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A contribution to the knowledge of charophytes in Myanmar; morphological and genetic identification and ecology notes

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ABSTRACT

Information on the distribution and species composition of charophytes in Myanmar is scarce. Only a few studies on charophytes in ponds were conducted in Myanmar at the end of the nineteenth and first half of the twentieth century and lake habitats were not included in these studies. To increase the knowledge, we investigated *Chara* spp. from seven Myanmar lakes and reservoirs. In a polyphasic approach using morphological traits and DNA barcoding the specimens found were classified as *Chara zeylanica* and *Chara fibrosa. Chara zeylanica* is the most common of the two species found in Myanmar and was observed in five lakes, while *Chara fibrosa* was only found in three lakes. *Chara zeylanica* seems to prefer calcareous lakes while *C. fibrosa* was found in both highly and moderate alkaline lakes. Both species were recorded in low-impacted lakes only, with total phosphorous (TP) concentrations below 20 µg L^{-1}. Increased human impact on freshwater habitats must therefore be considered as a factor reducing *Chara* biodiversity in Myanmar.

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Myanmar; *Chara zeylanica*; *Chara fibrosa*; DNA barcoding; *matK*; *rbcl*

Introduction

Charophytes (Charales, Charophyceae) are macroscopic green algae, included among the submerged macrophyte vegetation in fresh and brackish waters. They have a world-wide distribution and comprise six genera (Wood 1965), of which *Chara* and *Nitella* are the most species rich. In total, 678 charophyte species are identified (Guiry and Guiry 2020).

Most *Chara* species utilize bicarbonates (HCO_3) as a carbon source for photosynthesis and are limited to calcareous water bodies. They seem to have higher light demands than the vascular plants (Blindow 1992) and are vulnerable to eutrophication (e.g. Penning et al. 2008). Hence, the preferable habitat for the *Chara* species are oligotrophic-slightly mesotrophic calcareous lakes or ponds. In the Water Framework Directive (WFD 2000) charophytes are recognized as sensitive species and the occurrence of *Chara* in a lake is considered as indicative of low trophy and high ecological status (e.g. Penning et al. 2008). However, due to anthropogenic stressors, e.g. water abstraction and eutrophication, freshwater habitats with charophyte vegetation are decreasing and hence, red listed in Asia as well as in Europe (Allen et al. 2012; Janssen et al. 2016). Correspondingly, the distribution of many *Chara* taxa is decreasing and they are considered as threatened in several countries (Blaženčić et al. 2006; Henriksen and Hilmo 2015).

Several taxonomy studies concerning charophytes have been conducted, with most of the recent studies including molecular analyses (e.g. Borges and Necchi 2017). Charophytes are widespread in the temperate zone, but are also known in several countries in West and South Asia, including India (Groves 1924; Pal et al. 1962; Subramanian 2002), Pakistan (Faridi 1955; Langangen and Leghari 2001), Bangladesh (Naz et al. 2011; Naz and Diba 2012), Saudi Arabia (Khoja and Hussain 1990; Hussain et al. 1996), Iran (Ahmadi et al. 2012; Ghaemmaghami et al. 2012) and China (Ling et al. 2000). From Afghanistan, only old scattered information is available (Braun and Nordstedt 1882; Vilhelm 1928; Corillion 1957).

Studies of charophytes in Myanmar are very scarce, including the comprehensive study published in 1932 by B.P. Pal: "Burmese Charophyta". In that study, 24 charophyte species were reported from Myanmar including twelve *Chara*-species. In our study the species are updated to be in accordance with Guiry and Guiry (2020) and the number of species are reduced to nine. In case of changed status, the names given by Pal (1932) are in brackets.

Chara wallichii A. Braun, *C. corallina* Klein ex Willdenow, *C. nuda* B.S. Pal, *C. hydropithus* Reichenbach, *C. fibrosa* f. *erythrogyna* (Griffith) R.D. Wood. (*C. erythrogyna* (Griffith) R.D. Wood, *C. fibrosa* var. *burmanica* (Pal) van Raam. (*C. burmanica* Pal), *C. fibrosa* Agardh ex Bruzelius (*C. flaccida* A.Br., *C. gymnopitus* A.Br.), *C. grovesii* Pal, *C. handae* Pal, *C. setosa* Klein ex Willdenow (*C. brachypus* A. Br.), *C. zeylandica* Klein ex Willdenow.

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The distribution areas included paddy-fields, ponds, drains and marshy areas, and similar habitats, but no lakes were mentioned in the study by Pal (1932). Little attention has been paid to charophyte studies in Myanmar after that study, and no molecular analyses have been conducted on charophytes from Myanmar. As in the rest of the world, freshwater habitats in Myanmar are increasingly impacted by anthropogenic stressors, and today the status of species richness and diversity of *Chara* in Myanmar is therefore highly uncertain.

The goals of this study are 1) to contribute to the knowledge about *Chara* species distribution in lakes in Myanmar, 2) to identify *Chara* species, using a polyphasic approach including both traditional morphological methods and DNA barcoding, and 3) to indicate some ecological demands for the detected *Chara* species. As far as we know, our study is the first study concerning charophytes in the lakes of Myanmar.

Materials and methods

The studied Chara-lakes are part of an ongoing biological investigation of lakes and reservoirs in Myanmar, which for now includes 17 water bodies (Table 1). The overall goal of the project, which ends in 2024, is to improve the knowledge on the biological parameters, including charophytes, as a basis for the development of a biological monitoring system in Myanmar. *Chara* species are recorded in seven of these waterbodies, Inlay Lake, Meiktila Lakes (north and south), Yezin Dam, Ngalaik Dam, Kyet Mauk Taung, and Wethtigan Lake.

Studied lakes with Chara vegetation

Inlay Lake is a shallow lake (average depth around 3–3.5 m) located in Nyaung Shwe township, Taunggyi district, Southern Shan State. It is the second-largest natural lake in Myanmar (lake surface ca. 116 km^2, however with decreasing open areas) and located 884 m above sea level. It is a clear, very calcareous lake (Table 1) (Ballot et al. 2018). Most of the lake area is covered by a luxurious and diverse community of submerged macrophytes. *Chara* is a common taxon in the macrophyte community, especially in the northern part of the lake.

Meiktila Lake is a shallow reservoir, divided into two separate basins, and located close to Meiktila city in the Mandalay region in central Myanmar. The lake basins are located at an altitude of 230 m and cover an area of around 9 km^2 with a maximum water depth of 10 m. The lake water is moderate alkaline and slightly turbid (Table 1), with the southern basin more turbid than the northern basin. The *Chara* vegetation was common in both basins, whilst the northern basin had in addition extensive stands of the floating leaved *Nelumbo nucifera*.

Yezin Dam is a reservoir used for irrigation and drinking water. It is located in the Zeyar Thiri township, Nay Pyi Taw, Mandalay region at 128 m above sea level and covers an area of around 6.5 km^2. The maximum water depth is more than 10 m. The reservoir is a low-moderate alkaline and turbid lake (Table 1) with large water level fluctuations. At the time of investigation, the coverage of aquatic macrophytes was very low, and only a few specimens of *Chara* were found.

Table 1. Lakes in Myanmar, visited in 2014–2019, including characteristic physico-chemical data and coverage of *Chara* spp. The data for each lake include average values from all visited years and sites. *Chara* coverage; 1 = seldom, 2 = common, 3 = large stands. Lake area can vary considerable between wet and dry season. Lake areas measurements are based on Google Earth where satellite photo dates can vary from region to region.

Lake	State/Region/ Division	Latitude	Longitude	Altitude m	Lake area km^2	Calcium mg L^{-1}	TOC mg L^{-1}	Tot P µg L^{-1}	Tot N µg L^{-1}	*Chara*-coverage
Inlay Lake	Shan State	20,563,046	96,918,640	884	116*	45	5.0	15.4	448	3
Sakar Inn	Shan State	20,169,411	96,932,716	884	3	40	3.9	7	430	-
Pekon Lake	Shan State	19,879,100	97,032623	884	134	45	5.5	9	440	-
Indawgyi Lake	Kachin State	25,116,667	96,316,667	170	123	10	2.3	20	532	-
Meiktila Lake, North	Mandalay Region	20,886,560	95,852,966	230	4.8	19	4.9	21	428	2
Meiktila Lake, South	Mandalay Region	20,863,464	95,854,511	230	4.3	14	4.1	15	415	3
Yezin Dam	Mandalay Region	19,855,852	96,276,798	128	6.4	8	5.1	15	402	1
Nga Laik Dam	Mandalay Region	19,861,665	96,005058	163	5.5	22	-	20	510	2
Moeyingyi Reservoir	Bago Region	17,570,721	96,596,947	10	15	2	16.4	103	702	-
Taung Taman Lake, North	Mandalay Region	21,900,833	96,060556	61	3	45	13	520	2900	-
Kantawgyi Lake, South	Mandalay Region	21,936,389	96,065833	66	1.8	18	3.5	35	520	-
Pyu Kan Lake	Mandalay Region	21,768,056	95,891,111	102	2	18	2.2	45	500	-
Khu Le Inn	Mandalay Region	22,592,222	95,980,000	76	2.5	7	3.8	31	560	-
Sunye In Tank	Mandalay Region	21,679,722	96,230,000	91	4	21	6.1	26	490	-
Pauk In	Mandalay Region	21,326,944	95,048056	55	0.15	24	10.3	190	2000	-
Kyetmauk Taung Dam	Mandalay Region	20,812,222	95,250,833	279	4.5	40	5.1	19	1900	2
Wethtigan Lake	Magwe Division	20,575,833	94,641,111	66	1.7	44	3.5	8	530	3

*: Inlay lake area is reduced. Today open water area is measured to 46 km^2.

Ngalaik Dam is a medium-large irrigation reservoir, located in a boreal area in Ottara Thiri Township, Nay Pyi Taw, Mandalay region. The reservoir has a surface area of around 5.5 km^2, is situated 163 m above sea level, and is a calcareous, clear water lake. The aquatic vegetation consists of a moderate coverage of a few submerged species, including charophytes.

Kyet Mauk Taung is a medium-large reservoir and is mainly used for irrigation (Pa 1983). It is located in a boreal area in the Kyaukpadaung Township, Mandalay region. The reservoir is situated 279 m above sea level, close to Popa National Park, and covers an area of about 7.3 km^2. The reservoir is a calcareous and slightly humic water body.

Wethtigan (Whattae) Lake is included in the Wethtigan Wildlife Sanctuary, situated in Salin Township, Magway Region. The lake is situated 66 m above sea level and has a surface area of around 1.7 km^2. It is a clear, calcareous lake (Table 1) with luxurious growth of both floating leaved vegetation (mainly *Nelumbo nucifera*) and submerged vegetation, dominated by *Chara*.

Integrated water samples were taken (1 m steps up to max. 3 m water depth) for the analysis of chemical parameters (ammonium, nitrate, total nitrogen (TN), soluble reactive phosphorous, total phosphorous (TP), Ca, turbidity and total organic carbon (TOC)). The *Chara* specimens were collected from a boat, using an aquascope and a rake. In total, 11 *Chara* samples were analysed from 7 lakes. All specimens were preserved as herbarium samples directly after collection, for later morphological and molecular analysis.

Morphological analysis

The species were determined following the nomenclature in Wood (1965).

DNA- barcoding

Genomic DNA from *Chara* material was isolated after Schneider et al. (2016). PCR for the *matK* gene and the *rbcl* gene was performed on a Bio-Rad CFX96 Real-Time PCR Detection System (Bio-Rad Laboratories, Oslo, Norway) using the iProof High-Fidelity PCR Kit (Bio-Rad Laboratories, Oslo, Norway). Amplification of the *matK* gene region was conducted using the primers F-Chara (agaatgagcttaaacaaggat) and R-Chara (acgatttgaacatccactataata) and for the *rbcl* gene using the primer rbclaf (atgtcaccacaaacagagactaaagc) and rbclar (gtaaaatcaagtccaccrcg). The following cycling protocol was used for *matk* and *rbcl*: one cycle of 5 min at 94°C, and then 35 cycles each consisting of 10 s at 94°C, 20 s at 62°C, and 20 s at 72°C, followed by a final elongation step of 72°C for 5 min. PCR products were visualized by 1.5% agarose gel electrophoresis with GelRed staining (GelRed Nucleic Acid Gel Stain, Biotium, Fremont, CA, USA) and UV illumination. For sequencing the same primers and for *matk* additionally the intermediate primers charaintF (gatggctattcaagcagga), charaintR (ctaccgataagttcgtcct), charaBt2F (datatggcaacaycaaaagac) and charaBT2R (atacagaccatgcagcytt) were used. For each PCR product, both strands were sequenced on an ABI 3730 Avant genetic analyser using the BigDye terminator V.3.1 cycle sequencing kit (Applied Biosystems, Thermo Fisher Scientific Oslo, Norway) according to the manufacturer's instructions.

The sequence data were deposited at the National Center for Biotechnology Information (NCBI) under the accession numbers given in Table 2.

Phylogenetic analyses

Sequences were analysed and aligned using Seqassem (version 04/2008) and Align (version 03/2007) MS Windows-based manual sequence alignment editor (SequentiX – DigitalDNA Processing, Klein Raden Germany) to obtain DNA sequence alignments, which were then corrected manually. A *matK* set containing 11 *Chara* samples from Myanmar (Table 2) and 24 other *Chara* sequences, and 953 nucleotide positions were used for the phylogenetic analysis. *Nitellopsis obtusa* (AY170447) was used as an outgroup taxon in the *matK* phylogenetic tree. For the *rbcl* phylogenetic tree, seven samples from Myanmar and 32 other *Chara* sequences were used and 567 Nucleotide positions. The datasets were analysed using maximum likelihood (ML), maximum parsimony (MP) and distance (neighbour-joining (NJ)) in MEGA version x (Kumar et al. 2018). GTR+G was selected as the best-fitting evolutionary model for the *matK* gene region and T92 + G for the *rbcl* region. ML, MP, and distance analyses were performed with 1000 bootstrap replicates in MEGA version X (Kumar et al. 2018).

Table 2. *Chara* samples, origin, sampling date and NCBI accession numbers.

ID	Lake	date	*matK*	*rbcl*
MMYA-1	Inlay Lake	05.11.2014	MT739758	MT739769
MMYA-2	Inlay Lake	05.11.2014	MT739759	MT739774
MY-32	Yezin Dam	23.05.2017	MT739768	-
MY-33	Yezin Dam	23.05.2017	MT739760	-
MY-45	Meiktila North	16.11.2017	MT739767	-
MY-34	Meiktila South	16.11.2017	MT739761	-
MY-35	Ngalaik Dam	17.11.2017	MT739762	MT739772
MY-58	Kyet Mauk Taung	22.11.2019	MT739765	MT739773
MY-59	Kyet Mauk Taung	22.11.2019	MT739763	MT739775
MY-60	Wethtigan Lake	23.11.2019	MT739766	MT739771
MY-61	Wethtigan Lake	23.11.2019	MT739764	MT739770

- = not analysed

Results

Morphological determination

The collected specimens include two charophyte species: *Chara zeylanica* Klein ex Willdenow and *Chara fibrosa* Agardh ex Bruzelius. (*C. fibrosa* was first determined as *C. flaccida* A. Braun, based on the yellow/brown colour of the oospore).

Chara zeylanica was found in Inlay lake, Meiktila Lake south, Ngalaik Dam, Kyet Mauk Taung Reservoir, and Wethtigan Lake. *C. fibrosa* was found in Yezin Dam, Kyet Mauk Taung Reservoir, and Weththigan Lake (Table 3).

Description of *Chara zeylanica* collected in Myanmar

15–40 cm high. Axis 0.35–0.8 mm in diameter. Internodes up to 10 cm long, but most commonly less than 4 cm. Slightly encrusted with lime. Stem cortex regularly triplostichous, isostichous (The examined specimens, all collected in November have stems strongly overgrown with diatoms, and therefore difficult to see). Spine-cells papillous to short, acute, from papillous up to 0.4 mm long, solitary. Stipulodes in two rows (Diplostephanous), upper row with cells to 0.5 mm long and lower row to 0.4 mm long, acute. 8–12 branchlets in a whorl, 20–50 mm long, 0.5x to 1.0x times the length of the internodes. 8–11 segments on each branchlet, first segment *ecorticate* (Gymnopodous). End segments 2–4, ecorticate. *End-cell* acute.

Description of *Chara fibrosa* collected in Myanmar

Specimens 5–25 cm high, commonly lower to 15 cm. Axis diameter 0.4–0.6 mm. Internodes up to 3 cm long. Slightly to moderate encrusted with lime. Cortex diplostichous, tylacanthous to isostichous. Spine-cells scattered, solitary 0.2 mm to 0.6 mm long, acute. Stipulodes one row (Haplostephanous), 0.8 mm –2.0 mm long. Branchlets 8–10 in each whorl, up to 15 mm long and 0.25 to 1 times the length of internodes. All branchlets ecorticate and with 3–5 segments. Bract-cells verticillate up to 0.8 mm long. In two of three localities the plants were richly fertile. Oogonia 0.7 mm long, 0.3–0.5 mm wide. Oospore up to 0.5 mm long and 0.35 mm wide, yellow to brown. Antheridia up to 0.3 mm in diameter. The species are further described by Langangen and Leghari (2001) and Langangen (2015).

Phylogenetic analyses

In the matk phylogenetic tree, seven *Chara* samples, morphologically determined as *C. zeylanica*, from the Myanmar lakes Inlay Lake, Meiktila Lake, Nga Laik Dam, Kyat Mauk Taung Reservoir, and Weththigan Lake, formed a monophyletic cluster together with the Brazilian *Chara* species; *C. hydropithys, C. guairensis* R. Bicudo, *C. rusbyana* M. Howe, *C. haitensis* Turpin and *C. braunii* var. *schweinitzii* (A.Braun) Zaneveld (Figure 1). The whole cluster was supported by a bootstrap value of 100%. *C. hydropithys* was the closest related *Chara* species to *Chara zeylanica* from Myanmar, which was supported by a bootstrap value of 57%. Although the *Chara* sample MY-45 was not investigated morphologically its matk sequence was 100% identical with those from the other *C. zeylanica* samples. It was therefore also assigned to *C. zeylanica*.

Four *Chara* specimens from Yezin Dam, Wethtigan Lake and Kyat Mauk Taung Reservoir were determined as *Chara fibrosa*. They clustered together with *C. braunii* Gmelin from Lake Kasumigaura (Japan), which was supported by a bootstrap value of 100%. In the phylogenetic tree based on partial rbcl gene of *Chara* spp. only five *Chara zeylanica* from Myanmar were included. They grouped in a separate subcluster but clearly clustered together with other *C. zeylanica* from Japan, USA (California), New Caledonia and New Zealand (Figure 2). The rbcl phylogenetic tree therefore confirmed the assignment of the *Chara* samples MMYA-1, MMYA-2, MY-34, MY-35, MY-45, MY-59, and MY-61 to *C. zeylanica*. Only two of the four *C. fibrosa* from Myanmar were analysed for the rbcl gene and grouped in a subcluster. They clustered together with *C. fibrosa* from Japan and *C. braunii* from Japan, Hawaii (USA) and New Zealand (Figure 2).

Ecological notes

Chara zeylanica is the most common of the two species found in Myanmar and was observed in five lakes.

Table 3. Comments to the collected *Chara* specimens from Myanmar.

No.	Lake	Comments	Specimens quality
Chara zeylanica			
MMYA-1,2	Inlay Lake	-	Poorly developed and preserved
MY-34	Meiktila North	Broken specimens but have been long. Special locality.	Poorly developed
MY-35	Ngalaik Dam	Typical specimens. Richly fertile with ripe, black oospores.	Well developed
MY-45	Meiktila South	-	-
MY-59	Kyet Mauk Taung	Richly fertile with ripe, black oospores.	Well developed
MY-61	Wethtigan Lake	With very long internodes. Sterile.	Well developed
Chara fibrosa			
MY-32,33	Yezin	Small specimens. Fertile	Well developed
MY-58	Kyet Mauk Taung	Richly fertile specimens	Well developed
MY-60	Wethtigan Lake	Sterile specimens	Well developed

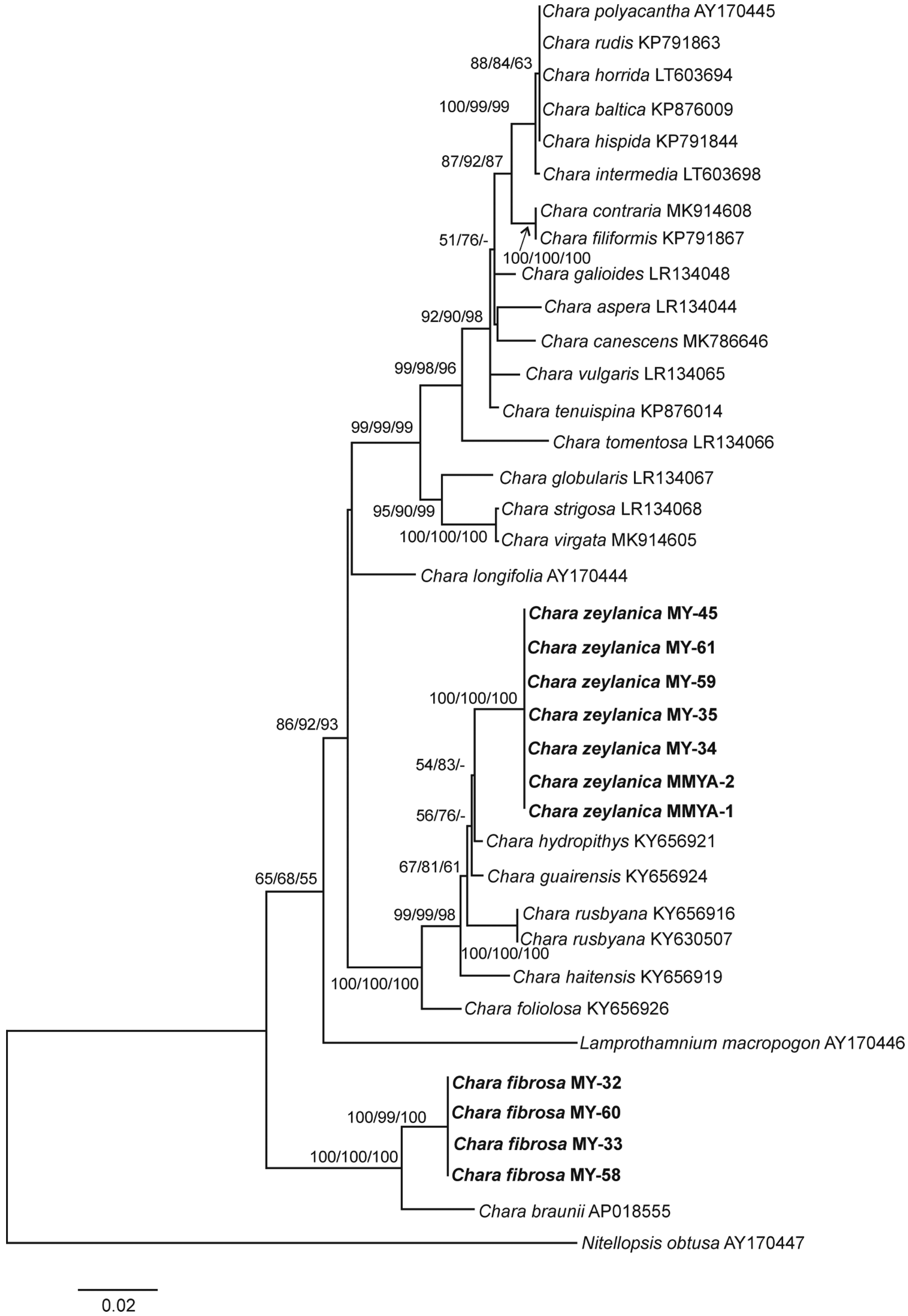

Figure 1. Maximum likelihood tree of the *matK* gene of *Chara* spp. Bootstrap values (ML/MP/NJ) above 50 are included. Strains from this study are marked in bold. The scale bar indicates 2 % sequence divergence.

It is present in different habitat types; from the medium-altitude and calcium-rich natural Inlay Lake in Shan state to lowland lakes in the dry zone area close to Mandalay. The species is growing in Ngalaik Dam and Kyet Mauk Taung Reservoir, but it was not recorded in the heavily regulated Yezin Dam.

Chara fibrosa had a sparser distribution and was only found in three lakes in the dry zone. In Yezin Dam only a few small specimens were recorded, while it was more common in Kyet Mauk Taung reservoir and in the natural Wethtigan Lake, where it grew in between mass stands of *C. zeylanica*. Both Kyet Mauk Taung Reservoir and the natural Wethtigan Lake are calcareous lakes with calcium levels around 40 mg L^{-1}, while Yezin Dam is characterised by more moderate calcium concentrations of around 8 mg L^{-1}.

Both species were recorded in low-impacted lakes only, with TP concentrations below 20 µg L^{-1}.

Discussion

Two *Chara* species, *C. zeylanica* and *C. fibrosa* were recorded in our lake survey in 2014–2019. The morphological determination of both species has been

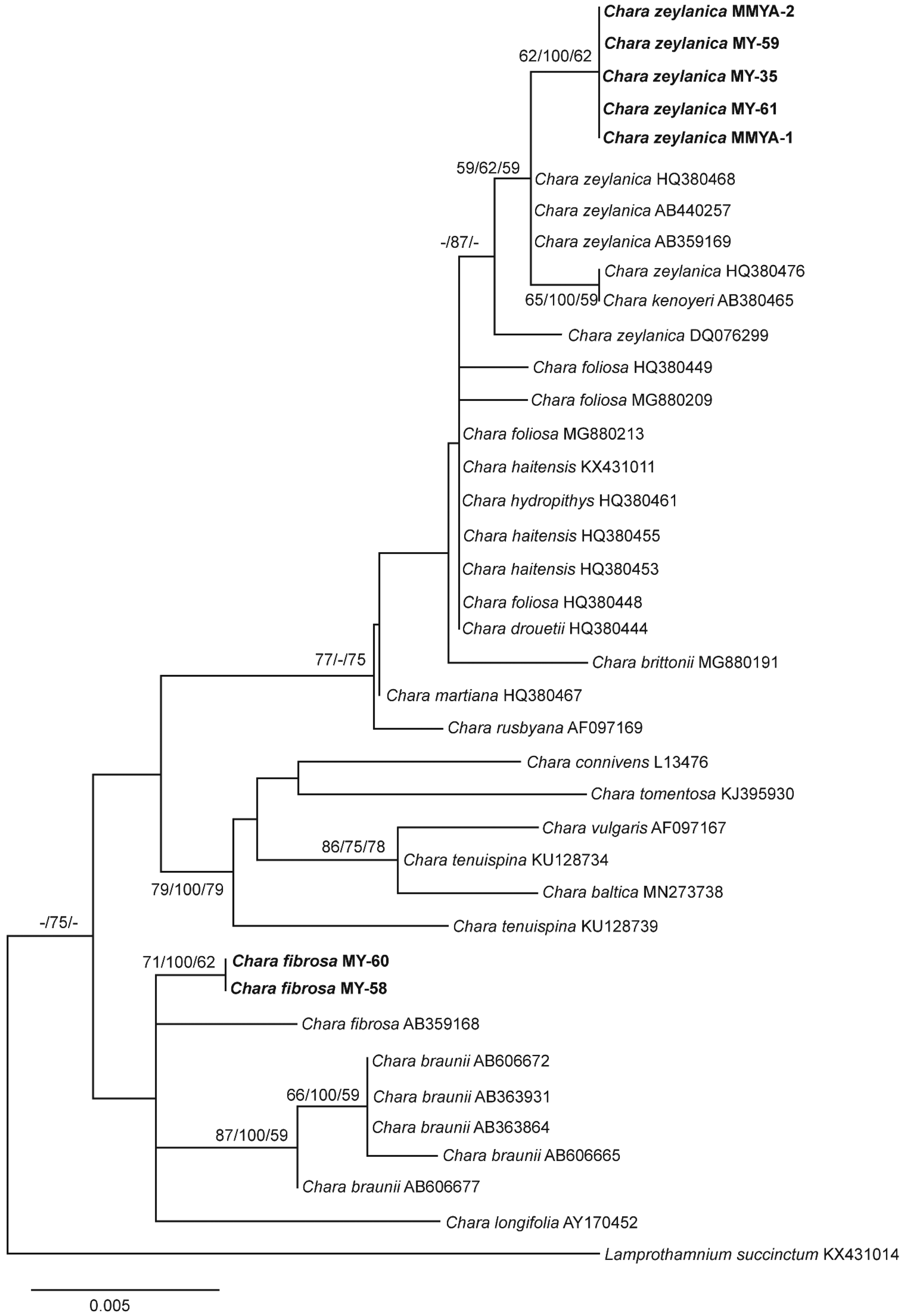

Figure 2. Maximum likelihood tree of the *rbcl* gene of *Chara* spp. Bootstrap values (ML/MP/NJ) above 50 are included. Strains from this study are marked in bold. The scale bar indicates 0.5 % sequence divergence.

supported by phylogenetic studies using matk and rbcl genes. Although the *Chara* sample MY-45 was not determined morphologically, the phylogenetic analysis confirms its assignment to *C. zeylanica*.

In the 1920s and the beginning of the 1930s, 12 *Chara* species were recognised in Myanmar (Pal 1932). However, four of these species are today recognised as variants or synonyms to *C. fibrosa*, i.e. *C. erythrogyna, C. burmanica, C. flaccida* and *C. gymnopitys* (Guiry and Guiry 2020).

Both *C. zeylanica* and *C. fibrosa* are commonly distributed species, especially *C. zeylanica*, which is common in tropical and sub-tropical areas in southern parts of Africa, Asia, Southern Australia, Oceania (Hawaii), and Central- and South-America, but also in North-America (Wood 1967). *Chara fibrosa* is common in Africa, South-Asia and Australia (Wood and Imahori 1959). Both species have a wide distribution and are not considered as red-listed *Chara*-species.

The study by Pal (1932) on charophytes was very comprehensive and included several localities in different regions and areas in Myanmar. These include the large delta area around Yangon, the dry zone area around Mandalay, the intermediate zone between

Naypyitaw and Yangon, the large Shan Plateau in the east, and the extreme south-eastern part. The study focused on small freshwater habitats like paddy-fields, ponds, drains and marshy areas.

Our study is less comprehensive than the study conducted by Pal (1932). However, it includes only lakes, a habitat which was not investigated by Pal (1932). We have so far visited 17 lakes, situated in different regions, Mandalay region, Magwe region, Bago region, Shan State, and Kachin state, i.e. covering the same areas as in Pal (1932), except from the extreme southeast area. *Chara* species were recorded in seven of the visited lakes.

There may be several reasons why we have recognised so few species compared to the 1930s:

- different survey seasons
- different habitat preferences
- increased impacts on habitats
- taxonomic changes since the 1930s

Pal (1932) found that the best season for the charophyte flourishment in Myanmar was between the months of August and March. In our study all lakes were surveyed twice, February–March and November, which covers the best season for charophyte growth indicated by Pal (1932).

In contrast to the study of Pal (1932) our study focuses on lakes only. Surveys conducted in different habitats can be a reason for the discrepancy between the two observations. His surveys were conducted in small habitats, like paddy-fields and ponds. It can be argued that these habitats are the preferable habitats for charophytes. In other countries, most *Chara* species including *C. zeylanica* and *C. fibrosa*, inhabit different types of water bodies, including lakes (Siong and Asaeda 2006; Penning et al. 2008). However, the existence of *Chara* species with special preferences for ditches and ponds cannot be excluded.

In our study, *C. fibrosa* appears in both high and moderate alkaline lakes. In the high alkaline lakes, they grow with larger specimens and more vigorous stands. This agrees with Asaeda et al. (2014) who indicated 40–80 mg Ca L^{-1} as the optimum range for this species, and is similar to the results from Vaidya (1967). The smaller *C. fibrosa* specimens observed in Yezin Dam can be the result of stress caused by water level regulations (Ellawala et al. 2011). *C. zeylanica* also seems to prefer calcareous lakes. All investigated lakes in our study except for one had calcium concentrations >19 mg L^{-1}, which is also in agreement with Vaidya (1967). We have recorded both species only in low-impacted lakes, with TP concentrations below 20 µg L^{-1}. This is supported by several European studies (e.g. Blindow 1992), with *Chara* recognised as a species sensitive to eutrophication (Penning et al. 2008).

Increased human impact on freshwater habitats must be considered as a factor reducing *Chara* biodiversity in Myanmar as elsewhere. The destruction of small freshwater habitats, increased urbanization and enhanced agricultural activities, followed by increased eutrophication and reduced light conditions, are already mentioned by Pal (1932). These activities are today considered as the main impacts on freshwater habitats and recognised as reasons for decreased occurrence of *Chara* vegetation in Asia as well as in Europe (Allen et al. 2012; Janssen et al. 2016).

The "taxonomic development" during the last 90 years has also caused a considerable change in the assignment of *Chara* species to certain taxa.

In addition to "taxonomic development", surveys in different habitats may be a reason for the discrepancy between the two surveys. However, we believe that increased human impact on freshwater habitats must be considered as a factor reducing *Chara* biodiversity, in Myanmar as elsewhere.

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Disclosure statement

The authors declare that there is no conflict of interest.

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Author contributions

Marit Mjelde: study design, field work, manuscript writing.
Thida Swe: field work, manuscript writing
Anders Langangen: morphological studies, manuscript writing,
Andreas Ballot: study design, field work, genetic and phylogenetic analyses, manuscript writing.

References

Ahmadi, A, H Riahi, M Sheidai, J Van Raam. 2012. Some charophytes (Characeae, Charophyta) from central and western of Iran including *Chara kohrangiana* species nova. Cryptogamie Algologie. 33(4):359–390. doi:10.7872/crya.v33.iss4.2012.359.

Allen, DJ, KG Smith, WRT Darwall. 2012. The status and distribution of freshwater biodiversity in Indo-Burma. Cambridge, UK and Gland, Switzerland: IUCN; x +158pp+4pp cover.

Asaeda, T, M Senavirathna, Y Kaneko, MH Rashid. 2014. Effect of calcium and magnesium on the growth and calcite encrustation of *Chara fibrosa*. Aquat Bot. 113:100–106. doi:10.1016/j.aquabot.2013.11.002.

Ballot, A, M Mjelde, T Swe 2018. Integrated water resources management in Myanmar. Assessing ecological status in Inlay Lake. NIVA Report No.: 7301–2018. Norwegian Institute for Water Research, Oslo, Norway.

Blaženčić, J, B Stevanović, Z Blaženčić, V Stevanović. 2006. Red data list of charophytes in the Balkans. Biodivers Conserv. 15(11):3445–3457. doi:10.1007/s10531-005-2008-5.

Blindow, I. 1992. Decline of charophytes during eutrophication - comparison with angiosperms. Freshwater Biol. 28 (1):9–14. doi:10.1111/j.1365-2427.1992.tb00557.x.

Borges, FR, O Jr Necchi. 2017. Taxonomy and phylogeny of *Chara* (Charophyceae, Characeae) from Brazil with emphasis on the midwest and southeast regions. Phytotaxa. 302(2):101–121. doi:10.11646/phytotaxa.302.2.1.

Braun, A, O Nordstedt. 1882. Fragmente einer Monographie der Characeen. Nach den hinterlassenen Manuscripten A. Brauns's herausgegeben von Dr. O. Nordstedt. Abh K Akad Wiss Berlin. Verlag der Königlichen Akademie der Wissenschaften, Berlin, pp. 1–211.

Corillion, R. 1957. Les Charophycées de France et d'Europe occidentale. Trav Lab Fac Sc Angers. 11–12:1–259.

Ellawala, C, T Asaeda, K Kawamura. 2011. Influence of flow turbulence on *Chara fibrosa*: growth, stress, and tissue carbon content. J Freshwater Ecol. 26(4):507–515.

Faridi, M. 1955. A contribution to the Charales of West Pakistan. Biologia. 1(1):70–81.

Ghaemmaghami, SS, S Afsharzadeh, GR Balali. 2012. Characterization of genetic diversity in three *Chara* L. species (Characeae) in Iran using RAPD-PCR technique. Iran J Bot. 18(2):319–326.

Groves, J. 1924. Notes on Indian charophyta. Bot J Linn Soc. 46(310):359–376. doi:10.1111/j.1095-8339.1924.tb00493.x.

Guiry, M, G Guiry 2020. AlgaeBase. World-wide electronic publication. Galway: National University of Ireland. [accessed 2020 Jun 30]. https://www.algaebase.org.

Henriksen, S, O Hilmo. 2015. The 2015 Norwegian red list for species. Norway: Norwegian Biodiversity Information Centre.

Hussain, MI, TM Khoja, M Guerlesquin. 1996. Chemistry, ecology and seasonal succession of charophytes in the Al-Kharj Irrigation Canal, Saudi Arabia. Hydrobiologia. 333 (2):129–137. doi:10.1007/BF00017575.

Janssen, JAM, JS Rodwell, M García Criado, S Gubbay, T Haynes, A Nieto, N Sanders, F Landucci, J Loidi, A Ssymank, T Tahvanainen, M Valderrabano, A Acosta, M Aronsson, G Arts, F Attorre, E Bergmeier, RJ Bijlsma, F Bioret, C Biţă-Nicolae, I Biurrun, M Calix, J Capelo, A Čarni, M Chytrý, J Dengler, P Dimopoulos, F Essl, H Gardfjell, D Gigante, G Giusso del Galdo, M Hájek, F Jansen, J Jansen, J Kapfer, A Mickolajczak, JA Molina, Z Molnár, D Paternoster, A Piernik, B Poulin, B Renaux, JHJ Schaminée, K Šumberová, H Toivonen, T Tonteri, I Tsiripidis, R Tzonev, M Valachovič.. 2016. European red list of habitats: part 2. Terrestrial and freshwater habitats. European Union. Luxembourg: Publications Office of the European Union. doi: 10.2779/091372

Khoja, TM, MI Hussain. 1990. Preliminary studies on the distribution of charophytes in Saudi-Arabia. Cryptogamie Algologie. 11(3):187–196.

Kumar, S, G Stecher, M Li, C Knyaz, K Tamura. 2018. MEGA X: molecular evolutionary genetics analysis across computing platforms. Mol Biol Evol. 35(6):1547–1549. doi:10.1093/molbev/msy096.

Langangen, A. 2015. Some finds of charophytes from East-Africa (Zambia, Tanzania, Kenya and Somalia). Acta Musei Nationalis Pragae Ser B Hist Nat. 71(3–4):239–248.

Langangen, A, SM Leghari. 2001. Some charophytes (Charales) from Pakistan. Stud Bot Hung. 32:63–85.

Ling, YJ, SL Xie, A Langangen. 2000. Charales of China. Nowa Hedwigia. 71(1–2):69–94.

Naz, S, N Diba. 2012. Some morphological observations of charophytes (Characeae) from Bangladesh. J Life Earth Sci. 7:71–77. doi:10.3329/jles.v7i0.20124.

Naz, S, N Diba, H Schubert. 2011. Monograph on charophytes of Bangladesh: taxonomy, diversity, distribution, charophyta, Northern Bangladesh. Saarbrücken (Germany): VDM Publishing Dr. Müller.

Pa, UY 1983. Economic aspects of the Kyet Mauk Taung irrigation project in Central Burma [Master Thesis]. Canberra (Australia): Australian National University.

Pal, B. 1932. Burmese charophyta. Bot J Linn Soc. 49 (327):47–92.

Pal, B, B Kundu, V Sunderlingam, G Venkataraman. 1962. Charophyta. New Delhi: ICAR Monograph on Algae.

Penning, WE, M Mjelde, B Dudley, S Hellsten, J Hanganu, A Kolada, M van den Berg, S Poikane, G Phillips, N Willby, F Ecke. 2008. Classifying aquatic macrophytes as indicators of eutrophication in European lakes. Aquat Ecol. 42(2):237–251. doi:10.1007/s10452-008-9182-y.

Schneider, SC, P Nowak, U von Ammon, A Ballot. 2016. Species differentiation in the genus Chara (Charophyceae): considerable phenotypic plasticity occurs within homogenous genetic groups. Eur J Phycol. 51(3):282–293. doi:10.1080/09670262.2016.1147085.

Siong, K, T Asaeda. 2006. Does calcite encrustation in *Chara* provide a phosphorus nutrient sink? J Environ Qual. 35 (2):490–494. doi:10.2134/jeq2005.0276.

Subramanian, D. 2002. Monograph on Indian charophyta. Dehra Dun (India): Bishen Singh Mahendra Pal Singh.

Vaidya, B. 1967. Study of some environmental factors affecting the occurrence of charophytes in western India. Hydrobiologia. 29(1–2):256–262.

Vilhelm, J. 1928. Characeae Europae orientalis et Asiae ex herbario instituti cryptogamici horti botanici Reipublicae rossicae (ante Petropolitani). Prir Fak Karlovy Univ. 80:1–24.

WFD. 2000. The EU water framework directive - integrated river basin management for Europe - directive 2000/60/EC. [accessed 2020 Jul 03]. https://ec.europa.eu/environment/water/water-framework/index_en.html.

Wood, RD. 1965. Monograph of the characeae. In R D Wood, K Imahori, editors. A revision of the characeae (Vol. 1). Weinhein (Germany): Verlag J. Cramer; p. 1–904.

Wood, RD, K Imahori. 1959. Geographical distribution of characeae. Bull Torrey Bot. 86(3):172–183. doi:10.2307/2482517.

Wood, RD. 1967. Charophytes of North America. West Kingston (Rhode Island): Stella's Printing; p. 72.