The Natural History Journal of Chulalongkorn University 7(1): 75-85, May 2007

Comparative Anatomy of the Genus *Pyrrosia* Mirbel (Polypodiaceae) in Thailand

KANOKORN KOTRNON, ACHRA THAMMATHAWORN* AND PRANOM CHANTARANOTHAI

Applied Taxonomic Research Center, Department of Biology, Faculty of Science, Khon Kaen University, Khon Kaen 40002, Thailand.

ABSTRACT.– Lamina epidermal peels and transections of leaves, stipes, rhizomes and roots of 17 *Pyrrosia* species in Thailand were investigated. The characteristics found to be of most use in distinguishing the species were: presence/absence of hydathodes, trichome type, stomatal type, shape and wall structure of the epidermal cells, presence/absence of hypodermis, presence/absence of sclerenchyma in the midrib and leaf margins, arrangement of the mesophyll, stipe shape in transection, distribution of sclerenchyma strands and presence/absence of sclerenchyma sheaths in the parenchyma ground tissue of the rhizomes.

KEY WORDS: *Pyrrosia*; Polypodiaceae; Plant anatomy; Thailand

INTRODUCTION

The genus *Pyrrosia* Mirbel (Polypodiaceae) comprises c. 100 species, widely distributed from tropical and subtropical Asia to Africa and Australia (Holttum, 1968). Tagawa & Iwatsuki (1989) recorded 18 species of the genus in Thailand. The genus is well circumscribed by the presence of stellate hairs. Most species are epiphytic in lowland and montane forests; others are terrestrial at low to high elevations. Holttum (1968) noted that *Pyrrosia* is closely related to *Drymoglossum* but differs in sori shape.

Anatomical studies of this genus has been carried out by many authors: Nayar & Chandra (1966); van Cotthem (1970); Ogura (1972); Lin & Devol (1977); Sen & Hennipman (1981); Hovenkamp (1986) and Schneider (1996). Hovenkamp (1986) recognized 51 species worldwide and noted the importance of anatomical characteristics for the delimitation of the genus and species. These included the presence/absence of hydathodes, the wall structure of the epidermal cells, the type and level of stomata, the presence/absence of hypodermis, the structure of the mesophyll, the presence/absence of sclerenchyma sheaths, and the scattering pattern of sclerenchyma strands in rhizomes. Nayar & Chandra (1966) studied the morphology and anatomy of 13 species of Indian *Pyrrosia* and divided the genus into six morphological groups namely: Flocculosa, Heteractis, Mannii, Nayariana, Obovata and Varia. The purpose of the present paper is to investigate the leaf,

* Corresponding author:
Tel: (6643)-342-908
Fax: (6643)-364-169
E-mail: achara @kku.ac.th

stipe, rhizome and root anatomy in Thai *Pyrrosia*. Our study will serve to fill in gaps on the anatomy of the organs where published information on the genus is lacking. Once our data are combined with other information on the comparative anatomy of Polypodiaceae, they will add to a comprehensive analysis of this species-rich family. This report, then, represents a partial effort towards that goal.

MATERIALS AND METHODS

Mature and fresh leaves, stipes, rhizomes and roots of 17 taxa of *Pyrrosia* were obtained from the field in Thailand (Table 1), sectioned for anatomical studies using the sliding microtome, and stained. Transverse sections were cut from the middle part of the midrib, leaf margin, stipe, rhizome and root. Two to five specimens were used to represent each species. For epidermal studies, samples were prepared by mechanically scraping midway between the base and the apex of the lamina and stained. Permanent slides were mounted in DePeX artificial mounting medium. Photographs were taken with the aid of an Olympus BH_2 light microscope. Voucher specimens are kept at Khon Kaen University Herbarium (KKU).

RESULTS

General anatomical description

Lamina

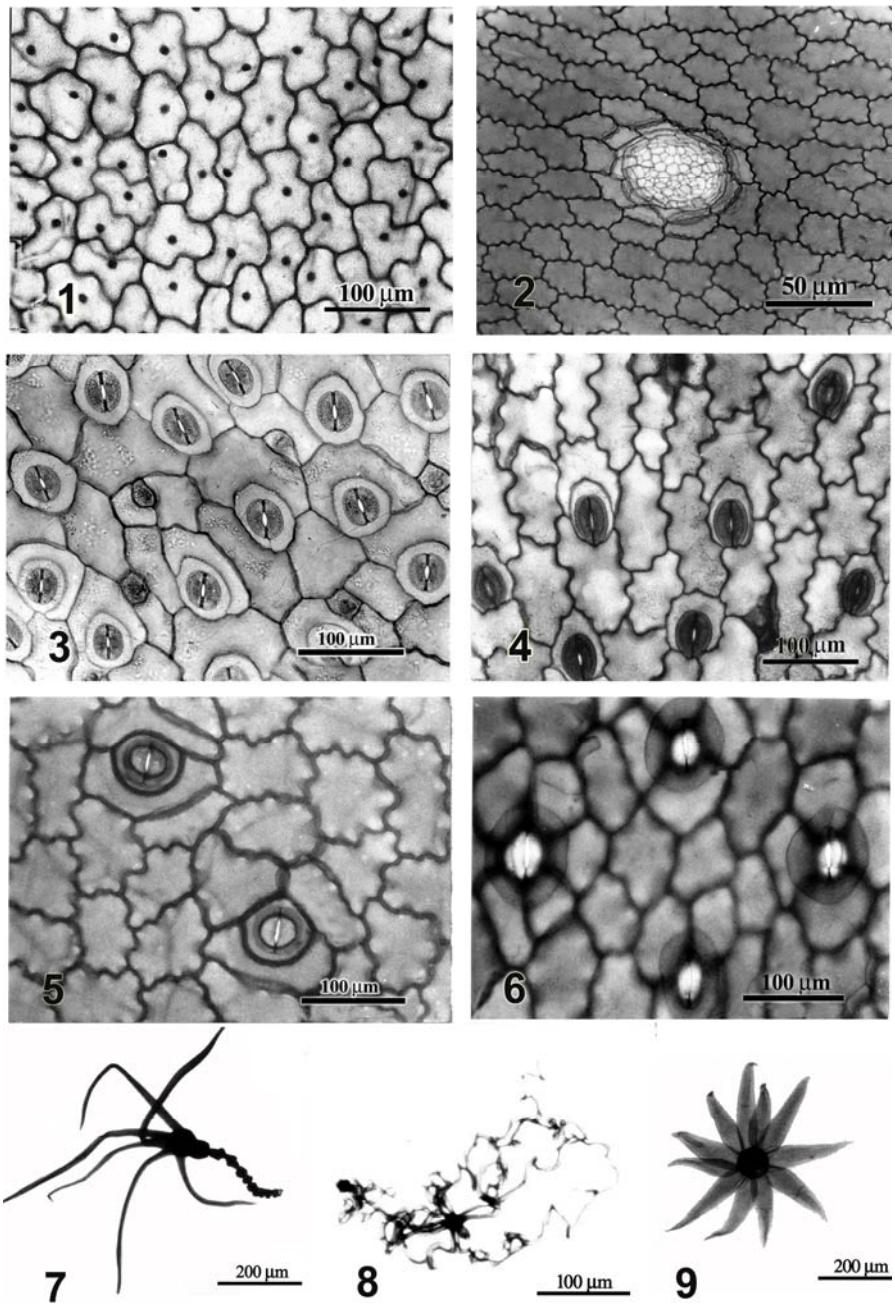
Epidermis: In surface view, the cells of the upper epidermis of most species are 5-6-sided and orientated longitudinally (Fig. 2). However, *P. angustata*, *P. floccigera*, *P. flocculosa*, *P. heteractis* var. minor, *P. longifolia*, *P. mollis*, *P. nummularifolia*, *P. penangiana* and *P. piloselloides* have irregularly-shaped sinuous walls (Fig. 1) with straight, anticlinal walls in *P. adnascens*, *P. lanceolata*, *P. nuda* and *P. stigmosa*. In transverse section, the outer walls of the epidermis are flat or convex and project outwards to varying degrees. The epidermal cells on the midrib are smaller than those on the blade except in *P. piloselloides* and *P. tonkinensis* where their sizes are similar. The cells of the lower epidermis are similar to those of the upper, in having sinuous walls. *P. adnascens*, *P. lanceolata* and *P. longifolia* have straight epidermal cell walls. The outer wall of the lower surface is thicker than that of the upper surface except in *P. mollis*, *P. penangiana*, *P. stigmosa* and *P. varia* where it is thinner. The cutinized epidermis on both surfaces of the leaf are distinct in *P. adnascens, P. angustata, P. heteractis, P. heteractis* var. *minor, P. nummularifolia, P. stigmosa* and *P. varia.*

Hydathodes: Hydathodes are scattered all over the upper leaf surface (Fig. 2) in all species but absent in *P. adnascens, P. angustata, P. floccigera, P. lanceolata, P. longifolia, P. nuda* and *P. nummularifolia*.

Stomata: Stomata are restricted to the lower leaf surface. Most species have pericytic stomata (with vary narrow guard cells encircling the stomata) (Figs. 3, 5, 6) and polocytic stomata (with stomata attached to the wall of the guard cells at the anterior end) occur only in *P. penangiana* (Fig. 4). Deeply sunken stomata occur in *P. adnascens, P. angustata, P. eberhardtii, P. floccigera, P. heteractis, P. heteractis* var. *minor, P. lanceolata, P. longifolia, P. nuda* and *P. varia*. Superficial stomata are present in *P. flocculosa, P. mollis, P. nummularifolia, P. penangiana, P.*

TABLE 1. Anatomical characters of leaf of Thai *Pyrrosia*. (*=Group I, **=Group II, ***=Group III, QU=5-6-sided, IR=irregular, SI=sinuous, ST=straight, PR=pericytic, PO=polocytic, DE=deeply sunken, SUP=superficial stomata, LA=lanceolate ray, AC=acumiate ray, RI=ribbon ray, DI=combination of ribbon and lanceolate rays, SC=scale-like, GL=glandular hairs, DIF=differented into palisade and spongy parenchyma, UN=undifferentiated, +=presence, -=absence)

Species	Epidermis				Hydathodes	Stomata		Trichome		Leaf			Mid rib
	Shape		Anticlinal wall										
	Upper	Lower	Upper	Lower		Type	Level	Upper	Lower	Hypodermis	Mesophyll	Sclerenchyma of margin	Sclerenchymatous sheath
P. adnascens (Sw.) Chin *	QU	QU	ST	ST	-	PR	DE	LA	LA	+	DIF	+	+
P. angustata (Sw.) Ching *	IR	IR	SI	SI	-	PR	DE	LA, AC	LA, AC, RI, DI	+	DIF	+	+
P. eberhardtii (H.Christ) Ching***	QU	IR	SI	SI	+	PR	DE	LA	LA, DI, GL	+	DIF	-	+
P. floccigera (Blume) Ching *	IR	QU	SI	SI	+	PR	DE	AC	AC, RI, GL	+	DIF	-	+
P. flocculosa (D.Don) Ching **	IR	IR	SI	SI	-	PR	SUP	AC	AC, RI	-	UN	-	+
P. heteractis (Mett. ex Kuhn) Ching ***	QU	IR	SI	SI	+	PR	DE	LA	LA, GL	+	DIF	+	+
P. heteractis var. *minor* (C. Chr) Ching **	IR	IR	SI	SI	+	PR	DE	LA	LA, GL	+	DIF	+	+
P. lanceolata (L.) Farwell ***	QU	QU	ST	ST	-	PR	DE	LA, SC	LA	+	DIF	+	+
P. longifolia (Burm.f.) C.V. Morton *	IR	QU	SI	ST	-	PR	DE	LA, SC	LA	-	UN	+	+
P. mollis (Kunze) Ching **	IR	IR	SI	SI	+	PR	SUP	LA, AC	AC, RI, GL	-	DIF	-	+
P. nuda (Giesenh.) Ching *	QU	IR	ST	SI	-	PR	DE	LA	LA	+	DIF	-	+
P. nummularifolia (Sw.) Ching **	IR	IR	SI	SI	-	PR	SUP	AC	AC, RI, GL	+	DIF	-	+
P. penangiana (Hook.) Holtt. **	IR	IR	SI	SI	+	PO	SUP	LA, AC	AC, GL	-	DIF	-	+
P. piloselloides (L.) M.G. Price ***	IR	IR	SI	SI	+	PR	SUP	LA	LA	-	UN	-	-
P. stigmosa (Sw.) Ching ***	QU	IR	ST	SI	+	PR	SUP	AC	AC, RI	-	DIF	-	+
P. tonkinensis (Giesenh.) Ching **	QU	IR	SI	SI	+	PR	SUP	LA, AC	AC, RI, GL	-	DIF	-	+
P. varia (Kaulf.) Farwell *	QU	QU	SI	SI	+	PR	DE	LA, SC	LA	+	DIF	-	+



FIGURES 1-9. Upper epidermis (1-2) lower epidermis showing stomata (3-6) and surface trichomes (7-9): 1. *P. angustata* 2. *P. tonkinensis* 3. *P. eberhardtii* 4. *P. penangiana* 5. *P. piloselloide* 6. *P. varia* 7. *P. angustata* 8. *P. mollis* 9. *P. adnascens*.

piloselloides, P. stigmosa and *P. tonkinensis*.

Trichomes: Stellate hairs develop on both surfaces in all species, but are dense on the lower surface. Four forms of stellate rays are observed. Lanceolate rays (Fig. 9) with 1-7 stalk cells and 6-15 rays on both surfaces occur in *P. adnascens, P. angustata, P. eberhardtii, P. heteractis, P. heteractis* var. *minor, P. lanceolata, P. nuda, P. piloselloides* and *P. varia*. Acuminate rays (Fig. 7) (narrower and longer than lanceolate rays) with 1-18 stalk cells and 6-10 rays on both surfaces occur in *P. angustata, P. floccigera, P. flocculosa, P. mollis, P. nummularifolia, P. penangiana, P. stigmosa* and *P. tonkinensis*. Ribbon (or woolly) rays (Fig. 8) (long, strongly and irregularly curled, closely appressed rays) with 1 stalk cell and 5-15 rays occur only on the lower surface in *P. angustata, P. floccigera, P. flocculosa, P. mollis, P. nummularifolia, P. stigmosa,* and *P. tonkinensis*. Finally, both ribbon rays and lanceolate rays occur on the lower surface in *P. angustata* and *P. eberhardtii*. Glandular hairs are unicellular and uniseriate and occur on the lower surface in *P. eberhardtii, P. floccigera, P. heteractis, P. heteratctis* var. *minor, P. mollis, P. nummularifolia, P. penangiana* and *P. tonkinensis*. Scale-like hairs occur on the upper suface in *P. lanceolata P. longifolia* and *P. varia*.

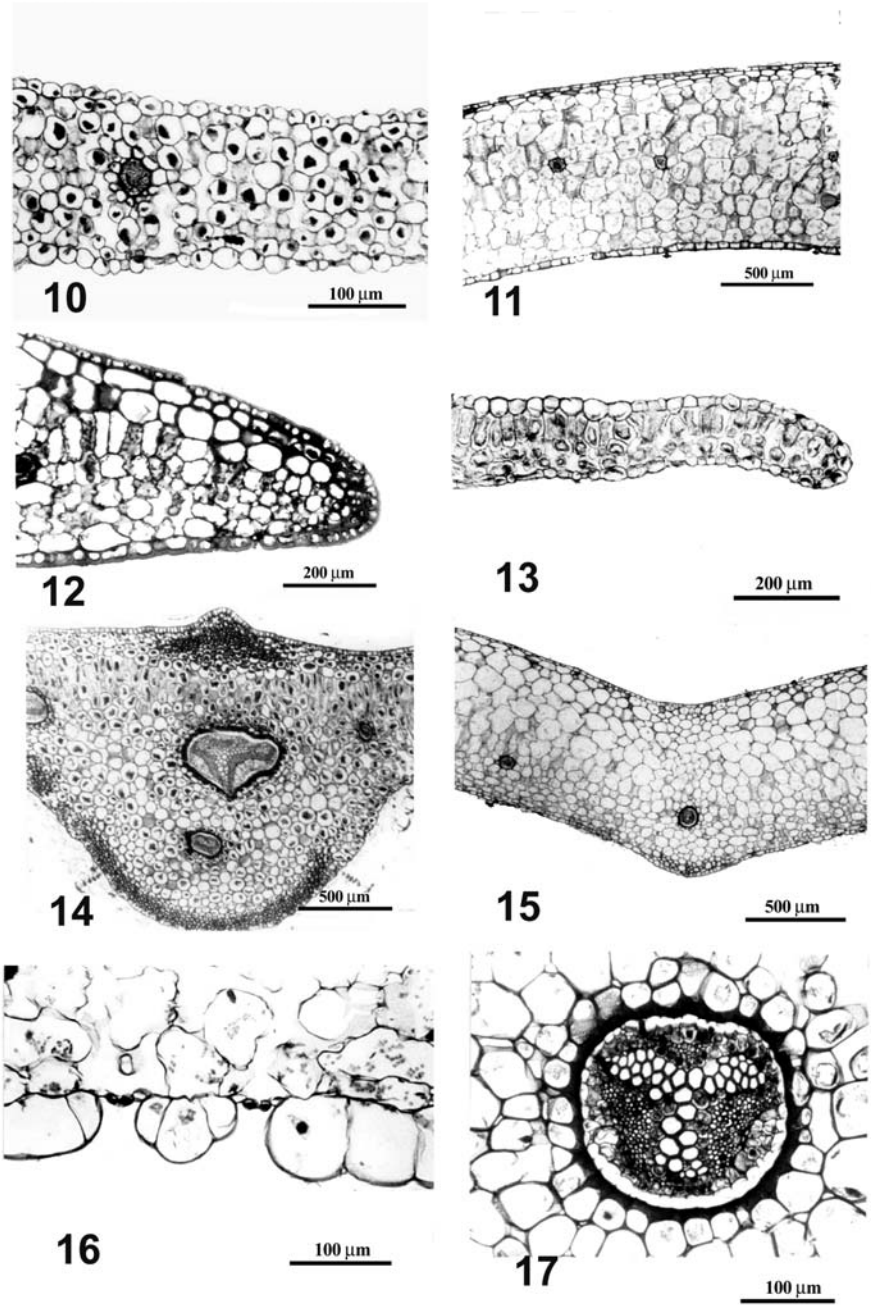
Hypodermis: The hypodermis covers the area of the leaf blade and develops on the upper surface alone in all species except *P. flocculosa, P. longifolia, P. mollis, P. penangiana, P. piloselloides, P. stigmosa* and *P. tonkinensis*, where hypodermal cells are absent from the leaf margin and midrib. The hypodermis has 1-4 layers, and cells in each layer are larger with a distinctly thinner wall than the epidermal cells. Hypodermal cells in *P. eberhardtii* are filled with tannin.

Mesophyll: Dorsiventral in most species, but undifferentiated mesophyll only occur in *P. flocculosa, P. longifolia* and *P. piloselloides* (Figs. 10-11) which are composed of parenchymatous cells, in which all the cells are large, thin-walled and filled with tannin and chloroplasts. The cells on each side are round and have 4-11 layers with large intercellular spaces. The palisade parenchyma has 1-5 layers and is composed of narrow, short cells in all species except *P. nummularifolia, P. floccigera* and *P. nuda* in which the cells are large and broad. Spongy parenchyma has 2-7 layers and is irregularly shaped with small cells and large intercellular spaces. In all species, the palisade and spongy cells contain chloroplasts.

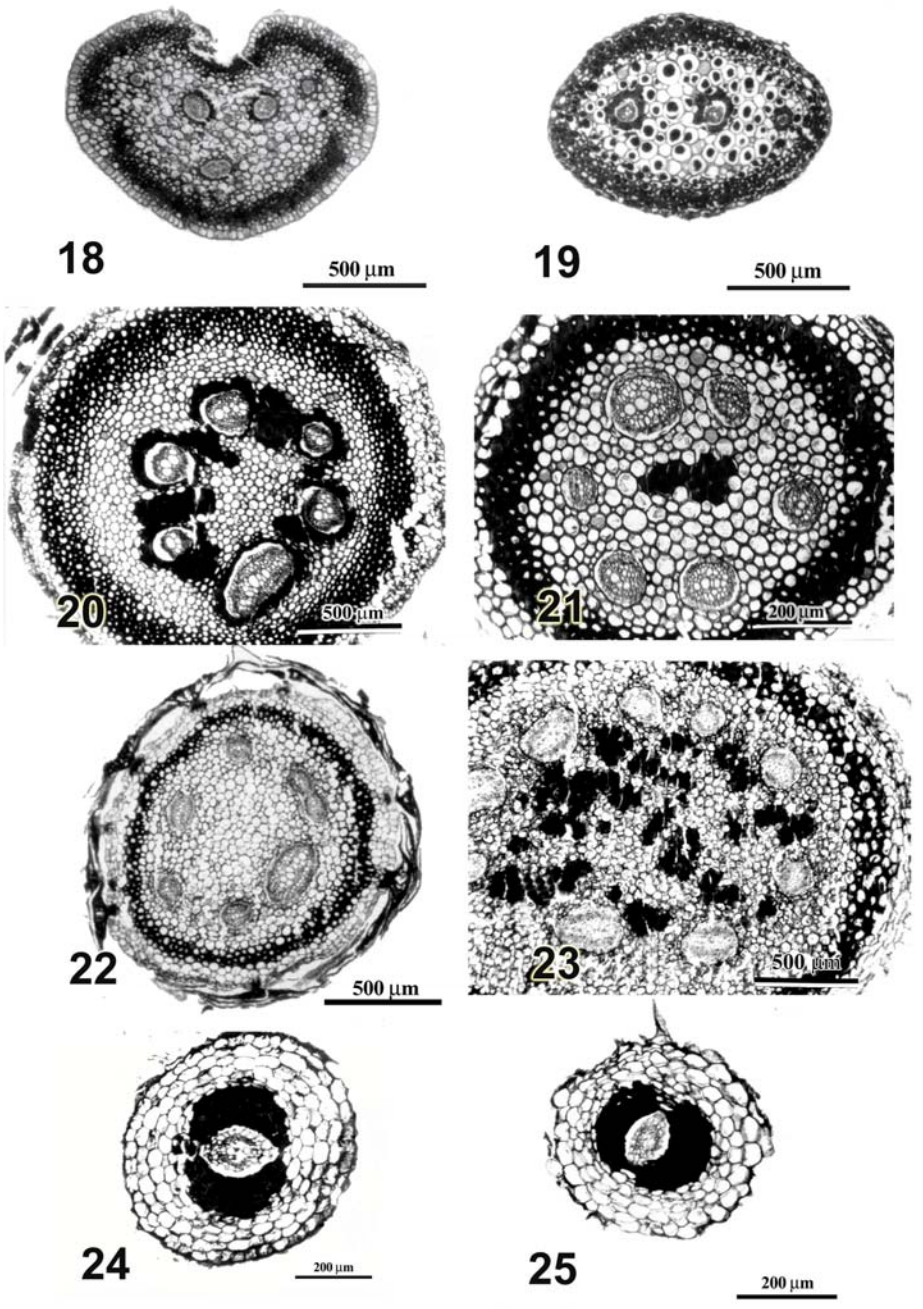
Leaf margin: Cells in the leaf margin are slightly downwards in all species (Figs. 12-13), and sclerenchymatous in *P. adnascens, P. angustata, P. heteractis, P. heteractis* var. *minor, P. lanceolata* and *P. longifolia*.

Midrib: The ground tissue is parenchymatous and contains chloloplasts in all species except *P. flocculosa* and *P. piloselloides* in which it is densely filled with tannin. The sclerenchymatous sheath is below the lower and upper epidermis in the midrib of all species except *P. piloselloides* (Figs. 14-15).

Vascular tissue: Vascular bundles range in number from 1-5 through out the width of the midrid, with concentric T-shaped xylem surrounded by phloem and pericyclic cells comprising 1-3 layers of parenchyma. The vascular bundles are surrounded by endodermis. (Fig. 17).



FIGURES 10-17. TS of leaf (10-11) TS of leaf margins (12-13) and TS of leaf midribs (14-15): 10. *P. flocculosa* 11. *P. longifolia* 12. *P. angustata* 13. *P. stigmosa* 14. *P. eberhardtii* 15. *P. nummularifolia* 16. *P. floccigera*, showing stomata level 17. *P. mollis,* showing vascular bundle.



FIGURES 18-25. TS of stipes (18-19) TS of rhizomes (20-23) TS of roots (24-25): 18. *P. nuda* 19. *P. piloselloides* 20. *P. angustata* 21. *P. nummularifolia* 22. *P. floccigera* 23. *P. mollis* 24. *P. penangiana* 25. *P. tonkinensis*.

Stipe

The outlines of the stipe in transverse sections (TS) are mostly rounded, while heart-shaped (Fig. 18) in *P. adnascens, P. lanceolata, P. nuda* and *P. varia*, elliptic in *P. piloselloides* (Fig. 19) and spindle-shaped in *P. tonkinensis*. The epidermal cells of stipes contain stomata. Below the epidermis is the sclerenchymatous sheath, formed by 3-10 layers. Dense starch grains in many species are present in the parenchymatous ground tissue.

Vascular tissue: Three shapes of vascular bundle arrangement in the stipes are recognized: U-shaped in *P. adnascens, P. angustata, P. eberhardtii, P. floccigera, P. flocculosa, P. heteractis, P. lanceolata, P. longifolia, P. penangiana, P. stigmosa, P. tonkinensis* and *P. varia*; V-shaped (Fig. 18) in *P. mollis, P. heteractis* var. *minor* and *P. nuda*, and Linear-shaped in P. *nummularifolia* and *P. piloselloides*. The vascular tissue system consists of 2-13 vascular bundles with extended ends. Vascular bundles are round or oval in TS, with plate-like xylem and phloem on both sides. The vascular bundle has 1 - 3 layers of pericycle and is enclosed by the endodermis.

Rhizome

The ground tissue of rhizomes is parenchymatous and the cells contain dense starch grains. In most species, the sclerenchymatous sheaths are distinct and situated peripherally to the vascular tissue. They have 2-8 layers and are absent from the sheath in P. flocculosa, *P. penangiana* and *P. stigmosa*. The sclerenchyma strands may be divided into 4 groups. Sclerenchyma strands scattered irregularly through the inner parenchyma (Fig. 23) occur in *P. longifolia, P. mollis, P. penangiana* and *P. tonkinensis*. In *P. angustata*, sclerenchyma strands are restricted to the peripheral zone and occur in between the vascular bundles (Fig. 20). Sclerenchyma strands are situated centrally in the inner parenchyma (Fig. 21) in *P. adnascens, P. eberhardtii, P. heteractis, P. heteractis* var. *minor, P. lanceolata, P. nuda, P. nummularifolia* and *P. varia*. Sclerenchyma strands are absent (Fig. 22) in *P. floccigera, P. flocculosa, P. piloselloides* and *P. stigmosa*. The structure of the cell walls in sclerenchyma strands is similar to that of the walls of sclerenchymatous sheaths, but is often distinctly darker.

Vascular tissue: In all species, rhizomes are dictyostelic, with meristeles arranged in a ring. The number of meristeles ranges from 4-10. Each meristele is oval, with plate-like xylem surrounded by phloem and endodermis, and 1-3 layers of parenchymatous pericycle.

Root

The root consists of a single layer of epidermis. Bristle-like root hairs are present in all species. The cells of the outer cortex are thin-walled and parenchymatous, consisting of 2-5 layers, while those of the inner cortex form sclerenchymatous sheaths, consisting of 5-10 layers (Figs. 24-25).

Vascular tissue: The stele is a diarch surrounded by endodermis and a layer of parenchymatous pericycle.

DISCUSSION AND CONCLUSIONS

The results show that a combination of some characteristics is useful in the identification of Thai *Pyrrosia* (Tables 1 & 2). It is impossible to identify the

TABLE 2. Anatomical characters of stipes, rhizomes and roots in section of Thai *Pyrrosia* (-=absence, TS=transverse sections, VB=vascular bundle)

Species	Stipe				Rhizome			Root	
	Shape of TS	Shape of VB	Vascular bundle (no.)	Sclerenchymatous sheaths (no. of layers)	Arrangement of sclerenchymatous strands	Sclerenchymatous sheaths (no. of layers)	Vascular bundle (no.)	Parenchyma (no. of layers)	Sclerenchyma (no. of layers)
P. adnascens	Heart-shaped	U	8	3-5	central	3-4	8	2-3	2-3
P. angustata	Globose	U	6-7	4-6	vacular	5-7	6	1	2-3
P. eberhardtii	Globose	U	10	7-9	central	5-8	7-8	3-4	2-8
P. floccigera	Round	U	6-7	4-5	-	2-4	6-7	1-2	2-3
P. flocculosa	Globose	U	7-8	3-4	-	-	5-6	2-3	2-3
P. heteractis	Globose	U	7-9	7-9	central	5-8	7-8	2-3	2-8
P. heteractis var. *minor*	Globose	V	3	4-6	central	4-6	4-5	1	5-10
P. lanceolata	Heart-shaped	U	6	4-6	central	4-6	5-6	1	1
P. longifolia	Globose	U	6	5-7	scattered	6-8	4	2	2-4
P. mollis	Round	V	6	5-8	scattered	3-5	9-10	3-4	2-5
P. nuda	Heart-shaped	V	5	4-5	central	4-6	5-6	1	2-3
P. nummularifolia	Globose	L	2	4-6	central	3-5	6	1	3-6
P. penangiana	Globose	U	10-12	8-10	scattered	-	7-10	4-5	2-6
P. piloselloides	Elliptic-shaped	L	3	4-6	-	2-4	4	1	2-3
P. stigmosa	Globose	U	12-13	6-8	-	-	7-10	5	2-3
P. tonkinensis	Spindle-shaped	U	8-9	4-5	scattered	3-4	3-4	3-4	2-6
P. varia	Heart-shaped	U	7	4-6	central	5-6	2-3	2-3	5-6

sclerenchymatous sheaths in the mibrib, stipe, rhizome and root of all species. The sclerenchymatous strands in rhizomes may be divided into four groups based on their position: (1) scattered irregularly through the inner parenchyma; (2) restricted to the peripheral zone; (3) situated centrally in the inner parenchyma; and (4) absent. Hovenkamp (1986) suggested that the arrangement of sclerenchymatous strands and their position in parenchyma is of taxonomic value in *Pyrrosia*.

According to Sen & Hennipman (1981); Ogura (1972), pericytic stomata are restricted to the genus *Pyrrosia*. Price (1974, cited in Hovenkamp, 1986) notes that *Drymoglossum piloselliodes* should therefore be transferred to *Pyrrosia*.

Using a combination of anatomical characteristics, only two taxa (*P. heteractis* and *P. heteractis* var. *minor*) could not be separated because they are morphologically similar (Tagawa & Iwatsuki, 1989). The latter differs from the typical variety in its smaller stipe, 1-3 cm in length, and its fronds 3-6 cm by up to 2 cm. Both taxa also grow at the same altitude. Further study is needed to elucidate their status.

Pyrrosia lanceolata and *P. adnascens* are similar in morphology. The former can be readily distinguished by the scale-like hairs on its upper lamina surface and the cortical parenchyma with dense deposits of starch in the rhizomes. The latter lacks scale-like hairs on the upper surface, and has cortical parenchyma with dense deposits of tannin in the rhizomes.

Based on ecological and lamina anatomical characteristics, the genus can be divided into three groups.

Group I: *P. angustata, P. longifolia, P. nuda* and *P. varia*

This group grows on tree-trunks in open places or mixed deciduous forest and dry evergreen forest, and has thick cuticles on both surfaces, deeply sunken stomata and mesophyll with few intercellular spaces.

Group II: *P. floccigera, P. flocculosa, P. mollis, P. nummularifolia, P. penangina* and *P. tonkinensis*

This group grows on mossy tree-trunks, especially along stream in evergreen forest, and has thin or very thin cuticles, superficial stomata and mesophyll with large intercellular spaces. *P. floccigera* differs by having sunken stomata.

Group III: *P. adnascens, P. eberhardtii, P. heteractis, P. heteractis* var. *minor P. lanceolata P. piloselloides* and *P. stigmosa*

This group grows on tree-trunks in dry evergreen forest or on mossy tree-trunks in moist evergreen, and has thick cuticles on both surfaces and sunken stomata. *P. piloselloides* and *P. stigmosa* differ by having superficial stomata.

However, this study indicates that the level of stomata may be a genetically inherited characteristic and not a response to environmental conditions.

SPECIMES EXAMINED

All specimens examined are deposited at KKU. *Pyrrosia adnascens*, Kanokorn 35/1999. – *P. angustata*, Kanokorn 67/1999. – *P. eberhardtii*, Kanokorn 41/1999. – *P. floccigera*, Kanokorn 39/1999. – *P. flocculosa*, Kanokorn 31/1999. – *P. heteractis*, Kanokorn 67/2000. – *P. heteractis* var. *minor*, Kanokorn 80/2000. – *P. lanceolata*, Kanokorn 51/2000. – *P longifolia*, Kanokorn 36/1999. – *P. mollis*, Kanokorn 70/2000. – *P. nummularifolia* Kanokorn

59/2000. – *P. nuda*, Kanokorn 54/2000. – *P. penangiana*, Kanokorn 64/2000. – *P. piloselloides*, Kanokorn 10/1999. – *P. stigmosa,* Kanokorn 40/1999. – *P. tonkinensis*, Kanokorn 52/2000. – *P. varia*, Kanokorn 37/1999.

ACKNOWLEDGEMENTS

We would like to thank Dr. David A. Simpson for reviewing the manuscript. This work was supported by the TRF/BIOTEC Special Program for Biodiversity Research and Training grant (BRT 543001).

LITERATURE CITED

Holttum, R.E. 1968. Flora of Malaya (ferns of Malaya). Government printing office, Singapore.

Hovenkamp, P.H. 1986. A monograph of the fern genus *Pyrrosia* (Polypodiaceae). Leiden Netherlands.

Lin, B.L and Devol, C.E. 1977. The use of stipe characters in fern taxonomy Part 1. Taiwania, 22: 91-99.

Nayar, B.K. and Chandra, S. 1965. Ferns of India-XV, *Pyrrosia*. Bulletin of the National Botanic Gardens, 117: 1-98.

Ogura, Y. 1972. Comparative anatomy of vegetative organs of the Pteridophytes. Berlin: Gebruder Barntraeger.

Schneider, H. 1996. The root anatomy of ferns: a comparative study. In: Camus, J.M., Gibby, M and Johns, R.J (Eds). Pteridology in Perspective. Royal Botanic Gardens, Kew, 271-283 pp.

Sen, U. and Hennipman, E. 1981. Structure and ontogeny of stomata in Polypodiaceae. Blumea, 27: 175-201.

Tagawa, M. and Iwatsuki, K. 1989. Polypodiaceae. In: Smitinand, T and Larsen, K (Eds). Flora of Thailand (Pteridophytes) 3(4). Tistr, Bangkok.

van Cotthem, W.R.J. 1970. A classification of stomatal type. Botanical Journal of the Linnean Society, 63: 235-246.

Received: 22 November 2006
Accepted: 10 May 2007