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**RESEARCH ARTICLE**

## Plant taxonomy

# First record of *Thottea duchartrei* Sivar., A. Babu & Balach. (Aristolochiaceae) in Sri Lanka

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**Abstract:** The occurrence of *Thottea duchartrei* Sivar., A. Babu & Balach. (Aristolochiaceae), a shrub species previously recorded as endemic to India, has been reported in Sri Lanka for the first time. Previously, only one species of *Thottea* (*T. siliquosa* (Lam.) Ding Hou) has been recorded in the Revised Handbook to the Flora of Ceylon. Two new populations of *T. duchartrei* were discovered in two isolated sites of disturbed, mid country secondary forest at Polthenna and Ihalagama in Hatharaliyadda, Sri Lanka. The taxonomic treatment includes a species description of *T. duchartrei,* its distribution, and phenology. A comparative account of the morphological differences between *T. duchartrei* and *T. siliquosa* is provided for easy identification of the two species of *Thottea*. Further, floral characteristics which are unique to the original collection of *T. duchartrei* from Sri Lanka are also reported. The gene sequences, *rbcL*, *t RNA-Lys (trnK)* and *psba-transH* were submitted to the GenBank database as the first record of this species. Iconography of floral variations, habitats, and local and global distribution maps are also included. Identification using morphological characters, DNA barcoding to confirm the species, and its conservation status are discussed. Biogeographical influences such as habitat fragmentation, soil erosion, invasive species, the low density of natural pollinators and human settlement are also discussed. Based on morphological characters and DNA analysis, we confirmed the first known collections of *T. duchartrei* from Sri Lanka.

**Keywords:** Biodiversity, DNA barcodes, Flora of Sri Lanka, GenBank, *Thottea.*

## INTRODUCTION

*Thottea* Rottb. (Aristolochiaceae) is a genus of about 43 species distributed in the Andaman Islands, Bangladesh, Borneo, Hainan, India, Jawa, Laos, Malaya, Myanmar, The Nicobar Islands, The Philippines, Sri Lanka, Sulawesi, Sumatera, Thailand, and Vietnam (POWO, 2021). The genus occurs rarely in tropical lowland forests, up to an altitude of ca. 1000 m (Hou, 1981). Diversification of *Thottea* species have been reported in two biogeographic regions: the Western Ghats in India and the Malesian region. High degrees of species endemism were found in both regions and only one species is shared in both regions.

In 1783 Rottboll named the genus *Thottea*. Since then, seven further genera have been published and used by various authors, *Apama* Lam (1783), *Bragantia* Lour (1790), *Ceramium* Blume (1826-27), *Trimeriza* Lindl (1832), *Asiphonia* Griff (1845), *Lobbia* Planch (1847) and *Strakaea* C. Presl (1851). These genera have been

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classified under different tribes and sections by Klotzch (1859), Duchartre (1864), Hooker (1890), Solereder (1894), Hou (1981) and Huber (1985). Duchartre (1864) recognized only two genera, *Thottea* and *Bragantia* and synonymised the others. Hooker (1890) accepted this but also noted these genera displayed morphological similarities and could be united. Solereder (1894) renamed *Bragantia* as *Apama* and later Hou (1981) also concluded that *Thottea* and *Apama*, cannot be separated and should be merged into a single genus *Thottea.* In 1985 Huber excluded one genus *Asiphonia* Griff. from *Thottea* due to differences in seed anatomy. Recent phylogenetic studies did not support this exclusion and authors decided to follow Hou (1981) with *Asiphonia piperiformis* being included in the genus *Thottea*. These studies have strongly supported the genus *Thottea* sensu lato as a monophyletic taxon with *Thottea piperiformis* a sister to the other *Thottea* species. The most widely distributed species, *Thottea tomentosa* was identified as a subsequent sister to the rest of the *Thottea* species (Oelschlägel *et al*., 2011; Madhumita, 2013).

*Thottea* species are used for the treatment of coughs, malaria, healing wounds, toothache, gum diseases, mouth ulcers and poisonous snake bites in rural and tribal communities in Malaysia, Indonesia, India and Sri Lanka (Perry & Judith, 1980; Udayan *et al*., 2005; Anilkumar *et al*., 2014; Sabran *et al*., 2016; *Unpublished data, T.M.S.G. Tennakoon*). In Sri Lanka, *T. siliquosa* leaves are chewed with betel leaves to experience mild stimulant effect by rural people (*Unpublished data, T.M.S.G. Tennakoon*). Tribal communities in India used to apply crushed roots of *T. duchartrei* externally to treat abscesses, inflammation, and poisonous snake bites (Anilkumar *et al*., 2014). There have not been any reported uses of *T. duchartrei* in Sri Lanka.

*Thottea* species have been reported to possess several biological activities such as antibacterial, antioxidant, anticancer, and anti-inflammatory properties (John *et al*., 2008; Merrine & Ramesh, 2012; Nusaiba & Murugan, 2013; Moorthy *et al*., 2015; Fatimah *et al*., 2018; Koottasseri *et al*., 2020). Biologically active endophytic *Streptomyces* bacteria have been isolated and described from *Thottea grandiflora* (Zin *et al.,* 2007). Aristolochic acid sequestration by *Pachliopta neptunus* and *Troides amphrysus* larvae that feed on *T. tricorns* and *T. dependens* leaves have also been reported (Nishida *et al*.,1993).

Ten species of *Thottea* Rottb. have been recorded in India (Sunil & Kumar, 2014). In Sri Lanka, *Thottea* has been represented by only one species, *T. siliquosa* (Heart *et al*., 1999) with the vernacular name *thapasara bulath* (Sinhala) (Wijesundara *et al*., 2021). It is distributed in wet lowland evergreen forests. Here we report the first recorded occurrence of *T. duchartrei* in Hatharaliyadda, Kandy, Sri Lanka. Originally described from Western Ghats, India (Nazarudeen & Sabu, 2002; Shaiju & Omanakumari, 2009; 2010; Madhumita 2013) *T. duchartrei* was not found in any other known locality of *T. siliquosa*, during a survey of the wet lowland evergreen forests of the Galle, Kalutara, Kandy, Kegalle and Ratnapura districts in Sri Lanka.

## MATERIALS AND METHODS

### Field sampling and morphological investigation

During a field visit to the disturbed secondary midland forest in Hatharaliyadda, Kandy, the first author found populations of a *Thottea* species on a small rocky and steeply sloped hill at Polthenna with approximately 70 plants and a slightly sloped hill at Ihalagama with 5 plants. Herbarium specimens, including flowers and fruits preserved in alcohol, were prepared, and sent to the Herbarium of the Royal Botanic Gardens, Kew (K) for taxonomic verification as those were not matched with the specimens of *T. siliquosa* nor its related species deposited at the National Herbarium, Peradeniya (PDA) and Research and development Centre, Link Natural Products (Pvt) Ltd, Sri Lanka (LNP). The *Index Herbariorum* was followed for herbarium acronyms (it is updated continuously). In addition, 1,118 flowers, 50 fruits and 50 leaves were preserved in alcohol for further investigation and photographs were taken. The morphological and anatomical characters were observed using stereo microscope BMS7045 Biobase and Axio Lab A1 series and digital Carl Zeiss Microscopy GmbH. Plant photographs were taken by digital phone camera (iPhone Xs, Apple Inc., USA). Beentje (2016) and World Checklist of Vascular Plants (WCVP, 2021) were followed for morphological terms and taxonomy, respectively. Characters listed in the protologues, and descriptions were compared to those present in herbarium and spirit material. Turland *et al*. (2018) was followed for nomenclatural practices. The geographical measurements were recorded using a GARMIN eTrex 30 x device. The phenological details, associated species, and soil characteristics were noted in the field. The herbarium specimens were deposited at LNP, K and PDA.

**Table 1:** Oligonucleotide primers used to amplify barcoding sequences

| Primer name | Direction | Sequence 5′- 3′ | Reference |
|---|---|---|---|
| *rbcL* | Forward | ATGTCACCACAAACAGAGACTAAAGC | Lee *et al*., 2016 |
| *rbcL* | Reverse | CTTCTGCTACAAATAAGAATCGATCTC | Lee *et al*., 2016 |
| *psbA3'f* | Forward | GTTATGCATGAACGTAATGCTC | Lee *et al*., 2016 |
| *trnHf_05* | Reverse | CGCGCATGGTGGATTCACAATCC | Lee *et al*., 2016 |
| *matk390F* | Forward | CGATCTATTCATTCAATATTTC | Cuénoud *et al*., 2002 |
| *matk1326R* | Reverse | TCTAGCACACGAAAGTCGAAGT | Cuénoud *et al*., 2002 |

### DNA barcoding

Total genomic DNA was extracted from fresh leaf tissues using a Phytospin D™ plant genomic extraction kit (CEYGEN Biotech). Barcoding regions of chloroplast genome were amplified by PCR using primers given in Table 1.

Amplified PCR products were purified by Wizard PCR cleanup kit (Promega) and bidirectionally sequenced with BigDye Terminator Cycle Sequencing Kits, version 3.1 (Applied Biosystems) and ABI 3500Dx automated DNA sequencer.

### Analysis of sequences

Bidirectional DNA sequences of each amplicon were assembled using BioEdit version 4 software and the contiguous sequences were searched over the GenBank database at the National Center for Biotechnology Information, USA and BOLD system version 3 using Basic Local Alignment Search Tool (BLAST). The sequences were deposited in GenBank database.

## RESULTS AND DISCUSSION

### Taxonomic treatment

*Thottea duchartrei* Sivar., A. Babu & Balach. Indian J. Forest. 8(4): 267. (1986). Type: India, Kerala, Malappuram District, Herbal Garden, Arya Vaidya Sala, Kottakkal, *Indu* 1191 (holotype MH; isotypes CALI & CMPR).

### Specimens examined

Sri Lanka: Hatharaliyadda, Polthenna, Kandy, N 7 º 19' 39" E 80 º 29' 7", 200 – 213 m, 2 July 2018, *Tennakoon* 11-001-21 (K! [K0003423376]) (Figure 3); 29 May 2020, *Tennakoon* 11-001-23 (PDA! [PDA 11-001-23]) Hatharaliyadda, Ihalagama, Kandy, N 7 º 19' 44" 80 º 30'38", 265 m, 29 May 2020, *Tennakoon* 11-001-46 (LNP! [LNP 11-001-46]).

### Description of *T. duchartrei*

Shrub 0.5 – 1.5 m tall. Mature stem woody, greyish brown, 6.5 – 14 mm in diameter; vegetative shoots light to deep green, often flexuous, branching profusely, with 17 – 43 swollen nodes. Leaves alternate; petiole 8 – 12 mm long; blade lanceolate, 16 – 28 × 4 – 7 cm, base cuneate, apex acuminate, margin entire; strongly 3-veined from base, lateral veins exceeding ¾ of the lamina; young leaves greenish yellow, mature leaves shiny green and pubescent beneath, minutely pubescent above; crushed leaves emit strong aromatic smell. Inflorescence cymose, 1 – 3 partial inflorescences arising from the foliar axis or from the lower leaf nodes, 1 – 6 flowered; flowers emit mild rotten fish smell, peduncle 4 – 12 mm long, pubescent; basal bracts (prophylls) entire, 1.5 – 3.0 mm long, pubescent; floral bracts 2 – 12 mm long, entire, pubescent; flowers reddish purple, deep purple or yellow; perianth trimerous, fused up to the middle, pubescent, bell-shaped; tepal lobes 3, orbicular – obovate, apex acute, broader than long, stamens 8 – 12, fused, commonly arranged in three groups of 3+3+3, 4+3+3, and 4+4+3 around the stylar column opposite to perianth lobes, anthers slightly curved, dithecous, four lobed, extrorse and fully adnate to the filaments, connectives slightly extended at the apex, 1.2 – 2 mm long; gynostemium with 0 – 3 tooth like floral appendages alternate to filaments; ovary 6 – 12 mm long, inferior, densely pubescent, syncarpous, 4 carpels, 4 locules; stylodia 6 – 12, sometimes bifid. Fruit a dehiscent capsule, 34 – 80 × 3.06 – 3.67 mm, subterete, cylindrical to squared, yellowish green at maturity, straight or slightly curved, narrowed at base and apex, reflexed, fruit dehiscence acropetal, fruit valves persistent after dehiscing, twisted spirally. Seeds numerous, light yellow, 2 – 2.64 mm long, trigonous, rugose, attached to furrows of placenta longitudinally (Figure 1 and Figure 2).

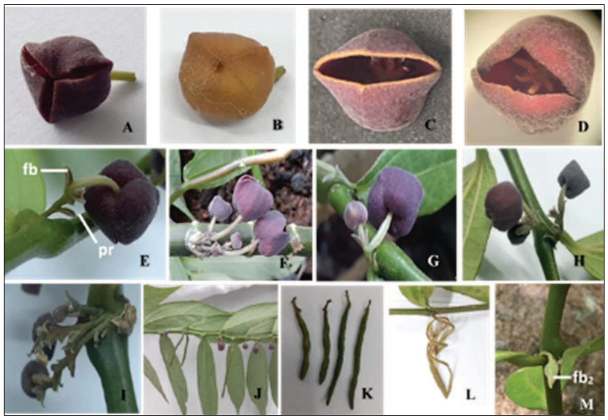



**Figure 1:** Floral, inflorescence and fruit morphology of *T. duchartrei*. A: purple flower; B: yellow flower; C – D: perianth with two lobes; E: cymose partial inflorescence (fb: floral bract, pr: prophyll); F: inflorescence arising from lower leaf node; G: single partial inflorescence from foliar axis with two flowers); H: two partial inflorescences from the same foliar axis; I: three partial inflorescences from the same foliar axis; J: abaxial view of partial inflorescence and capsules of a branch; K: capsule; L: capsule after dehiscing; M: abaxial view of leaf like floral bracts ($fb_2$).

## Key to species

Young shoots light to deep green, leaf blade 16 – 28 cm long. Peduncle 4 – 12 mm long, floral bract 2 – 12 mm long, stamen arrangement variable (3+3+3, 4+3+3 or 4+4+3), perianth fused up to the middle ………………………..........….. ***T. duchartrei***

Young shoots light to deep purple, leaf blade 7 – 25 cm long. Peduncle 3 – 6 mm long, floral bract 0.5 – 1 mm long, stamen arrangement usually 3+3+3, perianth free to the base ……………………………………………… ………..........………. ***T. siliquosa***

**Notes:** Differences in the morphological characters of *T. duchartrei* and *T. siliquosa* are given in Table 2.

## Morphological variations

The morphological variations that are observed in the inflorescences and flowers of *T. duchartrei* growing in Sri Lanka and India may be due to geographical and environmental factors (Caruso, 2006). In this study, we examined 1,118 individual flowers and revealed several modifications of previously reported characters in *T. duchartrei* from Sri Lanka. These are described for the first time in this article.

**Figure 2:** A mature *T. duchartrei* plant observed in Hatharaliyadda, Polthenna, Kandy

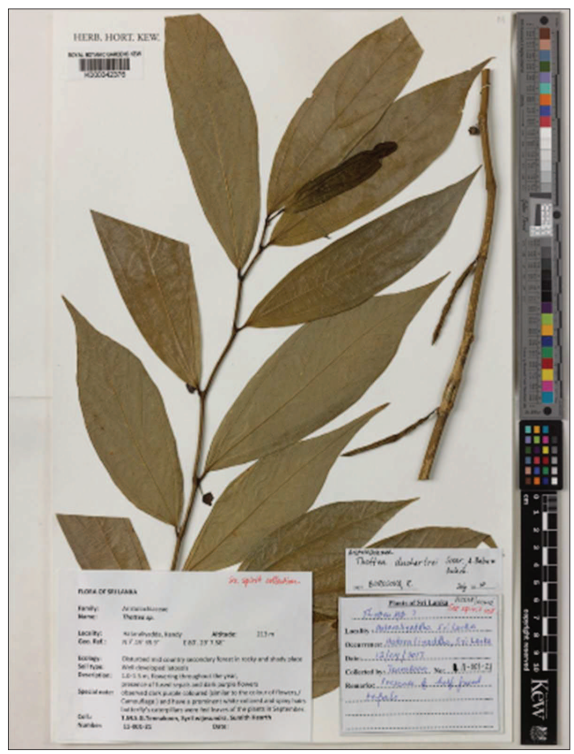



**Figure 3**: Specimen of *T. duchartrei* collected from Sri Lanka at the Herbarium, Royal Botanic Gardens, Kew

**Table 2:** Comparison of the morphological characters of *T. duchartrei* and *T. siliquosa*

| Character | *T. duchartrei* | *T. siliquosa* |
|---|---|---|
| Habit | Shrub, 0.5 – 1.5 m | Shrub, 1 – 3 m |
| Young shoot | Light to deep green | Light to deep purple |
| Leaf blade length | 16 – 28 cm | 7 – 25 cm |
| Flowers | Perianth fused up to the middle, bell shaped | Perianth free to the base, cup shaped |
| Arrangement of stamens | Variable (3+3+3, 4+3+3 or 4+4+3) | Usually, 3+3+3 |
| Stylodia | 6 – 12 | 6 – 10 |
| Floral appendages | 0 – 3 | 0 – 10 |
| Peduncle length | 4 – 12 mm | 3 – 6 mm |
| Capsule length | 3.4 – 8 cm | Up to 19 cm |
| Floral bract length | 2 – 12 mm | 0.5 – 1 mm |
| Prophyll length | 1.5 – 3 mm | 1 – 2 mm |

**Inflorescence morphology:** The occurrence of two partial inflorescences arising from the same foliar axis and a peduncle of less than 5 mm were reported as common morphological characters in the *T. duchartrei* growing in India (Shaiju & Omanakumari, 2009). However, a single partial inflorescence is very common in this species growing in Sri Lanka and only a few plants (n = 5) had 2 or 3 partial inflorescences arising from the same foliar axis, prominent leaf like floral bracts (10 – 12 mm long) and longer peduncles (10 – 12 mm long).

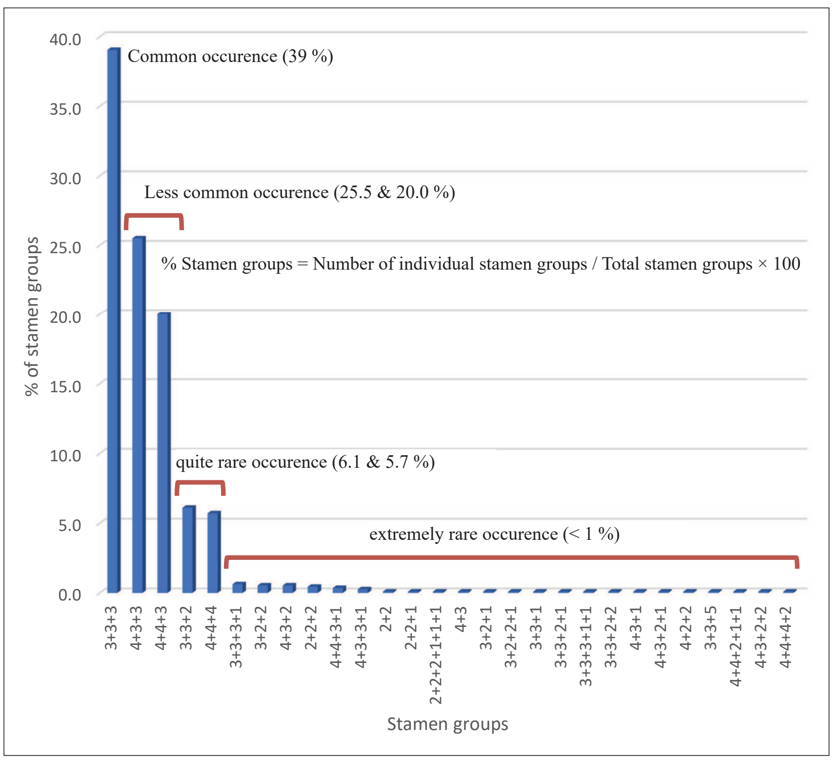
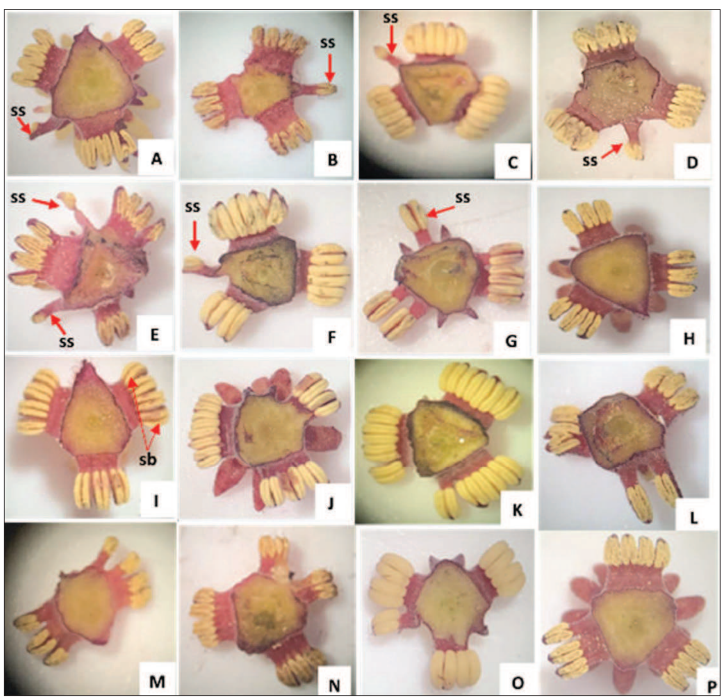



**Figure 4:** Frequency of occurrence of the 28 different stamen arrangements in the flowers of *T. duchartrei* collected in Sri Lanka.

**Perianth morphology:** Large bell shaped perianths with three completely fused tepal lobes and small cup shaped perianths with two tepal lobes fused to halfway from the base are reported here for the first time in *T. duchartrei* (Figure 1: C, D).

**Stamen morphology:** Our study highlights variations in the morphology, arrangement, and the number of stamens of the Indian and Sri Lankan plants. Although flowers with single stamens were rare in the Indian specimens (Renuka & Swarupanandan, 1986) they were found occasionally in our specimens (Figure 5: A – G). In some specimens we found appendiculate stamens with or without sterile remnants of anthers (Figure 6: 1 – 8), stamen-stylodium fusion (Figure 6: 9 – 13) and a fertile stylodium which carried an abaxial, monothecous anther extrorsely (Figure 6: 14 – 18). Stamen-perianth fusion (Figure 6: 19), fusion of anthers with adjacent stamens (Figure 6: 20 – 22), undeveloped anthers (Figure 6: 23 – 26), free stamens with monothecous anthers and dithecous anthers (Figure 6: 30 – 31) were observed. Anther connectives of free stamens (Figure 6: 32 – 33) and fused stamens (Figure 6 : 34 – 35) in the main bundles were occasionally separated half-way to the apex and the free stamens in the main bundles (Figure 6: 37).

The arrangement of stamen bundles in the Indian plants varied according to the records of different authors. Renuka & Swarupanandan (1986) reported the presence of 4+3+3 and 3+2+2 stamen bundles as common and the occurrence of a single stamen or pairs of stamens as very rare. According to Shaiju and Omanakumari (2010), 3+3+3 was more common but 4+3+3 and 3+2+2 were very rare. The percentage variation of stamen bundles in Indian plant has been reported as 40% for 3+3+3, 37% for 4+4+3, 12% for 4+3+3, and 7% for 4+4+4 (Nazarudeen & Sabu, 2002). Our study has revealed that the arrangement of stamens is different from what has been previously reported (Figure 5). The most common stamen arrangement in Sri Lankan species is 3+3+3, occurring in 39 % of specimens. Less common arrangements are 4+3+3 (25.5 %) and 4+4+3 (20 %) and quite rare arrangements are 4+4+4 (5.7 %) and 3+3+2 (6.1 %). In addition, this is the first time that 28 different combinations of stamen groups from the flowers of *T. duchartrei* were reported (Figure 4). Variation and high plasticity of androecial patterns of the genus *Thottea*




ss – single stamen, sb – stamen bundle

**Figure 5:** Stamen arrangements of *T. duchartrei* flower (View of transections at the base of androecium). A: 4+4+3+1, B: 4+3+3+1, C: 4+3+3+1, D: 3+3+3+1, E: 4+4+2+1+1, F: 4+3+2+1, G: 3+2+1, H: 3+3+3, I: 4+4+3, J: 4+3+2, K: 4+4+4, L: 3+3+2, M: 4+3, N: 4+ 3+2+2, O: 3+2+2, P: 4+3+3.

has been previously reported and emphazied. However those androecium characters were not appropriated for the delimition of *Thottea* species (Leins *et al.,* 1988; Nazarudeen & Sabu, 2002).

Floral appendages are present in two whorls, as an outer series, and an inner series. Renuka & Swarupanandan (1986) describe the outer series as having 3 and the inner series as having 6 – 12 appendages. In the Sri Lankan specimens, the authors describe the outer series as having 0 – 4 and, the inner 0 – 5 appendages (Figure 6: 36). In addition, stamens were found with vestigial anther sacs (Figure 6: 27 – 29). The morphology of vestigial anther sacs was shown to completely deviate from the original arrangement of anthers in size, bearing monothecous anthers, and anthers containing fewer pollen grains (Figure 6:28). This observation is supported by the gradual evolutionary transition of stamens to sterile floral appendages or stamonoides and the reduction of stamen appendages in the family Aristolochiaceae (Leins *et al.,* 1988*;* Kelly, 2001; Shaiju and Omanakumari, 2010).

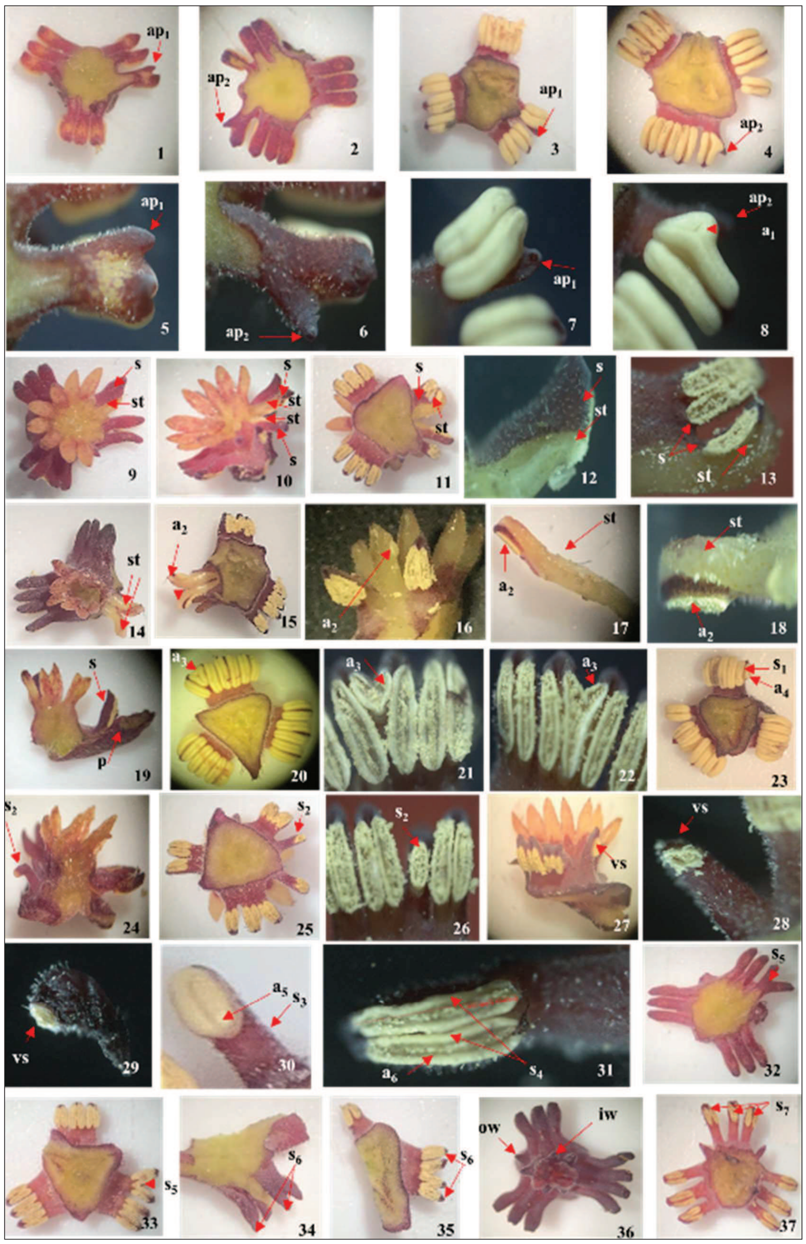



**Figure 6:** Stamen morphology

**Connective appendage:** 1. Appendage (**$ap_1$**) is positioned inside of the stamen bundle - view of transection at the base of the gynostemium, 2. Appendage (**$ap_2$**) is positioned outside of the stamen bundle - view of transection at the base of the gynostemium, 3. Appendage (**$ap_1$**) is positioned inside of the stamen bundle - view of transection at the base of the androecium, 4. Appendage (**$ap_2$**) is positioned outside of the stamen bundle - view of transection at the base of the androecium, 5. Top view of enlarged connective appendage (**$ap_1$**) positioned inside of the stamen bundle, 6. Top view of enlarged connective appendage (**$ap_2$**) positioned outside of the stamen bundle, 7. Bottom side view of enlarged connective appendage (**$ap_1$**) positioned inside of the stamen bundle, 8. Bottom side view of enlarged connective appendage (**$ap_2$**) positioned outside of the stamen bundle with a sterile remnant of an anther (**$a_1$**); **Stamen (s) and stylodium (st) fusions (arrows point to fusion):** 9. Inner top view of the androecium and pistil, 10. Lateral view of the androecium and pistil, 11. Transection view at base of the androecium, 12. Upper position - lateral view of enlarged **s** & **st** fusion, 13. Lateral view - lower position of **s** & **st** fusion; **Anther ($a_2$) bearing stylodium (st):** 14. Inner top view of the androecium, 15. Bottom view of transection at the base of the androecium, 16. Lateral view of the androecium and stylodium,17. Lateral view of enlarged anther **($a_2$)** bearing stylodia **(st)**, 18. Lateral view of anther **($a_2$)** bearing enlarged tip of stylodia **(st)**; **Stamen(s)-perianth** (p) **fusion:** 19. Longitudinal section of flower; **Anther lobes ($a_3$) of adjacent stamens partially fused by theca:** 20. Bottom view of transection at the base of the androecium, 21 & 22. Bottom view of enlarged stamens bundles. **Undeveloped fused anther ($a_4$), stamen bundle ($s_1$):** 23. Bottom view of transection at base of the androecium; **Undeveloped anther on free stamen ($s_2$):** 24. Lateral view of the androecium and pistil, 25. Bottom view of transection at the base of the androecium, 26. Bottom view of enlarged undeveloped anther **($s_2$)** in a free stamen; **Vestigial anther (vs):** 27. Lateral view of androecium and pistil, 28 & 29. Bottom view of enlarged vestigial stamens; **Free stamen ($s_3$) with monothecous anther ($a_5$):** 30. Bottom view of enlarged monothecous anther **($a_5$)**; **Free stamen ($s_4$) with dithecous anther ($a_6$):** 31. Lower view of enlarged dithecous stamen. **Free stamen divided half-way ($s_5$):** 32. Top view of transection at base of the gynostemium, 33. Bottom view of transection at base of the androecium; **Fused stamens divided half-way ($s_6$):** 34. Top view of transection at base of the gynostemium, 35. Bottom view of transection at base of the androecium; **Inner (iw) and outer (ow) whorls of floral appendage:** 36. Inner top view of the androecium; **Free stamens bundle ($s_7$):** 37. Bottom view of transection at the base of the androecium.

**Phenology:** Flowering and fruiting were observed throughout the year.

**Molecular Analysis:** The barcode sequences were deposited in the GenBank database, as partial sequences *matK* (Acc. No. MK074729), *rbcL* (Acc. No. MK524718), and *psba-tranH* (Acc. No. MK074730). The *matK* sequence was aligned with 100 % identity with *T. duchartrei* (Acc. No. JN415678) which is the first sequence of *T. duchartrei* in the GenBank database. This further confirmed the identification of the species in this study based on morphological characters.

**Phytogeography**

Sri Lanka is a tropical island country located between 5° 55′′– 9° 51′′ N and 79° 41′′– 81°54′′ E, in the Indian Ocean, off the southern tip of the Indian subcontinent. The total area of the country is 65,610 $km^2$ and consists of three peneplains, namely, lowland (up to 300 m altitude), upland (300 – 900 m altitude) and highland (more than 900 m altitude). The three major climatic zones are defined based on the rainfall, namely, dry zone (annual rainfall less than 1900 mm), intermediate zone (annual rainfall between 1900 – 2500 mm) and wet zone (annual rainfall more than 2500 mm). There are three unique mountain ranges in the Island, which have been described as the Central hill massif, the Rakwana range in the south-west, and the Knuckles range to the north of the Central massif (Ashton *et al*., 1997).

The flora of Sri Lanka consists of 3087 angiosperm species belonging to 186 families, with 28 % of them (863) recorded as endemic, belonging to 98 of the families. A total of 1496 of the species (48.4 %) are threatened. Most of the endemic species are found in the central and southwest regions of Sri Lanka (Wijesundara, 2020).

The flora of Sri Lanka is a relic of Deccan flora which evolved during the Tertiary period and the late Cretaceous epoch. The Indian plate broke away from Gondwana and migrated north of the equator. With the aridification of the Deccan flora during the Miocene epoch, most of the rainforest taxa gradually disappeared from the Deccan plate and only remained restricted in isolated areas of Asia. The Western Ghats and the Southwest of Sri Lanka have similar geology, climate, and evolutionary history, and the present-day rainforest taxa persisted as “relicts and vicariants or endemics” in these biodiversity hotspots. Moreover, approximately 433 plant species and 3 genera are confined to Sri Lanka and Western Ghats in India (Ashton & Gunatilleke 1987; Bossuyt *et al*., 2004; Mittermeier *et al*., 2004; Gunawardene *et al*., 2007; Gunatilleke *et al*., 2017).

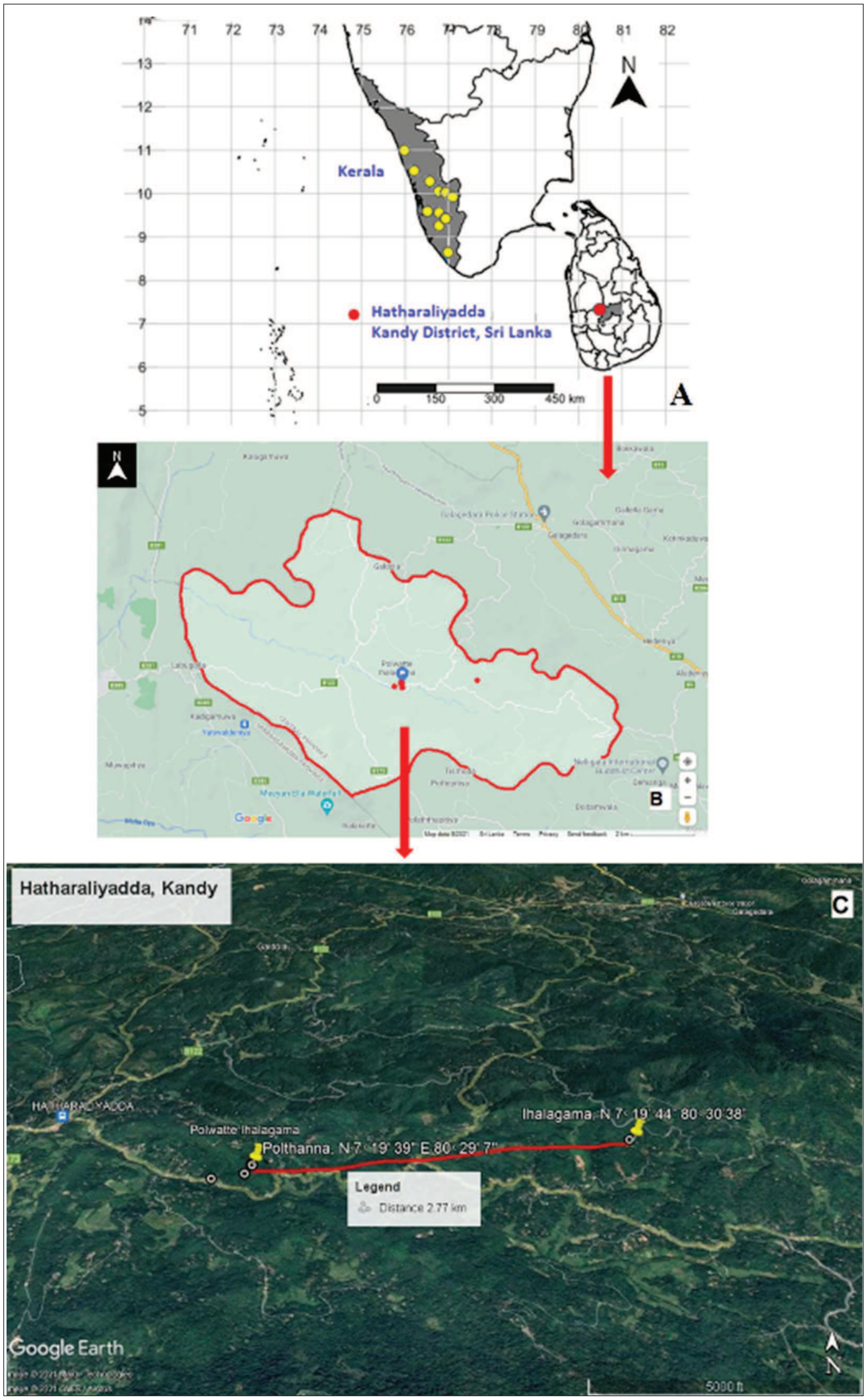



**Figure 7:** Geographical locations of *T. duchartrei* in India and Sri Lanka. **A**: Previous locations in Kerala state, India, and new records at Hatharaliyadda, Kandy, Sri Lanka; **B**: Satellite image of the two new locations at Polthenna and Ihalagama villages in Hatharaliyadda, Kandy; **C**: Expanded satellite image representing the topography of the two new locations, Polthenna and Ihalagama villages in Hatharaliyadda, Kandy.

The global distribution of the genus *Thottea* is mainly confined to the Western Ghats in India, and Malaysia. A single species *T. celebica* occurs across the whole distribution area of *Thottea,* from India to the Philippines via the Greater Sunda Islands. The Western Ghats and Sri Lanka shared one species, *Thottea siliquosa* (Oelschläge *et al*., 2011) until the discovery of *T. duchartrei* in Sri Lanka. Fewer species crossed the ocean from mainland India and Malaysia to the more remote islands. Most of the time single species crossed from the mainland of Asia to the islands of southeast Asia. The presence of *T. duchartrei* in Sri Lanka could be an indication of the existence of similar flora in the two land masses before the last disruption happened via Palk Isthmus 10,000 years before present (ybp).

The discovery of *T. duchartrei* in both locations, Polthenna and Ihalagama in the Kandy district is a novel addition to the known flora of Sri Lanka. Previously *T. duchartrei* has been reported in the Western Ghats of India and was considered an endemic species to the region. (Nazarudeen & Sabu, 2002; Shaiju & Omanakumari, 2009; 2010) (Figure 7: A). Since our discovery of *T. duchartrei* in Sri Lanka the species can no longer be considered as an endemic taxon to India. The topography and anthropogenic activities are the most important factors affecting the distribution of vegetation (Wang *et al*., 2015). It was globally recognized that progressive habitat fragmentation caused by human activities has resulted in a reduction of biodiversity (Mullu, 2016; Rogan & Lachen, 2018). The dominant soil type of the Kandy district is reddish brown laterite which is more prone to erosion (Moormann & Panabokke, 1961). The new locations of *T. duchartrei* were rocky, steep hills with the same soil type and high soil erosion. In addition, the existence of exotic invasive species, anthropogenic habitat modifications, and fragmentation of the landscape may also have affected the dispersal of *T. duchartrei* over large areas.

The influence on the distribution of vegetation by reduction in propagation, availability of pollinators, dispersal vectors, and survival capacity of seedlings has been well documented in fragmented areas (Benitez-Malvido & Martinez-Ramos, 2003). Similar low abundance of animal pollinators and a high rate of seedling mortality were observed in our study areas. These natural geological and biological factors, including human activities could be a hindrance to species richness and survival in both locations.

**Ecology:** Polthenna is a rocky and steeply sloped hill area at an altitude 200 – 213 m. Seventy plants were observed growing with *Cinnamomum* species, *Panicum maximum* Jacq., *Atrocarpus nobilis* Thw., *Atrocarpus heterophyllus* Lam., *Piper nigrum* L., *Hevea brasiliensis* (A. Juss) Muell. Arg., *Coffea arabica* L., *Macaranga peltata* (Roxb.) Muell. Arg., *Clidemia hirta* (L.) D. Don, *Alstonia macrophylla* Wall. ex G. Don and *Adiantum* species. Ihalagama is a slightly sloped hill area at an altitude 265 m. *Adiantum* species, *C. arabica*, *Costus speciosus* (J. Koenig) Sm. and naturalized ornamental plants were the common species in the area (Figure 7: B & C).

**Conservation status**

*T. duchartrei* could be considered as a critically endangered species based on the IUCN Red list categories and criteria, version 3.1, and present records. It is not currently protected by legislation despite the high conservation value to the flora of Sri Lanka. Therefore, for the future conservation of this species, it is suggested that an official Red List assessment and further studies using *in situ* and *ex situ* conservation need to be undertaken. The related species, *T. siliquosa* is found in low country and mid country wetland secondary forest and it is currently in the least concern category of the National red list of Sri Lanka (Wijesundara *et al*., 2020). We observed isolated patches of *T. siliquosa* in human settlement areas adjacent to the forest reserves, in agricultural fields, and beside streams and foot trails. However, gradual but not immediate elimination of the common species *T. siliquosa* from natural ecosystems was noted. *Ex situ* conservation measures are in place at Link Natural nursery in Sri Lanka.

## CONCLUSION

The discovery of *T. duchartrei* marks a new addition to the known flora of Sri Lanka. Modifications of the flower characters and androecium plasticity in the Sri Lankan specimens were highly significant and reconfirmed the high variability of morphological characters of the genus *Thottea.* This is the first report of *T. duchartrei* occurring outside of India and it increases the total number of Aristolochiaceae species from 4 to 5 and *Thottea* species from 1 to 2 in the flora of Sri Lanka.

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## REFERENCES

Anilkumar E.S., Mathew D., Nishanth K.S., Dileep K.B.S. & Latha P.G. (2014). A Comparative study on the in-vitro antimicrobial activity of the roots of four *Thottea* species. *International Journal of Pharmacy and Pharmaceutical Sciences* **6**(10): 444–447.

Ashton P.S. & Gunatilleke C.V.S. (1987). New light on the plant geography of Ceylon. I. Historical plant geography. *Journal of Biogeography* **14**: 249–285. DOI: https://doi.org/10.2307/2844895

Ashton P.M., Gunatilleke C.V.S., Zoyza N., Dassanayake M.D., Gunatilleke I.A.U.N. & Wijesundara S. (1997). Introduction to the flora. In: *A Field Guide to the Common Trees and Shrubs of Sri Lanka*, pp.1–3. WHT Publications (Pvt) Ltd., Sri Lanka.

Beentje H. (2010). *The Kew Plant Glossary: An Illustrated Dictionary of Plant Identification Terms*, 1st edition, pp.1–160. Royal Botanic Gardens, Kew, Richmond, Surrey, TW9 3AB, UK.

Benitez-Malvid J. & Martinez-Ramos M. (2003). Impact of forest fragmentation on seedling abundance in a tropical rain forest. *Conservation Biology* **12**: 380–389. DOI: https://doi.org/10.1111/j.1523-1739.1998.96 295.x

Bossuyt F. *et al.* (13 authors) (2004). Local endemism within the Western Ghats - Sri Lanka Biodiversity Hotspot. *Science* **306**: 479–481.

Caruso C.M. (2006). Plasticity of inflorescence traits in *Lobelia siphilitica* (Lobeliaceae) in response to soil water availability. *American Journal of Botany* **93**(4): 531–538. DOI: https://doi.org/10.3732/ajb.93.4.531

Cuénoud P., Savolainen V., Chatrou L.W., Powell M., Grayer R.J. & Chase M.W. (2002). Molecular phylogenetics of Caryophyllales based on nuclear 18S rDNA and plastid rbcL, atpB, and matK DNA sequences. *American Journal of Botany* **89**(1): 132–144.

Duchartre P.E.S. (1864). Aristolochiaceae. In: *Prodromus Systematis Naturalis Regni Vegetabilis* 15 (ed. A.P. de Candolle), pp. 421–498. Sumptibus Sociorum Treuttel & Würtz, Paris, France.

Fatimah S.S., Maryati M., Mohd Fadzelly A. & Cuevas A.L. (2018). Phytochemical profiling and antimycobacterial activity of ethnomedically selected *Thottea* species (Aristolochiaceae) from Peninsular Malaysia. *Journal of Engineering and Applied Sciences* **13** (Spec.Iss.3): 1–9. DOI: http://doi.org/10.36478/jeasci.2018.3110.3118

Gunatilleke N., Gunatilleke S. & Ashton P.S. (2017). South-west Sri Lanka: a floristic refugium in South Asia. *Ceylon Journal of Science* **46**(5): 65–78. DOI: http://doi.org/10.4038/cjs.v46i5.7454

Gunawardene N.R. *et al*. (11 authors) (2007). A brief overview of the Western Ghats-Sri Lanka biodiversity hotspot. *Current Science* **93**: 1567–1572.

Heart T.R., Somaratne S. & Wickremasinghe K.L. (1999). Aristochiaceae. In: *A Revised Handbook to the Flora of Ceylon*, volume 13 (eds. M.D. Dassanayake & W.D. Clayton), pp. 11–22. Oxford and IBH Publishing Co. Pvt. Ltd, New Dehli, India.

Hooker J.D. (1890). *The Flora of British India* 5, pp. 72–74. L. Reeve & Co., London, UK.

Hou D. (1981). Florae Malesianae Praecursores LXII On the genus *Thottea* (Aristolochiaceae). *Blumea* **27**: 301–332.

Huber H. (1985). Samenmerkmale und Gliederung der Aristolochiaceen. *Botanische Jahrbücher fur Systematik, Pflanzengeschichte und Pflanzengeographie* **107**: 277–320.

IUCN (2001). *IUCN Red List Categories and Criteria*: *Version 3.1*, IUCN Species Survival Commission, IUCN, Gland, Switzerland and Cambridge, UK.

John J,A., Jose J.O., George V., Pradeep N.S. & Sethuraman M.G. (2008). Volatile constituents and antibacterial activity of leaf oil of *thottea ponmudiana* Sivar. *Journal of Essential Oil Research* **20**(5): 460–463.

Kelly L.M. (2001). Taxonomy of *Asarum* section *Asarum* (Aristolochiaceae). *Systematic Botany* **26**: 17–53. DOI: https://doi.org/10.1043/0363-6445-26.1.17

Klotzsch F. (1859). Die Aristolochiaceae des Berliner Herbariums. *t.1. Monatsberichte der Königlichen Preusischen Akademie der Wissenschaften zu*, Berlin, pp. 571–626.

Koottasseri A., Babu A., Augustin A., Job J.T. & Narayanankutty A. (2020). Antioxidant, anti-inflammatory and Anticancer activities of methanolic Extract of *Thottea siliquosa*: an in vitro study. *bioRxiv*.

Lee S.Y., Ng W.L., Mahat M.N., Nazre M. & Mohamed R. (2016). DNA barcoding of the endangered *Aquilaria* (Thymelaeaceae) and its application in species authentication of agarwood products traded in the market. *PloS one* **11**(4): e0154631. DOI: https://doi.org/10.1371/journal.pone.0154631

Leins P., Erbar C. & Heel W.A. (1988). Note on the floral development of *Thottea* (Aristolochiaceae). *Blumea* **33**: 357–370.

Madhumita M. (2013). Taxonomic revision of the family Aristolochiaceae Juss. in India. *PhD thesis*, Kalyan University, West Bengal, India.

Merrine R. & Ramesh B. (2012). Phytochemical investigation and pharmacological activity in the root extracts of *Thottea siliquosa* Lam. *Asian Journal of Biological and Life Sciences* **1**(1): 72–75.

Mittermeier R.A., Gil P.R., Hoffman M., Pilgrim J., Brooks T., Mittermeier C.G., Lamoreux J. & da Fonseca G.A.B. (2004). *Hotspots Revisited: Earth's Biologically Richest and Most Threatened Terrestrial Ecoregions*. CEMEX, Mexico City, Mexico.

Moormann F.R. & Panabokke C.R. (1961). *Soils of Ceylon, A New Approach to the Identification and Classification of the Most Important Soil Groups of Ceylon*. The Government Press, Ceylon.

Moorthy K., Punitha, T., Vinodhini, R., Mickymaray S., Shonga A., Tomass Z. & Thajuddin N. (2015). Efficacy of different solvent extracts of *Aristolochia krisagathra* and *Thottea ponmudiana* for potential antimicrobial activity. *Journal of Pharmacy Research* **9**(1): 35–40.

Mullu D. (2016). A Review on the effect of habitat fragmentation on ecosystem. *Journal of Natural Sciences Research* **6**: 1–16.

Nazarudeen A. & Sabu T. (2002). Staminal instability in *Thottea duchartrei*. *Indian Journal of Forestry* **25**(2): 194–195.

Nishida R., Weintraub J.D., Feeny P. & Fukami H. (1993). Aristolochic acids from *Thottea* spp. (Aristolochiaceae) and the osmeterial secretions of *Thottea*-feeding troidine swallowtail larvae (Papilionidae). *Journal of Chemical Ecology* **19**: 1587–1594.
DOI: https://doi.org/10.1007/BF00984899

Nusaiba S.A.W. & Murugan K. (2013). In vitro analysis on bactericidal screening and antioxidant potentiality of leaf and root extracts of *Thottea siliquosa* (Lam.) Ding Hou. An ethnobotanical plant. *Asian Pacific Journal of Tropical Biomedicine* **3**(11): 859–865.

Oelschlägel B., Wagner S., Salomo K., Pradeep N.S., Yao T.L., Isnard S., Rowe N., Neinhuis C. & Wanke S. (2011). Implications from molecular phylogenetic data for systematics, biogeography and growth form evolution of *Thottea* (Aristolochiaceae). *Gardens' Bulletin Singapore* **63**(1&2): 259–275.

Perry L.M. & Metzger J. (1980). *Medicinal Plants of East and Southeast Asia: Attributed Properties and Uses*, pp. 620. MIT Press, USA.

POWO (2021). Plants of the World Online. Facilitated by the Royal Botanic Gardens, Kew. Available at *http://www.plantsoftheworldonline.org/*, Accessed 23 August 2021

Renuka C. & Swarupanandan K. (1986). Morphology of the flower in *Thottea siliquosa* and the existence of staminodes in Aristolochiaceae. *BLUMEA* **31**: 313–318.

Rogan J.E. & Lacher T.E. (2018). Impacts of habitat loss and fragmentation on terrestrial. In: *Reference Module in Earth Systems and Environmental Sciences* (ed. S.A. Elias), pp. 1–18. Elsevier, Oxford, UK.
DOI: http://dx.doi.org/10.1016/B978-0-12-409548-9.10913-3

Sabran S.F., Mohamed M. & Abu Bakar M.F. (2016). Ethnomedical knowledge of plants used for the treatment of tuberculosis in Johor, Malaysia. *Evidence-Based Complementary and Alternative Medicine* **2016**: 2850845.
DOI: https://doi.org/10.1155/2016/2850845

Shaiju P.N. & Omanakumari N. (2009). Inflorescence morphology and systematics of the genus *Thottea* Rottb. (Aristolochiaceae) from the Western Ghats, India. *Systematics and Biodiversity* **7**(4): 445–451.
DOI: https://doi.org/10.1017/S1477200009990181

Shaiju P.N. & Omanakumari N. (2010). Floral morphology and systematics of the genus *Thottea* Rottb. (Aristolochiaceae) from the Western Ghats, India. *Plant Systematics and Evolution* **288**: 213–225.
DOI: https://doi.org/10.1007/s00606-010-0326-x

Sivarajan V.V., Balachandran I. & Babu A. (1986). A new species of *Thottea* Rottb. (Aristolochiaceae) with notes on the identity of *Thottea siliquosa* (Lam.) Ding Hou. *Indian Journal of Forestry* **8**(4): 265–268.

Solereder H. (1894). Aristolochiaceae. In: *Die natürlichen Pflanzenfamilien* 3 (eds. A. Engler & K. Prantl), pp. 264–273.

Sunil C.N. & Kumar V.V.N. (2014). *Thottea adichilthottiana* (Aristolochiaceae), a new species from Ernakulam, Western Ghats, India. *Webbia* **69**(2): 239–242.
DOI: https://doi.org/10.1080/00837792.2014.951205

The World Checklist of Vascular Plants (WCVP). Checklist Data set. Royal Botanic Gardens, Kew, Available at *https://doi.org/10.15468/6h8ucr*, Accessed 10 December 2020.

Thiers B. (continuously updated). *Index Herbariorum*: A global directory of public herbaria and associated staff. New York Botanical Garden's Virtual Herbarium. Available at *http://sweetgum.nybg.org/science/ih/*, Accessed February 2020.

Turland N.J. *et al.* (15 authors) (2018). International Code of Nomenclature for algae, fungi, and plants (Shenzhen Code) adopted by the Nineteenth International Botanical Congress Shenzhen, China, July 2017. Koeltz Botanical Books, Germany.

Udayan P.S., George S., Tushar K.V. & Balachandran I. (2005). Medicinal plants used by the Kaadar tribes of Sholayar forest Thrissur district, Kerala. *Indian Journal of Traditional Knowledge* **4**(2): 159–163.

Wang B.W., Zhang G.H. & Duan J. (2015). Relationship between topography and the distribution of understory vegetation in a *Pinus massoniana* forest in Southern China. *International Soil and Water Conservation Research* **3** (4): 291–304.
DOI: https://doi.org/10.1016/j.iswcr.2015.10.002

Wijesundara S., Ranasinghe S., Jayasinghe H., Gunawardena N., Fonseka G. & Wijesooriya S. (2020). Angiosperms in Sri Lanka, In: *The National Red List 2020 of Sri Lanka*. Biodiversity Secretariat of the Ministry of Environment and National Herbarium, Department of National Botanic Gardens, Peradeniya, Sri Lanka.

Zin N.M., Sarmin N.I., Ghadin N., Basri D.F., Sidik N.M., Hess W.M. & Strobel G.A. (2007). Bioactive endophytic streptomycetes from the Malay Peninsula. *FEMS Microbiology Letters* **274**(1): 83–88.
DOI: https://doi.org/10.1111/j.1574-6968.2007.00819.x