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Original Article

Leaf micromorphological adaptation of *Pogostemon* spp. (section *Eusteralis*) in Thailand

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ABSTRACT

Pogostemon section *Eusteralis* is comprised of diverse taxa found in a range of habitats, from terrestrial to aquatic/marshland. However, few studies have examined the leaf micromorphology of *Pogostemon* and the micromorphological adaptations of this section to their habitats. Therefore, leaf micromorphological characters of the section *Eusteralis* in Thailand were investigated to assess the relationships between leaf micromorphological characters and environmental conditions and to explore alternative taxonomic characters for supporting the infrageneric classification. Common leaf micromorphological characters of this section were striate cuticular ornamentation, capitate trichomes with two apical cells, peltate trichomes with a large head cell, a convex outline of the abaxial side of the midrib and crescent collateral vascular bundles with a parenchymatous sheath. Variable leaf micromorphological characters were observed and appeared to be associated with environmental habitat conditions. The correlation between leaf micromorphology and environmental factors were considered and it was suggested that the major factor causing different leaf micromorphology is water availability. The important leaf micromorphological trait of *Eusteralis* that can distinguish this section from the section *Pogostemon* is the type of non-glandular trichomes; the section *Eusteralis* possesses only simple unicellular and bicellular trichomes, but no simple multicellular trichomes were found in the section *Eusteralis*, unlike in the section *Pogostemon*. This might be used as an additional character to define the infrageneric classification of *Pogostemon*.

Introduction

Pogostemon Desf. is the largest genus of the tribe Pogostemoneae within the subfamily Nepetoideae (Lamiaceae). This genus contains approximately 80 species discontinuously distributed throughout the Indian subcontinent and Southeast Asia, as well as a few species sporadically found in Japan, Northern Australia and southern tropical Africa (Press, 1982; Ingrouille and Bhatti, 1998). *Pogostemon* plant forms are diverse, ranging from terrestrial subshrubs to aquatic herbs and the common characteristics of this genus is the presence of exerted stamens bearing moniliform hairs. Formerly, several taxonomists have treated the genus as two separate groups based fundamentally on habitat (Bentham, 1833; Hooker, 1885; Airy Shaw, 1967), comprising the terrestrial group of *Pogostemon* s.s., and the aquatic/marshland group of *Dysophylla* El Gazzar & L. Watson ex Airy Shaw (El-Gazzar and Watson, 1967). The species within the terrestrial group are commonly opposite petiolate with broad leaves covered more or less by hairs (El-Gazzar and Watson, 1967), while the species within the aquatic/marshland group ordinarily possess verticillate sessile leaves with a linear to filiform lamina shape and are usually glabrous (El-Gazzar and Watson, 1967).

Ten years later, these two groups were combined into a single genus, *Pogostemon* s.l., but were sub-classified into two sections based principally on phyllotaxy, that is section *Pogostemon* with opposite leaves, and section *Eusteralis* (Rafin.) Keng with verticillate leaves (Keng, 1978). This classification was then followed by Press (1982), who also recognized the two sections based on phenetic analysis of morphological characters. Afterward, Bhatti and Ingrouille (1997) revised the infrageneric classification of *Pogostemon* s.l. and divided the genus into three subgenera—*Allopogostemon* Bhatti & Ingr., *Dysophyllus* (Blume) Bhatti & Ingr., and *Pogostemon* sensu Bhatti & Ingr. —based mainly on the inflorescence type and the calyx morphology, using a total of 135 morphological character states.

Historically, the infrageneric classifications of this genus have been contradictory due to morphological variability and putative convergent evolution within the genus (Yao et al., 2016). The trait evolution of the characters previously used for infrageneric

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classification showed that the species with verticillate phyllotaxy are grouped within a clade of species that possess opposite phyllotaxy (Yao et al., 2016). Similarly, neither the terrestrial nor the aquatic/marshland species formed monophyletic clades (Yao et al., 2016). Thus, phyllotaxy and habitat might be inappropriate characters for infrageneric classification (Yao et al., 2016). Moreover, the phylogenetic distribution of taxa based on calyx size and secondary calyx venation resemble the taxa distribution based on habitats (Yao et al., 2016). This evidence suggests that these related characters might have undergone parallel evolution, resulting from adaptation to similar habitats (Yao et al., 2016).

The taxonomic significance of micromorphology is well established and is often useful at various taxonomic levels within the Lamiaceae (Metcalfe and Chalk, 1950; El-Gazzar and Watson, 1970; Abu-Asab and Cantino, 1987; Cantino, 1990; Salmaki et al., 2009, 2011; Xiang et al., 2010; Celep et al., 2011; Hu et al., 2012; Rashid and Parnell, 2013). Some taxa of the section *Pogostemon*, which mostly grow in terrestrial habitats, have been examined in terms of micromorphology (Bhatti and Ingrouille, 1997; Guo et al., 2013; Pradeep and Murugan, 2013; Rusydi et al., 2013). In contrast, the micromorphology of the section *Eusteralis* has never been studied. Yet such research may reveal important insights into micromorphological adaptation, given that *Eusteralis* is comprised of taxa growing in diverse habitats, from terrestrial to aquatic/marshland, and the obligate aquatic species are perhaps the only lamiaceous taxa exhibiting this trait (Yao et al., 2016). Therefore, the leaf micromorphological characters of a representative sample of Thai *Pogostemon* section *Eusteralis sensu* Keng (1978) and Press (1982), consisting of both terrestrial species and aquatic/marshland species, were described to interpret the relationships between the examined micromorphological characters and environmental conditions. Furthermore, leaf micromorphological traits that were considered likely to be useful in supporting the infrageneric classification were also investigated and emphasized.

Materials and methods

Plant collections

Mature leaves of four *Pogostemon* species—*P. cruciatus* (Benth.) Kuntze, *P. globulosus* Phuong ex Suddee & A.J. Paton, *P. helferi* (Hook.f.) Press, and *P. trinervis* Chermsir. ex Press (Table 1) —were taken from herbarium specimens deposited at Khon Kaen University Herbarium, Thailand (KKU), as well as being collected from their natural habitats. Voucher specimens were deposited at the Forest Herbarium (BKF), Thailand. The leaf transversal method was not conducted with *P. cruciatus* as fresh samples could not be obtained.

Clearing method of leaf laminas

Leaves from herbarium specimens were boiled in water for rehydration. All leaf samples were soaked in 10% (w/v) potassium hydroxide, rinsed in water, soaked in 50% (v/v) commercial bleaching agent (Haiter bleach; KAO Corporation; Bangkok, Thailand), and stained with Chlorazal Black E.

Scanning electron microscopy

Ethanol-fixed leaf samples were sonicated twice (10 min each) to remove contaminants. After that, the samples were soaked in 95% ethanol, dried and fixed on stubs. The samples were then coated with platinum palladium and photographed using a scanning electron microscope (S-2500; Hitachi; Tokyo, Japan). The terminology used in this study mainly followed Dilcher (1974), Abu-Asab and Cantino (1987), Cantino (1990) and Navarro and El-Oualidi (2000). The frequency and index of stomata and trichomes were determined following Dilcher (1974).

Transverse section of leaves

For the transverse section method, 5×5 mm^2 of 70% ethanol-fixed leaf samples were dehydrated using a tertiary butyl alcohol series, immersed in 60 °C melted paraffin and embedded in paper molds. Transverse sections of about 10 μm thickness were cut using a sliding microtome (SM2000R; Leica; Wetzlar, Germany). The sections were pre-stained by immersion in xylene, xylene with absolute ethanol (1:1), absolute ethanol with ether (1:1), absolute ethanol, 95% (v/v) ethanol and 70% (v/v) ethanol, respectively. They were then stained with Safranin-O, counterstained with Fast green, dehydrated and mounted with DePeX. The leaf micromorphological characters were observed and photographed using a light microscope (BX 43 with a camera set DP21; Olympus; Tokyo, Japan).

Results

The leaf micromorphological characters of *Pogostemon* section *Eusteralis* in Thailand are summarized in Tables 2 and 3. Selected light microscope and scanning electron microspore (SEM) micrographs are presented in Figs. 1–3.

Leaf surfaces

The leaf epidermal characters of each species were similar on both surfaces. Striate cuticular ornamentation on the epidermal cells was found on both sides in all species (Fig. 1A). Irregularly shaped epidermal cells were found in *P. globulosus* (Figs. 1D, 2E,F), *P. helferi* (Fig. 2I,J) and *P. trinervis* (Fig. 2M,N), while pentagonal to hexagonal shapes were present in *P. cruciatus* (Fig. 2A,B). The anticlinal cell walls could be divided into two types: straight to curved in *P. cruciatus* (Fig. 2A,B) and undulated in *P. globulosus* (Fig. 2E,F), *P. helferi* (Fig. 2I,J) and *P. trinervis* (Fig. 2M,N). The length of epidermal cells ranged from 42.12 ± 2.23 μm in *P. cruciatus* to 75.14 ± 6.11 μm in *P. globulosus* (Table 2).

The stomatal occurrences of *P. helferi*, *P. glubulosus*, and *P. trinervis*, were amphistomatic, while *P. cruciatus* had

Table 1
List of examined specimens of Thai *Pogostemon*, section *Eusteralis*.

Species	Locality	Voucher specimen
P. cruciatus (Benth.) Kuntze	Loei, Phu Kradueng	B. Bongcheewin 444
	Chaiyaphum, Phu Kiao	B. Bongcheewin 449
P. globulosus Phuong ex	Ubon Ratchathani, Khong Chiam	K. Pramali et al. 26
Suddee & A.J. Paton	Ubon Ratchathani, Khong Chiam	B. Bongcheewin 446
	Ubon Ratchathani, Khong Chiam	B. Bongcheewin 469
P. helferi (Hook.f.) Press	Kanchanaburi, Sang Khla Buri	K. Pramali et al. 46
	Kanchanaburi, Sang Khla Buri	K. Pramali et al. 50
P. trinervis Chermsir. ex Press	Sakon Nakhon, Phu Phan	B. Bongcheewin 770
	Sakon Nakhon, Phu Phan	K. Pramali et al. 28

Table 2
Leaf epidermal characters of *Pogostemon* sect. *Eusteralis* in Thailand.

Character	*P. cruciatus*	*P. globulosus*	*P. helferi*	*P. trinervis*
Leaf epidermal characters				
Epidermal cell				
Adaxial leaf surface				
Cuticular ornamentation	Unable to be observed	Striate	Striate	Striate
Shape	Penta- to hexagonal	Irregular	Irregular	Irregular
Anticlinal cell wall	Straight to curved	Undulated	Undulated	Undulated
Length (μm)	55.08 ± 8.94	60.65 ± 10.12	70.76 ± 7.61	68.11 ± 6.32
Abaxial leaf surface				
Cuticular ornamentation	Unable to be observed	Striate	Striate	Striate
Shape	Penta- to hexagonal	Irregular	Irregular	Irregular
Anticlinal cell wall	Straight to curved	Undulated	Undulated	Undulated
Length (μm)	42.12 ± 2.23	75.14 ± 6.11	67.09 ± 5.03	49.00 ± 7.09
Stomata				
Stomatal occurrences	Hypostomatic	Amphistomatic	Amphistomatic	Amphistomatic
Adaxial leaf surface				
Type	Not found	DIA + DIAL	DIA + ANO	DIA + DIAL
Length (μm)	–	27.60 ± 1.56	27.81 ± 1.36	30.87 ± 1.40
Frequency (stomata/mm^2)	–	2.16 ± 0.68	0.65 ± 0.53	2.80 ± 0.53
Stomatal index	–	2.68 ± 0.83	0.48 ± 0.39	6.54 ± 1.10
Abaxial leaf surface				
Type	DIA + DIAL	DIA + DIAL	DIA + ANO	DIA + DIAL
Length (μm)	21.49 ± 0.87	24.18 ± 2.07	27.13 ± 2.08	26.20 ± 1.72
Frequency (stomata/mm^2)	22.21 ± 1.46	7.12 ± 1.10	0.65 ± 0.53	20.27 ± 1.26
Stomatal index	27.38 ± 0.86	10.49 ± 1.19	0.47 ± 0.38	35.10 ± 2.49
Non-glandular trichomes				
Apical cell ornamentation	Striate	Micropapillae	Not found	Micropapillae
Type of non-glandular trichome	Simple unicellular	Simple uni- and bicellular	Not found	Simple uni- and bicellular
Number of basal cell	Single cell	Single or two cells	Not found	Single or two cells
Adaxial leaf surface				
Length (μm)	417.66 ± 121.56	67.04 ± 10.40	–	151.74 ± 26.00
Frequency (trichome/mm^2)	62.18 ± 5.67	1.62 ± 1.32	–	40.56 ± 6.62
Non-glandular trichome index	26.10 ± 2.46	0.82 ± 0.67	–	28.69 ± 3.50
Abaxial leaf surface				
Length (μm)	434.98 ± 118.63	62.96 ± 9.01	–	123.36 ± 12.27
Frequency (trichome/mm^2)	114.64 ± 15.35	2.16 ± 1.08	–	101.66 ± 16.63
Non-glandular trichome index	43.56 ± 4.44	1.41 ± 0.71	–	51.60 ± 4.43
Glandular trichomes				
Capitate trichomes				
Adaxial leaf surface				
Diameter (μm)	20.74 ± 1.76	22.20 ± 0.91	28.78 ± 1.94	Not found
Frequency (glands/mm^2)	6.47 ± 5.28	34.50 ± 26.76	105.67 ± 4.31	–
Glandular trichome index	0.91 ± 0.74	4.15 ± 3.09	7.30 ± 0.21	–
Abaxial leaf surface				
Diameter (μm)	Not found	23.33 ± 1.97	27.65 ± 2.08	17.10 ± 0.68
Frequency (glands/mm^2)	–	19.41 ± 8.07	92.73 ± 11.00	6.47 ± 5.28
Glandular trichome index	–	3.10 ± 1.22	6.45 ± 0.54	1.67 ± 1.36
Peltate trichomes				
Adaxial leaf surface				
Diameter (μm)	Not found	Not found	Not found	Not found
Frequency (glands/mm^2)	–	–	–	–
Glandular trichome index	–	–	–	–
Abaxial leaf surface				
Diameter (μm)	53.64 ± 4.02	60.86 ± 1.85	Not found	38.20 ± 0.68
Frequency (glands/mm^2)	12.94 ± 4.31	12.94 ± 4.31	–	8.63 ± 4.31
Glandular trichome index	2.13 ± 0.61	2.10 ± 0.71	–	2.19 ± 1.10

abEP = abaxial epidermis; adEP = adaxial epidermis; ANO = anomocytic; DIA = diacytic; DIAL = diallelocytic.

hypostomatic leaves. Stomatal types were typically diacytic (Fig. 1D) intermixed with diallelocytic (Fig. 1E), except in *P. helferi* which had diacytic with anomocytic stomata (Fig. 1F). The shortest guard cells were found in *P. cruciatus* (21.49 ± 0.87 μm), while the longest was found in *P. trinervis* (30.87 ± 1.40 μm). Stomatal density ranged from 0.65 stomata/mm^2 in *P. helferi* to 22.21 stomata/mm^2 in *P. cruciatus*. Stomata on the abaxial surface were denser than those of the adaxial surface for all species. *Pogostemon helferi* had the lowest stomatal index, while *P. trinervis* had the highest stomatal index (Table 2).

Non-glandular trichomes and glandular trichomes were observed in some species. Generally, non-glandular trichomes were composed of apical and basal cells (Figs. 1G and 2M). The apical cells were usually covered by micropapillae (Fig. 1C), except in *P. cruciatus*, which had striation on the surface of apical cells (Fig. 1B). Based on the number of apical cells, non-glandular trichomes could be categorized as simple unicellular and simple bicellular trichomes. Simple unicellular trichomes with a single basal cell were present in *P. cruciatus* (Fig. 2C,D), while simple uni- and bicellular trichomes with single or two basal cells were found in *P. globulosus* and *P. trinervis* (Fig. 2M,N). In contrast, *P. helferi* had no non-glandular trichomes on either leaf surface. Statistically, *P. globulosus* possessed the shortest non-glandular trichomes (62.96 ± 9.01 μm), the lowest non-glandular trichomes density (1.62 ± 1.32 trichomes/mm^2) and the lowest trichome index (0.82 ± 0.67). In contrast, *P. cruciatus* presented the longest non-

Table 3
Leaf transverse section characters of *Pogostemon* sect. *Eusteralis* in Thailand.

Character	*P. cruciatus*	*P. globulosus*	*P. helferi*	*P. trinervis*
Leaf transverse section				
Margin				
Outline	No material available	Straight	Curved, slightly downward	Curved, slightly downward
Minor veins	No material available	Present	Present	Present
Peltate trichomes	No material available	Present	Present	Present
Midrib				
Outline of adaxial surface	No material available	Flat or convex	Convex	Flat or convex
Outline of abaxial surface	No material available	Convex	Convex	Convex
VA (μm)	No material available	448.45 ± 93.34	675.49 ± 271.67	302.11 ± 7.28
DVA (μm)	No material available	435.59 ± 6.08	755.05 ± 224.79	293.05 ± 4.17
Non-glandular trichomes	No material available	Present	Not found	Present
Type of vascular bundle	No material available	Collateral	Collateral	Collateral
Outline of vascular bundle	No material available	Curved and opened to the adaxial surface	Curved and opened to the adaxial surface	Curved and opened to the adaxial surface
Parenchymatous sheath	No material available	Present	Present	Present
DVB (μm)	No material available	210.85 ± 15.56	168.51 ± 29.84	142.53 ± 23.48
Lamina				
Type	No material available	Dorsiventral	Isobilateral	Dorsiventral
Thickness (μm)	No material available	145.00 ± 8.84	134.41 ± 34.15	174.47 ± 2.90
Thickness of adEP (μm)	No material available	17.50 ± 1.15	13.97 ± 5.35	33.03 ± 6.22
Thickness of abEP (μm)	No material available	11.21 ± 2.92	12.62 ± 4.60	14.08 ± 0.79
Distinctness between PMS and SMS	No material available	Well distinguished	No differentiation	Well distinguished
Thickness of PMS (μm)	No material available	34.76 ± 8.36	No differentiation	64.54 ± 18.12
Thickness of SMS (μm)	No material available	75.87 ± 1.64	No differentiation	60.72 ± 10.49

abEP = abaxial epidermis; adEP = adaxial epidermis; DVA = dorsiventral axis; DVB = diameter of vascular bundle; PMS = palisade mesophyll; SMS = spongy mesophyll; VA = ventral axis.

glandular trichomes (434.98 ± 118.63 μm) and the highest non-glandular trichomes frequency (114.64 ± 15.35 trichomes/mm^2) as shown in Table 2.

Two types of glandular trichomes (capitate and peltate) were identified. Capitate trichomes were observed in most species (Fig. 1E). The largest capitate trichomes occurred in *P. helferi* (28.78 ± 1.94 μm), and the smallest occurred in *P. trinervis* (17.10 ± 0.68 μm). Moreover, *P. helferi* had the highest density of capitate trichomes (105.67 ± 4.31 glands/mm^2; trichome index = 7.30 ± 0.21), while *P. cruciatus* had the lowest density (6.47 ± 5.28 glands/mm^2; trichome index = 0.91 ± 0.74). Peltate trichomes (Figs. 1H and 2H) were less common than capitate trichomes. The largest (60.86 ± 1.85 μm) and smallest (38.20 ± 0.68 μm) peltate trichomes were found in *P. globulosus* and *P. trinervis*, respectively. In addition, the highest frequencies of peltate trichomes were found in *P. cruciatus* and *P. globulosus*, while *P. trinervis* possessed the lowest frequency. However, trichome indices among the three species were very similar (Table 2).

Leaf transverse section

The leaf margin outline was straight in *P. globulosus* (Fig. 3C), while outlines of *P. helferi* and *P. trinervis* curved slightly downwards (Fig. 3F,I). Minor veins and peltate trichomes frequently occurred at the leaf margins of all three species. The adaxial surface of the midrib was flat or convex, while the abaxial surface of the midrib was totally convex for the three species. The length of the ventral axis ranged from 302.11 ± 7.28 μm (*P. trinervis*) to 675.49 ± 271.67 μm (*P. helferi*). Similar to the ventral axis, the width of the dorsiventral axis varied between 293.05 ± 4.17 μm in *P. trinervis*, and 755.05 ± 224.79 μm in *P. helferi* (Table 3). The ground tissue was usually comprised of parenchymatous cells. The widest parenchymatous tissue occurred in the middle region between the vascular bundle and the abaxial surface. The vascular bundle was of the collateral type, ranging in width from 142.53 ± 23.48 μm to 210.85 ± 15.56 μm for *P. trinervis* and *P. globulosus*, respectively (Table 3). It was usually curved and opened to the adaxial surface. A layer of parenchyma was found surrounding the vascular bundle (Fig. 3A).

Laminas of *P. trinervis* and *P. globulosus* were dorsiventral, except for *P. helferi* which had isobilateral leaves. The thickness of the leaf blade transverse sections ranged from 134.41 ± 34.15 μm in *P. helferi* to 174.47 ± 2.90 μm in *P. trinervis* (Table 3). In all species, the epidermis was composed of a single layer of polygonal to rounded epidermal cells in which the adaxial epidermis was usually thicker than the abaxial epidermis. However, the thickness of the abaxial epidermis of all taxa was quite similar (Table 3). The thickest adaxial epidermis was found in *P. trinervis* (33.03 ± 6.22 μm) and the thinnest ones were recorded in *P. helferi* (13.97 ± 5.35 μm). The long palisade and loose spongy cells in the mesophyll were well distinguished in two species (*P. trinervis* and *P. globulosus*), whereas in *P. helferi*, there was no differentiation of short palisade and dense spongy cells (Fig. 3E). The palisade mesophyll was composed of one to two parenchymatous cell layers. Internal glands were also embedded in this region (Fig. 3H). Spongy mesophyll was the thickest region, composed of two to six parenchymatous cell layers with intercellular spaces lying beneath the abaxial epidermis. Non-glandular trichomes could occur on the midrib, as well as on both leaf surfaces, except in *P. heferi*. These trichomes were composed of uni- or bicellular apical cells reaching from the epidermis and a single basal cell embedded in the epidermis (Figs. 1G and 3H,I). Capitate trichomes were frequently observed on both epidermises of all species. The capitate trichomes comprised three parts: a foot cell located in the epidermis, a short stalk and two head cells extending from the epidermal surface (Fig. 1I). Peltate trichomes were often found on the abaxial surface. All parts of these trichomes (one-to-two foot cells, a short stalk cell, and a large head cell) were sunk into the epidermis (Fig. 1H).

Discussion

Leaf micromorphological adaptations of *Pogostemon* section *Eusteralis* in Thailand were investigated via leaf anatomy and SEM observation of leaf epidermal surfaces. This study revealed that the leaf micromorphological characters of the section are typical of the

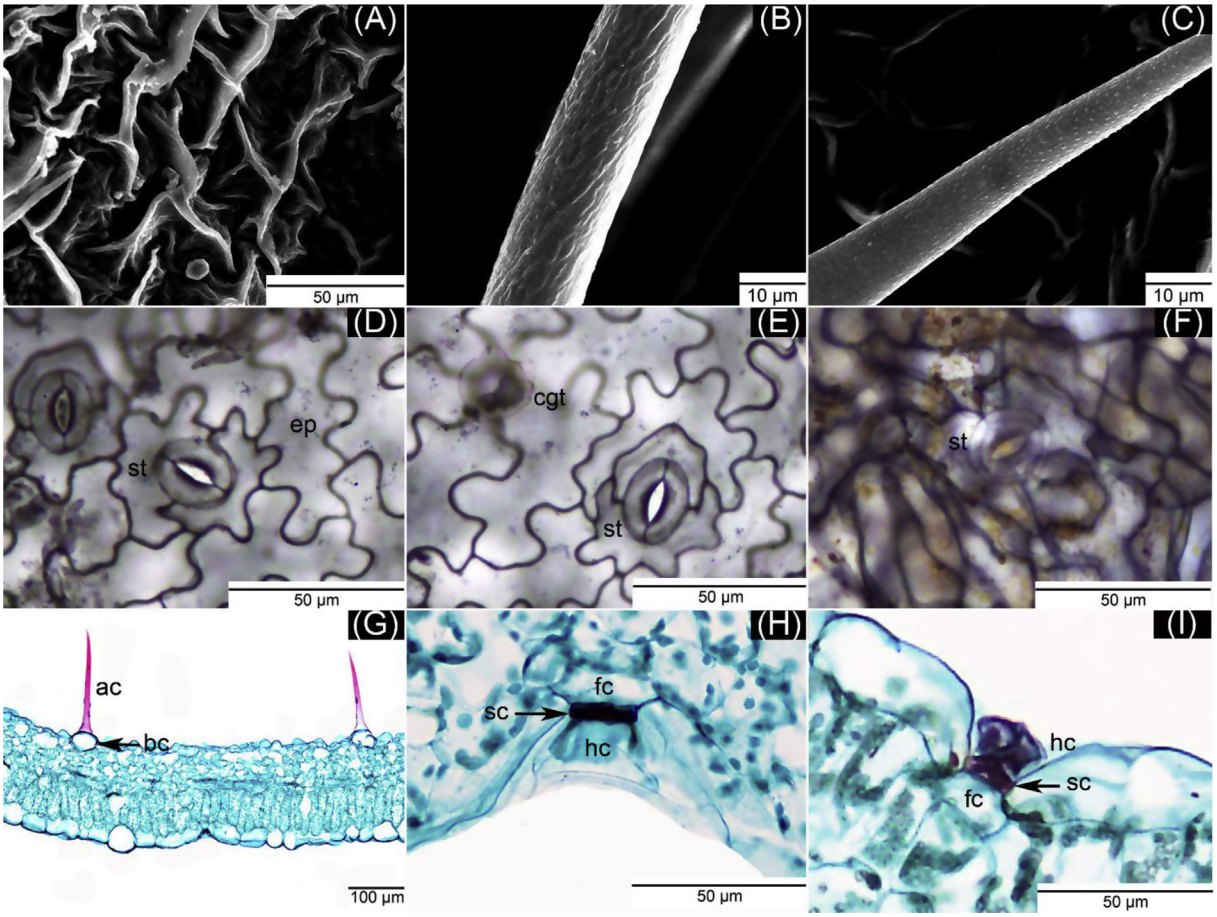

Fig. 1. General leaf micromorphological characters of *Pogostemon* section *Eusteralis* in Thailand: (A) Striate cuticular ornamentation on the adaxial leaf epidermal cells of *P. trinervis*; (B) striate ornamentation on the surface of a simple unicellular trichome in *P. cruciatus*; (C) micropapillae on a simple unicellular trichome of *P. trinervis*; (D) diacytic stomata on the abaxial leaf surface of *P. globulosus*; (E) diallellocytic stoma on the abaxial leaf surface of *P. globulosus*; (F) anomocytic stoma on the adaxial leaf surface of *P. helferi*; (G) simple unicellular trichomes on the abaxial leaf surface of *P. trinervis*; (H) a peltate trichome on the abaxial leaf surface of *P. globulosus*; (I) a capitate trichome on the adaxial leaf surface of *P. globulosus* (ac = apical cell, bc = basal cell, cgt = capitate glandular trichome, ep = epidermal cell, fc = foot cell, hc = head cell, sc = stalk cell, st = stoma).

Lamiaceae, as reported by Metcalfe and Chalk (1950); Harley et al. (2004). Some micromorphological characters of *Eusteralis* that are constant throughout the genus *Pogostemon* include: 1) the striate cuticular ornamentation on the epidermal cells; 2) the presence of capitate trichomes with two head cells; 3) the presence of peltate trichomes with a large head cell; 4) the convex abaxial outline of the midrib; and 5) the collateral vascular bundle with a parenchymatous sheath. However, the distinct leaf micromorphological traits of *Pogostemon* section *Eusteralis* that can distinguish it from *Pogostemon* section *Pogostemon* are the type of non-glandular trichomes (Metcalfe and Chalk, 1950; Harley et al., 2004; Pramali, 2017).

Variable leaf micromorphological characters were observed within *Eusteralis* and appear to be associated with the environmental conditions of the habitats. The characteristics of epidermal cells were different within the section. *Pogostemon cruciatus* exhibited straight-to-curved anticlinal cell walls and the shapes of the epidermal cells are pentagonal to hexagonal. Conversely, the other three taxa—*P. helferi*, *P. globulosus* and *P. trinervis*—possessed undulate anticlinal cell walls and irregular epidermal cells. Previous work has shown that environmental conditions such as humidity play an important role in defining the undulation of anticlinal cell walls (Watson, 1942; Stace, 1965); epidermal cells with straight or curved anticlinal cell walls were especially found in species growing in dry habitats, while undulate walls were identified as being characteristic of species growing in areas with higher humidity (Solereder, 1908; Stace, 1965). Therefore, the differences in anticlinal cell walls between *P. cruciatus* and the other species might be influenced by the humidity of their habitats. *Pogostemon cruciatus*, which exhibited straight-to-curved anticlinal cell walls, was collected from drier areas such as grasslands, montane forests or coniferous forests. In contrast, the other three species (*P. globulosus*, *P. herferi* and *P. trinervis*) showed irregular epidermal cell shapes because they grow in damp areas, such as streams or wetlands occurring in rice fields (Bongcheewin, 2005).

Stomata were present on both leaf surfaces, except in *P. cruciatus*, where stomata were restricted to the abaxial surface. The occurrence of stomata was less correlated with environmental conditions, with the exception of water availability (Parkhurst, 1978); therefore, habitat humidity might have influenced the stomatal occurrence of these four *Pogostemon* species. *Pogostemon cruciatus* possessed hypostomatous leaves, as is commonly found in drier areas, while the other three species exhibited amphistomatous leaves that are typical of moist habitats (Bongcheewin, 2005). Amphistomatous leaves are characteristic of plants that have high photosynthetic capacities growing in full-sun environments with available water (Mott et al., 1982). This trait is an adaptation to reduce the intercellular distance of carbon dioxide diffusion (Parkhurst, 1978), improving stomatal conductance that is necessary for sun leaves that have high photosynthetic rates (Mott and

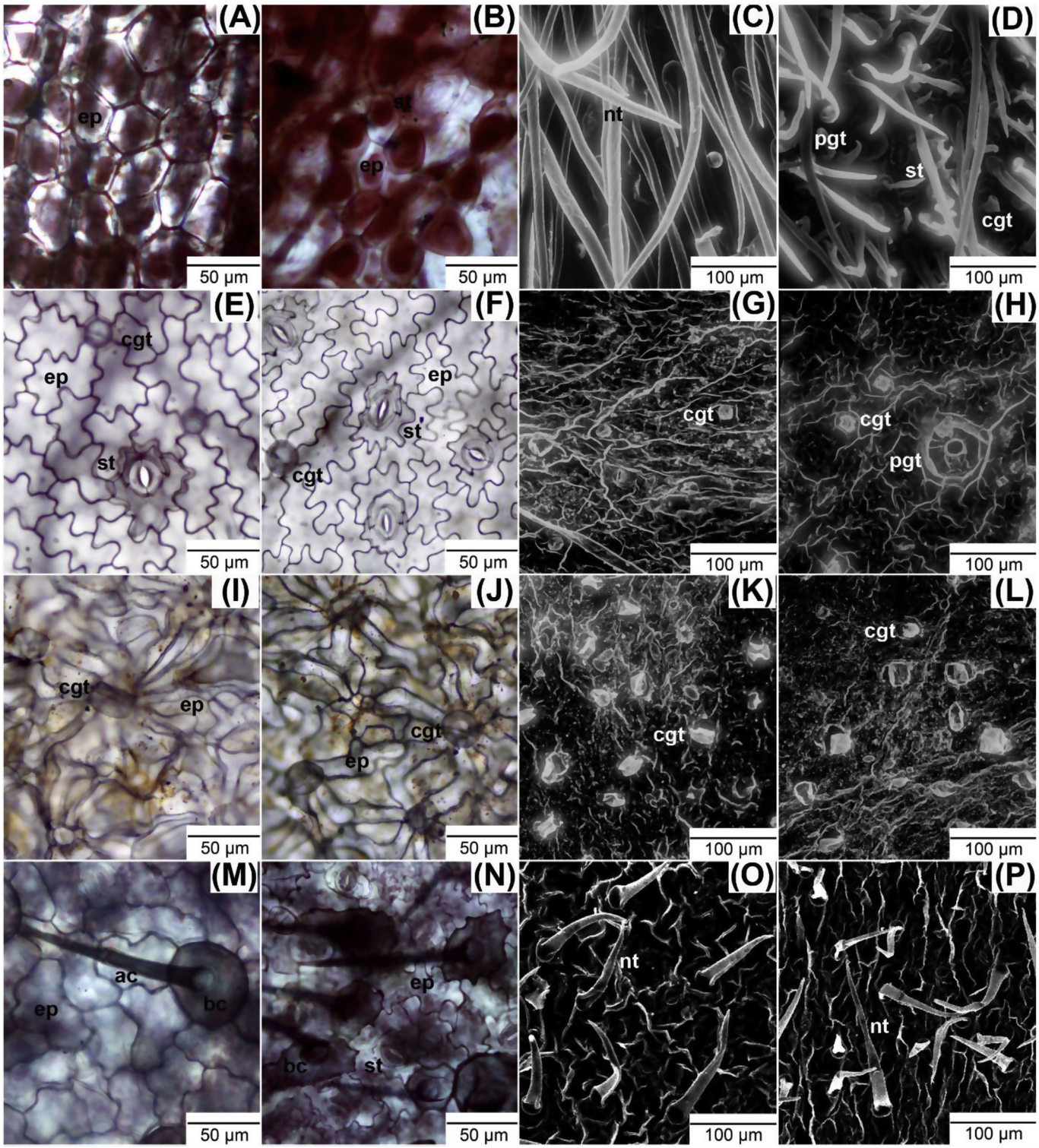

Fig. 2. Leaf epidermal surfaces of (A–D) *P. cruciatus*; (E–H) *P. globulosus*; (I–L) *P. helferi*; (M–P) *P. trinervis*; (A, E, I, M) light microscope micrographs of adaxial leaf surface; (B, F, J, N) light microscope micrographs of abaxial leaf surface; (C, G, K, O) scanning electron micrographs of adaxial leaf surface; (D, H, L, P) scanning electron micrographs of abaxial leaf surface (ac = apical cell, bc = basal cell, cgt = capitate trichome, ep = epidermal cell, nt = non-glandular trichome, pgt = peltate trichome, st = stoma).

Michaelson, 1991). Although *P. cruciatus* grows in grasslands exposed to full sunlight, it possesses hypostomatous leaves. This raises the question, why do stomata occur only on the abaxial leaf surface of *P. cruciatus*. This trait may have evolved to prevent stomatal pores on the adaxial leaf surface from being obstructed by dust or rainwater among other things, which could be more likely to occur than on those of the abaxial leaf surface (Parkhurst, 1978). Another possibility is to decrease evaporation rates from adaxial leaf surfaces that are exposed directly to the sun (Parkhurst, 1978). Since the saturation of water vapor pressure is exponential

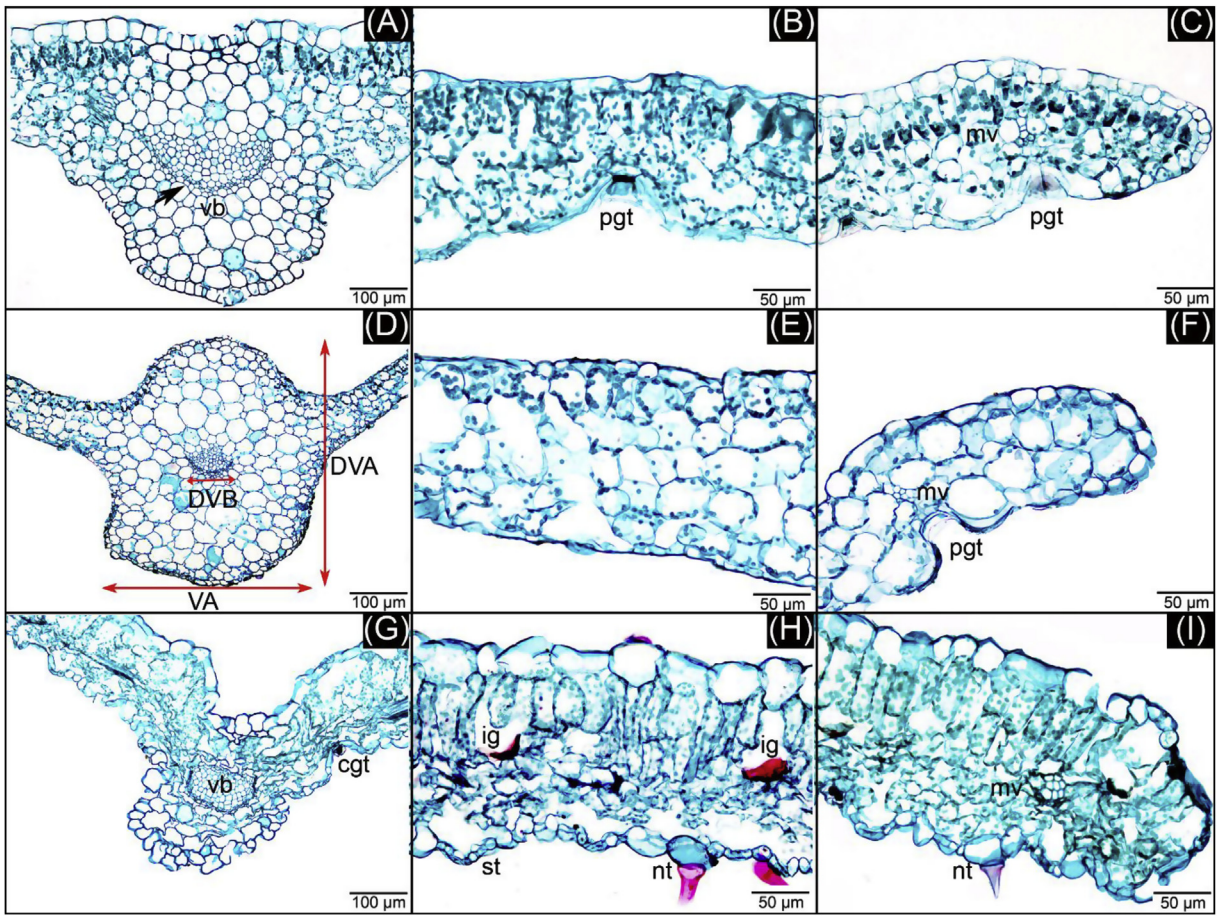

Fig. 3. Leaf transverse sections of (A–C) *P. globulosus*; (D–F) *P. helferi*; (G–I) *P. trinervis*; (A, D, G) midrib; (B, E, H) lamina; (C, F, I) leaf margins (arrow = paranchymatous sheath, cgt = capitate trichome, DVA = dorsiventral axis, DVB = diameter of vascular bundle, ig = internal gland, mv = minor vein, nt = non-glandular trichome, pgt = peltate trichome, st = stoma, VA = ventral axis, vb = vascular bundle).

depending on tissue temperature (Parkhurst and Loucks, 1972), the adaxial leaf surface is slightly warmer, leading to greater evaporation from the adaxial side compared with the abaxial side (Parkhurst, 1978). Therefore, hypostomatous leaves in *P. cruciatus* might be due to the dry and full-sun conditions of its habitat, which select for leaf adaptations to reduce water evaporation.

Observed stomatal types were typically diacytic and diallelocytic; however, *P. helferi* had some anomocytic stomata mixed with the diacytic type. The presence of the diacytic stomata type in *Pogostemon* section *Eusteralis* corresponded to a previous report by Metcalfe and Chalk (1950); however, they did not mention anomocytic or diallelocytic types. Later, Cantino (1990) noted that the diallelocytic type was found in all species of the tribe Pogostemoneae. Moreover, Harley et al. (2004) mentioned that anomocytic and diacytic types were most commonly encountered in the Lamiaceae, while the diallelocytic type was most frequently observed in the subfamily Nepetoideae. Previous work has shown that the spacing of the stomatal complex is non-random and primarily forced by a cell lineage-dependent mechanism, in which the distribution of guard cells is regulated by a series of asymmetric cell divisions for the differentiation of guard cells and subsidiary cells (Larkin et al., 1997; Glover, 2000). However, stomatal density fluctuates within leaves, within plants, and across individuals of the same species (Al Afas et al., 2006). It can be influenced by environmental conditions (Casson and Hetherington, 2010) such as drought (Elias, 1995), atmospheric CO_2 concentration (Ferris and Taylor, 1994; Ceulemans et al., 1995; Jarvis et al., 1999), as well as light (Gay and Hurd, 1975). In this study, adaxial stomata were often looser than abaxial stomata, similar to the observations of other lamiacious species (Abdulrahaman and Oladele, 2005). This difference may occur because the majority of photosynthetic gas exchange occurs through abaxial stomata (Glover et al., 1998), which is an adaptation to decrease water evaporation from the adaxial side since it is directly exposed to solar radiation (Parkhurst, 1978). Moreover, the highest density of abaxial stomata occurred in *P. cruciatus*. This finding may be related to stomatal conductance (g_s), which is measured by determining the rate of CO_2 entering, or H_2O exiting, stomatal apertures (Lawson and Blatt, 2014). The g_s is constrained by the size and density of stomata on the plant epidermis, and the maximum rate of g_s is estimated based on these stomatal traits (Dow et al., 2014). Indeed, stomatal apertures in the real environment tend to function far below the maximum opening rate, meaning that guard cells rarely function at maximum turgor pressure, which can decrease the sensitivity of guard cell response (Dow and Bergmann, 2014). Therefore, plants subsequently alter gas exchange capacity via stomatal development to increase stomatal density, while maintaining stomata under optimum functional turgor pressure (Dow and Bergmann, 2014). Consequently, *P. cruciatus*, which has hypostomatous leaves and the shortest guard cells, must compensate with increased stomatal density in order to optimize stomatal conductance under dry and full-sun conditions.

The leaf surfaces of the examined species were covered by a combination of non-glandular and glandular trichomes, except in *P. helferi*, which had no non-glandular trichomes. Uni- and

bicellular non-glandular trichomes co-occurred on both leaf surfaces, which is concordant with Lamiaceae anatomical evidence from Harley et al. (2004); Pramali (2017). The non-glandular trichomes of all investigated *Pogostemon* section *Eusteralis* can be distinguished from *Pogostemon* section *Pogostemon* in terms of the number of apical cells. Although previous leaf micromorphological studies of *Pogostemon* reported simple multicellular trichomes as general non-glandular trichomes (Press, 1982; Tahir et al., 1995; Pradeep and Murugan, 2013), simple multicellular trichomes were absent in *Pogostemon* section *Eusteralis* in this study (Pramali, 2017). Thus, this trait may be an alternative taxonomic character to support the classification of Keng (1978) and Press (1982), in which the infrageneric classification of *Pogostemon* included two taxonomic levels based on leaf arrangement (opposite or verticillate). These results were inconsistent with the phylogenetic study of Yao et al. (2016), which showed poor support for both leaf arrangement and habitat characters; for example, species that had verticillate leaves were nested within a clade (B) comprised of species with opposite leaves (Yao et al., 2016). In order to better understand the relationships of *Pogostemom* spp., the examination of more characters is required. The current study has offered additional micromorphological characters that may be useful.

The highest density of non-glandular trichomes occurred in *P. cruciatus*, which might be related to its dry and full-sun habitats. As plants respond to water shortages by closing their stomata, the CO_2 availability in chloroplasts is reduced, resulting in progressively decreasing photosynthesis and photosynthetic capacity (Cornic and Massacci, 1996). Consequently, photosynthesis under low-water conditions does better in lower light intensity compared to that of well-watered conditions (Cornic and Massacci, 1996), since high light intensity increases the susceptibility of drought-stressed plants to photoinhibition (Ma et al., 2015). Therefore, it is necessary for plants exposed to the combination of low water availability and high solar radiation to protect against photoinhibition (Galmés et al., 2007). Non-glandular trichomes are one photo-protective mechanism that functions as a mechanical barrier for leaf surfaces by increasing radiation reflectance (Ehleringer et al., 1976). This reasoning may explain why *P. cruciatus* growing under low water availability and full sunlight has a higher density of non-glandular trichomes than the other three species inhabiting full sunlight, yet damp, habitats.

The glandular trichomes observed in this study were capitate trichomes with two head cells and peltate trichomes with a single large head cell. The former was more frequently observed than the latter in all investigated species. In accordance with Ascensão et al. (1999), short-stalked capitate trichomes generally appear in Lamiaceae. Moreover, the capitate type was also recorded in *P. cablin* (Blanco.) Benth., which belongs to *Pogostemon* section *Pogostemon* (Guo et al., 2013; Rusydi et al., 2013). Due to the presence of this similar character in both sections, capitate trichomes appear to have minimal taxonomic significance for Thai *Pogostemon* section *Eusteralis*. In contrast, peltate trichomes are taxonomically significant in *Pogostemon* at the generic level in that the head cells are unicellular. They are unique for the genus *Pogostemon* and the closely related genus *Anisomeles* (Cantino, 1990). The current results revealed substantial spatial variation in the density of capitate trichomes, exhibiting different densities among diverse environmental conditions. *Pogostemon cruciatus* found in arid habitats had the lowest density of capitate trichomes, while *P. herferi* growing in habitats with high water availability possessed the highest density of capitate glandular trichomes. The positive correlation between water availability and glandular trichome density suggests that glandular trichome density is directly influenced by water availability, in accordance with previous studies (Martínez-Natarén et al., 2011; Yadav et al., 2014; Thitz et al., 2017). It has been proposed that habitats with low-resource availability, such as in arid areas, reduce photosynthetic processes that are required for the production of trichomes (Martínez-Natarén et al., 2011; Thitz et al., 2017).

The observed vascular bundles in this study were usually arranged in a crescent collateral shape and were always surrounded by a parenchymatous sheath, as reported by Metcalfe and Chalk (1950). The mesophyll of two species (*P. trinervis* and *P. globulosus*) showed a clear-cut boundary between the palisade and spongy layers, unlike in *P. helferi*, in which the border between the mesophyll layers was unclear. Due to its aquatic habit, the mesophyll of *P. helferi* may reflect an adaptation to the environment (Boeger and Poulson, 2003; Evert, 2006; Scremin-Dias, 2009). However, *P. helferi* exhibited dimorphic leaves, consisting of emerged leaves that are less durable than submerged leaves. The emerged leaves were only presented near inflorescence during a very short flowering period, while submerged leaves were consistently found on stems year-round. Therefore, submerged leaves were investigated in this study because this leaf type is usually found in both fresh and herbarium specimens and might be more useful for taxonomic purposes than emerged leaves. This study also found internal glands in the palisade mesophyll, which was in agreement with Maeda and Miyake (1997) and Guo et al. (2013) who reported that internal glands or large sac-like secretory cells occurred in various species within the Lamiaceae (Metcalfe and Chalk, 1950).

Although the examined species were adapted to a range of habitats, from terrestrial to aquatic/marshland, the type of non-glandular trichomes observed was constant through all investigated samples. Therefore, this trait might be an additional taxonomic character used for sectional delimitation of the genus *Pogostemon*, which conforms to the infrageneric classification by Keng (1978) and Press (1982). The species in the section *Eusteralis* possess only simple unicellular and simple bicellular trichomes, while the taxa in section *Pogostemon* have simple multicellular trichomes in addition to simple unicellular and simple bicellular trichomes (Pramali, 2017). Furthermore, the observed anatomical characters might be useful for facilitating the subgeneric classification of the genus in further evolutionary studies. However, additional species throughout *Pogostemon* s.l. should be added and examined in the future to affirm the taxonomic usefulness of leaf anatomical traits.

Conflict of interest

There is no conflict of interests.

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