Wawelia argentea and *W. microspora*, two new species of xerophilous fungi on rabbit and hare dung in Britain

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Two new species of *Wawelia* are described from rabbit and hare dung incubated under conditions of restricted water supply. A key to the five known species of *Wawelia* is provided.

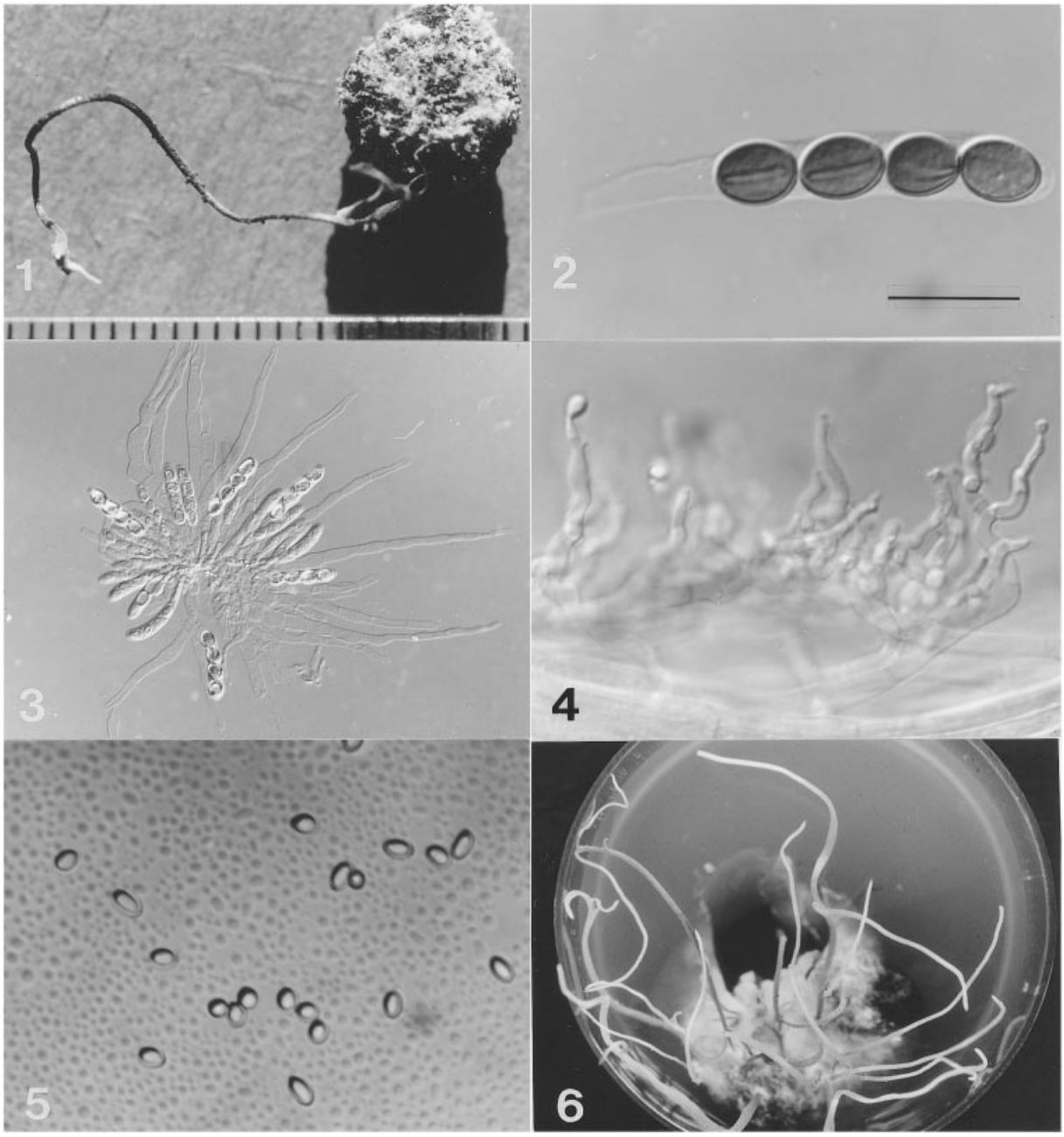
When Namyslowski (1908) described *Wawelia regia*, collected on rabbit dung near Kraków, Poland, he placed it in the Hypocreaceae but it has since been classified in the Xylariaceae (Müller, 1959; Müller & Arx, 1973). Laessøe (1994), however, considered its status within the Xylariaceae as somewhat precarious although Whalley (1996) and Ju & Rogers (1996) accepted *Wawelia* as a 'good' member of the family. Minter & Webster (1983) described *W. octospora* on rabbit dung which had been incubated for several months over saturated salts solutions giving relative humidities in the range from 90 to 95%. Lundqvist (1992) has since described *W. effusa* on partly dried material of hare dung from Sweden and from deer dung in Hungary. Richardson & Watling (1997) provided a key to four species of *Wawelia*: two of them were as yet unnamed and they are the new species described in this paper.

MATERIALS AND METHODS

Species of *Wawelia* developed fruit bodies on droppings of rabbit (*Oryctolagus cuniculus* L.) and hare (*Lepus capensis* L.) after incubation for several weeks under conditions of restricted water supply. Samples, mostly of rabbit dung, collected from a wide range of habitats and locations, were incubated in the light either in Petri dishes lined by moist filter paper or in deeper dishes with transparent lids containing beach sand and supplied intermittently with small amounts of water. After 2–4 wk, thread-like stromata appeared on a few of the samples. These stromata formed conidia near their apices and, later, scattered superficial perithecia along their length. Cultures were prepared in three ways. The tips of young stromata were removed aseptically and transferred to Petri dishes of tap water agar (TWA) supplemented with a mixture of antibiotics giving a final concentration of *ca* 50 µg penicillin and 30 µg streptomycin ml^{-1} of medium. After a few days, transfers were made to fresh media from hyphal growths from the cut ends and growing tips of the excised stromata. In a second method the tips of stromata bearing conidia were held in sterile forceps and wiped over the surface of TWA. The conidia germinated within 24–48 h and transfers were then made to fresh media. The ascospores of *Wawelia* are not violently discharged and accumulate at the ostioles of the perithecia. Clumps of such ascospores, or ascospores released by crushing a ripe perithecium, were suspended in a saturated solution of filter-sterilized pigs' pancreatin (Sigma) at pH 9 in cavity microscope slides placed in moist chambers and incubated for 5 h at 37 °C. The treated ascospore suspension was streaked or spotted out onto dung extract agar (DEA, a filtered extract of 1% dried cow dung in 2% agar) or yeast extract sucrose agar (YESA, 4 g yeast extract, 20 g sucrose, 1 g KH_2PO_4, 0·5 g $MgSO_4$, 15 g agar l^{-1} H_2O) and incubated at room temperature. Good germination (about 50%) occurred within 12 h. Untreated ascospores or spores treated by exposure to 60° for 1 h failed to germinate. Germinated ascospores were transferred to fresh media. Following isolation by all these methods, growth took place on a variety of laboratory media such as PDA, DEA, yeast extract agar (YEA, 1% yeast extract, 2% agar) and YESA. The production of stromata was stimulated by growing cultures of both fungi in slopes or Petri dishes on these media with the addition of a few sterilized (autoclaved) rabbit pellets. The cultures were incubated in diffuse light at room temperature. In some cases, small stromata developed on agar media without rabbit pellets.

Wawelia argentea J. Webster, sp. nov. (Figs 1–6)

Stroma peritheciale filiforme, cum vel sine ramis, ad 30 mm longa et 0·1–0·5 mm diam. curvum et contortum, cylindricale vel leviter planum, argenteum-glaucum sed album ad acutum apicem, leve et lucidum prope basim, pruinosum supra. *Perithecia* superficialia singula aut corymbis per majorem partem longitudinis stromatis, late pyriformia cum lata plana basi et brevi collo, ad 400 µm diam. et 400 µm alta, primum argentea-glauca, postea nigriora-glauca. *Asci* 4-sporati, late clavati, unitunicati, sine apicali apparatu, cum longa desinente basi et cylindrica superiore parte continenti dense

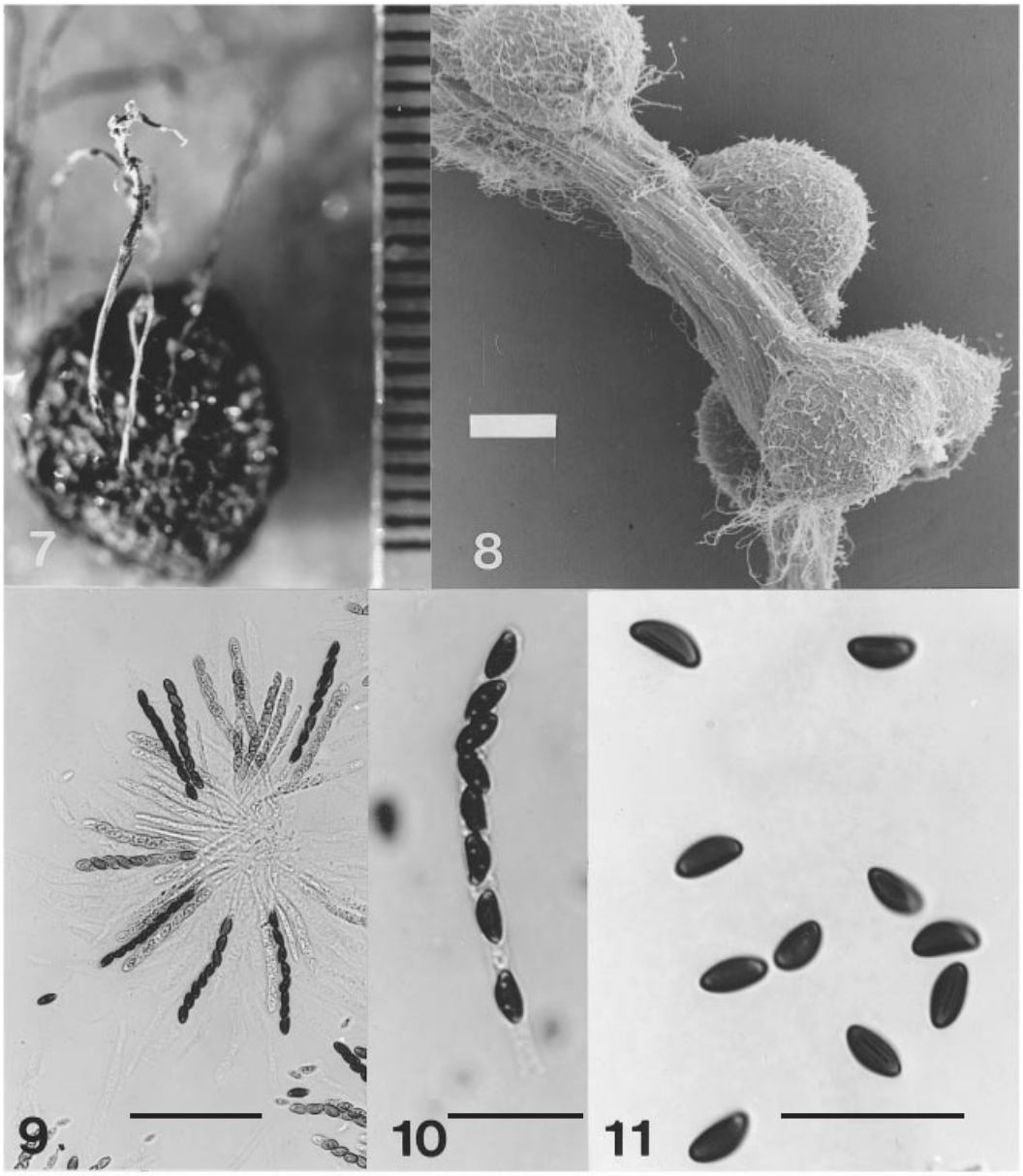


Figs 1–6. *Wawelia argentea*. **Fig. 1.** Rabbit dung pellet with perithecial stroma (mm scale). The stroma bears superficial perithecia scattered along its base and conidia at the paler apex. **Fig. 2.** Ascus. Note the cut-off base, the absence of an apical apparatus and the longitudinal germ slit visible on the face of three of the ascospores. **Fig. 3.** Paraphyses and immature asci. **Fig. 4.** Conidiophores. **Fig. 5.** Conidia. **Fig. 6.** Culture grown in a 9 cm Petri dish on YEA with sterilized rabbit pellets. The stromata have developed conidia near their tips. Bar: Figs 2, 4 and 5 = 20 µm; Fig. 3 = 100 µm.

congregatas ascosporas: 87–93 × 10–12 µm. *Ascosporae* uniseriatae, late ellipticae vel paulo inaequilaterales, levibus parietibus, nigro-glaucae ad nigrae, cum hyalina rima, 15–18 × 9–12 µm. *Paraphyses* tenuissimis parietibus, desinentes, septatae, multo longiores quam asci, compositae ex vacuis cellis ad 12 µm latis. *Conidiophorae* cum vel sine ramis, multa longitudinis varietate, rectae vel contortae, desinentes ad apicem, 2–3 µm diam. Conidia sicca, elliptica-fusoidea, levia, paulo protuberanti desinente basi, et cum lata cicatrice, 3–4 × 2–2·5 µm.

In sicco cuniculorum stercore post longam incubationem, collectum ad Cornworthy, Dumnonia, mense Julio 1995. Herb. **K** (M) 59520 typus est.

Perithecial stromata up to 30 mm long and 0·1–0·5 mm diam., thread-like, branched or unbranched, wavy and contorted, cylindrical to slightly flattened, silvery grey along most of their length, white at the pointed tip, smooth and lustrous near the base, pruinose in the upper portions due to the development of conidiophores and conidia (Fig. 1). The outer layer of the stroma is made up of *textura porrecta* hyphae, 4–5 µm diam. with grey walls 0·5–1·0 µm thick. *Perithecia* up to 400 µm diam. and 400 µm high, superficial, single or in clusters along much of the length of the stroma, broadly pyriform, with wide, flattened bases and short, cylindrical,



Figs 7–11. *Wawelia microspora*. **Fig. 7.** Rabbit dung pellet with perithecial stromata (mm scale). **Fig. 8.** Portion of a perithecial stroma bearing superficial perithecia (SEM). Bar = 200 µm. **Fig. 9.** Squash preparation of asci and paraphyses. Bar = 50 µm. **Fig. 10.** Ascus. Note the cut-off base. Bar = 25 µm. **Fig. 11.** Ripe ascospores. Bar = 25 µm.

ostiolate necks. They are at first silvery grey and appear pruinose because of a covering of conidiophores, at maturity a darker grey. *Asci* 4-spored, cylindrical to broadly clavate, unitunicate, 87–93 µm × 10–12 µm, lacking an apical apparatus, with long tapering bases and cylindrical upper portions containing the closely packed ascospores (Fig. 2). *Ascospores* uniseriate, broadly elliptical in face view, slightly inequilateral when viewed from the side, smooth walled, dark grey to black, with a hyaline germ slit running the length of the spore, 15–18 × 9–12 µm (Fig. 2). *Paraphyses* thin-walled, tapering, septate, greatly exceeding the asci in length and composed of empty cells up to 12 µm wide (Fig. 3). *Conidiophores* developing directly from the outer cells of the stroma, branched or unbranched, variable in length, straight or contorted, sometimes zig-zag like, tapering to the tip, 2–3 µm diam. (Fig. 4). Conidiogenous cells integrated. Conidiogenesis holoblastic, conidiogenous cell proliferation sympodial. Conidial secession is indicated by a flattened, slightly thickened, scar. *Conidia* dry, ellipsoid-ovate, hyaline, smooth, with a slightly protruding tapering base and a flattened scar, 3–4 × 2–2·5 µm (Fig. 5).

Cultures. On YEA slow-growing, with a silky appressed mycelium on the agar until it made contact with the rabbit pellets. Within a few days of making contact with the pellets numerous positively phototropic stromata developed. They resembled those formed on incubated pellets, but were often longer (Fig. 6). Conidia were formed within a few days on the tips of the stromata. Perithecial rudiments were first observed near the bases of the stromata 14 d after inoculation. They did not mature over a period of observation of several weeks.

The type material was from a sample of about 60 pellets, collected by J. Webster at Cornworthy, near Totnes, S. Devon (Map ref. SK 847554) on 13 July 1995 and incubated on filter

paper in a Petri-dish on a window sill at a temperature of about 20–25°. After 3 wk stromata were noted on eight of the pellets. When examined on 18 Sep. it was found that the perithecia contained 4-spored asci, a feature of *W. regia*, the type species of the genus. The shape of the stromata, the dimensions of the conidia, asci and ascospores differ, however, from those of *W. regia*. If a ripe perithecium is crushed in a drop of water, detached asci and separate ascospores escape, and examination of the bases of these asci shows that they are truncate. It is believed that the ascus walls deliquesce in the body of the perithecium.

Other material examined. Several other collections have been made by J.W. on rabbit dung:

From the same site near Cornworthy, 4 Aug. 1998, **K**(M)59521. Ripe perithecia were seen on 26 Sep. The asci and ascospores were somewhat smaller than in the earlier collection: 60–68 × 10–11 μm and 12·5–13 × 8–10 μm respectively. In culture, on PDA slopes with sterilized rabbit pellets, perithecial stromata were formed freely.

High Land of Orcombe, near Exmouth, Devon (SY 023796), 14 Sep. and 12 Oct. 1998. Plentiful material of *W. argentea* was found among samples collected on the edge of sea cliffs. In both collections the stromata grew to up to 50 mm tall. Cultures were established from excised stromatal tips on TWA which went on to develop ripe perithecia within 25 d. Asci were 50–67 × 10–11 μm and the ascospores 12–15 × 9·5–12·5 μm.

Wawelia microspora J. Webster, sp. nov. (Figs 7–11)

Stromata 20–30 mm longa, 0·1–0·3 mm lata, primum sine ramis, cylindrica, pallide fusca, cum roseis acutis apicibus, postea contorta et ramata, glauca vel fusca ad nigra, luculenta, plana, vel longis striis per longitudinem. Rami distantes, desinentes in acutum album apicem, pulverulentes. *Conidiophora* crescentes de superficialibus cellis stromatis et de superficie peritheciali, hyalina, desinentes, ad 45 μm longae et 2·5–3 μm latae. *Conidia* hyalina, elliptica ad cylindrica, cum basali plana cicatrice, 3–4 × 2 μm. *Perithecia* sparsa, late separata per longitudinem stromatis, primum levia et pallida flava-fusca, postea glauca-fusca ad nigra, pilosa, subglobosa ad late obpyriformia, cum lata plana basi et brevi papilliformi ostiola: 230–360 μm diam. et circa 250 μm alta. *Asci* octospori, cylindricales, longo culmo, sine manifesto apicali apparatu, 75–88 × 5 μm. *Paraphyses* longiores quam asci, ramatae, compositae ex cellis parietibus tenuis, ad 8 μm latae. *Ascosporae* uniseriatae, vel paulo superjacentes, inaequilaterales vel ellipticae, glaucae vel nigrae cum longitudinali hyalina rima, primo biguttulatae, terminis rotundis, 7·5–8 × 3–4 μm.

In siccis cuniculorum stercoribus post longam incubationem. Collectum in Cawsand Beacon, prope Belstone, Dumnonia, mense Septembri, 1995. Herb. **K**(M)59516 typus est.

Stromata 20–30 mm long, 0·1–0·3 mm wide, at first unbranched, cylindrical, pale brown, with pink, pointed tips which are phototropic, later becoming contorted and branched, greyish-brown to black, glistening, flattened or with longitudinal ridges, branches distant, tapering to a fine white point, powdery due to the production of conidia (Fig. 7). Outer layer of stroma made up of thick-walled *textura porrecta* hyphae up to 75 μm long and 2–4 μm, wide near the branch tips, widening to 10 μm in older parts. *Perithecia* sparse, widely separated along the length of the stroma, at first smooth and pale yellowish-brown, later greyish-brown to black and villose due to the development of conidiophores, sub-globose to broadly obpyriform with a wide flat base and a short papilla-like ostiole, or occasionally with two ostioles, 230–360 μm diam. and *ca* 250 μm high, (Fig. 8). The outer wall of the perithecium composed of *textura prismatica* of brown polygonal cells 10–20 μm across. *Asci* 8-spored, cylindrical, without obvious apical apparatus, I/KI negative, 75–88 × 5 μm (Figs 9, 10). *Ascospores* uniseriate or slightly overlapping, inequilateral or elliptical in outline, grey to black with a longitudinal hyaline germ slit, at first biguttulate, ends rounded, 7·5–8 × 3–4 μm (Fig. 11). There is no evidence that the ascospores are discharged: they accumulate around the ostiole. *Paraphyses* exceeding the asci, branched, composed of thin-walled sac-like cells up to 8 μm wide. *Conidiophores* developing from the surface cells of the stroma and the perithecial wall, hyaline, tapering, up to 45 μm long and 2·5–3 μm wide. The apex of the conidiophore is 'zig-zag' like with a series of flattened scars. Conidiogenesis holoblastic with a basal scar of secession. *Conidia* hyaline, elliptic to cylindrical with a basal flattened scar, 3–4 × 2 μm.

The type material was collected by J.W. at Cawsand Beacon, near Belstone, Dartmoor, Devon, SK 636915, on 3 Sep. 1995, following a period of prolonged dry weather. The dry pellets were incubated as described above. Within 3 wk some of the pellets produced thread-like, pointed, pink stromata which were strongly phototropic. Two months after collection scattered perithecia were noted along the length of some of the stromata and when one was crushed 10 wk after incubation, ripe 8-spored asci were found with small ascospores.

Other material examined. Several other collections have been made by J.W. on rabbit dung:

Hay Tor, near Bovey Tracey, Devon (SX 755768), 14 July, 1998 **K**(M)59517. perithecial stromata observed 13 d later and ripe

***Key to species of* Wawelia**

1. Stromata effuse, applanate, in patches < 10–15 mm across. Perithecia 650–960 × 615–720 μm. Asci 8-spored 110–140 × 13–15 μm. Ascospores 15–19 × 9–10 μm . . . . . *W. effusa*
1′. Stromata filiform or cylindrical-conical 2
   2. Stromata cylindrical-conical 3–5 × 1–1·5 mm. Perithecia 240–400 μm. Asci 4-spored, 60–80 μm. Ascospores 6–8 × 4–6 μm, conidia 4–6 × 2 μm . . . . . *W. regia*
   2′. Stromata filiform 3
3. Asci 4-spored, 50–93 × 10–12 μm. Ascospores 12–18 × 9–12 μm, conidia 3–4 × 2–2·5 μm. Stromata < 30 × 0·1–0·3 mm. Perithecia up to 400 × 400 μm . . . . . *W. argentea*
3′. Asci 8-spored 4
   4. Ascospores 9–12 × 6–8 μm, conidia 8–12 × 2–4 μm. Asci 70–90 × 6–8 μm. Stromata 2–25 × 0·1–0·3 mm. Perithecia hairy, 300–500 μm, with setose ostioles . . . . . *W. octospora*
   4′. Ascospores 7·5–10·5 × 3–5 μm, conidia 3–5 × 2–3·5 μm. Asci 75–88 × 5 μm. Stromata 20–50 × 0·1–0·3 mm. Perithecia 230–360 × 250 μm . . . . . *W. microspora*

perithecia on 8 Sep. Cultures derived from a single ascospore and from multi-ascospore inoculum formed stromata with numerous perithecia, preserved as **K**(M)59518.

Hound Tor, near Manaton, Devon (SX 741789), 14 July, 1998. Cultures prepared from stromatal tips formed stromata but without perithecia.

Hew Down, near Watern Tor, Gidleigh, Devon (SX 632868), 12 Aug. 1998. **K**(M)59519. Wildtor Well, near Wild Tor, Gidleigh, Devon (SX 627875), 12 Aug. 1998.

One collection on brown hare dung (M.J.R.): Exposed cliff-top heath at Hobbister, Orkney Mainland (HY 396064), 27 Sep. 1994. Immature perithecia present on incubated material on 15 Nov. 1994, which subsequently matured after being passed to A.J.S.W.

Cultures of the two species of *Wawelia* described here have been deposited in the following culture collections: CBS, Baarn, The Netherlands, CABI (IMI), Egham, and ATCC, U.S.A. CABI accession numbers are *W. argentea* IMI 380160 (1), *W. microspora* IMI 380161 (2).

DISCUSSION

Kuthubutheen & Webster (1986*a*) have pointed out that the conditions under which coprophilous fungi are usually studied in the laboratory, in which dung samples are supplied with plentiful water and are incubated at high humidity and at room temperature, differ greatly from the conditions to which such fungi are exposed in the field, where fluctuating and often low water contents of the substratum are the norm. When rabbit dung is deliberately incubated for long periods (several weeks) at r.h. values below saturation a number of fungi not normally observed on moist dung samples may develop. These include several species of *Aspergillus* and their *Eurotium* teleomorphs (*A. candidus*, *A. repens*), *Penicillium claviforme*, *Stilbella erythrocephala*, *Doratomyces stemonitis*, *D. nanus* and *Isaria felina*. Two species were originally discovered by incubating rabbit dung at low humidity, *Onychophora coprophila* (Gams, Fisher & Webster, 1984) and *Wawelia octospora* (Minter & Webster, 1983). It seems that there is a specialised group of xerophilous coprophilous fungi adapted to growth and fruiting on substrata with a relatively low water content. Kuthubutheen & Webster (1986*b*) have shown that some of these fungi, such as *O. coprophila* and *I. felina*, fruit more profusely when incubated at low r.h. The same is probably true of *W. octospora* and the two new species of *Wawelia* described here, but physiological studies are desirable. This is the most likely explanation for the apparent rarity of *Wawelia* as discussed by Lundqvist (1992). As far as we are aware there are no reports of field collections of *Wawelia* spp.: all the species known have been described from incubated dung samples. It is of interest that the three species reported from Britain seem to grow, although not exclusively, on leporid dung from exposed sites such as moorland, exposed sea cliffs, wind-swept heaths and sand dunes and, in view of the incubation conditions under which they occur, these are the kind of situations where fruiting might be expected. We have noted in material of all three species of *Wawelia* reported from Britain that, even under the relatively dry conditions of incubation, young stromata accumulate beads of liquid along their length. It is possible that these represent condensed water and we speculate that the stromata may be able to absorb water vapour as well as being able to absorb water in the liquid state.

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