Entylomaster typhonii gen. et sp. nov. (Ustilaginomycetes) from Australia

Kálmán Vánky [1]* & Roger G. Shivas [2]

[1] Herbarium Ustilaginales Vánky (H.U.V.), Gabriel-Biel-Str. 5, D-72076 Tübingen, Germany
[2] Plant Pathology Herbarium, Queensland Department of Primary Industries and Fisheries, 80 Meiers Road, Indooroopilly, Queensland 4068, Australia

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Abstract. The four recognised smut fungi of Araceae are studied. A new genus and species, *Entylomaster typhonii,* are described and illustrated on *Typhonium brownii* from Australia. A new combination proposed is *Entylomaster dietelianus* on *Ambrosina bassii* from Italy.

Key words: Araceae, Doassansiales, *Entylomaster dietelianus, E. typhonii,* new genus, new species, new combination, smut fungi

Introduction

There is an interesting parasitic fungus in the Queensland Department of Primary Industries and Fisheries, Plant Pathology Herbarium (BRIP 8669), filed under the name *Entyloma* sp., on the endemic Australian *Typhonium brownii* Schott (Araceae). A detailed examination of this specimen showed that it was not an *Entyloma.*

Three smut fungi are known on members of the Araceae: **1.** *Melanustilospora ari* (Cooke) Denchev, type on *Arum maculatum;* **2.** *M. arisari* (Peglion) Denchev, type on *Arisarum proboscideum;* and **3.** *Entyloma dietelianum* Bubák, type on *Ambrosina bassii.* All three of these species have sori on the leaves (and petioles), the spores are embedded in the host tissues and the spore mass is not powdery. *Melanustilospora ari* and *M. arisari* are closely related species, earlier treated under the generic name *Melanotaenium* (comp. Vánky 1994: 144 & 147). Based on ultrastructural characters (Bauer *et al.* 1997), these two species were recently placed into the new genus *Melanustilospora* Denchev (2003: 476) within the Urocystaceae Begerow, R. Bauer & Oberw. Whereas the spores of the two *Melanustilospora* species are dark, those in *Entyloma dietelianum* are yellow to pale yellowish brown (for other characters see below).

The elaboration of the new, phylogenetic classificatory system of the smut fungi and related taxa (Bauer *et al.* 1997), led not only to a more correct placement of many of these fungi but also to several more general conclusions which have led to profuse changes and the transfer of a great number of species into different, often new genera. Some examples are: **1.** Species of *Ustilago* and *Sporisorium* are restricted to host plants in the Gramineae only (Vánky 1999; Bauer *et al.* 2000). **2.** All *Ustilago* species on dicotyledonous host plants and with a violet tint of the spores belong to the genus *Microbotryum* (Microbotryales) within the Urediniomycetes, whereas those without such a violet tint belong to other, often new genera (Vánky 1998). **3.** Dark-spored *Entyloma* and *Melanotaenium* species on Gramineae belong to *Eballistra, Jamesdicksonia* or *Phragmotaenium,* within the Georgefischeriales (Bauer *et al.* 2001). **4.** True *Entyloma* and *Melanotaenium* species are restricted to dicotyledonous host plants only. This last conclusion is important in determining the correct generic placement of the Australian '*Entyloma*' species on the monocotyledonous *Typhonium.*

*Corresponding author: e-mail: vanky.k@cityinfonetz.de

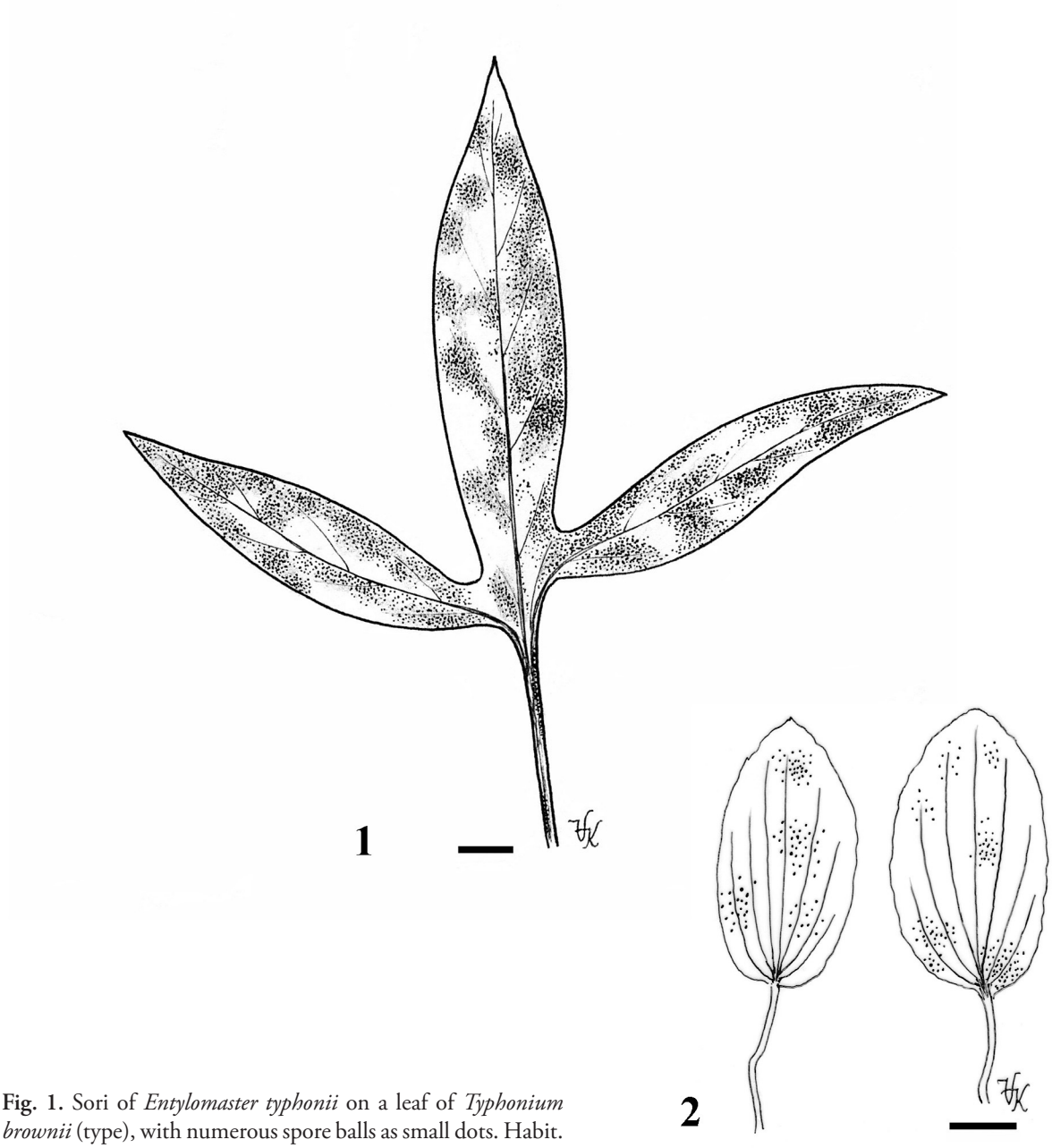


Fig. 1. Sori of *Entylomaster typhonii* on a leaf of *Typhonium brownii* (type), with numerous spore balls as small dots. Habit. Bar = 1 cm

Fig. 2. Sori of *Entylomaster dietelianus* on two leaves of *Ambrosina bassii* (type), with spore balls as small dots. Habit. Bar = 1 cm

Materials and Methods

Sorus and spore characteristics were studied using dried herbarium specimens. For light microscopy (LM), small pieces of the sori were rehydrated in a few droplets of a mixture of distilled water and lactophenol, on a microscope slide, followed by gently heating to boiling point, and lightly squashing with a lancet prior to examination. Thin, hand-cut sections were made with a razor blade. The sections were placed in lactophenol and covered with a cover glass. For scanning electron microscopy (SEM), dry sori were hand-sectioned, or dry spore balls were squashed, placed on double-sided adhesive tape, mounted on a specimen stub, sputter-coated with gold-palladium, c. 20 nm, and examined in a SEM at 10 kV. For transmission electron microscopy (TEM), the rehydrated material was fixed in 3 % glutaraldehyde and 4 % paraformaldehyde in a phosphate buffer at pH 7.2 for 4

days under vacuum at 4 °C. Following four transfers in 0.1 M sodium cacodylate buffer, the material was postfixed in 1 % osmiumtetroxide in the same buffer for 24 hours in the dark, washed in distilled water, and stained in 1 % uranyl acetate solution for 1 h in the dark. After 5 washes in distilled water, the material was dehydrated through 10 minute changes in 30 %, 40 %, 50 %, 60 %, 70 %, 80 %, 90 % and two changes in 100 % acetone. The material was embedded in Spurr's plastic, sectioned with glass knives and poststained for 5 minutes with lead citrate at room temperature, and washed again with distilled water. Semithin or thin sections were examined in LM or in a transmission electron microscope operated at 60 or 80 kV.

Results

The characters of the '*Entyloma*' species on *Typhonium* do not fit with any of the presently recognised 85 genera of true smut fungi (comp. Vánky 2002, 2004a: 102, 2004b: 172, 2005a: 106, 2005b: 113 & 114; Denchev 2003: 476; Piątek 2005: 34; Bauer *et al.* in press). A new genus is proposed:

Entylomaster Vánky & R.G. Shivas, **gen. nov.**

Sori *in foliis plantarum nutrientium familiae Araceae formantes maculas sporas in glomerulis irregularibus, in telis plantae nutrientis immersis continentes.* ***Glomeruli sporarum*** *e sporis tantum compositi sine cortice fungali, sine cellulis sterilibus.* ***Sporae*** *pallide coloratae (flavae, pallide flavidobrunneae), non colore nigrescente vel rubrobrunneo tinctae, in apice hypharum sporogenarum formatae.* ***Hyphae*** *intercellulares.* ***Porus septalis*** *simplex, pileolis membranaceis duobus clausus.* ***Conjunctio hospitis et parasiti*** *per apparatum complexum conjunctionis cum portionibus cytoplasmaticis.* ***Haustoria*** *nulla.*

Typus generis: E. typhonii.

Sori on host plants in the Araceae forming leaf spots containing spores agglutinated in irregular balls, embedded in the host tissue. **Spore balls** composed of spores only, without a fungal cortex, without sterile cells. **Spores** pale coloured (yellow, pale yellowish brown or orange-yellow, without blackish- or reddish brown tint), produced on the top of sporogenous hyphae. **Hyphae** intercellular. **Septal pore** simple, enclosed by two membrane caps. **Host-parazite interaction** by complex interaction apparatus with cytoplasmic portions. **Haustoria** absent.

Type of the genus:

Entylomaster typhonii Vánky & R.G. Shivas, **sp. nov.**

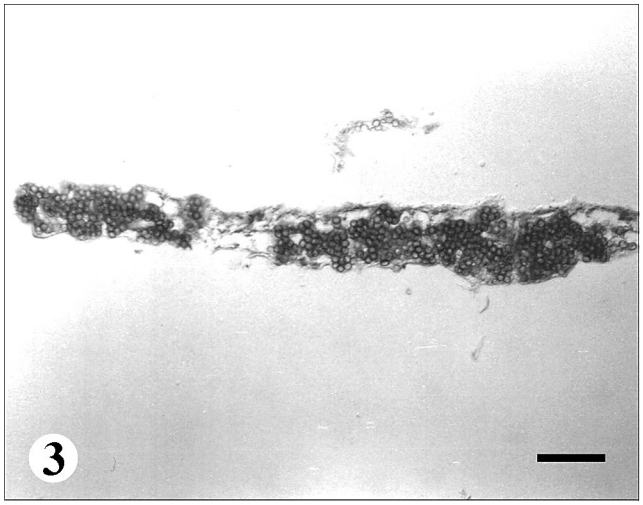
Typus *in matrice: Typhonium brownii Schott, Australia, Queensland, cca. 250 km NNW urbe Brisbane, 55 km WNW urbe Gympie, Mt. Sinai pr. oppid. Kilkivan, cca. 26°03' S, 152°09' E, 13.IV.1968, leg. J.M. Simmonds 18 995.* ***Holotypus*** *in BRIP 8669, isotypi in IMI 153 737 et in Herbario Ustil. Vánky, H.U.V. 20 221.*

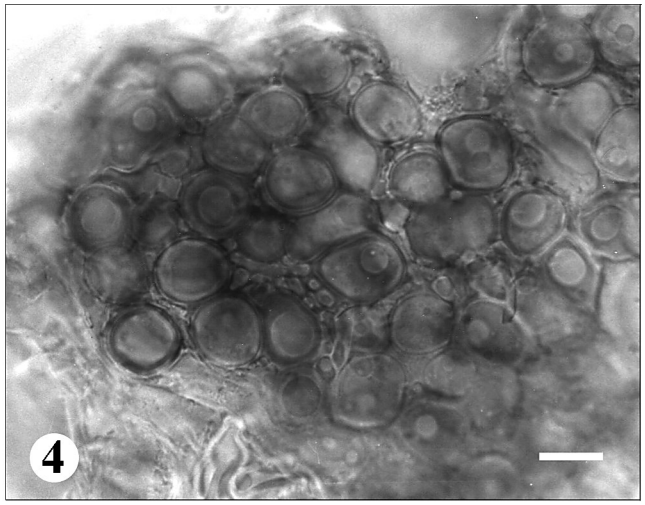
Sori *in maculis rotundis vel ellipsoidalibus foliorum diametro cca. 0,5-1 cm vel majores propter confluentiam nonnunquam fere totam laminam folii occupantes, flavidobrunnei, cum punctis multis, dense, irregulariter distributis, minutis, atrobrunneis, perspicue primarie in latere foliorum abaxiali evolutis. Maculae atrobrunneae glomeruli peculiares sporarum in telis foliorum plantae nutrientis immersi, disintegratione telarum plantae nutrientis liberi.* ***Glomeruli sporarum*** *e catervis nonnullis sporarum aggregatarum formati. Catervae minutae, globosae vel ellipsoidales, diametro cca. 30-60 µm, melleoflavae, e sporis decem vel nonnullis decem, arcte adherentibus, vel residuis hypharum fungalium separatis formatae. Catervae parvae in glomerulis magnis sporarum irregularibus, diametro 100-200 µm, vel propter reliquam agglomerationem majoribus, pallide usque atro-aurantiacoflavis, usque colore laterum, e sporis pluries centum, pressu separabilibus compositi. Cortex fungalis circum glomerulos sporarum nullis, sed glomeruli sporarum majores forte strato tenue cellularum compressarum plantae nutrientis circumdati.* ***Sporae*** *globosae, subglobosae, ellipsoidales raro irregulares, cum latere uno vel lateribus duobus deplanatis, 11-14,5 (–16) × 11-15 (–17,5) µm, limonio-, melleo- usque aurantiacoflavae; pariete plus minusve aequali, 1-1,5 µm crasso, leve; imago obliqua sporae saepe parum undulata propter residua hypharum inanium circumdantium. Sporae in apice hypharum sporogenarum intercellularum formatae.* ***Germinatio sporarum*** *non nota.* ***Cellulae sterilae*** *inter sporas et glomerulos sporarum nullae.*

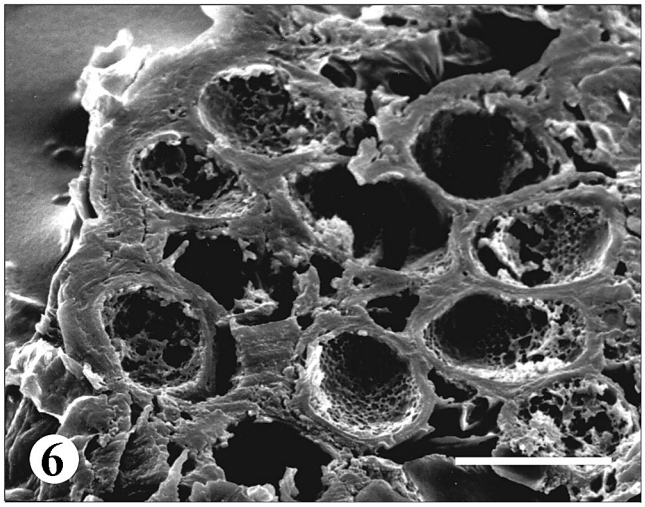
Sori (Fig. 1) as rounded or ellipsoidal leaf spots, c. 0.5-1 cm in diameter or larger by confluence, sometimes covering almost the whole lamina, yellowish brown, with numerous, densely, irregularly situated, minute, dark brown dots, evident especially on the abaxial side of the leaves. The dark brown dots are peculiar spore balls embedded in the leaf tissues, liberated by the disintegration of the host tissues. **Spore balls** (Figs 3, 4, 6) formed of several, small groups of aggregated spores. Small groups globose or ellipsoidal, c. 30-60 µm in diameter, honey-yellow, composed of ten to several tens of spores which are tightly adhering or separated by remnants of fungal hyphae. Small balls grouped into large, irregular balls of 100-200 µm in diameter, or larger by further agglomeration; pale to dark orange-yellow to brick-red, composed of hundreds of spores which separate by pressure. Fungal cortex around the balls lacking, but the large spore balls sometimes surrounded by a thin layer of compressed host cells. **Spores** (Figs 4-6) globose, subglobose, ellipsoidal, rarely irregular, with one or two flattened sides, 11-14.5 (–16) × 11-15 (–17.5) µm, lemon-, honey- to orange-yellow; wall more or less even, 1-1.5 µm thick, smooth; spore profile often slightly undulate due to the remnants of surrounding empty hyphae. Spores formed on the top of intercellular sporogenous hyphae (Fig. 5). **Spore germination** not known. **Sterile cells** between the spores or spore balls absent.

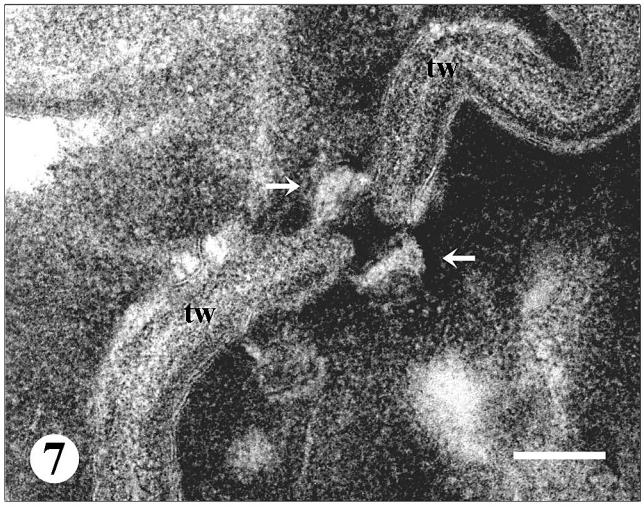
On Araceae: *Typhonium brownii* Schott.

Distribution: Australia. Known only from the type collection.









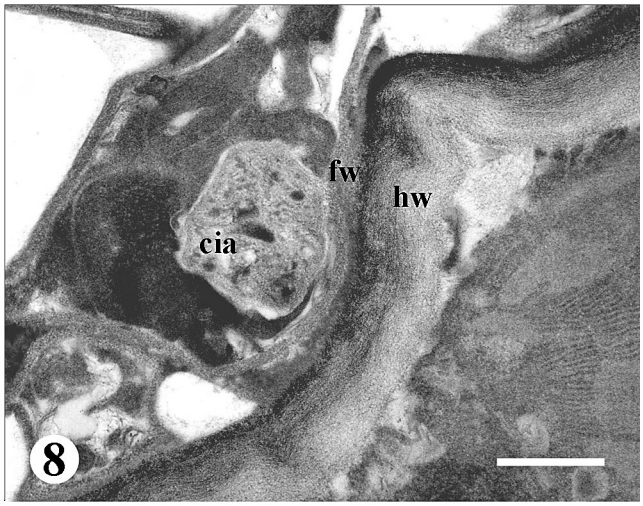


Fig. 3. Hand-cut transversal section of a leaf of *Typhonium brownii* with spore balls of *Entylomaster typhonii* embedded in the host tissue (type), in LM. Bar = 100 µm. **Fig. 4.** Spore balls and spores of *Entylomaster typhonii* (type), in LM. Bar = 10 µm. **Fig. 5.** Sectioned spores of *Entylomaster typhonii* (type), in TEM. To the left part of a young spore on the top of a sporogenous hypha (arrows) (Photo Ms T. Desley). Bar = 5 µm. **Fig. 6.** A sectioned spore ball and dried (empty) spores of *Entylomaster typhonii* (type), in SEM. Bar = 10 µm. **Fig. 7.** Simple septal pore enclosed by two membrane caps (arrows) of *Entylomaster typhonii* (type), in TEM (Photo Dr R. Bauer) – tw = transversal wall (septum). Bar = 0.1 µm. **Fig. 8.** Complex interaction apparatus of *Entylomaster typhonii* (type), in TEM (Photo Dr R. Bauer): cia = complex interaction apparatus, fw = fungus cell wall, hw = host cell wall. Bar = 0.5 µm

Etymology: *Entylomaster,* from *Entyloma,* a genus of smut fungi, and the Latin *aster, -a, -um* = such as, similar to but not identical; as suffix to nouns indicating incomplete likeness, in this case to the genus *Entyloma.*

Knowing that *Entyloma* species are restricted to dicotyledonous host plants, we examined '*Entyloma*' *dietelianum.* This revealed that it is best to place it into the genus *Entylomaster* as:

Entylomaster dietelianus (Bubák) Vánky & R.G. Shivas, **comb. nov.**

Basionym: *Entyloma dietelianum* Bubák, Ann. Mycol. **1**: 255, 1903. — *Melanotaenium dietelianum* (Bubák) Ciferri 1924: 59. — Type on *Ambrosina bassii* L., Italy, Island of Sardigna (Sardinia), Sinnai, Punto da Corsetta, 31 Dec 1896, leg. Martelli (BPI 176 989!).

Sori (Fig. 2) on the leaves as small, brown dots or pustules, scattered or gregarious, evident on the abaxial side, formed by spore balls embedded in the host tissue. **Spore balls** globose, ellipsoidal or elongated, 120-200 (–350) × 140-250 (–500) µm, honey- to orange-yellow, composed of hundreds of spores which separate by hard pressure. No fungal cortex around the balls, no sterile cells between the spores. **Spores** densely packed, varying in shape and size, globose, ovoid, broadly ellipsoidal to usually subpolyhedrally irregular, with one or several flattened sides by mutual pressure, 8-16 × 9-20 µm, when young hyaline, later yellow to pale yellowish brown, content usually finely granular; wall smooth, 1-3 (–3.5) µm thick, two-layered, inner layer thin, even (0.5-1 (–1.5) µm), outer layer even or unevenly thick, subhyaline, sometimes with a papilla or with a hyphal appendage. According to Bubák (*l.c.*), the spores are formed intercellularly on the top of sporogenous hyphal branches of which remnants may be attached to one side of the spores as thin, hyaline, appendage.

On Araceae: *Ambrosina bassii* L.

Distribution: S. Europe (Italy). Unamuno (1942: 367) reported it on an unidentified Araceae from Morocco which represents *Entyloma ficariae* A.A. Fisch. Waldh. on *Ranunculus* sp. from *ficaria* group (Vánky 1991: 159).

Entylomaster dietelianus and *E. typhonii* seem to be closely related species. Both parasitise plants in the Araceae, and both have spores agglutinated into smaller or larger balls. In *E. dietelianus* the spore balls are larger and the spores more tightly packed than those in *E. typhonii. E. dietelianus* has mostly subpolyhedrally irregular spores, whereas in *E. typhonii* they are mostly rounded. In *E. dietelianus* no remnants of fungal hyphae between the spores could be observed.

For comparison, the two *Melanustilospora* species, belonging to the Urocystaceae, are also presented:

Melanustilospora ari (Cooke) Denchev 2003: 476.

Protomyces ari Cooke 1872: 7. — *Melanotaenium ari* (Cooke) Lagerheim 1899: 98. — Type on *Arum maculatum* L., England, Chichester, May 1872, F.V. Paxton.

Ustilago plumbea Rostrup, in Thümen, Mycotheca universalis no. 531, 1876. – *Melanotaenium plumbeum* (Rostr.) Pirotta 1889: 312 (as '*Melanothaenium*'). — Type on *Arum maculatum* L., Denmark, Funen, Tiselholt, Jun 1875, E. Rostrup; isotypes in Thümen, Mycoth. univ. no. 531, H.U.V 1382!

Sori in leaves and petioles as lead-coloured pustules with irregular, distinct margins, often clustered or in rows, covered by the epidermis. **Spores** globose, ovoid, mostly irregular, angular, 12-15 × 12-20 µm, yellowish brown to dark olivaceous brown; wall two-layered, endospore even, c. 1.5 µm thick, dark olivaceous brown, exospore uneven, 0.5-3.5 µm thick, lighter coloured (subhyaline to pale yellowish brown), often pierced by one to several narrow, thin-walled protuberances of the endospore (germ pores?).

On Araceae: *Arum* (?)*elongatum* Steven, *A. italicum* Miller, *A. maculatum* L., *A. orientale* Bieb. (incl. *A. besseranum* Schott), *Biarum tenuifolium* (L.) Schott.

Distribution: Europe.

Melanustilospora arisari (Peglion) Denchev 2003: 476.

Melanotaenium plumbeum (Rostr.) Pirotta f. *arisari* Peglion 1894: 424. — *Melanotaenium arisari* (Peglion) Ciferri 1924: 57. — Type on *Arisarum proboscideum* (L.) Savi, Italy, Avellino, 'torrente di Capriglia', May 1894.

Sori in the leaves, petioles and floral bracts as black, round spots, up to 1 cm in diameter, covered by the epidermis. **Spores** irregular, often angular, occasionally ovoid or globose, 18-20 × 20-26 µm, dark reddish brown; wall thick, smooth.

On Araceae: *Arisarum proboscideum* (L.) Savi.

Distribution: Europe (Italy). Known only from the type locality.

Material not seen. Description taken from the original and Zundel (1953: 274). Ciferri (1924: 57), in connection with this species wrote: 'Differisce nettamente dal *M. ari*', but he later (Ciferri 1963: 174) treated *Melanotaenium arisari* as a synonym of *M. ari*. A critical study of the type is desired.

Melanustilospora arisari is similar to *M. ari* but, according to the original description (Peglion 1894: 424), the spores are larger (18-20 × 20-26 µm).

Discussion

The placement of *Entylomaster* in the classification of the smut fungi requires knowledge of either its spore germination or its ultrastructure and DNA sequencing data. Without this data we can only speculate as to its phylogenetic position. The appearance of the sori, and the fact that the spores are agglomerated into spore balls, indicate a relationship to the *Doassansiaceae* (Azbukina & Karatygin) R.T. Moore ex P.M. Kirk, P.F. Cannon & J.C. David (of the order Doassansiales R. Bauer & Oberw.), a family with 10 genera and 40 species, of which all but one form spore balls. These smut fungi parasitise aquatic or paludal plants of widely different genera, both mono- and dicotyledonous ones. Indeed, the host plant of

E. typhonii, Typhonium brownii, 'grows on rainforest margins, in sheltered gullies and along creek banks' (Harden 1993: 35). The ultrastructure of the septum pore and host-parasite interaction of *E. typhonii,* kindly checked by Dr R. Bauer, demonstrated without doubt that the fungus is a member of the Doassansiaceae. Its septal pore (Fig. 7) is simple, with two membrane caps (arrows) closing the pore. The host-parasite interaction of the intercellular hyphae (Fig. 8) is a complex interaction apparatus (cia), containing cytoplasmic compartments.

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