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Biosystematic Studies on *Enicostema axillare* (Lam.) A. Raynal subsp. *Axillare* (Gentianaceae) in Peninsular India

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Abstract: The pantropical genus *Enicostema* (Gentianaceae) has three species and two sub species world over, namely, *E. verticillatum* (L.) Engl. (America), *E. elizabethae* Veldkamp (Madagascar) and *E. axillare* having 3 subsp. viz., subsp. *axillare* (Lam.) A. Raynal (India), subsp. *latilobum* (N.E. Br.) A. Raynal (East Africa) and subsp. *littorale* (Blume) A. Raynal (Indonesia). The present study aims to delimit the Indian taxa based on field and herbarium studies. Comparative morphology is studied using live as well as consulting wide range of specimens housed at various herbaria. The anatomy of leaf, stem and root is studied using free hand sections and from epidermal peelings. The seed and pollen morphology are studied under SEM. Information on anatomy, palynology and seed micromorphology of *E. axillare* subsp. *axillare* is provided for the first time.

Key words: *Enicostema*, *E. axillare* subsp. *axillare*, morphology, anatomy, palynology, seed micromorphology

INTRODUCTION

The genus *Enicostema* was established by Blume in 1826 to include a single species *E. littorale* from Java. Subsequently three more species, *E. verticillatum* (L.) Engl. (*Gentiana verticillata* L.), *E. hyssopifolium* (Willd.) I.C. Verdoon (*Exacum hyssopifolium* Willd.) and *E. latilobum* N.E. Br. were added to the genus. Veldkamp (1968) while providing a synopsis of the genus reduced *E. littorale* as conspecific to *E. hyssopifolium* but described yet another species *E. elizabethae* from Madagascar. Raynal (1969) in his revision of *Enicostema*, transferred *Gentiana axillaris* Lam. to *Enicostema* and considered *E. hyssopifolium* as conspecific but reduced *E. latilobum* and *E. littorale* as sub species of *E. axillare*. At present the genus is represented by 3 species and 2 subsp. *viz.*, *E. verticillatum* (America), *E. elizabethae* Veldkamp (Madagascar) and *E. axillare* having 3 subsp. viz., subsp. *axillare* (Lam.) A. Raynal (India), subsp. *latilobum* (N.E. Br.) A. Raynal (East Africa) and subsp. *littorale* (Blume) A. Raynal (Indonesia) (Raynal, 1969).

The Indian species of *Enicostema* was first described as *Gentiana verticillata* by Linnaeus in 1781 having the same name assigned by his father in 1759 for an American species (Veldkamp, 1968). It was Willdenow (1798) who placed them in the genus *Exacum* and gave *E. verticillatum* (L.) Willd. for the American species and *E. hyssopifolium* Willd. for the Indian species. However, the Indian taxa were differently treated by various authors. Hooker (1885), Nairne (1894), Cooke (1908), Rama Rao (1914) and Gamble (1923) treated it as *E. littorale* while Chandrabose and Nair (1987) as *E. hyssopifolium* and still others like Matthew (1982, 1999) and Pullaiah and Moulali (1997) as *E. axillare* subsp. *axillare*. Our studies in consultation with type and protologue conclusively identified the Indian specimen as *E. axillare* subsp. *axillare*.

The word '*Enicostema*' is derived from three words 'en' means inside, 'icos' means 20, 'stema' means wreath, referring to the arrangement of about 20 flowers around every node of the stem. The genus is remarkable for its dense axillary cymes, unique androecium in which the staminal tube formed by the coalescence of net at their base and short filaments furnished with a double hooded scale at the base, bilocular, apiculate anthers and capitate stigma.

In the present study, the morphology, taxonomy, anatomy, palynology and seed micromorphology of *E. axillare* subsp. *axillare* is discussed.

MATERIALS AND METHODS

Morphology: Comparative morphology is studied using fresh and herbarium specimens. In order to understand the variation pattern, field trips are conducted across peninsular India and different populations are examined. Data sheets are prepared for each population studied. The materials collected from different localities are introduced in the botanical garden and characters were observed. More than 100 specimens housed at BLAT, BSD, BSI,

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CAL, CALI, DD, DEV, FRC, KFRI, MH, RHT, SNC, SKU and TBGT are also consulted (only representative specimens are cited in the study). Voucher specimens are deposited at DEV. The identification of the taxa is done in consultation with type and protologue. Author citations follow Brummit and Powell (1992) and citations are restricted to the basionyms and essential synonyms. Classification of *Enicostema* follows Struwe *et al*. (2002). Illustrations of the floral parts are made with the help of Camera lucida attached to Leica MZ75 Stereo Zoom Microscope and photographs are taken with the help of Nikon D50 Camera.

Anatomy: The anatomy of leaf, stem and root is studied using fresh samples. To study the type and distribution of stomata epidermal peelings are taken from the basal, medial, apical and margins of leaf blades by making an oblique incision with the help of a sharp razor blade. Free hand sections of leaf, stem and root are taken to obtain the details. Stems were sampled at the 2nd and 3rd internodes. Sections are stained with Toluidine blue for 5 min and mounted in Glycerine. Characters are recorded and photographs were taken using Leica DFC 290 Camera attached to Leica DM 1000 Trinocular Microscope.

Palynology: The pollen is studied under Scanning Electron Microscope. For SEM dried flowers or buds are rehydrated for 1-2 h. The anthers are picked out from the flowers and the tips of the anthers are removed with a razor blade to facilitate rehydration of the locules. The pollen grains are then gently removed from the opened locules with a fine needle and mounted on aluminium stubs using double adhesive tape and coated with gold in sputter coater. Micrographs are obtained under Hitachi Su 6600 Scanning Electron Microscope.

Seed micromorphology: Seeds taken from mature fruits are dried and directly fixed to aluminium stubs, sputter coated with gold. The samples are examined using Hitachi Su 6600 Scanning Electron Microscope and micrographs are taken. Terminology for seed morphology follows Radford *et al*. (1974).

RESULTS

Systematic treatment: *Enicostema axillare* subsp. *axillare* (Lam.) (Raynal, 1969), Matthew, Fl. Tamilnadu Carnatic 973. (Matthew, 1982), Pull., Ali Moulali, Fl. Andhra Pradesh 2: 606. 1997. *Gentiana axillaris* Lam., Illustr. Gen. 1: 487. 1793. Type: India, Pondicherry, Sonnerat s.n. (P!).

Exacum hyssopifolium Willd., Sp. Pl. 1: 640. 1798; Chandrab. and N.C. Nair, Fl. Coimbatore 183. 1987.

***Enicostema littorale* auct.:** C.B. Clarke in Hook. f., Fl. Brit. India 4: 101. 1883; Nairne,Fl. Plants. W. India 191. 1894; T. Cooke, Fl. Pres. Bombay 2: 255. (1908; Rama Rao, Fl. Pl. Travancore 270. (1914; Gamble, Fl. Pres. Madras 2: 875. 1923, non Blume 1826.

Erect or ascending, unbranched-profusely branched herbs, 3-25 cm tall. Roots 2-6 cm long, reddish-white. Stem terete or 4-angled; internode 0.5-1.2 cm long. Leaves simple, opposite, sessile-sub sessile; lamina narrowly linear-oblong or linear lanceolate or elliptic-oblong, 2-5×0.5-2 cm, pale-dark green with 3 distinct basal nerves. Flowers sessile, 5-10 at every node. Bracts 2, 0.05×0.025 cm, green. Sepals 5 or rarely 6, 0.2-0.4×0.1 cm, green, persistent, margins hyaline, basally fused; lobes ovate-lanceolate, apex acute-acuminate. Petals 5 or rarely 6, white; tube 0.5×0.15 cm; lobes 0.2 cm long, ovate, acute at apex. Stamens 5 or rarely 6; staminal tube formed of narrow white nets fused to the corolla; anthers 0.2 cm long, yellow, erect, shortly apiculate, filaments short, 0.15 cm long, white, furnished with a double hooded scale at the insertion of each anther. Ovary oblong, 0.3 cm long; style 0.2 cm long; stigma capitate. Capsule obovoid; 0.3-0.4×0.3 cm, dehiscence longitudinal. Seeds many in a capsule, subglobose, 0.5 mm diam., testa brown, pitted reticulate (Fig. 1, 2).

Phenology: September-December.

Ecology: In grasslands and sea coasts.

Specimens examined: Andhra Pradesh: Anantapur district, Anantapur, 19/07/2011, Sheba M. Joseph and Santhosh Nampy 4335 (DEV). Kurnool district, Omkaram, 14/02/1999, S. Sumitha and A. Madhu 21345 (SKU). Nellore district, Nellore, 02/1885, J.S. Gamble 20411 (BSI). Karnataka: Adilabad district, Pangidilodhi, 20/09/1985, P. Ravishankar 83127 (MH). Kerala: Kollam district, Kollam, 25/01/1980, N. Valsalakumar 102 (SNC). Thiruvananthapuram district, Thiruvananthapuram, 29/12/1968, G. Balasubramaniam 203 (CALI). Thrissur district, Keralavarma College Campus, 17/11/2011, Shahina Shanavas and Santhosh Nampy 4922 (DEV). Madhya Pradesh: Rewa district, Rewa, 03/08/1986, R. Prasad 38103 (CAL). Maharashtra: Amravati district, Amravati university Campus, 28.10.2012, Shahina P.M. and Asharaf P.M. 4938; Ibid., 29.10.2012, Pramod and Prabhukumar 4941 (DEV). Mumbai district, Bandra, 05/1919, B.N. Vakil 35325 (BLAT). Pune district, Pimpri, 03/09/2010, Shahina Shanavas and Santhosh Nampy

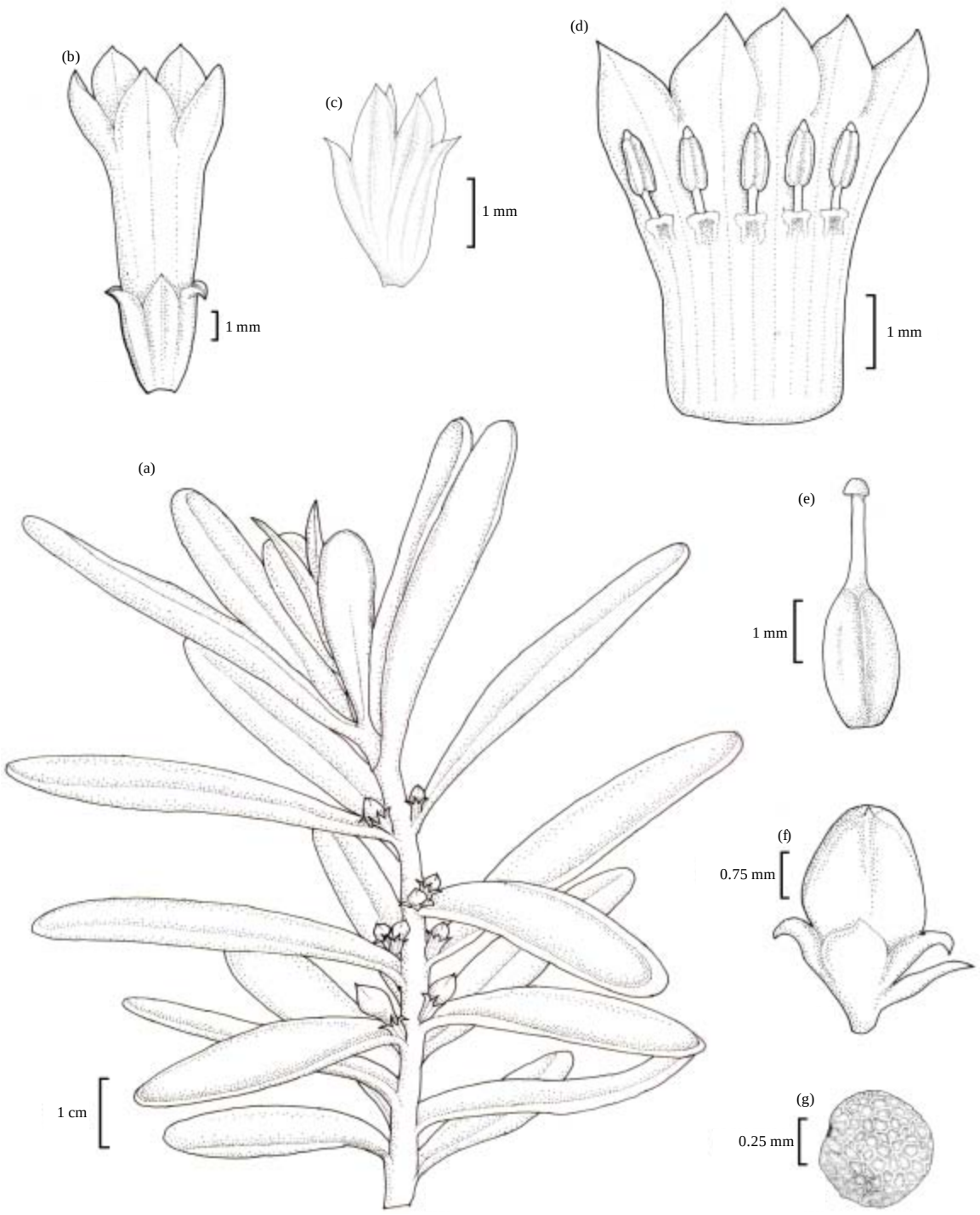

Fig. 1(a-g): Illustration of *E. axillare* subsp. *axillare* (Lam.), (a) Raynal: A. Habit, (b) Single flower, (c) Calyx, (d) Corolla opened, (e) Pistil, (f) Fruit and (g) Seed (from Shahina P.M. and Santhosh Nampy 3335)

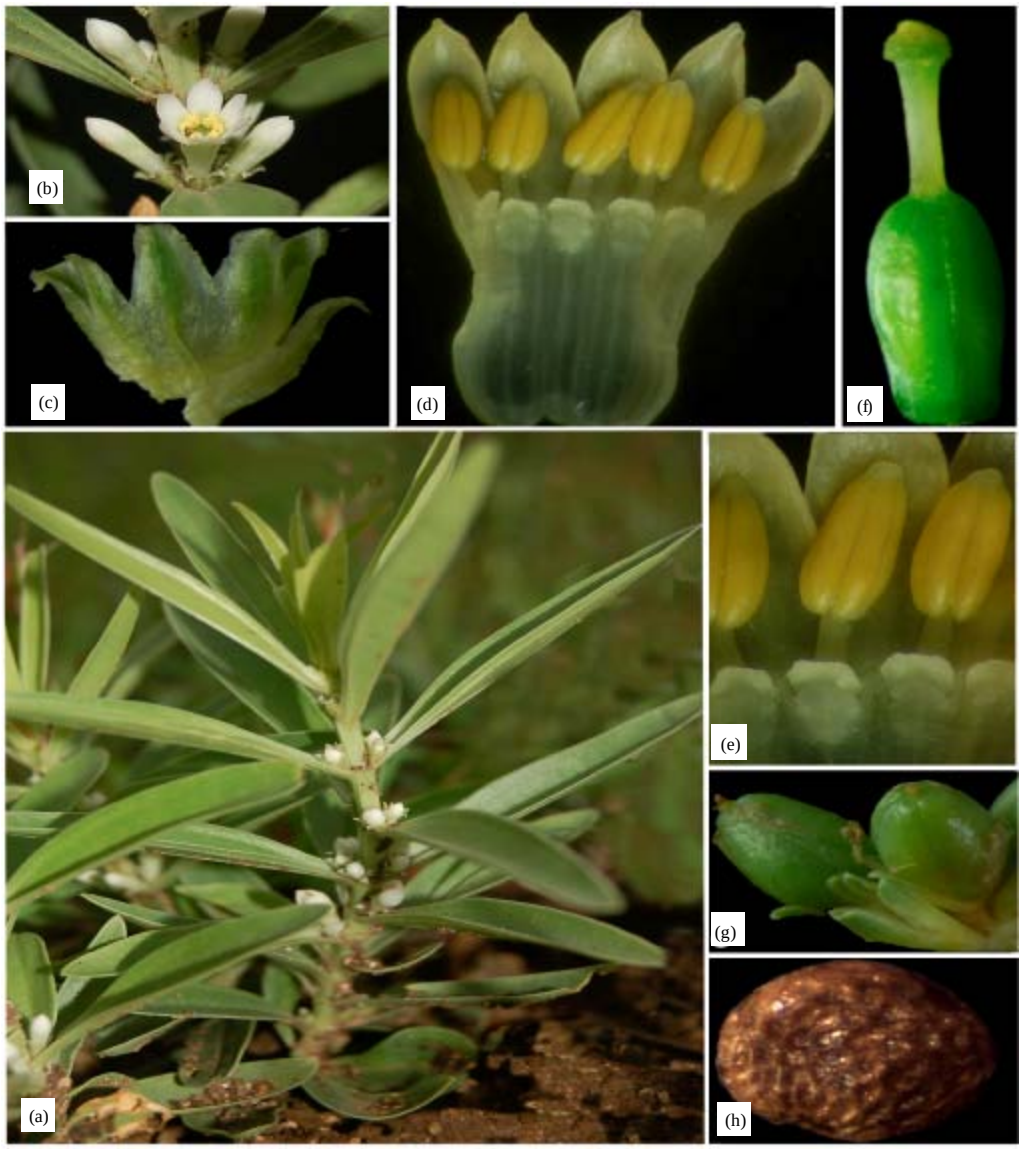

Fig. 2(a-h): Morphological characters of *E. axillare* subsp. *axillare* (Lam.), (a) Raynal: A. Habit, (b) Axillary cyme showing opened flower, (c) Calyx, (d) Corolla opened, (e) Stamen, (f) Pistil, (g) Fruit and (h) Seed (from Shahina P.M. and Santhosh Nampy 3335)

3335 (DEV). Thane district, Dharampur, 13/06/1981, P.S. Tour 39012 (BSI); Ghandgaon, 08/1985, Rajendra Shinde 476 (BLAT). Tamil Nadu: Chennai district, 1921, J.S. Gamble 87 (MH); Layola College Campus, 07/1956, J. Pallithanam 1976 (RHT); Madras Christian College Campus, 06/10/2010, Manudev and Santhosh Nampy 3962 (DEV). Erode district, Bhavanisagar, 03/10/1987, N. Venkatasubramamian 728 (FRC). Thirunelveli district, Thavalai, 22.06.1984, T.K. Abraham, P. Mohan Kumar and P.J. Mathew 291 (TBGT). Uttar Pradesh: Etawah district, Etawah, 23/01/1924, P.C. Kanjilal 37407 (DD). Mahoba district, Mahoba, 19/07/1962, C.L. Malhotra 21459 (BSD).

Palynology: Pollen grains radially symmetrical, isopolar, sub oblate to prolate, spheroidal, circular-oval in lateral view, circular and tri lobate in polar view, 17.5-19.6×16.5-19.2 µm, tricolporate, colpi meridional. Exine striate; striae with numerous minute perforations. Orbicules absent (Fig. 3a, b).

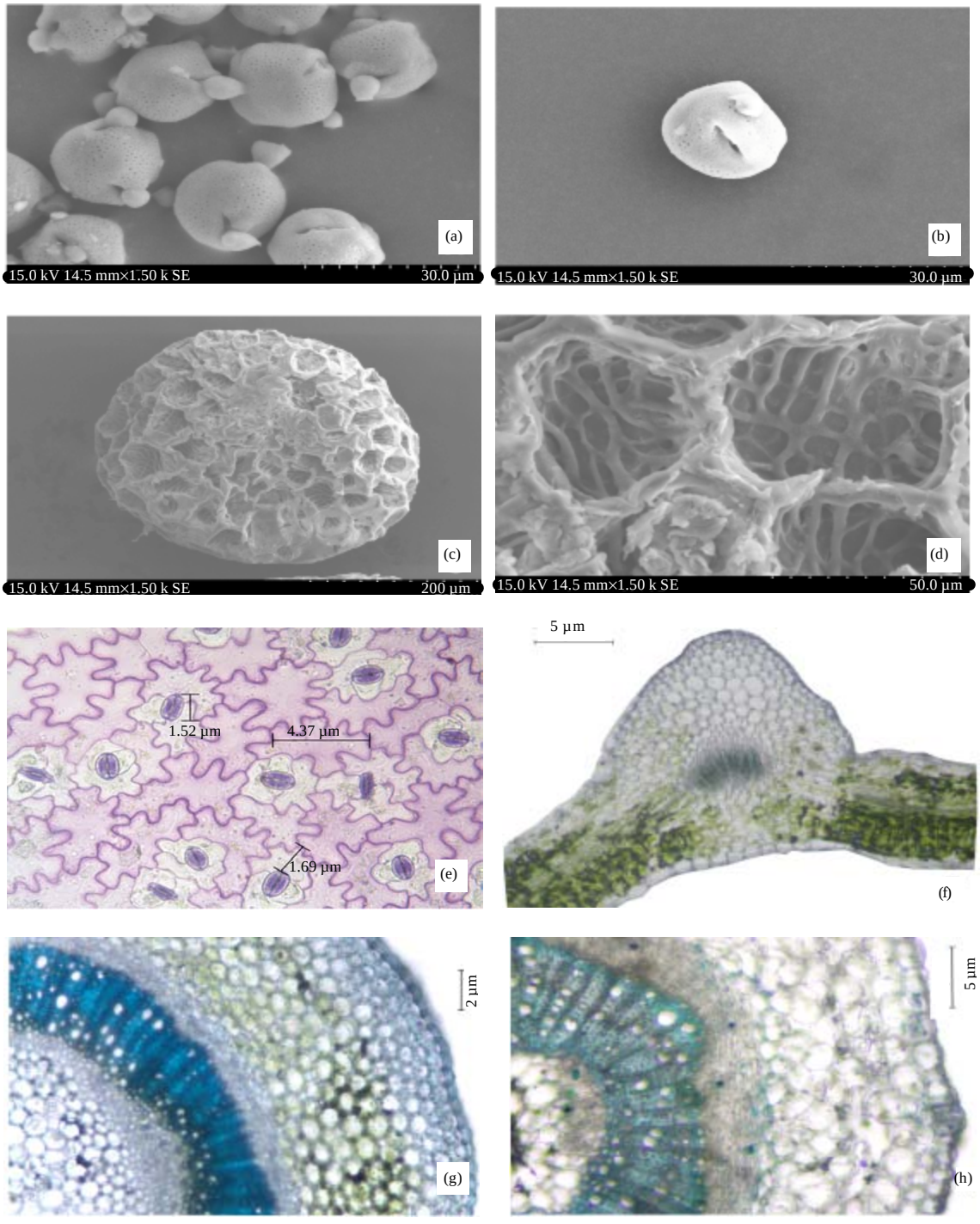

Fig. 3(a-h): Anatomical details of *E. axillare* subsp. *axillare* (Lam.) (a) Raynal: A. Pollen grains, (b) Pollen grains-equitorial view, (c) Seed, (d) Seed-testa ornamentation, (e) Stomata on abaxial epidermis, (f) T.S. of leaf, (g) T.S. of stem and (h) T.S. of root

Seed micromorphology: Seeds subglobose, 39-443 μm diam.; testa brown, irregularly oriented, pitted reticulate; granular exudates present on cuticle that are fused near the anticlinal boundaries (Fig. 3a-c).

Anatomy

Leaf: Epidermis 1-layered. Stomata more on the abaxial surface, anisocytic. Subsidiary cells 3. Hypodermis chlorenchymatous. Mesophyll without mucilage cells. Vascular bundles conjoint, collateral and open. Bundle sheath extension chlorenchymatous. Minor leaf veins with phloem transfer cells present (Fig. 3e-f).

Stem: Stem terete or quadrangular. Epidermis 1-layered, cells round, parenchyamtous, without intercellular spaces, covered with cuticle. Hypodermis collenchymatous, 2-layered. Cortex, parenchymatous, chlorophyllous, subepidermal sclerieds present. Phloem both external and internal (included phloem). There is an initial secondary growth with the addition of secondary xylem towards the inner side. Vascular cylinder consists of secondary xylem, secondary phloem, vascular cambium and pith (Fig. 3g).

Root: Epidermis 1-layered, cells irregularly shaped. Outer cortex broadly parenchymatous. Fibres many layered, arranged between the cortical parenchyma and the secondary phloem. Root hairs absent (Fig. 3h).

DISCUSSION

E. axillare subsp. *axillare* shows variation in its vegetative features influenced by habitat and soil. The plants growing in open grass land with limy soil (Anantapur-Andhra Pradesh) are very small (c. 3.5 cm tall), unbranched and erect with terete stem, pale green, sessile, linear-oblong leaves and very short (0.1-0.2 cm) internodes while that from sandy soil of sea coasts (Chennai-Tamil Nadu) are large (10-25 cm tall) highly branched and straggling with quadrangular stem, dark green, petiolate, linear lanceolate leaves and long (1-3 cm) internodes. Plants from the lateritic soil (Pimpri-Maharashtra) are characterized by its moderate size (5-10 cm) tall, 1-2 branched and erect with quadrangular stem, pale green, sub sessile elliptic-oblong leaves and short (0.5-1.5 cm) internodes. This corroborates Raynal (1969) observation that plants of *Enicostema* are endowed with great power variation within the population.

Anatomical peculiarities include anisocytic stomata (Fig. 3e) and minor leaf veins with phloem transfer cells in the leaf and included phloem in the stem (Fig. 3g). Included phloem is a major distinguishing feature between Gentianaoideae and Menyanthoideae and the position of *Enicostema* in the former is justifiable.

The taxonomy and nomenclature of *Enicostema* is very complex and was summerized by Veldkamp (1968) and Raynal (1969). Hooker (1885) treated the genus in the tribe Chironiae along with other genera like *Erythraea*, *Hoppea* and *Canscora*. Struwe *et al*. (2002) placed it under the subtribe Faroinae in tribe Potalieae along with Faroa, Neurotheca and Urogentias.

Vinckier and Smets (2003) studied the morphological and ultrastructural diversity of Orbicules in 53 species of Gentianaceae. They analyzed *E. verticillatum* within *Enicostema* and found no orbicules. Our study in *E. axillare* subsp. *axillare* also corroborates (Vinckier and Smets, 2003) in that no orbicules are found in *Enicostema* and its inclusion in the subtribe Faroinae is thus justified (Fig. 3a, b).

Seed micromorphology of *E. axillare* subsp. *axillare* shows reticulate testa (Fig. 3c-d). Testa cells are irregularly oriented. Anticlinal walls curved to form pits. The inner wall is further reticulate. According to Struwe *et al*. (2002) crystallized structures are found upon and under the exotestal cuticle of several genera of the Chironieae (*Chironia*, *Coutoubea*, *Orphium*) and Faroinae (*Neurotheca*). Our studies on the seed micromorphology, also clearly evidenced the absence of crystallized structures in *E. axillare* subsp. *axillare* but the cuticle has granular exudates that are fused near the anticlinal boundaries.

CONCLUSION

The present study concludes that *Enicostema axillare* subsp. *axillare* alone occurs in Peninsular India and exhibits considerable variation in the habit. The leaf possess anisocytic stomata, minor leaf veins and phloem transfer cells. In the current study no orbicules were reported for the Indian taxa.

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