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Taxonomy and phylogenetic position of *Fimbristylis fusiformis*, a new species of *Cyperaceae* from Thailand

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Key words

conservation status
Fimbristylis
phylogeny
taxonomy

Abstract *Fimbristylis fusiformis*, an unusual new species of *Cyperaceae* from Thailand, is described and illustrated. This taxon has a single terminal spikelet per culm with a semi-distichous glume arrangement, bisexual flowers that lack perianth parts, and pistil with persistent style whose base is slightly swollen and trigonous nutlets with pubescent ribs. Phylogenetic reconstruction using ITS sequence data places this taxon in *Abildgaardieae* and sister to the rest of *Fimbristylis*. The species has a conservation status of Least Concern (LC).

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INTRODUCTION

The genus *Fimbristylis* Vahl, comprising c. 300 species, occurs mostly in the tropics and subtropics with some species occurring in warm temperate regions (Govaerts et al. 2007). The genus is morphologically diverse, with inflorescences ranging from complex umbel-like structures to a solitary spikelet. The glumes are generally spirally arranged in ellipsoid spikelets, more rarely distichously arranged, the bisexual flowers lack perianth parts and their deciduous styles have distinctly thickened base, and the nutlets often have distinct surface patterns. *Fimbristylis* is currently (e.g., Govaerts et al. 2007) treated to include the segregate genus *Abildgaardia* Vahl, the latter only differing in having a distichous glume arrangement (Goetghebeur 1998).

The genus was revised for Thailand by Simpson & Koyama (1998), who enumerated 60 species for that country. A study of undetermined material by one of us (DAS) suggested a previously undescribed species was present in eastern Thailand (Map 1). Further herbarium studies, together with associated fieldwork, confirmed that the species was new. We inferred the phylogenetic position of this new species using DNA sequence data, and describe and illustrate it here (Fig. 1–3).

MATERIALS AND METHODS

Specimens were examined from herbaria at BK, BKF, K, KKU and QBG. Fieldwork was undertaken in September 2008 and November 2011. Observations of inflorescences and fruits were made with an Olympus SZ-PT binocular microscope. Mature nutlets from the spikelet of the holotype were collected for scanning electron microscopy. Selected material was mounted on stubs with double-sided adhesive tape and coated with gold using a SC7620 mini sputter-coater (Polaron range). Micrographs were generated using a JSM6460LV scanning electron microscope (JEOL Ltd.)

Using a silica gel dried sample of the new taxon, DNA was extracted using the CTAB method and the ITS marker amplified and sequenced using standard protocols and primers (Muasya et al. 2014). Contigs of forward and reverse sequences were assembled using the STADEN package (Staden 1996). Additional ITS DNA sequences, primarily taken from studies of *Abildgaardieae* (Ghamkhar et al. 2007) and *Cypereae* (Yano et al. 2012), were downloaded from GenBank. The matrix thus assembled (Table 1) included a total of 90 taxa, with representatives of subfamily *Cyperoideae*, tribes *Abildgaardieae*, *Cypereae*, *Eleocharideae*, *Fuireneae*, *Scirpeae* with *Cladium* as outgroup. The matrix was aligned using Muscle (v. 3.8.31; Edgar 2004), and further manually aligned in BioEdit (v. 7.0.9; Hall 1999). The aligned matrix was analysed using maximum parsimony in PAUP* (Swofford 2002), with heuristic searches using the random-addition-sequence method with 10 000 replicates, Tree-Bisection-Reconnection (TBR) branch-swapping with the Multrees option in effect and no maximum tree number set. Node support was evaluated using bootstrap analyses with 500 replicates, repeating the heuristic search procedure (with 10 replicates) above.

RESULTS AND DISCUSSION

Although the new species was easily identifiable as a member of subfamily *Cyperoideae* (absence of the spicoid floral structure (Simpson et al. 2003), individual flowers comprising stamens and gynoecium subtended by a glume), its generic placement within *Cyperoideae* was not immediately obvious. The combination of morphological characters, especially in the structure of the gynoecium, with the scabrous style, the apparent lack of a disjunction between the style base and nutlet, the fusiform shape of the nutlet and the fimbriate hairs at its apex and base (Fig. 1), suggested a genus other than *Fimbristylis*. Indeed, some aspects of the plant's gross morphology (e.g., a single, terminal spikelet) were superficially similar to genera such as *Trichophorum* (tribe *Scirpeae*) whereas the lack of perianth parts and possession of a persistent style with an indistinct base on the nutlet, were reminiscent of features observed in *Isolepis* (tribe *Cypereae*).

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Table 1 List of taxa sampled for the phylogenetic study.

Tribe	Taxon	Voucher	Accession number
Abildgaardieae	*Arthrostylis aphylla* R.Br.	*Clarke 183* (NE)	AY506757
	Bulbostylis barbata (Rottb.) C.B.Clarke	*Clarke 113* (NSW)	AY506764
	Bulbostylis densa (Wall.) Hand.-Mazz.	*Klaphake 1411* (NSW)	AY506763
	Bulbostylis funckii (Steud.) C.B.Clarke	*Roalson 1384* (RSA)	AF190616
	Crosslandia setifolia W.Fitzg.	*Clarke 246* (NSW)	AY506768
	Fimbristylis aestivalis Vahl	*Arai & Hirahara 18166* (OKAY)	AB250626
	Fimbristylis bisumbellata (Forssk.) Bubani	*Clarke 107* (NE)	AY506778
	Fimbristylis complanata (Retz.) Link	*Muasya 1029* (EA, K)	AY242051
	Fimbristylis composita Latz	*Clarke 186* (NE)	AY506756
	Fimbristylis composita Latz	*Clarke 213* (NE)	AY506755
	Fimbristylis composita Latz	*Clarke 214* (NE)	AY506754
	Fimbristylis cymosa R.Br.	*Wilson 10041* (NSW)	AY506798
	Fimbristylis densa S.T.Blake	*Clarke 119* (NE)	AY506781
	Fimbristylis dichotoma (L.) Vahl	*Katayama 17519* (OKAY)	AB250630
	Fimbristylis diphylloides Makino	*J. Jung 909179* (AJOU)	JX644883
	Fimbristylis ferruginea (L.) Vahl	*Hodgon 445* (NSW)	AY506797
	Fimbristylis fusiformis Wangwasit & D.A.Simpson	*K. Wangwasit 080927-17* (K)	KY652919
	Fimbristylis globulosa (Retz.) Kunth	*J. Jung 1010275* (AJOU)	JX644885
	Fimbristylis lanceolata C.B.Clarke	*Wilson 10113* (NSW)	AY506786
	Fimbristylis laxiglumis Latz	*Clarke 106* (NE)	AY506785
	Fimbristylis littoralis Gaudich.	*Orel 10* (NSW)	AY506790
	Fimbristylis longispica Steud.	*Katsuyama et al. 19910* (OKAY)	AB250636
	Fimbristylis miliacea (L.) Vahl	*J. Jung 909180* (AJOU)	JX644886
	Fimbristylis neilsonii F.Muell.	*Wilson 10051* (NSW)	AY506784
	Fimbristylis ovata (Burm.f.) J.Kern.	*Klaphake 1410* (NSW)	AY506758
	Fimbristylis oxystachya F.Muell.	*Clarke 165* (NE)	AY506762
	Fimbristylis pachyptera S.T.Blake	*Clarke 181* (NT)	AY506760
	Fimbristylis pauciflora R.Br.	*Clarke 50* (NE)	AY506783
	Fimbristylis pierotii Miq.	*Hoshino et al. 20053* (OKAY)	AB250639
	Fimbristylis polytrichoides (Retz.) R.Br.	*Clarke 91* (NE)	AY506796
	Fimbristylis rara R.Br.	*Clarke 105* (NE)	AY506780
	Fimbristylis schultzii Boeckeler	*Clarke 108* (NE)	AY506791
	Fimbristylis sericea (Poir.) R.Br.	*Wilson 10042* (NSW)	AY506801
	Fimbristylis sieberiana Kunth	*S. Jacobs 8659* (NSW)	AY506801
	Fimbristylis sieboldii Miq.	*J. Jung 1010277* (AJOU)	JX644884
	Fimbristylis squarrosa Vahl	*Arai & Hirahara 18165* (OKAY)	AB250641
	Fimbristylis stauntonii Debeaux & Franch.	*S.-M. Yun s.n.* (AJOU)	JX644888
	Fimbristylis subbispicata Nees	*J. Jung 1010018* (AJOU)	JX644889
	Fimbristylis tetragona R.Br.	*Clarke 173* (NE)	AY506799
	Fimbristylis tristachya R.Br.	*Clarke 3* (NE)	AY506802
	Fimbristylis vaginata (R.Br.) Domin	*Bruhl 2057* (NE)	AY506759
	Fimbristylis velata R.Br.	*Wilson 10028* (NSW)	AY506792
Cariceae	*Carex esenbeckii* Kunth	*Ikeda et al. 20814080* (TI)	AB643648
Cypereae	*Afroscirpoides dioeca* (Kunth) García-Madr.	*Muasya 3062* (BOL)	GU012394
	Cyperus ascocapensis Bauters	*Muasya 1009* (EA, K)	AB685858
	Cyperus brevifolius (Rottb.) Hassk.	*Hirahara & Yano 18135* (OKAY)	AB261669
	Cyperus cyperoides (L.) Kuntze	*Morimoto 17532* (OKAY)	AB261665
	Cyperus involucratus Rottb.	*Kew Acc. 6136603* (K)	AY242052
	Cyperus isolepis (Nees) Bauters	*Muasya 1217* (K)	AB685866
	Cyperus papyrus L.	*Hepper 4213* (K)	AY242048
	Cyperus sanguinolentus Vahl	*Komagine & Masyo 17655* (OKAY)	AB261671
	Erioscirpus microstachyus (Boeckeler) Palla	*Noltie 2001562* (E)	AB643639
	Erioscirpus comosus (Wall.) Palla	*Ikeda et al. 20814007* (TI)	AB643639
	Ficinia bergiana Kunth	*Muasya 2337* (BOL)	AB685861
	Ficinia esterhuyseniae Muasya	*Muasya 2312* (BOL)	GU012400
	Ficinia gracilis Schrad.	*Faden et al. 96/433* (K)	AB685862
	Ficinia nodosa (Rottb.) Goetgh., Muasya & D.A.Simpson	*Wilson 9455* (K)	GU012383
	Ficinia rigida Levyns	*Muasya 2319* (K)	AB685863
	Ficinia spiralis (A.Rich.) Muasya & de Lange	*HUG 2003-0699* (GENT)	GU012395
	Ficinia trichodes (Schrad.) Benth. & Hook.f.	*Muasya 2328* (K)	AB685864
	Hellmuthia membranacea (Thunb.) R.W.Haines & Lye	*Muasya 3081* (BOL)	GU012384
	Isolepis cernua (Vahl) Roem. & Schult.	*Muasya 3073* (BOL)	GU012413
	Isolepis crassiuscula Hook.f.	*Hirahara & Hoshino 19165* (OKAY)	AB261668
	Isolepis hystrix (Thunb.) Schrad.	*Muasya 2971* (BOL)	GU012388
	Isolepis levynsiana Muasya & D.A.Simpson	*Muasya 1151* (K)	AB685865
	Isolepis marginata (Thunb.) A.Dietr.	*Muasya 2973* (BOL)	GU012418
	Isolepis setacea (L.) R.Br.	*Ikeda et al. 20814046* (TI)	AB643644
	Isolepis venustula Kunth	*Muasya 1189* (K)	GU012421
	Scirpoides holoschoenus (L.) Soják	*HBUG 2003-1536* (GENT)	AB685867
	Scirpoides thunbergii (Schrad.) A.Spreng.	*Muasya 1205* (K)	AB685868
Dulichieae	*Dulichium arundinaceum* (L.) Britton	*Waterway 2003.052* (MTMG)	DQ998949
Eleocharideae	*Eleocharis mamillata* (H.Lindb.) H.Lindb.	*Bures et al. s.n.* (BRNU)	GU977089
	Eleocharis neozelandica C.B.Clarke ex Kirk.	*Gardner et al. AK284635* (AK)	DQ385566
	Eleocharis pusilla R.Br.	*Gardner et al. AK284890* (AK)	DQ385564
	Eleocharis quinqueflora (Hartmann) O.Schwarz	*Bures et al. s.n.* (BRNU)	GU977095.1
	Eleocharis wichurae Boeckeler	*Hoshino et al. 17616* (OKAY)	AB180715

Table 1 (cont.)

Tribe	Taxon	Voucher	Accession number
Fuireneae	*Actinoscirpus grossus* (L.f.) Goetgh. & D.A.Simpson	*Katsuyama et al. 19915* (OKAY)	AB261672
	Schoenoplectus hotarui (Ohwi) Holub	*Katayama 17521* (OKAY)	AB180720
Schoeneae	*Cladium chinense* Nees ex Hook. & Arn.	*H.-K. Choi 2006 s.n.* (AJOU)	GQ130342
Scirpeae	*Amphiscirpus nevadensis* (S.Watson) Oteng-Yeb.	*Helmkamp s.n.* (RSA)	AF190618
	Eriophorum angustifolium Honck.	*Waterway 2001.018* (MTMG)	DQ998950
	Eriophorum gracile Koch	*Hoshino et al. 17382* (OKAY)	AB261684
	Eriophorum vaginatum L.	*Starr 98007 & Scott* (FHO)	AY242008
	Scirpus mitsukurianus Makino	*J. Jung 808304* (AJOU)	GQ130354
	Scirpus wichurae Boeckeler	*J. Jung 808322* (AJOU)	GQ130357
	Trichophorum alpinum (L.) Pers.	*Sato 13260* (OKAY)	AB206270
	Trichophorum dioicum (Y.N.Lee & Y.C.Oh) J.Jung & H.K.Choi	*J. Jung 804015* (AJOU)	FJ797641
	Trichophorum pumilum (Vahl) Schinz & Thell.	*Ikeda et al. 20814101* (TI)	AB643647
	Trichophorum subcapitatum (Thwaites & Hook.) D.A.Simpson	*Ikeda et al. 10042360* (TI)	AB679909

The aligned matrix yielded 837 characters, of which 419 were parsimony-informative, 116 were variable but parsimony-uninformative and 302 were constant.

Parsimony analysis recovered 16 equally parsimonious trees (Fig. 4), and the ITS phylogeny is similar to results previously published using plastid (e.g., Ghamkar et al. 2007, Muasya et al. 2009) and nuclear (e.g., Yano & Hoshino 2006, Ghamkar et al. 2007) markers. These strongly supported the *Abildgaardieae* clade as having two subclades, one comprising *Bulbostylis* (including *Actinoschoenus* and *Arthrostylis*) and the other comprising *Fimbristylis*. The new taxon was sister to the rest of *Fimbristylis* in all the 16 trees generated in our study, but there was a lack of bootstrap support for the backbone topology in *Fimbristylis* in this study as well as previous studies (e.g., Ghamkhar et al. 2007) using *trnL*-F and ITS sequence data.

On present evidence, the best placement for the new species is in *Fimbristylis*. However, we were unable to assign the new taxon to any of the sections in *Fimbristylis* s.l. recognized by previous researchers (e.g., Kern 1974) and further studies are needed to elucidate the precise relationships of the new taxon.

TAXONOMIC TREATMENT

Fimbristylis fusiformis Wangwasit & D.A.Simpson, *sp. nov.* — Fig. 1–3; Map 1

Superficially similar to *F. pauciflora* R.Br. but distinguished by the glumes 6.5–8.5 mm long (vs 2.5–3 mm long in *F. pauciflora*), nutlets fusiform, fimbriate at apex and base, with 3 longitudinal costae (vs nutlets obovate and glabrous in *F. pauciflora*). — Type: *K. Wangwasit 080927-17* (holo K; iso BK, KKU), Thailand, Ubon Ratchathani, Pha Taem National Park, 27 Sept. 2008.

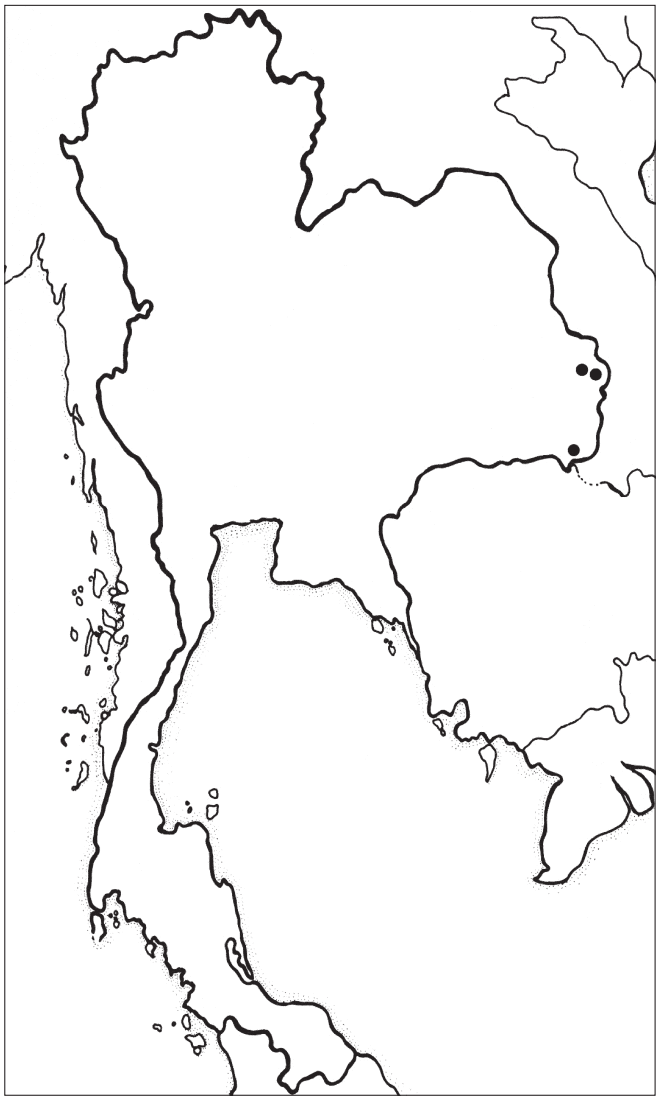
Etymology. Named after the shape of the nutlets.

Rhizomatous perennial. *Culms* densely tufted, 12–23 cm by 0.1–0.4 mm, trigonous, glabrous. *Leaves* basal; blade narrowly linear, 4–5 cm by 0.5 mm, obtuse, thickly crescentiform in cross-section, laterally flattened, glabrous; sheath 1–1.5 cm long, stramineous, sides membranous; ligule absent. *Involucral bracts* glume-like. *Inflorescence* a single terminal spikelet. *Spikelet* linear-cylindric, terete, 1 cm by 1.2–1.5 mm. *Glumes* 9 per spikelet, spirally arranged, lower glume glabrous, uppermost strigose, oblong elliptic, 6.5–8.5 by 1.5–2.3 mm, acuminate, sides membranous, stramineous, reddish brown tinged, keel obtuse, 1-nerved. *Stamens* 3; anthers 2–2.5 by 0.1–0.2 mm. *Stigma branches* 3; style persistent; style base rather indistinct, somewhat elongate, gradually widening into the nutlet, scabrous. *Nutlets* fusiform, trigonous, fimbriate, especially at apex and base, 2.5–3.8 by 0.5–0.85 mm, apex brown to dark brown, with minute hexagonal epidermal cells, longitudinal costae present.

Distribution — Endemic to eastern Thailand. Mostly seen in Pha Taem National Park but also observed in Phu Chong Na Yoi National Park, both in Ubon Ratchathani.

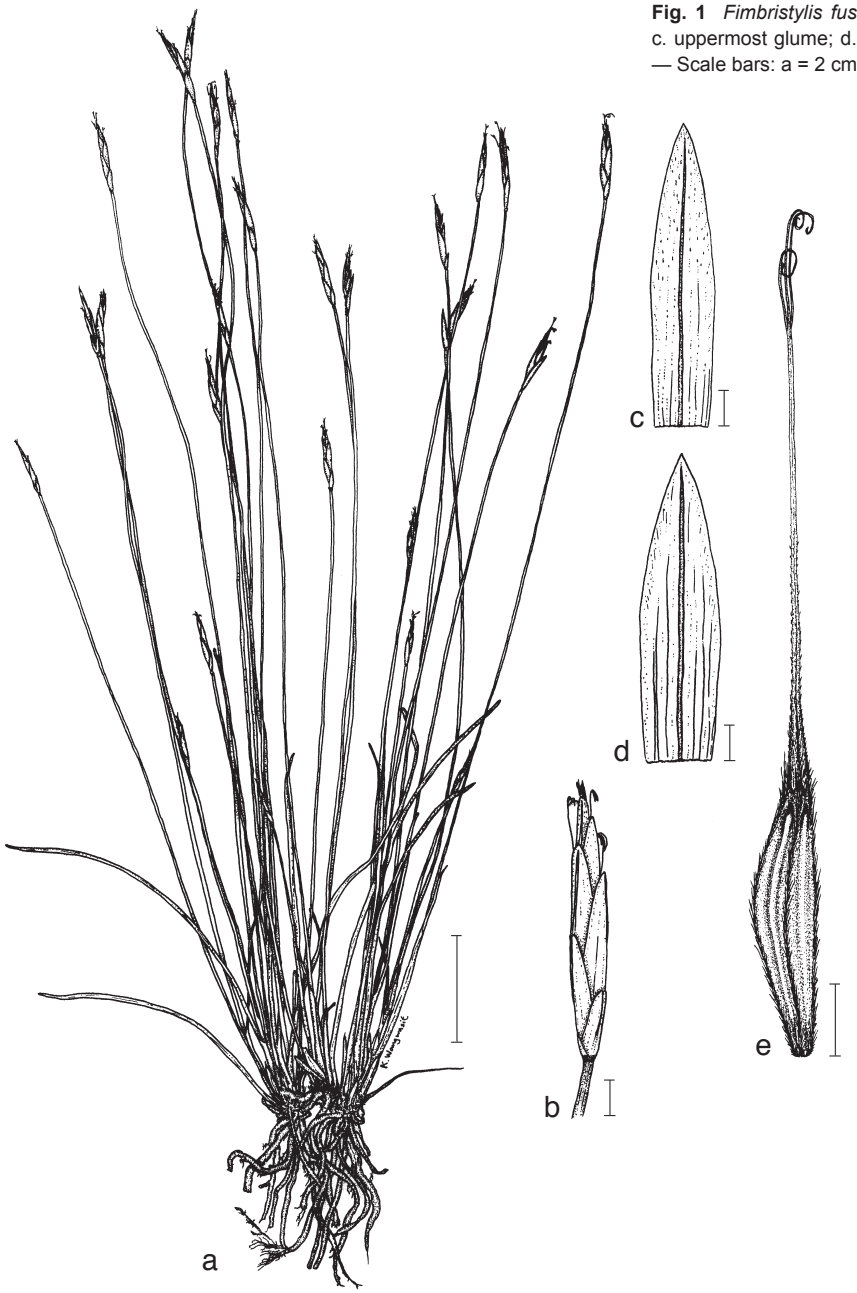
Habitat & Ecology — Open, stony places with scattered tree cover on seasonally wet, sandy soils. Altitude 227 m (Google Earth 2016).

Conservation status — Least Concern (LC; IUCN 2012). The species occurs in protected areas and is locally abundant in these localities.



Map 1 Distribution of *Fimbristylis fusiformis* Wangwasit & D.A.Simpson.

Fig. 1 *Fimbristylis fusiformis* Wangwasit & D.A.Simpson. a. Habit; b. spikelet; c. uppermost glume; d. lower glume; e. nutlet (all: *K. Wangwasit 080927-17*, K). — Scale bars: a = 2 cm; b = 2 mm; c–e = 1 mm. — Drawn by Khanit Wangwasit.



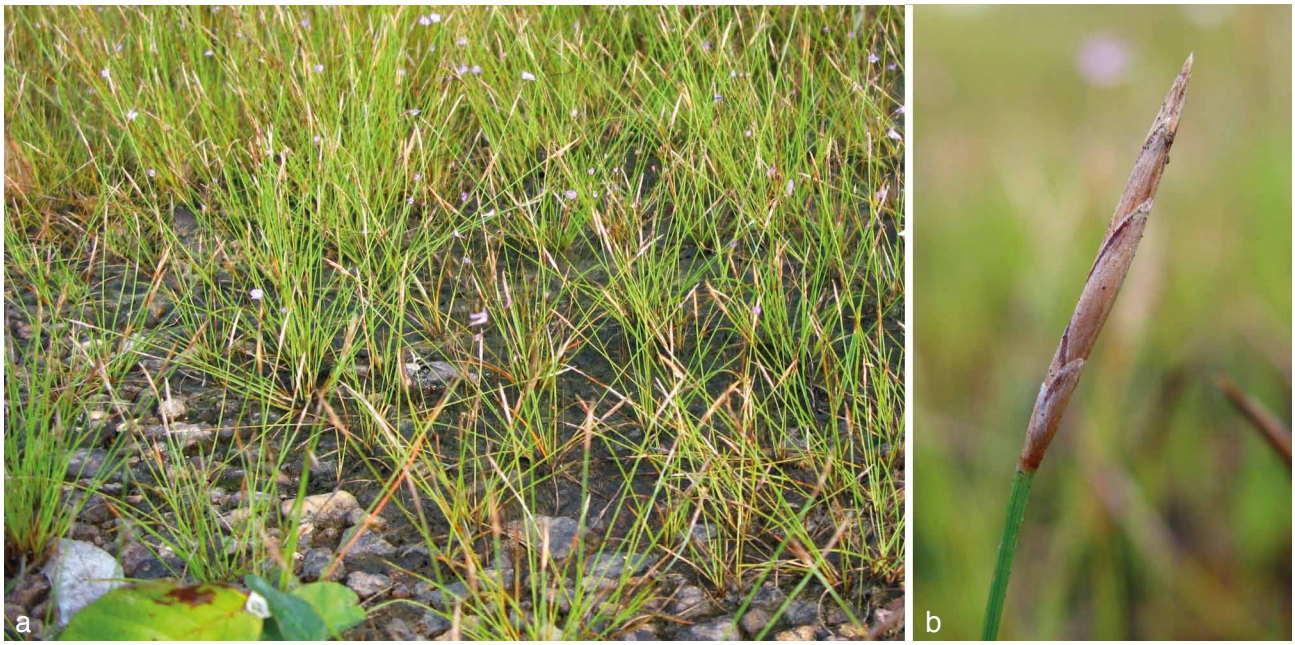


Fig. 2 *Fimbristylis fusiformis* Wangwasit & D.A.Simpson. a. Plants in habitat; b. close-up of spikelet. — Photos by D.A. Simpson.

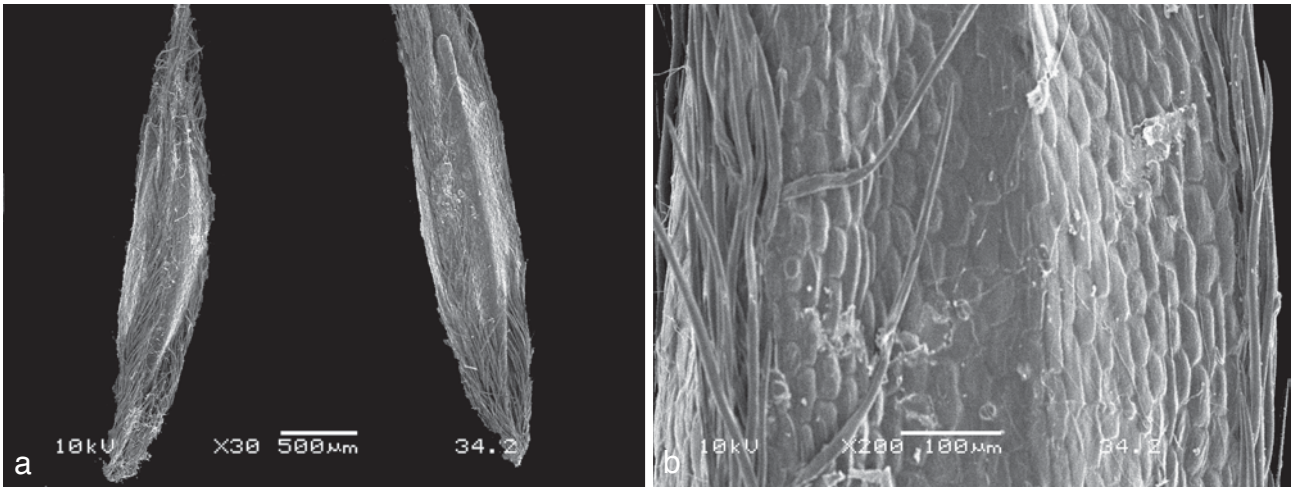


Fig. 3 SEM micrographs of the nutlet of *Fimbristylis fusiformis* Wangwasit & D.A.Simpson. a. Whole nutlet; b. nutlet surface (from *K. Wangwasit 080927-17*, K).

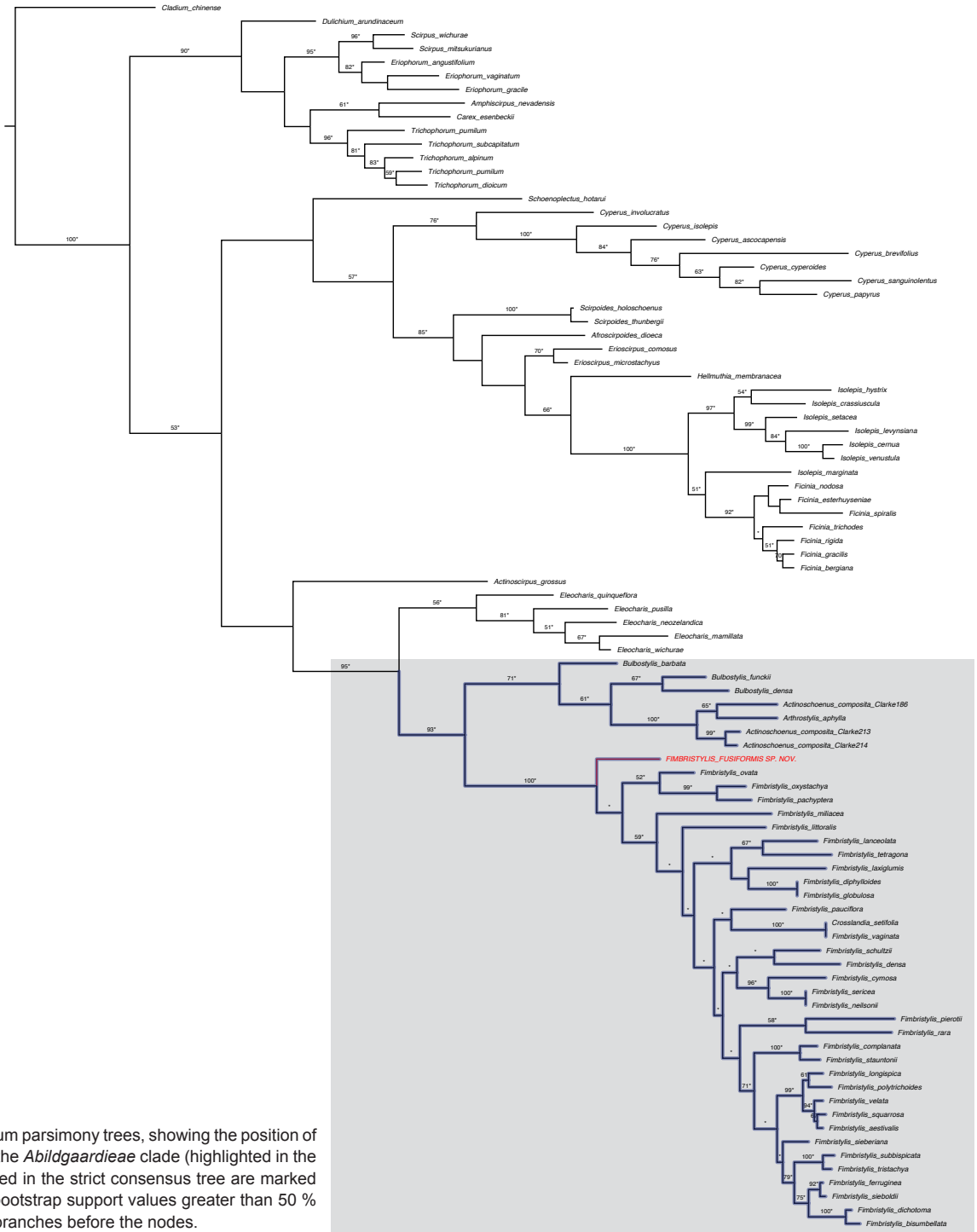


Fig. 4 One of 16 maximum parsimony trees, showing the position of *Fimbristylis fusiformis* in the *Abildgaardieae* clade (highlighted in the grey box). Nodes observed in the strict consensus tree are marked with an asterisk (*) and bootstrap support values greater than 50 % are indicated above the branches before the nodes.

Additional specimens examined. Thailand, Ubon Ratchathani: Khong Chiam, Pha Taem National Park, Khua Nangnee Falls (N15°24'11" E105°31'02"), *Pooma, De Wilde & Duyfjes 2270* (BKF); Sribunreung District, 27 Sept. 2008, *K. Wangwasit 080927-6* (KKU).

Note — *Fimbristylis fusiformis* is superficially similar to *F. pauciflora*, in having an inflorescence with a single terminal linear-cylindric spikelet bearing a glume-like bract. We interpret these similarities to be due to morphological convergence, as the two taxa are not sister in our phylogeny.

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