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Entoloma sequestratum, a new species from northern Thailand, and a worldwide key to sequestrate taxa of *Entoloma* (*Entolomataceae*)

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Abstract: Based on our study of the morphology and genetics of sporocarps collected in the mountains of northern Thailand, we herein describe *Entoloma sequestratum* as a new sequestrate member of the *Entolomotaceae*. This serves as the first report of a sequestrate member of the genus from Thailand. In addition, we provide a worldwide key to all of the described sequestrate members of the genus.

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INTRODUCTION

Sequestrate fungi are widespread in a diversity of habitats around the world. They are commonly reported from temperate or semi-arid regions, but there are a growing number of species and genera being discovered in the wet tropics (Castellano *et al*. 2011, Trappe *et al*. 2013, Verbeken *et al*. 2014, Smith *et al*. 2015, Castellano *et al*. 2016a, b, Sulzbacher *et al*. 2020). Sequestrate fungi belonging to at least eight families have now been reported from the mostly wet and tropical habitats of Thailand. These include the genus *Leucogaster* in the *Albatrellaceae* (Dissing 1963); members of the genus *Descolea* (syn. *Descomyces*) in the *Bolbitiaceae* (Ellingsen 1982); the genera *Mycoamaranthus, Octaviania, Rhodactina*, and *Spongiforma* in the *Boletaceae* (Pegler & Young 1989, Lumyong *et al*. 2003, Yang *et al*. 2006, Desjardin *et al*. 2009, Choeyklin *et al*. 2012, Vadthanarat *et al*. 2018); the genus *Elaphomyces* in the *Elaphomycetaceae* (in Castellano *et al*. 2016a, Elliott, unpubl. data); the truffle-like *Radiigera tropica* in the *Geastraceae* (Orihara *et al*. 2008); the genus *Hymenogaster* in the *Hymenogastraceae* (Dissing 1963, Elliott, unpubl. data); and the genus *Tuber* and in the *Tuberaceae* (Suwannarach *et al*. 2015, Suwannarach *et al*. 2016, Elliott, unpubl. data). Various sequestrate representatives of the *Russulaceae* have also been collected, but not all of their generic relationships have been resolved (Heim 1959, Ellingsen 1982, Verbeken *et al*. 2014). Dissing (1963) also listed the presence of *Melanogaster* (*Paxillaceae*) in Thailand; however, from the illustrations of spores and the description (we were unable to examine the collection), it appears that he was describing the genus *Mycoamaranthus* (*Boletaceae*), which was erected much later (Lumyong *et al*. 2003).

It has gradually become apparent through the application of newly available molecular techniques and morphological re-assessments that many of the closely allied sequestrate and non-sequestrate genera are poly- or paraphyletic; numerous nomenclatural changes have been made to resolve these issues (*e.g.*, Peintner *et al*. 2001, Geml 2004, Lebel & Tonkin 2007, Lebel & Syme 2012, Lebel 2013, Braaten *et al*. 2014, Kuhar *et al*. 2017, Lebel 2017, Elliott & Trappe 2018).

Taxa in the cosmopolitan family *Entolomataceae* fulfill a wide range of functions in the environment, including ectomycorrhizal associations with plants, decomposition of organic material, and parasitic associations with other fungi or plants (Noordeloos 2004). Among the more than 1 500 species in the family, there is a great diversity of macromorphologies that range from gymnocarpic/agaricoid to entirely sequestrate/gastroid forms (Cribb 1956, Co-David *et al*. 2009, Baroni & Matheny 2011, Gates 2012). The large diversity of species and variety of morphologies led early mycologists to erect a number of generic names that have not withstood the test of time or the "genetic era" in mycology. In the *Entolomataceae*, sequestrate species were once placed in the genera *Rhodogaster* and *Richoniella*; however, many mycologists provided morphological and genetic evidence to show that these two genera do not form monophyletic lineages and should be combined with *Entoloma* (Dodge & Zeller 1934, Horak 1964, Dring & Pegler 1978, Beaton *et al*. 1985, Horak & Moreno 1998, Co-David *et al*.

2009, Kinoshita *et al*. 2012). Our genetic and morphological data further confirm this, and we have made the decision to place the new species described herein in the genus *Entoloma*.

MATERIALS AND METHODS

Sporocarp collection and morphological observation methods

Species of sequestrate *Entoloma* typically develop within the leaf litter or partially below ground, so sporocarps are collected by raking away the leaf and upper soil layers in suitable habitats or by looking for areas where the soil surface is partially exposed. Occasionally, specimens are partially emerged from the soil in eroded or disturbed environments such as road banks or trail edges. Descriptions of macromorphological characters are based on fresh material. Colors are described in general terms based on the observations of the authors. Tissues and spores from dried specimens were rehydrated and examined in water mounts for study of microscopic characters. Spore dimensions were taken from 20 randomly selected basidiospores measured from the holotype collection (there was no observable variation in spore sizes between collections). For scanning electron microscopy of the basidiospores, fragments of the gleba were mounted on aluminum stubs with double-sided adhesive tape, coated with gold palladium alloy, and then observed under an SEM (Hitachi S4800).

DNA extraction, PCR amplification, and sequencing

Approximately 0.02 g of fungal tissue was removed from the interior of a sporocarp and placed into a sterile 1.5 mL microcentrifuge tube. The sample was homogenized with a sterile pestle. DNA extraction was carried out using the NucleoSpin Plant II kit (Macherey-Nagel, Bethlehem, PA) following the manufacturer's protocol. To amplify the internal transcribed spacer (ITS), partial large subunit nrRNA gene (LSU), partial DNA-directed RNA polymerase II second largest subunit gene (*RPB2*), and partial mitochondrial small subunit (mtSSU), PCR conditions and primers were set under standard conditions as shown in Table 1. The PCR product was verified via electrophoresis in a 1.5 % agarose gel in 0.5× TAE buffer and stained by SYBR Safe DNA gel stain (Invitrogen, Carlsbad, CA). MassRuler Express Forward DNA Ladder Mix (Thermo Scientific, Waltham, MA) was used as a molecular size standard. Finally, single-pass Sanger sequencing (GENEWIZ, Danvers, MA) was used to obtain sequence data for further analysis.

Phylogenetic analysis

The sequence data generated in this study were analyzed with closely related taxa retrieved from the GenBank database (www. http://blast.ncbi.nlm.nih.gov/) based on BLAST searches and recent publications (Kinoshita *et al*. 2012, Morgado *et al*. 2013) (Table 2). Single gene sequence datasets were aligned using the MAFFT v. 7.215 website (Katoh *et al*. 2016) and manually edited in BioEdit v. 7.0 and Geneious v. 10.2.3 when necessary (Hall 2004). Single sequence alignment datasets were combined using BioEdit v. 7.2.5 (Hall 2004). The alignment of combined datasets in FASTA format was converted to PHYLIP and NEXUS formats using the Alignment Transformation Environment (ALTER) website (Glez-Peña *et al*. 2010).

A Maximum likelihood (ML) phylogenetic analysis was performed on the combined 4-gene alignment using RAxML-HPC2 v. 8.2.4 (Stamatakis 2014) on XSEDE via the CIPRES science gateway (Miller *et al*. 2010; www.phylo.org) with 1 000 bootstrap replications. The resulting ML tree was visualized with the program FigTree v. 1.4 (http://tree.bio.ed.ac.uk/software/figtree/). Maximum likelihood values equal to or greater than 50 are reported in the final tree. The phylograms were reorganized using Microsoft Office PowerPoint 2007 and Adobe Illustrator CS3 (Adobe Systems Inc., USA). The sequences generated in this study were submitted to GenBank (Table 2).

Taxonomy

Entoloma sequestratum T.F. Elliott, S.L. Stephenson, Karun. & D. Nelsen, ***sp. nov***. MycoBank MB825011. Fig. 1.

Etymology: The name "sequestratum" refers to the enclosed (sequestrate) sporocarp.

Fresh *Sporocarps* up to 15 mm × 10 mm, globose to irregularly globose to somewhat oblong. *Stipe* absent. *Peridium*/*Pileus* with occasional irregular pits, overall smooth, sometimes with small invaginated pit or stub at the base, overall white to off-white and thin in section (< 1 mm), white and appearing solid in color throughout. *Gleba*/*Hymenophore* loculate to labyrinthiform, when young off-white to faintly pinkish but darkening with maturation, lamellae fused to form compact and stuffed locules with whitish hyphae when young (Fig. 1B), but with maturation locules becoming empty and labyrinthiform and reaching nearly 0.5 mm broad, hymenophoral trama darker in color than the hymenial layer (Fig. 1C). *Overall odor* not distinctive. *Peridiopellis/Pileipellis* 75–162.5 µm thick, with two layers, outer layer thinner (28–50 µm), reddish brown, composed of compacted interwoven hyphae up to 5 µm broad, thin-walled, not gelatinized, with intermixed irregular granules, inner layer hyaline to pale yellow, interwoven with irregular hyphae up to 5 µm broad, thin-walled, not gelatinized. *Hymenophoral trama* 12.5–27.5 µm thick, consisting of hyaline to pale yellow hyphae, densely interwoven and irregularly shaped hyphae up to 5 µm broad, thin-walled, not gelatinized, with occasional clusters of inflated thin-walled globose cells 2.5–12.5 µm broad. *Clamp connections* not observed. *Basidia* irregularly to broadly clavate,

Table 1. Details of genes/loci with PCR primers and protocols.

Gene/loci	PCR primers (forward/reverse)	References
ITS	ITS5/ITS4	White *et al*. (1990)
LSU	LROR/LR5	Vilgalys & Hester (1990)
RPB2	fRPB2-5f/fRPB2–7cR	Liu *et al*. (1999)
mtSSU	ms1/ms2	Skovgaard *et al*. (2002)

Table 2. Taxa used in the phylogenetic analysis and their corresponding GenBank accession numbers.

Taxa names	Isolate	mtSSU	*RPB2*	LSU	ITS	Locality	References
Calocybe carnea	CBS 552.50	AF357097	DQ825407	AF223175	AF357028	–	Hofstetter *et al.* (2002)
Clitocybe dealbata	–	–	DQ825407	AY207152	MK214399	–	–
Clitopilus fallax	isolate 37	GQ289350	GQ289276	GQ289210	–	Slovakia, EU	Co-David *et al.* (2009)
Clitopilus hirneolus	isolate 263	GQ289352	GQ289278	GQ289211	KC710132	Italy, EU	Co-David *et al.* (2009)
Clitopilus nitellinus	isolate 400	GQ289355	GQ289282	GQ289215	–	Austria, EU	Co-David *et al.* (2009)
Entocybe haastii	strain 617	KC710174	–	–	KC710089	–	Morgado *et al.* (2013)
	strain 126	KC710173	–	KC710144	KC710086	Tasmania, Australia, AA	Morgado *et al.* (2013)
En. myrmecophilum	isolate 231	GQ289314	GQ289245	GQ289174	KC710120	The Netherlands, EU	Co-David *et al.* (2009)
En. nitida	strain 2006201	–	–	–	KC710100	Austria, EU	Morgado *et al.* (2013)
	strain 8376	–	–	–	KC710076	Scotland, EU	Morgado *et al.* (2013)
En. prismaticum	TNS F-46866	–	–	NG_042335	AB691999	–	–
En. trachyospora	strain 405	–	–	–	KC710088	–	Morgado *et al.* (2013)
En. turbidum	strain 27	–	–	–	KC710060	–	Morgado *et al.* (2013)
Entoloma aff. prunuloides	strain 628	KC710189	KC710159	–	–	California, USA, NA	Morgado *et al.* (2013)
E. aff. prunuloides	53901	KC710168	–	KC710139	KC710071	California, USA, NA	Morgado *et al.* (2013)
E. aff. sinuatum	TRTC156542	–	–	–	JN021020	Québec, Canada, NA	Dentinger *et al.* (2011)
	TRTC156546	–	–	–	JN021019	Québec, Canada, NA	Dentinger *et al.* (2011)
E. afrum	isolate Tle1416	–	–	–	KP191914	–	Unpublished
E. albidum	strain 620	KC710180	–	KC710151	KC710102	Québec, Canada, NA	Morgado *et al.* (2013)
E. albomagnum	strain 427	KC710165	–	KC710137	KC710065	Tasmania, AA	Morgado *et al.* (2013)
E. alcedicolor	isolate 210	GQ289292	GQ289224	GQ289152	KC710123	The Netherlands, EU	Co-David *et al.* (2009)
E. araneosum	isolate 14	GQ289293	GQ289225	GQ289153	KC710056	Belgium, EU	Co-David *et al.* (2009)
E. asterosporum	TENN064538	–	JF706312	–	JF706309	–	–
E. baronii	strain L644	–	–	–	KC710093	Tasmania, Australia, AA	Morgado *et al.* (2013)
E. bloxamii	isolate 219	GQ289294	GQ289226	GQ289154	KC710087	Austria, EU	Co-David *et al.* (2009)
	strain 8003	–	–	–	KC710083	Italy, EU	Morgado *et al.* (2013)
	RBG Kew K(M)128736	–	–	–	EU784208	UK, EU	Brock *et al.* (2009)
	strain 13	–	–	–	KC710082	France, EU	Morgado *et al.* (2013)
	strain 619	KC710166	–	–	KC710066	Germany, EU	Morgado *et al.* (2013)
E. caccabus	isolate 17	GQ289295	GQ289227	GQ289155	KC710063	Belgium, EU	Co-David *et al.* (2009)
E. caesiolamellatum	strain 626	KC710187	–	KC710157	KC710126	Canary Islands, Spain, EU	Morgado *et al.* (2013)
	strain TB6117	–	–	AF261289	KC710128	California, USA, NA	Baroni *et al.* (2011); Morgado *et al.* (2013)
E. callidermum	strain 512	KC710183	–	KC710153	KC710115	Malaysia, AA	Morgado *et al.* (2013)
E. calongei	BRACR30482	MK530244	–	MK531556	–	–	Unpublished

Table 2. (Continued).

Taxa names	Isolate	mtSSU	*RPB2*	LSU	ITS	Locality	References
E. cf. griseoluridum	LNM221111	–	–	–	KC710118	Portugal, EU	Morgado *et al.* (2013)
E. cf. subsinuatum	strain 633	KC710190	KC710160	–	–	New York, USA, NA	Morgado *et al.* (2013)
E. chilense	MES 1012	–	–	–	KY462399	–	Truong *et al.* (2017)
E. clypeatum	strain 41	KC710164	–	KC710136	KC710059	The Netherlands, EU	Morgado *et al.* (2013)
E. coeruleogracilis	strain 216	–	–	–	KC710069	–	Morgado *et al.* (2013)
E. coeruleoviride	strain 609	KC710162	–	KC710134	KC710057	Malaysia, AA	Morgado *et al.* (2013)
E. conferendum	strain 30	KC710161	KC710191	KC710133	KC710055	Slovakia, EU	Morgado *et al.* (2013)
	isolate 6	GQ289300	GQ289231	GQ289160	–	Belgium, EU	Co-David *et al.* (2009)
E. corneri	strain 607	KC710163	–	KC710135	KC710058	Malaysia, AA	Morgado *et al.* (2013)
E. cretaceum	strain 2010039	–	–	–	KC710090	Newfoundland, Canada, NA	Morgado *et al.* (2013)
	strain 2011022	–	–	–	KC710074	Tasmania, AA	Morgado *et al.* (2013)
	isolate 213	GQ289302	GQ289233	GQ289162	KC710064	Tasmania, AA	Co-David *et al.* (2009)
E. eulividum	TB6807	–	–	AF261295	–	USA, NA	Moncalvo *et al.* 2002
E. flavifolium	strain 621	KC710179	–	KC710150	KC710097	Québec, Canada, NA	Morgado *et al.* (2013)
E. flavifolium	TB6215	–	GU384644	AF261301	–	USA, NA	Baroni *et al.* (2011)
E. fumosobrunneum	MEN 2005113	KC710185	–	KC710155	KC710124	Newfoundland, Canada, NA	Morgado *et al.* (2013)
	strain 2005120	KC710186	–	KC710156	KC710125	Newfoundland, Canada, NA	Morgado *et al.* (2013)
E. gasteromycetoides	isolate 180	GQ289304	GQ289235	GQ289164	–	–	Co-David *et al.* (2009)
E. gelatinosum	isolate 212	GQ289305	GQ289236	GQ289165	–	–	Co-David *et al.* (2009)
	strain E792	GQ289305	GQ289236	GQ289165	KC710103	Tasmania, Australia, AA	Morgado *et al.* (2013)
E. gracilior	strain 2011043	–	–	–	KC710079	Tasmania, AA	Morgado *et al.* (2013)
	strain 217	–	–	–	KC710112	–	Morgado *et al.* (2013)
E. haastii	isolate 216	GQ289308	GQ289239	GQ289168	–	–	Co-David *et al.* (2009)
	isolate 126	GQ289307	GQ289238	GQ289167	–	–	Co-David *et al.* (2009)
E. hypogaeum	K382	–	AB692019	–	NR_119416	–	Kinoshita *et al.* (2012)
	TNS F-46869	–	AB692019	–	NR_119416	–	Kinoshita *et al.* (2012)
E. indigoticoumbrinum	isolate 83	GQ289311	GQ289242	GQ289171	–	Tasmania, Australia, AA	Co-David *et al.* (2009)
E. kermandii	isolate 222	GQ289313	GQ289244	GQ289173	–	Tasmania, Australia, AA	Co-David *et al.* (2009)
	strain 703	–	–	–	KC710075	Tasmania, AA	Morgado *et al.* (2013)
E. lividoalbum	strain 233	KC710182	–	KC710152	KC710114	Belgium, EU	Morgado *et al.* (2013)
E. lividum	TB5034	–	–	AF261294	–	USA, NA	Baroni *et al.* (2011)
E. luridum	strain 2005108	KC710175	KC710192	KC710146	KC710091	Newfoundland, Canada, NA	Morgado *et al.* (2013)

Table 2. (Continued).

Taxa names	Isolate	mtSSU	*RPB2*	LSU	ITS	Locality	References
	strain 634	KC710170	–	KC710141	KC710080	Newfoundland, Canada, NA	Morgado *et al*. (2013)
E. madidum	strain 221	KC710188	–	KC710158	KC710127	The Netherlands, EU	Morgado *et al*. (2013)
	strain 67195	–	–	–	KC710130	Norway, EU	Morgado *et al*. (2013)
E. manganaense	strain 215	KC710172	–	KC710143	KC710085	Tasmania, Australia, AA	Morgado *et al*. (2013)
E. myrmecophilum	strain 231	–	–	–	KC710120	–	Morgado *et al*. (2013)
E. nitidum	isolate 24	GQ289315	GQ289246	GQ289175	KC710122	Slovakia, EU	Co-David *et al*. (2009)
	TB7526	–	GU384655	GU384626	–	New York, USA, NA	Baroni *et al*. (2011)
E. ochreoprunuloides	strain 15721	–	–	–	KC710111	Corsica, France, EU	Morgado *et al*. (2013)
	strain 632	KC710176	–	KC710147	KC710092	Germany, EU	Morgado *et al*. (2013)
E. ochreoprunuloides f. hyacinthinum	strain 6	–	–	–	KC710105	UK, EU	Morgado *et al*. (2013)
E. perbloxamii	strain 2010037	–	–	–	KC710095	Tasmania, Australia, AA	Morgado *et al*. (2013)
	isolate 71	GQ289318	GQ289249	GQ289178	KC710117	Tasmania, Australia, AA	Morgado *et al*. (2013)
E. prismaticum	isolate K381	–	AB692016	–	AB691998	–	Kinoshita *et al*. (2012)
E. prunuloides	isolate 40	GQ289324	GQ289255	GQ289184	KC710073	Slovakia, EU	Co-David *et al*. (2009)
	AFTOL-ID 523	–	DQ385883	–	DQ206983	New York, USA, NA	Matheny *et al*. (2007)
E. pseudoprunuloides	strain 627	KC710169	–	KC710140	KC710078	Newfoundland, Canada, EU	Morgado *et al*. (2013)
E. sequestratum*	**MFLU 12-2045**	**MT345061**	**MT349886**	**MT344186**	**MH323431**	Thailand	Present study
E. sinuatum	strain 182	KC710184	–	KC710154	KC710116	Finland, EU	Morgado *et al*. (2013)
	isolate 50	GQ289333	GQ289264	GQ289193	KC710109	The Netherlands, EU	Co-David *et al*. (2009)
E. sordidulum	isolate 1	GQ289334	GQ289265	GQ289194	KC710062	Belgium, EU	Co-David *et al*. (2009)
	strain L700	–	–	–	KC710119	Portugal, EU	Morgado *et al*. (2013)
Entoloma sp. 2	AK 2012 K479	–	AB692017	–	AB691990	–	Kinoshita *et al*. (2012)
Entoloma sp. 3	AK-2012 K389	–	AB692018	–	AB691993	–	Kinoshita *et al*. (2012)
E. sphagneti	isolate 209	GQ289335	–	GQ289195	KC710061	The Netherlands, EU	Co-David *et al*. (2009)
	strain 209	–	–	–	KC710061	–	Morgado *et al*. (2013)
E. subsinuatum	strain YL2269	KC710178	–	KC710149	KC710096	Québec, Canada, NA	Morgado *et al*. (2013)
	strain 624	KC710167	–	KC710138	KC710067	Newfoundland, Canada, NA	Morgado *et al*. (2013)
E. trachyosporum	isolate 405	GQ289338	–	GQ289198	KC710088	Canada, NA	Co-David *et al*. (2009)
	isolate 414	GQ289339	–	GQ289199	KC710121	Canada, NA	Co-David *et al*. (2009)
	strain 414	–	–	–	KC710121	–	Morgado *et al*. (2013)
E. turbidum	isolate 27	GQ289341	GQ289269	GQ289201	KC710060	Slovakia, EU	Co-David *et al*. (2009)
E. whiteae	strain 629	KC710171	–	KC710142	KC710084	Québec, Canada, NA	Morgado *et al*. (2013)

Table 2. (Continued).

Taxa names	Isolate	mtSSU	*RPB2*	LSU	ITS	Locality	References
E. zuccherellii	isolate 242	GQ289346	–	GQ289206	–	Italy, EU	Co-David *et al.* (2009)
E. aff. sinuatum	TRTC156542	–	–	–	JN021020	–	Dentinger *et al.* (2011)
	TRTC156546	–	–	–	JN021019	–	Dentinger *et al.* (2011)
Lyophyllum leucophaeatum	–	–	DQ367434	AF223202	MK966521	–	–
	Hae251.97	AF357101	DQ367434	AF223202	AF357032	–	Hofstetter *et al.* (2002)
Rhodocybe trachyospora	DAVFP:28111	–	–	–	JF899553	Canada, NA	Guichon *et al.* (2011)
	strain TB5856	–	GU384658	GU384629	–	USA, NA	Baroni *et al.* (2011)

*Sequences derived from this study are in black bold type.

up to 7.5 × 12.5 µm, infrequent when mature, two sterigmata observed per basidium; however, mature basidia almost entirely absent, apparently collapsing after producing spores. *Basidiospores* (Fig. 1D, E) hyaline, complex heterodiametrical, cuboid, most often 4–5 angles in side-view, 7.5–12.5 × 7.5–11(–12) µm excluding the apiculus (up to 2.5 µm), spore outer wall nearly 2.5 µm thick, average spore size 11.75 × 11µm (*n* = 20).

Typus: **Thailand**, *ca*. 50 km north of the city of Chiang Mai between Pa Pae and Mae Taeng, on the grounds of the Mushroom Research Centre, just past the dining hall (19° 07.200' N, 98° 44.044' E), 17 Jun. 2012, *T.F. Elliott* (**holotype** MFLU 12-2045).

Additional materials examined: All collections examined were made by the first author within a few meters of the type, but all were collected on different days. **Thailand**, *ca*. 50 km north of the

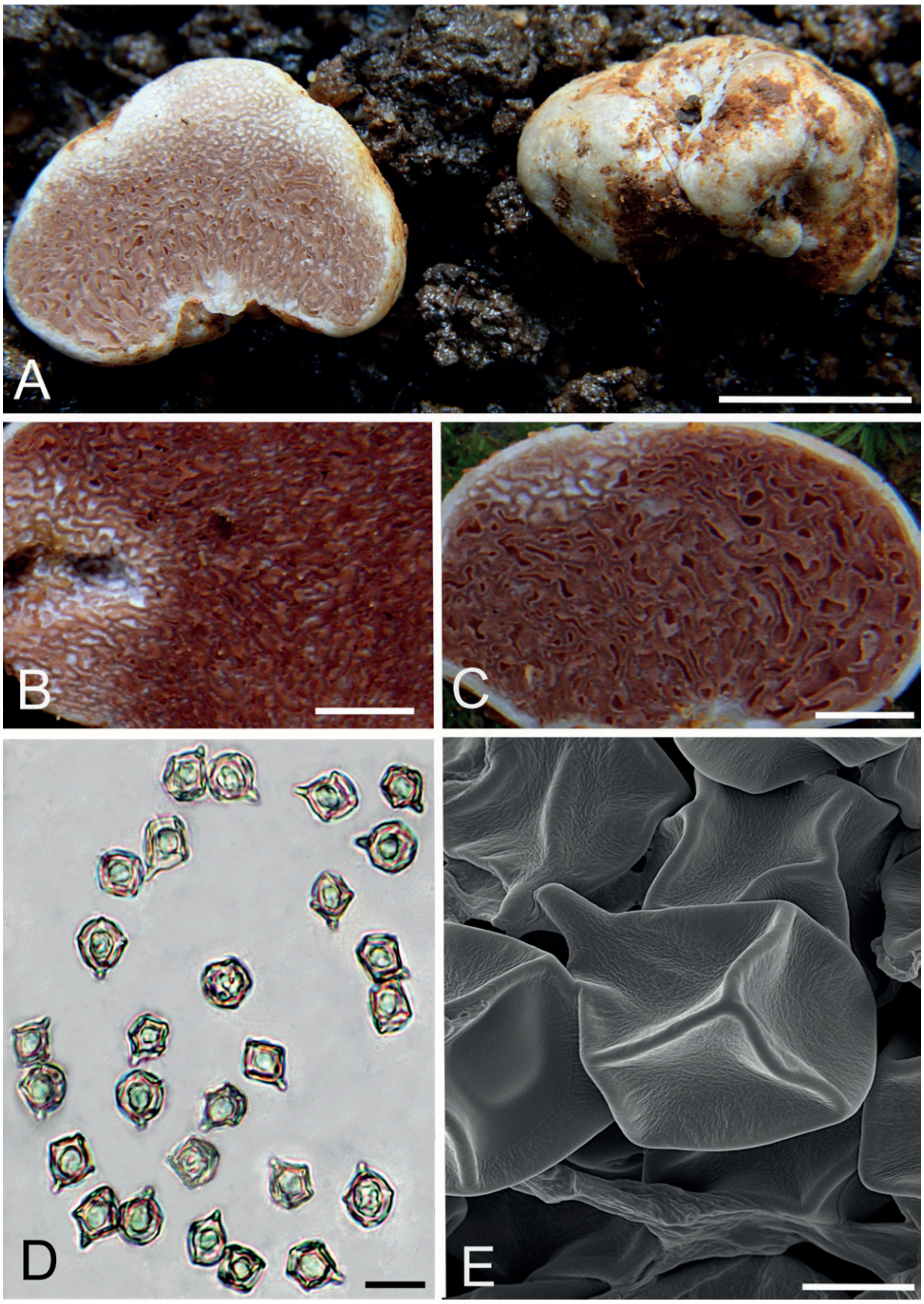

Fig. 1. *Entoloma sequestratum* (MFLU 12-2045, **holotype**). **A.** Fresh sporocarp of *E. sequestratum* from the type locality with bits of clay still adhering to outer peridial surface (note the range of development in the gleba within a single sporocarp). **B.** Locules in the gleba of a young sporocarp, compact and still stuffed with whitish hyphae. **C.** Mature locules expanded and empty, more clearly labyrinthiform. **D.** Basidiospores cuboid and showing 4–5 angles in side view (note the prominent apiculus). **E.** Scanning electron micrograph of basidiospores of *Entoloma sequestratum*. Scale bars: A = 10 mm, B = 1 mm, C = 2 mm, D = 10 µm, E = 2.5 µm.

city of Chiang Mai, between Pa Pae and Mae Taeng on the grounds of the Mushroom Research Centre, just past the dining hall (19° 07.200' N, 98° 44.044' E). 1 Jun. 2012, *T.F. Elliott*, MFLU 12-2085; *idem*., 1 Jun. 2012, *T.F. Elliott*, MFLU 12-2088; *idem*., 27 Jun. 2012, *T.F. Elliott*, MFLU 12-2080.

Notes: We have no direct evidence of the role of this fungus in forest ecosystems and whether or not it is mycorrhizal. There is not a lot known about the ecology of its close genetic relatives, so we can only hypothesize about its ecological function. It may be a decomposer or it may form ectomycorrhizal associations with trees in one or more of the following genera: *Lithocarpus*, *Dipterocarpus*, or *Castanopsis* (all of which occur in the area around the type collection).

Many of the sequestrate species in the genus *Entoloma* are relatively similar; however, there are several factors that make this novel species distinct. The most morphologically similar species to *E*. *sequestratum* is *E*. *gasteromycetoides* (syn. *Richoniella pumila* and *R*. *pumila f*. *bispora*). These two species are most easily separated on the basis of geography, with *E*. *gasteromycetoides* known only from Australia and New Zealand and *E*. *sequestratum* only from northern Thailand. They also differ genetically and in morphology. *Entoloma gasteromycetoides* has larger sporocarps that can be up to 25 mm diam, whereas *E*. *sequestratum* has sporocarps no bigger than 15 mm × 10 mm. In Cunningham's original description of *E*. *gasteromycetoides*, he describes that the peridium/pileus often disappears with maturation, leaving the gleba/hymenophore exposed (Cunningham 1940); this is a feature never observed in *E*. *sequestratum*. Cunningham also reports that the tramal plates of *E. gasteromycetoides* were 75–150 µm, whereas in *E*. *sequestratum* they are 12.5–27.5 µm. For further clarification on species differences, see the key and discussion provided in the following section.

Key to the described sequestrate *Entolomataceae* of the world

(Note: for full descriptions of the following species, please refer to the original publications).

1. Pileus-like portion of sporocarp sequestrate, pale brownish to grey-brown (not white), with a pronounced stipe 2
1. Sporocarp sequestrate, white, without a pronounced stipe, sometimes with a columella 3

2. From Pacific rainforests of Chile, ellipsoid nodular/cruciform spores (9.5–11.5 × 6.5–7.5 µm) .... ***Entoloma chilense***
2. From mixed conifer hardwood forests of northern Spain, spores cuboid (side length of cube ranging from 6–10 µm) .... ***Entoloma calongei***

3. Sporocarp smaller than 4.5 cm 4
3. From France, sporocarp larger than similar species (4.5–6 cm), spores cuboid/obtuse-angled (8–13 × 7–10.5 µm) .... ***Entoloma leptoniisporum***

4. Spores smaller than 15.5–19 × 9.5–12 µm 5
4. From Australia, spores cuboid/obtuse-angled, larger than related species (15.5–19 × 9.5–12 µm) .... ***Entoloma macrosporum***

5. Spores cuboid or more oval with angular warts 6
5. From southeastern USA and possibly Japan, spores cruciform/acute-rounded (8–12 × 6.4–9.8 µm) .... ***Entoloma asterosporum*** [Note: also see *Entoloma* sp. 2 (spores 5.6–7.5 × 5.6–7.5 µm) Kinoshita *et al*. 2012]

6. Spores cuboid 7
6. From Japan, spores oval in shape (7.7–10.0 × 6.8–8.6 µm) but with angular warts, (not cuboid as in similar species) .... ***Entoloma hypogaeum***

7. From Japan, spores cuboid (7.8–11.3 ×6.1–10.8 µm) with apiculus centered in a pentagonal face .... ***Entoloma prismaticum***
7. Apiculus not centered in a pentagonal face 8

8. Lacking columella or a basal pad at the base 9
8. From Ghana, short white columella-like base, spores cuboid (9.5–13 × 8–10.5 µm) .... ***Entoloma afrum***

9. From Australia, New Zealand, and possibly Japan, sporocarps up to 25 mm broad, spores cuboid (8.5–11 × 7.5–9 µm), peridium often disappearing with maturity .... ***Entoloma gasteromycetoides*** [Note: also see *Entoloma* sp. 3 (spores 7.5–12.5 × 7.5–10 µm) Kinoshita *et al*. 2012 and the Discussion]
9. From Thailand, sporocarps up to 15 mm × 10 mm, spores cuboid (7.5–12.5 × 7.5–11 (12) µm) .... ***Entoloma sequestratum***

DISCUSSION

Based on the geographic distribution, morphology, and genetics provided herein for *Entoloma sequestratum*, this new taxon appears to be distinct from all other described species in the genus. Based on combined data from the ITS, LSU, *RPB2*, and mtSSU genes, the phylogenetic relationships between *E*. *sequestratum* and other similar sequestrate taxa support the decision to distinguish this taxon as distinct from other sequestrate species in the genus (Fig. 2).

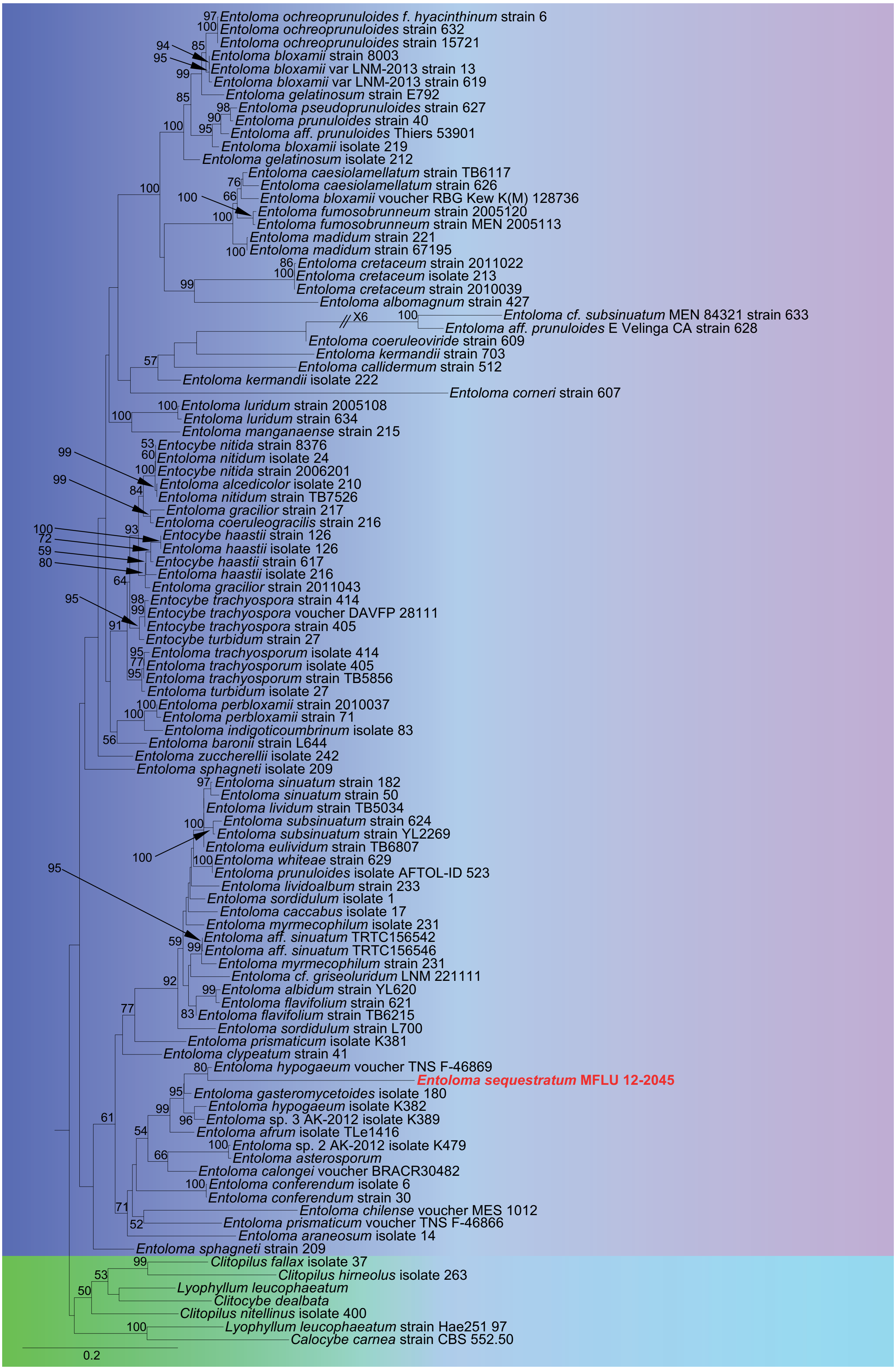

Fig. 2. Maximum Likelihood (ML) tree generated using RAxML based on the combined dataset of ITS, LSU, *RPB2* and mtSSU sequences. The analysis ran for 1 000 bootstrap replications; ML bootstrap support values ≥ 50 % are given above each of the branches. The new species *Entoloma sequestratum* is indicated in red bold font.

Our genetic analysis indicates that *E*. *sequestratum* clearly occurs on a separate branch and appears to be most closely related to *E*. *hypogaeum* and *Entoloma* sp. 3 (Kinoshita *et al*. 2012) collected in Japan and *E*. *gasteromycetoides* collected from New Zealand. Genetically, the most similar of these species is the taxon called *E*. *hypogaeum* in the study by Kinoshita *et al*. (2012). *Entoloma hypogaeum* is described as having peridiopellis up to 300 mm thick, with two layers: superficial layer 16.5 × 20 mm thick, composed of narrowly interwoven thin-walled hyphae 2.9–4.2 mm broad; inner layer pseudoparenchymatous, of inflated hyaline cells 15.8–22 × 10.5–12.5 mm, and basidia and basidiospores that are 26.5–43.4 × 6.5–8.5 µm and 7.7–10.0 × 6.8–8.6 µm, respectively (Kinoshita *et al*. 2012). Key morphological characters of *E*. *sequestratum* that separate it from *E. hypogaeum* include a thinner outer peridiopellis layer that is 75–162.5 µm thick (versus up to 200 µm in *E. hypogaeum*) with intermixed irregular granules, the presence of irregular inflated cells in the inner and outer layers (up to 5 µm broad), much smaller basidia (7.5 × 12.5 µm), and larger basidiospores 7.5–12.5 × 7.5–11(–12) µm. It is sometimes difficult to find morphological features that support clear genetic-based distinctions in species complexes. In other taxonomic studies of sequestrate and non-sequestrate species complexes (the *Megacollybia platyphylla* and *Tuber gibbosum* complexes, for example), the microscopic structures in the pileipellis and peridium provide the best non-genetic features to separate the various species (Hughes *et al*. 2007, Bonito *et al*. 2010); we suspect that it may prove to be a similar case among these sequestrate species of *Entoloma*.

Sequestrate fungi are important food resources for a diversity of animals, and their spores generally remain viable after passage through the digestive system (Fogel & Trappe1978, Caldwell *et al*. 2005, Elliott *et al*. 2018, 2019a, b, c, Elliott & Vernes 2019). It is possible that animals co-occurring with *E. sequestratum* also use it for food, but scat samples would need to be collected near the type collection and then analyzed in order to determine if this is true. There are limited reports of sequestrate *Entoloma* species occurring in animal diets, but the Australian long-footed potoroo (*Potorous longipes*) has been reported to eat fungal species in the genus (Nuske *et al*. 2017). No close relatives to this potoroo occur in Thailand, but many rodents found in the region likely eat fungi. This study highlights the need for further research into the diversity of fungi associated with the tropical forests of northern Thailand, and we hope it inspires future investigation into the diversity of sequestrate fungi endemic to Southeast Asia.

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