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REGULAR PAPER

Morphological analysis of vessel elements for systematic study of three Zingiberaceae tribes

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Abstract Zingiberaceae containing over 1,000 species that are divided into four subfamilies and six tribes. In recent decades, there has been an increase in the number of studies about vessel elements in families of monocotyledon. However, there are still few studies of Zingiberaceae tribes. This study aims to establish systematic significance of studying vessel elements in two subfamilies and three tribes of Zingiberaceae. The vegetative organs of 33 species processed were analysed by light and scanning electron microscopy and Principal Component Analysis was used to elucidate genera boundaries. Characteristics of vessel elements, such as the type of perforation plate, the number of bars and type of parietal thickening, are proved to be important for establishing the relationship among taxa. Scalariform perforation plate and the scalariform parietal thickening are frequent in Zingiberaceae and may be a plesiomorphic condition for this taxon. In the Principal Component Analysis, the most significant characters of the vessel elements were: simple perforation plates and partially pitted parietal thickening, found only in Alpinieae tribe, and 40 or more bars composing the plate in *Elettariopsis curtisii, Renealmia chrysotricha, Zingiber spectabile, Z. officinale, Curcuma* and *Globba* species. Vessel elements characters of 18 species of Alpinieae, Zingibereae and Globbeae were first described in this work.

Keywords Alpinoideae · Globbeae · Monocotyledon · Root · Xylem · Zingiberoideae

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Introduction

Zingiberales comprises approximately 2,000 species of herbaceous and rhizomatous plants distributed among eight families. Zingiberaceae containing 50 genera and over 1,000 species with pantropical distribution (Judd et al. 2009; Souza and Lorenzi 2012).

Previous morphological analysis divided Zingiberaceae into four tribes, Alpinieae, Globbeae, Hedychieae and Zingibereae (Holttum 1950; Larsen et al. 1998; Petersen 1889). The addition of molecular data generated a new proposal to split into four subfamilies (Siphonochiloiedeae, Tamijioideae, Alpinioideae and Zingiberoideae) and six tribes (Siphonochileae, Tamijieae, Alpinieae, Riedelieae, Zingibereae and Globbeae) (Kress et al. 2002). However, the evolutionary relationships within the larger genera (*Alpinia, Amomum, Etlingera, Curcuma* and *Globba*) are still not well understood (Pedersen 2004; Xia et al. 2004). Kress et al. (2005) expanded the studies on Zingiberaceae and proposed a new classification for *Alpinia*, taxonomically difficult and complex genus, dividing it into six clades: *Alpinia Fax, Alpinia Galanga, Alpinia*

Carolinensis, Alpinia Zerumbet, Alpinia Eubractea and *Alpinia Rafflesiana*.

Pedersen (2004) studied the phylogeny of Alpinioideae subfamily, based on nuclear and plastid DNA analysis. The author provides strong support that *Amomum, Geocharis, Hornstedtia* and *Etlingera* form a monophyletic group. Xia et al. (2004) confirmed the polifiletism of *Amomum* and proposed the division of this genus into three large groups, one of them a sister group containing *Paramomum* and *Elettariopsis*. For Globbeae tribe, an infragenetic classification was proposed based on analysis of the shape and number of anther appendages, characteristics of the inflorescence and fruit morphology. This classification divided the tribe into three subgenera, seven sections and two subsections (Williams et al. 2004).

Studies on vessel elements in monocotyledons began in the 1940s and have been enhanced over the last several years with an evolutionary and functional approach for different taxa (Carlquist 2012, 2005; Carlquist and Schneider 1998, 2006, 2007, 2010a, b, 2014; Cheadle 1942; Cheadle and Kosakai 1980, 1982; Pace et al. 2011; Rodrigues et al. 2007; Rodrigues and Estelita 2009; Schneider and; Thorsch 2000; Thorsch and Cheadle 1996; Wagner 1977).

Carlquist (2012) revisited phylogenetic, functional and ecological data within the aforementioned studies on monocotyledon xylem, to understand how this tissue would have evolved. The author found that the vessel elements are generally not present in the stems and leaves of monocotyledons.

To date, few studies have been reported on the distribution and specialization of tracheary elements in Zingiberaceae (Carlquist 2012; Gevú et al. 2014; Thorsch 2000; Tomlinson 1956, 1969; Wagner 1977). Therefore, this study aims to answer the following questions: (1) are there different degrees of specialization of these vessel elements in the roots? (2) Can characteristics of vessel elements be used as diagnostics for the distinction of two subfamilies (Alpinioideae and Zingiberoideae) and three tribes (Alpineae, Zingibereae and Globbeae) of Zingiberaceae?

Materials and methods

Selected species and study areas

The present study includes 33 native, naturalized and exotic species that are found in the protected and cultivated areas. Table 1 shows the list of the species in Zingiberaceae that were selected for the study and their vouchers in the Greenhouse (G), Herbarium of the Federal Rural University of Rio de Janeiro (RBR) and Herbarium of the Federal University of Acre (UFACPZ).

Some species of *Alpinia, Curcuma* and *Zingiber* were collected in the municipal district of Seropédica, located in the west of the state of Rio de Janeiro. This region has a warm and dry climate with an average rainfall of 100.2 mm per month. Collections were made at two distinct locations: Botanical Garden of UFRRJ, which comprises an area of 16.5 ha, located at 22°55′45″S × 42°58′54″W coordinates, with an average elevation of 27 m (Cysneiros et al. 2011; Miranda and Colombini 2009); Fazendinha Agroecológica, which is located at Embrapa Agrobiology, covering an extent of 59 ha, and has low fertility soil devoted to agroecology practice, at the 49 km of old Rio/São Paulo highway.

Renealmia chrysotricha and *Hedychium coronarium* were collected in the National Park of Itatiaia, located in the southwestern of Rio de Janeiro state, between the coordinates 22°30′ and 22°33′S × 42°15′ and 42°19′W (Lima and Guedes-Bruni 2004). The samples used in the present study were collected at 760 m asl. Local vegetation is classified as Montane Ombrophilous Dense Forest. *Etlingera elatior* and *E. fulgens* were collected in the Botanical Garden Research Institute of Rio de Janeiro (JBRJ).

Renealmia breviscapa and *R. nicolaioides* were collected in the Experimental Farm Catuaba (10°4′40 S × 67°37′35W), 1281 ha of area and in the Forest Reserve Humaitá (9°45′17S × 67°40′15W), 3,665 ha of area, located in the Rio Branco, Acre state (Storck-Tonon et al. 2009).

Twenty-one species of the genera *Alpinia, Etlingera, Amomum, Aframomum, Elettariopsis, Curcuma, Hedychium, Zingiber* and *Globba* were provided by Dr. John Kress, a researcher in the Botany Department of the Smithsonian's Research Institute (Washington DC, USA). Species are from the greenhouse (Botany Greenhouse/Smithsonian Institution, Suitland, MD).

Anatomy of the vegetative organs

Roots, rhizomes and leaves were sampled from mature individuals with approximately 2.0 m tall. The root fragments were collected 3.0 cm from root cap. The fragments of them were fixed in 70% FAA (Johansen 1940) and subsequently conserved in 70% alcohol (Jensen 1962).

To dissociate the tracheary elements of these organs, the modified Franklin (1945) method was used. The obtained fragments were stained with 1% aqueous safranin and then mounted on semi-permanent slides.

For scanning electron microscope analysis, dissociated tracheary elements were affixed to supports using carbon adhesive tape, dried at room temperature, covered with a 20 nm gold layer (Sputter Coater SCD050; Bal-Tec AG, Balzers, Liechtenstein), and examined with a DSM 962 scanning electron microscope (Zeiss, Jena, Germany).

Table 1 List of the species used in the evaluation of vessel elements, accompanied of the taxonomic categorization, origin and vouchers in the G, HRJ, RBR and UFACPZ

	Species	Subfamilie/tribe	Study area	Vouchers
1.	*Aframomum angustifolium* (Sonn.) K. Schum	Alpi/Alp	GH	92-039(G)
2.	*Alpinia purpurata* (Vieill.) K. Schum	Alpi/Alp	AF	34622(RBR)
3.	*A. zerumbet* (Pers.) B.L. Burtt & R. M. Sm	Alpi/Alp	CS	349739(RBR)
4.	*A. elegans* Ridl	Alpi/Alp	GH	94-756(G)
5.	*A. nigra* (Gaertn.) B.L. Burtt	Alpi/Alp	GH	02-078(G)
6.	*A. galanga* (L.) Willd	Alpi/Alp	GH	00-221(G)
7.	*Amomum delbatum* Roxb	Alpi/Alp	GH	06-033(G)
8.	*Elettariopsis curtisii* Baker	Alpi/Alp	GH	99-078(G)
9.	*Etlingera elatior* (Jack) R.M. Sm	Alpi/Alp	BGRJ	35796(RBR)
10.	*E. fulgens* (Ridl.) C.K.Lim	Alpi/Alp	BGRJ	35797(RBR)
11.	*E. yunnanensis* (T.L. Wu & S.J. Chen) R.M. Sm	Alpi/Alp	GH	95-229(G)
12.	*Renealmia breviscapa* Poepp. & Endl	Alpi/Alp	FH/EC	6645/6647 (UFACPZ)
13.	*R. chrysotricha* Petersen	Alpi/Alp	IN	33416(RBR)
14.	*R. nicolaiodes* Loes	Alpi/Alp	EC	6646(UFACPZ)
15.	*Curcuma longa* L	Zing/Zin	BGUF	34971(RBR)
16.	*C. zedoaria* (Christm.) Roscoe	Zing/Zin	BGUF	34972(RBR)
17.	*C. comosa* Roxb	Zing/Zin	GH	96-229(G)
18.	*C. domestica* Valeton	Zing/Zin	GH	01-072(G)
19.	*C. petiolata* Roxb	Zing/Zin	GH	00-040(G)
20.	*Hedychium borneense* R.M.Sm	Zing/Zin	GH	94-781(G)
21.	*H. coronarium* J. König	Zing/Zin	IN	33417(RBR)
22.	*H. greenei* W.W. Sm	Zing/Zin	GH	94-776(G)
23.	*H. thyrsiforme* Sm	Zing/Zin	GH	1994-773(G)
24.	*Zingiber officinale* Roscoe	Zing/Zin	BGUF	34975(RBR)
25.	*Z. spectabile* Griff	Zing/Zin	BGUF	34922(RBR)
26.	*Z. cassumunar* Roxb	Zing/Zin	GH	2000-070(G)
27.	*Z. corallinum* Hance	Zing/Zin	GH	95-199(G)
28.	*Z. wrayii* Prain ex Ridley	Zing/Zin	GH	94-844(G)
29.	*Z. mioga* (Thunb.) Roscoe	Zing/Zin	GH	1999-282(G)
30.	*Globba curtisii* Holttum	Zing/Glo	GH	89-050(G)
31.	*G. magnifica* M.F. Newman	Zing/Glo	GH	2004-011 (G)
32.	*G. sherwoodiana* W.J. Kress & V. Gowda	Zing/Glo	GH	97-141(G)
33.	*G. schomburgkii* Hook. f	Zing/Glo	GH	98-186(G)

Subfamilies: *Alpi* Alpinioideae, *Zing* Zingiberoideae; Tribes: *Alp* Alpinieae, *Zin* Zingibereae, *Glo* Globbeae, *GH* greenhouse, *AF* agroecological farm, *CS* cultivated in the municipality of Seropédica, *BGRJ* botanical garden at RJ, *FH* Forest Reserve Humaitá, *EC* experimental farm Catuaba, *IN* Itatiaia National Park, *BGUF* Botanical Garden at UFRRJ

Vessel elements analysis

To characterize and evaluate the degree of specialization of the vessel elements, the following characteristics were used: perforation plates, inclination of the end wall, number of bars and types of parietal thickening. The most matured tracheal elements in the metaxylem were chosen for the vessel elements analysis.

The reconstruction of a phylogenetic tree of the genera of Zingiberaceae, formerly proposed by Kress et al. (2002), was performed by using the software Mesquite (Maddison and Maddison 2011), and included new data on vessel elements. The mapping of the three characters, parietal thickening, arrangement and type of perforation plate, is presented in the phylogenetic trees shown in Figs. 2, 3 and 4. All analyzed species in this study are marked with a square in the tree and the branches ending striped line indicate clades that were not evaluated in this work.

The some species of *Alpinia* included in this study were placed, as in molecular studies (Kress et al. 2005), in the following clades: (1) *A. zerumbet—Alpinia Zerumbet* clade, (2) *A. purpurata* and *A. elegans—Alpinia Eubractea* clade, and (3) *A. galanga* and *A. nigra—Alpinia Galanga* clade.

Otherwise, *Amomum delbatum* was included into *Amomum* II group.

Statistical analysis

A Principal Component Analysis (PCA) correlation was performed to select taxonomically significant qualitative characters and to detect outliers. Pair-wise relationship was estimated by the Euclidean distance coefficient. Statistical test was performed with the R Development Core Team (2015).

Results

Vessel elements are restricted to the roots of the 33 species studied, which belong to the tribes Alpinieae, Zingibereae and Globbeae. However, tracheids were also found in this organ in *Elettariopsis curtisii* (Alpinieae), *Hedychium borneensis, H. greenei* (Fig. 2a) and *Zingiber cassumunar* (Zingibereae).

In Alpinieae (subfamilie Alpinioideae), the genera *Aframomum, Alpinia, Amomum, Elettariopsis, Etlingera* and *Renealmia* were evaluated, totalizing 14 species (Table 2). Partially pitted parietal thickening (Fig. 1c, i) were found in *Alpinia* and *Etlingera* species, *Amomum delbatum, Elettariopsis curtisii, R. breviscapa*, and *R. nicolaioides*, while the reticulate parietal thickening were observed in *Aframomum angustifolium* and *R. chrysotricha*. In this tribe, the perforation plates were found to be simple (Fig. 1d–f, j) and scalariform (Fig. 1a–c, g–i), usually with a few bars (≤20). However, *Elletariopsis curtisii* and *R. chrysotricha* have in common the presence of markedly oblique perforation plate with more than 40 bars. In some species can occur both simple and scalariform perforation plates in end wall, for example in *Alpinia purpurata* and *Etlingera fulgens*.

In Zingibereae tribe (subfamilie Zingiberoideae), 15 species of *Curcuma, Hedychium* and *Zingiber* were studied (Table 3). All species studied of Zingibereae tribe have scalariform perforation plate (Fig. 2b-k). Generally, the vessel elements have slightly and long oblique scalariform perforation plates carrying a varied number of bars (Fig. 2b, c, e–k). *Curcuma species, H. coronarium, Z. officinale* and *Z. spectabile* showed scalarifom perforation plate occurring in both transverse (Fig. 2d) and oblique position. Plates

Table 2 Characterization of vessel elements present in roots of Alpinieae tribe

	Alpinia					*Etlingera*			*Amomum*	*Aframomum*	*Elletariopsis*	*Renealmia*		
	purp	zeru	eleg	gala	nigr	fulg	elat	yunn	delb	angu	curt	brev	chry	nico
Parietal thickening														
Scalariform	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Reticulate	−	−	−	−	−	−	−	−	−	+	−	−	+	−
Partially pitted	+	+	+	+	+	+	+	+	+	−	+	+	−	+
Helical	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Perforation plate														
Scalariform	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Simple	+	+	+	+	+	+	+	+	+	+	+	+	−	+
Simple/scalariform	+	+	+	+	+	+	+	+	+	+	+	+	−	+
Inclination of the terminal vessel walls														
Transverse/transverse	+	+	+	+	+	+	−	+	+	+	−	+	−	+
Transverse/slightly oblique	+	+	+	+	+	+	−	+	+	+	−	+	+	+
Transverse/long oblique	+	−	+	+	+	+	−	+	+	+	−	+	−	+
Slightly oblique/slightly oblique	−	+	−	+	+	+	+	+	−	−	+	+	+	+
Slightly oblique/long oblique	−	+	−	+	−	−	+	+	−	−	+	+	+	+
Long oblique/long oblique	−	+	−	−	−	−	+	+	−	−	+	−	+	−
Bar numbers														
≤10	+	+	+	+	+	+	+	+	+	+	+	+	+	+
10–20	+	+	+	+	+	+	+	+	−	−	+	+	+	−
20–40	−	−	−	−	−	−	+	+	−	−	+	−	+	−
≥40	−	−	−	−	−	−	−	−	−	−	+	−	+	−

Legend: purp (*purpurata*), zeru (*zerumbet*), eleg (*elegans*), gala (*galanga*), nigr (*nigra*), fulg (*fulgens*), elat (*elatior*), yunn (*yunnanensis*), delb (*delbatum*), angu (*angustifolium*), curt (*curtisii*), chry (*chrysotricha*). Symbol: (+) presence; (−) absence

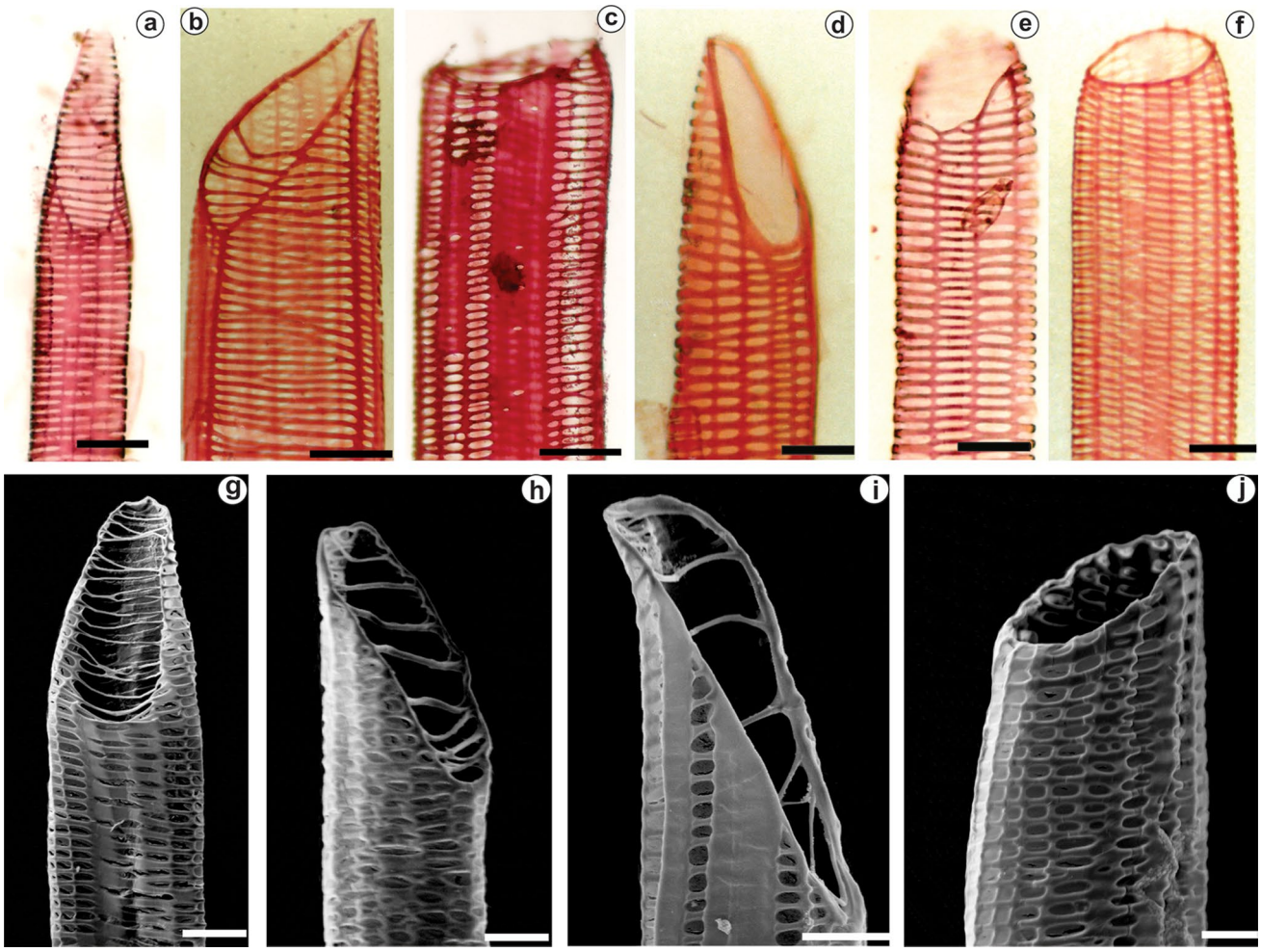

Fig. 1 Vessel elements present in roots of Alpinieae tribe. **a–f** Light microscopy (LM); **g–j** scanning electron microscopy (SEM); **a–c, g–j** scalariform perforation plates; **a, b, d–h, j** Scalariform parietal thickening; **c, i** partially pitted parietal thickening; **a** long oblique perforation plate in *Renealmia chrysotricha*; **b** perforation plate with 7 bars in *Amomum delbatum*; **c** transverse scalariform perforation plate in *Alpinia zerumbet*; **d** long oblique simple perforation plate in *Elettariopsis curtisii*; **e** slightly oblique simple perforation plate in *Etlingera fulgens*; **f** transverse simple perforation plate in *Amomum delbatum*; **g** long oblique perforation plate in *Etlingera elatior*; **h** long oblique perforation plate in *Aframomum angustifolium*; **i** Perforation plate with 7 bars in *Alpinia purpurata*; **j** Simple perforation plate in *Renealmia nicolaioides*. **b, c, f** 100 µm; **a, d, e** 75 µm; **g, h, j** 25 µm; **i** 40 µm

with more than 40 bars were found in some *Zingiber* and *Hedychium* species. *Zingiber officinale* (Fig. 2f), *Z. cassumunar* and *Hedychium coronarium* showed reticulate parietal thickening, and *Z. coralinum, Z. mioga* and *Z. wrayii* (Fig. 2e) showed helical type.

Four species of *Globba*, from Globbeae tribe (subfamily Zingiberoideae), were analyzed (Table 4). These species differ from the others studied here because they do not fit the description of giant herbs proposed for Zingiberaceae, presenting heights of approximately 40 cm. The root tracheary elements of this genus contain two cell types: tracheids (Fig. 3a) and vessel elements (Fig. 3b–d). The vessel elements have slightly and long oblique scalariform perforation plates and scalariform parietal thickening (Fig. 3b–d). In *G. curtisii* and *G. sherwoodiana*, these elements in general have perforation plates porting 20 bars (Fig. 3c–d), whereas in *G. schomburgkii* and *G. magnifica*, vessel elements with fewer number of bars (≤10) were found. These information about *G. curtisii, G. magnifica* and *G. sherwoodiana* were first described in this work.

The scalariform parietal thickening was observed in all species in this study (Tables 2, 3, 4).

The reticulate parietal thickening was observed in Alpinieae and Zingibereae tribes, and the helical secondary wall thickening was found only in the latter tribe (Fig. 4; Tables 2, 3, 4). The partially pitted parietal thickening was found in several species of Alpinieae tribe, except in

Table 3 Characterization of vessel elements present in roots of Zingibereae tribe

	Curcuma					*Hedychium*				*Zingiber*					
	como	zedo	long	peti	dome	coro	gree	thyr	born	spec	offi	cass	cora	miog	wray
Parietal thickening															
Scalariform	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Reticulate	–	–	–	–	–	+	–	–	–	–	+	+	–	–	–
Partially pitted	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Helical	–	–	–	–	–	–	–	–	–	–	–	–	+	+	+
Perforation plate															
Scalariform	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Simple	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Simple/scalariform	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Inclination of the terminal vessel walls															
Transverse/transverse	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Transverse/slightly oblique	–	+	+	–	–	+	–	–	–	+	+	–	–	–	–
Transverse/long oblique	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Slightly oblique/slightly oblique	+	+	+	-	+	+	+	+	+	+	+	–	–	+	+
Slightly oblique/long oblique	+	–	–	+	+	+	+	+	+	–	–	+	–	+	+
Long oblique/long oblique	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–
Bar numbers															
≤10	+	+	+	+	+	+	–	–	–	+	+	+	+	+	+
10–20	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
20–40	+	–	–	+	+	+	+	+	+	+	+	+	+	–	–
≥40	–	–	–	–	–	+	+	+	+	+	+	–	–	–	–

Legend: como (*comosa*), zedo (*zedoaria*), long (*longa*), peti (*petiolata*), dome (*domestica*), coro (*coronarium*), gree (*greenei*), thyr (*thyrsiforme*), born (*bornense*), spec (*spectabile*), offi (*officinale*), cass (*cassumunar*), cora (*coralinum*), miog (*mioga*), wray (*wrayii*). Symbol: (+) presence; (–) absence

Aframomum angustifolium and *Renealmia chrysotricha* (Fig. 4; Table 2).

All species analyzed in this study presented scalariform perforation plates, and simple perforation plates were also found in the representatives of Alpinieae tribe (Fig. 5).

The arrangement of the perforation plates varied greatly among the studied species. The most significant character states were selected: (1) vessel elements with at least one transverse end wall and (2) vessel elements with both oblique end walls. In *Amomum delbatum, Aframomum angustifolium, Alpinia purpurata* and *Alpinia elegans* only type (1) was observed; in *Elettariopsis curtisii* and *Globba* species, only type (2); and both types were found in other taxon (Fig. 6).

According to the PCA, features correlated to axes I and II explained almost 60% of total variance (Fig. 7). *Alpinia* and *Amomum* showed the same characters states so these clades are overlayed. Axis I was responsible for 38% of total variance, segregating part of Alpinieae (*Alpinia, Amomum*, and *Etlingera*) species, and the species belonging to *Zingiber* and *Hedychium*, of tribe Zingibereae. Axis II contributed to explain about 20% of total variance, gathering Alpinieae (*Renealmia* spp. and *Aframomum angustifolium*) species, as well as all of Zingibereae and Globbeae investigated.

The characters of vessel elements which explained 38% of the variance were the presence of simple perforation plates, a partially pitted parietal thickening, a helical wall thickening, and 40 or more bars composing the plate.

Discussion

The obtained results from the vegetative organ analysis show that the vessel elements are present only in the roots of the studied species. Previous studies carried out with monocotyledon species already indicated the restriction of these elements to that organ, showing that there is no continuous progression and xylem specialization in aerial organs (Carlquist 2001, 2012; Carlquist and Schneider 1998; Cheadle and Kosakai 1982; Gevú et al. 2014; Thorsch 2000). Ewers et al. (1997) and Fisher et al. (1997) correlate this fact with the pronounced positive root pressure in this group of plants. This pressure acts

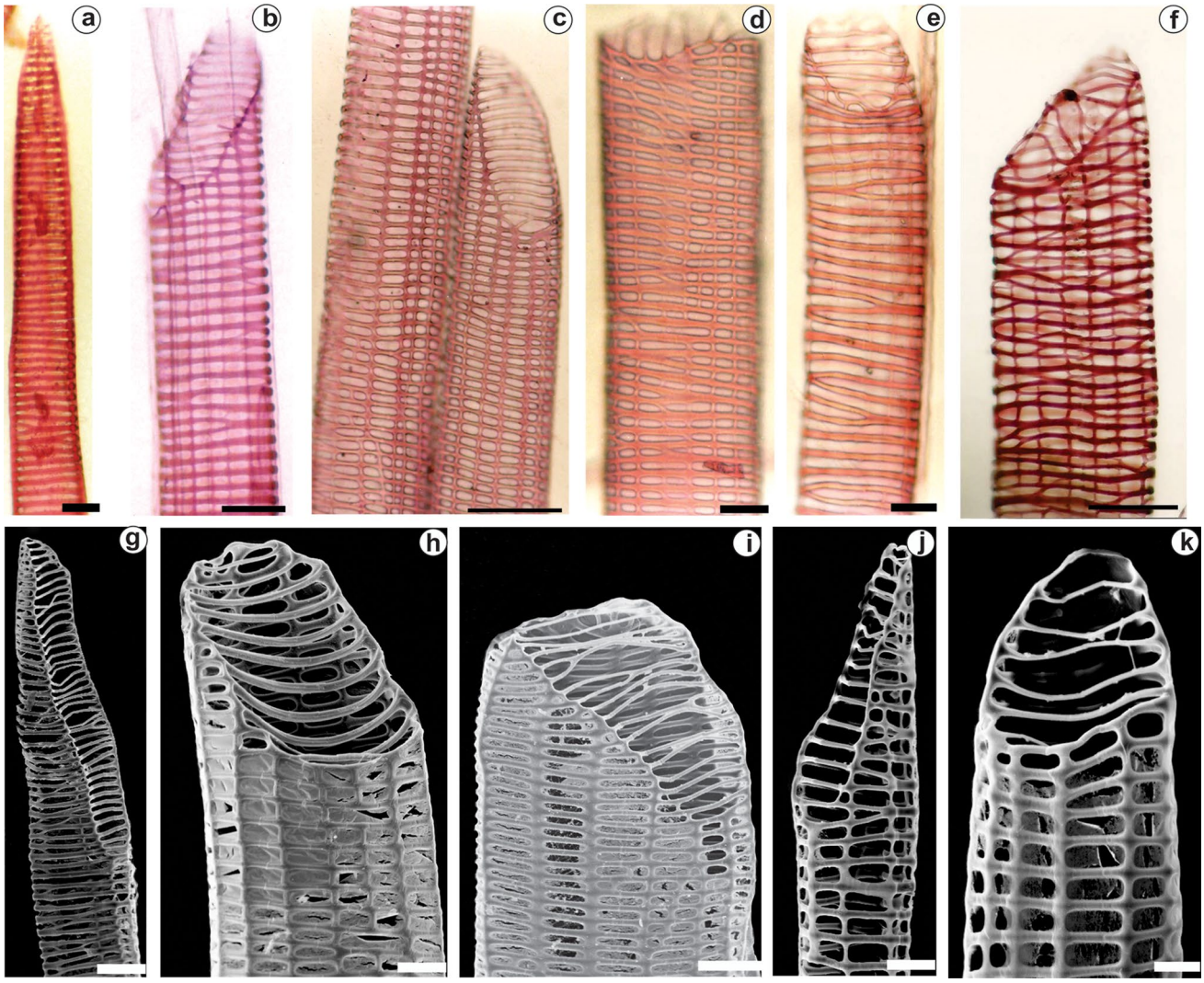

Fig. 2 Tracheary elements present in roots of Zingibereae tribe. **a** Tracheid in *Hedychium greenei* (LM); **b–k** vessel elements with scalariform perforation plates; **a–f** light microscopy (LM); **g–k** scanning electron microscopy (SEM); **b–d, g–k** scalariform parietal thickening. **b** Long oblique perforation plate in *Hedychium thyrsiforme*; **c** two vessel elements in *Z. wrayii*; **d** transverse perforation plate in *Curcuma comosa*; **e** helical parietal thickening in *Zingiber wrayii*. **f** Reticulate parietal thickening in *Zingiber officinale*; **g** markedly long oblique perforation plate in *Zingiber spectabile*; **h** perforation plate with 10 bars in *Z. spectabile*. **i** Long oblique perforation plate in *Curcuma zedoaria*; **j** long oblique perforation plate with 13 bars in *Z. wrayii*. **k** Slightly oblique perforation plate with 5 bars in *Z. wrayii*. *LM* light microscopy, *SEM* scanning electron microscopy. **a, b, g** 50 µm; **c, d, f** 100 µm; **e** 75 µm; **h, i** 20 µm; **j, k** 10 µm

in repair of embolized vessels (Brodersen and McElrone 2013). In dicots, vessel embolism can be replaced by cambial growth (Cochard et al. 1994; Sperry 1986; Wang et al. 2011; Yang et al. 2012).

Wagner (1977) cited that Zingiberaceae would be the only order to present vessel elements with more derived features, primarily in roots. Until now, the occurrence of these elements in the stem was restricted to *Renealmia*, according to Tomlinson (1956). Different results were found in this study for this genus, which presented tracheids in the rhizome and aerial stem only.

The six genera and 13 species studied of Alpinieae tribe present metaxylem vessels with both simple and scalariform perforation plates. Tomlinson (1969) had already noted the presence of both types for *Alpinia*. Uma and Muthukumar (2014) observed only vessel elements with scalariform perforation plate in *A. purpurata* and *A. zerumbet*. According to the obtained results, *Elettariopsis* and *Amomum* share the same character states, corroborating the results of Tomlinson (1956, 1969). Uma and Muthukumar (2014) reported only reticulate perforation plates in *Etlingera elatior* vessel elements, which was not seen in this work.

Table 4 Characterization of vessel elements present in roots of Globbeae tribe

	Globba			
	curtisii	*schomburgkii*	*sherwoodiana*	*magnifica*
Parietal thickening				
Scalariform	+	+	+	+
Reticulate	–	–	–	–
Partially pitted	–	–	–	–
Helical	–	–	–	–
Perforation plate				
Scalariform	+	+	+	+
Simple	–	–	–	–
Simple/scalariform	–	–	–	–
Inclination of the terminal vessel walls				
Transverse/transverse	–	–	–	–
Transverse/slightly oblique	–	–	–	–
Transverse/long oblique	–	–	–	–
Slightly oblique/slightly oblique	+	+	+	+
Slightly oblique/long oblique	+	+	+	+
Long oblique/long oblique	+	+	+	+
Bar numbers				
≤10	–	+	–	+
10–20	+	+	+	+
20–40	+	+	+	+
≥40	+	+	+	+

Symbol: (+) presence; (–) absence

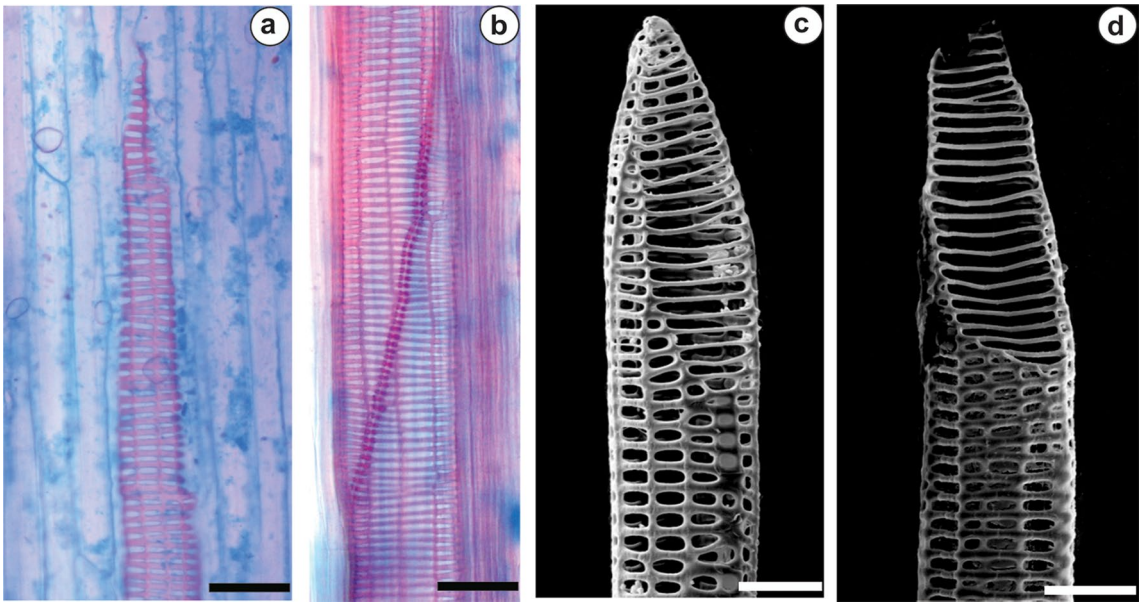

Fig. 3 Tracheary elements present in roots of Globbeae tribe. **a, b** Roots in longitudinal section in *Globba schomburgkii* (LM). **a** Tracheid. **b** Junction of two markedly long oblique scalariform perforation plates in vessel elements. **c, d** Vessel elements with scalariform perforation plates and scalariform parietal thickening in *Globba curtisii* (SEM). *LM* light microscopy, *SEM* scanning electron microscopy. **a, b** 50 µm; **c, d** 150 µm

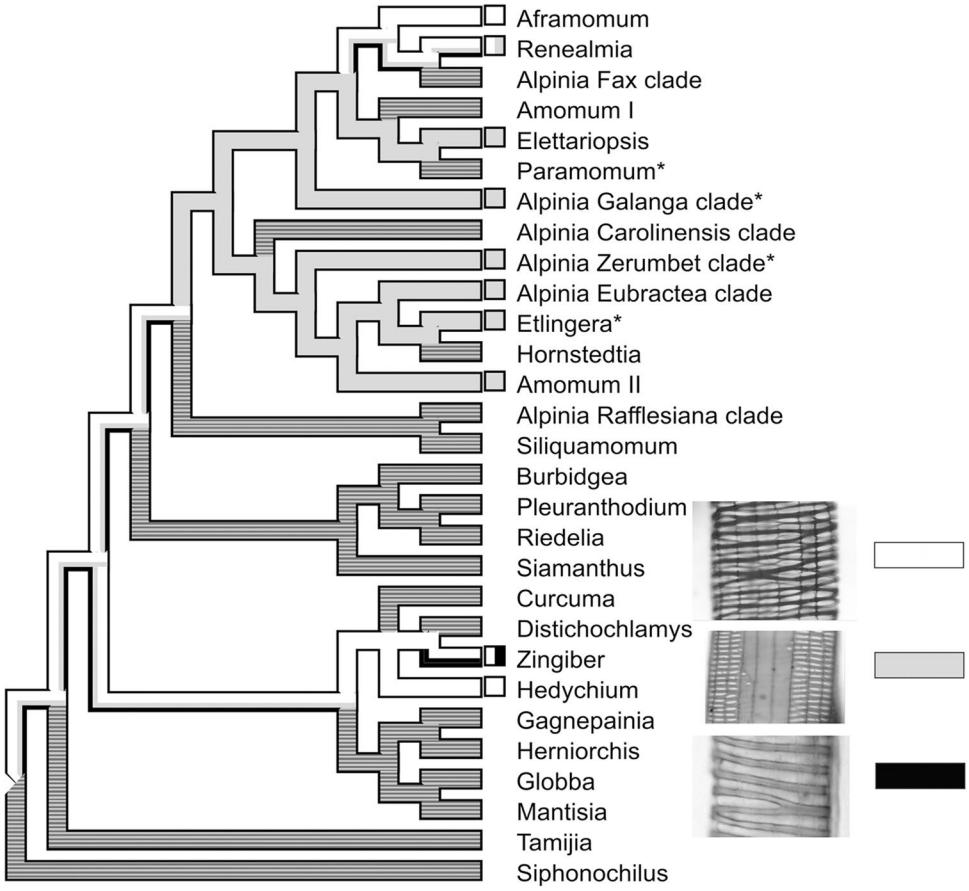

Fig. 4 Types of parietal thickening of vessel elements on phylogenetic tree of Kress et al. 2005. *White color* indicates reticulate parietal thickening, *gray color* refers to partially pitted parietal thickening and *black color* indicates helical parietal thickening. The *squares* indicates clades that were evaluated and the branches ending *striped line* indicate clades that were not evaluated in this work

The *Amomum delbatum, Aframomum angustifolium* and *Alpinia Eubractea* clade always present with one of the end walls being transversal and the other ranging from oblique to transversal. This feature was found in *Cyperus* species, according to Rodrigues et al. (2007). The obtained results show the convergence of this character state in the aforementioned taxa and validate the categorization of groups from the end wall inclination analysis, as suggested by Chalk (1989).

Kress et al. (2005) considered *Alpinia* is a highly polyphyletic genus. These authors also highlighted the close phylogenetic relationship of species of Alpinia to other genera in Alpinieae, such as *Renealmia, Aframomum, Amomum, Etlingera, Hornstedtia, Leptosolena, Plagiostachys, Siliquamomum*, and *Vanoverberghia*.

In the phylogenetic tree proposed by Kress et al. (2002), Alpinieae was considered a derivate clade in comparison to Zingibereae and Globbeae tribes. Our results showed that derived characters of the vessel elements are found in Alpinieae, including a simple perforation plate and pitted parietal thickening.

Oblique end walls predominate the vessel elements of Zingibereae tribe. According to Chalk (1989), the perforation plates with large number of bars can be originated from tracheids with scalariform pits in their end walls. *Hedychium* and *Zingiber* species studied here have tracheids and vessel elements with scalariform perforation plates that carry many bars, suggesting a more basal position compared with *Curcuma* species. The results found corroborate the observations of Tomlinson (1956), who found tracheids and vessel elements, respectively, in *Hedychium*.

The presence of only scalariform perforation plates in the vessel elements of *C. longa, H. coronarium* and *Z. officinale* and the wide variation in the number of bars in these plates corroborate the results of Thorsch (2000) and Uma and Muthukumar (2014). Thorsch (2000) considered the vessel elements in Zingiberaceae to be less specialized than others families of Zingiberales.

The four Globba species studied show uniformity in the vessel elements, scalariform parietal thickening and perforation plate, and both oblique end wall. Thorsch (2000) and Uma and Muthukumar (2014) described only the presence of vessel elements with scalariform perforation plates in *Globba schomburgkii*. However, the present study showed that tracheids are also present in the roots of this species. According to Kress et al. (2002), *Globba* is a polyphyletic genus and the relationships among its species are not yet well defined. *Globba curtissi* and *G. schomburgkkii* are part

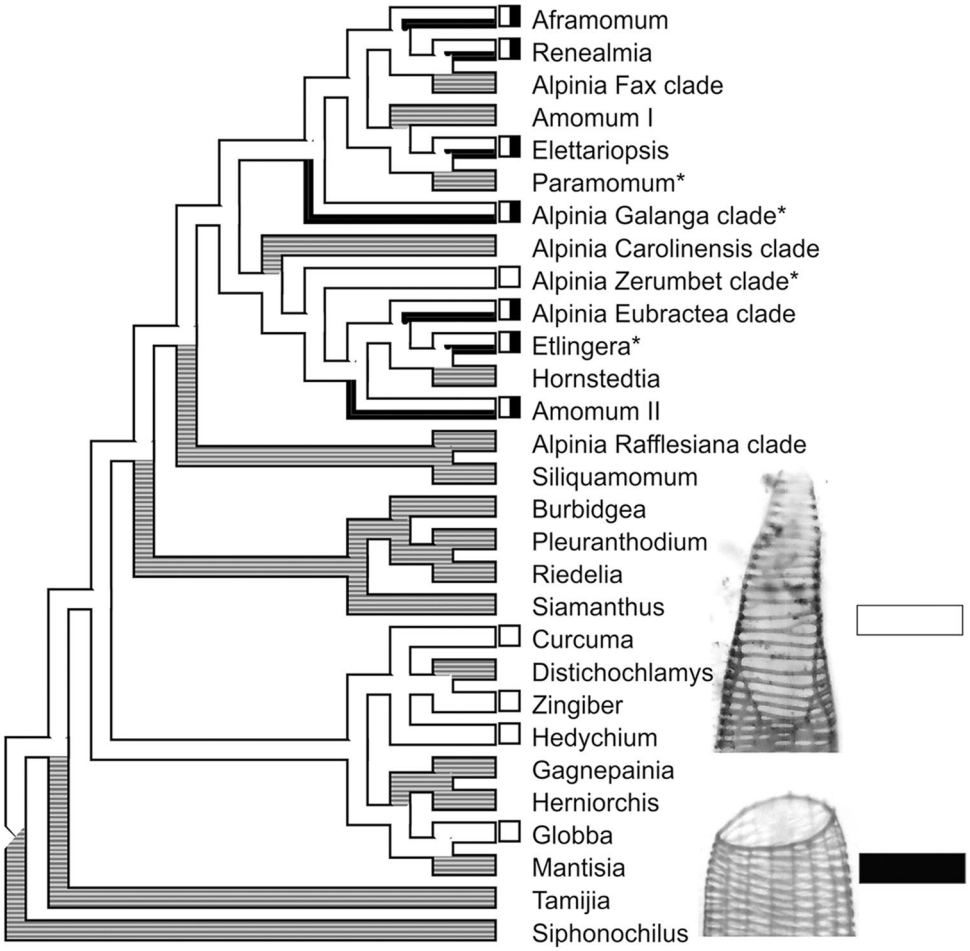

Fig. 5 Types of perforation plates of vessel elements on phylogenetic tree of Kress et al. 2005. *White color* indicates scalariform perforation plate and *black color* refers to simple perforation plate. The *squares* indicates clades that were evaluated and the branches ending *striped line* indicate clades that were not evaluated in this work

of the same section and are supported by these evaluated characters (Williams et al. 2004).

Benedict et al. (2015) successfully distinguished the subfamilies Alpinioideae, Siphonochiloideae, Tamijioideae, and Zingiberoideae based on multiple seed characters. Our results strengthen the separation of the Alpinieae, Zingibereae and Globbeae tribes (Kress et al. 2002), based on vessel elements characters.

In conformity with the classification proposed by Kress et al. (2002), Globbeae and Zingibereae tribes present synapomorphies that place them in the subfamily Zingiberoideae. The found results confirm the previous proposition because they gather the following basal characters: scalariform perforation plates, variation in the end wall inclination from markedly to slightly oblique and scalariform parietal thickening.

The presence of tracheids in the roots of all *Globba* species and in some species of Zingibereae tribe reaffirms the pre-established taxonomic position.

The analysis of tracheary elements that belong to different tribes of Zingiberaceae allowed the conclusion that the vessel elements are restricted to roots and that the occurrence of scalariform perforation plate and the scalariform parietal thickening are frequent, which may be a plesiomorphic condition for this taxon.

In the PCA performed by Uma and Muthukumar (2014), the most significant morphological characters of the Zingiberaceae roots were: multilayered exodermis, exodermis layers, exodermis thickening, inner cortex layers, intercellular spaces in outer and inner cortex, endodermis and thickening, vascular cylinder and intercellular spaces in pith.

The present work analyzed and described the vessel elements of seven species of Alpinieae, eight of Zingibereae and three of Globbeae adding new information to these taxa. Our results showed that vessel elements represent an important marker of evolutionary trends in these three tribes of Zingiberaceae. More studies on the tracheary elements in other two subfamilies and its tribes can be conducted.

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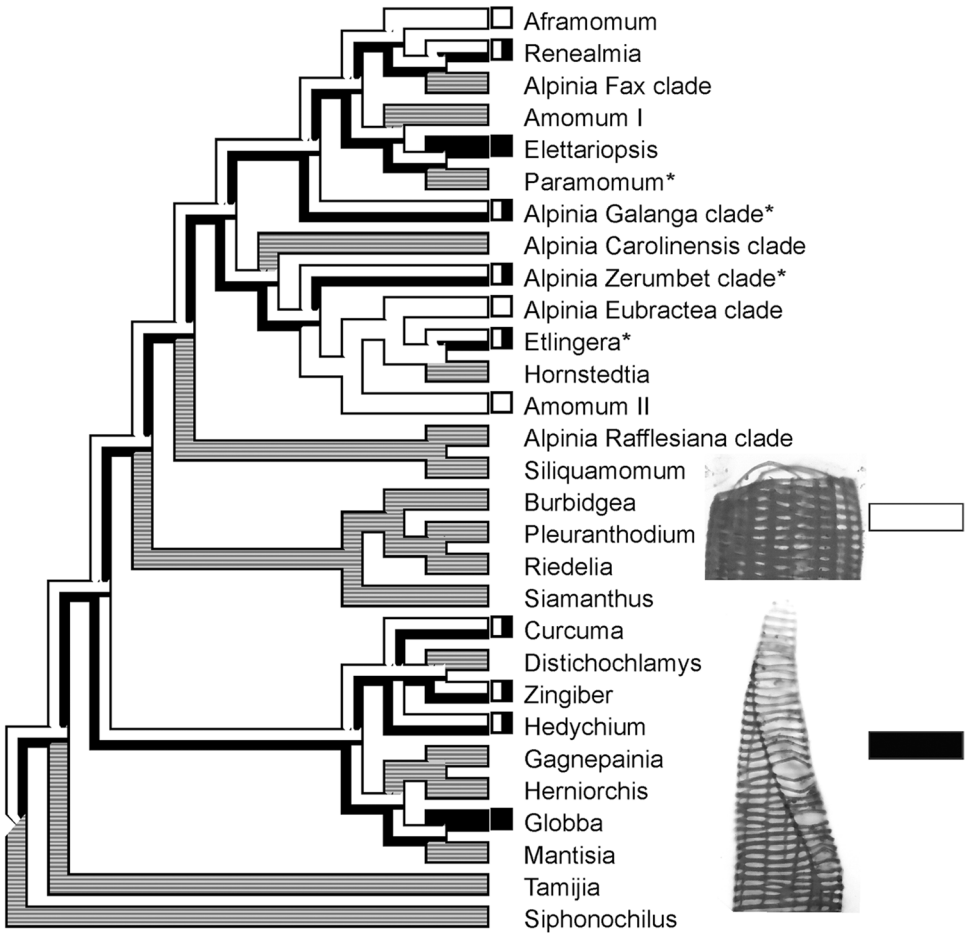

Fig. 6 Types of the vessel end walls on phylogenetic tree of Kress et al. 2005. *White color* indicates on least one of end transverse walls and *black color* refers to oblique inclination on both as end walls. The *squares* indicates clades that were evaluated and the branches ending *striped line* indicate clades that were not evaluated in this work

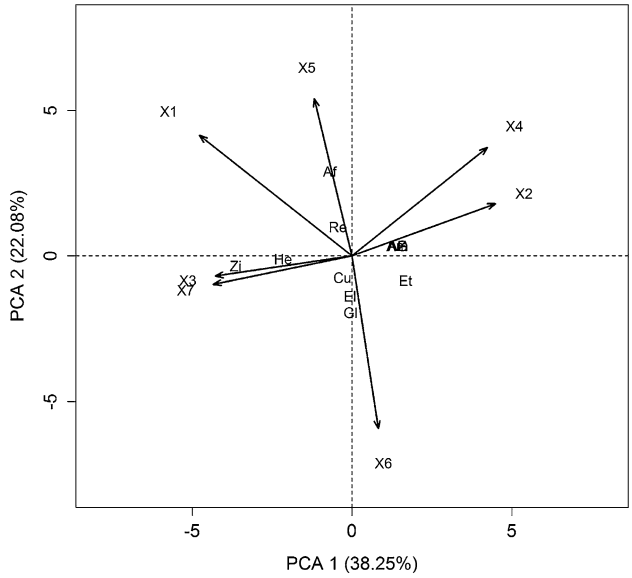

Fig. 7 Principal component analysis (PCA). *X1* Reticulate parietal thickening, *X2* partially pitted parietal thickening, *X3* helical parietal thickening, *X4* simple perforation plate, *X5* transverse terminal vessel walls, *X6* bar numbers (10–40), *X7* bar numbers (≥40). Af, *Aframomum*; Re, *Renealmia*; Am, *Amomum*; Al, *Alpinia*; Et, *Etlingera*; El, *Elettariopsis*; Gl, *Globba*; Cu, *Curcuma*; He, *Hedychium*; Zi, *Zingiber*

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