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Microfungal Biodiversity on *Fagus grandifolia* in an Old-growth Forest of Eastern North-America

By

Vladimir VUJANOVIC*) and Jacques BRISSON

With 1 Figure

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Summary

VUJANOVIC V. & BRISSON J. 2002. Microfungal biodiversity on *Fagus grandifolia* in an old-growth forest of Eastern North-America. – *Phyton* (Horn, Austria) 42 (2): 315–328, 1 figure. – English with German summary.

Microfungi are one of the most neglected groups of organisms in biodiversity studies. Yet, they are overly important in representation and could be one of the best indicators of ecological integrity of old-growth forests. We made an inventory of the microfungi associated with woody aerial parts of *Fagus grandifolia* in the Boisé-des-Muir, an old growth beech-maple forest of Southern Quebec, Canada. Our inventory revealed a remarkable diversity, with 60 genera and 76 species isolated (*Ascomycota* 31, *Hyphomycetes* 23, *Coelomycetes* 20 and *Zygomycota* 2), including many species never previously found on beech as well as two species never described before. Due to the high biological interest of old-growth forests and their potential for diverse niches for microfungi, we believe that this type of ecosystem should particularly be the focus of microfungi inventories efforts.

Zusammenfassung

VUJANOVIC V. & BRISSON J. 2002. Kleinpilz-Biodiversität auf *Fagus grandifolia* in einem Urwald des östlichen Nordamerika. – *Phyton* (Horn, Austria) 42 (2): 315–328, 1 Abbildung. – Englisch mit deutscher Zusammenfassung.

In Biodiversitäts-Studien werden Kleinpilze oft vernachlässigt. Sie sind jedoch wichtige Bestandteile von Ökosystemen und könnten als ausgezeichnete Indikatoren

*) Dr. Vladimir VUJANOVIC, Institut de recherche en biologie végétale & Département de sciences biologiques, Université de Montréal, 4101 east, Sherbrooke St., Montréal, Québec, Canada, H1X 2B2 ; e-mail : vujanovv@magellan.umontreal.ca

für ökologische Unversehrtheit von Urwäldern dienen. Wir haben Kleinpilze inventarisiert, die auf den verholzten, oberirdischen Organen von *Fagus grandifolia* in Boisé-des-Muir, einem von Buche und Ahorn dominierten Urwald im Süden von Québec, Kanada, vorkommen. Unsere Studie hat eine bemerkenswerte Vielfalt zu Tage gebracht. Wir haben 60 Gattungen und 76 Arten (*Ascomycota* 31, *Hyphomycetes* 23, *Coelomycetes* 20 und *Zygomycota* 2) isoliert, unter welchen sich zahlreiche Arten befinden, die nie zuvor auf Buche gefunden wurden, sowie zwei, die bisher völlig unbekannt waren. Da Urwälder biologisch gesehen von grossem Interesse sind und eine beträchtliche Mannigfaltigkeit an verschiedenen Nischen für Kleinpilze aufweisen, glauben wir, dass sich Bemühungen, Kleinpilze zu inventarisieren, besonders auf diese Art von Ökosystem konzentrieren sollten.

1. Introduction

Many studies or inventories have identified species that are closely associated with undisturbed forest ecosystems (DAVIS 1996), but knowledge about biological diversity, of different life forms in old-growth forests, is covered unequally. The majority of studies characterizing old-growth forests or their comparisons with disturbed ecosystems are based on vascular plant diversity, sometimes only woody species (HARDT & SWANK 1997, CHESTER & al. 1995, ROOVERS & SHIFLEY 1997). There has been also interest in vertebrates, especially birds (HANEY & SCHAADT 1996, WEBSTER & ADAMS 1973) and amphibians (BONIN 1991, POUGH & al. 1987, BUREY 1983, WELSH 1990). The number of inventories of lichens or mosses also has increased significantly over the last two decades (GUSTAFSSON & HALLINGBACK 1988, LESICA & al. 1991, SELVA 1996, COOPER-ELLIS 1998). Despite the growing number of studies in old-growth forests, we still know next to nothing about certain groups of invertebrates or microorganisms, simply because they are hard to study or identify. This ignorance does not imply that these groups have a low diversity or do not play an important ecological role. One of the most neglected yet potentially important groups are the microfungi (BILLS 1994, STONE & al. 1996). It is estimated that there are six times more species of microfungi in nature than vascular plants (HAWKSWORTH 1991). In old-growth forests, microfungal diversity could be even more important considering the larger number of functional ecological niches: larger, older trees, greater number of phytopathological relationships, large logs in various stages of decay, etc. In fact, because of the importance of decomposition processes and pathology in old-growth forests, it would not be surprising that microfungi diversity would be one of the best measures of ecological integrity of old-growth systems.

The Boisé-des-Muir Ecological Reserve is an 11 ha old-growth beech-maple forest located at the northern limit of the Deciduous Forest of North America (BRISSON & al. 1992). Although it has never been cut and is now protected from any human exploitation, it is not shielded from indirect human disturbances such as introduced species and pathogens, pollution

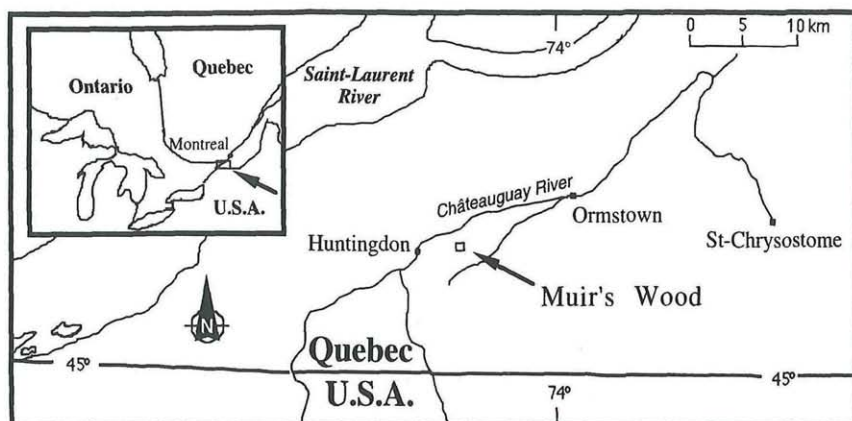


Fig. 1. Geographical location of Bois  -des-Muir (Muir's Wood) old-growth forest in Canada, North America.

or global warming due to increasing atmospheric CO_2 . The last of such disturbances, first observed in 1990 in the Bois  -des-Muir, is the beech bark disease (VUJANOVIC & al. 1999a), caused by an introduced pathogen [*Nectria coccinea* (PERS.: FR.) FR.] that may kill more than 50% of the beech trees in a forest over a period of several decades (HOUSTON 1994). Anthropogenic disturbances can affect the integrity of the forest and may well modify its microfungal diversity in ways that we cannot evaluate now since we know so little about microfungi to begin with.

As a first step toward improving our knowledge of microfungal diversity in old-growth forests, we inventoried the microfungi associated with aerial parts of American beech in the Bois  -des-Muir. Our approach is based on the methodology proposed by the All-Taxa Inventories of Fungal Biodiversity (ATIFB), a major component of the All-Taxa Biodiversity Inventories (ATBI) (ROSSMAN & al. 1998). The ATIFB is a new scientific strategy that is particularly appropriate for the study of microfungal diversity in a habitat of limited surface area (VUJANOVIC & al. 1997). Although preliminary in nature, our inventory nonetheless revealed a remarkable diversity of caulosphere and allowed the discovery of new species as well as species never found on beech before, confirming the need for more microfungi inventories in general, particularly in old-growth forests.

2. Study Area

The Bois  -des-Muir Ecological Reserve is located 70 km south-west of Montreal (Fig. 1), in the Haut-Saint-Laurent of the Qu  bec Province, Canada. This region is characterized by extensive lowlands covered with Champlain Sea clays deposited 12,000 to 10,000 years B.P. These lowlands

are interrupted by morainic ridges lying parallel to the Saint-Lawrence River. The clay plain has been almost entirely deforested for agricultural uses while morainic deposits still support forest communities. These forests, generally of small area, have suffered anthropogenic disturbances of varied intensity and nature since European settlement, some 200 years ago.

The Bois  -des-Muir is located on a morainic ridge, with a maximum slope of 10%, and a drainage ranging from moderate to good. The soil is a brown stony loam with a pH ranging from 6.1 to 7.0 in the B horizon. Sugar maple (*Acer saccharum* MARSH.) and American beech (*Fagus grandifolia* EHRH.) are the dominants, followed by Ironwood [*Ostrya virginiana* (MILL.) K. KOCH], American basswood (*Tilia americana* L.), Hemlock [*Tsuga canadensis* (L.) CARR.] and Butternut [*Carya cordiformis* (WANG) K. KOCH]. The larger canopy trees range in age from 150 to 320 years. The understory is typical of a beech-maple forest, with a low percentage of ground cover, and the presence of acidophilic species such as *Polystichum acrostichoides* (MICHX.) SCHOTT and *Maianthemum canadense* DESF. The forest has been exceptionally protected from important human disturbances; only very restricted partial cuts have occurred until it was acquired by the provincial government and protected under the status of an Ecological Reserve in 1993. A more complete description of the vegetation and history of the Bois  -des-Muir can be found in BRISSON & al. 1992.

3. Methodology

3.1. Sampling

Our interest was solely on microfungi, which do not represent a single systematic group, but include species of all 4 phyla of *Eumycota*, those mostly detectable at the microscopic scale and with reproductive structures that are less than a few millimeters in size (ELLIS & ELLIS 1985, BILLS 1994). Many species produce fruiting bodies on samples in the nature, but not necessarily on synthetic media in culture. Because of that, diagnostics of their presence and/or their identification from the culture becomes a very difficult task (BRIDGE & SPOONER 2001). To avoid this obstacle, we proceeded with a visual observation and sampling of characteristic fruiting bodies and symptomatic samples of the present microfungi on beech in the ecological reserve. This allowed us to identify several species that produced only sterile mycelium in culture. We assessed also with other diagnostic indications *in situ* (HARTMANN & al. 1988, SINCLAIR & al. 1993) to distinguish the samples with symptoms caused by other (abiotical and biotical) harmful agents in order to facilitate the interpretation of our obtained results. However for all microfungi, required sampling was carried out on different plant parts followed by an isolation of fungi on growth culture under laboratory conditions. Thus, to harmonize the cultural inventory, we chose 45 trees for further study. Among them 15 were chosen

in each of the following categories: canopy tree, subcanopy tree and sapling. Because microfungal diversity may vary according to local edaphic or phytosociological conditions (VUJANOVIC 1995, VUJANOVIC & VUCKOVIC 1994), trees were chosen in such a way that the full spectrum of local conditions in the Boisé-des-Muir was covered. Bark of both living and dead plant parts was sampled in order to cover parasitic and saprobic fungi. Sampling has been restricted to still attached, not yet decorticated woody aerial parts. In all, 1911 samples were collected from different locations on the tree: trunk (pieces of bark of 36 mm²); large branches (diameter > 3 cm; portions of branches from 10 to 20 cm in length); small branches (diameter from 0.5 to 3 cm; portions of 5 to 10 cm in length); and twigs (diameter < 0.5 cm, portions of 1 to 5 cm in length). The samples were put into sterile boxes or bags, and the date of sampling and location in the forest were noted (MYREN & al. 1994). Microfungi found on other aerial plant parts such as leaves, fruits or flowers were not surveyed.

3.2. Isolation of Microfungi on Growth Culture

Samples were kept 24h in an incubator at 4 °C until isolation. The isolation procedure consists of: (i) disinfection of the surfaces of the bark samples and branches with ethyl alcohol 70% (10 sec.); (ii) excision of bark pieces with a scalpel and sterilization of the pieces with sodium hypochlorite (0.25%) during 2 min., followed by immersion in sterilized distilled water (5 min., twice); (iii) incubation and isolation of microfungi on growth culture (PDA, CZ, MA) at 22 °C in darkness; (iv) purification and transfer of the isolates on culture media for preservation following the procedure developed by DHINGRA & SINCLAIR 1987 and BOOTH 1971.

3.3. Identification

Identification of the isolates was done using the following keys: AINSWORTH & al. 1973, ELLIS & ELLIS 1985, LANIER & al. 1978, and VON ARX 1981; for Mitosporic Fungi: BARNETT & HUNTER 1987, ELLIS 1971, 1976, GERLACH & NIRENBERG 1982, KIFFER & MORELET 1997, NAG RAJ 1993, SUTTON 1980, and WANG & ZABEK 1990; for *Ascomycota*: BARR 1978, 1987, 1990a, 1990b, BREITENBACH & KRÄNZLIN 1981, DENNIS 1978, HANLIN 1990, JU & ROGERS 1996, RAPPAZ 1987, SIVANESAN 1984 and SHOEMAKER 1964. The taxonomy and phylogeny adopted is based on HAWKSWORTH & al. 1995. Given the complexity inevitably associated with these groups, many identifications were made to the genus level only, and many others remained unidentified. We were conservative in our identifications, so that doubtful specimens were considered unidentified. The specimens are kept at the Marie-Victorin Herbarium of the Université de Montréal (MT: 10311–13260).

3.4. Species Status

So little is known about microfungal biodiversity in North America that it is difficult to make a definite assessment on the rarity of all the taxa found at the Boisé-des-Muir ecological reserve. We compared our inventory with the most recent fungal-host indexes of species found in Québec (SPPQ 1996) in Canada (GINNS 1986) and in the United States (FARR & al. 1989; COTTER & BLANCHARD 1982). Finally, we ranked the taxa found in the Boisé-des-Muir according to their frequency in the samples.

4. Results

4.1. Inventory

We obtained 315 isolates, of which 83% were identified to the species or genus level. In all, 60 genera including 76 species were isolated and identified (Tables 1 and 2). Three phyla are represented in our inventory, the *Ascomycota*, the *Zygomycota* and the Mitosporic Fungi (= *Deuteromycota*). The *Deuteromycota* are represented by 37 genera and 43 species, of which 19 genera and 20 species belong to the *Coelomycetes*, and 18 genera and 23 species to the *Hyphomycetes* (Table 1). The most frequent genera encountered in the Boisé-des-Muir are *Alternaria*, *Asterosporium*, *Cladosporium*, *Cytospora*, *Libertella*, *Cylindrocarpon*, *Epicoccum*, *Microdiplodia*, *Neohendersonia*, *Phomopsis*, and *Trichoderma*. The phylum *Zygomycota* is represented by two species, *Mucor racemosus* and *Rhizopus* sp. (Table 1). The phylum *Ascomycota* is represented by 21 genera and 31 species belonging to 15 families (Table 2). The family *Xylariaceae* is the most diverse, with 3 genera (*Hypoxylon*, *Ustulina* and *Xylaria*) and 6 species, followed by the *Diatrypaceae*, with 3 genera and 5 species. The genus *Nectria* is represented by 5 species (including one unidentified) followed by the genera *Hypoxylon* (4 species), *Diatrype* (3 species) and *Valsa* (2 species). Because of our limited resources, the inherent taxonomic complexity of microfungi and lack of knowledge and expertise, nearly 40% of our isolates were not identified to the species level, especially in the *Coelomycetes*.

4.2. Microfungal Biodiversity in Relation to Position in the Tree

Most of the species in the *Hyphomycetes* showed no preference in terms of position in the tree, but the majority of the other species were closely associated with a specific location (Table 1 and 2). At the base of the trunk, especially on old trees with a diameter larger than 40 cm, fruiting bodies of the pathogenic species *Ustulina deusta* were frequent. We found 10 taxa preferentially colonizing the trunk, primarily two species from the *Nectria* complex. Six species were preferentially found on the first branches of the crown, with a good representation of the genus

Table 1.

Fungal taxa in Mitosporic Fungi (= *Deuteromycota*) and *Zygomycota* from the bark of *Fagus grandifolia* in Muir Ecological Reserve, Quebec, Canada. Frequency (Freq) of fungal isolations: A: 1 or 2 isolation; B: more than 2 but fewer than 1.0% of bark samples; C: 1-10%; D: > 10%. Preferred colonized location on the tree (Pref): Tr: Trunk; Lb: Large branch; Sb: Small branch; Tw: Twigs; Ub: Ubiquitous; Ind: Indeterminate, too uncommon. (See Methods section for details).

Mitosporic fungi (43 taxa)					
Hyphomycetes (23)	Freq	Pref	Coelomycetes (20)	Freq	Pref
<i>Acronium</i> sp.	B	Ub	<i>Asterosporium asterospermum</i> (PERS. : FR.) HUGH.	C	Tr
<i>Alternaria alternata</i> (FR. : FR.) KESSL.	D	Ub	<i>Camarosporium</i> sp.	C	Tw
<i>Ampelomyces</i> sp.	B	Ub	<i>Coniothyrium fagi</i> TECHON	B	Ub
<i>Aureobasidium pullulans</i> (DE BARY) ARNAUD	B	Ub	<i>Cryptosporiopsis</i> sp.	A	Ind
<i>Botrytis cinerea</i> PERS. : FR	B	Ub	<i>Cytospora</i> sp.	D	SbTw
<i>Cladosporium herbarum</i> (PERS. : FR.) LINK	D	Ub	<i>Diplodia</i> sp.	C	Sb
<i>Cladosporium cladosporioides</i> (FR.) DE VRIES	D	Ub	<i>Diarimella laurentidae</i> VUJANOVIC & al.	A	Ind
<i>Cylindrocladium</i> sp.	B	Ub	<i>Dinemasprium</i> sp.-1	B	Sb
<i>Cylindrocarpon destructans</i> (ZIN.) SCH.	D	Ub	<i>Dinemasprium</i> sp.-2	B	Tw
<i>Cylindrocarpon</i> sp.	C	Ub	<i>Fusicoccum</i> sp.	B	SbTw
<i>Endophragmiella</i> sp.	B	Ub	<i>Hendersonia</i> sp.	C	Ub
<i>Epicoccum nigrum</i> LINK	D	Ub	<i>Libertella faginea</i> DESM.	D	SbTw
<i>Epicoccum</i> sp.	C	Ub	<i>Microdiplodia</i> sp.	C	TrLb
<i>Fusarium oxysporum</i> SCHL.	C	Ub	<i>Neohendersonia kickxii</i> (WEST) SUTTON & POLLACK	D	LbSb
<i>Fusarium redolens</i> WOLLENWEB.	C	Ub	<i>Phoma</i> sp.	C	SbTw
<i>Gliocladium</i> sp.	B	Ub	<i>Phomopsis oblonga</i> (DESM.) TRAVERSO	C	SbTw
<i>Leptographium microsporum</i> R.W. DAVIDSON	A	Ind	<i>Phyllosticta</i> sp.	C	Tw
<i>Penicillium</i> sp.	C	Ub	<i>Polynema mairii</i> VUJANOVIC & al.	A	Ind
<i>Paeclomyces farinosus</i> (HOLM.) BROWN & SM.	A	Ind	<i>Pseudolachnea</i> sp.	A	Ind
<i>Rhinocladia</i> sp.	A	Ind	<i>Scoliosporium</i> sp.	B	Sb
<i>Trichoderma harizanum</i> RIFAI	C	Ub			
<i>Trichoderma viride</i> PERS. : FR.	D	Ub			
<i>Verticillium lecanii</i> (ZIMM.) VIÉGAS	B	Ub			
Zygomycota (2 taxa)					
<i>Mucor racemosus</i> FRESN.	B	Ub			
<i>Rhizopus</i> sp.	A	Ind			

Table 2.

Fungal taxa in Ascomycota from the bark of *Fagus grandifolia* in Muir Ecological Reserve. Taxa are grouped according to their families. For legend, see Table 1.

Ascomycota (31 taxa)	Freq	Pref
<i>Ascodichaenaceae</i>		
<i>Ascodichaena rugosa</i> BUTIN	C	Tr
<i>Botryosphaeriaceae</i>		
<i>Botryosphaeria quercuum</i> (SCHWEIN.) SACC.	A	Ind
<i>Dermateaceae</i>		
<i>Mollisia</i> sp.	A	Ind
<i>Diatrypaceae</i>		
<i>Diatrype disciformis</i> (HOFFM. : FR.) FR.	D	LbSb
<i>Diatrype stigma</i> (HOFFM. : FR.) FR.	D	Sb
<i>Diatrype bullata</i> (HOFFM. : FR.) FR.	D	Sb
<i>Eutypella</i> sp.	D	SbTw
<i>Quaternaria quaternata</i> (PERS.) SCHRÖTER	D	LbSb
<i>Erysiphaceae</i>		
<i>Uncinula</i> sp.	B	Tw
<i>Hyaloscyphaceae</i>		
<i>Dasyscyphus</i> sp.	A	Ind
<i>Hypocreaceae</i>		
<i>Nectria coccinea</i> (PERS : FR.) FR.	C	Tr
<i>Nectria episphaeria</i> (TODE : FR.) FR.	B	Ge
<i>Nectria cinnabarina</i> (TODE : FR.) FR.	A	Ind
<i>Nectria galligena</i> BRES.	C	Tr
<i>Nectria</i> sp.	A	Ind
<i>Leotiaceae</i>		
<i>Durella</i> sp.	A	Ind
<i>Lophiostomataceae</i>		
<i>Massarina</i> sp.	B	Tr
<i>Melanommataceae</i>		
<i>Karstenula</i> sp.	A	Ind
<i>Trematosphaeria cariosa</i> (FAIRM.) BOISE	A	Ind
<i>Microthyriaceae</i>		
<i>Microthyrium</i> sp.	A	Ind
<i>Pleomassariaceae</i>		
<i>Asteromassaria macrospora</i> (DESM.) HÖHNEL	B	Sb
<i>Pleosporaceae</i>		
<i>Pleospora herbarum</i> (PERS. : FR.) RABENH.	A	Ind
<i>Valsaceae</i>		
<i>Diaporthe fagi</i> Wehmeyer	C	Lb
<i>Valsa ambiens</i> (PERS. : FR.) FR.	D	SbTw
<i>Valsa</i> sp.	D	SbTw
<i>Xylariaceae</i>		
<i>Hypoxyton fragiforme</i> (SCOP : FR.) J. KICKX.	D	SbTw
<i>Hypoxyton mammatum</i> (WAHL.) J. H. MILL.	C	SbTw
<i>Hypoxyton rubiginosum</i> (PERS. : FR.) FR.	D	Tr
<i>Hypoxyton cohaerens</i> (PERS. : FR.) FR.	D	TrLb
<i>Ustulina deusta</i> (FR.) PETRAK	C	Tr
<i>Xylaria polymorpha</i> (PERS : FR.) GREV.	C	Tr

Hypoxylon. The microfungal community associated with small branches (0.5 to 3 cm in diameter) is diverse, with 19 taxa preferring this location. *Diatrype* pathogens are characteristic of small branches. Finally, 18 species preferred twigs, with a dominance by *Valsa* species.

4.3. New Species of Microfungi

We found two new species never described before, one attributed to the genus *Diarimella* SUTTON and the other in the genus *Polynema* LÉVEILLÉ. The *Diarimella* found in the Boisé-des-Muir is the first of the genus found in temperate forest. Previously, the genus was known from only 2 species colonizing plants of tropical environment, in Argentina, Brazil and India (NAG RAJ 1993). The new species, *Diarimella laurentidae* VUJANOVIC & al. was described in VUJANOVIC & al. 1998 [specimens are kept at the Marie-Victorin Herbarium in Montreal, MT:11205 (holotype) and at the Agriculture Canada Herbarium in Ottawa, DAOM: 11205 (isotype)]. The genus *Polynema* was known in North America by only one species, represented by a single specimen collected in Pennsylvania more than a century ago (Harvard University Herbaria, FH: 97-5539, dated 2-12-1887). The species was named *Polynema muirii* VUJANOVIC & al. (VUJANOVIC & al. 1999b). The holotype is kept at the Marie-Victorin Herbarium (MT:12615).

4.4. New Taxa of Microfungi on *Fagus grandifolia*

Generally, there is a close relationship between a species of microfungi and the host or hosts that it can infect or colonize. Many taxa found on American beech in the Boisé-des-Muir had previously never been found before on any species of beech. This is specifically the case for all the members of the appendage coelomycetous taxa of sub-family *Phialotromatinae*, according to SUTTON's classification (1980), i.e. 4 genera and 5 species. Other than the taxa never found on beech species before, 61% of the *Hyphomycetes*, 45% of the *Coelomycetes* and 25% of the *Ascomycota* identified at the Boisé-des-Muir were new on *Fagus* in North America.

5. Discussion

The inconspicuous microfungi are generally ignored from biological surveys in old-growth forests or in other ecosystems because their identification, when possible, is often time consuming and requires a distinctive expertise. This unfortunate omission leaves out one of the most important elements of the biodiversity in old-growth forests. For example, alone from the branches and the bark of a single tree species in the Boisé-des-Muir, we isolated 76 species of microfungi. Considering the number of unidentified isolates and the fact that the leaves, flowers and fruits were not sampled, we guess that the actual number of fungi species may well be more than three times over. Our simple inventory allowed us to significantly increase

the number of species known to colonize *Fagus*, particularly *Fagus grandifolia*, and to discover two new species as well as one sub-family never before found on *Fagus*. Furthermore, the integration of their asexual form genera with their sexual counterparts should be an important goal for the future mycological research.

While an important part of the discoveries we made could be simply attributed to our poor general knowledge of microfungi distribution and abundance, we have no doubt that the old-growth Boisé-des-Muir has an exceptionally rich and diverse biodiversity. This large number of species we found (in spite of the restriction to corticate, still attached material), including rare or unknown species, and their complex vertical distribution support the hypothesis that old-growth forests have a high microfungi biodiversity due to a large number of active ecological niches (ARNOLDS 1988). They may therefore represent a large reservoir of inter- and intraspecific genotypic diversity (STONE & al. 1996). Other than the intrinsic value of this exceptional diversity, microfungi may also have a large commercial potential, as a source of biochemical products, for biotechnological applications or in biological control (VUJANOVIC 2000).

The number of specialists available to collect and document the world microfungi is insufficient and complete inventories require considerable efforts and resources. However, there are approaches and indirect isolation methods that allow basic inventories to be made more rapidly and by researchers or technicians with only limited taxonomic training (BILLS 1994). Because we know so little about microfungi biodiversity and their ecological function, any inventory could bring a real scientific contribution. Due to their high biological interest and the number of potential niches for microfungi, we believe that old-growth forests are one of the ecosystems that should particularly focus efforts for such inventories.

6. Acknowledgment

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Autor(en)/Author(s): Vujanovic Vladimir, Brisson Jaques

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