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DISTRIBUTION AND CONSERVATION STATUS OF SPECIES OF *PRONEPHRIUM s.l.* (THELYPTERIDACEAE) FERNS IN PENINSULAR MALAYSIA

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Pronephrium s.l. (Thelypteridaceae) has its highest species diversity in Malesia. The distributional range and conservation status for many species have not been evaluated. Members of *Pronephrium* have been repeatedly collected from different parts of Peninsular Malaysia, nevertheless, their distribution has never been updated since Holttum's account in 1955, and the conservation status of the species reported from Peninsular Malaysia has never been assessed. Considering this, we provide an up-to-date account of the distribution and evaluate the conservation status of all the species reported for Peninsular Malaysia. The evaluation was based on five years of field observation and reviewing all herbarium collections. The conservation status of all species in the current study was assessed at a sub-national level confined to Peninsular Malaysia. Assesments were conducted based on IUCN Conservation Criteria and Categories. Two species were categorised as threatened, one species assessed as Endangered, one as Vulnerable, two as Near Threatened and four as Least Concern. One variety is endemic in Peninsular Malaysia. Various threats to the extant populations are identified and discussed.

Keywords: Malaysia Plant Red List, threatened taxa, endemic

INTRODUCTION

Pronephrium sensu Holttum or *sensu lato* in this manuscript (Thelypteridaceae) (Holttum 1972, 1980, 1982) is a fern genus best represented in tropical and subtropical Asia, from India and Sri Lanka to South China, throughout Malesia, reaching as far east as Fiji in the Pacific. Less than 2% of its species occur in the temperate regions (Smith 1990). The highest species diversity is within Malesia, with about 82% of the diversity or 68 species (Holttum 1982). In Peninsular Malaysia, *Pronephrium s.l.* is represented by eight species with one variety and possibly one hybrid (Holttum 1980, Parris & Latiff 1997). Of these, *Pronephrium peltatum* var. *peninsulare* Holttum is endemic in Peninsular Malaysia. This variety is recognised by its hairy indusia (Holttum 1982).

Members of *Pronephrium* are all terrestrial and shade-loving ferns, growing on dry forest ground to wet stream banks where a small number of them are rheophytic (Holttum 1982). In Peninsular Malaysia, almost all members are found in lowland to hill dipterocarp forests, except for *Pronephrium peltatum* var. *peninsulare* which is restricted to lower montane forest.

The latest phylogenomic results by Fawcett and Smith (2021) clearly indicate that *Pronephrium s.l.* is polyphyletic and propose the recognition of four separate genera: *Abacopteris* Fée, *Grypothrix* (Holttum) S.E.Fawc. & A.R.Sm. (with hammate hairs on various plant parts), *Menisciopsis* (Holttum) S.E.Fawc. & A.R.Sm., and *Pronephrium s.s* (dimorphic fronds and sporangia with yellow glands). Meanwhile, a number of formerly *Pronephrium s.l.* species were transferred to other genera, including *Sphaerostephanos* by Fawcett and Smith (2021). In accordance with this, the Peninsular Malaysian species are now placed in *Abacopteris* (*Abacopteris aspera* and *A. repanda*; both species have monomorphic fronds and lacking of yellow glands on indusia),

Grypothrix (*Grypothrix rubicunda*, *G. salicifolia* and *G. triphylla*; all three species have hammate hairs on their lamina and sporangia), *Pronephrium s.s.* (*Pronephrium menisciicarpon* and *P. peltatum* var. *peninsulare*; both species with dimorphic fronds) and *Sphaerostephanos* (*Sphaerostephanos glandulosus*; adaxial laminae with appressed hairs and yellow glands on lamina beneath). At the global scale, the conservation status of most of the *Pronephrium s.l.* species has yet to be assessed. Likewise, at the regional scale, only a handful of species were evaluated, for instance, *Grypothrix longipetiolata* (K.Iwats.) S.E.Fawc. & A.R.Sm. (formerly *Pronephrium longipetiolatum* (K.Iwats.) Holttum) was classified as Critically Endangered in the preliminary Red List of Chinese lycophytes and ferns (Dong et al. 2017).

The lowland areas of Peninsular Malaysia have been subjected to many large-scale land conversions since the country's independence in 1957. Deforestation, fragmentation and habitat degradation are the biggest threats to Malaysia's lowland forests (Lau 2014). Forested area, especially in the lowland, has been declining significantly since the 1960s, from more than 70% of the total land area covered by forest before 1957 to only 63% by 1972 (Forestry & Forest Industries Development Malaysia, 1973), to 49.4% in 1980 (Forest Statistics Peninsular Malaysia 1979–1985), and 43.5% in 2021 (Forestry Department of Peninsular Malaysia 2022). The large-scale conversion of lowland forest to rubber and oil palm plantations, particularly in the states of Pahang, Terengganu, Johor and Kelantan, have altered the peninsular landscape and threatened the lowland biological diversity (Abdullah & Nakagoshi 2008, Wicke et al. 2011, Miyamoto et al. 2014, Hamdan et al. 2016).

Furthermore, there has been no global assessment of the conservation status of the many fern species in Malaysia (IUCN 2022a). In a recent conservation assessment of Polypodiaceae ferns in Peninsular Malaysia, two out of 122 species were classified as Extinct (EX) in Peninsular Malaysia (Parris 2010, Saw et al. 2010, Hovenkamp 2013, Yong et al. 2021). Thus, there is an urgent need to assess the conservation status of the *Pronephrium s.l.* species using the International Union for the Conservation of Nature (IUCN) categories and criteria version 3.1 (IUCN 2012a), Guidelines for Using the IUCN Red List Categories and Criteria (IUCN 2022b) and Guidelines for Application of IUCN Red List Criteria at Regional and National Levels (IUCN 2012b). Information generated from this will contribute to the global assessment.

This study aims to produce up-to-date species distribution maps, identify the threats to all *Pronephrium s.l.* species reported for Peninsular Malaysia, and assess their conservation status. Information obtained from this research can later be incorporated into the conservation action plan and contribute to the Malaysia Plant Red List project to ensure the continued survival of the *Pronephrium s.l.* species.

MATERIALS & METHODS

Sources of distribution data

Specimens deposited in KEP, KLU, SING and UKMB herbaria were examined (herbarium acronyms follow Thiers 2016). Images of type specimens were downloaded from online resources, including Global Plants on JSTOR and Plants of the World Online by Royal Botanic Gardens Kew. A total of 333 accessions of *Pronephrium s.l.* were verified and the data were assembled using the Botanical Research and Herbarium Management System (BRAHMS Version 7.9 2017) software. Historical materials deposited in oversea herbaria e.g., BR, BM, UC, S and FI with good image quality where identification to species level was possible, were included in this study.

Field observations and collections were conducted by the first author in 13 different forested and limestone areas between 2015 and 2020 (Figure 1, 3 & 4). The newly obtained field data, together with the information obtained from the herbarium studies were further analysed. The newly documented populations or locations were remarked in the distribution map using "★" symbol (Figure 3 and 4).

These surveys aimed to verify some doubtful reports and investigate previously unexplored areas. In addition, data on the following aspects: ecology, altitude, habitat preferences, and threats to each population, were recorded for later analysis.

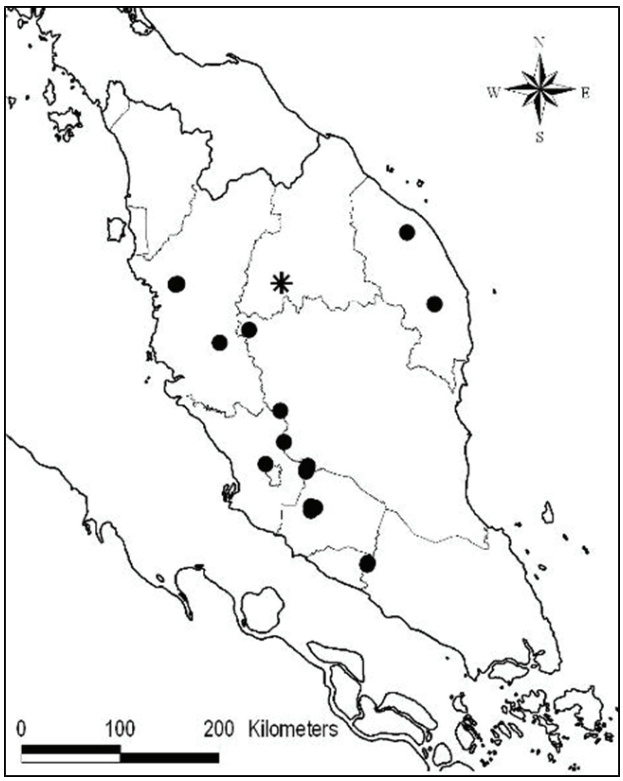


Figure 1 Forested (•) and limestone (*) areas in Peninsular Malaysia where field observations and collections were conducted between 2015 and 2020

Distribution maps

BRAHMS database includes information such as location, Global Positioning System (GPS) reading, collection date, habitat notes and altitude for each specimen. Geographical coordinates of the newly obtained samples were read directly from the GPS, meanwhile, the latitude and longitude of the older specimens were reconfirmed by referring to the botanical gazetteer (Hamidah et al. 2011). The distribution map of each species was generated using the QGIS 3.28.3 software.

Conservation status assessments

According to the IUCN Red List Categories and Criteria version 3.1 (IUCN 2012), nine categories are recognized: Extinct (EX); Extinct in the wild (EW); Critically Endangered (CR); Endangered (EN); Vulnerable (VU); Near Threatened (NT); Least Concern (LC); Data Deficient (DD) and Not Evaluated (NE). Criteria used to assign conservation status include population reduction, present geographic range, very small or restricted population, and quantitative analysis showing the probability of wild extinction. Fulfilment of any of the above criteria entitled a taxon to be given the threatened category, *i.e.*, VU, EN and CR.

The documentation of every species' conservation status followed the guidelines in the Malaysia Plant Red List Guide (Chua & Saw 2006). A Taxon Data Information Sheet (TDIS) (Chua & Saw 2006) was prepared for every species that is a document modified from the IUCN Red List Assessment Questionnaire suited to the Malaysian context. It comprises five parts: Taxon Attributes; Geographical Range and Demographic Details of Population(s); Red List Category & Criteria Assessment; Current Conservation Measures for the Taxon; and Utilisation.

The extent of occurrence (EOO), area of occupancy (AOO) and reduction analysis for every species were calculated using Geospatial Conservation Assessment Tool (GeoCAT 2023). In order to assess habitat decline, the distribution map was overlaid with the forest cover map based on the Third National Forest Inventory (1991–1993) (Forestry Department of Peninsular Malaysia) and the Google Earth map. The status of the historical and recent collection sites was also reviewed through this method.

In order to assign a conservation status, the following criteria were considered: the total number of past and recent collecting locations, the continual decline of extent of occurrence or area of occupancy, and the habitat quality related to recent threats and disturbances. Additionally, habitat preferences, endemism, and species occurrence in the network of Totally Protected Areas are also considered while assigning the status.

RESULTS

Distribution of *Pronephrium s.l.* species in Peninsular Malaysia

Results of the reduction analysis, including the comparison between the past and the current extent of occurrence, area of occupancy and

number of locations are represented in Table 1. All *Pronephrium s.l.* species evaluated in this analysis showed a drop in area of occupancy (about 10% or more) before and after the reduction analysis, even though the extent of occurrence remained largely unchanged (Table 1). Some of the historical collecting sites were no longer covered by forest, or had been converted for other land use purposes, indicating the loss of the original habitats (Table 1).

Peninsular Malaysia has a total land area of 132,490 km^2. At present, most species show an extent of presence in less than half of the Peninsular Malaysia land area, except for *Abacopteris repanda*, *Grypothrix rubicunda*, *G. triphylla* and *Pronephrium menisciicarpon* with extent of occurrence approaching or larger than 66,245 km^2 (Table 1). Two species show an extent of occurrence less than a quarter of the Peninsular Malaysia land area, viz., *Pronephrium peltatum* var. *peninsulare* and *Sphaerostephanos glandulosus*, and both these species have a very restricted geographical range in Peninsular Malaysia (Figure 3A & B).

The most remarkable decrease in area of occupancy before and after the reduction analysis is observed for *Pronephrium peltatum* var. *peninsulare* (42.9%) then *P. menisciicarpon* (18.2%), followed by *Sphaerostephanos glandulosus* (12.5%).

A few species were recollected several times from forest reserves and protected areas by different researchers. For example, *Abacopteris aspera* was recollected from Bukit Lagong forest reserves, Selangor, and Sungai Tahan, National Park, Pahang. *Abacopteris repanda* was recollected from Kledang Saiong forest reserves, Perak, *Grypothrix salicifolia* from Bubu forest reserves, Perak, while *G. triphylla* was recollected from Bukit Larut forest reserves, Perak, and Endau-Rompin State Park, Pahang. *Sphaerostephanos glandulosus* was collected from the National Park, Pahang, and *Pronephrium menisciicarpon* from Ulu Gombak forest reserves, Selangor, and the National Park, Pahang. Nevertheless, under the National Forestry Act 1984, any permanent forest reserve can be excised by the state authority if needed, unless a protection status has been gazetted.

Ecology of *Pronephrium s.l.* species in Peninsular Malaysia

In Peninsular Malaysia, members of *Pronephrium s.l.* are mostly species of lowland to hill dipterocarp forest. Only *Pronephrium peltatum* var. *peninsulare* is restricted to highland habitats, while *Grypothrix triphylla* is known to occupy a wide elevation range from lowland to lower montane forest (Table 2). Most of the *Pronephrium s.l.* members are confined to natural forests, except for *Abacopteris repanda* and *Grypothrix triphylla*, which are known to be present in both primary and secondary vegetation. In terms of the substrate, all *Pronephrium s.l.* members occur on forest soil and rocks (granitic materials) and sometimes on riverine or alluvial sediments, where four of them, viz., *Abacopteris aspera, A. repanda*, *Grypothrix triphylla* and *Sphaerostephanos glandulosus* are also present in limestone habitats (Table 2).

Implications of conservation status

Table 3 summarises the conservation status of *Pronephrium s.l.* members for Peninsular Malaysia. They were assigned under four different IUCN categories, i.e., one species *Pronephrium peltatum* var. *peninsulare* was assigned a status of Endangered B1ab(iii,iv)+2ab(iii,iv), *Sphaerostephanos glandulosus* as Vulnerable B2ab(i,ii,iv), two species (*Abacopteris aspera* and *Pronephrium menisciicarpon*) as Near Threatened (NT) and four species (*Abacopteris repanda*, *Grypothrix rubicunda*, *G. salicifolia* and *G. triphylla*) as Least Concern (LC).

Endangered

Pronephrium peltatum var. *peninsulare* has an EOO of 4,921 km^2 and AOO of 28 km^2 (Table 1 & 3) and has experienced a drastic decline of AOO (42.9%) and only represented by nine collections from four locations (Table 1), therefore qualified for the status Endangered Criterion B1ab(iii, iv) and Criterion B2ab (iii,iv). The species has been collected only from four isolated mountain locations in Perak, Pahang and Negeri Sembilan (Figure 3A). Its reliance on shady and wet streams in pristine montane

Table 1 Reduction analysis of each species

Species	EOO before reduction analysis (km^2)	Current EOO (km^2)	AOO before reduction analysis (km^2)	Current AOO (km^2)	Percentage loss of AOO (%)	Total number of locations before reduction analysis	Total number of locations after reduction analysis
Abacopteris aspera	43,964.797	43,964.797	80	72	10	20	18
Abacopteris repanda	146,954.047	146,954.047	268	240	10.4	59	52
Grypothrix rubicunda	100,186.871	100,186.871	176	160	9.1	39	35
Grypothrix salicifolia	48,228.700	48,228.700	76	68	10.5	19	17
Grypothrix triphylla	79,020.616	79,008.562	120	108	10	30	27
Pronephrium menisciicarpon	67,501.449	66,542.254	88	72	18.2	21	17
Pronephrium peltatum var. *peninsulare*	4921.086	4921.086	28	16	42.9	6	4
Sphaerostephanos glandulosus	27,082.327	25,581.925	64	56	12.5	12	10

EOO = Extent of Occurrence, AOO = Area of Occupancy

Table 2 The ecology of *Abacopteris, Grypothrix, Pronephrium s.s.* and *Sphaerostephanos* species in Peninsular Malaysia

Species	Habitat	Altitude (m above sea level)	Forest type	Survival in primary/ secondary forest	Substrate
Abacopteris aspera (C.Presl) Ching	T. Sometimes close to rivers.	50–700	LD, HD	1°	G, LS
Abacopteris repanda (Fée) S.E.Fawc. & A.R.Sm.	T. Sometimes riverine.	20–950	LD, HD	1°, 2°	G, LS, A
Grypothrix rubicunda (Alderw.) S.E.Fawc. & A.R.Sm.	T, rarely L. Sometimes riverine and forest edge.	50–850	LD, HD	1°	G, A
Grypothrix salicifolia (Wall. ex Hook.) S.E.Fawc. & A.R.Sm.	L. By river banks.	70–914	LD, HD	1°	G
Grypothrix triphylla (Sw.) S.E.Fawc. & A.R.Sm.	T. By roadsides and in plantations.	10–1400	LD, HD, LM	1°, 2°	G, LS
Pronephrium menisciicarpon (Blume) Holttum	L, sometimes T. By stream. Only once collected from a location between rubber plantations and forest (*A. Abd. Samat 439*)	120–610	LD, HD	1°	G, A
Pronephrium peltatum var. *peninsulare* Holttum	L, only once T along dried stream bed (*A. Abd. Samat 425*)	920–1465	LM	1°	G
Sphaerostephanos glandulosus (Blume) S.E.Fawc. & A.R.Sm.	T, L. Often nearby rivers.	66–700	LD, HD	1°	G, LS, A

T = terrestrial, L = lithophyte, LD = lowland dipterocarp, HD = hill dipterocarp, LM = lower montane; 1° = primary forest, 2° = secondary forest; G = granite, LS = limestone, A = alluvial

Table 3 The conservation status of *Abacopteris, Grypothrix, Pronephrium s.s.* and *Sphaerostephanos* species in Peninsular Malaysia

Species	Threats	IUCN Category	IUCN Criteria
Abacopteris aspera	Rapid development surrounding limestone areas; mining.	NT	-
Abacopteris repanda	Not affected by land use change.	LC	-
Grypothrix rubicunda	Oil palm plantations; urban areas.	LC	-
Grypothrix salicifolia	Tourism/recreation	LC	-
Grypothrix triphylla	Not affected by land use change.	LC	-
Pronephrium menisciicarpon	Oil palm plantations; urban areas.	NT	-
Pronephrium peltatum var. *peninsulare*	Agricultural activities and rapid development in montane areas	EN	B1ab(iii,iv) + 2ab(iii,iv)
Sphaerostephanos glandulosus	Oil palm plantations; clearing and burning at base of limestone hill; mining.	VU	B2ab(i,ii,iv)

forest places it at a high risk of extinction. All the reported locations of *Pronephrium peltatum* var. *peninsulare* do not lie within the protected area, unless they are present in forest at elevations above 1000 m, where any forested area above that elevation has been promoted to strict protection status after the 14th National Forestry Council Meeting (Kumaran et al. 2011). Herbarium data recorded 78% of the specimens of this endemic variety as collected before the 1980s (Figure 2), and it has not been re-collected from all its former localities in Pahang (Cameron Highlands: Boh Plantations, Brinchang, Telom Valley; Genting Highland: Gunung Ulu Kali; Fraser's Hill: Jeriau Falls) and Perak (Maxwell's Hill), even though several reappraisal trips have been carried out to above-mentioned area (e.g., Razali et al. 2011 and present survey). Rapid agricultural expansion and tourism development in the highlands have been drastically altering the montane habitats in Peninsular Malaysia and have threatened the survival of this endemic variety. The species was re-discovered by the first author during her survey to Gunung Telapak Buruk in the year 2019 (Figure 3A), but unfortunately the population was found at elevation 920 m, an elevation where no protection has been granted and could be deforested for commercial purposes.

Vulnerable

Sphaerostephanos glandulosus with its low area of occupancy of 64 km^2 and continuous decline of extent of occurrence, area of occupancy and number of locations in recent years, corresponding to IUCN Criterion B2ab(i,ii,iv) (Table 1 & 3) and thus assigned the status Vulnerable. The large-scale conversion of forested areas to oil palm plantations in central Pahang (Shaharum et al. 2020) may have completely wiped out the species in that area, therefore the population of *S.* g*landulosus* that was encountered in 1931 at Mentakab and 1963 at Jengka may have long disappeared (Figure 3B). The species is also known to occur in limestone habitats and has been collected from several limestone areas including Gunung Senyum and Gua Telinga in Pahang, and Gua Renayang in Kelantan. All these habitats are subjected to anthropogenic disturbances. Even though Gua Telinga is located within the National Park, but it is heavily visited by tourists and subjected to the trampling effect. Gunung Senyum and Gua Renayang, just like many of the limestone areas in the country, lie on state land and are not under any form of protection and may subjected to quarrying activities (Liew et al. 2016). As an example, this survey noted the population in Gua Renayang, Kelantan, has been threatened by the clearing and burning of the foothill area for banana and papaya plantations.

Near Threatened

Abacopteris aspera and *Pronephrium menisciicarpon* are assigned a status of Near Threatened (NT), according to the IUCN criteria based on the following justification:

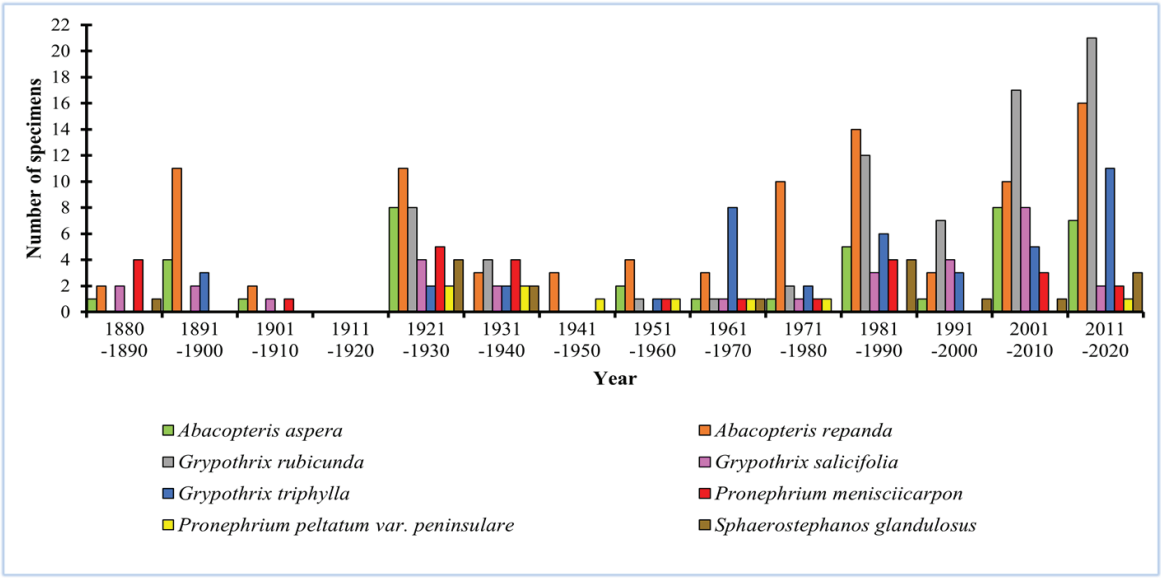


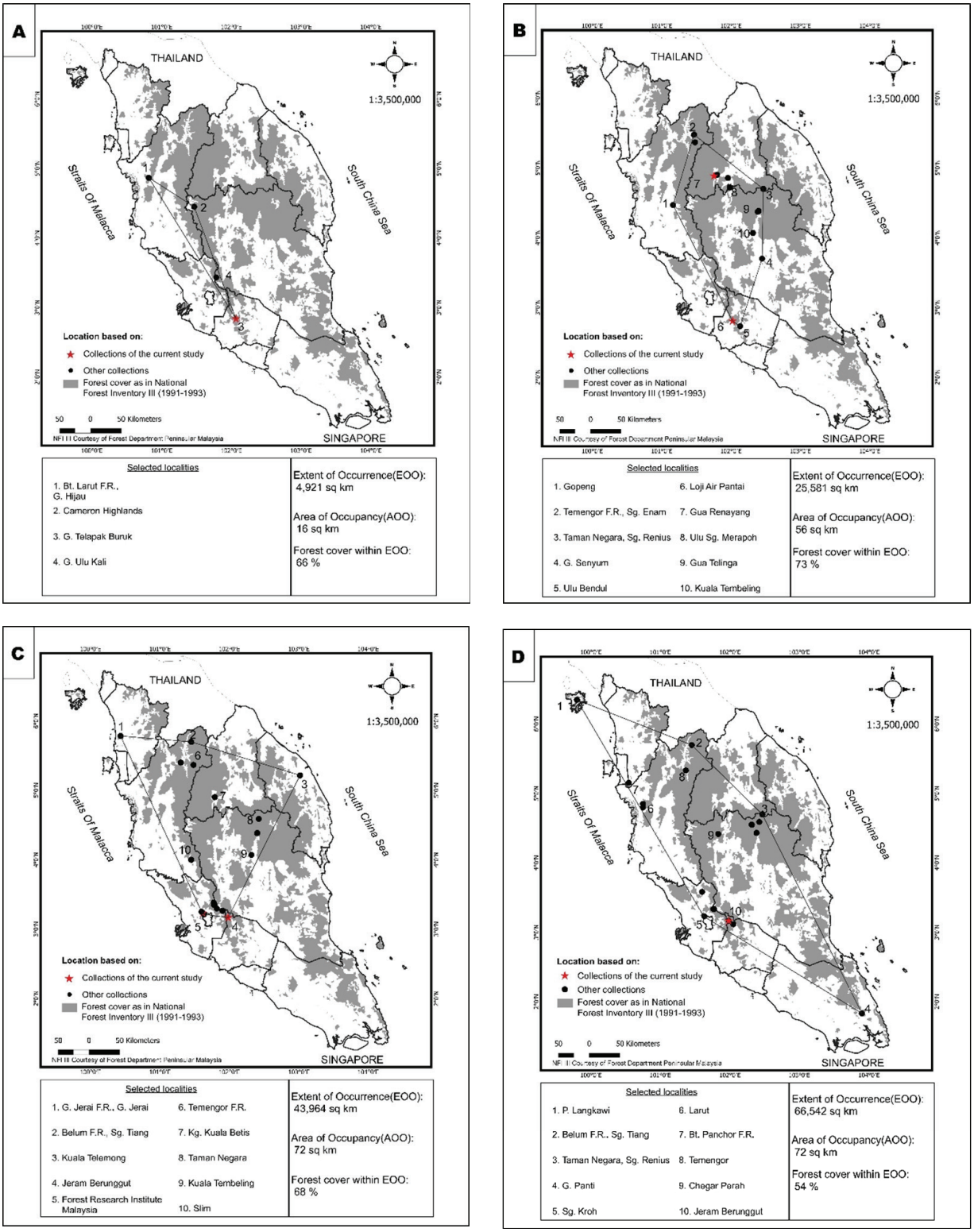
Figure 2 Number of specimens collected in every decade

Abacopteris aspera has more than 10 locations and an extent of occurrence beyond 20,000 km^2 thus does not qualify for threatened categories. However, this species has a narrow distribution range, confined to the area between Gunung Jerai FR at the north, and Jeram Berunggut in Sungai Kenaboi Valley, Negeri Sembilan, at the southern end of the Main Range (Figure 3C) with an area of occupancy of 72 km^2. There are collections of this species from limestone habitats, for instance, Batu Caves, Selangor, and Gua Panjang, Kelantan, between 1890–1930. The later large-scale land conversion and rapid development surrounding the above limestone areas has greatly impacted the local biological diversity (Kiew 2014, Liew et al. 2016, Kiew & Rafidah 2021, Liew et al. 2021). Therefore, a continuing decline in area of occupancy is suspected. Recent attempts to rediscover the species from limestone areas, including the above two historical sites, met with no success indicating that the species may have disappeared from the limestone habitats, particularly those subjected to anthropogenic disturbance. Although the species has been collected a few times from Totally Protected Areas, but many other populations occur in state land or areas without protection status, for instance, Kuala Telemong, Terengganu and Slim, Perak. These areas have been subjected to plantation expansion, rural development and habitat fragmentation, posing significant threats to the sub-populations there. Therefore, this species is assessed as Near Threatened nearly meeting VU B2b(ii, iii).

The second species assigned to this category, *Pronephrium menisciicarpon* is assessed as Near Threatened nearly meeting VU B2b(iii), shows a decrease of 18.2% area of occupancy, from 88 to 72 km^2 in the reduction analysis, besides continuous decline of extent of occurrence and the number of locations (Table 1). It is restricted to riverine areas with semi-riparian behaviours and requires shaded and humid habitats, thus vulnerable to disturbance. Even though *Pronephrium menisciicarpon* has been collected several times from Totally Protected Areas, the species was seldom recollected after 1990 (Figure 2). Besides, later visits to the historical collecting sites, for instance, Langkawi Islands by Maideen and Damanhuri (2015), failed to rediscover the species. Several of the past collecting areas have either been converted into oil palm plantations (Kota Glanggi, Mentakab, and Sungai Cheka, Pahang) (Shaharum et al. 2020), or been developed into urban housing and commercial areas (Damansara, Selangor), where the species can no longer exist (Figure 3D).

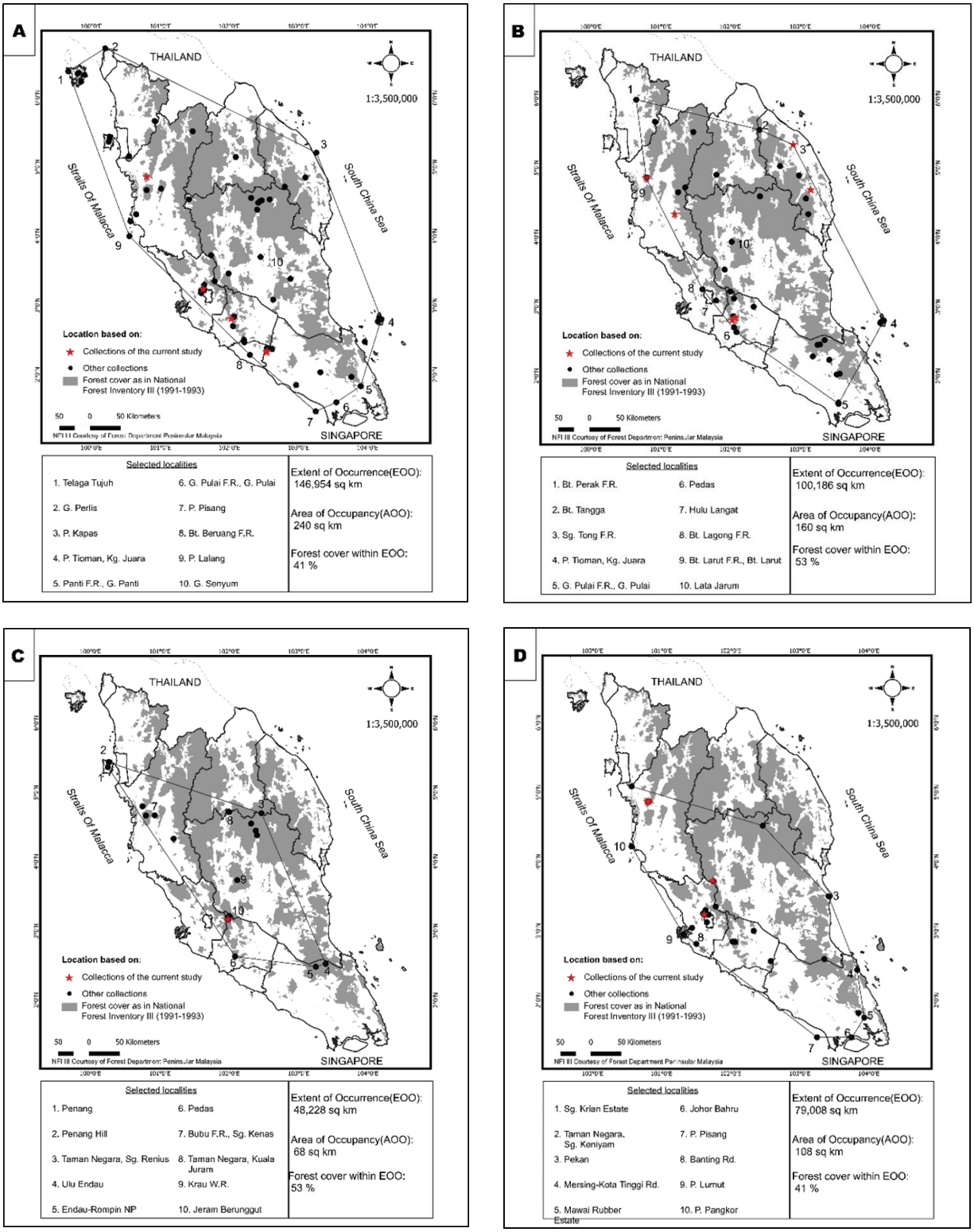
Least Concern

These four species: *Abacopteris repanda*, *Grypothrix rubicunda*, *G. salicifolia* and *G. triphylla*, are proposed a status of Least Concern (LC), according to the IUCN criteria.



G. = Gunung = mountain, P. = Pulau = island, Bt. = Bukit = hill, Sg. = Sungai = river, Kg. = Kampung = village; ★ = newly added collections from the recent survey trips, ● = other collections

Figure 3 Distribution maps of the species categorised as threatened; Endangered: A, *Pronephrium peltatum* var. *peninsulare* and Vulnerable: B, *Sphaerostephanos glandulosus* and Near Threatened. C, *Abacopteris aspera* and D, *Pronephrium menisciicarpon*



G. = Gunung = mountain, P. = Pulau = island, Bt. = Bukit = hill, Sg. = Sungai = river, Kg. = Kampung = village, Rd. = Road; ★ = newly added collections from the recent survey trips, ● = other collections

Figure 4 Distribution maps of the species categorized as Least Concern. A, *Abacopteris repanda*; B, *Grypothrix rubicunda*; C, *Grypothrix salicifolia* and D, *Grypothrix triphylla*

The extent of occurrence of the four species is almost equivalent or more than half of the Peninsular Malaysia land area (Table 1) and many of the surviving populations of these four species are within the network of Totally Protected Areas (Figure 4A–D). Even though a continuous decline in area of occupancy and number of collection locations of the four species are shown in the reduction analysis (Table 1), in the last 20 years, these four species were frequently encountered and collected by different researchers from different parts of Peninsular Malaysia (Figure 2, Figure 4A–D). Therefore there is no clear indication that any of the above species is under threat.

Beside being commonly encountered on the mainland, *Abacopteris repanda*, *Grypothrix rubicunda* and *G. triphylla* have been repeatedly collected from the offshore islands, for instance, Pulau Kapas, Pulau Tengah and Pulau Tioman on the east coast of Peninsular Malaysia, and from Pulau Pisang and Pulau Langkawi on the west coast of Peninsular Malaysia (Figue 4A, B & D), which suggests that these species can disperse over a wide geographical range and across a physical barrier, for instance, ocean, therefore with successful living strategy and with a higher capability to colonize new habitats.

Among the four species, *Grypothrix salicifolia* has the lowest extent of occurence, area of occupancy and the number of locations, nevertheless this species occurs mainly within the network of Totally Protected Areas. Thus, no major threats have been identified to its habitats and populations are well protected. Therefore, it is appropriate to categorize it under LC.

DISCUSSION

Threats to habitat loss

In Peninsular Malaysia, members of *Pronephrium s.l.* are primarily species of lowland to hill dipterocarp forests, while *Pronephrium peltatum* var. *peninsulare* is the only species restricted to highland habitats. In contrast, *Grypothrix triphylla* tolerates a wide range of elevations, from lowland to lower montane elevations. Most *Pronephrium s.l.* species in Peninsular Malaysia are forest dwellers that prefer shady and humid habitats, with a few (*Abacopteris repanda*, *Grypothrix rubicunda*) also occurring in old-growth rubber estates or secondary forests, and *Grypothrix triphylla* that can survive in disturbed habitats (Table 2).

Fern species that are restricted to undisturbed habitats such as the pristine lowland and montane forests, are more prone to extinction (Mehltreter 2010). Forest reliance is one of the characteristics of plant species most prone to extinction (Sodhi et al. 2008). In Peninsular Malaysia, the tropical forest areas are rapidly shrinking and causing depletion in biological diversity. Habitat disturbance resulting from land conversion causes reduced species richness and lower densities of forest-dependent taxa (Sodhi et al. 2010), in which forest-dependent taxa cannot live and survive in disturbed conditions. Thus, even though a forest-dwelling species has been reported with high extent of occurance, but a low forest cover area, clearly indicating it would have a smaller extension range that is equivalent to the remaining forest size (e.g., *Abacopteris aspera*, *Grypothrix salicifolia*, *Pronephrium menisciicarpon*, *Pronephrium peltatum* var. *peninsulare*, *Sphaerostephanos glandulosus*) (Figure 3A–D & 4C). Further conversion of forested land to other land use will continue to threaten forest-dwelling species, eventually driving them to extinction.

In limestone areas, quarrying is a major threat. Malaysia is one of the top five cement exporters in the world (Hughes 2017) and most limestone areas in Peninsular Malaysia are not within the protected area network but are subjected to cement extraction and various anthropogenic disturbances such as land conversions, urban development (Kiew 2014), encroachment and fire (Kiew & Rafidah 2021). The species inhabiting limestone are under constant threat (Liew et al. 2016, Rafidah & Kiew 2018) due to the elimination of the pristine forest surrounding the limestone area, thus causing significant changes to the original ecosystem and causing the loss of species sensitive to environmental change.

In the highland areas, the establishment of resorts, tourism and urban expansion are among the main threats. In Cameron Highlands, Pahang, urban expansion especially near the town areas in Tanah Rata and Brinchang contributed to 100% increase in land use changes between 1966 to 2010 (Rozimah & Khairulmaini 2016, Razali et al.

2018). In Genting Highlands in Pahang, the development of tourism infrastructure has led to the deforestation and removal of almost all its upper montane cloud forest (Kumaran et al. 2011, Entalai et al. 2020). Rapid recreational and business complex development on the lower slopes of Gunung Ulu Kali has resulted in the loss of forested areas and left only minimal forested areas on some isolated summits (Chua & Saw 2001). The expansion of urban centres and the establishment of golf courses in the lower montane forest of Fraser's Hill in Pahang have caused deforestation (Peh et al. 2011). Golf resort development in the Jeriau Valley in Fraser's Hill has resulted in heavy siltation, threatening the Sungai Jeriau stream system, flora, fauna, and surrounding area (Kiew 1992). Similar highland resort development projects were carried out or proposed on other hill stations in Peninsular Malaysia, portraying potential disruption to the highland habitats and threatening the highland biodiversity (Saw et al. 2010).

Apart from urban expansion, Cameron Highlands, Pahang is highly disturbed by agricultural activities. Rozimah & Khairulmaini (2016) and Razali et al. (2018) indicated that the increase in land-use changes, primarily for agricultural and urbanization in several parts of the highlands were correlated with the decline of the forest areas from 67,381 ha in 1966 to 58,855 ha in 2010. Continuous deforestation in Cameron Highlands is further shown by How et al. (2020) using remote sensing data. An increase of farmland and agriculture plots by 7.71% and urban area by almost 1.70% between 2009 and 2019 correlates with the decrease in primary forest cover of 8.88% within those ten years.

Comparison with other assessed fern genera of Peninsular Malaysia

The conservation status of a total of 329 taxa of ferns and lycophytes reported in the Flora of Peninsular Malaysia Series I (Volumes 1–3) was recently reassessed by Yong et al. (2021), showing that the highest percentage of threatened species (excluding the genus with a single species) are the Polypodiaceae genera *Tomophyllum*, *Calymmodon*, *Radiogrammitis*, *Platycerium* and *Lecanopteris* with 100%, 80%, 80%, 66.7%, 66.7% respectively. Meanwhile, in the current study, the percentage of threatened taxa is 25% based on one EN species, *Pronephrium peltatum* var. *peninsulare* and one VU species, *Sphaerostephanos glandulosus*. This percentage is equivalent to the rate of threatened species in the ferns *Antrophyum* (Pteridaceae) and *Dasygrammitis* (Polypodiaceae). *Antrophyum* species are epiphyte or lithophyte, while *Dasygrammitis* species are commonly epiphyte or lithophyte (only *Dasygrammitis brevivenosa* (Alderw.) Parris is sometimes are terrestrial).

CONCLUSION

Recommendation for conservation of endangered species

For endangered species that occur outside of Totally Protected Areas such as *Pronephrium peltatum* var. *peninsulare*, priority must be given to habitat conservation. A species Conservation Action Plan needs to be developed to provide clear direction for priority setting, as has been done in Peninsular Malaysia for *Begonia herveyana* (Chan & Chua 2020). This Conservation Action Plan is crucial for *Pronephrium peltatum* var. *peninsulare* because it provides detailed information on the population biology of the species that helps better understand the effect of the threats to the species' survival, clarifies the role of each implementing agency in achieving the *in situ* and *ex situ* conservation objectives and sets up the timeline towards achieving these objectives.

Ballesteros and Pence (2018) reported the storage of spores in liquid nitrogen at -196ºC as the most long-lasting storage method, which may last up to 21 years with most species able to germinate to the gametophyte. A good candidate for this would be the endangered *Pronephrium peltatum* var. *peninsulare*, with limited surviving sub-populations. Propagation using spores and rhizomes was initiated by FRIM to rescue a rare fern *Calciphilopteris alleniae* (Pteridaceae) from highly threatened habitats (Tan 2012), after which several young individuals were successfully produced. *In vitro* propagation using gametophytes and sporophytes has been proven effective for *ex situ* conservation (Ballesteros & Pence 2018). Then, the young, new individuals can be replanted back in their original habitat to prevent extinction.

In conclusion, this study highlights the distributional range and conservation status of *Pronephrium s.l.* ferns that had not yet been assessed in Peninsular Malaysia. In this study we assessed two taxa in the threatened category, two as Near Threatened and four as Least Concern. The current conservation status of each taxon reported in this study are provided with essential information for setting up priorities for further conservation efforts.

Conversion of forests to other land uses in the lowlands and habitat disturbance in limestone and montane areas of Peninsular Malaysia have an adverse effect on most of the *Pronephrium s.l.* species. On the other hand, the effectiveness of *in situ* conservation in protecting the species is proven by the rediscovery of many of the species from the protected areas. In a nutshell, a combination of both *in situ* and *ex situ* conservation is recommended here to ensure the species' continued survival. At the same time, sustainable development that considers the importance of biodiversity is highly recommended.

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