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First record of *Artomyces microsporus* in Europe

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Abstract – Previously known from only two collections from Japan, *Artomyces microsporus* has been repeatedly collected in a forest near Kiev (Ukraine). The identification of these collections has been confirmed by sequence analysis of the nuclear ribosomal internal transcribed spacer region (nrITS). The specimens are described and a comparison with *A. pyxidatus* is given. Data concerning the genus *Artomyces* in Ukraine have been checked; herbarium specimens have been revised and literature has been screened. All the collected data may be referred to *A. pyxidatus*, except the collections of *A. microsporus* presented here.

***Artomyces microsporus* / *Artomyces pyxidatus* / Ukraine / taxonomy / chorology**

Résumé – Uniquement connu jusqu'ici de deux récoltes japonaises, *Artomyces microsporus* a été récolté à plusieurs reprises dans une forêt près de Kiev (Ukraine). L'identification de ces récoltes a été confirmée par le séquençage du nrITS. Les spécimens sont décrits et une comparaison avec *A. pyxidatus* est présentée. Les données concernant le genre *Artomyces* en Ukraine ont été rassemblées ; les spécimens d'herbier ont été révisés et la littérature a été dépouillée. Toutes les données récoltées peuvent être rapportées à *A. pyxidatus*, à l'exception des récoltes de *A. microsporus* présentées ici.

***Artomyces microsporus* / *Artomyces pyxidatus* / Ukraine / taxonomie / chorologie**

INTRODUCTION

During a mycological foray in the forest near Novobilychi (Kiev, Ukraine), the first two authors (AF and VH) collected a clavarioid fungus, growing on a *Pinus* stump. The pyxidate branching pattern was indicative of

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Artomyces pyxidatus (Pers.: Fr.) Jülich, a species known to occur in Europe, but the small size of the spores and the compact habit of the sporophore was not consistent with that species. Suspecting that it could be *Artomyces microsporus* (Qiu X. Wu & R.H. Petersen) Lickey, they sent a piece of the sporophore to Ronald Petersen (Univ. of Tennessee, Knoxville), co-author of the species. The third author (EL, from the same laboratory), realized an analysis of the nuclear ribosomal internal transcribed spacer region (nrITS), which confirmed the hypothesis. The nrITS sequence has been deposited in GenBank.

Artomyces pyxidatus and *A. microsporus* are morphologically very similar species, and are mainly distinguished by spore size. However, mating tests showed that they are incompatible (Wu & Petersen, 1992). Laccase enzyme studies show similar results for the two species (Wu *et al.*, 1995), and phylogenetic study based on nrITS sequences indicates that the two species are sister taxa (Lickey *et al.*, 2003).

Before its discovery in Ukraine, *Artomyces microsporus* was only known from two collections from Japan :

– Tochigi Pref., Mashiko, 29.IX.1989, herb. *R.H. Petersen TFB 2349* (TENN 48856, holotypus).

– Our efforts to obtain more information about the second collection (K.Y. 5352) failed. It was probably collected at the same site as TFB 2349, but the date of collection is unknown, and no herbarium specimen has been kept except for a spore print, which was used for pure culture isolation (strain K.Y. 5352). It seems that this strain has been lost.

Some information has been published on the development of *Artomyces* species *in vitro*. Wu *et al.* (1995) note that *A. microsporus* rapidly fruits on agar media. In his monograph on the genus *Clavicorona*, Dodd (1972: 762) reports that he obtained fruit body development on cultures of *A. pyxidatus*, using Brodie's medium (Brodie, 1948) or 2% malt extract. Basidiocarps developed 50 days after inoculation. Many other data concerning the culture of the two species can be found in Dodd (1972), Doty (1947), James & McLaughlin (1988), Wu & Petersen (1992) and Wu *et al.* (1995). The structure of the spore wall of *A. pyxidatus* was studied by Clémençon (1970).

DESCRIPTION

The description hereunder has been written based on the specimens of *Artomyces microsporus* collected in Ukraine (see list below), particularly the collection *A. Fraiture 3067*. More detailed descriptions of the species are given by Wu & Petersen (1992) and Lickey *et al.* (2003) and information about sulphovanillin reaction in gloeocystidia and gloeoplerous hyphae is given by Hjortstam (1989).

Sporophores (Fig. 1-2) up to 9-10 (-14) × 5 (-12) cm, clavarioid, profusely and pyxidately branched from the base, devoid of a conspicuous stipe. Branches thin, densely crowded, forming up to 6-7 ranks, with a coronate tip, cream to pale pinkish ochraceous (4A2, 5A2), becoming orange brown to dark brown (7D7, 7F8) from the tips with age or desiccation (colour codes after Kornerup & Wanscher, 1978). Spore print whitish.

Basidiospores 3.0-3.35-3.8 × 2.1-2.45-2.9 µm, Q = 1.10-1.35-1.56, ellipsoid to largely ellipsoid, amyloid, appearing smooth under light microscope

Fig. 1. *Artomyces microsporus*. Sporophore (specimen *A. Fraiture 3067*, photo A. De Kesel).

Fig. 2. *Artomyces microsporus*. Sporophore, → details showing the pyxidate ramification (specimen *A. Fraiture 3067*, photo A. De Kesel).

(immersion; magnification 1560×), verrucose under SEM (Fig. 4). *Basidia* 12-16 × 3-4 µm. *Gloeocystidia* cylindrical, 3.5-5.0 µm wide, not or slightly protruding, appearing dark grey with black granules in sulphovanillin. Hymenium pale lilac in sulphovanillin. Generative hyphae clamped, thin-walled, 4.5-7.0 µm diam.

Ecology: In a *Pinus sylvestris* forest containing many *Quercus robur*, on podzolic sand. The locality is situated in a part of the forest which was partly destroyed by fire a few years ago. All the observed specimens were growing on dead wood of *Pinus sylvestris* (stumps, dead logs lying on the ground or still standing trunks).

DISTRIBUTION AND ECOLOGY OF THE GENUS *ARTOMYCES* IN UKRAINE

A survey of the data concerning the genus *Artomyces* in Ukraine has been made. Herbarium specimens were checked and literature consulted. Ukrainian mycologists working with macromycetes were contacted in order to

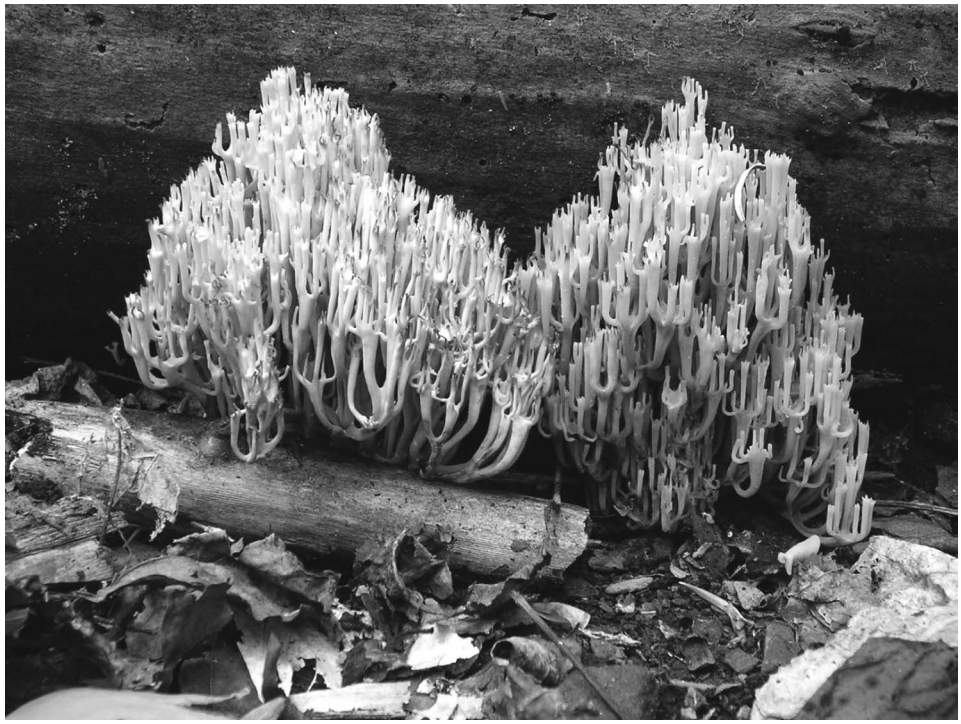

Fig. 3. *Artomyces pyxidatus*. Sporophores (photo E. Lickey).

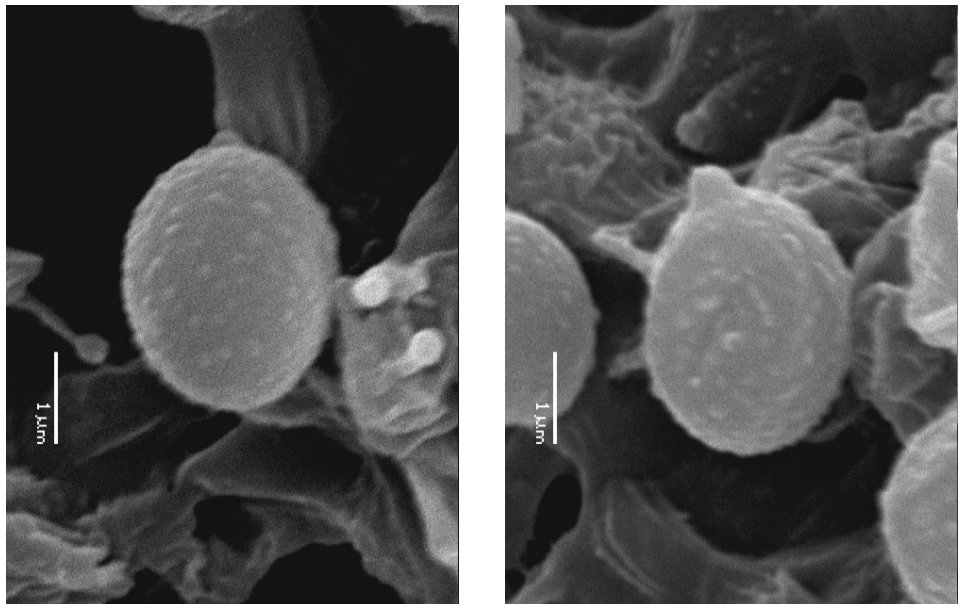

Fig. 4. *Artomyces microsporus*. Spores seen with the SEM (specimen *A. Fraiture 3067*). Bars = 1 µm.

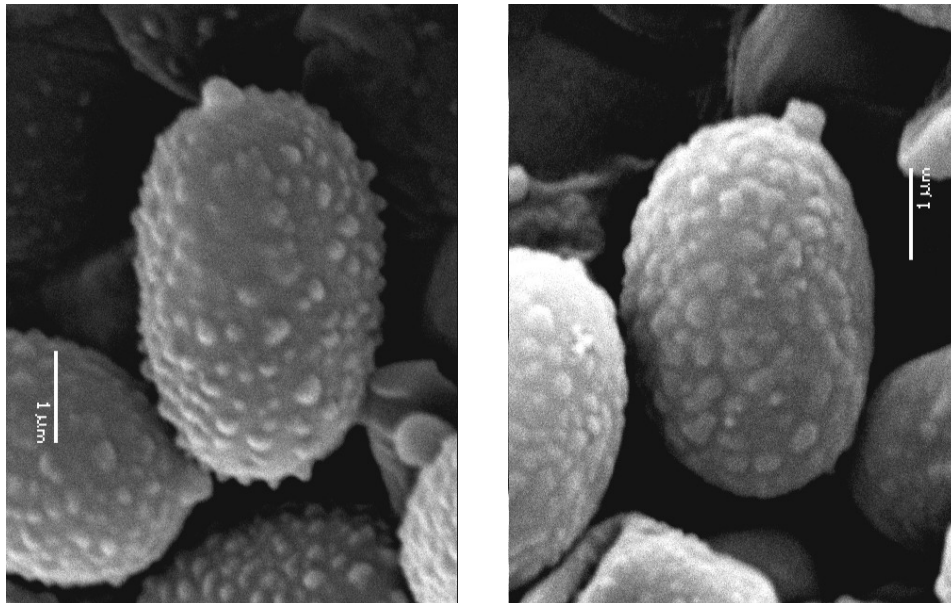

Fig. 5. *Artomyces pyxidatus*. Spores seen with the SEM (specimen *A. Fraiture 2880*). Bars = 1 µm.

obtain appropriate information. Apart from the collections we made in Novobilychi (Kiev), all other specimens we studied are identified as *Artomyces pyxidatus*. All accessible bibliographic data also seem to concern that species. A list of the studied specimens for both species, as well as a list of found bibliographic information is given below. A distribution map of *A. microsporus* and *A. pyxidatus* in Ukraine is presented in figure 6.

1) *Artomyces microsporus*

***Artomyces microsporus* (Qiu X. Wu & R.H. Petersen) Lickey**, *Sydowia* 55 (2): 227 (2003).
≡ *Clavicorona microspora* Qiu X. Wu & R.H. Petersen, *Mycotaxon* 45: 124 (1992).

Specimens observed and collected in Ukraine

Currently, *A. microsporus* is known from a single locality in a forest, near Novobilychi (Kiev), Ukraine. AF & VH observed and collected the species there several times. The fruit bodies occurred during a period between 28 June and 3 October. They were always growing on dead wood of *Pinus sylvestris* (mainly stumps but also dead trunks, either still standing or lying on the ground). This species was not observed in this area prior to a brush fire, which did not kill the trees but left superficial traces on the barks. We do not know if that circumstance had an influence on the occurrence of the species.

Specimens studied: Kiev, near Novobilychi, 50°29'N 30°19'E, 16.IX.2004, on stumps of *Pinus sylvestris*. Herb. *A. Fraiture 2943* (BR) and *V. Hayova* (KW 32263). DNA sequence analysis has been made (by EL) on a part of this collection – Ibid., 3.X.2004, on *Pinus sylvestris.* Herb. *V. Hayova* (KW 32264). – Ibid.,

Fig. 6. Distribution map of *Artomyces pyxidatus* ▲ and *A. microsporus* □ in Ukraine, from herbarium and literature data.

19.VII.2005, on dead wood of *Pinus sylvestris* (1 stump, 2 lying trunks and 1 dead trunk still standing). The carpophores were looking burnt or dry (top of the branches blackish brown). – Ibid., 04.VIII.2005. Herb. *V. Hayova* (KW 32265). – Ibid., 19.IX.2005, carpophore of 14 x 12 cm, the largest one we saw on this site. Herb. *V. Hayova* (KW 32266). – Ibid., 28.VI.2006. Herb. *A. Fraiture 3067* (BR) and *V. Hayova* (KW 32267).

Pure cultures were obtained by putting small pieces of branches of *A. microsporus* (specimen *A. Fraiture 3067 = V. Hayova KW 32267*) on MA2-50-2 agar in Petri dishes (prepared by MUCL). It is a malt (2 %) agar with an addition of 50 ppm chloramphenicol and 2 ppm benomyl. The strain is now preserved in MUCL culture collection, under the number 47701.

2) *Artomyces pyxidatus*

***Artomyces pyxidatus* (Pers.: Fr.) Jülich**, *Bibliotheca mycologica* 85: 399 (1981).

≡ *Clavaria pyxidata* Pers., *Neues Magazin für die Botanik* 1: 117 (1794). [= 1st version of the "*Tentamen*"]

≡ *Clavaria pyxidata* Pers.: Fr., *Systema Mycologicum* 1: 470 (1821).

≡ *Clavicorona pyxidata* (Pers.: Fr.) Doty, *Lloydia* 10 (1): 43 (1947).

= *Clavicorona coronata* (Schwein.) Doty, *Lloydia* 10 (1): 42 (1947).

≡ *Clavaria coronata* Schwein., *Transactions of the American Philosophical Society*, *NS*, 4 (2): 182 (1834).

= *Clavaria petersii* Berk. & M.A. Curtis, *Grevillea* 2 (n°13): 7 (1873). – *Clavaria petersii* Berk. & M.A. Curtis, in Ravenel, *Fungi Caroliniani Exsiccati* 5: 33 (1860) [invalid, Art. 41.3].

Specimens collected in Ukraine

The abbreviations for the herbaria are: Kholodny Institute of Botany, Kiev (KW) ; Mycological Department, National Museum, Praha (PRM) ; V.N. Karasin National University, Kharkov (CWU). Apart from the specimen Pilát 496045, all the collections cited hereunder have been revised by us.

- Zakarpatska oblast, Rakhiv region, Dilove village, 47°56'N 24°12'E ["Carpatorossia, in the valley of the river Lišenka near the town of Trebušany"], VIII.1936. In mixed virgin forests (*Abies alba*, *Picea excelsa*, *Fagus silvatica*, etc.), alt. 800-1000 m. On *Abies alba* and/or *Fagus sylvatica* [The text of Pilát (1958: 148) gives only *Abies alba* as substrate and the three legends of the illustrations only *Fagus sylvatica*. The substrate given by Pilát (1940: 41) for the same collections is only *Abies*]. Specimens *Albert Pilát 28764*, *29040*, *29049*, *29056* (PRM), collections cited by Pilát (1958: 148 + fig. 2, tab. 18b and tab. 19, and 1940: 41). Notes : The specimen *29056* is cited under the n°*29055* in Pilát (1958). For the localisation of Pilát's collecting sites in the Eastern Carpathians (Ukraine), see Holec (2002).

– Tchernigiv oblast, Korop region, near Rozlioty village, 51°43'N 33°08'E 18.VIII.2004. In a *Quercus* forest with *Corylus*, *Acer* and *Tilia*, in a very humid biotope. On remnants of very rotten wood lying on the ground. Specimen *Karpenko K.K. s.n.* (KW 30598) (coll. Golubtsova J.I., det. Karpenko K.K.).

– Sumy oblast, near Sumy, on the bank of the river Psiol, Berezniak forest, 50°58'N 34°52'E 26.VI.2000. Forest of *Salix alba*. On fallen and rotten trunk of *Salix alba*. Specimen *Karpenko K.K. s.n.* (KW 30597).

– Sumy oblast, near Sumy, on the bank of the river Psiol, 50°59'N 34°57'E 3.IX.2000. In a plantation of broadleaved trees. On rotten wood of *Populus* sp. Specimen *Karpenko K.K. s.n.* (KW 23241).

– Dnipropetrovsk oblast, Dniprovsko-Orilskyi nature reserve, sector 59, 48°31'N 34°47'E 24.IX.1996. In an association with *Populus nigra*, *Amorpha*, *Chelidonium* and *Elytrigia*. On a fallen trunk of *Populus nigra*. Specimen *Prydiuk M.P. 11* (KW 30595). Collection cited in Prydiuk (2000).

– Kharkiv oblast, Zmiïv region, Homolchanski National nature park, near the biological station of the University of Kharkiv, 49°37'N 36°20'E 10.VII.2004. In a *Quercus* forest, with *Acer* and *Tilia*. On rotten wood. Specimen *Akulov A. s.n.* (CWU).

– Kharkiv oblast, Zmiïv region Homolchanski Forest, in the direction of Korobovi Khutory, 49°36'N 36°21'E 21.VI.2005. In a forest of *Populus tremula* and *Acer platanoides*, with *Corylus* and *Asarum*. On rotten wood (*Acer* ?). Specimen *Prydiuk M.P. s.n.* (KW 30596).

Additional bibliographic data concerning *Artomyces pyxidatus* in Ukraine

– Zakarpatska oblast, Rakhiv region, Carpathians, Svidovec [Khrebet Svidovets'] : Jalinka, near Kosovská Polana [Kosivs'ka polyana], in the valley of the river Kosovská Rika [Kosivs'ka], VII.1930, 48°04'N 24°08'E. On *Fagus sylvatica*. Herb. *Pilát 496.045 (*not seen). (Pilát, 1940: 41 and 1958: 148).

– Ternopil oblast, Berezhany region, village Lisnyky, 1903-1905, 49°28'N 24°52'E (Bobiak, 1907).

– Zhytomyr oblast, Malyn district, Malyn, 50°45'N 29°15'E. On rotten wood (Girzitska, 1929: 40).

– Kiev oblast, Kiev, Sviatoshyn, 50°27'N 30°23'E. On rotten wood (Girzitska, 1929: 40).

– Kiev oblast, Kiev, Pushcha-Vodytsia, 50°32'N 30°25'E. On rotten wood (Girzitska, 1929: 40).

– Kiev oblast, Irpin', 50°32'N 30°15'E. On rotten wood (Girzitska, 1929: 40).

– Kiev oblast, Kiev, Holosiiv, 50°22'N 30°30'E. On rotten wood (Girzitska, 1929: 40).

– Cherkasy oblast, Kaniv region, Kaniv nature reserve, 1976-1977, 49°44'N 31°29'E. On fallen twig of *Populus tremula* (Solomakhina, 1979).

– Donetsk oblast, Krasnyi Lyman district, Yampol, along Severskiy Donets river, 48°53'N 37°56'E, 27.VI.1989. In a flood plain forest. On *Tilia cordata* and soil, coll. V.P. Isikov (YALT).

– Republic of Crimea, Alushta region, Crimean nature reserve, VII.1936, 44°41'N 34°10'E (Gutsevich, 1940, as *Clavaria pyxidata*).

DISTINCTION BETWEEN *A. MICROSPORUS* AND *A. PYXIDATUS*

Mating experiments have shown that all isolates of *A. microsporus* and *A. pyxidatus* were incompatible and revealed strong antagonism phenomena (Wu & Petersen, 1991). They undoubtedly constitute two distinct species, even if they are closely related. As it was mentioned above, phylogenetic studies indicate that these two species are sister taxa (Lickey *et al.*, 2003).

Only a very restricted number of specimens of *A. microsporus* have been available for study and therefore it is difficult to determine the range of morphological variability of the species. However, all examined specimens from Ukraine exhibited a special character which could be used to distinguish the species from *A. pyxidatus*: the branches remain strait and densely crowded, giving the fruit body a compact appearance, as if it had been forced to grow in a restricted space, without possibility to spread its branches (Fig. 1-2). In addition, the branches remain more cylindrical and less progressively enlarged towards the top compared with those of *A. pyxidatus*. On the contrary, all the specimens of *A. pyxidatus* we observed were expanded, with their branches curved from the base and well separated from each other. Moreover, the branches were also very narrowly conical, being progressively enlarged from the base towards the top. Those characteristics of the latter species are illustrated in figure 3 and can be observed in published pictures as well (a.o. Cetto 4: pl. 1618, Imazeki & Hongo 1989: pl. 670, Jahn 1966: pl. 4, Jahn 1979: pl. 41, Michael & Hennig 1960: pl.122, Pilát 1958: 149 and tab. 18b and 19, Riva 1980, Ryman & Holmåsen 1984: 114).

Another difference between the two species is that the branch tips of the sporophores of *A. microsporus* have a tendency to become orange or even brownish, a character previously reported by Wu & Petersen (1992). The colour of *A. pyxidatus* fruit bodies is rather variable, ranging from cream to very bright yellow to tan (Wu *et al.*, 1995: 116), but it seems that the branch tips remain more or less concolorous with the rest of the fruit body.

A third morphological difference between the two species is the size of the basidiospores. The average width is nearly identical but the spores of *A. microsporus* are shorter, as can be seen in table 1 and figures 4 and 5. We also present in this table the spore-sizes given by Lickey *et al.* (2003), which fit very well with Ukrainian specimens. It can be seen that the ratio length/width (= Q or E_m) is different between the two species where the spores are mainly ellipsoid to broadly ellipsoid in *A. microsporus* while they are elongate to ellipsoid in *A. pyxidatus* (nomenclature of the spore shape after Bas, 1969: 321). It is interesting to note that Corner (1970: 148) points out that "Most collections [of

Table 1 : Spore size in *Artomyces microsporus* and *A. pyxidatus*. Source : AF = our observations on Ukrainian specimens (2 coll. of *A. microsporus* and 10 coll. of *A. pyxidatus*), EL = descriptions by Lickey *et al.* (2003). Underlined figures are means.

	Artomyces microsporus	*Artomyces pyxidatus*	*Source*
Spore size (µm)	3.0-<u>3.35</u>-3.8 × (2.0-) 2.1-<u>2.45</u>-2.9 (-3.0)	(3.5-) 4.0-<u>4.32</u>-4.8 (-5.1) × (2.2-) 2.4-<u>2.58</u>-2.8 (-3.0)	AF
	3.2-3.4-3.6 (-4.0) × 2.0-2.8	3.6-4.1-4.8 (-5.0) × (2.0) 2.4-2.8 (-3.0)	EL
Ratio length/width	1.10-<u>1.35</u>-1.56	1.48-<u>1.68</u>-1.93	AF
	1.41 +/- 0.14	1.65 +/- 0.05	EL

A. pyxidatus] have ellipsoid spores 4-5 x 2.5 µ, but a few have subglobose spores 3.5-5 × 3-3.7 µ." A similar observation had already been made by Doty (1947), who reports that he "has found many shorter (to nearly subglobose) spores in many of the collections examined." However, it is not likely that those collections could belong to *A. microsporus*.

Very little is said in the literature about the ecology of the two Japanese collections of *A. microsporus*. The original description of the species (Wu & Petersen, 1992) mentions "on rotten wood", without any indication on the host species, and Wu *et al.* (1995) do not discuss ecology. Lickey *et al.* (2003) determined that the substrate included with the type (TENN 48856) was "rotten hardwood". All the specimens we had the opportunity to observe in Ukraine were growing on dead wood of *Pinus sylvestris* (see the list of specimens seen and collected).

The ecology of *A. pyxidatus* is better known (see a.o. Jahn, 1966 and 1979, Krieglsteiner, 1985, Michael & Hennig, 1960, Dománski, 1975, Kotlaba & Pouzar, 1964, Pilát, 1958, Riva, 1980). It can be found growing on big pieces of rotten wood (stumps, logs) of different tree species. The most frequent host in Europe seems to be *Populus tremula*. Jahn (1966) even described a mycosociological association ("Pilzgesellschaft") on rotten trunks of *Populus tremula*, characterized by *A. pyxidatus* accompanied by (a.o.) *Leptopodia elastica*, *Chlorosplenium aeruginosum* and *Peziza repanda* (which probably corresponds with what is now frequently called *P. micropus*). However, *A. pyxidatus* has also been found growing on other hardwoods, like *Salix* (*S. alba*) and *Fagus*, and on coniferous trees as well. Kotlaba & Pouzar (1964), Jahn (1979), Krieglsteiner (1985) and Pilát (1958) cite *Abies alba* as a host; Krieglsteiner (1985) and Jahn (1966, 1979) mention *Pinus* (*P. sylvestris*).

MOLECULAR ANALYSIS

To determine the genetic diversity among the collections of *A. microsporus*, DNA was extracted from approximately 250 mg dried herbarium material (*A. Fraiture 2943*) following the protocol described in Hughes *et al.* (1999). Amplification and sequencing of the nuclear ribosomal internal transcribed spacer region (nrITS) followed the procedure outlined in Lickey *et al.* (2002). The sequence was manually corrected, deposited in GenBank (Accession # DQ449944) and aligned with sequences of other *A. microsporus* and *A. pyxidatus* cited in Lickey *et al.* (2002) using MacClade (Maddison & Maddison, 2005). Divergence estimates were calculated based on this alignment of 679 bp.

The estimated sequence divergence between the two Japanese *A. microsporus* collections (GenBank Accession # AF336138 and AF336137) is 0.1%, but is 1.0% between the Ukrainian and Japanese collections. This is consistent with the levels of divergence found in *A. pyxidatus* which exhibits a similar pattern and is probably due to limited gene flow among widely separated populations as well as the potential effect of past glacial cycles (Lickey *et al.*, 2002). The estimated divergence reported among *A. pyxidatus* collections from Sweden, Russia, and China is 0.3%, while the divergence between eastern North American and western North American collections is 0.6% (Lickey *et al.*, 2002). Comparatively, the estimated divergence between North American and Eurasian

A. pyxidatus collections is 2.5% and is 6.9% between *A. microsporus* and *A. pyxidatus*.

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