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## Sporal characters in *Gomphales* and their significance for phylogenetics

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Margarita Villegas<sup>1\*</sup>, Joaquín Cifuentes<sup>1</sup> and Arturo Estrada-Torres<sup>2</sup>

<sup>1</sup>Herbario, Facultad de Ciencias, UNAM. A.P. 70-399, Coyoacán 04510 D.F., México

<sup>2</sup>Centro de Investigación en Ciencias Biológicas, Universidad Autónoma de Tlaxcala, San Felipe Ixtacuixtla, Tlaxcala C.P. 90120, México

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Traditionally, sporal characters, such as color, shape and ornamentation, have been important in differentiating the various genera within the *Gomphales*. In some instances, however, no precise analyses have been made that would allow us to build primary homologies between these and other spore features. For this study, the characteristics of the basidiospores of 14 taxa of *Gomphales* were examined, using both photonic and electronic microscopy. These examinations clearly demonstrated that spore ornamentation is a very variable character and data, such as the base shape of the spore and the hilar appendix, previously not considered in the taxonomy of this group, can be very informative at this level.

**Key words:** basidiospores, *Gomphales*, spore ornamentation.

### Introduction

The selection of homologous characters is a fundamental process within any systematic study, although there is a great debate regarding their recognition at different levels of organization (Patterson, 1982; Roth, 1988; Hall, 1994; Brower, 2000; Hawkins, 2000). Phylogenetic homology is the historical link between structures or processes of different organisms that share a common ancestry and is the most relevant concept in systematics and comparative anatomy. Since homology allows us to recognize historical connections between modified structures in organisms, phylogenetic homology is pivotal in the study of patterns of similarity at various levels. Taxonomy based on the formal study of characters as a hypothesis of a homology is the best framework for understanding biological diversity (De Luna and Mishler, 1996).

Homology cannot be assessed directly, but needs to be inferred through several analytical approaches that permit the study of the variation patterns of

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\*Corresponding author: Margarita Villegas; e-mail: mvr@ciencias.unam.mx

discernible features from different viewpoints and using various techniques. Cladistic methods, for example, provide criteria and explicit procedures to determine homologies (Roth, 1988; Bryant, 1989; Mabee, 1989; De Pinna, 1991; Hall, 1994; De Luna and Mishler, 1996; Rieppel and Kearney, 2002).

Spores, like any other fungal structures, contain potential phylogenetic information, and the comparison of characters allows one to arrive at an initial conjecture of homology (primary homology). Despite the fact that systematists have shown the greatest interest in spores when proposing relationships between fungal groups at different taxonomic levels (Pegler and Young, 1971, 1979, 1981; Kühner, 1976), only a few sporal characters have been considered in the descriptions of families comprising the traditional order *Apyllophorales* (Donk, 1964). Nevertheless, a few of these characters have been used for quite a long time.

Donk (1961, 1964) described the *Gomphaceae* for the genera *Beenakia*, *Gomphus*, *Kavinia*, *Lentaria* and *Ramaria*. One of the characters that allowed him to differentiate this group from others within the *Apyllophorales* was the presence of almost invariably ellipsoid, oblong or amygdaliform spores, often ochre-colored and only rarely colorless, not amyloid and with variable ornamentation (rarely smooth), which vigorously absorbed cotton blue. Jülich (1981) proposed that the *Gomphales* order consisted of four families (*Beenakiaceae*, *Gomphaceae*, *Lentariaceae* and *Ramariaceae*), also emphasizing the presence of yellow-ochre, ellipsoid spores. The majority of these spores displays a cyanophilic ornamentation and are rarely smooth. The spore characters of the *Lentariaceae* are particularly different from those of the remaining *Gomphales* members since they vary from being elongated, ellipsoid to cylindrical or sigmoid and are generally hyaline. The spore wall is thin and slightly amyloid in a few taxa, but never dextrinoid or cyanophilic. Nevertheless, other features, such as the hyphal construction of the basidia, suggest a close affinity with *Ramariaceae*, similarities that had been already questioned by Corner (1950, 1970). These findings, in turn, led some authors, such as Petersen (1988) and Estrada-Torres (1994), to reconsider *Gomphaceae sensu* Donk (1961) and to propose the exclusion of *Lentaria* from this group.

Maas Geesteranus (1963) reported that the spores of *Beenakia dacostae* (*Beenakiaceae*) are very similar to those of *Ramaria* (*Ramariaceae*) in terms of their color, ornamentation and form of the hilar appendix. Similarly, Jülich and Star (1983), after considering the ultrastructure of the basidiospores of *B. dacostae*, concluded that it was very similar to others in the *Gomphales*, such as *Gomphus* and *Ramaria*.

Petersen (1967, 1971a, 1981) and Petersen and Pearman (1973) demonstrated the existence of a wide margin of spore ornamentation within

*Ramaria* and *Gomphus*, and discussed the similarity of the spores between these two genera.

The order *Gomphales* was also recognized by Hawksworth *et al.* (1995), with modifications for the *Ramariaceae* (including also *Delentaria*, *Kavinia* and *Ramaricium*), leaving *Lentaria* as the sole member of the *Lentariaceae* family. Hawksworth *et al.* (1995) included *Beenakia* as the only member of the *Beenakiaceae*, and transferred it to the *Boletales*. He may have arrived at this idea because of the similarities between *Beenakia* and some *Boletales* in terms of the color and shape of their spores, similarities that are also found between *Austroboletus*, *Strobilomyces* and several members of *Gomphaceae sensu* Donk (Petersen, 1971a; Pegler and Young, 1981). The genera *Pseudogomphus* and *Terenodon* (described later) are also included in the *Gomphaceae* (Heim, 1970; Maas Geesteranus, 1971). Recent systematic investigations (Hibbett *et al.*, 1997; Pine *et al.*, 1999; Villegas *et al.*, 1999; Hibbett and Thorn, 2001) have shown some agreements between the Jülich (1981), Hawksworth *et al.* (1995) and Estrada-Torres (1994) classifications.

More precise definitions of certain features, such as spore ornamentation and the shapes of the hilar appendix, have not yet been made. Studies that can focus on these features with detail are of great value in understanding their homology (Patterson, 1982; Roth, 1988; Rieppel and Kearney, 2002). Furthermore, the comparative description of different types of ornamentation, shapes of the hilar appendix and macrochemical reactions could reveal new characters, which may be potentially useful at different taxonomic levels.

Taking into account the various hypotheses regarding the relationships within *Gomphales* drawn partially from spore morphology, this study examined spore structures of representative taxa of *Beenakia*, *Gloeocantharellus*, *Gomphus*, *Kavinia*, *Lentaria*, *Ramaria* and *Ramaricium*. Data on spore morphology available in the literature for this group were also considered. Based on these observations, hypotheses of primary homology are proposed.

## Materials and methods

The study looked at around 150 samples of representative taxa of the genera *Beenakia*, *Gloeocantharellus*, *Gomphus*, *Kavinia*, *Lentaria*, *Ramaria* and *Ramaricium*. Thirty of these samples were further selected for microscope scanning, based on the availability of materials and the way of preservation (see list in Table 1). The genera *Delentaria*, *Pseudogomphus* and *Terenodon*, which are monospecific, could not be observed due to the lack of available material. A total of fourteen species, corresponding to ten genera, were

**Table 1.** List of representative specimens examined in *Gomphaceae sensu lato*.

<b>Taxon</b>	<b>Specimens examined with SEM</b>
<i>Beenakia dacostae</i>	E. Horak 68/448 (L)
<i>Gloeocantharellus purpurascens</i>	F. Tapia 209 (XAL); E. Horak 13977 (TENN)
<i>Gomphus clavatus</i>	G. Guzmán 6845 (ENCB); A.H. Smith s.n. (TENN)
<i>Gomphus floccosus</i>	A. Estrada-Torres 3539 (TLXM); F.R. Bralish 1015 (TENN)
<i>Kavinia alboviridis</i>	A. Estrada-Torres 3058 (TLXM); H.S. Khara 4070 (TENN)
<i>Lentaria surculus</i>	R.E. Halling 5472 (TENN); R.H Petersen 597 (TENN)
<i>Ramaria abietina</i>	R.H. Petersen 2961 (TENN); L. Hernández Díaz 34 (TLXM)
<i>Ramaria cf. botrytis</i>	López & Villegas R.M. 575 (FCME); R. Lamothe & E Pérez-Silva 70 (MEXU)
<i>Ramaria cf. fennica</i>	Villegas R.M. 364 (FCME); S. López & Villegas R.M. 437 (FCME); Gavito y Santiago 21 (FCME)
<i>Ramaria gracilis</i>	Villegas R.M.1353 (FCME); W.B. Cooke 5000 (TENN)
<i>Ramaria grandis</i>	R.H. Petersen s.n. (TENN); Villegas R.M. 1691 (FCME)
<i>Ramaria rubripermanens</i>	Scates & Petersen 45911 (TENN); A. Estrada-Torres 3244 (TLXM); A. Kong Luz 2594 (TLXM)
<i>Ramaria stricta</i>	Smith & R.H.P. 31891 (TENN); L. Guzmán-Dávalos 3595 (IBUG); E.Pérez, R. Hernández y E. Aguirre (MEXU)
<i>Ramaricium polyporoideum</i>	E.D. Farr & J. Ginns 9732 (NFC); E.D. Farr & J. Ginns 9733 (NFC)

Herbaria: FCME = Facultad de Ciencias, UNAM, México; TENN = University of Tennessee, Knoxville, EUA; ENCB = Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional; TLXM = Centro de Investigación en Ciencias Biológicas, Universidad Autónoma de Tlaxcala; IBUG = Instituto de Botánica, Universidad de Guadalajara; XAL = Instituto de Ecología, A.C., Xalapa, México; MEXU = Instituto de Biología, UNAM, México; NFC = National Fungus Collection, New York.

investigated (Table 1). These samples are located in the ENCB, FCME, IBUG, L, MEXU, NFC, TENN, TLXM and XAL herbaria.

Techniques proposed by Kotlaba and Pouzar (1964), Marr and Stuntz (1973) and Petersen (1967) were followed for cyanophily observation. Samples placed under a scanning microscope were observed by desiccation of the critical point according to the modified techniques proposed by Espinoza (pers. comm.), using a critical point desiccator BAL-TEC CPD030. The scanning microscope observations (SEM) were made at the Facultad de Ciencias, UNAM, using a JEOL JSM-5310LV electron microscope with an integrated using a magnification range of 5000 to 10000.

Historically, it has not been an easy task to describe spore ornamentation with any accuracy. Therefore, a large number of descriptive terms for spore ornamentation have been introduced, but with little consensus among them. In this study, an attempt was made to employ terminology used in glossaries and dictionaries (Largent *et al.*, 1977; Vellinga, 1988; Hawksworth *et al.*, 1995; Kirk *et al.*, 2001). Images in figures may, however, convey differences

between one type of ornamentation and another with greater clarity. The terminology proposed by Hawksworth *et al.* (1995), Largent *et al.* (1977), Pegler and Young (1971) and Vellinga (1988) was used for the descriptions.

There is no way to ensure that a character possesses phylogenetic information before the similarity patterns between taxa have been analyzed. Therefore, in this study, the characters were evaluated taking into account empirical criteria of similarity, discrete variation between taxa, conjunction, inheritability and independence (De Pinna, 1991; De Luna and Mishler, 1996).

## Results

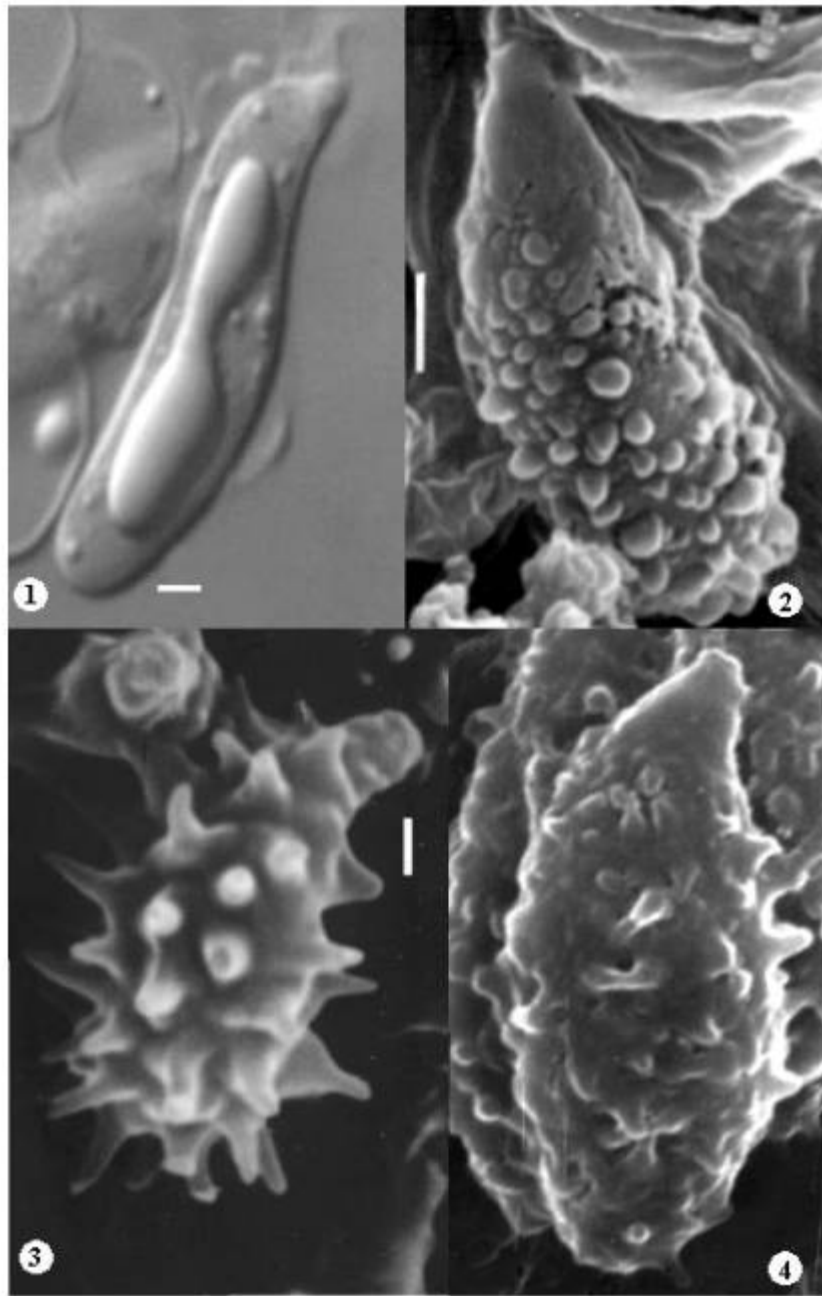
The following are the results obtained in our review of the qualitative characters of spores:

### *Spore color*

Color is one of the characters that, because of its nature, tends to be subjective. Nevertheless, in many basidiomycetes and in our particular case, this feature can be distinguished more clearly in a spore print, which is not always easily obtained. *Gomphales* spores have been described as yellow to ochraceous (Estrada-Torres, 1994), ochraceous-brown, sometimes but rarely straw colored with shades of olive green, cream colored, or colorless (Donk, 1961, 1964; Jülich, 1981). It is evident that color can vary even within the same genus. In *Ramaria*, for example, spores of the subgenus *Echinoramaria* have darker shades than those of *Lentoramaria*.

Under certain circumstances, for example, depending on the features of the sporome and/or environmental conditions, the color of the spore may become either lighter or darker than normal (Largent *et al.*, 1977), rendering color comparison difficult. Since very few spore prints of specimens could be obtained during our study, direct observations were made using a photonic microscope. Despite the use of field guides, descriptions were problematic. Even though the spores examined displayed a gradient of hues, it was evident that the color fluctuated between yellow and brown. This is the case for taxa such as *Beenakia dacostae*, *Gloeocantharellus purpurascens*, *Gomphus clavatus*, *G. floccosus*, *Kavinia alboviridis*, *Ramaria abietina*, *R. cf. botrytis*, *R. cf. fennica*, *R. gracilis*, *R. grandis*, *R. stricta* and *Ramaricium polyporoideum*.

The only samples to have hyaline spores (observed with a microscope at a magnification of 125×) were *Lentaria surculus*.



**Figs. 1-4.** Characteristics of spores. **1.** Nomarski interference contrast of smooth spore and hilar appendix obtuse in *Lentaria surculus*. Scanning electron micrograph (SEM) of: **2.** *Ramaria abietina* with spore tuberculate-nodulose and hilar appendix confluent. **3.** Spore echinulate of *Ramaria grandis*. **4.** Spores echinulate and hilar appendix acuminate in *Kavinia alboviridis*. Bars = 1 µm.

### ***Spore shape***

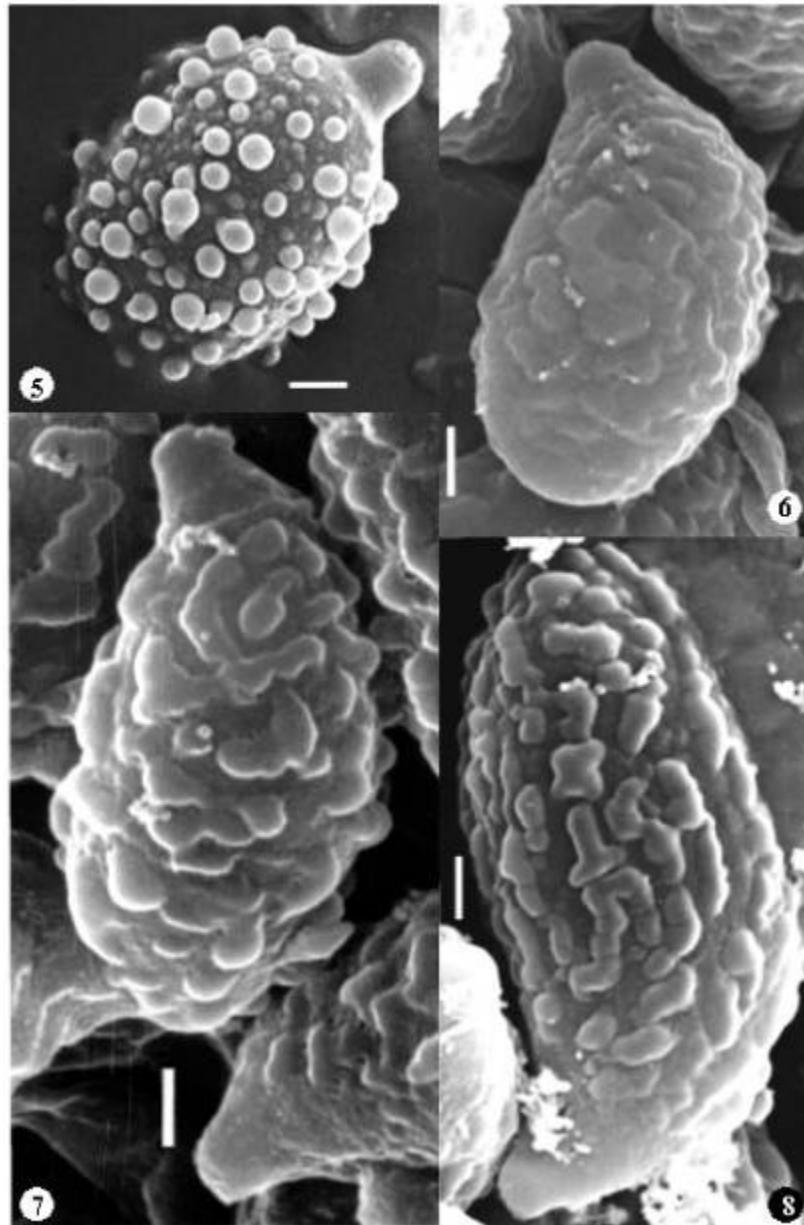
With regard to their symmetry, nearly all taxa observed possessed equilateral spores, with the exception of *Lentaria surculus* (Fig. 1). If the entire spore body is taken into account, the range of spore shapes varied from subglobose to ellipsoid, or more elongated with various gradations in *Ramaria abietina* (Fig. 2), *R. cf. botrytis*, *R. grandis* (Fig. 3), *Kavinia alboviridis* (Fig. 4), *Ramaricium polyporoideum* (Fig. 5), *R. gracilis* (Fig. 6), *R. cf. fennica* (Fig. 7), *R. rubripermanens* (Fig. 11), *R. stricta*, *Gomphus clavatus* (Fig. 8), *G. floccosus*, *Gloeocantharellus purpurascens* (Fig. 9), *Beenakia dacostae* (Fig. 10), and to sigmoid and almost cylindrical in *Lentaria surculus* (Fig. 1).

### ***Hilar appendix***

The hilar appendix, which is the protuberance that connects the spore to the sterigma of the basidium, may take a variety of shapes at the base of the spore. According to the terminology proposed by Pegler and Young (1971), these shapes may be: (i) obtuse, in which the base of the spore is broadly round and the hilar appendix is completely different from the rest of the spore; (ii) acuminate, with the base of the spore distinctively attenuated towards the hilar appendix to form a basal prolongation, although the tip is still distinguishable from the rest of the spore, and (iii) confluent, in which the base of the spore gradually becomes thinner. Here, the continuity between the spore and its hilar appendix is more evident and it is very difficult to distinguish between the base of the spore and the hilar appendix. (In this case, the term clavate is also used: elongated spores with a tapering base). With *Lentaria surculus* and *Ramaricium polyporoideum*, the hilar appendix was observed to be obtuse (Figs. 1 and 5), whereas *Beenakia dacostae*, *Gloeocantharellus purpurascens*, *Gomphus clavatus*, *G. floccosus*, *Kavinia alboviridis*, *Ramaria cf. botrytis*, *R. cf. fennica*, *R. gracilis*, *R. rubripermanens* and *R. stricta* had a hilar appendix with an acuminate base (Figs. 4, 6-11). Only *Ramaria abietina* and *R. grandis* exhibited a hilar appendix with a confluent base (Figs. 2 and 3).

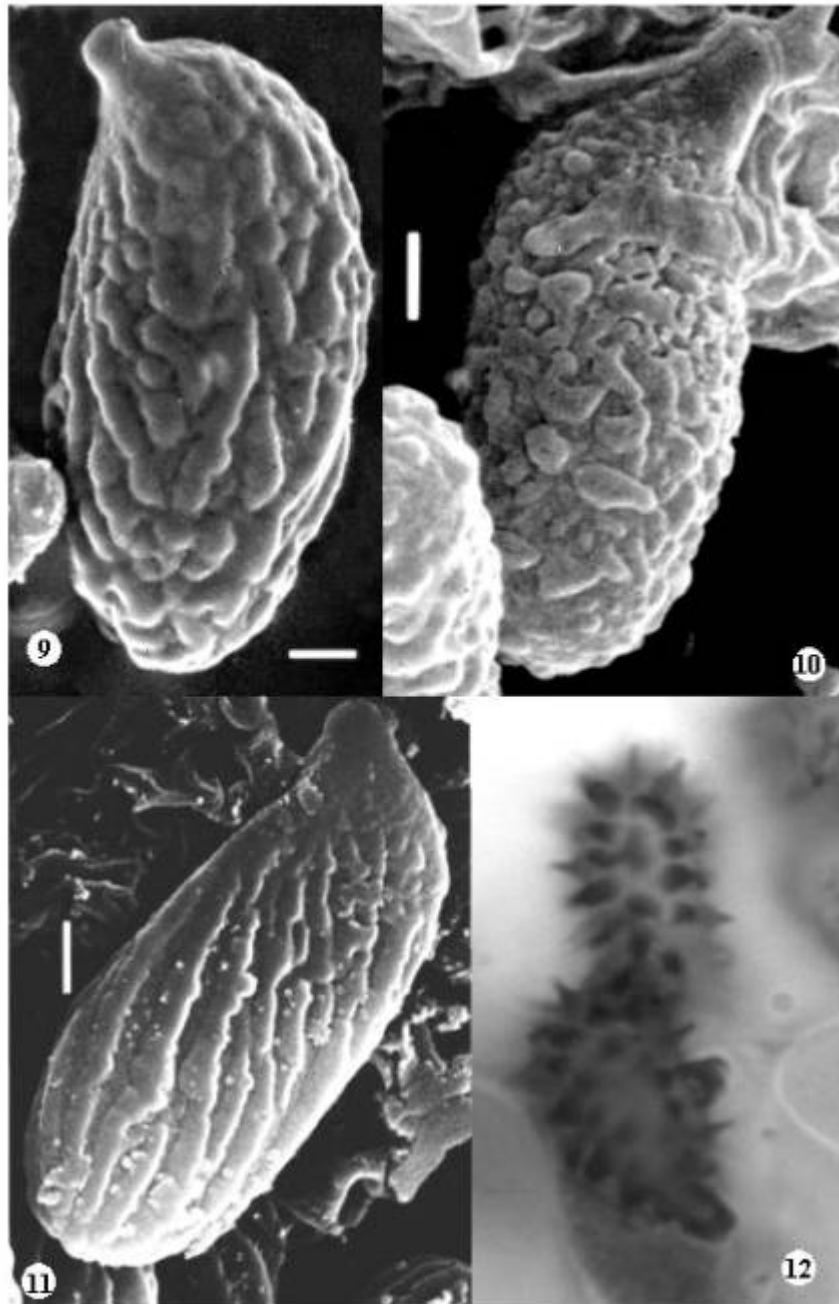
### ***Spore ornamentation***

A great amount of terminology has been introduced to describe spore ornamentation (Largent *et al.*, 1977; Petersen, 1988; Vellinga, 1988; Hawksworth *et al.*, 1995). After making careful comparisons and noting which terms agreed with one another, we arrived at the resulting descriptions:



**Figs. 5-8.** SEMs of spores. **5.** *Ramaricium polyporoideum*: tuberculate-nodulose spore and hilar appendix obtuse. **6.** *Ramaria gracilis*: verrucose ornamentation and hilar appendix acuminate. **7.** *Ramaria cf. fennica*: hilar appendix acuminate and verrucose spore with shorter and varied unions. **8.** *Gomphus clavatus*: verrucose spore. Bars = 1 µm.





**Figs. 9-12.** SEMs of spores in: **9.** *Gloeocantharellus purpurascens* and **10.** *Beenakia dacostae* with verrucose ornamentation and hilar appendix acuminate. **11.** *Ramaria rubripermanens*: striate spore. **12.** Nomarski interference contrast of cyanophilic ornamentation in *Ramaria grandis*. Bars = 1 µm.

### **Smooth**

The spore has no ornamentation. Only *Lentaria surculus* (Fig. 1) showed this feature.

### **Echinulate**

The spore surface is covered with broad-based spines, which can be subacute or blunt. The spines may be connected (connate) at their basal portion in the form of reliefs or isthmuses in such a way that these ornamentations are seen as extensions at the spore surface: they do not have an apparent origin at different layers of the wall. This type of ornamentation can be seen in *Kavinia alboviridis* and *Ramaria grandis* (Figs. 4 and 3).

### **Tuberculate-nodulose**

The spore surface has prominent excrescences, such as individual warts, more or less equidistant from, or rarely connected to one another. This type of ornamentation was observed only in *Ramaria abietina* and *Ramaricium polyporoideum* (Figs. 2 and 5).

### **Verrucose**

The spore surface is covered by obvious warts or round lobes. These ornamentations can be connected to each other in different degrees. In taxa such as *Gloeocantharellus purpurascens* (Fig. 9) and *Gomphus floccosus*, the connection is quite extensive, forming waves or irregular 'cords', although not forming striae. In *Beenakia dacostae*, *Gomphus clavatus*, *Ramaria* cf. *fennica*, *R. gracilis* and *R. stricta*, on the other hand, the warts tend to have shorter and varied connections (Figs. 10, 8, 7 and 6). They can also be isolated. Species such as *R. gracilis* and *R. stricta* exhibit smaller and more separate warts in comparison to the other taxa considered in this group.

### **Striate**

The spore ornamentation is organized in shallow lines or grooves, which apparently arise from the external layer(s) of the wall, following a divergent spiral pattern that runs from the abaxial to the adaxial part of the spore. A few round warts can be seen in the frontal portion, especially in immature spores. This suggests that the ornamentation might be formed from the union of these warts or, more likely, we are dealing with two different ornamental elements. This is a characteristic of *Ramaria* cf. *botrytis* and *R. rubripermanens* (Fig. 11). When considering the fundamental principles for the selection of primary homologies, the collective presence of two discrete states of a character in the same organism (isolated warts and continuous striate) allows us to infer that

there are two independent characters instead of just one (De Pinna, 1991; De Luna and Mishler, 1996; Hawkins *et al.*, 1997). It is considered, therefore, that the striate condition is independent from the verrucose one.

In *Kavinia alboviridis*, for example, even though the spore ornamentation is mainly characterized by sharp projections, it also tends to present round tips, similar to warts. In *Ramaria abietina*, some nodules or tubercles tend to become slightly slender towards the tip. It is evident that these elements involve variations at another level. For this reason, these patterns were considered as more evident modalities at a generic level.

### ***Cyanophilic reaction***

According to Kotlaba and Pouzar (1964), Petersen (1967) and Largent *et al.* (1977), a spore is cyanophilic when its wall absorbs a great concentration of cotton blue, acquiring a more intense color than the cytoplasm. When the wall or the ornamentation stands in distinct contrast to the internal portion of the spore, the former are called cyanophilic.

With the exception of *Lentaria surculus*, all the remaining taxa studied in this work are cyanophilic. Nevertheless, some differences do exist in the site of reaction. Spores of *Beenakia dacostae*, *Gloeocantharellus purpurascens*, *Gomphus clavatus*, *G. floccosus*, *Kavinia alboviridis*, *Ramaria abietina*, *R. cf. botrytis*, *R. cf. fennica*, *R. gracilis*, *R. grandis*, *R. rubripermanens*, *R. stricta* and *Ramaricium polyporoideum* absorb the dye rapidly but the reaction takes place only in the ornamentation; the wall does not absorb the dye (Fig. 12). The extent of the cyanophilic reaction varies from species to species depending on the ornamentations dimension and density. The reaction is very evident in species such as *Beenakia dacostae*, *Gomphus clavatus*, *G. floccosus*, *Kavinia alboviridis*, *Ramaria abietina*, *R. cf. fennica* and *R. grandis*, among others, and less evident in *R. gracilis* and *R. stricta*. In *Ramaria cf. botrytis* and *R. rubripermanens*, the cyanophilic reaction follows the pattern of the ‘grooves’ more than the crests.

In general, it can be said that the taxa showing a conspicuous ornamentation under the light microscope clearly reacted strongly to cotton blue, whereas those with smooth spores or inconspicuous ornamentation had a less noticeable reaction.

### **Discussion**

Spore characters are widely used in classification across a range of basidiomycetes (e.g. Desjardin *et al.*, 2003; Das *et al.*, 2004). In this study we

examined the stability of spore characters in the *Glomerales*, but the results may apply across a broad range of fungi.

### ***Spore color***

The results obtained in this study show that, although color was described in a qualitative manner, it could vary considerably according to the species and their developmental stages. Methodological problems have not allowed us to consider this variation in descriptive or quantifiable terms.

### ***Spore shape***

If we consider spore shape to be a qualitative feature, it is difficult to represent the great variation in general shapes as discrete character states. The transformation of spore shapes into quantitative characters in morphometric studies may enable us to use them at the desired level explained above. Nevertheless, using characters such as symmetry and the shape of the spore base, it is interesting to note that, according to Domínguez de Toledo (1994) and Largent *et al.* (1977), it is possible to identify taxa such as *Lentaria surculus*, which present a clearly un-equilateral symmetry, while others consistently exhibit a more regular (equilateral) symmetry.

### ***Hilar appendix***

It is evident that the hilar appendix has inherent variations in its shape; however, the only available data obtained in this study refers to external morphology and position in relation to the rest of the spore. In general, we know that the hilar appendix is always present at the base of the basidiospores. But, unlike with spore ornamentation, it is not known with certainty if this structure is made up of only one or several wall layers. The consensus at the moment is that it is probably made up of the basic structure of spore layers, since a thinner wall is generally observed (Keller, 1992, 1997). Despite this, at present, it is difficult to distinguish if a hilar appendix with an obtuse base is ultrastructurally and ontogenetically different from one that is acuminate or confluent. There is even the possibility that this feature is independent from the structure. The current evidence allows us to acknowledge only that there is a positional similarity with different base shapes. The base shape of the spore and the hilar appendix seen as a hypothesis of 'primary homology' (De Pinna, 1991) might suggest that they vary altogether (round base with obtuse hilar appendix and a pointed base with an acuminate hilar appendix). Nevertheless,

the conjunction of the spore base, the sharp tip and a confluent hilar appendix in *Ramaria abietina* and *R. grandis* leads us to suggest that they are non-homologous features, implying at least two events of change or, in other words, two independent characters.

### ***Spore ornamentation***

It is evident that ornamentation exhibits great variation. Despite the existence of different patterns, these may be present without distinction in some genera such as *Ramaria*. Indeed, it is within the *Gomphales* that the greatest diversity of spore ornamentation can be seen: from the verrucose to echinulate.

Petersen (1981) classified spore ornamentation in *Ramaria* subgenus *Echinoramaria* into five different types: (i) with long and sharp spines, (ii) a mixture of long, sharp spines and shorter elements, (iii) with telephoroid ornamentation, (iv) with truncate ('volcanic') spines, and (v) with rounded warts. If this classification is examined in detail, comparing the images of our results with those presented by Petersen (1981), it becomes evident that types 1 to 4 correspond to what we consider as tall ornamentations with projections at the tip, but with variations in dimension or shapes of the tips (volcanic or telephoroid).

On the other hand, it is evident that, in the case of *Ramaria abietina* (Fig. 2), as is generally assumed in the descriptions arrived at using a light microscope, the ornamentation is in the form of round warts, not sharp spines, a feature which Petersen (1981) also revealed in *Ramaria myceliosa*. Both of these *Ramaria* are classified in the *Flaccidae* section of the subgenus *Echinoramaria*. Petersen (1981) also speculated on the possibility that the sections *Flaccidae* and *Dendrocladium* have very little in common, considering, among other aspects, spore ornamentation. Although the cladistic hypotheses currently proposed for the *Gomphales* (Villegas *et al.*, 1999; Humpert *et al.*, 2001) suggest *Ramaria* to be polyphyletic, there has been no additional study to clarify the generic classification in this group. It is important to point out that Villegas *et al.* (1999) showed that the two species of *Echinoramaria* (*R. abietina* of section *Flaccidae* and *R. grandis* of section *Dendrocladium*) are not grouped using the criteria presented in this study.

A strong resemblance in spore ornamentation has been observed for various species of *Gomphus* and *Ramaria* (Corner, 1950; Eriksson, 1954; Petersen, 1971a). It is important to point out that this similarity in spore ornamentation is indeed surprising. Up to now, it has not been suggested that this similarity corresponds to any discrete pattern or definitive grouping, but

has been observed without distinction in several species traditionally classified in different sub-generic levels. Examples include *G. clavatus* and *R. cf. fennica* (Petersen, 1971a, 1981, 1988; Pegler and Young, 1981), *Gomphus guadelupensis* and *G. retisporus* and species of subgenus *Echinoramaria* such as *R. cristatospora* and *R. pancaribbea*, among others. We can also see similarities between species such as *Gloeocantharellus purpurascens* and *Gomphus floccosus*, or between *Gomphus clavatus* and *Ramaria flava*, *R. flavobrunescens*, and *R. fumigata* (Corner, 1966; Petersen, 1967, 1971a; Keller, 1997). For these latter works, hypotheses of intergeneric relationships have been based on these features.

Ornamentation has been considered equally important in the differentiation of the subgenera within *Ramaria* (Corner, 1970; Marr and Stuntz, 1973; Petersen, 1988). Researchers using a light microscope described the spores of the subgenus *Lentoramaria* as having a 'lighter' ornamentation than those of subgenus *Laeticolora* (Petersen, 1975). The results of this investigation show that there is no clear distinction that consistently separates these subgenera because the form and position of the ornamentation are very similar. Moreover, we must bear in mind that the spore dimensions in the subgenus *Lentoramaria* are smaller than those of the subgenus *Laeticolora*. In addition, this group has less conspicuous ornamentation, as pointed out by Keller (1997) in his work with *Ramaria flavosalmonicolor*. For the subgenus *Ramaria*, on the other hand, the spores have a homogeneous ornamentation pattern, with a conspicuous longitudinal arrangement of parallel striae, totally or slightly discontinuous, with occasional isolated warts.

Corner (1950) also suggested a possible relationship between *Lentaria* and some taxa of the *Ramaria gracilis* group, considering, among other aspects, the scarce spore ornamentation in the latter as an indication. As is shown in Figs. 1 and 6, combined with descriptions by Petersen (1967, 1968, 1971b, 1975), for instance, the spores in *Lentaria* are smooth, whereas the species of the group of *Ramaria gracilis* present ornamented spores.

The same ornamentation pattern found in our investigation for *Beenakia dacostae* is also found in the study of Parmasto and Ryvarden (1990) on *Beenakia fuliginosa* and *B. informis*. Therefore, it is likely that is a group with spores of homogeneous ornamentation.

In recent decades, spore wall construction has received a great deal of attention (Keller, 1997). Studies carried out on this and other groups of hymenomycetes have shown that spores may have a variable number of layers in the walls (between three and five) and that the ornamentation may originate from different strata (Clémenton, 1997; Keller, 1997). The results obtained by Jülich and Star (1983) show that the spore wall in *Beenakia dacostae* is

identical to the structure known for the species of *Gomphus* and *Ramaria* studied by Perreau (1967), where it became evident that the ornamentation arises from the outermost layer of the wall, whose width is greater than the inner layers. Similar characteristics are shown by Vázquez *et al.* (1995) for some species of *Ramaria*.

Authors such as Perreau (1967), Clémenton (1970, 1973) and Keller (1986, 1997) have demonstrated that the layers of the spore wall are not always arranged in the same order. Keller (1997), studying some species of *Gomphus* and *Ramaria*, described the spore wall in *Gomphaceae* (*sensu* Donk) as thick and irregular. It was constituted of (i) a coriotunica, which is the fundamental basis of all spores, which could be granular, fibrillar or stratified; (ii) a tunica, represented by opaque material; (iii) a podostratum, seen as a transparent thin layer which does not react to iodine; and (iv) a mucostratum, which reacts to cotton blue.

In *Gomphaceae*, it has been suggested that the ornamentation arises from the mucostratum layer (Keller, 1986). In absence of ontogenetic studies that would allow us to recognize the order of formation and aggregation of the layers of the spore wall, and in view of the fact that up to this point this process is known in only relatively few taxa of *Gomphales*, there is not enough evidence to propose homologies between these characters or to establish correlations between the ornamentation patterns. More studies of this group, using electron micrographs, may enable us to understand more about the similarity observed in these ornamentation patterns.

All the data obtained for this study lead us to conjecture that, at least within *Gomphales*, there are four hypotheses of homology: (i) spores without ornamentation; (ii) ornamentation with pointed projections that can be differentiated into conical shapes with or without anastomosed bases; (iii) round ornamentation with the following distinguished states: (a) tuberculate-nodulose and (b) verrucose; and (iv) striate ornamentation. At a first glance, this last character could be considered as belonging to 2. However, the presence of striae and warts in the same spore lead us to believe that they are two different characters. It is clear, therefore, that it is not feasible to consider the whole ornamental character under only one hypothesis of transformational homology.

### ***Cyanophilic reaction***

The cyanophilic reaction of the spore ornamentation was considered as a primary taxonomic feature by Donk (1964). From this perspective, authors such as Petersen (1967) considered that *Gomphus* and some species of

*Ramaria* are closely related. A similar connection has been suggested for *Beenakia* and *Ramaria* (Maas Geesteranus, 1963).

Petersen (1967) observed that the spore surface in *Ramaria grandis* is conspicuously more colored than the areas between the spines. Marr and Stuntz (1973), in a comparative study of spores of *Ramaria* treated with cotton blue, noted that the ornamentation was always cyanophilic, but that it may or may not be distinct from the rest of the wall. In *Ramaria* cf. *botrytis*, the cyanophilic reaction is less conspicuous. Petersen and Pearman (1973) showed that, contrary to what happens in species of other *Ramaria* subgenera, the cyanophilic reaction is lighter in the crests of the striae and more intense in the areas between those striae, a feature that, from our perspective, is probably related to the number and the thickness of the wall layers.

It is clear that, within *Gomphales*, there is a group of taxa whose spores react with cotton blue, albeit not in a uniform manner (Marr and Stuntz, 1973; Petersen and Pearman, 1973). But, as with ornamentation, we are not convinced that the variations in the cyanophilic reaction are due to different ultrastructural, chemical or ontogenetic causes. Because of this, the deductive, inferential process allows us to consider only the presence or absence of cyanophily as a likely taxonomical character at this level. In preliminary studies (Villegas *et al.*, 1999), cyanophily has been postulated as a synapomorphy for the group including *Beenakia*, *Gloeocantharellus*, *Gomphus*, *Kavinia*, *Ramaria* and *Ramaricium*. Here the polarization of characters, by means of external groups, suggests a phylogenetic transformation from absence of cyanophily towards a condition of derived cyanophily, possibly phylogenetically correlated with features of ornamentation and spore color. These results suggest that cyanophily could be interpreted as a synapomorphy that could unite the aforementioned genera into a monophyletic group.

Finally, it is clear that all these hypotheses must be evaluated by their phylogenetic congruence with other characters, as proposed by De Pinna (1991), Roth (1988) and De Luna and Mishler (1996), including mating studies where feasible (e.g. Peterson and Hughes, 2003; Mata *et al.*, 2004) and molecular phylogenetics (e.g. Keirle *et al.*, 2004; Zhang *et al.*, 2004).

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