peltigera* show different chemical patterns. Some species contain lichen substances produced by two major biochemical pathways: the acetate-polymalonate pathway (tridepside, tenuiorin-aggregate) and the mevalonic acid pathway (triterpenoids of the hopane series). Other species have secondary compounds generated only by one of these

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two pathways, whereas some species lack secondary substances detectable by thin-layer chromatography.

Although *Peltigera* is one of the earliest described lichen genera (Willdenow 1787), it is still poorly understood compared to other macrolichens. Recently, 28 *Peltigera* species have been reported for North America (Goward et al. 1995) and 29 for Europe (Vitikainen 1994b). Depending on the taxonomic authority, 45 to 60 taxa are recognized worldwide (Goffinet and Hastings 1994; Hawksworth et al. 1995). Seventeen of these taxa have been described within the past 18 yr (Awasthi and Joshi 1982; Vitikainen 1985, 1986, 1994a, 1994b, 1995; Holtan-Hartwig 1988, 1993; Purvis and James 1993; Stenroos et al. 1994; Goffinet and Hastings 1995; Goward et al. 1995; Goffinet and Miadlikowska 1999; Goward and Goffinet 2000). The great majority of revisionary studies on *Peltigera* are focused on the Northern Hemisphere (table 1).

The first modern chemotaxonomic study of the genus was done by Kurokawa et al. (1966). In 1983, Tønsberg and Holtan-Hartwig described the secondary compound composition for the *P. aphthosa* group in Norway, and White and James (1985, 1987a) studied the chemistry of several taxa of *Peltigera* from Great Britain. Secondary substances were also included in a taxonomic revision of European *Peltigera* by Vitikainen (1994b). Detailed chemotaxonomic work describing infra-chemotype variation for triterpenoids found in *Peltigera* taxa was carried out by Holtan-Hartwig (1993) in Norway, by Martínez (1999) in the Iberian Peninsula, and by Miadlikowska (1999) in Poland. A total of 35 chemotypes were reported in these three publications. A total of 42 different terpenoids have been found in *Peltigera* thalli, but the chemical structure is known for only 10 of them (table 2).

The traditional taxonomy of *Peltigera* was based on vegetative features of the thallus, i.e., cortex structure, venation type, rhizine morphology and arrangement, cephalodium or

propagule presence/absence, and occasionally on secondary substance composition. A wide range of morphological and chemical variation at both the intra- and interspecific levels is present in this genus, causing major problems for species delimitation and identification, especially for taxa within the *P. canina* complex. Single terpenoid differences or chemosyn-dromic variations between species (e.g., *P. hymenina*, *P. polydactylon*, and *P. neopolydactyla*) have been documented. Some taxa are represented by several chemotypes (e.g., *P. malacea*, *P. aphthosa*, and *P. neopolydactyla*). Morphologically similar species, such as *P. elisabethae* and *P. horizontalis*, often share common chemical features. Intermediate pheno- and chemotypes are frequently observed within a population or even within an individual thallus. Goffinet and Hastings (1995) proposed introgressive hybridization as the potential cause of such patterns of variation.

Past molecular systematic studies on lichens included only a few exemplar taxa (*P. canina*, *P. membranacea*, and *P. neopolydactyla*), mainly to resolve phylogenetic relationships at the suprafamily level, and were based strictly on sequences of the small subunit nuclear ribosomal DNA (nrDNA) (Eriksson and Strand 1995; Lutzoni et al. 1996). The results from these molecular studies did not support Hawksworth's (1982) hypothesis that the origin of this particular group of lichens was especially ancient. This hypothesis was based on the observation that a remarkable number of lichenicolous fungi occur exclusively on *Peltigera* and also on the ascotal ontogeny, ascus structure, and wide geographical distribution of many *Peltigera* species. Other molecular studies were designed to determine if the same mycobiont was responsible for both photomorphs within the *P. aphthosa* group and to study phylogenetic relationships among photomorphs (Goffinet and Bayer 1997). Only recently was the genetic variation of those cyanobacteria associated with *Peltigera* investigated at the population level and its possible implications for the system-

Table 1

Recent General Taxonomic Studies on Lichens (including *Peltigera*) or Exclusively on the Genus *Peltigera* from Different Areas of the Northern Hemisphere

| Area                            | Reference                          | No. of species |
|---------------------------------|------------------------------------|----------------|
| Newfoundland, Canada            | Ahti and Vitikainen 1977           | 19             |
| India and Nepal                 | Awasthi and Joshi 1982             | 15             |
| Denmark                         | Alstrup 1986                       | 16             |
| Farö Islands                    | Alstrup 1986                       | 10             |
| Great Britain and Ireland       | Purvis and James 1992              | 19             |
| Italy                           | Nimis 1993                         | 20             |
| Norway                          | Holtan-Hartwig 1993                | 17             |
| Slovakia                        | Pisut et al. 1993                  | 15             |
| Alberta, Canada                 | Goffinet and Hastings 1994         | 24             |
| Belgium and Luxembourg          | Goffinet et al. 1994               | 17             |
| Europe                          | Vitikainen 1994b                   | 29             |
| British Columbia, Canada        | Goward et al. 1995                 | 28             |
| Czywczyńskie Mountains, Ukraine | Miadlikowska 1996                  | 17             |
| Iberian Peninsula               | Martínez 1999                      | 22             |
| Oregon and Washington, U.S.A.   | McCune and Geiser 1997             | 15             |
| Ukraine                         | Kondratyuk et al. 1998             | 20             |
| Romania                         | Bartók 1998                        | 17             |
| Lithuania                       | Motiejunaite and Miadlikowska 1999 | 15             |
| Poland                          | Miadlikowska 1999                  | 20             |

**Table 2**  
**Terpenoids of Known Chemical Structures Reported from *Peltigera* Thalli**

| TLC spot number | Common name    | Chemical structure                                    | Reference   |
|-----------------|----------------|---|---|
| 10              | Peltidactylin  | 7 $\beta$ -acetoxyhopan-22-ol                         | Zopf 1909; Corbett and Young 1966a; Huneck et al. 1973                          |
| 12              | Dolichorrhizin | 15 $\alpha$ -acetoxyhopan-22-ol                       | Zopf 1909; Corbett and Young 1966b; Kurokawa et al. 1966; Takahashi et al. 1970 |
| 15              | Zeorin         | Hopane-6 $\alpha$ ,22-diol                            | Zopf 1909; Kurokawa et al. 1966   |
| 16              | Phlebic acid A | 28-acetoxy-22-hydroxyhopan-23-carboxylic acid         | Kurokawa et al. 1966; Takahashi et al. 1969                                     |
| 17              | Phlebic acid B | 22-hydroxyhopan-23-carboxylic acid                    | Kurokawa et al. 1966; Takahashi et al. 1970                                     |
| 20              | ?              | Hopane-6 $\alpha$ ,7 $\beta$ ,22-triol                | White and James 1985  |
| 34              | ?              | Hopane-7 $\beta$ ,22-diol                             | Corbett and Young 1966a; White and James 1985; Holtan-Hartwig 1988              |
| 35              | ?              | Hopane-15 $\alpha$ ,22-diol                           | Huneck et al. 1973; Tønsberg and Holtan-Hartwig 1983                            |
| ?               | Phlebic acid C | 7 $\beta$ -acetoxy-22-hydroxyhopan-27-carboxylic acid | Bachelor et al. 1990  |
| ?               | Phlebic acid D | 22-hydroxyhopan-27-carboxylic acid                    | Bachelor et al. 1990  |

Note. References are given for the first report of particular terpenoids from general taxonomic studies on lichens or restricted to *Peltigera*. TLC spot numbers are according to Holtan-Hartwig (1993).

atics of this genus explored (Miao et al. 1997; Paulsrud et al. 1998).

Most infrageneric classifications of *Peltigera* were exclusively based on the photobiont composition of the thallus. Species associated with a green alga as their main photobiont (trimembered thalli) usually have been included in a different group (section, or even genus) than *Peltigera* species associated strictly with cyanobacteria, i.e., bimembered thalli (Nylander 1863, 1866; Jatta 1893). Among cephalodiate taxa, *P. venosa* was most problematic and often segregated to form a monospecific group. Gyelnik (1933) created four groups based on a combination of photobiont types and presence of rhizines versus single rhizopt. In 1966, Kurokawa et al. used, for the first time, a combination of photobiont and secondary metabolite compositions (i.e., presence/absence of phlebic acids A and B as well as dolichorrhizin) to circumscribe two infrageneric groups within *Peltigera* (*Phlebia* and *Emprostea*). They also distinguished three species groups within the section *Emprostea*: *P. polydactylon*, *P. malacea*, and *P. canina*. In 1993, Holtan-Hartwig came to the same conclusion as Gyelnik, and Kurokawa and coworkers, when he wrote that because *Peltigera* species can establish symbiotic interactions with both a green alga and a cyanobacterium, it disqualifies a dichotomous classification based solely on the photobiont composition. Using a combination of apothecial, thalline, and chemical characters, he presented a practical subdivision of the Norwegian species into seven groups: *P. aphthosa*, *P. canina*, *P. horizontalis*, *P. polydactylon*, *P. scabrosa*, *P. retifoveata*, and *P. venosa* (fig. 1). Holtan-Hartwig (1993) assumed that this informal classification also likely reflected phylogenetic relationships within this genus. This is the only subdivision of *Peltigera* that has been widely adopted by contemporary lichenologists.

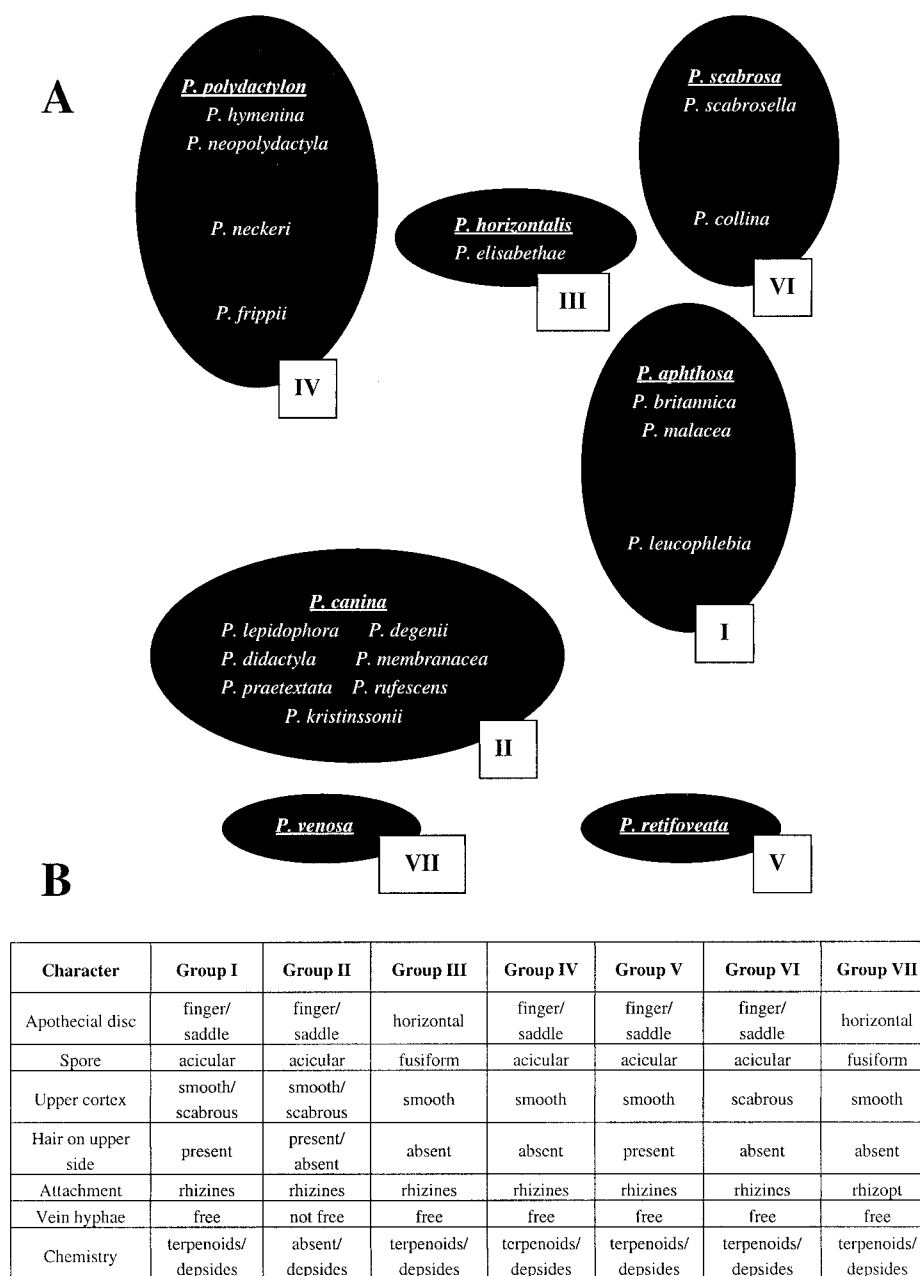
The genus *Peltigera* was never subjected to a broad and exhaustive phylogenetic study. All taxonomic revisions of this genus were exclusively based on phenotypic characters. The

main goal of this study is to elaborate an integrated phylogenetic synthesis of morphological, chemical, and molecular data for members of the lichen-forming genus *Peltigera*. To reach this goal, we have conducted a detailed reassessment of morphological-anatomical and chemical features, and sequenced a 1.4-kb fragment at the 5' end of the large subunit nrDNA (LSU nrDNA) for 46 putative species of *Peltigera*, mainly from the Northern Hemisphere, and nine outgroup species. In this article we test the monophyletic status of this genus and establish, for the first time, a classification for the genus *Peltigera* that is based on a phylogenetic approach. This new infrageneric classification provides a stable framework for future systematic work on this genus and closely related genera.

## Material and Methods

### Taxon Sampling

The 38 published species of *Peltigera* included here represent the entire known flora of peltigerous lichens from North America and almost all known European species, as well as some representatives from Asia (*P. nigripunctata*, *P. laciniata*, and *P. continentalis*), Africa (*P. lambinonii* and *P. polydactyloides*), and South America (*P. frigida*, *P. pulverulenta*, and *P. laciniata*). In addition, 15 specimens representing eight potential species currently under study by T. Goward were selected, for a total of 86 specimens from 46 putative species of *Peltigera* (table 3). By including at least two specimens for almost every taxon, this selection encompasses virtually all known phenotypic and biogeographic variation for this genus. Three interesting European taxa, two of which were described in 1993 by Purvis and James (*P. melanorrhiza* Purvis, P. James & Vitik., and *P. dissecta* Purvis, P. James & Vitik.) and one that was recognized only recently but described in 1932 by Gyelnik (*P.*



**Fig. 1** A, Informal grouping of *Peltigera* species from Norway (dark ovals) after Holtan-Hartwig (1993). In bold and underlined are the prominent species after which each group was named. B, List of main diagnostic characters for each group according to Holtan-Hartwig (1993).

*lyngae* Gyeln.), could not be included because of their rarity. Fresh or herbarium material for these three species was not available for sequencing. To produce a stable root for the ingroup and to provide a better delimitation of the genus *Peltigera*, 10 specimens representing nine species from seven putatively closely related genera from the suborder Peltigerineae were selected, for a grand total of 96 specimens representing 55 taxa. Voucher information for each specimen used in this study is listed in table 3.

#### Morphological Data

Morphological characters are described and documented in the “Results” section. They are derived from J. Miadlikowska’s studies of various collections in European and North American herbaria, including a voucher study set of herbarium specimens listed in table 3, field observations, literature, and unpublished information by J. Miadlikowska.

A total of 26 morphological characters were recognized here, describing various features of the thallus and apothecium for

Table 3

## Voucher Specimens and GenBank Accession Numbers for 96 LSU nrDNA Sequences included in This Study

| Taxon   | Voucher   | GenBank accession no. |
|---|---|-----------------------|
| <i>Peltigera aphthosa</i> (L.) Willd.   | Canada, <i>Lutzoni</i> 97.06.29 (F)                         | AF286759              |
| <i>P. britannica</i> (Gyeln.) Holt.-Hartw. & Tønsberg 1 (cyanomorph)              | Norway, <i>Tønsberg</i> L-24759 (BG)                        | AF286754              |
| <i>P. britannica</i> 2  | U.S.A., <i>Sharnoff &amp; Sharnoff</i> 103102 (CANL)        | AF286758              |
| <i>P. "boreorufescens"</i> 1*   | Canada, <i>Goward &amp; Ceska</i> 82-1084 (UBC)             | AF286820              |
| <i>P. "boreorufescens"</i> 2*   | Canada, <i>Goward &amp; Ceska</i> 82-562 (UBC)              | AF286781              |
| <i>P. canina</i> (L.) Willd. 1  | Canada, <i>Wong &amp; Wong</i> 4297 (CANL)                  | AF286821              |
| <i>P. canina</i> 2  | Poland, <i>Czyzewska</i> 009205 (LOD-L)                     | AF286822              |
| <i>P. cinnamomea</i> Goward 1*  | Canada, <i>Brodo &amp; Hamilton</i> 21921 (CANL)            | AF286787              |
| <i>P. cinnamomea</i> 2*   | Canada, <i>Goward, Miller &amp; Nelson</i> 85-305 (UBC)     | AF286784              |
| <i>P. cinnamomea</i> 3  | Canada, <i>Goward</i> 82-1416 (UBC)                         | AF286811              |
| <i>P. collina</i> (Ach.) Schrad.  | Norway, <i>Vitikainen</i> 11485 (H)                         | AF286765              |
| <i>P. continentalis</i> Vitik.  | Mongolia, <i>Humeck</i> 88-151 (H)                          | AF286777              |
| <i>P. degenii</i> Gyeln. 1  | Poland, <i>Abti &amp; Drozdowicz</i> 45565 (UGDA-L)         | AF286794              |
| <i>P. degenii</i> 2   | Canada, <i>Gowan</i> 2582 (CANL)                            | AF286789              |
| <i>P. degenii</i> 3   | Finland, <i>Vitikainen</i> 10836 (H)                        | AF286793              |
| <i>P. didactyla</i> var. <i>didactyla</i> (With.) J.R. Laundon 1                  | Poland, <i>Miadlikowska</i> 5233 (UGDA-L)                   | AF286806              |
| <i>P. didactyla</i> var. <i>didactyla</i> 2                                       | Poland, <i>Miadlikowska</i> 5234 (UGDA-L)                   | AF286805              |
| <i>P. didactyla</i> var. <i>didactyla</i> 3*                                      | Poland, <i>Lesiak &amp; Czyzewska</i> 009202 (LOD-L)        | AF286804              |
| <i>P. didactyla</i> var. <i>didactyla</i> 4                                       | Poland, <i>Wroclawska &amp; Toborowicz</i> 74 (KTC)         | AF286807              |
| <i>P. didactyla</i> var. <i>extenuata</i> (Nyl. ex Vainio) Goffinet & Hastings 1* | Poland, <i>Faltynowicz &amp; Miadlikowska</i> 5235 (UGDA-L) | AF286809              |
| <i>P. didactyla</i> var. <i>extenuata</i> 2                                       | Poland, <i>Cieslinski</i> 1296 (KTLC)                       | AF286808              |
| <i>P. didactyla</i> var. <i>extenuata</i> 3                                       | Poland, <i>Butkus</i> 5236 (UGDA-L)                         | AF286810              |
| <i>P. elisabethae</i> Gyeln. 1  | Poland, <i>Bielczyk</i> 42135 (KRAM-L)                      | AF286762              |
| <i>P. elisabethae</i> 2   | Italy, <i>Vitikainen</i> 10292 (H)                          | AF286763              |
| <i>P. evansiana</i> Gyeln. 1  | Canada, <i>Goward</i> 94-972 (UBC)                          | AF286819              |
| <i>P. evansiana</i> 2   | Canada, <i>Goward</i> 89-145 (UBC)                          | AF286818              |
| <i>P. frigida</i> R. Sant.  | Argentina, <i>Stenroos</i> 2158 (H)                         | AF286780              |
| <i>P. frippii</i> Holt.-Hartw.  | Canada, <i>Scotter</i> 6032 (H)                             | AF286755              |
| <i>P. "fuscoponojensis"</i> *   | Canada, <i>Goward &amp; Bringhurst</i> 94-1017 (UBC)        | AF286795              |
| <i>P. "fuscopraetextata"</i> 1*   | Canada, <i>Goward</i> 93-434 (UBC)                          | AF286817              |
| <i>P. "fuscopraetextata"</i> 2*   | U.S.A., <i>Goward &amp; Knight</i> 90-154 (UBC)             | AF286816              |
| <i>P. horizontalis</i> (Huds.) Baumg. 1   | Canada, <i>Zoladecki &amp; Lutzoni</i> 11336-L7 (QFA)       | AF286761              |
| <i>P. horizontalis</i> 2  | Canada, <i>Goward</i> 81-1663 (CANL)                        | AF286760              |
| <i>P. hymenina</i> (Ach.) Delise 1  | Poland, <i>Budzbon</i> 2215 (UGDA-L)                        | AF286744              |
| <i>P. hymenina</i> 2  | Poland, <i>Czarnota</i> 1.10.1994 (GPN)                     | AF286745              |
| <i>P. kristinssonii</i> Vitik. 1  | Canada, <i>Goward</i> 81-1718 (UBC)                         | AF286779              |
| <i>P. kristinssonii</i> 2   | Canada, <i>Goward &amp; Findlay</i> 83-506 (UBC)            | AF286778              |
| <i>P. laciniata</i> (G. Merr. ex Riddle) Gyeln.                                   | Ecuador, <i>Kalb</i> 511 (H)                                | AF286799              |
| <i>P. lambinonii</i> Goffinet   | Australia, <i>Tibell</i> 12401 (H)                          | AF286803              |
| <i>P. "latopraetextata"</i> 1   | Canada, <i>Goward &amp; Lea</i> 90-876 (UBC)                | AF286785              |
| <i>P. "latopraetextata"</i> 2   | Canada, <i>Goward &amp; Clark</i> 81-1576 (UBC)             | AF286786              |
| <i>P. lepidophora</i> (Vain.) Bitter 1  | Canada, <i>Nuyt</i> 10.083-L26 (QFA)                        | AF286798              |
| <i>P. lepidophora</i> 2   | Canada, <i>Goward</i> 97-293 (UBC)                          | AF286797              |
| <i>P. leucopplebia</i> (Nyl.) Gyeln.  | China, <i>Koponen</i> 45314 (H)                             | AF286753              |
| <i>P. malacea</i> (Ach.) Funck 1  | Poland, <i>Faltynowicz</i> 5237 (UGDA-L)                    | AF286756              |
| <i>P. malacea</i> 2   | Poland, <i>Cieslinski</i> 29.08.1988 (KTLC)                 | AF286757              |
| <i>P. membranacea</i> (Ach.) Nyl. 1*  | Canada, <i>Gowan</i> 2134 (CANL)                            | AF286792              |
| <i>P. membranacea</i> 2   | Poland, <i>Olszewski</i> 5238 (UGDA-L)                      | AF286791              |
| <i>P. membranacea</i> 3   | Canada, <i>Bastian &amp; Dignard</i> 575 (QFA)              | AF286790              |
| <i>P. monticola</i> Vitik. 1  | Poland, <i>Faltynowicz</i> 5239 (UGDA-L)                    | AF286768              |
| <i>P. monticola</i> 2   | Yugoslavia, <i>Vitikainen</i> 7196 (H)                      | AF286770              |
| <i>P. monticola</i> 3   | Poland, <i>Toborowicz</i> 13.08.1976 (KTLC)                 | AF286769              |
| <i>P. neckeri</i> Hepp ex Müll. Arg.  | Poland, <i>Miadlikowska</i> 5240 (UGDA-L)                   | AF286766              |
| <i>P. "neocanina"</i> 1   | Canada, <i>Goward</i> 95-689 (UBC)                          | AF286782              |
| <i>P. "neocanina"</i> 2   | Canada, <i>Goward &amp; Clement</i> 94-233 (UBC)            | AF286783              |
| <i>P. neopolydactyla</i> (Gyeln.) Gyeln.  | Canada, <i>Zoladecki &amp; Lutzoni</i> 1136-L10 (QFA)       | AF286737              |
| <i>P. "neorufescens"</i> 1  | Canada, <i>Goward</i> 95-688 (UBC)                          | AF286796              |
| <i>P. "neorufescens"</i> 2  | Canada, <i>Goward &amp; Clark</i> 81-1591 (UBC)             | AF286801              |

**Table 3**  
(Continued)

| Taxon   | Voucher                                     | GenBank accession no. |
|---|---|-----------------------|
| <i>P. nigripunctata</i> Bitter 1              | China, Koponen 47011 (H)                    | AF286752              |
| <i>P. nigripunctata</i> 2                     | China, Koponen 47470 (H)                    | AF286751              |
| <i>P. occidentalis</i> (E. Dahl) Krist.       | Canada, Goward 93-420 (UBC)                 | AF286742              |
| <i>P. pacifica</i> Vitik. 1                   | Canada, Abti 38539 (H)                      | AF286749              |
| <i>P. pacifica</i> 2                          | Canada, Abti 38528 (H)                      | AF286748              |
| <i>P. "pallidorufescens"</i> 1*               | Canada, Goward & Clement 94-232 (UBC)       | AF286815              |
| <i>P. "pallidorufescens"</i> 2*               | Canada, Goward & Lea 92-214 (UBC)           | AF286812              |
| <i>P. phyllidiosa</i> Goffinet & Miadlikowska | U.S.A., Reeb VR 10-X-97/6 (F)               | AF286764              |
| <i>P. polydactylon</i> (Neck.) Hoffm. 1       | Poland, Kukwa 5241 (UGDA-L)                 | AF286739              |
| <i>P. polydactylon</i> 2                      | Australia, Streiman 43811 (H)               | AF286741              |
| <i>P. polydactylon</i> 3*                     | Poland, Toborowicz 2751 (UGDA-L)            | AF286740              |
| <i>P. polydactyloides</i> Nyl.                | Tanzania, Koponen 44127 (H)                 | AF286767              |
| <i>P. ponojensis</i> Gyeln. 1                 | Canada, Goward 82-1233 (CANL)               | AF286773              |
| <i>P. ponojensis</i> 2                        | Poland, Bielczyk 42116 (KRAM-L)             | AF286771              |
| <i>P. ponojensis</i> 3                        | Poland, Kiszka 2.09.1988 (KRAP-L)           | AF286772              |
| <i>P. praetextata</i> (Sommerf.) Zopf 1       | Poland, Cieslinski 1208 (KTC)               | AF286813              |
| <i>P. praetextata</i> 2                       | Poland, Gos 5242 (UGDA-L)                   | AF286814              |
| <i>P. pulverulenta</i> (Tayl.) Nyl. 1         | Venezuela, Abti 37205 (H)                   | AF286747              |
| <i>P. pulverulenta</i> 2                      | Ecuador, Ardivisson 4627 (H)                | AF286746              |
| <i>P. retifoveata</i> Vitik. 1                | Canada, Goward & Burger 94-1004 (UBC)       | AF286776              |
| <i>P. retifoveata</i> 2                       | Canada, Goward & Goward 83-514 (UBC)        | AF286775              |
| <i>P. rufescens</i> (Weiss) Humb. 1           | Canada, Wong 4067 (CANL)                    | AF286802              |
| <i>P. rufescens</i> 2                         | Poland, Faltynowicz 5243 (UGDA-L)           | AF286800              |
| <i>P. scabrosa</i> Th. Fr.                    | Finland, Vitikainen 9743 (H)                | AF286738              |
| <i>P. scabrosella</i> Holt.-Hartw.            | Finland, Abti 38058 (H)                     | AF286743              |
| <i>P. "scotteri"</i> 1*                       | Canada, Goward 81-1289a (UBC)               | AF286774              |
| <i>P. "scotteri"</i> 2*                       | Canada, Goward & Miede 95-1153 (UBC)        | AF286788              |
| <i>P. venosa</i> (L.) Hoffm.                  | Canada, Goward 97-294 (UBC)                 | AF286750              |
| <i>Hydrothyria venosa</i> J.R. Russell        | U.S.A., LaGreca 492 (DUKE)                  | AF286823              |
| <i>Lobaria pulmonaria</i> (L.) Hoffm.         | Switzerland, Zoller V4 (personal herbarium) | (AF183934)            |
| <i>L. quercizans</i> Michx.                   | U.S.A., 17-III-1992 (DUKE)                  | (AF279397)            |
| <i>Massalongia carnosa</i> (Dickson) Körber   | U.S.A., McCune 21508 (OSU)                  | AF286827              |
| <i>Nephroma arcticum</i> (L.) Torss.          | U.S.A., Sharnoff & Sharnoff 1484.27 (CANL)  | AF286828              |
| <i>N. resupinatum</i> (L.) Ach. 1             | U.S.A., Sharnoff & Sharnoff 1192.22 (CANL)  | AF286829              |
| <i>N. resupinatum</i> 2                       | U.S.A., Sharnoff & Sharnoff 1098.34 (CANL)  | AF286830              |
| <i>Pseudocyphellaria crocata</i> (L.) Vain.   | U.S.A., McCune 22989 (OSU)                  | AF286826              |
| <i>Stictia fuliginosa</i> (Hoff.) Ach.        | U.S.A., McCune 23489 (OSU)                  | AF286825              |
| <i>Solorina crocea</i> (L.) Ach.              | U.S.A., McCune 23785 (OSU)                  | AF286824              |

Note. Abbreviations of herbaria follow Holmgren et al. (1990) and Mirek et al. (1997). Taxa in quotation marks are under study by T. Goward but are not formally published. Specimens followed by an asterisk are morphologically atypical or represent species that are poorly delimited and, therefore, are treated as separate OTUs throughout this study. Accession numbers in parentheses represent sequences obtained from GenBank (Bhattacharya et al. 2000; Zoller et al. 1999); all other sequences were generated by this study.

all 55 taxa. Five additional characters describing nonmorphological attributes (e.g., ecology and geography) were also part of the morphological data matrix. Anatomical structure of veins was investigated using scanning electron microscopy (SEM; Amray 1810). Specimens were scored in a global way; i.e., the scores represent morphological features for the species as a whole rather than for the specific specimen. This was true for all specimens except for the putative species proposed by T. Goward and four other atypical specimens that were scored as separate OTUs (see table 3). For trimembered taxa that can develop into different photomorphs, both morphs were considered part of the same taxonomic and biological unit.

#### Chemical Data (Terpenoids)

The chemical data set presented here is from chromatographic analyses carried out by J. Miadlikowska and from literature reports. Secondary metabolites were examined using standard thin-layer chromatography (TLC) techniques described by Culberson and Kristinsson (1970) and Culberson (1972), modified by Menlove (1974). Secondary compounds were extracted in hexane or acetone for ca. 5 min and spotted onto precoated Merck silica gel 60 F<sub>254</sub> aluminum plates and eluted two or three times in the solvent system TA and EHF (Tønsgberg and Holtan-Hartwig 1983). The chromatograms

were air dried between each elution. For two-dimensional chromatography, EHF was used for the first direction and TA for the second direction; the chromatograms were eluted twice in both directions. These plates were then sprayed with 10% sulfuric acid and heated in an oven at 110°C for 3–5 min. Triterpenoids were studied in daylight and in ultraviolet light (350 nm) for observation of characteristic colors. Identification of substances was made by comparison with other species with known chemical composition, mostly with material from the study by Holtan-Hartwig (1993). The literature consulted and the chemical characters used to build the data matrix are presented in the “Results” section.

Depside, from the tenuiorin complex, occur in all *Peltigera* members outside of the *canina* complex. With few exceptions, they are uniformly distributed within each of these species. Therefore, each depside has virtually no taxonomic value within *Peltigera*, and they were pooled to form one character (presence/absence of depsides). To demonstrate the contribution of terpenoids alone (the most informative secondary compounds within *Peltigera*) to resolve phylogenetic relationships among peltigeroid species, we decided to restrict the chemical data set to terpenoids and to transfer the presence/absence of depsides to the morphological matrix. The chemical data set includes 42 terpenoids, all scored as present/absent. The numbering of these terpenoids throughout this study follows the system of Holtan-Hartwig (1993).

For all taxa with multiple chemotypes, where specific terpenoids occurred only in some chemotypes within a species, all lineages of such polymorphic species were considered to have (or to have had) the potential to synthesize that specific terpenoid. These terpenoids were scored as present for these taxa. Substances of unknown chemical structure that were recognized and numbered by Holtan-Hartwig (1993) and Miadlikowska and Holtan-Hartwig (1997) were treated as missing data for taxa that have not been studied extensively by at least one of these authors. Presence/absence of individual terpenoids were scored globally, as for the morphological study, except for taxa with uncertain circumscriptions (see table 3).

#### Molecular Data

Well-preserved lichen thalli without any visible damage or fungal infection were chosen for DNA isolation. All extraneous plant material attached to the targeted portion of the thallus was removed under a dissecting microscope. Small thallus fragments from subterminal parts of lobes from fresh or dried herbarium collections (up to 20 yr old) were sampled for DNA isolation. DNA was isolated using the Purgene Kit (GENTRA Systems) from fragments containing both the photo- and mycobiont, following the manufacturer’s protocol for filamentous fungi. DNA concentration was determined by spectrophotometry or by visual comparison with a positive control ( $\lambda$  100 ladder, concentration 10, 20, 40 Ng) on an ethidium-bromide-stained TBE agarose gel.

Symmetric polymerase chain reaction (PCR) was performed to amplify both strands of a 1.4-kb fragment at the 5’ end of the LSU nrDNA. The amplification reaction was prepared for a 50- $\mu$ L final volume containing 33.7  $\mu$ L of sterile double-distilled water, 5.0  $\mu$ L of 10  $\times$  Taq polymerase reaction buffer (Behringer-Mannheim), 5.0  $\mu$ L dNTP, 0.3  $\mu$ L Taq DNA poly-

merase (Behringer-Mannheim), 2.5  $\mu$ L for each of the 10  $\mu$ M fungi-specific primers LROR and LR7 (Vilgalys and Hester 1990; Moncalvo et al. 1993), and 1.0  $\mu$ L of template genomic DNA. PCR was performed on Peltier Thermal Cyclers PTC-200 (MJ Research) under the following conditions: one cycle of 1 min at 95°C linked to 34 cycles of 30 s at 95°C, 30 s at 52°C, and 1.5 min at 72°C. Samples were held for a final 4 min at 72°C to complete primer extensions, after which the samples were kept at 4°C until electrophoresis was performed on agarose gels. PCR symmetric amplification products were cleaned using low-binding regenerated cellulose 30,000 NMWL (nominal molecular weight limit) filter units (Millipore). Both strands of purified PCR products were sequenced using the following primers: LIC24R (5’-GAAACCAACAGGGATTG-3’), LROR, LR7, LR6, LR5, LR3, and LR3R (Vilgalys and Hester 1990; Moncalvo et al. 1993). The sequencing reaction was performed in a 10- $\mu$ L final volume using d-Rhodamine Terminator (ABI PRISM, Perkin-Elmer, Applied Biosystems) following the manufacturer’s instructions. Sequenced product was precipitated with 10  $\mu$ L of deionized sterile water, 2  $\mu$ L of 3 M NaOAc, and 50  $\mu$ L of 95% EtOH. Polyacrylamide gel electrophoresis was conducted using Long Ranger Singel packs (FMC BioProducts) and an ABI 377A automated DNA sequencer (Perkin-Elmer, Applied Biosystems). Sequence fragments were assembled using Sequencher 3.0 (Gene Codes). The resulting consensus sequences were aligned using Sequencher 3.0 and optimized by eye. The secondary structure of the LSU nrDNA of *Saccharomyces cerevisiae* (Larsen et al. 1993) was used to verify the alignment and to provide an additional criterion to delimit ambiguously aligned regions (Lutzoni et al. 2000).

#### Phylogenetic Analyses

All phylogenetic analyses were performed using maximum parsimony as the optimization criterion as implemented in PAUP\* 4.0d61a (Swofford 1998). The following five analyses were carried out on the morphological, chemical, and molecular data sets: morphology only; chemistry only; LSU nrDNA only; chemical, morphological, and nrDNA LSU data combined for all 96 specimens; and combined analysis for a subsample of 54 specimens. For the separate phylogenetic analyses of the chemical and morphological data sets, all changes among character states were weighted equally. To simultaneously accommodate taxa with multiple character states resulting from uncertainty or polymorphism, the “variable” option in PAUP\* was used.

The phylogenetic analysis of the LSU nrDNA data set alone involved several step matrices. Ambiguously aligned regions were integrated in the phylogenetic analyses without violating positional homology using the program INAASE (Lutzoni et al. 2000); i.e., these regions were replaced by unequivocally coded characters that were subjected to specific step matrices. The unambiguous portions of the alignment were subjected to one additional step matrix that was obtained as follows: the dinucleotide frequency option of PAUP\* was implemented on the unambiguous sites only; the average frequencies of changes were calculated from pairwise comparisons of sequences using the program Excel 5.0 (Microsoft Corp.); the absolute average frequencies for all 10 pairs of possible probabilities of recip-

rocal changes from one state to another (including unambiguous gaps) were converted to costs of changes using the negative natural logarithm of the probability (Felsenstein 1981; Wheeler 1990; Maddison and Maddison 1992); and these costs were rounded off to a single decimal point and then inserted in the symmetric step matrix. Triangle inequality was tested for each step matrix using MacClade 3.05 (Maddison and Maddison 1992). When triangle inequality was violated, the highest cost in the step matrix between two states was lowered by increments of one step, using MacClade's Type Edit dialog box and retesting triangle inequality. Constant sites were excluded from all analyses (including bootstrap). Gaps were used as a fifth character state for the unambiguous portions of the alignment. Two additional molecular characters corresponding to presence/absence of spliceosomal and group I introns are also included in the molecular data matrix.

For all three separate analyses, a first round of heuristic searches was performed with 1000 random-addition-sequence replicates, NNI branch swapping, MULPARS option in effect, saving no more than two trees greater than or equal to five steps for each replicate, and collapsing zero-length branches. The trees generated from this analysis were used as starting points for a second round of heuristic searches, but with TBR branch swapping and saving all most parsimonious trees without any restrictions. This procedure was necessary because of the large number of equally most parsimonious trees resulting from the inclusion of many representative specimens for species nested within the *P. canina* complex that could not be resolved by any of these data sets when analyzed separately.

The detection of potential conflicts among partitions was performed by inspecting bootstrap scores above 70% (Mason-Gamer and Kellogg 1996). If bootstrap analyses on two different partitions provided support  $\geq 70\%$  for two different phylogenetic relationships for the same set of taxa, this was interpreted as a potential incongruence between two partitions. The morphological, chemical, and molecular characters for the 96 OTU data matrix were treated the same way in the combined analysis as when analyzed separately. The search was performed in two rounds, as for the three separate analyses. The phylogenetic relationships revealed by the combined analysis of the 96 OTU data set was summarized using a strict consensus tree. Bootstrap support for these four analyses was obtained using the fast-heuristic search option for 2000 replicates.

Because we wanted to confirm the validity of sequences as well as the validity of species used in our study, for most taxa more than one specimen (often three) was included in the complete data matrix (96 OTUs). Many of these sequences were almost identical within species and generated a large number of equally most parsimonious trees. All redundant within-species sequences, including undescribed taxa from the *P. canina* complex, were removed from the fifth analysis of 54 out of 96 OTUs. This second combined analysis was identical in procedure to the combined analysis of 96 OTUs, except that it consisted of only one round of searches with 1000 random-addition-sequences, TBR swapping, and MULTREES selected. Branch support for this combined analysis of 54 sequences was estimated by 1000 bootstrap replicates (Felsenstein 1985) and implemented full heuristic searches with four random-addition-sequences per bootstrap replicate. Unequivocal syn-

apomorphic character states for the main clades were recognized using PAUP\*.

To determine if some reconstructed phylogenetic relationships among species could be a result of sampling error, alternative phylogenetic relationships were tested: (1) *Peltigera*, including *Hydrothyria venosa*, is not monophyletic; (2) *H. venosa* is not sister to *Peltigera* or nested within *Peltigera*; (3) trimembered *Peltigera* species are monophyletic; (4) *P. venosa* is not sister to the rest of *Peltigera* species; (5) "*P. scotteri*" is monophyletic. For each hypothesis, the search was done by constraining the appropriate nodes to test a particular phylogenetic alternative and performing maximum parsimony analyses as described above for the fifth analysis on 54 OTUs. If the length of the most parsimonious constrained tree was longer than 95% of the 1000 tree lengths resulting from 1000 bootstrap replicates and scored according to the original data matrix, the tree lengths of the constrained most parsimonious tree(s) and the unconstrained maximum parsimony tree(s) were considered to be significantly different (see O'Donnell et al. 2000; Wu et al. 2000), and the tested hypothesis was rejected. Each alternative hypothesis was also tested using the Templeton test available in PAUP\*.

## Results

### *Morphological Characters*

Character scores in the morphological data matrix (table 4) are derived from specimen-based studies conducted by J. Miadlikowska and descriptions from the following publications: Bitter (1909), Vainio (1915), Santesson (1944), Thomson (1955, 1979), Hale (1957), Wetmore (1960), Lindhal (1962), Henssen (1963), Yoshimura (1971), James and Henssen (1976), Vitikainen (1985, 1987, 1994a, 1994b), James and White (1987), White and James (1987b), Galloway (1988), Swinscow and Krog (1988), Holtan-Hartwig (1988, 1993), Ott (1988), Goffinet and Hastings (1994, 1995), Goward et al. (1994, 1995), Stenroos et al. (1994), Kondratyuk and Galloway (1995), Miadlikowska (1998), Goffinet and Miadlikowska (1999), and Martínez (1999). Most of the characters included in this matrix are routinely used for the determination and description of *Peltigera* species. Because many specimens have intermediary and chimeric features and most species are known to exhibit high interpopulation phenotypic variation, defining characters and character states was often problematic. This broad range of morphological variation found in *Peltigera* at the species and population levels often required the use of multistate characters. Only eight out of the 31 morphological characters were binary. The following characters were included in the morphological data matrix. In parentheses are the "tracking" numbers for these characters as they are presented in tables 4 and 5 and figure 9 (as part of the complete data matrix):

1. (43) Cyanomorph: 0 = not seen; 1 = seen. Early developmental stages during the ontogeny of trimembered taxa (e.g., *Solorina crocea*) were not taken into consideration, even though reports of this phenomenon exist in the literature (see James and Henssen 1976; Holtan-Hartwig 1996).

2. (44) Photobiont type: 0 = cf. *Nostoc* sp. only; 1 = cf. *Coccomyxa* sp. + cf. *Nostoc* sp.; 2 = grass-green alga + cf.



*Nostoc* sp. The term “grass-green alga” was used for cases where an alga other than *Coccomyxa* was found in the thallus.

3. (45) Cephalodium: 0 = absent; 1 = present, external on upper surface of thallus; 2 = present, external on under surface of thallus; 3 = present, internal.

4. (46) Lower cortex of thallus: 0 = absent; 1 = present. For *Hydrothyria venosa* and the cyanomorph of *P. venosa*, their homoiomerous thalli consisting of paraplectenchymatic fungal + cyanobacterial tissue surrounded by layers of fungal hyphae were considered to have a lower cortex.

5. (47) Cortex on fertile lobes beneath apothecia: 0 = absent; 1 = present as an almost continuous layer; 2 = present as distinctly discontinuous patches or warts.

6. (48) Apothecium: 0 = not seen; 1 = seen.

7. (49) Apothecial disk color: 0 = black; 1 = brown (medium brown, dark brown, chestnut brown, reddish brown); 2 = pale brown. Because the color of the apothecial disk can vary according to the age of the apothecium (disks usually become darker with age), only well-developed and mature ascomata were taken into consideration. Observations were made from dry specimens.

8. (50) Apothecium orientation: 0 = vertical; 1 = horizontal. The orientation of apothecia is relative to the general plane of the thallus.

9. (51) Apothecium habit: 0 = planar; 1 = folded downward (distally revolute, finger or saddle shaped); 2 = folded upward (concave or involute).

10. (52) Venation on underside of thallus: 0 = absent; 1 = present and distinctly visible; 2 = present but indistinct (always with only a few interstices between veins).

11. (53) Vein prominence: 0 = not prominent; 1 = prominent (i.e., elevated compared to the rest of the lower surface of the thallus).

12. (54) Vein structure: 0 = hyphae completely unordered; 1 = hyphae mostly unordered, but with small groups of parallel and conglutinated hyphae; 2 = hyphae mostly to completely ordered in a parallel and conglutinated fashion. These three main types of vein structure were recognized based on a SEM study (figs. 2, 3). Character state 2 corresponds to the pilema type reported by Holtan-Hartwig (1993) for members of the *P. canina* group. The central part of the vein (if not the entire vein) forms a distinct compact core composed of parallel hyphae in which the hyphal walls are completely or mostly conglutinated (fig. 2). This type of vein is usually well differentiated and convex. The extreme form of this vein type occurs in *H. venosa* (fig. 2a). All members of the *P. canina* group have this type of vein, as well as *P. venosa* (fig. 2d) and some members of the *P. polydactylon* group (fig. 2b). The pilema of veins scored as character state 0 forms broad flat veins to a thick layer covering the entire lower surface of the thallus. Hyphae are totally or mostly unordered and nonconglutinated (fig. 3a, 3c, 3e). This vein type occurs only in the *P. aphthosa* group and *P. pulverulenta*. The veins scored as character state 1 are found in most members of the *P. polydactylon* group, in *P. elisabethae* (fig. 3d, 3f), *P. retifoveata* (fig. 3b), *P. leucophlebia*, and *P. nigripunctata*. In this case, the small groups of parallel and distinctly conglutinated hyphae are embedded in a matrix of unoriented and nonconglutinated hyphae. No distinct core is present (fig. 3). Some intermediary forms between character states 1 and 2 were observed.

13. (55) Vein tomentum: 1 = erect; 2 = not erect.

14. (56) Rhizine: 0 = absent; 1 = present, numerous; 2 = present as a single rhizopt.

15. (57) Rhizine tomentum: 1 = erect; 2 = not erect.

16. (58) Rhizine development: 1 = separate; 2 = confluent.

17. (59) Rhizine morphology: 1 = simple; 2 = fasciculate; 3 = fibrillose, flocculent or penicillate.

18. (60) Rhizine arrangement: 0 = unordered; 1 = concentric rows; 2 = radiating rows; 3 = clusters.

19. (61) Upper cortex scabrosity: 0 = absent; 1 = distinctly present over most of the surface; 2 = sparse on some parts only.

20. (62) Pruina on upper surface of thallus: 0 = not seen; 1 = seen.

21. (63) Maculae: 0 = not seen; 1 = seen, weak, almost only on the margin of thallus; 2 = seen, conspicuous, also in the center of thallus.

22. (64) Upper cortex tomentum: 0 = absent; 1 = present only at the margin; 2 = present also on other parts of thallus; 3 = present as scattered patches at the margin of sterile lobes and adjacent to apothecia, if apothecia present.

23. (65) Upper cortex tomentum type: 1 = arachnoid (felt-like with appressed and entangled tips); 2 = erect (the ends of hyphae are erect and discrete).

24. (66) Soralium: 0 = absent; 1 = present, marginal only; 2 = present, laminal only; 3 = present, marginal and laminal.

25. (67) Vegetative propagule (excluding soredia): 0 = absent; 1 = isidia or phyllidia present; 2 = schizidia present.

26. (68) Chemistry: 0 = no secondary compounds detected by TLC; 1 = depsides and triterpenoids present; 2 = only triterpenoids present; 3 = only depsides present.

27. (69) Ecological preference: 0 = xeric; 1 = mesic; 2 = subhygric; 3 = aquatic. These scores should not be seen as absolutes. Because we included a truly aquatic species in this study, this had the effect of shifting the relative scale of moisture. For example, a species that was considered to be hygric is now subhygric. Because it is difficult to distinguish between classes of moisture preferences, we preferred to have fewer well-defined classes than many classes forming a continuum.

28. (70) Distribution: 0 = Europe; 1 = North America; 2 = Asia; 3 = South America; 4 = Africa; 5 = Australia or New Zealand.

29. (71) Lichenicolous fungi *Corticifraga* spp.: 0 = not seen; 1 = seen.

30. (72) Lichenicolous fungi other than *Corticifraga* spp.: 0 = not seen; 1 = seen.

31. (73) Pycnidium: 0 = not seen; 1 = seen, *Peltigera* type (VII, according to Vobis 1980); 2 = seen, *Lobaria* type (II, according to Vobis 1980); 3 = seen, other type.

#### Chemical Characters (Terpenoids)

Data for the outgroups and *Peltigera* taxa that are rare or insufficiently known (i.e., rarely collected) were taken from the following literature and unpublished sources: Wetmore (1960), Kurokawa et al. (1966), Yoshimura (1971), Ohlsson (1973), Maass (1975a, 1975b), Tønsberg and Holtan-Hartwig (1983), Vitikainen (1985, 1994a, 1994b), James and White (1987), White and James (1987a, 1987b), Galloway (1988), Holtan-Hartwig (1988, 1993), Feige et al. (1989), Purvis and James

Table 4

Data Matrix for Chemical (1–42) and Morphological (43–73) Characters

| OTU                         | 1          |            | 2          |            | 3          |            | 4          |            | 5          |            | 6          |            | 7          |     |
|-----------------------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|-----|
|                             | 1234567890 | 1234567890 | 1234567890 | 1234567890 | 1234567890 | 1234567890 | 1234567890 | 1234567890 | 1234567890 | 1234567890 | 1234567890 | 1234567890 | 1234567890 | 123 |
| <i>P. neopolydactyla</i>    | 0000000011 | 0010000101 | 0000000000 | 0010101000 | 00000001E0 | 11A12121E0 | B110?001EM | 110        |            |            |            |            |            |     |
| <i>P. scabrosa</i>          | 0000000011 | 0010000100 | 0000000000 | 0010001??? | ??00000110 | 11A22121J0 | 1000?0011H | 110        |            |            |            |            |            |     |
| <i>P. polydactylon 1</i>    | 0000000011 | 0010000100 | 0000010000 | 1000101000 | 0000000110 | 1101212EE0 | 0000?0A11W | 111        |            |            |            |            |            |     |
| <i>P. polydactylon 3</i>    | 0000000011 | 0010000100 | 0000010000 | 1000101000 | 000000?0?? | ?1012121E0 | 0000?00110 | 000        |            |            |            |            |            |     |
| <i>P. polydactylon 2</i>    | 0000000011 | 0010000100 | 0000010000 | 1000101000 | 0000000110 | 1101212EE0 | 0000?0A11W | 111        |            |            |            |            |            |     |
| <i>P. occidentalis</i>      | ????????11 | 0?100??0?? | ??0????0?0 | 0???0????? | ??00000110 | 1101212130 | 0100?001E1 | ???        |            |            |            |            |            |     |
| <i>P. scabrosella</i>       | 0000010001 | 0010010000 | 0000000000 | 0000000??? | ??00000110 | A102212110 | 1000?0012A | 000        |            |            |            |            |            |     |
| <i>P. hymenina 1</i>        | 0000000011 | 0010000000 | 0000000001 | 0000100000 | 0000000110 | 11012121E0 | 0010?001EK | 110        |            |            |            |            |            |     |
| <i>P. hymenina 2</i>        | 0000000011 | 0010000000 | 0000000001 | 0000100000 | 0000000110 | 11012121E0 | 0010?001EK | 110        |            |            |            |            |            |     |
| <i>P. pulverulenta 2</i>    | ????????11 | ??100??0?? | ?????????0 | 0????????? | ??000001E0 | 1110211110 | 1000?00113 | ?1?        |            |            |            |            |            |     |
| <i>P. pulverulenta 1</i>    | ????????11 | ??100??0?? | ?????????0 | 0????????? | ??000001E0 | 1110211110 | 1000?00113 | ?1?        |            |            |            |            |            |     |
| <i>P. pacifica 2</i>        | ????????11 | 0?100??0?? | ??0????0?0 | 0???0????? | ??00000110 | 1111212120 | 0000?0A121 | ???        |            |            |            |            |            |     |
| <i>P. pacifica 1</i>        | ????????11 | 0?100??0?? | ??0????0?0 | 0???0????? | ??00000110 | 1111212120 | 0000?0A121 | ???        |            |            |            |            |            |     |
| <i>P. venosa</i>            | 0000000010 | 0111100100 | 0000000100 | 0011001000 | 001ABA0111 | B1A222???? | 0000?00AEH | 010        |            |            |            |            |            |     |
| <i>P. nigripunctata 2</i>   | ??????1100 | ??101?10?? | ??1?????0? | 0????????? | ??011021A1 | 01A12121E0 | 0?012001H2 | ???        |            |            |            |            |            |     |
| <i>P. nigripunctata 1</i>   | ??????1100 | ??101?10?? | ??1?????0? | 0????????? | ??011021A1 | 01A12121E0 | 0?012001H2 | ???        |            |            |            |            |            |     |
| <i>P. leucophlebia</i>      | 0000001100 | 1101101000 | 0010000000 | 0000000000 | 00011021A0 | 1EA12121E0 | 010A2001HH | 010        |            |            |            |            |            |     |
| <i>P. britannica 1</i>      | 0000000001 | 0111100000 | 0000000010 | 1000000000 | 001AA01110 | AE10212EE0 | 012A20012A | 010        |            |            |            |            |            |     |
| <i>P. frippii</i>           | 0000000000 | 0111100000 | 0000000000 | 0000100??? | ??0000?0?? | ?1A1212E22 | 0020?001HH | 000        |            |            |            |            |            |     |
| <i>P. malacea 1</i>         | 0000000111 | 1110001000 | 0000000000 | 0000100000 | 0000000110 | 1E10212E23 | B1012001AH | 110        |            |            |            |            |            |     |
| <i>P. malacea 2</i>         | 0000000111 | 1110001000 | 0000000000 | 0000100000 | 0000000110 | 1E10212E23 | B1012001AH | 110        |            |            |            |            |            |     |
| <i>P. britannica 2</i>      | 0000000001 | 0111100000 | 0000000010 | 1000000000 | 001AA01110 | AE10212EE0 | 012A20012A | 010        |            |            |            |            |            |     |
| <i>P. aphthosa</i>          | 0000001101 | 0111101000 | 0100100010 | 1000000000 | 001AA01110 | AE10212EE0 | 01212001AP | 110        |            |            |            |            |            |     |
| <i>P. horizontalis 2</i>    | 1111100000 | 0010000000 | 0000011001 | 0100010111 | 11000001A1 | 010Y212121 | 0000?0A1EH | 010        |            |            |            |            |            |     |
| <i>P. horizontalis 1</i>    | 1111100000 | 0010000000 | 0000011001 | 0100010111 | 11000001A1 | 010Y212121 | 0000?0A1EH | 010        |            |            |            |            |            |     |
| <i>P. elisabethae 1</i>     | 1111100000 | 0010000000 | 0000011001 | 0100010111 | 11000001A1 | 0E01212121 | 0100?0211H | 010        |            |            |            |            |            |     |
| <i>P. elisabethae 2</i>     | 1111100000 | 0010000000 | 0000011001 | 0100010111 | 11000001A1 | 0E01212121 | 0100?0211H | 010        |            |            |            |            |            |     |
| <i>P. phyllidiosa</i>       | ????????00 | 0?100??0?? | ??0????0?? | ?????0???? | ??00000100 | 110Y2121E0 | 0110?01111 | ???        |            |            |            |            |            |     |
| <i>P. collina</i>           | 0000000011 | 0010000100 | 0000000000 | 0000101000 | 0000000100 | 1E0Y212EG0 | B110?G01EM | 111        |            |            |            |            |            |     |
| <i>P. neckeri</i>           | 0000000001 | 0010000010 | 1101100001 | 0000100000 | 0000000100 | 1E0Y212EJ0 | 0110?0011M | 111        |            |            |            |            |            |     |
| <i>P. polydactyloides</i>   | ????????01 | 0?100??0?? | 1?0????0?0 | 0????1???? | ??00000100 | 1E1Y212E30 | 0110?0A1E4 | ???        |            |            |            |            |            |     |
| <i>P. monticola 1</i>       | 0000000000 | 0000000000 | 0000000000 | 0000000000 | 0000000120 | A1A2212EG0 | B10A10A0AA | 001        |            |            |            |            |            |     |
| <i>P. monticola 2</i>       | 0000000000 | 0000000000 | 0000000000 | 0000000000 | 0000000120 | A1A2212EG0 | B10A10A0AA | 001        |            |            |            |            |            |     |
| <i>P. monticola 3</i>       | 0000000000 | 0000000000 | 0000000000 | 0000000000 | 0000000120 | A1A2212EG0 | B10A10A0AA | 001        |            |            |            |            |            |     |
| <i>P. ponojensis 2</i>      | 0000000000 | 0000000000 | 0000000000 | 0000000000 | 0000000110 | H1122121G0 | B10M1000AH | 111        |            |            |            |            |            |     |
| <i>P. ponojensis 3</i>      | 0000000000 | 0000000000 | 0000000000 | 0000000000 | 0000000110 | H1122121G0 | B10M1000AH | 111        |            |            |            |            |            |     |
| <i>P. ponojensis 1</i>      | 0000000000 | 0000000000 | 0000000000 | 0000000000 | 0000000110 | H1122121G0 | B10M1000AH | 111        |            |            |            |            |            |     |
| <i>P. scotteri 1</i>        | 0000000000 | 0000000000 | 0000000000 | 0000000000 | 0000000110 | 111221Y1G0 | 0003100011 | 001        |            |            |            |            |            |     |
| <i>P. retifoveata 2</i>     | 0000000011 | 0010000000 | 0100000000 | 0000000000 | 00000001A0 | 11111111E0 | 000E10011H | 000        |            |            |            |            |            |     |
| <i>P. retifoveata 1</i>     | 0000000011 | 0010000000 | 0100000000 | 0000000000 | 00000001A0 | 11111111E0 | 000E10011H | 000        |            |            |            |            |            |     |
| <i>P. continentalis</i>     | 0000000000 | 0000000000 | 0000000000 | 0000000000 | 0000000110 | B112212130 | 000E101012 | 01?        |            |            |            |            |            |     |
| <i>P. kristinssonii 2</i>   | 0000000000 | 0000000000 | 0000000000 | 0000000000 | 0000000110 | 21A211E120 | 210120001H | 01?        |            |            |            |            |            |     |
| <i>P. kristinssonii 1</i>   | 0000000000 | 0000000000 | 0000000000 | 0000000000 | 0000000110 | 21A211E120 | 210120001H | 01?        |            |            |            |            |            |     |
| <i>P. frigida</i>           | 0000000000 | 0000000000 | 0000000000 | 0000000000 | 0000000111 | 01122121G0 | 0100?000Y3 | ???        |            |            |            |            |            |     |
| <i>P. boreorufescens 2</i>  | 0000000000 | 0000000000 | 0000000000 | 0000000000 | 0000000110 | 1112212230 | 0002100011 | 000        |            |            |            |            |            |     |
| <i>P. neocanina 1</i>       | 0000000000 | 0000000000 | 0000000000 | 0000000000 | 0000000110 | 1102212130 | 0002100011 | ???        |            |            |            |            |            |     |
| <i>P. neocanina 2</i>       | 0000000000 | 0000000000 | 0000000000 | 0000000000 | 0000000110 | 1102E12130 | 0002100011 | ???        |            |            |            |            |            |     |
| <i>P. cinnamomea 2</i>      | 0000000000 | 0000000000 | 0000000000 | 0000000000 | 0000000110 | 11A2212110 | 0002100011 | 000        |            |            |            |            |            |     |
| <i>P. latopraetextata 1</i> | 0000000000 | 0000000000 | 0000000000 | 0000000000 | 0000000110 | 11A22121G0 | 000E10A011 | ???        |            |            |            |            |            |     |
| <i>P. latopraetextata 2</i> | 0000000000 | 0000000000 | 0000000000 | 0000000000 | 0000000110 | 11A22121G0 | 000E10A011 | ???        |            |            |            |            |            |     |
| <i>P. cinnamomea 1</i>      | 0000000000 | 0000000000 | 0000000000 | 0000000000 | 0000000110 | 11A2212110 | 0002100021 | 000        |            |            |            |            |            |     |
| <i>P. scotteri 2</i>        | 0000000000 | 0000000000 | 0000000000 | 0000000000 | 0000000120 | 1112E121G0 | 0003100011 | 001        |            |            |            |            |            |     |
| <i>P. degenii 2</i>         | 0000000000 | 0000000000 | 0000000000 | 0000000000 | 0000000120 | 1112E12110 | 000C10A0EH | 110        |            |            |            |            |            |     |
| <i>P. membranacea 3</i>     | 0000000000 | 0000000000 | 0000000000 | 0000000000 | 00000001E0 | 1112E11110 | 000H1000EH | 011        |            |            |            |            |            |     |
| <i>P. membranacea 2</i>     | 0000000000 | 0000000000 | 0000000000 | 0000000000 | 00000001E0 | 1112E11110 | 000H1000EH | 011        |            |            |            |            |            |     |
| <i>P. membranacea 1</i>     | 0000000000 | 0000000000 | 0000000000 | 0000000000 | 0000000110 | 1112E1Y110 | 0001100011 | 001        |            |            |            |            |            |     |

Table 4  
(Continued)

| OTU                                    | 1           |            | 2           |             | 3          |            | 4          |            | 5          |            | 6          |            | 7   |  |
|--|-------------|------------|-------------|-------------|------------|------------|------------|------------|------------|------------|------------|------------|-----|--|
|  | 1234567890  | 1234567890 | 1234567890  | 1234567890  | 1234567890 | 1234567890 | 1234567890 | 1234567890 | 1234567890 | 1234567890 | 1234567890 | 1234567890 | 123 |  |
| <i>P. degenii</i> 3                    | 0000000000  | 0000000000 | 0000000000  | 0000000000  | 0000000000 | 0000000000 | 0000000120 | 1112E12110 | 000C10A0EH | 110        |            |            |     |  |
| <i>P. degenii</i> 1                    | 0000000000  | 0000000000 | 0000000000  | 0000000000  | 0000000000 | 0000000000 | 0000000120 | 1112E12110 | 000C10A0EH | 110        |            |            |     |  |
| <i>P. fuscoponojensis</i>              | 0000000000  | 0000000000 | 0000000000  | 0000000000  | 0000000000 | 0000000000 | 00000000?? | ?112212110 | 0102100011 | ??1        |            |            |     |  |
| <i>P. neorufescens</i> 1               | 0000000000  | 0000000000 | 0000000000  | 0000000000  | 0000000000 | 0000000000 | 0000000110 | 1112212E3B | 00021000X1 | ??1        |            |            |     |  |
| <i>P. lepidophora</i> 2                | 0000000000  | 0000000000 | 0000000000  | 0000000000  | 0000000000 | 0000000000 | 0000000110 | 1102212EG0 | B00210100U | 110        |            |            |     |  |
| <i>P. lepidophora</i> 1                | 0000000000  | 0000000000 | 0000000000  | 0000000000  | 0000000000 | 0000000000 | 0000000110 | 1102212EG0 | B00210100U | 110        |            |            |     |  |
| <i>P. laciniata</i>                    | ?????????00 | 0?100??0?? | ??0?????0?0 | 0??0??????? | ??00000110 | 11A2E12130 | 00021002AF | ??1        |            |            |            |            |     |  |
| <i>P. rufescens</i> 2                  | 0000000000  | 0000000000 | 0000000000  | 0000000000  | 0000000000 | 0000000000 | 0000000110 | 11A221223B | 01021000AW | 111        |            |            |     |  |
| <i>P. neorufescens</i> 2               | 0000000000  | 0000000000 | 0000000000  | 0000000000  | 0000000000 | 0000000000 | 0000000110 | 1112212E3B | 00021000X1 | ??1        |            |            |     |  |
| <i>P. rufescens</i> 1                  | 0000000000  | 0000000000 | 0000000000  | 0000000000  | 0000000000 | 0000000000 | 0000000110 | 11A221223B | 01021000AW | 111        |            |            |     |  |
| <i>P. lambinonii</i>                   | 0000000000  | 0000000000 | 0000000000  | 0000000000  | 0000000000 | 0000000000 | 0000000110 | 1102212E30 | 000212031D | ???        |            |            |     |  |
| <i>P. didactyla</i> var. <i>did.</i> 3 | 0000000000  | 0000000000 | 0000000000  | 0000000000  | 0000000000 | 0000000000 | 0000000110 | 1102E121G0 | 0001120310 | 001        |            |            |     |  |
| <i>P. didactyla</i> var. <i>did.</i> 2 | 0000000000  | 0000000000 | 0000000000  | 0000000000  | 0000000000 | 0000000000 | 0000000110 | 1102E12EG0 | 000A1200AW | 111        |            |            |     |  |
| <i>P. didactyla</i> var. <i>did.</i> 1 | 0000000000  | 0000000000 | 0000000000  | 0000000000  | 0000000000 | 0000000000 | 0000000110 | 1102E12EG0 | 000A1200AW | 111        |            |            |     |  |
| <i>P. didactyla</i> var. <i>did.</i> 4 | 0000000000  | 0000000000 | 0000000000  | 0000000000  | 0000000000 | 0000000000 | 0000000110 | 1102E12EG0 | 000A1200AW | 111        |            |            |     |  |
| <i>P. didactyla</i> var. <i>ext.</i> 2 | 0000000000  | 0000000000 | 0000000000  | 0000000000  | 0000000000 | 0000000000 | 0000000110 | 1102212EG0 | 000A12031H | 111        |            |            |     |  |
| <i>P. didactyla</i> var. <i>ext.</i> 1 | 0000000000  | 0000000000 | 0000000000  | 0000000000  | 0000000000 | 0000000000 | 00000000?? | ?102212E30 | 20021B0010 | 000        |            |            |     |  |
| <i>P. didactyla</i> var. <i>ext.</i> 3 | 0000000000  | 0000000000 | 0000000000  | 0000000000  | 0000000000 | 0000000000 | 0000000110 | 1102212EG0 | 000A12031H | 111        |            |            |     |  |
| <i>P. cinnamomea</i> 3                 | 0000000000  | 0000000000 | 0000000000  | 0000000000  | 0000000000 | 0000000000 | 0000000110 | 11A2212110 | 0002100021 | 000        |            |            |     |  |
| <i>P. pallidorufescens</i> 2           | 0000000000  | 0000000000 | 0000000000  | 0000000000  | 0000000000 | 0000000000 | 0000000110 | 1102212E30 | 2001101011 | 000        |            |            |     |  |
| <i>P. praetextata</i> 1                | 0000000000  | 0000000000 | 0000000000  | 0000000000  | 0000000000 | 0000000000 | 00000001E0 | 1112E1E110 | B10M10A0EN | 110        |            |            |     |  |
| <i>P. praetextata</i> 2                | 0000000000  | 0000000000 | 0000000000  | 0000000000  | 0000000000 | 0000000000 | 00000001E0 | 1112E1E110 | B10M10A0EN | 110        |            |            |     |  |
| <i>P. pallidorufescens</i> 1           | 0000000000  | 0000000000 | 0000000000  | 0000000000  | 0000000000 | 0000000000 | 00000000?? | ?112212E30 | 0002100011 | 011        |            |            |     |  |
| <i>P. fuscopraetextata</i> 2           | 0000000000  | 0000000000 | 0000000000  | 0000000000  | 0000000000 | 0000000000 | 0000000110 | 1112E1223B | 2102100011 | 000        |            |            |     |  |
| <i>P. fuscopraetextata</i> 1           | 0000000000  | 0000000000 | 0000000000  | 0000000000  | 0000000000 | 0000000000 | 00000000?? | ?112E1Y110 | 0002100011 | 000        |            |            |     |  |
| <i>P. evansiana</i> 1                  | 0000000000  | 0000000000 | 0000000000  | 0000000000  | 0000000000 | 0000000000 | 00000001E0 | 11122121G0 | B102101011 | 01?        |            |            |     |  |
| <i>P. evansiana</i> 2                  | 0000000000  | 0000000000 | 0000000000  | 0000000000  | 0000000000 | 0000000000 | 00000001E0 | 11122121G0 | B102101011 | 01?        |            |            |     |  |
| <i>P. boreorufescens</i> 1             | 0000000000  | 0000000000 | 0000000000  | 0000000000  | 0000000000 | 0000000000 | 0000000110 | 11A221223B | 0102100011 | 001        |            |            |     |  |
| <i>P. canina</i> 1                     | 0000000000  | 0000000000 | 0000000000  | 0000000000  | 0000000000 | 0000000000 | 0000000110 | 11A22122J0 | B1021000AW | 111        |            |            |     |  |
| <i>P. canina</i> 2                     | 0000000000  | 0000000000 | 0000000000  | 0000000000  | 0000000000 | 0000000000 | 0000000110 | 11A22122J0 | B1021000AW | 111        |            |            |     |  |
| <i>Hydrothyria venosa</i>              | 0000000000  | 0000000000 | 0000000000  | 0000000000  | 0000000000 | 0000000000 | 0000010111 | A1A222???  | 0000?00C31 | 000        |            |            |     |  |
| <i>Solorina crocea</i>                 | 0000000000  | 0000000000 | 0000000000  | 0000000000  | 0000000000 | 0000000000 | 00013001A1 | 0112212EEB | B000?003HL | 110        |            |            |     |  |
| <i>Sticta fuliginosa</i>               | 0000000000  | 0000000000 | 0000000000  | 0000000000  | 0000000000 | 0000000000 | 00000111EX | B0??00???  | 0000?01C2R | 012        |            |            |     |  |
| <i>Pseudocyphellaria crocata</i>       | ?????????00 | ??000??1?? | ?????????1? | 0?????????? | ??00011111 | B0??00???  | 0000?3012W | 012        |            |            |            |            |     |  |
| <i>Lobaria quercizans</i>              | 0000000000  | 0000000000 | 0000000000  | 0000000000  | 0000000000 | 0002311111 | 00??12EJ0  | 0000?00311 | 012        |            |            |            |     |  |
| <i>L. pulmonaria</i>                   | 0000000000  | 0000000000 | 0000000000  | 0000000000  | 0000000000 | 0002C11111 | 00??121J0  | 0000?C03EN | 012        |            |            |            |     |  |
| <i>Massalonia carnosa</i>              | 0000000000  | 0000000000 | 0000000000  | 0000000000  | 0000000000 | 00000001E1 | 00??12110  | 0000?0A02T | 013        |            |            |            |     |  |
| <i>Nephroma arcticum</i>               | ?????????00 | ??100??0?? | ?????????20 | 0?????????? | ??1AC11111 | 00??00???  | 0020?001EH | 013        |            |            |            |            |     |  |
| <i>N. resupinatum</i> 1                | 0000000000  | 0000000000 | 0000000000  | 0000000000  | 0000000000 | 000001112X | B0??00???  | B00220102H | 013        |            |            |            |     |  |
| <i>N. resupinatum</i> 2                | 0000000000  | 0000000000 | 0000000000  | 0000000000  | 0000000000 | 000001112X | B0??00???  | B00220102H | 013        |            |            |            |     |  |

Note. Taxon vouchers are listed in table 3. Characters and character states are described in the "Results" section. ? = character state unknown or not applicable. For certain characters, some taxa were assigned multiple character states because they were highly polymorphic and were analyzed as such: A = 0&1; B = 0&2; C = 0&3; D = 4&5; E = 1&2; F = 2&3; G = 1&3; H = 0&1&2; J = 1&2&3; K = 0&1&4; L = 0&1&5; M = 0&1&2&3; N = 0&1&2&4; P = 0&1&2&5; R = 0&3&4&5; T = 0&1&3&5; U = 0&1&2&3&5; W = 0&1&2&3&4&5. For a few characters, we were partially uncertain on how to score a particular taxon, but some of the potential character states could be excluded: X = 0/1; Y = 1/2; Z = 0/3. These polymorphisms and uncertainties were analyzed differently using the "variable" option in PAUP\*.

(1993), Goward et al. (1995), Kondratyuk and Galloway (1995), Huneck and Yoshimura (1996), Martínez and Burgaz (1997), Martínez et al. (1997), Miadlikowska and Holtan-Hartwig (1997), Goffinet and Miadlikowska (1999), Martínez (1999), J. Miadlikowska (unpublished data), B. Goffinet (unpublished data), and O. Vitikainen (unpublished data).

The chemical data matrix contains two groups of terpenoids: terpenoids with well-known chemical structures (table 2) and terpenoids for which the chemical structure is unknown but are recognizable using TLC (see Holtan-Hartwig 1993). For

the purpose of this study, all terpenoids are numbered in accordance with Holtan-Hartwig's (1993) system. Five terpenoids observed in chemotype I of *P. horizontalis* and *P. elisabethae* are newly added here to this list (numbered 50–54; fig. 4). They consistently occur in detectable concentrations in terpenoid-rich races of chemotype I for these two taxa. Because the variation in concentration of these newly and previously reported terpenoids can form a continuum within populations of chemotype I for these two taxa, including extreme patterns with only six out of potentially 15 terpenoids present, it was

Table 5

Shared Derived Chemical and Morphological Character States for the Eight Infrageneric Sections and Four Subgroups of *Peltigera* Revealed by the Combined Phylogenetic Analysis of the Reduced (54 OTUs) Data Set (See Figs. 8 and 9)

| Characters | A.1           | A.2 | A             | B             | C  | D.1           | D.2 | D  | E                | F             | G             | H             |
|------------|---------------|-----|---------------|---------------|----|---------------|-----|----|------------------|---------------|---------------|---------------|
| 1          | 0             | 0   | 0             | 0             | 0  | $\frac{1}{1}$ | 0   | 01 | 0                | 0             | 0             | 0             |
| 2          | 0             | 0   | 0             | 0             | 0  | $\frac{1}{1}$ | 0   | 01 | 0                | 0             | 0             | 0             |
| 3          | 0             | 0   | 0             | 0             | 0  | $\frac{1}{1}$ | 0   | 01 | 0                | 0             | 0             | 0             |
| 4          | 0             | 0   | 0             | 0             | 0  | $\frac{1}{1}$ | 0   | 01 | 0                | 0             | 0             | 0             |
| 5          | 0             | 0   | 0             | 0             | 0  | $\frac{1}{1}$ | 0   | 01 | 0                | 0             | 0             | 0             |
| 6          | 0             | 01  | 01            | 0             | 0  | 0             | 0   | 0  | 0                | 0             | 0             | 0             |
| 7          | 0             | 0   | 0             | $\frac{1}{1}$ | 01 | 0             | 0   | 0  | 0                | 0             | 0             | 0             |
| 8          | 0             | 0   | 0             | $\frac{1}{1}$ | 01 | 0             | 0   | 0  | 0                | 0             | 0             | 0             |
| 9          | 1             | 01  | 01            | 0             | 01 | 0             | 01  | 01 | 0                | $\frac{1}{1}$ | 1             | 0             |
| 10         | 1             | 1   | $\frac{1}{1}$ | 0             | 01 | 0             | 01  | 01 | 0                | $\frac{1}{1}$ | 0             | 0             |
| 11         | 0             | 0   | 0             | 1             | 01 | 0             | 0   | 0  | 0                | 0             | 0             | 0             |
| 12         | 0             | 0   | 0             | 1             | 1  | 0             | 0   | 0  | 0                | 0             | 1             | 0             |
| 13         | 1             | 1   | 1             | 01            | 1  | 1             | 1   | 1  | $\frac{0[1]}{0}$ | 1             | 1             | 0             |
| 14         | 0             | 0   | 0             | 01            | 01 | 0             | 0   | 0  | 0                | 0             | $\frac{1}{1}$ | 0             |
| 15         | 0             | 0   | 0             | $\frac{1}{1}$ | 01 | 0             | 0   | 0  | 0                | 0             | $\frac{1}{1}$ | 0             |
| 16         | 0             | 01  | 01            | 0             | 0  | 0             | 0   | 0  | 0                | 0             | 0             | 0             |
| 17         | 0             | 0   | 0             | $\frac{1}{1}$ | 01 | 0             | 0   | 0  | 0                | 0             | 0             | 0             |
| 18         | 1             | 01  | 01            | 0             | 0  | 0             | 01  | 01 | 0                | 0             | 1             | 0             |
| 19         | 0             | 0   | 0             | 0             | 0  | 0             | 01  | 01 | 0                | 0             | 0             | 0             |
| 20         | 01            | 0   | 01            | 0             | 0  | 0             | 0   | 0  | 0                | 0             | 0             | 0             |
| 21         | 0             | 0   | 0             | 0             | 0  | 0             | 01  | 01 | 0                | 0             | 0             | 0             |
| 22         | 0             | 0   | 0             | 0             | 01 | 0             | 01  | 01 | 0                | $\frac{1}{1}$ | 0             | 0             |
| 23         | 0             | 0   | 0             | $\frac{1}{1}$ | 0  | 0             | 0   | 0  | 0                | 0             | 0             | 0             |
| 24         | 0             | 0   | 0             | 0             | 0  | 0             | 01  | 01 | 0                | 0             | 0             | 0             |
| 25         | 0             | 0   | 0             | 0             | 01 | 0             | 01  | 01 | 0                | 0             | 0             | 0             |
| 26         | 0             | 01  | 01            | 0             | 0  | $\frac{1}{1}$ | 0   | 01 | 0                | 0             | 0             | 0             |
| 27         | 0             | 0   | 0             | 0             | 0  | $\frac{1}{1}$ | 0   | 01 | 0                | 0             | 0             | 0             |
| 28         | 0             | 0   | 0             | 0             | 0  | 0             | 0   | 0  | 0                | 0             | $\frac{1}{1}$ | 0             |
| 29         | 0             | 0   | 0             | 0             | 01 | 0             | 0   | 0  | 0                | 0             | 0             | 0             |
| 30         | 0             | 01  | 01            | 0             | 0  | $\frac{1}{1}$ | 01  | 01 | 0                | 0             | 0             | 0             |
| 31         | 0             | 01  | 01            | 0             | 01 | 0             | 0   | 0  | 0                | 0             | 0             | 0             |
| 32         | 0             | 0   | 0             | 0             | 0  | $\frac{1}{1}$ | 0   | 01 | 0                | 0             | 0             | 0             |
| 33         | $\frac{1}{1}$ | 0   | 01            | 0             | 0  | 0             | 0   | 0  | 0                | 0             | $\frac{1}{1}$ | 0             |
| 34         | 0             | 0   | 0             | 0             | 0  | 0             | 0   | 0  | 0                | 0             | $\frac{1}{1}$ | 0             |
| 35         | 01            | 01  | 01            | 0             | 01 | 0             | 01  | 01 | 0                | 0             | 0             | 0             |
| 36         | 0             | 0   | 0             | 0             | 0  | $\frac{1}{1}$ | 0   | 01 | 0                | 0             | 0             | 0             |
| 37         | 1             | 01  | 01            | 0             | 0  | 0             | 01  | 01 | 0                | 0             | 1             | 0             |
| 38         | 0             | 0   | 0             | 0             | 0  | $\frac{1}{1}$ | 0   | 01 | 0                | 0             | 0             | 0             |
| 39         | 0             | 0   | 0             | 0             | 0  | $\frac{1}{1}$ | 0   | 01 | 0                | 0             | 0             | 0             |
| 40         | 0             | 0   | 0             | 0             | 0  | $\frac{1}{1}$ | 0   | 01 | 0                | 0             | 0             | 0             |
| 41         | 0             | 0   | 0             | 0             | 0  | $\frac{1}{1}$ | 0   | 01 | 0                | 0             | 0             | 0             |
| 42         | 0             | 0   | 0             | 0             | 0  | $\frac{1}{1}$ | 0   | 01 | 0                | 0             | 0             | 0             |
| 43         | 0             | 0   | 0             | 0             | 01 | 0             | 0   | 0  | 0                | 0             | $\frac{1}{1}$ | 0             |
| 44         | 0             | 0   | 0             | $\frac{1}{1}$ | A0 | 0             | 0   | 0  | 0                | 0             | A             | 0             |
| 45         | 0             | 0   | 0             | $\frac{1}{1}$ | A0 | 0             | 0   | 0  | 0                | 0             | B             | 0             |
| 46         | 0             | 0   | 0             | 0             | 0  | 0             | 0   | 0  | 0                | 0             | A             | $\frac{1}{1}$ |
| 47         | 0             | 0   | 0             | $\frac{2}{1}$ | 01 | 0             | 0   | 0  | 0                | 0             | 0             | 0             |
| 48         | 1             | 01  | 01            | 1             | 01 | 1             | 1   | 1  | 1                | 1             | 1             | 1             |

**Table 5**  
(Continued)

| Characters | A.1      | A.2    | A        | B  | C   | D.1       | D.2      | D           | E            | F        | G | H        |
|------------|----------|--------|----------|----|-----|-----------|----------|-------------|--------------|----------|---|----------|
| 49         | E1       | E1     | E1       | A  | 1   | A         | 0        | A0          | E12          | A        | 1 | 1        |
| 50         | 0        | 0      | 0        | 01 | 0   | <u>1</u>  | 0        | 01          | 01           | 0        | 1 | 1        |
| 51         | 1        | A1     | A1       | 01 | A1  | <u>0</u>  | 1        | 01          | ABH012       | 1        | B | A        |
| 52         | 1        | 1      | 1        | E1 | E1  | <u>E1</u> | E1       | E1          | 1            | 1        | 1 | 1        |
| 53         | A        | 01     | A01      | A  | A1  | 0         | 01       | <u>0[1]</u> | A01          | 1        | A | A        |
| 54         | 12       | 012    | 012      | 1  | 01  | Y1        | Y        | <u>Y1</u>   | <u>2</u>     | 1        | 2 | 2        |
| 55         | 2        | 2      | 2        | 2  | 2   | 2         | 2        | 2           | E12          | <u>1</u> | 2 | 2        |
| 56         | 1        | 1      | 1        | 1  | 1   | 1         | 1        | 1           | 1            | 1        | 2 | 2        |
| 57         | 2        | 12     | 12       | 2  | 2   | 2         | 2        | 2           | EY12         | <u>1</u> | ? | ?        |
| 58         | 1        | E1     | E1       | 1  | E   | 1         | E1       | E1          | E12          | 1        | ? | ?        |
| 59         | EJ       | E123   | EJ123    | E  | E2  | 2         | EGJ3     | EGJ23       | 3GJ12        | E        | ? | ?        |
| 60         | 0        | 0      | 0        | 0  | 023 | <u>1</u>  | 0        | 01          | B0           | 0        | ? | ?        |
| 61         | B1       | 01     | B01      | 0  | B0  | <u>0</u>  | B0       | B0          | B02          | 0        | 0 | 0        |
| 62         | 01       | 01     | 01       | 1  | 01  | 01        | 1        | 01          | 01           | 0        | 0 | 0        |
| 63         | 01       | 01     | 01       | 0  | 02  | 0         | <u>1</u> | 01          | 0            | 0        | 0 | 0        |
| 64         | 0        | 0      | 0        | A1 | A01 | 0         | <u>0</u> | <u>0</u>    | ACEHM0123    | E        | 0 | 0        |
| 65         | ?        | ?      | ?        | 2  | 2   | ?         | ?        | ?           | 12           | 1        | ? | ?        |
| 66         | 0        | 0      | 0        | 0  | 0   | 0         | G0       | G0          | B02          | 0        | 0 | 0        |
| 67         | 0        | A0     | A0       | 0  | 0   | A2        | A01      | A012        | A01          | 0        | 0 | 0        |
| 68         | 1        | 1      | 1        | 1  | 1   | 1         | 1        | 1           | <u>0[23]</u> | 1        | A | C        |
| 69         | E1       | E12    | E12      | H  | AH2 | E1        | E1       | E1          | AEXY012      | 1        | E | 3        |
| 70         | HM       | AKUO13 | AHKMU013 | H2 | AHP | H         | M14      | HM14        | ADFHNU0123   | H        | H | 1        |
| 71         | <u>1</u> | 01     | 01       | 0  | 01  | 0         | 1        | 01          | 01           | 0        | 0 | 0        |
| 72         | <u>1</u> | 01     | 01       | 1  | 01  | 1         | 1        | 1           | 01           | 0        | 1 | 0        |
| 73         | 0        | 01     | 01       | 0  | 0   | 0         | 01       | 01          | <u>1[0]</u>  | 0        | 0 | <u>0</u> |

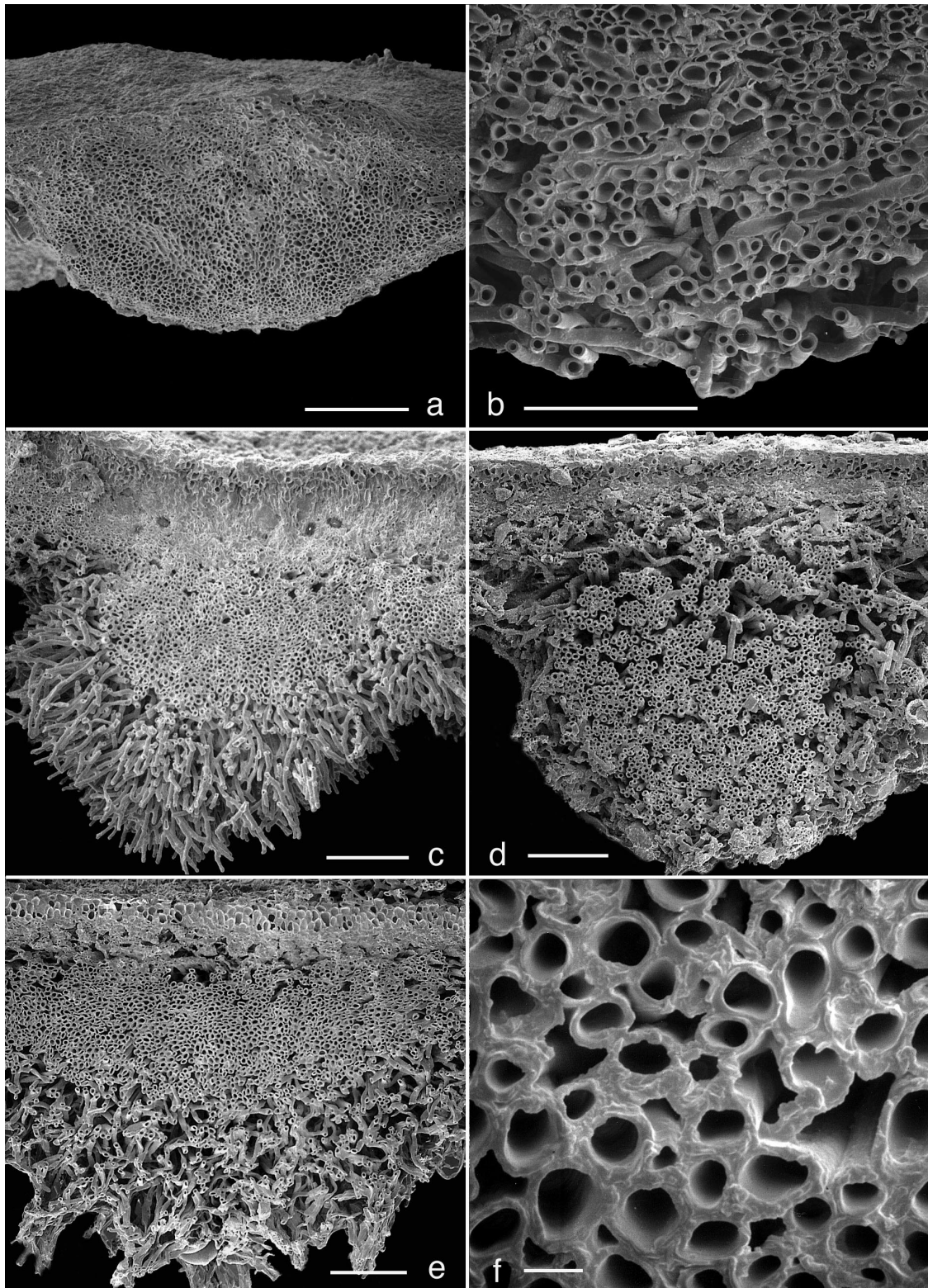
Note. The first 42 characters are presence/absence of 42 different terpenoids. Characters 43–73 form the morphological data set. Underlining indicates unequivocal synapomorphies for each group. Reversals for each group are shown in brackets. Question marks indicate that the character did not apply in this group. For a description of the character coding, see the “Results” subsections entitled “Morphological Characters” and “Chemical Characters (Terpenoids).”

not possible to split this chemotype into two or more well-delimited new chemotypes.

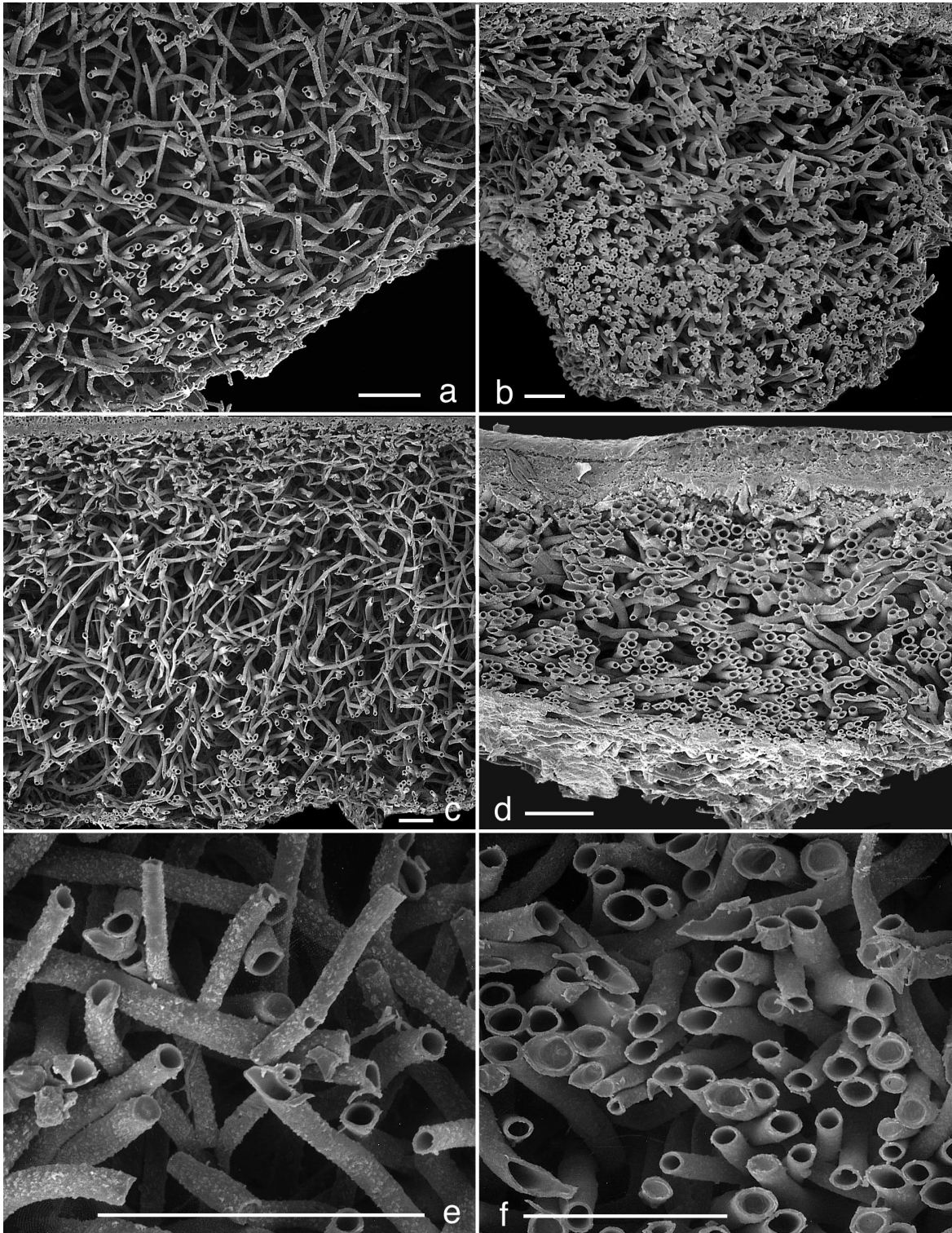
New chemotypes also are reported here for the first time from specimens of *P. leucophlebia* (II), *P. malacea* (V), *P. collina* (IV), and *P. hymenina* (II and III), collected mainly in Poland (fig. 5). Chemotype II of *P. leucophlebia* is characterized by the following set of compounds: unidentified terpenoids 7, 14 (trace), 25, and 13. Chemotype V of *P. malacea* is characterized by the following set of terpenoids: peltidactylin (terp. 10), zeorin (terp. 15), and unidentified terpenoid 14. Two new chemotypes were recognized from *P. collina* thalli. Chemotype III corresponds to specimens with zeorin (terp. 15) reported by Holtan-Hartwig (1993), from Norway. The same specimens were later shown also to contain peltidactylin (terp. 10, trace) and the unidentified terpenoid 39 (fig. 5). This chemotype with these three terpenoids has been also found in Poland and Scotland. In the new chemotype IV of *P. collina*, unidentified terpenoid 39 is absent and two additional terpenoids (terps. 20 and 41) are present (fig. 5). Chemotype IV was reported only from Norway. The new chemotypes II and III of *P. hymenina* differ from the known chemotype I by the lack of dolichorhizin (terp. 12) and peltidactylin (terp. 10), respectively (fig. 5). In contrast to Martínez's (1999) report from the Iberian Peninsula, *P. hymenina* thalli collected in Poland and Norway do not produce hopane-7 $\beta$ ,22-diol (terp. 34). Instead, unidentified terpenoid 39 is consistently reported for specimens collected outside the Iberian Peninsula. Martínez also reported

variation in chemotype I of *P. hymenina* from the Iberian Peninsula. This variation partly reflects chemotypic patterns found in the Norwegian and Polish populations of this species. Chemotypes I of *P. elisabethae* and *P. horizontalis* reported here correspond to specimens from Norway examined by Holtan-Hartwig (1993), except that the newly identified terpenoids (50–54) are added to the list of terpenoids detected so far. A similar composition of terpenoids in chemotype I for both species was found in other European and American material.

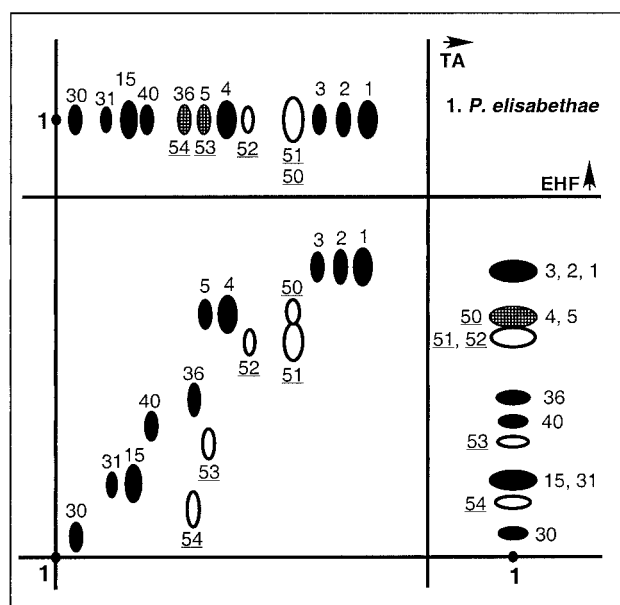
The following 42 terpenoid characters were scored as present (1) or absent (0) in the chemical data matrix (table 4). In parentheses are abbreviations that have been used by Vitikainen (1994b) and Martínez (1999), as well as common names or chemical structures, if available: 1, terp. 1; 2, terp. 2; 3, terp. 3; 4, terp. 4; 5, terp. 5; 6, terp. 6; 7, terp. 7 (Plc-3); 8, terp. 9 (Plc-1); 9, terp. 10 (peltidactylin; T1); 10, terp. 12 (dolichorhizin; T2); 11, terp. 13; 12, terp. 14 (Pbr-1); 13, terp. 15 (zeorin; T3); 14, terp. 16 (phlebic acid A; phlA); 15, terp. 17 (phlebic acid B; phlB); 16, terp. 18; 17, terp. 19 (Plc-2); 18, terp. 20 (hopane-6 $\alpha$ ,7 $\beta$ ,22-triol; T6); 19, terp. 21; 20, terp. 22; 21, terp. 23; 22, terp. 24; 23, terp. 25; 24, terp. 27; 25, terp. 28; 26, terp. 30 (Phr-1); 27, terp. 31; 28, terp. 32; 29, terp. 33; 30, terp. 34 (hopane-7 $\beta$ ,22-diol; T4); 31, terp. 35 (hopane-15 $\alpha$ ,22-diol; T5); 32, terp. 36; 33, terp. 37; 34, terp. 38; 35, terp. 39 (Pnp-1); 36, terp. 40; 37, terp. 41; 38, terp. 50; 39, terp. 51; 40, terp. 52; 41, terp. 53; 42, terp. 54.



**Fig. 2** Scanning electron micrographs documenting vein structure and depicting cross sections of veins corresponding to character state 2 (i.e., hyphae mostly to completely ordered in a parallel and conglutinated fashion) of morphological character 12 (54 in tables 4 and 5). Scale bar = 100  $\mu\text{m}$ . *a*, *Hydrothyria venosa*, USA, Bratt 4757 (UGDA-L ex OSU). *b*, *Peltigera scabrosella*, Sweden, Vitikainen 8580 (H). *c*, *P. kristinssonii*, Austria, Vitikainen 8085a (UGDA-L ex H). *d*, *P. venosa*, Canada, Goward 97-294 (UBC). *e*, *P. didactyla* var. *didactyla*, USA, Schuster 854 (CANL). *f*, Central part of a vein in *P. membranacea*, Canada, Schueler 18243 (CANL).



**Fig. 3** Scanning electron micrographs documenting vein structure and depicting cross sections of veins. *a, c, e* correspond to character state 0 (hyphae completely unordered) of morphological character 12 (54 in tables 4 and 5). *b, d, f* correspond to character state 1 (hyphae mostly unordered but with small groups of parallel and conglutinated hyphae) of morphological character 12 (54 in tables 4 and 5). Scale bar = 100  $\mu\text{m}$ . *a*, *Peltigera aphthosa*, Canada, Lutzoni 97.06.29 (F). *b*, *P. retifoveata*, Canada, Scotter 19147 (CANL). *c*, *P. pulverulenta*, Venezuela, Abti 37205 (H). *d*, *P. elisabethae*, Austria, Vitikainen 8915 (H). *e*, Close-up of central part of a vein in *P. aphthosa* shown in *a*. *f*, Close-up of central part of a vein in *P. elisabethae* shown in *d*.



**Fig. 4** Schematic two-dimensional thin-layer chromatography plate for *P. elisabethae* chemotype I. Terpenoids previously recognized are represented by black ovals. Terpenoids newly reported are represented by white ovals (numbers 50–54 underlined). Previously and newly recognized terpenoids sharing the same position in unidirectional displays are represented by checked ovals.

#### Phylogenetic Analyses of the Genus *Peltigera*

**Morphological data set alone.** All 31 characters of the morphological data set were parsimony informative. The heuristic searches on this data set resulted in so many equally most parsimonious trees that the analyses were stopped during the first TBR swapping when the memory was filled with trees. Consistency Index (CI) and Retention Index (RI) values were very high (fig. 6A). Because of the low resolving power of morphology for this selection of 96 OTUs, bootstrap values above 50% were rare. The level of phylogenetic information was not sufficient to provide strong broad delimitations within the ingroup. Bootstrap support  $\geq 95\%$  was restricted to bipartitions at the base of conspecific samples. Based on morphology alone, the genus *Peltigera* is not monophyletic. However, this is only because of the placement of *P. venosa* (G) within the outgroup, sister to *H. venosa* (H) (fig. 6A).

**Chemical data set alone.** A total of 35 out of 42 terpenoids were parsimony informative. The large number of trees resulting from this heuristic search also did not allow completion of the first TBR swapping. The CI value and the level of resolution achieved were distinctly lower compared to the phylogenetic analysis restricted to morphology (fig. 6B). However, the RI value was slightly higher. Bootstrap values above 50% were associated with only a few internodes supporting relationships between specimens from the same taxon or species characterized by a unique or almost identical terpenoid composition. All taxa without detectable terpenoids were placed as a large polytomy outside of a clade that includes taxa with terpenoids. Based on terpenoids alone, the genus *Peltigera* is not phylogenetically delimited.

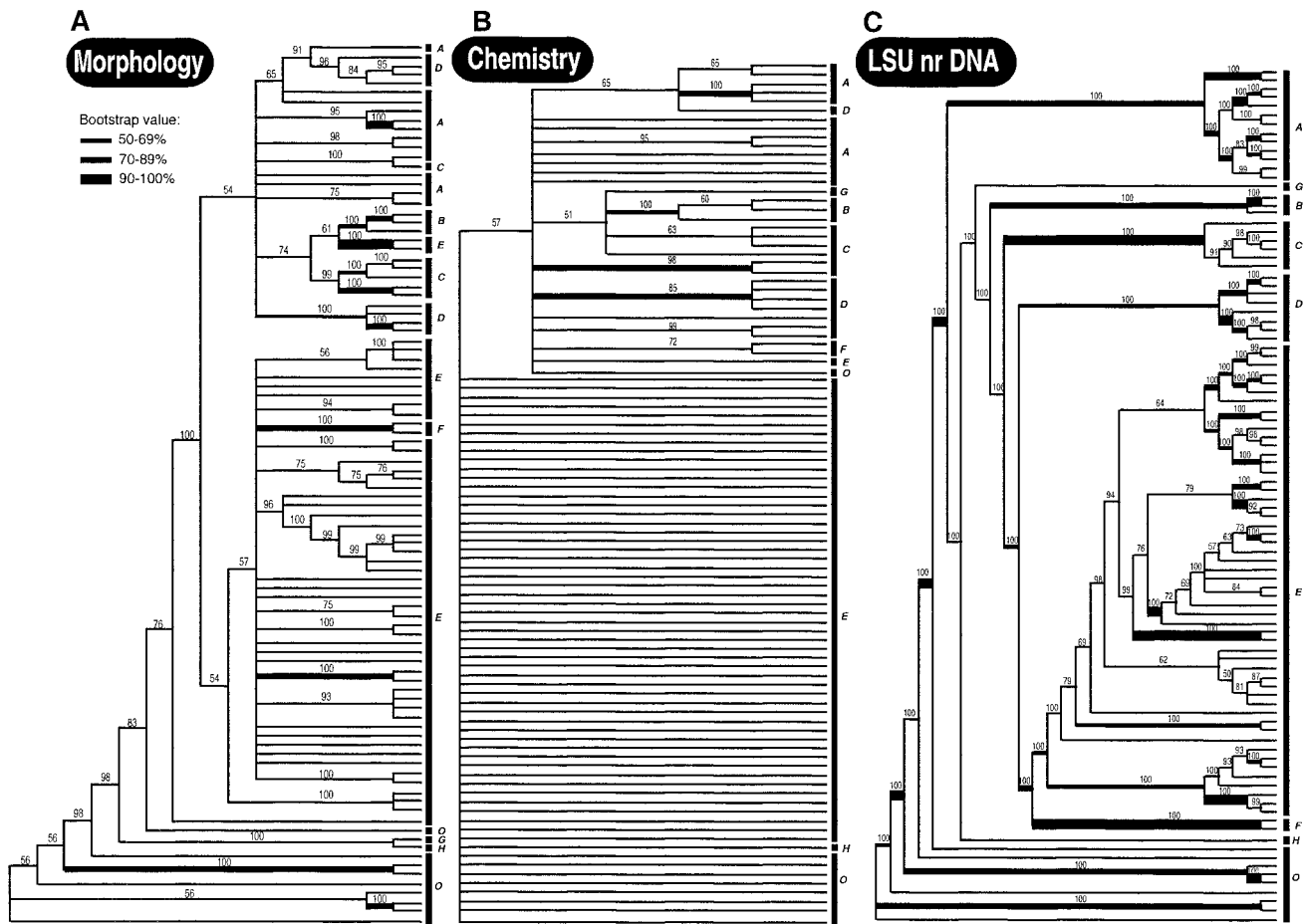
**LSU nrDNA data set alone.** The final alignment for the 96 LSU nrDNA sequences consisted of 1643 sites. A total of 20 ambiguously aligned regions were delimited, resulting in the exclusion of 232 nucleotide sites. The presence of one spliceosomal intron site in sequences of *Lobaria pulmonaria* (Zoller et al. 1999) and *L. quercizans*, as well as a group I intron site in *Nephroma arcticum* and *Massalongia carnososa*, resulted in the exclusion of 296 additional sites, for a total of 1113 nucleotide sites included in the phylogenetic analysis. The 20 ambiguously aligned regions were recoded using INAASE (Lutzoni et al. 2000), and the two intron sites were recoded as present/absent, providing 22 additional characters, for a grand total of 1135 characters included in the phylogenetic analysis of the LSU nrDNA data set. Of these, 905 characters were constant and 147 were parsimony informative.

As with the two other data sets when analyzed separately, this analysis could not be completed beyond the first TBR swapping because of the large number of equally most parsimonious trees recovered. Contrary to the morphological and chemical data sets, however, the molecular data, when analyzed separately, provided a very high level of resolution with many internodes having bootstrap values above 50% (fig. 6C). The CI value was intermediate between values from the morphological and chemical studies, but the RI value was the highest of the three separate analyses. Together, *Peltigera* and *Hydrothyria* (H) form a monophyletic group with bootstrap support of 100%. Relationships among taxa in the outgroup are partly well supported. Within *Peltigera*, seven monophyletic groups were revealed (A–G). For the five groups with more than one representative, bootstrap values were  $>50\%$ . For most groups, the relationships among species were fairly well resolved but bootstrap support was low, especially in group E. Although topologies obtained from the three separate analyses were very different, no conflict was detected using our reciprocal 70% bootstrap support criterion (see “Material and Methods”), and therefore, the three data sets were combined.

**Combined data set for 96 specimens.** The final combined data matrix for the 96 samples consisted of 1208 characters, 213 of which were parsimony informative. When analyzing the combined data set it was possible to complete TBR swapping from seven different starting trees. However, the number of equally most parsimonious trees distributed on multiple islands was too high to include them all in one file and to summarize them with a consensus tree. We took the largest pool of equally most parsimonious trees generated from one of the TBR searches and generated a strict consensus tree (fig. 7). The CI value for the combined data is significantly higher than for the chemistry and LSU nrDNA data sets alone but lower compared to morphology. The RI value remains almost the same as for the chemical data but greater than for morphology alone and lower than for LSU nrDNA alone. The topology of the strict consensus tree based on combined data sets, except for minor changes, reflects the phylogenetic relationships revealed by the molecular data when analyzed separately. Contrary to the three separate analyses, *Peltigera* forms a monophyletic entity. The LSU nrDNA, when analyzed separately, supported *H. venosa* (H) as being nested within *Peltigera* (fig. 6C). However, there is no strong support for the precise relationship (i.e., sister to or nested within *Peltigera*)





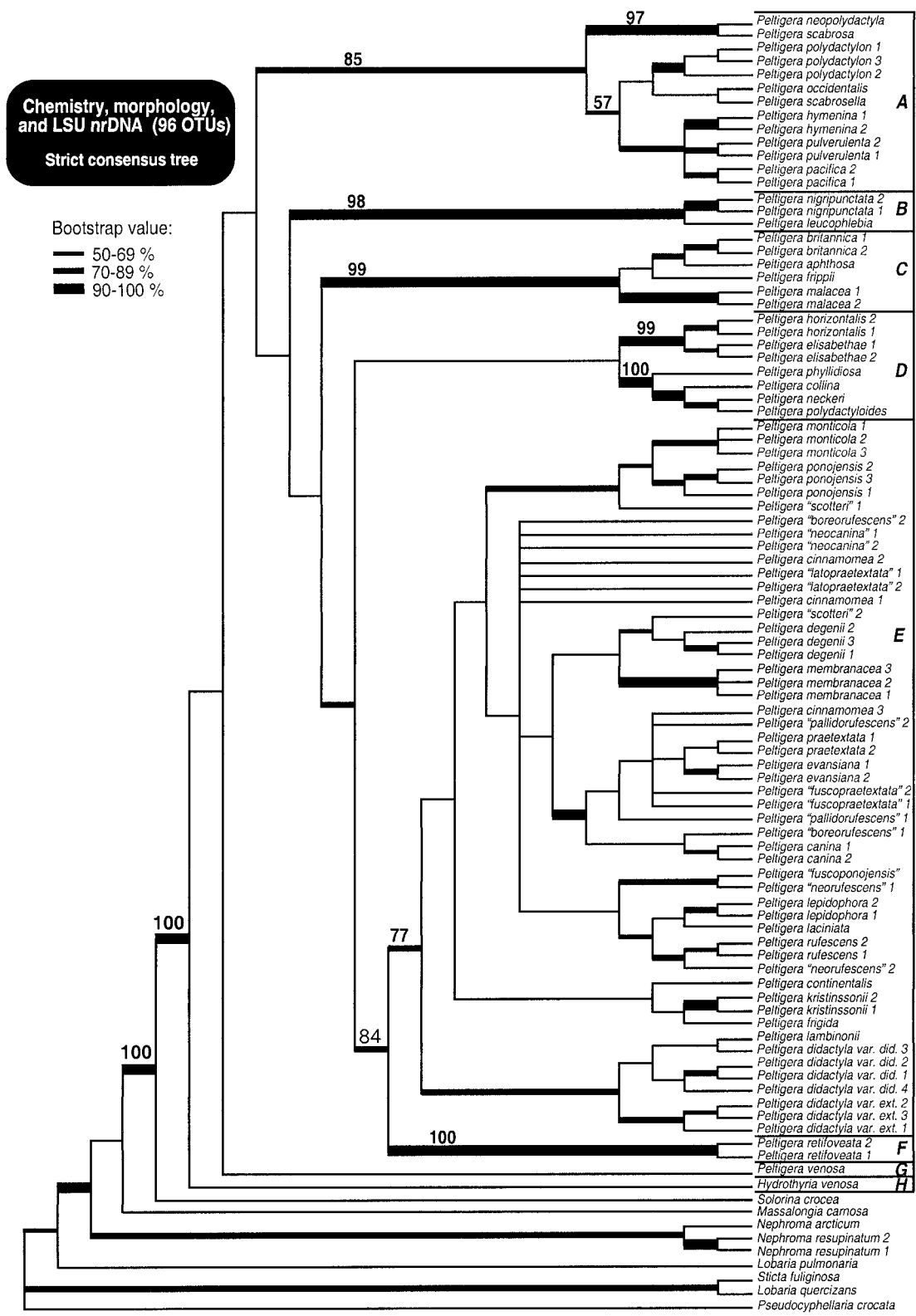


**Fig. 6** Phylogenetic relationships among 96 individuals representing 46 putative *Peltigera* species and nine outgroup species using maximum parsimony as the optimization criterion and summarized by majority rule consensus trees. Values above branches correspond to percentages of equally most parsimonious trees with that specific bipartition. Bootstrap values greater than 50% are represented by increasingly thicker branches as indicated in A. The names of taxa have been replaced by sections as delimited in figs. 7 and 8. Letter O indicates outgroup taxa. Letters A–H represent infrageneric sections proposed here (see fig. 8). A, Majority rule consensus of 139,100 trees based on morphological data alone. Tree length = 619 steps, CI (excluding uninformative characters) = 0.903, RI = 0.802. B, Majority rule consensus of 87,304 trees based on chemical data alone. Tree length = 68 steps, CI (excluding uninformative characters) = 0.574, RI = 0.869. C, Majority rule consensus for 137,700 trees based on LSU nrDNA alone. Tree length = 1055.2 steps, CI (excluding uninformative characters) = 0.644, RI = 0.890.

E). The data matrix for this reduced combined analysis provided 193 parsimony informative characters. By simply eliminating redundancy, this analysis revealed a single most parsimonious tree (fig. 8). The topology of this tree is virtually identical to the strict consensus tree derived from the 96 OTU combined data matrix. The bootstrap support was generally higher, but the CI and RI values were slightly lower than the latter. Phylogenetic relationships between *Peltigera* and the outgroup as well as within the outgroup are highly supported. The closest relative to the genus *Peltigera* remains *H. venosa* with 100% bootstrap support. The most parsimonious constrained tree to not have *Peltigera* and *Hydrothyria* as monophyletic (constrained tree length = 1463.9 steps) was much longer than the unconstrained most parsimonious tree (unconstrained tree length = 1429.0 steps). The result of the Tree Length Distribution nonparametric Bootstrap (TLDB; O'Donnell et al. 2000; Wu et al. 2000) test ( $P = 0.14$ ) indicates

that the null hypothesis cannot be rejected at the  $P = 0.05$  level. According to Templeton's test, the nonmonophyly of *Peltigera-Hydrothyria* clade can be rejected ( $P = 0.0002$ ). Therefore, it is very likely that this monophyletic relationship revealed by the combined data set is not a result of sampling error. Based on the combined analyses and these two tests we concluded that *Hydrothyria* and *Peltigera* form a monophyletic group and should be recognized as one genus. A constrained tree forcing *H. venosa* to be nested within *Peltigera* (tree length = 1431.5) versus an unconstrained tree (tree length = 1429.0 steps) could not be rejected (Templeton test,  $P = 0.46$ ; TLDB test,  $P = 0.99$ ). The exact relationship of *Hydrothyria* to members of *Peltigera* is still uncertain.

The sister group to the *Hydrothyria-Peltigera* clade is *Solorina*, with 100% bootstrap support. Compared to the combined analysis of the 96 specimen data matrix, the bootstrap value for the branch supporting the *Massalongia-Solorina-*



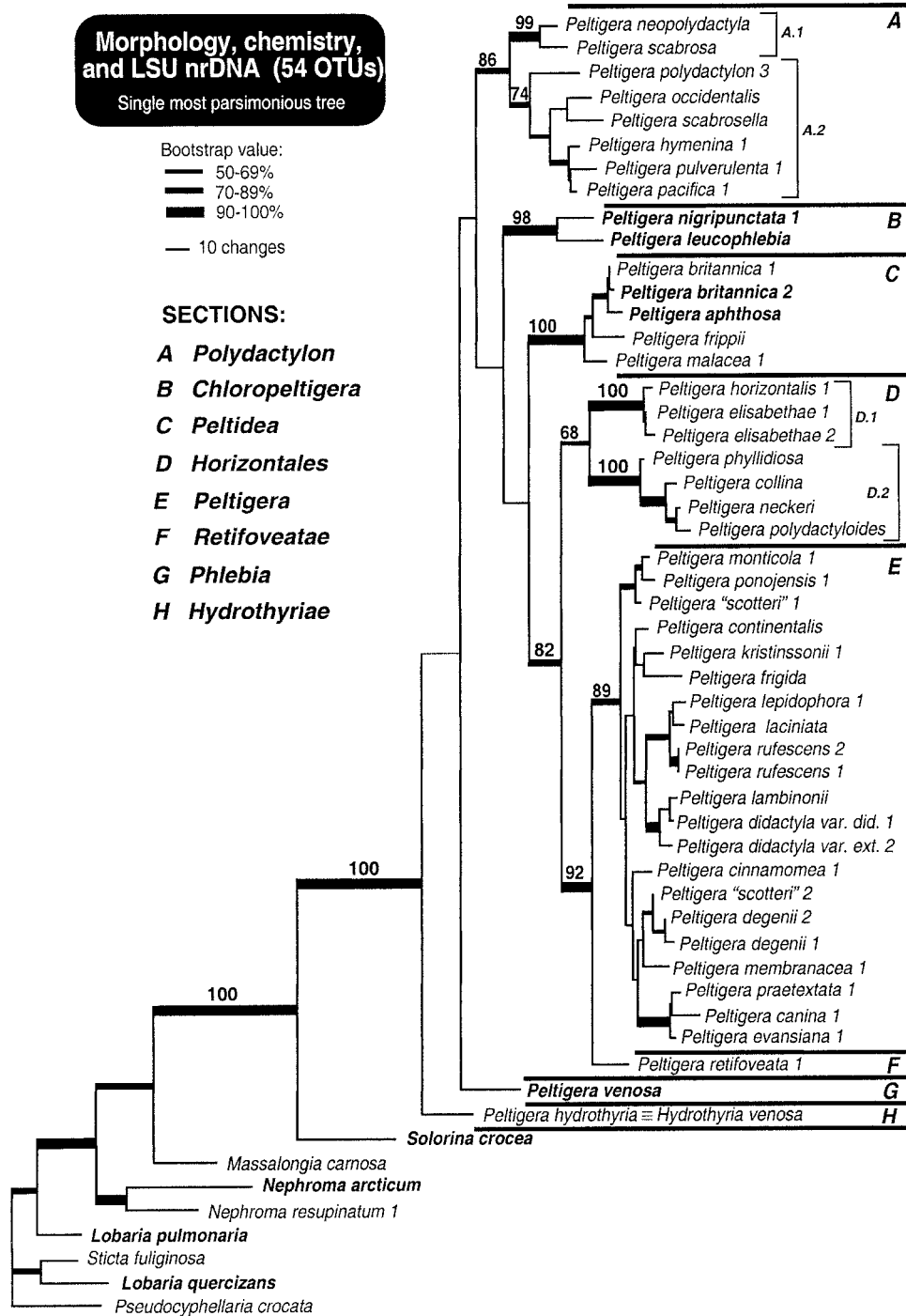
**Fig. 7** Strict consensus of 9400 trees resulting from a combined analysis of chemistry, morphology, and LSU nrDNA data for 96 OTUs. Tree length = 1815.5 steps, CI (excluding uninformative characters) = 0.691, RI = 0.854. Bootstrap values greater than 50% are shown with increasingly thicker branches. Bootstrap values supporting the main monophyletic entities revealed by this study are given above these critical internodes. Groups A-H represent infrageneric sections proposed here (see fig. 8). Names in quotation marks indicate putative *Peltigera* species proposed by T. Goward (see table 3).

**Morphology, chemistry,  
and LSU nrDNA (54 OTUs)**  
Single most parsimonious tree

Bootstrap value:  
 — 50-69%  
 — 70-89%  
 — 90-100%  
 — 10 changes

**SECTIONS:**

- A Polydactylon**
- B Chloropeltigera**
- C Peltidea**
- D Horizontales**
- E Peltigera**
- F Retifoveatae**
- G Phlebia**
- H Hydrothyriae**



**Fig. 8** Single most parsimonious tree resulting from a combined analysis of chemical, morphological, and LSU nrDNA data for a subsample of 54 OTUs. Tree length = 1429.0 steps, CI (excluding uninformative characters) = 0.682, RI = 0.819. Bootstrap values greater than 50% are shown with increasingly thicker branches. Bootstrap values supporting the main monophyletic entities revealed by this study are given above these critical internodes. Groups A–H correspond to the same infrageneric sections as shown in figs. 6 and 7. A.1, A.2 and D.1, D.2 represent main clades within sections A and D, respectively. Names in quotation marks indicate putative *Peltigera* species proposed by T. Goward (see table 3). Names in bold indicate trimembered symbiotic entities.

*Hydrothyria-Peltigera* clade increased drastically (from <50% to almost 80%). The two *Lobaria* species did not form a monophyletic group. This could be because of the low number of taxa within the outgroup in all analyses of this study.

This analysis revealed the same seven groups within *Peltigera* as the combined analysis for 96 OTUs. Bootstrap support is distinctly higher for these major clades, ranging from 68% for group *D* to 100% for group *C* (fig. 8). The most distinct increase in bootstrap values was noticed for group *D* (from <50% to 68%) and for group *E* (from 77% to 89%). Two distinct clades are present in group *A*: the *P. neopolydactyla*–*P. scabrosa* clade (group *A.1*), with a bootstrap value of 99%, and the *P. polydactylon*–*P. pacifica* clade (group *A.2*), with a bootstrap value much higher than for the combined analysis of the 96 OTUs data matrix (from 57% to 74%). Group *D* is also represented by two very well defined clades. Both of them, the *P. horizontalis*–*P. elisabethae* clade (group *D.1*) and the *P. phyllidiosa*–*P. polydactyloides* clade (group *D.2*), are supported by bootstrap values of 100%. Within the *P. canina* complex (group *E*), the bootstrap values are still relatively low, as well as the interspecies level of divergence. “*Peltigera scotteri*” is a nonmonophyletic putative species. One of the specimens of this species is closely related to *P. degenii* and the other is sister to the *P. monticola*–*P. ponojensis* clade. However, this study does not allow us to reject the null hypothesis of “*P. scotteri*” being monophyletic (constrained tree length = 1439.6 vs. unconstrained tree length = 1429.0 steps; Templeton test,  $P = 0.422$ ; TLDB test,  $P = 0.91$ ).

#### Infrageneric Classification (Sections) of *Peltigera*

The highly resolved topologies (figs. 7, 8) derived from these multiple data sets and the high bootstrap values for the main clades within *Peltigera* allow us to propose a new infrageneric classification using monophyly as the grouping criterion. We propose eight monophyletic sections corresponding to the eight main groups (*A*–*H*) within *Peltigera*: section *Polydactylon* Miadlikowska & Lutzoni (group *A*), section *Chloropeltigera* Gyeln. (group *B*), section *Peltidea* (Ach.) Vain. (group *C*), section *Horizontales* Miadlikowska & Lutzoni (group *D*), section *Peltigera* (group *E*), section *Retifoveatae* Miadlikowska & Lutzoni (group *F*), section *Phlebia* Wallr. (group *G*), and section *Hydrothyriae* Miadlikowska & Lutzoni (group *H*). The last section includes the newly transferred monospecific genus *Hydrothyria* to the genus *Peltigera*. The infrageneric groupings of *Peltigera* species are characterized by distinct sets of unequivocal synapomorphies derived from the morphological and chemical data sets (table 5; fig. 9). This result was unexpected given that chemical and, especially, morphological characters are highly variable within *Peltigera*. Only *C*, section *Peltidea*, and subgroup *A.2* did not have a unique combination of unequivocal synapomorphies (table 5). The chemical data set provided more synapomorphies for the delimitation of *Peltigera* sections than did the morphological data set (table 5).

Some of the changes among character states (characters 44 and 45; fig. 9) describe aspects of the symbiotic nature of these taxa. Trimembered taxa do not form a monophyletic group within the genus *Peltigera*. *Peltigera venosa* (section *G*) occupies a basal position within *Peltigera* (fig. 8), whereas the other tripartite members belong to two distinct paraphyletic

groups (sections *B* and *C*) that are clearly nested within bi-membered *Peltigera* species. The monophyly of cephalodiate taxa (tripartite *Peltigera* species) could not be rejected (constrained tree length = 1446.6 vs. unconstrained tree length = 1429.0 steps; Templeton test,  $P = 0.3574$ ; TLDB test,  $P = 0.78$ ). The phylogenetic position of *P. venosa* is not stable, as indicated by very low bootstrap support (less than 50%) in the combined analyses and a paraphyletic relationship with other trimembered *Peltigera* species (groups *B* and *C*) is revealed by LSU nrDNA when analyzed separately. Both tests confirmed that the hypothesis of *P. venosa* being nested within *Peltigera* cannot be rejected (constrained tree length = 1430.6 vs. unconstrained tree length = 1429.0 steps; Templeton and TLDB tests,  $P = 1$ ). Groups *A* and *D*–*F* comprise only bimembered taxa with cyanobacteria (cf. *Nostoc*) as the photobiont. Two bimembered species, *P. malacea* and *P. frippii*, are placed together with trimembered taxa in section *Peltidea* (group *C*). Groups *G* and *B* include exclusively trimembered *Peltigera* species.

*Peltigera* Willd. *Fl. Berol. Prodr.*: 347. 1787—Type species. *P. canina* (L.) Willd.—Lichen caninus L.  
*Sp. Pl.*: 1149. 1753.

*Peltigera* sect. *Peltigera*. Type section. *Peltigera canina* (L.) Willd.—*Peltidea* [sect.] \**Emprostea*  
*Ach. Meth. Lich.*: 282. 1803.

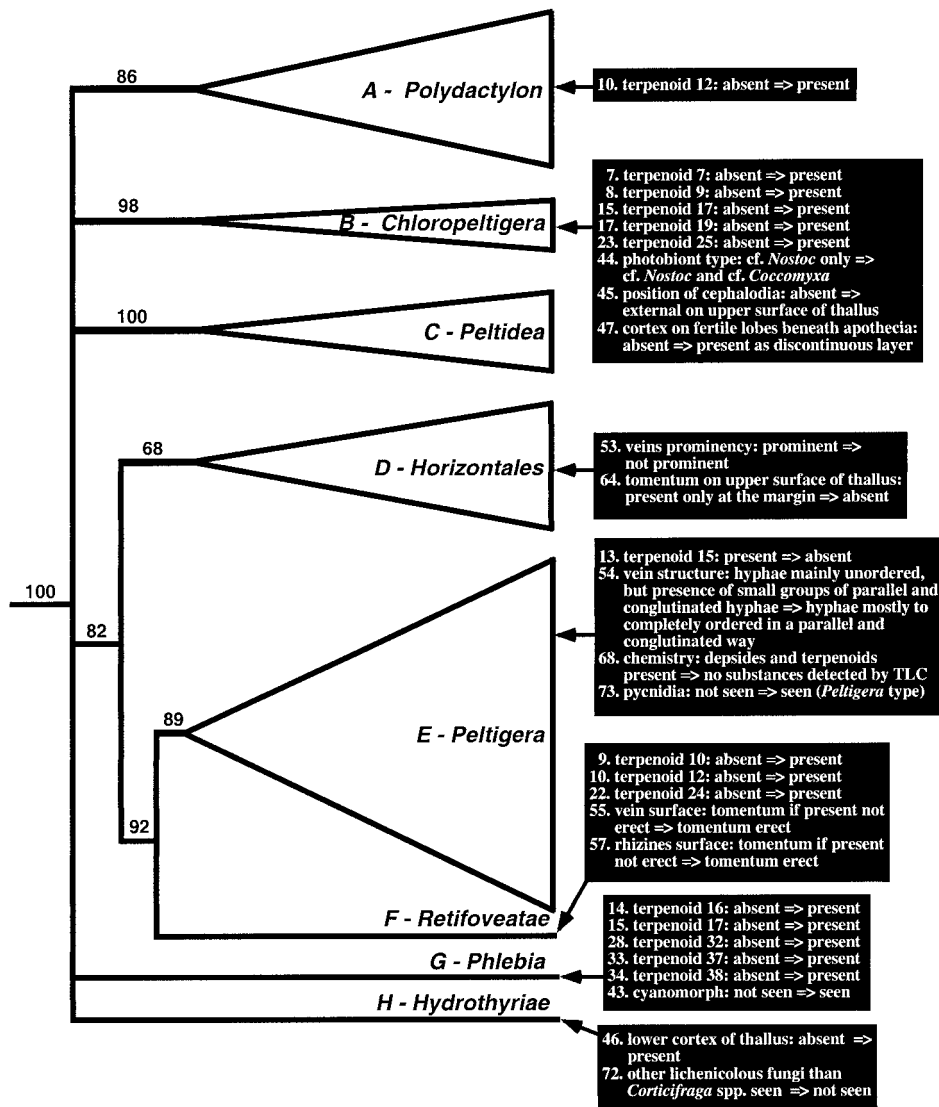
Exclusively bimembered species with cyanobacteria (cf. *Nostoc*). Upper cortex of the thallus arachnoid tomentose (exception: glabrous in *P. degenii* and *P. frigida*, erect tomentose in *P. kristinssonii*). Veins usually distinct, mostly bulging with compact internal core composed of parallel and conglutinated hyphae (character state 12.2 [54.2], fig. 2; and 54.2, table 5). Rhizines often fibrillose, flocculent or penicillate rarely fasciculate or simple. Apothecia pale to dark brown, vertical, mainly saddle shaped (exception: horizontal in *P. frigida*). Vegetative propagules (isidia, phyllidia, and soredia) often occur. Pycnidia very common (character state 73.1, table 5).

*Chemistry*. Usually lacking (character states 13.0 and 68.0, table 5) (exception: triterpenoid zeorin present in *P. laciniata*), tridepsides methyl gyrophorate and gyrophoric acid present in sorediate taxa: *P. didactyla* var. *extenuata* and *P. lambinonii*. Terpenoids never associated with depsides.

Section *Peltigera* includes 16 species: *Peltigera canina* (L.) Willd., *P. cinnamomea* Goward, *P. continentalis* Vitik., *P. degenii* Gyeln., *P. didactyla* var. *didactyla* (With.) J.R. Laundon, *P. didactyla* var. *extenuata* (Nyl. ex Vainio) Goffinet & Hastings, *P. evansiana* Gyeln., *P. frigida* R. Sant., *P. kristinssonii* Vitik., *P. laciniata* (G. Merr. ex Riddle) Gyeln., *P. lambinonii* Goffinet, *P. lepidophora* (Vain.) Bitter, *P. membranacea* (Ach.) Nyl., *P. monticola* Vitik., *P. ponojensis* Gyeln., *P. praetextata* (Sommerf.) Zopf, and *P. rufescens* (Weiss) Humb.

*Peltigera* sect. *Polydactylon* Miadlikowska & Lutzoni, sect. nov. Type Species. *Peltigera polydactylon* (Neck.) Hoffm. *Descr. Adumbr. Lich.* 1: 19. 1790.

A sectione *Horizontales* chimice differt. Dolichorrhizinum praesens et substantia terpenoidea ignota 24 deficiens. Thallus



**Fig. 9** Schematic representation of the eight infrageneric sections of *Peltigera* resulting from the combined analysis of morphological, chemical and LSU nrDNA data sets (see fig. 8). The size of the isosceles triangles is proportional to the number of taxa included in each section. Numbers above branches are bootstrap values. Black boxes highlight unequivocal changes for each of the sections as shown in table 5.

superne non tomentosus, glaber, laevigatus vel scabrosus. Soredia desunt.

Exclusively bimembered species with cyanobacteria (cf. *Nostoc*). Upper surface of the thallus nontomentose, smooth or scabrid (*P. scabrosa*, *P. pulverulenta*, *P. neopolydactyla* p.p., and *P. scabrosella*). Apothecia finger or saddle shaped, almost never black. Soredia are lacking. Marginal and laminal isidioid squamules and phyllidia absent (exception: present in *P. pacifica* and occasionally in *P. polydactylon*). More or less distinct venation present (exception: absent in *P. pulverulenta*). Veins broad, pale, mostly ochraceous, diffuse, becoming slightly or distinctly darker toward center of the thallus (*P. hymenina*, *P. scabrosella*, *P. pacifica*) or entirely distinctly pigmented, pale brown to black (*P. scabrosa*, *P. neopolydactyla*, and *P. occidentalis*). Veins do not exceed the marginal part of the thallus

(exception: distinct through the whole thallus in *P. pacifica* and *P. polydactylon*). Taxa with scabrous upper cortex (*P. scabrosa* and *P. scabrosella*) have veins with distinct central compact core (exception: pilema composed of entirely unoriented hyphae in *P. pulverulenta*) (character state 12.2 [54.2], fig. 2; and 12.0 [54.0], fig. 3). Taxa with smooth upper cortex have veins with partly parallel and conglutinated and partly unoriented hyphae (fig. 3b, 3d, 3f).

**Chemistry.** Terpenoids and tridepsides always present; chemotypical composition highly variable. Dolichorrhizin is present at least in one chemotype from each of the species (character state 10.1, table 5, fig. 9) and is never associated with unidentified terpenoid 24. If dolichorrhizin is absent, then zeorin and unidentified terpenoid 39 or zeorin alone is always present (in *P. scabrosa* chemotype II; fig. 5). In this section, three species (*P. hymenina*, *P. neopolydactyla*, and *P. scabrosa*)

are represented by several chemotypes. According to the results obtained based mainly on material from Central Europe and North America, the other taxa are chemically rather constant, with only minor qualitative and quantitative differences among terpenoids within the same chemotype.

In most cases the qualitative and quantitative variation of four specific terpenoids (zeorin, dolichorrhizin, peltidactylin, and unidentified terpenoid 39) provide diagnostic characters for species identification. The accessory substances found below zeorin on TLC plates in chemotype I of *P. neopolydactylon*, *P. scabrosa*, and *P. polydactylon* also can be very helpful if their concentration is sufficient to detect them with TLC. Based on qualitative and quantitative patterns of the four main terpenoids (fig. 10), we provide here a practical key for chemotypes and species within this section.

Key to Chemotypes of the Section Polydactylon (Fig. 10)

1. More than one terpenoid is present .....2  
 Only zeorin (15) is present ..... *P. scabrosa* II
2. Zeorin (15) and other terpenoids are present .....3  
 Only zeorin (15) and unidentified terpenoid 39 are present;  
 $\uparrow 15 > 39$  ..... *P. neopolydactyla* IV
3. Zeorin (15), unidentified terpenoid 39, and peltidactylin (10) are present .....4  
 Zeorin (15) and dolichorrhizin (12) are present .....6
4.  $10 + 39 = 15$  ..... *P. hymenina* II or *P. neopolydactyla* III  
 (morphological characters must be applied to distinguish these two chemotypes)  
 $10 + 39 \neq 15$  .....5
5.  $10 + 39 > 15$  ..... *P. hymenina* II  
 $10 + 39 < 15 \uparrow$  ..... *P. neopolydactyla* III
6. Peltidactylin (10) or unidentified terpenoid 39 are lacking .....7  
 Peltidactylin (10) and unidentified terpenoid 39 are present .....8
7. Peltidactylin (10) is lacking;  $\uparrow 12 = 15 > 39$  ..... *P. hymenina* III  
 Unidentified terpenoid 39 is lacking; unidentified terpenoids 37, 20, and 41 are present;  $\uparrow 10 > 12 > 15$  ..... *P. scabrosa* I
8. Peltidactylin (10) and unidentified terpenoid 39 have the highest concentration .....9  
 Peltidactylin (10) and unidentified terpenoid 39 have the same or lower concentration than dolichorrhizin (12) .....10
9. Concentration of dolichorrhizin (12) is distinctly higher than concentration of zeorin (15) and smaller than peltidactylin (10) and unidentified terpenoid 39;  $\uparrow 10 + 39 > 12 > 15$  ..... *P. occidentalis*  
 Concentration of dolichorrhizin (12) is similar to concentration of zeorin (15);  $\uparrow 10 + 39 > 12 = 15$  ..... *P. neopolydactyla* I
10. Concentration of peltidactylin (10) and unidentified terpenoid 39 are almost identical with concentration of dolichorrhizin (12); zeorin (15) has the lowest concentration;  $\uparrow 10 + 39 = 12 > 15$  (*P. hymenina* I or *P. pacifica* or *P. neopolydactyla* I + II; morphological characters must be applied to distinguish these chemotypes) .....11  
 Concentration of peltidactylin (10) and unidentified ter-

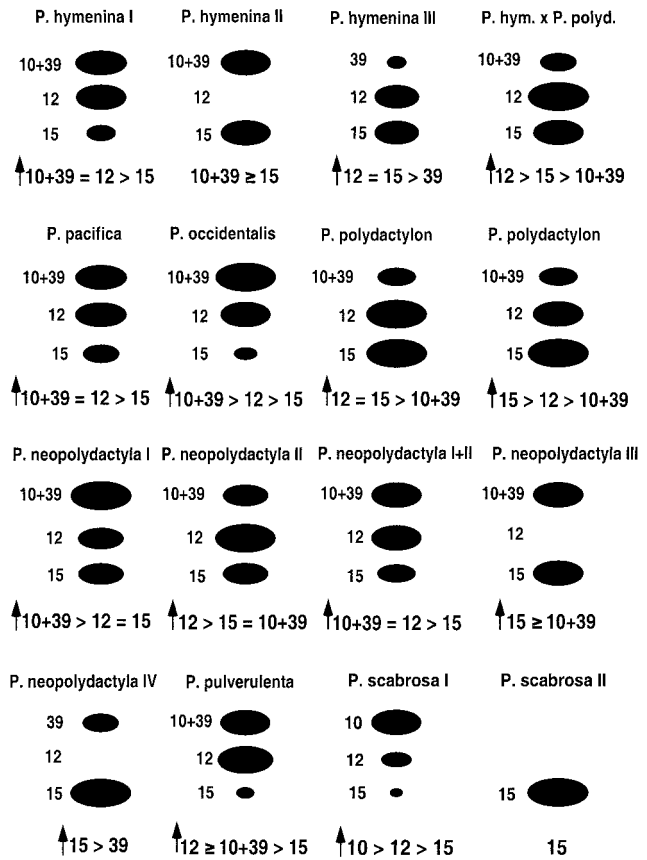


Fig. 10 Quantitative and qualitative comparison of four main terpenoids (peltidactylin [terp. 10], dolichorrhizin [terp. 12], zeorin [terp. 15], and unidentified terpenoid 39) among chemotypes in members of the section *Polydactylon* (*P. hymenina*, *P. neopolydactyla*, *P. occidentalis*, *P. pacifica*, *P. polydactylon*, *P. pulverulenta*, and *P. scabrosa*). Terpenoid positions are shown after elution in EHF solvent. Terpenoids 10 and 39 are represented by a single oval because they share the same position (similar Rf values) in this solvent. Relative quantitative comparisons among these four terpenoids are summarized below each chemotypic pattern. Arrows indicate terpenoids with the highest concentration.

- penoid 39 are lower than concentration of dolichorrhizin (12) .....12
11. Concentration of zeorin (15) is only slightly smaller than concentration of the other terpenoids;  $\uparrow 10 + 39 = 12 > 15$  ..... *P. neopolydactyla* I + II  
 Concentration of zeorin (15) is distinctly smaller than concentration of the other terpenoids;  $\uparrow 10 + 39 = 12 > 15 \downarrow$  ..... *P. hymenina* I or *P. pacifica*
12. Concentration of peltidactylin (10) and unidentified terpenoid 39 is smaller, equal, or higher than concentration of zeorin (15) .....13  
 Concentration of dolichorrhizin (12) is similar to concentration of zeorin (15); concentration of peltidactylin (10) and unidentified terpenoid 39 is distinctly lower than concentration of the other terpenoids;  $\uparrow 12 = 15 > 10 + 39 \downarrow$  ..... *P. polydactylon*
13. Concentration of peltidactylin (10) and unidentified ter-

- penoid 39 is similar to concentration of zeorin (15) and only slightly lower than concentration of dolichorrhizin (12); dolichorrhizin (12) has the highest concentration;  $\uparrow 12 > 10 + 39 = 15$  ..... *P. neopolydactyla* II  
 Concentration of peltidactylin (10) and unidentified terpenoid 39 is higher or lower than concentration of zeorin (15) ..... 14
14. Concentration of peltidactylin (10) and unidentified terpenoid 39 is distinctly higher than concentration of zeorin (15) and only slightly lower or sometimes similar to concentration of dolichorrhizin (12); dolichorrhizin (12) usually has the highest concentration;  $\uparrow 12 \geq 10 + 39 > 15 \downarrow$  ..... *P. pulverulenta*
15. Concentration of peltidactylin (10) and unidentified terpenoid 39 is lower than the concentration of zeorin (15); dolichorrhizin (12) has the highest concentration;  $\uparrow 12 > 15 > 10 + 39$  ..... *P. hymenina*  $\times$  *P. polydactylon*
16. Concentration of peltidactylin (10) and unidentified terpenoid 39 is lower than concentration of zeorin (15); zeorin (15) has the highest concentration;  $\uparrow 15 > 12 > 10 + 39$  ..... *P. polydactylon*

Section *Polydactylon* (group A in fig. 8) includes eight species belonging to two distinct sister clades: (1) *P. neopolydactyla* Gyeln. and *P. scabrosa* Th. Fr. (chemical synapomorphies present; see table 5); (2) *P. hymenina* (Ach.) Delise, *P. occidentalis* (E. Dahl) Krist., *P. pacifica* Vitik., *P. polydactylon* (Neck.) Hoffm., *P. pulverulenta* (Tayl.) Nyl., and *P. scabrosella* Holt.-Hartw.

*Peltigera* sect. *Chloropeltigera* Gyeln. *Rev. Bryol. Lichenol.* 5: 69. 1933. Type species. *Peltigera leucophlebia* (Nyl.) Gyeln. *Magyar Bot. Lapok* 24: 79. 1926—*Peltigera apthosa* var. *leucophlebia* Nyl. *Syn. Lich.*: 323. 1860.

Exclusively trimembered species with green algae (cf. *Coccomyxa*) as the main photobiont and cyanobacteria (cf. *Nostoc*) in external cephalodia on the upper surface of the thallus (character states 44.1 and 45.1, table 5, fig. 9). Apothecia flat and horizontal in *P. nigripunctata* or vertical and saddle shaped in *P. leucophlebia*. Fertile lobes beneath apothecia discontinuously corticated (character state 47.2, table 5). Underside of the thallus often with a distinct network of dark veins and broad pale marginal zone. Veins composed of groups of parallel and conglutinated hyphae in a network of unoriented hyphae (character state 12.1 [54.1], fig. 3). Cyanomorphs have never been found (see Goffinet and Bayer 1997).

**Chemistry.** Terpenoids and depsides always present. Unidentified terpenoid 7 might be a good feature to distinguish members of this section from members of section *Peltidea* (character state 7.1, table 5, fig. 9). However, in Iceland, three specimens that are morphologically in agreement with *P. apthosa* according to Orange (1990) were found to have terpenoid 7. In Europe and in North America, two chemotypes have been detected for *P. leucophlebia*. Both of them are characterized by the absence of phlebic acids A and B, which are usually present in thalli of other trimembered taxa. However, in Japanese material, phlebic acids A and B (character state 15.1, table 5, fig. 9) were reported for specimens identified as

*P. leucophlebia* (Kurokawa et al. 1966). *Peltigera nigripunctata* contains only phlebic acid B (character state 15.1, table 5, fig. 9) and has horizontal apothecia, and its distribution is restricted to Eastern Asia. This taxon needs further taxonomic study in terms of its delimitation from *P. leucophlebia*.

Section *Chloropeltigera* includes two species: *P. leucophlebia* (Nyl.) Gyeln. and *P. nigripunctata* Bitter.

*Peltigera* sect. *Peltidea* (Ach.) Vain. *Etud. Lich. Brésil. Vol. 1*: 179. 1890. Type species. *Peltigera apthosa* (L.) Willd. *Fl. Berol. Prodr.*: 347. 1787—*Lichen apthosus* L. *Sp. Pl.*: 1148. 1753.

Bimembered species with cyanobacteria (cf. *Nostoc*) as the only photobiont and trimembered species with green algae (cf. *Coccomyxa*) inside the thallus and cyanobacteria in external cephalodia on the upper surface of the thallus. At least marginal parts of the lobes usually with erect hairy tomentum (exception: glabrous lobes in *P. frippii*). Venation indistinct, veins broad and fused, or pilema covering almost the entire lower surface of the thallus (exception: distinct narrow veins in *P. frippii*). Apothecia saddle shaped (exception: not seen in *P. frippii*);<sup>2</sup> fertile lobes beneath apothecia entirely corticated (exception: not corticated in *P. malacea*).

**Chemistry.** Terpenoids and depsides always present; great chemotypical variation especially in *P. apthosa* and *P. malacea*. Some terpenoids are considered to be taxonomically informative: unidentified terpenoids 14 and 9, or only one of them, as well as both phlebic acids (A and B) have been found in three chemotypes out of five for *P. apthosa* and *P. malacea*. These terpenoids also occur in *P. frippii*.

Section *Peltidea* includes four species: trimembered *P. apthosa* (L.) Willd. and *P. britannica* (Gyeln.) Holt.-Hartw. & Tønsb. and bimembered *P. frippii* Holt.-Hartw. and *P. malacea* (Ach.) Funck.

*Peltigera* sect. *Horizontales* *Miadlikowska & Lutzoni*, sect. *nov.* Type species. *Peltigera horizontalis* (Huds.) Baumg. *Fl. Lips.*: 562. 1790—*Lichen horizontalis* Huds. *Fl. Angl.*: 453. 1762.

Thallus superne etomentosus, glaber, laevigatus vel raro scabrosus. Soredia et phyllidia frequenter adsunt. Subtus nervis planis confluentibus vel discretis, fuscus vel atratus. Apothecia horizontalia, plana et fusca vel verticalia, digitiformia et nigra. Materia chimica adsunt.

Exclusively bimembered species with cyanobacteria (cf. *Nostoc*). Upper cortex of the thallus nontomentose and smooth (exception: sometimes scabrid in *P. collina*; character state 64.0, table 5, fig. 9). Apothecia brown, horizontal, and flat (in *P. horizontalis* and *P. elisabethae*) or black, vertical, and finger shaped. Vegetative propagules frequent: schizidia in *P. elisabethae*, marginal and laminal phyllidia in *P. phyllidiosa*, as

<sup>2</sup> A specimen of *P. frippii* with a vertical apothecium was found in Siberia (J. Holtan-Hartwig via O. Vitikainen, personal communication). However, this information was obtained too late to include it in our study.



well as marginal and laminal soredia in *P. collina*. Venation distinct and dense with numerous white interstices in *P. horizontalis*, *P. phyllidiosa*, and often in *P. collina*, versus almost continuous pilema with only a few broad veins and numerous or sparse interstices in *P. elisabethae*, *P. neckeri*, and *P. polydactyloides*. Veins mostly flat (character state 53.0, table 5, fig. 9). The central part of the veins with core that is never as well delimited and compact as in section *Peltigera* (character state 12.1 [54.1], fig. 3). Broad veinless and pale zone along the lobe margin on the under side of the thallus consistently present. Rhizines usually black and fasciculate. Maculae commonly occur.

*Chemistry.* Terpenoids and depsides always present; chemotypic variation occurs.

Section *Horizontales* includes six species belonging to two distinct sister clades (group *D* in fig. 8): *D.1. Peltigera horizontalis* (Huds.) Baumg. and *P. elisabethae* Gyeln. (unequivocal chemical and morphological synapomorphies support this clade; see table 5) and *D.2. P. collina* (Ach.) Schrad., *P. neckeri* Hepp ex Müll. Arg., *P. polydactyloides* Nyl., and *P. phyllidiosa* Goffinet & Miadlikowska (one unequivocal morphological synapomorphy supports this clade; see table 5).

*Peltigera* sect. *Retifoveatae* Miadlikowska & Lutzoni, sect. nov. *Type species.* *Peltigera retifoveata* Vitik. *Ann. Bot. Fennici* 22: 296. 1985.

A sectione *Peltigera* chimice differt. Materia chimica (peltidactylum, dolichorrhizinum, zeorinum et substancia terpenoidea ignota 24) semper adsunt. Thallus superne in partibus marginalibus crassitomentosus, centrum versus glabrescens. Venae et rhizinae tomentosae et dense breviter ramosae; interstitia foveata.

Bimembered lichen with cf. *Nostoc* as the photobiont. Upper cortex of the thallus arachnoid tomentose. Marginal part of lobes upturned. Morphologically similar to *P. canina*, but rhizines and veins erect tomentose (character states 55.1 and 57.1, table 5, fig. 9). Veins without compact internal core (character state 12.1 [54.1], fig. 3). Differs from *P. membranacea* by deeply pitted interstices.

*Chemistry.* Terpenoids and depsides always present. Unique terpenoid composition: peltidactylin (10), dolichorrhizin (12), zeorin (15), and unidentified terpenoid 24 (character states 9.1, 10.1, and 22.1, table 5, fig. 9).

Section *Retifoveatae* includes only *Peltigera retifoveata* Vitik.

*Peltigera* sect. *Phlebia* Wallr. *Fl. Crypt. Germ. II*: 556. 1831. *Type species.* *Peltigera venosa* (L.) Hoffm. *Descr. Adumb. Lich.* 1: 31. 1789—Lichen *venosus* L. *Sp. Pl.*: 1148. 1753.

Trimembered species with green algae (cf. *Coccomyxa*) as the main photobiont and cyanobacteria (cf. *Nostoc*) in external cephalodia on the lower surface of the thallus. Rhizines absent; single rhizopt present. Vein structure similar to section *Peltigera* with a distinct compact central core (character state 12.2 [54.2], fig. 2). Apothecia horizontal, plane, or concave. Cyanomorphs often present, outgrowing from cephalodia, at-

tached or detached from main thallus; similar to small *Leptogium* with an homoiomerous thallus (character state 43.1, table 5, fig. 9).

*Chemistry.* Terpenoids and depsides always present. Unique terpenoids composition: peltidactylin (10), phlebic acid A (occasionally), phlebic acid B, hopane-6 $\alpha$ ,7 $\beta$ ,22-triol, and unidentified terpenoids 14, 32, 37, 38, and 41 (character states 14.1, 15.1, 28.1, 33.1, and 34.1, table 5, fig. 9).

Section *Phlebia* includes only *Peltigera venosa* (L.) Hoffm.

*Peltigera* sect. *Hydrothyriae* Miadlikowska & Lutzoni, sect. nov. *Type species.* *Peltigera hydrothyria* Miadlikowska & Lutzoni, nom. nov.  $\equiv$  *Hydrothyria venosa* Russ. *Proc. Essex Inst.* 1: 188. 1856. Non *Peltigera venosa* (L.) Hoffm.

Species aquatilis; thallus homoiomereus, superne corticatus. Rhizinae desunt. Hyphae in parte venorum interiori perfecte conglutinatae et parallelae. Apothecia submarginalia.

Bimembered species with cyanobacteria (cf. *Nostoc*) as the only photobiont. Thallus gelatinous, nonstratified, and corticated (character state 46.1, table 5, fig. 9). Rhizopt and distinct venation composed entirely of parallel and conglutinated hyphae (character state 12.2 [54.2], fig. 2a). Apothecia submarginal. Aquatic; North America. Lichenicolous fungi not reported (character state 72.0, table 5, fig. 9).

*Chemistry.* Typically lacking secondary substances; chemodeme with depsides (methyl gyrophorate and methyl lecanorate) occurs (Feige et al. 1989).

#### Key to Sections of *Peltigera*

1. Aquatic. Bimembered species with nonstratified, corticated, and gelatinous thallus. Single rhizopt and distinct venation present. Veins entirely composed of parallel and conglutinated hyphae. Apothecia subterminal. Secondary substances absent (only occasionally depsides present). North America ..... Sect. *Hydrothyriae*  
Nonaquatic. Bi- or trimembered species with stratified, non-gelatinous thalli (exception: the cyanomorph of *P. venosa* is gelatinous). Apothecia terminal ..... 2
2. Trimembered species with cephalodia on the upper or under surface of the thallus; always with terpenoids detectable by TLC. Cyanomorphs present or absent. Exceptions: A, bimembered species without cephalodia, with erect tomentose lobes (*P. malacea*), or B, bimembered species without cephalodia, thallus smooth, phlebic acids present (*P. frippii*) ..... 3  
Bimembered species without cephalodia. Thallus tomentose or smooth, with or without secondary compounds detectable by TLC ..... 5
3. Cephalodia on the lower surface of the thallus. Single rhizopt present. Apothecia horizontal. Cyanomorph similar to small *Leptogium*, veinless, with homoiomerous thallus structure. Veins with distinct core composed of parallel and conglutinated hyphae. Unidentified terpenoids 32 and 38

- always present .....Sect. *Phlebia*  
Cephalodia on the upper surface of the thallus; if cephalodia absent, see exceptions A and B in couplet 2 .....4
4. Cephalodia always present. Apothecia horizontal, flat, or vertical; saddle-shaped, fertile lobes beneath apothecia discontinuously corticated or not corticated. Lower side mostly with a distinct network of dark veins, composed of a mixture of unoriented hyphae and aggregations of parallel and conglutinated hyphae. Unidentified terpenoid 7 is always present. Cyanomorphs have never been reported .....Sect. *Chloropeltigera*  
Cephalodia present or absent. Apothecia, if present, saddle shaped and fertile lobes beneath apothecia entirely corticated. Veins mostly broad and fused into dark pilema with only few interstices. Veins consist of randomly oriented and nonconglutinated hyphae. Unidentified terpenoid 14 is never associated with unidentified terpenoid 9, and unidentified terpenoid 7 is only occasionally present (in the material from Iceland). If cephalodia absent, then exceptions A or B in couplet 2 are applicable. Cyanomorphs present, but never the *Leptogium* type ..... Sect. *Peltidea*
5. Thallus arachnoid tomentose. Terpenoids and depsides detectable by TLC are absent. Veins bulging with compact core of parallel and conglutinated hyphae. Exceptions: A, secondary compounds absent, thallus nontomentose (*P. frigida* and *P. degenii*); B, secondary compounds absent, tomentum consists of erect hairs (*P. kristinssonii*); C, thallus arachnoid tomentose, pycnidia common and bigger than usual, zeorin (15) is present, and depsides are lacking (*P. laciniata*); D, thallus arachnoid tomentose, soredia usually occur, depsides detectable by TLC are present (*P. didactyla* and *P. lambinonii*) ..... Sect. *Peltigera*  
Thallus nontomentose or tomentose. Secondary compounds (terpenoids and depsides) are present .....6
6. Thallus tomentose. Veins and rhizines densely and shortly branched, and interstices deeply pitted. Zeorin (15), peltidactylin (10), dolichorrhizin (12), and unidentified terpenoid 24 are present; occasionally only zeorin (15) is detected ..... Sect. *Retifoveatae*  
Thallus nontomentose, glabrous to glossy or scabrous. Phyllidia often present. Lower side of the thallus different from section *Retifoveatae* .....7
7. Marginal and laminal soredia or schizidia often present. Rhizines mostly fasciculate, dark brown to black. Veins flat, brown or black, distinct and narrow or broad, and fused into a continuous pilema with sparse to few interstices. Apothecia horizontal and plane or vertical, finger shaped, and black. Zeorin (15) and unidentified terpenoid 30 or unidentified terpenoids 23 and 24 are often present. If apothecia absent, thallus has vegetative propagules; soredia (*P. collina*) or phyllidia (*P. phyllidiosa*) .....Sect. *Horizontales*  
Soredia and schizidia never present. Rhizines rarely fasciculate, brown to black. Venation well developed, distinct, dark colored, or venation diffused and ochraceous, occasionally forming a continuous pilema (*P. pulverulenta*). Apothecia vertical, finger or saddle shaped, never black. Dolichorrhizin (12) often occurs but never together with unidentified terpenoid 24. If dolichorrhizin (12) is absent, then in most cases unidentified terpenoid 39 and zeorin (15)

are present. If dolichorrhizin (12) and unidentified terpenoid 39 are lacking, upper cortex of the thallus is scabrous (*P. scabrosa* chemotype II) .....Sect. *Polydactylon*

## Discussion

### *Relative Contributions of Morphological, Chemical, and Molecular Data Sets to the Final Combined Analysis*

Because of the frequent overlapping variability among species and occurrence of chimeric thalli in many *Peltigera* species, particularly in the *P. canina* group, reconstructing phylogenetic relationships within the genus based solely on morphological and chemical features was very limited (fig. 6A, 6B), even with the reduced 54 OTU data set (results not shown). Morphology, chemistry, and LSU nrDNA data sets when analyzed alone do not confirm the monophyly of *Peltigera*. The monophyletic groups that include more than one species (i.e., A–E, fig. 8) resulting from the analyses of the LSU nrDNA and the combined data set were not reconstructed as monophyletic entities when the morphological and chemical data sets were analyzed separately, with the exception of section B (recovered by all phylogenetic analyses) and subsections D.1 and D.2 (recovered by the morphological phylogenetic analysis).

Based on morphological characters alone (fig. 6A), *Peltigera* is divided into two artificial clades. One includes all taxa with tomentose thalli belonging to groups E and F and the second contains members of groups A–D, as well as one species with a hairy upper cortex from group E. Broad phylogenetic relationships within both groups are not resolved. *Peltigera venosa* (group G) was the only member of the ingroup to be nested within the outgroup, sister to *Peltigera hydrotheria* (group H).

Fifty-seven percent of trees derived from the separate analysis of terpenoids (fig. 6B) grouped taxa containing terpenoids (i.e., all groups except most members of group E) in one clade. This clade also contains *Nephroma arcticum*, an outgroup species (O) with some secondary substances similar to *Peltigera*. *Peltigera laciniata*, a clear member of the *P. canina* complex (group E), was also included in this terpenoid clade. The unresolved clade, outside of the terpenoid clade, includes all other species from group E and the remaining outgroup species.

Topologies based on LSU nrDNA data alone (fig. 6C) reveal the same broad relationships among groups as the combined analyses (figs. 7, 8). One exception is the paraphyletic *H. venosa*–*P. venosa* group, which is nested between group A and the rest of *Peltigera* species in the LSU nrDNA phylogenetic analysis rather than being basal to the rest of *Peltigera*, as in the combined analyses. All reconstructed *Peltigera* groups derived from the combined analyses are also monophyletic entities in the LSU nrDNA tree (fig. 6C). Within the *P. canina* complex (group E), different relationships among taxa are obtained compared to the results derived from the combined data sets.

In spite of the small amount of phylogenetic information provided by morphology and chemistry when analyzed separately, both data sets contribute to increasing the resolution and bootstrap support in the combined analyses. In this study, adding morphological and chemical data to the LSU nrDNA data almost never decreased bootstrap support.

### Monophyly of *Peltigera* and Status of *Hydrothyria*

The traditional delimitation of *Peltigera* based exclusively on morphological characters has not previously been disputed, except when Nylander (1863) proposed to segregate the tripartite species. Including *Hydrothyria* in *Peltigera* or not, depends on whether this monospecific genus is nested within *Peltigera* or is sister to *Peltigera*. In the latter case, the final decision would depend on the taxonomic circumscription of the genus *Peltigera* and the ranking of *Hydrothyria*. From the results presented here based on phylogenetic analyses of LSU nrDNA (where *Hydrothyria* is nested within *Peltigera*; fig. 6C) and the combined data set (where *Hydrothyria* is sister to *Peltigera*; figs. 7, 8), as well as on two monophyly tests (Templeton and TLDB), we cannot reject either hypothesis with confidence. Although the hypothesis that *Hydrothyria* does not form a monophyletic group with *Peltigera* can be rejected only based on the Templeton test ( $P = 0.0002$ ), both monophyly tests revealed that there is no strong evidence to reject the hypothesis that *Hydrothyria* is nested within *Peltigera*.

*Hydrothyria venosa* is a foliose aquatic North American endemic cyanolichen. This monospecific genus shares many diagnostic features with *Peltigera*, e.g., distinct cylindrical veins with compact internal structure, presence of a rhizopt similar (probably homologous) to *P. venosa*, presence of chemodeme with depsides (Feige et al. 1989), 3-septate spores, and nonstratified paraplectenchymatous thallus that internally resembles the cyanomorph of *P. venosa*. Although *Hydrothyria* has submarginal apothecia, compared to *Peltigera* with marginal apothecia, the ascus structure in both taxa is virtually identical (J. Hafellner, personal communication). Based on the results from our phylogenetic analyses, tests of monophyly, and the long internode supporting the *Peltigera-Hydrothyria* group (fig. 8) with a bootstrap value of 100%, we concluded that *H. venosa* is an aquatic member of the genus *Peltigera*. There are several genera that include a mixture of terrestrial and aquatic species (e.g., *Verrucaria*, *Hymenelia*, and *Dermatocarpon*). Therefore, the *nomen novum* *Peltigera hydrothyria* Miadlikowska & Lutzoni is introduced, and a new section *Hydrothyriae* Miadlikowska & Lutzoni within *Peltigera* is proposed. The same arguments can be used to reject the establishment of a new monospecific genus to accommodate *P. venosa*.

If *H. venosa* was clearly established to be sister to *Peltigera*, an alternative classification could consist of including *H. venosa* and *P. venosa* in a genus outside *Peltigera*. Several morphological and anatomical similarities would support this classification (fig. 6A); however, if monophyly is the grouping criterion, their paraphyletic relationship as revealed by our combined analysis prevents the inclusion of these two species into one genus (fig. 8).

### Stability of Proposed Sections within *Peltigera*

The phylogeny presented here (fig. 8) is the first to be constructed for the genus *Peltigera*. This phylogeny is based mainly on North American and European taxa, with some representatives from other parts of the world. Most of these species have a wide geographical distribution, and they cover broad phenotypical and chemical variation within *Peltigera*. No geographical pattern was detected on our best trees, but

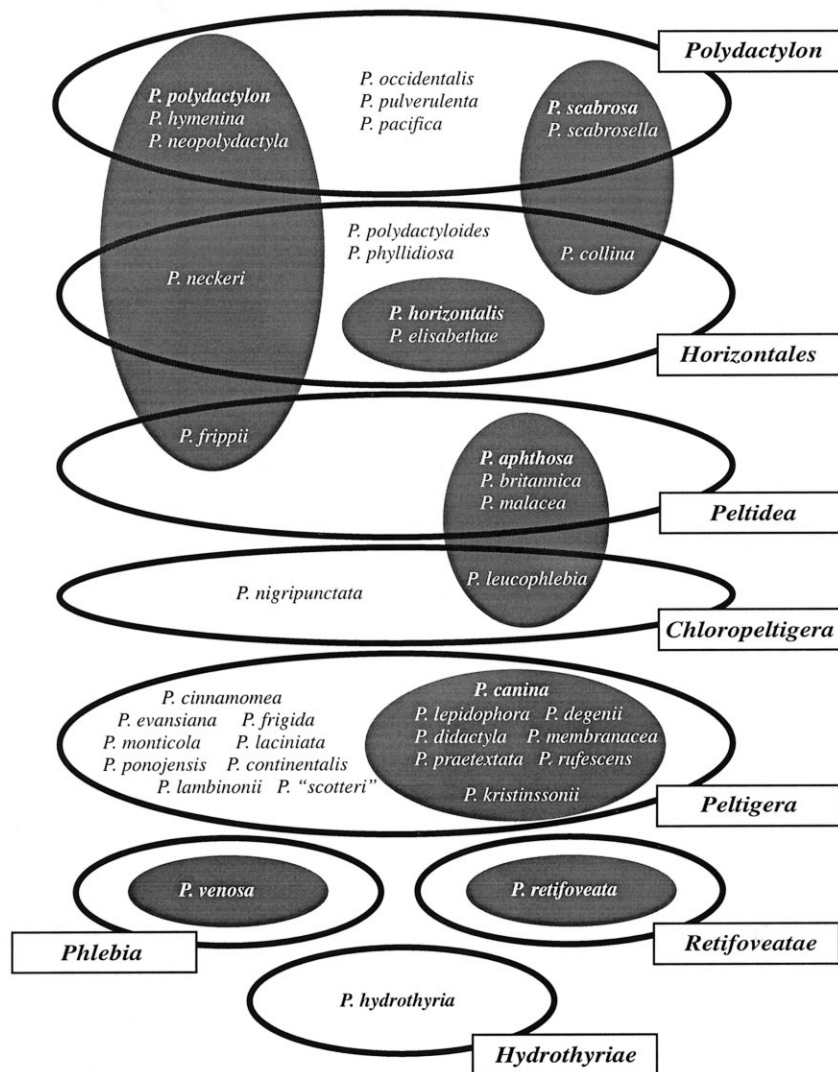
a broader taxon sampling and populations from distinct geographical areas for each species would be needed for a thorough biogeographical study. The results presented here provide a robust phylogenetic framework for further systematic, evolutionary, and biogeographical studies on *Peltigera* and suborder Peltigerineae. We believe that by adding taxa, the main groups will not change drastically, although their relationships could change. For example, the monophyly of cephalodiate taxa from sections *Chloropeltigera*, *Peltidea*, and *Phlebia* could not be rejected because the polyphyletic result shown on the best tree (fig. 8) could be the result of sampling error. We can say with a relatively high degree of confidence that section *Retifoveatae* is sister to section *Peltigera* and that section *Horizontales* shares a most recent common ancestor with sections *Retifoveatae* and *Peltigera* (bootstrap = 82%). To gain confidence in the broad relationships among members of *Peltigera*, adding taxa will not be sufficient. Additional molecular characters are needed.

### Comparison of Our Sections with Past Infrageneric Classifications

Holtan-Hartwig's (1993) grouping of Norwegian taxa is only partly supported by our sections (fig. 11). His circumscription of three *Peltigera* groups (*P. canina*, *P. retifoveata*, and *P. venosa*) was confirmed by our study. His *P. scabrosa*, *P. polydactylon*, and *P. aphthosa* groups are polyphyletic. His *aphthosa* group consisted of members found in two of our sections (*Chloropeltigera* and *Peltidea*). The paraphyletic relationship of these two sections to one another and the high level of divergence between *P. aphthosa* and *P. leucophlebia* (fig. 8) were unexpected. The latter two species are often difficult to distinguish morphologically and sometimes also chemically. Goffinet and Bayer (1997) already pointed out the relatively high genetic divergence between *P. leucophlebia* and *P. aphthosa* compared to *P. britannica* and *P. aphthosa*. One feature that demarcates section *Peltidea* (*P. leucophlebia* and *P. nigripunctata*) is their inability to produce cyanomorphs. Moreover, section *Chloropeltigera* is supported by eight unequivocal morphological and chemical synapomorphies (table 5; fig. 9).

Another trimembered species, *P. venosa* (section *Phlebia*) holds a basal position in our best trees derived from the combined analyses (figs. 7, 8). However, when the LSU nrDNA data set (fig. 6C) was analyzed separately, this section (with *Hydrothyriae*) formed a paraphyletic group with the two other sections (*Peltidea* and *Chloropeltigera*) containing trimembered taxa. Our data do not allow us to reject this paraphyletic relationship nor do they allow us to reject the monophyly of cephalodiate taxa. However, we predict that the latter null hypothesis will be rejected as more data will be available. *Peltigera venosa* was included in a separate section by Wallroth (1831) and later on was adopted as such in Gyelnik's (1933) classification. Because of the unique morphology of its cyanomorphs, as well as its exceptional chemistry (five terpenoids are unequivocal synapomorphies for this section; table 5; fig. 9), *P. venosa* is very easy to distinguish from other species and sections within *Peltigera*.

The newly described section *Horizontales* is a fusion of members from three groups according to Holtan-Hartwig's



**Fig. 11** Comparison of Holtan-Hartwig's (1993) subdivisions of Norwegian *Peltigera* species (black ovals) and infrageneric sections newly proposed here (white ovals) based on combined analyses of morphological, chemical and molecular data sets. Sections (in boxes) correspond to the main groups A–H as shown in figs. 7 and 8.

classification (1993): the entire *P. horizontalis* group, *P. neckeri* (from his *P. polydactylon* group), and *P. collina* (from his *P. scabrosa* group). Holtan-Hartwig (1993) established the *P. horizontalis* group to accommodate two species (*P. horizontalis* and *P. elisabethae*) with fusiform spores and planar apothecial disks that are situated at an angle to the lobes. The presence of a scabrous upper cortex, which was considered by Holtan-Hartwig (1993) to be the main criterion for circumscribing the *P. scabrosa* group, seems to have had at least two independent origins. The *P. scabrosa* group was therefore the artificial assemblage of three taxa characterized by the scabrosity of their thalli.

Molecular and combined data sets indicate that *P. collina*, *P. neckeri*, and two additional taxa that were not studied by Holtan-Hartwig (*P. polydactyloides* and *P. phyllidiosa*), together are sister to the former *P. horizontalis* group. Holtan-Hartwig (1993) pointed out that the affinities of *P. collina* with

the *P. scabrosa* group might be artificial, and he suggested the alternative placement of this taxon in the *P. polydactylon* group. Vitikainen (1994b) also expressed his disagreement about the inclusion of *P. collina* together with *P. scabrosa* and *P. scabrosella* as part of one natural taxonomic entity. *Peltigera polydactyloides* is known so far only from East Africa and shows only minor morphological (undulating and phyllidiose margins, broader lobes, more dense rhizines) and ecological (epiphytic muscicolous) differences from the widely distributed (Europe, Asia, North and South America) *P. neckeri* (Swinscow and Krog 1988; Vitikainen 1994b). The terpenoid pattern revealed by TLC for *P. polydactyloides* is similar to that of *P. neckeri*. An in-depth species-level systematic study is needed for these two species.

*Peltigera phyllidiosa* was recently described by Goffinet and Miadlikowska (1999) from the southern Appalachian Mountains. It is most closely related to the *P. collina*–*P. neckeri*–*P.*

*polydactyloides* group. The phenotypic similarity of *P. phylloidioides* to *P. collina* was independently noted by Goffinet and Miadlikowska (1999). Although *P. horizontalis* and *P. elisabethae* are widely accepted as two distinct species, based on ecological and morphological features, they share several unique features, including an identical pattern of chemotypical variation. The level of divergence among individuals selected from both species is virtually nil (fig. 8). Delimitation of Holtan-Hartwig's (1993) *P. polydactylon* group was changed by this study to include two taxa from the artificial *P. scabrosa* group and to exclude *P. neckeri* and *P. frippii* (fig. 11). We found that three additional taxa (*P. occidentalis*, *P. pacifica*, and *P. pulverulenta*) were also members of this section. Holtan-Hartwig considered the nontomentose and smooth upper side of the thallus as the common diagnostic feature for all members of his *P. polydactylon* group. According to our results, this group also contains taxa with scabrous cortices. The chemistry and morphology of three rare European species (*P. lyngei*, *P. melanorrhiza*, and *P. dissecta*) indicate a probable close affiliation to other members of this section (Purvis and James 1993; Vitikainen 1994b; Holtan-Hartwig 1988; Martínez 1999). *Peltigera melanorrhiza* and *P. dissecta* are phenotypically similar to *P. hymenina* and together probably form a monophyletic group. *Peltigera lyngei*, with a scabrous upper surface, may represent a sister taxon to *P. scabrosa*, as was suggested by Vitikainen (1994b).

In general, all acid-deficient and tomentose species form the well-defined section *Peltigera* that includes *P. canina*, the type species for the genus. This clade includes all members of the *P. canina* group as delimited by Holtan-Hartwig (1993). Because of the inclusion of the Neotropical species *P. laciniata*, which contains zeorin, as well as *P. didactyla* var. *extenuata* and *P. lambinonii*, the only species with depsides in the section *Peltigera*, the chemical variability within this section is broader than anticipated by Holtan-Hartwig. Seven additional species (not known from Norway and therefore beyond Holtan-Hartwig's study) are shown here to belong to this section. *Peltigera degenii* and *P. frigida*, with their glabrous upper cortex, a feature shared with members of the sections *Horizontales* and *Polydactylon*, belong to this group. Also, *P. kristinssonii* with an erect tomentum formerly found exclusively in members of the section *Chloropeltigera*, is clearly nested within section *Peltigera*.

Vitikainen (1994b) suggested that *P. retifoveata*, classified as a monospecific group by Holtan-Hartwig (1993), should be included in the *P. canina* complex in spite of its complex chemistry (triterpenoids and tridepsides). Our results show this species as a sister taxon to the *canina* group. Therefore, the inclusion of *P. retifoveata* within the *canina* group becomes a question of rank. Members of section *Peltigera* share some morphological features with *P. retifoveata*, such as an arachnoid tomentum on the upper surface of the thallus. However, based on other characters, section *Retifoveatae* should not be fused with section *Peltigera*. In addition to its distinct and unique chemistry, *P. retifoveata* possesses veins in which the hyphal walls are more or less nonconglutinated (fig. 3b), whereas in all members of section *Peltigera*, a distinct conglutinated core in the central part of the veins is present (fig. 2). Because the LSU nrDNA along with the chemical and morphological data did not allow us to completely resolve rela-

tionships among taxa in section *Peltigera*, we are currently gathering data from the ITS region. With this additional data set we will revisit the status of newly proposed taxa that are nested within the *P. canina* complex.

#### *Symbiotic Composition of the Thallus as a Criterion for an Infrageneric Classification of Peltigera*

Infrageneric groups within *Peltigera* based strictly on the symbiotic composition of the thallus are not in agreement with the monophyly criterion used here. Although the monophyly of cephalodiate taxa cannot be rejected, the *Peltidea* section (bootstrap = 100%) clearly includes both bi- and trimembered species (figs. 7, 8). The monophyly of the trimembered symbiotic section *Peltidea*, as defined by Acharius (1814), Nylander (1863), and Jatta (1893), and the monophyly of section *Phlebia*, as defined by Kurokawa et al. (1966), were not confirmed by our phylogenetic study.

Holtan-Hartwig (1993) noted that the capability of some *Peltigera* mycobionts to develop bi- and trimembered thalli is the main reason why a dichotomous classification of this genus based solely on photobiont composition is artificial. Results based on ITS sequences from *Peltigera* (Goffinet and Bayer 1997), as well as on RFLP analysis of the LSU nrDNA and Southern hybridization from *Sticta* and *Pseudocyphellaria* (Armaleo and Clerc 1991), support the hypothesis that the same fungus can form a bi- and trimembered symbiotic association. However, more sensitive methods or more variable portions of the fungal genome could reveal, for a given species, that the fungus interacting with the green alga is different from the one associated with the cyanobacterium. Even if this was the case, the fact that bimembered *Peltigera* (e.g., *P. malacea* and *P. frippii*) are more closely related to some trimembered *Peltigera* species than to other bimembered species is sufficient to reject a classification based solely on the photobiont composition.

One possible explanation for this mixed composition of bi- and trimembered species in the section *Peltidea* could be that *P. malacea* and *P. frippii* are derived from a trimembered ancestor. In this scenario, they would have been cyanomorphs of these ancestral (extinct) tripartite species. Another possibility is that the ancestor was a bimembered cyanopeltigera. In this case, an independent acquisition of a green alga would have led to *P. britannica* and *P. aphthosa*. Recent *in vivo* studies on early developmental stages in trimembered Peltigerineae demonstrated a bimembered phase with a cyanobacterium followed by the subsequent acquisition of a green alga as a second developmental phase (Stocker-Wörgötter and Türk 1994; Yoshimura et al. 1994; Holtan-Hartwig 1996). If this second hypothesis is true, this would exemplify a case where ontogeny recapitulates phylogeny. To gain more insight and to determine which hypothesis is more likely, the ancestral symbiotic states (bi- vs. trimembered) need to be reconstructed for a broad selection of Peltigerineae species. This is the subject of our current research.

Group I of Holtan-Hartwig (1993) is a good example of a subgeneric division that includes a mixture of bipartite (*P. malacea*) and tripartite species (fig. 1A). *Peltigera malacea* is morphologically very similar to the cyanomorph of *P. aphthosa* and *P. britannica*. Evidence for a close relationship between *P. aphthosa* and *P. malacea* was recently provided by a phylo-

genetic analysis of ITS sequence data (Goffinet and Bayer 1997). Our results not only confirm this relationship but also add another closely related bipartite cyanopeltigera (*P. frippii*; figs. 8, 11). Holtan-Hartwig (1993) included the latter species in the *P. polydactylon* group because of its glabrous thallus. However, he was uncertain about this decision mainly because of the chemical similarity of *P. frippii* to *P. aphthosa* chemotype I. Phlebic acids A and B, as well as unidentified terpenoid 14, which are present in thalli of *P. frippii*, have not been found in any members of *P. polydactylon*. These three substances commonly occur in tripartite species from section *Peltidea* and *Phlebia*, whereas unidentified terpenoid 14 alone is present in other trimembered taxa from section *Chloropeltigera* and in *P. malacea* (another bimembered species from the section *Peltidea*).

#### *Morphology and Chemistry as Diagnostic Characters for Peltigera Sections*

Morphology has been for a long time the only criterion for species and infrageneric group delimitation within *Peltigera*. Most diagnostic morphological features in *Peltigera* are notoriously highly variable. Morphological features that were used by Holtan-Hartwig (fig. 1B) as main indicators for *Peltigera* groups (e.g., horizontal apothecia, scabrous cortex, and veins with internal compact core) are not likely to be homologous across the genus. In the combined data set (54 OTUs) analysis, several synapomorphies reconstructed for our newly proposed sections refer to morphological characters (table 5; fig. 9). Section *Peltigera*, which in general lacks secondary compounds, is characterized by two unequivocal morphological synapomorphic character states; however, in both cases reversals are present. Synapomorphic characters alone do not provide sufficient information for circumscribing sections. Therefore, the most practical approach includes a combination of synapomorphies and other morphological features that can be used to define each section.

Chemistry, especially triterpenoid composition in *Peltigera*, was only recently considered as a taxonomically valuable tool in addition to morphology (e.g., Kurokawa et al. 1966; Holtan-Hartwig 1993). In this study, chemical characters greatly contributed to the list of unequivocal synapomorphies that circumscribed each section and the main internal clades within sections (table 5; fig. 9). Each of the monotypic sections *Phlebia* and *Retifoveateae* is characterized by a unique composition of terpenoids. Therefore, it is possible to distinguish them based exclusively on chemistry. In section *Peltigera*, only one species contains a terpenoid. As such, the lack of terpenoids is a good indicator for this group. In other sections the secondary compound composition is more complex because of frequent chemotypic variants.

Conducting chemotaxonomic studies on *Peltigera* requires a broad knowledge of the chemical patterns occurring among and within species. Secondary compound composition of *Peltigera* individuals from different areas of the world, as well as complete interpretation of the observed chemotypical variation at the population and species level, still remains to be done. Triterpenoids in *Peltigera* cannot be studied using one-dimensional thin-layer chromatography because some terpenoids share the same position on TLC plates (similar Rf values),

thereby masking one another. Some terpenoids and depsides occur in very low concentrations, which makes them difficult or even impossible to detect with TLC.

Most reports on secondary substances found in *Peltigera* include only terpenoids with known chemical structures. These terpenoids are not necessarily taxonomically informative. Not using a common system for terpenoid identification can prevent comparison among different reports. Therefore, it is possible that chemical patterns reported by different authors for *Peltigera* species (fig. 5) are only variations within already recognized chemotypes or might even represent the same chemotype. Because the chemical structure is known only for a few terpenoids (table 2), the positions of the terpenoids in the metabolic pathways and the pathways themselves are unknown. Therefore, it is often impossible to evaluate if the presence/absence of a particular terpenoid or differences in concentration within a species deserve recognition at the chemotypic level or are only a result of environmental conditions.

Variation in terpenoid pattern may also result from recombination in sexually reproducing species (all *Peltigera* species, except *P. frippii*, form apothecia) as well as from mechanical fusion of chemically and/or genetically different individuals (Jahns 1987; Rikkinen 1995). Presence of hybrids has previously been documented in lichens in general and suggested as possibly occurring in the genus *Peltigera* (Laundon 1978; Culberson et al. 1988; Letrouit-Galinou and Asta 1994; Goffinet and Hastings 1995). Some of the material examined by J. Miadlikowska showed atypical chemotypic patterns that could be regarded as intermediate between two chemotypes from the same or different species (fig. 10). Within section *Polydactylon*, specimens morphologically in agreement with the phenotype of *P. hymenina* possess a chemical pattern intermediate between chemotypes I of *P. hymenina* and *P. polydactylon* in terms of terpenoid concentration. A similar case was found in two specimens of *P. neopolydactyla* representing mixed chemotypes with a concentration of zeorin (15) appropriate for chemotype II and a concentration of peltidactylin (10) reflecting chemotype I. Although within *P. aphthosa*, *P. malacea*, and *P. neopolydactyla* some chemotypes are also ecologically and morphologically well defined and could be considered as separate "sibling" species (Holtan-Hartwig 1993; Martínez 1999), further molecular studies are needed before these chemotypes can be recognized as distinct species.

Miadlikowska (1999) and Goffinet and Miadlikowska (1999) suggested that the terpenoid profile for *P. collina* is more complex than was described in past publications (fig. 5). Martínez (1999) assumed that chemotype I of *P. collina* from the Iberian Peninsula is similar to the terpenoid composition found in populations of Great Britain (White and James 1987a). In her opinion, chemotype II of *P. collina* collected in the Iberian Peninsula is identical to the chemistry of this species reported from Norway by Holtan-Hartwig (1993). Both Iberian chemotypes contain dolichorrhizin (12), a terpenoid that was not found in the material from Norway or from Great Britain. The only other report of dolichorrhizin in *P. collina* thalli was from Canada, by Goward et al. (1995). Holtan-Hartwig (1993) reported zeorin (15) as the only terpenoid present in *P. collina* from Norway. He intentionally neglected the other terpenoids that were present in smaller concentrations (J. Holtan-Hartwig, personal communication). A re-

examination of specimens from Norway and Scotland revealed that two chemotypes of *P. collina* occur in Norway (chemotypes III and IV) and one in Scotland (chemotype III). None of the investigated specimens from these two countries contained dolichorrhizin. Therefore, we assume that the chemotype of *P. collina* reported from Canada (Goward et al. 1995) is conspecific with chemotype II from the Iberian Peninsula (Martínez 1999), while chemotype III from Norway is the same as reported by White and James (1987a) and Vitikainen (1994b), except for the presence of unidentified terpenoid 41. Terpenoid 41 is easily overlooked because it usually occurs in very small concentrations. Absence of peltidactylin and dolichorrhizin, as well as presence of zeorin as the only identified terpenoid, was detected in some populations of *P. collina* by B. Goffinet (Goffinet and Miadlikowska 1999). Whether this chemical pattern represents an additional chemotype (chemotype V) for *P. collina* needs further work. To have a better insight into the chemical variation of *P. collina*, further studies must be conducted. In this sorediate species, it is possible that heterothallic individuals have resulted from the mechanical fusion of soredia originating from chemically different populations (see Stocker-Wörgötter and Türk 1990, Goffinet and Hastings 1995).

#### *Relationships within the Outgroup*

The relationships described in this section are based on the assumption that *Pseudocyphellaria*, *Sticta*, and *Lobaria* are the most basal genera from our selection of taxa, as revealed in a broader preliminary study (result not shown). The genera *Solorina* and *Massalongia* represented on the trees by *S. crocea* and *M. carnososa*, respectively, are the closest relatives to *Peltigera* (including *P. hydrothyria*). Their relationship to *Peltigera* is well supported, with bootstrap values of 100% and 79%, respectively. *Solorina* has been traditionally considered as a closely related taxon to *Peltigera* and was often classified as a member of the family Peltigeraceae (Poelt 1974; Hawksworth et al. 1995; Tehler 1996). Other classifications have proposed the establishment of the monotypic family Solorinaceae (Eriksson 1981; Hafellner 1988; Hafellner et al. 1993). Eriksson and Winka's (1998) results based on the small subunit (SSU) nrDNA data do not justify a need for this new family. The taxonomic placement of *Massalongia* has been rather problematic. Galloway (1991), Eriksson and Hawksworth (1991), and Tehler (1996) placed this genus in the family Peltigeraceae together with *Solorina*, *Peltigera*, and *Hydrothyria* (Tehler also included *Siphulina* in this family). Hafellner et al. (1993) included *Massalongia* in the suborder Peltigerineae but within the artificial "group G," which included genera with a caplike apical ascus structure. Our study sup-

ports a close (paraphyletic) relationship between *Massalongia* and *Nephroma*. Nephromataceae, in all contemporary classifications, and based on SSU nrDNA (Eriksson and Winka 1998), is a monotypic family. The long internodes leading to *Solorina* and *Peltigera* (fig. 8) indicate that a high level of divergence occurred between *Massalongia* and *Solorina*, as well as between *Solorina* and *Peltigera* (including *P. hydrothyria*). Our taxon sampling for this study was not designed to address questions at the suprageneric level within the outgroup. Phylogenetic studies at the family and ordinal levels, including the suborder Peltigerineae, are in progress.

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