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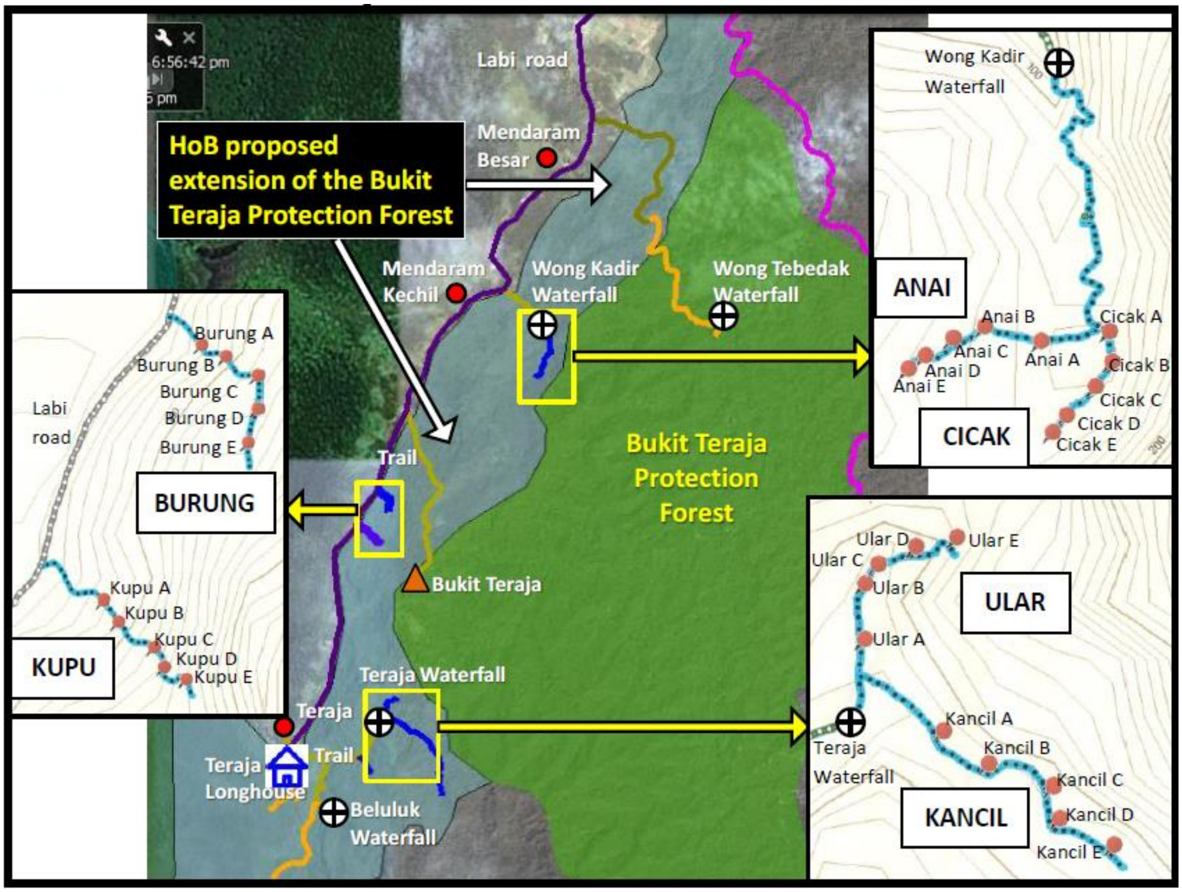

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Cover photo: Map of the Bukit Teraja Protection Forest (green) and its proposed extension (blue).
(Courtesy: Hanyrol H. Ahmad Sah and T. Ulmar Grafe).

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Effects of *Acacia* invasion on leaf litter nutrient and soil properties of coastal Kerangas forests in Brunei Darussalam

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Abstract

Exotic *Acacia* were introduced to Brunei Darussalam in the 1990s for plantation forestry and land rehabilitation but are now regarded as invasive. We assessed the effects of *Acacia* on litter nutrient composition and soil physicochemical properties of Brunei's coastal Kerangas (heath) forests. Soil and litter samples were collected from ten 20 x 20 m plots in *Acacia*-invaded Kerangas forests (IKF) and ten uninvaded (UKF) plots. Soil samples were analyzed for pH, gravimetric water content and nutrient concentrations whereas litter samples were analyzed for total nutrients only. We recorded significantly higher concentrations of litter total N and P in the IKF than the UKF plots. In contrast, no significant differences were detected in soil properties, except for topsoil available P and subsoil exchangeable Ca which were both lower in the IKF plots. A significant positive correlation was detected between litter N and topsoil N in the IKF plots. We suggest that the fairly recent timescale of *Acacia* invasion (< 25 years) of the IKF sites resulted in the lack of significant increase of soil nutrients. In conclusion, *Acacia* invasion into Brunei's forests can potentially alter both leaf litter and soil physicochemical properties of Kerangas forests, in particular affecting nutrient availability. This alteration of ecosystem may further enhance the invasion success of *Acacia*, making restoration attempts more challenging.

Index Terms: alien invasive plants, heath forests, *Acacia mangium*, *Acacia auriculiformis*, nutrient cycling, ecosystem services

1. Introduction

Invasive plant species are a well-known threat to the biodiversity, services and functions of natural ecosystems.[1-3] The negative ecological effects of invasive plants are often irreversible and once established, invasives are typically difficult to control and eradicate.[4] One crucial factor for successful plant invasion is the ability of invasive plants to alter soil physicochemical properties and litter input, and modify decomposition rates and nutrient fluxes.[5-8] Such alterations often promote further spread of the invasive plant and competitive reduction of native species, resulting in changes in community structure over the long term,[9] and possibly even local extinction of native species.[2]

Among invasive plant species, *Acacia* species are widely regarded as one of the most successful alien species to have invaded many areas worldwide.[10-13] Although their centre of diversity and distribution is in Australia,[14,15] *Acacia* have been successfully planted in varying climatic conditions globally for commercial supply of tree products, as well as for land rehabilitation.[16,17] Similarly, *Acacia* were introduced to Brunei Darussalam, northwest Borneo in the 1990s to increase timber productivity and as roadside plantings,[18] but has since spread into disturbed forest habitats, in particular the coastal heath forests.[19-21]

As a nitrogen-fixing legume, *Acacia* are known to alter nutrient cycles[22,23] and modify litter and soil physicochemical properties.[24-26] Several studies in South African fynbos, Portugal coastal dunes, China and India have recorded three- to four-fold increases in litter mass in *Acacia*-invaded habitats compared to non-invaded ones,[25,27] and double the average N concentration in leaf litter and soil of invaded ecosystems.[11,28,29] The higher litter mass in *Acacia*-invaded habitats appear to enable more nutrients to be released during litter decomposition, leading to nutrient enrichment and eventually changing the nutrient quantity in invaded ecosystems.[24,27,30-32]

The detrimental effects of *Acacia* may be particularly significant in nutrient-poor tropical ecosystems, such as heath forests. Tropical heath (Kerangas) forests are rare in Brunei Darussalam[19,33] and their distribution throughout Borneo is now highly threatened by land conversion and habitat fragmentation.[33] With repeated fire incidences in coastal heath forests[21,33,34] coupled with the nitrogen-fixing capabilities of *Acacia*, there is increasing concern that *Acacia* invasion may modify the physicochemical properties of soils in invaded habitats.[35-37]

We investigated the effects of *Acacia* invasion on the soil physicochemical properties and litter nutrients in disturbed coastal Kerangas (heath) forests (KF) in Brunei Darussalam. Two research questions were formulated: (1) Does *Acacia* invasion alter the nutrient concentrations of leaf litter in coastal Kerangas forests? and (2) Does *Acacia* invasion alter soil physicochemical properties in coastal Kerangas forests?

2. Experimental approach

2.1 Study site

The study was conducted in coastal Kerangas forests surrounding Universiti Brunei Darussalam (4°58'30.37"N, 114°53'37.93"E), Brunei-Muara District. Ten plots (20 x 20 m) were established in *Acacia*-invaded Kerangas forests, located within a 200 ha area that was affected by forest fires in 2009 (henceforth referred to as invaded Kerangas Forest, IKF). Ten additional 20 x 20 m plots were established randomly in intact coastal, remnant Kerangas forests that have not experienced forest fires since 2009, referred to as uninvaded Kerangas forest (UKF) plots. Pairs of UKF plots were spaced along the coastline as a representative sample of the surrounding forest composition. Plots were set up at a minimum distance of 300 m apart from each other. UKF plots were located at distances of 300 m to 22 km from IKF plots, and reached 5 km inland (see ***Figure 1***). Within the 10 IKF plots, 1588 trees of ≥ 1 cm dbh were recorded, most of which were *Acacia* trees. In contrast, within the 10 UKF plots, 1286 trees were recorded, most of which were native heath tree species, in particular *Buchanania sessifolia*, and *Ixonathis reticulata* (RS Sukri, unpublished data).

2.2 Collection of litter and soil samples

Leaf litter and soil samples were collected from the IKF and UKF plots in early December 2013 to mid-February 2014, during the typical wet season for Brunei Darussalam.[38] Within each plot, leaf litter were collected using 0.25 m^2 quadrats at four points at the midpoint along the diagonals from the plot centre, and bulked to give one sample per plot. Similarly, soil samples at topsoil (0 – 15 cm) and subsoil (30 – 50 cm) depths were collected using a soil auger at four random sampling points per plot and bulked, giving a total of 20 topsoil samples and 20 subsoil samples.

2.3 Litter and soil analyses

Fresh soils were subsampled to determine soil pH and gravimetric water content (GWC).[39] Fresh soils were mixed with distilled water in a 2:1 water-to-soil ratio and pH measured using a benchtop pH meter (Hanna instruments Ltd, UK). For GWC, a 10g sample of fresh soil was oven-dried for 24 hours at 105^{o}C until a constant weight was obtained, weighed and GWC determined.[39] Soil organic matter was measured using a muffle furnace (Gallenkamp Size 2, Apeldoorn, Netherlands) set at 550°C for two hours.[39] The remaining fresh soil samples were air-dried at room temperature for two months. Air-dried samples were sieved through a 2.0 mm sieve, ground using a pestle and mortar and 200 g of the processed soil samples were further ground using a ball mill (Retch mixer mill mm 400, Germany) to obtain fine soil samples for nutrient and OM analyses. Three replicates from each soil depth (topsoil and subsoil) per plot were sub-sampled randomly for this purpose.

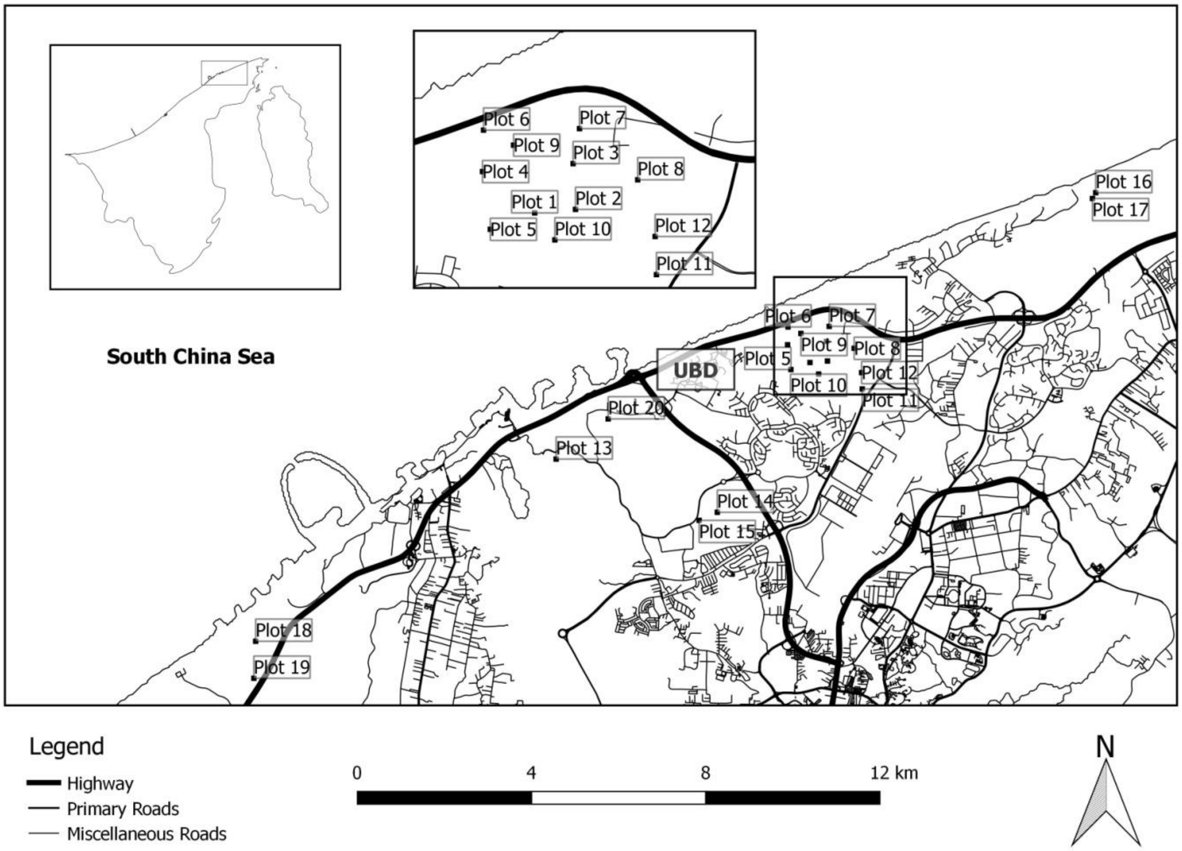

Figure 1. Map of study sites selected in Coastal Kerangas Forest, close to Universiti Brunei Darussalam (4°58'30.37"N, 114°53'37.93"E), Brunei Muara District. Ten 20 x 20 m plots were established within locations of disturbance caused by fire in 2009 (Plots 1- 10), and ten 20 x 20 m plots were established randomly within intact coastal Kerangas forests that have not experienced forest fires since 2009 (Plots 11-20).

Leaf litter samples collected were wiped clean using 70% ethanol to remove mineral soil and oven-dried at 60°C for 2-3 days to constant mass.[39] A total of 50 g litter samples per plot were subsampled and ground using a ball mill (Retch mixer mill mm 400, Germany) to fine powder. Three replicates of litter samples from each plot were used for nutrient analysis.

Ground soil samples were analyzed for the concentrations of total N, P, Mg, K, exchangeable Mg, Ca, K and available P, while ground leaf litter samples were analyzed for total N, P, K, Mg and Ca concentrations. Total N and P concentrations were determined using the Kjeldahl method by digesting each soil sample in concentrated sulphuric acid, and analyzed using a Flow Injector Analyser (FIAstar 5000, Hoganas, Sweden). For analysis of total Mg and K concentrations, air-dried soil samples were acid-digested using a microwave digestor (Multiwave 3000 Anton Paar, Austria) following Allen *et al*.[39] We attempted to analyse for total Ca in soil but the total Ca concentration levels were below the instrument's detection limit and thus were not included in the final data analysis. Concentrations of soil exchangeable Ca, Mg and K were extracted using 1 N neutral ammonium acetate.[40] Total and exchangeable Mg, Ca and K concentrations were measured using a Flame

Atomic Absorption Spectrophotometer (AAS; Thermo Scientific iCE 3300, Sydney, Australia). Soil available P concentrations were extracted using Bray's solution (0.03 N ammonium fluoride in 0.025 N HCl) and mixed with ascorbic acid and molybdate reagent.[39] The absorbance of each solution was read at 880 nm wavelength using UV-spectrophotometer (UV-1800, Shimadzu, Kyoto, Japan).

2.4 Statistical analysis

All statistical analyses were conducted using R 3.2.2 software.[41] Differences in litter nutrient properties and soil physicochemical properties between the IKF and UKF plots (n = 10 samples each) were determined using t-tests. Assumptions of normality and equality of variances were checked, and where necessary, response variables were $\log_{10}$ transformed. For the concentrations of Total K, the non-parametric Mann-Whitney U test was used. GWC and OM values were arc-sine transformed prior to the t-tests. Simple linear regressions were also used to assess the relationships of total N and total P in the topsoil with litter N and litter P, respectively.

3. Results

3.1 Differences in leaf litter properties between Acacia invaded Kerangas forest (IKF) plots and uninvaded Kerangas forest (UKF) plots.

Leaf litter from the IKF plots was significantly higher in total dry mass, as well as in total N and P concentrations, than litter from the UKF plots ($p < 0.05$; see ***Table 1***). In contrast, total Ca concentrations was significantly higher in litter from the UKF plots than the IKF plots ($p < 0.05$; see ***Table 1***), but total K and total Mg concentrations did not differ.

3.2 Differences in soil physicochemical properties between Acacia invaded Kerangas forest (IKF) plot and uninvaded Kerangas forest (UKF) plots

Available P concentrations in topsoil were significantly lower in the IKF plots than in the UKF plots ($p < 0.001$; see ***Table 1***). However, no significant differences in available P concentrations in subsoils were detected ($p > 0.05$) between the UKF and IKF plots. Concentrations of exchangeable Ca in subsoil were significantly higher in the UKF than the IKF plots ($p < 0.01$; see ***Table 1***), but topsoil exchangeable Ca concentrations were not significantly different ($p > 0.05$). There were no significant differences in the concentrations of other soil nutrients (Total N, P, Ca, Mg, K and exchangeable Mg and K) either in topsoil or subsoil samples from the UKF and IKF plots. Similarly, no significant differences were detected in GWC, OM content and pH for topsoil and subsoil in the IKF and UKF plots (see ***Table 1***).

3.3 Relationships between leaf litter nutrient (N and P) and topsoil nutrient properties

Litter N concentrations were highly significantly and positively related to topsoil N concentrations in the IKF plots ($R^2 = 0.41$ $p = 0.001$**; see ***Figure 2***). However, there were no significant linear relationships between the litter N and topsoil N concentrations in the UKF plots, and no significant litter P-topsoil P relationships were detected in either the IKF or UKF plots ($p > 0.05$; see ***Figure 2***).

4. Discussion

4.1 The effects of Acacia invasion on leaf litter and soil physicochemical properties

Leaf litter dry mass at the invaded Kerangas forest (IKF) plots were significantly higher than at the uninvaded Kerangas forest (UKF) plots. This is consistent with the findings of similar studies conducted in South African fynbos, coastal dunes in Portugal, China and India where litter mass values were three to four times greater in *Acacia*-invaded areas compared to non-invaded areas.[25,27-29,42,43] Higher litter mass indicates higher litterfall production in the IKF plots, and this can potentially lead to nutrient enrichment as more nutrients may be released during litter decomposition.[24,27,30-32,44] Indeed, we recorded significantly higher litter N and P concentrations from the IKF plots (see ***Table 1***). Moreover, as a nitrogen fixing species, *Acacia* produce phyllodes with higher N content than native tropical trees.[45]

Table 1. Summary of litter properties and soil physicochemical properties for top soil (0-15cm depth) and subsoil (15-30cm) depth of Invaded Kerangas forest (IKF) and uninvaded Kerangas forest (UKF) in Brunei. Litter and soil nutrients are expressed as mg g^{-1}, Gravimetric water content (GWC) and Organic matter (OM) in %. Significant differences were detected at α = 0.05 level. (ns - not significant; *p < 0.05; **p < 0.01;***p < 0.001).

Component	Variables	Habitat		p value
		IKF	UKF	
Litter	Total N	10.07 ± 1.16	7.91 ± 0.74	*
	Total P	1.57 ± 0.08	1.16 ± 0.12	**
	Total K	0.58 ± 0.09	0.57 ± 0.08	ns
	Total Ca	1.76 ± 0.19	2.96 ± 0.44	**
	Total Mg	1.17 ± 0.18	1.13 ± 0.15	ns
Topsoil	Total N	0.87 ± 0.10	1.05 ± 0.13	ns
	Total P	0.03 ± 0.003	0.04 ± 0.01	ns
	Total K	1.11 ± 0.30	1.39 ± 0.42	ns
	Total Mg	0.13 ± 0.02	0.26 ± 0.09	ns
	Exchangeable K	0.07 ± 0.01	0.09 ± 0.02	ns
	Exchangeable Ca	0.02 ± 0.002	0.02 ± 0.003	ns
	Exchangeable Mg	0.02 ± 0.003	0.07 ± 0.04	ns
	Available P	0.18 ± 0.003	0.22 ± 0.005	**
	GWC	31.9 ± 2.81	35.49 ± 3.54	ns
	pH	4.59 ± 0.04	4.51 ± 0.06	ns
	OM	4.87 ± 0.55	5.38 ± 0.51	ns
Subsoil	Total N	0.27 ± 0.06	0.37 ± 0.11	ns
	Total P	0.04 ± 0.01	0.04 ± 0.01	ns
	Total K	1.23 ± 0.36	1.83 ± 0.51	ns
	Total Mg	0.14 ± 0.03	0.46 ± 0.23	ns
	Exchangeable K	0.05 ± 0.01	0.06 ± 0.02	ns
	Exchangeable Ca	0.02 ± 0.002	0.03 ± 0.002	**
	Exchangeable Mg	0.01 ± 0.002	0.08 ± 0.07	ns
	Available P	0.22 ± 0.004	0.23 ± 0.004	ns
	GWC	21.15 ± 3.69	20.38 ± 2.92	ns
	pH	4.87 ± 0.06	4.70 ± 0.10	ns
	OM	2.83 ± 0.26	2.95 ± 0.41	ns

Despite the higher N concentrations in the IKF leaf litter, we did not detect a significant increase in soil N concentrations in the IKF plots. This is contradictory to the results of other *Acacia* invasion studies that have all detected increased soil N concentrations under *Acacia* invasion.[25,27,42,46] In Brunei Darussalam, Matali and Metali[47] recorded significantly higher total N concentrations and lower GWC, total Ca, K and exchangeable Ca concentrations in *Acacia* plantation soils than in nearby intact Kerangas forest soils. In contrast, we did not detect significant changes in soil physicochemical properties in our IKF plots, except for lower available P and exchangeable Ca concentrations in the IKF plots.

We suggest that our findings may be partly a reflection of the invasion time scale in these coastal KF habitats. *Acacia* invasion may be regarded as a fairly recent event in Brunei's coastal KF landscape, as the start of invasion is thought to have occurred in the 1990s.[18] The effects of *Acacia* on soil nutrient properties may take time to be significantly effective and can become more profound after a longer period of invasion.[29,48] Concentrations of C and N in *Acacia longifolia*-invaded soils in Portuguese coastal dune systems were higher in areas long invaded (>20 years) than in recently invaded areas (>10 years).[29] Similarly, *Acacia saligna* invasion in South African fynbos appeared to alter N-cycling regimes in poor nutrient soils through long term invasion of well over three decades.[25]

Further, our IKF plots were established within coastal Kerangas habitats which experienced forest fires in 2009. As fires are known to cause volatilization of many soil nutrients including N,[29,49,50] there would have been considerable loss of nutrients during those fire events. Similarly, lower C and N pools were detected in a burned plot in a Mediterranean coastal dune ecosystem, reflecting nutrient loss from the initial fire event coupled with repeated fire occurrence.[29] In that dune ecosystem, both C and N pools have since increased as a result of *A. longifolia* invasion.[29] Thus, *Acacia* invading these burnt Kerangas habitats would need time to recover from the initial and substantial loss of soil nutrients. We suggest *Acacia* invasion into burnt Kerangas habitats[19] would eventually increase soil N concentrations in our IKF plots more than that in the UKF plots.

It is also possible that the lack of increased soil nutrients in the IKF plots may be partly due to the allelopathic ability of *Acacia*,[51,52] which is known to affect decomposition processes and slow down the release of nutrients from decomposed leaf litter into soils. The lack of a significant increase in soil N pools by litter of *Acacia dealbata* in the Iberian Peninsula, Portugal was attributed to the presence of secondary compounds in *A. dealbata* litter that inhibit microbial activity.[53] Moreover, rapid mobilization of nutrients to plant biomass and leaching of nutrients in sandy Kerangas habitats may also lead to further lowering of soil nutrient concentrations.[45]

It should be highlighted that the distance between the IKF and UKF plots differed, as the IKF plots were closer together compared to the UKF plots which were more spread out along the coastline. However, we suggest that any distance effects would be minimal as the whole of this coastal landscape has the same underlying sandy heath soils.[21]

4.2 The relationship between litter and soil nutrient concentrations

We detected a highly significant positive correlation between litter N and topsoil N concentrations in the IKF plots. This finding highlights the role of litter input in the availability of soil nutrients, as topsoil nutrients in particular are highly influenced by litter quality.[54,55] Our findings indicate the potential of *Acacia* invasion in altering soil properties such as total soil N particularly through the process of nutrient enrichment from their N-rich litter. Studies of other *Acacia* species in South Africa and China have recorded increased input of N into soil through higher litterfall rates, in combination with N rich litter from the invasive *Acacia* species.[25,27,46] Enrichment of N in soil may generate negative feedback that increases the competitive superiority of invasive species, promoting further invasion and subsequently resulting in changes to the community composition of invaded habitats.

5. Conclusion

In conclusion, our study recorded increased total N and P concentrations in the litter of *Acacia*-invaded coastal Kerangas forests, and a significant relationship between litter N and topsoil N concentrations in these invaded habitats. Our findings suggest that successful *Acacia* re-invasion of the IKF plots after fires and its competitive superiority can be attributed to present status of low topsoil N contents present in these Kerangas habitats due to the short invasion time period.

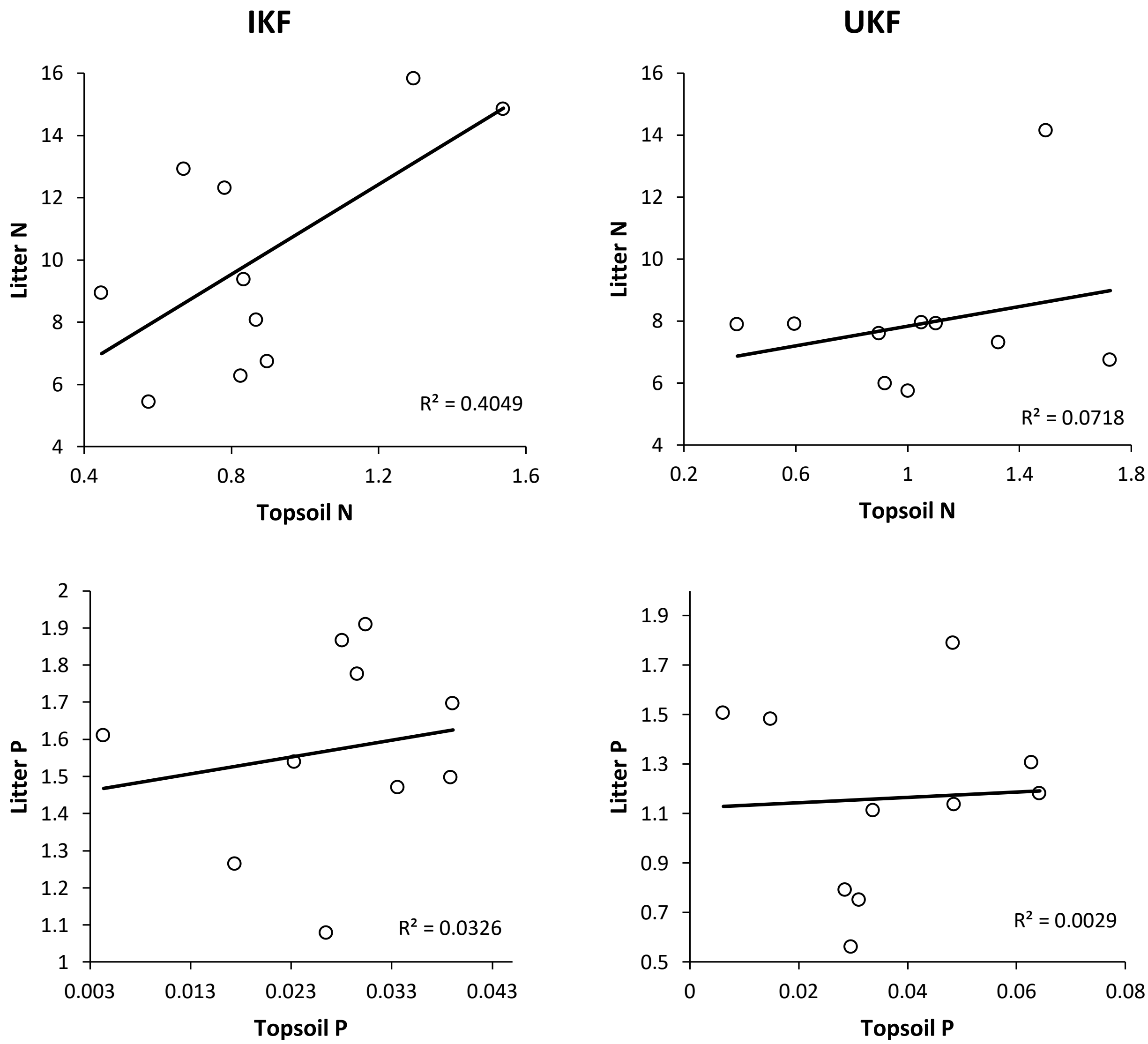

Figure 2. Simple linear regressions between litter nutrients (Tot N and Tot P) against topsoil nutrients (Tot N and Tot P) from the IKF (n = 10) and UKF (n = 10) plots.

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Amphibian species diversity in the proposed extension of the Bukit Teraja Protection Forest, Brunei Darussalam

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Abstract

Amphibian species diversity was investigated within the lowland mixed-dipterocarp forest of the proposed extension of the Bukit Teraja Protection Forest (BTPF), Brunei Darussalam in north-west Borneo. A combination of both visual encounter and acoustic sampling techniques were conducted opportunistically as well as in six selected stream transects, each containing five 5x10m plots. A total of 39 species of frogs from seven families (Bufonidae, Ceratobatrachidae, Dicroglossidae, Megophryidae, Microhylidae, Ranidae, and Rhacophoridae) were identified from the BTPF. Most notably, four new records for Brunei were discovered: *Hylarana nicobariensis*, *Kaloula baleata*, *Limnonectes malesianus*, and *Microhyla perparva*. This brings the total number of amphibian species in Brunei Darussalam to 84. Canonical correspondence analysis showed that out of 13 measured environmental variables maximum stream depth and % soil/sand of ground cover were significantly correlated with anuran assemblage composition. Species overlap between the proposed extension of the Bukit Teraja Protection Forest and the Ulu Temburong National Park (UTNP) determined by the coefficient of biogeographic resemblance (CBR) showed low species complementarity between these two regions. This emphasizes the need to upgrade the proposed extension of the BTPF to a protection forest to ensure the conservation of regional amphibian biodiversity.

Index Terms: anurans, biodiversity, conservation, species assemblages, tropical lowland rainforest

1. Introduction

The distributions of terrestrial vertebrates have informed global and local conservation priorities.[1] However, more fine-scale and targeted surveys will be necessary to identify priorities at a scale practical for local action. In the case of amphibians, not all regions have been equally targeted, with amphibian distributions on Borneo remaining particularly incomplete.[2] Small-ranged amphibians with specific habitat requirements are particularly vulnerable to falling through the grid of large-scale meta-analyses. There is a recognized need for conservation actions particularly in Borneo where the rates of deforestation are high due to logging and land conversion for oil-palm plantations and urbanization.[3,4] Furthermore, studies that explore the effects of both environmental variables and biotic effects on amphibian assemblage composition in the tropics are limited.[5,6] Such information serves to predict distribution patterns of data-deficient species and can direct conservation efforts.

Borneo provides an excellent model for studying amphibian species diversity and factors influencing diversity. Borneo, the third largest island in the world, is widely known as an area of high biodiversity[7,8] hosting nearly 200 species of amphibians.[2] The level of endemism of frogs in Borneo is high with about two-thirds of its frog species endemic to the island.[2] This number will continue to rise as additional frog species are being discovered every year.[2] Wildlife inventories are the basic tool for conservation, laying the foundation for prioritizing areas for conservation.[9-13]

Research on amphibian assemblages regarding spatial and environmental effects have produced contrasting outcomes. In sub-tropical eastern Australia, dominant environmental effects dominated anuran species composition on a regional scale.[14] In contrast, anuran assemblages in pristine and disturbed forests of the afro- and neotropics were mostly affected by pure spatial effects with pure environmental effects controlling assemblages in disturbed areas only.[15-16] However, within pristine habitats, it was found that it was impossible to predict assemblage compositions on a species-specific level with habitat variables.[16] It was deduced that in pristine habitats, priority effects and lottery recruitment were more important than species-specific responses to the environment, even though species have, for instance, explicit breeding habitats. Furthermore, investigations on the community assemblages of leaf-litter and canopy frogs in pristine forests identified the importance of spatial effects and spatially structured environments.[16-18]

The current study was undertaken in Brunei Darussalam located on the north coast of Borneo. Brunei's land area (5,766 km^2), although representing only less than 1% of the whole of Borneo, is still approximately 56% forested.[19] Seventeen percent of Brunei's forests are undisturbed or pristine and are currently protected as either protection or conservation forest or national park. A variety of forests exist including mixed-dipterocarp rainforest, lower montane forest, upper montane forest, tropical heath forest and others.[20] These forests are among the richest in the world[21] and they support many different frog species. For example, Grafe and Das[19] list a total of 70 species of frogs of seven families (Bufonidae, Cetatobatrachidae, Dicroglossidae, Megophryidae, Microhylidae, Ranidae and Rhacophoridae) for the lowland mixed-dipterocarp forest of Ulu Temburong National Park alone. However, as more changes occur as a result of increased road and dam constructions, new settlement schemes, and fire, the forest cover will continue to be reduced.[20] Therefore, there is an urgent need to study these habitats to ensure conservation of frogs in this small but highly diverse country.

Riparian frogs were chosen for this study because most frog species on Borneo are stream-associated and the environmental variables affecting their assemblages tend to be group-specific, i.e. correlated with differences in life-history patterns as well as habitat affiliation.[2, 15]

The main objectives of the study were to (1) provide an inventory of amphibians within the proposed extension of the Bukit Teraja Protection Forest, (2) investigate whether environmentally similar sites have similar species assemblages, and (3) evaluate the area for its conservation value in particular its complementarity to other forested areas, such as Ulu Temburong National Park.

2. Materials and Methods

2.1 Study area

The study was conducted within the lowland mixed-dipterocarp forest of the proposed extension of the Bukit Teraja Protection Forest (see ***Figure 1***) Belait District, Brunei Darussalam from April 2010 to May 2011, as well as on 8 June 2014 and 25 January 2018. Only opportunistic surveys were conducted in 2014 and 2018. Mean air temperature during fieldwork was 25.5˚C (± SD of 0.9˚C) and mean water temperature was 25.2˚C (± SD of 0.8˚C). The area comprises Bukit Teraja Protection Forest and the Heart of Borneo proposed extension of the Bukit Teraja Protection Forest (BTPF). This area covers an elevational range from under 30–415 m above sea level with Bukit Teraja being the highest point. The Bukit Teraja Protection Forest has a total area of more than 6000 hectares of undisturbed lowland mixed-dipterocarp forest. While the BTPF has approximately 2,500 hectares of mostly undisturbed mixed-dipterocarp forest, about 325 ha of the BTPF is peat swamp forest that borders the peat swamp forest of the Ulu Mendaram Conservation Forest[22] (see ***Figure 1***).

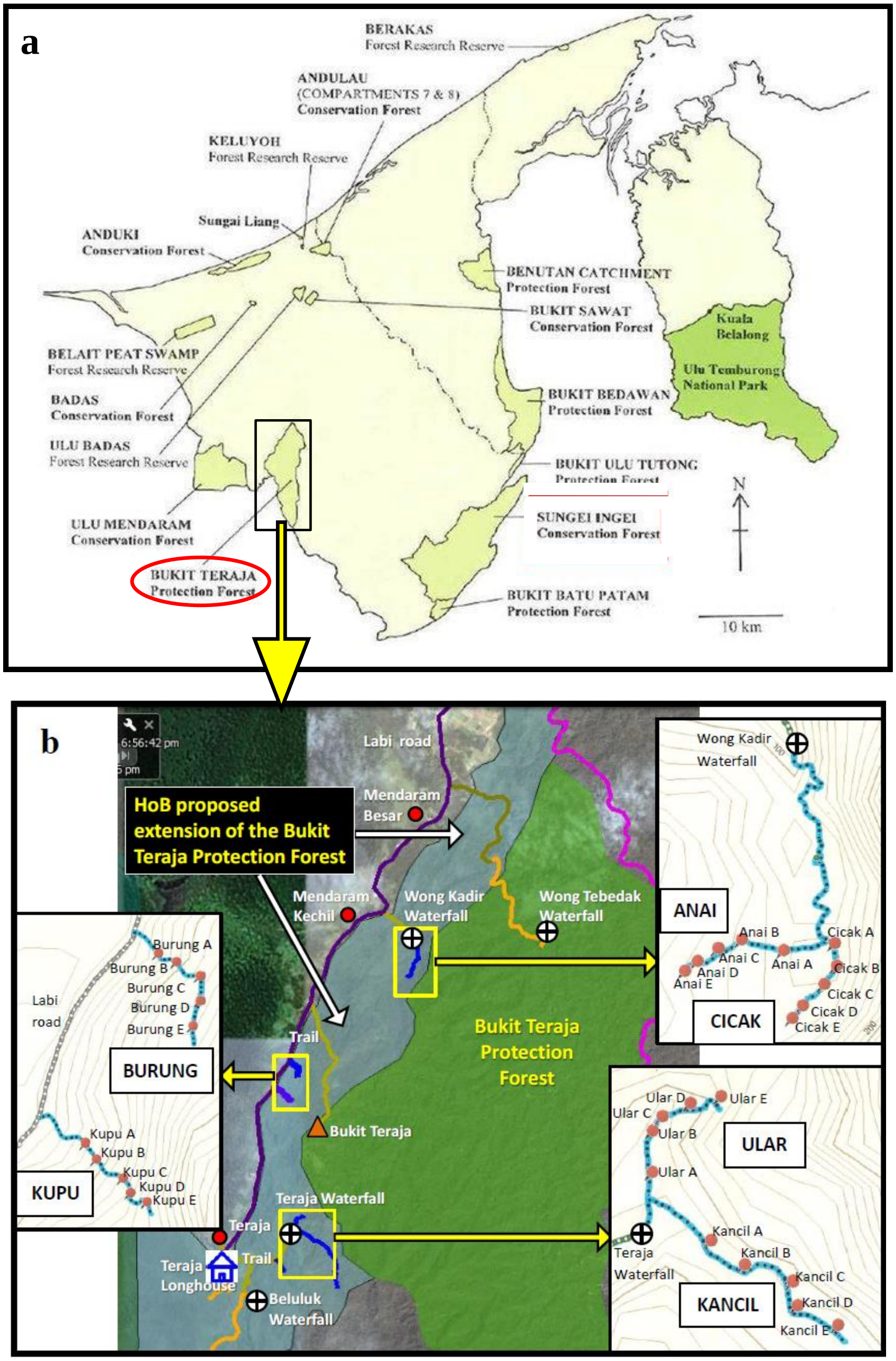

Figure 1. Maps showing (a) the location of the Bukit Teraja Protection Forest and its surrounding area[21] and (b) the close-up view of the Protection Forest (green) and the proposed extension of the Bukit Teraja Protection Forest (blue), showing all the stream transects and 30 study plots (Google map by Peter Engbers)

Six stream transects of small to medium sizes were selected within the BTPF. These streams were labelled Ular, Kancil, Anai, Cicak, Kupu and Burung. Ular and Kancil were located upstream of the Teraja waterfall, Anai and Cicak were upstream of the Wong Kadir waterfall, and Kupu and Burung ran perpendicular and upstream from the Labi-Teraja road (see ***Figure 1***). Five 5 x 10 m plots were established within each stream transect. Plot locations were selected haphazardly with irregular intervals to cover stream heterogeneity. Distance between neighbouring plots was at least 20 m. Each stream plot was visited eight times during the study period. We visited each plot at irregular intervals timing our visits to insure an even spread of plot visits throughout the study period.

2.2 Species capture and identification

A combination of both visual and acoustic encounter surveys were conducted to sample frogs between 1900–2330 h. Transect walks were conducted by 1-4 people. Survey effort was normalized by increasing or decreasing search time per plot according to the number of surveyors. The mean time spent within each plot searching for frogs was 10.6 min (± 2.9 min SD). Any encountered individuals were identified and when identification was not possible photographs were taken or samples were brought back to be identified. Frogs removed were released back the next day roughly at the spot of capture. The substrates on which the frogs were encountered (leaf, branch, ground) and height from the ground were recorded. Opportunistic surveys required actively searching for animals over large areas in order to increase the probability of encountering as many different species as possible. Samples of each new species were preserved in 75% ethanol and kept in the UBD Natural History Museum for future reference. Identification of frogs was facilitated by Malkmus[23], Das[24] and Inger et al..[2] Scientific names follow Frost[25] except for the genus *Hylarana* as we do not accept the splitting of this monophyletic genus.

2.3 Environmental characterization

We measured maximum stream width and depth, stream slope, density of riparian vegetation and canopy cover as these have been shown to influence the assemblage structure of riparian frogs.[14,26,27,28,29] Density of vegetation was measured by counting each individual plant within five height categories; < 10 cm, 10 cm–1 m, 1–2 m, 2–3 m and >3 m. Canopy cover at each plot was determined using a spherical densiometer. To determine ground cover in each plot, the ground was divided into % soil/sand, % rock, % gravel and % log roughly by eye. Maximum stream width and depth were measured on each visit and averaged over the study period, whereas the other largely invariant parameters were determined only once during the project. Descriptive statistics are given as mean ± SD unless stated otherwise.

2.4 Species diversity

Species accumulation curves for all six transects combined were plotted. To get an estimate of the true species richness of frogs for the BTPF the values of abundance-based coverage estimator (ACE) and Chao 1 were determined using EstimateS Win 8.20.[30] ACE and Chao 1 are non-parametric estimators that predict species richness based on species abundance. Since toe clipping was not done to mark each individual species found, the maximum number of individuals of each species captured on a single night per stream transect was assumed to be the abundance of that species in each stream. Rank abundance graphs were ploted and frogs categorized as abundant, common or rare, depending on whether the they were encountered in all 6 streams, in 2-5 transects or in only one stream transect. Species diversity in each stream transect was calculated by Fishers' alpha index using EstimateS Win 8.20[30] and similarity between streams was determined by Morisita's similarity index in Krebs[31]. The Kruskal-Wallis test, a non-parametric test, was used to determine the differences in stream characteristics among the six streams using SPSS (version 17).

2.5 Community analysis

Canonical correspondence analysis (CCA), a multivariate direct gradient analysis, in the program CANOCO (version 4.5)[32] was used to analyze the relationship between environmental variables and species abundance of frogs. Species with one individual only (i.e. *Philautus tectus*, *Chaperina fusca* and *Rhacophorus nigropalmatus*) were excluded from this analysis. The environmental variables utilized in the CCA were therefore, mean stream depth and width, stream slope, canopy cover, density of vegetation (< 10 cm, 10 cm-1 m, 1-2 m, 2-3 m and >3 m vegetation) and percentage ground cover (% soil/sand, % rock, % gravel and % log). In this technique, the ordination axes are constrained by linear combinations of the selected environmental variables.[33,34,35] CCA was chosen as it is robust to analysis with numerous correlated variables[37] and allows the identification of variation patterns that are best explained by the particularized environmental variables.[34] To determine the relative importance of the variables, the forward step-wise selection procedure was then performed. In this procedure, each of the variables was analyzed separately for individual explanatory power (marginal effects) as well as the effect that each variable had in addition to the variables that have already been chosen (conditional effects).[38] Monte Carlo permutation tests (with 999 random permutations) were run to test the significance of these effects as well as the statistical significance of the first canonical axis and of all canonical axes. We used Moran's *I* (R package *ade4*) to test environmental parameters for spatial autocorrelation for all plots and within streams.

2.6 Complementarity analysis

Species overlap between the proposed extension of the Bukit Teraja Protection Forest and the Ulu Temburong National Park (UTNP) was determined by the coefficient of biogeographic resemblance (CBR).[38,39,40] Complementarity analysis gives an indication of the degree of similarity in the species composition between two geographically separated areas.[40] The index was calculated as: Overlap/Similarity = 2C/(N1 + N2) where C is the number of species in common to the two regions (i.e., the BTPF and the UTNP) and N1 and N2 are the number of species in the first and the second region, respectively. The value of CBR ranges from 0 (no species in common i.e. low complementarity) to 1 (all species are shared in both regions).[39] The checklist of frog species for the UTNP was obtained from Grafe and Das.[19]

3. Results

3.1 Species diversity

In total, 39 species of frogs representing 46% of frog species in Brunei were recorded from the proposed extension of the Bukit Teraja Protection Forest (see ***Table 1***). These anurans belong to seven families: Bufonidae (4 spp), Ceratobatrachidae (1 sp), Dicroglossidae (10 spp), Megophryidae (2 spp), Microhylidae (6 spp), Ranidae (8 spp), and Rhacophoridae (8 spp). All individuals were identified to the species level except for one individual of *Ansonia*. According to the Global Amphibian Assessment,[41] twenty-five species are classified as least concern, nine species are near threatened and only one species i.e. *Philautus tectus* is listed as Vulnerable. Only two individuals of *P. tectus* were encountered during the study period; one individual outside a riparian plot at the Teraja Waterfall and another inside a riparian plot at the Wong Kadir Waterfall. The majority of the frogs encountered in BTPF were either adults or juveniles except for *Leptobrachium abbotti* and *Microhyla borneensis*, which were a young metamorph and a tadpole, respectively.

Out of the 36 frog species, 22 species were encountered in varying habitats outside the riparian plots within the BTPF via opportunistic surveys. The four species *Hylarana nicobariensis*, *Kaloula baleata*, *Limnonectes malesianus*, and *Microhyla perparva* were new records for Brunei. The four new records bring the total number of frog species in Brunei to 84.

The overall species accumulation curve for all six streams combined at the BTPF started to level off on the 27th survey and reached an asymptote on the 46th survey (see ***Figure 2***). This suggests that most of the riparian anuran community have now been detected. Both ACE and Chao 1 computed only 14 species in the stream transects. This implies that if additional surveys were to be conducted, no new species will likely be encountered.

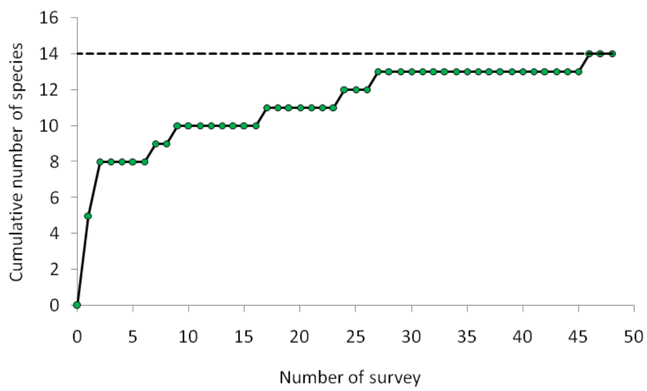

Figure 2. Species accumulation curve for the proposed extension of the Bukit Teraja Protection Forest during 48 visits. Dotted line represents both values of ACE and Chao 1.

3.2 Species richness, compositions and rank abundance at different streams

Overall, the most abundant species in the BTPF was *Limnonectes* aff. *kuhlii* with a total of 38 individuals (see ***Figure 3***). Species that were encountered only once within plots were considered rare (i.e. *Chaperina fusca*, *Philautus tectus* and *Rhacophorus nigropalmatus*). The rest of the species had intermediate abundances and thus were considered common.

Values of the Morisita's similarity index between Ular-Kancil, Ular-Cicak, Anai-Kupu, and Kupu-Burung were 1 or slightly more than 1. Thus, these streams showed complete similarity in species composition. Cicak-Kancil, Cicak-Anai and Burung-Anai also showed strong similarity. On the contrary, poor similarities were detected between Ular-Kupu, Ular-Burung, Kancil-Kupu and Kancil-Burung.

Moran's *I* showed a lack of spatial autocorrelation for all plots and within streams. All combinations of streams and environmental parameters were not significant ($P>0.05$) and distance did not explain much of the environmental variance (mean $I = -0.14 \pm 0.09$).

The Canonical correspondence analysis explained only 49% of the total variation indicating that some other important variance was not included. Axis 1 and Axis 2 showed high correlations between species and environmental variables ($r = 0.888$ and $r = 0.877$), and explained 31.8 % and 26 % of the total variance, respectively. Only mean depth and % soil/sand showed very significant difference ($P = 0.005$ and $P = 0.006$, respectively) when all of the variables were combined and tested for conditional effects.

Both canonical coefficients and intraset correlations show that Axis 1 is a gradient of increasing depth of stream and % sand/soil, whereas Axis 2 is a gradient of increasing depth and decreasing % soil/sand.

The assemblage of *Hylarana baramica*, and *Ingerophrynus divergens* was influenced by stream depth (see ***Figure 4***). Both species are known to breed in stream-side pools.[2] *Limnonectes leporinus* showed preference for shallow streams. *Staurois guttatus* preferred areas with less sand. Conversely, the presence of *Phrynoidis asper* was influenced by the presence of soil or sand. In contrast, the *Hylarana glandulosa, H. megalonesa, H. signata, A. baluensis* and *Limnonectes* aff. *kuhlii* assemblage was clustered in the centre of the ordination diagram. This suggests that these species had no habitat preferences based on the chosen ecological variables. Thus, these species were grouped as generalists.

3.3 Species complementarity between Teraja and Ulu Temburong National Park

The BTPF with 39 and the UTNP with 70 species had 30 species of frogs in common. The two regions had a low complementarity (CBR = 0.550).

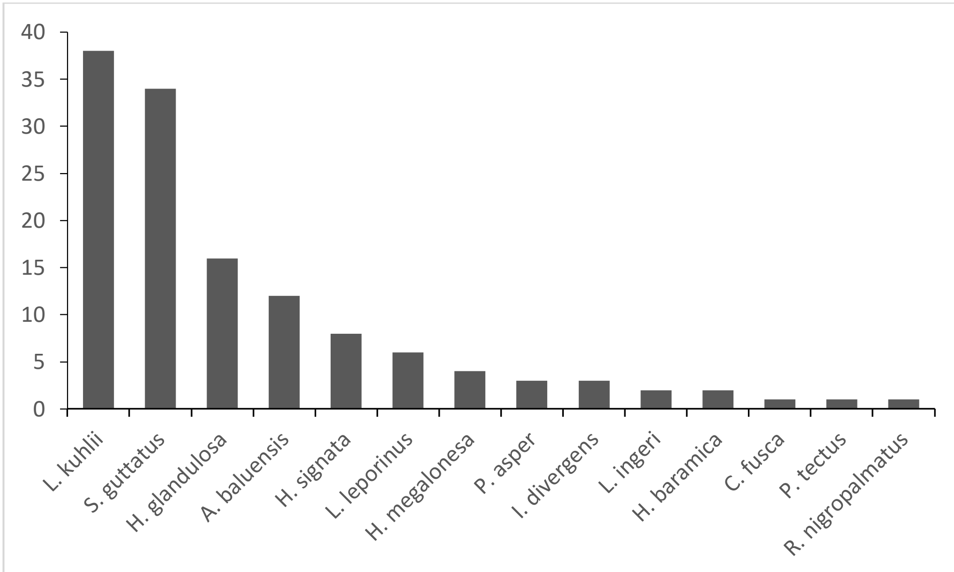

Figure 3. Rank abundance of species found in all riparian plots.

4. Discussion

4.1 Species diversity

Extensive studies of the amphibians of Brunei have only been done in restricted parts of the country including Ulu Temburong National Park[24,27,42] and Tasek Merimbum Heritage Park.[44] This is the first detailed study of anuran diversity in the proposed extension of the Bukit Teraja Protection Forest. The current survey identified 39 species of frogs and thus provides a baseline inventory of anuran species diversity of this area. Anuran diversity of the BTPF is relatively low when compared to other sites with similar lowland forest type within Borneo such as the Ulu Temburong National Park[19] (70 species), Nanga Tekalit in Sarawak[44] (60 species) and Crocker Range National Park in Sabah[46] (59 species) and Danum Valley (50 species).[46] However, among these sites the BTPF has the lowest elevational profile possibly providing a lower variety of suitable breeding sites.

Several more promising areas within the BTPF remain unexplored including the Beluluk Waterfall, Talingan Waterfall and Tebedak Waterfall as well as the Bukit Teraja Protection Area itself. Species such as *Ingerophrynus quadriporcatus*, and frogs from the genus *Kalophrynus* might occur in area but were not encountered during the surveys. Further surveys will most likely result in the rise of the number of amphibians in the BTPF.

4.2 New records

The current study extends the knowledge of the range distribution of frogs in Borneo. The four frogs (*Hylarana nicobariensis*, *Kaloula baleata*, *Limnonectes malesianus*, and *Microhyla perparva*) found in this study have already been known to occur in the Malaysian and the Indonesian parts of Borneo as well as in other parts of south-east Asia. It can now be confirmed that these species occur in Brunei and that the BTPF might possibly be one of the few places in Brunei where these species can be found. This finding also emphasizes that the inventory of Brunei's amphibians is far from complete.

4.3 Species richness, distribution and abundance of riparian frogs

Fisher's alpha indicated that Kancil was the most diverse stream and the Burung was the least diverse although Burung had more species than

Kancil (nine and seven species, respectively). This is probably because of the difference in species evenness. Burung might have more species but only a few species were dominating in abundance.

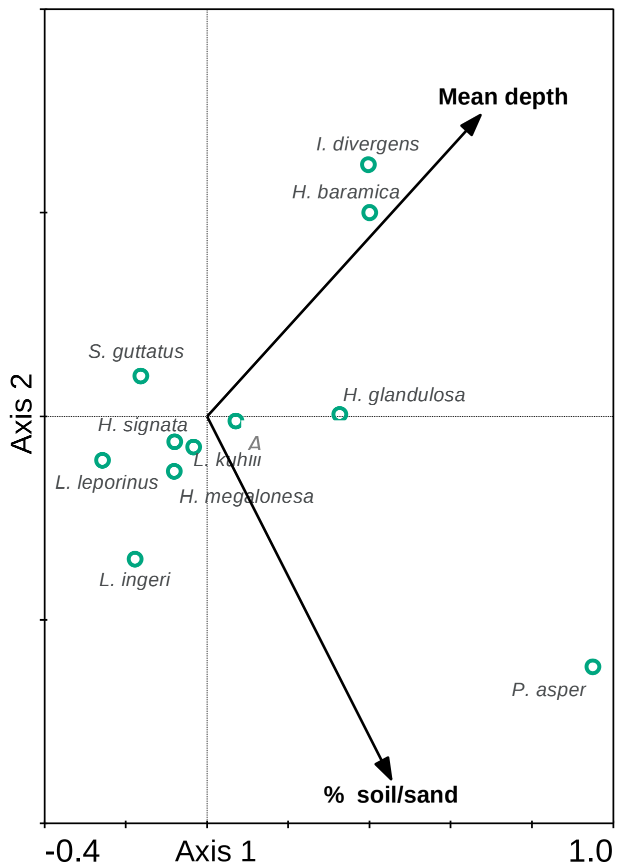

Figure 4. CCA biplot of the relations between environmental variables and frog assemblage. Arrows are environmental variables. Direction of arrow indicates correlation with that axis and length indicates the strength of the correlation.

Hylarana glandulosa, *Alcalus baluensis* and *Limnonectes* aff. *kuhlii* were the three most widely distributed species in all six streams. *Hylarana glandulosa* is generally found in peat swamps.[2, 47] The close proximity of the peat swamp forest of the Ulu Mendaram Conservation Area might explain its occurrence in Teraja. Moreover, the presence of some juveniles of *H. glandulosa* in streams reflected that this species might use streams as dispersal routes. *Limnonectes* aff. *kuhlii* is probably the most common frog species in the lowland rainforest of Brunei, reportedly found in small streams with rocky bottom.[24,47] However, the individuals of *L.* aff. *kuhlii* in this study were encountered at streams with sandy bottom. As this species is likely to be a complex of cryptic species, it cannot be ruled out that the differences in habitat use between study areas reflect habitat preferences of two different species within the complex. Alternatively, this reflects its ability to occupy and survive in many different habitats. Another species that showed wide distribution at all studied streams except for Anai was *Limnonectes leporinus*. *Limnonectes leporinus* has been known to dwell along medium and large streams.[2,48] Thus, Anai being the smallest stream it might not be preferred by *L. leporinus*. *Staurois guttatus* and *Hylarana signata* were both absent in Kupu and Burung. This may reflect the intermittent nature of these streams (i.e. both streams are short and probably dry out in periods without rainfall with a concomitant drop in humidity to which *S. guttatus* is sensitive).

Abundance for most species at streams was very low (see ***Figure 3***). *Chaperina fusca*, *Philautus tectus* and *Rhacophorus nigropalmatus* were only found once inside riparian plots. *P. tectus* was also found outside a plot in Ular. Given that *P. tectus* is listed as vulnerable occurring only in primary forest streams (see ***Table 1***) its presence in Ular and Cicak indicate that these stream habitats were pristine. *C. fusca* were also found in Kancil, Burung and Kupu but all individuals were found outside plots. *Rhacophorus nigropalmatus* is known to live very high in the canopy of primary lowland rainforest and only comes down from the trees to breed in forest ponds such as pig or rhino wallows.[2,47] Interestingly, the only individual of *R. nigropalmatus* in this study was found above a pool at one of the waterfalls in Burung.

4.4 Environmental influences on frog assemblages

Canonical correspondence analysis of the anuran assemblages in the BTPF revealed that species composition was influenced by two stream characteristics: stream depth and the presence of soil/sand. Stream depth can be translated to occurrence of stream-side pools. The edges of a pool might be used by frogs as oviposition sites. *Hylarana baramica* and *Ingerophrynus divergens* showed preference for pool areas. *I. divergens* is known to use stream-side pools as breeding sites.[2] In contrast, there have been no reports of *H. baramica* using stream-side pools as oviposition sites. The assemblage of *H. glandulosa, H. megalonesa, H. signata, A. baluensis* and *L.* aff. *kuhlii* were clustered in the centre of the CCA biplot suggesting that these species had no habitat preference and are thus likely to be habitat generalists within streams. This is in contrast to the previous study by Keller et al.[27] in the UTNP. For instance, *Hylarana signata* was found to be associated with large to mid-sized streams. However, in Teraja, *H. signata* was also encountered at Anai which was the narrowest studied stream. *Limnonectes.* aff. *kuhlii* was previously found to be associated with the presence of riffles and runs[27] and thus did show microhabitat specificity. *Limnonectes ingeri* is a pond breeder but was not grouped together with *H. baramica* and *I. divergens*. *Staurois guttatus* is known to be associated with rocky streams and the presence of waterfalls. Thus, it showed preference for less sandy or muddy areas. On the other hand, *Phrynoides asper*, which is a forest litter toad, preferred sandy or muddy areas.

The results of this study are different from that of Parris[14] in Queensland, Australia and Keller et al.[27] in UTNP. Both studies revealed that anuran assemblages in streams were affected by three stream characteristics and their environment including the density of understorey vegetation, stream size and the presence of waterfalls or slope. Riparian vegetation is an important structural component used by frogs as calling or resting sites.[14] However, the density of vegetation among the streams in Teraja showed little variation and thus no significant correlation with species assemblage although several species such as *A. baluensis, I. divergens* and *S. guttatus* were often found sitting on the leaves of understorey vegetation. Moreover, stream size was also important in explaining the differential species composition in other studies.[27] Many frogs prefer large streams due to their ability to retain water for a very long period of time for tadpoles to metamorphose.[48] In contrast, stream size was not a determinant of anuran assemblages in the BTPF probably because streams were not variable enough. There was no large stream sampled in the BTPF. Furthermore, the presence of waterfalls, riffles, and runs as well as the general steepness of streams also influenced frog assemblage composition in the UTNP.[27] Although the BTPF had a lot of waterfalls, this factor showed no influence on species composition.

4.5 Complementarity between Teraja and Ulu Temburong

Complementarity analysis showed that there was a low complementarity or resemblance in anuran species richness between BTPF and UTNP. This is due to the low number of species overlap between both regions. Species occurring in the BTPF not found in the UTNP include all of the new records (*H. nicobariensis*, *K. baleata*, *L. malesianus*, *M. perparva*). Conversely, many of the litter frogs (Megophryidae) and all of the species in the genera *Ansonia*, *Kalophrynus* and *Meristogenys* did not occur in BTPF. Low complementarity between the two regions might be due to the differences in topography, differences in habitat structure, biogeographical distance or a combination of these factors. For example, high complementarity was found between UTNP and lowland forests in Mulu, as they are much closer in terms of biogeographic distance (CBR = 0.636, based on[7] and pers. observations).

It is important to conserve areas with high biodiversity, but areas with complementary fauna must also be considered for conservation.[49] Although the BTPF has a lower frog diversity than the UTNP, the low species overlap between the regions suggests that the proposed extension of the Bukit Teraja Protection Forest harbours a distinctive fauna that should be conserved. It is thus lauded that the Forestry Department of Brunei

Darussalam has been tasked to gazette the proposed area, following the proposal to take action passed by the Heart of Borneo National Council.

On a larger regional scale, the diversity of stream-associated frogs within lowland mixed-dipterocarp rainforest sites in northern Borneo shows significant turnover in species richness and composition with stream width a good predictor of frog assemblages both locally and regionally.[7,28] Thus, beta diversity plays a significant role in maintaining regional diversity of stream-associated frogs in Brunei and neighbouring Malaysia.[19] In regard to its high beta diversity, and in order to protect the diversity of riparian anurans of northwestern Borneo, it is necessary not only to focus on a few hotspots, such as the Ulu Temburong National Park, but to put conservation efforts on other, less diverse, sites as well. The rate of species turnover in north-western Borneo is comparable to that of New Guinea and Bolivia and this calls for a network of protected forests.[28]

5. Conclusion

In conclusion, this study confirms the presence of a substantial amount of anuran diversity in the BTPF, providing a baseline inventory for future amphibian research in this area. The four new records increase the anuran diversity in Brunei and extend the range of frog distributions in Borneo. Spatial and environmental effects both had an effect on the species assemblage of riparian frogs in the BTPF. The low species complementarity detected between the BTPF and the UTNP emphasizes the importance of conserving areas with not only high species richness but also areas with unique fauna. Since the forest is under threat of development, upgrading the proposed extension of the Bukit Teraja Protection Forest to a protection forest is strongly recommended.

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Table 1. Anuran amphibian species recorded from the proposed extension of the Bukit Teraja Protection Forest. Data sources (P = encountered in plots, O = encountered outside of plots). Conservation status (Con. status) follows the Global Amphibian Assessment listings of IUCN 2004 Red List Categories (LC = Least Concern, NT = Near Threatened, VU = Vulnerable). New records are underlined.

Species	Data source	Habitat	Con. status	Total abundance at streams	Rank abundance at streams
Bufonidae					
Ansonia sp.	O	Pond behind Teraja Longhouse	-	-	-
Ingerophrynus divergens	P	On leaves and branches	LC	3	8.5
Phrynoidis asper	P	On rock at Cicak	LC	3	8.5
Phrynoidis juxtasper	O	Near Wong Kadir Waterfall	LC	-	
Ceratobatrachidae					
Alcalus baluensis	P	Mostly on rocks and small plants	LC	12	4
Dicroglossidae					
Fejervarya limnocharis	O	On forest road	LC	-	-
Limnonectes ibanorum	O	On sand in front of Teraja Waterfall	NT	-	
Limnonectes ingeri	P	On rocks at Burung	NT	2	10.5
Limnonectes aff. *kuhlii*	P	Mostly in water, on rocks and ground	LC	38	1
Limnonectes laticeps	O	Waterfall	LC	-	

Limnonectes leporinus	P	In water, on ground and rocks	LC	6	6
Limnonectes malesianus	O	On forest trail to Teraja Waterfall	NT	-	-
Limnonectes paramacrodon	O	On forest trail to Teraja Waterfall	NT	-	-
Occidozyga baluensis	O	In puddle on trail to Teraja Waterfall	NT	-	-
Occidozyga sumatrana	O	In pond behind Teraja Longhouse	LC	-	-
Megophryidae					
Leptobrachium abbotti	O	Tadpoles above Teraja Waterfall	LC	-	-
Leptolalax gracilis	O	Forest above the Teraja Waterfall	NT	-	-
Microhylidae					
Chaperina fusca	P	On ground in Kancil	LC	1	13
Kaloula baleata	O	Rain pool behind Teraja Longhouse	LC	-	-
Metaphrynella sundana	O	In a tree hole near Teraja Waterfall	LC	-	-
Microhyla borneensis	O	Pond behind Teraja Longhouse	LC	-	-
Microhyla perparva	O	Pond behind Teraja Longhouse	NT	-	-
Microhyla petrigena	O	Pond behind Teraja Longhouse	NT	-	-
Ranidae					
Hylarana baramica	P	On rocks, leaves in Kupu and Burung	LC	2	10.5
Hylarana erythraea	O	Road side near ditch	LC	-	-
Hylarana glandulosa	P	On ground, rocks, leaves and branches	LC	16	3
Hylarana megalonesa	P	Mostly on roots and rocks	LC	4	7
Hylarana nicobariensis	O	Forest up Teraja Waterfall	LC	-	-
Hylarana signata	P	On roots, branches, rocks and ground	LC	8	5
Staurois guttatus	P	Mostly on leaves, branches and rocks	LC	34	2
Staurois latopalmatus	O	On rock near Teraja Waterfall	LC	-	-
Rhacophoridae					
Kurixalus appendiculatus	O	Forest behind Teraja Longhouse	LC	0	13
Nyctixalus pictus	O	Vegetation behind Teraja Longhouse	NT	-	-
Philautus tectus	P	On leaves of low vegetation, Cicak	VU	1	13
Polypedates leucomystax	O	Vegetation behind Teraja Longhouse	LC	-	-
Polypedates macrotis	O	Vegetation behind Teraja Longhouse	LC	0	-
Rhacophorus nigropalmatus	P	Above pool in Burung	LC	1	13
Rhacophorus pardalis	O	Vegetation behind Teraja Longhouse	LC	0	-
Zhangixalus dulitensis	O	Vegetation behind Teraja Longhouse	NT	0	-

215 new seed plant species recorded for Brunei Darussalam

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Abstract

We provide a checklist of new seed plant species (Angiosperms and Gymnosperms) in Brunei Darussalam with detailed information. The plant database for Brunei, extracted from the Global Biodiversity Information Facility (GBIF) was compared with the existing plant checklist for Brunei and plant holdings of the UBD herbarium (UBDH). 215 species of seed plants, belonging to 59 families, were found in the GBIF list that were absent in both the Brunei checklist and UBDH collections. The plant family that recorded the highest number of new plant species was Orchidaceae, but overall, tree species dominate the list. Only 8% of the new species were IUCN Red-List evaluated. Most new species collections came from Tutong district, but the highest number of new species was from Temburong district. Almost all new species collections came from areas with easy access such as near roads and around the Kuala Belalong Field Study Centre. As much of the Brunei rainforest remains unexplored, new discoveries are likely to occur.

Index Terms: Brunei Darussalam, Brunei checklist, new species records, GBIF, seed plants, threat status

1. Introduction

Nearly 20 years has elapsed since the first checklist of seed plants for Brunei Darussalam was published[1] and it is to be expected that quite a number of new plant species have been collected since. This expectation is supported by an additional 234 plants species recorded for Brunei Darussalam in 2018, based on the relatively small plant species collections of the UBDH.[2] Here we use the Global Biodiversity Information Facility (GBIF) database,[3] to check for additional plant species not listed in Coode *et al.*[1] or Zamri and Slik.[2]

GBIF is a global network and research organization funded by the governments of the world and designed to provide access to collection data on every type of life on Earth.[3] As GBIF allows data-holding institutions to share their collection data, the information can originate from numerous sources, i.e., from an 18th century museum collection to a recent geotagged cell phone photograph. For that reason, a plant species might be available for the world to see but still be absent from the Brunei seed plant checklist that was published more than 20 years ago.[1] Although the plant collections in GBIF have been identified by experienced botanists working at the participating institutions, we were unable to see the actual specimens themselves, since they were located in multiple herbaria around the world.

2. Methods and Materials

2.1 Compiling the new species list

On 22 November 2018 we extracted all seed plants collections originating from Brunei Darussalam from GBIF,[3] resulting in a total of 6238 seed plant collections consisting of 679 fully identified seed plant species. The GBIF data for Brunei Darussalam came from the following institutions: Nationaal Herbarium Nederland, Leiden University branch (L); Royal Botanic Gardens Kew (K); Bishop Museum (BISH); Royal Botanic Garden Edinburgh (E); British

Museum of Natural History (BM); Naturalist Biodiversity Center (WAG); University of Zimbabwe (CAH); Harvard University Herbaria (A); Australian Tropical Herbarium (CNS); and Missouri Botanical Garden (MO). This list of species was then compared with the Brunei Checklist[1] and the UBDH checklist[2] to detect species not mentioned in those two data sources. This resulted in a preliminary list of new seed plant species for Brunei Darussalam.

Because the original Brunei Checklist[1] was over two decades old, its nomenclature was partly outdated. This meant that some species in our preliminary new species list might be present in the Brunei Checklist under an older synonym name, or under a different family. To overcome this, all synonyms of the species in the preliminary list of new species for Brunei Darussalam, as well as their previous family classifications, were compared with the Brunei checklist[1] and UBDH checklist.[2] The synonyms were provided by both "The Plant List"[4] and "The Plants of the World Online".[5] Plant species that were labeled "unresolved" by both websites were removed from our preliminary list. After resolving the synonym and classification issues we were left with a final list of 215 new seed plant species for Brunei Darussalam.

2.2 Species information

Species names were listed in the following format:

[Family Name]

[Genus] [Species] [Author] [Reference] [Native or Not][Threat status]

[Local Name] [Growth Form] [Location] [Habitat] [Elevation] [Collection numbers]

Family, Genus, Species, Author, Reference, and Collection Number were obtained from the original GBIF database,[3] with an additional check for up to date taxonomy.[4,5] Native or Non-Native status was checked using the following websites.[3,5,6] Threat status (critically endangered (CR), endangered (EN), vulnerable (VU), near threatened (NT) and least concern (LC)) was determined using the IUCN Redlist website.[7] Local names and growth form were derived from literature and websites.[6,8-19] Location was available as geographic coordinates in GBIF, these coordinates were then entered into GooglePro[20] to find the elevation and the district in which they were located. The habitat was determined by overlaying the geographic coordinates with the forest type map in Coode *et al*.[1] Some geographic coordinates from the GBIF list were obviously wrong (located in the sea). In these cases, the location descriptions from the collectors were used to get the correct geographic coordinate. To study the distribution of all new species collections, a scatter plot of collection coordinates was made overlaying the map of Brunei Darussalam using GooglePro.[20]

3. Results and Discussion

From a total of 8247 plant specimens present in the original GBIF database for Brunei, 6238 specimens belonged to seed plants. This number contained 679 species names, which was further reduced to 380 species after comparing the species names and their synonyms with Coode *et al.*[1] After comparing with the checklist by Zamri and Slik,[2] and after removing unresolved species names, we were left with 215 species of seed plants that were not found in any existing checklist, and thus form new records for Brunei Darussalam. The complete list of species and their details is presented in ***Table 1*** and ***Appendix 1***.

3.1 Most collected plant families

The new records for Brunei were distributed over a total of 52 plant families (see ***Table 1***). The plant family with highest number of new species was Orchidaceae, the orchid family, which recorded about 29 new species followed by Annonaceae, the soursop family and Araceae, the arum family which contained 17 and 16 new species respectively (see ***Figure 1***). The list continues with Rubiaceae (14), Euphorbiaceae (12), Phyllanthaceae (10), Fabaceae (9), Zingiberaceae (9), Apocynaceae (8), Elaeocarpaceae (7), Myrtaceae (7), Poaceae (6), Primulaceae (6), Moraceae (5) and another 37 families with 4 or less species (see ***Table 1*** and

Appendix 1). That the Orchidaceae family recorded the highest number of new species occurrences might be due to the fact that Borneo is known to have a high orchid diversity with more than 2,500 species.[17,21,22]

3.2 Plant species distribution

Most plant species in this study were collected at locations where access was easy such as the coastal area and near the Kuala Belalong Field Study Centre in Temburong (see ***Figure 2***). Therefore the collections only include a limited part of Brunei Darussalam. For example, plant collections in Belait district where mainly concentrated near the main road from Mendaram Kechil to Bukit Teraja, whereas few plants species were collected in the isolated areas of the district. It is likely that many more species will be added to the Brunei flora when these isolated areas are explored in the future.

In Temburong district the highest number of new species (97) were found, followed by Belait district (92) and Tutong district (46) (see ***Figure 3***). In Brunei Muara district on the other hand, only 8 of the new species were present. This outcome may correspond to the difference in the number and size of forest types in every district.[23] In addition, development is more concentrated in Brunei Muara where the main city Bandar Seri Begawan is located, and most areas in Muara district have already been cleared or are secondary regrowth forests.

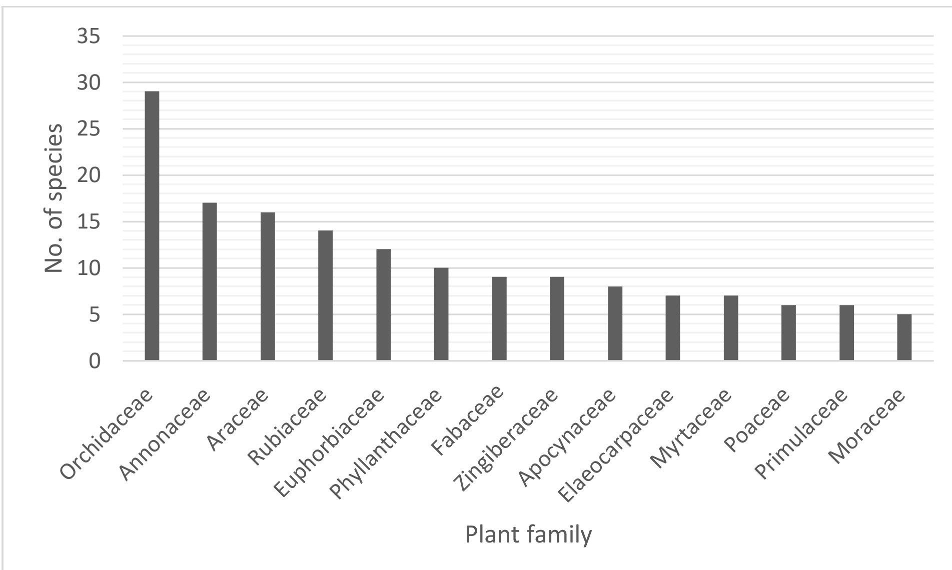

Figure 1. The 15 families that had the highest number of new species.

3.3 Plant growth form

The new seed plant species were also grouped according to their plant growth form (see ***Figure 4***). The highest number of species were trees, while the lowest number of species were creepers. However, herbs, climbers, epiphytes and shrubs also recorded quite a number of species with 39, 36, 26 and 23, respectively. All 26 species of epiphytes belonged to one family, the Orchidaceae. That tree species dominated the number of new species is not surprising as trees are the dominant plants in all types of forest. Furthermore, they also highly control where other vegetation can grow due to their height (providing shade) and resource utilization. Additionally, many ecologists and botanists are specialized on trees, which might also lead to more trees being identified.

3.4 Threatened and Invasive Species

Around 93% of the 215 new seed plant species were not yet evaluated by the IUCN red list[7] and therefore their threat status remains unknown (see ***Figure 5***). Of the assessed species, nine were included in a Threat category, with three being “Critically Endangered” (*Anodendron* *coriaceum* (Climber), *Dolichandrone spathacea* (Tree) and *Ficus trichocarpa* (Climber). Of the other six threatened species one was classified as "Endangered" (*Nageia wallichiana* (Tree)), and five were classified as "Vulnerable" (*Gnetum acutum* (Liana), *Actephilla excelsa* (Shrub), *Eusideroxylon zwageri* (Tree), *Aglaia rivularis* (Tree), and *Dacrydium medium* (Tree)). The lower risk categories included "Near Threatened" (*Ormosia stipulacea* (Tree), *Gnetum diminutum* (Liana), *Aglaia oligophylla* (Tree) and *Falcatifolium falciforme* (Tree)) and "Least Concern" (*Mimosa pigra* (Shrub), *Gnetum raya* (Liana), *Dacrydium beccarii* (Tree), and *Persicaria barbata* (Herb)). In addition, according to the Global Invasive Species Database,[24] three of the newly recorded seed plant species were considered invasive species. These species were *Mimosa pigra*, *Spermacoce verticillata*, and *Antigonon leptopus.*

The red list is crucial for conservation efforts because it provides information on which species (both plants and animals) need to be protected.[25,26] Red lists are also used as an authoritative way to encourage governments to protect threatened species. Unfortunately, only 17 (8%) of the new seed plant species recorded for Brunei were assessed for the IUCN Red List, which means that there is not enough data to evaluate the risk of extinction of the other 198 (92%) plant species. More effort will have to be spent on assessing the threat status of plants in Asia to make the IUCN Red List more useful for plants in the tropics. The fact that 9 of the 17 IUCN Red List assessed species were categorized as threatened (critically endangered = 3, endangered = 1, vulnerable =5) indicates that Brunei, with its relatively intact forests, plays an important role in the conservation of tropical plants. All critically endangered plant species in this study were found in Brunei Muara district where development is currently taking place. For that reason, Brunei needs to step up its conservation efforts, to prevent that development will be at the expense of threatened species.

3.5 Conclusion

Most of Brunei's dense rainforests remain under-explored today and the full range of plant species is most likely not yet documented. Thus in the future, more plant species will likely be discovered.

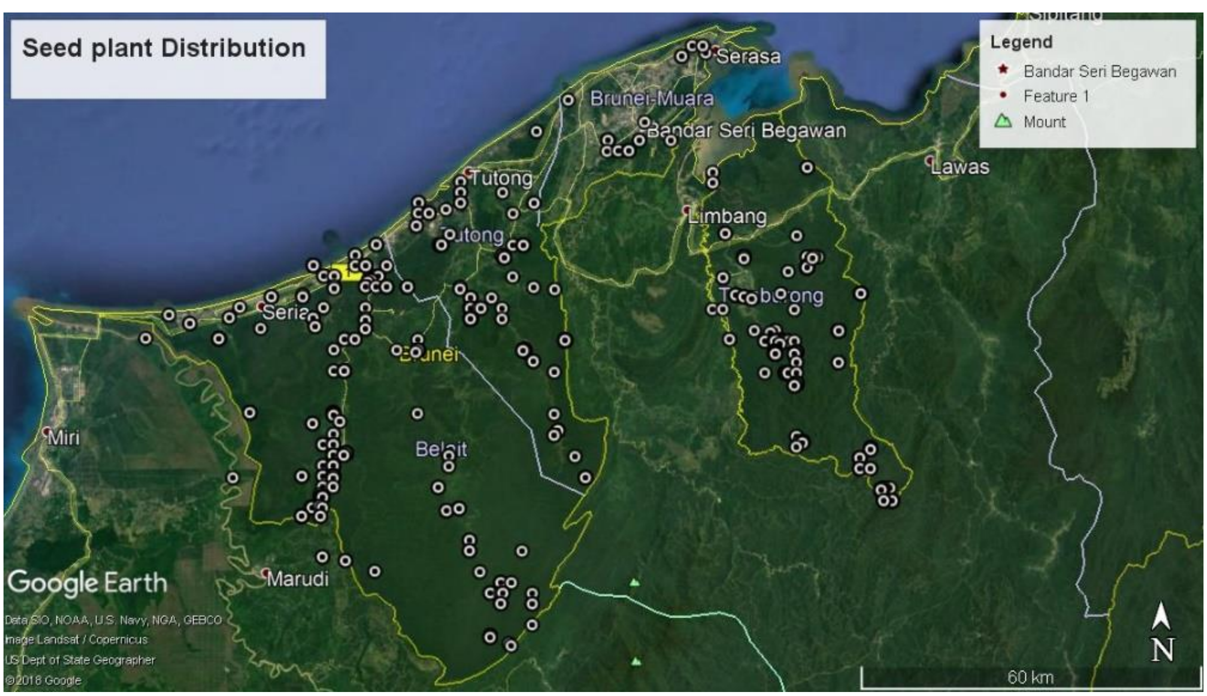

Figure 2. Collection localities (white open dots) of the new seed plant species in Brunei Darussalam.

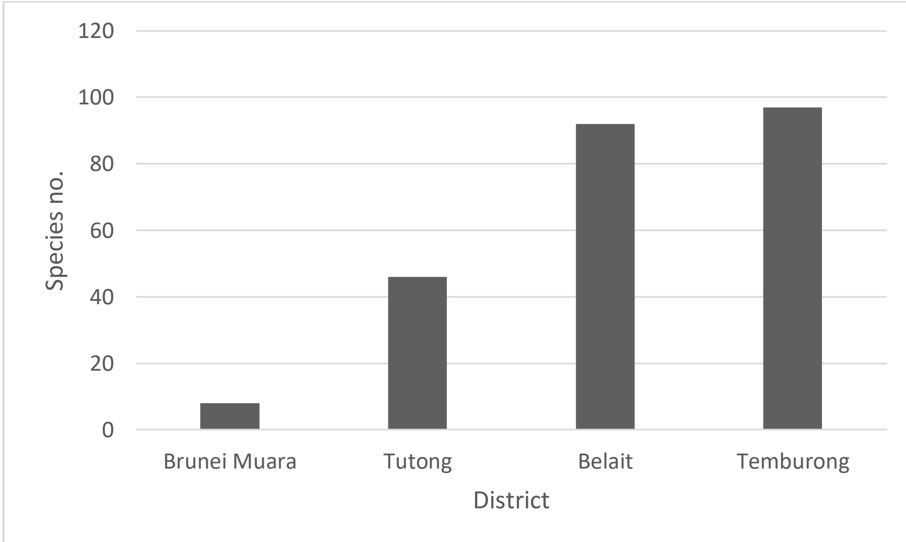

Figure 3. Distribution of the new seed plant species across the four districts of Brunei Darussalam.

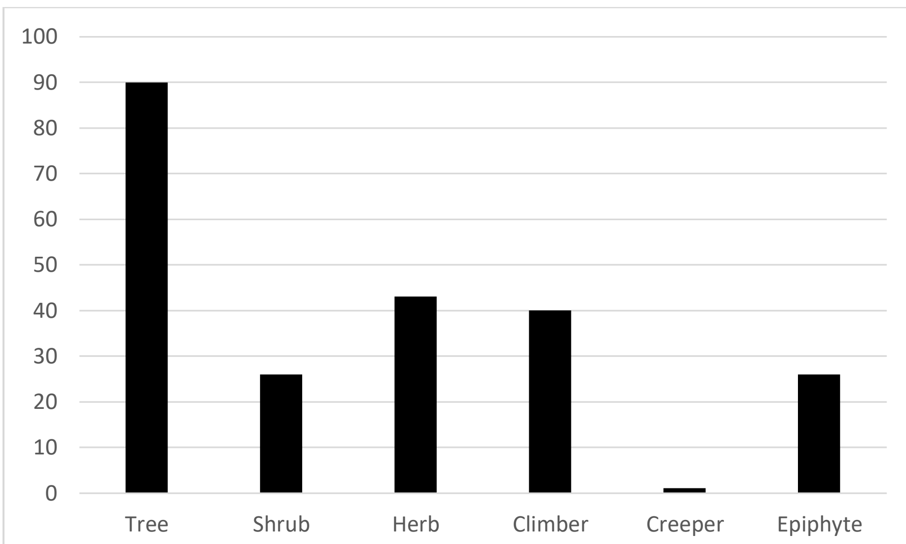

Figure 4. The new species numbers according to their growth form.

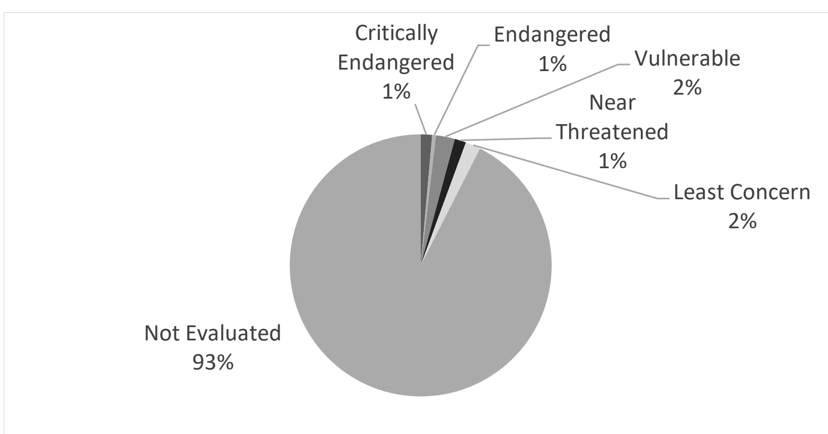

Figure 5. IUCN Red List results for the 215 new species, with no data available for 93% of the species.

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Table 1. Summary of plant species present in GBIF data for Brunei, but absent in the Brunei[1] and UBDH[2] checklists. For more details on these species see ***Appendix 1***.

FAMILY	**GENUS**
ANNONACEAE	*Anaxagorea borneensis*
	Artabotrys hirtipes
	Artabotrys lanuginosus
	Dasymaschalon ellipticum
	Drepananthus ridleyi
	Fissistigma kingii
	Friesodielsia latifolia
	Goniothalamus bygravei
	Mitrephora maingayi
	Monocarpia borneensis
	Monocarpia kalimantanensis
	Polyalthia charitopoda
	Polyalthia miliusoides
	Popowia hirta
	Popowia odoardi
	Pseuduvaria bruneiensis
	Xylopia kuchingensis
APOCYNACEAE	*Alyxia mujongensis*
	Anodendron coriaceum
	Chilocarpus rostratus
	Dischidia cochleata
	Dischidia complex
	Dischidia punctata
	Hoya imperialis
	Strophanthus singaporianus
AQUIFOLIACEAE	*Ilex tamii*
ARACEAE	*Amorphophallus borneensis*
	Hapaline brownii
	Hestia longifolia
	Ooia kinabaluensis
	Pedicellarum paiei
	Pothos chinensis

	Pothos lancifolius
	Rhaphidophora cylindrosperma
	Rhaphidophora latevaginata
	Schismatoglottis asperata
	Schismatoglottis motleyana
	Schismatoglottis patentinervia
	Schismatoglottis pectinervia
	Schismatoglottis petri
	Schismatoglottis trifasciata
	Scindapsus treubii
ARECACEAE	*Calamus maiadum*
	Calamus semoi
	Licuala olivifera
BIGNONIACEAE	*Dolichandrone spathacea*
BURSERACEAE	*Canarium latistipulatum*
	Canarium odontophyllum
	Canarium sarawakanum
	Dacryodes kingii
CENTROPLACACEAE	*Bhesa indica*
CLUSIACEAE	*Garcinia acuminata*
COMMELINACEAE	*Dictyospermum conspicuum*
CONNARACEAE	*Ellipanthus tomentosus*
CONVOLVULACEAE	*Ipomoea mauritiana*
CUCURBITACEAE	*Trichosanthes beccariana*
CYPERACEAE	*Isolepis setacea*
	Scirpus radicans
DIOSCOREACEAE	*Dioscorea moultonii*
ELAEOCARPACEAE	*Elaeocarpus beccarii*
	Elaeocarpus cristatus
	Elaeocarpus cumingii
	Elaeocarpus glaber
	Elaeocarpus jugahanus
	Elaeocarpus palembanicus
	Elaeocarpus valetonii
EUPHORBIACEAE	*Acalypha hispida*
	Croton carrii
	Euphorbia atoto
	Hancea stipularis
	Macaranga bancana
	Macaranga didymocarpa
	Macaranga havilandii
	Mallotus aureopunctatus
	Mallotus connatus

APPENDIX 1. Species information for the 215 new species for Brunei Darussalam.

Species names are listed in the following format:

[Family Name]

[Genus] [Species] [Author] [Reference] [Native or Not][IUCN Threat status][Local Name] [Growth Form] [Location] [Habitat] [Elevation] [Collection numbers]

ANNONACEAE

***Anaxagorea borneensis* (Becc.) J.Sinclair** (Sarawak Mus. J. 5: 598 [1951]) [Native] {NE} **Growth Form:** Shrub, Small tree; **Location:** Belait; **Habitat:** Keranga Forest; Elevation**:** 52 m asl; **Collections:** L.1744733.

***Artabotrys hirtipes* Ridl.** (Bull. Misc. Inform. Kew 1912: 383 [1912]) [Native] {NE} **Growth Form:** Climber; **Location:** Temburong; **Habitat:** Mixed Dipterocarp Forest; **Elevation:** 171 m asl; **Collections:** L.1744834.

***Artabotrys lanuginosus* Boerl.** (Icon. Bogor. 2: 52 [1903]) [Native] {NE} **Growth Form:** Climber; **Location:** Temburong; **Habitat:** Mixed Dipterocarp Forest; **Elevation:** 81 m asl; **Collections:** L.1749591, L.1749593.

***Dasymaschalon ellipticum* Nurmawati** (Floribunda 2: 78 [2003]) [Native] {NE} **Growth Form:** Small tree; **Location:** Belait; **Habitat:** Mixed Dipterocarp Forest; **Elevation:** 293 m asl; **Collections:** L.1756264.

***Drepananthus ridleyi* (King) Survesw. & R.M.K.Saunders** (Taxon 59: 1731 [2010]) [Native] {NE} **Growth Form:** Tree; **Location:** Muara; **Habitat:** Mixed Dipterocarp Forest; **Elevation:** 63 m asl; **Collections:** L.1755655, L.1755654.

***Fissistigma kingii* (Boerl.) Burkill** (Bull. Misc. Inform. Kew 1935: 317 [1935]) [Native] {NE} **Growth Form:** Creeper; **Location:** Temburong; **Habitat:** Mixed Dipterocarp Forest; **Elevation:** 371 m asl; **Collections:** L.1754157.

***Friesodielsia latifolia* (Hook.f. & Thomson) Steenis** (Blumea 12: 360 [1964]) [Non-Native] {NE} **Growth Form:** Climber; **Location:** Belait; **Habitat:** Peat Swamp Forest; **Elevation:** 15 m asl; **Collections:** L0333695.

***Goniothalamus bygravei* I.M.Turner & R.M.K.Saunders** (Nordic J. Bot. 26: 329 [2009]) [Native] {NE} **Growth Form:** Small tree; **Location:** Belait; **Habitat:** Mixed Dipterocarp Forest; **Elevation:** 331 m asl; **Collections:** L.3965635, L.3965637, L.3965632, L.3965636, L.3965633, L.3965641, L.3965634, L.3965598, L.3965657, L.3965607, L.3965656, L.3965599, L.3965605, L.3965600.

Mitrephora maingayi (Natuurk. Tijdschr. Ned.-Indië 31: 12 [1870]) [Native] {NE} **Growth Form:** Tree; **Location:** Temburong; **Habitat:** Mixed Dipterocarp Forest **Elevation:** 78 m asl; **Collections:** L.1757167.

***Monocarpia borneensis* Mols & Kessler** (Bot. Jahrb. Syst. 122: 235 [2000]) [Native] {NE} **Growth Form:** Subcanopy Tree; **Location:** Belait; **Habitat:** Mixed Dipterocarp Forest; **Elevation:** 289 m asl; **Collections:** L.1762375.

***Monocarpia kalimantanensis* P.J.A.Kessler** (Rheedea 3: 73 [1993]) [Native] {NE} **Local name:** Banitan, Karai-man, Mehawai, Pisang-pisang, Semukau; **Growth Form:** Subcanopy tree; **Location:** Belait; **Habitat:** Mixed Dipterocarp Forest; **Elevation:** 11-81 m asl; **Collections:** L.2067122, L.1757782, L.1757771.

Polyalthia charitopoda **I.M.Turner** (Folia Malaysiana 9: 90 [2008]) [Native] {NE} **Growth Form:** Understory tree; **Location:** Belait; **Habitat:** Mixed Dipterocarp Forest; **Elevation:** 101-190 m asl; **Collections:** barcode-00295751, K000381536.

Polyalthia miliusoides **I.M.Turner** (Folia Malaysiana 9: 86 [2008]) [Native] {NE} **Growth Form:** Shrub; **Location:** Tutong; **Habitat:** Mixed Dipterocarp Forest; Elevation**:** 52 m asl; **Collections:** L.1767113.

Popowia hirta Miq. (Ann. Mus. Bot. Lugduno-Batavi 2: 21 [1865]) [Native] {NE} **Growth Form:** Understory tree; **Location:** Temburong; **Habitat:** Mixed Dipterocarp Forest; **Elevation:** 180 m asl; **Collections:** 09-2955, 01-3216, 04-1703, 10-2900, L.1759545, L.1759549.

Popowia odoardi **Diels** (Notizbl. Bot. Gart. Berlin-Dahlem 11: 82 [1931]) [Native] {NE} **Local name:** Pisang-pisang; **Growth Form:** Understory tree; **Location:** Belait; **Habitat:** Peat Swamp Forest; Elevation**:** 16 m asl; **Collections:** L.1759476.

Pseuduvaria bruneiensis **Y.C.F.Su & R.M.K. Saunders** (Syst. Bot. Monogr. 79: 62 [2006]) [Native] {NE} **Growth Form:** Tree; **Location:** Temburong; **Habitat:** Mixed Dipterocarp Forest; **Elevation:** 317-356 m asl; **Collections:** 2377750, L0046964, L.1764397, K000574577, S14-54677.

Xylopia kuchingensis **I.M.Turner & D.M.Johnson** (Harvard Pap. Bot. 14: 129 [2009]) [Native] {NE} **Growth Form:** Tree; **Location:** Belait; **Habitat:** Peat Swamp forest; **Elevation:** 43 m asl; **Collections:** L.1775350.

APOCYNACEAE

Alyxia mujongensis **Markgr.** (Blumea 23: 384 [1977]) [Native] {NE} **Growth Form:** N/A; **Location:** Temburong; **Habitat:** Montane Forest, **Elevation:** 995 m asl; **Collections:** L.2711377, L.2711378, WAG.1475212, WAG.1475213.

Anodendron coriaceum **(Blume) Miq.** (Fl. Ned. Ind. 2: 455 [1857]) [Native] {CR} **Growth Form:** Climber; **Location:** Belait; **Habitat:** Peat Swamp Forest; **Elevation:** 25 m asl; **Collections:** L.3718352.

Chilocarpus rostratus **Markgr.** (Blumea 19: 165 [1971]) [Native] {NE} **Growth Form:** Climber; **Location:** Tutong-Temburong; **Habitat:** Mixed Dipterocarps Forest; **Elevation:** 9-850 m asl; **Collections:** WAG.1491590, WAG.1491591, WAG.1491592, L.2712580, L.2712575.

Dischidia cochleata **Blume** (Bijdr. 1060 [1826]) [Native] {NE} **Growth Form:** Epiphyte; **Location:** Belait; **Habitat:** Mixed Dipterocarps Forest; **Elevation:** 173 m asl; **Collections:** L.3739366.

Dischidia complex **Griff.** (Not. Pl. Asiat. 4: 50 [1854]) [Native] {NE} **Growth Form:** Epiphyte; **Location:** Belait; **Habitat:** Mixed Dipterocarps Forest; **Elevation:** 19 m asl; **Collections:** L.3739367.

Dischidia punctata **(Blume) Decne.** (Prodr. 8: 631 [1844]) [Native] {NE} **Growth Form:** Epiphyte; **Location:** Belait; **Habitat:** Mixed Dipterocarps Forest; **Elevation:** 69 m asl; **Collections:** L.3739329.

Hoya imperialis **Lindl.** (Edwards's Bot. Reg. 32: t. 68 [1846]) [Native] {NE} **Growth Form:** Climber; **Location:** Tutong; **Habitat:** Mixed Dipterocarps Forest; **Elevation:** 53-358 m asl; **Collections:** L.2726621, L.2726625, L.2726624, L.2726622.

Strophanthus singaporianus **(Wall. ex G.Don) Gilg** (Monogr. Afrik. Pflanzen-Fam. 7: 11 [1903]) [Native] {NE} **Growth Form:** Climber, Shrub; **Location:** Belait; **Habitat:** Peat Swamp Forest; **Elevation:** 21 m asl; **Collections:** L.4284180, L.4284042, L.2708044, WAG.1605701.

AQUIFOLIACEAE

Ilex tamii **T.R. Dudley** (Hollies 244 [1997]) [Non-Native?] {NE} **Growth Form:** Small tree; **Location:** Temburong; **Habitat:** Montane Forest; **Elevation:** 1151 m asl; **Collections:** L.2269305, 2823900.

ARACEAE

Amorphophallus borneensis **(Engl.) Engl. & Gehrm.** (Pflanzenr. IV, 23C: 79 [1911]) [Native] {NE} **Common Name:** Voodoo Plant, Voodoo Lily, **Growth Form:** Tuberous herb; **Location:** Belait; **Habitat:** Secondary Forest; **Elevation:** 31 m asl; **Collections:** L.1409091.

Hapaline brownii **Hook.f.** (Fl. Brit. India 6: 521 [1893]) [Non-Native] {NE} **Growth Form:** Herb; **Location:** Temburong; **Habitat:** Disturbed mixed dipterocarp forest; **Elevation:** 318 m asl; **Collections:** L.1416226.

Hestia longifolia **S.Y.Wong & P.C.Boyce** (Bot. Stud. (Taipei) 51: 252 [2010]) [Native] {NE} **Growth Form:** Herb; **Location:** Muara-Belait; **Habitat:** Disturbed mixed dipterocarp forest; **Elevation:** 22-36 m asl; **Collections:** L.1422654, L.1422656.

Ooia kinabaluensis **S.Y.Wong & P.C.Boyce** (Bot. Stud. (Taipei) 51: 548 [2010]) [Native] {NE} **Growth Form:** Herb; **Location:** Temburong; **Habitat:** Montane Forest; **Elevation:** 995 m asl; **Collections:** L.1417512.

Pedicellarum paiei **M.Hotta** (Acta Phytotax. Geobot. 27: 61 [1976]) [Native] {NE} **Growth Form:** Climber; **Location:** Temburong; **Habitat:** Mixed Dipterocarp Forest; **Elevation:** 318 m asl; **Collections:** L.1417228.

Pothos chinensis **(Raf.) Merr.** (J. Arnold Arbor. 29: 210 [1948]) [Non-native] {NE} **Growth Form:** Liana; **Location:** Temburong; **Habitat:** Mixed Dipterocarp Forest; **Elevation:** 171 m asl; **Collections:** E00348661.

Pothos lancifolius **Hook.f.** (Fl. Brit. India 6: 554 [1893]) [Non-native] {NE} **Growth Form:** Climber; **Location:** Temburong; **Habitat:** Mixed Dipterocarp Forest; **Elevation:** 526 m asl; **Collections:** L.1418135.

Rhaphidophora cylindrosperma **Engl. & K.Krause** (Pflanzenr. IV, 23B: 28 [1908]) [Native] {NE} **Growth Form:** Climber; **Location:** Belait; **Habitat:** Secondary Forest; **Elevation:** 14 m asl; **Collections:** L.1422062, L.1422061.

Rhaphidophora latevaginata **M.Hotta** (Acta Phytotax. Geobot. 22: 4 [1966]) [Native] {NE} **Growth Form:** Climber; **Location:** Temburong; **Habitat:** Mixed Dipterocarp Forest; **Elevation:** 318 m asl; **Collections:** L.1422416.

Schismatoglottis asperata **Engl.** (Bull. Soc. Tosc. Ortic. 4: 297 [1879]) [Native] {NE} **Growth Form:** Herb; **Location:** Belait; **Habitat:** Mixed Dipterocarp Forest; **Elevation:** 147 m asl; **Collections:** L.1422876.

Schismatoglottis motleyana **(Schott) Engl.** (Pflanzenr. IV, 23Da: 102 [1912]) [Native] {NE} **Growth Form:** Herb; **Location:** Belait; **Habitat:** Mixed Dipterocarp Forest, Secondary Forest; **Elevation:** 31-147 m asl; **Collections:** L.1422668, L.1422664, L.1422666.

Schismatoglottis patentinervia **Engl.** (Pflanzenr. IV, 23Da: 90 [1912]) [Native] {NE} **Growth Form:** Robust herb; **Location:** Belait; **Habitat:** Secondary Forest; **Elevation:** 31 m asl; **Collections:** L.1422638.

Schismatoglottis pectinervia **A.Hay** (Telopea 9: 138 [2000]) [Native] {NE} **Growth Form:** Herb; **Location:** Temburong; **Habitat:** Mixed Dipterocarp Forest; **Elevation:** 171 m asl; **Collections:** K000291717.

Schismatoglottis petri **A.Hay** (Telopea 9: 162 [2000]) [Native] {NE} **Growth Form:** Lithophytic herb; **Location:** Belait; **Habitat:** Mixed Dipterocarp Forest; **Elevation:** 89 m asl; **Collections:** K000291720.

Schismatoglottis trifasciata **Engl.** (Pflanzenr. IV, 23Da: 106 [1912]) [Native] {NE} **Growth Form:** Herb; **Location:** Tutong; **Habitat:** Mixed Dipterocarp Forest; **Elevation:** 53 m asl; **Collections:** L.1422742, L.1422741.

Scindapsus treubii **Engl.** (Bot. Jahrb. Syst. 25: 13 [1898]) [Native] {NE} **Growth Form:** Perennial herb; **Location:** Temburong; **Habitat:** Mixed Dipterocarp Forest; **Elevation:** 318 m asl; **Collections:** L.1418713.

ARECACEAE

Calamus maiadum **J.Dransf.** (Rattans Brunei 193 1997 [1998]) [Native] [Endemic] {NE} **Growth Form:** Climber; **Location:** Belait; **Habitat:** Mixed Dipterocarps Forest; **Elevation:** 89 – 191 m asl; **Collections:** K000114121- JD7027, K000114120-6593.

Calamus semoi **Becc.** *(Ann. Roy. Bot. Gard. (Calcutta) 11(App.): 129 [1913]*) [Native] {NE} **Growth Form:** Climber; **Location:** Temburong; **Habitat:** Mixed Dipterocarp Forest; **Elevation:** 716 m asl; **Collections:** K000114124-7117, K000114125-1407.

Licuala olivifera **Becc.** (*Malesia 3: 78 [1889]*) [Native] {NE} **Growth Form:** Tree; **Location:** Tutong-Temburong; **Habitat:** Secondary Forest Mixed Dipterocarp Forest; **Elevation**: 107 – 348 m asl; **Collections:** L.4179734, L.4179735, L.4179733, K000113146- BRUN17368, 2319669-900.

BIGNONIACEAE

Dolichandrone spathacea **(L.f.) Seem.** (J. Bot. 1: 226 [1863]) [Native] {CR} **Growth Form:** Understory tree; **Location:** Belait; **Habitat:** Peat-swamp Forest; **Elevation:** 9 m asl; **Collections:** QRS 57700.1-394, QRS 57701.3-3945.

BURSERACEAE

Canarium latistipulatum **Ridl.** (Bull. Misc. Inform. Kew 1930: 81 [1930]) [Native] {NE} **Growth Form:** Tree; **Location:** Temburong; **Habitat:** Mixed Dipterocarp Forest; **Elevation:** 112 m asl; **Collections:** 1415170-14463.

Canarium odontophyllum **Miq.** (Fl. Ned. Ind. 1(2 Suppl.): 525 [1859]) [Native] {NE} **Growth Form:** Tree; **Location:** Temburong; **Habitat:** Mixed Dipterocarp Forest; **Elevation:** 56 m asl; **Collections:** 1415172-14448.

Canarium sarawakanum **Kochummen** (Sandakania 5: 73 [1994]) [Native] {NE} **Growth Form:** Tree; **Location:** Temburong; **Habitat:** Mixed Dipterocarp Forest; **Elevation:** 224 m asl; **Collections:** 1415291-14454.

Dacryodes kingii **(Engl.) Kalkman** (Fl. Males. 5: 224 [1956]) [Non-native] {NE} **Growth Form:** Shrub, Tree; **Location:** Brunei Temburong; **Habitat:** Mixed Dipterocarp Forest; **Elevation:** 147 – 186 m asl; **Collections:** 1415197-14428, 1415198-14457.

CENTROPLACACEAE

***Bhesa indica* (Bedd.) Ding Hou** (Blumea, Suppl. 4: 152 [1958]) [Non-native] {NE} **Growth Form:** Tree; **Location:** Tutong-Belait; **Habitat:** Secondary Forest, Mixed Dipterocarp Forest; **Elevation:** 23 – 149 m asl; **Collections:** L.3750816, L.3750449, L.3748479, L.3748347, L.3748478, L.2282693, L.2282692, L.3750811.

CLUSIACEAE

***Garcinia acuminata* Planch. & Triana** (Ann. Sci. Nat., Bot. IV, 14: 355 [1860]) [Non-native] {NE} **Growth Form:** Tree; **Location:** Tutong; **Habitat:** Secondary Forest; **Elevation:** 32 m asl; **Collections:** L.2417671.

COMMELINACEAE

***Dictyospermum conspicuum* (Blume) J.K.Morton** (J. Linn. Soc., Bot. 59: 436 [1966]) [Non-native] {NE} **Growth Form:** Herb; **Location:** Temburong; **Habitat:** Mixed Dipterocarp Forest; **Elevation:** 109 m asl; **Collections:** L.3757617.

CONNARACEAE

***Ellipanthus tomentosus* Kurz** (J. Asiat. Soc. Bengal, Pt. 2, Nat. Hist. 41(2): 305 [1872]) [Native] {CR} **Growth Form:** Shrub; **Location:** Belait; **Habitat:** Secondary Forest; **Elevation:** 92 m asl; **Collections:** L.3762477, L.3762476.

CONVULVULACEAE

***Ipomoea mauritiana* Jacq.** (Collectanea 4: 216 [1790]) [Non-native] {NE} **Growth Form:** Climber; **Location:** Belait; **Habitat:** Secondary Forest; **Elevation:** 22 m asl; **Collections:** L.2724985, 2812749.

CUCURBITACEAE

***Trichosanthes beccariana* Cogn** (Monogr. Phan. 3: 380 [1881]) [Non-native] {NE} **Growth Form:** Climber; **Location:** Temburong; **Habitat:** Mixed Dipterocarp Forest, Peat-swamp Forest; **Elevation:** 2 – 171 m asl; **Collections:** barcode-00261847-788, L.2988407.

CYPERACEAE

***Isolepis setacea* (L.) R.Br.** (Prodr. Fl. Nov. Holl. 222 [1810]) [Non-native] {NE} **Growth Form:** Herb; **Location:** Temburong; **Habitat:** Mixed Dipterocarp Forest; **Elevation:** 569 m asl; **Collections:** L.1402789.

***Scirpus radicans* Schkuhr** (Ann. Bot. (Usteri) 4: 49 [1793]) [Non-native] {NE} **Growth Form:** Herb; **Location:** Belait; **Habitat:** Secondary Forest; **Elevation:** 33 m asl; **Collections:** 2377761-5668.

DIOSCOREACEAE

***Dioscorea moultonii* Prain & Burkill** (Bull. Misc. Inform. Kew 1925: 62 [1925]) [Native] {NE} **Growth Form:** Tuberous herbaceous vine; **Location:** Tutong; **Habitat:** Mixed Dipterocarp Forest; **Elevation:** 43 m asl; **Collections:** K001144440-16467.

ELAEOCARPACEAE

***Elaeocarpus beccarii* DC.** (Bull. Herb. Boissier II, 3: 367 [1903]) [Native] {NE} **Local name:** Perdu; **Growth Form:** Mid-canopy tree; **Location:** Belait; **Habitat:** Secondary Forest; **Elevation:** 29 m asl; **Collections:** CNS 143540.1-264.

***Elaeocarpus cristatus* Coode** (Kew Bull. 53: 88 [1998]) [Native] {NE} **Growth Form:** N/A; **Location:** Temburong; **Habitat:** Mixed Dipterocarp Forest; **Elevation:** 171 m asl; **Collections:** E00679257-833.

***Elaeocarpus cumingii* Turcz** (Bull. Soc. Imp. Naturalistes Moscou 19(2): 491 [1846]) [Non-native] {NE} **Growth Form:** Tree; **Location:** Temburong; **Habitat:** Mixed Dipterocarp Forest; **Elevation:** 850 m asl; **Collections:** L.3790356.

***Elaeocarpus glaber* Blume** (Catalogus 78 [1823]) [Native] {NE} **Local name:** Bangkinang, Bengkinang, Bengkining hutan, Kemurik, Pabom, Surugam, Tamang; **Growth Form:** Tree; **Location:** Tutong-Belait-Temburong; **Habitat:** Mixed Dipterocarp Forest, Secondary Forest; **Elevation:** 26 – 63 m asl; **Collections:** A.846, A.1366, A.1350, A.1126, L.3788950, CNS 143532.1-234.

***Elaeocarpus jugahanus* Coode** (Kew Bull. 53: 118 [1998]) [Native] {NE} **Growth Form:** Tree; **Location:** Temburong; **Habitat:** Mixed Dipterocarp Forest, Montane Forest; **Elevation:** 850 – 1693 m asl; **Collections:** L.3790237, L.3790593, L.3790236, L 0537785.

***Elaeocarpus palembanicus* (Miq.) Corner** (Gard. Bull. Straits Settlem. 10: 323 [1939]) [Native] {NE} **Growth Form:** Tree; **Location:** Tutong-Belait; **Habitat:** Mixed Dipterocarp Forest; **Elevation:** 18-63 m asl; **Collections:** A.463, A.707, A.1105, L.3790596.

***Elaeocarpus valetonii* Hochr.** (Pl. Bogor. Exs. 29 [1904]) [Native] {NE} **Local name:** Kungkurad, Peredu, Sengkurat, Tamang; **Growth Form:** Mid-canopy tree; **Location:** Temburong; **Habitat:** Mixed Dipterocarp Forest; **Elevation:** 145 m asl; **Collections:** CNS 143538.1-261.

EUPHORBIACEAE

***Acalypha hispida* Burm.f.** (Fl. Indica 303 [1768]) [Non-native] {NE} **Local name:** Pokok ekor kucing; **Growth Form:** Shrub; **Location:** Belait; **Habitat:** Keranga Forest; **Elevation:** 17 m asl; **Collections:** L.2180378.

***Croton carrii* Airy Shaw** (Kew Bull. 27: 82 [1972]) [Non-native] {NE} **Growth Form:** N/A; **Location:** Belait; **Habitat:** Secondary Forest; **Elevation:** 76 m asl; **Collections:** L.2210750, L.2210749.

***Euphorbia atoto* G.Forst.** (Fl. Ins. Austr. 36 [1786]) [Non-native] {NE} **Growth Form:** Shrub; **Location:** Belait; **Habitat:** Secondary Forest; **Elevation:** 7 m asl; **Collections:** L.2215929, L.2215928.

***Hancea stipularis* (Airy Shaw) S.E.C.Sierra, Kulju & Welzen** (Blumea 52: 364 [2007]) [Native] {NE} **Growth Form:** Tree; **Location:** Temburong; **Habitat:** Mixed Dipterocarp Forest; **Elevation:** 572 m asl; **Collections:** L.2223510.

***Macaranga bancana* (Miq.) Müll.Arg.** (Prodr. 15(2): 990 [1866]) [Native] {NE} **Local name:** Benuah, Layang-layang, Purang, Sedaman, Sedaman laki; **Growth Form:** Understory tree; **Location:** Tutong; **Habitat:** Secondary Forest; **Elevation:** 31 m asl; **Collections:** L.2236791.

Macaranga didymocarpa **Whitmore** (Kew Bull. 39: 607 [1984]) [Native] {NE} **Growth Form:** Tree; **Location:** Tutong; **Habitat:** Secondary Forest; **Elevation:** 32 m asl; **Collections:** L.2241706, L.2241708, L.2241707, L.2241711.

Macaranga havilandii **Airy Shaw** (Kew Bull. 23: 112 [1969]) [Native] {NE} **Growth Form:** Small tree; **Location:** Tutong-Belait; **Habitat:** Mixed Dipterocarp Forest, Peat-swamp Forest; **Elevation:** 40 – 107 m asl; **Collections:** L.2230845, L.2230849, L.2230848.

Mallotus aureopunctatus **(Dalzell) Müll.Arg.** (Prodr. 15(2): 973 [1866]) [Non-native] {NE} **Growth Form:** Tree; **Location:** Belait; **Habitat:** Mixed Dipterocarp Forest, **Elevation:** 24 m asl; **Collections:** L.3784712.

Mallotus connatus **M.Aparicio** (Blumea 52: 54 [2007]) [Native] {NE} **Growth Form:** N/A; **Location:** Belait; **Habitat:** Peat-swamp Forest; **Elevation:** 33 m asl; **Collections:** L.2243537.

Mallotus leucodermis **Hook.f.** (Fl. Brit. India 5: 441 [1887]) [Native] {NE} **Local name:** Galungan, Galunggung, Perupuk, Perupuk Batu; **Growth Form:** Mid-Canopy Tree**; Location:** Belait-Temburong; **Habitat:** Keranga Forest, Mixed Dipterocarp Forest, Submontane Forest; **Elevation:** 111 - 180 m asl; **Collections:** CP-1042, CAH-318, 10-5187, 05-5045.

Ptychopyxis grandis **Airy Shaw** (Kew Bull. 14: 367 [1960]) [Native] {NE} **Growth Form:** Treelet, Tree; **Location:** Tutong; **Habitat:** Secondary Forest, Mixed Dipterocarp Forest; **Elevation:** 64 m asl; **Collections:** L0584266, L.2253417.

Trigonostemon aurantiacus **(Kurz ex Teijsm. & Binn.) Boerl.** (Handl. Fl. Ned. Ind. 3(1): 284 [1900]) [Non-native] {NE} **Growth Form:** N/A; **Location:** Temburong; **Habitat:** Mixed Dipterocarp Forest; **Elevation:** 318 m asl; **Collections:** L.2259027.

FABACEAE

Bauhinia lambiana **Baker f.** (J. Bot. 76: 19 [1938]) [Native] {NE} **Growth Form:** Tendrilled liana; **Location:** Belait; **Habitat:** Mixed Dipterocarp Forest; **Elevation:** 87 m asl; **Collections:** L.1976121.

Crudia caudata **Prain** (J. Asiat. Soc. Bengal, Pt. 2, Nat. Hist. 66: 219 [1897]) [Native] {NE} **Growth Form:** N/A; **Location:** Belait; **Habitat:** Secondary Forest; **Elevation:** 31 m asl; **Collections:** L.3884887.

Dalbergia bintuluensis **Sunarno & Ohashi** (J. Jap. Bot. 72: 202 [1997]) [Native] {NE} **Growth Form:** N/A; **Location:** Tutong; **Habitat:** Secondary Forest; **Elevation:** 14 m asl; **Collections:** L.1984399.

Dalbergia borneensis **Prain** (J. Asiat. Soc. Bengal, Pt. 2, Nat. Hist. 70: 44 [1901]) [Native] {NE} **Growth Form:** N/A; **Location:** Muara; **Habitat:** Secondary Forest; **Elevation:** 22 m asl; **Collections:** L.1984403

Derris montana **Benth.** (F.A.W.Miquel, Pl. Jungh.: 253 [1852]) [Native] {NE} **Growth Form:** Liana; **Location:** Belait; **Habitat:** Peat-swamp Forest; **Elevation:** 21 m asl; **Collections:** L.2039719.

Millettia borneensis **Adema** (Blumea 45: 407 [2000]) [Native] {NE} **Local Name:** Babai, Biansu, Binsu, Marbahai, Merbatrai, Tonudou. **Growth Form:** Upper canopy rree; **Location:** Belait; **Habitat:** Peat-swamp Forest; **Elevation:** 21 m asl; **Collections:** L.2049382.

Mimosa pigra **L.** (Cent. Pl. I: 13 [1755]) [Non-native] {LC} **Growth Form:** Woody invasive shrub; **Location:** Belait; **Habitat:** Peat-swamp Forest; **Elevation:** 6 m asl; **Collections:** L.2041626.

Ormosia stipulacea **Meeuwen** (Reinwardtia 6: 234 [1962]) [Native] {NT} **Growth Form:** N/A; **Location:** Tutong-Belait; **Habitat:** Mixed Dipterocarp forest, Secondary Forest; **Elevation:** 10 – 43 m asl; **Collections:** L.3885408, K000628248, K000628247.

Spatholobus auricomus **Ridd.-Num.** (Reinwardtia 10: 165 [1985]) [Native] {NE} **Growth Form:** N/A; **Location:** Tutong; **Habitat:** Mixed Dipterocarp forest; **Elevation:** 47 m asl; **Collections:** L.3885390.

FAGACEAE

Castanopsis paucispina **Soepadmo** (Reinwardtia 7: 398 [1968]) [Native] {NE} **Growth Form:** Tree; **Location:** Belait; **Habitat:** Mixed Dipterocarp Forest; **Elevation:** 87 m asl; **Collections:** L.3792180.

Lithocarpus mariae **Soepadmo** (Reinwardtia 8: 258 [1970]) [Native] {NE} **Growth Form:** Tree; **Location:** Tutong; **Habitat:** Secondary Forest, Mixed Dipterocarp Forest; **Elevation:** 44 m asl; **Collections:** L.1570350.

GENTIANACEAE

Fagraea ceilanica **Thunb.** (Kongl. Vetensk. Acad. Nya Handl. 3: 132 [1782]) [Native] {NE} **Local Name:** Akar sempirai, Kayu alah, Penungpang, Salang mapit, Seraya. **Growth Form:** Shrub, Liana, Small tree; **Location:** Belait; **Habitat:** Mixed Dipterocarp Forest; **Elevation:** 65 m asl; **Collections:** L.2687914.

Fagraea gardenioides **Ridl.** (J. Fed. Malay States Mus. 5: 42 [1914]) [Non-native] {NE} **Growth Form:** N/A; **Location:** Belait; **Habitat:** Secondary forest; **Elevation:** 7 m asl; **Collections:** L.2674651.

GESNERIACEAE

Cyrtandra phoenicoides **Hilliard & B.L.Burtt** (Edinburgh J. Bot. 61: 177 [2004]) [Native] {NE} **Growth Form:** Herb, Shrub; **Location:** Temburong; **Habitat:** Mixed Dipterocarp Forest; **Elevation:** 268 m asl; **Collections:** E00188886-811, E00188887-811.

Cyrtandra splendens **C.B.Clarke** (Monogr. Phan. 5: 209 [1883]) [Native] {NE} **Growth Form:** Herb, Shrub; **Location:** Belait; **Habitat:** Secondary Forest; **Elevation:** 115 m asl; **Collections:** L.3794218.

GNETACEAE

Gnetum acutum **Markgr.** (in Fl. Males. 6: 947 [1972]) [Native] {VU} **Growth Form:** Liana; **Location:** Tutong-Belait; **Habitat:** Peat-swamp forest, Keranga Forest; **Elevation:** 19 – 46 m asl; **Collections:** L.3875127, K000454208, K000454206, K000454207, K000454210, K000454211, K000454209.

Gnetum diminutum **Markgr.** (Bull. Jard. Bot. Buitenzorg III, 10: 483 [1930]) [Native] {NT} **Growth Form:** Liana; **Location:** Tutong-Belait; **Habitat:** Peat-swamp forest, Keranga Forest; **Elevation:** 7 – 53 m asl; **Collections:** K000454620, K000454619, K000458511.

Gnetum raya **Markgr.** (Blumea 14: 284 [1967]) [Native] {LC} **Growth Form:** Liana; **Location:** Tutong; **Habitat:** Secondary Forest; **Elevation:** 75 m asl; **Collections:** K000454873.

LAMIACEAE

***Clerodendrum disparifolium* Blume** (Bijdr. 809 [1826]) [Native] {NE} **Local Name:** Patah ayam, Petah ringan, Sipang; **Growth Form:** Shrub; **Location:** Muara-Belait; **Habitat:** Mixed Dipterocarp Forest, Secondary Forest, Peat-swamp Forest, Keranga Forest; **Elevation:** 5 – 191 m asl; **Collections:** K000785325-890726, K000785560-22, K000785557-7615, K000785561-2419b, K000785559-BRUN665, K000785556-209, K000853733-15393.

***Teijsmanniodendron bullatum* Rusea** (Kew Bull. 64: 595 2009 [2010]) [Native] {NE} **Growth Form:** Herb; **Location:** Tutong-Belait; **Habitat:** Mixed Dipterocarp Forest; **Elevation:** 24 – 188 m asl; **Collections:** L.2754840, L.2754839, K000721852-17471, K000721848-7827, K000721854-6828, K000721855-256, K000721853-16469, K000721856-253.

LAURACEAE

***Cryptocarya griffithiana* Wight** (Icon. Pl. Ind. Orient. 5: t. 1830 [1852]) [Native] {NE} **Local name:** Madang, Medang; **Growth Form:** Subcanopy tree; **Location:** Muara-Tutong-Belait; **Habitat:** Peat-swamp Forest, Mixed Dipterocarp Forest, Secondary Forest; **Elevation:** 17 – 41 m asl; **Collections:** L.3903636, L.3903840, L.3908199, L.3887626, L.3903833.

Eusideroxylon zwageri Teijsm. & Binn. (Natuurk. Tijdschr. Ned.-Indië 25: 292 [1863]) [Native] {VU} **Local Name:** Belian, Belian timun, Betian, Talion bening, Tebelian geriting, Telianoii, Teluyan, Ulin, Ulin bening, Ulion; **Growth Form:** Mid-canopy tree; **Location:** Belait; **Habitat:** Mixed Dipterocarp Forest; **Elevation:** 81 m asl; **Collections:** QRS 102256.1-14965, QRS 102257.1-14966, QRS 104233.2-14965.

***Litsea fulva* (Blume) Villar** (Blanco, Fl. Philipp. ed. III Nov. App. 181 [1880]) [Native] {NE} **Growth Form:** Tree; **Location:** Temburong; **Habitat:** Mixed Dipterocarp Forest, Montane Forest; **Elevation:** 47 – 781 m asl; **Collections:** 19-3471, 01-0911, 01-0183, L.3883431, L.3912887, L.3912882, L.3912888.

LORANTHACEAE

***Scurrula parasitica* L.** (Sp. Pl.: 110 [1753]) [Non-native] {NE} **Growth Form:** Parasitic shrub; **Location:** Tutong; **Habitat:** Mixed Dipterocarp Forest; **Elevation:** 358 m asl; **Collections:** L.3890694, L.3890695, L.3923679.

LOWIACEAE

***Orchidantha longiflora* (Scort.) Ridl.** (Fl. Malay Penins. 4: 292 [1924]) [Non-native] {NE} **Growth Form:** Perennial herb; **Location:** Belait; **Habitat:** Secondary Forest; **Elevation:** 31 m asl; **Collections:** L 0673560, L 0673559.

MALVACEAE

***Clappertonia ficifolia* (Willd.) Decne.** (J.P.B.Delessert, Icon. Sel. Pl. 5: 1 [1846]) [Non-native] {NE} **Growth Form:** Shrub; **Location:** Belait; **Habitat:** Secondary Forest; **Elevation:** 45 m asl; **Collections:** L.4204872, L.4204845.

***Sterculia lanceolata* Cav.** (Diss. 6: 287, pl. 143, f. 1 [1788]) [Non-native] {NE} **Growth Form:** Tree; **Location:** Temburong; **Habitat:** Secondary Forest; **Elevation:** 43 m asl; **Collections:** L.4146549.

MARANTACEAE

Schumannianthus benthamianus **(Kuntze) Veldkamp & I.M.Turner** (Kew Bull. 71(4)-47: 2 [2016]) [Native] {NE} **Growth Form:** Herb; **Location:** Belait; **Habitat:** Peat-swamp Forest; **Elevation:** 10 m asl; **Collections:** L.3916274.

MELIACEAE

Aglaia rivularis **Merr.** (Univ. Calif. Publ. Bot. 15: 125 [1929]) [Native] {VU} **Growth Form:** Tree; **Location:** Temburong; **Habitat:** Mixed Dipterocarp Forest; **Elevation:** 348 m asl; **Collections:** L.2144165, L.2150070, L.2150071, L.2150014.

MENISPERMACEAE

Diploclisia glaucescens **(Blume) Diels** (H.G.A.Engler (ed.), Pflanzenr., IV, 94: 225 [1910]) [Non-native] {NE} **Growth Form:** Climber; **Location:** Temburong; **Habitat:** Mixed Dipterocarp Forest; **Elevation:** 318 m asl; **Collections:** L.3913893, L.3913892.

MORACEAE

Ficus callosa **Willd.** (Mém. Acad. Roy. Sci. Hist. (Berlin) 1798: 102 [1798]) [Native] {NE} **Growth Form:** Tree; **Location:** Tutong; **Habitat:** Secondary Forest; **Elevation:** 14 m asl; **Collections:** L.1605990, 2791281.

Ficus depressa **Blume** (Catalogus: 35 [1823]) [Native] {NE} **Growth Form:** Tree; **Location:** Temburong; **Habitat:** Mixed Dipterocarp Forest; **Elevation:** 149 m asl; **Collections:** L.1606376, 2796641.

Ficus eumorpha **Corner** (Gard. Bull. Singapore 17: 439 [1960]) [Native] {NE} **Growth Form:** Shrub; Tree; **Location:** Temburong; **Habitat:** Montane Forest; **Elevation:** 1693 m asl; **Collections:** L.1601143, 2799122.

Ficus heteropleura **Blume** (Bijdr. Fl. Ned. Ind.: 466 [1825]) [Native] {NE} **Growth Form:** Climber; **Location:** Belait; **Habitat:** Mixed Dipterocarp Forest; **Elevation:** 18 m asl; **Collections:** L.1602464, 2804137.

Ficus trichocarpa **Blume** (Bijdr. Fl. Ned. Ind.: 448 [1825]) [Native] {CR} **Growth Form:** Climber; **Location:** Temburong; **Habitat:** Mixed Dipterocarp Forest; **Elevation:** 348 m asl; **Collections:** L.1613092, 2835380.

MYRTACEAE

Eugenia media **(Sagot) Nied. ex T.Durand & B.D.Jacks.** (Index Kew. Suppl. 1: 164 [1902]) [Non-native] {NE} **Growth Form:** N/A; **Location:** Temburong; **Habitat:** Mixed Dipterocarp Forest; **Elevation:** 348 m asl; **Collections:** L.2508498.

Syzygium claviflorum **A.M.Cowan & Cowan** (Trees N. Bengal: 67 [1929]) [Native] {NE} **Local name:** Jambu arang, gelam, lenceh, Obah, Ubah; **Growth Form:** Canopy tree; **Location:** Tutong; **Habitat:** Secondary Forest; **Elevation:** 14 m asl; **Collections:** K000259546, K000259547.

Syzygium creaghii **(Ridl.) Merr. & L.M.Perry** (Mem. Amer. Acad. Arts, n.s., 18: 164 [1939]) [Native] {NE} **Local name:** Obah, Obah paya; **Growth Form:** Canopy tree; **Location:** Belait-Temburong; **Habitat:** Mixed Dipterocarp Forest, Peat-swamp forest; **Elevation:** 31 – 850 m asl; **Collections:** L.2507640, L 0526841, L.2507639, L.2507641.

***Syzygium cymosum* (Lam.) DC.** (Prodr. 3: 259 [1828]) [Non-native] {NE} **Growth Form:** Tree; **Location:** Temburong; **Habitat:** Mixed Dipterocarp Forest; **Elevation:** 530 m asl; **Collections:** L.2507503.

***Syzygium jambos* (L.) Alston** (H.Trimen, Handb. Fl. Ceylon 6(Suppl.): 115 [1931]) [Native] {NE} **Growth Form:** Tree; **Location:** Temburong; **Habitat:** Mixed Dipterocarp Forest, Secondary forest; **Elevation:** 81 – 196 m asl; **Collections:** L.3932135, L.3932088.

***Syzygium panzeri* Merr. & L.M.Perry** (Mem. Amer. Acad. Arts, n.s., 18: 162 [1939]) [Native] {NE} **Growth Form:** N/A; **Location:** Temburong; **Habitat:** Mixed Dipterocarp Forest; **Elevation:** 850 m asl; **Collections:** L.2529736.

***Xanthomyrtus flavida* (Stapf) Diels** (Bot. Jahrb. Syst. 57: 366 [1922]) [Native] {NE} **Growth Form:** N/A; **Location:** Temburong; **Habitat:** Montane Forest; **Elevation:** 1152 m asl; **Collections:** L.3919133.

OLACACEAE

***Ximenia Americana* L.** (Sp. Pl. 1193 [1753]) [Non-native] {NE} **Growth Form:** Shrub, Small tree; **Location:** Tutong; **Habitat:** Secondary Forest; **Elevation:** N/A; **Collections:** L.4163880.

OLEACEAE

***Jasminum longipetalum* King&Gamble** (J. Asiat. Soc. Bengal, Pt. 2, Nat. Hist. 74: 262 [1905]) [Non-native] {NE} **Growth Form:** Shrub; **Location:** Belait; **Habitat:** Peat-swamp Forest; **Elevation:** 18 m asl; **Collections:** L.2683778.

ORCHIDACEAE

***Aphyllorchis montana* Rchb.f.** (Linnaea 41: 57 [1876]) [Native] {NE} **Growth Form:** Saprophyte, Terrestrial herb; **Location:** Temburong; **Habitat:** Mixed Dipterocarp Forest; **Elevation:** 569 m asl; **Collections:** L 0663766, L.1482916.

***Bulbophyllum calceolus* J.J.Verm.** (Orchids Borneo 2: 157 [1991]) [Native] {NE} **Growth Form:** Perennial epiphyte; **Location:** Temburong; **Habitat:** Peat-swamp Forest; **Elevation:** 32 m asl; **Collections:** L.1492646.

***Bulbophyllum coniferum* Ridl.** (J. Fed. Malay States Mus. 4: 67 [1909]) [Native] {NE} **Growth Form:** Perennial epiphyte; **Location:** Temburong; **Habitat:** Montane Forest; **Elevation:** 1014 m asl; **Collections:** L.1492959, L 0538708.

***Bulbophyllum hymenanthum* Hook.f.** (Fl. Brit. India 5: 767 [1890]) [Native] {NE} **Growth Form:** Perennial epiphyte; **Location:** Temburong; **Habitat:** Montane Forest; **Elevation:** 1014 m asl; **Collections:** L 0538705.

***Bulbophyllum multiflexum* J.J.Sm.** (Mitt. Inst. Allg. Bot. Hamburg 7: 66 [1927]) [Native] {NE} **Growth Form:** Perennial epiphyte; **Location:** Temburong; **Habitat:** Montane Forest; **Elevation:** 1014 m asl; **Collections:** L.1495162, L.1495161.

***Bulbophyllum obtusum* (Blume) Lindl.** (Gen. Sp. Orchid. Pl. 56 [1830]) [Native] {NE} **Growth Form:** Perennial epiphyte; **Location:** Temburong; **Habitat:** Montane Forest; **Elevation:** 1014 m asl; **Collections:** L.1495021.

***Bulbophyllum papillatum* J.J.Sm.** (Bull. Dép. Agric. Indes Néerl. 43: 60 [1910]) [Native] {NE} **Growth Form:** Perennial epiphyte; **Location:** Temburong; **Habitat:** Montane Forest; **Elevation:** 1014 m asl; **Collections:** L.1488756.

Bulbophyllum salaccense **Rchb.f.** (Bonplandia (Hannover) 5: 57 [1857]) [Native] {NE} **Growth Form:** Perennial epiphyte; **Location:** Temburong; **Habitat:** Montane Forest; **Elevation:** 1014 m asl; **Collections:** L 0538703.

Bulbophyllum tothastes J.J.Verm. (Orchids Borneo 2: 277 1991.) [Native] {NE} **Growth Form:** Perennial epiphyte; **Location:** Temburong; **Habitat:** Peat-swamp Forest; **Elevation:** 32 m asl; **Collections:** L.2109017.

Bulbophyllum trigonopus **Rchb.f.** (Gard. Chron. n.s., 16: 71 [1881]) [Native] {NE} **Growth Form:** Perennial epiphyte; **Location:** Tutong-Temburong; **Habitat:** Mixed Dipterocarp Forest, Montane Forest; **Elevation:** 357 – 1014 m asl; **Collections:** L 0662975, L.4172636, L.1489231.

Chelonistele unguiculata **Carr** (Gard. Bull. Straits Settlem. 8: 77 [1935]) [Native] {NE} **Growth Form:** Epiphyte, Lithophyte; **Location:** Tutong; **Habitat:** Mixed Dipterocarp Forest; **Elevation:** 358 m asl; **Collections:** L.1500126, L.4171687.

Coelogyne craticulilabris **Carr** (Gard. Bull. Straits Settlem. 8: 214 [1935]) [Native] {NE} **Growth Form:** Sympodial epiphyte; **Location:** Temburong; **Habitat:** Montane Forest; **Elevation:** 1014 m asl; **Collections:** L.1500859.

Coelogyne monilirachis **Carr** (Gard. Bull. Straits Settlem. 8: 206 [1935]) [Native] {NE} **Growth Form:** Sympodial epiphyte; **Location:** Temburong; **Habitat:** Montane Forest; **Elevation:** 1014 m asl; **Collections:** L 0821910.

Coelogyne verrucosa **S.E.C.Sierra** (Blumea 45: 309 [2000]) [Native] {NE} **Growth Form:** Sympodial epiphyte; **Location:** Belait-Temburong; **Habitat:** Mixed Dipterocarp Forest; **Elevation:** 123 – 569 m asl; **Collections:** L 0663763, L.1496917.

Crepidium ramosum **(J.J.Sm.) Marg. & Szlach.** (Polish Bot. J. 46: 69 [2001]) [Native] {NE} **Growth Form:** Spur epiphyte; **Location:** Belait; **Habitat:** Peat-swamp Forest; **Elevation:** 52 m asl; **Collections:** L.1506112.

Dendrobium corallorhizon **J.J.Sm.** (Bull. Jard. Bot. Buitenzorg III, 11: 140 [1931]) [Native] {NE} **Growth Form:** Epiphyte, Lithophyte; **Location:** Temburong; **Habitat:** Montane Forest; **Elevation:** 967 – 1014 m asl; **Collections:** L.1498055, 76778-s.n.

Dendrobium indragiriense **Schltr.** (Repert. Spec. Nov. Regni Veg. 9: 164 [1911]) [Native] {NE} **Growth Form:** Epiphyte, Lithophyte; **Location:** Tutong; **Habitat:** Peat-swamp Forest; **Elevation:** 20 m asl; **Collections:** L.1502430, L.4266678, 78172-1782.

Dendrobium pseudoaloifolium **J.J.Wood** (Kew Bull. 39: 82 [1984]) [Native] {NE} **Growth Form:** Epiphyte, Lithophyte; **Location:** Temburong; **Habitat:** Secondary Forest; **Elevation:** 23 m asl; **Collections:** L.1506609.

Dendrobium rosellum **Ridl.** (J. Linn. Soc., Bot. 31: 268 [1896]) [Native] {NE} **Growth Form:** Epiphyte, Lithophyte; **Location:** Tutong; **Habitat:** Mixed Dipterocarp Forest; **Elevation:** 358 m asl; **Collections:** L.1506421, L.1506422, L.4172818.

Dendrobium subulatum (Blume) **Lindl.** (Gen. Sp. Orchid. Pl. 91 [1830]) [Native] {NE} **Growth Form:** Epiphyte, Lithophyte; **Location:** Muara; **Habitat:** Secondary Forest; **Elevation:** 10 m asl; **Collections:** L.1507307.

Dendrochilum imitator **J.J.Wood** (Check-list Orchids Borneo 179 [1994]) [Native] {NE} **Growth Form:** Epiphyte, Lithophyte; **Location:** Temburong; **Habitat:** Montane Forest; **Elevation:** 958 m asl; **Collections:** K000392335.

Mycaranthes citrina **(Ridl.) Rauschert** (Feddes Repert. 94: 455 [1983]) [Native] {NE} **Growth Form:** Epiphyte; **Location:** Temburong; **Habitat:** Secondary Forest; **Elevation:** 22 m asl; **Collections:** L.1510494.

***Neuwiedia inae* de Vogel** (Blumea 17: 333 [1969]) [Native] {NE} **Growth Form:** Terrestrial herb; **Location:** Tutong; **Habitat:** Mixed Dipterocarp Forest; **Elevation:** 107 m asl; **Collections:** L.1534350**.**

***Peristylus gracilis* Blume** (Bijdr. 404 [1825]) [Native] {NE} **Growth Form:** Terrestrial herb; **Location:** Temburong; **Habitat:** Mixed Dipterocarp Forest; **Elevation:** 348 m asl; **Collections:** L.1519117.

***Phalaenopsis fuscata* Rchb.f.** (Gard. Chron. n.s., 2: 6 [1874]) [Native] {NE} **Growth Form:** Epiphyte; **Location:** Tutong; **Habitat:** Mixed Dipterocarp Forest; **Elevation:** 358 m asl; **Collections:** L.1519460.

Spathoglottis aurea Lindl. (J. Hort. Soc. London 5: 34 1850.) [Native] {NE} **Growth Form:** Terrestrial herb; **Location:** Belait; **Habitat:** Keranga Forest; **Elevation:** 87 m asl; **Collections:** L.1533691.

***Thrixspermum canaliculatum* J.J.Sm.** (Bull. Jard. Bot. Buitenzorg II, 13: 42 [1914]) [Native] {NE} **Growth Form:** Monopodial epiphyte; **Location:** Belait; **Habitat:** Peat-swamp Forest; **Elevation:** 43 m asl; **Collections:** L.1540345.

***Trichoglottis vandiflora* J.J.Sm.** (Bull. Dép. Agric. Indes Néerl. 22: 49 [1909]) [Native] {NE} **Growth Form:** Epiphyte; **Location:** Temburong; **Habitat:** Montane Forest; **Elevation:** 1014 m asl; **Collections:** L.1529322.

***Trichotosia lawiensis* (J.J.Sm.) J.J.Wood** (Check-list Orchids Borneo 223 [1994]) [Native] {NE} **Growth Form:** Epiphyte; **Location:** Temburong; **Habitat:** Montane Forest; **Elevation:** 1014 m asl; **Collections:** L.1535327, L 0303134.

PHYLLANTHACEAE

***Actephila excelsa* (Dalzell) Müll.Arg.** (Linnaea 32: 78 [1863]) [Native] {VU} **Growth Form:** Shrub; **Location:** Belait; **Habitat:** Peat-swamp Forest; **Elevation:** 18 m asl; **Collections:** K000187077-BRUN 16892.

***Baccaurea edulis* Merr.** (Univ. Calif. Publ. Bot. 15: 149 [1929]) [Native] {NE} **Growth Form:** Tree; **Location:** Belait; **Habitat:** Mixed Dipterocarp Forest; **Elevation:** 39 m asl; **Collections:** L.2188488, L.2188487.

***Baccaurea maingayi* Hook.f.** (Fl. Brit. India 5: 370 [1887]) [Native] {NE} **Growth Form:** Tree; **Location:** Belait; **Habitat:** Peat-swamp Forest; **Elevation:** 43 m asl; **Collections:** L.2191475.

***Baccaurea parviflora* (Müll.Arg.) Müll.Arg.** (Prodr. 15(2): 462 [1866]) [Native] {NE} **Local name:** Rambai hutan; **Growth Form:** Tree; **Location:** Temburong; **Habitat:** Mixed Dipterocarp Forest; **Elevation:** 99 m asl; **Collections:** 153823-Schatz 3251.

***Baccaurea ramiflora* Lour.** (Fl. Cochinch. 661 [1790]) [Non-native] {NE} **Local name:** Tampoi; **Growth Form:** Tree; **Location:** Tutong; **Habitat:** Secondary Forest; **Elevation:** 64 m asl; **Collections:** L.2189311.

***Baccaurea trigonocarpa* Merr.** (Univ. Calif. Publ. Bot. 15: 152 [1929]) [Native] {NE} **Growth Form:** Shrub; Tree; **Location:** Temburong; **Habitat:** Secondary Forest; **Elevation:** 81 m asl; **Collections:** L.2197234.

***Cleistanthus pubens* Airy Shaw** (Kew Bull. 21: 365 [1968]) [Native] {NE} **Local name:** Komuning; **Growth Form:** Understory tree; **Location:** Belait; **Habitat:** Keranga Forest; **Elevation:** 57 m asl; **Collections:** L.2206079.

***Phyllanthus lanceilimbus* (Merr.) Merr.** (Philipp. J. Sci. 30: 402 [1926]) [Native] {NE} **Growth Form:** N/A; **Location:** Tutong; **Habitat:** Peat-swamp Forest; **Elevation:** 5 m asl; **Collections:** L.2219870.

***Phyllanthus microcarpus* (Benth.) Müll.Arg.** (Linnaea 32: 51 [1863]) [Non-native] {NE} **Growth Form:** Rare shrub; **Location:** Temburong; **Habitat:** Mixed Dipterocarp Forest; **Elevation:** 140 m asl; **Collections:** L.2059435.

***Phyllanthus ruber* (Lour.) Spreng.** (Syst. Veg. 3: 22 1826.) [Non-native] {NE} **Growth Form:** Shrub, Treelet; **Location:** Tutong-Temburong; **Habitat:** Mixed Dipterocarp Forest; **Elevation:** 39 – 318 m asl; **Collections:** L.2227970, L.2228072, L.2228085, L.2227971.

POACEAE

***Digitaria fuscescens* (J.Presl) Henrard** (Meded. Rijks-Herb. 61: 8 [1930]) [Native] {NE} **Growth Form:** Perennial herb; **Location:** Belait; **Habitat:** Secondary Forest; **Elevation:** 53 m asl; **Collections:** CANB 67187.1, L.1244347, 2800873.

***Digitaria violascens* Link** (Hort. Berol. 1: 229 [1827]) [Native] {NE} **Growth Form:** Herb; **Location:** Belait; **Habitat:** Secondary Forest; **Elevation:** 6 – 28 m asl; **Collections:** L.3801733, L.1246709, L.1246707, 2838547, 2838578.

***Garnotia stricta* Brongn.** (Voy. Monde Phan.: 133 [1832]) [Native] {NE} **Growth Form:** Perennial herb; **Location:** Temburong; **Habitat:** Mixed Dipterocarp Forest; **Elevation:** 171 – 348 m asl; **Collections:** L 0398769, L 0398772, L 0580825.

***Imperata cylindrica* (L.) Raeusch.** (Nomencl. Bot. ed. 3: 10 [1797]) [Non-native] {NE} **Local name:** Lalang; **Growth Form:** Herb; **Location:** Temburong; **Habitat:** Mixed Dipterocarp Forest; **Elevation:** 349 m asl; **Collections:** CANB 67170.1-5624.

***Pogonatherum crinitum* (Thunb.) Kunth** (Enum. Pl. 1: 478 [1833]) [Non-native] {NE} **Growth Form:** Perennial herb; **Location:** Temburong; **Habitat:** Mixed Dipterocarp Forest; **Elevation:** 349 m asl; **Collections:** CANB 67288.1-5598.

***Schizostachyum khoonmengii* S.Dransf.** (Kew Bull. 55: 491 [2000]) [Native] {NE} **Growth Form:** Perennial herb; **Location:** Temburong; **Habitat:** Mixed Dipterocarp Forest; **Elevation:** 302 m asl; **Collections:** K000795370-SD 998, K000290709-998, K000290710-998, K000290708-998.

PODOCARPACEAE

***Dacrydium beccarii* Parl.** (Prodr. 16(2): 494 [1868]) [Native] {LC} **Growth Form:** Shrub, Tree; **Location:** Temburong; **Habitat:** Montane Forest; **Elevation:** 520 m asl; **Collections:** K000288671-BRUN15823.

***Dacrydium medium* de Laub.** (Blumea 23: 98 [1976]) [Non-native] {VU} **Growth Form:** Tree; **Location:** Tutong; **Habitat:** Mixed Dipterocarp Forest; **Elevation:** 37 m asl; **Collections:** L.4174274, L.4174275.

***Falcatifolium falciforme* (Parl.) de Laub.** (J. Arnold Arbor. 50: 309 [1969]) [Native] {NT} **Growth Form:** Tree; **Location:** Temburong; **Habitat:** Montane Forest; **Elevation:** 520 – 1430 m asl; **Collections:** K000288838-S8743, K000288832-BRUN1066.

***Nageia wallichiana* (C.Presl) Kuntze** (Revis. Gen. Pl. 2: 800 [1891]) [Native] {EN} **Growth Form:** Mid-canopy tree; **Location:** Temburong; **Habitat:** Secondary Forest, Mixed Dipterocarp Forest; **Elevation:** 79 – 716 m asl; **Collections:** K000289003-7201, K000289002-6953.

POLYGONACEAE

***Antigonon leptopus* Hook. & Arn.** (Bot. Beechey Voy. 308 [1838]) [Non-native] {NE} **Growth Form:** Invasive climber; **Location:** Belait; **Habitat:** Secondary Forest; **Elevation:** 8 m asl; **Collections:** L.4190730.

***Persicaria barbata* (L.) H.Hara** (in Fl. E. Himal. 70 1966.) [Non-native] {LC} **Growth Form:** Perennial herb; **Location:** Belait; **Habitat:** Peat-swamp Forest; **Elevation:** 7 m asl; **Collections:** L.4190729.

PRIMULACEAE

***Ardisia belaitensis* C.M.Hu** (Blumea 47: 504 [2002]) [Native] [Endemic] {NE} **Growth Form:** N/A; **Location:** Belait; **Habitat:** Peat-swamp Forest, Mixed Dipterocarp Forest; **Elevation:** 36 – 53 m asl; **Collections:** L.2627368, L 0064528, K001089875-153.

***Ardisia forbesii* S.Moore** (J. Bot. 52: 291 [1914]) [Native] {NE} **Local name:** Decar flanak, Kubi, Merjemah; **Growth Form:** Understory tree; **Location:** Tutong-Belait-Temburong; **Habitat:** Mixed Dipterocarp Forest, Montane Forest; **Elevation:** 39 – 793 m asl; **Collections:** L.2624152, L.2624180, L.2624172, L.2624292, L.2624302.

***Ardisia fuliginosa* Blume** (Bijdr. 692 [1826]) [Native] {NE} **Growth Form:** Shrub, Small tree; **Location:** Belait; **Habitat:** Mixed Dipterocarp Forest; **Elevation:** 148 m asl; **Collections:** L.2624558, L.2624559.

***Ardisia lepidotula* Merr.** (Philipp. J. Sci., C 13: 115 [1918]) [Native] {NE} **Growth Form:** N/A; **Location:** Temburong; **Habitat:** Mixed Dipterocarp Forest, Montane Forest; **Elevation:** 572 – 1693 m asl; **Collections:** L.2632054, L.2632051.

***Ardisia obscurinervia* Merr.** (Philipp. J. Sci., C 13: 112 1918.) [Native] {NE} **Growth Form:** N/A; **Location:** Belait; **Habitat:** Mixed Dipterocarp Forest; E**levation:** 29 m asl; **Collections:** L.2632133.

***Ardisia polygama* (Roxb.) A.DC.** (Prodr. 8: 138 [1844]) [Non-native] {NE} **Growth Form:** N/A; **Location:** Muara-Belait-Temburong; **Habitat:** Secondary Forest, Mixed Dipterocarp Forest; **Elevation:** 18 – 572 m asl; **Collections:** L.4175542, L.4175543, L.4163134, L.2677500.

RHAMNACEAE

***Alphitonia excelsa* (Fenzl) Reissek ex Benth.** (Fl. Austral. 1: 414 [1863]) [Non-native] {NE} **Growth Form:** Tree; **Location:** Tutong-Belait-Temburong; **Habitat:** Secondary Forest, Mixed Dipterocarp Forest; **Elevation:** 6 – 81 m asl; **Collections:** L.2326557, L.2326471, L.4209991, L.4206667, L.2326559, L.2326558.

***Ziziphus rugosa* Lam.** (Encycl. 3(1): 319 [1789]) [Non-native] {NE} **Growth Form:** Tree; **Location:** Temburong; **Habitat:** Mixed Dipterocarp Forest; **Elevation:** 140 – 318 m asl; **Collections:** L.2328607, L.2328606, L.4202734.

RUBIACEAE

***Aidia beccariana* (Baill.) Ridsdale** (Blumea 41: 159 [1996]) [Native] {NE} **Growth Form:** Shrub, Small tree; **Location:** Belait; **Habitat:** Freshwater swamp forest, Mixed Dipterocarp Forest, Secondary forest; **Elevation:** 18 – 147 m asl; **Collections:** L.2847147, L.2847144, L.2847149, L.2847148.

***Argostemma propinquum* Ridl.** (J. Straits Branch Roy. Asiat. Soc. 57: 53 [1911]) [Non-native] {NE} **Growth Form:** Herb; **Location:** Temburong; **Habitat:** Mixed dipterocarp forest; **Elevation:** 572 m asl; **Collections:** L.2849563.

***Discospermum abnorme* (Korth.) S.J.Ali & Robbr.** (Blumea 35: 300 [1991]) [Native] {NE} **Growth Form:** Shrub, Small tree; **Location:** Temburong; **Habitat:** Mixed dipterocarp forest; **Elevation:** 318 m asl; **Collections:** L.2904647, L.2904646.

***Lasianthus griffithii* Wight** (Calcutta J. Nat. Hist. 6: 505 [1846]) [Native] {NE} **Growth Form:** Shrub; **Location:** Tutong; **Habitat:** Mixed dipterocarp forest; **Elevation:** 189 m asl; **Collections:** L.2922780.

***Neonauclea pseudocalycina* Ridsdale** (Blumea 34: 204 [1989]) [Native] {NE} **Growth Form:** N/A; **Location:** Belait; **Habitat:** Mixed dipterocarp forest; **Elevation:** 19 m asl; **Collections:** L.2937417.

***Oxyceros bispinosus* (Griff.) Tirveng.** (Nordic J. Bot. 3: 466 [1983]) [Native] {EN} **Growth Form:** Climber; **Location:** Belait-Temburong; **Habitat:** Secondary forest, Peat swamp forest; **Elevation:** 27 – 36 m asl; **Collections:** L.2938631, L.2938609, L.2938608, L.2938610.

***Praravinia parviflora* Bremek.** (Recueil Trav. Bot. Néerl. 37: 266 [1940]) [Native] {NE} **Local name:** Kopi-kopi; **Growth Form:** Shrub; Location**:** Tutong-Temburong; **Habitat:** Mixed dipterocarp forest; **Elevation:** 55 - 340 m asl; **Collections:** L.2939025, L.2939015, L.2938992, L.2939019.

***Psychotria sarmentosoides* Valeton** (Icon. Bogor. t. 292 [1909]) [Non-native] {NE} **Growth Form:** N/A; **Location:** Muara; **Habitat:** Secondary forest; **Elevation:** 49 m asl; **Collections:** L.2944427.

***Spermacoce baileyana* Domin** (Biblioth. Bot. 89: 628 [1929]) [Non-native] {NE} **Growth Form:** Herb; **Location:** Belait; **Habitat:** Secondary forest; **Elevation:** 53 m asl; **Collections:** CANB 67205.1-5672.

***Spermacoce verticillata* L.** (Sp. Pl. 102 [1753]) [Non-native] {NE} **Growth Form:** Invasive shrub; **Location:** Tutong; **Habitat:** Secondary forest; **Elevation:** N/A; **Collections:** CBG 7803088.1-3740.

***Tarenna debilis* Ridl.** (J. Bot. 72: 272 [1934]) [Native] {NE} **Growth Form:** N/A; **Location:** Belait; **Habitat:** Peat swamp forest; **Elevation:** 8 m asl; **Collections:** L.3971575.

***Tarenna gibbsiae* Wernham** (J. Linn. Soc., Bot. 42: 93 [1914]) [Native] {NE} **Growth Form:** N/A; **Location:** Temburong; **Habitat:** Mixed dipterocarp forest; **Elevation:** 171 m asl; **Collections:** L.3970889.

***Timonius lasianthoides* Valeton** (Bull. Dép. Agric. Indes Néerl. 26: 48 [1909]) [Native] {NE} **Local name:** Bar, Bulu udok, Kemudok, Mulong udok, Rentap, Selembuca, Turang Oyung; **Growth Form:** Understory tree; **Location:** Belait; **Habitat:** Peat swamp forest; **Elevation:** 19 m asl; **Collections:** L.2957078.

***Wendlandia densiflora* (Blume) DC.** (Prodr. 4: 412 [1830]) [Native] {NE} **Growth Form:** Tree; **Location:** Temburong; **Habitat:** Mixed dipterocarp forest; **Elevation:** 340 m asl; **Collections:** L.2938871.

RUTACEAE

***Citrus* x *aurantiifolia* (Christm.) Swingle** (J. Wash. Acad. Sci. 3(18): 465 [1913]) [Non-native] {NE} **Growth Form:** Small tree; **Location:** Muara; **Habitat:** Secondary forest; **Elevation:** 13 m asl; **Collections:** 5514-64, 2012872.

***Melicope accedens* (Blume) T.G. Hartley** (Sandakania 4: 67 [1994]) [Non-native] {NE} **Local name:** Kulampapa, Pahau, Pau; **Growth Form:** Understory tree; **Location:** Tutong; **Habitat:** Secondary forest; **Elevation:** 31 m asl; **Collections:** L.4194960, L.4194959.

SALICACEAE

***Casearia loheri* Merr.** (Philipp. J. Sci., C 9: 327 [1914]) [Non-native] {NE} **Growth Form:** N/A; **Location:** Belait; **Habitat:** Peat swamp forest; **Elevation:** 49 m asl; **Collections:** L.2451654.

SAPOTACEAE

***Madhuca malaccensis* (C.B.Clarke) H.J.Lam** (Bull. Jard. Bot. Buitenzorg III, 7: 167 [1925]) [Native] {NE} **Growth Form:** Tree; **Location:** Belait; **Habitat:** Mixed dipterocarp forest; **Elevation:** 259 m asl; **Collections:** L.4222070.

***Payena acuminata* (Blume) Pierre** (Bull. Mens. Soc. Linn. Paris 1: 528 [1885]) [Native] {NE} **Local name:** Bee-taul, Malau pedara, Natu, Nyatoh, Nyatoh merah, Nyatoh rian; **Growth Form:** Upper canopy tree; **Location:** Temburong; **Habitat:** Mixed dipterocarp forest; **Elevation:** 302 m asl; **Collections:** K000009271-176.

SMILACACEAE

***Smilax corbularia* Kunth** (Enum. Pl. 5: 262 [1850]) [Native] {NE} **Growth Form:** Climbing vine; **Location:** Belait; **Habitat:** Keranga Forest; **Elevation:** N/A; **Collections:** L.3808994.

SOLANACEAE

***Lycianthes laevis* (Dunal) Bitter** (Lycianthes 484–485 [1919]) [Native] {NE} **Growth Form:** Shrub; **Location:** Belait; **Habitat:** Secondary forest; **Elevation:** 31 m asl; **Collections:** L.2881514.

URTICACEAE

***Poikilospermum micranthum* (Miq.) Merr.** (Contr. Arnold Arbor. 8: 50 [1934]) [Native?] {NE} **Growth Form:** N/A; **Location:** Belait-Temburong; **Habitat:** Secondary forest, Mixed dipterocarp forest; **Elevation:** 12 – 340 m asl; **Collections:** L.1637799, L.1637800, L.1637797, L.1637798.

VITACEAE

***Ampelocissus pauciflora* Merr.** (Philipp. J. Sci., C 11: 126 [1916]) [Non-native] {NE} **Growth Form:** Climbing herb; **Location:** Belait; **Habitat:** Keranga Forest; **Elevation:** N/A; **Collections:** L.4254274.

WINTERACEAE

***Drimys membranea* F.Muell.** (Fragm. 5: 175 [1866]) [Non-native] {NE} **Growth Form:** N/A; **Location:** Temburong; **Habitat:** Montane forest; **Elevation:** 608-1693 m asl; **Collections:** L.1748232, L.1748251.

ZINGIBERACEAE

***Boesenbergia armeniaca* Cowley** (Kew Bull. 55: 669 [2000]) [Native] {NE} **Growth Form:** Perennial herb; **Location:** Belait; **Habitat:** Secondary forest; **Elevation:** 188 m asl; **Collections:** 61944-13, K000255387-13, K000255389-13, K000255388-13.

***Boesenbergia bruneiana* Cowley** (Kew Bull. 53: 626 [1998]) [Native] {NE} **Growth Form:** Perennial herb; **Location:** Belait; **Habitat:** Peat swamp forest; **Elevation:** 53 m asl; **Collections:** 64083-185, K000255382-185, K000255383-185, K000255385-185, K000255384-185.

Epiamomum augustipetalum(Edinburgh J. Bot. 55: 49 [1998]) [Native] {NE} **Growth Form:** Perennial herb; **Location:** Belait; **Habitat:** Mixed dipterocarp forest; **Elevation:** 78 m asl; **Collections:** E00830248-2974.

***Etlingera aurantia* A.D.Poulsen** (Etlingera Borneo 39 [2006]) [Native] {NE} **Growth Form:** Perennial herb; **Location:** Temburong; **Habitat:** Mixed dipterocarp forest; **Elevation:** 170 m asl; **Collections:** K000433057-169.

***Etlingera belalongensis* A.D.Poulsen** (Nordic J. Bot. 19: 141 [1999]) [Native] {NE} **Growth Form:** Perennial herb; **Location:** Temburong; **Habitat:** Mixed dipterocarp forest; **Elevation:** 171 m asl; **Collections:** E00177148-130, E00177149-130, E00177150-130, K000255132-130.

***Etlingera brevilabrum* (Valeton) R.M.Sm.** (Notes Roy. Bot. Gard. Edinburgh 43: 243 [1986]) [Native] {NE} **Growth Form:** Perennial herb; **Location:** Belait-Temburong; **Habitat:** Peat swamp forest, Mixed dipterocarp forest; **Elevation:** 27 – 171 m asl; **Collections:** E00190075-170, L.1478671, L.1478669.

***Etlingera rubromarginata* A.D.Poulsen & Mood** (Nordic J. Bot. 19: 139 [1999]) [Native] {NE} **Growth Form:** Perennial herb; **Location:** Temburong; **Habitat:** Mixed dipterocarp forest; **Elevation:** 170 m asl; **Collections:** K000255117-35, K000255118-35.

***Plagiostachys breviramosa* Cowley** (Kew Bull. 54: 154 [1999]) [Native] {NE} **Growth Form:** Perennial herb; **Location:** Temburong; **Habitat:** Undisturbed mixed dipterocarp forest; Elevation**:** 125-339 m asl; **Collections:** 64081-42, K000292329-42, K000292327-42, K000292330-42, K000292328-42.

***Tamijia flagellaris* S.Sakai & Nagam.** (Edinburgh J. Bot. 57: 245 [2000]) [Native] {NE} **Growth Form:** Perennial herb; **Location:** Belait; **Habitat:** Kerangas forest, Mixed dipterocarp forest, **Elevation:** 48 m asl; **Collections:** 61903-142.

Oleanane- and ursane- type triterpenoids from *Eugenia grandis*

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Abstract

Arjunolic acid (an oleanane-type triterpenoid) and asiatic acid (an ursane-type triterpenoid) were obtained as an inseparable mixture from a chloroform extract of stem-bark of *Eugenia grandis* (Myrtaceae). They were characterised mainly by analysis of their spectral data. Arjunolic acid and asiatic acid are reported to have a variety of biological and pharmacological activities, which include antibacterial, anti-inflammatory, antioxidant, antidiabetic, antihyperlipidemic, cardiacprotective, antitumor, anticancer and hepatoprotective activities. Arjunolic acid has been used as a cardio-protective phytotonic in traditional Ayurvedic medicine for centuries.

Index Terms: Eugenia grandis, Eugenia, Myrtaceae, arjunolic acid, asiatic acid, pentacyclic triterpenoids, ^{13}C NMR, HMBC, HSQC-DEPT, MS

1. Introduction

The genus Eugenia consists of approximately 600 species in the tropics.[1] They are either trees or shrubs, which can yield edible fruits[1] and are often planted for ornaments in warm regions.[1] Triterpenoids have been reported as the main constituents from these species.[2-5] Examples of triterpenoids isolated from Eugenia plants include lupeol, betulinic acid, methyl arjunolate, α-amyrin , β-amyrin, methyl asiatate, methyl 2α-acetoxy-3β-hydroxyolean-12-en-28-oate, methyl 2α-acetoxy-3β-hydroxyurs-12-en-28-oate, arjunolic acid, 2α-hydroxyursolic acid, methyl maslinate (methyl 2α,3β-dihydroxyolean-12-en-28-oate), asiatic acid, methyl 2α,3β-dihydroxyurs-12-en-28-oate, oleanolic acid, epioleanolic acid, ursolic acid, epiursolic acid, crategolic acid, friedelin, erythrodiol and 3β-friedelinol.[2-5] The only steroid isolated has been β-sitosterol. *Eugenia caryophyllata*, *Eugenia mooniana*, *Eugenia aromatica*, *Eugenia biflora Jambosa caryophyllus*, *Eugenia jambolana Eugenia maire*, *Eugenia fructicosa*, *Eugenia wallichii* and *Eugenia javanica* are some examples of species belong to the Myrtaceae family.[6] Among the Eugenia species, *Eugenia caryophyllata* has been most extensively studied due to its medicinal properties and economic value.[6] Extracts from this plant have been used in treating toothache and gum diseases, dandruff control, scalp-treatment, transdermal and antitumour pharmaceuticals, food flavourings and as antioxidants for fats.[6]

Called locally as Sea apple or Jambu Laut, *Eugenia grandis* (synonym: *Syzygium grande*) is a common seashore tree but often planted along roadsides to give shade.[6] *E. grandis* grows to 30 m height and has an irregular crown.[6] The leaves are large, shiny, dark green, elliptic in shape and have a distinct down-turned tip.[6] The flowers are oblong, large, white and fluffy. Our literature search showed that this plant has not been explored well for phytochemical studies.[6] Castalagin, vescalagin and ellagitannin (1-*O*-

galloyl castalagin) have previously been isolated from the leaves of *E. grandis*.[7] Additionally, we reported the isolation and structural elucidation of a lupane- type triterpenoid *viz*. 2α,3β-dihydroxylup-12-en-28-oic acid **(1)** as a new compound from the chloroform extract of stem-bark of *E. grandis* together with six known compounds *viz*. 3β-hydroxylup-12-en-28-oic acid, fridelin, 3β-friedelinol, β-sitosterol, oleanolic acid and betulinic acid.[8] Herein, we report the isolation and identification of two pentacyclic terpenoids *viz*. arjunolic acid (**2**) (an oleanae- type terpenoid) and asiatic acid (**3**) (an ursane- type terpenoid) which were obtained as an inseparable mixture from the same chloroform extract of stem-bark of *E. grandis*. Compounds **2** and **3** are reported to have a variety of biological and pharmacological activities, which include antibacterial, anti-inflammatory, antioxidant, antidiabetic, antihyperlipidemic, cardiac-protective, antitumor, anticancer and hepato-protective activities.

2. Experimental Method

2.1 Plant materials

The plant material was collected in Singapore along Kent Ridge Road and identified by Associate Prof. Hugh Tan Tiang Wah, Dept. of Biological Sciences, NUS and Chua Keng Soon, Senior Laboratory Officer (RMBR), Herbarium, NUS. A voucher specimen (KM20041122) was deposited in the herbarium, Department of Biological Sciences, National University of Singapore, Singapore.

2.2 Extraction and Isolation

A whole plant weighing about 20 kg (wet weight) was cut. The leaves were removed using a knife and the stem-bark was chopped into small pieces. The air-dried stem-bark was then exhaustively extracted with chloroform under reflux conditions. The combined extract was chromatographed over silica gel column using hexane and eluted with the solvents of increasing polarity. Purification of eluted fractions by repeated column chromatography and/or preparative TLC afforded 2α,3β-dihydroxylup-12-en-28-oic acid (**1**), 3β-hydroxylup-12-en-28-oic acid, oleanolic acid, betulinic acid, β-sitosterol, friedelin and 3β-friedelinol.[8] All these seven compounds have previously been reported.[8] Additionally, we also obtained two pentacyclic terpenoids *viz*. arjunolic acid (**2**) and asiatic acid (**3**) as an inseparable mixture and the characterisation of this mixture is discussed in this article.

2.3 Instruments and chemicals used

Silica gel 60 (Merck, 0.063- 0.200 m) was used for column chromatography. Precoated silica gel plates (Merck, Kieselgel 60F 254, 0.25 mm or Baker Si250F, 0.25 mm) were used for preparative TLC and/or analytical TLC. Spots were detected using UV light or staining with iodine or by spraying with 50% H_2SO_4 followed by heating at 110˚C for 5 minutes. Lichroprep RP-18 (Merck, 40-63 μm) was used for separation and/or purification. HPLC was carried on a Waters associates, μ-Porasil (300 x 5 mm) column with a Shimadzu RID-10A, refractive index detector. ^{1}H, ^{13}C NMR and 2D NMR spectra were recorded on Bruker, 300 and/or 500 MHz spectrometers. Standard microprograms supplied by Bruker were used to run 1D and 2D NMR spectroscopy. Chemical shifts are reported in parts per million (ppm) with TMS as a reference standard or with reference to solvent peaks and coupling constants (J) expressed in hertz. LREIMS were measured on a Finnigan/MAT MAT 95 XL-T or VG Micromass 7035. HREIMS were measured on Finnigan/MAT MAT 95 XL-T mass spectrometers. IR spectra were recorded on a Bio Rad, Class II Laser product.

2.4 List of spectral data

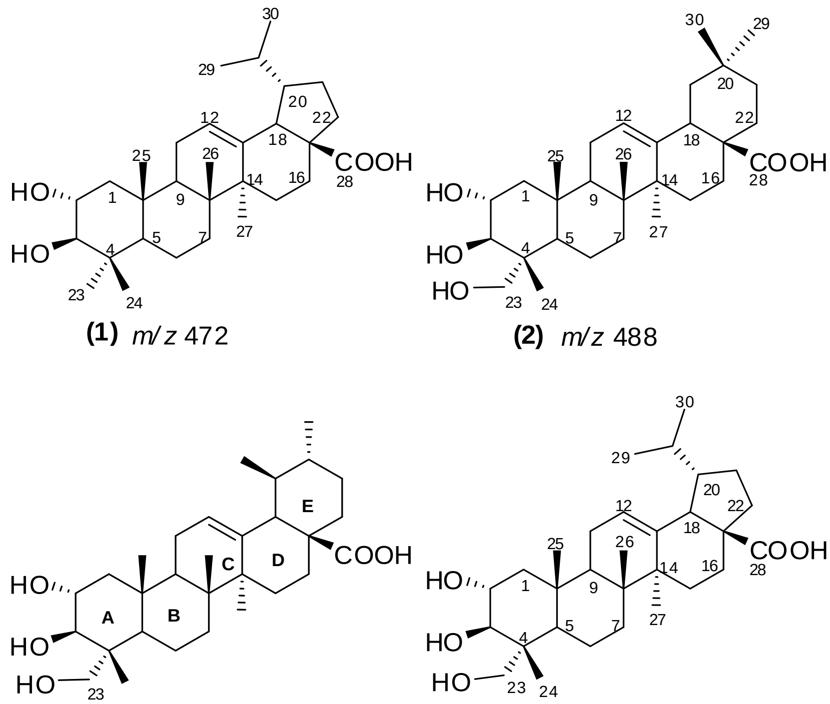
Arjunolic acid and asiatic acid

Colourless crystals; IR (KBr) v_{max} 3535, 3372, 2925, 2856, 1694, 1639, 1458, 1389, 1306, 1270, 1047 cm^{-1}; MS (EI, 70eV), *m/z* (rel.int. %): 488 $[M]^+$ (4), 470 (3), 452 (10), 248 (100), 203 (88), 189 (34), 133 (54), 119 (24), 69 (26), 41 (16); HREIMS m/z 488.3497 (calcd. for $C_{30}H_{48}O_5$, 488.3501); ^{1}H NMR (80% $CDCl_3$:20% CD_3OD, 300 MHz) δ 3.79, 3.79 (1H, ddd, J = 4.5, 10, 11.5 Hz, H-2; 1H, ddd, J = 4.5, 10, 11.5 Hz, H-2); 3.48, 3.48 (1H, d, J = 10 Hz, H-3; 1H, d, J = 10

Hz, H-3); 5.24, 5.28 (1H, t, J = 4 Hz, H-12; 1H, t, 4 Hz, H-12); 2.20, 2.88 (1H, d, J = 12 Hz, H-18; 1H, dd, J = 13.5, 4.0 Hz, H-18); 3.44, 3.43, 3.71, 3.70 (1H, d, J = 10.5 Hz, H_α-23; 1H, d, J = 10.5 Hz, H_β-23; 1H, d, J = 10.5 Hz, H_α-23; 1H, d, J = 10.5 Hz, H_β-23); 0.68, 0.71 (3H, s, H-24; 3H, s, H-24); 0.99, 1.00 (3H, s, H-25; 3H, s, H-25); 0.83, 0.86 (3H, s, H-26; 3H, s, H-26); 1.04, 1.09 (3H, s, H-27; 3H, s, H-27); 0.89, 0.90 (3H, s, H-29; 3H, d, H-29); 0.90, 0.91 (3H, s, H-30; 3H, d, H-30); ^{13}C NMR (80% $CDCl_3$: 20% CD_3OD, 125.7 MHz) δ 46.1, 47.1 (C-1), 66.7, 66.8 (C-2), 75.1, 75.2 (C-3), 42.7, 43.5 (C-4), 48.4, 48.4 (C-5), 18.6, 18.6 (C-6), 32.4, 33.1 (C-7), 39.4, 40.1 (C-8), 47.3, 48.5 (C-9), 38.5, 38.5 (C-10), 23.3. 23.8 (C-11), 122.6, 125.9 (C-12), 139.0, 144.6 (C-13), 42.0, 42.4 (C-14), 27.9, 28.3 (C-15), 23.9, 24.1 (C-16), 47.9, 47.9 (C-17), 43.5, 52.8 (C-18), 39.2, 46.3 (C-19), 30.8, 39.0 (C-20), 33.8, 30.6 (C-21), 30.5, 34.2 (C-22), 63.3, 63.4 (C-23), 180.1, 181.1 (C-28), 13.1, 13.2, 16.8, 17.0, 17.2, 17.3, 17.4, 23.6, 23.7, 23.7, 26.1, 33.2 (C-24, C,25, C-26, C-27, C-29, C-30).

3. Results and Discussion

Arjunolic acid **(2)** and asiatic acid **(3)** were obtained as colourless crystals. The IR spectrum gave absorption peaks at v_{max} 3535 & 3372, 1694 and 1639 cm^{-1}, which are characteristics of hydroxyl, carboxyl and double bond respectively.[8] Both compounds have the same molecular formula, $C_{30}H_{48}O_5$, deduced from HREIMS *m/z* 488.3497. The EIMS gave a single molecular ion peak at *m/z* 488. We have confirmed this molecular ion peak with ESI and FAB mass spectra and gave $[M-1]^+$ ion at *m/z* 487. However, there was an inconsistency in the observed molecular weight and the number of ^{13}C NMR signals. In the ^{13}C NMR spectrum, we observed more than forty peaks. Some peaks displayed doublets with unequal intensities, which were not due to incomplete ^{1}H-decoupling. For example, a peak at about δ 181, which indicated the carboxyl carbon, showed two very close signals at δ 180.9 and 181.1. Further, both HMBC and HSQC-DEPT gave peaks with stacking one over another or with very close chemical shift values. Based on these observations, we understood that it was a mixture of two closely related compounds. Additionally, we also confirmed that these two compounds were pentacyclic triterpenoids since a fragment ion was observed at *m/z* 248 in the MS, which was also a base peak. A fragment ion at *m/z* 248 in the MS was a clear and unambiguous indication of the presence of pentacyclic triterpenoids (refer to ***Figures 2*** and ***3***). Other fragmentation peaks were observed at *m/z* 203, 189 and 133.[8] These are characteristics of a oleanane- or ursane- or lupane-type triterpenoids.[8, 9-11] Furthermore, the major MS fragmentation pattern of the previously reported new compound **1** was found to be *m/z* = 472, 454, 243 (base peak), 203, 189 and 133.[8] The major MS fragmentation pattern of **2** and **3** were also very similar to **1** (refer to ***Figures 2*** and ***3***) except a mass difference of 16 *amu*. This observation allowed us to place an extra hydroxyl group in the mixture of pentacyclic terpenoids relative to compound **1**. As expected, on inspection of its chemical shift values in ^{13}C and DEPT spectra at the hydroxyl region, three oxygenated carbons were observed, one at δ 75.2 another at δ 66.8, both attached to methine carbons with the third one at δ 63.3 attached to a methylene carbon. However, each of the three peaks displayed two peaks with a negligibly small chemical shift difference. We have placed one hydroxyl group at C-2 and another group at C-3 based on the spectral correlations obtained in the previously reported compound **1**. According to the number of different types of carbons, the third hydroxyl group, as it attached to methylene carbon, should replace any one of methyl hydrogens, so that it will form $-CH_2OH$ group. It is impossible to use all other positions which will make the carbon either methine or quaternary.



(1) *m/z* 472 **(2)** *m/z* 488 **(3)** *m/z* 488 **(4)** *m/z* 488

Figure 1. Structures of compounds **1**, **2**, **3** and **4**.

As mentioned previously, EIMS gave a base peak at *m/z* 248 and other fragment peaks at *m/z* 203, 189 and 133. These are indicative of lupene or oleanene or ursene type triterpenoids with a double bond between C-12 and C-13 and a carboxyl group at C-17 position.[8] This observation allowed us to exclude the presence of a hydroxyl group in rings C, D and E. Any changes in any one of the position in these rings will not follow this fragmentation pattern. In other words, it is impossible to utilise the methyl group present in these rings for the formation of –CH_2OH. The only possibility is to utilise one of the methyl groups present in ring A or B i.e. C-23, C-24, C-25 and C-26. Inspection of its HMBC revealed that the single proton at C-3 position correlates with an oxygenated methylene carbon at C-23 position. This allowed us to exclude the possibility of C-24, C-25 and C-26 positions. This is consistent with previous reports that NMR signals of methyl groups of pentacyclic triterpenoids will have oxygen functions at 2, 3 and 23 positions.[12] For this interpretation, three structures are possible viz. 2α,3β,23-trihydroxyolean-12-en-28-oic acid (arjunolic acid, **2**, an oleanane-type triterpenoid), 2α,3β,23-trihydroxyurs-12-en-28-oic acid (asiatic acid, **3**, an ursane-type triterpenoid) and 2α,3β,23-trihydroxylup-12-en-28-oic acid **(4,** a lupane type- triterpenoid**)** (refer to ***Figure 1***).

We were unable to get the two possible structures based on their 2D NMR spectral correlations, due to their extreme complexity, particularly at the high-field region. Fortunately, analysis of its ^{13}C NMR or DEPT spectrum in the olefinic region showed that peaks were not stacked one over another; we observed four independent peaks with reasonably good chemical shift difference. Additionally, it was also observed that two of them were methine and the other two were quaternary carbons. These peaks were due to C-12 and C-13 positions. The chemical shift values of one of the methine and a quaternary carbon were observed at δ 122.6 and 144.6, respectively. These values were in very good agreement with chemical shift values of arjunolic acid (**2**) at its C-12 and C-13 positions.[13-17] Similarly the chemical shift values of another methine and a quaternary carbon were observed at δ 125.9 and δ 139.0, respectively. These values were in very good agreement with chemical shift values of asiatic acid (**3**) at its C-12 and C-13 positions.[13-17] As stated previously that the ^{13}C NMR and HMBC spectra were very complex at the high field region; we were unable to get the chemical shift values for the individual compounds. Thus, we tentatively assigned that the mixture of two compounds were arjunolic acid (**2**) and asiatic acid (**3**). Our literature search indicated that reports of a mixture containing two compounds are very common, particularly in the case of the pentacyclic triterpenoids.[18,19] Our literature search also revealed that the existence of the compound, 2α,3β,23-trihydroxylup-12-en-28-oic acid (**4**) has not been reported so far. Further, oleanane- and ursane-type compounds have previously been reported as a mixture[18,19] rather than lupane & oleanane or lupane & ursane types. The NMR chemical shift values for the individual compounds and/or their methyl esters are available in the literature.[13-17]

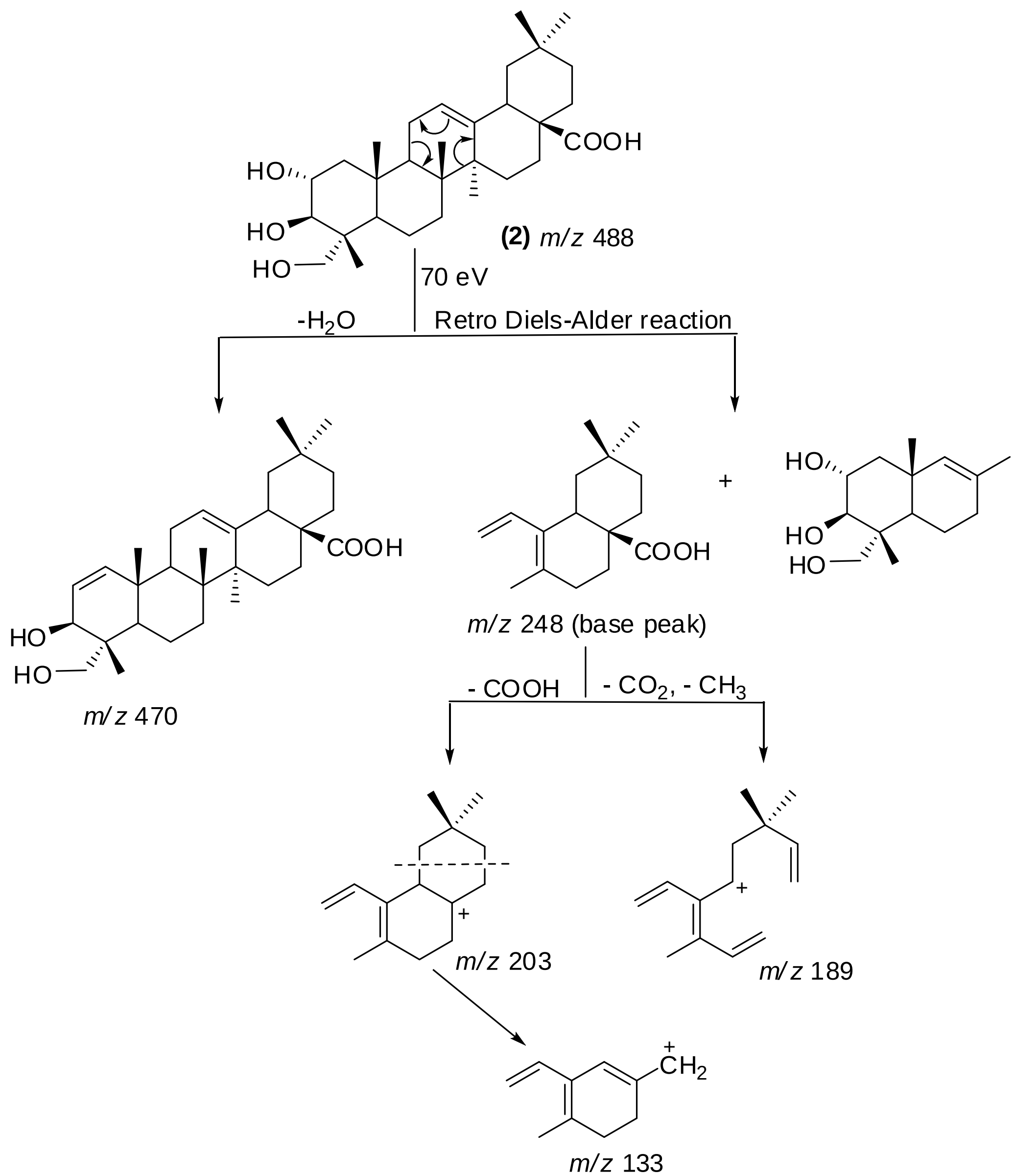


Figure 2. Proposed major MS fragmentation pattern of arjunolic acid (**2**).

In general, pentacyclic triterpenoids are reported to have a wide range of biological activities.[20] Arjunolic acid (**2**) has been used as a cardio-protective tonic in traditional Indian medicine for centuries.[21] Compound **2** is reported to have many beneficial effects to humans such as lowering of blood pressure, cholesterol levels and heart rate.[21] Compound **2** protects against myocardial necrosis, platelet coagulation and aggregation.[21] Compound **2** protects the cells from metal induced toxicity.[21] Compound **2** possesses antidiabetic, anti-inflammatory, antitumor and antimicrobial activity.[21] Compound **2** also serves as a potent free radical scavenger and antioxidant.[22] Compound **2** and its semisynthetic derivatives were shown to exhibit inhibitory effects on Epstein-Barr virus (EBV) activation in Raji cells.[6] Arjunolic acid derivatives could be valuable compounds as antitumour-promoters.[6] Their inhibitory effects on skin tumour promoters were greater than those of previously studied natural products.[23,24] Compound **2** decrease Ehrlich Ascites Carcinoma cell viability and increases cell toxicity in experimental animals. Compound **2** reduced cell count and tumour volume.[25] Overall, **2** is regarded as a phytochemical with multifunctional therapeutic applications.[21,26]

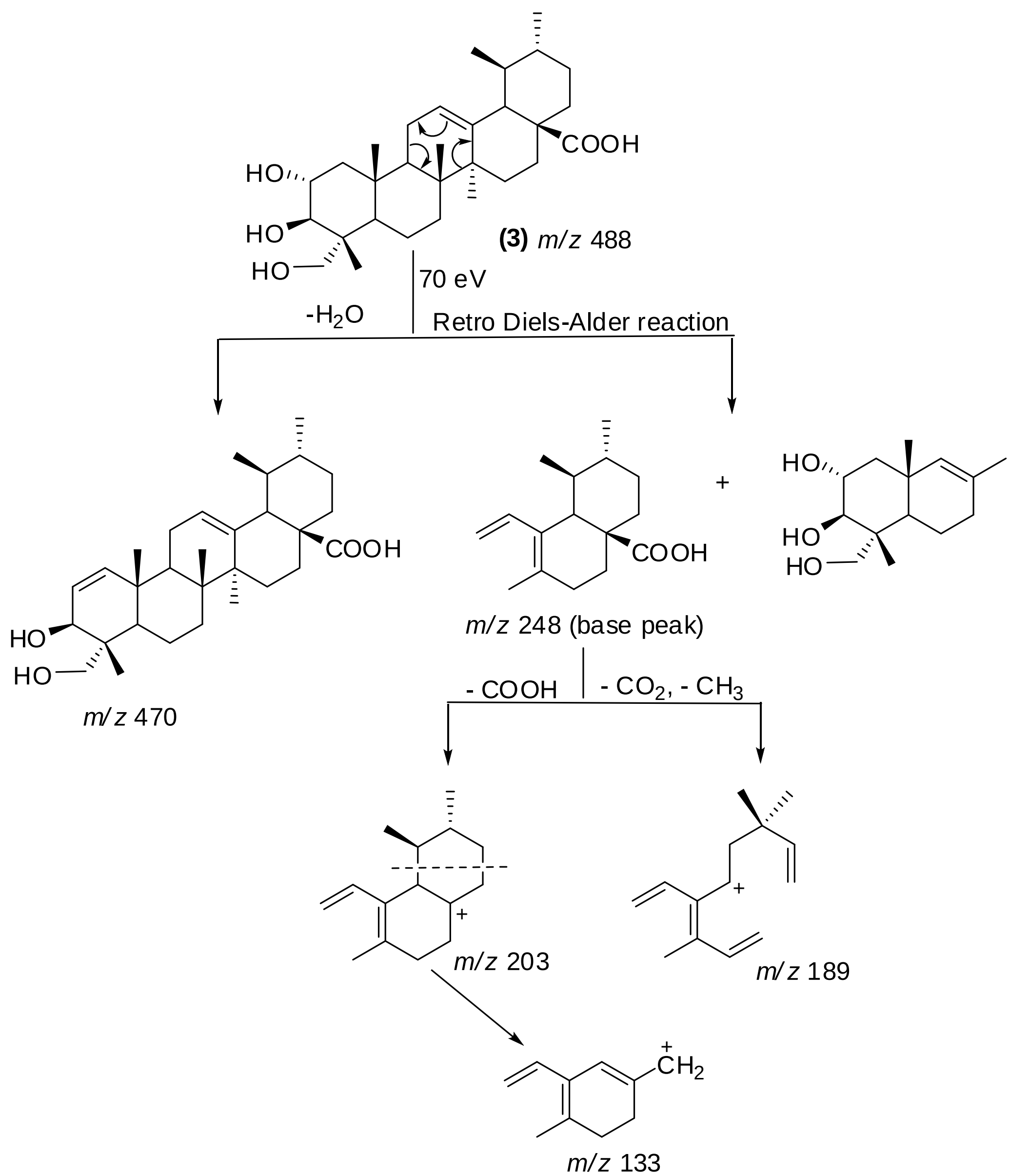


Figure 3. Proposed major MS fragmentation pattern of asiatic acid (**3**).

Asiatic acid (**3**) also exhibits a wide variety of biological and pharmacological activities, which include antioxidant, anti-inflammatory and hepatoprotective activities.[27-29] Compound **3** showed antidiabetic and antihyperlipidemic activities in experimental animals.[30-32] The antihyperlipidemic activity of **3** was found to be comparable to glibenclamide, a well-known antihyperglycemic prescription drug. The anticancer effect of asiatic acid in two human breast cancer cell lines, MCF7 and MDA-MB-231 has previously been reported.[6] Compound **3** exhibited effective cell growth inhibition by inducing cancer cells to undergo S-G2/M phase arrest and apoptosis.[33] Compound **3** decreased viability and induced apoptosis in SK-MEL-2 (Human melanoma cells) and HepG2 (Human hepatoma cells) in a time- and dose dependent manner.[34,35] Compound **3** dose-dependently showed cytotoxicity in HT29 (Human colon adenocarcinoma cell lines). The structural relationships of **3** and its derivatives to cytotoxicity and antihepatofibrotic activity in HSC-T6 cells have been reported. Modification of the carboxylic acid group at C28 also reduced the cytotoxicity in HSC-T6 cancer cell lines.[36]

4. Conclusion

Arjunolic acid (**2)** and asiatic acid (**3)** were obtained as an inseparable mixture from the chloroform extract of stem-bark of *Eugenia grandis*. They were characterised mainly by analysis of their IR, NMR and MS spectral data. Compounds **2** and **3** exhibit a variety biological and pharmacological activities. Additionally, we proposed the possibility of the existence of compound **4** in the mixture based on rational analysis. Interestingly, our literature search showed that **4** has never been reported not only from the natural product kingdom but also by synthesis. Therefore, **4** could be a potential synthetic target molecule. Due to its structural similarity to **2** and **3**, it is expected that **4** could potentially exhibit a variety of biological and pharmacological activities in line with **2** and **3**. Species from the genus, Eugenia reported to have many therapeutic applications. Further studies on *E. grandis* is required to explore this plant for its therapeutic applications.

Conflict of interest

The authors have no conflicts of interest to declare.

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An example of an equation is shown for ***Equation 1***, Weber Morris intraparticle diffusion:

$$q_t = k_{id}t^{1/2} + C \qquad (1)$$

and Boyd model (***Equation 2***):

$$F = 1 - \frac{6}{\pi^2} exp(-B_t) \qquad (2)$$

where F = q_t / q_e, F is the fraction of solute adsorbed at any time, t and B_t is mathematical function of F.

In-line citation style

The in-line citation should be in IEEE referencing format (numbers with square brackets), generated using a reference manager. Example:

> Even though various studies have shown this [1]–[5], there are others that have contradicted this [6]–[10]. Data was obtained from [11].

Please note that brackets should go **before** punctuation.

References

The reference/bibligraphic list should only include references cited in the text and should be listed in the references section in the following format:

<u>Journal article</u>

[1] J. H. Surname and J. E. Doe, “Title,” *Journal*, Vol., No., Pages, Year. DOI: (number)

Textbook/Chapter of a book
[2] J. H. Surname and J.E. Doe, *Title of Textbook*, Publication House, Year.

Dissertation/Thesis
[3] J.H. Surname, "Title of Thesis," University, Year

Webpages/Online Databases
[4] "Website/Database name/body," Year. Available: http;//www.weburl.com. [Accessed 18-Apr-2017]

These should be generated automatically if a reference manager software has been used. When there are **three or more authors**, just state the name of the first author, e.g. J. H. Surname, et. al. Please include the digital object identifier (DOI) for journal articles.

Figures and Tables

A list of tables, figures and captions should be given at the end of the manuscript after the reference list. These must be appropriately numbered in the order that they appear in the paper. Each table and figure must be adequately discussed and referenced in the text. It is important that you do not include tables and figures in the main body of text of your submitted manuscript.

Sizing

Please keep tables/figures/images/illustrations to have a **maximum width of either 8.4 cm (single column) or 17.5 cm (double column)**, with enough clarity that the images does not appear blurred, skewed or pixelated (unless the pixelation is unavoidable from the raw data collection).

Text

Texts in figures and tables should be 10pt, using either Times New Roman, Arial or Calibri font, with consistent font size and style throughout the manuscript's figures/tables/artwork/images/illustrations. Please ensure that texts do not fall below 8pt size as this will greatly affect readability of said text.

Colour

Colour images are highly encouraged for the on-line issue, however they should be designed such that the information is still obvious in grey-scale too for the print version.

Graphs

Graphs could either be saved as an embedded graph format in DOCX or as an image (JPEG or TIFF). Graphs should have clearly-labelled axes and lines that can be distinguished in both color for on-line and grey-scale for print version. You can use dotted and dashed lines etc, or you can use different data point types when appropriate to discriminate between data sets.

Tables

Tables could either be saved as an embedded table format in DOCX or as an image (JPEG or TIFF), with appropriate captions/titles.

Captions

All figures and tables should be appropriately captioned. The caption should be sufficiently able to explain the figure/table without the reader having to refer to the main text. The words "figure" and/or "table" should be bold. italicized and spelled out fully (not abbreviated), followed by a full stop (also bold and italicized). The rest of the caption should not be bold and italicized (unless it is a scientific genus or species).

Example format for figure/table caption:

Figure 1. *Periophthalmus malaccensis* collected in Sungai Bunga, Brunei (UBDM MBu081013mal); a. freshly dead specimen, lateral view; b. live specimen; c. freshly dead specimen, ventral view, detail (scale bars are 10 mm long).

Manuscripts that do not conform to the above instructions will be returned without review.