## Asplenium alleniae (Aspleniaceae), a new fern species from Borneo and New Guinea

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#### Key words

conservation Malaysia Malesia Mount Kinabalu Papua New Guinea Sabah taxonomy

Abstract A new species, Asplenium alleniae, is described from high elevation habitats in Sabah (Malaysia) and Papua New Guinea. Previous phylogenetic analyses of chloroplast loci determined that A. alleniae was most closely related to A. pauperequitum from New Zealand. Asplenium alleniae differs from A. pauperequitum most obviously by the acuminate apices of its longer pinnae. The combination of pinnate fronds with few pairs of primary pinnae and dark red-brown axes distinguishes A. alleniae from superficially similar species of Asplenium in Malesia. Asplenium alleniae is provisionally assessed as Endangered.

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#### INTRODUCTION

Asplenium L. is one of the largest fern genera, with over 700 species (Ohlsen et al. 2014a, Xu et al. 2020). Its species are morphologically and ecologically diverse, with fronds varying from undivided, such as the birds' nest ferns, to deeply divided (4-pinnate-pinnatifid), and plants that occur terrestrially, rupestrally, or epiphytically. Nevertheless, species of Asplenium can generally be recognised by their clathrate scales, and sori that are usually elongate along veins and protected by elongated indusia, and often away from the margins (Brownsey & Perrie 2018); the sori often have a 'herringbone' appearance, at least in larger species. In a global phylogeny of the genus compiled by Xu et al. (2020), an unidentified collection from Malaysia (Chen Wade 4599) was shown to be the closest known relative of A. pauperequitum Brownsey & P.J.Jacks. Asplenium pauperequitum is endemic to New Zealand, where it has a remarkably disjunct distribution, occurring on the Poor Knights Islands off the northern coast of the North Island and c. 1200 km away to the southeast on the Chatham Islands (Brownsey & Jackson 1984, Cameron et al. 2006, Brownsey & Perrie 2018). Asplenium pauperequitum is only distantly related to other New Zealand Asplenium species, and its closest relative had been thought to be the Australasian Asplenium flabellifolium Cav. (Ohlsen et al. 2014a, 2020).

The collection Chen Wade 4599 originated from Mount Kinabalu, Sabah, Malaysia, on the island of Borneo. Among the Asplenium species from Mount Kinabalu listed by Parris et al. (1992) was an undescribed species, A. allenae Parris ined. (see also Parris & Latiff 1997, Said 2005). The collections cited

by Parris et al. (1992) matched the morphology of Chen Wade 4599. Here, we name this species as A. alleniae Parris ex Parris & Perrie. We provide a morphological description, distinguish it from related or superficially similar species, map its known distribution, and suggest a conservation status.

#### **MATERIAL AND METHODS**

Collections of A. alleniae in CANB, K, and TAIF were seen by us in person, as were collections of A. pauperequitum in AK, CHR, and WELT. Duplicates and additional collections of A. alleniae were sought in AK, B, L, LAE, SING, and US via their respective online catalogues (accessed May 2020). Collections seen by us only as online images are indicated with \*. Listed duplicates not located by us are indicated with ^. Herbarium abbreviations follow Thiers (2020).

A morphological description for A. alleniae was prepared in the format of Brownsey & Perrie (2018). The mean exine length and width for the TAIF specimen of Chen Wade 4599 was determined by measuring 30 spores.

A distribution map was generated using QGIS 3.10.2 (QGIS 2020) with layers from Koordinates and Viewfinder Panorams (www.koordinates.com, www.viewfinderpanoramas.org/dem3. html; accessed 14 May 2020). For assessment against the IUCN Red list criteria (IUCN 2012), Extent of Occurrence and Area of Occupancy (with a cell width of the default 2 km) were calculated using GeoCAT (geocat.kew.org, accessed 14 May 2020; Bachman et al. 2011).

The DNA sequences for the chloroplast loci rbcL, rps4-trnS, and trnL-trnF for samples of A. alleniae, A. flabellifolium, and A. pauperequitum from Ohlsen et al. (2014a) and Xu et al. (2020) were downloaded from GenBank, and compared by aligning them with Clustal X v. 2.1 (Larkin et al. 2007). Sample details, including GenBank numbers, are given in the Appendix.

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#### **RESULTS**

A morphological description for *A. alleniae* is given in the Taxonomy section below, and the diagnosis indicates how *A. alleniae* differs from *A. pauperequitum*. The former is distinguished in the Discussion from Malesian species of *Asplenium* that have superficially similar morphology. Figure 1 shows the holotype of *A. alleniae*, along with the lamina, sori, rhizome scales, and spores of *Chen Wade 4599*. Based on its morphological similarity, we include a collection – *Croft 713* – from New Guinea's Mount Giluwe in our circumscription of *A. alleniae* (see Discussion). Map 1 is a distribution map of *A. alleniae* and *A. pauperequitum*. GeoCAT calculated for *A. alleniae* an Area of Occupancy of 20 km² and an Extent of Occurrence of 33 839 km². There is some uncertainty with these values, particularly the Area of Occupancy, because of imprecise localities for some of the collections.

The analysed DNA sequences of *A. alleniae* and *A. pauperequitum* differed from one another by 11 substitutions. Polarising these using *A. flabellifolium* as an outgroup, five were autapomorphies for *A. alleniae* and six for *A. pauperequitum*. Two autapomorphic insertion/deletion events further distinguished *A. alleniae*. The sample of *A. flabellifolium* differed by 48 substitutions and two insertion/deletion events.

#### **DISCUSSION**

Asplenium alleniae is described as a new species because of its morphological and genetic distinctiveness, especially from A. pauperequitum, its closest known relative. The spore exine of A. alleniae (c. 40 by 27  $\mu$ m) appears substantially smaller than that of the octoploid A. pauperequitum (49–54 by 34–38  $\mu$ m; Brownsey & Perrie 2018). Given the correlation of spore size with ploidy (Barrington et al. 1986), A. alleniae may have a lower ploidy, but this needs confirmation. To compare the perispores of A. alleniae shown in Fig. 1 with those of A. pauperequitum, see Brownsey & Jackson (1984) and Large & Braggins (1991).

In the phylogeny of Xu et al. (2020), A. alleniae was nested among the diversity of A. pauperequitum. However, we believe this was because there are only partial sequences for the AK 290307 'cellophane' sample of A. pauperequitum, and, within these reduced data, it has the plesiomorphic state at three characters that are autapomorphic in other samples of A. pauperequitum, including another sample from the same sheet (Cameron et al. 2006). Although this sequence is slightly anomalous, the plant's morphology is entirely consistent with A. pauperequitum, and it notably shares no synapomorphies with A. alleniae. The 11 substitution differences between the samples, for which we have complete sequences for the three loci, is consistent with species-level distinctiveness for the majority of Asplenium species (e.g., Ohlsen et al. 2014a, Xu et al. 2020). Within the sampling of Asplenium by Xu et al. (2020), A. alleniae and A. pauperequitum belong to one of the 11 major clades that otherwise includes only the morphologically-distinct A. flabellifolium, and the southern South American A. dareoides Desv. (Moore 1983), which is also noticeably different in having 3-pinnate fronds.

Asplenium pauperequitum is restricted to more-or-less coastal habitats, often associated with seabirds, on offshore islands of temperate New Zealand (Cameron et al. 2006, Brownsey & Perrie 2018). Perrie & Brownsey (2005) noted its isolated phylogenetic position and speculated that a species more closely related than A. flabellifolium might be found in the Pacific. That did not eventuate with further sampling of Asplenium from the Pacific (Ohlsen et al. 2014a). Instead, the analyses of Xu et al.

(2020) linked *A. pauperequitum* to what is here named *A. alleniae*, a species from the highlands (3160–3500 m elevation) of tropical Malesia. This markedly disjunct connection (Map 1) between temperate Australasia and the mountains of Malesia, with an intervening absence in tropical Australia and the Pacific Islands, is replicated somewhat by each of the ferns *Blechnum fraseri* (A.Cunn.) Luerss. and *B. fluviatile* (R.Br.) E.J.Lowe ex Salomon and the lycophytes *Huperzia australiana* (Herter) Holub and *Lycopodium scariosum* G.Forst.; however, all four are more widespread in both Malesia and New Zealand, with *B. fraseri* also in Taiwan and the other three also in southeastern Australia (Parris et al. 1992, Chambers & Farrant 2001, Brownsey & Perrie 2020).

The disjunct distribution in Malesia of *A. alleniae* is shared by another fern, *Oreogrammitis clemensiae* Copel., in similar habitats. It is known from Mount Kinabalu (type locality) and seven peaks in Papua New Guinea at elevations of 3400–4450 m, in upper montane and subalpine forest and above the treeline (Parris 1983).

Several other *Asplenium* species have recently been described for Malesia (e.g., Salgado 2004, Dong et al. 2012, Jaman et al. 2017). While Van Alderwerelt van Rosenburgh (1909, 1917) listed 94 *Asplenium* species for 'Malaya' (equivalent to Malesia), the total number of *Asplenium* species in Malesia is probably around 130, based on recent publications, past treatments and checklists (e.g., Copeland 1960, Holttum 1968, Salgado 1990, Parris & Latiff 1997, Ohlsen in Cámara-Leret et al. 2020), and putatively new or unrecognised species. By comparison, Lin & Viane (2013) listed 90 species for China. A revised treatment of *Aspleniaceae* for Malesia is currently being prepared (D.J. Ohlsen).

Of the many Asplenium species in Malesia, only a few are even superficially similar to A. alleniae. Members of the Asplenium polyodon G.Forst. group that are pinnate with tapering pinnae, such as A. falcatum Lam. and A. listeri C.Chr., resemble A. alleniae but can be distinguished by having more than four pairs of pinnae, sharply serrate or doubly serrate margins, and narrowly-triangular or lanceolate scales on the rachis (Salgado & Fraser-Jenkins 2013, Ohlsen et al. 2014b). Asplenium salignum Blume has larger fronds with green rachises and pinnae with caudate apices. It can alternatively have simple fronds, and grows at low to medium elevations (Holttum 1968, Lindsay & Middleton 2020; e.g., Indonesia, Blume s.n. date unknown, US 00135115\*). Asplenium diversifolium Blume (= A. horizontale Baker) is morphologically similar to A. salignum but the primary pinnae are sessile (e.g., Indonesia, Hancock 59, date unknown, US 00135091\*). Asplenium finlaysonianum Wall. ex Hook. has fronds with an enlarged terminal segment that is often trifid, green rachises, comparatively broader and more elliptic primary pinnae, and longer sori (e.g., Malaysia, collector unknown, 1822, K 000639701\*; Nepal, Fraser-Jenkins 1798 & Chhetri, 27 Dec. 1997, US 01514899\*).

The single collection of *A. alleniae* from Mount Giluwe (Papua New Guinea) has smaller fronds, which may be more membranous, compared with most of the collections from Mount Kinabalu. Determining whether this is representative of morphological differences between the populations requires more collections from New Guinea. In the meantime, we believe the Mount Giluwe collection is best included within the circumscription of *A. alleniae*, based on overall morphological similarity, rather than being left unplaced or described as a separate taxon. Nevertheless, it will be desirable to investigate the relationships with DNA analyses between the populations on Mount Kinabalu and Mount Giluwe when additional collections are made from the latter.

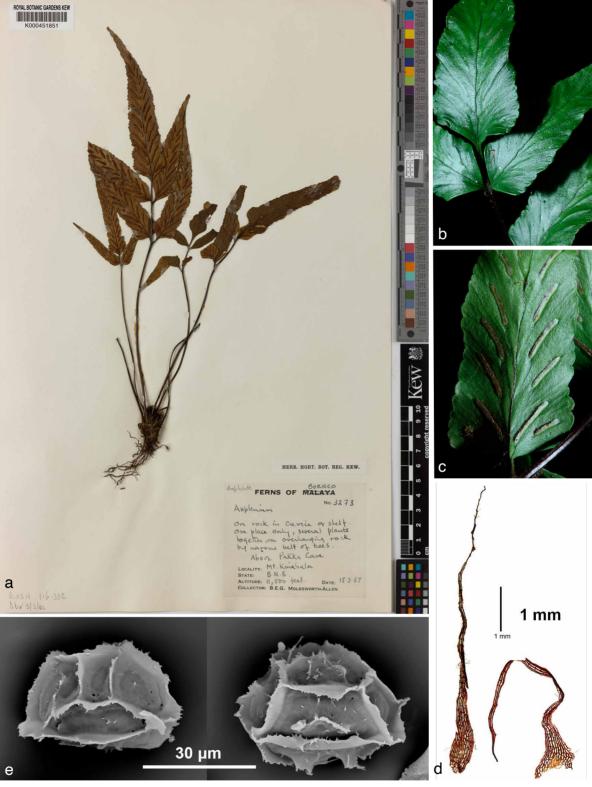


Fig. 1 Asplenium alleniae Parris ex Parris & Perrie. a. Holotype; b. adaxial lamina; c. sori; d. rhizome scales; e. SEM of spores (a. *Molesworth-Allen 3273* (K 000451851); b–e. *Chen Wade 4599*). — Holotype image from K; other images by Cheng-Wei Chen.

### **TAXONOMY**

# Asplenium alleniae Parris ex Parris & Perrie, sp. nov. — Fig. 1; Map 1

Asplenium alleniae resembles A. pauperequitum in having ovate or triangular, glossy, thin, brittle and coriaceous fronds with few pinnae, dark red-brown axes, and stipes longer than, or as long as, the laminae. However, A. alleniae differs most obviously by the acuminate apices of its longer primary pinnae that are up to 105 mm long; those of A. pauperequitum are obtuse or rounded, and up to 37 mm long. Additionally, A. alleniae has larger, 1-pinnate fronds,

with laminae up to 175 mm long and 165 mm wide, and 1–3 (rarely 4) pairs of primary pinnae, whereas *A. pauperequitum* has 1- or 2-pinnate fronds, laminae up to 100 by 75 mm, and 1–5 pairs of primary pinnae. The spores of *A. alleniae* have smaller exines, and winged and ridged perispores; those of *A. pauperequitum* have larger exines, and perispores with short reticulate projections. *Asplenium alleniae* grows at elevations of 3100–3500 m in the tropics, while *A. pauperequitum* occurs below 200 m elevation on temperate offshore islands. — Type: *B.E.G. Molesworth-Allen 3273* (holo K000451851), Malaysia, Mount Kinabalu, above Pakka Cave, 11 500 feet (3500 m) elevation, 18 Feb. 1957.

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Etymology. Named for Betty E.G. Molesworth-Allen (1913–2002), who made the earliest collection of Asplenium alleniae known to us. She was a New Zealand-born pteridologist who collected intensively in Malaysia between 1948 and 1963. We follow Article 60.8b of Turland et al. (2018) in using the iae termination.

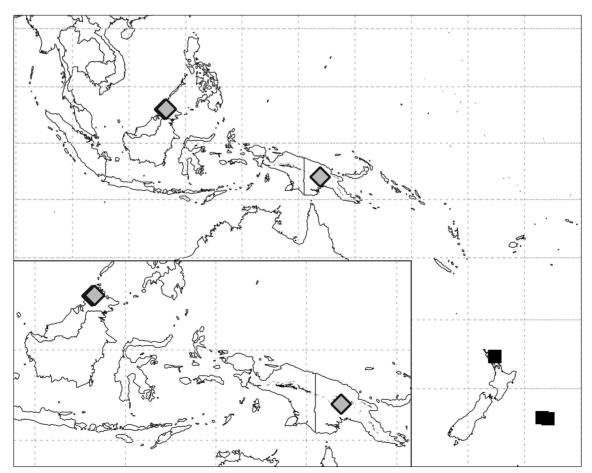
Rupestral or terrestrial ferns. Rhizomes short, erect, bearing scales. Rhizome scales acicular or narrowly ovate, with long filiform apices, 3-6.5 by 0.3-0.8 mm, dark brown, clathrate. Fronds 105-390 mm long. Stipes 65-300 mm long, dark redbrown, shiny, scaly. Rachises dark red-brown, scaly; scales hair-like, occasionally with a small triangular base, to c. 1 mm long. Laminae 1-pinnate, ovate or triangular, 45-175 by 45-165 mm, glossy dark green adaxially, paler abaxially, thin, brittle, coriaceous, ± glabrous. Primary pinnae in 1-3 (rarely 4) pairs below conformal or rarely 2- or 3-partite apical segment, not or only slightly overlapping, lanceolate or narrowly ovate; the longest at the base, 25-105 by 9-21 mm; pinna apices acuminate, margins irregularly crenate, bases stalked. Sori away from margins; indusia 4-13 mm long, curved away from midribs; free margins of indusia entire. Spore exine c. 40 by 27 µm; perispores winged and ridged.

Distribution — *Asplenium alleniae* is known only from Mount Kinabalu, Sabah, Malaysia, on the island of Borneo, where there have been multiple collections, and one collection from Papua New Guinea's volcanic Mount Giluwe (Map 1).

Habitat & Ecology — Found in upper montane forest (on Mount Kinabalu, comprising *Dacrydium gibbsiae* Stapf (*Podocarpaceae*), *Leptospermum recurvum* Hook.f. (*Myrtaceae*), and *Rhododendron* L. (*Ericaceae*)), often within rock crevices, small caves, overhangs and outcrops that are often dry but occasionally damp, or occasionally in tree boles. On granite at Mount Kinabalu (Borneo) and volcanic substrates at Mount Giluwe (New Guinea). Elevation: 3160–3500 m.

Conservation — We provisionally assess A. alleniae as Endangered via pathway B2ab(iii) of IUCN (2012). Asplenium alleniae is known from only two locations. Mount Kinabalu and Mount Giluwe, with its estimated Area of Occupancy of 20 km<sup>2</sup> well under the threshold of 500 km<sup>2</sup> for this pathway. On Mount Kinabalu its sites are at least 5 km apart, but the only comments about population size are 'Locally common' on one collection, and 'several plants' on another. On Mount Giluwe it has been collected just once. It was not seen by one of us (BSP) during a separate trip to a nearby site on Mount Giluwe. It was also not seen during trips by BSP to high elevation sites on Mounts Hagen and Wilhelm in Papua New Guinea. Similarly, Jim Croft, who made the Mount Giluwe collection, seemingly did not collect it during trips to other mountains in Papua New Guinea, such as the Star Mountains, Mount Saruwaged, Victoria Range, and Mount Albert Edward (based on specimens in CANB). It is not among collections made on Mount Jaya in Indonesian New Guinea (Johns et al. 2006). Consequently, and with areas above 3000 m in Malesia being very limited (Map 1), particularly outside New Guinea, we believe the few known sites reflect genuine scarcity. Moreover, with species confined to tropical mountains considered particularly susceptible to global warming (La Sorte & Jetz 2010, Costion et al. 2015), we infer a likely decline in the extent and quality of habitat available for A. al-

Specimens examined (Paratypes). Malaysia, Borneo, Sabah, Mount Kinabalu, southern slope, crest between eastern tributary of Sungai Mesilau and uppermost part of Sungai Mentaki, 3160 m, 4 Sept. 1963, S.H. Collenette 21525 (L 3526087\*, US 01517375\*); Mount Kinabalu, Upper Mesilau Valley, 3480 m, 30 July 1978, J.M.B Smith 535 (L 3526066\*); Mount Kinabalu, summit track, 3270 m, 25 Oct. 1980, B.S. Parris 8743 & J.P. Croxall (K 000451852, K 000451853, SNP); Mount Kinabalu, summit trail, 3300 m, 20 May 1988, B.S. Parris 11587 (K 000451877, SNP); Mount Kinabalu, along main climbing trail from Laban Rata to Low's Peak, 3400 m, 23 Dec. 1997, M. Kato,



Map 1 Distribution map of Asplenium alleniae (♠) and its closest relative, A. pauperequitum (■). The few areas of land above 3000 m elevation are indicated in black (mostly in New Guinea). The grid is 10 degrees of latitude and longitude.

H. Okada, R. Imaichi, M. Sugiura & N. Takamoto 1499 (L 3526073\*, TI^); Mount Kinabalu, summit trail, 3259 m, 4 Mar. 2016, C.-W. Chen Wade 4599 et al. (SAN, SNP, TAIF 500088, UC 2071133). – PAPUA NEW GUINEA, northwest slopes of Mount Giluwe, above Kagoba, 3500 m, 21 Jan. 1979, J.R. Croft 713 (CANB 8903663, L 3525875\*, K^, LAE^, NSW^).

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**Appendix** Collection details for *Asplenium* samples whose DNA sequences were compared. GenBank numbers are in the order: *rbc*L, *rps4-tmS*, and *tmL-tmF*.

- A. alleniae: C.-W. Chen Wade 4599 et al. (SAN, SNP, TAIF 500088, UC 2071133), Malaysia, Mount Kinabalu, summit trail, 4 Mar. 2016; MK826977, MK827488, MK827976.
- A. flabellifolium: D.J. Ohlsen 185 (MELU P105747), Australia, Tasmania, Snug Falls, 5 Jan. 2010; KP774850, KP835460, KP851902.
- A. pauperequitum: L. Forester s.n. (WELT P020513), New Zealand, Poor Knights Islands, Tawhiti Rahi Island, 23 Jan. 2003; AY283233, KP888648, KP888649.