Modern Phytomorphology **7**: 47–53, 2015

LEAF EPICUTICULAR AND POLLEN ULTRASTRUCTURAL COMPARISONS OF *SONNERATIA APETALA* BUCH.-HAM. AND *S. CASEOLARIS* (L.) ENGLER (SONNERATIACEAEA)

Saikat Naskar

Abstract. *Sonneratia apetala* and *S. caseolaris* are easily distinguishable by leaf epicuticular and pollen ultrastructure. *S. apetala* shows fine, distinct, intermingled rodlets of wax crystalloids on leaf surface with sunken stomata. In the contrary *S. caseolaris* shows wide, indistinct, rodlets of wax crystalloids with exposed stomata. Epicuticular structures enlighten an important adaptation toward transpiration control according to their area of occupancy in saline habitat. Pollens of the both species show the phenomenon of harmomegathy. Harmomegathic effect is pronounced in *S. apetala*.

Key words: *Sonneratia apetala, Sonneratia caseolaris,* leaf epicuticular ultrastructure, pollen ultrastructure, harmomegathy

Post Graduate Department of Botany, Barasat Govt. College, Kolkata-700124, West Bengal, India; saikatnaskar@rediffmail.com

Introduction

The genus *Sonneratia* L.f. (Sonneratiaceae) is a major mangrove component and is confined to mangrove communities of Indo-Malayan region (Tomlinson 1986). Members of this genus are characterized by their solitary, vestigial or apetalous flowers with numerous stamens, along with conical pneumatophores up to 1.5 m height. Recent phylogentic studies treated the genus Sonneratia as a member of Lythraceae, in which *Trapa* is the sister of *Sonneratia* (Huang & Shi 2002; Graham *et al.* 1998, 2005). Tomlinson (1986) has described five distinct mangrove species of *Sonneratia*, namely *S. caseolaris* (L.) Engler, *S. alba* J. Smith, *S. apetala* Buch.-Ham., *S. griffithii* Kurz, and *S. ovata* Backer. The first four species are distributed widely in the mangrove habitats of Indian Sub-continent (Backer & van Steenis 1951). Naskar (2004) reported three species, namely *S. caseolaris*, *S. apetala* and *S. griffithii* from Indian Sundarban. *S. apetala* is a common species on the river facing intertidal mud flat, whereas *S. caseolaris* occurs in inner estuary, prefers less salinity and is inundated only with spring tide. It is also found growing in the junctions of urban sewage disposal cannels and saline rivers, and along the edges of saline water feed prawn cultivation fisheries (Fig. 2).

The interrelationship among the species of *Sonneratia* has not got adequate attention till date. Generally gross morphological descriptors are used to distinguish the species of *Sonneratia* (Tomlinson 1986). Data from other sources may strengthen the species discrimination and indicate their evolutionary trends.

Inter-specific variations are readily captured in Scanning electron microscopy (SEM). The SEM provides high-quality resolution and excellent depth of focus of surfaces and therefore it gives apparently three-dimensional images of surface features (Lane 1985). SEM is widely applied to demonstrate epidermal features (Barthlott 1981; Knight *et al.* 2004; Wang *et al.* 2005; Carpenter 2006) and external morphology of pollen (Halbritter & Hesse 2004; Sauquet & Cantrill 2007). Though pollen morphology of *S. caseolaris* and *S. alba* has been studied in details (Patel *et al.* 1984), still there has been inadequate records of leaf epicuticular ultrastructural analysis of *Sonneratia*. Keating (1984) described the guard cells of stomata of *Sonneratia*, which are partially enclosed by large epidermal cells. This histological observation was not sufficient to reveal the pattern of epicuticular wax crystalloid

Fig. 1. *Sonneratia apetala*: **A** – plant; **B** – flower; **C** – fruits.

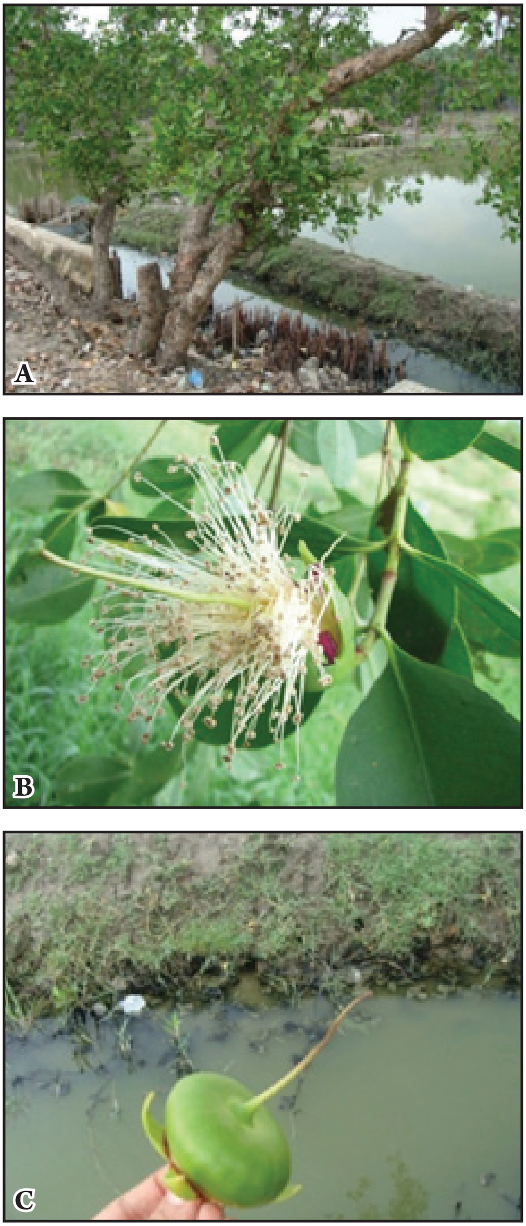

Fig. 2. *Sonneratia caseolaris*: **A** – plant; **B** – flower; **C** – fruit.

deposition on leaf and it is not obvious that all species of *Sonneratia* will show similar kind of epidermal structure.

Therefore, in the present study the leaf epicuticular surface and pollen surface ultrastructure of two mangrove species of Indian Sundarban under the genus *Sonneratia* has been examined through scanning electron microscope and a comparative account has been given. Pollens were also studied under light microscope.

Material and methods

Leaves and pollens of *S. apetala* Buch.-Ham. and *S. caseolaris* (L.) Engler (Figs. 1, 2) were

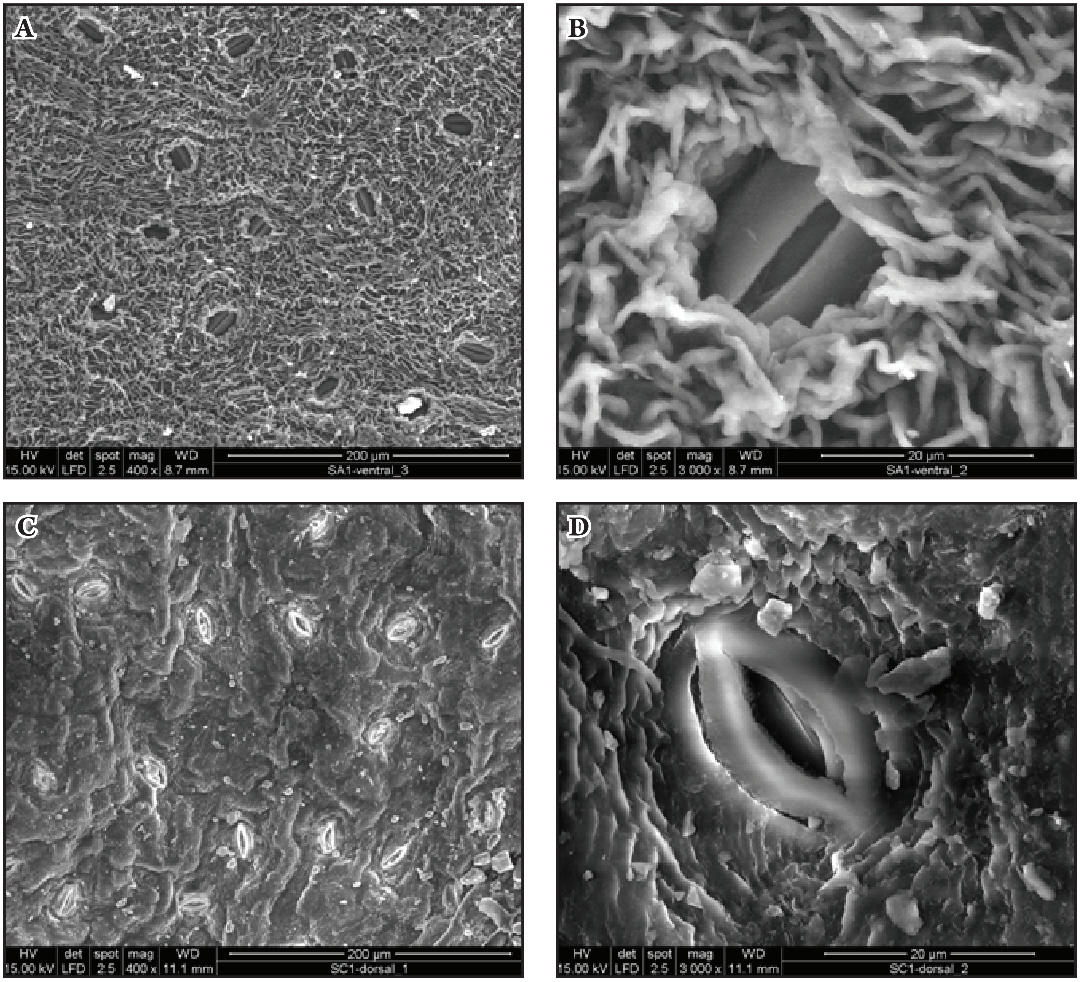

Fig. 3. SEM of leaf surface of *Sonneratia apetala* (**A, B**) and *S. caseolaris* (**C, D**). **B** and **D** show stomata (NASKAR 2014).

collected from Jharkhali (22.01° 88' 96" N, 88.68° 23' 63" E), located at north-west of Indian Sundarban.

Mature leaves were fixed in Gluteraldehyde-Heps buffer immediately after collection. Desirable portion was obtained from treated leaf and processed for dehydrated through ethanol series. Leaf portion was then mounted on a metal stub and a thin layer of gold was applied with automated sputter coater. Leaf surface morphology was captured under a SEM (FEI Company Make Quanta 200).

Pollens for light microscopic study were prepared following Erdtman's Acetolysis method. Measurements were taken with micrometers (Erma-Japan). Anthers fixed in 70% ethanol were crushed on a slide to get pollens which then transferred on metal stub, followed by Gold coating applied. Pollen morphology was studied under Scanning Electron Microscope.

Epicutcular descriptions and pollen terminology were made following BARTHLOTT *et al.* (1998) and HESSE *et al.* (2009) respectively.

Result and discussion

Sonneratia apetala **Buch.-Ham.**

Leaf surface (Fig. 3 A, B):

Cuticular surface covered by waxy terete or hooked rodlets type of crystalloids, rodlets

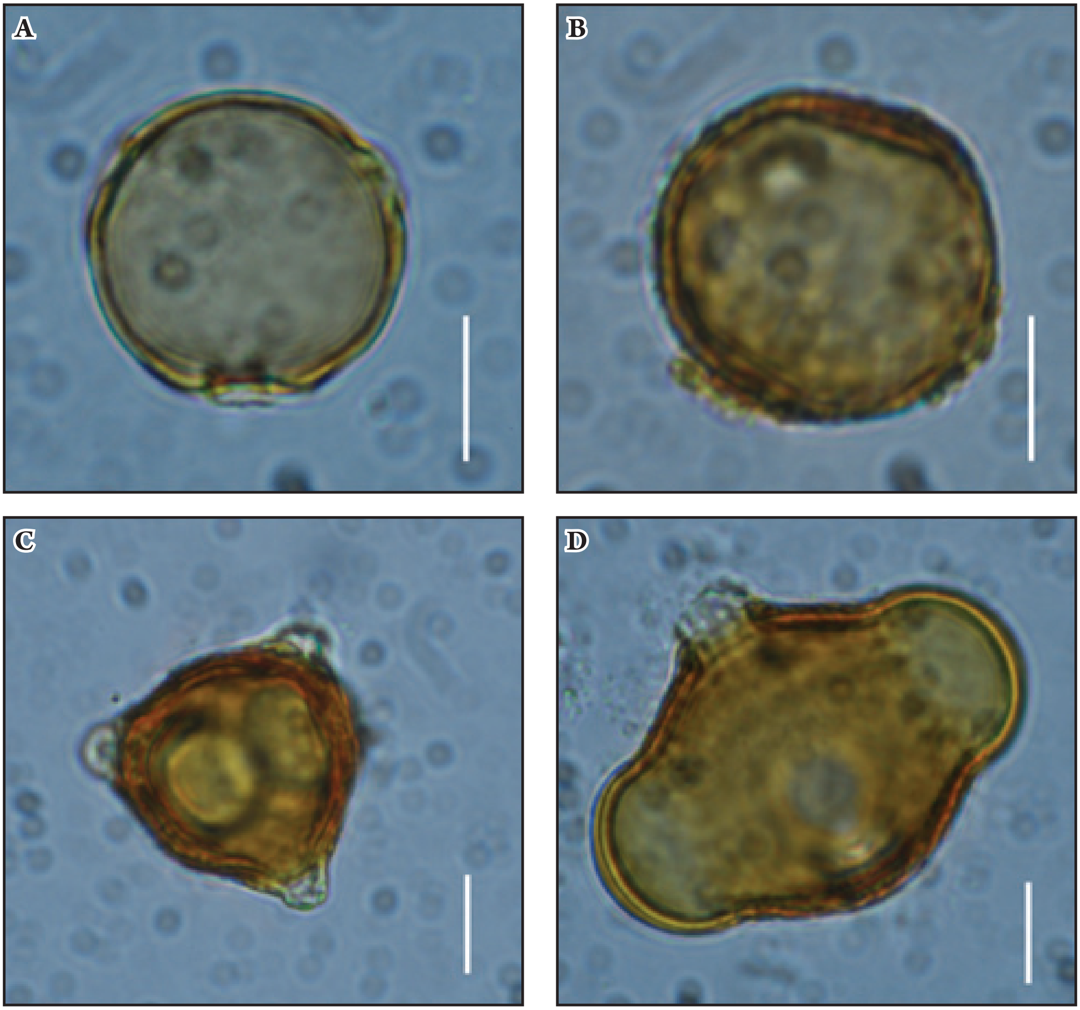

Fig. 4. Pollen grains in LM: *Sonneratia apetala* pollen in polar view (**A**) and in equatorial view (**B**); *S. caseolaris* pollen in polar view (**C**) and in equatorial view (**D**). Scales: 10 µm.

edges more or less entire with irregular shape. Stomata sunken and stomatal chimney may be present.

Pollen (Fig. 4 A, B; Fig. 5 A-C):

Shape: spheroidal; circular in polar view. Size: 27.75±0.69 µm diameter. Structure: exine tectate, ca. 2 µm, ektexine thicker than endecxine. Sculpture: areolate. Aperture: triporate, zonoaperturate, pore diameter ca. 3.4 µm, pores are not prominent. Peculiarities: small portion of polar regions psilate. Harmomegathy is observed in pollen grains. Different infoldings of wall are observed due to hermomegathic effect.

***Sonneratia caseolaris* (L.) Engler**

Leaf surface (Fig. 3C, D):

Cuticular surface covered by waxy irregularly arranged rodlets type of crystalloids, rodlets thicker and irregular in shape, irregular orientation form crust like appearance. Stomata exposed on waxy crystalloids and stomatal chimney absent.

Pollen (Fig. 4C, D; Fig. 5D-F):

Shape: prolate; triangular, obtuse and convex in polar view. Size: 37.75±1.46 µm (P), 27.25±1.08 µm (E). Structure: exine tectate, ca. 2.5 µm, ektecxine thicker than endexine. Sculpture: areolate. Aperture: triporate,

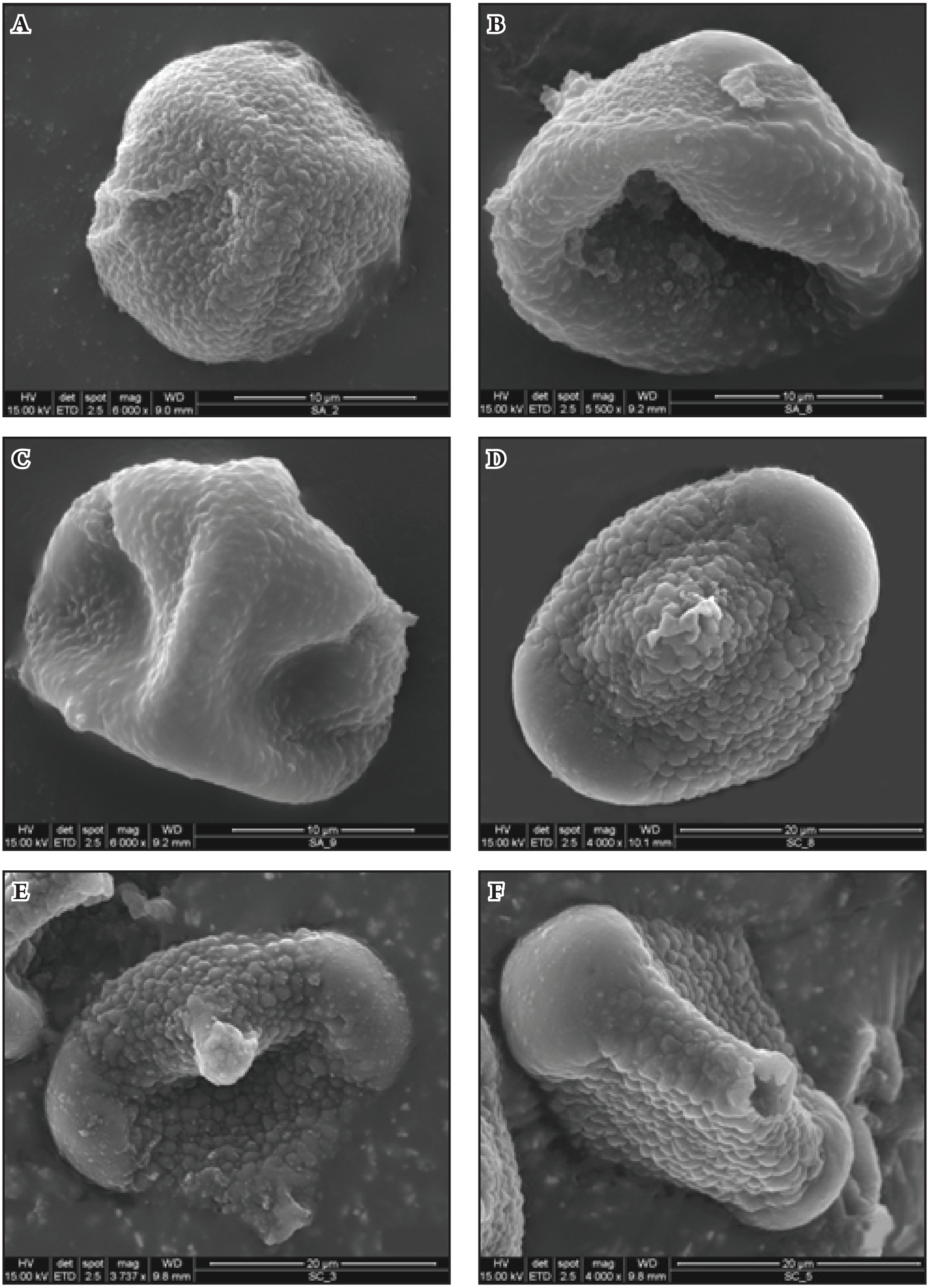

Fig. 5. Pollen grains in SEM: *Sonneratia apetala* pollens (**A-C**); *S. caseolaris* pollens (**D-F**).

zonoaperturate, pore diameter ca. 6.3 µm, pores prominent. Peculiarities: polar area as a whole psilate. Harmomegathy is observed. Infoldings of wall are observed in equatorial region due to hermomegathic effect.

Thus, the epicuticular and pollen ultrastructural differences between *S. apetala* and *S. caseolaris* are easily distinguishable. Both the attributes are to be considered as key characters to distinguish them taxonomically. Interestingly there has some sort of similarity between the leaf epicuticular wax crystalloid deposition pattern between *Trapa* (Nedukha 2012) and *Sonneratia*. Combined morphological and molecular cladistic analysis suggested *Trapa* (Trapaceae) to be sister to Sonneratiaceae (Huang & Shi 2002). Besides taxonomic utility, epicuticular characters focus on some physiological aspects. Riederer (2006) stated that "cuticle is a non-living though highly multifunctional structure into which numerous functions have been integrated". The functions of cuticle include transpiration control, control of loss and uptake of polar solutes, control of gases exchange, reduction of UV radiation, provision of mechanical support to cell wall, protection against the invasion by microbes, etc. Water conservation is crucial in *Sonneratia* being a dweller of physiologically dry soil. The higher salinity in soil causes to more attenuate uptake of water. Epicuticular structure of the two species of *Sonneratia* may enlighten an important adaptation towards transpiration control. *S. apetala*, regularly flushed by saline river water, shows fine, distinct, even covering of intermingled rodlets of wax crystalloids with sunken stomata. In the contrary, tidal flush is rare to the *S. caseolaris* shows wide, indistinct, uneven covering of rodlets of wax crystalloids with exposed stomata.

Small, spheroidal pollens are observed in *S. apetala* (ca. 27 µm diameter) as compared with medium size, prolate pollens of *S. caseolarisa* (ca. 38×27 µm). The ultrastructural details of ornamentation patter of these two species are non-significant. Pollens of the both species show the phenomenon of harmomegathy, i.e. infolding of exine wall (Fig. 5). Harmomegathy is considered as an adaptive feature of pollen grains to escape from full desiccation and death (Katifori *et al.* 2010). This process ensures the survival of protoplast of pollen until it reaches the stigma of a flower (Volkova *et al.* 2013). The infolding of pollen is found to be pronounced in *S. apetala* with location and number of depression on the exine varying greatly, whereas *S. caseolaris* shows the depression on exine between the pores.

Conclusions

The leaf epicuticular and pollen surface ultrastuctural details are useful to discriminate *S. apetala* and *S. caseolaris*. If the same parameters from other species of *Sonneratia* and closely related genera are worked out it will be the good markers of the evolutionary trends among the members of the genus *Sonneratia*. Besides taxonomic importance, the studied characters show some important physiological adaptation towards water retention mechanism according to their area of occupancy in mangrove habitat.

References

Backer C.A., van Steenis C.G.G.J. 1951. Sonneratiaceae. In: van Steenis C.G.G.J. (ed.), Flora Malesiana. **I (4)**: 280–289.

Barthlott W. 1981. Epidermal and seed surface characters of plants: systematic application and some evolutionary aspects. *Nord. J. Bot.* **1**: 345–355.

Barthlott W., Neinhuis C., Cutler D., Ditsch F., Meusel I., Theisen I., Wilhelmi H. 1998. Classification and terminology of plant epicuticular waxes. *Bot. J. Linn. Soc.* **126**: 237–260.

Carpenter K.J. 2006. Specialized structures in the leaf epidermis of Basal Angiosperms: morphology, distribution, and homology. *Am. J. Bot.* **93**: 665–681.

Graham S.A., Hall J., Sytsma K., Shi S. 2005. Phylogenetic analysis of the Lythraceae based on four gene regions and morphology. *Int. J. Plant Sci.* **166**: 995–1017.

Graham S.A., Thorne R.F., Reveal J.L. 1998. Validation of subfamily names in Lythraceae. *Taxon* **47**: 435–436.

Halbritter H., Hesse M. 2004. Principal modes of infoldings intricolp(or)ate Angiosperm pollen. *Grana* **43**: 1–14.

Hesse M., Halbritter H., Zetter R., Weber M., Buchner R., Frosch-Radivo A., Ulrich S. 2009. Pollen terminology: An illustrated handbook. Springer Wien, New York.

Huang Y., Shi S. 2002. Phylogenetics of Lythraceae *sensu lato*: a preliminary analysis based on chloroplast *rbcL* gene, *psaA-ycf3* spacer, and nuclear rDNA internal transcribed spacer (ITS) sequences. *Int. J. Plant Sci.* **163**: 215–225.

Katifori E., Alben S., Cerda E., Nelson D.R., Dumais J. 2010. Foldable structures and the natural design of pollen grains. *PNAS* **107**: 7635–7639.

Keating R.C. 1984. Leaf histology and its contribution to relationships in the Myrtales. *Ann. Mo. Bot. Gard.* **71**: 801–823.

Knight T.G., Wallwork M.A.B., Sedgley M. 2004. Leaf epicuticular wax and cuticle ultrastructure of four *Eucalyptus* species and their hybrids. *Int. J. Plant Sci.* **165**: 27–36.

Lane M.A. 1985. Features observed by electron microscopy as generic criteria. *Taxon* **34**: 38–43.

Naskar K. 2004. Manual of Indian Mangroves: 93–97. Daya Publishing House, Delhi.

Naskar K. 2014. Histo-Physiological and biochemical studies of some salt tolerant plants of Indian Sundarbans. *Ph.D. Thesis.* University of Calcutta, Kolkata, India

Nedukha O.M. 2012. Ultrastucture of epidermal surface in floating and submerged leaves of *Trapa natans* L. *Mod. Phytomorphol.* **1**: 77–80.

Patel V.C., Skvarla J.J., Raven P.H. 1984. Pollen characters in relation to the delimitation of Myrtales. *Ann. Mo. Bot. Gard.* **71**: 858–969.

Riederer M. 2006. Introduction: biology of the plant cuticle. In: Riederer M., Müller C. (eds), Biology of the plant cuticle. *Ann. Plant Rev.* **23**: 1–10.

Sauquet H., Cantrill D.J. 2007. Pollen diversity and evolution in Proteoideae (Proteales: Proteaceae). *Syst. Bot.* **32**: 271–316.

Tomlinson P.B. 1986. The botany of mangroves. Cambridge University Press, Cambridge.

Volkova O.A., Severova E.E., Polevova S.V. 2013. Structural basis of harmomegathy: evidence from Boraginaceae pollen. *Plant Syst. Evol.* **299**: 1769–1779.

Wang Y., Guignard G., Thévenard F., Dilcher D., Barale G., Mosbrugger V., Yang X., Mei S. 2005. Cuticular anatomy of *Sphenobaiera huangii* (Ginkgoales) from the Lower Jurassic of Hubei, China. *Am. J. Bot.* **92**: 709–721.