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Ionomidotis irregularis (Ascomycota, *Helotiales*) in the Czech Republic with comments on its distribution and ecology in Europe

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The first collections of the rare ascomycete *Ionomidotis irregularis* from the Czech Republic are described and illustrated with colour photos and drawings of the most important microscopic characters. Data on ecology and occurrence at 17 localities throughout Europe including some unpublished data are summarised. Evidently, *I. irregularis* prefers strongly decayed trunks of *Fagus sylvatica* as its substrate within the European beech distribution area. In North-eastern Europe, where *Fagus* is missing, the occurrence of the fungus is documented on decayed wood of several other deciduous tree species (*Alnus incana*, *Betula* sp., *Carpinus betulus*, *Ulmus glabra*). Because of its strong preferences for unmanaged, old-growth forests, *I. irregularis* should be considered an indicator and flagship species of such habitats throughout Europe.

Key words: *Ionomidotis irregularis*, distribution, ecology, indicator species, old-growth forests.

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Uveřejňujeme první české nálezy vzácné vřeckaté houby *Ionomidotis irregularis*. Popis nalezených plodnic je doplněn fotografiemi a kresbami nejdůležitějších mikroskopických znaků. Shromážděny byly údaje o výskytu a ekologii druhu na 17 evropských lokalitách včetně několika dosud nepublikovaných nálezů. Uvnitř evropského areálu rozšíření buku lesního (*Fagus sylvatica*) preferuje *I. irregularis* silně rozložené kmeny nebo silné větve buku. V severovýchodní Evropě, kde buk chybí, byl druh sbírán na tlejícím dřevě několika dalších druhů listnatých dřevin (bříza, habr, jilm, olše). Vzhledem k jeho vyhraněným stanovištním nárokům se domníváme, že může být považován za vlajkový druh pralesních a přírodě blízkých bukových a hemiboreálních lesů a že jeho nálezy mají vysokou bioindikační hodnotu.

INTRODUCTION

The representatives of the genus *Ionomidotis* E.J. Durand (Ascomycetes, *Helotiales*) form small to large, sessile to short-stipitate, dark-coloured discoid or ear-shaped apothecia, often arising from a common base. All members of this genus are lignicolous saprotrophs, some of them are associated with other fungi from the group of pyrenomycetes (Zhuang 1988). The genus comprises seven species worldwide, but only two of them are found in Europe.

I. irregularis was originally described from North America as *Peziza irregularis* Schwein. in 1832. Later on, it was placed in *Cordierites* by Cooke (1875). In 1923, two species of *Cordierites*, including *C. irregularis*, were transferred to the newly established genus *Ionomidotis* E.J. Durand (Durand 1923). On the basis of studies by Gamundí (1991), who discovered that *Ionomidotis chilensis* E.J. Durand is conspecific with *Ameghiniella australis* Speg. and discussed the differences between the genera *Ameghiniella* Speg. and *Ionomidotis* E.J. Durand, Kirk et al. (2008) and Robert et al. (2005) accepted the generic name *Ameghiniella* Speg. and treated *Ionomidotis* E.J. Durand as a synonym. However, since no new combinations for the other members of the *Ionomidotis* genus have been proposed, we use the well-established name *Ionomidotis irregularis* in this article.

The other *Ionomidotis* species reported from Europe, *Ionomidotis fulvotingens* (Berk. & M.A. Curtis) E.K. Cash, is originally also a North American taxon described from Pennsylvania, USA. In Europe it was first found in Switzerland (Luthi 1969) and since then this comparatively small and inconspicuous species has been collected in several other European countries (Vesterholt 2000, Lohmeyer & Kasparek 2002).

The genus *Ionomidotis* was placed in the family *Leotiaceae*, subfamily *Encoelioidae* by Korf (1973) and Zhuang (1988) and is characterised by a ionomidotic reaction of apothecial tissues (a purplish brown pigmentation appears when treated with an aqueous KOH solution). However, molecular studies have indicated that the genus *Encoelia* belongs in the *Sclerotiniaceae* (see e.g. Johnston & Park 2005). Although *Ionomidotis* is currently considered a member of the family *Helotiaceae* (Robert et al. 2005), the taxonomy of the order *Helotiales* including *Ionomidotis* and related taxa is unsettled and further molecular study is needed (Kirk et al. 2001, Baral 2003).

The aim of this article is to describe the first records of this remarkable species in the Czech Republic and to summarise available data on its distribution and ecology in Europe including some new, unpublished information.

MATERIAL AND METHODS

Colour photographs of all Czech collections were taken in situ. All voucher specimens studied are deposited in the herbarium of the National Museum in Prague, Czech Republic (PRM). Other herbarium abbreviations used: CNF – Croatian Mycological Society Croatian National Fungarium, Zagreb, Croatia; HB – herbarium Hans Otto Baral; LE – herbarium of the V.L. Komarov Botanical Institute, Saint Petersburg, Russia; RPK – herbarium R.P. Korf (housed in CUP – Plant Pathology Herbarium, Ithaca, USA); RW – herbarium Ruben Walleyn, Gent, Belgium; TAAM – fungal herbarium of the Institute of Agricultural and Environmental Sciences, Tartu, Estonia; TU – Herbarium of Tartu University, Estonia; TUR – Herbarium of the University of Turku, Finland; WU – Herbarium of Vienna University, Austria.

The description of macroscopic characters is based on fresh material from Holý kopec Nature Reserve, Czech Republic (PRM 859942, PRM 859943). The ionomidotic reaction was examined both in fresh and dried material by applying a 5% aqueous solution of NaOH. Microscopic features were briefly observed on fresh apothecia and subsequently more precisely studied on dried material in Melzer's and Lugol's reagents, a 5% aqueous solution of NH_4OH, Cotton blue and ammoniac Congo-red under a light microscope at magnifications of 400× and 1000×. Unless otherwise specified, the description of microscopic characters is given as observed in ammoniac Congo-red. A total of 20 ascospores were measured. Terminology of the degree of forest naturalness follows Vrška & Hort (2003).

Specimens studied

Czech Republic. Bohemia, Šumava National Park, central part of Boubínský prales National Nature Reserve, on lying decaying trunk of *Fagus sylvatica* in natural fir-beech forest, ca 1000 m alt., 10. IX. 2009 leg. Z. Egertová et M. Kříž, det. S. Glejdura (PRM 899989). – Moravia, Chřiby hills, Buchlovice, Holý kopec Nature Reserve, ca. 530 m NNW of the top of Holý kopec, on the upper side of a moist, rather decayed lying trunk of *Fagus sylvatica* (ca. 70 cm in diameter) covered with mosses (*Brachythecium* sp.) in mesophilous near-natural beech forest (*Carici pilosae-Fagetum* Oberdorfer 1957), in a moist place with a rich understorey, 420 m alt., 27. IX. 2011 leg. et det. J. Běťák (PRM 859942); Ibid., 26. X. 2011 leg. et det. J. Běťák (PRM 859943).

Poland. Hajnówka, Bialowieża virgin forest, quadrate no. 343, on decaying trunk of *Carpinus betulus*, 3. IX. 1973 leg. V. Holubová, det. Z. Pouzar (PRM 902629); Ibid., 3. IX. 1973 leg. V. Holubová, det. Z. Pouzar (PRM 902623). – Hajnówka, Bialowieża virgin forest, quadrate no. 283, on decaying trunk of *Ulmus* sp., 4. IX. 1973 leg. et det. Z. Pouzar (PRM 902617).

Slovakia. Nízké Poloniny, Nová Sedlica, Stužica National Nature Reserve, on decaying trunk of *Fagus sylvatica*, 22. X. 1987 leg. et det. Z. Pouzar (PRM 854508).

RESULTS

At the end of September 2011 during a field excursion to the Chřiby hills in south-east Moravia, the first author found interesting blackish apothecia on a decaying trunk of beech. The fruitbodies were too young and presumably sterile, therefore part of the collection was left at the locality until a next visit. In late October, well-developed ascocarps were collected at the same site. After studying the material in the laboratory, the fungus was identified as *Ionomidotis irregularis* (Schwein.) E.J. Durand – a very rare species, which had presumably not been reported from Czech Republic before. Its characters are described below.

Ionomidotis irregularis (Schwein.) E.J. Durand, Proc. Amer. Acad. Arts & Sci. 59: 9 (1923). Figs. 1–4

≡ *Peziza irregularis* Schwein., Trans. Am. Phil. Soc., Ser. 24(2): 171 (1832).
≡ *Cordierites irregularis* (Schwein.) Cooke, Bull. Buffalo Soc., Nat. Sci. 3: 26 (1875).
≡ *Midotis irregularis* (Schwein.) Cooke in Sacc., Syll. Fung. 11: 42 (1895).
= *Peziza doratophora* Ellis et Everh., J. Mycol. 1: 90 (1885).
≡ *Otidea doratophora* (Ellis et Everh.) Sacc., Syll. Fung. 8: 96 (1889).
= *Poloniodiscus fischeri* Svrček et Kubička, Česká Mykol. 21(3): 154 (1967).

Description. Apothecia of tough, gelatinous consistency, at first discoid, soon ear-shaped or irregularly lobed and fan-shaped when old (resembling small clustered apothecia of *Otidea* spp.), with short stalk arising from the common base joining 2–5 apothecia, solitary or more often gregarious to clustered, (10)15–30 mm in diameter (Fig. 1a, b). Hymenium smooth, first dark purplish black, later blackish. Outer surface blackish, covered with slightly pustulate to powdery dark olive-brown, easily removable coating. Flesh up to 4 mm thick, leathery gelatinous and dark purplish black in fresh condition, blackish and fragile when dry. Smell and taste inconspicuous. Fresh and dried tissue exuding purplish brown pigmentation in 5% NaOH (ionomidotic reaction).

Asci eight-spored, ca. 70 × 7 µm, narrowly clavate, tips not turning blue in Melzer's or Lugol's reagents (I-, IKI-). Ascospores 7–9 × 2.8–3.5 µm, narrowly ellipsoid, usually with two large and several small guttules. Paraphyses of two types: filiform, up to 3 µm broad, usually two– to four-septate, slightly broadened in upper part (up to 4 µm), with conspicuous, lanceolate, acute apex (Fig. 4a); other type similar, ± narrowly cylindrical, irregularly curved and sometimes shortly branched, not pointed at apex. Ectal excipulum consisting of thin-walled, rounded to irregularly shaped cells (10–25 µm in diameter), with brown to purplish brown intracellular pigment (in 5% NH_4OH), embedded in a thin gel layer in fresh material. Medullary excipulum of densely arranged, slightly gelified, septate hyphae up to 6 µm thick, with purplish brown contents (Fig. 4b).

Fig. 1. a – young, sterile ascomata of *Ionomidotis irregularis* in Holý kopec Nature Reserve, Czech Republic, 27. IX. 2011 (PRM 859942). **b** – mature apothecia of *Ionomidotis irregularis* at the same locality, 26. X. 2011 (PRM 859943). Photo J. Běťák.

Fig. 2. Old ascomata of *Ionomidotis irregularis* from Žofínský prales National Nature Reserve (Czech Republic) on decaying beech trunk, 5. X. 2004 (herbarium R. Walleyn, Gent, no. 3710). Photo P. Škubla.

Fig. 3. Mature ascomata of *Ionomidotis irregularis* from Boubínský prales National Nature Reserve (Czech Republic) on lying decaying beech trunk, 10. IX. 2009 (PRM 899989). Photo Z. Egertová.

Other finds from the Czech Republic. Later, when gathering information on the distribution of the species, three older, to date unpublished collections from the Czech Republic were re-discovered. In October 2004 the species was found in Boubínský prales National Nature Reserve in the Šumava Mountains by J. Burel during a mycological workshop held in Frymburk and was subsequently identified by the Croatian mycologist N. Matočec. A voucher specimen is deposited in the Croatian National Fungarium (CNF-2/7103). On the same day, another locality was discovered by the now deceased Belgian mycologist R. Walleyn during a concurrent field excursion to the Žofínský prales Nature Reserve (RW 3710, see Fig. 2). These two finds therefore represent the first collections of *I. irregularis* in the Czech Republic. Five years later, the species was re-collected in Boubínský prales by Z. Egertová and the third author of this paper (PRM 899989, see Fig. 3).

Notes on studied specimens. A thick greyish fibrous-powdery covering was present on the hymenium of old fruitbodies from Stužica National Nature Reserve (PRM 854508). Microscopically, a process of septal strangulation and subsequent disintegration of upper parts of the lanceolate paraphyses, and formation of thick-walled conidia-like cells was observed on this material. A thin greyish covering on the hymenium was present also in material from the Chřiby hills (PRM 859943, see Fig. 1b), but microscopically only a few conidium-like cells were observed.

DISCUSSION

Taxonomy

Except for the presence of conidium-like cells in the two collections mentioned above, all specimens studied agree well in all main features with descriptions and illustrations of the species as given in the literature (Svrček & Kubička 1967, Zhuang 1988, Pärtel & Põldmaa 2008, Tabarés et al. 2010). More detailed morphological and anatomical characteristics of the ascomata are presented there.

When mature, *I. irregularis* is easily recognised by both macroscopic and microscopic characters (large ear-shaped blackish apothecia with powdery, olive-brown coating on the outer surface, ionomidotic reaction of the tissues, lanceolate paraphyses). Very young ascomata may macroscopically resemble other members of the genus. However, the only other species occurring in Europe – *I. fulvotingens* – forms discoid apothecia (when fresh), not exceeding 10 mm in diameter at maturity. It also differs in its ecological requirements (Lohmeyer & Kasparek 2002). Microscopically, *I. irregularis* differs from other members of the genus especially by having paraphyses with acute, lanceolate tips. *Diplocarpa bloxamii* (Berk. ex W.

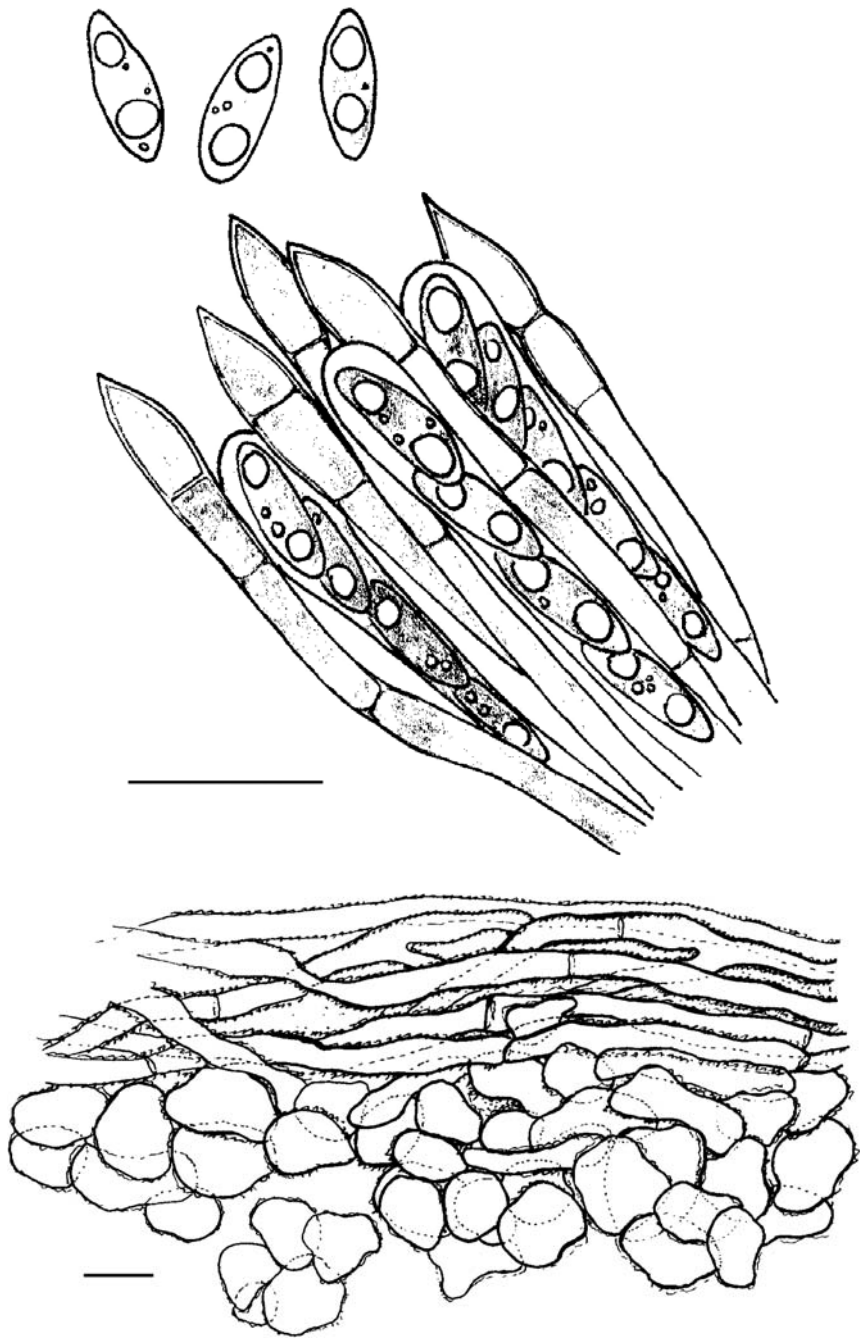

Fig. 4. *Ionomidotis irregularis* (PRM 859943). **a** – ascospores, lanceolate paraphyses and asci. **b** – ectal and medullary excipulum. Scale bars = 10 µm. J. Běťák del.

Phillips) Seaver is microscopically almost identical and shows the ionomidotic reaction as well, but has much smaller and more stipitate apothecia with an olive-green hymenium and hairy margin (Seaver 1937, Baral 2003, Petersen & Læssøe online: species, *Diplocarpa bloxamii*). For a more detailed discussion on the similarities between *Diplocarpa* and *Ionomidotis*, see Baral (2003).

Distribution

Ionomidotis irregularis was collected in central Europe for the first time in 1966 by the German mycologist Wolfgang Fischer during the field excursion of the 4th Congress of European Mycologists in the Bialowieża virgin forest in Poland (Svrček & Kubička 1967). Fischer kindly gave part of the material to Czech mycologists. Due to an unsuccessful search for this fungus in the literature, M. Svrček and J. Kubička described a new genus, *Poloniodiscus*, naming the species after its collector – *Poloniodiscus fischeri* Svrček et Kubička. Korf (1973) discovered the species to be conspecific with *Ionomidotis irregularis* (Schwein.) E.J. Durand and *Poloniodiscus fischeri* became its synonym.

During the following years the fungus was discovered in several other Central European countries – Austria (Zhuang 1988, Dämon et al. on-line), Slovakia (Kuthan et al. 1999, Heilmann-Clausen & Christensen 2004, Adamčík et al. 2007, Kunca pers. comm. 2012) and Switzerland (H.O. Baral pers. comm. 2011).

The first record of *I. irregularis* in Northern Europe originates from Estonia, where Ain Raitviir collected it from a nemoral bog island in the Nigula Nature Reserve in south-west Estonia in 1963. Although published (Raitviir 1974), the specimen was located only recently, as it had been misplaced in the TAAM fungal collection. The record from 1988 originates from the same Nature Reserve (Kalamees & Vaasma 1989) but more precise collection data and a voucher specimen are lacking. In 2011 the species was recollected in the northern part of Nigula, ca. 3 km N of the original location (K. Põldmaa, pers. comm.). During 2006–2009 *I. irregularis* was also found in north-east Estonia (Muraka Nature Reserve), and in Alam-Pedja Nature Reserve in central Estonia (Pärtel & Põldmaa 2008, Anonymus 2011a). In 2005 the fungus was collected in Etelä-Häme county, central Finland (Huhtinen & Halme 2007) and in 2011 another locality was discovered in the newly established Sipoonkorpi National Park near Helsinki, south Finland (Huhtinen pers. comm. 2011).

There are only two known localities of *I. irregularis* in southern Europe, both of them situated in mountainous regions of Spain. The first record from 2005 in the Catalan Pyrenees at an altitude of 1400 m a.s.l., represents the highest location of the species in Europe (Tabarés et al. 2010). The other collection originates from Asturia in northern Spain (Lechat on-line). For more detailed information on European records of *Ionomidotis irregularis*, see Tab. 1.

Tab. 1. European records of *Ionomidotis irregularis*.

Year of collection	Country	Locality	Substrate	Habitat	References	Voucher specimen
1963, 1988? (no voucher), 2011	Estonia	Nigula Nature Reserve	broadleaved trees (decayed trunk)	natural hemiboreal forest (*Populus tremula, Alnus, Betula, Tilia*)	Raitviir (1974), Kalamees & Vaasma (1989), Anonymus (2011a)	TAAM194929, TU112908
1966, 1973	Poland	Bialowieża virgin forest	broadleaved tree (decayed trunk), *Carpinus betulus* (decayed trunk), *Ulmus* sp. (decayed trunk)	virgin/natural hemiboreal forest	Svrček & Kubička (1967), this paper	PRM 902629, PRM 902623, PRM 902617
1973	Austria	Scharnstein	conifer needles mixed with mosses	not known	Zhuang (1988)	RPK 4161
1987–89, 2003	Slovakia	Stužica National Nature Reserve (two microlocalities)	*Fagus sylvatica* (2 decaying trunks)	natural beech-fir forest	Kuthan et al. (1999), Heilmann-Clausen & Christensen (2004), Adamčík et al. (2007)	PRM 854508, PRM 893592, RW 3251
1996	Austria	Rotwald Nature Reserve	*Fagus sylvatica*	natural beech-fir-spruce forest	Dämon et al. (on-line), Kovacs (2000)	WU 16946
2000, 2001, 2006	Switzerland	Graubünden	*Fagus sylvatica* (decaying trunk and branch)	mixed ravine forest (*Fagus*, *Picea*, *Alnus incana*)	Baral (pers. comm. 2011)	HB 7014, HB 7015, HB 8233,
2004, 2009	Czech Republic	Boubínský prales National Nature Reserve	decaying tree trunk (*Fagus/Abies/Picea*?); *Fagus sylvatica* (well-decayed trunk)	natural beech-fir-spruce forest	Matočec, Burel (pers. comm. 2012); this paper	CNF-2/7103, PRM 899989
2004	Czech Republic	Žofínský prales National Nature Reserve	*Fagus sylvatica* (decaying tree trunk)	natural beech-fir forest	Škubla (pers. comm. 2012); this paper	RW 3710

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Tab. 1. – continuation.

Year of collection	Country	Locality	Substrate	Habitat	References	Voucher specimen
2005	Finland	Etelä-Häme (central Finland), Kuusimäki Nature Reserve	*Betula* sp. (fallen trunk)	natural spruce-dominated mixed forest	Huhtinen & Halme (2007)	TUR
2005	Spain	Arriu de Bordius (Val d'Aràn)	*Fagus sylvatica*	montane beech-fir forest	Tabarés et al. (2010)	HB 8190
2006	Estonia	Muraka Nature Reserve, Heinassaar	decaying tree trunk (strongly rotten wood)	natural hemiboreal forest with *Populus tremula, Alnus, Betula, Picea abies*	Anonymus (2011a)	TU 104011
2007, 2008	Estonia	Alam-Pedja Nature Reserve, near Utsali	deciduous tree (rotten wood); *Alnus incana* (decaying trunk)	natural hemiboreal forest (*Populus tremula, Picea abies, Alnus, Betula)*	Pärtel & Põldmaa (2008), Anonymus (2011a)	TU 104143
2009	Estonia	Alam-Pedja Nature Reserve, Ummniidu	*Ulmus glabra* (decaying trunk)	natural hemiboreal forest, elm-dominated mixed with *Fraxinus excelsior*	Anonymus (2011a)	TU 104445
2009	Spain	Valgrande (Asturia)	*Fagus sylvatica* (rotten wood)	near-natural(?) beech forest	Lechat (on-line)	
2011	Finland	Sipoo, Sipoonkorpi National Park	*Alnus incana* (three fallen trunks)	natural boreal nemoral forest	Huhtinen (pers. comm. 2011)	
2011	Slovakia	Vihorlat National Nature Reserve	*Fagus sylvatica* (fallen trunk)	natural beech-fir forest	Kunca (pers. comm. 2012)	
2011	Czech Republic	Chřiby hills, Holý kopec Nature Reserve	*Fagus sylvatica* (decayed trunk)	near-natural beech forest	this paper	PRM 859942, PRM 859943

Besides Europe, where the species is relatively rare, it has been collected several times in the United States and Canada (Zhuang 1988). There are also a few localities known from the Asian part of Russia. Besides a single record by W.Y. Zhuang from Sudzukhe Nature Reserve (Zhuang 1988), two unpublished specimens from the Russian Far East are preserved in the TAAM fungal collection in Estonia, Tartu (Anonymus 2011a). Another two more recent collections from Russia originate from western Siberia and from Altai National Park in the lower course of the Atkechu river (Shiryaev pers. comm. 2011, LE 247578, LE 247719).

Ecology

Brief ecological characteristics of substrates and habitats of European collections of *I. irregularis* are summarised in Tab. 1. Altogether there are 17 localities of the species in Europe known to us, nearly all of them situated in old-growth forests in nature reserves.

In central Europe the species evidently prefers strongly decayed beech trunks or thick branches as a substrate – the only exception is represented by the specimen from Austria (Zhuang 1988), where it was collected in "duff" (the original label information is written in French and refers to conifer needles mixed with mosses).

In northern Europe, where beech is missing, the species substrate preferences are not so strict. It has been found on lying trunks of several deciduous trees: birch, alder, elm and hornbeam (Huhtinen & Halme 2007, Huhtinen pers. comm. 2011, Anonymus 2011a, PRM 902617). In four cases detailed information about the substrate is missing – due to the late stage of decay of the logs serving as substrate, the host's identity often remains unknown. In the case of Estonia, all localities are in natural forests, two of them being situated on islands in the middle of a raised bog (Nigula and Muraka bogs), in nemoral forests where human activity has been minimal for centuries. The Alam-Pedja Nature Reserve, where four specimens have been collected at different localities, is a vast wilderness area which covers over 300 km^2 and consists of a complex of five large bogs separated by unregulated rivers, their floodplains and extensive forests.

Because of its rareness throughout Europe, localities of *I. irregularis* should be protected in all European countries where its occurrence has been documented. In Estonia *I. irregularis* was included in the Red List of Threatened Species as a vulnerable species in 2008 (Anonymus 2011b), in Finland it is considered critically endangered (Huhtinen et al. 2010). Although the number of its finds has slightly increased during the past years, it still represents one of the strictest specialists of old-growth forests and should therefore be considered an indicator and flagship species of such habitats (see also Adamčík et al. 2007). Permanent natural, unmanaged habitats seem to be the most important prerequisite for the occurrence of the species in Europe.

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