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Anatomical Patterns of Aerenchyma in Aquatic and Wetland Plants

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A well-developed aerenchyma is a major characteristic of aquatic plants. However, because such tissues are also found in wetland and terrestrial plants, it is not always possible to use their presence or absence to distinguish aquatic species. Whereas patterns of aerenchyma in roots have been studied in detail, those of the shoots have not. We collected and tested 110 species of various aquatic and wetland plants, including ferns (5), basal angiosperms (5), monocots (65), and eudicots (35). Three common and two rare types of aerenchyma were observed in their roots (three schizogeny and two lysigeny), plus five types of schizogeny in their shoots. We re-confirmed that, although a well-developed aerenchyma is more common in most organs of aquatic plants than in wetland plants, this presence cannot be used as strict evidence for the aquatic quality of vascular plants. Here, aerenchyma patterns were stable at the genus level, and the consistency of pattern was stronger in the roots than in the shoots. Furthermore, significant trends were verified in several higher taxa, and those consistencies of patterns partially coincided with their phylogeny.

Keywords: aerenchyma, aquatic plants, lysigeny, schizogeny, wetland plants

Aerenchyma is a large intercellular space that acts as a mediator of internal gas exchange and maintains strength with the least tissue. This structure is critical under hypoxic conditions or during processes of normal development in aquatic species (Williams and Barber, 1961; Jackson and Armstrong, 1999; Evans, 2004). Because these structures are easily observed in aquatic plants, they are a major characteristic for identification. Aquatic plants include angiosperms derived from various independent origins of basal flowering plants, monocots, eudicots, and ferns, such as monilophytes (Azollaceae and Marsileaceae) and lycophytes (Isoetaceae) (Arber, 1920; Sculthorpe, 1967; Tomlinson, 1982; Choi, 1985; Cook, 1996; Les et al., 2003). Most aquatic plants form aerenchyma constitutively in their roots, leaves, and stems, whereas others, including certain amphibious and land plants, produce aerenchyma in response to poor aeration (Justin and Armstrong, 1987; Jackson and Armstrong, 1999). Therefore, the adaptive boundaries among these plant types, based on the presence of aerenchyma, are unclear.

Schenck (1890) initially used the term 'Aërenchym'. A few years earlier, De Bary (1877) had described the processes that form enlarged air chambers as either 'schizogenetic' or 'lysigenetic'. The current view is that a schizogenous aerenchyma (or schizogeny) is created by cell separation and differential cell expansion, which forms spaces among parenchyma cells, whereas a lysigenous aerenchyma (or lysigeny) is created via programmed cell death (Jackson and Armstrong, 1999; Evans, 2004). Schizogeny and lysigeny can be further distinguished depending on the shape or direction in which the aerenchyma forms. Therefore, Justin and Armstrong (1987) have classified the primary cortex and their associated gas-space feature (aerenchyma) into 3 major types, with 14 lower categories based on the cell arrangements in roots, while Seago et al. (2005) have characterized root aerenchyma into 6 major types and have introduced a new term, "expansigeny" (Fig. 1). In contrast, Kaul (1971) has investigated the aerenchyma in the culm of *Scirpus validus*, and reported the anatomical characteristics of floating leaves from angiosperms and ferns, including their aerenchyma (Kaul, 1976). Additionally, Sculthorpe (1967) has reported that aquatic vascular plants, e.g., those in the genera *Isoetes*, *Ceratophyllum*, *Myriophyllum*, and *Nuphar*, have specific patterns of shoot aerenchyma. Schussler and Longstreth (1996) have investigated different patterns within *Sagittaria lancifolia*, which shows lysigeny in its roots but schizogeny in its shoots. Although these studies demonstrate various and specific aerenchyma patterns within each taxon and organ, they have not classified shoot aerenchyma into reliable types.

Lysigeny occurs in the roots of crops such as rice (Kawai et al., 1998; Colmer, 2003; Colmer et al., 2006) and maize (Campbell and Drew, 1983; He et al., 1996; Gunawardena et al., 2001). Aerenchyma formation has also been characterized in several *Carex* species (Moog, 1998; Visser et al., 2000a), *Acorus calamus* L. (Soukup et al., 2005), Nymphaeaceae and related taxa (Seago, 2002), and other plants (Seago et al., 1999). Other research has identified developmental processes related to flooding and, concomitantly, anatomy. However, on the aerenchyma of aquatic plants has been examined extensively by Kaul (1976), Justin and Armstrong (1987), and Seago et al. (2005).

Pohl and Lersten (1975) have applied such characterization for taxonomic purposes, showing that the aerenchyma within stems of *Hymenachne* can be a useful tool for distinguishing this genus from *Sacciolepis* (Poaceae). Seago et al. (2005) have attempted to compare the distribution of aerenchyma patterns with the angiosperm phylogeny tree from the Angiosperm Phylogeny Group (2003), although this investigation has focused only on patterns in the roots.

Our study took an anatomical approach with three objec-

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tives: i) to distinguish aquatic plants from wetland plants, ii) to determine specific aerenchyma patterns in roots and shoots, and iii) to expand our understanding of shoot aerenchyma. We also attempted to reveal phylogenic relationships based on aerenchyma patterns.

MATERIALS AND METHODS

Plant Materials

Aquatic and wetland plant materials classified into 21 orders, 42 families, 65 genera, and 110 species were collected in South Korea from 2004 to 2008.

For the root samples, sections were taken at least 20 mm above the root tip to observe fully developed aerenchyma. Whereas most aquatic plants have only adventitious roots that could be examined, taproots were obtained from a few wetland plants, including *Rorippa islandica* (Brassicaceae) and *Bidens tripartite* (Asteraceae). We also verified morphological patterns in root aerenchyma by comparing our results with those previously reported (Justin and Armstrong, 1987; Seago et al., 2005; Fig. 1).

To observe aerenchyma patterns in shoots, we investigated several organs, e.g., stems or culms, rhizomes, petioles, peduncles, and leaves, in order to discover variations within a species or, in many cases, where species had reduced, condensed, or specialized stems. In particular, we tested sporophylls as the alternative shoots in ferns.

Light Microscopy

All plant samples were fresh and mature. Immediately after collection, tissues from the roots and shoots were separately fixed overnight at room temperature in FAA (50% ethanol, 5% glacial acetic acid, 10% formalin, and 35% water). Samples were dehydrated through a graded ethanol series, then replaced with xylene and embedded in paraffin. Blocks were cut into 8- to 15-µm-thick sections with a rotary microtome (Microm, model HM325, Germany). After the paraffin was removed with xylene, the sections were stained with Safranin O and Fast Green FCF or Toluidine Blue O (Ruzin, 1999). They were then mounted with Poly-Mount (Polysciences, Inc., USA) and examined under a light microscope (Olympus, model IX50, Japan). In addition, some fresh plant samples were sectioned manually with a two-sided razor blade, and stained with Toluidine Blue O or Safranin O.

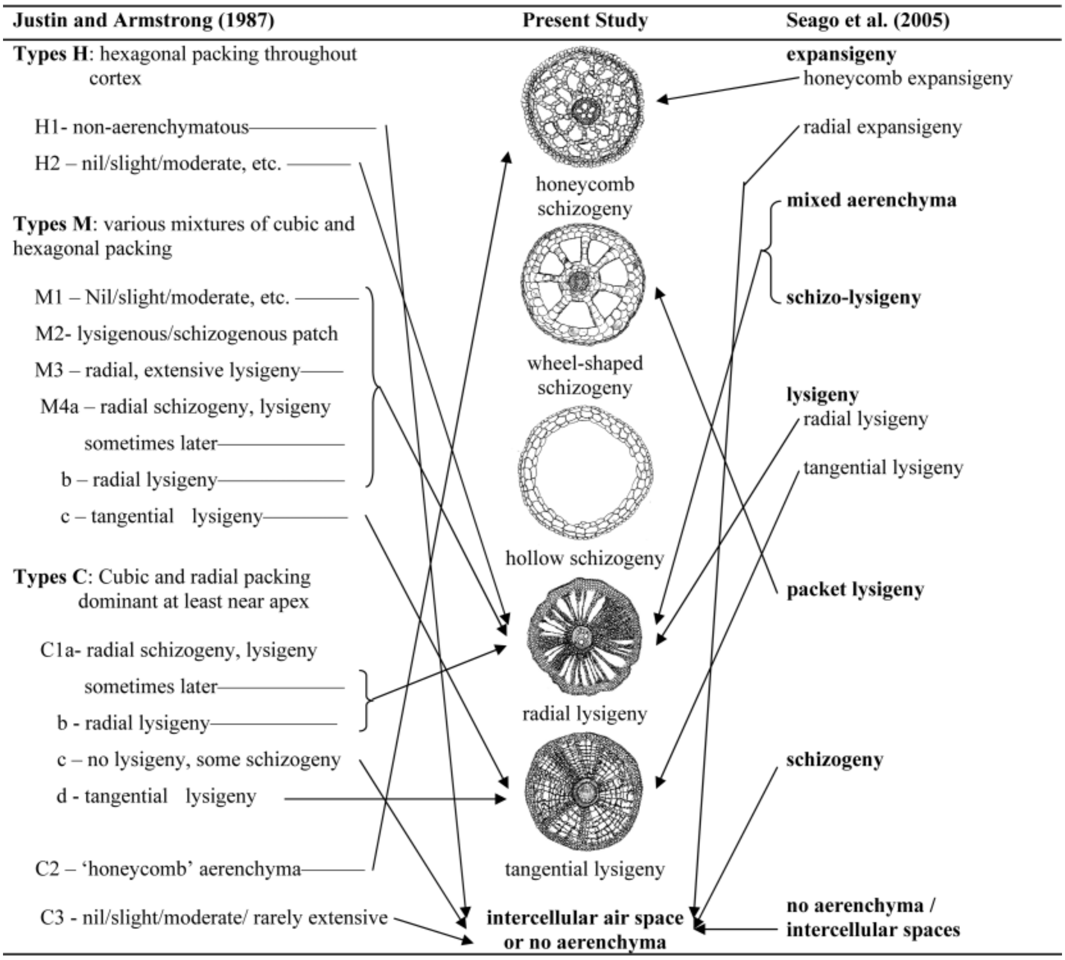

Figure 1. Comparison of root aerenchyma characterization from previous and present studies. Justin and Armstrong (1987) classified aerenchymas into 3 major types (Types H, M, and C), with 14 lower categories based on cell arrangements in roots. Seago et al. (2005) characterized root aerenchymas into 6 major types. Arrows indicate rearrangements of type among these and present studies.

Scanning Electron Microscopy

For observations via scanning electron microscope (SEM), fresh tissues were fixed for 4 h at 4°C, using a modified Karnovsky's fixative (2% paraformaldehyde and 2% glutaraldehyde in 0.05 M sodium cacodylate buffer). These primary tissues were then post-fixed for 2 h at 4°C using 1% osmium tetroxide in 0.05 M sodium cacodylate buffer before being washed with distilled water. Afterward, they were dehydrated in a graded ethanol series, then placed in a critical point dryer (Cosmos SP1, USA) and liquid carbon dioxide. The dried tissues were mounted on stubs and sputter-coated with gold under vacuum before viewing in an SEM (JSM-6380; JEOL, Japan).

Arrangement of Taxa

A total of 110 aquatic and wetland species examined here, plus 2 species from a previous study (Tomlinson, 1982), were arranged into 5 habitat types: emergent-, submerged-, free-floating aquatic plant, aquatic plant with free-floating leaves, and wetland plant (Choi, 1985). The arrangement order for ferns was based on suggestions by Pryer et al. (2004). For angiosperms, we followed the categorization of the Angiosperm Phylogeny Group (2003).

RESULTS

The ferns were classified into three species of Isoetales and two of Equisetales and Marsileales. Angiosperms were placed into either basal angiosperms (5 species), monocots (66 species), Ceratophyllales (1 species), or eudicots (35 species). Their aerenchyma patterns and habits are presented in Table 1.

Table 1. Aquatic and wetland plants examined in the present study and their aerenchyma patterns in which angiosperms are arranged according to the APG II classification system.

Taxon*	Habit**	Aerenchyma pattern***	
		Root	Shoot
LYCOPHYTES			
Order Isoetales			
Family Isoetaceae			
Isoetes coreana Y. H. Chung et H.-K.Choi	A_{em}	HW	WA
I. hallasanensis H.-K. Choi, C. Kim et J. Jung	A_{em}	HW	WA
I. jejuensis H.-K. Choi, C. Kim et J. Jung	A_{em}	HW	WA
MONILOPHYTES			
Order Equisetales			
Family Equisetaceae			
Equisetum arvense L.	W	I	WA
Order Marsileales			
Family Marsileaceae			
Marsilea quadrifolia L.	A_{em}, A_{fl}	RL	WA
ANGIOSPERMS			
Basal angiosperms			
Order Nymphaeales			
Family Cabombaceae			
Brasenia schreberi J.F. Gmel.	A_{fl}	HA	HA~WA
Family Nymphaeaceae			
Nuphar pumilum (Timm.) DC. var. *pumilum*	A_{fl}	HA	HA
N. pumilum var. *ozeense* (Miki) Hara	A_{fl}	HA	HA
Nymphaea tetragona Georgi	A_{fl}	HA	HA+SA
Euryale ferox Salisb.	A_{fl}	HA	SA
Monocots			
Order Acorales			
Family Acoraceae			
Acorus calamus var. *angustatus* Besser	A_{em}	HA	HA+LA
Order Alismatales			
Family Araceae			
Lemna paucicostata (L.) Hegelm.	A_{ff}	I	LA
Family Hydrocharitaceae			
Ottelia alismoides (L.) Pers.	A_{sm}	WA	HA
Hydrocharis dubia (Blume) Backer	A_{fl}	WA	HA
Vallisneria natans (Lour.) Hara	A_{sm}	HA	HA
Hydrilla verticillata (L. f.) Royle	A_{sm}	I	HA

Table 1. Continued.

Taxon*	Habit**	Aerenchyma pattern***	
		Root	Shoot
Najas graminea Delile	A_{sm}	HA	WA
N. marina L.	A_{sm}	HA	WA
Family Alismataceae			
Alisma canaliculatum A. Braun & Bouché	A_{em}	n.a.	HA+HW
A. orientale (Samuelsson) Juz. in Kom.	A_{em}	RL	HA
Sagittaria pygmaea Miq.	A_{em}	n.a.	HA
S. sagittifolia subsp. *leucopetala* (Miq.) Hartog var. *leucopetala*	A_{em}	RL	HA
S. sagittifolia subsp. *leucopetala* var. *edulis* (Schlecht) Rataj	A_{em}	RL	HA
Family Juncaginaceae			
Scheuchzeria palustris L.	W	RL†	HA†
Triglochin maritimum L.	A_{em}	I	HA
T. palustre L.	A_{em}	RL†	HA†
Family Ruppiaceae			
Ruppia maritima L.	A_{sm}	I	HA
Family Zosteraceae			
Zostera marina L.	A_{sm}	n.a.	LA
Z. nana Roth	A_{sm}	n.a.	LA
Family Potamogetonaceae			
Potamogeton pectinatus L.	A_{sm}	n.a.	HA
P. maackianus A. Benn.	A_{sm}	RL	HA
P. natans L.	A_{sm}, A_{fl}	n.a.	HA
P. gramineus L.	A_{sm}, A_{fl}	n.a.	HA
P. distinctus A. Benn.	A_{sm}, A_{fl}	RL	HA
P. malaianus Miq.	A_{sm}, A_{fl}	RL	HA
P. alpinus Balb.	A_{sm}	n.a.	HA
P. perfoliatus L.	A_{sm}	n.a.	HA
P. cristatus Regel & Maack	A_{sm}, A_{fl}	I	HA
P. octandrus Poir.	A_{sm}, A_{fl}	n.a.	HA
P. crispus L.	A_{sm}	RL	HA
P. oxyphyllus Miq.	A_{sm}	n.a.	HA
P. pusillus L.	A_{sm}	n.a.	HA
Zannichellia palustris L.	A_{sm}	RL	HA
Order Asparagales			
Family Iridaceae			
Iris pseudacorus L.	W	I	LA
Order Poales			
Family Sparganiaceae			
Sparganium erectum L.	A_{em}	RL	LA
S. japonicum Rothert	A_{em}	RL	HA
Family Typhaceae			
Typha orientalis C. Presl	A_{em}	RL	LA
T. angustifolia L.	A_{em}	RL	LA
T. laxmannii Lepech.	A_{em}	RL	LA
T. latifolia L.	A_{em}	RL	LA
Family Juncaceae			
Juncus effusus var. *decipiens* Buchenau	W	RL	HW
J. krameri Franch. & Sav.	W	RL	HW
Family Cyperaceae			
Eleocharis dulcis Trin. ex Hensch.	A_{em}	TL	HW
E. kuroguwai Ohwi	A_{em}	TL	HW

Table 1. Continued.

Taxon*	Habit**	Aerenchyma pattern***	
		Root	Shoot
E. acicularis for. *longiseta* (Svenson) T. Koyama	A_{em}	TL~RL	LA
E. congesta D. Don	A_{em}	TL	LA
E. mamillata var. *cyclocarpa* Kitag.	A_{em}	TL	LA
E. equisetiformis (Meinsh.) B. Fedtschenko	A_{em}	TL	LA
Carex transversa Boott ex A. Gray	A_{em}	TL	HA
C. dickinsii Fr. et Sav.	W, A_{em}	TL	LA
C. iria L.	W, A_{em}	TL	HA
Bolboshoenus planiculmis (F. Schmidt) T. V. Egorova	A_{em}	TL	HA+LA
B. fluviatilis (Torr.) Sojak	A_{em}	TL	HA+LA
Schoenoplectus mucronatus (L.) Palla	A_{em}	TL	HA+LA
S. triqueter (L.) Palla	A_{em}	TL	HA
Scirpus radicans Schkuhr	A_{em}	TL	LA
Family Eriocaulaceae			
Eriocaulon sieboldianum Siebold & Zucc. ex Steud.	A_{em}	RL	HA+LA
Family Poaceae			
Leersia japonica Makino	W, A_{em}	RL	HW + LA
Zizania latifolia Turcz.	A_{em}	RL	HW + LA
Oryza sativa subsp. japonica S. Kato	A_{em}	RL	HW + LA
Phragmites australis (Cav.) Trin. ex Steud.	A_{em}	RL	HW + LA
Echinochloa oryzicola (Vasinger) Vasinger	A_{em}	RL	HW + LA
Beckmannia syzigachne (Steudel.) Fernald	W, A_{em}	RL	HW + LA
Order Commelinales			
Family Commelinaceae			
Aneilema keisak Hassk.	A_{em}	TL	HA
Family Pontederiaceae			
Monochoria korsakowii Regel & Maack	A_{em}	RL	HA+HW+LA
M. vaginalis var. *plantaginea* (Roxb.) Solms	A_{em}	n.a.	HW+LA
Order Ceratophyllales			
Family Ceratophyllaceae			
Ceratophyllum demersum L.	A_{sm}	n.a.	HA
Eudicots			
Order Ranunculales			
Family Ranunculaceae			
Ranunculus kadzusensis Makino	A_{sm}	I	HA+HW
R. yezoensis Nakai	A_{sm}	I	HW
R. sceleratus L.	A_{em}	RL	HA
R. cantoniensis DC.	W	RL	HA+HW
Order Proteales			
Family Nelumbonaceae			
Nelumbo nucifera Gaertn.	A_{fl}	HA	SA
Order Caryophyllales			
Family Polygonaceae			
Persicaria amphibia (L.) Gray	A_{fl}	HA	HW
P. thunbergii Siebold & Zucc.	W	HA	HA
Rumex conglomeratus Murr.	W	HA	HA
Family Amaranthaceae			
Suaeda japonica Makino	A_{em}	RL	WA
Order Saxifragales			
Family Haloragaceae			
Myriophyllum spicatum L.	A_{sm}	RL	WA
Order Myrtales			

Table 1. Continued.

Taxon*	Habit**	Aerenchyma pattern***	
		Root	Shoot
Family Lythraceae			
Rotala indica (Willd.) Koehne	A_{sm}, A_{em}	n.a.	HA
R. koreana Nakai	A_{sm}, A_{em}	n.a.	HA
Trapa incisa Siebold & Zucc.	A_{fl}	n.a.	HA
T. japonica Flerow	A_{fl}	HA	HA
Family Onagraceae			
Ludwigia ovalis Miq.	A_{sm}, A_{em}	HA	HA
Order Malpighiales			
Family Elatinaceae			
Elatine triandra Schkuhr	A_{sm}, A_{em}	n.a.	WA
Order Cucurbitales			
Family Cucurbitaceae			
Actinostemma lobatum (Maxim.) Maxim. ex Franch. & Sav.	W	I	I
Order Brassicales			
Family Brassicaceae			
Nasturtium officinale R. Br.	A_{em}	RL	I
Rorippa islandica (Oeder) Borbas	W	I	HW
Order Lamiales			
Family Scrophulariaceae			
Limnophila sessiliflora (Vahl) Blume	A_{sm}	RL	HA~WA+WA
Veronica peregrina L.	A_{em}	RL	HA
V. undulata Wall. ex Jack	A_{em}	RL	HA
Lindernia procumbens (Krock.) Philcox	W, A_{em}	RL	HA~WA
L. dubia (L.) Pennell	W, A_{em}	RL	HA~WA
Family Lamiaceae			
Lycopus lucidus Turcz.	W	RL	HA
Family Phrymaceae			
Mazus pumilus (Burm. f.) Steenis	W	RL	I
Family Pedaliaceae			
Trapella sinensis Oliv.	A_{fl}	RL	HA
Family Lentibulariaceae			
Utricularia tenuicaulis Miki	A_{ff}	n.a.	WA
Order Apiales			
Family Apiaceae			
Oenanthe javanica (Blume) DC.	A_{em}	RL	HA+HW
Order Asterales			
Family Companulaceae			
Lobelia chinensis Lour.	W	n.a.	HA~WA
Family Menyanthaceae			
Menyanthes trifoliata L.	A_{em}	HA	HA
Nymphoides peltata (S.G. Gmel.) Kuntze	A_{fl}	RL	HA
N. indica (L.) Kuntze	A_{fl}	RL	HA
N. coreana (H. Lév.) H. Hara	A_{em}, A_{fl}	n.a.	WA
Family Asteraceae			
Bidens tripartita L.	W	RL	HA

*Taxa were arranged according to the Angiosperm Phylogeny Group (2003), while fern arrangement followed that of Pryer et al. (2004).

**Habit abbreviations: Aem, emergent aquatic; Asm, submerged aquatic; Afl, aquatic with free-floating leaves; Aff, free-floating aquatic; W, wetland.

***Pattern abbreviations: HA, honeycomb aerenchyma; TL, tangential lysigeny; RL, radial lysigeny; SA, schizogenous air canal; WA, wheel-shaped aerenchyma; HW, hollow aerenchyma; LA, leafy aerenchyma; I, intercellular air space, or non-aerenchyma; '+' indicates a case where two or more types are observed in a species; '∼' means an intermediate shape was observed; n.a., data not available.

[†] Refer to Tomlinson (1982).

Aerenchyma Patterns in Roots

Three common types -- honeycomb aerenchyma, tangential- and radial lysigeny -- and two rare types -- wheel-shaped and hollow aerenchyma -- were found in the roots (Fig. 1). Both honeycomb aerenchyma and tangential lysigeny were homologous to those described previously (Seago et al., 2005), while radial lysigeny was only considered to be a similar feature (Fig. 1). All examined basal angiosperms, i.e., *Brasenia schreberi* (Cabomaceae) and two species of *Nuphar* (Nymphaeaceae), had honeycomb aerenchyma formed via schizogeny (Table 1; Fig. 2A, B). That same pattern was noted in the monocots, including *Acorus* (Acoraceae), and *Vallisneria* and *Najas* (Hydrocharitaceae); and in the eudicots *Nelumbo* (Nelumbonaceae), *Persicaria* and *Rumex* (Polygonaceae), *Trapa* (Lythraceae), *Ludwigia* (Onagraceae), and *Menyanthes* (Menyanthaceae) (Table 1; Fig. 2C, D). In contrast, tangential lysigeny was observed in only two groups: *Aneilema keisak* (Commelinaceae) and all of the Cyperaceae examined (Table 1; Fig.

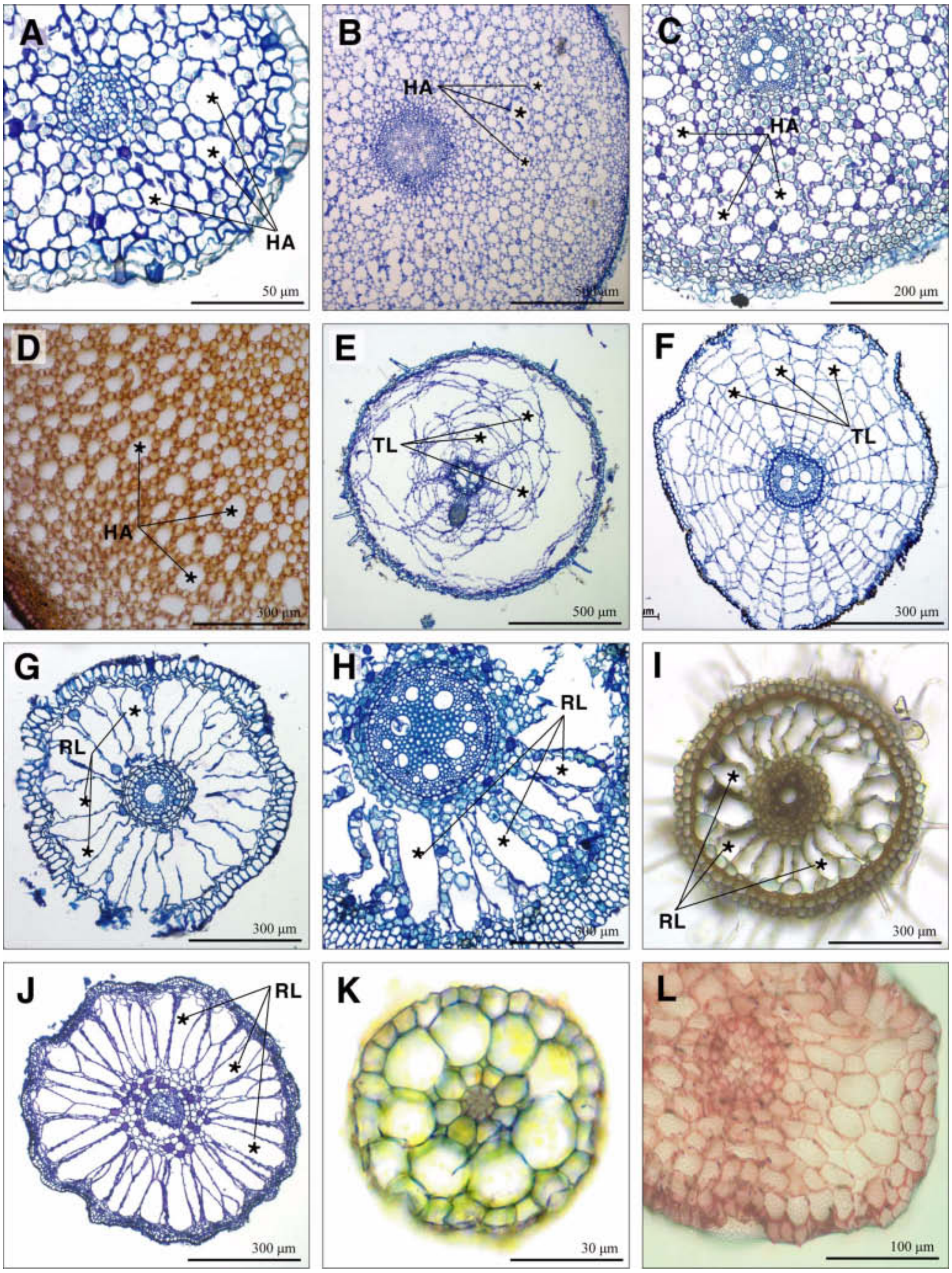

Figure 2. Cross-sections of root organs in aquatic vascular plants. Honeycomb aerenchyma (HA): **A**, *Brasenia schreberi*; **B**, *Nuphar pumilum* var. *pumilum*; **C**, *Acorus calamus* var. *angustatus*; **D**, *Menyanthes trifoliate*. Tangential lysigeny (TL): **E**, *Aneilema keisak*; **F**, *Eleocharis kuroguwai*. Radial lysigeny (RL): **G**, *Juncus krameri*; **H**, *Typha latifolia*; **I**, *Oryza sativa* subsp. *japonica*; **J**, *Trapella sinensis*. Intercellular air space or no aerenchyma (I): **K**, *Lemna paucicostata*; **L**, *Ruppia maritima*.

2E, F). Radial lysigeny was the most common type in the sampled roots, and was also found in numerous taxa, including *Marsilea* (Marsileaceae) of the monilophytes; and in monocots, such as *Alisma* (Alismataceae), *Potamogeton* (Potamogetonaceae), and all of the Poaceae tested (Table 1; Fig. 2G-I). Radial lysigeny was also abundant in several eudicots, e.g., *Nasturtium* (Brassicaceae), *Limnophila* (Scophulariaceae), and *Nymphoides* (Menyanthaceae) (Table 1; Fig. 2J). The less-common wheel-shaped aerenchyma was observed only in two Hydrocharitaceae, *Ottelia alismoides* and *Hydrocharis dubia* (Table 1). This type was homologous with the packet lysigeny described by Seago et al. (2005; Fig. 1). Hollow aerenchyma, characterized by a large cylindrical lacuna in the center, was found in three species of *Isoetes* (Isoetaceae) of the lycophytes (Table 1). However, we found no aerenchyma in the roots of aquatic plants, including *Lemna paucicostata* (Araceae), *Hydrilla verticillata* (Hydrocharitaceae), *Ruppia maritima* (Ruppiaceae), and *Ranunculus kadzuensis* and *R. yezoensis* (Ranunculaceae), or in the root tissues of several wetland plants, e.g., *Equisetum arvense* (Equisetaceae) and *Rorippa islandica* (Brassicaceae) (Table 1; Fig. 2K, L).

Aerenchyma Patterns in Shoots

Among the shoots examined here, five types of aerenchyma were observed -- honeycomb, wheel-shaped, hollow, or leafy aerenchyma, and schizogenous air canals (Fig. 3). Two types of shoot aerenchyma were morphologically different from those in the roots, including schizogenous air canals and leafy aerenchyma. Those types with identical names were not necessarily homologous to one another in their developmental processes or origins. Although honeycomb aerenchyma was the major type seen in our tested shoots (Table 1; Figs. 3A, B, 4A-C), it was not seen in the lycophytes or monilophytes. Within a species, honeycomb aerenchyma was often observed together with other types, such as schizogenous air canals or hollow aerenchyma (Table 1; Fig. 4D). Occasionally, this type was found as an intermediate form with wheel-shaped aerenchyma in *Lobelia chinensis* (Campanulaceae) and three species of Scrophulariaceae: *Limnophila sessiliflora*, *Lindernia procumbens*, and *L. dubia* (Table 1; Fig. 4E, F). Schizogenous air canals were found in two basal angiosperms -- *Nymphaea tetragona* and *Euryale ferox* (Nymphaeaceae) -- and in one eudicot, *Nelumbo nucifera* (Nelumbonaceae) (Table 1; Fig. 3C).

Wheel-shaped aerenchyma comprised wide air spaces of regular size that were circular or polygonal. This wheel-shaped type was also found in the shoots of ferns, such as *Isoetes coreana* (Isoetaceae) and *Marsilea quadrifolia* (Marsileaceae); and in angiosperms, e.g., two species of *Najas* (Hydrocharitaceae) and *Utricularia tenuicaulis* (Lentibulariaceae) (Table 1; Figs. 3D, 4G-I). Hollow aerenchyma was observed in the shoots of Poales plants, *Eleocharis* (Cyperaceae), *Juncus* (Juncaceae), and in Poaceae species (Table 1; Fig. 4J). This type was also recorded for the monocot *Alisma canaliculatum* (Alismataceae) and eudicots, the *Ranunculus* species (Ranunculaceae) and *Oenanthe javanica* (Apiaceae) (Table 1; Figs. 3E, 4J). We examined leaves from plants with condensed or reduced stems, such as *Acorus* (Acoraceae), *Lemna* (Araceae), *Zostera* (Zosteraceae), and *Typha* (Typhaceae), as well as from several monocots with stems or culms, including several Cyperaceae and Poaceae members, plus two from *Monochoria* (Pontederiaceae). Most of the above had leafy aerenchyma, such as in the leaves and culms of *Typha orientalis* (Typhaceae) and those in Cyperaceae, e.g., *Eleocharis congesta*, *E. equisetiformis*, and *Bolboschoenus planiculmis* (Table 1; Figs. 3F, 4K-M).

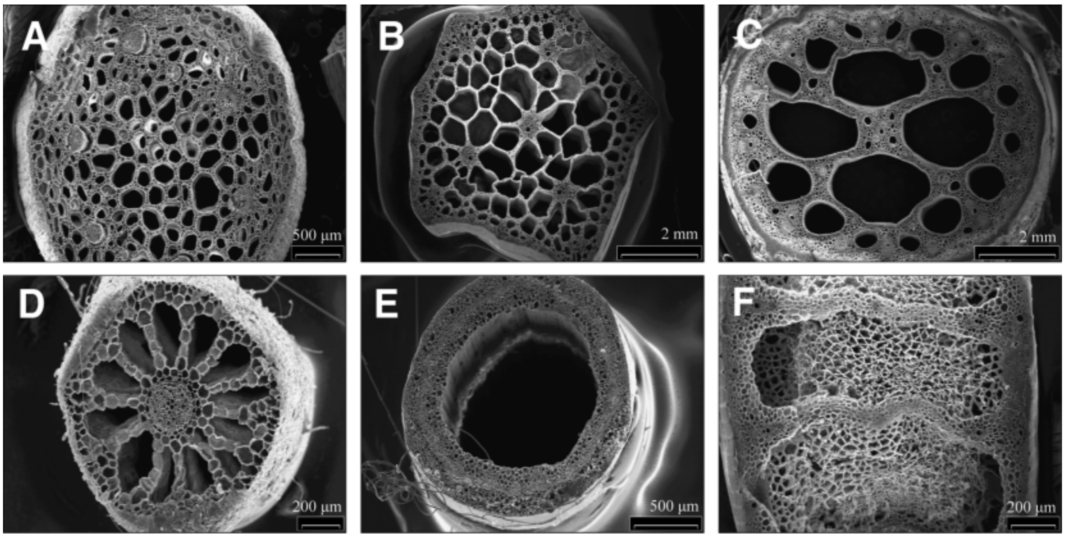

Figure 3. Morphological patterns of shoot aerenchyma in aquatic vascular plants. **A**, Honeycomb aerenchyma (HA), *Menyanthes trifoliata*; **B**, *Sagittaria sagittifolia* subsp. *leucopelata* var. *edulis*; **C**, Schizogenous air canal (SA), *Euryale ferox*; **D**, Wheel-shaped aerenchyma (WA), *Myriophyllum spicatum*; **E**, Hollow aerenchyma (HW), *Ranunculus cantoniensis*; and **F**, Leafy aerenchyma (LA), *Typha latifolia*.

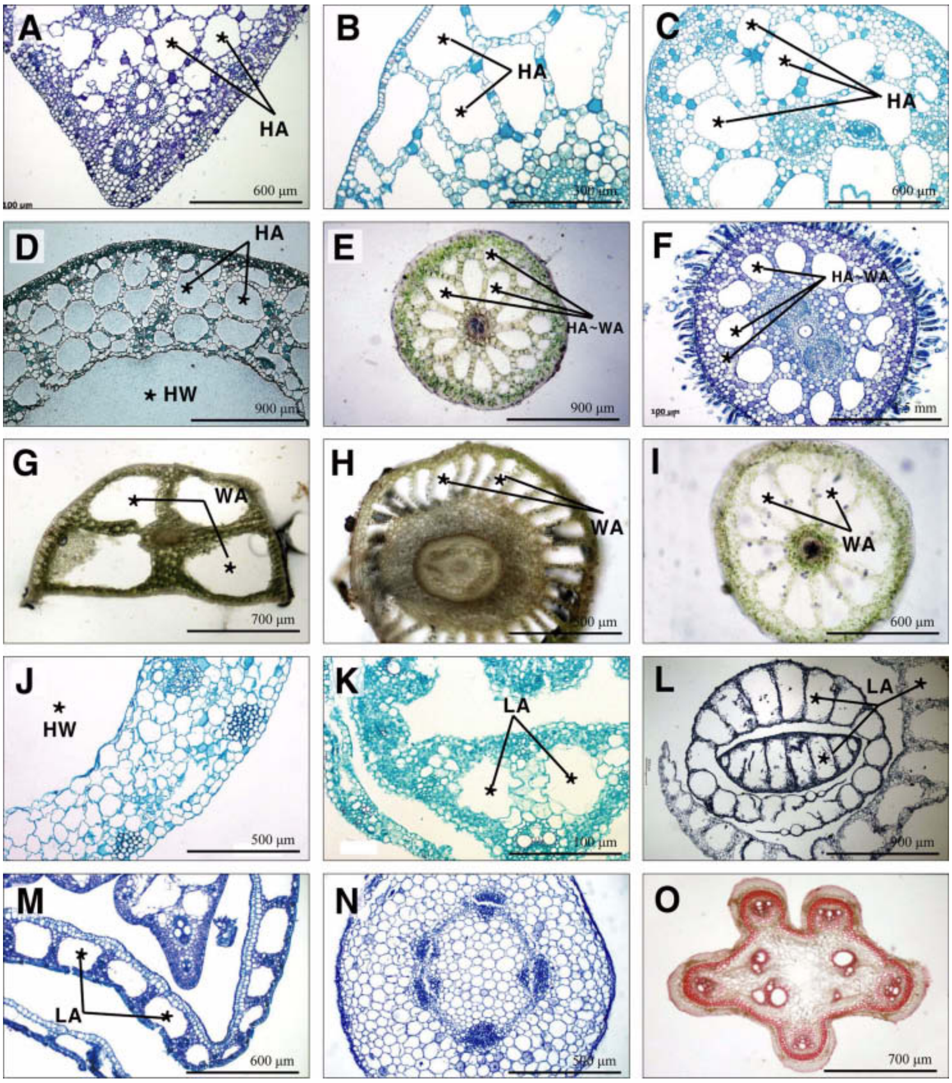

Figure 4. Cross sections of shoot organs in aquatic vascular plants. Honeycomb aerenchyma (HA): **A**, *Sagittaria sagittifolia* subsp. *leucopelata* var. *edulis*; **B**, *Potamogeton distinctus*; **C**, *Nymphoides peltata*; **D**, *Monochoria korsakowii*. Intermediate type: **E**, *Limnophila sessiliflora*; **F**, *Brasenia schreberi*. Wheel-shaped aerenchyma (WA): **G**, *Isoetes coreana*; **H**, *Marsilea quadrifolia*; **I**, *Myriophyllum spicatum*. Hollow aerenchyma (HW): **J**, *Oenanthe javanica*. Leafy aerenchyma (LA): **K**, *Oryza sativa* subsp. *japonica*; **L**, *Typha orientalis*; **M**, *Schoenoplectus mucronatus*; **N**, *Nasturtium officinale*; **O**, *Actinostemma lobatum*.

DISCUSSION

Presence of Aerenchyma as a Morpho- Anatomical Criterion for Aquatic versus Wetland Plants

All but a few species of the aquatic plants tested here had well-developed aerenchyma. Likewise, except for *Nasturtium officinale* (Brassicaceae, Table 1; Fig. 4N), all shoots had aerenchyma. In contrast, wetland species, such as *Iris pseudacorus* (Iridaceae) and *Rorippa islandica* (Brassicaceae), had aerenchyma in their shoots but not in their roots (Table 1); others showed such structures in both organs. In the roots of typical aquatic plants, i.e., *Lemna paucicostata* (Araceae), *Ruppia maritima* (Ruppiaceae), and *Hydrilla verticillata* (Hydrocharitaceae), aerenchyma was not observed but only small intercellular spaces. These plants frequently had very thin adventitious roots. Some non-aquatic plants form aerenchyma even when not in environments that experience flooding (Hejnowicz and Barthlott, 2005; Lee et al., 2007), and some wetland species have induced aerenchyma when faced with flooding (Visser et al., 2000b). Even though aquatic plants require aerenchyma to survive long-term submergence, this presence cannot be used to generate a strict boundary between aquatic vascular plants and wetland plants. However, through our extensive study, we have now re-confirmed that well-developed aerenchyma is an important feature of the former. This fact is strongly supported by Justin and Armstrong (1987), who have measured the root porosity of numerous plants.

Categorization of Root Aerenchyma

We characterized root aerenchyma into three common types (honeycomb aerenchyma, radial-, and tangential lysigeny) and two rare types (wheel-shaped- and hollow aerenchyma) (Fig. 1). In previous studies, aerenchyma had been

Table 2. Summary of aerenchyma patterns in roots and shoots of aquatic and wetland plants.

Aerenchyma type		Occurrence within each taxonomic rank*							
		Root				Shoot			
		Order (16)	Family (32)	Genus (52)	Species (96)	Order (20)	Family (40)	Genus (63)	Species (109)
Schizogeny	Honeycomb	7	9	13	17	12	22	38	66
	Wheel-shaped	1	1	2	2	8	9	9	13
	Hollow	1	1	1	3	8	10	14	19
	Air canal	-	-	-	-	2	2	3	3
	Leafy	-	-	-	-	5	10	19	28
Lysigeny	Radial	11	20	30	58	-	-	-	-
	Tangential	2	2	6	16	-	-	-	-
Sum		22	33	52	96	35	53	83	129

*Value is the number of taxa having this aerenchyma type at each taxonomic rank. Numbers in parentheses indicate total taxa having aerenchyma in the present study.

merged or divided into larger or smaller categories based on cell arrangement or developmental process (Justin and Armstrong, 1987; Seago et al., 2005). This difference arose because that group of aquatic plants is polyphyletic (Les et al., 2003), i.e., their developmental processes are independent of morphological similarities because, in many cases, the aerenchyma within polyphyletic groups may be products of convergence.

Here, we did not consider small air spaces, created by intercellular gaps, to be aerenchyma. This is in contrast to the conclusion of Justin and Armstrong (1987), who characterized those spaces as various types of aerenchyma. Our reasoning was that land plants, under special conditions, often create similar structures as temporary paths for gas diffusion (Justin and Armstrong, 1987; Colmer, 2003; Thomas et al., 2005). Therefore, we separated root aerenchyma patterns into five types, which included the novel hollow aerenchyma, but excluded small intercellular air spaces. Finally, schizogenous aerenchymas occurred less frequency than lysigenous aerenchyma in the roots. Of the 96 species that had aerenchyma in their roots, honeycomb-, hollow-, and wheel-shaped aerenchyma of schizogeny were found in 17, 3, and 2 species, respectively, whereas radial- and tangential lysigeny were observed in 58 and 16 species, respectively (Table 2). These proportions in patterning were similar at the genus, family, and order level (Table 2).

Diversity and Categorization of Shoot Aerenchyma

Shoot aerenchymas were arranged into five categories (Fig. 3). In all, the most common was the honeycomb type, found in 66 of 109 species having shoot aerenchyma; this was followed by hollow-, wheel-shaped-, and leafy aerenchyma in 19, 13, and 28 species, respectively (Table 2). The most rarest type, the schizogenous air canal, was found in 3 species (Table 2). Another intermediate aerenchyma form, between honeycomb and wheel-shaped, was observed in 5 species; 19 species had more than one type. However, this trend in patterns seen at the species level did vary at the levels of family and order because of either the lower consistency within each taxon or a scattered distribution of shoot aerenchyma patterns (Table 2).

As seen in *Sagittaria* of Alismataceae (Schussler and Longstreth, 1996; Liang et al., 2008), the shoot is a complex structure consisting of various organs such as stems, leaves, and peduncles. Because of this complexity, shoot aerenchymas are more varied than those in the roots. Honeycomb and wheel-shaped aerenchyma were morphologically similar in both shoots and roots (Figs. 1, 3). In addition, an intermediate form between those two types occurs in the shoots of *Brasenia schreberi* (Cabombaceae) and several species of Scrophulariaceae, thereby suggesting a relationship between the two. However, we cannot assume that similarities between root and shoot aerenchyma were homologous because of the unknown formation processes for shoot aerenchymas and the different origins of each organ. For example, honeycomb aerenchyma in the roots was evenly sized while that of the shoots was irregular and larger. Moreover, we need more consideration for similarities among aerenchymas of taxa within a single type, because those taxa may not share a common ancestor.

Nonetheless, we suggest that shoot aerenchyma patterns can be morphologically characterized into five types, and that shoot aerenchyma has intermediate forms and variety within a species.

Distinction between Schizogenous and Lysigenous, and Other Processes

In contrast to the leafy aerenchyma of *Typha latifolia* that is formed by cell lysis, *Sagittaria lancifolia* has schizogenous aerenchyma that develops via cell elongation and separation (Kaul, 1974; Schussler and Longstreth, 1996). Kausch and Horner (1981) have reported that aerenchyma formation in the young leaves of *Typha angustifolia* occurs through schizolysigenous development, and is associated with crystal idioblast formation around an air space. In addition to lysigeny and schizogeny, Seago et al. (2005) have suggested that expansigeny is another process of root aerenchyma formation. This phenomenon refers to aerenchyma that forms by expansion but without further cell separation or cell death. Seago et al. (2005) also have shown that more than one process takes place during the formation of schizo-lysigeny and mixed aerenchyma. Our study identified additional types of aerenchyma in the shoots that were not found in the roots. Unfortunately, because the developmental mechanisms responsible for their formation is ambiguous (Lee et al., 2007), we cannot determine whether shoot aerenchyma undergoes schizogeny, lysigeny, or another process. Nonetheless, we are certain that each aerenchyma type has a characteristic morphology and developmental mechanism.

Phylogenic Comparison

Through phylogenic comparisons, we found consistencies in aerenchyma patterns within each genus as well as significant, similar trends among higher taxa. However, it was not possible to simply relate aerenchyma patterns of the taxa with their phylogenies for the following reasons. First, these aquatic plants were derived from various origins. Moreover, the aerenchyma patterns of several taxa were determined from only a few species here, and our results cannot be considered representative of an entire taxon. Finally, as discussed previously, we found ambiguous homologies among aerenchymas of other taxa within a single category. Therefore, our discussion will focus on the relationship between aerenchyma patterns and phylogeny only within monophyletic groups.

Initially, we noted that aerenchyma patterns of roots and shoots were consistent in most genera, although there were small differences in the shoots depending on what organs were present or what part of the shoot was examined (Table 1). This also was true among the 52 genera with root aerenchyma. Patterns for shoot aerenchyma were consistent for 55 (87%) of 63 genera (Table 2).

Furthermore, trends for aerenchyma patterns were significant among taxa, higher than they were at the genus level (see Table 1). Briefly, all ferns had wheel-shaped aerenchyma in their shoots even though lycophytes and monilophytes are paraphyletic. The root aerenchymas of Nymphaeales of basal angiosperms, typical aquatic plants, were homogeneous, while several types were observed in the characteristic air circular systems of their shoots (Matthews and Seymour, 2006). Morphological trends were clear for both organs in three families -- Alismataceae, Potamogetonaceae, and Typhaceae. The rare wheel-shaped aerenchyma in roots was seen only in two species of Hydrocharitaceae (*Ottelia alismoides* and *Hydrocharis dubia*). This contrasts with reports by Seago et al. (1999, 2005), who discovered packet lysigeny (wheel-shaped aerenchyma) in *Pistia stratiotes* (Araceae), *Hydrocharis*, and *Stratiotes* of Hydrocharitaceae. The tangential lysigeny in the roots of Cyperaceae observed here is consistent with results from other studies (Moog, 1998; Visser et al., 2000a). In addition, this type was homogeneous within root samples from that family. Tangential lysigeny was also found in the roots of *Aneilema keisak* of Commelinaceae (Fig. 2E), and its morphology was very similar to that of species within the family Cyperaceae. Both Commelinaceae and Cyperaceae are included in the Commelinids. However, the root aerenchymas of all other Commelinids plants -- such as from Poaceae, Juncaceae, and Pontederiaceae -- were of radial lysigeny. In contrast, leafy and hollow shoot aerenchymas could not yield phylogenic information because of their scattered distribution.

In root aerenchymas, consistency of patterns within a family was found in 29 (91%) of 32 families having aerenchyma; those patterns in 11 (69%) of 16 orders were consistent within each order (Table 2). This consistency was also discovered in the shoots, although it was not as strong as that in the roots. For shoot aerenchymas, patterns were consistent in 29 (73%) of 40 families, and in 12 (60%) of 20 orders (Table 2). Therefore, our results suggest that, even though these relationships are not always clear, this consistency of aerenchyma patterns in aquatic plants reflects their phylogenies.

We tried to determine a morpho-anatomical criterion between aquatic and wetland plants. Although no clear-cut boundary seemed to exist, we found several tendencies for aquatic plants with specific types of aerenchyma that was based on tissues and hierarchical ranks. Specific aerenchyma patterns are a type of plant adaptation to flooding stress (Lee et al., 2007) and can be a useful character for inferring the taxonomy and phylogeny of aquatic plants.

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