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A New Macrofungi Record for Turkey and Asia with Molecular Characterization: *Xerocomellus redeuilhii* (*Boletales, Basidiomycota*)

Fuat BOZOK[1*], Boris ASSYOV[2], Hatıra TAŞKIN[3],Saadet BÜYÜKALACA[4]
**Corresponding author: fbozok@osmaniye.du.tr*

[1*]Department of Biology, Faculty of Arts and Science, Osmaniye Korkut Ata University, 80000 Osmaniye, Turkey
Orcid ID:0000-0002-9370-7712 / fbozok@osmaniye.edu.tr
[2]Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, 2 Gagarin Str., 1113 Sofia, Bulgaria
Orcid ID: 0000-0002-7365-2443 / contact@boletales.com
[3,4]Department of Horticulture, Faculty of Agriculture, Çukurova University, 01330 Adana, Turkey
[3]Orcid ID: 0000-0002-1784-4731 / hatirataskin1@gmail.com
[4]Orcid ID: 0000-0002-1129-2729 / sbircan@cu.edu.tr

Abstract: *Xerocomellus redeuilhii* A.F.S. Taylor, U. Eberh., Simonini, Gelardi & Vizzini, an uncommon southern bolete, is recorded for the first time from Asia and Turkey, based on phylogenetic analysis of ITS rDNA sequence and morphological characters. Description and illustrations of the species collected from Osmaniye province (East Mediterranean region) of Turkey are given. An updated key to the Turkish and European members of the genus *Xerocomellus,* based on macroscopic and microscopic characters, is also presented.

Key words: *Boletaceae*, boletoid fungi, *Xerocomus*, Turkish mycobiota, xerocomoid boletes

Moleküler Karakterizasyonla Türkiye ve Asya'dan Yeni Bir Makromantar Kaydı: *Xerocomellus redeuilhii* (*Boletales, Basidiomycota*)

Öz: Güney kesimlerde nadiren bulunan bir Bolet olan *Xerocomellus redeuilhii* morfolojik karakterlere ve ITS rDNA diziliminin filogenetik analizine dayanarak Türkiye ve Asya kıtasından ilk kez kayıt altına alınmıştır. Türkiye'nin Osmaniye ilinden (Doğu Akdeniz bölgesi) toplanan türün fotografları ve betimlemeleri verilmiştir. *Xerocomellus* cinsinin Türkiye ve Avrupa üyeleri için makroskobik ve mikroskobik karakterelere dayanarak güncel bir teşhis anahtarı sunulmuştur.

Anahtar kelimeler: *Boletaceae*, boletoid mantar, *Xerocomus*, Türkiye mikobiyotası, xerocomoid boletes

Introduction

The genus *Xerocomellus* Šutara unites about ten species in Europe, characterized by their relatively small, xerocomoid basidiomata, tubulate hymenium with angular pores, encrusted pileipellis hyphae, smooth or longitudinally striate and sometimes truncate basidiospores; particular morphologically distinct type of amyloid hyphae ('pruinatus'-type) are also typical for some members (Ladurner and Pöder 2000, Ladurner and Simonini 2003, Peintner et al. 2003, Šutara 2008, Ariyawansa et al. 2015, Moreno et al. 2016). In Turkey four species (*X. zelleri, X. truncatus, X. chrysenteron, X. porosporus*) have been recorded so far (Sesli & Denchev 2008), while others are yet to be recognized. In November 2017, the first author collected a boletoid fungus with distinct appearance, which after morphological and molecular assessment was determined to belong to *X. redeuilhii* A.F.S. Taylor, U. Eberh., Simonini, Gelardi & Vizzini. It is presented here as first record for Turkey and Asia.

Material and methods

Macrofungal samples were collected under *Arbutus andrachne* L. from Osmaniye province of Turkey in 2017 (Figure 1). The samples were photographed in the field and salient characters and habitat features were recorded. The specimens were dried by dehydrator for 24 h at 60°C and deposited in the Fungarium of Osmaniye Korkut Ata University, as accession number FBozok00136. The microscopic study was held with AmScope T360B compound microscope, fitted with AmScope MU900 digital camera. Microscopic slides for observation of basidiospores were prepared with tap water. The remaining microscopic structures were studied on slides prepared by submerging sections of dry material in 10% KOH. Preparations were left for approximately one minute and Congo red in ammonia was added. Measurements were conducted with Piximetre v. 5.9 on calibrated microphotographs. The size of basidiospores is based on 50 measurements of random, normally developed spores in lateral view (with the apiculus clearly visible). The sizes of the remaining structures are derived from 15 measurements. In addition, preparations with Melzer's reagent (Langeron's modification) were also observed for peculiar iodine reactions of any structure.

Total genomic DNA was extracted from dried samples by using Eurx GeneMatrix Plant & Fungi DNA Purification Kit with slight modifications (increasing the concentration (100 mg/mL and 20 mg/mL) and the volume (20 µL) of RNase A and Proteinase K, respectively (Bozok et al 2018). ITS1F–ITS4 primers were used for PCR amplification of ITS rDNA (White et al., 1990). PCR conditions were set as follows: 94°C for 5 min, followed by 30 cycles of 45 s at 94°C, 60 s at 51°C and 90 s at 72°C and final extension 10 min at 72°C. PCR amplification was verified by electrophoresis on a 1.5% agarose gel. DNA sequencing of successful amplification was performed using the BigDye Terminator v3.1 Sequencing Kit, again with ITS1F–ITS4 (for ITS rDNA) primers. An ABI 3730XL Sanger Sequencer (Applied Biosystems, Foster City, CA, USA) was used for running of sequencing reactions. Raw sequence chromatogram was edited and aligned using Sequencher version 5.4.5 (Gene Codes, Ann Arbor, MI, USA). The sequence obtained from this study was deposited in GenBank as accession MH472623.

The phylogenetic tree was drawn by using the Maximum Likelihood method based on the Tamura-Nei model (Tamura and Nei, 1993) in Mega7.0 software by using *Suillus lakei* (Murrill) A.H. Sm. & Thiers as outgroup (Figure 2) (Kumar et al. 2016). The highest log likelihood of the tree with is -5096.76. Bootstrap values are shown next to the branches. Initial tree for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach and then selecting the topology with superior log likelihood value. The analysis involved 31 nucleotide sequences. All positions containing gaps and missing data were eliminated. The final dataset contained a total of 1221 positions.

Figure 1. Macro and microscopic features of *Xerocomellus redeuilhii* (a, b, c: Basidiomata, d: Cystida, e: Basidia, f: Basidiospores). Scale bars = 20 µm.

Results and discussion

Phylogenetic affiliation

As a result of morphological assessment, the bolete samples collected under *Arbutus andrachne* were identified as *Xerocomellus redeuilhii*. This was further verified through phylogenetic analysis based on ITS rDNA region. When GenBank and UNITE databases were searched for *X. redeuilhii*, nine sequences (KU721022, KU721023, KU721024, KX889920, KX905051, MH011842, MH011929, NR155981, UDB000448) were found – unpublished or submitted by Simonini et al (2016) and Loizides et al (2019). When compared to those sequences, the one obtained from the Turkish specimen was found to show similarity at rate of 99%. The sequences downloaded for comparison from GenBank and UNITE databases are shown in Table 1.

Table 1. Taxa, their accession numbers from GenBank and Unite databases for specimens used in the phylogenetic tree

Taxa	Location	Accession No	References
Xerocomellus chrysenteron	Cyprus,	MH011839	Loizides et al. 2019
Xerocomellus chrysenteron	Cyprus	MH011845	Loizides et al. 2019
Xerocomellus chrysenteron	Italy	UDB000441	Unpublished
Xerocomellus chrysenteron	Germany	UDB000439	Unpublished
Xerocomellus sarnarii	France	MH011926	Loizides et al. 2019
Xerocomellus sarnarii	Cyprus	MH011930	Loizides et al. 2019
Xerocomellus porosporus	Italy	KT271744	Unpublished
Xerocomellus porosporus	United Kingdom	UDB000475	Unpublished
Xerocomellus poederi	Spain	KU355479	Crous et al. 2016
Xerocomellus poederi	Spain	KU355480	Crous et al. 2016
Xerocomellus pruinatus	United Kingdom	UDB000477	Unpublished
Xerocomellus pruinatus	United Kingdom	UDB000479	Unpublished
Xerocomellus riparielllus	France	UDB000484	Unpublished
Xerocomellus riparielllus	Denmark	UDB001397	Unpublished
Xerocomellus aff. *redeuilhii*	Greece	KU721022	Unpublished
Xerocomellus redeuilhii	Cyprus	MH011929	Loizides et al. 2019
Xerocomus dryophilus	Italy	UDB000448	Unpublished
Xerocomellus aff. *redeuilhii*	Spain	KU721023	Unpublished
Xerocomellus aff. *redeuilhii*	Croatia	KU721024	Unpublished
Xerocomellus redeuilhii	Italy	KX905051	Unpublished
Xerocomellus redeuilhii	Cyprus	MH011842	Loizides et al. 2019
Xerocomellus redeuilhii	Italy	NR155981	Simonini et al. 2016
Xerocomellus redeuilhii	Italy	KX889920	Simonini et al. 2016
Xerocomus cisalpinus	Estonia	UDB023759	Unpublished
Xerocomus cisalpinus	Estonia	UDB011445	Unpublished
Xerocomus communis	Denmark	UDB001386	Unpublished
Xerocomus communis	Denmark	UDB001387	Unpublished
Xerocomus rubellus	Denmark	UDB001405	Unpublished
Xerocomus rubellus	Denmark	UDB001406	Unpublished
Suillus lakei	Turkey	MG279701	Akata et al. 2018

The phylogenetic tree (Figure 2) revealed two highly supported clades, corresponding to the genera of *Xerocomellus* and *Hortiboletus* Simonini, Vizzini & Gelardi, which representatives were included in the analysis. The clade of *Hortiboletus* holds basal position to *Xerocomellus* and includes the sequences of *H. engelii* (Hlaváček) Biketova & Wasser (originally accessioned as *Xerocomus communis*) and *H. rubellus* (Krombh.) Simonini, Vizzini & Gelardi. The *Xerocomellus* lineage further splits into seven clades, each one corresponding to the species which sequences were included in the analysis – *X. chrysenteron* (Bull. : Fr.) Šutara, *X. cisalpinus* (Simonini, H. Ladurner & Peintner) Klofac, *X. poederi* G. Moreno, Heykoop, Esteve-Rav., P. Alvarado & Traba, *X. porosporus* (Imler ex Watling) Šutara, *X. pruinatus* (Fr. & Hök) Šutara, *X. redeuilhii, X. riparielllus* (Redeuilh) Šutara, *X. sarnarii* Simonini, Vizzini & U. Eberh, with all above clades receiving high statistical support. The topology of the tree is generally congruent with the one, published by Ariyawansa et al. (2015) with *X. cisalpinus* holding basal position within its generic clade. The sequence from the Turkish specimen of *X. redeulhii* nests firmly in a clade with the publicly available sequences of this taxon from Italy, Greece and Cyprus, including the sequences from type materials of the species.

Description of species

Xerocomellus redeuilhii A.F.S. Taylor, U. Eberh., Simonini, Gelardi & Vizzini, Rivista di Micologia 59: 2 (2016); *Xerocomus dryophilus* auct. Eur. nonnul., non *Boletus dryophilus* Thiers, California Mushrooms, p. 82 (1975).

Pileus up to 9 cm, at first hemispherical, then more or less flat, dry, velvety, viscid when wet, red, dark red, pinkish red, paler towards the margin; surface pruinose to very finely cracked (lens), unchanging on touch. **Stipe** 6–12 × 1.5–2 cm, slender, cylindrical, straight or curved, tapering at the base, lemon yellow in the upper part, downwards becoming yellowish orange and sometimes spotted reddish brown, dark red in the lower part when young, blackish red, purplish or brownish red when old; surface smooth or in places somewhat fibrillose. **Context** bright yellow in the pileus and the upper part of stipe when young, then pale yellow, dark red to brownish red or blackish red up to half of stipe, unchanging or slightly blueing when exposed to air. **Tubes** up to 14 mm long, adnate, lemon yellow to bright yellow when young, olive yellow when old, unchanging or slightly blueing when bruised or cut. **Pores** up to 2 mm diam., concolorous with the tubes, unchanging to slightly blueing when bruised. **Basidiospores** 12.4–(13.9±0.7)–15.7 × 5.5–(6.6±0.3)–7.5 µm, Q=1.9–(2.1±0.1)–2.5 (n=50), thick-walled (ca 0.7 µm), ellipsoid, brownish yellow in water and KOH, inamyloid, smooth, with one or two large guttules. **Basidia** 30.9–37.3 × 10.7–14.2 µm, 4-spored (2-spored basidia also seen), clavate, hyaline. **Pleurocystidia** abundant, 50.7–82.3 × 13.7–18.6 µm, fusoid-ventricose, hyaline. **Cheilocystidia** similar to pleurocystidia. **Hymenophoral trama** phylloporoid. **Hyphae** of context in the stipe base inamyloid; 'pruinatus'-type hyphae not seen. Hyphae of pileus hyaline, 6–28 µm broad, many distinctly inflate. **Pileipellis** a trichodermium of somewhat interwoven, 6–15 µm broad, finely encrusted in KOH and Melzer's reagent hyphae; terminal elements 23.5–48.2 × 6.7–13.7 µm, not or slightly encrusted, mostly with tapering, but also with obtuse apex, occasionally slightly inflate or ampuliform.

Specimen examined: Turkey, Osmaniye, Amanos Mountains, 37º01'32"N, 36º13'58"E, 444 m elev., under *Arbutus andrachne* (sandal ağacı in Turkish), 12 November 2017, leg. Fuat Bozok (FBozok00136; GenBank MH472623).

The macroscopic and micromorphological features of the Turkish specimen agree well with the available descriptions of *X. redeuilhii* from Europe (Simonini 1994, Ladurner and Simonini 2003, Galli 2007, Simonini et al. 2016; in some of the works as *Xerocomus dryophilus*). Furthermore, the phylogenetic analysis confirmed the identity of the sequence obtained from the Turkish specimen to the publicly available sequences of this species, including such from type materials.

Xerocomellus redeuilhii appeared previously in the European mycological literature as *X. dryophilus* (Thiers) Singer, a bolete described originally from North America (Thiers 1975, as *Boletus dryophilus* Thiers). The two species indeed share a certain degree of similarity, but Simonini et al (2016) found that *X. redeuilhii* is different from *X. dryophilus* morphologically (as well as phylogenetically) on account of the usually slenderer habit and pileipellis terminal cells with more acute apex and less evident epiparietal incrustations. *X. redeuilhii* also shows some similarity to other European species of the genus. Due to the red-coloured pileus it may appear similar to *X. fennicus* (Harmaja) Šutara and *X. riparielllus* (Redeuilh) Šutara. Both of them are however easily set apart due to their basidiospores, which are longitudinally striate and also truncate in the former (Ladurner and Simonini 2003). *H. rubellus* (Krombh.) Simonini, Vizzini & Gelardi is superficially similar, due to the red-coloured pileus, but it may be distinguished undoubtedly in the field due to the very characteristic orange-red dots in the context of the stipe base.

Xerocomellus redeuilhii seems to be less common southern species, spread in the Mediterranean area of Europe and Asia (incl. some Mediterranean islands), but absent elsewhere. Published records (some as *Xerocomus dryophilus*) are known from Croatia (Ladurner and Simonini 2003), Cyprus (Loizides et al. 2019), France (Galli 2007), Greece (Polemis et al. 2012), Italy (Simonini 1994, Ladurner and Simonini 2003, Galli 2007, Simonini et al. 2016), Malta (Briffa 2002), Spain (Ladurner and Simonini 2003, Muñoz et al. 2008, Siquier et al. 2011) and Turkey (this paper).

In Turkey, *X. redeulhii* is so far known from a single locality in the East Mediterranean region. However, it may well appear to be more widespread in the country and should be further looked for. Reddish-coloured pileal surface, yellow in the upper and dark red in the lower part of stipe and similarly tinted stipe context, coupled with thermophilous habitats, are useful field characters pointing towards this species.

The members of the genus *Xerocomellus* seem scarcely presented in the Turkish mycological literature and apparently need further attention. Together with the addition of this paper a total of five species are so far recorded in this country, which is about half of the species currently recognized in Europe. Among them, *X. truncatus* (Singer, Snell & E.A. Dick) Klofac and *X. zelleri* (Murrill) Klofac, species described from North America and previously reported from Europe and Turkey, need to be carefully revisited as records under those names may represent other taxa. For further details on this topic the reader is referred to Ladurner & Simonini (2003).

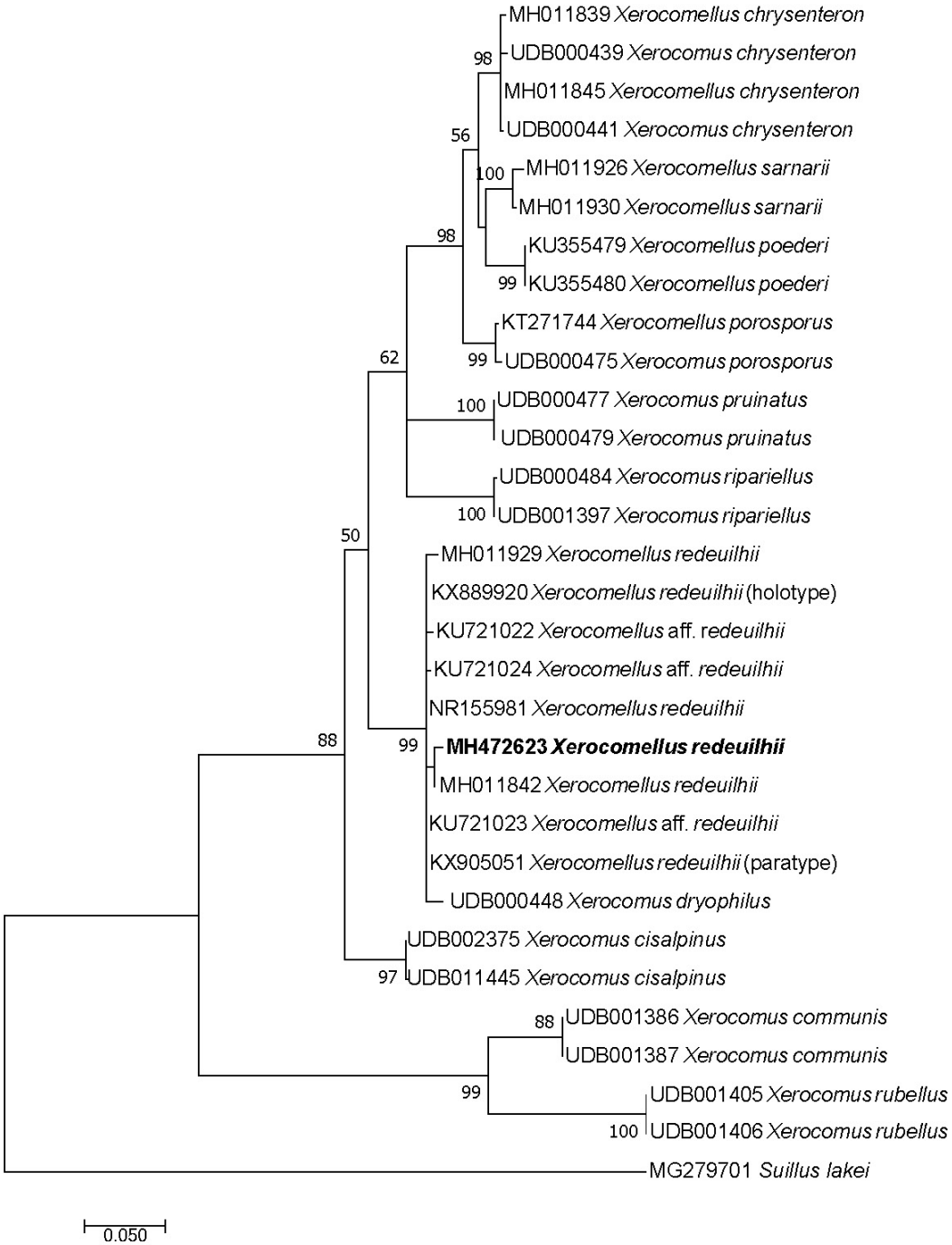

Figure 2. Maximum likelihood phylogenetic tree of ITS rDNA sequences of *Xerocomellus redeuilhii* obtained from the present study and related species selected from GenBank database. Bootstrap values (>50%) are given near the branches. *X. redeuilhii* sequence in this study is indicated as bold in phylogenetic tree.

A key to the Turkish and European species of *Xerocomellus*

1. At least part of the basidiospores truncate.....2
1* Basidiospores not truncate.....5
2. Pileus coloured red or reddish at least in young basidiomata.....3
2* Pileus differently coloured, but lacking reddish tints.....4
3. Basidiospores striate (under SEM or in LM with heated lactophenol+Cotton blue and magnification ≥1000×). Species associated with *Betula* and *Alnus* in Northwestern and Central Europe, not yet known from Turkey..... ***X. fennicus***
3* Basidiospores smooth. Species allegedly associated with *Quercus.* Considered by some authors to represent a synonym of *X. porosporus,* but molecular evidence of its phylogenetic position is pending. Records exist from the Czech Republic and Hungary.....***X. marekii***
4. Context usually blueing evidently, in stipe base with vinaceous or purple colour and amyloid 'pruinatus'-type hyphae. Not yet recorded in Turkey.....***X. sarnarii***
4* Context usually unchanging or blueing slightly and patchy, in stipe base more or less brownish to greyish or blackish, sometimes pinkish red, but lacking vinaceous or purple tints, and amyloid 'pruinatus'-type hyphae absent... ***X. porosporus***
5. Context of stipe blueing slowly and often strongly, with 'pruinatus'-type hyphae 6
5* Context of stipe usually not blueing or turning faint blue (mostly patchy) relatively quickly after exposure to air; 'pruinatus'-type hyphae absent.....8
6. Pileus with obvious red colours, sometimes partly discolouring to brownish; species found in more or less humid habitats, often associated with *Salix, Populus* or *Betula.* Not yet recorded from Turkey.....***X. ripariellus***
6* Pileus with prevailing brownish colours; species associated with Pinaceae or Fagaceae.....7
7. Pileus initially finely velutinous, but still in young stage becoming strongly and finely cracked; basidiospores on average <5 µm broad. Species mostly associated with *Quercus,* but also with *Fagus, Pinus* and *Cedrus* in thermophilous habitats. Not yet found in Turkey.....***X. cisalpinus***
7*Pileus long time finely velutinous, even in stage of maturity rarely with very few cracks; basidiospores on average >5 µm broad. Species associated with Fagaceae (*Fagus, Castanea*) or Pinaceae (*Picea, Pinus*), mostly found in cooler or mountain habitats. Not yet recorded in Turkey .....***X. pruinatus***
8. Pileus with prevailing red colours, sometimes discolouring brownish or somewhat olivaceous in places; stipe surface smooth, finely flocculose to somewhat fibrillose; context in stipe base blood red to reddish brown, in rest of the stipe bright yellow; basidiospores on average >6 µm broad and with Qm<2.4; species from termophilous habitats.....***X. redeuilhii***
8* Pileus with prevailing brownish colours; stipe surface at least in the lower part with coloured granules or fine scales; context in stipe base reddish, pinkish red or vinaceous red coloured, in rest of the stipe pale yellow or whitish; basidiospores on average <6 µm broad and with Qm>2.5.....9
9. Basidiospores on average ≥5 µm broad; pleurocystidia up to 100 µm long; species associated with Pinaceae or *Fagus* in ± cooler or mountain environment.....***X. chrysenteron***
9* Basidiospores on average <5 µm broad; pleurocystidia up to 60 µm; species associated with *Quercus* in ± thermophilous habitats. So far only known from the Iberian Peninsula.....***X. poederi***

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