Article

Paraphyletic Species of Podostemaceae: *Cladopus fallax* and *Polypleurum wallichii*

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Abstract

All species of Podostemaceae grow only in rocky rapids and waterfalls and usually consist of separate populations. Previous studies found paraphyletic species among such species and suggested that possibly more species are paraphyletic. Molecular phylogenetic analyses and comparative morphology using samples from different populations found that *Cladopus fallax* C.Cusset and *Polypleurum wallichii* (R.Br. ex Griff.) Warm. are paraphyletic to *C. taiensis* C.Cusset and *P. schmidtianum* Warm., respectively. It is interpreted that the daughter species markedly differentiated morphologically from a population of the mother species, and perhaps paraphyletic speciation is associated with allopatric speciation. Molecular and morphological data indicate that *P. wallichii* is conspecific with *P. stylosum* (Wight) J.B.Hall, so the redefined *P. wallichii* is widely distributed in southern and southeastern Asia.

Key words: *Cladopus taiensis*, molecular (*matK*, ITS) phylogeny, *Polypleurum schmidtianum*, *Polypleurum stylosum* (*syn. nov.*)

Introduction

Species are monophyletic or paraphyletic in terms of phylogeny (Levin 1993; Rieseberg & Brouillet 1994; Gottlieb 2003; Coyne & Orr 2004). Crisp & Chandler (1996) provided comprehensive data on paraphyletic species, mainly based on cladistic analyses of morphological and molecular data (enzyme and DNA polymorphism). In an estimation by Crisp & Chandler (1996), 20−50% of plant species are paraphyletic, and in an estimation by Ross (2014), 19% of animal species are paraphyletic. Paraphyletic species can be produced when populations are reproductively isolated. In addition, there are metaphyletic species, which have unresolved (metaphyletic) relationships with monophyletic daughter species (Crisp & Chandler 1996).

Podostemaceae, or river-weeds, grow only in specialized habitats such as rocky waterfalls and rapids in fast currents in the seasonal tropics and subtropics. The plants adhere to rock surfaces, which are submerged during the rainy season, and then become exposed and flower in the dry season. The exposed plants wither and die while dispersing seeds from their capsules. They are annual, but perennial when submerged all year round. The habitats are narrow and far from each other over the distribution area. This scattered distribution of populations may promote local speciation resulting in paraphyletic species. Koi *et al.* (2015) enumerated eight paraphyletic species in five genera and paraphyletic African populations of one species (Table 1), but further unrecognized paraphyletic species are possible. Analysis of DNA sequences of specimens representing multiple populations will be able to provide exact phylogenetic data to discover paraphyletic species.

The genus *Cladopus* H.A.Möller is characterized by ribbon-like roots with reduced, rarely long shoots, digitate, rarely lobed or trifid bracts, one, rarely two stamens, and smooth-surfaced capsules (Kato 2006, 2018; Cook & Rutishauser 2007; Koi & Kato 2012). It is distributed in southeastern and eastern Asia, Malesia and Australia. Phylogenetically *Cladopus* with *Paracladopus* M.Kato is sister to *Hydrobryum* Endl. and allied *Hanseniella* C.Cusset, *Hydrodiscus* Koi & M. Kato and *Thawatchaia* M.Kato, Koi & Y. Kita (Koi *et al.* 2012; Koi & Kato 2012). *Cladopus fallax* C.Cusset is sister to *C. taiensis* C.Cusset, while *C. austrosinensis* M.Kato & Y.Kita is paraphyletic to *C. fukienensis* (H.C.Chao) H.C.Chao, and *C. pierrei* (Lecomte) C.Cusset to *C. doianus* (Koidz.) Koriba (Koi *et al.* 2015).

TABLE 1. Paraphyletic species of Podostemaceae.

Paraphyletic species	Daughter species	Source
Cladopus austrosinensis M.Kato & Y.Kita	*Cladopus fukienensis* (H.C.Chao) H.C.Chao	Koi *et al*. 2015
Cladopus fallax C.Cusset	*Cladopus taiensis* C.Cusset	present study
Cladopus pierrei (Lecomte) C.Cusset	*Cladopus doianus* (Koidz.) Koriba	Koi *et al*. 2015
Dicraeanthus africanus Engl.	*Dicraeanthus zehnderi* H.E.Hess	Koi *et al*. 2015
Hydrobryum japonicum Imamura	*Hydrobryum floribundum* Koriba	Koi *et al*. 2015
Hydrobryum vientianense (M.Kato & Fukuoka) Koi & M.Kato	*Hydrobryum loeicum* M.Kato	Koi *et al*. 2015
Podostemum distichum Wedd.	*Podostemum irgangii* C.T.Philbrick & Novelo	Koi *et al*. 2015
Polypleurum wallichii (R.Br. ex Griff.) Warm.	*Polypleurum schmidtianum* Warm.	present study
Terniopsis malayana (J.Dransf. & Whitmore) M.Kato	*Terniopsis australis* (C.Cusset & G.Cusset) M.Kato	Koi *et al*. 2015
Terniopsis savannaketensis Koi & M.Kato	*Terniopsis sessilis* (H.C.Chao) M.Kato	Koi *et al*. 2015
Old World populations of *Tristicha trifaria* (Willd.) Spreng.	New World populations of *Tristicha trifaria*	Koi *et al*. 2015

Polypleurum (Tul.) Warm. is characterized by the ribbon-like roots with reduced, rarely long shoots, simple bracts, one or two stamens and 8-ribbed capsules (Mathew & Satheesh 1997; Kato 2006, 2018; Cook & Rutishauser 2007; Koi & Kato 2012). It is distributed in southern and northeastern India, Sri Lanka and Southeast Asia. Based on morphological and phylogenetic data, the genus is divided into two groups; one group is characterized by the shoots borne on the flank of internodes of root branches and comprises *P. stylosum* (Wight) J.B.Hall, *P. wallichii* (R.Br. ex Griff.) Warm. and *P. schmidtianum* Warm. (Kato 2006, 2018; Koi *et al.* 2012). The species are phylogenetically unresolved, although *P. schmidtianum* is monophyletic. The other group is characterized by the shoots borne only in the sinuses of root branches, although *P. longistylosum* M.Kato is isolated from the rest of the group and has a similar root-shoot character with the first group.

To test whether there are additional paraphyletic species in *Cladopus* and *Polypleurum*, we performed molecular phylogenetic analysis and compared the morphology of *C. fallax* with *C. taiensis* and other allied species, and the *P. stylosum-P. wallichii* complex.

Material and Methods

Material

Materials used in molecular phylogenetic analysis were collected in northeastern India and Southeast Asia (Appendix; see also Results). In addition, we used published sequence data (Kita & Kato 2001, 2004; Koi *et al.* 2008, 2012; Koi & Kato 2010, 2012; Khanduri *et al*. 2015; Kato *et al.* 2017; Werukamkul *et al.* 2018). We examined the morphology of five or more samples per specimen in 46 specimens of five species of *Cladopus* and in 27 specimens of five species of *Polypleurum* (see footnotes of Tables 2, 3). Vouchers were deposited in the herbarium, National Museum of Nature and Science (TNS). We also used published morphological data of species of *Cladopus* (Kato 2006, 2009) and *Polypleurum* (Philcox 1996; Mathew & Satheesh 1997; Kato 2006; Koi & Kato 2012).

Morphological observations

The species of *Cladopus* and *Polypleurum* are characterized by a variety of characters in roots, shoots and flowers/ fruits, which were described in floristic and taxonomic studies (Willis 1902; Cusset 1992; Philcox 1996; Mathew & Satheesh 1997; Kato & Kita 2003; Kato 2006, 2009, 2013; Koi & Kato 2012). In this study, the characters of *Cladopus fallax* and *Polypleurum wallichii* were selected (see Tables 2, 3), based on floristic studies of Podostemaceae of China (Kato & Kita 2003), Thailand (Kato 2006) and Laos (Koi & Kato 2012) and Malesia (Kato 2009). Molecular data (e.g. Koi *et al*. 2012) were also used for identification.

TABLE 2. Character states in the *Cladopus fallax* clade.

Species*	Root (width; mm)	Flowering shoot (length; mm)	Bract (form)	Bract-segment (cross-sectional view)	Bract-segment (surface)	Number of bracts	Stamen [length; mm (relative length to ovary)]	Stigma (length; mm)	Ovule (number per ovary)	Capsule (length; mm)	Stalk of capsule (length; mm)
C. fallax-1	1–2	1–2	digitate	subterete	papillate	6–12	1.2–1.6 (1–1.5)	0.3–0.6	~50	0.9–1.2	1.2–3
C. fallax-2	2–2.5	≤1	digitate	subterete	papillate or moderately papillate	4–6	≤1.5 (≥1)	~0.3	20–40	1–1.2	≤1.7
C. fallax-3	2–3	1–1.5	digitate	subterete	papillate	6–8	1.3–1.5 (~1.3)	0.5–1	45–50	1–1.2	1–1.8
C. taiensis	2–2.5	≤1	3–4-lobed	semicircular and flattened	smooth	4(–6)	≤1.5 (~1)	0.3–0.5	35–46	1–1.2	≤2
C. nymanii	3–5	3–10	digitate	subterete	papillate	8–24	2–3 (1–2.5)	0.5–1	≤100	1–2	2–3
C. queenslandicus	1.5–2.5	30–90	3-lobed	middle segment much longer than lateral projections	smooth	20–50	2–3 (1–2.5)	~0.5	50–80	1.8–2.5	0.5–4.5
C. javanicus	2–4	30–70	digitate	subterete	moderately papillate	30–60	1.5–3 (~1)	1–1.5	~100	2–3	≤6

TABLE 3. Character states in the *Polypleurum wallichii* complex.

Species[*1]	Root (habit[*2])	Root (length [cm] × width [mm])	Bract (form)	Bract (number)	Pedicel (length; mm)	Stamen (number)	Ovary (length; mm)	Stigma (length; mm)	Ribs on capsule (number)	Stalk of capsule (length; mm)
P. wallichii-1	S type	7–10 × 4–7	ovate, apex acuminate	2–4	10.5	2	1.3–2.5	n.a.	8	6–21
P. wallichii-2	S type & W type	5–10 (sometimes shorter) × 2–7	ovate to lanceolate	2–4	5–13	2	1.2–3	0.3–1	8(–10)	4–18
P. wallichii-3	W type	≤5 × 4–7	ovate, apex subacute or acuminate	2–4	5–8	2	2–2.5	0.8–1.2	8	6–20
P. stylosum	S type	≤52 × 4–23	ovate, apex acute	2–4(–6)	2–20	2	1.5–3	0.8–1	8	1–45
P. schmidtianum	W type	≤3 × 1.2–4	lanceolate, ovate, apex linear	3–4(–6)	3–7	1	1.2–2	0.3–1	8–9	3–15
P. elongatum	S type	≤50–60 × 0.8–2.5	ovate	4(–6)	n.a.	2	~2	~1	8	7–12
P. munnarense	W type	≤5 × 5–8	ovate, apex subacute or acute	2	3.5–8	2	2–3	0.8–1.3	8	3–9

[*1] *P. wallichii*-1 = specimens CAM-03, 11 (from southwestern Cambodia); *P. wallichii*-2 = TPK-114, 122; TL-55, 601, 1902; CP-07, 22, 24, 27, 29 (Thailand), L-03, 508; LK-09, 127, 238 (Laos) and specimens of Thailand (Kato 2006); *P. wallichii*-3 = IND-1431, 1431A, 1503, 1504, 1505 (northeastern India); *P. stylosum* = specimens from southern India and Sri Lanka (Appendix), specimens deposited at TNS, and data of Mathew & Satheesh (1997); *P. schmidtianum* = CAM-05, 06, 11, 12, 16, 17, 19b (southwestern Cambodia) and specimens given in Appendix (Koi *et al.* 2012); *P. elongatum* = SL-12 (Sri Lanka, Koi *et al.* 2012) and specimens deposited in TNS; *P. munnarense* = Cu-90004, 90007; IN-1205 (southern India).

[*2] S type = floating but adhering at base; W type = adhering the full length.

DNA extraction, amplification and sequencing

DNA extraction, PCR amplification and sequencing of the chloroplast *matK* gene followed the methods of Koi *et al*. (2012). The methods of the analyses for internal transcribed spacer (ITS) regions of the nuclear ribosomal RNA (nrRNA) gene (including ITS1, the 5.8S rRNA gene, and ITS2) also followed Kita & Kato (2004). ITS analysis was conducted to examine a hybridization possibility that is suggested by chloroplastic data (Kita & Kato 2004) and used selected samples.

Phylogenetic analysis

The sequences obtained in this study and those deposited in GenBank were used for phylogenetic analysis (Appendix). In the *matK* analyses, the sequences were aligned by CLUSTAL X (Thompson *et al*. 1997) and refined manually with Mesquite 3.31 (Maddison & Maddison 2017). Gaps were treated as missing data. The program MrModeltest 2.3 (Nylander 2004) determined a general time reversible (GTR) + G (shape parameter of the gamma distribution) + I (proportion of invariable sites) substitution model as the best fitting model of substitution. In the former analysis, nucleotide frequencies were A = 0.3329, C = 0.1436, G = 0.1255, T = 0.3980; the substitution rate matrix was A to C = 1.1549, A to G = 1.0643, A to T = 0.2151, C to G = 0.4735, C to T = 0.9508, G to T = 1.0000; the proportion of invariable sites was 0.3124; and the gamma distribution shape parameter was 1.0733. In the Bayesian analysis, Markov chain Monte Carlo (MCMC) iterations with four chains were conducted under the GTR + G + I model for 2,000,000 generations, sampling the trees every 100 generations, with the program MrBayes 3.2.5 (Ronquist *et al*. 2012). Potential scale reduction factors (PSRF) of all parameters approached 1.000 or 1.001 as runs converge. The first 5,000 trees were discarded as burn-in and the remaining 15,000 trees were used to determine the posterior probabilities for branches. Maximum likelihood (ML) and maximum parsimony (MP) analyses were conducted using the program PAUP* Version 4.0a159 (Swofford 2002). In ML analyses, heuristic searches were conducted with 100 random addition replicates involving tree-bisection-reconnection (TBR) branch swapping. Bootstrap values were calculated for 1,000 replicates with 10 random addition replicates involving nearest-neighbor-interchange (NNI) branch swapping, and 'MulTrees' option was not in effect. In MP analyses, all characters were equally weighted, and heuristic searches were conducted with 100 random addition replicates involving TBR branch swapping. A strict consensus tree was built with the best-scored trees obtained. Bootstrap values were calculated for 1,000 replicates with 10 random addition replicates involving NNI branch swapping, and 'MulTrees' option was not in effect. Based on the phylogeny of Koi *et al*. (2012), a *matK* tree was rooted on a branch between the clade of *Cladopus*, *Paracladopus*, *Hanseniella*, *Hydrobryum*, *Hydrodiscus* and *Thawatchaia* and the clade of *Farmeria* Willis ex Hook.f., *Griffithella* (Tul.) Warm., *Hydrobryopsis* Engl., *Polypleurum*, *Willisia* Warm. and *Zeylanidium* Engl. (Appendix).

Analyses of ITS sequences were conducted with the same software described above. The regions aligned ambiguously were removed and gaps were treated as missing data. A GTR + G + I substitution model was selected as the best fitting model of substitution: nucleotide frequencies were A = 0.3320, C = 0.1496, G = 0.1326, T = 0.3858; the substitution rate matrix was A to C = 0.9481, A to G = 1.1018, A to T = 0.1215, C to G = 0.2571, C to T = 0.7550, G to T = 1.0000; the proportion of invariable sites was 0.4628. The gamma distribution shape parameter was 0.9746. In the Bayesian analysis, MCMC iterations with four chains were conducted under the GTR + G + I model with the same conditions as above. Potential scale reduction factors (PSRF) of all parameters approached 1.000 as runs converged. The first 5,000 trees were discarded as burn-in and the remaining 15,000 trees were used to determine the posterior probabilities for the branches. In the ML analysis, heuristic searches were conducted with 100 random addition replicates involving NNI branch swapping. Bootstrap values were calculated for 1,000 replicates with 10 random addition replicates involving NNI branch swapping. The 'MulTrees' option was not in effect. In the MP analyses, all characters were equally weighted and heuristic searches were conducted with 100 random addition replicates involving TBR branch swapping. A strict consensus tree was built with the best-scored trees obtained. Bootstrap values were calculated for 1,000 replicates with 10 random addition replicates involving NNI branch swapping. Based on the phylogeny of Koi *et al*. (2012), an ITS tree was rooted on a branch between the clade of *Cladopus*, *Hanseniella* and *Hydrobryum* and the clade of *Griffithella*, *Hydrobryopsis*, *Polypleurum*, *Willisia* and *Zeylanidium* (Appendix).

Analyses of concatenated *matK* and ITS sequences were conducted with the specimens, of which both sequences were analyzed. A GTR + G + I substitution model was selected as the best fitting model of substitution: nucleotide frequencies were A = 0.2976, C = 0.1996, G = 0.1728, T = 0.3300; the substitution rate matrix was A to C = 1.0934, A to G = 1.2114, A to T = 0.5534, C to G = 0.6601, C to T = 1.6968, G to T = 1.0000; the proportion of invariable sites was 0.4239; and the gamma distribution shape parameter was 0.6902. In the Bayesian analysis, MCMC iterations with four chains were conducted under the GTR + G + I model with the same condition above. Potential scale reduction factors (PSRF) of all parameters approached 1.000 to 1.002 as runs converge. The first 5,000 trees were discarded as

burn-in and the remaining 15,000 trees were used to determine the posterior probabilities for branches. In ML analysis, heuristic searches were conducted with 100 random addition replicates involving TBR branch swapping. Bootstrap values were calculated for 1,000 replicates with 10 random addition replicates involving NNI branch swapping, and 'MulTrees' option was not in effect. In MP analyses, all characters were equally weighted, and heuristic searches were conducted with 100 random addition replicates involving TBR branch swapping. Strict consensus tree was built with the best-scored trees obtained. Bootstrap values were calculated for 1,000 replicates with 10 random addition replicates involving TBR branch swapping. A combined *matK* and ITS tree is rooted on a branch between *Polypleurum* and a clade of *Cladopus*, *Hanseniella* and *Hydrobryum* (Koi *et al.* 2012).

Results

The *matK* analyses examined 90 samples, and the length of the matrix consisted of 1,527 base pairs (including gaps and excluding ambiguously aligned regions). Of these, 954 characters were constant and 573 were variable, of which 313 characters were parsimony informative. The ITS analyses examined 77 samples, and the length of the matrix consisted of 592 base pairs (including gaps and excluding ambiguously aligned regions). Of these, 270 characters were constant and 322 were variable, of which 282 characters were parsimony informative. The analyses of concatenated *matK* and ITS sequences examined 46 samples, and the length of the combined matrix consisted of 2,119 base pairs (including gaps and excluding ambiguously aligned regions). Of these, 1,494 characters were constant and 625 were variable, of which 479 characters were parsimony informative.

Cladopus fallax
Cladopus fallax was distinguished from other congeners by combined characters. It differs from *C. taiensis* in the form (surface-viewed and in cross section), surface and number of the bract-segments, from *C. nymanii* H.Möller in the length of the stamens and the number of ovules, from *C. queenslandicus* (Domin) C.D.K.Cook & Rutish. in the length of the flowering shoots and the number and form of the bracts, and from *C. javanicus* M.Kato & Hambali in the length of the flowering shoots, the number of the bracts, and the length of the capsules and capsule stalks (Table 2).

In the chloroplast *matK* tree (Fig. 1), *Cladopus* was divided into two clades with robust support. One clade (upper in Fig. 1) was subdivided into three subclades, i.e. a subclade consisting of *C. fallax*-1 from Cambodia, *C. fallax*-2 from Thailand and *C. taiensis*; a subclade of *C. javanicus*, *C. nymanii* and *C. queenslandicus*; and *C. fallax*-3 from Cambodia. *Cladopus fallax*-1 had the same sequence as the Thai specimens of *C. taiensis* (TL-101, TL-102, TL-604), the two were sister to the other *C. taiensis* from Cambodia and Thailand, and all were sister to *C. fallax*-2. Geographically, *C. fallax*-1 is adjacent to *C. taiensis* (CAM-07, CAM-14) of Cambodia (4.2 or 12.2 km apart) and far from the Thai populations.

The nuclear ITS tree (Fig. 2) showed that there are variations in the ITS regions of *C. fallax* CAM-13, *C. fallax* CAM-19 and *C. taiensis* CAM-14, while uniform in others (e.g. *C. fallax* CAM-26, *C. fallax* CAM-41, *C. fallax* TKF-109, *C. taiensis* TL-604). The variants of CAM-13 and CAM-19 of *C. fallax*-1, and CAM-14 of *C. taiensis* were grouped in each clade, although one *C. fallax* CAM-13 was isolated. These samples of *C. fallax*-1 and *C. taiensis* formed a monophyletic clade, with low support, which was sister to *C. fallax*-2 and together sister to *C. fallax*-3.

In the combined *matK* and ITS tree, *C. fallax*-1 and *C. taiensis* were monophyletic and sister to *C. fallax*-2 (Fig. 3). Then, *C. fallax*-1, *C. fallax*-2, *C. fallax*-3 and *C. taiensis*, together with *C. javanicus*, were monophyletic.

Polypleurum wallichii
In the *Polypleurum wallichii* group, *P. stylosum* differs from *P. wallichii* in the root habit, the length and width of the root and the length of the pedicel and stalk of the capsule, although the ranges of variation in the characters are similar (Table 3; Fig. 4). *Polypleurum elongatum* (Gardner) J.B.Hall differs from the two species by the root being long, adhering only at the base and floating nearly its full length. *Polypleurum schmidtianum* differs from *P. wallichii* in one stamen (versus 2) and indistinctly in the root being relatively short and narrow and adhered to the rock for its full length, the pedicel short, in which the ranges of variations overlap to some extents.

In the *matK* phylogenetic tree (Fig. 1), *Polypleurum wallichii*, *P. stylosum*, *P. elongatum* and *P. schmidtianum* were monophyletic and sister to *P. munnarense* Nagendran & Arekal, although the relationships within the clade were not well resolved. *Polypleurum wallichii* was divided into three subgroups. *Polypleurum wallichii*-1 and *P. wallichii*-3 with *P. stylosum* KI-109 each were robustly monophyletic, whereas *P. wallichii*-2 was an unresolved subgroup.

Polypleurum wallichii-2 was defined by its distribution in Laos and Thailand (while *P. wallichii*-1 and 3 occur in Cambodia and India, respectively). The three subgroups were morphologically variable and inseparable (Table 3).

Polypleurum wallichii-1 (from one site in Cambodia [Koh Kong Province]) was sister to *P. schmidtianum* (Fig. 1). Geographically, the *P. wallichii*-1 plants (CAM-03, CAM-11) were sympatric with CAM-05 and CAM-12 of *P. schmidtianum*, and they grew in adjacent subpopulations in the same habitat. *Polypleurum wallichii*-2 comprised specimens from eastern and central Thailand and northern central Laos. *Polypleurum wallichii*-3 comprised specimens from Meghalaya, northeastern India, and had the same sequences as southern Indian KI-109. *Polypleurum stylosum* (specimens of which were collected from southern India and Sri Lanka) was also not monophyletic and divided into several subclades, of which Cu-90003, IND-1401 and IND-1413 were monophyletic, with low support, with *P. wallichii*-3 and KI-109 of *P. stylosum*, and as well as *P. elongatum*.
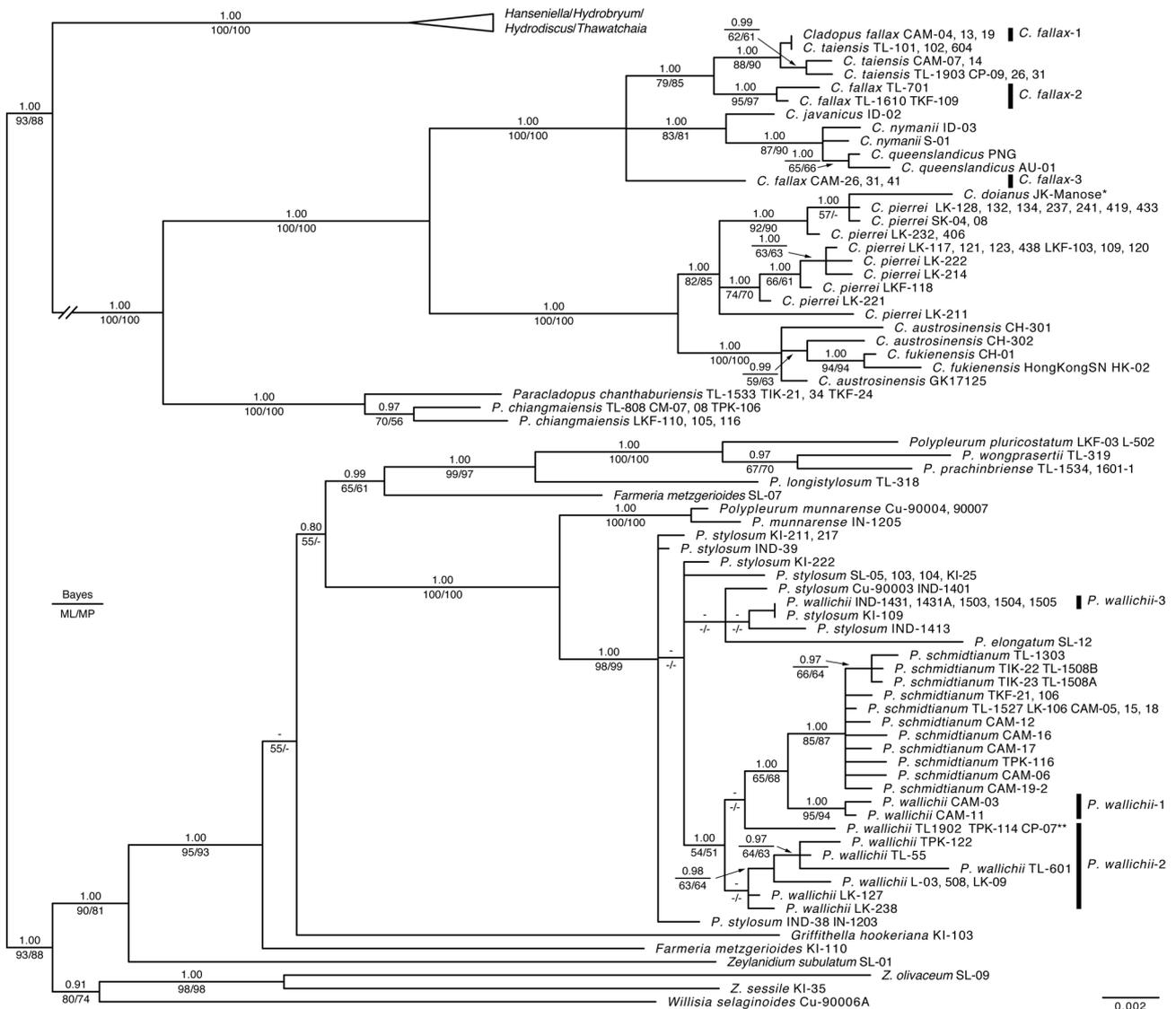

FIGURE 1. Bayes phylogenetic consensus tree deduced from *matK* sequences. Numbers above and below branches are Bayesian posterior probabilities (≥0.80) and bootstrap values (≥50 %) of ML (left) and MP (right), respectively. Single asterisk (*) indicates that specimen JK-Manose is followed by additional specimens with the same sequence, JK-02, JK-Yamazaki, JK-Anraku, JP-127, JK-Mawatari, and CH-02. Double asterisk (**) indicates that specimen CP-07 is followed by additional specimens with the same sequence, CP-22, 24, 27, and 29. For simplicity, species of *Hanseniella*, *Hydrobryum*, *Hydrodiscus* and *Thawatchaia* examined are united as a single clade (for materials see Appendix). The tree is rooted on a branch between a clade of *Cladopus*, *Hanseniella*, *Hydrobryum*, *Hydrodiscus*, *Paracladopus* and *Thawatchaia*, and a clade of *Polypleurum*, *Griffithella*, *Hydrobryopsis*, *Willisia* and *Zeylanidium*.
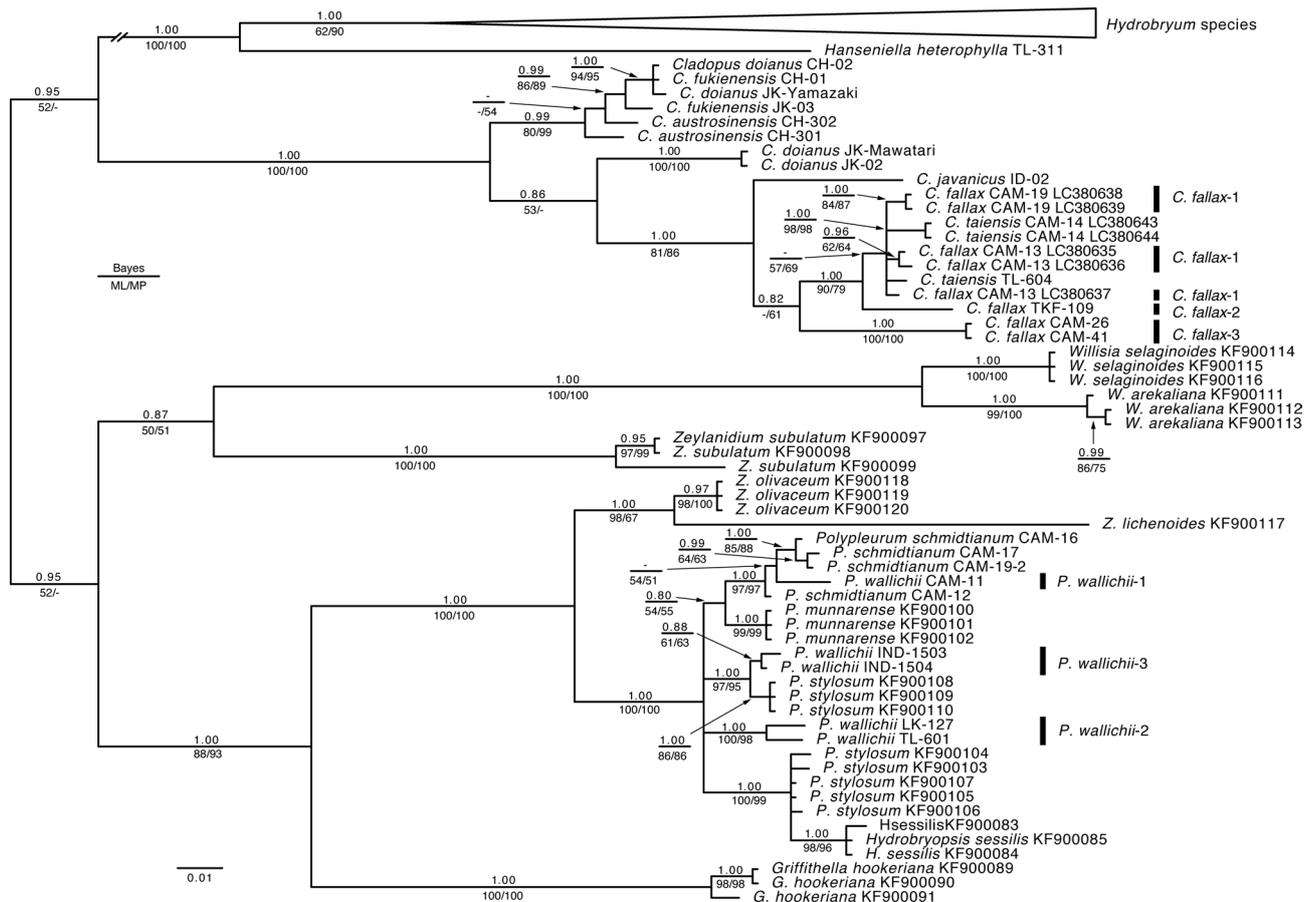
FIGURE 2. Bayes phylogenetic consensus tree deduced from ITS sequences. Numbers above and below branches are Bayesian posterior probabilities (≥0.80) and bootstrap values (≥50 %) of ML (left) and MP (right), respectively. For simplicity, species of *Hydrobryum* examined are united as '*Hydrobryum* species' (see Appendix). The tree is rooted on a branch between a clade of *Cladopus*, *Hanseniella* and *Hydrobryum* and a clade of *Polypleurum*, *Griffithella*, *Hydrobryopsis*, *Willisia* and *Zeylanidium*.

The relationships deduced from the ITS sequences, like the *matK* tree, showed that *P. wallichii*-1 and *P. schmidtianum* were monophyletic (Fig. 2). This *P. wallichii*-1 clade, *P. wallichii*-2 and *P. wallichii*-3 were separated from each other. As a whole, *P. schmidtianum*, *P. stylosum* and *P. wallichii*, together with *P. munnarense* and *Hydrobryopsis sessilis* (Willis) Engl., formed an unresolved complex.

In the combined *matK* and ITS tree, *P. wallichii*-1 and *P. schmidtianum* were monophyletic (Fig. 3). The clade was sister to *P. wallichii*-2 and both were sister to *P. wallichii*-3.

Discussion

Paraphyly of *Cladopus fallax*

From morphological comparison with ample specimens (i.e. in the number, form and surface of bract-segments)., we estimate that *C. fallax* and *C. taiensis* are different species. Considering this, results of the phylogenetic analysis indicate that *C. fallax* is paraphyletic to *C. taiensis*, with Cambodian *C. fallax*-1 sharing the sequences with Thai plants of *C. taiensis*. The populations are allopatric throughout the distributional area and the closest populations of *C. fallax* and *C. taiensis* are 4.2 km apart in Cambodia. It is suggested that paraphyletic speciation is associated with allopatric speciation, or a daughter species is derived from one of the populations of a mother species. As stressed by Kruckenhauser *et al.* (2014) and speculated for Podostemaceae (Kita & Kato 2004; Cheek *et al.* 2017), it might be possible that paraphyly in *Cladopus* is due to hybridization/introgression, because of the shared chloroplast sequences. The hybridization hypothesis of Cheek *et al.* (2017) was based on morphological data, and that of Kita & Kato (2004)

used molecular data, which may be consistent with chasmogamic as well as cleistogamic flowers of *Cladopus doianus* (M. Kato unpubl. data). On the contrary, pairs of ITS sequences in each sample did not present any sign of hybridization. The present paraphyletic interpretation, together with that of the *Polypleurum wallichii-P. stylosum* group noted below, should be tested by further analysis using more datasets (e.g. Katayama *et al.* 2016).

In the *Cladopus nymanii* subclade, *C. javanicus* is sister to the monophyletic group of *C. nymanii* and *C. queenslandicus*. *Cladopus javanicus* and *C. queenslandicus* are remarkable in their long flowering shoots with many bracts, but differ in the form of bracts (Table 2). By contrast, *C. nymanii* agrees in the short shoot with digitate/lobed bracts with, or slightly differs from, *C. fallax* and *C. taiensis* (Table 2) and also species of the other *C. pierrei* clade (Kato & Kita 2003; Kato 2008; Koi & Kato 2012). Because of such phylogenetic relationships of *C. javanicus* and *C. queenslandicus* within *Cladopus*, and comparative morphology that *C. nymanii*, like many other congeners, retains plesiomorphic characters, *C. javanicus* and *C. queenslandicus* may be products of saltational evolution. In contrast, perhaps due to subtle differences, Cusset (1992) synonymized *C. fallax* with *C. nymanii* in a very broad sense, including local species of China and Japan together, although *C. nymanii* and allies are placed in the other *C. pierrei* clade (Koi *et al.* 2012; present study). The phylogeny of *C. fallax* and the Malesian and Oceanian species requires further analysis.
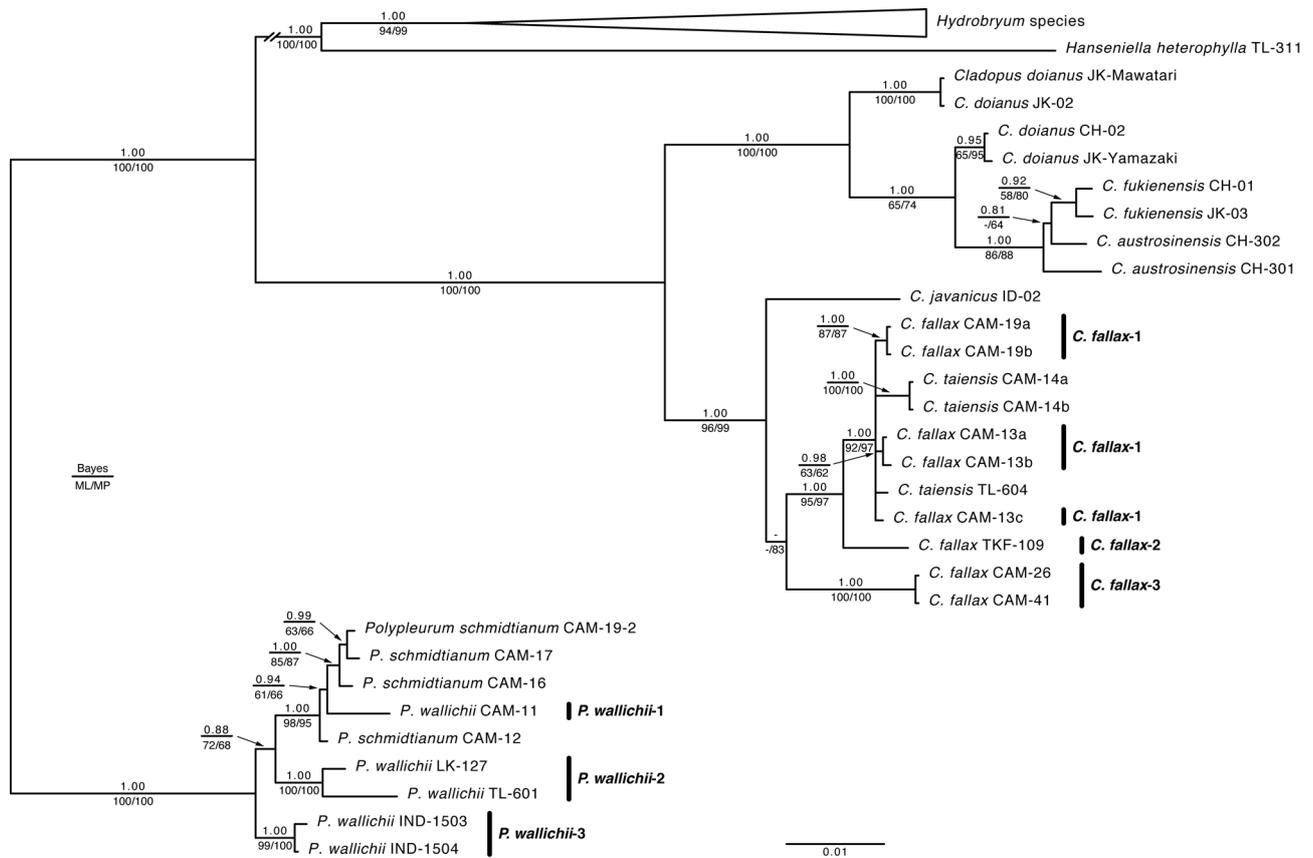

FIGURE 3. Bayes phylogenetic consensus tree deduced from concatenated *matK* and ITS sequences. Numbers above and below branches are Bayesian posterior probabilities (≥0.80) and bootstrap values (≥50 %) of ML (left) and MP (right), respectively. For simplicity, the species of *Hydrobryum*, both *matK* and ITS sequences of which were examined, are united as '*Hydrobryum* species' (Appendix). *C. fallax* CAM-13a = LC380594 + LC380635; *C. fallax* CAM-13b = LC380594 + LC380636; *C. fallax* CAM-13c = LC380594 + LC380637; *C. fallax* CAM-19a = LC380595 + LC380638; *C. fallax* CAM-19b = LC380595 + LC380639; *C. taiensis* CAM-14a = LC380602 + LC380643; *C. fallax* CAM-14b = LC380602 + LC380644. The tree is rooted on a branch between a clade of *Cladopus*, *Hanseniella* and *Hydrobryum*, and *Polypleurum*.

Paraphyly of *Polypleurum wallichii*

The previous *matK* analysis (Koi *et al.* 2012) showed that *Polypleurum stylosum*, *P. wallichii* and *P. schmidtianum*, along with *P. elongatum* and *P. munnarense*, form a monophyletic clade. The monophyly of this group agrees with the morphological consistency in the flowering and vegetative shoots borne between the nodes of the root branches (Willis 1902; Philcox 1996; Mathew & Satheesh 1997; Kato 2006; Koi & Kato 2012; Koi *et al.* 2012). All previous morphology-based classifications have recognized these five species (Willis 1902; Cusset 1992; Philcox 1996; Mathew & Satheesh 1997).

FIGURE 4. Root habits of *Polypleurum*. Roots are reproductive (with flowers or fruits borne on flanks, indicating root habit; vegetative in A) and exposed in the dry season. A, B, E, F, adhering roots (W type); C, D, floating roots (S type). A−C. *P. wallichii* (A = *P. wallichii*-2, Khao Yai National Park, Thailand; B = *P. wallichii*-3, Meghalaya, India; C = *P. wallichii*-1, Koh Kong, Cambodia). D, E. *P. stylosum* (D, E, Kerala, India). F. Dried-up root remains of *P. schmidtianum* (at top, light brown) with those of *Cladopus fallax* (at bottom and right, white gray) in Koh Kong, Cambodia.

Based on current knowledge, *Polypleurum wallichii* ranges from northeastern India (Assam and Meghalaya) to Myanmar, Thailand, Laos and Cambodia (Cusset 1992; Kato 2011). It was also recorded from southern India (Raveendran & Mathew 1994). *Polypleurum stylosum* is in southern India and Sri Lanka (Cusset 1992), but Rao & Hajra (1979) reported it from northeastern India. Among the other allies, *P. schmidtianum* occurs in southeastern and eastern Thailand, southwestern Cambodia and central Laos, but not in India. *Polypleurum elongatum* is endemic to Sri

Lanka, and *P. munnarense* is endemic to Kerala, southern India. The distributions of these two species are included in the range of *P. stylosum*. Thus, the group shows a north-south pattern of distribution in Asia (Koi *et al*. 2012; Kato 2013).

In the *matK* phylogenetic relationships, a Cambodian population of *P. wallichii* is sister to *P. schmidtianum*, and in the relationships of ITS and combined *matK* and ITS sequences, the two are close, whereas the other plants of *P. wallichii* are far from them. The consistency of the chloroplast and nuclear DNA phylogenies and absence of variations in the ITS region do not provide evidence for hybridization of *P. schmidtianum* and *P. wallichii*. Most likely, *P. schmidtianum* was derived from a local (possibly Cambodian) population of *P. wallichii* and extended to neighboring Thailand and Laos. It may be possible that *P. elongatum* is also derived from part of *P. wallichii* (= *P. stylosum*), which is revised below.

Polypleurum wallichii and *P. schmidtianum* are sympatric and grow in neighboring subpopulations in one waterfall in southwestern Cambodia (M. Kato unpubl. observation), whereas they are allopatric in Thailand (Kato 2006) and Laos (Koi & Kato 2012). These patterns of distribution and phylogeny can be interpreted to be a result of sympatric or allopatric speciation. In a scenario of sympatric speciation, *P. schmidtianum* was derived from a co-existing subpopulation of *P. wallichii*-1 (in Koh Kong, southwestern Cambodia) and extended to the surrounding areas. *Polypleurum wallichii* is facultatively autogamous (Okada & Kato 2002) and its populations may consist of isolated subpopulations, one of which possibly differentiated into *P. schmidtianum*. In an allopatric scenario, *P. schmidtianum* became secondarily sympatric during extension into Southeast Asia. This is less parsimonious than the sympatric scenario. In either case, the daughter *P. schmidtianum* is adapted to a corresponding environment as the mother *P. wallichii*.

In summary, *Cladopus fallax* and *Polypleurum wallichii* are added to the paraphyletic species of Podostemaceae (Table 1). Among them, the morphological differences between the parental species and derived species examined are clear in the bracts and in the length of roots and the number of stamens. The species consist of far separate populations. Gene flow between populations may be limited, as found for *Hydrobryum japonicum* Imamura (Katayama *et al*. 2016). It is interpreted that part of the populations differentiated into daughter species, accompanied with prominent phenotypic changes, while the other populations remained little changed. Nonetheless, speciation took place in quite similar habitats. Paraphyletic species may be the result of allopatric speciation.

Taxonomic revision of *Polypleurum wallichii*

Polypleurum stylosum is inseparable from *P. wallichii* by the sequence data. Specimen *IND-1503* collected from the type locality of *P. wallichii* (Mawsmai, Cherrapunji, Meghalaya) and specimens from Quinine north of it (Meghalaya) had the same *matK* sequences as the specimen *KI-109* from Kerala, southern India, and they together merge within *P. stylosum*. The nuclear ITS data also shows a close affinity of *P. wallichii*-3 and a specimen of *P. stylosum*. These molecular data do not support the taxonomic separation of *P. wallichii* and *P. stylosum*.

Previous morphology-based classifications have recognized *P. wallichii* and *P. stylosum* (Willis 1902; Cusset 1992; Philcox 1996; Mathew & Satheesh 1997). Rao & Hajra (1979) recorded *P. stylosum* from northeastern India. On the contrary, Raveendran & Mathew (1994) reported *P. wallichii* as occurring in southern India (Kerala) and distinguished it from *P. stylosum* mainly by the root habit, which is a diagnostic character of the species. The root of *P. stylosum* is up to 40 (or 52) cm long and attached at the base, distally free (this habit is called S type), while the root of *P. wallichii* is usually less than 10 cm long and adheres along the full length or in the proximal part (W type) (Raveendran & Mathew 1994). The roots of plants examined of *P. wallichii* from northeastern India (*P. wallichii*-3) are of W type, while those of plants from Thailand (Kato 2006) and Laos (Koi & Kato 2012) are of W type or occasionally S type (*P. wallichii*-2), and those from Cambodia (*P. wallichii*-1) are of S type (Table 3). The two root habits transition in Southeast Asian plants. Furthermore, the roots of some southern Indian plants are of W type (Raveendran & Mathew 1994), while others are of S type. Thus, *P. wallichii* and *P. stylosum* are not clearly separable by root habit. Rao & Hajra (1979) described the young root of *P. stylosum* to be "firmly attached to the rock all along the ventral surface," while the old root is "attached only at base." The difference in root habit seems ontogenetic and heterochronic, while the two root habits are distributed at different frequencies in the distribution areas.

Although Cusset (1992) described that the roots and pedicels of *P. stylosum* are longer than those of *P. wallichii*, in this study it is difficult to clearly separate them by these characteristics and others (Table 3). Based on the morphological and molecular data, we conclude that *P. wallichii* is conspecific with *P. stylosum*. The redefined species is the most widely distributed of Asian Podostemaceae. *Polyplerum stylosum* is referred to *P. wallichii* var. *wallichii* of the two varieties (Kato 2006).

Polypleurum wallichii (R.Br. ex Griff.) Warm. var. *wallichii*, Warm., Dansk Vidensk. Selsk. Skrift. Ser. 6, Nat. Math. 11: 15. 1901; Cusset, Bull. Mus. Natl. Hist. Nat. Paris, 4ᵉ sér. 14: 42, f. 8. 1992; Kato, Acta Phytotax. Geobot. 57: 36, f. 14. 2006; Fl. Thai. 14(1): 97. 2018; Koi & Kato, Kew Bull. 67: 338, f. 3. 2012 ≡ *Podostemum wallichii* R.Br. & Griff., Asiat. Res. 19: 103, tab. 17. 1836. Type: prope Moosmai et Mamloo, Cherrapunji, Meghalaya, India, *Griffith s.n.* (K!).

= *Polypleurum stylosum* (Wight) J.B.Hall, Kew Bull. 26: 131. 1971; Cusset, Bull. Mus. Natl. Hist. Nat. Paris, 4ᵉ sér. 14: 39, f. 7. 1992; Mathew & Satheesh, Aquat. Bot. 57: 257, f. 9−13. 1997 ≡ *Dicraeia stylosa* Wight, Icon. Pl. Ind. Orient. 5: 33, tab. 1917, II. 1852. Type: Malabar, India, *Johnson s.n.* (K, not seen). **Syn. nov.**

Distribution. Sri Lanka, India (southern, northeastern), Myanmar (Cusset 1992), Thailand, Cambodia, Laos.

Acknowledgments

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APPENDIX. Sequence data of *Cladopus fallax*, *Polypleurum wallichii* and allied species used in this study. Species names are followed by localities, collectors, vouchers, herbarium acronyms, and DDBJ accession numbers (*matK*; ITS). (--) means absence of data. For species with published sequences, voucher numbers and accession numbers are given.

Cladopus austrosinensis M.Kato & Y.Kita. CHINA. Deng, Y.E. *et al.* CH-301 (TNS) (AB104560; AB104608*[1]); Deng, Y.E. *et al.* CH-302 (TNS) (AB104559; AB104607*[1]); Kokubugata GK17125 (TNS) (LC144911*[2]; --).

Cladopus doianus (Koidz.) Koriba. JAPAN. Kato JK-02 (TNS) (AB038189*[3]; --); Kato JK-Yamazaki (TNS) (AB038189; AB104603*[1]); Kato JK-Anraku (TNS) (AB038189*[1]; --); Kato JK-Mawatari (TNS) (AB179655*[1]; --); Kato JK-Manose (TNS) (AB179656*[1]; --); Kato & Katayama JP-127 (TNS) (AB698209*[4]; --). CHINA. Kato CH-02 (TNS) (AB179654; AB104602*[1]).

Cladopus fallax C.Cusset. THAILAND. Koi, Fujinami & Wongprasert TKF-109 (TNS) (AB698210*[4]; LC380634*[5]); Kato & Wongprasert TL-701 (TNS) (AB293561*[6]; --); Kato, Koi, Tsutsumi *et al.* TL-1610 (TNS) (AB537378*[7]; --). CAMBODIA. Koh Kong Prov.: Ruessei Chrum, Thma Bang, Kato CAM-04 (TNS) (LC380593*[5]; --); *ibid.*, Kato CAM-13 (TNS) (LC380594; LC380635, LC380636, LC380637*[5]); Chhay Areng rapid, Kato CAM-19 (TNS) (LC380595; LC380638, LC380639*[5]). Mondulkiri Prov.: Romnear 1 waterfall, Kato CAM-26 (TNS) (LC380596; LC380640*[5]); *ibid.*, Kato CAM-31 (TNS) (LC380597*[5]; --); Kbal Prah waterfall, Kato CAM-41 (TNS) (LC380598; LC380641*[5]).

Cladopus fukienensis (H.C.Chao) H.C.Chao. CHINA. Kato CH-01 (TNS) (AB179653; AB104601*[1]); Hong Kong, Kato HongkongSN (TNS) (LC144912*[2]; --); *ibid.*, Kato HK-02 (TNS) (LC144913*[2]; --). JAPAN. Kato JK-03 (TNS) (AB048371*[3]; AB104604*[1]).

Cladopus javanicus M.Kato & Hambali. INDONESIA. Kato, Koi & Hambali ID-02 (TNS) (AB066175; AB104609*[1]); Ciawi, West Java, Kato & Hambali ID-301 (TNS) (LC380599*[5]; --); Bogor Botanic Gardens, Kato & Hambali ID-302 (TNS) (LC380600*[5]; --).

Cladopus nymanii H.Möller. INDONESIA. Kato FL-02 (TNS) (AB698211*[4]; --); Kato ID-03 (TNS) (AB104561*[1]; --); Kato S-01 (TNS) (AB104577*[1]; --).

Cladopus pierrei (Lecomte) C.Cusset. THAILAND. Sakaew Prov.: Mantara Waterfall, Werukamkul & Ampornpan SK-04 (TNS) (LC151304*[5]; --); Kaeng Dan Krating, Werukamkul & Ampornpan SK-08 (TNS) (LC151305*[5]; --). LAOS. Koi, Katayama & Wongprasert LK-128 (TNS) (AB610219*[8]; --); Koi, Katayama & Wongprasert LK-132 (TNS) (AB610220*[8]; --); Koi, Katayama & Wongprasert LK-134 (TNS) (AB610221*[8]; --); Koi & Wongprasert LK-237 (TNS) (AB610222*[8]; --); Koi & Wongprasert LK-241 (TNS) (AB610223*[8]; --); Koi, Katayama & Wongprasert LK-117 (TNS) (AB610213*[8]; --); Koi, Katayama & Wongprasert LK-121 (TNS) (AB610214*[8]; --); Koi, Katayama & Wongprasert LK-123 (TNS) (AB610215*[8]; --); Koi & Wongprasert LK-211 (TNS) (AB610224*[8]; --); Koi & Wongprasert LK-221 (TNS) (AB610226*[8]; --); Koi & Wongprasert LK-222 (TNS) (AB610227*[8]; --); Koi & Wongprasert LK-214 (TNS) (AB610224*[8]; --); Koi & Wongprasert LK-232 (TNS) (AB610228*[8]; --); Koi, Fujinami & Wongprasert LKF-103 (TNS) (AB537379*[7]; --); Koi, Fujinami & Wongprasert LKF-109 (TNS) (AB610217*[8]; --); Koi, Fujinami & Wongprasert LKF-118 (TNS) (AB537380*[7]; --); Koi, Fujinami & Wongprasert LKF-120 (TNS) (AB610218*[8]; --).—Salavan Prov.: Kaeng Koo rapid, Kato, Koi & Thawatchai LK-406 (TNS) (LC151297*[5]; --).—Attapeu Prov.: Tad Nam Pa (=Tad Jo) waterfall, Kato, Koi & Thawatchai LK-419 (TNS) (LC151299*[5]; --); Se Lamong stream, Kato, Koi & Thawatchai LK-433 (TNS) (LC151302*[5]; --).—Champasak Prov.: Tad Champy waterfall, Kato, Koi & Thawatchai LK-438 (TNS) (LC151303*[5]; --).

Cladopus queenslandicus (Domin) C.D.K.Cook & Rutish. AUSTRALIA. Kato AU-01 (TI) (AB038199*[3]; --). PAPUA NEW GUINEA. Akiyama 16803 (TNS) (AB300702*[6]; --).

Cladopus taiensis C.Cusset. THAILAND. Kato, Imaichi & Wongprasert TL-101 (TNS) (AB048372*[3]; --); Kato, Imaichi & Wongprasert TL-102 (TNS) (AB698212*[4]; --); Kato & Wongprasert TL-604 (TNS) (AB698213*[4]; LC380642*[5]).— Chaiyaphum Prov.: Forest Protection Phu Kiew Unit 5, Phu Khieo wildlife sanctuary, Koi, Werukamkul, Ampornpan & Kato TL-1903 (TNS) (LC151309*[5]; --); Forest Protection Phu Kiew Unit 5, Nong Bua Daeng, Werukamkul & Ampornpan CP-09 (TNS) (LC151306*[9]; --); Kaeng Wang Konsak, Nong Bua Daeng, Werukamkul & Ampornpan (TNS)CP-26 (LC151307*[9]; --); Kaeng Paktok, Nong Bua Daeng, Werukamkul & Ampornpan CP-31 (TNS) (LC151308*[9]; --). CAMBODIA. Koh Kong Prov.: Pro Pang Khna, Thma Bang, Kato CAM-07 (TNS) (LC380601*[5]; --); *ibid.*, Kato CAM-14 (TNS) (LC380602; LC380644*[5]).

Farmeria metzgerioides Willis. INDIA. Kato, Koi & Pradeep KI-110 (TNS) (AB698231*[4]; --). SRI LANKA. Kato, Imaichi, Okada & Akiyama SL-07 (TNS) (AB104580*[1]; --).

Griffithella hookeriana (Tul.) Warm. INDIA. Kato, Koi & Pradeep KI-103 (TNS) (AB698233*[4]; --); Khanduri gf3103 (-; KF900089, KF900090, KF900091*[10]).

Hanseniella heterophylla C.Cusset. Thailand. Kato, Kita & Wongprasert TL-311 (TNS) (AB104562; AB104584*[1]).

Hydrobryopsis sessilis (Willis) Engl. India. Khanduri hs3114 (--; KF900083, KF900084, KF900085*[10]).

Hydrobryum bifoliatum C.Cusset. Thailand. Kato, Kita & Wongprasert TL-310 (TNS) (AB1046583*[1]; --).

Hydrobryum chiangmaiensis M.Kato. Thailand. Kato, Koi, Kita & Wongprasert TL-423 (TNS) (AB104591*[4]; --); Kato, Koi, Kita & Wongprasert TL-429 (TNS) (AB104590*[1]; --).

Hydrobryum floribundum Koidz. Japan. Kato JK-Anraku (TNS) (AB104600*[1]; --); Kato JK-Mae (TNS) (AB104600*[1]; --).

Hydrobryum griffithii (Wall. ex Griff.) Tul. Thailand. Kato, Koi & Wongprasert TL-205 (TNS) (AB104592*[1]; --); Katayama & Kato CH-102 (TNS) (AB104593*[1]; --).

Hydrobryum japonicum Imamura. Japan. Kato JK-01 (TI) (AB104597*[3]; --); Kato, Koi, Kita & Wongprasert TL-415 (TNS) (AB104598*[7]; --). China. Katayama & Kato CH-101 (TNS) (AB104599*[1]; --).

Hydrobryum kaengsophense M.Kato. Thailand. Kato, Kita & Wongprasert TL-312 (TNS) (AB104585*[1]; --).

Hydrobryum koribanum Imamura ex Nakayama & Minamitani. Japan. Kato JK-05 (TI) (AB104596*[3]; --).

Hydrobryum loeicum M.Kato. Thailand. Kato, Koi & Wongprasert TL-210 (TNS) (AB104586*[1]; --).

Hydrobryum micrantherum (P.Royen) C.D.K.Cook & Rutish. Thailand. Kato & Imaichi TL-58 (TNS) (AB104589*[1]; --); Kato, Kita & Wongprasert TL-306 (TNS) (AB104588*[1]; --).

Hydrobryum puncticulatum Koidz. Japan. Kato JK-Yaku (TNS) (AB104595*[1]; --).

Hydrobryum tardhuangense M.Kato. Thailand. Kato, Koi & Wongprasert TL-208 (TNS) (AB104587*[1]; --).

Hydrodiscus koyamae (M. Kato & Fukuoka) Koi & M. Kato. Laos. L-06 (AB537381*[7]; --)

Paracladopus chanthaburiensis Koi & M.Kato. Thailand. Kato, Koi & Wongprasert TL-1533 (TNS) (AB293558*[6]; --); Imaichi, Nishida, Koi *et al.* TIK-21 (TNS) (AB293559*[6]; --); Imaichi, Koi, Fujinami *et al.* TIK-34 (TNS) (AB300701*[6]; --); Imaichi, Nishida, Koi *et al.* TKF-24 (TNS) (AB698346*[4]; --).

Paracladopus chiangmaiensis M.Kato. Thailand. Kato, Imaichi & Wongprasert TL-808 (TNS) (AB293560*[6]; --); Ampornpan & Werukamkul CM-07 (TNS) (LC151393*[5]; --); Ampornpan & Werukamkul CM-08 (TNS) (LC151394*[5]; --); Koi, Werukamkul & Ampornpan TPK-106 (TNS) (LC151395*[5]; --); Koi, Fujinami & Wongprasert LKF-110 (TNS) (AB537419*[7]; --); Koi, Fujinami & Wongprasert LKF-105 (TNS) (AB610256*[8]; --); Koi, Fujinami & Wongprasert LKF-116 (TNS) (AB610257*[8]; --).

Polypleurum elongatum (Gardner) J.B.Hall. Sri Lanka. Kato, Imaichi, Okada & Akiyama SL-12 (TNS) (AB048376*[3]; --).

Polypleurum longistylosum M.Kato. Thailand. Kato, Kita & Wongprasert TL-318 (TNS) (AB104578*[1]; --).

Polypleurum munnarense Nagendran & Arekal. India. Pradeep Cu-90004 (TNS) (AB610267*[8]; --); Pradeep Cu-90007 (TNS) (698371*[4]; --); Khanduri pm3108 (--; KF900100, KF900101, KF900102*[10]).—Kerala State: Verala near Irumpupalam and Adimali, Idukki, Kato IN-1205 (TNS) (LC380603*[5]; --).

Polypleurum pluricostatum M.Kato. Laos. Koi, Katayama, Fujinami & Wongprasert LKF-03 (TNS) (AB610273*[8]; --).—Bolikhamsai Prov.: Nam Mang 3 Dam, Phou Khao Khouay NPA, Kato L-502 (TNS) (LC380604*[5]; --).

Polypleurum prachinburiensis M.Kato & Koi. Thailand. Kato, Koi & Wongprasert TL-1534 (TNS) (AB698377*[4]; --); Kato, Koi, Tsutsumi *et al.* TL-1601-1 (TNS) (AB610276*[8]; --).

Polypleurum schmidtianum Warm. Thailand. Koi, Katayama & Wongprasert LK-106 (TNS) (AB610279*[8]; -); Imaichi, Nishida, Koi *et al.* TIK-22 (TNS) (AB698382*[4]; --); Imaichi, Nishida, Koi *et al.* TIK-23 (TNS) (AB698383*[4]; --); Koi, Fujinami, Katayama & Wongprasert TKF-21 (TNS) (AB610463*[8]; --); Koi, Fujinami & Wongprasert TKF-106 (TNS) (AB698384*[4]; --); Kato, Koi & Wongprasert TL-1303 (TNS) (AB610280*[8]; --); Kato, Koi & Wongprasert TL-1508A (TNS) (AB610281*[8]; --); Kato, Koi & Wongprasert TL-1508B (TNS) (AB610282*[8]; --); Kato, Koi & Wongprasert TL-1527 (TNS) (AB698385*[4]; --).—Sisaket Prov.: Wang Yai waterfall, Phanom Dongrak Wildlife Sanctuary, Koi, Werukamkul & Ampornpan TPK-116 (TNS) (LC380605*[5]; --). Cambodia. Koh Kong Prov.: Ruessei Chrum, Thma Bang, Kato CAM-05 (TNS) (LC380606*[5]; --); *ibid.,,* Kato CAM-12 (TNS) (LC380607; LC380645*[5]); Pro Pang Khna, Thma Bang, Kato CAM-06 (TNS) (LC380608*[5]; --); *ibid.*, Kato CAM-15 (TNS) (LC380609*[5]; --); *ibid.*, Kato CAM-16 (TNS) (LC380611; LC380646*[5]); Chhay Areng rapid, Areng River, Kato CAM-17 (TNS) (LC380612; LC380647*[5]); *ibid.*, Kato CAM-18 (TNS) (LC380610*[5]; --); *ibid.*, Kato CAM-19-2 (TNS) (LC380613; LC380648*[5]).

Polypleurum stylosum (Wight) J.B.Hall. India. Pradeep Cu-90003 (TNS) (AB610465*[8]; --); Kato & Imaichi KI-25 (TNS) (AB698386*[4]; --); Kato, Koi, Mathew & Pradeep KI-109 (TNS) (AB610466*[8]; --); Kato, Katayama & Pradeep KI-211 (TNS) (AB610467*[8]; --); Kato, Katayama & Pradeep KI-217 (TNS) (AB698387*[4]; --); Kato, Katayama & Pradeep KI-222 (TNS) (AB610468*[8]; --); Khanduri psl3102, Khanduri psl3102_2, Khanduri psl3102_3 (--; KF900103, KF900104, KF900105, KF900106, KF900107*[10]); Khanduri pss3112 (--; KF900108, KF900109, KF900110*[10]).—

Kerala State: Punthikuzha, Mannarkkad, Kato & Koi IND-1401 (TNS) (LC380614[5]; --); Meenmutty waterfall, Thiruvananthapuram, Kato & Koi IND-1413 (TNS) (LC380615[5]; --); Verala near Irumpupalam and Adimali, Idukki, Kato IN-1203 (TNS) (LC380616[5]; --); Pooyamkutti, Ernakulam, Tsutsumi & Kato IND-38 (TNS) (LC380617[5]; --); *ibid*., Tsutsumi & Kato IND-39 (TNS) (LC380618[5]; --). Sri Lanka. Kato, Imaichi, Okada & Akiyama SL-05 (TI) (AB066174[1]; --); Kato & Katayama SL-103 (TNS) (AB698388[4]; --); Kato & Katayama SL-104 (TNS) (AB698389[5]; --).

Polypleurum wallichii (R.Br. ex Griff.) Warm. **var.** *wallichii.* Laos. Kato, Koi, Tsutsumi *et al.* L-03 (TNS) (AB610470[7]); Koi & Wongprasert LK-09 (TNS) (AB610471[8]; --); Koi, Katayama & Wongprasert LK-127 (TNS) (AB610472[8]; --); Koi & Wongprasert LK-238 (TNS) (AB610473[8]; --). Thailand. Kato & Imaichi TL-55 (TNS) (AB038204[3]; --); Kato & Wongprasert TL-601 (TNS) (AB610469[8]; LC380650[5]).—Chaiyaphum Prov.: Forest Protection Phu Kiew Unit 5, Phu Khieo wildlife sanctuary, Koi, Werukamkul, Ampornpan & Kato TL-1902 (TNS) (LC380619[5]; --); Forest Protection Phu Khiew Unit 5, Saphungnuea Bon, Phu Khiew Wildlife Sanctuary, Werukamkul & Ampornpan CP-07 (BKF, TNS) (LC380620[5]; --); Kaeng Wang Hin Pake, Phu Khiew Wildlife Sanctuary, Werukamkul & Ampornpan CP-22 (BKF, TNS) (LC380621[5]; --); Kaeng Wang Khon Sak, Phu Khiew Wildlife Sanctuary, Werukamkul & Ampornpan CP-24 (BKF, TNS) (LC380622[5]; --); Kaeng Sabmakuaepoung, Phu Khiew Wildlife Sanctuary, Werukamkul & Ampornpan CP-27 (BKF, TNS) (LC380623[5]; --); Kaeng Pake Tok, Phu Khiew Wildlife Sanctuary, Werukamkul & Ampornpan CP-29 (BKF, TNS) (LC380624[5]; --); Forest Protection Phu Kiew Unit 5, Koi & Werukamkul TPK-114 (TNS) (LC380625[5]; --). Nakhon Nayok Prov.: Haew Suwat waterfall, Khao Yai National Park, Koi & Werukamkul TPK-122(TNS) (LC380626[5]; --). Cambodia. Koh Kong Prov.: Ruessei Chrum, Thma Bang, Kato CAM-03 (TNS) (LC380627[5]; --); *ibid*., Kato CAM-11 (TNS) (LC380628; LC380651[5]). India. Meghalaya State: Mawsmai waterfalls, Cherrapunji, Kato IND-1503 (TNS) (LC380629; LC380652[5]); Quinine, Ribhoi, south of Nongpoh, Kato IND-1431 (TNS) (LC380630[5]; --); *ibid*., Kato IND-1431A (TNS) (LC380631[5]; --); *ibid*., Kato IND-1504 (TNS) (LC380632; LC380653[5]); *ibid*., Kato IND-1505 (TNS) (LC380633[5]; --).

Polypleurum wongprasertii M.Kato. Thailand. Kato, Kita & Wongprasert TL-319 (TNS) (AB104579[1]; --).

Thawatchaia trilobata M.Kato, Koi & Y. Kita. Thailand. Kato, Koi, Kita & Wongprasert TL-419 (AB104563[1]; --)

Willisia arekaliana Shivam. & Sadanand. India. Khanduri wa3101 (--; KF90011, KF900112, KF900113[10]).

Willisia selaginoides (Bedd.) Warm. ex Willis. India. Khanduri ws3106 (--; KF900114, KF900115, KF900116[10]).

Zeylanidium lichenoides (Kurz) Engl. India. Khanduri zl3105 (--; KF900117[10]).

Zeylanidium olivaceum Engl. Sri Lanka. Kato, Imaichi, Okada & Akiyama SL-09 (AB038207[1]; --).

Zeylanidium sessile (Willis) C.D.K.Cook & Rutish. India. Kato & Imaichi KI-35 (AB048828[1]; --)

Zeylanidium subulatum (Gardner) C.Cusset. India. Khanduri ps3104, Khanduri ps3105 (--; KF900097, KF900098, KF900099[10]).

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[1] Kita & Kato 2004, [2] Kato *et al.* 2017, [3] Kita & Kato 2001, [4] Koi *et al.* 2012, [5] this study, [6] Koi *et al.* 2008, [7] Koi & Kato 2010, [8] Koi & Kato 2012, [9] Werukamkul *et al.* 2018, [10] Khanduri *et al.* 2015.