

Variation of *Pyrrrosia heterophylla* (L.) Price and Evidence for Occurrence of Two Other *Pyrrrosia* Species in Sri Lanka

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ABSTRACT. *Pyrrrosia heterophylla* (L.) Price (Panam Pethi) is a common creeping fern species belonging to the family Polypodiaceae found naturally in South India and Seychelles Island and Sri Lanka. It is used in indigenous medicine. Although detailed information is scanty for *P. heterophylla*, a wide range of morphological variation exists within the species. The objective of this study was to identify variation of *P. heterophylla* in Sri Lanka. The study was carried out in Kalutara, Kandy, Kegalle, Kurungala, Matale and Ratnapura districts. Variable characters such as habit and habitats of the species, shape, color, venation, thickness, width, length of sterile fronds, length, width and spore arrangement of fertile fronds, scale and cross sections of rhizomes were assessed in 28 samples.

Three major habits were identified for the species on the basis of arrangement of fronds on the substrate. The cross sections of rhizome and venation pattern of sterile fronds were not significantly different within and among samples. Leaf shape, leaf length, leaf width and thickness of sterile fronds and length and width of fertile fronds were variable within and among samples. Out of 28 samples, four samples deviated from normal spore arrangement of *P. heterophylla*. In three samples, a sub marginal spore arrangement was observed whilst the other samples showed interrupted spore arrangement. Out of four samples, three closely resembled *P. piloselloides* whilst the other sample closely resembled *P. niphoboloides*. This is the first record of occurrence of *P. niphoboloides* and *P. piloselloides* in Sri Lanka, and continuation of the same observations for at least another season and research on cytology of the samples are required for confirmation of these findings.

INTRODUCTION

Pteridophyte flora is an important component of the biodiversity of Sri Lanka. At present, 343 Pteridophyte species have been recorded from Sri Lanka (Fernando, 2002). Except for few species of Pteridophytes, the majority is distributed in terrestrial habitats. Terrestrial Pteridophytes usually prefer moist and shady environment and thus a majority of them are concentrated in various habitats of tropical rainforests. Although less recognized, Pteridophytes have the ability to provide food, medicine, fiber, craft, building material, abrasive and material for decoration (Winter and Amoroso, 2003).

Pyrrrosia is a relatively common fern genus in the family Polypodiaceae. It contains about 50 species. Natural distribution of the genus extends from Central Africa

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through South and South East Asia to Oceania and New Zealand (Winter and Amoroso, 2003). Six *Pyrrosia* species have been recorded from Sri Lanka, namely *Pyrrosia heterophylla* (L.) Price, *P. gardneri* (Mett.) Sledge, *P. lanceolata* (L.) Farwell, *P. ceylanica* (Giesenh.) Sledge, *P. pannosa* (Mett.) Ching and *P. porosa* (Presl.) (Fernando, 2002). *P. heterophylla* (which was previously called as *Drymoglossum heterophyllum* (L.) Trimen) has renamed as *Pyrrosia heterophylla* by Price (Hovenkamp, 1986). It is a local common species which is restricted to South India, Sri Lanka and Seychelles and grows on tree trunks and branches, shrubs and rocks in lower and middle altitude areas (Hovenkamp, 1986). It is a creeping fern, which has a long and slender rhizome usually covered by multi cellular, fine structures called scales. It has dimorphic fronds. The sterile frond is mainly responsible for photosynthesis and shows a wide range of variation in terms of length, width, thickness and shape, whilst the fertile frond is longer, thin and linear and its spores are arranged on the lower surface of the lamina (Santhosh and Madhusoodanan, 1998). *P. heterophylla* is used in indigenous medicine to prepare oil treatment, and leaves are used as a styptic to arrest capillary haemorrhages and eczema (Jayaweera, 1981).

P. heterophylla has first been collected from Sri Lanka by Paul Hermann in the period of 1672-1675 and first described by Linnaeus (1749) in *Flora Zeylanica* (Sledge, 1960). Later, Thwaites (1864) has described the species in the *Plantarum Zeylanicae*. The last description of *P. heterophylla* was compiled by Beddome (1883) in his Handbook to the ferns of British India, Ceylon and Malay Peninsula. Hovenkamp (1986) has compiled a monograph for all *Pyrrosia* species in the world, which contains description of *P. heterophylla*. However, such description is mainly based on South Indian materials and only a few samples have been collected before 1900 from Sri Lanka. Santhosh and Madhusoodanan (1998) have prepared a detailed description of *P. heterophylla* for South India. As in any other Sri Lankan Pteridophytes, detailed information of *P. heterophylla* is scanty. Information on ecological, morphological and anatomical variation and reproductive biology of *P. heterophylla* is not well documented, although such information is vital for conservation and sustainable uses of *P. heterophylla*. Therefore, the objective of this study was to identify the morphology, habitats and host plant variations of *P. heterophylla* populations in Sri Lanka.

MATERIALS AND METHODS

Sampling of *Pyrrosia heterophylla*

Review of literature, observation of herbarium samples at the Royal Botanical Gardens, Peradeniya and individuals in Kandy district revealed that the species is distributed in and around Kandy district including Matale and Kegalle. Thus, samples for identification of variation in *P. heterophylla* were collected from Kandy, Matale, Kegalle Kurunegala, Ratnapura and Kalutara districts on a random basis. Details of each collection positions are given in Appendix 1. Natural forest margins, homegardens and roadsides of each district were visited for collection of samples. After initial observations, undisturbed habitats of natural forests were excluded because the occurrence of *P. heterophylla* is limited due to low light intensity inside the forest. At each place where *P. heterophylla* was observed, sample specimens with fertile and sterile fronds were collected for further observation.

Data collection

Data was collected in the field and in the laboratory. Plant habit and its microclimatic environment were identified at each sampling location. Plant habit of each sample was identified on the basis of arrangement of fronds along the rhizome on the substrate. Microclimate of each sampling location was identified using three light levels namely, direct, medium and low sunlight. At each location, the habitat was identified based on existing vegetation as natural forest margins, homegardens or roadsides. Then, the substrates of *P. heterophylla* were identified based on epiphytic and lithophytic nature. If epiphytic samples were observed host plant species was identified to species level by using preserved specimens of host plants.

In the laboratory, a representative specimen from each sample was preserved to be deposited at the National Herbarium, the Royal Botanical Garden, Peradeniya. The remaining specimens were observed for morphological variation as described in Table 1. Initially, character variations were obtained and analyzed and the data were analyzed using hierarchical cluster analysis procedure of SAS (SAS Software, 1999). Out of the 28 collected samples, only 12 samples had fertile fronds, and only these samples were used for the cluster analysis. Further, for cluster analysis, instead of length and width, length to width ratio was used.

Table 1. Morphological characters observed to identify variation in *Pyrrosia heterophylla*.

Character	Method of observation
1. Rhizome characters	
(a) Cross section	Rhizome was sectioned from 3 places and observed using a dissecting microscope (2×10) and line diagrams drawn.
(b) Thickness	Three places of rhizome were selected from each samples and thickness measured using a vernier caliper.
(c) Scales of rhizome	Shape and color and transparency of scales were observed using a light microscope (10×10) and line diagrams drawn.
2. Sterile frond	
(a) Length, width and thickness	Thirty-five leaves were selected from each sample and length, width and thickness measured using a vernier caliper.
(b) Shape	Shape of sterile fronds was assessed using 1-4 scale (1-rounded, 2-oblong, 3-lanceolate, 4-linear) developed after observation of a large number of samples.
(c) Color	Color of leaf was measured using a Munsel color chart.
(d) Venation	Venation pattern of 54 sterile fronds of 28 samples was observed, after obtaining transparent appearance by keeping fronds 10 minutes in boiling water.
3. Fertile frond	
(a) Length and width	Eighty four fertile fronds were selected from 12 samples and length and width of fertile frond were measured in the same way as sterile fronds.
(b) Spore arrangement	Eighty four fertile fronds were selected from 12 samples and spore arrangement and sequence of the spore development were observed at different maturity levels (from spore development to spore release from sporangia) using a dissecting microscope (2×10).

RESULTS AND DISCUSSION

Morphological variation of *Pyrosia heterophylla*

Rhizome characters

P. heterophylla has a long, creeping, slender rhizome. The diameter of rhizome varied from 0.5-0.9 mm. It was covered with brown color scales. The cross section of rhizome varied from terete to cylindrical shape. Cortical sclerenchyma sheath, and two or three vascular bundles were clearly observed from all samples (Fig. 1). The rhizome was covered by multi cellular, fine structures called scales. Shape of the scale varied from round to linear and their margins varied from dentate to ciliate (Fig. 2). Some scales had long cilia (Fig. 2a). The central part of the scale was blackish brown in color and middle and outer parts were usually brown and light brown, respectively. However, transparent outer parts were observed in scales of four samples.

Frond characters

Sterile fronds of *P. heterophylla* were simple and fleshy. Variation of sterile frond characters observed in the study is given in Table 2. Four frond shapes namely, rounded, oblong, lanceolate and linear were identified in sterile fronds (Fig. 3). Fronds had 0.1-0.3 cm long stripes. Veins of sterile fronds were initiated from anastomosis and formed large costal areoles and small marginal areoles (Fig. 4). Free veins of areoles were forked or simple. Midribs were presented in two thirds of the leaf and except midribs other veins were immersed. Color of the sterile frond varied from yellow green to dark green. Fertile fronds of *P. heterophylla* were simple, longer and linear than sterile fronds. Variation of fertile fronds length and width of samples is given in Table 2. In fertile fronds, apexes were rounded. Length of stripes ranged from 0.3-0.4 cm and showed little variation within and among samples (Fig. 5).

Table 2. Variation of frond characters of *Pyrosia heterophylla*.

Leaf characters	Mean (\pm SE)	Range	Within plant variation (%)	Between plant variation (%)
Sterile frond				
Length (cm)	2.67 (\pm 0.12)	1.30 - 5.80	45	55
Width (cm)	1.43 (\pm 0.02)	1.10 - 1.90	62	38
Thickness (cm)	0.12 (\pm 0.01)	0.01 - 0.25	41	59
Fertile frond				
Length (cm)	5.62 (\pm 0.34)	2.30 - 21.00	23	77
Width (cm)	3.45 (\pm 0.05)	3.00 - 04.00	32	68

Note: SE - Standard Error

Arrangement of sori

Sori of *P. heterophylla* were arranged on lower surface of the fertile frond. At the initial stage spores were white, when maturing, they became brownish. Lower surface of fertile fronds of 24 samples were totally covered by the sori (acrostichoid spore arrangement) (Fig. 5a). Three samples showed the sub marginal spore arrangement where the mid rib was clearly visible (Fig. 5b), whilst another sample showed interrupted spore arrangement where the mid rib was visible in some areas and sori were not uniformly distributed (Fig. 5c).

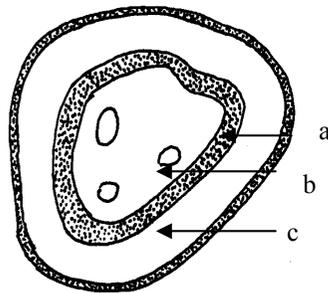


Fig. 1. Cross section of rhizome of *Pyrrosia* species ($\times 100$).
Note: a - cortical sclerenchyma sheath, b - vascular bundle and c - epidermis.

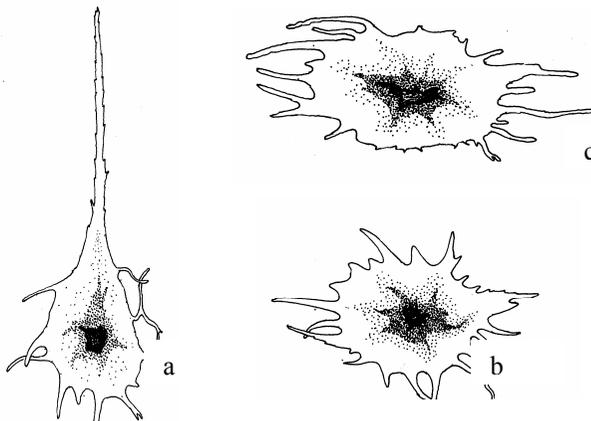


Fig. 2. Shapes of rhizome scales of *Pyrrosia* species ($\times 100$).
Note: a - triangular, b - rounded and c - linear.

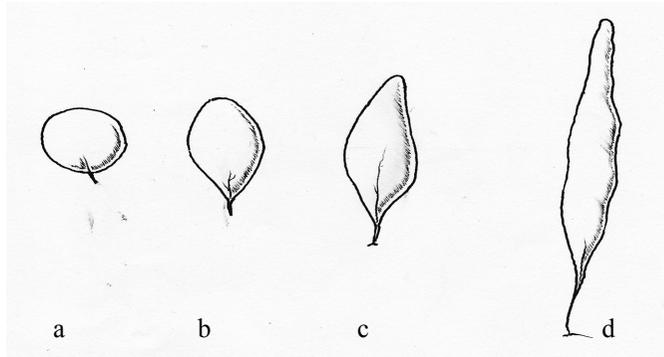


Fig. 3. Shape of sterile fronds of *Pyrrrosia* species ($\times 1$).
Note: a - rounded, b - oblong, c - lanceolate, d - linear.

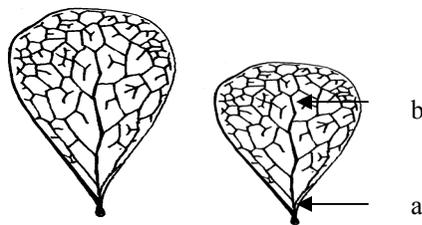


Fig. 4. Venation pattern of sterile fronds of *Pyrrrosia* species ($\times 2$).
Note: a - stipe, b - areoles.

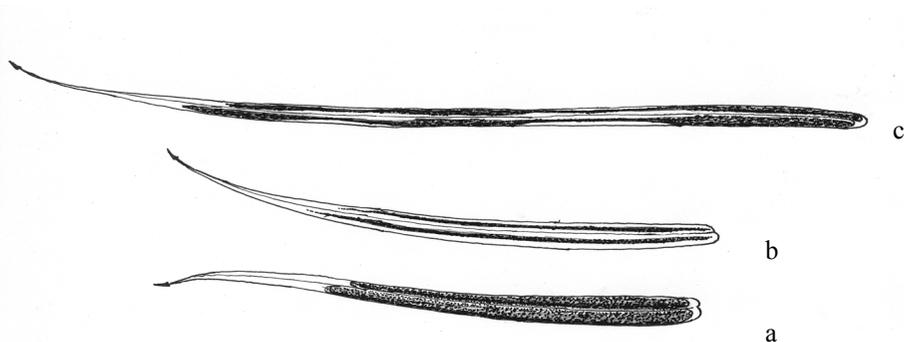


Fig. 5. Three spore arrangements of *Pyrrrosia* species ($\times 1$).
Note: a - acrostichoid, b - sub-marginal, c - interrupted spore.

Habits and habitats of *Pyrrosia heterophylla*

Three major plant habits were observed on the basis of arrangement of fronds along the rhizome on substrates. They were: leaves arranged along the rhizome and one leaf partially covered by another leaf (Habit I); leaves arranged on both side of the rhizome and they grow parallel to the substrate (Habit II); and leaves arranged one side of the rhizome and grows away from the substrate (Habit III) (Fig. 6; Table 3).

A majority of the plants occurred in homegardens (Table 3). Most of the ferns preferred homegarden as habitats, because of high moisture and shady environment. In comparison with roadside habitats, homegardens are cool and shaded. Thus, a majority of *P. heterophylla* was observed in homegardens. Except in forest boundaries, *P. heterophylla* was rarely observed inside natural forests. Variation of host plants of *P. heterophylla* is given in Table 4. Host plants ranged from small ornamental shrubs of one meter height such as *Acalypha wilkissiana* to tall tree of over 20 m height such as, *Mangifera indica*, *Artocarpus heterophyllus* and *Peltophorum pterocarpum* (Table 4). It grows well even on tree trunks, which have smooth bark such as *Areca catechu* as well as rough bark (*Mangifera indica*) and grows on both monocots (*Caryota urenus*) and Dicots (*Citrus aurantium*).

Table 3. Different habitats, plant habits and light levels of observed samples.

Habitat	Light Level				Plant habit			
	Low	Medium	High	Total	I	II	III	Total
Homegarden	1	11	5	17	-	5	12	17
Roadside	7	2	2	11	4	6	1	11
Total	8	13	7	28	4	11	13	28

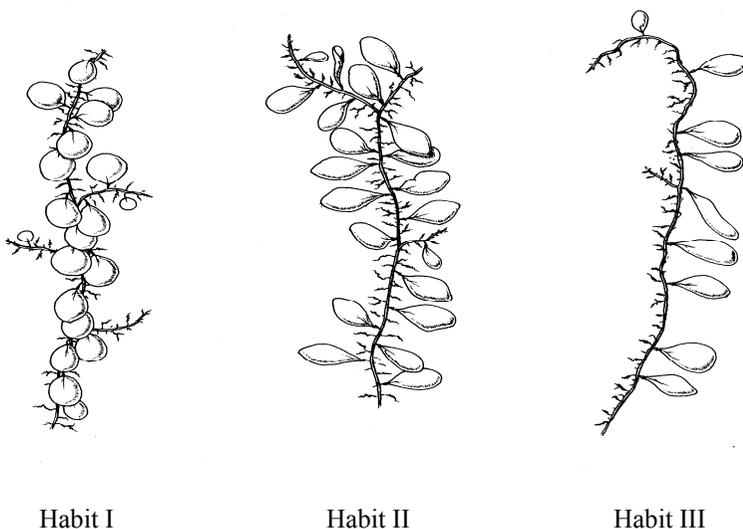


Fig. 6. Variation of plant habits of *Pyrrosia heterophylla* ($\times 0.5$).

Note: Habit I - leaves arranged along the rhizome; Habit II - leaves arranged both sides of the rhizome; Habit III - leaves arranged one side of the rhizome.

Table 4. Variation of host plant of *Pyrrosia heterophylla*.

Host plants	Common name	Habit of host plant	Occurrence (%)
<i>Codiaeum verigatum</i>	Croton (E)	Shrub	17
<i>Peltophorum pterocarpum</i>	Pare mara (S)	Tree	11
<i>Mangifera indica</i>	Mango (E)	Tree	11
<i>Artocarpus heterophylla</i>	Jak fruit (E)	Tree	11
<i>Acalypha wilkisia</i>	Acalypha (E)	Shrub	11
<i>Pithecelobium dulce</i>	Andara (S)	Shrub	7
<i>Gliricidia sepium</i>	Gliricidia (E)	Shrub	7
<i>Areca catechu</i>	Arecunut (E)	Tree	7
<i>Myristica fragrans</i>	Nutmeg (E)	Tree	4
<i>Dracaena fragrans</i>	Dracaena (E)	Shrub	4
<i>Crescentia cujete</i>	Rum tree (E)	Shrub	4
<i>Citrus aurantium</i>	Sour orange (E)	Tree	4
<i>Caryota urenus</i>	Kitul (S)	Tree	4

Note: E - English name; S - Sinhala name

Cluster analysis

The dendrogram developed from cluster analysis using all observed variables is shown in Figure 7. It is clear from Figure 7 that all samples can be divided into three significant clusters at the distance of 1.0054. Sample 26 is clustered and separated from all other samples. Length and width of fertile fronds of sample 26 is comparatively high (Table 5). The interrupted spore arrangement was unique to this sample and rhizome scale had transparent outer parts. Such characters are deviations from normal characters of *P. heterophylla* (Table 5). According monograph of world *Pyrrosia* species, this sample closely resembled *P. niphoboloides*, which has been restricted to North West and East Madagascar (Hovenkamp, 1986). *P. niphoboloides* had not previously been recorded in Sri Lanka.

Three samples (3, 5 and 6) are also grouped into a separate cluster and are different from all others, due to the sub marginal spore arrangement. They are different from are *P. heterophylla* due to transparent outer part of scales. Literature revealed that sub marginal spore arrangement and transparent scales are more close to *P. piloselloides* than *P. heterophylla*. *P. piloselloides* has also not previously been recorded in Sri Lanka. This is the first record in Sri Lanka for the occurrence of *P. niphoboloides* and *P. piloselloides*. However, it is important to note that this identification of *P. niphoboloides* based on only one sample is questionable. Hence, observation of samples for at least one more season is necessary for the confirmation of these findings. Such information will provide consistency of characters observed in identification of new species. Further, investigations on cytological molecular and biochemical information will support the conformation of such occurrences in Sri Lanka.

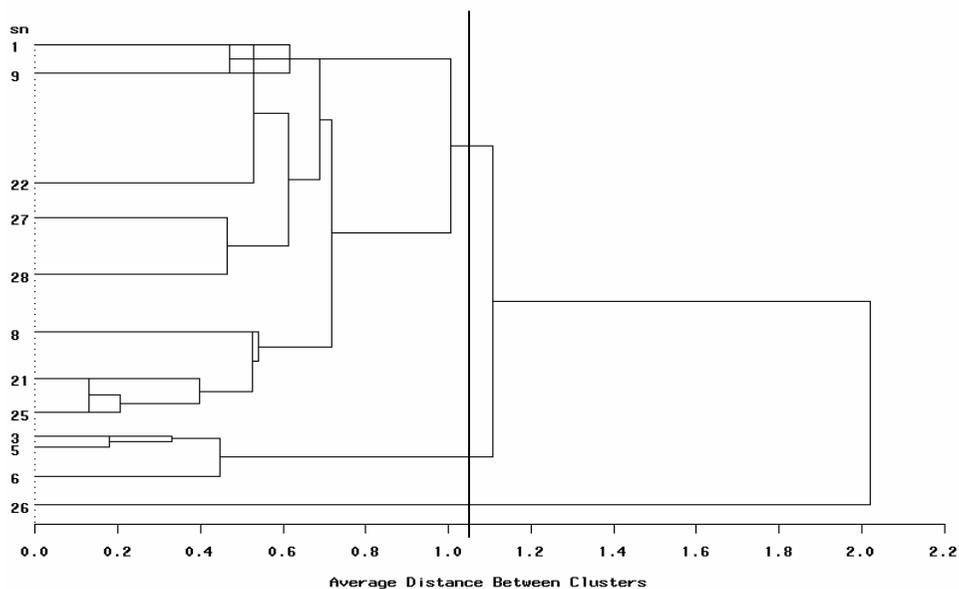


Fig. 7. Dendrogram of hierarchical cluster analysis for all variables.

Note: sn - sample number.

Table 5. Significant differences in spores arrangement, scale color and ratio of length to width of fertile fronds of three *Pyrrisia* species.

Characters	<i>P. heterophylla</i>	<i>P. piloselloides</i>	<i>P. niphoboloides</i>
Spores arrangement	Acrostichoid	Sub marginal	Interrupted
Scale color	Light brown	Transparent	Transparent
Ratio between length and width of fertile fronds	1.56	0.92	5.45

CONCLUSIONS

P. heterophylla shows a wide range of variation for characters of sterile and fertile fronds, habit and habitats. Cluster analysis revealed that *P. heterophylla* samples could be separated into three groups. Majority of the samples belong to *P. heterophylla* whilst one sample was suggested as *P. niphoboloides* and three samples as *P. piloselloides*. This is the first record of the occurrence of *P. niphoboloides* and *P. piloselloides* in Sri Lanka.

ACKNOWLEDGEMENTS

Authors wish to thank Mr. W.P. de Winter, Editor of Plant Resources of Southeast Asia, Cryptogams, Netherlands and Mr. Tom Stuart, Coordinator of American Ferns Society, USA for their generosity in providing current information with respect to identification of *Pyrrrosia* spp. Special word of thanks to Mr. Janaka Kandepola for preparation of illustrations.

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APPENDICES

Appendix 1. Locations of samples of *Pyrrosia* species collected for the study.

Sample No.	Species	Districts	Location
1	<i>P. heterophylla</i>	Kandy	University premises, University of Peradeniya
2	<i>P. heterophylla</i>	Kandy	University premises, University of Peradeniya
3	<i>P. piloselloides</i>	Kandy	University premises, University of Peradeniya
4	<i>P. heterophylla</i>	Kandy	Udispattuwa, Teldeniya
5	<i>P. piloselloides</i>	Kandy	University premises, University of Peradeniya
6	<i>P. piloselloides</i>	Kandy	Horticultural Research and Development Institute, Gannoruwa.
7	<i>P. heterophylla</i>	Kandy	University premises, University of Peradeniya
8	<i>P. heterophylla</i>	Kandy	Kandy to Colombo road, Kadugannawa
9	<i>P. heterophylla</i>	Kegalle	Weradeniya, Nelumdeniya
10	<i>P. heterophylla</i>	Kegalle	Mangala gama, Molagoda
11	<i>P. heterophylla</i>	Ratnapura	Elapatha, Amuwala
12	<i>P. heterophylla</i>	Ratnapura	Elapatha, Amuwala
13	<i>P. heterophylla</i>	Ratnapura	Gurupaskada watta, Amuwala
14	<i>P. heterophylla</i>	Ratnapura	Ilanga watta, Amuwala
15	<i>P. heterophylla</i>	Ratnapura	Ilanga watta, Amuwala
16	<i>P. heterophylla</i>	Ratnapura	Arambe gedara, Amuwala
17	<i>P. heterophylla</i>	Kandy	Sinhapitiya, Gampola
18	<i>P. heterophylla</i>	Kandy	University premises, University of Peradeniya
19	<i>P. heterophylla</i>	Kurunegala	Godawela, Polgahawela
20	<i>P. heterophylla</i>	Kurunegala	Kiribamuna, Ibbagamuwa
21	<i>P. heterophylla</i>	Kurunegala	Walakumbura, Alauwa
22	<i>P. heterophylla</i>	Kandy	University premises, University of Peradeniya
23	<i>P. heterophylla</i>	Matale	Galwadukumbura, Kawdupellella
24	<i>P. heterophylla</i>	Matale	Galwadukumbura, Kawdupellella
25	<i>P. heterophylla</i>	Matale	Aranwatta, Alupothuwala
26	<i>P. niphoboloides</i>	Kalutara	Gonaduwa, Moronthuduwa
27	<i>P. heterophylla</i>	Kandy	Udurawana, Wattegama
28	<i>P. heterophylla</i>	Kandy	Getambe, Peradeniya