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Leaf Anatomical Studies in Indian Aponogetonaceae

Rupali N. Chougule, Shrirang R. Yadav and Manoj M. Lekhak*
Angiosperm Taxonomy Laboratory, Department of Botany, Shivaji University, Kolhapur-416004, Maharashtra, India.

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ABSTRACT

The present study was designed to explore the anatomical features of the leaf and petiole of the Indian *Aponogeton* species and a Madagascan species, *A. decaryi*. Species investigated here comprised floating, emergent, and submerged leaves. Floating and emergent leaves were dorsiventral whereas submerged leaves were more or less isobilateral. Anatomy of the leaf showed the presence of paracytic stomata only on the adaxial side in all studied species except *A. bruggenii* and *A. natans*, which had amphistomatic leaves. Transverse sections passing through midrib of a leaf and petiole showed single layered epidermis, hydropoten tissue (on the abaxial surface of floating leaves and both abaxial and adaxial surface of submerged leaves), cortex and vascular bundles. The vascular system of the midrib and petiole had five to seven vascular bundles present in a single row. Central tissue consisted of air canals. Besides these features, observations on petiole outline and leaf lamina (midrib region), and the number of peripheral vascular bundles have also been discussed.

Key words: Anatomy, *Aponogeton*, diaphragm, leaf lamina

***Author for correspondence:** Manoj M. Lekhak, e-mail: mml_botany@unishivaji.ac.in

Introduction

Aponogeton L.f. (Aponogetonaceae Planch.) is an aquatic genus that is represented by 60 species that are distributed in the Old World (van Bruggen, 1985; POWO, 2022). In India the genus is represented by nine species of which five are endemic (Yadav & Gaikwad, 2003; Dey et al., 2021). Most of the species are restricted in their distribution and endemic to the continents in which they appear and some species are even endemic to the islands (Madagascar, Papua New Guinea, and Sri Lanka) (van Bruggen, 1985). On account of its aquatic nature, the genus has always attracted the attention of anatomists (Tomlinson, 1982; Gaikwad, 2000). The anatomical features of species show a well-developed system of air lacunae in the leaf, petiole, and inflorescence axis (Tomlinson, 1982). The leaf anatomy of *Aponogeton* species can vary according to their ecological conditions under which they thrive. For instance, species with floating and emergent leaves have paracytic stomata on the adaxial surface, well-developed mesophyll, and multiseriate palisade tissue whereas the submerged leaves have no stomata, reduced mesophyll and there is no differentiation of palisade tissue (Tomlinson, 1982). Based on the anatomical characters combined with information from embryology, shoot construction and floral morphology, Tomlinson (1982) concluded that the *Aponogeton* belongs to the order Helobiae, and can be placed between the group of Alismataceae Vent., Butomaceae Mirb. and Hydrocharitaceae Juss. on the one hand and Scheuchzeriaceae F.Rudolphi, Juncaginaceae Juss. and Liliaceae Juss. group on the other hand. According to the recent classification, *Aponogeton* is placed in the order Alismatales R.Br. ex Bercht. & J.Presl (Angiosperm Phylogeny Group, 2016).

Here, stomata and transverse sections (TS) passing through midrib region of leaf lamina and petiole, structure of the diaphragm and other anatomical features of Indian *Aponogeton* species and *A. decaryi* (a Madagascan species) have been studied. The main aim was to find out the structural differences in the leaf and petiole anatomy and stomatal parameters among the *Aponogeton* species.

Materials and Methods

All species of *Aponogeton* reported from India were collected from different localities and the germplasm is being maintained in the Lead Botanical Garden, Department of Botany, Shivaji University, Kolhapur. Fresh plant material was used for anatomical studies. Hand-cut sections of the middle portion of the petiole and leaves passing through the midrib region were taken for anatomical studies. Sections were stained by

1% phosophoglucinol ethanol solution. A drop of conc. HCl was then poured on the section and a cover slip placed. For cuticular studies, epidermal peels were taken from the middle of the both upper and lower surface of mature leaves. The peel was mounted in a drop of glycerin on a glass slide and covered with a cover slip. Slides were then observed and microphotographs were taken under Leica DM 750 microscope with an attached camera at 10X and 40X magnification. For stomatal studies, ten random fields were selected for analysis and readings were noted. Stomatal index was calculated following Salisbury's formula (Salisbury, 1928) and all the analysis was done and values represented as mean ± standard error (SE). The data were subjected to a one-way analysis of variance and significant differences between mean values were determined by Duncan's multiple range test (DMRT) ($p < 0.05$) using SPSS (version 16.0). The stomata and other anatomical features of the investigated species were illustrated as line drawings. Microphotographs were used to illustrate structures. Line drawings were made on Gateway tracing paper by using a Rotring isograph pen having 0.2 mm thick nib.

Results

Transverse sections of petiole and leaf lamina (passing through midrib) of *A. appendiculatus, A. bruggenii, A. crispus, A. decaryi, A. lakhonensis, A. microphyllus, A. natans, A. nateshii, A. satarensis* (female), *A. undulatus* and *A. wolfgangianus* were examined. The anatomical features are described under the following heads:

Lamina: Floating and emergent leaves were dorsiventral and the submerged leaves were isobilateral. Both floating and submerged leaves were present in *A. crispus, A. lakhonensis, A. microphyllus, A. undulatus* and *A. wolfgangianus*. *A. bruggenii, A. decaryi, A. satarensis* and *A. natans* had only floating or emergent leaves whereas only submerged leaves were observed in *A. appendiculatus* and *A. nateshii*. Stomata were paracytic in all the species but subsidiary cells were sometimes obscure because the lateral one or both the subsidiary cells got segmented by a median anticlinal division (Fig. 1H, J, N, P). Submerged leaves lacked stomata. Epistomatic leaves were observed in *A. crispus, A. decaryi, A. lakhonensis, A. satarensis, A. microphyllus, A. wolfgangianus,* and *A. undulatus* whereas *A. bruggenii* and *A. natans* had amphistomatic leaves (Fig. 1A, B, K, L). In amphistomatic leaves, the frequency of stomata on the abaxial surface was much lower as compared

Table 1. Cuticular features of amphistomatic floating leaves of *Aponogeton* species. L = Length and B = Breadth.

Sr. No.	Species	Stomata type	Stomatal frequency/mm^2±SE		Epidermal cell frequency/mm^2±SE		Stomatal index (SI)±SE		Size of stomatal cell (µm)		Size of epidermal cell (µm)	
			abaxial surface	adaxial surface	abaxial surface	adaxial surface	abaxial surface	adaxial surface	abaxial (L±SE×B±SE)	adaxial (L±SE×B±SE)	abaxial (L±SE×B±SE)	adaxial (L±SE× B±SE)
1	*A. bruggenii*	Paracytic	21.20 ± 2.86^{b}	146.40 ± 6.56^{a}	526.40 ± 24.38^{c}	1372.80 ± 20.90^{a}	4.00 ± 0.61^{b}	9.63 ± 0.40^{a}	32.09 ± 0.60^{b} × 21.47 ± 0.82^{ab}	27.72 ± 0.61^{c} × 22.19 ± 0.35^{a}	77.09 ± 7.61^{a} × 31.24 ± 3.24^{a}	44.80 ± 4.69^{b} × 19.60 ± 1.11^{b}
2	*A. natans*	Paracytic	20.00 ± 2.53^{b}	153.60 ± 6.37^{a}	627.20 ± 44.86^{b}	1333.60 ± 38.01^{a}	3.10 ± 0.36^{b}	10.42 ± 0.56^{a}	37.93 ± 1.10^{a} × 19.80 ± 0.41^{c}	36.44 ± 0.87^{a} × 20.11 ± 0.31^{bc}	70.01 ± 8.33^{a} × 26.25 ± 1.76^{a}	40.00 ± 3.24^{b} × 19.17 ± 1.55^{b}

Mean values with different alphabets in column showed statistically significant differences ($p<0.05$) according to Duncan's multiple range test

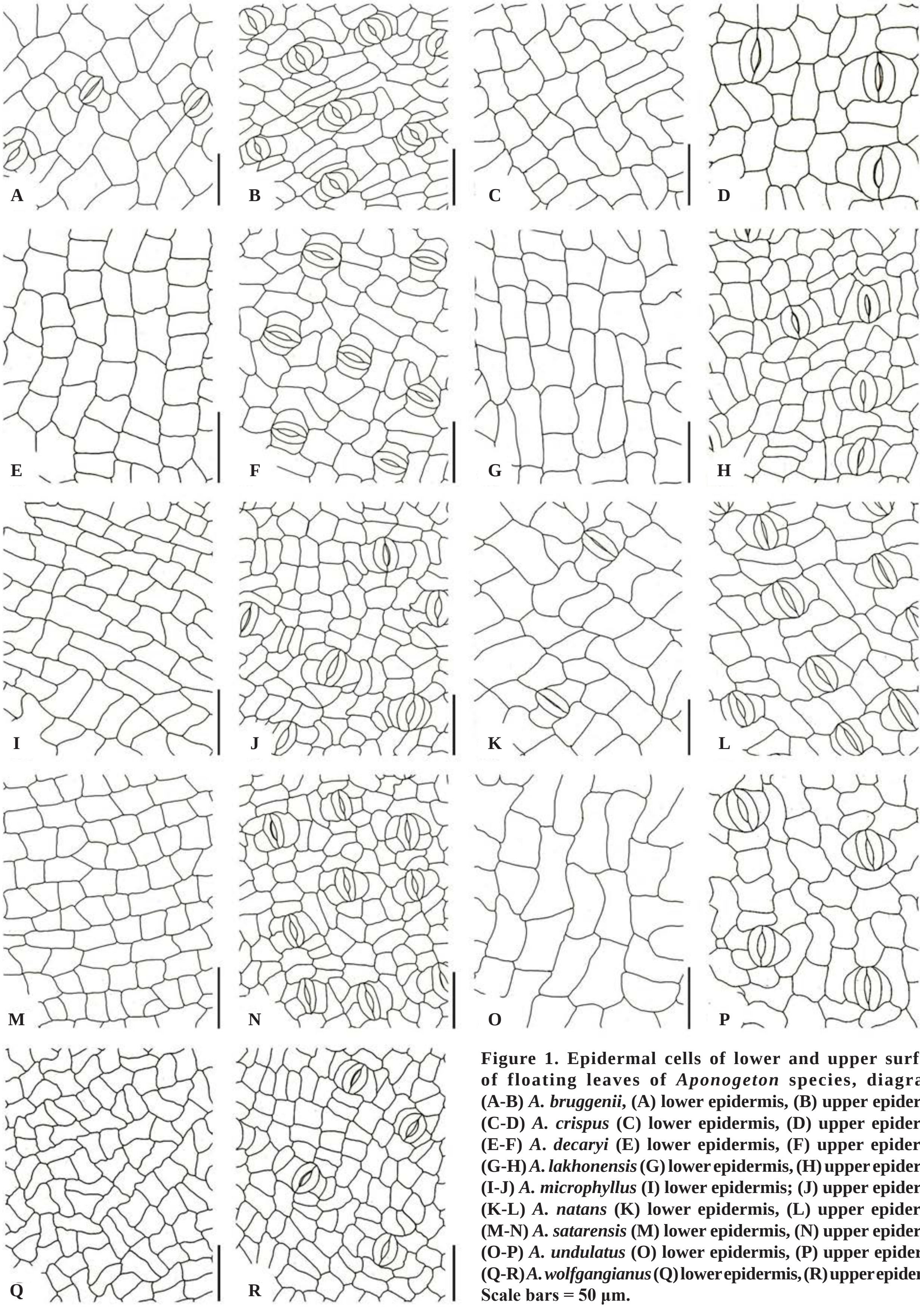


Figure 1. Epidermal cells of lower and upper surfaces of floating leaves of *Aponogeton* species, diagrams. (A-B) *A. bruggenii*, (A) lower epidermis, (B) upper epidermis; (C-D) *A. crispus* (C) lower epidermis, (D) upper epidermis; (E-F) *A. decaryi* (E) lower epidermis, (F) upper epidermis; (G-H) *A. lakhonensis* (G) lower epidermis, (H) upper epidermis; (I-J) *A. microphyllus* (I) lower epidermis; (J) upper epidermis; (K-L) *A. natans* (K) lower epidermis, (L) upper epidermis; (M-N) *A. satarensis* (M) lower epidermis, (N) upper epidermis; (O-P) *A. undulatus* (O) lower epidermis, (P) upper epidermis, (Q-R) *A. wolfgangianus* (Q) lower epidermis, (R) upper epidermis. Scale bars = 50 µm.

Table 2. Cuticular features of epistomatic floating leaves of Aponogeton (adaxial surface) species. L = Length and B = Breadth.

S. No.	Species	Stomata type	Stomatal frequency/ mm^2±SE	Epidermal cell frequency/ mm^2±SE	Stomatal index (SI)±SE	Size of stomatal cell (LXB) µm	Size of epidermal cell (LXB) µm
1	*A. crispus*	Paracytic	191.60 ± 8.16^b	2894.40 ± 54.62^b	6.21 ± 0.22^a	$28.86 \pm 2.20^b \times 15.42 \pm 1.18^d$	$24.59 \pm 2.10^c \times 14.58 \pm 1.12^{cd}$
2	*A. decaryi*	Paracytic	158.80 ± 3.43^c	2072.00 ± 48.90^d	7.15 ± 0.22^a	$30.74 \pm 0.63^b \times 16.78 \pm 0.48^c$	$39.17 \pm 3.84^b \times 18.33 \pm 1.11^{ab}$
3	*A. lakhonensis*	Paracytic	234.80 ± 10.08^a	3336.00 ± 125.72^a	6.58 ± 0.15^{ab}	$29.28 \pm 1.02^b \times 20.21 \pm 0.42^b$	$26.78 \pm 1.64^c \times 14.38 \pm 0.94^{cd}$
4	*A. microphyllus*	Paracytic	143.20 ± 6.79^c	2568.00 ± 42.15^c	5.28 ± 0.23^c	$30.95 \pm 0.78^b \times 19.90 \pm 0.53^b$	$29.49 \pm 1.84^c \times 16.15 \pm 0.39^{bc}$
5	*A. satarensis*	Paracytic	240.00 ± 6.05^a	3305.60 ± 92.04^a	6.81 ± 0.26^{ab}	$30.01 \pm 0.56^b \times 16.15 \pm 0.32^{cd}$	$15.77 \pm 1.21^d \times 11.92 \pm 0.90^d$
6	*A. undulatus*	Paracytic	70.00 ± 2.00^e	952.00 ± 15.87^e	6.87 ± 0.25^{ab}	$38.76 \pm 0.71^a \times 22.40 \pm 0.32^a$	$52.31 \pm 4.37^a \times 19.61 \pm 1.56^a$
7	*A. wolfgangianus*	Paracytic	88.00 ± 3.68^d	2774.40 ± 103.01^{bc}	3.11 ± 0.17^d	$29.91 \pm 0.73^b \times 20.21 \pm 0.47^b$	$28.03 \pm 2.44^c \times 16.05 \pm 1.10^{bc}$

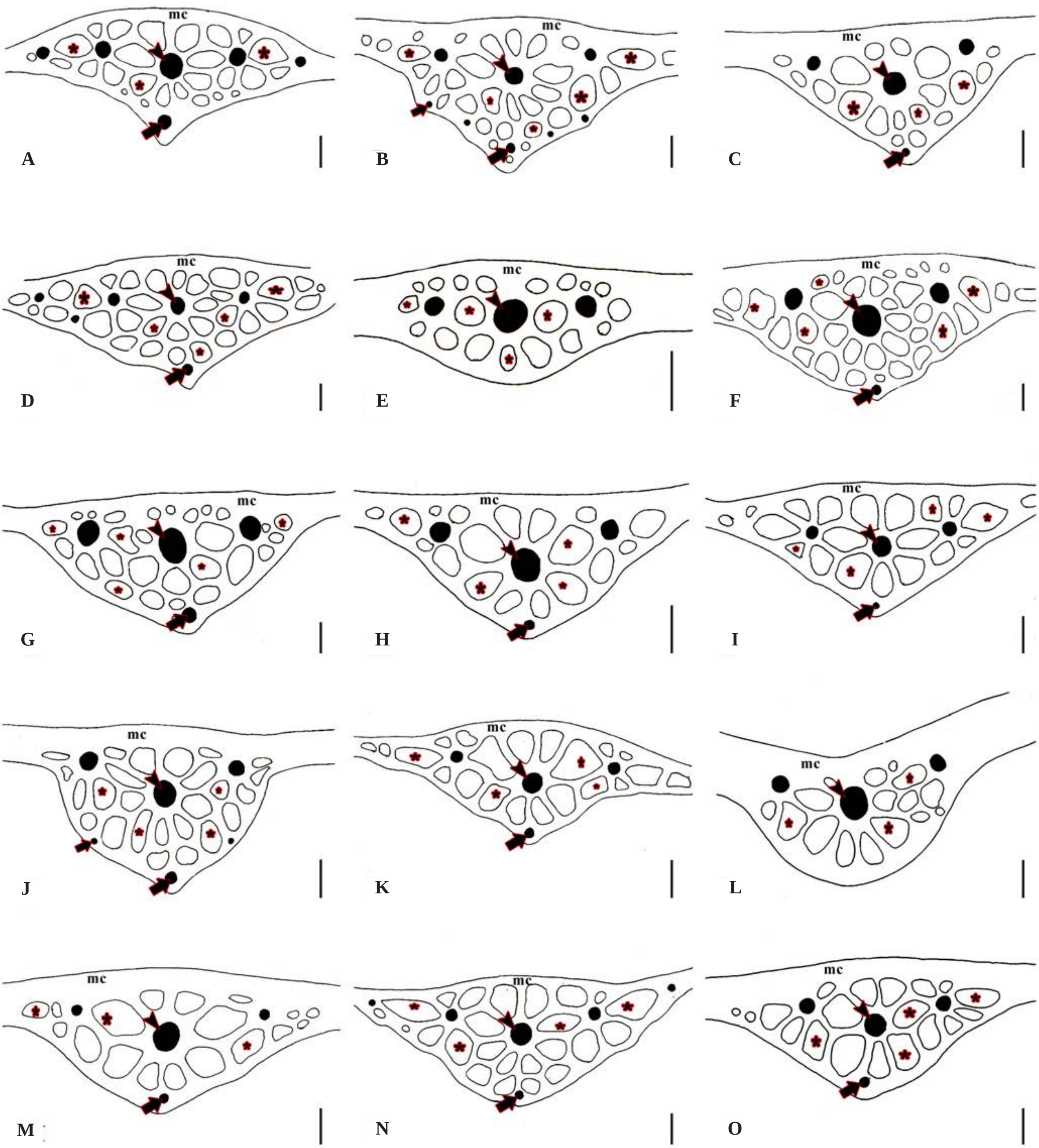
Mean values with different alphabets in column showed statistically significant differences ($p<0.05$) according to Duncan's multiple range test

to the adaxial surface whereas stomatal and epidermal cell sizes were larger on the abaxial surface (Table 1). The details of stomatal parameters such as stomatal and epidermal cell frequency, stomatal index, stomata and epidermal cell size are provided in Tables 1 and 2.

Stomatal parameters: The stomatal frequency ranged from 240.00 to 70.00 stomata mm^{-2} with *A. satarensis* (female) showing the highest stomatal frequency and *A. undulatus* the lowest (Table 2). Stomatal frequencies (70 mm^{-2} - 240 mm^{-2}) and stomatal index (3.11 mm^{-2} - 10.42 mm^{-2}) differed significantly across the species (Table 1, 2). Amongst the studied species, *A. undulatus* had the largest stomatal cell (38.76 × 22.40 µm) and epidermal cell (52.31 × 19.61 µm). *A. bruggenii* had the smallest stomatal cell (27.72 × 22.19 µm) and *A. satarensis* the smallest epidermal cell (15.77 × 11.92 µm).

Transverse sections of leaves: Transverse sections (TS) passing through the midrib region (of floating, and submerged leaves) of all the studied species are shown in Fig. 2A-P. Outline of the TS was sub-trigonous except for *A. decaryi* and *A. satarensis* where it was planoconvex (Fig. 2E, L). Epidermis was single layered made up of small colorless, irregularly arranged parenchymatous cells. The epidermal cells of the abaxial surface are usually larger than adaxial epidermal cells (Fig. 1). Thin cuticle was observed in *A. bruggenii* and *A. satarensis*. The lower epidermis of all studied species exhibited the presence of hydropoten tissue. Mesophyll was as much as five layered. Hypodermis on the adaxial side consisted of 1-2 layered palisade cells that contained numerous large discoid chloroplasts. Palisade cells were elongated in shape and compactly arranged but interrupted by a well-developed chamber below each stoma (Fig. 3). This was followed by 1-3 layered spongy mesophyll cells that were circular to polygonal in shape and compactly arranged. Honeycomb-type aerenchyma, characterized by irregular and large air spaces, was observed in the midrib. Lacunose tissue consisted of air canals separated by uniseriate plates of parenchyma cells. Air cavities at intervals were traversed by small stellate diaphragm cells. The vascular system of the midrib consisted of usually three to five vascular bundles in a single arc. The central median vascular bundle was large and lateral bundles successively narrower (Fig. 2). Each of the main vascular bundles consisted of a large xylem lacuna and few metaxylem cells which were surrounded by phloem. Cells surrounding the phloem are somewhat thick-walled and collenchymatous. Many peripheral abaxial subsidiary vascular bundles were present in *A. bruggenii* and *A. natans* while a single peripheral abaxial subsidiary vascular bundle was observed in *A. crispus, A. lakhonensis, A. microphyllus, A. undulatus* and *A. wolfgangianus* (Figs. 2 & 3). Peripheral subsidiary vascular bundles consisted of narrow metaxylem elements (see Tomlinson, 1982). Peripheral abaxial subsidiary vascular bundles were found to be completely absent in *A. decaryi* and *A. satarensis*. Adaxial subsidiary vascular bundles were absent in floating leaves of all the studied species (Figs. 2 & 3).

Anatomical characteristics of submerged leaves were more or less similar to floating leaves except for some variations such as leaf nature, absence of stomata and size of the epidermal cells. The epidermis was single-layered. The epidermal cells of the abaxial surface are usually larger than the adaxial epidermal cells. Mesophyll was 2-5 layered. In mesophyll layers, spongy cells were irregularly polygonal to circular whereas palisade cells were indistinct or absent. The structure of vascular bundles was similar to that of floating leaves. The single peripheral abaxial subsidiary



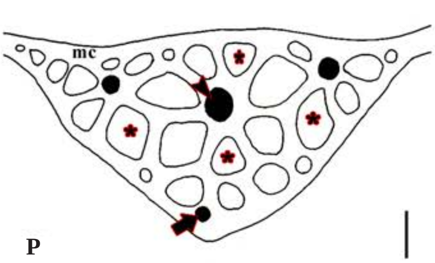


Figure 2. Transverse sections of the laminae of floating and submerged leaves of *Aponogeton* species, outline diagrams. (A) *A. appendiculatus,* submerged; (B) *A. bruggenii,* emergent; (C-D) *A. crispus,* (C) floating, (D) submerged; (E) *A. decaryi,* floating; (F-G) *A. lakhonensis* (F) floating, (G) submerged; (H-I) *A. microphyllus* (H) floating, (I) submerged; (J) *A. natans,* floating; (K) *A. nateshii,* submerged; (L) *A. satarensis,* floating; (M-N) *A. undulatus* (M) floating, (N) submerged; (O-P) *A. wolfgangianus* (O) floating, (P) submerged. Arrowheads indicate the central vascular bundle, asterisks air cavity, blank portions of aerenchyma cells and arrows abaxial subsidiary vascular bundle. Abbreviations: mc = mesophyll cells (Scale bars = 250 µm).

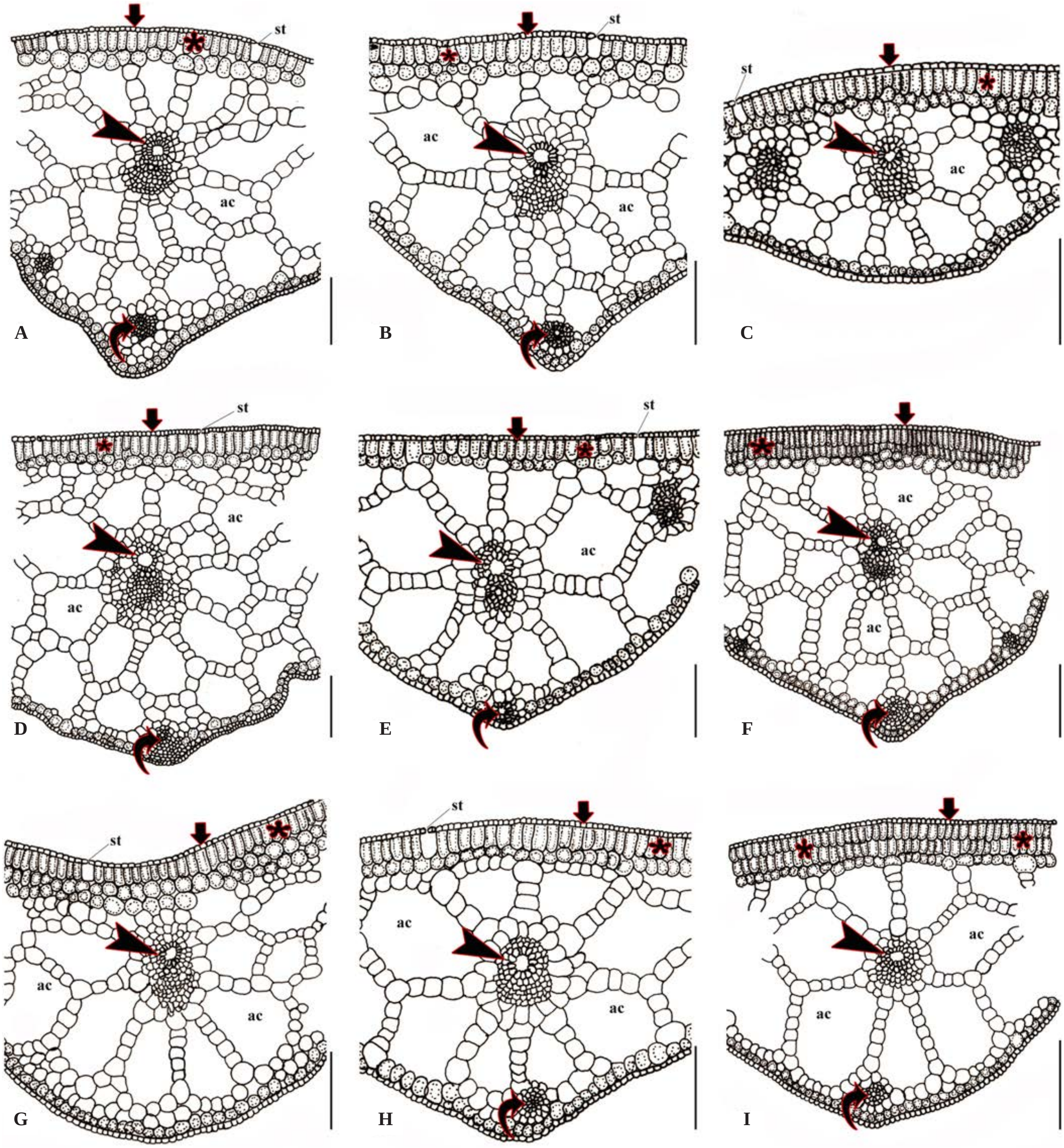


Figure 3. Transverse sections of laminae of floating leaves of *Aponogeton* species, schematic diagrams. (A) *A. bruggenii*; (B) *A. crispus*; (C) *A. decaryi*; (D) *A. lakhonensis*; (E) *A. microphyllus*; (F) *A. natans*; (G) *A. satarensis*; (H) *A. undulatus*; (I) *A. wolfgangianus*. Arrows show adaxial epidermis, arrowheads central vascular bundle, asterisks palisade cells and curved arrows abaxial subsidiary vascular bundle. Abbreviations: st = stomata, ac = air cavity. Scale bars = 250 µm.

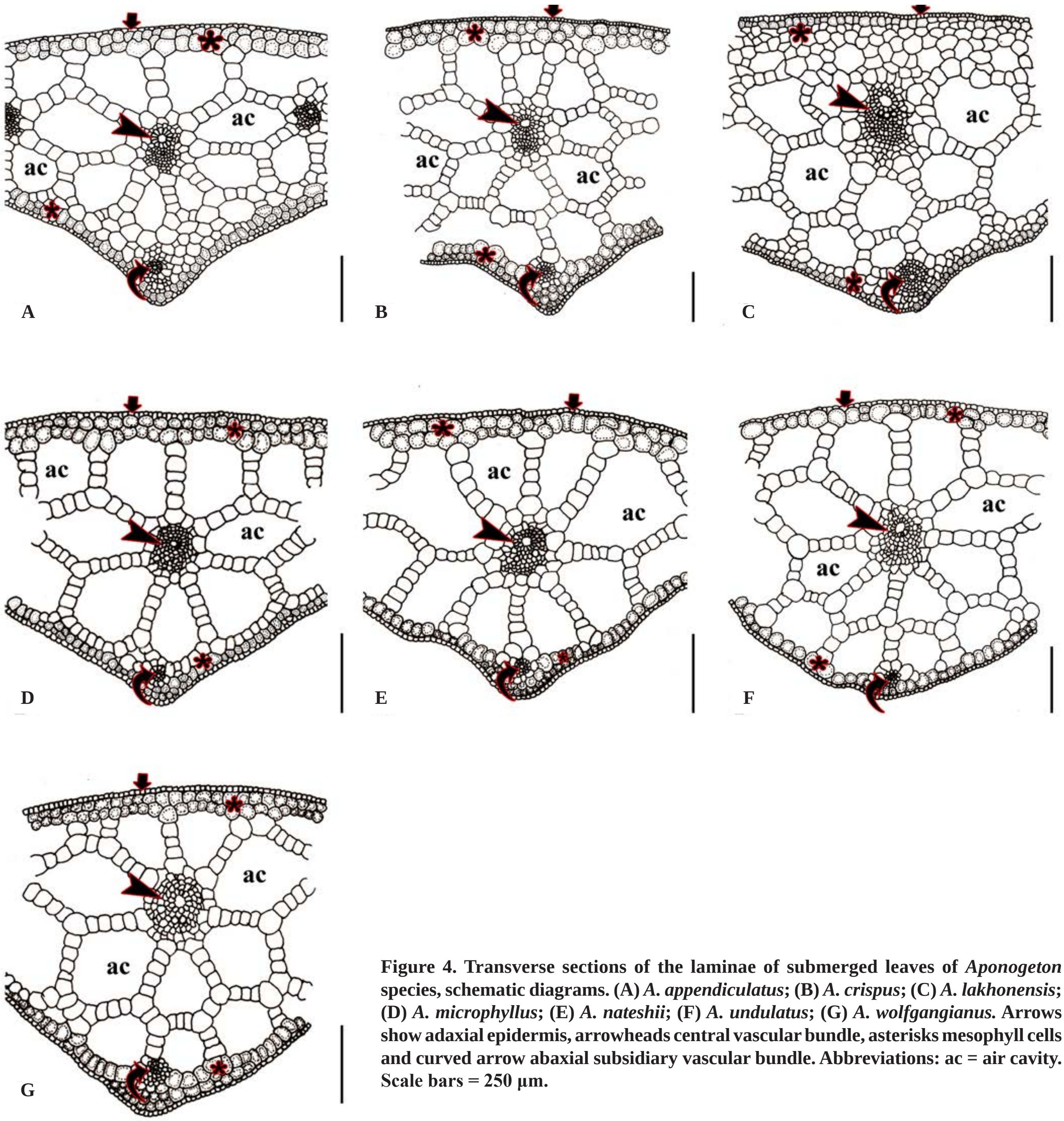


Figure 4. Transverse sections of the laminae of submerged leaves of *Aponogeton* species, schematic diagrams. (A) *A. appendiculatus*; (B) *A. crispus*; (C) *A. lakhonensis*; (D) *A. microphyllus*; (E) *A. nateshii*; (F) *A. undulatus*; (G) *A. wolfgangianus.* Arrows show adaxial epidermis, arrowheads central vascular bundle, asterisks mesophyll cells and curved arrow abaxial subsidiary vascular bundle. Abbreviations: ac = air cavity. Scale bars = 250 µm.

vascular bundle was present in submerged leaves of all the studied species whereas adaxial subsidiary vascular bundles were absent in submerged leaves of all the studied species (Figs. 2 & 4).

***Transverse sections of petioles**:* Outline of TS of the petiole of *A. decaryi* and *A. satarensis* (Fig. 5 E & L) was planoconvex whereas petioles of all other species were sub-trigonous (Fig. 5). In all the species, the single-layered epidermis was present which was made up of more or less rectangular thin-walled cells arranged in longitudinal files. Hypodermis consisted of 1-4 layers of compact parenchymatous cells. A honeycomb type of aerenchyma was observed. Lacunose tissue consisted of air canals separated by uniseriate plates of parenchyma cells. Air cavities at intervals were traversed by small stellate diaphragm cells. The vascular system of the petiole consisted of usually five to seven vascular bundles in a single arc. The central median vascular bundle was large and lateral bundles successively narrower. Each of the main vascular bundles consisted of a large xylem lacuna and a few metaxylem cells which were surrounded by phloem. Cells surrounding the phloem were somewhat thick-walled and collenchymatous (Figs. 6 & 7). Peripheral subsidiary vascular bundles consisted of narrow metaxylem elements. Many peripheral abaxial subsidiary vascular bundles were present in *A. bruggenii*, *A. crispus* (in both floating and submerged leaves), *A. lakhonensis* (in both floating and submerged leaves) and *A. natans* (Figs. 5, 6, & 7). A single peripheral abaxial subsidiary vascular bundle was present in *A. appendiculatus*, *A. nateshii* and both floating and submerged petioles of *A. microphyllus*, *A. undulatus* and *A. wolfgangianus* (Figs. 5, 6, & 7). Peripheral abaxial subsidiary vascular bundles were absent in *A. decaryi* and *A. satarensis* (Figs. 5 & 6). Few (1-3) adaxial subsidiary vascular bundles were present in the petioles of floating leaves of *A. crispus*, *A. lakhonensis* and *A. natans* (Fig. 5C, F, J & Fig. 6B, D, F). These were absent in the petiole of floating leaves of the remaining species and petiole of all submerged leaves (Figs. 5, 6 & 7).

***Diaphragm**:* The intercellular spaces or pores of diaphragms slightly differed in shape and size across the species. Pore shape was bluntly triangular in all species except *A. satarensis* where it was more or less oval or rounded in shape and larger (Fig. 8).

Discussion

Baas (1985) described the vegetative anatomy of Aponogetonaceae and reported that hairs were absent on the leaf surfaces but observed squamules in leaf axils. Singh (1965) also observed two large squamules in the leaf axils of *A. crispus*, *A. natans* and *A. distachyos*. For the first time, Kam et al. (2016) documented glandular trichomes on both the abaxial and adaxial surfaces of leaves and along the petiole of *A. ulvaceus*. Hydropoten tissues were observed on both the surfaces of submerged leaves, the abaxial surfaces of floating leaves, and the petioles of both types of leaves (Baas, 1985). Our study also recorded hydropoten tissues on both the adaxial and abaxial surfaces of submerged leaves and the abaxial surfaces of floating leaves. *Aponogeton* species are characterized by the presence of floating or emergent, submerged or both types of leaves. Stomata were absolutely absent in submerged leaves but present only on the adaxial surface of floating leaves because in the floating leaves the adaxial surface is exposed to the atmosphere and the abaxial surface is completely submerged in water. Therefore, gas exchange occurs mainly from the adaxial leaf surface (Shtein et al., 2017). In the present study, we observed epistomatic leaves in all the studied species except *A. bruggenii* and *A. natans* which had amphistomatic leaves. Stomata were paracytic in all the species studied. Similar observations were made by Gaikwad (2000). Submerged leaves generally lack stomata entirely (Evert, 2007). Earlier workers had observed stomata in submerged leaves of *Aponogeton* but those stomata were with closed pores and it is assumed that they were non-functional (Riede, 1921). In the present study, we did not observe the stomata in submerged leaves. The emergent and floating leaves of some species such as *A. bruggenii*, *A. distachyos* and *A. satarensis* exhibit waxy coating or thin cuticle (Sergueeff, 1907; Mujawar, 1991; Gaikwad, 2000). We have also observed thin cuticles in *A. bruggenii* and *A. satarensis*.

Dash et al. (2013) observed that the midrib and petiole were flat on the adaxial side and broadly V-shaped on the abaxial side in *A. natans*. Also, the abaxial epidermis was comparatively thick with narrowly oblong cells. The TS of leaf lamina (passing through midrib) and petiole in all studied species was bluntly triangular or sub-trigonous in outline except for species, i.e. *A. satarensis* and *A. decaryi* where it was planoconvex. Similar observations were made by Mujawar (1991) and Gaikwad (2000). Kam et al. (2016) observed the presence of at least seven vascular bundles in *A. ulvaceus*. In our study, we observed five to seven vascular bundles in a single row in which the central median vascular bundle was relatively larger in size while lateral vascular bundles were successively narrower in both midrib and petiole. Similar observations were made in some Indian species of *Aponogeton* by Gaikwad (2000). The number of peripheral abaxial subsidiary vascular bundles varied across the studied species and a few adaxial subsidiary vascular bundles were present only in the petiole of floating leaves of *A. crispus*, *A. lakhonensis* and *A. natans*. Tomlinson (1982) reported a single arc of vascular bundles with wide median and successively narrower lateral bundles and several abaxial and few adaxial subsidiary vascular bundles in the petiole of floating leaves of *A. spathaceum*. Three main vascular bundles and several small peripheral bundles were observed in the leaves of *A. spathaceum* var. *junceum*

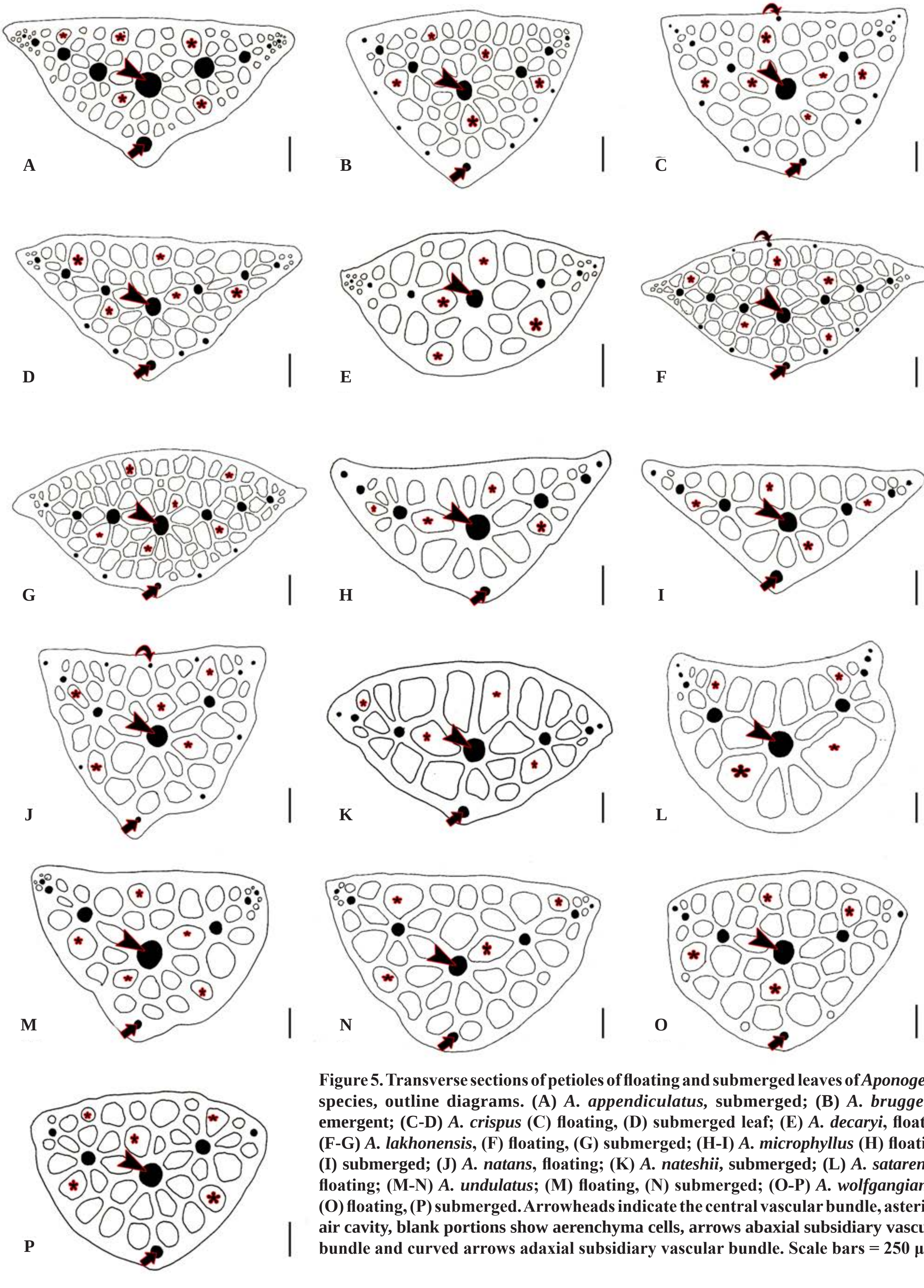


Figure 5. Transverse sections of petioles of floating and submerged leaves of *Aponogeton* species, outline diagrams. (A) *A. appendiculatus,* submerged; (B) *A. bruggenii*, emergent; (C-D) *A. crispus* (C) floating, (D) submerged leaf; (E) *A. decaryi*, floating (F-G) *A. lakhonensis*, (F) floating, (G) submerged; (H-I) *A. microphyllus* (H) floating, (I) submerged; (J) *A. natans*, floating; (K) *A. nateshii*, submerged; (L) *A. satarensis*, floating; (M-N) *A. undulatus*; (M) floating, (N) submerged; (O-P) *A. wolfgangianus*, (O) floating, (P) submerged. Arrowheads indicate the central vascular bundle, asterisks air cavity, blank portions show aerenchyma cells, arrows abaxial subsidiary vascular bundle and curved arrows adaxial subsidiary vascular bundle. Scale bars = 250 µm.

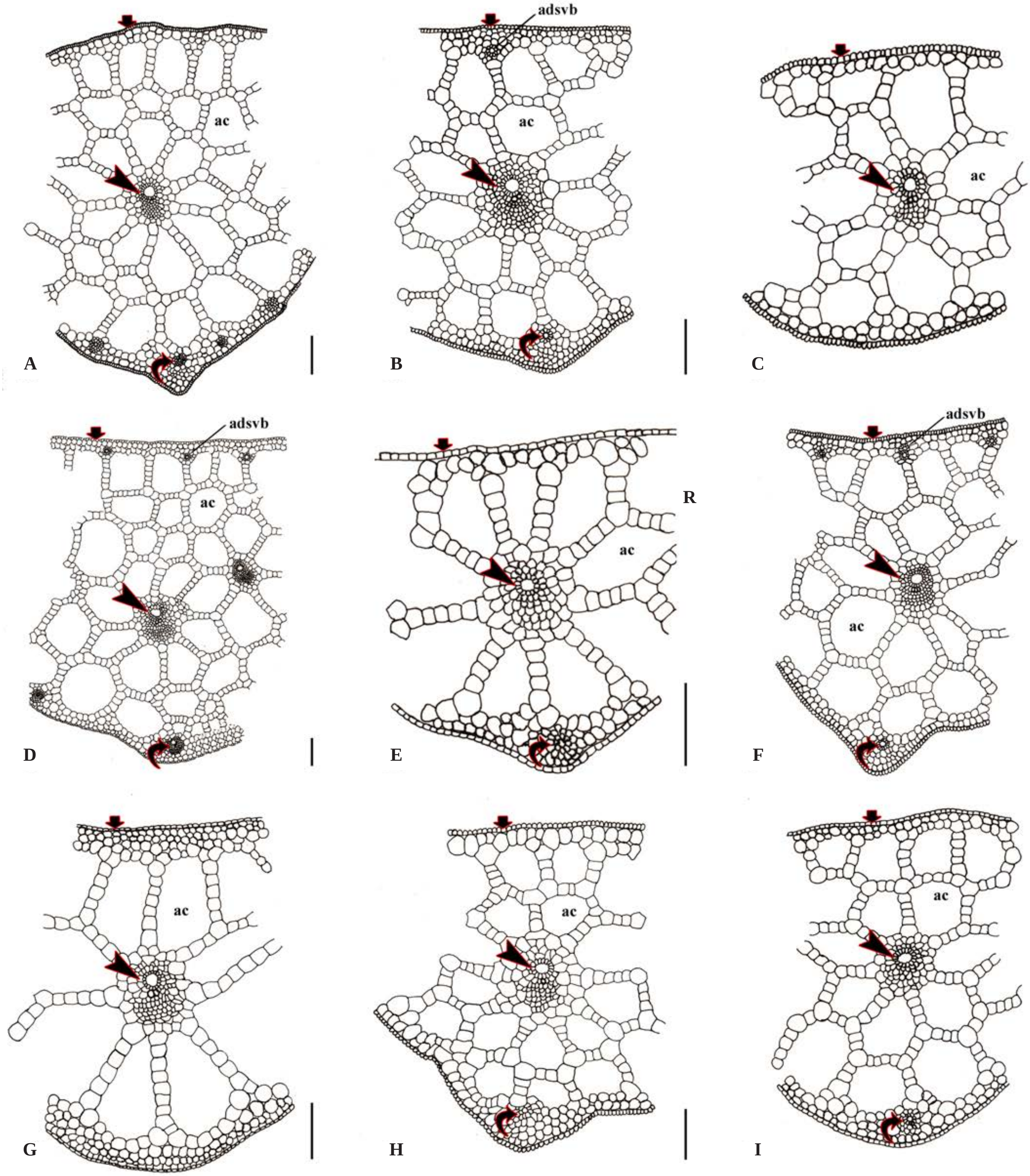


Figure 6. Transverse sections of petioles of floating leaves of *Aponogeton* species, schematic diagrams. (A) *A. bruggenii*; (B) *A. crispus*; (C) *A. decaryi*; (D) *A. lakhonensis*; (E) *A. microphyllus*; (F) *A. natans*; (G) *A. satarensis*; (H) *A. undulatus*; (I) *A. wolfgangianus.* Arrows show adaxial epidermis, arrowheads central vascular bundle and curved arrows abaxial subsidiary vascular bundle. Abbreviations: ac = air cavity, adsvb = adaxial subsidiary vascular bundle. Scale bars = 250 µm.

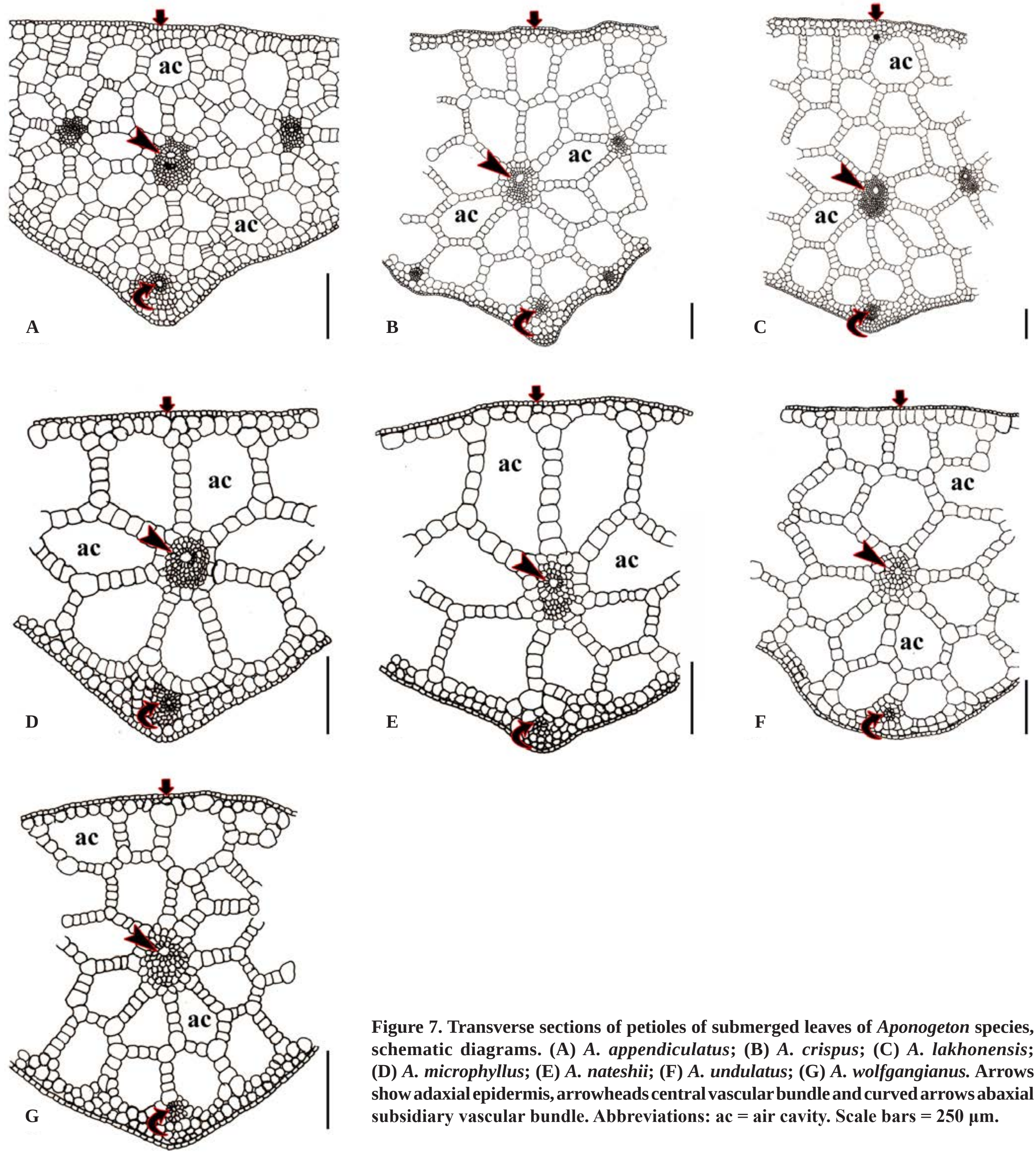
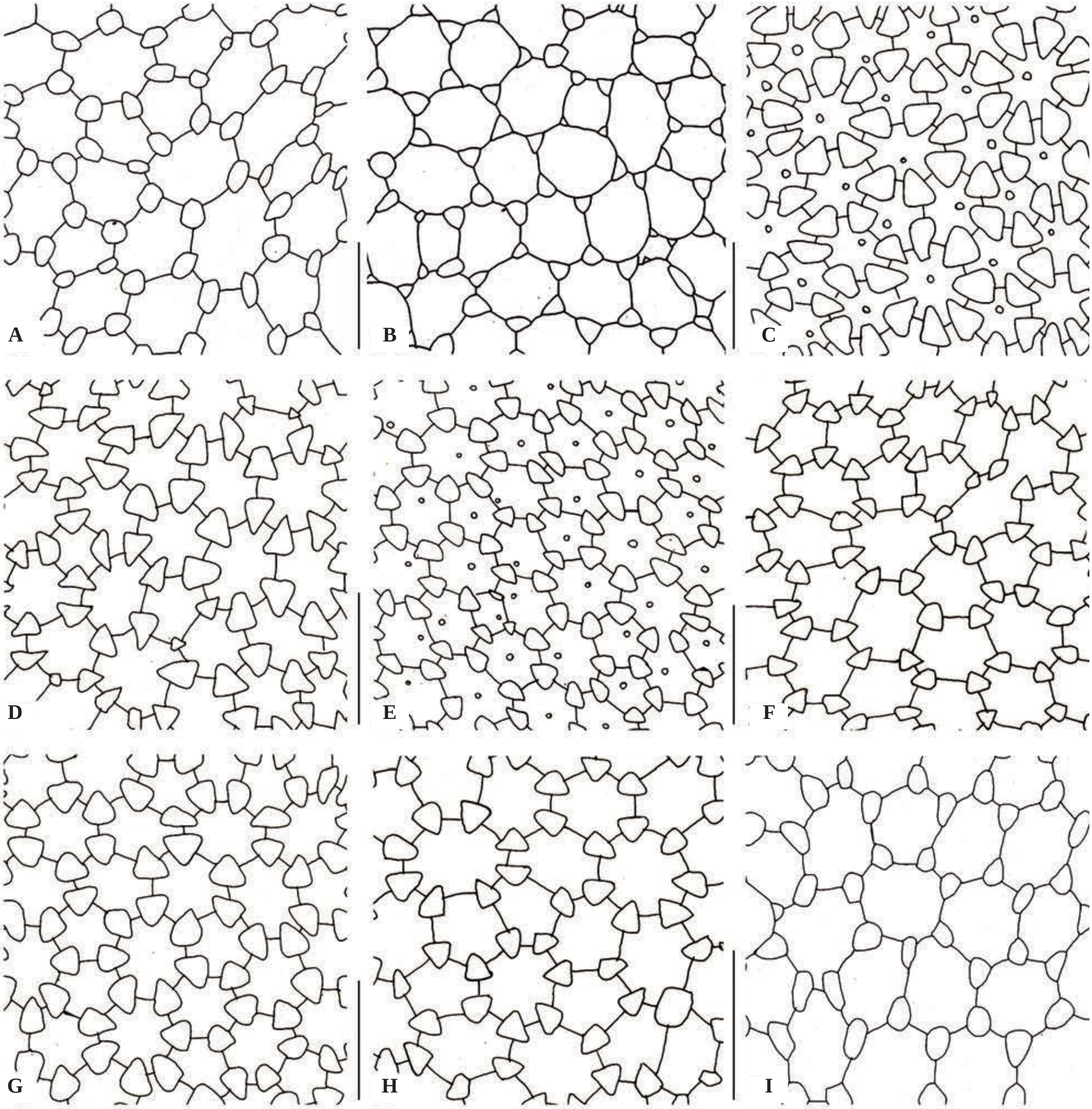


Figure 7. Transverse sections of petioles of submerged leaves of *Aponogeton* species, schematic diagrams. (A) *A. appendiculatus*; (B) *A. crispus*; (C) *A. lakhonensis*; (D) *A. microphyllus*; (E) *A. nateshii*; (F) *A. undulatus*; (G) *A. wolfgangianus.* Arrows show adaxial epidermis, arrowheads central vascular bundle and curved arrows abaxial subsidiary vascular bundle. Abbreviations: ac = air cavity. Scale bars = 250 µm.

by Arber (1921). Sergueeff (1907) also reported 1-2 small abaxial bundles in the petiole of *A. natans* and well-developed subsidiary vascular bundles observed in the petiole of submerged leaves of *A. fenestralis.* The abaxial subsidiary vascular bundles were entirely absent in *A. decaryi* and *A. satarensis* indicating that they are closely related species. Earlier, Yadav (1995) has shown that the species hybridize and hence, are genetically close. A honeycomb type of aerenchyma and an extensive lacunae system was observed in the shoot of *A. ulvaceus* by Kam et al. (2016). In our study, we also observed a honeycomb type of aerenchyma



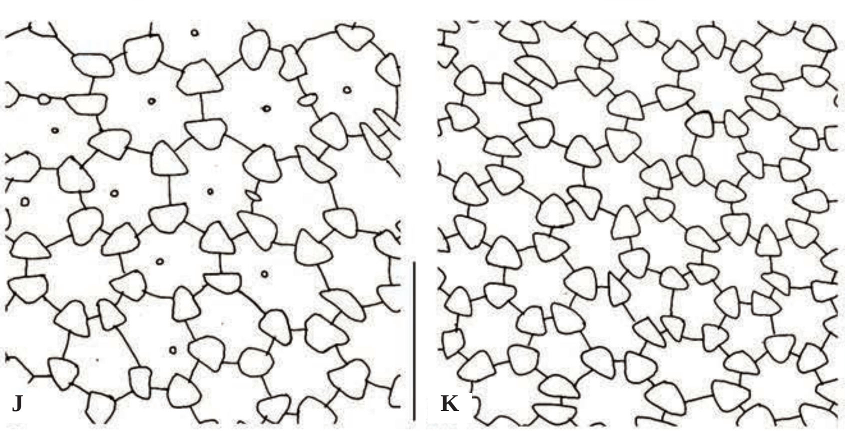


Figure 8. Diaphragms in *Aponogeton* species, schematic diagrams. (A) *A. appendiculatus*; (B) *A. bruggenii*; (C) *A. crispus*; (D) *A. decaryi*; (E) *A. lakhonensis*; (F) *A. microphyllus*; (G) *A. natans*; (H) *A. nateshii*; (I) *A. satarensis*; (J) *A. undulatus*; (K) *A. wolfgangianus*. Scale bars = 50 µm.

and an extensive lacunae system in the midrib and petiole of the all studied species. Diaphragms are unique anatomical features and have been reported in many aquatic plants such as *Scirpus* Tourn. ex L., *Sagittaria* Ruppius ex L., *Hippuris* L., *Ceratophyllum* L., *Sparganium* L. and documented in some grasses also (Snow, 1914; Kaul, 1973; Liang et al., 2008). Snow (1914) observed that the shape of the diaphragm varied from polygonal with tiny air spaces at the corners to stellate with very much elongated arms in *Scirpus* species. In *A. ulvaceus* small hexagonal diaphragm cells were observed in some lacunae by Kam et al. (2016). We recorded diaphragms in the vegetative parts of all the *Aponogeton* species and their shape was stellate. These diaphragms provide gaseous continuity along the lacunae, support the stem, cross bundles and some diaphragms are photosynthetic, carry laticifers, store tannins, food as well as conduct food material from the cross bundles to the partition walls of the space. They also prevent internal flooding when stems are damaged (Snow, 1914; Liang et al., 2008; Kam et al., 2016).

The present study reveals that the anatomical features of *Aponogeton* species are nearly similar in all the studied species except for some differences such as the presence or absence of stomata, outline of a transverse section of leaf lamina and petiole, numbers of peripheral vascular bundles, etc. Anatomical characters along with morphological and embryological characters may prove useful in the identification of *Aponogeton* species. Chen et al. (2015) estimated a phylogenetic tree of 42 species of *Aponogeton* based on a maximum likelihood analysis of combined nuclear (ITS) and plastid (trnK/matK) sequence data. This tree included only six Indian species, which were placed in two clades, Clade I and Clade VI. Clade I comprised *A. bruggenii, A. crispus, A. lakhonensis, A. natans* and *A. undulatus* and tropical Australian species. Clade VI contained *A. satarensis*, along with the Madagascan *A. decaryi* and *A. dioecus*. These clades are supported to some extent by the anatomical characters observed in the present investigation. Members of Clade I tend to be characterized by a sub-trigonous outline of the leaf lamina, while members of Clade VI tend to have a planoconvex outline. Similarly, members of Clade I tend to have one to many peripheral abaxial subsidiary vascular bundles, while these are absent in members of Clade VI. As more Indian species are included in phylogenetic analyses, we may get a complete picture of the systematic patterns of variation in anatomy in *Aponogeton*.

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