THE LIMITS BETWEEN *LYSURUS CRUCIATUS* AND *L. CRUCIATUS* VAR. *NANUS*. A COMPARATIVE DNA SEQUENTIAL STUDY

by

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Summary. MARTÍN, M.P., F.D. CALONGE & B. MARCOS (2005). The limits between *Lysurus cruciatus* and *L. cruciatus* var. *nanus*. A comparative DNA sequential study. *Bol. Soc. Micol Madrid* 29: 31-36.
After knowing the macroscopic differences between both taxa, the present paper was intended to look for possible correlations at a DNA sequential level.

Key words: *Phallales, Lysurus,* taxonomy, ITS nr DNA sequences.

Resumen. MARTÍN, M.P., F.D. CALONGE & B. MARCOS (2005). Los límites entre *Lysurus cruciatus* y *L. cruciatus* var. *nanus*. Estudio comparativo a nivel de la secuenciación de ADN. *Bol. Soc. Micol. Madrid* 29: 31-36.
Conocidas las diferencias macroscópicas entre ambos táxones, el presente artículo ha pretendido buscar una posible correlación también a nivel de biología molecular.

Palabras clave: *Phallales, Lysurus,* taxonomía, secuenciación, ADN nr ITS.

INTRODUCTION

Lysurus cruciatus var. *nanus* was proposed by CALONGE & MARCOS (1992) based on several macroscopic characters which separate it from *L. cruciatus* type. These characters are basically as follows; smaller size of the basidioma, slightly shorter spores and living associated with grasses.

The purpose of the present article is trying to find a possible correlation between the macroscopic features and the DNA sequencing in both taxa. The terms used in morphology are those published by CALONGE (1998).

MATERIAL AND METHODS

The material studied is preserved at the herbarium MA-Fungi and consists of the following collections:

Lysurus cruciatus (Lepr. & Mont.) Lloyd

GERONA: Amer (La Selva), 200 m, entre la hierba de un jardín, 9-IX-1995, leg. A. Torrent, AT- 950909; MA-Fungi 59262. (TORRENT, 1999)

MADRID: Cuatro Vientos, en suelo mezclado con estiércol de caballo, 7-XI-1997, leg. F.D. Calonge, MA-Fungi 37872.

SEVILLA: Puebla del Río, junto a la Venta del Cruce, sobre cascarilla de arroz, 17-I-1987, leg. F.D. Calonge, MA-Fungi 22359.

Fig. 1.- *Lysurus cruciatus*. A colony of basidiomata in different degree of development, showing volva, pseudostipe and receptacle, with the black gleba among the arms. MA-Fungi 37872. (Photo: F.D. Calonge)

Lysurus cruciatus* var. *nanus Calonge & Marcos

SALAMANCA: Césped de piscina municipal, VIII-1985, leg. M.L. Montilla, MA-Fungi 33809; Proximidades de la piscina del Campo de Tiro y Deportes, entre el césped, VII-1997, leg. M.L. Montilla, MA-Fungi 39591; Campo de Tiro y Deportes, creciendo entre la hierba de un céped, 3-25-IX-1991, leg. M.L. Montilla, MA-Fungi 26792 (HOLOTYPE).

A small quantity (less than 10 mg) of each collection was subjected to molecular analysis of the internal transcribed spacer regions of rDNA (ITS1 and ITS2), including the 5.8S. Total DNA was isolated using E.Z.N.A. Fungal MiniPrep Kit (Omega-Biotech, Doraville, USA) as described in MARTÍN & GARCÍA-FIGUERES (1999). Primer pair ITS1F and ITS4 was used to obtain amplifications of both ITS regions (WHITE & *al.*, 1990). Amplifications were done using Ready-to-Go® PCR Beads (Amersham-Biosciences, UK) as mentioned in WINKA & *al.* (1998). Sequences of both ITS regions, including the 5.8S of the ribosomal RNA gene cluster were obtained at the Automatic Sequencing Service (CIB-CSIC, Madrid) with primers mentioned above. SEQAPP software for multiple sequences was used to compare these sequences.

RESULTS

The new sequences have been logged in the EMBL database with the Accesion Numbers AJ878733 (*Lysurus cruciatus*, MA-Fungi 37872), AJ878734 (*L. cruciatus*, MA-Fungi 22359), AJ878735 (*L. cruciatus* MA-Fungi 59262), AJ878736 (*L. cruciatus* var. *nanus*, MA-Fungi 33809), AJ878737 (*L. cruciatus* var. *nanus*, MA-Fungi 39591), AJ878738 (*L. cruciatus* var. *nanus*, MA-Fungi 26792). All sequences obtained have the same length (586 bp).

Fig. 2.- *Lysurus cruciatus* var. *nanus*. Several basidiomata in their natural habitat showing reddish arms. MA-Fungi 39592. (Photo: B. Marcos)

The alignment of the six sequences is shown in Table 1, where they are coded with the DNA isolation code (CRULYS plus a number). Alignment resulted in 221 sites in the ITS1 region, 159 in the 5.8S nrDNA gene and 216 in the ITS2, without ambiguous areas. There were not differences in the ITS1 region; neither in 5.8S nrDNA gene. Only three sites were different in ITS2 (marked with ! in Table 1). There are two genotypes: One shared by two sequences of *L. cruciatus* (CRULYS1; CRULYS2) and one sequence of *L. cruciatus* var. *nanus* (CRULYS5); and another identical between two sequences of *L. cruciatus* var. *nanus* (CRULYS4 and CRULYS6) and one *L. cruciatus* (CRULYS3).

DISCUSSION

Lysurus cruciatus and *L. cruciatus* var. *nanus* can be identified by a number of macroscopic characters; *L. cruciatus* shows eggs of 2-4 cm diam., pseudostipe cylindrical, 5-8 x 1.5-2 cm, whitish, receptacle made of 5-8 arms, usually white (Fig. 1) but sometimes with yellowish red tones and spores of 4-5 x 1.5-2 µm. It grows on rich soils with plant debris, during spring and autumn. It is widespread in the world, being found in the provinces of Cáceres, Duero Litoral, Madrid, Pontevedra and Seville, within the Iberian Peninsula (CALONGE, 1998).

On the other hand, *L. cruciatus* var. *nanus* is smaller showing eggs of 0.7-1 cm diam., pseudostipe 0.8-5 x 0.3-0.5 cm, receptacle with 5-6 arms, reddish to orange (Figs. 2-3) and spores 3.5-4 x1.8-2 µm. It grows among grasses in summer, being collected in the province of Salamanca (CALONGE & MARCOS, 1992).

The molecular data (from only a small portion