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CONTRIBUTIONS TO THE KNOWLEDGE OF CAMBODIAN CYPERACEAE

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To date, there are very few sequence data for Cyperaceae from mainland Southeast Asia. The aim of the present study was to contribute nuclear ribosomal internal transcribed spacer (ITS) sequences of selected species of Cambodian Cyperaceae to the overall phylogeny of the family. We generated ITS sequences of 38 accessions representing 26 species from Cambodia and used these sequences for phylogenetic analysis together with similar sequences from the National Center for Biotechnology Information GenBank. Our results corroborate recent phylogenetic work in the family and largely confirm established tribal relationships. The backbone of the phylogenetic tree of species-rich genera that have undergone rapid radiations is often weakly resolved (e.g. in *Fimbristylis* and in the C_4 clade of *Cyperus*). Cryptic variation was revealed in the taxonomically difficult group of *Fimbristylis dichotoma*, with samples of this taxon appearing in two distinct clades within *Fimbristylis*. Further addition of geographically spread accessions of taxa will improve our understanding of the complex biogeographical history of the genera in the family. *Eleocharis koyamae* Tremetsb. & D.A.Simpson is proposed as a new name for *E. macrorrhiza* T. Koyama.

Keywords. *Cyperus*, *Fimbristylis*, internal transcribed spacer, mainland Southeast Asia, phylogeny, structure-based alignment.

Introduction

Cyperaceae (sedges) are characterised by leaves that are arranged in three (eventually twisted) rows ([spiro]tristichous phyllotaxis), a reduced or lacking perianth, fruit an achene (nutlet), and unique conical silica bodies (Judd *et al.*, 2016). Inflorescences are a complex arrangement of small spikes (spikelets). The family has a worldwide distribution and includes about 5600 species (Govaerts *et al.*, 2018). Spalink *et al.* (2016) suggested that Cyperaceae originated in South America in the late Cretaceous. Two major genera besides *Carex* L. (~2000 spp.), which has a cosmopolitan distribution with a concentration in cold Holarctic regions, are *Cyperus* L. (~950 spp.) and *Fimbristylis* Vahl (~300 spp.), two mainly tropical genera extending towards the temperate regions (Govaerts *et al.*, 2018). Several Cyperaceae genera follow the C_4 photosynthetic pathway (Bruhl & Wilson, 2007), which is associated with Kranz anatomy, i.e. the presence of a green sheath of Kranz cells around the vascular bundles. Multiple origins of C_4 photosynthesis out of C_3 lineages associated with variants of the Kranz anatomy (e.g. in *Cyperus* and *Fimbristylis*; Goetghebeur, 1998) have recently been confirmed by phylogenetic analysis (e.g. Besnard *et al.*, 2009).

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There has been much recent phylogenetic work on Cyperaceae worldwide. Muasya *et al.* (2009), based on an analysis of the plastid *rbcL*, *trnL* intron and *trnL–F* intergenic spacer regions, found two primary clades in Cyperaceae, which led them to revise the previous subfamilial classification based on morphological characters (Bruhl, 1995; Goetghebeur, 1998). They recognised two subfamilies, Mapanioideae and Cyperoideae, and so merged the subfamilies Cyperoideae, Caricoideae and Sclerioideae of Goetghebeur (1998) into a single large subfamily, Cyperoideae, which is sister to Mapanioideae. Larridon *et al.* (2011a, 2013) performed phylogenetic studies in the large genus *Cyperus* using the nuclear ETS 1f and the plastid *rpl32–trnL* and *trnH–psbA* regions. They established that the eucyperoid C_3 lineages of *Cyperus* are distributed over several basally branching clades, and that the clade containing all C_4 *Cyperus* species, which are of the chlorocyperoid anatomy type, is nested in the C_3 grade. However, the species sampled for the molecular phylogenetic studies so far are not evenly distributed among tribes (Muasya *et al.*, 2009) nor among regions of the world. To date, there are few sequence data for Cyperaceae from mainland Southeast Asia (the Indochinese Peninsula) and none from Cambodia.

In September 2010, during an excursion to Cambodia by members of the Institute of Botany of the University of Natural Resources and Life Sciences, Vienna, we had the opportunity of sampling specimens of Cyperaceae. The taxa sampled belong to various tribes of the large subfamily Cyperoideae. Our aim was to contribute DNA sequences of these taxa to the overall phylogeny of the family by focusing on the rapidly evolving nuclear ribosomal internal transcribed spacer (ITS), a useful marker for species-level phylogenetic studies (Nieto Feliner & Rosselló, 2007). This region has already been widely used in Cyperaceae phylogenetics, for example in *Carex* (Global *Carex* Group, 2016), *Cyperus* (Reid *et al.*, 2014), Abildgaardieae (Ghamkhar *et al.*, 2007) and Schoeneae (Viljoen *et al.*, 2013). Based on the integration of the new sequence data with existing data, we were specifically interested in the phylogenetic placement and the biogeographical relationships of the Cambodian taxa. We also aimed to detect taxonomically unclear groups with need for further investigation. Finally, our study should help advance floristic knowledge of Cambodian Cyperaceae.

Materials and Methods

Plant material

The field trip was carried out in the framework of a university course for master's degree students in Landscape Architecture and Landscape Planning. The excursion was led by Karl-Georg Bernhardt, Klaus Hackländer, Steffen Hameister and Hermann Schacht. Thirty-one people, including 23 students, participated. Various regions of Cambodia were visited, from Sihanoukville Province in the south to Banteay Meanchey Province in the northwest and Ratanakiri Province in the northeast. Habitats explored included wetlands, secondary forests, agricultural and forestry land, beaches and mangroves, and historic temple complexes. Species of Cyperaceae were collected from 9 to 25 September 2010 (Table 1). Leaf material was preserved in silica gel and vouchers of all samples were

TABLE 1. Samples of Cambodian Cyperaceae species used for DNA sequencing in the present study[a]

Accepted name (*World Checklist of Cyperaceae*[b]) and name used in the *Flora of Thailand*[c] (in parentheses)	Locality (collection no., herbarium accession no.)	European Nucleotide Archive accession no.
Actinoscirpus grossus (L.f.) Goetgh. & D.A.Simpson	Ratanakiri, SE Banlung, 13.569°N, 107.232°E (Cyp63, WHB 52012)	LS999522
	Banteay Meanchey, NW Siem Reap city, 13.791°N, 103.317°E (Cyp23, WHB 52013)	LS999523
Bulbostylis barbata (Rottb.) C.B.Clarke	Siem Reap, NNE Siem Reap city, Pre Rup temple site, 13.435°N, 103.921°E (Cyp7, WHB 53774)	LS999524
Cyperus babakan Steud.	Banteay Meanchey, NW Siem Reap city, 13.689°N, 103.291°E (Cyp21, WHB 53977)	LS999525
Cyperus diffusus Vahl (*Cyperus laxus* Lam.)	Siem Reap, NE Siem Reap city, Beng Mealea temple site, 13.472°N, 104.229°E (Cyp25, WHB 53989)	LS999526
	Siem Reap, N Siem Reap city, Preah Khan temple site, 13.465°N, 103.872°E (Cyp10, WHB 53990)	LS999527
Cyperus haspan L.	Banteay Meanchey, NW Siem Reap city, 13.689°N, 103.291°E (Cyp13, WHB 54010)	LS999528
	Kratié, N Kratié city, 13.288°N, 106.105°E (Cyp76, WHB 54066)	LS999529
Cyperus leptocarpus (F.Muell.) Bauters (*Lipocarpha microcephala* (R.Br.) Kunth)	Kratié, NE Kratié city, 12.511°N, 106.070°E (Cyp34, WHB 51950)	LS999530
Cyperus leucocephalus Retz.	Preah Vihear, S Koh Ker temple site, 13.768°N, 104.543°E (Cyp31, WHB 54063)	LS999531
Cyperus nutans Vahl	Ratanakiri, S Banlung, Katieng waterfall, 13.666°N, 106.976°E (Cyp45, WHB 54018)	LS999532
Cyperus cf. *paniceus* (Rottb.) Boeckeler	Preah Vihear, S Koh Ker temple site, 13.731°N, 104.553°E (Cyp27, WHB 54014)	LS999533
Cyperus pulcherrimus Willd. ex Kunth	Banteay Meanchey, NW Siem Reap city, 13.689°N, 103.291°E (Cyp19, WHB 54013)	LS999534
	Ratanakiri, NNW Banlung, 13.916°N, 106.887°E (Cyp66, WHB 54064)	LS999535
Cyperus pumilus L. (*Pycreus pumilus* (L.) Nees)	Kratié, NE Kratié city, 12.511°N, 106.070°E (Cyp37, WHB 53988)	LS999536

TABLE 1. *(Continued)*

Accepted name (*World Checklist of Cyperaceae*[b]) and name used in the *Flora of Thailand*[c] (in parentheses)	Locality (collection no., herbarium accession no.)	European Nucleotide Archive accession no.
Cyperus rotundus L.	Kampong Cham, E Kampong Cham city and E Mekong, 11.980°N, 105.480°E (Cyp33b, WHB 54007)	LS999537
Cyperus tenuispica Steud.	Kampong Speu, W Phnom Penh, 11.480°N, 104.598°E (Cyp89, WHB 53997)	LS999538
Cyperus trialatus (Boeckeler) J.Kern	Ratanakiri, NNE Banlung, between 13.864°N, 107.044°E and 13.883°N, 107.019°E (Cyp51, WHB 54001)	LS999539
Cyperus cf. *trialatus*	Ratanakiri, S Banlung, Katieng waterfall, 13.666°N, 106.976°E (Cyp46, WHB 53982)	LS999540
Eleocharis koyamae Tremetsb. & D.A.Simpson (*Eleocharis macrorrhiza* T.Koyama, nom. illeg.)	Banteay Meanchey, NW Siem Reap city, 13.791°N, 103.317°E (Cyp24, WHB 53643)	LS999541
Fimbristylis acuminata Vahl	Banteay Meanchey, NW Siem Reap city, 13.689°N, 103.291°E (Cyp15, WHB 53645)	LS999542
Fimbristylis cf. *bisumbellata* (Forssk.) Bubani	Kratié, NE Kratié city, 12.511°N, 106.070°E (Cyp81, WHB 51959)	LS999543
	Preah Vihear, S Koh Ker temple site, 13.755°N, 104.548°E (Cyp33, WHB 51961)	LS999544
Fimbristylis dichotoma (L.) Vahl	Kampong Speu, near Kirirom National Park, 11.321°N, 104.107° E (Cyp82, WHB 51955)	LS999545
	Ratanakiri, SE Banlung, 13.569°N, 107.232°E (Cyp71, WHB 51975)	LS999546
Fimbristylis dichotoma group	Preah Vihear, S Koh Ker temple site, 13.731°N, 104.553°E (Cyp28, WHB 51980)	LS999547
	Kratié, N Kratié city, 13.288°N, 106.105°E (Cyp75, WHB 51984)	LS999548
Fimbristylis hookeriana Boeckeler	Ratanakiri, N Banlung, 13.833°N, 107.002°E (Cyp57, WHB 51926)	LS999549
Fimbristylis insignis Thwaites	Ratanakiri, NNW Banlung, 13.916°N, 106.887°E (Cyp65, WHB 51956)	LS999550

TABLE 1. *(Continued)*

Accepted name (*World Checklist of Cyperaceae*[b]) and name used in the *Flora of Thailand*[c] (in parentheses)	Locality (collection no., herbarium accession no.)	European Nucleotide Archive accession no.
Fimbristylis quinquangularis (Vahl) Kunth subsp. *quinquangularis*	Banteay Meanchey, NW Siem Reap city, 13.689°N, 103.291°E (Cyp18, WHB 51969)	LS999551
(*Fimbristylis miliacea* (L.) Vahl)	Kratié, NE Kratié city, 12.511°N, 106.070°E (Cyp40, WHB 51971)	LS999552
	Ratanakiri, NNW Banlung, 13.873°N, 106.928°E (Cyp62, WHB 51974)	LS999553
Fimbristylis schoenoides (Retz.) Vahl	Kratié, NE Kratié city, 12.511°N, 106.070°E (Cyp41, WHB 51912)	LS999554
Fimbristylis umbellaris (Lam.) Vahl	Kampong Speu, W Phnom Penh, 11.480°N, 104.598°E (Cyp87, WHB 51964)	LS999555
	Banteay Meanchey, NW Siem Reap city, 13.689°N, 103.291°E (Cyp16, WHB 51966)	LS999556
Fuirena umbellata Rottb.	Ratanakiri, NNW Banlung, 13.916°N, 106.887°E (Cyp69, WHB 51928)	LS999557
Scleria levis Retz.	Kratié, N Kratié city, 13.288°N, 106.105°E (Cyp79, WHB 53646)	LS999558
Scleria poiformis Retz.	Banteay Meanchey, NW Siem Reap city, 13.689°N, 103.291°E (Cyp20, WHB 53773)	LS999559

[a] All samples collected by K.-G. Bernhardt and M. M. Wernisch. Vouchers are deposited at WHB. The internal transcribed spacer sequences are available from the European Nucleotide Archive browser at http://www.ebi.ac.uk/ena/data/view/PRJEB27967.
[b] Govaerts *et al.* (2018).
[c] Simpson & Koyama (1998).

deposited in the herbarium of the Institute of Botany of the University of Natural Resources and Life Sciences, Vienna (WHB). We determined the specimens using the *Flora of Thailand* (Simpson & Koyama, 1998) and verified the determination by direct comparison of our vouchers with vouchers deposited at the herbarium of the Royal Botanic Gardens, Kew (K). We then updated the species names with the accepted names by accessing the *World Checklist of Cyperaceae* (Govaerts *et al.*, 2018).

DNA extraction and sequencing

We ground silica gel–dried leaf material with glass beads in a TissueLyser II and extracted DNA from the ground material using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany). For amplification of ITS, we used the primer combinations ITS5/ITS4 (White *et al.*, 1990) or, alternatively, ITSL (Hsiao *et al.*, 1994)/ITS4.

The PCR mix contained 2.5 µL of 10× DreamTaq Buffer, 0.125 µL of 5 U/µL DreamTaq DNA Polymerase and 0.25 µL of 100 mM dNTP Mix (25 mM each; Thermo Scientific; Thermo Fisher Scientific, Waltham, Massachusetts, USA), 1 µL of forward primer and 1 µL of reverse primer (each at 10 µM), 19.125 µL of water and 1 µL of template DNA. The PCR program involved initial denaturation at 95°C for 3 min; 35 cycles of 95°C for 1 min, 51°C for 1 min and 72°C for 2 min; and a final extension at 72°C for 7 min. Samples that produced single bands on the agarose gel were treated with 0.5 µL of Exonuclease I (20 U/µL) and 1 µL of FastAP Thermosensitive Alkaline Phosphatase (1 U/µL; Thermo Scientific). Single bands of samples that produced multiple bands on the agarose gel were extracted from the gel with the Hi Yield Gel/PCR DNA Fragment Extraction Kit (Süd-Laborbedarf, Gauting, Germany).

Cycle sequencing was done in both directions with the same primers that were used in the PCR. The cycle-sequencing reaction mix contained 1.5 µL of BigDye Terminator v3.1 5× Sequencing Buffer and 1 µL of BigDye Terminator v3.1 Ready Reaction Mix (Applied Biosystems; Thermo Fisher Scientific), 1 µL of 3.5 µM forward or reverse primer, and 6.5 µL of purified PCR product. The program for cycle sequencing was 96°C for 1 min followed by 35 cycles of 96°C for 10 s, 50°C for 5 s and 60°C for 4 min. Ten microlitres of the cycle-sequencing products was purified on Sephadex G-50 Fine (GE Healthcare; General Electric, Boston, Massachusetts, USA) columns prepared in MultiScreen-HV (Merck Millipore; Merck, Darmstadt, Germany) filter plates. The flow-through was resuspended in 10 µL of Hi-Di Formamide and loaded on a 3500 Genetic Analyzer (Applied Biosystems).

Sequence analysis

Forward and reverse sequences were assembled and edited in Geneious version 8.0.4 (Biomatters, Auckland, New Zealand). Hidden Markov Models were used for ITS2 delimitation by comparison with a conserved structural motif in the 5.8S/26S rDNA regions (proximal stem of the ITS2) of the Viridiplantae model available via the ITS2 Database at http://its2.bioapps.biozentrum.uni-wuerzburg.de/ (Keller *et al.*, 2009). To exclude possible pseudogenic sequences, we verified the presence of the conserved angiosperm 5.8S rDNA motifs M1, M2 and M3 and reconstructed the 5.8S rRNA secondary structure following Harpke & Peterson (2008). The reconstructions were done at a folding temperature of 37°C using the RNA-folding form of the mfold version 4.7 web server (http://unafold.rna.albany.edu/?q=mfold/RNA-Folding-Form; Zuker, 2003), with the same constraints for base pairings for the helices B4–B8 as denoted in Harpke & Peterson (2008).

We then merged our sequences with highly similar sequences downloaded from the National Center for Biotechnology Information GenBank for phylogenetic analysis (Appendix I), applying taxon names to the downloaded sequences according to the *World Checklist of Cyperaceae* (Govaerts *et al.*, 2018). We carried out global alignments of ITS1 and ITS2 separately by using the Q-INS-i strategy, which incorporates structural information of non-coding RNAs (Katoh & Toh, 2008), implemented in the online version

of MAFFT version 7 (https://mafft.cbrc.jp/alignment/server/; Katoh *et al.*, 2017), with the following settings: unalignlevel = 0.0, try to align gappy regions anyway, scoring matrix for nucleotide sequences = 200PAM/κ = 2, gap opening penalty = 1.53, offset value = 0.0, and n is treated like a wildcard. We then used RAxML version 8.2.10 for maximum likelihood phylogenetic inference (1000 searches starting from 1000 maximum parsimony starting trees; Stamatakis, 2014), specifying that individual base frequencies, GTR rates (Rodríguez *et al.*, 1990), and shape parameters α of the gamma model of rate heterogeneity (i.e. individual GTR+G models of nucleotide substitution) should be estimated separately for the partitions 18S rDNA, ITS1, 5.8S rDNA, ITS2 and 26S rDNA. We also conducted 1000 standard non-parametric bootstrap replicate searches.

FigTree version 1.4.2 (Rambaut, 2006–2014) was used to display the bootstrap values on the best tree and to root it at midpoint. Despite some obviously misaligned positions, manual modifications of the alignment did not substantially improve the resolution of the phylogenetic tree, so we used the MAFFT alignment without further modification. The alignment and tree are available from TreeBASE (www.treebase.org; Piel *et al.*, 2009; Vos *et al.*, 2012) under the study accession number S23576.

For the biogeographical interpretation of the phylogenetic tree, we extracted species distributions from the *World Checklist of Cyperaceae* (Govaerts *et al.*, 2018). The regions considered are Africa and the western Indian Ocean (Madagascar and nearby islands), tropical and subtropical Asia and Oceania (Australia and islands of the eastern Indian Ocean and western Pacific), temperate Asia, and America. Extensions of a few species into Europe are not delineated.

Results

A new name in Eleocharis

Eleocharis macrorrhiza T.Koyama (Koyama, 1979), the name of one of the taxa included in our analysis, was shown by Govaerts *et al.* (2018) to be illegitimate. However, no supporting information for this designation was provided. Further investigation showed it to be illegitimate under Article 53.2 of the *International Code of Nomenclature for Algae, Fungi and Plants* (Turland *et al.*, 2018) because of the earlier published *Eleocharis macrorhiza* Boeckeler (1858), a 'confusingly similar name' as defined by the Code, which refers to an unrelated American species. Therefore, the following new name is proposed.

Eleocharis koyamae Tremetsb. & D.A.Simpson, **nom. nov.** – *Eleocharis macrorrhiza* T. Koyama, Brittonia 31: 285 (1979), nom. illegit., non *Eleocharis macrorhiza* Boeckeler, Flora 41: 413 (1858). – Type: Thailand, Thung Salaeng Luang, *Shimzu* T-11303 (holo KYO).

New records for Cambodia

We collected several species in Cambodia which have not been recorded in this country by Govaerts *et al.* (2018), namely *Cyperus babakan* Steud. along a ditch in an area of large-scale rice cultivation; *C. leucocephalus* Retz. in a temple complex; *C. pulcherrimus* Willd. ex Kunth in the same place as *C. babakan* (LS999534) and in an open regeneration

area of dipterocarp forest (LS999535); *C. pumilus* L. in another open regeneration area of dipterocarp forest; *C. tenuispica* Steud. in a wet place near a body of stagnant water; *C. trialatus* (Boeckeler) J.Kern along a 'bamboo trail' in partly cultivated, partly wooded, hilly landscape (LS999539) and in woods near a waterfall (LS999540 [*C.* cf. *trialatus*]); *Eleocharis koyamae* along a ditch; *Fimbristylis acuminata* Vahl in the same place as *C. babakan*; *F. dichotoma* (L.) Vahl in various open regeneration areas of dipterocarp forest (all samples, including also those assigned to the group of *F. dichotoma*); *F. hookeriana* Boeckeler on a lava field; *F. insignis* Thwaites in the same place as *C. pulcherrimus* LS999535; *F. quinquangularis* (Vahl) Kunth in the same place as *C. babakan* (LS999551), in the same place as *C. pumilus* (LS999552) and along a path in a community forest (LS999553); and *F. schoenoides* (Retz.) Vahl in the same place as *C. pumilus*. The identity of two species that are difficult to identify, *Cyperus paniceus* (Rottb.) Boeckeler, found in the same place as the sample of the *Fimbristylis dichotoma* group LS999547, and *F. bisumbellata* (Forssk.) Bubani, found in the same place as *C. pumilus* (LS999543) and in a temple complex (LS999544), still needs to be confirmed after more detailed taxonomic work has been done. Cambodia lies within the previously described range of all these species except *Eleocharis koyamae*.

DNA sequences

The 5.8S rDNA motifs M1 (CGATGAAGAACGTAGC), M2 (GAATTGCAGAATCC) and M3 (TTTGAAYGCA, whereby Y is represented by C in our sequences), which may serve to identify pseudogenes in angiosperms (Jobes & Thien, 1997; Harpke & Peterson, 2008), are invariably present in all sequences. The conserved helices B4–B8 of angiosperm 5.8S rRNA are also present in all sequences (exemplified for *Cyperus babakan* in Appendix II; Harpke & Peterson, 2008), the GC content of the 5.8S rDNA is rather uniform among all sequences (Appendix III), and there are no polymorphic sites in our 5.8S rRNA sequences. These results gave us no reasons to assume that non-functional gene copies (i.e. pseudogenes) were to be found among our sequences, so we were able to use all the sequences for phylogenetic analysis.

The GC content as well as the length of the sequences of ITS1 and ITS2 varies greatly among the genera (Appendix III). In ITS1, there was one polymorphic site with two nucleotide peaks in *Bulbostylis barbata* (Rottb.) C.B.Clarke (LS999524), one in *Cyperus leptocarpus* (F.Muell.) Bauters (LS999530), three in *C. leucocephalus* (LS999531), one in *C. pumilus* (LS999536), two in *C. rotundus* L. (LS999537) and one in each of two accessions of *Fimbristylis quinquangularis* (LS999551, LS999553). In ITS2, there were three such polymorphic sites in *Bulbostylis barbata* (LS999524), one in *Cyperus leptocarpus* (LS999530), one in *C. leucocephalus* (LS999531), three in *C. rotundus* (LS999537), one in *Fimbristylis umbellaris* (Lam.) Vahl (LS999556) and one in *Scleria poiformis* Retz. (LS999559). The infrequent occurrence of polymorphic sites suggests that the presence of paralogous variants is not common in the Cyperaceae genera studied (Nieto Feliner & Rosselló, 2007).

Phylogenetic analysis

We provided new ITS sequences for the following species that have not previously been included in a molecular phylogenetic analysis: *Cyperus babakan*, *C. leucocephalus*, *C.* cf. *paniceus*, *C. pulcherrimus*, *C. trialatus*, *Eleocharis koyamae*, *Fimbristylis hookeriana*, *F. insignis* and *Scleria levis* Retz. The sequences of both accessions of *Actinoscirpus grossus* (L.f.) Goetgh. & D.A.Simpson (LS999522, LS999523) are identical, as are the sequences of both accessions of *Cyperus diffusus* Vahl (LS999526, LS999527), *C. haspan* L. (LS999528, LS999529), *Fimbristylis* cf. *bisumbellata* (LS999543, LS999544) and *F. dichotoma* (LS999545, LS999546). The sequences of the two accessions of *Fimbristylis umbellaris* (LS999555, LS999556) are almost identical.

The MAFFT structure-based alignment of ITS sequences in conjunction with maximum likelihood phylogenetic estimation yielded a highly resolved phylogenetic tree (Figs 1, 2).

Discussion

Generic relationships

The order of appearance of the genera analysed on the phylogenetic tree reflects the order shown in Jung & Choi (2013), except for *Scleria* P.J.Bergius (tribe Sclerieae). As one of the early-diverged genera in subfamily Cyperoideae, it should be sister to the remaining genera we analysed, all of which belong to the FAEC clade consisting of four tribes (Fuireneae, Abildgaardieae, Eleocharideae and Cypereae). In our midpoint-rooted tree, however, *Scleria* is sister to *Fuirena*, *Eleocharis*, *Bulbostylis* and *Fimbristylis* but not to *Actinoscirpus*, *Schoenoplectus* or *Cyperus*. We suggest that this discrepancy results from the choice of molecular markers. Whereas the more slowly evolving *rbcL* and *trnL–F* used in addition to ITS by Jung & Choi (2013) would be better suited to resolving deeper phylogenetic relationships in the family, our phylogeny based on ITS would only be able to resolve species relationships within genera. However, deeper relationships, such as those among *Scleria* and the genera of the FAEC clade, might be distorted due to higher levels of homoplasy.

Tribe Fuireneae is split into four clades (Jung & Choi, 2013), two of which are represented in our sample (the *Fuirena* Rottb. clade and the *Actinoscirpus* (Ohwi) R.W.Haines & Lye and *Schoenoplectus* (Rchb.) Palla clade). Eleocharideae (*Eleocharis* R.Br.) and Abildgaardieae (represented by *Bulbostylis* Kunth and *Fimbristylis* in our sample) are sister to each other, and together they are sister to *Fuirena*. The clade comprising *Actinoscirpus*, *Schoenoplectus* and Cypereae is characterised by a 3-bp deletion in the 5.8S rRNA gene (see also Yano *et al.*, 2012).

In the following, we discuss the phylogenetic placement and biogeographical relationships of the Cambodian taxa, with special emphasis on taxonomically unclear groups with need for further evaluation. The genera are discussed in the order in which they appear on the phylogenetic tree.

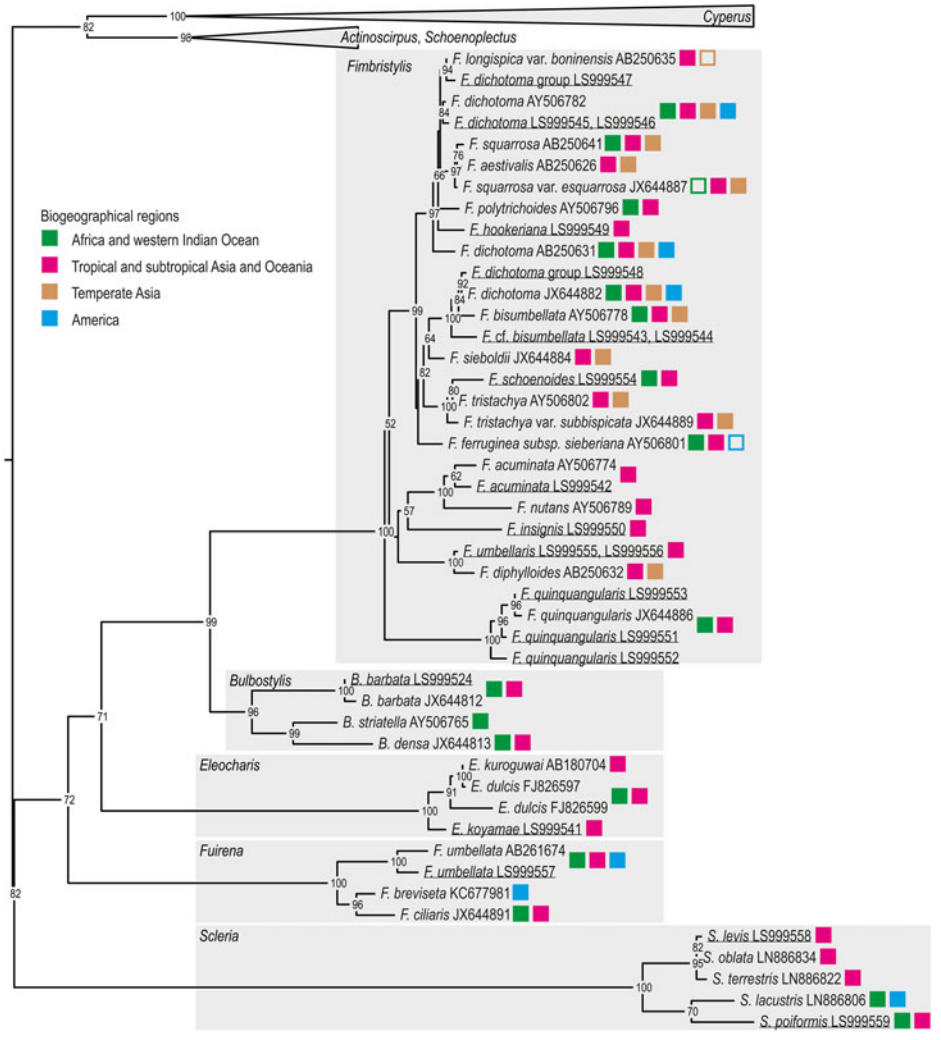

FIG. 1. Midpoint-rooted best maximum likelihood tree of concatenated 18S rDNA (3′ end), internal transcribed spacer (ITS) 1, 5.8S rDNA, ITS2, and 26S rDNA (5′ end) sequences with individual GTR+G models of nucleotide substitution optimised for each partition. Node labels are bootstrap support values (1000 replicates). Only values ≥ 50 are shown. Newly provided sequences are underlined. Coloured boxes denote the main distribution of species in biogeographical regions. For subspecies or varieties, boxes for regions occupied by the species, but not the inferior taxon, are not filled. The clades comprising *Actinoscirpus*, *Schoenoplectus* and *Cyperus* are collapsed.

Scleria

Scleria is a pantropical genus, locally extending to warm temperate regions (Goetghebeur, 1998), with a presumed origin in South America (Spalink *et al.*, 2016). Our sequence of *Scleria poiformis* is almost identical to the sequence of the same species with the GenBank

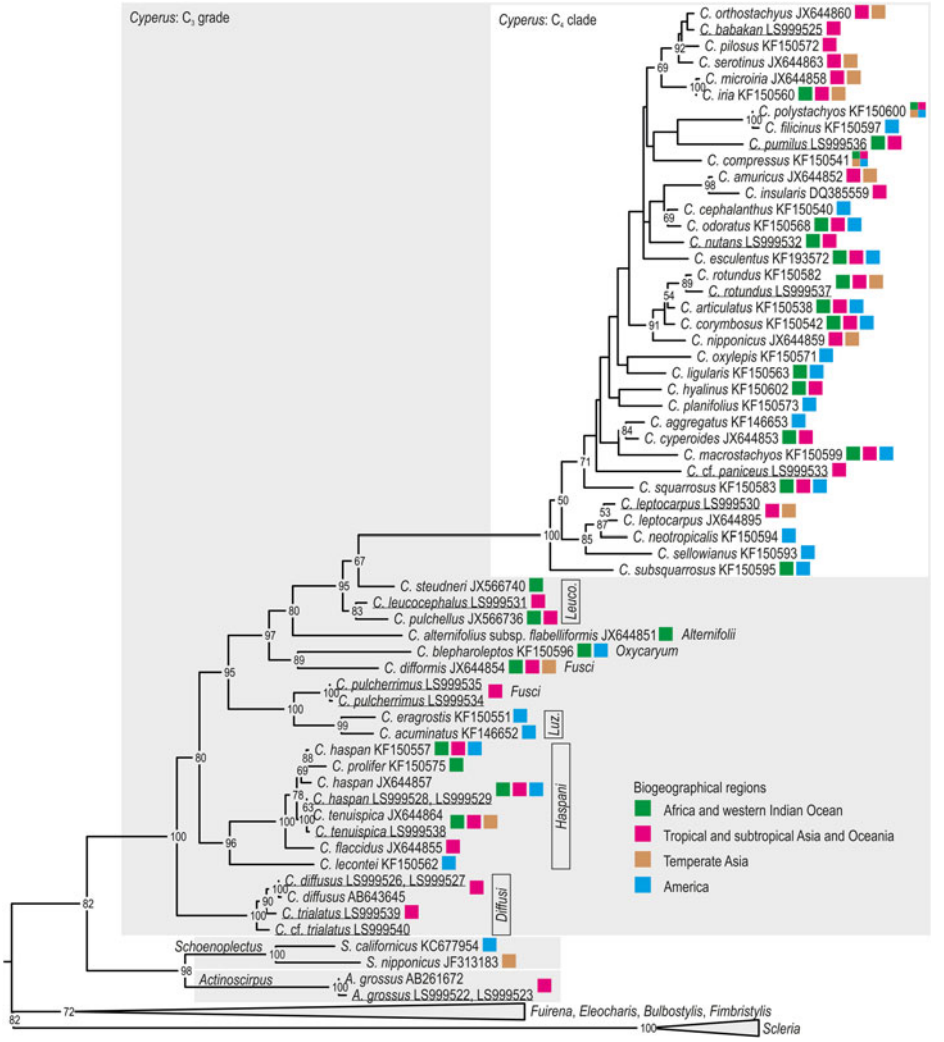

FIG. 2. The same tree as in Fig. 1, but this time the clades comprising *Actinoscirpus*, *Schoenoplectus* and *Cyperus* are expanded, and the clades shown in Fig. 1 are collapsed.

accession number LN886914 (Bauters *et al.*, 2016), which was therefore not included in the analysis. Together with *Scleria lacustris* C.Wright, the species belongs to section *Margaleia* of subgenus *Scleria*. The hypothetical area of origin of this section is in Africa, where both species occur. The African origin is also supported by the sister group relationship of sections *Margaleia* and *Acriulus* of subgenus *Scleria*, as the basally branching species of section *Acriulus* are solely distributed in Africa (with one species also occurring in Madagascar; Bauters *et al.*, 2016). We may thus hypothesise that *Scleria poiformis* expanded from Africa to tropical and subtropical Asia and Australia.

Scleria levis is nested within subgenus *Scleria*, section *Elatae*, as suggested by Bauters *et al.* (2016) based on morphology. The species of this section occur in tropical and subtropical Asia and Oceania (with one species extending to the Seychelles), as does *Scleria levis*.

Fuirena

Fuirena occurs mostly in the tropics and subtropics, with the centre of diversity and presumed origin in Africa (Spalink *et al.*, 2016). Phylogenetic analysis revealed some sequence variation among the available sequences of *Fuirena umbellata* Rottb. (Hirahara *et al.*, 2007; Jung & Choi, 2013 [GenBank accession number JX644892, not included in analysis]), the only species in the genus with a worldwide tropical and subtropical distribution, and no close relatives. However, few species of this genus have yet been sequenced.

Eleocharis

Eleocharis is a cosmopolitan genus with a marked concentration of taxa in (sub)tropical America (Goetghebeur, 1998) and a presumed origin in North America (Spalink *et al.*, 2016). *Eleocharis koyamae* is closely related to *E. dulcis* (Burm.f.) Trin. ex Hensch. and belongs to the *E. dulcis* clade of *Eleocharis* subgenus *Limnochloa* (González-Elizondo & Peterson, 1997; Roalson & Friar, 2000; Yano *et al.*, 2004; Hinchliff & Roalson, 2009). Both species have round, transversely septate culms (Simpson & Koyama, 1998). The distinguishing character is the shape of the glume apex. It is acute in *Eleocharis koyamae* versus obtuse to rounded-truncate in *E. dulcis*. The morphologically and genetically variable *Eleocharis dulcis* complex is distributed in the tropical and subtropical Old World. *Eleocharis koyamae* was thought to be endemic to Thailand (Simpson & Koyama, 1998; Govaerts *et al.*, 2018) and is now also known from Cambodia. The species is distinct from *Eleocharis dulcis* and *E. kuroguwai* Ohwi in our tree, but sequencing of further individuals of the complex is necessary for a clearer picture.

Bulbostylis

Bulbostylis is distributed worldwide, mainly in the tropics and subtropics, with a primary centre of diversity and presumed origin in Africa and a secondary centre of diversity in America (Reutemann *et al.*, 2018). Only a few species occur in Asia. Based on the presently available sequences, phylogenetic analysis reveals an isolated, rather early-branching position within the genus and no close relatives of *Bulbostylis barbata*. This is one of the few species in the genus with a large, intercontinental distribution spanning tropical and subtropical Africa, Southeast Asia and Oceania.

Fimbristylis

In contrast to *Bulbostylis*, species of *Fimbristylis* are concentrated in the tropical and subtropical regions of Southeast Asia, Malesia and Northeast Australia (Goetghebeur, 1998).

Some phylogenetic analyses have been undertaken (Yano & Hoshino, 2006; Ghamkhar *et al.*, 2007; Jung & Choi, 2013; Wangwasit *et al.*, 2017), but a comprehensive phylogenetic study that could be used to test the sectional classification on morphological grounds proposed by Kern (1974) does not exist. The backbone of the phylogenetic tree of *Fimbristylis* is weakly resolved, which suggests a rapid radiation.

Sequence analysis revealed some variation among the Cambodian accessions of *Fimbristylis quinquangularis*. The Korean (GenBank accession number JX644886) and Japanese (AB250637; not shown) sequences are nested within the Cambodian sequences. The species has a wide distribution across tropical Africa, tropical and subtropical Asia, and Australia (Govaerts *et al.*, 2018). Phylogenetic analysis did not reveal any close relative. A relationship with *Fimbristylis littoralis* Gaudich. and some other species has been suggested based on morphology (section *Miliaceae*; Kern, 1974) and combined ITS and plastid DNA sequences, but without bootstrap support (Jung & Choi, 2013). Based on the sequences available to date, *Fimbristylis quinquangularis* thus appears to be a rather isolated early-diverged species within the genus.

The Cambodian sequences of *Fimbristylis umbellaris* (also section *Miliaceae*; Kern, 1974) are related to the available sequence of *F. umbellaris* with the GenBank accession number JX644885 (not used in our analysis), which is almost identical to sequences of *F. diphylloides* Makino (JX644883 [not used] and AB250632). The two species are closely related (Jung & Choi, 2013). *Fimbristylis umbellaris* is a perennial occurring in tropical and subtropical Asia and Oceania, whereas *F. diphylloides* is an annual or short-lived perennial occurring in more temperate regions of eastern Asia (southern and eastern China, southern Korea, and central and southern Japan; Lunkai *et al.*, 2010; Govaerts *et al.*, 2018).

Phylogenetic analysis did not conclusively support any close relative of *Fimbristylis insignis*, a species with a wide distribution in tropical and subtropical Asia and Oceania (section *Cymosae*; Kern, 1974), among the sequences available to date.

The Cambodian sequence of *Fimbristylis acuminata* groups with the available sequence from Australia (AY506774), but with some sequence divergence. The species is related to *Fimbristylis nutans* (Retz.) Vahl (Ghamkhar *et al.*, 2007), as also suggested by morphology (section *Nutantes*; Kern, 1974). Both species are widely distributed across tropical and subtropical Asia and Oceania.

The remaining taxa analysed in this study form a well-supported group. *Fimbristylis schoenoides* is related to *F. tristachya* R.Br., as also suggested by morphology (section *Dichelostylis*; Kern, 1974; Simpson & Koyama, 1998). The two species co-occur in tropical and subtropical Asia and Oceania, but *Fimbristylis schoenoides* extends its range to tropical Africa and *F. tristachya* to temperate regions of eastern Asia.

Two sequences (LS999543, LS999544) have been tentatively assigned to *Fimbristylis bisumbellata*, which is, however, difficult to differentiate from *F. merrillii* J.Kern. These Cambodian sequences are related to *Fimbristylis bisumbellata* (AY506778), a widespread species in tropical to warm temperate regions of the Old World (Simpson & Koyama, 1998). They also group with sequences assigned to the complex of *Fimbristylis dichotoma* (JX644882, LS999548), which resembles *F. bisumbellata* morphologically (section *Fimbristylis*; Kern, 1974). Other sequences of the *Fimbristylis dichotoma* complex

(AB250631, AY506782, LS999545–LS999547), however, appear in a different but likewise well-supported group. *Fimbristylis dichotoma* is distributed in tropical to warm temperate regions worldwide (Simpson & Koyama, 1998). Further investigations are necessary to unravel the cryptic variation in this complex, which is known for its wide range of morphological variation and taxonomic difficulty (Kern, 1974; Yano & Hoshino, 2006; Yarrayya & Ratna Kumar, 2018). *Fimbristylis hookeriana*, a species distributed from Assam to Southwest Korea (Govaerts *et al.*, 2018), is also part of the group containing some sequences of the *F. dichotoma* complex as well as sequences of other species which all occur in Southeast Asia and partly extend to Africa or temperate Asia (e.g. *F. polytrichoides* and *F. squarrosa*).

Actinoscirpus

Actinoscirpus is a monotypic genus related to *Schoenoplectus* (Shiels *et al.*, 2014). Whereas *Schoenoplectus* is cosmopolitan, *Actinoscirpus grossus* occurs from tropical and subtropical Asia to North Australia (Govaerts *et al.*, 2018).

Cyperus

This genus occurs worldwide from tropical to temperate regions, with a concentration of species and presumed origin in tropical Africa (Goetghebeur, 1998; Spalink *et al.*, 2016). Our results corroborate recent phylogenetic analyses, which show that the C_4 clade (corresponding to subgenus *Cyperus*) is derived from the C_3 species that branch off basally within the genus (C_3 grade corresponding to subgenus *Anosporum*; Besnard *et al.*, 2009; Larridon *et al.*, 2011a, 2011b, 2013; Jung *et al.*, 2016).

Among the C_3 species, *Cyperus trialatus* and *C. diffusus* resemble each other morphologically (Simpson & Koyama, 1998 [In the *Flora of Thailand*, *C. diffusus* is treated as a synonym of *C. laxus* Lam.]; Lunkai *et al.*, 2010). They are also closely related in the phylogenetic analysis. Both species belong to section *Diffusi* (Larridon *et al.*, 2011a), which is distributed across (sub)tropical regions in Africa, America and Asia. *Cyperus diffusus* grows in tropical and subtropical Asia and Oceania. *Cyperus trialatus* occurs sympatrically but has a narrower distribution from Southeast China to West Malesia. A comprehensive sampling of species in the section is needed to infer biogeographical relationships among the species.

Cyperus haspan and *C. tenuispica* are similar morphologically. *Cyperus haspan* may form creeping rhizomes so that the culms may be tufted or scattered, whereas the culms of *C. tenuispica* are always tufted (Goetghebeur, 1998; Simpson & Koyama, 1998; Lunkai *et al.*, 2010). Both species belong to section *Haspani* (Larridon *et al.*, 2011a), which inhabits (sub)tropical regions in Africa, America and Asia, as does section *Diffusi*. The greatest concentration of species of section *Haspani* is in Africa. *Cyperus haspan* is widely distributed across tropical and subtropical regions worldwide. *Cyperus tenuispica* also occupies a large and mostly sympatric range but is absent from the New World and extends

to more temperate regions in Asia. The phylogenetic analysis reveals that *Cyperus haspan* is paraphyletic with respect to *C. tenuispica* and *C. prolifer* Lam. The African *Cyperus prolifer* appears to be derived from *C. haspan*. A re-evaluation of specific limits based on morphological and molecular characters seems to be needed especially for the highly similar *Cyperus haspan* and *C. tenuispica*. The ITS sequences also suggest that the Australian *Cyperus flaccidus* R.Br. should be transferred from section *Graciles* (Larridon *et al.*, 2011a) to section *Haspani* (see also Jung & Choi, 2013; Reid *et al.*, 2014). As for section *Diffusi*, more comprehensive sampling is needed to resolve species limits and biogeographical relationships in section *Haspani*.

Interestingly, *Cyperus pulcherrimus* from tropical Asia and Oceania is more closely related to species of section *Luzuloidei* (represented here by *C. acuminatus* Torr. & Hook. and *C. eragrostis* Lam.; all species of the section occur in America) than to species of sections *Fusci* (represented here by *C. difformis* L.), in which it is placed on morphological grounds, or *Oxycaryum* (represented here by *C. blepharoleptos* Steud.; Larridon *et al.*, 2011a; Reid *et al.*, 2014), apparently suggesting long-distance dispersals from the presumed African area of origin (Spalink *et al.*, 2016).

Internal transcribed spacer analysis confirms the close relationship of *Cyperus leucocephalus* and *C. pulchellus* R.Br., which are both placed in section *Leucocephali* (Larridon *et al.*, 2011a). The section occurs with a few species across tropical regions in Africa, Asia and America. *Cyperus leucocephalus* (Indian Subcontinent to Indochina) and *C. pulchellus* (Africa, Malesia and Australia) are the only members of the section occurring in Southeast Asia.

Among the C_3 *Cyperus*, section *Leucocephali* sensu Larridon *et al.* (2011b), i.e. including *Kyllingiella* R.W.Haines & Lye (represented here by *C. steudneri* (Boeckeler) Larridon) is closest to the C_4 clade (Larridon *et al.*, 2011a; Muasya *et al.*, 2014). Based on the distribution of species, Larridon *et al.* (2013) supposed that the C_4 species originated in East Africa. Basal relationships among C_4 *Cyperus* are poorly resolved, largely impeding a sectional classification (Larridon *et al.*, 2011b, 2013; Reid *et al.*, 2014). Nevertheless, some species show strong relationships.

Our sequence of *Cyperus leptocarpus* groups with the GenBank sequence of the same species (accession number JX644895). This plant from Asia and Oceania is related to species from Africa and America represented here by *Cyperus neotropicalis* Alain (Bauters *et al.*, 2014).

Cyperus paniceus is morphologically similar to *C. cyperinus* (Retz.) Valck.Sur. and *C. cyperoides* (L.) Kuntze (Simpson & Koyama, 1998). These species often cannot be distinguished easily (Prasad & Singh, 2002). *Cyperus cyperoides* is widely distributed across Africa, Asia and Oceania, and *C. cyperinus* across Asia and Oceania. *Cyperus paniceus* has a narrower distribution from the Indian subcontinent to Indochina (Govaerts *et al.*, 2018), but all three species occur sympatrically in Indochina. Our sample is probably *Cyperus paniceus* and is clearly distinct from *C. cyperoides* in the ITS tree. For *Cyperus cyperinus*, no sequence is available to date. A thorough revision of these variable and often-mistaken species is needed (Bryson & Carter, 2008).

Cyperus rotundus is also a variable species (Bryson & Carter, 2008). Our sequence groups with the available sequence of the same species (accession number KF150582). It is related to the pantropical species *Cyperus articulatus* L. and *C. corymbosus* Rottb. (Reid *et al.*, 2014).

Our sequence of *Cyperus nutans* Vahl from Africa, Asia and Oceania is not resolved close to any of the available sequences.

No close relative of *Cyperus pumilus* was detected among the available sequences. It occurs in tropical and subtropical Africa, Asia and Oceania and belongs to the 'basal *Pycreus* lineages' represented here by *Cyperus macrostachyos* Lam., *C. filicinus* Vahl and *C. polystachyos* Rottb. (Larridon *et al.*, 2013).

Cyperus babakan occurs from Southeast Tibet to New Guinea and is closely related to *C. orthostachyus* Franch. & Sav., *C. pilosus* Vahl, which it resembles morphologically, and *C. serotinus* Rottb. These species are centred in Southeast Asia, from where they extend to other regions. *Cyperus orthostachyus* occurs from temperate Asia to Vietnam, *C. pilosus* from tropical and subtropical Asia to East Australia, and *C. serotinus* from temperate Eurasia to Indochina (Govaerts *et al.*, 2018).

Conclusions

More work is necessary to resolve the phylogeny and taxonomy of Cyperaceae of mainland Southeast Asia, as there are still many species from this region that have not yet been sequenced. A detailed phylogenetic analysis based on comprehensive sampling of species across their ranges and the use of additional, highly resolving markers in combination with a re-evaluation of morphological characters is necessary for species delimitation in some variable species groups (e.g. *Fimbristylis dichotoma* and *Cyperus haspan*). Several groups have supposedly speciated in Southeast or East Asia (e.g. *Fimbristylis schoenoides* and *F. tristachya*, *Cyperus diffusus* and *C. trialatus*, as well as *C. babakan*, *C. orthostachyus*, *C. pilosus* and *C. serotinus*). Some supposed speciation events included range extension from tropical to temperate Asia (e.g. in *Fimbristylis umbellaris* and *F. diphylloides*). Many species or species groups, however, have large intercontinental distributions, which relates to their high dispersal ability (Kern, 1974; Goetghebeur, 1998; Bryson & Carter, 2008; Viljoen *et al.*, 2013).

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APPENDIX I

Sequences downloaded from GenBank

Appendix table 1 shows the taxon names of the sequences downloaded from the National Center for Biotechnology Information GenBank for phylogenetic analysis in the present study. The names have been determined according to the *World Checklist of Cyperaceae* (Govaerts *et al.*, 2018).

APPENDIX TABLE 1. Taxon names of sequences downloaded from Genbank for phylogenetic analysis in the present study

Taxon	GenBank accession no.	Reference
***Actinoscirpus* (Ohwi) R.W.Haines & Lye**		
Actinoscirpus grossus (L.f.) Goetgh. & D.A.Simpson	AB261672	Hirahara *et al.* (2007)
***Bulbostylis* Kunth**		
Bulbostylis barbata (Rottb.) C.B.Clarke	JX644812	Jung & Choi (2013)
Bulbostylis densa (Wall.) Hand.-Mazz.	JX644813	Jung & Choi (2013)
Bulbostylis striatella C.B.Clarke	AY506765	Ghamkhar *et al.* (2007)
***Cyperus* L.**		
Cyperus acuminatus Torr. & Hook.	KF146652	Reid *et al.* (2014)
Cyperus aggregatus (Willd.) Endl.	KF146653	Reid *et al.* (2014)
Cyperus alternifolius L. subsp. *flabelliformis* Kük.	JX644851	Jung & Choi (2013)
Cyperus amuricus Maxim.	JX644852	Jung & Choi (2013)
Cyperus articulatus L.	KF150538	Reid *et al.* (2014)
Cyperus blepharoleptos Steud.	KF150596	Reid *et al.* (2014)
Cyperus cephalanthus Torr. & Hook.	KF150540	Reid *et al.* (2014)
Cyperus compressus L.	KF150541	Reid *et al.* (2014)
Cyperus corymbosus Rottb.	KF150542	Reid *et al.* (2014)
Cyperus cyperoides (L.) Kuntze	JX644853	Jung & Choi (2013)

APPENDIX TABLE 1. *(Continued)*

Taxon	GenBank accession no.	Reference
Cyperus difformis L.	JX644854	Jung & Choi (2013)
Cyperus diffusus Vahl	AB643645	Yano *et al.* (2012)
Cyperus eragrostis Lam.	KF150551	Reid *et al.* (2014)
Cyperus esculentus L.	KF193572	Reid *et al.* (2014)
Cyperus filicinus Vahl	KF150597	Reid *et al.* (2014)
Cyperus flaccidus R.Br.	JX644855	Jung & Choi (2013)
Cyperus haspan L.	JX644857	Jung & Choi (2013)
	KF150557	Reid *et al.* (2014)
Cyperus hyalinus Vahl	KF150602	Reid *et al.* (2014)
Cyperus insularis Heenan & de Lange	DQ385559	Gardner *et al.*[a]
Cyperus iria L.	KF150560	Reid *et al.* (2014)
Cyperus lecontei Torr. ex Steud.	KF150562	Reid *et al.* (2014)
Cyperus leptocarpus (F.Muell.) Bauters	JX644895	Jung & Choi (2013)
Cyperus ligularis L.	KF150563	Reid *et al.* (2014)
Cyperus macrostachyos Lam.	KF150599	Reid *et al.* (2014)
Cyperus microiria Steud.	JX644858	Jung & Choi (2013)
Cyperus neotropicalis Alain	KF150594	Reid *et al.* (2014)
Cyperus nipponicus Franch. & Sav.	JX644859	Jung & Choi (2013)
Cyperus odoratus L.	KF150568	Reid *et al.* (2014)
Cyperus orthostachyus Franch. & Sav.	JX644860	Jung & Choi (2013)
Cyperus oxylepis Nees ex Steud.	KF150571	Reid *et al.* (2014)
Cyperus pilosus Vahl	KF150572	Reid *et al.* (2014)
Cyperus planifolius Rich.	KF150573	Reid *et al.* (2014)
Cyperus polystachyos Rottb.	KF150600	Reid *et al.* (2014)
Cyperus prolifer Lam.	KF150575	Reid *et al.* (2014)
Cyperus pulchellus R.Br.	JX566736	Muasya *et al.* (2014)
Cyperus rotundus L.	KF150582	Reid *et al.* (2014)
Cyperus sellowianus (Kunth) T.Koyama	KF150593	Reid *et al.* (2014)
Cyperus serotinus Rottb.	JX644863	Jung & Choi (2013)
Cyperus squarrosus L.	KF150583	Reid *et al.* (2014)
Cyperus steudneri (Boeckeler) Larridon	JX566740	Muasya *et al.* (2014)
Cyperus subsquarrosus (Muhl.) Bauters	KF150595	Reid *et al.* (2014)
Cyperus tenuispica Steud.	JX644864	Jung & Choi (2013)
***Eleocharis* R.Br.**		
Eleocharis dulcis (Burm.f.) Trin.	FJ826597	Hinchliff & Roalson (2009)
ex Hensch.	FJ826599	Hinchliff & Roalson (2009)
Eleocharis kuroguwai Ohwi	AB180704	Yano *et al.* (2004)
***Fimbristylis* Vahl**		
Fimbristylis acuminata Vahl	AY506774	Ghamkhar *et al.* (2007)
Fimbristylis aestivalis (Retz.) Vahl	AB250626	Yano & Hoshino (2006)
Fimbristylis bisumbellata (Forssk.) Bubani	AY506778	Ghamkhar *et al.* (2007)
Fimbristylis dichotoma (L.) Vahl	AB250631	Yano & Hoshino (2006)
	AY506782	Ghamkhar *et al.* (2007)
	JX644882	Jung & Choi (2013)
Fimbristylis diphylloides Makino	AB250632	Yano & Hoshino (2006)

APPENDIX TABLE 1. *(Continued)*

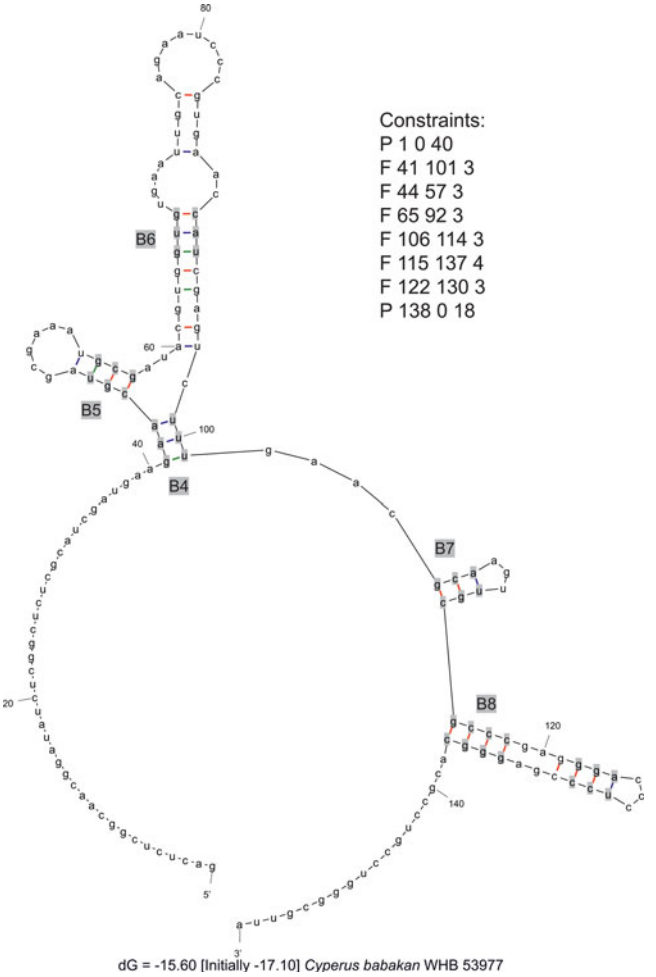
Taxon	GenBank accession no.	Reference
Fimbristylis ferruginea (L.) Vahl subsp. *sieberiana* (Kunth) Lye	AY506801	Ghamkhar *et al.* (2007)
Fimbristylis longispica Steud. var. *boninensis* (Hayata) Ohwi	AB250635	Yano & Hoshino (2006)
Fimbristylis nutans (Retz.) Vahl	AY506789	Ghamkhar *et al.* (2007)
Fimbristylis polytrichoides (Retz.) Vahl	AY506796	Ghamkhar *et al.* (2007)
Fimbristylis quinquangularis (Vahl) Kunth	JX644886	Jung & Choi (2013)
Fimbristylis sieboldii Miq. ex Franch. & Sav.	JX644884	Jung & Choi (2013)
Fimbristylis squarrosa Vahl	AB250641	Yano & Hoshino (2006)
Fimbristylis squarrosa Vahl var. *esquarrosa* Makino	JX644887	Jung & Choi (2013)
Fimbristylis tristachya R.Br.	AY506802	Ghamkhar *et al.* (2007)
Fimbristylis tristachya R.Br. var. *subbispicata* (Nees) T.Koyama	JX644889	Jung & Choi (2013)
***Fuirena* Rottb.**		
Fuirena breviseta (Coville) Coville	KC677981	Shiels *et al.* (2014)
Fuirena ciliaris (L.) Roxb.	JX644891	Jung & Choi (2013)
Fuirena umbellata Rottb.	AB261674	Hirahara *et al.* (2007)
***Schoenoplectus* (Rchb.) Palla**		
Schoenoplectus californicus (C.A.Mey.) Soják	KC677954	Shiels *et al.* (2014)
Schoenoplectus nipponicus (Makino) Soják	JF313183	Jung & Choi (2011)
***Scleria* P.J.Bergius**		
Scleria lacustris C.Wright	LN886806	Bauters *et al.* (2016)
Scleria oblata S.T.Blake ex J.Kern	LN886834	Bauters *et al.* (2016)
Scleria terrestris (L.) Fassett	LN886822	Bauters *et al.* (2016)

[a] To be published in *A New Zealand Biodiversity Database* (authors: R. C. Gardner, J. Keeling, P. J. de Lange, S. D. Wright and E. K. Cameron).

Appendix II

5.8S rRNA secondary structure of Cyperus babakan (LS999525)

The conserved helices B4–B8 (highlighted in grey in Appendix figure 1) were identified according to Harpke & Peterson (2008). The constraints used as input for the RNA-folding form of the mfold version 4.7 web server (Zuker, 2003) are denoted.



Appendix figure 1. Model of the 5.8S rRNA secondary structure of *Cyperus babakan* (LS999525).

Appendix III

GC content of sequences used

Appendix table 2 shows the GC content of the sequences used for phylogenetic analysis in the present study (Figs 1, 2).

Appendix table 2. GC content in percentage and length in bases (mean and standard deviation) of sequences used for phylogenetic analysis in the present study

Group (no. of sequences)	ITS1	5.8S rDNA	ITS2
Cyperus: C_3 grade (22)	GC: 54.0 ± 3.3 Length: 188 ± 2	GC: 57.2 ± 0.4 Length: 155 ± 0	GC: 61.5 ± 2.8 Length: 251 ± 2
Cyperus: C_4 clade (35)	GC: 71.2 ± 2.7 Length: 186 ± 3	GC: 57.4 ± 0.2 Length: 155 ± 0	GC: 73.9 ± 2.2 Length: 218 ± 6
Actinoscirpus (2)	GC: 59.1 ± 0.7 Length: 215 ± 0	GC: 55.8 ± 0.5 Length: 155 ± 0	GC: 64.9 ± 0.2 Length: 222 ± 1
Schoenoplectus (2)	GC: 67.1 ± 0.4 Length: 193 ± 17	GC: 56.8 ± 0.0 Length: 155 ± 0	GC: 72.5 ± 3.8 Length: 207 ± 6
Fuirena (4)	GC: 57.1 ± 1.0 Length: 214 ± 1	GC: 54.7 ± 0.4 Length: 158 ± 0	GC: 62.3 ± 1.3 Length: 250 ± 1
Eleocharis (4)	GC: 63.4 ± 0.6 Length: 222 ± 0	GC: 56.6 ± 0.4 Length: 158 ± 0	GC: 68.7 ± 0.7 Length: 225 ± 0
Bulbostylis (4)	GC: 55.4 ± 2.3 Length: 221 ± 11	GC: 57.0 ± 0.0 Length: 158 ± 0	GC: 61.6 ± 2.9 Length: 231 ± 5
Fimbristylis (29 [ITS1 and ITS2], 28 [5.8S rDNA])	GC: 50.3 ± 2.3 Length: 223 ± 11	GC: 55.7 ± 0.2 Length: 158 ± 0	GC: 56.9 ± 1.3 Length: 237 ± 2
Scleria (5)	GC: 66.1 ± 0.9 Length: 217 ± 1	GC: 56.5 ± 0.3 Length: 158 ± 0	GC: 66.5 ± 2.9 Length: 243 ± 5

ITS, internal transcribed spacer.