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DOI: https://doi.org/10.3897/arphapreprints.e105209

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Morphological, anatomical, phytochemical, and phylogenetic evidences reveal into a new *Derris* species (Fabaceae) with rare flowers and reddish midribs, from Peninsular Thailand

Punvarit Boonprajan [1], Charan Leeratiwong [2], Yotsawate Sirichamorn [1]

1 Department of Biology, Faculty of Science, Silpakorn University, Sanam Chandra Palace Campus, Nakhon Pathom 73000, Thailand

2 Division of Biological Science, Faculty of Science, Prince of Songkla University, Songkhla 90112, Thailand

Corresponding author: Yotsawate Sirichamorn (sirichamorn_y@su.ac.th)

Abstract

Derris erythrocosta Boonprajan & Sirich., sp. nov., a new species of the genus *Derris* Lour. (Fabaceae) was discovered from Peninsular, Thailand. The taxon was considered as a distinct species in terms of integrative taxonomy approach. The overall morphology demonstrated that this species most resembled *D. pubipetala*. According to the macro-morphological and leaves micro-morphological studies, this taxon has several autapomorphies distinctively different from other *Derris* species, e.g., the presence of reddish midribs of the mature leaflets, sparsely hairy filaments, prominent hairs at base of anthers, and presence of glandular trichomes along the midrib. Additionally, HPLC fingerprints from phytochemical study of this species were also distinct. Results from molecular phylogenetic analyses strongly supported and clearly confirmed its status as a new of the genus *Derris*.

Keywords

anatomy, *Derris*, HPLC fingerprint, molecular phylogeny, morphology, phytochemical

Introduction

The genus *Derris* Lour. is a papilionoid–legume, consisting of approximately 50 species (Adema 2000, 2003; POWO 2023; Sirichamorn 2020). It is a taxonomically complex genus in the most problematic tribe Millettieae of Fabaceae. Species of *Derris* are quite well known as important sources of a toxic substance, "Rotenone", which is traditionally and commercially used as insecticide and fish poison. However, many species of *Derris* are also used as local medicines for a variety of treatments in many South-East Asian countries (Hamid 1999).

Derris was first described by Loureiro, João de (Loureiro 1790), based on the type species *D. trifoliata* Lour. It was once recognised as a large genus called *Derris* in a broader sense (sensu lato) and divided into several sections by Bentham (1860). After revisions of the genus based mainly on morphological and molecular phylogenetic analyses, the concept of *Derris* became subsequently narrower (Geesink 1985; Adema 2000, 2003; Sirichamorn et al. 2012a; Sirichamorn et al. 2012b; Sirichamorn et al. 2014b).

All *Derris* species are liana, with imparipinnate leaves and opposite leaflets, pseudoracemes/ pseudopanicles, or rarely panicles, monadelphous and generally glabrous stamens, indehiscent, flat, thin but leathery pods usually with marginal wings. Some genera in the tribe Millettieae such as the Asiatic *Aganope* Miq. and *Brachypterum* (Wight & Arn.) Benth. or the American *Deguelia* Aubl. are, however, morphologically very similar to *Derris* and so-called as *Derris*–like plants (Sirichamorn et al. 2012a). Species of *Derris* are found mainly in South-East Asia. Seventeen species were reported in Flora of Thailand (Sirichamorn 2020). However, unknown Thai specimens have been still continuously discovered from different parts of the country.

Thailand is a part of the ancestral center area of *Derris* (Sirichamorn 2014a). The country situates on the meeting point of three major floristic regions: Indo–Chinese, Indo–Burmese and Malesian elements. Southern Thailand is on Malay's peninsula and bounded to the north by Kra Isthmus. The majority of the region is encompassed by the Tenasserim-South Thailand semi-evergreen rain forests ecoregion, extending from north to south along the western coast of the Andaman Sea and the eastern coast of the Gulf of Thailand. The southernmost part along the border with Malaysia, on the other hand, is covered by the Peninsular Malaysian rain forests and Peninsular Malaysian montane rain forests ecoregions (Wikramanayake et al. 2002). Several karsts formed by erosion of limestone are also found along the western coast. The forest areas at Thai-Malaysian border such as Hala-Bala Wildlife Sanctuary is, moreover, regarded as one of Thailand's richest forests and home of various rare and endemic flora and fauna. Due to the violence situation in Thailand's three southern border provinces, the forest there has become mostly inaccessible and with low anthropogenic disturbance. Up to now, a variety of new plant taxa have been discovered and reported successively in this area. For example, *Clerodendrum angustipetalum* Satthaphorn, A.J.Paton & Leerat. and *C. peninsulare* Satthaphorn, A.J.Paton & Leerat. (Lamiaceae; Satthaphorn et al. 2021, 2022), *Goniothalamus roseipetalus* Leerat.,

Chalermglin & R.M.K. Saunders and *G. sukhirinensis* Leerat., Chalermglin & R.M.K. Saunders (Annonaceae; Leeratiwong et al. 2021), *Khaosokia caricoides* D.A. Simpson, Chayam. & J. Parn. (Cyperaceae; Simpson et al. 2005) or *Lasianthus ranongensis* Sinbumr. & Napiroon (Theaceae; Napiroon et al. 2021) were discovered in this area.

During the field expedition in 2019, the co- and corresponding authors of this article discovered and collected an unrecognised "*Derris*-like" species along the stream at Pha Dam Forest Ranger Unit (Ton Nga Chang Wildlife Sanctuary), Songkhla province. However, the plant showed some unusual morphological characteristics compared with other known species of *Derris* and *Derris*-like taxa. After comprehensive field surveys for years, two more populations were later found by the stream at the entrance of Tone Prew waterfall (Ton Nga Chang Wildlife Sanctuary, also in Songkhla province) and Krung Ching waterfall (in Khao Luang National Park in Nakhon Si Thammarat province). The latter two populations had very similar morphological characters vegetatively but unfortunately, never produced flowers. Only the type specimen from Pha Dam Forest Ranger Unit only once produced inflorescences and flowers in 2019, but with no pod setting. The plant specimens were observed and then compared with the type specimens of all *Derris*-like taxa, including the description from several taxonomic literatures such as the Flora of Thailand (Sirichamorn 2020) and neighboring floras such as the Flora of the Malay peninsula (Ridley 1922), Flora of China (Wei et al. 2008), Flora of India (Thothathri 1982), Flora of British India (Baker 1878) and taxonomic revision of the genus in India (Thothathri 1961). Preliminary results from detailed morphological studies, revealed it to be an undescribed species of a *Derris*-like genus, most possibly *Derris*.

The main objectives of this work are to investigate and verify the exact taxonomic status of this unknow *Derris*-like species using more critical macro- and micro-morphological studies, molecular phylogenetic analyses, and phytochemical evidences from HPLC fingerprint. The full descriptions, key to the species recognised are provided with the illustration in the taxonomic part.

Materials and Methods

Taxon collections, preparations, and taxonomic study

Three unknown samples which are morphologically similar from 3 localities (representing 3 accessions), also 3 samples of *D. pubipetala* from 3 different localities were collected for morphological, anatomical, phytochemical, and molecular investigation. Standard methods for plant taxonomy were used in the preparation of voucher specimens (Table 1), then were carefully examined before being deposited at BKF and other herbaria. Three mature leaflets per accession were fixed in liquid preservatives (70% ethyl alcohol) for leaves anatomical study. Young leaves

of 3 unknown samples were collected and then stored in a silica gel for DNA extraction. Stems and roots were also collected, cleaned, cut into smaller parts prior to dried in a 50–60°C hot air oven, and immediately stored in a dark at room temperature for phytochemical study.

Voucher specimens were examined by stereo-microscope. The description was prepared using the format of Flora of Thailand (Sirichamorn 2020). Morphological measurement and comparison of the collected specimens with the (type) specimens of *Derris*-like plants conserved in Thai-herbaria (BK, BKF, and PSU) or digital images available online in abroad herbaria (K, L, and P) were carried out.

Table 1. Species taxa, localities, and vouchers of the material in Thailand used in morphological and phytochemical study.

Accession No.	Species	Locality	Voucher specimens	Herbarium
1	*Derris* sp.	Songkhla, Sadao district, Padang Besar sub-district (Pha Dam Forest Ranger Unit, Ton Nga Chang Wildlife Sanctuary)*	C. Leeratiwong 19–1666	BKF
2	*Derris* sp.	Songkhla, Rattaphum district, Kamphaeng Phet sub-district (Tone Prew waterfall, Ton Nga Chang Wildlife Sanctuary)	YSM2021–15	BKF
3	*Derris* sp.	Nakhon Si Thammarat, Nopphitam district, Nopphitam sub-district (Krung Ching waterfall)	YSM2021–16	BKF
4	*D. pubipetala*	Surat Thani, Ko Samui district, Ang Thong sub-district (Hin Lad Waterfall)**	Leeratiwong et al. 18–1192	PSU
5	*D. pubipetala*	Nakhon Si Thammarat, Khanom district, Khanom sub-district (Samedchun Waterfall)	YSM2022–5	BKF
6	*D. pubipetala*	Nakhon Si Thammarat, Khanom district, Khanom sub-district (Hin Lad Waterfall)**	YSM2022–6	BKF

Note: * type locality; ** The name of both waterfalls is the same but they are different places.

Scanning electron microscopy (SEM)

Three lateral leaflets of mature leaves were cut in the midrib and area between margin and midrib for each accession (5 mm × 5 mm). Then, they were cleaned, dehydrated in a series of ethyl alcohol, dried by critical point drying (CPD) techniques, and preserved in a desiccator for subsequent observation by SEM technique. Upper and lower leaves samples were mounted directly on aluminum stubs using double-sided carbon tape, then sputter-coated with gold using an SPI module sputter coater. The samples were observed and photographed by SEM Tescan Mira3 scanning electron microscope at the Scientific and Technological Equipment Center, Faculty of Science, Silpakorn University.

Anatomical study of the leave

Leaf epidermis was studied using leaf scrapping technique. Mesophyll was directly scrapped off from the upper and lower surfaces with a razor blade, following by epidermal bleaching using sodium hypochlorite. Samples were cleaned 3 times in distilled water, then stained with 1% Safranin-O before washing the pieces with water again and dehydrated in a graded series of ethyl alcohol and series of xylene. Samples were mounted on slides using DePeX mounting agent.

In order to observe transverse sections. Three leaflets from 3 mature leaves for each accession were free-hand transversely sectioned, stained with 1% Safranin-O, then, dehydrated in a graded series of ethyl alcohol and series of xylene. Finally, the sections were mounted on slides using DePeX. All leaf epidermal surfaces and sectioned parts were observed and digitally photographed with an Olympus BX53 microscope with a camera attachment Olympus DP27. Each leaf anatomical character was measured using ImageJ v. 1.50i.

Phytochemical analyses

Dried materials of the stems and roots were ground into powder using a high-speed blender. The plant sample (25 g) was then placed in an Erlenmeyer flask and maceration was performed in 250 ml of Dichloromethane at dark, room temperature for 72 hrs. The solvent extract of each accession was then filtered and evaporated in a dark fume hood at room temperature for 24 hrs. The crude extract was transferred into a well-closed plastic tube, protected from light and stored in a refrigerator at 0–5°C for further analysis.

The qualitative phytochemical analysis of rotenone and deguelin were analyzed using high-performance liquid chromatography (HPLC) (Agilent Technologies, Germany) consisting of a 1260 Infinity II LC system controller fitted with 1260 Infinity II Quaternary, 1260 Infinity Binary pump, 1260 Infinity II degasser, and 1260 Infinity II Diode Array UV-Visible detector. An Agilent 5 TC-C18 (4.6 x 150 mm, particle size 5 µm) reversed-phase analytical column was used. The isocratic method was performed for separating chemical substances using a mobile phase of acetonitrile/water (60 : 40; v/v) in 35 min. The injection volume was 10 µL, and the flow rate was 1.0 ml/min. The liquid chromatography system had to be stabilized for 15 min with the mobile phase before injecting the analyte. The analysis was performed at a wavelength of 294 nm. The control and data elaboration used Agilent OpenLAB ChemStation Edition.

Approximately 0.01 g of crude extract was dissolved and diluted with 1 mL of acetonitrile in a 2 ml microcentrifuge tube. The extracted solution was filtered using a syringe filter nylon membrane of 0.22 µm pore size. The chemical patterns and contents of the main two chemical markers were carried out by the HPLC system and determined as above.

Molecular analyses

Taxa sampling used in this study were followed the phylogeny reconstructed by Sirichamorn et al. (Sirichamorn et al. 2012a) (Suppl. material 1: Table S1). Additional DNA samples were extracted from dried young leaves from three populations of the unknown plants using DNeasy Plant mini kit and modified protocol (Qiagen, Hilden, Germany). Two chloroplasts [*trnL–F* intergenic spacer (IGS) and *trnK–matK*] and one nuclear ribosomal internal transcribed spacer (ITS/5.8S) were amplified using universal primers (Table 2). PCRs reagents were carried out in a 25 µL reaction mixture which contained 1 µL (10 µM) of each forward and reverse primer, 12.5 µL GoTag Green Master Mix (Promega), 2 µL (<250 ng) of total DNA and Nuclease-free water to 25 µL. PCR conditions for *trnL–F* IGS and *trnK–matK* followed the protocol modified from Hu et al. (2000). ITS/5.8S region was amplified using similar conditions described in Wojciechowski et al. (1993, 1999). Quality, quantity, and size of PCR products were tested by Gel electrophoresis and genomic absorption. Then they were sent to Macrogen (http://www.macrogen.com) for purifying and sequencing. Each purified fragment was treated by Barcode-Tagged Sequencing (BTSeq^{TM}) technique of Next-Generation Sequencing (NGS) technology for automated dsDNA sequencing. Ultimately, all newly generated sequences in this study have been deposited in GenBank (Table 5).

Sequence alignments were performed using the program Bioedit v. 7.0.9 (Hall 1999) with the CLUSTAL W multiple alignment in the software (default settings; Thompson et al. 1994) together with subsequent manual adjustment. Sequences of each marker were firstly aligned separately. Then, the combined data matrix was made by concatenating those already aligned datasets. Phylogenetic relationship was reconstructed using Maximum Parsimony (MP), Maximum Likelihood (ML), and Bayesian Inference (BI). For MP analyses, phylogenetic trees were constructed with the program PAUP* v. 4.0a169 (Swofford 2002) under heuristic search with 10000 replicates of random taxon additions combined with inferred using the option with Tree Bisection-Reconnection (TBR) branch swapping and Multrees activated, with all parsimonious trees were saved. Bootstrap percentages analysis was used for the evaluation of MP clade support and calculated using the same settings (Felsenstein 1985). A clade with Maximum Parsimony Bootstrap Support (MPBS) ≥ 85%, 75–84%, 50–74%, or <50% was considered strongly, moderately, weakly, or no supported, respectively. The jModelTest v. 2 (Darriba 2012) on the CIPRES web portal was used to find the best-fitting substitution model chosen by the Akaike Information Criterion (AIC) scores (Akaike 1974). The General Time Reversible (GTR) (Tavaré 1985) nucleotide substitution model with a gamma distribution for among-site rate

variation was selected for all DNA regions. ML analyses were performed for the combined data sets using the program IQ-TREE v. 2.2.0 (Nguyen 2015) under GTR+G partition models implemented with the "-p" command. Bootstrap (Felsenstein 1985) clade support was calculated using a non-parametric bootstrap resampling with 2000 replicates. Maximum Likelihood Bootstrap Support (MLBS) values are described as high (85–100%), moderate (75–84%), low (50–74%), or no (<50%) support. Bayesian Markov chain Monte Carlo (MCMC) (Yang and Rannala 1997) phylogenetic analyses were reconstructed using the program MrBayes v. 3.2.7a (Ronquist et al. 2012) via the CIPRES Science Gateway v. 3.3 (Miller 2010). The majority-rule consensus tree was started from random trees and run for 10000000 generations until stationarity, with MCMC sampled every 1000 generations. The first 25% of all trees were discarded as burn-in and each BI clade was supported by posterior probabilities (PP) estimation from the remaining sampled trees. PP are described as high (0.95–1), moderate (0.9–0.94), low (0.5–0.89), or no (<0.5) support.

Table 2. Sequences of the primers used for PCR amplification and sequencing.

The abbreviation Ch means chloroplast marker, and Nr means nuclear marker.

Primers	Amplified region	Direction	Sequence (5′ to 3′)	References
trnKIL	*trnK–matK* (Ch)	forward	CTC AAT GGT AGA GTA CTC G	Hu et al., 2000
trnK2R	*trnK–matK* (Ch)	reverse	AAC TAG TCG GAT GGA GTA G	Hu et al., 2000
e	*trnL–F* IGS (Ch)	forward	GGT TCA AGT CCC TCT ATC CC	Taberlet et al., 1991
f	*trnL–F* IGS (Ch)	reverse	ATT TGA ACT GGT GAC ACG AG	Taberlet et al., 1991
ITS1	ITS/5.8S (Nr)	forward	TCC GTA GGT GAA CCT GCG G	White et al., 1990
ITS4	ITS/5.8S (Nr)	reverse	TCC TCC GCT TAT TGA TAT GC	Wojciechowski et al., 1993

Results

Morphological Study

Three unknown *Derris* samples were taken photos and morphologically studied as shown in Figs 1–3. They were collected from 3 localities in Sonkhla and Nakhon Si Thammarat provinces (Fig. 4, a yellow square and 2 light-blue triangles). As previously mentioned, their overall-vegetative morphology is undistinguishable and presumable the same species. The most remarkable characteristics found in all three samples are the reddish midribs of the mature leaflets. Only the sample collected from the type locality in Songkhla province produced flowers and thus the floral characteristics were observed from it. The most similar species distributed in Peninsular Thailand is *D. pubipetala*, however, several morphological characters were distinctly different.

For example, the unknown *Derris* samples have paler bark and more leaflets in general. The inflorescence of the flowering type specimen is longer than *D. pubipetala*'s. Shape, recurvation, and presence of the lower auricle of the wing petals were also different. Stamens of the unknown *Derris* show hairiness on free part of the filaments and more importantly, the hairs at the base of the anthers which were not present in *D. pubipetala* or any species of *Derris*. All comparative morphological characters between the unknown *Derris* and *D. pubipetala* were summarized in Table 3.

Table 3. Comparative morphological characters of the unknown *Derris* samples and its morphologically most similar species, *D. pubipetala*.

Morphological characters	**Species**	
	The unknown *Derris*	***D. pubipetala***
Vegetative parts		
Colour of roots	brownish to black-gray	slightly pinkish or reddish
Colour of bark	pale brownish-gray to gray	dark reddish-brown
Colour of leaves when young	reddish	light green to brownish
Number of leaflets	9–11	5–9
Colour of midrib on leaves when mature	reddish	green
Adaxial leaf surface	glabrous but slightly strigose along midrib and lateral veins	glabrous to slightly hairy
Abaxial leaf surface	glabrous to thinly strigose along midrib and leaf surface, sometime slightly strigose to glabrous at margin	strigose to almost glabrous
Reproductive parts		
Length of inflorescence (cm)	40–50	5–28
Number of flowers per a brachyblast	2–8	5–7
Length of pedicels (mm)	3.5–3.2	2–6
Position of bracteoles	at the base of calyx	on pedicel
Colour of corolla	pale pink to pink	white
Shape of wing petals	blade elliptic narrowly ovate	elliptic to semi-hastate
Apex of wing petals	obtuse-long	rounded
Lower auricle of wing petals	absent	present
Recurvation of wing petals	straight	curved backward.
Apex of keel petals	retuse	rounded
Anthers with some basal hairs	present	absent
Free part of filaments with hairs	present	absent
Shape of floral disc	indistinct or more or less	annular

Indumentum on style	sericeous at the base and gradually become glabrous apically	glabrous
Shape of floral disc	lobed	annular

Figure 1. *Derris erythrocosta*, sp. nov. (**A**) habit and habitat; (**B**) leaves; (**C**) stem; (**D**) young reddish leaflets; (**E**) reddish midribs of mature leaflets; (**F, G**) closed up midrib on adaxial and abaxial surface respectively; (**H**) flowers and inflorescence; (**I, J**) closed up inflorescence, showing flower buds and brachypblasts; (**K**) closed up flowers. All photos were taken from the type living plants in Songkhla province. Photos by Punvarit Boonprajan (**A, B, D–G**) and Charan Leeratiwong (**C, H–K**).

Figure 2. Comparative macro- and micro-morphological characters of leaflets and flowers. (**A, B**) a branch with leaves and inflorescence; (**A1, B1**) outside and (**A2, B2**) inside standard petal; (**A3, B3**) outside and (**A4, B4**) inside keel petals; (**A5, B5**) outside and (**A6, B6**) inside wing petals; (**A7.1, B7.1**) stamens and (**A7.2, B7.2**) closed up stamens; (**A8.1, B8.1**) pistil and (**A8.2, B8.2**) closed up pistil's apex; (**A9, B9**) adaxial and (**A10, B10**) abaxial leaf surface. (**A–A10**) *D.*

erythrocosta and (**B–B10**) *D. pubipetala*. Scale bars = 1 mm. Photos by Charan Leeratiwong (**A, B**) and Punvarit Boonprajan (**A–A10, B–B10**).

Figure 3. *D. erythrocosta*. (**A**) inflorescence with leaf; (**B**) closed up inflorescence; (**C**) flower (top and side view); (**D**) outside and (**E**) inside standard petal; (**F**) outside and (**G**) inside keel petals; (**H**) outside and (**I**) inside wing petals; (**J**) stamens; (**K**) apical stamens with anthers; (**L**) pistil; (**M**) adaxial and (**N**) abaxial leaf surfaces. Scale bars: (**A, B**) 5 mm; (**C–N**) 1 mm. Drawn by Punvarit Boonprajan from C. Leeratiwong 19–1666 (BKF).

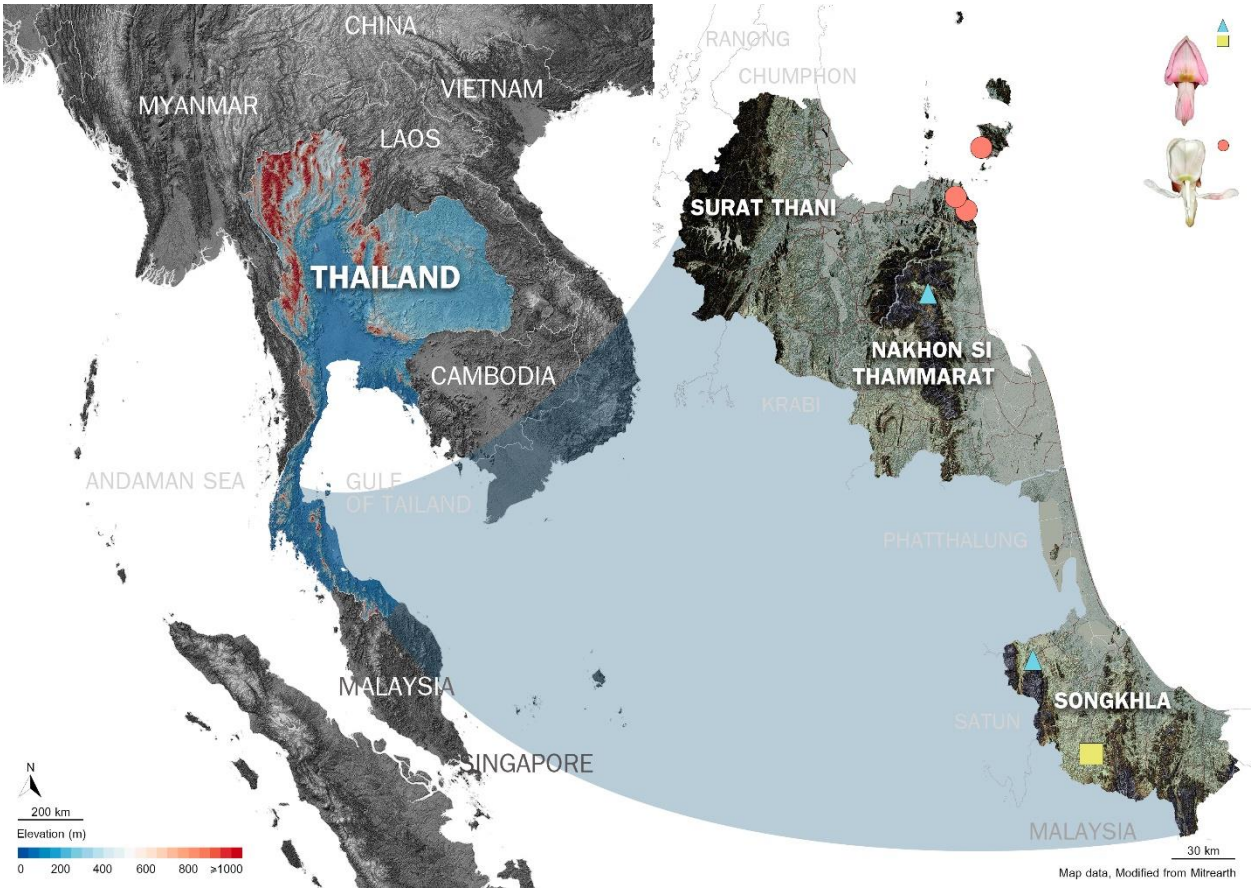

Figure 4. Collection sites of the unknown *Derris* samples, represented by yellow square (type locality) and light-blue triangle and *D. pubipetala* (orange circle) in Peninsular Thailand.

Anatomical study of the leaflets

The micro-morphological and anatomical characters of the unknown *Derris* samples and *D. pubipetala* studied from the light microscope and scanning electron microscope (SEM) are summarized in Table 4 and presented in Figs 5–7.

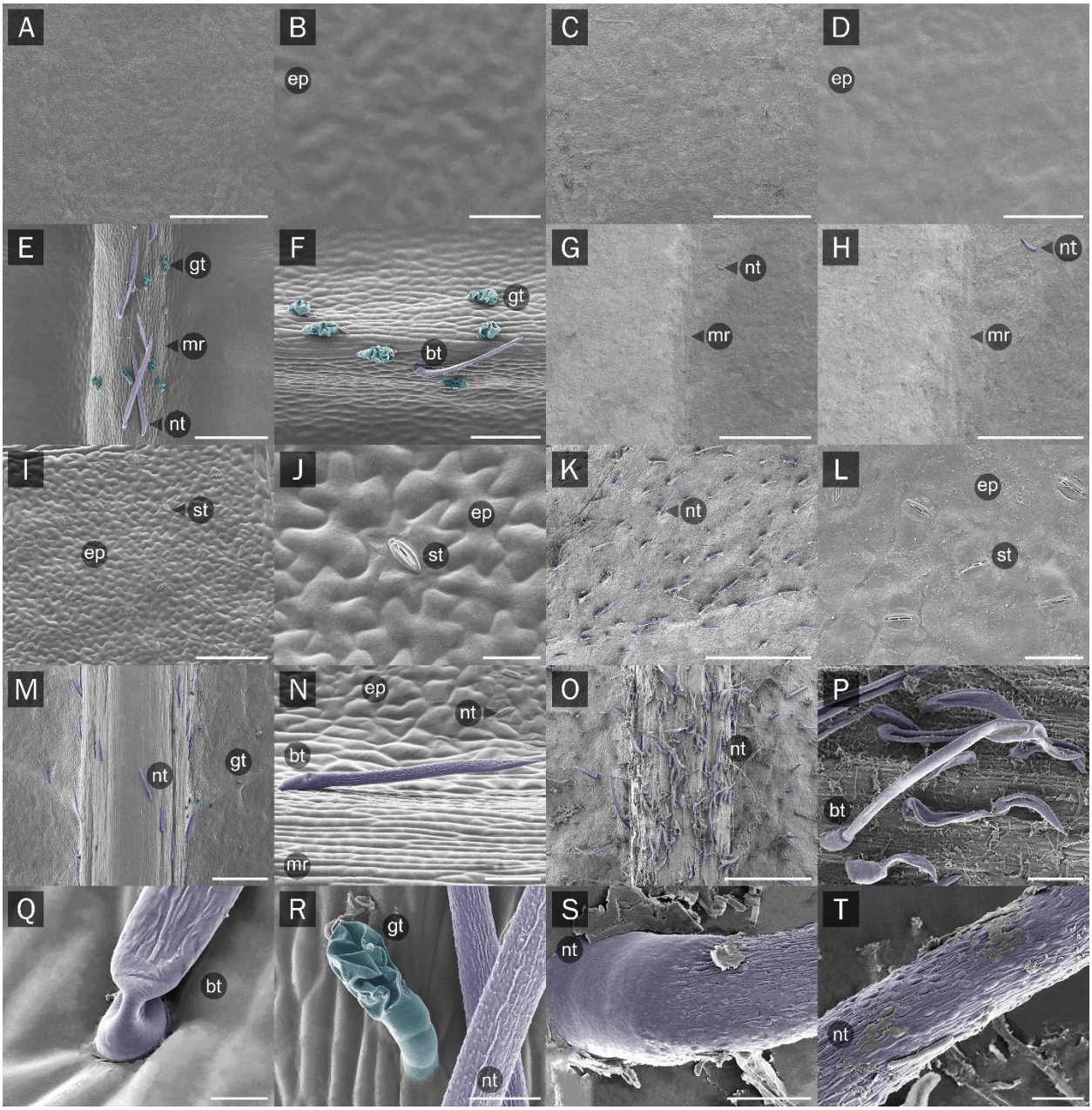

Figure 5. Scanning electron microscope (SEM) micromorphology of leaves. (**A–D**) adaxial and (**I–L**) abaxial surfaces of lamina; (**E–H**) adaxial and (**M–P**) abaxial surfaces of midrib; (**Q–T**) non-glandular and glandular trichomes; (**A, B, E, F, I, J, M, N, Q, R**) The unknown *Derris* samples and (**C, D, G, H, K, L, O, P, S, T**) *D. pubipetala*; bt, bi-cellular non-glandular trichome; ep, epidermal cell; gt, glandular trichome; mr, midrib; nt, non-glandular trichome; st, stoma. Scale bars: (**T**) 5 µm; (**Q, S**) 10 µm; (**J, L, R**) 20 µm; (**B, D, N, P**) 50 µm; (**F, I**) 100 µm; (**E**) 200 µm; (**A, C, H, K, M, O**) 500 µm; (**G**) 1000 µm.

Leaf epidermis (Fig. 6A–L): Leaf epidermal cells of 3 unknown *Derris* samples and *D. pubipetala* on both leaf surfaces were quite similar, i.e., lobed or jigsaw-liked in shape because of

their undulate anticlinal cell walls (slightly more deeply undulate in the unknown *Derris* samples). The width and length of the epidermal cells on both surfaces ranged between 21.91–41.53 µm and 31.15–56.60 µm, respectively. Ratios of epidermal cell size (width : length) of the unknown *Derris* were 0. 77 (±0.20) for adaxial and 0. 66 (±0.12) for abaxial surface, whereas the ratio of *D. pubipetala* were 0.83 (±0.09) for adaxial and 0.78 (±0.36) for abaxial surface respectively. Leaves of all taxa were hypostomatic with commonly paracytic and rarely anomocytic stomata. The width and length of stomata in the unknown *Derris* (w = 17.47±1.01 µm; l = 22.95±1.03 µm) was greater than *D. pubipetala* (w = 12.43±0.73 µm; l = 15.04±0.52 µm), whereas the stomatal density of *D. pubipetala* (230±15.62 per mm^2) was higher than the unknown *Derris* (104. 66±3.05 per mm^2). The stomatal index of the unknown *Derris* was also higher (17. 02±0. 75) than *D. pubipetala*' s (16.52±1.04). Guard cells size of the unknown *Derris* was c. 22.65 µm long and c. 8.24 µm wide (ratio w : l = 0.36±0.01). For *D. pubipetala*, guard cells were obviously smaller, c. 15.41 µm long and c. 5.17 µm wide (w : l = 0.33±00). Only unicellular non-glandular trichomes occurred on both leaf surfaces in the unknown *Derris*. In *D. pubipetala*, unicellular non- glandular trichomes occurred only on the adaxial side and three lengths (large: more than 400 µm, medium: >100–400 µm, and small: less than 100 µm) of bicellular non-glandular trichomes were found only on abaxial surface. No significant differences in leaf epidermis can be found among three accessions of the unknown *Derris* or populations of *D. pubipetala*.

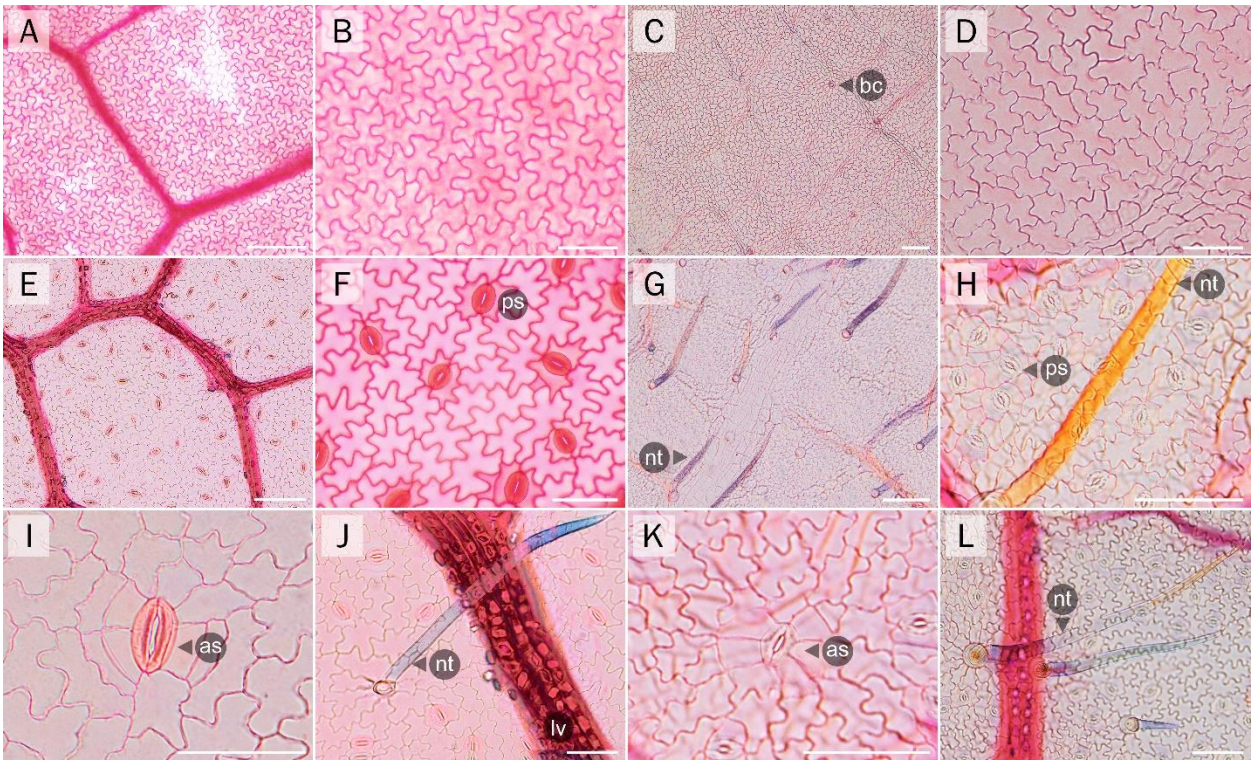

Figure 6. Comparative anatomical characters of leaf epidermis. (**A–D**) adaxial leaf epidermis; (**E–H**) abaxial leaf epidermis; (**A, B, E, F, I, J**) The unknown *Derris* samples and (**C, D, G, H, K, L**)

D. pubipetala; as, anomocytic stoma; bc, basal cell of trichome; nt, non-glandular trichome; ps, paracytic stoma; Scale bars: (**B, D, F, H–L**) 50 µm; (**A, C, E, G**) 100 µm.

Transverse section (Fig. 7A–P): Midrib of the unknown *Derris* samples showed a single convex on the abaxial side, while the adaxial side of *D. pubipetala*' s midrib was quite flat or slightly concave. The midrib transverse sectional outline size of the unknown *Derris*'s was larger (w = 1200.15±234.88 µm; h = 1241.50±244.61 µm) than *D. pubipetala* (w = 616.44±85.36 µm; h = 593.48±80.23 µm). Height of epidermal cells on adaxial and abaxial surface of midrib ranged from 8.41–13.9 µm. in all species. Vascular tissues in all taxa formed into one bundle, semi-circular to rounded shape. Perivascular fibers were found around the vascular bundle. Vascular tissues in the bundle presented into two groups: the smaller fan-shaped on the adaxial side and the larger horseshoe-shaped on the abaxial side. Vascular bundle of all samples of the unknown *Derris* was larger (w = 1067.22±240.35 µm; h = 923.20±240.47 µm) than what found in *D. pubipetala* (w = 481.48±64.20 µm; h = 406.98±52.31 µm). Bicellular non-glandular and glandular trichomes were restricted along the midrib of the unknown *Derris* (Fig. 5E, F, M, N). In *D. pubipetala*, on the other hand, only bicellular non-glandular trichomes were found (Fig. 5O, P and Fig. 7C, D).

All taxa had dorsiventral leaves (Fig. 7M, O), with palisade mesophyll adaxially and spongy mesophyll abaxially. The unidentified *Derris* showed greater leaf thickness (204.27±37.90 µm) than *D. pubipetala* (150.35±8.43 µm). The epidermal cell heights on adaxial and abaxial surfaces of leaf blades in all studied samples were between 9.22–23.49 µm. Furthermore, the mesophyll of the unknown *Derris* showed the thicker 2–3 layered palisade parenchyma (105.14±19.70 µm) than *D. pubipetala* (59.62±7.51 µm). However, spongy parenchyma in *D. pubipetala* was thicker (69.85±5.19 µm) than what was examined in the unknown *Derris* samples (64.48±20.24 µm). Interestingly, spongy mesophyll of *D. pubipetala* consisted of variously-shaped cells, loosely arranged with larger intercellular air spaces when compared with the unknown *Derris*, which possessed relatively fewer and smaller intercellular air spaces.

Transverse sections of leaf margin in all samples were slightly revolute (Fig. 7N, P). Leaf margin of the unknown *Derris* was thicker (143.98±5.86 µm) than *D. pubipetala*'s (138.04±4.19 µm). Hight of epidermal layer on adaxial and abaxial surfaces ranged from 7.73–14.10 µm. Besides, thickness of palisade and spongy parenchyma at leaf margin of the unknown *Derris* samples and *D. pubipetala* were (74.57±6.01 vs. 31.88±2.27 µm) and (49.54±7.35 vs. 54.42±11.82 µm), respectively.

Leaves transverse section of all samples demonstrated the accumulation of prismatic crystals, associated generally with vascular bundles and particularly in the midribs. Rhomboidal prism and styroid prism were two types of crystal observed in the unknown *Derris*' s leaves. Whereas only rhomboidal dipyramid prisms could be observed in *D. pubipetala* with higher

density. The unknown reddish substances present in cortical parenchyma cells of the midrib abaxially is a unique characteristic observed only in the unknown *Derris*.

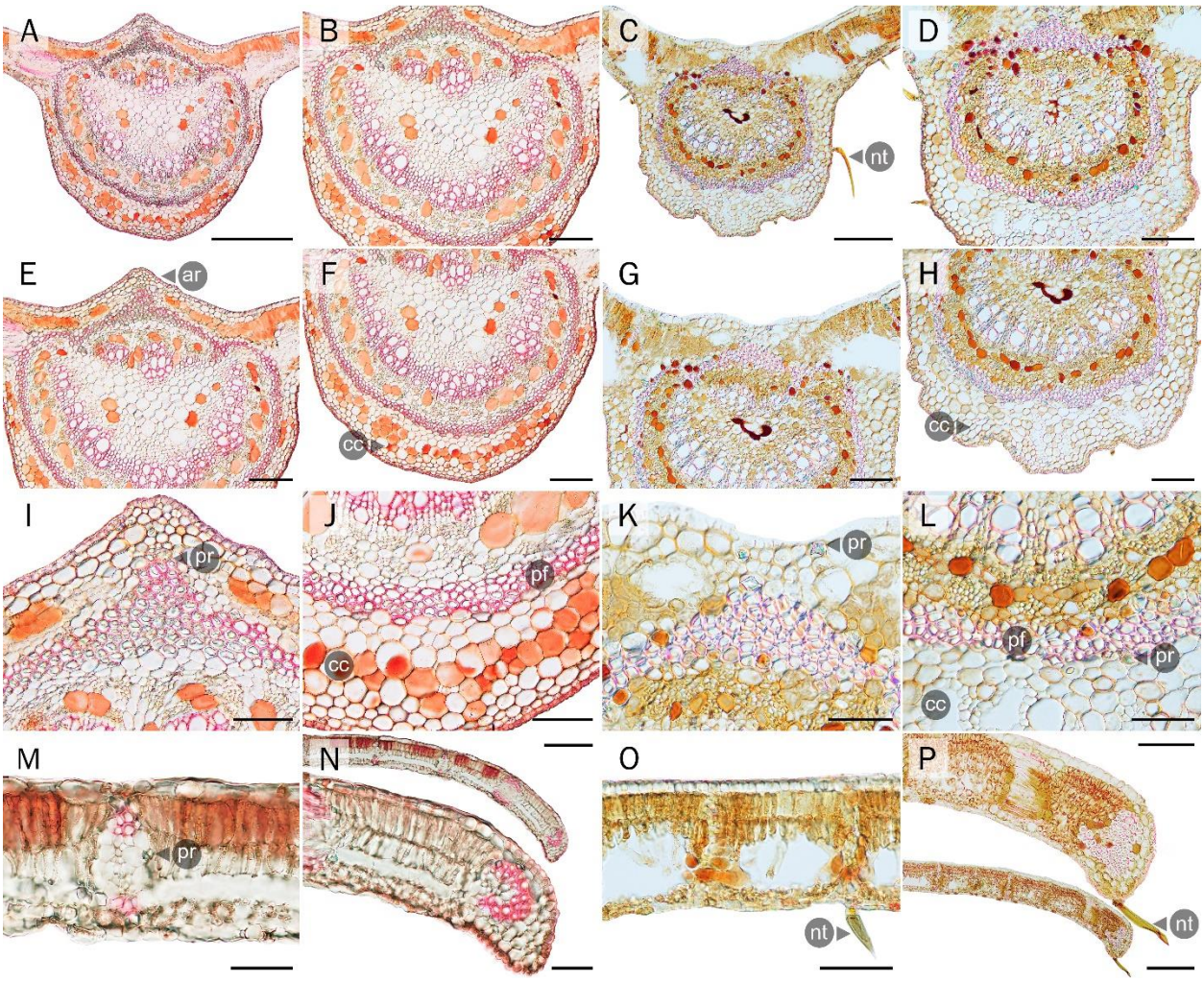

Figure 7. Comparative anatomical characters of leaf transverse sections. (**A–L**) midrib; (**M, O**) leaf blade and (**N, P**) leaf margin; (**A, B, E, F, I, J, M, N**) The unknown *Derris* samples and (**C, D, G, H, K, L, O, P**) *D. pubipetala*; ar, adaxial ridge; cc, cortical cells; nt, non-glandular trichome; pf, perivascular fibers; pr, prism. Scale bars: (**M, N**(below)**, O, P**(above)) 50 µm; (**I–L**) 100 µm; (**B, D, E–H, N**(above)**, P**(below)) 200 µm; (**A, C**) 500 µm.

Table 4. Comparison of selected anatomical characters of the unknown *Derris* samples and *D. pubipetala*. [Quantitative data; Mean value ± standard deviation (SD)]

Anatomical characters	Species	
	The unknown *Derris*	*D. pubipetala*
Leaf epidermal		

Adaxial leaf surface		
Patterns of epidermal cell walls	undulate	undulate
Width of epidermal cell walls (µm)	41.53±5.52	35.73±3.94
Length of epidermal cell walls (µm)	55.05±6.50	43.22±4.71
Indumentum	glabrous, slightly strigose along midrib and lateral veins	thinly strigose
Ratio of epidermal cell walls size (Width : Length)	0.77±0.20	0.83±0.09
Abaxial leaf surface		
Patterns of epidermal cell walls	undulate	undulate
Width of epidermal cell walls (µm)	37.28±2.71	21.91±2.42
Length of epidermal cell walls (µm)	56.60±6.81	31.15±10.43
Ratio of epidermal cell walls size (Width : Length)	0.66±0.12	0.78±0.36
Types of stomata	Pa., rarely An	Pa., rarely An
Width of stomata (µm)	17.47±1.01	12.43±0.73
Length of stomata (µm)	22.95±1.03	15.04±0.52
Ratio of stomatal size (Width : Length)	0.76±0.04	0.82±0.07
Stomatal density (per mm^2)	104.66±3.05	230±15.62
Number of epidermal cells per unit area (mm^2)	511.33±38.27	1161.33±20.42
Stomatal Index (SI)	17.02±0.75	16.52±1.04
Width of guard cell (µm)	8.24±0.76	5.17±0.08
Length of guard cell (µm)	22.65±1.46	15.41±0.34
Ratio of guard cell size (Width : Length)	0.36±0.01	0.33±0.00
Indumentum	glabrous to thinly strigose	hairy strigose
Types of indumentums	Ut	Bt
Length of Indumentum (µm)	204.70±80.00	ST=60.10±16.26 MT=265.02±39.15 LT=457.25±30.70
Ratio of epidermal cells width on adaxial leaf surface to epidermal cells width on abaxial leaf surface (Width : Width)	1.11±0.07	1.64±0.25
Ratio of epidermal cells length on adaxial leaf surface to epidermal cells length on abaxial leaf surface (Length : Length)	0.97±0.07	1.54±0.72
Leaf transverse sections		
Transverse section of Midribs		
Outline of midribs	convex on the abaxial surface with an adaxial ridge	convex on the abaxial surface with flat or slight concavity on the adaxial surface
Width of midrib (µm)	1200.15±234.88	616.44±85.36
Height of midrib (µm)	1241.50±244.61	593.48±80.23
Ratio of midrib size (Width : Height)	0.96±0.00	1.04±0.10
Height of epidermal cells on adaxial leaf surface (µm)	9.96±3.59	13.9±0.92

Height of epidermal cells on abaxial leaf surface (µm)	8.55±0.38	8.41±0.72
Width of vascular bundle (µm)	1067.22±240.35	481.48±64.20
Height of vascular bundle (µm)	923.20±240.47	406.98±52.31
Ratio of vascular bundle size (Width : Height)	1.16±0.05	1.18±0.08
Types of indumentums	Ut, Bt, Gt	Bt
Transverse section of Leaf Blades		
Thickness of leaf blade (µm)	204.27±37.90	150.35±8.43
Height of epidermal cells on adaxial leaf surface (µm)	23.49±5.80	11.59±1.03
Height of epidermal cells on abaxial leaf surface (µm)	13.06±0.99	9.22±1.49
Thickness of palisade mesophyll (µm)	105.14±19.70	59.62±7.51
Thickness of spongy mesophyll (µm)	64.48±20.24	69.85±5.19
Thickness ratio of palisade mesophyll to spongy mesophyll	1.68±0.31	0.85±0.13
Number of palisade mesophyll layers	2 or rarely 3	2
Transverse section of Margins		
Outline of leaf margin	downward	downward
Thickness of leaf margin (µm)	143.98±5.86	138.04±4.19
Height of epidermal cells on adaxial leaf margin (µm)	10.84±1.33	14.10±2.61
Height of epidermal cells on abaxial leaf margin (µm)	7.73±1.19	10.34±0.21
Thickness of palisade mesophyll (µm)	74.57±6.01	49.54±7.35
Thickness of spongy mesophyll (µm)	31.88±2.27	54.42±11.82
Thickness ratio of palisade mesophyll to spongy mesophyll	2.35±0.34	0.95±0.31
Number of palisade mesophyll layers	2	2
Angle of leaf margin apex	9.16±1.68	16.01±3.04

Note: BT, bicellular non-glandular trichome; GT, glandular trichome; LT, long trichome; MT, medium trichome; ST, short trichome; Ut, unicellular non-glandular trichome

Chemical fingerprint

HPLC chromatograms (Fig. 8) showed intra- and inter-specific differences in pattern of chemical compounds of each sample's root and stem crude extract in this study. Under the optimal HPLC conditions, the peaks of two investigated chemical markers showed acceptable resolution (Fig. 8A). The standard peaks of rotenone and deguelin were detected at 6.1 (peak I) and 6.7 (peak II) min, respectively. Most of HPLC chromatograms (8 from 12) of the unknown *Derris* (Fig. 8B) and *D. pubipetala* (Fig. 8C) showed the presence of two common chemical markers, i.e., Rotenone (I) and Deguelin (II), except the unknown *Derris* accession no. 2 (both root and stem extract), stem extract of the unknown *Derris* accession no. 1, and stem extract of *D. pubipetala* accession no. 5). Peaks of unknown chemical compounds (III–XI) were detected only in *D. pubipetala* but absent in all samples of the unknown *Derris*.

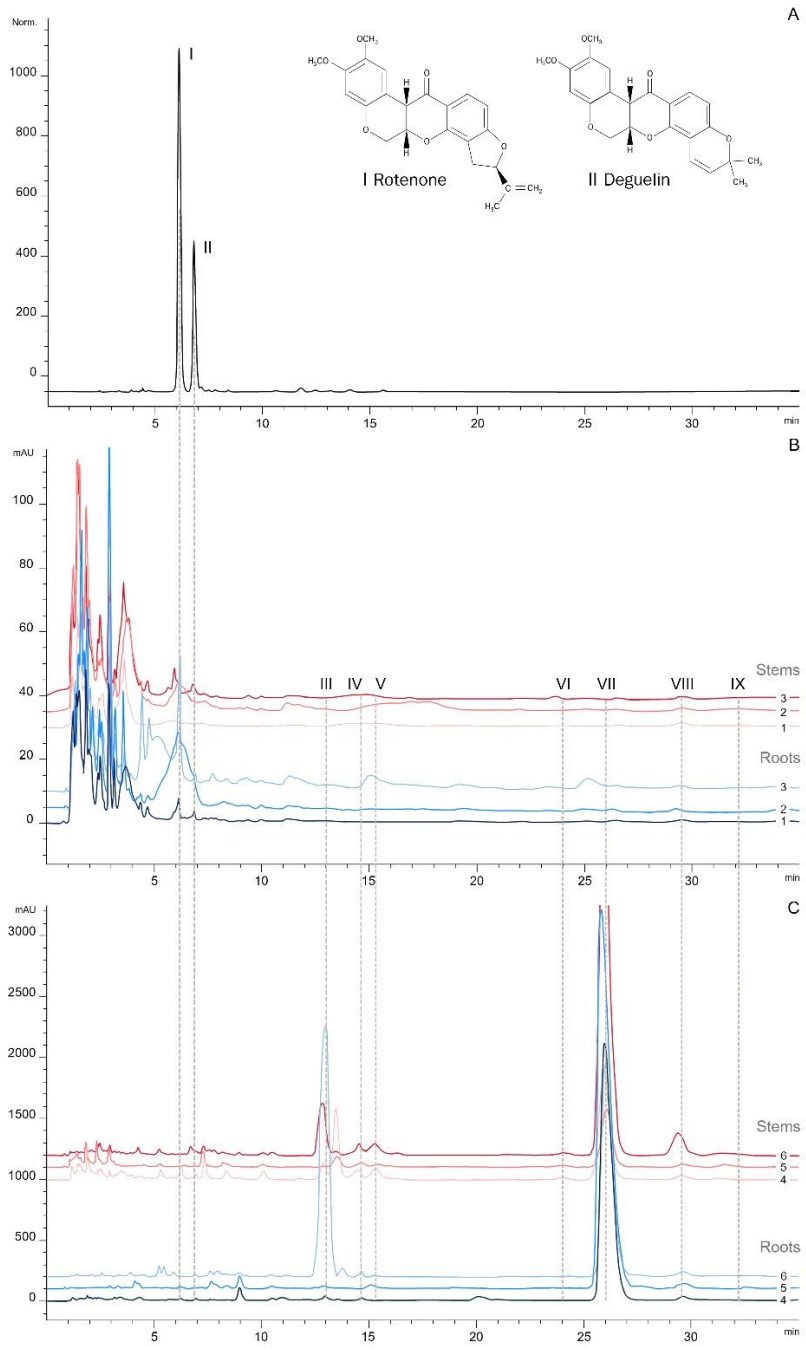

Figure 8. High-performance liquid chromatography (HPLC) chromatograms of unknown *Derris* samples and *D. pubipetala*'s roots (the lower, blueish lines) and stems (the upper, reddish lines) extract for fingerprint detection; (**A**) two standard compounds; (**B**) The unknown *Derris* samples; (**C**) *D. pubipetala*; Each Arabic numeral represents the accession number of each sample. Each Roman numeral represents each peak of HPLC chromatograms (chemical compound) with its retention time, i.e. (**I**) RT, 6.1 min; (**II**) DG, 6.7 min; (**III**) UK1, 13.3 min; (**IV**) UK2, 14.4 min; (**V**) UK3, 15.3 min; (**VI**) UK4, 24.0 min; (**VII**) UK5, 26.1 min; (**VIII**) UK6, 29.5 min; (**IX**) UK7, 32.2 min; RT, rotenone; DG, deguelin; UK, unknown.

ARPHA Preprints *Author-formatted, not peer-reviewed document posted on 24/04/2023* DOI: https://doi.org/10.3897/arphapreprints.e105209

Molecular phylogeny

Phylogeny based on combined nuclear and chloroplast sequences using three analyses (MP, ML, and BI) demonstrates different degrees of resolution and support, but still compatible tree topologies. According to the cladogram of the Bayesian analysis (Fig. 9), the clade of the genus *Derris* has very high support (PP 1.00, MLBS 99%, MPBS 98%) and is sister to the clade consisting of members of the genus *Brachypterum* including *Millettia pinnata* (L.) Panigrahi and *Fordia splendidissima* (Blume ex Miq.) Buijsen. *Derris* clade splits into several subclades with a variety of clade supports. Three samples of the unknown *Derris* from 3 populations, however, did not form a clade in Maximum Likelihood and Bayesian analyses. The sample from Nakhon Si Thammarat province was separated from the others. Whereas two samples from different localities in Songkhla province formed a strongly supported clade in all three analyses (PP 1.00, MLBS 100%, MPBS 100%), clearly separated from other species of *Derris*, especially its morphologically closely species, *D. pubipetala*. Slightly different topology found in cladogram from Maximum Parsimony (MP) analysis, those 3 samples formed a moderately supported clade (MPBS 72%, please see the Suppl. material 2: Fig. S1). These 3 accessions of the unknown *Derris* species also had an ambiguous relationship with several species, i.e. *D. laxiflora* Benth., *D. rubrocalyx* Verdc., *D. reticulata* Craib, *D. ferruginea* Benth., *D. pubipetala*, *D. glabra* Sirich., *D. elegans* Benth., *D. spanogheana* Blume ex Miq., *D. laotica* Gagnep., and *D. trifoliata* as they were in the same clade (PP 0.97, MLBS 51%).

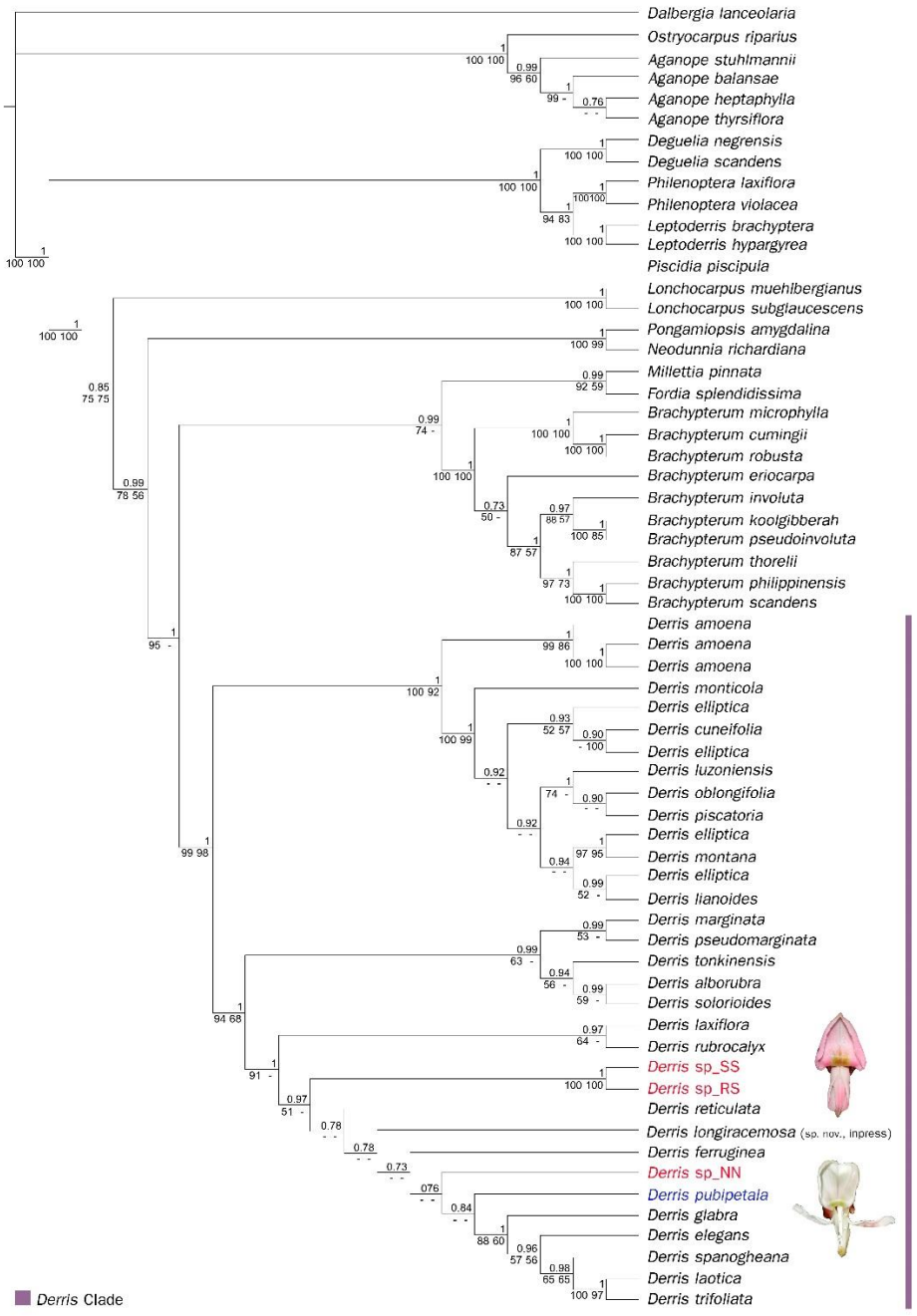

Figure 9. Bayesian consensus tree from Bayesian inference analysis (BI) of concatenated two plastids and one nuclear dataset. The posterior probabilities (PP) are shown in above the branches. Bootstrap percentage supports values of Maximum likelihood (MLBS) and Maximum parsimony (MPBS) are shown below the branches, respectively (- = MPBS and/or MLBS < 50%). Abbreviations and numbers after scientific name indicate localities: NN, Nopphitam district (Nakhon Si Thammarat province); RS, Rattaphum district (Songkhla province); SS, Sadao district (Songkhla province) Thailand. Red and blue highlighted the three samples of the unknown *Derris* collected from three localities and *D. pubipetala*, respectively.

Discussion

Macro- and micro-morphologically, this unknown *Derris* samples are most similar to *D. pubipetala*. They also share distribution area and thrive in very similar ecology. (Fig. 4; Southern Thailand) (Sirichamorn et al. 2014a; Sirichamorn 2020). However, many morphological differences can also be observed as explained in Table 3. The permanent presence of a reddish colour of midrib of leaflets is the most prominent vegetative part detected in the unknown *Derris* samples, which has never been reported in any *Derris*' s species. Red substance in plants is probably anthocyanins in general, usually exhibits on young leaves of almost all members of the genus *Derris* and many species of legumes (Sirichamorn et al. 2012a; Sirichamorn 2020). Pigments are hypothesized that they are provisionally accumulated to protect the tissue from light-intensity damage or defense against herbivory (Chalker-Scott 1999; Steyn et al. 2002; Neill 2003; Gould 2004; Hatier and Gould 2009; Gould et al. 2018). Mature Leaves generally turn completely green by rapid degrading pigments after their functions have been accomplished (Chalker-Scott 1999; Steyn et al. 2002; Close and Beadle 2003; Oren-Shamir 2009). Delay decolorization of this plant is still unknown and require future investigation. However, it might be a result of anthocyanin stabilities from their molecular structure properties (Lachman and Hamouz 2005; Woodward et al. 2009). Obviously, two populations from Songkhla province growing in the area exposed to more sunlight showed very distinct red midrib and some mature, shaded leaves from the same plants showed fader red midribs. The sample from Nakhon Si Thammarat province thriving in less sunlight area than Songkhla province showed less red substance accumulations in its mature leaves' midribs. Therefore, the pigment accumulation might be related to the light intensity but many *Derris* species growing in areas exposed to more sunlight did not show delayed decolorization as found in these unknown samples.

Microscopic characters of leaflets surface from SEM observation demonstrated additional unique character of these unknown samples. Small-sized glandular trichomes distributed mainly on both sides of the midrib, including with uni- and bi-cellular non-glandular hairs (Fig. 5E, F, M, N, Q, R). Generally, the biological function of trichomes is related to several aspects of plant physiology and ecology (Wagner et al. 2004; Peiffer et al. 2009; LoPresti 2016; Li et al. 2018). Glandular trichomes usually contribute to deterring herbivores and protect against oxidative stress from the ability to produce, store, or secrete chemical substances (Peiffer et al. 2009; LoPresti 2016; Murungi et al. 2016; Stojičić et al. 2016; Zhao and Chen 2016; Tozin and Rodrigues 2017; Jachuła et al. 2018; Li et al. 2018) as reported in many plants but their presence in species of *Derris* was quite rare. Few species such as *D. elegans*, *D. elliptica* (Wall.) Benth., *D. ferruginea*, and *D. pubipetala* were reported as species with hairy leaves but only non-glandular trichomes were found. Glandular trichomes found in samples of these unidentified samples are small, scentless, and thinly distributed. From the shape and pattern of distribution, they might be external secretory structures for plant protection, not for accumulation or attraction. Interestingly, the

position of glandular trichomes occurrence is the same as the red pigment accumulation, associated with the midribs, which are the main vascular system of leaves.

Additionally, several characteristics of reproductive parts of the unknown samples were different from *D. pubipetala* (Fig. 2). Prominent hairs on filaments and below the anthers, for example, have never been reported in any *Derris*'s species including *D. pubipetala*. Hence, hairy stamen was a very useful diagnostic character for this taxon. Incidentally, presence of hairs at the tip of filaments (base of the anthers) demonstrated homoplasy with some members of the genus *Millettia* Wight & Arn., e.g. *M. extensa* (Benth.) Benth. ex Baker and *M. pinnata* (Mattapha 2020; Sirichamorn 2020). Existence of this trait was possibly related to the enhancing the effectiveness of the pollen export function (as pollen adherence position or wilted zone below anthers after pollens were released for facilitating the transfer of pollen to the pollinator) or possibly relate to the essential functional feature of the secondary pollen presentation of the pollination biology process. The certain role of hairy filaments needs further investigation. Unfortunately, the type plant flowered once in 2019 and has never produced flowers again until 2022 when the type plant and its thriving area were killed and cleared for building solar cell system to support tourism and the new building of the forest ranger unit. Other individuals in the type localities are still safe but they have never flowered unlike other *Derris*'s species which flower yearly. Hardness of flower production in this plant causes difficulties in study of reproductive parts.

Although the pods are one of the most important characters for identifying the *Derris* plant, such as the number of wings on the margin of the pods or the density of the hairs covering the pod surfaces. Most species of *Derris* have two-winged pods, e.g. the pods of *D. pubipetala* are also two-winged, densely covered with golden-brown hairs (Sirichamorn 2020). In 2019, the type plant produced only 4 inflorescences and two of them were collected as types (holo- and isotype), leaving about the rest 2 inflorescences for pods. Unfortunately, there were no pods production and thus we are still not able to collect them until now, causing a lack of information in this part. But we assumed from its phylogenetic position that its pods are also two-winged.

Based on leaf anatomical characteristics, they revealed several taxonomic significances (Fig. 6 and Fig. 7), especially those pertaining to the leaf epidermis and transverse sections of leaf blades. According to the epidermal cells size of the unknown *Derris* samples, it was larger than *D. pubipetala*, notably on the lower leaf surface. Additionally, larger stomata were also observed. The larger size of epidermal cells and stomata in the unknown *Derris* samples also resulted in much lower stomatal density per area. Moreover, the Stomatal Index (SI) of *D. pubipetala* is lower because the size of epidermal cells and stomatal are both smaller than the unknown *Derris*. These results reflected different environmental conditions. Although all *Derris* sample in this study were found nearby stream or waterfall, the unknown samples *Derris* thrive in more sun-light condition, while samples of *D. pubipetala* thrive in shadier and more humid habitat. As previously reported (Bosabalidis and Kofdis 2002; Raven 2002; Woodward et al. 2002; Hetherington and Woodward

2003; Franks and Farquhar 2007), size of guard cells of plants under drier condition was larger than the one growing under shadier environment because opening and closure of larger stomata would be slower, which demonstrated a greater potential of water storage (Aasamaa et al. 2001; Hetherington and Woodward 2003; Lawson and Blatt 2014).

Size of midrib and vascular bundle of the unknown *Derris* and *D. pubipetala* were also obviously different. In *D. pubipetala*, they were smaller. The larger size of the midrib and vascular tissues in the unknown *Derris* species are likely corresponding to higher-temperature or more light-exposed environments where the three populations grow compared with another's. All samples of *D. pubipetala* thrives in locations where the potential of water transport does not need to be increased because the plants receive enough quantities of water. It is probable that the vascular tissue is not very large. The unknown *Derris*, on the other hand, grow in a more sunlight and more water-stressed environment. As a result, water transport must be modified to be more efficient, probably by developing a larger vascular bundle.

Leaf thickness of the unknown *Derris* samples were positively correlated with the height of epidermal cells and palisade mesophylls, which were approximately more than those found in *D. pubipetala*. In contrast, height of the spongy mesophyll in *D. pubipetala* was higher. The result was straightforward and similar to what we previously found for other leaf anatomical characters mentioned above. Because the unknown *Derris* samples were found in a more exposed area whereas *D. pubipetala* grew in shady areas. Palisade mesophylls on the upper side are the primary site of photosynthesis and cells of this layer are firstly directly exposed to light. The plants growing in more sunlight areas may develop thicker palisade layer not only to increase photosynthesis but also prevent the deeper leaf tissue from sunlight damage (Hanson at al. 1917; Lee and Gates 1964; Wilson and Cooper 1969, 1970; Vogelmann 1993; Smith et al. 1997; Mendes et al. 2001; Niinemets 2001; Terashima et al. 2011; Wang et al. 2002; Kozuka et al. 2011; Ho et al. 2016; Gotoh et al. 2018). On the other hand, the shady and moisture-loving plant like *D. pubipetala* develops thicker spongy mesophylls because there are more intercellular spaces which facilitate gas exchange as often found in aquatic plants.

Phytochemical investigations of *Derris* from a variety of geographic regions of southern Thailand in our study are the first report on the HPLC fingerprint profile of the unknown *Derris* species and *D. pubipetala*. Previously, some articles mentioned the uses of HPLC fingerprint in legumes for Pharmaceutical (drug quality control) or chemotaxonomic purposes (Xie et al. 2014; Yang et al. 2014; da Costa et al. 2016; Orak et al. 2016; Stefanucci et al. 2022), also in the genus *Derris* (Vittaya et al. 2014; Chen et al. 2015; Zabairi et al. 2015; Saraf and Shinede 2016; Saraf and Shinede 2018). The results showed peaks of unique chemical markers with reasonable heights and acceptable resolution, that can be used for the rapid identification of the unknown *Derris* species and *D. pubipetala*. Intra-specific variation of chemical constituents could be found among populations, but it was a common phenomenon found generally in other researches (Chen et al.

2015; Saraf and Shinede 2016; Saraf and Shinede 2018). Production and accumulation of phytochemicals are not only dependent on the plant species but also the environmental factors (Qaderi et al. 2023), which were concerned in this study. The roots and stems were thus collected from the plants which were more or less the same age, same size and in the same rainy season. However, some environmental factors such as the soil type and nutrient are uncontrollable. Those differences were a consequence of infra-specific variations of HPLC fingerprints in this study but they were still acceptable because remarkable similarities within the same species were still detected. The unknown *Derris* samples could be recognised in this study by the absence of chemical peaks. Meanwhile, several chemical markers could be detected in *D. pubipetala*. The unknown chemical compound UK1 to UK7 (peak III to IX) were distinctive and thus selected as characteristic peaks for the fingerprint (Fig. 8C), used as chemical markers for the *D. pubipetala* species. Interestingly, the UK5 (peak VII) was dominant substances in *D. pubipetala* with the highest intensity and peak area in all root and stem extracts. It is interesting for further research about what it is or its beneficial properties.

Cladograms from all phylogenetic analyses, based on 62 taxa (55 species, 14 genera) exhibit similar topologies to the previous study by Sirichamorm et al. (2012a) (Fig. 9). *Derris* is a monophyletic group with strong support. The phylogenetic positions of 3 accessions demonstrated that they were members of the genus *Derris*, however and surprisingly, not a single taxon as they were not monophyletic. Their phylogenetic relationship with other species in the genus was also still obscure. Those 3 accessions from 3 localities divided into two groups i.e. one from Songkhla province (consisting of *Derris* sp_SS and *Derris* sp_RS) and another from Nakhon Si Thammarat province (*Derris* sp_NN). Vegetative morphology of these 3 samples was almost identical. They showed similar leaf shapes, the number of leaflets, hairiness, and the most remarkable character, the reddish midrib of the mature leaves (although less distinct in *Derris* sp_NN from Nakhon Si Thammarat). Unfortunately, two samples (*Derris* sp_SR and *Derris* sp_NN) still lacked reproductive structures, which hampers taxon recognition. Unlike other species of *Derris* which usually flower annually, only a sample (*Derris* sp_SD) produced once inflorescences in 2019, then has never flowered again afterward. Species of *Derris* are vegetatively similar in common and cause taxonomic problems sometimes. Without flowers or fruits, *Derris cuneifolia* Benth. and *D. monatna* Benth. are indistinguishable, for example (Sirichamorn et al. 2012a). Such a phenomenon also occurs in a very well-known and commercially used species, *D. elliptica*, which interspecific hybridization between this species and *D. montana* is possible and may cause non-monophyletic species (Toxopeus 1952a, 1952b; Sirichamorn et al. 2012b). It might be assumed there were more than one taxon in the studied samples. This complexity can be clarified in near future when more reproductive parts are collected from those non-flowering samples. At this time, we accept 2 samples from Songkhla province i.e., one is the type with flowers and inflorescence and another without reproductive part as a new species to science. But the sample from Nakhon Si Thammarat province was still recognised as an unknown status, waiting for its flowers to determine its taxonomic status and species boundaries in future.

Although fruit characters of this species are lacking as plants have never produced pods, molecular phylogenetic analysis can be used to predict the most possibility. Comparing our phylogenetic results with the evolution of pod wings optimized onto the molecular phylogeny by Siricharmon et al. (2014b), The presence of two wings along both sutures was more commonly found among closely related species of *Derris* sp. (only one-winged pods found parallelly in *D. trifoliata* and *D. elegans*). Consequently, two-winged pods are presumed to be characteristic of this new species's pods.

Results from morphological and molecular phylogenetic analyses including the distribution area indicated that *D. pubipetala* is most closely related to *Derris* sp. but several morphological diagnostics for separation can be found as mentioned in taxonomic treatment.

Taxonomic treatment

Derris erythrocosta Sirich. & Boonprajan, sp. nov.

Figs 1–3

Type. THAILAND. Songkhla, Sadao district, Padang Besar sub-district, Pha Dam Forest Ranger Unit, Ton Nga Chang Wildlife Sanctuary, c. 150 m elevation, GPS coordinate 6°47'16. 7" N 100°13'51.8"E, 22 January 2019, C. Leeratiwong 19–1666 (holotype BKF!; isotypes K!).

Diagnosis. This species has several autapomorphies compared with other *Derris*'s species. It is the only species that has reddish midribs of mature leaflets. Style is sericeous at base and gradually becomes glabrous apically (vs. thinly hairy at base and mostly glabrous throughout in other *Derris*'s species). This species possesses prominent hairs below the anthers (vs. glabrous in all *Derris* species). It is morphologically highly similar to *D. pubipetala* Miq., but differs from that species by colour of roots (brownish to black-gray vs. reddish), the presence of reddish midribs of mature leaflets (reddish vs. green), number of leaflets (9–11 vs. 5–9), colour of corolla (pale pink to pink vs. white), wings petal folding (straight vs. revolute), filament hairiness (thinly hairy vs. glabrous), indumentum presence below the anthers (present vs. absent), disc shape (lobed vs. annular), etc. (see Table 3)

Description. Woody climber; *Bark* pale brownish-gray to gray, lenticellate. *Leaves* with 9–11 leaflets, reddish when young, chartaceous to sub-coriaceous. *Stipules* caducous (thus not present in specimens); petiole 6–10.8 cm long, grooved above, thinly strigose to almost glabrous;

rachis 10–18 cm long, grooved above, thinly strigose to glabrous; pulvinus 9–15 mm long, thinly strigose; stipellae absent; terminal one elliptic to obovate or narrowly oblong, 10–18.3 × 3.0–4.3 cm, length/ width ratio 3. 2– 4. 3, base cuneate, apex acuminate, acumen 7. 2– 18 mm long, emarginate, upper surface glabrous but slightly strigose along midrib and lateral veins, lower surface glabrous to thinly strigose along midrib, lateral veins and lamina, sometime slightly strigose to glabrous at margin, midrib (with reddish colour of the living mature leaflets) and veins slightly raised or flat above, raised below, veins 7–9 per side, 0.7–2.7 cm a part, curving towards the apex and almost reaching the margin, sometimes anastomosing near the margin, venation reticulate, pulvinus 5.0–6.5 mm long, thinly strigose; lateral one mostly like terminal one, narrowly elliptic to obovate, rarely ovate, 10–18.8 × 3–4.4 cm, length/ width ratio 3–4.3; pulvinus of petiolules 4. 5– 6. 5 mm long, thinly strigose to sericeous. *Inflorescence* psedoracemes/ psedopanicles, axillary or terminal, 40–50 cm long; peduncle 2–7 cm long, lenticellate, strigose; bracts subtending inflorescence 4, triangular, 2–2.6 × 1.8–2.5 mm, outside with some hairs at base and along margin apically, inside glabrous; bracts subtending lateral branches 4, triangular, ovate, 2–2.5 × 1.8–2.4 mm, outside with some hairs at base and along margin apically, inside glabrous; lateral branches 3.4–15 cm long, thinly strigose at base, lenticellate; bracts subtending brachyblasts ovate-triangular, 1–2.5 × 0.8–1.4 mm, outside with some hairs at base and along margin apically, inside glabrous. *Brachyblasts* knob- like to elongated cylindrical, 1– 12 mm long, with 2– 8 flowered, strigose; bracts subtending flowers ovate-triangular, 0.7–0.9 × 0.7–1 mm, outside thinly hairy at base and along margin, inside glabrous; pedicels 3.5–3.2 mm long, strigose; bracteoles 2, at base of calyx, ovate, semi-circular orbicular to narrowly, narrowly triangular, 0.6–0.9 × 0.5–0.7 mm, outside thinly strigose, with some hairs along the margin, inside glabrous. *Calyx* red- to maroonish, cup-shaped 3.4–4.2 mm long, outside thinly strigose, with some hairs along the margin, inside glabrous; tube 3–3.2 mm; upper lip with 2 short lobes, 0.2–0.4 × 1.5–2 mm; lateral lobes short triangular, 0.2–0.6 × 0.7–1 mm; lower lobes triangular 0.3–1.3 × 0.8–1.2 mm. *Corolla* pale pink to pink; standard orbicular or broadly ovate, with a green-yellowish spot at the base, 8.5–10 × 8.6–9.3 mm, apex emarginate, basal callosities absent, outside hairy from the middle part to apex, inside with some hairs near apex, claw 1.5–2.8 mm long; wings elliptic to narrowly ovate, 7.3–8.2 × 3.1–4 mm, apex obtuse, upper auricle pubescent, 0.5–0.9 mm long, lower auricle absent, lateral pocket 1.4–2.2 mm long, outside hairy in the middle part of the petal to the apex, inside hairy near the apex, claw 1.8–3.5 mm long; keels boat- shaped 7–7.8 × 2.3–3 mm, apex retuse, upper auricle pubescent, 0.5–1 mm long, lower auricle absent, lateral pocket 1–2.1 mm long, outside and inside hairy near the apex, sometimes also with thinly hairs along the veins ventrally, claw 1–2.9 mm long. *Stamens* 10 monadelphous, 2.8–4.6 mm long, free part 1.5–3.1 mm long, thinly hairy, anthers 0.5–0.6 × 0.2–0.3 mm, with some basal hairs. *Disc* indistinct or more or less 10-lobed, glabrous. *Ovary* 3.5–5 mm long, sericeous; stipe usually indistinct, sericeous; style 5.6–7.4 mm long, sericeous at base and gradually become glabrous apically. *Pod* and Seed unknown.

Phenology. Flowering from November-February and fruiting possibly in March-April.

Vernacular names. Khruea lai leeratiwong (เครือไหลลีรติวงศ์)

Etymology. The specific epithet refers to the remarkable reddish colour of the midrib of the mature leaflets which is rarely found in other species of *Derris*. The Thai name "Khruea lai leeratiwong" (means "Leeratiwong's *Derris*", in Thai) honors Associate Professor Dr. Charan Leeratiwong, the discoverer and collector of the type specimens.

Distribution. PENINSULA: Songkhla (Rattaphum district, Sadao district). (Fig. 4)

Habitat and Ecology. Usually scattered near the stream, in semi-shaded to a fully exposed areas to sunlight, tropical evergreen rainforest. This species, especially in the type locality, thrives on sandy or sandy-loam soil.

Proposed IUCN conservation assessment. This new species is only known from two locations in Songkhla province. The total number of mature individuals is less than 10,000. The Area of Occupancy (AOO) is about 2,000 km^2. Although its type locality and distribution are located in conserved areas, the species is still threatened by human disturbance. Therefore, we provisionally considered its conservation status as "Vulnerable category, (VU) B2 b(ii)c(ii)", according to IUCN Standards and Petitions Committee (2022, v. 15.1).

Representative Specimens Examined (paratypes). THAILAND. Songkhla: Rattaphum district, Kamphaeng Phet sub-district, 27 October 2021, YSM2021–15 (BKF!); Sadao district, Padang Besar sub-district, Pha Dam Forest Ranger Unit, 27 October 2021, YSM2021–14 (BKF!).

Key to the species of *Derris erythrocosta*

The new taxa were inserted as couplet 7 in the modified key to *Derris* species in the Flora of Thailand (Sirichamorn, 2020; 391–392).

6. Brachyblasts variable in shape and length, usually with more than 3 flowers throughout. Standard less than 10 mm long, rarely with basal callosities

7. Mature leaflets with reddish midribs. Filament thinly hairy. Anthers' base with turf of hairs. Pod unknown **18. *D. erythrocosta***

7'. Mature leaflets without reddish midribs. Filament glabrous. Anthers' base glabrous. Pod known 8

8. Pods one-winged or wingless

9. Leaflets hirsute to velvety underneath; stipels present 4. *D. elegans*

9’. Leaflets glabrous underneath; stipels usually absent

10. Leaflets 3.3–7.5 by 0.9–3.5 cm; petiolules 3–5 mm long 8. *D. laotica*

10’. Leaflets 3.5–16 by 1.5–8.5 cm; petiolules 5–10 mm long 17. *D. trifoliata*

8’. Pods two-winged

11. Pods velvety or sericeous

12. Leaflets slightly strigose to velvety below, apex rounded, obtuse or cuspidate to short acuminate. Pods with upper wing 4–10 mm wide, lower wing 4–7 mm wide. North-eastern Thailand 6. *D. ferruginea*

12’. Leaflets slightly strigose to almost glabrous below, apex distinctly acuminate, Pods with upper wing 5–9 mm wide, lower wing 2–4 mm wide. Southen-eastern Thailand 13. *D. pubipetala*

11’. Pods mostly glabrous

13. Leaflets 9–11, narrowly obovate, base narrowly cuneate to attenuate 11. *D. monticola*

13’. Leaflets 9–7 (–9), elliptic ovate or obovate, base cuneate to obtuse

14. Leaflets sometime glabrous below, Lateral veins reaching leaf margin 2. *D. amoena*

14’. Leaflets never glabrous below. Lateral veins not reaching leaf margin but curving toward leaf apex, sometime forming an intramarginal-like vein

15. Leaflets 3–5. Terminal leaflets distinctly longer and wider than lateral ones. Calyx glabrous outside 7. *D. glabra*

15’. Leaflets 5–7. Terminal leaflets slightly longer but not wider than lateral ones. Calyx thinly sericeous outside 12. *D. pseudomarginata*

Acknowledgments

We would like to thank all staffs of the Khao Luang National Park and Ton Nga Chang Wildlife Sanctuary for their help during fieldwork and authorization to carry out research in the areas. The authors wish to express gratitude to Dr. Tanawat Chaowasku for his advice with phylogenetic analysis. We also thank the three anonymous reviewers for their helpful comments

which greatly improved the text. This research was also financially supported by the Faculty of Science, Silpakorn University, grant number SCSU-STA-2564-02 (awarded to P.B.) and SRIF-JRG-2564-06 (awarded to Y.S.).

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