

# New species and records of orbiliaceous fungi from Georgia, USA

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## Abstract

*Orbilia georgiana* and *O. renispora* are described as new species to science, in addition, *O. eucalypti*, *O. occulta*, and *O. xanthoguttulata* are reported based on collections taken from the state of Georgia, USA. *Orbilia xanthoguttulata* is a new record for North America. The morphology of all species is described based on both sexual and asexual morphs. Asexual morphs were obtained from ascospore isolates, from which also DNA was extracted and used to amplify rDNA genes (ITS and LSU) for phylogenetic comparison.

**Keywords** Orbiliomycetes · *Orbilia* · *Trinacrium* · *Dicranidion* · phylogenetic analysis · Taxonomy

## Introduction

Members of Orbiliaceae are characterized by producing small, usually light-colored (rarely dark), and semi-translucent apothecia with often minute asci and ascospores which usually contain KOH-soluble spore bodies (SBs), a refractive vacuolar organelle inside the ascospores which is only visible in living spores, and frequently swollen paraphysis apices. In the traditional classification system, the family has been assigned to the order Helotiales. Molecular evidences placed the family Orbiliaceae in a new order Orbiliales and new class Orbiliomycetes (Eriksson et al. 2003). It includes at present three validly published teleomorph genera, *Orbilia* Fr., *Hyalorbilia* Baral & G. Marson, and *Pseudorbilia* Ying Zhang bis et al. (further so far unpublished or asexually typified genera are listed in Jaklitsch et al. 2016 and Baral et al. 2017). Among them, *Orbilia* is the largest genus with much over 300–400 detected species (Quijada et al. 2012,

2016; Jaklitsch et al. 2016; Baral et al. 2017). The class is widely distributed from subarctic to tropical areas and in humid as well as arid climates. During the recent years, various new species, new records, and new anamorph-teleomorph relationships have been reported (e.g., Karasch et al. 2005; Liu et al. 2005, 2006; Mo et al. 2005; Yu et al. 2006, 2011; Su et al. 2011; Qiao et al. 2012; Quijada et al. 2012, 2015, 2016; Ren et al. 2014; Qiao et al. 2015; Zhang et al. 2015; Akata and Dogan 2015; Zhang et al. 2016; Haelewaters et al. 2018).

Along with the development of molecular techniques, phylogenetic analysis for the classification of fungi became popular, also among scientific researchers of Orbiliaceae, based on single or multiple gene data in addition to morphological studies. Phylogeny mainly focuses on sequence analysis of internal transcribed spacer (ITS1 and ITS2) gene regions, 5.8S rDNA, 18S and 28S rDNA,  $\beta$ -tubulin, but also on several other genes (Li et al. 2005, Zhang et al. 2015, 2016, Baral et al. 2017). ITS region is used as the primary barcode for fungal identification, and also in Orbiliomycetes its suitability in species delimitation has been proved.

The state of Georgia is part of the subtropical southeast of North America and is rich in biodiversity including fungi. Nevertheless, the diversity and distribution of Orbiliaceae are poorly known around this area. In this study, a survey of orbiliaceous fungi from several localities in Georgia was done in May (South Georgia, strain number with TF) and September 2015 (Northwest and South Georgia, strain numbers with GA). With the aim to provide extensive information about the holomorph, the present study includes descriptions of both the sexual and asexual morph. The latter

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was established in pure culture in the laboratory in Georgia for teleomorph collections, whereas microscopic study of each teleomorph was done in Guangxi University. We combined morphological and molecular data to identify five species. Sequences were obtained from six strains that represent these five species. In the present paper, two new species are described and illustrated. Additional three species are also reported.

## Materials and methods

### Collection of teleomorph, isolation and characterization of anamorph

Moist or dry apothecia of Orbiliaceae were collected from bark and wood of decaying, fallen, hygric, or xeric, branches of broad-leaved or sometimes coniferous trees. All herbarium materials were deposited at the Herbarium of University of Guangxi (GXU) in Nanning, China, and all pure cultures are deposited in University of Georgia in Tifton, USA (strain numbers in Table 1). Observations, measurements, and photographs were taken with an Olympus BX41 microscope and an Olympus SZX16 stereo microscope with integrated camera. To establish cultures, fresh apothecia were fixed to the lid of a petri dish with their hymenia facing downward allowing the asci to shoot on the surface of water agar (WA: 18 g agar, 1000 ml distilled water). The spore deposits were then transferred to PDA as described by Liu et al. (2005) in order to get a pure culture. Elements of the apothecia were predominantly studied in the dead state while those of the asexual morph were documented in the living state. The biometrics for ascospore and conidial size and septation as well as conidiophore size were calculated by measuring around 10 elements. The following abbreviations were adopted from Baral (1992): \*, living state; †, dead state while without symbol is not sure.

### DNA extraction, PCR amplification, and sequencing

Fungal mycelium and conidia were observed from pure cultures grown on potato-dextrose agar (PDA) after 2 weeks at 25 °C in the dark. The cultures were scraped and mechanically disrupted by grinding them with sterilized sand and liquid nitrogen using a mortar and pestle. Total DNA was extracted using DNeasy Plant Mini Kit following the manufacturer's instructions. Sequence data were generated from the internal transcribed spacer region of nuclear ribosomal DNA (ITS) and large subunit of rDNA gene (LSU) amplified with primer pairs ITS4/ITS5 (White et al. 1990) and LROR/LR5 (Vilgalys and Hester 1990). PCR reaction conditions were as follows: initial denaturation 5 min at 94 °C, followed by 35 cycles of 94 °C for 30 s, annealing at 58 °C for 1 min, and extension at 72 °C for 1 min, followed a final extension at 72 °C for 5 min. PCR products were visualized under UV light on 1.0% agarose gels stained with ethidium bromide. The purified PCR products were directly sequenced on both strands with the same pair of primers that were used for amplification.

### Phylogenetic analysis

We performed phylogenetic analyses with related sequences from GenBank using the ITS region as well as LSU rDNA data. The maximum-likelihood (ML) analyses were performed with MEGA version 6 using Kimura 2-parameter model, including transitions and transversions with pairwise gap deletion. The branch support was inferred from 500 replicates of bootstrap. GenBank accession numbers are given in Table 1 and the phylogenetic trees. The combined analysis is shown in Fig. 6, the separate analyses of ITS and LSU in the supplement files S1 and S2.

**Table 1** Strains treated in the present study, sorted according to collection number (in bold: new species described here). \* culture and sequence only (without data on asexual and sexual morph, both mixture with *O. georgiana*)

Species	Specimen number (Guangxi University)	Strain number (Georgia University)	GenBank accession/ITS	GenBank accession/LSU
<i>Orbilia occulta</i>	GXU1467	TF018	MG742398	MG742399
<b><i>Orbilia georgiana</i></b>	GXU1472	GA006	MG742408	MG742396
<b><i>Orbilia cf. georgiana</i></b>	GXU1478	GA013		
<b><i>Orbilia georgiana</i></b>	GXU1493	GA026a		
<i>Orbilia cf. xanthoflexa</i> *		GA026b	MG742405	MG742400
<i>Orbilia eucalypti</i>	GXU1484	GA028	MG742397	MG742409
<b><i>Orbilia renispora</i></b>	GXU1486	GA031		
<b><i>Orbilia renispora</i></b>	GXU1487	GA038	MG742403	MG742404
<b><i>Orbilia georgiana</i></b>	GXU1488	GA040	MG742394	MG742395
<i>Orbilia xanthoguttulata</i>	GXU1491	GA047	MG742406	MG742407
<b><i>Orbilia georgiana</i></b>	GXU1497	GA055a		
<i>Orbilia sp.</i> *		GA055b	MH520123	MH520122

## Results

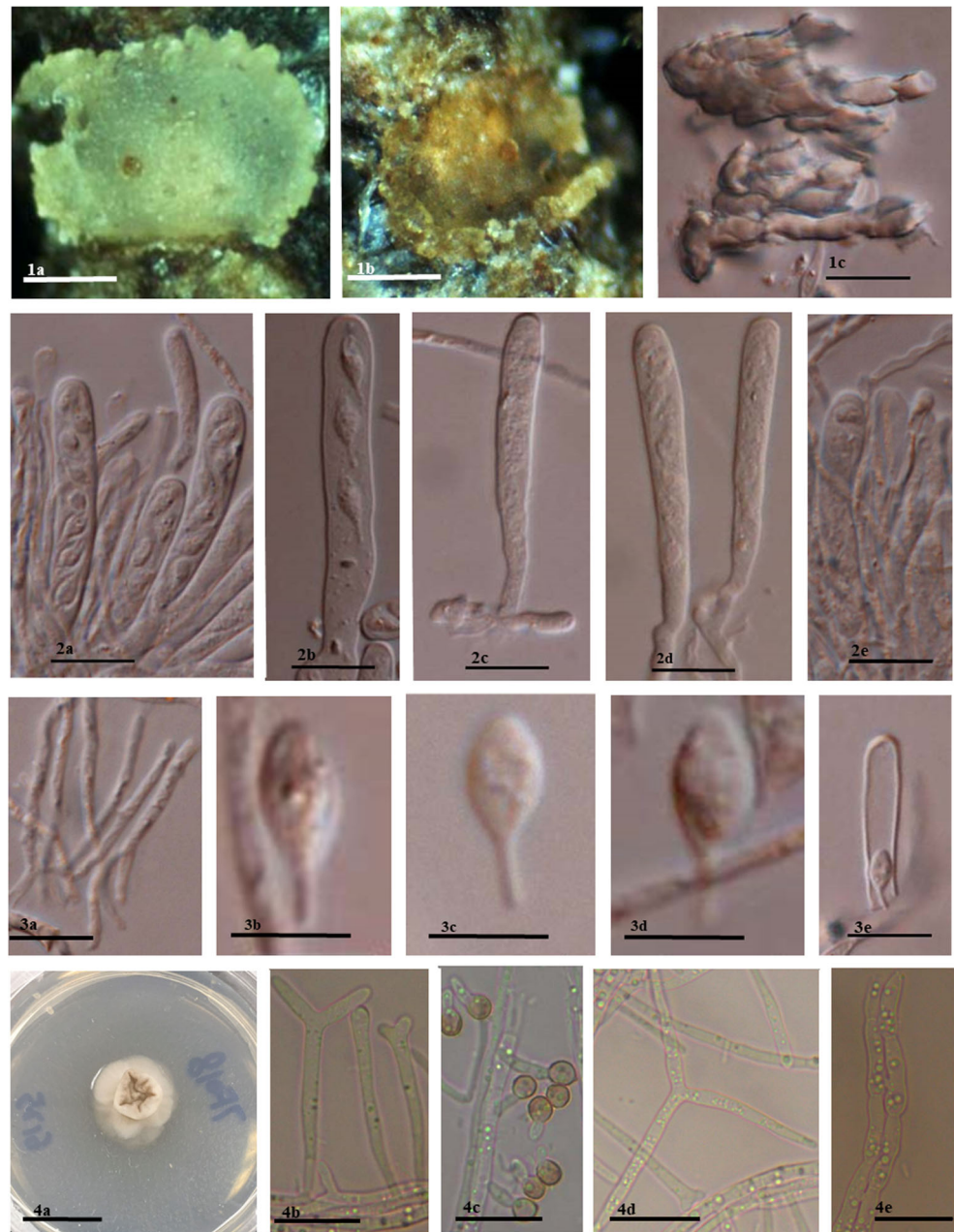
### Taxonomic description

*Orbilbia occulta* (Rehm) Sacc., *Syll. Fung.* 8: 623 (1889) (Fig. 1).

**Teleomorph:** Apothecia superficial, with distinctly denticulate margin; gregarious in small groups, waxy, translucent, appressed to the surface of decayed bark, disc 0.25–0.35 mm diameter, pale grayish-cream to light orange. Ectal excipulum composed of *textura globulosa*, cells thin-walled,  $\dagger 7\text{--}10\ \mu\text{m}$  diameter; marginal denticles composed of refractive glassy processes,  $8\text{--}14 \times 2\text{--}3\ \mu\text{m}$ . Asci

cylindric-clavate,  $\dagger 43\text{--}57 \times 4.5\text{--}6.0\ \mu\text{m}$ , tapering below to a flexuous, bifurcate stipe, apex hemispherical to slightly truncate, with a  $\dagger 0.5\text{--}0.8\ \mu\text{m}$  thick-apical wall. Ascospores  $\dagger 8\text{--}9 \times 2.8\text{--}3.3\ \mu\text{m}$ , with an ellipsoid (to fusoid) head ( $\dagger 5.5\text{--}6.5\ \mu\text{m}$  long) and a thin and basally not inflated, straight tail ( $2\text{--}2.7\ \mu\text{m}$  long); smooth, uniseriate, containing 1–2 small, globose lipid bodies in the center of spores; spore body tear-shaped, visible as indistinct transparent region in spore apex. Paraphyses hyaline, cylindrical, not or slightly inflated at clavate apex,  $\dagger 1.5\text{--}2.7\ \mu\text{m}$  wide, mostly covered by a thin layer of rough exudate. Anamorph: Colonies whitish, light brown in the center of PDA plate, 20 mm diameter after incubating for

**Fig. 1** *Orbilbia occulta* with trinacrium-like asexual morph (GXU1467). 1a, rehydrated apothecium; 1b, dry apothecium; 1c, marginal glassy processes; 2a–e, asci and paraphyses; 3a, paraphyses; 3b, ascospore in broken ascus; 3c–e, ascospores; 4a, colony on PDA medium; 4b, young conidia formed on short conidiophores on mycelium; 4c, chlamydospore-like bodies formed on side branches; 4d, young and mature conidium; 4e, conidiophores with septate. Dead state, except for 4b–d. Scale bars 1a–b = 0.1 mm; 1c, 2a–e, 3a, 3e, 4b–e = 10  $\mu\text{m}$ ; 3b–d = 5  $\mu\text{m}$ ; 4a = 2 cm



10 days, appressed to the agar but with sparse aerial mycelium, hyphae hyaline, septate, branched,  $*2.2\text{--}3.5\ \mu\text{m}$  wide. Conidiophores reduced, integrated, forming a short nodule of ca.  $*0.5 \times 1\ \mu\text{m}$  or a  $*4 \times 1.2\ \mu\text{m}$  large cell. Conidia triradiate, with a 3–4-septate main axis (stipe) of  $*14\text{--}20 \times 1.5\text{--}2.0\ \mu\text{m}$  and two  $\pm$  straight, 3–5-septate arms formed by dichotomous branching, of equal length or slightly different,  $*13\text{--}24 \times 1.8\text{--}2.3\ \mu\text{m}$ ; Chlamydospore-like bodies subglobose,  $*2.5\text{--}3 \times 2.4\text{--}2.8\ \mu\text{m}$ , with smooth, thick, light-ochre wall, formed on hyaline lateral branches  $*3.5\text{--}6.5 \times 1.1\text{--}1.3\ \mu\text{m}$ .

**Specimens examined:** USA, GA, Tifton, Black Shank Farm, on the bark of a corticated branch of deciduous tree lying on the ground, altitude 105 m, Y.Y. Shao, 8 V 2015, GXU1467.

***Orbilina georgiana* Y.Y. Shao, Baral & Bin Liu, sp. nov. (Fig. 2)**  
—Mycobank MB826737

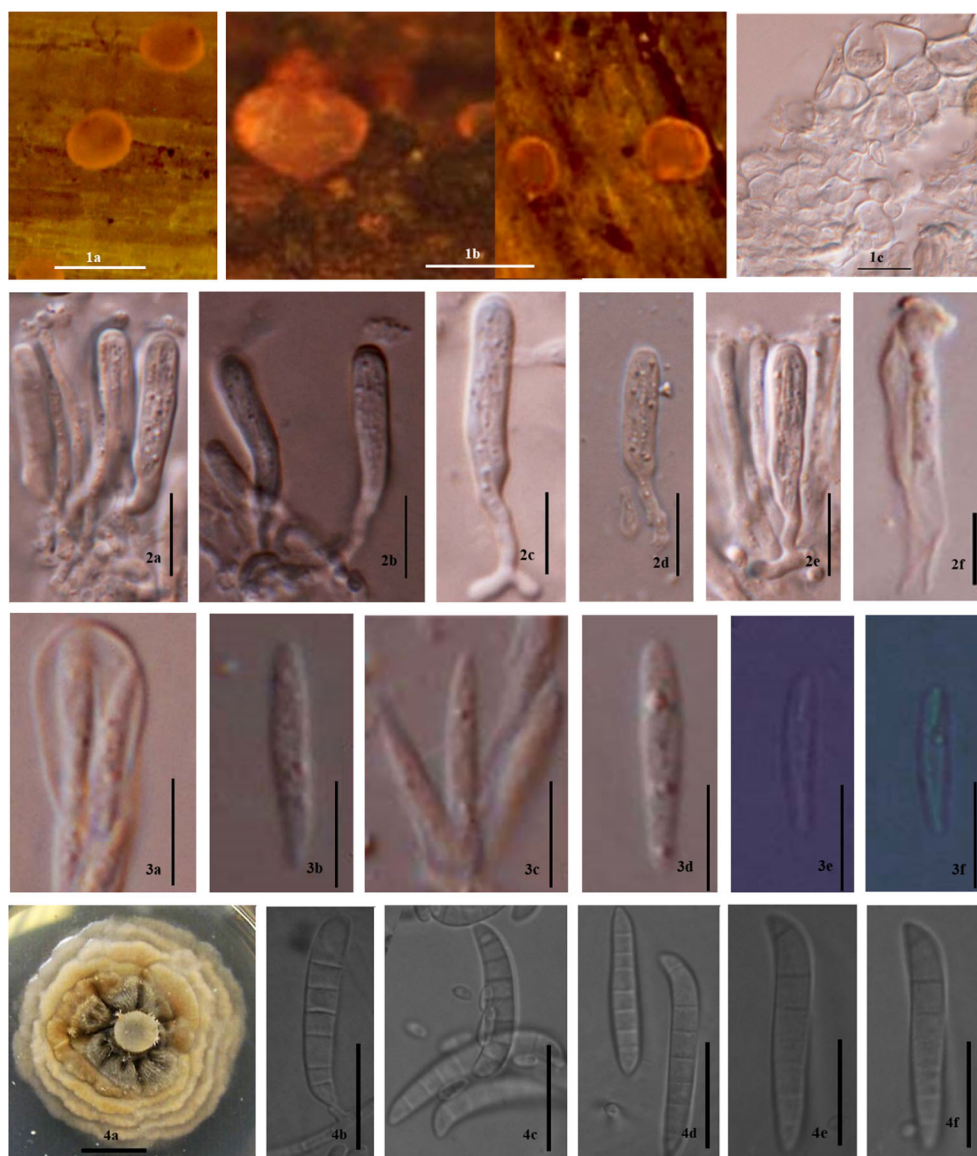
**Etymology:** the specific epithet refers to the locality, the state of Georgia (USA) where the species was collected.

**Holotype:** USA, GA, Albany, Chehaw Park, altitude 60 m, on decayed wood of decorticated branch of *Quercus* lying on the ground, 3 IX 2015, Y. Y. Shao, P. S. Ji & H. F. Zheng, GXU1472 (holotype), ex-holotype culture GA006; GenBank: MG742408 ITS, MG742396 nLSU rDNA.

**Diagnosis:** Similar to *Orbilina aprilis* but with slightly longer and narrower ascospores and very different ITS and LSU rDNA data.

**Teleomorph:** Apothecia rehydrated (0.15–)0.25–0.65(–0.8) mm diameter, 0.08 mm high, pale to light yellow-orange or

**Fig. 2** *Orbilina georgiana* with vermisporea-like asexual morph (1a from GXU1488; 1b, 3a–d, 4b–f from GXU1472; 1c, 2a–f from GXU1493, 3e–f *Orbilina* cf. *georgiana* from GXU1478). 1a, b, rehydrated apothecia; 1c, ectal excipulum near base; 2a–f, asci and paraphyses; 3a–f, ascospores (3a inside of ascus); 4a, colony on PDA medium; 4b–f, conidia. Dead state, except for 3e–f, 4b–f. Scale bars 1a–b = 0.5 mm; 1c, 2a–e, 4b–f = 10  $\mu\text{m}$ ; 2f, 3a–f = 5  $\mu\text{m}$ ; 4a = 2 cm



cream-reddish or orange-rose when fresh, rose-red when dry, slightly to strongly translucent, round, scattered to subgregarious; disc flat to slightly convex, margin distinct, not protruding, smooth to very finely rough; sessile on a broad base, superficial or somewhat erumpent between fibers; dry slightly contracted. Ectal excipulum at base up to mid flanks of vertically oriented *textura angularis* to *t. prismatica*, 25  $\mu\text{m}$  thick at base, cells thin-walled,  $\dagger 6\text{--}13$   $\mu\text{m}$  wide, marginal cortical cells elongate,  $\dagger 3\text{--}6.7$   $\mu\text{m}$  wide, without glassy processes. Medullary excipulum 25  $\mu\text{m}$  thick. Asci 8-spored,  $\dagger 28\text{--}38 \times 4\text{--}5$   $\mu\text{m}$ , cylindrical-clavate, apex rounded to slightly truncate, with apical wall thickening of ca.  $\dagger 0.9$   $\mu\text{m}$  and small apical chamber, base tapered below in a short to medium long, flexuous stalk, sometimes forked (L- or Y-shaped). Ascospores narrowly fusoid to very slightly clavate, with obtuse apex, straight or sometimes slightly inequilateral, non-septate, smooth,  $*7.5\text{--}11 \times 1.4\text{--}1.5$   $\mu\text{m}$ ,  $\dagger 9\text{--}10.5(-11.5) \times 1.3\text{--}1.6(-1.8)$   $\mu\text{m}$ , 2–3-seriate in the asci; spore body elongated tear-shaped, refractive,  $*1.5\text{--}1.8 \times 0.8$   $\mu\text{m}$ , attached to the wider apical spore end. Paraphyses filiform with uninflated or slightly clavate to capitate apex, terminal cells  $\dagger 1.5\text{--}3.0$   $\mu\text{m}$  wide, cells below  $\dagger 1.8\text{--}2.3$   $\mu\text{m}$ , exudate over paraphyses granular, hyaline to pale orange-yellow, 0.3–1.5  $\mu\text{m}$  thick. Anamorph: Colonies brown to dark yellow, slowly growing on PDA medium, attaining less than 30 mm diameter in 10 days at 25 °C, appressed to agar on PDA medium. Aerial mycelium sparse, hyaline, septate, branched,  $*2.6\text{--}3.4$   $\mu\text{m}$  wide. Vegetative hyphae branched and septate, producing many conidia under natural light after 7 days at room temperature. Conidiophores reduced, non-septate, unbranched, initially erect,  $*1.4\text{--}2 \times 0.8\text{--}1$   $\mu\text{m}$ . Conidia unbranched, straighter in the center, cylindrical but tapering, and slightly curved towards the ends, apex obtuse, indistinctly truncate at the base, 6–7(–8)-septate,  $*(12\text{--})13.5\text{--}19(-22.5) \times 2.5\text{--}3.2$   $\mu\text{m}$ .

*Additional specimens examined:* USA, GA, Fargo, Stephen C. Foster State Park, altitude 40 m, on decayed wood of decorticated branch of *Quercus* lying on the ground, 24 IX 2015, Y. Y. Shao, GXU1488; *ibid.*, decorticated branch of *Acer*, 24 IX 2015, Y. Y. Shao, GUX1493; Canyon State Park, altitude 310 m, on decayed wood of decorticated branch of *Acer* lying on the ground, 30 IX 2015, Y. Y. Shao, GXU1497. Not included: USA, GA, Albany, Chehaw Park, altitude 60 m, on decayed wood of decorticated branch of *indet. deciduous tree*, 3 IX 2015, Y. Y. Shao, P. S. Ji & H. F. Zheng, GXU1478.

*Notes:* Among the known species of *Orbilbia*, *O. georgiana* is morphologically similar to *O. aprilis*, but differs from it in slightly longer and narrower ascospores ( $*7.5\text{--}11 \times 1.4\text{--}1.5$   $\mu\text{m}$ ,  $\dagger 9\text{--}10.5 \times 1.3\text{--}1.6$   $\mu\text{m}$ ) vs. ( $*6.5\text{--}9.5 \times 1.6\text{--}2$   $\mu\text{m}$ ,

$\dagger 6.2\text{--}9 \times 1.4\text{--}1.7$   $\mu\text{m}$ ). *O. georgiana* is also very similar to *O. nothoaprilis* *ined.*, from which it differs by narrower spores ( $*1.4\text{--}1.5$   $\mu\text{m}$  vs.  $1.7\text{--}1.8$   $\mu\text{m}$ ) and in shorter and wider SBs.

*Orbilbia eucalypti* (W. Phillips & Harkn.) Sacc., Syll. Fung. 8: 628 (1889) (Fig. 3).

Apothecia rehydrated 0.25–1.0 mm diameter, orange red when fresh, not or slightly translucent, round, scattered to subgregarious; disc slightly concave, margin distinct, smooth, not protruding, sessile on a broad base, superficial or somewhat erumpent between fibers; when dry slightly contracted. Ectal excipulum at base up to mid flanks of vertically oriented *textura globulosa* to *t. angularis*, cells thin-walled,  $*13.5\text{--}28 \times 9\text{--}19$   $\mu\text{m}$ . Asci 8-spored,  $\dagger 30\text{--}40.5 \times 3.8\text{--}4.3$   $\mu\text{m}$ , cylindrical, apex rounded or strongly truncate, thin-walled, tapered below in a very short to long stipe, L- to h- or often H-shaped. Ascospores ellipsoid (–fusoid), straight, non-septate, smooth,  $*3.2\text{--}4.5 \times 1.8\text{--}2.7$   $\mu\text{m}$ , germinating spores swollen to  $*5.3\text{--}5.8 \times 3.9\text{--}4.6$   $\mu\text{m}$ , seemingly forming yeast-like spores by budding; spore body is visible, each side with a strongly refractive, globose lipid body of 0.4–1.1  $\mu\text{m}$  diameter. Paraphyses apical cell slightly to strongly capitate,  $\dagger 2.8\text{--}5.3$   $\mu\text{m}$  diameter, lower cells  $\dagger 1.0\text{--}2.8$   $\mu\text{m}$  wide, apex covered with thin layer of granular exudate. Anamorph: Colonies whitish to pale cream, brownish-yellowish in center, slowly growing on PDA, attaining 4 mm diameter in 10 days at 25 °C. Aerial mycelium sparse, hyaline, septate, branched,  $*2.5\text{--}3.5$   $\mu\text{m}$  wide; vegetative hyphae branched and septate, appressed to agar on PDA medium, producing a great number of conidia under natural light after 7 days at room temperature. Conidiophores unbranched, erect,  $*20\text{--}30$   $\mu\text{m}$  long, 2–3-septate,  $*2.5\text{--}3.3$   $\mu\text{m}$  wide near base,  $*2\text{--}2.4$   $\mu\text{m}$  wide near apex. Conidia U-shaped with a conical base and indistinct nodulous protuberance,  $*(10.5\text{--})12.5\text{--}15.5 \times 6.3\text{--}7.5$   $\mu\text{m}$ , the two arms equal or distinctly unequal in length, parallel,  $*5.5\text{--}10.5 \times 3\text{--}3.7$   $\mu\text{m}$ , 1–2 septate (each arm with an oblique basal septum). Also with unbranched subcylindrical conidia with 1–3 septa, e.g.,  $*16.5 \times 4$   $\mu\text{m}$ .

*Specimen examined:* USA, GA, Nicholls, General Coffee State Park, on bark of an attached, decayed coniferous branch, 2 m above the ground, altitude 70 m, 24 IX 2015, Y. Y. Shao & H. F. Zheng, GXU1484.

***Orbilbia renispora* Y.Y. Shao, Quijada, Baral, Haelew. & Bin Liu, sp. nov. (Fig. 4)—Mycobank MB826739**

*Etymology:* according to the reniform ascospore shape.

*Holotype:* USA, GA, Fargo, Stephen C. Foster State Park, on decayed *Quercus* branch on the ground, altitude 40 m, 24 IX 2015, Y. Y. Shao & H. F. Zheng, GXU1487, ex-holotype culture GA038; GenBank: MG742403 ITS, MG742404 nLSU rDNA.

**Fig. 3** *Orbilbia eucalypti* with dicranidion-like asexual morph (*Dicranidion fragile*, from GXU1484). 1a, 1b, rehydrated apothecia; 1c, ectal excipulum near base in median section; 1d, colony on PDA medium; 2a–c, Asci; 2d, paraphyses; 3a, 3e, ascospores; 3b, 3c, ascospores germinating; 4a–e, conidia. Dead state, except for 1e, 3b–d, 4a–e, spores in 2a–c. Scale bars 1a–b = 0.5 mm; 1c = 20  $\mu$ m; 1d = 2 cm; 2a–d, 4a–e = 10  $\mu$ m; 3a–e = 5  $\mu$ m

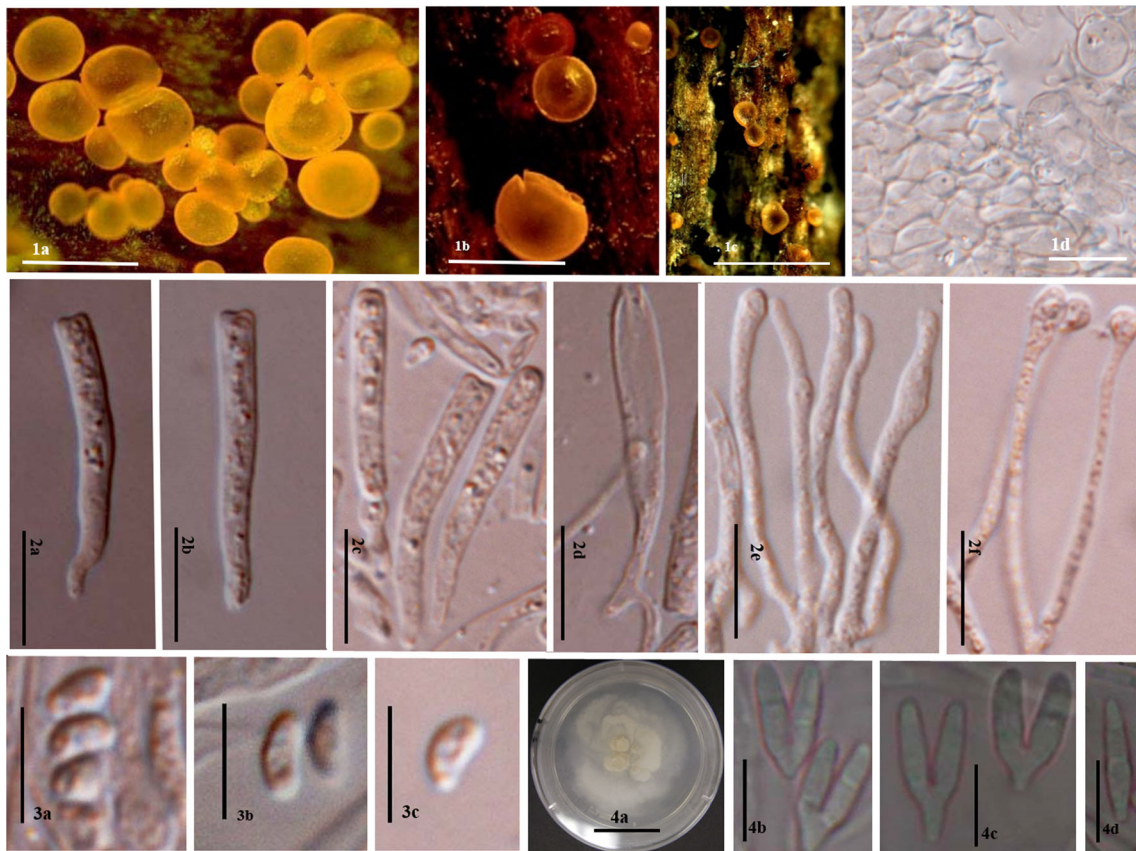


**Diagnosis:** Differs from *Orbilbia xanthostigma* and *O. leucostigma* in larger, much less curved, smooth ascospores.

**Teleomorph:** Apothecia yellow to orange when fresh, (0.25–)0.45–1(–1.6) mm diameter, waxy and translucent, round, subgregarious to gregarious; disc slightly concave, margin distinct, smooth, not protruding, sessile on a broad base, superficial or somewhat erumpent between fibers; when dry slightly contracted. Ectal excipulum at base up to mid flanks of vertically oriented *textura angularis* to *t. prismatica*, marginal cortical cells †3–7  $\mu$ m diameter, glassy processes not seen. Asci 8-spored, †29–39  $\times$  3–3.8  $\mu$ m, cylindrical, apex rounded or strongly truncate, thin-walled, base tapered in a short to long flexuous stalk, L-, Y- or often H-shaped. Ascospores ellipsoid-clavate, slightly to medium curved (reniform), apex rounded to obtuse, base slightly to medium attenuated, non-septate, smooth, \*/†(2.8–)3–3.6(–3.9)  $\times$  1.5–1.8(–2)  $\mu$ m; spore body globose, present in the broader end. Paraphyses filiform, with medium to strongly capitate apex, †(2–)2.5–3  $\mu$ m wide at apex, sometimes bent, lower cells †1.2–2.2  $\mu$ m wide, apical and particularly lower cells

containing many droplets (probably lipid), covered with a hyaline, firmly attached layer of thin exudate. Anamorph: Colonies colorless to light brown on PDA, growing slowly, appressed to agar. Aerial mycelium sparse, hyaline, septate, branched, \*1–2.5  $\mu$ m wide; vegetative hyphae branched and septate, producing a large number of conidia after 7 days at 25  $^{\circ}$ C. Conidiophores unbranched, initially erect, \*3.5  $\mu$ m long, 1.0–2.8  $\mu$ m wide at base, 1.0–1.5  $\mu$ m at apex. Conidia Y-shaped, with two straight, parallel or distinctly diverging arms of  $\pm$  equal length, total size \*9.3–12.3  $\times$  4–5.8  $\mu$ m, stipe \*1.5–3  $\times$  1.4–1.6  $\mu$ m, obconical, aseptate, arms \*5.5–8.5  $\times$  1.8–2  $\mu$ m, cylindrical but slightly tapering above, ca. 2-septate; some conidia unbranched, narrowly fusoid, e.g., \*15.5  $\times$  2.6  $\mu$ m, 3-septate.

**Additional specimens examined:** USA, GA, Nicholls, General Coffee State Park, on decorticated, decayed deciduous branch on the ground, altitude 70 m, Y. Y. Shao & H. F. Zheng, 24 IX 2015, GXU1486. Massachusetts, Boston, Hingham, World's End peninsula, 5 m, on wood of stump of *Quercus*, 29 IX 2013, D. Haelewaters (bhi-F097, H.B. 9997,



**Fig. 4** *Orbilia renispora* with dicranidion-like asexual morph (1a, 1c, 1d, 2a–f, 3a–c: from GXU1486; 1b, 4a–d: from GXU1487). 1a–b, rehydrated apothecia; 1c dry apothecia; 1d, ectal excipulum near base; 2a–c, asci; 2d,

emptied ascus; 2e–f, paraphyses; 3a–c, ascospores; 4a, colony on PDA medium; 4b–d, conidia. Dead state, except for 4b–d. Scale bars 1a–c = 1 mm; 1d, 2a–f, 4b–d = 10  $\mu$ m; 3a–c = 5  $\mu$ m; 4a = 3 cm

GenBank MH445964). Ibid., on wood of log of *Acer*, 29 IX 2013, D. Haelewaters (bhi-F108a, H.B. 9998, GenBank MF161173).

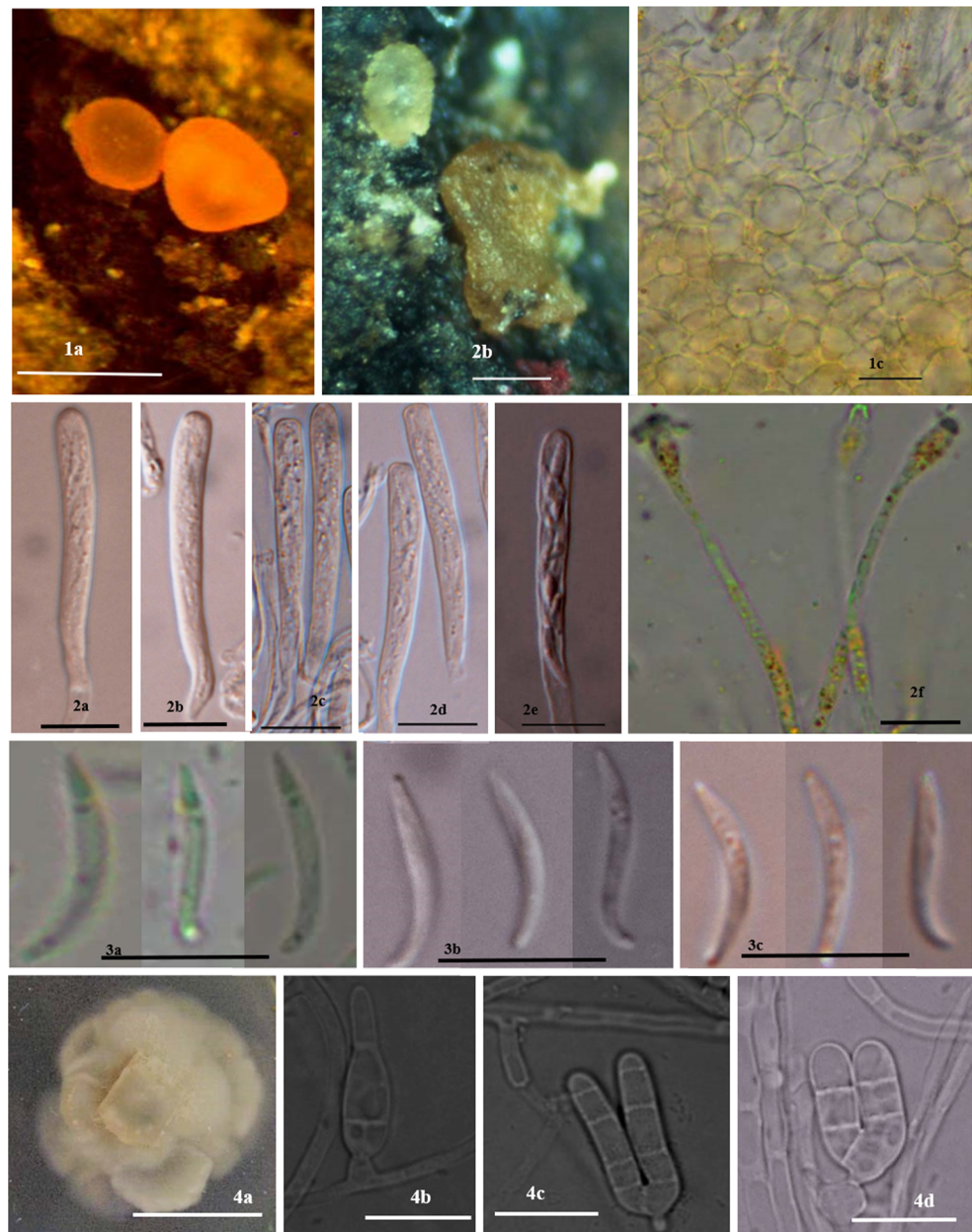
**Notes:** *Orbilia renispora* is similar to *O. xanthostigma* (Fr.: Fr.) Fr. and *O. leucostigma* (Fr.: Fr.) Fr. in the shape and size of ascospores and the dicranidion-like asexual morph, but differs from these species in larger, much less curved, smooth ascospores. *O. leucostigma* and *O. xanthostigma* are extraordinary within Orbiliomycetes in having warted ascospores. Macroscopically *O. renispora* and *O. xanthostigma* appear to be indistinguishable in their yellow-orange color. Further, more recent collections from Massachusetts referable to *O. renispora* were studied by L. Quijada (personal communication) in the living state. They were not included in the description, but their documentation will be published in a future paper.

***Orbilia xanthoguttulata* Baral, in Quijada et al., *Phytotaxa* 175: 12 (2014) (Fig. 5)**

**Teleomorph:** Apothecia (0.2–)0.3–1(–1.5) mm diameter, light to bright yellow to orange when fresh, not or slightly translucent, round, scattered to subgregarious; disc flat, margin distinct, smooth, not protruding, sessile on a broad base,

superficial; when dry slightly contracted. Ectal excipulum at base up to mid flanks of vertically oriented *textura angularis* to *t. prismatica*, cells thin-walled,  $\dagger$ 8–14  $\times$  6–9  $\mu$ m, marginal cortical cells  $\dagger$ 2.6–7.8  $\mu$ m diameter. Asci 8-spored,  $\dagger$ 35–44  $\times$  3.4–4.1, cylindrical, apex rounded or strongly truncate but not notched, tapered below in a medium long flexuous stalk, sometimes L-shaped. Ascospores narrowly fusiform, slightly helicoid, \*12–14  $\times$  1.5–1.7  $\mu$ m,  $\dagger$ 10.5–13.5  $\times$  1.3–1.5  $\mu$ m, apex acute, base strongly curved, attenuated in a tail-like end, 2–3-seriate, lower spores inverted in the asci; spore body narrowly tear-shaped, \*2.5–3.2  $\times$  0.7–0.9  $\mu$ m; Paraphyses filiform, apically moderately to strongly ellipsoid-spathulate to sublageniform,  $\dagger$ 3.0–4.5  $\mu$ m wide, lower cells  $\dagger$ 1.3–1.7  $\mu$ m wide, apex mostly covered by a thick, glassy, hyaline, cap-like exudate; orange-yellow lipid bodies present in the paraphyses and ectal excipulum. Anamorph: Colonies colorless to light brown, appressed to agar, growing slowly on PDA, attaining 25 mm diameter in 10 days at 25  $^{\circ}$ C. Aerial mycelium sparse, hyaline, septate, branched; vegetative hyphae branched and septate, \*1.0–2.5  $\mu$ m wide, producing a few conidia after 10 days at 25  $^{\circ}$ C. Conidiophores unbranched, initially erect, \*2.0–2.8  $\mu$ m wide at base and 1–1.5  $\mu$ m at apex. Conidia U-shaped,

**Fig. 5** *Orbilbia xanthoguttulata* with dicranidion-like asexual morph (from GXU1491). 1a, rehydrated apothecia; 1b, dry apothecia; 1c, ectal excipulum near base; 2a–e, asci; 2f, paraphyses; 3a–c, ascospores; 4a, colony on PDA medium; 4b–d, conidia and conidiophores. Dead state, except for 3a, 4b–d. Scale bars 1a = 1 mm, 1b = 0.1 mm; 1c, 2a–f, 3a–c, 4b–d = 10  $\mu$ m; 4a = 2 cm



with a stalk-like nodulous protuberance, the two arms  $\pm$  equal in length,  $*15\text{--}19.5 \times 9\text{--}10 \mu\text{m}$  (values from two conidia), arms  $9.5\text{--}14 \times 3.5\text{--}5 \mu\text{m}$ , with 2–5 septa (one arm with oblique basal septum), also with unbranched subcylindrical conidia with 1–3 septa.

*Specimen examined:* USA, GA, Tifton, Black Shank Farm, on bark of corticated branch of indet. deciduous tree on the ground, altitude 105 m, Y. Y. Shao, 16 IX 2015, GXU1491.

### Phylogenetic analysis

Maximum-likelihood trees were produced based on separate and combined data sets of ITS and LSU sequences gained from the ascospore isolates from Georgia and from

sequences retrieved from GenBank to compare the phylogenetic relationships among the species. The phylogenetic analyses based on the combined ITS+LSU sequences and separate datasets of ITS and LSU D1-D2 regions formed several well-supported lineages (Figs. 6, S1–2). The positions of our five isolates are more or less in accordance with these three trees. Among the different sections of *Orbilbia* proposed by Baral et al. (2017), some were not recognized as supported clades according to their combined phylogenetic inference, which is confirmed by the present analyses. In the combined analysis the bootstrap values were generally higher, but the paraphyly of sections *Hemiorbilbia* and *Aurantiorubrae*, which are mainly based on morphological data, remained evident.





European *O. xanthoguttulata*, although *O. succulenticola* differs from European *O. xanthoguttulata* by only 1–3 nt.

GA026b and GA055b, two strains that were identified from their sexual morph as *O. georgiana* (GXU1493 and GXU1497), clustered in section *Orbilbia* instead of section *Hemiorbilbia*, the former near the North American *O. xanthoflexa* ined., the latter unresolved, which suggests mixed populations. Because the sexual morph from which they originate remained obscure, we refrained from including their sequences in the trees.

## Discussion

Two species new to science (*O. georgiana*, *O. renispora*) and one species newly reported for North America (*O. xanthoguttulata*) are presented in the genus *Orbilbia*. For these as well as *O. occulta* and *O. eucalypti*, the asexual morph was obtained in pure culture, from which also rDNA sequences were gained. This is the first report of the holomorph of *O. occulta* (asexual and sexual morph) linked with a sequence.

The name *Orbilbia occulta* was applied based on a reexamination of type material (Baral et al. [in prep](#)). Because of the thick-walled, only slightly truncate ascus apex and not or only slightly inflated paraphyses, *O. occulta* belongs in subgenus *Hemiorbilbia* of which it is the type species (Baral 1994). Its most remarkable character is the thin spore tail that forms the basal end of the ellipsoid(-fusoid) ascospore. This tail is shorter in the present collection compared to previous samples, including the not yet formally designated lectotype (Baral et al. [in prep](#)), except for J.B. Ellis NAF 438 (as *Peziza regalis*) which concurs well with our sample. In the tailed spores, *O. occulta* resembles *O. brevicauda* Y. Zhang, Baral & K.Q. Zhang and *O. aristata* (Velen.) Velen., but differs from the former by wider spore heads and from the latter by shorter spore tails.

The Y-shaped conidia of the anamorph of *Orbilbia occulta* are similar to those of *Trinacrium subtile* Riess but much smaller and cannot be identified with a known species. In pure culture also small, subglobose, light brown chlamydospore-like bodies developed on hyaline side branches. Although brown-walled spores have so far never been seen in pure culture of Orbiliomycetes, it seems from the single available photo that these structures emerged from the hyphae of the cultured *Orbilbia*.

This is the first report of the asexual morph of *Orbilbia occulta*, and it is the first sequence presented for this species. *O. occulta* was so far only known from a few authentic collections on bark of *Malus* and *Vitis* in New Jersey, and a more recent sample on bark of *Cornus* in North Carolina studied in the living state (Baral et al. [in prep](#)). *O. occulta* deviates from *O. spermoides* ined. by a 9% distance in the ITS region.

Our collections of *Orbilbia georgiana* were distinguished from the other species of this study by narrowly fusoid to very slightly clavate ascospores with an elongated tear-shaped spore body in the apex, asci with hemispherical, thick-walled apices, and not or only slightly inflated paraphyses covered by a layer of exudate. Ascus and paraphysis features reveal the species as a member of subgenus *Hemiorbilbia*. Because of a similar ascospore shape, *O. georgiana* can be confused with distantly related species, such as *O. luteorubella* (Nyl.) P. Karst. and *O. rectispora* (Boud.) Baral, which differ by having a thin-walled, strongly truncate ascus apex in the dead state, the former species also by longer and thinner spore bodies as well as capitate paraphyses, and the latter by globose spore bodies. Among the species with a thick-walled, hemispherical apex, *O. vinosa* (Alb. & Schwein.) P. Karst. differs by much longer ascospores, whereas *O. aprilis* Velen. matches quite well *O. georgiana*. However, *O. aprilis* and *O. georgiana* are quite distantly related when comparing ITS and LSU data, whereas *O. nothoaprilis* ined., which is morphologically also very similar, clustered with *O. georgiana* in a strongly supported clade in the phylogenetic analyses (Figs. 6, S1–2), though with a distance of 4.5% in the ITS and 2% in the LSU D1–D2. One of the five samples of *O. georgiana* from the type locality (GXU1478, Fig. 2: 3e–f) had distinctly smaller spores (\*5.5–6.7 × 1–1.2 μm) and was, therefore, not included in the description.

*Orbilbia eucalypti* has previously been mostly reported under the names *O. coccinella* Fr. or *O. alnea* Velen., but the type material of *O. coccinella* in UPS turned out to be a rare North European species with 16-spored asci and strongly curved spores (Baral et al. [in prep](#), see Quijada et al. 2016). Further misidentifications include the asexual morph *Dactylella coccinella* Ying Yang & Xing Z. Liu, which has unbranched conidia. It was based on “*O. coccinella*” CBS 916.72 and was erroneously reported as the asexual morph of *O. coccinella* s. auct. (= *O. eucalypti*) which has *Dicranidion fragile* as asexual morph (Baral et al. 2017, [in prep](#)).

*Orbilbia renispora* resembles *O. xanthostigma* and *O. eucalypti* in having similar dicranidion-like conidia, but *O. xanthostigma* differs by smaller, strongly curved, warted ascospores, and *O. eucalypti* by ellipsoid, straight ascospores. *O. renispora* also resembles *O. umbilicata* Baral, Y. Zhang bis & K.Q. Zhang, which differs by longer and narrower ascospores and narrowly lanceolate protruding paraphyses (Zhang et al. 2009), or *O. dryadum* (Velen.) Baral & E. Weber, which differs by narrower, straight spores, unbranched conidia, and whitish apothecia (Baral et al. [in prep](#)). *Orbilbia renispora* is referred to as *Orbilbia* sp. 2 in the checklist of fungi at the Boston Harbor Islands (collections bhi-F097, bhi-F108a; Haelewaters et al. 2018).

*Orbilbia xanthoguttulata* is easily recognized by its spatulate paraphyses covered by glassy caps of exudate and the content of abundant yellow-orange lipid bodies. Yet, *O. succulenticola* is morphologically very similar, differing by shorter spores and spore bodies, and quite another

ecology compared to *O. xanthoguttulata* in Europe (Quijada et al. 2014).

The above molecular results on this species, together with the comprehensive analysis of the ITS region of *O. xanthoguttulata* and *O. succulenticola* in Baral et al. (in prep), appear to indicate that the specimen from Georgia belongs to a different, cryptic taxon, even though European *O. xanthoguttulata* showed a rather high variation in the ITS. Further North American collections are required to clarify the status of the specimen from Georgia.

*O. xanthoguttulata* and *O. succulenticola* resemble *O. aurantiorubra* Boud. and *Orbilina vermiformis* Baral et al. (Yu et al. 2007) in having similarly curved, helicoid ascospores and narrowly tear-shaped SBs. Yet, the former differs by orange-rose apothecia, capitate-clavate paraphyses, and the absence of pigmented lipid bodies, while instead KOH-soluble cytoplasmic bodies are present, and the latter differs by whitish apothecia, hardly inflated paraphyses, absence of pigmented lipid bodies, and smaller spores. Besides, both species differ by their unbranched conidia.

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

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