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Molecular phylogenetic analyses reveal two new synonyms of *Xanthoria parietina*

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Abstract. Molecular analyses of the recently described *Xanthoria polessica* collected from the type locality and of the Australian species *X. coomae* were used to determine whether these taxa are distinct species or fall within the wide phenotypic variation of the well-known and cosmopolitan *X. parietina*. Our results clearly indicate that both taxa should be considered as synonyms of *X. parietina* since the infraspecific morphological range is accommodated by the observed variation in the thallus and lobe size, their color, position of apothecia, the shape of ascospores, and width of ascospore septum.

Key words: taxonomy, phylogeny, ITS, *Teloschistaceae*, lichens

Introduction

The well-known lichen genus *Xanthoria* is currently represented by at least nine species, namely *X. aureola*, *X. calcicola*, *X. ibizaensis*, *X. mediterranea*, *X. monofoliosa*, *X. parietina*, *X. resendei*, *X. steineri* and *X. stiligera* (Arup et al. 2013; Kondratyuk et al. 2020), but there are many unresolved names and the taxonomy of the genus is in need of molecular evaluation. Recently, two new species were described in this genus, namely *X. polessica* and *X. juniperina* (Kondratyuk et al. 2013), but their descriptions were based only on morphological characters and were never supported by molecular data. Phylogenetic analyses by Arup et al. (2013) indicated that the Australian species *X. coomae* (Kondratyuk et al. 2007) probably belongs to *X. parietina*.

In our study, we focused on the species *X. polessica* and *X. coomae*, the former described by Kondratyuk et al. (2013) based on an old herbarium specimen from 1967 stored in MSK herbarium in Minsk. In addition, 29 specimens from rather different areas of Belarus, Russia and Ukraine have been cited giving an idea of the widespread occurrence of this species. Fieldwork conducted by the second author (PB) at the well-preserved type locality yielded fresh material of *X. polessica*, allowing us to obtain ribosomal DNA sequences (ITS) of the species. The performed phylogenetic analyses indicate that *X. polessica* is conspecific with *X. parietina*. In the case of *X. coomae*, the previous suggestion by Arup et al. (2013) that the species belongs to *X. parietina* has been confirmed in this study. Therefore, we propose *X. polessica* and *X. coomae* to be synonymous with *X. parietina*. The high morphological variability of *X. polessica* is discussed.

Material and methods

Morphological examination

Morphology and anatomy were studied using Nikon SMZ-745 and Nikon Eclipse 80i microscopes (Tokyo, Japan). Handmade sections of ascomata were studied in water and 5% KOH (K). Measurements of ascospores size and length/width ratio (l/w) are given as (minimum–) X–SD – X+SD (–maximum), where X represents the arithmetic mean and SD the corresponding standard deviation, followed by the number of measurements (n).

DNA amplification, sequencing, alignment and phylogenetic analysis

Two ITS sequences were obtained from the two collections of *X. polessica* using direct PCR following the procedure described in detail in Arup et al. (2015). Primers for amplification were ITS1F (Gardes & Bruns 1993) and ITS4 (White et al. 1990). An alignment was created to include the two newly generated ITS sequences of *X. polessica* together with 34 sequences downloaded from GenBank (Table 1) of *X. aureola*, *X. calcicola*, *X. coomae*, *X. ectaneoides* (according to the name in Genbank, but the relationship to *X. aureola* is unclear), *X. mediterranea*,

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Table 1. Location, collector and GenBank accession numbers of sequences of species of *Xanthoria* used in the analyses. Specimens in bold were newly produced for this study.

Species	Location and collector	GenBank Accession Number
Dufourea flammea	South Africa, Feuerer and Thell 60488a (HBG)	KC179357
X. aureola	Sweden, Lindblom X188 (priv.herb.)	AY438276
X. aureola	United Kingdom, 2002, Coppins s. n. (E)	AY438275
X. calcicola	Sweden, Arup L97372, (LD)	AF353944
X. calcicola	Sweden, Lindblom 1199CL (BG)	AY438282
X. coomae	Australia, Kondratyuk 204116 (KW)	EU681291
X. coomae	Australia, Kondratyuk 20455 (KW)	EU681292
X. coomae (holotype)	Australia, Kondratyuk 20494 (CANB)	KC179410
X. ectaneoides	France, Unknown s.n. (G)	AJ320131
X. mediterranea	Italy, Honegger s.n. (G)	AM408410
X. monofoliosa	South Africa, Thell 00196, (LD)	EU681293
X. parietina	Italy, Arup L97905 (LD)	AF353943
X. parietina	Switzerland, Unknown 98 (??)	AJ320118
X. parietina	Russia, Honegger 213+4 (Z+XT)	AM292826
X. parietina	Sweden, Lindblom & Blom L111 (BG)	AY438310
X. parietina	Sweden, Lindblom 1/1 (BG)	AY926499
X. parietina	Norway, Lindblom 1/3 (BG)	AY926500
X. parietina	Norway, Lindblom 1/15 (BG)	AY926502
X. parietina	Norway, Lindblom 2/36 (BG)	AY926504
X. parietina	Norway, Lindblom 2/37 (BG)	AY926505
X. parietina	Norway, Lindblom 2/52 (BG)	AY926509
X. parietina	Norway, Lindblom 11/340 (BG)	AY926513
X. parietina	United Kingdom, Unknown s.n. (KEW)	FR799309
X. parietina	United Kingdom, Unknown s.n. (KEW)	KJ027704
X. parietina	Germany?, Unknown s.n. (??)	KF590005
X. parietina	Germany?, Unknown s.n. (??)	KF590014
X. parietina	Unknown, Grande & Singh s.n. (??)	KJ027703
X. parietina	Unknown, Unknown s.n. (??)	KY379230
X. parietina	Norway, Arnstein Lye O-L-206852 (O)	MK811702
X. parietina	Norway, Bendiksby O-L-196081 (O)	MK812134
X. parietina	Norway, Timdal O-L-195782 (O)	MK812349
X. parietina	Denmark, Søchting s.n. (C)	KC179411
X. parietina	Chile, Frödén 1620 (L)	KC179412
X. polessica	**Belarus, Bely s.n. (LD, GSU, MSKH)**	**MT928332**
X. polessica	**Belarus, Bely s.n. (LD, GSU, MSKH)**	**MT928333**
X. resendei	Unknown origin, Lich 13259 (BCN)	AF101285
X. cf. *stiligera*	Spain, *Moestrup* s.n. (C)	KC179409

X. monofoliosa, *X. parietina*, *X. resendei* and *X.* cf. *stiligera*. *Dufourea flammea* was used to root the tree (Arup et al. 2013). The 37 sequences were aligned using MAFFT ver. 7.450 (Katoh & Standley 2013; Katoh et al. 2002) as implemented in Geneious 11.0.5 and then improved manually, including a total of 523 positions, of which 53 were phylogenetically informative.

A suitable model of molecular evolution was selected using the Bayesian Information Criterion (BIC) as implemented in jModeltest ver. 2.1.4 (Guindon & Gascuel 2003; Darriba et al. 2012) to evaluate only the 24 models available in MrBayes 3.2.4 (Ronquist et al. 2012). The SYM+G model was found to be optimal. Bayesian tree inference was carried out using Markov chain Monte Carlo (MCMC) as implemented in MrBayes 3.2.4. The number of discrete categories used to approximate the gamma distribution was set to 4. The following priors were used: beta (1, 1) on the transition-transversion rate, fixed on the state frequencies, uniform (0·001, 200) for the gamma shape parameter, and all trees *a priori* equally likely. The prior on branch lengths for the analyses was set to an exponential with a mean of 1/10. Three parallel runs were performed, each with 6 chains, 5 of which were incrementally heated with a temperature of 0·10. Analyses were diagnosed every 100,000 generations and automatically halted when convergence was reached. Convergence was defined as a standard deviation of splits (with frequency $b \geq 0{\cdot}1$) between runs below 0·01. Every 1,000th tree was sampled and the first 50% of runs were removed as burn-in. FigTree 1.4 (http://tree.bio.ed.ac.uk/software/figtree/) and Adobe Illustrator CS4 were used to construct and illustrate a phylogenetic consensus tree.

Results

A 50% majority rule consensus tree from the post burn-in trees is presented in Figure 1. The phylogenetic tree shows a monophyletic and well delimited *Xanthoria parietina*

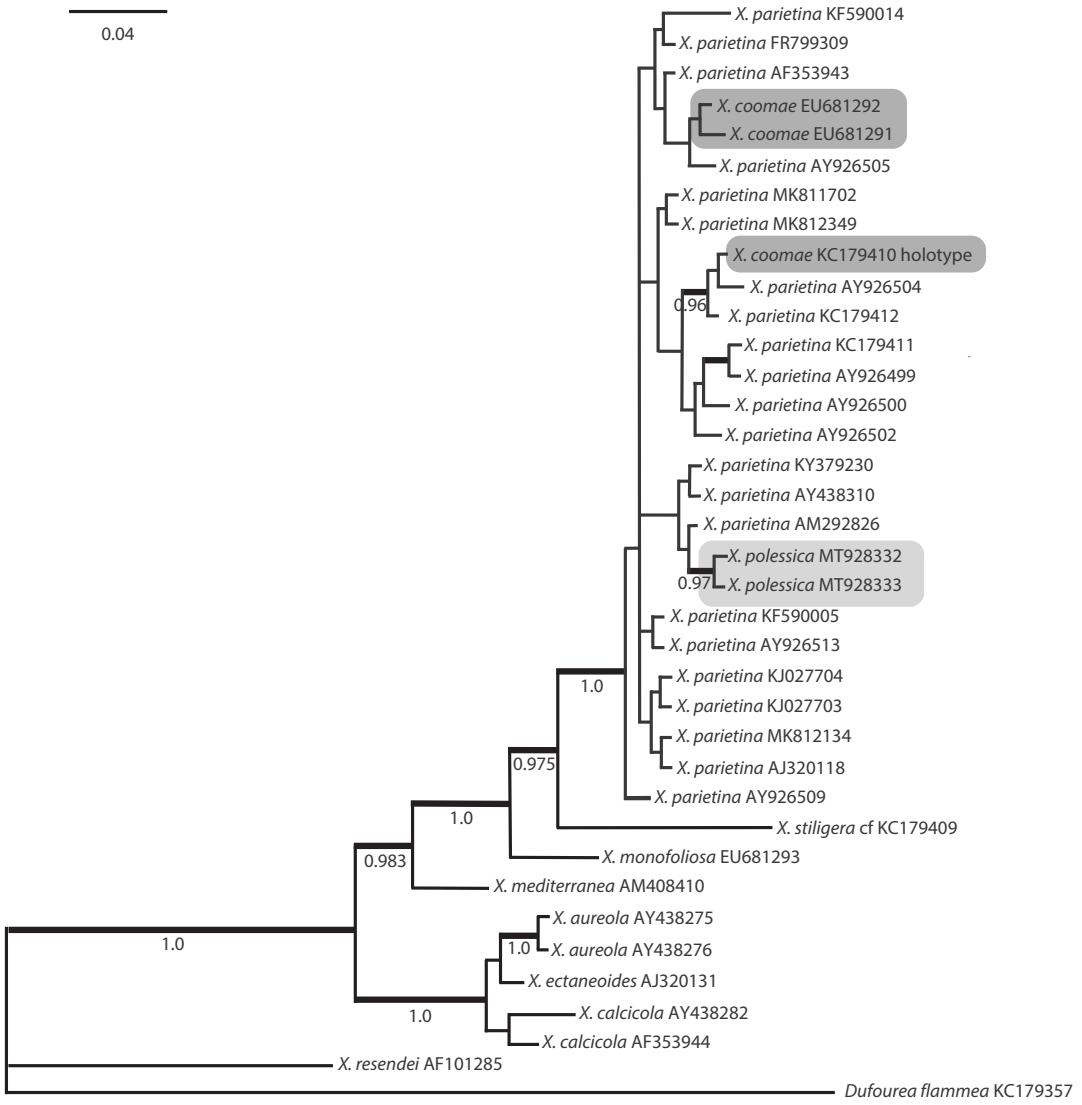

Figure 1. A majority-rule consensus tree based on an analysis of 36 ITS *Xanthoria* sequences using Bayesian MCMC. *Dufourea flammea* was used to root the tree. The tree shows *Xanthoria coomae* and *X. polessica* (highlighted in grey) nested within the *X. parietina* clade. Numbers below internodes indicate PP values ≥0.95.

(represented by 22 sequences) including *X. polessica* (represented by two sequences from the type locality) and *X. coomae* (represented by three sequences including the holotype) nested in this highly supported clade. *X. coomae* appeared polyphyletically divided into two clades, with the type material clustered with *X. parietina* sequences from Norway and Chile (PP = 0.96). The remaining two sequences of *X. coomae* form a weakly-supported clade with *X. parietina* sequences from Norway. The two identical sequences of *X. polessica* cluster with two sequences of *X. parietina* from Russia and Sweden, and an additional sequence of unknown origin.

Discussion

Lindblom & Ekman (2005) investigated the phylogenetic relationship of *Xanthoria parietina* to *X. aureola* and *X. calcicola* using sequences of two molecular markers, one of which was the ITS. A total of 15 haplotypes differing mostly by a single point mutation were recognized within *X. parietina*. We have partially used sequences from their data set, but also included sequences from additional specimens representing a much wider geographical range. We observed a similar, low level of genetic variation within the ITS region of *X. parietina* including *X. polessica* and *X. coomae*, ranging from identical sequences to a single or rarely a few nucleotide difference among the specimens from different continents.

Based on morphology, *X. polessica* and *X. coomae* represent small forms of *X. parietina* differing in a few additional characters. *X. polessica* has a thicker thallus, smaller thalline lobes which are well-developed only in the peripheral zone, apothecia developing mainly in the central part of the thallus, which are surrounded by thick thalline margins, and smaller ascospores with a narrower ascospore septum (Kondratyuk et al. 2013). The fresh specimens collected from the type locality perfectly match the morphological and anatomical characters given above. The vouchers have small, rosette-like thalli, mainly 1–1.5 cm in diameter, with lobes not exceeding 2 mm in width and length, seen in peripheral zone, thallus in section 240–450 μm thick, abundant apothecia in the

central part, with thalline margin to 0.2 mm thick, and widely ellipsoid ascospores (10.0–)11.0–12.9(–15.0) × (6.0–)6.5–7.5(–10.0) µm, l/b (1.1–)1.4–1.8(–2.2), n = 23, with narrow septum (2.5–)3.6–5.5(–8.0) µm in width. *X. coomae* differs from *X. parietina* by its more horizontally orientated lobes, slightly shiny, wrinkled and uneven central portion of the thallus, much paler yellowish peripheral zone, and more ellipsoid ascospores although of similar size and width of septum (Kondratyuk et al. 2007).

The phylogenetic analyses of the ITS region do not support the recognition of *X. polessica* and *X. coomae* as two independent species outside of *X. parietina.* Their nested placement within a monophyletic *X. parietina* justifies their synonymification and indicates that the observed morphotypes of *X. polessica* and *X. coomae* should be considered as infraspecific variation within *X. parietina*.

Specimens examined. Belarus, Gomel region, Kalinkovichi district, Ozarichi village, on wooden fence, 6 Oct. 2018, P. Bely (MSKH, GSU, LD).

Xanthoria parietina (L.) Th. Fr., Lich. Arct.: 69. 1860.

= *Xanthoria coomae* S.Y. Kondr. & Kärnefelt, Bibl. Lichenol. 96: 167. 2007, syn. nov.

Type: Australia, New South Wales, vicinity of Cooma township, on roadside *Populus nigra* and *Pinus radiata* trees, on introduced trees in Cooma Lions Park, 31 Jan. 2004, Kondratyuk 20494 (CANB – holotype!, MEL, PERTH, HO – isotypes).

= *Xanthoria polessica* S.Y. Kondr. & A.P. Yatsyna, Acta Bot. Hung. 55(3–4): 355. 2013, syn. nov.

Type: Belarus, Gomel region, Kalinkovichsky district, Ozarichi village, on wooden fence, 16 May 1967, N.V. Gorbach (MSK-L 5686 – holotype!, MSK-L 5652 – isotype).

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