

**MOLECULAR PHYLOGENY OF CONVALLARIOIDEAE
(ASPARAGACEAE), WITH EMPHASIS ON VIETNAMESE SPECIES**

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ABSTRACT

With the aim of inferring phylogenetic relationships among 86 species (including 45 species from Vietnam) mostly of the subfamily Convallarioideae (=Nolinoideae) (Asparagaceae *sensu* APG IV), we analyzed their chloroplast DNA sequences (*rbcL* and *trnL-F*) by both Bayesian inference (BI) and maximum likelihood (ML) methods. Our dataset included six of the seven tribes classified in this subfamily; Convallarieae, Dracaeneae, Liriopeae Nolineae, Polygonateae and Rusceae (Eriospermeae not examined). Our study supported the sisterhood between Convallarioideae and Asparagoideae and the monophyly of all the tribes except Polygonateae. Within the Convallarioideae we examined, *Dracaena* formed the basalmost clade. *Theropogon* did not positively nest in any of the tribes including Convallarieae in which it had often been classified. It was weakly defined as the second basalmost branch. *Ruscus* (Rusceae) nested in Polygonateae as the sister to *Maianthemum*, hence Polygonateae was recognized here as paraphyletic. Nolineae was discordant in position between BI and ML analyses, probably reflecting the limited molecular markers we examined. In both BI and ML analyses, all genera of Liriopeae and Convallarieae were monophyletic and their intergeneric relationships were consistent. In Liriopeae, *Liriope* was sister to the clade of *Ophiopogon* + *Peliosanthes*. In Convallarieae, *Aspidistra* + *Tupistra* formed the sister clade to *Reineckea* + *Rohdea*. Interspecific relationships within these genera were, however, not clearly resolved, except for several pairs of sister species. We also briefly discussed some of the resultant phylogenetic relationships from the morphological and/or evolutionary aspects.

Keywords: Convallarieae, Liriopeae, Nolinoideae, Ophiopogoneae, phylogeny, *Theropogon*.

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INTRODUCTION

Plants of Asparagaceae Juss. (nom. cons.) circumscribed by Chase et al. (2009) had been variously classified. Some botanists classified all of them in one family Liliaceae (Krause, 1930; Cronquist, 1981; Chen et al., 2000), whereas others recognized a number of families in them (Dahlgren & Bremner, 1985; Conran, 1989; Nguyen, 2007; Takhtajan, 2009). Classification schemes later presented by such botanists as Reveal (2012) and Fischer (2015) differed from the scheme by Chase et al. (2009). Thus, the taxonomic delimitation of the plants is still unstable and controversial.

Based on combined molecular and morphological studies, Chase et al. (2009), Reveal & Chase (2011) and APG IV (2016) subdivided their Asparagaceae into seven subfamilies; Agavoideae Herb. (1837), Aphyllanthoideae Lindl. (1846), Asparagoideae Burmeist. (1837), Brodiaeoidae Traub (1972), Lomandroideae Thorne & Reveal (2007), Convallarioideae Herb. (1837) (replaced name: Nolinoideae Burnett 1835. For this replacement, see Tanaka & Nguyen, 2023), and Scilloideae Burnett (1835). In the present paper, we focus on the Convallarioideae circumscribed by them.

With the aim of inferring phylogenetic relationships among taxa of Asparagaceae, several phylogenetic studies have so far been conducted (Rudall et al., 2000; Tamura et al., 2004; Kim et al., 2010, 2012; Seberg et al., 2012; Wang et al., 2014; Meng et al., 2021 a & b; Wang et al., 2022; Ji et al., 2023). In several reports (Kim et al., 2010, 2012, 2017; Seberg et al., 2012; Ji et al., 2023), it was shown that Convallarioideae is sister to Asparagoideae. Stevens (2001 onward) listed seven tribes in Convallarioideae: Eriospermeae Endl. ex Meisn. (1842: tab. diagn. 397, 400), Dracaeneae Dumort. (1829: 60), Rusceae Dumort. (1829: 60), Liriopeae Baker (1875: 509) (this name is adopted here as the correct name for a group of genera including *Liriope* instead of Ophiopogoneae that has often been used), Nolineae S. Watson (1879: 218), Convallarieae Dumort. (1827:

138), and Polygonateae Benth. & Hook. f. (1883: 749) (we added publication data to the list). In our research, we examine samples from all of the tribes except Eriospermeae.

Regarding the intertribal and intergeneric phylogenetic relationships within Convallarioideae, the results of previous molecular analyses were rather inconsistent (Jang & Pfosser, 2002; Kim et al., 2010; Wang et al., 2016; Meng et al., 2021 a & b; Ji et al., 2023). For example, Kim et al. (2010) showed that *Speirantha* is sister to the clade of *Convallaria* + (*Aspidistra* + (*Reineckea* + (*Campylandra* + (*Rohdea* + *Tupistra*)))), while Ji et al. (2023) reported that *Convallaria* is sister to the clade of *Speirantha* + other genera of Convallarieae (excluding *Theropogon*). Thus, we still need to more accurately resolve these unsettled phylogenetic relationships in this subfamily. Further, as one of the causal factors for topological discordance, limited taxon sampling has been suggested (Heath et al., 2008; Nabhan & Sarkar, 2011; Wiens & Tiu, 2012). This factor should be taken into account when one attempts to infer the process of phyletic diversification by building phylogenetic trees based on molecular data.

Vietnam and its neighboring Indochinese countries have many species of Liriopeae and Convallarieae (e.g. Averyanov et al., 2016, 2017 a, 2021; Nguyen et al., 2021; Tillich, 2023). However, except for a study by Nguyen et al. (2020), no phylogenetic studies on them have been performed. To properly infer the phylogeny of this subfamily, we need to incorporate such Indochinese species into studies. Considering our insufficient knowledge about the phylogeny of this subfamily and the possible impacts of limited taxon sampling on building phylogenetic trees, we undertook our own study, using many species of Liriopeae and Convallarieae occurring in Vietnam. Here we report the research results obtained by analyzing their chloroplast DNA sequences (*rbcL* and *trnL-F*).

In the present paper, we briefly discuss some of the resultant phylogenetic

relationships from the morphological and/or evolutionary aspects.

MATERIALS AND METHODS

Taxon sampling, DNA extraction, PCR amplification, and sequencing

Of the 92 samples studied, 46 (Appendix 1) represent 45 species in five genera of Convallarieae and Liriopeae (Convallarioideae) collected from Vietnam: - *Aspidistra* (19 species), *Tupistra* (7), *Rohdea* (3), *Peliosanthes* (11) and *Ophiopogon* (5), and the remaining 46 (Appendix 2) represent 41 species in 19 genera of Asparagaceae (18 of Convallarioideae) and one species of Liliaceae (*Disporum*) from outside Vietnam (mostly China). Totally 86 species (one overlapped species is deducted from the above summation) including one species of *Disporum* (as the outgroup) were examined here. Since the reconstructed phylogenetic trees of Asparagaceae have often been discordant between studies based on nuclear and chloroplast DNA sequences (Kim et al., 2010; Wang et al., 2014; Floden & Schilling, 2018), we examined two chloroplast DNA markers (*rbcL* and *trnL-F*) that have been widely employed for inferring the phylogeny of this family (Kim et al., 2010, 2012; Wang et al., 2014; Ji et al., 2023).

Total genomic DNAs of the samples were extracted from silica gel-dried leaves using the CTAB method (Doyle & Doyle, 1987). Primers of *rbcL* and *trnL-F* were from Taberlet et al. (1991) and Zurawski et al. (1981) respectively. Procedures of polymerase chain reaction (PCR) amplification and sequencing followed our previous work (Nguyen et al., 2020).

Sequences newly generated from this study were edited in Sequencher version 4.1.4 (Gene Codes Corporation, Ann Arbor, MI USA) and submitted to NCBI GenBank (Appendix 1). These sequences and other 46 samples downloaded from the NCBI GenBank (Appendix 2) were aligned in MAFFT v7.505 with default parameters (Katoh & Standley, 2013).

Phylogenetic analyses

Substitution models with free-rate heterogeneity were identified through the ModelFinder (Kalyaanamoorthy et al., 2017). Phylogenetic analyses were performed by both maximum likelihood (ML) and Bayesian inference (BI) methods. The ML phylogeny was reconstructed in IQ-TREE 2 (Bui et al., 2020) under the best-fit model of substitution TPM2+F+G4, branch support of 1000 ultrafast bootstraps (UFBS) replicates was determined in UFBoot2 (Hoang et al., 2018). MrBayes v3.2 (Ronquist et al., 2012) plugin PhyloSuite v.1.2.3 (Zhang et al., 2020) was used to infer the BI analysis under the GTR+I+G4 model. Two independent runs with four chains each of the Markov Chain Monte Carlo (MCMC) simulations were set to perform 10000000 generations with the sampling frequency of every 5000 generations and the first 25% of generations discarded as burn-in. The convergence of the running results was assessed by Tracer v. 1.7.2 (Rambaut et al., 2018). After reaching the stationary state when the average standard deviation of the split frequencies was < 0.05, the two independent runs were combined to obtain the majority rule consensus trees and to calculate posterior probabilities (PP). The output trees were edited in FigTree v.1.4.4 (Rambaut, 2018).

Terminology on phylogenetics/cladism

We followed Lincoln et al. (1987).

RESULTS AND DISCUSSION

DNA sequence characteristics

All *rbcL* sequences here analyzed comprised 1122 base pairs (bp) in length with 97 (8.6%) parsimony-informative sites, and *trnL-F* sequences consisted of 1156 bp with 132 (11.4%) parsimony-informative sites. The combined data matrix of 2278 characters including 229 parsimony-informative sites (10%) was used to build phylogenetic trees.

Phylogenetic relationships

Intertribal relationships

Eriospermeae, which was omitted in our analyses, has been included as one of the

seven tribes in Convallarioideae (Stevens, 2001 onward). Its phylogenetic position has been inferred as the sister to the remainder of Convallarioideae (Jang & Pfosser, 2002; Kim et al., 2010; Wang et al., 2016; Meng et al., 2021 a & b; Ji et al., 2023). In our analysis of Convallarioideae (excluding Eriospermeae), we recognized five monophyletic tribes (Convallarieae, Dracaeneae, Liriopeae, Nolineae, Rusceae) and one paraphyletic tribe (Polygonateae; for details see below) (Fig. 1). Within this subfamily, Dracaeneae formed the basalmost clade (PP = 1, UFBS = 100%), agreeing with previous reports (Wang et al., 2016; Floden & Schilling, 2018; Meng et al., 2021 a & b; Ji et al., 2023).

In our study, phylogenetic trees built by BI and ML methods showed a high similarity in topology among the tribes of Convallarioideae, except for Nolineae which formed the sister clade to Convallarieae (PP < 0.5) in the BI tree (Fig. 1 a) or to the clade (UFBS = 66%) of Liriopeae + Convallarieae (UFBS = 70%) in the ML tree (Fig. 1 b). This inconsistency in the position of Nolineae may be ascribed to the insufficient numbers of markers and parsimony-informative sites (10%) in our data matrix, and/or to the different methods of building trees (Planet, 2006; Urantowka et al., 2017).

The relationships between Nolineae and other tribes of Convallarioideae have been inconsistent among studies. Namely, in a study by Seberg et al. (2012), Nolineae was positioned closer to Convallarieae and Rusceae than to Liriopeae. In a study by Meng et al. (2021a), which was based on a comprehensive transcriptome data (covering 2126 genes), Nolineae was sister to the clade (BS 100%, PP=1) of (*Liriope* + *Theropogon*) + (Convallarieae + Polygonateae). In their study based on five markers (ITS, *psbA-trnH*, *trnC-petN*, *rbcL*, and *matK*), Wang et al. (2016) showed that Liriopeae is sister (PP < 0.95) to the clade of Nolineae + Convallarieae. Ji et al. (2023), who analyzed 68 plastid protein-coding genes, also confirmed that Liriopeae is sister (BS = 48%, PP = 0.83) to the clade of Nolineae +

Convallarieae (BS = 52%, PP = 0.93). Thus, the results of both Wang et al. (2016) and Ji et al. (2023) agreed with the BI tree in our analysis. Judging from these results, Nolineae is likely to have originated at least before the beginning of the diversification of Convallarieae.

In our analysis, *Theropogon*, which is a genus of Convallarioideae (Chase et al., 2009), resided as the second basalmost branch sister to the clade (PP = 0.51, UFBS = 58%) consisting of Liriopeae, Nolineae, Convallarieae, and Polygonateae + Rusceae (Fig. 1), implying that the origin of *Theropogon* preceded the beginning of diversification of these tribes. It was phyletically distinct from any other tribes of Convallarioideae, including Convallarieae to which it had often been assigned (Engler, 1887; Hooker, 1892; Conran & Tamura, 1998; Takhtajan, 2009; Fischer, 2015). Our data hence raise doubt about the placement of this genus in Convallarieae. In previous phylogenetic studies, *Theropogon* was variously positioned; it resided as the sister to *Ruscus* + (*Sansevieria* + *Dracaena*) in Wang et al. (2016), to *Liriope* in Meng et al. (2021a), to *Maianthemum* (Polygonateae) in Ji et al. (2023), or to the clade including Dracaeneae and Rusceae (Kim et al., 2010; Meng et al., 2021 b). Thus, none of these studies showed that *Theropogon* nests in Convallarieae.

Ruscus resided as the sister to *Maianthemum* with moderate supporting values (PP = 0.83, UFBS = 64%, Fig. 1). Polygonateae is hence interpreted here as a paraphyletic group. Rusceae was unstable as to its position among previous studies (Rudall et al., 2000; Kim et al., 2010; Seberg et al., 2012). In recent studies (Wang et al., 2016; Floden & Schilling, 2018; Meng et al., 2021a&b; Ji et al., 2023) it was inferred as the sister to Dracaeneae. Judging from all these results, Rusceae also appears to be of old origin within Convallarioideae.

Our study supported the monophyly of Liriopeae (PP = 0.93, UFBS = 70%, Fig. 1), agreeing with previous reports (Kim et al.,

2010; Seberg et al., 2012; Wang et al., 2014, 2016; Floden & Schilling, 2018; Meng et al., 2021b; Ji et al., 2023). On the other hand, its phyletic position has been discordant among studies; i.e., Liriopeae was sister to the clade of Convallarieae + Polygonateae (Floden & Schilling, 2018; Meng et al., 2021a) or formed a polytomous clade with Nolineae and Convallarieae (Meng et al., 2021 b). In our BI analysis, Liriopeae was inferred as the sister clade (PP = 0.93) to Nolineae + Convallarieae (Fig. 1a), agreeing with Kim et al. (2010), Wang et al. (2016) and Ji et al. (2023).

Intergeneric relationships

As in Ji et al. (2023), our data showed that *Asparagus* and all the genera belonging to the tribes of Convallarioideae are monophyletic: *Maianthemum*, *Polygonatum*, *Disporopsis*, *Heteropolygonatum* - Polygonateae; *Rohdea*, *Reineckea*, *Aspidistra*, *Tupistra*, *Speirantha*, *Convallaria* - Convallarieae; *Liriope*, *Peliosanthes*, *Ophiopogon* - Liriopeae; and *Dracaena* - Dracaeneae (Fig. 1). In our paper, *Theropogon* is treated separately from these tribes because of its phyletic independency as mentioned earlier.

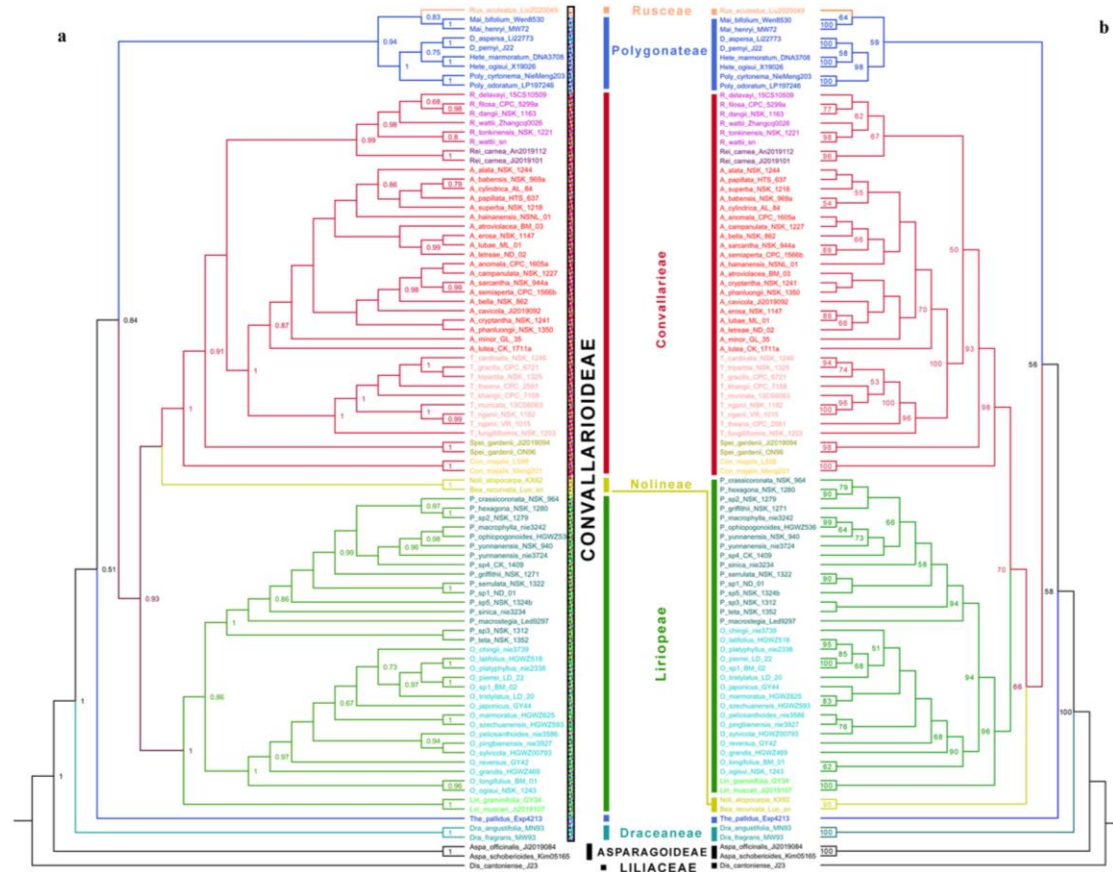


Figure 1. Phylogenetic trees of Convallarioideae (Asparagaceae) based on chloroplast DNA sequence data (*rbcl* and *trnL-F*). a. Bayesian (BI) tree, numbers near nodes represent Bayesian posterior probabilities (PP, values < 0.5 not presented), b. Maximum likelihood (ML) tree, numbers near nodes indicate ultrafast bootstrap percentage (UFBS%, values < 50% not presented). Abbreviation used: A = *Aspidistra*, Aspa = *Asparagus*, Bea = *Beaucarnea*, Con = *Convallaria*, D = *Disporopsis*, Dis = *Disporum*, Dra = *Dracaena*, Hete = *Heteropolygonatum*, Liri = *Liriope*, Mai = *Maianthemum*, Noli = *Nolina*, O = *Ophiopogon*, P = *Peliosanthes*, Poly = *Polygonatum*, R = *Rohdea*, Rei = *Reineckea*, Rus = *Ruscus*, Spei = *Speirantha*, T = *Tupistra*, The = *Theropogon*

Within Liriopeae, it was strongly supported that *Liriope* is sister to the clade (PP=1, UFBS = 96%) consisting of *Peliosanthes* + *Ophiopogon* (PP = 0.86, UFBS = 94%) (Fig. 1). This finding disagrees with earlier studies in which very few samples of *Peliosanthes* were analyzed (Rudall et al., 2000; Tamura et al., 2004; Kim et al., 2010; Seberg et al., 2012; Wang et al., 2016; Meng et al., 2021b; Ji et al., 2023). For example, in studies using only one sample of *Peliosanthes macrostegia*, *Peliosanthes* was recognized as the sister to the clade (BS > 95%, PP > 0.95) of *Liriope* + *Ophiopogon* (Kim et al., 2010; Wang et al., 2016; Meng et al., 2021b; Ji et al., 2023). In Wang et al. (2014), where both

nuclear (ITS) and chloroplast DNA sequences (*psbA-trnH*, *rbcL*, *matK*, *trnL-F*) were analyzed for six species of *Peliosanthes*, four species of *Liriope* and over 30 species of *Ophiopogon*, the three genera formed a polytomous clade. The topological discordance between genera of Liriopeae in these studies may stem from the insufficient numbers of used markers, limited taxon sampling, and/or different methods of building phylogenetic trees. The probable involvement of these factors in phylogenetic inference has been suggested in several works (Zwickl & Hillis, 2002; Crawley & Hillu, 2012; Wiens & Tiu, 2012; Floden, 2017; Russo et al., 2017).



Figure 2. *Ophiopogon* sp.1. a) Habit, b) Flower with three perianth lobes removed

In Convallarieae, we recognized six genera (Fig. 1): *Convallaria* as the basalmost clade (PP = 1, UFBS = 98%); *Speirantha* as the sister to the clade (PP = 0.91, UFBS = 93%) consisting of two subclades (PP < 0.5, UFBS = 50%), one comprising *Tupistra* + *Aspidistra* (PP = 1, UFBS = 100%) and the

other consisting of *Reineckea* + *Rohdea* (PP = 0.99, UFBS = 67%). These results agree with Ji et al. (2023). In our study, *Aspidistra* proved to be more closely related to *Tupistra* than to *Rohdea*, and this relationship is also corroborated by our morphological observations. Namely, compared with *Rohdea*,

Aspidistra and *Tupistra* usually possess a relatively larger stigma, a longer style nearly as broad as the ovary (vs. distinctly narrower), and warty, brownish or blackish (vs. smooth, orange or red) mature fruits (Tanaka, 2003a&b, 2010a&b; Averyanov et al., 2019b). In these respects, *Reineckea* agrees with *Rohdea* in our preliminary and previous observations (e.g. Wang et al., 1978).

Here we add some notes on the identities of several samples used in previous phylogenetic analyses of *Convallarieae*. The species used under the names of *Campylandra fimbriata* and *Tupistra aurantiaca* in Kim et al. (2010) are classified under *Rohdea* by Tanaka (2003a, 2010a&b). In Kim et al. (2010), the clade of *Reineckea* + (*C. fimbriata* + (*Rohdea japonica* + *T. aurantiaca*)) is sister to *Aspidistra*. In Tanaka's classification, the former clade is equivalent to *Reineckea* + *Rohdea*, so their result does not contradict our result. Likewise, in Meng et al. (2021b), *Aspidistra* sp. was sister to *Tricalistra ochracea*, and *Reineckea carnea* was sister to *Tupistra fimbriata* + *Tupistra* sp. The species of *Tricalistra* was transferred to *Tupistra* as *Tricalistra ochracea* (Ridl.) N.Tanaka (Tanaka, 2003b, 2010b), and *Tupistra fimbriata* belongs to *Rohdea* (Tanaka, 2010a). If *Tupistra* sp. (voucher: *G.W.Hu 208*, KUN) in Meng et al. (2021 b) represents a species of *Rohdea*, the relationships among *Aspidistra*, *Tupistra*, *Reineckea* and *Rohdea* in Meng et al. (2021b) are compatible with Ji et al. (2023) and our study. Thus, in interpreting the relationships among taxa, we need to pay careful attention to identifications made for the samples used.

Interspecific relationships

Ophiopogon Ker Gawler

Phylogenetic relationships between species were poorly resolved as reflected in the discordance between BI and ML trees and/or in the weak to moderate bootstrap supports (PP < 0.5–0.8, UFBS < 50–80%, Figs. a & b). However, in samples of *Ophiopogon* from Vietnam, we found that

Ophiopogon sp. 1 is most closely related to *O. pierrei* L.Rodr. (PP = 1, UFBS = 100%). *Ophiopogon* sp. 1 (sample *BM 02*, Fig. 2) and *O. pierrei* (Tanaka, 2000) share such character states as an elongate stem with several rigid prop roots, linear leaf blades clustered in the distal part of the stem, narrowly lanceolate bracts, and a nearly hemispheric semi-inferior ovary. Their phyletic closeness is thus also supported by morphological observations. However, the former has some morphological differences from the latter (e.g. leaf width, length of peduncle, flower number per inflorescence, etc.), so our decisive identification of *Ophiopogon* sp. 1 is postponed until our closer study is completed. On the other hand, *O. ogisui* M.N.Tamura & J.M.Xu formed a branch sister to *O. longifolius* Decne in both trees (Fig. 1), though the bootstrap support was moderate in the ML tree. These two species differ markedly in various morphological traits; *O. ogisui* has elliptic leaf blades with a distinct petiole (Tamura & Xu, 2007), whereas *O. longifolius* has significantly narrower elliptic to linear blades (Tanaka, 1998). This disparity between phylogeny and morphology apparently necessitates further investigation of their relationship. In *Ophiopogon* sampled from China, we found close relationships between *O. latifolius* L.Rodr. and *O. platyphyllus* Merr. & Chun, and between *O. marmoratus* Pierre ex L. Rodr. and *O. szechuanensis* F.T.Wang & Tang (PP = 1, UFBS > 80%). Detailed analyses and discussion on the interspecific relationships of *Ophiopogon* from China were made by Wang et al. (2014).

Peliosanthes Andrews

In our study, some close specific relationships supported by moderate to strong bootstrap values were found. Namely, we found sister relationships between *Peliosanthes serrulata* L.Rodr. and *Peliosanthes* sp. 1 (PP = 1, UFBS = 90%); between *P. crassicornata* K. S. Nguyen, Aver. & N. Tanaka and *P. hexagona* Aver., N. Tanaka & K. S. Nguyen + *Peliosanthes* sp. 2 (PP = 1, UFBS = 90%); and between

Peliosanthes yunnanensis F. T. Wang & Tang sampled from Vietnam and *P. macrophylla* Wall. ex Baker + *P. ophiopogonoides* W. T. Wang & Tang from China (Fig. 1). The identity of the sample identified as *P. macrophylla* (Nie3242 in Wang et al., 2014) from China may need re-examination because this species has been known only from Nepal, Bhutan and India (Borah et al., 2020).

As in *Ophiopogon*, relationships among many *Peliosanthes* species, however, remained largely unclear as reflected in the discordance between BI and ML trees and the weak bootstrap supports (Fig. 1).

It appears noteworthy that *P. macrostegia* Hance and *P. teta* Andrews resided basally in both trees (Fig. 1). It has been known that they are both widespread (from India, south to the Malay Peninsula, east to Taiwan and/or China) and highly polymorphic (Tanaka, 2018; Averyanov et al., 2016, 2021). This fact might reflect their long history after being established as a species, for it is likely that it takes a certain amount of time for such perennials to spread over vast areas of Asia and to increase their diversity (or variation) as a result of adaptation to diverse environmental conditions. In contrast, species such as *P. hexagona* (Averyanov et al., 2015), *P. ophiopogonoides* (Wang & Tang, 1978) and *P. yunnanensis* (Wang & Tang, 1978; Nguyen et al., 2017) resided as the terminalmost branches of the trees (Fig. 1). These species are reported to be local in distribution (southern Yunnan, China, and/or northern Vietnam) and not to be polymorphic in particular, implying their shorter history after being established as a species. Apparently, we need to accumulate more data to test this inference.

In this study we used five, as yet unidentified species of this genus (*Peliosanthes* sp. 1–sp. 5, Appendix 1, Fig. 1). They will be dealt with elsewhere after their morphological features become more amply available.

***Aspidistra* Ker Gawler**

We found that *Aspidistra semiaperta* Aver. & Tillich is sister to *A. sarcantha* Aver., Tillich, T. A. Le & K. S. Nguyen (PP = 0.99, UFBS = 89%, Fig. 1). They share similar morphological traits such as urceolate perianths, broadly ovate anthers and peltate stigmas (Averyanov & Tillich, 2015; Averyanov et al., 2019 a). We also found that *A. letreae* Aver., Tillich & T. A. Le is sister to the clade (PP = 0.99, UFBS = 66%) of *A. lubae* Aver. & Tillich + *A. erosa* Aver., Tillich, T. A. Le & K. S. Nguyen (PP < 0.5, UFBS = 89%) in both trees. *Aspidistra lubae* and *A. erosa* are similar in having an ascending rhizome with prop roots, lanceolate leaf blades, slender rigid peduncles, horizontal flowers, campanulate perianths with a cupulate or slightly urceolate tube, anthers attached to the lower half of the perianth tube, and slender cylindrical styles (Averyanov & Tillich, 2014; Averyanov et al., 2019a). These two species somewhat resemble *A. letreae* (Averyanov et al., 2017b) in having a similar habit, an elongate rhizome with prop roots, and triangular perianth lobes, but *A. letreae* strikingly differs from them in the size and shape of leaves and flowers. Concerning these three species, the results of our molecular analysis were well-compatible with morphological observations. We scarcely found any definite relationships among other species because of low supporting values (Fig. 1).

***Tupistra* Ker Gawler**

In our study, *Tupistra nganii* K. S. Nguyen, Aver., N. Tanaka & Nuraliev and *T. muricata* (Gagnep.) N. Tanaka were found to have a sister relationship (PP = 1, UFBS = 96%, Fig. 1). It was earlier suggested by Averyanov et al. (2020) from a morphological standpoint that these two species are closely similar, sharing a campanulate perianth, recurved triangular-ovate perianth lobes, and a cylindrical pistil with a small shallowly lobed thin (non-incrassate) stigma. In both BI and ML trees (Fig. 1), *T. cardinalis* Aver., N. Tanaka & T. S. Hoang, *T. gracilis* Aver. & N. Tanaka and *T. tripartita* Aver., N. Tanaka & K. S. Nguyen formed a monophyletic clade,

though the topology differed between the trees; i.e., in the BI tree (Fig. 1a), *T. cardinalis* was sister to the clade (PP = 1) of *T. gracilis* + *T. tripartita* (PP < 0.5), while in the ML tree (Fig. 1 b) *T. gracilis* formed a clade sister (UFBS = 74%) to *T. cardinalis* + *T. tripartita* (UFBS = 94%). Phenotypically *T. cardinalis* (Averyanov et al., 2018) differs markedly from both *T. gracilis* (Nguyen et al., 2017) and *T. tripartita* (Averyanov et al., 2019 b) mainly by its perianth lobes of different coloration and larger, less exerted, dentate, brown to dull purple stigmas nearly covering the anthers (vs. trilobed, white or yellowish stigmas scarcely covering the anthers). The phenotypical difference thus appears greater between *T. cardinalis* and the other two species, which is more compatible with the topology of the BI tree (Fig. 1a).

It seems notable that *Tupistra fungilliformis* F. T. Wang & S. Yun Liang formed the basalmost branch (PP = 1, UFBS = 96%) in both trees (Fig. 1), for it has been suggested that species with a pendulous floriferous stem-like *T. fungilliformis* and its allies (e.g. *T. clakei* Hook.f., *T. pingbianensis* J. L. Huang & X. Z. Liu, *T. tupistroides* (Kunth) Dandy) are morphologically close to *Aspidistra* (Tanaka, 2010b: 87). Like many species of *Aspidistra* (e.g. Li et al., 2004), at least both *T. pingbianensis* and *T. tupistroides* have repent rhizomes. Recently, a few more species were deemed as close to this group, such as *T. natmataungensis* Y. H. Tan. & H. B. Ding (Ding et al., 2019) and *T. annamensis* N. Tanaka, N. S. Ly, K. S. Nguyen & T. S. Hoang (Ly et al., 2022), have been discovered. To deepen our understanding of the evolutionary relationship between *Tupistra* and *Aspidistra*, it seems desirable to investigate them more in detail from both molecular and morphological aspects.

Tupistra theana Aver. & N. Tanaka, which is unique in having a small pistil among species of this genus (Averyanov & Tanaka, 2012), formed the second basalmost branch (UFBS = 100%) in the ML tree, but this phyletic position was not supported in the BI tree (Fig. 1).

Rohdea Roth

We found that *Rohdea dangii* K.S.Nguyen, N. Tanaka & Aver. is sister to *R. filosa* Aver. & N. Tanaka with a moderate support (PP = 0.99, UFBS = 77%, Fig. 1). This clade of *R. dangii* + *R. filosa* resided as the sister to *R. delavayi* (Franch.) N. Tanaka with a weak support (PP = 0.68, UFBS < 50%). On the other hand, *R. tonkinensis* (Baill.) N. Tanaka formed a branch sister to *R. wattii* (C.B.Clarke) Yamashita & M. N. Tamura (voucher: *s.n.* in Ji et al., 2023) with a strong support (PP = 0.8, UFBS = 98%), but its relationship to another sample of *R. wattii* (*Zhangcq0026*) was unresolved (Fig. 1). This topological deviation in the use of the sample *Zhangcq0026* may come from its short sequence of *rbcL* gene with only 615 bp and missing *trnL-F* sequence. The closeness in phylogeny between *R. tonkinensis* and *R. wattii* is also strongly supported by their morphological similarity; they share, for example, an elongate stem and elliptic to narrowly ovate leaf blades (Tanaka, 2010 a). Morphologically *R. dangii* is near to both *R. tonkinensis* and *R. wattii*, but has some marked differences from them (Nguyen et al., 2021). The molecular data supported the specific distinctness of *R. dangii*.

CONCLUSION

It is evident from previous and our studies that the subfamily Convallarioideae (Asparagaceae *sensu* APG IV) includes seven tribes: Eriospermeae (not examined here), Dracaeneae, Rusceae, Polygonateae, Liriopeae, Nolineae and Convallarieae. Our data supported the monophyly of all these tribes except Polygonateae. Within this subfamily we examined, Dracaeneae formed the basalmost clade. *Theropogon*, a genus of this subfamily, did not positively nest in any of the tribes including Convallarieae in which it had often been classified. It was weakly defined as the second basalmost branch, implying that it is an isolated lineage of old origin having weak relationships with the tribes of the sister clade. It is desirable to conduct further multidisciplinary studies on the taxonomic identity and phylogenetic

position of this genus. Though not examined here, it is also desirable to clarify the phylogenetic position of *Comospermum* Rauschert, another genus of Convallarioideae (Chase et al., 2009), toward a better understanding of the phylogeny of this subfamily. Though *Ruscus* formed a branch sister to *Maianthemum* of Polygonateae, further analyses may be needed to more accurately resolve their relationship.

The present study supported the monophyly of all the genera belonging to Polygonateae, Convallarieae, Liriopeae and Dracaeneae (*Dracaena*). Intergeneric relationships within Liriopeae and Convallarieae were concordant between BI and ML trees. In Liriopeae, *Liriope* was resolved to be sister to the clade of *Ophiopogon* + *Peliosanthes*. We may need to test this inference by studies from other angles (e.g. morphology). In Convallarieae, the clade of *Aspidistra* + *Tupistra* was resolved to be sister to the clade *Reineckea* + *Rohdea*. This phylogenetic inference agreed with our morphological observations.

Phylogenetic relationships of species occurring in Vietnam were, however, not clearly resolved, except for some pairs of sister species such as *Ophiopogon pierrei* and *Ophiopogon* sp.1, *Aspidistra semiaperta* and *A. sarcantha*, *A. lubae* and *A. erosa*, *Tupistra nganii* and *T. muricata*, and *Rohdea tonkinensis* and *R. wattii*. Further analyses are thus needed to more accurately resolve interspecific relationships within genera of such tribes as Liriopeae and Convallarieae from Vietnam.

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REFERENCES

- APG (Angiosperm Phylogeny Group) IV, 2016. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG IV. *Botanical Journal of the Linnean Society* 181: 1–20.
- Averyanov L. V., Tanaka N., 2012. New species of *Peliosanthes* and *Tupistra* (Asparagaceae) from eastern Indochina. *Taiwania*, 57(2): 153–167.
- Averyanov L. V., Tillich H. J., 2014. *Aspidistra albopurpurea*, *A. khangii*, *A. lubae* and *A. stellata* spp. nov. (Asparagaceae, Convallariaceae s.s.) from Indochina. *Nordic Journal of Botany*, 32: 752–760.
- Averyanov L. V., Tillich H. J., 2015. *Aspidistra laotica*, *A. multiflora*, *A. oviflora* and *A. semiaperta* spp. nov. (Asparagaceae, Convallariaceae s.s.) from eastern Indochina. *Nordic Journal of Botany*, 33: 366–376.
- Averyanov L. V., Tanaka N., Nguyen K. S., T. H. Nguyen, 2016. New species of *Ophiopogon* and *Peliosanthes* (Asparagaceae) from Laos and Vietnam. *Taiwania*, 61(3): 201–217.
- Averyanov L. V., Tanaka N., Nguyen K. S., Q. N. Nguyen, T. V. Maisak, T. H. Nguyen, 2017 a. New species of *Peliosanthes*, *Rohdea* and *Tupistra* (Asparagaceae) from Laos and Vietnam. *Nordic Journal of Botany*, 35: 697–710.
- Averyanov L. V., Tillich H. -J., Le T. A., Pham V. T., Maisak T. V., Vu T. C., 2017 b. *Aspidistra letreae* (Asparagaceae), a new species from central Vietnam. *Phytotaxa*, 308(1): 137–140.
- Averyanov L. V., Tanaka N., Son H. T., Nguyen K. S., Maisak T. V., Nguyen T. H., Peng C. I., 2018. *Tupistra cardinalis* (Asparagaceae), a new species from limestone areas in northern Vietnam. *Phytotaxa*, 334(1): 060–064.
- Averyanov L. V., Le T. A., Nguyen K. S., Tillich H. J., Nguyen D. D., Hoang L. T. A., Tran H. D., Dat P. T. T., Maisak T. V., 2019 a. *Aspidistra erosa*, *A. sarcantha*, and *A. verruculosa* (Asparagaceae), three new species from Vietnam. *Phytotaxa*, 404(3): 102–110.
- Averyanov L. V., Tanaka N., Nguyen K. S., Maisak T. V., 2019 b. A new species and a

- new combination in *Tupistra* (Asparagaceae). *Taiwania*, 64(3): 280–284.
- Averyanov L. V., Nguyen K. S., Nuraliev M. S., Vislobokov N. A., Tanaka N., Yury O. K. G., Lyskov D. F., Maisak T. V., Hieu N. Q., Kuznetsov A. N., Kuznetsova S. P., Thai T. H., 2020. *Tupistra nganii* (Asparagaceae), a new species with greenish yellow flowers from northern Vietnam and southwestern China. *Phytotaxa*, 449(2): 173–180.
- Averyanov L. V., Tanaka N., Nguyen K. S., Maisak T. V., Nuraliev M. S., Vislobokov N. A., Romanov M. S., Son H. T., 2021. New and noteworthy species of *Ophiopogon* and *Peliosanthes* (Asparagaceae) from Laos, Vietnam and Thailand. *Nordic Journal of Botany*, 2021: e03130. <https://doi.org/10.1111/njb.03130>
- Borah D., Taram M., Tangjang S., Upadhyaya A., Tanaka N., 2020. *Peliosanthes macrophylla* var. *assamensis* (Asparagaceae), a new variety from Behali Reserve Forest in Assam, Northeast India. *Blumea*, 65: 121–125.
- Bui Q. M., Schmidt H. A., Chernomor O., Schrepf D., Woodhams M. D., Haeseler A. V., Lanfear R., 2020. IQ-TREE 2: New models and efficient methods for phylogenetic inference in the genomic era. *Molecular Biology and Evolution*, 37(5): 1530–1534.
- Chase M. W., Reveal J. L., Fay M. F., 2009. A subfamilial classification for the expanded asparagalean families Amaryllidaceae, Asparagaceae and Xanthorrhoeaceae. *Botanical Journal of the Linnean Society*, 161: 132–136.
- Chen X. Q., Liang S. Y., Xu J. M., Boufford D. E., Gilbert M. G., Kamelin R. V., Kawano S., Koyama T., Mordak E. V., Noguchi J., Soukup V. G., Takahashi H., Tamanian K. G., Tamura M. N., Turland N. J., 2000. Liliaceae. In: Wu Z. Y., Raven P. H. (eds.) *Flora of China* 24. Science Press, Beijing & Missouri Botanical Garden Press, St. Louis, pp. 73–263.
- Conran J. G., 1989. Cladistic analyses of some net-veined *Liliitlorae*. *Plant Systematics and Evolution*, 168: 123–141.
- Conran J. G., Tamura M. N., 1998. Convallariaceae. In: Kubitzki K. (ed.) *The Families and Genera of Vascular Plants III. Flowering Plants, Monocotyledons, Liliaceae (except Orchidaceae)*. Springer-Verlag, Berlin, Heidelberg, pp. 186–198.
- Crawley S. S., Hilu K. W., 2012. Impact of missing data, gene choice, and taxon sampling on phylogenetic reconstruction: the Caryophyllales (angiosperms). *Plant Systematics and Evolution*, 298: 297–312.
- Cronquist A., 1981. *An integrated system of classification of flowering plants*. Columbia University Press, New York, USA, 1262 p.
- Dahlgren F., Bremner K., 1985. Major clades of Angiosperms. *Cladistics*, 1(4): 349–368.
- Ding H. B., Yang B., Zhou S. S., Maw M. B., Maung K. W., Tan Y. H., 2019. New contributions to the flora of Myanmar I. *Plant diversity*, 41(3): 135–152.
- Doyle J. J., Doyle J. L., 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemistry Bulletin*, 19: 11–15.
- Engler A., 1887. Liliaceae. In: Engler A., Prantl K. (eds.), *die Natürlichen Pflanzenfamilien*, II(5). Verlag von Wilhelm Engelmann, Leipzig, pp. 10–91.
- Fischer E., 2015. Magnoliopsida (Angiosperms) p.p.: Subclass Magnoliidae [Amborellanae to Magnolianae, Liliaceae p.p. (Acorales to Asparagales)]. In: Frey W. (ed.) *Syllabus of plant families*. Adolf Engler's *Syllabus der Pflanzenfamilien*, 13th ed., part 4. Borntraeger Science Publishers, Stuttgart, pp. 111–466.
- Floden A. J., 2017. *Molecular phylogenetic studies of the genera of tribe Polygonateae (Asparagaceae: Nolinoideae): Disporopsis, Heteropolygonatum, and Polygonatum*. PhD dissertation, University of Tennessee, Knoxville, Tennessee, USA, pp. 102.

- Floden A., Schilling E. E., 2018. Using phylogenomics to reconstruct phylogenetic relationships within tribe Polygonateae (Asparagaceae), with a special focus on *Polygonatum*. *Molecular Phylogenetics and Evolution*, 129: 202–213.
- Heath T. A., Hedtke S. M., Hillis D. M., 2008. Taxon sampling and the accuracy of phylogenetic analyses. *Journal of Systematics and Evolution*, 46(3): 239–257.
- Hoang D. T., Chernomor O., Haeseler A. V., Minh B. Q., Vinh L. S., 2018. UFBoot2: Improving the Ultrafast Bootstrap Approximation. *Molecular Biology and Evolution*, 35(2): 518–522.
- Hooker J. D., 1892. Flora of British India, 6. L. Reeve & Co., London, 792 pp.
- Jang C. G., Pfosser M., 2002. Phylogenetics of Ruscaceae sensu lato based on plastid *rbcL* and *trnL-F* DNA sequences. *Stapfia*, 80: 333–348.
- Ji Y. H., Landis J. B., Yang J., Wang S. Y., Zhou N., Lou Y., Liu H. Y., 2023. Phylogeny and evolution of Asparagaceae subfamily Nolinoideae: new insights from plastid phylogenomics. *Annals of Botany*, 131: 301–312.
- Kalyaanamoorthy S., Bui Q. M., Wong T. K. F., Haeseler A. V., Jermiin L. S., 2017. ModelFinder: Fast model selection for accurate phylogenetic estimates. *Nature Methods*, 14(6): 587–589.
- Katoh K., Standley D. M., 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution*, 30: 772–780.
- Kim J. H., Kim D. K., Forest F., Fay M. F., Chase M. W., 2010. Molecular phylogenetics of Ruscaceae sensu lato and related families (Asparagales) based on plastid and nuclear DNA sequences. *Annals of Botany*, 106: 775–790.
- Kim D. K., Kim J. S., Kim J. H., 2012. The Phylogenetic relationships of Asparagales in Korea based on five plastid DNA regions. *Journal of Plant Biology*, 55: 325–341.
- Kim C. K., Cameron K. M., Kim J. H., 2017. Molecular systematics and historical biogeography of *Maianthemum* s.s. *American Journal of Botany*, 104(6): 939–952.
- Krause K., 1930. Liliaceae. In: Engler A., Prantl K. (eds.) Die Natürlichen Pflanzenfamilien 15a. Engelmann, Leipzig, Germany, pp. 227–386.
- Li G. Z. (chief ed.), Lang K. Y., Wang R. X., Wei Y. G., Zhao D. Y., Tang S. Q., Li S., Li F. Y., Wang Y. G., Qi X. X., Tang W. X., Tang S. C., Qi S. H., Su H. L., 2004. The genus *Aspidistra*. Guangxi Science & Technology Publishing House, Nanning, Guangxi, China, pp. 229 (in Chinese).
- Lincoln R., Boxshall G. & Clark P., 1998. A Dictionary of Ecology, Evolution and Systematics, 2nd ed. Cambridge University Press, Cambridge, UK, pp. 361.
- Ly N. S., Hoang T. S., Nguyen K. S., Tanaka N., 2022. *Tupistra annamensis* (Asparagaceae), a new species from central Vietnam. *Phytotaxa*, 567(2): 173–180.
- Meng R., Luo L. Y., Zhang J. Y., Zhang D. G., Nie Z. L., Meng Y., 2021 a. The deep evolutionary relationships of the morphologically heterogeneous Nolinoideae (Asparagaceae) revealed by transcriptome data. *Frontiers in Plant Science*, 11: 584981. <http://dx.doi.org/10.3389/fpls.2020.584981>.
- Meng R., Meng Y., Yang Y. P., Nie Z. L., 2021 b. Phylogeny and biogeography of *Maianthemum* (Asparagaceae: Nolinoideae) revisited with emphasis on its divergence pattern in SW China. *Plant Diversity*, 43: 93–101.
- Nabhan A. R., Sarkar I. N., 2011. The impact of taxon sampling on phylogenetic inference: a review of two decades of controversy. *Briefings in Bioinformatics*, 13(1): 122–134.
- Nguyen K. S., Averyanov L. V., Tanaka N., Konstantinov E. L., Maisak T. V., Nguyen

- H. T., 2017. New taxa of *Peliosanthes* and *Tupistra* (Asparagaceae) in the flora of Laos and Vietnam and supplemental data for *T. patula*. *Phytotaxa*, 312 (2): 199–212.
- Nguyen K. S., Averyanov L. V., Tanaka N., Quang B. H., Hai D. V., Binh T. D., Qao Q., 2020. *Peliosanthes crassicoronata* (Asparagaceae), a new species from southern Vietnam. *Phytotaxa*, 429(1): 39–47.
- Nguyen K. S., Tanaka N., Averyanov L. V., Nguyen P. H., Tran D. B., 2021. *Rohdea dangii* (Asparagaceae), a new species from northwestern Vietnam. *Phytotaxa*, 482(1): 65–72.
- Nguyen T. D., 2007. Flora of Vietnam, Liliales Perleb, Vol. 8. Science and Technics Publishing House, Hanoi, 510 p (in Vietnamese).
- Planet P. J., 2006. Tree disagreement: Measuring and testing incongruence in phylogenies. *Journal of Biomedical Informatics*, 39: 86–102.
- Rambaut A., 2018. FigTree v.1.4.4. <http://tree.bio.ed.ac.uk/software/figtree/> accessed: 25/7/2023.
- Rambaut A., Drummond A. J., Xie D., Baele G., Suchard M. A., 2018. Posterior summarisation in Bayesian phylogenetics using Tracer 1.7. *Systematic Biology* 67(5): 901–904.
- Reveal J. L., 2012. An outline of a classification scheme for extant flowering plants. *Phytoneuron*, 2012-37: 1–221.
- Reveal J. L., Chase M. W., 2011. APG III: Bibliographical information and synonymy of Magnoliidae. *Phytotaxa*, 19: 71–134.
- Ronquist F., Teslenko M., Mark P. V. D., Ayres D. L., Darling A., Höhna S., Larget B., Liu L., Suchard M. A., Huelsenbeck J. P., 2012. MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology*, 61(3): 539–542.
- Rudall P. J., Conran J. G., Chase M. W., 2000. Systematics of Rusceae/Convallariaceae: a combined morphological and molecular investigation. *Botanical Journal of the Linnean Society*, 134: 73–92.
- Russo C. A. M., Aguiar B., Selvatti A. P., 2017. Selecting molecular markers for a specific phylogenetic problem. *MOJ Proteomics & Bioinformatics*, 6(3): 295–301.
- Seberg O., Petersen G., Davis J. I., Pires J. C., Stevenson D. W., Chase M. W., Fay M. F., Devey D. S., Jorgensen T., Sytsma K. J., Pillon Y., 2012. Phylogeny of the Asparagales based on three plastid and two mitochondrial genes. *American Journal of Botany*, 9: 875–889.
- Stevens P. F., 2001 onwards. Angiosperm phylogeny website. Version 14. <http://www.mobot.org/MOBOT/research/APweb/>, accessed: 25/7/2023.
- Taberlet P., Gielly L., Pautou G., Bouvet J., 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molecular Biology*, 17(5): 1105–1109.
- Takhtajan A., 2009. Flowering Plants. Springer, Dordrecht, Netherlands, 871 pp.
- Tamura M. N., Xu J. M., 2007. A new species of *Ophiopogon* (Asparagaceae) from Guangxi, China. *Acta Phytotaxonomica et Geobotanica*, 58(1): 39–41.
- Tanaka N., 1998. Taxonomic notes on *Ophiopogon* of South Asia I. *The Journal of Japanese Botany*, 73: 301–313.
- Tanaka N., 2000. Taxonomic notes on *Ophiopogon* of South Asia V. *The Journal of Japanese Botany*, 75: 69–79.
- Tanaka N., 2003 a. New combinations in *Rohdea* (Convallariaceae). *Novon*, 13(3): 329–333.
- Tanaka N., 2003 b. Inclusion of *Tricalistra* and *Gonioscypha muricata* in *Tupistra* (Convallariaceae). *Novon*, 13(3): 334–336.

- Tanaka N., 2010 a. A taxonomic revision of the genus *Rohdea* (Asparagaceae). *Makinoa, New Series*, 9: 1–54.
- Tanaka N., 2010 b. A taxonomic revision of the genus *Tupistra* (Asparagaceae). *Makinoa, New Series*, 9: 55–93.
- Tanaka N., 2018. Taxonomic revision of *Peliosanthes bakeri* and *P. violacea* (Asparagaceae), with description of two new species from Bangladesh and India. *Phytotaxa*, 356(1): 34–48.
- Tanaka N., Nguyen K. S., 2023. Nolinoideae (Asparagaceae) in APG III needs replacing with Convallarioideae. *Phytotaxa*, 583(3): 297–299.
- Tillich H.-J., 2023. 200 years *Aspidistra* (Asparagaceae), and now more than 200 species: a new comprehensive determination key, and an annotated bibliography of the genus. *Nordic Journal of Botany*, 2023(3): e03818. <https://doi.org/10.1111/njb.03818>
- Urantowka A. D., Krocak A., Mackiewicz P., 2017. The influence of molecular markers and methods on inferring the phylogenetic relationships between the representatives of the (parrots, Psittaciformes), determined on the basis of their complete mitochondrial genomes. *BMC Evolutionary Biology*, 17: 166. <https://doi.org/10.1186/s12862-017-1012-1>
- Wang F. T., Tang T. (eds.), 1978. Flora Reipublicae Popularis Sinicae 15. Science Press, Beijing, China, 280 pp (in Chinese with Latin addenda).
- Wang G. Y., Meng Y., Huang J. L., Yang Y. P., 2014. Molecular phylogeny of *Ophiopogon* (Asparagaceae) inferred from nuclear and plastid DNA sequences. *Systematic Botany*, 39: 776–784.
- Wang J. J., Yang Y. P., Sun H., Wen J., Deng T., Nie Z. L., Meng Y., 2016. The biogeographic south-north divide of *Polygonatum* (Asparagaceae tribe Polygonateae) within eastern Asia and its recent dispersals in the Northern Hemisphere. *PLoS One* 11:e0166134. <https://doi.org/10.1371/journal.pone.0166134>
- Wang J., Qian J., Jiang Y., Chen X. C., Zheng B. J., Chen S. L., Yang F. J., Xu Z. C., Duan B. Z., 2022. Comparative analysis of chloroplast genome and new insights into phylogenetic relationships of *Polygonatum* and tribe Polygonateae. *Frontiers in Plant Science*, 13: 882189. <http://dx.doi.org/10.3389/fpls.2022.882189>
- Wiens J. J., Tiu J., 2012. Highly incomplete taxa can rescue phylogenetic analyses from the negative impacts of limited taxon sampling. *PLoS ONE*, 7(8): e42925. <https://doi.org/10.1371/journal.pone.0042925>
- Zhang D., Gao F. L., Jakovlić I., Zou H., Zhang J., Li W. X., Wang G. T., 2020. PhyloSuite: An integrated and scalable desktop platform for streamlined molecular sequence data management and evolutionary phylogenetics studies. *Molecular Ecology Resources*, 20(1): 348–355.
- Zurawski G., Perrot B., Bottomley W., Whitfeld P. R., 1981. The structure of the gene for the large subunit of ribulose 1,5-bisphosphate carboxylase from spinach chloroplast DNA. *Nucleic Acids Research*, 9(14): 3251–3270.
- Zwickl D. J., Hillis D. M., 2002. Increased taxon sampling greatly reduces phylogenetic errors. *Systematic Biology*, 51(4): 588–598.

Appendix 1. List of species of *Convallarieae* and *Liriopeae* (*Asparagaceae*) from Vietnam examined, their voucher specimens and sources, and NCBI GenBank accession of newly sequenced samples in this study

Species	Voucher (Herbarium)	Collected place	GenBank accession	
			<i>rbcL</i>	<i>trnL-F</i>
<i>Aspidistra</i> Ker Gawler				
<i>A. alata</i> Tillich	NSK 1244 (HN)	Cao Bang	OQ680631	OQ658020
<i>A. anomala</i> Aver. & Tillich	CPC 1605a (LE)	Thanh Hoa	OQ680632	OQ658021
<i>A. atroviolacea</i> Tillich	BM 03 (HN)	Thue Thien Hue	OQ680633	OQ658022
<i>A. babensis</i> K. S. Nguyen, Aver. & Tillich	NSK 969a (HN)	Bac Kan	MN165130	MN153047
<i>A. bella</i> Aver., Tillich & K. S. Nguyen	NSK 862 (HN)	Ha Giang	OQ680634	OQ658024
<i>A. campanulata</i> Tillich	NSK 1227 (HN)	Tuyen Quang	OQ680635	OQ658025
<i>A. cryptantha</i> Tillich	NSK 1241 (HN)	Cao Bang	OQ680636	OQ658026
<i>A. cylindrica</i> Vislobokov & Nuraliev	AL 84 (LE)	Lam Dong	OQ680637	OQ658027
<i>A. erosa</i> Aver., Tillich, T. A. Le & K. S. Nguyen	NSK 1147 (HN)	Quang Binh	OQ680638	OQ658028
<i>A. hainanensis</i> W.Y.Chun & F. C. How	NSNL 01 (HN)	Hoa Binh	OQ680639	OQ658029
<i>A. letreae</i> Aver., Tillich & T. A. Le	ND 02	Hue	OQ680640	OQ658030
<i>A. lubae</i> Aver. & Tillich	ML 01 (HN)	Son La	OQ680641	OQ658031
<i>A. lutea</i> Tillich	CK 1711a (HN)	Hoa Binh	OQ680642	OQ658032
<i>A. minor</i> Vislobokov, Nuraliev & M. S. Romanov	GL 35 (HN)	Gia Lai	OQ680650	OQ658033
<i>A. papillata</i> G. Z. Li	HTS 637 (HN)	Lang Son	OQ680643	OQ658034
<i>A. phanluongii</i> Vislobokov	NSK 1350 (HN)	Dong Nai	OQ680644	OQ658035
<i>A. sarcantha</i> Aver., Tillich, T. A. Le & K. S. Nguyen	NSK 944a (HN)	Ha Tinh	OQ680645	OQ658036
<i>A. semiaperta</i> Aver. & Tillich	CPC 1566b (LE)	Hoa Binh	OQ680646	OQ658037
<i>A. superba</i> Tillich	NSK 1218 (HN)		OQ680647	OQ658038
<i>Ophiopogon</i> Ker Gawler				
<i>O. longifolius</i> Decne	BM 01 (HN)	Hue	OQ969134	OQ658040
<i>O. ogisui</i> M. N. Tamura & J. M. Xu	NSK 1243 (HN)	Cao Bang	OQ969135	OQ658041
<i>O. pierrei</i> L. Rodr.	LD 22 (HN)	Lam Dong	OQ969136	OQ658042
<i>O. tristylatus</i> Aver., N. Tanaka & Luu	LD 20 (HN)	Lam Dong	OQ969137	OQ658043
<i>O. sp. 1</i>	BM 02 (HN)	Hue	OQ969132	OQ658039
<i>Peliosanthes</i> Andrews				
<i>P. crassicornata</i> K. S. Nguyen, Aver. & N. Tanaka	NSK 964 (HN)	Gia Lai	MN263921	MN263920
<i>P. griffithii</i> var. <i>breviracemosa</i> Aver. & N. Tanaka	NSK 1271 (HN)	Cao Bang	OQ969138	OQ658045
<i>P. hexagona</i> Aver., N. Tanaka & K. S. Nguyen	NSK 1280 (HN)	Hoa Binh	OQ969140	OQ658047
<i>P. serrulata</i> L. Rodr.	NSK 1322 (HN)	Kien Giang	OQ969142	OQ658049
<i>P. teta</i> Andrews	NSK 1352 (HN)	Ha Noi	OQ969144	OQ658051
<i>P. yunnanensis</i> F. T. Wang & Tang	NSK 940 (HN)	Lao Cai	OQ969146	OQ658053
<i>P. sp. 1</i>	ND 01 (HN)	Hue	MZ476866	OQ658044
<i>P. sp. 2</i>	NSK 1279 (HN)	N. Vietnam	OQ969139	OQ658046
<i>P. sp. 3</i>	CK 1312 (HN)	Tuyen Quang	OQ969141	OQ658048
<i>P. sp. 4</i>	CK 1409 (HN)	Tuyen Quang	OQ969143	OQ658050
<i>P. sp. 5</i>	NSK 1324b (HN)	Kien Giang	OQ969145	OQ658052

Species	Voucher (Herbarium)	Collected place	GenBank accession	
			<i>rbcL</i>	<i>trnL-F</i>
Rohdea Roth				
<i>R. dangii</i> K. S. Nguyen, N. Tanaka & Aver.	NSK 1163 (HN, LE)	Son La	OQ969147	OQ658054
<i>R. filosa</i> Aver. & N. Tanaka	CPC 5299a (LE)	Cao Bang	OQ969148	OQ658055
<i>R. tonkinensis</i> (Baill.) N. Tanaka	NSK 1221 (HN)	Ha Noi	OQ969149	OQ658056
Tupistra Ker Gawler				
<i>T. cardinalis</i> Aver., N. Tanaka & T. S. Hoang	NSK 1246 (HN)	Cao Bang	OQ969150	OQ658057
<i>T. fungilliformis</i> F. T. Wang & S. Yun Liang	NSK 1203 (HN)	Ha Giang	OQ969151	OQ658058
<i>T. gracilis</i> Aver. & N. Tanaka	CPC 6721 (LE)	Thanh Hoa	OQ969152	OQ658059
<i>T. khangii</i> Aver., N. Tanaka & Vislobokov	CPC 7158 (LE)	Son La	OQ969153	OQ658060
<i>T. nganii</i> K. S. Nguyen, Aver., N. Tanaka & Nuraliev	NSK 1182 (HN)	Ha Giang	OQ969154	OQ658061
<i>T. nganii</i> K. S. Nguyen, Aver., N. Tanaka & Nuraliev	VR 1015 (HN, LE)	Ha Giang	OQ969155	OQ658062
<i>T. theana</i> Aver. & N. Tanaka	CPC 2581 (LE)	Quang Binh	OQ969156	OQ658063
<i>T. tripartita</i> Aver., N. Tanaka & K. S. Nguyen	NSK 1325 (HN)	Son La	OQ969157	OQ658064

Appendix 2. List of samples representing 42 species (Asparagaceae and *Disporum* of Liliaceae) from outside Vietnam used in this study, and information about their voucher specimens, sources and NCBI GenBank accession

Species	Voucher	Country of origin	GenBank accession	
			<i>rbcL</i>	<i>trnL-F</i>
<i>Disporum cantoniense</i> (Lour.) Merr.	J23	CN	NC_065360	NC_065360
<i>Asparagus officinalis</i> L.	JiY 2019084	Yunnan, CN	ON872702	ON872702
<i>A. schoberioides</i> Kunth	Kim 05-165	-	JF972888	KY909046
<i>Dracaena angustifolia</i> Roxb.	<i>s.n.</i>	Yunnan, CN	MN200193	MN200193
<i>D. fragrans</i> (L.) Ker Gawl.	<i>s.n.</i>	Hainan, CN	MW123093	MW123093
<i>Disporopsis aspersa</i> (Hua) Engl.	Li 22773	Yunnan, CN	EU850072	EU850172
<i>D. pernyi</i> (Hua) Diels	J22	CN	OL587681	OL587681
<i>Heteropolygonatum marmoratum</i> (H.Lév.) Floden	DNA3708	-	MH891735	MH891735
<i>H. ogisui</i> M. N. Tamura & J. M. Xu	X19026	CN	MZ150833	MZ150833
<i>Maianthemum bifolium</i> (L.) F. W. Schmidt	Wen 8530	Beijing, CN	EU850093	EU850197
<i>M. henryi</i> (Baker) LaFrankie	<i>s.n.</i>	CN	MW429372	MW429372
<i>Polygonatum cyrtoneura</i> Hua	Nie-Meng 203	Chongqing, CN	EU850071	EU850170
<i>P. odoratum</i> (Miller) Druce	LP197246	CN	MZ150859	MZ150859
<i>Ruscus aculeatus</i> L.	LiuC 2020049	Yunnan, CN	ON872723	ON872723
<i>Theropogon pallidus</i> Maxim.	Exp. 4213	Tibet, CN	ON872724	ON872724
<i>Beaucarnea recurvata</i> Lem.	Lou <i>s.n.</i>	Yunnan, CN	ON872730	ON872730
<i>Nolina atopocarpa</i> Bartlett	<i>s.n.</i>	-	KX931462	KX931462
<i>Convallaria majalis</i> L.	Liu M et al 598	Heilongjiang, CN	ON872704	ON872704
<i>C. majalis</i> L.	Nie 201	Heilongjiang, CN	KJ745528	EU850171
<i>Speirantha gardenii</i> Baill.	JiY 2019094	Yunnan, CN	ON872718	ON872718

Species	Voucher	Country of origin	GenBank accession	
			<i>rbcL</i>	<i>trnL-F</i>
<i>S. gardenii</i> Baill.	<i>s.n.</i>	Anhui, CN	ON872696	ON872696
<i>Aspidistra cavicola</i> D. Fang & K. C. Yen	<i>JiY 2019092</i>	Yunnan, CN	ON872717	ON872717
<i>Tupistra muricata</i> (Gagnep.) N. Tanaka	<i>13CS6063</i>	Laos	ON872699	ON872699
<i>Rohdea delavayi</i> (Franch.) N. Tanaka	<i>15CS10509</i>	Yunnan, CN	ON872710	ON872710
<i>R. watti</i> (C.B.Clarke) Yamashita & M. N. Tamura	<i>Zhangcq0026</i>	CN	JF941120	–
<i>R. watti</i> (C.B.Clarke) Yamashita & M. N. Tamura	<i>s.n.</i>	CN	MW822041	MW822041
<i>Reineckea carnea</i> Kunth	<i>AnH 2019112</i>	Yunnan, CN	ON872727	ON872727
<i>R. carnea</i> Kunth	<i>JiY 2019101</i>	Yunnan, CN	ON872715	ON872715
<i>Liriope graminifolia</i> Baker	<i>GY 34</i>	Guangdong, CN	KF671513	KF671374
<i>L. muscari</i> (Decne.) L. H. Bailey	<i>JiY 2019107</i>	Yunnan, CN	ON872721	ON872721
<i>Ophiopogon chingii</i> W. T. Wang & Tang	<i>Nie3739</i>	Yunnan, CN	KF671468	KF671329
<i>O. grandis</i> W.W. Sm.	<i>HGWZ469</i>	Hunan, CN	KF671466	KF671327
<i>O. japonicus</i> (Thunb.) Ker Gawl.	<i>GY44</i>	Guangdong, CN	KF671478	KF671339
<i>O. latifolius</i> L. Rodr.	<i>HGWZ518</i>	Yunnan, CN	KF671497	KF671359
<i>O. marmoratus</i> Pierre ex L. Rodr.	<i>HGWZ625</i>	Yunnan, CN	KF671487	KF671349
<i>O. peliosanthoides</i> W. T. Wang & Tang	<i>Nie3586</i>	Yunnan, CN	KF671507	KF671369
<i>O. pingbienensis</i> W. T. Wang & L. K. Dai	<i>Nie3927</i>	Yunnan, CN	KF671508	KF671370
<i>O. platyphyllus</i> Merr. ex Chun	<i>Nie2338</i>	Guangxi, CN	KF671498	KF671360
<i>O. reversus</i> C. C. Huang	<i>GY42</i>	Guangdong, CN	KF671474	KF671335
<i>O. sylvicola</i> W. T. Wang & Tang	<i>HGWZ00793</i>	Yunnan, CN	KF671494	KF671356
<i>O. szechuanensis</i> W. T. Wang & Tang	<i>HGWZ593</i>	Yunnan, CN	KF671486	KF671348
<i>Peliosanthes macrophylla</i> Wall. ex Baker	<i>Nie3242</i>	Yunnan, CN	KF671525	KF671387
<i>P. macrostegia</i> Hance	<i>Led9297</i>	Guangxi, CN	ON872701	ON872701
<i>P. ophiopogonoides</i> W. T. Wang & Tang	<i>HGWZ536</i>	Yunnan, CN	KF671526	KF671388
<i>P. sinica</i> W. T. Wang & Tang	<i>Nie3234</i>	Yunnan, CN	KF671522	KF671384
<i>P. yunnanensis</i> W. T. Wang & Tang	<i>Nie3724</i>	Yunnan, CN	KF671528	KF671390

Notes: “-” missing data; CN = China.