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## *Balsamia* (Sequestrate *Helvellaceae*, *Ascomycota*) in western North America

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*Pezizomycotina*  
*Pseudobalsamia*  
sequestrate fungi  
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**Abstract:** *Balsamia*, a hypogeous, sequestrate genus in the *Helvellaceae*, has been characterized variously as having three to eight species in North America, and these have been considered either different from or conspecific with European species. No available modern systematic treatment of *Balsamia* exists to allow for accurate identification at the species level. We sequenced DNA from recent western North American *Balsamia* collections, assessed relationships by sequence similarity, and identified molecular taxonomic units. From these data, we determined which matched descriptions and types of named species. ITS sequences supported 12 *Balsamia* species in western North America, five originally described by Harkness and Fischer and seven new species that we describe here. No sequences from *Balsamia* collections in western North America were nested among those of European species. We found no clear evidence for separation of *Balsamia* into multiple genera.

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## INTRODUCTION

*Balsamia* is a genus of hypogeous, ectomycorrhizal, sequestrate fungi (truffles) in the *Helvellaceae* (O'Donnell *et al.* 1997, Læssøe & Hansen 2007, Lumbsch & Huhndorf 2007). No comprehensive molecular phylogeny of *Helvellaceae* exists. The few phylogenetic studies of *Pezizales* that include the *Helvellaceae* agree that *Balsamia* is sister to *Helvella sensu stricto* (e.g. O'Donnell *et al.* 1997, Læssøe & Hansen 2007, Hansen & Pfister 2006).

The historical literature on *Balsamia* raises important questions regarding the integrity of the genus and the number of species: One genus or two? How many *Balsamia* species occur in western North America? Do European *Balsamia* species occur in North America? Vittadini (1831) described the genus *Balsamia* initially from Europe based on *B. vulgaris*, to which Fischer (1897) and Harkness (1899) added species from North America. Fischer (1907) later described the genus *Pseudobalsamia* based on *P. setchellii* from western North America. Gilkey (1916) accepted *Pseudobalsamia* and concluded that *Balsamia* was restricted to Europe whereas *Pseudobalsamia* was restricted to North America. However, Trappe (1975) synonymized *Pseudobalsamia* with *Balsamia*.

Harkness (1899) recognized seven *Balsamia* species from California. Four he described as new: *B. alba*, *B. filamentosa*, *B. magnata*, and *B. nigrans* (as *nigrens*); and three he considered as species described from Europe: *B. platyspora*, *B. polysperma*, and *B. vulgaris*. In addition, W.A. Setchell sent a Californian specimen to E. Fischer (1907) who described it as *Pseudobalsamia setchellii* to increase the total to eight species in California.

Gilkey (1916, 1954) synonymized *Balsamia alba*, *B. filamentosa*, and *Pseudobalsamia setchellii* with *P. magnata*, and reduced *B. nigrans* to *P. magnata* var. *nigrans* (as *nigrens*). Later, however, Gilkey re-elevated her var. *nigrans* to species level as *P. nigrans* (Gilkey 1954). Gilkey (1916) reclassified the three collections identified by Harkness as *B. platyspora*, *B. polysperma*, and *B. vulgaris* to *P. magnata*. Later Gilkey (1939) identified *B. platyspora* in a Canadian collection. Thus at various times, Gilkey recognized one, two, or three *Balsamia* species in North America. Based on Gilkey's revisions (1916, 1939, 1954) and Trappe's (1975) synonymizing of genera, all collections of *Balsamia* and *Pseudobalsamia* from California and the Pacific Northwest would be identified as one of three species: *B. magnata*, *B. nigrans*, or *B. platyspora*.

Taxonomic uncertainty has limited our ability to recognize fungal species and confirm the identity of rare fungal species (Molina 2008). No modern systematic treatment of *Balsamia* has been published, leading to earlier generic and species names being retained *ad hoc* and applied to collections from California and the Pacific Northwest. For example, Grubisha *et al.* (2005) reported collections of *B. magnata*, *B. nigrens* (sic), and *B. setchellii* from the Channel Islands (California). Frank *et al.* (2006) submitted an ITS sequence to GenBank from an oak-associated specimen from Oregon under the name *B. alba*. Palfner & Agerer (1998) recognized an ectomycorrhizal morphotype formed by *B. alba* on roots of *Pinus jeffreyi*. Bonito *et al.* (2013) used DNA sequences of *Balsamia* cf. *setchellii* (as *Balsamia* sp. in Smith *et al.* 2007) as the outgroup collection for a multi-locus phylogeny of *Tuberaceae*. Allen *et al.* (1999) identified a *Balsamia* associated with *Adenostoma fasciculatum*

(*Rosaceae*) in southern California as *B. vulgaris*. In California and the Pacific Northwest, *B. alba*, *B. nigrans*, and *B. platyspora* have been recognized as rare, special status, or sensitive species (Castellano *et al.* 1999, ORBIC 2016).

Molecular data are critical to verify *Balsamia* species distinctions and to appropriately apply names that should be used for these collections. The nuclear ribosomal internal transcribed spacer (ITS) region has been recognized as a standard of comparison among fungal species (Schoch *et al.* 2012, Køljalg *et al.* 2013, Ryberg 2015).

Our goals were to (1) determine whether ITS sequences support three species for western North America as determined by Gilkey (1916, 1939, 1954) or more than three species as described by Harkness (1899) and Fischer (1907); (2) correlate molecular data with morphological characters that can be used to identify and differentiate *Balsamia* species from western North America; and (3) determine whether *Pseudobalsamia* is distinct from *Balsamia* and should be retained as a separate genus. We sequenced ITS ribosomal DNA from recent collections, assessed relationships by sequence similarity, and then correlated sequence groups with type descriptions. Because we did not obtain ITS sequences from type specimens, we selected epitypes and emended species descriptions based on fresh collections. For collections that did not match one of the named species, we described new species.

## MATERIALS AND METHODS

### DNA extraction, PCR amplification, sequencing and phylogenetic analysis

DNA was obtained from fresh or dry specimens. Samples were extracted in 2 % cetyltrimethyl ammonium bromide (CTAB) with chloroform and amplified in PCR with fungal primers ITS1F and ITS4 (White *et al.* 1990, Gardes & Bruns 1993). An initial 3 min at 93 °C was followed by 30 cycles of 30 s at 95 °C, 2 min at 54 °C and 3 min at 72 °C, with a final cycle for 10 min at 72 °C, modified from Frank *et al.* (2010). PCR products were purified with QIAquick PCR Purification kits (QIAGEN, Valencia, CA), prepared with BigDye Terminator Ready Reaction Mix 3.1 and sequenced in an ABI 310 Genetic Analyzer (Applied Biosystems, Foster City, CA) at the Biotechnology Center at Southern Oregon University. We obtained molecular data by sequencing the ITS region, including ITS1, the 5.8S ribosomal DNA gene, and ITS2, with forward primers ITS1F and ITS1, and reverse primer ITS4 (Frank *et al.* 2010).

Sequences were edited with Chromas v. 1.45 (McCarthy 1998); contigs were assembled with Sequencher v. 4.7 (Gene Codes Corp. Ann Arbor, MI). Sequences were compared to fungal sequences in GenBank ([www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov)) with BLAST (Altschul *et al.* 1990, Thompson *et al.* 1997). Multiple sequence alignments of the ITS region, from our sequences and from GenBank, were generated with MAFFT v. 7 (Katoch *et al.* 2002, Katoch and Standley 2013) and the sequences reviewed in Clustal X and edited in Mesquite (Maddison & Maddison 2011). DNA sequences were deposited in GenBank (Accession numbers KF983477–KF983491, KM115877–KM115881, KU170027–KU170042, and KY706376–KY706390).

Phylogenetic trees, using parsimony and maximum likelihood with 1000 bootstrap replicates and 1000 jack-knife replicates, were generated using PAUP v. 4.10b10 (Swofford 2002).

Consensus trees with 50 % majority-rule were generated using a tree-bisection-reconnection branch-swapping algorithm. All characters were given equal weight; gaps were treated as missing. Maximum likelihood trees with 100 bootstrap replicates were also generated using PhyML through the phylogeny.fr portal using the substitution model HKY85 (Dereeper *et al.* 2008). Consensus trees were examined to confirm branch positions.

### Morphology

We examined specimens from western North America obtained from the following sources (<http://sweetgum.nybg.org>): Oregon State University (OSC), Southern Oregon University (SOC), University of California, Berkeley (UC), National Fungus Collections (BPI) and the Florida Museum of Natural History (FLAS), as well as permanent microscopic sections of Harkness collections from OSC (duplicates at BPI). We also obtained fresh specimens collected from southern Oregon and California. We compared these with descriptions in Tulasne & Tulasne (1851), Harkness (1899), Fischer (1907, 1908), Gilkey (1916, 1939, 1954), Ławrynowicz (1988), Pegler & Spooner (1993), and Montecchi & Sarasini (2000).

Emended descriptions were based on epitypes selected by D. Southworth. The International Code of Nomenclature for algae, fungi and plants (Melbourne Code) allows for designation of an epitype when the holotype or isotype cannot be “critically identified” because of a lack of diagnostic features or when no molecular sequences could be obtained from the type to confirm the current application of the name (McNeill *et al.* 2012). No material was available for sequencing from type collections, which were in too poor condition for a comprehensive morphological assessment. We assigned Harkness species names to MOTUs that segregated by ITS sequences based on original descriptions, on tissue and spores prepared from Harkness collections, and on habitat and location of the holotype.

For the Harkness type specimens and other collections that Harkness identified, we examined resin-embedded sections, stained with toluidine blue O or safranin-fast green, housed at OSC with duplicates at BPI. From fresh and dried specimens, hand sections were mounted in water and ascospores measured (20 randomly selected spores per collection) at 1000 × with a Leica DMLB microscope and SPOT RT software (Diagnostic Instruments Inc., Sterling Heights, MI). Digital images were captured with a SPOT RT camera. Because means and medians did not differ (paired *t*-test,  $P = 0.853$ , Minitab v. 15), spore dimensions were reported as mean and range (in parentheses) for length, width, and the ratio of length to width (Q). Terminology for description of ascomata tissues follows Ulloa & Hanlin (2012); dimensions of peridial warts are reported as height × width. Holotypes, epitypes, and additional specimens were deposited in the OSC herbarium.

## RESULTS

### Phylogenetic analyses

The ITS data matrix consisted of 15 taxa with 63 sequences, 58 from vouchered specimens of *Balsamia*, four from ectomycorrhizas, and one from the outgroup *Barssia* (Table 1). The edited alignment consisted of 1 109 characters: 484 parsimony informative, 442 constant, 183 non-informative.

**Table 1.** Collections of *Balsamia* species and *Bassia oregonensis* with ITS GenBank Accession numbers from which sequences were used in phylogenetic analyses.

Taxon	Fungarium <sup>a</sup>	Voucher <sup>b</sup>	Collection locale	ITS GenBank	Type designation	
<i>Balsamia alba</i>	OSC157708	T37489	USA: CA: Placer Co.	KY706381		
	OSC130666	T6253	USA: OR: Jackson Co.	KF983482		
	OSC157720	NS1740	USA: CA: Modoc Co.	KY706376		
	OSC80952	T19926 <sup>g</sup>	USA: CA: Fresno Co.	AY558741		
	UC1999421	T17852 <sup>g</sup>	USA: CA: Fresno Co.	AY558742		
	OSC157711	T37496	USA: CA: Placer Co.	KY706384		
	OSC157712	T37499	USA: CA: Placer Co.	KY706385		
	OSC157715	T37509	USA: CA: Placer Co.	KY706387		
	OSC157709	T37490	USA: CA: Placer Co.	KY706382	epitype	
	OSC157719	T37520	USA: CA: Shasta Co.	KY706390		
<i>Balsamia cascadenis</i>	OSC151392	S1522	USA: OR: Jackson Co.	KF983483	holotype	
	OSC151391	S1521	USA: OR: Jackson Co.	JN022501	paratype	
<i>Balsamia filamentosa</i>	OSC157710	T37491	USA: CA: Placer Co.	KY706383		
	OSC157713	T37502	USA: CA: Placer Co.	KY706386		
	OSC157716	T37511	USA: CA: Placer Co.	KY706388	epitype	
	OSC130728	T19467	USA: CA: Contra Costa Co.	KM115880		
	OSC131295	T18019	USA: CA: Ventura Co.	KF983484		
<i>Balsamia latispora</i>	OSC148026	AM-CA11-021	USA: OR: Riverside Co.	MF098665	paratype	
	OSC157702	T37338	USA: CA: Siskiyou Co.	KY706378	paratype	
	OSC157704	T37345	USA: CA: Siskiyou Co.	KY706380	paratype	
	OSC66585	T35434	USA: OR: Jefferson Co.	KF983487	holotype	
	OSC157701	T37336	USA: CA: Siskiyou Co.	KY706377	paratype	
	OSC49763	T11671	USA: CA: El Dorado Co.	KU170033	paratype	
	OSC48551	T9530	USA: CA: Sierra Co.	KU170037	paratype	
	OSC130672	T8082	USA: CA: Sierra Co.	KU170036	paratype	
	OSC131289	T7574	USA: CA: Siskiyou Co.	KF983486	paratype	
	OSC130676	T11394	USA: CA: San Bernardino Co.	KU170032	paratype	
	OSC151399	S1648	USA: OR: Jackson Co.	KF983485	paratype	
	OSC157703	T37340	USA: CA: Siskiyou Co.	KY706379	paratype	
	OSC62003	T25894	USA: OR: Jackson Co.	KU170034	paratype	
	OSC80116	T26983	USA: OR: Jackson Co.	KU170035	paratype	
	<i>Balsamia lazyana</i>	OSC130670	T19348	USA: OR: Josephine Co.	KM115879	holotype
	<i>Balsamia limuwensis</i>	OSC130981	LG1115	USA: CA: Santa Barbara Co.	KU170027	holotype
OSC49229		T11111	USA: CA: Marin Co.	KU170029	paratype	
<i>Balsamia magnata</i>	OSC157718	T37519	USA: CA: Shasta Co.	KY706389		
	OSC151398	S1843	USA: OR: Jackson Co.	KF983477		
	OSC151397	S1386	USA: OR: Jackson Co.	KF983478	epitype	
		JLF3324	USA: CA: Mendocino Co.	KU170038		
	OSC151393	F1943	USA: OR: Marion Co.	KF983479		
	OSC130663	T2050	USA: OR: Columbia Co.	KF983480		
	OSC151395	S1860	USA: OR: Jackson Co.	KF983481		
	<i>Balsamia nigrans</i>	OSC151401	S1061	USA: OR: Josephine Co.	FJ789590	
	OSC130695	T8431	USA: CA: Mariposa Co.	KP859264		
	SOC871	S871	USA: CA: Tehama Co.	GU184098		
	OSC130700	T19921	USA: CA: Fresno Co.	EU669383	epitype	
<i>Balsamia pallida</i>	OSC130727	T17945	USA: CA: Riverside Co.	KU170040	holotype	

Table 1. (Continued).

Taxon	Fungarium <sup>a</sup>	Voucher <sup>b</sup>	Collection locale	ITS GenBank	Type designation
<i>Balsamia platyspora</i>	O-F245397		Norway: Ostfold	KP149493	
<i>Balsamia quercicola</i>	OSC151402	S558	USA: OR: Jackson Co.	DQ453695	paratype
	FLAS_F58857	MES84	USA: CA: Yolo Co.	KF983490	paratype
	FLAS_F58860	SRC868	USA: CA: Yuba Co.	KF983491	holotype
<i>Balsamia setchellii</i>	FLAS_F58859	SRC395	USA: CA: Yuba Co.	DQ974730	epitype
	OSC80893	SRC396	USA: CA: Yuba Co.	KU170028	
	OSC140476	L5470	USA: OR: Josephine Co.	KP859277	
	OSC49779	T11706	USA: AZ: Gila Co.	KU170030	
<i>Balsamia trappei</i>	OSC149793	D3000	Canada: Alberta	KU170041	paratype
	OSC131300	T20842	USA: WA: Pend Oreille Co.	KU170042	holotype
<i>Balsamia vulgaris</i>	AH44223 <sup>h</sup>	M2404	Italy: Reggio Emilia	KM243646	
	AH44224 <sup>h</sup>	M2403	Italy: Reggio Emilia	KM243647	
	OSC149589	S2036	Cyprus: Kelefos Bridge	KM115881	
<i>Balsamia ectomycorrhiza</i>		7992.6.R <sup>f</sup>	UK: England	EU668245	
	UE_ITA248 <sup>c</sup>		Italy: Perugia	JX474845	
	AZA731 <sup>e</sup>		France	JX989935	
	AR1166 <sup>d</sup>		Canada: NW Terr.	JX630958	
<i>Barssia oregonensis</i>	OSC151400	F2161	USA: OR: Marion Co.	KF983489	

<sup>a</sup>Index Herbariorum <http://sweetgum.nybg.org/ih/>

<sup>b</sup>AM, A Mujic; D, RM Danielson; F, JL Frank; G, HM Gilkey; H, HW Harkness; L, S Loring; LG, L Grubisha; M, A Montecchi; MES, ME Smith; NS, N. Siegel; S, D Southworth; SRC, Sierra Research Center; T, JM Trappe.

<sup>c</sup>GMN Benucci, unpublished; Benucci *et al.* (2014).

<sup>d</sup>Timling *et al.* (2012).

<sup>e</sup>Roy *et al.* (2013).

<sup>f</sup>Bidartondo & Read (2008).

<sup>g</sup>Izzo *et al.* (2005). Note: The accession numbers of these two collections are reversed in GenBank.

<sup>h</sup>Crous *et al.* (2014).

ITS sequences supported 12 ITS molecular operational taxonomic units (MOTUs) in North America (Fig. 1). Bootstrap analysis separated these into two major branches, one with eight MOTUs from North America and one with European species and two MOTUs from North America (Fig. 1). We assigned five MOTUs to described species: *B. alba*, *B. filamentosa*, *B. magnata*, *B. nigrans*, and *B. setchellii* (Harkness 1899, Fisher 1907); and five to new species: *B. cascadiensis*, *B. latispora*, *B. lazyana*, *B. limuwensis*, and *B. trappei*. In addition we described *B. pallida* and *B. quercicola* with ambiguous positions. The position of *B. quercicola* was driven mainly by a large deletion. We did not attempt to verify identification of European species.

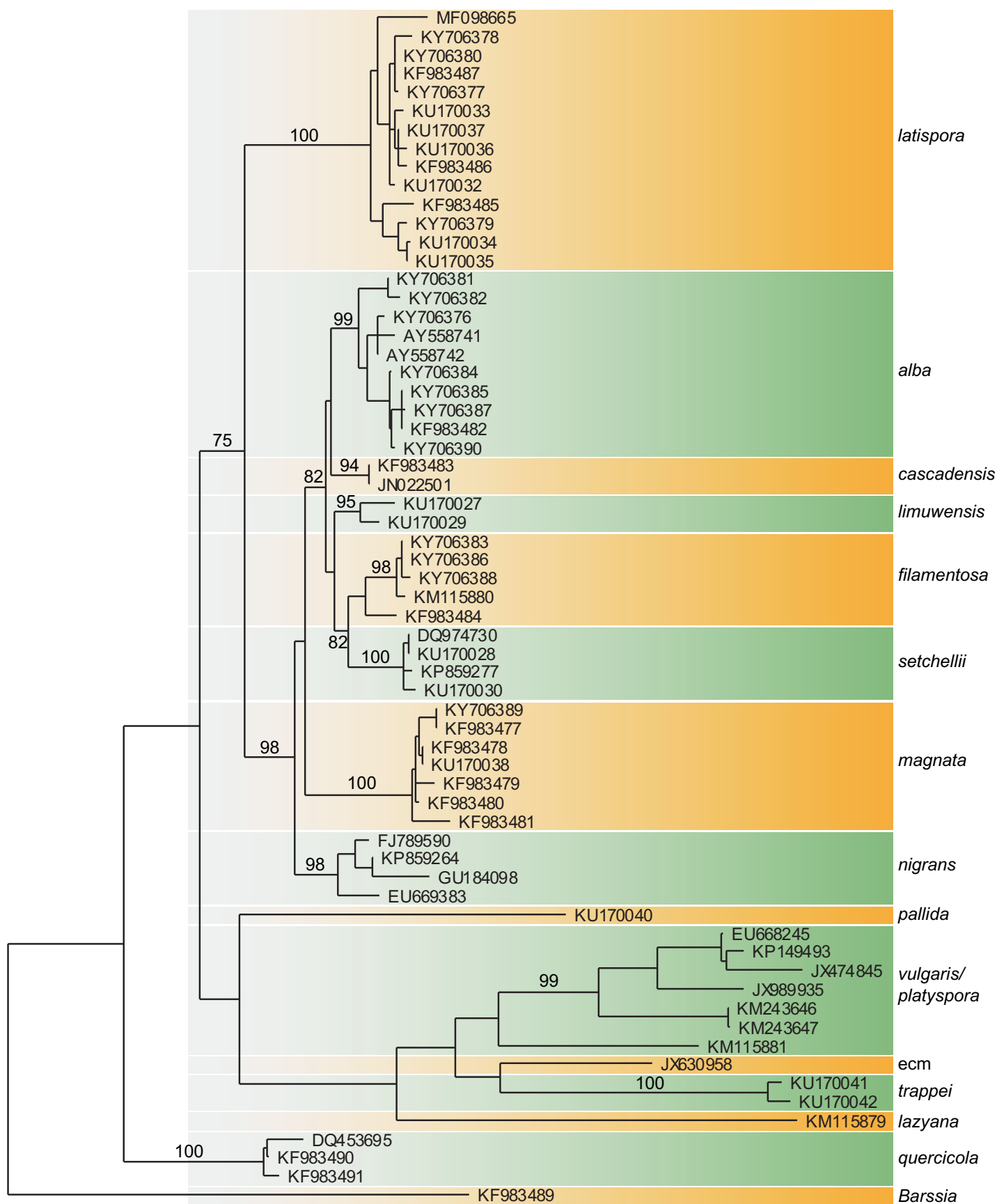
### Morphological variation

Morphological distinctions among species were sometimes subtle as a consequence of inherent variation, environmental effects, and uncertain developmental stage. Relative maturity clearly differed among and within collections, though developmental stages were difficult to assess. As specimens dried, the gleba color of immature specimens sometimes changed from white to golden brown and shrank, while the gleba color of more mature specimens remained white and did not shrink. The shrinking of glebal tissue in immature specimens resulted in enlarged glebal

chamber openings and narrowed the spacing of peridial warts.

Because ascospores in *Balsamia* species are not actively discharged from asci, the relative maturity of asci and ascospores is difficult to determine precisely. However, hand-sectioned specimens differed in the ease with which spores dispersed from asci (as viewed under the compound microscope). We interpreted specimens with readily dispersed spores as more mature than those in which most spores remained inside the asci. Ascospores in less mature specimens had thinner spore walls and were shorter than in fully mature specimens. The number of guttules per spore varied among collections within a species, possibly due to developmental stage.

Dimensions of ascomata, warts, asci, and ascospores varied considerably. For example, width of peridial warts on individual specimens ranged from 200–700 µm. Although spore dimensions tended to be similar within a given ascus, spore size varied by up to 30 % within a single ascoma for some species. Furthermore, different regions in an individual ascoma often differed in spore dimensions. This may have been due to differences in maturity or to normal size variation. Ascoma size was a poor indicator of developmental stage. Although all ascomata must have grown from a small initial structure to the final size, some small (<1 cm) ascomata were fully mature while some large ones (1–2 cm) were immature.



**Fig. 1.** Maximum likelihood tree inferred from ITS rDNA showing relationships among North American species of *Balsamia*. Bootstrap values (>50%) from the parsimony analysis (1000 replicates) using PAUP\* are shown above branches.

## TAXONOMY

***Balsamia*** Vittad., *Monogr. Tubercac.* (Milano): 30. 1831, *emend.* D. Southw. & Trappe

*Type species: Balsamia vulgaris* Vitt.

*Original type description* (Vittadini 1831, translation D. Southworth): Characters. *Fruiting body* pliant, sessile, rootless [hyphal cords], always closed, chambered-fleshy [gleba]. *Sporangia* [asci] oblong, membranous, stalked, 8-spored, embedded in the walls of the chambers and arranged in rows. *Spores* cylindrical, smooth, hyaline.

*Observations: Fruiting body* hypogeous, lacking root and branch, exterior warty, interior chambered, with the peridium inserted from the surface and variously folded on itself, chambered-fleshy. *Flesh* [gleba] always white, with veins markedly pallid when immature, at last nearly liquifying; the inside of the chambers lined with short hairs. *Spores* white, hyaline, containing 1–2 large vesicles (perhaps little spores?) and as many smaller ones. Natural History. The fungi fleshy-soft, not very persistent. Habit altogether of the genus *Tuber*, with which they are still commonly confused but distinct by the softness of the fruiting body, the form and nature of the spores, *etc.* They grow together with true truffles and frequently with those produced in autumn and winter. Edible, but less valued than truffles and often even noxious.

*Emended description: Ascomata* sequestrate, subglobose, often compressed, irregularly lobed and invaginated to form an apical depression, 5–30 × 5–30 mm; peridial warts pale orange, reddish brown, brown, or black; warts pointed to rounded or nearly flat, 100–350 × 150–700 μm; gleba white, drying to golden when immature and remaining white when mature; chambers open or closed - a condition in which the chamber walls are pressed together to create narrow channels; some of the channels open to the exterior (venae externae) in the area of the apical depression. *Peridium* surface with sparse, pointed cystidium-like cells, ±8 μm wide at base, protruding up to 25 μm, with walls 1 μm thick; outer peridial layer 2–4 cells thick; cells isodiametric, 20–25 μm wide, to radially elongate, 11–20 μm × 22–33 μm; with walls 2–4 μm thick, red-brown; inner layer of hyaline, isodiametric cells 10–15 μm wide, with walls 1 μm thick; with scattered brown cells up to 27 μm wide, with walls 2 μm thick. *Gleba* of loosely interwoven hyphae, 4–5 μm wide. Chambers when immature lined with a palisade of paraphyses, at maturity the hyphal lining of interwoven paraphyses. *Asci* hyaline, citriform when immature, maturing to ovoid, 65–100 × 30–35 μm, with walls 1–1.5 μm, stalk 5 × 25 μm visible in immature specimens, 8-spored. *Ascospores* hyaline, smooth (but see Commentary), ellipsoid to cylindrical, 14.5–40 × 8–20 μm, Q = 1.3–3.0 with 1–3 guttules.

*Habit, habitat, and distribution:* Hypogeous under diverse host trees including conifers (*Abies*, *Pinus*, *Pseudotsuga*), hardwoods (*Alnus*, *Arbutus*, *Corylus*, *Quercus*, *Populus*), or shrubs (*Adenostoma*, *Arctostaphylos*) widespread in the PNW and western USA and Canada.

*Commentary:* In some specimens of *B. setchellii*, *B. filamentosa*, and *B. nigrans* collected 1898 to 1904, spores exhibited a pattern

of transverse, slightly oblique ridges (0.9 μm wide) visible at 100× (oil immersion) with Nomarski optics. We interpret these as artifacts of shrinkage due to a combination of uncertain factors, *e.g.*, developmental stage, specimen condition, and fixation and drying procedures. They may reflect an underlying structure, *e.g.*, of cell wall microfibril orientation and/or the subtending cytoskeleton. We attempted to induce this pattern in recent specimens by fixation in formalin and/or ethanol followed by air-drying. Less than 1 % of spores and only those with broken cell walls exhibited transverse ridges; no ridges were observed in fully developed spores with filled guttules.

***Balsamia alba*** Harkn., *Proc. Calif. Acad. Sci. Bot.* 1: 264. 1899, *emend.* D. Southw. & Castellano, Fig. 2.

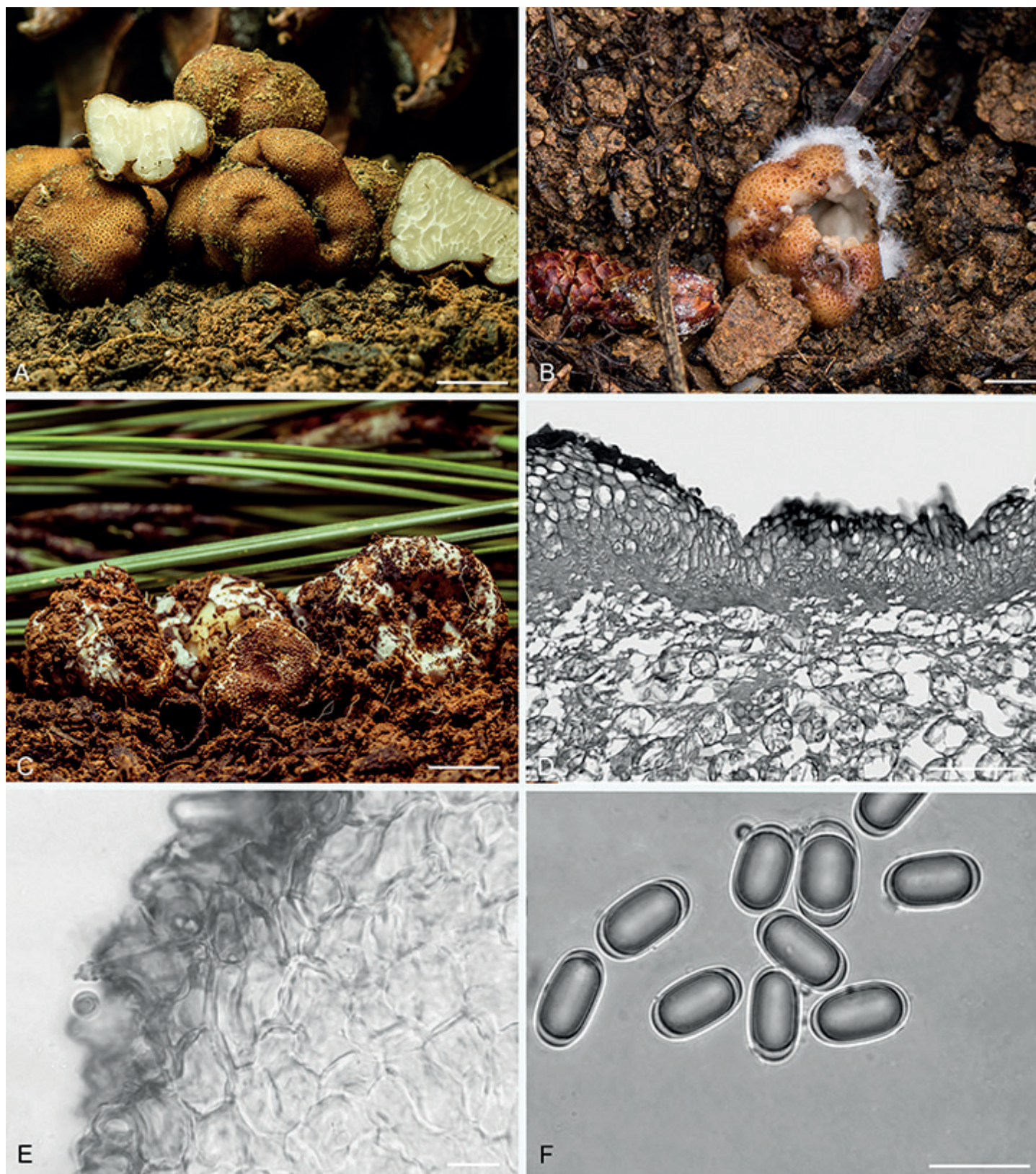
*Diagnosis:* Peridium of red-brown warts often covered with white hyphae to which soil particles adhere thus rendering it “dirty white” as described by Harkness. The warts of *B. alba* are low, rounded to pointed, less red than in *B. magnata*. In addition, the spores of *B. alba* have a slightly narrower aspect (mean Q = 2.1) and the inner peridial tissue is significantly thicker than in *B. magnata*.

*Isotype:* **USA**, California, Placer Co., Wire Bridge, south of Auburn at end of Rattlesnake Lane, at Rattlesnake Bar, Folsom Lake State Recreation Area, under *Quercus*, Feb. c. 1898, *H.W. Harkness 129, Gilkey 1033* (OSC81203).

*Epiletype:* **USA**, California, Placer Co., Auburn State Recreation Area, Foothill Rd., 0.8 km from Upper Lake Clementine Day Use area to the right after entering at trailhead entering meadow, 38°55.0'N 120°58.4'W, 540 m, under *Ceanothus*, *Pinus ponderosa*, *P. sabiniana*, 11 Dec. 2016, *M. Castellano* (designated here OSC157709, MBT381870), GenBank accession number ITS: KY706382.

*Original type description* (Harkness 1899, p. 264): “Large, color dirty white, subglobose, fissured, deeply verrucose; gleba firm; asci ellipsoidal, 8-spored; spores cylindrical, guttulate, 12 × 18 μ. Type: Harkness 129. Under oaks, Wire Bridge, Placer County, Calif., February. This fungus is remarkable for the density of the gleba and the small number of its asci”.

*Emended description: Ascomata* sequestrate, subglobose to reniform with invaginations, 7–20 × 6–12 mm, warts dark reddish brown, pale tan between warts. Peridial surface often covered with white hyphae between or over warts with adhering soil particles; thus the surface may appear “dirty white;” peridial warts low, flat to rounded or obtusely pointed, 100–220 × 260–430 μm. Under a compact white hyphal layer, warts flattened to create a brown layer, 50–60 μm thick; gleba pale yellow when fresh, chambers mostly appressed. *Peridium* wart surface partially covered with white mycelium comprised of loosely woven, branched, hyaline hyphae either 5–6.5 μm wide, lacking clamp connections, but bulging on one side of septum, or 2.5–3 μm wide, with clamp connections and encrusted walls. Under the white hyphae are warts comprised of 4–6 layers of reddish brown cells that are ± isodiametric (20 × 17 μm) to radially elongate, 22–30 × 11–14 μm, with walls 6–7 μm thick, intergrading to radially elongated yellow to hyaline cells of similar size, with walls 4 μm thick, intergrading to cells



**Fig. 2.** *Balsamia alba*. **A.** Fresh ascomata showing peridial surface, gleba, and peridium in section (OSC 157712). **B.** Ascoma *in situ* with white alien hyphae (OSC 157714). **C.** Ascoma with white alien mycelium covering the peridium (OSC 157708). **D.** Peridium showing outer wart layer, inner peridial layer and glebal structure. **E.** Peridium with outer warty layer of thick-walled cells. **F.** Ascospores. C–F (OSC 130666). Bars A = 10 mm, B–C = 5 mm, D = 100  $\mu$ m, E–F = 25  $\mu$ m.

with walls 1  $\mu$ m thick. On warts lacking white surface hyphae are scattered pointed cystidium-like cells up to 35  $\mu$ m long. *Gleba* of loosely interwoven hyphae (*textura intricata*), 4–7  $\mu$ m wide; chambers lined with a palisade of paraphyses 70–120  $\times$  4–5  $\mu$ m.

*Asci* hyaline, elongate to ellipsoid, 23–25  $\times$  30–70  $\mu$ m, 8-spored. *Ascospores* hyaline, smooth, ellipsoid, 24.9  $\times$  12.4 (21–28  $\times$  11–13.5)  $\mu$ m, Q = 2.1 (1.7–2.3), with 1–3 guttules.

**Habit, habitat, and distribution:** Hypogeous in variously mixed forests under *Abies concolor*, *Picea engelmannii*, *Pinus jeffreyi*, *P. lambertiana*, *P. ponderosa*, *P. sabiniana*, *Pseudotsuga menziesii*, *Quercus garryana*, *Q. kelloggii*, and *Quercus* species; Sierra Nevada and Klamath-Siskiyou mountains and foothills.

**Additional materials examined:** USA, California, Fresno Co., Sierra National Forest, McKinley Grove Rd. junction with FS Rd. 10S13, 37°0.8'N 119°4.8'W, 2050 m, under *Abies concolor*, *Pinus jeffreyi*, 17 Aug. 1995, T. Bruns SNF222 (OSC130690, UC1999421), GenBank accession number ITS: AY558742; Ross Creek Drainage, Turtle Creek, 36°57'N 119°6'W, 1463 m, under *Abies concolor*, *Pinus lambertiana*, *P. ponderosa*, 25 June 1997, L. Criley (OSC80952), GenBank accession number ITS: AY558741; Kern Co., Sequoia National Forest, Kern Gulch Campground, 35°40.3'N 118°28.3'W, 665 m, under *Arctostaphylos*, *Pinus ponderosa*, *Quercus*, 24 Apr. 1996, M. Castellano (OSC131296); Modoc Co., Modoc National Forest, Warner Mtns., 0.8 km north of Hwy 299, road to Stough Reservoir, under *Abies concolor*, 11 Jun. 2016, N. Siegel 1740 (OSC157720), GenBank accession number ITS: KY706376; Placer Co., Auburn State Recreation Area, Foothill Blvd., 0.8 km from Upper Lake Clementine Day Use Area at trailhead entering meadow, 38°57.0'N 120°58.4'W, 540 m, under *Ceanothus*, *Pinus ponderosa*, *P. sabiniana*, *Pseudotsuga menziesii*, *Quercus*, 11 Dec. 2016, M. Castellano (OSC157708), GenBank accession number ITS: KY706381; Old Foothill Blvd. at Mammoth Bar OHV Area, downhill, 38°55.3'N 121°0.9'W, 350 m, under *Ceanothus*, *Pinus ponderosa*, *P. sabiniana*, *Quercus*, 11 Dec. 2016, M. Castellano (OSC157711), GenBank accession number ITS: KY706384 (OSC157712), GenBank accession number ITS: KY706385 (OSC157714); (OSC157715), GenBank accession number ITS: KY706387; Shasta Co., I-5 Exit 707 in median strip, headed north, at state transportation property turnoff, 40°56.2'N 122°25.1'W, 395 m, in disturbed soil and gravel and under *Pinus ponderosa*, *Pseudotsuga menzeisii*, 12 Dec. 2016, M. Castellano (OSC157719), GenBank accession number ITS: KY706390; Oregon, Jackson Co., Jacksonville Cemetery, 425 m, under *Pinus ponderosa*, *Quercus garryana*, *Q. kelloggii*, 1 Apr. 1981, M. Castellano (OSC130666), GenBank accession number ITS: KF983482.

**Commentary:** Recent collections from Placer Co., CA, near the original Harkness site included specimens with and without the “dirty white” peridial coating. This supports the interpretation that the white mycelium, which sometimes has clamp connections, consists of secondary fungi parasitic on some, but not all specimens of *B. alba*. We were unable to obtain clean sequences of the white mycelium. No spores of other fungi, e.g., *Sepedonium* or *Hypomyces*, have been observed on the “dirty white” specimens. Specimens of other *Balsamia* species, for example, *B. cascadenis* and *B. nigrans*, are sometimes covered with white hyphae.

The isotype of *B. alba* (OSC81203) is in poor condition and stored in an unknown liquid. Ascospores from sections prepared at BPI from the type collection measured 19–25 × 11–13 μm, longer than those reported in the original description and closer to spore sizes in the epitype. Peridial surface warts observed on the type specimen measured 100 × 200 μm, dimensions consistent with the epitype.

***Balsamia cascadenis*** D. Southw., J.L. Frank & Castellano, *sp. nov.* MycoBank MB822496. Fig. 3.

**Etymology:** Named for the Cascade Range in southern Oregon.

**Diagnosis:** Peridium of red-brown warts sometimes covered with white hyphae to which soil particles adhere. *Balsamia*

*cascadenis* is distinguished by narrow spores (mean width, 11.6 μm; Q = 2.1) from all other *Balsamia* species except *B. lazyana*, which has even narrower spores (mean width = 10.1 μm).

**Holotype:** USA, Oregon, Jackson Co., Bureau of Land Management, Cascade-Siskiyou National Monument, Chinquapin Mtn., T39S R3E section 35, 42°8.3'N 122°26.0'W, 1860 m, under *Abies concolor*, 17 Jun. 2010, C. Reha (OSC151392), GenBank accession number ITS: KF983483.

**Ascomata** sequesterate, subglobose, 7–12 × 5–12 mm; warts brown 110–160 × 200–360 μm, rounded to pointed; with white hyphae between or over warts with adhering soil particles, thus the surface appears “dirty white;” gleba white, chamber walls pressed together. **Peridium** wart surface partially covered with white mycelium comprised of loosely woven, branched, hyaline hyphae 5–6.5 μm wide, lacking clamp connections, but bulging on either side of septum; or hyaline hyphae, 2.5–3 μm wide, with clamp connections and encrusted walls; or brown hyphae, 5 μm wide, smooth, with clamp connections. Warts of 4–6 layers of reddish brown cells, radially elongate, 18 × 22 μm, with walls 4 μm thick, intergrading to radially elongated yellow to hyaline cells, 20–22 × 10–12 μm, with walls 1 μm thick. **Gleba** hyphae branched, loosely interwoven, 4–7 μm wide; chambers lined with a palisade of paraphyses 110–120 × 2–8 μm. **Asci** hyaline, obovoid, 29 × 50 μm, 8-spored. **Ascospores** hyaline, smooth, ellipsoid to nearly cylindrical, 23.7 × 11.6 (21–27 × 11–13.5) μm, Q = 2.1 (1.6–2.3); 1–3 guttules.

**Habit, habitat, and distribution:** Hypogeous under *Abies concolor*, *Pinus jeffreyi*, or *Quercus garryana*, southern Oregon mountains.

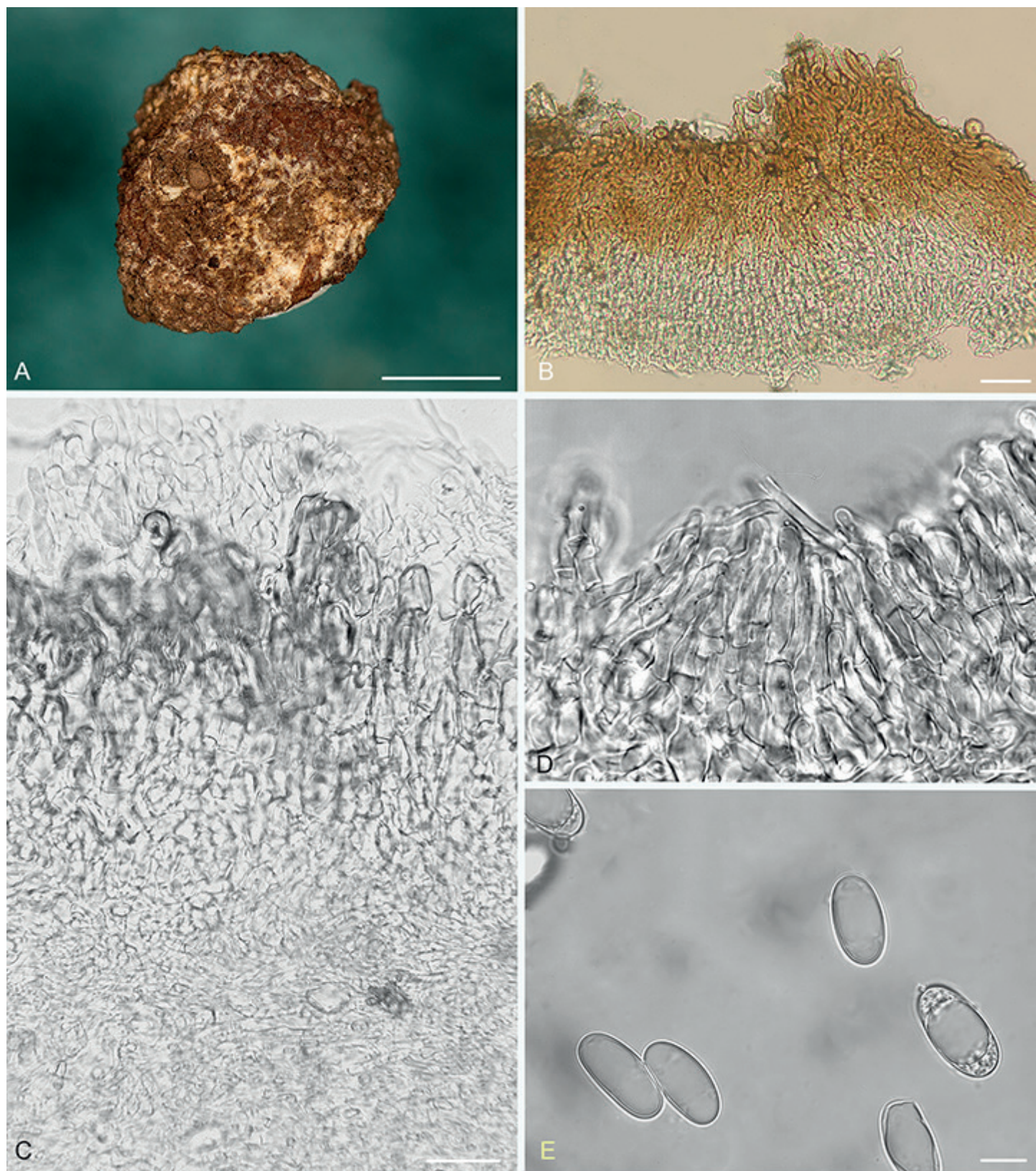
**Additional materials examined:** USA, Oregon, Jackson Co., Bureau of Land Management, Cascade-Siskiyou National Monument, Chinquapin Mtn., T39S R3E section 35, 42°8.3'N 122°26.0'W, 1860 m, under *Abies concolor*, 17 Jun. 2010, C. Hill (OSC151391), GenBank accession number ITS: JN022501; Josephine Co., Bureau of Land Management, Grants Pass Resource Area, French Flat, T40S R8W, section 15, NAD83 N447555 E4660130, alt. 450 m, under *Pinus ponderosa*, *Quercus garryana*, 29 May 2011, D. Southworth 1832 (OSC151390).

**Commentary:** *Balsamia cascadenis* is morphologically similar to *B. alba* with a white hyphal coating that is “dirty white”. However, ITS sequences support its separation as a new species. The diverse nature of the white hyphae on the peridium supports the interpretation that the white mycelium is a parasitic fungus or group of fungi. Two of the hyphal types resembled those seen on *B. alba* at the type locality. In addition, there was a third hyphal type (brown with clamp connections). Attempts to sequence alien mycelia were not successful.

***Balsamia filamentosa*** Harkn., *Proc. Calif. Acad. Sci. Bot.* 1: 264. 1899, emend. D. Southw., J.L. Frank & Castellano, Fig. 4.

**Diagnosis:** *Balsamia filamentosa* is similar to *B. magnata*, but with warts slightly wider, more rounded, and browner, and with slightly larger spores (25.6 × 12.8 μm; Q = 2.0) than *B. magnata* (24.5 × 12.1 μm; Q = 2.0). *Balsamia filamentosa* differs from *B. limuwensis* which has shorter and narrower spores (24.1 × 12.1 μm; Q = 2.0) and from *B. setchellii* which has slightly shorter spores with a wider aspect (24.6 × 13.1 μm; Q = 1.9).





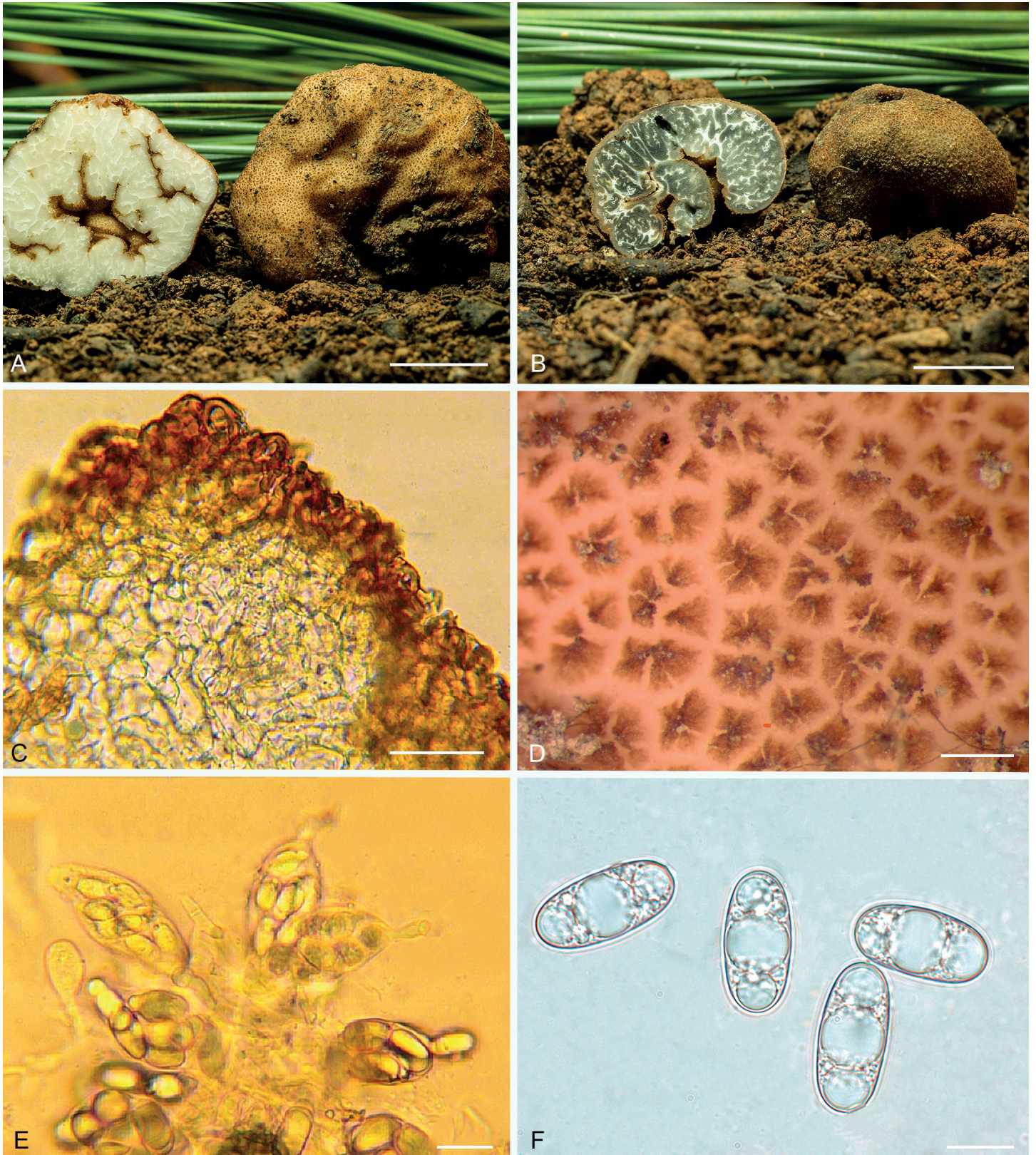
**Fig. 3.** *Balsamia cascadensis*. **A.** Dried ascoma showing peridial surface partially covered by white alien mycelium (holotype; OSC 151392). **B.** Peridium showing outer wart layer and inner peridial layer (OSC 151390). **C.** Peridium showing outer wart layer covered by alien hyphae, and inner peridial layer (OSC 151392). **D.** Paraphyses lining glebal cavity (OSC 151392). **E.** Ascospores (OSC 151390). Bars A = 5 mm, B = 25  $\mu$ m, C–E = 10  $\mu$ m.

*Isotype:* **USA**, California, Placer Co., Auburn, under *Heteromeles arbutifolia*, Feb. 1898, *H.W. Harkness* 236 (OSC157736).

*Epitype:* **USA**, California, Placer Co., Auburn State Recreation Area, Old Foothill Blvd. at Mammoth Bar OHV Area, downhill from main road near fence line, 38°55.3'N 121°0.9'W, 280 m,

under *Ceanothus*, *Pinus ponderosa*, *P. sabiniana*, 11 Dec. 2016, *M. Castellano* (designated here OSC157716, MBT381871), GenBank accession number ITS: KY706388.

*Original type description* (Harkness 1899, p. 265): “Large, oblong or irregularly globose, color ferruginous brown; densely



**Fig. 4.** *Balsamia filamentosa*. **A.** Ascoma showing peridial surface, gleba, and peridium in section (epitype; OSC 157716). **B.** Parasitized ascoma showing peridial surface, gleba and peridium (OSC 157717). **C.** Peridium with outer wart layer with red-brown outer cells and hyaline inner cells (OSC 131291). **D.** Peridial surface showing wart arrangement (OSC 157713). **E.** Immature asci (OSC 131291). **F.** Ascospores (OSC 157713). Bars A–B = 10 mm, C = 50  $\mu$ m, D = 300  $\mu$ m, E = 30  $\mu$ m, F = 10  $\mu$ m.

verrucose; gleba filamentous; veins irregular; parenchyma pellucid; asci ellipsoid, markedly stipitate,  $42 \times 24 \mu$ ; spores cylindrical, having two to three oil globules,  $18 \times 12 \mu$ . The oil globules disappear from view when placed in a medium denser than that of water".

*Emended description:* *Ascomata* sequestrate, subglobose,  $10 \times 10$ – $30$  mm, invaginated at apex; peridial warts reddish brown to brown; warts rounded, sometimes ribbed,  $80$ – $100 \times 200$ – $520 \mu$ m; gleba white, chamber walls appressed; a few appressed channels open through the peridium (*venae externae*) at the

invaginations. *Peridium* outer pigmented layer 2–4 cells thick, of brown, isodiametric cells, 20–25  $\mu\text{m}$  wide, or elongate cells,  $33 \times 15 \mu\text{m}$ , with walls 2–4 (–6.5)  $\mu\text{m}$  thick; occasional surface cells protrude as pointed cystidium-like cells, up to 25  $\mu\text{m}$  from peridial surface, with walls 1  $\mu\text{m}$  thick; outer pigmented layer intergrades to an inner hyaline layer of isodiametric cells, 10–15  $\mu\text{m}$  wide or elongate cells  $25\text{--}33 \times 11\text{--}17 \mu\text{m}$ , with walls 1  $\mu\text{m}$  thick, with scattered brown cells, up to 27  $\mu\text{m}$  wide, with walls 2  $\mu\text{m}$  thick. *Gleba* of branched, interwoven hyphae, 4–5  $\mu\text{m}$  wide; when immature, chambers lined with paraphyses, up to 66  $\mu\text{m}$  long; at maturity, opposing chamber walls pressed against each other. *Asci* hyaline, ellipsoid to citriform,  $55\text{--}65 \times 30\text{--}35 \mu\text{m}$ , 8-spored, with walls 1  $\mu\text{m}$  thick. *Ascospores* hyaline, smooth, ellipsoid,  $25.6 \times 12.8 (24\text{--}28 \times 11.5\text{--}14.5) \mu\text{m}$ ,  $Q = 2.0 (1.6\text{--}2.4)$ , containing 1–3 guttules.

**Habit, habitat, and distribution:** Hypogeous in mixed conifer-hardwood stands with *Arbutus menziesii*, *Pinus ponderosa*, *P. sabiniana*, *Pseudotsuga macrocarpa*, *Ps. menziesii*, *Quercus douglasii*, *Q. kelloggii*, and *Q. wislizeni* in foothills of the Sierra Nevada and Klamath Mountains in California and in the Pinal Mountains of Arizona.

**Additional materials examined:** **USA:** Arizona, Gila Co., Pinal Mountains, Signal Peak, 2135 m, under *Arctostaphylos*, *Pseudotsuga macrocarpa*, *P. menziesii*, 8 Mar. 1991, J.M. Trappe 11706 (OSC49779), GenBank accession number ITS: KU170030; California, Los Angeles Co., Angeles National Forest, Upper Shake campground, under *Pseudotsuga macrocarpa*, *Quercus*, 14 Mar. 1993, K. Schaffroth (OSC131291), GenBank accession number ITS: KM115877; Placer Co., Auburn State Recreation Area, Old Foothill Blvd. at Mammoth Bar OHV Area, downhill,  $38^{\circ}55.3'N$   $121^{\circ}0.9'W$ , 350 m, under *Ceanothus*, *Pinus ponderosa*, *P. sabiniana*, 11 Dec. 2016, M. Castellano (OSC157713), GenBank accession number ITS: KY706386, something is missing here (OSC157717); along Foothill Blvd. 0.8 km from Upper Lake Clementine Day Use area, at trailhead entering meadow, 540 m, under *Ceanothus*, *Pinus sabiniana*, *Pseudotsuga menziesii*, *Quercus*, 11 Dec. 2016, M. Castellano (OSC157710), GenBank accession number ITS: KY706383; San Diego Co., Fry Creek campground near Palomar observatory, 1585 m, under *Pinus*, *Quercus*, 3 Mar. 1990, G. Menser (OSC130677); Trinity Co., Shasta-Trinity National Forest, Hwy 299 west of Willow Creek, Burnt Ranch roadside west of Weaverville, 10 Dec. 2014, S. Loring, L. Sims, (OSC149794); Yuba Co., Sierra Research Station, near Englebright Reservoir, Koch Natural Area, 685 m, under *Pinus sabiniana*, *Quercus douglasii*, *Q. wislizeni*, 5 Feb. 2002, M.E. Smith SRC396 (OSC80893), GenBank accession number ITS: KU170028; Oregon, Douglas Co., Azalea exit I-5, under *Pseudotsuga menziesii*, 26 Nov. 1996, M. Amaranthus (OSC131299); Josephine Co., 605 m, under *Arbutus menziesii*, *Pseudotsuga menziesii*, *Quercus kelloggii*, 13 Dec. 2009, S. Loring 5470 (OSC140476), GenBank accession number ITS: KP859277.

**Commentary:** *Balsamia filamentosa*, *B. limuwensis*, and *B. setchellii* form a species complex distinguished by ITS sequences. Morphological separation of these species requires measurements of at least 20 spores to get average spore dimensions with which to compare species. In original descriptions (Harkness 1899, Fischer 1907), spore lengths given for *B. filamentosa* ( $18 \times 12 \mu\text{m}$ ) were shorter than those given for *P. setchellii* ( $21\text{--}28 \times 10\text{--}12 \mu\text{m}$ ). However, spore dimensions given for *B. filamentosa* were similar to those for *B. magnata*, which were also shorter than those of modern collections (Harkness 1899). Thus the Harkness spore measurements

appear imprecise. Morphological characters combined with ITS barcodes (Fig. 1) supported the distinction between *B. filamentosa* and *B. magnata* made by Harkness (1899) and rejected the synonymy of *B. filamentosa* with *P. magnata* as proposed by Gilkey (1916).

***Balsamia latispora*** D. Southw., J.L. Frank & Castellano, *sp. nov.* MycoBank MB807885. Fig. 5.

**Etymology:** *Lati* (Latin) = wide + *spora*, referring to the relatively wide spores.

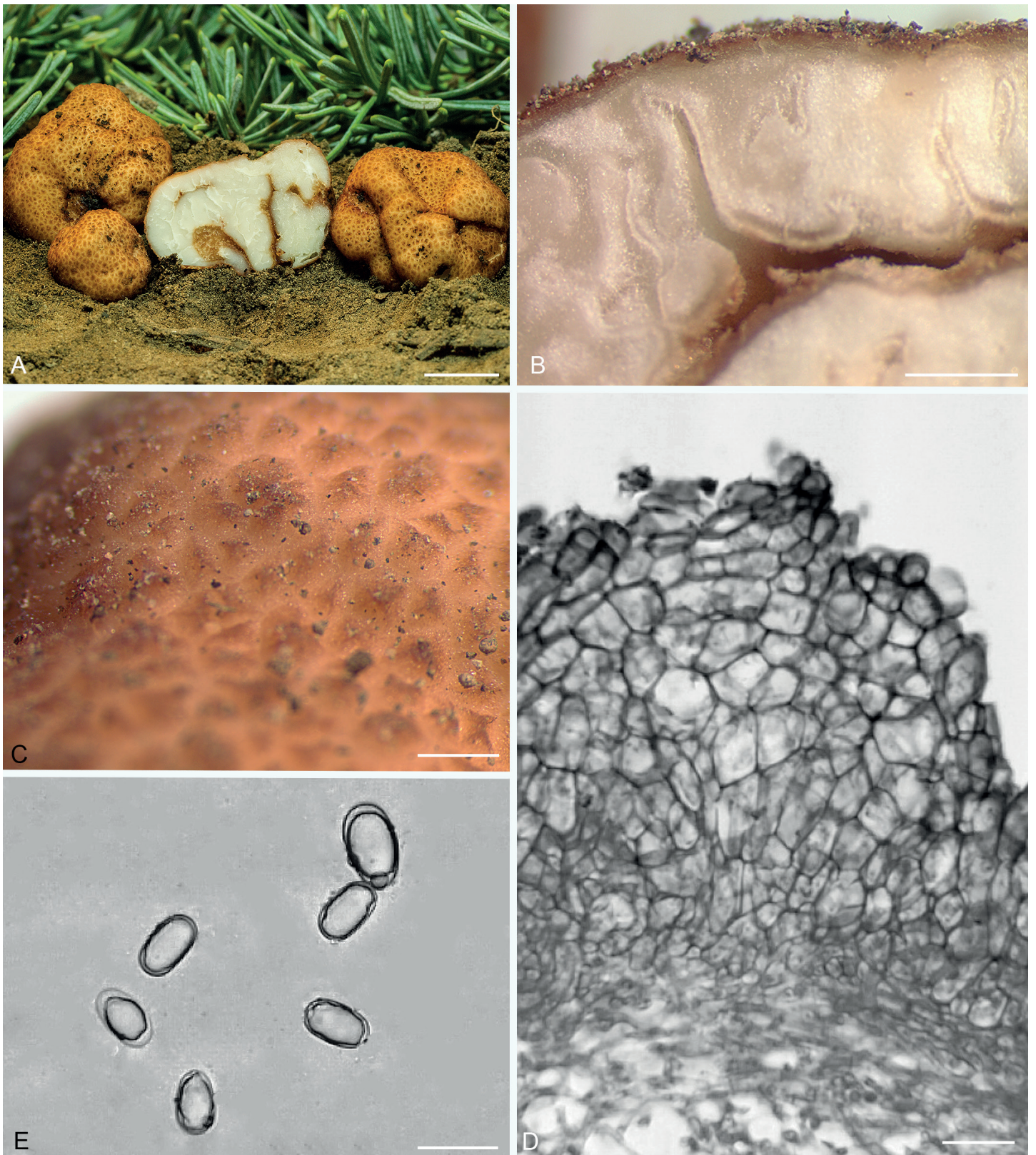
**Diagnosis:** *Balsamia latispora* is distinguished by low warts and broad spores,  $13.7 (11.5\text{--}15.5) \mu\text{m}$  wide, and the lowest length-to-width ratio ( $Q = 1.7$ ) of any *Balsamia* species in western North America.

**Holotype:** **USA**, Oregon, Jefferson Co., Deschutes National Forest, Jack Creek headwaters,  $44^{\circ}29' N$   $121^{\circ}42' E$  940 m, under *Abies grandis*, 21 May 1998, B. Fondrick, T. O'Dell (OSC66585), GenBank accession numbers ITS: EU837226, KF983487.

*Ascomata* sequestrate, subglobose, irregularly lobed and invaginated  $7\text{--}10 \times 10\text{--}25 \text{ mm}$ ; peridial warts red to reddish-brown, flat to rounded,  $100\text{--}250$  tall  $\times$   $750 \mu\text{m}$ , some tripartite; pale reddish between warts; gleba white to pale yellow; chambers mostly laterally compressed, partly open, occasional chambers open to exterior in deep invaginations. *Peridium* wart surface uneven with rounded cells or pointed cystidium-like cells,  $15 \times 18 \mu\text{m}$ ; wart outer layer 2–3 cells thick, of brown cells,  $15\text{--}20 \mu\text{m}$  wide, with walls 2–3  $\mu\text{m}$  thick, grading into hyaline cells,  $15\text{--}30 \mu\text{m}$  wide, with walls 1  $\mu\text{m}$  thick; space between warts with 1–2 pigmented cell layers over 1–2 hyaline inflated cell layers, subtended by a layer  $30\text{--}40 \mu\text{m}$  thick of isodiametric cells,  $4\text{--}6 \mu\text{m}$  in wide, grading into the trama. *Gleba* of interwoven hyphae (*textura intricata*),  $4\text{--}6 \mu\text{m}$  wide; chambers lined with a weft of irregularly branched, septate hyphae,  $3\text{--}5 \mu\text{m}$  wide. *Asci* hyaline, subglobose to citriform,  $45 \times 35 \mu\text{m}$ , 8-spored, stalked. *Ascospores* hyaline, smooth, ellipsoid,  $22.0 \times 13.1 (19\text{--}25.5 \times 11\text{--}15.5) \mu\text{m}$ ,  $Q = 1.7 (1.5\text{--}2.0)$ , containing one guttule.

**Habit, habitat, and distribution:** Hypogeous under *Abies grandis*, *A. x shastensis*, *A. procera*, *Pinus jeffreyi*, *P. ponderosa*, *Pseudotsuga macrocarpa*, *Ps. menziesii*, *Quercus garryana*, and *Q. kelloggii*, widespread from the Sierra Nevada range in southern California through the Klamath Mountains in California and Oregon, and the central Cascade Range of Oregon and in Canada.

**Additional materials examined:** **USA**, California: El Dorado Co., Ice House Resort, 1645 m, under *Pseudotsuga menziesii*, 15 May 1990, C. Thayer (OSC49763), GenBank accession number ITS: KU170033; Placer Co., Auburn Dec. c. 1898, H.W. Harkness 214b (OSC157742); under *Ceanothus*, in sandy soil, Dec. 1898, H.W. Harkness 220b (OSC130980); Placer Co., Auburn, among shrubs under vegetable mould, Dec. 1898, H.W. Harkness 222 (OSC157743); west of Yuba Gap I-80 exit 160,  $39^{\circ}18.6'N$   $120^{\circ}37.8'W$ , 1920 m, under *Abies concolor*, *Pinus jeffreyi*, *P. ponderosa*, *Quercus*, 27 June 1996, M. Castellano (OSC131298); Riverside Co., Hwy 74 at McGaugh Rd., under *Pseudotsuga macrocarpa*, *Quercus agrifolia*, *Q. dumosa*, *Q. wislizeni*, 24 May 2011, A. Mujic CA11-021 (OSC148026), GenBank accession number ITS: MF098665; San Bernardino Co., San Bernardino Mountains, Camp Seeley, under



**Fig. 5.** *Balsamia latispora*. **A.** Ascomata showing peridial surface, gleba, and peridium in section (OSC 157702). **B.** Ascoma in section showing peridium and gleba (OSC 157702). **C.** Peridial surface showing wart arrangement (OSC 157702). **D.** Peridial outer warty layer (OSC 131289). **E.** Ascospores (holotype; OSC 66585). Bars A = 10 mm, B = 1 mm, C = 250  $\mu$ m, D = 30  $\mu$ m, E = 20  $\mu$ m.

*Pinus ponderosa*, *Pseudotsuga macrocarpa*, *Quercus chrysolepis*, 29 May 1983, D. Hayward (OSC130676), GenBank accession number ITS: KU170032; Shasta Co., Whiskeytown National Recreation Area, T32N R7W section 25, under *Pinus attenuata*, *Quercus kelloggii*, 11 Mar. 2008, J.L. Frank, Southworth 1161 (OSC157733); (OSC157732); (OSC157730);

(OSC157731); 14 Apr. 2008, J.L. Frank, (OSC157734); Sierra Co., Plumas National Forest, San Francisco State University field campus, Hwy 49, under *Abies concolor*, *A. magnifica*, 15 Jun. 1984, Y. Wang (OSC130672), GenBank accession number ITS: KU170036; Wild Plum campground, before bridge near entrance downhill towards main road,

39°34.1'N 120°36.2'W, 1355 m, under *Abies concolor*, *Pseudotsuga menziesii*, *Quercus kelloggii*, 13 Jun. 1987, *M. Amaranthus* (OSC48551), GenBank accession number ITS: KU170037; Tahoe National Forest, Yuba Pass campground, 2045 m, under *Abies concolor*, *A. magnifica*, *Pinus contorta*, 9 Jun. 1987, *M. Castellano* (OSC48552); Siskiyou Co., Klamath National Forest, Carter Meadows summit 1890 m, under *Abies concolor*, *A. magnifica* var. *shastensis*, 1 Oct. 1983 *J.M. Trappe 7574* (OSC131289), GenBank accession number ITS: KF983486; Shasta-Trinity National Forest at Harris Springs Fire Station, 41°27.4'N 121°47.1'W, 1510 m, under *Abies concolor*, *P. ponderosa*, 18 May 2016, *M. Castellano* (OSC157705); along Forest Service road 15, 1 km from upper entrance to Harris Springs campground, north side of road, 41°28.1'N 121°46.9'W, 1565 m, under *Abies concolor*, *Pinus lambertiana*, *P. ponderosa*, 18 May 2016, *M. Castellano* (OSC157701), GenBank accession number ITS: KY706377; (OSC157702), GenBank accession number ITS: KY706378; (OSC157703), GenBank accession number ITS: KY706379; (OSC157706); (OSC157707); (OSC157738); (OSC157704), GenBank accession number ITS: KY706380; Oregon, Deschutes Co., Cold Springs campground, under *Pinus ponderosa*, 7 May 1970, *J.M. Trappe 2165* (OSC130671); 15 June 1975, *R. Fogel 1165* (OSC157741); Douglas Co., Umpqua National Forest, Demonstration site, Watson Falls block, T27S R3E section 2, 43°14.8'N 122°26.1'W, 945 m, under *Abies concolor*, *Pseudotsuga menziesii*, 14 May 1996, *D. Luoma 8831* (OSC123230); *D. Luoma 8852* (OSC123232); Dog Prairie block, T27S R4E section 26, 43°11.7'N 122°18.9'W, 1585 m, under *Abies procera*, *Pseudotsuga menziesii*, 28 May 1996, *D. Luoma 8894* (OSC123235); Jackson Co., west of Brown Mountain, 42°21.3'N 122°22.7'W, 1705 m, under *Abies concolor*, *Pinus ponderosa*, *Pseudotsuga menziesii*, June 1997, *D. Laury* (OSC80116), GenBank accession number ITS: KU170035; (OSC62003), GenBank accession number ITS: KU170034; China Gulch, under *Pseudotsuga menziesii*, *Quercus garryana*, 26 Jan. 2011, *C. Reha* (OSC151399), GenBank accession number ITS: KF983485; Jefferson Co., Riverside Campground, 16 km west of Sisters, 44°26.7'N 121°38.1'W, under *Pinus ponderosa*, *Pseudotsuga menziesii*, 28 May 2009, *M. Castellano* (OSC149004); Wasco Co., 5 km west of Pine Grove, under *Pinus ponderosa*, *Quercus garryana*, 2 May 1969, *J.M. Trappe 1828* (OSC27826).

**Commentary:** Sequences of the ITS separated the western North American species *B. latispora* from the European species *B. platyspora*, which also has broad spores. *Balsamia platyspora* was described as “globose, about the size of a horse-bean, rufous, with the interstices of the minute warts of a light yellow tint, from the exposure of the internal substance. Cells minute, sporidia at first oblong-elliptic with a large globose nucleus and a number of minute granules” (Berkeley 1844). Dimensions of *B. platyspora* spores have been reported as 20–24  $\mu\text{m}$ ,  $Q = 1.6$ – $1.7$  (Montecchi & Sarasini 2000) and 21–26  $\times$  13–15  $\mu\text{m}$ ,  $Q = 1.5$ – $1.7$  (Pegler *et al.* 1993). Two California collections identified by Harkness as *B. platyspora* (H222) and *B. polysperma* (H220b) have similar ascospores [20–24  $\times$  9–11(–13)  $\mu\text{m}$  and 21  $\times$  13  $\mu\text{m}$ , respectively], minute warts, and chambers and canals partly filled with paraphyses suggesting that these collections are *B. latispora*.

*Balsamia latispora* may be a species complex. However, the difference in base pairs among branches is not consistently  $> 2\%$ . Thus there is not sufficient support within *B. latispora* to merit splitting.

***Balsamia lazyana*** Trappe, D. Southw. & Amaranthus, *sp. nov.* MycoBank MB807880. Fig. 6A–D.

**Etymology:** *lazyana* refers to the type locality, the Lazy A Ranch in the Applegate Valley near Grants Pass, Oregon.

**Diagnosis:** The combination of minute warts, open glebal chambers, and narrow spores ( $Q = 2.3$ ) distinguishes *B. lazyana* from other *Balsamia* species.

**Holotype:** USA, Oregon, Josephine Co., Ruch, 12108 North Applegate Rd., Missouri Flat, Lazy A Ranch 42°19'N 123°15'W, under *Pinus ponderosa* and *Pseudotsuga menziesii*, 30 Nov. 1996, *M. Amaranthus* (OSC130670), GenBank accession numbers ITS: KF983488, KM115879.

**Ascomata** sequesterate, irregularly lobed to indented and reniform, 6–11  $\times$  4–5 mm; peridial warts orange-red to reddish-brown, reddish between warts, flat or rounded to acute, 250–400  $\mu\text{m}$  wide  $\times$  300  $\mu\text{m}$  tall, often compound with 3–4 sections; surface of immature specimens mostly smooth with widely spaced warts, 100  $\times$  100  $\mu\text{m}$ ; gleba white to pale tan; chambers open, 0.5–1 mm wide narrowing at maturity; chamber openings to exterior rare. **Peridium** wart surface uneven with rounded cells, 25  $\times$  30  $\mu\text{m}$  (Fig); wart outer layer of reddish-brown cells, 2–3 cells thick, wide, with walls 2–4(–9)  $\mu\text{m}$  thick, spheroidal to elongate, grading into yellow to hyaline, isodiametric cells, 25–30  $\mu\text{m}$ , with walls 1  $\mu\text{m}$  thick; inner layer of interwoven, hyaline hyphae, 10  $\mu\text{m}$  wide, with walls 1  $\mu\text{m}$  thick, grading into gleba matrix. **Gleba** of densely interwoven hyphae (*textura intricata*), 3–4  $\mu\text{m}$  wide; chambers lined with a palisade of uniseriate paraphyses, 3–4  $\times$  50  $\mu\text{m}$ , with rounded or clavate tips; paraphyses tightly aligned in immature specimens becoming disorganized at maturity. **Asci** hyaline, elongate to citriform, 60  $\times$  40  $\mu\text{m}$ , stalked up to 20  $\mu\text{m}$  long, 8-spored. **Ascospores** hyaline, smooth, ellipsoid, 23.5  $\times$  10.1 (19.5–27  $\times$  8–11.5)  $\mu\text{m}$ ,  $Q = 2.3$  (2.0–2.7), 1(–3) guttules.

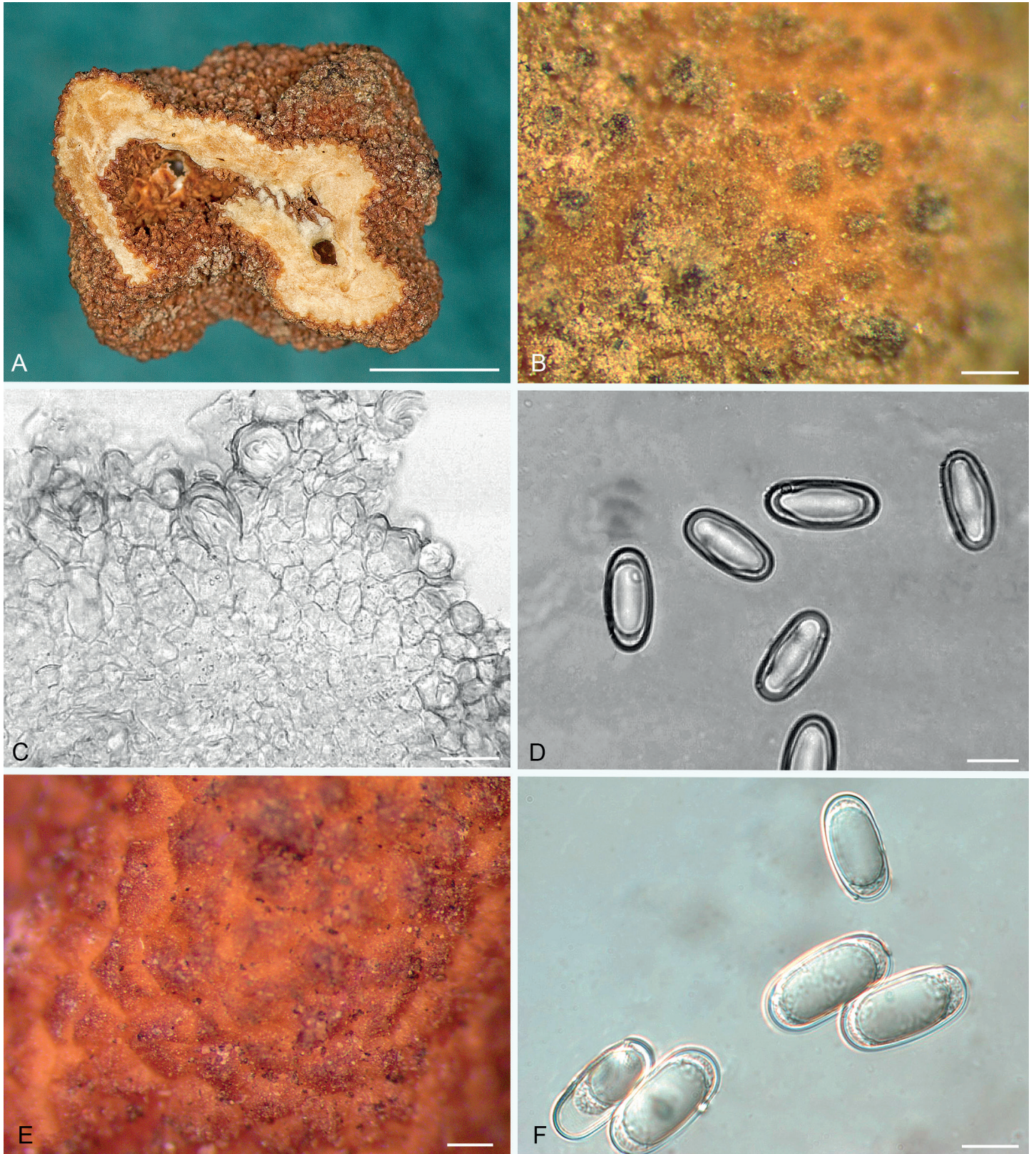
**Habit, habitat, and distribution:** Hypogeous under variously mixed conifer-hardwoods: *Arbutus menziesii*, *Arctostaphylos*, *Pinus ponderosa*, *Pseudotsuga menziesii*, *Quercus garryana*, and *Q. kelloggii*.

**Additional materials examined:** USA, California, Sonoma Co., Lake Ralphine, under *Arctostaphylos*, 20 Mar. 1977, *R. Heblack* (OSC131292); Oregon, Josephine Co., north of Selma, under *Quercus kelloggii*, 26 Oct. 1971, *J.M. Trappe 2969* (OSC130665); Ruch, 12108 North Applegate Rd., Missouri Flat, Lazy A ranch, 415 m, under *Arbutus menziesii*, *Pinus ponderosa*, *Pseudotsuga menziesii*, Feb. 1988, *D. Parrish* (OSC48654); under *Arbutus menziesii*, *Pseudotsuga menziesii*, 24 Mar. 1991, *D. Arthur* (OSC130668); under *Pinus ponderosa*, *Pseudotsuga menziesii*, *Quercus garryana*, 25 Jan. 1990, *M. Amaranthus* (OSC130667); 20 Mar. 1996, *M. Amaranthus* (OSC130669), GenBank accession number ITS: KM115878.

**Commentary:** *Balsamia lazyana* has the narrowest spores (mean width 10.1  $\mu\text{m}$ ;  $Q = 2.3$ ) of any North American *Balsamia* species. *Balsamia cascadenensis* is closest in spore width to *B. lazyana* but has spores that are significantly wider (mean width 11.6  $\mu\text{m}$ ).

***Balsamia limuwensis*** D. Southw., J.L. Frank & Castellano, *sp. nov.* MycoBank MB815758. Fig. 6E–F.

**Etymology:** Limuw, the Native American Chumash name for Santa Cruz Island.



**Fig. 6.** *Balsamia lazyana* (A–D; holotype; OSC 130670) and *B. limuwensis* (E–F; holotype; OSC 13098). **A.** Dried ascoma showing peridial surface, gleba, and peridium in section. **B.** Peridial surface showing wart arrangement. **C.** Peridial outer wart layer. **D.** Ascospores. **E.** Peridial surface with warts. **F.** Ascospores. Bars A = 5 mm, B = 400  $\mu$ m, C = 30  $\mu$ m, D = 10  $\mu$ m, E = 200  $\mu$ m, F = 10  $\mu$ m.

**Diagnosis:** *Balsamia limuwensis* (spore mean = 24.1 x 12.1  $\mu$ m) is similar to *B. filamentosa* (spore mean = 25.6 x 12.8  $\mu$ m) and *B. setchellii* (spore mean = 24.6 x 13.1  $\mu$ m) but differs from them in slightly shorter and narrower spores.

**Holotype:** USA, California, Santa Barbara Co., Santa Cruz Island, 34°01'N, 119°46'W, 150 m, associated with *Pinus muricata* and *Quercus*, Mar. 2001, *L. Grubisha 1115* (OSC130981), GenBank accession number ITS: KU170027.

*Ascomata* sequestrate, subglobose, 10 × 10–20 mm, invaginated at apex; peridial warts reddish-brown to brown, yellow-brown to brown between warts; warts rounded to obtusely pointed, 75–150 × 230–380 µm; gleba white, chambers closed, chamber walls appressed. *Peridium* outer pigmented layer 2–4 cells thick, of brown, isodiametric cells, 20 µm wide, with walls 2–4 µm thick, some cells radially aligned; sparse, pointed cystidium-like cells on surface, ±8 µm wide at base, protruding up to 25 µm from surface, with walls 1 µm thick; inner layer of hyaline, isodiametric cells, 10–15 µm wide, with walls 1 µm thick; with scattered brown cells, up to 27 µm wide, with walls 2 µm thick. *Gleba* composed of loosely interwoven hyphae surrounding asci and extending into chambers as paraphyses, 3–5 µm wide, some branched. *Asci* hyaline, citriform to ovoid, 27–32 × 48–58 µm, 8-spored. *Ascospores* hyaline, smooth, ellipsoid, 24.1 × 12.1 (20–31 × 10–14.5) µm, Q = 2.0 (1.7–2.4); 1–3 guttules.

**Habit, habitat, and distribution:** Hypogeous under *Pinus muricata*, *Pinus* spp., or *Quercus agrifolia* and other low elevation coastal *Quercus* spp., at low elevations in coastal California.

**Additional materials examined:** USA, California, Marin Co., Inverness, 150 m, under *Quercus*, 13 Feb. 1989, C. Thayer (OSC49229), GenBank accession number ITS: KU170029; Santa Barbara Co., Santa Cruz Island, Pelican Bay, under *Pinus*, *Quercus*, 18 Jan. 2002, J.M. Trappe 28212 (OSC130713).

**Commentary:** ITS sequences support the separation of this species from *B. filamentosa* and *B. setchellii*.

***Balsamia magnata*** Harkn., *Proc. Calif. Acad. Sci. Bot.* 1: 264. 1899, emend. D. Southw. & Castellano, Fig. 7.

**Synonym:** *Pseudobalsamia magnata* (Harkn.) Gilkey, *Univ. Calif. Publ. Bot.* 6: 292. 1916.

**Diagnosis:** Red-brown, relatively large pointed warts, often appearing compound (tripartite) and longitudinally ribbed. *Ascospores* 20–28.5 × 10–14.5 µm; Q = 1.7–2.4.

**Isotype:** USA, California, Placer Co., Auburn, in forests, May ca. 1898, Harkness 185 (OSC 81168).

**Epitype:** USA, Oregon, Jackson Co., Bureau of Land Management, Wellington Butte 2 km below saddle, 42°17.65'N, 123°5.20'W, 840 m, under *Quercus kelloggii*, 7 May 2010, C. Reha, D. Southworth 1386 (designated here OSC151397, MBT381872), GenBank accession number ITS: KF983478.

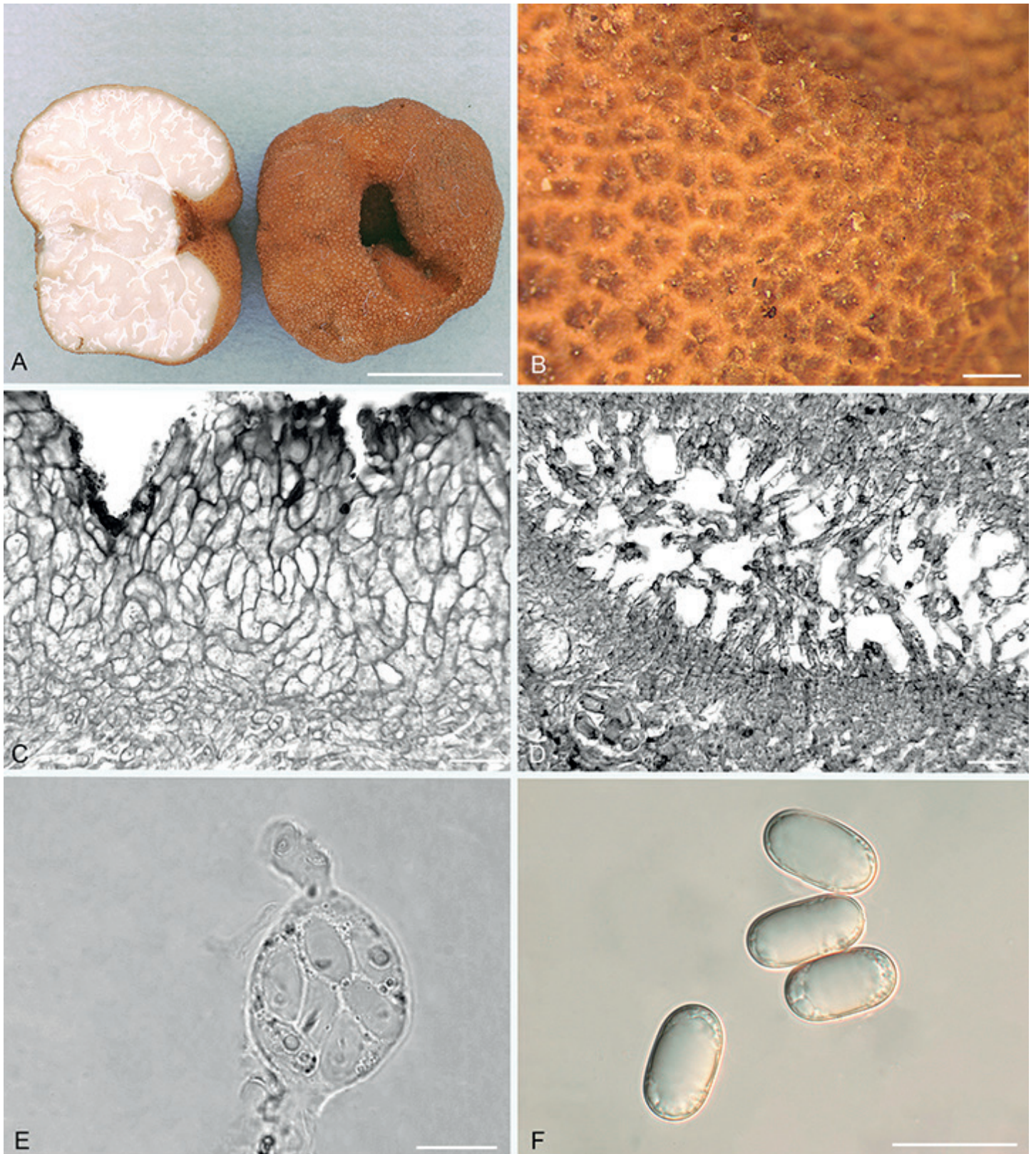
**Original type description** (Harkness 1899, p. 264): Subglobose, densely verrucose, 2.5 cm in diam., color orange red; cavity gyrose; gleba white, firm; asci subovate, 8-spored, 50 × 38 µ; spores cylindrical, containing from one to three oil globules, 18 × 8 µ".

**Emended description:** *Ascomata* sequestrate, subglobose to irregularly lobed, often deeply invaginated, 8–20 × 6–10 mm; peridial warts reddish-brown, pale reddish-brown between warts; acutely to obtusely pointed, 150–300 × 250–500 µm, often compound of 3–5 segments and longitudinally ribbed; gleba white when fresh, immature gleba becomes brown when dry; chamber walls appressed, some opening to the exterior

in deep folds. *Peridium* warty pigmented outer 2 to 4 layers of reddish-brown cells, 10 × 15 µm, with walls 4–8 µm thick, sometimes radially elongate; grading into a hyaline layer four cells thick, cells 10 × 15 µm, with walls 4 µm thick, then to an inner layer of interwoven hyphae, up to 10 µm wide, with walls 1 µm thick. *Gleba* of pseudoparenchyma (*textura angularis*) with cells 12–20 µm wide, grading to tightly to loosely interwoven hyphae (*textura intricata*), 4–6 µm wide; chambers lined with branched paraphyses, 3 µm wide × 60–100 µm long. *Asci* hyaline, obovate, 60–70 × 30–40 µm, with stalk 5 µm wide × 25 µm long with a swollen base, 8-spored. *Ascospores* hyaline, smooth, ellipsoid 24.5 × 12.1 (20–28.5 × 10–14.5) µm, Q = 2.0 (1.7–2.4), 1(–3) guttules.

**Habit, habitat, and distribution:** Hypogeous commonly under *Pseudotsuga menziesii*, but also in conifer-hardwood forests: *Abies concolor*, *Alnus*, *Arbutus menziesii*, *Pinus coulteri*, *P. contorta* var. *contorta*, *P. jeffreyi*, *P. muricata*, *P. ponderosa*, *P. sabiniana*, and *Quercus agrifolia*, *Q. chrysolepis*, or *Q. kelloggii*, widespread in mountains of the PNW, California, Montana and Arizona.

**Additional materials examined:** USA, Arizona, Coconino Co., Kaibab Plateau, Jacob Lake, under *Pinus ponderosa*, 6 June 1981, J. States (OSC40562); California, Butte Co., near Inskip, 1370 m, under *Abies concolor*, *Pseudotsuga menziesii*, *Quercus kelloggii*, 16 June 1980, R. Molina (OSC131286); El Dorado Co., University of California, Blodgett forest, in mixed forest, 6 May 1983, J.M. Trappe 7297 (OSC131288); Fresno Co., Sierra National Forest, Ross Creek Drainage, Turtle Creek, 36°54'N 119°6'W, 1465 m, under *Abies concolor*, *Pinus lambertiana*, *P. ponderosa*, 25 June 1997, B. Feno 13 (OSC130693); Los Angeles Co., Angeles National Forest, Angeles Forest Hwy milepost 14.21, Baughman Spring, 34°21.4'N 118°6.6'W, 1210 m, under *Pinus coulteri*, *Populus*, 24 Apr. 1996, M. Castellano (OSC130692); near Upper Shake campground, under *Pseudotsuga macrocarpa*, *Quercus*, 18 Apr. 1993, M. Castellano (OSC130687); Marin Co., Mill Valley, N.L. Gardner, Gilkey 1190 (OSC130977, UC46); 28 Dec. 1902, W. Setchell (OSC130982, UC33); Mariposa Co., Yosemite National Park, 26 June 2006, M. Meyer, M.E. Smith 691 (FLAS-F-58858); Mendocino Co. Mendocino National Forest, Croney Ridge 1340 m, under *Abies concolor*, *Pinus ponderosa*, *Pseudotsuga menziesii*, *Quercus kelloggii*, 8 May 2014, N. Siegel, J.L. Frank 3324, GenBank accession number ITS: KU170038; Napa Co., Pope Valley, Cleary Reserve, under *Pinus*, *Quercus*, 19 Dec. 1967, D. Largent 3490 (OSC157737); Placer Co., Auburn, H.W. Harkness 221b (OSC157739); under shrubby thickets, Dec. 1898, H.W. Harkness 231; Wire Bridge, under *Ceanothus*, May c. 1898, H.W. Harkness, 185 (OSC81168 – isotype); Plumas Co., Lassen National Forest, Jennie Springs Study site, 40°24.2'N 121°5.3'W, 1850 m, under *Abies concolor*, 9 June 1994, J. Waters (OSC58431); 14 June 1994, J. Waters (OSC58456); Riverside Co., San Bernardino National Forest, Hwy 243, milepost 15.50, under *Arctostaphylos*, *Pinus attenuata*, *P. coulteri*, *Quercus agrifolia*, 24 Apr. 1995, M. Castellano (OSC61547); San Francisco Co., sand dunes, under *Ceanothus*, 23 Mar. 1905, N.L. Gardner, H.W. Harkness 280 (OSC130979); Santa Clara Co., Alma, Tevis Ranch, under *Pinus*, 14 Jan. 1921, H.E. Parks (OSC146628); Shasta Co., I-5 Exit 707 in median strip, headed north, at state transportation property turnoff, 40°56.2'N 122°25.1'W, 395 m, in disturbed soil and gravel and under *Pinus ponderosa*, *Pseudotsuga menzeisii*, 12 Dec. 2016, M. Castellano (OSC157718), GenBank accession number ITS: KY706389; Stanislaus Co., Minnear Camp, Del Puerto Creek rd., milepost 11.8, under *Pinus sabiniana*, *Quercus wislizeni*; 28 Mar. 1985, D. Luoma (OSC46919);



**Fig. 7.** *Balsamia magnata*. **A.** Ascogonium showing peridial surface, gleba, and peridium in section (OSC 56611). **B.** Peridial surface showing wart arrangement (OSC 130724). **C.** Peridium showing outer wart layer and inner peridial layer (OSC 130724). **D.** Glebal opening with scattered hyphae (OSC 131286). **E.** Immature ascus with stipitate base and capitate apex (OSC 130724). **F.** Ascospores (OSC 151395). Bars A = 10 mm, B = 500  $\mu$ m, C = 15  $\mu$ m, D = 25  $\mu$ m, E = 20  $\mu$ m, F = 25  $\mu$ m.

(OSC131290); Tuolumne Co., Stanislaus National Forest, Evergreen Rd., 1370 m, under *Pinus ponderosa*, 28 Apr. 1991, C. Thayer (OSC130691); Montana, Gallatin Co., Bozeman, Wiegand Garden, 30 June 1958, F.B. Cotner (OSC146631); Oregon, Benton Co., Oregon State University McDonald Forest, Soap Creek, 215 m, under *Pseudotsuga menziesii*, 8

Jan. 1983, D. Grandorff, R. Housen (OSC41111); Oak Creek gate, 180 m, under *Pseudotsuga menziesii*, 18 Apr. 1976, B. Ames (OSC130724); under *Pseudotsuga menziesii*, 14 Nov. 1982, P. Rawlinson (OSC131287); Philomath, Evergreen Rd., under *Pseudotsuga menziesii*, 4 Jan. 1992, F. Morris (OSC56611); Columbia Co., Scappoose, 105 m, under *Alnus*,



*Pseudotsuga menziesii*, 8 Nov. 1969, H & R Oswald (OSC130663), GenBank accession number ITS: KF983480; west of Scappoose, 105 m, 29 Oct. 1971, D. & C. Goetz, H. & R. Oswald (OSC131235); near Scappoose, 105 m, 20 Oct. 1972, H. & R. Oswald (OSC131237); Douglas Co., Bureau of Land Management, Beatty Creek Research Natural Area, near reference point 51050, under *Arbutus menziesii*, *Pinus jeffreyi*, 26 Oct. 1997, J.M. Trappe 21422 (OSC61685); under *Pseudotsuga menziesii*, 24 Nov. 1997, G. Palfner (OSC60785); (OSC60841); upper Beatty Creek, under *Arbutus menziesii*, *Pinus jeffreyi*, 26 Oct. 1997, J.M. Trappe 21442 (OSC61680); North Bank Habitat Management Area, under *Abies grandis*, *Pseudotsuga menziesii*, *Tsuga heterophylla*, 2 Dec. 1997, J.M. Trappe 22328 (OSC61681); Jackson Co., Butte Falls R32S T2E sec20, under *Quercus garryana*, 9 Apr. 2015, S. Loring 13911 (OSC149590); Cascade-Siskiyou National Monument, Mariposa Reserve, under *Pinus ponderosa*, *Pseudotsuga menziesii*, *Quercus garryana*, 8 June 11, J.L. Frank (OSC151398), GenBank accession number ITS: KF983477; Cove Rd., under *Pseudotsuga menziesii*, *Quercus garryana*, 10 June 2011, D. Southworth 1860 (OSC151395), GenBank accession number ITS: KF983481; Heppsie Mountain, under *Abies concolor*, 1 June 2010, J.D. Scelza (OSC151394); Phoenix, 42°15.6'N 122°53.3'W, 625 m, under *Arbutus menziesii*, *Quercus*, *D. Laury* (OSC81162); Josephine Co., Deer Creek Center, under *Pinus ponderosa*, *Quercus garryana*, 7 Jan. 2007, S. Gladish (OSC151396); Ruch, 12108 North Applegate Rd., Missouri Flat, Lazy A ranch, 415 m, under *Arbutus menziesii*, *Pseudotsuga menziesii*, 4 Jan. 1987, D. Parrish (OSC48655); 2 Dec. 1995, D. Arthur (OSC49789); 24 Mar. 1991, J.M. Trappe 17560 (OSC55429); Lane Co., Willamette National Forest, H.J. Andrews Experimental Forest, stand L104, 44°13.5'N 122°12'W, 625 m, under *Pseudotsuga menziesii*, *Tsuga heterophylla*, 21 May 1992, D. Luoma 1181 (OSC56959); Winberry Park, under *Cornus*, *Pseudotsuga menziesii*, 23 Dec. 1973, H. & B. Pruitt (OSC40204); under *Pseudotsuga menziesii*, 24 Dec. 1975, H. Pruitt (OSC131285); Fall Creek Dam, under *Pseudotsuga menziesii*, mixed forest, 2 Dec. 1971, H. & B. Pruitt (OSC131236); Linn Co., Brownsville, Kirk drive, 120 m, under *Pinus*, *Pseudotsuga menziesii*, 3 Dec. 1988, N. Wedam (OSC49120); Marion Co., Willamette National Forest, Detroit, under *Pseudotsuga menziesii*, *Quercus garryana*, 4 May 2011, J.L. Frank 1943 (OSC15139), GenBank accession number ITS: KF983479; Polk Co., Silver Falls State Park, 5 Nov. 1926, S. Zeller 6994 (OSC146629); Wasco Co., Sunflower Flat, southeast of Bear Springs Ranger Station, 2970 m, under *Pinus ponderosa*, *Quercus garryana*, 3 May 1969, J.M. Trappe 1827 (OSC27827); Yamhill Co., Yamhill River, Flying M Ranch, under *Pseudotsuga menziesii*, 2 Mar. 1985, H. Pavelek (OSC130674).

**Commentary:** Ascospores from the isotype (Harkness 185) measured 19–29 × 10–14.5 μm, Q = 1.4–2.0 and thus were larger than originally reported (18 × 8 μm) in an unknown mounting medium. Gilkey (1916, 1939, 1954) reported ascospore dimensions of 20–24 × 12–14 μm for the type collection. Warts from the type specimen measured 300–400 μm wide × 250–500 μm tall.

***Balsamia nigrans*** Harkn., *Proc. Calif. Acad. Sci. Bot.* **1**: 264. 1899, emend. D. Southw. & Castellano, Fig. 8.

**Synonyms:** *Pseudobalsamia magnata* var. *nigrens* (Harkn.) Gilkey, *Univ. Calif. Publ. Bot.* **6**: 294. 1916.

*Pseudobalsamia nigrens* (Harkn.) Gilkey, *North American Flora* **2**: 18. 1954.

**Diagnosis:** Black to dark reddish brown peridium composed of warts that are 250–500 × 300–600 μm, pointed, often longitudinally ribbed. Spore means 25.4 × 13.2 μm, Q = 1.9.

**Isotype:** USA, California, Placer Co., Auburn, beneath *Ceanothus*, May, ca. 1898, Harkness 180 (OSC 38607).

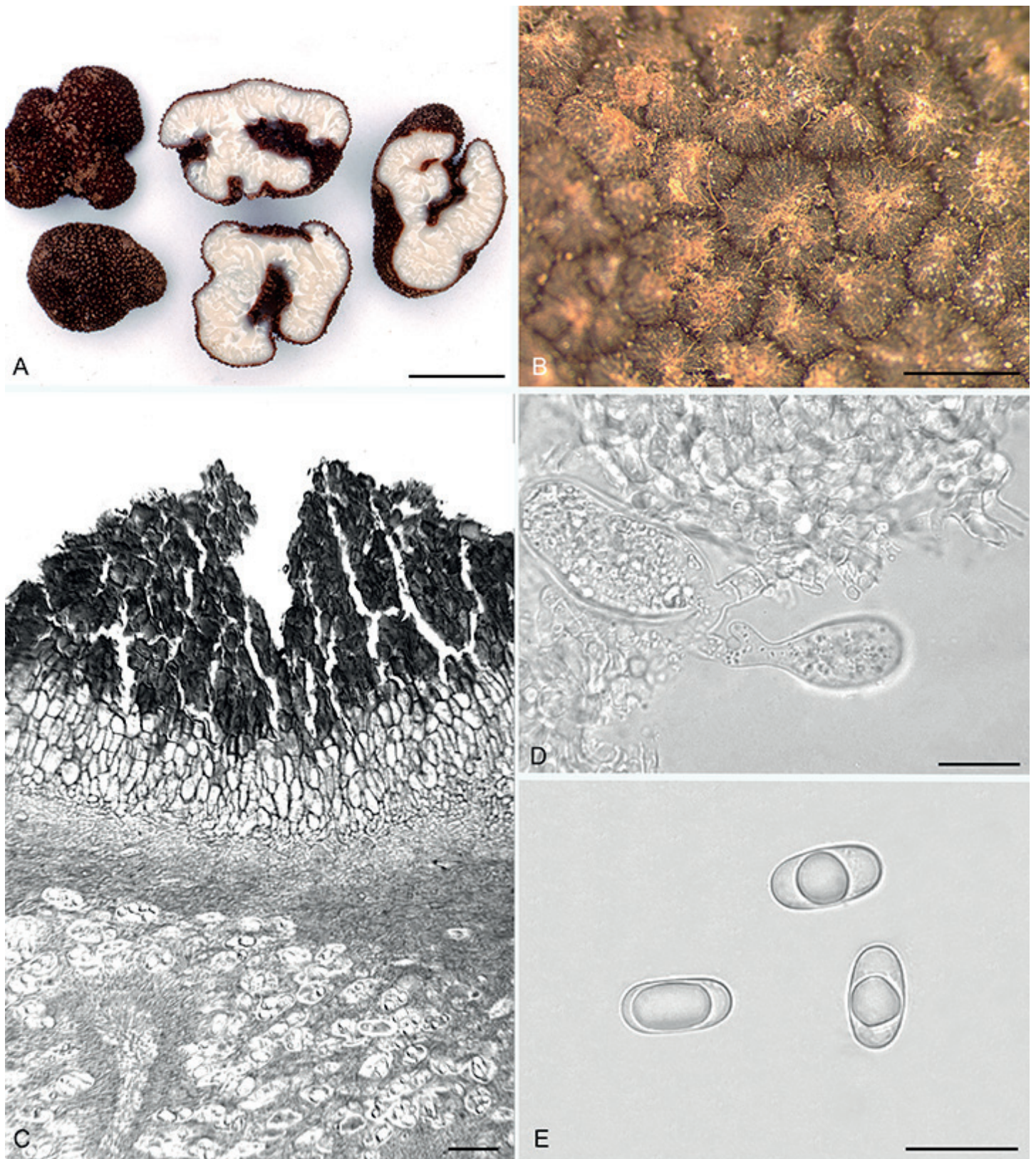
**Epitype:** USA, California, Fresno Co., Sierra National Forest, Ross Creek drainage, Turtle Creek, 36°57'N, 119°6'W, 1465 m, under *Abies concolor*, *Pinus lambertiana*, *P. ponderosa*, 25 Jun. 1997, L. Criley (designated here OSC130700, MBT381873), GenBank accession number ITS: EU669383.

**Original type description** (Harkness 1899, p. 264): “Medium, semiglobose, irregular, black, verrucose, warts with polygonal base; openings stellar, sometimes extending through the mass; cavities large and somewhat regular; gleba white, firm, crossed by white wavy lines. Asci semiglobose or ellipsoid, briefly stipitate, 8-spored, 48 × 32 μ; ascospores oblong-elliptic, guttulate, 26 × 12 μ”.

**Emended description:** *Ascomata* sequestrate, subglobose or irregularly lobed with deep or convoluted invaginations, 10–40 × 9–20 mm; *peridial warts* black or dark reddish-brown, some with reddish-brown hyphal tufts at the apex, brown to black between warts; warts pointed, 300–600 μm wide × 250–500 μm tall, often with longitudinal ribs. *Gleba* white when fresh becoming yellow when dry; chambers with white lining, open to appressed, some appressed chambers open to the exterior. *Peridium* wart surface uneven with rounded cells and scattered, pointed cystidium-like cells, 23–35 μm long, 10 μm at base; outer pigmented layer 4–8 cells thick, of brown cells, 20 × 30 μm, with walls 6 μm thick, grading to yellow to hyaline isodiametric cells, 30 μm wide, with walls 1–4 μm thick, pigmented and hyaline cells radially aligned, especially under the wart apex; warts subtended by a *textura angularis* with cells 7–9 μm wide. *Gleba* of loosely interwoven hyphae (*textura intricata*), 4–6 μm wide; chambers lined with paraphyses, 60–65 μm long × 4 μm wide, arranged in a palisade when immature, becoming disordered when mature. *Asci* hyaline, globose to citriform, 50 × 30 μm, with walls 1.5 μm thick, stalked, 8-spored. *Ascospores* hyaline, smooth, ellipsoid, 25.4 × 13.2 (22–29 × 10.5–15.5) μm, Q = 1.9 (1.5–2.5); one guttule.

**Habit, habitat, and distribution:** Hypogeous under *Abies concolor*, *Pinus attenuata*, *P. jeffreyi*, *P. lambertiana*, *P. ponderosa*, *P. sabiniana*, *Pseudotsuga macrocarpa*, *Ps. menziesii*, *Quercus chrysolepis*, *Q. garryana*, *Q. kelloggii*, and *Q. wislizeni*; widespread in Sierra Nevada and Cascade Mountains and foothills.

**Additional specimens examined:** USA, California, Fresno Co., Sierra National Forest, Ross Creek Drainage, Turtle Creek, 36°54'N 119°6'W, 1465 m, under *Abies concolor*, *Pinus lambertiana*, *P. ponderosa*, 25 Jun. 1997, K. Pendleton 15 (OSC130693); Teakettle Research Natural Area, 2040 m, under *Abies magnifica*, 24 Jun. 1996, M. Castellano (OSC131297); Lake Co., Boggs Mountain State Forest, intersection of roads 400 and 410, T11N R8W, sec. 12, 1005 m, under *Pinus ponderosa*, *Pseudotsuga menziesii*, *Quercus kelloggii*, 1 Mar. 1990, M. Castellano, D. Luoma (OSC157740); Los Angeles Co., Angeles National Forest, Upper Shake campground, under *Pseudotsuga macrocarpa*, *Quercus*, 14 Mar. 1993, K. Schaffroth (OSC130698); 24 Mar. 1993 (OSC131294); Mariposa Co., Greely Hill Rd. Hwy 120, under *Lithocarpus densiflora*, *Pinus sabiniana*, *Quercus chrysolepis*, *Q. wislizeni*, 27 Mar. 1985, M. Castellano (OSC130695), GenBank accession number ITS: KP859264; Placer Co., Auburn, under *Ceanothus*, *Quercus* May 1898, H.W. Harkness 180 (OSC38607 – isotype); H.W. Harkness 186; Auburn State



**Fig. 8.** *Balsamia nigrans*. **A.** Ascomata showing peridial surface, gleba, and peridium in section (OSC 157740). **B.** Peridial surface warts (epitype; OSC 130700). **C.** Peridium showing outer wart layer, inner peridial layer and gleba (holotype; OSC 38607). **D.** Immature asci (OSC 157740). **E.** Ascospores (OSC 130983). Bars A = 20 mm, B = 600  $\mu$ m, C = 25  $\mu$ m, D = 20  $\mu$ m, E = 25  $\mu$ m.

Recreation Area, Foothill Blvd., 0.8 km from Upper Lake Clementine Day Use area at trailhead entering meadow, 38°57.0'N 120°58.4'W, 540 m, under *Ceanothus*, *Pinus ponderosa*, *P. sabiniana*, 14 Jan. 2017, *N. Siegel* (OSC157728); *N. Siegel* (OSC157727); Old Foothill Blvd. past Mammoth Bar OHV Area south side, trail on small hill, 38°55.6'N 121°0.4'W, 420 m, under *Ceanothus*, *Pinus sabiniana*, *Quercus*, 14 Jan. 2017, *N. Siegel* (OSC157725);

*N. Siegel* (OSC157726); Tehama Co. Whiskeytown National Recreation Area, under *Pinus attenuata*, *Quercus kelloggii*, 1 May 2006, *J.L. Frank* (OSC151400), GenBank accession number ITS: GU184098; Tuolumne Co., Hwy J20, east of Groveland, south of old Hwy 120, under *Pinus lambertiana*, *P. ponderosa*, 27 Mar. 1985, *M. Castellano* (OSC130673); Oregon, Benton Co, near Philomath, milepost 4.2 along Beaver Creek rd.,

south of Decker Rd., 400 m, under *Pseudotsuga menziesii*, 6 Jun. 2009, S. Donovan (OSC158069); west of Philomath along Beaver Creek rd., 6.6 km from Decker rd., 380 m, under *Pseudotsuga menziesii*, 3 June 2006, S. Donovan (OSC112221); Woods Ck Rd, 460 m, under *Pseudotsuga menziesii*, 9 Jun. 1972, R. Fogel (OSC130983); Jackson Co., Applegate T37S R4W sec 33 sw1/4, 6 Dec. 1998, R. Young (OSC61957); Josephine Co., Missouri Flat, Ramsgate Ranch, 380 m, under *Pinus ponderosa*, 24 Mar. 1991, M. Amaranthus (OSC130697); Bureau of Land Management, Waldo Hill, 610 m, under *Pinus jeffreyi*, 19 Oct. 1968, J.M. Trappe 1737 (OSC130699); Rogue River-Siskiyou National Forest, Eight Dollar Mountain Rd., Josephine camp, across green bridge, T38S R8W, section 19, NAD83 443146 4677394, 400 m, under *Pinus ponderosa*, *Quercus garryana*, 11 May 2007, S. Gladish (OSC151401), GenBank accession number ITS: FJ789590; Polk Co., Mill Creek, 170 m, 19 Jun. 1989, W. Bushnell (OSC158068); Yamhill Co., Yamhill River, Flying M Ranch, under *Pseudotsuga menziesii*, 2 Mar. 1985, H. Pavelek (OSC130696).

**Commentary:** Ascospores from the isotype (Harkness 180) measured 23–29 × 13–17 μm, Q = 1.5–2.2, slightly wider than in the original description. *Balsamia nigrans* is an orthographic correction of the original spelling, *B. nigrens*, to its proper Latin spelling *nigrans* and does not affect authorship of the name.

***Balsamia pallida*** D. Southw., J.L. Frank & Castellano, *sp. nov.* MycoBank MB815825. Fig. 9A–C.

**Etymology:** *pallid* refers to the pale orange peridium.

**Diagnosis:** The low warts (30–50 μm tall) and pale orange peridium differentiate this species from all other *Balsamia* species, yet DNA clearly places it in the genus. Open glebal chambers resemble those of *B. lazyana*, but the paraphyses lining the chambers are shorter. The habitat in chaparral under *Adenostoma fasciculatum* is distinctive.

**Holotype:** USA, California, Riverside Co., Skinner Lake, 33°35'N 117°03'W, 450 m, under *Adenostoma fasciculatum*, 18 Jun. 1996, M.F. Allen (OSC130727), GenBank accession number ITS: KU170040.

**Ascomata** sequestrate, subglobose, flattened, irregularly lobed and invaginated, 10–15 × 5–8 mm; *peridial warts* and space between warts pale orange with occasional pale tan streaks, warts pointed, 100 μm wide × 30–50 μm tall; gleba yellow-brown to pale yellow-tan, with open chambers to 0.5 mm wide and 2 mm long; where appressed, chambers open to the exterior. *Peridium* wart surface with protruding cells; outer pigmented layer 1–2 cells thick, yellow, isodiametric to elongate, 19 × 29 μm wide, with walls 2–3.5 μm thick, grading to hyaline isodiametric to elongate cells, 16 × 26 μm, with walls 1 μm thick. *Gleba* of interwoven hyphae, ±4 μm wide; chambers lined with paraphyses, 3 × 100 μm. *Asci* hyaline, ampulliform when immature, becoming ovoid to citriform at maturity, 60–80 × 30 μm, stipitate 6 μm wide × 11–15 μm long, with walls 1–2 μm thick, 8-spored. *Ascospores* hyaline, smooth, ellipsoid, 26.9 × 12 (25–29 × 11–13) μm, Q = 2.2 (2.0–2.5); with one guttule.

**Habit, habitat, and distribution:** Hypogeous under *Adenostoma fasciculatum* (*Rosaceae*) in southern California chaparral.

**Commentary:** This species is known only from the type collection. Other hypogeous sequestrate fungi were collected

with *B. pallida* in chaparral (Allen *et al.* 1999). *Adenostoma fasciculatum* belongs to *Rosaceae*, a group of plants which is not typically considered to be ectomycorrhizal, but Allen *et al.* (1999) found evidence of some ectomycorrhizal roots with unusual morphologies. Thus, *B. pallida* is putatively an ectomycorrhizal associate of *A. fasciculatum*.

***Balsamia quercicola*** D. Southw., M.E. Smith & J.L. Frank, *sp. nov.* MycoBank MB807879. Fig. 9D–F.

**Etymology:** *Quercus* and *-icola* (Latin) = inhabitant of oak woodlands.

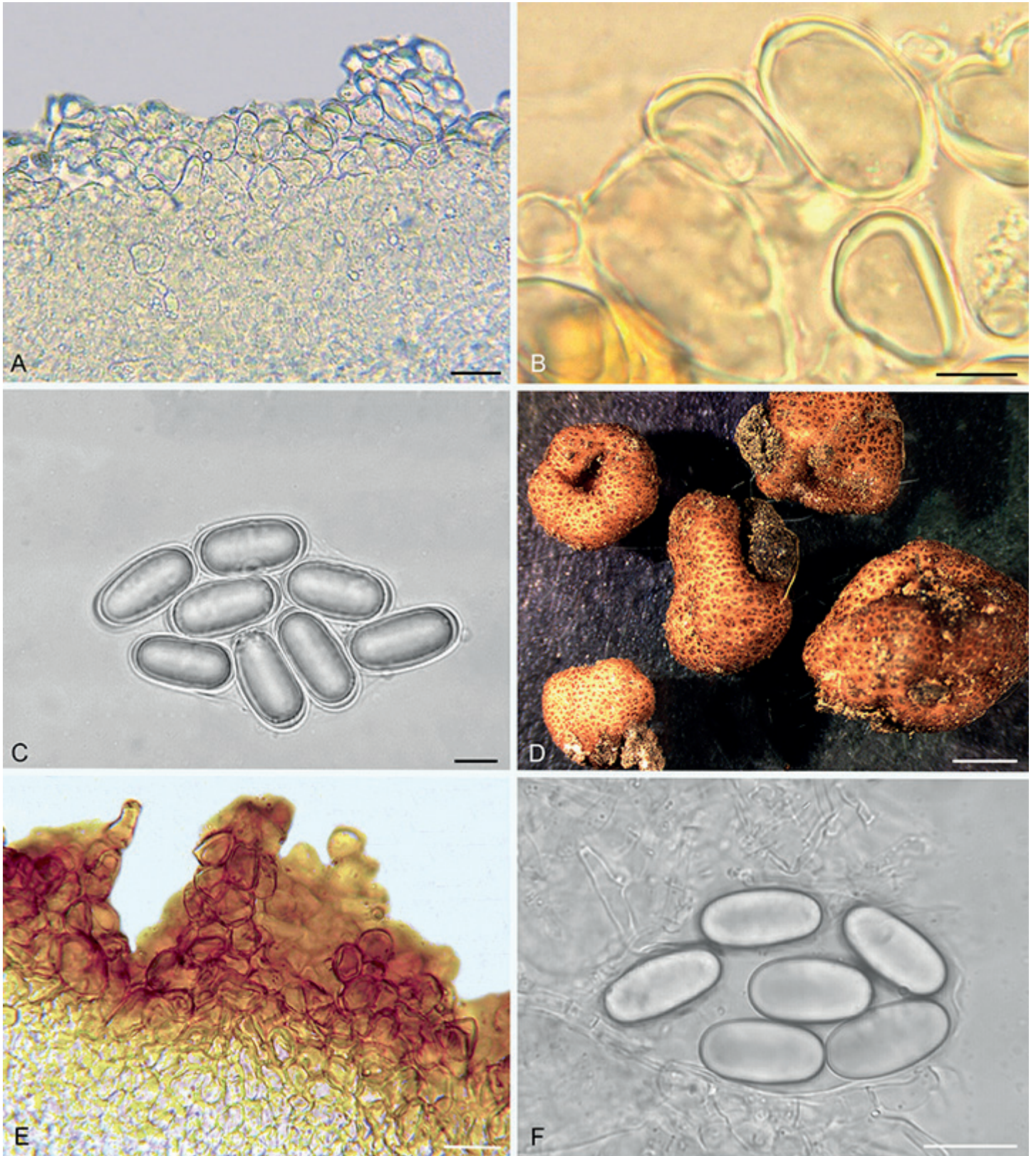
**Diagnosis:** *Balsamia quercicola* can be distinguished by the brown color of the peridium and the compact gleba. Mature ascospores of *B. quercicola* (mean spore length = 28.7 μm) are the longest of any *Balsamia* species in western North American species.

**Holotype:** USA, California, Yuba Co., University of California Sierra Foothill Research and Extension Center, Koch Natural Area, 39°15'N 121°18'W, 370 m, under *Pinus sabiniana*, *Quercus douglasii*, *Q. wislizeni*, 15 Jan. 2005, M.E. Smith SRC 868 (FLAS-F-58860). GenBank accession number ITS: KF983491.

**Ascomata** sequestrate, subglobose to irregularly lobed, often deeply invaginated, 4–11 × 3–8 mm; peridial warts reddish-brown, pale reddish-brown between warts; warts rounded to pointed, 100–200 × 200–700 μm; gleba white when fresh, pale tan when dry; chamber walls appressed. *Peridial* wart surface with sparse cystidium-like cells protruding about ±8 μm, wart outer pigmented layer 3–4 cells thick, reddish-brown, isodiametric to radially elongate cells, 20–25 μm wide, with walls 2–4 μm thick, grading to hyaline isodiametric cells, 15–20 μm wide, with walls 1 μm thick, then grading to smaller cells, 8 μm wide. *Gleba* of loosely interwoven hyphae, ±4 μm wide. *Asci* hyaline, subglobose to citriform, 60 × 30 μm, 8-spored. *Ascospores* hyaline, smooth, cylindrical, 28.7 × 12.9 (25.5–33 × 10.5–16) μm, Q = 2.2 (2.0–2.5); with three guttules.

**Habit, habitat, and distribution:** Hypogeous under *Pinus ponderosa*, *P. sabiniana*, *Quercus douglasii*, *Q. garryana*, or *Q. wislizeni*; in the Sierra Nevada foothills and in interior valleys of California and Oregon.

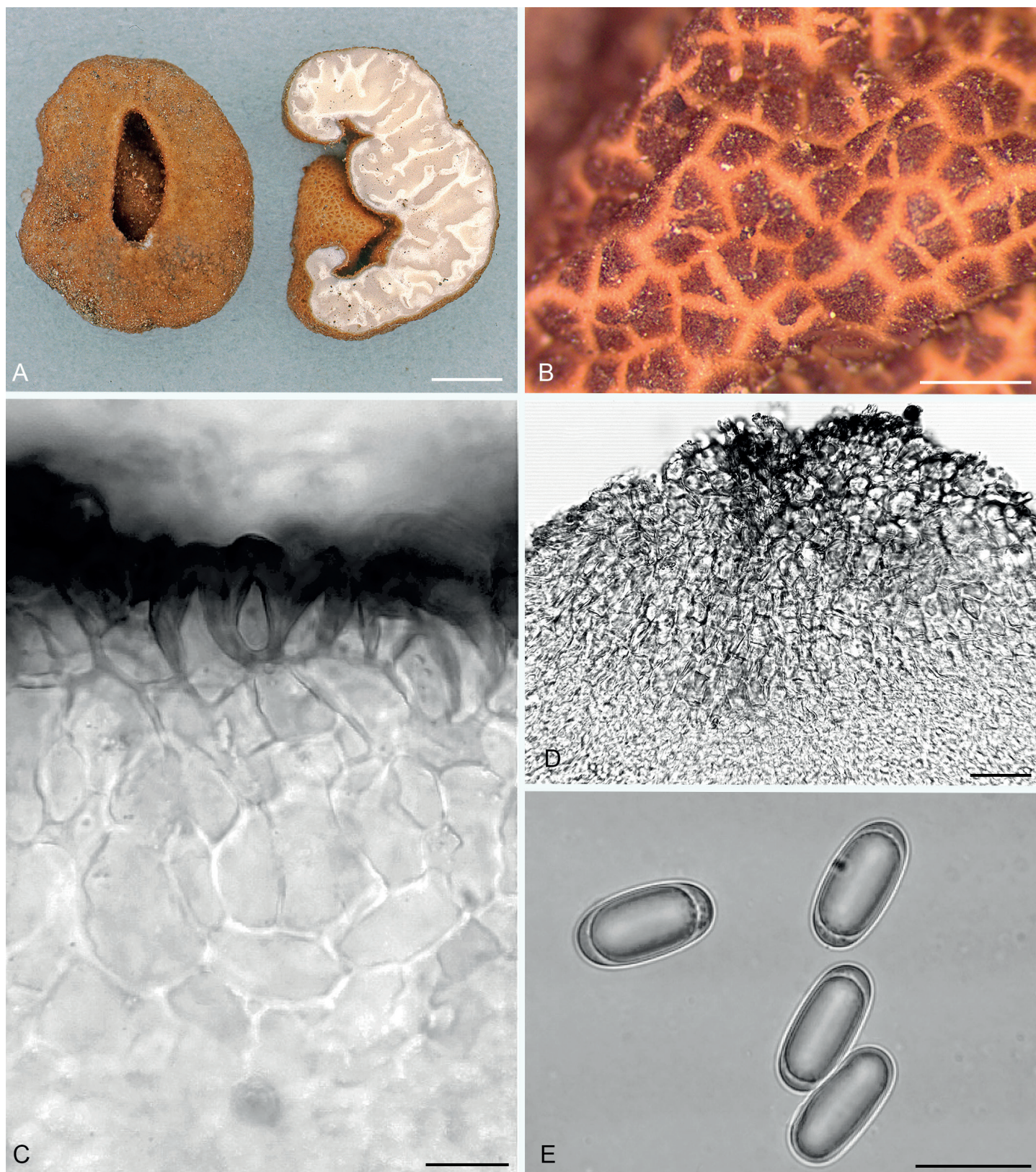
**Additional materials examined:** USA, California, Los Angeles Co., Angeles National Forest, Upper Shake campground, under *Pseudotsuga macrocarpa*, *Quercus*, 14 Mar. 1993, K. Schaffroth (OSC130688); (OSC130689); Placer Co., Auburn State Recreation Area, Old Foothill Blvd. at Mammoth Bar OHV Area, downhill next to road, 38°55.3'N 121°0.9'W, 350 m, under *Ceanothus*, *Pinus ponderosa*, *P. sabiniana*, 14 Jan. 2017, N. Siegel (OSC157723); pullout past Mammoth Bar OHV Area, uphill south side, east side of huge rock, 38°55.3'N 121°0.8'W, 375 m, under *Pinus sabiniana*, *Quercus*, 14 Jan. 2017, N. Siegel (OSC157724); 0.8 km from Upper Lake Clementine Day Use area at trailhead entering meadow, 38°57.0'N 120°58.4'W, 540 m, under *Ceanothus*, *Pinus ponderosa*, *P. sabiniana*, 14 Jan. 2017, N. Siegel (OSC157729); Yolo Co., Davis, under *Pinus ponderosa*, *Quercus*, 6 Nov. 2004, M.E. Smith 84 (FLAS-F-58857), GenBank accession number ITS: KF983490; Oregon, Jackson Co., Whetstone Savanna Preserve, under *Quercus garryana*, 22 Nov. 2003, M.E. Smith (OSC151402), GenBank accession number ITS: DQ453695.



**Fig. 9.** *Balsamia pallida* (A–C; holotype; OSC 130727) and *B. quercicola* (D–F). **A.** Peridium showing wart layer and inner layer in section. **B.** Peridial warts with thick-walled cells. **C.** Ascospores. **D.** Ascomata showing surface warts (OSC 151402). **E.** Wart structure showing thick-walled cells (FLAS 58860). **F.** Ascospores (FLAS 58860). Bars A = 25  $\mu$ m, B = 10  $\mu$ m, C = 10  $\mu$ m, D = 3 mm, E–F = 20  $\mu$ m.

*Commentary:* *Balsamia quercicola* is differentiated by the large deletion in the ITS1 region yielding an ITS of  $\sim$ 600 bp, in contrast to  $\sim$ 700 bp in most other *Balsamia* species. In addition, mature ascospores (mean length = 28.7  $\mu$ m) of *B. quercicola*, the longest

of any western North American species, are comparable in length to those of *B. vulgaris* from Europe reported as 28  $\mu$ m (Tulasne & Tulasne 1851); 26–32  $\mu$ m (Pegler *et al.* 1993); and 25–30  $\mu$ m (Montecchi & Sarasini 2000).



**Fig. 10.** *Balsamia setchellii*. **A.** Ascoma showing peridial surface, gleba, and peridium in section (OSC 79995). **B.** Peridial surface showing wart pattern in surface view (OSC 131295). **C.** Wart structure showing thick-walled cells. **D.** Cross-section of peridial surface showing wart structure (isotype; OSC 130978). **E.** Ascospores (OSC 131295). Bars A = 5 mm, B = 750  $\mu$ m, C = 20  $\mu$ m, D = 30  $\mu$ m, F = 20  $\mu$ m.

*Balsamia setchellii* (E. Fisch.) Trappe, D. Southw. & Castellano, **comb. nov.** MycoBank MB825537. Fig. 10.

**Basionym:** *Pseudobalsamia setchellii* E. Fisch., *Ber. Deutsch. Bot. Ges.* **25**: 374. 1907.

**Diagnosis:** *Balsamia setchellii* (spore means 24.6 x 13.1  $\mu$ m, Q = 1.9) is distinguished from *B. filamentosa* (means 25.6 x 12.8  $\mu$ m; Q = 2.0) by its slightly shorter and wider spores. *Balsamia setchellii* is distinguished from *B. limuwensis* (mean spore width = 12.1  $\mu$ m) by its broader spores. ITS sequences clearly separate *B. setchellii*, *B. filamentosa*, and *B. limuwensis*.

**Isotype:** USA, California, Alameda Co., Berkeley, under *Pinus radiata*, 19 Nov. 1904, N.L. Gardner 212 (OSC130978).

**Epitype:** USA, California, Yuba Co., University of California Sierra Foothill Research and Extension Center, Koch Natural Area 39°14'N, 121°19'W, 370 m, under *Pinus sabiniana*, *Quercus douglasii*, and *Q. wislizeni*, 15 Dec. 2004, M.E. Smith SRC395 (designated here FLAS-F-58859, MBT381874), GenBank accession number ITS: DQ974730.

**Original type description** (Fischer 1907 p. 374; transl. D. Southworth and J. M. Trappe): "*Pseudobalsamia setchelli* gen. et sp. nov. The fruiting bodies are penetrated by venae externae [external veins] which either radiate from a pitted depression on the outer surface to the interior or show an irregular course and open to several points on the outer surface and which are lined by a more or less distinct hyphal palisade. The fruiting body surface is covered with a warty pseudoparenchymatous rind (peridium). The asci generally appear irregularly distributed between the venae externae that are evenly situated in the fruit body tissue (gleba). They are mostly ellipsoid to citriform or broadly fusiform, and contain in an irregular arrangement eight ellipsoid ascospores with slightly thickened, colorless, smooth membranes (cell walls). In *Ps. setchelli* the asci are 50–70  $\mu$  long and 25–35  $\mu$  in cross section. The ascospores are 21–28  $\times$  10–12  $\mu$ m".

**Emended description:** *Ascomata* sequestrate, subglobose, invaginated at apex, 10  $\times$  10–20 mm; peridial warts reddish-brown to brown, pale reddish between warts; warts rounded, 200–350  $\times$  300–750  $\mu$ m, ribbed from tip to base; gleba white when fresh, drying to yellow-brown; most chamber walls appressed, some open; a few appressed channels open through the peridium (venae externae) at the invaginations. *Peridium* outer pigmented layer 2–4 cells thick, of brown, isodiametric cells, 20  $\mu$ m wide, or elongate cells, 12  $\times$  24  $\mu$ m, with walls 4–6  $\mu$ m thick, some cells radially aligned; sparse, pointed cystidium-like cells on surface,  $\pm$ 8  $\mu$ m wide at base, protruding up to 25  $\mu$ m from surface, with walls 1  $\mu$ m thick; inner layer of hyaline, isodiametric cells, 13–17  $\mu$ m wide, with walls 1  $\mu$ m thick; with scattered brown cells, up to 27  $\mu$ m wide, with walls 2  $\mu$ m thick. *Gleba* chambers lined with white hyphae; trama surrounding asci of loosely interwoven hyphae, 4–5  $\mu$ m wide, extending into chambers as paraphyses, up to 80  $\mu$ m long with rounded or capitate ends. *Asci* hyaline, stalked, citriform to ovoid, 65–100  $\times$  30–35  $\mu$ m; with walls 1–1.5  $\mu$ m; 8-spored. *Ascospores* hyaline, smooth, ellipsoid, 24.6  $\times$  13.1 (21–28  $\times$  11–15)  $\mu$ m, Q = 1.9 (1.6–2.4); 1–3 guttules.

**Habit, habitat, and distribution:** Hypogeous under *Arbutus menziesii*, *Arctostaphylos* spp., *Pinus coulteri*, *P. contorta* var. *contorta*, *P. muricata*, *P. radiata*, *P. sabiniana*, *Pseudotsuga menziesii*, *Quercus agrifolia*, *Q. chrysolepis*, *Q. douglasii*, *Q. kelloggii*, and *Q. wislizeni*, in the Sierra Nevada foothills, Klamath Mountains, and California Coast Range.

**Additional specimens examined:** USA, California, Contra Costa Co., Mount Diablo State Park, 305 m, under *Pinus coulteri*, *P. contorta* var. *contorta*, *P. sabiniana*, *Quercus agrifolia*, 2 Feb. 1996, C. Thayer (OSC130728), GenBank accession number ITS: KM115880; Kern Co., Hwy 218 at gate 5 km from summit, under *Pinus ponderosa*, *Quercus kelloggii*, 19 Apr. 1993, M. Castellano (OSC79995), GenBank accession number ITS: KU170031; Lake Co., 3 km west of Middletown, 17 Jan. 1936, H.L. Mason (OSC130712); McLaughlin Reserve, under *Pinus*

*sabiniana*, *Quercus durata*, 18 Jan. 2004, M.E. Smith 62 (FLAS-F-58856); Ventura Co., Los Padres National Forest, milepost 40 on Hwy 33, 34°37.5'N 119°22.3'W, 1920 m, under *Arctostaphylos*, *Pinus muricata*, *Quercus chrysolepis*, 23 Apr. 1996, M. Castellano (OSC131295), GenBank accession number ITS: KF983484.

**Commentary:** Fischer (1908) distinguished *P. setchelli* from *B. filamentosa* despite a note from W.A. Setchell, "I have examined the types of Harkness's *Balsamia filamentosa*, *B. alba* and *B. nigrens*. Externally and in asci, shape of spores, etc., these species are all very close and resemble our No. 212 (p. 155)". ITS barcodes support Fischer's claim that these are distinct taxa.

***Balsamia trappei*** D. Southw., J.L. Frank & Castellano, *sp. nov.* MycoBank MB815757. Fig. 11.

**Etymology:** Named in honor of James Martin Trappe who collected the type specimen as well as many other *Balsamia* specimens in the Pacific Northwest.

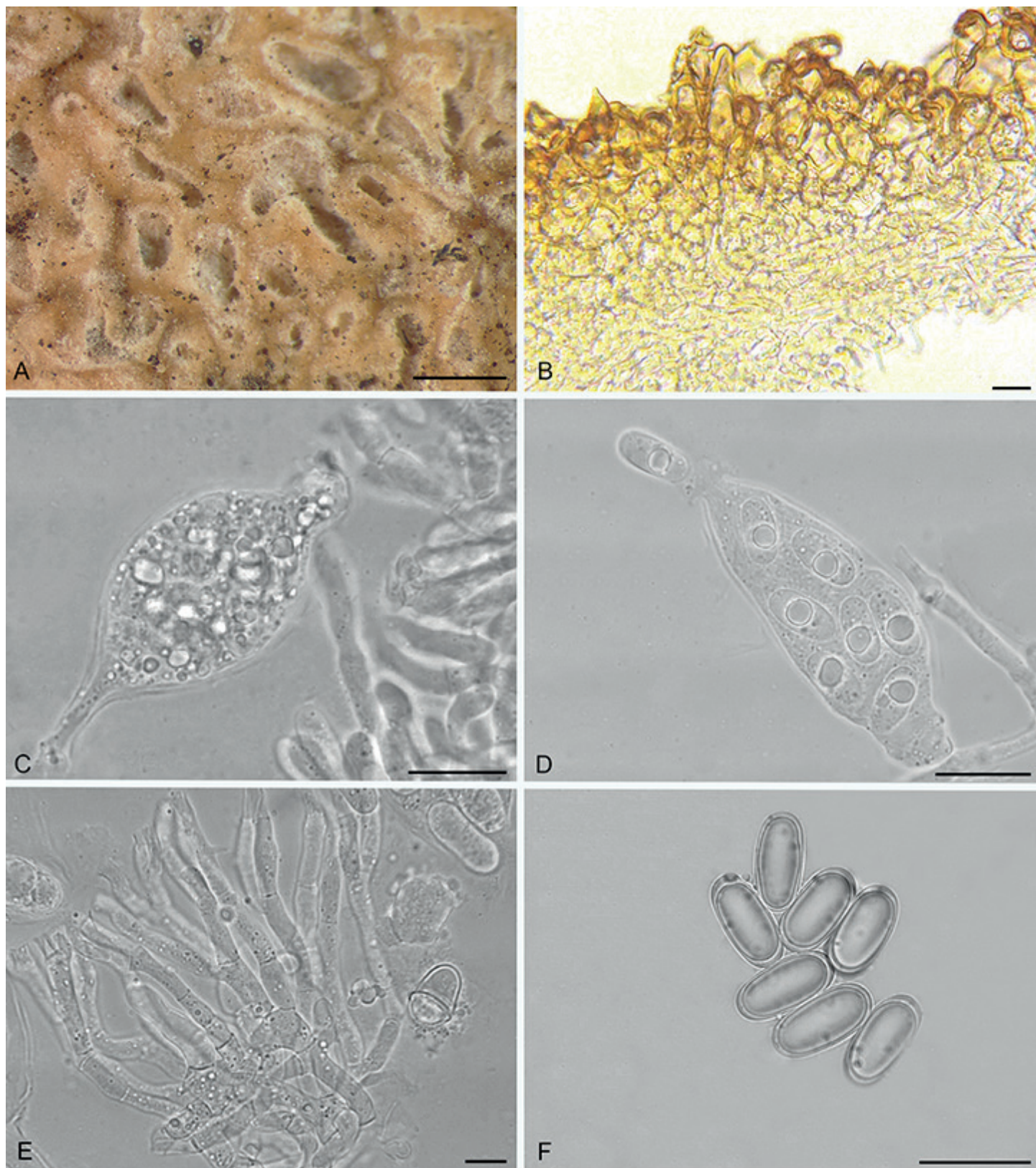
**Diagnosis:** *Balsamia trappei* is distinguished by glebal chambers that remain open at maturity. Ascospores of *B. trappei* (mean = 24.8  $\times$  12.3  $\mu$ m, Q = 2.0) are similar to those of *B. alba* (mean = 24.9  $\times$  12.4  $\mu$ m, Q = 2.1) and *B. magnata* (mean = 24.5  $\times$  12.1  $\mu$ m, Q = 2.0), but *B. alba* has larger warts (260–430  $\mu$ m wide  $\times$  100–220  $\mu$ m tall) that are rounded to obtuse, never pointed, and dark reddish brown in color with pale tan between warts. *Balsamia magnata* has wider and taller warts (250–550  $\mu$ m wide  $\times$  150–300  $\mu$ m tall) that are pointed and longitudinally ribbed, and reddish brown in color with pale reddish brown between warts. Warts in *B. trappei* are the narrowest and shortest (175–350  $\mu$ m wide  $\times$  100–120  $\mu$ m tall) of the three; warts are also rounded to pointed, and brown in color with reddish brown between warts.

**Holotype:** USA, Washington, Pend Oreille Co., Pend Oreille State Park, 48°05'N 117°20'W, under *Abies grandis*, 13 July 1997, J.M. Trappe 20842 (OSC131300), GenBank accession number ITS: KU170042.

*Ascomata* sequestrate, subglobose to irregularly lobed, 15–20 mm; peridial warts brown, reddish brown between warts; warts pointed to rounded, 175–350  $\mu$ m wide  $\times$  100–120  $\mu$ m tall; gleba white to tan, chambers sinuous, open to 1 mm. *Peridial wart* surface with occasional protruding cells, rounded or with pointed tips to  $\pm$ 8  $\mu$ m long; outer pigmented layer 4–6 cells thick, of reddish-brown cells, 25  $\times$  45  $\mu$ m, with walls 2–6  $\mu$ m thick, and then into hyaline cells approximately 18  $\times$  24  $\mu$ m. *Gleba* of loosely interwoven hyphae, 4  $\mu$ m wide; chambers lined with paraphyses, 2–7  $\mu$ m wide, with clavate to acuminate end cells. *Asci* hyaline, saccate-capitate with stipitate base, with walls 1  $\mu$ m thick, 35  $\times$  75  $\mu$ m wide, 8-spored. *Ascospores* hyaline, smooth, ellipsoid, 24.8  $\times$  12.3 (24–26  $\times$  11.5–13.5)  $\mu$ m, Q = 2.0 (1.7–2.2), with one guttule.

**Habit, habitat, and distribution:** Hypogeous under *Abies grandis* and *Pseudotsuga menziesii* or with hardwoods, e.g., *Alnus*, *Populus* and *Salix*; in the intermountain region of Idaho, Oregon, Washington, and Alberta, Canada; and in Alaska.

**Additional specimens examined:** Canada, Alberta, Calgary, under *Populus balsamifera*, 7 Sept. 1980, J. Zak, R. Danielson 3000 (OSC149793), GenBank



**Fig. 11.** *Balsamia trappei* (holotype; OSC 131300). **A.** Glebal chambers in section. **B.** Peridial surface showing wart structure. **C.** Immature asci with stipitate base and capitate apex. **D.** Ascus showing release of ascospore. **E.** Paraphyses. **F.** Ascospores. Bars A = 1 mm, B = 25  $\mu$ m, C–D = 20  $\mu$ m, E = 10  $\mu$ m, F = 25  $\mu$ m.

accession number ITS: KU170041. **USA**, Alaska, Palmer, Old Palmer Hwy, near Eklutna power plant, under *Alnus*, *Salix*, 3 Sept. 1974, V. Wells 6216 (OSC149792); Idaho, Idaho Co., near Florence, under *Abies*, 13 Aug. 1964, A.H. Smith 69265 (OSC149791); Oregon, Union Co., Starkey Experimental Forest and Range, Battle Creek, 1430 m, under *Abies grandis*, *Pseudotsuga menziesii*, 15 Oct. 1976, J.M. Trappe 4769 (OSC131284).

*Commentary:* Of the North American species, *B. trappei* most closely resembles the European *B. vulgaris* in morphology and ITS barcode.

**Table 2.** Morphological characters that delineate species of *Balsamia* based on specimens for which we have ITS sequences.

Species	Wart color, width x depth-to-cleft ( $\mu\text{m}$ ), shape	Peridium pigmented cell size, wall thickness; surface features	Spore size ( $\mu\text{m}$ ); Q (ratio L/W)	Gleba chambers open or appressed
<i>alba</i>	white hyphae over brown to red-brown warts, 170–350 x 100–200, flat to rounded or pointed	1–4 layers, 15 x 20–25 $\mu\text{m}$ , 3–5 $\mu\text{m}$ , surface hyphae hyaline, 6–7 $\mu\text{m}$ wide or with scattered pointed cystidium-like cells to 35 $\mu\text{m}$	24.9 (21–28) x 12.4 (11–13.5) Qav 2.1 (1.7–2.3)	partly open
<i>ascadensis</i>	white hyphae over brown warts, 200–360 x 110–160, rounded to pointed	4–6 layers, 18 x 22, 4 $\mu\text{m}$ ; surface hyphae hyaline, 6–7 $\mu\text{m}$ wide	23.7 (21–27) x 11.6 (11–13.5) Q 2.1 (1.6–2.3)	appressed
<i>filamentosa</i>	red-brown, 200–520 $\mu\text{m}$ x 80–200, rounded to obtusely pointed	2–4 layers, isodiametric 20–25 or elongate 15 x 33, 2–4 $\mu\text{m}$ ; sparse cystidium-like cells to 25 $\mu\text{m}$	25.6 (24–28) x 12.8 (11.5–14.5) Q 2.0 (1.6–2.4)	appressed
<i>latispora</i>	red to red-brown, 240–300 x 100–250, flat to rounded	2–3 layers, 15–20 $\mu\text{m}$ , 2–3 $\mu\text{m}$ ; pointed or rounded cystidium-like cells	22.0 (19–25.5) x 13.1 (11–15.5) Q 1.7 (1.5–2)	mostly appressed
<i>lazyana</i>	orange-red to red-brown, 250–400 x (50–)100–300, flat to rounded, often compound 3–4 lobed	2–3 layers, 22–28 $\mu\text{m}$ , 2–4 $\mu\text{m}$ ; pointed cystidium-like cells to 25 $\mu\text{m}$	23.5 (19.5–27) x 10.1 (8–11.5) Q 2.3 (2–2.7)	open to 1 mm
<i>limuwensis</i>	red-brown to brown, 230–380 x 75–150, rounded to obtusely pointed	2–4 layers, isodiametric 20 $\mu\text{m}$ , 2–4 $\mu\text{m}$ , pointed cystidium-like cells	24.1 (20–31) x 12.1 (10–14.5) Q 2.0 (1.7–2.4)	appressed
<i>magnata</i>	red-brown, 250–500 x 150–300, pointed, often compound of 3 small warts	2–4 layers, 10–15 $\mu\text{m}$ , 4–8 $\mu\text{m}$	24.5 (20–28.5) x 12.1 (10–14.5) Q 2.0 (1.7–2.4)	slightly open to appressed
<i>nigrans</i>	black to dark red-brown, 300–600 x 250–500, pointed, often ribbed	4–8 layers, 20 x 30 $\mu\text{m}$ , 6 $\mu\text{m}$ ; cystidium-like cells sparse 8 $\mu\text{m}$ wide up to 90 $\mu\text{m}$ long	25.4 (22–29) x 13.2 (10.5–15.5) Q 1.9 (1.5–2.5)	appressed
<i>pallida</i>	pale orange to pale tan, 30–50 x 100, pointed	3–4 layers, isodiametric to elongate 16–26 $\mu\text{m}$ , 2–3.5 $\mu\text{m}$	26.9 (25–29) x 12.0 (11–13) Q 2.2 (2–2.5)	open to 2 mm
<i>quercicola</i>	red-brown, 200–700 x 100–200, rounded to pointed	3–4 layers isodiametric to elongate 20–25 $\mu\text{m}$ , 2–4 $\mu\text{m}$ ; cystidium-like cells 8 $\mu\text{m}$ long	28.7 (25.5–33) x 12.9 (10.5–16) Q 2.2 (2.0–2.5)	appressed
<i>setchellii</i>	red-brown to brown, 300–750 x 200–350, rounded to obtusely pointed	2–4 layers elongate 12 x 24 $\mu\text{m}$ or isodiametric 20 $\mu\text{m}$ , 4 $\mu\text{m}$ , sparse cystidium-like cells to 25 $\mu\text{m}$	24.6 (21–28) x 13.1 (11–15) Q 1.9 (1.6–2.4)	appressed
<i>trappei</i>	brown to red-brown, 170–350 x 100–120, pointed to rounded	4–5 layers, 25 x 45 $\mu\text{m}$ , 2–6 $\mu\text{m}$	24.8 (24–26) x 12.3 (11.5–13.5) Q 2.0 (1.7–2.2)	open to 1 mm, sinuous

## DISCUSSION

Using ITS rDNA we were able to resolve the differences in the number of *Balsamia* species proposed by Harkness (1899) and by Gilkey (1916, 1939, 1954). Phylogenetic analysis supported recognition of multiple *Balsamia* species for western North America as described by Harkness (1899), rather than one to three species as proposed by Gilkey (1916, 1939, 1954). We described seven new species (*B. cascadenis*, *B. latispora*, *B. lazyana*, *B. pallida*, *B. limuwensis*, *B. quercicola*, and *B. trappei*) from North America. Thus, based on ITS barcodes, morphological characters, distribution, and habitat factors, 12 *Balsamia* species occur in western North America (Table 2).

Identification of *Balsamia* species was challenging due to variability in morphological characters, particularly among specimens of different developmental stages. The best indicator of ascoma maturity appeared to be the immediate release of ascospores from cut specimens. In mature specimens, ascospores dispersed quickly; the gleba remained white and did not shrink as the specimen dried leaving peridial warts fully

expanded. In contrast, in immature specimens the ascospores remained in the asci. After drying, immature gleba tissue turned golden brown and shrank, thus opening cavities and moving peridial warts closer together.

We revisited the question of whether *Balsamia* and *Pseudobalsamia* are separate genera, and observed no consistent pattern based on ITS sequences to show separation between the two genera. Fischer's original description of *Pseudobalsamia* (translated above) did not clearly distinguish between *Balsamia* and *Pseudobalsamia* though he later described *Balsamia* as having open chambers and *Pseudobalsamia* as having chambers more or less filled with hyphae (Fischer 1907, 1908, 1938). Gilkey (1916) applied *Pseudobalsamia* to Harkness's *Balsamia* collections from California, but reported that there was no evidence for separation into two "distinct phylogenetic lines". We did not observe morphological distinctions between the *Pseudobalsamia* isotype and the *Balsamia* species that we examined. Here we take the conservative position and recognize a single genus, *Balsamia*. This is appropriate because this has been the status quo accepted by most taxonomists since



*Pseudobalsamia* was synonymized with *Balsamia* by Trappe (1975). However, it should be noted that the basal nodes in our phylogeny received moderate to weak bootstrap support so sequencing of additional molecular markers in the future should be useful to test this hypothesis.

The relative paucity of *Balsamia* collections and the rarity of certain species have limited our systematic analyses. The ITS barcodes proved useful for distinguishing *Balsamia* species. ITS data suggest that the patterns of endemism between North America and Europe in *Balsamia* parallel patterns found in *Tuber* and related *Tuberaceae* whereby species are rarely or never shared across continents (Bonito *et al.* 2013). However, further sampling of additional specimens from Asia and Europe is needed to clarify evolutionary patterns within *Balsamia* and among the other genera of *Helvellaceae*.

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