

## Three new species of *Oidiodendron* Robak from Spain

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**Abstract:** Three new *Oidiodendron* species, isolated from litter and soil samples collected in Spain, are described and illustrated. *Oidiodendron muniellense* is characterized by conidiophores with dematiaceous, seta-like branches fertile above, and by its globose to subglobose, roughened conidia. *Oidiodendron ramosum* is distinguished by its long, broadly recurved, fertile conidiophore branches, and by its subglobose to ellipsoidal, smooth to slightly roughened conidia. *Oidiodendron reticulatum* bears simultaneously two types of conidiophores: one of them with a network of anastomosed, brown and very ornamented hyphae which arises from the upper part of the conidiophores forming a reticulum, and another without such a complex structure. Both conidiophores produce ellipsoidal and roughened conidia. The sequence analysis of rDNA fragments, which included partial sequences of the genes 5.8 and 28S and the complete sequence of ITS2, of the three new species, and of other *Oidiodendron* species and other members of *Myxotrichaceae*, confirmed that the herein proposed species are genetically distant from the other species tested. A dichotomous key to all the accepted species of *Oidiodendron* is provided.

**Taxonomic novelties:** *Oidiodendron muniellense* Calduch, Stchigel, Gené & Guarro sp. nov., *O. ramosum* Calduch, Stchigel, Gené & Guarro sp. nov., *O. reticulatum* Calduch, Stchigel, Gené & Guarro sp. nov.

**Key words:** hyphomycetes, litter, *Oidiodendron*, soil, Spain, taxonomy.

## INTRODUCTION

During a survey of hyphomycetes from plant debris and soil in Spain, we isolated three interesting hyphomycetes which belong to the genus *Oidiodendron* Robak. One of them was from a soil sample collected in the Canary Islands, and the other two from litter collected in the Asturias province.

*Oidiodendron* is a cosmopolitan genus whose members can usually be found in soil or colonizing different cellulose substrates, i.e. litter, wood pulp, bark, mosses, paper, although occasionally they can also be isolated from lichens or from air (Domsch *et al.* 1980). Additionally, some *Oidiodendron* species have also been reported as ericoid mycorrhizal fungi (Couture *et al.* 1983, Dalpé 1989, Hambleton & Currah 1997, Lacourt *et al.* 2001). The genus was erected by Robak in 1932 with three species: *O. fuscum* Robak, *O. nigrum* Robak and *O. rhodogenum* Robak, all isolated from wood pulp in Norway (Robak 1932). The first two species are currently synonyms of *O. tenuissimum* (Peck) Hughes and *O. cerealis* (Thüm.) G.L. Barron, respectively. Key morphological features of *Oidiodendron* are erect and well differentiated dematiaceous conidiophores, profusely branched above, forming fertile hyphae which fragment basipetally to form arthroconidia. The teleomorphs of *Oidiodendron* are members of *Myxotrichum* Kunze and *Byssoascus* (G.L. Barron & C. Booth) Arx, both belonging to the ascomycetous

family *Myxotrichaceae* (Sigler & Carmichael 1976, Currah 1985) of uncertain taxonomic position (Sugiyama *et al.* 1999, Sugiyama & Mikawa 2001). Molecular studies confirmed these anamorph-teleomorph connections (Hambleton *et al.* 1998). The genus *Oidiodendron* has been reviewed by Barron (1962), Ellis (1976) and Domsch *et al.* (1980). More recently Udagawa & Toyazaki (1987) emended the genus to include specimens with setiform sterile hyphae which arise from the conidiophore stipe. Currently, a total of 20 *Oidiodendron* species have been accepted, excluding the anamorphs of *Byssoascus* and *Myxotrichum*. The most recent addition to *Oidiodendron* has been *O. myxotrichoides* Calduch *et al.* (2002), which provides a novel character for the genus, i.e. the development of conidiophores from globose or subglobose, dark brown sporodochia.

In this study, based on the mentioned isolates, we propose three new species of *Oidiodendron*. A comparative study employing rDNA sequences of these taxa and other members of *Myxotrichaceae* provide further support for this proposal.

## MATERIALS AND METHODS

### Fungal isolation and characterisation

Numerous plant debris samples, including wood, bark, leaves and fruits of deciduous trees and herbaceous plants were collected from the Muniellos Integral

Nature Reserve. The Muniellos forest is located in the southwest of Asturias province (northern Spain). It has a surface area of 5542 ha and an altitude ranging from 650 to 1642 m. The average annual temperature is 6–10 °C and the annual mean rainfall is 1400–2300 mm. The vegetation is composed of forests of *Quercus petraea* (Matt.) Liebl., *Q. pyrenaica* Willd., *Betula celtiberica* Rothm & Vasc., *Fagus sylvatica* L., river-side forests and mixed forests of *Fraxinus excelsior* L. and *Acer pseudoplatanus* L. (Fernández & Bueno 1996).

**Table 1.** List of names, strain numbers and accession numbers of the species analysed.

Species	Strain	Accession No. EMBL
<i>Oidiodendron cerealis</i> (Thüm.) G.L. Barron	UAMH 1522	AF062788
<i>O. chlamydosporicum</i> Morall	UAMH 6520 (T)	AF062789
<i>O. citrinum</i> G.L. Barron	UAMH 1525 (T)	AF062790
<i>O. echinulatum</i> G.L. Barron	IMI 110132	AF062791
<i>O. flavum</i> Szilvinyi	UAMH 1524	AF062792
<i>O. griseum</i> Robak	CBS 249.33	AF062793
<i>O. maius</i> G.L. Barron	UAMH 1540 (T)	AF062798
<i>O. muniellense</i> Calduch, Stchigel, Gené & Guarro	IMI 391998 (T)	AJ634700 <sup>1</sup>
<i>O. myxotrichoides</i> Calduch, Gené & Guarro	IMI 388795 (T)	AJ635314
<i>O. periconoides</i> Morrall	DAOM 197506 (T)	AF062802
<i>O. pilicola</i> Kobayasi	UAMH 7526	AF062787
<i>O. ramosum</i> Calduch, Stchigel, Gené & Guarro	IMI 391997 (T)	AJ634701 <sup>1</sup>
<i>O. reticulatum</i> Calduch, Stchigel, Gené & Guarro	IMI 391999 (T)	AJ634699 <sup>1</sup>
<i>O. rhodogenum</i> Robak	UAMH 1405	AF062803
<i>O. scytaloides</i> Gams & Söderström	UAMH 6521 (T)	AF062804
<i>O. setiferum</i> Udagawa & Toyazaki	UAMH 5715 (T)	AF062805
<i>O. tenuissimum</i> (Peck) Hughes	CBS 238.31 (T)	AF062807
<i>Oidiodendron</i> sp.	CBS 315.95	AF062806
<i>O. truncatum</i> G.L. Barron	UAMH 1399 (T)	AF062809
<i>Myxotrichum arcticum</i> Udagawa, Uchiyama & Kamiya	UAMH 7565	AF062810
<i>M. cancellatum</i> Phillips	UAMH 1911	AF062811
<i>M. setosum</i> (Eidam) Orr & Plunkett	UAMH 3835	AF062815
<i>Bysoascus striatosporus</i> (G.L. Barron & C. Booth) Arx	UAMH 3572 (T)	AF062817
<i>Gymnostellatospora japonica</i> Udagawa, Uchiyama & Kamiya	UAMH 8899	AF062818
<i>Pseudogymnoascus roseus</i> Raïllo	UAMH 9163	AF062819
<i>Aphanoascus keratinophilus</i> Punsola & Cano	IMI 319010	AJ133436

<sup>1</sup>Strains sequenced for this study. CBS = Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; DAOM = Canadian collection of fungal cultures, Ottawa, Canada; IMI = International Mycological Institute, Egham, United Kingdom; FMR = Facultat de Medicina de Reus culture collection, Spain; UAMH = University of Alberta micro-

fungus collection and herbarium, Edmonton, Alberta, Canada. (T) = ex-type culture.

Soil samples were also collected in Gran Canaria Island (Canary Islands), located in the Atlantic Ocean, to the north west of the African continent. This island has an area of 1532 km<sup>2</sup> and a maximum altitude of 1949 m. It is of volcanic origin and mainly composed of basaltic terrain. The area is dominated by a temperate to subtropical climate, with an annual temperature ranging 19–23 °C at 200 m of alt, and from 12–16 °C at 1500 m. The annual mean rainfall is 150–200 mm.

Plant material and soil samples were processed as in Calduch *et al.* (2002) and Stchigel *et al.* (2001), respectively. The macro- and microscopic features of the fungi were studied on oatmeal agar (OA; 30 g flakes, 20 g agar, 1 L distilled water, home-made), potato-carrot agar (PCA; 20 g potatoes, 20 g carrots, 20 g agar, 1 L distilled water, home-made) and potato-dextrose agar (PDA; Difco Laboratories, Detroit, Michigan) at 15, 25 and 37 °C under 12 h of darkness alternating with 12 h under cool white fluorescent light. Colour notations in parentheses are from Kornerup & Wanscher (1984) (M. = Methuen). Photomicrographs were obtained under a Leitz Dialux 20 light microscope and scanning electron images with a Jeol JSM-6400 scanning electron microscope.

### Molecular study

In addition to the sequences of the herein proposed *Oidiodendron* species and that of *O. myxotrichoides* sequenced previously (Calduch *et al.* 2002), a total of 21 sequences accessed from the GenBank belonging to other *Oidiodendron* species, *Myxotrichum* and *Bysoascus* species with *Oidiodendron* anamorphs, and other members of *Myxotrichaceae* were included in the molecular study (Table 1). *Aphanoascus keratinophilus* (AJ133436) was used as outgroup.

Fungal DNA extraction, amplification and sequencing of the rDNA region were performed as described in Solé *et al.* (2002). Fragments of 311–320 bp, comprising the 5.8S RNA gene (partial sequence), ITS2 (complete sequence) and 28S RNA gene (partial sequence), obtained with the primers ITS3 and ITS4 were aligned using the Clustal X (version 1.81), computer programme for multiple sequence alignment (Thompson *et al.* 1997). We performed maximum parsimony analysis (MP), using the PAUP software (4.0 Beta version 10). Heuristic searches were conducted under the following conditions: simple addition sequence with tree-bisection reconnection (TBR) branch swapping algorithm, starting trees obtained by stepwise addition, MULPARS option in effect, steepest descent option not in effect, MAXTREES setting 100, and branches having minimum length zero allowed to collapse yielding polytomies. Branch robustness was evaluated using 100 bootstrap replicates. The sequences have been deposited in the European Molecular Biology Laboratories (EMBL).

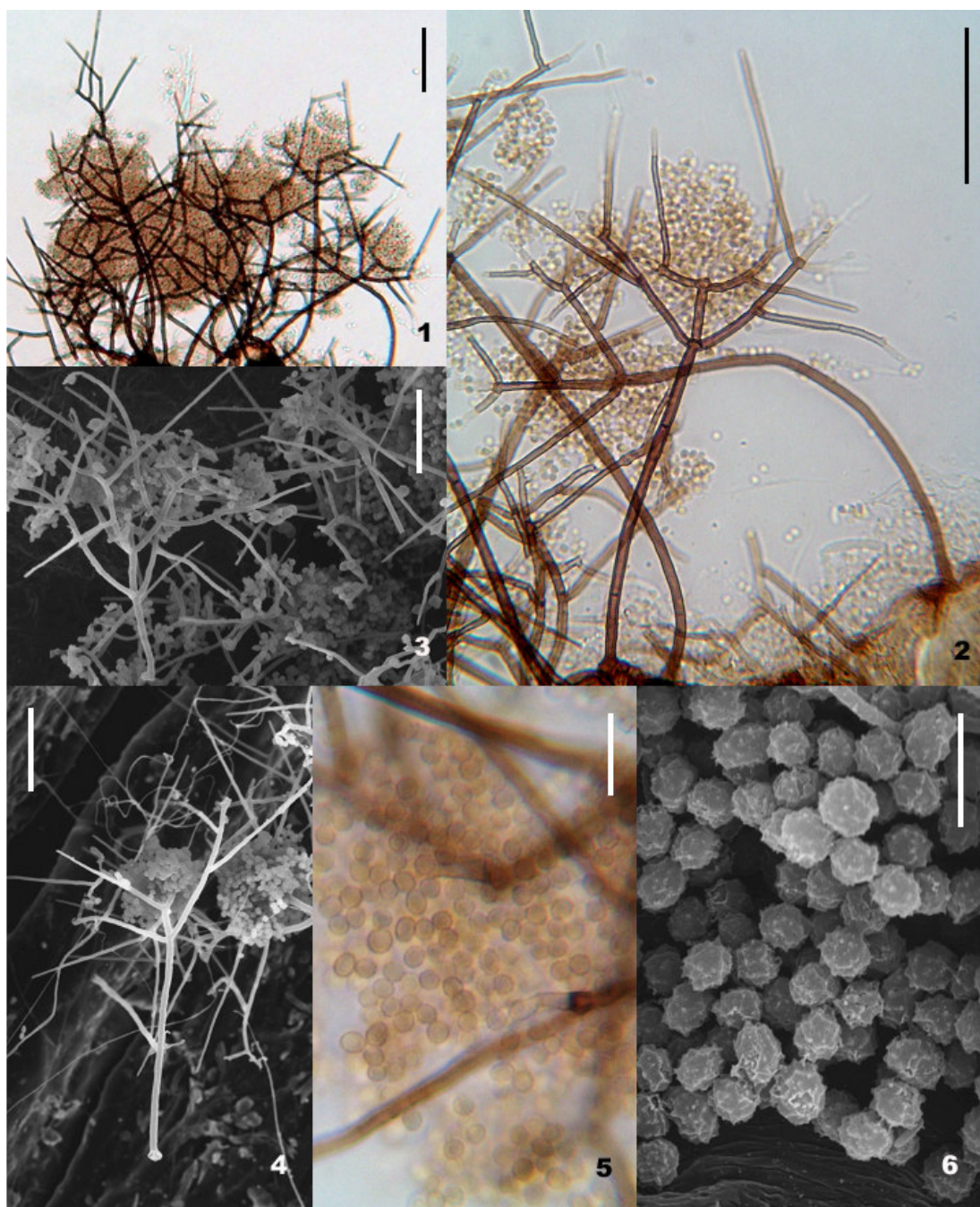
## TAXONOMY

*Oidi dendron muniellense* Calduch, Stchigel, Gené & Guarro, **sp. nov.** MycoBank MB500040. Figs 1–10.

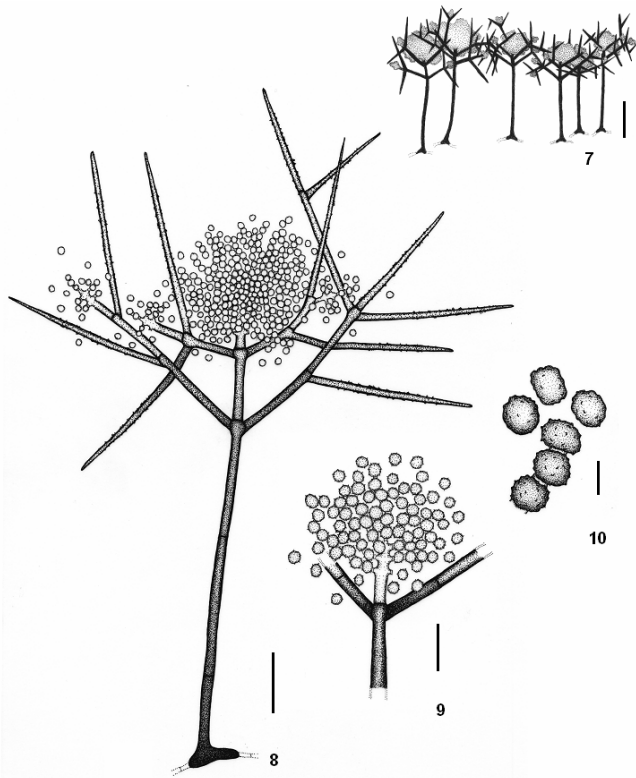
*Etymology*: Referring to the place where the fungus was collected.

Ad Hyphomycetes pertinens. Coloniae in basidiomate putrido substrato naturali effusae, pilosae, olivaceo-brunneae coloratae. Hyphis pallide brunneis vel brunneis, septatis, 1–2  $\mu\text{m}$  latis. Conidiophora macronematosa,

mononematosa, cum stipite simplici, recto, septato, brunneo, laevi, crassitunicato, usque ad 200  $\mu\text{m}$  longo, 2–3.5  $\mu\text{m}$  lato ad basim, rami sursum in hyphas fertiles exeuntes; rami numero 4–6, identidem subdichotome vel trichotome ramosi, recti, septati, brunnei, acuti ad apicem, laevi et crassitunicati ad basim, leniter asperi et tenuitunicati ad apicem, usque ad 60  $\mu\text{m}$  longi, 1.5–2.5  $\mu\text{m}$  lati ad basim. Hyphae fertiles terminales vel laterales, ramosae, septatae, subhyalinae, laeves, cylindricae, 1–2.5  $\mu\text{m}$  latae. Conidia sicca, catenata, unicellularia, globosa vel subglobosa, ochracea, rugosa vel echinulata, tenuitunicata, 1.5–2.5  $\mu\text{m}$  diam. Teleomorphosis ignota.



**Figs 1–6.** *Oidi dendron muniellense* (IMI 391998). 1. Colony growing on the natural substrate. 2–4. Conidiophores showing distal fertile ramifications, bearing a central mass of arthroconidia. 5, 6. Arthroconidia. Scale bars: 1, 2 = 50  $\mu\text{m}$ , 3, 4 = 40  $\mu\text{m}$ , 5 = 10  $\mu\text{m}$ , 6 = 5  $\mu\text{m}$ .



**Figs 7–10.** *Oidiiodendron muniellense* (IMI 391998). 7. Habit sketch. 8. Conidiophore. 9. Fertile hyphae bearing arthroconidia. 10. Arthroconidia. Scale bars: 7 = 50  $\mu\text{m}$ , 8, 9 = 10  $\mu\text{m}$ , 10 = 2.5  $\mu\text{m}$ .

**Hyphomycetes.** *Colonies* on the natural substrate effuse, hairy, greenish brown. *Mycelium* partly immersed in the substrate. *Hyphae* pale brown to brown, septate, 1–2  $\mu\text{m}$  wide. *Conidiophores* macronematous, mononematous, each composed of a long stipe and a repeatedly branched upper part from where fertile hyphae are borne; stipe straight, septate, brown, smooth- and thick-walled, up to 200  $\mu\text{m}$  long, 2–3.5  $\mu\text{m}$  wide at the base; upper part with 4–6 verticillate branches, each branch several times subdichotomously or trichotomously branched, seta-like, straight, septate, brown, paling distally, smooth- and thick-walled, becoming roughened and thin-walled towards the apex, pointed at the tip, up to 60  $\mu\text{m}$  long, 1.5–2.5  $\mu\text{m}$  wide near the base. *Fertile hyphae* terminal or lateral on the dark hyphae of the conidiophore upper part, branched, septate, subhyaline, smooth-walled, cylindrical, 1–2.5  $\mu\text{m}$  wide, fragmenting to form arthroconidia. *Conidia* dry, catenate, one-celled, globose to subglobose, ochraceous, roughened (echinulate under SEM), thin-walled, 1.5–2.5  $\mu\text{m}$  diam. *Teleomorph* unknown.

**Cultural characteristics:** The optimal colony growth was at 25 °C. At this temperature and after 4 wk of incubation, colonies on OA were 30–35 mm diam, brownish beige to brown (M6E3–6E8), flat, velvety,

irregularly folded, with a darkening brown (M6D8–6F8) reverse. Colonies on PCA were 26–30 mm diam, olive-brown (M4E4–4F5), flat, velvety, with an olive-brown (M4E4–4F5) reverse. Colonies on PDA were 37–40 mm diam, greyish orange to greyish brown (M5B3–5F3), slightly fasciculate at the centre, radially folded, with a brownish orange to yellowish brown (M5C5–5F5) reverse. The fungus showed good sporulation on the three culture media mentioned. Microscopic features under these conditions did not vary from those on the natural substrate. At 15 °C the fungus grew more slowly than at 25 °C on all media assayed, with colonies attaining 18–26 mm diam after 14 wk. A diffusible brownish orange to brown (M6C7–6D8) pigment was produced only on OA. No growth was observed at 37 °C.

**Specimen examined:** **Spain**, Asturias, Muniellos Integral Nature Reserve, on a decaying basidiome, 26 June 1999, col. A.M. Stchigel & M. Calduch, **holotype** IMI 391998, ex-type culture CBS 113932 = FMR 6765.

**Notes:** The present species is similar to *O. hughesii* Udagawa & Uchiyama (Udagawa & Uchiyama 1998) and *O. setiferum* (Udagawa & Toyazaki 1987) in the morphology of the terminal part of the conidiophores. However, *O. hughesii* differs mainly by the development of a reticulum of dark olive-brown and conspicuously ornamented hyphae with spine-like appendages at the periphery and by the production of only lateral fertile hyphae from the hyphal reticulum. The upper part of the conidiophore of *O. setiferum* is characterized by a subterminal whorl of dark brown, sterile setiform branches, which surrounds a terminal whorl of subhyaline penicillate fertile hyphae. Moreover, conidia of *O. muniellense* are globose to subglobose, roughened and ochraceous, while those of *O. setiferum* and *O. hughesii* are ovoid to ellipsoidal, and smooth-walled and brown, paler in the former, and asperate and hyaline in the latter.

***Oidiiodendron ramosum* Calduch, Stchigel, Gené & Guarro, sp. nov.** MycoBank MB500041. Figs 11–22.

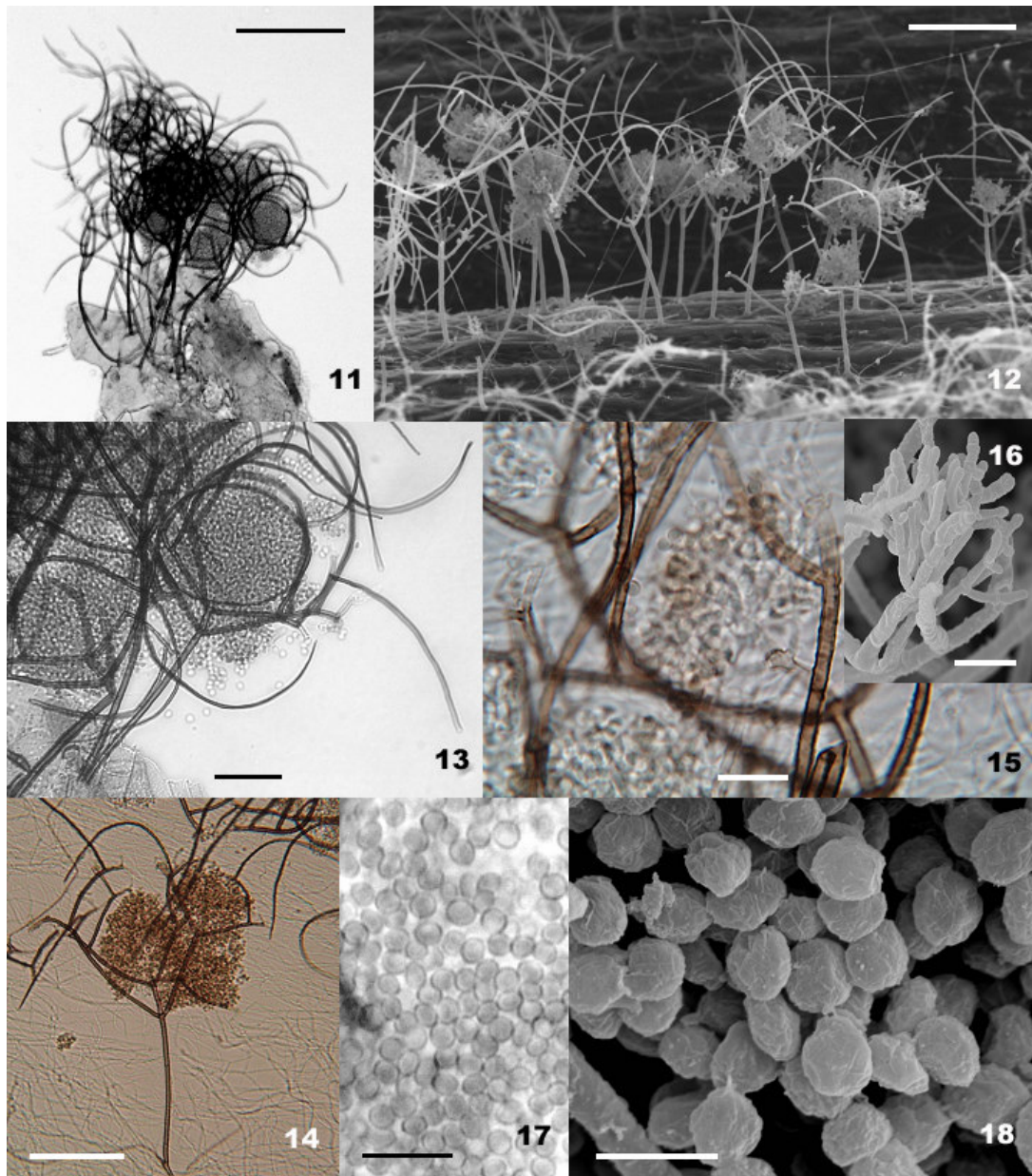
**Etymology:** Referring to the branched conidiophores.

Ad Hyphomycetes pertinens. Coloniae in basidiomate putrido, pilosae, olivaceo-brunneae coloratae. Hyphis pallide brunneis vel brunneis, septatis, 1.5–2  $\mu\text{m}$  latis. Conidiophora macronematosa, mononematosa, cum stipite simplici, recto, septato, brunneo, plerumque verrucoso, crassitunicato, usque ad 150  $\mu\text{m}$  longo, 2–3  $\mu\text{m}$  lato ad basim, rami sursum in hyphas fertiles terminantes; rami numero 2–6, subdichotomi, septati, longi recurvati, apicem versus attenuati, verruculosi vel verrucosi, pallidiores brunnei ad basim, pallidore ad apicem, usque ad 130  $\mu\text{m}$

longi, 1.5–2.5  $\mu\text{m}$  lati ad basim. Hyphae fertiles terminales vel laterales, ramosae, septatae, laeves, cylindricae, subhyalinae, 1–1.5  $\mu\text{m}$  latae. Conidia sicca, catenata, unicellularia, subglobosa vel ellipsoidea, pallide brunnea, laevia vel leniter rugosa, tenuitunicata, 1.5–3  $\times$  1–2  $\mu\text{m}$ . Teleomorphosis ignota.

Hyphomycetes. Colonies on a decaying basidiome, hairy, greenish brown. Mycelium partly immersed in the substrate. Hyphae pale brown to brown, septate, 1.5–2  $\mu\text{m}$  wide. Conidiophores macronematous, mononematous, composed of a stipe bearing at the upper part a whorl of dematiaceous, fertile seta-like branches which surrounds a dense head of fertile hyphae; stipe straight, septate, brown, verruculose,

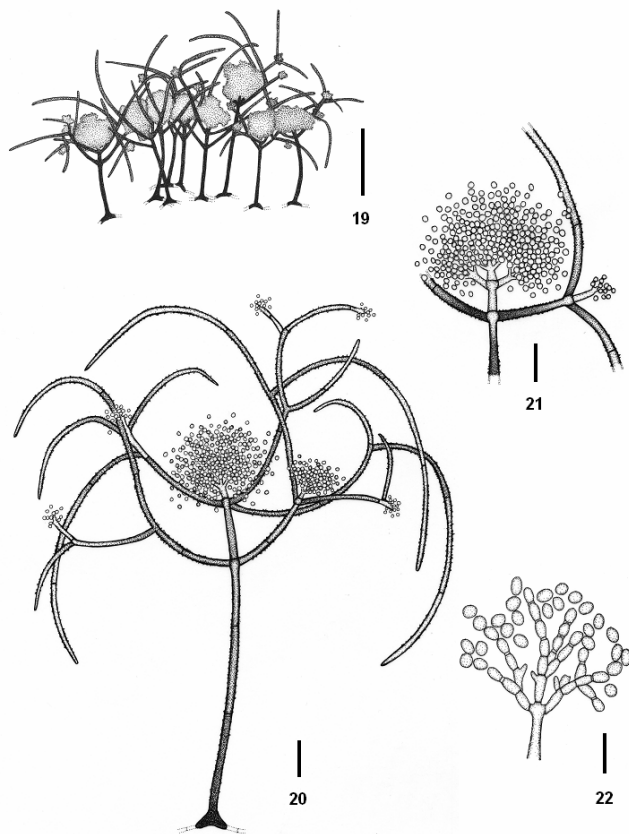
thick-walled, up to 150  $\mu\text{m}$  long, 2–3  $\mu\text{m}$  wide at the base; upper part with 2–6 verticillate branches, each branch subdichotomously branched, seta-like, septate, broadly recurved, attenuate at the tip, verruculose to verrucose, pale brown and becoming subhyaline distally, up to 130  $\mu\text{m}$  long, 1.5–2.5  $\mu\text{m}$  wide near the base, from which fertile hyphae also develop. Fertile hyphae terminal or lateral on the dematiaceous branches of the conidiophore upper part, but also on the terminal portion of the conidiophore stipe, forming a dense whorl of penicillate hyphae, septate, smooth-walled, subhyaline, cylindrical, 1–1.5  $\mu\text{m}$  wide, fragmenting to form arthroconidia.



**Figs 11–18.** *Oidiiodendron ramosum* (IMI 391997). 11, 12. Conidiophores on the natural substrate. 13. Detail of a conidiophore apical part with fertile ramifications that surround arthroconidial masses. 14. Conidiophore *in vitro*. 15, 16. Penicillate fertile hyphae. 17, 18. Arthroconidia. Scale bars: 11, 12 = 100  $\mu\text{m}$ , 13 = 25  $\mu\text{m}$ , 14 = 50  $\mu\text{m}$ , 15, 17 = 10  $\mu\text{m}$ , 16 = 5  $\mu\text{m}$ , 18 = 2.5  $\mu\text{m}$ .

*Conidia* dry, catenate, one-celled, subglobose to ellipsoidal, pale brown, smooth to slightly roughened (roughened under SEM), thin-walled,  $1.5\text{--}3 \times 1\text{--}2 \mu\text{m}$ . *Teleomorph* unknown.

**Cultural characteristics:** The optimal colony growth was at 25 °C. Colonies on OA were 20–25 mm diam after 4 wk, olive-brown (M4F8), with greyish margins, flat, powdery, concentrically radiate, with an olive-brown (M4D5–4E5) reverse. On PCA colonies were 18–20 mm diam after 4 wk, olive (M2F4) with whitish margins, flat, velvety, with an olive (M2E4) reverse. On PDA, they were 27–30 mm diam after 4 wk, orange-white to greyish orange (M5A2–5B3), cerebriform, radially folded, with a greyish orange (M5B4–5B5) reverse. The microscopic features of the fungus did not vary from those observed on the natural substrate. At 15 °C the fungus grew slower (13–17 mm diam after 4 wk) and sporulation was poorer than at 25 °C on all the media assayed. No growth was observed at 37 °C.



**Figs 19–22.** *Oidi dendron ramosum* (IMI 391997). 19. Habit sketch. 20. Conidiophore. 21, 22. Fertile hyphae bearing arthroconidia. Scale bars: 19 = 100  $\mu\text{m}$ , 20, 21 = 20  $\mu\text{m}$ , 22 = 4  $\mu\text{m}$ .

**Specimen examined:** Spain, Asturias, Muniellos Integral Nature Reserve, on an unidentified rotten basidiocarp, 26 June 1999, coll. A.M. Stchigel & M. Calduch, **holotype** IMI 391997, ex-type cultures CBS 113931 = FMR 6764.

**Notes:** *Oidi dendron setiferum* (Udagawa & Toyazaki 1987) is most similar to *O. ramosum*. Both have at the

upper part of the conidiophore stipe a dense head of fertile hyphae surrounded by a whorl of dematiaceous, seta-like branches. However, in the former, these branches are sterile, while in *O. ramosum* they are fertile. Moreover, the conidia of *O. setiferum* are smooth-walled and separated each other by thick and dark connectives. *Oidi dendron muniellense* also resembles *O. ramosum* in conidiophore morphology, but in the former the peripheral branches of the conidiophore upper part are straight and smooth-walled, becoming roughened distally. Additionally, the conidial morphology of *O. muniellense* is different.

***Oidi dendron reticulatum*** Calduch, Stchigel, Gené & Guarro, **sp. nov.** MycoBank MB500042. Figs 23–35.

**Etymology:** Referring to the morphology of the upper part of the conidiophores.

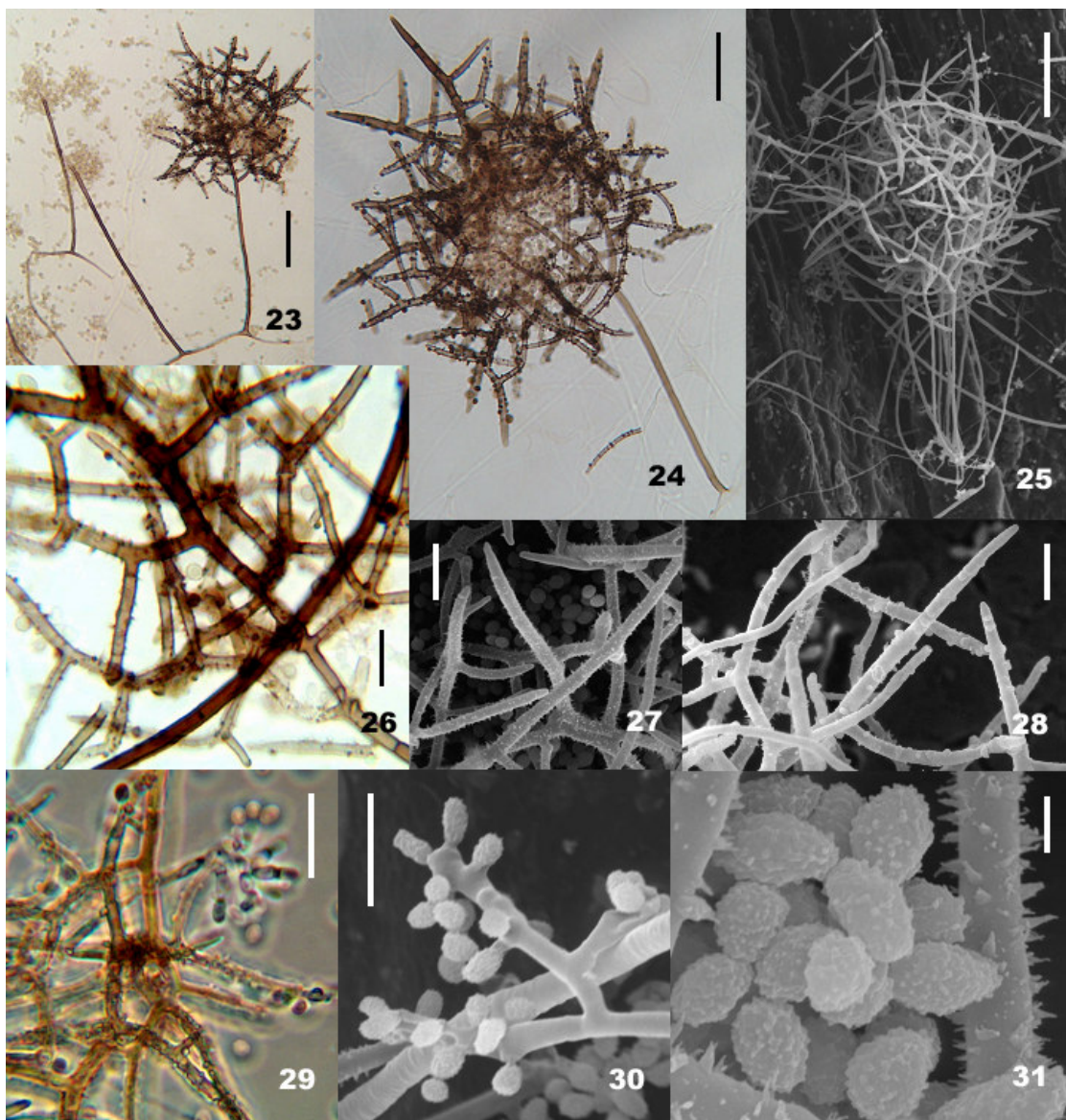
Ad Hyphomycetes pertinens. Coloniae *in vitro* 22–25 °C restrictae, planae, olivaceo-griseae coloratae. Hyphis pallide brunneis, septatis, 1–1.5  $\mu\text{m}$  latis. Conidiophora macronematosa, mononematosa, stipite simplici, reticulum globosum in parte apicale formantes et hyphis lateralibus fertilibus; stipites reticulum e ramis anastomosantibus, 90–150  $\mu\text{m}$  diam, ramis apicem versus attenuatis et rotundatis, septatis, fortiter verrucosis et spinulosis, brunneis, 5–60  $\mu\text{m}$  longis, 2–3  $\mu\text{m}$  latis ad basim. Hyphae fertiles ex hyphis reticuloriundae, ramosae, septatae, laeves, subhyalinae vel pallide griseo-brunneae, cylindricae, 5–15  $\mu\text{m}$  longis, 1.5–2  $\mu\text{m}$  latae. Conidia sicca, catenata, unicellularia, ellipsoidea, pallide brunnea vel pallide olivaceo-brunnea, rugosa vel echinulata, tenuitunicata,  $1.5\text{--}4 \times 1.5\text{--}2.5 \mu\text{m}$ . Conidiophora altera simplicia; co-formantia, stipites recti, septati, leniter crassitunicati, pallide brunnei, usque ad 350  $\mu\text{m}$  longi, 1.5–2.5  $\mu\text{m}$  lati ad basim; capitulum compactum hypharum fertilium ferentes. Teleomorphosis ignota.

Hyphomycetes. Colonies *in vitro* at 22–25 °C restricted, plane, olive-grey in colour. Mycelium partly immersed in the substrate, composed of pale brown, septate, 1–1.5  $\mu\text{m}$  wide hyphae. Conidiophores macronematous, mononematous, of two types: i) a stipe ending in a reticulate network of hyphae from which lateral fertile hyphae are produced; stipe straight, septate, brown, smooth-walled to slightly verruculose, thick-walled, up to 200  $\mu\text{m}$  long, 1.5–2.5  $\mu\text{m}$  wide at the base, upper part several times branched and anastomosing to form a more or less globose reticulate network, measuring 90–150  $\mu\text{m}$  diam, with seta-like peripheral branches; branches of the reticulum recurved or sinuous, attenuate and rounded at the tip, septate, strongly verrucose and spinulose throughout, brown, 5–60  $\mu\text{m}$  long, 2–3  $\mu\text{m}$  wide at the base; and ii) conidiophores simple, stipe straight, septate, slightly thick-walled, pale to brown,

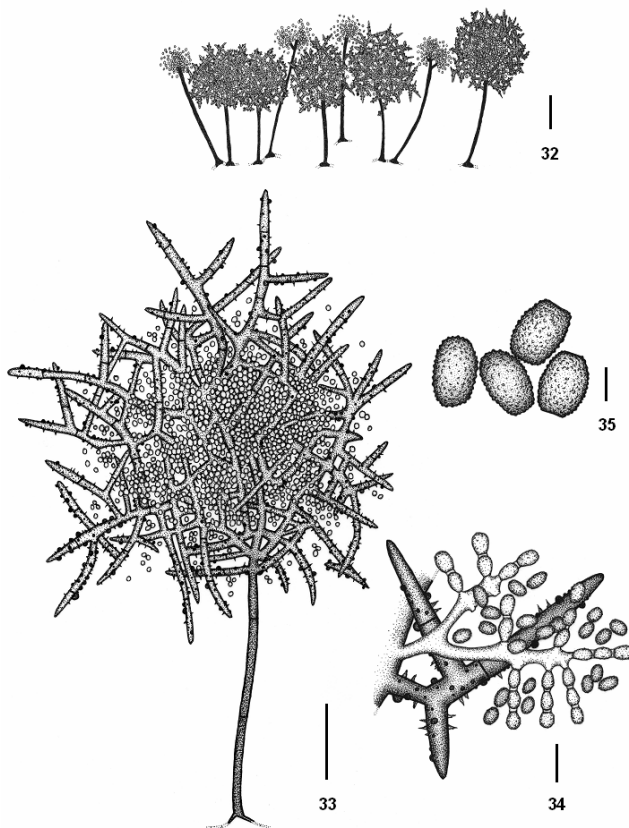
up to 350  $\mu\text{m}$  long, 1.5–2.5  $\mu\text{m}$  wide at the base, producing terminally a dense head of fertile hyphae. *Fertile hyphae* arising laterally from branches of the reticulum or terminally from the conidiophore stipe, branched, septate, smooth-walled, subhyaline to pale greyish brown, cylindrical, 5–15  $\mu\text{m}$  long, 1.5–2  $\mu\text{m}$  wide, fragmenting to form arthroconidia. *Conidia* dry, catenate, one-celled, pale brown to pale olivaceous-brown, roughened (echinulate under SEM), thin-walled, ellipsoidal, 1.5–4  $\times$  1.5–2.5  $\mu\text{m}$ , with one or both ends truncate. *Teleomorph* unknown.

*Cultural characteristics*: The optimal colony growth was at 25 °C. Colonies on OA were 29–32 mm diam after 4 wk, with abundant conidiophores arranged in concentric rings, brownish grey (ME3–4F3), flat,

slightly granulose; reverse brownish grey (M4E3–4F3); a diffusible pigment brownish orange to brown (M6C6–6E8). On PCA, colonies 20–25 mm diam after 4 wk, olive-grey (M3E2–3F2), with whitish margins, flat, velvety; reverse olive-grey. On PDA, 30–35 mm diam after 4 wk, pale yellow to greyish yellow (M1A3–1B4), olive (M1E4–1F4) at the centre, slightly fasciculate, irregularly folded; reverse orange-grey to greyish orange (M5B2–5B3). At 15 °C colony growth rates on OA and PDA were similar to those at 25 °C, while on PCA they were restrictedly (12–16 mm in 4 wk), sporulation was scarce and the diffusible pigment was absent in all media assayed. No growth was observed at 37 °C.



**Figs 23–31.** *Oidiiodendron reticulatum* (IMI 391999). 23–25. Two different type of conidiophores, simple on the left and with an apical reticulate network of hyphae on the right. 26–28. Network wall ornamentation. 29, 30. Fertile hyphae producing arthroconidia. 31. Arthroconidia. Scale bars: 23, 25 = 50  $\mu\text{m}$ , 24 = 30  $\mu\text{m}$ , 26–28, 30 = 10  $\mu\text{m}$ , 29 = 20  $\mu\text{m}$ , 31 = 2  $\mu\text{m}$ .



**Figs 32–35.** *Oidiiodendron reticulatum* (IMI 391999). 32. Habit sketch. 33. Conidiophore. 34. Fertile hyphae bearing laterally from a hyphae of the reticulum. 35. Arthroconidia. Scale bars: 32 = 50  $\mu\text{m}$ , 33 = 25  $\mu\text{m}$ , 34 = 5  $\mu\text{m}$ , 35 = 2  $\mu\text{m}$ .

*Specimen examined:* **Spain**, Canary Islands, Gran Canaria Island, from soil sample, 7 Mar. 1997, coll. B. Acosta & A.M. Stchigel, **holotype** IMI 391999; ex-type culture CBS 113933 = FMR 6766.

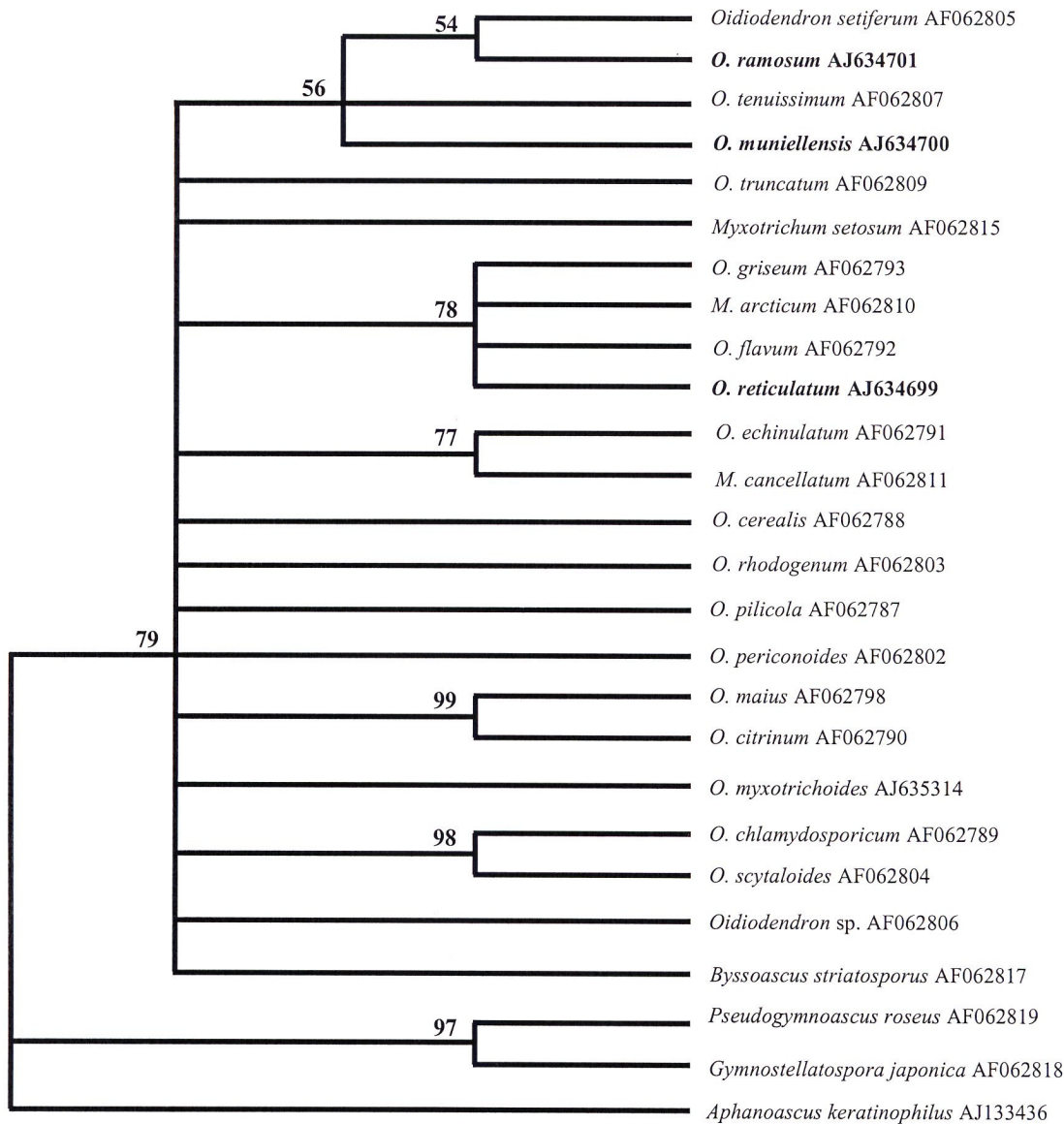
*Notes:* *Oidiiodendron hughesii* and *O. myxotrichoides* are the species showing a dematiaceous, branched and anastomosed reticulum of hyphae similar to that present in the conidiophore upper part of *O. reticulatum*. However, in *O. myxotrichoides* the reticulate structure is never borne terminally on an erect stipitate conidiophore, as happens in *O. hughesii* and *O. reticulatum*. Moreover, conidia of *O. myxotrichoides* are predominantly globose or subglobose and smooth or finely rugose under SEM, while those of *O. reticulatum* are ellipsoidal, with roughened or echinulate walls. *Oidiiodendron hughesii* differs from *O. reticulatum* mainly in the ornamentation of the hyphae of the reticulum, which are asperate and smooth-walled distally, conidia being hyaline and rather thick-walled. Additionally, *O. hughesii* is psychrophilic, growing better at 15 °C than at 25 °C, in contrast to *O. reticulatum*, which is mesophilic.

### Phylogeny

The amplicons from the three *Oidiiodendron* strains were consistent with those of other *Oidiiodendron* species and their relatives studied by Hambleton *et al.* (1998). The majority rule consensus tree, resulting from 1000 bootstrap replications of maximum parsimony analysis of the *Oidiiodendron* species and related taxa data set, using the heuristic search algorithm.

The topology of the phylogenetic tree supports the proposal of the three new species. All *Oidiiodendron*, *Myxotrichum* and *Bysoascus* strains included in the study fall into a monophyletic clade supported by a bootstrap value of 79 %. These results agree with Hambleton *et al.* (1998), who demonstrated that the three genera conformed a well-supported monophyletic group within the *Myxotrichaceae*, and that this group diverged considerably from other members of the family, i.e. *Gymnostellatospora japonica* and *Pseudogymnoascus roseus*. In our study, the ingroup was split into many subclades, some of them unispecific, e.g. that formed by the *O. myxotrichoides* strain, a species recently described by Calduch *et al.* (2002) which develops a structure similar to those present at the upper part of the conidiophore of *O. hughesii* (Udagawa & Uchiyama 1998) and of the new species *O. reticulatum*. Unfortunately, *O. hughesii* could not be included in this molecular study because ex-type cultures are not available. *Oidiiodendron reticulatum* was placed in a clade together with *O. griseum*, *O. flavum* and *M. arcticum*, which was supported by a moderate bootstrap value (78 %). However, none of these species develop the typical complex conidiophores of *O. reticulatum*. The four members of this clade develop simple conidiophores, but the other three species can be easily distinguished from *O. reticulatum* by some morphological features of their conidiophores and conidia. The conidiophores of *O. flavum* are shorter (maximum up to 100  $\mu\text{m}$  long) and the conidia larger (3.5–6  $\times$  2.5–3.5  $\mu\text{m}$ ) (Ellis 1976). The conidia of *O. griseum* and those of the anamorph of *M. arcticum* are pale greyish green and nearly smooth (Ellis 1971, Udagawa *et al.* 1994). The other two new species, *O. muniellense* and *O. ramosum*, were grouped with *O. setiferum* and *O. tenuissimum* in a separate clade supported by a low bootstrap value (56 %). *Oidiiodendron tenuissimum* differs from the other species included in the clade because it only develops the unbranched stipitate conidiophores typical of the genus (Ellis 1971, 1976). As expected, *O. ramosum* and *O. setiferum*, two species with some similar features, clustered close each other, but with a low bootstrap support, which confirms that they are two different species.





**Fig. 36.** Parsimony heuristic phylogram obtained for 5.8S and internal transcribed spacer (ITS2) regions of the species listed in Table 1. Bootstrap probabilities (1000 replicates) are indicated on the nodes. The tree statistics for the analysis are as follows: tree length = 252 steps; consistency index = 0.5357; retention index = 0.4730, rescaled consistency index = 0.2534 and homoplasy index = 0.4643.

### Key to *Oidiiodendron* species

1. Presence of globose or subglobose, dark brown sporodochia ..... *O. myxotrichoides*
1. Sporodochia absent ..... 2
2. Teleomorph present ..... 3
2. Teleomorph absent ..... 4
3. Ascospores with a dark reticulate peridium; ascospores ellipsoidal or fusiform, longitudinally striate, hyaline to pale yellow ..... *Oidiiodendron* anamorphs of *Myxotrichum* spp.\*
3. Ascospores with a white, cottony peridium; ascospores fusiform, striate, greenish yellow ..... *Oidiiodendron* anamorph of *Byssosascus striatosporus*
4. Conidiophores with dematiaceous seta-like branches in the upper part ..... 5
4. Conidiophores otherwise ..... 9

5. Branches anastomosing to form a more or less globose, reticulate network, with the conidial mass in the centre; simple conidiophores produced simultaneously ..... 6
5. Branches otherwise; simple conidiophores absent ..... 7
6. Psychrophilic; branches basally asperate, smooth towards the apex; conidia hyaline ..... *O. hughesii*
6. Mesophilic; branches coarsely verrucose and spinulose along their length; conidia pale brown to olivaceous-brown ..... *O. reticulatum*
7. Upper part of the conidiophores with dematiaceous sterile branches ..... *O. setiferum*
7. Upper part of the conidiophores with dematiaceous fertile branches ..... 8
8. Branches long (up to 130  $\mu\text{m}$ ), verruculose to verrucose, broadly recurved; conidia subglobose to ellipsoidal, slightly roughened under SEM,  $1.5\text{--}3 \times 1\text{--}2 \mu\text{m}$  ..... *O. ramosum*
8. Branches shorter (up to 60  $\mu\text{m}$ ), smooth and becoming slightly roughened distally, straight; conidia globose to subglobose, echinulate under SEM,  $1.5\text{--}2.5 \mu\text{m}$  diam ..... *O. muniellense*
9. Conidiophores 250–870  $\mu\text{m}$  long; conidia cylindrical, with truncate ends,  $5\text{--}11 \times 2.5\text{--}3.5 \mu\text{m}$  ..... *O. robustum*
9. Conidiophores shorter; conidia not combining the above characteristics ..... 10
10. Conidia lens-shaped, with a thick-walled ring,  $3\text{--}5.5 \times 2\text{--}3 \mu\text{m}$ ; colonies violaceous to purple-black. *O. cerealis*
10. Conidia and colonies otherwise ..... 11
11. Pigmented chlamydospores present in culture ..... 12
11. Pigmented chlamydospores absent ..... 14
12. Chlamydospores  $4.5\text{--}14 \times 3.5\text{--}12.5 \mu\text{m}$ ; conidia  $4\text{--}15 \times 3\text{--}13 \mu\text{m}$ , usually ellipsoidal or cylindrical, sometimes two-celled ..... *O. terrestre*
12. Chlamydospores and conidia otherwise ..... 13
13. Chlamydospores 4–9  $\mu\text{m}$  diam; conidia fusiform or doliiform,  $2\text{--}6 \times 1.2\text{--}2 \mu\text{m}$  ..... *O. chlamydosporicum*
13. Chlamydospores  $3\text{--}5 \times 2.5\text{--}3 \mu\text{m}$ ; conidia cylindrical, guttuliform or ellipsoidal,  $2\text{--}4 \times 1\text{--}2 \mu\text{m}$ ... *O. scytaloides*
14. Colonies yellowish ..... 15
14. Colonies coloured otherwise ..... 17
15. Conidiophores up to 300  $\mu\text{m}$  long; conidial chains often undulate; conidia  $2\text{--}4 \times 1.5\text{--}3 \mu\text{m}$  ..... *O. citrinum*
15. Conidiophores shorter ..... 16
16. Conidiophores up to 100  $\mu\text{m}$  long; conidial chains straight; conidia  $3.5\text{--}6 \times 2.5\text{--}3.5 \mu\text{m}$  ..... *O. flavum*
16. Conidiophores rather short or absent; conidial chains curved; conidia  $3.8\text{--}5 \times 2.5\text{--}3.3 \mu\text{m}$  ..... *O. sulphureum*
17. Conidia doliiform or cylindrical, truncate at the ends ..... 18
17. Conidia otherwise ..... 19
18. Conidia olivaceous-brown, often bearing darkened frills terminally,  $3\text{--}6.5 \times 1.5\text{--}3 \mu\text{m}$  ..... *O. truncatum*
18. Conidia hyaline, with inconspicuous frills,  $3\text{--}3.5 \times 1.5\text{--}2 \mu\text{m}$  ..... *O. pilicola*
19. Conidia mostly globose to subglobose, thick-walled ..... 20
19. Conidia variable in shape, mostly thin-walled ..... 21
20. Conidia with alternating dark and light brown bands in the wall, 2.5–5  $\mu\text{m}$  diam; colonies isabelline to greyish sepia ..... *O. periconioides*
20. Conidia distinctly echinulate with dark spines, pale to mid brown, 2.5–4  $\mu\text{m}$  diam; colonies olivaceous or purplish black ..... *O. echinulatum*

21. Colonies with a red pigment diffusing into the agar; conidia ellipsoidal or subspherical,  
 2–4 × 1.5–2 µm ..... *O. rhodogenum*
21. Colonies without a red pigment diffusing into the agar ..... 22
22. Colonies pale grey or off-white; conidia subglobose or short cylindrical, 2.5–4 × 2–2.5 µm,  
 pale olive, often forming curled chains ..... *O. maius*
22. Colonies grey to olivaceous brown; conidia otherwise ..... 23
23. Conidia smooth or minutely verruculose, paler, oblong or ellipsoidal ..... 24
23. Conidia distinctly verruculose, brown, globose, subglobose or ellipsoidal, 2–4 × 1.5–2.5 µm..... *O. tenuissimum*
24. Conidia hyaline, 1.5–2.5 × 1.3–.7 µm ..... *O. gracile*
24. Conidia pale greyish green, 2–3.5 × 1.5–2 µm ..... *O. griseum*

\*The morphological differences among the *Oidiodendron* anamorphs of *Myxotrichum* spp. are difficult to establish. They are always associated with the corresponding teleomorphs. These species are differentiated from each other by the morphology of their ascomata.

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