

Two new species of *Minutoexcipula* (mitosporic fungi) from Spain

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Abstract: Two new species of *Minutoexcipula* (mitosporic fungi) are described and illustrated, *M. mariana* and *M. calatayudii*. *M. mariana* grows on thalli of *Pertusaria heterochroa* and is known from two collections made in the “Parque Natural Devesa-Albufera” (Valencia, Spain). *Minutoexcipula calatayudii* grows on thalli of *Hypogymnia tubulosa* and it is known only from the type locality (Badajoz, Spain). The separation of this two new species from *Minutoexcipula tuckeræ* and *M. tuerkii* is discussed. A key and a table summarising their differences is also included.

Introduction

Since the introduction of the mitosporic genus *Minutoexcipula* V. Atienza & D. Hawksw. (ATIENZA & HAWKSWORTH 1994) only two species have been described in the genus: *Minutoexcipula tuckeræ* V. Atienza & D. Hawksw. (op. cit.) and *M. tuerkii* Hafellner (HAFELLNER 1994). This paper describes two new *Minutoexcipula* species, *M. mariana* and *M. calatayudii*.

Material and methods

The material was examined by means of standard microscopic techniques. Drawings were made with help of a drawing tube. All measurements were made in water. The most extreme values are given in parenthesis, and the other values give the range after rejecting 10% of the highest and 10% of the lowest values measured. The SEM micrographs were taken on the Hitachi S 4100 field emission from material with a critical point techniques treatment. Terminology follows HAWKSWORTH et al. (1995). The material examined is deposited in VAB-lich.

Additional specimen examined for comparison: *Minutoexcipula tuerkii*
Austria: Kärnten, Nationalpark Nockberge, Klamnack N von Bad Kleinkirchheim, ca. 2250 m, auf *Pertusaria glomerata* (Ach.) Schaer., J. Hafellner 33423 (GZU).

Results

Minutoexcipula mariana V. Atienza sp. nova (Figures 1-14)

Etym.: mariana, after my mother's name María.

Minutoexcipula tuckeræ similis, sed differt praecipue conidiomatibus concavis vel convolutis et conidiophoris simplicibus vel rarissimo ramosis. Fungus in thallis lichenis *Pertusaria heterochroa* vicens. Conidiomata sporodochioidea, superficialia, concava vel convoluta, atrobrunnea, 90-115 (-200) μm diam. Conidiomata basaliter pseudoparenchymatica pallidebrunnea. Conidiophora macronemata, cylindrica, simplicia vel rarissimo ramosa, septata, (10.5-) 11.25-13.75 x (1.25-) 2-3 μm . Stratum conidiophorum excipulo pseudoparenchymatico circumdatum. Cellulae conidiogenae terminales, cylindricae, enteroblasticae, annellatae, pallidebrunneae, 4.5-5-6.25 x 1.5-2 μm . Conidia singularia, fusca, ellipsoidea, basaliter truncata, 1-septata, 6.25-7-7.5 x 2.5-3.75 μm .

Typus: ESPAÑA, Valencia, Dehesa del Saler, Puchol nou, 30SYJ314595, at sea level, on *Pertusaria heterochroa* (Müll. Arg.) Erichsen on *Pinus halepensis*, 25/1/1998, V. Atienza (VAB 12001, holotypus).

Mycelium hyaline, not clearly seen. Conidiomata sporodochia-like, superficial, rounded to irregularly shaped, concave at first but becoming confluent and convoluted with age, arising from the upper cortex of the host lichen; scattered singly, occasionally confluent and not clearly delimited, dark brown to black, granular, 90-115 (-200) μm diam; in section composed of a basal stromatic tissue formed of 2-3 (-4) irregular layers of pseudoparenchymatic cells. Cells isodiametric to irregularly polyhedral, hyaline to pale brown, 2.5-5 μm diam. on the conidioma base but forming an exciple-like structure at the margins, the cells being elongated and having swollen dark brown, more intensely pigmented unevenly thickened tips. Conidiophores arising from the basal pseudoparenchymatous tissue, macronematous, mainly erect, but close to the excipula sometimes slightly flexuous, acroauxic, mainly unbranched although occasionally 2(-3) branched, cylindrical, pale brown, smooth walled, septate, mainly (10.5-) 11.25-13 x (1.25-) 2-3 μm . Conidiogenous cells integrated, terminal, proliferation percurrent and enteroblastic, with 2-4 (-5) annellations, depending on age, subhyaline, smooth, cylindrical, mainly 4.5-6.25 x 1.5-2 μm . Conidia holoblastic arising singly, acrogenous, dry, abundant, the youngest pale brown, smooth, simple, sometimes obpyriform mostly ellipsoid, apex rounded the base truncate, the most mature brown, 1-euseptate, sometimes the upper cell

Figures 1-6: *Minutoexcipula mariana* (VAB 12001).

Figure 1: Sporodochia-like structures on *Pertusaria heterochroa* host thallus.

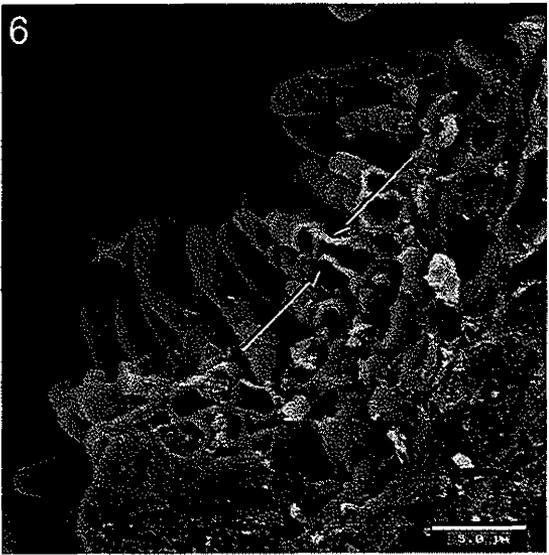
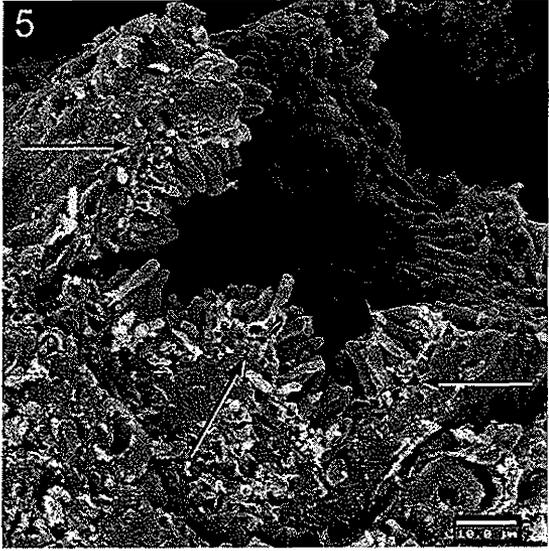
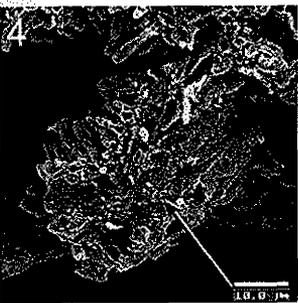
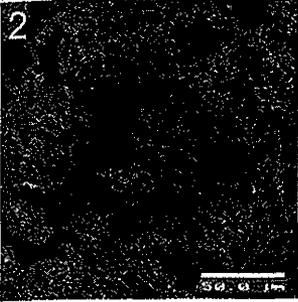
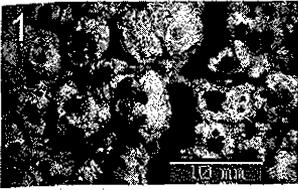
Figure 2: Concave sporodochia-like structure surface view.

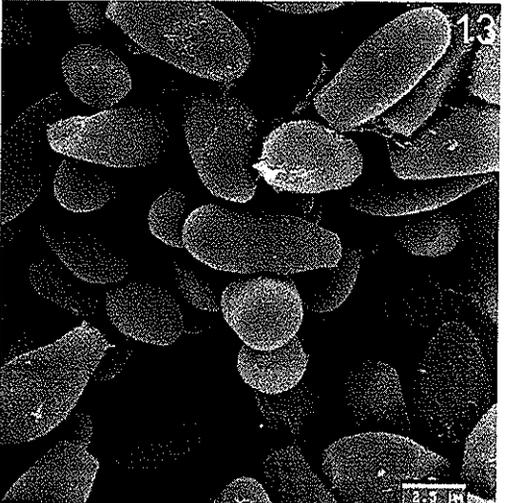
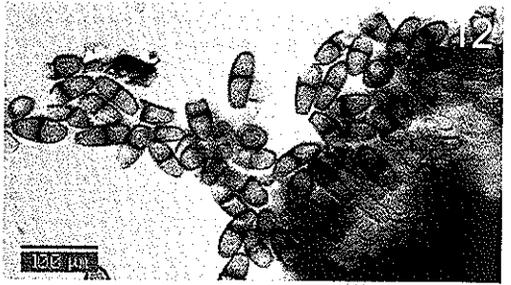
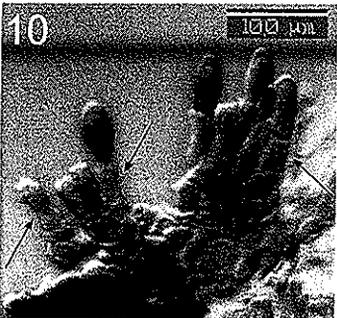
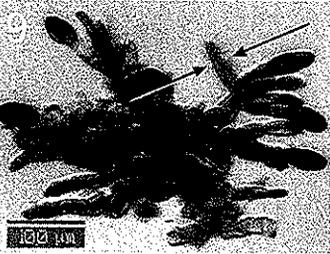
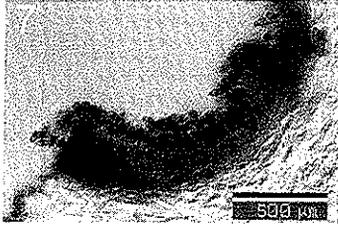
Figure 3: Surface view of the pseudoparenchymatous cells of the exciple-like margin, see swollen cell tips (arrow).

Figure 4: Detail of the exciple-like margin (arrow).

Figure 5: Convoluted sporodochia-like structure, section showing pseudoparenchymatous basal tissue (arrows).

Figure 6: Detail of conidia and conidiogenous cells showing an annellation (arrows).





swollen and the lower attenuated below, septum dark brown, walls darker than content, 6.25-7.75 x 2.5-3.75 μm , lacking a gelatinous sheath.

Ecology and distribution: *Minutoexcipula mariana* is known from two collections infecting the thallus and apothecia verrucae of *Pertusaria heterochroa*. The host thallus is not marked by any discoloration of the thallus surface and in sections the thallus retains its original structure. The fungus appears to be commensalistic rather than pathogenic. The host species is a rather common epiphytic lichen species. It occurs growing on *Pinus halepensis*, *Quercus ilex*, *Q. coccifera* or *Pistacia lentiscus* in open maquis or woodlands (degraded stages of *Quercus ilex* forest) mainly in thermomediterranean dry to subhumid localities lower than 400 m altitude. From the Iberian peninsula it has been reported principally along the east coast. The holotypus locality, the "Parque Natural Devesa-Albufera" (Valencia, Spain), is a special sand place with little dunes situated near the seashore. In this area woodlands (phorophytes, lichen epiphytes and lichenicolous fungi) are directly exposed to the sea water spray. *Minutoexcipula mariana* should be searched for elsewhere within this ecology.

Additional material studied: ESPAÑA, Valencia, Dehesa del Saler, Tancat de la Creu, on *Pertusaria heterochroa* on *Pinus halepensis*, 19/IV/2000, 2 m, S. Fos. (VAB 10731).

Minutoexcipula calatayudii V. Atenza spec nova (Figures 15-22)

Etym.: *calatayudii*, dedicated to Vicent Calatayud, discoverer of the fungus.

Minutoexcipula tuerkii similis, sed differt praecipue conidiomatibus concavis minoribus, absentia conidiophorum et cellulis conidiogenis ampulliformibus latoribus. Fungus in thallis lichenis *Hypogymniae tubulosae* vigens. Conidiomata sporodochioidea, superficialia, concava, atrobrunnea, 75-90 μm diam. Conidiomata basaliter pseudoparenchymatica hyalina. Conidiophora desunt. Cellulae conidiogenae terminales, ampulliformes, enteroblasticae, annellatae, hyalinae, 5.5-6.25 x 3-5 μm . Stratum cellularum conidiogenarum excipulo pseudoparenchymatico circumdatum. Conidia singularia, fusca, ellipsoidea, basaliter truncata, 1-septata, 7.5-8.25-10.6 (-11.25) x (2.5-) 3-3.75-4 μm .

Typus: ESPAÑA, Extremadura, Badajoz, Salvaleón, Lomo Lozano, on *Hypogymnia tubulosa* (Schaer.) Hav., on *Quercus ilex*, 5.IV.2000, V. Calatayud (VAB 12002, holotypus).

Mycelium hyaline, not clearly seen. Conidiomata sporodochia-like, superficial, rounded to irregularly shaped, plane to slightly concave and wart-like,

Figures 7-13: *Minutoexcipula mariana* (VAB 12001).

Figure 7: Vertical section of a concave sporodochia-like structure.

Figure 8: Vertical section of a convoluted sporodochia-like structure.

Figure 9: Conidiophores, conidiogenous cells showing series of annellations (arrows) young pale and mature dark conidia.

Figure 10: as Figure 9. Detail of conidiogenous cells showing series of annellations (arrows) and different stages of conidiogenesis, young pale and mature dark conidia.

Figure 11: as Figure 7. Detail of the exciple-like structure showing the swollen and pigmented cells, conidiogenous cells showing series of annellations and pseudoparenchymatous basal tissue (arrows).

Figure 12: Detail of conidia.

Figure 13. Conidia surface view, showing strongly truncate base.

arising from the upper cortex of the host lichen, scattered, single, rarely confluent, dark brown to black, granular, 75-90 μm diam; in section composed of a basal stromatic tissue formed of 1-2 (-3) irregular layers of pseudoparenchymatic cells. Cells isodiametric to irregularly polyhedral, hyaline to pale brown, 2.5-4.5 μm diam. on the conidioma base, but forming an exciple-like structure at the margins, with elongated cells and swollen dark brown, unevenly thickened, and more intensely pigmented tips. Conidiophores absent. Conidiogenous cells sitting directly on the pseudoparenchymatic cells of the basal layer, proliferation percurrent and enteroblastic, with 1-2 (-3) annellations, depending on age, hyaline, smooth, ampulliform to broadly subcylindrical, mainly 5.5-6.25 x 3-5 μm . Conidia holoblastic arising singly, acrogenous, dry, abundant, the youngest aseptate, pale brown, smooth, sometimes sphaerical but mostly ellipsoid to obpyriform, with the apex rounded and truncate at the base, brown, 1-euseptate when mature, sometimes the upper cell swollen and the lower attenuated below, septum dark brown, walls darker near the septum, (5.5-) 7.5-8.25-10.6 (-11.25) x (2.5-) 3-3.75-4 μm , without a gelatinous sheath.

Ecology and distribution: *Minutoexcipula calatayudii* is only known from the type collection, growing on a thallus of *Hypogymmia tubulosa*. In the host thallus surface, discoloration or spots, in response to the infection were not observed, and in sections the thallus retains its original structure. The fungus appears to be commensalistic rather than pathogenic. *Hypogymmia tubulosa* is a common pioneer and widespread lichen in Spain and also in the rest of Europe. In Spain it grows mainly on bark and less often saxicolous occurring normally in humid sites higher than 400 m altitude.

Key to the species of *Minutoexcipula*

- 1 Conidiophores present 2
- 1a Conidiophores mainly absent 3
- 2 Conidiomata convex, (75-) 100-275 (-300) μm diam.
Conidiophores 2-3-branched, (8-) 12.5-14 (-19.5) x 2.5-3 μm *M. tuckerae*
- 2a Conidiomata concave to convolute, 90-115 μm diam.
Conidiophores mainly simple to 2-branched, (10.5-) 11.25-13 x
1.25-2.5-3 μm *M. mariana*
- 3 Conidiomata plane to convex, 150-300 μm diam. Conidiophores
mainly absent or one-celled, (2.5-) 3.75-4.4 μm . Conidiogenous
cells cylindrical, 5-7(-10) x 2-2.5-3 μm *M. tuerkii*
- 3a Conidiomata plane to concave, 75-90 μm diam.
Conidiophores absent. Conidiogenous cells ampulliform, 5.5-
6.25-10 x 3.1-5 μm *M. calatayudii*

Discussion

A summary of the main distinguishing characteristics of the four species currently known in the genus *Minutoexcipula* is presented in Table 1. For the conidiomata of *Minutoexcipula* the exciple-like margins are particularly

diagnostic, were interpreted as sporodochia-like structures by ATIENZA & HAWKSWORTH (1994).

All species accepted in the genus have in common the formation of sporodochia-like structures with a well differentiated exciple and a basal stromatic tissue, conidiogenous cells with distinct annellations and brown 1-septate conidia. Their differences are discussed below. The sporodochia-like conidiomata are quite characteristic in *M. mariana* since they are concave at first but become confluent to convolute with age (Figures 5, 7, 8). This is unlike the sporodochia-like conidiomata described in the first two species included in the genus: *M. tuckerae* which has convex conidiomata, and *M. tuerkii* which has sporodochia-like conidiomata that are flat at first, becoming convex with age. *M. calatayudii* also has flat sporodochia-like conidiomata at first which become slightly concave with age as in *M. mariana*, but they are never convoluted (Figures 16-18). All four species have a stromatic tissue at the base of the sporodochia-like structures consisting of a few irregular layers of pseudoparenchymatic cells. The stroma cell walls are pale brown except in *M. calatayudii* which are hyaline and formed mainly only by 1 or 2 layers of cells (Figures 17, 18). Regarding the size *M. tuerkii* has the larger sporodochia-like structures which are 150-300 μm whereas *M. calatayudii* has the smaller which are 75-90 μm .

As HAFELLNER (1994) mentioned, in *M. tuerkii* conidiophores are mainly absent, if they exist they are one celled and unbranched. *M. calatayudii* recalls *M. tuerkii* in the absence of conidiophores, since the conidiogenous cells arise

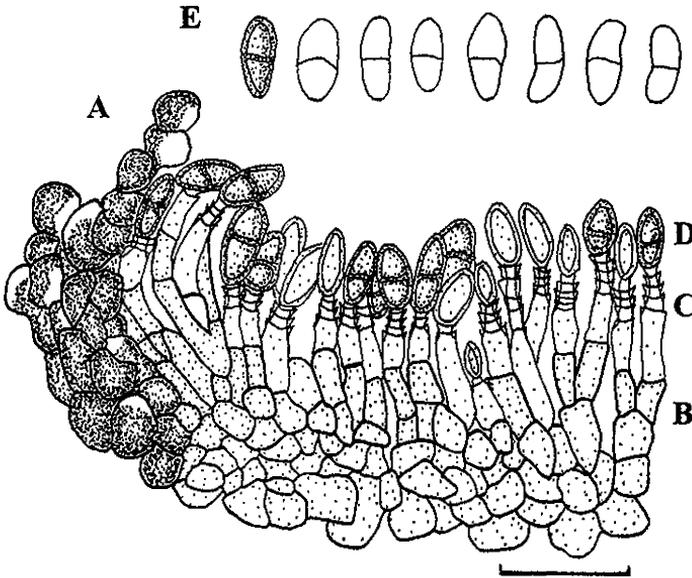


Figure 14: *Minutoexcipula mariana* VAB 12001, vertical section of a conidioma showing A: a detail of the exciple-like structure B: the conidiophores C: the conidiogenous cells showing series of annellations D: young pale and mature dark conidia E: mature conidia and several conidia outlines. Bar = 10 μm .

directly from the pseudoparenchymatous cells of the basal layer (Figures 17, 19, 22B). *M. mariana* has conidiophores that are mainly simple, erect, one or two celled (Figures 9, 10), but 2(-3) branched and slightly flexuous close to the exciple (Figures 11, 14B). In *M. tuckeræ* the conidiophores are clearly septate, formed by 2-4 cells, mainly 2-3 branched and longer, ((8-) 12.5-14 (-19.5) x 2.5-3 µm), than in *M. mariana* which are 10.5-11.25-13 x 1.25-2.5-3 µm, in length.

Table 1: Characters distinguishing the four currently known *Minutoexcipula* species.

	<i>M. tuckeræ</i>	<i>M. mariana</i>	<i>M. tuerkii</i>	<i>M. calatayudii</i>
Conidiomata, Sporodochia-like (µm diam.)	Superficial, convex (75-)100-275 (-300) µm	Concave to convoluted 90-115(-200) µm	Plane to convex 150-300 µm	Plane to slightly concave 75-90 µm
Basal stromatic tissue (µm diam.)	4-5 layers of subhyaline to pale brown cells. Cells 3-4.5 µm	2-3(-4) layers of subhyaline to pale brown cells. Cells 2.5-5µm	3-4 layers of subhyaline to pale brown cells. Cells 3-5 µm	1-2(-3) layers of hyaline cells. Cells 2.5-.5 µm
Conidiophores (µm diam.)	2-3 branched (8-)12.5-14(19.5) x 2.5-3µm	Simple or 2(-3)-branched (10.5-)11.25-13 x 1.25-2-3µm	Mainly absent	Absent
Conidiogenous cells (µm diam.)	Subhyaline 2-3(-5) annellations 4.5-10x2-2.5 µm	Subhyaline 2-4(-5) annellations 4.5-6.25 x 1.5-2 µm	Subhyaline, cylindrical 1-3 annellations 5-7-(10)x2-2.5-3 µm	Hyaline, ampulliform 1-2(-3) annellations 5.5-6.25 x 3-5 µm
Conidia (µm diam.)	Ellipsoidal, brown (5-)6.5-8x(2.5-) 3-4 µm	Ellipsoidal, brown 6.25-7(-7.5)x(2.5-)3.75 µm	Sphaerical to obpyriform, brown 7-9-10 x (3-)3.5-4 µm	Ellipsoid to obpyriform, brown (7.5-)8.25-10.6(-11.25) x (2.5-) 3- 3.75-4.4 µm
Host	<i>Pertusaria texana</i>	<i>Pertusaria heterochroa</i>	<i>Pertusaria glomerata</i> , <i>Pertusaria schizostomella</i>	<i>Hypogymnia tubulosa</i>
Descriptions	Atienza & Hawksworth (1994)	this treatment	Hafellner (1994)	this treatment

Figures 15-21: *Minutoexcipula calatayudii* (VAB 12002).

Figure 15: Sporodochia-like structures on *Hypogymnia tubulosa* host thallus.

Figure 16: Sporodochia-like structure surface view showing a detail of the exciple-like structure showing the swollen and pigmented cells (arrows).

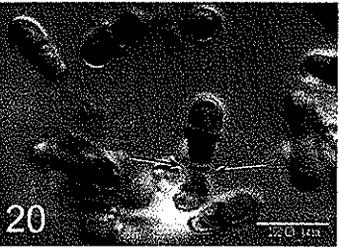
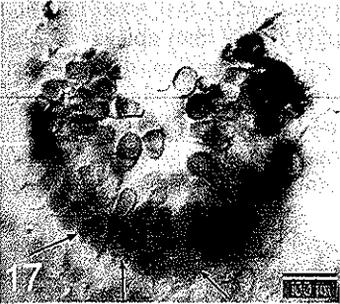
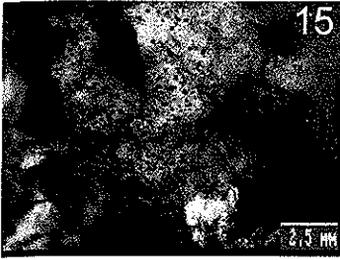
Figure 17: Vertical section of a concave sporodochia-like structure showing a detail of the conidiogenous cells and the pseudoparenchymatous basal tissue (arrows).

Figure 18: as figure 17, detail of the exciple-like margin, conidia, conidiogenous cells and the pseudoparenchymatous basal tissue (arrows).

Figure 19: Conidiogenous cells showing series of annellations and different stages of conidiogenesis, young pale brown (arrow) and mature dark conidia.

Figure 20: Detail of conidia and conidiogenous cell showing an annellation (arrows).

Figure 21: Mature conidia showing strongly truncated base.



In all the species the proliferation of the conidiogenous cells is percurrent and enteroblastic and therefore produces different number of annellations depending on age. The number of annellations are mainly 1-3 in *M. tuerkii*, 2-3 or even 5 in *M. tuckerae* and *M. mariana* (Figures 6, 9, 10, 11, 14C), but only 1 or 2 in *M. calatayudii* (Figures 19, 20, 22B). In the latest the microscopic observation of the annellations proved to be very difficult, even using erythrosin in ammonia solution. The conidiogenous cells are subhyaline and cylindrical except in *M. calatayudii*, which are hyaline and ampulliform (Figures 18, 20, 22B) and wider than in any of the others, 3-5 μm in width. However *M. tuckerae* has the longer ones, being 4.5-10 μm in length.

The conidia in all the species arise singly from the apices of the conidiogenous cells, and become pigmented prior their separation, the youngest are simple, pale brown, and the most mature brown, 1-septate lacking gelatinous sheaths. *M. tuckerae* and *M. mariana* (Figures 6, 12, 13, 14D-E) have ellipsoid, mature conidia similar in size, but a little longer in *M. tuckerae*. *M. tuerkii* and *M. calatayudii* (Figures 20, 21, 22C-D) have obpyriform mature conidia bigger in *M. calatayudii* (Table 1).

Due to the absence of conidiophores other brown-spored lichenicolous mitosporic fungi such as *Lichenocodium*, *Laeviomycetes* or *Lichenodiplis* species can be mistaken for *M. tuerkii* or *M. calatayudii*. However these can be

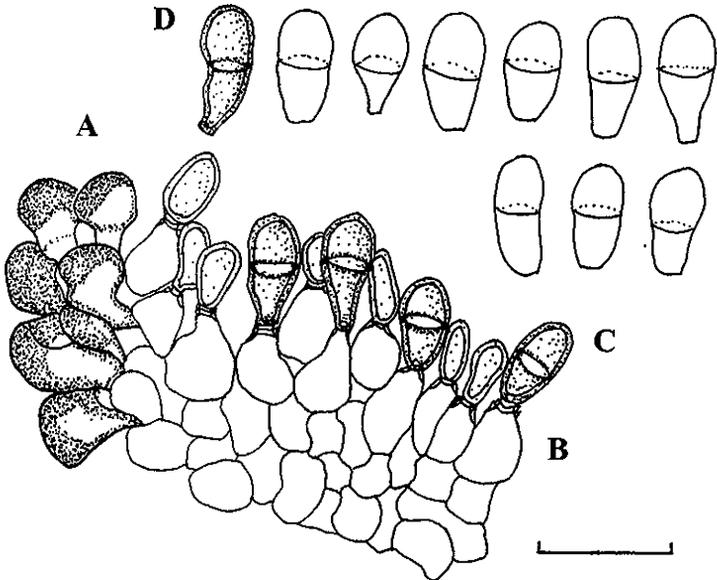


Figure 22: *Minutoexcipula calatayudii* VAB 12002, vertical section of a conidioma showing **A:** a detail of the exciple-like structure **B:** the conidiogenous cells showing series of annellations **C:** young pale and mature dark conidia **D:** mature conidia and several conidia outlines. Bar = 10 μm .

recognized by their pycnidial conidiomata, even though they have not a well-defined ostiole (HAWKSWORTH 1977, HAWKSWORTH & DYKO 1979, HAWKSWORTH 1981) they never form sporodochia-like structures with a very well differentiated exciple. Furthermore conidia of the *Lichenoconium* species are simple, globose and with verruculose ornamentation, and on the other hand *Laeviomycetes* species have simple conidia with a basal frill. *Lichenodiplis* species (*L. lichenicola* Dyko & D. Hawksw. and *L. lecanorae* (Vouaux) Dyko & D. Hawksw.) are the closest to *Minutoexcipula calatayudii* and *M. tuerkii*, but they can be easily separated by the 1-septate, cylindrical conidia and often with a distinct basal frill. *Lichenodiplis lichenicola* grows on *Rinodina* species and has longer conidia (9.5-13 x 4.45 µm), and *L. lecanorae* has shorter conidia (4-7.5 x 2-3 µm), than *Minutoexcipula*. Moreover, they are mainly reported from different hosts than *Pertusaria* or *Hypogymnia*, they are mainly on *Lecanora* and *Caloplaca* species (HAWKSWORTH & DYKO 1979).

Although the genus *Minutoexcipula* was first described as having branched conidiophores two species later included in the genus, *M. tuerkii* and *M. calatayudii* lack these and the conidiogenous cells arise simply from the basal pseudoparenchymatic layer of the sporodochia-like structures. Yet we believe that these taxa are congeneric and that the main feature distinguishing *Minutoexcipula* from other lichenicolous mitosporic fungi is the presence of exciple-like margins of the sporodochia-like structures.

Acknowledgements

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References

- ATIENZA, V. & HAWKSWORTH, D.L. (1994): *Minutoexcipula tuckeriae* gen. et sp. nov. a new lichenicolous deuteromycete on *Pertusaria texana* in the United States. - *Mycological Research* 98: 587-592.
- HAFELLNER, J. (1994): Beiträge zu einem Prodromus der lichenicolen Pilze Österreichs und angrenzender Gebiete. I. Einige neue oder seltene Arten. - *Herzogia* 10: 1-28.
- HAWKSWORTH, D.L. (1977): Taxonomic and biological observations on the genus *Lichenoconium* (Sphaeropsidales). - *Persoonia* 9: 159-198.
- HAWKSWORTH, D.L. (1981): The lichenicolous Coelomycetes. - *Bulletin of the British Museum (Natural History, Botany)* 9: 1-98.

- HAWKSWORTH, D.L., & DYKO, B.J. (1979): *Lichenodiplis* and *Vouauxiomyces*: two new genera of lichenicolous Coelomycetes. - *Lichenologist* 11: 51-61.
- HAWKSWORTH, D.L., KIRK, P., SUTTON, B.C. & PEGLER, D.N. (1995): *Ainsworth & Bisby's Dictionary of the fungi*. 8th edition. Wallingford.