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Leaf anatomical characters of four epiphytic orchids of Sempu Island, East Java, Indonesia: The importance in identification and ecological adaptation

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Abstract. *Rindyastuti R, Nurfadilah S, Rahadiantoro A, Hapsari L, Abywijaya IK. 2018. Leaf anatomical characters of four epiphytic orchids of Sempu Island, East Java, Indonesia: The importance in identification and ecological adaptation. Biodiversitas 19: 1906-1918.* Leaf anatomy features are important characters to support species identification and classification, and they are related to ecological adaptation of species. The aims of the present study were: (i) to investigate leaf anatomical characters of four epiphytic orchids of Sempu Island (*Ascochilus emarginatus, Dendrobium subulatum, Thrixspermum subulatum,* and *Thrixspermum acuminatissimum*) in relation to the significance in species identification and ecological adaptation in coastal habitats of Sempu Island, (ii) to compare the adaptive ability of the four species in coastal habitats based on adaptive anatomical characters. The procedure of leaf anatomical studies as follows: orchid leaves were fixed in ethanol 70% and sliced into thin pieces with a microtome, and stained with 1% Safranin. The leaf anatomical organization of orchids (stomata, epidermis, mesophyll, vascular bundles, and other characters such as hypodermis, fibre bundles, raphide bundles, and spiral thickenings) was observed under light microscope. The results showed the comparable data of leaf anatomical characters among the orchids. There was distinct variation in the anatomical characters of the orchids including stomata anomocytic, tetracytic, and cyclocytic; the presence or absence of hypodermis, spiral thickenings, fibre bundles, raphide bundles, and bundle sheaths; homogenous and heterogenous mesophyll; and variation in vascular bundle arrangement. Detailed leaf anatomical characters can be used to distinguish a species from others, which are important to support species identification. The similarity of anatomical characters among these orchids were they possessed relatively thick cuticle and other specific anatomical characters as a structural adaptation to coastal habitat with high irradiation to reduce leaf transpiration. *D. subulatum* can be considered as the most adaptive orchid species to coastal habitats based on adaptive anatomical characters as it possessed the largest number of adaptive anatomical characters. The implication of this study is the importance of leaf anatomical features to support species identification and to increase understanding of orchid biology and ecology which are important in orchid conservation.

Keywords: Anatomy, ecology, environment, Orchidaceae, small island

INTRODUCTION

Orchidaceae is one of the most successful plant families in evolution and speciation, resulting in approximately 25.000 species across the world with highly various morphological and anatomical characters (Dressler 1993). Anatomical characters of orchids have been widely studied in a wide range of species within tribes, subtribes, and genera levels, such as tribe *Calypsoeae* (Stern and Carlsward 2008); subtribes *Laeliinae* (Stern and Carlsward 2009); *Aeridinae*, *Angraecinae* and *Aerangidinae* (Carlsward et al. 2006); *Stanhopeinae* (Stern and Whitten 1999), *Orchidinae* (Stern 1997); *Habenariinae* (Stern 1997), *Oncidiinae* (Stern and Carlsward 2006); and genera *Caladenia* (Pridgeon 1993), *Dendrobium* sections *Aporum* and *Rhizobium* (Carlsward et al. 1997), *Ophrys, Orchis* and *Dactylorhiza* (Aybeke et al. 2010).

Anatomical features are important characters to support identification and classification in Orchidaceae (Pridgeon 1982; Aybeke et al. 2010, Fan et al. 2014). Pridgeon (1982) and Pridgeon and Norris (1979) showed diagnostic characters to distinguish genera within subtribe *Pleurothallidinae* (*Acostaea, Barbosella, Brachinionidium, Cryptophoranthus, Dracula, Dresslerella, Dryadella, Lepanthes, Masdevallia*, and other genera within the subtribe) using anatomical characters of leaf such as cuticle, epidermis, hypodermis, spiral thickenings, and vascular bundle number. Fan et al. (2014) reported that *Holcoglossum* could be distinguished from the related genera, such as *Ascocentrum, Luisia, Papilionanthe, Rhynchostylis,* and *Vanda* based on leaf cuticular wax characteristics. Furthermore, Aybeke et al. (2010) demonstrated the use of anatomical characters to distinguish species within genera of *Ophrys,* such as *O. speculum, O. fusca, O. lutea, O. sphegodes, O. cornuta, O. umbilicata, O. bucephala, O. apifera* and *Orchis,* such as *O. coriophora*, *O. tridentata, O. militaris, O. papilionacea, O. mascula,* and *O. palustris*. The species had particular comparable anatomical characters that can be used to distinguish it from others using anatomical characters, such as thickness of epidermal walls and shape of chlorenchyma cells.

Anatomical characters have been shown to have the relationship with the ecological adaptation of orchids. Moreira et al. (2013) showed the leaves of *Epidendrum secundum* growing in the luminous area had a relatively thick cuticle, indicating adaptation to the environment with intense solar radiation, with cuticle functions as a barrier to reduce transpiration because of the high intensity of sunlight. Fan et al. (2014) demonstrated other anatomical characters showing structural adaptation in other orchids. They reported that *Holcoglossum* had structural adaptations to strong winds and ample rains in subalpine region of the Hengduans Mountains by having laterocytic and polarcytic stomata in their leaf epidermal layer.

Previous studies on orchids anatomy in Indonesia have been conducted including anatomical characters of roots of orchids of Sempu Island (Nurfadilah et al. 2016), leaf anatomy of nine species of *Bulbophyllum* (Orchidaceae) (Betty 2011), comparative leaf and root anatomy of two species of *Dendrobium* (Metusala et al. 2017). The present study of orchid leaf anatomy provides additional data to support species identification and to increase understanding of orchid biology and ecology, concerning structural adaptation of orchids in coastal habitats in Sempu Island. The clear species identification, biology and ecology data of orchids are required in the management of orchid conservation. Furthermore, understanding of the adaptive ability of orchids to coastal habitats based on adaptive anatomical characters also supports the assessment of the susceptibility of orchid species to the environmental alteration, which is important for species conservation priority.

Various orchids can be found in Sempu island, especially on coastal areas exposed to irradiation. Approximately 15 orchid species have been recorded in Sempu island, that consisted of mostly epiphytic orchids (14 species) and one terrestrial orchid (Rindyastuti et al. 2018). The most common orchids that could be found in Sempu island included *Ascochilus emarginatus* (Blume) Schuit., *Dendrobium subulatum* (Blume) Lindl., and *Thrixspermum subulatum* (Blume) Rchb.f. (Figure 1). The present study aimed (i) to investigate the anatomical characters of these orchids of Sempu Island (*A. emarginatus, D. subulatum, T. subulatum*)., as well as *Thrixspermum acuminatissimum* (Blume) Rchb.f. (ii)) to compare the adaptive ability of the four orchids to coastal habitats based on adaptive anatomical characters.

Figure 1. Four orchids species which grow in coastal habitats of Sempu Island. A. *Ascochilus emarginatus* (Blume) Schuit., B. *Dendrobium subulatum* (Blume) Lindl., C. *Thrixspermum subulatum* (Blume) Rchb.f., D. *Thrixspermum acuminatissimum* (Blume) Rchb.f. Courtesy: Figure 1.A. Siti Nurfadilah, Figure 1.B. Nina Dwi Yulia, Figure 1.C. Apriyono Rahadiantoro, Figure 1.D. Comber (1990)

MATERIALS AND METHODS

Study area

Sempu Island is a small island located off the south coast of East Java, Indonesia (Figure 1). The island is a nature reserve under the Ministry of Forestry with an area of approximately 877 ha. The island altitude ranges between 0 and 102 m asl. The epiphytic orchids were collected in the coastal habitat in Waru-Waru and Air Tawar, Sempu Island with temperatures around 27-29 ^{o}C, high humidity 90-94 %, and sunlight intensity around 52-163 lux. The epiphytic orchids grew on some host trees (phorophytes), such as *Terminalia catappa* L., *Streblus asper* Lour., *Sophora tomentosa* L. in the coastal habitats that were exposed to high irradiation.

Anatomical sample preparations

The leaves were fixed in ethanol 70%, and sliced into thin pieces with a microtome, and stained with 1 % Safranin (Stern and Judd, 2000). The leaf anatomical organization of orchids was observed under a light microscope (Olympus CX21) with three replication of slices of leaf for each orchid species. The leaf anatomical features of all orchids were characterized, including stomata, epidermis, mesophyll, vascular bundles, and other characters (hypodermis, fibre bundles, raphide bundles, and spiral thickenings). The size of stomata, epidermis, and cuticle thickness was measured using micrometer.

Assessment of the adaptive ability of orchid species to coastal habitats

Adaptive anatomical characters, such as smaller stomata, larger epidermis, the presence of hypodermis, many layers of mesophyll, the presence of fibre bundles, spiral thickenings and bundle sheaths were used to assess the adaptive ability of orchid species to coastal habitats (Aybeke et al. 2010; Fahn 1982; Guan et al. 2011; Roth 1984; Reginato et al. 2009; Hsiao 1973; Metusala et al. 2017; Vincent 2000; Richter et al. 2011; Placet et al. 2014; Leroux et al. 2010; Lack and Evans 2001). The data of adaptive anatomical characters were obtained by comparing the size of stomata and epidermis, the presence of hypodermis, many layers of mesophyll, the presence of fibre bundles, spiral thickenings and bundle sheaths among four orchid species. Assessment of the most adaptive orchid species was established through the largest number of adaptive anatomical characters it had.

Data analyses

Data of stomata size, cuticle thickness, and epidermis size among four orchid species were analyzed using Analysis of Variance (ANOVA) and Tukey post-test in confidence level of 95% using MINITAB 15.0. Comparison of the adaptive ability of orchid species to coastal habitats was based on the presence and absence of adaptive anatomical characters, such as smaller stomata, larger epidermis, the presence of hypodermis, more layers of mesophyll, the presence of fibre bundles, spiral thickenings, and bundle sheaths.

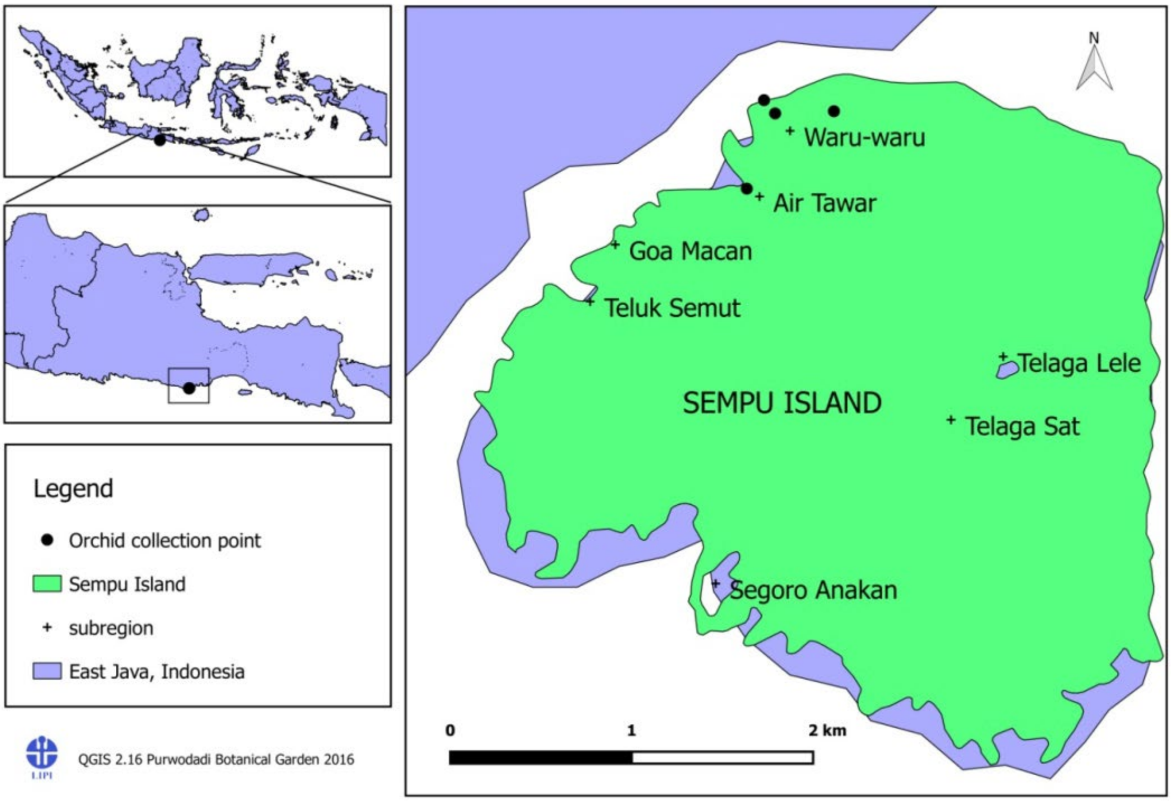

Figure 2. The study site of epiphytic orchid species in Waru-Waru and Air Tawar within Sempu Island, East Java, Indonesia

RESULTS AND DISCUSSION

The results of this study showed leaf anatomical features of orchids of Sempu Island and comparable data among the orchids to support species identification and to understand their structural adaptation to coastal habitat in Sempu Island.

Leaf anatomical features

The leaf anatomical structures showed organization of components forming leaves consisted of stomata, cuticle, epidermis, mesophyll, and vascular bundles. There were specific characters, such as hypodermis, spiral thickenings, fibre bundles, and raphide bundles in epidermal cells in particular orchid species (Table 1).

Ascochilus emarginatus

Leaf transverse section of *Ascochilus emarginatus* showed the leaf anatomical structures including stomata with anomocytic and tetracytic configuration. Cuticle was striate, relatively thick ranging from 6.3-11 µm. The epidermis was uniseriate, composed of elongated-shaped cells. Hypodermis was absent or not clear. Mesophyll was homogenous, consisted of 10-14 layers and consisting of thin-walled and rounded-shaped parenchymatous cells. Parenchymatous cells contained chlorophylls (chlorenchyms). Spiral thickenings were present in parenchymatous cells of mesophyll. Vascular bundles consisted of xylem and phloem arranged collaterally. There were 9-10 arches of vascular bundles arranged in a single row embedded in parenchymatous cells of mesophyll. There were no sclerenchyma cells associated with vascular bundles. Thin-walled bundle sheath surrounded the vascular bundle (Table 1; Figure 2).

Dendrobium subulatum

Dendrobium subulatum anatomical characters showed that stomata configuration was cyclocytic. Cuticle was striate, relatively thick ranging from 2.9-5.4 µm. The epidermis was a single layer composed of elongated-shaped cells. There was a specific anatomical character of *D. subulatum*, fibre bundles subtended epidermal layer, forming hypodermal layers (Figure 3). Mesophyll was 19-22 layers, heterogeneous, consisted of elongated-shaped parenchymatous cells in the outer mesophyll and polygonal-shaped parenchymatous cells in the inner mesophyll. Chlorophylls were present in the parenchymatous cells of mesophyll. Vascular bundles were arranged radially within parenchymatous areas of mesophyll, consisted of 10-12 arches of vascular bundles. Sclerenchymatic cells were associated with phloem. Bundle sheath was thin-walled surrounding vascular bundles (Table 1; Figure 3).

Thrixspermum subulatum

Thrixspermum subulatum had anomocytic and tetracytic stomata configuration, smooth and thick cuticle layer ranging from 9.4-11.8 µm. The epidermis was uniseriate composed of polygonal-shaped cells. Hypodermis was absent. Mesophyll was 9-12 layers; homogenous consisted of polygonal-shaped parenchymatous cells. There were chlorophylls in parenchymatous cells of mesophyll. Spiral thickenings were present in parenchymatous mesophyll. Vascular bundles consisted of xylem and phloem arranged collaterally. No sclerenchyma cells were associated with vascular bundles. Bundle sheath was indistinct. There were 11-13 arches of vascular bundles arranged in a single row embedded within parenchymatous cells of mesophyll (Table 1; Figure 4).

Table 1. Leaf anatomical characters of four epiphytic orchids in Sempu Island

Anatomical characters	*Ascochilus emarginatus*	*Dendrobium subulatum*	*Thrixspermum Subulatum*	*Thrixspermum acuminatissimum*
Leaf shape	blade-like	cylindric	blade-like	blade-like
Leaf thickness	512.4-584.4 µm	1225-1773 µm	523.4-585.5 µm	681.6-923 µm
Stomata				
Stomata configuration	anomocytic, tetracytic	cyclocytic	anomocytic, tetracytic	tetracytic
Cuticle				
Thickness (µm)	6.3-11	2.9-5.4	9.4-11.8	5.7-14.9
Cuticle Pattern	striate	striate	smooth	smooth
Epidermis				
Number of epidermis cell layer	uniseriate	uniseriate	uniseriate	uniseriate
Epidermis cell shape	elongated	elongated	polygonal	elongated
Hypodermis	absent or indistinct	single layer, thin-walled	absent or indistinct	absent or indistinct
Mesophyll				
Number of mesophyll layer	10-14	19-22	9-12	14-17
Mesophyll cell shape	rounded	elongated, polygonal	polygonal	polygonal
Mesophyll cell thickening	no	no	no	no
Mesophyll homogenous	yes	no	yes	yes
Vascular bundles				
Bundle sheath	thin-walled	thin-walled	indistinct	thin-walled
Arch number	9-10	11-12	10-11	10
Vascular bundle arrangement	single row	radial	single row	single row
Specific characters				
Fibre bundles	absent	present	absent	absent
Raphide bundles in epidermis	absent	absent	absent	present
Spiral thickening	absent	absent	present	present

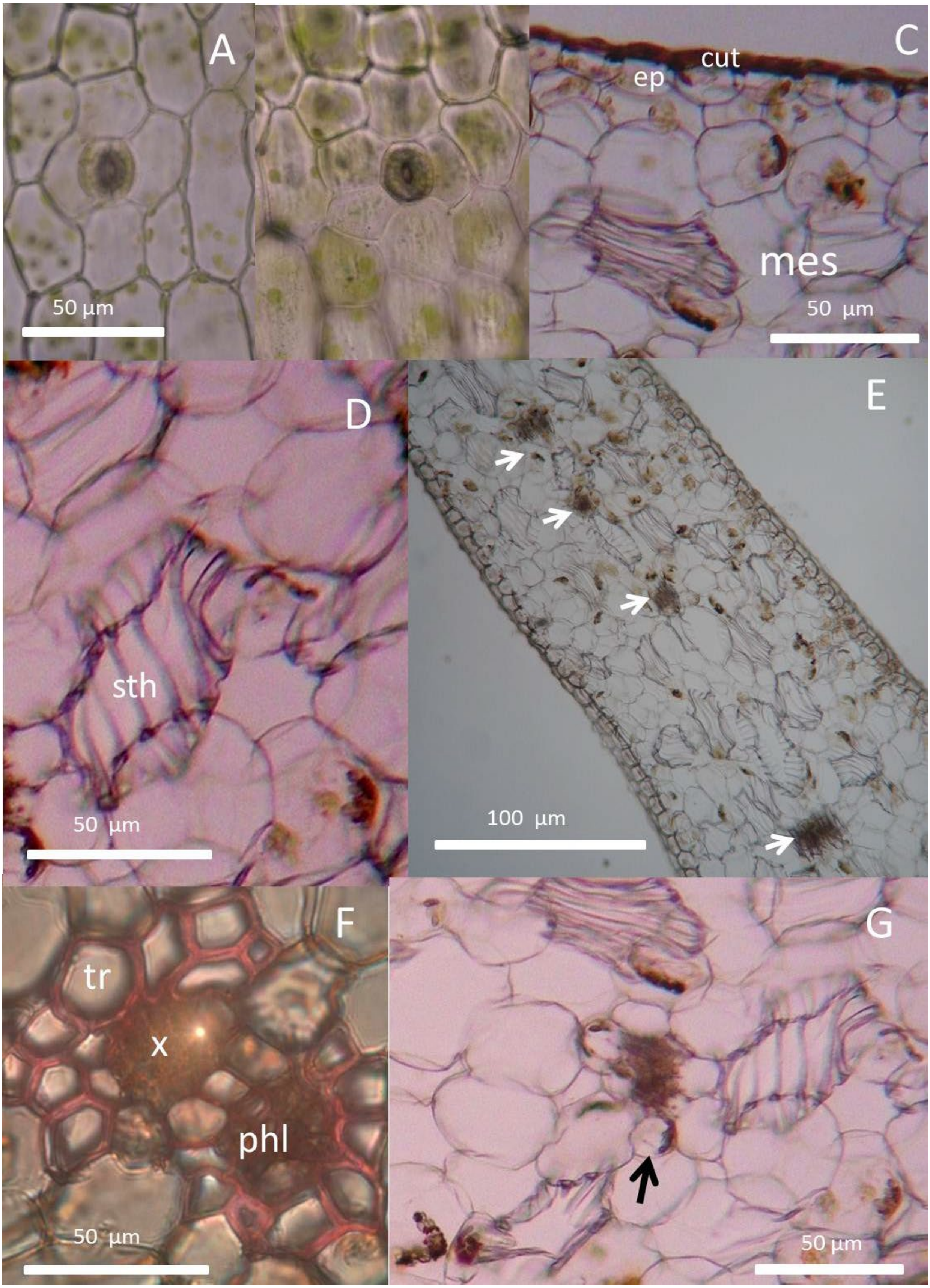

Figure 2. Transverse section of *Ascochilus emarginatus* leaf. A. Stomata, tetracytic; B. Stomata anomocytic; C. Cuticle (cut), epidermis (ep), mesophyll (mes); D. Spiral thickening in parenchymatous cell (Sth) within mesophyll (mes); E. Homogenous mesophyll and vascular bundles arranged in a single row, vascular bundle (arrow); F. Xylem (x), phloem (phl), tracheid (tr); G. Thin-walled cells of bundle sheath surrounding the vascular bundle (black arrow).

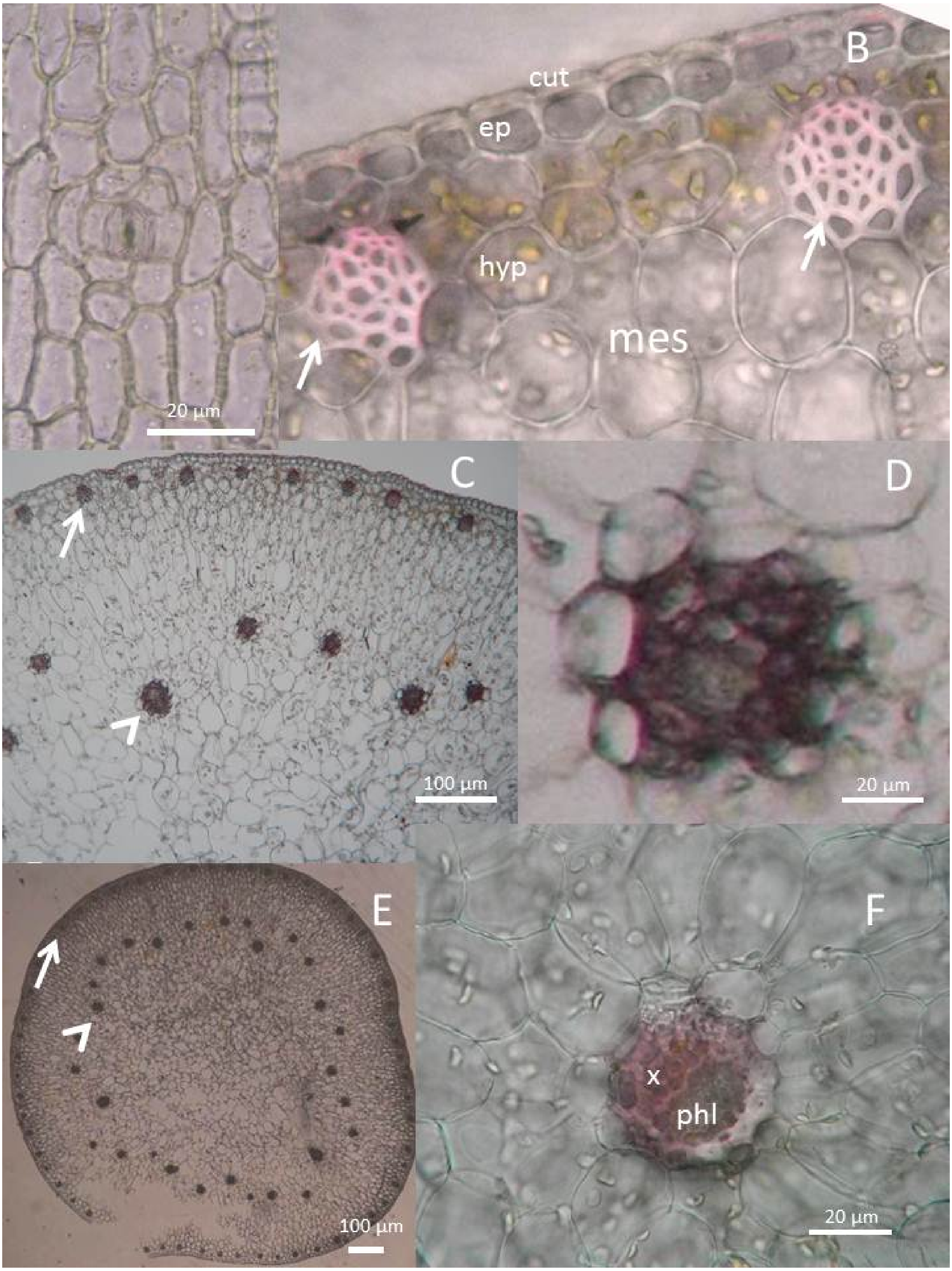

Figure 3. Transverse section of *Dendrobium subulatum* leaf. A. Cyclocytic Stomata; B. Cuticle (cut), sclerenchym (arrow), epidermis (ep), hypodermis (hyp), fibre bundle (arrow), mesophyll (mes); C. Heterogenous mesophyll, fibre bundle (arrow), vascular bundle (arrowhead); D. Thin-walled bundle sheath surrounding vascular bundle; E. Vascular bundles arranged radially, vascular bundle (arrowhead), fibre bundle (arrow); F. Xylem (x), phloem (phl), sclerenchyma associated with phloem (under phloem).

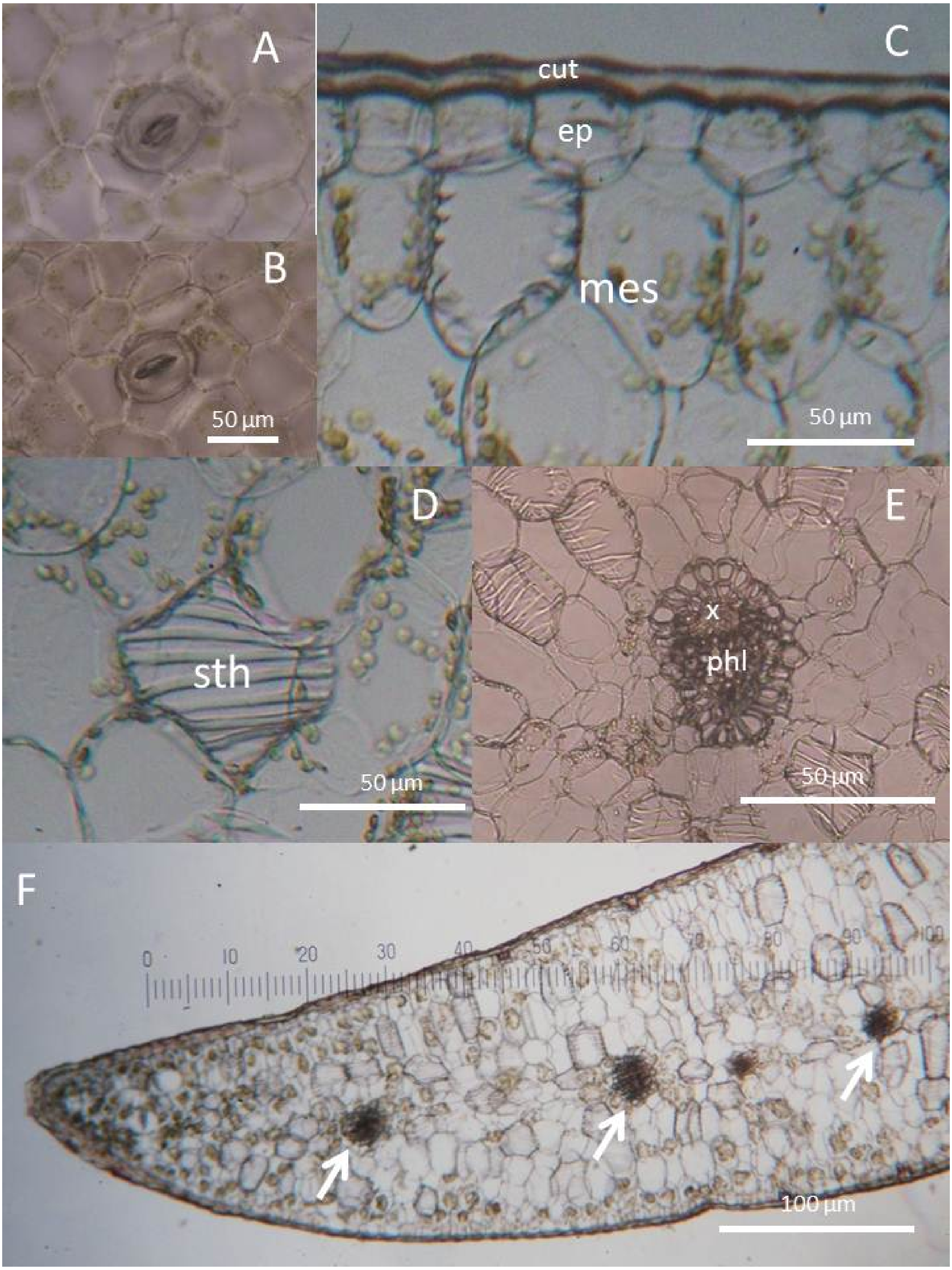

Figure 4. *Thrixspermum subulatum* leaf transverse section. A. Tetracytic stomata; B. Anomocytic stomata; C. Cuticle (cut), epidermis (ep), mesophyll (mes); D. Spiral thickening in parenchymatous cell (Sth); E. Xylem (x), phloem (phl), indistinct. bundle sheath; F. Homogenous mesophyll and vascular bundles arranged in a single row, vascular bundle (arrow)

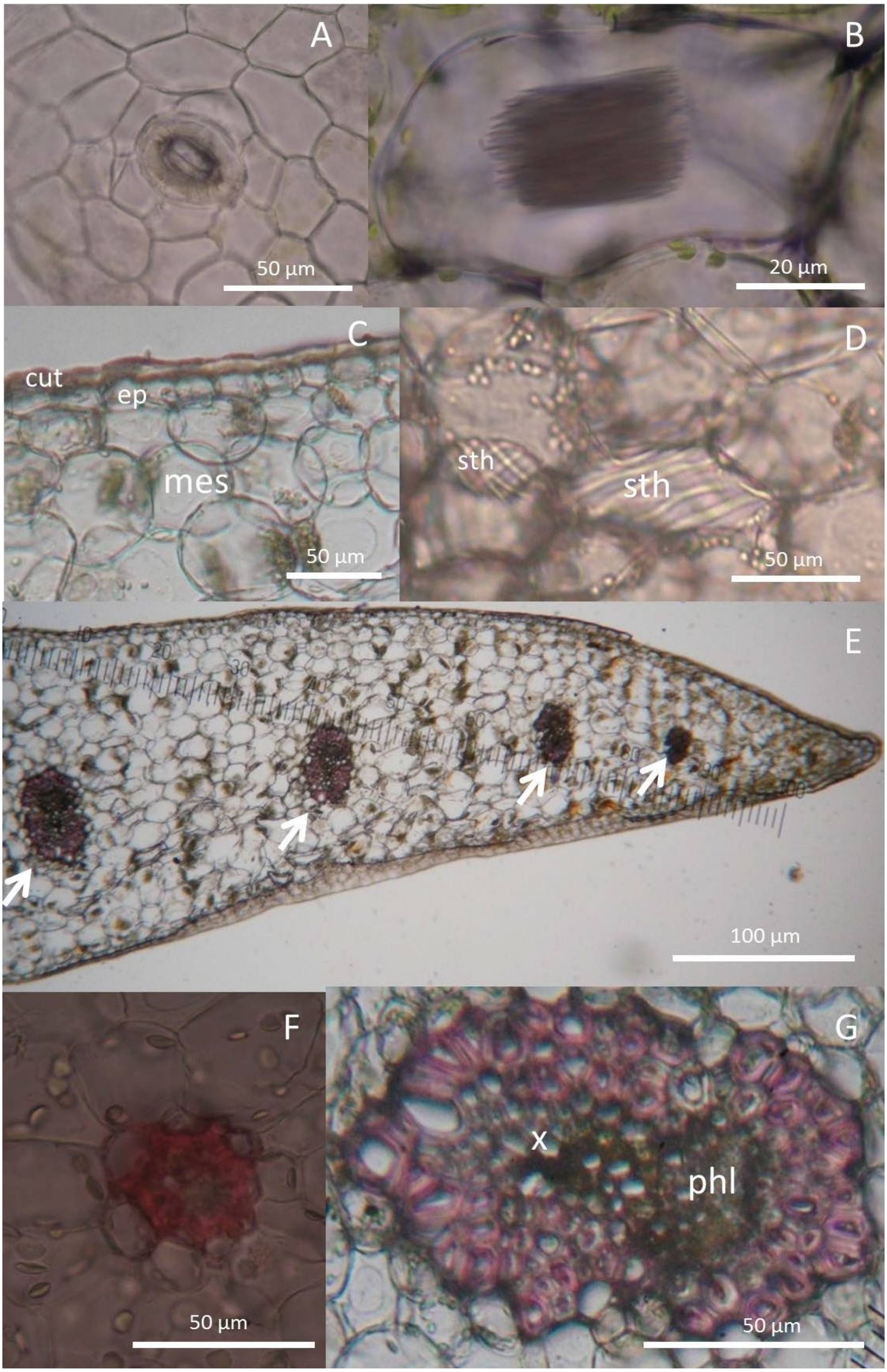

Figure 5. Leaf transverse section of *Thrixspermum acuminatissimum*. A. Stomata, anomocytic; B. Raphide bundles in epidermis cell; C. Cuticle (cut), Epidermis (ep), mesophyll (mes); D. Spiral thickening (Sth); E. Mesophyll homogenous, vascular bundles arranged in a single row, vascular bundle (arrow); F. Thin-walled bundle sheath surrounding vascular bundle; G. Xylem (x), phloem (phl), sclerenchyma cells associated with xylem and phloem.

Table 2. Means of stomata size, cuticle thickness and epidermis size of orchids Sempu Island

Anatomical characters	*Ascochilus emarginatus*	*Dendrobium subulatum*	*Thrixspermum subulatum*	*Thrixspermum acuminatissimum*
Stomata				
Stomata width (mean±SE)	36.83 ±3.35[b]	36.50 ±0.72[b]	55.82 ±2.05[a]	59.70 ±0.47[a]
Stomata length (mean±SE)	44.10 ±2.1[b]	37.40 ±2.07[b]	75.92 ±2.63[a]	79.65 ±1.02[a]
Cuticle				
Cuticle thickness (mean±SE)	8.82 ±0.96[a]	3.880 ± 0.45[b]	10.00 ± 0.32[a]	9.99 ± 0.84[a]
Epidermis				
Epidermis width (mean±SE)	11.73 ±1.18[ab]	8.98 ±1.635[b]	9.71 ±0.26[b]	21.38 ±4.74[a]
Epidermis length (mean±SE)	25.54 ±2.73[a]	13.02 ±1.59[b]	31.74 ±1.34[a]	31.26 ±1.94[a]

Note: Size was performed in µm; Different letters indicate significant differences among orchid species at $p < 0.05$ based on ANOVA and Tukey test

Thrixspermum acuminatissimum

Thrixspermum acuminatissimum had anomocytic stomata configuration and cuticle with 5.7-14.9 µm thick. Epidermis had one layer composed of elongated-shaped cells. Raphide bundles were present in epidermis cells. Hypodermis was absent. Mesophyll was homogenous, 14-17 layers consisting of polygonal shaped-parenchymatous cells. Chlorophylls and spiral thickenings were present in mesophyll parenchymatous cells. Vascular bundles consisted of xylem and phloem arranged collaterally. There were 8-11 vascular bundles arches arranged in a single row embedded in the mesophyll. Sclerenchyma cells associated with xylem and phloem. Thin-walled bundle sheath is surrounding vascular bundle (Table 1; Figure 5).

Anatomical features of the four epiphytic orchid species of Sempu Island

The leaf anatomical characters of four orchids of Sempu Island was revealed. There were similarities and differences in their anatomical features which showed variations in ecological adaptation among orchid species (Table 1 and Table 2).

Stomata

Stomata are small pores on leaves surfaces and have role of facilitating the gases movement in and out of leaves and, thus, the gas exchange in plants as a whole. Stomata have significant importance in the plant physiology, evolution, and ecology (Hetherington and Woodward 2003). Stomata configuration of plants varied, such as anomocytic, tetracytic, cyclocytic, and diacytic. The stomata configuration of four epiphytic orchids studied were various, anomocytic and tetracytic in *Ascochilus emarginatus* and *Thrixspermum subulatum,* (Figure 2 and Figure 4), cyclocytic in *Dendrobium subulatum* (Figure 3), and anomocytic in *Thrixspermum acuminatissimum* (Figure 5).

The stomata size (width and length) of the orchids was also significantly different among species (Table 2). According to Guan et al. (2011), larger stomata are slower to close and have greater potential for hydraulic dysfunction under drought condition. Small stomata is more tolerant to drought than the larger stomata. In the present study, *A. emarginatus* and *D. subulatum* had smaller stomata size than two other species (*T. subulatum T. acuminatissimum*). The smaller stomata of *Ascochilus emarginatus* and *D. subulatum* can support the species to be more adaptive to coastal habitat with warmer temperature and high irradiation.

Cuticle

The cuticle is the outermost layer of orchid leaves, deposited on the surface of epidermal cells. Plant cuticle plays an important role in the interaction of plants with the environment, such as to reduce absorbed solar radiation and temperature by reflecting the sunlight and to reduce transpiration (Domínguez et al. 2011; Fahn 1982; Rosso 1966). Cuticle contains polysaccharides, flavonoids and cutin matrix. These characters provide structural and chemical modifications for surface wetting, ranging from super hydrophilic to superhydrophobic to adapt to the environment (Koch and Barthlott 2009). Furthermore, according to Pridgeon (1993), cuticular patterns varies among species but are a consistent feature within each of them.

Orchidaceae has a large variation in cuticle thickness. In the present study, the orchids of Sempu Island had relatively thick cuticle (> 2.5 or 3 µm) (Carlsward et al. 2006), with various cuticle thickness, 8.82 µm in *A emarginatus;* 3.88 in *D. subulatum*; 10.005 in *T. subulatum,* and 9.99 in *T. acuminatissimum* (Table 2). The thick cuticle is an ecological adaptation to reduce transpiration (Moreira et al. 2013) and dry condition (Guan et al. 2011). The thick leaf cuticle of the orchids is significantly different among species based on the variance analysis. The cuticle of *D. subulatum* is thinner than that of three other species (*A. emarginatus, T. subulatum* and *T. acuminatissimum*). The thicker cuticle of these three species can also support for more adaptive capability of these species in coastal habitats. There are some types of cuticle including smooth, striate, papillate, or pitted, verrucose along the contours of epidermal cells. In the present study, two types of cuticle were recorded on the orchid leaf of Sempu Island, striate and smooth. *Ascochilus emarginatus* and *D. subulatum* had striate cuticle, while *T. subulatum* and *T. acuminatissimum* had smooth cuticle.

Epidermis

The epidermis is the outer cell layer of a plant leaf that functions as a barrier interconnecting and separating leaves from the environment, and is important in the response of plants to external stimulus. Ecophysiologically, important functions of leaf epidermis either in the direct interaction with environmental factors (Dietz and Hartung 1996). According to Darling (1989), the epidermis is important to protect mesophyll and chlorenchyma from high solar radiation and reduce the heat load on leaves. The thicker the epidermal cells, the more radiation and heat can be reflected and reduced. Moreover, large epidermal cells in many species of orchids serve as water storage (Guan et al. 2011). Based on the variance analysis, the width and length of the epidermal cells significantly different among species. *T. acuminatissimum* has the largest epidermal cells among the species in this study, while *D. subulatum* has the smallest epidermal cells. The larger epidermal cells of *T. acuminatissimum* can support the species to be more adaptive to the warmer environment compared to other species. There are various shapes of leaf epidermis of orchids such as polygonal, isodiametric, rectangular, and elongated (Aybeke et al. 2010). The epidermis of orchids of Sempu Island (*A. emarginatus, D. subulatum*, *T. subulatum,* and *T. acuminatissimum*) in the present study had various shape of epidermal cells. *A. emarginatus, D. subulatum,* and *T. acuminatissimum* had elongated epidermal cells, while *T. subulatum* had polygonal epidermal cells (Table 1; Figure 2-5).

Hypodermis

The hypodermis is a structure beneath the epidermis. This structure can be present or absent in orchid species (Pridgeon 1982; Stern 1997). Together with the epidermis, it has a role to protect the underlying layers, parenchymatous mesophyll, from too high sun radiation especially UV A and UV B and reduce the heat load in the leaves (Roth 1984; Darling 1989). Another role of hypodermis is for water storage (Roth 1984; Reginato et al. 2009).

The hypodermis is an important anatomical character. The development of hypodermis is useful in distinguishing monocotyledon genera and species. For example, within *Pleurothallidinae*, such as *Acostaeae, Barbosella, Dryadella*, *Lepanthes, Masdevallia* and *Pleurothallis* had hypodermis, while other genera within the same section such as *Brachionidium* and *Dracula* did not exhibit to posses hypodermis (Pridgeon 1982).

In the present study, hypodermis was only present in *D. subulatum* with a single layer hypodermic cell, while it was absent or not clear in other species (*A. emarginatus, T. subulatum,* and *T. acuminatissimum*). Hypodermis in *D. subulatum* is a single layer and composed of thin-walled hypodermic cell. The thin-walled hypodermic cells usually have a role in water storage (Carlsward et al. 2006; Stern and Carlsward 2008). Hypodermal layers in orchids also serve as additional structures on leave thickness and succulence (Metusala et al. 2017). The presence of hypodermis in *D. subulatum* in the present study is one of the important structures to store water and as a structural adaptation in coastal habitat in Sempu Island, with relatively high irradiation to reduce water loss because of leaf transpiration. Some other orchids of Sempu Island (*A. emarginatus, T. subulatum,* and *T. acuminatissimum*) that did not possess hypodermis, had another additional structure for adaptation in relatively high illumination in coastal habitat in Sempu Island (spiral thickening; discussed below).

Mesophyll

The mesophyll is an important structure in leaves containing a vital component for photosynthesis (chlorophyll) to assimilate nutrients. The number of mesophyll layers in the present study varied; *A. emarginatus* (10-14 layers), *D. subulatum* (19-22 layers), *T. subulatum* (9-12 layers), and *T. acuminatissimum* (14-17 layers). The thicker mesophyll layers also support the leaves succulence. The succulence level of leaves related to the parenchymal capacity of mesophylls to provide water supply for photosynthetic process and leaves cells turgor especially in the arid environment (Hsiao 1973; Lack and Evans 2001; Metusala et al. 2017). In the present study, *D. subulatum* had more mesophyll layers than the other three orchid species indicating that *D. subulatum* was more succulent compared to the other three orchid species and had more capability to store water, which is important to survive in the habitats with high irradiation.

The mesophyll parenchymatic cells are known to be homogenous for some orchids and heterogeneous for some others (Carlsward et al. 2006; Stern and Whitten 1999). In the present study, there was variation in the homogeneity and heterogeneity of mesophyll of orchids of Sempu Island. *A. emarginatus, T. subulatum,* and *T. acuminatissimum* possessed homogenous mesophyll, while *D. subulatum* had heterogenous mesophyll. Homogeneity and heterogeneity of mesophyll of other orchids were also reported to be varied.

Vascular bundles

The vascular bundle is a transport system containing xylem and phloem that are important in the water and nutrients transport (Lack and Evans 2001; Fahn 1982). The vascular bundles arrangement in the mesophyll of orchids of Sempu Island varied. *A.emarginatus, T. subulatum,* and *T. acuminatissimum* had vascular bundles aligned in one row in the center of mesophyll, while the vascular bundles of *D. subulatum* arranged radially in the mesophyll (Figure 2-5).

Other orchids were also reported to have variation in the alignment of vascular bundles in the mesophyll. Vascular bundle arrangement in a single row was reported in *Campylocentrum micranthum* (Carlsward et al. 2006); in *Habenaria cornuta, H. holothrix, H. monorrhiza, H. occidentalis, H. odontopetala, H. snowdenii, H. vaginatum, Stenoglottis fimbriata, S. longifolia,* and *S. woodii* (Stern 1997); in *Calypso bulbosa, Govenia tingens, Tipularia discolor* (Stern and Carlsward 2008). Another vascular bundle arrangement, symmetric arrangement, was reported in *Dendrobium anceps* (Carlsward et al. 1997).

The presence or absence of sclerenchyma associated with vascular bundles (xylem and phloem) can be one of the anatomical characters in the differentiation between species (Stern and Carlsward 2009). In the present study, sclerenchyma cells associated with xylem and phloem were recorded in *Thrixspermum acuminatissimum*, associated with phloem only in *D. subulatum;* while *A. emarginatus* and *T. subulatum* did not exhibit to possess sclerenchyma associated with xylem or phloem. Other studies also showed the presence and absence of sclerenchyma associated with vascular bundles (xylem and or phloem) in some other orchid species.

Other orchids which were reported to possess schlerenchyma associated with vascular bundles were *Octomeria* sp. (Pridgeon 1982), *Campylocentrum micranthum* (Carlsward et al. 2006), *Epidendrum secundum* (Moreira et al. 2013), *Dendrobium leonis, D. anceps, D. brevimentum, D. aloifolium, D. distichum, D. indivisum, D. mannii, D. rosellum,* and *D. nathaniele* (Carlsward et al. 1997); *Arpophyllum giganteum* (Stern and Carlsward 2009), while other orchids which reported to not exhibit schlerenchyma cells associated with vascular bundles were *Barbosella cucullata* (Pridgeon 1982) and *Calypso* (Stern and Carlsward 2008).

Bundle sheath consisted of cells surrounding the vascular bundle. Bundle sheath is an anatomical structure of leaf which indicates that leaf contains two photosynthetic enzymes both Rubisco and PEP carboxylase to catalyze the CO_2 fixation in photosynthetic reactions. This feature is important to adapt to the higher temperature through the photosynthetic effectiveness in maintaining plant productivity (Lack and Evans 2001). Of four orchids of Sempu Island, three orchids (*A. emarginatus, D. subulatum,* and *T. acuminatissimum*) had thin walled bundle sheath; while the bundle sheath in *T. subulatum* was indistinct.

Other studies also reported variation in the bundle sheath features of other orchid species. Distinct and thin-walled bundles sheath were reported in *Ophrys iricolor, O. heldreichii, O. bucephala, Orchis coriophora, O. fragrans, O. punctulata, O. purpurea, O. morio subsp. morio, O. papilionacea var. papilionacea, O. laxiflora,* and *Ophrys tenthredinifera* (Aybeke et al. 2010), *Cynorkis fastigiata* (Stern 1997); *Platanthera flava* (Stern 1997); *Dendrobium leonis, D. anceps, D. brevimentum, D. aloifolium, D. distichum, D. indivisum, D. mannii, D. rosellum,* and *D. nathaniele* (Carlsward et al. 1997). Bundle sheath of other orchids was reported indistict, such as in *Ancistrorhynchus clandestinus*, *A. refractus, Rhipidoglossum curvatum, R. kamerunense* and *R. obanense* (Carlsward et al. 2006).

Specific characters

Fibre bundles

Fibre is elongated cells, typically sclerenchyma cells, with thick-walled cells composed of cellulose and lignin. The sclerenchymatic fibres organized, glued together by a pectin interface form fibre bundles. The thick-walled sclerenchymatic cells in fibre or fibre bundles act as mechanical protection and a barrier to reduce water loss (Vincent 2000; Richter et al. 2011; Placet et al. 2014).

In the present study, fibre bundles were only present in *D. subulatum* (Figure 3), forming hypodermal layers under epidermis, while they were absent in *A. emarginatus, T. subulatum,* and *T. acuminatissimum*. The presence of fibre bundles can be a diagnostic character in species identification and differentiation. Other studies also reported the presence and absence of fibre bundles in some other orchid species, such as the presence of fibre bundles in *Ionopsis utricularioides, Helcia sanguinolenta, Aspasia lunata, Oncidium boothianum* (Stern and Carlsward 2006), *Gongora portentosa, G. truncata,* and *Cirrhaea dependens* (Stern and Whitten 1999), *Epidendrum secundum* (Moreira et al. 2013), *Cattleya skinneri, C. forbesii, Laelia anceps, Arpophyllum giganteum* (Stern and Carlsward 2009); while fibre bundles were absent in some others, such as in *Campylocentrum micranthum* (Carlsward et al. 2006), *Brachtia andina, Erycina echinata, Lemboglossum maculatum, Odontoglossum cordatum* (Stern and Carlsward 2006), *Caladenia* (Pridgeon 1993), *Epidendrum anceps, Prosthechea boothiana,* and *P. radiata* (Stern and Carlsward 2009).

Raphide bundles

Raphides are bundles of narrow, elongated needle-shaped crystals containing calcium oxalate (Prychid and Rudall 1999). The raphide function is as a storage form for calcium or oxalate, regulation levels of calcium oxalate, mechanical support, and osmotic regulation (Franceschi and Horner 1980; Paiva and Machado 2005).

Raphide bundles in parenchymatous cells are common in Orchidaceae and are of little or no systematic value (Carlsward et al. 1997). However, raphide bundles in epidermal cells are of taxonomic value (Carlquist 1961; Tomlinson 1961,1969). In this study, raphide bundles can be used in species identification as they were only present in leaf epidermal cells in *T. acuminatissimum*, while they were absent in other orchids (*A. emarginatus, D. subulatum,* and *T. subulatum*). Pridgeon (1982) also showed similar results of useful characters of raphide bundles in epidermal cells in the systematic and identification within subtribe Pleurothallidinae.

Spiral thickening

Spiral thickening is spiral-shaped cell wall thickening in parenchymatic cells that function as mechanical stabilization of parenchymatic cells, prevention from desiccation, and for water storage (Leroux et al. 2010). Spiral thickenings were present in parenchymatic mesophyll of *A. emarginatus, T. subulatum,* and *T. acuminatissimum,* while they were absent in *D. subulatum* (Table 1, Figure 2, Figure 4, Figure 5). The spiral thickenings presence in *A. emarginatus, T. subulatum,* and *T. acuminatissimum* is a structural adaptation to high illumination in coastal forests of Sempu Island to reduce water loss because of transpiration. Although *D. subulatum* did not exhibit to posses spiral thickenings in the mesophyll, it had fibre bundles that have the same function as spiral thickenings (discussed above). In the previous study, spiral thickenings were also found in the cortical roots of *A. emarginatus* (Nurfadilah et al. 2016).

The presence or absence of spiral thickenings in orchids of Sempu Island in the present study can be used in species differentiation. *D. subulatum* is characterized by spiral thickenings absence, while they were present in other orchids of Sempu Island. Pridgeon (1982) also reported that spiral thickening is one of useful diagnostic anatomical characters in orchids systematic and identification.

Assessment of the adaptive ability of four orchid species to coastal habitats

To assess the adaptive ability of orchid species to coastal habitats for the classification of the most to the less adaptive species was based on adaptive anatomical characters. The adaptive anatomical characters used included: (i) smaller stomata; smaller stomatas are faster to close under the dry condition that leads to high capacity to reduce transpiration rates (Fahn 1982; Guan et al. 2011); (ii) thicker cuticle; the thicker cuticle the more ability to reflect sunlight and to reduce water loss (Dominguez et al. 2011; Fahn 1982; Rosso 1966); (iii) larger epidermal cell; the larger epidermal cell; the more water can be stored in epidermal cells which is important for water storage in dry condition (Guan et al. 2011); (iv) thicker leaf, this character is related to the succulence level for the adaptation in dry condition (Hsiao 1973; Lack and Evans 2001; Metusala et al. 2017); (v) presence of hypodermis; presence of hypodermis is important for water reservation to increase adaptive ability in warmer environment (Roth 1984; Reginato et al. 2009); (vi) thicker layer of mesophyll; thicker layer also supports the leaves succulence which is important to provide water supply for photosynthetic process and leaves cells turgor especially in arid environment (Hsiao 1973; Lack and Evans 2001; Metusala et al. 2017); (vii) presence of fibre bundles; fibre bundles composed of cellulose, pectin and lignin which is important to prevent from water loss (Vincent, 2000; Richter et al. 2011; Placet et al. 2014); (viii) presence of bundle sheath; bundle sheath containing PEP carboxylase to catalyze the CO_2 fixation in photosynthetic reactions under higher temperature condition for the efficacy of photosynthetic in dry area (Lack and Evans 2001).

Orchids of Sempu Island had various adaptive anatomical characters to ecologically adapt to coastal habitats with high irradiation that can induce high transpiration (Table 3). *A. emarginatus* had three adaptive anatomical characters including smaller stomata, the presence of bundle sheath surrounding vascular bundles, and spiral thickenings in the mesophyll to reduce water loss from leaves. *D. subulatum* had seven adaptive anatomical characters including smaller stomata, thicker cuticle, thicker leaf, the presence of hypodermis, thicker layer of mesophyll, the presence of fibre bundles and bundle sheath to reduce leaf transpiration. *T. subulatum* had two adaptive anatomical characters including thicker cuticle and the presence of spiral thickenings in mesophyll to inhibit water loss. *T. acuminatissimum* had four adaptive anatomical characters including thicker cuticle, larger epidermal cells, the presence of bundle sheath surrounding vascular bundles and spiral thickenings in mesophyll to prevent from the high rate of transpiration.

Table 3. Adaptive anatomical characters of four epiphytic orchids of Sempu Island, East Java, Indonesia

Adaptive anatomical characters	*Ascochilus emarginatus*	*Dendrobium subulatum*	*Thrixspermum subulatum*	*Thrixspermum acuminatissimum*
Smaller stomata	+	+	-	-
Thicker cuticle	-	+	+	+
Larger epidermal cell	-	-	-	+
Thicker leaf	-	+	-	-
Presence of hypodermis	-	+	-	-
Thicker layer of mesophyll	-	+	-	-
Presence of fibre bundles	-	+	-	-
Presence of bundle sheath	+	+	-	+
Presence of spiral thickenings	+	-	+	+

Note: + indicates the presence of the character, - indicates the absence of the character

Dendrobium subulatum had more adaptive anatomical characters (7 adaptive anatomical characters) compared to other orchid species *T. acuminatissimum* (4 adaptive anatomical characters), *A. emarginatus* (3 adaptive anatomical characters), and *T. subulatum* (2 adaptive anatomical characters) (Table 3). This result indicates that *D. subulatum* had more adaptive ability to coastal habitats compared to other orchid species in Sempu Island. Other studies also showed that other orchids species have adaptive anatomical characters, such as *Epidendrum secundum* growing in luminous area had relatively thick cuticle (Moreira et al. (2013); *Holcoglossum* growing in subalpine region of mountainous area possessed laterocytic and polarcytic stomata in their leaf epidermal layer showing structural adaptation to strong winds and ample rains (Fan et al. 2014).

Implication for conservation

This study implied the importance of anatomical characters in species identification. According to Aybeke et al. (2010), detailed anatomical characters are useful to support species identification. Taxonomic knowledge and species identification are required in the conservation. Selecting target species for conservation, identifying endangered species, inventory for biodiversity assessments, and monitoring, all depend on taxonomy and species identification (Schuiteman and de Vogel 2003).

Furthermore, orchids anatomical characters of Sempu Island explain ecological adaptation to the habitat with high irradiation in the coastal habitats. Relatively thick cuticle, smaller stomata, larger epidermis, the presence of hypodermis, many layers of mesophyll, the presence of fibre bundles, spiral thickenings and bundle sheaths, in these orchids are anatomical structures that have key roles to adapt to coastal habitats with high intensity of sunlight.

Of four epiphytic orchids, *D. subulatum* can be considered as the most adaptive species to coastal habitat in

Sempu Island based on the larger number of anatomical adaptive characters it possessed. As epiphytic orchids need higher humidity for growth, four orchid species in Sempu island are facing greater threats of global climate change especially temperature rising. A more adaptive orchid species will be less threatened by extinction. On the other hand, *A. emarginatus, T. subulatum,* and *T. acuminatissimum* were potentially influenced by even slight environmental alteration. Therefore, these orchids species need more attention in conservation than the more adaptive species, for their future survival.

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