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and first record from outside Australia

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***Nitella sonderi* A.Braun (Charales, Charophyceae) – a new record for South America, and first record from outside Australia**

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ABSTRACT

Nitella sonderi A.Braun is newly reported from the Patagonian Plateau, Argentina. This species was only reliably known from Australia until now. The morphological and genetic studies carried out here found no difference between Argentinian and Australian specimens. The species is dioecious, mucous-bearing on fertile whorls, with two to three-forked homeoclemous, macrodactylous branchlets with a long primary ray, an evident central secondary ray, bicellulate dactyls with a shortly narrowing tip of the penultimate cell, which is confluent with a small discoloured conical cell and large antheridia. Although only male plants were found in Patagonia, this combination of traits allowed its differentiation from other species of *Nitella* C.Agardh known from South America, but pointed towards the similarity with *N. papillata* F.S.Han & W.Q.Chen found only in South-East China. *Nitella sonderi* is the first dioecious species from the section *Gioallenia* R.D.Wood found in South America. Additions to the description of *N. sonderi* were suggested here based on specimens from Argentina and Australia. Doubt concerning a species record of *N. morongii* Allen for Australia published elsewhere was outlined. The new species record for South America highlights an unexpected biogeographical pattern, rare for dioecious species, or may suggest a case of invasion. Future taxonomic research is desirable to clarify the affinity of *N. sonderi* and *N. papillata*.

KEY WORDS

Argentina,
Patagonia,
Characeae,
genetics,
new record.

RÉSUMÉ

Nitella sonderi A.Braun (Charales, Charophyceae) – un nouvel enregistrement pour l'Amérique du Sud, et un premier signalement en dehors de l'Australie.

Nitella sonderi A.Braun est nouvellement signalée sur le plateau patagonien, en Argentine. Cette espèce n'était connue de manière fiable que d'Australie. Les études morphologiques et génétiques menées ici n'ont révélé aucune différence entre les spécimens argentins et australiens. L'espèce est dioïque, avec des mucosités sur les verticilles fertiles, des rameaux macrodactyles homéoclémeux à deux ou trois fourches avec un long rayon primaire, un rayon secondaire central évident, des dactyles constitués de deux cellules avec une pointe peu rétrécie de l'avant-dernière cellule, qui est confluite avec une petite cellule conique et hyaline et de grandes anthéridies. Bien que seules des plantes mâles aient été trouvées en Patagonie, cette combinaison de traits a permis sa différenciation des autres espèces de *Nitella* C.Agardh connues d'Amérique du Sud, mais a pointé des similitudes avec *N. papillata* F.S.Han & W.Q.Chen trouvée uniquement dans le sud-est de la Chine. *Nitella sonderi* est la première espèce dioïque de la section *Gioallenia* R.D.Wood trouvée en Amérique du Sud. Des ajouts à la description de *N. sonderi* ont été suggérés ici sur la base de spécimens étudiés d'Argentine et d'Australie. Des doutes concernant le premier enregistrement de l'espèce de *N. morongii* Allen en Australie, publié ailleurs, sont émis. Le nouvel enregistrement de l'espèce pour l'Amérique du Sud met en évidence un schéma biogéographique inattendu, rare pour les espèces dioïques, ou peut suggérer un cas d'invasion. Des recherches taxonomiques futures sont souhaitables pour clarifier l'affinité entre *N. sonderi* et *N. papillata*.

MOTS CLÉS

Argentine,
Patagonie,
Characeae,
génétique,
signalement nouveau.

INTRODUCTION

Knowledge about charophytes distribution in different regions of South America is sparse, which is also true for many large areas of the world. Studies of Argentinian charophytes have mostly focused on northern and central provinces of the country (Spegazzini 1883; Nordstedt 1888; Fries 1905; Hasslow 1934; Carl de Donterberg 1938; Whelden 1946; Horn af Rantzien 1951; Carl de Donterberg 1960, 1970, 1971, 1984; Wood & Imahori 1964; Wood 1965; Carl de Donterberg & Rotman 1973; Cáceres 1975, 1978, 1979, 1985; Tell 1985; Cáceres & García 1989; Cáceres *et al.* 1990; García 1990, 1993a, b, 1999; D'Ambrosio *et al.* 2012; Blindow *et al.* 2018a, b). Only seven species and one variety of *Chara* L., and three species of *Nitella* C.Agardh, were reported from the Argentinian part of Patagonia from a few localities (Table 1; Spegazzini 1883; Hasslow 1934; Cáceres 1985; Cáceres & García 1989; Cáceres *et al.* 1990; García 1990, 1993a, 1999; Blindow *et al.* 2018a), and some of these records need confirmation. An evidently longer and mostly dissimilar species list could be compiled for the Chilean part of Patagonia (Table 1; Braun & Nordstedt 1882; Horn af Rantzien 1950, 1951; Blindow *et al.* 2018a, b). It includes eight species of *Chara*, 13 species of *Nitella* and one species of *Tolypella* (A.Braun) A.Braun. This discrepancy could be due to a significant difference in the environment but also could partly be attributed to the poor knowledge of charophytes from Argentinian Patagonia. Here, we report a new record of *Nitella sonderi* A.Braun from the north of the Patagonian Plateau, confirmed with integrative taxonomy, and we outline some perspectives for future studies of this species and similar species.

MATERIAL AND METHODS

MORPHOLOGICAL STUDY

The plants were collected in the Alicurá Reservoir, Limay River in April 2019. They were pressed and parts of the plant were fixed with 70% ethanol. The Australian specimens of *N. sonderi* also stored in LE (the herbarium of the Komarov Botanical Institute of the Russian Academy of Sciences) were studied as well. They were identified as this species by M. Casanova in March 2019. The photos were taken with a stereo microscope Leica M 165C equipped with a camera Leica DDMC2900, and with a microscope Zeiss Axio Scope A1. The terms for the morphological description of plants are in agreement with Wood (1965) and Casanova (2009).

DNA EXTRACTION, AMPLIFICATION AND SEQUENCING

The genetic analyses presented here were performed by two different working groups: 1) INIBIOMA, CONICET-Universidad Nacional del Comahue, Bariloche, Río Negro, Argentina; and 2) Federal Scientific Center of the East Asia Terrestrial Biodiversity of the Far Eastern Branch of the Russian Academy of Sciences.

Accordingly, two different methods for DNA extraction and PCR amplification were used:

1) The DNA isolation was carried out with standard 2× CTAB method. Amplification of part of the *rbcl* gene was obtained with primers 1 forward and 889 reverse, described in Vidal-Russell & Nickrent (2008). The nuclear ribosomal internal transcribed spacer region (ITS1 + 5.8S + ITS2) was amplified with universal primers ITS1 and ITS4. Standard polymerase chain reactions (PCRs) were used for both regions (Vidal-Russell & Nickrent 2007), and the PCR products were sent for sequencing to Macrogen Inc. (Korea);

and 2) Total genomic DNA was extracted as described previously by Echt *et al.* (1992) with some modifications (Kiselev *et al.* 2015). Part of the *rbcL* gene was amplified and sequenced in two fragments, following Romanov *et al.* (2022). The ITS region was amplified with primers ITS-36F and ITS-R (Hall *et al.* 2010). The PCR products were purified by ExoSAP-IT PCR Product Cleanup Reagent (Affymetrix Inc., United States) and sequenced in both directions using an ABI 3500 genetic analyser (Applied Biosystems, United States) with a BigDye terminator v.3.1 sequencing kit (Applied Biosystems, United States). The same primers were used for PCR. Sequences were assembled with the Staden Package v.1.4 (Bonfield *et al.* 1995). The obtained sequences were submitted to GenBank under the accession numbers MW727279, OM311638, OM311639 for *rbcL* and MW723615, MW723616, OM338646 for ITS.

PHYLOGENETIC ANALYSES

The *rbcL* and ITS sequences for *Nitella* were downloaded from GenBank, along with two sequences for *Chara* used as outgroup. Alignment for each region was done automatically in AliView (Larsson 2014) and checked manually in the SeaView program (Galtier *et al.* 1996). Bayesian phylogenetic inference (BI) was carried out in MrBayes v.3.2.6 (Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003). The model of sequence evolution selected was GTR + I + G; MrBayes was set to run two independent analyses with four chains each for five million generations, sampling trees and parameters every 100th generation. Convergence was analysed by examining the average standard deviation of split frequencies between analysis (below 0.05), graphically looking stationarity in the likelihood scores versus generations; the log files were examined in Tracer v.1.6 (Rambaut *et al.* 2014) to check that parameter effective sample sizes were above 200. The first 25% was discarded as burn-in. *Chara vulgaris* L. (KX216498) and *C. connivens* Salzm. ex A.Braun (KX216499) were used as outgroups for the *rbcL* analysis and *C. haitensis* Turpin (KR080215) and *C. hornemannii* Wallman (KR080216) for ITS.

RESULTS

Family CHARACEAE Gray
Genus *Nitella* C.Agardh

Nitella sonderi A.Braun

SPECIMENS EXAMINED. — **Argentina.** Neuquén Province, Alicurá Reservoir, 40°35'19"S, 70°52'34"W, 690 m a.s.l., 07.IV.2019, P. Quiroga & R. Vidal-Russell (LE[A0000321, A0000322], in spirit) (Figs 1-3).

Australia. Queensland, 3.5 mi [5.6 km] S of Stanthorpe post office, W of road, SW shore of metal (gravel) quarry, in clear water, 20.XI.1960, R.D. Wood 60-11-20-22 (LE[A0001490], GenBank accession: *rbcL*[OM311638]); 50.9 mi [81.9 km] S of Miriam Vale on road to Gin Gin, abundant in c. eight inches of water, mud bottom, creek in deep valley (Black Creek?), 30.XI.1960, R.D. Wood 60-11-30-10 (LE[A0001491], GenBank accessions: *rbcL*[OM311639], ITS[OM338646]); 3.5 mi [6.5 km] S of Stanthorpe, post office, W of road, selected slender specimens common

TABLE 1. — Species of charophytes known from Patagonia (Argentina and Chile). Evidently it is impossible to include here complete bibliography; therefore, we cited mostly important references covering all species records in Argentina (Spegazzini 1883; Hasslow 1934; Cáceres 1985; Cáceres & García 1989; Cáceres *et al.* 1990; García 1990, 1993a, 1999; Blindow *et al.* 2018a; this study) and Chile (Braun & Nordstedt 1882; Horn af Rantzien 1950, 1951; Blindow *et al.* 2018a, b). The species known from Australia (Wood 1972; Casanova 2005, 2007; this study) are indicated with an asterisk. The records of *C. baltica* Bruzelius from South America seem to be based on another species (Romanov *et al.* 2020a).

Species	Argentina	Chile
<i>Chara andina</i> (A.Braun) R.D.Wood & Imahori	+	-
<i>C. baltica</i> Bruzelius	+	-
<i>C. braunii</i> C.C.Gmel.*	-	+
<i>C. bulbiflora</i> (C.Donterberg) Adr.García	+	-
<i>C. contraria</i> A.Braun ex Kütz.*	+	+
<i>C. contraria</i> var. <i>nitelloides</i> A.Braun & Nordstedt	+	-
<i>C. globularis</i> Thuill.*	+	-
<i>C. hispida</i> var. <i>major</i> (Hartman) R.D. Wood n. inv.	+	-
<i>C. leptosperma</i> A.Braun	-	+
<i>C. leptospora</i> Sakayama	-	+
<i>C. longifolia</i> C.B.Rob.	+	-
<i>C. magellanica</i> (A.Braun) Blindow & Schubert	-	+
<i>C. poopóensis</i> G.O.Allen	-	+
<i>C. squamosa</i> Desf.*	-	+
<i>C. vulgaris</i> L.*	-	+
<i>Nitella acuminata</i> A.Braun ex Wallmann*	-	+
<i>N. arechavaletae</i> Arechav. ex Speg.	-	+
<i>N. asagrayana</i> W.Schaffn. ex Nordstedt	-	+
<i>N. bonaërensis</i> Speg.	-	+
<i>N. clavata</i> Kütz.	+	+
<i>N. flexilis</i> (L.) C.Agardh	-	+
<i>N. gracilis</i> (Sm.) C.Agardh	-	+
<i>N. hyalina</i> (DC.) C.Agardh*	+	+
<i>N. lechleri</i> A.Braun ex Horn	-	+
<i>N. mucronata</i> (A.Braun) Miq.	+	+
<i>N. opaca</i> (C.Agardh ex Bruzelius) C.Agardh	+	+
<i>N. pygmaea</i> A.Braun	-	+
<i>N. sonderi</i> A.Braun*	+	-
<i>N. tenuissima</i> (Desv.) Kütz.	-	+
<i>Tolypella glomerata</i> (Desv.) Leonh.*	-	+
Sum	12	22

along S shore of metal (gravel) quarry, 20.XI.1960, R.D. Wood 60-11-20-20 (LE[A0001488]); Victoria, Benalla, N arm of Broken River, c. 300 yd. [c. 274 m] N of bridge (behind swimming pool), N shore of river, common at water's edge, in c. 3 inches of fairly clear water, sandy gray mud bottom, 26.III.1963, R.D. Wood 61-3-26-2 (LE[A0001489]).

PHENOLOGY. — Male plants of *N. sonderi* were observed in the Patagonian locality during summer and autumn. A single plant producing antheridia has been successfully growing in an indoor aquarium since March 2020.

DESCRIPTION OF PATAGONIAN PLANTS

Plants dioecious (only male plants were found), green, not encrusted with lime, unbranched, longer than 13 cm, with isolated homeoclemous whorls, neither condensed nor clumped at the apex (Fig. 1), with obvious and wide, somewhat diffluent mucilage envelope of upper part of plants covering fertile whorls and internodes with a layer (Fig. 2D, E, G), approximately as thick as the diameter of the internodes and uniting whole branchlets within common envelope, sometimes resulting in lax apical heads (Figs 1; 2C, D). Stem diameter is (196-)337-509(-540) µm, increasing towards the basal part.



FIG. 1. – Apical parts of *Nitella sonderi* A. Braun from Argentina with sterile and fertile whorls (LE). Note: **arrowhead**, sterile whorls; **double arrowhead**, fertile whorls of male plants. Photo taken by R. E. Romanov. Scale bar: 1 cm.

The transversely elliptical cells extending from the base of the branchlet and forming a ring surrounding it are easily recognisable at appropriate magnification (Fig. 3A, B). The pair of these cells is commonly present below the branchlet base, one of the cells as an exception can be missing. These cells contain chloroplasts, not starch, and are not discoloured, 42-109 μm in length, 71-164 μm in width. Their growth seems to be able to culminate in nodal bulbil formation, recognisable as overgrowth of stem nodes inside and outside of the whorl before destruction of branchlets.

The sterile and fertile branchlets are macrodactylous, with similar principal arrangement and quite uniform appearance within the same whorl. However, fertile ones have shorter penultimate rays, resulting in a tassel-like appearance at the ends of the central rays because of aggregated dactyls (cf. Figs 1; 2A-D).

The sterile branchlets are 2-2.8-times shorter than the internodes; the length of internodes and the proportion between internode and branchlet length decrease towards the apex. The whorls of sterile branchlets are 32-44 mm in span, more

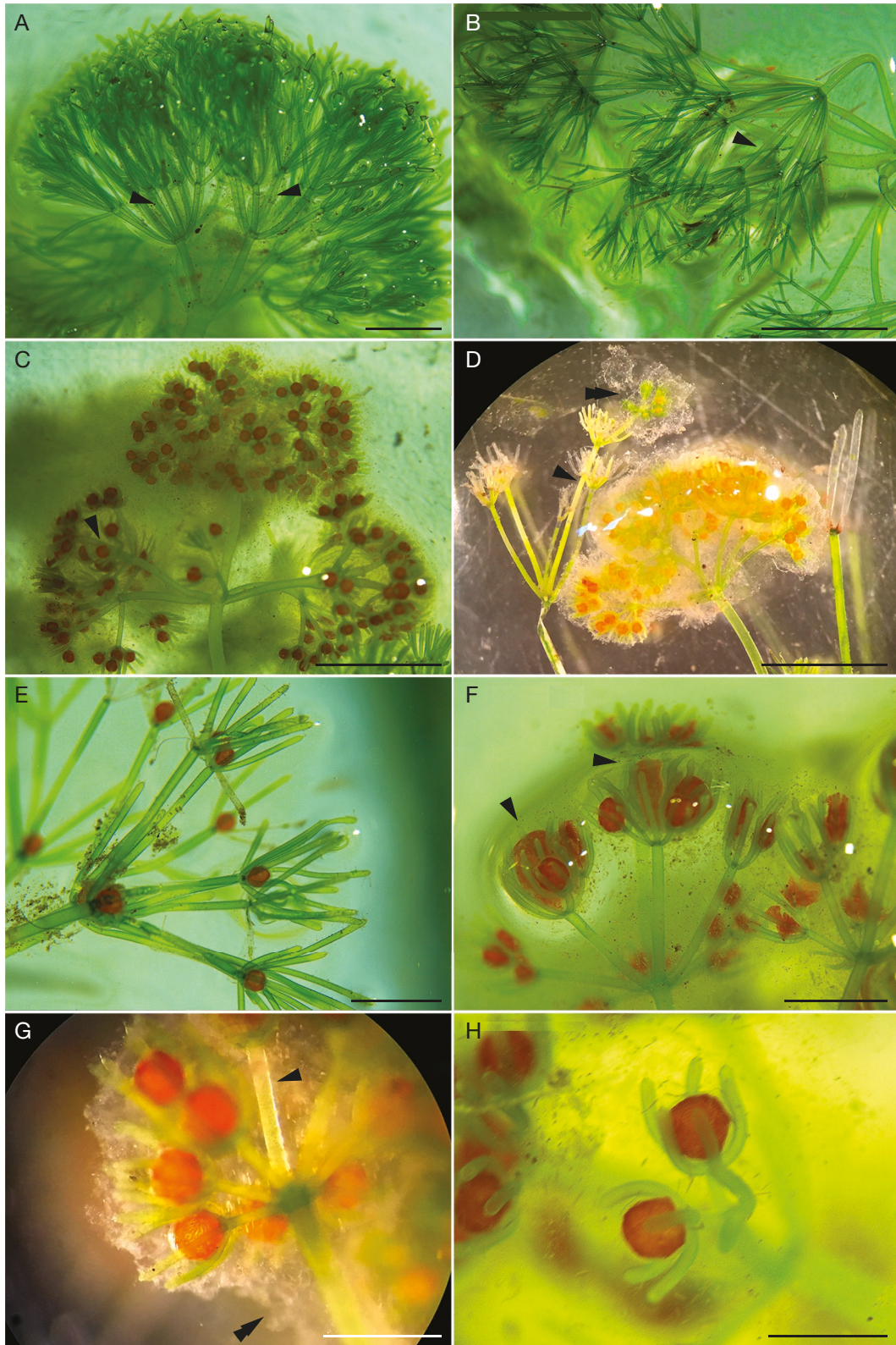


FIG. 2. – Male plants of *Nitella sonderi* A.Braun from Argentina (LE): **A**, whorl of sterile branchlets; **B**, sterile branchlet; **C**, apex with whorls of fertile branchlets forming lax head; **D**, lax apical head consisting of fertile branchlets, embedded in mucilage 3-furcate "proliferation" looking as a fertile branchlet without a central secondary ray from the lateral tertiary ray at the furcation of the central secondary ray (**double arrowhead**); **E**, last furcations of fertile branchlets with long straight dactyls; **F**, fertile branchlet with antheridia obviously unequal each other; **G**, mucilage cover of fertile branchlet (**double arrowhead**), mucilage cover of branchlet primary ray; **H**, antheridia at last furcations of branchlet surrounded with short dactyls, arcuate at their basal parts. Note: **A-C**, **arrowheads** represent central secondary ray; **D, F, G**, **arrowheads** represent surface of mucilage cover. Photos taken by R. Vidal-Russell. Scale bars: A, E, F, 2 mm; B-D, 5 mm; G, H, 1 mm.

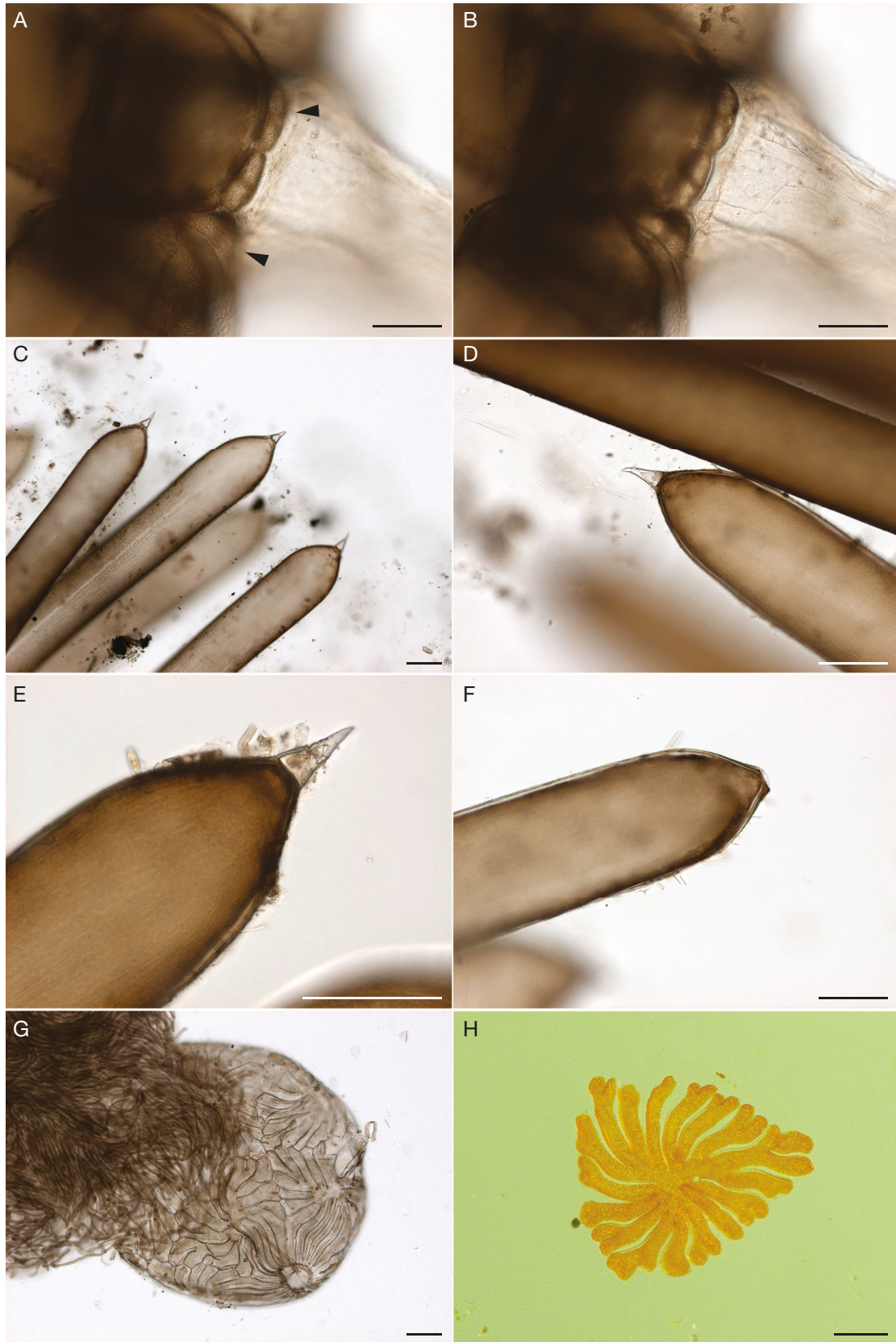


FIG. 3. – Male plants of *Nitella sonderi* A. Braun from Argentina (LE): **A, B**, cells extending from the node at the base of the lowest branchlet cell at different focus (**arrowheads at A**); **C-E**, shortly narrowing ends of bicellulate dactyls with discoloured tiny confluent end cells having thickened cell walls at the tip; **F**, end of dactyl after loss of end cell; **G, H**, triangular shields of antheridia. All photos taken by R. E. Romanov, but H by R. Vidal-Russell. Scale bars: 100 µm.

lax and diffuse, spreading more in contrast to fertile ones, which are shorter and compact at their ends, with a diameter of (10-)16-29 mm. The length of sterile branchlets is (10.9-)12.5-21.5(-23) mm, with primary rays (5-)7.7-9.5(-10) mm long, i.e., half of the total branchlet length or slightly shorter. The lateral secondary rays of sterile branchlets are 3.5-6(-6.5) mm in length, whereas the central secondary ones are approximately 4.5-5 mm.

The fertile branchlets form at apical parts of the plants (Fig. 1). The length of fertile branchlets is 9-15 mm, with primary rays having a length of 5-8.5 mm, i.e., mostly somewhat exceeding half of the total branchlet length. The primary ray at the basal part is 155-382 µm in diameter, and in the apical part, in fertile whorls, it is 110-235 µm in diameter. The lateral and central secondary rays of fertile branchlets are shorter in absolute values (approximately two times less in case of the latter ones) in comparison with sterile branchlets.

Each branchlet is 2-3-times forked, with a central secondary ray, surrounded by 6-8 more or less equal or somewhat shorter lateral secondary rays (sometimes *c.* 0.4-0.7 the length of the central secondary ray), mostly not differing in width from the central one, but sometimes obviously more robust (Fig. 2A, C, F, G). As an exception, the branchlet can produce 3-furcate “proliferation”, looking like a fertile branchlet without a central secondary ray. It forms from the lateral tertiary ray at the central secondary ray furcation (Fig. 2D). The lateral secondary rays are 1- or 1- and 2-times forked; the latter is less frequent at sterile branchlets in contrast with the common pattern of fertile ones. The lateral secondary rays are longer than the dactyls in sterile whorls and longer, nearly equal to mostly slightly or obviously shorter than the dactyls in fertile whorls (to approximately 0.8 of dactyl length). The dactyls of 1- and 2-times forked lateral secondary rays in sterile whorls are 1.8-2-times shorter than those in tertiary rays.

The central tertiary ray is neither formed at the furcation of lateral rays nor at the central secondary ray; the tertiary rays are 1-3 (or more?) in furcation and, as a rule, shorter than dactyls. The central secondary rays are 2-times furcated.

The dactyls are 2-4(-5) in sterile branchlets, and 5-6 in fertile branchlets, strictly bicellular, 0.8-2.8(-3) mm in length in sterile branchlets, 0.8-1.3 mm long in fertile branchlets, at second and third furcations, and at the first furcation of sterile branchlets, straight at sterile and fertile nodes in case of dactyls significantly longer of the antheridium, usually more or less arcuate in their basal parts at nodes with the antheridium in case of short dactyls, 1.2-2-5-times longer than the antheridium (Fig. 2C, E-H). The penultimate cell is long cylindrical with a shortly narrowing end (Fig. 3C-E). The base of the end cell is confluent with the tip of penultimate cells. The end cell is small, conical, pointed, straight or somewhat curved through the whole length or only at the tip, mostly discoloured in living, pressed and fixed states. The dactyls appear single-celled at lower magnification because of tiny and mostly discoloured confluent end cells. The width of dactyls at their base (above the antheridium) is (112-)130-250 µm, at the apical part (below the shortly narrowing end) it is 123-187 µm. The end cells are 46-68 µm

in length and 15-31 µm in width. The tip of the end cell has an obviously thickened cell wall (Fig. 2C-D). The end cells can be lost over time (Fig. 3F).

The antheridia are solitary, strictly terminal at the second and third furcations of the branchlet, never at the first one, sessile, round with a slightly attenuated base, octoscutate (i.e., with triangular shields; Fig. 3G, H), frequently looking robust in contrast with the length and width of dactyls surrounding them (Fig. 2E-H), (370-)399-655(-762) µm in diameter; their diameter can be different within the same branchlet (Fig. 2F).

PHYLOGENETIC ANALYSES

The *rbcL* alignment was 1193 bp long and the ITS alignment was 1648 bp long including indels. Both, nuclear (ITS rRNA) and the chloroplast (*rbcL*) markers recovered congruent topologies. In the *rbcL* phylogeny (Fig. 4), *N. sonderi* is a sister to *N. morongii*, with high support (100/1.00) within a clade with *N. hyalina* (DC.) C. Agardh, *N. pseudoflabellata* A. Braun, *N. comptonii* Groves, *N. elegans* Pal, *N. imperialis* (Allen) Sakayama, *N. vieillardii* (A. Braun) Sakayama, *N. singaporensis* R.E. Romanov, R.S.W. Yeo, B.C. Ho, V.Y. Nikulin & A.A. Gontcharov, and *N. megaspora* (Groves) Sakayama, i.e., in clade (4) in Group A (Fig. 4) following Sakayama (2008).

With the ITS dataset the same clade is recovered, albeit with fewer species. *Nitella sonderi* again was resolved as a sister of *N. morongii* within a clade composed of *N. comptonii*, *N. elegans*, *N. pseudoflabellata*, and *N. megaspora* (89/0.96, Fig. 5).

DISCUSSION

The monoecy/dioecy, details of whorl and branchlet arrangement, i.e., homoclemous/heteroclemous, presence/absence of secondary rays, brachydactylous/macrodactylous, proportions between branchlet segment length, cell number in dactyl, size and shape of dactyl end cells, mucilage presence in combination with oospore surface pattern, are the most important traits for the contemporary taxonomy of *Nitella* (Wood 1965; Sakayama 2008; Casanova 2009). According to the branchlet and dactyl arrangement as well as phylogenetic affinity, *N. sonderi* belongs to the subgenus *Tieffallenia* R.D. Wood section *Gioallenia* R.D. Wood (Karol 2004; Casanova 2007; authors pers. comm.). Few dioecious species of *Nitella* are known from South America (Carl de Donterberg 1970; Guerlesquin 1981; Picelli-Vicentini *et al.* 2004; Araújo *et al.* 2010; Rodríguez *et al.* 2012; Bueno *et al.* 2016; Blindow *et al.* 2018b; Ribeiro *et al.* 2021), but none of them belongs to the section *Gioallenia*. Although only male plants were found, the combination of traits in the case of our plant is unknown for nearly all other species of *Nitella* reported from South America, but it points towards identity of vegetative traits with *N. sonderi*, known formerly from Australia and Tasmania only, although the latter records are unverifiable (Casanova 2007, 2009).

The vegetative traits of Patagonian plants are the same as described for *N. sonderi* across the distribution range (Casanova 2007), but with a narrower variation in the number of branch-

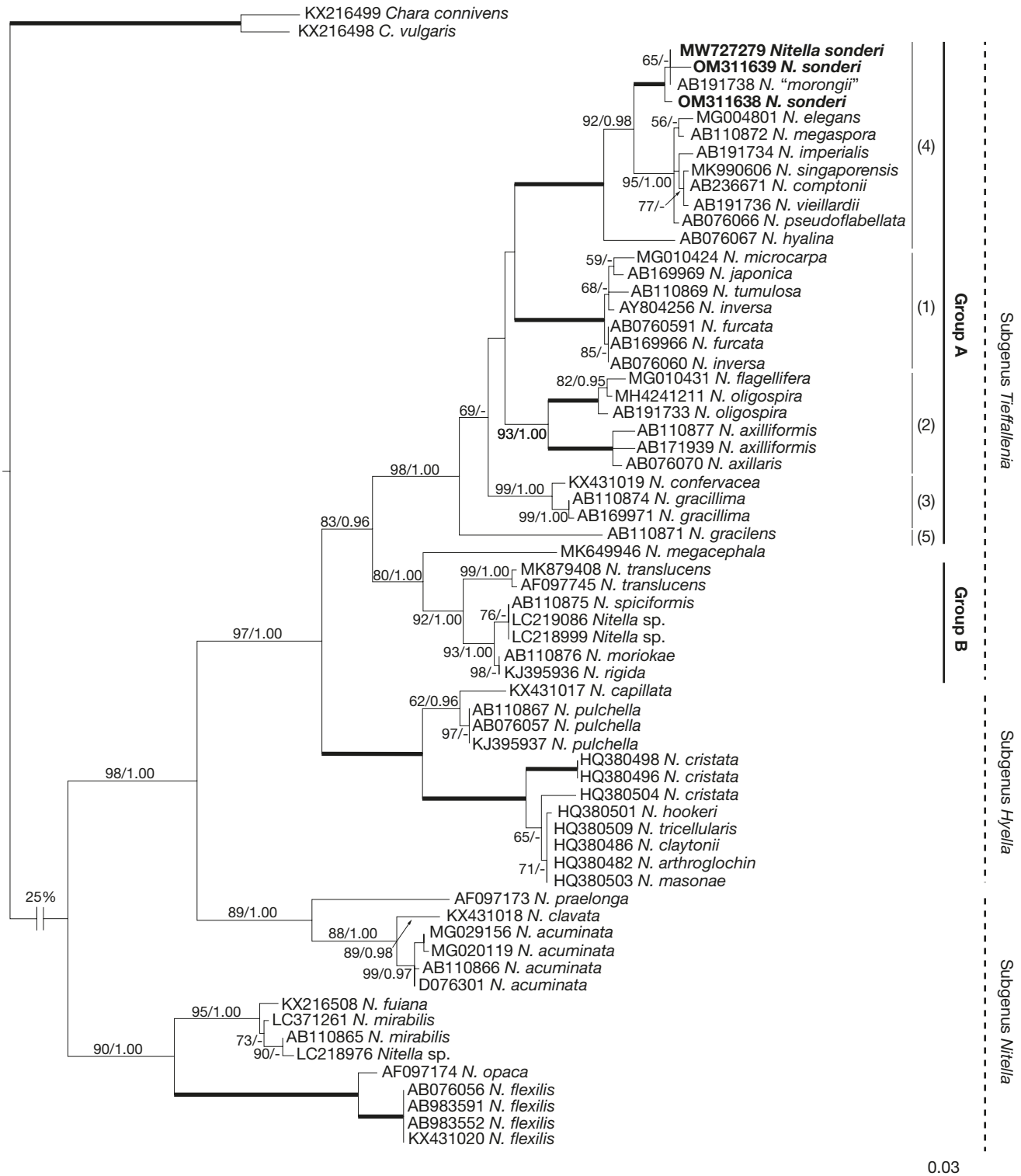


FIG. 4. — ML phylogenetic tree inferred in PAUP with GTR + I + G nucleotide substitution model from 66 *rbcL* sequences of *Nitella* C.Agardh. Support (ML/BI, BP ≥ 50% and PP ≥ 0.95) are given above/below the branches. Branches with 100% BP, 1.00 PP and sequences obtained for this study are shown in boldface. The branch leading to the outgroup was reduced by three quarters in length. Clade designation is according to Sakayama (2008).

let rays, being closer to the maximal values. The diameter of antheridia has overlapping values for Australian and Argentinian populations (up to 450 μm vs (370-)399-655(-762) μm), but Patagonian plants produce mostly larger ones, and lower

values characterize unripe antheridia. They are octoscutate in the case of Patagonian plants and probably have the same number and shape of antheridial shields in the case of Australian ones, but this does not seem to be indicated in their

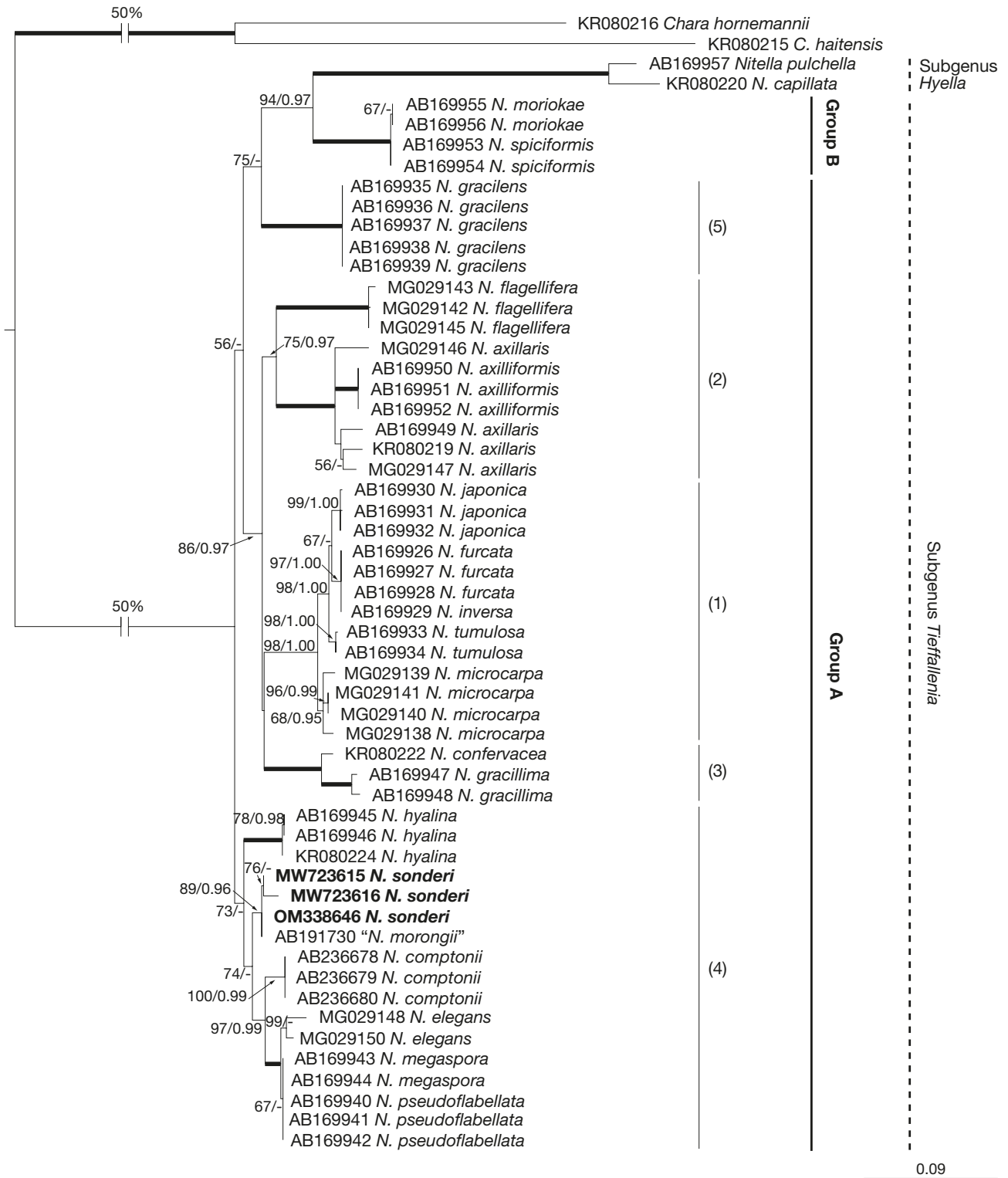


FIG. 5. – ML phylogenetic tree inferred in PAUP with GTR + I + G nucleotide substitution model from 56 ITS sequences of *Nitella* C. Agardh. Support (ML/BI, BP \geq 50% and PP \geq 0.95) are given above/below the branches. Branches with 100% BP, 1.00 PP and sequences obtained for this study are shown in **boldface**. The branch leading to the outgroup was reduced by 50% in length. Clade designation is according to Sakayama (2008).

descriptions. Unfortunately, female plants of *N. sonderi* were not found at the new site, even after thorough searches.

According to the available descriptions, Patagonian plants differ from Australian ones in having a higher number of

branchlet secondary rays: 7-9 including central one in contrast with 4-6 of the latter, as well as the number of dactyls at the third furcation of fertile branchlets: 5-6 vs 2-4 (Casanova 2007). The clear indication of a central secondary ray in

branchlets of *N. sonderi* is noted in descriptions by Wood & Imahori (1964) and Wood (1965) and traceable in drawings by Imahori (Wood & Imahori 1964). This trait is of primary importance for the taxonomy of *Nitella* (Wood 1965). Therefore, it should be present in all specimens of *N. sonderi*, although branchlets without it were described and illustrated for male plants (Wood & Imahori 1964: icon 296, 4, 5).

All the traits known for Australian plants were observed in specimens from this region stored in LE (Romanov pers. comm.). Moreover, their thorough study found overlapping values for all features of Patagonian plants that seemed to be different to Australian plants according to published descriptions of them. The antheridia of Australian plants are octoscutate too. The number of branchlet secondary rays is 6-9 including central one. The number of dactyls is 4-5(6). The central secondary ray is a stable trait for female and male Australian plants. The occurrence of branchlets without a central secondary ray in male plants illustrated by Imahori (Wood & Imahori 1964: fig. 296, 5) was also confirmed in Australian specimens at our disposal. It seems to occur only at lowermost whorls. Therefore, these additions to the description of *N. sonderi* (see above) suggested here are based on the specimens from Argentina and Australia.

Nitella papillata F.S.Han & W.Q.Chen, known from South-West China (Han & Chen 1982: 360-362, pl. 3, figs 6-13; Han & Li 1994), is similar to *N. sonderi*, with dioecy and branchlet arrangement as well as presence of mucilage, but it has smaller antheridia diameter (230-360 µm in the case of *N. papillata*) and differs in having a thick central secondary ray, as well as more elongated and longer branchlet end cells and shorter branchlets, although overlapping with minimal values known for *N. sonderi*.

Phylogenetic relationships between *Nitella* species recovered in our analyses are in agreement with previous studies (Sakayama *et al.* 2004, 2005; Sakayama 2008; Borges & Necchi 2018; Lee *et al.* 2019; Romanov *et al.* 2020b). *Nitella sonderi* was resolved in a clade with other members of the section *Gioallenia* in agreement with their morphology. Unfortunately, no sequence for *N. papillata* is available and sequence from another Australian plants of *N. sonderi* are not publicly available either (cf. Karol 2004). Therefore, these could not be included in our phylogenetic analyses.

The genetically similar species, forming clade 4 (cf. Figs 4; 5), are mainly known from Eastern and South-Eastern Asia, Australia and New Caledonia. With both markers, dioecious *N. sonderi* shows neither significant difference with the Australian specimen referred to as *N. morongii* Allen by Sakayama *et al.* (2005) (Fig. 4) nor with other species in the clade.

Nitella morongii is a North American species clearly differing from *N. sonderi* by its monoecy, general habit because of the sharp difference between sterile and richly fertile branchlets, numerous heads per shoot and their shape, the presence of axillary heads, less branchlet furcations (mostly 1-2), the number of secondary rays (3-5) and of dactyls (2-3), the absence of a central secondary ray in a branchlet, the presence of mucronate end cells of the branchlet, longer and wider branchlet end cells, very small (up to 190 µm) and shortly

stipitate antheridia (Allen 1887; Wood 1948, 1949, 1965; Wood & Muenscher 1956; Wood & Imahori 1964). Differences in oospores taken from type specimen of *N. morongii* and Australian plant referred to this species are notable. The type specimen has oospores that are closely granulate to vermiferous at the surface, described with the help of a light microscopy (Wood & Imahori 1964). Later, it was recognised as spongy according to an SEM study (Frame 1977). The oospores of the Australian plant referred to *N. morongii* (the sequence used in this study) have a different surface because of the tuberculate pattern with a spongy background in SEM (Sakayama *et al.* 2005). The oospores of *N. sonderi* have surface appearing verrucate in immature state, the verrucae extending into tuberculate at maturity, occasionally joining to form ridges perpendicular to the striae (Casanova 2007). This is clearly dissimilar with pattern described for Australian plant referred to *N. morongii* (cf. Sakayama *et al.* 2005). Therefore, its thorough morphological study is desirable.

Any suggestion of affinity between morphologically distinct *N. sonderi* and *N. morongii* without results of phylogenetic analyses (Figs 4; 5) is problematic given the insufficient evidence. Their close clustering can be explained by misidentification of the GenBank accession for *N. morongii*, which means that the sequence deposited in the database actually refers to another taxon. This could be possible because this data represents the first report of *N. morongii* in Australia (Sakayama *et al.* 2005). ITS, rRNA and *rbcL* sequences suggested that the two taxa are closely related. However, that is unlikely from a morphological perspective. A closer examination of the morphology of the Australian specimen collected by Sakayama *et al.* (2005) should be conducted to have a better understanding of the taxa and their biogeographic history.

Dioecious *Nitella lhotzkyi* (A.Braun) A.Braun belongs to the *Decandollea* section. It was not sequenced yet, but it might be a member of the clade 4 (cf. Figs 4; 5). It differs from *N. sonderi* with presence of few accessory branchlets in a whorl and less number of secondary rays in a branchlet, absence of gametangia at third furcation of branchlet and, probably, absence of central secondary ray (Wood 1965).

Some morphological traits described for the Patagonian population of *N. sonderi* are notable. The difference in antheridia diameter within the same branchlet (Fig. 2F) can be recognised as a sign of uneven ripening. Therefore, the lower values of antheridial size can be due to the measurement of unripe antheridia, which is important for separation from *N. papillata*. The small, discoloured end cell of the branchlet, recognised as a pointed hyaline end of a long penultimate cell (Fig. 3C-E), i.e., the dactyls, could be erroneously recognised as single-celled with the naked eye or at low magnification. This seems to be a possible source of confusion with species having unicellular dactyls. The cells extending from the base of branchlets and surrounding them described for *N. sonderi* (Fig. 3A, B) are known for species of *Nitella* from different sections from Europe and Asia (cf. Romanov *et al.* 2020b; Romanov pers. comm.). At the moment, this trait seems to be of little taxonomic value for the genus, probably being a common step of node development, but its occurrence and

expression by different species and populations needs to be studied in detail.

As a rule, dioecious species of charophytes have smaller distribution ranges in comparison with monoecious ones. This was suggested for *Chara*, but it is applicable for *Nitella* representatives as well (Proctor 1980). Lack of intercontinental migration and exchange of dioecious taxa between the Western Hemisphere and Australia was concluded from worldwide species distribution patterns (Khan & Sarma 1984). The dispersal of charophytes across the Andean chain seems to be restricted as well (Blindow *et al.* 2018b), but it might be not so significant in case of Patagonia because of less mountain heights there. However, occurrence of *N. sonderi*, known from the region east of the Andes, is not in agreement with these hypotheses. The list of charophyte species common for Patagonia and Australia includes nine species only (Table 1), mostly having wide distribution areas. All of them are monoecious except *N. sonderi*. The charophytes from vast territory of Patagonia and especially areas south of Tierra del Fuego (cf. Schubert *et al.* 2015) are still underexplored, and this could be an explanation of this unexpected new record of the species far from Australia where it mostly resides. The record of a species from the group of *Nitella hookeri* A. Braun was expected in Patagonia, because it was assumed as having a circumpolar distribution in the Southern Hemisphere (Casanova *et al.* 2007). This biogeographical pattern, implying contemporary speciation, could be suitable for *N. sonderi* too. Only few dioecious, not parthenogenetic, species of charophytes have so large native disjunctions in their distribution range. *Nitella lhotzkyi* is known both from Australia and Brasil (Picelli-Vicentini & Bicudo 1990; Casanova 2005), and *Chara fulgens* Fil. has been recorded from Indonesia (Bali) and Chile only (Blindow *et al.* 2018a), although its environment, a caldera lake with very low conductivity, comparable with rain water during sampling of *C. fulgens* (Green *et al.* 1978), vs saline coastal lakes (Blindow *et al.* 2018a), is evidently different between these regions, remote from each other.

The invasion of *N. sonderi* seems to be less probable but could be possible, and population consisting of male plants could be an argument for this point of view, although an example of dioecious *N. cristata* A. Braun having populations of female plants restricted to the North Island of New Zealand and male plants primary known from the South Island (Wood & Mason 1977) softens this suggestion. The dispersal of *Nitellopsis obtusa* (Desv.) Groves from Eurasia to North America (Larkin *et al.* 2018) and *C. canescens* Loisel. from North to South Hemisphere (Casanova & Nicol 2009) are good examples of charophyte invasions at long distance.

Some perspectives for further studies can be suggested based on our results. A polyphasic approach should be implemented to *N. papillata* to clarify its possible close relationship with *N. sonderi*. The search and careful examination of the female plant of *N. sonderi* in South America, including scanning electron microscopy of its oospores for updating its description, are also essential for further studies. The Australian plants have remarkable stability of oospore traits across the continent (Casanova 2007). The population consisting of male plants

only with vegetative reproduction established from single or few oospores in the Patagonian locality of *N. sonderi* could be an explanation of unsuccessful searches for female plants. The section *Gioallenia*, which this species belongs to, covers species with many fertile nodes in a branchlet. Therefore, if fertile plants with abundant gametangia are available (as in our case), it seems to be unlikely that oogonia or antheridia can be missed during the study. The formation of different gametangia at different seasons at the same plant is unknown for charophytes, although frequent protandry of monoecious species like *N. flexilis* (L.) C. Agardh might complicate the perspective.

Ecology of *N. sonderi* in Argentina is also a challenge for future research. At the moment, it is known from a single locality on the Patagonian Plateau, in a large permanent freshwater hydro-power reservoir created at the Limay River, originating from the lake in the foothills of the Patagonian Andes and situated in the Patagonian Steppe. In Australia *N. sonderi* is generally an annual species of temporary edges and temporary wetlands, requiring drying to stimulate good germination of oospores (Casanova & Brock 1999).

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