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Phylogeny, morphology, and ecology resurrect previously synonymized species of North American *Stereum*

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Abstract

Stereum is a globally widespread genus of basidiomycete fungi with conspicuous shelf-like fruiting bodies. Several species have been extensively studied due to their economic importance, but broader *Stereum* taxonomy has been stymied by pervasive morphological crypsis in the genus. Here, we provide a preliminary investigation into species boundaries among some North American *Stereum*. The nominal species *Stereum ostrea* has been referenced in field guides, textbooks, and scientific papers as a common fungus with a wide geographic range and even wider morphological variability. We use ITS sequence data of specimens from midwestern and eastern North America, alongside morphological and ecological characters, to show that *Stereum ostrea* is a complex of at least three reproductively isolated species. Preliminary morphological analyses show that these three species correspond to three historical taxa that were previously synonymized with *S. ostrea*: *Stereum fasciatum*, *Stereum lobatum*, and *Stereum subtomentosum*. *Stereum hirsutum* ITS sequences taken from GenBank suggest that other *Stereum* species may actually be species complexes. Future work should apply a multilocus approach and global sampling strategy to better resolve the taxonomy and evolutionary history of this important fungal genus.

Introduction

The fungi are a remarkably diverse kingdom, and the Russulales is one of the most morphologically diverse fungal orders. While the Russulales have been extensively studied at the family level and above, the evolutionary and biogeographic histories of the order are mostly unknown, and fine scale explorations of relationships below the family level are still needed (Miller et. al. 2006). Most described fungi still have no published DNA sequence data, and this applies to many of the genera within the Russulales (Miller et. al. 2006, Hawksworth & Lücking 2017). Similarly, many species have yet to be discovered or formally described.

New species of fungi are often discovered as cryptic species lumped under single, well-established names (Hawksworth & Lücking 2017). Many well-known fungi, such as *Amanita muscaria* and *Cantharellus cibarius*, have been found to contain multiple species (Geml et. al. 2006, Buyck and Hofstetter 2011). While the members of some species complexes may initially be difficult to separate morphologically, distinguishing features can become clear after phylogenetic analysis and trait-mapping, as was shown with *Fomes fomentarius* and its neglected sister species *F. inzengae* (Peintner et. al. 2019). Critically, differences among cryptic or apparently cryptic species can economically relevant, such as with the fungal plant pathogen *Magnaporthe grisea* and the morphologically indistinguishable *M. orzyae*, which infect crabgrass and rice, respectively (Couch & Kohn, 2002).

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Stereum is a common genus of white-rot fungi, and the type genus of the Stereaceae, a family within the diverse Russulales order (Krah et. al. 2018). Though *Stereum* has been the focus of extensive bioprospecting (Doljak et. al. 2006, Hybelhauerová et. al. 2008), and some economically important species are relatively well-researched (Čermák et. al. 2004, Stenlid & Vasiliauskas 1998), the below-genus level taxonomy of *Stereum* has not been subjected to phylogenetic analysis. This disconnect between the slow progress toward molecular taxonomy in *Stereum* and interest in the potentially useful properties of *Stereum* species can be problematic, especially where species descriptions include uninformative or deceptive morphological characters that may lead to misidentification of study specimens.

Stereum ostrea is an exemplar of a species with a fraught taxonomic history. While the name *S. ostrea* has been applied to collections around the world, it is unlikely that these varied collections are from a single species given pre-Anthropocene obstacles to dispersal. First used to describe a collection from the island of Java in Indonesia, the name *S. ostrea* has been consistently applied to specimens from North America since a publication by Lentz (1955) placed it in synonymy with two other putative species, *S. fasciatum* and *S. lobatum*, with which Welden (1971) and Chamuris (1985, 1988) concurred. Even before this, *S. ostrea* was considered either a synonym of *S. fasciatum* by some authors (Burt 1920, Pilát 1930, Banerjee 1935, Hendrickx 1948) or of *S. lobatum* by other authors (Massee 1890, Cooke 1892, Hohnel & Litschauer 1907, Reinking 1920, Boedijn 1940). Critically, Demoulin (1985) argued in favor of *S. fasciatum* and *S. lobatum* as distinct species, outlining their differences, but the literature remained divided (Eicker & Louw 1998). Currently, *S. fasciatum* is considered a synonym of *S. ostrea* (according to Index Fungorum in October 2020, http://indexfungorum.org/), and despite being an accepted name *S. lobatum* is rarely used. Another similar species, *S. subtomentosum*, has both been confused with *S. fasciatum* and proposed to be in a complex with *S. hirsutum* (Pouzar 1964, Welden 1971, Chamuris 1988, Ginns & Lefebvre 1993).

We hypothesize that *S. ostrea* in North American is not a single species but a complex of several species. To test this, and to begin developing a more integrated approach to *Stereum* taxonomy, we collected over 50 *Stereum* specimens from eastern North America and used internal transcribed spacer (ITS) data to create a phylogenetic tree. Because sequence information from a single gene can be in conflict with true species boundaries, we coupled our phylogenetic approach with morphological and ecological data. Following our hypothesis that *S. ostrea* is a species complex, we predicted that differences in ITS sequences would correlate with differences in morphology and ecology (e.g., substrate), and as such would reveal evidence of more than one reproductively isolated *Stereum* species.

Methods

Collection and Identification

We collected *Stereum* basidiocarps from locations in midwestern and eastern North America, with the most intensive sampling in Iowa and some supplemental collections made in Alabama and Florida (Table 1). For identification we used the dichotomous key and

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morphological descriptions from Chamuris (1988), the most comprehensive and recent publication on *Stereum.* We identified our collections as *S. ostrea*, *S. hirsutum*, *S. complicatum*, *S. gausapatum*, *S. sanguinolentum*, and *S. striatum*. We used iNaturalist to record photographs, dates of collection, and approximate GPS coordinates of collection location. We also recorded whether the substrate from which samples were collected was hardwood or conifer, and if the hymenium changed color when bruised. We air dried all collections and preserved samples in polyethylene plastic bags. In total, we collected and analyzed 49 samples, 36 of which are included in this paper (Table 1).

All *Stereum* species mentioned are found primarily on angiospermous wood, except for *S. sanguinolentum* which is primarily found on conifers. *Stereum* are occasionally reported on rare hosts and have been sequenced from atypical host species (Ginns and Lefebvre 1993, Jusino 2014). SD50 *S. complicatum* was found growing on a dead branch of loblolly pine still attached to the tree.

DNA Extraction, PCR and Sequencing

We used a CTAB and liquid nitrogen method adapted from Chen et. al. (2010) to extract DNA from 3 x 3 mm pieces of basidiocarp from each collection, taking care to exclude as much dirt and debris as possible. We used DNA diluted 1:20 with molecular grade water for PCR amplification using ITS1-F (CTTGGTCATTTAGAGGAAGTAA) and ITS4 (TCCTCCGCTTATTGATATGC) primers (Gardes and Bruns 1993, White et al. 1990), with the following thermocycler program: 3min @ 94C, 20-30 cycles (30s @ 94C, 30s @ 55C, 30s @ 72C), 2min @ 72C. We cleaned PCR products with Exo-SAP following manufacturer protocols. We sequenced PCR products in forward and reverse directions on an Applied Biosystems ABI 3730 DNA Analyzer (Thermo Fisher Scientific, Waltham, MA, USA) housed in the Roy J. Carver Center for Genomics in the University of Iowa Biology Department.

Phylogenetic Trees

We used Geneious 8.1.7 (http://www.geneious.com/) for alignment of forward and reverse sequences for each collection, which we then manually checked and trimmed. We chose *Xylobolus subpileatus*, another member of the Stereaceae, as an outgroup. This specimen was collected in Florida by S.G.D. and sequenced by the Smith Lab at the University of Florida. We also obtained North American and Eurasian *S. hirsutum* sequences from BOLD and GenBank. We used MAFFT (Katoh et. al. 2002) via the CIPRES server (Miller et. al. 2010) to align chosen sequences, then generated a maximum likelihood tree using RAxML (Stamatakis 2014) via CIPRES. For computation of the Bayesian tree, we used MrBayes 3.2.7 (Ronquist et. al. 2012) with a GTR+I+Γ substitution model for 200,000 generations, with sampling every 100 generations.

Results

Maximum likelihood (Figure 1) and Bayesian trees (Supplemental Figure 1) were largely in agreement, except for the placement of the single *Stereum striatum* sequence. Both trees show *Stereum* sorting out into several clades. Specimens initially identified as *S. ostrea* were

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monophyletic but split into three well-differentiated clades (Clades 1-3, Fig. 1). Sequences within the same clade differed from one another by 1-3%, while sequences among the three clades differed from one another by 7-14%.

The "*S. ostrea*" specimens in the first clade (Figure 1) all share features of the previously synonymized species *S. fasciatum*, as described by many authors including Burt (1920) and Demoulin (1985). These specimens feature a cap clothed in coarse hair that is resistant to wearing off in bands, and these hairs gather in individual clumps that are best observed with a hand lens or dissecting microscope. If bruised (when basidiocarps are fresh) or wet (when dried) the hymenium (undersurface) does not stain color (Figure 2a, b).

Similarly, the "*S. ostrea*" specimens in the second clade (Figure 1) showed features consistent with the previously synonymized *S. lobatum* (Burt 1920, Demoulin 1985). The cap is clothed in matted, felted hairs that quickly begin wearing off, from the edge of the cap inwards, in concentric bands exposing the chestnut-brown context beneath. When bruised or wet the hymenium stains a bright yellow color (Figure 2c, d).

"*S. ostrea*" specimens in the third clade shared some features with both *S. fasciatum* and *S. lobatum*, however the basidiocarps were usually thicker, more irregular, and a richer brown color both cap and hymenium. The cap is covered clumping hairs that are typically longer and woolier than specimens of the first clade (*S. fasciatum*), and wear off in bands more readily but not to the extent of specimens in the second clade (*S. lobatum*). When bruised or wet the hymenium stains a bright yellow color (Figure 2e, f). Overall, the morphology agrees with descriptions of *S. subtomentosum* (Pouzar 1964, Chamuris 1988).

Specimens identified before sequencing as belonging to other species of *Stereum* formed clades with ITS sequences differing within clades by 1-5%. These included *S. complicatum*, *S. gausapatum*, and *S. sanguinolentum.* The single sequence of *S. striatum* was placed differently in the two trees but differed from all other sequences by more than 7%. *S. hirsutum* sequences acquired from GenBank and BOLD databases differed from one another by 1-6%, but only as much as 4% within the two geographically isolated (North America vs. Eurasia) clades.

Discussion

Together, our results support the hypothesis that the North American fungi that have been lumped under the name "*S. ostrea*" are actually at least three distinct species, reproductively isolated and differing in morphology. The inference of isolation is based on the observation that while all three species can be found growing together on the same substrate, there is strong concordance between ITS sequence identity and morphology, such that they appear not to be hybridizing despite often living in sympatry. Demoulin (1985) argued that *S. fasciatum* and *S. lobatum* were distinct species based on differing morphology and lack of fusion while growing on the same substrate, and our results support this claim with new genetic evidence.

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As a consequence of these new data, the *Stereum* in the three "*S. ostrea*" clades should be recognized as distinct species, and those in synonymy with *S. ostrea* should revert to their original names. Our Figure 2 along with images for each specimen on iNaturalist (Table 1) are provided to assist future naturalists and researchers in distinguishing among these taxa. Most importantly, these three species in midwestern and eastern North America can be differentiated by 1) texture of cap hairs, 2) presence and extent of banding, and 3) color staining of the hymenium when bruised or wet.

A more general conclusion of this research is that an integrative approach to *Stereum* taxonomy that includes DNA sequence data will be helpful in resolving species boundaries. In many cases, original descriptions are only a few sentences long or overly vague. Further, *Stereum* taxonomy has a long history of synonymization efforts such that descriptions of *Stereum* species from different authors are often conflicting. Historically, *Stereum* has been used as a genus name to describe many corticioid fungi, many of which have been transferred to other genera both in the Stereaceae and to other genetically distant families (Larsson & Larsson 2003). Many *Stereum* are accepted as synonyms of better-known *Stereum* species, but if our study is of any indication, some of those names may also need to be resurrected.

Our work here demonstrates a need to explore the rest of the *Stereum* genus, so that we may better understand their ecological roles, how they might be useful to us, and how they evolved. Molecular phylogenetic approaches will be necessary as a supplemental tool to delineate *Stereum* species, as morphology alone has proved inadequate. By using a multilocus approach and worldwide sampling strategy, we can achieve greater resolution within this genus and other members of the Stereaceae.

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Table 1. Specimens used in this study. "Original ID" is the species name assignment based on current morphological taxonomy for *Stereum*. "Revised ID" refers to the species name recommended based on the outcomes of this study.

Collector Number/Voucher	Revised ID	Original ID	Location	GPS Coordinates	iNaturalist ID	GenBank (or BOLD) Accession #	Substrate
SD0002	*Stereum fasciatum*	*Stereum ostrea*	Cedar Rapids, IA	42.00530704 N 91.56669945 W	8671795	Awaiting processing	*Quercus alba*
SD0005	*Stereum complicatum*	*Stereum complicatum*	Cedar Rapids, IA	42.006663 N 91.569884 W	59879686	Awaiting processing	Hardwood
SD0007	*Stereum lobatum*	*Stereum ostrea*	Toddville, IA	42.09934134 N 91.75594183 W	9705348	Awaiting processing	*Carya ovata*
SD0008	*Stereum complicatum*	*Stereum complicatum*	Toddville, IA	42.09901 N 91.755838 W	19024153	Awaiting processing	Hardwood
SD0010	*Stereum complicatum*	*Stereum complicatum*	Mt. Vernon, IA	41.89896304 N 91.48367087 W	9564870	Awaiting processing	Hardwood
SD0012	*Stereum subtomentosum*	*Stereum hirsutum*	Central City, IA	42.2133460819 N 91.5435951673 W	19024235	Awaiting processing	Hardwood
SD0013	*Stereum complicatum*	*Stereum complicatum*	Central City, IA	42.21380938 N 91.54359371 W	19024271	Awaiting processing	Hardwood
SD0019	*Stereum fasciatum*	*Stereum ostrea*	Cedar Rapids, IA	41.9703498 N 91.5875476 W	13708432	Awaiting processing	Hardwood
SD0020	*Stereum subtomentosum*	*Stereum ostrea*	Cedar Rapids, IA	41.97007352 N 91.59099844 W	13708507	Awaiting processing	Hardwood
SD0022	*Stereum fasciatum*	*Stereum ostrea*	Cedar Rapids, IA	41.96928863 N 91.59225843 W	8317624	Awaiting processing	*Populus deltoides*
SD0023	*Stereum gausapatum*	*Stereum gausapatum*	Cedar Rapids, IA	41.96930212 N 91.58904619 W	13750325	Awaiting processing	*Quercus alba*
SD0025	*Stereum lobatum*	*Stereum ostrea*	Mt. Vernon, IA	41.89833209 N 91.48480468 W	12494830	Awaiting processing	Hardwood
SD0026	*Stereum lobatum*	*Stereum ostrea*	Central City, IA	42.22393886 N 91.54316937 W	13151425	Awaiting processing	*Quercus rubra*
SD0027	*Stereum lobatum*	*Stereum ostrea*	Cedar Rapids, IA	42.00945632 N 91.57635104 W	13992043	Awaiting processing	Hardwood
SD0028	*Stereum complicatum*	*Stereum complicatum*	Toddville, IA	42.08706334 N 91.76554189 W	14109784	Awaiting processing	Hardwood
SD0029	*Stereum fasciatum*	*Stereum ostrea*	Cedar Rapids, IA	42.00992866 N 91.5733616 W	13992061	Awaiting processing	*Quercus alba*

SD0030	*Stereum subtomentosum*	*Stereum ostrea*	Cedar Rapids, IA	42.02686135 N 91.72864418 W	14201337	Awaiting processing	*Betula*
SD0031	*Stereum lobatum*	*Stereum ostrea*	Vina, AL	34.45011069 N 87.98298005 W	12653541	Awaiting processing	Hardwood
SD0032	*Stereum subtomentosum*	*Stereum hirsutum*	Cedar Rapids, IA	41.96937897 N 91.59167423 W	13750363	Awaiting processing	Hardwood
SD0034	*Stereum subtomentosum*	*Stereum hirsutum*	Homestead, IA	41.76767421 N 91.87976291 W	14575926	Awaiting processing	Hardwood
SD0035	*Stereum lobatum*	*Stereum ostrea*	Homestead, IA	41.76716015 N 91.88214488 W	14581271	Awaiting processing	*Tilia americana*
SD0036	*Stereum sanguinolentum*	*Stereum sanguinolentum*	Toddville, IA	42.09754684 N 91.7560792 W	14652976	Awaiting processing	*Pinus strobus*
SD0039	*Stereum gausapatum*	*Stereum gausapatum*	Central City, IA	42.21451457 N 91.54637958 W	14725260	Awaiting processing	Hardwood
SD0040	*Stereum complicatum*	*Stereum complicatum*	Central City, IA	42.21421407 N 91.54698225 W	14725532	Awaiting processing	Hardwood
SD0041	*Stereum fasciatum*	*Stereum ostrea*	Cedar Rapids, IA	41.96788977 N 91.58194713 W	15178270	Awaiting processing	Hardwood
SD0042	*Stereum complicatum*	*Stereum complicatum*	Cedar Rapids, IA	41.96937751 N 91.58662002 W	15178294	Awaiting processing	Hardwood
SD0043	*Stereum fasciatum*	*Stereum ostrea*	Grant, AL	34.44427785 N 86.33917893 W	12779982	Awaiting processing	Hardwood
SD0046	*Stereum lobatum*	*Stereum ostrea*	Mt. Vernon, IA	41.90488893 N 91.50849332 W	15250205	Awaiting processing	*Carya ovata*
SD0047	*Stereum lobatum*	*Stereum ostrea*	Central City, IA	42.21695895 N 91.54493417 W	15320607	Awaiting processing	Hardwood
SD0048	*Stereum gausapatum*	*Stereum hirsutum*	Maquoketa, IA	42.11860587 N 90.77452929 W	16110487	Awaiting processing	Hardwood
SD0049	*Stereum sanguinolentum*	*Stereum sanguinolentum*	Goshen, AL	31.71356163 N 86.0591069 W	19559179	Awaiting processing	*Pinus taeda*
SD0050	*Stereum complicatum*	*Stereum*	Goshen, AL	31.67680582 N 86.19746112 W	19774388	Awaiting processing	*Pinus taeda*
SD0051	*Stereum striatum*	*Stereum*	Goshen, AL	31.6292607941 N 86.0024681297 W	19774395	Awaiting processing	Hardwood
SD0052	*Stereum lobatum*	*Stereum ostrea*	Mary Esther, FL	30.4134526 N 86.6658315 W	19922353	Awaiting processing	Hardwood
SD0053	*Stereum lobatum*	*Stereum ostrea*	Guntersville, AL	34.38312861 N 86.20634405 W	19559158	Awaiting processing	Hardwood

SD0054	*Stereum lobatum*	*Stereum ostrea*	Goshen, AL	31.69701004 N 86.05894787 W	19559171	Awaiting processing	Hardwood
LCM8004	*Xylobolus subpileatus*	*Xylobolus subpileatus*	Gainesville, FL	29.646684 N 82.358474 W	26613386	Awaiting processing	Hardwood
PIMO 23	*Stereum hirsutum*	*Stereaceae*	MT	N/A	N/A	JF705934.1	*Pinus monticola*
KARE1330	*Stereum hirsutum*	*Stereum hirsutum*	CA	N/A	N/A	MN218206.1	*Prunus dulcis*
Mushroom Observer.org/312763	*Stereum hirsutum*	*Stereum hirsutum*	CA	37.8462 N 122.068 W	N/A	MH160727	*Quercus agrifolia*
N/A	*Stereum hirsutum*	*Stereum hirsutum*	Germany	N/A	N/A	KX838369.2	*Arrhenatherum elatius*
N/A	*Stereum hirsutum*	*Stereum hirsutum*	India	N/A	N/A	KX440605.1	N/A
O-F-256436	*Stereum hirsutum*	*Stereum hirsutum*	Norway	60.292 N 10.69 E	N/A	NOBAS768-15 (BOLD)	N/A
NHMO-DFL-1045	*Stereum hirsutum*	*Stereum hirsutum*	Norway	59.866 N 5.524 E	N/A	NOBAS7709-19 (BOLD)	N/A

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Figure 1. Maximum likelihood phylogeny generated from ITS sequence data. Bootstrap values are above branches. Scale bar represents the number of nucleotide changes per site. The first three clades show strong support, suggesting *S. ostrea* in midwestern and eastern North America consists of three distinct species, which we identify as *S. fasciatum*, *S. lobatum*, and *S. subtomentosum*.

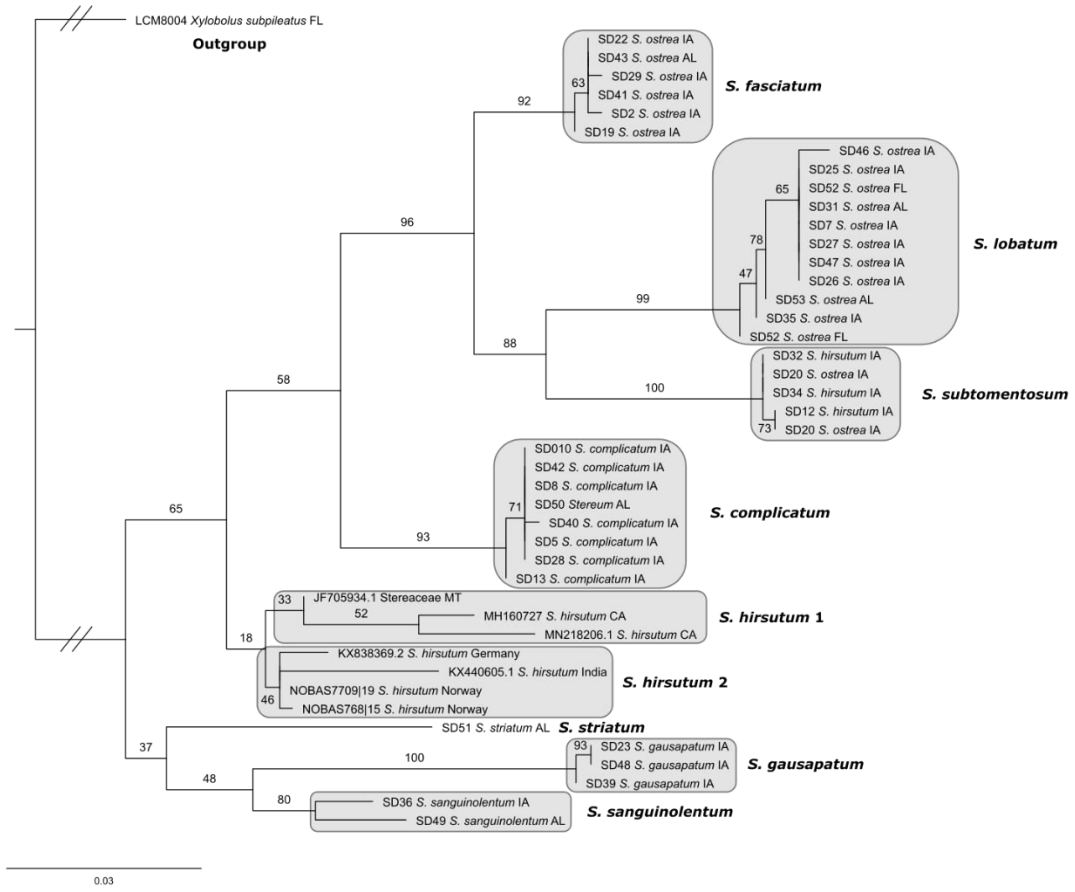

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Figure 2. Morphological comparison of a-b) SD01 *Stereum fasciatum*, c-d) SD03 *S. lobatum*, e-f) SD12 *S. subtomentosum*. Arrows point to areas where hairs have worn away in concentric bands, exposing the chestnut-brown context; a) few very thin bands, c) wide bands, starting from cap edge, and e) intermediate, irregular banding. Note differences between species in cap hair texture; b) coarse and clumped, d) matted and felted, and f) long and wooly.

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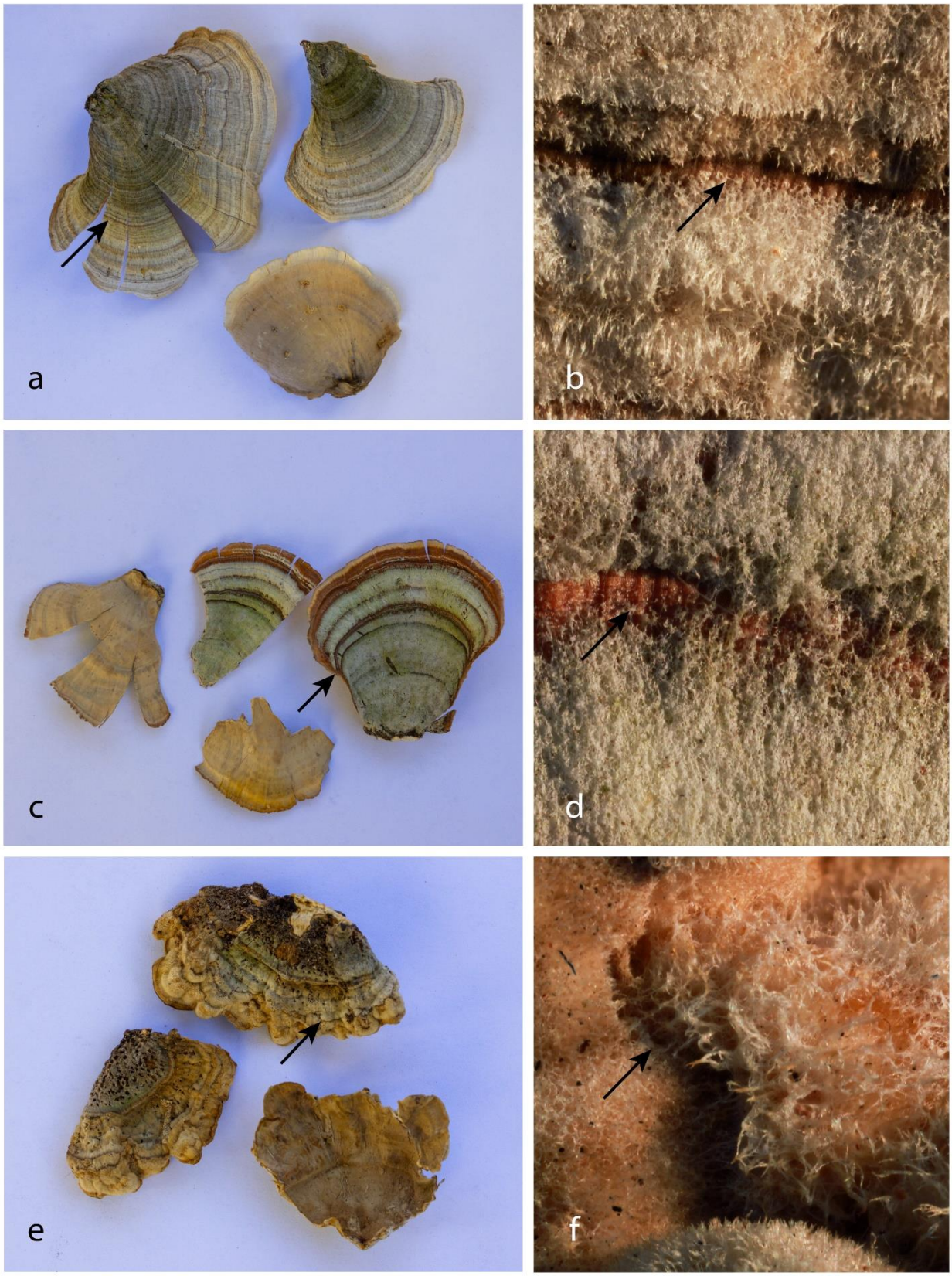

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