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Geodina (Pezizomycetes: Wynneaceae) has a single widespread species in tropical America

D.H. Pfister*, L. Quijada, K.F. LoBuglio

Farlow Library and Herbarium, Harvard University, 22 Divinity Ave., Cambridge, MA 02138 USA

*Corresponding author: dpfister@oeb.harvard.edu

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Abstract: *Geodina salmonicolor* is shown to be a synonym of *G. guanacastensis*, the type and only species of the genus. Comparisons of ITS rDNA sequences of a paratype and two recent collections of *G. guanacastensis* with published ITS sequences of *G. salmonicolor*, from the Dominican Republic, show that these are nearly identical. When *G. salmonicolor* was erected no sequences of the type species were available. Morphological comparisons supports the conspecificity. Details regarding the description of *G. salmonicolor* are pointed out. A four-gene phylogeny places *Geodina* and *Wynnea* as a supported sister group to the rest of the *Sarcoscyphaceae*. Species in these genera share morphological traits of cyanophobic spore markings, dark angular outer excipular cells that give rise to hairs and the origin of several apothecia from a common basal stalk. Their occurrence on soil rather than on wood or plant material distinguish them from other *Sarcoscyphaceae*. Based on morphology, phylogenetic relationships and trophic interactions we erect a new family, *Wynneaceae*, for *Geodina* and *Wynnea*.

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INTRODUCTION

The genus *Geodina* (Denison 1965), described more than 50 years ago, has remained an enigmatic taxon. The original and only species, *G. guanacastensis*, seemingly was not recollected until recently. It was placed in the *Sarcoscyphaceae* because of ascus form, anatomical details of the ascoma and hymenial coloration but, unlike other members of the *Sarcoscyphaceae*, it is distinctive because of its occurrence on soil. Other *Sarcoscyphaceae* are found on wood and plant material. In recent years a few collections of *G. guanacastensis* have come to light. Originally described from collections from Costa Rica there have been records and postings of *G. guanacastensis* from Florida, USA, and Mexico (Ortega-Lopez *et al.* 2019, Mushroom Observer: Observations 187260, 296893, 357225). These recent findings provide some insight into the distribution and the taxonomy of this species. This investigation was undertaken to determine the identity of several recent collections and to evaluate the recently described species, *G. salmonicolor* (Angelini *et al.* 2018), from the Dominican Republic.

Geodina salmonicolor was said to be distinguished from *G. guanacastensis* by hymenial color, pink salmon to pale pink-orange vs. pale orange to light yellow-orange, and larger ascospores ($35\text{--}39 \times 11\text{--}13 \mu\text{m}$ vs. $22\text{--}25 \times 11\text{--}13 \mu\text{m}$) (Angelini *et al.* 2018). Angelini *et al.* (2018) did not report examining the holotype or other material of *G. guanacastensis*. Using ITS and 28S rDNA sequences from the Dominican collections they were able to place their species in a clade with *Wynnea* in the *Sarcoscyphaceae*. In this paper we report on a recently collected specimen of *G. guanacastensis* and an ITS sequence obtained from a paratype of *G. guanacastensis*. In light of sequence data and a detailed morphological study we reconsider the

identity of *G. salmonicolor*. We provide a revised description and illustrations of *G. guanacastensis*. We have studied and compared species of *Wynnea* and *G. guanacastensis*. Our goals in this study were to determine the species identity of these collections; to further investigate the relationship between *Wynnea* and *Geodina*; and to highlight the shared phylogenetic and morphological characteristics of the species in these two genera.

MATERIALS AND METHODS

Material studied

Bahamas: Hardwood forest near Albany, New Province, Nassau, 2 Dec. 2017, *D. Maillis*. Specimen deposited at Leon Levi Native Plant Preserve, Banks Road, Eleuthera, Bahamas.

Costa Rica: Kilometre 135, Pan American Highway no[rth] of Punta Arenas, Guanacaste Province, Denison *et al.* 2278, 13 Sep. 1964 (CUP-CA 81, **holotype**); Playa del Coco, Guanacaste, alt 200 ft, Denison *et al.* 2310, 14 Sep. 1964 (CUP-CA 84); Caña, Guanacaste Province, alt 150 ft., Denison *et al.* 2294, 13 Sep. 1964 (CUP-CA 83, CUP-CA 82). [A note on these collections. Even though we were unable to study these collections because of their ravaged condition we cite them to aid future researchers. A small fragment of CUP-CA 84 was used in the molecular work]. A collection of *Geodina* from Everglades National Park, Florida, USA was reported on the Mushroom Observer website (https://mushroomobserver.org/observer/show_observation/357225). The ITS DNA sequence data from this specimen, determined by Arian Farid, University of Southern Florida, was included in our study.

Morphological methods

Macro-photography of apothecia on their substrate were taken and apothecia examined in living condition. Hand sections of the apothecia were mounted and described in tap water or using 3 % potassium hydroxide (KOH), and then stained with Congo Red (CR) or Melzer's reagent (MLZ). Micro-photography was done using a Motic B1 (MoticEurope S.L.U., Spain) compound light microscope with a USB MotiCam 2500 camera. Motic Images Plus v. 2.0 processing software, calibrated for the optical devices of Motic B1, was used to carry out the biometry of each microscopic features. Measurements are given as follows: (smallest single measurement–) smallest mean–largest mean (–largest single measurement). The small and large means are based on 10–30 measurements on individual specimens and obtained doing 95 % confidence intervals. Photographic plates were assembled using Illustrator CC (Adobe Systems, San José, CA). The following abbreviations were adopted (Baral 1992): * = living state; † = dead state. Color coding refers to Anonymous (1976).

Molecular techniques

The new DNA sequences that contributed to this phylogenetic study were obtained from either DNA extraction of ascomata or previously extracted genomic DNA stocks that had been kept at -20 °C (Table 1). DNA extraction from ascomata utilized the Qiagen DNeasy Plant Mini kit (Germantown, MD cat #69104) as previously described (Pfister & LoBuglio 2018). PCR amplification utilized the following primer combinations: primer pair ITS1F, ITS2, ITS3 and ITS4 (Gardes & Bruns 1993, White *et al.* 1990) for the internal transcribed spacer region plus 5.8S gene (ITS); primer pair LROR and LR7 (Moncalvo *et al.* 2000) for the large subunit ribosomal RNA gene (28S); primers NS1, NS4, SL344, SL122 and NS8 (Landvik *et al.* 1997, White *et al.* 1990) for the small subunit ribosomal RNA gene (18S); primers RPB2-P6Fa and RPB2-P7Ra (Hansen *et al.* 2005) for the DNA-directed RNA polymerase II second largest subunit gene (*RPB2*); and either primer pairs EF1-983F and EF1-2218R or EF1-983F and EF1-1567R (Rehner 2001, Rehner & Buckley 2005) for the translation elongation factor 1- α gene (*EF*).

The *RPB2* and *EF* gene regions were difficult to amplify from the herbarium samples of *Wynnea* species included in this study. Since a phylogenetic relationship between *Wynnea* and *Geodina* was initially established from phylogenetic analysis of the 28S rDNA, it allowed specific primers to be designed and successfully implemented in PCR. For the *RPB2* region specific primers were designed based on the *Geodina* *RPB2* DNA sequence. The sequence for the *RPB2* specific primers are: GG3_RPB2-6F 5'-GCGTACATAAGTGTGGGAACA-3', GG3_RPB2-7R 5'-CCATGGCCGATTGATATGTGT-3. The *EF* gene was successfully amplified for *Wynnea macrotis*. This sequence was used to design *Wynnea* specific primers for the *EF* gene. The sequences for the *EF* specific primers are: WM2_EF-983F 5'-TACTGGTACATCCCAGGCT-3', and WM2_EF-1567R 5'-GCGTACATAAGTGTGGGAACA-3'. The NCBI primer designing tool (<https://www.ncbi.nlm.nih.gov/tools/primer-blast/>) was used to design these specific primers.

PCR amplifications were carried out in a BIO-RAD thermocycler. For PCR, 5 μ L of 1/10 and 1/100 dilutions of the DNA extracts were used as templates in a total reaction volume of 25 μ L. BIO-RAD iProof High-Fidelity PCR Master Mix (Hercules, CA, cat

#1725310) was used for PCR amplification of the ITS, 28S, 18S, and *EF* gene regions. For the ribosomal DNA regions the PCR cycling parameters followed the BIO-RAD recommendations for iProof High-Fidelity DNA Polymerase and were as follows: 94 °C for 3 min, then 35 cycles of 94 °C for 5 seconds, a primer annealing step at 53 °C for 30 s, an extension at 72 °C for 30 s, followed by a final elongation step at 72 °C for 5 min and a 4 °C soak. The PCR reactions of the *RPB2* region were most successful using Invitrogen Platinum *Taq* DNA Polymerase High Fidelity (Invitrogen, Thermo Fisher Scientific, Waltham, MA, cat # 11304011). For *RPB2*, the PCR cycling parameters were as follows: 94 °C for 3 min, then 35 cycles of 94 °C for 45 s, a primer annealing step at 52 °C for 45 s, an extension at 72 °C for 1:30 min, followed by a final elongation step at 72 °C for 10 min and a 4 °C soak.

Multiple bands were often present from *RPB2* and *EF* PCR products. In these cases, the bands of interest were excised from 2 % agarose gels and purified using the Qiagen Gel extract kit (Germantown, MD cat # 28704). PCR product of all genes were sent to GeneWiz Inc. sequencing facilities (Cambridge, MA) for Sanger Sequencing. The forward and reverse sequences from each PCR product were edited using Sequencher v. 5.1 (GeneCodes, AnnArbor, Michigan). Sequences are deposited in GenBank and listed in Table 1.

The QIAamp DNA micro kit (Qiagen cat. #56304) was used to extract material from a paratype of *G. guanacastensis* (CUP-CA 84) because tissue sampling was very limited (approximately 1 mm square). PCR amplification of the ITS utilized the same procedures as above using the primer pairs ITS1F-ITS2 and ITS3-ITS4 and the BIO-RAD iProof High-Fidelity PCR Master Mix. The ITS DNA sequence of this type specimen was compared to our recent *Geodina* collection and to the sequence of *G. salmonicolor* reported by Angelini *et al.* (2018) using the alignment feature in NCBI BLAST (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>).

DNA sequence and phylogenetic analyses

A phylogenetic analysis of the four-gene (*EF*, 28S, *RPB2*, and 18S) concatenated data was assembled. DNA sequences were aligned using MUSCLE v. 3.7 and the model of nucleotide substitution was determined using jModelTest2 on XSEDE through the CIPRES Science Gateway (Miller *et al.* 2010). The four-gene data matrix included 23 taxa from the families *Sarcoscyphaceae*, *Sarcosomataceae*, *Chorioactidaceae* and the outgroup taxa *Morchella elata* and *Gyromitra californica* (Table 1). Phylogenetic analyses were performed using Maximum likelihood (ML) and Bayesian analyses through the CIPRES Science Gateway (Miller *et al.* 2010). Both phylogenetic methods used the GTR+I+G model of sequence evolution. The ML analyses used RAxML-HPC2 on XSEDE (v. 8.2.12) (Stamatakis 2014) using the default parameters, and branch support was determined by 1 000 bootstrap replicates. Bayesian inference used Mr Bayes v. 3.2.6 (Huelsenbeck and Ronquist 2001) and consisted of four Markov chain Monte Carlo (MCMC) chains initiated from random trees for 10 000 000 generations and with tree sampling every 200 generations. The first 25 % of trees were discarded as the burn-in phase of each analyses, and posterior probability (PP) values were determined from the remaining trees. Single gene ML trees were determined for each of the 4 gene regions for comparison with the four-gene phylogeny to determine conflicting results.

ITS DNA sequence data for the two isolates of *G. salmonicolor* (MG597289 and MG597290/holotype), *G. guanacastensis* from the Bahamas and Florida and a paratype were phylogenetically

Table 1. Specimens included in phylogenetic analyses. The * indicates sequences obtained in this study from the collection listed. NA denotes not available. Collection numbers are given for taxa where a collection or DNA stock was used to generate DNA sequences.

Taxon Name	Collection Number	GenBank accession numbers				
		ITS	28S	18S	RPB2	EF
<i>Chorioactis geaster</i>			KC012672	AF104340	DQ017609	KC109211
<i>Cookeina tricholoma</i>	MH 686 DNA Stock		AY945860	AF006311	MN103428*	MN103423*
<i>Desmazierella acicola</i>			AY945854	AF104341	DQ017603	LN794774
<i>Donadinia nigrella</i>			AY945853	NA	DQ017592	KC109214
<i>Galiella rufa</i>			AY945850	AF004948	DQ017594	KC109213
<i>Geodina guanacastensis</i>	CUP-CA 84	MN096938*	NA	NA	NA	NA
	Bahama collection	MN096939*	MN096940*	MN096941*	MN103424*	MN090946*
<i>Geodina salmonicolor</i>	JBSD127408	MG597289	MG597287	NA	NA	NA
	JBSD127409	MG597290	MG597288	NA	NA	NA
<i>Gyromitra californica</i>			AY544673	AY544717	DQ470891	DQ471059
<i>Komposocypha phyllogena</i>	DHP 10-690 DNA Stock		JQ260810	JQ260820	MN103430*	MN103416*
<i>Morchella elata</i>			U42667	U42641	AF107810	HM756737
<i>Neournula pouchetii</i>			AY307940	AF104666	DQ017601	NA
<i>Phillipsia carnicolor</i>	DHP 7126 DNA Stock		JQ260811	JQ260821	MN103426*	MN090948*
<i>Pithya cupressina</i>	FH 00465472		JQ260818	AF006316	MN103429*	MN103415*
<i>Plectania melastoma</i>	MH 679 DNA Stock		JX669850	MN102128*	MN103434*	MN103422*
<i>Pseudopithyella minuscula</i>			AY945849	AF006317	DQ017600	FJ238387
<i>Pseudoplectania nigrella</i>	KH.97.28 DNA Stock		AY945852	MN096942*	MN103433*	MN103421*
<i>Pseudosarcosoma latahense</i>			FJ176860	FJ176806	NA	FJ238392
<i>Rickiella edulis</i>	FH 01146895		JQ260809	JQ260819	MN103425*	MN090947*
<i>Sarcoscypha austriaca</i>	MH 670 DNA Stock		AY945856	AF006318	MN103427*	KC109210
<i>Sarcosoma globosum</i>	KH.07.04 DNA Stock		FJ499393	U53386	JX943753	KC109215
<i>Urnula craterium</i>			AY945851	AF104347	DQ017595	KC109216
<i>Wolfina aurantiopsis</i>			AY945859	AF104664	DQ017605	KC109212
<i>Wynnea americana</i>	FH 00445979 DNA Stock	MK599141	AY945848	MK592785	MN103435*	MN103417*
<i>Wynnea macrospora</i>	FH 00445975 DNA Stock	MK335784	MK335803	MK335793	MN103432*	MN103419*
<i>Wynnea macrotis</i>	CUP 2684 DNA Stock		MK335804	MK335795	NA	MN103420*
<i>Wynnea sparassoides</i>	FH 00445986 DNA Stock		EU360917	MK335796	MN103431*	MN103418*

analyzed as described above with *Wynnea macrospora* (MK335784) and *W. americana* (MK599141) as outgroups. The percent identity of ITS DNA sequence among the *Geodina* specimens was analyzed using the MUSCLE alignment program in Geneious v. 10.2.3 (<https://www.geneious.com>).

RESULTS

Taxonomy

Geodina guanacastensis Denison, *Mycologia* **57**: 650. 1965. Figs 1A–G, 2C.

Synonym: *Geodina salmonicolor* Angelini & Medardi, *Mycosphere* **9**: 172. 2018.

Apothecia 10–30 mm diam, up to (10–)25–40 mm high, terricolous, solitary or gregarious in small groups with 2–4(–6) stipitate apothecia, with shared rooting stipes, wider near the receptacle tapering downwards. **Receptacle** cyathiform to funnel-shaped, irregularly undulate at maturity, alternating

smooth and hairy concentric rings, smooth areas light greyish brown (60.l.gy.Br) to light brown (57.l.Br), hairy concentric rings medium brown (58.m.Br) to dark greyish brown (62.gy.Br). **Margin** differentiated, with long hairs aggregated in triangular fascicles. **Disc** smooth, light yellowish pink (28.l.yPink) to medium orange (53.m.O), strongly depressed in the centre. **Stipe** strongly differentiate from the receptacle, dark greyish-brown (62.d.gy.Br) to black (267.Black), longitudinally wrinkled and tapering downwards. **Hairs** cylindrical, 118–516 µm long at margin, 66–205 µm long at flanks, apex tapering to 2–3.5 wide, apex blunt, rounded to obtuse; medium brown (58.m.Br) to deep brown (59.d.Br), walls darker and refractive, up to 1 µm thick at the apex, up to 2 µm in the lower cells; unbranched and sparsely septate, distance between septa more than 50 µm. **Ectal excipulum** from base to flank of *textura angularis* to *t. prismatica*, inner layer light greyish brown (60.l.gy.Br), *60–86 µm thick at flanks; margin composed of *textura porrecta* to *t. oblita*, inner layer light yellowish brown (76.l.yBr), outermost layer of the ectal excipulum from base to margin medium brown (58.m.Br) to deep brown (59.d.Br). **Ectal cells** *(7–)11–13(–16) × (5.5–)7–8(–10.5) µm at middle flank, isodiametric, wall

slightly thickened up to $*1\ \mu\text{m}$; cells at margin long rectangular, running parallel, cohering, $*(16.5\text{--}20\text{--}28.5\text{--}30) \times 2.5\text{--}3.5\ \mu\text{m}$, walls refractive and strongly thickened up to $*2\ \mu\text{m}$. Medullary excipulum of *textura intricata*, $*64\text{--}240\ \mu\text{m}$ thick at flanks, cells $*(14.5\text{--}27.5\text{--}43.5\text{--}91.5) \times (3.5\text{--}4.5\text{--}5.5\ \mu\text{m})$, thin-walled, intercellular spaces present or absent, without gel. Asci $*(440\text{--}467\text{--}536\text{--}575) \times 21.5\text{--}23\text{--}24\ \mu\text{m}$, $†(421\text{--}425\text{--}466\text{--}488) \times 16.5\text{--}19\ \mu\text{m}$; cylindric, 8-spored, uniseriate, pars sporifera $*161\text{--}192\ \mu\text{m}$, lateral walls thick, up to $†3.5\ \mu\text{m}$, asci inamyloid in MLZ and LUG, with or without KOH pre-treatment, wall at apex thinner up to $†2.5\ \mu\text{m}$ thick, operculum eccentric, $†8\text{--}9.5\ \mu\text{m}$ diam; base curved and gradually tapered arising from a constricted simple septa. Ascospores $*(21.7\text{--}24\text{--}25.4\text{--}28.3) \times (11.2\text{--}12.2\text{--}12.8\text{--}14.5)\ \mu\text{m}$, $†(21.1\text{--}23.1\text{--}24.7\text{--}27) \times (10.4\text{--}11.3\text{--}12.1\text{--}13.5)\ \mu\text{m}$; ellipsoid, straight, inequilateral, hyaline, cyanophobic, sculptured, ornamentation consisting of coarse, irregular, longitudinal ridges ($0.5\text{--}1.5\ \mu\text{m}$ high) which anastomose to form an irregular reticulum, not lost after KOH pre-treatment (spore walls sometimes loosening in KOH), with apiculi, $1.8\text{--}3.6\ \mu\text{m}$ in height; oligo- to multi-guttulate (lipid bodies), guttules pale yellow (89.p.Y), 2–3(–5) large guttules ($3\text{--}6.5\ \mu\text{m}$ diam.) surrounded by several smaller guttules ($0.5\text{--}2.5\ \mu\text{m}$ diam.), oil content inside the spores 75–90 %. Paraphyses cylindrical to slightly enlarged at the apex, apical cells $*17.5\text{--}55.5 \times 2.5\text{--}4\ \mu\text{m}$, cells below $*20\text{--}33 \times 1.5\text{--}3\ \mu\text{m}$; septate, simple to bifurcate toward the base, thin-walled, with one or several yellow grey (93.yGrey) guttules in the apical cells (vacuolar bodies).

Remarks: Our description of the specimens generally agree with Dennison (1965), Angelini *et al.* (2018) and Ortega-López *et al.* (2019). We were unable to study the type material of *G. salmonicolor* from the Jardín Botánico Nacional, Dr. Rafael Ma. Moscoso (JBSD). But some differences were noted. These three authors described the length of the asci in a range between 300–450 μm , but we found the asci to be $*440\text{--}575\ \mu\text{m}$ or $†421\text{--}488\ \mu\text{m}$. This difference may have resulted from the study of dead asci, but also asci are deeply rooted in the subhymenium often curved and twisted. It is likely that they did not measure the complete asci. Our ascospore size range agrees with Dennison (1965) and Ortega-López *et al.* (2019), but not with Angelini *et al.* (2018). In our study we noticed that some spores can swell after pre-treatment with some reagents (KOH + CR), this produces a deviation of the maximum length of the spore up to $7\ \mu\text{m}$, from $27\ \mu\text{m}$ to $34\ \mu\text{m}$. This probably accounts for their measurement of spore length up to $33.5\ \mu\text{m}$. Treatment with KOH can also loosen the outer wall causing it to detach and expand several μm with respect to the inner wall. In our measurements such ascospores were excluded. Spore size was a primary feature used by Angelini *et al.* (2018) to distinguish *G. samonicolor*. The previous reports and our observations also differ in the interpretation

of the excipulum and hairs. Angelini *et al.* (2018) seem to have interpreted hair morphology differently and confused fasciculate hairs with single hairs, therefore their description and measures are misleading and refer mostly to fasciculate hairs not to single hairs. The medullary excipulum is described by all authors as *textura intricata*, but there are inconsistencies regarding the interpretation of the ectal excipulum. None of the previous reports describe the ectal excipulum at the margin, flanks and base, and clearly differences exist between the tissues in these regions (Fig. 1: E1–E2). Denison (1965) described the ectal excipulum as *textura prismatica* to *t. angularis*, Angelini *et al.* (2018) noted that it was composed of *t. globulosa* to *t. angularis* and Ortega-López *et al.* (2019) wrote of *textura angularis* to *t. epidermoidea*. We disagree with the interpretation of these tissues as *textura globulosa* or *t. epidermoidea*. *Textura angularis* to *t. prismatica* are found at the base (Fig. 1: E4), whereas in the margin *textura porrecta* to *t. oblita* (Fig. 1: E3) are observed. Finally, the morphology of the ascospores is well described in all the papers, but none of them specifically mentioned the apiculi (Fig. 1: G1). Previous studies of *Geodina* specimens all mention that the spores have 1–2 guttules. We have observed in fresh material that ascospores are multiguttulate and that the pattern is lost when they are dead in which condition small guttules merge to form one or two large bodies (Fig. 1: G1–G2).

Molecular results

The ITS sequence of the *Geodina* specimen collected in the Bahamas was 100 % identical to the ITS sequence of the holotype of *G. salmonicolor* JBSD 127409 (GenBank # MG597290) and 99.8 % identical to *G. salmonicolor* JBSD 127408 (GenBank # MG597289) (Fig. 2B). The *G. salmonicolor* collections were determined by Angelini *et al.* (2018). Comparison of the ITS sequence data indicated that the three specimens of *Geodina*, the one from the Bahamas and the two isolates of *G. salmonicolor* (JBSD 127408 and JBSD 127409, Angelini *et al.* 2018) had respectively 99.7 %, 99.5 % and 99.6 % sequence identity to *G. guanacastensis* and is presented in Fig. 2B. Thus, the ITS phylogeny does not support the recognition of two *Geodina* species.

Phylogenetic analyses using ML and Bayesian analyses of the combined *EF*, 28S, *RPB2*, and 18S data set (Fig. 2A) indicated that *Geodina* formed a highly supported sister group with *Wynnea* species. The sister group relationship between *Geodina* and *Wynnea* was resolved in each single gene phylogenetic analyses. This relationship was furthermore supported in all single gene phylogenies, except for the *EF* phylogeny. The families *Sarcosomataceae*, *Sarcoscyphaceae* and *Chorioactidaceae* were highly supported as monophyletic in analyses of the combined data set and for individual gene analyses of the 28S, *RPB2* and 18S data sets.

Fig. 1. Morphological features for *Geodina guanacastensis*. **A.** Fresh apothecia in their natural conditions. **B.** Transverse section of an apothecium. **C.** Asci: C1–2. Mature and immature living asci with ascospores, C3–4. Details of the apex on living (slightly refringent thick walls) and dead asci (thick walls), C5–6. Eccentric operculum. **D.** Paraphyses: D1. Living paraphyses with long or short guttules (vacuolar bodies) inside the apical cells, D2. Dead branched paraphyses without guttules. **E.** Excipular tissues: E1 & E3. Details of *textura porrecta* to *t. oblita* in the margin and upper flanks, E2 & E4. Details of *textura angularis* to *t. prismatica* in the lower flanks and base; E5. Medullary excipulum. **F.** Hairs: F1–F2. Single hairs with thick refractive walls, F3. Fascicled hair. **G.** Ascospores: G1. Living multiguttulate ascospores with apiculi in the extremes, G2. Dead ascospore with merged guttules, G3. Details of wall ornamentation. Mounted media: CR = C2; E1; H₂O = B, C1 & C3, D1, E2–3, E5, F1–3, G1; KOH = C4 & C6, G2; KOH + CR = C5, E4, G3. Scale bars: A = 1 cm; B = 100 μm ; C1–2, E1–2, F1 = 50 μm ; C3–6, D1–2, E3–5, F2–3, G1–G3 = 10 μm . All the photographs are from *Geodina guanacastensis* from Bermuda.

Fig. 2. A. Phylogenetic relationships of *Geodina* and *Wynnea* among the families *Sarcosomataceae*, *Sarcoscyphaceae* and *Chorioactidaceae* in the *Pezizales*. The tree was determined from Maximum Likelihood (ML) analysis of combined *EF*, *RPB2*, 18S and 28S DNA sequence data. The tree was rooted with *Morechella elata* and *Gyromitra californica*. Branches in bold indicate ML bootstrap support > 75 % and Bayesian posterior probabilities > 95 %. **B.** Phylogenetic relationships of the four specimens of *Geodina*, Bahamas, the two isolates of *G. salmonicolor* (JBSD 127408 and the holotype JBSD 127409, Angelini *et al.* 2018) from the Dominican Republic and the paratype of *G. guanacastensis* (CUP-CA 84) from Costa Rica. The tree was determined from ML analysis of ITS rDNA sequence data and rooted with *Wynnea americana* and *Wynnea macrospora*. Branches in bold indicate support values as given for A. **C.** Drawings of *Geodina* (on left) and *Wynnea* (on right) indicating shared morphological features for the family *Wynneaceae*.

DISCUSSION

Our results indicate that there is a single widespread species in the genus *Geodina*. The species seems to have been collected primarily

in lowland, dry tropical or subtropical forests (Denison 1965, Angelini *et al.* 2018, Ortega-López *et al.* 2019). The occurrence in these areas may account for the seeming rarity of this species since such areas may not be as frequently collected as other habitats.

Geodina and *Wynnea* occupy together a position on a long branch sister to members of the *Sarcoscyphaceae*. Molecular phylogenetic studies based on 18S and combined 28S and 18S rDNA have resolved *Wynnea* as an independent sister clade to the other genera of the *Sarcoscyphaceae* (Harrington *et al.* 1999, Romero *et al.* 2012, Angellini *et al.* 2018). In the current study and in Angellini *et al.* (2018) the *Geodina* collections consistently formed a highly supported sister clade with *Wynnea* species in the individual and combined gene analyses (Fig. 2A). Pfister in Romero *et al.* (2012) proposed the tribe *Wynneae* to accommodate *Wynnea*. Unlike the current analysis, which includes three species of *Wynnea*, this earlier analysis included only *Wynnea americana*. Even with this more expanded sample the long branch relationship is supported.

The species of the *Wynnea* and *Geodina* share certain morphological features (Fig. 2C). The outer excipula in both are composed of dark-walled angular cells that give rise to hairs. In *Geodina* the hairs are composed of adherent hyphae which form multifilament hairs, but in *Wynnea* the hairs are generally separate or only loosely associated (Pfister 1979). In both cases the walls of the hairs are thick and can appear to be refractive. Ascospores of species of these genera are multiguttulate, and are marked with cyanophobic ornamentations. In *Wynnea* species the ornamentations take the form of longitudinal ridges; in *G. guanacastensis* the ornamentation is of robust interconnected ridges that form a reticulum, as beautifully illustrated with SEM by Ortega-López *et al.* (2019). The ascospores of both genera are apiculate to a greater or lesser degree. *Wynnea* species are characterized by forming several ear-shaped, or cup-shaped apothecia on a common stipe. The color of the hymenium of species of both genera range from pink to orange tones. Korf (1949) observed that in fresh material the hymenium of *W. americana* was rose-pink, but in older material the hymenial color was deep purple-red. Although generally overlooked it is clear from the photograph in Ortega-López *et al.* (2019) that in *G. guanacastensis* several cupulate apothecia arise from a common stipe as in *Wynnea* species. These species also are found on soil. All other species of the sister family *Sarcoscyphaceae* are found on wood or other plant materials such as twigs, leaves and fruits. *Geodina guanacastensis* ascomata have been assumed to originate from buried wood. The long stalks of the ascomata show signs of having been detached from a longer structure. All photographs and specimens show signs of a broken base. *Wynnea* species on the other hand produce sclerotium-like structures that are composed of hyphae of the *Wynnea* species and hyphae and rhizomorphs of *Armillaria* species (Xu *et al.* 2019). Whether like *Wynnea* species, the *Geodina* species is associated with another fungus or whether it arises from deeply buried wood or roots remains to be demonstrated through thorough and careful excavation of the ascomatal base.

Given the affinities of the species of these two genera and the phylogenetic relationships we propose to recognize this group as a family.

Wynneaceae Pfister & Quijada **fam. nov.** MycoBank MB833213.

Type genus: *Wynnea* Berk. & M. A. Curtis

Diagnosis: Ascomata medium to large with multiple apothecia arising from a common base or stalk, spatulate or cupulate, ectal excipulum of dark angular cells, thick-walled in the outer

layers, giving rise to pustules and/or hairs, hairs brown thick-walled, sometimes joining to form multifilament hairs, asci thick-walled with an eccentric, thickened operculum, ascospores with two or more large guttules and several smaller one, ornamented with cyanophobic longitudinal ribs or ribs interconnecting to form a reticulum, apiculate.

Collected on soil but trophic status unknown. *Wynnea* is associated with *Armillaria*.

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