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Notes on the systematics, chemistry and distribution of European *Parmelia* and *Punctelia* species (lichenized ascomycetes)

Anmerkungen zur Systematik, Chemie und Verbreitung europäischer *Parmelia*- und *Punctelia*-Arten (lichenisierte Ascomyceten)

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Key words: *Parmelia, Punctelia*, systematics, chemistry, distribution, Europe.

Schlagwörter: *Parmelia, Punctelia*, Systematik, Chemie, Verbreitung, Europa.

Summary: Some European species of the genera *Parmelia* and *Punctelia* are analysed using molecular, morphological and chemical characters and notes on their distribution are presented. ITS sequences were compared from a total of 31 representative specimens. *Parmelia* ernstiae and *P. serrana* form a sister group to *P. pinnatifida* and *P. saxatilis*, whereas *P. discordans* and *P. omphalodes* are closely related and form a sister group to all former four species. Bootstrap support values from the PAUP and PHYLIP analyses are used to confirm relationships between *Parmelia* and *Punctelia* species. *Parmelia ernstiae, P. serrana, Punctelia jeckerii* and *P. subrudecta* are reported from additional countries and provinces. *Parmelia ernstiae* is reported from Bosnia-Herzegovina and Czech Republic for the first time, and *P. serrana* is new to Germany, Russia and Ukraine. The chemical compounds present in *Parmelia ernstiae, P. saxatilis* and *P. serrana* were analysed and compared using HPLC. These morphologically similar species are chemically different.

Zusammenfassung: Einige europäische Arten der Gattungen *Parmelia* und *Punctelia* werden systematisch und chemisch untersucht, zusätzliche Funde werden angegeben. ITS-Sequenzen von insgesamt 31 Belegen werden verglichen. *Parmelia ernstiae* und *P. serrana* bilden die Schwestergruppe von *P. pinnatifida* und *P. saxatilis*. Diese vier Arten bilden die Schwestergruppe von *P. discordans* und *P. omphalodes*, die nahe verwandt sind. Bootstrap-Werte von PAUP- und PHYLIP-Analysen werden verglichen um die Verwandschaft von *Parmelia*- und *Punctelia*-Arten zu klären. *Parmelia ernstiae, P. serrana, Punctelia jeckeri* und *P. subrudecta* werden von zusätzlichen Ländern und Bundesländern gemeldet. *Parmelia ernstiae* wird erstmals von Bosnien-Herzegowina und Tschechien angegeben, *P. serrana* ist neu für Deutschland, Russland und die Ukraine. Die sekundären Inhaltstoffe von *Parmelia ernstiae, P. saxatilis* und *P. serrana* wurden mit HPLC untersucht und verglichen. Diese morphologisch ähnlichen Arten sind chemisch unterschiedlich.

Introduction

Although *Parmelia* ACH. s. str. is one of the most frequently investigated and well-known genera, three new European species have been discovered in the past decade: *Parmelia barrenoae* DIVAKAR, M.C. MOLINA & A. CRESPO, a close relative of *P. sulcata* TAYLOR (DIVAKAR et al. 2005), and *P. ernstiae* FEUERER & A. THELL and *P serrana* A. CRESPO, M.C. MOLINA & D. HAWKSW., which were segregated from *P. saxatilis* (L.) ACH. (DIVAKAR et al. 2005; FEUERER & THELL 2002; MOLINA et al. 2004). Furthermore, according to a recent molecular study, it is clear that further species will be segregated from *P. saxatilis* (HEDSTRÖM 2006). Twelve of the ca. 60 known species of *Parmelia* occur in Europe, whereas approximately two-thirds of the species are restricted to Asia and Australasia (HALE 1987). Most likely, the European species belong to a single monophyletic group. *Parmelia* is typically a boreal-temperate genus in Europe.

The phylogenetic status of the entire genus *Parmelia* is still poorly known. The three new species mentioned above are known exclusively from Europe, except for *P. serrana,* which also occurs on the Canary Islands. Previous molecular studies have supported three separate species within the *P. omphalodes* (L.) ACH. group, namely *P omphalodes, P. pinnatifida* KUROK. and *P skultii* HALE (MOLINA et al. 2004; THELL et al. 2004). The chemically distinct fourth species, *P. discordans* NYL., has not previously been investigated by molecular methods.

There are six representatives of the genus *Punctelia* KROG in Europe, a minor part of its entire diversity [ca. 30 species], most of which occur in Africa and South America (THELL et al. 2005). The genus was segregated from *Parmelia* because of the development of punctiform pseudocyphellae and differences in chemistry and conidial characters (KROG 1982). *Punctelia* has a temperate-subtropical distribution in Europe. The distribution of three species extends into the Nordic countries (SANTESSON et al. 2004). New localities are reported for Denmark and southernmost Sweden. The extended distribution is perhaps due to

the warmer climatic conditions (CHRISTENSEN & SØCHTING 2007; MALMQVIST pers. comm.).

Punctelia is most probably a monophyletic genus (THELL et al. 2005). *Parmelia* and *Punctelia* appeared as sister groups in an earlier phylogenetic analysis of the family Parmeliaceae (THELL et al. 2004). This present study is a further contribution to an understanding of the systematics of the *Parmelia* and *Punctelia* species in Europe, and is complemented by new data on their relationships, chemistry and distribution.

Material and Methods

Selection of specimens

The material, mainly collected by the authors in recent years for the purpose of molecular analyses, produced 14 new sequences. Seventeen sequences were downloaded from GenBank, www.ncbi.nlm.nih.gov. Additional herbarium material was selected for morphological and chemical studies (Table 1).

DNA phylogeny analyes

Extraction and amplification: Genomic DNA of the specimens studied was extracted employing MagAttract 96 DNA Plant Extraction Kit from Qiagen (08/2003). The samples were vigorously shaken in a vortex machine in 300 ml extraction buffer incorporating one steel bead in each microtube; otherwise, the manufacturer's protocol for manual DNA purification was followed. The nuclear ITS1-5.8S-ITS2 ribosomal DNA region was amplified, using the primers ITS1F (GARDES & BRUNS 1993) and ITS4 (WHITE 1990), and standard methods described by THELL et al. (2007b).

Phylogeny: The phylogenetic analyses of the manually aligned ITS sequences were done with PAUP version 4.0b (SWOFFORD 1998). Trees were calculated using the general heuristic search option, saving 100 trees. Gaps were treated as missing characters. Bootstrap analyses with 1000 replicates were performed using the same settings.

In addition to the examination of the sequences with PAUP, the data were subjected to bootstrap analysis using programs from the PHYLIP package (FELSENSTEIN 2005). 100 bootstrapped data sets where created with "seqboot", whereafter the most parsimonious trees for each data set were computed using "dnapars" The consensus tree (Fig. 1) was built using the "consensus" program. Support values of 50 or above, obtained from both methods, are marked and compared in a combined consensus tree (Fig. 1).

Finally, the data set was examined using the Two-Tree-Method as presented by SCHÜLER (1998), based on an approach used by CAROLL & PRUZANSKY (1980). It turned out that the first dimension resulted in a tree very similar to the

trees obtained using parsimony or neighbour joining, whereas the tree for the second dimension showed no significant phylogenetic signals.

Table 1: Material used in the study.

Species	DNA-number	Specimen-voucher	GenBank Accession
Flavopunctelia borrerioides	1521	China, Yunnan, 2002-10-29, VAN HERK (ABL)	AY773129*
Flavopunctelia flaventior	1285	Germany, Bayern, Dachau, 2002-03-31, FEUERER (HBG)	AF251420*
Parmelia discordans	–	United Kingdom, Scotland, Hawksworth (MAF-Lich 10232)	AY583212*
Parmelia ernstiae	1929	Bosnia-Herzegovina, Sarajevo, Weckesser (GOET)	EF611286
Parmelia ernstiae	1697	Czech Republic, W. Bohemia, V.D. BOOM (herb. V.D. BOOM)	EF611289
Parmelia ernstiae	1923	Denmark, Bornholm, Olsker, 2005-10-31, THELL (LD-1003288)	EF406113*
Parmelia ernstiae	–	Denmark, N. Jutland, Vindblæs S of Hadsund, saxicolous!, 1999, SØCHTING (C); THELL (LD-1274821)	
Parmelia ernstiae	1924	Denmark, S. Jutland, Agerskov, 2005-07-01, THELL (LD-1062891)	EF406114*
Parmelia ernstiae	1939	Estonia, Hiiu Co., 2005-07-06, SUIJA & NÖMM 748 (TU-33948)	EF611291
Parmelia ernstiae	–	Estonia, Pärnu Co., 1999-07-29, JÜRIADO 265/7 (TU-33949)	
Parmelia ernstiae	–	France, Bretagne, 1949-06-16, ALMBORN (LD-1151119)	
Parmelia ernstiae	1299	Germany, Brandenburg, Prignitz, 2002-06-15, SCHULTZ 02127 (herb. SCHULTZ)	
Parmelia ernstiae	836	Germany, Niedersachsen, Soltau-Fallingbostel, 2000-04-14, ERNST (HBG)	AF410833*
Parmelia ernstiae	858	Germany, Schleswig-Holstein, Großsolt, 2001-01-15, FEUERER & THELL (HBG)	AF410834*
Parmelia ernstiae	1928	Germany, Saarland, Mallert, 2005-08-26, FEUERER (HBG)	EF611292
Parmelia ernstiae	–	Luxembourg, Lorrain, 2003-05-10, DIEDERICH 15601; 8621 (LD-1067698)	
Parmelia ernstiae	–	The Netherlands, Gelderland, 1941-10-24, MAAS GEESTERANUS 1581 (LD-1123383)	
Parmelia ernstiae	–	Sweden, Gotland, 2003-05-24, ARUP L03103 (LD-1103325)	
Parmelia ernstiae	–	Sweden, Dalsland, 2006-05-25, WESTBERG 06-004 (LD-1172315)	
Parmelia ernstiae	865	Sweden, Skåne, Eslöv, 2003-03-01, THELL 0101 (LD-1026466)	AY247007*
Parmelia ernstiae	1930	Sweden, Skåne, Norra Rörum, 2005-10-28, ARUP L05080 (LD-1256223)	EF421713*
Parmelia ernstiae	–	Sweden, Skåne, Tåssjö, 2006-08-09, ARUP	

		06038 (LD-2006-08-29)	
Parmelia omphalodes	1028	Finland, Varsinais-Suomi, Turku, 2001-10-15, THELL 01101 (TUR)	AY251440*
Parmelia omphalodes	2240	United Kingdom, Scotland, 2005-05-23, FEUERER (HBG)	EF611295
Parmelia pinnatifida	985	Austria, Tyrol, 2001-08-08, FEUERER & THELL 64609 (HBG)	EF611300
Parmelia pinnatifida	–	Russia, Murmansk obl., Kola Peninsula (MAF-7274)	AY036987*
Parmelia saxatilis	518	Chile, Region XII, Peine, FEUERER 29542 (TUR)	AF410672*
Parmelia saxatilis	–	Denmark, N. Jutland, 1999-08-26, SØCHTING (C)	
Parmelia saxatilis	534	Finland, Varsinais-Suomi, 1999-08-23, THELL 9926 (TUR)	AF410835*
Parmelia saxatilis	–	Sweden, Skåne, Örkened, 2000-09-09, THELL SK0010 (LD-1024482)	
Parmelia serrana	914	Germany, Bayern, München, FEUERER (HBG)	EF611297
Parmelia serrana	915	Germany, Bayern, München, FEUERER (HBG)	EF611296
Parmelia serrana	2213	Germany, Bayern, München, FEUERER (HBG)	EF611298
Parmelia serrana	2225	Germany, Bayern, München, FEUERER (HBG)	EF611293
Parmelia serrana	2242	Germany, Bayern, München, FEUERER (HBG)	EF611299
Parmelia serrana	1327	Russia, Adygei, OTTE L2742 (herb. OTTE)	EF611294
Parmelia serrana	–	Ukraine, Tauria, Distr. Aluschta, 1955-08-25, E. KOPACZEWSKAJA (LD-1123323)	
Parmelia serrana	–	Spain, Prov. Madrid, 2005-10-08, THELL SP0509, FEUERER & HAWKSWORTH (LD-1052249)	
Parmelia sulcata	517	Sweden, Skåne, 1999-07-27, THELL & MARTH 9920 (TUR)	AF410840*
Punctelia borreri	945	Italy, South Tyrol, 2001-05-15, FEUERER & THELL (HBG)	AY773113*
Punctelia jeckerii	1507	Belgium, Liege, 2003-05-01, APTROOT 57873 (ABL)	AY773121*
Punctelia jeckerii	1979	Sweden, Skåne, Lund, 2007-01-03, ARUP et al. (LD-1188067)	EF611288
Punctelia subrudecta	944	Germany, Schleswig-Holstein, Frörup, 2001-01-15, FEUERER & THELL (HBG)	AY773116*
Punctelia subrudecta	1978	Sweden, Skåne, Lund, 2005-07-21, WESTBERG (LD-1193242)	EF611287

*Sequences downloaded from GenBank, http://www.ncbi.nlm.nih.gov.
**DNA-numbers refer to the collections of the working group stored in LD and HBG.

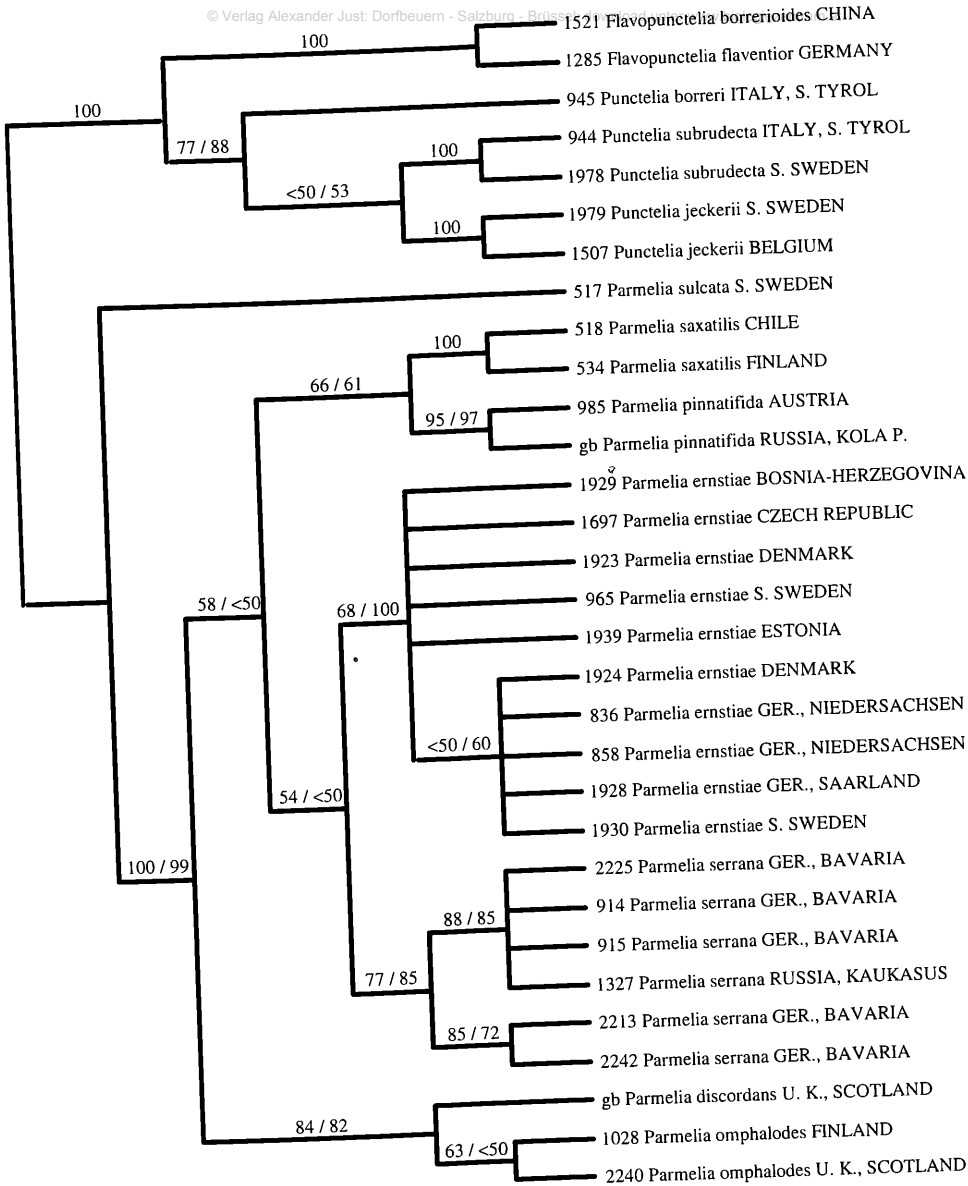

Fig. 1: Consensus tree from parsimony analysis showing estimated relationships of some European species of *Parmelia* and *Punctelia* inferred from ITS-sequences. Bootstrap support values are indicated above the branches. If different, those resulting from the PAUP-analysis are indicated to the left and those from the PHYLIP-analysis to the right.

Chemistry

Secondary lichen compounds were detected by means of high performance liquid chromatography (HPLC) and thin-layer chromatography (ELIX et al. 2003).

Results

ITS matrix and phylogenetic analysis

Thirty-one terminal taxa were examined. The final matrix consisted of 515 aligned positions, including gaps, of which 99 positions were parsimony informative. Gaps were treated as missing characters (tree length=208, CI=0.7692, RI=0.8763). The ITS region of the separate sequences measured between 479 to 503 nucleotides. These differences are explained by small insertions or deletions in some of the sequences. One small insertion unique for *P. sulcata* was removed prior to the alignment.

On examination of the data using the Two-Tree-Method, the first dimension resulted in a tree very similar to the trees obtained using parsimony or neighbour joining, whereas the tree of the second dimension showed no significant phylogenetic signals. As the data set is not too big for this method, an alternative history, for example a hybridization event, presumably never happened in the phylogeny of this group.

Monophyletic groups

The support values of the branches in the phylogenetic tree obtained from PAUP and PHYLIP are compared. The support values resulting from the PHYLIP analysis are generally higher (Fig. 1), although support levels above the species level are generally low within *Parmelia*. The genera *Flavopunctelia* and *Parmelia* are distinguished by high support values. The species of *Parmelia* are divided in two groups, 1. the single species *P. sulcata*, which is separated from 2., the *P. saxatilis* group, by a 100/99 % bootstrap value. The *P. saxatilis* group is in turn divided into three sister groups, all by moderate or weak bootstrap values, except for *P. ernstiae*, which is supported by 100 % in the PHYLIP-analysis. The group comprising *P. discordans* and *P. omphalodes* received higher support than each of the two separate species of the group, whereas the opposite is the case for the other sister groups in *Parmelia* (Fig. 1). Except for *P. omphalodes*, the PHYLIP-analysis gives stronger support for accepted species and genera, compared with the PAUP-analysis, but lower support values for species groups within genera.

Table 2: Chemistry of the investigated species detected by HPLC complemented by literature data.

compound	Pa di	Pa er	Pa om	Pa pi	Pa sa	Pa se	Pu je	Pu su
atranorin	+	+	+	+	+	+	+	+
chloroatranorin	–	+	–	–	+	+	–	–
salazinic acid	–	++	+	+	++	++	–	–
consalazinic acid	–	+	+	+	+	+	–	–
galbinic acid	+	–	(+)	(+)	–	–	–	–
lecanoric acid	–	–	–	–	–	–	+	+
lobaric acid	+	+	+	–	+	–	–	–
lichesterinic acid	–	+	–	–	–	+	–	–
protolichesterinic acid	–	+	+	+	–	++	–	–
nephrosterinic acid	–	+	–	–	–	+	–	–
isonephrosterinic acid	–	+	–	–	–	++	–	–
fumarprotocetraric acid	+	–	(+) / –	(+) / –	–	–	–	–
protocetraric acid	+	(+)	(+) / –	(+) / –	(+)	– / (+)	–	–

++ = major, + = minor, (+) = trace, – = absent. *Parmelia ernstiae*, *P. saxatilis* and *P. serrana* were HPLC-analyzed within the present study. Secondary compounds of the other species were detected by TLC (HALE 1987; VAN HERK & APTROOT 2000).

Chemical analysis

The chemistry of three morphologically similar species, *Parmelia ernstiae*, *P. saxatilis* and *P. serrana*, was compared (Table 2). The chemistry of *P. ernstiae* was analyzed for the first time. All four specimens of *P. ernstiae* (Denmark, LD-1003288, LD-1062891, 1999-08-26 Søchting (C) and Sweden, LD-1026466) contained the same chemical constituents: atranorin (minor), chloroatranorin (minor), salazinic acid (major), consalazinic acid (minor), lobaric acid (minor), lichesterinic acid (minor), protolichesterinic acid (minor), nephrosterinic acid (minor), isonephrosterinic acid (minor) and traces of protocetraric acid. A single specimen of *P. serrana* from Spain (LD-1052249) exhibited very similar chemistry except for the absence of lobaric acid (Table 2). For comparison, a typical *P. saxatilis* from southernmost Sweden (LD-1024482) was investigated. It was found to differ from both *P. ernstiae* and *P. serrana* by the absence of the fatty acids, protolichesterinic, lichesterinic, nephrosterinic and isonephrosterinic acids (Table 2).

Distribution

The above phylogenetic analysis and new data on the chemistry in combination with morphological studies resulted in a better understanding of the distribution of several of the species investigated. *Parmelia ernstiae* is reported for the first time from Bosnia-Herzegovina, the Czech Republic and several new provinces of Germany and Sweden, while *P. serrana* is new to Germany, Russia and Ukraine. Several interesting, previously published localities, have been confirmed by molecular or chemical data: *Parmelia ernstiae* in Estonia, and *Punctelia jeckerii* (ROUM.) KALB and *P. subrudecta* (NYL.) KROG from southernmost Sweden (Table 1). Results on distribution of lichens are continuously collected by FEUERER (2007) at http://www. checklists.de

Discussion

The phylogenetic species concept

The phylogenetic species concept, based on molecular characters, has led to the recognition of cryptic species, i. e., morphologcally identical but genetically different species (GRUBE & KROKEN 2000). The discovery of cryptic species sometimes leads to the discovery of new distinguishing morphological or chemical characters. The seven species discussed below are more or less difficult to distinguish from their closest relatives by morphology alone. However, their characteristic chemistry or DNA-sequence, most commonly an ITS sequence, may assist in confirming their identity. Of particular interest was the fact that *Parmelia discordans* hardly was distinguished phylogenetically from *P. omphalodes*, whereas all seven species are chemically distinct (Fig. 1; Table 2).

Notes on the species

Parmelia discordans

Parmelia discordans appears to be a chemical species, although most specimens are also morphologically distinct. In the past it has frequently been treated as a variety of *P. omphalodes*. *P. discordans* is generally smaller and has narrower, more overlapping lobes when compared with *P. omphalodes*. The colour is more uniformly dark, and the pseudocyphellae are indistinct. However, this morphology overlaps with that of the highly variable *P. omphalodes* (HALE 1987: 23).

The DNA-analysis of the single sample of *P. discordans* did not distinguish it from *P. omphalodes* (Fig. 1). The status of *P. discordans* as a separate species therefore depends on which species concept is used, at least until a more intensive phylogenetic analysis is undertaken.

Parmelia ernstiae

This species is distinguished from *P. saxatilis* by the pruinose upper surface. The thallus is usually composed of small rounded, non-overlapping lobes and is often lobulate, particularly in central parts, where the lobules are intermixed with the isidia (FEUERER & THELL 2002). Subsequently described and similarly pruinose *P. serrana* differs from *P. ernstiae* by the large rounded and commonly overlapping lobes (MOLINA et al. 2004). *P. ernstiae* is a corticolous species in the nemoral-atlantic part of Europe from Spain to Estonia (FEUERER & THELL 2002; VAN HERK & APTROOT 2004; MOLINA et al. 2004; SÉRUSIAUX et al. 2003; SØCHTING et al. 2007; SUIJA et al. 2007; THELL 2003; THELL et al. 2007a). In addition to the morphological characters, all of which overlap with *P. saxatilis* to some extent, FEUERER & THELL (2002) demonstrated several characters in the ITS-region of the rDNA which differ from the latter species.

New data on their respective chemical products assist in distinguishing the three species. *P. ernstiae* exhibits the largest set of secondary compounds. *P. saxatilis* lacks the fatty acids, lichesterinic, protolichesterinic, nephrosterinic and isonephrosterinic acids observed in the other two species. No lobaric and protocetraric acids were detected in *P. serrana* in our investigation, but MOLINA et al. (2004: 48) previously reported traces of protocetraric acid for this species. New localities in Bosnia-Herzegovina, the Czech Republic and eastern Germany extend the distribution of this species eastwards.

One saxicolous specimen of *P. ernstiae* from Denmark (SØCHTING 1999-08-26 p. p. [C]), of this otherwise corticolous species, was chemically analyzed and its contents agreed with *P. ernstiae.* This material was collected before *P. ernstiae* was described (FEUERER & THELL 2002), and is still the single known epilithic occurrence. The locality was revisited in July 2007 and, notably, the species was found to be frequent on granite stones of the stone fence surrounding the church yard in Vindblæs parish south of Hadsund in eastern Jutland (LD-1274821).

Parmelia pinnatifida

HALE (1987) did not recognize *P. pinnatifida* as a separate species because of the overlapping morphological characters, particularly between North American populations of *P. pinnatifida* and *P. omphalodes.* However, *P. omphalodes* and *P. pinnatifida* were shown to be phylogenetically distinct following DNA-analyses based on ITS and β-tubulin sequences (MOLINA et al. (2004). The latter authors highlighted three distinguishing characters for *P. pinnatifida:* the narrower lobes, marginal pseudocyphellae, and the absence of lobaric acid.

Our phylogenetic analysis supports that performed by MOLINA et al. (2004): in Europe *P. pinnatifida* is well-distinguished from *P. omphalodes.* However, further work would be needed to establish the status of the North American material.

Parmelia saxatilis

P. saxatilis s. str. is a morphologically rather variable, subcosmopolitian species with no phylogeographic divergences (THELL et al. 2004). Two species have been segregated from *P. saxatilis* in recent times, *P. ernstiae* and *P. serrana*, and further species are expected to be segregated following recent molecular analyses (HEDSTRÖM 2007). *P. saxatilis* s. str. is still not strictly delimited, so all previously published data on its secondary products may include other species.

Chemical constituents for two specimens (LD-1024482 and C: SØCHTING 1999-08-26 p. p.), were investigated for comparison with the segregates *P. ernstiae* and *P. serrana*. Atranorin, chloroatranorin, salazinic acid, consalazinic acid, lobaric acid and protocetraric acid were detected in both specimens. The presence of lobaric acid and the absence of lichesterinic, protolichesterinic, nephrosterinic and isonephrosterinic acids distinguishes *P. saxatilis* from *P. serrana* and *P. ernstiae* respectively (Table 2).

Parmelia serrana

P. serrana is characterized by its broad, rounded and often overlapping lobes. It is less adnate to the substratum and is paler coloured than *P. saxatilis*. *P. ernstiae* has a similar colour but usually produces small lobules and the lobes are rarely overlapping and narrower. The distribution of *P. serrana* remains to be clarified. Compared with the atlantic-nemoral *P. ernstiae*, it seems to prefer more mountainous and continental areas. However, it has been collected in quite dissimilar localities, from saxicolous as well as corticolous substrata in the Canary Islands, to Spain and Austria and semi-boreal parts of Sweden (MOLINA et al. 2004, MATTSSON et al. 2006). It has a broader distribution than *P. ernstiae*, as the latter is not known from the Canary Islands.

New localities in Bavaria, Russia, and Ukraine are reported here, thus strengthening the hypothesis that *P. serrana* prefers more continental habitats. Except for the absence of lobaric acid, the secondary compounds in this species are the same as in *P. ernstiae* (Table 2; MOLINA et al. 2004: 48). According to this and earlier phylogeny analyses, P. serrana is clearly distinguished from *P. ernstiae*, *P. saxatilis* as well as other Parmelia species.

Punctelia jeckerii

P. jeckerii was raised to species level as *P. ulophylla* by VAN HERK & APTROOT (2000), a name based on *Parmelia caperata* var. *ulophylla* (ACHARIUS 1810). However, KALB (2007) discovered an older species name, *Sticta jeckerii* ROUM., and therefore proposed the new combination *P. jeckerii* (ROUM.) KALB. Until this species was resurrected by van HERK & APTROOT (2000), it had been treated as a synonym of *P. subrudecta* for many years and as a consequence its distribution is still poorly known; SANTESSON et al. (2004) retained this species. An old specimen of *P. subrudecta* from Uppland in southern-central Sweden has now been

shown to be *P. jeckerii*. *P. subrudecta*, and thereby *P. jeckerii*, were listed as being extinct in Sweden. However, both species were recently collected in Lund and confirmed within this study by their respective ITS-sequences. The two species differ in several morphological characters. *P. jeckerii* has a thicker thallus, its upper surface is more greenish and shiny in the centre, whereas the margins are pruinose (VAN HERK & APTROOT 2000). A phylogenetic analysis based on ITS-sequences shows that the two species are not very closely related (THELL et al. 2005). *P. jeckerii* is probably common in southern and central Europe but rare in the north, where there are a few records from Denmark and western Norway, in addition to the new Swedish locality (CHRISTENSEN & SØCHTING 2007; GAUSLAA 2000).

Punctelia subrudecta

This is a common species in southern and central Europe and its distribution extends to the Baltic Sea; it is also reported from Denmark and westernmost Norway (CHRISTENSEN & SØCHTING 2007; GAUSLAA 2000). The newly discovered Swedish locality for *P. subrudecta* is the first, following the redetermination of the old specimen as *P. jeckerii* (MALMQVIST pers. comm.). The corticolous habit and distribution are very similar to that of *P. jeckerii*. Although the distribution of the latter is still poorly known, *P. subrudecta* seems to be more common.

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