Folia Cryptog. Estonica, Fasc. 43: 51–56 (2007)

The substrate preferences of epiphytic *Lepraria* species in old-growth forests in Estonia

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Abstract: The study is based on herbarium specimens from the lichenological herbarium of the Natural History Museum of University of Tartu (TU). Altogether about 260 specimens were examined. Eight *Lepraria* species were found: *L. borealis*, *L. eburnea*, *L. elobata*, *L. incana*, *L. jackii*, *L. lobificans*, *L. umbricola* and *L. vouauxii*. Statistically significant substrate preferences were discovered both on generic and species levels using Chi-square test and Dufrene-Legendre indicator species analyses. On the genus level, norway spruce was favoured as a substrate. *L. incana* was shown to prefer norway spruce, *L. elobata* and *L. lobificans* the bark of deciduous trees and *L. jackii* was not associated with any substrate group analysed. *L. borealis*, *L. eburnea*, *L. umbricola* and *L. vouauxii* did not show any affinities in this study, probably due to low number of records.

Kokkuvõte: Epifüütsete *Lepraria* liikide substraadieelistused Eesti vanades metsades.

Töös kasutati Tartu Ülikooli loodusmuuseumi lihhenoloogilise herbaariumi (TU) herbaareksemplare. Ligikaudu 260 eksemplari hulgast määrati kaheksa *Lepraria* liiki: *L. borealis*, *L. eburnea*, *L. elobata*, *L. incana*, *L. jackii*, *L. lobificans*, *L. umbricola* and *L. vouauxii*. Leiti statistiliselt olulisi substraadieelistusi nii perekonna kui liigi tasemel. Kasutati χ^2-testi ja Dufrene-Legendre'i indikaatorliikide analüüsi. Perekonna tasemel osutus eelistatud substraadiks harilik kuusk. Tuvastati, et *L. incana* eelistab harilikku kuuske, *L. elobata* ja *L. lobificans* lehtpuude koort. Liigil *L. jackii* ei ilmnenud seotust ühegi uuritud substraadirühmaga. Liikidel *L. borealis*, *L. eburnea*, *L. umbricola* ja *L. vouauxii* ei leitud eelistusi tõenäoliselt eksemplaride väikese arvu tõttu.

INTRODUCTION

The taxonomy of the sorediate sterile lichen genus *Lepraria* has been rather intensively studied especially in recent years (Aptroot 2002, Baruffo et al. 2006, Bayerová et al. 2005, Crespo et al. 2006, Ekman & Tønsberg 2002, Elix 2005, 2006, Elix et al. 2005, Knudsen & Elix 2007, Knudsen et al. 2007, Kukwa 2002, 2006a, 2006b, Lendemer 2005, Orange & Wolseley 2005, Sipman 2003, 2004, Tønsberg 2002, 2004, 2007, Tønsberg & Zhurbenko 2006, Wirth et al. 2004). However, the quantitative ecological studies of the *Lepraria* species are much fewer (Wirth & Heklau 1995, Lõhmus et al. 2003, Baruffo *et al.* 2006). Lõhmus et al. (2003) explored the substrate preferences of epiphytic *Lepraria* species in old peatland forests in Estonia, reporting different habitat use of *L. incana* compared to other species that were treated collectively. Since then more data have become available and a more detailed treatment is possible.

MATERIAL AND METHODS

This study is based on herbarium specimens from the lichenological herbarium of the Natural History Museum of the University of Tartu (TU) particularly the collections of Piret Lõhmus from Alam-Pedja Nature Reserve and of Inga Jüriado from Valgamaa, Pärnumaa, Ida-Virumaa and Saaremaa counties. Altogether about 260 specimens were examined. Morphology and anatomy were studied using a stereomicroscope Olympus SZ40. Thin layer chromatography (TLC) was carried out according to Orange et al. (2001). Mostly solvent A, sometimes additionally C, was used.

Two datasets were compiled for the statistical analyses.

The first dataset contained information on collections from old peatland forests (age of dominant trees >60 years for deciduous and >80 years for coniferous species) of the Alam-Pedja Nature Reserve in east-central Estonia. In randomly selected plots the occurrence of *Lepraria* specimens was recorded on four tree species – the coniferous *Picea abies* and *Pinus sylvestris* and the deciduous *Betula pubescens* and *Alnus glutinosa* – as these are the most common in the studied habitats. Two types of trunks from each tree species were selected – living trees and snags without bark. In each plot two pairs of trunk types from each tree species

were analysed. For further details of sampling design see Lõhmus & Lõhmus (2001). The data matrix contained entries for 114 specimens on 288 trunks, including the trunks that had no *Lepraria* thalli on them. Some data from this dataset were analysed also in Lõhmus et al. (2003).

The first dataset was used to test the tree species preference of the genus *Lepraria* as a whole. The Chi-square test was used in STATISTICA 6.0 (StatSoft, Inc. 2001).

The second dataset was compiled using the first matrix and the results of lichenological inventories of old-growth forests from different parts of Estonia (age of dominant trees >50 years for deciduous and >80 years for coniferous species). Besides *Picea abies*, *Pinus sylvestris*, *Betula* spp. and *Alnus glutinosa,* also *Populus tremula* was among the common tree species in the studied stands, although much less frequent in the analysed dataset. Other tree species were represented by few records only in the matrix (Appendix 1). The trunks of different tree species were selected at random within the sampled forest stands. For further details see Viilma et al. (2001) and Jüriado et al. (2003). The entries of uninhabited trunks were excluded. The abundance of species on trunks was not evaluated. The matrix included 220 specimens.

The second dataset was used to investigate the substrate preferences of *Lepraria* species separately. The Dufrene-Legendre indicator species analysis was carried out using PC-ORD 4.0 and the significance values were calculated with Monte Carlo test (Legendre & Legendre 1998, McCune & Mefford 1999). For tree species preference analysis infrequent substrata with 1–4 records were excluded. When analysing corticolous specimens, the records on wood were omitted (Appendix 1).

The nomenclature follows Randlane & Saag (1999, 2004) and Santesson et al. (2004).

RESULTS

Eight *Lepraria* species were found from the studied trunks: *L. borealis* Loht. & Tønsberg, *L. eburnea* J.R. Laundon, *L. elobata* Tønsberg, *L. incana* (L.) Ach., *L. jackii* Tønsberg, *L. lobificans* Nyl., *L. umbricola* Tønsberg and *L. vouauxii* (Hue) R.C. Harris (Appendix 1). *L. incana* was by far the most frequent (141 records of total 220), which is sometimes not the case in other Estonian forests (L. Saag, unpublished). Several species belonging to other genera, such as *Lecidea nylanderi* (Anzi) Th. Fr., *Loxospora elatina* (Ach.) A. Massal., *Lecanora expallens* Ach., *Haematomma ochroleucum* var. *porphyrium* (Pers.) J.R. Laundon etc. were identified in the material and excluded from the data matrix.

In the first dataset 40% of trunks (115 of 288) were inhabited by *Lepraria*. *Picea abies* was significantly favoured as substrate on the genus level ($p<0{,}0000$; $\chi^2=64{,}6$; df=3; Table 1), in accordance with Lõhmus et al. (2003).

Table 1. Occurrence of *Lepraria* species on four tree species ($p<0{,}0000$; $\chi^2=64{,}6$; df=3)

	Picea	*Pinus*	*Betula*	*Alnus*
inhabited	54	11	19	31
uninhabited	18	70	47	37
altogether	72	81	66	68

The indicator species analysis was used to discover the preferences of studied species for different substrate classes: (1) tree species; (2) bark of coniferous trees and bark of deciduous trees; (3) bark of coniferous trees, bark of deciduous trees and wood of all trees. No distinction was made between the wood of coniferous and deciduous tree species as their pH and the composition of lichen flora are considered to be similar (Lõhmus & Lõhmus 2001).

L. incana prefers norway spruce and the bark of coniferous trees in general (Table 2). This species is overwhelming on wood, compared to other taxa, although majority of thalli grow on bark (Fig. 1, 2). Notably most of the wood samples came from coniferous trees, mainly spruce (Appendix 1). *L. elobata* is a species of deciduous bark in studied forests. *L. lobificans* mostly grows on the bark of deciduous trees but this preference was statistically significant only in corticolous specimens. *L. jackii* does not have a preference for any substrate analysed. *L. borealis*, *L. eburnea*, *L. umbricola* and *L. vouauxii* also did not show any affinities in this study, probably due to low number of records.

Table 2. Substrate preferences of studied *Lepraria* species according to indicator species analysis (the method of Dufrene and Legendre), the significance values calculated with Monte Carlo test

	Coniferous bark / Deciduous bark	Coniferous bark / Deciduous bark / Wood	Tree species	Total no. of specimens
L. incana	coniferous p=0.000	wood p=0.011	*Picea abies* p=0.024	141
L. jackii	-	-	-	30
L. lobificans	deciduous p=0.003	-	-	21
L. elobata	deciduous p=0.003	deciduous bark p=0.001	-	16
L. eburnea	-	-	-	5

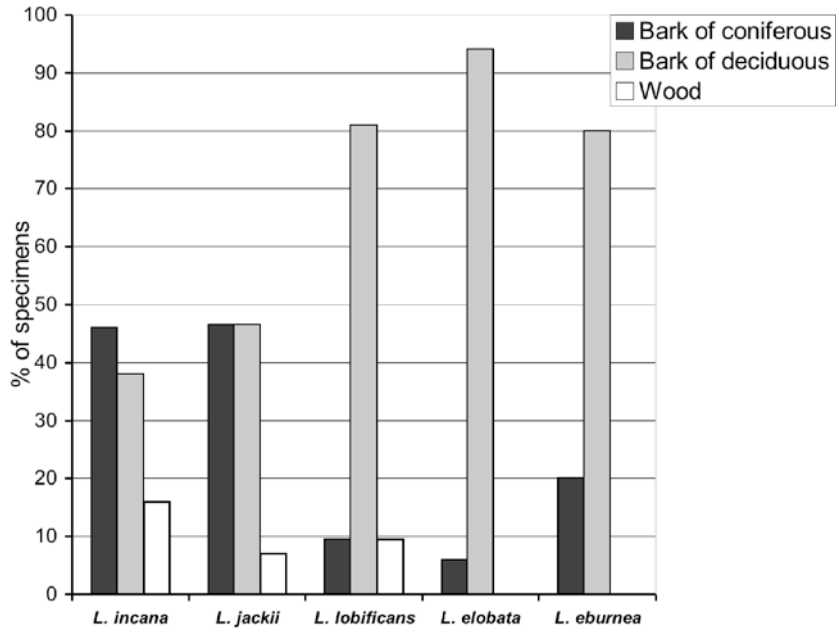

Fig. 1. Relative frequencies of studied *Lepraria* species on different substrates.

DISCUSSION

As stated by Lõhmus et al. (2003), treatment of *Lepraria* on generic level in ecological studies would be justified if the species had similar requirements for the substrate or if the samples were likely to contain only one species. Although *L. incana* was by far the most abundant species in this study, being the main cause for g. *Lepraria* (if treated collectively) preferring spruce, 36% of thalli in the randomly collected samples belonged to other taxa with different habitat uses. The number of taxa in a random sample depends on species richness and sample

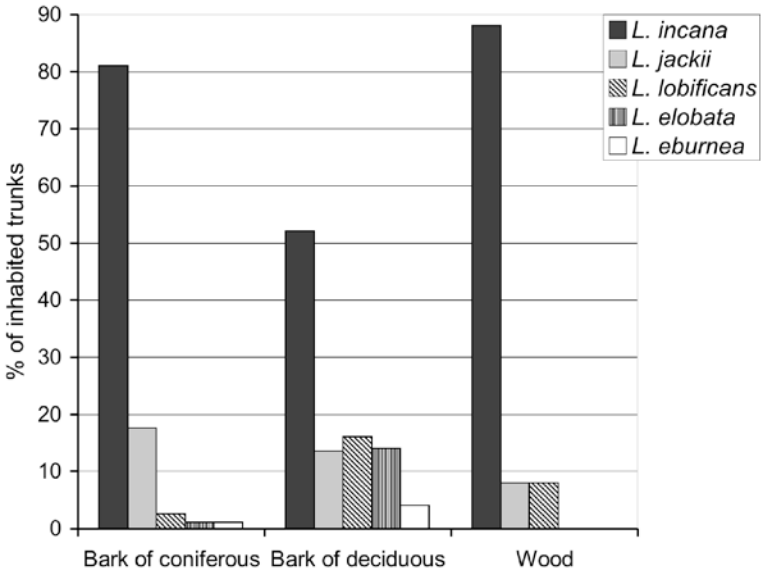

Fig. 2. Relative frequencies of studied substrates inhabited by different *Lepraria* species.

size. In this study relatively high diversity was found in most of the studied areas.

15% of the specimens which were determined as *Lepraria* in the field by collectors were demonstrated to belong to other genera. Such percentage of initial misidentifications has also been mentioned earlier (Lõhmus 2003, Baruffo 2006). This further demonstrates the need for more detailed determination.

The low frequency of *L. lobificans* compared to *L. incana* in the studied forests is unexpected as *L. lobificans* is very common across Estonia in different habitats (Saag & Saag 1999). The old spruce dominated forests may be especially suitable for *L. incana* because of the shade, relatively high humidity and low pH of spruce bark. This species often grows abundantly on the bases of norway spruce in Estonia. Also Baruffo et al. (2006) consider that it prefers acid substrata in humid places, other authors report it from various substrates (Laundon 1992, Tønsberg 1992, Lohtander 1994, Wirth & Heklau 1995, Kukwa 2006b). Additionally, Baruffo et al. (2006) report that in Italy both *L. eburnea* and *L. lobificans* are substratum indifferent on broader scale but corticolous specimens of the latter are still more frequent on neutral bark; that *L. jackii* is mainly epiphytic, also preferring neutral substrate and that *L. elobata* belongs to the epiphyte group together with *L. jackii* but is more often acidophytic. The present study was designed to compare coniferous and deciduous trees rather than acidic and neutral substrata. Nevertheless, given that conifers and birch have lower bark pH than other studied trees (Kuusinen 1996, Lõhmus & Lõhmus 2001), our data from Estonian old forests are in accord with the results of Baruffo et al. from Italy as far as *L. eburnea* and *L. lobificans* are concerned, while *L. jackii* and *L. elobata* show slightly different tendencies – *L. jackii* is more frequent on acidic bark and *L. elobata* is equally represented in both pH groups (Appendix 1). Lõhmus et al. (2003) noted that *L. incana* inhabited conifers slightly more frequently than deciduous trees. However, this tendency was not statistically significant – unlike in the present study. They treated other *Lepraria* species collectively, documenting the preference of this group for deciduous trees. This result is only partly supported here as *L. jackii* does not share this feature.

ACKNOWLEDGEMENTS

Piret Lõhmus, Inga Jüriado and Ave Suija are thanked for providing the collections and environmental data. The study was partly supported by Estonian Science Foundation Grant no. 5823.

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Appendix. The numbers of specimens of recorded *Lepraria* species on different substrates

		L. incana	*L. borealis*	*L. jackii*	*L. elobata*	*L. eburnea*	*L. lobificans*	*L. umbricola*	*L. vouauxii*	No. of specimens
Picea abies	Bark	52	1	6	1	1	0	0	0	61
	Wood	14	0	1	0	0	1	1	0	17
Pinus sylvestris	Bark	13	0	7	0	0	2	0	0	22
	Wood	2	0	1	0	0	0	0	0	3
Betula spp.	Bark	25	0	12	7	0	4	0	2	50
	Wood	0	0	0	0	0	1	0	0	1
Alnus glutinosa	Bark	23	0	1	6	1	7	0	1	39
	Wood	6	0	0	0	0	0	0	0	6
Populus tremula	Bark	1	0	0	2	3	2	0	0	8
Quercus robur	Bark	3	0	0	0	0	0	0	1	4
Fraxinus excelsior	Bark	1	0	0	0	0	1	0	0	2
Sorbus aucuparia	Bark	0	0	0	0	0	2	0	0	2
Acer platanoides	Bark	0	0	0	0	0	1	0	0	1
Alnus incana	Bark	0	0	1	0	0	0	0	0	1
Juniperus communis	Bark	0	0	1	0	0	0	0	0	1
Sorbus intermedia	Bark	1	0	0	0	0	0	0	0	1
Unknown deciduous	Bark	0	0	0	0	0	0	1	0	1
Total		141	1	30	16	5	21	2	4	220