

repeated thrice. The cultures were grown at 22–25°C and a maximum relative humidity of 60% and under 16 h light of 3200 lux provided by Philips fluorescent day tubes. The detection of artemisinin in roots, shoot cultures and callus, was done following the method of Nair *et al.*<sup>2</sup>

Callus was induced on lamina and petiole segments derived from field grown plants as well as from aseptic seedlings. However, the explants from field grown plants exhibited better callusing (figure 1).

The auxins, IAA, IBA and NAA (1–2 mg/l) when used alone induced roots from cut ends of leaves and did not induce callus formation. On medium supplemented with NAA (2 mg/l)+Kn(1mg/l) the callus was very compact, greenish but turned brown after 8 weeks. The calli were greenish and less compact in the presence of BA (0.5, 1 mg/l+NAA (0.5–2 mg/l). The callus did not turn brown and could be subcultured and maintained in an undifferentiated state in the presence of BA (1 mg/l)+NAA (1.2 mg/l). In these hormonal combinations, callus induced on petiole explants was less compact than those induced from lamina. In the presence of NAA and Kn, the calli tend to form roots. Profuse rooting occurred with high concentration of NAA (4 mg/l) and low concentration (1–2 mg/l) of Kn (figure 2).

In combinations with low BA (0.5 mg/l) and high NAA (2 mg/l), the callus induced root formation. With a combination of high BA (2 mg/l) and low NAA (0.5 mg/l), the callus showed profuse shoot bud regeneration (figure 3). The regenerating calli were maintained on BM+BA (2 mg/l). Clusters of shoots (5–10 shoots/cluster) with a little basal callus were used for further shoot multiplication or for rooting to regenerate whole plants.

Shoot cultures were established from the regenerating shoots and from shoot apices derived from sterile seedlings. They were allowed to grow in BM+2 mg/l BA for 4 weeks. Subsequently shoots were subcultured in liquid MS medium with 2 mg/l BA. Shake culture (agitated) induced profuse multiplication of shoots (80–100 shoots/flask) within 4 weeks (figure 4). Subculturing was done at 3–4 weeks interval as prolonged treatment showed browning of surface shoots. Shoots have been multiplied and thus maintained for over a year.

The shoots excised from proliferating shoot cultures or callus cultures were rooted on BM+2 mg/l IBA (figure 5). On the medium, well established roots developed within 4–6 weeks. Rooted plants were allowed to grow in MS liquid medium without

any hormone for 4–6 weeks. The cultures were then kept at room temperature (28–30°C) for hardening the plants for 6 weeks. The plants were then thoroughly washed in tapwater and transferred to pots containing soil and with polythene bags for 7–10 days initially. All the plants survived (figure 6) and produced viable seeds within 4–5 months. The plants could be successfully transferred to the field throughout the year.

Preliminary studies revealed the presence of artemisinin in roots, unrooted shoots and callus. This is interesting as Nair *et al.*<sup>2</sup> did not detect the compound in callus and unrooted shoots of *A. annua*. Further work on isolation of artemisinin and its possible analogues from callus cultures is in progress.

The method of shoot multiplication from shoot apices of aseptic plants or from shoots regenerated from calli ensures a constant supply of plant material and is also suitable for propagation of the plant. The plants transferred to soil have survived and studies on their growth, morphology and artemisinin content are now being pursued.

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### VITTARIA HIMALAYENSIS CHING, A NEW RECORD FOR WESTERN HIMALAYAS

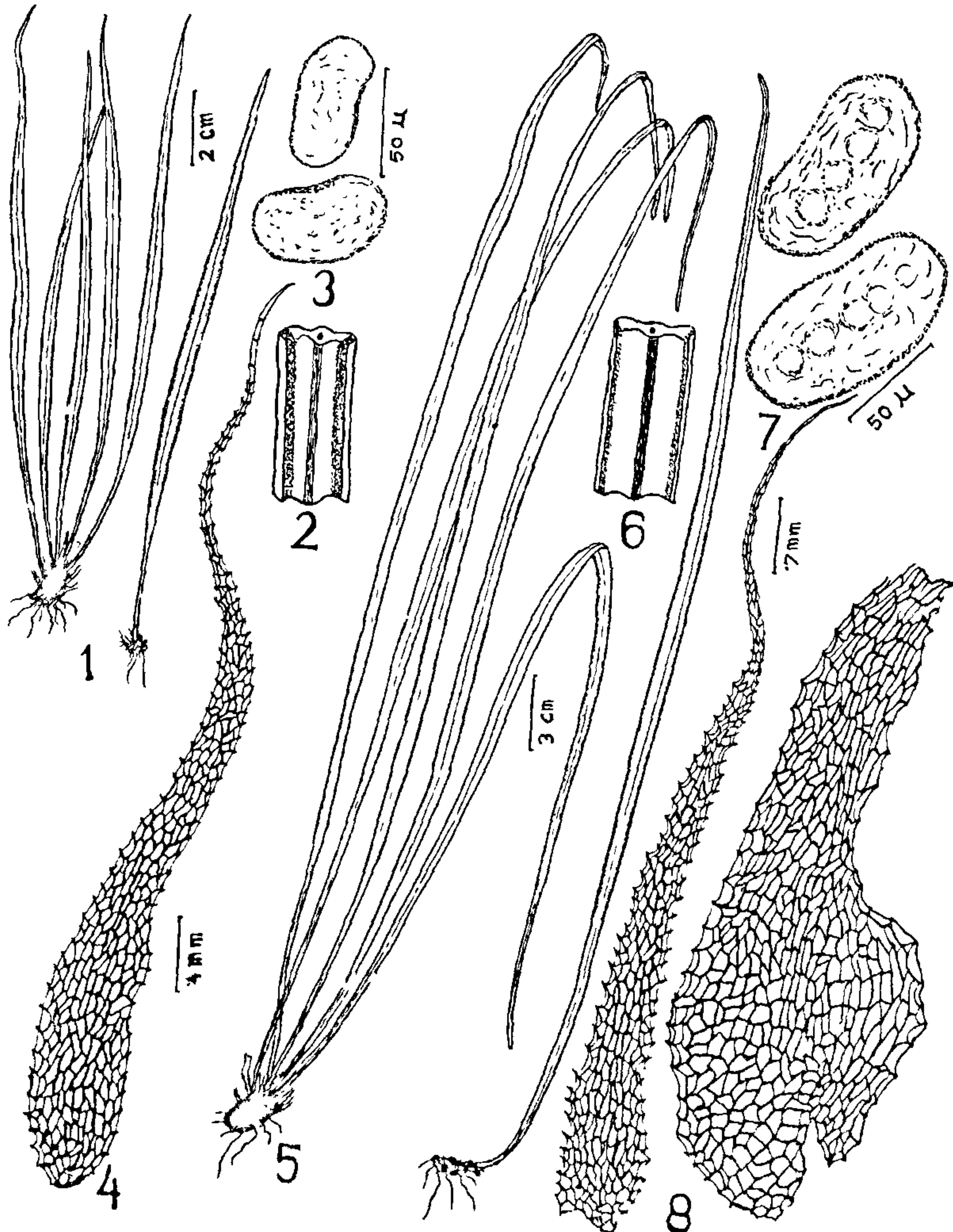
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FROM the taxonomy point of view, the himalayan ferns have received considerable attention during the last three decades. However, most of the work concerns with the hill stations situated on the outer and middle Himalayan ranges. Information on the

fern vegetation of these regions is so far based on the classical but pioneering work of Clarke<sup>1</sup>, Beddome<sup>2</sup>, Hope<sup>3</sup> and Duthie<sup>4</sup>. The inner ranges need thorough exploration; amongst these are the interior regions of the Kumaon hills in north-west Himalayas. The district of Pithoragarh lying in Kumaon hills, forms the easternmost limit of western Himalayas of the Indian territory. Apparently both the eastern and western himalayan elements

are found here, and the district is rich in fern vegetation. The epiphytic ferns in the Pithoragarh District are as common as the mesophytic ferns. The common epiphytic ferns of this region are numerous Polypodiaceous ferns, *Loxogramme lanceolata* (Sw.) Presl and *Vittaria flexuosa* Fee. These commonly grow during the rainy season and prefer mossy substrata on the tree trunks of oak trees.

During our studies on the Pteridophytic Flora of



Figures 1-4. *V. himalayensis*, 1. Habits, 2. Part of lamina, 3. Spores, 4. Rhizome scale; 5-8. *V. flexuosa*, 5. Habit, 6. Part of lamina; 7. Spores, 8. Rhizome scales.



the Pithoragarh District, we collected *Vittaria himalayensis* Ching from near Munsyari (Kalamuni 3000 m) which is a new record for the western Himalayas.

A genus of about 80 species<sup>5</sup>, *Vittaria* J. Smith, is widely distributed in the tropical parts of the world. In the western Himalayas it is so far known to be represented by only one species, *V. flexuosa* Fee<sup>1-7</sup> but in the eastern Himalayas the genus is represented by as many as seven species. Of these *V. doniana*, *V. himalayensis* and *V. ophipogonoides* were recorded for the first time from the Indian part of the Himalayas<sup>5</sup>. Most of the species are epiphytes or lithophytes. The fronds are simple, fleshy with two rows of marginal or intramarginal linear coenosori.

Presently the two species of the region are described. Key to the species:

1a. Fronds erect, small, less than 25 cm long; sori half way between the margins and the midrib; rhizome scales with cuneate or obtuse base

*V. himalayensis*

1b. Fronds pendent, large, 30–50 cm long; sori near the margins; rhizome scales with cordate base

*V. flexuosa*

1. *V. himalayensis* Ching, *Sinensia*, 1, 190, f. 5B, 1931.

Rhizome creeping or suberect, densely covered with linear, pale yellow scales; lamina simple, linear, broad in the middle, gradually tapering towards both ends, 8–25 × 0.3–0.5 cm, leathery, apex acute, midrib restricted to the central part of lamina; veins simple, oblique; sori intramarginal, in grooves, half way between margin and midrib; spores 62 × 32 μ (figures 1–4).

Specimens examined: PUN 4184, Punetha 584, 585.

2. *V. flexuosa* Fee, 3 me. Mem., 16, 1851–52.

Rhizome short, creeping, densely scaly; stipe 0.5–1 cm long; lamina simple, linear, coriaceous, 25–50 × 0.3–0.5 cm, wide at the middle, gradually narrowed towards both ends, apex acute, a distinct midrib is present from the base to the apex; veins simple, immersed, parallel; sori intramarginal, in shallow furrows, near to margins, spores 100 × 50 μ (figures 5–8).

Although some earlier workers<sup>2,3</sup> described the north Himalayan *V. flexuosa* Fee as *V. lineata* (L.) J. Smith, Bir<sup>5</sup> clarified that the Himalayan fern is *V. flexuosa* Fee and that *V. lineata* (L.) J. Smith is a tropical American species.

In Pithoragarh District it is very common at Didihat (1700 m), Gini (2200 m) and Kalamuni (2800 m). Specimens examined: PUN 4185; Punetha 324, 325, 586, 587, 588, 589, 590.

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## LONG-STYLED FORM OF WATER HYACINTH IN INDIA

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WATER hyacinth (*Eichhornia crassipes* (Mart.) Solms), the most troublesome aquatic weed, is a tristylous species of family Pontederiaceae<sup>1</sup>. The mid-styled form is widely distributed throughout the tropics and subtropics where it was introduced from its native home in South America<sup>2</sup>. The short-styled form is so far known only from Amazonia in Brazil. The long-styled form has been found in many places both in its native and adventive range except Australia<sup>2</sup>. In India, the long-styled form was first reported from Madras and Bangalore by Haldane<sup>3</sup> who called for more field surveys to assess the distribution of the three forms. Later, Das<sup>4</sup> reported it from Sagar and Bhopal in Madhya Pradesh, and Reddy and Bahadur<sup>5</sup> collected this form from a