

*Candelaria asiatica*, New record from PakistanHira Wahab\*<sup>1</sup>, Siraj Uddin<sup>1</sup>, Afshan Wahab<sup>1</sup> & Abdul Nasir Khalid<sup>2</sup><sup>1</sup>\* Department of Botany, University of Peshawar, Pakistan<sup>2</sup>Fungal Biology and Systematics Laboratory, Institute of Botany, University of the Punjab, Quaid-e-Azam Campus-54590, Lahore, Pakistan**ABSTRACT**

An investigation of the morphology, chemistry, and molecular phylogenetics of a *Candelaria* specimen was undertaken when the lichens of Malakand were being surveyed. With a pulverulent surface, a tiny yellow lobate thallus, and a delicate lobe edge featuring lobules that resemble phyllidia, the taxon that is new to Pakistan is identified as *Candelaria asiatica*. The morpho-anatomical, ecological, and distribution details are provided.

**KEYWORDS** Biota, Candelariaceae, taxonomy, phylogeny**INTRODUCTION**

Pakistan is widely recognized for its diverse landscape and climate, which is associated with an abundance of biological diversity (IUCN, 2006). Because there haven't been many studies conducted in many areas, it's likely that the lichen diversity in this region is quite great. (Aptroot & Iqbal, 2012). So far, up to 400 lichens have been reported from Pakistan (e.g. Aptroot & Iqbal, 2012; Fatima *et al.*, 2021; Habib *et al.*, 2021; Kousar *et al.*, 2021; Fayyaz *et al.*, 2022; Nadeem *et al.*, 2022).

*Candelaria* A. Massal. is a small lichen genus characterized by a micro-foliose to micro-fruticose yellowish thallus and 8- or polyspored asci (Westberg & Frödén, 2007). *Candelaria concolor* (Dicks.) Arnold, is reported from Pakistan (Aptroot & Iqbal, 2012). A *Candelaria* specimen was collected during a survey of the lichen flora in Malakand. The specimen, when subjected to morphological, chemical, and phylogenetic investigations, showed a strong resemblance to *Candelaria asiatica*.

## MATERIALS AND METHODS

### Morphological and Chemical Investigations

Lichen specimen was collected from Malakand, Khyber Pakhtunkhwa in 2018. Meiji Techno's EMZ-5TR stereomicroscope and the Swift M4000-D compound microscope, both equipped with a 9MP camera system, were used to study the specimen macro and micromorphologically. Hand-made sections of apothecia were investigated in 10% KOH and water for anatomical study. Each diagnostic feature from specimen was measured in the water at least twenty times. Spot tests using sodium hypochlorite solution (C) and KOH (10%; K) were used to assess the secondary chemistry. Standard procedures for Thin Layer Chromatography were followed while utilizing Solvent System C. (Orange *et al.*, 2010).

### Extraction of DNA, amplification by PCR, and sequencing

Using a modified 2% CTAB technique, genomic DNA was directly isolated from thallus (Gardes & Bruns, 1993). The ITS-nrDNA region (Internal Transcribed Spacer of the nrDNA) was amplified using the primer pair ITS1F (forward primer) (Gardes & Bruns, 1993) and ITS4 (reverse primer) (White *et al.*, 1990). The PCR results were visualized using ethidium bromide on a 1% agarose gel. (Sambrook & Russel, 2001). PCR products were sent to Tsingke, China for sequencing.

BLAST analysis was used to obtain extremely comparable ITS region sequences (Altschul, 1990). Related taxa were indicated, along with the sequence maximum query coverage and percent identity. Sequences from published literature and GenBank were used in an initial alignment, and then web-PRANK was used to realign the data using its default parameters. (Dong Liu & Jae-Seoun Hur, 2018). Phylogenetic relationships were investigated using Maximum Likelihood bootstrapping, as implemented in RAxML-HPC2 v. 8.1.11 (Stamatakis, 2014), hosted on the CIPRES Science Gateway. The HYK+G+I substitution model and quick bootstrapping with 1000 iterations were employed in the analyses. Version 1.4.3 of FigTree (Rambaut *et al.*, 2014) was used for displaying trees from the ML analysis.

## RESULTS

### Phylogenetic analysis

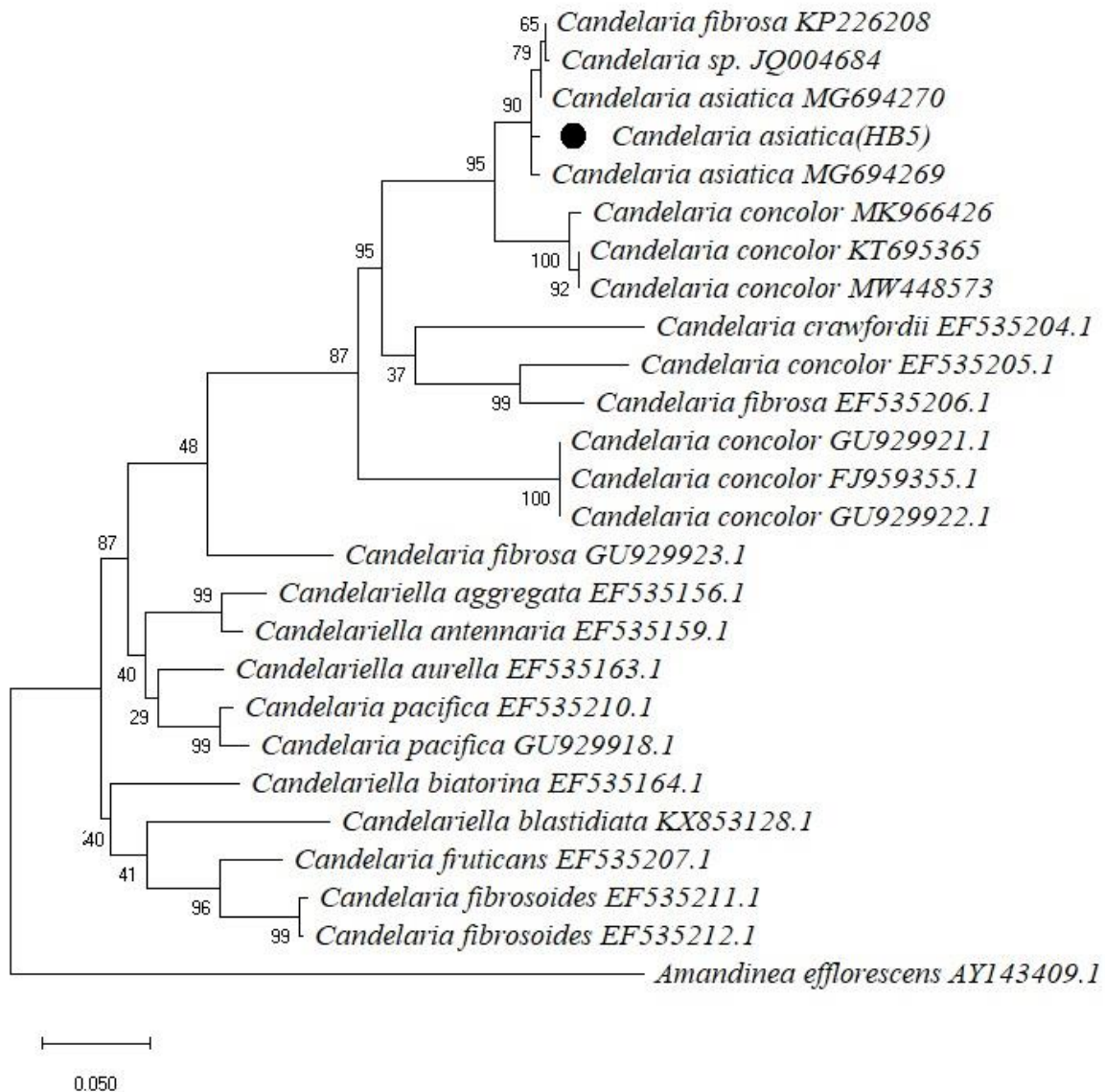
In this study, the ITS on nrDNA of *Candelaria* species obtained from Buner was successfully amplified and generated. The phylogenetic analysis sample of taxa was based on collecting closely related sequences from GenBank. MAFFT v7.450 (Katoh & Standley, 2013) The ITS sequences were aligned using Mafft v7's 'automatic' option, with all other parameters set to default. Each one-locus alignment's ambiguous spots were deleted. Maximum Likelihood was used to do the phylogenetic analysis (ML). ML trees were generated using RAxML 8.2.11 on GENEIOUS (Stamatakis, 2014), with 1000 bootstrap pseudoreplicates and the GTR+G+I substitution model. FigTree 1.4.2 was used to visualise the tree files (Rambaut, 2014).

Analysis involved 26 nucleotide sequences. Pakistani collection *C. asiatica* is clustered with *C. asiatica* (MG694269, MG694270).

**Table 1.** Sequences used in the ITS phylogenetic analyses of *Candelaria* species.

<b>Taxon</b>	<b>Origin</b>	<b>Specimen Voucher</b>	<b>GenBank Number (ITS)</b>
<i>Amandinea efflorescens</i>	Sweden	SE-901 87	AY143409
<i>Candelaria asiatica</i>	Pakistan	Bot-99	PP091273
<i>Candelaria asiatica</i>	South Korea	D. Liu 171446 (KoLRI)	MG694269
<i>Candelaria asiatica</i>	South Korea	D. Liu 171454 (KoLRI)	MG694270
<i>Candelaria concolor</i>	Albany	APBP017A	MK966426
<i>Candelaria concolor</i>	Canada	BIOUG24047-G11	KT695365
<i>Candelaria concolor</i>	USA	PUL F27065	MW448573
<i>Candelaria concolor</i>	Mexico	Westberg 454 (LD)	EF535205
<i>Candelaria concolor</i>	Italy	Arup L07018 (LD)	GU929921
<i>Candelaria concolor</i>	Sweden	SAR07	FJ959355
<i>Candelaria concolor</i>	Italy	Arup L07001 (LD)	GU929922
<i>Candelaria fibrosa</i>	USA	Worthington 21240 (COLO)	EF535206
<i>Candelaria fibrosa</i>	Argentina	Froeden 1670 (LD)	GU929923
<i>Candelaria fibrosoides</i>	Peru	Froden 1513 (LD)	EF535211
<i>Candelaria fibrosoides</i>	Peru	Froden 1512 (LD)	EF535212
<i>Candelaria fruticans</i>	Ecuador	ECU352	EF535207
<i>Candelaria pacifica</i>	Turkey	28.IV.1992 John [John, Lich. Anatol. Exs. no. 16] (ASU)	EF535210
<i>Candelaria pacifica</i>	USA	Westberg 967 (LD)	GU929918
<i>Candelariella aurella</i>	USA	Westberg 150 (LD)	EF535163

<i>Candelariella biatorina</i>	USA	Westberg 1181 (LD)	EF535164
<i>Candelariella blastidiata</i>	Russia	ALTB:E.A. Davydov 7716	KX853128



**Figure 1.** Molecular phylogenetic analysis of ITS sequences of *Candelaria* inferred by using the maximum likelihood method.

## TAXONOMY

*Candelaria asiatica* D. Liu & J.S. Hur, *Mycobiology* 46(4): 308 (2018).

**Thallus:** corticolous, scattered, and squamulose to foliose or subfruticose, 0.3–1.5 cm, irregular, rosettes, aggregated into extensive colonies covering the substrate. **Lobes:** linear and irregular branched, 0.1–0.59 mm, adnate to erect, lobe tip slightly inverted, crenate, lobe surface color yellow to greenish yellow in the center, then becoming bright yellow toward the lobe tip, phyllidia-like lobules. **Margins:** 50–130 mm wide loosely adnate lobe tips. **Upper cortex:** 11–25  $\mu\text{m}$  thick. paraplectenchymatous, texture globularis. **Medula:** white but not well-developed; the lower cortex white, covered with white rhizines; **Algae cells:** chlorococcoid, cells 8–12  $\mu\text{m}$  in diam. **Medulla:** white but undeveloped, the lower cortex is present at the center of the thallus. **Lower surface:** white, covered with white rhizines. **Rhizines:** white, densely present. **Apothecia:** not observed.

**Chemistry:** K–, KC–, C–, PD–; **Secondary metabolites:** calycin and pulvinic acid detected.

**Habitat:** On bark in coniferous forest, an altitude is 994 m a.s.l., dominant plants are *Picea smithiana* (Wall.) Boiss. *Cedrus libani* subsp. *Stenocoma*. *Juniperus communis* subsp. *Hemisphaerica*, *Taxus wallichiana* Zucc. *Quercus dilatata* Royle. *Acer cappadocicum* Gled. And *Betula utilis* var. *jacquemontii* (Himalayan Birch). Himalayan region, maximum and minimum temperature of 35°C and -10°C respectively, annual rainfall varying between 600–800 mm.

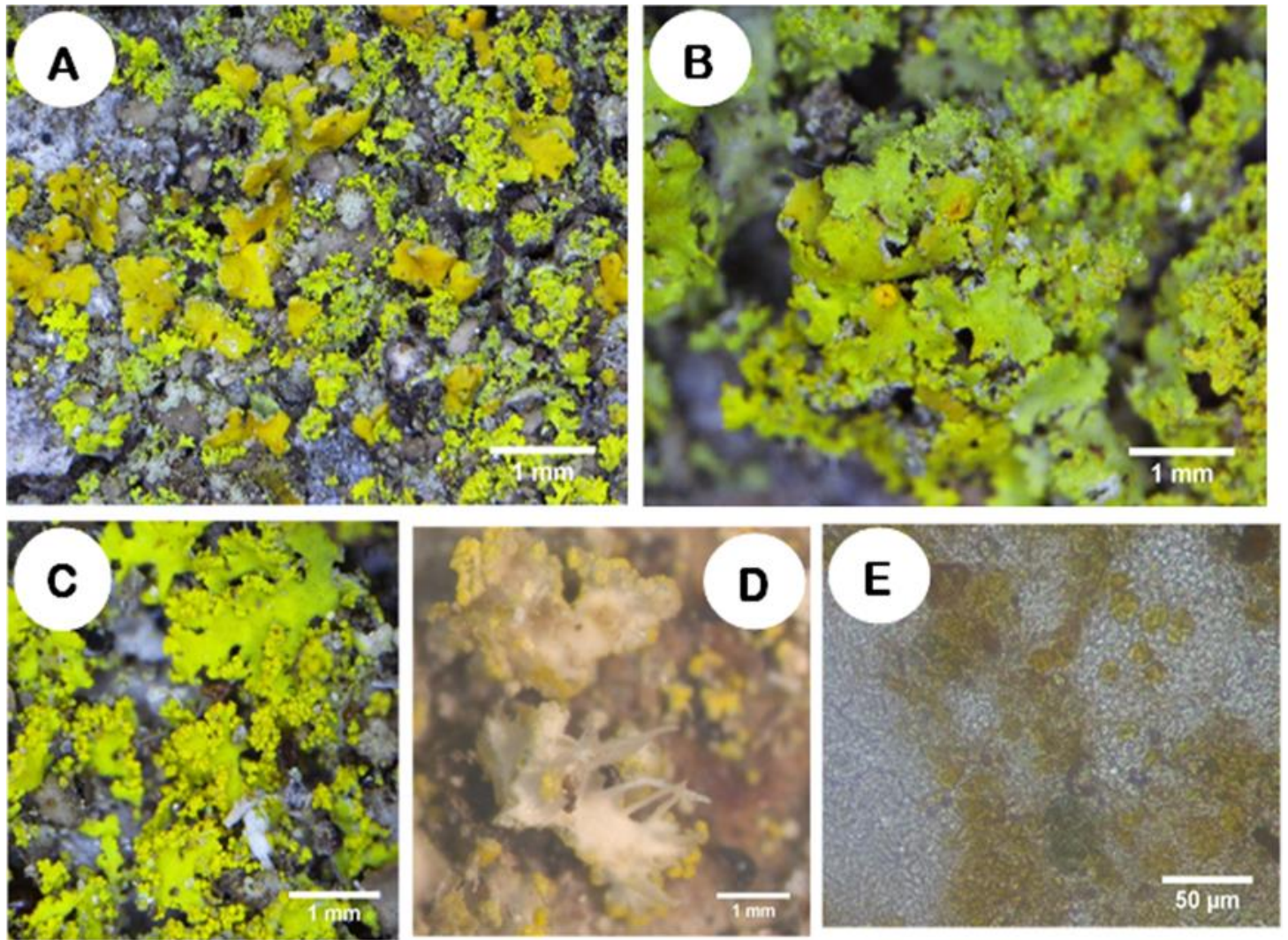
**Material Examined:** PAKISTAN. Khyber Pakhtunkhwa: District Buner, Daggar; at 994 m a. s. l., May, 04, 2018, H. Wahab, A. N. Khalid. HB–5. ITS GenBank accession number PP091273.

## REMARKS:

A small lichen genus *Candelaria* A. Massal. is distinguished by its 8- or polyspored asci and micro-foliose to micro-fruticose yellowish thallus. There are eight species in this genus: *Candelaria Antarctica* (Js. Murray) Poelt, *C. crawfordii* (M€ull. Arg.) P.M.Jørg. & D.J. Galloway, *C. concolor* (Dicks.) Arnold, *C. fibrosa* (Fr.) M€ull. Arg., *C. fibrosoides* M. Westb. & Fr€oden, *C. fruticans* Poelt & Oberw., *C. murrayi* Poelt, and *C. pacifica* M. Westb. & Arup, around the world (Dong Liu & Jae-Seoun Hur, 2018). Only *C. concolor* is reported from Pakistan by Aptroot and Iqbal in 2012. The Pakistani collection of *Candelaria asiatica* is grouped with South Korean

specimens (MG694269, MG694270) in a branch with a strong bootstrap value. Their designation as *Candelaria asiatica* is confirmed by morpho-anatomical and chemical characters.





**FIG. 2.** *Candelaria asiatica* **A**, habitat; **B**, lobes margins after blastidia or phyllidia like lobules are dropped; **C**, young thalli; **D**, lower surface **E**, transverse section of the thallus.

**Table 2.** Morphological difference among the species of *Candelaria* species

<b>Taxon</b>	<b>Lobe</b>	<b>Upper surface</b>	<b>Asexual propagules</b>	<b>Distribution</b>
<i>C. asiatica</i>	linear and irregular branched	rough	phyllidia-like lobules present	Pakistan
<i>C. asiatica</i>	Shallowly to deeply branched, narrower (up to 0.47 mm)	rough	Blastidia or phyllidia-like lobules present	South Korea
<i>C. concolor</i>	Deeply branched, narrower (up to 0.5 mm)	smooth	Soredia granular	Asia, America, and Europe
<i>C. crawfordii</i>	Shallowly branched, wider (up to 0.7 mm)	smooth	Soralia labriform, with soredia on the upturned lower surface.	Asia, Australia
<i>C. fibrosa</i>	Wider (up to 2 mm)	Smooth or wrinkled	Soredia absent	Pantropical region

### CONCLUSIONS

The ITS rDNA phylogenetic trees had similar topologies with *Candelaria* species forming a separate clade with *C. asiatica* (Fig. 1). The morphological characters are similar to *Candelaria asiatica* (Dong Liu & Jae-Seoun Hur, 2018).

However, information regarding this species is still limited and the abilities of this species should be investigated further.

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