FUSE

Fungal Systematics and Evolution

VOLUME 8
DECEMBER 2021
PAGES 39–47

doi.org/10.3114/fuse.2021.08.04

Morphological and molecular characterization of *Langdonia walkerae sp. nov*. infecting *Aristida stricta* and *A. beyrichiana* in longleaf pine-grassland ecosystems in the southeastern USA

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Key words:
new taxa
plant pathogens
smut fungi
spore balls
Ustilaginaceae

Abstract: A smut fungus that hinders wiregrass restoration efforts in longleaf pine-grassland ecosystems was collected from *Aristida stricta* and *A. beyrichiana* (*Poaceae*) in three states in the southeastern USA. Morphological and phylogenetic characteristics of this fungus were examined. These data show that the specimens from both plant species were infected by the same fungus and represent a new species of *Langdonia*. The new species differs morphologically from other species of *Langdonia* by teliospores being solitary and not compacted into spore balls. Spore wall ornamentation and teliospore size also differ from other *Langdonia* species. Phylogenetic analyses of DNA sequences of the ITS, LSU, and *EF-1α* supported separation of the species from *A. stricta* and *A. beyrichiana* from other *Langdonia* species. Based on these results, a new species, *Langdonia walkerae*, is proposed.

Citation: Alqurashi AS, Kerrigan J, Savchenko KG (2021). Morphological and molecular characterization of *Langdonia walkerae sp. nov.* infecting *Aristida stricta* and *A. beyrichiana* in longleaf pine-grassland ecosystems in the southeastern USA. *Fungal Systematics and Evolution* **8**: 39–47. doi: 10.3114/fuse.2021.08.04
Received: 8 December 2020; **Accepted:** 14 April 2021; **Effectively published online:** 15 June 2021
Corresponding editor: P.W. Crous

INTRODUCTION

Aristida, a member of the *Poaceae* and the *Aristidoideae* (Barkworth 2003, Cerros-Tlatilpa *et al.* 2011), is distributed worldwide with approximately 300 species (Barkworth 2003) primarily located in Central, North and South America, Australia, and Africa (Cerros-Tlatilpa *et al*. 2011). This genus is distinct from other grass genera by its three-awned lemma (Clewell 1989, Barkworth 2003). In the southeastern United States, two *Aristida* species, *A. stricta* and *A. beyrichiana*, are common grasses found in the ground layer of longleaf pine, *Pinus palustris*, forests (Peet 1993), which extend from the border of Texas and Louisiana eastward to the Atlantic Ocean and from the middle of Florida northward into Virginia (Brockway *et al*. 1998, Frost 2006). *Aristida stricta,* called Carolina wiregrass or the pineland threeawn, occurs in North Carolina and the northern part of South Carolina, and is distinguished morphologically by having hairs along the length of the blades, wider culms, and shorter ligules (Peet 1993, Van Eerden 1997). *Aristida beyrichiana,* called southern wiregrass or Beyrich threeawn, grows in the southern part of South Carolina into Florida and west to Mississippi, and is distinguished morphologically by its soft, short hairs at the base of the blades and the glumes are more unequal in length (Peet 1993, Van Eerden 1997).

These perennial bunchgrasses are adapted to fire, enabling them to persist and reproduce despite frequently recurring fires (Platt 1999, Means 2007) as well as to survive for decades without burning (Shearman *et al*. 2019). Flowers and seed production are most abundant within a year of burning; without fire, few, if any, are produced each subsequent year (Clewell 1989, Van Eerden 1997, Fill *et al*. 2012). These native wiregrasses are considered keystone species in the longleaf pine-grass ecosystem as they produce the fine fuels needed for frequent burning, a requirement for sustained ecosystem structure and function (Clewell 1989, Duever 1989, Noss 1989). Thus, the seed viability of wiregrasses is important for ecosystem restoration efforts (Van Eerden 1997) in projects that focus on wiregrass as a primary understory species.

The production of *A. stricta* and *A. beyrichiana* seeds has been observed to be affected by a smut fungus that replaces the developing ovaries with teliospores (Van Eerden 1997). Smut fungi are pathogens that primarily infect grasses and occur world-wide. They produce sori, sporocarps in which teliospores are produced, in different organs of their host (Vánky 2013). Previously, many grass-infecting smut fungi, including *Sporisorium*, were united into the genus *Ustilago* that has proved to be polyphyletic after the introduction of molecular phylogenetic studies (Begerow *et al*. 1997, 2006). In the taxonomy of smut fungi, the host plant is one of the criteria usually used to classify groups (Bauer *et al*. 2001, Begerow *et al*. 2004). Consequently, McTaggart *et al*. (2012b) emended the description of the genus *Sporisorium* and separated all species that occur on *Aristida* into a new genus, *Langdonia,* based on the host plant and morphological and phylogenetic differences.

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Langdonia is a monophyletic group within the *Ustilaginaceae* (McTaggart *et al*. 2012b). Currently, eight species have been described on other *Aristida* species from various countries - including Argentina, Australia, Bolivia, Madagascar, Mexico, Thailand, and the USA (Durán 1987, McTaggart *et al*. 2012b, Vánky 2012, Denchev *et al*. 2015). The defining morphological characteristics of the genus *Langdonia* are sori that can be found in some or all ovaries of the panicle, lack of columella and sterile cells, teliospores compacted into spore balls, and *Ustilago*-type germination (McTaggart *et al*. 2012b, Vánky 2013). Based on these criteria, the tentative identification of the fungus causing smut on *A. stricta* and *A. beyrichiana* was a species of *Langdonia*. Therefore, the objectives of this study were to identify the fungus that infects *A. stricta* and *A. beyrichiana* and to determine if the same fungus species infects both *A. stricta* and *A. beyrichiana*.

MATERIALS AND METHODS

Sample collection and documentation

Naturally infected plants were collected from seven locations in North Carolina, South Carolina, and Florida during the seed production stage (Table 1). In 2017, samples were collected from two locations - one in South Carolina and one in Florida, and in 2018 samples were collected from six locations in all three states, with only one location being common to both years. All sampled sites had been burned by prescription within the year. The locations and the wiregrass species from which each sample was obtained are included in the "specimens examined" section and in Table 1. For collection, samples of infected wiregrass culms, erect stems bearing inflorescences, were cut at ground level, placed in paper bags, and returned to the laboratory where the specimens were kept at room temperature for subsequent use. Plant voucher specimens were deposited in the Clemson University Herbarium (CLEMS), and fungus specimens were deposited in the Washington State University Mycological Herbarium (WSP) and the U.S. National Fungus Collections - Herbarium (BPI) and kept as voucher specimens.

Morphological examination

The characteristics of the sori and teliospores were examined on infected plants. Pictures of sori were taken using a Nikon D5100 camera. Teliospore characteristics were studied using a compound microscope (LM; BX60F; Olympus optical Co. Ltd., Japan) equipped with ProgRes C5 camera (JENOPTIK, Germany) and CapturePro software; and a scanning electron microscope (SEM; Hitachi SU6600) at 5.0 kV at the Clemson University Electron Microscopy Facility. For LM, teliospores were mounted in lactic acid (85–90 %, VWR, International, LLC) (Savchenko *et al*. 2014) and examined at 1 000 × magnification. The diameters of 30 teliospores, oriented in plane view so that they appeared globose, were measured from each sample collection. The colors of the sori and the teliospores were described according to Rayner (1970). For SEM examination, teliospores were dusted on double-sided adhesive carbon tape, mounted on aluminum stubs, and sputter-coated with platinum using a Cressington sputter-coater (*ca*. 30 nm in 6 min).

Table 1. Smut samples on *Aristida beyrichiana* and *A. stricta* collected and examined for this study**.**

Site	Voucher	Isolate	State	City/County	*Aristida* spp.	Date
Silver Bluff Audubon Center	CLEMS0080381	JK 5017	SC	Jackson/Aiken	*A. beyrichiana*	10 Nov. 2017
	Holotype: WSP74240					
	Isotype: BPI911222					
	CLEMS0080382	JK 6017				
	CLEMS0080383 CLEMS0080384	SB 1218				6 Dec. 2018
Aiken Gopher Tortoise Heritage Preserve	CLEMS0080385	AGT 1218	SC	Aiken/Aiken	*A. beyrichiana*	6 Dec. 2018
Savannah River Site	CLEMS0080388 WSP74241	SRS 1218	SC	Barnwell/Barnwell	*A. beyrichiana*	4 Dec. 2018
	BPI 911220					
Apalachicola Bluffs and Ravines Preserve	CLEMS0080387	FLW 1017	FL	Bristol/Liberty	*A. beyrichiana*	31 Oct. 2017
Austin Cary Forest	CLEMS0080386	FL 1218	FL	Gainesville/Alachua	*A. beyrichiana*	3 Dec. 2018
Carolina Sandhills National Wildlife Refuge	CLEMS0080389	CSN 1118	SC	Hoffman/Richmond	*A. stricta*	27 Nov. 2018
	WSP74242					
	BPI 911221					
North Carolina Sandhills Game Land	CLEMS0080390	NC 1018	NC	Chesterfield/Chesterfield	*A. stricta*	29 Nov. 2018
	WSP74243	NC 2018				
	BPI 911219	NC 3018				
		NC 5018				

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Growth on artificial medium

Sori were carefully removed from infected plants and surface-sterilized by immersing in 70 % ethanol for 30 s, rinsing with sterile distilled water, immersing in 3 % sodium hypochlorite for 10 s, and rinsing twice with sterile distilled water. Each sorus was handled individually using aseptic techniques; one sorus was placed in a 2.0 mL centrifuge tube and gently ground with a tissue grinder pestle to release the teliospores. To make a spore suspension, 500 µL sterile distilled water was added to a tube, the contents were thoroughly mixed, 100 µL was pipetted onto a petri dish containing Difco™ malt extract agar (MEA; Becton, Dickinson and Co., Sparks, MD) and the suspension was spread with a L-shaped cell spreader over the agar surface. The MEA was made according to the manufacturer's instruction and amended with streptomycin (MEA+S; 200 ppm = 200 mg/L). Plates were placed at 28 °C for 2–3 d; and then individual colonies were aseptically subcultured onto MEA+S and incubated for 10 d at 28 °C. The morphological characteristics of shape, color, and diameter of the colony were recorded. The color of the colony was defined according to Rayner (1970).

DNA Extraction, Polymerase chain reaction (PCR), and sequencing

DNA was extracted from 10-d-old cultures using a DNeasy Plant Mini Kit (QIAGEN GmbH, Hilden, Germany) according to the manufacturer's instructions. DNA concentration was determined using a Thermo Scientific Nanodrop 2000 /2000c spectrophotometer. All DNA samples were stored at -20 °C until used for amplification. Genomic DNA was amplified using an Eppendorf Mastercycler Gradient thermocycler with Thermo Scientific Phusion High-Fidelity DNA Polymerase following the manufacturer's cycling and reaction conditions. Two nuclear ribosomal DNA regions and one gene locus were amplified and sequenced: the internal transcribed spacer (ITS) region, the large subunit region (LSU), and translation elongation factor-1α locus (*EF-1α*). The ITS region was amplified with primers M-ITS1 (Stoll *et al*. 2003) and ITS4 (White *et al*. 1990) and annealed at 58 °C (McTaggart *et al*. 2012a); the LSU region was amplified with primers LROR and LR5 (Vilgalys & Hester 1990) at 60 °C; and the *EF-1α* locus was amplified with primers EF-1αF and EF-1αR (McTaggart *et al*. 2012a) at 64 °C. The PCR products were analyzed using gel electrophoresis on a 1.0 % agarose gel and sent to Arizona State University Core Laboratories for purification and sequencing using the same forward and reverse PCR primers. AB1 sequence trace files were assembled using Geneious Prime® v. 2020.0.5 software, and a BLAST search was conducted for all resulting sequences to confirm accurate species identification. The sequences were deposited in GenBank and their accession numbers are shown in Table 2.

Phylogenetic analysis

The phylogenetic analysis was conducted using Geneious Prime® v. 2020.0.5 software. The relationships among the isolates of *Langdonia* species sequenced in this study and other taxa in the *Ustilaginaceae* were inferred from a phylogenetic tree based on the ITS, LSU, and *EF-1α* data sets. The sequences for all taxa were first concatenated for each taxon and then aligned using the MUSCLE algorithm. The final data set contained sequences from 12 isolates collected in this study and 43 reference sequences obtained from GenBank (Table 2). Alignments were uploaded to NGphylogeny.fr (https://ngphylogeny.fr; Lemoine *et al*. 2019) and curated using BMGE (Criscuolo & Gribaldo 2010) to remove poorly aligned positions. The final super matrix contained 1 465 characters, including gaps.

The phylogenetic analysis was conducted using Bayesian inference and maximum likelihood; a GTR model with GAMMA distribution (Nylander 2004) was selected for both analyses. For Bayesian inference, MrBayes v. 3.2.6 was used to conduct a Markov Chain Monte Carlo (MCMC) (Huelsenbeck & Ronquist 2001). Four runs, each consisting of four chains, were conducted for 1 000 000 generations, and the cold chain was heated at a temperature of 0.25. Trees were sampled every 1 000 generations. The standard deviation of split frequencies was 0.01. RAxML v. 8 (Stamatakis 2014) was used for the maximum likelihood using a bootstrapping analysis. RAxML analyses were run with a rapid bootstrap analysis to search for the best-scoring likelihood tree using a random starting tree and 1 000 maximum likelihood bootstrap replicates. Trees were rooted using *Melanotaenium euphorbiae* (HUV17733). The final alignment and trees were deposited in TreeBASE 23819.

RESULTS

Growth on artificial medium

When teliospores were cultured on MEA+S, germ-tube formation occurred after 6 h incubation at 28 °C, and the germination rate increased steadily over a period of 2–3 d. Spore germination was the *Ustilago*-type, making a phragmobasisium on which ovoid sporidia (*i.e*., basidiospores) were produced. Colonies grew slowly; most colonies were visible after 3–5 d, and when subcultured, a colony reached 3–4 cm diam after 14 d. However, more than 50 % of the teliospores remained dormant throughout the incubation period. Young colonies first appeared pale luteous but later became ochreous with age. Colony shape was convex and irregular with undulate edges. At a later stage of growth, the colony surface in some isolates became wrinkled and formed cerebriform sectors (Ulloa & Hanlin 2000).

Phylogenetic analysis

A total of 12 isolates from the culture were sequenced for two nuclear ribosomal DNA regions, ITS and LSU and one gene locus, *EF-1α*, and included in the final data set with 43 reference sequences obtained from GenBank. The list of taxa, their host plants and geographic sources, and the GenBank accession numbers for these sequences are shown in Table 2. The phylogenetic relationships between the isolates from *A. stricta* and *A. beyrichiana* exhibited full support to recognize them as one species based on the MrBayes and RAxML analyses, with values of 1.0 and 100, respectively. The topology of the MrBayes and RAxML trees (Figs 1, 2) indicate that the new species described below forms a highly supported sister group to other species of *Langdonia* as found from ITS+LSU+*EF-1α* analyses. Estimates for posteriori probabilities are indicated on branches of the tree (Fig. 1). In both analyses, isolates collected in this study clustered with values of 1.0 and 100 for MrBayes and RAxML, respectively (Figs 1, 2). The ITS+LSU+*EF-1α* dataset showed that the monophyly of these isolates within the *Ustilaginaceae* was fully supported. All taxa of *Langdonia*

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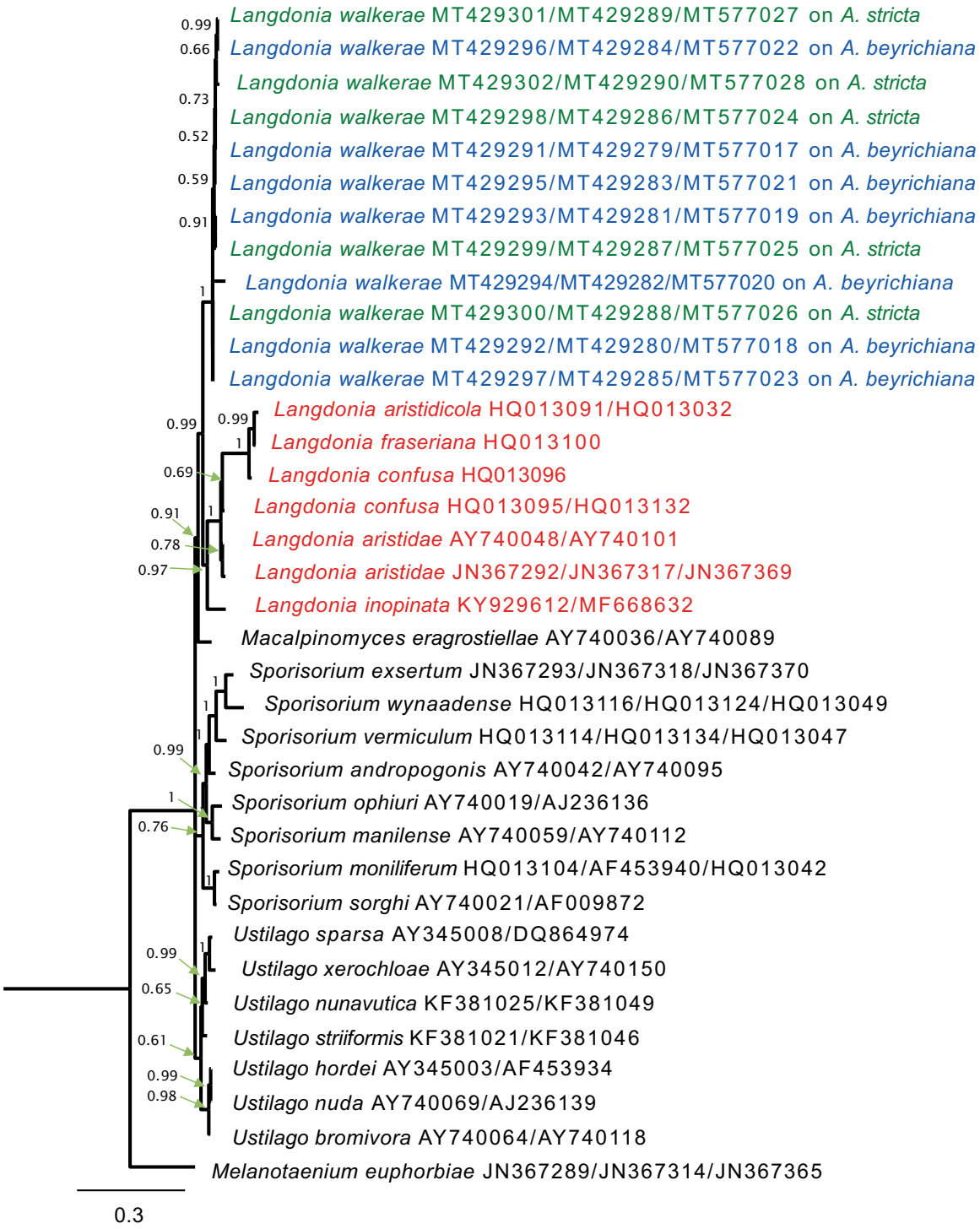

Fig. 1. Bayesian inference of phylogenetic relationships resulting from the analysis of ITS, LSU and *EF-1α* sequences. Numbers on the branches are estimates for Posterior Probability from Bayesian inference. The tree was rooted using *Melanotaenium euphorbiae.*

on *Aristida* spp. formed a well-supported monophyletic clade in the *Ustilaginaceae* and were well separated from the representatives of species in other genera of this family. Within this clade of *Langdonia* species, all isolates from both *Aristida* species clustered with strong support. In MrBayes and RAxML analyses of the alignment, *Macalpinomyces eragrostiellae* was sister to the clade of *Langdonia* species (Fig. 2).

Taxonomy

Langdonia walkerae Alqurashi, J. Kerrigan & K. G. Savchenko, ***sp. nov.*** MycoBank MB 839451. Fig. 3A–F.

Etymology: Named after Dr. Joan L. Walker (USDA-Forest Service), a plant ecologist and conservationist who observed and contemplated the ecological significance of the sori in *A. stricta* and *A. beyrichiana* inflorescences as early as 1979 and brought that to our attention for study.

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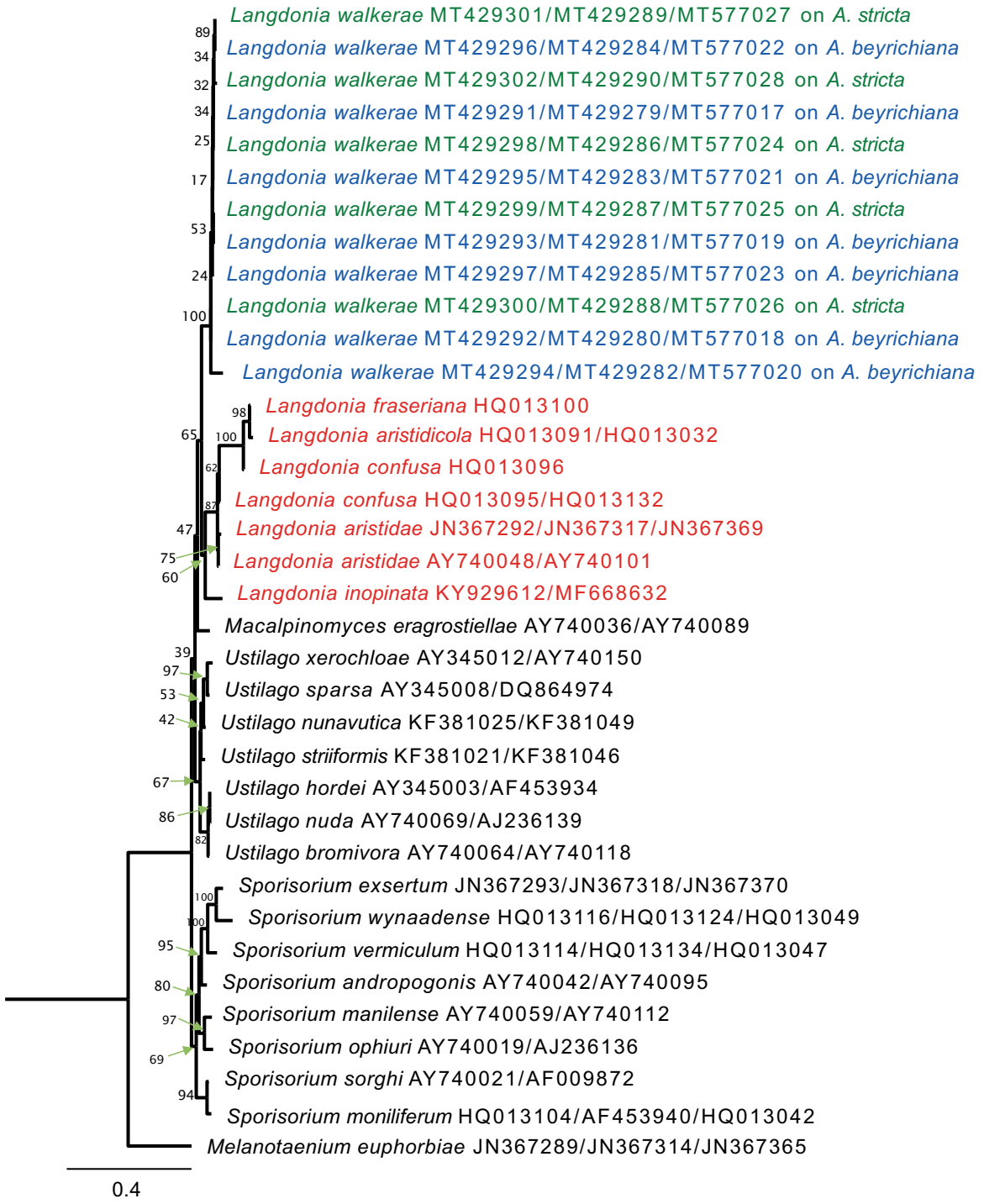

Fig. 2. Maximum likelihood of phylogenetic relationships resulting from the analysis of ITS, LSU and *EF-1α* sequences. Numbers on the branches are estimates of bootstrap support values. The tree was rooted using *Melanotaenium euphorbiae*.

Typus: **USA**, South Carolina, Silver Bluff Audubon Center, on *Aristida beyrichiana*, 10 Nov. 2017, *J. Kerrigan* & *A.S. Alqurashi*, CLEMS 0080381, CLEMS 0080382 (holotype WSP 74240; isotype BPI 911222).

Sori in some ovaries, covered at first by a dark herbage green and later by a dark brick peridium. *Sori* are conspicuous, ovoid or symmetrically fusiform with an acute tip and sized between 1–1.5 × 2.5–7 mm. *Teliospores* are solitary, not compacted into spore balls, dark sienna in color. In the plane view, teliospores are globose, subglobose, or ellipsoidal and sized 8–13 µm diam. In the side view, teliospores are slightly flattened tangentially. *Spore walls* are uniformly thick, aculeate, and have dense conical spines (Ulloa & Hanlin 2000). *Columella*, *spore balls,* and *sterile cells* are lacking.

Host: *Aristida beyrichiana* (*Poaceae*).

Distribution: USA, southern part of South Carolina to southern Florida.

Additional specimens examined: On *Aristida beyrichiana*. **USA**, South Carolina, Silver Bluff Audubon Center, 6 Dec. 2018, *A.S. Alqurashi* & *A.H. Alqurashi*, CLEMS 0080383 & CLEMS 0080384; South Carolina,

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Table 2. Species of fungi used in phylogenetic analyses for this study, including host plant and country of origin.

Species	Voucher/Isolate	Host	Country	GenBank accession no.		
				ITS	LSU	*EF-1α*
Langdonia aristidae	H.U.V.19145	*Aristida urugayensis*	Germany	AY740048	AY740101	n/a
	HUV19145	*Aristida urugayensis*	Argentina	JN367292	JN367317	JN367369
Langdonia aristidicola	BRIP 26930	*Aristida jerichoensis*	Australia	HQ013091	n/a	HQ013032
Langdonia confusa	BRIP 42670	*Aristida queenslandica*	Australia	HQ013095	HQ013132	n/a
	BRIP 52755	*Aristida sp.*	Australia	HQ013096	n/a	n/a
Langdonia fraseriana	BRIP 49668	*Aristida nitidula*	Australia	HQ013100	n/a	n/a
Langdonia inopinata	M-0215944	*Aristida adscensionis*	Zambia	KY929612	MF668632	n/a
Langdonia walkerae	JK 5017	*Aristida beyrichiana*	USA, SC	**MT429291**	**MT429279**	**MT577017**
	JK 6017	*Aristida beyrichiana*	USA, SC	**MT429292**	**MT429280**	**MT577018**
	SB 1218	*Aristida beyrichiana*	USA, SC	**MT429293**	**MT429281**	**MT577019**
	AGT 1218	*Aristida beyrichiana*	USA, SC	**MT429294**	**MT429282**	**MT577020**
	SRS 1218	*Aristida beyrichiana*	USA, SC	**MT429295**	**MT429283**	**MT577021**
	FLW 1017	*Aristida beyrichiana*	USA, FL	**MT429296**	**MT429284**	**MT577022**
	FL 1218	*Aristida beyrichiana*	USA, FL	**MT429297**	**MT429285**	**MT577023**
	CSN 1118	*Aristida stricta*	USA, SC	**MT429298**	**MT429286**	**MT577024**
	NC 1018	*Aristida stricta*	USA, NC	**MT429299**	**MT429287**	**MT577025**
	NC 2018	*Aristida stricta*	USA, NC	**MT429300**	**MT429288**	**MT577026**
	NC 3018	*Aristida stricta*	USA, NC	**MT429301**	**MT429289**	**MT577027**
	NC 5018	*Aristida stricta*	USA, NC	**MT429302**	**MT429290**	**MT577028**
Macalpinomyces eragrostiellae	Ust. Exs. 960	*Eragrostiella bifaria*	India	AY740036	AY740089	n/a
Melanotaenium euphorbiae	HUV17733	*Euphorbia heterophylla*	Papua New Guinea	JN367289	JN367314	JN367365
Sporisorium andropogonis	56588 (M)	*Bothriochloa saccharoides*	Ecuador	AY740042	AY740095	n/a
Sporisorium exsertum	KVU965	*Themeda triandra*	Australia	JN367293	JN367318	JN367370
Sporisorium manilense	Ust.Exs.854 (M)	*Sacciolepis indica*	India	AY740059	AY740112	n/a
Sporisorium moniliferum	BRIP 52504	*Heteropogon contortus*	Australia	HQ013104	n/a	HQ01302
Sporisorium ophiuri	HB 20	*Rottboellia cochinchinensis*	Unknown	AY740019	AJ236136	n/a
Sporisorium sorghi	MP 2036a	*Sorghum bicolor*	Nicaragua	AY740021	AF009872	n/a
Sporisorium vermiculum	BRIP 49748	*Sorghum plumosum*	Australia	HQ013114	HQ013134	HQ01307
Sporisorium wynaadense	BRIP 27640	*Sarga leiocladum*	Australia	HQ013116	HQ013124	HQ01309
Ustilago bromivora	H.U.V.19322	*Bromus catharticus*	Argentina	AY740064	AY740118	n/a
Ustilago hordei	Ust.exs.784	*Hordeum vulgare*	Iran	AY345003	AF453934	n/a
Ustilago nuda	H.U.V.17782	*Hordeum leporinum*	Greece	AY740069	AJ236139	n/a
Ustilago nunavutica	DAOM 91211	*Puccinellia angustata*	Canada	KF381025	KF381049	n/a
Ustilago sparsa	Ust.exs.892	*Dactyloctenium aegyptium*	India	AY345008	DQ864974	n/a
Ustilago striiformis	HAI 4610	*Milium effusum*	Ukraine	KF381021	KF381046	n/a
Ustilago xerochloae	Ust. Exs. 1000	*Xerochloa imberbis*	Australia	AY345012	AY740150	n/a

Note: Species and accession numbers in bold are new sequences from this study.

Aiken Gopher Tortoise Heritage Preserve, 6 Dec. 2018, *A.S. Alqurashi* & *A.H. Alqurashi*, CLEMS 0080385; South Carolina, Savannah River Site, 4 Dec. 2018, *L. Lee,* CLEMS 0080388, WSP 74241, BPI 911220; Florida, Apalachicola Bluffs and Ravines Preserve, 31 Oct. 2017, *J. Walker*, CLEMS 0080387; Florida, Austin Cary Forest, 3 Dec. 2018, *J. Hong*, CLEMS 0080386. On *Aristida stricta*. **USA**, North Carolina, North Carolina Sandhills Game Land, 27 Nov. 2018, *A.S. Alqurashi, A.H. Alqurashi* & *B. Beck*, CLEMS 0080389, WSP 74242, BPI 911221;

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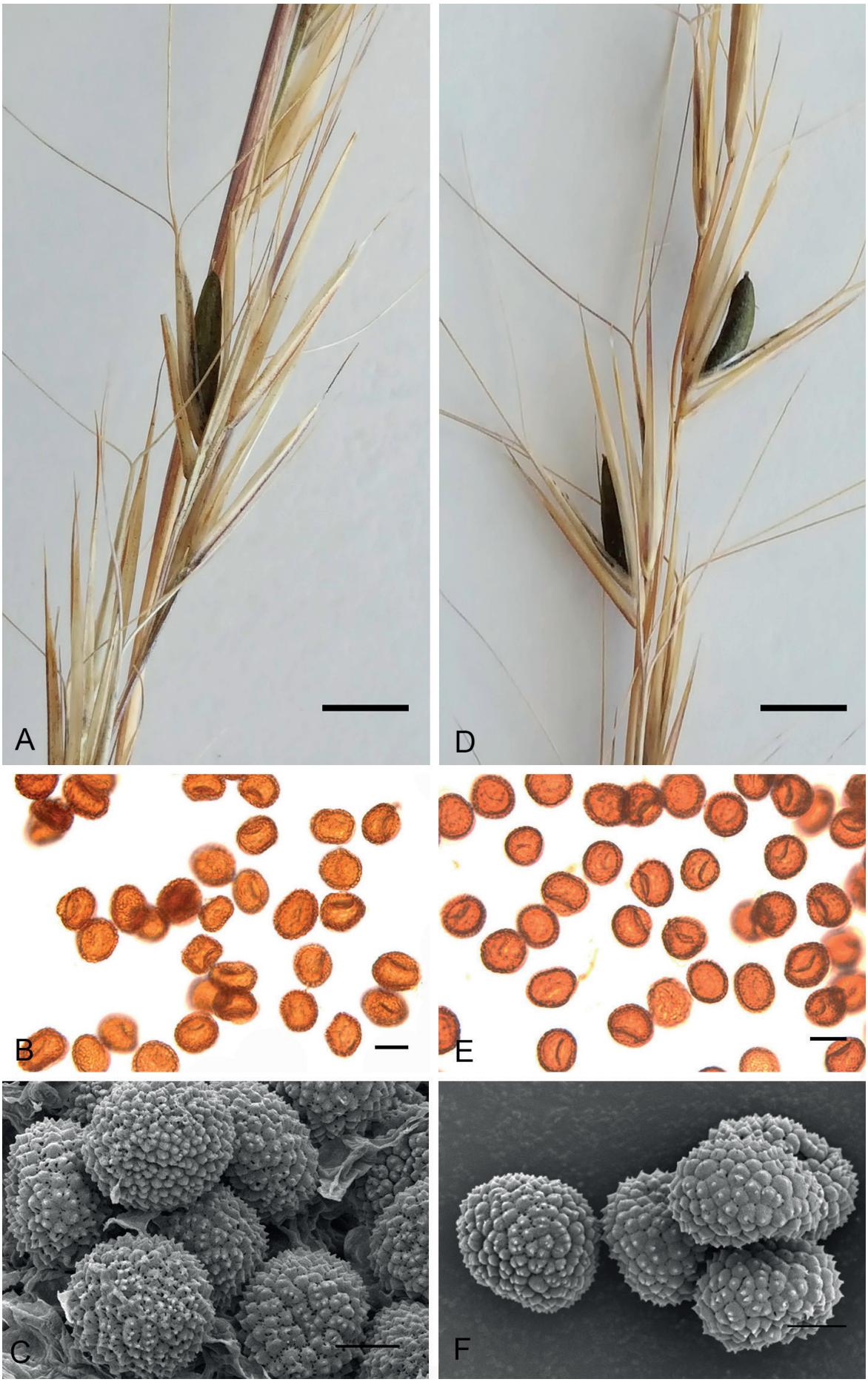

Fig. 3. Sori and teliospores of *Langdonia walkerae sp. nov.* on *Aristida beyrichiana* (A–C) and on *Aristida stricta* (D–F). **A.** Sori (WSU 74240). **B.** Teliospores viewed with transmitted light. **C.** Scanning electron micrograph (SEM) of teliospores. **D.** Sori. **E.** Teliospores viewed with transmitted light. **F.** SEM of teliospores. Scale bars: A, D = 2 mm; B, E = 10 µm; C, F = 5 µm.

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South Carolina, Carolina Sandhills National Wildlife Refuge, 29 Nov. 2018, *A.S. Alqurashi* & *A.H. Alqurashi*, CLEMS 0080390, WSP 74243, BPI 911219.

Notes: Morphologically, *Langdonia walkerae* is distinguished from other species of *Langdonia* by teliospores that are not compacted into spore balls (McTaggart *et al*. 2012a, b). Phylogenetically, there is a strong support to recognize *L. walkerae* as a separate species.

DISCUSSION

Smut specimens from two species of wiregrass, *A. stricta* and *A. beyrichiana*, collected from longleaf pine ecosystems in three southeastern states — South Carolina, North Carolina, and Florida — were examined and compared in terms of morphology and DNA sequence data. Our findings determined both *Aristida* species were infected by the same pathogen, which was identified as a new species. *Langdonia walkerae* is designated as a new species based on its morphological and phylogenetic differences from the other described species of *Langdonia*. Only eight other species of *Langdonia* have been erected, including *L. aristidae*, *L. aristidaria, L. aristidicola*, *L. clandestina, L. confusa, L. fraseriana, L. goniospora,* and *L. inopinata*. All of these species have been reported from two or more *Aristida* species. For example, *L. confusa,* originally named *Sporisorium confusum,* was reported from several species of *Aristida* including *A. dichotoma*, *A. fendleriana*, *A. spiciformis*, and *A. wrightii* from several US states (Farr & Rossman 2020). In contrast, one host has been reported to be infected by different *Langdonia* species. For example, *Aristida adscensionis* is infected by *Sporisorium aristidicola* ≡ *Langdonia aristidicola*, *S. consanguineum* ≡ *L. aristidae*, and *S. inopinatum* ≡ *L. inopinata* in Zambia, India, and Zimbabwe, respectively (Farr & Rossman 2020). In the present study, sori were found in a proportion of the ovaries of *A. stricta* and *A. beyrichiana*. This is similar to four species of *Langdonia*, namely *L. aristidaria* (Durán 1987), *L. aristidicola, L. clandestina* and *L. inopinata* (Vánky 2012). In the other four species of *Langdonia*, namely *L. confusa*, *L. aristidae*, *L. fraseriana* and *L. goniospora,* the sori are produced in all ovaries of an inflorescence on a culm of the host plants (Vánky 2012).

Langdonia walkerae teliospores were solitary in all specimens examined on *A. stricta* and *A. beyrichiana*, and no spore balls were observed. This finding conflicts with that from McTaggart *et al*. (2012b), who considered this an apomorphic characteristic among the species of *Langdonia*. Based on our data, the genus *Langdonia* seems to be sharing the recent common ancestor with *Macalpinomyces eragrostiellae*, another species with well-defined spore balls. It is possible that the ancestors of *L. walkerae* have had spore balls, but they were lost in the process of species evolution.

The absence of spore balls leads us to infer that this fungus is a new species of *Langdonia*, and the genus description, which states that teliospores are compacted into spore balls (McTaggart *et al*. 2012b), needs to be emended. The size of teliospores is distinct from *L. confusa* and *L. aristidae*, both of which have larger teliospore diameters, and from *L. aristidaria, L. aristidicola*, *L. clandestina, L. fraseriana, L. goniospora,* and *L. inopinata,* all of which have smaller teliospore diameters (Durán 1987, Vánky 2012, Denchev *et al*. 2015). Furthermore, the teliospore wall ornamentation of *L. walkerae* is distinguishable from other described species by having dense conical spines whereas the teliospore wall ornamentation in other species of *Langdonia* varies from smooth to verruculose (Vánky 2012). For example, the wall ornamentation in *Sporisorium confusum* ≡ *L. confusa* on *A. dichotoma* was densely verrucose-echinulate and in *S. consanguineum* ≡ *L. aristidae* on *A. arizonica*, the teliospores wall were almost smooth to finely verruculose (Vánky 2012). Teliospore germination of *L. walkerae* is *Ustilago*-type, and columella and sterile cells are absent, fitting the description of the genus *Langdonia*. No data are available from previous studies to compare the culture growth of *L. walkerae* with other species of *Langdonia*.

Phylogenetic analysis demonstrated that all 12 isolates included in this study, five on *A. stricta* and seven on *A. beyrichiana,* form a highly supported monophylum, clustering in one separate phylogenetic clade within the *Ustilaginaceae*. These isolates were sister to other species of *Langdonia* and together formed a monophyletic group supporting the results of McTaggart *et al*. (2012b).

Although many species of *Langdonia* were reported on different species of *Aristida* in different regions around the world, only limited molecular data are available in GenBank for some of these species. Thus, additional sequences are needed for a more complete understanding of the phylogenetic relationships within this genus. The current study shows that *L. walkerae* on *A. stricta* and *A. beyrichiana* is clearly distinguishable morphologically and phylogenetically from other species of *Langdonia* on *Aristida* spp. More material from additional locations in longleaf pine ecosystems should be collected and analyzed to determine if *L. walkerae* occurs throughout the natural range of longleaf pine.

ACKNOWLEDGEMENTS

Research was supported by the USDA–FS. Technical Contribution No. 6910 of the Clemson University Experiment Station. This material is based upon work supported by NIFA/USDA, under project number SC-1700560. We thank the curators of Aiken Gopher Tortoise Heritage Preserve, Carolina Sandhills National Wildlife Refuge, North Carolina Sandhills Game Land, and Silver Bluff Audubon Center for permissions to sample. Special thanks to Brady Beck, the southern piedmont management biologist, for his extreme field assistance in collecting the samples. Also, thanks to Dr. Joan Walker, Jessica Hong and Linda Lee for providing samples from Apalachicola Bluffs and Ravines Preserve, Austin Cary Forest, and Savannah River Site, respectively. We thank Dayton Cash and George Wetzel at Clemson University electron microscopy facility. We also thank Karen Bryson, Andrew Gitto and Linus Schmitz for technical assistance and Xinyuan Ma for his assistance in molecular data analysis. Also, thanks to the curators of CLEMSON, BPI and WSP herbaria for their help in depositing the samples. We thank Drs Steven Jeffers, Guido Schnabel, and Joan Walker for their valuable reviews and suggestions for the manuscript.

Conflict of interest: The authors declare that there is no conflict of interest.

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