

Two new species of *Digitodesmium* from Euskadi (Spain)

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Abstract – Two new species of *Digitodesmium*, *D. intermedium* and *D. macrosporum*, found respectively on plant debris and from a soil sample collected in Euskadi (the North of Spain), are described and illustrated. *Digitodesmium intermedium* is characterized by conidia with up to 11 arms and *D. macrosporum* by large conidia, reaching 130-145 µm long. Both species are compared with the closest species of the genus. A key to *Digitodesmium* species is also provided.

Anamorphic fungi / cheirosporous hyphomycetes / *Digitodesmium* / Taxonomy

Résumé – Deux nouvelles espèces de *Digitodesmium*, *D. intermedium* et *D. macrosporum*, collectés au Pays Basque (nord de l'Espagne) respectivement sur débris végétaux et sol, sont décrites et illustrées. *Digitodesmium intermedium* est caractérisée par des conidies possédant jusque 11 bras et *D. macrosporum* par des conidies de grande taille, atteignant 130-145 µm de long. Une analyse comparative des principales caractéristiques de ces deux espèces est effectuée, et une clé d'identification des espèces de *Digitodesmium* est fournie.

INTRODUCTION

Spain is considered one of the most important European reservoirs of biodiversity. However, its mycobiota, especially of the anamorphic fungi, is poorly known. During our ongoing survey of fungi in Northern Spain, two interesting anamorphic species were found on plant debris and soil, in different localities in Euskadi. Both species belong to the genus *Digitodesmium* P. M. Kirk (1981) and considered sufficiently different from all previously described taxa to be described as new.

MATERIALS AND METHODS

Site and sample procedure. The samples were collected in the Aramaio Valley and the Pagoeta Natural Park, Euskadi. The former locality has an annual average temperature of 4.7°C in January and 19.1°C in August and an annual

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rainfall of 800-1500 mm. The local ecosystem is dominated by meadows, although towards the mountains the vegetation comprises also pine groves mixed with oak and beech forests. The Pagoeta Natural Park is located in the Diputación Foral of Guipúzcoa, between the coast and the interior valleys, and has a very sharp relief. The annual average temperature is approximately 12-13°C and the annual rainfall exceeds 1.600 mm. The vegetation comprises mainly beech groves, with oak trees standing along the watercourse and alder groves at the bottom of the valleys.

The samples were collected and put into polyethylene bags, labeled, and taken to the laboratory. Material was kept in the refrigerator at 4-7°C until processing.

Isolation and identification of fungi. A soil bait technique was used for recovering the soil-borne fungi. The soil samples were placed in sterile Petri dishes, moistened with sterile distilled water, covered with several pieces of sterile wood (approx 2 cm²) usually of angiosperms, and incubated at room temperature (22-25°C). All the plates were checked weekly under the stereomicroscope for a 6-month period.

Plant debris was placed into moist chambers, incubated at room temperature and examined periodically under the stereomicroscope for a 2-month period. The fungi growing on the natural substrate were mounted on 85% lactic acid and examined under light microscopy for identification.

Pure cultures were obtained by transferring conidia on Potato Carrot Agar (PCA; 20 g potatoes, 20 g carrots, 20 g agar, 1 L distilled water) and Oatmeal Agar (OA; 30 g flakes, 20 g agar, 1 L distilled water), and incubated at 25°C and 37°C in darkness. Colour notations in parentheses are from Kornerup & Wanscher (1984).

Photomicrographs were obtained with a Leitz Dialux 20 EB microscope and a Jeol JSM-6400 scanning electron microscope.

RESULTS

***Digitodesmium intermedium* J. Mena, Silvera, Gené & Guarro sp. nov.**

Mycobank: 513298

Figs 1, 2

Conidiomata sporodochia, punctiformia, pulvinata, atrobrunnea vel atra. Mycelium in substrato plerumque immersum, ex hyphis ramosis, laevibus, septatis, subhyalinis vel pallide brunneis, 1.8-4 µm latis compositum. Conidiophora semi-macronematosa, mononematosa, pallide brunnea, laevia, tenuitunicata non-ramosa, brevia, flexuosa, 5-6 µm lata compositum. Cellulae conidiogenae monoblasticae, in conidiophoris incorporatae, terminales, pallide brunneae, laeves, tenuitunicatae. Conidia acrogena, solitaria, cheiroidea, brunnea vel modice atro-brunnea, laevia, 39-76 × 25-35 µm, cum 3-11 rami praedita cylindrica, 6-8 µm lata, divergentia, 7-13-euseptata, septa modici constricti, cellula basilaris et apicalis 3.5-6 µm lata. Conidiorum secesio schizolytica.

Etymology: “*intermedium*” referred to the morphological affinities with *Digitodesmium elegans* P.M. Kirk and *Digitodesmium macrosporum* Silvera, Mercado, Gené & Guarro.

Conidiomata sporodochial, punctiform, pulvinate, dark brown to black. *Mycelium* mostly immersed in the substratum, composed of subhyaline to very pale brown, smooth-walled, septate, branched hyphae, 1.8-4 µm wide.

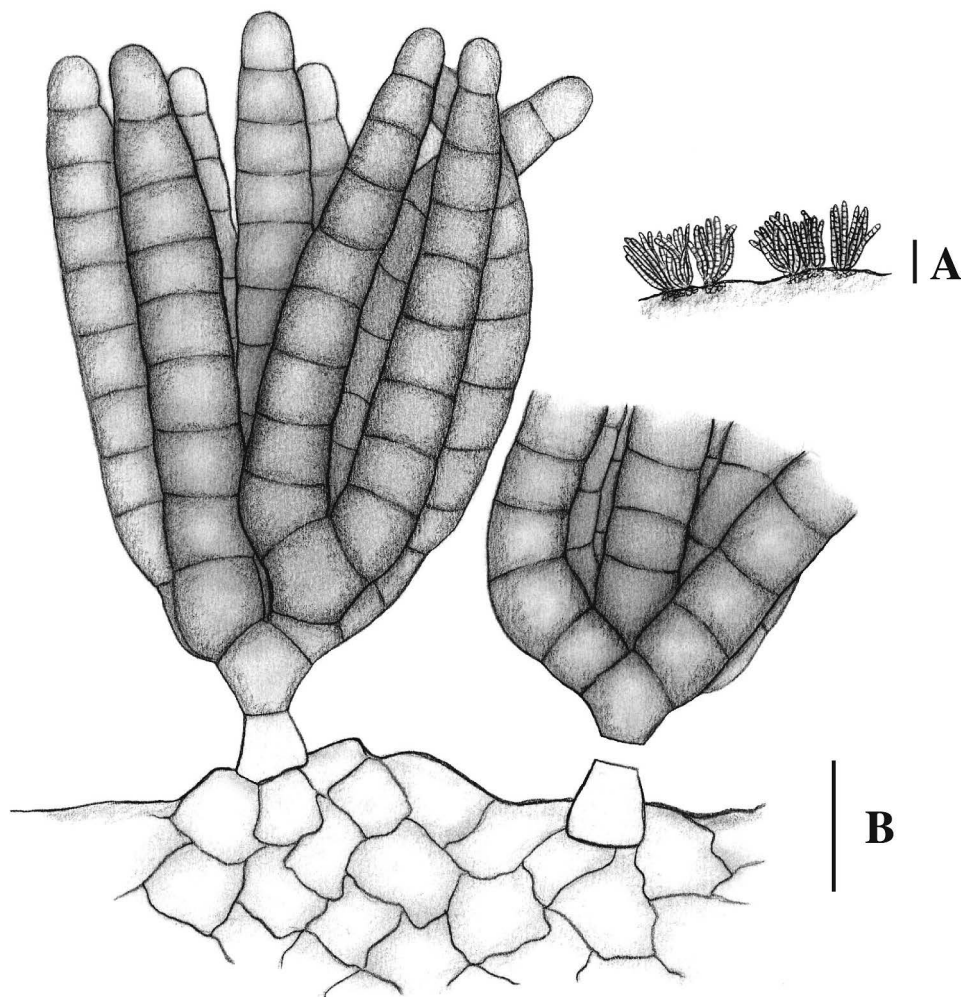


Fig. 1. *Digitodesmium intermedium* (IMI 396699). A. Habitat. B. Cheiroid conidia with divergent rows. Bars: A = 40 μm ; B = 10 μm .

Conidiophores semi-macronematous, mononematous, pale brown, smooth and thin-walled, unbranched, short, flexuous, up to 15 μm long, 5-6 μm wide at the inflate apex. *Conidiogenous cells* monoblastic, integrated, terminal, pale brown, smooth and thin-walled. *Conidia* acrogenous, solitary, cheiroid, euseptate, brown to dark brown, smooth-walled, 39-76 \times 25-35 μm , with 3-11 divergent arms inserted on basal cells in different planes; arms discrete, unbranched, cylindrical, 6-8 μm wide, 7-13-euseptate, slightly constricted at septa; apical and basal cells 3.5-6 μm wide. *Conidial secession* schizolytic.

Habitat: plant debris.

Known distribution: Spain, Euskadi.

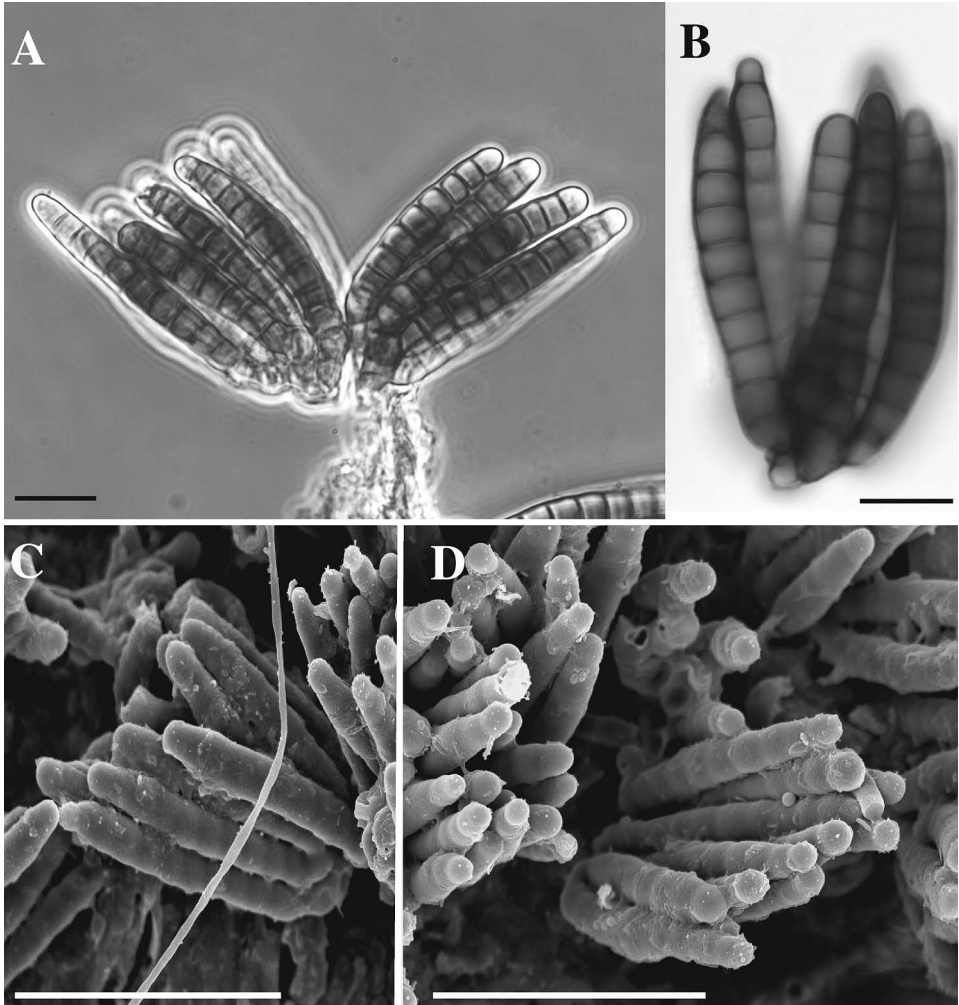


Fig. 2. *Digitodesmium intermedium* (IMI 396699). A-D. Cheiroid conidia with divergent rows. Bars: A, B = 25 μ m; C, D = 50 μ m.

Material examined: Guipúzcoa, the Pagoeta Natural Park, on unidentified dead bark, April 2008, J. Mena & C. Silvera (holotypus IMI 396699, isotypus FMR 10088).

Cultural characteristics: The fungus did not grow in vitro.

***Digitodesmium macrosporum* Silvera, Mercado, Gené & Guarro sp. nov.**

Mycobank: 513328

Figs 3, 4

Conidiomata sporodochia, sparsa, punctiformia, pulvinata, atrobrunnea vel atra. Mycelium plerumque in substrato immersum, ex hyphis ramosis, laevibus, septatis, pallide brunneis, 2-4 μ m latis compositum. Conidiophora semi-

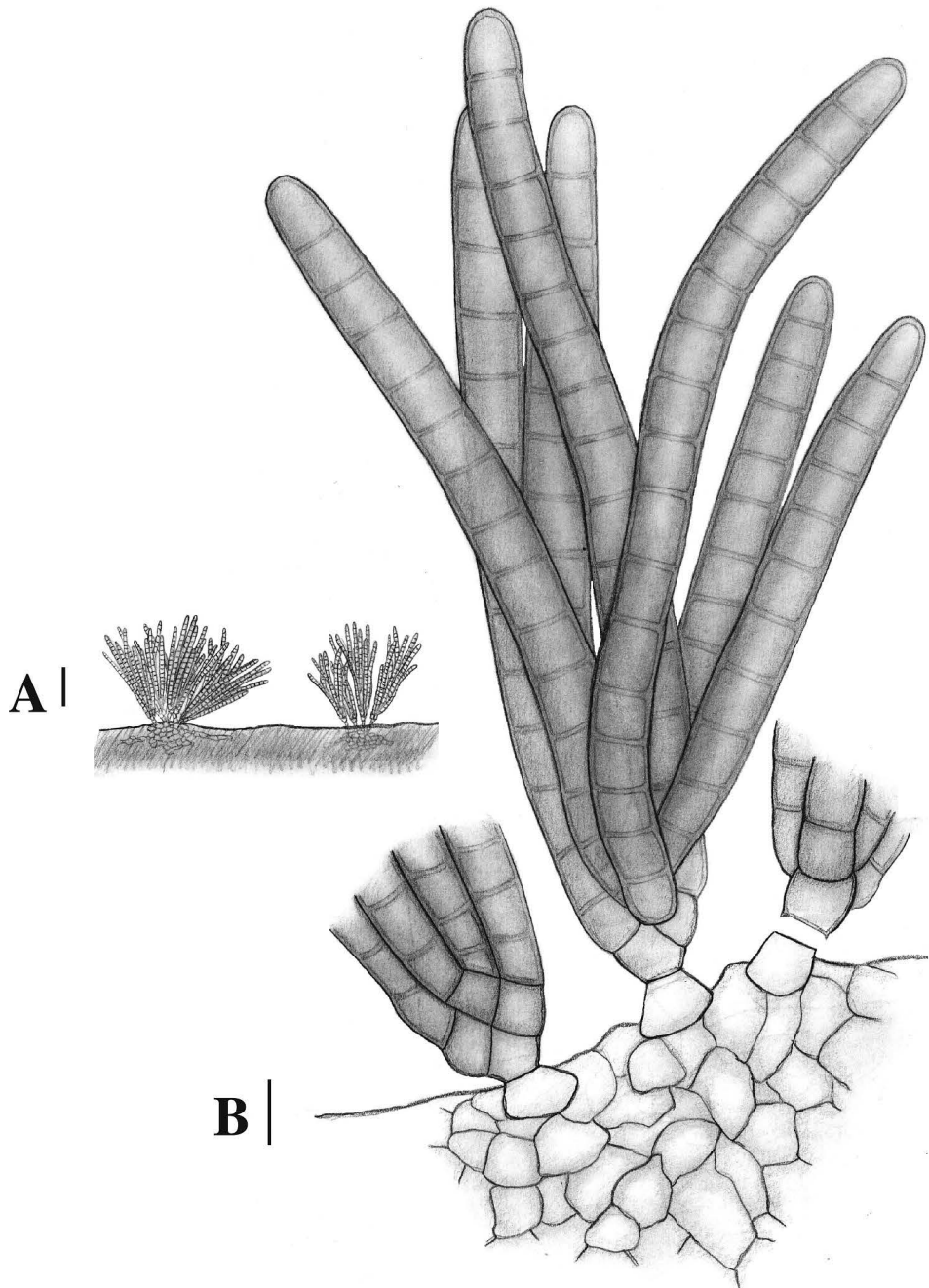


Fig. 3. *Digitodesmium macrosporum* (IMI 394094). A. Habitat. B. Cheiroid conidia with divergent rows. Bars: A = 40 μm ; B = 10 μm .

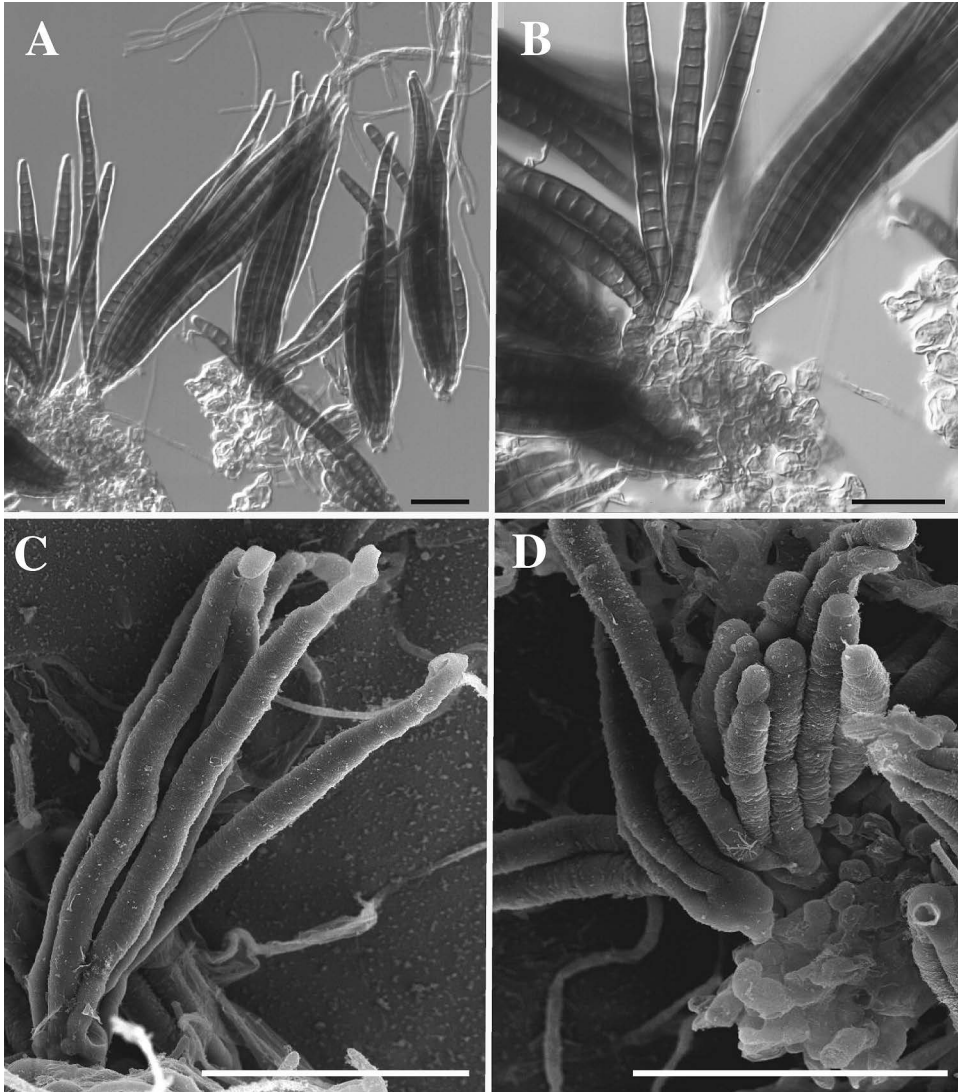


Fig. 4. *Digitodesmium macrosporum* (IMI 394094). A-D. Cheiroid conidia with divergent rows. Bars: A, B = 10 µm; C, D = 40 µm.

macronematosa, mononematosa, pallide brunnea, laevia, tenuitunicata, non-ramosa, brevia, flexuosa, 5-6 µm lata compositum. Cellulae conidiogenae monoblasticae, in conidiophoris incorporatae, terminales, pallide brunneae, laeves, tenuitunicatae. Conidia acrogena, solitaria, cheiroidea, brunnea vel atro-brunnea, laevia, 130-145 × 19-26 µm, cum 5-8 rami praedita, divergentia, cylíndrica 8-10 µm lata, 17-19-euseptata, ad septa modici constricti, cellula basilaris et apicalis 4-6 µm lata. Conidiorum secesio schizolytica.

Etymology: “*macrosporum*” referred to the great size of the conidia.

Conidiomata sporodochial, scattered, punctiform, pulvinate, dark brown to black. *Mycelium* mostly immersed in the substratum, composed of pale brown, smooth-walled, septate, branched hyphae, 2-4 μm wide. *Conidiophores* semi-macronematous, mononematous, pale brown, smooth and thin-walled, unbranched, short, flexuous, 5-6 μm wide. *Conidiogenous cells* monoblastic, integrated, terminal, pale brown, smooth and thin-walled. *Conidia* acrogenous, solitary, cheiroid, euseptate, brown to dark brown, smooth-walled, 130-145 \times 19-26 μm , with 5-8 arms inserted on basal cells in different planes; arms divergent, cylindrical, 8-10 μm wide, sometimes curved at the apex, 17-19-euseptate, slightly constricted at septa with conspicuous septal pores at the central part of each septum; apical and basal cells approximately 4-6 μm . *Conidial secession* schizolytic.

Habitat: soil.

Known distribution: Spain, Euskadi.

Material examined: Valle de Aramaio, Ibarra, on soil, March 2004, J. Matías (holotype IMI 394094, ex-type cultures: CBS 119741 and FMR 8972).

Cultural characteristics: colonies on OA at 25°C attaining a diameter of 28 mm in 2 wks, cottony to lanose, orange yellow (4A6), white at the periphery, margin irregular, slightly lobulate, with production of a yellow (3A6-7) diffusible pigment; reverse orange (4A7), yellow (3A7) at the periphery. On PCA at 25°C, colonies attaining a diameter of 32 mm in 2 wks, cottony to lanose, greyish at the centre (5-6D3), white to pale brown (5B2) towards the periphery, margin irregular, with production of a brownish orange (6C8) to brown (6D8) exudation and a yellow (3A6-7) diffusible pigment; reverse yellow (3A8). No growth was observed at 37°C on the three culture media tested. *Conidiogenesis* abundant after 2 wks of incubation, with numerous conidia, 75-125 \times 23-29 μm , brown, smooth-walled, composed of 5-8 divergent arms; arms cylindrical, 8-9 μm wide, 17-21-euseptate, sometimes curved at the apex.

DISCUSSION

The genus *Digitodesmium* was erected by Kirk (1981) to accommodate *D. elegans* P.M. Kirk, a species with cheiroid, euseptate conidia. These conidia are similar to those produced by members of *Dictyosporium* Corda or *Cheiromyces* Berk. & M.A. Curtis. However, these genera differ either by their conidial septation or secession. In *Digitodesmium*, the conidial secession was described as schizolytic while it is rhexolytic in *Dictyosporium*. In addition, the conidial arms are divergent at maturity in *Digitodesmium*, while they remain closely appressed in *Dictyosporium* (Kirk, 1981; Goh & Hyde, 1999; Cai *et al.*, 2002). On the basis of the type of septation in *D. elegans*, Sutton (1985) argued on a possible synonymy of *Digitodesmium* and *Cheiromyces*. However, as evidenced by Ho *et al.* (1991, 2000), the conidial septation is distoseptate in *Cheiromyces*. It is euseptate in *Digitodesmium*.

In order to clarify the boundaries among cheiросporous anamorphic fungi, the phylogenetic relationships of *Dictyosporium* and allied genera were inferred based on different ribosomal DNA gene sequences (Tsui *et al.*, 2006; Cai *et al.*, 2008). However, results were inconclusive and the authors (op. cit.) preferred to maintain the current circumscription of the genera compared, among them *Dictyosporium* and *Digitodesmium*, until a more complete taxon sampling could be achieved. We agree with Cai *et al.* (2008) that the pertinence of the

morphological characters used to distinguish these genera should be evaluated using multiple-gene phylogenies. However, in order to perform such studies, it is necessary to compare a representative number of strains of the different taxa, something that has important limitations because, as in our case, several of these fungi fail to grow *in vitro*.

In addition to the type species (*D. elegans*), three other species are currently accepted in *Digitodesmium*: *D. recurvum* W. H. Ho, K. D. Hyde & Hodgkiss (Ho *et al.*, 1999), *D. bambusicola* L. Cai, K. Zhang, Mc Kenzie, W. H. Ho & K. D. Hyde (Cai *et al.*, 2002) and *D. heptasporum* L. Cai & K. D. Hyde (Cai *et al.*, 2003).

Digitodesmium macrosporum can be distinguished from the other species of the genus by its large conidia with more septate arms. The second new species, *D. intermedium*, has the morphological features intermediate between *D. elegans* and *D. macrosporum*: the conidia of *D. elegans* are slightly shorter (45-60 µm long) and have usually 3-4 arms (Kirk, 1981); the conidia of *D. macrosporum* are longer and with 5-8 arms.

The diagnostic morphological features of the six species of *Digitodesmium* are keyed out below.

KEY TO SPECIES OF *DIGITODESMIUM*

- | | |
|---|-----------------------|
| 1 Conidia longer than 100 µm | <i>D. macrosporum</i> |
| 1 Conidia shorter than 100 µm | 2 |
| 2 Conidia with up to 11 arms | <i>D. intermedium</i> |
| 2 Conidia with less than 8 arms | 3 |
| 3 Arms with apical cells curved | 4 |
| 3 Arms with apical cells not curved | 5 |
| 4 Conidia 30-45 × 12-21 µm, with 4-7 arms | <i>D. recurvum</i> |
| 4 Conidia 50-75 × 32.5-70 µm, with (6)-7 arms | <i>D. heptasporum</i> |
| 5 Conidia with 3-4 arms; arms sometimes with a gelatinous cap at the apex | <i>D. elegans</i> |
| 5 Conidia with 3 arms; arms with hyaline appendages | <i>D. bambusicola</i> |

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