A new species of *Glutinoglossum* (*Geoglossaceae*, *Ascomycota*) from Europe, *G. persoonii*

Salvatore SAITTA
Agustin SIERRA
Viktor KUČERA
Pablo ALVARADO

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Abstract: *Glutinoglossum persoonii* is here described as new to science. It is regularly found in acidic soils of Italy and Spain near *Pinus* sp., *Quercus* sp., *Cupressus* sp., *Arbutus unedo* and *Erica* sp. plants. It is characterised by the apically branched hyphae of its stipe surface and relatively long spores and asci. Morphologically, the most similar species is *G. pseudoglutinosum* since both taxa present a huge gelatinous layer on the stipe surface and similar ascospores with 3 and 7 septa. The phylogenetic analyses based on ITS and 28S rDNA confirm that *G. persoonii* and *G. pseudoglutinosum* are closely related, and show they represent a sister clade to *G. glutinosum*.
Keywords: acidic soil, biodiversity, distribution, earth tongues, phylogeny.

Introduction

Molecular studies, based on ITS and 28S (LSU) rDNA regions and the nuclear genes rpb2 and mcm7 (Hustad *et al.*, 2013), showed that *Geoglossum glutinosum* Pers. formed a significantly distinct clade, different from the one containing the type species of *Geoglossum* Pers., *G. glabrum* Pers. As a result, the genus *Glutinoglossum* Hustad, A.N. Mill., Dentinger & P.F. Cannon was coined by Hustad *et al.* (2013) to accommodate *G. glutinosum* and the newly described taxon *G. heptaseptatum* Hustad, A.N. Mill., Dentinger & P.F. Cannon. This genus is macroscopically characterised by viscid-gelatinous black ascocarps which are easily recognizable in the field, and microscopically by its straight paraphyses with enlarged apical cells that extend beyond the hymenium down to the stipe surface, where they form a distinct gelatinous layer.

Later, Hustad & Miller (2015) added four more taxa: *Glutinoglossum americanum* Hustad & A.N. Mill. present in North America, and *G. australasicum* Hustad & A.N. Mill., *G. exiguum* Hustad & A.N. Mill. and *G. methvenii* Hustad & A.N. Mill., with an Australasian distribution. More recently, Fedosova *et al.* (2017) added seven more species to the genus, based on morphological and molecular data: *Glutinoglossum circinatum* Fedosova (from Russia), *G. lumbricale* Fedosova (from Russia and China), *G. orientale* Fedosova, E.S. Popov & A.V. Alexandrova (from Russia and Vietnam), *G. peregrinans* Fedosova & V. Kučera (from Russia, Finland, France and Slovakia), *G. proliferatum* V. Kučera (from Romania), *G. pseudoglutinosum* V. Kučera (from Central Europe) and *G. triseptatum* V. Kučera (from Slovakia).

The aim of the present study is to describe a newly found species of *Glutinoglossum*, the first to be found in the Mediterranean region (Italy, Spain).

The Italian samples were found in Sicily, in Monte Dinnammare, also known as Antennamare, one of the highest peaks of Peloritani Mountains (elev. 1127 m). It dominates the city of Messina, on the northeastern edge of Sicily, and its vegetation reaches the suburbs. Near Salice, a village of Messina municipality, there is an interesting forest with two very well known trails called Candelara and Candelara 2 (elev. 350 m). The second one contains multiple Mediterranean tree species, together with the typical shrubs and plants that occur in these areas. The samples of *Glutinoglossum* are regularly found in a small mossy corner, in the same spot where *Geoglossum brunneipes* Arauzo, Lebre & M. Becerra and *Colus hirudinosus* Cavalier & Séchier are also found. Not far, the rare *Pseudoplectania ericae* Donadini was collected too (Saitta, 2020). The putatively new species was also found in a second Sicilian locality, Bosco della Contessa, a small forest near the town of Buccheri, in Siracusa province, in the southern part of the island. Located at 880 m asl, the forest is very mossy and mainly composed of *Pinus* sp. and *Cupressus* sp. Ascomycetes are quite diverse there, with many species of *Geoglossaceae* and the notable presence in the area of the rare species *Donadinia lusitanica* (Torrend & Boud.) M. Carbone, Agnello & P. Alvarado (Saitta, 2020).

The Spanish samples were found in two localities too. The first ones, in the mountains of the Fembra Morta (elev. 768 m) a continuation of the Catalan Pre-Coastal Range (elev. 1236 m), in the Montserrat Natural Park (Barcelona, Catalonia). The area has a small calcareous zone and a wider one made up of siliceous slates, where the samples of *Glutinoglossum* were located. Mediterranean trees and shrubs are the main plants found, and ascomycetes are especially diverse in moist areas. Another Spanish collection was located in acidic soils of Casas de Miravete (Cáceres, Extremadura), in humus of a mixed forest made up of *Eucalyptus globulus*, *E. camaldunensis*, *Quercus suber*, *Q. ilex*, *Cupressus* sp., *Rubus* sp. and *Cistus ladanifer*.

Material and methods

Morphological studies. — The macroscopical characters of the ascomata were studied in fresh state. The microscopical structures were studied both on fresh and dried material using a light microscope with an oil immersion lens. Fragments of ascomata were examined in tap water, 5% KOH, Melzer's reagent (MLZ), Lugol's solution (IKI) and a solution of Congo red in ammonia. Dimensions of microscopical structures are reported as an average plus and minus standard deviation calculated from 30 measurements (with minimum/maximum values indicated inside parentheses) taken from at least 3 specimens from each collection studied. Collection sites of all samples are georeferenced with the WGS84 system. Description is based on voucher specimens that are deposited in the herbarium of the Museum of Natural History of Venice (MCVE), the private herbarium of Agustin Sierra (ASS) and the herbarium of the Institute of Botany, Slovak Academy of Sciences (SAV).

Molecular phylogeny. — DNA extraction, amplification and sequencing.— Total DNA was extracted from dried specimens using a standard CTAB method (Murray & Thompson, 1980). PCR reactions (Mullis & Faloona, 1987) included 35 cycles with an annealing temperature of 54° C. Primers ITS1F and ITS4 (White *et al.*, 1990; Gardes & Bruns, 1993) were employed to amplify and sequence the ITS, while LR0R and LR5 (Vilgalys & Hester, 1990; Cubeta *et al.*, 1991) were used for a portion of the 28S rDNA (LSU). PCR products were checked in 1% agarose gels, and positive reactions were sequenced with one or both PCR primers. Chromatograms were edited by hand to correct reads at heteromorphic sites and other putative errors using MEGA 5.0 (Tamura *et al.*, 2011).

Phylogenetic analyses. — A single dataset with ITS and LSU sequences of the genus *Glutinoglossum* was constructed using *Sabuloglossum arenarium* (Rostr.) Hustad, A.N. Mill., Dentinger & P.F. Cannon as outgroup (Table 1). Sequences of each DNA marker were first aligned in MEGA 5.0 followed by manual correction, and then concatenated into a single dataset. The final alignment was loaded in PAUP* 4.0B10 (Swofford, 2003), divided into 2 partitions (ITS, 28S), and subjected to MRMODELTEST 2.3 (Nylander, 2004) to infer the best fitting evolutionary model of each partition. MrBayes 3.2.6 (Ronquist *et al.*, 2012) was employed to conduct a Bayesian inference (BI) analysis using model GTR+G+I, two simultaneous runs, four chains, temperature set to 0.2, and sampling every 100th generation. Convergence between runs (SD<0.01) was achieved after 0.22 M generations. A consensus phylogram was obtained from sampled trees after removing the first 25% as burn-in. Finally, a full search for the best-scoring maximum likelihood (ML) tree was performed in RAXML 8.2.10 (Stamatakis, 2014) using the standard search algorithm with the GTRGAMMAI model as recommended by the manual for datasets <50 taxa, data partitioned as for bayesian analysis, and 2000 bootstrap replications. All analyses were run locally. The significance threshold was set above 0.95 posterior probability (PP) for BI, and above 70% bootstrap proportions (BP) for ML.

Table 1 – Specimens and NCBI GenBank accession numbers of DNA sequences used in this study, in bold the newly generated. (T) means "type collection"

Species	Herbarium voucher	GenBank Accession numbers	
		ITS	LSU
Glutinoglossum americanum	ILLS:64444	KP690086	KP690098
Glutinoglossum americanum	ILLS:67352 (T)	KC222128	KC222141
Glutinoglossum australasicum	PDD:103619	KP690087	KP690099
Glutinoglossum australasicum	PDD:103623 (T)	NR155129	NG059545
Glutinoglossum circinatum	LE:303993 (T)	KX694149	KX694187
Glutinoglossum exiguum	PDD:103574 (T)	NR155130	NG059546
Glutinoglossum exiguum	PDD:103611	KP690090	KP690102
Glutinoglossum glutinosum	LE:222165 (T)	KX694157	KX694196
Glutinoglossum glutinosum	ILLS:72217	KP690091	KP690103
Glutinoglossum glutinosum	LE:303994	KX694158	KX694197
Glutinoglossum glutinosum	SAV:F11258	KX694155	KX694194
Glutinoglossum heptaseptatum	ILLS:63754 (T)	NR132024	NG060644
Glutinoglossum heptaseptatum	K:M165359	KC222131	KC222144
Glutinoglossum lumbricale	LE:303987 (T)	NR158499	KX694202
Glutinoglossum methveni	PDD:103604	KP690097	KP690109
Glutinoglossum methveni	PDD:103629 (T)	KP690096	NG059547
Glutinoglossum orientale	LE:222166 (T)	KX694166	NG060681
Glutinoglossum orientale	LE:291818	KX694167	KX694204
Glutinoglossum peregrinans	LE:303988 (T)	KX694170	KX694208
Glutinoglossum peregrinans	SAV:F11246	KX694174	KX694211
Glutinoglossum persoonii	**ASS2198**	**MW901450**	**MW901458**
Glutinoglossum persoonii	**ASS2651**	**MW901453**	**–**
Glutinoglossum persoonii	**MCVE:31360 (T)**	**MW901454**	**MW901459**
Glutinoglossum persoonii	**MCVE:31361**	**MW901455**	**MW901460**
Glutinoglossum persoonii	**MCVE:31362**	**MW901456**	**MW901461**
Glutinoglossum persoonii	**SAV:F11599**	**MW901457**	**MW901462**
Glutinoglossum proliferatum	SAV:F11249 (T)	KX694175	KX694212
Glutinoglossum pseudoglutinosum	SAV:F10406	KX694182	KX694219
Glutinoglossum pseudoglutinosum	SAV:F10903 (T)	KX694178	KX694215
Glutinoglossum pseudoglutinosum	SAV:F11196	KX694179	KX694216
Glutinoglossum pseudoglutinosum	SAV:F11243	KX694176	KX694213
Glutinoglossum pseudoglutinosum	SAV:F11251	KX694184	KX694222
Glutinoglossum pseudoglutinosum	SAV:F11255	KX694177	KX694214
Glutinoglossum pseudoglutinosum	SAV:F11264	KX694180	KX694217
Glutinoglossum pseudoglutinosum	SAV:F11265	KX694181	KX694218
Glutinoglossum triseptatum	SAV:F10262	KX694186	KX694224
Glutinoglossum triseptatum	SAV:F9828 (T)	KX694185	KX694223
Sabuloglossum arenarium	ILLS:61043	JQ256426	JQ256440

Phylogenetic results

The analysis of ITS and 28S rDNA sequences of *Glutinoglossum* available in public databases and those obtained from the samples analysed in the present work (Fig. 1) shows that the latter are significantly similar between them, and to a lesser extent to the clades of *Glutinoglosum glutinosum*, *G. pseudoglutinosum* and *G. triseptatum*, suggesting that they represent a distinct species related to these taxa. Therefore, a new name is proposed below to accommodate them.

Taxonomy

Glutinoglossum persoonii S. Saitta, A. Sierra & V. Kučera, *sp. nov.* Figs. I-III – MycoBank: MB839266

Diagnosis: Besides its unique genetic profile, it differs from other species of *Glutinoglossum* by the following combination of characters: some fruitbodies have a globose or spathulate fertile part, mostly 3- and 7-septate ascospores, asci up to 320 µm long, as well as a thick mucilaginous layer on the stipe composed of long hyphae with globose to subglobose apical elements and short lateral branches. Occurring on acidic soils in the Mediterranean area.

Holotype: Italy, Sicily, Messina (ME), Monti Peloritani, Dinnammare, Candelara 2 trail, 38°14.119′ N 15°30.919′ E, 350 m a.s.l., in the woods near the trail, on acidic soil covered with moss near *Cupressus* sp., *Pinus* sp., *Quercus* sp., *Erica arborea*, 2 January 2020, *leg*. S. Saitta, MCVE:31360, Genbank: ITS MW901454, LSU MW901459.

Etymology: Named to honour the famous mycologist and botanist Christiaan Hendrik Persoon (1761-1836), who conducted important works on the systematics of *Geoglossaceae* fungi.

Description: Ascomata occurring in small, scattered groups or in dense clusters, ranging from clavate to lanceolate, 26–47 mm long × 3–8 mm wide, entirely glutinous. **Fertile part** black, clavate, rounded or flattened, sometimes lanceolate club-shaped or trilobed, oval to lobed in cross section, measuring 8–17 × 8 mm, smooth, slightly less glutinous than the stipe. **Stipe** cylindrical, oval in cross section, 19–30 × 0.8–3 mm, straight to more or less curved, glutinous, reddish-brown to brown or dark brown, always lighter than the fertile part. **Flesh** waxy, concolorous with the surface, lacking any distinctive smell.

Asci thin-walled, slender, clavate, measuring (250–)260–315 (–320) × (13–)14–17(–18) µm, Q = 17–20, 8-spored, inoperculate, with an euamyloid apical ring and inamyloid wall in MLZ and IKI, arising from croziers. **Ascospores** cylindrical, more or less rounded at the apex, straight to slightly curved, (70–)75–89(–96) × (4.5–)4.7–5.7(–6.3) µm, Q = (11.2–)13.5–18(–20), Qm=14.5, light brown, (0) 3

Figure I – *Glutinoglossum persoonii* holotype MCVE:31360. Scale bar = 1 cm. Photo by S. Saitta.

Figure II – *Glutinoglossum persoonii in situ*. 1: Coll. ASS2198, 2: Holotype MCVE:31360, 3: Coll. MCVE:31362, 4: Coll. ASS2644. Photos 1,4 by A. Sierra; 2,3 by S. Saitta.

or 7-septate. **Ascoconidia** not observed. **Paraphyses** slightly protruding above the asci, numerous, fragile, hyaline and cylindrical at the base which measures (1.5–)1.9–3.4 µm diam., septate, constricted at the septa, sometimes inflated at the tip, frequently straight but rarely slightly curved or hooked. **Apical cells of paraphyses** swollen, globose, subglobose, pyriform or utriform, 16.5–33.6 × 3.3–10.5 µm, pale brown to hyaline. **Hyphae of stipe surface** immersed in a gelatinous matrix, of two main types: 1) light brown near the tip, 1.5–2.4 µm diam., moderately septate, straight or slightly curved, terminal elements globose or subglobose 2.8–4.7 µm wide, sometimes branched; 2) narrow hyaline hyphae 1–2 µm diam., terminal elements 1.8–3.4 µm wide, cylindrical or sometimes globose, rarely with side branches up to 8.2 × 1 µm.

Additional material examined: Italy. Sicily, Messina (ME), Monti Peloritani, Dinnammare, Candelara 2 trail, 38°14′7.14″ N 15°30′55.14″ E, 350 m a.s.l., in the woods near the trail, on soil and moss, with *Cupressus* sp., *Pinus* sp., *Quercus* sp. and *Erica arborea*, 7 February 2019, *leg*. S. Saitta (MCVE:31362). Sicily, Buccheri (SR), Bosco della Contessa, 37°06′46.74″ N 14°51′55.74″ E, 870 m a.s.l., in *Pinus* sp. and *Cupressus* sp. woods, on soil and moss, 5 February 2020, *leg*. S. Saitta, M. Carbone & V. Avola (MCVE:31361). Spain. Catalonia, Barcelona, Piera, Macizo de la Fembra Morta, near the trail, 41°33′33″ N 1°43′36″ E, 630 m a.s.l., on soil covered with moss near *Quercus* sp., *Pinus* sp., *Arbustus unedo* and *Erica* sp., 13 February 2020, *leg*. A. Sierra (ASS2198). *Ibidem*, 23 February 2020, *leg*. A. Sierra (ASS2644). Catalonia, Girona, Bescanó, Can Rauric, 41°55′43.6″N 2°43′06.2″E, 150 m a.s.l., among mosses, in a mixed oak and pine forest, 29 December 2018, *leg*. M.À. Pérez-De-Gregorio (SAV:F-11599). Extremadura, Cáceres, Casas de Miravete, 39º43´56.11″ N 5º45´ 20.21″ W, 450 m a.s.l., in humus of a mixed forest made up of *Eucalyptus globulus*, *E. camaldunensis*, *Quercus suber*, *Q. ilex*, *Cupressus* sp., *Rubus* sp. and *Cistus ladanifer*, 20 February 2021, *leg*. J.A. Suárez (ASS2651).

Habitat: On acidic soil, among mosses growing near *Pinus* sp., *Quercus* sp., *Eucalyptus* sp., *Cupressus* sp., *Arbutus unedo* or *Erica* sp. plants.

Distribution: Currently known only from the Mediterranean area, in Italy (Sicily) and Spain (Catalonia).

Discussion

According to Fedosova *et al.* (2017), one of the most important characters for identification of *Glutinoglossum* species is the number of ascospores septa inside the asci. Based on this character they recognised three groups: 1) species with 3-septate ascospores: *G. glutinosum*, *G. triseptatum* and *G. methvenii*; 2) species with 7-septate ascospores: *G. heptaseptatum*, *G. australasicum*, *G. americanum*, and *G. exiguum*; 3) species with both 3- and 7-septate ascospores in asci: *G. circinatum*, *G. lumbricale*, *G. orientale*, *G. proliferatum*, *G. pseudoglutinosum*, and *G. peregrinans*. *Glutinoglossum persoonii* belongs to the third group, although this classification is not related with genetic data. The main differences with the other species of *Glutinoglossum* with similar ascospores are discussed below. Data used for comparision were taken from original descriptions (Fedosova *et al.*, 2017).

G. circinatum has shorter and wider asci (217–249 × 15.5–21 µm vs. 260–315 × 14–17 µm in *G. persoonii*) with curved to circinate (vs. straight to slightly curved in *G. persoonii*) hyphae of the stipe surface.

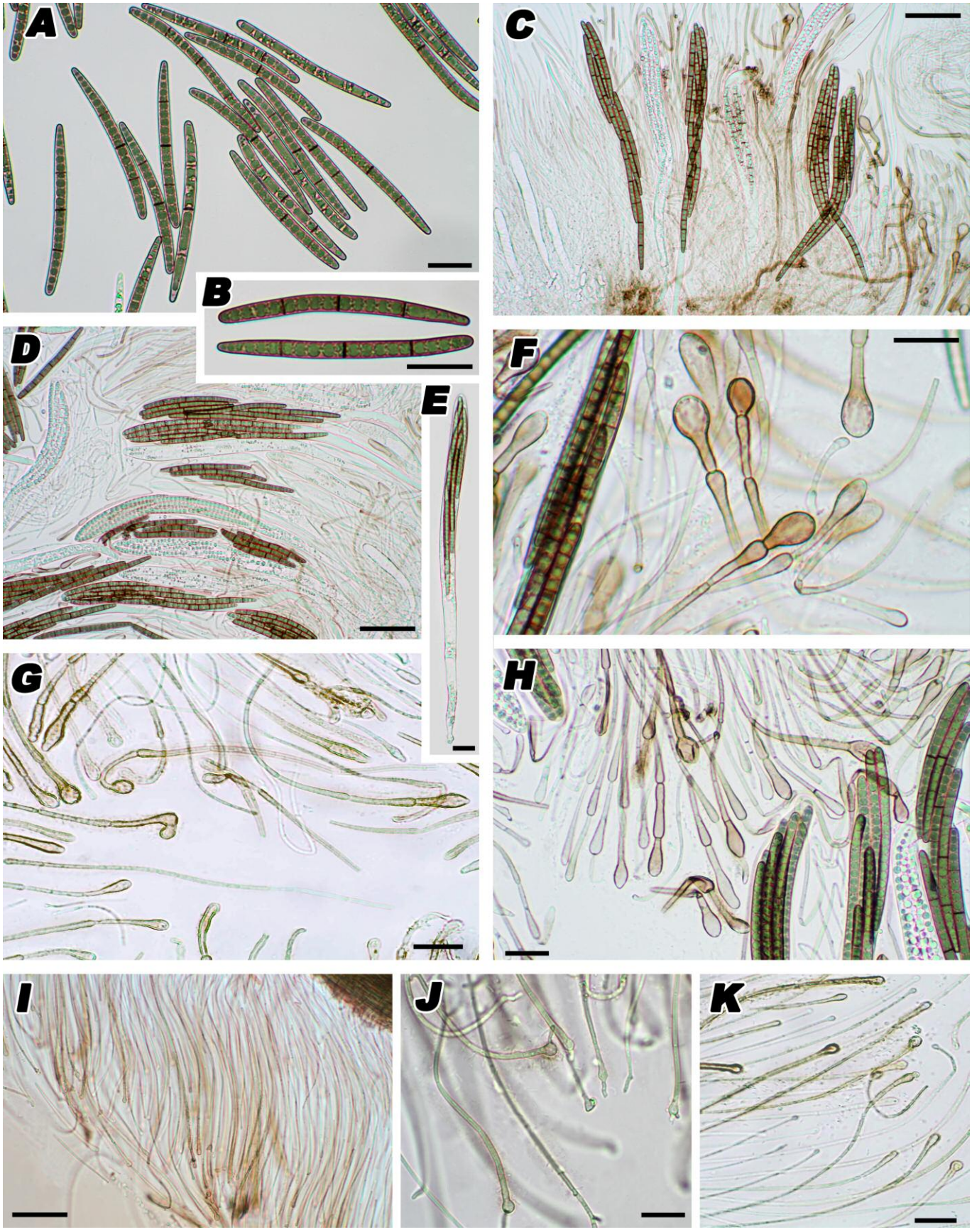

Figure III – *Glutinoglossum persoonii*, microscopical elements in water. A, B: Ascospores from fresh sporal deposit. C, D, E: Asci. F, G, H: Paraphyses. I, J, K: Hyphae of the stipe surface. Scale bars = 20 µm, except C, D, I = 50 µm. Photos A-F, H-J by S. Saitta; G, K by A. Sierra.

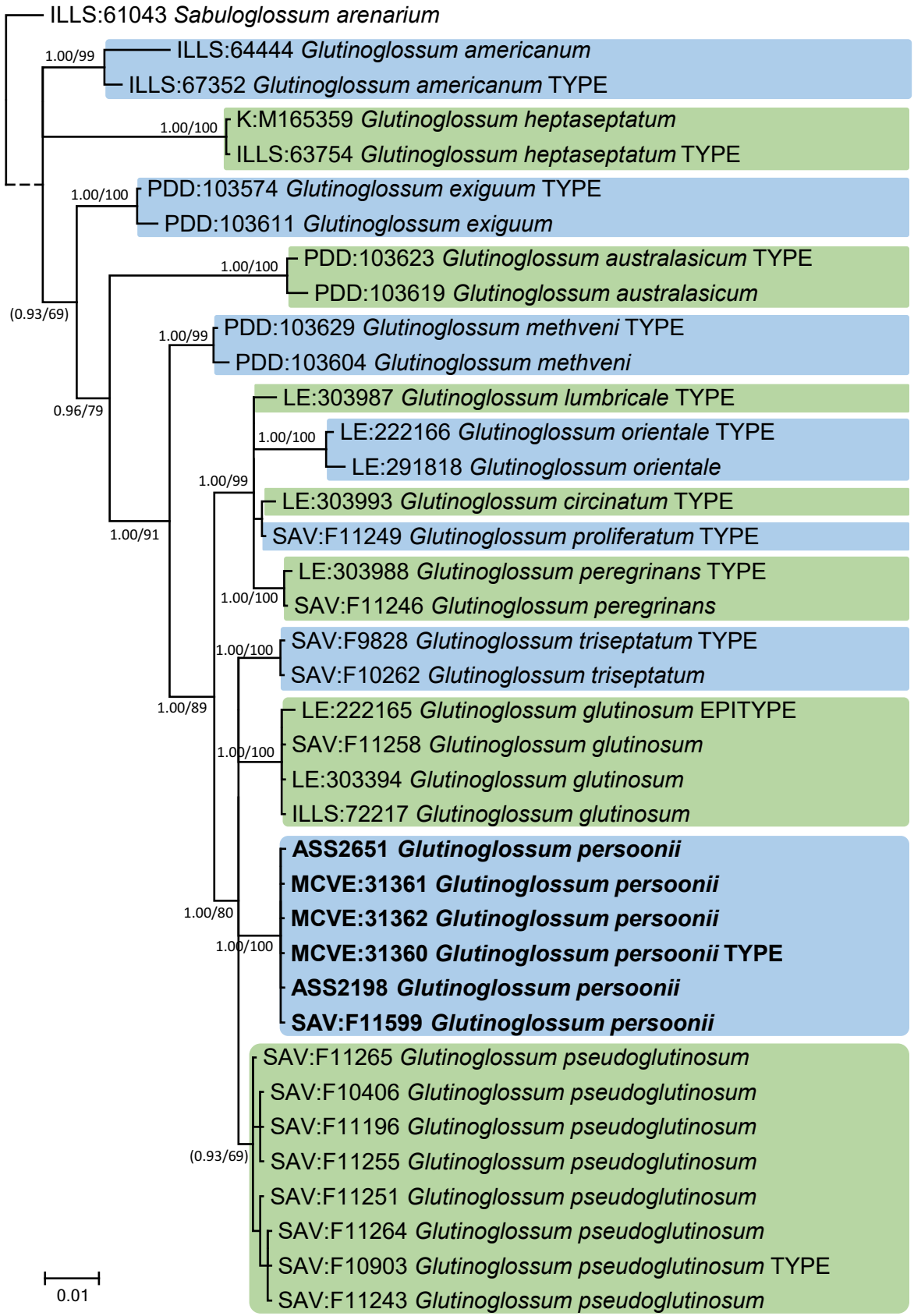

Figure IV – A 50% majority rule ITS rDNA-28S rDNA consensus phylogram of the genus *Glutinoglossum* (*Geoglossaceae*), with *Sabuloglossum arenarium* as outgroup, obtained using MrBayes from 1650 sampled trees. Nodes were annotated if they were supported by ≥ 0.95 Bayesian posterior probability (left) or ≥ 70% maximum likelihood bootstrap proportions (right). Sequences newly generated in this study are in bold.

G. lumbricale has shorter and thinner asci (202–231 × 13.5–14 µm), with a Q = (14.4–)15.8(–16.9) vs. Q = 17–20 in *G. persoonii*, narrower ascospores (73.9 × 4 µm vs. 75–89 × 5–6 µm in *G. persoonii*) and thinner apical cell of paraphyses (20 × 4 µm vs. 17–34 × 3–10.5 µm in *G. persoonii*).

The slightly shorter ascospores (75–89 × 5–6 µm) and longer asci (260–315 × 14–17 µm) separate *G. persoonii* from the similar *G. orientale* (83.6–94.2 × 4.6–4.7 µm respectively 237–280 × 14.5–16.5 µm).

G. proliferatum has apical cells of paraphyses pale brown to brown, pyriform, globose or swollen, measuring 12 × 9 µm, frequently proliferated, while *G. persoonii* has these cells brown to hyaline, pyriform or utriform and longer (17–34 × 3–10.5 µm).

G. pseudoglutinosum has thinner apical cells of paraphyses (2.1–5 µm vs. 3.3–10.5 µm in *G. persoonii*) and occurs preferably on soils developed over calcareous bedrocks while *G. persoonii* occurs on acidic soils.

G. peregrinans has slightly shorter asci (225–255 × 13.5–17 µm vs. 260–315 × 14–17 µm in *G. persoonii*) and has sometimes branched apical elements in its paraphyses, while these are unbranched in *G. persoonii*. Similar paraphyses (globose to pyriform, brown at the tip) have been observed also in *G. glutinosum* and *G. heptaseptatum*, but the first has predominantly 3-septate ascospores and smaller asci, and the second has ascospores with 7 septa.

Hyphae on the stipe surface of *G. persoonii* are of two types, a feature that is probably present also in other species of *Glutinoglossum*, but not mentioned by previous authors. Hyphae of the first type are light brown in the tip, moderately septate, straight or slightly curved, with globose or subglobose terminal cells, sometimes branched. Those of the second type are narrow, hyaline, sometimes laterally branched, with cylindrical or globose tips. Hyphae are branched laterally in their middle part or in the upper part when apical cells proliferate.

To our knowledge, *G. persoonii* is the first species of *Glutinoglossum* apparently endemic to the Mediterranean region. However, additional studies focused on *Geoglossaceae* species with sticky and gelatinous fruitbodies are needed to clarify the taxa present in this ecoregion. Up to now, thirteen species of *Glutinoglossum* have been described (Hustad & Miller, 2015; Fedosova *et al.*, 2017) and only two of them are present in the Mediterranean region (*G. glutinosum* and *G. persoonii*). Before 2013, when the genus *Glutinoglossum* was established (Hustad *et al.*, 2013), many of these collections were determined as *Geoglossum glutinosum*. Reports are known from Bulgaria (Hinkova & Stoichev, 1983), Spain (Iglesias, 2006; Arauzo & Iglesias, 2014), Greece (Zervakis *et al.*, 2002), Italy (Massee, 1897), Portugal (Massee, 1897; Arauzo & Iglesias, 2014), France (Massee, 1897; Priou, 1992; Moingeon & Moingeon, 2003; Fedosova *et al.*, 2017). Some of these collections could actually represent *Glutinoglossum persoonii* because it can be easily confused with *G. glutinosum*.

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Authors' contributions

Salvatore Saitta provided the first collections and was responsible for the preliminary morphological observations and descriptions, pictures editing, figures and plates design. Viktor Kučera provided the first draft of the manuscript and deep morphological comparisons between the species of the genus. Agustin Sierra provided new voucher specimens giving life to the project, contributing with rich microscopical studies, morphological observations and invaluable fixes, comments and enhancements. Pablo Alvarado performed all the molecular analyses and provided a critical revision of the manuscript. All authors equally contributed to the update of the first draft of the manuscript, read and approved the final version.

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1: S. Saitta – Via Di Anfuso, pal. 28, 98147 Messina, Italy – s.saitta@outlook.it

2: A. Sierra – 2C/ Santa Maria de Miralles, 2 - 3º 1ª, 08700 Igualada, Spain – agustingallur@gmail.com

3: V. Kučera – Plant Biology and Biodiversity Centre, Institute of Botany, Slovak Academy of Sciences, Dúbravská cesta 9, 845 23 Bratislava, Slovakia – viktor.kucera@savba.sk

4: P. Alvarado – Alvalab, Dr. Fernando Bongera st., Severo Ochoa bldg. S104, 33006 Oviedo, Spain – pablo.alvarado@gmail.com