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Lophium arboricola (*Mytilinidiales*, *Ascomycota*) from conifer resins

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Abstract. *Lophium arboricola* is known as a saprotrophic fungus from different substrates, but it was mainly reported from conifers. In this study, two strains of fungi found growing on resin were molecularly identified as *Lophium arboricola*. The species was isolated from the resin of *Picea abies* and *Abies alba* in Poland. It is the first report of *L. arboricola* from resin substrate and, simultaneously, the first indication of a resinicolous lifestyle of this species. Isolated strains were morphologically characterized. The phylogenetic analysis was conducted based on ITS and LSU rDNA regions. Moreover, it is the first published report of *L. arboricola* from Poland.

Key words: *Lophium*, *Lophium arboricola*, morphology, *Mytilinidiales*, phylogeny, resinicolous fungi

Introduction

Resins are produced as a defense barrier against external dangers including microbial infections (Langenheim 2003). Because of antimicrobial properties and chemical composition, resins constitute a specific and harsh habitat for microorganisms. However, there are some fungi which can live in such an environment. Fungi living on resin exudates are called resinicolous fungi (Mitchell 2021). The knowledge about this ecological group is limited and data are sporadic. The best comprehensive study about resinicolous fungi was presented by Mitchell (2021). The author mentioned that resinicolous fungi are represented by approx. 50 species included in the phylum *Ascomycota*. Some of them belong to the order *Mytilinidiales*. To date, *Mytilinidion resinicola* (Lohman 1933) and *Mytilinidion resinae* (Speer 1986) are the only resinicolous representatives in this order which can be found in published studies (Mitchell 2021). Both species were described on resin and are well documented on this substrate (Lohman 1933; Speer 1986; Mitchell 2021). However, Mitchell (2021) mentioned that based on personal observation – *Lophium mytilinum* (belonging to the order *Mytilinidiales*) also may grow on resin. It is an interesting observation because members of the genus *Lophium* are mainly collected from conifers (Mathiassen et al. 2015; Hernández-Restrepo et al. 2016). The observation of representatives of this genus on resin may indicate that the resinicolous lifestyle may also exist in the genus *Lophium*. The genus accommodates numerous species, but only *Lophium arboricola* and *Lophium zalerioides* are supported by molecular data from holotype material (Buczacki 1972; Hernández-Restrepo et al. 2016; Hyde et al. 2017). A few strains described as *Lophium elegans* and *Lophium mytilinum* are sequenced as well, but phylogenetic analyses indicated that the genus is polyphyletic and needs further molecular investigations (Boehm et al. 2009; Mathiassen et al. 2015; Delgado et al. 2019).

Interestingly, similar to personal observations of Mitchell (2021), unidentified specimens of the genus *Lophium* were found on resin exudates in this study. Specimens were isolated and identified as *Lophium arboricola* using molecular and morphological approaches.

Materials and methods

Samples of resin covered by a dark fungal coat was collected in July 2021 in Koszarawa (the resin sample of *Abies alba*) and Przyborów (the resin sample of *Picea abies*) – two small villages in Southern Poland, Silesian Province, Żywiec County. Samples were collected in two separate sterile containers. In the laboratory, under sterile conditions, fungal hyphae from samples of resin surfaces were collected using tweezers and smeared on a Petri dish (Ø 90 mm) containing malt extract agar (MEA). After a period of two weeks at room temperature, light-brown mycelia grew on MEA. Several single species cultures were established from the initial culture and the only one of them was processed for molecular study. The above steps were applied to both samples.

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Table 1. List of species, strains, country of origin, isolation source and GenBank accession numbers of sequences used for phylogenetic analyses. Data on analyzed strain with newly generated sequences are indicated in bold. T – ex-type strain.

Species	Strain	Country	Isolation source	GenBank accession numbers	
				ITS	LSU
Cenococcum geophilum	1-17-2	USA	–	–	JN860135
Glonium circumserpens	CBS 123343	Australia	saxicolous on limestone	–	FJ161200
Lophium arboricola	NW-FVA 6260	Germany	wood of *Acer pseudoplatanus*	ON710911	–
Lophium arboricola	CBS 758.71 T	United Kingdom	canker of *Larix decidua*	NR_153447	NG_064094
Lophium arboricola	CBS 102826	Spain	on dung	KU705825	KU705842
Lophium arboricola	ZK52b/08	Czech Republic	needles of *Picea abies*	FR837917	FR837917
Lophium arboricola	MF6282	Norway	stem wound of *Picea abies*	AF169308	–
Lophium arboricola	**P98**	**Poland**	**resin of *Picea abies***	**OR754901**	**OR754923**
Lophium arboricola	**P99**	**Poland**	**resin of *Abies alba***	**OR754902**	**OR754924**
Lophium mytilinum	CBS 123344	United States	dead wood of *Pinus strobus*	–	FJ161203
Lophium mytilinum	CBS 269.34	United States	*Pinus* sp.	EF596817	EF596817
Lophium mytilinum	CBS 114111	Sweden	*Pinus sylvestris*	EF596819	EF596819
Lophium zalerioides	MFLUCC 14-0417 T	Italy	–	MF621583	MF621587
Mytilinidion resinicola	CBS 304.34 T	United States	*Larix laricina*	MH855535	MH867038
Mytilinidion rhenanum	CBS 135.45	–	–	–	FJ161175
Mytilinidion rhenanum	EB 0341	France	–	–	GU323207
Mytilinidion scolecosporum	CBS 305.34 T	USA	*Pinus strobus*	NR_160069	NG_057808
Pseudocamaropycnis pini	CBS 115589 T	Hong Kong	leaf of *Pinus elliotii*	KU728518	KU728557
Slimacomyces isiolus	FP1465	Japan	–	AB597207	AB597217
Slimacomyces isiolus	P10436	Japan	–	AB597213	AB597220

The material for microscopic observations was taken from about one-year old culture (the only culture where conidia appeared). Mycelia were mounted in 80% lactic acid. Observations of morphological characters were made under a Nikon SMZ1500 stereoscopic microscope (Tokyo, Japan) and a Nikon Eclipse E-600 light microscope equipped with a Nikon DS-Fi1 digital camera head. Measurements and photographs were conducted on the strain P98 using imaging software NIS D-Elements 4.30 (Nikon).

The isolation of DNA was performed based on slightly modified CTAB protocol (Owczarek-Kościelniak & Sterflinger 2018) and the process of amplification and sequencing described in Czachura et al. (2021). Taxonomic affinities of obtained sequences were found based on MegaBLAST searches of the GenBank nucleotide database (Zhang et al. 2000). Sequences used for the phylogenetic reconstruction of analyzed strains were selected from a phylogenetic analysis conducted by Delgado et al. (2019). All sequences used in this study are listed in Table 1. Sequences of both loci were aligned independently using MAFFT v7.490 (Katoh & Standley 2013) and then combined to construct the two-gene matrix. The final two-gene alignment resulted in 1,571 positions (ITS: 652, LSU: 919) including gaps. Prior to phylogenetic analyses, the best-fitting evolutionary models were calculated using PartitionFinder v2.1.1 (Lanfear et al. 2017) with relevant partition schemes, and the best selected models were HKY+G and TRN+G for ITS and LSU, respectively. The phylogenetic analyses were performed under Maximum likelihood (ML) and Bayesian inference (BI). ML analysis was conducted using RAxML-NG v1.2.0 (Kozlov et al. 2019) at CIPRES Science Gateway (Miller et al. 2010). BI analysis was performed with MRBAYES v3.2.3 (Ronquist et al. 2012). Four Markov chain Monte Carlo (MCMC) analyses were run for 10×10^6 generations and the first 25% were discarded as burn-in. Phylogenetic trees were visualized with FIGTREE 1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/).

Results and discussion

Lophium arboricola (Buczacki) Madrid & Gené, in Hernandez-Restrepo et al., Sydowia 68: 208. 2016 (Figs 1–2)

Description. Mycelium consisting of branched, septate, mostly pale brown, brown or subhyaline (hyaline when young), smooth or verruculose, 2–4.5 µm diam. Conidiophores reduced to conidiogenous cells. Conidiogenous cells cylindrical, subcylindrical or broadly ellipsoidal, hyaline, subhyaline or pale brown, smooth or verruculose, 6.5–8 × 3–3.5 µm, intercalary or terminal on hyphae. Conidia solitary, having an irregular shape, pale brown to brown or dark brown, smooth or verruculose, 9–21.5 × 7–18 µm, multi-celled, composed mostly of 2–11 (occasionally more) globose or subglobose, 3.5–8 × 3–7 µm cells.

Culture characteristics. Colony on MEA umbonate with dense aerial mycelium, margin fimbriate, grayish brown with whitish margin, reaching 41 mm diam. at 15°C after one month, reverse grayish brown with whitish margin.

Notes. Sequences of strains from this study clustered with sequences of the type of *L. arboricola*, as well as together with sequences of all *L. arboricola* strains which have been already published and supported by molecular data (Fig. 3). Sequences of all strains of *L. arboricola* were not sufficiently supported by Maximum likelihood bootstrap (MLB) and Bayesian posterior probabilities (BPP) (MLB < 70%, BPP < 0.9), which was presumably

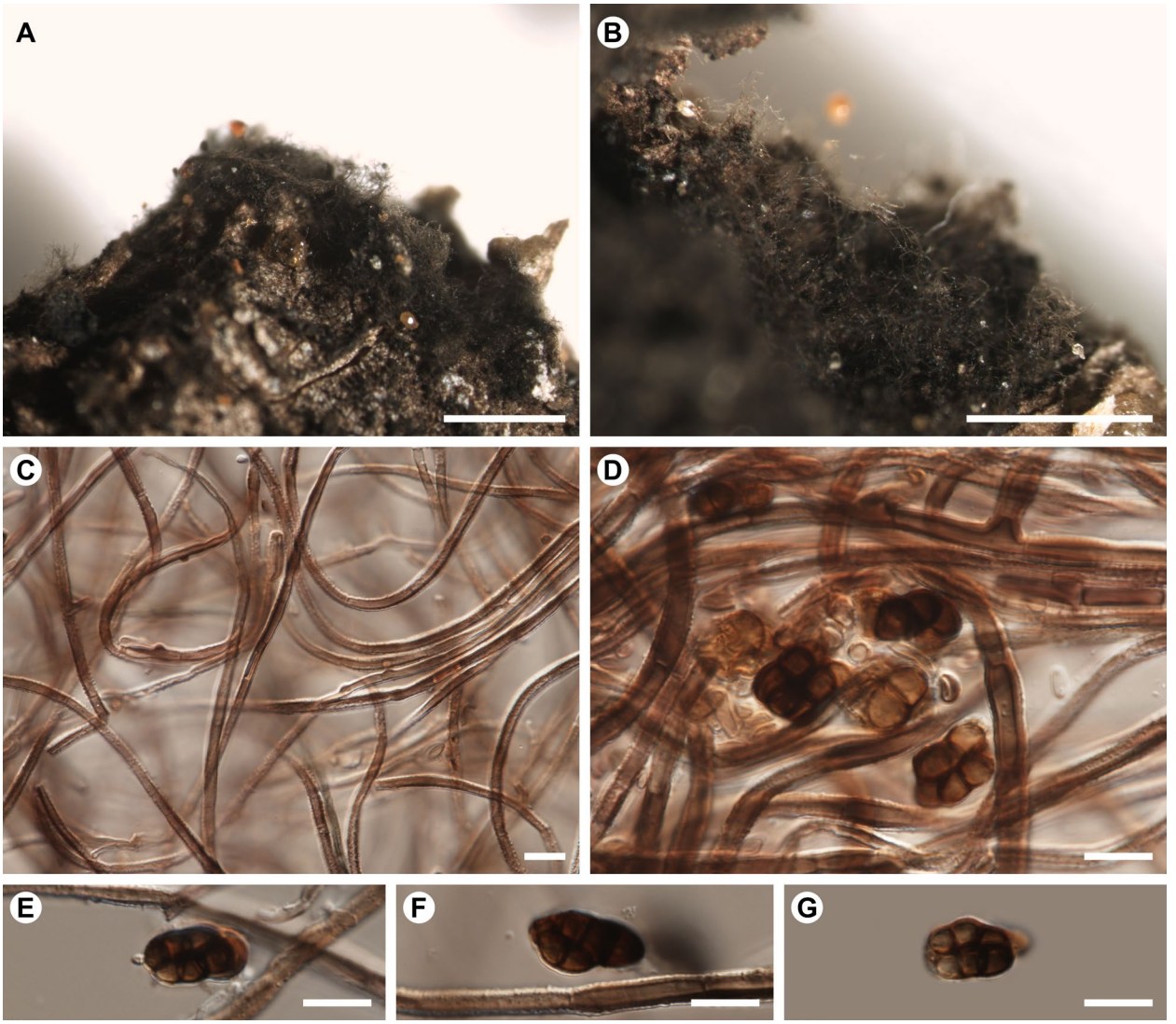

Figure 1. *Lophium arboricola* on the resin of *Picea abies*. A–B – dense dark hyphae growing on resin; C – hyphae; D – hyphae and conidia; E–G – conidia. Scales: A–B = 1 mm; C–G = 10 µm.

affected by molecular variability of *L. arboricola* and scarcity of molecular data in this group. However, sequences of all strains of *L. arboricola* formed a distinctive and sister clade to the type specimen of *L. zalerioides* (BPP ≥ 0.9), which formed a noticeably distant branch (Fig. 3).

Lophium arboricola is known as a saprobic species on bark and wood (Buczacki 1972). It was described as *Zalerion arboricola* from canker on bark of *Larix decidua* in the United Kingdom (Buczacki 1972) and belonged to one of the most frequently isolated fungi from larch cankers (Buczacki 1973). After molecular analyses of the type specimen, the species was included in the genus *Lophium* (Hernández-Restrepo et al. 2016). Moreover, the species was reported several times from stem wounds of *Picea abies* in Norway (Bills et al. 1999), needles of *P. abies* in the Czech Republic (Koukol et al. 2012), dung in Spain (Hernández-Restrepo et al. 2016) and wood of *Acer pseudoplatanus* in Germany (Schlößer et al. 2023).

Generally, the morphology of previously examined strains of *L. arboricola* and the strain from resin are nearly identical. Some differences can be found in the shape of conidia – the strain P98 formed conidia composed of cluster of cells, whereas remaining strains of *L. arboricola* formed elongated conidia (composed of chains of cells) (Buczacki 1972; Bills et al. 1999; Hernández-Restrepo et al. 2016). Moreover, the strain P98 formed slightly larger (up to 8 µm) individual cells forming conidia in comparison to the holotype (up to 5.3 µm) (Buczacki 1972). However, individual cells forming conidia of strain P98 were identical or nearly identical to other morphologically examined strains of *L. arboricola* (Bills et al. 1999; Hernández-Restrepo et al. 2016). All four *L. arboricola* strains differ significantly from the type specimen of closely related species – *L. zalerioides*. The latter formed conidia composed of cluster of cells similar to the strain P98 (Hyde et al. 2017), but conidia of *L. zalerioides* are bigger (reaching 41.6 µm) and composed of more cells than conidia of the type of *L. arobricola* (Buczacki 1972) and the strain analyzed in this study (Fig. 2). Moreover, *L. zalerioides* has smaller conidiogenous cells (2.1–2.6 × 1.8–2.2 µm) than *L. arboricola* (6.5–8 × 3–3.5 µm).

Based on conducted research, *L. arboricola* was reported from resin substrate for the first time. The presence of *L. arboricola* on resin exudates was confirmed in the following way – two separate resin samples covered

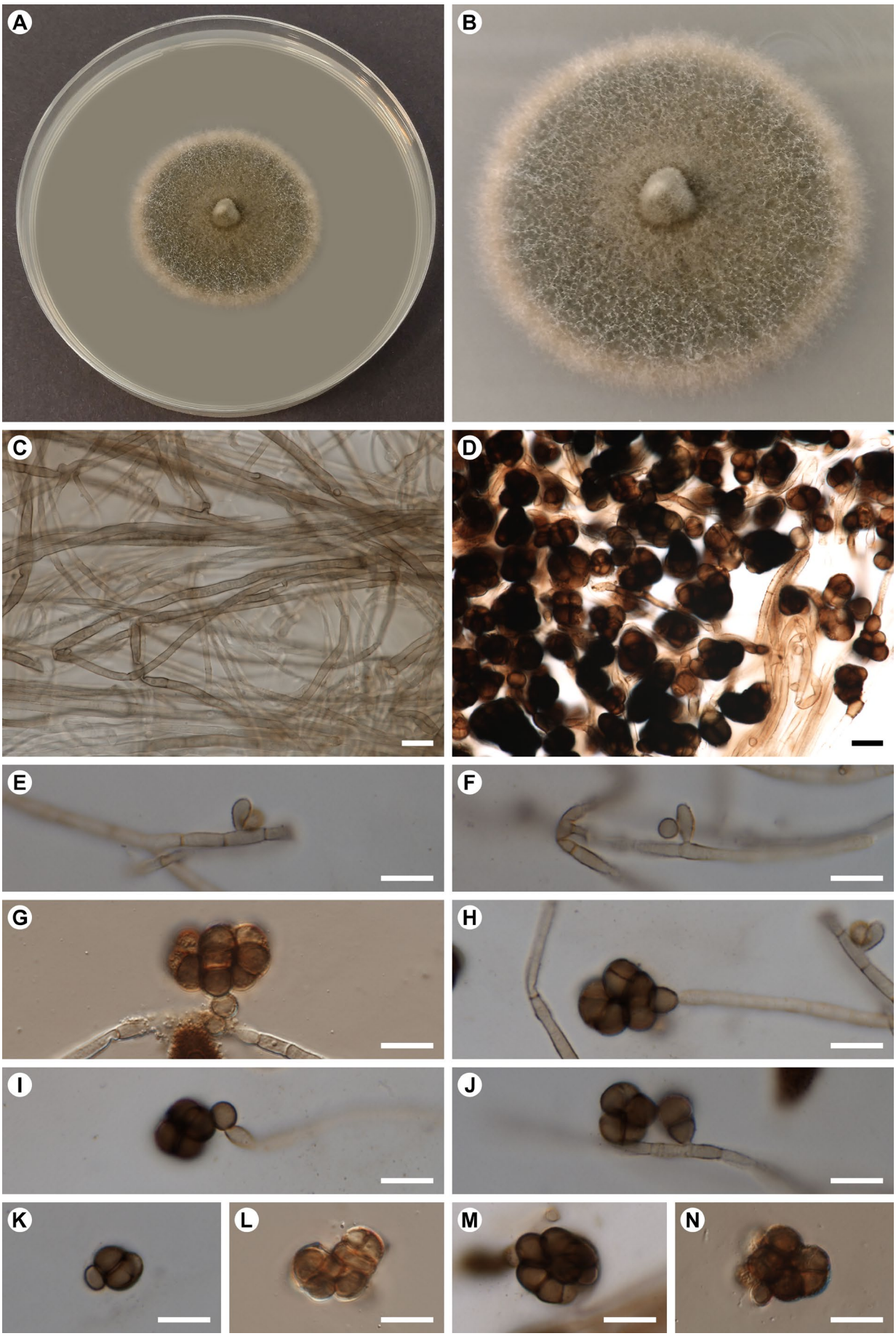

Figure 2. Morphology of *Lophium arboricola* (strain P98). A–B – general view and detailed view of upper side of colony on MEA after 1 month of growth at 15°C; C – hyphae; D – hyphae and conidia; E–F – hyphae with conidiogenous cells; G–J – hyphae with conidia arising from conidiogenous cells; K–N – conidia. Scale = 10 µm.

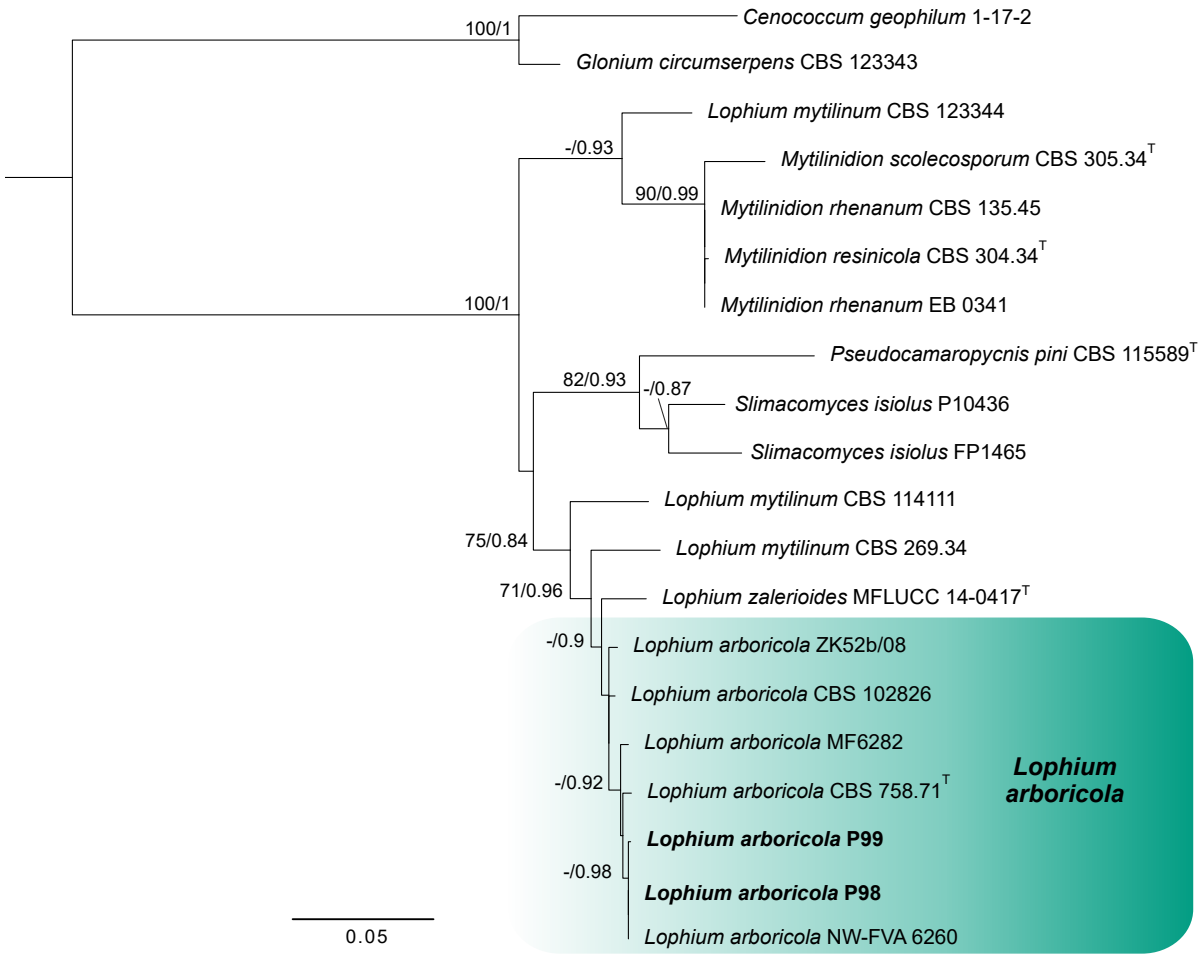

Figure 3. Phylogenetic placement of *Lophium arboricola* within closely related members of the order *Mytilinidiales*, inferred with maximum likelihood analysis using combined ITS and LSU sequences. Maximum likelihood bootstrap (MLB) support values ≥70% and Bayesian posterior probabilities (BPP) ≥0.9 are given above branches (MLB/BPP). Type specimens are indicated by 'T'. The scale bar represents the average number of substitutions per site.

by dense fungal hyphae were collected from two conifers – *Abies alba* and *Picea abies* in two different locations. Fungal hyphae grown on resins was examined under a stereoscopic microscope. Subsequently, the species was isolated into cultures, characterized morphologically and analyzed by molecular studies. Based on the applied methodology, it can be assumed that the occurrence of this species was not accidental and the species may actively grow on resin substrate. However, other reports of *L. arboricola* from resin will be helpful to confirm this assertion. The presence on coniferous resin may indicates that *L. arboricola* may be considered as facultative resinicolous fungi because the species was also recorded from different substrates such as bark, wood, needles and even dung (Buczacki 1972, 1973; Bills et al. 1999; Koukol et al. 2012; Hernández-Restrepo et al. 2016; Schlößer et al. 2023). Presumably, *L. arboricola* together with *Mytilinidion resinae*, *Mytilinidion resinicola* and *Lophium mytilinum* may be included into resinicolous species within the order *Mytilinidiales* (Mitchell 2021). Mitchell (2021) indicated that the resinicolous lifestyle within the order *Mytilinidiales* may be more common, but further investigation focusing on this poorly known group of fungi is needed. It is also the first published report of *L. arboricola* from Poland.

Specimens examined. POLAND. Silesian Province, Żywiec County: Przyborów, isolated from the resin sample of *Picea abies*, 10 Jul. 2021, leg. P. Czachura (KRAM F-59985, strain P98); Koszarawa, isolated from the resin sample of *Abies alba*, 11 Jul. 2021, leg. P. Czachura (KRAM F-59986, strain P99).

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References

Bills, G. F., Platas, G., Peláez, F. & Masurekar, P. 1999. Reclassification of a pneumocandin-producing anamorph, *Glarea lozoyensis* gen. et sp. nov., previously identified as *Zalerion arboricola*. *Mycological Research* 103: 179–192. https://doi.org/10.1017/S095375629800687X

Boehm, E. W., Mugambi, G. K., Miller, A. N., Huhndorf, S. M., Marincowitz, S., Spatafora, J. W. & Schoch, C. L. 2009. A molecular phylogenetic reappraisal of the *Hysteriaceae*, *Mytilinidiaceae* and *Gloniaceae* (*Pleosporomycetidae*, *Dothideomycetes*) with keys to world species. *Studies in Mycology* 64: 49–83. https://doi.org/10.3114/sim.2009.64.03

Buczacki, S. T. 1972. *Zalerion arboricola*, a new helicosporous hyphomycete from conifer stems. *Transactions of the British Mycological Society* 59: 159–161.

Buczacki, S. T. 1973. A microecological approach to larch canker biology. *Transactions of the British Mycological Society* 61: 315–329.

Czachura, P., Owczarek-Kościelniak, M. & Piątek, M. 2021. *Salinomyces polonicus*: A moderately halophilic kin of the most extremely halotolerant fungus *Hortaea werneckii*. *Fungal Biology* 125: 459–468. https://doi.org/10.1016/j.funbio.2021.01.003

Delgado, G., Koukol, O., Miller, A. N. & Piepenbring, M. 2019. *Septonema lohmanii* G. Delgado & O. Koukol, sp. nov., a new species in *Mytilinidiales* (*Dothideomycetes*) and the phylogenetic position of *S. fasciculare* (Corda) S. Hughes. *Cryptogamie, Mycologie* 40: 3–21.

Hernández-Restrepo, M., Schumacher, R. K., Wingfield, M. J., Ahmad, I., Cai, L., Duong, T. A., Edwards, J., Gené, J., Groenewald, J. Z., Jabeen, S., Khalid, A. N., Lombard, L., Madrid, H., Marin-Felix, Y., Marincowitz, S., Miller, A. N., Rajeshkumar, K. C., Rashid, A., Sarwar, S., Stchigel, A. M., Taylor, P. W. J., Zhou, N. & Crous, P. W. 2016. Fungal Systematics and Evolution: FUSE 2. *Sydowia* 68: 193–230. https://doi.org/10.12905/0380.sydowia68-2016-0193

Hyde, K. D., Norphanphoun, C., Abreu, V. P., Bazzicalupo, A., Chethana, K. W. T., Clericuzio, M., Dayarathne, M. C., Dissanayake, A. J., Ekanayaka, A. H., He, M. Q., Hongsanan, S., Huang, S. K., Jayasiri, S. C., Jayawardena, R. S., Karunarathna, A., Konta, S., Kušan, I., Lee, H., Li, J. F., Lin, C. G., Liu, N. G., Lu, Y. Z., Luo, Z. L., Manawasinghe, I. S., Mapook, A., Perera, R. H., Phookamsak, R., Phukhamsakda, C., Siedlecki, I., Mayra Soares, A., Tennakoon, D. S., Tian, Q., Tibpromma, S., Wanasinghe, D. N., Xiao, Y. P., Yang, J., Zeng, X. Y., Abdel-Aziz, F. A., Li, W. J., Senanayake, I. C., Shang, Q. J., Daranagama, D. A., de Silva, N. I., Thambugala, K. M., Abdel-Wahab, M. A., Bahkali, A. H., Berbee, M. L., Boonmee, S., Bhat, D. J., Bulgakov, T. S., Buyck, B., Camporesi, E., Castañeda Ruíz, R. F., Chomnunti, P., Doilom, M., Dovana, F., Gibertoni, T. B., Jadan, M., Jeewon, R., Jones, E. B. G., Kang, J. C., Karunarathna, S. C., Lim, Y. W., Liu, J. K., Liu, Z. Y., Plautz, Jr. H. L., Lumyong, S., Maharachchikumbura, S. S. N., Matočec, N., McKenzie, E. H. C., Mešić, A., Miller, D., Pawłowska, J., Pereira, O. L., Promputtha, I., Romero, A. I., Ryvarden, L., Su, H. Y., Suetrong, S., Tkalčec, Z., Vizzini, A., Wen, T. C., Wisitrassameewong, K., Wrzosek, M., Xu, J. C., Zhao, Q., Zhao, R. L. & Mortimer, P. E. 2017. Fungal diversity notes 603–708: taxonomic and phylogenetic notes on genera and species. *Fungal Diversity* 87: 1–235. https://doi.org/10.1007/s13225-017-0391-3

Katoh, K. & Standley, D. M. 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution* 30: 772–780. https://doi.org/10.1093/molbev/mst010

Koukol, O., Kolařík, M., Kolářová, Z. & Baldrian, P. 2012. Diversity of foliar endophytes in wind-fallen *Picea abies* trees. *Fungal Diversity* 54: 69–77. https://doi.org/10.1007/s13225-011-0112-2

Kozlov, A. M., Darriba, D., Flouri, T., Morel, B. & Stamatakis, A. 2019. RAxML-NG: a fast, scalable and user-frendly tool for maximum likelihood phylogenetic inference. *Bioinformatics* 35: 4453–4455. https://doi.org/10.1093/bioinformatics/btz305

Lanfear, R., Frandsen, P. B., Wright, A. M., Senfeld, T. & Calcott, B. 2017. PartitionFinder 2: new methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. *Molecular Biology and Evolution* 34: 772–773. https://doi.org/10.1093/molbev/msw260

Langenheim, J. H. 2003. *Plant resins: chemistry, evolution, ecology, and ethnobotany*. Timber Press, Portland, OR, USA.

Lohman, M. L. 1933. *Hysteriaceae*: Life histories of certain species. *Papers of the Michigan Academy of Sciences* 17: 229–288.

Mathiassen, G., Granmo, A. & Rämä, T. 2015. *Lophium elegans* (*Ascomycota*), a rare European species. *Mycotaxon* 129: 433–438. https://doi.org/10.5248/129.433

Miller, M. A., Pfeiffer, W. & Schwartz T. 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. Proceedings of the Gateway Computing Environments Workshop (GCE), 14 Nov. 2010, New Orleans, LA, pp. 1–8. https://doi.org/10.1109/GCE.2010.5676129

Mitchell, J. K. 2021. Resinicolous fungi on conifers: a review. In: Investigations into Resinicolous Fungi. Doctoral dissertation. Harvard University Graduate School of Arts and Sciences, pp. 1–105.

Owczarek-Kościelniak, M. & Sterflinger, K. 2018. First records of *Knufia marmoricola* from limestone outcrops in the Wyżyna Krakowsko-Częstochowska Upland, Poland. *Phytotaxa* 357: 94–106. https://doi.org/10.11646/phytotaxa.357.2.2

Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D. L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M. A. & Huelsenbeck, J. P. 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* 61: 539–542. https://doi.org/10.1093/sysbio/sys029

Schlößer, R., Bien, S., Langer, G. J. & Langer, E. J. 2023. Fungi associated with woody tissues of *Acer pseudoplatanus* in forest stands with different health status concerning sooty bark disease (*Cryptostroma corticale*). *Mycological Progress* 22: 13. https://doi.org/10.1007/s11557-022-01861-6

Speer, E. O. 1986. A propos de champignons du Brésil III. *Mytilidion resinae* sp. nov. (*Hysteriales*) et sa forme conidienne, *Camaroglobulus resinae* gen. et spec. nov. (*Sphaeropsidales*). *Bulletin de la Société Mycologique de France* 102: 97–100.

Zhang, Z., Schwartz, S., Wagner, L. & Miller, W. 2000. A greedy algorithm for aligning DNA sequences. *Journal of Computational Biology* 7: 203–214. https://doi.org/10.1089/10665270050081478