

MORPHOLOGICAL AND MOLECULAR DATA ON THE TAXONOMY OF GYMNOMYCES, MARTELLIA AND ZELLEROMYCES (RUSSULALES)

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Abstract. - This article reviews the current taxonomy of the family *Elasmomycetaceae*. After examining numerous Spanish specimens belonging to the genera *Gymnomyces*, *Martellia* and *Zelleromyces*, a series of suggestions are made to improve the delimitation among the three genera. Preliminary data based on molecular analysis of the rDNA gene, confirm the relationship between these genera and the epigeous Russulales.

Key words: *Elasmomycetaceae*, *Russulales*, taxonomy, ITS rDNA

Résumé. - Cet article est a tentative pour la clarification de la situation actuelle de la taxonomie dans la famille *Elasmomycetaceae*. Après l'examen de matériel espagnol des genres *Gymnomyces*, *Martellia* et *Zelleromyces*, nous proposons suggestions pour amender la délimitation entre tous les trois genres. Les premiers données basées sur l'analyse moléculaire de rDNA ont confirmé les relations entre ces genres et les Russulales épigés.

Mots clé: *Elasmomycetaceae*, *Russulales*, taxonomie, ITS rDNA

Resumen. - Este artículo intenta aclarar, en parte, la situación actual de la taxonomía de la familia *Elasmomycetaceae*. Después del estudio de numerosas muestras de material español, de los géneros *Gymnomyces*, *Martellia* y *Zelleromyces*, se proponen sugerencias, dirigidas a mejorar la delimitación entre los tres géneros. Los primeros datos, basados en el análisis de las secuencias del gen rDNA, confirman las relaciones filogenéticas entre estos géneros y los Russulales epigeos.

Palabras clave: *Elasmomycetaceae*, *Russulales*, taxonomía, ITS rDNA

INTRODUCTION

The taxonomy of fungi is a difficult task, and the gasteroid *Russulales* do not escape this problem. The order *Russulales* embraces two families: *Russulaceae* and *Elasmomycetaceae*, the basic differences being in the type of spores. The spores of *Russulaceae* are ballistosporic and asymmetric, while in *Elasmomycetaceae* the spores are statismosporic and symmetric. The latter family embraces four genera: *Elasmomyces*, *Gymnomyces*, *Martellia* and *Zelleromyces*. While *Elasmomyces* is easily separable by having basidioma stipitate and gleba exposed at

maturity, the other three genera are sessile with gleba not exposed at maturity (PEGLER & YOUNG, 1979).

Differentiating among these three genera is almost impossible when working with dried specimens. Thus, the present paper proposes some ideas to improve the identification of genera *Gymnomyces*, *Martellia* and *Zelleromyces*.

The use of molecular techniques in fungi provides new information in taxa where, as in this group, the morphological identification is ambiguous (MARTÍN & ROCABRUNA, 1999; JOHANNESSON & MARTÍN, 1999). Thus, in the present work, through the sequence analysis of the internal transcribed spacer regions of rDNA (ITS1 and ITS2, including the 5.8S), we examine the boundaries between the three genera and the relationships with *Lactarius* and *Russula* species.

MATERIAL AND METHODS

Material

The material used here have all been collected in Spain, and previously described in other papers (CALONGE & PEGLER, 1998; MORENO-ARROYO *et al.*, 1999). New collections of *Russula* and *Lactarius* have been included in the molecular analysis (Table 1). All the specimens are preserved at the Madrid Botanic Garden herbarium (MA-Fungi).

Table 1. List of specimens used in the molecular analysis.

Taxon name	MA-Fungi	GenBank AccNum.
<i>Gymnomyces ammophilus</i> Vidal & Calonge	40132	AF230890
<i>Gymnomyces ammophilus</i> Vidal & Calonge	40137	AF230891
<i>Martellia pila</i> (Pat.) Vidal	30667	AF230893
<i>Martellia pila</i> (Pat.) Vidal	30802	AF230894
<i>Russula foetens</i> Fr.	42065	AF230895
<i>Russula fragilis</i> (Pers.: Fr.) Fr.	42067	AF230898
<i>Russula integra</i> L.: Fr.	42066	AF230896
<i>Russula mairei</i> Sing.	42069	AF230899
<i>Russula postiana</i> Romagn.	42068	AF230897
<i>Zelleromyces giennensis</i> Calonge, Moreno-Arroyo & Gómez	38674	AF230800
<i>Zelleromyces hispanicus</i> Calonge & Pegler	37497	AF231911
<i>Zelleromyces hispanicus</i> Calonge & Pegler	37498	AF231912
<i>Zelleromyces hispanicus</i> Calonge & Pegler	38311	AF231913
Outgroup		
<i>Hygrophorus latitabundus</i> Britz.	42071	AF231915
<i>Suillus variegatus</i> (Swartz: Fr) O. Kuntze	42070	AF231914

Molecular methods

Total DNA was isolated with E.Z.N.A. Fungi DNA miniprep kit (Omega Biotech) as described in MARTÍN & GARCIA-FIGUERES (1999). Amplifications were done with Ready-To-Go® PCR Beads (Amersham-Pharmacia Biotech) (WINKA *et al.*, 1998) and the primer pair ITS1F/ITS4 (WHITE *et al.*, 1990). Amplification products were cleaned using E.Z.N.A. Clean kit (Omega Biotech) and both strands were sequenced separately using primers ITS1F and ITS4 with and

ABI Prism 377 Genetic Analyzer and the ABI Prism™ BigDye™ Terminator Cycle Sequencing Ready reaction Kit with AmpliTaq® DNA Polymerase (Perkin Elmer Applied Biosystem).

Sequencing and phylogenetic analysis

The software used for sequencing analysis is described in MARTÍN & GARCÍA-FIGUERES (1999). The new sequences have been logged in the EMBL database with the accession number indicated in Table 1. A sequence of *Lactarius deliciosus* was retrieved from the GenBank (U80999) and compared with the homologous sequence obtained in this study. Parsimony analysis was performed using the computer program PAUP 3.0s (Phylogenetic Program Using Parsimony) of SWOFFORD (1991). Isolates of *Suillus variegatus* and *Hygrophorus latitabundus* (Table 1) were used of outgroup. Branch robustness was estimated by bootstrap analysis (PELSENTEIN, 1985) of 1000 heuristic replicates.

RESULTS AND DISCUSSION

After the excellent work published by SINGER & SMITH (1960), where they established the basis to separate the genera *Gymnomyces*, *Martellia* and *Zelleromyces*, several other useful contributions have further clarified the taxonomy of these genera. PEGLER & YOUNG (1979) redefined the concept of the family *Elasmomycetaceae*, and BEATON *et al.* (1984) considered the anatomy of the hymenophoral trama and spore ornamentation as the main characters to be used. ZHANG & YU (1990) modified the systematics of the three genera reinforcing the importance of the spore ornamentation.

As a result of our own research (CALONGE & PEGLER, 1998; MORENO-ARROYO *et al.* 1998a-b, 1999), we consider that the main taxonomic characters to distinguish the three genera are the following:

- **Peridium:** In previous works, it seems to be considered as a character of minor importance, not withstanding the following comments by earlier authors: "...haud distinctum vel nullum ...", in *Gymnomyces* (MASSEE & RODWAY, 1898); "...enterely of filamentous hyphae or with scattered sphaerocysts or truly heteromerous in at least the inner layer...", in *Martellia* (SINGER & SMITH, 1960); "...well developed, the epicuticular layer distinctive by virtue of being a trichodermium or at least with numerous dermatocystidia...", in *Zelleromyces* (SINGER & SMITH, 1960). The peridium is almost forgotten by later authors (PEGLER & YOUNG, 1979; BEATON *et al.*, 1984; ZHANG & YU, 1990). As a matter of fact, the peridium may appear homomerous or heteromerous, and with or without sphaerocysts and laticiferous elements in the three genera.

- **Hymenophoral trama:** This is an important character to separate the three genera. In *Gymnomyces* it is heteromerous, with nests of sphaerocysts, while in *Martellia* and *Zelleromyces* there are not nests but some scattered sphaerocysts may be observed (SINGER & SMITH, 1960).

- **Columella and sterile base:** They are variable characters which may be present, absent or sometimes appear rudimentary, according to different species.

- **Spores:** They are variable in size, shape and ornamentation in the three genera making it difficult to establish a criterion, using spore morphology as the sole character, to separate these genera, as suggested by ZHANG & YU (1990). They vary from typically reticulate, as in *Zelleromyces* (Figs. 1a-b); warty or cresty, as in *Gymnomyces* (Figs. 1c-d), to typically warty, as in *Martellia* (Figs. 1e-f).

- **Basidia and sterigmata:** They are of a secondary importance, but of some help to differentiate related species, considering morphology and number of sterigmata on the basidia.

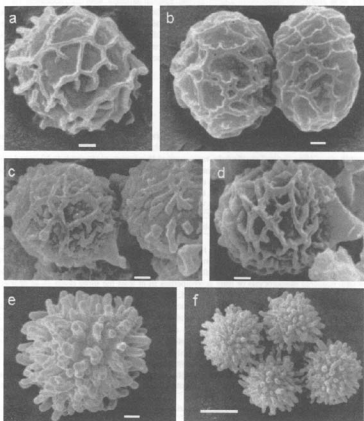


Fig. 1. Spores. (a) *Zelleromyces hispanicus*; (b) *Z. giennensis*; (c-d) *Gymnomyces dominguezii*; (e-f) *Martellia pila*.

- **Cystidia:** Two types of cystidia may be observed in these genera; leptocystidia, which are cylindrical, hyaline and thin-walled, and pseudocystidia, fusoid, with oleiferous contents (BEATON *et al.*, 1984). Their presence or absence help in some way to separate genera.

- **Latex, laticiferous and oleiferous hyphae:** We do agree with ZHANG & YU (1990) in their opinion concerning the relative taxonomic value of these characters. SINGER & SMITH (1960) decided to take the presence of laticiferous hyphae producing a copious latex as the main character to separate *Zelleromyces* from *Gymnomyces* and *Martellia*. However, PEGLER & YOUNG (1979) and BEATON *et al.* (1984) argued against this, since the presence or absence of latex rarely can be used to establish relationships among the *Russulales*. On the other hand, THIERS (1984) has observed a drastic variation in latex-production in *Arcangeliiella*, depending on the different developmental stages of the basidioma. If the collected basidiomata do not exude any latex, even though they have abundant laticiferous hyphae in peridium and trama, it may be due to aging, immaturity, dryness and so on. On the other hand, specimens belonging to the three genera included in the *Elasmomycetaceae* can develop a latex or laticiferous elements. Are they *Zelleromyces* or not? It is, indeed, difficult to say if we use only this character for the separation of the three genera.

On the other hand, when we observe the anatomy of the *Gymnomyces*-group under the light microscope, both, laticiferous and oleiferous hyphae may be present. But, how can they be distinguished? This is another interesting task.

Finally, habitat and ecology could be considered, but they are frequently overlooked. In the case of *Zelleromyces*, for instance, there are several species restricted to *Pinus* spp., such as *Z. cinnabarinus*, *Z. hispanicus*, *Z. giennensis*, etc., or to *Eucalyptus* spp., such as *Z. striatus*, *Z. daucinus*, *Z. australiensis*, etc., or in mixed forests, like *Z. stephensii*, *Z. josserandii*, etc.

The DNA sequence of the ITS regions of the rDNA gene helps not only to separate two close species but also to fuse them when the differences are too little. Regarding *Gymnomyces*, *Martellia* and *Zelleromyces*, we do not know any available publication where this subject is studied, that is why our assays could represent the first application of these new techniques to the clarification of the genetic identity of these three genera.

We performed a cladistic analysis of ITS regions, including the 5.8S rDNA, of the collections mentioned in Table 1 and the sequence of *Lactarius deliciosus* retrieved from the GenBank. Of the 685 characters, 166 were informative (77 in the ITS1 region and 89 in the ITS2 region). Using *Suillus variegatus* and *Hygrophorus latitabundus* as outgroup, the analysis produced three parsimonious trees with a length of 522, a consistency index (CI) of 0.812 and a retention index (RI) of 0.789, by using PAUP's branch-and-bound algorithm. The strict consensus tree is shown in Fig. 2. The three trees differ in the position of *Zelleromyces giennensis*. It may be either a sister group of the two *Lactarius deliciosus* or be a sister group of *Lactarius deliciosus* together with the three collections of *Zelleromyces hispanicus*. Morphological characteristics make the latter alternative most probable.

The monophyletic clade formed by all the ingroup taxa is not surprisingly very well supported (100 % bootstrap). *Martellia* and *Gymnomyces* species are closely related to *Russula* species, and the monophyletic clade of these genera is well supported (93 %). This is in agreement with morphological data. The clade formed by species of these genera is the sister group of the clade in which *Lactarius* and *Zelleromyces* are included; although this last clade is weakly supported (61 %).

HAWKSWORTH *et al.* (1995) retain two families in Russulales: *Elasmomycetacea* for the gasteroid genera and *Russulaceae* for the agaricoid ones. Based on our phylogenetic analysis these two families should not be maintained. Thus, we consider that the family *Elasmomycetaceae* is a synonymy of the *Russulaceae*. Attempts to amplify and sequence the DNA from *Martellia mistiformis* Mattirollo and *Zelleromyces cinnabarinus* Smith & C.A. Brown (type collections from NY and MICH herbaria respectively) are in course.

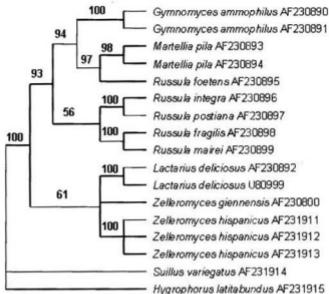


Fig. 2. Molecular analysis of the specimens included in Table 1 and *Lactarius deliciosus* retrieved from the Genbank. Strict consensus of three parsimonious trees under branch-and-bound algorithm. Percentages from 1000 bootstrap replications are given on the branches

As a conclusion of our morphological and molecular research we can state as follows:

- 1.- The family *Elasmomycetaceae* could be a synonym of the *Russulaceae*.
- 2.- The genera *Gymnomyces* and *Martellia* could well be considered as gasteroid forms of genus *Russula*.
- 3.- The genus *Zelleromyces* could represent gasteroid forms of the genus *Lactarius*.

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