



## Morphological and molecular study of *Dematophora buxi* (Xylariaceae) on *Buxus sempervirens* as a new record from Asia

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**Abstract:** In a survey of xylarialean fungi in the Northern part of Iran, some specimens that showed affinities to the rosellinoid *Xylariaceae* were collected. Morphological evidence, phylogenetic analyses of a combined ITS and *ACT1* genes dataset confirmed the presence of *Dematophora buxi* on *Buxus sempervirens* in Iran for the first time. In addition, to our knowledge, this is the first report of this species in Asia. *Dematophora buxi* is illustrated, described, and discussed.

**Keywords:** Biodiversity, Mycobiota, Phylogeny, Sordariomycetes, *Xylariales*

### INTRODUCTION

The *Xylariaceae* is one of the largest families of *Ascomycota*, with high biodiversity, rich in the production of secondary metabolites and distributed worldwide as saprobes or endophytes, while some species are well-known plant pathogens (Helaly et al. 2018, Becker & Stadler 2021). It is morphologically characterized by geniculosporium-like anamorphs and the absence of stromatal pigments according to the recent segregation by Wendt et al. (2018). Recently, 32 genera were accepted in the *Xylariaceae* (Hyde et al. 2020).

Hartig (1883) described the genus *Dematophora* with *D. necatrix* as the type species observing solely the conidial form. Berlese (1892a, 1892b) recognized affinities of *Dematophora* with *Rosellinia*, and subsequently, *D. necatrix* was accommodated in *Rosellinia* (Prillieux 1904). Wittstein et al. (2020) provided multi-gene analyses and segregated *Rosellinia* species with a synnematal *Geniculosporium* anamorph from *Rosellinia* species with non synnematal anamorph and resurrected the genus *Dematophora* for the former group. *Dematophora* species form superficial, large,

massive, uniperitheciate, brown to black stromata that do not release coloured pigments in 10% KOH. They are seated in a well-developed subiculum, embedding at least the lower part of the stromata. Asci are cylindrical, stipitate, evanescent, with a massive amyloid, higher than wide ascus apical plug, staining deep blue in Melzer's Reagent. Ascospores are dark brown, unicellular, with straight germ slit, short or of spore length, cellular appendages absent, slimy caps or sheath present. All species develop synnematal anamorph (Petrini, 2013, Wittstein et al. 2020).

In the framework of studying the xylarialean fungi of Guilan and Mazandaran Provinces, detailed morphological descriptions, illustrations, and phylogenetic information of *Dematophora buxi* are provided.

### MATERIALS AND METHODS

#### Fungal collection

During our survey of xylarialean fungi in the north of Iran, several fungal specimens were collected from Guilan and Mazandaran Provinces. Most of them have already been published elsewhere (Pourmoghaddam et al. 2022a, 2022b). One *Dematophora* species was gathered in two regions of Guilan Province (Siahkal County) and Mazandaran Province (Sisangan County) and identified using molecular and morphological characteristics.

#### Morphological observation

For light microscopy, specimens were examined according to Pourmoghaddam et al. (2018). Dried specimens were deposited in the University of Guilan Mycological Herbarium (GUM).

#### DNA extraction, PCR, and sequencing

Genomic DNA was extracted directly from one perithecium using Chelex 5% (Hirata & Takamatsu 1996, Khodaparast et al. 2020). DNA preparations were stored at -20 °C until used for PCR. The DNA amplification was obtained by polymerase chain reaction (PCR). A region spanning ITS1, 5.8S, and ITS2 of rDNA was amplified as described by Khodaparast et al. (2012) using the primers ITS5 and ITS4 (White et al. 1990). Part of the actin gene (*ACT1*) region was amplified with primers ACT-512F and ACT-783R (Carbone & Kohn 1999). The PCR product was purified and sequenced by Bioneer Inc. (Korea) using direct sequencing in an ABI 3730xl sequencer. Sequences derived from this study were

deposited in the NCBI GenBank nucleotide database (<http://www.ncbi.nlm.nih.gov>).

### Phylogenetic analyses

For the phylogenetic placement of the *Xylariaceae* taxa included in our analyses, a representative ITS-*ACT1* matrix including 55 members of the families was produced with two outgroups from *Hypoxylaceae* (*Hypoxylon fragiforme* (Pers.) J. Kickx f., *Hypoxylon howeanum* Peck). All alignments were produced with the server version of MAFFT (<http://www.ebi.ac.uk/Tools/msa/mafft>), checked and refined using MEGA7 (Kato et al. 2019, Kumar et al. 2016). After the exclusion of ambiguously aligned regions and long gaps, the final matrix contained 813 nucleotide characters, i.e. (543 from the ITS, and 270 from the *ACT1*). Available sequences were downloaded from GenBank; details on the sequences used in the phylogenetic analyses are provided in Table 1. Maximum Likelihood (ML) analyses were performed with RAXML (Stamatakis 2006) as implemented in raxmlGUI 1.3 (Silvestro and Michalak 2012), using the ML + rapid bootstrap setting and the GTRGAMMA substitution model with 1000 bootstrap replicates. The matrix was partitioned for the different gene regions.

Maximum Parsimony (MP) analyses were performed with PAUP v4.0a165 (Swofford 2002). All molecular characters were unordered and given equal weight; analyses were performed with gaps treated as missing data; the COLLAPSE command was set to MINBRLEN. MP analysis of the combined multilocus matrix was done using 1000 replicates of heuristic search with random addition of sequences and subsequent TBR branch swapping (MULTREES option in effect, steepest descent option not in effect). Bootstrap analyses with 1000 replicates were performed in the same way but using 10 rounds of random sequence addition and subsequent branch swapping during each bootstrap replicate. Bootstrap values  $\leq 70\%$  are considered low, between 70 and 90% intermediate, and  $\geq 90\%$  high in the discussion of the data that follows further below. Bootstrap values  $\leq 70\%$  are considered low, between 70 and 90% intermediate, and  $\geq 90\%$  high in the discussion of the data that follows further below.

## RESULTS

### Molecular phylogeny

The combined multilocus matrix used for phylogenetic analyses comprised 813 characters, of those, 422 were parsimony informative (257 from ITS, and 165 from *ACT1*). Figure 1 shows a simplified phylogram of the best ML tree ( $\ln L = -13,639.6577$ ) obtained by RAXML. Maximum parsimony analyses revealed 13 MP trees comprising

3018 steps (data not shown) that had a similar topology to the ML tree. The phylogenies reveal a monophyletic clade of *Dematophora*, like in previous studies (Wendt et al. 2018, Pourmoghaddam et al. 2022a, b, Voglmayr et al. 2022). It showed a closer affinity to *Rosellinia*, *Entoleuca*, and *Nemania* than to *Kretzschmaria*, some *Xylaria* species, and other xylariaceous genera. The sequences of the analyzed Iranian collection of *D. buxi* are identical to those retrieved from GenBank (JDR 99, from France) and they clustered together with maximum ML and MP BS support.

### Taxonomy

*Dematophora buxi* (Fabre) C. Lambert, K. Wittstein & M. Stadler, Stud. Mycol. 96: 14 (2020), Fig. 2

**Teleomorph.** Stromata superficial, semiglobose, sessile, solitary, 950–1550  $\mu\text{m}$  high  $\times$  900–1450  $\mu\text{m}$  wide, dark brown to black, embedded in a well-developed persistent subiculum. Synnemata dark brown, gathering around stromata. Ostioles finely papillate. Ectostroma 75–100  $\mu\text{m}$  thick, black. Entostroma not seen. Perithecia 1016–1143  $\mu\text{m}$  high  $\times$  1000–1100  $\mu\text{m}$  wide, usually separate from the stromatal wall. Asci with amyloid, urn-shaped apical apparatus, 5–6  $\mu\text{m}$  high  $\times$  4–5  $\mu\text{m}$  upper width  $\times$  3.5–4  $\mu\text{m}$  lower width, spore-bearing part 180–210  $\times$  8–10  $\mu\text{m}$ . Ascospores smooth, unicellular, dark brown to brown, narrowly ellipsoidal to broadly fusiform, 22–30  $\times$  6.5–8  $\mu\text{m}$ , with straight germ slit much less than spore-length; perispore indehiscent in 10% KOH.

**Specimens examined:** Iran, Guilan Province, Siahkal County, Ziaratgah forest, 37°08'07.34"N, 49°55'36.14"E, 260 m elev., on weaken and dead branch and trunk of *Buxus sempervirens*, 20 September 2021, leg. M.J. Pourmoghaddam (GUM 1910); Mazandaran Province, Sisangan County, 36°34'36.89"N, 51°48'37.17"E, 71 m elev., on weaken and dead branch and trunk of *Buxus sempervirens*, 23 October 2021, leg. M.J. Pourmoghaddam (GUM 1911).

**Notes:** *Dematophora buxi* was first described by Fabre (1878) from southern France. It is characterized by the presence of synnemata around stromata, ascospores with a germ slit shorter than spore length and the absence of a slimy sheath or caps (Petrini 2013). Most of the characters of the Iranian specimens are in accordance with this description (Petrini 2013), aside from variations in the size of ascospores (22–30  $\times$  6.5–8 vs. 19.8–30.1  $\times$  6–8.9  $\mu\text{m}$ ). It can be differentiated from *D. francisiae* by its smaller ascospores (24–30  $\times$  6.5–8 vs. 29–35  $\times$  8–13  $\mu\text{m}$ ). *Dematophora hsiehiae* and *D. samuelsii* also differs from it in ascospores with slimy sheath or caps and germ slit spore length.

**Table 1.** Vouchers and accession numbers of sequences used in the phylogenetic analyses. Type specimens are labeled with HT (holotype) and ET (epitype). Isolates/sequences in bold were isolated/sequenced in present study. N/A: not available.

Species/Status	Strain number	Origin	GenBank accession numbers		Reference
			ITS	ACT1	
<i>Amphirosellinia fushanensis</i> <sup>(HT)</sup>	HAST 91111209	Taiwan	GU339496	GQ452360	Hsieh et al. (2010)
<i>Amphirosellinia nigrospora</i> <sup>(HT)</sup>	HAST 91092308	Taiwan	GU322457	GQ452361	Hsieh et al. (2010)
<i>Astrocystis concavispora</i>	MFLUCC 14.0174	Italy	KP297404	N/A	Daranagama et al. (2015)
<i>Collodiscula japonica</i>	GMBC0034	China	MW732444	MW755350	Wu et al. (2021)
<i>Dematophora buxi</i>	JDR 99	France	GU300070	GQ398228	Hsieh et al. (2010)
<b><i>Dematophora buxi</i></b>	<b>GUM 1910</b>	<b>Iran</b>	<b>OP828512</b>	<b>OP828693</b>	<b>This study</b>
<i>Dematophora necatrix</i>	HAST 89062904	Taiwan	EF026117	EF025588	Hsieh et al. (2010)
<i>Dematophora necatrix</i>	MUCL 57709	Iran	OL635177	ON922888	Pourmoghaddam et al. (2022a)
<i>Entoleuca mammata</i>	JDR 100	France	GU300072	GQ398230	Hsieh et al. (2010)
<i>Hypocreodendron sanguineum</i>	JDR 169	Mexico	GU322433	GQ438747	Hsieh et al. (2005)
<i>Hypoxyton fragiforme</i>	YMJ 387	France	JN979419	AY951831	Hsieh et al. (2005)
<i>Hypoxyton howeanum</i>	YMJ 388	France	JQ009323	AY951839	Hsieh et al. (2005)
<i>Kretzschmaria clavus</i>	YMJ 114	French Guiana	EF026126	EF025596	Hsieh et al. (2010)
<i>Kretzschmaria deusta</i>	MUCL 57705	Iran	MH084755	MH056202	Pourmoghaddam et al. (2018)
<i>Kretzschmaria guyanensis</i>	HAST 89062903	Taiwan	GU300079	GQ408901	Hsieh et al. (2010)
<i>Kretzschmaria hedjaroudei</i> <sup>(HT)</sup>	MUCL 57706	Iran	MH084757	MH056204	Pourmoghaddam et al. (2018)
<i>Kretzschmaria lucidula</i>	YMJ 112	French Guiana	EF026125	EF025595	Hsieh et al. (2010)
<i>Kretzschmaria megalospora</i>	YMJ 229	Malaysi a	EF026124	EF025594	Hsieh et al. (2010)
<i>Kretzschmaria neocaledonica</i>	HAST 94031003	Taiwan	GU300078	GQ398236	Hsieh et al. (2010)
<i>Kretzschmaria pavimentosa</i>	JDR 109	Taiwan	GU300077	GQ398235	Hsieh et al. (2010)
<i>Kretzschmaria sandvicensis</i>	JDR 113	USA	GU300076	GQ398234	Hsieh et al. (2010)
<i>Linosporeopsis ischnothecca</i> <sup>(ET)</sup>	CBS 145761	Switzer land	MN818952	N/A	Voglmayr and Beenken (2020)
<i>Linosporeopsis ochracea</i> <sup>(ET)</sup>	CBS 145999	German y	MN818958	N/A	Voglmayr and Beenken (2020)
<i>Nemania abortiva</i> <sup>(HT)</sup>	BISH 467	USA	GU292816	GQ374123	Hsieh et al. (2010)
<i>Nemania beaumontii</i>	HAST 405	Martini que	GU292819	GQ389694	Hsieh et al. (2010)
<i>Nemania bipapillata</i>	HAST 90080610	Taiwan	GU292818	GQ389693	Hsieh et al. (2010)
<i>Nemania camelliae</i> <sup>(HT)</sup>	GMB0068	China	MW851889	MW836046	Pi et al. (2021)
<i>Nemania changningensis</i> <sup>(HT)</sup>	GMB0056	China	MW851875	MW836042	Pi et al. (2021)
<i>Nemania cyclobalanopsina</i> <sup>(HT)</sup>	GMB0062	China	MW851883	MW836038	Pi et al. (2021)
<i>Nemania diffusa</i>	HAST 91020401	Taiwan	GU292817	GQ389692	Hsieh et al. (2010)
<i>Nemania feicuiensis</i> <sup>(HT)</sup>	GMB0059	China	MW851880	MW836044	Pi et al. (2021)
<i>Nemania illita</i>	YMJ 236	USA	EF026122	EF025593	Hsieh et al. (2010)
<i>Nemania lishuicola</i> <sup>(HT)</sup>	GMB0065	China	MW851886	MW836048	Pi et al. (2021)

Table 1. *cont.*

Species/Status	Strain number	Origin	GenBank accession numbers		Reference
			ITS	ACT1	
<i>Nemania macrocarpa</i> <sup>(HT)</sup>	WSP 265	USA	GU292823	GQ389698	Hsieh et al. (2010)
<i>Nemania primolutea</i> <sup>(HT)</sup>	HAST 91102001	Taiwan	EF026121	EF025592	Hsieh et al. (2010)
<i>Nemania rubi</i> <sup>(HT)</sup>	GMB0064	China	MW851885	MW836040	Pi et al. (2021)
<i>Nemania serpens</i>	MUCL 57702	Iran	OP359334	N/A	Pourmoghaddam et al. (2022b)
<i>Nemania sphaeriosoma</i>	JDR 261	USA	GU292821	GQ389696	Hsieh et al. (2010)
<i>Podosordaria mexicana</i>	WSP 176	Mexico	GU324762	GQ455451	Hsieh et al. (2010)
<i>Podosordaria muli</i> <sup>(HT)</sup>	WSP 167	Mexico	GU324761	GQ455450	Hsieh et al. (2010)
<i>Rosellinia aquila</i>	MUCL 51703	France	KY610392	N/A	Wendt et al. (2018)
<i>Rosellinia corticium</i>	MUCL 57712	Iran	OL635178	ON922889	Pourmoghaddam et al. (2022a)
<i>Rosellinia corticium</i>	MUCL 57713	Iran	OL635179	ON922890	Pourmoghaddam et al. (2022a)
<i>Rosellinia nectrioides</i>	CBS 449.89	Sweden	MN984622	N/A	Wittstein et al. (2020)
<i>Stilbohoxylon elaeicola</i>	Y.M.J 173	French Guiana	EF026148	EF025601	Hsieh et al. (2010)
<i>Stilbohoxylon quisquiliarum</i>	Y.M.J 172	French Guiana	EF026119	EF025590	Hsieh et al. (2010)
<i>Xylaria acuminatilongissima</i> <sup>(HT)</sup>	HAST 95060506	Taiwan	EU178738	GQ853046	Hsieh et al. (2010)
<i>Xylaria adscendens</i>	J.D.R 865	Thailand	GU322432	GQ438746	Hsieh et al. (2010)
<i>Xylaria arbuscula</i>	HAST 89041211	Taiwan	GU300090	GQ421286	Hsieh et al. (2010)
<i>Xylaria bambusicola</i> <sup>(HT)</sup>	YMJ 205	Taiwan	EF026123	AY951873	Hsieh et al. (2010)
<i>Xylaria brunneovinosa</i> <sup>(HT)</sup>	HAST 720	Martinique	EU179862	GQ853041	Hsieh et al. (2010)
<i>Xylaria curta</i>	HAST 494	Martinique	GU322444	GQ449233	Hsieh et al. (2010)
<i>Xylaria hypoxylon</i>	HAST 152	Belgium	GU300096	GQ427196	Hsieh et al. (2010)
<i>Xylaria multiplex</i>	HAST 580	Martinique	GU300098	GQ427198	Hsieh et al. (2010)
<i>Xylaria polymorpha</i>	1012 (JDR)	USA	GU322460	GQ452364	Hsieh et al. (2010)

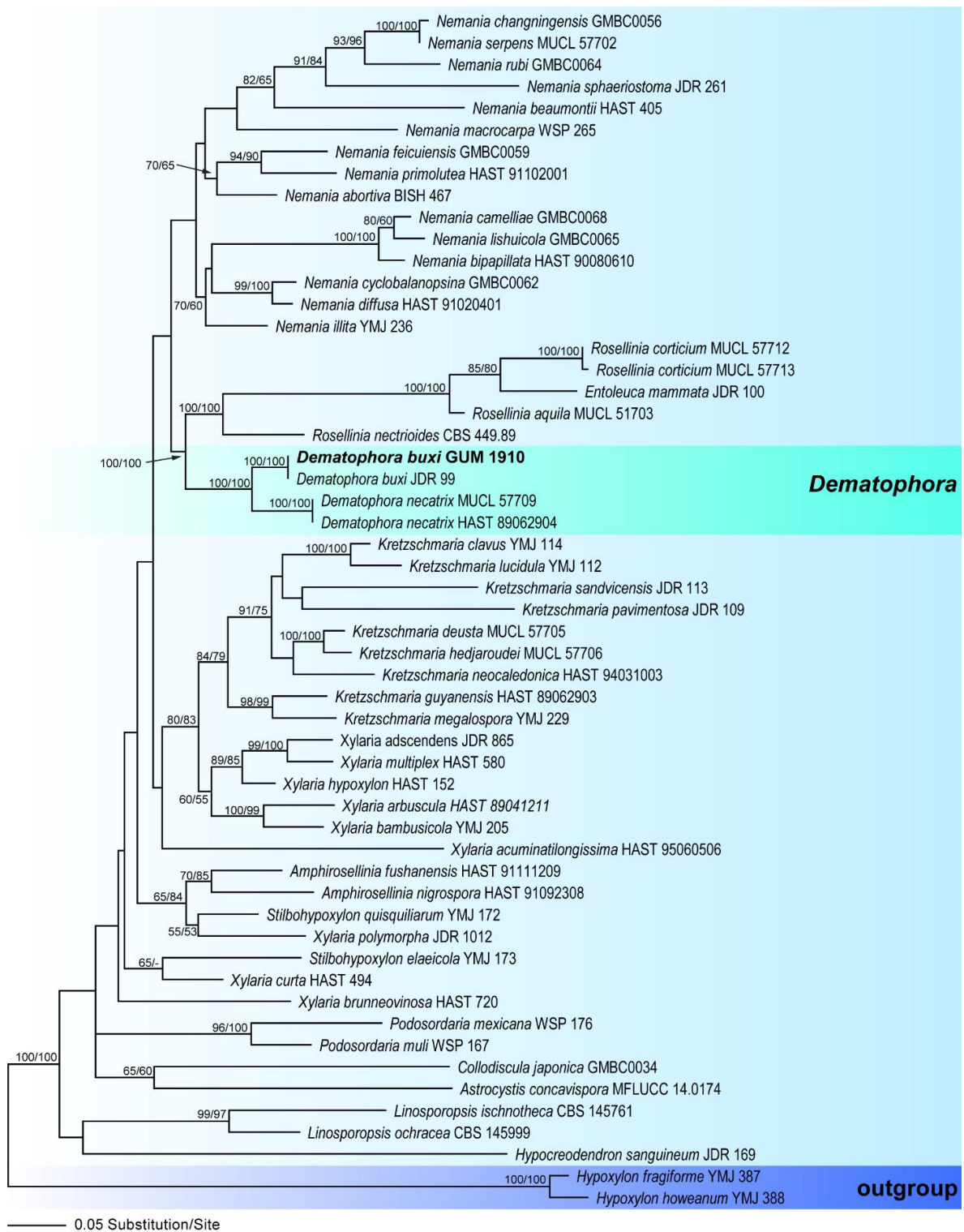
The ITS and *ACT1* sequences of the Iranian collection (OP828512/OP828693) are completely identical to sequences of European accessions of *D. buxi* (JDR 99), which this also supports the erection of novelties for the Iranian fungus.

## DISCUSSION

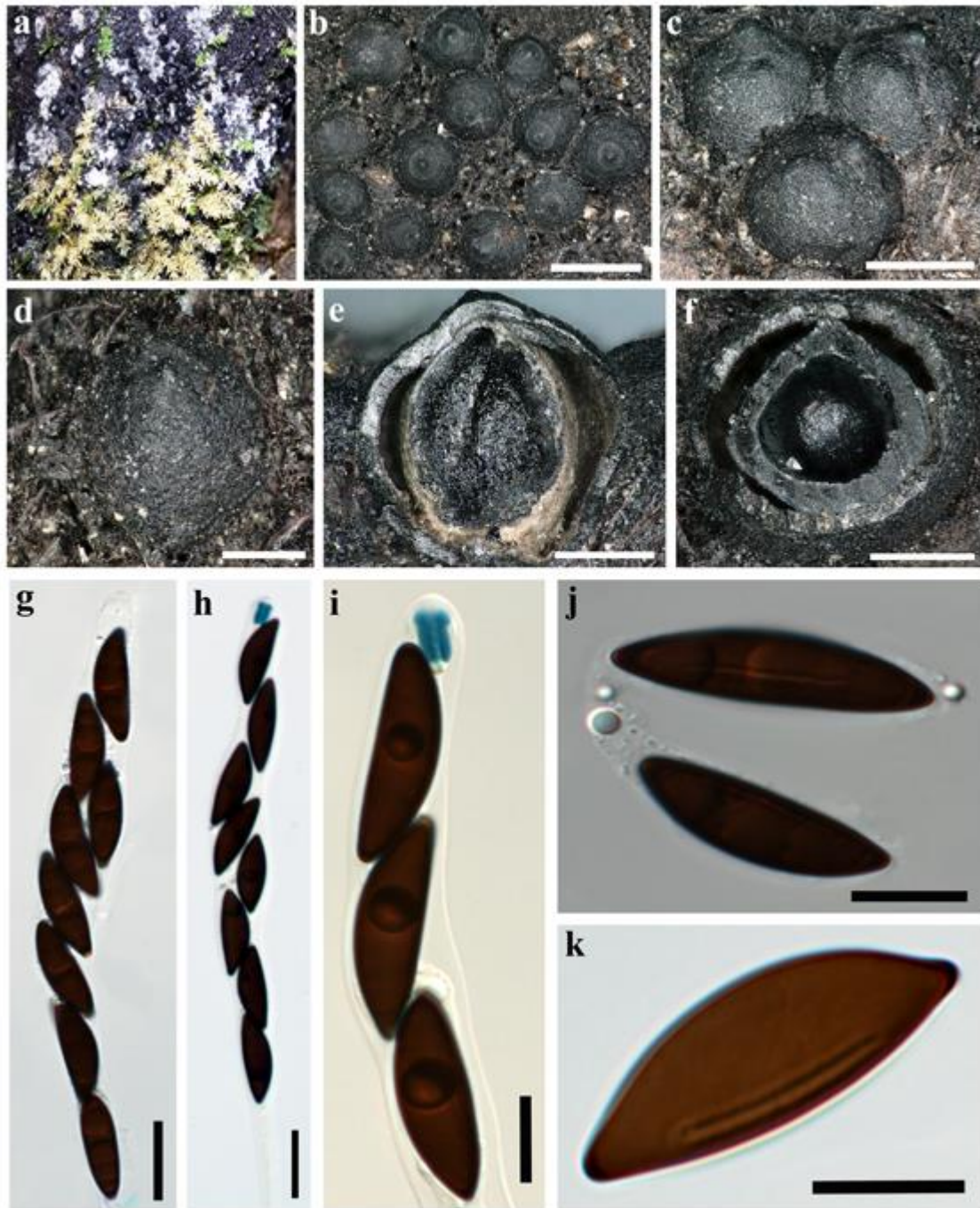
In this study, we examined the phylogenetic placement of our fresh collection with the available sequences of *Dematophora*. We have performed a multigene analysis using ITS and *ACT1* sequence

data to determine the phylogenetic placement of this species.

Species of the resurrected genus *Dematophora* can be assigned to two groups: those with length: width ratio >4, short germ slit, slimy sheath present (e.g. *D. necatrix*), and those with ratio <4 longer germ slit, slimy caps or sheath present (e.g. *D. buxi*). The *necatrix*-group comprises the well-known root pathogens (e.g. *D. necatrix*, *D. pepo*, *D. bunodes*), in the other is *D. buxi* which seems to be a pathogen as well, but it is not so well investigated because not destroying crop plants (Petrini, 2013; <http://pyrenomycetes.free.fr/>, accessed 22 Nov 2022).



**Fig. 1.** Phylogram of the best ML trees (lnL = - 13,639.6577) revealed by RAxML from an analysis of the combined ITS-*ACT1* matrix of selected *Xylariaceae*. Strain in bold was sequenced in the current study. ML and MP bootstrap support above 50% are given at the first and second positions, respectively, above or below the branches.



**Fig. 2.** *Dematophora buxi* (GUM 1910). **a.** stromatal habit; **b,c.** close-up view of stromatal surface; **d.** close-up view of stroma surface showing ostiole and synnemata around stroma; **e.** stroma in longitudinal section showing perithecia and ectostroma; **f.** stroma in horizontal section showing perithecia and ectostroma; **g.** mature ascus in water; **h.** mature ascus in Melzer's reagent; **i.** ascus apical plug in Melzer's reagent; **j,k.** ascospores in water showing straight germ slit much less than spore-length. Scales bars = (**b**) 1.5 mm; (**c,e**) 1 mm; (**d,f**) 0.5 mm; (**g-i**) 20  $\mu$ m; (**j,k**) 10  $\mu$ m.

The North of Iran has subtropical regions and thus houses numerous species of *xylariaceous* fungi indicating that the *Xylariaceae* is one of the dominant fungal families present in this area. Our exploration of *Xylariaceae* revealed many species of *Xylaria* (Hashemi et al. 2014, 2015), *Kretzschmaria* (Pourmoghaddam et al. 2018), *Rosellinia* (Pourmoghaddam et al. 2022a), and *Nemania* (Pourmoghaddam et al. 2022b). To our knowledge, *Dematophora buxi* fruits exclusively on *Buxus* spp. and is just reported from some parts of Europe, e.g., France and Great Britain (<http://pyrenomycetes.free.fr/>, accessed 22 Nov 2022). It is remarkable that *D. buxi* was found in two different Provinces in Iran and that the Iranian specimens match the European material on the molecular level, despite the geographical separation and different *Buxus* hosts. This is the first report of *D. buxi* outside Europe and the first report of *D. buxi* for the mycobiota of Asia and Iran.

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## مطالعه ریخت‌شناختی و مولکولی گونه *Dematophora buxi* (*Xylariaceae*) روی شمشاد خزری به عنوان آرایه جدیدی برای آسیا

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**چکیده:** طی نمونه برداری از قارچ‌های با خصوصیات راسته زایلاریالس در شمال ایران، تعدادی نمونه که دارای خصوصیات ریخت‌شناختی مشابه جنس رزلینیا بودند، جمع‌آوری شدند. خصوصیات ریخت‌شناختی، آنالیز فیلوژنتیکی شامل ترکیب دو ناحیه ژنی ITS و ACT1، ظهور گونه *Dematophora buxi* از روی شمشاد خزری (*Buxus sempervirens*) برای اولین بار از ایران را مورد تایید قرار داد. علاوه بر این، براساس یافته‌های ما، این گونه برای اولین بار از قاره آسیا گزارش می‌شود. در مقاله حاضر، تصویر گونه *Dematophora buxi* و شرح ریخت‌شناسی آن مورد بحث و بررسی قرار گرفته است.

**کلمات کلیدی:** آرایه بندی، تنوع ریستی، میکوبیوتا، فیلوژنی، *Xylariales*، *Sordariomycetes*