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Toniniopsis bartakii - A new species of lichenised fungus from James Ross Island (Antarctic Peninsula)

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Abstract: The new lichen species *Toniniopsis bartakii* is described from James Ross Island, the North-East Antarctic Peninsula region. It is phylogenetically most closely related to *T. bagliettoana* but differs mainly in forming a more developed, squamulose thallus. It is morphologically more similar to *T. coelestina* but differs mainly in the pigmentation in the proper exciple. An identification key to the known species of *Toniniopsis* is also provided.

Key words: Antarctica, biodiversity, lichenized fungi, *Ramalinaceae*, mtSSU, nrITS

1. Introduction

James Ross Island belongs to the North-East Antarctic Peninsula region and has a special location in the transition zone between maritime and continental climate types (Bednarek-Ochyra et al., 2000). The island has a large area (2600 km^2), and the deglaciated part (170 km^2) has a vegetation cover which is dominated by lichens and mosses. There are no vascular plants on James Ross Island. As the deglaciated areas are large on James Ross Island, the lichen biodiversity is high (Halıcı et al., 2018). Our knowledge of the lichen biodiversity in Antarctica is still insufficient. For instance, Dodge (1973) reported 415 species from this continent while describing many species and claiming that the endemism rate is rather high in Antarctica. Castello and Nimis (1997), however, examined most of the types for the species described by Dodge and reported that only 20% of the species were valid. Because of these reasons, the first author began collecting lichens on James Ross Island in 2016 and studied them by the aid of molecular techniques (Halıcı et al., 2017; 2018, Halıcı and Bartak 2019).

One of the collections, M. G. Halıcı and M. Bartak ERCH JR 209, morphologically similar to a squamulose species of *Toninia* A. Massal. or *Toniniopsis* Frey, turned out to be genetically close to the cosmopolitan, crustose lichen *Toniniopsis bagliettoana* (A.Massal. & De Not.) Kistenich & Timdal. The genus *Toniniopsis* was resurrected from the synonymy of *Toninia* by Kistenich et al. (2018a), but the species, although they are shown to belong in *Toniniopsis* by Kistenich et al. (2018a; as *Bacidia bagliettoana* [A. Massal. & De Not.] Jatta), was only recently transferred to *Toniniopsis* in Cannon et al. (2021). This paper discusses the identity of the collection from James Ross Island.

2. Materials and methods

2.1. Morphology and thin-layer chromatography

Lichen sample was collected from James Ross Island in Antarctica. The type specimen detailed below is deposited in Erciyes University Herbarium Kayseri, Turkey (ERCH). The specimen was examined by standard microscopic techniques. Hand-cut sections were studied in water, potassium hydroxide (KOH), and Lugol's solution (I). Measurements were made in water. Ascospores were measured from five different ascomata for each species. The measurement results are given as minimum–maximum, from n = 30 measurements. Thin-layer chromotography (TLC) was carried out to determine compounds, using solvent system C (Orange et al., 2010).

2.2. Molecular methods

2.2.1. DNA isolation, polymerase chain reaction (PCR), and sequencing

Total DNA was extracted from apothecia by using the DNeasy Plant Mini Kit (Qiagen) according to the manufacturer's instructions. Polymerase chain reaction (PCR) was carried out in 50 µL reaction volumes using 4

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µL of 10× reaction buffer, 4 µL $MgCl_2$ (50 mM), 0.5 µL each primer (ITS1F and ITS4), 2 µL dNTP (10 mM), 0.2 µL Taq DNA polymerase, 4 µL of template DNA and 34.8 µL dH_2O on a thermal cycler equipped with a heated lid. ITS4 (White et al., 1990) and ITS1-F (Gardes and Bruns, 1993) were used to amplify the ITS sequence. PCR amplification was performed under the following conditions: an initial denaturation for 7 min at 95 °C; six cycles for 1 min at 94 °C, 1 min at 56 °C and 1 min at 72 °C; and 30 cycles for 1 min at 94 °C, 1 min at 53 °C, and 2 min at 72 °C. A final extension step of 10 min at 72 °C was added, after which the samples were kept at 4 °C. The PCR products were visualised on 1.2% agarose gel as a band of approximately 600 bp or 800 bp.

Sequence analyses of the lichen sample obtained from the PCR products was performed by the BM Labosis Laboratory (Ankara, Turkey).

2.2.2. Additional sequences

40 ITS and 20 mtSSU DNA sequences were downloaded from GenBank, representing a selection of 22 taxa in the former, broad circumscription of the genus *Toninia* (i.e., the current genera *Bibbya* J.H. Willis, *Kiliasia* Hafellner, *Thalloidima* A. Massal., *Toninia* s. str., and *Toniniopsis*; cfr. Kistenich et al. (2018a) and the outgroup genus *Bellicidia* Kistenich et al. in press; Table 1).

2.2.3. Sequence alignment and phylogenetic analysis

Sequence editing was performed with the software Geneious R9 (Kearse et al., 2012). The mtSSU and ITS sequences were aligned separately using the MAFFT-plugin (Katoh and Standley, 2013) in Geneious. The E-INS-i algorithm was applied to our sequence datasets, setting the nucleotide scoring matrix to 1PAM /κ=2 and the gap open penalty to the default value of 1.53. We trimmed the 5'-end of the mtSSU alignment slightly to reduce the amount of missing data and trimmed the ITS alignment to comprise only the ITS-region, i.e. deleting the residual 18S and 28S sequence information. Each alignment was analysed by IQ-TREE v.1.6.7 (Nguyen et al., 2015) to find the best-fitting nucleotide substitution models and partitioning schemes (Chernomor et al., 2016; Kalyaanamoorthy et al,. 2017) among models implemented in MrBayes (i.e., 1-, 2-, and 6-rate models) and to infer a maximum likelihood tree with 1000 standard nonparametric bootstrap repetitions. We defined one subset for mtSSU and three subsets for ITS corresponding to the ITS1, 5.8S, and ITS2 regions, and analysed each dataset with the TESTMERGE function resembling PartitionFinder2. In addition, MrBayes v.3.2.6 (Altekar et al., 2004; Ronquist and Huelsenbeck, 2003) was used on each dataset as described in Kistenich et al. (2018b). The tree length α/β was set to 1 for mtSSU and 0.32 for ITS. We set the temperature increment parameter to 0.1 for both analyses. We projected the bootstrap support (BS) values from the IQ-TREE analysis onto the MrBayes consensus tree with posterior probabilities (PP) and collapsed branches with BS < 50 and PP < 0.8. The resulting trees were edited in TreeGraph2 (Stöver and Müller, 2010).

3. Results

3.1 Sequence alignment and phylogenetic analysis

The mtSSU alignment comprised 21 accessions (Table 1) with a length of 814 bp. The ITS alignment consisted of 41 accessions with a length of 605 bp. *Bellicidia incompta* (Borrer) Kistenich et al. was used for rooting of all generated trees. Both alignments are available at TreeBase (study no. 27456). The software IQ-TREE suggested the following nucleotide substitution models for the predefined subsets: HKY+I+Γ for mtSSU, GTR+Γ for the combined ITS1 and ITS2 and K2P+I for 5.8S.

The Bayesian phylogenetic analyses halted automatically after 2×10^6 generations for mtSSU and 4×10^6 for ITS, respectively, when the ASDSF in the last 50% of each run had fallen below 0.01. Following a burnin of 50%, we used 1,001 trees of the mtSSU analysis and 2001 trees of the ITS analysis for each of the final Bayesian majority-rule consensus gene trees. The phylogenetic topologies generated by IQ-TREE vs. MrBayes of each marker showed no strongly supported (i.e., BS > 70 and PP > 0.9) incongruences. The mtSSU extended majority rule consensus tree (Figure 1) showed good overall resolution, while the ITS extended majority rule consensus tree (Figure 2) showed poor resolution in the backbone but good resolution in more terminal groups. Both trees resolve the new species as sister to *Toniniopsis bagliettoana*. In the mtSSU tree (Figure 1), the genus *Toniniopsis* was resolved in a paraphyly with *Toninia*. In the ITS tree (Figure 2), *Toniniopsis* was resolved by forming three separate clades.

3.2. Taxonomy

Toniniopsis bartakii Halıcı, Kahraman, Kistenich & Timdal sp. nov. (Figure 3)

MycoBank No.: MB 838845

Type: Antarctic Peninsula, James Ross Island: Southern east part of Johnson Mesa, 63°49′46.2″S, 57°54′21.6″W, alt. 292 m, 14 January 2017, *ERCH JR 0.209* (ERCH—holotype).

Diagnosis: Similar to Toniniopsis coelestina (Anzi) Kistenich et al. but with smaller apothecia, which have a pale to colourless (not dark brown) inner part of the proper exciple. Differs from the phylogenetic sister species T. bagliettoana by having thicker, granulose to squamulose, and darker grey to brownish thallus; apothecial disc that apparently remains flat, and apothecial margin which is thicker and more persistent.

Etymology: Named in honour of Prof. Dr. Miloš Barták (Masaryk University Brno, Czech Republic), who conducted research on Antarctic terrestrial vegetation for

Table 1. mtSSU and ITS sequences used in the analyses.

Species		Authorship	Country	Year	Voucher	mtSSU	ITS
Bellicidia incompta		(Borrer) Kistenich et al.	Sweden	1997	Ekman 3144 (BG)	MG925849	AF282092
Bibbya albomarginata		(H. Kilias & Gotth. Schneid.) Kistenich et al.	Peru	2006	Timdal 10481 (O)	MG925927	MG926024
Bibbya bullata	I	(Meyen & Flot.) Kistenich et al.	New Zealand	2002	Bannister s.n. (O)	MG925928	MG926025
Bibbya bullata	II	(Meyen & Flot.) Kistenich et al.	Australia	1994	Elix & Streimann 40393 (O)	MG925929	MG926026
Bibbya ruginosa		(Tuck.) Kistenich et al.	Greenland	2005	Timdal 10087 (O)	MG925937	MG926033
Bibbya vermifera		(Nyl.) Kistenich et al.	Sweden	1998	Johansson 1619 (BG)	MG925852	AF282109
Kiliasia athallina		(Hepp) Hafellner	Austria	1994	Poelt, Pittoni & Köckinger (GZU)	-	MG926023
Kiliasia philippea		(Mont.) Hafellner	Norway	1994	Haugan & Timdal H3750 (O)	-	AF282112
Kiliasia sculpturata		(H. Magn.) Kistenich et al.	Russia	1992	Haugan & Timdal YAK17/30 (O)	MG925938	MG926034
Thalloidima candidum	I	(Weber) A. Massal.	Norway	2012	Hofton 12366 (O)	MG925931	MG926028
Thalloidima candidum	II	(Weber) A. Massal.	Norway	1997	Bratli & Timdal 8733 (O)	MG925932	AF282117
Thalloidima physaroides	I	(Opiz) Kistenich et al.	Norway	2013	Bendiksby et al. 12969 (O)	MG925935	MG926031
Thalloidima physaroides	II	(Opiz) Kistenich et al.	Norway	1995	Haugan & Timdal 8121 (O)	MG925936	MG926032
Thalloidima toninianum		(A. Massal.) A. Massal.	Spain	2015	Timdal 13773 (O)	MG925942	MG926036
Toninia cinereovirens		(Schaer.) A. Massal.	Norway	1994	Haugan & Timdal 7953 (O)	AY567724	AF282104
Toninia populorum		(A. Massal.) Kistenich et al.	Austria	1998	Ekman 3392 (BG)	MG925843	MG925950
Toninia sp.			Svalbard	2013	Zhang ZT2013191	-	KP314433
Toninia squalida		(Ach.) A. Massal.	Norway	1996	Haugan 4970 (O)	MG925940	AF282103
Toninia talparum		Timdal	USA	1998	Timdal SON120/01 (O)	-	AF282108
Toninia tristis		(Th. Fr.) Th. Fr.	Norway	1995	Haugan & Timdal 8109 (O)	-	AF282105
Toniniopsis aromatica	I	(Sm.) Kistenich et al.	Norway	1995	Haugan & Timdal 4819 (O)	MG925926	AF282126
Toniniopsis aromatica	II	(Sm.) Kistenich et al.	Norway	2009	Klepsland JK09-L101 (O)	-	MG838184
Toniniopsis aromatica	III	(Sm.) Kistenich et al.	Norway	2011	Haugan 10465 (O)	-	MG838161
Toniniopsis aromatica	IV	(Sm.) Kistenich et al.	Norway	2014	Klepsland JK14-L451 (O)	-	MG838189
Toniniopsis bagliettoana	I	(A. Massal. & De Not.) Kistenich & Timdal	Sweden	1997	Ekman 3150 (BG)	MG925847	AF282123

Table 1. (Continued).

Toniniopsis bagliettoana	II	(A. Massal. & De Not.) Kistenich & Timdal	Norway	2009	Haugan 8688 (O)	-	MG838180
Toniniopsis bagliettoana	III	(A. Massal. & De Not.) Kistenich & Timdal	Norway	2009	Klepsland JK09-L370 (O)	-	MG838178
Toniniopsis coelestina	I	(Anzi) Kistenich et al.	Norway	1997	Haugan 5985 (O)	MG925933	AF282127
Toniniopsis coelestina	II	(Anzi) Kistenich et al.	Norway	2010	Klepsland JK10-L342 (O)	-	MG838190
Toniniopsis obscura		Frey	Canada	1999	Westberg TNW2182 (UPS)	MG925943	MG926037
Toniniopsis bartakii			James Ross Island	2017	Halıcı & Bartak (ERCH ANT 209)	MW621866	MW620999
Toniniopsis subincompta	I	(Nyl.) Kistenich et al.	Sweden	1998	Ekman 3413 (BG)	MG925851	AF282125
Toniniopsis subincompta	II	(Nyl.) Kistenich et al.	Switzerland		WSL:DF231	-	KX098342
Toniniopsis subincompta	III	(Nyl.) Kistenich et al.	Norway	2014	Klepsland JK14-L020 (O)	-	MG838175
Toniniopsis subincompta	IV	(Nyl.) Kistenich et al.	Norway	2015	Klepsland JK15-L773 (O)	-	MG838176
Toniniopsis subincompta	V	(Nyl.) Kistenich et al.	Norway	2001	Løfall bpl-L7932 (O)	-	MG838165
Toniniopsis subincompta	VI	(Nyl.) Kistenich et al.	Norway	2013	Klepsland JK13-L056 (O)	-	MG838186
Toniniopsis subincompta	VII	(Nyl.) Kistenich et al.	Norway	2011	Timdal 12123 (O)	-	MG838157
Toniniopsis verrucarioides	I	(Nyl.) Kistenich, Timdal, Bendiksby & S. Ekman	Norway	1997	Bratli & Timdal 8709 (O)	-	AF282128
Toniniopsis verrucarioides	II	(Nyl.) Kistenich, Timdal, Bendiksby & S. Ekman	Norway	2013	Bendiksby et al. 12976 (O)	-	MG838167
Toniniopsis verrucarioides	III	(Nyl.) Kistenich, Timdal, Bendiksby & S. Ekman	Norway	2011	Klepsland JK11-L557a (O)	-	MG838179

decades and helped the first author in the field excursions on James Ross Island.

Description: Thallus granulose to squamulose; granules/squamules up to 1 mm in diam., contiguous, irregular, flat to weakly convex; upper surface ash grey to bluish grey, slightly pruinose, dull, lacking soredia, isidia, pores, pseudocyphellae and maculae; margin concolorous with upper side; upper cortex dark grey, 50–60 µm thick, lacking a well-defined epinecral layer, without crystals; algal layer green, 30–60 µm, containing trebouxioid, isodiametric algae up to 12 µm in diam.; medulla lacking crystals; lower cortex not well developed or almost excluded.

Apothecia single or aggregated, black, epruinose or weakly pruinose, up to 0.5 mm diam.; disc plane or weakly concave; margin persistent, concolorous with the disc, epruinose, becomes thicker when mature; proper exciple greenish black in the rim, pale to colourless in inner part, without crystals (polarized light!); epithecium greenish black, 40–110 µm, K– and N–, without crystals; hypothecium brown, 20–30 µm; hymenium hyaline except upper part brownish, 70–90 µm, K– and N+ violet-red, without crystals. Asci 8-spored, 45–60 × 7–12 µm. Ascospores bacilliform to acicular, hyaline, 3(–6)-septate, (17–) 24.5–31–37.5 (–41) × (1.5–) 2–2.5–3 (–5) µm, length/width ratio: (4–) 8–12–16 (–26.5) (n=30). Conidiomata not observed. Thallus and medulla K– and KI–. No lichen substances were detected in TLC.

4. Discussion

The new species differs from the phylogenetic sister species *T. bagliettoana* mainly in the development of the thallus. In *T. bagliettoana*, the thallus is crustose, forming a thin

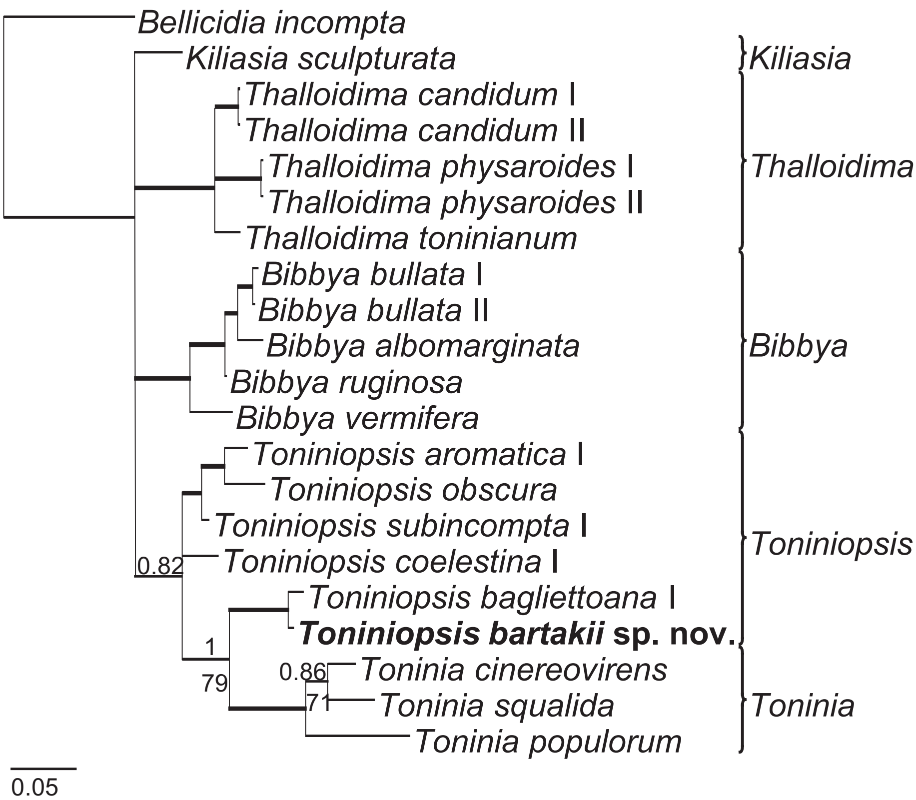

Figure 1. mtSSU molecular phylogenetic tree. Extended majority-rule consensus tree resulting from the IQ-TREE analysis of the mtSSU alignment with Bayesian PP ≥ 0.8 and/or IQ-TREE maximum likelihood BS ≥ 50 and branch lengths. Strongly supported branches (PP ≥ 0.95 and BS ≥ 80) are marked in bold; for other branches, support values are indicated. The new species is marked in bold. *Bellicidia incompta* was used for rooting.

greyish white crust over bryophytes, plant debris, and soil, whereas, in *T. bartakii*, the thallus is thicker, granulose to squamulose, and darker grey to brownish. The apothecial disc of *T. bagliettoana* is flat and thinly marginate when young, and later becomes convex with an excluded margin; in *T. bartakii* the disc apparently remains flat and has a thicker, more persistent margin (although this observation is based on the holotype, only). The thallus of *T. bartakii* actually resembles more that of the European species *T. coelestina*, a species that is recovered more distantly in our phylogenies (Figures 1–2). The apothecia of *T. coelestina* are larger (up to 1.6 mm) and have a dark brown inner part of the exciple (Timdal, 1992; as *Toninia coelestina*). The main differences of the related species are given in Table 2.

The two gene trees presented in this study (Figures 1–2) do not recover *Toninia* and *Toniniopsis* as two monophyletic genera but either rather unresolved (ITS) or *Toninia* paraphyletic with *Toniniopsis* nested within (mtSSU). Only a few individuals were used for the mtSSU tree (Figure 1), though. The split of *Toninia* s. lat. into five genera, including *Toninia* s. str. and *Toniniopsis*, was discussed in detail by Kistenich et al. (2018a) based on a five-marker phylogenetic analysis. Kistenich et al. (2018a) showed that several genetic markers are necessary for resolving *Toninia* as a monophyletic genus. On the other hand, they could not resolve *Toniniopsis* as a monophyletic genus using five genetic markers indicating that the taxonomy within this group might still not be fully resolved and understood.

An identification key to the species of *Toniniopsis*

1. Ascospores narrowly ellipsoid to bacilliform, 1- to 3-septate 2

1. Ascospores bacilliform to acicular, 3- to 7-septate .. 5

2. Thallus crustose 3

2. Thallus squamulose 4

3. On inland limestone; thallus finely warted to granular .... *T. coprodes* (Körb.) S. Ekman & Coppins

3. On coastal rocks; thallus rimose to areolate, not granular . *T. mesoidea* (Nyl.) Timdal.

4. Epithecium brown, N– .... *T. verrucarioides* (Nyl.) Kistenich, Timdal, Bendiksby & S. Ekman.

4. Epithecium green, N+ violet .... *T. aromatica* complex (including *T. fusispora*)

5. Thallus forming a thin crust 6

5. Thallus granulose to squamulose 9

6. On acidic rock; ascospores 11-19 µm long, 3 (-5) septate at maturity ..*T. inornata* (Nyl.) S. Ekman & Coppins

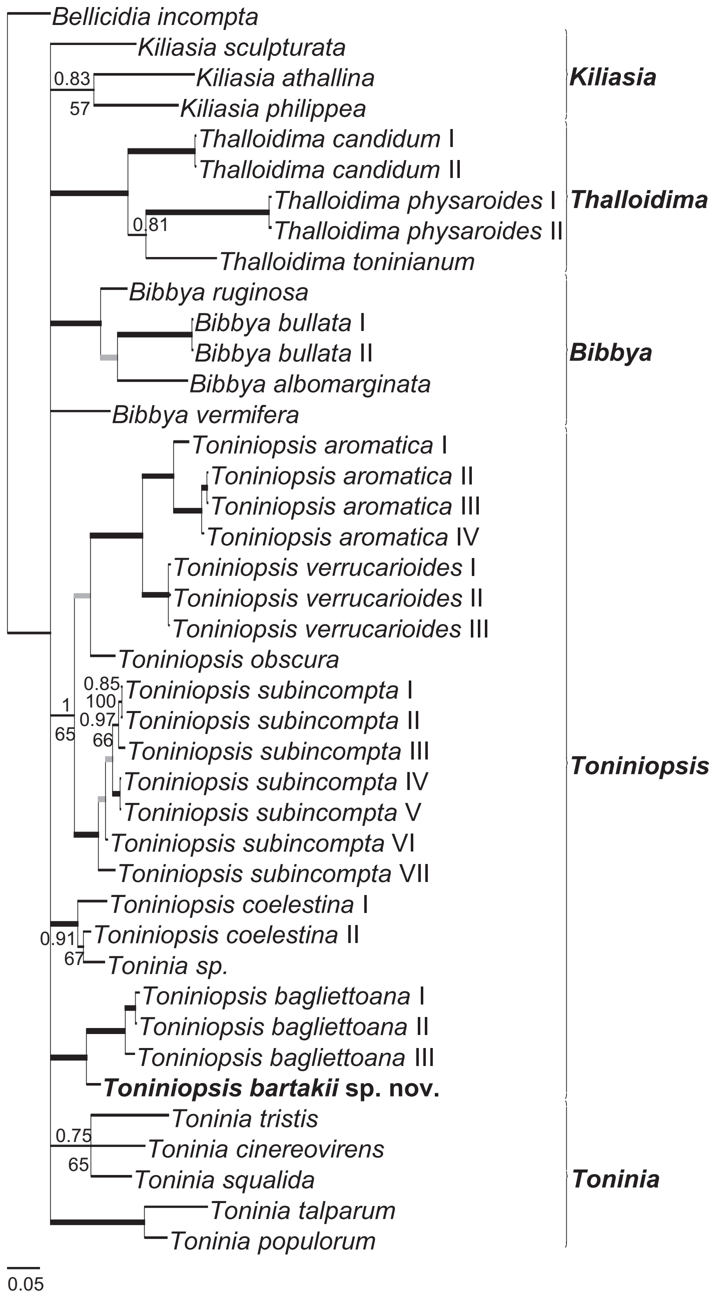

Figure 2. ITS molecular phylogenetic tree. Extended majority-rule consensus tree resulting from the IQ-TREE analysis of the ITS alignment with Bayesian PP ≥ 0.7 and/or IQ-TREE maximum likelihood BS ≥ 50 and branch lengths. Strongly supported branches (PP ≥ 0.95 and BS ≥ 80) are marked in bold black; bold grey branches show PP ≥ 0.9 or BS ≥ 70; for other branches, support values are indicated. The new species is marked in bold. *Bellicidia incompta* was used for rooting.

6. On bark, soil, or plant debris; ascospores > 20 µm long, 3- to 7-septate 7

7. On bark of tree trunks ....
T. subincompta Kistenich, Timdal, Bendiksby & S. Ekman.

7. On soil or plant debris 8

8. Hypothecium dark brown, at least in upper part; apothecia epruinose ....
T. bagliettoana (A. Massal. & De Not.) Kistenich & Timdal

8. Hypothecium pale brown to colourless; young apothecia often pruinose ....
T. illudens (Nyl.) Kistenich, Timdal, Bendiksby & S. Ekman.

9. Thallus containing a series of terpenoids; on limestone in the eastern Mediterranean region ....
.... *T. cretica* (Timdal) Timdal.

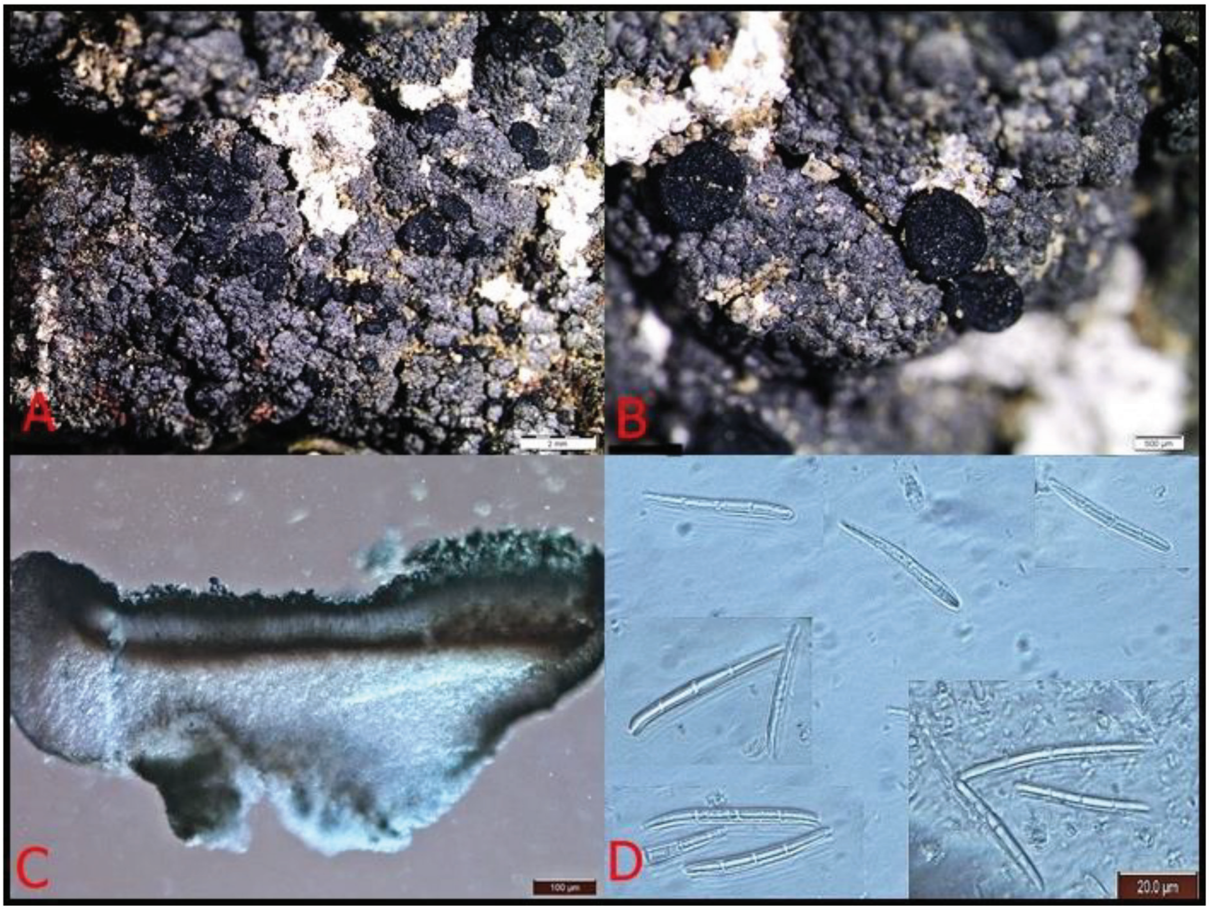

Figure 3. *Toniniopsis bartakii* sp. nov. A–B: Thallus with apothecia. C: Section through apothecium. D: Ascospores. Photos: M. G. Halıcı, M. Kahraman.

Table 2. Main taxonomical differences of *T. bartakii* from the morphologically related species.

Taxonomical character	*T. bagliettoana*	*T. bartakii*	*T. coelestina*
Growth form	crustose	granulose to squamulose	granulose to squamulose
Apothecia, in diam.	up 1.4 mm	up to 0.5 mm	up to 1.6 mm
Inner part of proper exciple	pale brown to colourless	pale brown to colourless	dark brown
Ascospores, length	25–45 µm	24.5–37.5 µm	20–40 µm
Chemistry	No lichen substances	No lichen substances	No lichen substances

9. Thallus lacking lichen substances 10

10. Inner part of proper exciple dark brown, confluent with hypothecium; apothecia up to 1.6 mm diam.; European montane to alpine region .... *T. coelestina* (Anzi) Kistenich, Timdal, Bendiksby & S. Ekman

10. Inner part of proper exciple pale brown to colourless, contrasting the dark brown hypothecium; apothecia up to 0.5 mm in diam.; Antarctic .... *Toniniopsis bartakii* sp. nova

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