

Reassessment of the University of Alcalá Herbarium (Madrid) reveals hidden diversity of the myxomycete genus *Lycogala* in Spain

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Abstract

The revision of collections, held in the Herbarium of the University of Alcalá (AH) reveals 94 specimens of the myxomycete genus *Lycogala*, mainly from Spain, but also from Mexico (3 specimens) and France (1). Morphological identification, based on recently developed criteria, allowed identification of 14 *Lycogala* species, 10 of which are new to Spain, further two are new for Mexico. All species found in Spain were already known from Europe, with the single exception of *L. cf. nigroconfusum*. The collections of *L. paraconfusum* and *L. guttatum* in Mexico represent the first records for the whole of North America. Due to the age of collections, the molecular identification was possible only for nine specimens, thus confirming the identification of three species. To provide reference material for further studies, representative specimens of all taxa were photographed, and the barcoding sequences were submitted to NCBI GenBank.

Key words: Amoebozoa, biodiversity, Eumycetozoa, Iberian Peninsula, molecular barcoding, Myxogastrea, species identification

Introduction

Lycogala epidendrum (L.) Fr. is the first myxomycete ever described by scientists (Ing and Stephenson, 2022). Today, it is regarded as a canonical species of the group and is routinely cited in academic sources as a representative example of myxomycetes. It was therefore particularly surprising to find that nearly half of the molecularly barcoded specimens identified as *L. epidendrum* actually belonged to other, as yet undescribed, species (Leontyev *et al.*, 2015). In 2022, it was demonstrated that *L. epidendrum* s. l. represents a complex taxonomic group consisting of dozens of species unknown to science (Leontyev *et al.*, 2022, 2023a). In the following year, fifteen of these species were formally described (Leontyev *et al.*, 2023b). In 2024–2025, two research teams—one working with material from Europe, the USA, China, and Japan, the other with material from subtropical China—described 24 and 10 additional new species, respectively (Leontyev *et al.*, 2025; Song *et al.*, 2025). Several classical species were reassessed based on the studies of type collections (Leontyev and Schnittler, 2023, 2025). As a result of this revision, in just two years, the number of known species in the genus *Lycogala* increased more than tenfold, from five to 54. Given the strict criteria for species delimitation proposed by Schnittler *et al.* (2025), this case is unprecedented.

Despite the considerable morphological homogeneity of the genus *Lycogala*, careful studies have revealed new taxonomic characters that allow reliable identification of well-preserved specimens based solely on morphology. Particular importance is given to the structural features of the peridium, specifically, the shape, coloration, and mutual arrangement of peridial vesicles, as well as the presence of crystalline, oily, or pigmented deposits within them. These characters are fairly stable within a biospecies and even may serve as phylogenetic predictors: the five sections recently established within this genus are distinguished primarily by the structure of the vesicles (Leontyev *et al.*, 2025).

The recent discovery of numerous species within the genus *Lycogala* has sparked renewed interest in this group among the scientific community. Several regional biodiversity surveys have been conducted in countries such as Belarus, China, Norway, and Ukraine, uncovering substantial hidden diversity within *Lycogala* (Johannesen and Vetlesen, 2024; Leontyev *et al.*, 2024; Song *et al.*, 2024; Yatsiuk *et al.*, 2024). These findings highlighted the considerable potential of critical re-examinations of herbarium collections, even within well-studied regions of Europe, based on the morphological characters of the peridium, which are preserved even in older herbarium collections with fragmented DNA.

Although recently published revisions have covered a significant portion of the European continent, some gaps remain, and one of the largest is the territory of Spain, where specialized studies of *Lycogala* myxomycetes using updated taxonomic approaches have not yet been conducted. Inspired by these considerations, we took the first step toward reassessing the diversity of *Lycogala* species in Spain by examining specimens from the University of Alcalá Herbarium (AH).

Subsequent to the reopening of the University of Alcalá in 1977, the Department of Botany initiated a systematic collection of material in 1978, thereby establishing the foundations for the AH Herbarium. The collection currently comprises over 35,000 specimens of vascular plants, 40,000 specimens of the fungi and approximately 5,000 specimens of myxomycetes, mostly from the Iberian Peninsula, the Balearic and the Canary Islands, supplemented by contributions from regions such as North Africa and North America. The myxomycete collection includes numerous taxonomic types.

The myxomycete collection of the AH herbarium contains 94 specimens of the genus *Lycogala*. 87% of the dated specimens were collected between 1970 and 2000. Since DNA degradation renders routine Sanger sequencing ineffective for molecular barcoding of such old collections, species identification had to rely primarily on morphological examination.

Materials and methods

The collections were gathered between 1978 and 2017 across various regions of Spain (Fig. 1), but as well in Mexico (3 specimens) and France (1 specimen). Morphological study and photographing of specimens was carried out at the University of Greifswald with a Keyence VHX 7000 digital microscope and a Leica DM2500 microscope in conjunction with a Flexacam C1 camera. Species identification was carried out by examining the microstructure of the peridium under reflected, transmitted, and polarized light, as well as the ornamentation of the capillitium and spores stained with methyl blue in lactic acid. The criteria used for identification followed those employed in identification keys and species diagnoses in recent publications (Leontyev *et al.*, 2023b, 2025).

To provide a molecular barcode for relatively fresh specimens (collected after 2000), we obtained a partial sequence of the nuclear 18S rDNA (=nucSSU); this is the only marker studied for nearly all described species of *Lycogala* (Leontyev and Schnittler, 2022). DNA extraction was done using the laboratory robot King Fisher Flex (Thermo Fisher Scientific, US) according to the procedure described previously (Leontyev *et al.*, 2023a). PCR was performed with primer pair S1a/RSIP using the following protocol: initial denaturation at 94 °C for 2 min; 34 cycles, including denaturation at 94 °C for 30 sec, annealing 56.3 °C for 30 sec, elongation at 72 °C for 2 min; final elongation at 72 °C for 5 min. The PCR product, purified by exonuclease and alkaline phosphatase (1:5), was sent to MacroGen Europe (Amsterdam, the Netherlands) for Sanger sequencing. Obtained chromatograms were visually checked and edited in Chromas 2.6.6 (Technelysium Pty Ltd, 1998) and collected in fasta files using BioEdit 7.2 (Hall, 1999). For all sequences we obtained, the Nucleotide BLAST has shown that the similarity threshold with the sequences stored in NCBI GenBank varied from 99.23 to 100% (0–2 bp substitution), with the query cover always reaching 100%. Sequences were deposited in GenBank under the accession numbers PX404417–PX404425. We obtained nine 18S rDNA sequences which were identified by Nucleotide BLAST web service (<https://www.ncbi.nlm.nih.gov/geo/query/blast.html>). The identification and numbering of ribogroups is based on the nomenclature proposed by Leontyev *et al.* (2025). Geographic coordinates were converted from UTM to decimal degrees format using Gemini AI service.

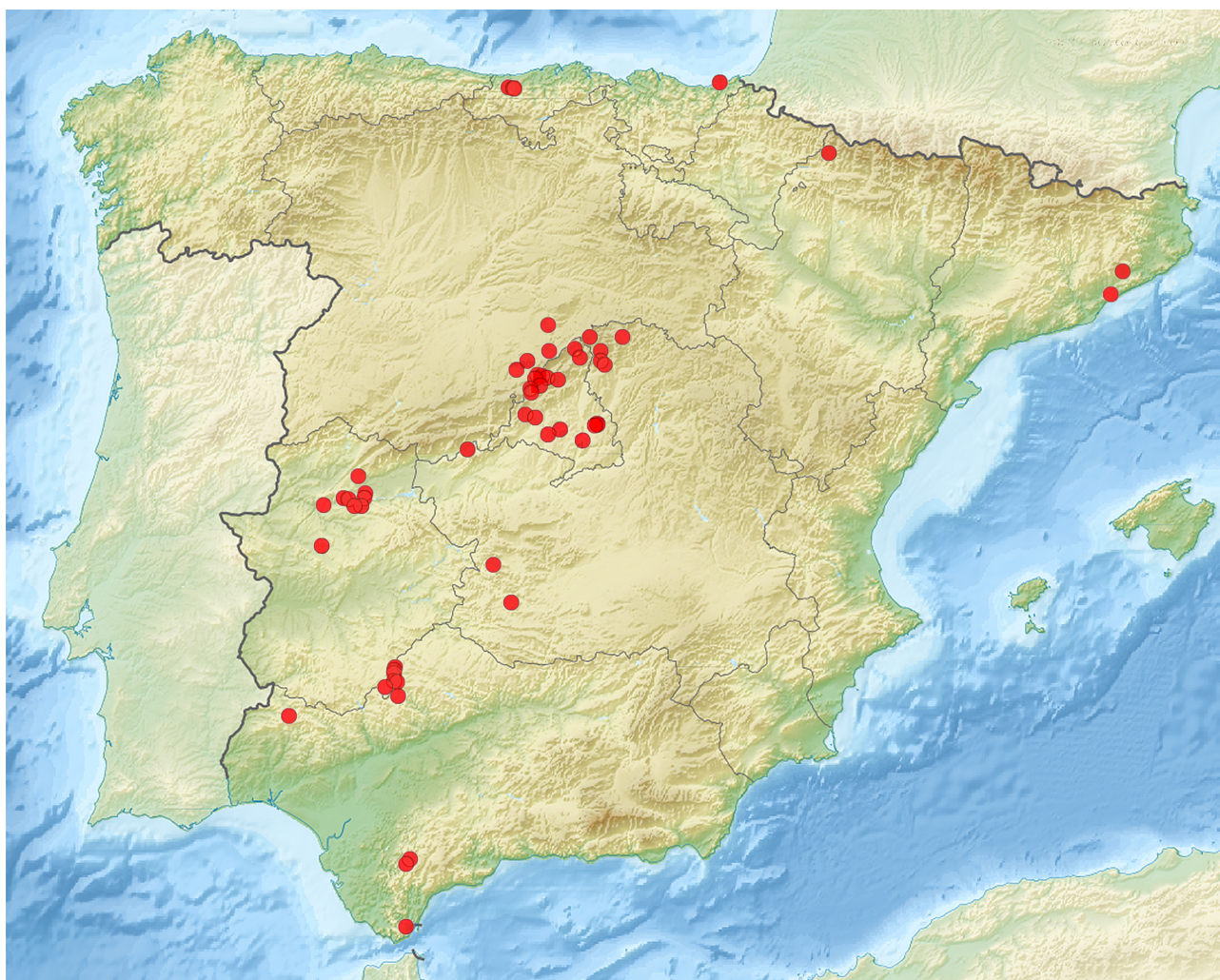


FIGURE 1. Collection sites of studied myxomycete specimens within Spain. Visualization was made in GPSVisualizer (https://www.gpsvisualizer.com/map_input?form=data).

Results

As a result of morphological study of 94 specimens (Figs. 2–4), we identified 14 species of the genus *Lycogala*. Of these, 10 are new for Spain (*L. caviaroides*, *L. confusum*, *L. irregulare*, *L. leopardinum*, *L. cf. nigroconfusum*, *L. olearium*, *L. palyianytsia*, *L. sphaeroconicum*, *L. succineum*, *L. umbrinum*). All species found in Spain were already known from Europe, with the single exception of *L. cf. nigroconfusum* (Leontyev *et al.*, 2025). Two of the three Mexican records were new for the country and for the whole of North America (*L. paraconfusum* and *L. guttatum*). Due to the age of collections, the molecular identification was possible only for nine, rather fresh, collections, thus confirming the identification of three species (*L. epidendrum* s. str., *L. olearium* and *L. succineum*). Fourteen specimens could not be identified due to their poor condition.

The list of species with collection data is given below. Substrate types are shown as follows: b—tree bark; br—branch; l—leaf litter; m—moss; w—decayed wood; wd—woody debris. For the molecularly barcoded specimens the NCBI accession number and the ribogroup code (see Leontyev *et al.*, 2025) are provided.

***Lycogala caviaroides* Leontyev, Schnittler, S.L. Stephenson & G. Konstantinides (Figs. 2A, 3A).** SPAIN: Cáceres, Torrejón el Rubio, Torrejón water reservoir, Monfragüe National Park, w *Pinus pinaster*, 39.875°N 6.145°W, 01.11.1990, leg. Ochoa C., Illana C., Esteve-Raventós F., Altés A. & Moreno G., AH13018; Huelva, La Nava, Múrtigas riverbanks, w *Alnus glutinosa*, 38.004°N 6.751°W, 22.11.1990, leg. Moreno G. & Galán R., AH13256; Madrid, San Ildefonso-Rascafría, Border Madrid-Segovia, descent from Cotos to Segovia, w *Pinus sylvestris*, 40.904°N 3.946°W, 28.05.1978, leg. Lambea E., AH5745.



FIGURE 2. Sporocarps of *Lycogala* species. A. *L. caviaroides* AH5745. B. *L. confusum* AH19007. C. *L. epidendrum* AH404. D. *L. epidendrum* AH11024. E. *L. epidendrum* AH47020. F. *L. flavofuscum* AH45985. G. *L. guttatum* AH20089. H. *L. irregulare* AH13019. I. *L. leopardinum* AH13020. J. *L. cf. nigroconfusum* AH12310. K. *L. olearium* AH10871. L. *L. palianytsia* AH11434. M. *L. sphaeroconicum* AH260 N. *L. succineum* AH10525. O. *L. umbrinum* AH47021. Bars: A–E, G–O—1 mm; F—1 cm.

Lycogala confusum Nann.-Bremek. ex Ing (Fig. 2B, 3B, 4A). SPAIN: Cantabria, Ruento, Monte Aa, wd *Fagus sylvatica*, 43.267°N 4.323°W, 23.09.1998, leg. Sánchez A., AH19007.

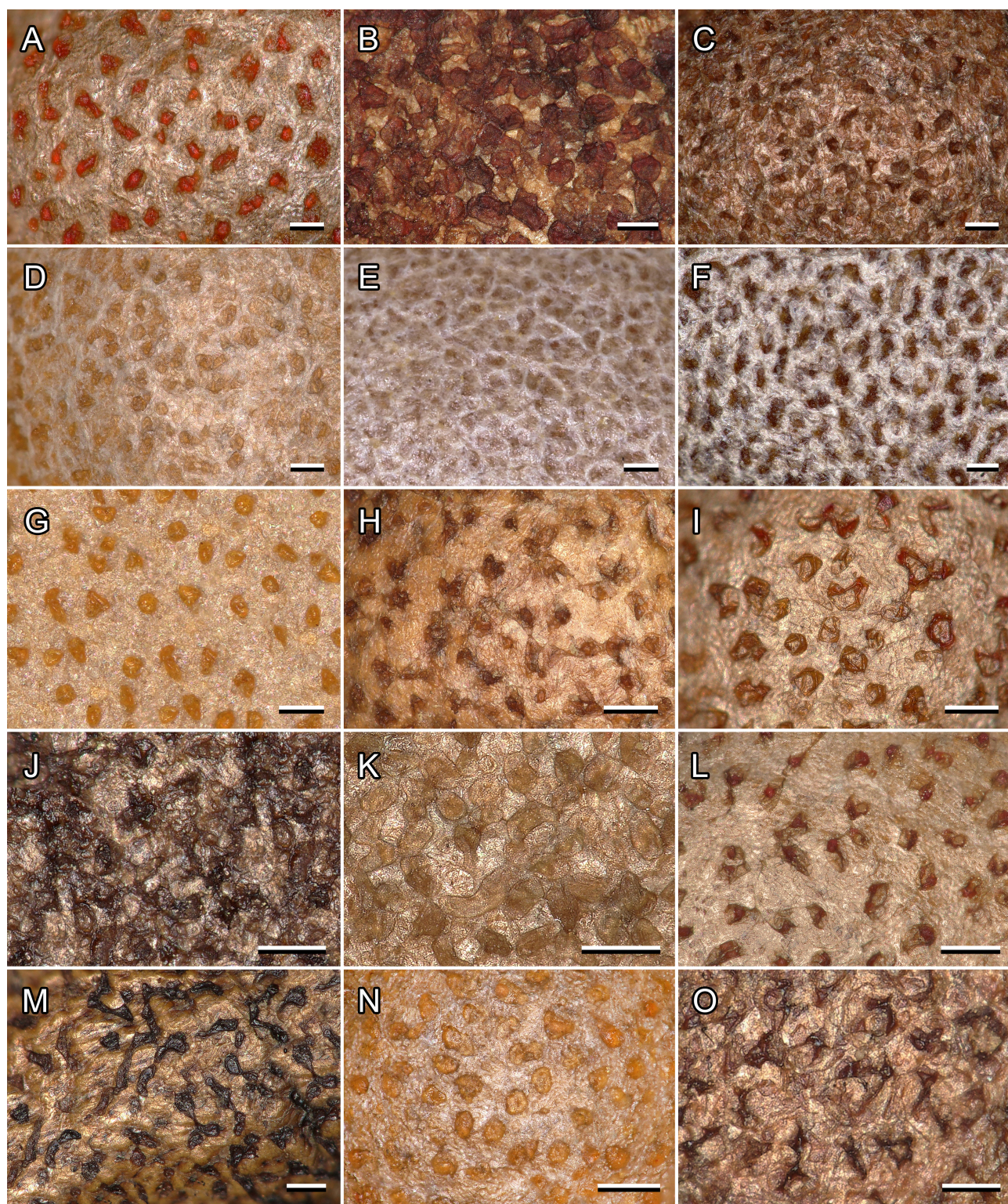


FIGURE 3. Peridium of *Lycogala* species under reflected light. A. *L. caviaroides* AH5745. B. *L. confusum* AH19007. C. *L. epidendrum* AH404. D. *L. epidendrum* AH11024. E. *L. flavofuscum* AH10872. F. *L. flavofuscum* AH10105. G. *L. guttatum* AH20089. H. *L. irregulare* AH13019. I. *L. leopardinum* AH13020. J. *L. cf. nigroconfusum* AH12310. K. *L. olearium* AH10871. L. *L. palianytsia* AH11434. M. *L. sphaeroconicum* AH260 N. *L. succineum* AH10525. O. *L. umbrinum* AH47021. Bars: A–D, F–O—200 μ m; E—500 μ m.

Lycogala epidendrum (L.) Fr. emend. Leontyev *et al.* 2023b (Fig. 2C–E, 3C, D, 4B, C). Ribogroup 01a: SPAIN: Madrid, Boadilla del Monte, wd *Pinus* sp., 40.414°N 3.876°W, 24.03.2014, leg. Mayoral A., AH49322, NCBI GenBank PX404423.

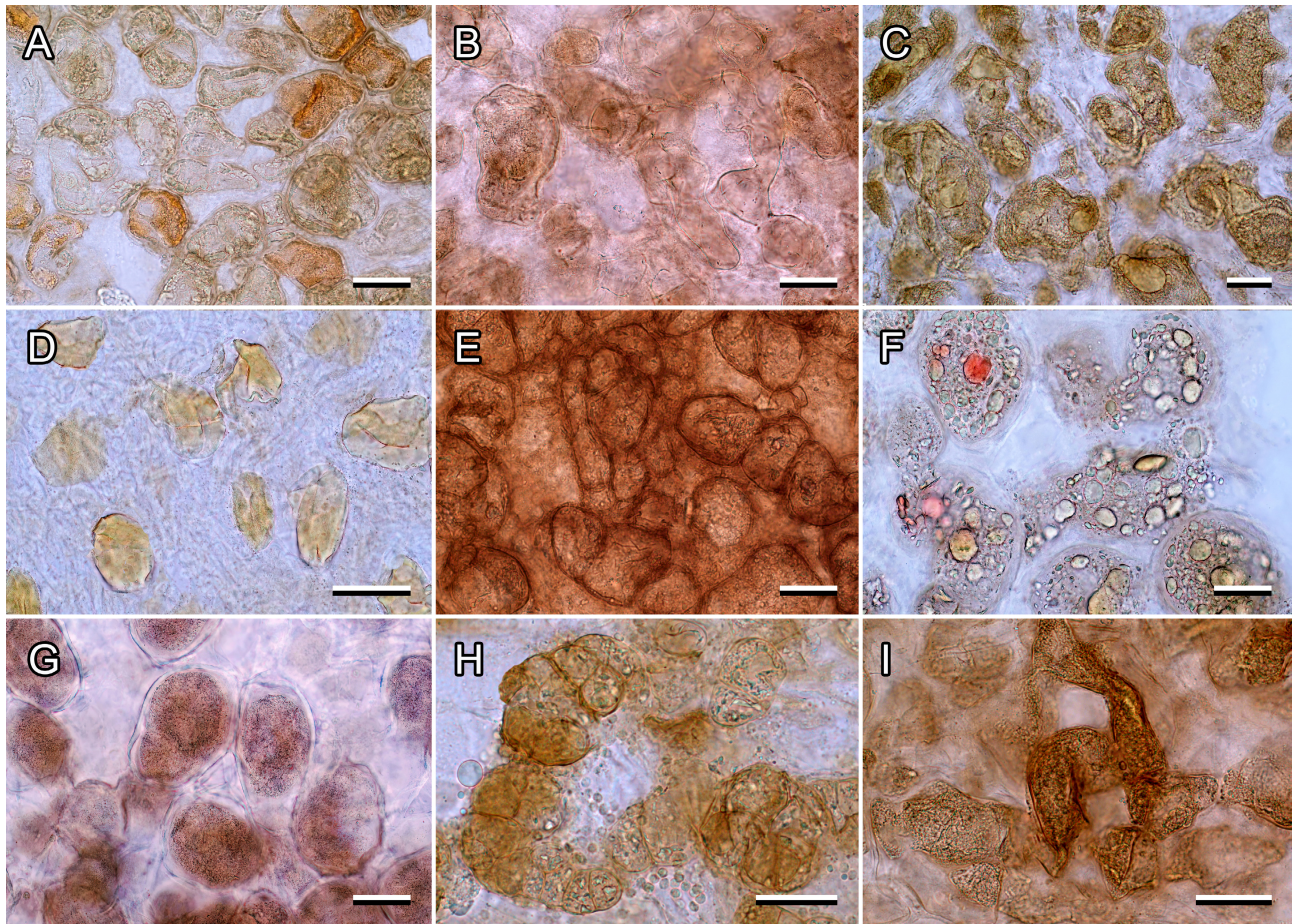


FIGURE 4. Peridium of *Lycogala* species under transmitted light. A. *L. confusum* AH19007. B. *L. epidendrum* AH13452. C. *L. epidendrum* AH47020. D. *L. guttatum* AH20089. E. *L. cf. nigroconfusum* AH12310. F. *L. olearium* AH10871. G. *L. olearium* AH47025. H. *L. paraconfusum* AH45593. I. *L. umbrinum* AH47021. Bars: 50 µm.

Ribogroup 01i: SPAIN: Cantabria, Riente, Saja-Besaya Natural Park, Uceda de Arriba, w, b *Fagus sylvatica*, 43.261°N 4.249°W, 17.07.2000, leg. Sánchez A., AH33412, NCBI GenBank PX404419.

Ribogroup 01u: SPAIN: Segovia, Cabañas de Polendos, Finca Mata de Quintanar, soil, 41.028°N 4.109°W, 31.03.2001, leg. Sánchez A., AH19065, NCBI GenBank PX404417.

Ribogroup 01w: SPAIN: Ciudad Real, Alcoba de los Montes, El Brezoso water reservoir, Cabañeros National Park, w *Quercus pyrenaica*, 39.308°N 4.486°W, 04.10.2001, leg. Moreno G. & Galán R., AH32830, NCBI GenBank PX404418; Córdoba, Fuente Obejuna, Finca El Río, w *Eucalyptus* sp., 38.429°N 5.57°W, 20.04.2000, leg. García J. R., AH33867, NCBI GenBank PX404420; Segovia, San Ildefonso, Valsain, w *Pinus sylvestris*, 40.877°N 4.023°W, 10.10.2012, leg. Sánchez A., AH45874, NCBI GenBank PX404422.

Ribogroup unknown (the molecular barcode not obtained): FRANCE: Hauts-de-France, Cappy, b *Fagus sylvatica*, 49.928°N 2.756°E, 22.06.1981, leg. Moreno G. & Manjón J. L., AH665;

SPAIN: Ávila, Casavieja, br *Pinus pinaster*, 40.284°N 4.768°W, 04.06.1988, leg. Francesch B., Sánchez P. & Illana C., AH11035; Badajoz, Azuaga, Argallón stream, w *Populus alba*, 38.176°N 5.54°W, 01.10.1991, leg. García J. R., AH33853; Old dehesa, w deciduous, 38.259°N 5.678°W, 22.10.1988, leg. García Martínez J. R., AH11453; b *Populus alba*, 38.259°N 5.678°W, 12.11.1990, leg. García J. R., AH30601; Granja de Torrehermosa, Chopped oak, margins of the Zujar river, w *Populus alba*, 38.302°N 5.55°W, 15.10.1993, leg. García J. R., AH30606; El Revuelo de Hermenegildo, w *Pinus pinaster*, 38.373°N 5.583°W, 07.12.1992, leg. García J. R., AH33868; Cáceres, Serradilla, Barbaón stream, Monfragüe National Park, w *Alnus glutinosa*, 39.865°N 6.082°W, 22.11.1985, leg. Blanco M. N & Moreno G., AH9539; Cañadilla mountain pass, Monfragüe, w *Pinus pinaster*, 39.808°N 5.939°W, 24.11.1988, leg. E.M.A.H. (Mycological group of the University), AH11443; Guadalajara, Majaerayo, b *Quercus pyrenaica*, 41.112°N 3.301°W, 25.06.1978, leg. Moreno G., AH47026; Huesca, Hecho, Fuente Churdana (Anchó-Hecho road), m, w *Pinus sylvestris*, 42.74°N 0.774°W, 02.05.1991, leg. Altés A., Esteve-Raventós F. & Illana C., AH13242; Madrid, Alcalá

de Henares, Quinta Cervantes, w *Coniferous*, 40.485°N 3.364°W, 11.1983, leg. Illana C., AH10098; Bustarviejo, Canencia mountain pass, w *Pinus sylvestris*, 40.869°N 3.765°W, 28.03.1978, leg. Manjón J. L., AH5743; Cercedilla, Dehesas de Cercedilla, w *Pinus sylvestris*, 40.764°N 4.068°W, 06.11.1981, leg. Moreno G., AH47022; Madrid, Ciudad Universitaria, w *Pinus halepensis*, 40.457°N 3.746°W, 11.04.1980, leg. Moreno G. & Manjón J. L., AH47024; Montejo de la Sierra, w *Fagus sylvatica*, 41.058°N 3.526°W, 09.07.1978, AH47513; Rascafria, El Paular, b *Pinus sylvestris*, 40.889°N 3.891°W, 16.11.1980, leg. Moreno G., AH47020; Somosierra, birch forest, w *Corylus avellana*, 41.132°N 3.577°W, 24.06.1988, leg. Illana C., Moreno G. & Esteve-Raventós F., AH11024; San Sebastián, b *Fagus sylvatica*, 43.31°N 1.983°W, 1978, leg. Huarte J., AH404; Segovia, El Espinar, Source of the Moros river, w *Pinus sylvestris*, 40.795°N 4.08°W, 04.06.1978, leg. Moreno G. & Manjón J. L., AH5744; Riofrío de Rianza, La Quesera mountain pass, w *Fagus sylvatica*, 41.228°N 3.42°W, 28.11.1979, leg. Checa J., Manjón J. L. & Moreno G., AH1914; San Ildefonso, Cotos mountain pass, w *Pinus sylvestris*, 40.821°N 3.971°W, 31.05.1991, leg. Castillo A. & Moreno G., Valenzuela E. & Ochoa C., AH13452; La Granja (on the path of the wells), w *Pinus* sp., 40.911°N 4.004°W, 25.10.1997, leg. García Amorena M., AH52619; Sebúlcór-Sepúlveda, Hoces del río Duratón Natural Park, b *Pinus* sp., 41.325°N 3.879°W, 10.11.1993, leg. Esteve-Raventós F., Escobar P. G. & Gómez Busutil S., AH19142.

***Lycogala flavofuscum* (Ehrenb.) Rostaf. (Fig. 2F, 3E, F).** MEXICO: Sonora, Álamos, Tropical Deciduous and Subdeciduous Forest, Secondary vegetation of shrubs and herbs, Mesa del Trigo, Biosfere Reserve Sierra de Álamos-Río Cuchujaqui, Loc6, w *Quercus* sp., 27.016°N 108.764°W, 14.11.2006, leg. Lizárraga M., Esqueda M., Chacón S., Valenzuela R. & Cifuentes J., AH31910;

SPAIN: Badajoz, Azuaga, Argallón stream, w *Nerium oleander*, *Populus nigra*, 38.176°N 5.54°W, 29.06.1997, leg. J.R. García, AH48746; Granja de Torrehermosa, Quejigo stream, w *Populus alba*, 38.308°N 5.578°W, 12.10.1992, leg. J.R. García, AH48683; Cáceres, Capital, l *Quercus* sp., 39.472°N 6.383°W, 18.07.2015, leg. Soc. Micol. Madrid, AH46526; Tejeda de Tiétar, Road from La Bazagona to Finca Las Cansinas, Monfragüe National Park, wd *Quercus suber*, 39.873°N 5.908°W, 18.05.1986, leg. Vena M. A. & Blanco M. N., AH10872; Cádiz, Algeciras, br *Quercus faginea*, 36.144°N 5.448°W, 29.10.1989, leg. Sogorb J. R., AH12312; Madrid, Alcalá de Henares, Campus Universitario, Fc. Biología, Univ. de Alcalá, b *Populus alba*, 40.505°N 3.338°W, leg. López Villalba A. & Moreno G., AH45696; AH46074; wd *Populus nigra*, 15.11.2012, leg. Moreno G. & Rosario P., AH42899; Finca La Oruga, b *Populus alba*, 40.493°N 3.327°W, 09.10.1986, leg. Illana C. & Heykoop M., AH10101; l *Populus* sp., 09.10.1986, leg. Heykoop M. & Illana C., AH10104; w *Populus alba*, 28.10.1986, leg. Illana C., AH10102; w *Populus nigra*, 22.09.1986, leg. Heykoop M. & Illana C., AH10105; w *Populus* sp., 26.09.1986, leg. Heykoop M., Moreno G. & Illana C., AH10100; AH10099; 31.10.1987, leg. Illana C., AH10944; Colmenarejo, Prado Ibarra, b *Fraxinus angustifolia*, 40.557°N 4.02°W, 29.11.2002, leg. Prieto F., AH40724; El Escorial, b *Fraxinus angustifolia*, 40.584°N 4.134°W, 09.06.2008, leg. Prieto F. & Moreno G., AH45788; Velilla de San Antonio, l *Populus* sp., 40.363°N 3.494°W, 18.04.2010, leg. Martín M., AH43909; b *Populus alba*, 15.11.2013, AH44065; Segovia, Santiuste de Pedraza, Requijada, w *Populus* sp., 41.116°N 3.865°W, 30.11.1997, leg. Sánchez A., AH33066; Valverde del Majano, w *Populus* sp., 40.957°N 4.234°W, 15.08.2015, leg. Sánchez A., AH45985.

***Lycogala guttatum* Leontyev, W.-L. Song, Shuang L. Chen, Ishchenko, D. Panayotova & V. Botyakov (Fig. 2G, 3G, 4D).** MEXICO: Baja California Sur, Loreto, Surroundings of Loreto, transpeninsular road México 1, w, 26.025°N 111.356°W, 27.01.1996, leg. Lizárraga M., Moreno G. & Illana C., AH20089.

***Lycogala irregulare* Leontyev, Schnittler, Ishchenko & G. Konstantinides (Fig. 2H, 3H).** SPAIN: Cáceres, Tejeda de Tiétar, Road from La Bazagona to Finca Las Cansinas, Monfragüe National Park, w *Quercus suber*, 39.873°N 5.908°W, 02.11.1990, leg. Altés A., Esteve-Raventós F., Illana C., Ochoa C. & Moreno G., AH13019; Madrid, Montejo de la Sierra, br *Fagus sylvatica*, 41.058°N 3.526°W, 09.06.1982, leg. E.M.A.H. (Mycological group of the University), AH2956.

***Lycogala leopardinum* Leontyev, Ishchenko, Schnittler & Johannesen (Fig. 2I, 3I).** SPAIN: Cáceres, Malpartida de Plasencia, La Bazagona, w *Pinus pinaster*, 39.922°N 5.901°W, 30.10.1990, leg. Illana C., Ochoa C., Esteve-Raventós F., Moreno G. & Altés A., AH13020; Serradilla, Barbaón stream, Monfragüe National Park, w *Alnus glutinosa*, 39.865°N 6.082°W, 15.11.1989, leg. E.M.A.H. (Mycological group of the University), AH12311; Cañadilla mountain pass, Monfragüe, w *Pinus pinaster*, 39.808°N 5.939°W, 09.04.1987, leg. E.M.A.H., AH10526; Madrid, Cercedilla, w *Pinus sylvestris*, 40.764°N 4.068°W, 10.05.1976, leg. Moreno G., AH47028.

***Lycogala* cf. *nigroconfusum* Leontyev, W.-L. Song, Shuang L. Chen & S.L. Stephenson (Fig. 2J, 3J, 4E).** SPAIN: Cáceres, Cañaveral, Los Castaños mountain pass, w *Eucalyptus camaldulensis*, 39.819°N 6.362°W, 19.01.1990, leg. Galán R., Blanco M. N., Moreno G. & Illana C., AH12310.

***Lycogala olearium* Leontyev, Schnittler & D. Panayotova (Fig. 2K, 3K, 4F, G).** SPAIN: Barcelona, La Roca del Vallès, Can Company de Dalt, w *Pinus pinea*, 41.586°N 2.347°E, 09.04.2017, leg. Sánchez Sánchez L., AH54941,

NCBI GenBank PX404425; Cáceres, Torrejón el Rubio, Torrejón water reservoir, Monfragüe National Park, w *Pinus pinaster*, 39.875°N 6.145°W, 22.04.1988, leg. E.M.A.H., AH10871; Guadalajara, Tamajón, El Vado water reservoir, br *Pinus pinaster*, 41.029°N 3.301°W, 20.06.1979, leg. Honrubia M., Manjón J. L. & Moreno G., AH47025.

***Lycogala palyianysia* Leontyev, Schnittler & Ishchenko (Fig. 2L, 3L).** SPAIN: Cáceres, Sierra de Bernabé, Cytisus area on Tormantos, w *Castanea sativa*, 40.062°N 5.982°W, 28.11.1988, leg. E.M.A.H., AH11412; Serradilla, Cañadilla mountain pass, Monfragüe, w *Pinus pinaster*, 39.808°N 5.939°W, 24.11.1988, AH11426; AH11434.

***Lycogala paraconfusum* Leontyev, Ishchenko & V. Dzizurova (Fig. 4H).** MEXICO: Chihuahua, Ojinaga, Pegüis II, Pegüis canyon, b *Yucca* sp., 29.57°N 104.441°W, 23.03.2009, leg. Lizárraga M. & Salazar C., AH45593.

***Lycogala sphaeroconicum* Leontyev, E. Moroz, Ishchenko, E. Johannesen, Schnittler, T. Kudryavtseva & Bortnikov (Fig. 2M, 3M).** SPAIN: Cantabria, Ruente, wd *Fagus sylvatica*, 43.258°N 4.267°W, 18.07.2000, leg. Sánchez A., AH26000.

***Lycogala succineum* Leontyev & Schnittler (Fig. 2N, 3N).** SPAIN: Barcelona, Fogars de Montclús, Pla de les Barraques, Santa Fe del Montseny, w *Fagus sylvatica*, 41.769°N 2.481°E, 07.07.2015, leg. Sánchez Sánchez L., AH54154, NCBI GenBank PX404424; Cáceres, Serradilla, Cañadilla mountain pass, Monfragüe, w *Pinus pinaster*, 39.808°N 5.939°W, 09.04.1987, AH10525; Guadalajara, Tamajón, w *Pinus pinaster*, 41°N 3.248°W, 31.10.2014, leg. Moreno G., AH45757, NCBI GenBank PX404421; San Sebastián, Donostia, w, 43.31°N 1.983°W, 00.00.1978, leg. Moreno G., AH47039.

***Lycogala umbrinum* Leontyev, P. Vetlesen, Schnittler, E. Moroz & E. Johannesen (Fig. 2O, 3O, 4I).** SPAIN: Madrid, El Escorial, b *Pinus pinaster*, 40.584°N 4.134°W, 05.02.1982, leg. Moreno G., AH47021.

Discussion

Our study demonstrates that, despite the intensive investigation of myxomycetes in Europe, the revision of herbarium collections using newly established taxonomic criteria can yield unexpected findings. The identification of 14 species, including 10 recorded for the first time in the country, underscores the potential of herbarium collections to serve as reservoirs of undocumented biodiversity. Our study also demonstrates that, in the absence of molecular data, *Lycogala* collections can be identified based solely on morphological characteristics. For three species from nine accessions we could confirm this identification by molecular barcodes. For the remaining collections, identifications based solely on morphology are provisional and may be revised in the future as molecular barcoding technology for fragmented DNA advances.

Several of the species identified in the AH herbarium are of particular interest. *Lycogala caviaroides* is a rather rare myxomycete, previously reported in Europe only from Germany and Greece (here and after: the distribution data are taken from Leontyev *et al.*, 2025). *L. guttatum*, a nearly cosmopolitan myxomycete, appears to represent a complex of at least three biological species with narrower distributions: a European (rg91), a North American (rg15), and a Southeast Asian (rg84). Since our specimen from Mexico was not barcoded, its ribogroup affiliation remains undetermined, although it is likely North American. *L. nigroconfusum* is represented by Asian and North American variants; our record, though not supported by molecular data, is the first from Europe. *L. paraconfusum*, *L. sphaeroconicum*, and *L. umbrinum* appear to be relatively common in Europe, although previously overlooked. Among these species, *L. sphaeroconicum* is of special interest as a taxon closely resembling the classical *L. conicum*. We recommend a re-examination of *L. conicum* collections to assess the presence of *L. sphaeroconicum*.

Molecular barcoding revealed that six specimens of *L. epidendrum* s. str. belong to four ribogroups (rg01a, l, u, w). All of these ribogroups were already known from Europe (Leontyev *et al.*, 2025). Rg01a and 01u have European distribution, rg01l occurs in Europe, Asia and Australia, rg01w—in Europe and North America. The ribogroup 01u seems to be rare: only two from 255 specimens analysed by Leontyev *et al.* (2025) belong to this group; both were collected in Norway.

An analysis of the substrate distribution for the genus *Lycogala* confirms its well-known preference for coarse woody debris, including stumps, trunks, and branches, across a variety of plant species. In contrast to other species, *L. flavofusum* often develops on living tree trunks, although it was also found on stumps, logs and woody debris. The data also show that rare species occur on unusual substrates, including *L. cf. nigroconfusum* found on *Eucalyptus* and *L. paraconfusum* on *Yucca* sp.

Our inability to sequence old herbarium material highlights a methodological limitation of the routine molecular barcoding of myxomycete collections. Although high throughput sequencing methods may potentially resolve this

problem, being able to read very short sequences (Jain *et al.*, 2016), these next generation methods remain too expensive for routine studies. Future investigations would benefit from integrating targeted field sampling of DNA with morphological studies to increase the availability of fresh material for molecular work.

This study demonstrates that even in well-explored regions such as Spain (e.g., Lado, 1991) the myxomycete diversity remains incompletely documented. The revision of the Alcalá de Henares Herbarium has not only expanded the known distribution of numerous *Lycogala* species but also reinforced the importance of herbarium collections in taxonomic research, particularly when re-examined through the lens of recent taxonomic advances. Our findings contribute to the ongoing global revision of *Lycogala* and serve as a call for similar investigations in other collections.

Acknowledgements

This study was supported by a MSCA4Ukraine grant (1208119) awarded to the first author. Lab procedures were in part supported by a grant within the DFG graduate school “Response” (RTG2010) to the third author. We wish to express our gratitude to Dr. F.J. Rejos, curator of the AH herbarium, for his kind assistance in selecting specimens for this study.

This project has received funding through the MSCA4Ukraine project, which is funded by the European Union. Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union, the European Research Executive Agency or the MSCA4Ukraine Consortium. Neither the European Union nor the European Research Executive Agency, nor the MSCA4Ukraine Consortium as a whole nor any individual member institutions of the MSCA4Ukraine Consortium can be held responsible for them.

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