Boletellus projectellus – an alien mycorrhizal bolete new to Europe

J. Motiejūnaitė, J. Kasparavičius & A. Kačergius

[1] Institute of Botany, Nature Research Centre, Žaliųjų ežerų 49, Vilnius, LT-08406, Lithuania.
E-mails: jurga.motiejunaite@botanika.lt, jonas.kasparavicius@botanika.lt, audrius.kacergius@botanika.lt

Motiejūnaitė J., Kasparavičius J. & Kačergius A. (2011) Boletellus projectellus – an alien mycorrhizal bolete new to Europe – Sydowia 63 (2): 203–213.

Boletellus projectellus (Murill) Singer is recorded for the first time from Europe, based on collections made in Lithuania, Curonian Spit. Morphological features and analysis of ITS sequences supporting the identity of the species are provided. Ecology, distribution and status of *B. projectellus* in Lithuania and in Europe are discussed.

Keywords: fungi, Basidiomycota, *Boletaceae*, phylogeography, invasions.

Within the past decades an unusual boletoid mushroom started to attract mushroom-hunters' attention at the northern part of the Curonian Spit (Baltic Sea coast, Lithuania) and was dubbed a "red bolete" by local people. During the last decade it has become common and abundant in this area and increasing number of the specimens started to arrive at the BILAS herbarium. Examination of the macro- and microscopical characters has shown that the fungus did not belong to any boletoid species known in Lithuania or Europe and subsequently it was identified as *Boletellus projectellus* (Murill) Singer, a species known from eastern part of North America and SE Asia (Taiwan) (Murrill 1938, Coker & Beers 1943, Singer 1945, Chen *et al.* 1998).

The purpose of this paper is to discuss morphological and anatomical characteristics of *B. projectellus* in Lithuania, its ecology and status as well as sequence data of the studied specimens.

Materials and Methods

Sampling site

All examined specimens were collected during the fall of 2007-2009 in the northern part of the Curonian Spit, in Smiltynė forest district of Curonian Spit National Park. Further field observations were made in September and first decade of October in 2010, in Smiltynė and Juodkrantė forest districts.

The Curonian Spit is characterized by maritime climate; it is strongly influenced by the Baltic Sea. The climate is milder compared to most continental parts of the country; average fall and winter temperatures are by 3.0 °C to 3.5 °C higher than in the continental part, thaws are common in winter and the number of sunny days is the highest in Lithuania. Air humidity reaches 82 % in winter and 76 % in summer (Gudelis 1998). A large part (70 %) of the land is covered by forests, more than half of them are planted. Conifers prevail (80 %) with 53 % local Scots pine (*Pinus sylvestris* L.) and 27 % introduced Mountain pine (*Pinus mugo* L.). Other introduced pines, like *P. banksiana* Lamb., *P. nigra* J.F. Arnold, *P. strobus* L. and others are found in small groups or as solitary trees. The Curonian Spit is situated in the migration route of the White and Baltic Seas, therefore millions of birds pass it every year (Kuršių nerijos nacionalinio parko direkcija: http://nerija.am.lt/VI/index.php#r/195). The northern part of the Spit is also influenced by sea transport: only several kilometres of the Curonian Lagoon separate it from Klaipėda, one of the largest seaports in the region.

Morphological and anatomical characteristics

Morphological features were observed and measurements were taken from fresh basidiomata. Macrophotographs were taken by a Fuji FinePix S5000 camera. Hymenium fragments taken from dried basidiomata were rehydrated and anatomical features measured in 3 % KOH using a Zeiss Jenaval microscope (oil immersion objective 100 x); microphotographs were taken by a Nikon Coolpix 995 camera. Measurements of pleurocystidia, basidia, spores and their length/width ratios (Q) are given as follows: (minimum) mean ± standard deviation (maximum); values were rounded to one decimal place. Colours were subjectively described and recorded following Kornerup & Wanscher (1978). Voucher specimens are deposited in the mycological collection of BILAS herbarium.

DNA extraction, amplification, sequencing and analysis

Genomic DNA was extracted from two frozen fresh fruiting bodies (collected in 2009, part of the collections BILAS 48765 and 48766) with NucleoSpin® Plant II Kit (Macherey–Nagel GmbH &Co. KG, Germany) according to manufacturer's instruction using approximately 100 mg wet weight of fruiting bodies. The internal transcribed spacers 1 and 2 of rDNA, including the 5.8S rDNA, were amplified in 25 µl reactions on TProfessional 96 Gradient Thermocycler (Biometra GmbH, Germany) in the following mixture: ~ 25 ng of template, 0.25 units of Taq polymerase (Fermentas UAB, Lithuania), 2.5 µl 10× PCR buffer with KCl and $MgCl_2$, 0.2 mM of each dNTP, 10 µM of primers ITS5 and ITS4 (White *et al.* 1990). PCR conditions: 5 min at 95 °C as initial denaturation, followed 35 cycles of 30 s at 94 °C, 30 s at 55 °C, and 45 s at 72 °C, with final extension of 10 min at 72 °C. Amplicons were visualised under UV light in 1.5 % agarose gels stained with AtlasSight DNA Stain (Bioatlas, Estonia). The PCR products were purified according to the Protocol for PCR Product Clean-up with Exonuclease I and FastAP™ Thermosensitive Alkaline Phosphatase (Fermentas UAB, Lithuania). Purified PCR products were sequenced by Macrogen (Macrogen Inc., Seoul, Korea) on an ABI 3730XL DNA sequencer. PCR products were successfully amplified with the estimated size (692 characters). Four different PCR products from each specimen with 2 repeats for each were sequenced from both ends (5' and 3') to confirm the sequence. The rDNA homology searches (BLAST) were performed through the internet at the National Center for Biotechnology Information (National Institutes of Health, Bethesda, USA). Sequences were aligned by using Clustal W, and the phylogenetic tree of the closely related sequences was constructed using the Lasergene software package (DNASTAR, Inc., Madison, USA) by Neighbour-Joining (NJ). Sequences derived in this study were deposited in the NCBI GenBank database. The accession numbers and data are provided in Table 1.

Results and Discussion

Morphological and anatomical description
Boletellus projectellus (Murill) Singer, Farlowia, 2: 129. 1945. – Figs. 1–5.

P i l e u s 3 cm to 14 cm broad, convex, almost plane when older, margin free, projecting and hanging straight down (Figs. 1–3), surface dry, smooth, subtomentose, not cracked, colour brownish orange (6C4) when young, becoming brownish red (8C8–9C8) with age. C o n t e x t thick, up to 1.5 cm above the stipe, pale brownish-greyish orange (5B3) turning slightly darker and flushed vinaceous near the cuticle when cut. T u b e s pale yellow (3A3) when young, later olivaceous (3C8), 1.2 cm to 2 cm long, depressed around the stipe, not staining when cut or bruised. P o r e s 1–2 mm broad, circular, yellow (3A4–3A5) when young, finally pale olive (3B7–3C8). S t i p e 3 cm to 12 cm long, 1 cm to 3 cm thick, mostly tapering upward, with a coarse reticulum from top to the bottom, base covered with white mycelium (Figs 2–3); surface yellowish (4A4) when young, later becoming brownish yellow (5B5) to reddish (7C7). F l e s h whitish to yellowish (3A2–3A3), buff (5B4) at the base, slightly darkening on exposure. Odour not distinctive, taste slightly acidic. S p o r e p r i n t olivaceous brown (4D5). S p o r e s (27.3) 30.1 ± 2.3 (36.4) x (7.8) 10.8 ± 1.2 (13.3) µm, Q = (2.1) 2.8 ± 0.3 (3.7) (n = 51), ovate to fusiform, with a prominent suprahilar depression, smooth, lacking a distinct apical pore, yellow in KOH (Fig. 4). B a s i d i a (36.4) 41 ± 3.9 (44.2) x (12.7) 14.9 ± 1.1 (17.9) µm (n = 28), cla-

Tab. 1. – Names, accession numbers and reference details of the isolates studied

Species	GenBank accession No.	Country of origin, reference
Aureoboletus thibetanus	DQ200917	China, Matheny *et al.* 2007
Boletus aereus	EU417845	Netherlands, Beugelsdijk *et al.* 2008
Boletus betulicola	EU417874	Netherlands, Beugelsdijk *et al.* 2008
Boletus betulicola	EU417846	Finland, Beugelsdijk *et al.* 2008
Boletus edulis	EU417857	Netherlands, Beugelsdijk *et al.* 2008
Boletus edulis	EU417852	Finland, Beugelsdijk *et al.* 2008
Boletus edulis	EU417873	Sweden, Beugelsdijk *et al.* 2008
Boletus edulis	EU417853	Sweden, Beugelsdijk *et al.* 2008
Boletus edulis	EU417856	Netherlands, Beugelsdijk *et al.* 2008
Boletus edulis	EU417870	Netherlands, Beugelsdijk *et al.* 2008
Boletus edulis var. *albus*	EU417862	Belgium, Beugelsdijk *et al.* 2008
Boletus edulis var. *aurantioruber*	EU231978	USA, Dentinger & McLaughlin 2007
Boletus edulis var. *clavipes*	EU231985	USA, Dentinger & McLaughlin 2007
Boletus edulis var. *pusteriensis*	AY680985	Italy, Leonardi *et al.* 2005
Boletus fechtneri	EU417872	Netherlands, Beugelsdijk *et al.* 2008
Boletus pinophilus	EU417865	Sweden, Beugelsdijk *et al.* 2008
Boletus quercicola	EU417869	Austria, Beugelsdijk *et al.* 2008
Boletus quercicola	EU417867	Austria, Beugelsdijk *et al.* 2008
Boletus reticulatus	EU417871	Netherlands, Beugelsdijk *et al.* 2008
Morphotaxon A	EU417850	Netherlands, Beugelsdijk *et al.* 2008
Morphotaxon B	EU417860	Netherlands, Beugelsdijk *et al.* 2008
Morphotaxon C	EU417863	Netherlands, Beugelsdijk *et al.* 2008
Boletellus elatus	AB509885	Japan, Sato *et al.* 2009
Boletellus elatus	AB531456	Japan, Sato *et al.* 2009
Boletellus emodensis	AB509761	Japan, Sato *et al.* 2009
Boletellus mirabilis	EU597076	Canada, Jones *et al.* 2008
Boletellus mirabilis	AF335451	Canada, Berbee *et al.* 2001
Boletellus mirabilis	DQ367893	Canada, Dural *et al.* 2006
Boletellus mirabilis	DQ384578	Canada, Berbee *et al.* 2006
Boletellus longicolis	AB509647	Japan, Sato *et al.* 2009
Boletellus longicolis	AB509927	Japan, Sato *et al.* 2009
Boletellus obscurococcineus	AB509989	Japan, Sato *et al.* 2009
Boletellus projectellus	AY789082	USA, Binder *et al.* 2004
Boletellus projectellus	JN996470 – JN996473	Lithuania, present study
Boletellus russeli	EU569284	Mexico, Morris *et al.* 2008
Boletellus shichianus	DQ200921	China, Matheny *et al.* 2007
Uncultured Boletellus	FJ196901	Mexico, Morris *et al.* 2009
Boletinellus exiquus	AJ419183	Brazil, Martin & Raidl 2002
Boletinellus exiquus	AJ419145	Brazil, Martin & Raidl 2002
Boletinellus merulioides	DQ200922	China, Matheny *et al.* 2007
Boletinellus rompelii	AJ419192	Brazil, Martin &Raidl 2002
Leccinum crocipodium	AF454589	Netherlands, den Bakker *et al.* 2004
Leccinum crocipodium	AF454590	Netherlands, den Bakker *et al.* 2004
Retiboletus nigerrimus	AB509860	Japan, Sato *et al.* 2009
Suillus americanus	AF166502	USA, Wu *et al.* 2000

vate, yellowish in KOH, 4-spored, a few 2-spored. Pleurocystidia (82.9) 84.2 ± 11.5 (85.8) x (15.6) 18.8 ± 2.5 (23.4) µm (n = 16), ventricose to subclavate, smooth, readily collapsing (Fig. 5).

Habitat. On soil, solitary to scattered under pines (*Pinus mugo* and *P. sylvestris*), occasionally abundant in *P. mugo* plantations.

Distribution in Lithuania. So far, the fungus was observed only in the Curonian Spit; no records for the continental part of the country.

Material examined. LITHUANIA, Curonian Spit National Park, Smiltynė forest district, forest square No 22, area No 27, N55°38'024" E21°07'652", among mosses under *P. sylvestris*, 5 Sep 2007, *leg.* J. Kasparavičius (BILAS 48763); Same locality and collector, 3 Sep 2008 (BILAS 48767); 7 Sep 2009 (BILAS 48764); 6 Oct 2009 (BILAS 48768); forest square No 22, area No 24, N55°38'074" E21°07'679", among mosses under *P. mugo*, 7 Sept 2009, *leg.* J. Kasparavičius (BILAS 48765 and 48766). Note: additional observations of the fungus (specimens not collected) were made: Curonian Spit National Park, Smiltynė forest district, forest square No 22, N55°37'877" E21°07'582", among mosses under *P. mugo* (massive appearance of basidiomata), 1 Sep 2010 (observed by J. Motiejūnaitė); Juodkrantė forest district, forest square No 54, N55°31'029" E21°06'297", among mosses under *P. sylvestris* (several solitary fruiting bodies), 2 Sep 2010 (observed by J. Motiejūnaitė).

Phylogenetic analysis

Four ITS sequences were produced in this study, each consisting of 692 characters. They were added to 43 sequences of boletoid species of the genera *Boletus, Boletellus, Aureoboletus, Boletinellus, Retiboletus* and *Leccinum*) (Tab. 1, Fig. 6). The sequence of *Suillus americanus* was used as outgroup. Sequences of the Lithuanian isolates nested in a *Boletus* s. str./*Boletellus* clade, subclade of smooth-spored *Boletellus* (section *Mirabilis*) (Fig. 6). They formed a discriminatory group with *B. projectellus* with a high bootstrap value (94 %), thus substantiating morphological and anatomical identification of the species.

Discussion

The genus *Boletellus* Murill encompasses ca. 50 described species worldwide, the majority with tropical distributions (Ortiz-Santana *et al.* 2007, Fulgenzi *et al.* 2008, Halling & Ortiz-Santana 2009). *Boletellus* has been variably defined, yet all definitions have the following features in common: spore deposit olivaceous brown, hymenophore yellow becoming olivaceous with time, and a boletoid tube trama (Fulgenzi *et al.* 2008). Singer (1986), e. g., accepted a wide definition of the genus, including species with both smooth and variously ornamented spores; meanwhile Smith and Thiers (1971) included only the species with longitudinally striate, winged or ridged spores. There is no exhaustive molecular research on the genus, however, the phylogram of Dentinger *et al.* (2010, p. 1286) demonstrated that *Boletellus* (including species with both smooth and ornamented spores) formed a separate clade together with *Aureoboletus*. *Boletellus projectellus,* with smooth and extremely large spores – the largest of all boletes (Singer 1945) belongs to the section *Mirabilis* that encompasses the smooth-spored species of the genus; its exceptional spore size and morphological characters make this fungus fairly easily identifiable. In Lithuania and in the adjacent countries there are no similar boletoid fungi that may be confused with *B. projectellus* and, as the region is comparatively well studied by mycologists, it is safe to assume that such a conspicuous species could not have escaped the attention of researchers or amateurs. An indirect support for the assumption that *B. projectellus* is a recent arrival to the region is the fact that from the Curonian Spit, so far the only Lithuanian locality of this fungus, a number of new alien fungi were recorded, especially recently (Markovskaja & Treigienė 2009, Markovskaja *et al.* 2011, Markovskaja and Kutorga pers. comm.).

Members of *Boletellus* are found in all pantropical regions and, to some extent, in temperate zones of North America (Pegler & Young 1981) and Asia (Nagasawa 1997). *Boletellus projectellus* is widely distributed in eastern North America from Canada to Mexico (Murrill 1938, Coker & Beers 1943, Singer 1945). It is also known from SE Asia, Taiwan (Chen *et al.* 1998). *Boletellus projectellus* is associated with pines and is apparently capable of switching hosts within the genus *Pinus* quite easily (Both 1993), thus making a good candidate for an invasive species. In Taiwan, however, it was reported from a broad-leaved forest (the presence of pines was not mentioned) (Chen *et al.* 1998).

In Lithuania, *B. projectellus* was recorded in stands of both local *Pinus sylvestris* and introduced *P. mugo*, though, according to our observations, production of basidiomata was obviously more abundant in the latter stands. It is difficult to tell when and how the species arrived to the area. Interviews with local people have revealed that first basidiomata were observed in the eighties of the 20th century and that they remained very much localized and scarce approximately for a decade. The fungus started to spread during the last decade, and at present it is one of the more common boletoid mushrooms in the northern part of the Curonian Spit, though it has not yet spread to the continental part of the country. Nothing is known about the rates of *Boletellus* spreading, but Pringle *et al.* (2009) reported in a well-documented study that the ectomycorrhizal *Amanita phalloides* (Vaill. ex Fr.) Link, alien to North America, may spread up to 10 km per year. Based on such an assumption and the information on the first sightings of *B. projectellus* in the Curonian Spit, it can be estimated that the fungus arrived about 30-35 years ago.

Figs. 1–5. – *Boletellus projectellus*: **1.** Mature basidioma. **2.** Young basidioma in longitudinal section. **3.** Old basidioma. **4.** Spores. **5.** Cystidium.


1
1 cm
2
1 cm
3
1 cm
4
10 µm
5
10 µm

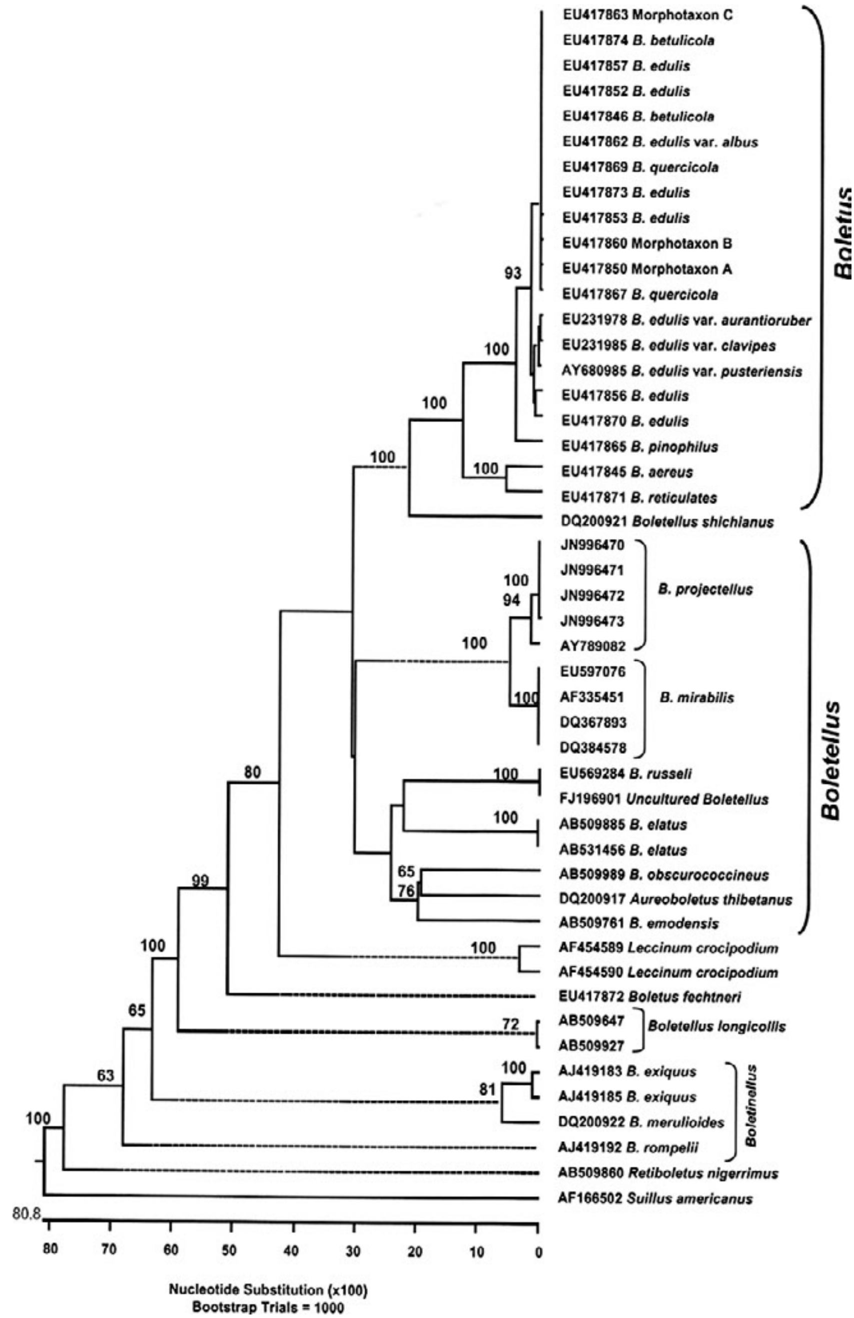

Fig. 6. – Neighbour-joining tree derived from ITS region of the rRNA gene, calculated in Lasergene without pairwise corrections. Numbers above branches are bootstrap values obtained from 1000 replications, shown only for branches supported by more than 50 %. Length of the branches is proportional of number of changes.

Though theoretically there may be several possible pathways of arrival as well as several vectors of introduction, the likeliest way for a mycorrhizal fungus is through roots and soil of introduced plant seedlings (Vellinga *et al*. 2009). The Curonian Spit holds extensive plantations of alien mountain pine, some of considerable age – the first seedlings of the introduced *P. mugo* were imported from Denmark and Belgium in the 19th century (Bučas 2001). The use of imported seedlings, however, was ceased after World War II. Few individuals of other alien pines were planted in the Curonian Spit at various periods, but their origins are difficult to track. Other possibilities may be an introduction through human transport (especially marine) or natural ones like wind or birds. These possibilities, however, would involve dispersal via spores, which is effective at a local scale, but not at wide distances (Vellinga *et al*. 2009). It is assumed that birds can play a role in distribution of lichen diaspores, e.g., the appearance and establishment of the arctic alpine lichen *Flavocetraria nivalis* (L.) Kärnefelt & A. Thell in Vistula Spit (Poland) is attributed to migrating birds (Fałtynowicz & Budzbon 1983), but, such assumptions do not exist so far for mycorrhizal fungi.

This is the first record of *B. projectellus* in Europe, and, assuming that it is an invasive fungus, it will be possible to track and evaluate its prospective spreading along geographic and climatic gradients. It is already known that the fungus invades sites harbouring alien plants and native forest as well. At this stage of invasion it would be worthwhile to study also its influence and relationships with indigenous mycorrhizal fungi.

Acknowledgments

The study was funded by a grant No LEK -23/2010 from the Research Council of Lithuania.

References

Berbee M. L., Inderbitzin P., Zhang G. (2001) Class project, University of British Columbia Biology 323, Structure and Reproduction of Fungi. http://www.ncbi.nlm.nih.gov/ (Last accessed: 19.04.2011).

Berbee M. L., Wright S. H. A., Lim S. (2006) University of British Columbia Biol. 323 class project. http://www.ncbi.nlm.nih.gov/ (Last accessed: 19.04.2011).

Beugelsdijk D. C. M., van der Linde S., Zuccarello G. C., den Bakker H. C., Draisma S. G. A., Noordeloos M. E. (2008) A phylogenetic study of *Boletus* section *Boletus* in Europe. *Persoonia* **20**: 1–7.

Binder M., Matheny P. B., Hibbett D. S. (2004) Assembling the Fungal Tree of Life. http://www.ncbi.nlm.nih.gov/ (Last accessed: 19.04.2011).

Both E. E. (1993) *The Boletes of North America*. Buffalo Museum of Science, New York.

Bučas J. (2001) *Kuršių Nerijos nacionalinis parkas*. Savastis, Vilnius.

Chen Ch. M., Yeh K. W., Hsu H. K. (1998) The Boletes of Taiwan (X). *Taiwania* **43**(2): 140–149.

Coker W. C., Beers A. H. (1943) *The Boletaceae of North Carolina*. North Carolina Press, Chapel Hill.

den Bakker H.C., Gravendeel B., Kuyper T.W. (2004) An ITS phylogeny of *Leccinum* and an analysis of the evolution of minisatellite-like sequences within ITS1. *Mycologia* **96**: 102–118.

Dentinger B. T. M. Ammirati, J. F., Both E. E., Desjardin D. E., Halling R. E., Henkel T. W., Moreau P.-A., Nagasawa E., Soytong K., Taylor A. F., Watling R., Moncalvo J.-M., McLaughlin D. J. (2010) Molecular phylogenetics of porcini mushrooms (*Boletus* section *Boletus*). *Molecular Phylogenetics and Evolution* **57**: 1276–1292.

Dentinger B. T. M., McLaughlin D. J. (2007) The evolution of specialization in ectomycorrhizal fungi: an example from porcini. http://www.ncbi.nlm.nih.gov/ (Last accessed: 19.04.2011).

Durall D. M., Gamiet S., Simard S. W., Kudrna L., Sakakibara S. M. (2006) Effects of clearcut logging and tree species composition on the diversity and community composition of epigeous fruit bodies formed by ectomycorrhizal fungi. http://www.ncbi.nlm.nih.gov/ (Last accessed: 19.04.2011).

Fałtynowicz W., Budzbon E. (1983) Drugie stanowisko *Cetraria nivalis* (L.) Ach. na niżu polskim. *Fragmenta Floristica et Geobotanica* **29** (3–4): 451–456.

Fulgenzi T. D., Mayor J. R., Henkel T. W., Halling R. E. (2008) New species of *Boletellus* from Guyana. *Mycologia* **100**: 490–495.

Gudelis V. (1998) Lietuvos įjūris ir pajūris. In: *Lietuvos mokslas* **17** (eds. Liekis A., Misevičius J.), Lietuvos mokslas, Vilnius: 1-444.

Halling R. E., Ortiz-Santana B. (2009) A revision of *Boletellu*s sect. *Ixocephali*. *Mycological Progress* **8**: 237–244.

Jones M. D., Twieg B. D., Durall D. M., Berch S. M. (2008) Location relative to a retention patch affects the ECM fungal community more than patch size in the first season after timber harvesting on Vancouver Island, British Columbia. *Forest Ecology and Management* **255**: 1342-1352.

Kirstukas M. (ed.) (2004) *Lietuvos gamta*. Lututė, Kaunas.

Kornerup A., Wanscher J. H. (1978) *Methuen handbook of colour*. 3rd edn. Eyre Methuen Ltd, London.

Kuršių Nerijos nacionalinio parko direkcija/Apie nacionalinį parką/Gamta. http://nerija.am.lt/VI/index.php#r/195 (Last accessed: 25.03.2011).

Leonardi M., Paolocci F., Rubini A., Simonini G., Pacioni G. (2005) Assessment of inter- and intra-specific variability in the main species of *Boletus edulis* complex by ITS analysis. *FEMS Microbiological Letters* **243**: 411-416.

Markovskaja S., Kačergius A., Treigienė A. (2011) Occurrence of new alien pathogenic fungus *Mycosphaerella dearnessii* in Lithuania. *Botanica Lithuanica* **17**(1): 29–37.

Markovskaja S., Treigienė A. (2009) New data on invasive pathogenic fungus *Dothistroma septosporum* in Lithuania. *Botanica Lithuanica* **15**(1): 41–45.

Martin M.P., Raidl S. (2002) The taxonomic position of *Rhizopogon melanogastroides* (Boletales). *Mycotaxon* **84**: 221–228.

Matheny P. B., Wang Z., Binder M., Curtis J. M., LimY. W., Henrik Nilsson R., Hughes K. W., Hofstetter V., Ammirati J. F., Schoch C. L., Langer E., Langer G., McLaughlin D. J., Wilson A. W., Froslev T., Ge Z. W., Kerrigan R. W., Slot J. C., Yang Z. L., Baroni T. J., Fischer M., Hosaka K., Matsuura K., Seidl M. T., Vauras J., Hibbett D. S. (2007) Contributions of rpb2 and tef1 to the phylogeny of mushrooms and allies (Basidiomycota, Fungi). *Molecular Phylogenetics and Evolution* **43**: 430–451.

Morris M. H., Perez-Perez M. A., Smith M. E. Bledsoe C. S. (2008) Multiple species of ectomycorrhizal fungi are frequently detected on individual oak root tips in a tropical cloud forest. *Mycorrhiza* **18**: 375–383.

Morris M. H., Perez-Perez M. A., Smith M. E., Bledsoe C. S. (2009) Influence of host species on ectomycorrhizal communities associated with two co-occurring oaks (*Quercus* spp.) in a tropical cloud forest. *FEMS Microbiology Ecology* **69**: 274–287.

Murrill W. A. (1938) New Boletes. *Mycologia* 30: 520–525.

Ortiz-Santana B., Lodge D. J., Baroni T. J., Both E. E. (2007) Boletes from Belize and the Dominican Republic. *Fungal Diversity* **27**: 247-416.

Nagasawa E. (1997) A preliminary checklist of the Japanese Agaricales. I. The Boletineae. *Reports of the Tottori Mycological Institute* **35**: 39–78.

Pegler D. N., Young T. W. K. (1981) A natural arrangement of the Boletales, with reference to spore morphology. *Transactions of the British Mycological Society* **76**(1): 103–146.

Pringle A., Adams R. I., Cross H. B., Bruns T. D. (2009). The ectomycorrhizal fungus *Amanita phalloides* was introduced and is expanding its range on the West Coast of North America. *Molecular Ecology* **18**: 817–833.

Sato H., Tsujino R., Kurita K., Yokoyama K. (2009) Use of ITS sequences for resolving species richness, ecological differentiation, and geographical distribution of macro-fungi. http://www.ncbi.nlm.nih.gov/ (Last accessed: 19.04.2011).

Singer R. (1945) The Boletinae of Florida with notes on extralimital species. *Farlowia* **2**(1): 97–141.

Singer R. (1986) *The Agaricales in modern taxonomy*. 4th edn., Koeltz Scientific Books, Koenigstein.

Smith A. H., Thiers H. D. (1971) *The boletes of Michigan*. University of Michigan Press, Ann Arbor.

Vellinga E. C., Wolfe B. E., Pringle A. (2009) Global patterns of ectomycorrhizal introductions. *New Phytologist* **181**: 960–973.

White T. J., Bruns T., Lee S., Taylor J. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: *PCR protocols: a guide to methods and applications* (eds. Innis M. A., Gelfand D. H., Sninsky S. S., White T. J.), Academic Press, San Diego, USA: 315–322.

Wu Q. X., Mueller G. M., Lutzoni F. M., Huang Y. Q., Guo S. Y. (2000) Phylogenetic and biogeographic relationships of eastern Asian and eastern North American disjunct *Suillus* species (fungi) as inferred from nuclear ribosomal RNA ITS sequences. *Molecular Phylogenetics and Evolution* **17**: 37–47.

(Manuscript accepted 11. November 2011; Corresponding Editor: R. Pöder)