

**A new species of *Ijuhya* on *Equisetum hyemale* and its *Acremonium* anamorph, with notes on *Hydropisphaera arenula*
Eine neue *Ijuhya*-Art auf *Equisetum hyemale* und ihre *Acremonium*-Anamorphe, mit Anmerkungen zu *Hydropisphaera arenula***

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Abstract: A previously unknown species of the genus *Ijuhya* was discovered in Germany. It is here described as *Ijuhya equiseti-hyemalis*, as it was only found on dead parts of standing stems of *Equisetum hyemale*. In pure culture the species developed an *Acremonium* anamorph. *Nectria equiseti-hyemalis* is re-examined from the holotype and found to be a synonym of the polyphagous *Hydropisphaera arenula*.

Zusammenfassung: Eine bislang unbekannte Art der Gattung *Ijuhya* wurde ausschließlich auf toten Teilen stehender Stengeln des Winterschachtelhalms (*Equisetum hyemale*) gefunden. Die Art wird deshalb als *Ijuhya equiseti-hyemalis* neu beschrieben. Sie besitzt winzige (0,12-0,2 mm), disco- und pyrenomycetenähnlich abgeplattete, haarig gezähnte, weißliche bis gelblich-braune, gesellig wachsende Perithezien. In Reinkultur entwickelte sich eine *Acremonium*-Anamorphe.

Die Art konnte über mehrere Jahre an verschiedenen Standorten Süd- und Norddeutschlands nachgewiesen werden, in feuchteren Perioden oft in großer Zahl. Der Winterschachtelhalm ist mit seinen ganzjährig grünen, von der Spitze her absterbenden, unverzweigten Halmen ein interessantes Pilzsubstrat, das teilweise von hochspezialisierten, aber auch von polyphagen Pilzen, hauptsächlich Disco- und Pyrenomyceten, besiedelt wird. Hierbei sind viele Arten trocken tolerant, d. h. sie fruchten auf den noch mehr oder weniger aufrechten toten Halmteilen. Während unter den an verschiedenen *Equisetum*-Arten vorkommenden Pyrenomyceten hauptsächlich dothideale Sippen mit völlig eingesenkten, schwarzen Fruchtkörpern bekannt sind, wachsen die blaß bis hell rötlichbraun gefärbten Perithezien der hier behandelten hypocrealen Arten *Ijuhya equiseti-hyemalis* und *Hydropisphaera arenula* völlig oberflächlich. Letztere Art ist unserer Ansicht nach polyphag und besonders von Stengeln verschiedener Kräuter bekannt. Die Nachuntersuchung des Holotypus von *Nectria equiseti-hyemalis* und zweier rezenter Funde an *E. hyemale* ergab keine Unterschiede zu Funden an Kräuterstengeln.

With its unbranched culms being permanently green throughout the year and dying back from the tip, *Equisetum hyemale* L. provides an interesting substrate for fungi, inhabited partly by highly specialised, but also polyphagous species, mainly disco- and pyrenomycetes. Most of the fungi seem to be xerotolerant, because their fruit-bodies are found on dead parts of more or less upright culms. Among those pyrenomycetes

collected on stems of various species of *Equisetum*, HOLM & HOLM (1981) reported exclusively dothidealean genera with entirely immersed, subepidermal, black ascomata. In contrast, the hypocrealean ascomycetes treated here develop superficial perithecia. The new species *Ijuhya equiseti-hyemalis* forms discomycete-like flattened, hairy to denticulate, whitish to yellowish-brown perithecia. In Germany the fungus has been recorded over several years during the more humid seasons, often in large numbers and exclusively on *E. hyemale*.

***Ijuhya equiseti-hyemalis* LECHAT & BARAL, spec. nova** (Figs 1, 2)

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Latin diagnosis: ¹Perithecia subglobosa, apice applanata, 120-200 µm diametro, subhyalina vel flavide ochracea, corona subapicalis pilis agglutinatis hyalinis, crasse tuni-catis, flexuosis composita, colore in KOH non mutanda. Asci †35-42 × 6-7 µm (in statu emortuo), octospori, unitunicati, inamyloidei. Ascosporae fusiformes vel ellipso-ideae, *8,5-12,5 × 2,5-3,5 µm (in statu vivo), uniseptatae, tetraguttulatae, sublaeves.

Anamorphic state: *Acremonium* spec.

Holotype: Germany, Baden-Württemberg, Tübingen-Pfrondorf, Tiefenbachtal, 26. 11. 2006, H.-O. BARAL C.L.L. 7026, deposited in the herbarium of the Museum National d'Histoires Naturelles de Paris (PC). Isotypes in private herbaria of H.-O. BARAL (H.B. 8377) and C. LECHAT (C.L.L. 7026).

Etymology: *equiseti-hyemalis* refers to the host plant.

Characters:

Perithecia: gregarious, solitary or densely crowded in groups of 2-6 and more, superficial, subglobose with flattened apex, 120-200 µm in diam. (excluding hair vestiture), 100-120 µm high, first white then yellowish to brownish-orange, becoming darker and collapsing cupulate when dry, not changing colour in 3 % KOH. Perithecial apex with short, thick-walled hairs in the centre, margin with fasciculate, thick-walled hairs, agglutinated to form teeth arranged in a stellate fringe around upper margin of perithecia.

Hairs: 28-55 or 50-80 µm long, varying among perithecia and collections, 2-2.5(-3) µm wide, hyaline to pale yellow, cylindrical, slightly flexuous, thick-walled (0.7-1 µm), with meandering lumina, rounded at tips, with at most a few septa.

Perithecial wall: 10-15 µm thick, strongly gelatinised, composed of two regions: outer region 8-10 µm wide, of 2-4 layers of globose to elongate cells 4-6 × 3-4 µm, cell walls pale yellow, 0.5-0.8 µm thick; inner region 5-6 µm wide, of ellipsoidal to flattened cells 5-8 × 1.5-2.5 µm, with hyaline walls 0.5 µm thick. Perithecia inamyloid in all parts (IKI or MLZ, with or without KOH-pretreatment).

Basal hyphae: hyaline, 1.5-2.2 µm in diam., thin-walled (0.2 µm), straight or flexuous, forming squamules on lateral parts of perithecia.

LBs (lipid bodies): in cells of subhymenium pale yellow-orange.

¹ * = living state, † = dead state

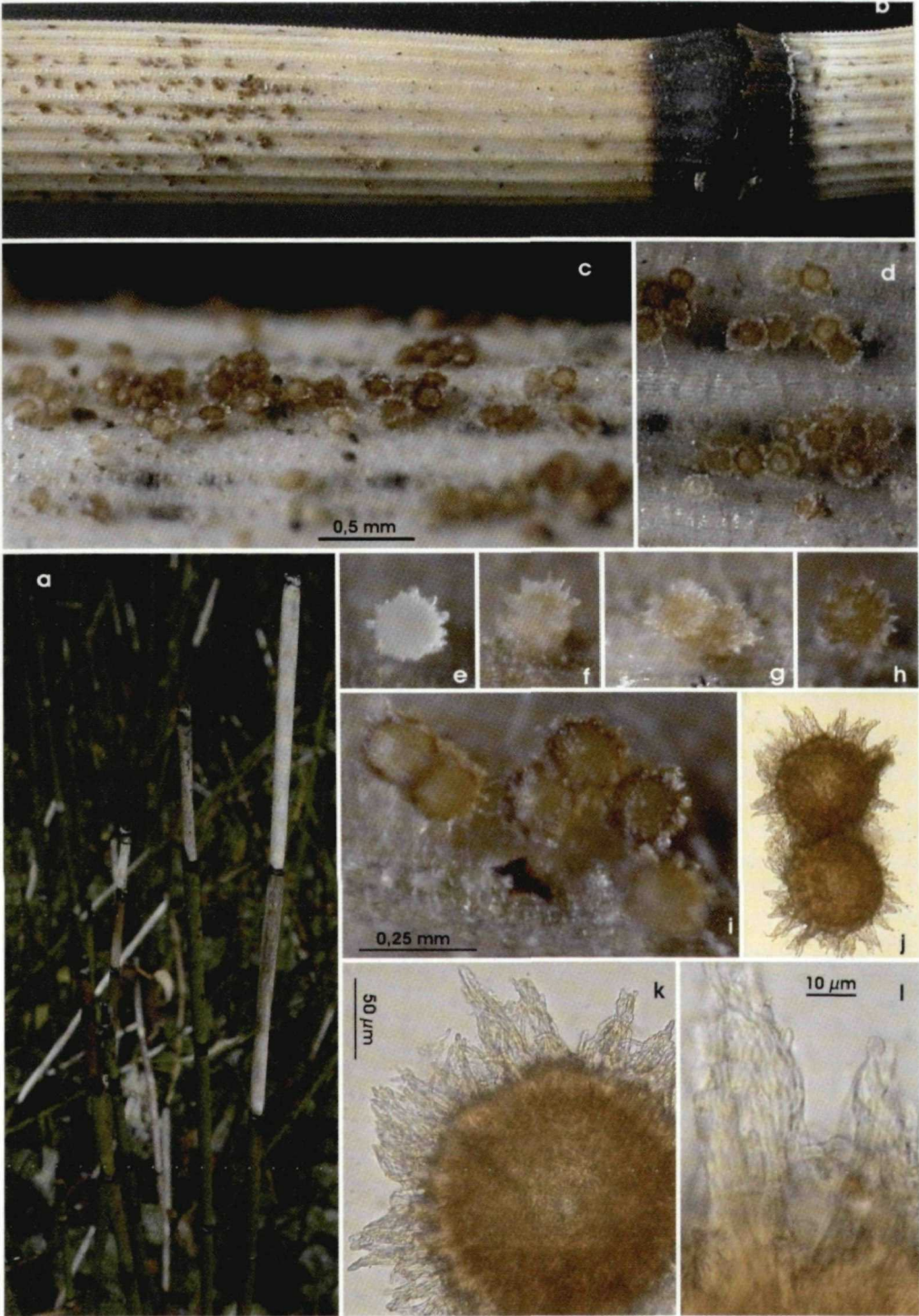


Fig. 1. *Ijuhya equiseti-hyemalis* on *Equisetum hyemale*. a Natural stand with dead, upright, periodically dry stem segments colonised by *I. equiseti-hyemalis*. b-i. rehydrated ascomata on natural substrate. e-i. pigmentation enhanced with maturity). j-l. apothecia in transmitted light (top view). a 5. 10. 2007 (Tübingen, Poppelesloch). b-i. topotypes (Tübingen, Schützenhaus, b-d H.B. 8072; e-i H.B. 8607a). j-l. H.B. 8458b (Parchim).

Asci: *50-52 × 8-9.5 μm {1}², †35-42 × 6-7 μm {2}, unitunicate, sessile, clavate to fusoid, apex ± long-conical, with an indistinct inamyloid ring (IKI or MLZ, KOH-pretreated), with 8 obliquely biseriata ascospores.

Ascospores: *(8.5-)9.5-11.5(-12.5) × (2.5-)2.8-3.2(-3.5) μm {3}, †(7.5-)9-12(-13) × 2.5-3(-3.3) μm {3}, hyaline, fusiform to fusoid-ellipsoidal, straight, equally 1-septate (exceptionally also 0- or 3-septate), overmature distinctly larger: *10-13 or rarely 13-19 × 3.5-4.5 μm (†3-3.8 μm wide), scarcely constricted at the septum but distinctly so when overmature, in dead state slightly less constricted, smooth, perhaps finely punctate-striate to striate (hardly visible at 1500x, in H₂O or CB), with 2(-3) LBs (1-1.7 μm in diam.) and several small ones in each cell. No interthecial elements seen.

Anamorph in pure culture (from holotype): Colony 2.5 cm in diam. after 9 days at 25 °C on PDA containing (5 mg/l) streptomycin, white to pale yellowish with aerial mycelium. Conidiophores mononematous, monophialidic, arising from hyphae 2-4 μm wide, straight, with one basal septum, 35-55 μm long, 2 μm wide at base, 1 μm wide at tip, hyaline, smooth.

Conidia: *3.5-7.2 × 1.8-2.2 μm, ellipsoidal to cylindrical, hyaline, unicellular, without basal scar, with a minute oil-drop at one or both ends.

Specimens examined (host always on dead xeric stems of *Equisetum hyemale*); C.L.L. specimens are deposited at LIP, Lille, except for the holotype): **Germany:** Baden-Württemberg, Schönbuch, Tübingen-Pfrondorf, Kirnbachtal, MTB 7420/1, 360 m s. m., Keuper (Stubensandstein), 16. 11. 1976, H.-O. BARAL (H.B. 1545); - *ibid.*, 5. 4. 1977 (not preserved, immature); - *ibid.*, Pfrondorf, Tiefenbachtal, Schützenhaus, MTB 7420/4, 350 m s. m., Keuper (Stubensandstein), 22. 2. 2006, H.-O. BARAL (H.B. 8072, C.L.L. 0771); - *ibid.* 3. 8. 2006 (not preserved, sterile); - *ibid.*, 26. 11. 2006 (C.L.L. 7026 - holotype in PC, isotypes in H.B. 8377, C.L.L.); - *ibid.*, 24. 1. 2007 (not preserved, overmature); - *ibid.*, 19. 8. 2007 (H.B. 8607a, not preserved); - *ibid.*, 16. 9. 2007 (H.B. 8622c); - *ibid.*, 7. 11. 2007 (H.B. 8673a); - *ibid.* 25. 2. 2008, C.L.L. 7171, culture deposited at the CBS no. 123499 (Utrecht, The Netherlands); - *ibid.*, Pfrondorf, Tiefenbachtal, Poppelesloch, MTB 7420/4, 320 m s. m., Keuper (Stubensandstein), 5. 10. 2007, H.-O. BARAL (not preserved). Mecklenburg-Vorpommern, Parchim, south of Weberin, valley towards Glambecksee, MTB 2335/4, 31 m, 1. 4. 2007, T. RICHTER & J. SCHWIK (H.B. 8458b, not preserved, overmature).

Ecology: Ascomata of *I. equiseti-hyemalis* occur on the dead whitish segments of still standing or hanging, dead stems of *Equisetum hyemale*, a host easily recognised by its rather unbranched, dark green, robust, winter-green stems. The ascomata are found at a height of ca. 10-100 cm above ground, thereby omitting the black nodes and living green parts. They are mostly found on the faintly brownish face of the stem, while the more white face is usually without perithecia. Mature asci and spores are found in autumn and winter, while perithecia collected during spring and summer are often overmature or empty. On several occasions, every second or third dead stem was found to be colonised by hundreds of perithecia at the type locality, while sometimes only every sixth or seventh stem bore perithecia. Not rarely, a number of other ascomycetes and mitosporic fungi were found growing in association with *I. equiseti-hyemalis*, partly on the same stem segment: the superficially growing *Epicoccum purpurascens* EHRENB., *Gibberella pulicaris* (FR.) SACC., *Hydropisphaera arenula* (BERK. & BROOME) ROSSMAN & SAMUELS, *Phaeostilbella atra* (DESM.) HÖHN., *Phialina anomala* RAITV. & SCHNELLER, *Psilachnum inquilinum* (P. KARST.) DENNIS s.l. *Stachybotrys dichroa* GROVE, and the subepidermally growing *Didymosphaeria spec.* and *Scirrhia castagnei* (MONT.) NITSCHKE.

² {x} = number of collections examined

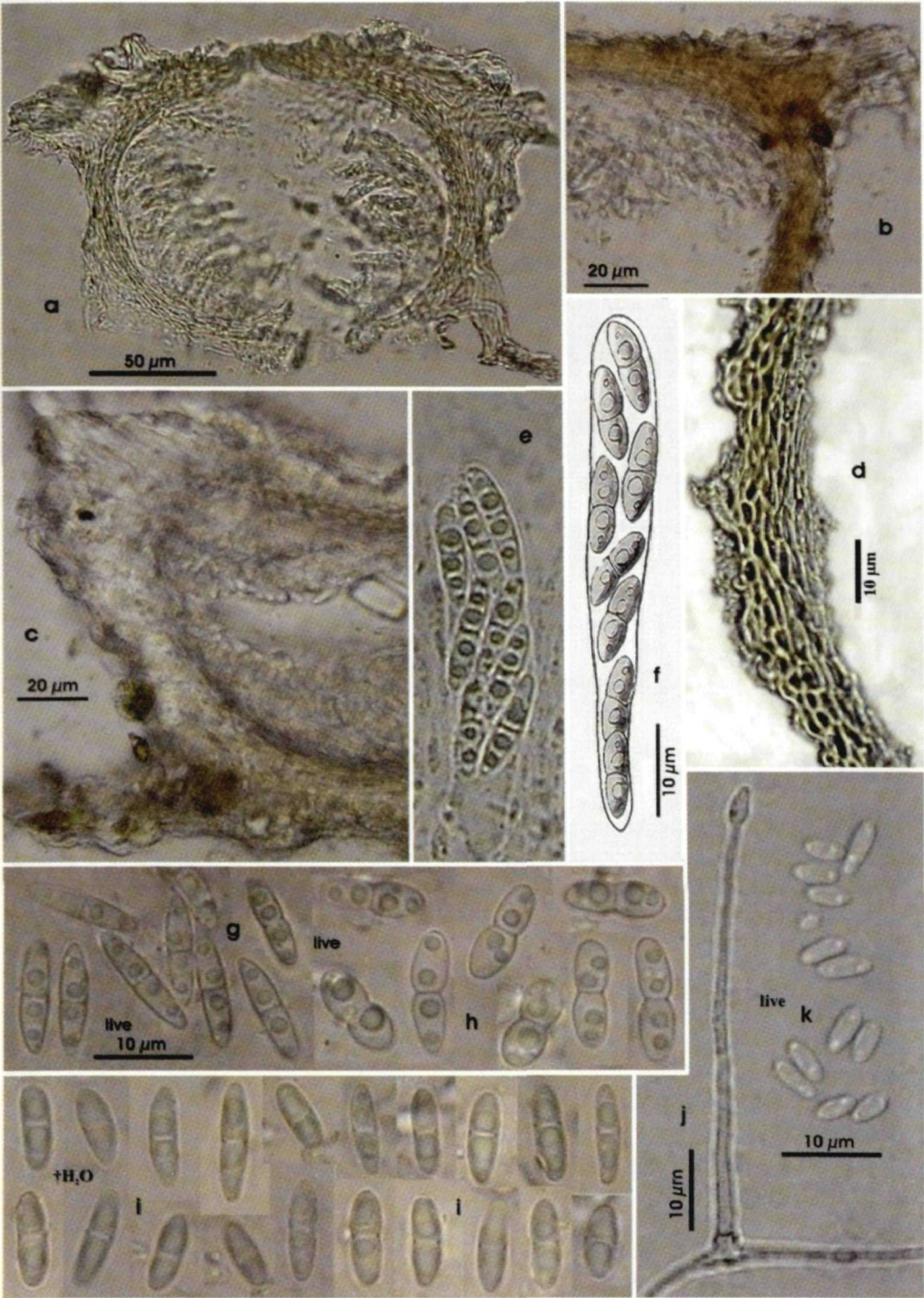


Fig. 2. *Ijuhya equiseti-hyemalis*. a-c ascomata (median section), d lateral perithecial wall, e, f dead asci containing living spores, g mature living ascospores, h overmature living ascospores (ascomata submerged in water for one day), i dead mature ascospores (after 10 months in the herbarium). j, k *Acremonium* anamorph (j conidiophore, k conidia). a, d-f, j, k holotype (Tübingen, H.B. 8377), b, c, g-i topotypes (Tübingen, b, c H.B. 8072, g H.B. 8607a, h, i H.B. 8622c).

Because of the air-exposed growth the perithecia dry out rather rapidly during dry weather, and thereby collapse to form flat cups. During drought immature asci survive complete desiccation for at least one week. *Ijuhya equiseti-hyemalis* is a xerotolerant fungus, which tolerates without damage the complete loss of its free intracellular water for some period of time. The fungus is neither found on permanently hydrated stems lying among moist litter, nor on dead stems hanging down into the streambed. Here two *Helotiales* species, *Phialina anomala* (a fungus confined to *E. hyemale*, see RAITVIIR & SCHNELLER 2003) and *Psilachnum* aff. *inquilinum*, occur which both, however, also fruit on the standing stems. Yet, if collected during dry weather on the air-exposed stems, these two species are frequently found to be no longer alive in most parts. Thus, we conclude that these helotialean species exhibit little xerotolerance, contrary to *I. equiseti-hyemalis*.

During summer perithecia of *I. equiseti-hyemalis* are found more and more infrequently. This could be explained by the fact that the perithecia are rather loosely attached to the stems and therefore are easily detachable, but only when the perithecia are moistened. The stellate crown at the upper edge of the perithecia may facilitate a connection to the fur of mammals, which may disperse them, in the way fruits of *Arctium* are dispersed. This appears to be reasonable since the asci are evidently incapable of active spore discharge; however, the phenomenon of disintegrating ascus walls is apparently not uncommon within the *Hypocreales*.

At the type locality of *I. equiseti-hyemalis* *Equisetum hyemale* forms extended, dense, pure stands on the relatively acid Stubensandstein [Late Triassic (Norian) sandstone] of the Middle Keuper formation. At the rather steep slopes in the shady gorge these stands cover a broad belt directly at the brook, while downwards they occur scattered on the flat bottom of the broader valley. The vegetation is an *Aceri-Fraxinetum* with mainly *Fraxinus* and *Fagus*. *I. equiseti-hyemalis* is observed only on stems growing several meters above the brook, never where the stems are flooded during high water periods. At the site in Mecklenburg-Vorpommern *Equisetum hyemale* grows in a *Chrysosplenio oppositifolii-Alnetum glutinosae* embedded in a *Fraxino-Alnetum*. It remains to be investigated, whether *I. equiseti-hyemalis* inhabits also periodically flooded flat banks along larger rivers, in which *Stammaria americana* MASSEE & MORGAN has been regularly found on *Equisetum hyemale* (KÜNKELE & al. 2005).

Discussion

STARBÄCK (1899: 30) erected the genus *Ijuhya* STARBÄCK, named after the Brazilian village Ijuí (Ijuhi) in Rio Grande do Sul (W. JAKLITSCH, pers. comm.), for a single species, *Ijuhya vitrea* STARBÄCK. SAMUELS (1976) considered the genus to be a synonym of *Nectria* (FR.) FR. However, ROSSMAN & al. (1999: 33) resurrected the genus *Ijuhya* and recognised ten predominantly tropical species. The type species was found to have an earlier valid name that they combined as *Ijuhya peristomialis* (BERK. & BROOME) ROSSMAN & SAMUELS.

Ijuhya is characterised by white to pale luteous perithecia, often with an apical disk from which fasciculate hairs extend. Because of a rather narrow wall structure the perithecia show a cupulate collapse when drying out. The species occur primarily on dead herbaceous stems but are also found on woody substrata. The ascospores vary from 1-septate to transversely multiseptate or muriform. The observed anamorphs have been placed in *Acremonium*.

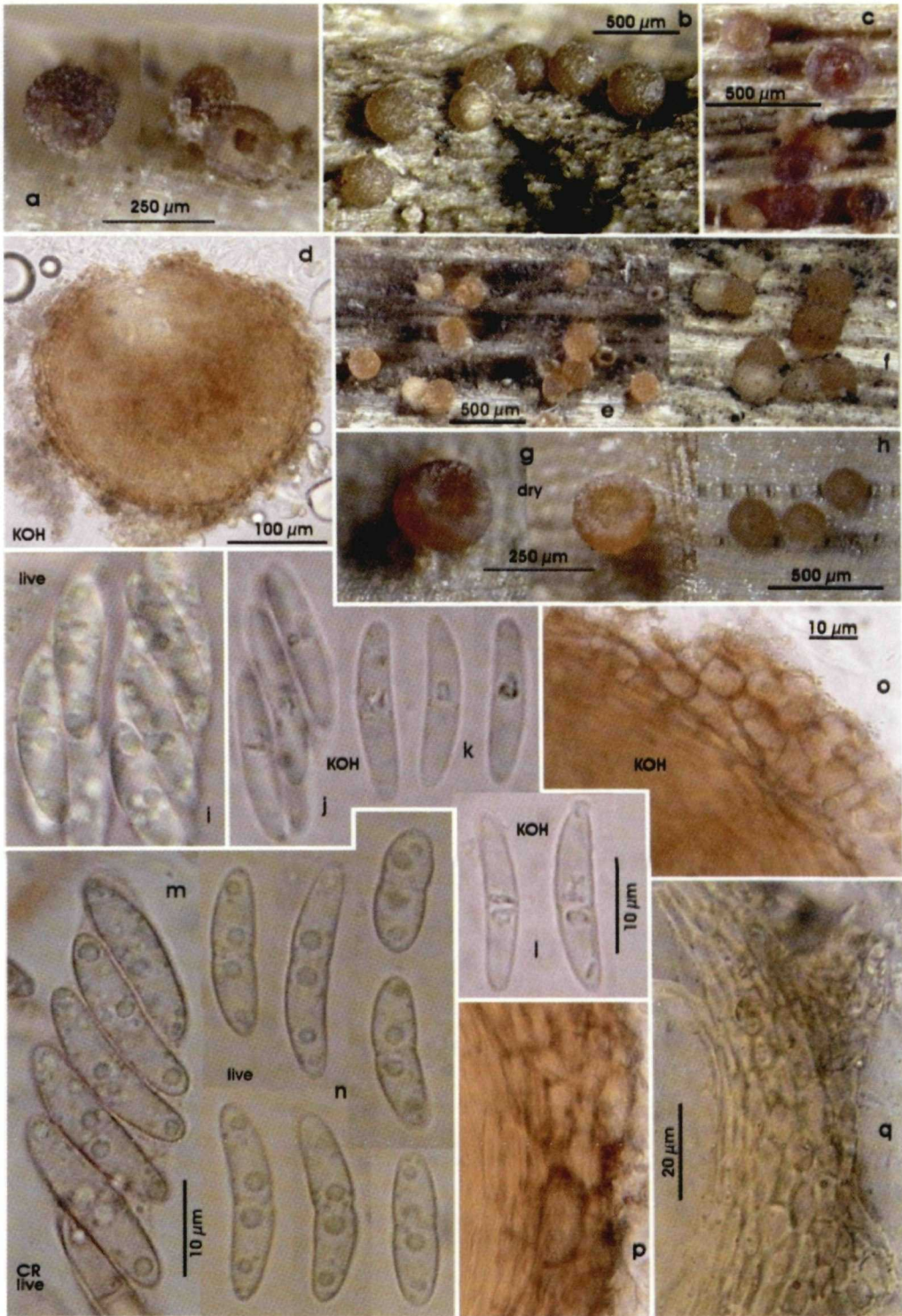


Fig. 3. *Hydropisphaera arenula*. a-c, e-h ascomata, d, o-q perithecial wall in median section, i-n ascospores (CR Congo Red). a, d, j-l, o, p holotype of *Nectria equiseti-hiemalis* (Kolding). g, h, m, n H.B. 8622b (Tübingen, *Equisetum hyemale*); b C.L.L. 649 (Deux-Sèvres, *Urtica dioica*); c syntype of *Peziza calamaria* (Piemonte, *Poaceae*); e, f, i, q H.B. 7869d (Luxembourg, *Heracleum sphondylium*).

Ijuhya equiseti-hyemalis is the second species of the genus *Ijuhya* so far discovered in Europe. The previously known species is *Ijuhya peristomialis*, which was reported from England by BERKELEY & BROOME (1866, on wood of *Ilex* spec., type of *Peziza peristomialis*) and from France by CROUAN & CROUAN (1867, type of *Helotium ciliatum* P. CROUAN & H. CROUAN, on stem of *Urtica dioica*). Recent collections of this species were made in France on rotting wood (Dept. Deux-Sèvres, Villiers en Bois, 12. 10. 2004, leg. C. LECHAT, not preserved; Dept. Ariège, Las Muros, 24. 9. 2006, leg. J. FOURNIER, Herb. C.L.L. 694).

Ijuhya equiseti-hyemalis is closely related to *I. dentifera* (SAMUELS) ROSSMAN & SAMUELS and *I. peristomialis* due to its stellate crown of fasciculate agglutinated hairs and the *Acremonium* anamorph. The characters separating these three species are listed in Table 1.

Table 1. Comparison between the new species and two apparently closely related taxa.

	<i>I. equiseti-hyemalis</i>	<i>I. peristomialis</i> (based on ROSSMAN & al. 1999)	<i>I. dentifera</i> (based on ROSSMAN & al. 1999)
Ascospores	*(8.5-)9.5-11.5 (-12.5) × (2.5-) 2.8- 3.2(-3.5) µm	(24-)30-60(-110) × 4-7 (-8) µm	6-8(-9) × 3-4 µm
	mostly 1-septate	mostly 5-7-septate	1-septate
Conidiophores	35-55 × 2 µm unbranched	20-50 × 2-3.5 µm unbranched	22-58 × 2-3 µm branched
Conidia	3.5-7.2 × 1.8-2.2 µm	5-8 × 1.7-2.5 µm	3-4 × 2.5-3 µm
Substrate	stems of <i>Equisetum</i> <i>hyemale</i>	wood, bark and debris of ferns, monocots and dicots	bark and wood

The genus *Lasionectria* (SACC.) COOKE is based on *L. mantuana* (SACC.) COOKE. *Lasionectria* shows similarities with the genus *Ijuhya*, but differs from it in the thick-walled peridial cells with narrow lumina, a peridial width exceeding 20 µm, and yellow- to dark brown ascomata (cf. ROSSMAN & al. 1999). Also the anamorphs are similar. *L. vulpina* (COOKE) ROSSMAN & SAMUELS resembles *I. equiseti-hyemalis* with its orange hairs and a similar ascospore size, but grows on wood and basidiocarps.

Also *L. sylvana* (COOKE) ROSSMAN & SAMUELS should be compared because it was reported from *Equisetum arvense*, but also from herbaceous stems of both dicotyledons and monocotyledons. It differs from *I. equiseti-hyemalis* by larger spores (11-15 × 3-4 µm) and by the well differentiated hairs emerging solitary or forming only partly triangular fascicles, while in *I. equiseti-hyemalis* the fasciculate hairs are just continuations of the hyphal cells that form the perithecial wall.

Whether or not two distinct genera are involved would require a phylogenetic analysis based on sequences of appropriate genes (W. JAKLITSCH, pers. comm.).

***Hydropisphaera arenula* (BERK. & BROOME) ROSSMAN & SAMUELS**, Stud. Mycol. 42: 30 (1999)

- ≡ *Sphaeria arenula* BERK. & BROOME, Ann. Mag. Nat. Hist. Ser. 2, 9: 320 (1982) – for further synonyms see ROSSMAN & SAMUELS (l.c.)
= *Orbilbia calamaria* (CES.) SACC., Syll. Fung. 8: 632 (1889)
≡ *Peziza calamaria* CES. in RABENHORST, Flora 13: 267 (1855)
= *Nectria equiseti-hiemalis* LARSEN & MUNK in MUNK, Dansk Bot. Ark. 17: 52 (1957)

Nectria equiseti-hiemalis LARSEN & MUNK (Fig. 3) was described from *Equisetum hyemale* (Denmark, Seest near Kolding, during October-January). In order to exclude a possible identity with *I. equiseti-hyemalis* we examined the type collection (see Fig. 3). The material is very sparse, with ca. 3-4 mature and some immature or empty ascospores. Ascospores orange-brown to dark red-brown, smooth, globose, glabrous, ca. 280 µm in diam., not changing colour in KOH, lateral wall 20-25 µm wide, of two layers, outer region of thin-walled, globose cells, inner region of elongate, hyaline cells. Ascospores (12-)16-22 × (3.2-)3.5-3.7(-4.2) µm in KOH, 1-septate, smooth (in KOH or CB). Although no striations were found on the ascospores, their inaequilateral, fusoid shape and the architecture of the perithecial wall suggest that *N. equiseti-hiemalis* is a synonym of *Hydropisphaera arenula* (for descriptions, see, e.g., BOOTH 1959, SCHMID & SCHMID 1991, as *Nectria arenula*). The ascospore size given by MUNK (1957) ("18-22 × 4-5 µm") does not very well match our measurements, particularly concerning the width. However, two recent collections of the same species on the same substrate prove that the spores strongly shrink when they loose turgor: while the living spores measured (13.5-)17-21(-22.5) × 4.3-5.2(-5.8) µm they were in the dead state only 16-19 × 3.9-4.1 µm. Also here no striations on the spore surface could be seen.

In comparison with collections of *Hydropisphaera arenula* on stems of various herbaceous plants we could not find any distinct differences. During a revision of types of names combined in *Orbilbia* FR., the type of *Peziza calamaria* CES. was examined and found to fit to *H. arenula*. The spores are here with 17-19 × 4.2-4.8 µm (in KOH) a bit wider than those in the type of *Nectria equiseti-hiemalis*. In all these specimens a distinct striation of the spores (in KOH or CB, oil immersion) could usually not be seen.

Specimens examined: Denmark: Jylland/Jutland, Kolding, Vyff's wood near Seest Mølleå, *Equisetum hyemale*, 29. 10. 1928, P. LARSEN (holotype of *Nectria equiseti-hiemalis*, C).

Germany: Baden-Württemberg, Schönbuch, Tübingen-Pfrondorf, Kirnbachtal, MTB 7420/1, 360 m s. m., Keuper (Stubensandstein), *Equisetum hyemale*, 16. 9. 2007, H.-O. BARAL (H.B. 8622b); - ibid. 7. 11. 2007 (not preserved).

Luxembourg: Lorentzweiler, Heederschleed, 252 m, *Equisetum hyemale*, 24. 9. 2007, G. MARSON (not preserved); - Howald, Zwickau, 285 m s. m., *Heracleum sphondylium*, 4. 8. 2005, G. MARSON (H.B. 7869d).

France: Deux-Sèvres, Clussay la Pommeraie, stems of *Urtica dioica*, 14. 4. 2005, B. COUE (C.L.L. 649).

Italy: Piemonte, Biella, *Festuca* or *Calamagrostis*, autumn 1850, V. DE CESATI (Rabh., Klotzschii herb. Viv. Mycol. ed. 2, no. 18, syntype of *Peziza calamaria*, H).

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