Folia Cryptog. Estonica, Fasc. 36: 1–6 (2000)

# EDITORIAL

## XIV Symposium of Baltic Mycologists and Lichenologists, September 3-8, 1999 in Järvselja, Estonia

This time more than 40 persons from six countries – Estonia, Latvia, Lithuania, Poland, Finland and Sweden – came to Järvselja, Tartumaa County (Fig. 1). Young students as well as *professor emeritus*, and all the intermediate generations were involved (Fig. 2). The short six days spent in a beautiful wooded locality of southeastern Estonia were flavoured with a tinge of the first meeting 40 years ago. We were lucky to have five persons with us who participated in 1959 and were still active in the XIV symposium – Leili Järva, Kuulo Kalamees, Erast Parmasto, Ain Raitviir and Anne-Liis Sõmermaa. In both meetings – in 1959 and 1999 – an excursion was organized to Taevaskoja, Põlvamaa County to visit the landscape reserve of the valley of Ahja River with wide pine forests and high cliffs of red devon sandstone. A photograph has been taken of participants during both meetings almost in the same place in Taevaskoja – in front of picturesque high sandstone cliffs called 'taevaskoda' ('heaven's hall' in direct translation). We managed to identify nearly all participants in 1959 (Fig. 3); on the photo from 1999 (Fig. 4) Kuulo Kalamees – standing – represents the elder generation while all the younger participants sit, squat or lie. Hopefully this does not symbolize the quality of scientific contributions.

The history of symposia of Baltic mycologists and lichenologists is long and variegated.

The first symposium of this kind took place in Tartu in 1959. The following meetings have been organized rather regularly in every second-third-fourth year: The role of these meetings has remained the same during last 40 years – to offer possibilities for the local mycologists and lichenologists from the three Baltic countries to meet each other and present the results of their latest research, and also to conclude the contacts with colleagues from other regions. While during the Soviet period our symposia were visited by mycologists-lichenologists from as far as the Caucasian republics and Russian Far East, then now we are glad to receive guests from other countries around the Baltic sea.

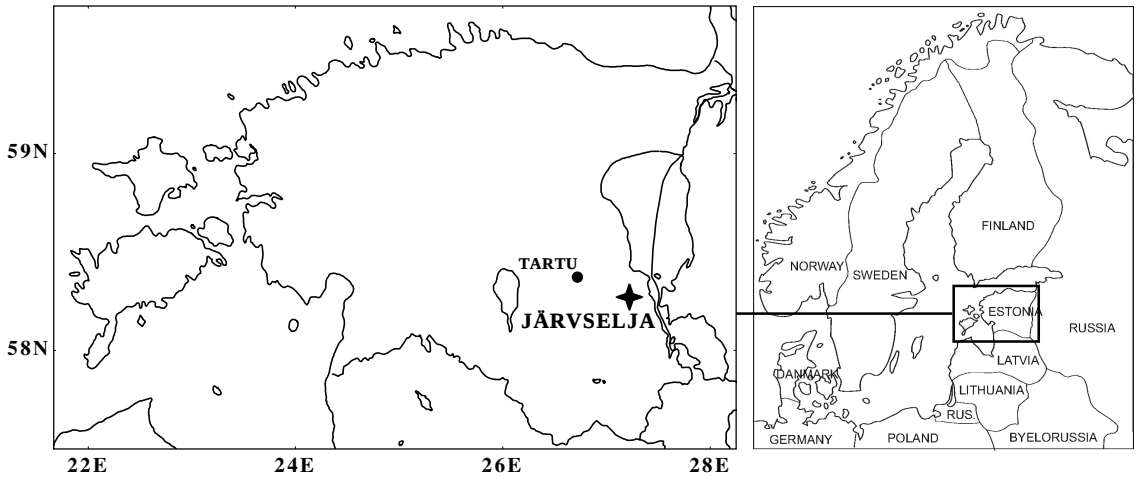



**Fig. 1.** Location of Järvselja, the site of the XIV Symposium of Baltic Mycologists and Lichenologists.

**Fig. 2.** Participants of the XIV Symposium of Baltic Mycologists and Lichenologists in Järvselja, September 1999 (photo by Helle Järv).
Sitting (from left): Ain Raitviir, Magda Kõljalg, Piret Lõhmus, Mall Vaasma, Tiina Randlane, Ernestas Kutorga, Martin Kukwa, Kaimo Kelder, Bellis Kullman.
Standing (from left): Maria Lawrynowicz, Leho Tedersoo, Urmas Kõljalg, Pekka Halonen, Arne Thell, Inga Jüriado, Irja Saar, Kadri Leenurm, Maarja Öpik, Tõnu Ploompuu, Olga Treikale, Mara Kilevica, Lauri Saag, Uve Ramst, Kuulo Kalamees, Andres Saag, Leili Järva, Marina Temina, Inguna Krastina, Anne-Liis Sõmermaa, Ilze Irbe, Ligita Liepina, Lelde Galovecka, Ljudmilla Martin, Jüri Martin, Eva Nilson, Nomeda Juceviciene, Jurga Motiejunaite, Grazina Adamonyte.

Some nice traditions have survived since 1959 – to interchange the presentation of reports and lectures with field works, and to publish the materials introduced during the symposium in a separate volume. Still, nothing remains quite the same, and this time we gave up the publication of abstracts before the meeting and decided to present contributions in their full length afterwards. The organizing committee also decided not to continue with the formal surveys concerning the latest developments in mycology or lichenology in the three Baltic countries but preferred to support the lectures and posters on most variable specific subjects – from the molecular studies of fungi to the possibilities of using these organisms for dying wool. As a result, in the present volume of FCE you can find 11 contributions that were introduced on the latest symposium either orally or in the form of posters. One paper indicates that lichenologists had spent their time extremely usefully – eagerly hunting for rare and interesting taxa during the field trips and excursions.

## THE LIST OF BALTIC SYMPOSIA OF MYCOLOGISTS AND LICHENOLOGISTS

I – 26–29 Sept. 1959, Tartu, Estonia.

Proceedings: *Papers on myco- and lichen flora of the Baltic region* (in Russian, summaries in English or German). Scripta Bot. 2. Institute of Zoology and Botany, Academy of Sciences of Estonian SSR, Tartu, 1962. 278 pp.

II – 11–14 Sept. 1961, Vilnius, Lithuania.

Proceedings: *Proceedings of the second symposium. On the investigations of the myco-lichen flore of the Baltic Republics* (in Russian, summaries in Lithuanian and English or German). The Academy of Sciences of the Lithuanian SSR, Botanical Institute, Vilnius,1963.155 pp.

III – 26–28 July 1963, Riga, Latvia.

Proceedings: *Proceedings of the Third Symposium of the Investigations of the Baltic Republics Myco-Lichen Flora* (in Russian, summaries in Latvian and English or German). P. Stuchka Latvian State University, Scientific works 74. Botany 2. Riga, 1966.155 pp.

IV – 7–12 Sept. 1965, Tartu, Estonia.

Proceedings: *Problems of studies on fungi and lichens* (in Russian, summary in English or German). Academy of Sciences of Estonian SSR, Tartu, 1965. 217 pp.

**Fig. 3.** Participants of the I Symposium of Baltic Mycologists and Lichenologists in Taevaskoja, Põlvamaa County, 1959.
Sitting (from left): Urve Haug, Hans-Voldemar Trass, Väino Lasting, Silvi Pärn (Eilart), Ülle Mägi (Kukk), Maria Aksel, Visolde Puusepp. Standing (from left): Tõnis Leisner, Kuulo Kalamees, Malvina Kasha, Alfons Piterans, Vija Medne (Lazdinja), Elena Florovskaja, Erast Parmasto, Nina Golubkova, Leili Järva, Anne-Liis Sõmermaa, Aire Meokas, Ain Raitviir, Ksenja Rassadina, Ilga Vimba, Edgars Vimba, Margarita Bondartseva, ?, ?, Antanas Minkevicius, Jonas Mazelaitis, ?.

**Fig. 4.** Participants of the excursion to Taevaskoja, Põlvamaa County, 1999 (from left): Olga Treikale, Ilze Irbe, Lelde Galovecka, Arne Thell, Jurga Motiejunaite, Laila Sica, Grazina Adamonyte, Andres Saag, Inguna Krastina, Kuulo Kalamees (standing), Ligita Liepina, Lauri Saag, Tiina Randlane, Nomeda Juceviciene, Ernestas Kutorga, Pekka Halonen, Martin Kukwa (photo by Piret Lõhmus).

V – 5–9 Sept. 1968, Vilnius, Lithuania.

Proceedings: *Proceedings of the V symposium on the investigations of the myco-lichen flora of the Baltic Republics* (in Russian, summaries in English or German). Academy of Sciences, Lithuanian SSR, Vilnius, 1968. 209 pp.

VI – 7–11 Sept. 1971, Riga, Latvia.

Proceedings: *Materials of the VI symposium of the Baltic Republics on mycology and lichenology*, 1-3 (in Russian, summaries in English or German). P. Stuchka Latvian State University, Riga, 1971-1973. 1 – 250 pp., 2 – 227 pp., 3 – 66 pp.

VII – 9–13 Sept. 1974, Lahemaa National Park, Estonia.

Proceedings: *Advances in mycology and lichenology in Soviet Pribaltics* (in Russian, summaries in English or German). Academy of Sciences of the Estonian SSR, Institute of Zoology and Botany, Tartu, 1974. 230 pp.

VIII – 21–23 Sept. 1977, Vilnius and Palanga, Lithuania.

*Ecological specialities of lown plants of Soviet Baltic region* (in Russian, summaries in English or German). Academy of Sciences of Lithuanian SSR, Institute of Botany, Vilnius, 1977. 286 pp.

IX – 17–19 Nov. 1982, Minsk, Byelorussia.

Proceedings: *Ecology and biology of lower plants* (in Russian). Academy of Sciences of the Byelorussian SSR, 1982. 271 pp.

X – 16–18 Sept. 1985, Madona, Latvia.

Proceedings: *Fungi and lichens in the ecosystem* I, II (in Russian, summaries in English or German). P. Stuchka Latvian State University, Riga, 1985. 1 – 117 pp., 2 – 133 pp.

XI – 23–26 May 1988, Kääriku, Estonia.

Proceedings: *11 symposium of the mycologists and lichenologists of the Baltic Republics and Byelorussia* (in Russian, summaries in English). Abstracts. Academy of Sciences of the Estonian SSR. Institute of Zoology and Botany, Tartu State University Chair of Botany and Ecology, Tallinn, 1988. 185 pp.

XII – 27 Sept.– 02 Oct. 1993, Vilnius, Lithuania.

Proceedings: *Fungi and lichens in the Baltic Region. 12 International Conference Abstracts*. Institute of Botany, Vilnius University, Vilnius, 1993. 151 pp.

XIII – 24–28 Sept. 1996, Kemeri, Latvia.

Proceedings: *Fungi and lichens in the Baltic Region. 13 International Conference Abstracts*. University of Latvia, Latvian Museum of Natural History, Institute of Biology, Latvian Academy of Sciences, Riga, 1996. 84 pp.

XIV – 3–8 Sept. 1999, Järvselja, Estonia.

Proceedings: *Folia Cryptog. Estonica* 36, 2000. 112 pp.

XV – 2002, to be held in Lithuania.

**Tiina Randlane, Andres Saag & Ain Raitviir**

Folia Cryptog. Estonica, Fasc. 36: 7–9 (2000)

# New Data on Estonian Myxomycete Biota

**Grazina Adamonyte**
Institute of Botany, 49 Zaliuju ezeru, LT-2021, Vilnius, Lithuania. E-mail: grazina@ktl.mii.lt

**Abstract:** 34 species of myxomycetes were recorded in four localities in the south-eastern Estonia on 4–7 September, 1999. 12 of them are new for the country: *Arcyodes incarnata, Arcyria affinis, A. stipata, A. ferruginea, Clastoderma debaryanum, Cribraria rufa, Fuligo leviderma, Hemitrichia serpula, Metatrichia floriformis, Oligonema flavidum, Stemonitis smithii,* and *Tubifera casparyi*. Each record is supplied with a brief information on the locality and substratum.

**Kokkuvõte:** G. Adamonyte. Limakute uusi leide Eestis.

4–7. septembril 1999. a. koguti Kagu-Eestis neljas erinevas leiukohas 34 liiki limaseeni. 12 liiki neist olid esmasleiud Eestis, need olid: *Arcyodes incarnata, Arcyria affinis, A. stipata, A. ferruginea, Clastoderma debaryanum, Cribraria rufa, Fuligo leviderma, Hemitrichia serpula, Metatrichia floriformis, Oligonema flavidum, Stemonitis smithii* ja *Tubifera casparyi*. Nimestikus on iga liigi juures antud tema kasvukoht ja substraat.

## INTRODUCTION

Myxomycetes, an important component of forest ecosystems, are being investigated for more than 200 years. During that time approximately 1000 their species have been described worldwide (Neubert, Nowotny, Baumann, 1995), the distribution of many of them being still unclear. So far, 89 species of myxomycetes have been reported from the territory of Estonia (Järva & Parmasto, 1980; Järva et al., 1998). During the 14th Symposium of Baltic mycologists and lichenologists on 4–7 September, 1999, myxomycete material was collected in mixed forests of the south-eastern part of Estonia. In this paper myxomycete species new for the country as well as new localities for the species reported earlier from Estonia are presented.

## INVESTIGATED SITES

J – Tartumaa County, Järvselja Virgin Forest Nature Reserve and adjacent squares (58°16'N, 27°19'E), old forests mainly of spruce, lime and black alder; material collected on 4th of September.

R – Tartumaa County, Järvselja towards Rõkka (58°13'N, 27°17'E), forests of *Oxalis-Myrtillus* and *Vaccinium uliginosum* types; material collected on 5th of September.

T – Põlvamaa County, Taevaskoja Landscape Reserve (58°07'N, 27°03'E), mixed forests of spruce and pine; material collected on 6th of September.

E – Tartumaa County, Emajõe Suursoo Reserve, the Lake Leegu towards Apnasaar mineral bog island (58°22'–58°21'N, 27°16'–27°17'E), old mixed forests of *Oxalis, Myrtillus* and *Oxalis-Myrtillus* types; material collected on 7th of September.

## LIST OF SPECIES

All the specimens were collected in the field by the author and other participants of the Symposium; moist chamber technique was not employed. The nomenclature follows B. Ing (1999). Myxomycete species reported for the first time in Estonia are marked with an asterisk (*). The voucher specimens are deposited in the Herbarium of the Institute of Botany (BILAS), Vilnius, Lithuania.

*ARCYODES INCARNATA (Alb. & Schwein.) O. F. Cook – E: on an aspen log.

*ARCYRIA AFFINIS Rostaf. – J: on a deciduous decorticated log and on a deciduous log together with *Trichia decipiens*; E: on a log together with *Trichia persimilis*.

A. CINEREA (Bull.) Pers. – J: on bark of a living deciduous tree; R: on plant debris.

A. DENUDATA (L.) Wettst. – J: on deciduous logs; R: on a log; E (abundant): on pine, alder, aspen logs.

*A. FERRUGINEA Saut. – E: on a decorticated pine log.

A. OBVELATA (Oeder) Onsberg – E: on an oak log.

*A. STIPATA (Schwein.) Lister – E: on side surface of an alder log.

CERATIOMYXA FRUTICULOSA (F. Muell.) T. Macbr. – J: on a deciduous log.

*CLASTODERMA DEBARYANUM A. Blytt – T: on a decorticated pine log.

CRIBRARIA ARGILLACEA (Pers.) Pers. – J: on a well-rotted moss-covered log.

C. PERSOONII Nann.-Bremek. – J: on a decorticated spruce log and on a well-rotted coniferous log; R: on side surface of a decorticated aspen log and on top of a stump.

C. PURPUREA Schrad. – J: on side surface of a decorticated spruce log.

*C. RUFA (Roth) Rostaf. – J: on a rotted coniferous log.

ENTERIDIUM LYCOPERDON (Bull.) M. L. Farr – T: on a coniferous log and on a burned log in a bonfire place.

*FULIGO LEVIDERMA Neubert, Nowotny et Baumann – E: on a birch stump.

HEMITRICHIA CLAVATA (Pers.) Rostaf. – J: on a moss-covered deciduous log together with *Hemitrichia serpula*.

*H. SERPULA (Scop.) Rostaf. – J: on a moss-covered deciduous log together with *Hemitrichia clavata*.

LYCOGALA TERRESTRE Fr. – J (abundant): on logs; E (abundant): on oak and unidentified deciduous logs.

*METATRICHIA FLORIFORMIS (Schwein.) Nann.-Bremek. – J: on a birch log together with *Trichia varia*; R: on a rotted aspen log and on a moss-covered aspen log together with *Trichia varia*.

*OLIGONEMA FLAVIDUM (Peck) Peck – E: on a decorticated rotted log.

PERICHAENA CORTICALIS (Batsch) Rostaf. – E: on bark of an aspen log.

PHYSARUM LEUCOPHAEUM Fr. – J: on litter; R: on litter; E: on top of a birch stump.

P. NUTANS Pers. – J: on a lower side of a decorticated spruce log.

STEMONITIS AXIFERA (Bull.) T. Macbr. – E: on bark of birch logs.

S. FUSCA Roth – R: on decorticated deciduous logs; E: on a decorticated pine log.

*S. SMITHII T. Macbr. – R: on a moss-covered log; E: on an upper side of a decorticated pine log and on an alder log.

STEMONITOPSIS HYPEROPTA (Meyl.) Nann.-Bremek. – E: on a decorticated rotted birch log.

S. TYPHINA (F. H. Wigg.) Nann.-Bremek. – J: on a rotted coniferous log; E: on the inner side of peeling bark of a birch log and on an oak log;

TRICHIA DECIPIENS (Pers.) T. Macbr. – J: on a deciduous log together with *Arcyria affinis* and on a fruit-body of *Fomes fomentarius* on a spruce log; E: on deciduous and coniferous logs.

T. FAVOGINEA (Batsch) Pers. – J: on logs; R: on a well-rotted moss-covered log.

T. PERSIMILIS P. Karst. – J: on a side of a decorticated spruce log; R: on a log; E: on a log together with *Arcyria affinis*.

T. VARIA (Pers.) Pers. – J: on a lime log, on a birch log together with *Metatrichia floriformis*; R: on an aspen log and on a moss-covered aspen log together with *Metatrichia floriformis*.

*TUBIFERA CASPARYI (Rostaf.) T. Macbr. – J: on a well-rotted decorticated coniferous log.

T. FERRUGINOSA (Batsch.) J. F. Gmel. – J: on a well-rotted birch log; T: on a well-rotted coniferous log; E: on a log.

Thus, in total 34 species of myxomycetes were recorded in the Tartumaa and Põlvamaa Counties on 4–7 September, 1999. Among them 12 species are reported here as new to Estonia. Because of dry and hot vegetation season, collections were not abundant. The richest sampling sites were the Järvselja Virgin Forest Nature Reserve with neighbouring forest squares, and forests of Emajõe Suursoo Reserve, both ecotopes rather humid and rich in fallen wood. Thus, microclimatic conditions, despite a prolonged drought of 1999, remained sufficiently favourable there for myxomycete sporulation. The largest part of the collection made lignicolous species, collected from coarse wood debris of various degree of decay. The substratum of this type is able to retain comparatively much water, thus favouring the development of plasmodia (Lado, 1993). The majority of collected myxomycete species are common cosmopolitans. Along with them, some species, such as *Arcyodes incarnata*, *Arcyria stipata*, *Oligonema flavidum*, *Tubifera casparyi*, which are considered to be not very common in Eu-

rope (Ing, 1999; Nannenga-Bremekamp, 1991), were collected. The present records contribute to the myxomycete species diversity in Estonia and to the general distribution patterns of the species.

## ACKNOWLEDGEMENTS

The author is greatly indebted to Mr. Yukinori Yamamoto (Japan) for the identification of *Arcyria affinis*, *Physarum leucophaeum*, *Stemonitis smithii* and *Tubifera casparyi*.

## REFERENCES

Ing, B. 1999. *The Myxomycetes of Britain and Ireland. An Identification Handbook*. The Richmond Publishing Co., Slough. 374 pp.

Järva, L. & Parmasto, E. 1980. *List of Estonian Fungi.* Institute of Zoology and Botany. Tartu. 331 pp.

Järva, L., Parmasto, I. & Vaasma, M. 1998. *List of Estonian Fungi. Supplement I.* Institute of Zoology and Botany. Tartu. 183 pp.

Lado, C. 1993. Myxomycetes of Mediterranean woodlands. In *Fungi of Europe: Investigation, Recording and Conservation* (eds Pegler, D. N., Boddy, L., Ing, B. & Kirk, P. M.), pp. 93–114. Kew.

Nannenga-Bremekamp, N. E. 1991. *A guide to temperate myxomycetes*. Biopress Limited, Bristol. 409 pp.

Neubert, H., Nowotny, W. & Baumann, K. 1995. *Die Myxomyceten. 2. Physarales*. Karlheinz Baumann Verlag. Gomaringen. 368 pp.

Folia Cryptog. Estonica, Fasc. 36: 11–15 (2000)

# Lichens of the 'Cisy w Czarnem' reserve (Western Pomerania, N Poland) with emphasis on old growth forest species

**Wieslaw Faltynowicz[1] & Martin Kukwa[2]**

[1]Department of Plant Systematic and Phytosociology, Institute of Botany, University of Wroclaw, 9 Maxa Bora, PL-50-204 Wroclaw, Poland

[2]Department of Plant Ecology and Nature Protection, University of Gdansk, 9 Al. Legionów, PL-80-441 Gdansk, Poland. E-mail: dokmak@univ.gda.pl

**Abstract:** The nature reserve 'Cisy w Czarnem' is situated ca 5 km E of Czarne on Western Pomerania (North Poland). The reserve was created to protect the population of *Taxus baccata*. Of the 59 lichen species found in the reserve, few are rare or very rare in northern Poland, e.g. *Arthonia vinosa*, *Bacidia arnoldiana* and *Lobaria pulmonaria*. 21 lichen species are included in the red list for Poland and eight species found in the reserve are considered to be 'old growth forest species'. The state of these taxa is discussed in relation to the conditions in the reserve.

**Kokkuvõte:** W. Faltynowicz ja M. Kukwa. 'Cisy w Czarnem' kaitseala (Lääne-Pomeraania, Põhja-Poola) samblikud.

Poola põhjaosas asuv 'Cisy w Czarnem' kaitseala on rajatud jugapuu (*Taxus baccata*) populatsiooni säilitamiseks. Kaitsealalt on leitud 59 samblikuliiki, neist mõned haruldased, näiteks *Arthonia vinosa*, *Bacidia arnoldiana* ja *Lobaria pulmonaria*. 21 liiki on kantud Poola punasesse raamatusse ja üheksat liiki võib pidada 'vanade metsade liikideks'. Artiklis vaadeldakse viimatinimetatud taksonite staatust seoses selle kaitseala tingimustega.

## INTRODUCTION

The nature reserve 'Cisy w Czarnem' is situated ca 5 km E of Czarne in the Pomorskie district of Western Pomerania (North Poland, ATPOL grid square Bc-50) (Fig. 1). It covers an area of 25.20 ha. The reserve was formerly alder bog forest *Ribo nigri-Alnetum*, only a small part of which remains. *Taxus baccata* L. was planted in the nineteenth century. These trees are now ca 120 years old with a few young and seedling trees present, growing mostly in acidophilous beech forest. Within the reserve there are also hornbeam-alder and birch-alder forests, and a very small area of pine-spruce forest (Faltynowicz & Markowski, 1997).

The present survey was undertaken to enumerate lichens found in the reserve and to locate 'old growth forest species' within the reserve. Some taxa (ca 20), mostly common in the country, were earlier reported in a general work about the nature reserves in the Slupsk district (Izydorek, 1996).

## METHODS

The investigations were carried out between 1997–1998. Lichens were collected from all types of habitats. Easily identified species were noted in the field. Lichens were identified using keys: Nowak and Tobolewski (1975), Purvis et al. (1992) and Tønsberg (1992). Specimens are kept in the Herbarium of the University of Gdansk (UGDA-L). Lichen substances were identified by TLC in solvent C (methods after White & James, 1985). The nomenclature mostly follows Faltynowicz (1993) and for naming of some taxa also Tønsberg (1992). Abbreviations of authors' names follow Kirk and Ansell (1992).

## RESULTS

Up to now 59 lichen species were found in the reserve. All taxa reported by Izydorek (1996) were refound except *Leproloma membranaceum* (Dicks.) Vain. In our opinion this species has

**Fig. 1.** Location of 'Cisy w Czarnem' in northern Poland.

been incorrectly reported. Probably it was mistaken for *Lepraria lobificans*, which occurs at present in the reserve. 13 taxa are totally protected by law (Faltynowicz, 1998) (marked with P in the list of species). Several species are included in the red list of lichens of Poland (Cieslinski et al., 1992), among them 8 taxa are considered endangered (marked with E in the list), and 13 – vulnerable (marked with V in the list).

## LIST OF SPECIES

E – endangered species (according to Cieslinski et al., 1992);
P – protected species (according to Faltynowicz, 1998);
V – vulnerable species (according to Cieslinski et al., 1992).

ARTHONIA VINOSA Leight. – on bark of *Quercus* sp.;
BACIDIA ARNOLDIANA Körb. – on bark of *Taxus baccata*;
B. GLOBULOSA (Flörke) Hafellner & V. Wirth [syn. *Catillaria globulosa* (Flörke) Th. Fr.] – on bark of *Quercus* sp.;
CALICIUM ADSPERSUM Pers. – (E), on bark of *Quercus* sp.;
CHAENOTHECA BRACHYPODA (Ach.) Tibell – (E), on bark of old *Fagus sylvatica*;
C. CHRYSOCEPHALA (Ach.) Th. Fr. – on bark of *Alnus glutinosa*;
C. FERRUGINEA (Turner & Borrer) Mig. – on bark of *Alnus glutinosa* and *Pinus sylvestris*;
C. FURFURACEA (L.) Tibell – (V), on bark of *Fagus sylvatica* in lower part of trunk;
C. TRICHIALIS (Ach.) Th. Fr. – (V), on bark of *Alnus glutinosa*;
CHRYSOTHRIX CANDELARIS (L.) J. R. Laundon – (E), on bark of *Alnus glutinosa, Fagus sylvatica* and *Quercus* sp.;
CLADONIA CHLOROPHAEA (Flörke ex Sommerf.) Spreng. – on soil, wood and on the base of trunks;
C. CONIOCRAEA (Flörke) Spreng. – on the base of trunks;
C. DIGITATA (L.) Hoffm. – on wood and on the base of trunks;
C. FIMBRIATA (L.) Fr. – on wood;
C. GLAUCA Flörke – on wood and on the base of trunks;
C. OCHROCHLORA Flörke – on bark of *Alnus glutinosa*;
EVERNIA PRUNASTRI (L.) Ach. – (P, V), on bark of *Fagus sylvatica*;
GRAPHIS SCRIPTA (L.) Ach. – (V), on bark of *Sorbus aucuparia*;
HYPOCENOMYCE SCALARIS (Ach.) M. Choisy – on bark of *Alnus glutinosa* and *Pinus sylvestris*;
HYPOGYMNIA PHYSODES (L.) Nyl. – on bark of *Alnus glutinosa, Fagus sylvatica, Quercus* sp., *Picea abies* and *Pinus sylvestris*;
H. TUBULOSA (Schaer.) Hav. – (P), on bark of *Fagus sylvatica* and *Padus avium*;
IMSHAUGIA ALEURITES (Ach.) S. L. F. Meyer – (P), on bark of *Pinus sylvestris*;
LECANORA ARGENTATA (Ach.) Malme – on bark of *Fagus sylvatica*;
L. CONIZAEOIDES Nyl. ex Cromb. – on wood, bark of *Frangula alnus* and *Pinus sylvestris*;
L. EXPALLENS Ach. – on bark of *Fagus sylvatica* and *Taxus baccata*;
L. GLABRATA (Ach.) Malme – on bark of *Sorbus aucuparia*;
LEPRARIA INCANA (L.) Ach. – on bark of *Fagus sylvatica;*
L. LOBIFICANS Nyl. – on bark of *Taxus baccata* and *Fagus sylvatica;*

LEPROLOMA VOUAUXII (Hue) J. R. Laundon – on bark of *Taxus baccata*;
LOBARIA PULMONARIA (L.) Hoffm. – (E, P), on bark of *Fagus sylvatica*;
MELANELIA FULIGINOSA (Fr. ex Duby) Essl. (c. ap.) – (P), on bark of *Fagus sylvatica*, *Sorbus aucuparia* and *Quercus* sp.;
MICAREA DENIGRATA (Fr.) Hedl. – on hard wood;
M. PRASINA Fr. – on wood and bark of *Alnus glutinosa*;
MYCOBLASTUS FUCATUS *(Stirt.) Zahlbr.* – on bark of *Sorbus aucuparia;*
OCHROLECHIA ANDROGYNA (Hoffm.) Arnold – (V), on bark of *Fagus sylvatica*;
OPEGRAPHA NIVEOATRA (Borrer) J. R. Laundon – (V), on bark of *Fagus sylvatica*;
O. RUFESCENS Pers. – (V), on bark of *Fagus sylvatica*;
O. VARIA Pers. – (V), on bark of *Fagus sylvatica*;
PARMELIA SAXATILIS (L.) Ach. – (P), on bark of *Corylus avellana*;
P. SULCATA Taylor – on bark of *Alnus glutinosa*, *Fagus sylvatica* and *Quercus* sp.;
PARMELIOPSIS AMBIGUA (Wulfen) Nyl. – (P), on bark of *Pinus sylvestris*;
PELTIGERA PRAETEXTATA (Flörke ex Sommerf.) Zopf – (E, P), at bases of many trunks of *Fagus sylvatica* and decaying logs;
PERTUSARIA ALBESCENS (Huds.) Choisy & Werner – on bark of *Fagus sylvatica*;
P. AMARA (Ach.) Nyl. – on bark of *Alnus glutinosa*, *Fagus sylvatica* and *Quercus* sp.;
P. COCCODES (Ach.) Nyl. var. *coccodes* – on bark of *Fagus sylvatica* and *Quercus* sp.;
P. FLAVIDA (DC.) J. R. Laundon – (V), on bark of *Fagus sylvatica*;
P. HEMISPHAERICA (Flörke) Erichsen – (V), on bark of *Fagus sylvatica*;
P. PERTUSA (Weigel) Tuck. – (V), on bark of *Fagus sylvatica*;
PHLYCTIS ARGENA (Spreng.) Flot. – on bark of *Alnus glutinosa* and *Fagus sylvatica*;
PLATISMATIA GLAUCA (L.) W. Culb. & C. Culb. – (P, V), on bark of *Alnus glutinosa*, and *Fagus sylvatica* and *Quercus* sp.;
PYRENULA NITIDA (Weigel) Ach. – (E), on bark of *Fagus sylvatica*;
PSEUDEVERNIA FURFURACEA (L.) Zopf – (P), on bark of *Quercus* sp.;
RAMALINA FARINACEA (L.) Ach. – (P, V), on bark of *Padus avium*;
ROPALOSPORA VIRIDIS (Tønsberg) Tønsberg – on bark of *Sorbus aucuparia*;
SCOLICIOSPORUM CHLOROCOCCUM (Graeve ex Stenh.) Vezda – on wood;
TRAPELIOPSIS FLEXUOSA (Fr.) Coppins & P. James – on wood;
T. GRANULOSA (Hoffm.) Lumbsch – on wood;
USNEA FILIPENDULA Stirt. – (E, P), on bark of *Quercus* sp.;
U. SUBFLORIDANA Stirt. – (E, P), on bark of *Quercus* sp.

## DISCUSSION

From 59 lichen species reported from the reserve, 21 taxa are included in the red list of Poland. Many of them are still common or quite common in northern Poland (e.g. *Platismatia glauca*), but some of them are rather rare, e.g. *Lobaria pulmonaria*, *Pertusaria hemisphaerica*.

*Lobaria pulmonaria* is the most interesting lichen species. It is becoming rare in northern Poland and is now on the endangered list. It is known from ca 40 localities but many of these are old records (Tobolewski & Kupczyk, 1976; Faltynowicz, 1992). The species prefers old growth deciduous forest. In the reserve the population was observed to consist of several mature thalli growing on trunks of old *Fagus sylvatica* in beech forest with many young thalli on the same trees. Young thalli are rarely found in Central Europe. Associated species included *Pertusaria* spp., *Opegrapha* spp., *Pyrenula nitida* and others.

*Bacidia arnoldiana* is a very rare lichen species in northern Poland, known in Western Pomerania from one locality only (Faltynowicz, 1992). The species is more common in north-eastern Poland (e.g. Cieslinski & Tobolewski, 1988, 1989), where there are better preserved deciduous forests (e.g. Bialowieza Primeval Forest). The species might be more common, but overlooked. In the reserve it was found on the trunk of an old *Taxus baccata* tree in beech forest. *Lepraria lobificans* was the only associated lichen taxon.

*Chaenotheca brachypoda* is another rare species in northern Poland, which is known from 9 localities only (Tobolewski & Kupczyk 1974; Faltynowicz, 1992, 1994; Cieslinski & Zielinska 1994). In the reserve it has been found

on lignum of old dead *Fagus sylvatica*. Other rare species include *Arthonia vinosa, Calicium adspersum, Pertusaria flavida, P. hemisphaerica, Usnea filipendula* and *U. subfloridana*.

Four lichen taxa, *Lepraria lobificans, Leproloma vouauxii, Ropalospora viridis* and *Mycoblastus fucatus* have been recently reported as new to Poland (Alstrup & Olech, 1990; Sliwa, 1996; Faltynowicz, 1997). Although there are at present few localities for these species in the country, they, especially *Lepraria lobificans*, are probably common.

Eight of the species found in the reserve are called 'primeval forest relicts' (Cieslinski et al., 1996), 'old forest indicator species' (Rose, 1976, 1992) or 'indicators of the ecological continuity' of a forest complex (Arup, 1997) (Table 1). In the reserve these species grow in completely anthropogenic habitats. The whole area of this stand has been covered in the past by black alder peat bog forest phytocoenosis *Ribo nigri-Alnetum*. This community is mostly replaced by acidophilous beech forest and others (Faltynowicz & Markowski, 1997). In this situation 'old growth forest' taxa can not be named relicts, in our opinion, but rather the indicators of continuity of forest conditions.

**Table 1.** Old growth forest species according to: 1 – Cieslinski et al. (1996), 2 – Rose (1976, 1992) and 3 – Arup (1997)

| Lichen species | Acc. to |
|---|---|
| *Arthonia vinosa* | 2 |
| *Bacidia arnoldiana* | 1 |
| *Chaenotheca brachypoda* | 1, 3 |
| *C. trichialis* | 2 |
| *Chrysothrix candelaris* | 1 |
| *Lecanora glabrata* | 3 |
| *Lobaria pulmonaria* | 1, 2, 3 |
| *Pyrenula nitida* | 1, 3 |

Species regarded as 'old growth forest lichens' may vary in adjacent countries or districts. This is, due to a range of ecological and biogeographic circumstances. Up to now there is no list of the 'old growth forest lichens' for all parts of Poland or neighbouring countries. Further studies on these lichens are needed.

**ACKNOWLEDGEMENTS**

The authors are greatly indebted to the Organizing Committee of the XIV Symposium of Baltic Mycologists and Lichenologists in Järvselja, Estonia, for the excellent organization of the symposium. The participation of the second author was partly supported by 'Fundacja im. Stefana Batorego' and 'Towarzystwo Naukowe Warszawskie'.

**REFERENCES**

Alstrup, V. & Olech, M. 1990. Addition to the lichen flora of the Polish Tatra Mountain. II. *Zesz. Nauk. Uniw. Jagiell., Pr. Bot.* 21: 211–217.

Arup, U. 1997. Indikatorarte och viktiga miljöer. In *Skyddsvärda lavar i sydvästra Sverige* (eds Arup, U., Ekman, S., Kärnefelt, I. & Mattsson, J.-E. ), pp. 96–103. Lund.

Cieslinski, S., Czyzewska, K. & Fabiszewski, J. 1992. Red list of threatened lichens in Poland. In *List of threatened plants in Poland* (eds Zarzycki, K., Wojewoda, W. & Heinrich, Z.). 2-nd ed., pp. 57–74. W. Szafera Institute of Botany, PAN, Kraków.

Cieslinski, S., Czyzewska, K., Falinski, J.B., Klama, H., Mulenko, W. & Zarnowiec, J. 1996. Relicts of primeval (virgin) forest. Relict phenomena. *Phytocoenosis* 8 (N.S.), *Archivum Geobotanicum* 6: 197–216.

Cieslinski, S. & Tobolewski, Z. 1988. Lichens of Bialowieza Primeval Forest and its western foreground (in Polish). *Phytocoenosis* 1 (N.S.), suppl. *Cartographiae Geobotanicae* 1: 1–216.

Cieslinski, S. & Tobolewski, Z. 1989. Lichens of north-eastern Poland I (in Polish). *Acta Mycol.* 25(1): 57–100.

Cieslinski, S. & Zielinska, J. (eds) 1994. A contribution to the lichen flora of Puszcza Knyszynska (in Polish). *Fragm. Flor. Geobot. Ser. Polonica* 1: 49-61.

Faltynowicz, W. 1992. The lichens of Western Pomerania (NW Poland). An ecogeographical study. *Polish Bot. Stud.* 4: 1–182.

Faltynowicz, W. 1993. A checklist of Polish lichens and lichenicolous fungi including parasitic and saprophytic fungi occurring on lichens. *Polish Bot. Stud.* 6: 1–65.

Faltynowicz, W. (ed.) 1994. The lichens of Wigierski National Park (NE Poland) (in Polish). *Parki nar. Rez. przyr.* 13(3): 9-28.

Faltynowicz, W. 1997. Lichen exsiccates from Poland including a list of the lichen taxa edited (in Polish). In *Dynamics and conservation of Pomeranian vegetation* (eds Faltynowicz, W., Latalowa, M. & Szmeja, J.), pp. 201–222. Bogucki Wyd. Nauk., Gdansk-Poznan.

Faltynowicz, W. 1998. The list of protected lichen species in Poland (in Polish). *Chronmy Przyr. Ojcz.* 54(1): 96–107.

Faltynowicz, W. & Markowski, R.1997. *Conditions of occurrence, state of preservation, dynamic of population of yew Taxus baccata L. in the reserve 'Cisy w Czarnem'* (in Polish). Manuscript in Department of Plant Ecology and Nature Protection, Gdansk.

Izydorek, I. 1996. Materials to lichen flora of the nature reserves in Slupsk district (northern Poland) (in Polish). *Parki nar. Rez. przyr.* 15.

Kirk, P. M. & Ansell, A. E. 1992. *Authors of fungal names*. C.A.B. International, Wallingford. 95 pp.

Nowak, J. & Tobolewski, Z. 1975. *Polish lichens* (in Polish). PWN, Warszawa-Kraków. 1177 pp.

Purvis, O. W., Coppins, B. J., Hawksworth, D. L., James, P. W. & Moore, D. 1992. *Lichen Flora of Great Britain and Northern Ireland*. Natural Museum Publications, London. 710 pp.

Rose, F. 1976. Lichenological indicators of age and environmental continuity in woodlands. In *Lichenology: progress and problems* (eds Brown, D. H., Hawksworth, D. L. & Bailey, R. H.), pp. 279–307. London.

Rose, F. 1992. Temperate forest management: its effects on bryophyte and lichen flora and habitats. In *Bryophytes and lichens in a changing environment* (eds Bates, W. & Farmer, A. M.), pp. 211–233. Oxford.

Sliwa, L. 1996. Note on *Mycoblastus fucatus* [= *M. sterilis*] (*Lichenes, Mycoblastaceae*) from Poland. *Fragm. Flor. Geobot.* 41(1): 491–492.

Tobolewski, Z. & Kupczyk, B. 1974. *Coniocybe sulphurea* (Retz.) Nyl. In *Atlas of geographical distribution of spore-plant in Poland* (Szweykowski, J. & Wojterski, T. ). Series III. Lichens (Lichenes). Part 2: 3–4.

Tobolewski, Z. & Kupczyk, B. 1976. *Lobaria pulmonaria* (L.) Hoffm. In *Atlas of geographical distribution of spore-plant in Poland* (Szweykowski, J. & Wojterski, T. ). Series III. Lichens (Lichenes). Part 3: 3–4.

Tønsberg, T. 1992. The sorediate and isidiate corticolous, crustose lichens in Norway. *Sommerfeltia* 14: 1–331.

White, F. J. & James, P. W. 1985. A new guide to microchemical techniques for the identification of lichen substances. *Br. Lichen Soc. Bull.* 57 (suppl.): 1–41.

Folia Cryptog. Estonica, Fasc. 36: 17–21 (2000)

# Notes on lichens and lichenicolous fungi found during the XIV Symposium of Baltic Mycologists and Lichenologists in Järvselja, Estonia

**Pekka Halonen[1], Martin Kukwa[2], Jurga Motiejunaite[3], Piret Lõhmus[4] & Ljudmilla Martin[5]**

[1] Department of Biology, Botanical Museum, P.O. Box 3000, FIN-90014 University of Oulu, Finland. E-mail: pehalone@sun3.oulu.fi

[2]Department of Plant Ecology and Nature Protection, University of Gdansk, 9 Al. Legionów, PL-80-441 Gdansk, Poland . E-mail: dokmak@univ.gda.pl

[3]Institute of Botany, 49 Zaliuju ezeru, LT-2021 Vilnius, Lithuania. E-mail: mikojm@ktl.mii.lt

[4]Institute of Botany and Ecology, University of Tartu, 38 Lai St., 51005 Tartu, Estonia. E-mail: pisi@ut.ee

[5]Eurouniversity, 4 Mustamäe St., 10621 Tallinn, Estonia. E-mail: ljuda@iceb.ee

**Abstract:** In total 43 lichen (including some threatened species) and lichenicolous fungus species, which have been regarded as rare in Estonia or were not previously known from the country, are reported. 17 taxa are recorded as new to Estonia. The lichen species *Absconditella lignicola*, *Japewia subaurifera*, *Porpidia soredizodes*, *Scoliciosporum pruinosum*, *Steinia geophana* and *Verrucaria praetermissa* are new to Estonia, and *Peltigera didactyla* var. *extenuata* represents a new variety to the country. The lichenicolous fungus species *Clypeococcum hypocenomycis*, *Corticifraga fuckelii*, *Hobsonia christiansenii*, *Lichenoconium lecanorae*, *Phaeopyxis punctum*, *Refractohilum peltigerae*, *Sphaerellothecium propinquellum*, *Syzygospora physciacearum*, *Tremella hypogymniae* and *T. ramalinae* are new to Estonia.

**Kokkuvõte:** P. Halonen, M. Kukwa, J. Motiejunaite, P. Lõhmus & L. Martin. Samblike ja lihhenikoolsete seente leidudest XIV Balti Mükoloogide ja Lihhenoloogide Sümpoosiumil Järvseljal, Eestis.

XIV Balti Mükoloogide ja Lihhenoloogide Sümpoosiumi ajal leiti Kagu-Eestist 43 Eestis haruldast või varem märkamata samblikku (s. h. mõned ohustatud) ja lihhenikoolset seeneliiki. 17 liiki ja liigisisest taksonit on Eestile uued. Nendeks on samblikuliigid *Absconditella lignicola*, *Japewia subaurifera*, *Porpidia soredizodes*, *Scoliciosporum pruinosum*, *Steinia geophana*, *Verrucaria praetermissa* ja uue varieteedina *Peltigera didactyla* var. *extenuata* ning lihhenikoolsed seeneliigid *Clypeococcum hypocenomycis*, *Corticifraga fuckelii*, *Hobsonia christiansenii*, *Lichenoconium lecanorae*, *Phaeopyxis punctum*, *Refractohilum peltigerae*, *Sphaerellothecium propinquellum*, *Syzygospora physciacearum*, *Tremella hypogymniae* ja *T. ramalinae*.

## INTRODUCTION

The study of the Estonian lichens has a long tradition (e.g., Bruttan, 1870; Mereschkowski, 1909, 1913; Räsänen, 1931; Ekman et al., 1991) and the flora is relatively well known. The first checklist of lichens was compiled by Trass (1970), and Trass & Randlane (1994) published a monograph of the Estonian macrolichens. Randlane & Saag (1999) have recently published the second checklist of lichens and allied fungi. It contains 790 lichenized species with 12 infraspecific taxa, and 39 lichenicolous and 22 other non-lichenized fungus species with notes on the ecology, distribution and frequency of each species. Their notes on frequencies are based on the numbers of known localities. Many of the species reported as rare, however, may be common, but overlooked or insufficiently known in Estonia.

The XIV Symposium of Baltic Mycologists and Lichenologists was arranged during September 3–8, 1999 in Järvselja, Tartumaa County, southeastern Estonia. Most of the excursions took place in the Järvselja area, except a field trip to Taevaskoja in Põlvamaa County. Estonia belongs to the hemiboreal vegetation zone (Ahti et al., 1968), and the study area is in the floodplain swamp region along the lower course of the River Emajõgi and southwestern coast of Lake Peipsi (Laasimer, 1965). The area is characterized by the extensive landscape with mires and old forests, which

are dominated by spruce and pine, sparse population and relatively low intensity of forest management. However, Järvselja has had a remarkable role in the forestry research during the last 150 years in Estonia.

The present paper contains notes on rare and/or new Estonian lichen and lichenicolous fungus species, which were found during the symposium. Four macrolichen species listed here have been regarded as threatened in Estonia by Randlane (1998): *Evernia divaricata* (vulnerable), *E. mesomorpha* (care demanding), *Flavoparmelia caperata* (extinct or probably extinct) and *Hypocenomyce sorophora* (rare).

## LOCALITIES

Altitudes of all localities are between 25–50 metres.

3–6 Sept. 1999

**1** Tartumaa County, Järvselja village (58°16'02''N, 27°18'06''E).

4 Sept. 1999

**2** Tartumaa County, Järvselja Virgin Forest Nature Reserve (forest squares 223–226), and forest squares 241, 242 and 227 in the vicinity of the Nature Reserve. Old growth forests mainly of *Picea*, *Tilia* and *Alnus* (58°16'50''N, 27°19'18''E).

5 Sept. 1999

**3a** Tartumaa County, Järvselja Dendrological park (58°16'02''N, 27°18'06''E).

**3b** Tartumaa County, Järvselja to Rõkka (58°15'N, 27°18'E). Old forests of *Oxalis-Myrtillus* and *Vaccinum uliginosum* types (forest squares 259–261), mixed forests of *Oxalis-Myrtillus* type (forest squares 273–274).

6 Sept. 1999

**4** Põlvamaa County, Kiidjärve (58°08'N, 27°02'E) to Taevaskoja (58°07'N, 27°03'E), Taevaskoja Landscape Reserve, east side of the valley of Ahja River. Coniferous forests mainly of *Pinus* and *Picea*, of *Oxalis*, *Myrtillus* and *Vaccinium uliginosum* types.

7 Sept. 1999

**5** Tartumaa County, Agali arboretum (58°17'4''N, 27°17'22''E).

**6** Tartumaa County, Emajõe Suursoo (Great Fen of Emajõgi) Reserve, from Lake Leegu (58°22'N, 27°17'E) to Apnasaar mineral bog island (58°21'N, 27°18'E). Old mixed forests of *Oxalis*, *Myrtillus* and *Oxalis-Myrtillus* types.

**7** Tartumaa County, Ahunapalu cemetery (58°19'N, 27°18'E).

## LIST OF SPECIES

! = new to Estonia, # = lichenicolous fungus. Collectors: PH = Pekka Halonen, MK = Martin Kukwa, PL = Piret Lõhmus, LM = Ljudmilla Martin, JM = Jurga Motiejunaite, TR = Tiina Randlane, AS = Andres Saag, AT = Arne Thell. Herbaria: BILAS = University of Vilnius, ICEB = International Centre for Environmental Biology, Tallinn Pedagogical University, OULU = University of Oulu, TU = University of Tartu, TUR = University of Turku, UGDA-L = lichen herbarium, University of Gdansk.

!Absconditella lignicola Vezda & Pisút – **2**: forest square 224, on decorticated wood in trench, LM (ICEB).

Arthonia byssacea (Weigel) Almq. – **6**: forest square 19, on *Quercus* sp., MK (TU, UGDA-L). – Rather rare in Estonia (Randlane & Saag, 1999).

Bacidina arnoldiana (Körb.) V. Wirth & Vezda – **6**: forest square 19, on stumps, MK (TU, UGDA-L). – Very rare in Estonia, previously known only from western islands (Randlane & Saag, 1999).

Biatora ocelliformis (Nyl.) Arnold – **2**: forest square 242, on trunk of *Tilia cordata* in mixed forest, JM (BILAS). – Rather rare in Estonia (Randlane & Saag, 1999).

Chaenotheca gracillima (Vain.) Tibell – **3a**: on rotten stump, PH & PL (OULU, TU). – This is the third locality for the species in Estonia (see Lõhmus, 1998).

Cladonia caespiticia (Pers.) Flörke – **2**: forest square 242, on fallen tree in mixed old growth forest, LM (ICEB). – This is the fourth locality for the species in Estonia.

Cladonia norvegica Tønsberg & Holien – **3b**: forest square 261, on stump, MK (UGDA-L). – Very rare in Estonia (Randlane & Saag, 1999).

!#Clypeococcum hypocenomycis D. Hawksw. – **3b**: forest square 261, on *Hypocenomyce scalaris* growing on *Pinus sylvestris*, MK (UGDA-L).

!#Corticifraga fuckelii (Rehm) D. Hawksw. & R. Sant. – **6**: forest square 17, on *Peltigera didactyla* growing on soil, MK (UGDA-L), JM (BILAS, TU).

Cybebe gracilenta (Ach.) Tibell – **2**: forest square 227, on lignum of old *Populus tremula*, PL (BILAS, TU, UGDA-L); **3b**: forest square 274, on lignum at base of *Picea abies*, PL (no specimen). – Before the symposium the species was known only from one locality in NE Estonia.

Evernia divaricata (L.) Ach. – **6**: forest square 19, on branch of *Picea abies*, AT & PH (TU). – The species has earlier been rather frequent in Estonia, but it has strongly decreased during recent decades (Randlane & Saag, 1999).

Evernia mesomorpha Nyl. – **7**: on base of *Betula* sp., PH & TR (TU). – Rather rare in Estonia (Randlane & Saag, 1999).

Flavoparmelia caperata (L.) Hale – **7**: on dead *Juniperus* tree, PH, TR, AT & AS (OULU, TU, TUR). – This is the fourth locality for *F. caperata* in Estonia (T. Randlane, personal comments) and three of them have been found only recently. Thus the category of threatened lichens concerning this species (i.e. extinct or probably extinct) should be changed.

Gyalecta truncigena (Ach.) Hepp – **3b**: forest square 261, on trunk of old *Populus tremula* in mixed old growth forest, JM (BILAS), MK (UGDA-L), LM (ICEB). – This is the second locality for *G. truncigena* in Estonia. The species has earlier been found from the vicinity of Tartu growing on *Quercus robur* (Thor & Nordin, 1998).

!#Hobsonia christiansenii B. L. Brady & D. Hawksw. – **1**: on *Physcia s. lat.* growing on branches of *Crataegus* sp., MK (UGDA-L); **6**: forest square 19, on *Physcia* sp. growing on *Populus tremula*, MK (UGDA-L).

Hypocenomyce sorophora (Vain.) P. James & Poelt – **3b**: forest square 260, on trunks of *Pinus sylvestris* in boggy pine forest, JM (BILAS, TU), MK (UGDA-L); **4**: 1 km SE of Kiidjärve, on *Pinus sylvestris*, MK (UGDA-L). – Reported as very rare in Estonia, previously known only from western islands (Randlane & Saag, 1999). The species is probably common in Estonian bog forests.

!Japewia subaurifera Muhr & Tønsberg – **6**: forest square 19, on trunk of *Alnus glutinosa* in swampy alder forest, JM (BILAS).

Lecidea nylanderi (Anzi) Th. Fr. – **2**: forest square 242, on *Betula* sp., MK (UGDA-L); **3b**: forest square 274, on *Betula* sp., MK (TU, UGDA-L). – Reported as rare in Estonia and previously known only from three localities in western regions, possibly overlooked (Suija, 1998).

Lepraria neglecta (Nyl.) Erichsen – **4**: 3.5 km SE of Kiidjärve, on granite stone, MK (UGDA-L), TLC: alectorialic acid and atranorin. – Reported as rare in Estonia, previously known only from five localities in western regions (Saag & Saag, 1999).

#Lichenoconium erodens M. S. Christ. & D. Hawksw. – **1**: on *Hypogymnia physodes* growing on *Betula* sp., JM (BILAS); **2**: forest square 226, on *Parmelia sulcata* growing on *Populus tremula*, MK (UGDA-L); forest square 241, on *Parmeliopsis ambigua* growing on *Pinus sylvestris*, MK (UGDA-L); forest square 242, on *P. sulcata* growing on *P. tremula*, and on *Cladonia ochrochlora*, MK (UGDA-L). – Reported as very rare in Estonia (Randlane & Saag, 1999), but the species is probably common in Estonia.

!#Lichenoconium lecanorae (Jaap) D. Hawksw. – **1**: on *Lecanora carpinea* growing on *Quercus robur*, JM (BILAS); **6**: forest square 19, on *Lecanora albella* growing on *Alnus incana*, MK (UGDA-L).

#Lichenoconium xanthoriae M. S. Christ. – **1**: on apothecia of *Xanthoria polycarpa* and *Physcia stellaris* growing on branches of *Crataegus* sp., MK (UGDA-L). – Very rare in Estonia (Randlane & Saag, 1999).

Micarea hedlundii Coppins – **6**: forest square 19, on rotten stump in pine forest, JM (BILAS). – Very rare in Estonia (Randlane & Saag, 1999).

Micarea misella (Nyl.) Hedl. – **2**: forest square 242, on rotten stump in mixed old growth forest, LM (ICEB). – This is the fourth locality for the species in Estonia.

MICAREA PELIOCARPA (Anzi) Coppins & R. Sant. – **4**: ca 1 km SE of Kiidjärve, on shaded side of siliceous stone in forest, JM (BILAS). – Rather rare in Estonia (Randlane & Saag, 1999).

!PELTIGERA DIDACTYLA var. EXTENUATA (Vain.) Goffinet & Hastings – **5**: over mosses on soil, MK (UGDA-L). – New variety to Estonia. *P. didactyla* var. *extenuata* differs from var. *didactyla* by the former's usually larger size and normally abundant soralia. Furthermore, var. *extenuata* contains gyrophoric acid (C+ red; seen on soralia), while var. *didactyla* does not contain secondary lichen substances. However, the morphological and chemical differences between these two varieties are not totally cleared yet (O. Vitikainen, personal comments). The world distribution of var. *extenuata* is very wide and the variety is common in northern Europe (O. Vitikainen, personal comments).

PERTUSARIA PUPILLARIS (Nyl.) Th. Fr. – **1**: on *Salix* sp., MK (UGDA-L). – Reported as rare in Estonia (Randlane & Saag, 1999).

!#PHAEOPYXIS PUNCTUM (A. Massal.) Rambold, Triebel & Coppins – **2**: forest square 242, on *Cladonia chlorophaea s. lat.* growing on wood, MK (UGDA-L).

PLACYNTHIELLA DASAEA (Stirt.) Tønsberg – **2**: forest square 242, on wood, MK (UGDA-L); **4**: 1 km SE of Kiidjärve, on humus, MK (UGDA-L). – Reported as very rare in Estonia, previously known only from two localities in western islands, possibly overlooked (Suija, 1998).

PORINA AENEA (Wallr.) Zahlbr. – **2**: forest square 226, on *Populus tremula*, MK (UGDA-L). – Very rare in Estonia, previously known only from western islands (Randlane & Saag, 1999).

!PORPIDIA SOREDIZODES (Nyl.) J. R. Laundon – **1**: on granite stone in open locality, MK (UGDA-L), TLC: stictic acid complex; **4**: ca 1 km SE of Kiidjärve, on siliceous stone in forest, JM (BILAS).

!#REFRACTOHILUM PELTIGERAE (Keissl.) D. Hawksw. – **6**: forest square 17, on *Peltigera didactyla* growing on soil, MK (UGDA-L).

RHIZOCARPON LAVATUM (Fr.) Hazsl. – **3b**: forest square 261, on granite boulder in mixed old growth forest near trench, LM (ICEB). – This is the second locality for the species in Estonia. Previously known from western islands (Randlane & Saag, 1999).

!SCOLICIOSPORUM PRUINOSUM (P. James) Vezda – **6**: forest square 19, on *Alnus incana*, MK (UGDA-L).

!#SPHAERELLOTHECIUM PROPINQUELLUM (Nyl.) Roux & Triebel – **1**: on apothecia of *Lecanora carpinea* growing on trunk of *Quercus robur*, JM (BILAS).

!STEINIA GEOPHANA (Nyl.) Stein – **6**: forest square 17, on sandy soil in old sandpit, JM (BILAS).

!#SYZYGOSPORA PHYSCIACEARUM Diederich – **5**: on *Physcia stellaris* growing on *Populus* sp., MK (UGDA-L).

!#TREMELLA HYPOGYMNIAE Diederich & M. S. Christ. – **1**: on *Hypogymnia physodes* growing on *Quercus robur*, JM (BILAS); **2**: forest square 242, on *H. physodes* growing on *Populus tremula*, MK (UGDA-L); **6**: forest square 19, on *H. physodes* growing on *Alnus glutinosa* in swampy alder forest, JM (BILAS).

!#TREMELLA RAMALINAE Diederich – **1**: on *Ramalina fraxinea* growing on *Tilia cordata*, MK (UGDA-L). – This is the third locality for the species in the world and second in Europe (see Diederich, 1996).

USNEA WASMUTHII Räsänen – **1**: on ground, fallen from tree, PH (OULU), TLC: usnic and barbatic acids. – Rather rare in Estonia (Randlane & Saag, 1999).

VERRUCARIA HYDRELA Ach. – **4**: ca 3 km SE of Kiidjärve, on siliceous boulders along riverbed, JM (BILAS). – Trass (1970) reported the species from Estonia, but any herbarium material has not been seen.

!VERRUCARIA PRAETERMISSA (Trevis.) Anzi – **4**: ca 3 km SE of Kiidjärve, on siliceous boulders along riverbed, JM (BILAS).

#VOUAUXIELLA LICHENICOLA (Linds.) Petr. & Sydow – **1**: on *Lecanora chlarotera* growing on *Abies* sp., MK (UGDA-L). – Very rare in Estonia, previously known only from NW regions (Randlane & Saag, 1999).

#VOUAUXIOMYCES SANTESSONII D. Hawksw. – **2**: forest square 227, on *Platismatia glauca* growing on dry branches of *Picea abies*, JM (BILAS); **3b**: forest square 260, on *P. glauca* growing on *Betula* sp., JM (BILAS); **4**: 1 km SE of Kiidjärve, on *P. glauca* growing on *Betula* sp., MK (UGDA-L); **6**: forest square

19, on *P. glauca* growing on dry branches of *P. abies*, MK (UGDA-L). – Reported as rather rare in Estonia (Randlane & Saag, 1999), but the species is probably very common in Estonia.

## ACKNOWLEDGEMENTS

We thank the Organising Committee of the symposium for the well-organised programme and excursions. All the other participants are also thanked for their nice company and co-operation.

## REFERENCES

Ahti, T., Hämet-Ahti, L. & Jalas, J. 1968. Vegetation zones and their sections in northwestern Europe. *Ann. Bot. Fennici* 5: 169–211.

Bruttan, A. 1870. Lichenen Est-, Liv- und Kurlands. *Arch. Naturk. Liv-, Ehst- u. Kurl. Zweiter Serie* 7: 163–326.

Diederich, P. 1996. The lichenicolous Heterobasidiomycetes. *Bibl. Lichenol.* 61: 1–198.

Ekman, S., Fröberg, L., Kärnefelt, I., Sundin, R. & Thor, G. 1991. New or interesting lichens from Estonia. *Folia Cryptog. Estonica* 28: 1–32.

Laasimer, L. 1965. *Vegetation of the Estonian S. S. R.* (in Estonian with English summary). Tallinn.

Lõhmus, P. 1998. List of Estonian calicioid lichens and fungi. *Folia Cryptog. Estonica* 32: 43–46.

Mereschkowski, K. 1909. *About the knowledge of lichens in the vicinity of Reval* (in Russian). Kazan.

Mereschkowski, K. 1913. *The checklist of lichens in the Baltic provinces* (in Russian). Kazan.

Randlane, T. 1998. Red list of Estonian macrolichens. *Folia Cryptog. Estonica* 32: 75–79.

Randlane, T. & Saag, A. (eds) 1999. Second checklist of lichenized, lichenicolous and allied fungi of Estonia. *Folia Cryptog. Estonica* 35: 1–132.

Räsänen, V. 1931. Die Flechten Estlands. *Ann. Acad. Sci. Fenn. Ser. A4*, 34(4): 1–163.

Saag, L. & Saag, A. 1999. The genus *Lepraria* (*Lichenes imperfecti*) in Estonia. *Folia Cryptog. Estonica* 34: 55–63.

Suija, A. 1998. Lecideoid lichens from Estonia. *Folia Cryptog. Estonica* 32: 107–112.

Thor, G. & Nordin, A. 1998. 16 lichens new to Estonia. *Folia Cryptog. Estonica* 32: 123–125.

Trass, H. 1970. The elements and development of the lichen flora of Estonia (in Russian). *Transactions of Tartu State Univ. 268, Pap. Bot.* 9: 5–233.

Trass, H. & Randlane, T. (eds) 1994. *Macrolichens of Estonia* (in Estonian). Tartu.

Folia Cryptog. Estonica, Fasc. 36: 23–29 (2000)

# Air quality assessment of Kaunas city using method of passive lichenoindication

**Nomeda Juceviciene & Vilma Valaikaite-Domarkiene**
Department of Environmental Sciences, Vytautas Magnus University, 8 Vileikos, LT-3035 Kaunas, Lithuania.
E-mail: nomeda_juceviciene@fc.vdu.lt

**Abstract**: Lichen indication studies were carried out in Kaunas city in 1996–1998. 62 taxa of epiphytic lichens were registered. Number of species and average coverage of epiphytic communities was determined on the base of 1x1 km grid. Lichen indication map of Kaunas city was compiled where three zones can be identified using the Index of Poleotolerance (I.P.). Results obtained allowed to propose modifications in the dislocation of air pollution monitoring stations in Kaunas.

**Kokkuvõte**: N. Juceviciene ja V. Valaikaite-Domarkiene. Kaunase linna õhu kvaliteedi hindamine passiivse lihhenoindikatsiooni meetodil.

Lihhenoindikatsiooniline uurimus Kaunase linnas viidi läbi aastatel 1996–1998. Registreeriti 62 taksonit epifüütseid samblikke. Määrati liikide arv ja epifüütsete koosluste keskmine katvus võrgustiku ruutudes sammuga 1 x 1 km. Poleotolerantsuse indeksit kasutades koostati Kaunase linna lihhenoindikatsiooniline kaart, milles eraldati kolm tsooni. Saadud tulemused võimaldasid esitada soovitusi Kaunases asuvate õhu saastatuse seirejaamade asukoha muutmiseks.

## INTRODUCTION

Epiphytic lichens are known as sensitive bioindicators of atmospheric air pollution for more than a century (Ferry et al., 1973). Since 1926 several indicational mapping techniques have been used to identify the distribution of air pollution in cities and industrial regions. Different characters of lichen species and communities have been applied for air quality assessment, such as morphological changes in transplanted lichen thalli, vitality, accumulation of pollutants in thalli, species composition and diversity in the communities, frequency of different species, general and specific coverage, etc. (Sernander, 1926; Skye, 1958; Brodo, 1961; Gilbert, 1965; de Wit, 1976). Passive lichen monitoring is one of the most widely implemented approaches (Stolte et al., 1993). This approach is based on quantitative analysis of occurrence of epiphytic lichen species and their coverage on the phorophytes in selected territories (Trass, 1973; Martin & Martin, 1974; Kauppi & Halonen, 1992; Straupe & Piterans, 1996). Lichen zones can be distinguished by calculation of indices of lichen communities (De Sloover & LeBlanc, 1968; Trass, 1968) or by using other mathematical methods and mapping techniques (McCune et al., 1997; Dietrich & Scheidegger, 1997 ).

In Lithuania lichens as bioindicators have been used only since 1995 by the authors of the present paper. At first, the green areas of Kaunas and a transect crossing the city were investigated (Skorobogataite & Valaikaite, 1996). The poleotolerance (*sensu* Trass, 1973) of 33 epiphytic lichens was determined and preliminary lichen zones were established for the studied area.

Kaunas, the second largest city in Lithuania with a territory of 157,15 km$^2$ and population close to 416 000 (in 1998), is located in the central part of Lithuania, on the confluence of the two largest Lithuanian rivers Nemunas and Neris (Fig. 1). The complex of river valleys and adjacent lowlands form a specific landscape. Kaunas is also an industrial centre, important junction of railway and highways. The industrial area, approximately 10 % of the territory of the city, is located in the southern part of it. Nitrogene compounds, dust and formaldehydes which are mainly produced by traffic (Juknys & Zukauskaite, 2000) are the main atmospheric pollutants in Kaunas.

Municipal Environmental Monitoring Program in Kaunas was launched in 1993. This program includes pollution assessment of atmospheric air, drinking and surface water, soil,

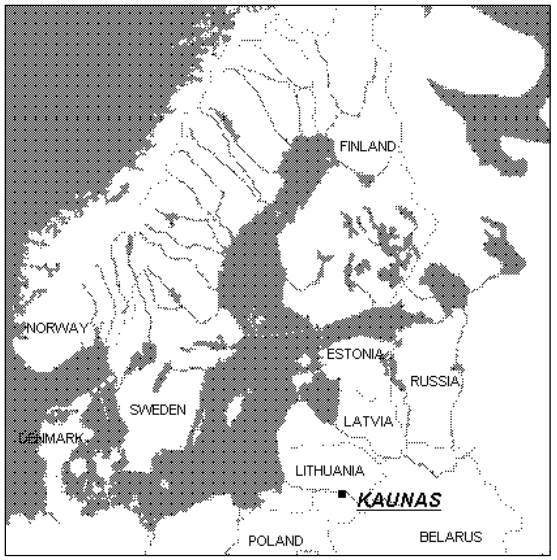



**Fig. 1.** Location of Kaunas city in the Baltic region

and vegetation. Atmospheric air quality is annually investigated in Kaunas in three stationary and twelve temporal stations for air monitoring (Kameneckas, 2000).

The purpose of the present study was to evaluate the state of complex atmospheric air pollution in the whole area of Kaunas city and to present the first results of bioindicational zonation.

## MATERIAL AND METHODS

The research for the present paper was carried out in 1996–1998. The grid of 1 x 1 km squares was used; 127 squares covered the whole territory of Kaunas city. In each square up to 10 trees were chosen, mostly *Tilia*, *Acer* and *Pinus* which are the dominating tree species in Kaunas area. *Tilia* and *Acer* are rather similar in the properties of bark (pH, bark structure) while *Pinus* has a different character of the bark with lower value of pH and softer squamulose structure (Barkman, 1958; Wirth, 1995). Data on Scots pines were included into the database because of the lack of deciduous trees in some areas of Kaunas (e.g. in Panemune and Kalnieciai districts). In study sites only separately standing trees with faultless, upright, solitary truncs with diameter of 20–40 cm were selected for the description of lichen communities. Epiphytic lichen communities were described by means of 20 x 20 cm sampling frame positioned at the northern side of a trunk at the breast height (1.3 m from base). All epiphytic lichen species were registrated for every sample as well as the specific and total coverage was identified.

Samples of all epiphytic lichens inside of the frame were collected for laboratory identification (about 3600 specimens in total). Lichen species were identified using traditional methodology which included morphological and anatomical observations as well as chemical spot tests and measurements under the transmission light microscope. Nomenclature of lichens used in the present paper follows R. Santesson (1993).

Classes (degrees) of poleotolerance for every species were established using the ordination of taxa according to the distances of the squares from the city centre where the corresponding species was registered (Martin & Martin, 1974 ). Considering the assumption that the squares in the centre of the city have the highest level of atmospheric pollution in the area and the squares in the outskirts of the city – the lowest, the appearance of registered lichen species was ordinated as shown in Table 1 and the numbers of classes of poleotolerance were conferred. In the studied area the lowest degree of poleotolerance was 3 and the highest was 8 while the degree 1 on the total scale corresponds to a pristine (non polluted natural area) and degree 10 – to heavily polluted area.

The Index of Poleotolerance (I.P.) proposed by Trass (1968, 1973) was used to characterize lichen communities and to reveal lichen zones. All the calculations were made using the following formula:

$$I.P. = \sum_{i=1}^{n} \frac{a_i c_i}{C_n}$$

where n – number of lichen species in sample frame, $a_i$ – class of poleotolerance of species i, $c_i$ – coverage of species i, and $C_n$ – total coverage of the lichen community.

I.P. was calculated for every sample (tree) and then averaged for each square of the grid. The average values of I.P. were used for zonation of the Kaunas territory.

**Table 1.** Definition of classes of poleotolerance ($a_i$) for lichen species recorded in Kaunas city

| No. | Lichen species | Distance from the centre of the city (km) 1 | 2 | 3 | 4 | 5 | 6 | $a_i$ |
|---|---|---|---|---|---|---|---|---|
| 1. | *Bacidia rubella* (Hoffm.) A. Massal. | | | | | | + | **3** |
| 2. | *Candelariella reflexa* (Nyl.) Lettau | | | | | | + | **3** |
| 3. | *Catillaria nigroclavata* (Nyl.) Schuler | | | | | | + | **3** |
| 4. | *Chaenoteca ferruginea* (Turner & Borrer) Mig. | | | | | | + | **3** |
| 5. | *Cladonia fimbriata* (L.) Fr. | | | | | | + | **3** |
| 6. | *Dimerella pineti* (Ach.) Vezda | | | | | | + | **3** |
| 7. | *Lecanora expallens* Ach. | | | | | | + | **3** |
| 8. | *Lecidea nylanderi* (Anzi) Th. Fr. | | | | | | + | **3** |
| 9. | *Micarea prasina* (Fr.) | | | | | | + | **3** |
| 10. | *Parmeliopsis ambigua* (Wulfen) Nyl. | | | | | | + | **3** |
| 11. | *Ramalina fastigiata* (Pers.) Ach. | | | | | | + | **3** |
| 12. | *Ramalina fraxinea* (L.) Ach. | | | | | | + | **3** |
| 13. | *Usnea hirta* (L.) F. H. Wigg. | | | | | | + | **3** |
| 14. | *Xanthoria candelaria* (L.) Th. Fr. | | | | | | + | **3** |
| 15. | *Evernia prunastri* (L.) Ach. | | | | | + | | **3** |
| 16. | *Graphis scripta* (L.) Ach. | | | | | + | | **3** |
| 17. | *Melanelia fuliginosa* (Fr. ex Duby) Essl. | | | | | + | | **3** |
| 18. | *Lecanora leptyrodes* (Nyl.) Degel. | | | | + | | | **3** |
| 19. | *Lecidella euphorea* (Flörke) Hertel | | | | + | | | **3** |
| 20. | *Phaeophyscia endophoenica* (Harm.) Moberg | | | + | | | | **3** |
| 21. | *Caloplaca holocarpa* (Hoffm. ex Ach.) A. E. Wade | | | | | + | + | **4** |
| 22. | *Physconia enteroxantha* (Nyl.) Poelt. | | | | | + | + | **4** |
| 23. | *Ramalina farinacea* (L.) Ach. | | | | | + | + | **4** |
| 24. | *Rinodina pyrina* (Ach.) Arnold | | | | + | + | | **4** |
| 25. | *Lecanora dispersa* (Pers.) Sommerf. | | | | + | | + | **4** |
| 26. | *Lecanora piniperda* Körb. | | | | + | | + | **4** |
| 27. | *Lecanora argentata* (Ach.) Malme | | | + | + | | | **4** |
| 28. | *Candelariella xanthostigma* (Ach.) Lettau | | | + | | | + | **4** |
| 29. | *Strangospora moriformis* (Ach.) Stein | | | + | | | + | **4** |
| 30. | *Melanelia exasperatula* (Nyl.) Essl. | | | + | | + | | **4** |
| 31. | *Phaeophyscia nigricans* (Flörke) Moberg | + | | | | | + | **4** |
| 32. | *Buellia punctata* (Hoffm.) A. Massal | | | | + | + | + | **5** |
| 33. | *Lecania cyrtella* (Ach.) Th. Fr. | | | | + | + | + | **5** |
| 34. | *Lepraria spp.* | | | | + | + | + | **5** |
| 35. | *Phlyctis argena* (Spreng.) Flot. | | + | | + | + | | **5** |
| 36. | *Physcia stellaris* (L.) Nyl. | | + | | | + | + | **5** |
| 37. | *Candelariella vitelina* (Hoffm.) Mull. Arg. | + | | | + | + | | **5** |
| 38. | *Physcia adscendens* (Fr.) H. Olivier | + | | | | + | + | **5** |
| 39. | *Melanelia subaurifera* (Nyl.) Essl. | + | + | + | | | | **5** |
| 40. | *Lecanora carpinea* (L.) Vain. | | | + | + | + | + | **6** |
| 41. | *Bacidia naegelii* (Hepp) Zahlbr. | | | + | + | + | + | **6** |
| 42. | *Lecanora symmicta* (Ach.) Ach. | | | + | + | + | + | **6** |
| 43. | *Lecidella elaeochroma* (Ach.) M. Choisy | | | + | + | + | + | **6** |
| 44. | *Scoliciosporum chlorococum* (Stenh.) Vezda | | + | + | + | | + | **6** |
| 45. | *Physcia dubia* (Hoffm.) Lettau | + | + | | | + | + | **6** |
| 46. | *Phaeophyscia orbicularis* (Neck.) Moberg | + | | + | | + | + | **6** |
| 47. | *Xanthoria polycarpa* (Hoffm.) Th. Fr. ex Rieber. | + | + | + | | + | | **6** |
| 48. | *Lecanora conizaeoides* Nyl. ex Cromb. | | + | + | + | + | + | **7** |
| 49. | *Hypogymnia physodes* (L.) Nyl. | + | | + | + | + | + | **7** |
| 50. | *Lecanora pulicaris* (Pers.) Ach. | + | | + | + | + | + | **7** |
| 51. | *Lecanora hagenii* (Ach.) Ach. | + | + | + | + | + | + | **8** |
| 52. | *Hypocenomyce scalaris* (Ach.) M. Choisy | + | + | + | + | + | + | **8** |
| 53. | *Lecanora chlarotera* Nyl. | + | + | + | + | + | + | **8** |
| 54. | *Parmelia sulcata* Taylor | + | + | + | + | + | + | **8** |
| 55. | *Physcia tenella* (Scop.) DC. | + | + | + | + | + | + | **8** |
| 56. | *Xanthoria parietina* (L.) Th. Fr. | + | + | + | + | + | + | **8** |

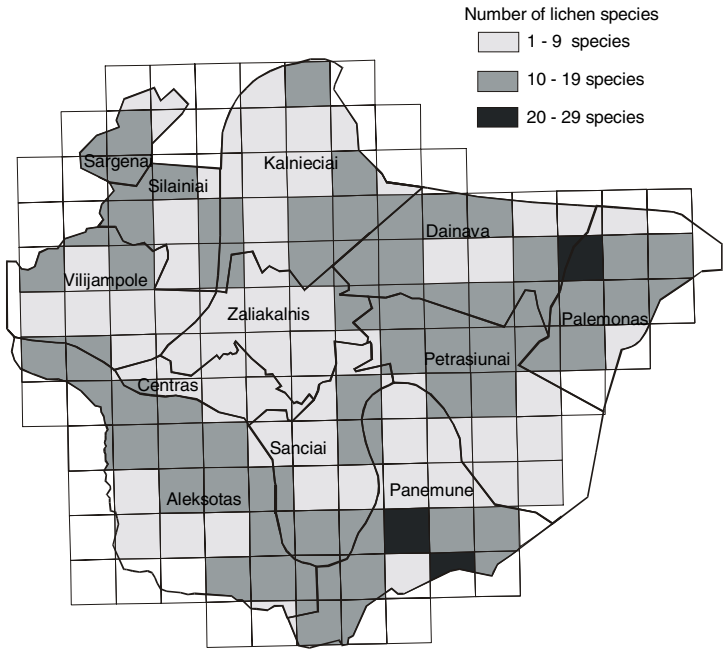



**Fig. 2.** Specific diversity of epiphytic lichens per assesment square.

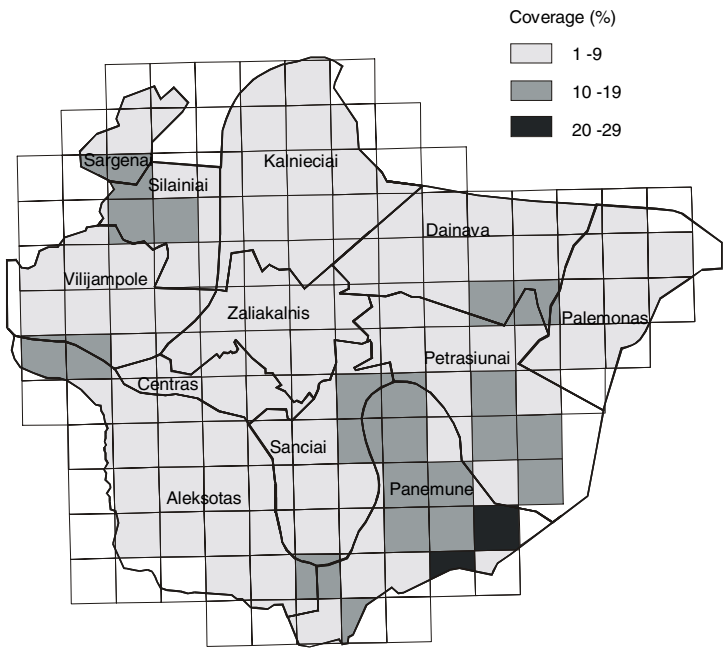



**Fig. 3.** Average coverage of epiphytic lichen communities in Kaunas city.

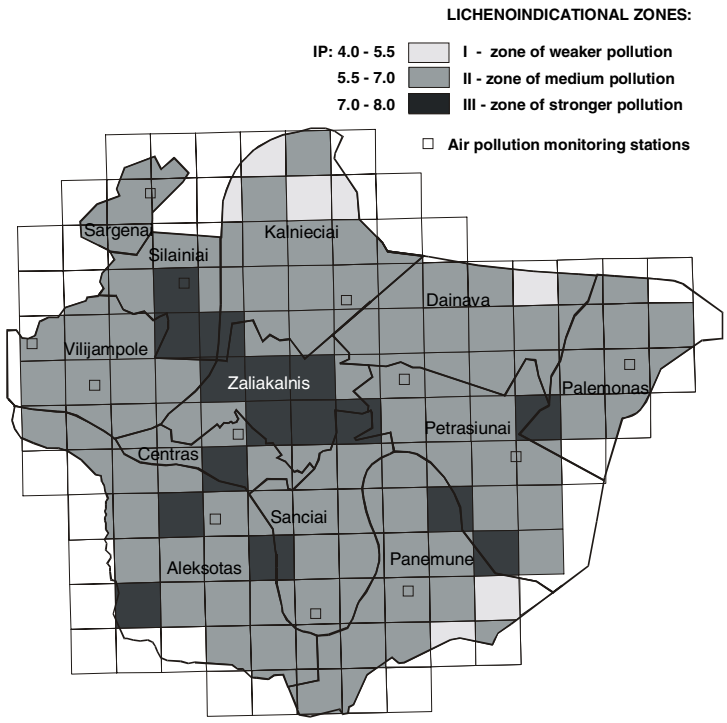



**Fig. 4.** Lichen indication zones of atmospheric air pollution in Kaunas city.

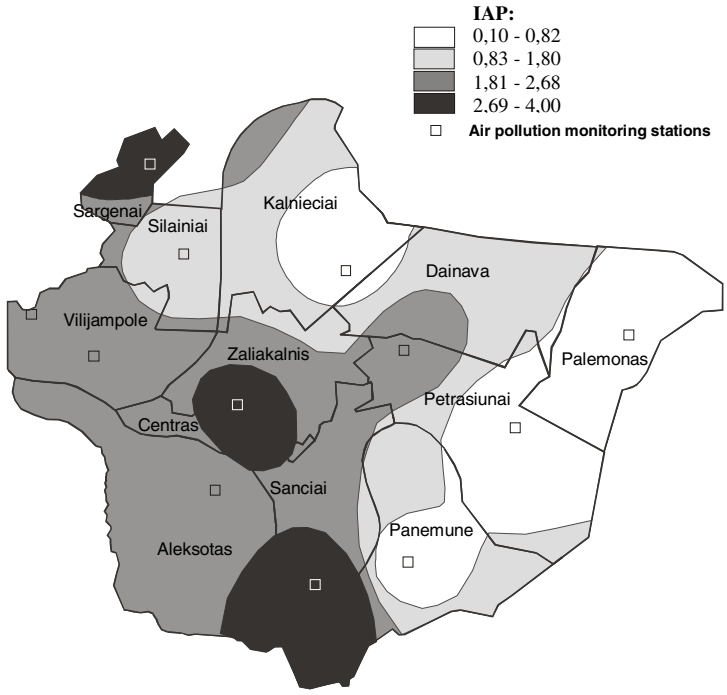



**Fig. 5.** Integrated zones of air pollution in Kaunas in 1998 (Kameneckas, 2000).

## RESULTS AND DISCUSSION

62 epiphytic lichen species were identified in Kaunas city (Table 1). Foliose and crustose lichens were met all over the investigated area while fruticose species were observed in five or more kilometers away from the centre of the city. The group of species tolerant to the air pollution (classes 7 and 8 in Table 1) occurring also in the central parts of the Kaunas city consists of nine species including *Lecanora conizaeoides, Hypogymnia physodes, Lecanora pulicaris, Lecanora hagenii, Hypocenomyce scalaris, Lecanora chlarotera, Parmelia sulcata, Physcia tenella* and *Xanthoria parietina*. The group of species sensitive to air pollution (class 3 in Table 1) includes 20 taxa of epiphytic lichens. Among them some fruticose taxa such as *Cladonia fimbriata, Ramalina fastigiata, R. fraxinea, Usnea hirta* and *Evernia prunastri* were registered. These species were found only in the periferial districts of the city (at least six km further from the centre of the city).

### Species diversity of the epiphytic lichen flora in the Kaunas area

The number of species per square was used for the mapping purposes. According to the map (Fig. 2), the number of lichen species increased gradually in the direction away from the centre of the city. The lowest numbers of species per one square was observed in the centre as well as in the northern and south-eastern areas of Kaunas. In the latter areas Scots pine forests are mainly distributed. Furthermore, these areas are located in the river valleys where the impact of air pollution on lichens is considerably higher due to the inversions in humid and cold seasons of the year. The highest number of epiphytic species was found in the outskirts of the city where the maximum number of taxa was 29 per square.

### Average coverage of epiphytic lichen communities

The average coverage of epiphytic lichens per grid square (1 x 1 km) varied from 1 to 29% in the studied area (Fig. 3). It is well seen that the communities with the highest values of coverage are located in the preriferial areas of the city (e.g. Panemune district) while the average coverage did not exceed 10% per grid square in the most parts of the territory.

### Lichen indication mapping

The lichen indication map of Kaunas city was compiled using the Index of Poleotolerance (I.P.) proposed by Trass (1968). I.P. expresses the weighted average coverage and poleotolerance of noted lichen species in the community. The estimation of classes of poleotolerance (degrees for lichen species) was explained in Material and Methods. In this study we used the data presented by Skorobogataite & Valakaite (1996) with the following amendments: (i) newly registered species were processed as described in Methods, (ii) lichen specimens identified to the level of genus only were not considered; therefore the list of lichen species presented in Table 1 includes 56 species (and not 62 as mentioned above), (iii) estimations of poleotolerance for some species were checked and changed according to the latest data.

Three zones of atmospheric pollution can be distinguished according to the lichen indication map of Kaunas city (Fig. 4). The variation of values of I.P. is rather limited exceeding from 4.0 to 8.0. The zone indicating lower pollution of air ( I.P. = 4.0–5.5) is very restricted. All the squares of this zone are located in the periferial districts around the city. The most part of the territory (105 squares from 127) belong to the zone of medium pollution. The zone of higher pollution which is mainly located in the central area of the city and valley of the Nemunas River occupies about 13% of the whole territory. Area without any lichen species ( “lichen desert”) has not been found in Kaunas.

Another map of Kaunas city has been compiled on the basis of the Integrated Index of Air Pollution (Fig.5, according to Kameneckas, 2000). The Integrated Index of Air Pollution is calculated using average daily concentrations of airborne pollutants such as dust, nitrogen oxides, sulphur dioxide, and formaldehydes (Kameneckas, 2000). The comparison of two maps (Figs 4 & 5) which had been composed using quite different methods demonstrated

considerable similarity. Some differences in contours of zones on these maps are evidently caused by the different methodological approaches: in our study the variables reflecting response of living organisms on pollution impact were considered while the map by Kameneckas is compiled using direct data of pollutants. On the base of the analysis of both maps, it is possible to propose a few corrections in dislocation of some air pollution monitoring stations in Kaunas: (1) it is sufficient to have only one station (instead of two) in Vilijampole district which is rather homogeneous regarding air pollution; (2) it is advisable to establish additional air monitoring stations in three other districts: Zaliakalnis, Dainava, and Panemune.

## ACKNOWLEDGEMENTS

We thank our supervisors dr. J. Motiejunaite and habil. dr. R. Juknys for their useful comments and support. We are also greatly indebted to dr. L. Martin and dr. J. Martin for inspiration to carry out this project.

## REFERENCES

Barkman, J. J. 1958. *Phytosociology and ecology of cryptogamic epiphytes, including a taxonomic survey and description of their vegetation units in Europe*. Assen. 598 pp.

Brodo, I. M. 1961.Transplant experiments with corticolous lichens using a new technique. *Ecology* 42: 838–841.

De Sloover, J. & LeBlanc, F. 1968. Mapping of atmospheric pollution on the basis of lichen sensitivity. In *Proc. of the Symposium on Advances in Tropical Ecology*, pp. 42–56. Varanasi.

de Wit, T. 1976. *Epiphytic Lichens and Air Pollution in the Netherlands*. J.Cramer, Vaduz. 226 pp.

Dietrich, M. & Scheidegger, C. 1997. Frequency, diversity and ecological strategies of epiphytic lichens in the Swiss Central Plateau and the Pre-Alps. *Lichenologist* 29 (3): 237–258.

Ferry, B. W., Baddeley, M. S. & Hawksworth D. L.(eds) 1973. *Air pollution and lichens*. The Athlone Press of the University of London, London. 389 pp.

Gilbert, O. L. 1965. Lichens as indicators of air pollution in the Tyne Valley. *Ecology and Industrial Society*. Oxford, 35–47.

Juknys, R. & Zukauskaite, I. 2000. Main trends of air pollution in Kaunas (in Lithuanian). In *Kaunas city ecological monitoring '99* (eds Juknys R., Kameneckas J. & Kilikevicius G.), pp. 7–15.

Kameneckas, J. 2000. The assesment of Kaunas high air polluted zones and percente of rezidents in these zone (in Lithuanian). In *Kaunas city ecological monitoring '99* (eds Juknys R., Kameneckas J. & Kilikevicius G.), pp. 16–27.

Kauppi, M. & Halonen, P. 1992. Lichens as indicators of air pollution in Oulu, northern Finland. *Ann. Bot. Fennici* 29: 1–10.

McCune, B., Dey, J., Peck, J., Heiman, K. & Will-Wolf, S. 1997. Regional gradients in lichen communities of the Southeast United States. *Bryologist* 100: 145–158.

Martin, L. & Martin, J. 1974. Comparison of two epiphytic lichen community indices reflecting air pollution. *Folia Cryptog. Estonica* 6: 47–48.

Santesson, R. 1993. *The lichen and lichenicolous fungi of Sweden and Norway*. SBT-förlaget, Lund. 240 pp.

Sernander, P. 1926. *Stockholms natur*. Uppsala.

Skorobogataite N. & Valaikaite, V. 1996. Identification of air pollution in Kaunas city (Lithuania) by the passive method of lichenoindication. In *Fungi and lichens in the Baltic region, 13th International Conference on Mycology and Lichenology, Abstracts*, pp. 81–82. Riga.

Skye, E. 1968. Lichens as biological indicators of air pollution. *Acta Phytogeogr. Suec.* 52: 1–123.

Stolte, K., Mangis, D., Doty, R. & Tonnessen, K. 1993. *Lichens as Bioindicators of Air Quality*. USDA Forest Service General Technical Report RM-224, 131 pp.

Straupe, I. & Piterans, A., 1996. Lichenoindicational analysis of Jelgava. In *Fungi and lichens in the Baltic region, 13th International Conference on Mycology and Lichenology, Abstracts*, pp. 82–83. Riga.

Trass, H. 1968. Samblikud – õhu saastatuse indikaatorid (in Estonian). *Eesti Loodus* 2: 80–83.

Trass , H. 1973. Lichen sensivity to the air pollution and index of poleotolerance (I.P.). *Folia Cryptog. Estonica* 3: 17–34.

Wirth, V. 1995. *Flechtenflora*. Ulmer, Stuttgart. 661 pp.

Folia Cryptog. Estonica, Fasc. 36: 31–46 (2000)

# Application of flow cytometry for measurement of nuclear DNA content in fungi

**Bellis Kullman**
Institute of Zoology and Botany, Estonian Agricultural University, 181 Riia St., 51014 Tartu, Estonia.
E-mail: bellis@zbi.ee

**Abstract:** Methods were developed for quantitative evaluation of the nuclear DNA content of fungal cells by flow cytometry. Cells of *Saccharomyces cerevisiae* (YAC M3 strain), the spore print of *Pleurotus ostreatus* (TAA 142824) and conidia of *Trichophaea hemisphaerioides* (TFC 97-71) are applicable as standards for fungal flow cytometry.

Measurements of the same specimens from different laboratories using different methods were compared. The value of nuclear DNA content of *P. ostreatus*, obtained through the primary standard *S. cerevisiae,* was 25.0 Mb with the use of laser , and 24.0 Mb with the use of HBO lamp.

Comparative measurements of *T. hemisphaerioides* were carried out with two species, *S. cerevisiae* and *P. ostreatus,* respectively using staining with PI. In the first case measurement was performed through intact cells and the obtained nuclear DNA content of *T. hemisphaerioides* was 22.2 Mb. In the second case measurment was performed through intact nuclei and the obtained nuclear content was 23.3 Mb. Measurements by experimental series, performed with the use of the same technique and fluorochrome, can be valued highly in estimation slight differences between aneuploids.

Flow cytometric studies show improved resolution when the complex fluorochrome DAPI SR101 was used. Two subpopulations in the spore print of *P. ostreatus*, differing in DNA content by 4.9 Mb (20%), were not detected when only PI staining or DAPI staining was used. Staining with DAPI SR101 is essential in the study of aneuploidy and especially in genetic characterizing of heteroploids (nonhaploids) derived by intraspecific hybridisation. Diversity of the nuclear DNA content of a spore print reflect the fate of chromosome length polymorphism of hybrid genomes during meiosis. This simple and precise method can prove highly promising in the analysis of DNA in the fundamental and applied cytogenetic research of the fungal cell as well as in the systematics of fungi.

**Kokkuvõte:** B. Kullman. Läbivoolu-tsütomeetria rakendamine seente rakutuuma DNA-sisalduse uurimisel.

Seene rakutuuma DNA-sisalduse kvantitatiivseks määramiseks arendati läbivoolu-tsütomeetriat. Leiva-pärmseene *Saccharomyces cerevisiae* (YAC M3) rakud, austerserviku *Pleurotus ostreatus* (TAA 142824) eosproov ja *Trichophaea hemisphaerioides* (TFC 97-71) koniidid puhaskultuurist on kasutatavad standarditena seente läbivoolu-tsütomeetrias.

Samu eksemplare mõõdeti erinevates laboratooriumites erinevate meetoditega ning võrreldi saadud tulemusi. Kasutades läbivoolu-tsütomeetriat erinevate värvidega (PI, DAPI) vastavalt laseri või HBO-lambiga mõõtes, saadi austerserviku tuuma DNA-sisalduseks 25.0 Mb ja 24.0 Mb, primaarseks standardiks olid pärmi rakud.

Liigi *T. hemisphaerioides* tuuma DNA-sisaldus mõõdetuna intaktsetest rakkudest või intaktsetest tuumadest saadi vastavalt 22.2 Mb või 23.3 Mb. Esimesel juhul kasutati standardina pärmi rakke, teisel juhul austerserviku eoseid. Aneuploidide vaheliste väikeste erinevuste kindlakstegemiseks tuleb teha seeriaviisilisi mõõtmisi samu meetodeid ja fluorokroome kasutades.

Erinevust austerserviku eosproovi kahe alampopulatsiooni vahel (4.9 Mb / 20%) ei avastatud, kui kasutati ainult üht fluorokroomi (PI või DAPI). Läbivoolutsütomeetria lahutusvõime paranes kompleksfluorokroomi DAPI SR101 kasutamisel. Värvimine kompleksiga DAPI SR101 on oluline aneuploidsuse uurimisel ja eriti liikidevahelise hübridisatsiooni käigus tekkivate heteroploidide (mittehaploidid) geneetiliseks iseloomustamiseks. Lahknemine eosproovi tuumade DNA-sisalduses peegeldab seene hübriidse genoomi saatust meioosi käigus. Kirjeldatud meetodit saab kasutada seeneraku fundamentaalsete ja rakenduslike tsütogeneetiliste uuringute jaoks ning seente süstemaatikas.

## INTRODUCTION

Nuclear DNA amount and genome size are important biodiversity characters, the study of which has both practical and theoretical uses in biology. DNA amount is relatively constant and tends to be highly characteristic of a species, making it a useful character in plant systematics (Bennett & Leitch, 1998). General correlation of genome size with cell size and mitotic cycle time indicates the pivotal role of genome size in many aspects of evolution and adaptation (see Dolezhel et al., 1998, for a review). The total DNA content of the unreplicated haploid nuclear genome is known as its 1C-value (Swift, 1950).

**Study of chromosome count.** Among the eucaryotes, fungi have the smallest nuclei, commonly with a diameter of 1–3 µm (Kamaletdinowa & Vassilyev, 1982). Fungal chromosomes are poorly studied due to their size, which is close to the resolution power limit of the light microscope (0.24 µm). As a result, chromosome count is difficult to determine directly, and the literature offers contradictory reports regarding chromosome numbers for various species. According to the data of light microscopy, the number of haploid chromosomes is small (2–28) (Rogers, 1973; Bresinsky et al., 1987b; Wittmann-Meixner, 1989), the suggested basic number being 4. Several authors (Rogers, 1973; Bresinsky et al., 1987b; Weber, 1992) regard the data on the fungal chromosome number, obtained by light microscopy, as doubtful. Pulsed-field gel electrophoresis (PFGE) is a complicated method for counting chromosomes in fungal taxonomy (Mills & McCluskey, 1990). Also, the largest chromosomes are close to the resolution limit for electrophoretic karyotyping (Kuldau et al., 1999). Hence there arises the need to determine the DNA content of the fungal genome (haploid chromosome set of the nucleus) for the establishment of ploidy levels.

**Indirect methods of studying genome size.** An indirect method to obtain ploidy levels consists in the analysis of nuclear DNA content after staining with a fluorochrome and comparative evaluation of the data of different species. Microfluorometry is used to estimate the amount of DNA in haploid nuclei, which allows to assess genome size for taxonomic purposes (Durán & Gray, 1989). Microspectrophotometry (cytophotometry) and microfluorometry (cytofluorometry) of nuclear DNA play an important role in providing quantitative data. Different fluorochromes are used. To determine relative DNA content, cytometric measurements of nuclear Feulgen-DNA content (Peabody et al., 1978; Peabody & Peabody, 1984; Tooley & Therrien, 1987; Therrien et al., 1989), DAPI-DNA content (Bresinsky et al., 1987a,b; Wittmann-Meixner, 1989; Wittmann-Meixner & Bresinsky, 1989; Wittmann-Meixner et al.1989; Weber & Bresinsky, 1992; Weber, 1992; Bresinsky & Wittmann-Bresinsky, 1995; Bresinsky et al., 1999), mithramycin-DNA content (Fischer, 1987; Baiman & Collins, 1990) and propidium iodide-DNA content (Tetsuka et al., 1984) have been used.

By means of these methods it is possible to measure relative DNA amount in the nucleus: the ratio of the result of the measurement of the organism to be studied to that of the standard organism is calculated. A fungus which chromosome number is known serves as the standard organism. After determining relative DNA content for neighbouring species in any genus, possible differences in ploidy levels in the karyotype can be found (Geber & Hsibeder, 1980).

Using cytofluorometric investigation of various fungi, Bresinsky et al., (1987b) showed a stability of the relative DNA content of nuclei within different strains and varieties; there are however, significant differences between species within a genus and between genera within an order. Correlation between relative nuclear DNA content, chromosome number and ploidy levels in several fungal divisions were tested by Wittmann-Meixner et al. (1989). Intraspecific variation in DNA amount, which is not exceptional in plant species, is quite rare in fungal species (Ascomycetes and Basidiomycetes) (Wittmann-Meixner, 1989; Weber, 1992).

Although cytofluorometry allows to study ploidy levels and taxonomic relationships it has specific limits (Wittmann-Meixner et al., 1989; Arnold, 1993; Bresinsky & Wittmann-Bresinsky, 1995).

To measure the size of the plant or animal genome, the flow cytometer (fluorescence-activated cell sorter – FACS) has found ever wider application. FACS is a convenient and rapid method for estimation of genome size. FACS has been used in case of some unicellular fungi. In case of yeast it was used to study its cell size and variability of DNA content (Talbot & Wayman, 1989; Smeraldi, 1994; Porro et al., 1995; Polacheck et al., 1995; Compagno et al., 1996; Gift, 1996; Joosten et al., 1996; Petit et al., 1996; Prudêncio et al., 1998). The genetic characteristics of the Dekkera yeast strain, isolated from sherries, as well as of a number of other *Brettanomyces* and *Dekkera* strains have been described on the bases of DNA content per cell (Ibeas, et al., 1996).

FACS was used to characterize the isolates of *Phialophora gregata* proceeding from the fluorescence intensity of propidium iodide

stained conidia. The isolates differed in their mean fluorescence intensity, ranging from 100.0 to 129.7 arbitrary units (a.u.) (Gourmet et al., 1997). FACS has also been used in the study of arbuscular mycorrhizal *(Glomus mosseae*) and non-mycorrhizal tomato roots infected or uninfected with *Phytophthora nicotianae* var. *parasitica*. Presence of the pathogen may result in DNA loss and condensation. Infection by either fungus (symbiotic or pathogen) reduced the ratio of 4C to 2C nuclei in the differentiated root (Lingua et al., 1996). Using flow cytometry, the *Scutellospora castanea* genome was estimated at 1 pg (965 Mb) (Zeze et al., 1996). Regrettably, no literature data are available on the earlier application of FACS to filamentous ascomycetes and basidiomycetes.

The aim of the present study was to adopt appropriate techniques and standards for estimation of genome size in fungi using flow cytometry.

## RESEARCH TECHNIQUE, DEVICES AND EQUIPMENT

**Flow cytometric analysis.** Detailed principles of flow cytometry are described in Gray et al. (1987), in Darzynkiewicz (1994) and in Bennett & Leitch (1995). To obtain a reliable result, about 100000 intact cells are needed. Nuclei are stained with fluorescence stains (fluorochromes) bound to DNA; a fluorochrome is bound to DNA quantitatively. In the cytometer, the cell suspension under study passes, as a fine jet, the laser beam, inducing excitation in the fluorochrome-DNA complex. The intensity of arising fluorescence is measured with the photodetector separately for each cell. The measured intensity is proportional to the amount of fluorescent substance and hence also to DNA content in cells.

Flow cytometric analysis of stained nuclei is used to characterize the diversity of specimens as well as to study their cell-cycle phase distributions.

The method relies on a single-time measurement of cell populations of one sample. Analysis can be either univariate, based on the measurement of DNA content alone, or multivariate, involving other cell characters besides DNA. Flow cytometric analysis of scattering light (FSC and SSC) depends on cell size, morphology and structure. Hence, although such measurements *per se* cannot reveal whether each individual cell actually progresses through the cell cycle or not, kinetic information can be inferred from DNA content (position of the cell cycle). Progression through the S-phase and mitosis (cytokinesis) is expressed by changes in cellular DNA content. The position of the cell in the cycle can therefore be estimated on the basis of measurement of DNA content (Fig. 4).

The resulting fluorescence histograms can be analysed for calculating the difference in nuclear DNA content between the specimens. By including an internal standard, relative DNA content is converted to absolute amount (Figs 5 and 6; 7 and 8). The absolute DNA content of an unknown specimen is obtained by dividing the mean relative DNA content of the unknown G1 population by the mean of the standard G1 population and by multiplying the result by the absolute DNA content of the standard. Flow cytometry is also useful in the measurement of cell and nuclear size with the aim to study the relationship between the two parameters and DNA amount (Fig. 11).

**Fluorochromes.** To analyse DNA content, mostly the fluorochromes 4,6'-diamidino-2-phenylindole (DAPI), which binds at AT-rich regions, and propidium iodide (PI), which intercalates into double-stranded DNA, are used. Dolezhel et al. (1992) found that the difference between the DNA contents measured with PI and DAPI, was statistically highly significant, and concluded that the use of base preferring fluorohromes can lead to errors.

Maximum excitation of DAPI, bound to DNA, is at 359 nm, and its maximum emission is at 461 nm. The HBO lamp supplied with a UG1 excitation filter serves as the source of excitation.

Maximum excitation of PI, bound to DNA, is at 536 nm and its maximum emission is at 617 nm. For excitation, an argon-ion laser emitting at 488 nm (blue light lines) appears optimal. A fluorescence detector is used to analyse the light emitted by stained cells at 610 nm.

PI has often been used to test nonviable cells. Dead cells with injured membranes can incorporate PI, which stains DNA in the nucleus. DAPI stains viable cells as well.

**Calibration standards for C-values of fungal DNA.** An organism in which DNA content has been measured by some other means, can be used as an standard. Applicability of chicken erythrocytes (2C=2.33pg) is generally accepted. (DNA amount is usually expressed in picograms (pg) or in megabase pairs of nucleotides (Mb) (NB 1pg=965 Mb, see Bennet & Leitch, 1995.) However, the C-value of chicken erythrocytes is too high for measuring fungal genome size. At setting a standard, it is desirable to select a taxon which DNA amount is similar to that of the unknown taxon, since larger than two-to threefold differences between the genome size of the standard and that of the unknown taxon may increase technical error (Dolezhel et al., 1992). In an ideal case, only one strain of a standard species from a single source should be used for calibration, which improves comparability of the results obtained at different laboratories.

Durán & Gray (1989) used *S. cerevisiae* as a benchmark organism in cytofluorometry (1.5 x $10^{10}$ Dalton ⇒ 22 Mb of DNA per haploid nucleus) and estimated that among 72 species of fungi haploid DNA ranged from 13.5 x $10^{9}$ to 95.8 x $10^{9}$ Dalton ⇒ 20 Mb to 142 Mb (1pg≈0.65 x $10^{12}$ Dalton, see Nagel, 1976). The complete sequence of *S. cerevisiae* was determined in 1996 in international collaboration within the project HUGO (ftp.ebi.ac.uk). The total genome size of *S. cerevisiae*, strain S288C, is 13.10502 Mb.

Another standard used for fungi is *Pleurotus ostreatus* (Bresinsky et al., 1987b; Wittmann-Meixner, 1989). Using various analytical techniques several authors have reported different chromosome numbers and genome sizes for *P. ostreatus*.

Horgen et al. (1984) reported that the haploid genome of *P. ostreatus* contains three classes of DNA sequences: (1) unique 23.4%; (2) repetitive 5.19%; and (3) foldback 5.19%. The unique DNA sequences was equal to 21 Mb. Using these data, Wittmann-Meixner (1989) calculated total genome size at 29.4 Mb.

Basing on PFGE experiments, Sagawa and Nagata (1992), Peberdy et al.(1993) and Larraya at al. (1999) reported the chromosome numbers of 6, 9 and 11; total genome size of 20.8 Mb, 31.3 Mb and 35.0; chromosome sizes ranging from 2.1 to 5.2 Mb, 1.1 to 5.7 Mb and 1.4 to 4.7 Mb per chromosome, respectively.

## MATERIAL

Cells of the yeast *Saccharomyces cerevisiae* (Meyen ex Reese) Hansen (YAC M3 strain), spore print of the oyster mushroom *Pleurotus ostreatus* (Jacq.: Fr.) P. Kumm. (TAA 142824; TAA 157761), *Phellinus punctatus* (P. Karst.) Pilát (TAA 152336), and conidia from a pure culture of the discomycete *Trichophaea hemisphaerioides* (Mouton) Graddon (TFC 97-71 from TAA 147708) deposited in Tartu, were used in the study. When the total genome size of *S. cerevisieae*, strain S288C, is 13.105 Mb, then the genome size of the YAC M3 strain, containing an artificial yeast chromosome of 0.55 Mb, is 13.665 Mb. This strain was provided by Dr. Th. Pötter (Westfälische Wilhelms-Universität, Institut für Strahlenbiologie), who obtained it from Max Planck Institute for Molecular Genetics in Berlin where it had arrived from ICRF (in London).

Polybead fluorescent microspheres (Cat# 17156, diameter 5.2 µm, SO=0.3) were used in the serial measurement of nuclear DNA amount. Measurement accuracy was enhanced by adding polybead fluorescent microspheres to the samples to be compared. In an ideal case, they remain in the same channel and can be used also for measurement of the size of nuclei and cells.

## EXPERIMENTAL DESIGN

At Rostock University and at Tartu University, the flow cytometer Becton Dickinson FACSort was employed. At Münster University, the flow cytometer Particle Analysing System (PAS) was employed which allowed to use both the laser (staining with PI) and the HBO lamp (staining with DAPI) in measurement of nuclear DNA content. The flow cytometer has linear fluorescence amplification with forward-scatter (FSC) and side-scatter (SSC) detection. Cytometers were equipped with the software package PAS (Partec) or CellQuest (Becton Dickinson). The number of nuclei in cells was determined using the fluorescence microscope Olympus.

## RESULTS

Five procedures were adapted (see appendix) for quantitative evaluation of DNA content of the intact cells, spores and conidia of fungi by

flow cytometry. Nuclear DNA content was measured in the same fungal specimens at different laboratories using different cytometers. Nuclei were stained in the cell or isolated chemically, or mechanically. A different set of reagents was used. The material was either fixed (procedures 3 and 5) or unfixed (procedures 1, 2, 4 and 6). For dissolving cell membrane lipids, for eliminating the cell cytoskeleton and nuclear proteins and for digesting cellular RNA, a nonionic detergent (procedure 3) and the enzymes zymolyase (procedures 2, 4), trypsin (procedure 3), proteinase K (procedure 1) and pepsin (procedure 5) were used. For stabilizing nuclear chromatin, spermine was used (procedure 5). Fluorochromes PI and DAPI SR101 are bound to isolated nuclei or/and to nuclei on cells. The fluorescence of PI is dependent on pH and on the ionic strength of solution. Stained samples should be analysed with the flow cytometer within three hours (procedure 3) or within some days (procedure 1, 2, 4) of staining with PI. After adding the fluorochrome DAPI SR101 (procedure 5), optimum time for measurement, yielding stable results, is 35±10 min.

**1. Staining with PI.**

1.1. Application of procedure 1 (PAS equipped with a laser, staining with PI, Münster University) (Figs 1–4). Use of *S. cerevisiae* (Y) as standard for measurement of the DNA content of *P. ostreatus* (P) and *T. hemisphaerioides* (T).

Measurement was performed by experimental series (Table 1). In the first experiment with PV and Y, two yeast cell suspensions, Y1 and Y2, were used. Cells in Y1 were identified as belonging to the subpopulations G1 and G2M of the cell cycle phase, since DNA content in the second subpopulation is two times larger than in the first one. Cells in Y2 are in the S-phase of the cell cycle, since they are larger by their DNA content and size than cells in Y1, which are in the G1 phase of the cell cycle. The nuclei of PV are also in the G1 phase (Fig. 13). The C-value of PV (G1) in relation to Y1 (G1) was calculated at 25 Mb.

The spore prints of two specimens of *P. ostreatus*, PV and PU, were studied comparatively. The C-value of PU in relation to the C-value of PV was determined as 27.9 Mb. The genome size of PU is 10% (2.9 Mb) and the volume 20 % larger than the respective parameters of PV.

In an experiment with T and Y1, the C-value of T in relation to the C-value of the YAC M3 strain was determined at 22.2 Mb. (Fig. 4. Nuclei in conidia of T were predominantly in the G1 phase of the cell cycle).

1.2. Application of procedure 2 (Becton Dickinson FACSort, University of Tartu). (Specimens are designed as in Table 1.) CV of DNA content per spore of PV were obtained routinely at 7%–11%.

Using linear or logarithmic signal gain the ratio the DNA content of PV to that of Y2 1.6 remained constant when different instruments (FACSort or PAS) and procedures (1 and 2) were used. Procedure 2: CV of DNA content per cell of PV was 7% and per cell of Y2, 10% (Figs 5, 6) (compare with the results of procedure 1, Table 1).

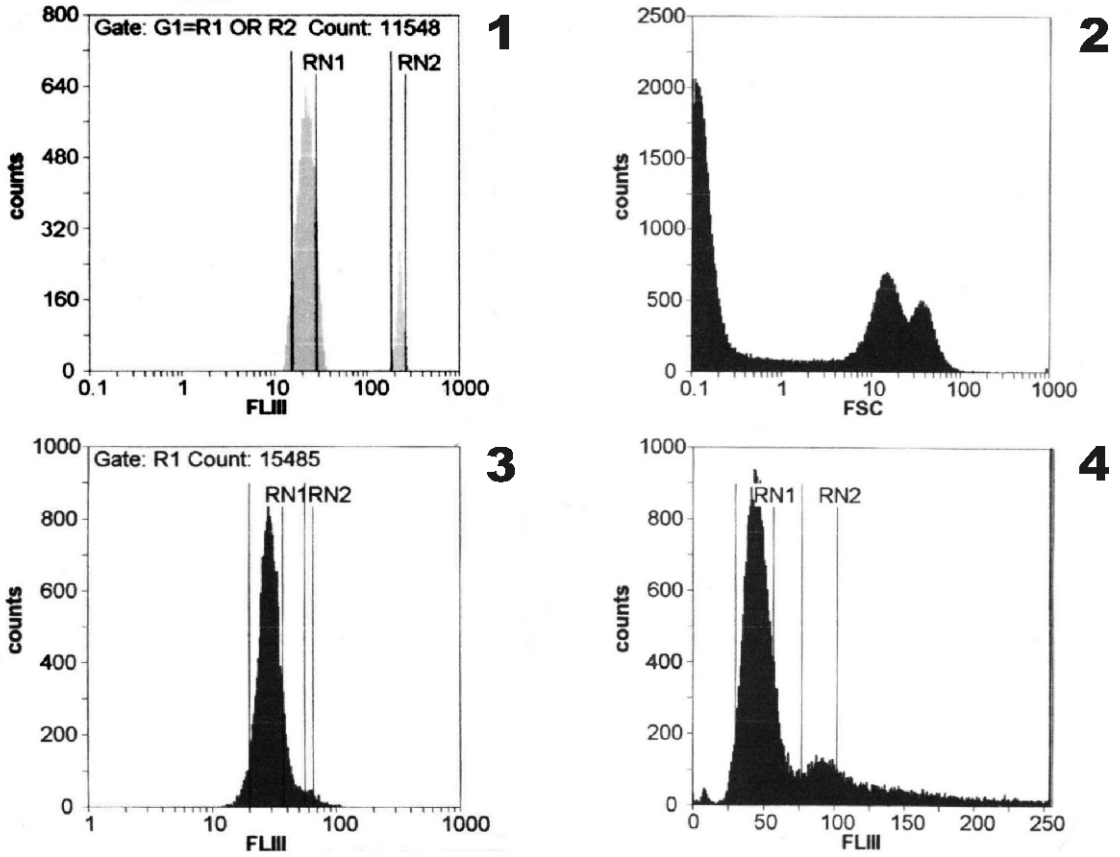
Spore prints of PV and *P. punctatus* were studied comparatively (Figs 7, 8). Spores of *P. punctatus* (M2) were mixed with the sample of the internal standard PV (M1) before staining (Fig. 8). The C-value of *P. punctatus* was identified to be 70.2 Mb.

1.3. Application of procedure 3 (Becton Dickinson FACSort, Rostock University). DNA analyses of *P. ostreatus* and *T. hemisphaerioides*, prepared with the Cycle TEST™ PLUS DNA Reagent Kit, were employed (Figs 9, 10). This reagent was used for PV as specified for unfixed cells. CV of DNA content per spore of PV was 15% and CV of DNA content per intact nucleus was 14%. Majority of spores remained whole. The ratio of the mean fluorescence intensity of spores to that of nuclei was 1.4, which means that cytoplasma was not clear. When zymolyase was added during this procedure, shaking gently, the number of released nuclei increased. CV of DNA content per spore increased to 22% (Fig. 9), while the ratio remained the same.

Gatings of nuclei were used for comparative analysis of the DNA content of PV and T in the same sample. The C-value of T was calculated as 23.3 Mb.

1.4. Application of procedure 4 (PAS equipped with a laser, Münster University) (Figs 11-14).

When procedure 4 was applied, one-third of nuclei were released from spores. The fluo-




**Figs 1–4.** Staining with PI using procedure 1. – **1.** Flow histogram of relative fluorescence intensity (FLIII) obtained from the analysis of *P. ostreatus* (RN1) and polybead fluorescent microspheres (RN2) using procedure 1B. Coefficient of variation (CV) of the mean value of the fluorescence of spores is 16%. – **2.** Volume-frequency histogram of *S. cerevisiae* cells (Y1). Cells in cell cycle phase G1 are represented by the first peak and cells in cell cycle phase G2M, by the second peak (see also Table 1). – **3.** DNA content-frequency histogram of *S. cerevisiae*, obtained using procedure 1C. Before fixing the cells were stored for some days at 4°C. Most of the cells are in cell cycle phase G1 (denoted as RN1, CV 14%). Ratio RN2/RN1=2.1. – **4.** DNA content-frequency histogram of *T. hemisphaerioides*, obtained using procedure 1A. Most of the cells are in cell cycle phase G1 (denoted as RN1, CV 15%). Ratio RN2/RN1=2.0.

rescence intensity of spores was somewhat higher than that of nuclei: 6.7 a.u. and 6.5 a.u., respectively. Mean spore size is 2.3 fold larger than mean nuclear size (Table 2: (1)).

Different fluorescence intensities were obtained when a mechanical method for release of nuclei was used after chemical treatment (Table 2: (2)). In the dot plot of FLIII and FSC (Fig. 11), groups of spores, nuclei and polybead fluorescent microspheres were separated with gating into regions, denoted as R1, R2 and R3, respectively, and analysed. Rinsing decreases the fluorescence intensity of both spores and nuclei, and increases the variability (CV) of spore fluorescence and size. It is evident that the relative size of damaged spores was 6.1 fold larger than that of nuclei: 10.4 µm and 1.7 µm, respectively (calculation of size was made in relation to polybead fluorescent microspheres with a diameter of 5.2 µm). The size of spores increases (up 15 a.u. to 30.7 a.u.), while that of clean nuclei decreases (up 6.5 a.u. to 4.7 a.u.). Mean spore size for the species *P. ostreatus* was measured at 8.8 x 3.3 µm (Hilber, 1982).

**Table 1.** Measurement of the C-values *of P. ostreatus* and *T. hemisphaerioides*. Y – cells of *S. cerevieae* YAC M3 strain, cell subpopulation Y1 in the cell cycle phase G1 was used as the standard (C-value=13.655Mb). PV and PU – spore prints of two specimens of *P. ostreatus, TAA* 142824 and 157761, respectively. T – conidia from a pure culture of *T. hemisphaerioides*. Measurements are presented as peak means on fluorescence intensity histograms (FLIII), or as region means on tot plots (FLIII/FSC). The accuracy of measurement is expressed as the coefficient of variation (CV) of the mean value of the fluorescence of a uniform population, e.g. CV of the DNA content of G1 cells.

| Cell subpopulation | DNA-mean (Mb) | CV-DNA % | Ratio of DNA means | Size mean | CV-size % | Ratio of size means |
|---|---|---|---|---|---|---|
| PV(G1) | 25.0 | ±23% | 1.8(PVG1/Y1G1) | 10.4 | ±33% | |
| Y1(G1) | 13.655 | ±18% | 1.6 (PVG1/Y2S) | 9.3 | ±22% | 1.1(PVG1/Y1G1) |
| Y1(G2M) | 28.7 | ±22% | 2.1(Y1G2M/Y1G1) | 20.6 | ±24% | 2.2(Y1G2M/Y1G1) |
| Y2(S) | 15.7 | ±21% | 1.2 (Y2S/Y1G1) | 9.9 | ±23% | 1.1 (Y2S/Y1G1) |
| PV(G1) | 25.0 | ±19% | | 10.4 | ±19% | |
| PU (G1) | 27.9 | ±14% | 1.1(PUG1/PVG1) | 12.1 | ±19% | 1.2(PUG1/PVG1) |
| Y1(G1) | 13.655 | ±18% | 2.0 (TG1/TG2M) | | | |
| T(G1) | 22.2 | ±15% | 1.6 (TG1/Y1G1) | | | |
| T(G2M) | 44.6 | ±12% | | | | |

When during procedure 4, steps including treatment with Proteinase K were introduced after treatment with zymolyase and RNase, then spores of *Phellinus igniarius* remained whole and spore fluorescence intensity was measured at 20.1 a.u. CV 20% instead of 20.3 a.u. CV18%, and the spore size was measured at 13.3 a.u. CV 21% instead of 14.3 a.u. CV 24%.

**2. Staining with the DNA and protein specific fluorochrome DAPI SR101.**

Application of procedure 5 (PAS equipped with a HBO lamp, Münster University) (Figs 15–19). *S. cerevisiae* (Y1) was used as the standard. The subpopulation of yeast cells in the cell cycle phase G1 was identified, and the C-value of PV was measured to be 24.0 Mb.

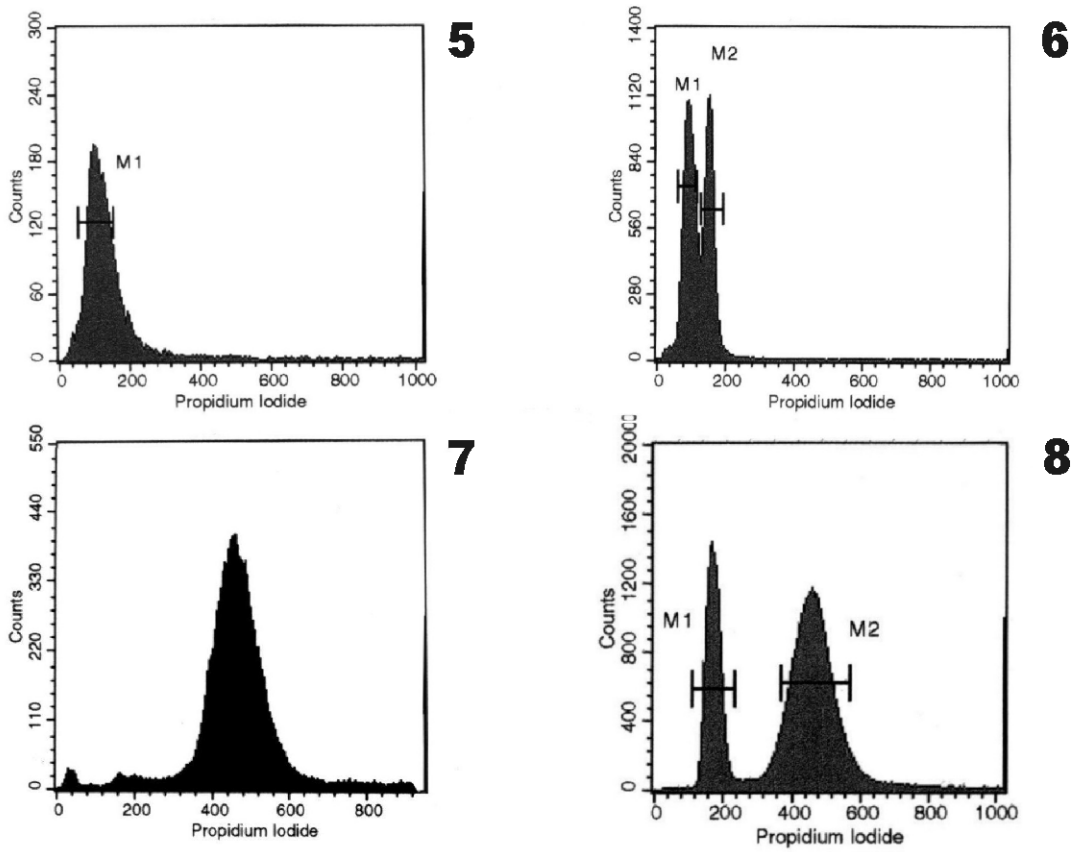
The results of the analysis of two *P. ostreatus* samples (PV and PU) are presented on Table 3 (Fig. 19). As the genome size of PV was measured as 24 Mb, then genome size in the first subpopulation of the spore print of PU was 30.2 Mb and in the second subpopulation 25.3 Mb (difference 4.9 Mb, or 19%). The spores of PV differ from one subpopulation of PU in a smaller DNA content and from the other

**Table 2**. Relative fluorescence intensity and relative size of spores (sp) and nuclei (nu) of *P. osteratus* (PV) stained with PI. Measurements: (1) – after application of procedure 4; (2) – the same sample after adding polybead fluorescent microspheres and careful rinsing of the suspension using the Pasteur pipette (Fig. 11); (3) – after more intensive rinsing.

| Sub-population | Fluo-rescence mean (a.u.) | CV-fluo-rescence % | Ratio of fluo-rescence means sp/nu | Size mean | CV-size % | Ratio of size means sp/nu |
|---|---|---|---|---|---|---|
| (1)PVsp | 6.7 | ±15% | | 15.0 a.u. | ±13% | |
| (2)PVsp | 4.7 | ±25% | | 10.4 μm | ±16% | |
| (3)PVsp | 4.2 | ±41% | | 10.4 μm | ±16% | |
| (1)PVnu | 6.5 | ±12% | 1.0 | 6.5 a.u. | ±13% | 2.3 |
| (2)PVnu | 5.8 | ±15% | 0.8 | 1.7 μm | ±21% | 6.1 |
| (3)PVnu | 5.8 | ±18% | 0.7 | 1.8 μm | ±33% | 5.7 |

**Table 3.** Analysis of the spore prints of two *P. ostreatus* specimens (PV and PU), stained with DAPI SR101. Subpopulation of spores are marked in the dot plot of DAPI SR101 as regions (R) for analysis of their relative DNA and protein contents (Fig. 19). The distribution of these parameters per sample is presented in Figs 15–18.

| Spore sub-population | DNA mean (Mb.) | CV-DNA % | Ratio of DNA means | Protein mean (a.u.) | CV-protein % | Ratio of protein means |
|---|---|---|---|---|---|---|
| PVR1 | 24.0 | ±11% | PUR2/PVR1 1.26<br>PUR3/PVR1 1.05 | 28.4 | ±20% | PUR2/PPV1 1.0<br>PUR3/PVR1 0.6 |
| PUR2 | 30.2 | ±10% | PUR2/PUR3 | 28.3 | ±15% | PUR2/PUR3 |
| PUR3 | 25.3 | ±9% | 1.2 | 17.9 | ±15% | 1.6 |




**Figs 5–8.** Staining with PI using procedure 2. – **5.** DNA content-frequency histogram of *S. cerevisiae* (Y2). Cells are in cell cycle phase S (denoted as M1, CV19%). – **6.** DNA content-frequency histogram of *S. cerevisiae* (Y2) and *P. ostreatus* (PV). Cells of *S. cerevisiae* (denoted as M1, CV10%) and spores of P. ostreatus (denoted as M2, CV 7%). – **7.** DNA content-frequency histogram of *Phellinus punctatus*. – **8.** DNA content-frequency histogram of *P. ostreatus* (PV) and *P. punctatus*. Spores of *P. ostreatus* (denoted as M1, CV 10%) and spores of *P. punctatus* (denoted as M2, CV 11%).

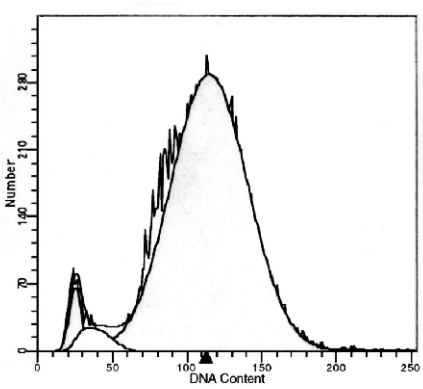



**Fig. 9.** DNA content-frequency histogram of *P. ostreatus* (PV), obtained using procedure 3 with treatment with zymolyase. CV of the mean value of the fluorescence of spores (bigger peak) is 22%.

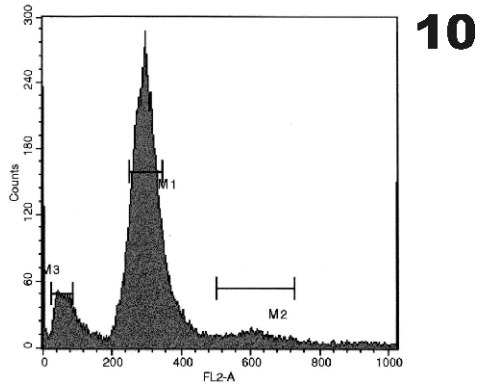



**Fig. 10.** DNA content-frequency histogram of *T. hemisphaerioides*. Before using procedure 3, conidia were fixed with Carnoy B. Most of the cells are in cell cycle phase G1 (denoted as M1, CV 8%). Ratio M2/M1=2.0.

subpopulation of PU in a smaller protein content. The subpopulation of PU differed more on the basis of protein content (1.6 times) than on the basis of DNA content (1.2 times).

Spores within the spore print of PU differ also microscopically: means of spore dimensions were 12.3 x 4.4 µm and 13.9 x 5.1 µm. Spores of the first group had a thin wall and granulated cytoplasma and spores of the second group had a thick wall and homogeneous cytoplasma.

## DISCUSSION

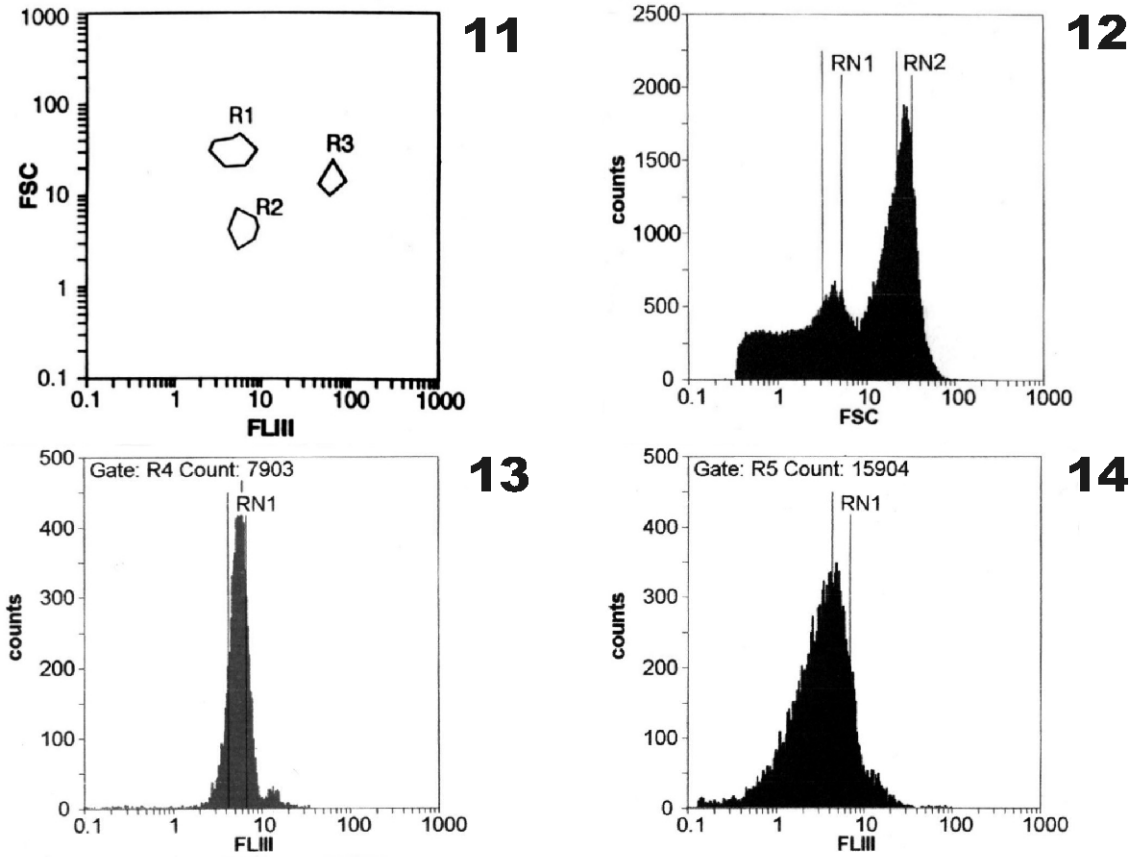
The resolution of the DNA histogram, i.e. coefficient of variation, of about 7–11% was obtained routinely from the spore print of *P. ostreatus* using procedures 2 (staining with PI, Figs 6, 8) and 5 (staining with DAPI SR101, Figs 15–19). The most important difference between procedure 2 and the other staining procedures with PI is the long-time slow continuous vertical rotation of the first. It ensures that all cells are treated similarly and remain whole. This method has been introduced in the study of polyploidy in the genera *Cystoderma* (Saar & Kullman, 2000) and the genera *Phellinus* (unpublished records).

**Using PI for relative measurement of nuclear DNA (procedures 1–4),** the result did not depend on the circumstance if fluorescence was measured on spores, or on nuclei isolated from spores; nor did it depend on the used fixator, enzyme, cytometer (PAS, FACSort), measurement scale (linear, logarithmic) or standard (*S. cerevisiae, P. ostreatus*).

The ratio of the spore DNA content of *P. ostreatus* (PV) to that of *S. cerevisiae* (Y2) remained the same (1.6) irrespective of the use of different fixators, enzymes, equipment and measurement scales (procedure 1 and 2; Table 1; Fig. 6).

Using two laser flow cytometers, PAS and Becton Dickinson FACSort, as well as different procedures (1 and 3), comparative measurements of *T. hemisphaerioides* were carried out with two standard species, *S. cerevisiae* and *P. ostreatus*, respectively. In the first case measurement was performed through intact cells, and in second case, through intact nuclei. The results displayed good agreement: in the first case the obtained nuclear DNA content of *T. hemisphaerioides* was 22.2 Mb (Table 1); in the second case 23.3 Mb (difference 5%).

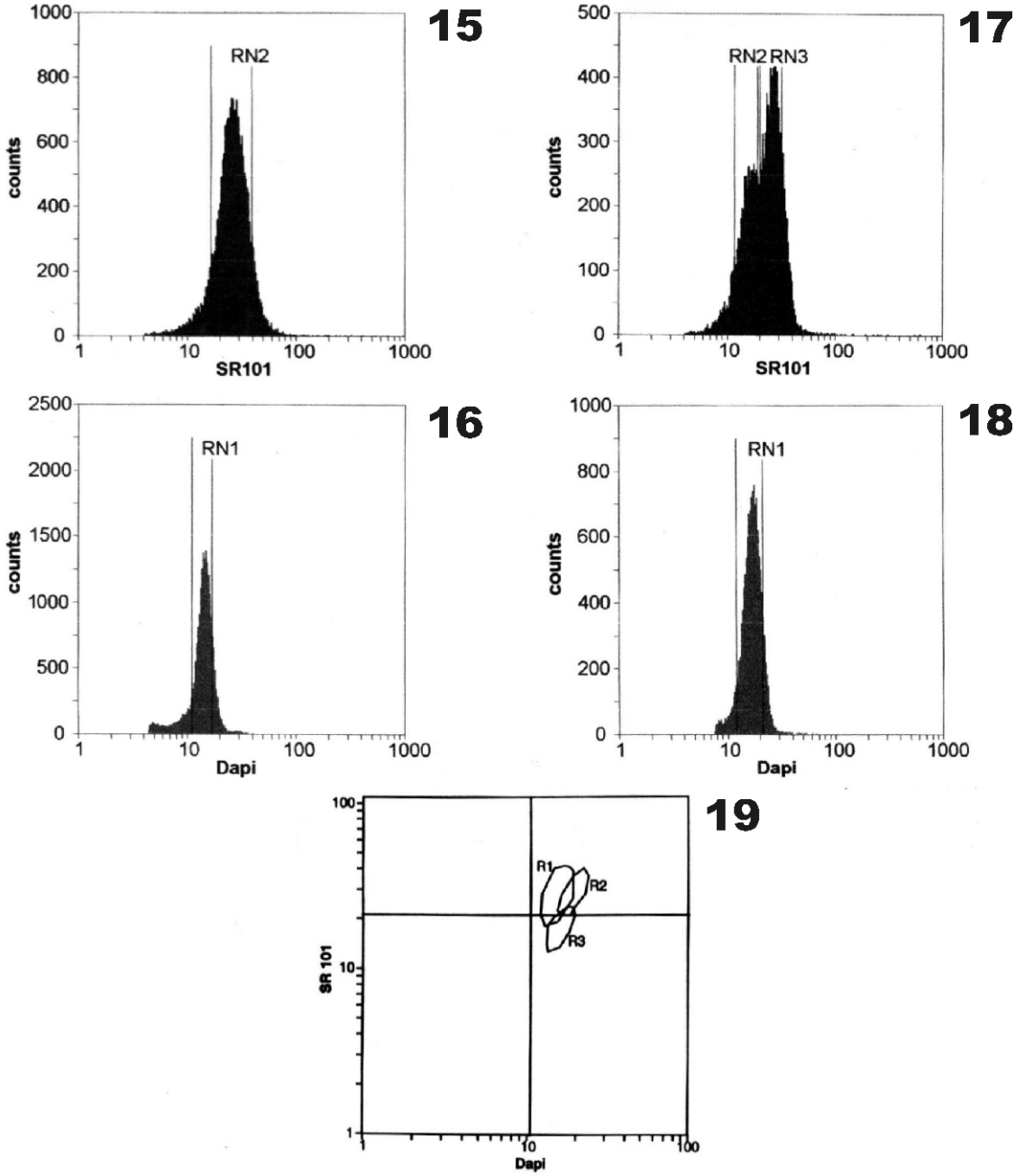
In case of relative measurement of DNA content in intact cells it is essential that the cells are subjected to an absolutely equal effect throughout the procedure. Hence it is required that both the standard specimen and the unknown specimen are kept in the same suspension. The problem lies, however, also in the different degree of interaction of PI into the strongly spiralling DNA of the spore nucleus and in different cellular RNA digesting – both resulting from minute variation of the proce-




**Figs 11-14.** Analysis of *P. ostreatus* (PV) using procedure 4. – **11.** Bivariate analysis of FLIII (fluorescence intensity) and FSC (forward scatter). FLIII represents interaction of PI with DNA. R1 – gate of spores, R2 – gate of released nuclei and R3 – gate of fluorescent microspheres. – **12.** Size-frequency histogram. Nuclei are represented by the first peak (RN1) and spores, by the second peak (RN2). – **13.** DNA content-frequency histogram of clean nuclei which have been gated on dot plot of FSC/SSC to exclude aggregates. Nuclei in cell cycle phase G1 are represented by the first peak (NR1). – **14.** DNA content-frequency histogram of damaged spores which have been gated on dot plot of FSC/SSC. Fluorescence intensity of clean nuclei is denoted by RN1 as in Fig. 13.

dure. Prolonged staining (34 h) yielded a good result (procedure 4). In an ideal case, when all enzymes are effective, the fluorescence intensity of spores is measured to be only very slightly higher from that of nuclei (Table 2: (1)).

In case of an unfixed sample (procedure 3) or a sample fixed with methanol or 96% ethanol/ice acetic acid (3:1) (procedure 2), enzymes did not act effectively and the difference between the fluorescence intensities of spores and the nuclei isolated from them was 1.4 – fold. When cells remain whole with the use of the above methods, the values of CV are low, and nuclear DNA content can be calculated on the basis of the peak means of the DNA content-frequency histogram. When, however, nuclei are released during preparation (procedure 3, Fig. 9), the CV value of peak mean is high (22%) and the results will prove inaccurate. In the latter case gate mean should be used. When calculating the relative values of specimen pairs either on the basis of nuclear gates or spore gates, respectively, the obtained results are the same. At the same time, application of FSC as the other parameter allows to study the relationship between nuclear and spore sizes and their DNA content. Parameter FSC described spore length relatively well. Measurement of *P.*




**Figs 15–19.** Bi-parametric analysis two specimens of *P. ostreatus* PV and PU stained with DAPI SR101 using procedure 5 (see Table 3). – **15.** Univariate DNA content-frequency histogram of PV. – **16.** Univariate protein content-frequency histogram of PV. – **17.** Univariate DNA content-frequency histogram of PU. – **18.** Univariate protein content-frequency histogram of PU. Two subpopulations of spores are denoted as RN2 and RN3 corresponding to gates R3 and R2 respectively on Fig. 19. – **19.** Two-dimensional presentation of measurement of nuclear DNA and protein of PV and PU. Unimodal spore print of PV (denoted as R1) and bimodal spore print of PU (denoted as R2 and R3 of the simultaneous analysis).

*ostreatus* (PU) spores by using the microscope, or in relation to fluorescent microspheres with a diameter of 5.2 µm by using the cytometer yielded similar results: 12.3–13.9 µm and 12.1, respectively.

For spores of basidiomycetes, fixation in ascending gradation (70% ethanol, Carnoy A, absolute ethanol, followed by gradual reduction of concentration) proved the most suitable. When the enzyme zymolyase was used after this, nuclei were released from spores. Measurement of chemically isolated nuclei is more reliable compared with that of mechanically isolated nuclei. Rinsing or vortexing after treatment with enzymes causes damage to spores and released nuclei, whose extent is difficult to predict (procedure 4; Table 2; Figs 11–14).

**Using DAPI SR101 for relative measurement of nuclear DNA (procedures 5).** Flow cytometric studies with fungal spores show improved resolution when the fluorochrome DAPI SR101 was used instead of PI. DAPI SR101 allows bi-parametric analysis of nuclear DNA and protein. Two subpopulations in the one spore print of *P. ostreatus* (PU), whose DNA content differs by 4.9 Mb (19%) (denoted in Fig. 19 as R2 and R3; Table 3), were not detected when only PI staining (Table 1) or DAPI staining (Fig. 18) was used. In meiosis, chromosomes of *P. ostreatus* with chromosome length polymorphism (CPL) (Larraya et al., 1999) may divide unequally, which will result in the appearance of aneuploidy and intraspecific variability. Staining with DAPI SR101 is essential in the study of aneuploidy and especially in the study of the fate of different genomes within a strain, which possess CLPs. Diversity of the nuclear DNA content of the spore print will reflect the fate of CLP during meiosis. This precise method can prove highly promising in the analysis of DNA in the fundamental and applied cytogenetic research of the fungal cell. The advantages of the procedure are also its simplicity and rapidity (no need for fixing, few steps, only one enzyme). Staining with PI involves more steps and enzymes.

It is evident that measurements by experimental series, performed with the use of the same technique and the same fluorochrome, can be valued highly in estimation of slight differences between aneuploids (Tables 1 and 3). Two parallel cytometric experiments, lamp cytometry (staining with DAPI SR101) and laser flow cytometry (staining with PI) are used as alternative methods for measurement of nuclear DNA content. The results of measurement obtained with laser cytometry were by 4% larger than those obtained with lamp cytometry. The nuclear DNA content of *P. ostreatus*, obtained through the standard species *S. cerevisiae* (13.655 Mb), was 25.0 Mb in the first case and 24.0 Mb in the second case (procedures 1 and 5). These values fall within the range of those published by Sagawa and Nagata (1992) 20.8 Mb, by Wittmann-Meixner (1989) 29.4 Mb and by Peberdy et al. (1993) 31.3 Mb.

The following three species were studied as standard candidates: *S. cerevisiae, P. ostreatus* and *T. hemisphaerioides*.

Uninucleate cells of *S. cerevisiae*, spores *P. ostreatus* and conidia of *T. hemisphaerioides* in the G1 phase of the cell cycle correspond to unreplicated haploid genome size (1C-value). DNA content per spore, calculated as having the 1C-value, can be used as the primary standard in estimation of fungal genome size (Figs 1, 3, 4, 10, 13).

Since the suspension of *S. cerevisiae* contains dividing cells, there may arise problems related to the obtaining the cells of the G1 phase of the cell cycle (Figs 2, 3, 5). In the spore print of *P. ostreatus*, division of nuclei stops in the G1 phase of the cell cycle (Fig. 13). In view of this, spores of *P. ostreatus* serve as a better standard than cells of *S. cerevisiae* (Figs 1, 8, 16). Besides, spores of *P. ostreatus* have no autofluorescence (their autofluorescence is not measurable by flow cytometry). However, not all spore prints of *P. ostreatus* can be employed as a standard due to intraspecific variability of genome size. The DNA content of the two specimens PV and PU may differ as much as 2.9 Mb (10%) measured with PI (Table 1). The spore print of PU includes two different groups whose DNA contents are different when measured with DAPI SR101 (Table 3, Figs 15–19). The genome size of *P. ostreatus* appears to be somewhat labile. For a standard, suitable is the specimen PV but not the specimen PU. Both species, *S. cerevisiae* and *P. ostreatus*, display chromosome length polymorphism (Larraya et al., 1999; Rustchenko et al., 1993) which makes them unsuitable as standard species. Only the spore print of a tested specimen can be used as a standard.

Conidia of *T. hemisphaerioides* are the most suitable standard in DNA measurement: vegetative reproduction serves as a guarantee of uniform DNA contet; the wall of conidia is thin and easily permeable by enzymes.

*T. hemisphaerioides* as well as 566 other ascomycetes were studied by Weber (1992) for establishment of their relative DNA content and ploidy levels. When the absolute DNA content of *T. hemisphaerioides*, obtained in the present study, is 23.3 Mb and the mean value of the same species, as measured in arbitrary units by Weber, is 54.4, then 1 a.u.=0.43 Mb. In this case it is possible to calculate the absolute values of all species studied by Weber (1992). Then the range of the value of the nuclear DNA content for ascomycetes is from 8.1 Mb for *Rhizina undulata* to 411-421 Mb for *Neotiella rutilans*. Majority of genome sizes reported earlier for other fungi fall in this range (Durán & Gray, 1989; Wittman-Meixner, 1989; Zolan, 1995; Kuldau et al., 1999).

As an alternative to chromosome number, and/or in addition to it, genome size (along with classical morphological criteria) should help the taxonomist distinguish more critically between morphologically similar taxa, especially in cases when the amount of nuclear DNA can be shown to be significantly different. Such information would prove highly useful in taxonomic studies of fungi, particularly in case of fungi with a dearth of definitive characteristics (Durán & Gray, 1989).

Flow cytometry is a useful method for measurement of genome size in intact cells – uninuclear spores and conidia. It is also a convenient tool in the measurement of relative spore dimensions and in the study of relationship between of spore/nuclear size and DNA content. Flow cytometric analysis of spores should prove powerful in determination of the DNA content of a large number of similar specimens, collected from different ecotypes and geographic areas, with the aim to study intraspecific variability and speciation.

The present study provided evidence that quantitative flow cytometry is an adequate method for characterisation of fungal species.

## ACKNOWLEDGEMENTS

The research was supported partly from the German Academic Exchange Service (DAAD) research grant A/98/07170 and Estonian Science Foundation grant No. 3580. I thank Prof. W. Göhde, Dr. E. Severin and Dr. B. Greve (Institut für Strahlenbiologie, Westfälische Wilhelms-Universität), Dr. W. Teterin (Institut für Medizinische Mikrobiologie, Universität Rostock), Dr. B. Nebe (Klinik für Innere Medizin, Universität Rostock), Dr. A. Kaare and Dr. J. Lehtmaa (Clinic of Hematology and Oncology, University of Tartu) and J. Popov (Institute of Molecular and Cell Biology, University of Tartu) for useful consultations and all-round help. I thank Dr. M. Rahi and Dr. A. Raitviir for critical comments on the manuscript. My sincere thanks are due to Mrs. E. Jaigma for revising the English text of the manuscript.

## REFERENCES

Arnold, N. 1993. Morphologisch-anatomische und chemische Untersuchungen an der Untergattung *Telamonia* (*Cortinarius, Agaricales*). *Libri Botanici* 7.

Bayman, P. & Collins, O.R. 1990. Meiosis and premeiotic DNA synthesis in a homothallic *Coprinus*. *Mycologia* 82: 170–174.

Bennett, M.D. & Leitch, I.J. 1995. Nuclear DNA amounts in angiosperms. *Ann. Bot.* (Oxford) 76: 113–176.

Bennett, M.D. & Leitch, I.J. 1998. Nuclear DNA amount and genome size in angiosperms. *Ann. Bot.* (Oxford) 82 (Supplement A): 1.

Bresinsky, A., Fischer, M., Meixner, B. & Paulus, W. 1987a. Speciation in *Pleurotus*. *Mycologia* 79: 234–245.

Bresinsky, A., Jarosch, M., Fischer, M. Schönberger, I. & Wittmann-Bresinsky, B. 1999. Phylogenetic relationships within *Paxillus* s.l. (Basidiomycetes, Boletales): Separation of southern hemisphere genus. *Plant Biology* 1: 327–333.

Bresinsky, A. & Wittmann-Bresinsky, B. 1995. Polyploidy levels and evolution in Boletales. *Beih. Sydowia* 10: 35–47.

Bresinsky, A., Wittmann-Meixner, B., Weber, E. & Fischer, M. 1987b. Karyologische Untersuchungen an Pilzen mittels Fluoreszenzmikroskopie. *Z. Mykol.* 53: 303–318.

Coleman, A.W., Maguire, M.J. & Coleman, J.R. 1981. Mitramycin – and 4'-6-Diamido-2-Phenylindole (DAPI) – DNA staining for fluorescence microspectrophotometric measurement of DNA in nuclei, plastids, and virus particles. *J. Histochem. Cytochem*. 29: 959–968.

Compagno, C., Porro, D., Radice, S., Martegani, E. & Ranzi, B.M. 1996. Selection of yeast cells with a higher plasmid copy number in a *Saccharomyces cerevisiae* autoselection system. *Yeast* 12 (3): 199–205.

Darzynkiewicz, Z. 1994. Cell cycle analysis by flow cytometry. In *Laboratory handbook*. Academic Press, Inc., pp. 261–271.

Dolezhel, J., Sorbati. S. & Lucretti, S. 1992. Comparison of three DNA fluorochromes for flow cytometric estimation of nuclear DNA content in plants. *Physiol. Pl.* 85: 652–631.

Dolezhel, J., Greilhuber, S., Lucretti, A., Meister, A.Lysák, L., Nardi, L. & Obermayer, R. 1998. Plant genome sise estimation by flow cytometry: inter-laboratory comparison. *Ann. Bot.* (Oxford) 82 (Supplement A): 17–26.

Durán, R. & Geay, P.M. 1989. Nuclear DNA, an adjunct to morphology in fungal taxonomy. *Mycotaxon* 36: 206–219.

Fischer, M. 1987. Biosystematische Untersuchungen an den Porlingsgattungen *Phellinus* Quél. und *Inonotus* Karst. *Biblioth. Mycol.* 107. 133.pp.

Geber,G. & Hsibeder, G. 1980. Cytophotometrische Bestimmung von DNA-Mengen: Vergleich einer neuen DAPI-Fluoreszenzmetode mit Feulgen – Absorptionsphotometrie. *Microscop. Acta, Supplementa* 4.

Gift, E.A., Park, H.J., Paradis, G.A., Demain, A.L. & Weaver, J.C. 1996. Facs-based isolation of slowly growing cells – double encapsulation of yeast in gel microdrops. *Nature Biotechnology* 14 (7): 884–887.

Gourmet, C., Gray, L.E., & Rayburn, A.L. 1997. Flow cytometric analysis of conidia of fungi isolated from soybean vascular tissue. *J. Phytopathol.* 145 (8-9): 405–408.

Gray, J.W., Dolbeare, F., Pallavicini, M.G. & Vandelaan, M. 1987. Flow cytokinetics. In *Techniques in cell cycle analysis*. (eds Gray, J. W. & Darzynkiewicz, Z.), pp. 95–133. New Jersey: Humana.

Hilber, O. 1982. *Die Gattung Pleurotus*. J. Cramer. 448. pp.

Horgen, P.A., Artur, R., David, O., Moun, A., Herr, F., Straus, N. &. Anderson J. 1984. The nucleotide sequence homologies of unique DNA's of some cultivated and wild mushrooms. *Canad. J. Microbiol.* 30: 587–593.

Ibeas, J.I., Lozano, I., Perdigones, F. & Jimenez, J. 1996. Detection of dekkera-brettanomyces strains in sherry by a nested pcr method. *Appl. Environm. Microbiol.* 62 (3): 998–1003.

Joosten H.M.L.J, Vandijck W.G.F.M, Spikker, J.W.M. & Traa, H.M. 1996. Detection of yeasts in quark by flow cytometry. *Milchwissenschaft-Milk Science International* 51 (4): 202–204.

Kamaletdinowa, F.I. & Vassilyev, A.E. 1982. *Cytology of Discomycetes*. Alma-Ata. 176 pp.

Kuldau, C.A., Tsai, H-F. & Schardl,C.L. 1999. Genome size of *Epichloë* species and anamorph hybrids. *Mycologia* 91 (5): 776–782.

Larraya, L.M., Pérez, G., Peòas, M.M., Baars, J.J.P., Mikosch, T.S.P., Pisabarro, A.G. & Ramirez, L. 1999. Molecular karyotype of white rot fungus *Pleurotus ostreatus*. *Appl. Environm. Microbiol*. 65: 3413–3417.

Lingua, G.; Varese, G. C.; Trotta, A.; Fusconi, A.; Gnavi, E., & Berta, G. 1996. Flow cytometry as a tool to investigate nuclear senescence in symbiotic and pathogenic systems. *Agronomie* 16: 663–670.

Mills, D., & McCluskey. K. 1990. Electrophoretic karyotypes of fungi: the new cytology. *Molec. Pl.-Microbe Interact.* 3: 351–357.

Nagel, W. 1976. *Zellkern und Zellzyklen*. Ulmer Verlag, Stuttgart.

Peabody, D.C., Motta, J.J. & Therrien, C.D. 1978. Cytophotometric evidence for heteroploidy in the life cycle of *Armillaria mellea*. *Mycologia* 70: 487–498.

Peabody, D.C. & Peabody, R.B. 1984. Microspectrophotometric nuclear cycle analyses of *Armillaria mellea*. *Exp. Mycol.* 8: 161–169.

Peberdy, J.F., Hanifah, A.H. & Jia, J.-H. 1993. New perspectives on the genetics of *Pleurotus*. In *Mushroom biology and mushroom products* (eds Chang, S.-T., Bruswell, J.A. & Chiu, S.W.), pp. 55–62. The Chinese University Press, Hong Kong.

Petit, P., Glab, N., Marie, D., Keffer, H. & Metezeau, P. 1996. Discrimination of respiratory dysfunction in yeast mutant by confocal microscope, image, and flow cytometry. *Cytometry* 23 (1): 28–39.

Polacheck, I., Antman, A., Barth, I., Sagi, E. & Giloh, H. 1995. Adherence of *Candida albicans* to epithelial cells: Studies using fluorescently labelled yeast and flow cytometry. *Microbiology* 141 (7): 1523–1533.

Porro, D, & Srience, F. 1995. Tracking of individual cell cohorts in asynchronous *Saccharomycetes cerevisiae* populations. *Biotehnol. Progr.* 11 (3): 342–347.

Prudêncio, C.P., Sansonetty, F. & Côrte-Real, M. 1998. Flow cytometric assessment of cell structural and functional changes induced by acetic acid in the yeast *Zygosaccharomyces* bailii and *Saccharomyces cerevisiae*. *Cytometry* 31: 307–313.

Rogers, J.D. 1973. Polyploidy in fungi. *Evolution* 27: 153–160.

Romeis, B. 1968. *Mikroskopische Technik*. München. Wien. 695 pp.

Rustchenko, E.P., Curran, T.M. & Sherman, F. 1993. Variation in the number of ribosomal DNA units in morphological mutants and normal strains of *Candida albicans* and in normal strains of *Saccharomyces cerevisiae*. *J. Bacteriol.* 174: 7189–7199.

Saar, I. & Kullman, B. 2000. Nuclear DNA content and spore dimensions in some species of the genus *Cystoderma*. *Folia Cryptog. Estonica* 36: 87-94.

Sagawa, I. & Nagata, Y. 1992. Analysis of chromosomal DNA of mushrooms in genus *Pleurotus* by pulsed field gel electrophoresis. *J. Gen. Appl. Microbiol.* 38: 47–52.

Smeraldi, C., Berard, E. & Porro, D. 1994. Monitoring of peroxisome induction and degradation by flow cytometric analysis of *Hansenula polymorpha* cells grown in methanol and glucose madia: Cell volume, refractive index and FITC retention. *Microbiology* 140 (11): 3161–3166.

Swift, H. 1950. The constancy of desoxyribose nucleic acid in plant nuclei. *Genetics* 36: 643–654.

Talbot, N.J. & Wayman, M. 1989. Increase in ploidy in yeast as a response to stressing media. *Appl. Microbiol. Biotechnol.* 32 (2): 167–169.

Tetsuca, Y., Ando. K., Yomaoka, Y. & Katsuya, K. 1984. The DNA content of the mycelial nuclei of rust fungi grown on artificial media. *Canad. J. Bot.* 62:189–194.

Therrien, C.D., Ritch, D., Davidse, L.C., Jespers, A.B.K. & Spielman, L.J. 1989. Nuclear DNA content, mating type and metalaxyl sensitivity of eighty-three isolates of *Phytophtora infestans* from the Netherlands. *Mycol. Res.* 92: 140–146.

Tooley, P.W. & Therrien, C.D. 1987. Cytophotometric determination of the nuclear DNA content of 23 Mexican and 18 non-Mexican isolates of *Phytophtora infestans*. *Exp. Mycol.* 11: 19–26.

Ulrich, I. & Ulrich, W. 1991. High-resulution flow cytometry of nuclear DNA in higher plants. *Protoplasma* 165: 212–215.

Weber, E. 1992. Untersuchungen zu Fortpflanzung und Ploidie verschiedener Ascomyceten. *Biblioth. Mycol.* 140: 1–186.

Weber, E. & Bresinsky, A. 1992. Polyploidy in Discomycetes. *Persoonia* 14: 553–563.

Wittmann-Meixner, B. 1989. Polyploidie bei Pilzen. *Biblioth. Mycol.* 131: 1–163.

Wittmann-Meixner, B. & Bresinsky, A. 1989. Quantitative differences of nuclear DNA contents in Paxillaceae compared with other families of Boletales. *Mycol. Res.* 93, 282–291.

Wittmann-Meixner, B., Weber, E. & Bresinsky, A.1989. Different grades of correlation between relative DNA content, ploidy level and chromosome number in various fungi. *Opera Bot.* 100: 267–274.

Zeze, A., Hosny, M., Gianinazzipearson, V. & Dulieu, H. 1996. Characterization of a highly repeated dna sequence (sc1) from the arbuscular mycorrhizal fungus *Scutellospora castanea* and its detection in plants. *Appl. Environm. Microbiol.* 62 (7): 2443–2448.

Zolan, M. E. 1995. Chromosome-length polymorphism in fungi. *Microbiol. Rev.* 59: 686–698.

## APPENDIX

### Solutions

Concentrated stock solutions of RNAse (10 mg/ml; Fluka), zymolyase (50 mg/ml; Seikagaku) and proteinase K (200 mg/ml; Sigma Chemical Co.) were prepared and stored at – 20°C. Concentrated stock solutions of Propidium iodide (1 mg/ml; Sigma Chemical Co); solution of Pepsin 0.5% pH 1.8; Zymolyase buffer: 218.6 mg D-Sorbitol (Sigma Chemical Co), 40 ml EDTA 0.5M, 10 ml 7.5 pH Tris 1M were prepared and stored at 4°C. Fixations: 96% ethanol/ice acetic acid (3:1) (Coleman et al., 1981); Carnoy A: absolute ethanol/chloroform/ice acetic acid (6:3:1); Carnoy B: ethanol/chloroform/ ice acetic acid (6:3:1) (Romeis, 1968) were prepared.

#### Staining Protocols

**Procedure 1.** The procedures for staining yeast nuclei with PI used in laboratory at Münster University (Westfählische Wilhelms-Universität, Institut für Strahlenbiologie) were modified (Table 1; Figs 1–4).

**Procedure 1A** (used for the pure culture of *T. hemisphaerioides*).

1. Collect mycelia from a liquid culture medium and fix in Carnoy B at 4°C until used.
2. To release conidia from hyphae, transfer a small piece of the cultured mycelium in a 1.5 ml Eppendorf tube and wash with sterile water shaking gently. Filter the suspension of released conidia through a 50 µm nylon mesh to remove hyphae. Centrifuge for 2.5 min at 3200 rpm and discard the supernatant. Resuspend conidia in 400 µl of 70% ethanol and store at 4°C until required.
3. Add sterile water little by little, vortexing intermittently to make sure it is homogeneous.
4. Centrifuge for 10 min at 9000 rpm and remove the supernatant (leave 50 µm suspension on the bottom of the tube).
5. Wash conidia twice with sterile water.
6. Add 400 µl sterile water and 63 µl of RNAse stock and mix to fully resuspend conidia. Incubate for 1 h at 50°C shaking slowly on a water bath.
7. Add 500 µl of PBS pH 7.4 and 5 µl of proteinase K stock and incubate 1 h at 50°C shaking slowly on a water bath.
8. Filter through 50 µm nylon mesh.
9. Cool down on ice and add 10 µl PI stock. The probe can be used after 15 min.

**Procedure 1B** (used for the spore print of *P. ostreatus*).
1. Fix the spore print in Carnoy A for 80 min on a shaker at room temperature.
2. Centrifuge for 10 min at 3200 rpm. Resuspend spores in absolute ethanol for 15 min on a shaker at room temperature. The probe can be stored at 4°C until required.
3. The same steps as in procedure 1A, 3–7.

**Procedure 1C** (used for the cell culture of *S. cerevisiae*).
1. Add 500 µl Carnoy B to 2 ml liquid cell culture.
2. Remove oil from the bottom of the Eppendorf tube with a syringe.
3. Centrifuge for 15 min at 3200 rpm. Resuspend spores in 1 ml 70% ethanol by vortexing.
4. Centrifuge for 15 min at 3200 rpm. Resuspend spores in 200 ml 70% ethanol.
5. The same steps as in procedure 1A, 3–7.

**Procedure 2** (used for staining nuclei in spore print and cell suspension) (Figs 5-8).
1. Fix the spores of the unknown and the reference species (*P. ostreatus)* in 95% ethanol/ice acetic acid (3:1) for 24 h on a slow rotator mixer at room temperature.
2. Add deionized water little by little in the amounts of 40 µl, 40 µl, 200 µl, 200 µl, and 200 µl, vortexing intermittently to make sure the solution is homogeneous.
3. Centrifuge for 5 min at 10000 rpm and remove the supernatant.
4. Wash the spores with Zymolyase buffer on a slow rotator mixer at room temperature for 15 min.
5. Centrifuge for 5 min at 10000 rpm and remove the supernatant.
6. Add 200 µl Zymolyase buffer and 18 µl Zymolyase stock and incubate at 37°C in a thermostat on a slow vertical rotator mixer for 24 h.
7. Add 100 µl PBS and 8 µl RNAse stock and mix gently to fully resuspend spores and incubate at 37°C in a thermostat on a slow vertical rotator mixer for 24 h.
8. Add PBS to 1 ml.
9. Incubate on ice and add 15µl PI stock for at least 15 min.
10. Filter through 85 µm nylon mesh before use.

**Procedure 3** (used for staining of the dried spore print of *P. ostreatus* and conidia of *T. hemisphaerioides* fixed in Carnoy A) (Figs 9, 10).
A slightly modified method, Cycle TEST ™ Plus DNA Reagent KIT (Cat. No. 340242), is used in which a set of reagents is required to isolate and stain cell nuclei from cell suspensions. The method involves the dissolving of cell membrane lipids with a nonionic detergent, eliminating the cell cytoskeleton and nuclear proteins with trypsin, digesting cellular RNA with enzyme, and stabilizing nuclear chromatin with spermine. The fluorochrome PI is bound to clean, isolated nuclei or to nuclei on cells.
Conidia are collected from a fixed culture as described in procedure 1, steps 1–5. 22.5 µl of Zymolyase stock is added or not added to trypsin buffer. All further steps are carried out as described in staining procedure with the Reagent KIT.

**Procedure 4** (used for the spore print of *P. ostreatus*) (Table 2; Figs 11–14).
1. Fix the spore print first in 70% ethanol and store for some days, then fix it in Carnoy B for 80 min on a shaker at room temperature.
2. Centrifuge for 10 min at 3200 rpm and discard the supernatant. Resuspend spores in absolute ethanol for 15 min on a shaker at room temperature.
3. Add sterile water little by little, vortexing intermittently to make sure the solution is homogeneous.
4. Centrifuge for 10 min at 9000 rpm and remove the supernatant.
5. Wash spores twice with sterile water.
6. Resuspend spores with 200 µl Zymolyase buffer and add 18 µl Zymolyase stock and incubate at 37°C in a thermostat for16 h.
7. Add 10 µm of RNAse stock and mix to fully resuspend spores. Incubate for 24 h at 37°C in a thermostat.
8. Add 1 ml of phosphate buffer saline (PBS), 0.1M pH 7.0 and 20 µm PI stock. Store at 4°C for 34 h.
9. Filter through 50 µm nylon mesh before use.

**Procedure 5** (DAPI in combination with the SR protein fluorochrome Sr 101 used for bivariate DNA and protein analysis) (Table 3; Figs 15–19).
For preparation and staining of fungal material, a slightly modified method is used (Ulrich & Ulrich, 1991; procedure used at the laboratory at Münster University/ Westfälische Wilhelms-Universität, Institut für Strahlenbiologie). 1 ml 0.5% Pepsin pH 1.8 is added to spore print by briefly vortexing and incubated for 3 min at room temperature. Then 3–5-fold volume of DAPI SR101 (Partec GmbH, Münster, Federal Republic of Germany) is added, and the sample is incubated for 20 min by vortexing intermittenly two times. Before use spores are filtered through a 50 µm nylon.

Folia Cryptog. Estonica, Fasc. 36: 47–55 (2000)

# The diversity and distribution of the Pezizales (Ascomycota) in Lithuania

**Ernestas Kutorga**
Department of Botany and Genetics, Vilnius University, 21/27 Ciurlionio, LT-2009, Vilnius, Lithuania.
E-mail: Ernestas.Kutorga@gf.vu.lt
Laboratory of Mycology, Institute of Botany, 49 Zaliuju ezeru, LT-2021, Vilnius, Lithuania

**Abstract:** This paper deals with the species diversity, distribution and ecology of the order Pezizales (including the Tuberales) in Lithuania. In total, 158 species and 5 varieties have been recorded in Lithuania, most of them are saprotrophs on soil, forest litter, wood, dung, and burned material. Serious damages to pine plantations in Lithuania caused by *Rhizina undulata* were strongly reduced by the appropriate forest management. 8 pezizalean species are included into The Red Data Book of Lithuania (1992), 13 species have been proposed for a new edition of it.

**Kokkuvõte:** E. Kutorga. Liudikulaadsete seente (Pezizales, Ascomycota) mitmekesisus ja levik Leedus.

Artiklis käsitletakse Leedu liudikulaadsete liigilist mitmekesisust, levikut ja ökoloogiat. Leedust on kokku leitud 158 liiki ja 5 varieteeti liudikulaadseid. Enamus neist on saproobid pinnasel, metsavarel, puidul, sõnnikul ja põlenud materialil. *Rhizina undulata* poolt tekitatud tõsiseid kahjustusi männikultuuridele Leedumaal on tugevasti vähendatud õige metsa hooldamisega. 8 liudikulaadset on kantud Leedu Punasesse raamatusse (1992), 13 liiki on esitatud selle uude väljaandesse.

## INTRODUCTION

The historical survey on investigations of Lithuanian discomycetes revealed that up to 1988 totally 69 pezizalean species were registered in the country (Kutorga, 1990). A number of papers concerning the distribution and ecology of operculate discomycetes have been published since that time (Kutorga, 1989a, b; 1991a; Prokhorov, Kutorga, 1991; Kutorga, Vishnjauskas, 1991). E. Kutorga (1991b) in the doctoral thesis listed 306 species of discomycetes for Lithuania, and 116 species of the order Pezizales among them. Subsequent investigations (Kutorga, 1992, 1993, 1994a, b, 1996a, b, 1997, 1999) added more knowledge about these fungi in the country.

The aim of this paper was to list all known Lithuanian species of the order Pezizales and provide a review of their distribution, ecology, significance, and conservation.

## MATERIAL AND METHODS

This study is based on the examination of *ca*. 1250 specimens of pezizalean fungi, mainly collected by the author from various districts of Lithuania in 1987–1999. Part of examined material (*ca*. 350 specimens) was collected by other persons (ca. 40), mainly by J. Mazelaitis, K. Raulinaitis, V. Urbonas, and L. Šveistyte. While doing the field work during various seasons of a year different habitats and substrates were searched for this group of discomycetes. Part of coprophilous fungi were recorded on animal dung which has been transferred to the laboratory and kept in damp conditions at room temperature. Hand made sections with razor blade or squashed material mounted in water, 2–5 % KOH, Melzer's Reagent, and solution of methyl blue in lactic acid (Cotton Blue) were examined microscopically. All material is deposited at the Institute of Botany, Vilnius (BILAS), Vilnius University (WI), and Moscow State University (MW). In the latter Herbarium the specimens of coprophilous discomycetes grown in moist chambers and identified by V. Prokhorov (Moscow) are preserved. Reliable literature data on the records of Lithuanian *Pezizales* was also taken into consideration during the compilation of the list of species.

## RESULTS AND DISCUSSION

**Diversity.** In total, 158 species and 5 varieties from 52 genera and 9 families of the order Pezizales have been recorded in Lithuania (Table 1). During several previous years the number of registered species in Lithuania increased from 1 to 3 per year, e.g. 2 species, *Miladina lechithina* and *Scutellinia minutella*, were found for the first time in Lithuania in 1999. Comparing the numbers of species recorded in other countries of Europe (Ukraine: 137 species (Smitskaya, 1980); Estonia: 172 species (Järva, Parmasto, 1980; Järva et al., 1998); Østfold county in Norway: 185 species (Kristiansen, 1983); Denmark: 221 species (Dissing, 1982), former Czechoslovakia: 360 species (Svrcek, 1981), it is possible to note, that on the one hand, the current knowledge about species diversity in Lithuania considerably represents the nature diversity and size of the country. On the other hand, there is a real possibility to expand the species list for additional 50–100 species in the future. Further investigations of the species of underinvestigated genera, e.g. *Lamprospora*, *Octospora*, *Otidea*, *Scutellinia*, *Sowerbyella* etc., and of hypogeous, coprophilous and bryophilous species will add more data for better understanding of the diversity and distribution of the *Pezizales* in Lithuania.

**Distribution, frequency.** The number of recorded species is different in various administrative districts of Lithuania (Fig. 1) and almost directly depends on the extent of carried investigations and collected specimens. More than a half of all identified species were recorded in Vilnius district (82 species), one third – in Varena district (52).

The species represent 6 frequency groups based on the number of known localities:

1. Common species (30 and more localities): 10 species – *Gyromitra esculenta* (over 50 localities), *Scutellinia scutellata* (50), *Rhizina undulata* (40), *Sarcoscypha austriaca* (35),

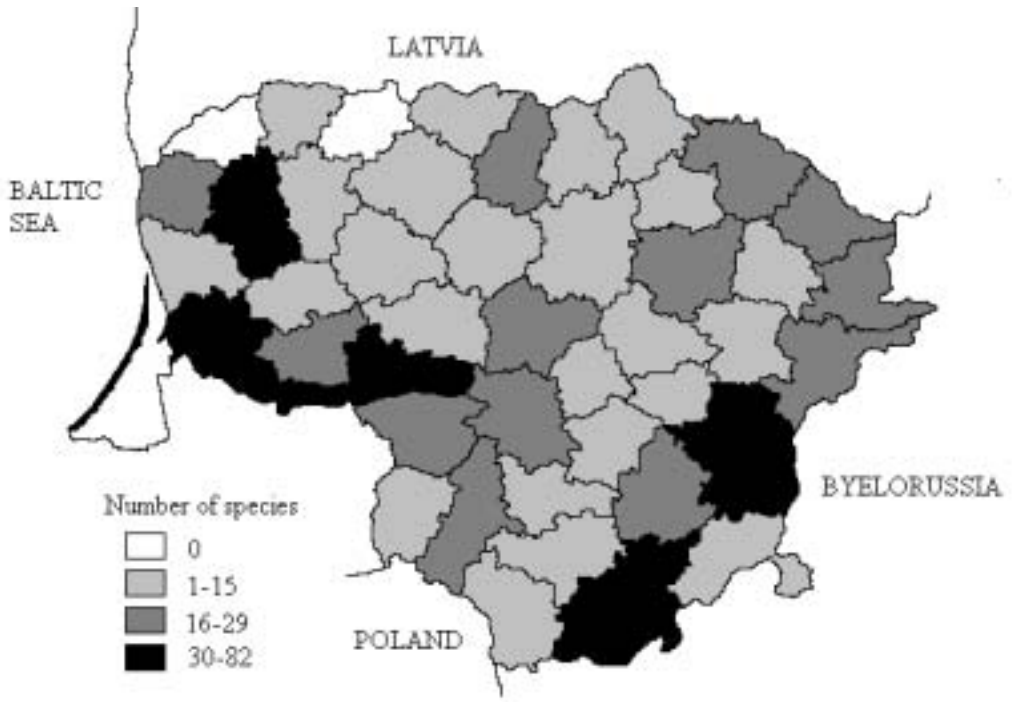



**Fig. 1.** The distribution of known species of the order Pezizales across administrative districts of Lithuania.

*Helvella crispa* (35), *H. macropus* (35), *H. elastica* (30), *H. lacunosa* (30), *Gyromitra infula* (30), and *Humaria hemisphaerica* (30).
2. Rather common species (20–29 localities): 9 species.
3. Rather rare species (10–19 localities): 15 species.
4. Rare species (5–9 localities): 35 species.
5. Very rare species (1–4 localities): 85 species, 5 varieties. Some of these species are really very rare in Lithuania, e.g. *Caloscypha fulgens*, *Helvella corium*, *H. pezizoides*, *H. cupuliformis*, *Hydnotrya tulasnei*, *Leucoscypha erminea*, *Morchella hortensis*, *M. esculenta* var. *crassipes*, *Peziza ammophila*, *P. ampelina*, *P. celtica*, *P. succosa*, *Rhodotarzetta rosea*, *Tarzetta catinus*, *T. gaillardiana*, and *Verpa conica*. Other species due to their small fruit-bodies and very short period of fructification are hardly located and, therefore, undercollected.
6. Species not recorded for a long period (1–5 localities before 1960 and not found afterwards): 4 species – *Tuber exiguum* and *T. rufum* (last time recorded in 1904), *Microstoma protracta* (in 1957), and *Sarcosoma globosum* (in 1960).

**Habitats.** Approximately a half of Lithuania's territory is occupied by arable land, lakes and rivers, buildings, or asphalt roads and is practically unsuitable for the development of pezizalean fungi. The majority of species develop in various types of woods (forest land area comprise 30.3% of Lithuania), large parks, in shrubby places, on forest paths and grounds, wood-cutting area, and forest quarter lines. In mixed forests the diversity of the *Pezizales* is bigger than in pure standings of coniferous or deciduous forests. Some species prefer a particular type of forest, e.g. *Pseudoplectania nigrella* was found only in spruce (*Picea abies*) standings, *Gyromitra esculenta*, *G. perlata*, *Rhizina undulata*, and *Desmazierella acicola* prefer pine (*Pinus sylvestris*) standings, *Verpa bohemica* was mainly associated with aspen (*Populus tremula*) trees.

Some species prefer wet banks of rivers and lakes, especially this is characteristic to several species of the genus *Scutellinia*. Such species as *Pachyella babingtonii* and *Miladina lechithina* were found on soaked wood and bark along the surface of the water or submerged into the water.

*Peziza ammophila* occurs only in the sand of coastal dunes between *Ammophila arenaria* and *Festuca arenaria*. Most species of the genera *Neottiella* and *Octospora* are associated with particular mosses. Pyrophilous species inhabit fireplaces in forests, banks of rivers and lakes, gardens and waste grounds. Coprophilous species which colonise the dung of wild animals were found mainly in forests, while those on the dung of domestic animals occur in pastures, farms, and meadows. Some species were found in the gardens, e.g. *Morchella hortensis*, *Disciotis venosa*, *Scutellinia scutellata*, and *Cheilymenia vitellina*. *Peziza cerea*, *P. ostracoderma* and *P. varia* were collected in greenhouses, plant pots, and mushroom beds.

**Ecological groups, substrates.** 3 ecological groups based on nutritional relations of the recorded species are recognized: saprotrophs, symbiotrophs and biotrophs.

I. Saprotrophs (saprobes). This group may be subdivided into such subgroups:
1. Soil and humus saprotrophs. Out of 90 species attributed to this group 54 species were found only on soil and decayed forest litter, and 36 species also inhabited other substrates.
2. Xylosaprotrophs. Out of 30 species only 8 species (*Ascobolus denudatus*, *A. lignatilis*, *Microstoma protracta*, *Miladina lechithina*, *Pachyella babingtonii*, *Peziza micropus*, *Sarcoscypha austriaca*, and *S. coccinea*) were recorded exclusively on dead wood and bark, and 22 species also inhabited other substrates.
3. Leaf and needle saprotrophs. Out of 6 species collected on dead leaves and needles only *Desmazierella acicola* is true pine needle inhabitant.
4. Pyrophilous fungi (post-fire or phoenicoid fungi). On burnt ground and charred plant remnants 18 species were identified, 12 of them grew only in bonfire places and in sites after the fire: *Anthracobia maurilabra*, *A. melaloma*, *Ascobolus carbonarius*, *Geopyxis carbonaria*, *Peziza echinospora*, *P. tenacella*, *Plicaria endocarpoides*, *Pulvinula carbonaria*, *Pyronema omphalodes*, *Rhodotarzetta rosea*, *Sphaerosporella brunnea*, and *Trichophaea hemisphaerioides*.
5. Coprophilous fungi. 43 species were recorded on the dung of wild animals (elks (*Alces alces*), red deer (*Cervus elaphus*), roe deer (*Capreolus capreolus*), wild boars (*Sus scrofa*), hares (*Lepus*

spp.), etc.) and domestic animals (cows and horses), of these 40 species were found only on excrements. *Ascobolus furfuraceus*, *A. sacchariferus*, *A. stercorarius*, *Cheilymenia granulata*, *C. stercorea*, *Lasiobolus ciliatus*, *Peziza fimeti*, *Saccobolus depauperatus*, and *Thelebolus microsporus* are the most common coprophilous species in Lithuania.

6. Saprotrophs on anthropogenic substrates. *Iodophanus carneus* was collected on decaying rags, fabric sacs, and paper, *Peziza cerea* – on rotting shoe leather, textile fabrics, and cardboard, *Pyronema domesticum* – on wet plaster wall.

II. Symbiotrophs. It is supposed, that hypogeous *Choiromyces venosus*, *Hydnotrya tulasnei*, *Tuber exiguum*, and *T. rufum* form ectomycorrhizas with trees and shrubs.

III. Biotrophs (parasites). *Rhizina undulata* (also found on needle litter and in post-fire sites) attack roots of pines and cause wilt of trees. Species of the genera *Neottiella* and *Octospora* are biotrophs on mosses.

**Phenology.** Under natural conditions in Lithuania the fungi of the *Pezizales* form fruitbodies from (February) March till November (December), they exhibit greatest species diversity in July, August, and September. According to the fruiting time 4 main phenological groups were distinguished:

1. Spring species (appear from March till mid June). *Ca*. 25 species.
2. Summer–autumn species (appear from June till October). *Ca*. 50 species.
3. Autumn species (start in August, most active in September, disappear in October–November). *Ca*. 50 species.
4. Spring–summer–autumn species (start in April–May, disappear in September–November). *Ca*. 20 species.

**Significance.** Most species are saprotrophs and play an important role in the decomposition of organic material and soil formation. Morels, truffles, even species of the *Sarcoscypha*, are suitable for food. Several species of the genera *Gyromitra* and *Helvella* are poisonous, however, *Gyromitra esculenta* is still sold in local markets for food, despite warnings of possible poisonings. Other species of the *Pezizales* have small and not tasty fruitbodies and, therefore, are not suitable for human consuming.

Fungus *Rhizina undulata* under suitable conditions is functioning as a pathogen and has caused serious damage to Lithuanian forestry, especially in the areas where slash burning has been performed. In the Curonian Spit serious attacks of *R. undulata* on the plantations of Swiss mountain pine (*Pinus mugo*) were observed since 1979 (Vasiliauskas, 1998). The area of dead and nearly wilt trees in 1979 comprised 3.4 ha, in 1983 – 12 ha ( 277 plots of infection). The appropriate forest management (localisation of slash burning and trenching) and some other unknown reasons (biology of fungus, climate conditions) have strongly reduced the area of infection (at least 11 plots of infection were observed in 1997).

**Conservation.** Some operculate discomycetes are rare and threatened in Lithuania. Special attention was paid to species with large fruitbodies. 8 species of the *Pezizales* were listed in the current Red Data Book of Lithuania (Balevicius et al., 1992): 1 category (endangered species) – *Choiromyces venosus*, *Peziza ammophila*, and *Sarcosoma globosum*; 2 category (vulnerable species) – *Morchella conica*; 3 category (rare species) – *Gyromitra perlata*, *Morchella elata*, *Verpa bohemica*, and *Verpa conica*. Gathering of fruit-bodies and destruction of habitats of these species is strictly prohibited by the law.

The inclusion of species into the Book and grouping into categories of threat have been performed on the basis of species frequency and partly by subjective judgment. The situation, when upon the urgent request of conservationists to select a limited number of species without detailed studies is still continuing. 13 pezizalean species were proposed in 1999 to be included into the forthcoming edition of the Red Data Book of Lithuania: *Caloscypha fulgens*, *Choiromyces venosus*, *Hydnotrya tulasnei*, *Microstoma protracta*, *Morchella conica*, *M. elata*, *M. semilibera*, *Otidea onotica*, *Peziza ammophila*, *Sarcosoma globosum*, *Urnula craterium*, *Verpa bohemica*, and *V. conica*. The knowledge about distribution, biology and ecology of threatened fungi of the *Pezizales* in Lithuania is still insufficient and pending further investigations.

**Table 1.** Habitats, ecological groups, fruiting time and number of known localities of species of the order Pezizales in Lithuania

Abbreviations. Habitats: B, buildings and their surroundings; C, cemetery; CF, coniferous forests; D, dunes; DD, data deficient; DF, deciduous forests; F, bonfire places, sites after the fire; FPG, forest paths and grounds; G, gardens; GH, greenhouses, mushroom beds, plant pots and flower gardens; MF, mixed forests; MP, meadows, pastures; P, parks; RP, ruderal places, gravel-sand-pits; RVD, road verges, ditches; S, shrubs; W, woods, forests; WCA, wood-cutting area, forest quarter lines; WB, water, banks of rivers and lakes.
Ecological groups: AST, saprotrophs on anthropogenic substrates (textile fabrics, paper, cardboard, leather, plaster etc.); BPH, bryophils; BT, biotrophs; CPH, coprophilous fungi; HST, herbosaprotrophs; MST, mycosaprotrophs; NLST, needle and leaf saprotrophs; PPH, pyrophils, post-fire fungi; SBT, symbiotrophs; SHST, soil and humus saprotrophs; XST, xylosaprotrophs.
Fruiting time (months): III, March; IV, April; V, May; VI, June; VII, July; VIII, August; IX, September; X, October; XI, November; XII, December; CULT, fungi cultivated in laboratory.

| Taxa | Habitats | Ecological groups | Fruiting time | No. of localities |
|---|---|---|---|---|
| **Ascobolaceae** | | | | |
| *Ascobolus albidus* P. Crouan & H. Crouan | DD | CPH | CULT | 1 |
| *A. carbonarius* P. Karst. | CF, F, P, S, WCA | PPH | VI–X | 9 |
| *A. denudatus* Fr. | CF | XST | VIII | 1 |
| *A. epimyces* (Cooke) Seaver | CF | CPH?, XST | X | 1 |
| *A. foliicola* Berk. & Broome | DF, MF | NLST, SHST | VII–IX | 3 |
| *A. furfuraceus* Pers.: Fr. | CF, DF, MP, P, S | CPH | VI–X | ~20 |
| *A. immersus* Pers.: Fr. | DD | CPH | CULT | 3 |
| *A. lignatilis* Alb. & Schwein.: Fr. | MF | XST | IX | 1 |
| *A. roseopurpurascens* Rehm | D | CPH | CULT | 1 |
| *A. saccharifer*us Brumm. | CF, DF | CPH | V–X | 9 |
| *A. stercorarius* (Bull. ex St. Amans) J. Schröt. | CF, MF | CPH | IV–X | 11 |
| *A. stictoideus* Speg. | DD | CPH | CULT | 1 |
| *A. viridis* Curr. | DF | SHST | VIII | 1 |
| *Saccobolus citrinus* Boud. & Torrend | DD | CPH | CULT | 1 |
| *S. depauperatus* (Berk. & Broome) E. C. Hansen | CF, MF | CPH | IV–VII, CULT | 10 |
| *S. glaber* (Pers.: Fr.) Lambotte | DD | CPH | CULT | 2 |
| *S. minimus* Velen. | W | CPH | VIII, CULT | 3 |
| *S. obscurus* (Cooke) W. Phillips | DD | CPH | CULT | 1 |
| *S. truncatus* Velen. | DD | CPH | CULT | 1 |
| *S. verrucisporus* Brumm. | CF | CPH | CULT | 1 |
| *S. versicolor* (P. Karst.) P. Karst. | CF | CPH | IV, CULT | 2 |
| *Thecotheus holmskjoldii* (E.C. Hansen) Chenant. | DD | CPH | CULT | 1 |
| *T. pelletieri* (P. Crouan & H. Crouan) Boud. | DD | CPH | CULT | 2 |
| **Helvellaceae** | | | | |
| *Choiromyces venosus* (Fr.) Th. Fr. | CF, DF | SBT?, SHST | VIII–X | 7 |
| *Gyromitra esculenta* (Pers.: Fr.) Fr. | CF, DF, FPG, MF, P, RVD, WCA | SHST, XST | IV–VI | >50 |
| *G. gigas* (Krombh.) Cooke | CF, MF, P, RVD | SHST, XST | IV–V | ~15 |
| *G. infula* (Schaeff.: Fr.) Quél. | CF, DF, F, GH, MF, P, RVD | PPH, SHST, XST | VIII–XI | >30 |
| *G. perlata* (Fr.) Harmaja | CF, FPG, P, WCA | SHST, XST | IV–V | ~20 |
| *Helvella acetabulum* (L.: Fr.) Quél. | CF, DF, FPG, P, RVD | SHST | V–VI | 10 |
| *H. albella* Quél. | CF, DF, FPG, KP | SHST | VII–X | 3 |
| *H. chinensis* (Velen.) Nannf. & L. Holm | C, CF, DF, FPG, RVD | SHST | VII–X | 4 |
| *H. corium* (O. Weberb.) Massee | FPG, RP, S | SHST | VI–IX | 2 |

Table 1 (continued)

| Taxa | Habitats | Ecological groups | Fruiting time | No. of localities |
|---|---|---|---|---|
| *H. crispa* (Scop.: Fr.) Fr. | CF, D, DF, FPG, MP, P, RVD, S | SHST | VII–IX(X) | 35 |
| *H. cupuliformis* Dissing & Nannf. | CF | SHST | IV | 1 |
| *H. elastica* Bull.: Fr. | C, CF, DF, F, FPG, MF, P, RVD, WB | SHST | VII–X | 30 |
| *H. ephippium* Lév. | DF, MF, P | SHST | VII–IX | 4 |
| *H. lacunosa* Afzel.: Fr. | CF, DF, F, P, RVD, S | NLST, SHST, XST | (V)VII–X | ~30 |
| *H. leucomelaena* (Pers.) Nannf. | C, CF, FPG, MF, RVD | SHST | V–VI | 3 |
| *H. macropus* (Pers.: Fr.) P. Karst. | CF, DF, F, MF, WB | NLST, SHST, XST | VII–IX | 35 |
| *H. nigricans* Pers. | CF, DF, FPG, MF, P | SHST | VII–IX | ~6 |
| *H. pezizoides* Afzel. | DF, FPG | SHST | IX | 1 |
| *H. solitaria* P. Karst. | P, RVD, S, W | SHST | VI | 6 |
| *Hydnotrya tulasnei* (Berk. & Broome) Berk. & Broome | CF, RVD | SBT?, SHST | VII | 1 |
| *Rhizina undulata* Fr.: Fr. | CF, F, WCA | BT, PPH, SHST | VII–X | ~40 |
| **Morchellaceae** | | | | |
| *Disciotis venosa* (Pers.: Fr.) Arnould | CF, DF, G, P, RVD, WB | SHST | V | 9 |
| *Morchella conica* Pers. | CF, DF, F, G, P, RVD | SHST | IV–V | 15 |
| *M. elata* Fr.: Fr. | CF, DF, F, P | SHST | IV–V | 9 |
| *M. esculenta* (L.) Pers.: Fr. var. *esculenta* | CF, F, G, MF, P, RVD, S | SHST | IV–VI | ~20 |
| *M. esculenta* var. *crassipes* (Vent.: Fr.) Kreisel | DF, MF, P | SHST | V | 2 |
| *M. esculenta* var. *rotunda* Pers. | S, W | SHST | V | 1 |
| *M. esculenta* var. *umbrina* (Boud.) S. Imai | P, W | SHST | V | 1 |
| *M. esculenta* var. *vulgaris* Pers. | S, W | SHST | V | 2 |
| *M. hortensis* Boud. | G | SHST | V | 1 |
| *M. semilibera* DC.: Fr. | DF, P, S | SHST | IV–V(VI) | 9 |
| *Verpa bohemica* (Krombh.) J. Schröt. | CF, DF, MF, P, WCA | SHST | IV–V | 12 |
| *V. conica* (O. F. Müll.) Sw.: Fr. | G, MP, S, W | SHST | IV–V | 4 |
| **Pezizaceae** | | | | |
| *Iodophanus carneus* (Pers.: Fr.) Korf | DF, RP | AST, CPH | VIII–X | 6 |
| *Pachyella babingtonii* (Berk. & Broome) Boud. | CF, WB | XST | VII–IX | 2 |
| *Peziza ammophila* Durieu & Mont. | D | SHST | IX–X | 2 |
| *P. ampelina* Quél. | DF, P | SHST | VI | 1 |
| *P. ampliata* Pers.: Fr. | DF, FPG, MF, RVD, WCA | SHST, XST | V–XI | 9 |
| *P. arvernensis* Boud. | CF, DF, WCA | SHST, XST | VII–VIII | 4 |
| *P. badia* Pers.: Fr. | CF, DF, FPG, MF, RP, RVD, WCA | SHST | VII–X | 25 |
| *P. badiofusca* (Boud.) Dennis | DF | SHST | X | 1 |
| *P. celtica* (Boud.) M. M. Moser | CF, F, FPG, MF, P | PPH?, SHST | VII | 3 |
| *P. cerea* Bull.: Fr. | B, GH, WB | AST, HST, SHST, XST | IV–IX | 9 |
| *P. echinospora* P. Karst. | F, P, RP, W, WCA | PPH | V–X | 22 |
| *P. fimeti* (Fuckel) Seaver | CF | CPH | IV–IX, CULT | 7 |
| *P. gerardii* Cooke | DF, P | SHST | VII | 1 |
| *P. howsei* Roze & Boud. | DF | SHST | VI | 1 |
| *P. lividula* W. Phillips | P, W | SHST | IX | 2 |
| *P. micropus* Pers.: Fr. | DF, MF, P, RP | XST | V–X | 9 |
| *P. ostracoderma* Korf | GH | SHST, XST | X | 3 |
| *P. phyllogena* Cooke | DF, FPG, MF, P, RVD | SHST | VI–VIII | 8 |
| *P. repanda* Pers. | B, CF, DF, G, MF, P, RVD, S | SHST | V–IX | 9 |
| *P. succosa* Berk. | CF, DF, P | SHST | VII–IX | 4 |

Table 1 (continued)

| Taxa | Habitats | Ecological groups | Fruiting time | No. of localities |
|---|---|---|---|---|
| *P. succosella* (Le Gal & Romagn.) M. M. Moser | DF | SHST | VIII | 1 |
| *P. tenacella* W. Phillips | F, P, RP, W, WB | PPH | IV–X | 24 |
| *P. varia* (Hedw.: Fr.) Fr. | B, G, GH, RP | HST, SHST, XST | IV–X | 6 |
| *P. vesiculosa* Bull.: Fr. | GH, MP, RVD | CPH, HST | VI–VIII | 5 |
| *Plicaria carbonaria* (Fuckel) Fuckel | CF, F | PPH | VII | 2 |
| *P. endocarpoides* (Berk.) Rifai | F, W | PPH | VII–X | 7 |
| *P. trachycarpa* (Curr.) Boud. | F, W | PPH | IX | 1 |
| **Pyronemataceae** | | | | |
| *Aleuria aurantia* (Pers.: Fr.) Fuckel | C, FPG, RP, W, WCA | SHST | VIII–XI | ~25 |
| *Anthracobia maurilabra* (Cooke) Boud. | CF, F | PPH | V–IX | 6 |
| *A. melaloma* (Alb. & Schwein.: Fr.) Arnould | F, W, WB | PPH | V–X | 7 |
| *Byssonectria fusispora* (Berk.) Rogerson & Korf | F, W | NLST, PPH, SHST | IV–VII | 5 |
| *Caloscypha fulgens* (Pers.: Fr.) Boud. | CF, MF, P | SHST | IV–VII | 2 |
| *Cheilymenia crucipila* (Cooke & W. Phillips) Le Gal ex Denison | FPG, W, WCA | SHST | V–IX | 12 |
| *C. fimicola* (De Not. & Bagl.) Dennis | W | CPH | VI–X | 5 |
| *C. granulata* (Bull.: Fr.) J. Moravec | MP, W, WB | CPH | IV–X | 8 |
| *C. stercorea* (Pers.: Fr.) Boud. | MP, W | CPH | IV–XI | 14 |
| *C. theleboloides*(Alb. & Schwein.: Fr.) Boud. | P, W | SHST, CPH | VI–IX | 4 |
| *C. vitellina* (Pers.: Fr.) Dennis | G, P, W | SHST | VI–IX | 4 |
| *Coprotus dextrinoideus* Kimbr., Luck-Allen & Cain | DD | CPH | CULT | 1 |
| *C. leucopocillum* Kimbr., Luck-Allen & Cain | MP, W | CPH | VI–VII, CULT | 3 |
| *C. luteus* Kimbr., Luck-Allen & Cain | W | CPH | VIII–X | 4 |
| *C. ochraceus* (P. Crouan et H. Crouan) J. Moravec | CF | CPH | VI | 1 |
| *C. sexdecimsporus* (P. Crouan & H. Crouan) Kimbr. & Korf | DD | CPH | VII | 1 |
| *Geopora arenicola* (Lév.) Kers | F, FPG, RP, W | SHST | VII–IX | 13 |
| *Geopyxis carbonaria* (Alb. & Schwein.: Fr.) Sacc. | F, P, W | PPH | IV–IX | 17 |
| *Humaria hemisphaerica* (Wigg.: Fr.) Fuckel | F, P, S, W | SHST, XST | VII–X | 30 |
| *Kotlabaea deformis* (P. Karst.) Svrèek | W, WCA | SHST | V–VI | 2 |
| *Lasiobolus ciliatus* (J. C. Schmidt: Fr.) Boud. | MP, P, S, W | CPH | IV–X | 22 |
| *L. cuniculi* Velen. | W | CPH | IX, CULT | 2 |
| *L. intermedius* J. L. Bezerra & Kimbr. | W | CPH | IX | 1 |
| *L. macrotrichus* Rea | W | CPH | VI | 1 |
| *L. ruber* (Quél.) Sacc. | CF | CPH | VI | 1 |
| *Leucoscypha erminea* (E. Bommer & M. Rousseau) Boud. | CF | SHST, XST | VII | 1 |
| *Melastiza chateri* (W.G. Sm.) Boud. | FPG, RVD, W, WCA | SHST, XST | VI–IX | 11 |
| *Miladina lechithina* (Cooke) Svrèek | CF, WB | XST | VII | 1 |
| *Neottiella hetieri* Boud. | F, W | BPH, PPH | VI–VII | 2 |
| *N. rutilans* (Fr.) Dennis | CF | BPH, SHST | VII–IX | 7 |
| *N. vivida* (Nyl.) Dennis | D, RP, W | BPH, SHST | IX–X | 3 |
| *Octospora humosa* (Fr.: Fr.) Dennis | W | BPH, SHST | X | 1 |
| *O. rubens* (Boud.) M. M. Moser | RP, W, WCA | BPH, SHST | IX–X | 2 |
| *O. rustica* (Velen.) J. Moravec | MP | BPH, SHST | IX | 1 |
| *O. tetraspora* (Fuckel) Korf | RK | BPH, SHST | IX | 1 |
| *Otidea alutacea* (Pers.) Massee | CF, DF, G, P | SHST | VII–IX | 8 |
| *O. bufonia* (Pers.) Boud. | FPG, P, W | SHST | VIII–X | 16 |

Table 1 (continued)

| Taxa | Habitats | Ecological groups | Fruiting time | No. of localities |
|---|---|---|---|---|
| *O. leporina* (Batsch) Fuckel var. *leporina* | CF | SHST | VIII–IX | 11 |
| *O. leporina* var. *minor* (Rehm) Sacc. | CF, MF | SHST | VIII–X | 2 |
| *O. onotica* (Pers.: Fr.) Fuckel | P, W | SHST, XST | VII–IX | 25 |
| *O. phlebophora* (Berk. & Broome) Sacc. | DF, P | SHST | VII | 1 |
| *Pseudombrophila porcina* (Svrèek & Kubièka) Brumm. | CF, D | CPH | VIII | 1 |
| *P. theioleuca* Rolland | CF | CPH | VIII | 1 |
| *Pulvinula carbonaria* (Fuckel) Boud. | CF, F | PPH | IX | 1 |
| *P. constellatio* (Berk. & Broome) Boud. | FPG, W | SHST | VII–IX | 6 |
| *Pyronema domesticum* (Sowerby: Fr.) Sacc. | B, F, W, WCA | AST, PPH | VII–IX | 8 |
| *P. omphalodes* (Bull.: Fr.) Fuckel | CF, F | PPH | VII–IX | 5 |
| *Rhodotarzetta rosea* (Rea) Dissing & Sivertsen | F, WCA | PPH | IX | 1 |
| *Scutellinia kerguelensis* (Berk.) Kuntze | FPG, W, WB | SHST, XST | VII–IX | 3 |
| *S. minutella* Svrèek & J. Moravec | W, WCA | SHST | V | 1 |
| *S. olivascens* (Cooke) Kuntze | W | SHST, XST | V–X | 5 |
| *S. scutellata* (L.: Fr.) Lambotte | B, G, P, RP, W, WB, WCA | HT, MST, NLST, PPH, SHST, XST | V–X | ~50 |
| *S. subhirtella* Svrèek | FPG, P, W, WB | SHST, XST | VI–IX | 9 |
| *S. trechispora* (Berk. & Broome) Lambotte | CF, DF | SHST | VII–IX | 2 |
| *S. umbrorum* (Fr.) Lambotte | W | SHST, XST | VI–IX | 4 |
| *Sepultaria semiimmersa* (P. Karst.) Massee | F, FPG, RVD, W | SHST | IX | 3 |
| *Sphaerosporella brunnea* (Alb. & Schwein.: Fr.) Svrèek & Kubièka | CF, F, WCA | PPH | VII–IX | 6 |
| *Tarzetta catinus* (Holmsk.: Fr.) Korf & J. K. Rogers | CF | SHST | VIII | 1 |
| *T. cupularis* (L.: Fr.) Lambotte | FPG, P, RVD, S, W, WB | SHST | V–IX | 12 |
| *T. gaillardiana* (Boud.) Korf & J. K. Rogers | W | SHST | VIII | 1 |
| *Tricharina cretea* (Cooke) K.S. Thind & Waraitch | GH | SHST | VII | 1 |
| *T. gilva* (Boud.) Eckblad | F, FPG, W, WCA | PPH, SHST | VI–IX | 3 |
| *Trichobolus sphaerosporus* Kimbr. | CF | CPH | VI | 1 |
| *Trichophaea gregaria* (Rehm) Boud. | CF, DF, F | SHST, XST | VIII–IX | 6 |
| *T. hemisphaerioides* (Mouton) Graddon | F, W | PPH | VII–X | 7 |
| **Sarcoscyphaceae** | | | | |
| *Desmazierella acicola* Lib. | CF | NLST | IV | 1 |
| *Microstoma protracta* (Fr.) Kanouse | CF | XST | III | 1 |
| *Pithya cupressina* (Batsch: Fr.) Fuckel | G, P | NLST, XST | IV–V | 2 |
| *Sarcoscypha austriaca* (Beck ex Sacc.) Boud. | P, S, W, WB | XST | III–V | ~35 |
| *S. coccinea* (Jacq.: Fr.) Lambotte | W | XST | IV | 1 |
| **Sarcosomataceae** | | | | |
| *Pseudoplectania nigrella* (Pers.: Fr.) Fuckel | CF | SHST, XST | IV–VI | 6 |
| *Sarcosoma globosum* (Schmidel: Fr.) Casp. | CF | SHST | IV, XII | 3 |
| *Urnula craterium* (Schwein.: Fr.) Fr. | P, W | SHST | III–V | 6 |
| **Thelebolaceae** | | | | |
| *Thelebolus caninus* (Auersw.) Jeng & J.C. Krug | W | CPH | VIII | 1 |
| *T. crustaceus* (Fuckel) Kimbr. | W | CPH | VI, CULT | 4 |
| *T. microsporus* (Berk. & Broome) Kimbr. | W | CPH | VI–X | 11 |
| *T. polysporus* (P. Karst.) Otani & Kanzawa | W | CPH | VI–VIII | 2 |
| *T. stercoreus* Tode: Fr. | W | CPH | VI, CULT | 4 |
| **Tuberaceae** | | | | |
| *Tuber exiguum* R. Hesse | MF | SBT, SHST | VIII | 1 |
| *T. rufum* Pico | MF | SBT, SHST | VIII | 1 |

## ACKNOWLEDGMENTS

I am grateful to Dr. Ain Raitviir for his valuable advise, assistance and comments on the manuscript. Thanks also to Dr. Brian Spooner for helpful suggestions concerning taxonomy and nomenclature. This study was partly funded by the Government of Lithuania, the International Science Foundation (USA) and the Nordic Scholarship Scheme for the Baltic Countries of the Nordic Council of Ministers.

## REFERENCES

Balevicius, K., Lapele, M. & Paltanavicius, S. (eds). 1992. *Lietuvos raudonoji knyga*. Vilnius, Lietuvos Respublikos aplinkos apsaugos departamentas. 364 pp.

Dissing, H. 1982. Operculate Discomycetes (Pezizales) from Greenland. In *Arctic and Alpine Mycology. The First International Symposium on Arcto-Alpine Mycology* (eds Laursen, G. A. & Ammirati, J. F.), pp. 56–81. Seattle, London, Univ. Washington Press.

Järva, L. & Parmasto, E. 1980. *List of Estonian Fungi.* Tartu. 331 pp.

Järva, L., Parmasto, I. & Vaasma, M. 1998. *List of Estonian Fungi.* Supplement 1. Tartu. 183 pp.

Kristiansen, R. 1983. Bidrag til Østfolds Ascomycetflora. 2. *Agarica* 4 (8): 220–264.

Kutorga, E. 1989a. Discomycetes from Curonian Spit. In *Jaunuju mokslininku straipsniø rinkinys (Botanikos institutui – 30 metu)*, pp. 39–47. Vilnius.

Kutorga, E. 1989b. Discomycetes inhabiting various parts of *Alnus*. In *X Congress of European mycologists. Abstracts*, p. 66. Tallinn.

Kutorga, E. 1990. Istoricheskii obzor izucheniya diskomicetov Litvi i napravleniya dal'neishikh ikh isledovanii. *Ekologija* 2: 40–52.

Kutorga, E. 1991a. Patogen *Rhizina undulata*: razvitie griba na kostrishshchakh. In *Problemi lesnoi fitopatologii i mikologii. Tezisi dokladov*, p. 36. Moskva–Kaunas.

Kutorga, E. 1991b. *Vakaru ir Pietvakariu Lietuvos diskomicetai (rušine sudetis, paplitimas ir struktura)* [Discomycetes of western and south-western parts of Lithuania (species composition, distribution and structure); doctoral thesis]. Vilnius.

Kutorga, E. 1992. Bobausiai. Valgyti ar ne? *Musu gamta* 4: 4–5.

Kutorga, E. 1993. Bobausiu plantacija. *Musu gamta* 5: 13.

Kutorga, E. 1994a. New records in the genus *Geopora* from Lithuania. *Ekologija* 2: 28–30.

Kutorga, E. 1994b. Paprastasis taukius Lietuvoje. *Musu gamta* 2: 3–4.

Kutorga, E. 1996a. Carbotrophic discomycetes in Lithuania. In *13th International Conference "Fungi and Lichens in the Baltic Region". Abstracts*, pp. 30–31. Riga.

Kutorga, E. 1996b. Mycological and lichenological investigations in the former soviet military forestries in Lithuania. Discomycetes. *Botanica Lithuanica* 2 (3): 221–232.

Kutorga, E. 1997. Briedzhiukiniu (*Morchellaceae*) šeimos grybai Lietuvoje. In *Lietuvos bioivairove (bukle, struktura, apsauga). Respublikines konferencijos dalyviu pranešimu santraukos*, pp. 81–82. Vilnius.

Kutorga, E. 1999. New records of discomycetes from Lithuania. *Botanica Lithuanica* 5 (1): 79–83.

Kutorga, E. & Vishnjauskas, A. 1991. Sistematiko-ekologicheskii analiz napochvennikh gribov poryadka *Pezizales* zapadnoi i yugo-zapadnoi Litvi. *Ekologija* 2: 3–12.

Prokhorov, V. & Kutorga, E. 1990. Koprotrofnie diskomiceti Litvi i Latvii. *Ekologija* 4: 23–32.

Smitskaya, M. F. 1980. *Flora gribov Ukraini. Operkulyatnie diskomiceti*. Kiev: Naukova dumka. 221 pp.

Svrcek, M. 1981. Katalog operkulatnich diskomycetu (*Pezizales*) Cekoslovenska. III. *Ceská Mykol.* 35 (3): 134–151.

Vasiliauskas, A. 1998. Kalniniu pušu dzhiuvimo priezhastys Kuršiu nerijoje. *Musu girios* 7 (603): 15–16.

Folia Cryptog. Estonica, Fasc. 36: 57–63 (2000)

# The ultrastructure of *Belonidium aeruginosum* Mont. & Durieu (Hyaloscyphaceae, Helotiales)

**Kadri Leenurm[1, 2] & Ain Raitviir[2]**

[1] Institute of Botany and Ecology, University of Tartu, 40 Lai St., 51005 Tartu, Estonia. E-mail: kadleen@ut.ee

[2] Institute of Zoology and Botany, Estonian Agricultural University, 181 Riia St., 51014 Tartu, Estonia. E-mail: ain@zbi.ee

**Abstract:** The ultrastructure of ascus apical apparatus and hair wall of *Belonidium aeruginosum* Mont. & Durieu has been studied. All significant ultrastructure features were found to be very similar to those in *Incrucipulum ciliare* (Fr.) Baral and a conclusion is made that *Belonidium* Mont. & Durieu is an earlier synonym of *Incrucipulum* Baral.

**Kokkuvõte:** K. Leenurm & A. Raitviir. Seene *Belonidium aeruginosum* Mont. & Durieu (Hyaloscyphaceae, Helotiales) ultrastruktuur.

Uuriti liudseene *Belonidium aeruginosum* Mont. & Durieu eoskoti tipuaparaadi ja karva seina ultrastruktuuri. Leiti, et kõik olulised ultrastruktuuri tunnused uuritud liigil ja liigil *Incrucipulum ciliare* (Fr.) Baral on väga sarnased. Sellest võib järeldada, et *Belonidium* Mont. & Durieu tuleb lugeda *Incrucipulum* Baral varasemaks sünonüümiks.

## INTRODUCTION

This paper is a continuation of our studies on the ultrastructure of the hyaloscyphaceous genera related to the genus *Lachnum* Retz. (Leenurm et al., 2000). The type and the only original species of the genus *Belonidium* Mont. & Durieu has been studied for the reason that its identity with *Incrucipulum* Baral has been suggested (Raitviir, 1991; Järv, 1995).

## MATERIAL

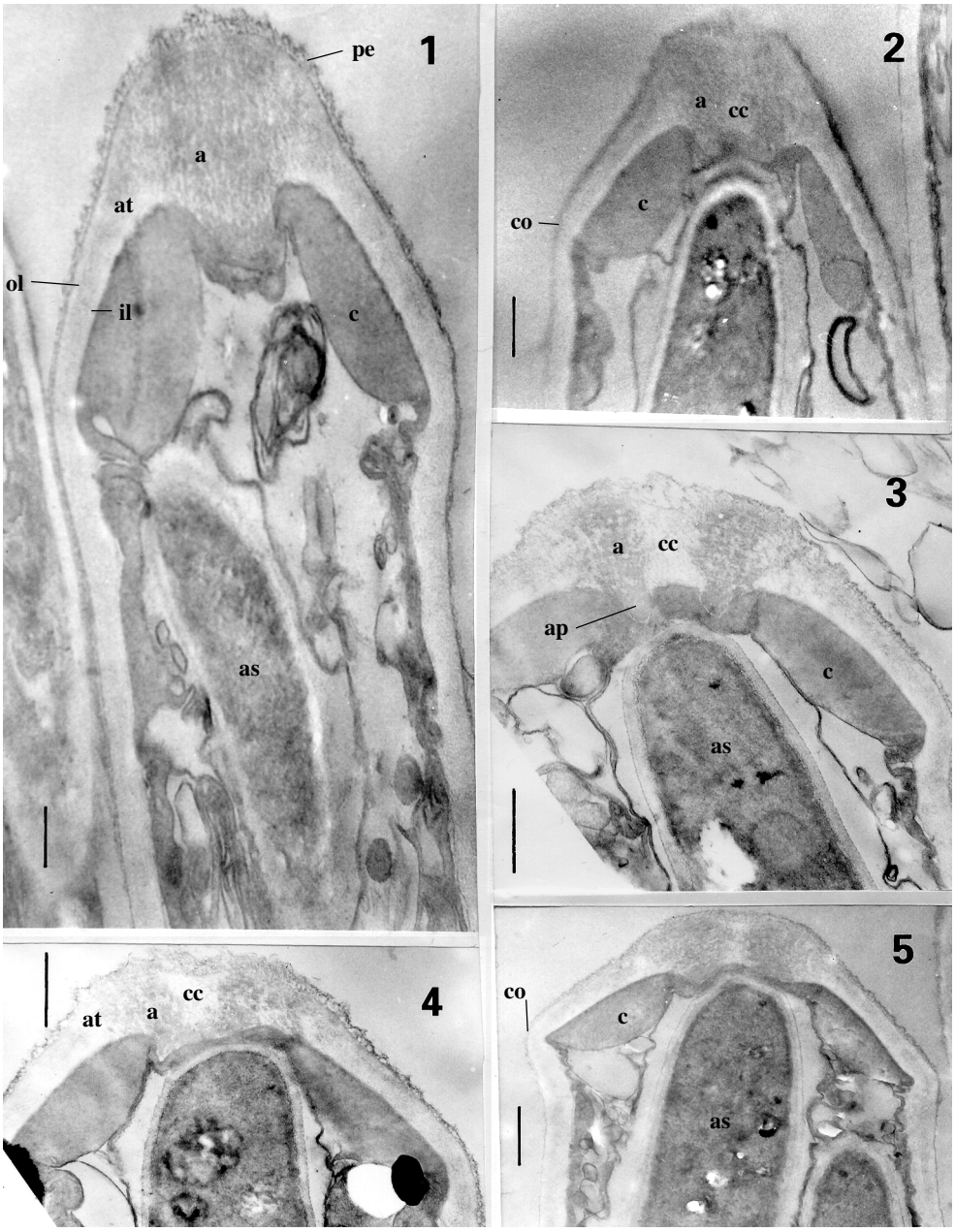
A living material for this study was sent us by Dr. Ricardo Galán. The collecting data of it are as follows: *Belonidium aeruginosum* Mont. & Durieu – on dead leaves of *Quercus ilex* subsp. *ballota*, Spain, Tamajón, Guadalajara, 26 May 1998, leg. R. Galán, AH–7177, TAA–137738.

## METHODS

Received by mail living material was kept in a small plastic box under humid condition for some days to become fully turgescent and then fixed.

The methods followed were those described by Curry & Kimbrough (1983) and Samuelson & Kimbrough (1978). For transmission electron microscopy, the fruitbodies were fixed for 2 h using 2% paraformaldehyde, 2.5% glutaraldehyde and 2 mM calcium chloride in 0.1M sodium cacodylate buffer (pH=7.2) at room temperature. Material was rinsed in 0,1M cacodylate buffer (pH=7.2) and postfixed for 45 min in 1% osmium tetroxide in the same buffer (pH=7.2) at room temperature. The material was buffer rinsed and dehydrated through a graded ethanol series from 10% to 90% (in 10% steps) followed by acetone, each 10 min. The material was embedded in Spurr's resin (ERL 4206) using infiltration series resin and acetone in 1:3, 1:1 and 3:1 proportions for 3 hours each and then polymerized. The material was thin-sectioned on a Reichert ultramicrotome Om2U using glass knives and the sections were stained with uranyl acetate and lead citrate. The material was examined using a JEOL-100S electron microscope.

The terminology for hair wall stratification based on cell wall terminology of Gooday (1995) and the terminology of Verkley (1995) for ascus apical apparatus are used following Leenurm et al. (2000).




**Figs 1–5.** Ascus apical apparatus of *Belonidium aeruginosum*
1. Young ascus. – **2–3.** Immature ascus. – **4.** Mature ascus (near median section). – **5.** Mature ascus. Bar = 1µm. Abbreviations: a – annulus, ap – annular protrusion, as – ascospore, at – apical thickening, c – apical cap, cc – central cylinder, co – collar, il – inner layer of ascus wall, ol – outer layer of ascus wall, pe – periascus.

## RESULTS

**Ascus** (Figs 1–5). The apex of a young ascus is almost lanceolate with an obtusely rounded tip (Fig. 1). During maturing, the apex shortens and widens becoming conical-truncate, in some cases with a characteristic shortly and sharply swollen collar just below the tip (Fig. 5). The apical thickening is gradually widening towards the ascus tip, mainly consisting of material continuous with the inner layer of the lateral ascus wall. The inner layer is electron-translucent, about three times thicker than the outer layer in the lateral ascus wall, becoming very thick in the young ascus tip. As the ascus matures, its tip becomes much thinner due to the compression of the material continuous with the inner layer. The outer layer is evenly thin at the ascus flanks and the tip. It consists of a very electron-translucent inner stratum and a very thin electron-dense outer stratum. An electron-dense, fibrillar stratum or periascus covers the ascus wall, becoming thicker and more loose at the ascus tip.

In the young ascus the developing apical apparatus is present as a cylindric-obconical area of sparsely electron-dense material comprising numerous microtubular structures and microvesicles; the central cylinder and apical chamber have not yet been differentiated, the annular protrusion is very long (Fig. 1). In the immature ascus (Figs 2, 3) the annulus, central cylinder and apical chamber are clearly differentiated. The ascus apical apparatus becomes shorter and wider during this stage. The annulus comprises electron-dense material, which is packed most densely in the annular protrusion. The annular protrusion is well delimited in the immature ascus and becomes compressed by the ascospore during the ascus maturation. The central cylinder comprises irregular electron-translucent fibrillar material, wider than the lateral ascus wall in the mature ascus. The apical chamber is convex and rather big in earlier stages of development, in the mature ascus it becomes shallow plane-concave under the pressure of the ascospore (Figs 4, 5).

In the epiplasma of the apical region there is an electron-dense, fine-granulated, quite homogenous structure, visible in section as cushion-shaped bodies, tightly pressed against the ascus wall, connected with a narrow band

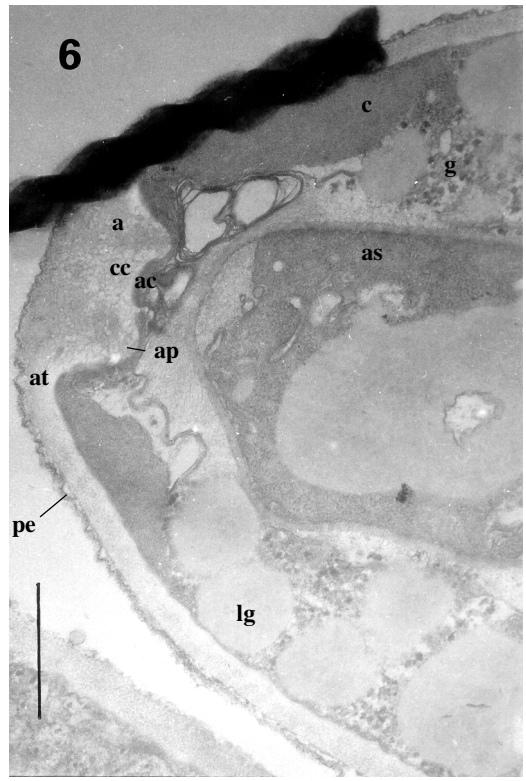
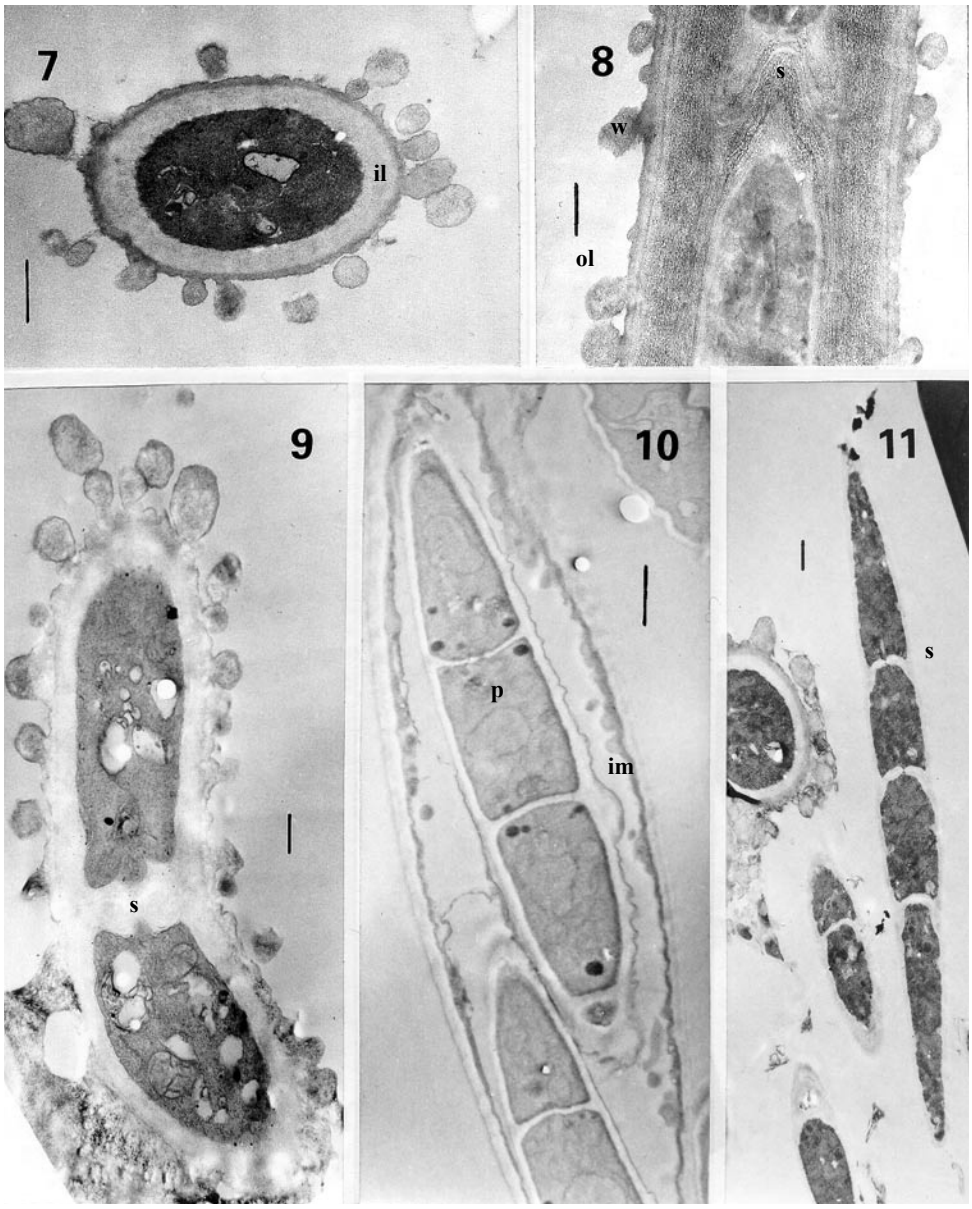


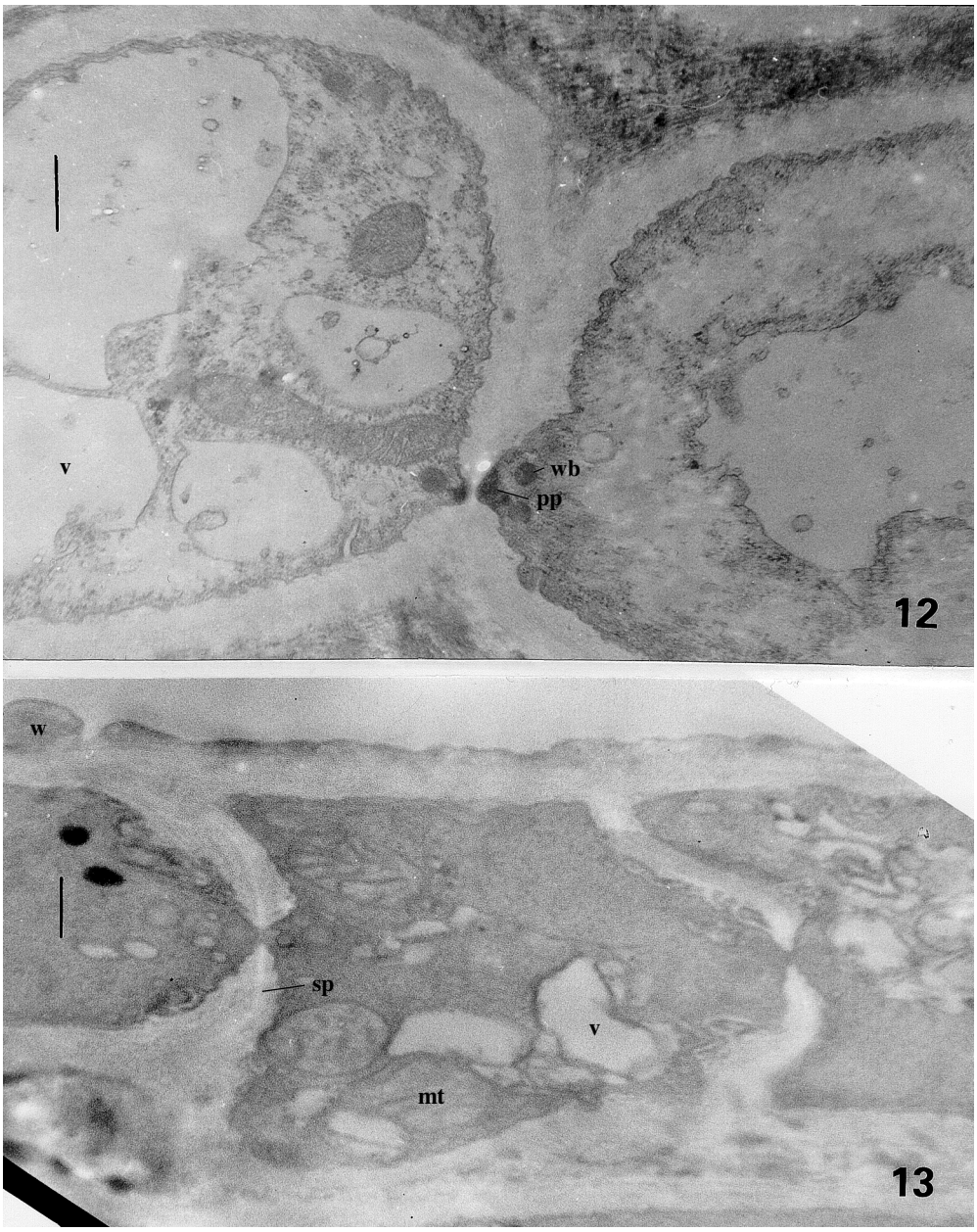

**Fig. 6.** Ascus apical apparatus of *Incrucipulum ciliare* (near median section). Bar = 1µm. Abbreviations: a – annulus, ac – apical chamber, ap – annular protrusion, as – ascospore, at – apical thickening, c – apical cap, cc – central cylinder, g – glycogen, lg – lipid globul, pe – periascus.

of the same material below the annulus. This structure forms a cap surrounding the ascus apical apparatus and wall in the conical part of the ascus above the collar and is here named apical cap. It seems, that the apical cap prevents strong elongation of the ascus tip under the growing osmotic pressure in the process of maturation and is responsible for the widely conical shape of the mature ascus apex and the presence of the collar.

**Hair wall.** (Figs 7–9, 13) The wall is well-stratified with papilliform warts. Warts are more crowded on the upper part (Figs 7, 8) and almost lacking on the base of the hair (Fig. 13).




**Figs 7–11.** – **7.** A cross section of a hair. – **8.** Hair wall (longitudinal section). – **9.** One-celled hair. – **10.** Two ascospores within ascus. – **11.** Liberated ascospore. Bar = 1µm. Abbreviations: il – inner layer of hair wall, im – investing membrane, ol – outer layer of hair wall, p – pore, s – septum, w – wart.




**Figs 12–13.** – **12.** Excipular cells. – **13.** Hair basal cells. Bar = 1µm. Abbreviations: mt – mitochondrion, pp – pore plug, sp – septal plate, v – vacuole, w – wart, Wb – Woronin body.

The most intensive forming of warts occurs at the hair tip as it is well seen in short one-celled hair (Fig. 9). It should be noted that the warts are not only present on the walls of hairs, but they cover also the external surface of the outermost excipular cells, as it is seen in the excipular cells just below the hairs (Figs 9, 13). The hair wall is composed of two basic layers. An inner layer, which is mostly more electron-translucent and more evenly arranged, granular; and the outer layer, which is differentiated into a granular-fibrillar inner stratum, and an outer stratum, which is clearly delimited, as it is more electron-dense, with a homogeneously fine-granular structure. Externally the wall and warts are covered with a thin electron-dense fibrillar layer.

**Ascospores** (Figs 10 – 11) are narrowly-fusoid, 4-celled, having septal pores (obviously with a pulley-wheel shaped plug) and small electron-dense spherical bodies close to the septa. Those bodies cannot be identified with certainty, but it is unlikely, that they are Woronin bodies because no distinct membrane is visible. The pulley-wheel shaped plug and Woronin bodies were, however, well observed near a pore between two excipular cells (Fig.12). The ascospore wall differentiation is observed. In young asci the ascospores are non-septate, with undifferentiated walls (Fig. 1). Later, the ascospore walls become two-layered and surrounded by a wavy investing membrane during maturation (Fig. 10). In mature spores the wall is distinctly two-layered (Figs 3, 5) and finally remarkably electron-translucent, as most clearly observed in a liberated ascospore (Fig.11).

## DISCUSSION

At the comparable stages of development the ascus apical apparatus structure of *Belonidium aeruginosum* is almost exactly the same as that of *Incrucipulum ciliare* (Fr.) Baral (Fig. 6), see also Leenurm et al. (2000). The only difference between the asci of *B. aeruginosum* and *I. ciliare* is the presence of a collar at the mature stage in the former but it is evidently a character at the species level.

The unique feature of this ascus type is the presence of an electron-dense apical cap, not observed in any other species of inoperculate Discomycetes. It bears in structure a striking resemblance to the lower ring in the ascus of an aquatic pyrenomycete *Annulatascus* (Sordariales) (Wong et al., 1999). Those two structures differ in the fact that the lower ring of *Annulatascus* is really a centrally open ring, but the apical cap of *Belonidium* forms a continuous cover below the annulus.

The hair wall structure and stratification, and the origin and structure of warts are also similar in *B. aeruginosum* and in *I. ciliare* (Leenurm et al., 2000). A characteristic feature of this hair wall type is the strongly thickened outer layer. The wall of excipular cells is warty in *B. aeruginosum*, too. This character was particularly stressed by Baral (Baral & Krieglsteiner, 1985) for his new genus *Incrucipulum*. The ultrastructural characters strongly support the idea that *Belonidium* and *Incrucipulum* are congeneric and *Belonidium* offers an earlier name for the genus *Incrucipulum*.

The development of the ascus apical apparatus was observed from its early to late phases of development. During this process the apical apparatus passes through a series of changes in its size and structure. Consequently the comparison of apical apparatuses in different taxa can be made only at the same stages of ascus development. as Verkley (1995) already concluded for three families of Helotiales.

## ACKNOWLEDGEMENTS

The authors are greatly indebted to Dr. Ricardo Galán (Universidad de Alcalá, Spain) for the living collection of *B. aeruginosum*. The study was financially supported by Estonian Science Foundation grant 2148 to A. Raitviir and K. Leenurm, by ESF grant 3921 to K. Leenurm and by the Estonian Government, Ministry of Education Research grant No. 0370204. We thank the staff of the Laboratory of General Zoology, University of Tartu for providing the facilities and technical support during this study.

## REFERENCES


Baral, H. O. & Krieglsteiner, G. J. 1985. Bausteine zu einer Ascomyceten-flora der Bundesrepublik Deutschland: in Süddeutschland gefundene inoperculate Discomyceten – mit taxonomischen, ökologischen, chorologischen Hinweisen. *Beih. Z. Mykol.* 6: 1–226.

Curry, K. J. & Kimbrough, J. W. 1983. Septal structures in apothecial tissues of the Pezizaceae (Pezizales, Ascomycetes). *Mycologia* 75: 781–794.

Gooday, G. W. 1995. The architecture of the fungal cells. In *The Growing Fungus* (eds Gow, N.A.R. & Gadd, G.M.), pp. 43–134. Chapman & Hall. London, Glasgow.

Järv, H. 1995. *Studies in the segregates of Lachnum Retz. (Hyaloscyphaceae, Leotiales) with thick-walled hairs.* Manuscript (M. Sc. thesis) in University of Tartu. 79 pp.

Leenurm, K., Raitviir, A. & Raid, R. 2000. Studies on the ultrastructure of *Lachnum* and related genera (Hyaloscyphaceae, Helotiales, Ascomycetes). *Sydowia* 52: 30–45.

Raitviir, A. 1991. The Helotiales. In *Plantae non Vasculares, Fungi et Bryopsida Orientis Extremi Sovietici, Fungi 2* (ed. Azbukina, Z.M.), pp. 254–363.

Samuelson, D. A. & Kimbrough, J. W. 1978. Asci of the Pezizales. V. The apical apparatus of *Trichobolus zukalii*. *Mycologia* 70: 1191–1200.

Verkley, G. J. M. 1995. The ascus apical apparatus in Leotiales: an evaluation of ultrastructural characters as phylogenetic markers in the families Sclerotiniaceae, Leotiaceae, and Geoglossaceae. *Proefschrift. Rijksherbarium*. Leiden. 209 pp.

Wong, S.-W., Hyde, K. D., Jones, E. B. G. & Moss, S. T. 1999. Ultrastructural studies on the aquatic ascomycetes *Annulatascus velatisporus* and *A. triseptatus* sp. nov. *Mycol. Res*. 103: 561–571.

Folia Cryptog. Estonica, Fasc. 36: 65–81 (2000)

# Lichens of Vormsi Island

**Ljudmilla Martin[1], Tiina Randlane[2] & Jüri Martin[1]**
[1]Eurouniversity, 4 Mustamäe St., 10621 Tallinn, Estonia. E-mail: juri@iceb.ee
[2]Institute of Botany & Ecology, University of Tartu, 38 Lai St., 51005 Tartu, Estonia. E-mail: randlane@ut.ee

**Abstract:** The list of lichenized and allied species presents locality and substrate data for 301 species and 4 infraspecific taxa identified in Vormsi Island; eight species are new to Estonia and 79 species belong to the group of rare taxa with 10 or less localities in Estonia. According to the substrate, nine ecological groups have been distinguished; the groups of epiphytes are the most numerous while rare taxa are more numerous among epilithic lichens on calcareous substrate and among epibryophytes.

Ten geographical elements have been identified among the lichens of Vormsi Island; multiregional, boreal, nemoral and holarctic elements are the biggest of them as expected. Relative abundance of southerly distributed lichens – xerocontinental and submediterranean taxa – is connected with the presence of various xeric habitats in Vormsi Island, e.g. alvars, moraine deposits, outcrops of limestone etc. Lichens of mainly northerly distribution often occur in the same habitats.

Concentration of different lichen species is biggest on Rumpo Peninsula where169 lichenized taxa have been recorded on a rather limited territory.

**Kokkuvõte:** L. Martin, T. Randlane ja J. Martin. Vormsi saare samblikud.

Vormsi saare samblike nimekiri sisaldab kohalikke leviku- ja kasvukohaandmeid 301 liigi ja 4 liigisisese taksoni kohta; neist kaheksa liiki on Eesti lihhenofloorale uued ja 79 liiki on Eestis seni leitud 10 või vähemast leiukohast. Substraadi järgi jagunevad Vormsi samblikud üheksasse ökoloogilisse rühma, neist kõige liigirikkamad on epifüüdid; haruldasi liike on kõige arvukamalt lubjakivil kasvavate epiliitide ja epibrüofüütide hulgas.

Geograafiliste elementide järgi jagunevad Vormsilt leitud samblikud kümnesse rühma; multiregionaalne, boreaalne, nemoraalne ja holarktiline element on neist ootuspäraselt liigirikkaimad. Suhteliselt arvukalt on lõunapoolse, eelkõige kserokontinentaalse levikuga liike; põhjapoolse levikuga – arktoalpiinseid ja hüpo-arktomontaanseid – taksone leidub Vormsil vähem. Mõlema rühma esindajad asustavad valdavalt loopealseid, rannavalle, graniit- ja lubjakive.

Üheks huvipakkuvamaks samblike leiukohaks Vormsil tuleb hinnata Rumpo poolsaart, kust seni on leitud 169 samblikuliiki.

## INTRODUCTION

Vormsi Island (Ormsö in Swedish) is situated in the north-eastern part of the Baltic Sea, on the western coast of Estonia (58°57'–59°02'N 23°07'–23°23'E) (Fig. 1). The shortest distance Estonian mainland of (Noarootsi Peninsula) is less than 3 km. It is the fourth biggest island of Estonia (besides Saaremaa, Hiiumaa and Muhu) with a territory of 93 sq. km.

Vormsi Island emerged from the sea about 3000 years ago during the Limnea II and Limnea III stages of the Baltic Sea. The oldest parts of the island are also its highest points (Lillkullbackan between Hullo and Rumpo villages, Högbackan) exceeding 12,7 and 11,9 m while the average altitude of Vormsi is 5–6 m above the sea level. The whole island is situated in an area of constant and rather fast neotectonic uplift about 2.2–3.5 mm per year (Zhelnin 1958; Raukas 1995). Quaternary sediments are rich in peb-

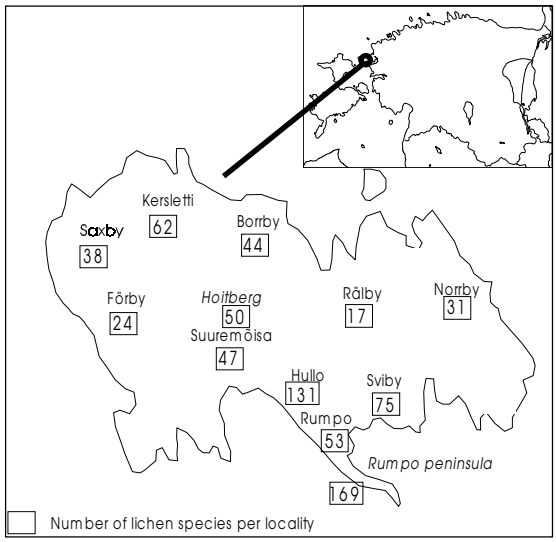



**Fig. 1.** Location of Vormsi Island on the western coast of Estonia and number of recorded lichen species per locality in Vormsi.

ble formed mostly of limestone. In the western part of Vormsi Island pebble is mainly composed of limestone and up to 5 % of crystalline rock while in the eastern part of the island the proportion of crystalline pebble in moraine material reaches 20–40 % (Raukas 1969). Moraine deposits are rich in granite erratic boulders of different size. Circumference of the largest erratic boulders is up to 20–25 m. Beach ridges originating from the Limnea stage as well as more recent ones are frequent on Vormsi Island. Some of these formations are connected to the moraines which are clearly visible on the coastline of Rumpo Peninsula.

The crystalline basement of Vormsi is overlain by the Ordovician carbonate rocks and limestones. The Quarternary cover is composed of glacial and glaciofluvial deposits of various thickness: it is thicker in the southern part (up to 10–12 m above the sea level in the vicinity of Hullo and Sviby) and very thin in the western part of the island (0,6 m in Saxby and Kersleti). The bedrock relief is predominantly flat. The elevation of Hoitberg (10 m high, relative elevation 2.5 m), where the bedrock outcrop composed of Pirgu stage limestone is exposed, is located in the western part of Vormsi. Marine sediments – sand and gravel – occur all over the island. The biggest coastal accumulative terraces are situated in the western part (Saxby) where the glacial deposits are thin and carbonate rocks close to the surface or even exposed. The radial eskers of NW–SE – orientation which are considerably abraded by the sea are the most characteristic glacial relief forms (Ratas 1977).

West-Estonian Archipelago is located in the area of maritime climate. Still, influence of the open Baltic is not so strong in Vormsi Island due to the distance and other islands around. The shallow Väinameri (inner sea of the West-Estonian Archipelago) also causes differences in air temperatures, humidity and precipitation if compared to with other Estonian islands and the mainland coast. The average yearly air temperature is 5.3? C in Vormsi which is lower than in Hiiumaa Island and in Haapsalu (western coast of mainland, 15 km east of Vormsi). The average annual wind speed is 5.6 m/s which is also higher than in the surrounding islands and the closest mainland coast. Southwestern and southern winds dominate (32%) in Vormsi. The average amount of precipitation is 536 mm per year in Vormsi. It is lower than in western Hiiumaa (Ristna, 561 mm) but higher than in southern Hiiumaa (Heltermaa, 526 mm) and Haapsalu. Duration of the frost free period is remarkable – it lasts 120–125 days and snow cover is present on 106 days (Raik 1967; Mardiste 1967; Ratas 1977).

Plant cover of Vormsi Island is similar to the vegetation of the western part of the Estonian mainland but also to that of Hiiumaa Island. The main types of vegetation are boreonemoral grasslands, coastal meadows and paludified grasslands. Alvar grasslands occur in rather limited territories only. About 34% of the territory are covered with different types of forests, mainly alvar forests and shrublands. Dry and fresh boreal forests and dry boreonemoral forests where the dominating tree species are *Pinus sylvestris* and *Picea abies* also occur. *Juniperus communis* is usual inhabitant in the second tree layer as well as *Corylus avellana, Frangula alnus, Lonicera xylosteum*. In paludified forests *Betula pendula* is dominating, occasionally with *Alnus glutinosa* and *Populus tremula*. *Acer platanoides, Aesculus hippocastanum, Fraxinus excelsior* and other broadleaved deciduous trees grow in parks of Hullo and Suuremõisa.

## HISTORICAL SURVEY OF LICHENOLOGICAL INVESTIGATIONS

Lichens of Vormsi Island have been studied since the 1930s by several persons but a general survey (including the list of lichens) has not been published yet. In the earliest publication – compiled by a Finnish lichenologist Ernst Häyren (1930) – in which the lichens of Vormsi Island have been mentioned only two very common species (*Parmelia sulcata* and *Xanthoria parietina*) are referred to. More thorough floristical data concerning lichens of Vormsi can be found in the manuscript of the doctoral dissertation by Hans Trass (1967) and later in the diploma paper by Raili Allmäe (1988). According to the latter, 210 lichen species grow in Vormsi Island.

Finding of *Flavocetraria cucullata* and other arctoalpine lichens in Vormsi on Rumpo Peninsula, which is the only locality of this species in Estonia (Trass 1963), and later observations concerning the richness of lichenized

taxa served as a motivation to establish a "Lichen Sanctuary" in the late seventies to protect rare and biogeographically interesting lichen species. It took more than ten years of discussions to go through different bureaucratic processes before the botanical reserve of Rumpo was officially established in 1987. In 1989, after the formation of the West Estonian Archipelago Biosphere Reserve, the botanical reserve of Rumpo was included in the former institution as one of the core areas.

Besides floristical studies some investigation projects concerning lichen populations and communities have been completed in this island. Studies on the structure and biomass of lichen vegetation inhabiting soil were carried out in connection with the estimation of radionuclides fallout after the accident in Tchernobyl Nuclear Power Station in 1986 (Martin et al. 1991). *Cladina mitis, C. rangiferina, Cetraria islandica, Flavocetraria cucullata* and *F. nivalis* appeared to be the dominant terricolous macrolichens on Rumpo Peninsula. Among them, *C. mitis* had the highest coverage (50 % in average) and the highest biomass (0.58 kg per $m^2$). Structure and biomass of investigated communities were quite comparable to those distributed in Arctic areas. This was the reason why studies on Rumpo Peninsula were included into the Circumarctic Program of vegetation contamination studies carried out by US Environmental Protection Agency. In 1992, *Flavocetraria cucullata* was sampled within the framework of this program as one of the indicator species.

The elemental analysis of dominant species demonstrated high concentrations of Zn, Mn and Pb in lichen biomass (Martin et al. 1991). Elevated concentrations of microelements mentioned above but also of Ni and Cd seem to be an impact of military air base which was located in Rohuküla, ca 15 km south of Vormsi Island. Furthermore, investigations concerning the accumulation of radionuclides have revealed that concentrations of radiocesium ($^{134,137}$ Cs) in terricolous lichens decreased exponentially. The higest concentration of radiocesium was detected in *Flavocetraria cucullata* (more than 4 kBq/kg). During four years (1986–1989) the concentration stabilized and reached the level close to that before radioactive fallout in 1986.

The impact of terricolous lichens on the soil microbial coenoses has also been investigated in Vormsi. The stimulating impact of lichen cover on the development of soil microflora was demonstrated while the antibiotic influence of lichens containing usnic acid could be discovered only in respect to the spore-forming bacteria and in the soil layer that was in close contact with lichens (Parinkina & Piin 1984, 1991).

The present survey was undertaken to enumerate all the lichenized taxa found in the island and to provide a general review of its lichen flora.

## MATERIAL AND METHODS

The herbarium materials used for the present review are kept in the University of Tartu (TU) – ca 550 specimens – and the International Centre for Environmental Biology (ICEB) – ca 420 specimens. Other, smaller collections of lichens of Vormsi Island are deposited in the Institute of Ecology (IE), Estonian Nature Museum (TAL) and Tallinn Botanical Gardens (TBA); these herbaria were taken into account to a lesser extent. Materials that were studied in preparing the present paper have been collected by different persons during a period of almost 40 years (1960-1999) (Table 1).

The taxa were identified using the routine morphological, anatomical and chemical methods (incl. hand-made cross-sections of fruit-bodies and color tests with p-phenylendiamine in ethanol and potassium hydroxide and sodium hypochlorite in ethanol). In a few cases secondary compounds were analyzed according to the standardized TLC methods.

## LIST OF TAXA

The list of lichenized and allied taxa found in Vormsi Island is presented in the alphabetical order (Table 2). From synonyms only these are included which have been used in earlier papers or manuscripts concerning lichens of Vormsi. Most of the taxa included were verified or identified by the authors of the present paper. In such cases, the names of herbaria (mainly ICEB or TU) where the respective materials are kept are reported. Where the her-

**Table 1**. Collectors of lichens in Vormsi.

| Collectors | Year | Herbarium | No of specimens |
|---|---|---|---|
| Group of students (M. Kubo, H. Ting et al.) guided by H. Trass | 1960 | TU | ca 40 |
| J. Martin & L. Martin | 1969, 1977, 1979, 1980, 1984, 1986, 1994, 1999 | ICEB | ca 420 |
| T. Randlane & A. Roosma | 1979 | TU | a few |
| T. Randlane | 1985 | TU | ca 60 |
| R. Allmäe | 1986-1987 | TU | ca 450 |
| H. Trass | 1986 | TU | a few |
| I. Jüriado & P. Lõhmus | 1994 | TU | a few |

barium material has not been seen and species are included according to literature data only, references of the papers cited are added.

## ANALYSES OF THE LICHEN FLORA

### Floristical analysis

Today, 301 lichen species and 4 infraspecific taxa (subspecies and varieties) have been identified in Vormsi Island. The first list of lichen species of the island (Allmäe 1988) presented 210 taxa. Still, during the last decade, not only approximately one hundred species new to the local flora have been found but several taxa should also be omitted from the list – due to both re-identifications and changes in the lichen systematics. According to our present knowledge the following lichen species (earlier mentioned as found in Vormsi) do not grow in the island: *Acarospora oligospora* (Nyl.) Arnold, *Bryoria chalybeiformis* (L.) Brodo & D. Hawksw., *Caloplaca cerinella* (Nyl.) Flagey, *Cladonia ecmocyna* Leight., *C. ramulosa* (With.) J. R. Laundon [syn. *Cladonia pityrea* (Flörke) Fr.], *C. scabriuscula* (Delise) Nyl., *Fulgensia fulgens* (Sw.) Elenkin, *Leproloma membranaceum* (Dicks.) Vain. [syn. *Crocynia lanuginosa* (Ach.) Hue], *Physcia clementei* (Sm.) Maas Geest., *Polyblastia albida* Arnold, *Porpidia cinereoatra* (Ach.) Hertel & Knoph [syn. *Huilia cinereoatra* (Ach.) Hertel], *Rhizocarpon alpicola* (Anzi) Rabenh., *R. badioatrum* (Flörke ex Spreng.) Th. Fr., *R. grande* (Flörke ex Flot.) Arnold, *R. riparium* Räsänen, *R. umbilicatum* (Ramond) Flagey, *Stereocaulon subcoralloides* (Nyl.) Nyl., *Usnea fulvoreagens* (Räsänen) Räsänen.

To evaluate the richness of the lichen flora of Vormsi Island, it is important to acknowledge that more than 850 lichenized and allied taxa have been reported in Estonia altogether lately (Randlane & Saag 1999). Thus the lichen flora of Vormsi constitutes about 35% of the whole flora of Estonia while its area is less than 5% of the total area of the country. The comparison of the numbers of lichen species found in other Estonian islands (see in Randlane & Jüriado 1999) is not representative because, firstly, the areas of different islands vary greatly and, secondly, most of these lichen lists have been compiled years ago and need contemporary revision and supplementation.

The lichen flora of different parts of Vormsi Island has been investigated quite unequally (Fig. 1). Southern areas of the island are surely more thoroughly studied than the localities in northern or central parts. Still, Rumpo Peninsula is not only the place where several lichenologists have made their collections but it is surely a very suitable locality for many lichens of different ecological requirements. Today, 169 lichenized taxa have been recorded from this small area of ca 100 ha.

Lichen species found in Vormsi Island can be divided into groups according to their general frequency of occurrence in Estonia which is stated according to the scale presented in

**Table 2**. Lichenized and allied taxa found in Vormsi Island. Localities: 1 – Rumpo Peninsula, 2 –Rumpo village, 3 – Hullo, 4 – Suuremõisa, 5 – Hoitberg, 6 – Förby, 7 – Saxby, 8 – Kersleti, 9 – Borrby, 10 – Rälby, 11 – Norrby, 12 – Sviby, 13 – others (Diby, Fällana, Hosby, Nyby, Söderby) (see also Fig. 1). Ecological groups according to substrata: F – growing on rusty iron, Lc – epilithic on calcareous rocks, Lm – growing on mortar, Ls – epilithic on siliceous rocks, M – growing on mosses and plant debris, S – growing on soil, Tc – epiphytic on coniferous trees (*Juniperus communis, Picea abies, Pinus sylvestris*), Td – epiphytic on deciduous trees, W – growing on wood. Frequency in Estonia (according to Randlane & Saag 1999): rr – very rare, 1-2 localities in Estonia; r – rare, 3-5 localities; st r – rather rare, 6-10 localities; st fq – rather frequent, 11-20 localities; fq – frequent, 21-50 localities; fqq – very frequent, 51 or more localities. Geographical elements: arc – arctic-alpine, hypm – hypo-arctic-montane, bor – boreal, nem – nemoral, xer – xerocontinental, smed – submediterranean, smon – submontane, soc – suboceanic, hol – holarctic, mul – multiregional, ? – general distribution undetermined.

| Taxa | Localities | Substrate | Frequency in Estonia | Herbaria or literature | Geogr. el. |
|---|---|---|---|---|---|
| *Absconditella lignicola* Vezda & Pisut | 8, 12 | W | rr | ICEB | bor |
| *Acarospora cervina*A. Massal. | 1 | Lc | st r | ICEB, TU | xer |
| *A. fuscata* (Nyl.) Arnold | 1, 12 | Ls | st fq | ICEB, TU | hol |
| *A. glaucocarpa* (Ach.) Körb. | 5 | Lc | r | TU | mul |
| *Amandinea coniops* (Wahlenb.) Scheid. & H. Mayrhofer [syn. *Buellia coniops* (Wahlenb.) Th. Fr.] | 1 | Ls | st r | TBA | soc |
| *A. punctata* (Hoffm.) Coppins & Scheid. [syn. *Buellia punctata* (Hoffm.) A. Massal.] | 1, 2, 4, 8 | Ls, Tc, Td, W | fq | ICEB, TBA, TU | mul |
| *Anaptychia ciliaris* (L.) Körb. var. *ciliaris* | 3, 4 | Td | fqq | ICEB, TU | nem |
| *A. ciliaris var. melanosticta* (Ach.) Boistel | 8 | Ls | st fq | ICEB | soc |
| *Arthonia lapidicola* (Taylor) Branth. & Rostr. | 1 | Lc | rr | ICEB | mul |
| *A. ligniaria* Hellb. | 3, 12 | Tc, W | NEW | ICEB | ? |
| *A. mediella* Nyl. | 3 | Tc | st r | ICEB | bor |
| *A. muscigena* Th. Fr. | 1 | Td | rr | ICEB | nem |
| *A. punctiformis* Ach. | ? | Td | st r | Trass 1967 | nem |
| *Arthopyrenia rhyponta* (Ach.) A. Massal. | 2 | Td | rr | ICEB | nem |
| *Aspicilia calcarea* (L.) Mudd. | 1, 7 | Lc | st fq | ICEB, TU | mul |
| *A. cinerea* (L.) Körb. | 1, 3, 5, 9 | Ls | fq | TU | mul |
| *A. contorta* ssp. *hoffmanniana* Ekman & Fröberg | 1, 3, 5, 7, 11 | Lc | fq | ICEB, TU | hol |
| *A. moenium* (Vain.) G. Thor & Timdal | 1, 8 | Lc, Lm | st r | ICEB | hol |
| *Bacidia bagliettoana* (A. Massal. & De Not.) Jatta | 1, 12 | Lm, M | st fq | ICEB | mul |
| *B. 'beckhausii'* Körb. | 3 | Td | st r | TU | nem |
| *B. fraxinea* Lönnr. | 4 | Td | fqq | Liiv & Piin 1996 | nem |
| *B. herbarum* (Stiz.) Arnold | 1 | M | r | ICEB | bor |
| *B. polychroa* (Th. Fr.) Körb. | 1 | Td | st fq | ICEB | nem |
| *B. rubella* (Hoffm.) A. Massal. | 3, 4 | Td | fq | TU | nem |
| *B. sabuletorum* (Schreb.) Lettau | 3 | M | fq | TU | mul |
| *Bacidina phacodes* (Körb.) Vezda | 1 | Td | st r | ICEB | ? |
| *Bryoria capillaris* (Ach.) Brodo & D. Hawksw. [syn. *B. setacea* (Ach.) Brodo & D. Hawksw.] | 3, 5, 6, 7, 8 | Tc | fqq | ICEB, TU | bor |

Table 2 (continued)

| Taxa | Localities | Substrate | Frequency in Estonia | Herbaria or literature | Geogr. el. |
|---|---|---|---|---|---|
| *B. fuscescens* (Gyeln.) Brodo & D. Hawksw. | 8, 12 | Tc | fqq | ICEB, TU | bor |
| *B. implexa* (Hoffm.) Brodo & D. Hawksw. | 8, 10 | Tc, W | st fq | ICEB, TU | bor |
| *B. nadvornikiana*(Gyeln.) Brodo & D. Hawksw. | 8 | W | st fq | ICEB | bor |
| *B. subcana* (Stizenb.) Brodo & D. Hawksw. | 3 | Tc | st fq | TU | hol |
| *Buellia disciformis* (Fr.) Mudd | 3, 9, 12 | Td | fqq | TU | mul |
| *Calicium abietinum* Pers. | 3 | W | fqq | TU | mul |
| *C. glaucellum* Ach. | 3 | W | fqq | TU | mul |
| *C. quercinum* Pers. | 2, 11 | W | st fq | ICEB, TU | nem |
| *C. salicinum* Pers. | 3, 11 | Tc, W | fq | Allmäe 1988 | mul |
| *C. viride* Pers. | 1, 3, 4, 12, 13 | Tc, Td, W | fqq | ICEB, TU | mul |
| *Caloplaca cerina* (Hedw.) Th. Fr. | 1 | M | fqq | ICEB | mul |
| *C. cerinelloides* (Erichsen) Poelt | 3 | Tc, Td | st r | ICEB, TU | nem |
| *C. citrina* (Hoffm.) Th. Fr. | 1, 4, 8, 12 | F, Lc, Lm | fq | ICEB, TU | mul |
| *C. decipiens* (Arnold) Blomb. & Forssell | 4 | Lc | fq | TU | mul |
| *C. flavorubescens* (Huds.) J. R. Laundon | 1, 3, 12 | Tc, Td | fqq | ICEB, TU | mul |
| *C. holocarpa* (Ach.) A. E. Wade | 1, 3, 12 | F, Tc, Td | fq | ICEB, TU | mul |
| *C. jungermanniae* (Vahl.) Th. Fr. | 1 | M | rr | TBA | arc |
| *C. lactea* (A. Massal.) Zahlbr. | 1, 7, 12 | Lc, Lm | fq | ICEB, TU | mul |
| *C. saxicola* (Hoffm.) Nordin | 4, 5, 6, 7, 12, 13 | F, Lc, Lm | fq | ICEB, TU | mul |
| *C. sinapispema* (Lam. & DC.) Maheu & Gillet [*C. leucoraea* (Ach.) Branth] | 1, 7, 8 | M | st fq | Allmäe 1988 | arc |
| *C. variabilis* (Pers.) Müll. Arg. | 1 | Lc | st fq | ICEB | hol |
| *C. vitellinula* auct. | 1, 10 | Lc, Lm, Ls | st fq | ICEB, TU | mul |
| *Candelariella aurella* (Hoffm.) Zahlbr. | 1, 7, 12 | Lc | fq | ICEB, TU | mul |
| *C. coralliza* (Nyl.) H. Magn. | 1, 3, 8 | Ls | fq | ICEB, TU | hol |
| *C. vitellina* (Hoffm.) Müll. Arg. | 1, 5, 7, 12 | F, Ls | fq | ICEB, TU | mul |
| *C. xanthostigma* (Ach.) Lettau | 2, 4 | Td | fq | ICEB | nem |
| *Catapyrenium squamulosum* (Ach.) Breuss | 1 | S | r | TU | xer |
| *Catillaria lenticularis* (Ach.) Th. Fr. | 1 | Lc | NEW! | ICEB | smon |
| *C. minuta* (A. Massal.) Lettau | 5 | Lc | rr | ICEB | smon |
| *Cetraria aculeata* (Schreb.) Fr. [syn. *Coelocaulon aculeatum* (Schreb.) Link] | 1, 3, 9, 11 | S | fqq | ICEB, TU | mul |
| *C. ericetorum* Opiz | 1, 6, 9, 11 | S | fqq | ICEB, TU | bor |
| *C. islandica* (L.) Ach. | 1, 3, 11 | S | fqq | ICEB, TU | mul |
| *C. muricata* (Ach.) Eckfeldt [syn. *Coelocaulon muricatum* Ach.) J. R. Laundon] | 1, 9 | S | st fq | ICEB, TU | mul |
| *C. sepincola* (Ehrh.) Ach. [syn. *Tuckermannopsis sepincola* (Ehrh.) Hale] | 1, 2, 3, 11 | Tc, W | fqq | ICEB, TU | mul |
| *Chaenotheca brunneola* (Ach.) Müll. Arg. | 3 | W | fq | TU | mul |
| *C. chrysocephala* (Ach.) Th. Fr. | 3, 4, 5, 13 | Tc | fqq | ICEB, TU | mul |
| *C. ferruginea* (Turner & Borrer) Mig. | 3, 5, 12 | Tc | fqq | ICEB, TU | mul |
| *C. trichialis* (Ach.) Th. Fr. | 1 | Td | fqq | ICEB | mul |

Table 2 (continued)

| Taxa | Localities | Substrate | Frequency in Estonia | Herbaria or literature | Geogr. el. |
|---|---|---|---|---|---|
| *Chaenothecopsis nana* Tibell | 8 | Tc | r | ICEB | bor |
| *Chromatochlamys muscorum* (Fr.) H. Mayrhofer & Poelt | 1 | M | NEW! | ICEB | xer |
| *Chrysothrix candelaris* (L.) J. R. Laundon | 3, 4 | Tc | fq | TU | mul |
| *Cladina arbuscula* (Wallr.) Hale & W. L. Culb. | 1, 7, 9 | S | fqq | ICEB, TU | bor |
| *C. ciliata f. tenuis* (Flörke) Ahti [*C. tenuis* (Flörke) Ahti] | 1, 9 | S | fq | ICEB, TU | soc |
| *C. mitis* (Sandst.) Hustich | 1, 3, 9, 11 | S | fqq | TU | mul |
| *C. rangiferina* (L.) Nyl. | 1, 9 | S | fqq | ICEB, TU | mul |
| *C. stellaris* (Opiz) Brodo | 3, 10 | S | fqq | TU | bor |
| *Cladonia bacillaris* (Leight.) Arnold | 1 | S | fq | Liiv & Piin 1996 | mul |
| *C. botrytes* (K. G. Hagen) Willd. | 3 | W | fqq | TU | bor |
| *C. cariosa* (Ach.) Spreng. | 1, 3, 13 | S | fqq | TU | mul |
| *C. carneola* (Fr.) Vain. | 1 | S | st fq | Liiv & Piin 1996 | bor |
| *C. cenotea* (Ach.) Schaer. | 3, 5, 7, 8, 13 | S, Tc, W | fqq | ICEB, TU | bor |
| *C. chlorophaea* (Sommerf.) Spreng. | 1, 3, 5, 6, 9, 13 | S, W | fqq | ICEB, TU | mul |
| *C. coccifera* (L.) Willd. | 11 | S | st fq | TU | mul |
| *C. coniocraea* (Flörke) Spreng. | 3, 7, 8, 12, 13 | Ls, S, Tc, Td, W | fqq | ICEB, TU | mul |
| *C. cornuta* (L.) Hoffm. | 1, 11 | S | fqq | TU | mul |
| *C. crispata* (Ach.) Flot. | 7 | S | fqq | TU | mul |
| *C. deformis* (L.) Hoffm. | ? | S | fqq | Trass 1967 | mul |
| *C. digitata* (L.) Hoffm. | 1, 3, 6, | Tc, W | fqq | TU | mul |
| *C. fimbriata* (L.) Fr. | 1, 3, 5, 9, 11 | Ls, S, Tc | fqq | ICEB, TU | mul |
| *C. floerkeana* (Fr.) Sommerf. | 1 | S | fqq | TU | mul |
| *C. foliacea* (Huds.) Willd. | 1 | S | st fq | Trass 1967, Liiv & Piin 1996 | xer |
| *C. furcata* (Huds.) Schrad. | 1, 3, 5, 6, 8, 9, 13 | S | fqq | TU | mul |
| *C. glauca* Flörke | 3 | W | fqq | TU | bor |
| *C. gracilis* (L.) Willd ssp. *gracilis* | 1, 11 | S | fqq | TU | mul |
| *C. gracilis* ssp. t*urbinata*(Ach.) Ahti | 3, 8, 9 | S, W | fqq | TU | mul |
| *C. macilenta* Hoffm. | 1, 2, 7 | S, W | fqq | ICEB, TU | mul |
| *C. macroceras* (Delise) Hav. | 1, 3 | S | r | TU | arc |
| *C. ochrochlora* Flörke | 3, 8 | W | fq | TU | mul |
| *C. phyllophora* Hoffm. | 9, 10 | S | fq | TU | mul |
| *C. pocillum* (Ach.) Grognot | 1, 3 | S | st fq | TU | xer |
| *C. pyxidata* (L.) Hoffm. | 1, 3, 8, 10 | Ls, S, Tc | fqq | TU | mul |
| *C. rangiformis* Hoffm. | 1, 6, 11 | S | fq | TU | xer |
| *C. rei* Schaer. | 1 | S | st r | Liiv & Piin 1996 | mul |
| *C. squamosa* Hoffm. | 7 | W | fqq | TU | mul |
| *C. subrangiformis* Sandst. | 1, 7, 9 | S | fq | TU | xer |
| *C. subulata* (L.) F. H. Wigg. | 1, 6, 9 | S | fqq | ICEB, TU | mul |
| *C. symphycarpa* (Flörke) Fr. | 1, 3, 5, 6, 7 | S | fq | TU | xer |
| *C. turgida* Hoffm. | 3, 7, 9, 13 | S | fq | TU | mul |
| *C. uncialis*(L.) F. H. Wigg. | 1, 10, 11 | S | fqq | ICEB, TU | mul |

Table 2 (continued)

| Taxa | Localities | Substrate | Frequency in Estonia | Herbaria or literature | Geogr. el. |
|---|---|---|---|---|---|
| *Clauzadea monticola* (Schaer.) Haffelner & Bellem. [syn. *Lecidea monticola* Schaer.] | 1, 3, 8 | Lc | fq | ICEB, TU | mul |
| *Cliostomum griffithii* (Sm.) Coppins | 1, 3, 8 | Tc, Td | st fq | ICEB, TU | bor |
| *C. leprosum* (Räsänen) Holien & Tonsberg | 5 | Tc | st r | ICEB | bor |
| *Collema cristatum* (L.) F. H. Wigg. | 1 | Lc | st fq | ICEB, TU | mul |
| *C. fuscovirens* (With.) J. R. Laundon [syn. *C. tuniforme* (Ach.) Ach.] | 4 | Lc | fq | TU | mul |
| *C. tenax* (Sw.) Ach. | 3 | Lc | st fq | TU | mul |
| *Dimerella pineti* (Ach.) Vezda | 1, 8, 12 | Tc, Td | fq | ICEB | bor |
| *Diploschistes muscorum* (Scop.) R. Sant. | 1 | M | st fq | ICEB, TU | xer |
| *D. scruposus* (Schreb.) Norm. | ? | Ls | st r | Trass 1967 | mul |
| *Diplotomma alboatrum* (Hoffm.) Flot. [syn. *Buellia alboatra* (Hoffm.) Th. Fr.] | 2, 4 | Td | st fq | ICEB, TU | mul |
| *D. ambiguum* (Ach.) Flagey | 1 | Ls | rr | ICEB | hol |
| *D. epipolium* (Ach.) Arnold | 1 | Lc | st fq | ICEB | hol |
| *Evernia prunastri* (L.) Ach. | 2, 4, 7, 8, 9 | Tc, Td, W | fqq | ICEB, TU | nem |
| *Flavocetraria cucullata* (Bellardi) Kärnefelt & A. Thell [syn. *Cetraria cucullata* (Bellardi) Ach.] | 1 | S | rr | ICEB, TBA, TU | arc |
| *F. nivalis* (L.) Kärnefelt & A. Thell [syn. *Cetraria nivalis* (L.) Ach.] | 1, 9, 11 | S | fqq | ICEB, TU | arc |
| *Fulgensia bracteata* (Hoffm.) Räsänen | 1, 3 | M, S | fq | ICEB, TU | arc |
| *Graphis scripta* (L.) Ach. | 1, 10 | Td | fqq | ICEB, TU | mul |
| *Hypocenomyce friesii* (Ach.) P. James & Gotth. Schneid. | 5 | Tc | st r | ICEB | bor |
| *H. scalaris* (Ach.) M. Choisy | 2, 3, 7, 11 | Tc, W | fqq | ICEB, Tu | mul |
| *Hypogymnia physodes* (L.) Ach. | 1,2,3,4,5, 6, 8, 9, 12 | Ls, Tc, Td, W | fqq | ICEB, TU | mul |
| *H. tubulosa* (Scher.) Hav. | 1, 8, 12 | Tc, Td, W | fqq | ICEB, TU | bor |
| *Imshaugia aleurites* (Ach.) S. L. F. Meyer [syn. *Parmeliopsis aleurites* (Ach.) Nyl.] | ? | Tc | fqq | Trass 1967 | bor |
| *Lecanactis abietina* (Ach.) Körb. | 3, 5, 8 | Tc | fq | ICEB | mul |
| *Lecania cyrtella* (Ach.) Th. Fr. | 3 | Tc | fq | ICEB | bor |
| *L. fuscella* (Schaer.) A. Massal. | 6, 12 | Td | st r | TU | bor |
| *L. naegelii* (Hepp) Diederich & Boom | 3 | Tc | fq | ICEB | bor |
| *Lecanora albella* (Pers.) Ach. [syn. *L. pallida* (Schreb.) Rabenh.] | 1, 8 | Tc | fq | ICEB, TU | mul |
| *L. albescens* (Hoffm.) Branth & Rostr. | 5, 7 | Lc | fqq | TU | hol |
| *L. allophana* Nyl. | 1 | Td | fqq | ICEB | nem |
| *L. argentata* (Ach.) Malme [syn. *L. subfuscata* H. Magn., *L. subrugosa* Nyl.] | 3, 4 | Tc, Td | fqq | TU | mul |
| *L. campestris* (Schaer.) Hue | 5, 7 | Ls | st r | TU | hol |
| *L. carpinea* (L.) Vain. | 1, 2, 4, 11, 12, 13 | Tc, Td | fqq | ICEB, TU | mul |
| *L. cenisia* Ach. | 1, 3 | Ls | fq | ICEB, TU | hypm |
| *L. chlarotera* Nyl. | 2, 3, 4, 8, 9, 12 | Td | fqq | ICEB, TU | mul |

Table 2 (continued)

| Taxa | Localities | Substrate | Frequency in Estonia | Herbaria or literature | Geogr. el. |
|---|---|---|---|---|---|
| *L. circumborealis* Brodo & Vitik. [syn. *L. coilocarpa* auct.] | 13 | W | r | TU | bor |
| *L. crenulata* Hook. | 1 | Lc | st fq | ICEB, TU | mul |
| *L. dispersa* (Pers.) Sommerf. | 1, 3, 12 | F, Lc, Lm, Ls | fqq | ICEB, TU | mul |
| *L. expallens* Ach. | 1 | Tc | fq | Liiv & Piin 1996 | mul |
| *L. hagenii* (Ach.) Ach. | 1, 2, 3, 4, 12 | Tc, Td, W | fqq | ICEB, TU | hol |
| *L. intricata* (Ach.) Ach. | 9 | Ls | st fq | TU | arc |
| *L. leptyrodes* (Nyl.) Degel. | 1, 3, 4, 8, 11, 12, 13 | Tc, Td | fqq | ICEB, TU | nem |
| *L. macrocyclos* (H. Magn.) Degel. | 1 | Ls | st fq | TU | hol |
| *L. muralis* (Schreb.) Rabenh. | 1, 3, 5, 8, 9 | Ls | fqq | ICEB, TU | mul |
| *L. piniperda* Körb. | 1, 2 | Td, W | r | ICEB | bor |
| *L. polytropa* (Hoffm.) Rabenh. | 1, 9, 12 | Ls | fq | ICEB, TU | mul |
| *L. pulicaris* (Pers.) Ach. [syn. *L. chlarona* (Ach.) Nyl.] | 1, 2, 3, 7, 10, 11, 12 | Tc, Td, W | fqq | ICEB, TU | bor |
| *L. rugosella* Zahlbr. | 2, 3, 4 | Td | fqq | ICEB, TU | nem |
| *L. rupicola* (L.) Zahlbr. | 1, 5, 7, 9, 12, 13 | Ls | fq | ICEB, TU | mul |
| *L. saligna* (Schrad.) Zahlbr. | 2 | W | fq | ICEB | hol |
| *L. sulphurea* (Hoffm.) Ach. | 1, 7 | Ls | fq | TU | smon |
| *L. symmicta* (Ach.) Ach. | 1, 2, 8, 12 | Td, W | fqq | ICEB, TU | mul |
| *L. varia* (Hoffm.) Ach. | 1, 2, 3, 8, 10 | Td, W | fqq | ICEB, TU | mul |
| *Lecidea fuscoatra* (L.) Ach. | 1, 3 | Ls | st fq | ICEB, TU | mul |
| *L. 'hypopta'* Ach. | 3 | Tc | rr | ICEB | bor |
| *L. lapicida* var. *pantherina* [syn. *L. lactea* Schaer.] | 1, 5 | Ls | st fq | ICEB, TU | mul |
| *L. 'nylanderi'*(Anzi) Th. Fr. | 3 | Tc | r | ICEB | bor |
| *L. 'turgidula'* Fr. | 3, 12 | Tc | fq | ICEB | mul |
| *Lecidella elaeochroma* (Ach.) M. Choisy | 1, 3, 4, 10, 12 | Tc, Td | fqq | ICEB, TBA, TU | mul |
| *L. euphorea* (Flörke) Hertel | 1, 2, 3, 4, 6 | Tc, Td | fqq | ICEB, TBA, TU | mul |
| *L. stigmatea* (Ach.) Hertel & Leuckert | 1, 4 | Lc, Ls | fq | ICEB, TBA, TU | mul |
| *Lepraria elobata* Tonsberg | 5 | Ls | r | TU | bor |
| *L. incana* (L.) Ach. | 3, 4, 5, 8, 9 | M, Tc | fq | ICEB, TU | mul |
| *L. lobificans*Nyl. | 12 | Tc | fq | ICEB | mul |
| *L. neglecta* (Nyl.) Erichsen | 13 | Ls | r | TU | arc |
| *Leptogium diffractum* Körb. | 1 | Lc | NEW! | ICEB | ? |
| *L. imbricatum* P. M. Jörg. | 1 | M | NEW! | ICEB | arc |
| *L. lichenoides* (L.) Zahlbr. | 1, 3, 5, 8 | Lc, M | st fq | ICEB, TU | hol |
| *L. schraderi* (Bernh.) Nyl. | 1 | M | rr | ICEB | xer |
| *Loxospora elatina* (Ach.) A. Massal. | 5 | Tc | r | ICEB | smon |
| *Melanelia disjuncta* (Erichsen) Essl. | 8, 12 | Ls | st fq | ICEB | hypm |
| *M. exasperata* (De Not.) Essl. [syn. *Parmelia exasperata* De Not.] | 2, 9 | Td | fq | ICEB, TU | nem |
| *M. exasperatula* (Nyl.) Essl. [syn. *Parmelia exasperatula* Nyl.] | 2, 6 | Td, W | fqq | ICEB, TU | nem |
| *M. fuliginosa* (Duby) Essl. [syn. *Parmelia fuliginosa* (Duby) Nyl., *P. glabratula* Lamy] | 3, 4, 6, 8, 9, 11, 12 | Ls, Td, W | fqq | ICEB, TU | hol |

Table 2 (continued)

| Taxa | Localities | Substrate | Frequency in Estonia | Herbaria or literature | Geogr. el. |
|---|---|---|---|---|---|
| *M. olivacea* (L.) Essl. [syn. *Parmelia olivacea* (L.) Ach.] | 1, 3, 6, 10, 11, 12 | Tc, Td | fqq | ICEB, TU | bor |
| *M. sorediata* (Ach.) Goward & Ahti [syn. *M. sorediosa* (Almb.) Essl.] | ? | Ls | fq | Liiv & Piin 1996 | hypm |
| *M. stygia* (L.) Essl. [syn. *Parmelia stygia* (L.) Ach.] | ? | Ls | st r | Trass 1967 | arc |
| *M. subaurifera* (Nyl.) Essl. | 1, 4, 8 | Tc, Td | fqq | ICEB, TU | bor |
| *Micarea denigrata* (Fr.) Hedl. | 2, 3, 8, 12 | Tc, W | st fq | ICEB | bor |
| *M. misella* (Nyl.) Hedl. | 2, 3, 12 | W | r | ICEB | bor |
| *M. peliocarpa* (Anzi) Coppins & R. Sant. | 8 | W | st r | ICEB | bor |
| *M. prasina* Fr. | 1, 3, 8, 12 | Tc, Td, W | fqq | ICEB | mul |
| *Mycobilimbia carneoalbida* (Müll. Arg.) comb. inedit. | 12 | Lm | st r | ICEB | bor |
| *M. hypnorum* (Lib.) Kalb & Hafellner | 1 | M | r | ICEB | ? |
| *M. microcarpa* (Th. Fr.) Lettau [syn. *Bacidia microcarpa*(Th. Fr.) Lettau] | 5 | M | r | ICEB, TU | bor |
| *M. tetramera* (De Not.) comb. inedit. [syn. *Bacidia obscurata* (Sommerf.) Zahlbr.] | 3 | M | st r | TU | bor |
| *Mycocalicium subtile* (Pers.) Szatala [syn. *Calicium subtile* Pers.] | 2, 3, 8, 12 | W | fqq | ICEB, TU | mul |
| *Mycomicrothelia confusa* D. Hawksw. | 1 | Td | rr | ICEB | ? |
| *Neofuscelia loxodes* (Nyl.) Essl. [syn. *Parmelia loxodes* Nyl.] | 1, 8, 9, 13 | Ls | fqq | ICEB, TU | bor |
| *N. pulla* (Ach.) Essl. var. *pulla* [syn. *Parmelia pulla* Ach.] | 1, 7, 8, 9, 12 | Ls | fqq | ICEB, TU | xer |
| *N. pulla* var. *delisei* (Duby) R. Sant. [syn. *Neofuscelia delisei* (Duby) Essl.] | 1 | Ls | st r | Liiv & Piin 1996 | xer |
| *Nephroma parile* (Ach.) Ach. | 12 | M | st fq | TU | bor |
| *Ochrolechia androgyna* (Hoffm.) Arnold | 3, 12 | Ls, M | fqq | TU | bor |
| *O. arborea* (Kreyer) Almb. | 3 | Tc | st fq | TU | bor |
| *Opegrapha rufescens* Pers. | 4, 6 | Td | fq | TU | nem |
| *O. varia* Pers. | 3, 4 | Td | fq | ICEB, TU | nem |
| *O. vulgata* var. *subsiderella* Nyl. | 3 | Tc | st fq | ICEB | mul |
| *Pachyphiale fagicola* (Hepp) Zwackh | 1, 2, 3 | Td | r | ICEB, TU | mul |
| *Parmelia saxatilis* (L.) Ach. | 6, 7, 8, 9 | Ls | fqq | ICEB, TU | mul |
| *P. sulcata* Taylor | 1, 2, 3, 4, 8, 9,11,12 | Ls, Td | fqq | ICEB, TU | mul |
| *Parmeliopsis ambigua* (Wulfen) Nyl. | 1, 3, 12, 13 | Tc, Td, W | fqq | ICEB, TU | bor |
| *Peltigera aphthosa* (L.) Willd. | 1, 3 | S | fqq | TU | hypm |
| *P. canina* (L.) Willd. | 1, 3, 4, 5, 7, 9 | S | fqq | ICEB, TU | mul |
| *P. didactyla* (With.) J. R. Laundon [syn. *P. spuria* (Ach.) DC.] | 1, 9 | S | fqq | TU | mul |
| *P. hymenina* (Ach.) Delise | 1 | S | st fq | ICEB | bor |
| *P. leucophlebia* (Nyl.) Gyeln. | 1 | S | fq | ICEB | hypm |
| *P. malacea* (Ach.) Funck | 1, 3, 11 | S | fq | ICEB, TU | bor |
| *P. polydactyla* (Neck.) Hoffm. | 3, 4, 6 | S | fqq | TU | mul |
| *P. rufescens* (Weiss) Humb. | 1, 3, 6, 9, 13 | S | fqq | ICEB, TU | mul |

Table 2 (continued)

| Taxa | Localities | Substrate | Frequency in Estonia | Herbaria or literature | Geogr. el. |
|---|---|---|---|---|---|
| *Pertusaria albescens* (Huds.) M. Choisy | 7, 8, 11 | Td | fqq | TU | nem |
| *P. amara* (Ach.) Nyl. | 3, 9, 10, 11 | Tc, Td | fqq | TU | nem |
| *P. coccodes* (Ach.) Nyl. | 3, 12 | Td | fqq | TU | nem |
| *P. leioplaca* DC. | 1, 3 | Td | fq | ICEB, TU | nem |
| *Phaeophyscia nigricans* (Flörke) Moberg | 1, 2, 12 | F, Lm, Td | fq | ICEB | hol |
| *P. orbicularis* (Neck.) Moberg | 1, 2, 3, 4, 12 | F, Lc, Lm, Td | fqq | ICEB, TU | mul |
| *Phlyctis argena* (Spreng.) Flot. | 1, 2, 3, 4, 7, 10, 12 | Tc, Td | fqq | ICEb, TU | nem |
| *Physcia adscendens* (Fr.) H. Olivier | 1, 2, 3, 4, 12 | Tc, Td | fqq | ICEB, TU | nem |
| *P. aipolia* (Humb.) Fürnr. | 3 | Td | fqq | TU | mul |
| *P. caesia* (Hoffm.) Fürnr. | 1, 8, 10 | Ls | fqq | ICEB, TU | mul |
| *P. dubia* (Hoffm.) Lettau | 1, 2, 8, 9, 13 | Ls, Td | fqq | ICEB, TU | mul |
| *P. stellaris* (L.) Nyl. | 2, 3, 12 | Td | fqq | ICEB, TU | mul |
| *P. tenella* (Scop.) DC. var. *tenella* | 2, 3, 4, 6, 12, 13 | Ls, Tc, Td | fqq | ICEB, TU | nem |
| *P. tenella* var. *marina* (E. Nyl.) Lynge | 2, 9 | Ls | fq | ICEB, TU | soc |
| *Physconia detersa* (Nyl.) Poelt | 4 | Td | r | Liiv & Piin 1996 | bor |
| *P. distorta* (With.) J. R. Laundon [syn. *P. pulverulacea* Moberg] | 2, 3, 4, 8, 11, 13 | Td | fqq | ICEB, TU | nem |
| *P. enteroxantha* (Nyl.) Poelt | 2, 3, 4, 12, 13 | Td | fqq | ICEB, TU | nem |
| *P. perisidiosa* (Erichsen) Moberg | 4 | Td | fq | Liiv & Piin 1996 | nem |
| *Placynthiella icmalea* (Ach.) Coppins & P. James | 1, 3, 5, 12 | M, S, Tc, W | fqq | ICEB, TU | mul |
| *P. uliginosa* (Schrad.) Coppins & P. James [syn. *Lecidea uliginosa* (Schrad.) Ach.] | 1, 2, 3, 8 | M, W | fq | ICEB, TU | mul |
| *Placynthium nigrum* (Huds.) Gray | 1, 3, 5 | Lc | fq | ICEB, TU | mul |
| *Platismatia glauca* (L.) W. L. Culb. & C. F. Culb. | 1, 2, 3, 4, 5, 8, 9, 12 | Tc, Td | fqq | ICEB, TU | mul |
| *Polyblastia cupularis* A. Massal. | 1 | Lc | rr | ICEB | smon |
| *P. gelatinosa* (Ach.) Th. Fr. | 5, 8 | M | rr | ICEB | xer |
| *Porpidia crustulata* (Ach.) Hertel & Knoph [syn. *Huilia crustulata* (Ach.) Hertel] | 3 | Ls | fq | Allmäe 1988 | mul |
| *P. macrocarpa* (DC.) Hertel & A. J. Schwab | 3 | Ls | st r | TU | mul |
| *P. tuberculosa* (Sm.) Hertel & Knoph [syn. *Huilia tuberculosa* (Sm.) P. James] | 3, 5 | Ls | r | TU | mul |
| *Protoblastenia calva* (Dicks.) Zahlbr. | 1 | Lc | st r | ICEB | hol |
| *P. incrustans* (DC.) J. Steiner | 5 | Lc | rr | ICEB | smed |
| *P. rupestris* (Scop.) J. Steiner | 1, 3, 5, 12 | Lc | fq | ICEB, TU | mul |
| *Pseudevernia furfuracea* (L.) Zopf | 1, 2, 3, 5, 8, 9, 11 | Ls, Tc, Td, W | fqq | ICEB, TU | mul |
| *Psora decipiens* (Hedw.) Hoffm. | ? | S | st fq | Trass 1967 | xer |
| *Ptychographa flexella* (Ach.) Coppins | 12 | W | NEW! | ICEB | ? |
| *Ramalina dilacerata* (Hoffm.) Hoffm. | 2 | Td | st r | ICEB | bor |
| *R. farinacea* (L.) Ach. | 2, 3, 4, 7, 12 | Tc, Td | fqq | ICEB, TU | nem |
| *R. fastigiata* (Pers.) Ach. | 1, 3, 4, 6, 12 | Tc, Td | fqq | ICEB, TU | nem |
| *R. fraxinea* (L.) Ach. | 2, 3, 4, 12 | Td | fqq | ICEB, TU | nem |

Table 2 (continued)

| Taxa | Localities | Substrate | Frequency in Estonia | Herbaria or literature | Geogr. el. |
|---|---|---|---|---|---|
| *R. pollinaria* (Westr.) Ach. | 4 | Td | fqq | Liiv & Piin 1996 | mul |
| *R. thrausta* (Ach.) Nyl. | 3, 4, 5 | Tc | fq | TU | bor |
| *Rhizocarpon distinctum* Th. Fr. | 1, 9 | Ls | st fq | ICEB, TU | bor |
| *R. geographicum* (L.) DC. | 1, 3, 6, 7, 13 | Ls | st fq | ICEB, TU | mul |
| *R. macrosporum* Räsänen | 5 | Ls | r | TU | smon |
| *R. obscuratum* (Ach.) A. Massal. | 3, 12 | Ls | st r | ICEB, TU | bor |
| *R. petraeum* (Wulfen) A. Massal. | 1 | Ls | r | ICEB | hol |
| *R. polycarpum* (Hepp) Th. Fr. | 10 | Ls | r | TU | bor |
| *Rimularia insularis* (Nyl.) Hertel & Rambold | 1 | on *Lecanora rupicola* | st fq | TU | mul |
| *Rinodina bischoffii* (Hepp) A. Massal. | 1, 10 | Lc | st fq | ICEB, TU | mul |
| *R. exigua* Gray | 1, 3 | Tc, Td | fq | ICEB, TU | mul |
| *R. immersa* (Körb.) Arnold | 1 | Lc | st r | ICEB, TU | smed |
| *R. sophodes* (Ach.) A. Massal. | 1, 2 | Td | fq | ICEB, TU | mul |
| *Sagiolechia protuberans* (Ach.) A. Massal. | 1, 5, 7 | Lc | st r | TU | arc |
| *Sarcogyne privigna* (Ach.) A. Massal. | 5 | Lc | rr | TU | mul |
| *S. regularis* Körb. | 1, 5, 13 | Lc | st fq | TU | mul |
| *Schismatomma pericleum* (Ach.) Branth & Rostr. | 5 | Tc | st r | ICEB, TBA, TU | bor |
| *Scoliciosporum chlorococcum* (Stenh.) Vezda | 1, 2 | Td, W | fq | ICEB | bor |
| *S. umbrinum* (Ach.) Arnold | 1 | Ls | st r | ICEB | bor |
| *Stereocaulon paschale* (L.) Hoffm. | 1, 3, 9, 11 | Ls | fqq | TU | bor |
| *S. tomentosum* Fr. | 6, 12 | S | fqq | TU | bor |
| *Strangospora moriformis* (Ach.) Stein | 2, 8 | W | rr | ICEB | bor |
| *Tephromela atra* (Huds.) Kalb [syn. *Lecanora atra* (Huds.) Ach.] | 1, 5, 7 | Ls | fqq | TU | mul |
| *Thelidium decipiens* (Nyl.) Kremp. | 1 | Lc | rr | ICEB | mul |
| *Toninia sedifolia* (Scop.) Timdal [syn. *T. caeruleonigricans* auct.] | 1 | S | st fq | TU | xer |
| *Trapeliopsis flexuosa* (Fr.) Coppins & P. James | 1, 3, 8, 12 | S, Tc, W | fq | ICEB, TBA, TU | mul |
| *T. granulosa* (Hoffm.) Lumbsch | 2, 3 | S, W | fqq | ICEB, TBA | mul |
| *Tuckermannopsis chlorophylla* (Willd.) Hale | 1, 3, 7, 11, 12 | Tc, Td | fqq | ICEB, TU | bor |
| *Umbilicaria deusta* (L.) Baumg. | 7, 12, 13 | Ls | fqq | ICEB, TU | smon |
| *U. polyphylla* (L.) Baumg. | 1, 7, 8, 12, 13 | Ls | fqq | ICEB, TU | smon |
| *U. torrefacta* (Light.) Schrad. | 13 | Ls | st fq | TU | arc |
| *Usnea filipendula* Stirt. | 2, 3, 5 | Tc | fqq | ICEB, TU | mul |
| *U. hirta* (L.) F. H. Wigg. | 1, 3, 5, 7, 8, 11, 12 | Tc, Td, W | fqq | ICEB, TU | bor |
| *U. scabrata* (Nyl.) [syn.*U. prostrata* Räsänen] | 3, 5 | Tc | fq | TU | bor |
| *U. subfloridana* Stirt. | 3, 6, 8 | Tc | fqq | ICEB, TU | bor |
| *Verrucaria acrotella* Ach. | 8 | Lc | NEW! | ICEB | ? |
| *V. caerulea*DC. | 1 | Lc | rr | ICEB | smon |
| *V. calciseda* DC. | 1, 5, 8 | Lc | st r | ICEB | smed |
| *V. fuscella* (Turner) Winch | 1 | Lc | r | ICEB | hol |

Table 2 (continued)

| Taxa | Localities | Substrate | Frequency in Estonia | Herbaria or literature | Geogr. el. |
|---|---|---|---|---|---|
| *V. glaucina* auct. | 1 | Lc | r | ICEB | mul |
| *V. maura* Wahlenb. | 10 | Lc | st r | TU | soc |
| *V. muralis* Ach. | 1, 3, 12, 13 | Lc | fq | ICEB, TU | hol |
| *V. nigrescens* Pers. | 1, 3, 5 | Lc | fq | ICEB, TU | hol |
| *V. umbrinula* Nyl. | 12 | Ls | NEW! | ICEB | mul |
| *V. viridula* (Schrad.) Ach. | 12 | Lm | rr | ICEB | mul |
| *Vulpicida juniperina* J.-E. Mattsson & M. J. Lai [syn. *Cetraria juniperina* (L.) Ach.] | 1, 3, 8, 13 | Tc, M | fq | ICEB, TU | hypm |
| *V. pinastri* (Scop.) J.-E. Mattsson & M. J. Lai [syn. *Cetraria pinastri* (Scop.) Gray] | 1, 2, 11, 12 | Tc, Td, W | fqq | ICEB, TU | bor |
| *V. tubulosa* (Schaer.) J.-E. Mattsson & M. J. Lai [syn. *Cetraria alvarensis* (Wahlenb.) Vain.] | 1 | Tc | st fq | TU | ? |
| *Xanthoparmelia conspersa* (Ach.) Hale [syn. *Parmelia conspersa* (Ach.) Ach., *P. plittii* auct.] | 1, 5, 7, 8, 9, 12, 13 | Ls | fqq | ICEB, TU | mul |
| *X. somloënsis* (Gyeln.) Hale [syn. *Parmelia taractica* auct.] | 3, 8, 12, 13 | Ls | fq | ICEB, TU | xer |
| *Xanthoria candelaria* (L.) Th. Fr. | 1, 2 | Ls, Td, W | fqq | ICEB | mul |
| *X. fulva* (Hoffm.) Poelt & Petutschnig | 2 | Td | st r | ICEB | ? |
| *X. parietina* (L.) Th. Fr. | 1, 2, 3, 9, 12 | F, Ls, Tc, Td, W | fqq | ICEB, TU | mul |
| *X. polycarpa* (Hoffm.) Rieber | 1, 2, 3, 4, 12 | Tc, Td, W | fqq | ICEB, TU | hol |

Randlane & Saag (1999) (see the legend to Table 2). Eight species are reported as new to the lichen flora of Estonia; 19 species belong to the group of very rare, 22 – to rare, and 30 – to rather rare lichens of Estonia. Thus there are 79 rare lichens in Vormsi altogether, which have been found in 10 or less localities in the whole Estonia. This constitutes about one quarter of the entire local lichen flora.

The following lichenized taxa are **new for Estonia**: *Arthonia ligniaria*, *Catillaria lenticularis*, *Chromatochlamys muscorum*, *Leptogium diffractum*, *L. imbricatum*, *Ptychographa flexella*, *Verrucaria acrotella* and *V. umbrinula*. ***Arthonia ligniaria*** was found on wood and branches of *Pinus sylvestris* in two localities (Hullo and Sviby) in the southern part of Vormsi Island. The taxon is rather rare in northern Europe – reported only from a few provinces of Sweden (Santesson 1993) but not known from Finland, Latvia, Lithuania or Norway. ***Catillaria lenticularis*** has been earlier reported from Estonia (Trass 1967: Island Abruka, on wood, V. Räsänen) but this record was a misidentification. Now the species was found on calcareous stones on Rumpo Peninsula. The lichen is common throughout Europe and is probably more widely spread in Estonia as well, but has been overlooked here. ***Chromatochlamys muscorum*** was collected from mosses also on Rumpo Peninsula. This is a widespread species which is easily recognized due to its pale perithecia and large brown ascospores. Still, the thallus is effuse and may be overlooked; furthermore, the suitable substrate (mosses) have not been sufficiently studied in Estonia. ***Leptogium diffractum*** and ***L. imbricatum*** both belong to the group of species with small thalli. The former is a rather rare taxon which is known in Sweden from Gotland only and is included into the Red Data Book of Sweden as a vulnerable lichen (Thor & Arvidsson 1999); it is not reported from Finland, Latvia, Lithuania or Norway. *L. imbricatum* was described recently and is evidently widely distributed in Europe on calcareous soil among mosses (Jørgensen 1994). Actually it has been reported from Estonia by Jørgensen (1994: Island Saaremaa, peninsula of Sõrve, Lõo, G. Thor 8003) already but this record was over-

looked while compiling "Second Checklist of lichenized, lichenicolous and allied fungi of Estonia" (Randlane & Saag 1999). Thus we report *L. imbricatum* again as "new" to Estonia. Both taxa were found in Vormsi on Rumpo Peninsula. ***Ptychographa flexella*** is a lirellate lichen that grows on wood. It is known from many European countries (Finland, Germany, Great Britain, Slovakia, Sweden) but it is not common anywhere and has been included in the list of endangered lichens e.g. in Germany (Wirth et al. 1996) and Slovakia (Pisut et al. 1996). In Vormsi the species was collected from Sviby. ***Verrucaria acrotella*** and ***V. umbrinula*** are both rather widely distributed in Northern Europe (Finland, Lithuania, Norway, Sweden). In Estonia the whole genus is poorly investigated and thus the finding of these taxa in Vormsi was not unexpected.

Nine ecological groups of lichens can be distinguished according to the substrate on which they grow (see the legend to Table 2). Among these, epiphytic species on deciduous trees and epiphytic species on coniferous trees are the most numerous (83 and 78 taxa accordingly). Still, among rare lichens (frequency groups very rare, rare and rather rare), the epilithic taxa on calcareous and siliceous rocks form the biggest groups (22 and 15 accordingly of 79 rare species). Almost half of all lichens growing on calcareous rocks belong to the category of rare taxa. The similar proportion is seen among lichens growing on mosses where 9 taxa of 22 are rare. Epiphytic lichens, although very numerous in the flora in general, contain less rare species. Rather a big group of taxa growing on soil (54 species) includes only a few rare lichens (5 species) (Fig. 2).

## Geographical analysis

The system of geographical elements proposed by H. Trass (1967, 1970) was used as a basis for the evaluation of lichen taxa according to their distribution. Recently published regional checklists and other reviews (Fröberg 1989; Kopaczevskaja et al. 1977; Pisut et al. 1996; Purvis et al. 1992; Santesson 1993; Vitikainen et al. 1997; Wirth 1995) were studied to obtain

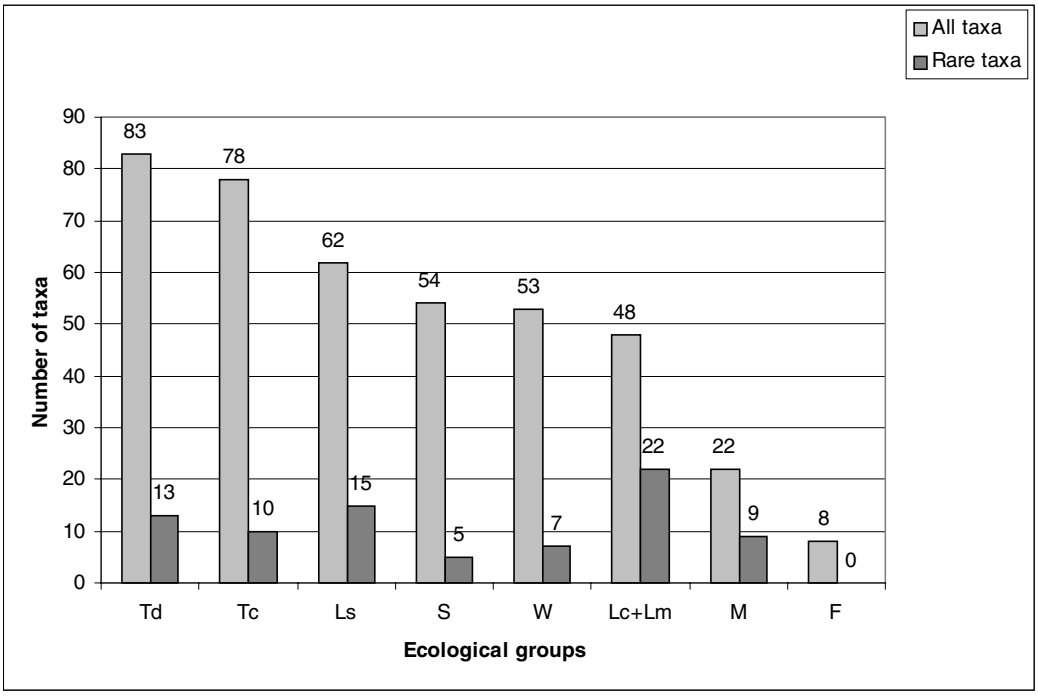



**Fig. 2.** Share of rare lichens in different ecological groups (according to the substrate). Abbreviations as in the legend to Table 2.

additional information concerning distributional data. More detailed subdivision of subelements and lower categories was not applied in the current study.

The following ten geographical categories (geographical elements or conelements *sensu* Trass) have been applied: arctic-alpine, hypo-arctic-montane, boreal, nemoral, xerocontinental, submediterranean, submontane, suboceanic, holarctic, multiregional. Some species of limited or poorly known distribution were included into the category "general distribution undetermined". Assignment of species to the elements listed above is presented in Table 2.

Multiregional (128 species), boreal (63 species), nemoral (33 species), and holarctic (23 species) elements are the biggest groups of lichen taxa recorded in Vormsi Island (Fig. 3). This kind of distribution of geographical elements is quite expected as Estonia is situated in the vegetation zone of mixed boreo-nemoral forests.

Relative abundance of southerly distributed lichens – xerocontinental and submediterranean taxa (16 and 2 species respectively) – is connected with the presence of various xeric habitats in Vormsi Island, e.g. alvars, moraine deposits, outcrops of limestone, and beach ridges composed of limestone, siliceous pebble and sand. Arctic-alpine and hypo-arctic-montane lichens of mainly northerly distribution are also numerous (12 and 5 species respectively) and they often occur in the same habitats. Trass (1963;1967) categorized some of them (e.g. *Flavocetraria cucullata* for which Vormsi is the only locality in Estonia) as relictoids because their invasion and occurrence here cannot be connected with the glaciers retreat or arctic period of climate in holocene ca 10-12 000 years ago. As mentioned above, the earliest fragments of Vormsi Island emerged from the sea only 3000 years ago. Rumpo Peninsula, which is one of the main localities where northern as well as southern species grow together, is still younger – its altitude is only 4–5 m above sea level – and, therefore, this part of the island is supposed to have emerged from the sea rather recently, about 1000-2000 years ago.

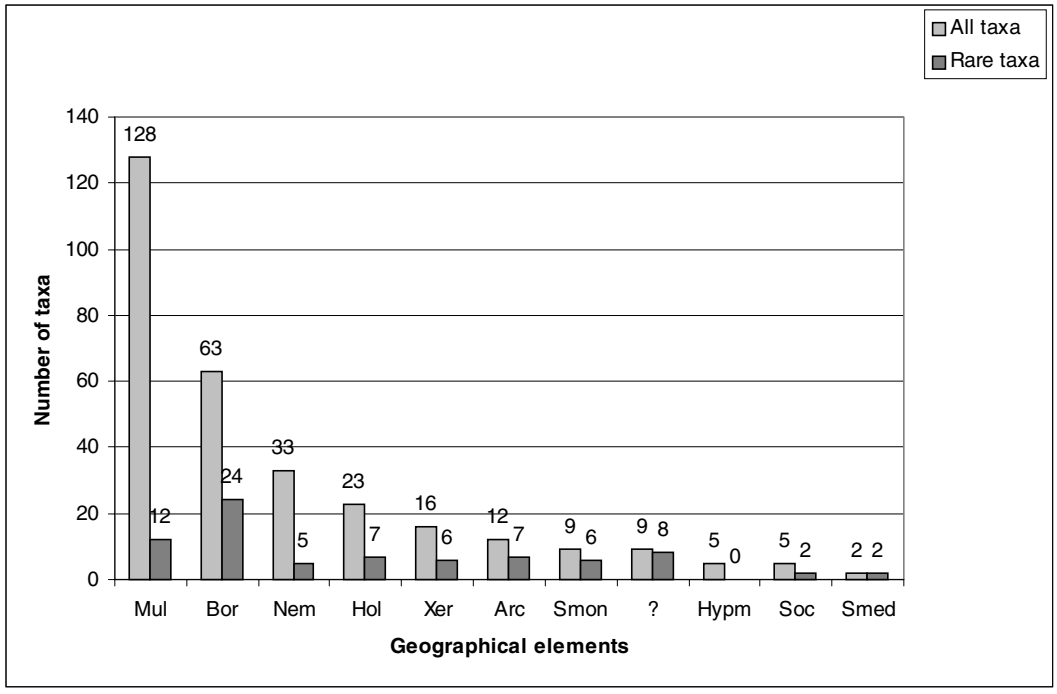



**Fig. 3.** Share of rare lichens in different different geographical elements. Abbreviations as in the legend to Table 2.

Lichens belonging to the true oceanic element, as treated by Degelius (1935) and later by Jørgensen (1996), are not present in the flora of Vormsi Island (or in the entire Estonian lichen flora). Still, some taxa grow in the close vicinity of the seashore only or prefer humid areas further in inland. Such species are considered suboceanic. The suboceanic element is surprisingly poor in Vormsi Island (5 taxa).

Species that mainly occur in the forest belts of high mountains but some of which also penetrate into the boreal or nemoral forests of plain territories are assigned to the submontane element (Makarevich 1963; Trass 1970). There are nine such species found in Vormsi Island and most of them are epilithic.

The distribution of geographical elements among rare (incl. very rare, rare and rather rare) taxa is quite different from that of all taxa (Fig. 3). The share of rare lichens is largest in the submediterranean, montane and arctic-alpine elements while quite numerous multiregional and nemoral elements include only a few rare lichens. The group of species with limited or poorly known distribution includes 8 rare taxa of 9 in total. Still, this is quite understandable as most of these species are rare also in other countries and therefore their general distribution is not clear.

## CONCLUSIONS

The lichen flora of Vormsi Island is rather rich including 301 species and 4 infraspecific taxa. Eight species are reported as new to the lichen flora of Estonia while 19 species belong to the group of very rare lichens with 1–2 localities only in the whole Estonia. Among them arctic-alpine *Flavocetraria cucullata* is the best known; the occurrence of it together with several other rare lichens on Rumpo Peninsula had served as a motivation to establish the botanical reserve there in 1987. Diversity of habitats including geologically young beach ridges seem to be a cause of concentration of different lichen species on Rumpo Peninsula where169 lichenized taxa have been recorded on a rather limited territory. This area must undoubtedly be continuously protected also in the future.

Among different ecological groups of lichens, distinguished according to the substrate where the taxa grow, epiphytic species are the most numerous. Still, the group of epilithic lichens includes considerably more rare species. This is partly due to the fact that epilithic taxa are less thoroughly investigated in the whole of Estonia. Furthermore, the calcareous and siliceous rocks probably serve as a suitable substrate for several lichens not quite usual in our area and therefore the share of really rare taxa might also be significant among epilithic species.

Distribution of geographical elements in the lichen flora of Vormsi Island is not unusual for Estonia. Species of both southerly and northerly distribution are present. The reason for the relative richness of lichens preferring xeric conditions is connected with the abundance of dry habitats on moraine formations, well drained rocky and sandy soils, and limestone outcrops. The occurrence of several arctic-alpine, hypo-arctic-montane and submontane lichens in Vormsi can be explained by the distribution of their propagules with migratory birds whose migratory tracks pass the western islands of Estonia. Abundance of geologically recently exposed substrates where only pioneer stages of vegetation succession have been developed might also be of importance.

## ACKNOWLEDGEMENTS

The authors are grateful to Inga Jüriado, Piret Lõhmus, Lauri Saag and Ave Suija for the identification of some species; Ivan Pisut is acknowledged for the confirmation of several identifications. Orvo Vitikainen kindly helped us with loans of many specimens from the Herbarium of the University of Helsinki. Elle Puurmann is thanked for initiating this survey. Financial support has been received from the grants No 3920 and 4144 of the Estonian Science Foundation.

## REFERENCES

Allmäe, R. 1988. *Lichen flora of Vormsi Island and its analysis* (in Estonian). Manuscript in Institute of Botany & Ecology, University of Tartu. 75 pp.

Degelius, G. 1935. Das ozeanische Element der Strauch- und Laubflechtenflora von Skandinavien. *Acta Phytogeogr. Suec.* 7: 1–411.

Fröberg, L. 1989. *The calcicolous lichens on the Great Alvar of Öland, Sweden*. Institutionen för Systematisk Botanik, Lund. 109 pp.

Häyren, E. 1930. Einige Flechtenfunde aus Estland. *Mem. Soc. pro Fauna et Flora Fenn.* 6: 135–136.

Jørgensen, P. M. 1994. Further notes on European taxa of the lichen genus *Leptogium*, with emphasis on the small species. *Lichenologist* 26 (1): 1–29.

Jørgensen, P. M. 1996. The oceanic element in the Scandinavian lichen flora revisited. *Acta Univ. Ups. Symb. Bot. Ups.* 31 (3): 297–317.

Kopaczevskaja, E. G., Makarevicz, M. F. & Oksner, A. N. 1977. *Handbook of the Lichens of the U.S.S.R. 4. Verrucariaceae-Pilocarpaceae* (in Russian). Nauka, Leningrad. 343 pp.

Liiv, S. & Piin, T. 1996. *Botanical motivation for founding nature reserve on Rumpo Peninsula* (in Estonian). Manuscript in Reg. Centre of Läänemaa Co. of West-Estonian Archipelago Biosphere Reserve.

Mardiste, M. 1967. Wind regime in straits of Muhu-Väin (in Russian). *Collection of papers THMO* 5: 5–11.

Martin, L., Nifontova, M. & Martin, J. 1991. Radionuclides variation in macrolichens in Estonia after the Chernobyl accident. *Proc. Estonian Acad. Sci.* 1 (1): 42–51.

Martin, L., Piin, T., Virolainen, V., Martin, J. & Hödrejärv, H. 1991. Lichen reserve on Rumpo Peninsula in Vormsi Island (in Estonian). In *Nature of Estonian islands and coastal areas. Abstracts of the reports on the XVI Day of Estonian Naturalists*. Tartu, pp. 15–21.

Parinkina, O. & Piin, T. 1984. Pecularities of microbial coenoses under terricolous lichen cover (in Russian with an English summary). In *Flora and groupings of lower plants in natural and anthropogenous extreme environment conditions*. Tallinn, pp. 51–71.

Parinkina, O. & Piin, T. 1991. Impact of lichens on soil microbocoenosis in tundra and forest. *Proc. Estonian Acad. Sci. Ecol.* 1 (4): 151–159.

Pisut, I., Lackovicova, A. & Lisicka, E. 1996. A second checklist and bibliography of Slovak lichens. *Biologia* (Bratislava) 51 / Suppl. 3: 1–79.

Purvis O. W., Coppins, D. L., Hawksworth, P. W., James, P. W. & Moore, D. M. (eds) 1992. *The lichen flora of Great Britain and Ireland*. Natural History Museum Publications. 710 pp.

Raik, A. 1967. Climatical districts of Estonia (in Estonian). *Eesti Loodus* 2: 65-70.

Randlane, T. & Jüriado, I. 1999. Lichen flora of Osmussaar Island (north-western Estonia). *Folia Cryptog. Estonica* 34: 47–53.

Randlane, T. & Saag, A. 1999. Second checklist of lichenized, lichenicolous and allied fungi of Estonia. *Folia Cryptog. Estonica* 35: 1–132.

Ratas, U. 1977. *Nature of Vormsi Island* (in Estonian). Valgus, Tallinn. 59 lk.

Raukas, A. 1969. Formation of moraine in Estonia (in Polish). *Zefz. Nauk UAM Geografia* (Poznan) 8: 167–176.

Raukas, A. (ed.) 1995. *Estonia. Nature* (in Estonian with an English summary). Valgus–Eesti Entsüklopeediakirjastus, Tallinn. 607 pp.

Santesson, R. 1993. *The lichens and lichenicolous fungi of Sweden and Norway*. Lund. 240 pp.

Thor, G. & Arvidson, L. 1999. *Rödlistade lavar i Sverige* (in Swedish). Artfakta–ArtDatabanken, SLU, Uppsala. 528 pp..

Trass, H. 1963. A plant from tundra in Vormsi Island (in Estonian). *Eesti Loodus* 2: 113–115.

Trass, H. 1967. *Analysis of the lichen-flora of Estonia* (in Russian). Manuscript (doctoral dissertation) in Institute of Botany & Ecology, University of Tartu. I-III.

Trass, H. 1970. The elements and development of the lichen-flora of Estonia (in Russian with an English summary). *Transactions of Tartu State Univ.* 268. *Pap. Bot.* 9: 5–233.

Vitikainen, O., Ahti, T., Kuusinen, M., Lommi, S. & Ulvinen, T. 1997. Checklist of lichens and allied fungi of Finland. *Norrlinia* 6:1-123.

Zhelnin, G. 1958. Rise of earth's crust in Estonia (in Estonian). *Eesti Loodus* 5: 269–274.

Wirth, V. 1995. *Flechtenflora: Bestimmung und ökologische Kennzeichnung der Flechten Südwestdeutschlands und angrenzender Gebeite*. Ulmer, Stuttgart. 661 S.

Wirth, V., Schöller, H., Scholtz, P. Ernst, G., Feuerer, T., Gnüchtel, A., Hauck, M., Jacobsen, P., John, V. & Litterski, B. 1996. Rote Liste der Flechten (*Lichenes*) der Bundesrepublik Deutschland. *Schr.-R.f. Vegetatsionskde*. 28: 307–368.

Folia Cryptog. Estonica, Fasc. 36: 83–86 (2000)

# Studies on lichenicolous fungi in Lithuania

**Jurga Motiejunaite**
Institute of Botany, 49 Zaliuju ezeru, LT-2021 Vilnius, Lithuania. E-mail: mikojm@ktl.mii.lt

**Abstract:** Studies on lichenicolous fungi in Lithuania are reviewed in the paper. A list of 44 fungus species occurring on lichens in this country is presented, the distribution of some species is discussed as well as the occurrence of the fungi on different host genera.

**Kokkuvõte:** J. Motiejunaite. Lihhenikoolsetest seentest Leedus.

Esitatakse lühike ülevaade Leedu lihhenikoolsetest seentest. Lisatud on nimekiri 44 seeneliigist, mis kasvavad samblikel. Käsitletakse mõnede liikide levikut ja esinemist erinevatel peremeesorganismidel.

## INTRODUCTION

Lichenicolous fungi are receiving continuously increasing attention in many countries during the recent years. Checklists of lichens, published during the last decade in various countries now frequently include lichenicolous fungi (e. g., checklists of Nordic countries (Santesson, 1993; Vitikainen et al., 1997), Poland (Faltynowicz, 1993), Ukraine (Kondratyuk et al., 1998), Estonia (Randlane & Saag, 1999). However, in spite of increasing study activities, the distribution of these fungi is still rather poorly known even in Europe. Lithuania makes no exception in this. Till the last decade of this century only 5 species of lichenicolous fungi were registered in a single paper dealing with this fungal group (Michalski, 1937). Especially during the last several years while preparing the checklist of lichens and allied fungi of Lithuania (Motiejunaite, in press) attention was paid also to the studies of lichenicolous fungi (e. g., Motiejunaite & Miadlikowska, 1998; Motiejunaite et al., 1998; Motiejunaite, 1999) which resulted in a rapid increase of the number of known species in this country.

## RESULTS AND DISCUSSION

In total 44 species of fungi occurring on lichens were registered so far in Lithuania, among them four species of *Hyphomycetes*, 13 species of *Coelomycetes*, 20 species of *Ascomycetes*, six taxa of *Basidiomycetes* and one member of *Myxomycetes* (see the species list below). These fungi were registered on 57 host species of 26 genera. Almost all fungi were found on common to ubiquitous lichen hosts, with the exception of *Muellerella pygmaea*, which was recorded on a rather rare *Porpidia cinereoatra* and *Rhizocarpon obscuratum* which is also not very common in Lithuania. Most of the lichenicolous fungi are more or less host-specific: e. g., *Xanthoriicola physciae* is found only on *Xanthoria parietina*, *Clypeococcum hypocenomycis* grows only on *Hypocenomyce scalaris*. Some of them are restricted to a certain host genus: e. g., *Karsteniomyces peltigerae* to *Peltigera* (in Lithuania registered on *P. praetextata* and *P. rufescens*), *Tremella cladoniae* to *Cladonia* (in Lithuania registered on *C. fimbriata* and *C. coniocraea*), *Sphaerellothecium propinquellum* to *Lecanora carpinea* group (in Lithuania found on *L. carpinea*). There are of course also species showing wide host spectra, and in fact these are often aggressive pathogens, e. g., *Athelia arachnoidea* which kills various epiphytic lichens, especially *Lecanora conizaeoides*, *Scoliciosporum chlorococcum* and species of the Xanthorion communities, and *Lichenoconium erodens*, which in Lithuania was found on eight host species belonging to six different genera. In most cases a single lichenicolous species was found on one thallus of the host, though some species were also found growing together: i. e., *Pronectria erythrinella* + *Nectria lecanodes* on *Peltigera canina*, *Lichenoconium lecanorae* + *L.*

*erodens* on *Hypogymnia physodes*, *Phacopsis oxyspora* + *Abrothallus cetrariae* on *Platismatia glauca*. Quite often also various unidentified species of *Hyphomycetes* were found growing on the same host thalli, being probably partly saprobic or a secondary infection.

Different genera of the hosts (Table 1.) differ in the number of lichenicolous fungi found on them. The widest range of lichenicolous species was registered on the genus *Peltigera*, which is explained by the fact that *Peltigera* represents an ancient group of lichens and therefore a large number of lichenicolous fungi was associated with this genus during the course of evolution (Hawksworth, 1982), also the lack of metabolite barrier (most of the hosts are tomentose, acid-deficient species), the fact which was noted by several authors (Vitikainen, 1994; Hawksworth & Miadlikowska, 1997 for *Peltigera*, and Kondratyuk & Galloway, 1995 for *Pseudocyphellaria*, *Peltigerales*) and partly by the fact that peltigericolous fungi are especially actively studied lately and therefore are relatively well-screened (e. g., Hawksworth, 1980; Miadlikowska & Alstrup, 1995; Hawksworth & Miadlikowska, 1997; Martinez & Hafellner, 1998). Many lichen genera shown in the table hosted only a single lichenicolous species. In some cases (e. g., *Buellia griseovirens*, *Scoliciosporum chlorococcum*, *Phlyctis argena*, *Lecidella elaeochroma*) the same species – *Athelia arachnoidea* – was registered on them, others (e. g., *Acarospora fuscata*, *Hypocenomyce scalaris*, *Usnea subfloridana*) hosted specific fungi (*Polycoccum microsticticum*, *Clypeococcum hypocenomycis*, and *Biatoropsis usnearum* respectively).

The known distribution of the lichenicolous fungi in Lithuania differs significantly from species to species. Whereas for example *Lichenoconium erodens* is known from 14 localities, *Clypeococcum hypocenomycis* is so far reported from five localities. On the other hand, *Polycoccum microsticticum* and *Vouauxiomyces santessonii* are known from single localities, *Phacopsis oxyspora* and *Abrothallus cetrariae* are also known from one locality, both from 60-year old collection. *Phoma peltigerae* and *Scutula miliaris* are known only from literature data (Michalski, 1937).

**Table 1.** Number of lichenicolous fungi species registered on the host genera

| Host genus | Number of lichenicolous species |
|---|---|
| *Acarospora* | 1 |
| *Anaptychia* | 1 |
| *Buellia* | 1 |
| *Candelariella* | 2 |
| *Chaenotheca* | 1 |
| *Cladonia* | 5 |
| *Hypocenomyce* | 1 |
| *Hypogymnia* | 4 |
| *Lecania* | 1 |
| *Lecanora* | 8 |
| *Lecidella* | 1 |
| *Parmelia* s. l. | 4 |
| *Parmeliopsis* | 1 |
| *Peltigera* | 12 |
| *Phaeophyscia* | 2 |
| *Phlyctis* | 1 |
| *Physcia* | 3 |
| *Physconia* | 1 |
| *Platismatia* | 3 |
| *Porpidia* | 1 |
| *Ramalina* | 1 |
| *Rhizocarpon* | 1 |
| *Scoliciosporum* | 1 |
| *Trapeliopsis* | 1 |
| *Usnea* | 1 |
| *Xanthoria* | 2 |

In all three Baltic countries studies of lichenicolous fungi are in their very beginnings. In Lithuania and Estonia almost equal numbers of species are known: in Lithuania 38 species of strictly lichenicolous fungi are registered (511 species of lichens and allied fungi in total), in Estonia – 47 species (866 species of lichens and allied fungi in total (Randlane & Saag, 1999; Halonen et al., 2000)). From Latvia only two species of lichenicolous fungi have been recorded so far (Motiejunaite & Piterans, 1998). Little is known about the distribution of the lichenicolous species and some groups of fungi (namely *Hyphomycetes* and *Basidiomycetes*) remain much understudied.

## List of lichenicolous fungi recorded in Lithuania

The nomenclature in the list follows mainly Santesson (1993), except for the recently monographed taxa and the listed myxomycete. Asterisk (*) marks the species facultatively occurring on lichens.

**HYPHOMYCETES**

BISPORA CHRISTIANSENII D. Hawksw.
ILLOSPORIUM CARNEUM Fr.
TRIMMATOSTROMA LICHENICOLA M. S. Christ. & D. Hawksw.
XANTHORIICOLA PHYSCIAE (Kalchbr.) D. Hawksw.

**COELOMYCETES**

EPICLADONIA SANDSTEDEI (Zopf) D. Hawksw.
KARSTENIOMYCES PELTIGERAE (P. Karst.) D. Hawksw. (anamorph of *Scutula miliaris*)
LIBERTIELLA MALMEDYENSIS Speg. & Roum.
LICHENOCONIUM ERODENS M. S. Christ. & D. Hawksw.
LICHENOCONIUM LECANORAE (Jaap) D. Hawksw.
LICHENOCONIUM PYXIDATAE (Oudem.) Petr. & Sydow
LICHENOCONIUM USNEAE (Anzi) D. Hawksw.
LICHENOCONIUM XANTHORIAE M. S. Christ.
LICHENODIPLIS LECANORAE (Vouaux) Dyko & D. Hawksw.
PHOMA CYTOSPORA (Vouaux) D. Hawksw.
PHOMA PELTIGERAE (P. Karst.) D. Hawksw.
VOUAUXIELLA LICHENICOLA (Linds.) Petr. & Sydow
VOUAUXIOMYCES SANTESSONII D. Hawksw. (anamorph of *Abrothallus cetrariae*)

**ASCOMYCETES**

ABROTHALLUS BERTIANUS De Not.
ABROTHALLUS CETRARIAE C. Kotte
ARTHONIA GALACTINARIA Leight.
CHAENOTHECOPSIS CONSOCIATA A. F. W. Schmidt
CLYPEOCOCCUM HYPOCENOMYCIS D. Hawksw.
CORTICIFRAGA FUCKELII (Rehm) D. Hawksw. & R. Sant.
CORTICIFRAGA PELTIGERAE (Nyl.) D. Hawksw. & R. Sant.
*DIPLOSCHISTES MUSCORUM (Scop.) R. Sant.
*LECANORA DISPERSA coll.
LICHENOCHORA OBSCUROIDES (Linds.) Triebel & Rambold
MUELLERELLA PYGMAEA (Körb.) D. Hawksw.
*MYCOBILIMBIA SABULETORUM (Schreb.) Hafellner
NECTRIA LECANODES Cesati
PHACOPSIS OXYSPORA (Tul.) Triebel & Rambold
POLYCOCCUM MICROSTICTICUM (Leight. ex Mudd) Arnold
PRONECTRIA ERYTHRINELLA (Nyl.) Lowen
*SARCOSAGIUM CAMPESTRE (Fr.) Poetsch & Schied
SCUTULA MILIARIS (Wallr.) Trevis.
SPHAERELLOTHECIUM PROPINQUELLUM (Nyl.) Roux & Triebel
TRICHONECTRIA HIRTA (Bloxam) Petch

**BASIDIOMYCETES**

*ATHELIA ARACHNOIDEA (Berk.) Jülich
BIATOROPSIS USNEARUM Räsänen
SYZYGOSPORA BACHMANII Diederich & M. S. Christ.
SYZYGOSPORA PHYSCIACEARUM Diederich
TREMELLA CLADONIAE Diederich & M. S. Christ.
TREMELLA PHAEOPHYSCIAE Diederich & M. S. Christ.

**MYXOMYCETES**

*LEOCARPUS FRAGILIS (Dicks.) Rostaf.

## AKNOWLEDGEMENTS

The author expresses her sincerest gratitude to an anonymous reviewer for the valuable comments on the manuscript.

## REFERENCES

Faltynowicz, W. 1993. A checklist of Polish lichen forming and lichenicolous fungi including parasitic and saprophytic fungi occurring on lichens. *Polish Bot. Stud.* 6: 1–65.

Halonen, P., Lõhmus, P., Kukwa, M., Motiejunaite, J. & Martin, L. 2000. Notes on lichens and lichenicolous fungi found during the XIV Symposium of Baltic Mycologists and lichenologists in Järvselja, Estonia. *Folia Cryptog. Estonica* 36: 17–21.

Hawksworth, D. L. 1980. Notes on some fungi occurring on *Peltigera* with a key to accepted species. *Trans. British Mycol. Soc.* 74: 363–386.

Hawksworth, D. L. 1982. Co-evolution and the detection of ancestry in lichens. *Journ. Hat. Bot. Lab.* 52: 323–329.

Hawksworth, D. L. & Miadlikowska, J. 1997. New species of lichenicolous fungi occurring on Peltigera in Ecuador and Europe. *Mycol. Research* 101: 1127–1134.

Kondratyuk, S. & Galloway, D. J. 1995. Lichenicolous fungi and chemical patterns in *Pseudocyphellaria*. *Bibl. Lichen.* 57: 327–345.

Kondratyuk, S. Ya., Khodosovtsev, A. Ye. & Zelenko, S. D. 1998. *The second checklist of lichen forming, lichenicolous and allied fungi of Ukraine*. Phytosociocentre, Kiev. 180 pp.

Martinez, I. & Hafellner, J. 1998. Lichens and lichenicolous fungi on *Peltigerales* in the Iberian peninsula and the Canary Islands. *Mycotaxon* 69: 271–310.

Miadlikowska, J. & Alstrup V. 1995. Some peltigericolous fungi and lichens mainly from Poland. *Graphis scripta* 7: 7–10.

Michalski, A. 1937. Grzybki pasozytnicze na porostach [Parasitic fungi on lichens]. *Acta Soc. Bot. Poloniae* 14(1): 45–48.

Motiejunaite, J. 1999. Dzukijos nacionalinio parko kerpes ir su jomis susijæ grybai [Lichens and allied fungi of Dzukija National Park]. *Botanica Lithuanica* 5(2): 137-153.

Motiejunaite, J. 1999. Checklist of lichens and allied fungi of Lithuania. *Botanica Lithuanica* 5(3): 251–269.

Motiejunaite, J. & Miadlikowska, J. 1998. Revised list of the genus *Peltigera* (lichenized *Ascomycotina*) and peltigericolous fungi from Lithuania. *Acta Mycol.* 33(2): 307–316.

Motiejunaite, J., Nordin, A., Zalewska, A., Bjelland, T., Hedens, H., Westberg, M., Heidmarsson, S. & Prigodina, I. 1998. Materials on lichens and allied fungi of Neringa National Park (Lithuania). *Botanica Lithuanica* 4(3): 285–305.

Motiejunaite, J. & Piterans, A. 1998. Materials on lichens and allied fungi of Kemeri National Park (Latvia). *Botanica Lithuanica* 4(2): 187–196.

Randlane, T. & Saag, A. (eds) 1999. Second checklist of lichenized, lichenicolous and allied fungi of Estonia. *Folia Cryptog. Estonica* 35: 1–132.

Santesson, R. 1993. *The lichens and lichenicolous fungi of Sweden and Norway*. SBT-förläget, Lund. 240 pp.

Vitikainen, O. 1994. Taxonomic revision of *Peltigera* (lichenized *Ascomycotina*) in Europe. *Acta Bot. Fennica* 152: 1–96.

Folia Cryptog. Estonica, Fasc. 36: 87–94 (2000)

# Nuclear DNA content and spore dimension in some species of the genus *Cystoderma*

**Irja Saar[1,2] & Bellis Kullman[2]**

[1] Institute of Botany and Ecology, University of Tartu, 40 Lai St., 51005, Tartu, Estonia. E-mail: irja@zbi.ee

[2] Institute of Zoology and Botany, Estonian Agricultural University, 181 Riia St., 51014, Tartu, Estonia. E-mail: bellis@zbi.ee

**Abstract:** Six species of the genus *Cystoderma* have been found in Estonia: *C. adnatifolium*, *C. amianthinum*, *C. carcharias, C. granulosum, C. jasonis* and *C. terreii*. Specimens have been collected in Estonia, Finland, Latvia, Norway and Sweden. Their genome size and ploidy levels were estimated. In this genus three ploidy levels (2x, 3x, 6x) were represented. The species *C. amianthinum* (29.5 Mb), *C. carcharias* (25.3 Mb) and *C. jasonis* (25.9 Mb) are on a 2x ploidy level; the species *C. adnatifolium* (35.9 Mb) and *C. granulosum* (37.3 Mb) are on a 3x ploidy level; the species *C. terreii* (65.4 Mb) is on a 6x ploidy level. The DNA content of the species on the 2x ploidy level (*C. amianthinum, C. carcharias* and *C. jasonis*) was statistically different only in case of *C. amianthinum*. The species *C. adnatifolium* & *C. granulosum* on 3x level did not have a statistically different DNA content. The spore length and width was measured in 303 specimens to estimate their variation between species. For all studied species differences in spore width and/or length became statictically significant. Correlation between DNA count and spore volume (r=-0.58), spore length (r=-0.55) and width (r=-0.61) within genus is negative; it means that species with larger spores have lower DNA content than species with smaller spores.

**Kokkuvõte:** Tuuma DNA sisaldus ja eoste suurus perekonna pisisirmik (*Cystoderma*) mõnedel liikidel.

Eestist on leitud kuus pisisirmiku (*Cystoderma*) liiki: *C. adnatifolium*, *C. amianthinum*, *C. carcharias, C. granulosum, C. jasonis* ja *C. terreii*. Herbaareksemplarid on kogutud Eestist, Soomest, Lätist, Norrast ja Rootsist. Neil liikidel mõõdeti DNA sisaldus ja tehti kindlaks ploidsusaste. Uuritud liikidel esines kolm ploidsusastet (2x, 3x, 6x). Ploidsusastmel 2x on liigid: *C. amianthinum* (29.5 Mb), *C. carcharias* (25.3 Mb) ja *C. jasonis* (25.9 Mb); ploidsusastmel 3x liigid *C. adnatifolium* (35.9 Mb) ja *C. granulosum* (37.3 Mb) ning ploidsusastmel 6x liik *C. terreii* (65.4 Mb). Ploidsusastmel 2x olevatest liikidest (*C. amianthinum, C. carcharias* ja *C. jasonis*) oli DNA sisaldus teistest statistiliselt erinev liigil *C. amianthinum*. Ploidsusastmel 3x olevate liikide *C. adnatifolium* ja *C. granulosum* DNA sisaldus polnud teineteisest statistiliselt erinev. 303 herbaareksemblaril mõõdeti eoste pikkus ja laius, et kindlaks teha nende liikidevahelist varieeruvust. Selgus, et kõigil uuritud liikidel oli eoste laius ja/või pikkus teistest liikidest statistiliselt erinev. Korrelatsioon DNA sisalduse ja eoste ruumala (r=-0.58), pikkuse (r=-0.55) ning laiuse (r=-0.61) vahel selles perekonnas on negatiivne, s. t suuremate eostega liikidel on väiksem DNA sisaldus kui väiksemate eostega liikidel.

## INTRODUCTION

Polyploidy in higher plants plays an important role in evolution and speciation (Stebbins, 1971; Grant, 1984). Evidence of polyploidy in fungi is known as well (Rogers, 1973; Bresinsky et al., 1987; Wittmann-Meixner & Bresinsky, 1989; Weber & Bresinsky, 1992). At the same time, studying chromosome numbers is difficult, because the fungal chromosomes are generally very small, therefore they are hard to separate and to count by the light microscope (Rogers, 1973). Alternative methods for obtaining ploidy levels use microspectrophotometry based on Feulgen staining (Peabody et al., 1978) and cytofluorometry based on fluorochrome staining (Bresinsky et al., 1987; Wittmann-Meixner & Bresinsky, 1989; Weber & Bresinsky, 1992; Bresinsky & Wittmann-Bresinsky, 1995). In the 90s flow cytometry is being used, as a convenient and rapid method for estimation of genome size, especially for plant research (Bennett & Leitch, 1995, 1997). Genome size as a quantitative character of an organism is relatively constant and tends to be highly characteristic of a species, which makes it a useful character in systematics (Bennett & Leitch, 1998).

The aim of the present study was to estimate genome size and to determine ploidy level as well as to measure spore dimensions to determine difference between the species of the genus *Cystoderma*.

## MATERIAL AND METHODS

All studied species have been found in Estonia, specimens used in this study have been collected in Estonia, Finland, Latvia, Norway and Sweden.

The specimens whose DNA content was measured are marked with an asterisk (*).

***C. adnatifolium*** (Peck) Harmaja

Estonia: Ida-Virumaa Co., Kassisare, 17.08.61, K. Kalamees (TAA 72586); Võrumaa Co., Meeksi, 30.09.97, K. Kalamees (TAA 147843)*; Võrumaa Co., Meeksi, 30.09.97, I. Saar (TAA 147335, 147339, 147340, 147341); Võrumaa Co., Meeksi, 30.09.97, K. Kalamees (TAA 147846); Võrumaa Co., Uigumäe, 01.10.97, I. Saar (TAA 147343)*; Tartumaa Co., Laeva, 23.10.97, M. Vaasma (TAA 171701)*. Finland: Punkaharju, 20.08.56, R. Tuomikoski (H); Vankalahti, 04.10.70, L. Fagerström (H); Lemland, Jungfruskär, 17.08.74, C-A. Hæggström (H); Kuusamo, Oulanka, 30.08.77, M. Korhonen, R. Tuomikoski (H); Lammi, Mataramäki, 24.09.77, R. Tuomikoski (H); Karkkila, Haavisto, 22.09.79, I. Kytövuori (H 79864); Melalahti, 29.08.81, I. Kytövuori (H); Liikasenvaara, 04.09.81, I. Kytövuori (H 811135); Liikasenvaara, Korvaswaara, 10.09.81, K. Kalamees (TAA 122115); Liikasenvaara, 11.09.81, K. Kalamees (TAA 122162); Susiniemi, 20.09.81, I. Kytövuori (H 811445); Kiuruvesi, Jynkkä, 19.09.82, I. Kytövuori (H 82497); Nilsiä, Kalkkiruukki, 08.09.83, I. Kytövuori (H 83229); Niinivaara, 29.08.85, I. Kytövuori (H); Italiankylä, 31.08.85, I. Kytövuori (H 85595); Nurmijärvi, 13.09.87, P. Askola, H. Harmaja (H 2227); Etelä-Savo, Karhunpää, 16.09.89, I. Kytövuori (H 89-918); Sompion Lappi, Tähtelä, 24.08.92, I. Kytövuori (H 92-957); Sompion Lappi, Viitaranta, 25.08.92, I. Kytövuori (H 92-1017); Sompion Lappi, Sadankylä, 27.08.92, I. Kytövuori (H 92-1155)*; Etelä-Karjala, Kaipiainen, 15.09.94, I. Kytövuori (H 94-531); Etelä-Savo, Karankamäki, 29.09.94, I. Kytövuori (H 94-1087); Tampere, 10.97, M. Rantala (TAA 147356)*; Tampere, 10.97, M. Rantala (TAA 147357); Nokia, Tottijärvi, 17.10.97, L. Kosonen (TAA 147360)*; Porvoo, Teissala, 09.10.97, P. Höijer (TAA 147381)*; Askola, 13.10.97, P. Höijer (TAA 147385). Latvia: Maczalaca, Ramota, 09.10.97, K. Kalamees (TAA 147895); Renceni, 07.10.97, K. Kalamees (TAA 147881).

***C. amianthinum*** (Scop.) Konrad & Maubl.

Estonia: Tartumaa Co., Variku, 09.10.46, N. Witkovski (TAA 142331, 114426); Põlvamaa Co., Laessaare, 25.09.55, K. Kalamees (TAA 70133); Põlvamaa Co., Taevaskoja, 16.10.55, K. Kalamees, Pärtelpoeg (TAA 70204); Põlvamaa Co., 08.09.56, K. Kalamees (TAA 70344); Harjumaa Co., Nissi, 18.09.57, K. Kalamees (TAA 70665); Võrumaa Co., Vungi, 27.09.57, K. Kalamees (TAA 70719); Lääne-Virumaa Co., Raeküla, 08.08.58, K. Kalamees (TAA 70966); Harjumaa Co., Külvandu, 18.09.58, U. Kalamees (TAA 50576); Põlvamaa Co., Veskimõisa, 14.09.59, K. Kalamees (TAA 71363); Lääne-Virumaa Co., Kolja, 16.09.59, U. Kalamees (TAA 51271); Harjumaa Co., between Pirga and Priske, 15.11.59, U., K. Kalamees (TAA 71557); Jõgevamaa Co., Aru, 24.09.60, K. Kalamees (TAA 71857); Põlvamaa Co., Saesaare, 10.09.61, S. Kask (TAA 263100); Tartumaa Co., Rannaküla, 04.10.61, K. Kalamees (TAA 72655); Jõgevamaa Co., Rünga, 25.09.65, U. Kalamees (TAA 52248); Jõgevamaa Co., Vooremaa, 70, K. Kalamees, A. Kollom (TAA 82455); Saaremaa Co., Kihelkonna, 07.09.79 (TAA 113400); Võrumaa Co., Saruküla, 29.09.80, M. Paas (TAA 142330); Saaremaa Co., Kaali, 07.10.83 (TAA 141097); Saaremaa Co., Kessulaid, 10.10.83, K. Kalamees (TAA 123304); Läänemaa Co., Vormsi, Fällarna, 20.09.86, K. Kalamees (TAA 143437); Harjumaa Co., Käsmu, 22.08.89, I. Kytövuori (H 89-222); Tartumaa Co., Ahunapalu, Apnasaar, 07.10.89, K. Kalamees (TAA 144411); Saaremaa Co., Harilaid, 12.09.93, I. Kytövuori (H 93-1062); Saaremaa Co., Selgase, 16.09.93, K. Kalamees (TAA 146052); Läänemaa Co., Dirhami, 06.10.93, K. Kalamees (TAA 146114); Põlvamaa Co., Taevaskoja, 20.10.93, K. Kalamees (TAA 146163); Tartumaa Co., Kabina, 14.10.95, I. Saar (TAA 147300)*; Järvamaa Co., Huuksi, 22.09.95, M. Vaasma (TAA 142717, 142718); Järvamaa Co., between Aela and Virla, 23.09.95, M. Vaasma (TAA 142733); Harjumaa Co., Paimetsa, 25.09.95, M. Vaasma (TAA 142741); Harjumaa Co., Järlepa, 27.09.95, M. Vaasma (TAA 142781); Harjumaa Co., Paunküla, 28.09.95, M. Vaasma (TAA 142786); Harjumaa Co., Paunküla, 28.09.95, M. Vaasma (TAA 142786); Põlvamaa Co., Taevaskoja, 07.10.95, K. Kalamees (TAA 146707); Põlvamaa Co., Kiidjärve, 15.10.95, I. Saar (TAA 147303); Tartumaa Co., Ahunapalu, 29.10.95, K. Kalamees (TAA 146736, 146737); Lääne-Virumaa Co., Tudu-Järvesoo, 96, T. Ploompuu (TAA 147241); Saaremaa Co., Audaku, 03.10.96, V. Liiv, J. Lumiste, I. Saar (TAA 147304); Saaremaa Co., Audaku, 03.10.96, I. Saar (TAA 147305); Saaremaa Co., Selgase, 05.10.96, I. Saar (TAA 147308); Tartumaa Co., Tõllassaare, 10.10.96, M. Vaasma (TAA 142905); Võrumaa Co., Hino, 12.10.96, I. Saar (TAA 147321)*; Võrumaa Co., Kuklase, 11.10.96, I. Saar (TAA 147311); Võrumaa Co., Luutsniku-Lehelaane, 11.10.96, I. Saar (TAA 147314, 147316, 147317); Võrumaa Co., Hino, 12.10.96, I. Saar (TAA 147320, 147322); Tartumaa Co., Sellisaare, 17.10.96, K. Leenurm, I. Saar (TAA 147325, 147326); Valgamaa Co., Hargla, 17.10.96, 18.10.96, K. Kalamees (TAA 147293, 147297,

147515, 147517, 147518); Põlvamaa Co., Taevaskoja, 02.11.96, K. Kalamees (TAA 147566, 147567, 147568); Võrumaa Co., Meeksi, 30.09.97, I. Saar (TAA 147342)*; Võrumaa Co., Kütiorg, 03.10.97, A. Kollom, V. Liiv, I. Saar (TAA 147347, 147348, 147350)*; Tartumaa Co., Selgase, 23.07.97, K. Kalamees (TAA 147983); Võrumaa Co., Kütiorg, 03.10.97, M. Vaasma (TAA 147351); Võrumaa Co., Meeksi, 30.09.97, K. Kalamees (TAA 147848, 147844, 147847); Võrumaa Co., Kütiorg, 03.10.97, K. Kalamees (TAA 171760); Jõgevamaa Co., Võiviku, 27.09.97, M. Vaasma (TAA 171618); Pärnumaa Co., Nigula Nature Reserve, Salupeaksi, 19.09.98, K. Kalamees (TAA 147989); Valgamaa Co., Aakre, 04.08.98, K. Leenurm (TAA 147386)*; Valga Co., Aakre, 09.08.98, B. Kullman (TAA 147388)*; Tartumaa Co., Trepimäe, 13.09.98, B. Kullman (TAA 147397); Põlvamaa Co., Mooste, 06.09.98, B. Kullman (TAA 157979). Finland: Tampere, 10.97, M. Rantala (TAA 147358)*; Kuhmalahti, 27.10.97, M. Rantala (TAA 147364); Luopioinen, 28.10.97, M. Rantala (TAA 147365); Kangasala, 10.10.97, M. Rantala (TAA 147371, 147372); Tampere, Kivitulampi, 01.11.97, M. Rantala (TAA 147376, 147377); Tampere, 22.08.98, I. Saar (TAA 147389, 147390). Latvia: Renceni, 07.10.97, K. Kalamees (TAA 147880)*; Saule, 08.10.97, K. Kalamees (TAA 147884); Saule, 08.10.97, K. Kalamees (TAA 147886)*; Maczalaca, Ramota, 09.10.97, K. Kalamees (TAA 147896); Puiku, 10.10.97, K. Kalamees (TAA 147897); Rozeni, 10.10.97, K. Kalamees (TAA 147898). Norway, between Trondheim and Heimdal, 08.08.95, K. Kalamees, V. Liiv (TAA 146531, 146543); between Troms and Metlevoll, 12.08.95 (TAA 146546). Sweden: Öden, 28.08.98, I. Saar (TAA 147395); Hudiksvall, 28.08.98, I. Saar (TAA 147396); Gotland, Kambs Gård, 04.10.98, K. Kalamees (TAA 147950); Gotland, Halla, 04.10.98, M. Vaasma (TAA 172016, 172019); Gotland, Åminne, 05.10.98, M. Vaasma (172029); Gotland, Russvätar, 06.10.98, M. Vaasma (172041).

***C. carcharias*** (Pers.) Konrad & Maubl.

Estonia: Põlvamaa Co., Taevaskoja, 06.11.55, K. Kalamees (TAA 70231); Harjumaa Co., Nissi, 18.09.57, K. Kalamees (TAA 70664); Lääne-Virumaa Co., Viitna, 18.09.59, U. Kalamees (TAA 51292); Harjumaa Co., Kongulaane, 14.11.59, U., K. Kalamees (TAA 71549); Jõgevamaa Co., Mõisamaa, 27.09.60, U., K. Kalamees (TAA 71897); Jõgevamaa Co., Lentsi, 27.12.60, K. Kalamees (TAA 72082); habitat unknown, 19.09.63, U. Kalamees (TAA 51638); Lääne-Virumaa Co., Äntu, 24.09.65, U. Kalamees (TAA 52255); Jõgevamaa Co., Vooremaa, 13.10.72, K. Kalamees, A. Kollom (TAA 114206, 114208); Lääne-Virumaa Co., Viitna, 25.10.77, L. Pihlik (TAA 95672); Võrumaa Co., Tõrvasoja, 16.08.80, M. Paas (TAA 142333); Võrumaa Co., Saruküla, 29.09.80, M. Paas (TAA 142332); Järvamaa Co., between Aela and Virla, 23.09.95, M. Vaasma (TAA 142730); Harjumaa Co., Paunküla, 28.09.95, M. Vaasma (TAA 142787); Harjumaa Co., Leistu, 28.09.95, M. Vaasma (TAA 142798); Harjumaa Co., Jüri, 03.10.95, K. Merisalu (TAA 142807); Põlvamaa Co., Taevaskoja, 07.10.95, K. Kalamees (TAA 146708, 146708A); Tartumaa Co., Muri, 08.10.95, K. Kalamees, J. Vauras (TAA 146721, 146722); Tartumaa Co., Kabina, 14.10.95, I. Saar (TAA 147301); Põlvamaa Co., Kiidjärve, 15.10.95, I. Saar (TAA 147302); Tartumaa Co., Ahunapalu, Apnasaar, 29.10.95, K. Kalamees (TAA 146735, 146733); Saaremaa Co., Audaku, 03.10.96, I. Saar (TAA 147306); Saaremaa Co., Kihelkonna, 04.10.96, I. Saar (TAA 147307); Saaremaa Co., Selgase, 05.10.96, K. Kalamees; I. Saar (TAA 147185, 147309); Saaremaa Co., Muhu, 06.10.96, N. Lundgvist (TAA 147310); Võrumaa Co, Luutsniku-Lehelaane, 11.10.96, I. Saar (TAA 147312, 147313, 147315); Võrumaa Co., Kokemäe, 12.10.96, I. Saar (TAA 147318); Võrumaa Co., Hino, 12.10.96, I. Saar (TAA 147319); Võrumaa Co., Järveküla, 12.10.96, K. Leenurm (TAA 147323); Tartumaa Co., Lootvina, 26.10.96, B. Kullman (TAA 147327); Põlvamaa Co., Taevaskoja, 02.11.96, K. Kalamees (TAA 147566); Võrumaa Co., Vällamägi, 13.10.96, I. Saar (TAA 147324)*; Tartumaa Co., Võiviku, 05.11.96, H. Järv (TAA 147328); Põlvamaa Co., Karisilla, 10.11.96, I. Saar (TAA 147329); Järvamaa Co., Peetri, 05.12.96, K. Kalamees (TAA 147330); Harjumaa Co., Viimsi, Ranna, 06.12.96, K. Kalamees (TAA 147331); Võrumaa Co., Meeksi, 30.09.97, I. Saar (TAA 147334)*; Võrumaa Co., Vällamägi, 02.10.97, P. Höijer (TAA 147345)*; Võrumaa Co., Kütiorg, 03.10.97, I. Saar (TAA 147346, 147349)*; Tartumaa Co., Variku, 20.10.97, B. Kullman (TAA 147353)*; Tartumaa Co., Laeva Forestry, 23.10.97, M. Vaasma (TAA 171700)*; Võrumaa Co., Meeksi, 30.09.97, K. Kalamees (TAA 147845); Saaremaa Co., Kihelkonna, 14.08.98, M. Vaasma (TAA 171965). Finland: Pirkkala, Säija, 10.10.97, R. Linkoaho (TAA 147367); Tampere, Tesoma, 10.10.97, M. Rantala (TAA 147368); Kangasala, 10.10.97, M. Rantala (TAA 147373); Askola, 13.10.97, P. Höijer (TAA 147384). Latvia: Vitrupe, 10.10.97, K. Kalamees (TAA 147900). Sweden: Gotland, Nyneshamn, 03.10.98, M. Vaasma (TAA 172011); Gotland, Halla, 04.10.98, M. Vaasma (TAA 172024); Gotland, Tjaukle, 07.10.98, M. Vaasma (TAA 172049); Gotland, Viklau, 04.10.98, K. Kalamees (TAA 147945, 147946); Gotland, Kambs Gård, 04.10.98, K. Kalamees (TAA 147948).

***C. granulosum*** (Batsch: Fr.) Kühner

Estonia: Lääne-Virumaa Co., Luusiku, Hanguse, 23.08.62, H. Kelder (TAA 73144); Tartumaa Co., Vasevere, 04.10.63, K. Kalamees (TAA 74956); Tartumaa Co., Kaagvere, Tõlgu, 11.10.68, K. Kalamees (TAA 77626); Jõgevamaa Co., Vooremaa, 22.09.72, K. Kalamees, A. Kollom (TAA 114037, 114061); Jõgevamaa Co., Vooremaa, 23.09.72, K. Kalamees, A. Kollom (TAA 114200); Jõgevamaa Co.,

Vooremaa, 02.10.72, K. Kalamees, A. Kollom (TAA 114036); Jõgevamaa Co., Vooremaa, 03.10.72, K. Kalamees, A. Kollom (TAA 114288); Põlvamaa Co., Valgesoo, Kriisa, 07.10.72, K. Kalamees (TAA 80411); Valgamaa Co., Karula, 25.08.82, S. Veldre (TAA 114912); Valgamaa Co., Karula, Kihati, 26.09.83, S. Veldre (TAA 141198); Valgamaa Co., Koikküla, Piiri, 21.07.84, S. Veldre (TAA 123550); Saaremaa Co., Audaku, 17.08.84, K. Kalamees (TAA 123631); Saaremaa Co., Järve, 04.10.88, K. Kalamees, M. Vaasma (TAA 144045); Tartumaa Co., Ahunapalu, Vaabnasaar, 25.07.90, K. Kalamees (TAA 144672); Tartumaa Co., Ihaste, 21.10.92, K. Kalamees (TAA 145644); Valgamaa Co., Hargla, 18.10.96, K. Kalamees (TAA 147500)*; Võrumaa Co., Meeksi, 30.09.97, I. Saar (TAA 147337)*; Võrumaa Co., Vällamägi, 02.10.97, S. Veldre, K. Kalamees (TAA 147344)*; Võrumaa Co., Vällamägi, 02.10.97, S. Veldre, K. Kalamees (TAA 147867); Võrumaa Co., Kütiorg, 03.10.97, E. Ohenoja (TAA 147352); Võrumaa Co., Meeksi, 30.09.97, K. Kalamees (TAA 147849); Tartumaa Co., Selgase, 23.07.98, K. Kalamees (TAA 147984); Valgamaa Co., Aakre, 04.08.98, K. Leenurm (TAA 147387)*; Saaremaa Co., Karjalasma, 12.08.98, M. Vaasma (TAA 171953). Finland: Tampere, 10.97, M. Rantala (TAA 147359)*; Tampere, Sitalahti, 01.11.97, M. Rantala (TAA 147375); Liikasenvaara, Korvasvaara, 10.09.81, K. Kalamees, E. Ohenoja, T. Ulvinen (TAA 122127); Pirkkala, 10.10.97, R. Lenkoaho (TAA 147366, 147369); Kangasala, 10.10.97, M. Rantala (TAA 147370); Helsinki, Annala, 07.10.97, P. Höijer (TAA 147379)*; Tampere, 22.08.98, I. Saar (TAA 147391). Latvia: Renceni, 07.10.97, K. Kalamees (TAA 147882)*; Vitrupe, 10.10.97, K. Kalamees (TAA 147901). Norway: Brennfjell, 26.08.98, A. Jakobson, I. Saar (TAA 147392).

***C. jasonis*** (Cooke & Massee) Harmaja

Estonia: Pärnumaa Co., Rannametsa, 28.08.89, P. Kytövuori (H 89)*; Valgamaa Co., Hargla, 17.10.96, K. Kalamees (TAA 147294, 147295, 147296); Valgamaa Co., Hargla, 18.10.96, K. Kalamees (TAA 147514); Võrumaa Co., Meeksi, 30.09.97, I. Saar (TAA 147336, 147338); Võrumaa Co., Meeksi, 30.09.97, K. Kalamees (TAA 147840); Tartumaa Co., Palupõhja, 23.10.97, M. Vaasma (TAA 171716)*; Valgamaa Co., Aakre, 30.07.98, M. Vaasma (TAA 171909). Finland: Oulanka, Sirkkapuro, 28.08.77, M. Korhonen, R. Tuomikoski (H); Lammi, Evo, Vahtervehmaa, 06.09.78, M. Korhonen, R. Tuomikoski (H); Tamminsaari, 19.09.78, M. Korhonen, R. Tuomikoski (H); Hindsby, 23.09.78, R. Saarenoksa (H 30978); Hindsby, 23.09.79, R. Saarenoksa (H 62779); Ristijärvi, 01.09.80, I. Kytövuori (H 80644); Kuhmo, 03.09.80, I. Kytövuori (H); Sauvo, 27.09.80, I. Kytövuori (H 801139); Mäntsälä, Levanto, 19.09.80, I. Kytövuori (H 801071); Oulanka, 09.09.81, K. Kalamees, E. Ohenoja, T. Ulvinen (TAA 122054); Rymättylä, Raula, 24.09.81, R. Tuomikoski (H); Annala, 27.09.81, R. Saarenoksa (H 57481); Rymättylä, Välimäki, 09.10.81, R. Tuomikoski (H); Kurikka, Vesiperä, 15.09.82, I. Kytövuori (H 82378); Kinnula, Mäkelä, 18.09.82, I. Kytövuori (H 82448); Kuusisto island, Juopinkrotti, 02.09.92, J. Vauras (Ex Herbario Universitatis Åbo Akademi-Tura); Porvoo, Teissala, 08.10.97, P. Höijer (TAA 147380)*; Porvoo, Teissala, 13.10.97, P. Höijer (TAA 147383)*; Kangasala, 10.10.97, M. Rantala (TAA 147374); Tampere, Sitalahti, 01.11.97, M. Rantala (TAA 147378). Latvia: Straume, 07.10.97, K. Kalamees (TAA 147879)*; Renceni, 07.10.97, K. Kalamees (TAA 147883)*; Saule, 08.10.97, K. Kalamees (TAA 147885)*; Mernieki, 10.10.97, K. Kalamees (TAA 147899). Norway, Brennfjell, 26.08.98, M. Öpik, I. Saar (TAA 147393)*.

***C. terreii*** (Berk. & Broome) Harmaja

Estonia: Põlvamaa Co., Taevaskoja, 08.09.57, K. Kalamees (TAA 70556); Võrumaa Co., Võru-Kubija, 25.09.57, K. Kalamees (TAA 70706); Valgamaa Co., Piiri, 01.10.57, K. Kalamees (TAA 70768); Järvamaa Co., Noku, 14.09.58, U. Kalamees (TAA 50592); Jõgevamaa Co., Mõisamaa, 04.09.60, K. Kalamees (TAA 71729); Jõgevamaa Co., Siimuste, 20.09.60, K. Kalamees (TAA 71809); Jõgevamaa Co., between Kurista and Kõpu, 03.10.60, K. Kalamees (TAA 71972); Ida-Virumaa Co., Jõetaguse, 07.08.61, K. Kalamees (TAA 72422); Saaremaa Co., Kihelkonna, 20.08.84, K. Kalamees, G. Štšukin, M. Vaasma (TAA 123714); Pärnumaa Co., Kabli, 23.09.90, K. Kalamees (TAA 144765); Saaremaa Co., Selgase, 10.09.93, K. Kalamees, K. Salo, V. Liiv (TAA 145958); Saaremaa Co., Audaku, 13.09.93, K. Kalamees, M. Vaasma (TAA 145991); Saare Co., Võhma, 16.09.93, I. Kytövuori (H 93-1328); Läänemaa Co., Dirhami, 06.10.93, K. Kalamees (TAA 146119); Läänemaa Co., Marimetsa, 24.09.94, K. Kalamees, I. Saar (TAA 146430); Pärnumaa Co., Ristiotsa, 27.09.94, M. Vaasma (TAA 142277); Hiiumaa Co., Ristna, 09.10.94, K. Kalamees (TAA 146415); Järvamaa Co., Huuksi, 20.09.95, M. Vaasma (TAA 142691); Harjumaa Co., Salmistu-Kaberneeme, 14.09.97, K. Jürgens (TAA 147332); Harjumaa Co., Rutja, 19.10.97, B. Kullman (TAA 147354, 147355)*; Võrumaa Co., Kütiorg, 03.10.97, K. Kalamees (TAA 171759)*; Lääne-Virumaa Co., Karepa, 27.07.98, B. Kullman (TAA 157823); Saaremaa Co., Kihelkonna, 14.08.98, M. Vaasma (TAA 171966); Lääne-Virumaa Co., Karepa, 20.08.99, B. Kullman (TAA 179144, 179145). Finland: Liikasenvaara, Korvasvaara, 10.09.81, K. Kalamees, E. Ohenoja, T. Ulvinen (TAA 122100). Latvia: Straume, 07.10.97, K. Kalamees (TAA 147878)*. Sweden: Överkalix, 27.08.98, I. Saar (TAA 147394)*.

The studied specimens are deposited in the herbarium of the Institute of Zoology and

Botany of the Estonian Agricultural University (TAA) or in the Botanical Museum of the University of Helsinki (H).

All specimens were determined by the author as well (303). Microscopy of specimens was studied in Melzer's reagent and 3% potassium hydroxide (KOH) (Swift M4000-D, x1000). For every specimen, 10 spores were measured. In this study the mean spore length and width of species, with standard deviation (d) are presented. For calculation of spore volumes, the formula for an ellipsoid of rotation was used: $V=4/3p\ L/2\ (W/2)^2 = 0.523\ LW^2 \sim 0.5\ LW^2$.

Number of nuclei in spores was counted by fluorescence microscope (Olympus Vanox, x1000).

**Flow cytometry:**
DNA measurement by flow cytometry is based on the quantitative binding of the DNA specific dye (fluorochrome) to DNA. Intercalating fluorochrome, e. g. propidium iodide (PI), binds on doublestranded DNA irrespective of the DNA sequence. Cytometry allows the measurement of DNA – fluorochrome complex fluorescence intensity separately for each cell (spore). Fluorescence intensity is proportional to the amount of fluorescent substance and hence to DNA content. To estimate DNA content it is necessary to compare the object of investigation with a standard whose DNA content is already known. Many DNA measurements in plants have been performed using animal standards, the most common being chicken erythrocytes. Often other plants whose DNA content is comparable to that of the studied object (i.e. not more than two or three times larger) have been employed as internal standards (Bennett & Leitch, 1995). Compared with plants or animals, fungi have small nuclei with a relatively low DNA content (about 100 times lower). For this reason it is not correct to use plant or animal standards for fungal measurements.

In this study the spore print of *Pleurotus ostreatus* (TAA 142824) served as the internal standard, with a DNA content of 25 Mb measured by Kullman (in print). Its DNA content was established using cells of *Saccharomyces cerevisiae* (strain YAC) as a standard.

**Preparation and staining:**
Spores were fixed in 96% ethanol: acetic acid (3:1) and incubated at room temperature for 20 h on a slow rotator mixer. The samples were washed slowly by gradually adding deionised water on a slow rotator mixer. Centrifuging for 5 minutes at 10.000 rpm, removed the supernatant. About 600 µl buffer (PBS) was added and samples were kept on a slow rotator mixer for at least 30 min. Centrifuging for 5 minutes at 10.000 rpm, removed the supernatant. 200 µl buffer (218.6 mg D-sorbitol, 40 ml 0.5 M EDTA, 10 ml 1 M TRIS, pH 7.5) and 18 µl zymolyase (Seikagaku) stock (50 mg/ml) was added, and samples were incubated at 37°C for 24 h on a slow rotator mixer. 100 µl buffer (PBS) and 6 µl RNase A (Fluka) stock (10 mg/ml) was added, and samples were incubated at 37°C for 24 h on a slow rotator mixer. The suspension was filtered through a 85 µm nylon mesh. 20 µl propidium iodide (Fluka) stock (1 mg/ml) was added. The samples were incubated on ice for at least 30 min; they were analyzed on a flow cytometer (Odam-Brucker or FACSort, Becton Dickinson, USA).

## RESULTS

All the studied species have dikaryotic spores. DNA content is given for one nucleus.

**Table 1.** Nuclear DNA content (in Mb) and ploidy (x) level in the species of the genus *Cystoderma* were measured from spore print

| Species | Measurings | DNA content, ±d | 1x DNA content | Ploidy level |
|---|---|---|---|---|
| C. carcharias | n=9 | 25.3 ± 2.4 | 12.7 | 2x |
| C. jasonis | n=9 | 25.9 ± 3.4 | 12.9 | 2x |
| C. amianthinum | n=13 | 29.5 ± 3.1 | 14.8 | 2x |
| C. adnatifolium | n=10 | 35.9 ± 3.3 | 12.0 | 3x |
| C. granulosum | n=10 | 37.3 ± 2.7 | 12.4 | 3x |
| C. terreii | n=6 | 65.4 ± 4.3 | 10.9 | 6x |

**Table 2.** Spore length and width in the species of genus *Cystoderma*

| Species | Specimens | Mean spore length ± d (µm) | Mean spore width ± d (µm) |
|---|---|---|---|
| C. carcharias | n=62 | 4.41 ± 0.34 | 3.05 ± 0.11 |
| C. jasonis | n=35 | 6.37 ± 0.35 | 3.16 ± 0.15 |
| C. amianthinum | n=103 | 5.56 ± 0.49 | 3.03 ± 0.18 |
| C. adnatifolium | n=38 | 4.26 ± 0.19 | 2.51 ± 0.15 |
| C. granulosum | n=36 | 4.10 ± 0.14 | 2.22 ± 0.20 |
| C. terreii | n=29 | 4.02 ± 0.13 | 2.19 ± 0.19 |

## DISCUSSION

The species of the genus *Cystoderma* reveal genome size differences in the range 25.3–65.4 Mb and three ploidy levels (2x, 3x and 6x) (Table 1; Fig. 1). Mean nuclear DNA content for the 1x-level of ploidy is 12.6 Mb. However, species with such a DNA content have yet to be discovered.

The species of the genus *Cystoderma* are divided into two groups according to their amyloidity of spores (Smith & Singer, 1945; Singer, 1986):

1. *C. amianthinum, C. jasonis* and *C. carcharias*
2. *C. adnatifolium, C. granulosum* and *C. terreii.*

In the first group, all species have a 2x ploidy level. *C. amianthinum* is significantly different from the others with respect to DNA content, *C. jasonis* & *C. carcharias* are not significantly different from one another. *C. carcharias* could be easily distinguished from the others by its morphological characters. Its spore length is significantly shorter from that of the others, spore width is not significantly different from that in the species *C. amianthinum* (Table 2; Fig. 2). *C. jasonis* is distinguished from *C. amianthinum* by deeper colours of cap, gills, stem, and flesh and microscopically by the significantly larger spore size.

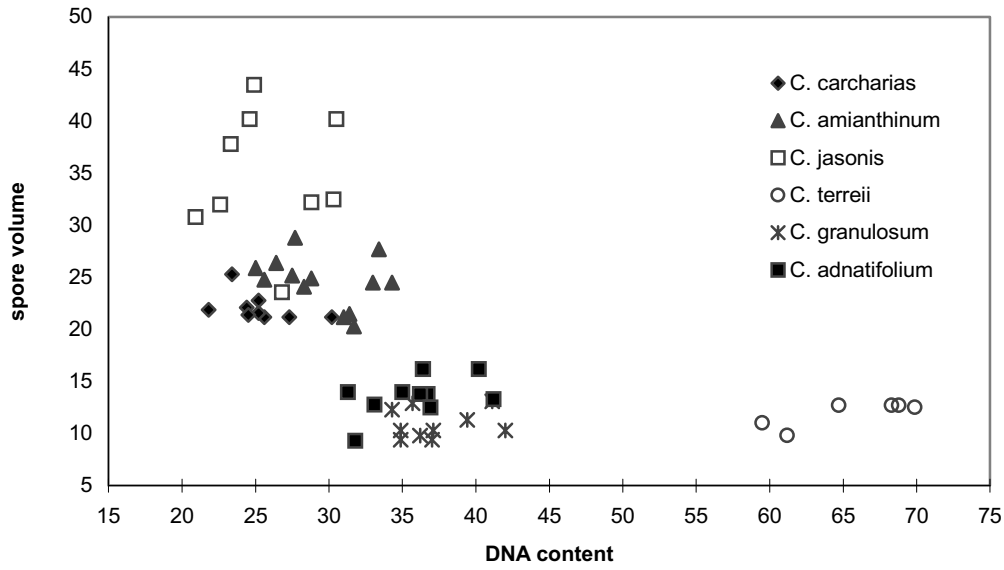



**Fig. 1.** Distribution of specimens' mean values of DNA content (Mb) and spore volume (µm$^3$) in species of the genus *Cystoderma*.

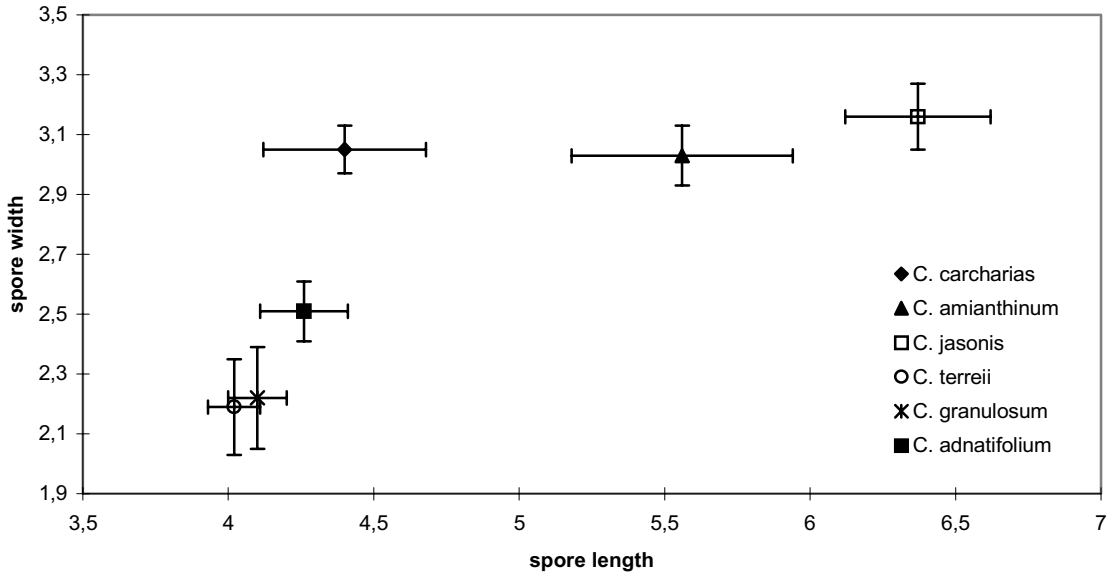



**Fig. 2.** Distribution of spore length and width values (µm) in the species of the genus *Cystoderma*. Standard error is given for the mean values of the species.

The second group includes the species *C. adnatifolium* & *C. granulosum*, which ploidy level is similar (3x) and DNA content is not significantly different (Table 2; Fig. 2). *C. adnatifolium* & *C. terreii* are morphologically more similar species, while *C. terreii* is distinct in having spear-like cystidia. *C. terreii* differs from the other species with respect to ploidy level (6x) and significantly different DNA content as well. Differences of mean spore length between species in the second group are statistically significant. At the same time, differences of mean spore width between the above species are not statistically significant between *C. granulosum* & *C. terreii* (Anova: Single Factor).

Correlation between DNA count and spore volumes (r=-0.58), length (r=-0.55) and width (r=-0.61) within genus is negative; it means that species with larger spores have smaller DNA content than species with smaller spores (Fig. 1).

The species of the genus *Cystoderma* are differentiated according to spore width and/or length, the differences of which are statistically significant between species, therefore these are good characters for differentiation of the species of this genus.

The DNA content of species *C. amianthinum* and *C. terreii* is statistically different from that of morphologically similar species; accordingly the species *C. amianthinum* differed from *C. jasonis* and *C. terreii* from *C. adnatifolium*. The difference between the species of the pair *C. adnatifolium* & *C. granulosum* as well as between the species of the pair *C. jasonis* & *C. carcharias* is not significant.

## ACKNOWLEDGEMENTS

This study was partially supported by the Estonian Science Foundation (grant no. 3580). The curators of the herbaria in the Botanical Museum of the University of Helsinki are thanked for arranging the loans of the specimens. Thank you to all those who collected specimens (P. Höijer, K. Kalamees, L. Kosonen, K. Leenurm, M. & M. Rantala, M. Vaasma et al). Many thanks to Ain Kaare and Jane Lehtmaa at the Clinic of Hematology and Oncology of the Tartu University Clinics for permission to use their flow cytometer, and also to Jevgeni Popov who assisted in the use of the flow cytometer at the Institute of Molecular and Cell Biology of the University of Tartu. We thank Ilmar Part for revising the English text.

## REFERENCES

Bennett, M. D. & Leitch, I. J. 1995. Nuclear DNA Amounts in Angiosperms. *Ann. Bot. (Oxford)* 76: 113–176.

Bennett, M. D. & Leitch, I. J. 1997. Nuclear DNA amounts in angiosperms – 583 new estimates. *Ann. Bot. (Oxford)* 80: 169–196.

Bennett, M. D. & Leitch, I. J. 1998. Nuclear DNA Amount and Genome Size in Angiosperms. *Ann. Bot. (Oxford)* 82: 1.

Bresinsky, A. & Wittmann-Meixner, B. 1995. Ploidy levels and evolution in *Boletales*. *Beih. Sydowia* 10: 35–47.

Bresinsky, A., Wittmann-Meixner, B., Weber, E. & Fischer, M. 1987. Karyologische Untersuchungen an Pilzen mittels Fluoreszenzmikroskopie. *Z. Mykol.* 53: 303–318.

Grant, V. 1984. *Plant Speciation*. Moskva. 616 p.

Peabody, D.C., Motta, J.J. & Therrien, C.D. 1978. Cytophotometric evidence for heteroploidy in the life cycle of *Armillaria mellea*. *Mycologia* 70: 487–498.

Rogers, J.D. 1973. Polyploidy in fungi. *Evolution* 27: 153–160.

Singer, R. 1986. *The Agaricales in Modern Taxonomy*. Koenigstein. 981 pp.

Smith, A.H. & Singer, R. 1945. A monograph on the genus *Cystoderma*. *Pap. Michigan Acad. Sci.* 30: 71–124.

Stebbins, G.L. 1971. *Chromosomal evolution in higher plants*. London. 216 pp.

Swift, H. 1950. The constancy of desoxyribose nucleic acid in plant nuclei. *Genetics* 36: 643–654.

Weber, E. & Bresinsky, A. 1992. Polyploidy in Discomycetes. *Persoonia* 14: 553–563.

Wittmann-Meixner, B. & Bresinsky, A. 1989. Quantitative differences of nuclear DNA contents in *Paxillaceae* compared with other families of *Boletales*. *Mycol. Res.* 93: 282–291.

Folia Cryptog. Estonica, Fasc. 36: 95–106 (2000)

# A DNA study of the *Cetraria aculeata* and *C. islandica* groups

**Arne Thell[1,2], Soili Stenroos[1] & Leena Myllys[1]**
[1]Herbarium, Department of Biology, University of Turku, FIN-20014 Turku, Finland
[2]Department of Systematic Botany, Lund University, 18–20 Östra Vallgatan, S-223 61 Lund, Sweden.
E-mail: Arne.Thell@sysbot.lu.se

**Abstract:** Group I intron (in nuclear SSU) and internal transcribed spacer (ITS) sequences of the *Cetraria aculeata* and *C. islandica* groups were compared using PAUP 4.0. ITS-sequences from the taxa *Cetraria islandica* ssp. *antarctica*, *C. islandica* ssp. *crispiformis*, *C. muricata* and *C. odontella*, are presented for the first time. The group I introns have a length of 218–224 nucleotides and occur in 11 of 20 studied samples of *Cetraria* s. str. Strict consensus trees based on the group I intron matrix, the ITS matrix and a combined data set were calculated and compared. *Cetraria odontella* forms a monophyletic line together with *C. aculeata* and *C. muricata*. Most species within the *Cetraria aculeata* and *C. islandica* groups are too closely related to be distinguished by group I intron or ITS sequences. *C. odontella* is reported from Australia for the first time.

**Kokkuvõte:** A. Thell, S. Stenroos & L. Myllys. *Cetraria aculeata* ja *C. islandica* rühmade DNA-st.

Võrreldakse *Cetraria aculeata* ja *C. islandica* rühmade I introni ja ITS sekventsidel põhinevaid fülogeneetilisi analüüse. Esmakordselt esitatakse *Cetraria islandica* ssp. *antarctica*, *C. islandica* ssp. *crispiformis*, *C. muricata* ja *C. odontella* ITS sekventsid. I intronite pikkus on 218–224 nukleotiidi ja nad esinevad 11-s *Cetraria* s. str. 20-st uuritud proovist. Esitatakse I introni ja ITS piirkonna andmemaatriksitel ning kombineeritud andmetel põhinevad ranged konsensuspuud, samuti nende võrdlus. *Cetraria odontella* moodustab monofüleetilise liini koos *C. aculeata* ja *C. muricata* eksemplaridega. Enamus *Cetraria aculeata* ja *C. islandica* rühmade liike on liiga lähedased I introni ja ITS sekventside alusel eristamiseks. Teatatakse *C. odontella* esmaleiust Austraaliast.

## INTRODUCTION

The major part of the 16 species within *Cetraria* Ach. s. str. (Parmeliaceae) could be divided into two closely related groups of hardly discernible species, centered on *Cetraria aculeata* (Schreb.) Fr. and *C. islandica* Ach. (Kärnefelt, 1979, 1986; Kärnefelt et al. 1993). *Cetraria aculeata*, *C. arenaria* Kärnefelt, *C. ericetorum* Opiz, *C. islandica*, *C. muricata* (Ach.) Eckfeldt and *C. odontella* Ach. are included in the study.

A group of four taxa, earlier comprising the genus *Coelocaulon* Link, were transferred to *Cetraria* by Kärnefelt et al. (1993). They differed from other *Cetraria* species by a fruticose habit with rounded, not dorsiventral lobes. However, one of the species, *C. aculeata*, occasionally has flattened branches similar to those found within the *C. islandica* group.

*Cetraria aculeata* and *C. muricata*, widespread in temperate and boreal regions of both hemispheres, are sometimes difficult to distinguish from each other. A typical *C. muricata* is a very small lichen with a shrubby habit, sparsely supplied with minute pseudocyphellae, which are hardly visible without a lens. Such specimens are easily distinguished from the usually larger and more loosely ramified *C. aculeata*. This species is, furthermore, frequently supplied with oblong and more pronounced pseudocyphellae (Kärnefelt, 1986).

*C. odontella* Ach., has a fragmented occurrence in the boreal part of the Northern Hemisphere. A report from Peru was the only known locality in the Southern Hemisphere until it recently was collected in Australia (Table 1). The lobes are usually flat, sometimes even slightly canaliculate, although the general habit – dense cushion-like tufts – is similar to *C. aculeata* or *C. muricata*. Yet, *C. odontella* is a distinct taxon, variable in minor characters only. The lobes are less flattened at high altitudes presumably caused by extreme conditions (Kärnefelt, 1986).

*Cetraria ericetorum* and *C. islandica*, two well-known and widely distributed species, have traditionally been considered very closely related. Formerly, the huge variation within *C. islandica* caused taxonomical overestimation of local morphotypes (Räsänen, 1943; Rassadina, 1945). Several of these were rejected in Kärnefelt's (1979) thorough study. *Cetraria ericetorum* is distinguished from *C. islandica* by the marginal pseudocyphellae, sometimes form-

**Table 1.** The 21 samples selected for this study
The sequences are available at the National Center for Biotechnology Information, NCBI, homepage: http://www.ncbi.nlm.nih.gov.

| Taxon, locality | Date | Collector, sample-ID | DNA# | Herb. | GeneBank accession |
|---|---|---|---|---|---|
| CETRARIA ACULEATA | | | | | |
| Canada. Nova Scotia, Shelburne Co. Tobeatic Wilderness Area | 1999-05-08 | T. Ahti 57221 | AT579 | TUR | AF228288 |
| Chile. Region XII de Magellanes y de la Ant. Chil., Prov. de Magellanes | 1999-02-06 | T. Feuerer 29320 | AT520 | HBG, TUR | AF228287 |
| Finland. Nylandia, Helsinki | 1999-11-15 | A. Thell FIN-9932 | AT703 | TUR | AF228289 |
| Germany. Niedersachsen, Landkreis Uelzen, Bullenberg D-9929 | 1999-09-25 | A. Thell & C. Marth | AT592 | TUR | AF228286 |
| CETRARIA ERICETORUM ssp. ERICETORUM | | | | | |
| Finland. Ostrobottnia kajanensis, Sotkamo, Vuokatti | 1999-08-04 | S. Stenroos 5173 | AT642 | TUR | AF228292 |
| Finland. Regio Aboënsis, Kaarina, Kuusisto | 1999-10-13 | A. Thell, S. Stenroos & A. Puolasma FIN-9937 | AT630 | TUR | AF228291 |
| Sweden. Scania, Åhus, Yngsjö SK-9928 | 1999-07-22 | A. Thell & C. Marth | AT544 | TUR | AF228290 |
| CETRARIA ISLANDICA ssp. ANTARCTICA | | | | | |
| New Zealand. South Island, Otago | 1999-02-07 | I. Kärnefelt 999201 | AT515 | TUR | AF228293 |
| CETRARIA ISLANDICA ssp. CRISPIFORMIS | | | | | |
| Finland. Nylandia, Helsinki, between Viikki and Herttoniemi | 1999-11-15 | A. Thell FIN-9930 | AT701 | TUR | AF228294 |
| Finland. Nylandia, Helsinki, Viikinmäki | 1999-11-15 | A. Thell FIN-9931 | AT702 | TUR | AF228295 |
| CETRARIA ISLANDICA ssp. ISLANDICA | | | | | |
| Estonia. Põlvamaa Co., Taevaskoja | 1999-09-06 | A. Thell EST-9901 | AT548 | TUR | AF228296 |
| Finland. Regio Aboënsis, Turku, Ruissalo | 1999-08-17 | A. Thell FIN-9925 | AT533 | TUR | AF228299 |
| Finland. Regio Aboënsis, Lieto Littoinen, broad-lobed form | 1999-08-21 | A. Thell FIN-9924 | AT532 | TUR | AF228298 |
| Finland. Regio Aboënsis, Lieto Littoinen, narrow-lobed form | 1999-08-21 | A. Thell FIN-9929 | AT546 | TUR | AF228300 |
| Sweden. Scania, Åhus, Yngsjö SK-9923 | 1999-07-22 | A. Thell & C. Marth | AT531 | TUR | AF228297 |
| CETRARIA MURICATA | | | | | |
| Canada. Newfoundland Ferryland Dist., Witless Bay Line | 1999-04-15 | T. Ahti & Scott 56948 | AT580 | TUR | AF228302 |
| Iceland. S Pingeyar sysla, Myvatn | 1997-08-11 | A. Thell ISL-9722 | AT547 | LD | AF228301 |
| CETRARIA ODONTELLA | | | | | |
| Australia. New South Wales Mount Kosuszko National Park | 1999-10-16 | S. Wall | AT704 | S. Wall | AF228285 |
| Finland. Ostrobottnia kajanensis, Sotkamo, Hiidenportti | 1999-08-04 | S. Stenroos 5174 | AT643 | TUR | AF228304 |
| Finland. Regio Aboënsis, Kaarina Kuusisto | 1999-10-13 | A. Thell, S. Stenroos & A. Puolasma FIN-9932 | AT628 | TUR | AF228303 |
| CETRARIELLA DELISEI | | | | | |
| Iceland. Nordur Mulasysla, Fljotsdalur | 1997-08-12 | A. Thell ISL-9713 | AT234 | LD | AF228305 |

ing a more or less continuous line near the margin of the lower surface and not spread over the thallus as in *C. islandica*. However, some material is difficult to determine either as *C. ericetorum* or *C. islandica*. Both these taxa contain a large infraspecific variation and seven subspecies were proposed by Kärnefelt (1979). None of the three subspecies of *C. ericetorum* overlap with each other geographically. *Cetraria ericetorum* ssp. *ericetorum* is a Eurasian taxon, ssp. *reticulata* is found in boreal North America whereas ssp. *patagonica*, not included here, is restricted to southern South America.

*Cetraria islandica* ssp. *islandica* includes an enormous variation in size and shape, although it is usually easily distinguished from ssp. *crispiformis* with which it grows side by side at some localities. They both have a circumpolar distribution in the northern continents. To separate *C. islandica* ssp. *crispiformis* from *C. ericetorum* ssp. *reticulata* is occasionally more problematic, in particular when laminal pseudocyphellae occur on the latter taxon (Kärnefelt, 1979). *Cetraria islandica* ssp. *antarctica* and ssp. *orientalis* are chemically and biogeographically distinct. The former is known from subantarctic and alpine parts of the Southern Hemisphere while the latter taxon, which was not included in this analysis, occurs in the Far East (Kärnefelt, 1979). *Cetraria arenaria* is a distinct lichen but closely allied to and separated from *C. islandica* by Kärnefelt (1979).

Although several unique, from *Cetraria* diverging characters, have been observed within the genus *Cetrariella*, a recently published study of the ITS regions revealed the two genera as closely related (Kärnefelt & Thell, 2000). A new sequence of the type species, *C. delisei*, was included in the analysis to represent a closely related genus.

The intention with this study is to compare ribosomal DNA sequences from the internal transcribed spacers, ITS, and group I introns in phylogenetic analyses to evaluate whether these parts of the DNA separate morphologically very similar, in some cases hardly distinguishable, species within the *Cetraria aculeata* and *C. islandica* groups.

This is a first report from a project carried out at the University of Turku, Finland, dealing with lichens and mosses with a bipolar distribution.

## MATERIAL AND METHODS

### I. Specimens included in the study

Group I intron and ITS sequences from eight taxa, *Cetraria aculeata*, *C. ericetorum* ssp. *ericetorum*, *C. islandica* ssp. *antarctica*, *C. islandica* ssp. *crispiformis*, *C. islandica* ssp. *islandica*, *C. muricata*, *C. odontella* and *Cetrariella delisei*, were produced from 21 specimens, collected for the herbaria H, HBG, LD, TU, TUR and the private herbarium of Staffan Wall (Table 1). Sequences from ten samples, available at the GenBank's homepage: http//:www.ncbi.nlm.nih.gov, were used to complement the new ones in the cladistic analyses (Table 2).

*Tuckermannopsis subalpina* was selected as outgroup. The taxonomic position of this species is, according to published investigations, clearly outside *Cetraria* s. str. (Kärnefelt, 1979; Kärnefelt et al., 1993; Thell, 1998). Furthermore, it contains a group I intron sequence that was easily alignable with the other intron sequences.

### II. Extraction

DNA was extracted from fresh material, not more than three years old, following the protocol enclosed in the DNEasy Plant Mini Kit for DNA isolation from plant tissue from QIAGEN (March, 1999). Minute fragments of thalli, approximately 2–3 mm in diameter, preferably lobe tips or, if present, apothecia, were ground carefully in 50 µl of the lysis buffer with pestles in 1.5 ml Eppendorf tubes. Different water-dilutions of the DNA-extractions were tested for PCR. Concentrated templates usually functioned as well as dilutions.

### III. Amplification and purification

The ITS regions, the 5.8S region and flanking parts of the small and large subunits (SSU, 18S and LSU, 28S) of the ribosomal DNA, including possible insertions at the 3'-end of the SSU, were amplified with a Perkin-Elmer Gene Amp PCR System 9700 thermal cycler. Ready To Go PCR beads (in 0.2 ml tubes) from Pharmacia Biotech Inc. were dissolved in 11.8 µl distilled water, 0.35 µl of a 16µM concentration of each of the primers ITS1F and ITS4 (Table 2) and 12.5 µl of the DNA extractions were added to

**Table 2.** Samples included in this study to complement the new sequences, available at the National Center for Biotechnology Information, NCBI, homepage: http://www.ncbi.nlm.nih.gov *TerraGen Diversity Inc., University of British Columbia

| GenBank accession | Taxon | Country; DNA #; herbarium | Reference |
|---|---|---|---|
| AF192409 | Cetraria aculeata | Sweden; AT11; LD | Thell & Miao (1998) |
| AF116176 | C. aculeata | Canada; AT156; TDI* | Thell & Miao (1998) |
| AF115758 | C. arenaria | Canada; AT173; TDI* | Thell (1999) |
| AF139033 | C. ericetorum ssp. e. | Finland; AT212; LD | Thell & Miao (1998) |
| AF139034 | C. ericetorum ssp. r. | Canada; AT44; LD | Thell & Miao (1998) |
| AF152466 | C. ericetorum ssp. r. | Canada; AT44; LD | Thell & Miao (1998) |
| AF139035 | C. islandica ssp. i. | Sweden; AT41; LD | Thell & Miao (1998) |
| AF072225 | C. islandica ssp. i. | Canada; AT93; TDI* | Thell & Miao (1998) |
| AF152467 | C. islandica ssp. i. | Iceland; AT94; LD | Thell & Miao (1998) |
| AF072237 | Tuckermannopsis subalpina | Canada; AT109; LD | Thell (1998) |

**Table 3.** Primers used in the analysis. *Fungal specific

| Primer | Reading direction | Part of the rDNA amplified | Primer sequence | Reference |
|---|---|---|---|---|
| ITS1-F* | 5' to 3' | Group I intron, (ITS) | CTTGTTCATTTAGAGGAAGTAA | Gardes & Bruns (1993) |
| ITS1-LM | 5' to 3' | ITS | GAACCTGCGGAAGGATCATT | Myllys et al. (1999) |
| ITS2-LM | 3' to 5' | Group I intron | AATGATCCTTCCGCAGGTTC | Lohtander et al. (in prep.) |
| ITS2-KL | 3' to 5' | ITS, (Gr. I intron) | ATGCTTAAGTTCAGCGGGTA | Lohtander et al. (1998) |
| ITS4 | 3' to 5' | ITS, (Gr. I intron) | TCCTCCGCTTATTGATATGC | White et al. (1990) |
| ITS5 | 5' to 3' | Gr. I intron, (ITS) | GGAAGTAAAAGTCGTAACAAGG | White et al. (1990) |

**Table 4.** Frequency of group I introns and number of nucleotides of the amplified regions having informative characters of *Cetraria* s. str.
*Including the samples listed in Table 1.

| Taxon | number of samples* | Gr. I intr. presence | Gr. I intr. length | ITS 1 length | ITS 2 length |
|---|---|---|---|---|---|
| Cetraria aculeata | 6 | - | - | 180–184 | 151–154 |
| C. arenaria | 1 | 1 | 219 | 182 | 152 |
| C. ericetorum | 6 | 3 | 219 | 181–183 | 152 |
| ssp. ericetorum | 4 | 2 | 219 | 181–182 | 152 |
| ssp reticulata | 2 | 1 | 219 | 182–183 | 152 |
| Cetraria islandica | 11 | 9 | 218–224 | 182 | 152 |
| ssp. antarctica | 1 | 1 | 224 | 182 | 152 |
| ssp. crispiformis | 2 | 2 | 219 | 182 | 152 |
| ssp. islandica | 8 | 6 | 218–220 | 182 | 152–153 |
| Cetraria muricata | 2 | - | - | 182 | 153–157 |
| C. odontella | 3 | 2 | 218 | 182 | 151 |
| In total | 29 | 16 | 217–224 | 180–184 | 151–157 |

the solution, resulting in final reaction volumes of ca. 25 µl. Each reaction will then, according to the enclosed component declaration, contain about 1.5 units of Taq DNA Polymerase, 10 mM Tris-HCl (pH 9.0 at room temperature), 50 mM KCl, 1.5 mM $MgCl_2$ and 200 µM of each dNTP.

The PCR started with 2 minutes at 95°C. Then a 30 cycle schedule followed using a denaturation temperature of 95°C for 1 min., an annealing temperature of 60°C for 1 min., and an extension temperature of 72°C for 1 min.

The PCR products were cleaned with QIAquick PCR purification kit from QIAGEN and diluted into 30 µl of the enclosed elution buffer (10 mM Tris-Cl, pH 8.5).

### IV. Sequencing

A 25 cycle sequencing PCR with a denaturation temperature of 96°C for 10 seconds, an annealing temperature of 50°C for 5 seconds, and an extension time of 60°C for 4 minutes was performed to amplify the DNA-fragments prior to the sequencing procedure. Deionized water, primers and template were added to BigDye Terminator Cycle Sequencing Ready Reaction Kit from Perkin Elmer according to the enclosed protocol (PE Applied Biosystems 1998).

Several different primer pairs were used for the sequencing PCR, depending on which product was desired (Table 3). If a sample did not contain an intron, the following alternative 5'-primers were used: ITS1-F, ITS1-LM or ITS5. These primers were combined with the 3'-primers ITS2-KL or ITS4. However, if the sample contained insertions at the rear end of the SSU, ITS1-LM was used together with either ITS2-KL or ITS4 to amplify only the ITS; for the intron ITS2-LM was used together with either ITS1F or ITS5. All primers worked equally successful in this study (Tables 3–4).

The sequences were produced using an automatic sequencer, ABI Prism 377 from Perkin-Elmer.

### V. Alignment and phylogenetic analysis

The sequences were easily aligned manually and a cladistic analysis was performed using the program PAUP 4.02b (Swofford, 1998). Gaps were treated as missing characters. The most parsimonious trees were obtained by using the heuristic search option with a maximum of 1000 random replicates. Starting trees were formed by stepwise addition. The swapping algorithm used was the tree-bisection-reconnection (TBR). Strict consensus trees from three separate data sets were calculated: one tree based on ITS data from all the samples (Tables 1–2), a second tree of taxa having group I intron sequences exclusively and, finally, one tree based on a combined ITS-group I intron data matrix. Bootstrap analyses using the heuristic search option and the TBR-algorithm in PAUP 4.02b were performed. Branch support values that exceed 50 (from 1000 replicates) are indicated at the nodes of the consensus trees (Tables 5–7, Figs. 1–3).

Congruence between different data sets (intron versus ITS data) was tested. Rather than comparing only the cladograms resulting from different data sets we wanted to use an approach that enables the comparison of the actual data sets. This was done using the beta version of the program KON and following the principles presented in Farris et al. (1994) and applied for example in Bruneau et al. (1995).

## RESULTS AND DISCUSSION

### I. The amplified genes and their variation

Group I intron and ITS sequences of the ribosomal DNA of four taxa, *C. islandica* ssp. *antarctica*, *C. islandica* ssp. *crispiformis*, *Cetraria muricata*, and *C. odontella*, are presented for the first time (Tables 5–7).

The group I introns are 218–224 nucleotides long and located between the positions 1516 and 1517 close to the 3' end of the SSU relative to *Escherichia coli* (DePriest & Been, 1992; Gargas et al., 1995; Gutell et al., 1994). They were easily aligned and thus presumably homologous. Introns occurred in 11 of the 20 investigated samples of *Cetraria* s. str., very frequent in species belonging to the *C. islandica* group but rare or absent in other taxa (Table 4).

Informative characters were restricted to the group I introns and the internal transcribed spacers, ITS 1 and ITS 2 (Tables 5–7), the ITS parts, measuring 180–184 and 151–157 nucleotides respectively. The ITS matrix con-

**Table 5.** Sequences and alignments from the group I introns of the new samples

```
                                5'       10          20          30          40          50
704 Cetraria odontella          GAATGGC-TT GCCTCCGGAA CGCCCCAGCA GCGACTCTAA ACAACTGCAC TAGTCGC
622/643 C. odontella            ......-T.. .......... .......... .......... .......... .......
544 C. eric. ssp. ericet.       ......GT.. .......... .......... .......... .........T .......
515 C. isl. ssp. antarctica     ......-T.. .......... .......... .......... .........T .......
701/702 C. isl. ssp. crisp.     ......-T.. .......... .......... .......... .........T .......
531 C. isl. ssp. islandica      ......-T.. .......... .......... .......... .........T .......
532 C. isl. ssp. islandica      ......GT.. .......... .......... .......... .T.......T .......
533 C. isl. ssp. islandica      ....A.-T.. .......... .......... ......T... .........T .......
548 C. isl. ssp. islandica      ......GT.. .......... .......... .......... .........T .......

                                60          70          80          90          100         110
704 Cetraria odontella          CCT T-AGCGGGCT GGCAACGCCG TCACTGTGCG CCGGG-AGTC CTCTGAAGTC GGG
622/643 C. odontella            ... .......... .......... .......... .......... .......... ...
544 C. eric. ssp. ericet.       ..- .G........ .......... .......... ....-A.... .C.....C.. ...
515 C. isl. ssp. antarctica     ..- .G......T. .......... .......... ....-A.... .C.....C.. ...
701/702 C. isl. ssp. crisp.     ..- .G........ .......... .......... ....AA.... .C.C...C.. ...
531 C. isl. ssp. islandica      ..- .G........ .......... .......... ....-A.... .C.....C.. ...
532 C. isl. ssp. islandica      ... .G........ .......... .......... ....-A.... .C....GC.. ...
533 C. isl. ssp. islandica      ..- .G........ .......... .......... ....-A.... .C.....C.. ...
548 C. isl. ssp. islandica      ..- .G........ .......... .......... ....AA.... .C.C...C.. ...

                                120         130         140         150         160         170
704 Cetraria odontella          GTCAACC AGCAGCTTCA GCCTTTT-GG AGTTCACAGA TCAAACGATA GCGGCCAC--
622/643 C. odontella            ....... .......... .......... .......... .......... ..........
544 C. eric. ssp. ericet.       ....... .......C.. ..T....... .......... .......... ..........
515 C. isl. ssp. antarctica     ....... .......C.. .TT....T.. .......... .......... ........TT
701/702 C. isl. ssp. crisp.     ....... G......C.. ..T....... .......... .......... ..........
531 C. isl. ssp. islandica      .T..... G......C.. ..T....... .......... .......... ..........
532 C. isl. ssp. islandica      ....... .......C.. ..T....... .......... .......... ..........
533 C. isl. ssp. islandica      ....... .......C.. ..T....... .......... .......... ..........
548 C. isl. ssp. islandica      ....... .......C.. ..T....... .......... .......... ..........

                                   180         190         200         210         220      3'
704 Cetraria odontella          ----TTCGGT GGTTGAGATA TGAACGGCCC CCGCTGTTAC CAGCTGGAGA CTTTGCG
622/643 C. odontella            .......... .......... .......... .......... .......... .......
544 C. eric. ssp. ericet.       ---....... .......... .......... .T........ .......... .......
515 C. isl. ssp. antarctica     CGAC...... .......... .......... .T........ .......... .......
701/702 C. isl. ssp. crisp.     .......... .......... .......... .T........ .......... .......
531 C. isl. ssp. islandica      .......C.. .......... .......... .T........ .......... .......
532 C. isl. ssp. islandica      .......... .......... .......... .T........ .......... .......
533 C. isl. ssp. islandica      .......... .......... .......... .T........ .......... .......
548 C. isl. ssp. islandica      .......... .......... .......... .T........ .......... .......
```

**Table 6.** Sequences and alignments from the ITS 1 region of the new samples

```
                                5'       10          20          30          40          50          60
520 Cetraria aculeata           CTGAGAGAGG GGCTTCGCGC TCTCGGGGGT CTCGGCCCCT AACTCTTCAC CCTTTGTGTA CC
579 C. aculeata                 .......... .......... ..C....... .......... .......... .......... ..
592 C. aculeata                 .......... .......... ..C......C .........C T.......C. .......... ..
703 C. aculeata                 .......... .......... ..C......C .........C T.......C. .......... ..
544 C. eric. ssp. eric.         .......... .......... ..C....... .......... .......... .......... ..
```

```
                          5'        10        20        30        40        50        60
630/642 C. e. ssp. e.     .......... .......... ..C....... .......... .......... .......... ..
515 C. isl. ssp. antarc.  .......... .......... ..CT...... .......... .......... .......... ..
701 C. isl. ssp. crisp.   .......... .......... ..C....... .......... .......... .......... ..
702 C. isl. ssp. crisp.   .......... .......... ..C....... .......... .......... ..C....... ..
531 C. isl. ssp. isl.     .......... .......... ..C....... .......... .......... .......... ..
532 C. isl. ssp. isl.     .......... .......... ..C....... ...A...... .......... .......... ..
533 C. isl. ssp. isl.     .......... .......... ..C....... .......... .......... .......... ..
546 C. isl. ssp. isl.     .......... .......... ..C....... .......... .......... .......... T.
548 C. isl. ssp. isl.     .......... .......... ..C....... .......... .......... .......... ..
547 C. muricata           .......... .......... ..C....... .........C .......... .......... ..
580 C. muricata           .......... .......... ..C....... .........C .......... .......... ..
628/643 C. odontella      .......... .......... ..C....... .......T.. .......... ......C... ..
704 C. odontella          .......... .......... ..C....... .......T.. .......... ......C... ..
234 Cetrariella delisei   .......... .......... ..C......C T......... .......... ..A....C.. T.

                          70        80        90        100       110       120
520 Cetraria aculeata     CACCTTTG TTGCTTTGGC GGGCCC-GAG GACCTCTCGC GCCGTGT-AC AAACCGGCGA GCGC
579 C. aculeata           ........ .......... .......... .......... .......... .......... ....
592 C. aculeata           ........ .......... .......... ....C..... ....C..... .......... ....
703 C. aculeata           ........ .......... .......... ....C..... ....C..... .......... ....
544 C. eric. ssp. eric.   ........ .......... ...T...... .......... ....CC-C.. .GG....... ....
630/642 C. e. ssp. e.     ........ .......... ...T...... .......... ....CC-C.. .GG....... ....
515 C. isl. ssp. ant.     ........ .......... ...T...... .......... ....CC-CC. .GG....... ....
701 C. isl. ssp. crisp.   ........ .......... ...T...... .......... ....CC-C.. .GG....... ....
702 C. isl. ssp. crisp.   ........ .......... ...T...... .......... ....CC-C.. .GG....... ....
531 C. isl. ssp. isl.     ........ .......... ...T...... .......... ....CC-C.. .CG....... ....
532 C. isl. ssp. isl.     ........ .......... ...T...... .......... ....CC-CG. .GG....... ....
533 C. isl. ssp. isl.     ........ .......... ...T...... .......... ....CC-CC. .GG....... ....
546 C. isl. ssp. isl.     ........ .......... ...T...... .......... ....CC-C.. .GG....... ....
548 C. isl. ssp. isl.     .....C.. .......... ...T...... .......... ....CC-C.. .GG....... ....
547 C. muricata           ........ .......... .......... ......C... ....CA.... .......... ....
580 C. muricata           ........ .......... .......... ......C... ....CA.... .......... ....
628/643 C. odontella      ........ .......... .......... .......... ....C..... .......... ....
704 C. odontella          ........ .......... .......... .......... .......... .......... ....
234 Cetrariella delisei   ........ .......... ...T..T... .......... ....GCGC.. CCG....... .T..

                          130       140       150       160       170       180       3'
520 Cetraria aculeata     CCGCCA GAGGCCCATT AAAACCTGCT TATTAGTGAT GTCCGAGCGA AAAAAACACA ATGAAT
579 C. aculeata           ...... .......... .......... .......... .......... ....-A.... ......
592 C. aculeata           ...... .......... ....T..... .......... .......... ....--.... ..C...
703 C. aculeata           ...... .......... ....T..... .......... .......... ....--.... ..C.T.
544 C. eric. spp. eric.   ...... .......... ....T...A. .......... .......... ....--.... ..A...
630/642 C. e. ssp. e.     ...... .......... .......... .......... .......... ....--.... ..A...
515 C. isl. ssp. antarc.  ...... .......... ....T..... .......... .......... ....--.... ..A...
701 C. isl. ssp. crisp.   ...... .......... ....T..... .......... .......... ....--.... ..A...
702 C. isl. ssp. crisp.   ...... .......... ....T..... .......... .......... ....--.... ..A...
531 C. isl. ssp. isl.     ...... .......... ....T..... .........C .......... ....--.... ..A...
532 C. isl ssp. isl.      ...... .......... ....T..... .......... .......... ....--.... ..A...
533 C. isl. ssp. isl.     ...... .......... ....T..... .......... .......... ....--.... ..A...
546 C. isl. ssp. isl.     ...... .......... ....T....C ...C...... .......... ....--.... ..A...
548 C. isl. ssp. isl.     ...... .......... ....T..... .......... .......... ....--.... ..A...
547 C. muricata           ...T.. .......... ....T..... ...C...... .......... ....--.... ..C...
580 C. muricata           ...T.. .......... ....T..... ...C...... .......... ....--.... ..C...
628/643 C. odontella      ...... .......... ....T..... .......... .......T.. ....--.... ..A...
704 C. odontella          ...... .......... ....T..... .......... .......T.. ....--.... ..A...
234 Cetrariella delisei   ...... .......... ....T..A.. .......-.. .......T.. ....--.--. ..A.T.
```

**Table. 7.** Sequences and alignments from the ITS 2 gene of the new samples

| | 10 | 20 | 30 | 40 | 50 | 60 | 70 | 80 |
|---|---|---|---|---|---|---|---|---|
| 520 aculeata | ATA-CCCCTC | AAGCGTAGCT | TGGTATTGGG | TCTCGCTCCC | -GTGGCGTGC | CCGAAAAACA | GTGGCGGTCC | GGGGCGACTT |
| 579 aculeata | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| 592 aculeata | ..... | ..... | ..... | .....C... | ..... | .....G.. | ..... | ..... |
| 703 aculeata | ..... | ..... | ..... | .....C... | ..... | .....G.. | ..... | ..... |
| 544 eri. eri. | ..... | ..... | ....G.... | C.....C... | ..... | .....T.. | ..... | ..... |
| 630/642 e. e. | .....T.. | ..... | ..... | C.....C... | ..... | .....G.. | ..... | ..... |
| 515 isl. ant. | ..... | ..... | ..... | CG....C... | ..... | .....G.. | ..... | ..... |
| 701/702 i. cr. | ..... | ..... | ..... | C.....C... | ..... | .....G.. | ..... | ....T..... |
| 531 isl. isl. | ..... | ..... | ..... | C.....C... | ..... | .....G.. | ..... | ..... |
| 532 isl. isl. | ..... | ..... | ..... | C.....C... | ..... | .....G.. | ..... | ..... |
| 533 isl. isl. | ..... | ..... | ..... | C.....C... | ..... | .....G.. | ..... | ..... |
| 546 isl. isl. | ..... | ..... | ..... | C.....C... | C..... | .....G.. | ..... | ..... |
| 548 isl. isl. | ..... | ..... | ..... | C.....C... | ..... | .....G.. | A...A..... | ....T..... |
| 547 muricata | ..... | ..... | ..... | .....C... | ........A. | .....GT. | ..... | ..... |
| 580 muricata | ..... | ..... | ..... | .....C... | ........A. | .....GT. | ..... | ..... |
| 628/643 odon. | ..... | ..... | ..... | .....C... | ..... | .....G.. | ..... | ...-..... |
| 704 odontella | .A..... | ..... | ..... | .....C... | ..... | .....G.. | ..... | ...-..... |
| 234 delisei | ..... | ..... | ..... | C.....C... | -..... | .....G.. | ..... | ..T..... |

| | 90 | 100 | 110 | 120 | 130 | 140 | 150 | 160 |
|---|---|---|---|---|---|---|---|---|
| 520 aculeata | TAAGCGTAGT | AAAA-CCAAT | CCCGCTTTGA | AAGTTCGCGT | CGTGGCCGGC | CAGACAACCC | C----GTACA | TTTCAAACCA |
| 579 aculeata | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| 592 aculeata | ..... | ....A..... | ..... | ..... | ..... | ....T..... | ..... | .....T.. |
| 703 aculeata | ..... | ....A..... | ..... | ..... | ..... | ....T..... | ..... | .....T.. |
| 544 eri. eri. | ..... | .....--C.. | ..... | .....C. | .....T.. | ..... | ..... | .....T.. |
| 630/642 e. e. | .....C... | ....T--T.. | ..... | .....T. | .....T.T | ....T..... | ..... | .....T.. |
| 515 isl. ant. | ..... | ....T--T.. | ..... | .....C. | .....T.. | .....T.. | ..... | ..... |
| 701/702 i. cr. | ..... | ....T--T.. | ..... | .....C. | .....T.. | ..... | ..... | .....T.. |
| 531 isl. isl. | ..... | ....T--T.. | ..... | .....C. | .....T.. | ..... | ..... | .....T.. |
| 532 isl. isl. | ..... | ....T--T.. | ..... | .....C. | .....T.. | .....T.. | ..... | .....T.. |
| 533 isl. isl. | ..... | ....T--T.. | ..... | .....C. | .....T.. | ..... | ..... | .....T.. |
| 546 isl. isl. | ..... | ....T--T.. | ..... | .....C. | .....T.. | ..... | ..... | .....T.. |
| 548 isl. isl. | ..... | ....T--T.. | ..... | .....C. | .....T.. | ..... | ..... | .....T.. |
| 547 muricata | ..... | ....T.-... | ..... | .....A. | ..... | ..... | .GTAC..... | .....T.. |
| 580 muricata | ..... | ....T.-... | ..... | .....A. | ..... | ..... | ..... | .....T.. |
| 628/643 odon. | ..... | ....T.--.. | ..... | .....T. | ..... | ..... | ..... | .....T.. |
| 704 odontella | ..... | ....T.--.. | ..... | .....T. | ..... | ..... | ..... | .....T.. |
| 234 delisei | ..... | ....T.--.. | ...A..... | ...A....C. | ..... | ..... | .ACAT----. | ..C....T.. |

tains 13% informative characters compared with 5% for the group I introns (Tables 5–7). The additional fragments amplified by the primers ITS1F and ITS4; the 3' end of the small subunit (18S), the 5.8S gene, and the first nucleotides of the large subunit (28S); did not contain any variation and were therefore excluded from the phylogeny analyses.

## II. Phylogeny

Strict consensus trees of 28 samples based on group I intron and ITS sequences separately were in the present study calculated and compared in PAUP 4.02b. A combined analysis based on both matrices was finally performed. Support values exceeding 50 (from 1000 replicates) based on bootstrap analyses were added to the strict consensus trees (Figs. 1–3).

Tree length for the intron tree=51, RI=1.0000 and CI=1.0000. The tree based on ITS characters has a tree length of 118, RI=0.8731 and CI=0.8559 and for the combined matrix we obtained a tree length of 175, RI=0.8750 and CI=0.8914.

Two of the high bootstrap support values are recognized in all three consensus trees: (1) the genera *Cetraria* and *Cetrariella* are distinguished from each other and (2) a *C. aculeata*

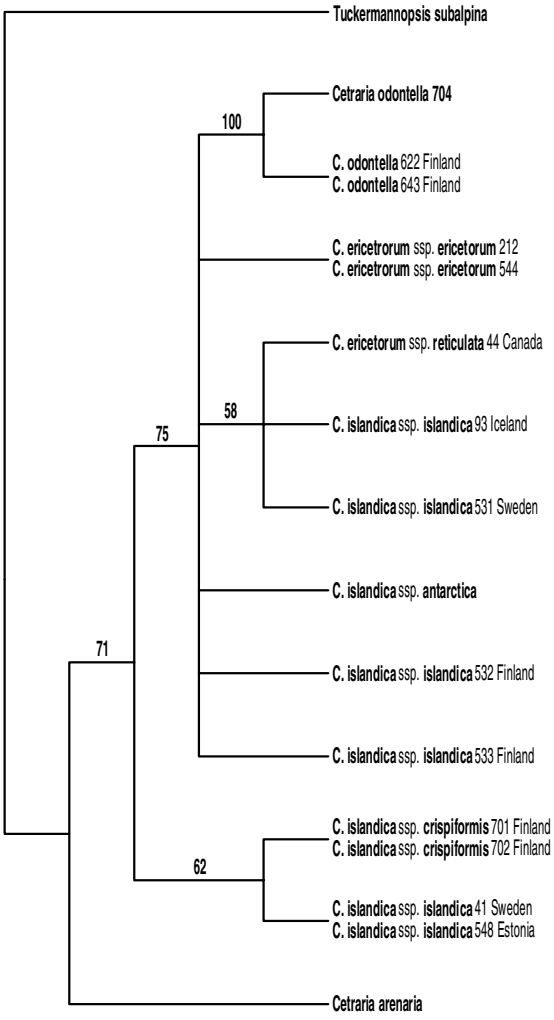



**Fig. 1.** Phylogeny of *Cetraria* s. str. based on group I intron sequences from table 5. Strict consensus tree based on 541 most parsimonious trees. Support values that exceed 50 based on bootstrap analysis are indicated.

species group, including *C. aculeata*, *C. muricata* and *C. odontella*, is recognized within *Cetraria*.

An interesting paraphyletic pattern for *C. aculeata* is strongly supported by the bootstrap analyses of the ITS data. The samples are divided among two groups according to the geographic origin. The European samples (DNA#AT11, 192, 592 and 703) form a clade together with the two *C. muricata* samples. The American material of *C. aculeata*, collected in Chile (DNA#AT520) and eastern and western North America (DNA#AT156, 579), interestingly form a clade together supported by a bootstrap value of 100. *Cetraria odontella* was never included in *Coelocaulon* and a very close relationship with *C. aculeata* and *C. muricata* was not expected (Figs. 2–3).

The strict consensus tree based on the combined matrix, calculated from 1000 random replicates, is leaving *C. ericetorum* as a paraphyletic and *C. islandica* as a polyphyletic taxon.

Congruence between the intron and ITS data was tested, using the program KON (Farris et al., 1994). According to the test data sets are totally congruent.

### III. Taxonomical conclusions

Sequences from different parts of the ribosomal DNA have provided congruent results in multigene studies. Data from the small subunit, group I introns and ITS were successfully combined within the families Parmeliaceae and Roccellaceae (Mattsson & Wedin, 1998; Myllys et al., 1999a, 1999b; Wedin et al., 1999). However, group I introns might be transposed between different positions in the rDNA (Thell, 1999). The applicability of group I introns is still very little investigated. Phylogeny comparisons between DNA-data and morphology and anatomy have, apart from multi-gene studies, provided incongruent information at family level (Myllys et al 1999b; Mattsson et al. in press).

In earlier molecular studies of cetrarioid lichens ITS sequences have been useful for distinguishing species regardless of their geographic origin of the samples (Thell & Miao, 1998). One single exception among cetrarioid lichens outside *Cetraria* s. str. had been detected until now. Two morphologically slightly overlapping species, *Platismatia herrei* (Imshaug) W. L. Culb. & C. F. Culb. and *P. stenophylla* (Tuck.) W. L. Culb. & C. F. Culb., contained minute variation within the ITS regions and did not appear as separate species in a cladistic analysis (Thell et al., 1998). The infraspecific variation of the ITS of *C. aculeata*, *C. ericetorum*, and *C. islandica* is, on the other hand, larger than what is usually observed in the Parmeliaceae. However, there are still no

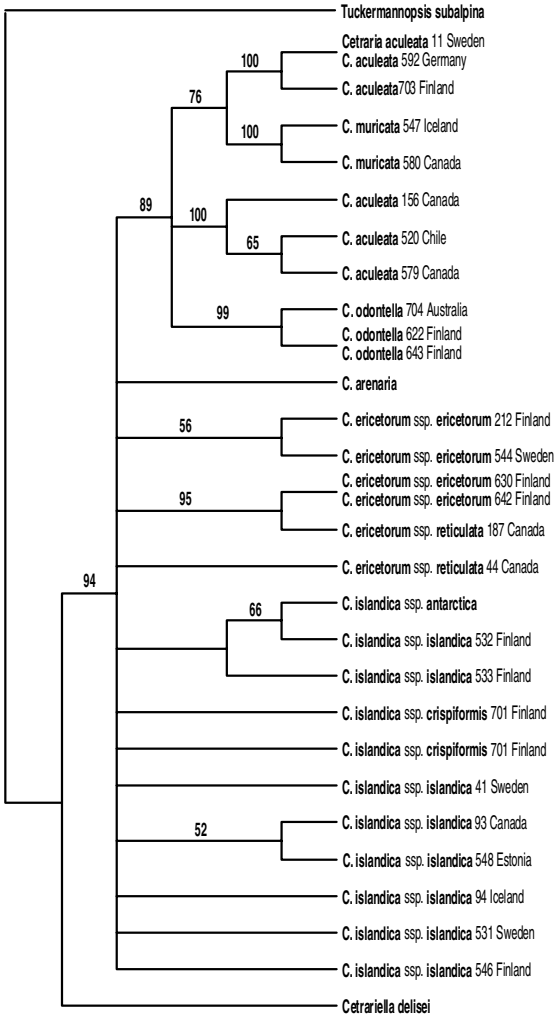



**Fig. 2.** Phylogeny of *Cetraria* s. str. based on internal transcribed spacer (ITS) sequences from tables 6–7. Strict consensus tree based on 1000 random replicates. Support values that exceed 50 based on a bootstrap analysis are indicated.

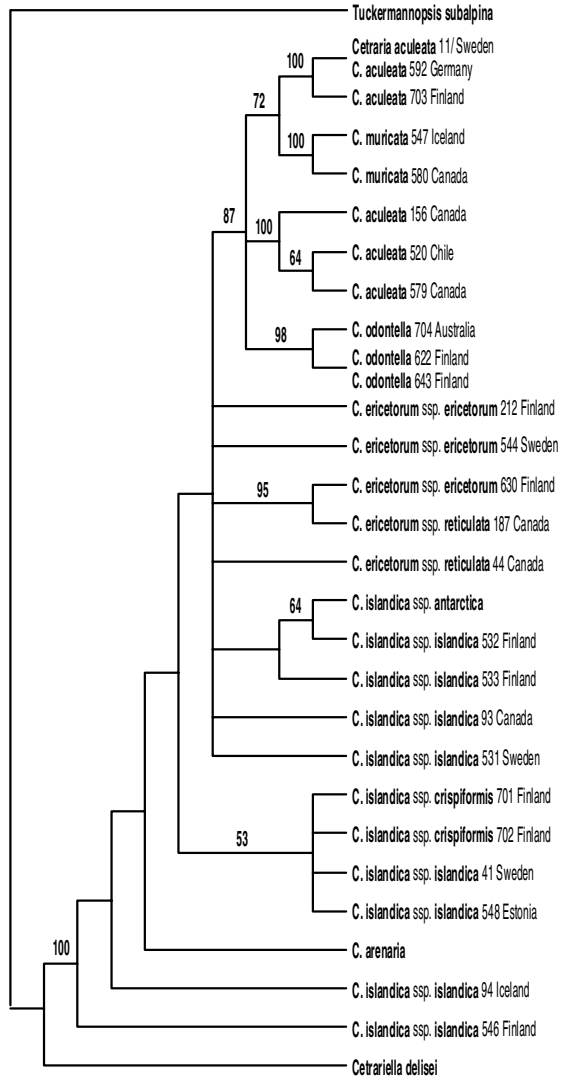



**Fig. 3.** Phylogeny of *Cetraria* s. str. based on a combined matrix of both group I intron and ITS sequences from tables 5–7. Strict consensus tree based on 1000 random replicates. Support values that exceed 50 based on a bootstrap analysis are indicated.

species typical sequences for *C. aculeata*, *C. ericetorum* or *C. islandica* and it is not evident whether there is a very close relationship between the species or if the poor separation is a result of a fast evolution at certain positions of the ITS. If *Cetraria aculeata* and *C. muricata* and the *C. islandica* group actually represent species groups or single species can not be decided on the basis of these results.

**ACKNOWLEDGEMENTS**

Many thanks to Teuvo Ahti, Tassilo Feuerer, Ingvar Kärnefelt and Staffan Wall for supplying us with fresh material from the Southern Hemisphere and North America. I. Kärnefelt is furthermore acknowledged for determining some of the specimens.

The authors thank Jaakko Hyvönen, who was involved in the data analyses with PAUP 4.0., and Steve Farris for placing the beta version of the program KON at our disposal.

The work was supported financially by a grant (no. 44079) from the Academy of Finland. Expenses to participate the 15th symposium of Baltic mycologists and lichenologists in Estonia were covered by the Botanical Society in Lund.

## REFERENCES

Bruneau, A. & Dickson, E. E. & Knapp, S. 1995. Congruence of chloroplast DNA restriction site characters with morphological and isozyme data in Solanum sect. Lasciocarpa. *Can. J. Bot.* 73: 1151–1167.

DePriest, P. T. & Been, M. D. 1992. Numerous group I introns with variable distribution in the ribosomal DNA of a lichen fungus. *J. Mol. Biol.* 228: 315–321.

Farris, J. S., Källersjö, M, Kluge, A. G. & Bult, C. 1994. Testing significance of incongruence. *Cladistics* 10: 315–319.

Gardes, M., & Bruns, T. D. 1993. ITS primers with enhanced specificity for basidiomycetes – Application to the identification of mycorrhizae and rusts. *Mol. Ecol.* 2: 113–118.

Gargas, A., DePriest, P. T. & Taylor, J. W. 1995. Positions of multiple insertions in SSU rDNA of lichen-forming fungi. *Mol. Biol. Evol.* 12: 208–218.

Gutell, R. R., Larsen, N. & Woese, C. R. 1994. Lessons from an evolving rRNA: 16S and 23S rRNA structures from a comparative perspective. *Microbiol. Rev.* 58: 10–26.

Kärnefelt, I. 1979. The brown fruticose species of *Cetraria*. *Opera Bot.* 46, 150 pp.

Kärnefelt, I. 1986. The genera *Bryocaulon*, *Coelocaulon* and *Cornicularia* and formerly associated taxa. *Opera Bot.* 86, pp.

Kärnefelt, I. & Thell, A. 2000. On the systematic position of the genus *Cetrariella* (Parmeliaceae: Ascomycotina) indicated by ITS rDNA data. In Contributions to cryptogamic botany. In *Honour of Ludger Kappen* (eds B. Schroeter, B., Schlensog, M. & Green, T. G. A.). *Bibl. Lichenol.* XX: xx–xx. J. Kramer in der Gebrüder Bortnträger Verlagsbuchhandlung, Berlin-Stuttgart, Germany (in press).

Kärnefelt, I., Mattsson, J.-E. & Thell, A. 1993. The lichen genera *Arctocetraria*, *Cetraria* and *Cetrariella* (Parmeliaceae) and their presumed evolutionary affinities. *Bryologist* 96: 394–404.

Lohtander, K., Myllys, L., Sundin, R., Källersjö, M. & Tehler, A. 1998. The species pair concept in the lichen *Dendrographa leucophaea* (Arthoniales): Analyses based on ITS sequences. *Bryologist* 101: 404–411.

Lohtander, K., Myllys, L., Källersjö, M., Moberg, R. & Tehler, A. 2000. The genus *Physcia*, a study based on ITS and group I intron sequences (in prep.).

Mattsson, J.-E. & Wedin, M. 1998. Phylogeny of the Parmeliaceae – DNA data versus morphological data. *Lichenologist* 30: 463–472.

Mattsson, J.-E., Thell, A. & Wedin, M. 2000. The importance of ascus structures when identifying monophyletic groups within the Parmeliaceae – an evaluation based on DNA data. *Mycologia* (in press).

Myllys, L., Lohtander, K., Källersjö, M & Tehler, A. 1999a. Sequence insertions and ITS data provide congruent information on *Roccella canariensis* and *R. tuberculata* (Arthoniales, Euascomycetes) phylogeny. *Mol. Phylogen. Evol.* 12: 295–309.

Myllys, L., Lohtander, K., Källersjö, M. & Tehler, A. 1999b. Applicability of ITS data in Roccellaceae (Arthoniales, Euascomycetes) phylogeny. *Lichenologist* 31: 461–476.

Räsänen, V. 1943. Petsamon Jäkäläkasvisto. Lisiä Fennoskandian arktisen alueen jäkäläkasviston tuntemiseen. Die Flechtenflora von Petsamo. Ein Beitrag zur Kenntnis der Flechtenflora des arktischen Gebietes in Fennoskandien. *Ann. Bot. Soc. Zool.-Bot. Fenn. Vanamo* 18 (1).

Rassadina, K. A. (1945). De specie nova et formis novis Cetrariae. *Bot. Materialy, Notul. System. Sect. Crypt. Inst. Bot. Nomine V. L. Komarovii Acad. Sci. URSS* 5: 133–137.

Swofford, D.L. 1998: *PAUP: Phylogenetic analysis using parsimony and other methods. Version 4.0 beta version.* Laboratory of Molecular Systematics. Smithsonian Institution, (preliminary version) 130 pp.

Thell, A. 1998. Phylogenetic relationships of some cetrarioid species in British Columbia with notes on *Tuckermannopsis*. *Folia Cryptog. Estonica* 32: 113–122.

Thell, A. 1999. Group I introns versus ITS sequences in phylogeny of cetrarioid lichens. *Lichenologist* 31: 441–449.

Thell, A., Berbee, M. & Miao, V. 1998. Phylogeny of the genus *Platismatia* based on rDNA ITS sequences (Lichenized Ascomycotina). *Cryptogamie, Bryol.-Lichénol.* 19: 43–54.

Thell, A. & Miao, V. 1998. Phylogenetic analysis of ITS and group I intron sequences from European and North American samples of cetrarioid lichens. *Ann. Bot. Fenn.* 35: 275–286.

Wedin, M., Döring, H. & Mattsson, J.-E. 1999. A multi-gene study of the phylogenetic relationships of the Parmeliaceae. *Mycol. Res.* 103: 1185–1192.

White, T. J., Burns T., Lee, S. & Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal DNA genes for phylogenetics. In *PCR protocols: A guide to methods and applications* (eds Innis, M., Gelfand, J., Sninsky, J. & White, T.), pp. 315–22. Academic Press, Orlando, Florida.

Folia Cryptog. Estonica, Fasc. 36: 107–112 (2000)

# *Sarcoscypha austriaca* (Pezizales) in Estonia

**Maarja Öpik[1], Bellis Kullman[2] & Anu Kollom[3]**

[1]Institute of Botany and Ecology, University of Tartu, 38 Lai St., 51005 Tartu, Estonia. E-mail: maarop@ut.ee

[2]Institute of Zoology and Botany, Estonian Agricultural University, 181 Riia St., 51014 Tartu, Estonia.
E-mail: bellis@zbi.ee

[3]Institute of Zoology and Botany, Estonian Agricultural University, 181 Riia St., 51014 Tartu, Estonia.
E-mail: anu@zbi.ee

**Abstract:** Recent Estonian *Sarcoscypha* collections were identified as *S. austriaca* on the basis of ascospore germination and anamorph characteristics. Ascospore germination and culture characters of *S. austriaca* were described, and a distribution map was compiled. The genus *Sarcoscypha* was previously known to be represented in Estonia by one species (*S. coccinea*). The latter species might occur in Estonia as it is known from Sweden, but present data of it's occurrence are questionable.

**Kokkuvõte:** Maarja Öpik, Bellis Kullman & Anu Kollom. *Sarcoscypha austriaca* (Pezizales) Eestis.

Viimastel aastatel Eestist kogutud karikseened osutusid eoste idanemise ja anamorfi tunnuste alusel liigiks *S. austriaca*. Kirjeldatakse *S. austriaca* eoste idanemist ja kultuuritunnuseid ning esitatakse liigi levikukaart. Karikseente (*Sarcoscypha*) perekonnast on Eestist varem märgitud vaid verev karikseen *(S. coccinea*). Viimane liik võiks Eestis esineda, kuna see leidub Rootsis, kuid senised andmed tema tegeliku esinemise kohta on kahtlased.

## INTRODUCTION

The genus *Sarcoscypha* contains a complex of macroscopically similar species known as *S. coccinea* s.l. (Baral, 1984). Three species of that complex are known in Europe: *S. austriaca* (Beck ex Sacc.) Boud., *S. coccinea* (Jacq.: Fr.) Lambotte and *S. emarginata* (Berk. & Broome) F.A. Harr. (=*S. jurana* (Boud.) Baral) (Baral, 1984; Harrington, 1990; Harrington & Potter, 1997). Earlier Estonian *Sarcoscypha* collections have been considered to be *S. coccinea* (see Järva & Parmasto, 1980; Järva et al., 1998). The species of the *S. coccinea* complex have been delimited on the basis of fruitbody micromorphological characters (Baral, 1984; Harrington, 1990; Harrington & Potter, 1997). Due to the plasticity of these characters, confusing misidentifications occur in literature (see Harrington, 1990). The aim of the present study is to identify the species of the genus *Sarcoscypha* growing in Estonia using pure culture and ascospore germination characters, and characterize the distribution of the species in Estonia.

## MATERIAL AND METHODS

54 collections from different parts of Estonia were used in the study, data on these have been published by Kullman et al. (1999). Some additional collections used are as follows: HARJUMAA Co.: Kiili Comm., Kangro Männiku (59°20'N 24°42'E), growing through ice, 7 Apr. 1998 K. Kalamees, det. M. Öpik (TAA 147930); Anija Comm., 6 km from Kose, near the Vetla-Voose crossroad (59°13'N 25°27'E), 7 Apr. 1998 K. Kalamees, det. M. Öpik (TAA 147931). PÕLVAMAA Co.: Väike-Munamägi (58°02.12'N 26°30.5'E), on wood, 4 Apr. 1998 L. Kalamees, det. M. Öpik (TAA 171121). TARTUMAA Co.: Tõravere (58°16'N 26°27'E), 5 May 1996 M. Öpik (TAA 165049, 165071, 165072), 11 May 1997 M. Öpik (TAA 165098, 165099).

Microscopical characters of fresh fruitbodies were observed and measured using light microscope, and tap water as mountant. Ten or more spores and conidia were measured for each specimen, using 100x immersion objective.

Ascospores from living fruitbodies were obtained by attaching a piece of apothecium to the inner surface of a Petri dish cover. The ascospores were allowed to discharge either onto the bottom of an empty sterile Petri dish, or onto the nutrient medium in a Petri dish, the cover being rotated from time to time. Ascospore germination was observed in a drop of sterile distilled water in the cavities of specific microscope slides. The slides were kept in mois-

ture chamber at room conditions (18–20°C). The ascospores on nutrient medium were allowed to germinate in the incubator at 25±2°C.

Ascospore germination on different nutrient media was studied in two collections during one week (TAA 179032, 179033). The following media were used: 1.5% malt extract 2% agar (MEA), potato dextrose agar (PDA), corn meal agar (CMA), water agar (WA). Ascospores were kept overnight in the refrigerator at 4°C prior to germination tests.

Pure cultures were isolated from the margins of colonies formed by the germinated ascospores and transferred to the fresh nutrient medium. Cultures were grown on 1.5% malt extract 2% agar (MEA) and potato-carrot agar (PCA). Culture characters were studied during 6 weeks growth of the cultures in incubator (25±2°C). Colours were described after Kornerup & Wanscher (1978).

For nuclear staining, spores and conidia were fixed in absolute ethanol:chloroform:ice acetic acid (6:3:1) for 80 minutes, then suspended in absolute ethanol for 15 minutes at room temperature on a shaker in a 1.5 ml Eppendorf tube. The samples were centrifuged for 2.5 minutes at 3200 rpm and the supernatant discarded. Resuspended samples were washed twice with sterile water, and hydrolysed with 5 N HCl at 24°C for 24 h on a shaker (Donadini, 1984). After that, the spores/conidia were washed twice with sterile water and once with PBS buffer. Nuclei were stained with propidium iodide (Fluka) (10 mg/ml) in PBS at 0°C for 1 h and observed under a fluorescence microscope.

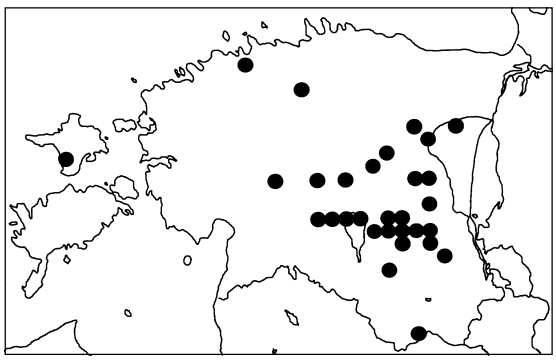


**Fig. 1.** Distribution of *S. austriaca* in Estonia.

The distribution map was generated using the software package DMAP. Recording units were given fixed size so that close localities appear as a single dot on the map.

## RESULTS

**Species identification and distribution.** The observed type of spore germination in water and on water agar, and size of conidia born on germinated spores or in pure culture represent those of *S. austriaca* as described by Harrington (1990). All collections used in the study were identified as *S. austriaca*. Apparently the species is common in southern and central Estonia, and rare in western and northern Estonia (Fig. 1).

**Ascospore germination.** The ascospores of *S. austriaca* germinated readily both in water and on nutrient media, usually within 1–4 days. One to four germ tubes were formed from ascospore tips but sometimes also laterally. Some germinating ascospores were traversed by one or two septae. Separation of endo- and epispore layer was observed. Ascospores germinating in water and on WA produced conidia on short and often branched germ tubes, infrequently directly on ascospores (Fig. 2, 3). Ascospores were also found to germinate while still in ascus or on the hymenium in senescing fruitbodies in humid conditions.

Rate and percentage of germination of ascospores of two collections studied (TAA 179032, TAA 179033) differed on different nutrient media. The best germination was obtained on PDA and CMA where most ascospores germinated in 1 to 2 days. On MEA and WA ascospore germination was slower (3 to 4 days) and number of germinated ascospores was much smaller. Only a few (2–3) germinated ascospores were detected during a week of incubation at room temperature (18–20°C). No conidia were produced on PDA, CMA and MEA.

**Number of nuclei.** Cytological studies revealed that both the spores (Fig. 4) and conidia of S. austriaca are multinucleate. Nuclear numbers in spores were 15–20, in conidia 5–9. Germinated spores were typically emptied of nuclei, which were apparently moved into the hyphae and conidia.

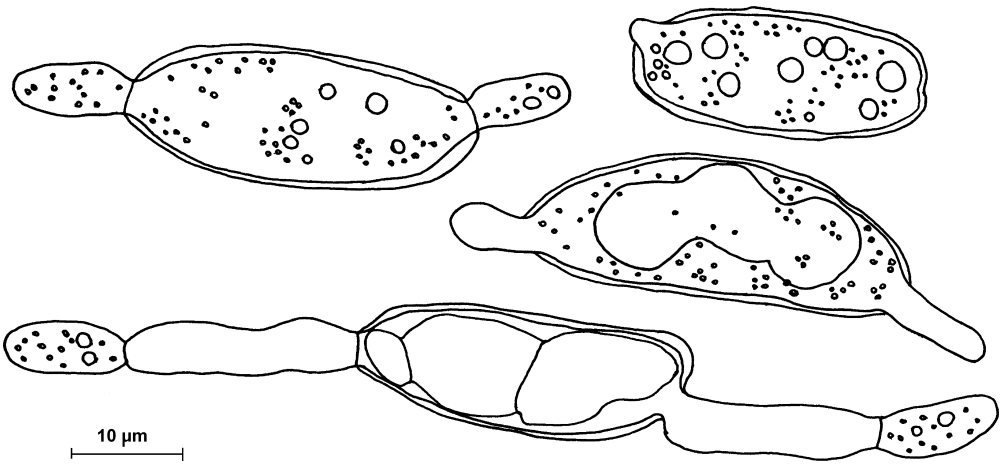



**Fig. 2.** Ascospores at different stages of germination.

**Culture description**

**Teleomorph:** *Sarcoscypha austriaca* (Beck ex Sacc.) Boud.

**Growth rate**: fast to very fast on MEA (26–60 mm/wk) and PCA(20–56 mm/wk).

**Culture appearance**: aerial mycelium white, woolly, low, sometimes floccose, later flattens into thin cover, sometimes raises to the walls of Petri dish, colony nontransparent to transparent. In some cultures (TFC 97-54 (on MEA), TFC 97-55 (on MEA and PCA), TFC 97-62 (on

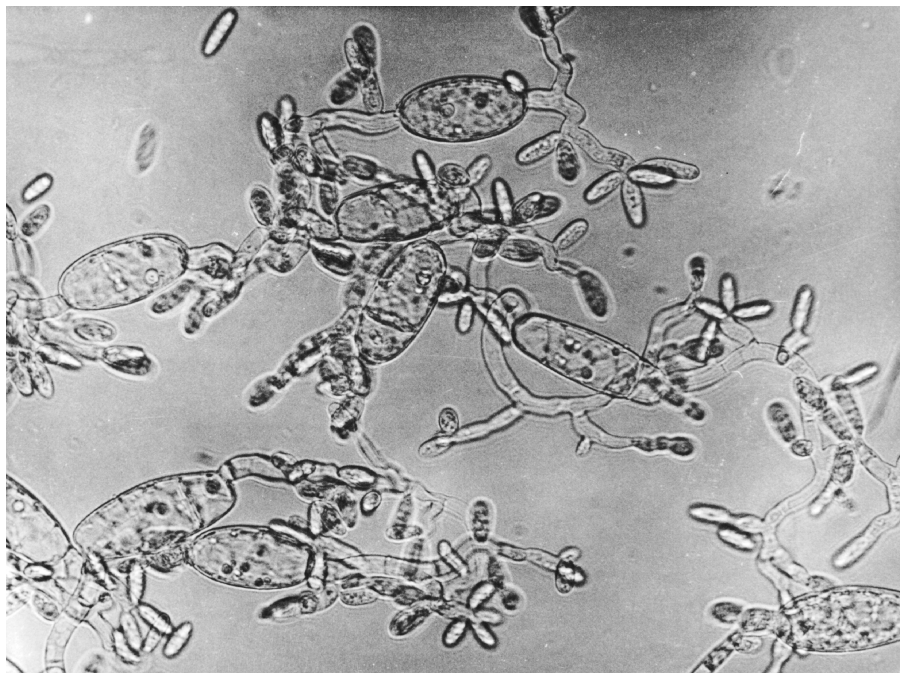


**Fig. 3.** Germinating ascospores with conidia.

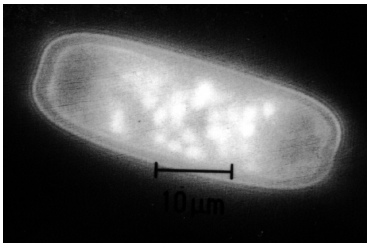



**Fig. 4.** Multinucleate ascospore stained with propidium iodide.

MEA)) brownish red (Kornerup & Wanscher: 10E8–10D8) areas of irregular shape ("stromata") developed after at least 6 week growth of cultures, paper-thin, tough, with reddish pigment in surrounding medium, covered with sparse white woolly mycelium. Marginal zone of colony even or bayed, hyphae appressed to raised, sparse to dense, intercalating. Reverse unchanged or lightened, blackish red under "stromata". Odour absent or aromatic (fruity) to sourish, may be absent in older culture.

**Microscopy**: Aerial mycelium consists of hyaline thin-walled hyphae, 2.5–7.5µm in diam., infrequently to moderately branched, cytoplasm of hyphal tip cells filled with vacuoles. Submerged mycelium consists of hyaline thin-walled hyphae, 1.0–3.5 µm in diam, straight, sinuous to coiled, infrequently branched, cytoplasm granular. "Stromata" consist of brown thick-walled hyphae, 2.5–4.0 µm in diam., very frequently branched, strongly interwoven, cells widened irregularly (*textura epidermoidea*), sometimes with aggregates of dark red substance around hyphae.

**Anamorph**: *Molliardiomyces coccinea* Paden. Conidiophores occurred in 6 isolates of 13 used (TFC 96-130 (on MEA), TFC 6-132 (MEA), TFC 96-133 (MEA), TFC 96-135 (PCA), TFC 97-54 (PCA), TFC 97-55 (MEA, PCA)) after 5–6 weeks to several months growth of cultures. Conidiophores arose in aerial mycelium, restricted to tufty areas, sometimes also on the walls of Petri dish, not abundant. Conidiophores erect, unbranched or irregularly branched; conidia ellipsoidal, oblong, or obovate, may be constricted in the middle, rounded or truncate at base, hyaline, cytoplasm granular, 8.2–14.6 x 3.8–5.1 µm(Fig. 5).

**Studied isolates**: TFC 96-123 (TAA 157461), TFC 96-124 (TAA 157452), TFC 96-126 (TAA 157456), TFC 96-130 (TAA 157462), TFC 96-131 (TAA 157464), TFC 96-132 (TAA 157465), TFC 96-133 (TAA 157466), TFC 96-135 (TAA 157477), TFC 97-51 (TAA 147675), TFC 97-52 (TAA 147678), TFC 97-54 (TAA 165091), TFC 97-55 (TAA 157606), TFC 97-62 (TAA 147683b).

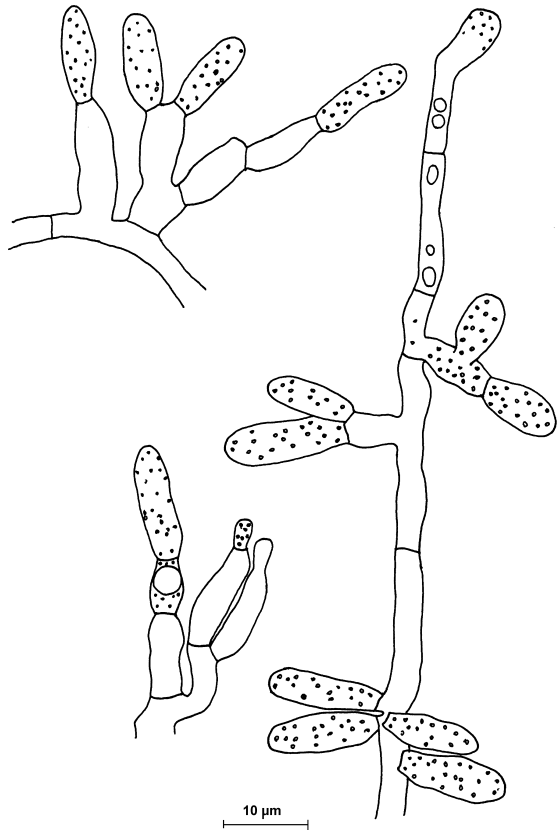



**Fig. 5.** Conidia in pure culture (anamorph *Molliardiomyces coccinea*).

## DISCUSSION

**Ascospore germination** by production of conidia observed in poor nutritional conditions (in water, on water agar) and on hymenium is most close to what is described for *S. austriaca* (Harrington, 1990). Germinated spores with conidia in ascus and on hymenium have been observed only in *S. austriaca* and occasionally in *S. occidentalis* (Harrington, 1990). The conidia of the latter species arise on hyphae from

the single polar end or both ends of a spore, and are smaller than in *S. austriaca* (see below) (Harrington, 1990).

Arisal of septa and separation of spore wall layers during spore germination was observed. The first phenomenon has been reported in *S. austriaca* by some authors (Rosinski, 1953; Baral, 1984; Fenwick, 1994), but has not been seen by others (Alexopoulos & Butler, 1949; Harrington, 1990). Separation of spore wall layers during germination was known before in *S. coccinea* (Harrington, 1990).

**Number of nuclei**. The ascospores of *S. austriaca* are known to be multinucleate (Berthet, 1964; Baral, 1984) and this was confirmed in the present study (Fig. 4). Also, it appeared that conidia of this species are multinucleate.

**Pure culture characters** of *S. austriaca* isolates studied were similar to those described earlier by Paden (1984, as *S. coccinea*) and Harrington (1990), except that blackish red patches of *textura epidermoidea* ("stromata") were found in some isolates. These structures are known in the cultures of *Sarcoscypha dudley* (Harrington, 1990), *S. coccinea* (Berthet, 1964; Paden, 1984), and in two other genera of the family Sarcoscyphaceae: *Phillipsia lutea* and *Pithya cupressina* (Paden, 1984).

The size of conidia born on germinating ascospores and in pure culture was 8.2–14.8 x 3.3–5.1 µm. Conidial size was variable within and between collections but fell into the limits given by Harrington (1990) for *Molliardiomyces coccinea*, the anamorph of *S. austriaca* – 8.5–15 x 3.5–5 µm. Conidial measurements after Butterfill & Spooner (1995) are 12–17.5 x 5–6.5µm, after Baral (1984) 13–18 x 5–5.5 µm. Anamorphs are known in three other *Sarcoscypha* species: *S. coccinea* (*M. eucoccinea*), *S. dudleyi* (*M. dudleyi*), and *S. occidentalis* (*M. occidentalis*). Their conidial measurements are 4.4–6.5 x 2.2–3 µm, 3.5–5.2 x 2.5–3 µm, and 4.5–7 x 3.8 µm, respectively (Harrington, 1990).

**Species identification and distribution.** According to the type of spore germination and the conidial measurements all *Sarcoscypha* specimens collected in Estonia from 1995 to 1999 belong to *S. austriaca*. Species of the *Sarcoscypha coccinea* complex differ in their shape of excipular hairs (straight or sinuous to coiled), shape of spores (ellipsoidal, truncate or indented), size of oil droplets in spores, form of paraphyses (not moniliform or often moniliform at base) (Baral, 1984; Harrington, 1990; Butterfill & Spooner, 1995). Still, considerable expertize is needed to recognize the *Sarcoscypha* species by fruitbody characters, especially if not using fresh (i.e. using herbarized) material (see Harrington, 1990). On the contrary, ascospore germination and anamorph morphology have been reported to distinguish well the species of the *S. coccinea* complex (Harrington, 1990).

First, only spores of *S. austriaca* and occasionally *S. occidentalis*, an American species, germinate by producing conidia; *S. coccinea*, *S. emarginata*, *S. macaronesica* (European species), *S. dudleyi* (an American species) germinate via germ tubes (Baral, 1984; Harrington, 1990). It has been shown that spores of *S. austriaca* can germinate either by conidia or germ tube (Harrington, 1990; Fenwick, 1994), depending mainly on the nutrients available (Fenwick, 1994). Both conditions can be seen in one Petri dish, showing different reactions of spores (Harrington, 1990; Fenwick, 1994). Second, the size of conidia born on germinated spores or in culture clearly delimits *S. austriaca* from *S. coccinea*.

*S. austriaca* was found to be common in Central and Southern Estonia, but it is apparently rare elsewhere (Fig. 1). All collections have been identified earlier as *S. coccinea* (see Järva & Parmasto, 1980; Järva et al., 1998). Both *S. austriaca* and *S. coccinea* are distributed in Europe and North America (Baral, 1984; Harrington, 1990), but in Europe do not occur together in the same biotope (Baral, 1984). In Great Britain *S. coccinea* is very rarely found now, though was common previously; *S. austriaca* has become more widespread than before (Butterfill & Spooner, 1995). In North America, the distribution of *S. coccinea* is restricted, and does not overlap with *S. austriaca* (Harrington, 1990). In Northern Europe, *S. austriaca* is found in Denmark, Norway, Sweden, and Finland, being occasional to rare. *S. coccinea* s. str. is occasional in Denmark, found in Norway and Sweden (distribution not known), while it's presence is questionable in

Finland (Hansen & Knudsen, in print). The above data suggest that *S. coccinea* s. str. might occur in Estonia, as it is found in Sweden, but present data are questionable.

## ACKNOWLEDGEMENTS

The study was financially supported by the Estonian Science Foundation grant no. 3580.

## REFERENCES

Alexopoulos, C.J. & Butler, E.E. 1949. Conidia-like structures in *Plectania coccinea*. *Mycologia* 41: 180–182.

Baral, H.O. 1984. Taxonomische und ökologische Studien über *Sarcoscypha coccinea* agg., Zinnoberrote Kelchbecherlinge. *Z. Mykol.* 50: 117–145.

Berthet, P. 1964. *Essai biotaxonomique sur les Discomycètes*. Thèse No. 340, Univ. Lyon.

Butterfill, G.B. & Spooner, B.M. 1995. *Sarcoscypha* (Pezizales) in Britain. *Mycologist* 9: 20–26.

Donadini, J.C. 1984. Techniques de coloration des noyaux cellulaires des spores et des paraphyses de discomycètes – application a l'etude du developpement des spores. *Bull. Soc. Linn. Provence* 35: 179–187.

Fenwick, G.A. (1994). Some observations on the germination of *Sarcoscypha austriaca* ascospores in the laboratory. *Mycologist* 8: 124–127.

Hansen, L. & Knudsen, H. (Eds). *Nordic Macromycetes*. Vol. 1. Ascomycetes. In print.

Harrington, F.A. 1990. *Sarcoscypha* in North America (Pezizales, Sarcoscyphaceae). *Mycotaxon* 38: 417–458.

Harrington, F.A. & Potter, D. 1997. Phylogenetic relationships within *Sarcoscypha* based upon nucleotide sequences of the internal transcribed spacer of nuclear ribosomal DNA. *Mycologia* 89: 258–267.

Järva, L. & Parmasto, E. 1980. *List of Estonian Fungi*. Tartu, 331 pp.

Järva, L., Parmasto, I. & Vaasma, M. 1998. *List of Estonian Fungi. Supplement 1*. Tartu. 183 pp.

Kornerup, A. & Wanscher, J.H. 1978. *Methuen Handbook of Colour*. 3rd ed. London.

Kullman, B., Öpik, M. & Jakobson, A. 1999. New Estonian records: Fungi. Pezizales. *Folia Cryptog. Estonica* 34: 81–85.

Paden, J.W. 1972. Imperfect states and the taxonomy of the Pezizales. *Persoonia* 6: 405–414.

Paden, J.W. 1984. A new genus of Hyphomycetes with teleomorphs in Sarcoscyphaceae (Pezizales, Sarcoscyphineae). *Canad. J. Bot.* 62: 211–218.

Rosinski, M.A. 1953. Two types of spore germination in *Sarcoscypha coccinea* (Scop. ex Fr.) Lambotte. *Mycologia* 45: 302–305.