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***Parmelia ernstiae*, *P. serrana* and *P. submontana*, three species increasing in the Nordic countries**

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Revised information on the morphology, chemistry and distribution of species of *Parmelia* s. str. in Fennoscandia is presented. Although *Parmelia ernstiae* was earlier believed to be chemically distinct from *P. serrana* in terms of lobaric acid, its presence in all studied samples detected by TLC means that these two semi-cryptic species can no longer be chemically separated. All three species are increasing in frequency and spreading northwards in Fennoscandia. *Parmelia serrana* is reported as new to Zealand in Denmark.

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Introduction

Parmelia Ach., is one of the most well-known of Nordic lichen genera. Its thallus is foliose, loosely to rather closely attached with a grey or brownish upper and blackish lower side. After the separation of the genera *Nipponoparmelia* (Kurok.) K. H. Moon, Y. Ohmura & Kashiw. and *Notoparmelia* A. Crespo, Ferencova & Divakar (Crespo *et al.* 2010, Ferencova *et al.* 2014), *Parmelia* is a genus of c. 40 species worldwide, mainly occurring in temperate-boreal areas of the Northern Hemisphere, nine of which occur in the Nordic countries (Thell *et al.* 2011). The genus can be divided into three morphological groups according to their vegetative propagules: (1) the *Parmelia sulcata* group having soredia or isidiate soredia (*P. sulcata* Taylor, *P. submontana* Nadv. ex Hale and *P. fraudans* (Nyl.) Nyl.) (2) the *Parmelia saxatilis* group, characterized by isidia (*P.*

saxatilis Ach., *P. ernstiae* Feuerer & A. Thell, and *P. serrana* A. Crespo, M. C. Molina & D. Hawskw.), and (3) the *P. omphalodes* group, without propagules (*P. omphalodes* (L.) Ach., *P. pinnatifida* Kurok. and *P. skultii* Hale).

Several species of *Parmelia* s. str. can be categorized as semi-cryptic, their distinguishing characters overlapping with other species, such as *Parmelia ernstiae* and *P. serrana*, which may be difficult to distinguish from each other and from *P. saxatilis* (Molina *et al.* 2004). Poorly developed or young specimens of *P. submontana* can be confused with *P. sulcata*, while *P. pinnatifida* may overlap morphologically with *P. omphalodes*. However, their species status is supported by phylogenetic analyses based on DNA-sequences (Thell *et al.* 2008). Two cryptic species were recently described in *Parmelia*, neither of which occur in the Nordic area: (1) *P. mayi* Divakar, A. Crespo & M. C. Molina, found at high altitudes in the Appalachian Mountains in North America, which differs from *P. saxatilis* by the presence of aliphatic acids (Molina *et al.* 2011a), and (2) *P. encryptata* A. Crespo, Divakar & M. C. Molina, found in Spain and Ireland, which has been segregated from *P. sulcata* (Molina *et al.* 2011b). The two species are otherwise identical, both in morphology and chemistry, according to current knowledge, but differ in their DNA-sequences. However, the discovery of cryptic species may in some cases lead to the discovery of earlier unknown distinguishing characters. Two additional species have been described very recently, earlier considered morphotypes of *P. saxatilis*, namely *P. sulymae* Goward, Divakar, M. C. Molina & A. Crespo and *P. imbricaria* Goward, Divakar, M. C. Molina & A. Crespo, that were revealed as independent monophyletic clades (Molina *et al.* 2016). The *P. sulcata* segregate, *P. barrenoae* Divakar, M. C. Molina & A. Crespo, was recently reported as new to Poland as a result of revision of herbarium material filed as *P. sulcata* (Ossowska & Kukwa 2016). It is possible that this semi-cryptic species, originally described from Spain but later found also in North America and Africa (Hodkinson *et al.* 2010), also occur in Scandinavia. *P. barrenoae* differs from *P. sulcata* by its simple rhizines, broader and apically rounded lobes which overlap (Divakar *et al.* 2005), and the pseudocyphellae are less abundant but develop into very distinct soralia which quickly release soredia.

This study is a contribution to our knowledge of three *Parmelia* species (*P. ernstiae*, *P. serrana* and *P. submontana*) with a more or less southern distribution in the Nordic area. New data on their secondary chemistry, distribution and morphology are provided, some of the specimens being confirmed by nuclear ITS rDNA-sequences.

Material and methods

Material used in the chemical and molecular analyses is listed in Table 1. Chemical compounds were detected by thin-layer chromatography (TLC) in solvents A, C and G according to methods described by Orange *et al.* (2001). ITS-sequences were amplified with the primers ITS5 and ITS4 (White *et al.* 1990), and extraction of total DNA and amplification of the ITS1-5.8S-ITS2 ribosomal DNA-region followed standard methods (*e.g.* Thell *et al.* 2008). BLAST searches were performed for the sequences (Altschul *et al.* 1990) which were aligned and compared with sequences from the GenBank (<http://www.ncbi.nlm.nih.gov>).

The species

Parmelia ernstiae Feuerer & A. Thell
Mitt. Inst. Allg. Bot. Hamb. 30–32: 52 (2002).

Parmelia ernstiae was described in 2002 and discovered in the Nordic area for the first time at Skåne in southernmost Sweden in 2003 (Feuerer & Thell 2002, Thell 2003). Since then it has been mapped for Denmark where it is relatively common in all regions except for western Jutland (Thell *et al.* 2007); it has also been collected in southernmost Finland, but not as yet observed in Norway. Except for young specimens and poorly developed thalli, the species is usually easily recognized by its pruinose lobes and many lobules. It cannot be distinguished chemically using TLC from its closest relative, *P. serrana*, as earlier believed, since lobaric acid was revealed also in the latter species (Table 1). The best distinguishing character is the clustering of the isidia (Fig. 3). *P. ernstiae* is increasing in southern Scandinavia and is currently the dominating epiphyte in some beech forests in Skåne (Figs. 1–2).

Chemistry. Cortex C–, K+ yellow, KC–, PD–; atranorin and chloroatranorin. Medulla C–, K+ red, KC–, PD+ orange; consalazinic, salazinic, lobaric, lichesterinic and protolichesterinic acids detected by TLC.

Distribution. Widespread in Denmark, including Bornholm, southern Finland (Regio aboënsis, Nylandia and Tavastia australis), southwestern Sweden (Skåne, Blekinge, Småland, Halland, Bohuslän, Dalsland and Västergötland), as well as Gotland, Uppland and Dalarna; unknown in Norway (<http://herbarium.emg.umu.se/databases.html>).



Figure 1. *Parmelia ernstiae* as the dominating epiphyte on a beech tree in Norra Rörum parish in central Skåne, the southernmost province of Sweden. Photo: Arne Thell 2016-07-02



Figure 2. *Parmelia ernstiae* (central thalli, with *P. serrana* in upper right-hand corner and *P. sulcata* in lower left-hand corner) competing for space on a maple tree in Billinge churchyard, central Skåne. Photo: Nataliya Thell 2016-06-09

Parmelia serrana A. Crespo *et al.*
Lichenologist 36: 48–49 (2004).

Although morphologically very variable, *P. serrana* is usually recognizable by the position of the isidia, being marginal or clustered as spots or along ridges (Fig. 3, Thell *et al.* 2011, Thell *et al.* 2014: 24). It was reported from Sweden later than *P. ernstiae* (Mattsson *et al.* 2006), and only recently in Denmark (Alstrup *et al.* 2013). *Parmelia serrana* has been searched for at many places in Denmark but only found twice, on both occasions in planted, coastal scrub forests dominated by *Pinus mugo* Turra. A sample from Omberg in Västra Tollstad parish, Östergötland, published by Thell *et al.* (2014) was rejected on the basis of its chemical characters (Motiejūnaitė *et al.*



Figure 3. *Parmelia serrana* on Mountain Pine, *Pinus mugo*, at the coast of northern Zealand, Denmark, x 6. Photo: Nataliya Thell 2016-08-27

2016), but according to this study, both the morphology, *i.e.* clustered and marginal isidia, and an ITS analysis, it corresponds with *P. serrana*. However, the discovery of lobaric acid in *P. serrana* makes it chemically indistinguishable from *P. ernstiae* when present. Preliminary studies show that the chemistry of *P. serrana* is more complicated than earlier believed.

Chemistry. Cortex C–, K+ yellow, KC–, PD–; atranorin and chloroatranorin. Medulla C–, K+ red, KC–, PD+ orange; consalazinic, salazinic, [usually] lobaric, lichesterinic and protolichesterinic acids detected by TLC.

Distribution. Rømø and North Zealand in Denmark (Fig. 4), southern Finland (Alandia, Regio aboënsis, Nylandia and Tavastia australis), Nord-Trøndelag in Norway, and southwestern Sweden (Skåne, Halland and Bohuslän) and Östergötland. (<http://herbarium.emg.umu.se/databases.html>)



Figure 4. *Parmelia serrana*, distribution in Denmark. The locality at Rømø was published in 2013 (Alstrup *et al.* 2013), whereas the locality on northern Zealand is new.

***Parmelia submontana* Nád. ex Hale**

Smithsonian Contr. Bot. 66: 44 (1987).

Parmelia submontana, formally described in a monograph of the genus (Hale 1987), was originally believed to have a southern distribution in Europe, but was discovered in Sweden 1989. In the 1990s it was also discovered in Denmark and Norway (Arvidsson 1989, Christensen 1997, Gauslaa 1999). Although the species has been overlooked, it is undoubtedly increasing in southern Scandinavia (Thell *et al.* 2011). It may be mixed with *P. sulcata* as young and poorly developed thalli, but when well developed, it has characteristic long lobes with down-rolled margins and isidia-like soredia, reminiscent of *Pseudevernia furfuracea* (L.) Zopf (Fig. 6). According to ITS-sequences, *Parmelia submontana* is more closely related to the *P. saxatilis* group than the *P. sulcata* group, whereas the secondary chemistry, presence of atranorin, salazinic and consalazinic acid, is identical with *P. sulcata* (Table 1).

Chemistry. Cortex C–, K+ yellow, KC–; atranorin. Medulla C–, K+ red, KC–, PD+ orange; salazinic and consalazinic acids detected by TLC.

Distribution. Southwestern Scandinavia: provinces of Skåne, Halland and Västergötland in Sweden, northern and eastern Jutland in Denmark, and Aust-Agder and West Agder in Norway (Christensen 1997, Alstrup *et al.* 2004, Thell *et al.* 2011, <http://nhm2.uio.no/lav/web/index.html>).

Concluding remarks

The three species studied were accorded species status in recent years due to their semi-cryptic habits, *Parmelia ernstiae* and *P. serrana* after confirmation by phylogenetic analyses based on

DNA-data. However, *P. ernstiae* and *P. serrana* are not chemically distinguishable in Fennoscandia as earlier believed (Thell *et al.* 2008). All three studied species are increasing in frequency and spreading northwards in Fennoscandia. Both morphology and BLAST searches of the ITS-sequences revealed which species the specimens belonged to, with one exception: the BLAST search of one *P. sulcata* specimen (KX961402) provided a better match with *P. encryptata*, but the species specific part of the sequence, ACCCGT, found at c. position 120 (Molina *et al.* 2011: 593), ultimately determined it as *Parmelia sulcata*.



Figure 5. An unusually rich locality for *P. submontana*. Sweden, Skåne, Eslöv, Kastberga. Photo: Nataliya Thell 2016-10-24.



Figure 6. Close up, showing interspersed *P. sulcata*. Photo: Nataliya Thell 2016-10-24.

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Table 1. Investigated specimens.

Species	Specimen voucher	GenBank-ID	Chemistry (TLC)
<i>P. ernstiae</i>	Sweden. Skåne Prov. Stehag par., Värlinge. 55°53'33"N, 13°22'52"E. On <i>Sorbus aucuparia</i> . 09.06.2016, A. Thell & E. Åsegård 16-005 (LD1884191)	–	consalazinic, salazinic, lobaric, protolicheterinic and licheterinic acids, atranorin
<i>P. ernstiae</i> and <i>P. saxatilis</i> – mixed sample	Denmark. Eastern Jutland. South of Hadsund, Vindblæs parish, on granite stones of the stone fence surrounding churchyard. 56°40'25.2"N, 10°8'38.0"E. 20.08.2015, A. Thell DK1501 (LD1760880)	–	<i>P. ernstiae</i> : consalazinic, salazinic, lobaric, protolicheterinic and licheterinic acids, atranorin <i>P. saxatilis</i> : consalazinic, salazinic and lobaric acids, atranorin
<i>P. ernstiae</i> and <i>P. serrana</i> – mixed sample	Sweden. Skåne Prov. Billinge par., 55°57'32.5"N, 13°20'8.9"E. On <i>Acer platanoides</i> in SE corner of churchyard. 01.06.2016, A. Thell & E. Åsegård 16-004 (GSU)	KX961401	both species: consalazinic, salazinic, lobaric, protolicheterinic and licheterinic acids, atranorin
<i>P. saxatilis</i>	Sweden. Skåne Prov. Örkened par., Tulla-Pellas, at the base of N side of a large <i>Fagus sylvatica</i> , N of barn. With apothecia! 01.11.2016, A. Thell 16-009 (LD1894843).	KY419142	consalazinic, salazinic, and lobaric acids, atranorin
<i>P. serrana</i>	Denmark. Jutland. Rømø. E of Bolilmark, on <i>Pinus mugo</i> . 55°9'16"N, 8°32'55.5"E, 05.04.2014, A. Thell 1201 (LD1575208)	–	consalazinic, salazinic, lobaric, protolicheterinic and licheterinic acids, atranorin
<i>P. serrana</i>	Denmark. Zealand. Grib Skov Distr. Tisvildeleje, northernmost part of Tisvilde Hegn forest, c. 50 m south of parking area, 3 m E of coastal path, on <i>Pinus mugo</i> 60 m from the beach. 56.0545°N, 12.0569°E. 27.08.2016, A. Thell 16-007 (LD1899143)	–	consalazinic, salazinic, lobaric, protolicheterinic and licheterinic acids, atranorin
<i>P. serrana</i>	Denmark. Zealand. Grib Skov Distr. Tisvildeleje, northernmost part of Tisvilde Hegn forest, c. 50 m south of parking area, 3 m E of coastal path, on <i>Pinus mugo</i> 60 m from the beach. 56.0545°N, 12.0569°E. 27.08.2016, A. Thell 16-006 (LD1895914)	KX961399	consalazinic, salazinic, lobaric, protolicheterinic and licheterinic acids, atranorin
<i>P. serrana</i> and <i>P. submontana</i> – mixed sample	Sweden. Skåne Prov. Bosarp par., Kärstorp, Lindnäs, på lind. 55.902567 °N, 13.366361°E. 06.09.2016, A. Thell 16-006 (LD1894124). Sample mixed with <i>P. Submontana</i>	KY419143	<i>P. serrana</i> : consalazinic, salazinic, lobaric, protolicheterinic and licheterinic acids, atranorin <i>P. submontana</i> : consalazinic and salazinic acids, atranorin
<i>P. serrana</i>	Sweden. Östergötland. V. Tollstad. Omberg, Hjässatorget, on bases of large <i>Fagus sylvatica</i> near parking area. 58°18'37.3"N, 14°39'17.0"E. 25.04.2013, A. Thell, I. Kärnefelt & A. Nordin (LD1637783)	KX961398	consalazinic, salazinic, lobaric, protolicheterinic and licheterinic acids, atranorin
<i>P. sulcata</i>	Sweden. Skåne Prov. Billinge par., 55°57'32.5"N, 13°20'8.9"E. On <i>Acer platanoides</i> in SE corner of churchyard. 09.06.2016, A. Thell & E. Åsegård 16-002	–	consalazinic and salazinic acids, atranorin

	(LD1884056)		
<i>P. sulcata</i>	Sweden. Skåne Prov. Billinge par., 55°57'32.5"N, 13°20'8.9"E. On <i>Acer platanoides</i> in SE corner of churchyard. 09.06.2016, A. Thell & E. Åsegård 16-003 (LD1884127)	KX961402	consalazinic and salazinic acids, atranorin
<i>P. submontana</i>	Sweden. Skåne Prov. Eslöv par., c. 200 m SW of Kastberga castle, 55°51'26.6"N, 13°20'41.2"E. On roadside <i>Acer pseudoplatanus</i> , 09.06.2016, Arne Thell & Emil Åsegård 16-001 (LD1895510)	KX961400	consalazinic and salazinic acids, atranorin

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