MACOWANITES AMMOPHILUS (*RUSSULALES*) A NEW COMBINATION BASED ON NEW EVIDENCE

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Work dedicated to the friend and colleage
August Rocabruna, in his 80 birthday

ABSTRACT. *Macowanites ammophilus* (*Russulales*) a new combination based on new evidence. After studying abundant new collections, *Gymnomyces ammophilus* is transferred to the genus *Macowanites* based on the emergence of new data on morphological and genetic characters. A redescription of this taxon is given.

Key words: *Russulales, Macowanites ammophilus*, taxonomy, Iberian Peninsula.

RESUMEN. *Macowanites ammophilus* (*Russulales*) una nueva combinación basada en nuevos datos. Después de estudiar abundante material procedente de nuevas recolecciones, *Gymnomyces ammophilus* se transfiere al género *Macowanites*, basándonos en los nuevos datos morfológicos y genéticos encontrados. Como consecuencia, se presenta una nueva descripción de este taxon.

Palabras clave: *Russulales, Macowanites ammophilus*, taxonomía, Península Ibérica.

INTRODUCTION

Recently, CALONGE & VIDAL (1999) published an article where they proposed *Gymnomyces ammophilus* as a new species. That new taxon was based on several collections without any presence of stipe-columella. As a result of a later foray to Portugal and South of Spain, during November 1999, new collections of basidiomata in all states of development were obtained, making possible to distinguish a clear stipe-columella. This fact induced us to study all the material and investigate carefully both, macro- and microscopic features, complemented with DNA sequencing. The results of this investigation suggest that our previous collections, named as *Gymnomyces ammophilus*, fit better within the genus *Macowanites*, following the generic key proposed by SINGER & SMITH (1960). Thus, a redescription is needed, which compiles all the main taxonomic characters.

MATERIAL AND METHODS

Samples from all the three places were dried up and the spores prepared for SEM, shadowing with gold and observed following the usual techniques. For the identification of colours we followed KORNERUP & WANSCHER´s colour guide (1978). Molecular analysis of the internal transcribed spacer of the rDNA (including the 5.8 S rDNA) were done following the DNA isolation, PCR amplification and sequencing protocols described by CALONGE & MARTIN (2000). The new sequences have been logged in the EMBL database with the accession number AJ438038 (MA-Fungi 51165), AJ438037 (MA-Fungi 51166) and AJ438036 (MA-Fungi 51167). These sequences were compared with the homologous sequences published by CALONGE & MARTIN (2000) and two new sequences obtained from *Macowanites vinaceodorus* collections MA-Fungi 46524 (AJ438035) and MA-Fungi 47416-2 (AJ438034). Sequence Navigator™ Sequence Comparison for pairwise comparisons and SEQAPP software for multiple sequences were used to search for the best alignment. Alignment gaps were marked "-" and unresolved nucleotides or unknown sequences were

indicated with "N". Parsimonium (branch-and-bound search) and maximum likelihood (substitutions model) analyses were performed using the computer program PAUP 4.0b* (Phylogenetic Program Using Parsimony) of SWOFFORD (1996). Branch robustness was estimated by bootstrap analysis (FELSENSTEIN, 1985) of 10.000 heuristic replicates using the fast stepwise-addition option.

DESCRIPTION

Macowanites ammophilus (Vidal et Calonge) Vidal et Calonge, comb. nov.

Basion.: *Gymnomyces ammophilus* Vidal et Calonge, in Calonge & Vidal, *Bol. Soc. Micol. Madrid* 24: 66 (1999)

Basidioma hypogeous, solitary or gregarious, subglobose to irregular, 1-3 cm diam., when unmature, angiocarpous. During ripening process it becomes semihypogeous and partially gymnocarpous, showing a well-developed stipe with rhizomorph. Pileus 2-7 cm diam., globose to hemisphaerical, not expanding, depressed to umbilicate, often gibbous. Peridium smooth, viscid, with adherent granules of sand, initially attached to stipe and finally separated from it and disintegrated in the perimarginal zone, orange white (K&W 5A2) at first, becoming light orange (K&W 5A4) and maculated of brownish orange (K&W 7C8) to dark brown (K&W 7F8) finally. Stipe-columella percurrent, prominent, cylindrical, 1-4 × 0.7-2.5 cm, initially white, then tipically punctate of brownish orange (K&W 7C8) from the basis, unchanging when bruished. Context white, initially solid, then cavernose in the stipe. Gleba loculate, labyrinthiform, annexed to the stipe-columella, the basal chambers later free and exposed after disintegration of the peridium, orange white (K&W 5A2) to pale orange (K&W 5A3). Spore-mass in the locules white to pale yellow (K&W 4A2). Odor fruity, taste very slowly acrid to sweetish.

Spores 7-9 × 5.5-7.5 µm (ornamentation excluded), 7.5-10 × 6-9 µm (ornamentation included), subglobose to broadly ellipsoidal or subovoid, heterotropic, with a prominent hilar appendage 2 µm long. Amyloid ornamentation consisting of isolated warts, sometimes forming short cristae, 0.25-0.75 µm high (Figs. 2-3). Suprahilar plage absent. Basidia clavate, hyaline in KOH, 35-45 × 8-11 µm, 2-4-spored. Macrocystidia abundant, 45-60 × 6-10 µm, lanceolate, with a sinuose mucronate apex. Subhymenium cellular, with globose cells, 15-25 µm diam. Hymenophoral trama heteromerous, with septate hyphae, 3-12 µm diam., and sphaerocysts 6-35 µm diam., with abundant oleiferous hyphae 2.5-10 µm diam. Context of pileus and stipe-columella typically heteromerous. Peridiopellis thin, 125-200 µm, suprapellis an intricate trichoderm of 15-40 × 2-5 µm cylindrical, sinuose, claviform, lanceolate or mucronate elements, without dermatocystidia; mediopellis an intricate ixocutis, composed of gelatinized repent hyphae, 2.25-7.5 µm diam., subpellis an intricate cutis of non-gelatinizated hyphae, 4-10 µm diam., with frequent enlargements up to 16-(25) µm, that connect with the context. Abundant oleiferous hyphae are present in the middle and internal layers. Stipitipellis 30-100 µm, consisting in cutis of repent incrustated hyphae, 2.5-7.5 µm diam., with bundles of long and semierected, septate, incrustate, yellow to red coloured hairs, 50-150 × 2-7.5 µm.

The alignment of the ITS rDNA sequences gives no ambiguous regions and 810 characters were included in the analyses. Among them, 257 were parsimony-informative. Under branch-and-bound search, 90 most parsimonious trees (MPTs) were obtained (tree length = 758, consistency index CI= 0.7784, retention index RI= 0.7908, rescaled consistency index RC = 0.6155. The strict consensus tree is shown in Fig. 3. Similar tree topology was generated by maximum likelihood analysis following FELSENSTEIN (1985) (not shown). Both analyses agree (with a 99 % of bootstrap), in placing in the same group collections described by CALONGE & VIDAL (1999) as *Gymnomyces ammophilus* and collections with a clear stipe-columella described in this paper.

COLLECTIONS EXAMINED. PORTUGAL: Beira Alta, Castelo Ventoso, road to Alcacer do Sal, coastal dunes, under *Pinus pinea,* and *Corema,* 26-11-1999, *leg.* J.M. Vidal and F.D. Calonge, MA-Fungi 51167.- SPAIN: Huelva, Mazagón, coastal dunes, under *P. pinea,* with *Halimium* and *Corema*, 27-11-1999, *leg.* J.M. Vidal and F.D. Calonge, MA-Fungi 51166.- Matalascañas, the same ecology, but in the presence of *Juniperus phoenicea*, 27-11-1999, *leg.* J.M. Vidal and F.D. Calonge, MA- Fungi 51165.

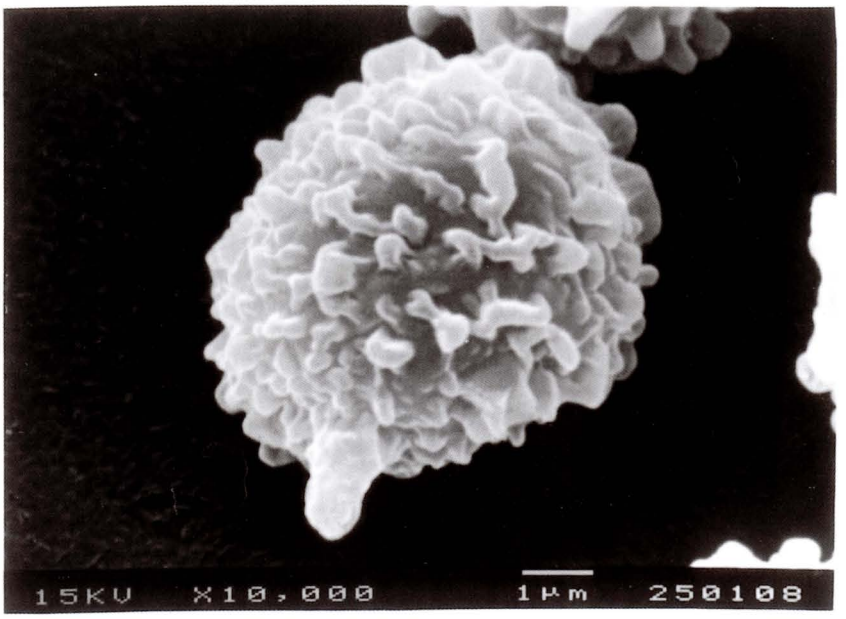

Fig. 1. *Macowanites ammophilus*. Spore showing a warty ornamentation forming some crests. Material collected in Castelo Ventoso, Portugal. MA-Fungi 51167.

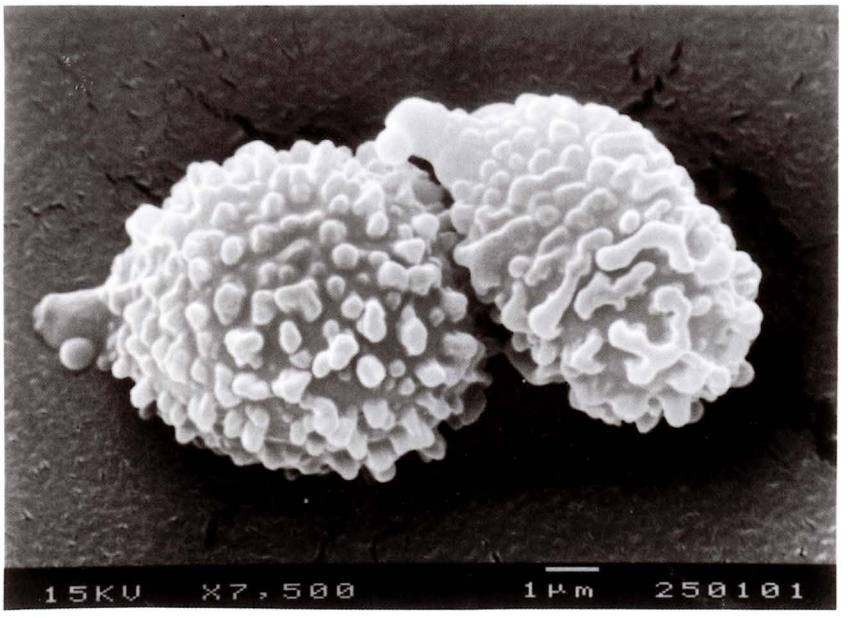

Fig. 2. *Macowanites ammophilus*. Spores showing warty and subreticulate ornamentation. Material collected in Mazagón, Spain. MA-Fungi 51166.

DISCUSSION. Following the work by PEGLER & YOUNG (1979) and according to our new data on this fungus (CALONGE & MARTIN, 2000; CALONGE & VIDAL, 2001), we consider that the genus in which our material fits better is *Macowanites* Kalchbr. In fact, the mature basidiomata look like a typical *Russula* at a first sight, and later observations, both under light and scanning electron microscopy, confirm this idea. Thus, they show a well-developed stipe, the gleba is exposed at base of peridium, at the margin, after ripening; the hymenophoral trama is heteromerous and macrocystidia of *Russula*-type are present. Thus, it can not be maintained in *Gymnomyces*, as previously thought (CALONGE & VIDAL, 1999) after the study of only unmatured material. In consequence, we propose the new combination *Macowanites ammophilus*.

M. ammophilus seems to be close to the North American *M. luteolus* A.H. Sm. et Trappe (SMITH, 1963). The microscopic characters are similar in both species, but the basidioma is larger in *M. ammophilus*, 2-7 cm, against 1-3 cm in *M. luteolus*. Also the brownish orange coloration of the stipe basis and the peridium in mature specimens are distinctive of *M. ammophilus*. Regarding their ecology, *M. ammophilus* is a psammophilous species associated with *Pinus pinea*, growing in the Atlantic coastal dunes of the South Iberian Peninsula, and probably it may be also present in similar habitats of the coast of North Africa, and rest of the Mediterranean littoral. *M. luteolus* is common in the Pacific coast of Oregon, Washington and California, under conifers (*Picea* and *Tsuga*) (SMITH, 1963; ARORA, 1986; PHILLIPS, 1991).

On the other hand, there is some mophological resemblance between *M. ammophilus* and *M. vinaceodorus* (CALONGE & VIDAL, 2001), which grows in the same ecological conditions, but, with the exception of several differences (see Table 1), after the phylogenetic analyses both species cluster in different clades (Fig. 3). Moreover, *Macowanites* seems to be polyphyletic. MILLER *et al.* (2001) used sequences of n-LSU (nuclear large subunit rDNA) to infer phylogenetic relationships among agaricoid, gasteroid and hypogaeous gasteroid taxa included in Russulales. In their study, *Macowanites, Gymnomyces, Cystangium* and *Martellia* grouped with *Russula* clades; whereas, *Zelleromyces* and *Arcangiella* cluster with *Lactarius* species, as first suggested by CALONGE & MARTÍN (2000). Moreover, *Cystangium, Gymnomyces* and *Martellia*, appear to be polyphyletic, too. This is in agreement with our results concerning *M. ammophilus* and *M. vinaceodorus*. Following KRETZER & BRUNS (1997), as many gasteroid and hypogeous gasteroid genera seem to be polyphyletic, MILLER *et al.* (2001) consider that a synonomisation of these genera into *Lactarius* and *Russula* is justifiable.

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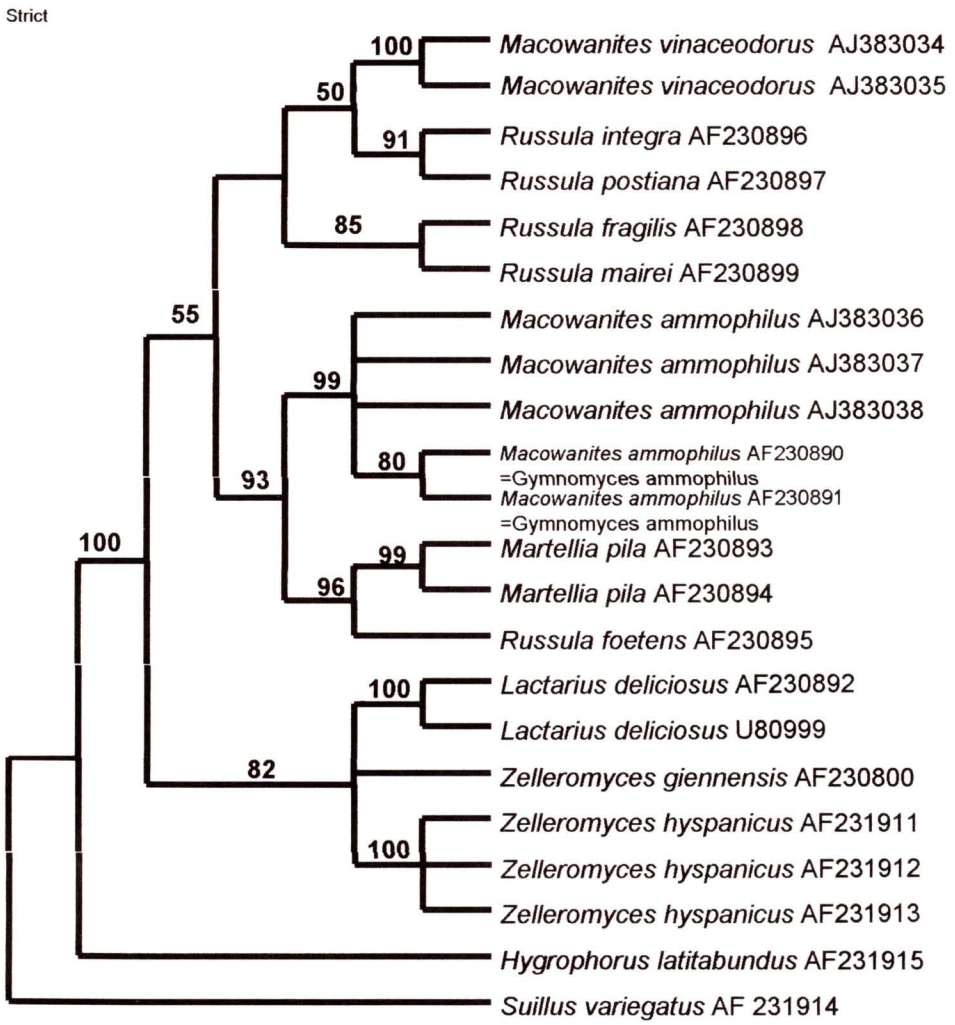

Fig. 3. Strict consensus tree of 90 MPTs under branch-and-bound algorithm. Percentages from 10.000 replications are given on the branches.

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Table 1. Comparison between some characters of *Macowanites ammophilus* and *M. vinaceodorus*

Characters	*M. ammophilus*	*M. vinaceodorus*
Spore mass	White-yellowish	Orange-yellowish
Spore ornamentation	0.25-0.75 µm thick	0.7-1.5 µm thick
Suprahilar plage	Absent	Present
Dermatocystidia	Absent	Present
Gleba	Loculate, whitish	Loculate-sublamellate, yellowish
Odour	Fruity	Vinaceous

Macowanites ammophilus (Vidal et Calonge) Vidal et Calonge (MA-Fungi 51167)