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Short Article

Two new records of *Lopadostoma* for mycobiota of Iran

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Abstract: Xylariaceous fungi are typically saprobes, but are also commonly isolated as endophytes and some species are pathogens. Two species of *Lopadostoma* (*Xylariaceae*, *Xylariales*) are reported for the first time from Iran. *L. dryophilum* was found from dead branches of *Quercus* sp. in East Azerbaiejan and *L. fagi* from dead branches of *Fagus* sp. in Ardabil province. Based on morphology and sequence data (ITS), the two species, *L. dryophilum* and *L. fagi* are confirmed as new records for mycobiota of Iran. A detailed description of the two species are provided. This is the first report of the genus in Iran.

Key words: *Lopadostoma*, *Xylariaceae*, systematic, ITS

INTRODUCTION

The *Xylariales* is a large order of perithecial ascomycetes that contains 209 genera and 2487 species (Kirk et al. 2008). *Xylariaceae* is the type and largest family of the *Xylariales* with 85 genera and a total of 1343 species (Kirk *et al*., 2008). The genus *Lopadostoma* (Nitschke) Traverso is a member of family *Xylariaceae* that was founded by Traverso (1906) and typified by *L. turgidum*. The genus is characterized by perithecial ascomata immersed in and erumpent from bark, standing on the wood, with only an ectostromatic disc visible or the disc surrounded by blackened bark surface; cylindrical asci with an flat ring-like part bluing in iodine reagent; ascospores which are oblong to narrowly ellipsoid, lack a dwarf cell, dark to blackish brown at maturity, with a straight germ slit across the entire spore length present on one side or circumferential side; and a *Libertella*-like anamorph (Jaklitsch et al. 2014).

Molecular delimitation of *Lopadostoma* and related genera, including *Anthostoma*, *Anthostomella* and *Barrmaelia* has been accomplished by sequencing three genes including the internal transcribed spacer (ITS) region, the nuclear large subunit rDNA (LSU) and the RNA polymerase II subunit B (rpb2) (Jaklitsch et al. 2014). According to revision of Jaklitsch et al. (2014), the genus consisted of twelve species: *L. turgidum*, *L. fagi*, *L. juglandinum*, *L. quercicola*, *L. gastrinum*, *L. lechatii*, *L. dryophilum*, *L. linospermum*, *L. ailanthi*, *L. insulare*, *L. americanum* and *L. meridionale*, which among them *L. ailanthi* and *L. juglandinum* are only known from morphology. *L. pouzarii*, *L. polynesium* and *L. amoenum* however are not included in *Lopadostoma* because molecular data do not support this conclusion (Jaklitsch et al. 2014). Vasilyeva and Stephenson (2014) also described a new species of *Lopadostoma* (*L. cryptosphaeroides*) on the bark of *Quercus* sp. in Virginia on the basis of morphological characters.

The aim of this study was to describe and illustrate two new records of *Lopadostoma* genus for Iranian mycobiota that have been collected during a taxonomic and phylogenetic study of *Diatrypaceae* in the northern Iran.

MATERIALS AND METHODS

Morphological studies

Branch samples were collected from Ardabil and East Azerbaijan provinces. Several unsuccessful attempts were made to isolate and culture the fungus from single ascospore on potato dextrose agar (PDA, Difco) and malt extract agar (MEA, Merck) at 25°C in darkness. Morphological measurements and photomicrographs were made according to Mehrabi et al. (2015). Dry specimens were deposited in the herbarium of Iranian Research Institute of Plant Protection (IRAN).

DNA extraction and amplification

For extraction of genomic DNA, the content of several perithecia with a sterile needle transferred to an empty 1500 µl Eppendorf tube. Thirty microliters of TE (10 mM Tris-HCl pH 7.5, 1 mM EDTA) was added to the tube, the tube was closed and transferred to liquid nitrogen. The mixture was grounded by a sterile peg. This was repeated several times. The tube was vortexed and then spun in a microfuge for 1 min at 12000 rpm. The supernatant liquid was collected and transferred to a clean Eppendorf tube. The supernatant contained genomic DNA and was used directly for PCR. PCR was performed in 25 µl reaction mixture containing 1 µl of each primer (10 pmol/µl, Takapouzist Inc.), 4 µl genomic DNA mentioned above (10 ng/µl), 2.5 µl 10× high yield

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PCR buffer (Jena Bioscience, Germany), 1.5 unit Taq polymerase (Jena Bioscience, Germany) ,1 mM $MgCl_2$ and 0.5 mM dNTP. Amplifications were performed by using primers ITS1 and ITS4 (White et al. 1990) in a PC-320 PCR System (ASTEC Co., Japan), which was programmed 4 min at 94˚C for denaturation , followed by 35 cycles at 94˚C/45 s, 58˚C/35 s and 72˚C/90 s, with a final elongation step at 72˚ C/10 min. The PCR products were sent out for sequencing in one direction (Macrogen company, South Korea).

Sequence analysis

The newly obtained nucleotide sequences were checked with FinchTV v. 1.4.0 (Geospiza Inc.). The sequences obtained were compared with those in the GenBank databases using the BLAST program. Based on the BLAST results, sequences were retrieved from GenBank for the comparative phylogenetic analysis. DNA sequences were aligned with Clustal W (Thompson et al. 1994), within the MEGA 6 (Tamura et al. 2013). Phylogenetic analyses of the aligned dataset were performed with Neighbor-Joining (NJ) method (Saitou and Nei 1987) with complete deletion option for gaps/missing data and the bootstrap test by 1000 replicates (Hillis and Bull 1993). Based on the Bayesian information criterion of MEGA 6, Kimura 2-parameter model with gamma distribution (K2 + G) was selected for the NJ analysis. *Xylaria hypoxylon* (AM993141) was used as the outgroup. The sequences of our isolates were deposited in GenBank.

RESULTS AND DISCUSSION

Molecular phylogeny

Two new ITS sequences were obtained in this study (GenBank accession numbers KR999997 and KR999998) and aligned with 13 sequences retrieved from GenBank (Table 1). Size of our sequences were 549 bp for *Lopadostoma dryophilum* and 516 bp for *L. fagi*. Based on both morphology and molecular sequence data, the occurrence of these two spices in Iran was confirmed with 100% bootstrap values.

Table 1: Isolates used in phylogenetic analysis, with data referring to the newly generated sequence marked in boldface.

Taxon name	Host	GenBank accession no.	Origin
Lopadostoma americanum	*Quercus* sp	KC774568	USA
Lopadostoma dryophilum	*Quercus petraea*	KC774570	Austria
Lopadostoma dryophilum	*Quercus petraea*	KC774571	France
Lopadostoma dryophilum	***Quercus* sp.**	**KR999998**	**Iran**
Lopadostoma fagi	*Fagus sylvatica*	KC774577	Austria
Lopadostoma fagi	*Fagus sylvatica*	KC774574	Austria
Lopadostoma fagi	***Fagus* sp.**	**KR999997**	**Iran**
Lopadostoma gastrinum	*Carpinus betulus*	KC774579	Austria
Lopadostoma insulare	*Quercus coccifera*	KC774588	Greece
Lopadostoma lechatii	*Carpinus betulus*	KC774590	France
Lopadostoma linospermum	*Pistacia lentiscus*	KC774591	Italy
Lopadostoma meridionale	*Quercus ilex*	KC774598	Croatia
Lopadostoma quercicola	*Quercus petraea*	KC774604	Austria
Lopadostoma turgidum	*Fagus sylvatica*	KC774616	Austria
Xylaria hypoxylon	*Sorbus aucuparia*	AM993141	Sweden

Lopadostoma dryophilum (G.H. Otth) Jaklitsch, J. Fourn.&Voglmayr, Persoonia 32: 61. 2014. Fig. 2.

Basionym: *Phaeosperma dryophilum* G.H. Otth, Mitt. Naturf.Ges. Bern Nr. 654–683: 42. 1868.

Synonyms are given by Jaklitsch et al. (2014).

Stromata immersed in the bark of dead branches (1.5 cm diam.), pustulate, erumpent, 2–3.5 mm diam., often with slightly projecting black ostioles, delimited by a black zone in the host tissues, the latter 100–200 µm thick, with groups of 8–20 perithecia. Ostioles dark, opening separately in the disc. Perithecia dark, circinately arranged, globoid to subgloboid, monostichous, 300–800 µm diam., surrounded by brownish entostroma. Asci narrow cylindric, containing (6–)8 uniseriate ascospores, (74–) 90–110 × 7–8 µm, with stalks up to 30 µm long. Ascospores (–9)10–15(–16.5) × 3.4–4.7 µm, narrowly ellipsoid or narrowly fusiform, aseptate, dark brown to nearly black, with straight, circumferential germ slit and 2 large and sometimes several small guttules.

Specimens examined: IRAN, EAST AZERBAIJAN, Aghoyeh, on dead branch of *Quercus* sp., 6 August. 2014, M. Mehrabi, IRAN 16685F.

Note: This taxon has suggested by Jaklitsch et al. (2014) as a new combination. The studied material fits with *L. dryophilum* as described by Jaklitsch et al. (2014). The phylogenetic analyses of the ITS sequences confirmed the morphological identification with 100% bootstrap value (Fig. 1). Based on a megablast search and the phylogenetic tree inferred

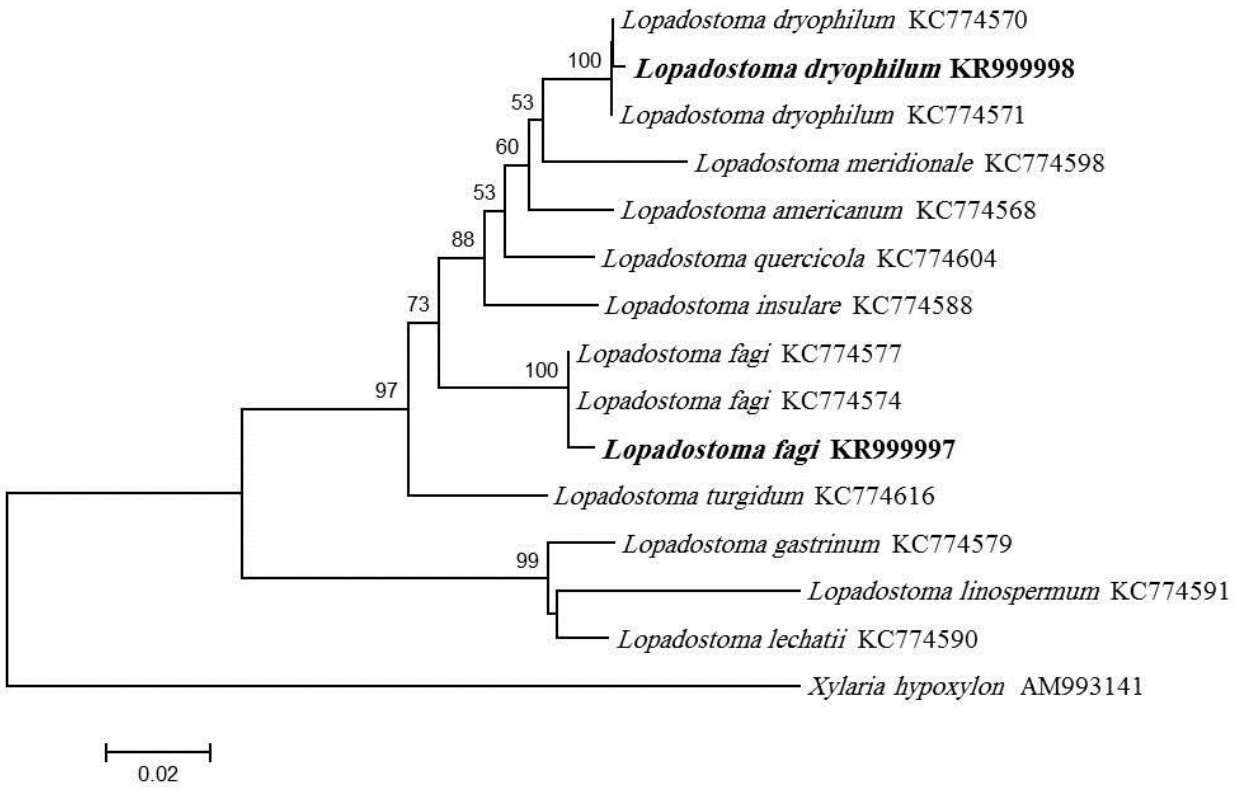

Fig. 1. Phylogenetic tree of *Lopadostoma* species inferred from ITS (ITS1–5.8S–ITS2) sequences using Neighbor Joining method in MEGA6 with 1000 bootstrap replications. The bootstrap values (>50%) are shown at the nodes. The new sequences obtained in this study are indicated in boldface.

from ITS sequences (Fig. 1), the closest sequence to our fungus is *L. dryophilum* (GenBank KC774570 and KC774571; Identities = 547/550(99%), Gaps = 1/550(0%)). This is the first report of this species from Iran.

Lopadostoma fagi Jaklitsch, J. Fourn. & Voglmayr, Persoonia 32: 63. 2014. Fig. 3.

Stromata densely immersed in the bark of dead branches (1.5 cm diam.), pustulate, covered by the epidermis which is not discolored, 1–1.5 mm diam., slightly erumpent with tiny, black, rounded or slightly elliptical ectostomatic disc, with groups of 3–7 perithecia. Ostioles dark, converging toward the disc; tissue between the ostioles blackish, opening separately in the disc. Perithecia dark, circinately arranged, globoid to subgloboid, monostichous, 300–800 µm diam., tissue surrounding perithecia yellowish brown. Asci cylindric, containing 8 uniseriate ascospores, 60–70 × 5–6 µm, with stalks up to 34 µm long. Ascospores 7–10.5(–11.3) × 3–4 µm, oblong or narrowly ellipsoid, aseptate, brown to nearly black, smooth, with straight, circumferential germ slit and 2 large guttules,.

Specimens examined: IRAN, ARDABIL, Khalkhal, on dead branch of *Faqus* sp., 5 August. 2014, M. Mehrabi, IRAN 16686F.

Note: This taxon has described by Jaklitsch et al. (2014) on the basis of material from Austria. The Iranian material was consistent with *L. fagi* as described by Jaklitsch et al. (2014). Based on both morphology and molecular sequence data, the occurrence of *L. fagi* in Iran was confirmed with 100% bootstrap values (Fig. 1). Based on a megablast search of NCBI GenBank nucleotide database, the closest sequence to our fungus is *L. fagi* (GenBank KC774577 and KC774574; Identities = 480/483(99%), Gaps = 0/483(0%)). This is the first report of this species from Iran.

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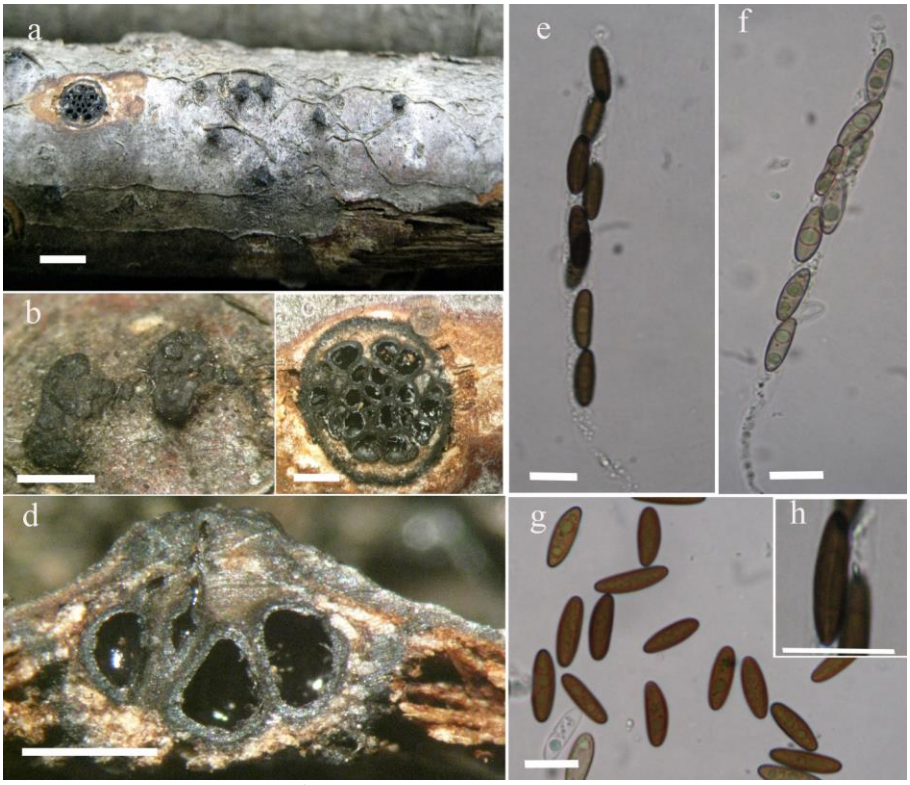

Fig. 2. *Lopadostoma dryophilum.* **a.** Habit of ascostromata on bark; **b.** Ectostromatic discs; c. Transverse section through the ascoma; **d.** Longitudinal section through the stroma; **e-f.** Asci; **g.** Ascospores; **h.** Ascospore with straight spore-length germ slit. — Scale bars: a = 3mm; b-d = 1 mm: e-h= 10 µm.

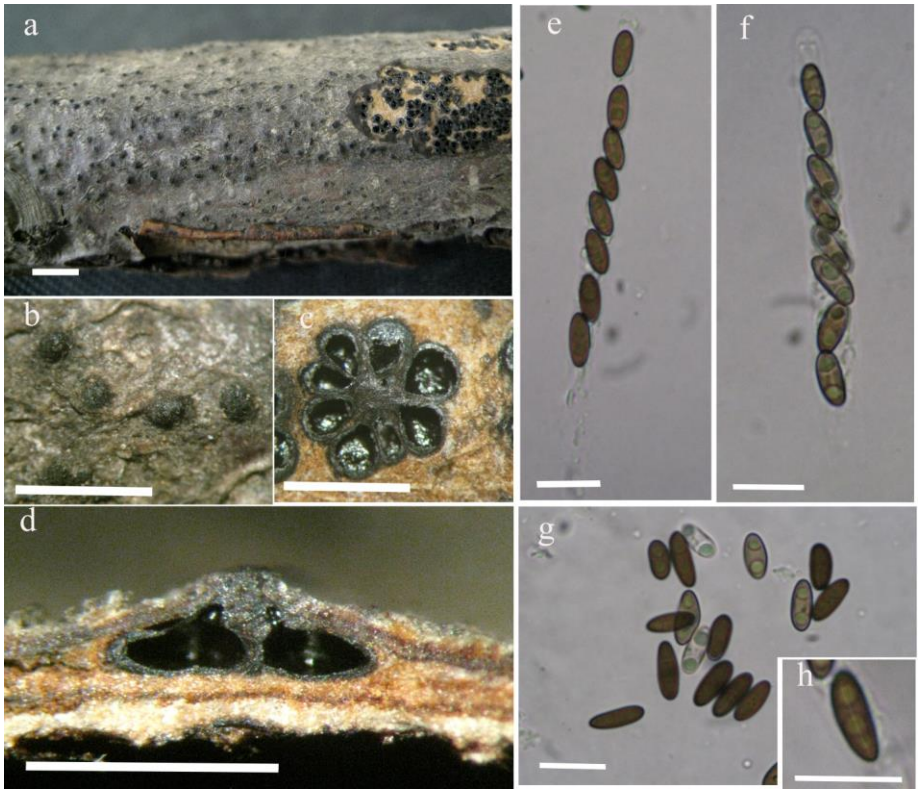

Fig. 3. *Lopadostoma fagi*. **a.** Habit of ascostromata on bark; **b.** Ectostromatic discs; **c.** Transverse section through the ascoma; **d.** Longitudinal section through the stroma; **e-f.** Asci; g. Ascospores; **h.** Ascospore showing germ slit. — Scale bars: a = 3mm; b-d = 1 mm: e-h= 10 µm.

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دو گزارش جدید از (Ascomycota) *Lopadostoma* برای میکوبیوتای ایران

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چکیده: قارچ های خانواده *Xylariaceae* اگر چه ساپرفیت می باشند ولی به صورت اندوفیت و پاتوژن هم جداسازی شده اند. در این تحقیق دو گونه از جنس *Lopadostoma* برای اولین بار از ایران گزارش می شوند. *L. dryophilum* روی شاخه مرده *Quercus* sp. در استان آذربایجان شرقی و *L. fagi* روی شاخه مرده *Fagus sp.* در استان اردبیل پیدا شدند. بر اساس مطالعات ریخت شناسی و توالی ناحیه ITS - rDNA، این دو گونه به عنوان گزارش‌های جدید برای میکوبیوتای ایران تایید شدند. ویژگی-های ریخت شناسی ماکروسکوپی و میکروسکوپی نیز تشریح شدند. این اولین گزارش از این جنس در این کشور می باشد.

واژه های کلیدی: *Lopadostoma*، *Xylariaceae*، سیستماتیک، ITS

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