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FULL PAPER

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Cuspidatispora xiphiago gen. et sp. nov. from an eastern North American creek

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Abstract An interesting aquatic ascomycete was found that appeared to possess characteristics of some members of the Sordariales. Subsequent sequencing of the 28S large subunit rDNA and β-tubulin genes confirmed the morphological data by placing it in the Sordariales but failed to provide support for placing this species in any recognized genus. Therefore, a new genus, *Cuspidatispora*, is erected to accommodate *Cuspidatispora xiphiago*, which is described as a new species based on a combination of morphological and molecular data. *Cuspidatispora xiphiago* is unique in possessing a central, melanized ascomal wall layer and apiosporous ascospores with a pronounced apical wall extension.

Key words β-Tubulin · LSU · Phylogenetics · Sordariales · Systematics

Introduction

An interesting aquatic ascomycete was discovered during an ecological experiment conducted in Jordan Creek, Illinois. The fungus possesses a unique combination of characters including superficial, villose ascomata with a central, melanized ascomal wall layer, an areolate outer wall layer, asci with a refractive, subapical ring, and apiosporous ascospores with a pronounced apical wall extension. These characters suggest that this taxon be placed in the Sordariales, but because it does not fit any recognized genus, it is described as a new genus and species.

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Materials and methods

Taxon sampling

GenBank accession numbers for all taxa can be found in Table 1. Two species of *Camarops* (Boliniales) were used to root trees based on previous phylogenetic analyses (Huhndorf et al. 2004; Miller and Huhndorf 2005). Voucher specimens are deposited in the Illinois Natural History Survey Mycology Herbarium (ILLS) and the University of Illinois Mycology Herbarium (ILL).

Morphological characterization

Ascomata were squash-mounted in water, and images of micromorphological structures were captured with a QImaging QColor 3 digital camera mounted on either a Leica MZ7.5 dissecting microscope with a Schott KL1500 fiber optics light source or an Olympus BX51 compound microscope using differential interference or phase-contrast microscopy. Images were processed using Adobe Photoshop 7.0 (Adobe Systems, Mountain View, CA, USA). A minimum of 30 measurements was taken for all morphological structures. Ascomata were sectioned at 5 µm according to Huhndorf (1991). Cultures of single spore isolates were obtained following Shearer et al. (2004).

DNA extraction, polymerase chain reaction (PCR) amplification, sequencing, and sequence alignment

Detailed protocols for the extraction, amplification, sequencing, and alignment of the LSU and β-tubulin sequences are fully described in Miller and Huhndorf (2005). DNA was extracted from cultures of *Cuspidatispora xiphiago* grown in potato dextrose broth (Difco).

Table 1. Taxa used in this study

Taxon	Source[a]	GenBank accession no.	
		LSU	β-Tubulin
Apiosordaria backusii	ATCC34568	AY780051	AY780085
Apiosordaria verruculosa	F-152365 (A-12907)	AY346258	AY780086
Bombardia bombarda	SMH3391	AY346263	AY780090
Bombardioidea anartia	HHB99-1	AY346264	AY780092
Camarops amorpha	SMH1450	AY780054	AY780093
Camarops tubulina	SMH4614	AY346266	AY780095
Cercophora sp.	SMH3200	AY780055	AY780098
Cercophora areolata	UAMH7495	AY587936	AY600252
Cercophora atropurpurea	SMH2961	AY780056[e]	AY780099
Cercophora coprophila	SMH3794	AY780058	AY780102
Cercophora costaricensis	SMH4021	AY780059	AY780103
Cercophora lanuginosa	SMH3819	AY436412	AY600262
Cercophora newfieldiana	SMH2622	AF064642	AF466019
Cercophora rugulosa	SMH1518	AY436414	AY600272
Cercophora scortea	GJS L556	AY780063	AY780107
Cercophora sparsa	JF00229 (a)	AY587937	AY600253
Cercophora striata	SMH3431	AY780065	AY780108
Cercophora sulphurella	SMH2531	AY587938	AY600254
Cercophora terricola	ATCC200395	AY780067	AY780109
Chaetomium globosum	SMH4214b	AY346272	AY780110
Chaetomium microascoides	F-153395 (A-12898)	AY346273	AY780111
Cuspidatispora xiphiago	A184-1A	DQ376251	DQ376252
Gelasinospora tetrasperma	ATCC96230	AY346281	AY780118
Immersiella caudata	SMH3298	AY436407	AY780101
Immersiella immersa	SMH4104	AY436409	AY780123
Jugulospora rotula	ATCC38359	AY346287	AY780120
Lasiosphaeria glabrata	TL4529	AY436410	AY600255
Lasiosphaeria ovina	SMH1538	AF064643	AF466046
Lasiosphaeria sorbina	GJS L555	AY436415	AY600273
Lasiosphaeris hirsuta	SMH1543	AY436417	AY780121
Lasiosphaeris hispida	SHM3336	AY436419	AY780122
Neurospora crassa	GenBank	AF286411	M13630
Neurospora pannonica	TRTC51327	AY780070	AY780126
Podospora appendiculata	CBS212.97	AY780071	AY780129
Podospora decipiens	CBS258.64	AY780073	AY780130
Podospora fibrinocaudata	TRTC48343	AY780074	AY780131
Podospora fimbriata	CBS144.54	AY780075	AY780132
Podospora fimiseda	CBS990.96	AY346296	AY780133
Schizothecium curvisporum	ATCC36709	AY346300	AY780136
Sordaria fimicola	SMH4106	AY780079	AY780138
Sordaria lappae	SMH4107	AY780080	AY780139
Sordaria macrospora	Buck s.n.	AY346301	AY780140
Strattonia carbonaria	ATCC34567	AY346302	AY780141
Triangularia mangenotii	ATCC38847	AY346303	AY780142
Triangularia tanzaniensis	TRTC51981	AY780081	AY780143
Zopfiella ebriosa	CBS111.75	AY346305	AY780146
Zygopleurage zygospora	SMH4219	AY346306	AY780147

LSU, large subunit

[a] ATCC, American Type Culture Collection; CBS, Centraalbureau voor Schimmelcultures, Netherlands; TRTC, Royal Ontario Museum, Toronto, Canada; UAMH, University of Alberta Microfungus Collection and Herbarium; Buck, William Buck; A, Carol A. Shearer; GJS, Gary J. Samuels; HHB, Harold H. Burdsall; JF, Jacques Fournier; SMH, Sabine M. Huhndorf; TL, Thomas Læssøe

Phylogenetic analyses

Maximum-parsimony (MP) and maximum-likelihood (ML) analyses were performed using PAUP 4.0b10 (Swofford 2002). Portions of the 5′- and 3′-ends of the LSU and β-tubulin genes were excluded from all analyses due to missing data in most taxa. Three ambiguously aligned regions were delimited in LSU, and a portion of the phylogenetic signal was recovered from all three of these regions by recoding them using the program INAASE (Lutzoni et al. 2000). The single ambiguously aligned intron, which occurred in the β-tubulin gene, was excluded from all analyses because of its size (Miller and Huhndorf 2005). The remaining unambiguously aligned characters were subjected to symmetrical step-matrixes to differentially weight nucleotide transformations following the methods of Miller and

Huhndorf (2005). Individual step-matrixes were generated for each of the three codon positions in β-tubulin, while all unambiguously aligned characters were subjected to a single step-matrix in LSU. Unequally weighted MP analyses were performed with 1000 random addition heuristic searches, TBR branch-swapping, Multrees option in effect, zero-length branches collapsed, constant characters excluded, and gaps treated as missing. Branch support was estimated by performing 1000 bootstrap replicates (Felsenstein 1985) each consisting of 100 random addition heuristic searches as above.

Modeltest 3.06 (Posada and Crandall 1998) was used to determine the best-fit model of evolution for each data set. Maximum-likelihood analyses were performed on separate and combined data sets using the best-fit model with 100 stepwise random addition replicates and TBR branch-swapping with a reconnection limit of 12. Constant characters were included and ambiguously aligned characters were excluded from all ML analyses. Bayesian analyses were performed using MrBayes 3.0b4 (Huelsenbeck and Ronquist 2001) as an additional means of assessing branch support. Constant characters were included, the above model of evolution was implemented, and 5 million generations were sampled every 100th generation, resulting in 50000 total trees. The Markov chain always achieved stationarity after the first 10000 generations (=1000 trees), so the first 10000 trees, which extended well beyond the burn-in phase of each analysis, were discarded. Posterior probabilities were determined from a consensus tree generated using the remaining 40000 trees. This analysis was repeated three times starting from different random trees to ensure trees from the same tree space were being sampled during each analysis.

Results

Phylogenetic analyses

Maximum-likelihood analyses performed on individual LSU and β-tubulin datasets produced phylogenetic trees with similar topologies (data not shown). These trees did not differ significantly from the single unequally weighted MP tree (data not shown) or the ML tree generated in the combined analyses (Fig. 1). Although *Cuspidatispora xiphiago* generally occurred as a sister-taxon to *Cercophora sulphurella* (Sacc.) R. Hilber and *C. sparsa* (Sacc. & Fairm.) R. Hilber, its placement was unsupported by both MP bootstrapping and Bayesian inference in all analyses. Both morphological and molecular data support the establishment of this species as a new genus in the Sordariales.

Cuspidatispora Shearer & Bartolata, gen. nov.

Ascomata ovoidea, erumpentia vel superficialia. Paries ascomatis tristratosus, strato medio scleroplectenchymatico. Paraphyses filiformes, septatae. Asci cylindrici-clavati vel clavati, stipitati, unitunicati, annulo apicali praediti, octospori. Ascosporae late fusiformes, extensione parietis apicalis, hyalinae, postremo septatae; cellula superior ellipsoidea, brunnea; cellula pedicelli conica, hyalina; extensio parietis apicalis apiculata, brunnea, rigida, persistens.

Ascomata ovoid, erumpent to superficial. Ascomal wall 3-layered, middle layer scleroplectenchymatous. Paraphyses filiform, septate. Asci cylindroclavate to clavate, stipitate, unitunicate, apical ring present, with eight ascospores. Ascospores broadly fusiform, with an apical wall extension, hyaline, finally septate; upper cell ellipsoid, brown; lower cell conical, hyaline; apical wall extension apiculate, brown, rigid, persistent.

Type species: *Cuspidatispora xiphiago* Shearer & Bartolata.

Etymology: Based on the pointed apical wall extension of the ascospore.

Cuspidatispora xiphiago Shearer & Bartolata sp. nov. Figs. 2–12

Ascomata ovoidea, 300–400 µm alta, 250–300 µm diametro, dispersa vel gregaria, erumpentia vel superficialia, villosa, pallide brunnea, collo conico, glabrata, nigra. Paries ascomatis superficialis textura angularis et textura prismatica, areolata, sectione longitudinali 21.5–43 µm crassa, tristratosus, strato interiori pseudoparenchymatico, strato medio scleroplectenchymatico, strato exteriori prosenchymatico. Paraphyses filiformes, septatae. Asci cylindrici-clavati vel clavati, 95–150 × 15.5–21 µm, stipitati, stipite 25.5–38 × 2.5–3 µm, unitunicati, annulo apicali praediti, octospori. Ascosporae biseriatae vel triseriatae in ascis, late fusiformes, extensione parietis apicali, aseptatae, 25.5–36.5 × 10–12 µm, deinde septatae; cellula superior ellipsoidea, 23–30.5 × 10–12 µm, deinde brunnea vel atrobrunnea, medio uniseptata; cellula pedicelli conica, 2.5–6 × 2.5–5.5 µm, hyalina; extensio parietis apicalis apiculata, 4.5–8.5 × 0.5–1.5 µm, deinde pallide brunnea vel brunnea, rigida, persistens.

Ascomata ovoid, papillate, 300–400 µm high, 250–300 µm in diameter, numerous, scattered to gregarious, erumpent to superficial, covered with villose hairs; hairs flexuous, beige to pale brown, sparse, thin-walled, septate, 1–2.5 µm wide; neck conical, 60–90 µm high, 70–100 µm wide, glabrous, black. *Ascomal wall* of *textura angularis* and *textura prismatica* in surface view, areolate, in longitundinal section 3-layered, 21.5–43 µm thick, inner layer pseudoparenchymatous, 8–11.5 µm thick, composed of 2–4 layers of elongate, flattened, hyaline, thin-walled cells, middle layer scleroplectenchymatous, 5.5–14 µm thick, composed of 2–3 layers of angular, dark brown, thick-walled melanized cells, outer layer prosenchymatous, 8–17.5 µm thick, composed of 2–5 layers of loosely interwoven hyphae that comprise the villose hairs. *Ascomal apex* with periphyses. *Centrum* hyaline. *Paraphyses* filiform, 120–250 µm long, 2.5–8 µm wide, longer than asci, hyaline, numerous, septate, unbranched. *Asci* cylindro-clavate to clavate, 95–150 × 15.5–21 µm, stipitate, stipe 25.5–38 × 2.5–3 µm, numerous, unitunicate, thin-walled, apex rounded; ring narrow, deep, refractive;

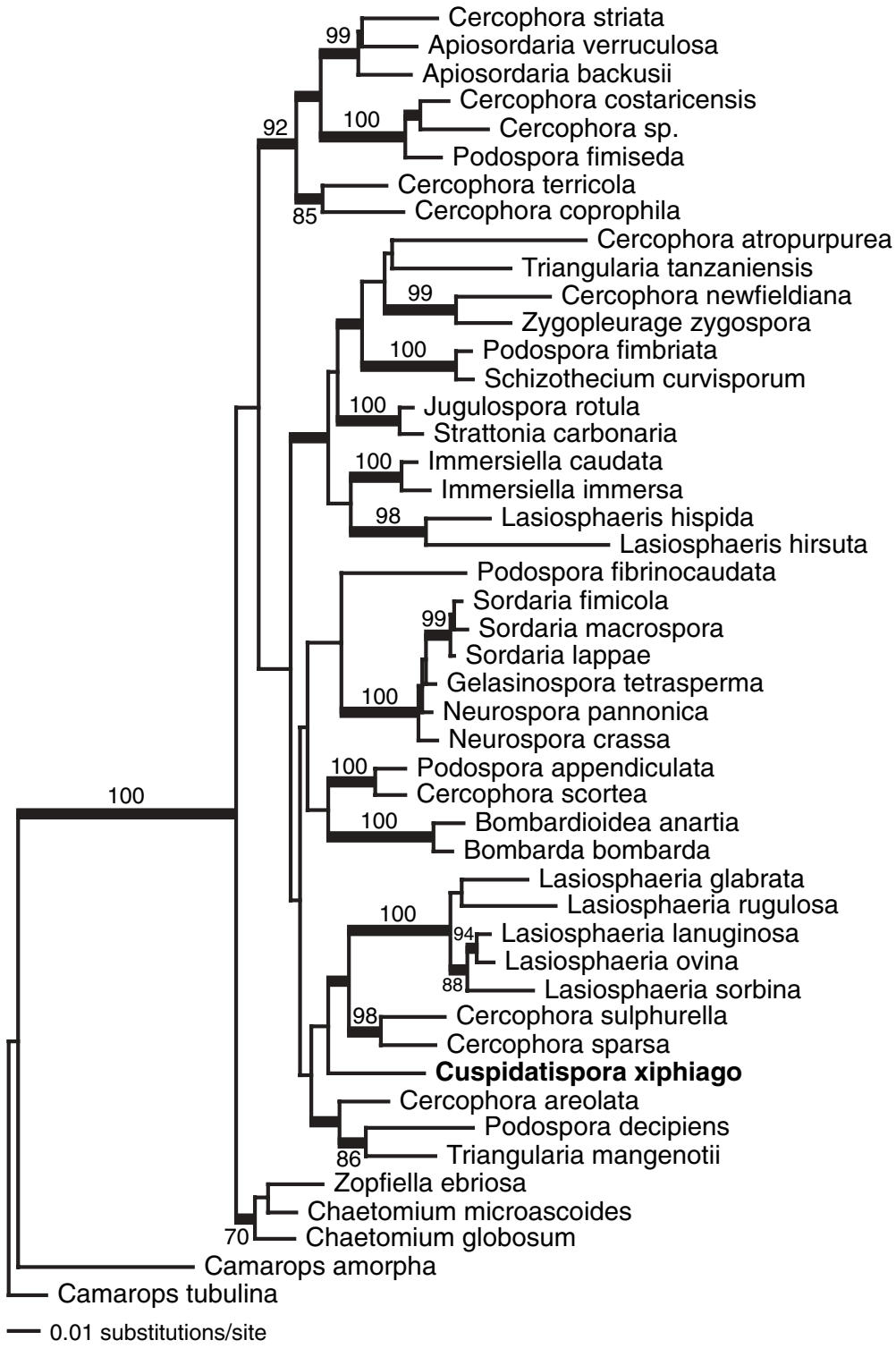


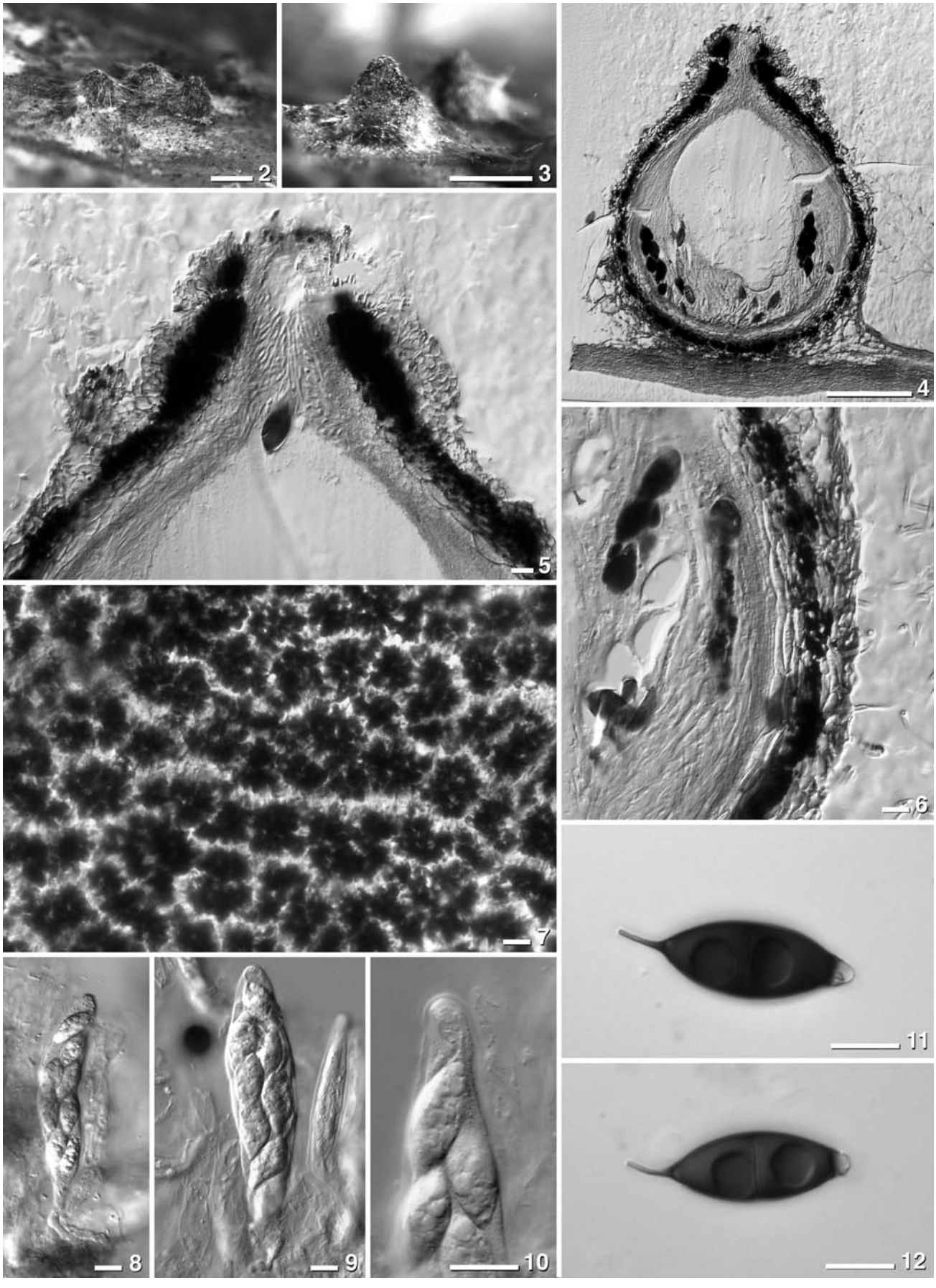
Fig. 1. Phylogram of the single most likely tree (–ln L = 13254.05) generated from a combined maximum-likelihood analysis of large subunit (LSU) and β-tubulin for 47 ascomycete sequences. *Thickened branches* indicate Bayesian posterior probabilities ≥95%; *numbers above branches* refer to maximum-parsimony bootstrap values ≥70%. Two species of *Camarops* are outgroups

with 8, biseriate to tetraseriate ascospores. *Ascospores* broadly fusiform, with an apical wall extension, initially one-celled, 25.5–36.5 × 10–12 µm; ascospore becoming septate; upper cell ellipsoid, 23–30.5 × 10–12 µm, conical at apex, truncate at base, becoming brown to dark brown and 1-medially septate; lower cell differentiated by a septum, conical, 2.5–6 × 2.5–5.5 µm, hyaline, commonly collapsing; apical wall extension apiculate, 4.5–8.5 × 0.5–1.5 µm, becoming pale brown to brown, rigid, persistent.

Anamorph: Unknown.

Etymology of species epithet: Based on the shape of the ascospore, which resembles a swordfish.

Material examined: USA, Illinois, Vermilion County,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Figs. 2–12. *Cuspidatispora xiphiago*. **2,3** Ascomata on the substrate. **4** Longitudinal section through ascoma. **5** Longitudinal section through ascomal neck. **6** Longitudinal section through ascomal wall. **7** Surface view of outer ascomal wall showing areolate pattern. **8,9** Asci. **10** Ascus apex. **11,12** Ascospores. All figures from holotype (A184-1). *Bars* **2,3** 500 µm; **4** 100 µm; **5–12** 10 µm

Jordan Creek, 40°49′50″ N, 87°49′50″ W, present on submerged maple wood test blocks preinoculated with *Pseudohalonectria lignicola* and *Tricladium angulatum*, March 14, 1994, C.A. Shearer, A184-1 (ILL, holotype designated here; isotype as ILLS 57515); February 3, 1994, C.A. Shearer, A184-2.
Distribution: Only known from type locality.
Cultures examined: A184-1A, B, C, D, E.

Discussion

Cuspidatispora xiphiago possesses several morphological characters attributable to the Sordariales, such as relatively large, superficial ascomata with large-celled membraneous or coriaceous walls and ascospores that often form an apical brown cell and basal hyaline cell (Huhndorf et al. 2004). Although some members of the Apiosporaceae possess a similar ascospore morphology, preliminary MP analyses of LSU placed this species in the Sordariales (data not shown). Subsequent phylogenetic analyses of the LSU and β-tubulin genes confirmed the placement of *C. xiphiago* in the Sordariales but did not provide support for its placement into any recognized genus (see Fig. 1). Although *C. xiphiago* clearly belongs in the Sordariales, it is uncertain in which family it belongs because familial delimitations in this order are currently unresolved (Huhndorf et al. 2004).

This taxon possesses a unique ascomal wall with a central, melanized wall layer (see Figs. 4–6) and an outer areolate wall layer (see Fig. 7). *Cercophora macrocarpa* (G.C. Carroll & Munk) O. Hilber & R. Hilber also possesses a melanized ascomal wall, but its outer wall layer, rather than its central wall layer, is melanized. Several species in the Sordariales possess an areolate ascomal wall, including *Cercophora areolata* N. Lundq., *Cercophora californica* (Plowr.) N. Lundq., *Cercophora coprogena* (Speg.) N. Lundq., and *Cercophora striata* (Ellis & Everh.) N. Lundq., among others (Lundqvist 1972). However, these species possess ascospores with a long, basal, hyaline cell, while *Cuspidatispora xiphiago* possesses ascospores with a short, hyaline cell (see Figs. 11, 12). Other genera in the Sordariales that possess ascospores with a short, basal, hyaline cell include *Apiosordaria* Arx & W. Gams, *Jugulospora* N. Lundq., and *Strattonia* Cif. (Lundqvist 1972). *Cuspidatispora xiphiago* did not cluster with representatives of any of these genera (Fig. 1) and differs from them morphologically in possessing a central melanized ascomal wall and ascospores with a pronounced apical wall extension.

In addition to having apiosporous ascospores, *Cuspidatispora xiphiago* also possesses a pronounced apical wall extension (see Figs. 11, 12), a character not found in other members of the Sordariales. Members of the Sordariales that have appendaged ascospores include *Arnium* Nitschke ex G. Winter, *Bombardia* (Fr.) P. Karst., *Cercophora* Fuckel, *Immersiella* A.N. Mill. & Huhndorf, *Lasiosphaeria* Ces. & De Not., *Lasiosphaeris* Clem., *Podospora* Ces., and *Zygopleurage* Boedijn. These taxa typically possess supple, gelatinous, hyaline appendages that are delimited from the ascospore by a septum. The apical wall extension in *C. xiphiago* is not delimited from the ascospore by a septum and differs in being rigid and brown.

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