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Research article

The eco-geographical and phylogenetic analysis of *Fomes fomentarius sensu lato* (Polyporales, Agaricomycetes) in Armenia

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Abstract

In the current article the revised molecular data of the taxonomic status, as well as eco-geographical distribution and host preferences of white rot medicinal fungus *Fomes fomentarius sensu lato* (Polyporaceae, Agaricomycetes) in Armenia widely distributed in the Northern Hemisphere was discussed. We carried out phylogenetic analysis of Internal Transcribed Spacers (ITS1 and ITS2) of nuclear rDNA and 5.8S rRNA obtained from 30 samples, including 18 mycelial collections of genetically heterogeneous medicinal fungus *Fomes fomentarius s.l.* collected in three floristic regions of Armenia (Ijevan, Aparan and Yerevan). Analysis showed that this species complex is comprises in two sympatric cryptic sublineages, corresponding to taxonomic species *Fomes fomentarius sensu stricto* and *Fomes inzengae*. The ratio of nucleotide divergence (D_{xy}) between ITS1-5.8S-ITS2 sequences and their nucleotide diversity (π) according to the Hudson fixation coefficient (F_{ST}=0.98) indicates a high genetic differentiation of *F. fomentarius s.s.* and *F. inzengae,* confirming that these two *Fomes* species are taxonomically distinct. In Armenia they were only found on deciduous trees: *F. fomentarius s.s.* on *Fagus orientalis* and *Carpinus betulus,* whereas *F. inzengae* on *Acer platanoides*, *C. betulus*, *F. orientalis*, *Fraxinus excelsior*, *Juglans regia*, *Populus nigra*, *Salix alba*, and *Ulmus laevis.* The altitudinal distribution of highland *F. fomentarius s.s.* in north-eastern part of Armenia (Aparan and Ijevan floristic regions) is about 680–1750 m a.s.l., while of lowland species *F. inzengae* is 680–1650 m a.s.l. (Yerevan, Ijevan and Aparan floristic regions). The contact (sympatry) zone where the studied populations of both cryptic species overlap is Ijevan floristic region on *C. betulus* at about 680–750 m a.s.l. Further studies of geographically different collections of *Fomes* species using morpho-ecological, physiological, biochemical and molecular methods are in progress.

Keywords
Fomes fomentarius s.s., Fomes inzengae, rDNA-ITS, host tree, sympatry, phylogeny

Introduction

The correct taxonomic identification and phylogenetic analysis of biotechnologically valuable medicinal mushrooms are challenging, particularly for morphologically similar, genetically heterogeneous, ecologically and geographically overlapped cryptic species. The majority of fungi differ with regard to their anatomical and morphological features (Taylor, 2006). Morphologically indistinguishable Agaricomycetes fungi, such as representatives of genus *Fomes* (Polyporales, Agaricomycetes) may belong to separate phylogenetic lineages (Judova et al., 2012; McCormick et al., 2013; Pristaš et al., 2013; Dresch et al., 2015, Mukhin et al., 2018; Peintner et al., 2019; Badalyan et al., 2022; Zhuykova and Mukhin, 2022; Tomšovský et al., 2023). Among described species names, only two - *Fomes fomentarius* (L.) Fr. and *Fomes fasciatus* (Sw.) Cooke - were taxonomically accepted (Dai, 2012; McCormick et al., 2013; Ryvarden and Melo, 2014; Rivoire, 2020).

Fomes fomentarius is a widely distributed white rot fungus growing on living or dead broadleaf trees, rarely on conifers (Gilbertson and Ryvarden, 1986; Peintner et al., 2019; Garrido-Benavent et al., 2020). The morphological study of *F. fomentarius* did not reveal any significant variability in different parts of its distribution area (Gáperová et al., 2016). Several forms and varieties of *F. fomentarius* have been described as its synonyms (Lécuru et al., 2019). However, further molecular data has shown that *F. fomentarius* is a genetically heterogeneous species complex *Fomes fomentarius sensu lato* comprising several cryptic lineages (clades) and sublineages (Judova et al., 2012; Gáper et al., 2016; McCormick et al., 2013; Dresch et al., 2015, Peintner et al., 2019). *Fomes fomentarius s.l.* are represented by two (A and B) lineages (clades) and four sublineages (A1, A2 and B1, B2) (Pristaš et al., 2013; Mukhin et al., 2018). The sublineage A1 was recorded only in North America (Gáper et al., 2016). However, recent findings proved its presence also in the Far East (Zhuykova and Mukhin, 2024). The range of distribution of the sublineage A2 covers the entire Boreal region from the Atlantic Ocean to the Pacific. The area of distribution of the sublineage B1 is Asia, B2 is subtropical regions of the European sub-continent with Eastern boundaries from the Southern Urals to Northern Kazakhstan (Mukhin et al., 2018; Zhuykova and Mukhin, 2022), as well as Armenia (Badalyan et al., 2022).

Based on 97% nucleotide similarity of the ITS rDNA sequences of both A and B genotypes, as well as the absence of morphologically distinguishable characteristics of basidiocarps and cohabitation areas, they may be considered two separate cryptic and sympatric species (Judova et al., 2012) identified as *Fomes fomentarius sensu stricto* and *Fomes inzengae* (Ces. & De Not.) Cooke (McCormick et al., 2013; Gáper et al., 2016; Peintner et al., 2019; Náplavová et al., 2020). Further phylogenetic analysis of partial *TEF1* gene and rDNA LSU clearly confirmed that *F. fomentarius s.s.* and *F. inzengae* are genetically distinct species (Pristaš et al., 2013). The genetic differences at the species level were further confirmed based on differences of ITS rDNA sequences (Garrido-Benavent et al., 2020; Tomšovský et al., 2023).

The analysis of 19 *F. fomentarius s.s.* and 31 *F. inzengae* ITS sequences from central Europe showed a high genetic differentiation confirming that these species are genetically diverse (Tomšovský et al., 2023). Although the ranges of their sympatric populations overlap the molecular data completely support genetic isolation, while the morphological characteristics of basidiocarps taxonomically are not reliable (Tomšovský et al., 2023) as previously suggested (Peintner et al., 2019). The phylogenetic analyses of ITS, LSU, *TEF1, RPB1* and *RPB2* sequences of *Fomes* species complex clearly showed that *F. fomentarius s.s.* and *F. inzengae* are genetically distinct and welldelimited species (McCormick et al., 2013). The six new *Fomes* clades were described in Iberian Peninsula (Garrido-Benavent et al., 2020) and their species names have been proposed (Peintner et al., 2019). *Fomes fomentarius* from North America may belong to geographically separated species *F. fomentarius* aff. USA, similarly to *F. inzengae* from China and Korea, as *Fomes* sp. Asia, which differ from other *Fomes* species and distinct evolutionary lineage close to *Globifomes graveolens* (Schwein.) Murill*,* a sister species of *F. inzengae* (Cristini et al., 2023; Tomšovský et al., 2023). Thus, at least two distinct genotypes corresponding to *F. fomentarius s.s.* (A2) and *F. inzengae* (B2) with different distribution areas, trophic spectra and ecological preferences were identified in Europe (Judova et al., 2012; Peintner et al., 2019; Náplavová et al., 2020; Tomšovský et al., 2023; Gáper et al., 2025). A new *Fomes* species - *Fomes hengduanensis* Y.C. Dai, Chao G. Wang & Y.J. Cui from *Quercus semecarpifolia* Sm. with basal position to *F. inzengae – G. graveolens* lineage has recently been reported in China (Cui et al., 2024). Thus, far, four *Fomes* species (*Fomes fasciatus, F. fomentarius s.s*., *F. inzengae* and *Fomes hengduanensis)* have been taxonomically described till now.

The morpho-ecological differences between *F. inzengae* and *F. fomentarius s.s.* basidiocarps correspond to the screening results of their mycelial collections (colony morphology, pigmentation, growth kinetics, growth temperature and pH, development of chlamydospores, cuticular cells and globular pseudomarenchyma, as well as fruiting bodies with mature meiospores) (data not shown). The taxonomically valuable cultural characteristics are particularly important for identification of *Fomes* cultures during their biotechnological cultivation. *Fomes inzengae* and *F. fomentarius s.s.* also differ by their metabolite profile, laccases (data not shown) and bioactivity (Dresch et al., 2015; Gharibyan et al., 2024; 2025). Therefore, further morpho-ecological, physiological and medicinal study of geographically different mycelial and basidioma collections of *Fomes* species with combination of molecular data are warranted.

The eco-geographical analysis of 50 samples of *F. fomentarius s.s.* and *F. inzengae* in central Europe showed that *F. fomentarius s.s.* is mainly distributed at 400–970 m, rarely < 400 m a.s.l. in association with *Fagus* and *Betula* trees, while *F. inzengae* at 154–490 m a.s.l. on wide spectrum of hosts (*Acer* L.*, Aesculus* L.*, Alnus* Mill.*, Betula* L.*, Fagus* L.*, Fraxinus* L.*, Quercus* L.*, Platanus* L.*, Populus* L.*, Salix* L.*, Sorbus* L. and *Tilia* L.). Their contact or sympatry zone was located in the upper-colline vegetation zone at 400–550 m a.s.l. within the mesophytic phyto-geographical region comprising *Carpinus betulus* L. forests. *Fomes inzengae* has a southern, Mediterranean distribution and was described as a lowland species, while *F. fomentarius s.s.,* as a highland species growing at higher altitudes in colder temperate woodlands, mesic and subalpine *Fagus* forests (Tomšovský et al., 2023).

In spite of wide distribution of *F. fomentarius s.s.* and *F. inzengae* almost all over Europe, no clear geographical segregation was found. These species showed different host preferences. They are developing basidiomata preferably on Angiosperms from more than 20 genera, rarely on Gymnosperms from genera *Abies, Picea, Larix* and *Pinus*. In central Europe, *F. inzengae* grows on hardwood (*Alnus*, *Aesculus*, *Acer*, *Betula*, *Carpinus*, *Castanea* Mill., *Cerasus* (Mill.) A. Gray, *Fraxinus*, *Festuca* Tourn. Ex L., *Juglans*, *Oreorchis* Lindl., *Platanus*, *Quercus*, *Sorbus*, rarely *Fagus*) (Peintner et al., 2019; Pirronitto et al., 2024; Tomšovský et al., 2023; Gáper et al., 2025) or softwood forests (*Salix*, *Tilia*, and *Populus*) (Zhuykova and Mukhin, 2022). *Fomes inzengae,* the only *Fomes* species on the Iberian Peninsula, was found on *Populus, Fraxinus* and *Salix* (Garrido-Benavent et al., 2020). The common hosts for *F. inzengae* in Europe are *Carpinus, Castanea, Cerasus, Platanus,* *Populus, Quercus,* and *Abies* (Peintner et al., 2019), while in the Southern Urals and Kazakhstan *Acer*, *Populus*, *Salix*, and *Tilia* (Zhuykova and Mukhin, 2022). *Fomes inzengae* has also been observed in England, Austria, Italy, Latvia, Slovakia, Slovenia, Spain, Czechia and Armenia (Mukhin et al., 2018; Garrido-Benavent et al., 2020; Badalyan et al., 2022; Zhuykova and Mukhin, 2022; Cristini et al., 2023; Tomšovský et al., 2023; Pirronitto et al., 2024).

The common hosts for *F. fomentarius s.s.* in Europe are *Fagus* and *Betula*, less common *Acer, Alnus, Populus* and *Picea* Dietrich (Peintner et al., 2019), while in the Urals (Russia) and Northern Kazakhstan *Alnus, Betula, Prunus* L., *Salix* and *Sorbus.* The distribution of *F. fomentarius s.s.* in the southern European area is likely determined by southern distribution area of *Betula pendula* Roth (Náplavová et al., 2020). Five genera (*Acer*, *Alnus*, *Betula*, *Fagus*, and *Populus*) have recently been reported as common hosts for *F. fomentarius s.s.* and *F. inzengae* in Europe (Gáper et al., 2025).

Based on ITS – rDNA sequence analysis, the studies of phylogenetic diversity of *F. fomentarius s.l.* in the Eurasian part of distribution area (the Urals, Siberia and the Far East) revealed three (A1, A2, B2) from four (A1, A2, B1, B2) *Fomes* sublineages. The two sublineages were identified as *F. fomentarius s.s.* (A2) and *F. inzengae* (B2) and suggested as sub–species of the polytypic *F. fomentarius s.l.* (Mukhin et al., 2018; Zhuykova and Mukhin, 2022; 2024). This was also shown in the phylogenetic analysis of previous collection of *F. fomentarius s.l.* from Armenia (Badalyan et al., 2022). However, recent molecular studies (Cristini et al., 2023; Tomšovský et al. 2023; Pirronitto et al., 2024) have necessitated the revision of taxonomic status of *F. fomentarius s.l.* sublineages described in Armenia. The current article discusses the revised molecular data of the taxonomic status of *F. fomentarius s.l.* sublineages, as well as their eco-geographical distribution and host preferences in Armenia.

Materials and Methods

Collection sites

Armenia is a mountainous landlocked country in the South Caucasus region located within the Armenian plateau and bordered by Georgia, Azerbaijan, Turkey and Iran (Fig. 1). The forest part of the country comprises 12.4%. The lowest point is at 380 m a.s.l. (the gorge of the Debed River) and the highest at 4095 m a.s.l. (Mt Aragats) with an average altitude of 1850 m. About 90% of the country territory lies at over 1000 m a.s.l. The lowland part (Tavush region) is at the altitude of about 500 m, while the highland part is 2300–2400 m high. The diversity of landscapes and climates (from dry subtropical to extreme alpine) are important determinants of flora and mycobiota of Armenia. Based on phyto-geographical characteristics, 12 floristic regions have been defined in Armenia (Takhtajyan, 1954) (Fig. 1).

Quercus, Fagus, Carpinus, *Fraxinus, Acer, Ulmus* L. and other trees predominantly occur in broadleaved forests at 500–2.100 m a.s.l. in the north and up to 2.500 m a.s.l. in the south. *Quercus* and *Fagus* forests are distributed at 500–2000 m and 1.000–2.100 m a.s.l., respectively; while *C. betulus* are less spread and occur at altitudes of 800 to 1.800 m (Fayvush et al., 2016). About 4000 fungi, including 1200 mushroom species have been described in the territory of Armenia (National Report to Convention on Biological Diversity, 2014). *Fomes fomentarius* is the only *Fomes* species were recorded in all floristic regions on deciduous trees (Melik-Khachatryan and Martirosyan, 1971; Badalyan and Gharibyan, 2008; 2016). Overall, 18 *Fomes* basidiomata collected from 2001 to 2025 in Armenia were used to create mycelial collections for the current study (Table 1). The collection sites of basidiocarps were located in three floristic regions: Ijevan, Aparan, and Yerevan with an average altitude of about 900, 1996 and 1000 m (Fig. 1).

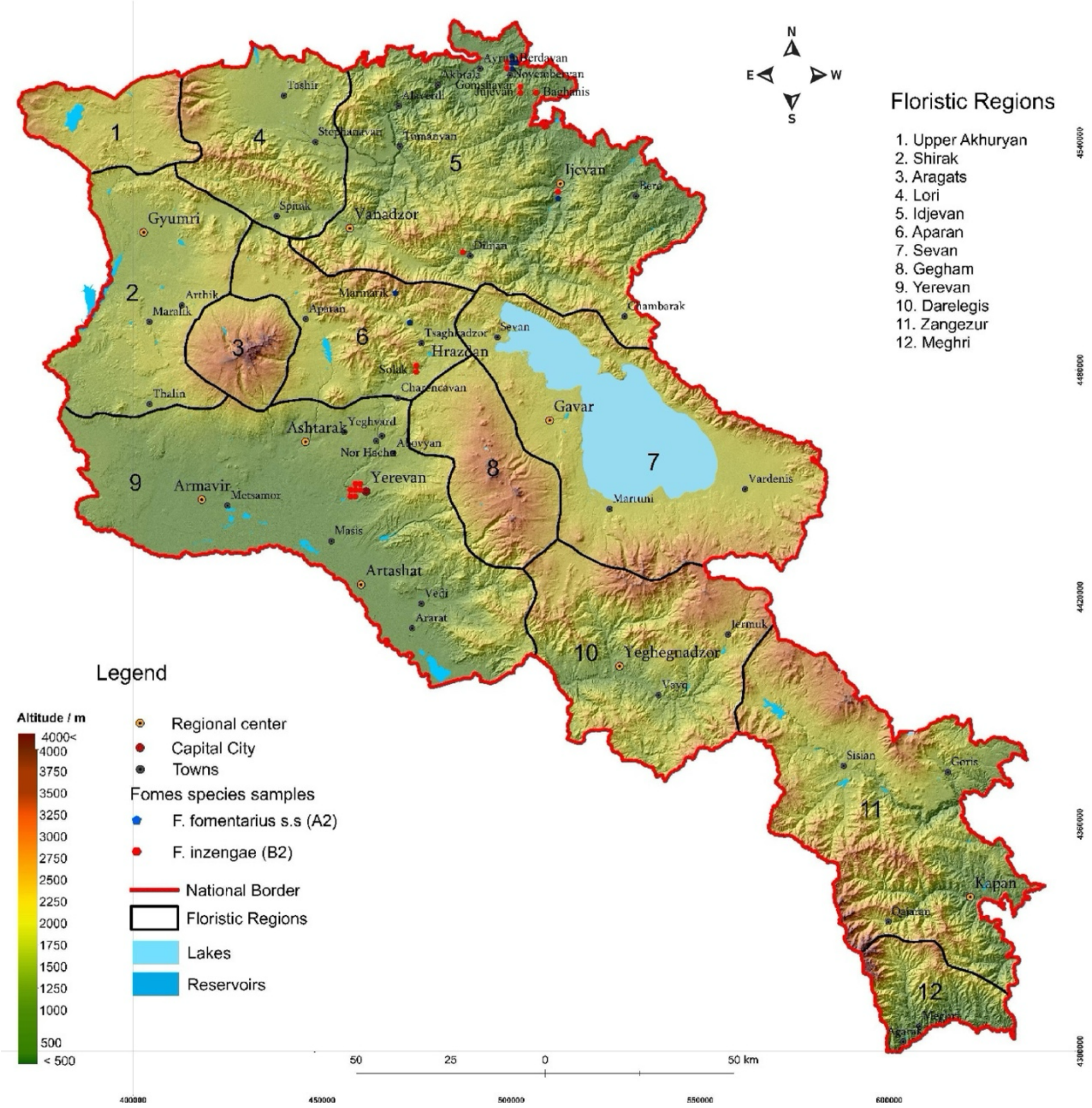

Fig. 1 – The distribution map of studied collections of *Fomes fomentarius s.l.* in Armenia.

The morphological identification of basidiocarps was performed using light microscope Omano OM157T, USA (10 × 40 and 15 × 40 ocular/objective) connected to a computer with Software program (OC View 7, version 7.1) and a digital camera (OptixCam Summit, Ser. OCS 1.3MP, USA), as well as traditional taxonomic keys (Bondarzew, 1953; Gilbertson and Ryvarden, 1986; Ryvarden and Melo, 2014) (Fig. 2). Dikaryotic tissue cultures were isolated on 1.5% malt-extract agar (MEA) medium and cultivated in Erlenmeyer flasks (surface growth) to obtain biomass for molecular studies, as previously described (Badalyan et al., 2023). In total, 30 samples of *F. fomentarius s.l.* (18 from Armenia and 12 from Russian Federation) were selected for current phylogenetic analysis (Table 1). The mycelial cultures and basidioma samples are preserved in the Fungal Culture Collection and Fungarium of the Laboratory of Fungal Biology and Biotechnology, Yerevan State University (FCC-YSU) (Badalyan and Gharibyan, 2017).

DNA isolation and PCR amplification

Overall, 30 ITS1–5.8S–ITS2 rDNA sequences from mycelial cultures of *F. fomentarius s.l.* were used in the current molecular study from which the sequences of 14 Armenian strains were originally received (Table 1).

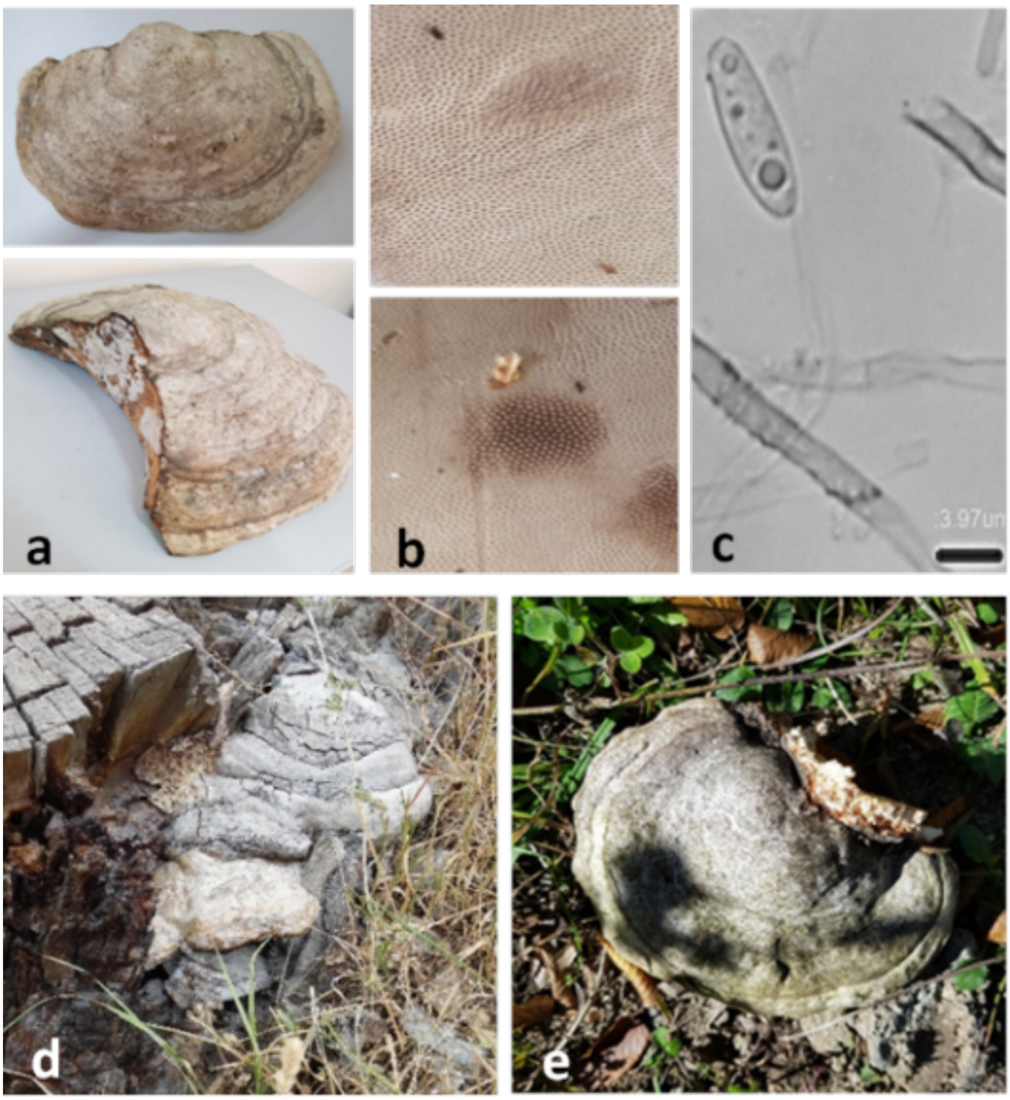

Fig. 2 – Basidiomata, hymenium and cylindric basidiospore of *Fomes inzengae* Fi/30 strain collected on trunk of *Fagus orientalis* (a, b, c) and Fi/16 strain, collected on trunk of *Salix alba* (d). Basidiomata of *Fomes fomentarius s.s.*, Ff/13 strain, collected on trunk of *F. orientalis* (e). Bars = 4 µm.

The DNA extraction and PCR amplification were performed in the Laboratory of Molecular Research of Plants and Fungi, Institute of Natural Sciences and Mathematics, Ural Federal University (Russian Federation) according to the previously described protocols (Badalyan et al., 2022). DNA was isolated by a diaGene (Diaem, Russian Federation) or FitoSorb (Sintol LLC, Russian Federation) for DNA extraction from mycelium. The sequences were amplified by primers ITS1-F and ITS4-B (Gardes and Bruns, 1993). The enzymatic purification of PCR products and sequencing in both directions were performed at Sintol LLC (Russian Federation). The primary data processing was carried out using Finch TV 1.4.0 (Geoprisa, Inc.) and MEGA 7.0.18 (Kumar et al., 2016). The sequence identification was performed with a search for similar sequences in GenBank using BLASTN algorithm. The obtained sequences were submitted to the GenBank database with the accession numbers reported in Table 1. The sequences of retyped strains were compared using MEGA 7.0.18 (Kumar et al., 2016) (Table 1).

Table 1 – Metadata of FCC-YSU of *Fomes fomentarius s.l.* used in this study.

Strain	**Host tree**	**Origin**	**Date of collection**	**GenBank code**	**Altitude**	**Reference**
***F. fomentarius s.s.* / Sublineage A2**						
Ffa-2	*Fagus orientalis* living tree	Berdavan, Tavush province, IJ	July 2011	KJ857248	680	Badalyan et al. 2015
Ff/11	*Fagus orientalis* living tree	Berdavan, Tavush province, IJ	April 2014	OL583666	680	Badalyan et al. 2022
Ff/12	*Fagus orientalis* living tree	Berdavan, Tavush province, IJ	April 2014	OL583667	680	Badalyan et al. 2022
Ff/13	*Fagus orientalis* stump	Berdavan, Tavush province, IJ	April 2014	PP972749	750	Original
Ff/9	*Carpinus betulus* living tree	Ijevan, Tavush province, IJ	July 2005	PP972751	750	Original
Ff/18	*Carpinus betulus* trunk	Marmarik, Kotayk province, AP	August 2015	OL583670	1750	Badalyan et al. 2022
Ff/1	*Betula pendula* fallen tree	Zvenigorod biostation, Moscow region, Russia	2009	KJ857249	160	Badalyan et al. 2022
Ff/2	*Betula pendula* fallen tree	Zvenigorod biostation, Moscow region, Russia	2009	JQ901965	160	Badalyan et al. 2015
Ff/4	*Betula pendula* living tree	Bitsevsky Park, Moscow region, Russia	2010	JQ901966	170	Badalyan et al. 2015
FF-T1	*Betula pendula* living tree	Zvenigorod biostation, Moscow region, Russia	2011	KJ857255 PP972753	167	Badalyan et al. 2015 Original
FF-T2	*Betula pendula* living tree	Troitsk, Moscow region, Russia	2011	PP972752	175	Original
FF-T3	*Alnus glutinosa* living tree	Zvenigorod biostation, Moscow region, Russia	2011	KJ857253	167	Badalyan et al. 2015
FF-T5	*Betula pendula* living tree	Zvenigorod biostation, Moscow region, Russia	2011	KJ857254	167	Badalyan et al. 2015
FA-1	*Populus tremula* living tree	Troitsk, Moscow region, Russia	2010	KJ857260 PP972755	175	Badalyan et al. 2015 Original
FA-3	*Populus tremula* living tree	Troitsk, Moscow region, Russia	2010	KJ857257 PP972754	175	Badalyan et al. 2015 Original
FF-G6	*Betula pendula* living tree	Troitsk, Moscow region, Russia	2010	KJ857256	160	Badalyan et al. 2015
Ff/Y23	*Populus balsamifera* living tree	Yekaterinburg, Russia	April 2016	PP972750	270	Original

***F. inzengae* / Sublineage B2**						
Fi/3	*Carpinus betulus* living tree	Ijevan, Tavush province, IJ	July 2005	PP972742	750	Original
Fi/26	*Carpinus betulus* living tree	Dilijan, Lake Parz, Tavush province, IJ	July 2016	OL583673	1220	Badalyan et al. 2022
Fi/VIII	*Fraxinus excelsior* living tree	Berdavan, Tavush province, IJ	August 2013	PP972746	680	Original
Fi/8	*Juglans regia* living tree	Berdavan, Tavush province, IJ	April 2014	OL583665	680	Badalyan et al. 2022
Fi/15	*Salix alba* stump	Solak village, Kotayk province, AP	July 2015	OL583668	1650	Badalyan et al. 2022
Fi/16	*Salix alba* stump	Solak village, Kotayk province, AP	July 2015	OL583669	1650	Badalyan et al. 2022
Fi/20	*Populus nigra* living tree	Yerevan, YR	July 2013	PP972744	860	Original
Fi/25	*Populus nigra* living tree	Yerevan, YR	July 2016	OL583672	860	Badalyan et al. 2022
Fi/21	*Ulmus laevis* living tree	Yerevan, YR	June 2014	PP972748	860	Original
Fi/24	*Fagus orientalis* living tree	Baghanis, Tavush province, IJ	July 2016	OL583671	920	Badalyan et al. 2022
Fi/30	*Fagus orientalis* trunk	Jujevan, Tavush province, March 2023; IJ	March 2023	PP972741	1050	Original
Fi/31	*Acer platanoides* living tree	Gomshavar, Tavush province, IJ	May 2023	PP972745	1564	Original
Fi/Y19	*Populus balsamifera* living tree	Yekaterinburg, Russia	April 2016	PP972747	270	Original

Note: (AP) Aparan, (IJ) Ijevan, and Yerevan (YR) floristic regions of Armenia

Phylogenetic and statistical analysis

The phylogenetic analysis of 30 ITS1–5.8S–ITS2 rDNA sequences (14 originals and 16 previously obtained; 18 from Armenia, 12 from Russian Federation and 16 references) was performed (Tables 1 and 2). The *Fomes fasciatus* sequence was selected as an out-group. The final dataset was composed by 543 positions, excluding sites with gaps/missing data 470. There were 426 monomorphic and 44 polymorphic sites, including 7 singleton variable and 37 parsimony informative sites.

The model selection was made based on minimal values of Akaike Information Criterion, corrected and Bayesian Information Criterion (Nei and Kumar, 2000). The phylogenetic tree was constructed using the Maximum Likelihood method based on the Hasegawa-Kishino-Yano model (Hasegawa et al., 1985). The tree was drawn to scale, with branch lengths measured in the number of substitutions per site. The evolutionary analysis was carried out in MEGA 7.0.18 (Kumar et al., 2016).

As an indicator of genetic polymorphism, similarity was calculated as the percentage of identical base pairs (bp) of all sites. The sequence similarity matrix was designed based on the alignment without polymorphic positions (removed from all sequences) in the Multiple Sequence Alignment EMBL-EBI (http://www.ebi.ac.uk/Tools/msa/clustalo/) with subsequent calculations in Microsoft Excel. The polymorphism indices, such as nucleotide diversity (π), average number of nucleotide differences per site between two sequences and nucleotide divergence (D_{xy}) – the average proportion of nucleotide differences between two groups of sequences – have also been calculated.

Table 2 – Metadata of *Fomes fomentarius s.s.* and *Fomes inzengae* reference sequences used in this study.

Origin	Host tree	GenBank code	Original clade	Reference
***F. fomentarius s.s.* / Sublineage A2**				
Austria	*Fagus sylvatica* L.	KM360127	*F. fomentarius s.s./* epitype	Peintner et al. 2019
Slovakia	*F. sylvatica*	HQ189534	Genotype A	Judova et al. 2012
Slovakia	*F. sylvatica*	GQ184603	Genotype A	Pristaš et al. 2013
Russia, Perm region	*Betula* sp.	OL546841	*F. fomentarius s.s./* sublineage A2	Zhuykova and Mukhin 2022
Russia, Perm region	*Betula* sp.	OL580752	*F. fomentarius s.s./* sublineage A2	Zhuykova and Mukhin 2022
Russia, Sverdlovsk region	*Betula* sp.	OL580751	*F. fomentarius s.s./* sublineage A2	Zhuykova and Mukhin 2022
Russia, Chelyabinsk region	*Betula* sp.	OL580749	*F. fomentarius s.s./* sublineage A2	Zhuykova and Mukhin 2022
***F. inzengae* / Sublineage B2**				
Italy	*Quercus cerris* L.	UDB034501*	*F. inzengae/* epitype	Peintner et al. 2019
Russia, Chelyabinsk region	*A. platanoides*	MF563981	*F. inzengae/* sublineage B2	Zhuykova and Mukhin 2022
Kazakhstan, Kostanay region	*Populus* sp.	OL579735	*F. inzengae/* sublineage B2	Zhuykova and Mukhin 2022
Russia, Orenburg region	*Alnus* sp.	MF563984	*F. inzengae/* sublineage B2	Zhuykova and Mukhin 2022
Russia, Sverdlovsk region	*Populus balsamifera*	OL579737	*F. inzengae/* sublineage B2	Zhuykova and Mukhin 2022
UK	Angiosperm tree	FN539043	Genotype B	Pristaš et al. 2013
Slovakia	*Cerasus avium* L.	HQ189535	Genotype B	Judova et al. 2012
F. fasciatus				
USA	*Platanus occidentalis* L.	JX126900	*F. fasciatus*	McCormick et al. 2013
USA	*Quercus* sp.	JX126901	*F. fasciatus*	McCormick et al. 2013

Note: * UNITE number

Only conservative sites and sites with unambiguous substitutions were used in their calculations. The aligned sequence sets with excluded polymorphic positions (R, M, Y and other ambiguous characters replaced with N) were used as a matrix in the DnaSP 6.12 (Rozas et al., 2017). The genetic isolation of phylogenetic lineages was estimated using the fixation index (F_{ST}): $F_{ST} = 1 - H_w / H_b$, where H_w and H_b are the mean number of differences between different sequences sampled from the same and two different lineages, respectively (Hudson et al., 1992). Their numeric value ranges from 0 (no genetic isolation) to 1 (complete genetic isolation).

Results and Discussion

Revision of phylogenetic analysis of Armenian collection of F. fomentarius s.l.
The phylogenetic analysis of the Armenian collection of *F. fomentarius s.l.* revealed two sublineages - *F. fomentarius s.s.* (A2) and *F. inzengae* (B2) - described as infraspecific taxa (Badalyan et al., 2022). However, further analyses of 30 ITS1–5.8S–ITS2 sequences derived from mycelial collections of *F. fomentarius s.l.* from Armenia and Russia showed that they divide into two groups: 17 (A2) and 13 (B2) (Table 1; Fig. 3). Among 18 Armenian sequences, six belong to the sublineage A2 (*F. fomentarius s.s.*) and 12 to sublineage B2 (*F. inzengae*) with ratio 1:2 which is significantly higher compared to the previous one (1:1) (Badalyan et al., 2022). Further phylogenetic analysis confirms the correspondence of sublineages to *F. fomentarius s.s.* (A2) and *F. inzengae* (B2) described in Europe as genetically separate cryptic sympatric species (Peintner et al., 2019; Tomšovský et al., 2023).

According to the ITS rDNA-based taxon delimitation technology, identification of species assesses the nucleotide similarity of ITS sequences of the studied fungal samples. The threshold value of nucleotide similarity indicating that they belong to different species is 97–99%, while higher similarity values typically indicate that they belong to the same species. The mean nucleotide similarity of ITS sequences of sublineages A2 and B2 of *F. fomentarius s.l.* in Armenian collections is 96.99% which indicates that differences between A2 and B2 reach the level of genetically distinct species (Table 3).

Table 3 – The average nucleotide similarity and its spread (in brackets) within (*) and between (**) ITS sequences of the sublineages A2 and B2 of *Fomes fomentarius s.l.* in Armenia, %.

Species/sublineages (number of sequences)	***F. fomentarius s.s.* / A2**	***F. inzengae* / B2**
F. fomentarius s.s./A2 (6)	99.67 (99.39–100)*	96.99 (96.58–97.18)**
F. inzengae/B2 (12)		99.84 (99.41–100)*

Moreover, the level of nucleotide diversity (π), an indicator of individual differences in sequences, is either 0 (sublineage B2) or 0.0006 (sublineage A2) (Table 4). The genetic differences between the sequences of sublineages A2 and B2, estimated by nucleotide divergence (D_{xy}), is 0.01296 or 1.29%. The same level of nucleotide differentiation (D_{xy} = 1.29%) was obtained when comparing the sequences of *F. fomentarius s.s.* (A2) from Armenia and *F. inzengae* (B2) the Urals (Zhuykova and Mukhin, 2022). The ratio of nucleotide divergence (D_{xy}) between sequences and their nucleotide diversity (π) according to the Hudson fixation coefficient (F_{ST}) indicates the level of genetic isolation of populations and species. The F_{ST} for ITS sequences *F. fomentarius s.s.* and *F. inzengae* from Armenia is 0.976851852 [1 − (0.0003:0.01296)]. The F_{ST} close to 1 indicates almost complete genetic isolation of sublineages A2 (*F. fomentarius s.s.*) and B2 (*F. inzengae*). Similar level of genetic isolation has been revealed between these species (F_{ST} = 0.991) in the forests of South Moravia (Central Europe) (Tomšovský et al., 2023).

Table 4 – The nucleotide divergence (D_{xy})** between ITS sequences A2 and B2 sublineages of *F. fomentarius s.l.* and their nucleotide diversity (π)* in Armenia

Species/sublineages (number of sequences)	***F. fomentarius s.s.* / A2**	***F. inzengae* / B2**
F. fomentarius s.s./A2 (6)	0.0006*	0.01296**
F. inzengae/B2 (12)		0*

The comparative analysis of nucleotide divergence showed that the sequences of *F. fomentarius s.s.* (sublineage A2) from Armenia show weak differences from similar sequences found in the Urals and the European part of Russia: D_{xy} = 0.03 and 0.05%, respectively. The nucleotide divergence between sequences of *F. inzengae* (sublineage B2) from Armenia and the Urals was 0.02%. In all three cases, π is equal to D_{xy} and Hudson's coefficient, is equal to 0, which shows the absence of genetic differentiation of Armenian populations of *F. fomentarius s.s.* and *F. inzengae* from their geo-ecologically distant Russian populations (Zhuykova and Mukhin, 2022). Thus, the obtained results clearly confirm the genetic heterogeneity of *F. fomentarius s.l.* in Armenia and the presence of phylogenetically different sublineages A2 and B2, taxonomically identified as two distinct *Fomes* species, *F. fomentarius s.s.* and *F. inzengae*, respectively.

Altitudinal distribution and substrate confinement

The sympatric nature of *F. fomentarius s.s.* and *F. inzengae* is well expressed in Armenia, as in other parts of the Eurasian distribution area of *F. fomentarius s.l.* Both species occur in three studied floristic regions. *F. fomentarius s.s.* and *F. inzengae* were represented in Aparan, Ijevan and Yerevan floristic regions by 1/5/0 and 2/7/3 number of records, respectively. The number of records of *F. inzengae* was higher in Ijevan and Yerevan regions, while *F. fomentarius s.s.* was not recorded in Yerevan collection (Table 1; Fig. 1).

The phylogenetic analysis of 18 Armenian collections in studied floristic regions showed that *F. fomentarius s.l.* is represented by two cryptic species: *F. fomentarius s.s*. and *F. inzengae* differing by altitudinal distribution and substrate confinement (Table 1). *Fomes fomentarius s.s.* basidiomata were collected on *F. orientalis* Lipsky and *C. betulus* trees at 680–1750 m a.s.l. (Aparan and Ijevan floristic regions), whereas *F. inzengae* on *Acer platanoides* L., *Fraxinus excelsior* L.*, Juglans regia* L., *Salix alba* L., *F. orientalis, Populus nigra* L., *Ulmus laevis* Pall. and *C. betulus* trees, at 680–1650 m a.s.l. *Juglans regia,* as a host tree of *F. inzengae* was reported originally (Badalyan et al., 2022). The sympatry zone, where the populations overlap, is at about 680–750 m a.s.l. on *C. betulus* (Ijevan floristic region; strains Ff/9 and Fi/3). Thus, the trophic competition between these species in Armenia is possible only with their common occurrence on the trunk and living wood of *F. orientalis* and *C. betulus,* respectively (Table 1; Fig. 1).

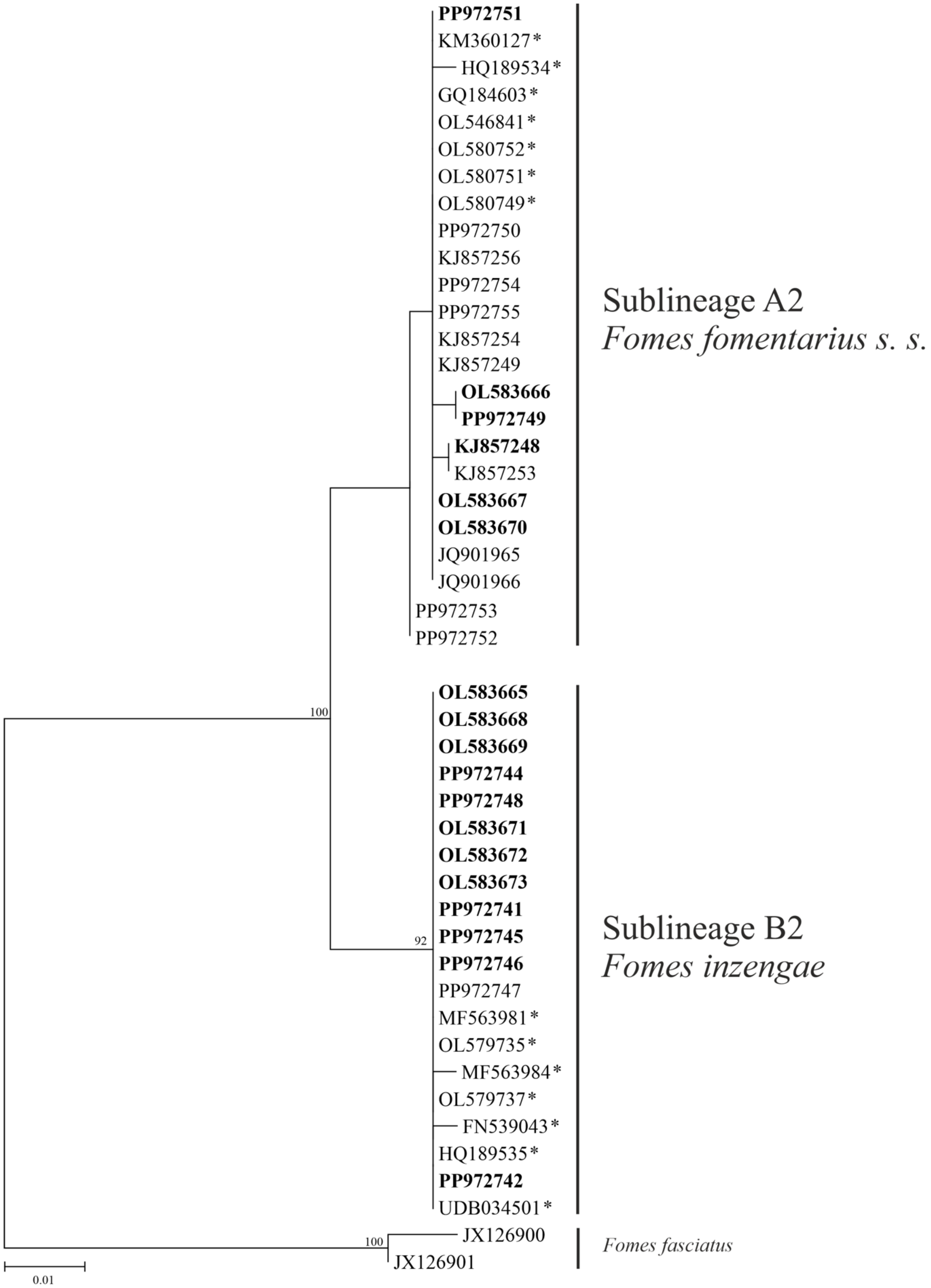

Fig. 3 – Phylogenetic tree of *Fomes fomentarius s.l.* collection from Armenia (bold), European part of Russia and reference ITS1–5.8S–ITS2 sequences (542 bp) inferred by using the Maximum Likelihood method based on the Hasegawa-Kishino-Yano model. The bootstrap values (1000 replicates) above 70 are shown next to the branches.

The distribution patterns of Armenian collections of *F. fomentarius s.s.* and *F. inzengae*, as well as host trees are consistent with data obtained from central Europe, where *F. fomentarius s.s.* is predominantly found in beech forests and *F. inzengae* has rare occurrence (Peintner et al., 2019; Pirronitto et al., 2024; Tomšovský et al., 2023). The native species, *F. fomentarius s.s.* and *F. inzengae*, as endophytes were recorded at the same *Fagus* forests in Ardennes, Belgium which showed that the potential direct competition between both sublineages is possible in the same environment (Pirronitto et al., 2024).

The co-occurrence of *F. fomentarius s.s.* (strains Ffa-2, Ff/11, Ff/12, and Ff/13) and *F. inzengae* (Fi/3, Fi/VIII, Fi/8) in Armenia was observed near Berdavan village on *C. betulus* and *F. orientalis,* as common hosts for both clades (species) at about 680–750 m a.s.l., while the area of sympatry was about 750 m a.s.l. on *C. betulus* (strains Ff/9, Ff/13, Fi/3) in North Eastern forest part of Armenia (Ijevan floristic region) (Table 1; Fig. 1). Thus, the contact zone for sublineages A2 and B2 in central Europe and Armenia comprises *C. betulus* forests at about 400–550 and 750 m a.s.l., respectively. Several *F. inzengae* strains (Fi/15, Fi/16, Fi/26, Fi/30, and Fi/31) have been isolated from basidiocarps collected on *C. betulus, F. orientalis* and *S. alba* from 1050 to 1650 m a.s.l. which are higher altitudes for lowland species *F. inzengae* compared to highland species *F. fomentarius s.s.* collected at up to 1750 m a.s.l.

Conclusions

The phylogenetic analysis of rDNA ITS1–5.8S–ITS2 sequences of white rot medicinal fungus *F. fomentarius s.l.* from the north-eastern forest part of Armenia showed that it is a genetically heterogenous species consisting of two (A and B) sympatric cryptic lineages (clades) represented by two A2 (*F. fomentarius s.s.*) and B2 (*F. inzengae*) sublineages. The fixation index (F_{ST}) for ITS sequences of *F. fomentarius s.s.* and *F. inzengae* from Armenia is close to 1 (F_{ST} = 0.976851852) which indicates their almost complete genetic isolation. A broad spectrum of substrate confinement was observed in *F. inzengae* comprising eight Angiosperm species from eight genera (*A. platanoides, C. betulus, J. regia, F. excelsior, F. orientalis, P. nigra, U. leavis,* and *S. alba*) vs. *F. fomentarius s.s.* – two species from two genera (*F. orientalis* and *C. betulus*).

Fomes species are distributed at about 680–1750 m a.s.l. in the floristic regions of Armenia: *F. fomentarius s.s.* at 680–1750 m a.s.l. (Aparan and Ijevan regions), while *F. inzengae* at 680–1650 m a.s.l. (Aparan, Ijevan and Yerevan regions). The geo-ecological distribution pattern of *F. fomentarius s.s.* and *F. inzengae* in Armenia and central Europe is not significantly different. The sympatry zone of both species is 680–750 m, while it is 400–550 m a.s.l in central Europe. *Carpinus betulus* (living tree) and *F. orientalis* (trunk) forests are considered geographical areas for co-occurrence of both species in Armenia and Europe. Further comprehensive studies of different *Fomes* collections from other regions of Armenia using the morpho-ecological, physiological, biochemical and molecular approaches are in progress.

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