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SYSTEMATIC STUDIES (MICRO-MORPHOLOGICAL, LEAF ARCHITECTURAL, ANATOMICAL AND PALYNOLOGICAL) OF GENUS *PHYSALIS* L. (SOLANACEAE) IN NORTHEAST INDIA

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Abstract

The present paper discusses the observations on morphology, anatomy, leaf architecture and palynological characteristics of *Physalis angulata* L. and *Physalis peruviana* L. in determining their taxonomic significance in species delimitation and classification. It was found that the *Physalis peruviana* L. is densely pubescent with large corolla and fruiting calyx as compared to *Physalis angulata* L. Both the species are amphistomatic and have anomocytic stomata with single-layered epidermis and bi-collateral vascular bundle. The other epidermal characters such as stomatal index (S.I), frequency (S.F), epidermal and stomatal cell measurements, etc. have also been described in detail. The two species are also varied by showing contrasting characters in anatomical and venation patterns. Several common pollen features *i.e.*, monads, radially symmetrical, eurypalynous, medium-sized (25.1–50 µm), 3-zonocolporate apertures with microechinate ornamentation were observed in both the species. These characters have taxonomic importance in distinguishing the genus *Physalis* L. at generic and species rank of the family.

Key words: Anatomy, Leaf architecture, Micro-morphology, Palynology, *Physalis*, Solanaceae.

Introduction

Physalis L., an American genus belonging to the family Solanaceae, consists of about 90 to 120 species inhabiting the tropical and temperate regions of the New World with centre of diversity in Mexico, the United States and Central America (Hunziker, 2001; Maggie 2005; Feng *et al.,* 2016). Several species of the genus have been widely introduced into cultivation in various parts of the world (Silva and Agra, 2005). About six species of the genus have been reported from India, of which neither are of Indian origin (Deb, 1979; Ganapathi *et al.,* 1991), but from the reported species, *P. minima* L. and *P. peruviana* L. are found growing naturally throughout the country (Hooker, 1885). The fruit of *Physalis* has a potential agricultural alternative with better projections for commercialization due to the high nutritional contents and the possibility to cultivate as an organic crop. It has immense economic significance in respect of its valuable chemical compounds (Tropane and Physalins) like the other species of the family *viz. Solanum, Withania, Atropa, Hyoscymus*. Numerous medicinal properties such as anti-bacterial, anti-inflammatory and anti-cancer have been reported from the genus (Hong *et al.,* 2015; Ji *et al.,* 2012). In the past, the species of the genus were poorly defined because of incorrect taxonomic description and nomenclature and of the strong morphological resemblance with other species (Menzel, 1951). The main character to distinguish the different species within the genus are inflated fruiting calyx, corolla colour, the hairiness of the leaves and the length and colour of the anthers (Menzel, 1951; Axelius, 1996). Anatomical, leaf epidermal and architecture features have immense taxonomic importance and implications in differentiating the taxa up to the species level and are considered as a significant source for the elucidation of phylogenetic relationships (Inamdar and Patel, 1969; Li and Tores, 1997; Thepsithar and Thongpukdee, 2013). The pollen morphology of 20 species of the family Solanaceae was reported by Perveen and Qaiser (2007) which have helped significantly in the identification of taxonomically related genera and species. Therefore the present paper attempts to assess the micromorphological, anatomical, leaf

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architectural and palynological studies for species identification, classification and also in establishing the taxonomic relationships between the species of *Physalis*.

Materials and Methods

A. Sample collection and herbarium specimen preparation

Fresh materials were collected and voucher specimens were prepared as standard plant taxonomy procedures (Jain and Rao, 1977). Identification was done by consulting relevant published literature (Kanjilal *et al.,* 1939; Hooker, 1885) and comparing with herbarium specimens, housed at ASSAM herbaria. The specimens have been deposited at herbaria of Botany Department of North Eastern Hill University, Shillong, Meghalaya.

B. Morphological and Leaf Architecture Investigation

Morphometric studies were carried out on freshly collected specimens of two *Physalis* species following the procedure of Mohanram and Nayyar (1977). Qualitative and quantitative characters of both the species were obtained with the aid of Labomed C × l Microscope and photographed with Canon camera (Power Shot SX720 HS). The terminologies used for describing the leaf architecture are that of Leaf Architecture Working Group (1999). The structure of the adaxial and abaxial epidermal cells *i.e.*, type, location and distribution of stomata; stomatal length and width were examined on semi-permanent slides. Stomatal index (SI) was calculated using the following equation (Salisbury, 1927).

$$Stomatal\,Index(SI)=\frac{S}{E+S}\times 100$$

Where S = Stomatal number per unit leaf area

E = Epidermal cell number in the same leaf area

Stomatal frequency was calculated by applying the formula as described by Ghosh and Davis (1973).

Stomatal frequency (SF) = Number of stomata per unit area.

The terminologies of Metcalfe and Chalk (1950) and Van Cotthem (1970) have been followed to describe the stomata.

C. Anatomical and Palynological Investigation

The anatomical details were studied by fixing the plant parts in FAA (formalin, acetic acid and ethyl alcohol, 10:5:85 v/v), followed by dehydration in the 85%, 95%, 100% ethanol and xylene, and then embedded in paraffin. The transverse section of petiole and stem of the species

Table 1: Morphological comparison between *P. angulata*, *P. minima* and *P. peruviana.*

Characters	*P. angulata* L.	*P. minima* L.	*P. peruviana* L.
Habit	Annual, herb, glabrescent, 40-60 cm tall	Annual, herb, pubescent with long many-celled hairs, 15- 45 cm tall	Annual-perennial, herb-shrub, densely pubescent, 50-100 cm tall
Leaf	Leaf blade ovate-lanceolate, 4-5.5×1.5-2.5 cm, glabrescent, base oblique, wavy margins, apex acuminate	Leaf blade ovate or ovate-lanceolate, 2-3 × 1-1.5 cm, pubescent along veins, base cuneate, apex acuminate	Leaf blade cordate, 5-15×3-8 cm, pubescent, base cordate, apex acuminate
Inflorescence	Flowers solitary & axillary, nodding	Flowers solitary & axillary	Flowers solitary & axillary, nodding
Pedicel	4-8 mm long	ca. 5 mm long	0.8-1.5 cm long
Calyx	Campanulate, 5-6.5 mm long, bell-shaped, glabrous	Campanulate, 2.5-3 mm long, pubescent; lobes deltate, densely ciliate	Broadly campanulate, 4-8 mm long, pubescent
Corolla	Yellow, bell/star-shaped, five dark brown spots near throat, glabrescent, 1.5-2×2-2.5 mm.	Corolla yellow, shortly tubular, ca. 5 mm., pubescent	Yellow, bell/star-shaped, five dark brown spots near throat, densely pubescent at base, 1.2 - 1.5 ×1.2-2 cm.
Stamens	Anther light blue, ca. 2.3 mm long, filament 3-3.5 mm long	Anther light yellow, 1-1.5 mm long, filament ca. 2 mm long	Anther dark blue, ca. 3 mm long, filament ca. 4 mm long
Fruit	Berry, fleshy, yellow, ca. 1.2 cm in diam.	Berry, fleshy, orange, ca. 6 mm in diam.	Berry, fleshy, yellow, 1-2 cm in diam.
Fruiting calyx	Glabrous, reticulate	Pubescent, densely ciliated lobes	Pubescent, reticulate
Seed	Yellow, 100-200 seeds per pulp, kidney-shaped, ca. 2 mm long, minutely reticulate	Brown, 100-200 seeds per pulp, subreniform, ca. 2 mm long, minutely reticulate-undulate	Yellow, 100-300 per pulp, discoid, ca. 2 mm long, minutely reticulate

Table 2a: Qualitative foliar architectural data of two *Physalis* L. species

Features	*P. angulata* L.	*P. peruviana* L.
Leaf attachment & Organization	Alternate, simple, lanceolate, variable	Alternate, simple, cordate, variable
Petiole outline	Reniform, base swollen	Reniform, base swollen
Blade class	Nanophyll, microphyll	Microphyll, notophyll
Laminar shape	Elliptic, ovate, oblong	Broad ovate
Laminar symmetry	Asymmetrical	Symmetrical
Apex angle	Acute	Acute
Apex shape	Acute, long-acuminate	Short acute
Base angle	Acute, obtuse	Obtuse, wide obtuse
Base shape	Convex	Lobate
Position of petiolar attachment	Marginal	Marginal
Margin type	Entire with few indistinct teeth	Entire with few indistinct teeth
Primary vein (1°) size	Moderate	Moderate
Primary vein (1°) category	Pinnate	Suprabasal
Secondary vein (2°) size	Moderate to weak	Moderate to weak
Secondary vein (2°) category	Semicraspedodromus	Semicraspedodromus
Angle between 1° & 2° veins	Acute	Acute
Tertiary vein (3°) category	Alternate percurrent	Alternate percurrent
Tertiary vein (3°) course	Sinuous	Sinuous
Quaternary vein (4°) category	Regular polygonal reticulate	Regular polygonal reticulate
Pentanary vein (5°) category	Dichotomizing	-
Areolation	Moderately developed	Moderately developed
Freely ending ultimate veins (F.E.V.S.)	Unbranched	2 or more branched

Table 2b: Quantitative foliar architectural data of two *Physalis* L. species

Features	*P. angulata* L.	*P. peruviana* L.
Petiole length (cm)	1-4	4-8
Lamina size range (mm^2)	238-1223	876-3675
No. of lateral veins/side	5-7	5-7
Highest vein order	5	4
Primary vein (1°) length (mm)	78	103
Secondary vein (2°) number (mm)	10	14
Secondary vein (2°) length (mm)	28	42
Tertiary vein (3°) length (mm)	12	14
Quaternary vein (4°) length (mm)	8	9
Pentanary vein (5°) length (mm)	1.5	-

were taken with a rotary microtome, stained with toluidine blue O, mounted in DPX (dibutyl phthalate xylene) (O'Brien *et al.,* 1964) and observed under Labomed Cxl Microscope.

For the palynological investigation, the pollen grains were acetolyzed according to the protocol of Erdtman (1960) with a slight modification by Nair (1970) and examined under the light microscope. Measurements of polar and equatorial axes were taken for each taxon with the help of the ocular micrometre inserted in the eyepiece of the microscope. Pollen grains for scanning electron microscopy were mounted on a stub with double-sided tape, coated with gold in a polaron JEC-1100E coating unit, and then photographed with HITACHI S-3000H SEM. The applied terminology was based on Erdtmann (1952) and Punt *et al.* (2007).

Results

Morphology

On the basis of field investigations, three species of *Physalis* L. were listed and compared (table 1). They share a number of morphological characteristics with each other such as campanulate calyx, yellow and bell-shaped corolla, fruit berry, as well as similar habitat and phenology. However, *P. peruviana* is readily distinguished from the other species due to its cordate leaf blade, large corolla and fruiting calyx which are densely tomentose (fig. 1). In addition, the calyx of *P. angulata* is glabrous as compared to *P. minima* L. which has pubescent and densely ciliated calyx lobes.

Foliar Architecture

Altogether a total of 32 leaf architectural features have been obtained and presented in the table along with the microphotographs (table 2 (a & b); fig. 2). This study ascertains various features like leaf organization; laminar size, shape and symmetry; blade class; apex angle and shape; base angle and shape; margin type; vein size, category, number and areolation. Striking differences were observed in leaf

Table 3a: Quantitative foliar micro-morphological data of two *Physalis* L. species.

Features	*P. angulata* L.		*P. peruviana* L.	
	Adaxial surface	Abaxial surface	Adaxial surface	Abaxial surface
EC cell length (µm)	59.54	63.22	67.92	82.32
EC cell breadth (µm)	44.29	43.52	51.64	54.87
No. of EC per mm^2	210.32	192.74	160.32	152.12
No. of St. per mm^2	55.43	72.51	29.68	43.33
St. length (µm)	32.57	28.64	32.69	26.44
St. width (µm)	19.82	18.33	16.92	18.05
St. index (SI)	6.35	7.87	5.92	6.33
St. Frequency (SF)	37.45	62.96	23.52	29.31
St. pore length (µm)	9.32	8.54	10.33	8.92
St. pore breadth (µm)	3.15	2.72	2.38	2.41

Legend: EC= Epidermal cell; St.= Stomata

Table 3b: Qualitative foliar micro-morphological data of two *Physalis* L. *s*pecies.

Name of the species	Surface	Epidermal cell shape	Coastal Area	Stomata type	Guard cell shape
P. angulata L.	Adaxial	Sinuous, unequal, Irregular	Distinct	Anomocytic	Elliptic
	Abaxial				
P. peruviana L.	Adaxial	Sinuous, unequal, Irregular	Distinct	Anomocytic	Elliptic
	Abaxial				

systematic way.

Foliar micro-morphological characteristics

Leaves of both the species are amphistomatic, and have anomocytic stomata with single layer epidermis, 3-4 layer spongy mesophylls and have distinct coastal areas. However, in *P. peruviana* the length of epidermal cells and stomatal pore observed was slightly higher than *P. angulata* but the stomatal Index (S.I), frequency (S.F) and stomatal pore breadth was observed greater in *P. angulata* than *P. peruviana* (fig. 2). The epidermal cells of both the species possess irregular cell shape with strongly angular sinuous distributed on both the leaf surfaces (table 3a & b).

Petiole and stem Anatomy

The comparative anatomical features of two species of *Physalis* are summarised in table 4 and displayed in the fig. 3. The petiole of *P. angulata* is composed of a thin, single layer epidermis made up of rectangular, anisodiametric shaped cells. Epidermal layer is followed by cortex section with 3-4 unequal layers of collenchymatous cells and the rest is composed of 5-layered parenchymatous cells which cover the vascular tissue. The vascular bundle is U-shaped, bi-collateral, four to five discrete vascular traces form a crescentic median arc and two additional traces are observed in lateral wings. The epidermis of *P. peruviana* petiole is one cell layer, anisodiametric with multicellular trichomes. The bi-collateral, U-shaped, seven to eight distinct vascular traces are observed. The vascular tissue layers of both the species are surrounded by small clusters of sclerenchymatous fibres (3-5 layered cells), which perform supporting functions. Pith area of both the species is very narrow consisting of few layers

Table 4: Anatomical features of the petiole and stem of the two *Physalis* L. species.

Plant part	Features	*P. angulata* L.	*P. peruviana* L.
Petiole	Epidermis	Single cell layered, trichomes absent	Single layered with multi-cellular trichomes
	Parenchyma	5 layered	5 layered
	Collenchyma	3-4 unequal layered	3-4 layered
	Vascular bundles	Bi-collateral,4-5 vascular traces	Bi-collateral,7-8 vascular traces
	Pith area	Narrow	Narrow
Stem	Epidermis	Single cell layered, trichomes absent	Single layered with multi-cellular trichomes
	Parenchyma	3 layers	2-3 layered
	Collenchyma	2-5 layers	4 layered
	Vascular bundles	Bi-collateral, 4-8 vascular traces	Bi-collateral,12-15 vascular traces
	Pith area	Broad	Broad

Table 5: Pollen morphological characters of the examined species of *Physalis* L.

Characters	*P. angulata* L.	*P. peruviana* L.
Pollen type	3-zonocolporate	3(4)-zonocolporate
Polar axis 'P' (µm)	33.58 ± 0.41	23.74 ± 0.52
Equatorial axis 'E' (µm)	27.08 ± 0.44	25.12 ± 0.96
(P/E)	1.24	0.94
Pollen shape	Prolate-spheroidal	oblate-spheroidal
Colpus Length (µm)	12.65-14.15	10.75-13.56
Exine ornamentation	Microechinate	Microechinate

base shape, 1^0 vein category, 2^0 vein angles, highest vein order, 5^0 vein category and F.E.V.S. These differences can be used to distinguish the two species in a more

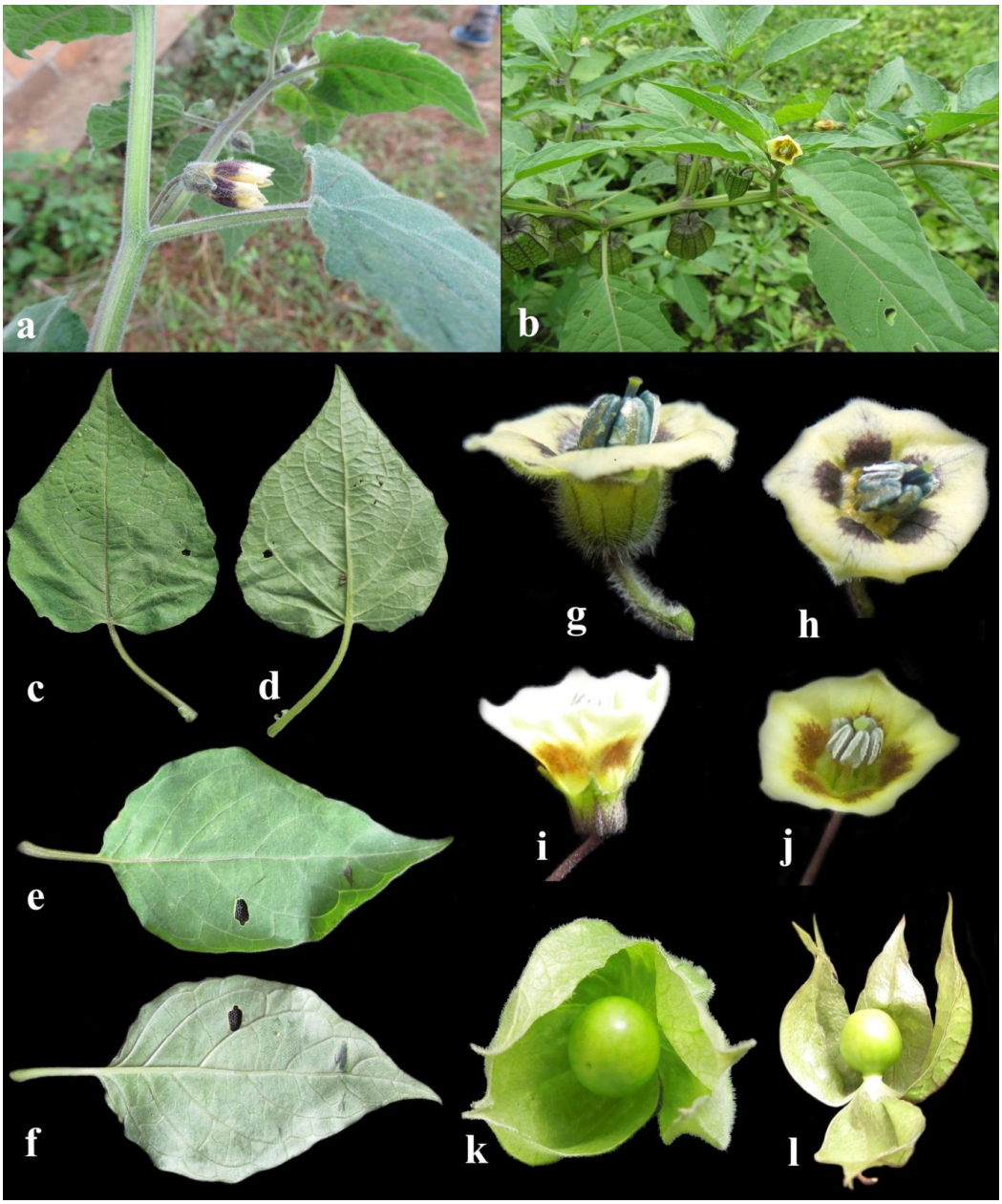

Fig. 1: *Physalis perviana*: **a.** habit, **c. d.** adxial & abaxial leaf, **g. h.** individual flower & **k.** fruiting calyx; *Physalis angulata*: **b.** habit, **e. f.** adaxial & abaxial leaf, **i. j.** individual flower & **l.** fruiting calyx.

of parenchymatous cells with intercellular space. The stem of *P. angulata* like the petiole is made of a single cell layered epidermis and the cells are rectangular or oval in shape. Multicellular trichomes are absent in the epidermis. The hypodermal region is composed of 2-5 layers of collenchymatous cells, followed by 3 layers of thin-walled parenchymatous cells. Endodermis is distinguishable and is made of a layer of barrel-shaped cells. The pericycle is located under the endodermis, composed of 3 to 4 cell-layers. Large irregular or rectangular cells of the bi-collateral vascular bundle are observed. The single-layered epidermis of *P. peruviana* is composed of numerous, simple, multicellular, glandular trichomes. The hypodermis is made of few layers of thick wall cells termed collenchymas and the rest of the general cortex is composed of large, thin-walled parenchymatous cells. The stem possesses two to three layers of sclerenchyma and single layer of parenchymatous cells surrounding 12 bi-collateral vascular bundles. The pith region of both the species is broad and is made of large polyhedral parenchymatic cells.

Pollen morphology

Both the species contributes some common pollen characters *i.e.*, monads, 3 (4)-zonocolporate apertures,

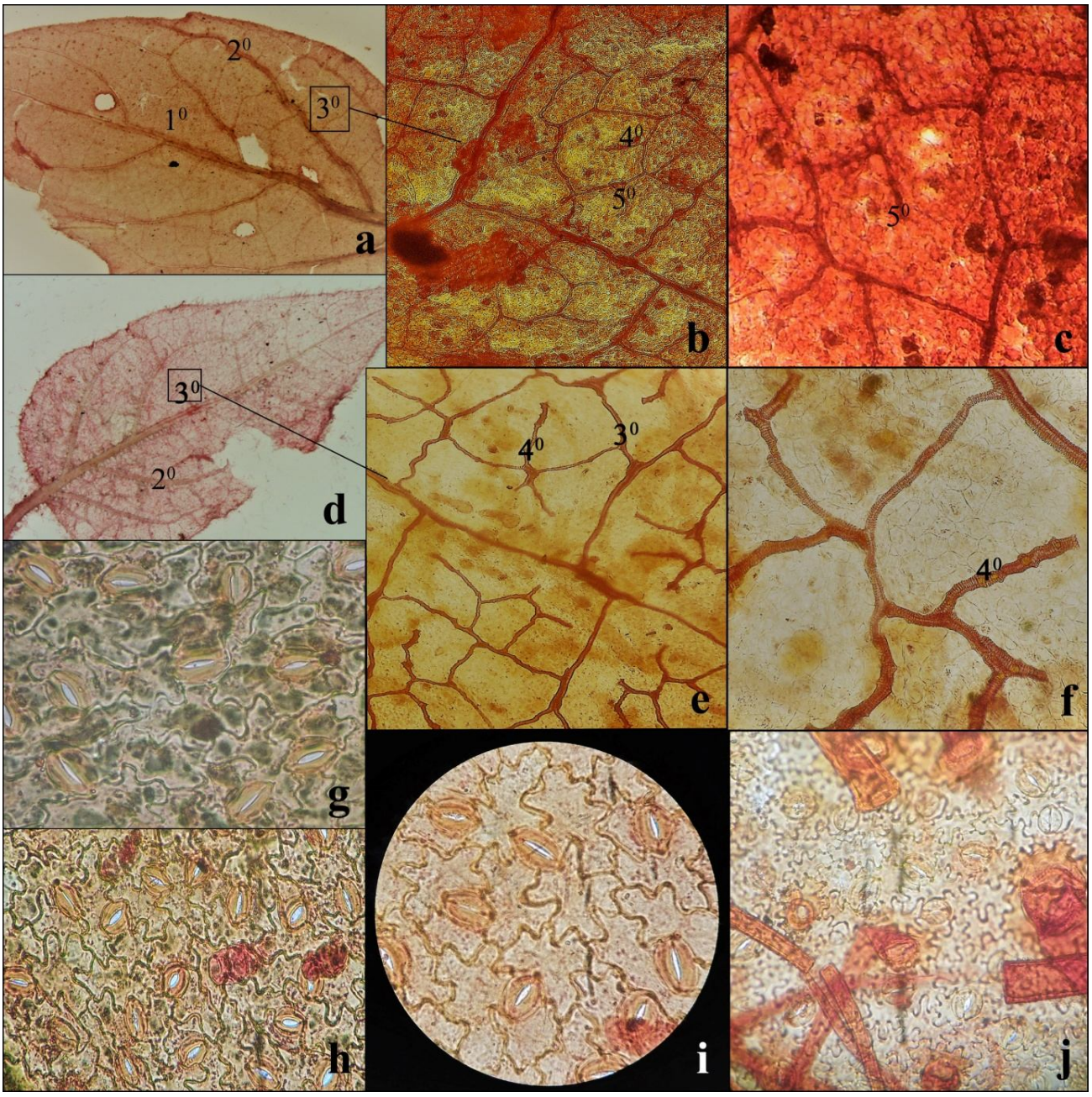

Fig. 2: Comparative leaf architecture of two *Physalis* species *P. angulata* (**a. - c**.), *P. peruviana* (**d. - f.**): (1^0, 2^0, 3^0, 4^0 & 5^0) = vein category; adaxial & abaxial leaf epidermis (**g., h.** = *P. angulata* & **i., j.** = *P. peruviana*).

radially symmetrical, microechinate, and according to Erdtman's (1952) pollen size classification, both were of medium size (25.1–50 µm). The smallest average length of the polar axis (P) was 23.74 ± 0.52µm for the pollen of *P. peruviana* and the largest (33.58 ± 0.41 µm) was found in *P. angulata*. The shortest mean equatorial diameter (E) was observed in the pollen of *P. peruviana* (25.12 ± 0.96 µm), while the longest was in *P. angulata* (27.08 ± 0.44µm). The average colpus Length (µm) was recorded 10.75-13.56 in *P. peruviana* and 12.65-14.15 in *P. angulata*. The species examined were most frequently Prolate-spheroidal and oblate-spheroidal (fig. 4). The P/E ratio was found 0.94 in *P. peruviana* and 1.24 in *P. angulata* (table 5).

Discussion

The studied *Physalis* L. species contribute a wide range of striking intra-specific morphological variations such as; *P. peruviana* is densely tomentose with cordate leaf blade as compared to the glabrous *P. angulata* and *P. minima* with pubescent and densely ciliated calyx lobes (Flora of China, 1994). The major venation pattern in

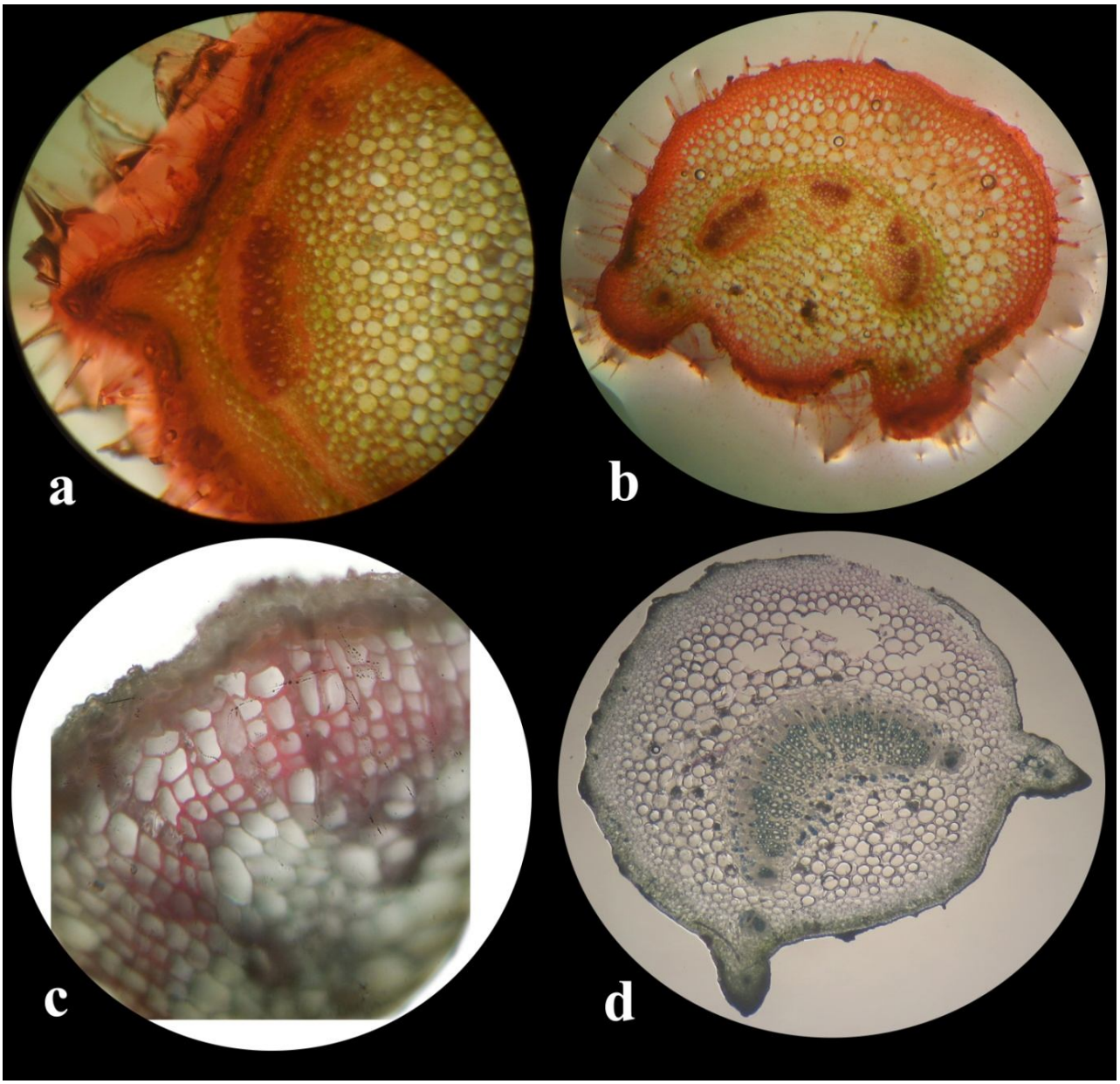

Fig. 3: Transverse section of stem and petiol of *Physalis.* A single epidermal layer with multi-cellular trichomes of *P. peruviana:* **a.** stem, **b.** petiole; **c.** enlarged view to show the stem layers & **d.** Outline of petiole in *P. angulata.*

both the species was pinnate Semi craspedodromus type, where the size of the primary vein was moderate and secondaries were moderate to weak. Areoles were moderately developed and revealed unbranched to branched free vein endings (Gupta, 1961; Verghese, 1969). The occurrence of more than one type of stomata on the same surface of leaves in the members of the family Solanaceae was reported by Metcalfe and Chalk (1950), Inamdar and Patel (1969) and Ahmad (1964d). In the present study, it was observed that leaves of both the species were amphistomatic and found anomocytic (Ranunculaceous) type of stomata. However, Zhang and Lu (1999) reported anomocytic stomata only on the abaxial surface in the leaves of *P. angulata*. Anatomical characters obtained from both the species are mostly similar; however, the epidermis of *P. peruviana* is densely covered with multicellular trichomes (Li and Tores, 1997). A bi-collateral vascular bundle is observed and is the characteristic feature for the family Solanaceae, formerly reported by Metcalfe and Chalk (1979). The secondary growth phase revealed vascular arc structure in the petiole, while the stem showed a complete ring structure of an open vascular system. The examined pollen grains were eurypalynous, trizonocolporate, prolate-spheroidal, oblate-spheroidal, medium-sized and microechinate which are quite similar to the findings as reported in the family

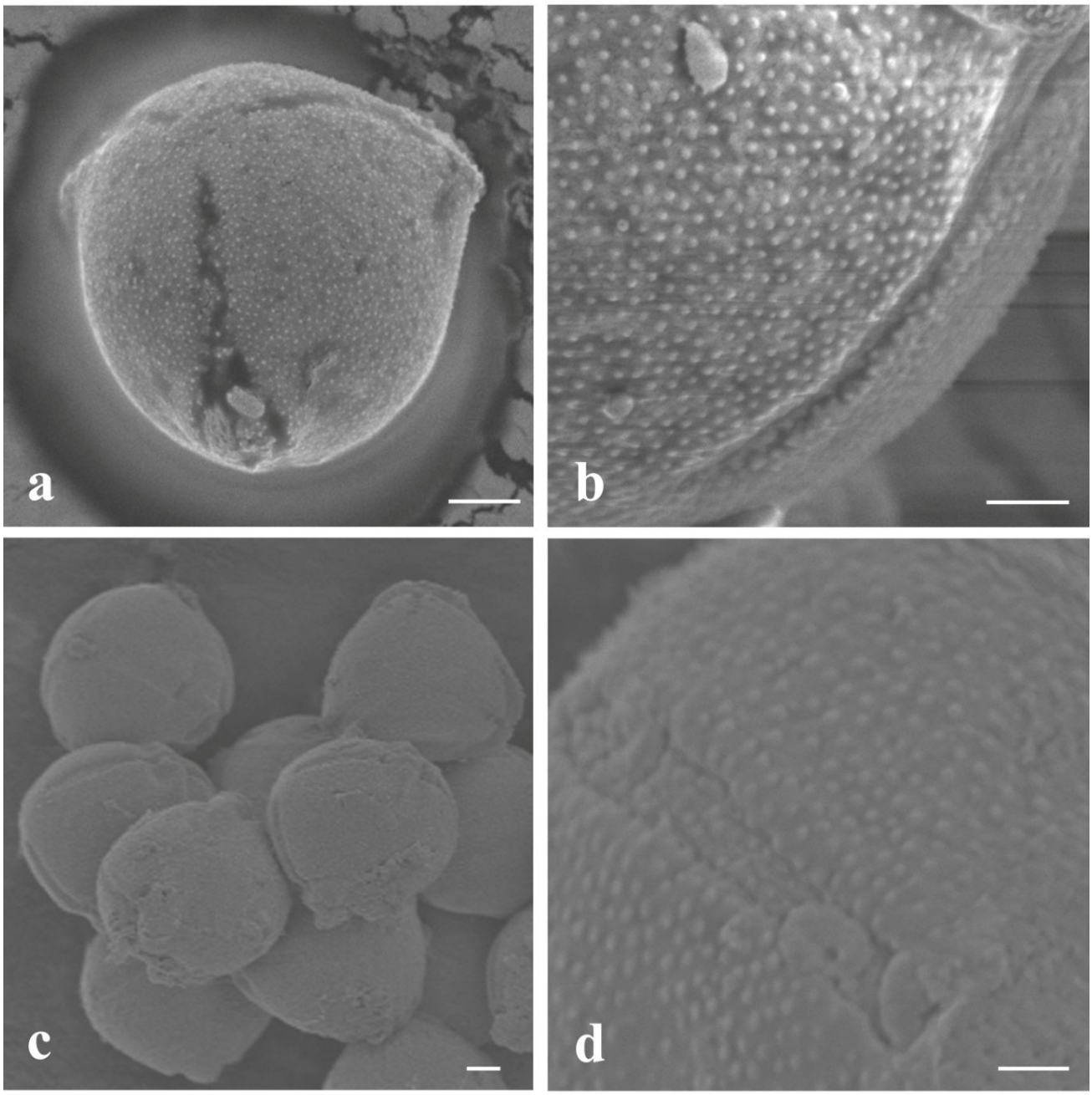

Fig. 4: Scanning electron micrograph showing the structure and exine sculpture of *Physalis* pollen grains: *Physalis angulata*: **a.** Polar view (scale bar = 5μm); **b.** exine structure (scale bar = 1μm); *Physalis peruviana*: **c.** polar view with compact granules (scale bar = 10μm); **d.** exine structure (scale bar = 1μm).

Solanaceae (Perveen and Qaiser, 2007; Kumar *et al.,* 2015). It was observed that light microscopy reveals size, shape and symmetry more clearly but with the help of SEM, it facilitates clearer picture of pollen wall surface which will be useful in identification of taxonomically related genera and species.

Conclusion

A number of traits (persistent calyx, bell-shaped flowers, poricidal anther dehiscence, amphistomatic and anomocytic type of stomata, bi-collateral vascular bundle etc.) of the members of the genus *Physalis* L. show a wide range of uniformity but the characters *viz.*, habit, 5^0 vein categories, distribution of stomata, stomatal index and frequency, eurypalynous pollen grains, etc. appears to be of taxonomically significant as they occur differently and constantly in diverse constant region from the studied species. The data obtained from the present study have apparently helpful for solving species delimitation problem and are useful for identification of morphologically closely

related species because these characters appear to be consistent and distinguishable between species. The study also helped in the correct identification of *P. angulata* which was earlier confused with *P. minima* as the former is a glabrous plant as compared to *P. minima* with pubescent and densely ciliated calyx lobes. Therefore the present study plays an important role in delimitation and identification of species of the genus *Physalis* L. of Solanaceae. The results obtained in the current study can also endorse revisionary studies of poorly known genera among all the families.

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