

Original Scientific Paper

## Considerations on the morphological features and phylogeny of the hypogeous gasteroid genera *Sclerogaster* and *Wakefieldia* (Basidiomycota) in North Macedonia

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### ABSTRACT:

The genera *Sclerogaster* and *Wakefieldia* are infrequently reported, especially from poorly studied regions of the Balkan Peninsula. Research on hypogeous fungi in North Macedonia has been advancing steadily in recent years, leading to a growing number of collections in the Macedonian Collection of Fungi. Molecular phylogenetic and morphological observations of deposited specimens of *Sclerogaster* and *Wakefieldia* were performed, and compared with collections from other areas and sequences in nucleotide databases. Molecular genetic diversity based on rDNA ITS and LSU markers and morphological characteristics of the specimens from two genera are presented, and information on their ecology is provided. Two species of *Sclerogaster*: *S. hysteroangioides*, reported for the first time for the Balkan Peninsula, and *S. compactus*, a second record for the Balkan Peninsula, were distinguished. *Sclerogaster hysteroangioides* was found in different habitat types at four sites, while *S. compactus* was present at only one site in a riparian community of *Populus alba* and *Ulmus laevis*. The genus *Wakefieldia* is represented with a single species, *W. macrospora*, in Europe. Our collections, which constitute the second records for the Balkan Peninsula, originate from mixed deciduous forest of *Quercus pubescens* and *Carpinus orientalis*. The phylogenetic analyses confirm the morphological identification of the voucher specimens as *S. hysteroangioides* and *W. macrospora*. The investigation of hypogeous fungi in North Macedonia demonstrates the rich diversity of this ecological group of fungi, and calls for future studies including comprehensive morphological observations and additional molecular markers.

**Keywords:** Balkan Peninsula, DNA-barcoding, fungi, morphology, mycodiversity, phylogenetics

Received:  
09 November 2025Revision accepted:  
13 February 2026

### INTRODUCTION

The genus *Sclerogaster* Hesse (Sclerogastraceae, Geastrales) comprises gasteroid fungi with small spherical basidiomata, a peridium that is white to light yellow, a gleba being initially white, then turning yellow or light olive-green and with very small chambers, and spherical basidiospores ornamented with small warts (PEGLER *et al.* 1993; HOSAKA & CASTELLANO 2008). Since the last review of this genus by ALFREDO *et al.* (2015), new species have been described (SULZBACHER *et al.* 2016a) and revisions have transferred certain species from *Sclerogaster* to other genera (VIDAL *et al.* 2019). Currently the genus *Sclerogaster* comprises 12 species, from the southern and northern hemispheres: *S. araripensis* Sulzbacher, J.O. Sousa & Baseia (SULZBACHER *et al.* 2016a), *S.*

UDC: 582.28:581.4+575.86(497.7)



*compactus* (Tul. & C. Tul.) Sacc. (SACCARDO 1895), *S. columellatus* (Zeller) Fogel (FOGEL 1990), *S. gastrosporoides* Pilát & Svrček (PILÁT & SVRČEK 1955), *S. hysteroangiooides* (Tul. & C. Tul.) Zeller & C.W. Dodge (ZELLER & DODGE 1935), *S. liospermus* (Tul. & C. Tul.) Soehner (SOEHNER 1924), *S. luteocarneus* (Bres.) Zeller & C.W. Dodge (ZELLER & DODGE 1935), *S. minor* Coker & Couch (COKER & COUCH 1928), *S. pacificus* Zeller & C.W. Dodge (ZELLER & DODGE 1935), *S. salisburyensis* Verwoerd (VERWOERD 1926), *S. siculus* Zeller & C.W. Dodge (ZELLER & DODGE 1935), and *S. xerophilus* Fogel (FOGEL 1977). The databases Index Fungorum (<https://www.indexfungorum.org>), Mycobank (<https://www.mycobank.org>) and Fungal Names (<https://nmdc.cn/fungal-names>) also list *S. lanatus* R. Hesse, which is regarded by most authors as synonymous with *S. compactus* (MONTECCHI & SARASINI 2000; ALFREDO *et al.* 2015), while *S. lanatus* sensu Mattiolo is considered a synonym of *S. siculus* (DODGE & ZELLER 1936). Furthermore, the names *S. porquerollensis* Donadini & G. Riouset and *S. rhizopogon* Donadini, Riouset & G. Riouset are regarded as synonyms of *Wakefieldia macrospora* (Hawker) Hawker (VIDAL 1997). Concerning their ecology, *Sclerogaster* species are saprotrophic, and most species occupy a hypogeous or semihypogeous habit, found among leaf litter, fallen branches or in thick humus layers. Only one species is known to be lignicolous, growing on white subiculum on decaying wood (HOSAKA & CASTELLANO 2008; ALFREDO *et al.* 2015).

The genus *Wakefieldia* Corner & Hawker is characterised by gasteroid basidiomata, which are spherical or irregular in shape, with a smooth, thin peridium, which is white and changes colour with age. The gleba consists of small empty chambers, and does not become gelatinous. The basidiospores are spherical, with low, broad verrucae or ridges, with a perisporium present (CORNER & HAWKER 1953; HAWKER 1954; PEGLER *et al.* 1993), HAWKER (1954) describes the ornamentation of the spores as plate-like or pitted, not echinate. The genus comprises two species, the type *W. striispora* Corner & Hawker [originally published as *W. striaespora*] described from Singapore, Southeast Asia (CORNER & HAWKER 1953) which has not been found in Europe; and *W. macrospora* (Hawker) Hawker as the sole species from this genus known in Europe. *Wakefieldia macrospora* was first described as *Sclerogaster macrosporus* Hawker (HAWKER 1951) and was transferred to *Wakefieldia* after the genus was established in 1953 (HAWKER 1954). The genus *Wakefieldia* was not formally assigned to a family in the original description by CORNER & HAWKER (1953). HAWKER (1954) proposed the classification of hypogeous species in Britain into four families, based on the developmental stages of the basidiocarps, and placed *Wakefieldia* in Hydnangiaceae, Agaricales. Later, PEGLER *et al.* (1993) and MONTECCHI & SARASINI (2000) placed the genus in the family Octavianiaceae, Cortinariales. However, molecular studies have included Octavianiaceae within Boletaceae, Boletales (BINDER & HIBBETT 2006), and the Index Fungorum currently lists *Wakefieldia* within Boletaceae. This placement reflects historical and secondary classifications rather than direct molecular evidence from the type species. Molecular analyses of European collections identified as *W. macrospora* place this taxon within the family Hymenogastraceae, order Agaricales (KAOUNAS *et al.* 2011). Nevertheless, the type species of the genus, *W. striispora*, has never been assessed using molecular data, therefore the placement of the genus *Wakefieldia* still remains unresolved.

To date, a total of 47 taxa of hypogeous fungi are known for the territory of North Macedonia, 25 taxa (21 species) of Ascomycota (KARADELEV *et al.* 2019) and 22 taxa of Basidiomycota (KARADELEV *et al.* 2018; TOFILOVSKA *et al.* 2019, 2023). Research into the diversity of hypogeous fungal species in the Republic of North Macedonia has intensified in recent years. A review of the collections deposited in the Macedonian Collection of Fungi (MCF) has been

carried out, and new material has been collected. This study focused on the rarely studied gasteroid genera *Sclerogaster* and *Wakefieldia* which led to the discovery of three species new for the country, one of which is the first, and two the second records for the Balkan Peninsula.

## MATERIALS AND METHODS

**Field data.** The materials examined were collected in the autumn of 2019 and 2020 in four biogeographical regions of the Republic of North Macedonia, namely the mountains of Bistra (biogeographic region code – BRC 10309), Shar Planina (BRC 10102) and Vodno (BRC 20617), and in the valley of Skopsko Pole (BRC 61976) (MELOVSKI *et al.* 2013). The basidiomata were retrieved by truffle hunters with trained dogs. For each collection, the data on habitat type, elevation, and locality were noted. These are presented under the description and ecology of the species.

**Morphological analysis.** The fresh basidiomata were photographed in the field or in the lab. The specimens were dried in an air dehydrator SIGG Dörrex (Switzerland) at 50°C and deposited in the MCF. The microscopic analyses were carried out on fresh and dried basidiomata. The peridium and gleba were examined on slides prepared from cross-sections and mounted in tap water, Melzer's reagent and 3% KOH (PEGLER *et al.* 1993). The slides were observed under a LW Scientific i4 microscope (Georgia, USA) and the photos taken using a LW Scientific MiniVID USB 1MP camera (Georgia, USA). Figures were assembled in Adobe Photoshop CC 2022. The slides prepared in 3% KOH were analysed under a ZEISS Primostar 3 microscope (Germany) and the photos were taken using a ZEISS Axiocam 208 colour microscope camera (Germany) and the ZEN 3.0 blue edition software. The measurements are based on randomly selected mature basidiospores, excluding spore ornamentation and apiculus; 50 basidiospores from one collection of *Sclerogaster compactus* and 30 mature basidiospores from two collections (total 60) each of *S. hysterangioides* and *Wakefieldia macrospora* were measured in mounting medium 3% KOH. The length and width of the basidiospores along with the minimum, maximum, and average values with standard deviation are presented; quotient (Q) and average quotient (Qav). The measurement of the peridium width is presented based on 10 cuttings per species, while the size of the hyphae or the spherical elements of the peridium are based on 20 measurements.

**Molecular genetic analysis.** DNA was extracted from a small piece of gleba using the Qiagen DNeasy Plant MiniKit (Qiagen, Hilden, Germany), following the manufacturer's instructions. The extracted DNA was re-suspended in prewarmed sterile milli-Q water to an approximate final concentration of 100 ng  $\mu\text{l}^{-1}$  and stored at -80°C at the Slovenian Forestry Institute DNA library and at the Department of Botany and Biodiversity Research, University of Vienna.

The complete nuclear Internal Transcribed Spacer (ITS) region of the rDNA was amplified using primer pairs ITS1F/ITS4 (WHITE *et al.* 1990). PCR reactions were performed as follows: 1.0  $\mu\text{l}$  DNA; 2.5  $\mu\text{l}$  PCR buffer 10 $\times$ ; 3.0  $\mu\text{l}$  dNTPs (1.5 mM); 2.0  $\mu\text{l}$  MgCl<sub>2</sub> (20 mM); 3.0  $\mu\text{l}$  of each primer (25 pmol); 0.5 U Taq polymerase (5 U  $\mu\text{l}^{-1}$ ); and 10.5  $\mu\text{l}$  of ultrapure water. The amplification conditions of the ITS region followed SULZBACHER *et al.* (2016b).

A fragment containing the complete ITS and a partial large subunit (LSU) of rDNA was amplified as a single stretch using the primer pair V9G (DE HOOG & GERRITS VAN DEN ENDE 1998)/LR5 (VILGALYS & HESTER 1990). PCR reactions were performed as follows: 5  $\mu\text{l}$  Phusion Plus 2.0 Master mix, 1.0  $\mu\text{l}$  DNA extract; 1.0  $\mu\text{l}$  of each primer (5 pmol); and 3  $\mu\text{l}$  of ultrapure water, using the following programme: 98°C 30 s, 35 cycles of 98°C 15 s, 55°C 15 s, 72°C 1

min; final extension 72° 5 min. All amplifications were done in a GeneAmp® PCR System 9700 thermal cycler (Applied Biosystems, Foster City, CA, USA).

For the ITS, the PCR products were purified from agarose gel using the Wizard SV Genomic DNA Purification System (Promega Corporation, Madison, WI, USA); for the ITS-LSU the enzymatic PCR cleanup (WERLE *et al.* 1994) as described in VOGLMAYR & JAKLITSCH (2008) was applied. For the ITS, both DNA strands were sequenced separately at MacroGen Europe B.V. (Amsterdam, The Netherlands) with the same primers used in the amplification; Sequencher v. 5.4.6 (Gene Codes Corporation, Ann Arbor, MI, U.S.A.) was used to assemble the consensus sequence. For the ITS-LSU fragment, DNA was cycle-sequenced using the ABI PRISM Big Dye Terminator Cycle Sequencing Ready Reaction Kit v. 3.1 (Applied Biosystems, Warrington, UK) with the same primers as for PCR; in addition, primers ITS4 (WHITE *et al.* 1990) and LR0R (MONCALVO *et al.* 1995) were also applied. Sequencing of the ITS-LSU fragment was performed on an automated DNA sequencer (3730xl Genetic Analyzer, Applied Biosystems) at the Department of Botany and Biodiversity Research, University of Vienna. The sequences were assembled using DNASTAR Lasergene SeqMan Pro v. 7.1 (Madison, MI, USA).

All consensus sequences generated in this study are deposited in GenBank (<https://www.ncbi.nlm.nih.gov/>). The sequences used in the phylogenetic analyses are listed in Table 1 and newly sequenced specimens are marked in bold.

**Phylogenetic analyses.** The phylogenetic analyses of ITS and LSU rDNA were based on the GenBank sequences included in the studies carried out by HOSAKA & CASTELANO 2008 (for *Sclerogaster*) and KAOUNAS *et al.* 2011 (for *Wakefieldia*), complemented with additional GenBank sequences retrieved by BLASTn searches. In addition, ITS sequences were also downloaded from the UNITE data base. Four sequences of *Geastrum* Pers. were used as the outgroup in the analyses of *Sclerogaster* (ALFREDO *et al.* 2015) and three of *Psilocybe* (Fr.) P. Kumm. in the analyses of *Wakefieldia* (KAOUNAS *et al.* 2011). Multiple sequence alignments of the three data sets, LSU and ITS for *Sclerogaster* and ITS for *Wakefieldia*, were performed in MAFFT7 (<https://mafft.cbrc.jp/alignment/server/index.html>; KATO *et al.* 2019) using the default settings. Maximum likelihood (ML) analyses based on single-gene datasets were performed in raxmlGUI 2.0.16 (EDLER *et al.* 2021), implementing the rapid bootstrap (BS) settings with 1000 BS replicates. Based on the Model Tests (DARRIBA *et al.* 2020) run in raxmlGUI using the corrected Akaike information criterion (AICc), the GTR + I + G model was selected for the *Sclerogaster* LSU matrix and the HKY85 +I +G model for the ITS matrices of *Sclerogaster* and *Wakefieldia*. The tree was visualised and rooted in PAUP\*4.0a (SWOFFORD 2003). For the evaluation and discussion of bootstrap support, values below 70% are considered low/weak, between 70 and 89% medium/moderate, and between 90 and 100% high.

## RESULTS

**Molecular phylogenetic results.** LSU and ITS sequences were obtained from two voucher specimens of *S. hysteroangioides*, and from one of *S. compactus*, while only ITS sequences were generated from two voucher specimens of *W. macrospora* (Table 1).

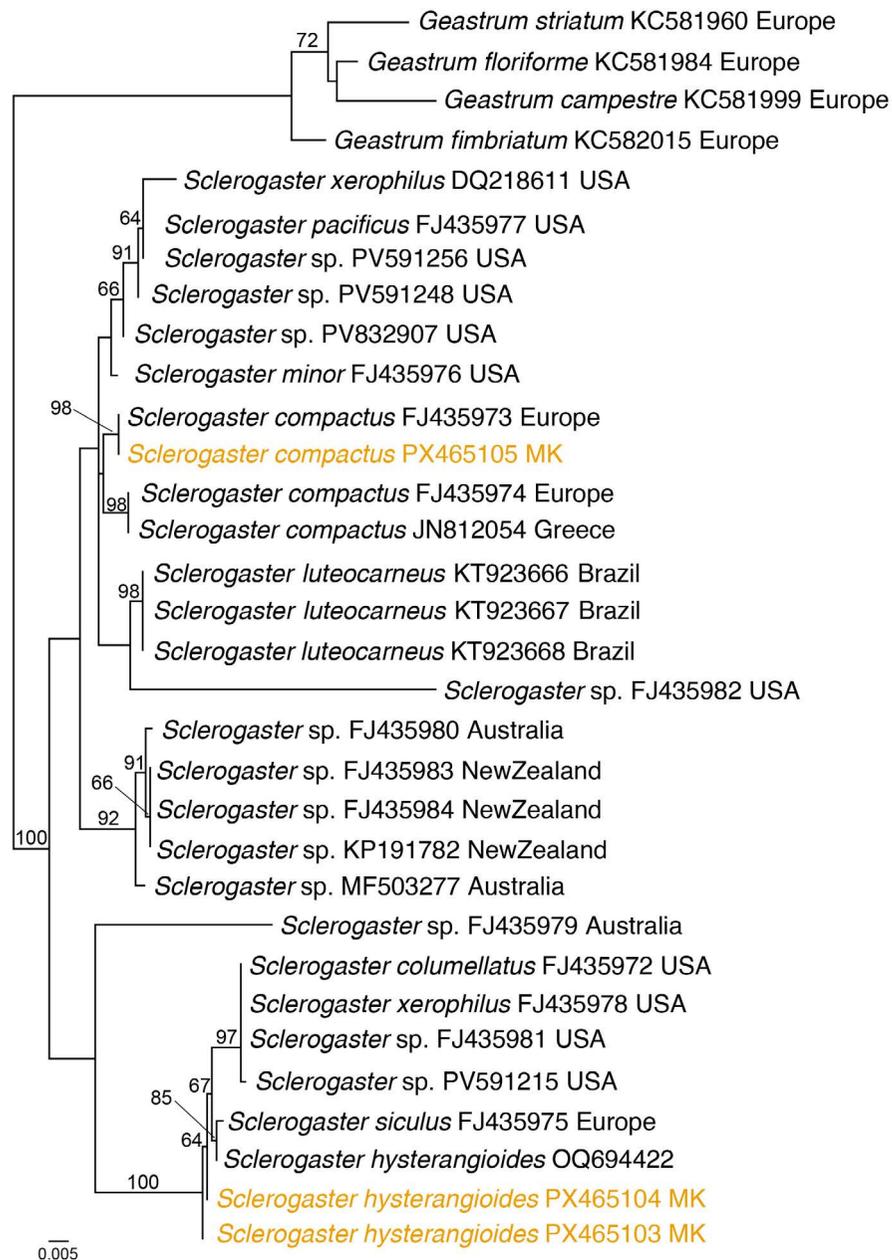
The LSU matrix of *Sclerogaster* consisted of 907 nucleotide characters from 28 accessions of *Sclerogaster* and four from the outgroup (*Geastrum* spp.), of which 765 characters were constant, 33 variable but parsimony uninformative and 109 parsimony informative. The ITS matrix of *Sclerogaster* comprised 732 nucleotide characters from 21 accessions of *Sclerogaster* and four from the

**Table 1.** Data on the LSU and ITS sequences used in the phylogenetic analyses. The sequences generated in this study are marked in bold.

Taxa	Origin	Material type	Specimen Voucher/ Isolate Number	GenBank/UNITE accession		
				LSU	ITS	Reference
<i>Alnicola badia</i>	Austria	basidioma	TU-EST:L7191		JN943936	SCHOCH <i>et al.</i> 2012
<i>Alnicola citrinella</i>	Estonia	basidioma	TU-EST:TU110341		JN943967	SCHOCH <i>et al.</i> 2012
<i>Alnicola citrinella</i>	France	basidioma	PAM08100405		HQ714733	ROCHET <i>et al.</i> 2011
<i>Alnicola escharioides</i>	France	basidioma	PAM03/99		AY900086	MOREAU <i>et al.</i> 2006
<i>Alnicola silvaenovae</i>	Estonia	basidioma	TU-EST:L3927		JN943941	SCHOCH <i>et al.</i> 2012
<i>Alnicola sp.</i>	USA	basidioma	WTU-F-073033		PV536737	direct submission
<i>Alnicola umbrina</i>	Estonia	basidioma	TU-EST:TU110268		JN943987	SCHOCH <i>et al.</i> 2012
<i>Geastrum campestre</i>	Europe	basidioma	MJBN061025	KC581999	KC581999	JEPSON <i>et al.</i> 2013
<i>Geastrum fimbriatum</i>	Europe	basidioma	MJ5706	KC582015	KC582015	JEPSON <i>et al.</i> 2013
<i>Geastrum floriforme</i>	Europe	basidioma	MJ5421	KC581984	KC581984	JEPSON <i>et al.</i> 2013
<i>Geastrum striatum</i>	Europe	basidioma	MJ8807	KC581960	KC581960	JEPSON <i>et al.</i> 2013
<i>Hebeloma birrus</i>	Netherlands	basidioma	KDAd580		AF124693	AANEN <i>et al.</i> 2000
<i>Hebeloma crustuliniforme</i>	China	basidioma	HKAS122681		ON794430	WANG <i>et al.</i> 2022
<i>Hebeloma crustuliniforme</i>	Sweden	basidioma	DKAm503-2		AF124668	AANEN <i>et al.</i> 2000
<i>Hebeloma danicum</i>	Poland	basidioma	IK-H0170		KX687198	KALUCKA <i>et al.</i> 2016
<i>Hebeloma heloides</i>	Netherlands	basidioma	DKAd665		AF124674	AANEN <i>et al.</i> 2000
<i>Hebeloma sinapizans</i>	Danmark	basidioma	DKAd514		AF124682	AANEN <i>et al.</i> 2000
						EBERHARDT <i>et al.</i> 2013
<i>Hebeloma syrjense</i>	Finland	basidioma	TURA:26197F		JQ751218	
<i>Hebeloma velutipes</i>	Sweden	basidioma	DKAd504-2		AF124677	AANEN <i>et al.</i> 2000
<i>Hymenogaster griseus</i>	England	basidioma	RBG Kew K(M)20266		EU784361	BROCK <i>et al.</i> 2009
<i>Hymenogaster populetorum</i>	Netherlands	basidioma	deVries 764		AF325637	PEINTNER <i>et al.</i> 2001
<i>Hymenogaster rehsteineri</i>	Hungary	basidioma	zb1503		GU479309	STIELOW <i>et al.</i> 2011
<i>Hymenogaster rehsteineri</i>	Hungary	basidioma	zb1842		GU479319	STIELOW <i>et al.</i> 2011
<i>Hymenogaster vulgaris</i>	England	basidioma	RBG Kew K(M)27363		EU784365	BROCK <i>et al.</i> 2009
						BOROVICKA <i>et al.</i> 2015
<i>Psilocybe atrobrunnea</i>	Sweden	basidioma	PRM:860905		HF912348	
						BOROVICKA <i>et al.</i> 2015
<i>Psilocybe semilanceata</i>	Czechia	basidioma	PRM:921860		HF912359	
	Unknown					
<i>Psilocybe semilanceata</i>	(USA?)	basidioma	UBC:F15293		OM276808	BRADSHAW <i>et al.</i> 2022
						HOSAKA & CASTELLANO 2008
<i>Sclerogaster columellatus</i>	USA	basidioma	Trappe 8098	FJ435972		
						HOSAKA & CASTELLANO 2008
<i>Sclerogaster compactus</i>	Europe	basidioma	WSL-KH01	FJ435973		
						HOSAKA & CASTELLANO 2008
<i>Sclerogaster compactus</i>	Europe	basidioma	Trappe 6136	FJ435974		
						CASTELLANO 2008
<i>Sclerogaster compactus</i>	Europe	basidioma	VK2040	JN812054		
						KAOUNAS <i>et al.</i> 2011
<b><i>Sclerogaster compactus</i></b>	<b>North Macedonia</b>	<b>basidioma</b>	<b>MCF17829</b>	<b>PX465105</b>	<b>PX523837</b>	<b>this study</b>
<b><i>Sclerogaster hysteroangioides</i></b>	<b>North Macedonia</b>	<b>basidioma</b>	<b>MCF17905</b>	<b>PX465103</b>	<b>PV170768</b>	<b>this study</b>
<b><i>Sclerogaster hysteroangioides</i></b>	<b>North Macedonia</b>	<b>basidioma</b>	<b>MCF17988</b>	<b>PX465104</b>	<b>PV170767</b>	<b>this study</b>
	Unknown					
<i>Sclerogaster hysteroangioides</i>	(USA?)		SCL2.BST	OQ694422	OQ694422	direct submission
<i>Sclerogaster luteocarneus</i>	Brazil	basidioma	UFRN-Fungos 1859	KT923666	KT923663	ALFREDO <i>et al.</i> 2015
<i>Sclerogaster luteocarneus</i>	Brazil	basidioma	UFRN-Fungos 2277	KT923667	KT923664	ALFREDO <i>et al.</i> 2015
<i>Sclerogaster luteocarneus</i>	Brazil	basidioma	UFRN-Fungos 2278	KT923668	KT923665	ALFREDO <i>et al.</i> 2015
						HOSAKA & CASTELLANO 2008
<i>Sclerogaster minor</i>	USA	basidioma	Trappe 8720	FJ435976		

<i>Sclerogaster pacificus</i>	USA	basidioma	Trappe 9011	FJ435977			HOSAKA & CASTELLANO 2008
<i>Sclerogaster siculus</i> (in GenBank as <i>S. lanatus</i> )	Europe	basidioma	Hintz 783	FJ435975			HOSAKA & CASTELLANO 2008
<i>Sclerogaster</i> sp.	Australia	basidioma	H4595	FJ435979			HOSAKA & CASTELLANO 2008
<i>Sclerogaster</i> sp.	Australia	basidioma	Trappe 15701	FJ435980			HOSAKA & CASTELLANO 2008
<i>Sclerogaster</i> sp.	Australia	basidioma	F44_S0059	MF503277	KY697609		NUSKE <i>et al.</i> 2018
<i>Sclerogaster</i> sp.	Australia	basidioma	44C_S0364b		KY697608		NUSKE <i>et al.</i> 2018
<i>Sclerogaster</i> sp.	New Zealand	basidioma	KH-NZ06-209	FJ435983			HOSAKA & CASTELLANO 2008
<i>Sclerogaster</i> sp.	New Zealand	basidioma	KH-NZ06-210	FJ435984			HOSAKA & CASTELLANO 2008
<i>Sclerogaster</i> sp.	New Zealand	basidioma	PDD 100944	KP191782	KP191950		direct submission
<i>Sclerogaster</i> sp.	USA	basidioma	Zeller 7425	FJ435981			HOSAKA & CASTELLANO 2008
<i>Sclerogaster</i> sp.	USA	basidioma	Zeller 8462	FJ435982			HOSAKA & CASTELLANO 2008
<i>Sclerogaster</i> sp.	USA	basidioma	HAD1433 iNat#242227452	PV591215	PV591127		direct submission
<i>Sclerogaster</i> sp.	USA	basidioma	HAD1022 iNat#195896136	PV832907	PV832741		direct submission
<i>Sclerogaster</i> sp.	USA	basidioma	HAD1379 iNat#242229237	PV591248	PV591164		direct submission
<i>Sclerogaster</i> sp.	USA	basidioma	HAD1366 iNat#242227451	PV591256	PV591174		direct submission
<i>Sclerogaster</i> sp.	USA	basidioma	JLF13696 AZ		PQ836643		direct submission
<i>Sclerogaster</i> sp.	USA	basidioma	JLF13620 AZ		PQ836642		direct submission
<i>Sclerogaster</i> sp.	USA	basidioma	TENN:F 075952		OK376732		direct submission
<i>Sclerogaster</i> sp.	USA	basidioma	FLAS-F-61806		MH399884		direct submission
<i>Sclerogaster</i> sp.	USA	basidioma	JLF13626 iNaturalist 247838971		PQ479640		direct submission
<i>Sclerogaster</i> sp.	USA	basidioma	FDS-CA-03812		PV742652		direct submission
<i>Sclerogaster xerophilus</i>	USA	basidioma	Wright 1956	FJ435978			HOSAKA & CASTELLANO 2008
<i>Sclerogaster xerophilus</i>	USA	basidioma	OSC 49777	DQ218611			HOSAKA <i>et al.</i> 2006
<i>Sclerogaster compactus</i>	Europe	basidioma	AT2001105		UDB001213		direct submission
<i>Wakefieldia macrospora</i> (in GenBank as uncultured <i>Hebeloma</i> )	France	ectomycorrhizal root tips	2008AIB11		HQ204662		RICHARD <i>et al.</i> 2011
<i>Wakefieldia macrospora</i> (in GenBank as uncultured <i>Hebeloma</i> )	France	ectomycorrhizal root tips	2008ALC10		HQ204659		RICHARD <i>et al.</i> 2011
<i>Wakefieldia macrospora</i>	Greece	basidioma	VK1379		JN812039		KAOUNAS <i>et al.</i> 2011
<b><i>Wakefieldia macrospora</i></b>	<b>North Macedonia</b>	<b>basidioma</b>	<b>MCF17950</b>		<b>PV170765</b>		<b>this study</b>
<b><i>Wakefieldia macrospora</i></b>	<b>North Macedonia</b>	<b>basidioma</b>	<b>MCF17977</b>		<b>PV170766</b>		<b>this study</b>
<i>Wakefieldia macrospora</i>	USA	basidioma	Trappe19331		OQ566918		direct submission
<i>Wakefieldia macrospora</i> (in GenBank as <i>W. sp.</i> ; in UNITE species hypothesis <i>W. macrospora</i> SH1563873.08FU)	Hungary	soil	ASV0939		OP042630		GEML <i>et al.</i> 2022

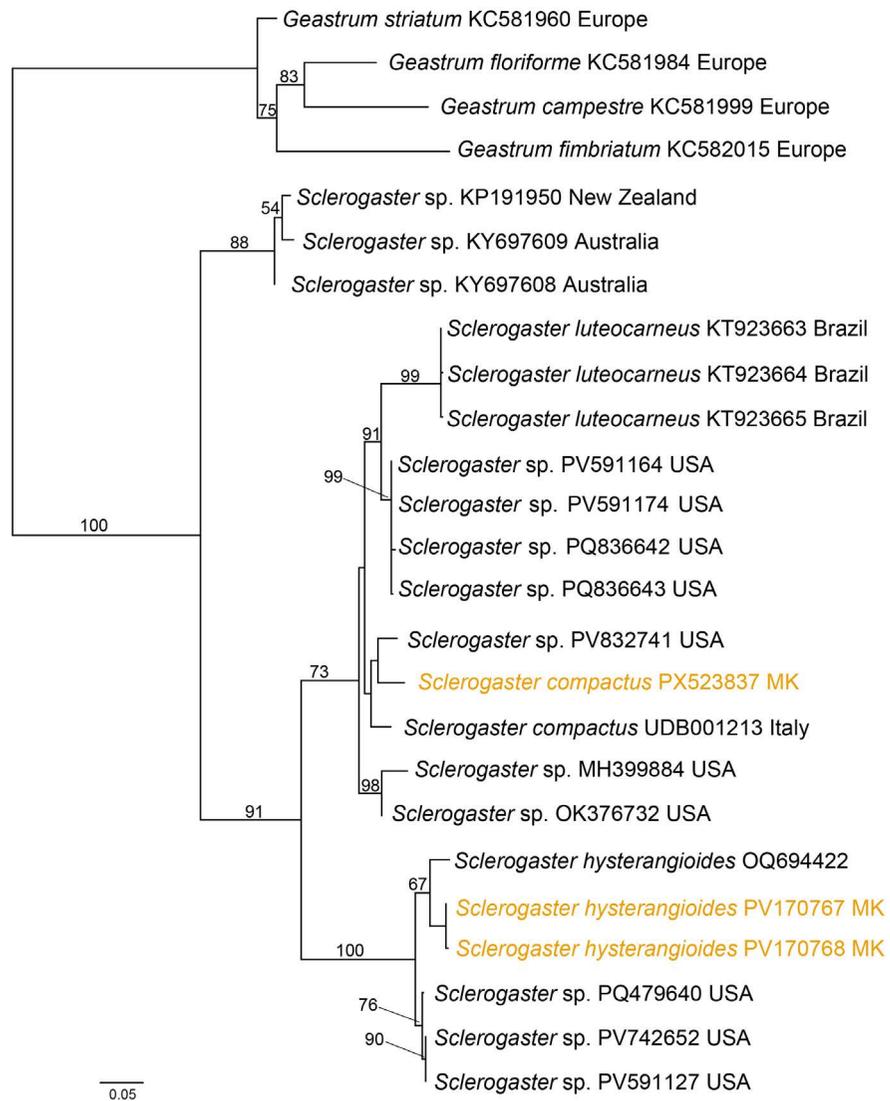
**Fig. 1.** Phylogram of the best ML tree revealed by RAxML from analyses of the LSU matrix of *Sclerogaster* (-lnL = 2576.45). GenBank accession numbers and country/area of origins are given following the names of the taxa; the accessions from North Macedonia are coloured in orange, the bootstrap values (>50%) are reported above each branch.



outgroup (*Gastrum* spp.), of which 421 characters were constant, 85 variable but parsimony uninformative and 226 parsimony informative.

Figures 1 and 2 show the phylograms of the best ML trees from the analyses of the LSU and ITS matrices of *Sclerogaster*, respectively. The phylogenetic analyses of the LSU matrix placed *S. compactus* from North Macedonia within an unsupported *S. compactus* clade where it formed a highly supported subclade (98% BS) with *S. compactus* accession FJ435973 from Europe. In contrast, the ITS analyses placed *S. compactus* from North Macedonia as sister to an unidentified *Sclerogaster* accession PV832741 from Oregon (USA), and both were sister to *S. compactus* accession UDB001213 from Italy; however, without bootstrap support. The three *S. hysterangioides* accessions included in the LSU matrix did not form a monophylum, but were contained within a highly supported clade (100% BS) also containing, amongst unidentified accessions, *S. columellatus*, *S. siculus* and *S. xerophilus*. In the ITS analysis, the three *S. hysterangioides* accessions included were placed in a poorly supported

**Fig. 2.** Phylogram of the best ML tree revealed by RAxML from analyses of the ITS matrix of *Sclerogaster* ( $-\ln L = 3618.73$ ). GenBank accession numbers and country/area of origins are given following the names of the taxa; the accessions from North Macedonia are coloured in orange, the bootstrap values ( $>50\%$ ) are reported above each branch.

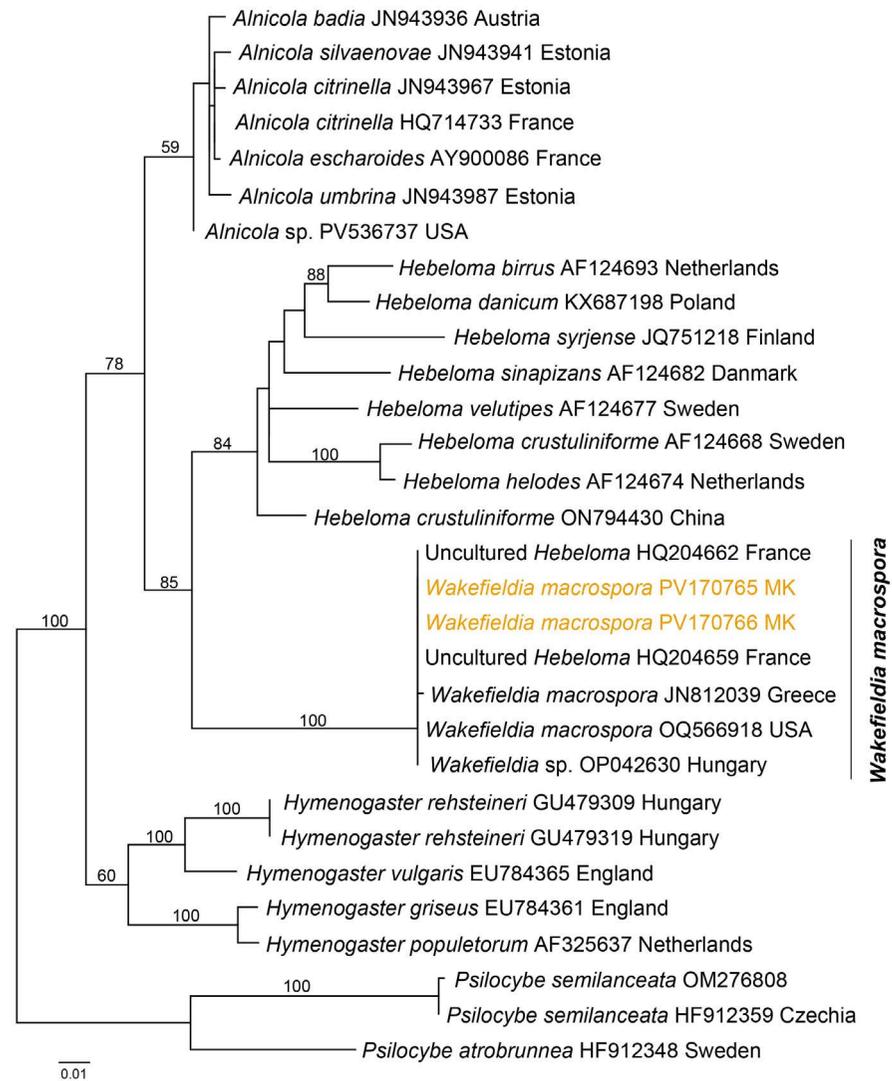


monophylum (67% BS), with the two North Macedonian accessions forming a highly supported subclade (98% BS).

The ITS matrix of *Wakefieldia* consisted of 30 representative accessions of Hymenogastraceae (from the genera *Alnicola*, *Hebeloma*, *Hymenogaster*, *Psilocybe*, and *Wakefieldia*). Of the 664 characters, 469 characters were constant, 53 variable but parsimony uninformative and 142 parsimony informative.

Figure 3 shows the phylogram of the best ML tree from the analysis of the ITS matrix of *Wakefieldia*. Most of the tree backbone receives bootstrap support; while support for the genera *Alnicola* (59% BS) and *Hymenogaster* (60% BS) is low and for *Hebeloma* moderate (84% BS), with the genus *Wakefieldia* receiving maximum support. Apart from four *W. macrospora* accessions, which represent isolated DNA from basidiomata (including the two from North Macedonia), the clade also contains three accessions of environmental samples; one from Hungary noted as *Wakefieldia* sp. in GenBank and as species hypothesis *W. macrospora* in UNITE, and two accessions from France identified as uncultured *Hebeloma* sp. As all ITS sequences of the *Wakefieldia* clade are fully or almost identical (99.83% with JN812039 from Greece; all others 100% identical), they all are confirmed to represent a single species, *W. macrospora*.

**Fig. 3.** Phylogram of the best ML tree revealed by RAxML from analyses of the ITS matrix of *Wakefieldia* (-lnL = 2835.41). GenBank accession numbers and country/area of origins are given following the names of the taxa; the accessions from North Macedonia are coloured in orange, the bootstrap values (>50%) are reported above each branch.



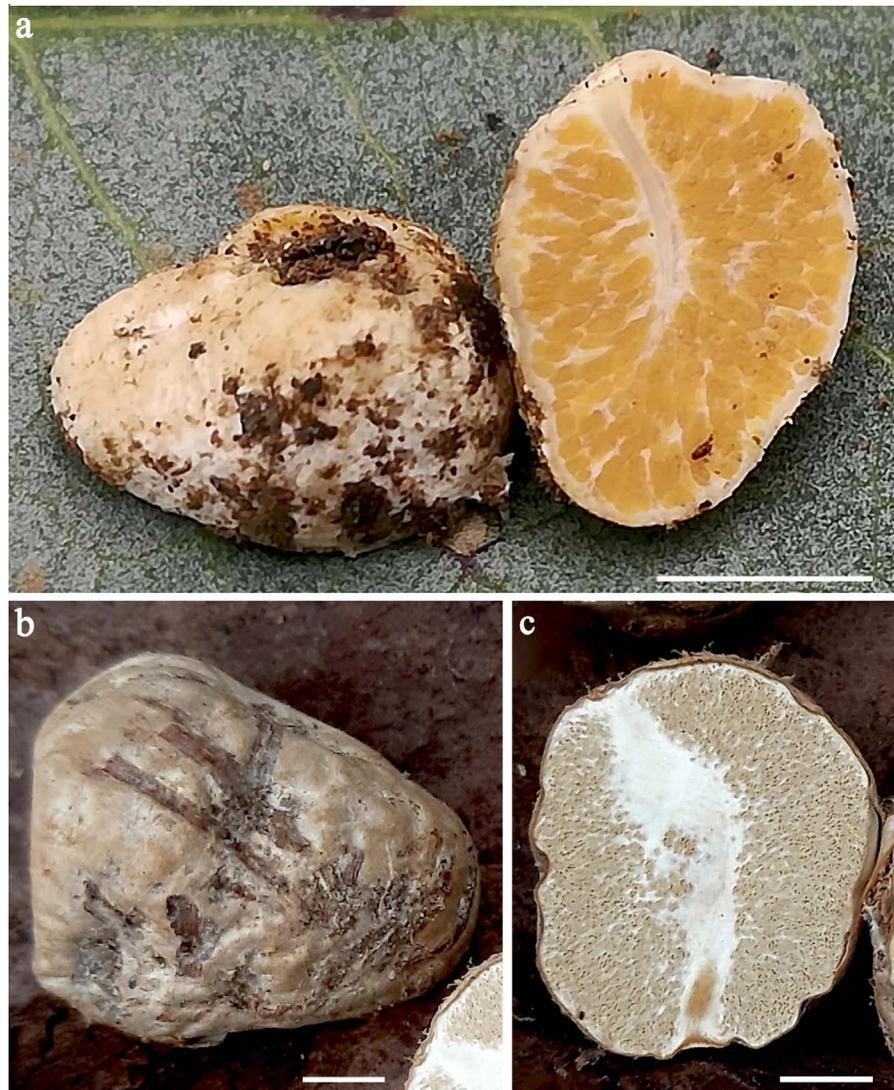
### Taxonomy and ecology

*Sclerogaster compactus* (Tul. & C. Tul.) Saccardo, *Sylloge Fungorum omnium hucusque cognitorum* 11: 170 (1895); Figs. 4a, 5a–d, 6a–b.

**Macroscopic features.** The basidioma is hypogeous, gasteroid, subglobose to ovoid, 6.6 mm long and 5 mm wide (Fig. 4a). The peridium is white, becoming yellow in places, dry, smooth with debris on the surface, and not separable from the gleba. The gleba is compact, yellow-orange with white veins, with a columella present, extending from the base to the centre, 0.3 mm wide, 3.2 mm long (around half of the length of the basidioma). The odour is indistinct.

**Microscopic features.** The peridium is two-layered, with a total average width of 200  $\mu\text{m}$ , varying between 130  $\mu\text{m}$  and 250  $\mu\text{m}$  (Fig. 5a–b). The external peridium layer averaged 50  $\mu\text{m}$  in thickness, varying between 30  $\mu\text{m}$  and 80  $\mu\text{m}$ , composed of thin, interwoven hyaline hyphae, 1–2  $\mu\text{m}$  wide with cell walls  $\pm$  0.5  $\mu\text{m}$  thick. The internal peridium layer averaged 150  $\mu\text{m}$  in thickness, varying between 70  $\mu\text{m}$  and 210  $\mu\text{m}$ , and was pseudoparenchymatous (Fig. 6a), composed of polygonal, subglobose, ovoid elements, sometimes more elongated, 4.5–30  $\times$  4–20  $\mu\text{m}$  (4.5  $\times$  4; 7.3  $\times$  7.3; 13  $\times$  10.5; 26  $\times$  17; 30  $\times$  10; 30  $\times$  20), wall  $\pm$  1  $\mu\text{m}$  (0.7–1.5) thick. The gleba was composed of irregularly shaped chambers, separated by trama (Fig. 5a). The chambers were filled with

**Fig. 4.** *Sclerogaster compactus* (a) and *S. hysterangioides* (b, c), basidiomata. Scale bars: 3 mm.



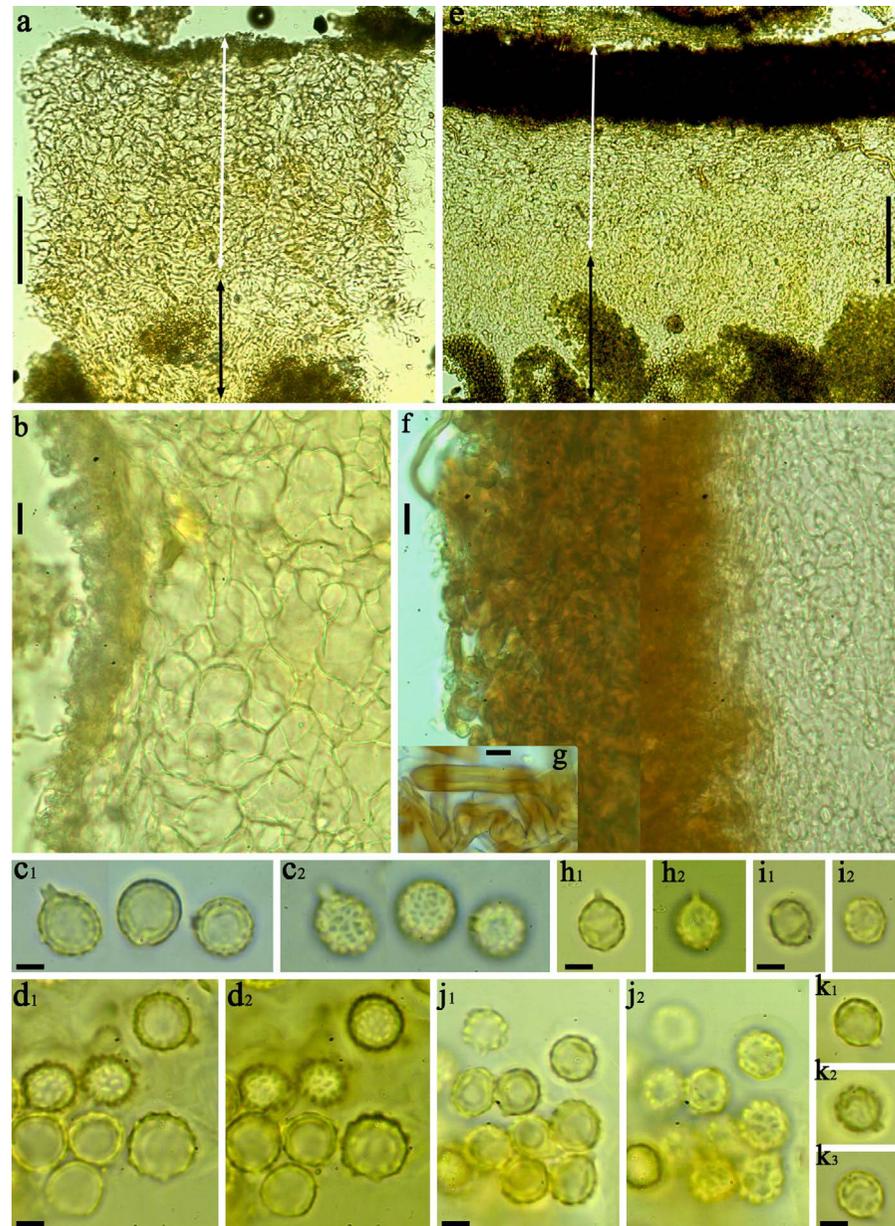
basidia and basidiospores. The trama comprised interwoven hyphae, 5–10  $\mu\text{m}$  wide, wall  $\pm 1 \mu\text{m}$  thick, with the presence of inflated cells in places (Fig. 6b). Basidia were not found. The basidiospores were mainly globose, some slightly subglobose, (4.5–)5.5–7.5(–8.5)  $\times$  (4.2–)5–7(–8.5)  $\mu\text{m}$ ,  $6.7 \pm 0.7 \times 6.3 \pm 0.7 \mu\text{m}$  on average ( $n = 50$ ),  $Q = 1–1.28$ ,  $Q_{\text{av}} = 1.05$ , spore wall  $\pm 1 \mu\text{m}$  thick, ornamented with small warts of different shapes, conical, rounded or triangular at the base. The warts were 0.5–1.2  $\mu\text{m}$  high, and the apiculus was present,  $\pm 1.5 \mu\text{m}$  wide, usually 1.5  $\mu\text{m}$  long, with the longest apiculus observed 4.2  $\mu\text{m}$  (Fig. 5c–d).

Specimen examined. North Macedonia: Skopsko Pole valley, under leaf litter in a riparian community of *Populus alba* L. and *Ulmus laevis* Pall., elev. 280 m.a.s.l., leg. Tome Jovanovski & Slavica Tofilovska, MCF 17829 (10.11.2020).

*Sclerogaster hysterangioides* (Tul. & C. Tul.) Zeller & C.W. Dodge, *Annals of the Missouri Botanical Garden* 22: 370 (1935); Figs. 4b–c, 5e–k, 6c–d.

Macroscopic features. The basidiomata was hypogeous, gasteroid, globose, subglobose or ovoid, 10–18 mm long and 8–15 mm wide (Fig. 4b). The peridium was initially white, soon changing colour to pale yellow-ochre when handled, with debris and mycelium cords present on the surface, attached to the gleba, separating while drying. The gleba was light yellow, compact with

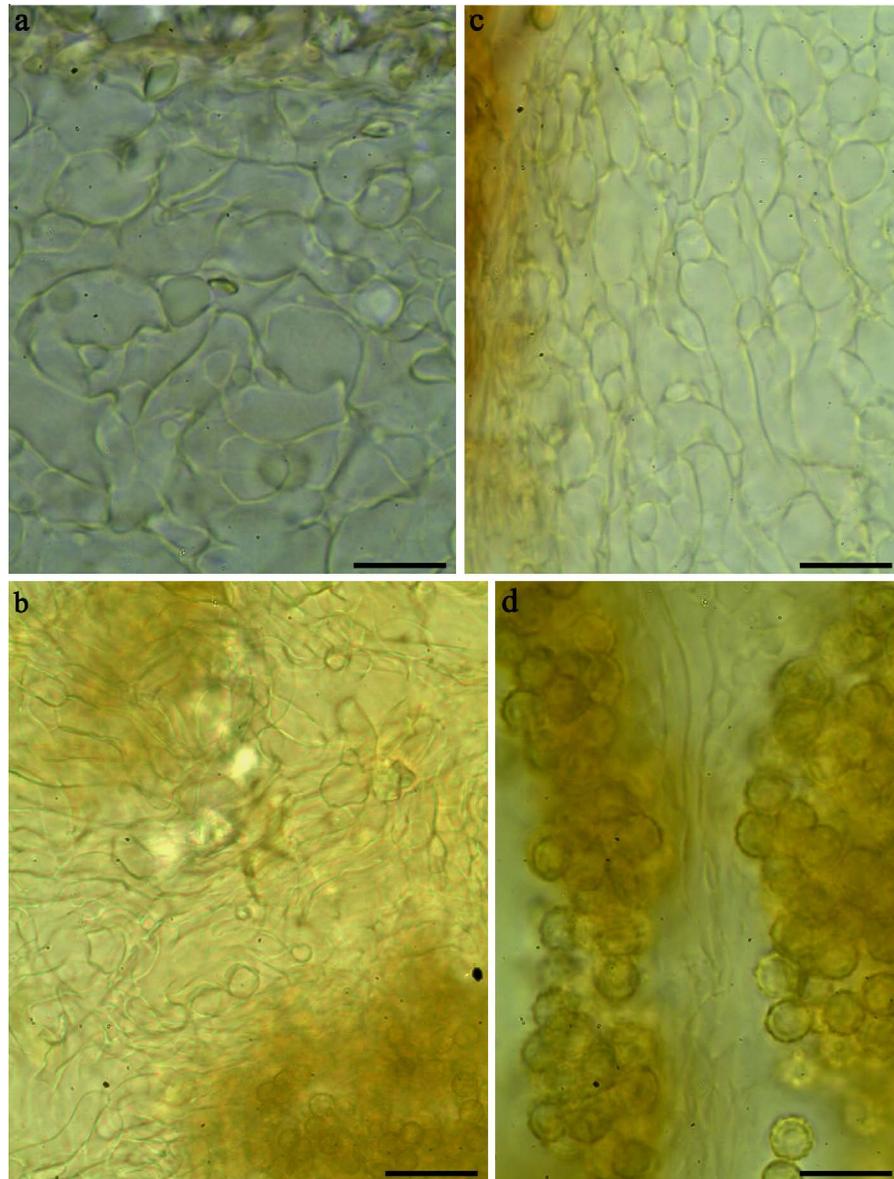
**Fig. 5.** *Sclerogaster compactus* (a-d) and *S. hysterangioides* (e-k) microscopic morphological features: **a.** cross-section, peridium (white arrow), gleba (black arrow); **b.** peridium external filamentous and internal pseudoparenchymatous layer; **c-d.** basidiospores; **e.** cross-section, peridium (white arrow), gleba (black arrow); **f.** peridium layers, external of thick-walled and internal of thin-walled hyphae; **g.** hyphae of external peridium; **h-k.** basidiospores. Scale bars: a, e, 100  $\mu\text{m}$ ; b, f, 10  $\mu\text{m}$ ; g, 5  $\mu\text{m}$ ; c, d, h-k, 3  $\mu\text{m}$ .



visible chambers, with white veins in some places. Columella were present in different shapes and sizes, spreading from the base to half of the length of the basidioma or along the whole basidioma, 0.4–2.7 mm wide (Fig. 4c), sometimes hardly visible in drying basidioma or even absent. A very strong odour was present, unpleasant, and reminiscent of naphthalene.

**Microscopic features.** The peridium was two-layered, with an average total width of 250  $\mu\text{m}$ , varying between 210  $\mu\text{m}$  and 380  $\mu\text{m}$  (Fig. 5e). The external peridium layer averaged 120  $\mu\text{m}$  thick, varying between 90  $\mu\text{m}$  and 200  $\mu\text{m}$ , composed of densely interwoven hyphae, 5–6.5  $\mu\text{m}$  wide with cell walls  $\pm 2$   $\mu\text{m}$  thick, dextrinoid (Fig. 5f–g). The internal peridium averaged 140  $\mu\text{m}$  thick, varying between 110  $\mu\text{m}$  and 180  $\mu\text{m}$ , and was pseudoparenchymatous (Fig. 6c), comprising mainly ovoid and subglobose as well as polygonal elements, 4–18  $\times$  2.5–13  $\mu\text{m}$  (4  $\times$  2.5; 5.5  $\times$  5.5; 9  $\times$  7; 18  $\times$  13), wall  $\leq 1$   $\mu\text{m}$  thick. The gleba comprised chambers of various shapes, filled with basidia and basidiospores, which were separated by trama composed of interwoven hyphae, 3  $\mu\text{m}$

**Fig. 6.** *Sclerogaster compactus* (a, b) and *S. hysterangioides* (c, d) microscopic morphological features: a. internal pseudoparenchymatous peridium; b. tramal hyphae; c. internal pseudoparenchymatous peridium; d. tramal hyphae. Scale bars: 10  $\mu$ m.



wide, wall 0.5  $\mu$ m thick (Fig. 6d). Basidia were not found. The basidiospores were subglobose, globose to ovoid (4.2–)4.3–5.4(–5.7)  $\times$  (3.9–)4–5(–5.5)  $\mu$ m,  $4.8 \pm 0.3 \times 4.5 \pm 0.3 \mu$ m on average ( $n = 60$ ),  $Q = 1-1.22$ ,  $Q_{av} = 1.06$ , wall  $\pm 1 \mu$ m thick, ornamented with small warts of different shapes, sometimes hardly visible. The warts were 0.44–0.88  $\mu$ m high, with the apiculus present but not easily observed, 0.8–1.5  $\mu$ m wide, usually 0.8  $\mu$ m long (Fig. 5h–k).

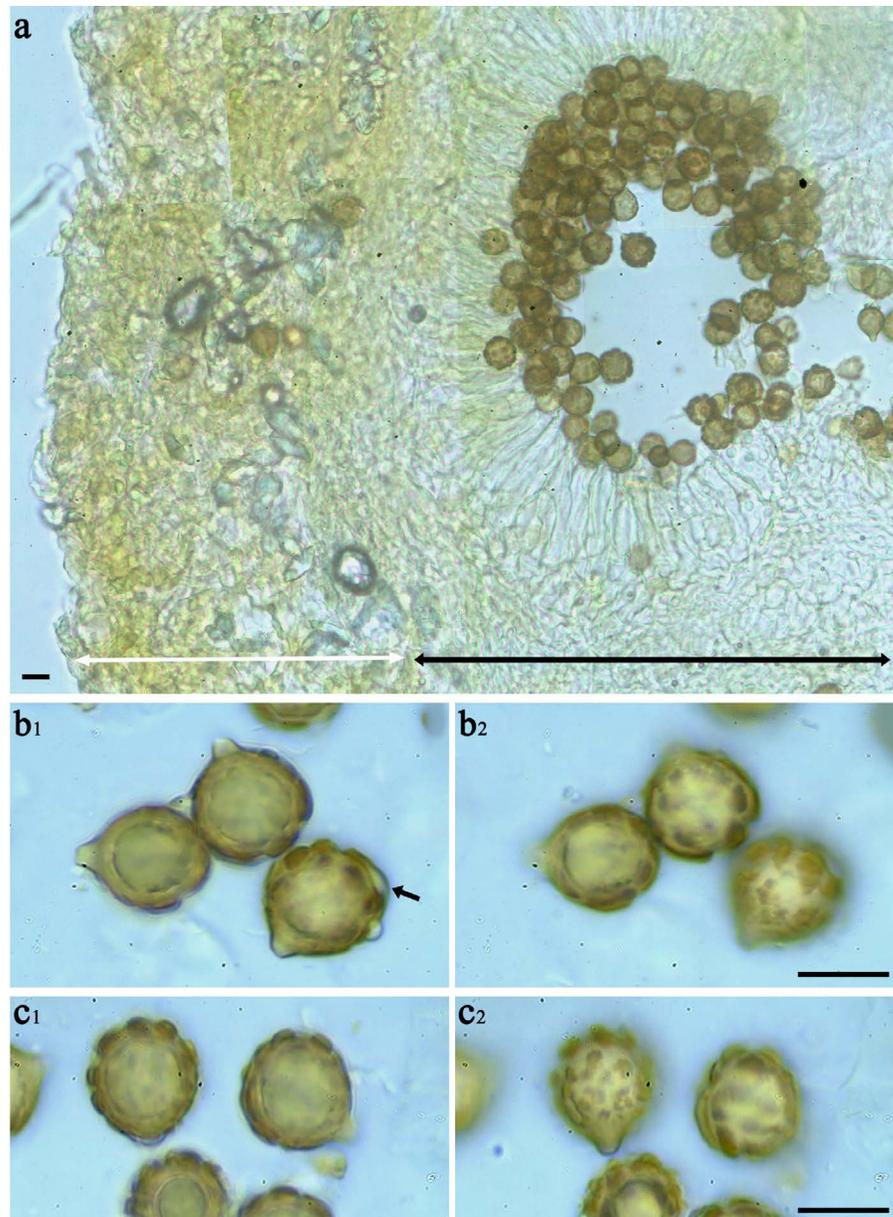
Specimens examined. North Macedonia: Mt. Bistra, hypogeous under leaf litter in a broadleaf forest of *Fagus sylvatica* L. and *Carpinus betulus* L., elev. 1300 m.a.s.l., leg. Zlatko Markoski & Marija Markoska, MCF 17646 (20.11.2019); Mt. Vodno, hypogeous under leaf litter in a mixed planted forest of *Pinus nigra* J.F. Arnold, *Tilia cordata* Mill., *Fraxinus excelsior* L., *Ulmus glabra* Huds., elev. 550 m.a.s.l., leg. Tome Jovanovski, MCF 17988 (04.10.2020); idem, leg. Tome Jovanovski, MCF 17822 (08.11.2020); Mt Shar Planina, hypogeous under needle litter in *Pinus sylvestris* L. forest, elev. 1400 m.a.s.l., leg. Tome Jovanovski, MCF 17905 (26.11.2020).

**Fig. 7.** *Wakefieldia macrospora*, basidiomata.  
Scale bars: 5 mm.



*Wakefieldia macrospora* (Hawker) Hawker, Philosophical Transactions of the Royal Society of London, Ser. B, Biological Sciences 237: 521 (1954); Figs. 7, 8. Macroscopic features. The basidiomata was solitary to gregarious, hypogeous, gasteroid, almost globose or tuberiform with lobes, 6.8–20.4 mm long and 6–15.8 mm wide (Fig. 7). The peridium was smooth, initially white, later cream white, attached to the gleba, and not separating after drying. The gleba was grey when fresh, becoming brown when drying, compact, and consisting of small chambers rounded to elongated in shape. The columella was absent. Microscopic features. The peridium was one-layered, with an average width of 150  $\mu\text{m}$ , varying between 100  $\mu\text{m}$  and 200  $\mu\text{m}$ , composed of hyaline thin-walled hyphae, wall 0.5–1  $\mu\text{m}$ , with elongated interwoven hyphae, 2.5–5  $\mu\text{m}$  wide, including some spherical to irregular inflated elements of 4.5–25  $\times$  3.5–15  $\mu\text{m}$  (4.5  $\times$  3.5; 9.5  $\times$  7; 19  $\times$  10; 25  $\times$  13), and clamp connections present (Fig. 8a). The gleba comprised chambers of various shapes, filled with basidiospores, with the hymenium at the edge of the chamber consisting of young

**Fig. 8.** *Wakefieldia macrospora*, microscopic morphological features: **a.** cross-section, peridium (white arrow) and gleba with chamber filled with basidiospores (black arrow); **b., c.** basidiospores with prominent apiculus and remnant of perisporium (black arrow). Scale bars: 10  $\mu\text{m}$ .



club-shaped to cylindrical basidia, followed by tramal plates. The trama was variable in width, 30–150  $\mu\text{m}$ , composed of hyaline interwoven hyphae forming small to larger spherical and polygonal elements, 3.5–11.5  $\times$  3.5–7  $\mu\text{m}$ , wall 1–1.5  $\mu\text{m}$  thick (Fig. 8a). Basidia were not frequent, cylindrical, 35–47  $\times$  7–11  $\mu\text{m}$ , and bearing two spores. The basidiospores were subglobose to fig-shaped (8.5–)10–14(–14.5)  $\times$  (8–)9–13(–13.5)  $\mu\text{m}$ ,  $12 \pm 1.2 \times 11 \pm 1 \mu\text{m}$  on average ( $n = 60$ ),  $Q = 0.96$ –1.18,  $Q_{av} = 1.08$ , wall 1.5–2  $\mu\text{m}$  thick, ornamented with easily visible, large warts of different shapes at the base, truncated at the top, 0.8–1.5  $\mu\text{m}$  high. A large apiculus was present, 3.5–4.5  $\mu\text{m}$  in width at the base, 2.5–3.5  $\mu\text{m}$  long, remnants of the perisporium were rarely visible (Fig. 8b–c). Specimens examined. North Macedonia: Mt. Skopska Crna Gora, hypogeous in a mixed broadleaf forest of *Quercus pubescens* Willd., *Q. frainetto* Ten., *Carpinus orientalis* Mill. and *Salix alba* L., elev. 700 m.a.s.l., leg. Tome Jovanovski, MCF 17950 (01.01.2021); idem, hypogeous in a mixed broadleaf forest of *Quercus pubescens*, *Q. frainetto* and *C. orientalis*, elev. 800 m.a.s.l., leg. Tome Jovanovski, MCF 17977 (23.02.2021).

## DISCUSSION

*Sclerogaster compactus* and *S. hysteroangioides* were treated as synonyms by PEGLER *et al.* (1993), but they clearly differ in morphology, macroscopically as well as microscopically, and are accepted as different taxa by other authors (VIDAL 1991; MONTECCHI & SARASINI 2000). From the presented observations of our collections, *S. compactus* is easily distinguished by its larger basidiospores ( $6.7 \times 6.3 \mu\text{m}$  on average) with verrucose to echinate ornamentation, compared with *S. hysteroangioides*, which has smaller basidiospores ( $4.8 \times 4.5 \mu\text{m}$  on average) and finer ornamentation. There are also differences in the structure of the peridium. In *S. compactus*, the hyphae of the external peridium are significantly narrower ( $1\text{--}2 \mu\text{m}$ ) than those of *S. hysteroangioides* ( $5\text{--}6.5 \mu\text{m}$ ), whereas in the internal peridium the elements are wider in *S. compactus* ( $4.5\text{--}30 \times 4\text{--}20 \mu\text{m}$ ) than in *S. hysteroangioides* ( $4\text{--}18 \times 2.5\text{--}13 \mu\text{m}$ ).

The morphological characters of the collection of *S. compactus* from North Macedonia are in accordance with the literature. The size of the basidioma being 5 mm wide and 6.6 mm long is within the usually reported size of 5 to 10 mm (DODGE & ZELLER 1936; MONTECCHI & SARASINI 2000; ELLIOTT *et al.* 2016), while KAOUNAS *et al.* (2011) reported a size of 20 mm as a rare finding and VIDAL (1991) presented a range of 4–14 mm. The same publications document that the columella may be absent, rudimental or centrally positioned, a variability explained by HAWKER (1954) as the columella is absent in mature basidiomata. In our collection, the columella of *S. compactus* extends from the base to the centre and is not as large as those observed in the collections of *S. hysteroangioides*. Concerning the basidiospore size of *S. compactus* in the original description of TULASNE & TULASNE (1845) measurements are not provided. It is only stated that the basidiospores are minute and spherical. Later TULASNE & TULASNE (1851) quoted a size of  $5.6\text{--}6.4 \mu\text{m}$ . Although an average spore size of 4 to 6  $\mu\text{m}$  in diameter is mostly reported (DODGE & ZELLER 1936; HAWKER 1954; MONTECCHI & SARASINI 2000), a range in spore size has also been recorded, including  $4\text{--}5.5\text{--}7 \mu\text{m}$  (MONTECCHI & SARASINI 2000),  $(5\text{--}) 6.2 \pm 0.7 (-7.5) \mu\text{m}$  (KAOUNAS *et al.* 2011), and  $5\text{--}7 \times 4\text{--}5.5 \mu\text{m}$  (VIDAL 1991). The values of both approaches correspond well with our specimens. The total peridium width in our observations varies between 130  $\mu\text{m}$  and 250  $\mu\text{m}$ , which is in line with the descriptions provided by HAWKER (1954), VIDAL (1991), and MONTECCHI & SARASINI (2000). The width of the peridium layers are usually not reported except by VIDAL (1991). The external layer of our specimen with a width of 30–80  $\mu\text{m}$  is in accordance with VIDAL (1991), who reports a variability between 10–60  $\mu\text{m}$ , while for the internal peridium we observed a higher variability between 70–210  $\mu\text{m}$  compared to 160–200  $\mu\text{m}$  noted by VIDAL (1991).

*Sclerogaster compactus* is widely distributed in Europe. It is reported from a few localities in Britain and France (PEGLER *et al.* 1993), Norway (BRANDRUD *et al.* 2021), Austria (JACQ CONSORTIUM 2004 ff.), Germany (HESSE 1891), Spain (VIDAL 1991; MORENO-ARROYO *et al.* 2005), Italy (MONTECCHI & SARASINI 2000), Turkey (UZUN *et al.* 2019), and Israel (KRAKHMALNYI *et al.* 2014) while on the Balkan Peninsula it has been found only once in Greece (KAOUNAS *et al.* 2011). Concerning the ecology, it occurs in various habitats in thermophilous areas, documented under *Pinus* L., *Quercus* L., *Fagus* L., *Tilia* L., grass roots and *Cistus monspeliensis* L. (VIDAL 1991; PEGLER *et al.* 1993). It is also reported from mixed forests of *Pinus pinaster* Aiton and *Quercus suber* L., *Pinus halepensis* Mill. and *Quercus coccifera* L., *Pinus brutia* Ten. with *Quercus coccifera*, as well as in clear stands of *Pinus pinea* L. and *Quercus ilex* L. and in habitats of *Pinus halepensis* and *Cistus monspeliensis* (MORENO-ARROYO *et al.* 2005; KAOUNAS *et al.* 2011; KRAKHMALNYI *et al.* 2014). In Norway, where the species has been documented in pine forest with stands of *Corylus*

L., it is assessed as endangered (BRANDRUD *et al.* 2021). In North Macedonia it was found only at a single site, representing the second record for the Balkan Peninsula. The basidioma was embedded in leaf litter under *Populus alba* and *Ulmus laevis* in a riverside community where the geological substrate comprises alluvium (PENDZERKOVSKI & HADZIMITROVA 1977), and the soil type is classified as fluvisol according to the World Reference Base for Soil Resources Classification System (MASIS 2015). This is not uncommon, since MONTECCHI & SARASINI (2000) reported that it can be found in the same environments as *Tuber magnatum* Picco.

The morphological characters of our collections of *S. hysteroangioides* correspond well to the literature. Basidiomata size mainly varies between 5–15 mm (VIDAL 1991; MONTECCHI & SARASINI 2000), which is consistent with our collections. Our observed spore measurements of  $4.3\text{--}5.4 \times 4\text{--}5 \mu\text{m}$ , with a rare minimum value of  $3.9 \mu\text{m}$  and a maximum of  $5.7 \mu\text{m}$  and an average size of  $4.8 \times 4.5 \mu\text{m}$ , are in line with TULASNE & TULASNE (1851), MONTECCHI & SARASINI (2000), and ELLIOTT *et al.* (2016), although DODGE & ZELLER (1936) and VIDAL (1991) reported a slightly larger maximum size of  $6.5 \mu\text{m}$ . The wart height of  $0.3\text{--}0.6 \mu\text{m}$  noted by VIDAL (1991) partly overlaps with our measurements of  $0.44\text{--}0.88 \mu\text{m}$ . In addition, our observation of total peridium width, varying between  $210\text{--}380 \mu\text{m}$ , corresponds well with the data provided by DODGE & ZELLER (1936), MONTECCHI & SARASINI (2000), and VIDAL (1991). All authors describe a two-layered peridium, noting that the external layer is composed of large interwoven hyphae (VIDAL 1991; MONTECCHI & SARASINI 2000), which also aligns well with our observations. RYDBERG & JEPSON (2014) and ELLIOTT *et al.* (2016) noted a dextrinoid reaction of the external peridium, also confirmed in our observations. The internal peridium layer has been described as pseudoparenchymatous (VIDAL 1991) or as composed of smaller hyaline, densely interwoven hyphae (MONTECCHI & SARASINI 2000; RYDBERG & JEPSON 2014) with the presence of inflated cells (ELLIOTT *et al.* 2016). Due to the presence of inflated cells clearly visible under higher magnification, we refer to it as pseudoparenchymatous, with the subglobose elements in our collections varying in size from  $4\text{--}18 \times 2.5\text{--}13 \mu\text{m}$ , similar to the values provided by other authors  $4\text{--}22 \times 3\text{--}12 \mu\text{m}$  (ELLIOTT *et al.* 2016) and  $8\text{--}25\text{--}(30)$  (VIDAL 1991).

*Sclerogaster hysteroangioides* is widely distributed with data available from Spain (VIDAL 1991), Italy (MONTECCHI & SARASINI 2000), Sweden (RYDBERG & JEPSON 2014), Norway (BRANDRUD *et al.* 2021), Poland (MLECZKO *et al.* 2020), and Turkey (ELLIOTT *et al.* 2016). Regarding its ecology, it has been found in various communities, in pure stands of *Pinus pinea*, in pure deciduous stands of *Fagus sylvatica*, as well as in mixed forests of *F. sylvatica* with *Picea abies* (L.) H. Karst., *Pinus nigra* and *Quercus*, *Quercus suber* with *Arbutus unedo* L., *Quercus ilex* and *Q. pubescens* Willd., and *Pinus pinea* and *Quercus ilex*, *Pinus halepensis* with *Quercus ilex* (VIDAL 1991; MONTECCHI & SARASINI 2000). In Sweden this thermophilous species has been observed in grassland on glacial gravel substrates, where young firs and pines are present, in July after a period of high temperatures, where other thermophilous species have also been found (RYDBERG & JEPSON 2014). In Norway it has been found at two localities in pine forests with stands of *Corylus*, and similarly to *S. compactus* it is assessed as endangered (BRANDRUD *et al.* 2021). In North Macedonia it has been documented at four sites, representing the first records of the species in the Balkan Peninsula. It is recorded in three different types of habitats as mentioned above, at an elevation range between 550–1400 m.a.s.l. In broadleaf forests of *Fagus sylvatica* and *Carpinus betulus*, as well as in *Pinus sylvestris* L. forests, the soil type is cambisol and the geological substrate consists of phyllitoids. In the mixed anthropogenic forests of *Pinus nigra*, *Tilia cordata*, *Fraxinus excelsior* and *Ulmus glabra* the soil type is rendzic leptosol,

while the geological substrate is represented by metamorphic rocks phyllite and greenschist (PENDZERKOVSKI & HADZIMITROVA 1977; MASIS 2015).

In addition to the two species reported here, *S. compactus* and *S. hysterangioides*, *S. gastrosporioides* and *S. liospermus* have also been documented in other European countries (MONTECCHI & SARASINI 2000; KREISEL 2001). *Sclerogaster gastrosporioides* occurs in arid and steppe environments, including abandoned fields (VIDAL 1991), habitats which have received little attention in North Macedonia with respect to hypogeous fungi, which may explain the lack of records from the country. Although *Sclerogaster liospermus* is associated with deciduous *Quercus* forests (DODGE & ZELLER 1936), one of the dominant habitat types in North Macedonia, despite numerous surveys, the species has not been observed to date.

*Wakefieldia macrospora* was first described as *Sclerogaster macrosporus* Hawker (HAWKER 1951). However, the author noted that its generic placement was provisional, since the thin peridium and relatively large basidiospores are not characteristic of this genus, and later transferred the species to a new genus to accommodate it together with *W. striispora* (CORNER & HAWKER 1953). No other species with the characteristics of this genus have been documented in Europe. However, three species are regarded as synonyms of *W. macrospora*, i.e. *S. porquerollensis* Donadini & G. Rioussset, *S. rhizopogon* Donadini, Rioussset & G. Rioussset (HAWKER 1954; VIDAL 1997) and *Hymenogaster vaccekii* Sviček (PEGLER *et al.* 1993; MONTECCHI & SARASINI 2000).

A comparison of the characters of our collection of *W. macrospora* with other descriptions reveals no significant dissimilarities. The size of the basidiomata mostly varies between 5–20 mm or 5–25 mm in diameter or occasionally smaller, between 5–8 mm (PEGLER *et al.* 1993; KRAKHMALNYI *et al.* 2014), which is consistent with our specimens. The spore size in our collections ((8.5–)10–14(–14.5) × (8–)9–13(–13.5) µm) is smaller compared to the original description, where HAWKER (1951) recorded a spore size of 13–18 µm in diameter. PEGLER *et al.* (1993) described a somewhat wider variability of 12–19 × 12–17.5 µm, while MONTECCHI & SARASINI (2000) noted even a greater range (12–12.9–15.4–19 × 8–9.6–11.9–13 µm), which is similar to our collections. The maximum spore size observed in our collections is 14.5 µm. However, other studies have also reported smaller maximum spore sizes than in the original description such as 16 µm (UZUN & KAYA 2020) and 17 µm (KAOUNAS *et al.* 2011). As noted by all authors, the peridium is composed of only one layer, and the reported width varies between 200–300 µm (MONTECCHI & SARASINI 2000; KAOUNAS *et al.* 2011), or 140–200 µm (PEGLER *et al.* 1993), while in our study it varies between 100–200 µm.

The molecular phylogenetic analyses of the ITS matrix confirm the species identification of the two North Macedonian accessions as *W. macrospora*. They are almost identical to the Greek accession JN812039, for which somewhat larger spore sizes have been recorded (KAOUNAS *et al.* 2011; see above), supporting the assumption that the spore size differences reported in the literature for the species are due to variability.

*Wakefieldia macrospora* is a rare species and is reported from a small number of sites; in Central Europe from Belgium, the Czech Republic, Germany, Switzerland (DE VRIES 1988; LUDWIG & SCHNITTLER 1996; RIVA 2009), as well as from the United Kingdom (HAWKER 1951). From the Mediterranean region it is more frequently reported in Italy and Spain (MONTECCHI & SARASINI 2000; MORENO-ARROYO *et al.* 2005), and it is found in Turkey and Israel (KRAKHMALNYI *et al.* 2014; UZUN & KAYA 2020), while on the Balkan Peninsula it is recorded from Greece (KAOUNAS *et al.* 2011). Based on ITS data, two environmental sequences (erroneously labelled as “uncultured *Hebeloma*”) obtained from ectomycorrhizal root tips of a mediterranean forest dominated by *Quercus ilex* in southern France (RICHARD *et al.* 2011) are also clearly re-

ferable to *W. macrospora* (see also Fig. 3). In the cited studies this species is reported from various habitat types, in both oak and beech forests mostly on calcareous substrate. It has been found in association with *Quercus pubescens*, *Q. ilex*, *Q. coccifera*, *Ostrya carpinifolia* Scop., and *Cistus albidus* L. and it shows little specificity for the host plant, as well as for the period of fructification as it has been found in winter, spring and summer. In North Macedonia at the two localities on Mt. Skopska Crna Gora it was found in association with *Q. pubescens* and *Carpinus orientalis*, on soils classified as a complex of cambisol, humic eutric and umbric regosol (umbrisol) (MASIS 2015), while according to the geological map the localities are found on biotite-muscovite schist and on marble with metamorphic calc-schist rocks (PENDZERKOVSKI & HADZIMITROVA 1977).

## CONCLUSION

The genus *Sclerogaster* comprises 12 species, most of them described from North America, while in Europe only four species are present. In addition to *S. compactus* and *S. hysterangioides* reported here, *S. gastrosporioides* and *S. liospermus* are also known from Europe. *Sclerogaster gastrosporioides* thrives in dry and steppe areas where surveys on the diversity of hypogeous fungi are lacking, while *S. liospermus* prospers in oak forests where frequent investigations are conducted and it has not yet been found in North Macedonia. However, despite being understudied due to their completely hypogeous life cycle, the genus *Sclerogaster* obviously comprises rare species. The habitats of *S. compactus* and *S. hysterangioides* are the most studied for hypogeous fungi, nevertheless the number of records remains small. Only one record for *S. compactus* and only four for *S. hysterangioides* have been noted for the country to date, while for the Balkan Peninsula *S. hysterangioides* is the first, while *S. compactus* and *W. macrospora* are the second records, respectively. Hence, further research is needed to extend the existing knowledge of the diversity of these species in the country and to gain in-depth knowledge of their ecology.

Concerning the phylogenetic analyses, although the inferences, particularly of the ITS marker, confirm (or at least do not contradict) the species identifications of the North Macedonian *Sclerogaster* collections, the available molecular reference data are currently insufficient to ensure reliable molecular species identification. This can be attributed to two factors: first, the lack of sufficient taxon sampling based on well-identified specimens from the entire distribution range, and second, the lack of additional high-resolution genetic markers to provide a sound reliable phylogenetic frame for molecular identification. It is therefore highly desirable to sequence additional markers from a broader range of well-identified accessions in future research. For *Sclerogaster*, more sequences of the LSU are available for comparison in GenBank than for ITS; however, the LSU is much more conserved and therefore contains far less phylogenetic information, and while allowing a rough placement within the genus it is not suitable for species identification. Another issue is the lack of well-identified reference sequences from multiple collections per species, which for the time being does not allow for unequivocal species identification based on molecular data alone. The difficulties in identifying species morphologically, as well as by sequence data, are illustrated by the fact that most *Sclerogaster* ITS sequences originate from accessions not identified to species level, showing that the genus has not yet been adequately studied to enable sound species identification on a world-wide scale. Therefore, combined phylogenetic analyses with additional markers in combination with a broader geographic sampling of morphologically well-identified reference specimens are necessary before sound conclusions about the geographical distribution and species delimitation of this challenging genus can be reached.

**Acknowledgements** – Some of the results were obtained within the framework of the bilateral project “Molecular phylogenetics, diversity and ecology of selected genera of hypogeous fungi within Ascomycota from Macedonia and Austria” (2024–2025), funded by the Macedonian and Austrian Ministries of Science and Education and the Austrian Agency for Education and Internationalisation (OeAD). The dedication of truffle hunters in the search for new and interesting hypogeous fungi is indispensable for making new discoveries, therefore we wish to acknowledge their contribution and to express our deepest appreciation to Tome Jovanovski and Zlatko Markovski for obtaining the collections examined. Furthermore, we would like to express our sincere gratitude to the reviewers for their devoted time, insightful feedback, valuable suggestions and comments, which greatly improved the manuscript.

## REFERENCES

- AAENEN DK, KUYPER TW, BOEKHOUT T & HOEKSTRA RF. 2000. Phylogenetic relationships in the genus *Hebeloma* based on ITS1 and 2 sequences, with special emphasis on the *Hebeloma crustuliniforme* complex. *Mycologia* **92**: 269–281. <https://doi.org/10.1080/00275514.2000.12061154>.
- ALFREDO DS, LAVOR P, HOSAKA K, BASEIA IG & MARTÍN MP. 2015. Rediscovery of *Sclerogaster luteocarneus* (Gastrales, Agaricomycetes): a forgotten species. *Global Journal of Advanced Biological Sciences* **1**: 30–37.
- BINDER M & HIBBET DS. 2006. Molecular systematics and biological diversification of Boletales. *Mycologia* **98**: 971–981. <https://doi.org/10.1080/15572536.2006.11832626>
- BOROVÍČKA J, OBORNÍK M, STRÍBRNÝ J, NOORDELOOS ME, PARRA SÁNCHEZ LA & GRYN-DLER M. 2015. Phylogenetic and chemical studies in the potential psychotropic species complex of *Psilocybe atrobrunnea* with taxonomic and nomenclatural notes. *Persoonia* **34**: 1–9. <https://doi.org/10.3767/003158515X685283>
- BRADSHAW AJ, BACKMAN TA, RAMÍREZ-CRUZ V, FORRISTER DL, WINTER JM, GUZMÁN-DÁVALOS L, FURCI G, STAMETS P & DENTINGER BTM. 2022. DNA authentication and chemical analysis of *Psilocybe* mushrooms reveal widespread misdeterminations in fungaria and inconsistencies in metabolites. *Applied and Environmental Microbiology* **88**: e0149822. <https://doi.org/10.1128/aem.01498-22>
- BRANDRUD TE, BENDIKSEN E, BLAALID R, HOFTON TH, JORDAL JB, NORDÉN J, NORDÉN B & WOLLAN AK. 2021. *Red List of Species Norway*. Fungi, Mushroom Expert Committee. Artsdatabanken. Available at: <http://www.artsdatabanken.no/lister/rodlisteforarter/2021> [Accessed 15 January 2025]
- BROCK PM, DÖRING H & BIDARTONDO MI. 2009. How to know unknown fungi: the role of a herbarium. *New Phytologist* **181**: 719–724. <https://doi.org/10.1111/j.1469-8137.2008.02703.x>
- COKER WC & COUCH NJ. 1928. *The Gasteromycetes of the eastern United States and Canada*. University of North Carolina Press, Chapel Hill, North Carolina, USA.
- CORNER EJH & HAWKER LE. 1953. Hypogeous fungi from Malaya. *Transactions of the British Mycological Society* **36**: 125–137. [https://doi.org/10.1016/S0007-1536\(53\)80057-4](https://doi.org/10.1016/S0007-1536(53)80057-4)
- DARRIBA D, POSADA D, KOZLOV AM, STAMATAKIS A, MOREL B & FLOURI T. 2020. ModelTest-NG: A new and scalable tool for the selection of DNA and protein evolutionary models. *Molecular Biology and Evolution* **37**: 291–294. <https://doi.org/10.1093/molbev/msz189>
- DE HOOG GS & GERRITS VAN DEN ENDE AHG. 1998. Molecular diagnostics of clinical strains of filamentous basidiomycetes. *Mycoses* **41**: 183–189. <https://doi.org/10.1111/j.1439-0507.1998.tb00321.x>
- DE VRIES GA. 1988. *Wakefieldia macrospora* (Hawker) Hawker, gastéromycète hypogée nouveau pour la mycoflore belge. *Lejeunia* **125**: 1–5.
- DODGE CW & ZELLER SM. 1936. *Hydnangium* and related genera. *Annals of the Missouri Botanical Garden* **23**: 565–598. <https://doi.org/10.2307/2394151>

- EBERHARDT U, BEKER HJ, VESTERHOLT J, DUKIK K, WALTHER G, VILA J & BRIME SF. 2013. European species of *Hebeloma* section *Theobromina*. *Fungal Diversity* **58**: 103–126. <https://doi.org/10.1007/s13225-012-0188-3>
- EDLER D, KLEIN J, ANTONELLI A & SILVESTRO D. 2021. RaxmlGUI 2.0: A graphical interface and toolkit for phylogenetic analyses using RAxML. *Methods in Ecology and Evolution* **12**: 373–377. <https://doi.org/10.1111/2041-210X.13512>
- ELLIOTT TF, TÜRKÖĞLU A, TRAPPE JM & GÜNGÖR MY. 2016. Turkish truffles 2: eight new records from Anatolia. *Mycotaxon* **131**: 439–453. <http://dx.doi.org/10.5248/131.439>
- FOGEL R. 1977. Additions to the hypogeous mycoflora of Colorado II. *Sclerogaster xerophilum* (Basidiomycetes, Hymenogastreales). *Mycologia* **69**: 980–986.
- FOGEL R. 1990. *Leucogaster columellatus* is a *Sclerogaster*. *Mycologia* **82**: 655–657. <https://doi.org/10.2307/3760058>
- FUNGAL NAMES (n.d.). Fungal Names: global nomenclatural repository for fungi. A database of scientific names of fungi coordinated by the Institute of Microbiology, Chinese Academy of Sciences (IMCAS). <https://nmdc.cn/fungalnames>
- GEML J, LEAL CM, NAGY R & SULYOK J. 2022. Abiotic environmental factors drive the diversity, compositional dynamics and habitat preference of ectomycorrhizal fungi in Pannonian Forest types. *Frontiers in Microbiology* **13**: 1007935. <https://doi.org/10.3389/fmicb.2022.1007935>
- HAWKER LE. 1951. Hypogaeous fungi I. A hypogaeous gasteromycete, *Sclerogaster macrosporus* n. sp. *Transactions of the British Mycological Society* **34**: 216–219.
- HAWKER LE. 1954. British hypogeous fungi. *Philosophical Transactions of the Royal Society of London, Series B, Biological Sciences* **237**: 429–546. <https://doi.org/10.1098/rstb.1954.0002>
- HESSE R. 1891. *Die hypogaeen Deutschlands, Band I, Die Hymenogastreen*. Ludwig Hofstetter, Halle, Germany.
- HOSAKA K, BATES ST, BEEVER RE, CASTELLANO MA, COLGAN III W, DOMÍNGUEZ LS, NOUHRA ER, GEML J, GIACHINI AJ, KENNEY SR, SIMPSON NB, SPATAFORA JW & TRAPPE JM. 2006. Molecular phylogenetics of the gomphoid-phalloid fungi with an establishment of the new subclass Phallomycetidae and two new orders. *Mycologia* **98**: 949–959. <https://doi.org/10.3852/mycologia.98.6.949>
- HOSAKA K & CASTELLANO MA. 2008. Molecular phylogenetics of Geastrales with special emphasis on the position of *Sclerogaster*. *Bulletin of the National Museum of Nature and Science, Series Botany* **34**: 161–173.
- INDEX FUNGORUM PARTNERSHIP (n.d.). IndexFungorum: Search for fungal names. A global database of scientific names in the fungal kingdom, coordinated by the Index Fungorum Partnership (Royal Botanic Gardens Kew, Landcare Research NZ, Institute of Microbiology CAS). <https://www.indexfungorum.org/Names/Names.asp>
- JACQ CONSORTIUM. 2004 ff. Virtual herbaria website. Available at: <https://www.jacq.org/> [Accessed 07 May 2025]
- JEPSON M, NILSSON RH & LARSSON E. 2013. European earthstars in Geastraceae (Geastrales, Phallomycetidae) – a systematic approach using morphology and molecular sequence data. *Systematics and Biodiversity* **11**: 437–465. <https://doi.org/10.1080/14772000.2013.857367>
- KALUCKA IL, JAGODZIŃSKI AM & NOWIŃSKI M. 2016. Biodiversity of ectomycorrhizal fungi in surface mine spoil restoration stands in Poland – first time recorded, rare, and red-listed species. *Acta Mycologica* **51**: 1080. <http://dx.doi.org/10.5586/am.1080>
- KAOUNAS V, ASSYOV B & ALVARADO P. 2011. New data on hypogeous fungi from Greece with special reference to *Wakefieldia macrospora* (Hymenogastreales, Agaricales) and *Geopora clausa* (Pyronemataceae, Pezizales). *Mycologia Balcanica* **8**: 105–113. <https://doi.org/10.5281/zenodo.2550663>
- KARADELEV M, RUSEVSKA K, KAJEVSKA I & MITIC KOPANJA D. 2019. Checklist of larger Ascomycetes in the Republic of Macedonia. *Contributions, Section of Natural, Mathematical and Biotechnical Sciences* **40**: 239–253. <https://doi.org/10.20903/csnmbs.masa.2019.40.2.148>

- KARADELEV M, RUSEVSKA K, KOST G & MITIC-KOPANJA D. 2018. Checklist of macrofungal species from the phylum Basidiomycota of the Republic of Macedonia. *Acta Musei Macedonici Scientiarum Naturalium* **21**: 23–112.
- KATO H, ROZEWICKI J & YAMADA KD. 2019. MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. *Briefings in Bioinformatics* **20**: 1160–1166. <https://doi.org/10.1093/bib/bbx108>
- KRAKHMALNYI M, WASSER SP & NEVO E. 2014. *Sclerogaster*, *Wakefieldia*, and *Setcheliogaster*: Hypogeous gasteroid basidiomycetes from Israel. *Plant Biosystems* **148**: 1239–1246. <https://doi.org/10.1080/11263504.2014.980364>
- KREISEL H. 2001. Checklist of the gasteral and secotioid Basidiomycetes of Europe, Africa, and the Middle East. *Austrian Journal of Mycology* **10**: 213–313.
- LUDWIG G & SCHNITTLER M. 1996. *Rote Listen gefährdeter Pflanzen Deutschlands. Schriftenreihe für Vegetationskunde* 28. Bundesamt für Naturschutz, Bonn - Bad Godesberg, Deutschland.
- MASIS. 2015: Macedonian Soils Information System. <http://www.maksoil.ukim.mk/masis/>
- MELOVSKI L, MARKOVSKI B, HRISTOVSKI S, JOVANOVSKA D, ANASTASOVSKI V, KLINCHAROV S, VELEVSKI M, VELKOVSKI N, TRENDAFILOV A, MATEVSKI V, KOSTADINOVSKI M, KARADELEV M, LEVKOV Z & KOLCHAKOVSKI D. 2013. Regional division of the Republic of Macedonia for the needs of biological databases. *Macedonian Journal of Ecology and Environment* **15**: 81–111. <https://doi.org/10.59194/MJEE13152081m>
- MLECZKO P, KOZAK M & KARPOWICZ F. 2020. New records of rare hypogeous fungi from Poland (Central Europe). *Acta Mycologica* **55**: 5529. <https://doi.org/10.5586/am.5529>
- MONCALVO JM, WANG HH & HSEU RS. 1995. Phylogenetic relationships in *Ganoderma* inferred from the internal transcribed spacers and 25S ribosomal DNA sequences. *Mycologia* **87**: 223–238. <https://doi.org/10.1080/00275514.1995.12026524>
- MONTECCHI A & SARASINI M. 2000. *Funghi Ipogei d'Europa*. Associazione Micologica Bresadola, Fondazione Centro Studi Micologici, Trento-Vicenza, Italy.
- MOREAU PA, PEINTNER U & GARDES M. 2006. Phylogeny of the ectomycorrhizal mushroom genus *Alnicola* (Basidiomycota, Cortinariaceae) based on rDNA sequences with special emphasis on host specificity and morphological characters. *Molecular Phylogenetics and Evolution* **38**: 794–807. <https://doi.org/10.1016/j.ympev.2005.10.008>
- MORENO-ARROYO B, GÓMEZ J & PULIDO E. 2005. *Tesoros de nuestro montes. Trufas de Andalucía*. Consejería de Medio Ambiente, Junta de Andalucía, Córdoba, Spain.
- MYCOBANK (n.d.). MycoBank: an online database of fungal names and nomenclatural novelties. Westerdijk Fungal Biodiversity Institute. <https://www.mycobank.org>
- NUSKE SJ, ANSLAN S, TEDERSOO L, BONNER MTL, CONGDON BC & ABELL SE. 2018. The endangered northern bettong, *Bettongia tropica*, performs a unique and potentially irreplaceable dispersal function for ectomycorrhizal truffle fungi. *Molecular Ecology* **27**: 4960–4971. <https://doi.org/10.1111/mec.14916>
- PEGLER DN, SPOONER BM & YOUNG TWK. 1993. *British truffles. A revision of British hypogeous fungi*. Royal Botanic Garden, Kew, London, Great Britain.
- PEINTNER U, BOUGHER NL, CASTELLANO MA, MONCALVO JM, MOSER MM, TRAPPE JM & VILGALYS R. 2001. Multiple origins of sequestrate fungi related to *Cortinarius* (Cortinariaceae). *American Journal of Botany* **88**: 2168–2179. <https://doi.org/10.2307/3558378>
- PENDZERKOVSKI J & HADZIMITROVA S. 1977. *Geological map of Republic Macedonia*. Council for Research in Mining, Geological Institute of Skopje, Department of Cartography “Geokarta”, Belgrade.
- PILÁT A & SVRCEK M. 1955. Über eine neue *Sclerogaster*-Art aus Böhmen: *Sclerogaster gastrosporoides* Pil. & Svr. *Sydowia* **9**: 289–291.
- RICHARD F, ROY M, SHAHIN O, STHULTZ C, DUCHEMIN M, JOFFRE R & SELOSSE MA. 2011. Ectomycorrhizal communities in a Mediterranean forest ecosystem dominated by *Quercus ilex*: seasonal dynamics and response to drought in the surface organic horizon. *Annals of Forest Science* **68**: 57–68. <https://doi.org/10.1007/s13595-010-0007-5>
- RIVA A. 2009. *Wakefieldia macrospora* e *Genea fragrans*, due funghi ipogei nuovi per il Ticino. *Schweizerische Zeitschrift für Pilzkunde* **87**: 119–121.

- ROCHET J, MOREAU PA, MANZI S & GARDES M. 2011. Comparative phylogenies and host specialization in the alder ectomycorrhizal fungi *Alnicola*, *Alpova* and *Lactarius* (Basidiomycota) in Europe. *BMC Ecology and Evolution* **11**: 40. <https://doi.org/10.1186/1471-2148-11-40>
- RYDBERG H & JEPSON M. 2014. *Sclerogaster hysteroangioides* (Basidiomycota, Geastrales) new to Sweden. *Svensk Mykologisk Tidskrift* **35**: 60–64.
- SACCARDO PA. 1895. *Sylloge Fungorum omnium hucusque cognitorum, Vol. XI, Supplementum Universale, pars III*. Padova.
- SCHOCH CL, SEIFERT KA, HUHNDORF S, ROBERT V, SPOUGE JL, LEVESQUE CA, CHEN W & FUNGAL BARCODING CONSORTIUM. 2012. Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. *Proceedings of the National Academy of Sciences U.S.A.* **109**: 6241–6246. <https://doi.org/10.1073/pnas.1117018109>
- SOEHNER E. 1924. Prodrömus der Fungi hypogei Bavariae. *Kryptogamische Forschung* **1**: 390–398.
- STIELOW B, BRATEK Z, ORCZÁN AK, RUDNOY S, HENSEL G, HOFFMANN P, KLENK HP & GÖKER M. 2011. Species delimitation in taxonomically difficult fungi: the case of *Hymenogaster*. *PLoS One* **6**: e15614. <https://doi.org/10.1371/journal.pone.0015614>
- SULZBACHER MA, GREBENC T, CABRAL TS, GIACHINI AJ, GOTO BT, SMITH ME & BASEIA IG. 2016b. *Restingomyces*, a new sequestrate genus from the Brazilian Atlantic rainforest that is phylogenetically related to early-diverging taxa in Trappeaceae (Phallales). *Mycologia* **108**: 954–966. <https://doi.org/10.3852/15-265>
- SULZBACHER MA, SOUSA JO, CORTEZ VG, GIACHINI AJ & BASEIA IG. 2016a. *Sclerogaster araripensis*, a new hypogeous fungus from the upland, wet forest enclaves of northeast Brazil. *Sydowia* **68**: 107–111. <https://doi.org/10.12905/0380.sydowia68-2016-0107>
- SWOFFORD DL. 2003. PAUP\*. Phylogenetic Analysis Using Parsimony (\*and Other Methods). Version 4. Sinauer Associates, Sunderland, Massachusetts. <https://phylosolutions.com/paup-test/>
- TOFILOVSKA S, KARADELEV M, JOVANOVSKI T & RUSEVSKA K. 2023. New data on the distribution of hypogeous species *Leucogaster nudus* (Basidiomycota) in the Republic of North Macedonia with note on its taxonomy and morphology. *Acta Musei Macedonici Scientiarum Naturalium* **26**: 45–53.
- TOFILOVSKA S, RUSEVSKA K, GREBENC T, KOST G & KARADELEV M. 2019. Contribution to the Checklist of Basidiomycota for the Republic of North Macedonia. *Acta Musei Macedonici Scientiarum Naturalium* **22**: 27–33.
- TULASNE L-R & TULASNE C. 1845. Fungi nonnulli hypogaei, novi v. minus cogniti. *Giornale Botanico Italiano* **1**: 55–63.
- TULASNE L-R & TULASNE C. 1851. *Fungi hypogaei: histoire et monographie des champignons hypogés*. Friedrich Klincksieck, Paris, France.
- UZUN Y & KAYA A. 2020. *Wakefieldia*, a new hypogeous basidiomycete genus record for Turkey. *Journal of Agriculture and Nature* **23**: 168–171. <https://doi.org/10.18016/ksutarimdog.vi.593676>
- UZUN Y, KAYA A & YAKAR S. 2019. A new record and new localities for the genus *Sclerogaster* R. Hesse in Turkey. *Journal of Natural and Applied Sciences* **23**: 9–12. <https://doi.org/10.19113/sdufenbed.429981>
- VERWOERD L. 1926. Addisionale beskrywings van enkele Suid-Afrikaanse Gasteromycetes. *South African Journal of Science* **23**: 290–294.
- VIDAL JM. 1991. Contribución al conocimiento de la flora micológica del Baix Empordà y zonas limítrofes (Catalunya). IV. Hongos hipogeos (Zygomycotina, Ascomycotina y Basidiomycotina). *Bulletin de Sociedad Catalana Micológica* **14–15**: 143–194.
- VIDAL JM. 1997. Algunos hongos hipogeos, nuevos o poco citados de Cataluña (Zygomycotina, Ascomycotina y Basidiomycotina). *Revista Catalana de Micologia* **20**: 25–62.
- VIDAL JM, ALVARADO P, LOIZIDES M, KONSTANTINIDIS G, CHACHUŁA P, MLECZKO P, MORENO G, VIZZINI A, KRAXHMALNYI M, PAZ A, CABERO J, KAOUNAS V, SLAVOVA M, MORENO-ARROYO B & LLISTOSELLA J. 2019. A phylogenetic and taxonomic revision of sequestrate Russulaceae in Mediterranean and temperate Europe. *Persoonia* **42**: 127–185. <https://doi.org/10.3767/persoonia.2019.42.06>

- VILGALYS R & HESTER M. 1990. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *Journal of Bacteriology* **172**: 4238–4246. <https://doi.org/10.1128/jb.172.8.4238-4246.1990>
- VOGLMAYR H & JAKLITSCH WM. 2008. *Prosthecium* species with *Stegonsporium* anamorphs on *Acer*. *Mycological Research* **112**: 885–905. <https://doi.org/10.1016/j.mycres.2008.01.020>
- WANG R, HERRERA M, XU W, ZHANG P, MORENO JP, COLINAS C & YU F. 2022. Ethnomycological study on wild mushrooms in Pu'er Prefecture, Southwest Yunnan, China. *Journal of Ethnobiology and Ethnomedicine* **18**: 55. <https://doi.org/10.1186/s13002-022-00551-7>
- WERLE E, SCHNEIDER C, RENNER M, VÖLKER M & FIEHN W. 1994. Convenient single-step, one tube purification of PCR products for direct sequencing. *Nucleic Acids Research* **22**: 4354–4355. <https://doi.org/10.1093/nar/22.20.4354>
- WHITE TJ, BRUNS T, LEE SH & TAYLOR JW. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: INNIS MA, GELFAND DH, SNINSKY JJ & WHITE TJ (eds.), *PCR Protocols: A Guide to Methods and Applications*, pp. 315–322, Academic Press, San Diego, California.
- ZELLER SM & DODGE CW. 1935. New species of Hydnangiaceae. *Annals of the Missouri Botanical Garden* **22**: 365–373. <http://dx.doi.org/10.2307/2394161>



## REZIME

### Razmatranja o morfološkim karakteristikama i filogeniji hipogejskih gasteroidnih rodova *Sclerogaster* i *Wakefieldia* (Basidiomycota) u Severnoj Makedoniji

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Rodovi *Sclerogaster* i *Wakefieldia* se retko pominju, posebno iz slabo procenjenih regiona Balkanskog poluostrva. Istraživanje hipogejskih gljiva u Severnoj Makedoniji stalno napreduje poslednjih godina, što je dovelo do sve većeg broja kolekcija. Izvršeno je molekularno filogenetsko i morfološko posmatranje deponovanih primeraka *Sclerogaster* i *Wakefieldia*, koji su upoređeni sa kolekcijama iz drugih područja i sekvencama u nukleotidnim bazama podataka. Prikazana je molekularno-genetska raznolikost zasnovana na rDNA ITS i LSU markerima i morfološkim karakteristikama primeraka iz dva roda, a date su i informacije o njihovoj ekologiji. Izdvojene su dve vrste *Sclerogaster*: *S. hysteroangiooides*, prvi put zabeležena na Balkanskom poluostrvu, i *S. compactus*, drugi nalaz na Balkanskom poluostrvu. *Sclerogaster hysteroangiooides* se nalazi u različitim tipovima staništa na četiri lokaliteta, dok je *S. compactus* prisutan samo na jednom lokalitetu u priobalnoj zajednici *Populus alba* i *Ulmus laevis*. Rod *Wakefieldia* je u Evropi predstavljen jednom vrstom, *W. macrospora*. Naše kolekcije, koje predstavljaju drugi nalaz na Balkanskom poluostrvu, potiču iz mešovite listopadne šume *Quercus pubescens* i *Carpinus orientalis*. Filogenetske analize potvrđuju morfološku identifikaciju uzoraka kao *S. hysteroangiooides* i *W. macrospora*. Istraživanje hipogejskih gljiva u Severnoj Makedoniji pokazuje bogatu raznolikost ove ekološke grupe gljiva i zahteva buduća istraživanja sa sveobuhvatnim morfološkim posmatranjima i dodatnim molekularnim markerima.

**Ključne reči:** Balkansko poluostrvo, DNA-barkoding, gljive, morfologija, mikodiverzitet, filogenija