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FACULTAT DE CIÈNCIES

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UNITAT DE BOTÀNICA

TRICOMICETS IBÈRICS



Memòria presentada per:

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per optar al grau de Doctor en Ciències Biològiques

Vist-i-plau del Director de la tesi:

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ECCRINIDUS Manier, Ann. Sci. Nat. Bot. Paris 10:469. 1970a

Generitypus.– *Eccrinidus flexilis* (Léger & Duboscq) Manier, Ann. Sci. Nat. Bot. Paris 10:469. 1970a.

Generitypus specimen.– vide *E. flexilis*.

Esporangiòspores primàries uninucleades, de paret prima, isodiamètriques i no sempre presents. En fases de muda es formen esporangiòspores primàries de tipus cist, biloculars o, més rarament, uniloculars. De cadascun dels lòculs es desprèn una espóra tetranucleada. Esporangiòspores secundàries cilíndriques, amb 4-8 nuclis. Ocasionalment, es poden produir altres tipus d'espores de funció desconeguda. Tal·lus fixat mitjançant un holdfast secretat, de morfologia ben definida.

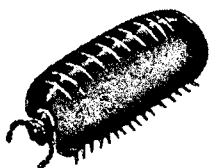
Una sola espècie, trobada a la Península Ibèrica. En Diplòpodes terrestres.

Eccrinidus flexilis (Léger & Duboscq) Manier, Ann. Sci. Nat. Bot. Paris 10:469. 1970a

≡ *Eccrina flexilis* Léger & Duboscq, Compt. Rend. Hebd. Acad. Sci. Paris 142:590. 1906 [basion.]

Typus.– Lame Léger et Duboscq 1850, conservé avec collection J.-F. Manier.

Tal·lus filamentosos, generalment corbats a la part basal i mitja, assolint els 4 mm de longitud total i amb un diàmetre de 5-25 µm. La zona basal apareix sovint una mica dilatada. Fixat a l'intestí mitjançant un **holdfast** de morfologia variable, en general estant format per una zona columnar de 17-35 x 13-17 µm, acabat en una expansió discoide, basal, de 16-25 µm de diàmetre. **Esporangiòspores primàries** tetranucleades, el·lipsoidals, de tipus cist bilocular, produint en cada lòcul una cèl·lula tetranucleada de 50-60 x 11-12 µm. Més rarament, són uniloculars i contenen una sola cèl·lula tetranucleada de 25-50 x 5-12 µm. Aquestes esporangiòspores es troben en les mudes dels hostes. **Esporangiòspores secundàries** de longitud variable i d'uns 10-22 µm de diàmetre, amb els septes transversals oblics o perpendiculars respecte a les parets del filament.



HOSTES I ECOLOGIA.– En la membrana interna del proctodeu de Glomèrids (Glomeridae, Diplopoda), terrestres.

MATERIAL ESTUDIAT

BALEARS (MALLORCA): Artà, vessant marítim de Talaia Moreia, 31S ED39, en *Glomeris* sp., leg. L. Guàrdia i Ll. Sáez, 4-Gen-01 [Tr1185-1187].

BARCELONA: El Brull, La Castanya, a les vores de la riera de la Castanya, 31T DG42, en *Glomeris*

ENTEROBRYUS Leidy, Proc. Acad. Nat. Sci. Philadelphia 4:225. 1849

Generitypus.– *Enterobryus elegans* Leidy, Proc. Acad. Nat. Sci. Philadelphia 4:225. 1849.

Generitypus specimen.– N.d.

Esporangiospores primàries mononucleades, de paret prima, isodiamètriques i no sempre presents. Esporangiospores secundàries cilíndriques, amb 4-8 nuclis. Ocasionalment, es poden produir altres tipus de d'espores de funció desconeguda. Tal·lus fixat mitjançant un holdfast secretat, de morfologia ben definida.

Vint-i-quatre espècies, de les quals, una ha estat identificada a la Península Ibèrica. En Diplòpodes.

Aquest fou el primer gènere de tricomicets en ésser descrit (LEIDY, 1849a). Ha esdevingut un dels més complexes per la quantitat d'espècies que conté (el més diversificat després de *Smittium*) i, sobretot, pels pocs caràcters morfològics dels que disposem, per la presència de cèl·lules accessòries de funció desconeguda, i per la no menys acusada variació intraespecífica (LICHTWARDT et al., 2001a). A tot això cal sumar-hi les nombroses descripcions de tàxons que hi ha associades, moltes d'elles poc acurades, que han incrementat de forma dramàtica la seva dificultat. Destaquem la presència de diversos tàxons que, sota noms ben diversos, han estat transferits a *Enterobryus* (MANIER & LICHTWARDT, 1968): *Andohaheloa* MANIER (1955b), *Capillus* GRANATA (1908), *Cestodella* TUZET, et al. (1957), *Daloala* TUZET et al. (1952), *Eccrina* LEIDY (1850), *Eccrinopsis* LÉGER & DUBOSCQ (1916), *Lactella* MAESSEN (1955), *Paratrichella* MANIER (1947), *Pistillaria* JEEKEL et al. (1959), *Recticoma* SCHEER (1935), *Trichella* LÉGER & DUBOSCQ (1929a) i *Trichellopsis* MAESSEN (1955).

Tant TUZET & MANIER (1948a) com MANIER (1950), en tractar espècies d'*Enterobryus* i altres *Eccrinals*, parlen d'un tipus de reproducció sexual mitjançant "espores durables" que correspondrien a les esporangiospores d'infestació 1^{ària}. Posteriorment (MANIER, 1970b) deixa de parlar d'espores sexuals ja que no observa evidències de cap procés d'aquesta mena. Aquestes espores durables són, de fet, les esporangiospores d'infestació 1^{ària}, que apareixen poc abans de la muda de l'hoste, en el moment en que l'*eccrinal* arriba a la fi del seu desenvolupament dins l'hoste. El fet que es parli de reproducció sexual té relació amb el comportament nuclear de les diferents espores. Les micròspores i les esporangiospores d'infestació 2^{ària} foren considerades espores haploides ja en el primer dels dos articles [que versa enterament sobre el tema de la sexualitat de les espores (TUZET & MANIER, 1948a)]. En aquest darrer article, les aurores expliquen com els tal·lus interrompen la producció d'esporengiospores d'infestació 2^{ària} (= macroconidis o macròspores) per iniciar el desenvolupament d'esporengiospores d'infestació 1^{ària} (= espores durables). Els nuclis dels filaments s'aparellarien i formarien un nucli diploide més gros. El protoplasma s'organitza en esporoblasts allargats, que s'escindeixen en elements uninucleats. Aquests, per contracció

citoplasmàtica, prenen una forma ovalada. D'aquesta manera, els tal·lus originats de la fixació i creixement dels "macroconidis" formen "espores durables" (TUZET & MANIER, 1948a).

Enterobryus leptoiuli (Manier) Manier, Ann. Sci. Nat. Bot. Paris 10:603.
1970b

≡ *Enterobryus leptoiuli* Manier, Ann. Sci. Nat. Bot. Paris 11:71. 1950 [nom. inval.]

Typus.- Lame SML 4-47. Collection J.-F. Manier.

Tal·lus majors (macrotal·lus) rectilinis o corbats, assolint una longitud total que pot superar els 1,5 mm, i un diàmetre de (7-)9-12 µm, homogeni en tota la seva llargada excepte en la zona proximal que apareix més eixamplada (fins a 17 µm). En l'extrem distal s'hi manté la cèl·lula mare. Estan fixats a l'intestí mitjançant un **holdfast** d'unes 10-17 µm de llarg, de morfologia acampanada, amb estries evidents, horitzontals, a la part mitja i superior, i amb microestriacions longitudinals en la zona inferior (FIGS.163.4, 163.5). Aquests tal·lus amples produeixen 2-20 espores d'**infestació 1^{ària}**, uninucleades, unes més aplanades (FIG. 162.1), de 6-8 x 9-12 µm, les altres isodiamètriques (FIG. 162.2), de 7-8 x 7-8 µm. **Tal·lus** més estrets i petits (microtal·lus), de 7-8 µm de diàmetre i amb un holdfast menys desenvolupat, cònic, produeixen **esporangiòspores d'infestació 2^{ària}** amb 4-8 nuclis cadascuna, de 60-80 x 7-8 µm (FIG. 162.4). Les esporangiòspores d'infestació 2^{ària} es desprenen per trencament de la paret en la zona basal de la cèl·lula corresponent (FIG. 162.3).



HOSTES I ECOLOGIA.- En la membrana interna del proctodeu de *Leptoiulus* sp. i altres Iulidae (Diplopoda), terrestres.

MATERIAL ESTUDIAT

ANDORRA: Parròquia d'Ordino, El Serrat, riu de Tristaina, Vall de Sorteny, 31T CH81, en *Leptoiulus* sp, leg. L. Guàrdia, 16-Maig-01 [Tr0760-0761].

BARCELONA: El Brull, La Castanya, vores Riera Castanya, Montseny, 31T DG42, en Iulidae indet., leg. L. Guàrdia, 3-Oct-00 [Tr0331-0334]; ídem, 18-Des-00 [Tr0437].

OBSERVACIONS.- MANIER va descriure a aquesta espècie l'any 1950 sense aportar una diagnosi llatina, en un article recopilatori on hi apareixen il·lustracions dels tàxons francesos i informació detallada i completa de cadascun d'ells. En la segona monografia de MANIER (1970b), es completa la descripció i es valida l'espècie amb l'aportació d'una diagnosi llatina i la designació del tipus.

Tots els caràcters tal·lials i espòrics justifiquen la classificació dels nostres espècimens com a *E. leptoiuli*, destacant només l'amplada una mica superior en alguns macrotal·lus ibèrics (9-15 µm en la regió basal, segons MANIER, 1970b). Les esporangiòspores d'infestació 2^{ària} que hem trobat nosaltres serien lleugerament més curtes. No obstant, en Eccrinals, les mides per

aquestes espores són sumament variables. La cèl·lula basal es correspon, en mides i morfologia, a la descripció del protòleg.

Només una altra espècie manté certa semblança amb els tal·lus que tractem, i és *E. duboscqii* [com "*E. dubosqui*"] (MANIER, 1968), que d'altra banda, es diferencia de les nostres mostres per la presència d'un espores primàries diferents a les d'*E. leptoiuli*. Per la resta de característiques hem de comentar que totes dues espècies són molt similars, i destaca la proximitat en la morfologia del holdfast. Malgrat tot, *E. duboscqii* no va ésser reconeguda per LICHTWARDT (1986), a causa de la presència d'unes esporangiòspores de paret gruixuda. L'any 1950 MANIER descriu en *E. leptoiuli* la presència d'unes espores de paret gruixuda. Posteriorment, la mateixa autora assigna aquesta observació a un possible defecte de visualització, i en desdú l'existència (LICHTWARDT et al., 2001a).

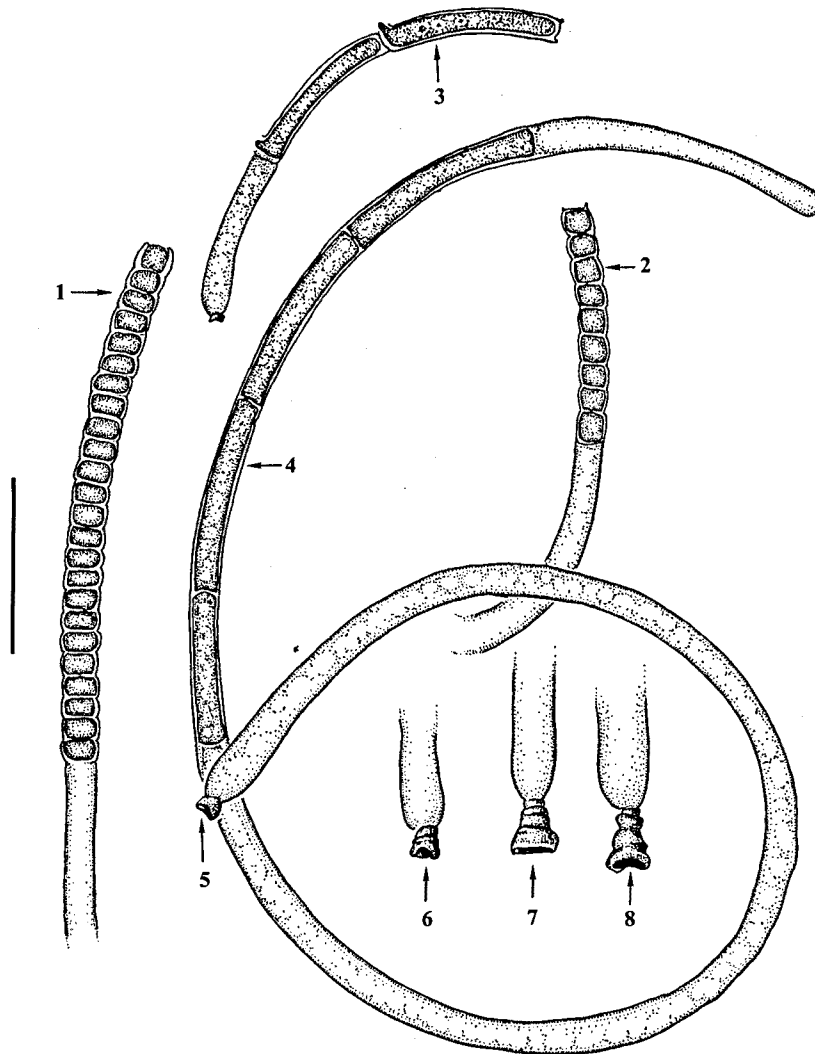


FIG. 162. *Enterobryus leptoiuli*. 1, 2, tal·lus amb esporangiòspores d'infestació 1^{ària}. 3, 4, tal·lus amb esporangiòspores d'infestació 2^{ària}. 5, holdfast de microtal·lus. 6-8, diverses morfologies de holdfasts de macrotal·lus. Escala = 50 µm.

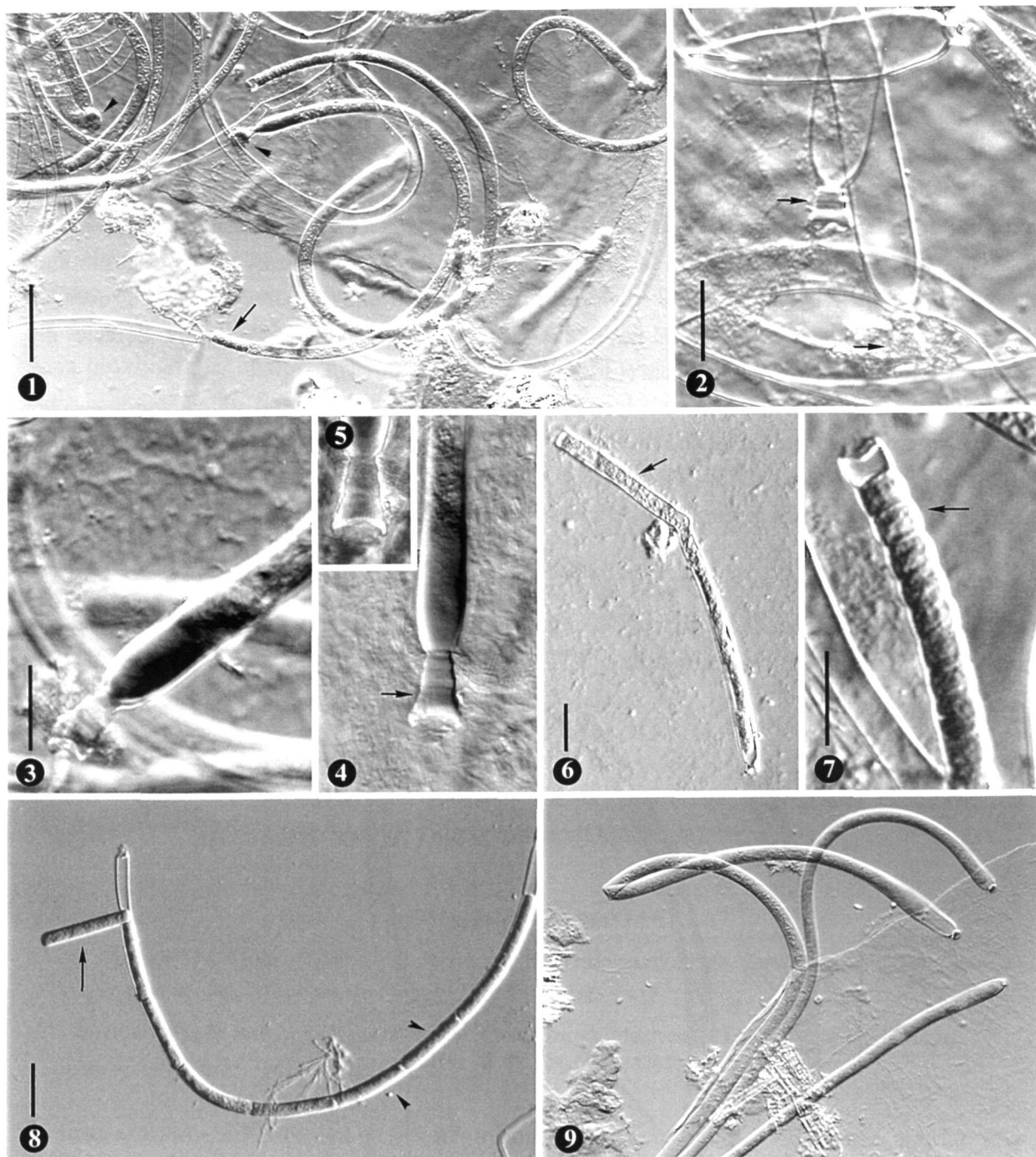


FIG. 163. *Enterobryus leptoiuli*. 1, visió general de diversos tal·lus (microtal·lus), s'hi diferencia alguna esporangiòspora 2^{ària} (fletxa) i holdfasts (puntes de fletxa). 2, 3, macrotal·lus: cèl·lules basals amb holdfasts (fletxes), on s'hi veuen les estries transversals. 4, 5, macrotal·lus: detall de holdfasts, amb cura hi podem observar, a part de les estries transversals, algunes estries longitudinals en la zona més proximal al budell. 6, microtal·lus jove amb la cèl·lula mare persistent a l'extrem distal (fletxa). 7, macrotal·lus amb esporangiòspores d'infestació 1^{ària} (fletxa). 8, microtal·lus amb esporangiòspores d'infestació 2^{ària}. (fletxa). 9, zona basal de microtal·lus amb holdfasts. Escales = 50 µm en 1; = 25 µm en 2, 3 (la mateixa escala per 3-5), 6, 7, 8 (la mateixa escala per 8, 9).

F. PARATAENIELLACEAE

MANIER & LICHTW., ANN. SCI. NAT. BOT. PARIS 9:525. 1968

Typus.— *Parataeniella* Poisson, Arch. Zool. Exp. Gén. 69:179. 1929.

Tal·lus holocàrpic, que funciona com un esporangi amb nombroses espores primàries uninucleades en la maturitat. Dos tipus d'espores funcionals.

Dos gèneres, dels quals, un trobat a la Península Ibèrica.

PARATAENIELLA Poisson, Arch. Zool. Exp. Gén. 69:179. 1929

Generitypus.— *Parataeniella mercieri* Poisson, Arch. Zool. Exp. Gén. 69:201. 1929.

Generitypus specimen.— N.d.

Presenta dos tipus de tal·lus: 1) tal·lus curts que formen espores de desenvolupament immediat, funcionant com un esporangi amb nombroses espores uninucleades; 2) tal·lus que poden formar, a més d'esporangiòspores primàries, esporangiòspores secundàries, normalment binucleades, i sovint amb el septes transversals lleugerament oblics. Fixats a la membrana intestinal mitjançant un holdfast en forma de petit embut discoide.

Cinc espècies descrites, de les quals, una trobada a la Península Ibèrica i Illes Balears. En isòpodes terrestres (Oniscidae).

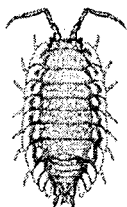
Parataeniella dilatata Poisson, Arch. Zool. Exp. Gén. 69:205. 1929

= *Parataeniella binucleata* Poisson, Arch. Zool. Exp. Gén., 69:207. 1929.

= *Parataeniella intermedia* Poisson, Arch. Zool. Exp. Gén., 69:204. 1929.

Ind. loc.— “J'ai observé cette forme chez des *Armadillo officinalis* capturés à Banyuls (Pyr.-Or.) (...) à la mare Reig et à la Fontaine des Chasseurs”.

Es diferencien dos tipus de tal·lus, fixats a l'intestí mitjançant un holdfast discoide poc desenvolupat, senzill: (1) tal·lus llargs, que assoleixen 300 x (8-)12-14 µm, formadors d'esporangiòspores d'infestació 2^{ària}, normalment 2-5 nucleades, de (20-)40-50 x 12-13 µm, o bé, esporangiòspores uninucleades més curtes, de 15-25 µm de longitud; (2) tal·lus més curts, de 100-230 x (12-)22-(28) µm, formadors d'esporangiòspores d'infestació 1^{ària} uninucleades o binucleades, que surten a l'exterior per l'apex del tal·lus holocàrpic.



HOSTES I ECOLOGIA.- En la membrana interna del proctodeu d'isòpodes terrestres Oniscidae (*Armadillo* spp.)

MATERIAL ESTUDIAT

BALEARS (MALLORCA): Palma, Castell de Bellver, 31S DD78, en *Armadillo* sp., leg. L. Guàrdia, 18-Abr-01 [Tr0695-0698]; ídem, 4-Gen-03, leg. L. Guàrdia [Tr0684-0685].

GIRONA: El Brull, la Castanya, riera de la Castanya, en Oniscidae, 31T DG42, leg. L. Guàrdia i S. Santamaria, 3-Oct-00 [Tr0373].

TARRAGONA: Tarragona, Móra, Punta de Na Móra, prop Platja, 31T CF65, en *Armadillo* sp., leg. M. Cafaro i L. Guàrdia, 4-Set-02 [Tr1523-1526].

OBSERVACIONS.- En la publicació original, POISSON (1929) va considerar que les espècies de *Parataeniella* que habitaven tres gèneres diferents d'Oniscids corresponien a tres espècies diferents. D'aquesta manera va descriure *P. dilatata* trobada en *Armadillo officinalis*, *P. intermedia* en *Trichoniscus roseus* i *P. binucleata* en *Porcelio laevis* (POISSON, 1929). Posteriorment diversos autors han considerat que no hi ha diferències entre les tres espècies. MANIER, (1970b) sinonimitzà *P. intermedia*; posteriorment, LICHTWARDT (1986), va incloure també *P. binucleata* dintre *P. dilatata*. Els escassos i variables caràcters morfològics que ens serveixen per identificar les espècies d'aquest gènere, no permeten fer descripcions excessivament estretes, sobretot pel que fa a morfologies espòriques, ja que, com hem vist en altres gèneres d'Eccrinals, la presència de diversos tipus d'espores en una mateixa espècie és reiterada.

Parataeniella dilatata ha estat localitzada també a França (POISSON, 1929; MANIER, 1950) Alemanya (SCHEER, 1976) i USA (LICHTWARDT, 1986).

Un tret diferencial d'aquesta espècie és la presència d'esporengiòspores secundàries relativament llargues i cilíndriques (FIGS. 164a, 165.1), així com la presència d'espores uninucleades, disposades generalment en una sola sèrie, dintre del tal·lus. Nosaltres hem pogut observar els quatre tipus descrits d'espores, essent les més habituals les uninucleades que es disposen en diverses sèries dins del tal·lus.

L'espècimen recol·lectat a la Castanya (Tr0373), té unes característiques una mica diferents respecte a la resta de material estudiat de *Parataeniella*. De fet, l'hoste no és el mateix que en les altres preparacions, tractant-se d'un Oniscidae no determinat, possiblement del gènere *Trichoniscus*. A pesar de lleus diferències tal·lials, creiem que pot ésser considerat dintre d'aquest mateix tàxon, i en tot cas, necessitariem més material per poder fer un estudi detallat. Val a dir que els tal·lus d'aquesta mostra (Tr0373) no són plenament desenvolupats.

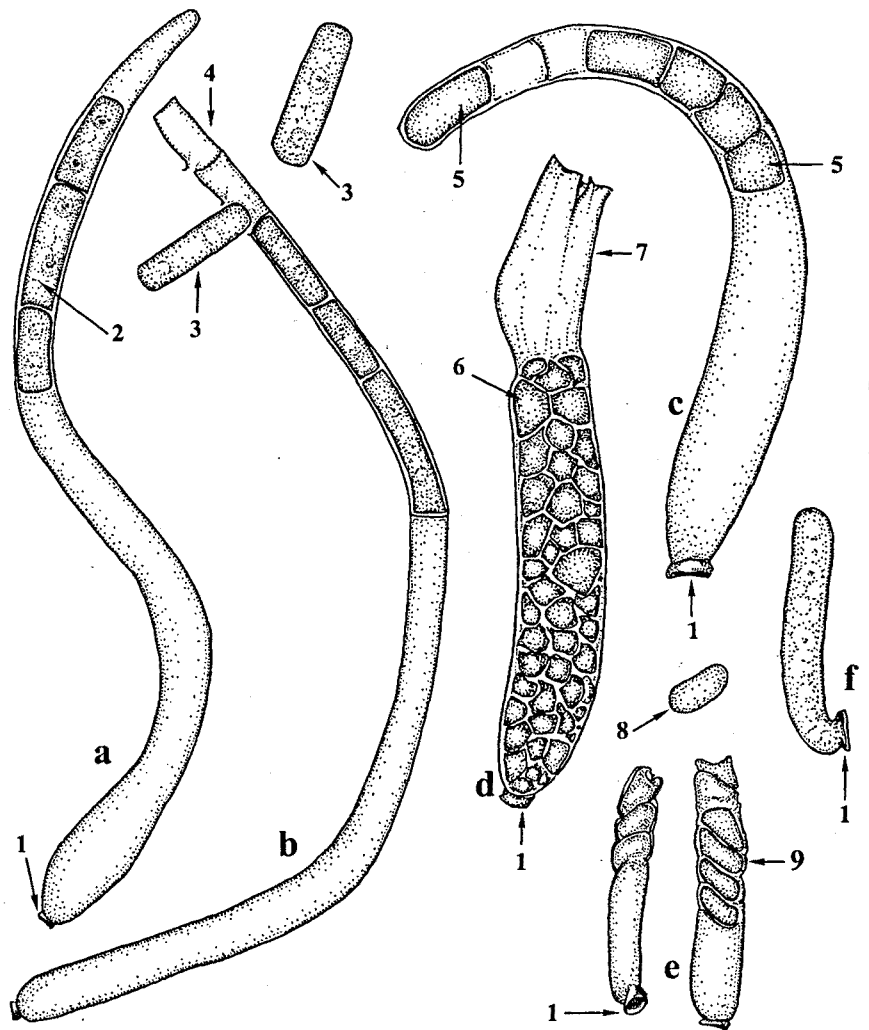


FIG. 164. *Parataeniella dilatata*. a, b, c: tal·lus amb esporangiòspores d'infestació 2^{ària}. d: tal·lus productors d'esporangiòspores uninucleades. e: tal·lus formadors d'esporangiòspores d'infestació 1^{ària}. 1, holdfasts. 2, 3, esporangiòspores d'infestació 2^{ària}. 4, cèl·lula buida d'on ha sortit una esporangiòspora d'infestació 2^{ària} trencant la paret. 5, esporangiòspores d'infestació 2^{ària} més isodiamètriques. 6, esporangiòspores d'infestació 1^{ària} formades a l'interior d'un tal·lus que actua com a esporangi. 7, zona apical del tal·lus per on surten les esporangiòspores. 8, esporangiòspora d'infestació 1^{ària} lliure al medi. 9, tal·lus amb esporangiòspores d'infestació 1^{ària} de paret prima.

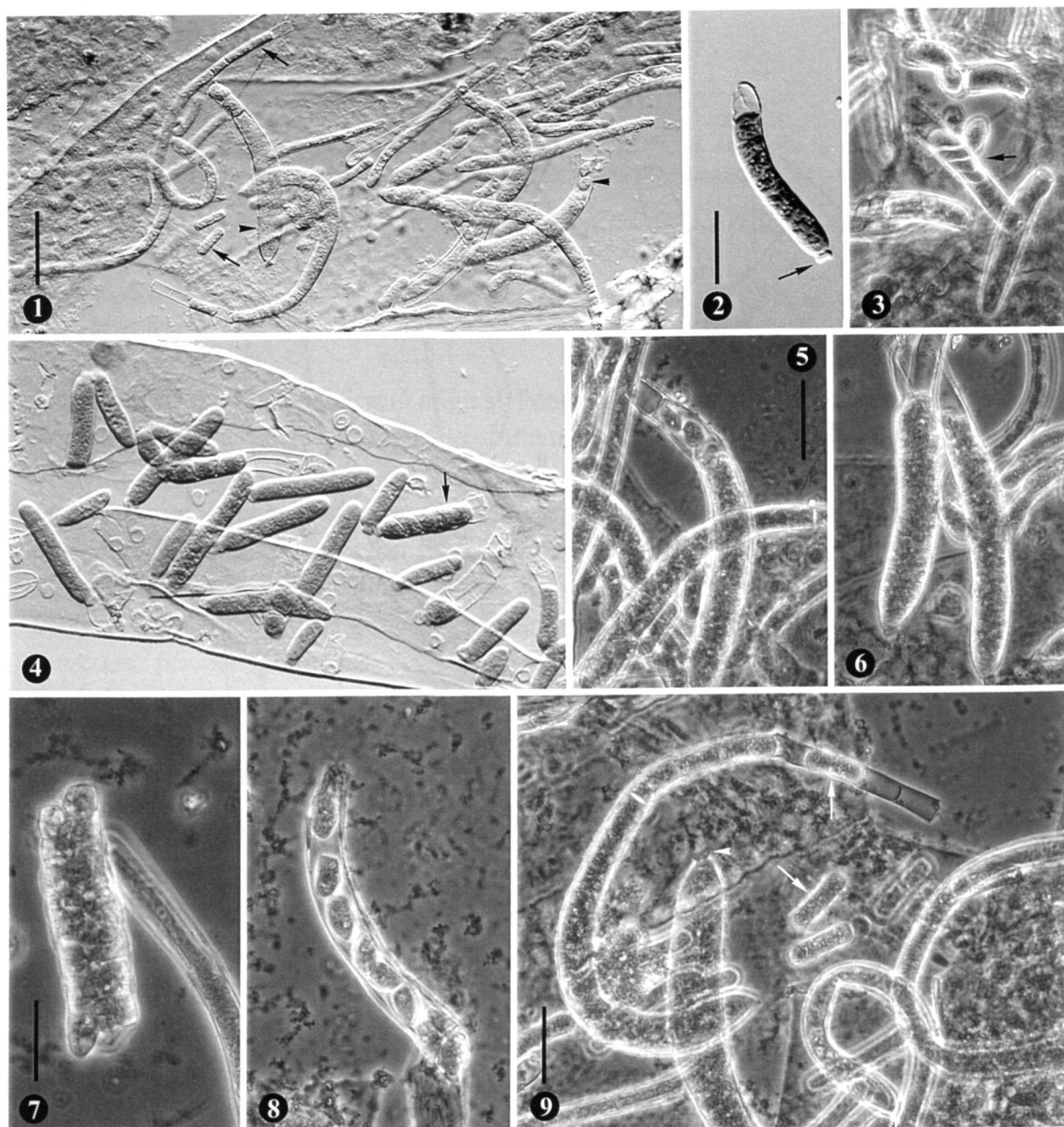


FIG. 165. *Parataeniella dilatata*. 1, Diversos tal·lus, els més estrets, formadors d'espores secundàries (fletxes), altres més amples produeixen espores primàries (puntes de fletxa) o tots dos tipus d'esporengiòspores alhora [Tr1523]. 2, una esporengiòspora acaba de formar un holdfast primari per a la seva fixació [Tr0373]. 3, tal·lus formadors d'esporengiòspores d'infestació 2^{ària} (fletxes) [Tr0684]. 4, tal·lus joves diferenciant esporengiòspores d'infestació 2^{ària}. [Tr0684]. 5, 6, tal·lus formant esporengiòspores d'infestació 1^{ària} [Tr1524]. 7, fragment d'un tal·lus amb esporengiòspores d'infestació 1^{ària} [Tr 1525]. 8, tal·lus formant espores d'infestació 1^{ària} uninucleades [Tr1525]. 9, tal·lus amb esporengiòspores d'infestació 1^{ària} en la zona basal i secundàries en l'apical [Tr1525]. Escales = 50 µm en 1; = 25 µm en 2 (la mateixa escala per 2, 3, 4), 5 (la mateixa escala per 5, 6), 7 (la mateixa escala per 7, 8), 9.

5 ANNEXES

ANNEX I (PÀGS. 285-292)

VALLE & SANTAMARIA. 2002a. *Baetimyces*, a new genus of Harpellales, and first report of *Legeriomyces ramosus* from the northeastern Iberian Peninsula. *Mycologia* 94:321-326.

***Baetimyces*, a new genus of Harpellales, and first report of *Legeriomyces ramosus* from the northeastern Iberian Peninsula**

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Abstract: *Baetimyces*, belonging to the Legeriomycetaceae, is described here as a new genus. Its single species, *B. ancorae*, has been found growing in the hindgut of mayfly nymphs of the genus *Baetis* (Ephemeroptera, Baetidae) inhabiting a Pyrenean Mountains canal-stream from the northeastern region of the Iberian Peninsula. The new genus is characterized by having trichospores with two thick appendages of unequal length, and by zygospores perpendicularly and medially attached to the zygosporophore. The new genus may be related to *Glottzia*, *Legeriomyces*, and *Zygopolaris*, and similarities with these and other genera are compared and discussed. The new species often coexists in the same gut with *Legeriomyces ramosus*, which is reported for the first time in Spain.

Key Words: Baetidae, gut fungi, Legeriomycetaceae, mayfly nymphs, taxonomy, Trichomycetes

INTRODUCTION

Trichomycetes (Zygomycota), growing within the digestive tracts of several orders of insects and other arthropods, have been reported from five continents. Current knowledge of Trichomycetes from the Iberian Peninsula is scant, with only twelve species previously reported (Santamaria 1997, Santamaria and Girbal 1997, 1998, Girbal and Santamaria 1998). We intensively surveyed the Iberian Peninsula to better understand its Trichomycete mycobiota. This research began in 2000 and is integrated with the project "Flora Mycologica Iberica."

Trichomycetes include three orders: Harpellales, Asellariales, and Eccrinales. The order Amoebidiales has been excluded from the class because molecular studies demonstrated its phylogenetic affinities are with protozoans (Benny and O'Donnell 2000). Har-

pellales, which is the most intensively studied order, includes two families: Harpellaceae and Legeriomycetaceae, the former for species with unbranched thalli living in the midgut, the latter for species with branched thalli living in the hindgut (Lichtwardt 1986).

Baetimyces, a new Trichomycete genus, is described from the hindgut of mayfly nymphs (Baetidae, Ephemeroptera). The single species, *B. ancorae*, was collected from a canal-stream of the northeastern Spain Pyrenean Mountains, during summertime. The presence of medially attached zygospores, and especially the peculiar trichospores bearing two thick appendages of unequal length, characterize the genus and represent a novel addition to the taxonomy of Legeriomycetaceae. *Baetimyces* is compared with *Glottzia* M. Gauthier ex Manier & Lichtw., *Legeriomyces* Pouzar and *Zygopolaris* S. T. Moss, Lichtw. & Manier on the basis of the ramification pattern of hyphae, on the shape of the holdfast apparatus, and on trichospore features.

MATERIALS AND METHODS

The description of the new taxon is based on material found in an unnamed artificial channel stream in Guils de Cerdanya (Lleida Province, Catalonia, Spain) in July 2000. Collections of insect nymphs were made by hand picking from rocks, wood, and leaves removed from streams and also by dragging aquatic nets. The insects were dissected to extract and clean the guts under a stereomicroscope, and the fungi were placed on a slide with water as a mounting medium and photographed using phase contrast or interference contrast optics. Afterwards, slides were fixed with lactophenol cotton-blue to be preserved and deposited in the herbarium BCB-Mycotheca, at the institutional address of the authors.

TAXONOMY

***Baetimyces* L.G. Valle et Santam., gen. nov.**

Trichosporae subcylindroides, cum duabus latus appendicibus, quarum prima elongata, secunda brevis. Appendices helictae dispositae intra cellulam genitalem ante trichosporarum liberationem. Zygosporae biconicae, zygosporophoro ad perpendiculum et in medio affixae. Thalli inaequaliter ramificantes. In unoquoque thallo duo magisve pedes laterales vel terminales. Ad cuticulam proctodaei nympharum Bactis affixi.

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Trichospores subcylindrical, bearing two thick appendages, one elongated and the other short. Appendages helically oriented within the generative cell before trichospore release. Zygosporangia biconical, perpendicularly and medially attached to zygosporophore. Thalli irregularly branched. More than one holdfast per thallus, in lateral and terminal positions. Attached to hindgut cuticle of *Baetis* (Ephemeroptera: Baetidae) nymphs.

Etymology. Latin, *Baetimyces*, fungus growing in *Baetis*, the host genus.

TYPUS generis. *Baetimyces ancorae* L.G. Valle et Santam.

***Baetimyces ancorae* L.G. Valle et Santam., sp. nov.**

FIGS. 1–8, 10–17

Cellulae hypharum 3.5–5 μm in laitudine. Trichosporae (40–)50(–58.5) \times (3.5–)4.5(–5.5) μm , 3–5 in unoquoque ramo fertili ortae. Zygosporae (34–)45(–54) \times 7–9 μm . Pedes cupulati. Zygosporophorae cum anchorali delineatione ubi conservatae et tinctae sunt.

Thalli sparsely branched, attached to the hindgut lining of *Baetis* nymphs by laterally secreted, cup-like holdfasts (FIGS. 7, 11), one of them in terminal position at the end of a sinuous basal cell. The presence of a main axis is not always evident due to the variable direction of projecting branches (FIG. 1). Hyphal cells 3.5–5 μm diam. Fertile branches bearing 3–5 trichospores each (FIGS. 1, 3–6, 8). Trichospores subcylindrical, (40–)50(–58.5) \times (3.5–)4.5(–5.5) μm , straight to slightly arcuate (FIGS. 1–6, 8, 15–17). Generative cells 12–18 \times 5–7 μm , distally swollen at the trichospore-formation area (FIGS. 3–6, 8). After release, the trichospores show two thick appendages: an elongated appendage, slightly wider at the proximal end, and a small, short appendage which contacts at the basal area and continues with the longest appendage (FIGS. 2, 15–17). A very small and inconspicuous collar can be seen in trichospore base with an accurate observation at high microscope magnification (FIG. 15). Both appendages are helically oriented within the generative cell before the tricho-

spore release, appearing as broad oblique bands (FIGS. 3–6). Zygosporangia biconical, (34–)45(–54) \times 7–9 μm , perpendicularly and medially attached to the zygosporophore (Type I; Moss et al 1975). The zygosporophore subtending a mature zygosporangium, when preserved and stained with lactophenol cotton-blue, contains cytoplasmic material restricted to a well-defined central zone showing cytoplasmic contractions with lateral expansions in the overall shape of an anchor (FIGS. 10, 12–13). Conjugation scalariform, with zygosporangia arising from the distal end of cells of either conjugating branches. After release, zygosporangia have an empty collar which is the zygosporophore remnant, and no appendages (FIG. 14).

Etymology. Latin, *ancorae*, referring to the anchor-like contraction of the cytoplasm in the zygosporophore when preserved and stained with lactophenol cotton-blue.

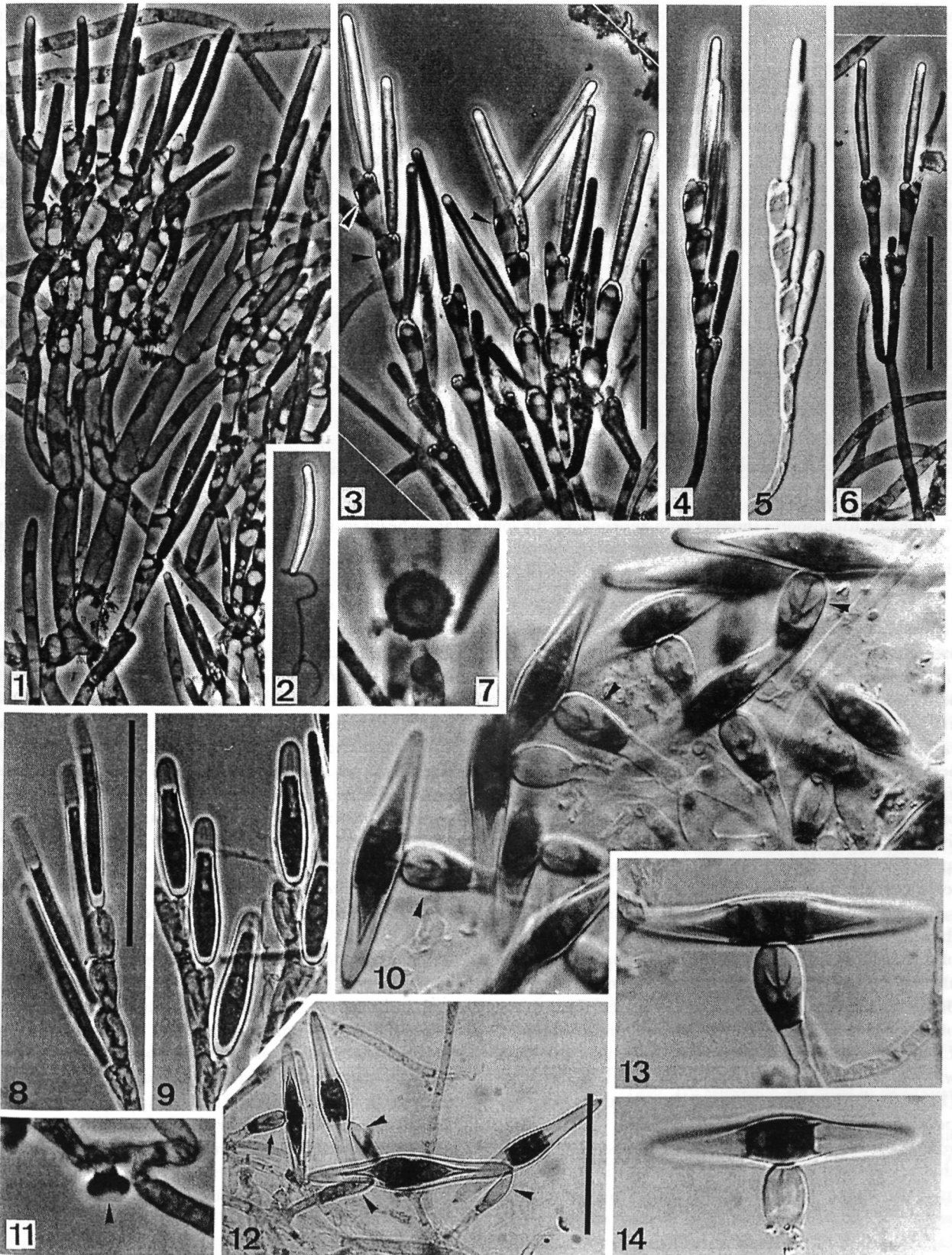
Specimens examined. SPAIN. CATALONIA: Prov. of Lleida, in an unnamed canal-stream of Guils de Cerdanya, on the way to Meranges lake, alt. 1700 m., UTM 31T DH0601, in the hindgut of *Baetis* sp. (Ephemeroptera, Baetidae), 13 Jul 2000, L. G. Valle [Tr0152, BCB (HOLOTYPE) that includes zygosporangia and trichospores] [Tr0153, Tr0158, Tr0159, Tr0160, Tr0161, Tr0162, Tr0163, Tr0164, Tr0168, Tr0171, Tr0172, Tr0176, Tr0178, Tr0179; BCB (ISOTYPES)].

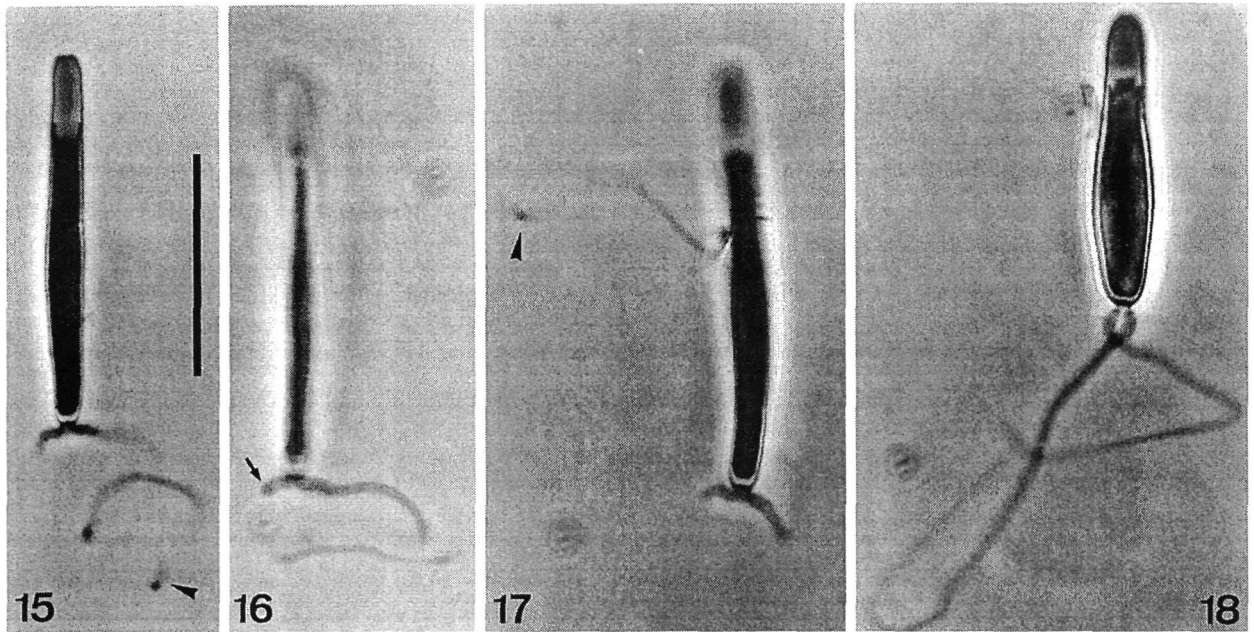
DISCUSSION

Among the examined nymphs of *Baetis*, a high percentage (nearly 70%) were infested with *Baetimyces ancorae*. *Legeriomyces ramosus* Pouzar was also present in 20% of these nymphs. When both species were present in the same gut, thalli grew in a dense net-like mass of hyphae, and were yet more compact when hyphae conjugated and zygosporangia of both species were fully developed (see FIG. 12).

Baetimyces ancorae resembles *Legeriomyces ramosus* in holdfast and thallus characteristics (lateral and broad holdfast, irregular branching without a clearly defined main axis and overall habit). However, the

FIGS. 1–14. *Baetimyces ancorae* [1–6, BCB-Tr0172; 7, 11, BCB-Tr0158; 8, BCB-Tr0161; 10, 13–14, BCB-Tr0152; 12, BCB-Tr0160]. FIGS. 9, 12. *Legeriomyces ramosus* [9, BCB-Tr0161; 12, BCB-Tr0160]. 1. Overall view of a branching and sporulating thallus. 2. Released trichospore showing the two appendages. 3. Fertile branches showing helically oriented appendages inside the generative cell (arrowheads). 4–6. Fertile branches. 7. Lateral holdfast in frontal view. 8–9. Fertile branches of *B. ancorae* and *L. ramosus*, respectively, both from the same slide. 10. Zygosporangia showing the "anchor-like" pattern in the zygosporophore (arrowheads). 11. Lateral holdfast in side view. 12. Zygosporangia of *B. ancorae* (arrow) and *L. ramosus* (arrowheads). Note the different attachment to zygosporophores. 13. Zygosporangium showing the "anchor-like" structure in zygosporophore. 14. Released zygosporangium with an empty collar. [FIGS. 1–6 in water-mounted slides; remaining FIGS. in lactophenol cotton-blue slides] [FIGS. 1–4, 6–9, 11 photographed with phase contrast optics; FIGS. 5, 10, 13–14 photographed with interference contrast optics; FIG. 12 photographed with light transmission optics] [Scale bar 3 = 50 μm for 3–5, or 25 μm for 7, 10–11, 13–14; Scale bar 6 = 50 μm for 1, 2, 6; Scale bar 8 = 50 μm for 8–9; Scale bar 12 = 50 μm for 12].





FIGS. 15–17. *Baetimyces ancorae* [15, BCB-Tr0163; 16–17, BCB-Tr0153]. FIG. 18. *Legeriomyces ramosus* [BCB-Tr0161]. 15–17. Released trichospores showing both appendages: the longer (arrowheads in 15 and 17) and the shorter which is entirely focused in 16 (arrow). 18. Released trichospore showing both appendages. [All from lactophenol cotton-blue slides and photographed with phase contrast optics] [Scale bar 15 = 50 μm for all Figs.]

presence of medially attached zygospores (Type I) and the appendaged trichospores bearing two appendages of unequal length, make *Baetimyces* a distinct genus among the family Legeriomycetaceae. In earlier examinations it was thought that the short appendage was a normal long appendage broken during the manipulation process, or that it was due to a premature release of trichospores from the generative cell. However, the persistence of this character in successive preparations makes it a diagnostic characteristic of the new genus.

Trichospores of *Legeriomyces ramosus* differ from those of *Baetimyces ancorae* not only by the presence of a second well-developed, elongate appendage, but also by its size ($27\text{--}31 \times 6.5\text{--}7.5 \mu\text{m}$ in our samples), being smaller, broader and more elliptical than those of *B. ancorae* (Figs. 9, 18). Moreover, appendages of *L. ramosus* appear evidently enlarged near the base without any trace of a collar in the trichospore base (Fig. 18). Differences between the length of both appendages were reported in *L. ramosus* (as *Genistella ramosa* L. Léger & M. Gauthier) by Moss (1979), but this asymmetry was not so extreme as in *B. ancorae* and the shape of these trichospores follow *Legeriomyces* characteristics. *Legeriomyces aenigmaticus* Lichtw. & M. C. Williams produces three ranges of trichospore size (Lichtwardt and Williams 1983), while in the species here described the size is invari-

able. *Legeriomyces rarus* Lichtw. & M. C. Williams has long-obpyriform to almost ellipsoidal trichospores ($25\text{--}31 \times 5.5\text{--}8 \mu\text{m}$ in Williams and Lichtwardt 1993) with 2 long appendages, thus differing from those of *B. ancorae*. Additionally, the genus *Legeriomyces* has thalli with a bulbous basal cell and type II zygospores (submedially and obliquely attached to the zygospore) (Fig. 12).

Glitzia M. Gauthier ex Manier & Lichtw., described (Gauthier 1936) from *Baetis* nymphs, possesses one long slender central appendage and two short broader divergent lateral appendages. Zygospores in *Glitzia* are of type II whereas in *Baetimyces* they are of type I. Trichospores of *Glitzia* and *Baetimyces* have some resemblance in shape, in both genera being subcylindrical, but sizes are rather different. Currently, the genus *Glitzia* includes four species: (1) *G. ephemeridiarum* Lichtw. has the longest trichospores ($45\text{--}70 \times 4.5\text{--}5 \mu\text{m}$; Lichtwardt 1972); (2) *G. centroptili* M. Gauthier ex Manier & Lichtw. has smaller trichospores ($40 \times 4 \mu\text{m}$; Gauthier 1936; Manier and Lichtwardt 1968) with the central appendage helically arranged around the two shorter, broader, somewhat rigid lateral appendages, and a lateral holdfast on a branched basal cell; (3) *G. coloradense* M. C. Williams & Lichtw. (Williams and Lichtwardt 1999) has longer trichospores than those of *B. ancorae*, and (4) *G. tasmaniensis* Lichtw. & M. C. Williams (Lichtwardt and Williams 1990) is sim-

ilar to *G. centroptili* and *G. ephemeridarum* but differs in holdfast structure.

Baetimyces ancorae shares characteristics with both species of the genus *Zygopolaris* S. T. Moss & Lichtw. that grow in Ephemeroptera: *Ephemerella* (Ephemerelellidae), *Epeorus* (Heptageniidae) and *Baetis*. Zygospores in *Zygopolaris* are of type IV, being attached to one pole in the same zygosporophore axis. These zygospores have been considered as typical biconical zygospores of Harpellales modified by the peculiar polar attachment (Moss et al 1975, Moss and Lichtwardt 1977). Moss et al (1975) described *Zygopolaris ephemeridarum* and stated that the released trichospores do not present defined appendages under the light microscope. Later ultrastructural studies on this species demonstrated that early in trichospore development an electron-opaque material accumulates between the cytoplasmic membrane and the wall of the generative cell, just below the trichospore (Moss and Lichtwardt 1977). Nevertheless, no further appendage differentiation has been observed.

The zygosporophore of *Zygopolaris* is separated from its conjugate by a septum, as in *Baetimyces*. The material contained inside the mature *Baetimyces* zygosporophore tends to remain in a rather constant position and in the shape of an anchor when treated with lactophenol cotton-blue. In *Zygopolaris*, the zygosporophores contain fibrous material that restricts the cytoplasm to the central region (Moss and Lichtwardt 1977). We have contrasted this character by studying two slides of *Z. ephemeridarum* borrowed from Lichtwardt's collection, and with several digitized images of this species as well as *Z. borealis* Lichtw. & M. C. Williams (Lichtwardt and Williams 1975). No structure similar to *B. ancorae* has been observed in this stained material. Also remarkable is the presence of both lateral and terminal types of holdfasts in *Baetimyces*, with the terminal one situated at the base of a sinuous cell such as that described for *Z. borealis*. *Zygopolaris ephemeridarum* has only lateral holdfasts. Despite these similarities, species of *Zygopolaris* can be easily distinguished from *B. ancorae* by zygospore and trichospore characters.

Another genus inhabiting Baetidae guts that has zygospores of type I is *Spartiella* Tuzet & Manier ex Manier, but trichospores bear one appendage attached medially to the proximal end. Also, the presence of a lobulate terminal holdfast cell is characteristic of this genus. Trichospores of *Spartiella barbata* Tuzet & Manier ex Manier measure 22–27 × 7.5–10 μm (Tuzet and Manier 1950), shorter and broader than the new species. Similarly, *Spartiella animae* Lichtw. differs in trichospore size, appendage formation and holdfast structure.

Lastly, we wish to promote the lactophenol cotton-

blue staining treatment in mounting slides for the observation of certain characters of taxonomic interest that otherwise are overlooked in water mounts. In *B. ancorae*, the distinctive structure seen in the zygosporophore is only observed in thalli preserved and stained with lactophenol cotton-blue (we have not studied the effect of other mounting media). However, *in vivo* studies with water mounts are required to observe some structures such as appendage arrangement inside generative cells and other fragile structures that are lost with fixation.

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ANNEX II

(PÁGS. 293-305)

VALLE & SANTAMARIA. 2005. *Bojamyces transfuga* sp. nov.
and new records of Trichomycetes from mayfly larvae in
Spain. Mycologia 97 (en premsa).

***Bojamyces transfuga* sp. nov. and new records of Trichomycetes from mayfly larvae in Spain**

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Abstract. The new species *Bojamyces transfuga* is described. It was found growing in the hindgut of mayfly nymphs and in their shed abdominal exoskeleton lumen. *Bojamyces transfuga* shows single appendaged trichospores and zygosporangia of type I, unlike *B. repens*, where appendages and zygosporangia are unknown. The generic limits of *Bojamyces* are emended accordingly to include this new taxon. *Bojamyces repens*, known only from the USA, has been found for the first time in the Iberian Peninsula on mayfly molts. We also note the presence of *Legeriomyces rarus* in Europe, on the Iberian Peninsula and Balearic Islands. Previously it was known only from Western Australia.

Key words: species distribution, Ephemeroptera, exuvia, gut fungi, Legeriomycetaceae, scanning electron microscopy, taxonomy.

INTRODUCTION

The species we report are included in the order Harpellales, which has both branched and unbranched species, separated into two families, Legeriomycetaceae and Harpellaceae, respectively. Legeriomycetaceae develop within the hindgut lining of their larval hosts, mainly Diptera, stoneflies (Plecoptera), mayflies (Ephemeroptera), and very rarely in beetles (Coleoptera), caddisflies (Trichoptera), and Isopoda (Lichtwardt et al 1999, 2001, White 1999). Harpellaceae grow within the peritrophic matrix of Diptera larvae (Lichtwardt et al 2001). Trichospores provide important taxonomic characters, used for the generic classification, including their size and shape, the number of appendages, and their arrangement on fertile branches. Also, zygosporangia characteristics are very important, especially their relative position on zygosporangium, which is the basis to designate the zygosporangium type (Moss et al 1975). The shape of the holdfast that anchors the thallus to the gut lining and the ramification pattern are other interesting features to consider in classification (Lichtwardt et al 2001). The presence of zygosporangia is by far more unusual than the presence of trichospores, and are considered of great significance for the comprehension of generic characteristics.

We have found *B. transfuga* growing and infecting *Caenis luctuosa* Burmeister molts and larvae. Both trichospores and zygosporangia were found, allowing us to study

and consider diagnostic characters that are different enough to describe a new taxon. This *Bojamyces* species was found in molts of *Habroleptoides confusa* Sartori & Jacobs (Ephemeroptera, Leptophlebiidae) with the typical characters of the species and without zygospores.

Longcore (1989) provided a generic diagnosis based on the single species, *B. repens*, but we believe that the new species must be included also in the genus *Bojamyces*, both by morphological and ecological traits. The generic description should be emended to account for characters discovered in the new species.

Legeriomyces rarus Lichtw. in *Caenis luctuosa* has not been reported after its description from Western Australia in an endemic genus of Caenidae, *Tasmanocoenis* (Williams and Lichtwardt 1993). In Spain, this species was found growing together with *B. transfuga*, although only in larvae and not in molts.

MATERIALS AND METHODS

The description of the new species is based on material found in localities from Northeast Spain and Balearic Islands. Collections of ephemeropteran nymphs were made by hand picking from the stream substrate with the help of entomological forceps, and also by dragging aquatic nets between rocks and sand in streams. Nymph's molts were collected between leaves and organic debris on the river shores, and transported in a small cooler to the laboratory. Insect nymphs were dissected to extract and clean the guts under a stereomicroscope using fine-pointed forceps and entomological needles. Molts containing the ectodermic hindgut remains were also examined. Fungi were placed on a slide with water as a mounting medium and photomicrographed using phase contrast and interference contrast optics (DIC). Slides were fixed and stained with lactophenol cotton blue to be preserved in the herbarium (BCB-Mycotheca, at the institutional address of the authors).

Some Caenidae individuals, collected from the locality where the infestation percentage was greatest (Marganell), were placed in an aquarium until molts were obtained. Some of the living larvae were also dissected just before molting.

For SEM, *Bojamyces* material together with the hindgut molt was fixed with 2.5% glutaraldehyde in cacodylate buffer 2M (pH 7). The thalli were washed with the same cacodylate solution, and dehydrated in an ethanol series (15 min in each 10--20--40--60--80 %) to absolute ethanol before the thalli were critical point dried and gold metalized. Samples were examined in a HITACHI S-570.

TAXONOMY

Bojamyces Longcore, *emend.* L.G. Valle & Santam.

Thallus with sparse and indeterminate branching, attached to the host hindgut cuticle. Generative cells scattered on the thallus, interspersed with vegetative cells. Trichospores elongate-ellipsoidal, with or without a single appendage, and with an outflared collar.

Zygospores perpendicularly and medially attached to the zygosporophore [type I (Moss et al 1975)], with a collar. In ephemeropteran nymphs.

Typus generis: *Bojamyces repens* Longcore.

Bojamyces transfuga L.G. Valle & Santam., sp. nov.

FIGS. 1--15

Trichosporae (24--30(--36) x 5--6 μ m, collare 1.5--2.5 x 2--2.5 μ m et appendicem, conspicuam in cellula genituali, ferentes. Trichosporae post liberatas appendicem confertim plicatam praebentes. Zygosporae (27--42(--50) x 8--9 μ m, basaliter inflato zygosporophoro ad perpendiculum et in medio affixae. Zygosporae post liberatas cum plane campaniformi collari 7--7.5 μ m longitudine et filiformi appendice.

Trichospores (24--30(--36) x 5--6 μ m, with a 1.5--2.5 μ m long and 2--2.5 μ m broad collar and a single appendage (FIG. 6), visible inside the generative cell before the trichospore release (FIG. 1, arrows). After trichospore release, the appendage appears compactly folded (FIG. 7, arrow). Trichospores arise from generative cells without a definite direction. Zygosporae (27--42(--50) x 8--9 μ m, perpendicularly and medially attached to the zygosporophore, which is inflated at the lower part (FIGS. 10, 11, arrows). Once released, a 7--7.5 μ m long outflared collar and a single filiform appendage can be observed. Later, typical scalariform conjugation bridges develop (Figs. 2, arrows, and 3, 5), and zygospores grow from the proximities of these bridges, from one of the conjugants (FIGS. 2, and 5, arrows).

Etymology. L., *transfuga* = transgressor, deserter; referring to its habitat, outside the living larval host.

Specimens examined. SPAIN. BARCELONA: Marganell, Riera de Marganell, prepared from *Caenis luctuosa* larvae and molts, 7-V-2001, L. G. Valle, BCB-Tr0729 (HOLOTYPE), BCB-Tr0707 to BCB-Tr0750 (ISOTYPES); same locality, 6-VI-2001, BCB-Tr0830, BCB-Tr0831; Cerdanyola del Vallès, Riera de St. Iscle, prepared from *Caenis luctuosa* larvae and molts, 7-VI-2001, L. G. Valle, BCB-Tr0832--0834.

The genus *Bojamyces* is so peculiar within Harpellales by its thallial morphology, development and also ecology that other features considered of generic importance in most of the Harpellales, such as appendage characteristics, here should be considered as secondary traits or specific features. The new species has been placed in this genus because all its distinctive features (disperse branching, the extremely long hypha irregularly septed in generative cells, disarranged trichospores, etc) are coincident with those of *Bojamyces repens*, the type species from which generic diagnosis was abstracted. Nonetheless, this new species of *Bojamyces* reveals the presence of specific characters not included in the original generic diagnosis (Longcore 1989), specifically the occurrence of the species in a different Ephemeropteran family, trichospore appendages, and also the presence of zygospores.

It is not rare within Harpellales the presence of different species from a same genus living within the gut of different insect genera or even different insect families. Moreover, Longcore (1989) reported the presence of a *Bojamyces*-like fungus in *Siphonurus* sp. (Ephemeroptera, Siphonuridae). Many genera of Harpellales have at least narrow generic host specificity, but some are not so specific, like those found in Dipteran larvae such as *Smittium*, *Stachylina*, and *Simuliomyces*, which include some species found both in Dipteran and Plecopteran nymphs (Lichtwardt et al 2001). *Zygopolaris* appears also in different ephemeropteran families (Lichtwardt and Williams 1984, Moss et al 1975). Therefore, it is not so unusual that a species of *Bojamyces* should be present in Caenidae rather than Leptophlebiidae or Siphonuridae.

Trichospores of *B. transfuga* are smaller and narrower than those of *B. repens*, and show an appendage (FIGS. 6, 7) that can be seen even inside the generative cell (FIG. 1, arrow) and, when just released, it is compactly folded (FIG. 7, arrow). It is not easy to observe the appendage completely unfolded after trichospore release.

The use of SEM has revealed the presence of a net-like skirt, of an unknown substance, covering the lower part of the zygosporophores (FIG. 10, arrow), and also located just at the lower margins of the trichospore collars (FIGS. 13, 14, arrows). This structure may be related with the dispersion and adhesion of the spores until being consumed by the hosts. The surface of the spores does not show any ornamentation (FIG. 9, 10). A similar structure, covering both trichospore surfaces and collars, was observed, also with SEM, in *Smittium culicis* Manier (Valle and Santamaria, 2004, FIGS. 62--63, in press). Images SEM have shown the possible three-dimensional view of *B. transfuga* inside the host, revealing its expansion outside the intestinal lumen to occupy the abdominal cavity of the molt (FIG. 8).

Bojamyces transfuga was found in high densities in Caenidae molt hindgut lumens, and in lesser proportion inside the nymph's hindguts, where thalli were mostly immature. Trichospores and zygosporangia were also found exclusively in molts. We could get thalli of *B. transfuga* from the Caenid larvae molts stored in the aquarium. Nearly 100% of these molts were infected with the gut fungi.

Bojamyces repens Longcore

FIGS. 16--18

This species has been found present in the hindgut molts of *Habroleptoides confusa* Sartori & Jacob, from a small calcareous mountain streams. The material examined matches the original description based on fungi collected in the American *Leptolebia intermedia* Traver (Longcore 1989), with unappendaged trichospores measuring (43--47(--51) x 7--8 μm , and with a collar of 2--3.5 x 2--3 μm (FIG. 18, arrow). The thallus disarticulates in small portions containing each 2--4 generative cells with their respective trichospores (FIGS. 16, 17). No zygosporangia were found.

Specimens examined. SPAIN. TARRAGONA: Ports de Beseit, Torrent del Mas de la Franqueta, prepared from *Habroleptoides confusa* (Leptophlebiidae) larvae molts, 24-V-2001, L.G. Valle, BCB-Tr0770--0771.

Legeriomyces rarus Lichtw. & M.C. Williams

FIGS. 19—28

Species found in the hindgut lining of *Caenis luctuosa* larvae, as well as in hindgut molts, where the thalli do not grow, but show some capacity to persist a period of time before they degrade completely, with only the trichospores remaining. The basal area of the mature thallus is a complex of rhizoid-like hypha, often pluricellular (2--3 cells) and occasionally ramified (FIG. 28). These basal branches are variable in number. In young, and more rarely in mature thalli, a discoid holdfast is observed. The Iberian individuals of this species are identical to those described from Australia (Williams and Lichtwardt 1993). The trichospores of our samples measure 21.5--30 x 7.5--9 μm , without a definite collar, although some deciduous remains (1.5--2 μm long) from the generative cells can be observed as a short-lived collar in some trichospores (FIG. 22, arrow). We have also observed the typical pair of appendages in free trichospores (FIGS. 24, arrow, and 25). We have neither noticed the presence of sexual processes nor zygospores in the peninsular individuals, but zygospores have been reported from nearly all the examined Majorcan specimens, measuring 40--53(--62) x 7--9 μm , with a zygosporophore of 14.5--19 x 3--5 μm (FIGS. 23, 26, arrows). The conjugation tube is a broad structure between the two conjugants, and the zygosporophore arises from the center of these conjugations, which can be present in a variable number between the same two conjugant hyphae. Released zygospores show a collar as long as the respective zygosporophore (FIGS. 23, 26, arrows).

Specimens examined. SPAIN. BARCELONA: Marganell, Riera de Marganell, prepared from *Caenis luctuosa* larvae, 10-IX-2001, L.G. Valle, BCB-Tr1178; 13-I-2002, L.G. Valle, BCB-Tr1201; 6-III-2002, L.G. Valle, BCB-Tr1303--1306, Tr1321, Tr1323. BALEARIC ISLANDS (MALLORCA): Sóller, Barranc de Biniaraix (Cases de L'Ofre), prepared from *Caenis luctuosa* larvae, 27-V-03, L.G. Valle, BCB-Tr1807--1810; Valldemossa, Torrent de Valldemossa, prepared from *Caenis luctuosa* larvae, 30-V-03, L. Sáez & L.G. Valle, BCB-Tr1813--1816.

DISCUSSION

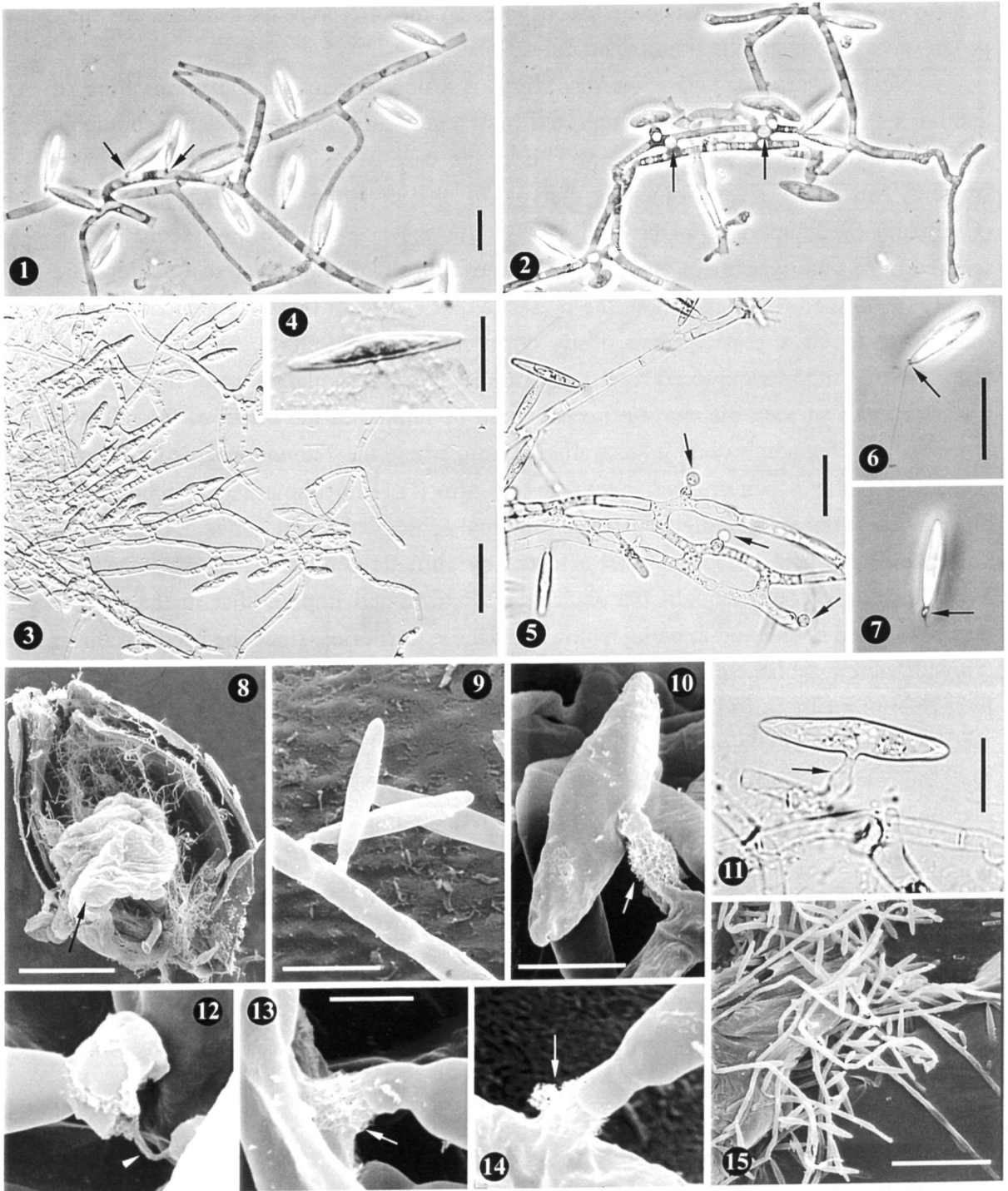
After Longcore (1989) described the genus *Bojamyces* with the single species *B. repens* from nymphs and molts of *Leptophlebia intermedia* Traver (Ephemeroptera, Leptophlebiidae) in the USA, no further occurrence of the taxon has been noticed. Lichtwardt and Williams (1992) reported the occurrence of immature thalli from Tasmanian leptophlebid nymphs, that resemble *B. repens*, but remained unnamed because of unsuitable material to provide an appropriate description (Lichtwardt and Williams 1992). The absence of appendages in trichospores may place this undescribed

fungus near *B. repens*. However, the authors mentioned the absence of thallial disarticulation in their specimens, which is coincident with observations of *B. transfuga* in Spanish samples. To answer the posed question by Lichtwardt and Williams (1992) about the generic or specific condition of the thallial disarticulation character, we consider it a specific attribute, as was already considered by Longcore (1989).

Infested Caenidae molts show long hyphal filaments of *B. transfuga* expanding within the shed exoskeleton (FIG. 8). Longcore (1989) also remarked on the prevalence of *B. repens* in molts, but did not report its expansion towards the outside limits of the internal gut lining. The ecological significance of this phenomenon is not well understood, but it might be that the development of the thalli and spores within the molt might improve fungal dispersion. It has been observed that other species of trichomycetes easily disintegrate (at least hyphal filaments, whereas zygospores and trichospores can persist) when present in insect exuviae, as happens with *Legeriomyces rarus*. It seems to be impossible for these trichomycetes to grow outside the larval body. In the example of *Bojamyces*, trichospores germinate inside the larval gut lumen, but sporulation and hyphal mass is greater in exuviae, where the thallus extrudes from the hindgut molt and extends throughout the lumen of the exoskeleton abdomen. The studied thalli from the hindgut of living host have never been observed to penetrate and trespass the internal gut membrane. Moreover, within larval hindguts, no mature thalli with zygospores have been noticed. After these observations, the only explanation for the presence of great amounts of hypal filaments with spores, outside the hindgut lining, and also between both the old external cuticle exuviae and the hindgut molt of almost all observed host exuviae, is by means of a fungic growth produced after the molt expulsion, when the new free extreme of the hindgut molt, opened towards the external digestive environment, allow the gut fungi to expand its hyphal filaments and produce large amounts of trichospores (FIG 8). Whereas this growth capacity outside the living host is by means of self-fungic reserved energy, or by a major nutritional and ecological plasticity that may allow this new species of *Bojamyces* the capacity to develop outside the living host as a saprophyte, is not clear.

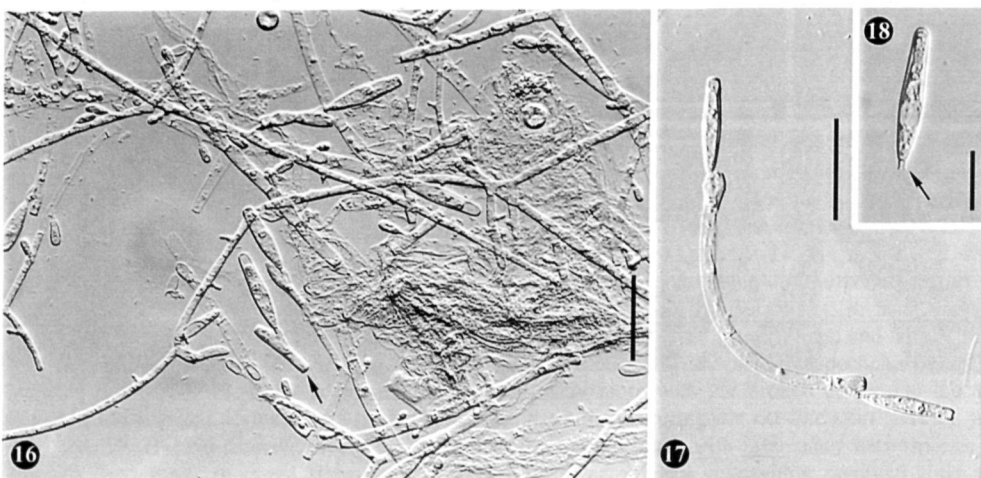
It is also controversial, the impossibility of axenic culturing of this fungi, at least with brain-heart infusion agar (following culture methods of Lichtwardt et al 2001).

→FIGS. 1--15. *Bojamyces transfuga*. 1. Fertile branches with intercalary trichospores showing the appendage sac inside the generative cell (arrows), and free trichospores [BCB-Tr1832]. 2--3. Conjugating thalli with bridges (arrows in FIG. 2), zygospores (immature in FIG. 2, immature and mature in FIG. 3) and few trichospores [BCB-Tr1832]. 4, 11. Mature zygospores of type I and zygosporophore (arrow in FIG. 11) [BCB-Tr0744]. 5. Thalli with young zygospores (arrows) and trichospores [BCB-Tr0745]. 6--7. Free trichospores with a diminutive collar (arrow in FIG. 6), an unfolded appendage in FIG. 6, partially folded in FIG. 7 (arrow) [BCB-Tr1832]. 8. Exuvia showing the abdominal exoskeleton lumen with hyphal filaments expanding throughout the internal area, and the hindgut molt in the center (arrow). 9. Trichospores showing a smooth surface. 10. Zygospore and zygosporophore with a net-like skirt covering it (arrow). 12. Basal segment of a released trichospore with the broken collar and the appendage (arrowhead). 13--14. Basal segment of a trichospore, with the collar carrying-the net-like skirt (arrow), just before being released. 15. Hyphal filaments from the outside of the hindgut molt with intercalary trichospores. All FIGS. photographed from water mounted slides, except FIG. 4 from lactophenol cotton-blue mounted slide, and SEM images. Illumination in FIGS. 3--4 by DIC, FIGS. 1--2, 6--7 by phase contrast, FIGS. 5, 11 by bright field. FIGS. 8--10, 12--15 are SEM. Scale bar: FIGS. 1 (use for FIG. 1 and 2), 4--5, 6 (use for FIGS. 6 and 7) and 11 = 25 µm; FIG. 3 = 50 µm; FIG. 8 = 0.3 mm; FIG. 9 = 14 µm; FIG. 10 = 9 µm; FIG. 13 = 3 µm (use for FIGS. 12, 14)

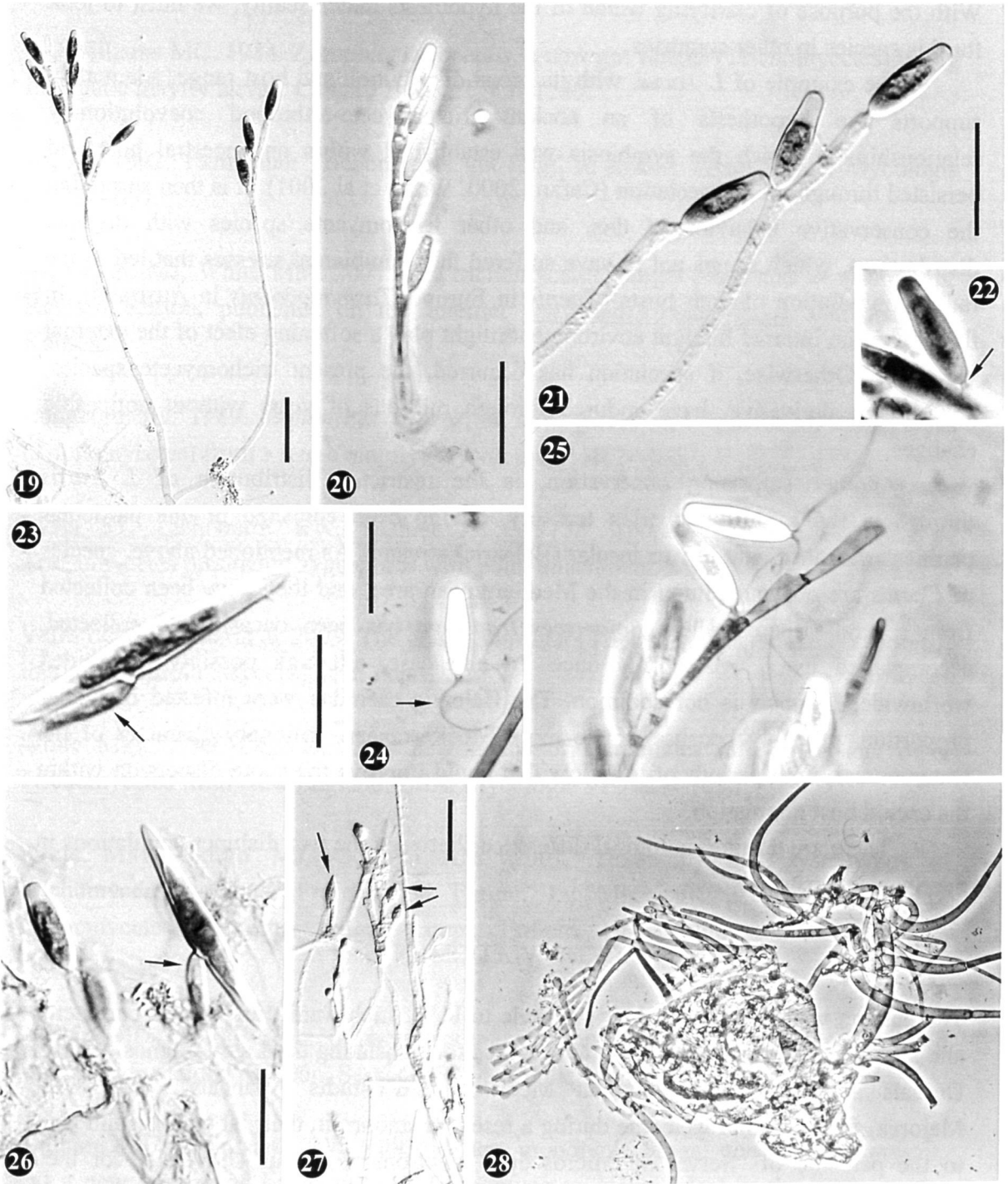


It will be interesting to determine if there are some behavioral patterns in host feeding which match the dispersal ecology of *B. transfuga*. It has been observed that during the molting stages of *Caenis*, large quantities of exuviae accumulate at the river shore, amongst leaves, aquatic herbs or other debris. Because the species was found in a fast-flowing zone, the presence of trichospores in molts represents a means for their progressive propagation in a protected quiet place.

No less surprising than the discovery of a new species, is the observation of another species that has only been reported from a very different and distant geographic territory, and within a different host. This is the case of *L. rarus*, described from a western Australian river, inhabiting larvae of the endemic genus *Tasmanocoenis* (Caenidae) (Williams and Lichtwardt 1993). The absence of any new record of this species from other countries is remarkable, especially in Europe, where Caenidae are widely distributed. In our opinion, the presence of *L. rarus* in Iberian Caenidae may be interpreted to be a consequence of its cosmopolitan distribution, although not yet reported from other unexplored countries. Another possible explanation for the presence of this species in such disjunct territories could be related to the effect of the climatic domain, since the southwestern Australian region, where the *Tasmanocoenis* hosts were collected, has a Mediterranean climate. The effect of the climate on trichomycete distribution is not well studied, since the principal ecological restriction is, logically, the presence of the host, which is most affected by climatic conditions. In any case, this hypothesis only could explain the actual distribution, and implies that in the past *L. rarus* occupied a wider area, being restricted to these territories, since the Mediterranean climates were established relatively late in geologic history, after the continents reached their present position, and after the main groups of animals and plants (mammals, birds, reptiles, insects and angiosperms) had evolved (Stebbins 1989). In this case, these areas play the role of climatic refugia and the species would be relict. With the purpose of clarifying which of the hypothesis match reality, we must to look for this species in other countries.



FIGS. 16—18. *Bojamyces repens* [BCB-Tr0770]. 16. Overview of a thallus with intercalary trichospores and a disarticulated segment with a trichospore (arrow). 17. A disarticulated segment with two trichospores. 18. Released trichospore with the collar (arrow) and no appendage. All FIGS. photographed from lactophenol cotton-blue mounted slides using DIC. Scale bar: FIGS. 16, 17 = 25 μ m; FIG. 18 = 10 μ m.



FIGS. 19--28. *Legeriomyces rarus*. FIGS. 19--21. Fertile terminal branches with trichospores [BCB-Tr1813]. 22. Released trichospore with the short-lived collar (arrow) [BCB-Tr1813]. 23. Released zygospore with collar (arrow) [BCB-Tr1813]. 24. Released trichospore with two fine appendages (arrow) [BCB-Tr1303]. 25. Fertile branch with trichospores attached to generative cells, and a released trichospore with two appendages [BCB-Tr1303]. 26. Zygospore with the zygosporophore (arrow), and a trichospore in focus [BCB-Tr1813]. 27. Young developing zygospores (arrows) [BCB-Tr1809]. 28. Overview of an immature thallus [BCB-Tr1303]. FIGS. 19, 21--23, 26, 27 photographed from lactophenol cotton-blue mounted slides. FIGS. 20, 24, 25, 28, photographed from water mounted slides. Illumination in FIGS. 19, 21, 23, 26--27 by DIC, FIGS. 20, 24--25 using phase contrast, FIGS. 22, 28 using bright field. Scale bar: FIGS. 19--20, 27 (use for FIGS. 27, 28) = 50 μ m; FIG. 21 (use for FIGS. 21--22, 25), 23, 24 and 26 = 25 μ m.

In this case, these areas play the role of climatic refugia and the species would be relict. With the purpose of clarifying which of the hypothesis match reality, we must to look for this species in other countries.

The example of *L. rarus*, with its broad distribution and host range, adequately supports the hypothesis of an ancient trichomycete-arthropod coevolutionary relationship, in which the symbiosis was established within an ancestral host and persisted throughout its speciation (Cafaro 2000, White et al 2001). It is then surprising the conservative behavior of this, and other trichomycete species with disjunct distributions, which seems not to have suffered those ambiental stresses that led to the radiative evolution of their hosts (*Caenis* in Europe, *Tasmanocoenis* in Australia). In this sense, the internal hindgut environment might play a softening effect of the external variations. Otherwise, if speciation has occurred, the present trichomycete species, ancestral or derivative, have endured through millions of years without noticeable changes.

Another surprising observation, is the restricted distribution of *L. rarus* throughout the examined Iberian territory, having been collected in one particular peninsular locality, and in two insular (Balearic) streams. As mentioned above, species of *Caenis* are not uncommon in the Mediterranean area, and they have been collected from several streams where *Bojamyces transfuga* has been occasionally collected, accompanied by *L. rarus* only once. In any case, although possibly distributed worldwide, *L. rarus* is not common. The Balearic caenidae were infested in higher proportion, probably because of the more Mediterranean stationary character of the watercourses, with periods of low flow that could improve the spore dispersion within the caenid host population.

There are no morphological differences between the two disjunct populations in the Spanish territories.

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