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Taxonomic studies on Encalyptaceae (Bryophyta) from Hunza, Karakoram Range, Pakistan

Suhail Karim[1], Jan Alam[1], Mazhar-ul-Islam[1], Zaheer Abbas[2], Gul Nawaz[3], Arshia Zia[4], Wasifa Rani[4], Syed Mohsan Raza Shah[2]

[1]Department of Botany, Hazara University, Mansehra, Pakistan
[2]Department of Botany, Division of Science and Technology, University of Education, Lahore, Pakistan
[3]Institute of Botany, Faculty of Life-Sciences, University of the Punjab, Lahore, Pakistan
[4]Department of Botany, University of Agriculture, Faisalabad, Pakistan

Abstract
The bryophytes of Pakistan are poorly explored, particularly in high altitude regions of the Karakoram ranges. In the present study, extensive field surveys were conducted in Hunza, Gilgit–Baltistan, Pakistan, from 2013-2017. The study deals with the taxonomic studies, particularly of the family Encalyptaceae. A total of six species were identified, belonging to the genus *Encalypta* within the elevation range of 2500 to 4100 m above sea level. Among these, *Encalypta cilliata* and *E. spathulata* were recorded for the first time from Pakistan. The relevant keys, description, currently known geographic distribution, color plates, location map, and precise ecological attributes in relation to habitat have been provided for each species. Consequently, the known diversity of Encalyptaceae in the country expanded to eight species, which underscores the significance of the Karakoram as a center of bryophyte diversity. Further surveys in remote valleys and higher altitudes are recommended to achieve a more comprehensive account of the national Bryoflora.

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Introduction

Encalyptaceae is an acrocarpic moss family that belongs to the division Bryophyta. Persistent calyptra is the distinguishing character of the family, which covers the whole capsule (Horton, 1982; Budke et al., 2018). In the diagnosis of habitat traits, spore morphology and leaf cells are also useful for family identification (Miller, 1968; Shah et al., 2021). Nevertheless, these characters are also found in other acrocarpic bryophytes except calyptra (Crandall-Stotler and Bartholomew-Began, 2007). The members of the family mainly prefer dry mountainous ranges and are distributed throughout the Northern Hemisphere, and a few species are cosmopolitan. So far, two genera (*Bryobrittonia* and *Encalypta*) with 34 species have been described globally (Horton, 1979). In the case of *Bryobrittonia,* the upper laminal cells bulge on both surfaces, while *Encalypta* has papillose cells present on one or both surfaces of the upper cells (Vitt and Hamilton, 1974).

Unfortunately, the Bryoflora of Pakistan is still underexplored. Furthermore, available literature

***CONTACT** Syed Mohsan Raza Shah, syedmohsan@ue.edu.pk; Department of Botany, Division of Science and Technology, University of Education, Lahore, Pakistan

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has only a few scattered checklists (Asghar, 1956; Nishimura et al., 1993; Townsend, 1993, 1994; 1995; Higuchi and Nishimura, 2003; Sollman, 2008; Alam et al., 2016; Gruber and Peer, 2021) and these checklists collectively represent about 500 species, including 6 species of the family Encalyptaceae. A major portion of the species is distributed in the Northern Areas of Pakistan. Generally, the floristic studies on the Valley of Hunza are limited. However, some literature is found on the ethnobotany and floristic diversity of higher plants, for instance, Akhtar et al. (2016) and Alam et al. (2023).

Previously known five species of the family for Pakistan have been documented by different taxonomists from various parts of the Northern Areas of Pakistan, e.g., *Encalypta alpina* Smith by Brotherus (1898) from Doyan and Gudhai, Astor Valley between 2727-3600 m.a.s.l.; *E. rabdocarpa* Schwägr. by Brotherus (1898) from Bari La, Baltistan at 4545 m.a.s.l., and by Higuchi and Nishimura et al. (1993) from Diamer, near Bial Camp, Gilgit Agency; *E. rabdocarpa* Schwägr. var. *leptodon* (Bruch.) Limpr. by Brotherus (1898) from Naltar valley between 2727-3300 m.a.s.l.; *E. streptocarpa* Hedw. by Higuchi and Nishmura et al. (1993) from Sikyan near Nadi, Mansehra District at 2020 m; *E. tibetana* Mitt. by Brotherus (1898) from Naltar Valley, Gilgit between 2727-3300 m.a.s.l.; *E. vulgaris* Hedw. by Brotherus (1898) from Satpara Nullah, Skardu, Balistan, and Pirni, Jhelum valley; *E. vulgaris* Hedw. *var. mutica* by Störmer (1954) from below Zapotili, Chital district at 3500 m.a.s.l.

It is widely accepted that plant richness is perturbed due to long-term climatic instability, thereby promoting species differentiation (Zhang et al., 2018). Temperature and rainfall are key drivers that affect the diversity and distribution of plant species. It is likely that the diversity of plant species of the Hunza Valley might have undergone a significant change with time due to changes in climate. Thus, the primary objective of the current study was to document species diversity and taxonomic attributes of the family Encalyptaceae in Hunza, an area with specific topography and climatic conditions.

Materials and Methods

Study Area

The Valley of Hunza is a district of Gilgit-Baltistan province, in the North of Pakistan. It is located in the central area (Hussain, 2021). Geographically, the study area lies between 36.7°-36.9° N and 75°-77.5° E with a total area of ca.100,00 km^2. It is bounded by China in the east, Afghanistan towards the north, the Ghizer district on the northwestern border, and the Nagar district in the south (**Figure 1**).

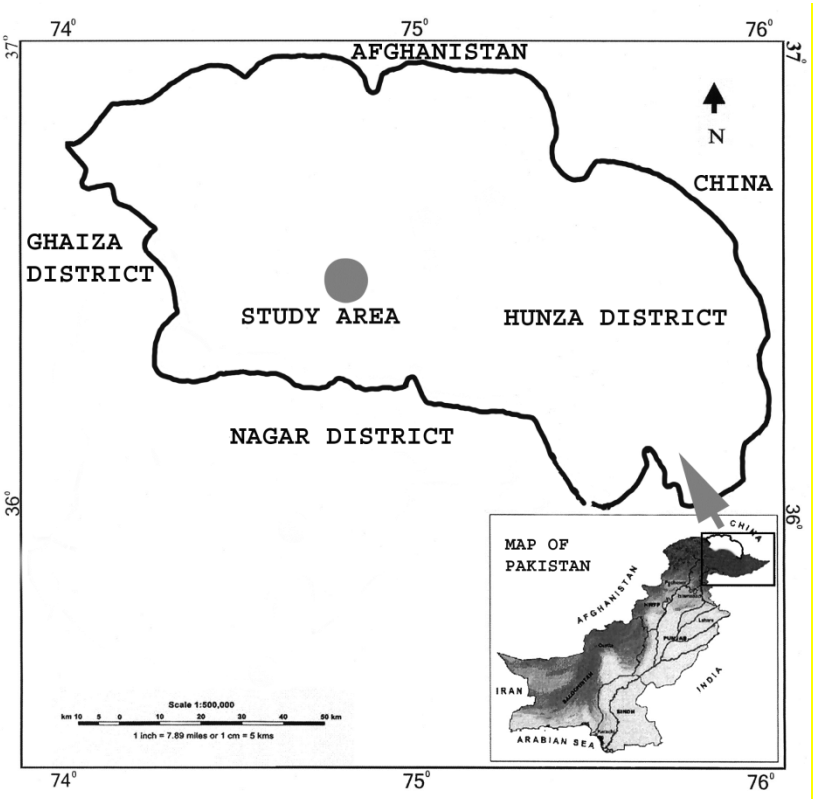

Figure 1: Location map of the study area

Topography is craggy and predominantly desolate. At the lower elevations, semi-arid desert zones are found along the banks of the river. But the geo-ecological features change with respect to the elevation, displaying many high peaks above 7000 m (Ultar peaks, etc.) and glaciers like Shishpar and Ultar glacier (Mustafa et al., 2024). River Hunza is the main water channel and one of the main tributaries of River Gilgit. The climate is dry, and little rainfall occurs in the early spring and late summer. The environmental conditions become cooler and wetter with elevation, shaping richer vegetation. Phytogeographically, the area falls in the Irano-Turanian region.

Experimental design

In order to assess the phytogeography and reports from Pakistan, previously published literature was reviewed thoroughly. Different Floras viz. Flora North America (Horton, 1982), Moss Flora of China (Blockeel, 2000), BBC and other literature (Duthie, 1898; Størmer, 1954; Nishimura et al., 1993; Higuchi and Nishimura, 2003; Gruber and Peer, 2010; Horton, 2012; Glime, 2013a & b; Glime and Wagner, 2013) were consulted. A comprehensive field program was chalked out based on the literature survey findings. Accordingly, many field trips were conducted to different localities of the study area in different seasons from 2013-2017. Specimens were collected using polyethene bags along with appropriate field information. Each specimen was separately tagged. Photographs were taken of the focusing substrate, habit, growth form, sporophyte, and gametophyte, etc. Specimens were dried on blotting papers, then transferred into the packets (14 x 10 cm) and properly labeled. Some amount of each specimen was examined under a stereoscope and microscope in wet condition and was identified using relevant literature. Research activities were carried out in the Cryptogrammic Laboratory, Department of Botany, Hazara University. Finally, the identified specimens were deposited in the Cryptogamic Laboratory, Hazara University Herbarium (HUH).

Results and Discussion

Single genus, i.e., *Encalypta* and its six species viz., *Encalypta alpina*, *E. ciliata*, *E. rhaptocarpa*, *E. spathulata*, *E. tibetica,* and *E. vulgaris* were recognized from the study area. A taxonomic outline has been provided, including an annotated key for identification of the species. Known species are restricted to 2500-4100 m. Of these, four species have been previously reported, while two species are new records for Pakistan.

Encalyptaceae

Plants are autoicous or delicious; the stem is short, erect, irregularly branched, loosely forked, or dense tufts. Leaves spreading and incurved in wet conditions, contorted and twisted when dried, elliptic to oblong-ovate, spathulate; costate, single, percurrent, excurrent, supercurrent or with awn, acute apex, obtuse, round, muticous or pointed awned, entire margin, sometimes crenate or serrate, rounded, rhombic or quadrate upper laminal cells. Cylindrical capsule, ovate to oblong, symmetric, smooth or furrowed; peristome double, single, undeveloped or absent; long beak on operculum. Calyptra completely covering the whole capsule, even at maturity, campanulate, conic-cylindric, rostrate, rostrum variable in size. Base lacerate, fringed, entire, entire-erose, lobed, smooth or pilose. Capsule erect, cylindrical, smooth or ribbed. Operculum with a rostrate beak. Peristome, single or double or absent, lanceolate to filiform, entire or divided, papillose. Spores spherical, small to large, with proximal and distal surfaces often differentiated.

Encalypta

Leaves oblong to ovate, lanceolate, lingulate, ligulate, oblong-ligulate, spathulate; leaf tip acute-obtuse, round, muticous, pointed-hair, mucronate, acuminate; entire leaf margin or serrate, sometimes margin incurve at base. Seta elongate, smooth, brown to red and dark red. Capsule cylindrical, oblong-ovate, narrow cylindrical, smooth, furrowed, capsule striate, weakly striate; operculum conic-rostrate with long beak. Smooth calyptra, rostrum long present, calyptra base entire, lacerate or fringed. Spore papillose, warty, brown, or ridged.

Key to Species

1. + Calyptra fringed at base.....1. ***E. ciliata***
   - Calyptra not fringed at base.....2
2. + Leaf gradually tapering towards apex; apex apiculus ..... 2 . ***E. alpina***
   - Leaf abruptly becomes narrower near apex; apex broadly acute-obtuse apex...3
3. + Leaf hair pointed at apex.....4

- Leaf without hair pointed at apex……5
4. + Capsule prominently straite; Peristome present, single……..3. ***E. rhaptocarpa***
- Capsule weakly straite; Peristome absent……4. ***E. spathulata***
5. + Costa percurrent; capsule oblong–ovate; peristome present……5. ***E. tibetana***
- Costa excurrent; capsule cylindrical; peristome absent or rudimentary……6. ***E. vulgaris***

Encalypta ciliata Hedwig

Plant 1.5-3.5 cm tall and dark-light green. Leaf, oblong, lanceolate to elliptical, lingulate, 2-5 x 1-1.5 mm. Apex acute-obtuse, mucronate; curved margin near base; excurrent costa; rectangular cells at laminal base, wall reddish and transverse; medial laminal cells quadrate; rounded to quadrate apical cells; marginal cells papillose. Seta 5-11mm, yellow. Capsule conical shape, cylindrical with swollen base, 2-3.5 mm, smooth, yellowish-brown; beak 1-1.5 mm; single peristome, 16 teeth, 0.3 mm, erect, lanceolate, papillose. Calyptra 3-5 mm, beak cylindrical, fringed base. Spores 30-35 µm, brownish-yellow (**Figure 2**).

Specimen examined: Pakistan: Gilgit-Baltistan: Hunza, Karimabad, 4030 m, 21-08-16, *Suhail Karim, Rehmat Karim, and Muhammad Baig 563* (HUP); Ibit Bululo, 2-08-16, 4100 m, *Suhail Karim, Rehmat Karim, and Muhammad Baig 564* (HUP); Ibit Bululo, 4000 m. 2-08-16, *Suhail Karim, Rehmat Karim, and Muhammad Baig 586* (HUP); Ultar, 3955 m, 4-9-2016, *Suhail Karim and Ghulam Baig 646* (HUP).

Distribution: Asia (Iran, Pakistan, China, Japan), Europe, South America, and North Africa.

This species is found in moist rock crevices at higher elevations from 3200-4100 m. However, the extreme basal portion of the capsule left the calyptras at maturity. *A new record for Pakistan*.

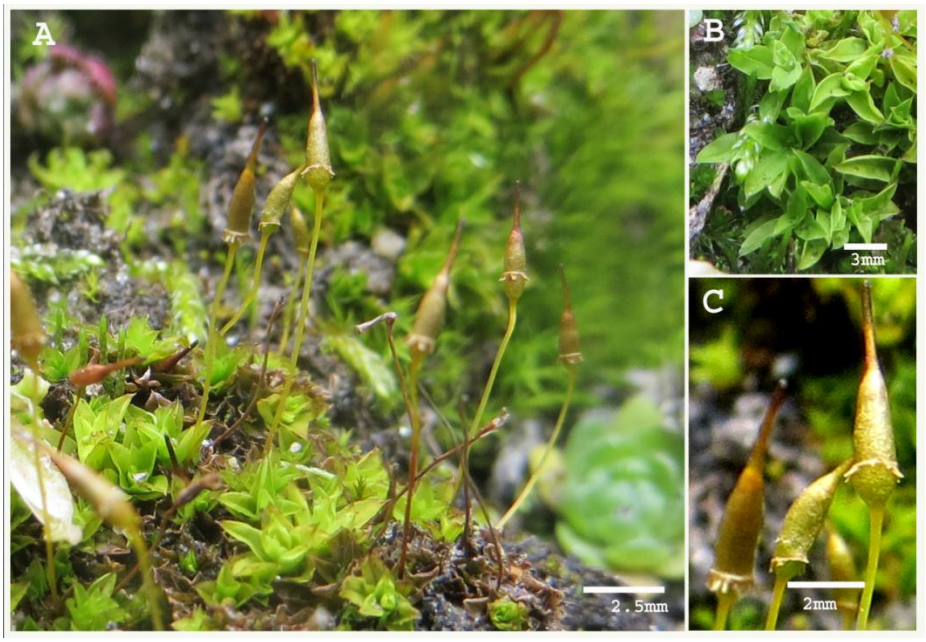

Figure 2: *Encalypta ciliata*: A, habit; B, gametophytes; C, sporophytes. Scale bars: A = 2.5, B = 3, C = 2 mm

Encalypta alpina Smith in Smith & Sowerby Engl.

Plant 1.0-1.5 cm tall. Leaves lanceolate or ovate, gradually acute apex tapering, acuminate, 2.5-3.1 mm long when moist; costa strongly excurrent; margin, entire or plane. Capsule 2-4 mm including beak, cylindrical, narrow cylindrical, smooth, without ribs, red at base; beak 1.3 mm; peristome. Seta twisted when dry, 11-13.5 mm long, brown. Calyptra cylindrical, smooth, light brown, 6-7 x 1-1.2 mm, base is lacerate; rostrum size is 25-30% of the calyptra, dark brown. Spore 30-33 µm in diam., yellowish brown, irregularly papillose. Some visual representations are presented in **Figure3**.

Specimen examined: Pakistan: Gilgit-Baltistan: Hunza, Karimabad, Ultar Nullah, 3900 m, 22-08-14, *Suhail Karim 336* (HUP); Pakistan - Gilgit-Baltistan: Hunza, Karimabad, Ultar Nullah, 3450 m, 08-07-14, *Jan Alam and Naik Alam 8088* (HUP).

Distribution: North America, Europe, China, Pakistan, Mongolia, Japan

Encalypta alpina is a distinct species. This species can be easily differentiated from other species in the area by means of its awnless, gradually tapering lanceolate leaves, long brown seta, and long, narrow, cylindrical calyptra.

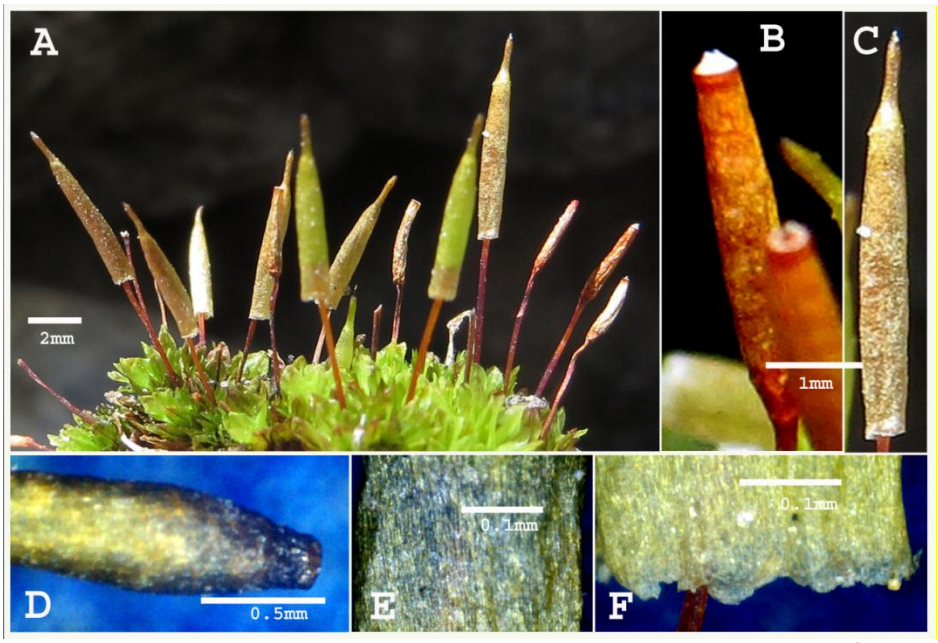

Figure 3: ***Encalypta alpina*****: A, habit; B, capsule; C, calyptra; D, rostrum; E, close-up of middle portion of calyptra; F, base of calyptra. Scale bars: A = 2 mm, B = 1 mm, C = 1 mm, D = 0.5 mm, E = 0.1 mm, F = 0.1 mm**

Encalypta rhaptocarpa Schweagr.

Plant 1.4-2 mm tall. Leaves lanceolate, oblong, oblong to lingulate, spathulate, oblong-ovate, curled when dry, 2-4 mm when moist; apex rounded, obtuse, obtuse–acute, hair pointed, awn ca. 0.4 mm; margin entire, incurved; costa single, percurrent, excurrent; rounded, quadrangular apical cells; basal cells rectangular-quadrangular; marginal cells rectangular. Seta yellow, brown, reddish brown, 2-6 mm. Cylindrical capsule, 2-3.5 mm x 0.5-0.6 mm, constricted when dry, longitudinally ribbed; beak ca. 1 mm long; peristome well developed, rudimentary. Calyptra cylindrical, smooth, yellow-brown, 4-4.2 mm; base entire, irregular; rostrum 1.5-1.6 mm, dark brown. Spore 32-38 µm, warty brown as illustrated in **Figure 4**.

Specimen examined: Pakistan: Gilgit–Baltistan: Hunza, Karimabad, Ultar Nullah, 3150 m, 18–10–2013, *Suhail Karim 158* (HUP); Pakistan. - Ibit, 2700 m, 08-7-2014, *Jan Alam and Suhail Karim 8070* (HUP); Ibit, 2700 m, 08-7-2014, *Jan Alam and Suhail Karim 8074* (HUP); Ibit, 2800 m, 08-7-2014, *Jam Alam and Suhail Karim 8085* (HUP). Pakistan. Gilgit-Baltistan: Hunza, Karimabad, Bullolo, 3700 m, 9-8-14, *Suhail Karim 293* (HUP); Ibit, 3700 m, 9-8-2014, *Suhail Karim* 289 (HUP).

Distribution: Europe, North and Central Asia, Hawaii, America, Greenland, Iceland.

It has been observed that *Encalypta rhaptocarpa* usually grows with *Encalypta alpina* at higher elevations (i.e., above 3700 m) and with *Encalypta vulgaris* at low altitudes; therefore, special care is needed during field collection of the species. Distribution-wise, this is a common species in the area and was found to occur between 2700 and 3780 m.

Encalypta spathulata Muller Ha., Syn. Muci. Frond, 1: 519. 1849.

Plant 12-15 mm tall. Leaves twisted when dry, 3-5 x 1 mm when moist, oblong, lingulate, oblong-lanceolate, ligulate; apex, obtuse, obtuse-acute, awn 0.6-0.7 mm; costa, single, excurrent; margin entire, undulate at middle, incurved at apex; basal cells rectangular; marginal cells linear, 2-papillose cell; apical cell rounded-quadrates. Seta 5-7 mm, angular. Capsule cylindrical, 3mm, ribbed; operculum with long beak ca. 0.8 mm; peristome absent. Calyptra brown, cylindrical, smooth 2-5 mm, lacerate at base; rostrum dark brown. Spore warty brown, 42-50 µm, as some specifications are given in **Figure 5**.

Specimen examined: Pakistan: Gilgit-Baltistan, Karimabad, Ultar Nullah, 3200 m, 18-10-2014, *Suhail Karim 610b* (HUP).

Distribution: North America, Europe, Asia.

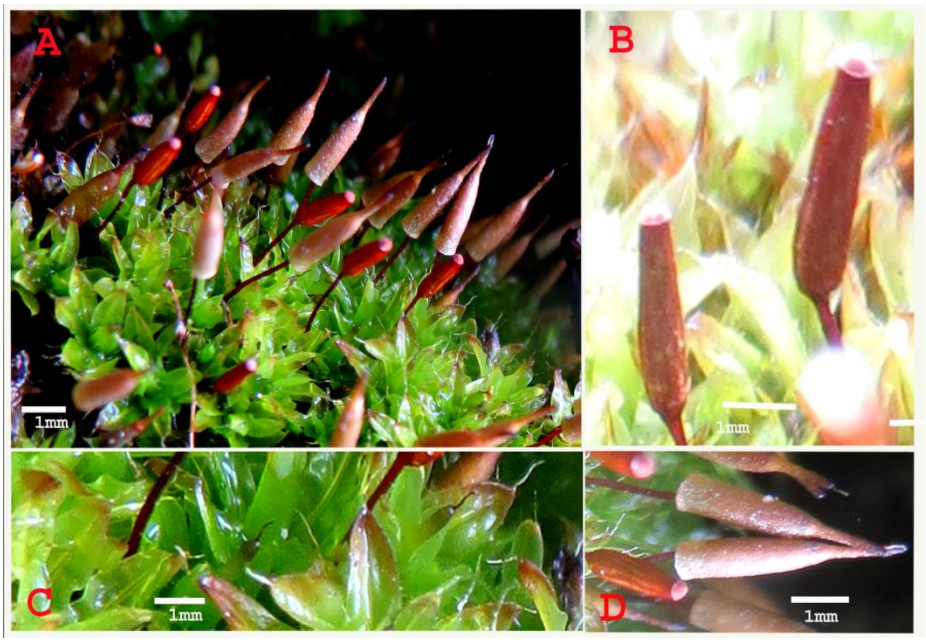

Figure 4: ***Encalypta rhaptocarpa***: **A, habit; B, capsules; C, gametophytes; D, calyptrae. Scale bars: A = 1 mm, B = 1 mm, C = 1 mm, D = 1 mm**

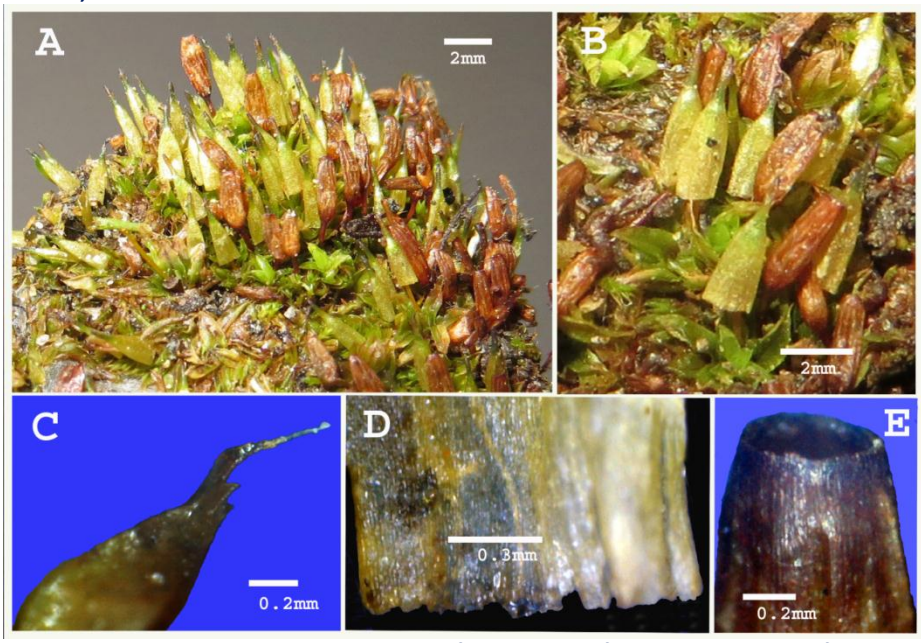

Figure 5: ***Encalypta spathulata***: **A, habit; B, close up of habit; C, leaf; D, basal portion of calyptra; E, orifice of capsule. Scale bars: A = 2 mm, B = 2 mm, C = 0.2 mm, D = 0.3 mm, E = 0.2 mm**

Encalypta tibetana Mitt.

Plant 6-15 mm tall. Leaves oblong, ligulate, brown; apex abruptly narrow, gradually narrow obtuse; margin entire, 1.5-2.2 x 0.5-0.9 mm, gemmae present at leaf base; costa single, percurrent; apical cells rounded-quadrates, 2-papillae; marginal cells linear, and basal cells rectangular. Seta 6-7 mm, reddish–brown. Capsule 2-2.3 mm; beak 1 mm; ribs prominent, red; peristome present. Calyptra yellow–brown, 2-2.2 mm; rostrum dark brown, 1 mm, base entire. Spore papillose, 30-38 x 28-35 µm (**Figure 6**).

Specimen examined: Pakistan: Gilgit-Baltistan: Hunza, Karimabad, Bullulo, 3750 m, 18-08-2014, *Suhail Karim and Abbas Ali Khan 316* (HUP); Ibit, 3200 m, 15-07-2014, *Jan Alam and Suhail Karim K-1* (HUP).

Distribution: China, Pakistan

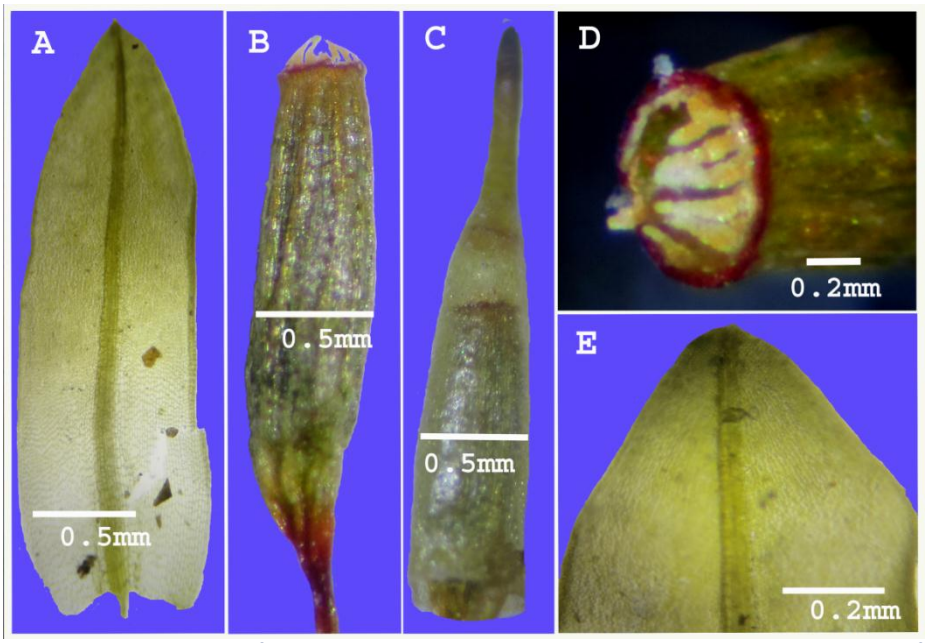

Figure 6: ***Encalypta tibetana*****: A, leaf; B, capsule; C, calyptras; D, peristome; E, apical portion of leaf. A = 0.5 mm, B = 0.5 mm, C = 0.5 mm, D = 0.2 mm, E = 0.2 mm**

Encalypta vulgaris Hedwig., Sp. Muci. Frond, 60. 1801.

Plant 8-15 mm tall. Leaves curled when dry, 2-3 mm x 1.2 mm when moist, lanceolate, oblong–ovate, lingulate, oblong-spathulate; apex obtuse, rounded, abruptly narrowed; margin entire, slightly incurved; base brown; costa single, percurrent, rarely excurrent; apical cells oval-hexagonal; marginal cells 2-papillae; Basal cells rectangular, brown. Seta 3-5 mm, reddish–brown. Capsule, smooth, rarely faintly plicate, 2-5 mm x 0.5 mm; peristome absent or rarely rudimentary; operculum with long beak, beak 1 mm. Calyptra cylindrical, smooth, yellow-brown, 1.6 mm; Rostrum 1.2 mm; base entire. Spores are slightly brown, rounded-ovate, papillose, spore 22-42 µm x 20-35 µm (**Figure 7**).

Specimen examined: Pakistan - Gilgit–Baltistan: Karimabad, Ultar Nullah, 3100 m, 1810–2013, *Suhail Karim* 185 (HUP); Ibit, 3050 m, 18-10-2013, *Suhail Karim 165* (HUP); Pakistan - Gilgit–Baltistan: Karimabad, Bullolo, 2700 m, 9-8-2014, *Suhail Karim 277* (HUP); Ibit, 2700 m, 08-07-14, Jan *Alam and Suhail Karim 8077* (HUP); Ibit, 3150 m, 08-07-2014, *Jan Alam and Noor Alam 8086* (HUP); Pakistan - Gilgit-Baltistan: Karimabad, Bullolo, 3400 m, 14-07-2014, *Jan Alam and Suhail Karim* s.n (HUP).

Figure 7: ***Encalypta vulgaris*****: A, habit; B, close-up of habit; C, capsule with calyptra; D, close-up of leaf; E, capsule; F, orifice of capsule. Scale bars: A = 2.5 mm, B = 2 mm, C = 1 mm, D = 0.6 mm, E = 1 mm, F = 0.5 mm**

Distribution: Asia, Central and South America, Europe, Africa, New Zealand, Australia.

Encalypta vulgaris is the most common species in the area and is found in lower to higher elevations (i.e., 2700-3400 m). Morphologically, the species shows variation in many aspects, i.e., leaf size, shape, capsule shape, etc.

Previously, five recognized species of the family for Pakistan have been reported from the drier regions of the Northern Areas of the country, i.e., Sheringal Valley, Upper Dir, Khyber Pakhtunkhwa, Chitral, and Gilgit-Baltistan province (Duthie, 1898; Størmer, 1954; Nishimura et al., 1993; Hazrat et al., 2020). However, of these, *E. streptocarpa* is also reported from Sharan Forest, Mansehra district, a moist region. In the current investigation, 6 species, viz., *Encalypta alpina*, *E. ciliata*, *E. rhaptocarpa*, *E. spathlata*, *E. tibetana,* and *E. vulgaris* have been documented from the study area. These species make up about 17.7 % of the total world-wide known species.

Enclypta ciliata and *E*. *spathulata* are the first records for Pakistan. *Enclypta ciliata* is a common species, which is distributed in Asia (China, Japan), North Africa, while *E. spathulata* is known from North America, Europe, and Asia (Horton, 1982). It has been observed that some species, i.e., *E. alpina*, *E. vulgaris,* and *E. rhaptocarpa,* usually grow intermixed. Therefore, special care is needed during collection, and for that, sufficient material should be collected even from a small microhabitat.

In addition, further comprehensive inventorying/ studies are needed in other remote localities in the study area, especially Shishpar Nullah and the adjacent areas. It is interesting to note that approximately 80% of the previously known species of the Encalyptaceae in Pakistan were documented in these surveys for the first time. As suggested elsewhere (Fengjiao et al., 2022), several additional areas should be investigated in the adjacent regions, particularly at higher elevations, considering different seasons.

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Conflict of interest
The authors declare no conflict of interest.

Contribution of authors
Research supervision: JA. Conceptualization and designing the study: JA, SK. Conduction of experiment: SK. Resource availability: SK. Moderation of laboratory activities: AZ, ZA, GN. Instrumentation and analysis: MUI, WR. Preparation of initial draft: SK, SMRS. Revision of final draft and proofreading: All authors.

Permissions and ethical compliance
This study does not involve human/animal subjects, and thus no ethical approval is required.

Handling of bio-hazardous materials
The authors certify that all experimental materials were handled with great care during collection and experimental procedures. After completion of the study, all materials were properly discarded to minimize/eliminate any types of bio-contamination(s).

Supplementary material
No supplementary material is included with this manuscript.

Availability of primary data and materials
As per editorial policy, experimental materials, primary data, or software codes are not submitted to the publisher/Journal management. These are available with the corresponding author (s) and/or with other author(s) as declared by the corresponding author (s) of this manuscript.

Authors' consent
All authors have critically read this manuscript and agreed to publish in IJAaEB.

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It is declared that the authors did not use any AI tools or AI-assisted services in the preparation, analysis, or creation of this manuscript submitted for publication in the International Journal of Applied and Experimental Biology (IJAaEB).

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