

Implications from molecular phylogenetic data for systematics, biogeography and growth form evolution of *Thottea* (Aristolochiaceae)

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ABSTRACT. The genus *Thottea* comprises about 35 species distributed from India throughout Southeast Asia. However, most of the species have a narrow distribution. A first molecular phylogeny based on the chloroplast *trnK* intron, *matK* gene and *trnK-psbA* spacer is presented and confirms the monophyly of the genus according to Hou in 1981. Earlier subdivisions into the sections or genera *Apama* and *Thottea* could not be substantiated since both proved to be paraphyletic with respect to each other. The taxonomic and systematic history of *Thottea* is discussed with respect to molecular and morphological data. *Thottea piperiformis* is sister to all other species, which gives limited recognition to *Asiphonia piperiformis* as proposed by Huber (1985). *Thottea tomentosa*, one of the smallest and most widespread species is subsequently sister to all remaining species. *Thottea* diversified in two biogeographic regions: the Western Ghats in India and the Indo-Malayan region. A high degree of endemism is observed resulting from the presence of very few species shared between islands, which might be the result of a single colonisation and subsequent radiation. Within Piperales, *Thottea* holds a key position between the herbaceous Asaroideae and the woody Aristolochioideae.

Keywords. Aristolochiaceae, biogeography, growth form evolution, molecular phylogeny, *Thottea*

Introduction

During the last two centuries, the number of accepted genera within Aristolochiaceae has differed according to the dataset investigated (e.g., seven by González & Stevenson 2002; five by Neinhuis et al. 2005 and Ohi-Thoma et al. 2006). Currently, the family Aristolochiaceae is divided into two subfamilies Aristolochioideae and Asaroideae and four genera are consistently recognised (*Saruma* Oliv., *Asarum* L., *Thottea* Rottb.

and *Aristolochia* L.) (Wanke et al. 2007a). However, Aristolochiaceae has turned out to be paraphyletic with respect to *Lactoris fernandeziana* Phil. and probably also Hydnoraceae (Nickrent et al. 2002; Wanke et al. 2007a), which will not be addressed here further. The subfamily Asaroideae contains small-sized herbaceous plants with flowers characterised by an actinomorphic perianth. It consists of two genera: the monotypic *Saruma* (*S. henryi* Oliv.), endemic to central China (Zhou et al. 2010) and *Asarum* with about 86 species from temperate areas of North America, Europe and Asia (Kelly 1998, Kelly & Gonzalez 2003, Wanke et al. 2006a). In contrast, the Aristolochioideae are distributed from tropical to temperate climate zones (Neinhuis et al. 2005). *Thottea* includes about 35 shrubby species with an actinomorphic perianth restricted to tropical Asia, while *Aristolochia* is the most species-rich genus with about 400 species representing geophytes, perennial herbs, climbers and shrubs (Wanke et al. 2006a).

Most likely due to the lack of *Thottea* in *ex situ* collections, only a few species have ever been included in molecular-based phylogenetic studies (e.g., Neinhuis et al. 2005, Ohi-Thoma et al. 2006, Wanke et al. 2006a, 2007a). However, all studies have assumed the monophyly of the genus based on morphological characters. In addition, traditional taxonomic concepts and infrageneric relationships have not yet been addressed using molecular data.

Since the genus *Thottea* was described by Rottböll (1783) seven further genera have been published and used by later authors at tribal or sectional levels:

Thottea Rottb. (type: *T. grandiflora* Rottb.), Nye Dansk. Vidensk. Selsk. Skrift. ii. (1783) 529. I. 2;

Apama Lam. (type: *A. siliquosa* Lam.), Encycl. (Lamarck) 1(1). (1783) 91;

Bragantia Lour. (type: *B. racemosa* Lour.), Fl. Cochinch. 2. (1790) 528;

Ceramium Blume (type: *C. tomentosum* Blume), Bijdr. Fl. Ned. Ind. 17. (1826-27) 1134, nom. illeg.: renamed as *Munnickia* Rchb., Consp. Regn. Veg. 85 (1828), *Vanhallia* Schult.f., Syst. Veg. 7 (1829) xviii & 166, and *Cyclodiscus* Klotzsch, Monatsb. Akad. Berl. (1859) 591.

Trimeriza Lindl. (type: *T. piperina* Lindl.), Edwards's Bot. Reg. 18. (1832) sub t. 1543;

Asiphonia Griff. (type: *A. piperiformis* Griff.), Trans. Linn. Soc. London 19. (1845) 333, t. 37;

Lobbia Planch. (type: *L. dependens* Planch.), London J. Bot. 6. (1847) 144, t. 3;

Strakaea C.Presl. (type: *S. melastomaefolia* C.Presl.), Epimel. Bot. (1851) 221;

Different taxonomic concepts are shown in Table 1. Klotzsch (1859) accepted three genera in two tribes, while Duchartre (1864) recognised only two: *Thottea* and *Bragantia*, and put all other genera in synonymy. He distinguished both genera by the number and arrangement of stamens. Whereas *Thottea* possesses 16–36 stamens arranged in two whorls (e.g., *T. abrahamii* M.Dan, P.J.Mathew, Unnithan & Pushp., Fig. 1A), *Bragantia* exhibits one whorl with only 6 to 10 stamens (e.g., *T. barberi* (Gamble) Ding Hou, Fig. 1B). Hooker adopted this classification but noted that both genera might “well be united” (Hooker 1890) because of their morphological similarity. Solereder (1894) also accepted the division into two genera but renamed *Bragantia*

Table 1. Traditional and current taxonomic concepts of the genus *Thottea* s.l.

Klotzsch (1859)	Duchartre (1864)	Hooker (1890)	Solereider (1894)	Hou (1981)	Huber (1985)
<u>Tribus Bragantiaceae</u>					
<i>Thottea</i> Rottb. incl. <i>Lobbia</i> Planch.; <i>Thottea</i> Rottb.	<i>Thottea</i> Klotzsch sect. I: THOTTEA incl. <i>Thottea</i> Rottb. sect. II: LOBBIA incl. <i>Lobbia</i> Planch.	<i>Thottea</i> Rottb. incl. <i>Lobbia</i> Planch.; <i>Thottea</i> Rottb.	<i>Thottea</i> Rottb. incl. <i>Lobbia</i> Planch.; <i>Thottea</i> Rottb.	<i>Thottea</i> Rottb. incl. <i>Apama</i> Lam.; <i>Asiphonia</i> Griff.; <i>Bragantia</i> Lour.; <i>Ceramium</i> Blume; <i>Cyclodiscus</i>	<i>Thottea</i> Rottb. sect. THOTTEA sect. APAMA
<i>Bragantia</i> Lour. incl. <i>Asiphonia</i> Griff.; <i>Bragantia</i> Lour.; <i>Trimeriza</i> Lindl.	<i>Bragantia</i> Lour. sect. I: EUBRAGANTIA incl. <i>Asiphonia</i> Griff.; <i>Bragantia</i> Lour.; <i>Strakaea</i> C.Presl. sect. II: TRIMERIZA incl. <i>Apama</i> Lamk.; <i>Trimeriza</i> Lindl.	<i>Bragantia</i> Lour. incl. <i>Asiphonia</i> Griff.; <i>Apama</i> Lamk.; <i>Bragantia</i> Lour.; <i>Ceramium</i> Blume; <i>Cyclodiscus</i> Klotzsch; <i>Strakaea</i> C.Presl.; <i>Trimeriza</i> Lindl.	<i>Apama</i> Lam. sect. I: BRAGANTIA incl. <i>Asiphonia</i> Griff.; <i>Bragantia</i> Lour.; <i>Strakaea</i> C.Presl. sect. II: TRIMERIZA incl. <i>Apama</i> Lamk., <i>Trimeriza</i> Lindl.	Klotzsch; <i>Lobbia</i> Planch.; <i>Strakaea</i> C.Presl.; <i>Thottea</i> Rottb.; <i>Trimeriza</i> Lindl.	<i>Asiphonia</i> Griff.
<u>Tribus Cyclodiscineae</u>					
<i>Cyclodiscus</i> Klotzsch incl. <i>Ceramium</i> Blume; <i>Cyclodiscus</i> Klotzsch	sect. III: CYCLODISCUS incl. <i>Ceramium</i> Blume; <i>Cyclodiscus</i> Klotzsch		sect. III: CYCLODISCUS incl. <i>Ceramium</i> Blume; <i>Cyclodiscus</i> Klotzsch		

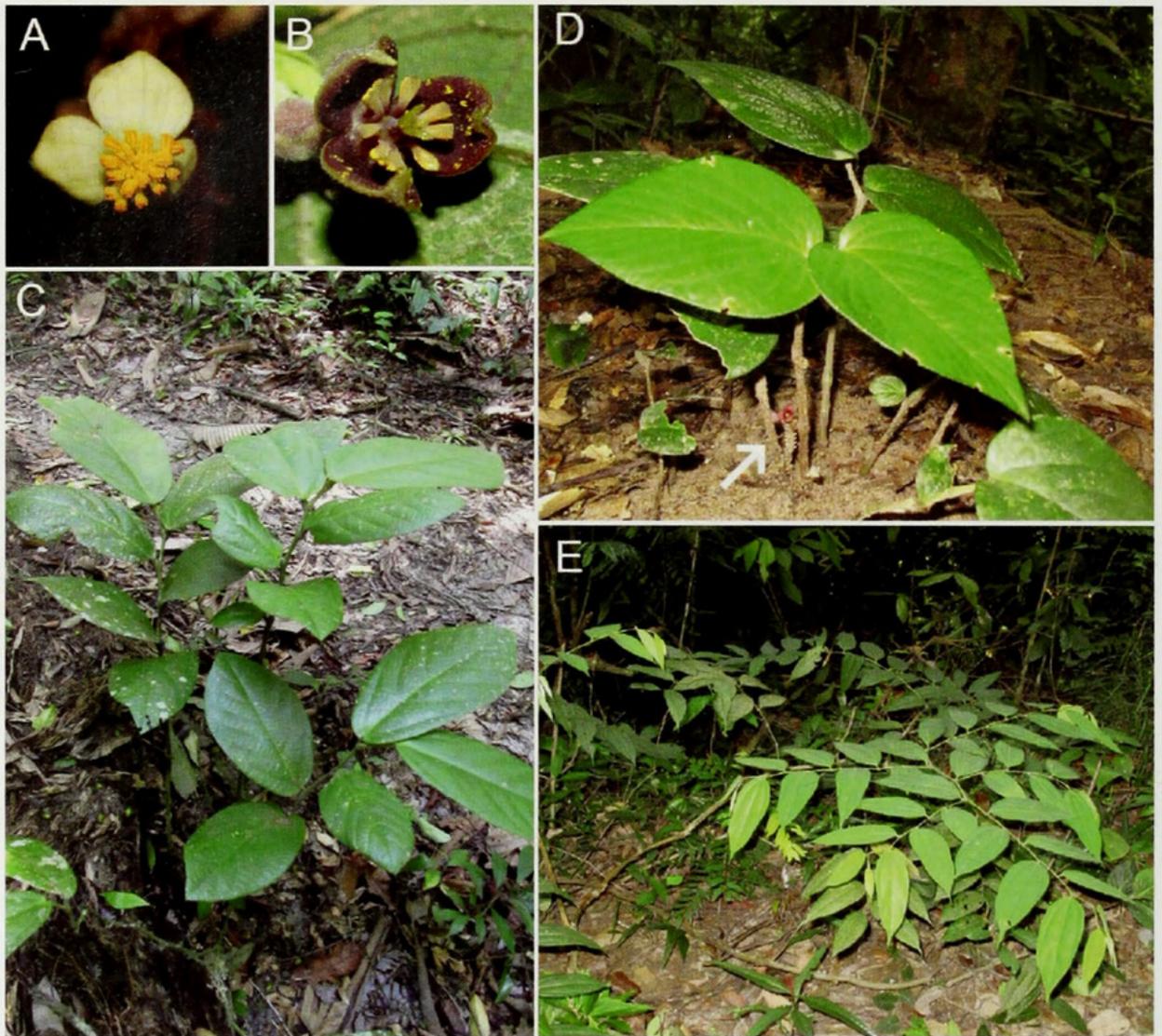


Fig. 1. Diversity of flowers and vegetative organisation within *Thottea* s.l. (Hou 1981). **A.** Flower of *T. abrahamii* M.Dan, P.J.Mathew, Unnithan & Pushp. (sect. *Thottea*) with stamens arranged in two whorls. **B.** Flower of *T. barberi* (Gamble) Ding Hou (sect. *Apama*), possessing 9 stamens in one whorl. **C.** Growth habit of *T. grandiflora* Rottb. **D.** *T. tomentosa* (Blume) Ding Hou, reaching only 40 cm in height. The flowers of this species appear at ground level (indicated by an arrow). **E.** *T. piperiformis* (Griff.) Mabb., growing up to 2 m or more with flowers borne in the axils of leaves. In addition to seed anatomy (Huber 1985), *T. piperiformis* also differs in growth form from all other species of the genus by its acrotonic branching.

as *Apama*, since this is the older name and has priority. About a century later, Hou published the most comprehensive study on the genus *Thottea* so far (Hou 1981). His study revealed that the differentiating characters of both genera were highly variable within species and even within one specimen. Based on these results he merged *Thottea* and *Apama* into one large genus *Thottea*. Furthermore, he explicitly pinpointed that no infrageneric subdivision is needed. A few years later, Huber (1985) again excluded one species, *Asiphonia piperiformis* Griff., due to the conspicuous seed anatomy. More recently six new species were described from India (Swarupandan 1983; Sivarajan, 1985; Sivarajan & Babu 1985; Pandurangan & Nair 1993; Dan et al. 1996; Kumar et

al. 2000). Although the flower and inflorescence morphology of the new Indian species were investigated from a systematic perspective (Shajiu & Omanakumari 2009, 2010), no further comprehensive study on the whole genus *Thottea* has been performed since then.

The aims of this study are to 1) test the monophyly of the genus *Thottea* sensu lato (s.l.) (Hou 1981) based on about half of the described species; 2) compare the most recent taxonomic concepts of Hou (1981) and Huber (1985), namely the treatment of the species as one single genus *Thottea*, and the separation of *Asiphonia* with the subdivision of the genus into the sections *Apama* and *Thottea* (Duchartre 1864), by means of molecular phylogenetic approaches; 3) compare results of a molecular phylogeny of the Indian species with the recently published results on flower and inflorescence morphology; and 4) provide a first molecular phylogenetic hypothesis as a starting point for more detailed studies addressing biogeographical questions, character evolution with respect to growth forms in *Thottea* and Aristolochiaceae and a revision of the genus reflecting natural relationships.

Methods

For this study, full sequences of three chloroplast regions (*trnK* intron, *matK* gene and *trnK-psbA* spacer) were generated for 15 *Thottea* species, as well as 21 species representing the other lineages of Aristolochiaceae and 3 outgroup genera of Saururaceae. For the latter, sequences of the *trnK* intron and the *matK* gene were derived from earlier studies (Wanke et al. 2006a, b, 2007a), whereas the *trnK-psbA* spacer was sequenced for this study. In Appendix A the origin of the material, voucher information and botanical garden accession numbers as well as GenBank accessions are provided. Total genomic DNA was isolated from herbarium specimens or leaves collected from the field or botanical gardens and dried in silica gel. A double-extraction approach with CTAB was used according to Borsch et al. (2003). After precipitation in ethanol and resuspension of the pellets in TE, DNA was cleaned by using the NucleoSpin® Extract II-Kit (Macherey-Nagel).

The amplification of the entire gene cluster was performed in one part for silica-dried material or in three parts with several 100 bp overlap for material from herbarium specimens. Primer sequences for amplification and sequencing are listed in Table 2. PCR products were obtained using a 25 µl reaction containing 1 µl template, 15.3 µl ddH₂O, 2.5 µl 10x Taq buffer (15 mM MgCl₂), 1 µl of 25 mM MgCl₂, 0.5 µl of each primer (10 pmol/µl), 4 µl dNTP mix (1.25 mM each), 0.2 µl Taq DNA polymerase (Promega). Amplification conditions were: one cycle of 1.5 min at 96°C, 1 min at 50°C, 2 min at 68°C, 34 cycles of 0.5 min at 95°C, 1 min at 48°C, 2 min at 68°C, and a final extension of 20 min at 68°C in a T3 Thermocycler (Biometra). PCR products were purified and extracted from a 1.2% agarose gel, using the NucleoSpin® Extract II-Kit. Sequences were run with an in-house Beckman Coulter 8000 capillary sequencer or sent to Macrogens' sequencing service (Macrogen Inc., Korea).

Table 2. Amplification and sequencing primers used.

Primer name	Direction	Sequence (5'-3')	Design	Primer used for
trnK-F	forward	GGG TTG CTA ACT CAA TGG TAG AG	Wicke & Quandt (2009)	all taxa
psbA-R	reverse	CGC GTC TCT CTA AAA TTG CAG TCA T	Steele & Vilgalys (1994)	all taxa
AR-matK-2400R	reverse	ATT TTC TAG CAT TTG ACT CC	Wanke et al. (2007a)	<i>Aristolochia</i>
AR-matK-2660F	forward	CTT ATG ATG AAG AAA TGG AAA TA	this study	<i>Aristolochia</i>
AR-psbA-3720R	reverse	CCC ATT TGY TAT TTC GGA T	this study	<i>Aristolochia</i>
AR-trnK-3480F	forward	ATT CTG AAA TGT TTA CRC AGT AGT	this study	<i>Aristolochia</i>
Th-matK-1510R	reverse	TAA ACT CCT GAA AGA GAA GTG G	this study	<i>Thottea</i>
Th-matK-2000F	forward	TTA TGG GCT ATC TTT CAA GTC G	this study	<i>Thottea</i>
Th-matK-2190R	reverse	TAT CAG AAT CAG ACG AAT CGG C	this study	<i>Thottea</i>
Th-matK-910F	forward	GAC TGT ATC GCA CTA TGT ATC G	this study	<i>Thottea</i>

Sequences were manually edited and aligned using the *Phylogenetic Data Editor* PhyDE[®] v0.995 (www.phyde.de) following alignment rules proposed by Kelchner (2000) and Borsch et al. (2003). Sixteen hotspots were excluded from the original dataset prior to the phylogenetic analyses due to ambiguous homology assessments. The dataset contained two inversions, one in the genus *Thottea* s.l. and one in *Asarum* and *Saruma*. To use both for phylogenetic reconstruction, the information on presence/absence of the inversion as well as the mutational events within, the inversions were coded in two additional columns at the end of the alignment and reversed in the alignment. Subsequently, indels were automatically coded using the simple indel coding approach according to Simmons & Ochoterena (2000) as implemented in SeqState (Müller 2005a), a PhyDE[®] plugin. The alignment and the indel matrix are available from TreeBASE (www.treebase.org). For phylogenetic reconstruction Maximum Parsimony and Bayesian Inference methods were employed. The most parsimonious trees were obtained by using the parsimony ratchet (Nixon 1999), as implemented in PRAP2 (Müller 2005b). Ratchet settings were set at 20 random addition cycles of 500 ratchet replicates up weighting randomly 25% of the characters during each iteration. A strict consensus tree was calculated and nodes were evaluated by bootstrapping

(BS) in PAUP* v.4.0 (Swofford 2002) using 1000 replicates. MrBayes v3.1 (Ronquist & Huelsenbeck 2003) was utilised for Bayesian Inference analysis. The GTR + Γ + I model was applied for sequence data, and the restriction site model ("F81") for the indel matrix after testing the best fitting model using jModeltest 0.1.1 (Posada 2008). Ten independent runs with 1,000,000 generations and 4 chains each were run simultaneously. Every 100th generation of each run was collected. The burnin was evaluated using Tracer v1.5 (Drummond & Rambaut 2007). A consensus tree and the posterior probabilities (PP) were calculated after discarding the first 50,000 sampled generations of each run as burnin.

Results

Characterisation of the molecular dataset

The total length of the alignment comprises 4440 bp, the mean sequence length 2887 bp (min = 2730 bp, max = 3272 bp), while the mean sequences in *Thottea* had a length of 2806 bp (min = 2763 bp, max = 2821 bp). Two inversions were detected. One in *Thottea* (position 226 to 253 in the *trnK* intron) forming a hairpin with a poly-T microsatellite as terminal loop (Wanke et al. 2007a) and one in *Asarum* and *Saruma* (position 4178 to 4185 in the *trnK-psbA* spacer). The dataset exhibited a large number of length mutations (indels), 222 of which were identified by SeqState. The combined data matrix (excluding hotspots) comprised a total number of 3802 characters, 1361 of which were variable and 906 parsimony-informative.

Phylogenetic reconstruction

The phylogram obtained by Bayesian Inference is shown in Fig. 2. Maximum Parsimony (MP) analyses resulted in 18 most-parsimonious trees of 2248 steps (CI = 0.736, RI = 0.901). The topology of the Bayesian and the MP strict consensus tree are virtually identical among early nodes and therefore only one tree is shown. However, within *Thottea* s.l. differences in branching patterns are observed for nodes lacking statistical support.

All *Thottea* species sampled form a single clade, which has maximum statistical support in both MP and Bayesian analyses (Fig. 2), as well as the sister group relationship of *Thottea* to *Aristolochia*. *Thottea piperiformis*, which Huber (1985) separated from *Thottea* as a monotypic genus (*Asiphonia piperiformis*), appears as sister to all other *Thottea* species. *Thottea tomentosa*, the most widespread species, is subsequent sister to the remaining species (PP 0.99, BS 97). The remaining species are found in two main clades. One is statistically highly supported (PP 1.00, BS 100), containing all Southeast Asian species, whereas the other is statistically unsupported and contains all Indian species. Within the clade of Indian species *T. abrahamii* and *T. dinghoui* are branching first, followed by *T. barberi*. *Thottea ponmudiana* is subsequent sister to the remaining species. Relationships among the rest are statistically supported for *T. dalzellii* being sister to *T. sivarajanii*, but their sistergroup relationship to *T. siliquosa*, *T. idukkiana*, *T. duchartrai* and a yet unidentified accession is unsupported.

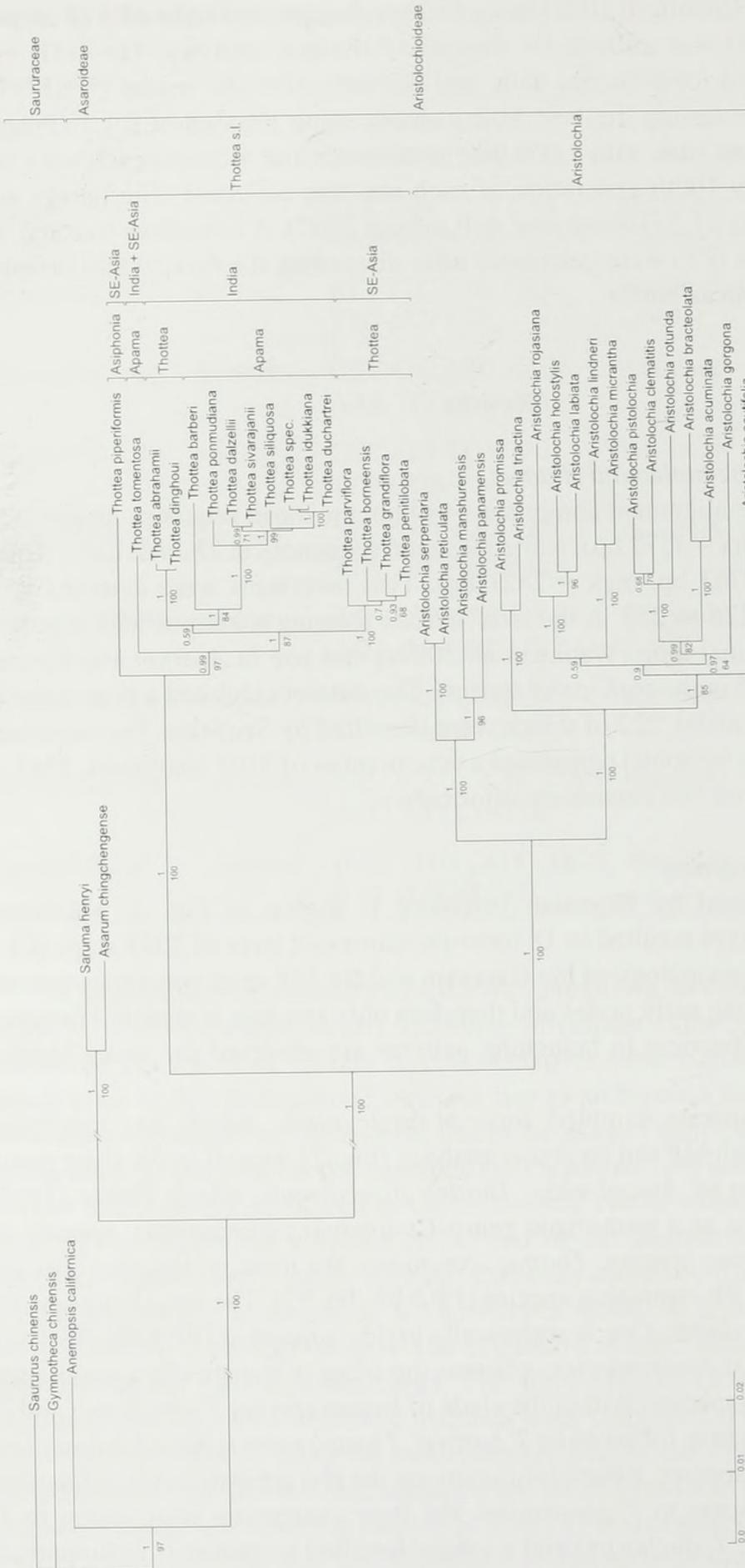


Fig. 2. Phylogram derived from Bayesian analysis, based on the chloroplast *trnK-matK-psbA* region, including coded length mutations. Posterior probabilities (PP) are given above and bootstrap values (BS) obtained from Maximum Parsimony analysis are shown below the branches. Probability values less than 0.5 and 50 for Bayesian and Maximum Parsimony, respectively, are not assigned. Branches interrupted by ‘//’ represent only 50 percent of the actual substitution rate observed. The subgeneric classification within *Thottea* s.l. (Hou 1981) represents the generic concepts of Duchartre (1864) and Huber (1985). The recently described species from India were assigned to these three groups based on their morphology.

The Southeast Asian clade contains four species (*T. parviflora*, *T. borneensis*, *T. grandiflora* and *T. penitilobata*).

Discussion

Monophyly of Thottea and taxonomic concepts

Phylogenetic analyses, based on half of the currently accepted species, demonstrate that *Thottea* s.l. is monophyletic. The study includes a small but biogeographically representative selection of the Southeast Asian species, as well as a complete taxon sampling of the Indian species, which were newly described since the last revision of *Thottea* by Hou in 1981. Furthermore, the specimens studied represent all traditional taxonomic subdivisions and all the morphological diversity of the genus. Therefore the systematic concept of Hou (1981) based on morphology is substantiated as well as the assumption of Hooker (1890), that both genera, *Apama* and *Thottea*, "...may well be united...".

We assigned species published after the treatment of Hou (1981) and Huber (1985) to the respective sections based on morphological characters that have traditionally been used to delimit the sections. Both sections are paraphyletic with respect to each other. Although the relationships between the different *Apama* and *Thottea* representatives are not highly supported in all nodes, a broader sampling or better resolution will not achieve monophyly. Consequently, the morphological characters used by Duchartre (1864), i.e. the number and arrangement of the stamens, are a result of parallel evolution. It is well known that the number of floral organs in basal angiosperms is not strictly determined (Soltis et al. 2009; Chanderbali et al., in press) and might therefore be of less systematic value at the species level. *Thottea* shows a comparatively high variability of flowers and especially a high plasticity of the androecium (Hou 1981, Leins et al. 1988, Shajiu & Omanakumari 2010). Whereas in earlier studies only two stamen whorls have been proposed, the detailed study of Hou (1981) revealed up to four whorls. Hou (1981) found 6 to 46 stamens and 2 to 20 styles per flower. Both, the number of styles and stamens per flower have been shown to vary between different individuals of the same species and even within one single individual (Hou 1981, Shajiu & Omanakumari 2010). However, species with low stamen numbers tend to have a lower stamen variability (e.g., *T. duchartrei*, 8–10 stamens) or the number is even constant (e.g., *T. tomentosa* 6 stamens), whereas in species with higher stamen numbers greater variability is observed (e.g. *T. dinghoui*, 15–30 stamens) (Hou 1981, Shajiu & Omanakumari 2010).

The sister relationship of *T. piperiformis* (Fig. 1E) to all other *Thottea* species (Fig. 2) may appear to lend support to the treatment by Huber (1985), who segregated this species from *Thottea* s.l. under its former name *Asiphonia piperiformis*. However, after comparing the sequences, the number of substitutions is not higher than in other *Thottea* species (Fig. 2). We therefore follow Hou (1981) in accepting only one single genus *Thottea* including *Asiphonia piperiformis*.

Morphological characters of the flowers and inflorescences investigated by Shajiu & Omanakumari (2009, 2010) substantiate the relationships within the Indian species complex. The sister group relationship of *Thottea abrahamii* and *T. dinghoui* to the remaining species is characterised by a racemose inflorescence and bi-lobed floral bracts (Shajiu & Omanakumari 2009) as well as a high number of (15–39) dorsifixed stamens that are arranged in two whorls (Shajiu & Omanakumari 2010). In contrast, all other Indian species show cymose inflorescence patterns (Shajiu & Omanakumari 2009) as well as a lower number of (mostly 9) ventrifixed stamens arranged in one whorl (Shajiu & Omanakumari 2010). The segregation of the next clade, *T. barberi*, is supported by the equal distribution of the stamens around the styles, whereas a pattern of 3+3+3 stamens substantiates the relationship of *T. duchartrei*, *T. idukkiana*, *T. ponmudiana*, *T. siliquosa* and *T. sivarajanii*. The close relationship of the latter five is also confirmed by the presence of a gynostemium that, in contrast, is absent in *T. abrahamii*, *T. dinghoui* and *T. barberi*. Furthermore, the affinity of *T. idukkiana* and *T. duchartrei* is retrieved in terms of the following morphological characters of their flowers and inflorescences: the very small prophyll in comparison to the floral bracts, fused sepals, the presence of sterile appendages on the gynostemium that are assumed to be staminodes and the co-occurrence of entire as well as bifid styler lobes (Shajiu & Omanakumari 2009, 2010). A morphological investigation of the undetermined species, which is cultivated in the Botanical Garden, Dresden, and resolved in the phylogeny together with *T. idukkiana* and *T. duchartrei*, revealed—besides a few differences—a high affinity to *T. idukkiana*. However, it has been used erroneously in our former studies as *T. siliquosa* (Neinhuis et al. 2005; Wanke et al. 2006a; Wanke et al. 2007a, b).

Outlook on biogeography and growth form evolution

From a biogeographic point of view, *Thottea* possibly represents an interesting case to study Southeastern Asian biogeography west of the Wallace line (Wallace 1859, 1863), as well as floristic affinities of this region to the Western Ghats in India, and island biogeography in general (Fig. 3). At first sight, the distribution of the genus seems rather constrained: from India to the Philippines and to the Greater Sunda Islands including one species crossing the Wallace line to Sulawesi (*T. celebica*). It is clear that *Thottea* diversified in two biogeographic regions: the Western Ghats in India and the Malesian region. In addition, a comparison of the biodiversity, distribution and similarity of species across the Islands, indicates that in most cases, only one species is shared between them, resulting in a high degree of endemism. Exceptions to this include only Sumatra, the Malay Peninsula, and Kra Isthmus, which share four species. However, floristic similarities of Sumatra and Malay Peninsula (Welzen et al. 2005) as well as Kra Isthmus and Malay Peninsula (Woodruff 2003) are well known. *Thottea tomentosa* (Fig. 1D) presents the link between the Indian species and the Southeast Asian species (excluding *T. piperiformis*). It is the smallest shrub within the genus that normally bears only 2 or 3 leaves per stem and is found throughout the western distribution area of the genus. Recently, Sumathi et al. (2004) reported the occurrence of *T. paucifida* from the Andaman Islands (not sampled in this study).

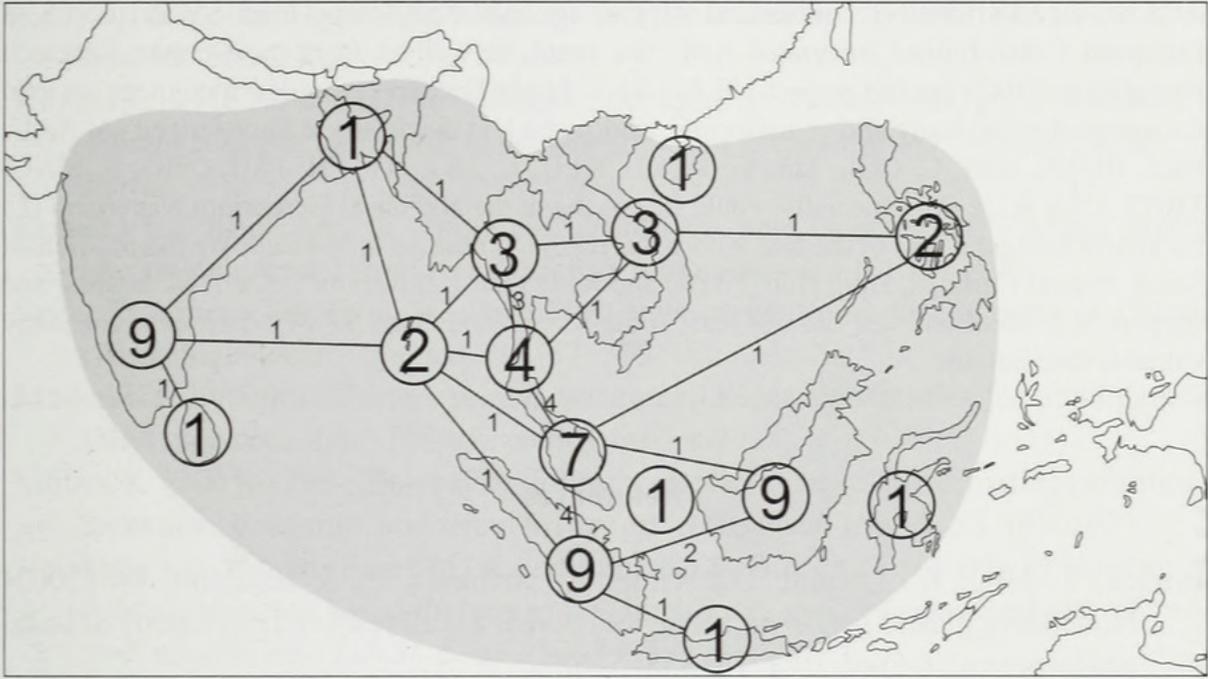


Fig. 3. Distribution and biodiversity similarity diagram for *Thottea* s.l. (Hou 1981) showing the number of species known for the respective biogeographic regions (in circles) and the number of species shared between them. Two diversity hotspots are observed: one in India (southern Western Ghats) and one in the Malesian region.

This species has only been reported from Borneo previously. This finding requires confirmation because *T. paucifida* and *T. tomentosa* can be confused due to superficial similarities. In addition, *T. paucifida* from the Andaman Islands is known only with fruits, whereas *T. paucifida* from Borneo was known only with flowers – which could exacerbate a comparison.

Thottea holds a potential key position with respect to growth form and woodiness evolution in the Piperales, being a potential link between the herbaceous Asaroideae and the woody Aristolochioideae. The genus *Aristolochia* is dominated by vines or lianas, but rarer shrub-like species are known. Close relationships between species having wide-ranging growth forms pose a number of questions concerning the processes by which highly different growth forms have evolved. Analysis of the developmental shifts in both primary and secondary development of the stem provides an implicit framework for identifying which structural and anatomical traits are adapted for life as herbs, shrub or lianas (Speck et al. 2003, Rowe & Speck 2005, Isnard et al. 2011, Wagner et al. in prep.). Ongoing studies are investigating to what extent heterochrony and relatively basic changes in developmental rate can radically influence the growth form and how specialisation and/or canalisation of developmental traits, play a role in modifying the overall size and growth form of species within the Aristolochiaceae and Piperales.

ACKNOWLEDGEMENTS. Financial support for this study came from SYNTHESYS, a European Union-funded Integrated Activities grant, as well as from the German Research Foundation (DFG)-funded project NE 681/11-1. Many Herbaria provided specimens on loan for morphological study and/or molecular work, which is thankfully acknowledged (A, AAU, BKF, BONN, BRI, C, COL, DR, G, K, KL, KLU, L, MO, NY, PH, PSU, QBG, S, SING, TBGT, USJ, WU). We especially would like to thank the Nationaal Herbarium Nederland (L) for kindly being the host of the first author during the Synthesys fellowship. We thank Michael Stech, Marcel Eurlings, Ding Hou (NHN) and René Glas (University Leiden) for hosting and support in Leiden, and last but not least Anna-Magdalena Barniske (TU Dresden) for many valuable discussions.

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Permanent URL of the alignment and analyses data on TreeBASE:

<http://purl.org/phylo/treebase/phylows/study/TB2:S11815>

Appendix A. Field origin or Botanical Garden (BG) accession numbers, voucher information and GenBank accessions used in the present study. * For these specimens sequences of the *trnK* intron and *matK* gene were derived from earlier studies. The *trnK* 3' exon and *trnK-psbA* spacer were newly generated and submitted to genbank as an update of the original sequences.

Taxon	Field origin / Botanical Garden accession no.	Voucher (herbarium)	Genebank accession no.	
			<i>trnK</i> intron, <i>matK</i> gene, <i>trnK-psbA</i> spacer	Source
<u><i>Saruma</i> Oliv.</u>				
<i>S. henryi</i> Oliv.	BG Bonn, 02618	Borsch 3456 (BONN)	DQ532033	Wanke et al. 2007a & this study*
<u><i>Asarum</i> L.</u>				
<i>A. chingchengense</i> C.Y.Cheng & C.S.Yang	BG Bonn, 02680	Neinhuis 90 (DR)	DQ882196	Wanke et al. 2007a & this study*
<u><i>Aristolochia</i> L.</u>				
<i>A. acuminata</i> Lam.	BG Bonn, 17417	Wanke & Neinhuis 146 (DR)	DQ532063	Wanke et al. 2007a & this study*
<i>A. acutifolia</i> Duch.	Colombia, Meta	González- 4176 (COL)	DQ532048	Wanke et al. 2006a & this study*
<i>A. bracteolata</i> Lam.	BG Bonn, 16714	Neinhuis 94 (DR)	DQ532059	Wanke et al. 2007a & this study*
<i>A. clematitis</i> L.	Croatia, Is Ilovik/ Asinello	Starmüller (KL)	DQ532060	Wanke et al. 2006a & this study*
<i>A. gorgona</i> M.A.Blanco	Heredia: Puerto Viejo de Sarapiquí, Costa Rica	Blanco 1752 (USJ)	DQ532051	Wanke et al. 2007a & this study*
<i>A. holostylis</i> F.González	BG Bonn, 02193	Neinhuis 116 (DR)	DQ532057	Wanke et al. 2007a & this study*
<i>A. labiata</i> Willd.	BG Bonn, 09867	Neinhuis 96 (DR)	DQ532055	Wanke et al. 2007a & this study*

<i>A. lindneri</i> A.Berger	Bolivia, San Jose de Chiquitos	Ibisch s.n. (DR)	DQ532047	Wanke et al. 2006a & this study*
<i>A. manshuriensis</i> Kom.	BG Bonn, 13085	Neinhuis 104 (DR)	DQ532040	Wanke et al. 2007a & this study*
<i>A. micrantha</i> Duch.	priv. coll. B.Westlund Texas, USA	Neinhuis 103 (DR)	DQ532046	Wanke et al. 2007a & this study*
<i>A. panamensis</i> Standl.	Panama, Panama	González-4018B (COL)	DQ532043	Wanke et al. 2006a & this study*
<i>A. pistolochia</i> L.	France, Cassis, Calenque d'En Veau	leg. Kreft, Wanke 37 (DR 25372)	DQ532062	Wanke et al. 2007a & this study*
<i>A. promissa</i> Mast.	BG Bonn, 13014	Neinhuis 118 (DR)	DQ532065	Wanke et al. 2007a & this study*
<i>A. reticulata</i> Nutt.	priv. coll. B.Westlund Texas, USA	Neinhuis 108 (DR)	DQ532037	Wanke et al. 2007a & this study*
<i>A. rojasiana</i> (Chodat & Hassl.) F.González	BG München s.n., Brazil, Mato Grosso	Wanke s.n. (DR)	DQ861635	Wanke et al. 2006a & this study*
<i>A. rotunda</i> L.	France, Corsica	Wanke 015 (DR)	DQ532061	Wanke et al. 2006a & this study*
<i>A. serpentaria</i> L.	priv. coll. B.Westlund Texas, USA	Neinhuis 112 (DR)	DQ532038	Wanke et al. 2007a & this study*
<i>A. triactina</i> Hook.f.	BG Bonn, 12767	Neinhuis 119 (DR)	DQ532066	Wanke et al. 2007a & this study*
<u>Thottea</u> Rottb.				
<i>T. abrahamii</i> M.Dan, P.J.Mathew, Unnithan & Pushp.	India, Kerala Trop. Bot. Garden	Pradeep s.n. (TBGT)	JN415669	this study
<i>T. barberi</i> (Gamble) Ding Hou	India, Kerala Trop. Bot. Garden	Pradeep s.n. (TBGT)	JN415675	this study
<i>T. borneensis</i> Valetton	Hort.Bogor XI.B.XIII.134, origin: Borneo	van Steenis 24294 (L 240977)	JN415668	this study
<i>T. dalzellii</i> (Hook.f.) Karthik. & Moorthy	India, Kerala Trop. Bot. Garden	Pradeep s.n. (TBGT)	JN415677	this study
<i>T. dinghoui</i> Swarupan.	India, Kerala Trop. Bot. Garden	Pradeep s.n. (TBGT)	JN415670	this study
<i>T. duchartrei</i> Sivar., A.Babu & Balach.	India, Kerala Trop. Bot. Garden	Pradeep s.n. (TBGT)	JN415678	this study

<i>T. grandiflora</i> Rottb.	Peninsular Malaysia, Selangor, Genting Sempah	B.C. Stone 6112 (PH 0961499)	JN415671	this study
<i>T. idukkiana</i> Pandur. & V.J.Nair	India, Kerala Trop. Bot. Garden	Pradeep s.n. (TBGT)	JN415680	this study
<i>T. parviflora</i> Ridl.	Thailand, Songkhla Province, Hat Yai	S.Chantanaorrapint 1265 (PSU)	JN415672	this study
<i>T. penitilobata</i> Ding Hou	Borneo, Brunei, Temburong, Batu Apoi Forest Reserve	Poulsen, A.D. 1 (AAU)	JN415673	this study
<i>T. piperiformis</i> (Griff.) Mabb.	Malaysia	Weber & Anthonysamy 870519-1/1 (WU)	DQ532036	Wanke et al. 2007a & this study*
<i>T. ponmudiana</i> Sivar.	India, Kerala Trop. Bot. Garden	Pradeep s.n. (TBGT)	JN415676	this study
<i>T. siliquosa</i> (Lamkey) Ding Hou	India, Kerala Trop. Bot. Garden	Pradeep s.n. (TBGT)	JN415679	this study
<i>T. sivarajanii</i> E.S.S.Kumar, A.E.S.Khan & Binu	India, Kerala Trop. Bot. Garden	Pradeep s.n. (TBGT)	JN415681	this study
<i>Thottea</i> sp.	BG Bonn, 09037, origin: India, Kerala, Thrissur District (Bogner 86-3421)	Neinhuis 121 (DR)	DQ532035	Wanke et al. 2007a & this study*
<i>T. tomentosa</i> (Blume) Ding Hou	Thailand, Phatthalung, Tha Mot	Larsen et al. 43958 (AAU)	JN415674	this study
<u>Saururaceae Rich.</u>				
<i>Anemopsis californica</i> (Nutt.) Hook. & Arn.	BG Bonn, 06422	Wanke 002 (DR)	DQ882198	Wanke et al. 2007a & this study*
<i>Gymnotheca chinensis</i> Decne.	BG Bonn, 17072	Wanke 004 (DR)	DQ882199	Wanke et al. 2007a & this study*
<i>Saururus chinensis</i> Hort. ex Loudon	BG Bonn, 00312	Wanke 001 (DR)	DQ212713	Wanke et al. 2006b & this study*



Oelschlägel, Birgit et al. 2011. "Implications from molecular phylogenetic data for systematics, biogeography and growth form evolution of *Thottea* (Aristolochiaceae)." *The Gardens' bulletin, Singapore* 63, 259–275.

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