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New record of *Trichoglossum rasum* from Asia

Prabhugaonkar A[1] and Pratibha J[2]

[1]*Botanical Survey of India, Eastern Regional Centre, Shillong 79300, India*
[2]*A4/G2, Vijaynagar Co-operative Housing Society, Corlim, Goa- 403110. Email: jalmipratibha@rediffmail.com*

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Abstract

Trichoglossum rasum, a rare species in the genus *Trichoglossum*, previously reported only from New Caledonia in 1909, was recently observed in Khasi hills, India. This paper illustrates important distributional record along with first molecular phylogenetic placement of the species. A review of all 19 hitherto accepted species in the genus is also provided.

Key words – Fungal diversity, Geoglossaceae, Khasi hills

Introduction

The Khasi hills in the Indian state of Meghalaya have a wide range of flora and fauna. The diverse vegetation is unique in having a mixture of Asiatic and Indian Peninsular elements. The Environment Report, Meghalaya, (2005) has expressed concerns that species that were common about 20 to 30 years ago have become rare due to anthropogenic pressures such as overexploitation, deforestation, mining, shifting cultivation and habitat destruction. In this regard, efforts are being made to document the biodiversity resources. In one of such effort, an ascomycetous fungus, commonly called 'earth tongue' and belonging to Geoglossaceae was collected. On microscopic examination, it was determined to belong to genus *Trichoglossum* Boud. (Boudier, 1885) with distinct trichomes on the surface and setae associated with Asci. The genus *Trichoglossum* has 19 accepted species (Table 1) till date (Index fungorum 2016). On careful morphological comparison, the species was identified as *Trichoglossum rasum* Pat. (Patouillard 1909) and thus forming a rare taxonomic record from the region. A first molecular phylogenetic analysis of species was performed to support this work.

Materials & Methods

Collection and culturing

Freshly gathered samples from botanical garden of Botanical Survey of India, Eastern Regional Centre, Shillong, Meghalaya, were photo-documented and examined under a stereomicroscope. Fungal fruiting bodies were hand-sectioned, mounted in lactophenol and observed under a light microscope. Repeated attempts of culturing fungus failed. Silica gel dehydrated fruiting bodies (aerial tissue) were used for DNA isolation and sequencing. Sequencing was done at Rajiv Gandhi Centre for Biotechnology, Thiruvananthpuram, Kerala, India. The holotype is deposited in the herbarium of the Indian Agricultural Research Institute, New Delhi,

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Corresponding Author: Ashish Prabhugaonkar – e-mail – ashishprabhugaonkar@gmail.com

India (HCIO) and isotype at herbarium of Botanical survey of India, Shillong (ASSAM). The taxonomic record has been deposited in 'Facesoffungi' database (Jayasiri et al. 2015).

DNA isolation and PCR Analysis

Silica gel dehydrated fruiting bodies (aerial tissue) were used for DNA isolation and sequencing. DNA isolation and PCR analysis was done according to Prabhugaonkar & Bhat (2011). The 5.8S nuclear ribosomal gene with the two-flanking internal transcribed spacers (ITS), 28S nrDNA sequence (LSU) were amplified and sequenced using the primer pairs ITS-1F + ITS-4R (White et al. 1990), LR5 + LROR (Crous et al. 2009) respectively. The sequence quality was checked using Sequence Scanner Software v1 (Applied Biosystems). Sequence alignment and required editing of the obtained sequences were carried out using Geneious Pro v5.1 (Drummond et al. 2010).

Sequence alignment and phylogenetic analysis

The sequences were blasted in GenBank with Blastn. ITS and LSU data sets generated based on the blasts and available literature (Hustad et. al. 2013). The combined data matrix was aligned using MEGA 7.0.18 to allow maximum alignment and maximum sequence similarity. A phylogenetic analysis was conducted using maximum likelihood (ML) in MEGA 7.0.18 (Kumar et al. 2008) with 1000 bootstrap replicates. The most suitable substitution models for the respective data sets were selected by using MEGA 7.0.18. Kimura 2-parameter model with Gamma distribution was used in analysis. Gaps were treated as a pair wise deletion and trees were viewed with MEGA 7.0.18. All newly generated sequences used in this study are deposited in GenBank.

Results

Taxonomy

Trichoglossum rasum Pat., Bull. Soc. mycol. Fr. 25: 130. 1909 Fig.2
Facesoffungi number: FoF02878

Saprobic in soil. Fruting body 3–4.5 cm, black, stipitate, erect, clavate to spatulate, with compressed ascogenous portion, ellipsoidal and inflated, with distinctly visible trichomes under hand lens; stipe 1.5–3.5 cm long, up to 3 mm thick. *Trichomes* dark brown, stiff. *Hymenium* with dark brown setae. *Setae* 200–250 × 5–12 µm, septate, smooth, straight to flexuous, dark brown. *Paraphyses* 1.5–4.5 µm wide, filamentous, septate, bulbous and curved at tips. *Asci* 200–240 × 20–30 µm, unitunicate, consistently 8-spored, cylindrical to clavate, rounded at apex, short-pedicellate at base. *Ascospores* 110–140 × 5–7 µm, mostly 7-septate, rarely 6–8-septate, straight or slightly curved, hyaline when young, dark brown when mature, narrowed and rounded at both ends, slightly wider in the middle portion, smooth.

Known distribution – Known from only collection in New Caledonia in 1909 and current collection in East Khasi hills, Meghalaya, India.

Material examined: India, Meghalaya, East Khasi hills District, Shillong, 4 July 2016, in garden soil amongst herbaceous vegetation, A. Prabhugaonkar, Herb. No. HCIO 52051; AVP-101 (ASSAM).

Discussion

The genus *Trichoglossum* Boud. was established in 1885 (Boudier, 1885) and is distinct from other genera in the family Geoglossaceae in having black projecting trichomes on surface of ascomata and setae amongst asci and paraphyses in transverse section. The genus is typified by *Trichoglossum hirsutum* (Pers.) Boud. 1907.

Trichoglossum rasum is a distinct species with asci up to 200 µm long and ascospores 115–140 × 7–9 µm and mostly 7–9-septate (Patouillard 1909). Our collection matches with the original description except that the asci are 200–240 µm (Patouillard 1909 has not given measurement

range) and ascospores are 7 to rarely 8-septate. We consider the new collection is the same species though they are geographically distant collections. This forms sixth species of Geoglossaceae reported from India with the other five being *Geoglossum glabrum* Pers. ex. Fr., *G. alveolatum* (Rehm.) Durand, *Trichoglossum hirsutum* (Pers. ex. Fr.) Bourd., *T. velutipes* (Peck) Durand (Agnihothrudu & Barua 1962) and *T. walteri* (Das 2009). Nannfeldt 1942 considered *T. wrightii* similar to *T. rasum* without treating it as a synonym. *T. wrightii* is distributed in Bermuda (Durand, 1908), China (Teng, 1934), Cuba (type locality of *T. hirsutum* f. *wrightii*), Java (Rifai, 1965), Panama (Nannfeldt, 1942) and India: Uttar Pradesh, Mussoorie (Thind & Singh 1965 as unidentified specimen). However, we consider *T. wrightii* as distinct species which is confirmed by its current treatment in Index fungorum/ MycoBank. *T. wrightii* is distinct from our collection with larger size of asci which is 240–265 µm and variation in spore septation which is mostly 8–9-septate, rarely 5–7-septate.

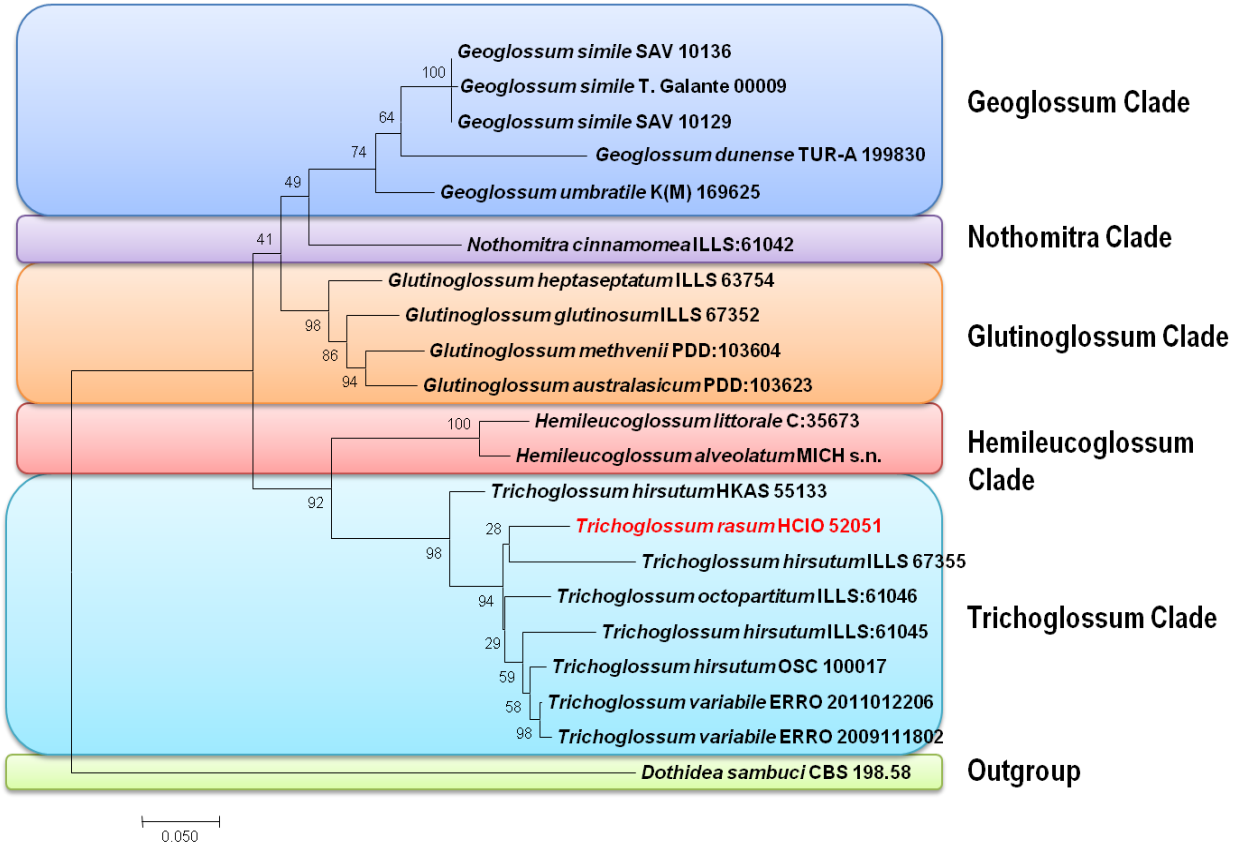

Figure 1 – Maximum likelihood (ML) tree inferred from ITS and LSU showing the relationship of *Trichoglossum rasum* with other species of *Trichoglossum* and related genera in family Geoglossaceae. Species sequenced and reported in the present paper is in red.

Molecular phylogeny confirms placement of the species in *Trichoglossum.* Only 4 out of 19 species described till date have molecular data. *T. hirsutum*, with good molecular data from collections around the world has been observed to be polyphyletic in nature (Hustad et. al. 2013). Current collection is morphologically distinct from *T. hirsutum* and same is confirmed by molecular phylogeny.

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Table 1 Comparison of *Trichoglossum* species

Species name	Fruiting body	Setae	Asci	Ascospores	References
Trichoglossum cheliense F.L. Tai	Clavate, 3–6 cm long, dark brown; ascogenous portion elliptic, compressed 0.5–1.4 cm long, 5–10 mm wide; stipe 3–4.5 cm long, 1.5–3 mm thick	Absent	Clavate, 200–231 × 18–22 µm, 8-spored	Clavate-cylindrical to subcylindrical, brown, 89–151 × 5–6 µm, mostly 15–septate, rarely 13–14-septate,	Tai, 1944
Trichoglossum confusum E.J. Durand	Black, clavate, 1.5–2.5 cm long; ascogenous portion furrowed to compressed; stipe 1–1.5 mm thick	Abundant, dark brown, acuminate	Narrowly clavate, 150–200 × 12–16 µm, 8-spored	Sub-cylindric, 45–75 × 5–6 µm, mostly 7-septate, rarely 3–7-septate	Durand, 1921
Trichoglossum farlowii (Cooke) E.J. Durand	Scatered or crowded, black or brownish black, clavate, 3–8cm long; ascogenous portion compressed 3–15 mm wide; stipe 1.5–4 mm thick	Abundant, dark brown	Clavate, 150–180 × 15–20 µm, 8-spored	Sub-cylindric to clavate-cylindric, 45–90 × 5–7 µm, mostly 3-septate, rarely 0–5 or more	Durand, 1908
Trichoglossum gracile Pat.	Black, 1.8–6 cm long; ascogenous portion elliptic, rounded, rarely slightly compressed, 2–4 mm diam; stipe 0.5–1 mm thick	Present	Clavate-cylindrical, 168–237 × 16–19 µm, 8-spored	Clavate-cylindrical, brown, 110–162 × 5–6 µm, 15-septate	Patouillard 1909
*Trichoglossum hirsutum (*Pers.) Boud.	Scatered, gregarious, black to brownish black, clavate to capitate, 1–8 cm long; ascogenous portion compressed upto 2 cm long, 2–5 mm wide; stipe 2–3 mm thick	Abundant, dark brown, acuminate	Clavate, 180–275 × 18–25 µm, 8-spored	Fusoid-clavate cylindric, 80-170 × 5–7 µm, 15-septate	Imai, 1941
Trichoglossum kunmingense F.L. Tai	1.5–3.5 cm long; ascogenous portion lanceolate to ellipticle, compressed 5.5 – 8 mm long, 3–4 mm wide; stipe 2–3 mm thick	Absent	Clavate, 175–225 × 19–25 µm, 8-spored	Clavate cylindrical to sub-cylindrical, brown, 104–144 × 6–8 µm, 7-septate, rarely 3–6-septate	Tai, 1944

Table 1 continued, comparison of *Trichoglossum* species

Species name	Fruiting body	Setae	Asci	Ascospores	References
Trichoglossum octopartitum Mains	Clavate, black, 1.5–4 cm long; ascogenous portion 2–4 mm wide; stipe 1–1.5 mm thick	Dark brown, acuminate	Clavate, 175–200 × 18–20 µm, 8-spored	Fusoid to fusoid-clavate, 80–150 × 4–5.5 µm, brown, 7-septate, rarely less	Mains, 1940
Trichoglossum persoonii F.L. Tai	Black, 2.5–4 cm long; ascogenous portion sub-globose to ovate, rounded, 5–9 mm long, 3-8 mm wide; stipe 1–3 cm long, 1–2 mm thick	Absent	Clavate, 225–275 × 18–23 µm, 8-spored	Clavate cylindrical, brown, 162–200 × 5–6 µm, mostly 15–19-septate, rarely 13–20-septate	Tai, 1944
Trichoglossum peruvianum E.K. Cash	Narrow-clavate to clavate-cylindrical, fuscous black, 1–2.5 cm long;ascogenous portion, occasionally compressed, 1.5–2 mm in diameter; stipe 5–10 × 1 mm	Dark brown, slightly constricted and paler at the base, acute at the tips	Long-cylindrical, narrowed sharply at the apex and gradually toward the base, 120–130 × 8–11 µm, 8-spored,	Elongate-fusoid, olivaceous, 50–75 × 2.5–3.5 µm, uniformly 7-septate	Cash, 1958
Trichoglossum qingchengense W.Y. Zhuang	Dark brown to black, 2–2.6 cm long; ascogenous portion broadly clavate, compressed 7–9 × 3–4 mm; stipe 6–12 × 1.5–1.8 mm	Very few, brown	Clavate, 190–230 × 20–24 µm, 8-spored, sometimes containing fewer than 8	Sub-cylindrical, brown, 76–117 × 6.5–7.7 µm, mostly 7–9-septate, rarely 6-septate	Zhuang & Wang, 1997
Trichoglossum rasum Pat.	Brownish black to black, clavate to broadly spathulate, 15 cm long; ascogenous portion compressed, 6-20 mm wide; stipe 2-3 mm thick	Present	Clavate, 200 × 20 µm, usually 8-spored	Mostly fusoid-clavate, to fusoid, 115–140 × 7–9 µm, mostly 7–9-septate	Patouillard, 1910
Trichoglossum rehmianum (Henn.) E.J. Durand	Scatered to crowded, black to brownish black, clavate, 3–10 cm long; ascogenous portion compressed 4–10 mm wide; stipe 2–6 mm thick	Abundant, dark brown, acuminate	Clavate, 165–200 × 15–18 µm, 8-spored	Sub-cylindric, narrowing below, 60–125 × 5–6 µm, light brown, mostly 7-septate	Durand, 1908

Table 1 continued, comparison of *Trichoglossum* species

Species name	Fruiting body	Setae	Asci	Ascospores	References
richoglossum sinicum F.L. Tai	Clavate, black, 5.5–7 cm long; ascogenous portion compressed 2.5–3 cm long, 0.8–1 cm thick; stipe 3–4 cm long, 3.5–5 mm thick	Absent	Clavate-cylindrical, 237–281 × 21–26 µm, 8-spored, rarely 4-spored	Cylindrical-clavate, brown, 147–175 × 6–7 µm, mostly 15-septate, rarely 7–15-septate	Tai, 1944
Trichoglossum tetrasporum Sinden & Fitzp.	Black, clavate, 3–8 cm long; ascogenous portion compressed; stipe 1–2 mm thick	Dark brown, acuminate	Clavate, 175–200 × 20–25 µm, 4-spored, ocassinally with fewer spores	Clavate to fusoid-clavate, 110–150 × 6–7 µm, dark brown, mostly 15-septate, rarely 0–17-septate	Sinden, & Fitzpatric, 1930
Trichoglossum variabile E.J. Durand) Nannf.	Dark brown to black, clavate, 2–4 cm long; ascogenous portion compressed; stipe 1 mm thick	Abundant, dark brown, acuminate	Clavate, 150–200 × 18–20 µm, 8-spored	Sub-fusoid or fusoid-clavate, 80–150 × 4.5–6 µm, mostly 10–13-septate, rarely 4–16-septate	Nannfeldt, 1942
Trichoglossum velutipes (Peck) E.J. Durand	Scatered to crowded, black to brownish black, clavate, 2–5 cm long; ascogenous portion compressed 2–12 mm wide; stipe 1.5–2.5 mm thick	Abundant, dark brown, acuminate	Clavate, 180–200 x 16–20 µm, 4-spored	Clavate, 90–160 × 6–7 µm, mostly 9-septate, rarely 0–13-septate	Durand, 1908
Trichoglossum walteri (Berk.) E.J. Durand	Up to 34 mm high; Clavula about 10 × 2.5 mm, ligulate, with median groove and obtuse apex, densely setose, dull, black-brown; Stipe 24 × 1 mm	Black to brown, thick walled	120–217 × 18–24 µm, 8-spored.	Cylindrical-clavate to acicuiar, 49–108 × 5–8 µm, 0–7-septate, brown	Imai, 1941
Trichoglossum wrightii (E.J. Durand) E.J. Durand	Black, velvety, clavate, variable in size; ascogenous portion irregular	Cystidia present	Clavate, cylindric, 250–265 × 20–25 µm, 8-spored	Clavate, brown, 105–145 × 7 µm, mostly 8-9–septate, rarely 5–6 or 7-septate	Durand, 1921
Trichoglossum yunnanense F.L. Tai	Black, 3.5–7.5 cm long; ascogenous portion ellipticle to subglobose, compressed; stipe 4.5–7 cm long, 2–3 mm thick	Absent	Cylindrical-clavate, 237–294 × 19–22 µm, 4-spored, rarely 2–8-spored	Clavate cylindrical, brown, 143–187 × 6–7 µm, 15-septate, rarely 16-septate	Tai, 1944

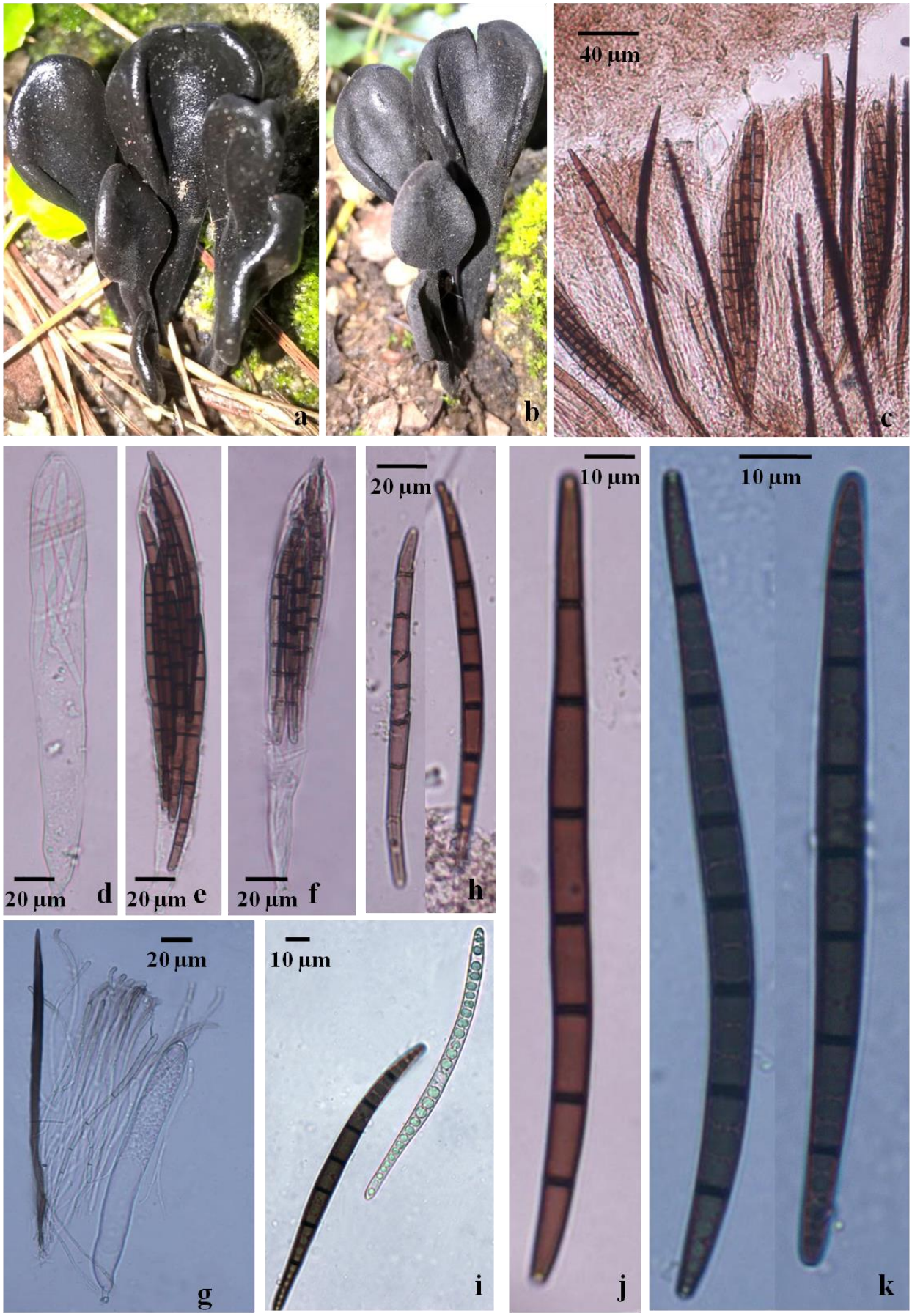

Figure 2 – *Trichoglossum rasum***.** a, b Habit, c Asci with setae, d Young ascus with ascospores, e, f Asci with ascospores, g Young ascus with setae and paraphyses, h–k Ascospores

Table 2 Sequence data used in combined ITS and LSU analyses. Newly deposited sequences are in bold

Taxon	Acession no.	ITS	LSU
Geoglossum simile	SAV 10136	KF854293	KF854301
Geoglossum simile	T. Galante 00009	KF944382	KF944384
Geoglossum simile	SAV 10129	KF854286	KF854294
Geoglossum dunense	TUR-A 199830	KP744516	KP744517
Geoglossum umbratile	K(M) 169625	KC222127	KC222140
Nothomitra cinnamomea	ILLS:61042	JQ256424	JQ256439
Glutinoglossum heptaseptatum	ILLS 63754	KC222130	KC222143
Glutinoglossum glutinosum	ILLS 67352	KC222128	KC222141
Glutinoglossum methvenii	PDD:103604	KP690097	KP690109
Glutinoglossum australasicum	PDD:103623	KP690088	KP690100
Hemileucoglossum littorale	C:35673	KP657561	KP657566
Hemileucoglossum alveolatum	MICH s.n.	KP657560	KP657565
Trichoglossum hirsutum	HKAS 55133	KC222133	KC222146
Trichoglossum hirsutum	ILLS 67355	KC222132	KC222145
Trichoglossum octopartitum	ILLS:61046	JQ256429	JQ256443
Trichoglossum hirsutum	ILLS:61045	JQ256428	JQ256442
Trichoglossum hirsutum	OSC 100017	NR_121205	NG_042405
Trichoglossum variabile	ERRO 2011012206	KP144106	-
Trichoglossum variabile	ERRO 2009111802	KP144105	-
Trichoglossum rasum	**HCIO 52051**	**KY457226**	**KY457227**
Dothidea sambuci	CBS 198.58	AY930109	AF382387

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