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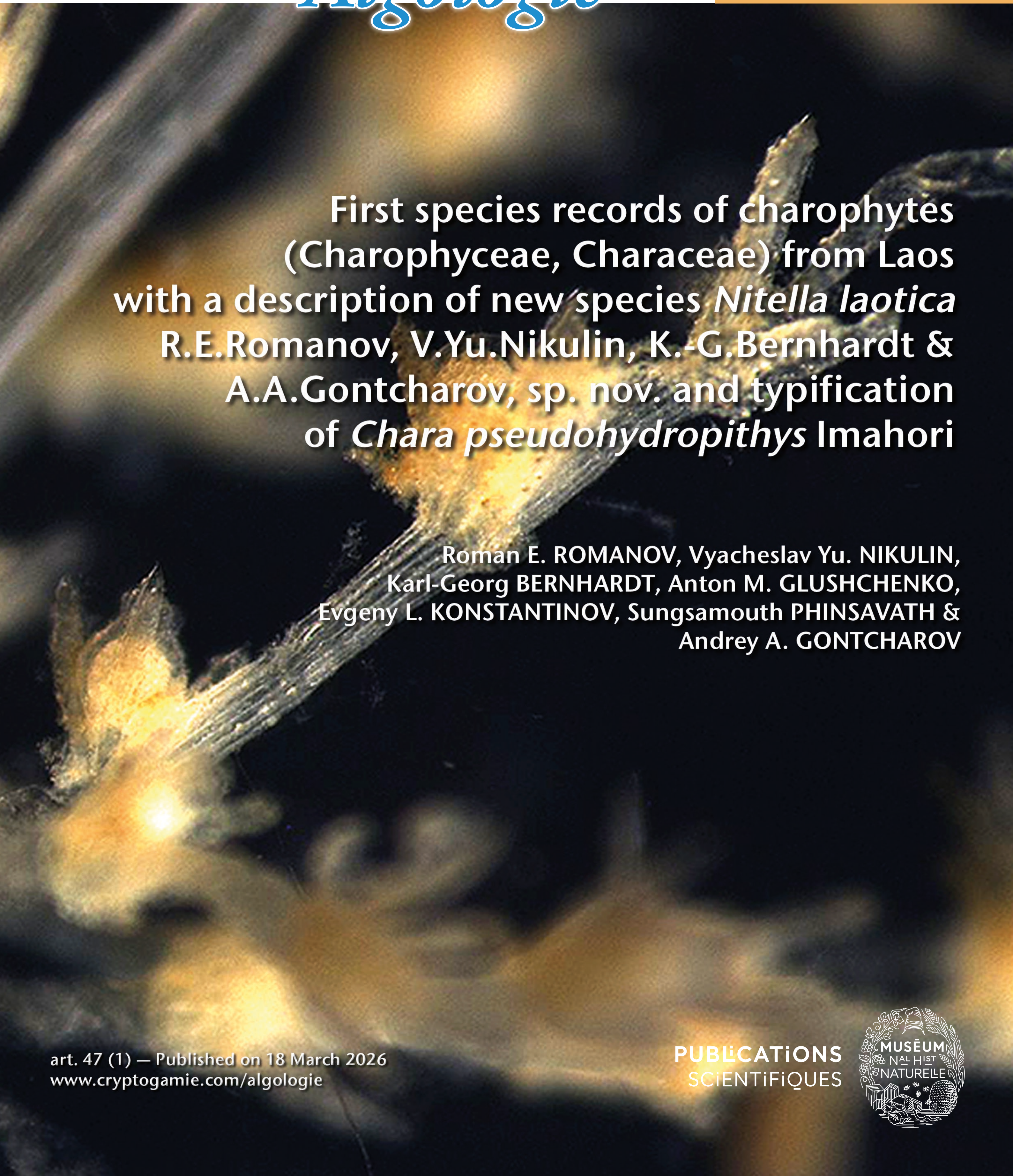

First species records of charophytes (Charophyceae, Characeae) from Laos with a description of new species *Nitella laotica* R.E.Romanov, V.Yu.Nikulin, K.-G.Bernhardt & A.A.Gontcharov, sp. nov. and typification of *Chara pseudohydropithys* Imahori

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ABSTRACT
Chara braunii C.C.Gmelin, *C. pseudohydropithys* Imahori, and *Nitella laotica* R.E.Romanov, V.Yu Nikulin, K.-G.Bernhardt & A.A.Gontcharov, sp. nov. are reported for Laos. These records seem to be the first species records of charophytes for the country. *Chara pseudohydropithys* is lectotypified here and described in detail. It is the first record for Southeast Asia, extending its distribution range from mainland China, Taiwan, India, and Brazil. We mention this first record of *C. braunii* which is a frequently reported species in well-studied regions of Southeast and East Asia, because of the complete absence of records of charophytes from Laos. *Nitella laotica* sp. nov. is described as a new species using integrative taxonomy. The plants of *N. laotica* sp. nov. are macrodactylous, homoclemous, with mostly two-furcated branchlets, some branchlets one-two- or two-three-furcated, with or without central secondary ray within the same thallus and the same whorl, without accessory branchlets originating from central secondary rays or any furcation of branchlets, with bicellular dactyls and conical and mucronate end cells, solitary conjoined gametangia at first and more frequently at second furcation of branchlets, with reticulate ornamentation on both oospore surface and low spiral striae. The formation of ripe oospore surface is tentatively outlined for *N. laotica* sp. nov. This species is similar to *N. oligospira* A.Braun, a species distinctly different in morphology, in *rbc*L and ITS rDNA data. Further studies of charophytes are essential for Laos because the three species found there do not correspond to the species richness that can be expected for charophytes in a tropical country with a humid climate.

KEY WORDS
Charales,
Chara braunii,
Nitella,
integrative taxonomy,
new species,
lectotypification.

RÉSUMÉ
Premiers signalements d'espèces de charophytes (Charophyceae, Characeae) au Laos, avec description d'une nouvelle espèce Nitella laotica *R.E.Romanov, V.Yu.Nikulin, K.-G.Bernhardt & A.A.Gontcharov, sp. nov. et typification de* Chara pseudohydropithys *Imahori.*
Chara braunii C.C.Gmelin, *C. pseudohydropithys* Imahori et *Nitella laotica* R.E.Romanov, V.Yu.Nikulin, K.-G.Bernhardt & A.A.Gontcharov, sp. nov. sont signalés pour le Laos. Ces signalements d'espèces de charophytes semblent être les premiers pour le pays. *Chara pseudohydropithys* est lectotypifié ici et décrit en détail. Il s'agit du premier signalement en Asie du Sud-Est, étendant son aire de répartition depuis la Chine continentale, Taiwan, jusqu'à l'Inde et le Brésil. Le premier signalement de *C. braunii*, qui est une espèce fréquemment signalée dans des régions bien étudiées d'Asie du Sud-Est et de l'Est, est dû à l'absence totale de signalements de charophytes du Laos. *Nitella laotica* sp. nov. est décrite comme une nouvelle espèce utilisant la taxonomie intégrative. Les plantes de *N. laotica* sp. nov. sont macrodactyles, homoclèmes, avec pour la plupart des rameaux deux fois furqués, certains rameaux mono-, bi- ou tri-, furqués, avec ou sans rayon secondaire central dans le même thalle et le même verticille, sans rameaux accessoires provenant des rayons secondaires centraux ou de tout autre furcation des rameaux, avec des dactyles bicellulaires et des cellules terminales coniques et mucronées, des gamétanges unies solitaires au premier et plus fréquemment à la deuxième furcation des rameaux, avec une ornementation réticulée à la fois sur la surface des oospores et sur les stries spirales basses. La formation de la surface des oospores mûres est provisoirement décrite pour *N. laotica* sp. nov. Cette espèce est similaire à *N. oligospira* A.Braun, une espèce qui se distingue nettement par sa morphologie et par ses données d'ADN ribosomique *rbc*L et ITS. Des études plus approfondies sur les charophytes sont essentielles pour le Laos car les trois espèces qui y sont trouvées ne correspondent pas à la richesse spécifique que l'on peut attendre pour les charophytes dans un pays tropical au climat humide.

MOTS CLÉS
Charales,
Chara braunii,
Nitella,
taxonomie integrative,
nouvelles espèces,
lectotypification.

INTRODUCTION

The charophytes (Characeae) are represented in extant flora with 511 currently accepted species (Guiry 2024). South and Southeast Asia can be one of the most charophyte species-rich areas in Eurasia. This could be expected partly based on charophyte species richness data from China (Qiu & Ling 2007) and a reported high diversity of the genus *Nitella* C.Agardh, the most species-rich genus of Characeae, reported from Asia (Khan & Sarma 1984; Sakayama 2008). An extensive bibliography for species records and a long list of charophyte taxa are notable for both India and China among Asian countries. The most recent synopsis of charophytes from India includes 205 taxa of extant charophytes with *c.* 140 species (Guiry & Guiry 2024). This number cannot be exactly evaluated taking into account different species concepts used in charophyte taxonomy by different authors. In any case, many taxa of charophytes have been described from India, including 22 endemic species (Khan 1991). The charophyte flora of China includes at least 205 taxa and 161 species (Han & Li 1994; Ling *et al.* 2000; Chen *et al.* 2018; Song *et al.* 2022) with 94 taxa, i.e., nearly half of the national taxonomic richness, being endemic to China (Qiu & Ling 2005; Chen *et al.* 2018; Song *et al.* 2022). *Nitella* and *Chara* L. are represented here by 55 and

35 endemic taxa, respectively (Qiu & Ling 2005; Chen *et al.* 2018; Song *et al.* 2022; Romanov *et al.* 2026). Taxonomic accounts for charophytes are available for South, East, and Southeast Asia, i.e., Pal *et al.* (1962) for South Asia, Faridi (1955), Langangen & Leghari (2001) for Pakistan, Subramanian (2002, 2018) for India, Naz *et al.* (2011) for Bangladesh, Pal (1932) for Myanmar, Zaneveld (1940) for the Indo-Malayan (Malesian) region, Imahori (1954), Imahori & Kasaki (1977) for Japan, Yongyuth (1977) for Thailand, Hô (1968) for Vietnam, Han & Li (1994) for China, and Choi & Kim (1997, 1998) for Korea. Recent studies on morphological and genetic polymorphism of selected species of *Chara*, *Lamprothamnium* J.Groves, and *Nitella* from Japan, Korea, India, and Myanmar are available (Nozaki *et al.* 1998; Kato *et al.* 2008, 2010, 2021; Sakayama *et al.* 2002, 2004a, b, 2005, 2006, 2009, 2015; Lee *et al.* 2016; Balakrishnan & Rani 2017; Mjelde *et al.* 2021), as well as some new distribution records for Taiwan (Chou *et al.* 2007; Chou & Wang 2014), Japan (Suzuki *et al.* 2025) and Nepal (Dhakal *et al.* 2025). In addition, new species of *Nitella* and *Chara* were described from Korea, China, Russia, and Singapore (Chen *et al.* 2018; Lee *et al.* 2019; Romanov *et al.* 2020, 2025; Song *et al.* 2022; Sakayama *et al.* 2024). Species checklists are available for India, Thailand, Vietnam, and Singapore (Suvatti 1978; Dang *et al.* 2002; Pham *et al.* 2011; Gupta 2012). In contrast, no species records of charophytes have been published for Laos. It can therefore be assumed that the local charophyte species richness in Southeast Asia is unevenly reported because local data are lacking and recent studies do not exist. Moreover, the identification and delineation of charophytes is complicated by the wide variability of morphological traits described for the few species among many known tropical species of charophytes (Zaneveld 1940; Wood & Imahori 1965). These obstacles could be overcome with detailed knowledge of the variability of species over their distribution ranges, as well as understanding variation within populations and even within the same plant, with a detailed knowledge of morphological traits of type specimens (cf. Casanova 2007; Romanov *et al.* 2020, 2023, 2026). The level of variability within the same population can be wide and underestimated in taxonomic research (Romanov *et al.* 2023, 2024). To fill this knowledge gap, we report three species for Laos with a focus on their morphological variability and describe the new species *Nitella laotica* R.E.Romanov, V.Yu.Nikulin, K.-G.Bernhardt & A.A.Gontcharov, sp. nov. on the basis of integrative taxonomy.

MATERIAL AND METHODS

Morphological study

The specimens were collected by hand from small water bodies in 2011, 2012, and 2018 during excursions organized by E. L. Konstantinov and S. Phinsavath. They were pressed or stored in a 4% solution of formalin. The studied specimens are stored in herbaria LE (Komarov Botanical Institute of the Russian Academy of Sciences; acronyms according to Thiers 2024) and WHB (Institute of Botany, Vienna). At least one specimen of unidentified *Nitella* from Laos is stored in the herbarium L (Naturalis Biodiversity Center: L.4104671) but it is almost impossible to guess its species identity without study in rewetted state.

The morphological features of the specimens studied were observed using a Carl Zeiss Stereo Discovery V12 stereo microscope equipped with an AxioCam MRs-5 digital camera (Carl Zeiss AG, Oberkochen, Germany), stereo microscopes Olympus SZ61 and SZX12, and microscope Olympus BH2 (Olympus Corporation, Shinjuku, Tokyo, Japan) equipped with Canon EOS80D digital camera (Canon Inc. Operations, Ohta-ku, Tokyo, Japan). Photographs of diagnostic traits were taken using a digital camera. We preferred using photographs instead of line drawings, because it allowed illustration of expression and variability of morphologic traits as they are, in other words, as they are visible directly to any researcher. Oospores taken from specimens of *Nitella* for scanning electron microscopy (SEM) were treated according to a previously described method (Romanov *et al.* 2015). The cleaned oospores were coated with gold and studied using a Zeiss EVO 40 scanning electron microscope (Carl Zeiss AG, Oberkochen, Germany). The terms for describing morphology for *Nitella* are in agreement with Wood & Imahori (1965), for *Chara* – with the book "Charophytes of Europe" (Schubert *et al.* 2024).

DNA extraction, amplification and sequencing

Total genomic DNA was extracted as described previously by Echt *et al.* (1992) with some modifications (Kiselev *et al.* 2015). Part of the *rbc*L gene and full nuclear ribosomal internal transcribed spacer region (ITS1 + 5.8S + ITS2) were amplified as described previously (Romanov *et al.* 2022b, 2022c, respectively). The PCR products were purified by ExoSAP-IT PCR Product Cleanup Reagent (Affymetrix, Santa Clara, California, United States) and sequenced in both directions using an ABI 3500 genetic analyzer (Applied Biosystems, Foster City, California, United States) at the Instrumental Center of Biotechnology and Gene Engineering of FSCEATB FEB RAS with a BigDye terminator v3.1 sequencing kit (Life Technologies Corporation, Austin, Texas, United States) and the same primers used for PCR. Sequences were assembled with Staden Package v.1.4 (Bonfield *et al.* 1995) and aligned manually in the SeaView program (Galtier *et al.* 1996).

Newly obtained *rbc*L and the ITS rDNA region sequences were deposited in GenBank (for details see "specimens examined" below, Appendix 1). Any attempts to obtain ITS for specimens of *N. laotica* sp. nov. collected in 2012 were unsuccessful.

Phylogenetic analyses

The *rbc*L and ITS datasets were assembled as described by Romanov *et al.* (2022b, 2022c). It is very likely that there are misidentifications among publicly available sequences. However, the comparison of each newly obtained sequence with already known ones is essential for showing their difference/similarity irrespective of their true identity as a first step of each integrative taxonomic research and allows detection of new entities, previously unknown from a genetic perspective. The comparison of sequences recovered from type specimens would be the best solution. Unfortunately, it is hardly if ever achievable because of the great age and the small size of many type specimens (cf. Wood & Imahori 1965; van Raam 2010). There is small hope for success and many herbaria exclude destructive sampling, although it has occurred in some cases, even for really old specimens (cf. Romanov *et al.* 2023). Maximum likelihood (ML) analyses were carried out using PAUP 4.0b10 (Swofford 2002). Bayesian inference (BI) was performed using MrBayes 3.1.2 (Huelsenbeck & Ronquist 2001). To determine the most appropriate DNA substitution models for our datasets, we used the Akaike information criterion (AIC; Akaike 1974) was applied with jModelTest 2.1.1 (Darriba *et al.* 2012). The GTR+I+G model was selected as the best fit for both datasets. ML analyses were carried out using heuristic searches with a branch-swapping algorithm (tree bisection-reconnection). In BI, four parallel MCMC runs were carried out for nine and four million generations for the *rbc*L and ITS datasets, respectively. Sampling was carried out every 100 generations. The convergence of the chains was assessed, and stationarity was determined according to the ‘sump’ plot (the first 25% of the samples were discarded as ‘burn-in’). The posterior probabilities were calculated from the trees sampled during the stationary phase. The robustness of the trees was estimated by bootstrap percentages (BP; Stamatakis *et al.* 2008) in ML and posterior probabilities (PP) in BI. A BP < 50% and PP < 0.95 were not considered. An ML-based bootstrap analysis was inferred using the web service RAxML version 7.7.1 (http://embnet.vitalit.ch/raxml-bb/; Kozlov *et al.* 2019).

RESULTS AND DISCUSSION

Taxonomic treatment

Three species were found in the available gatherings from Laos.

Family Characeae Gray
Genus *Chara* L.

Chara braunii C.C.Gmelin

Flora Badensis, Alsatica et confinium regionum cis et [...] 4: 646 (Gmelin 1826).

Neotype (designated by Wood *in* Wood & Imahori 1965: 262). — Prope Oldenico; aetate 1856; Malvinverni (NY945633, Macroalgal Herbarium Portal 2024).

Specimens examined. — **Laos** • Xiangkhouang Province, Plain of Jars, Site 2, in an inundated stone jar; *c.* 19°19’11.2”N, 103°09’14.8”E; pressed specimens; DNA voucher specimens for *C. braunii* C.C. Gmelin and *N. laotica*; 06.XII.2012; *A. M. Glushchenko, E. L. Konstantinov & other biologists of the Tsiolkovsky Kaluga State University* (LE[LEA0006486]; GenBank accession: *C. braunii rbc*L[PP470896]; *N. laotica* sp. nov.; *rbc*L[PP470898]) • Nordlaos, Provinz Xieng Khouang, Phonsavan, Ebene der Steinkrüge, Site 1, in wassergefülltem Steinkrug; 19°25’49.2”N, 03°09’19.0”E; 1109 m alt.; pressed specimens; DNA voucher specimen for *C. braunii* and *N. laotica* sp. nov.; 11.VIII.2018; *K.-G. Bernhardt & N. Stoeckl* (LE[LEA0006487], WHB[WHB72405]; GenBank accessions: *C. braunii rbc*L[PP470897], *N. laotica* sp. nov.; *rbc*L[PP470899], *rbc*L[PP470900], ITS[PP476817]), ITS[PP476818].

Distribution. — One of few genuinely cosmopolitan species of charophytes (Khan & Sarma 1984; Korsch 2018). It is known from north boreal to tropical regions, being a typical charophyte of paddy fields worldwide, although it can grow in different types of water bodies from freshwater to brackish including large lakes with diverse thermal regimes (Casanova & Doege 2024).

Variability. — Our specimens are quite uniform and showed no new or extreme expression of morphological traits from the perspective of morphological descriptions available for this species from different regions (see references in ‘Introduction’ and ‘Material and methods’). This uniformity of plants from individual populations was reported by Zaneveld (1940). The studied plants belong to the typical variety and form, in other words, var. *braunii* (A. Braun) Zaneveld f. *typica* Zaneveld *nom. inval.* (cf. Zaneveld 1940). Missing branchlets at most axial nodes of long plants can be recognized as evidence of long-term growth at the same site, probably indicating that these populations are represented by perennial plants. This assumption based on our numerous observations of perennial populations of other species requires direct field observations because it is widely accepted that *C. braunii* is mostly an annual species, although in permanent warm-water habitats, it can grow for a long time (Casanova & Doege 2024), likely repeatedly reproducing by germination of oospores in some cases (Proctor 1990), or probably growing as long-living individual plants having vegetative reproduction (Soulié-Märsche & Vautier 2004) . The annual populations are confirmed by our observations in North Asia, i.e., at the northern part of the species distribution range, including ice-free sites at Kamchatka receiving thermal effluents (Chernyagina 2018; Chernyagina O. A. & Romanov R. E., unpublished data).

Description

Rarely branched richly fertile ecorticate monoicous plants, 5.3 to 16-30 cm in length, moderately zonally lime-incrusted, bearing complete branchlets at upper ⅙-⅓ part, with decaying basal parts, bearing slightly enlarged stem nodes (Fig. 1A, B). Stipulodes are haplostephanous, acute, very short (Fig. 1A). Branchlets 10 in a whorl, 7-14 mm length, completely differentiated into nodes and internodes, consisting of three-four, mostly four segments and tiny coronula at the top (Fig. 1B), nearly equal or slightly shorter than internodes at parts with complete branchlets, slightly longer to 1.5-2-times longer of internodes at apical parts. Sometimes the basal segment is distinctly shorter than the segment above it. Gametangia are conjoined, mostly at the two basal nodes of the branchlets. Abaxial bract-cells are acuminate, short, adaxial bract-cells and bracteoles are longer but, anyway, they are shorter of ripe oogonia, usually *c.* ⅔-⅘ of oogonium length. Oogonia are 530-660 × 310-415 µm without coronula in a dry state. Coronulae are 110-135 × 90-162 µm in dry state. Ripe oospores are black.

Chara pseudohydropithys Imahori

Botanical Magazine, Tokyo 63: 262 (Imahori 1950), (‘*pseudohydropitys*’).

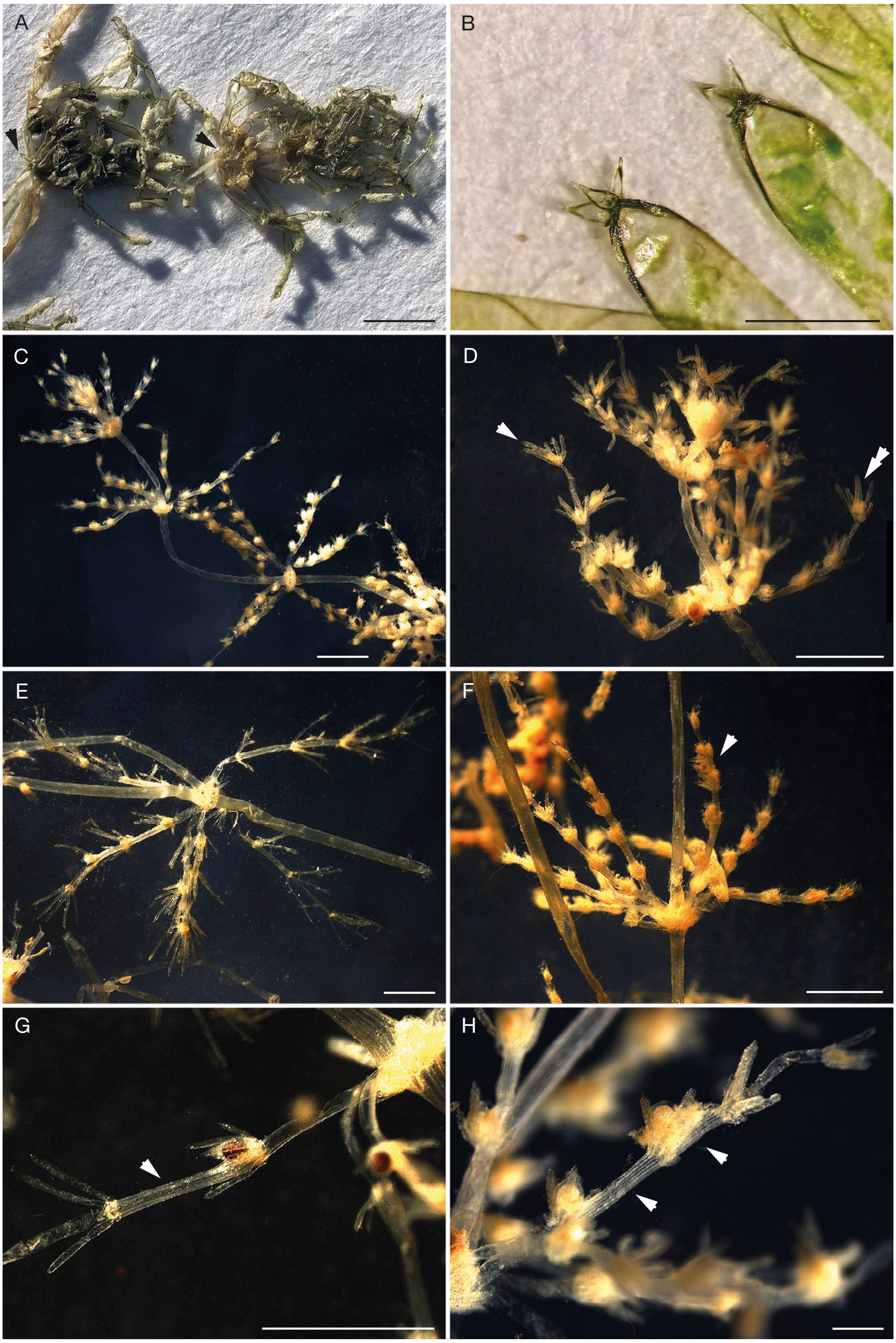

Fig. 1. — Morphological characters of *Chara braunii* C.C.Gmelin (**A**, **B**) and *C. pseudohydropithys* Imahori (**C**-**H**) from Laos (**A**, **C-H**, LE; **B**, WHB [72405]): **A**, apical branchlet whorls with partially lime-incrusted end segments of branchlets, short acute haplostephanous stipulodes (**arrowheads**) and conjoined gametangia (**double arrowheads**); **B**, coronulae of cells at ends of branchlets; **C**-**F**, different general appearance of branchlet whorls resulting from variable length of bract-cells; **C**, apical whorls of branchlets with short unilateral bract-cells, black oospores are visible at lower branchlets; **D**, apical branchlet whorls with verticillate and unilateral bract-cells of moderate length, **arrowhead** indicates end tassel of cells at the top of branchlet, **double arrowhead** indicates conjoined gametangia at fourth node of branchlet where end tassel became non-obvious; **E**, whorl of branchlets with long verticillate bract-cells; **F**, whorl of branchlet with short unilateral bract-cells, and three (**arrowhead**) and four corticate segments; **G**, branchlet with four nodes, well-developed verticillate bract-cells and single corticate segment (**arrowhead**) between ecorticate segments, brown oospores are visible; **H**, branchlet with four nodes, short unilateral to verticillate bract-cells and two corticate segments (**arrowheads**). Scale bars: A, C-G, 2 mm; B, H, 0.5 mm. Photo credits: R. E. Romanov.

Lectotype (designated here by R. E. Romanov). — Plate V based on syntypes from Taiwan at p. 134 in Imahori 1953.

SPECIEMEN EXAMINED. — **Laos** • Thakhek; 17°23.307'N, 104°50.026'E; 146 m alt.; submersed; stored in 4% formalin; 29.XI.2011; *A. M. Glushchenko* (LE[LEAW000054]); *Chara pseudohydropithys*.

LOCALITIES OF SYNTYPES (Imahori 1950). — Tantei Tairinsyo [Dairinsho *in* Imahori 1953], Prov. Tainan. Toseki Koryo [Kobiryo, Danrinsho? *in* Imahori 1953], Prov. Tainan. Kwandensyo [Kwandensho *in* Imahori 1953], Prov. Tainan, no date. The dates of collections were reported later (Imahori 1953): 12.VIII.1941 for Tantei and Kobiryo, 21.VIII.1941 for Kwandensho.

DISTRIBUTION. —This is the first record of *Chara pseudohydropithys* for Southeast Asia, extending its distribution range from Taiwan (Imahori 1950, 1953), mainland China (Han & Li 1994), India (Patel & Jawale 1979; Pundhir *et al.* 1994), and Brazil (Bueno & Bicudo 2021).

VARIABILITY. — The characteristics of the axial cortex, and length of both bract-cells and bracteoles are the most variable morphological traits of the studied specimens of *C. pseudohydropithys* from Laos. Different length of bract-cells and bracteoles results in different appearances of the branchlet whorls. The number of branchlet nodes and corticate segments, the number of fertile branchlet nodes, and the arrangement of stipulodes are less variable. The occasional presence of small accessory cells near spine-cells in the axial cortex has not been reported for this species before.

DESCRIPTION

Monoicous richly fertile plants are up to several cm in length, not incrusted with lime, green, and transparent after storage in formalin solution (Figs 1C-H; 2). The axis is smooth in general appearance, (280)300-510 µm in diameter. The axial cortex is triplostichous slightly tylacanthous to generally isostichous, to diplo-triplostichous slightly tylacanthous, and rarely even diplostichous and slightly tylacanthous (Fig. 2E-J). Secondary tubes contact each other obliquely or rarely at a right angle and can overlap each other (Fig. 2H). Some irregularity in axial cortex arrangement is not infrequent (Fig. 2G). Spine-cells are very short, rounded, solitary, and hardly visible (Fig. 2F), 27-40 µm in outline viewed from above, and 23-42 µm in length. Some spine-cells have small lateral accessory cells not protruding from the cortex, narrower, but a little longer than spine-cells (Fig. 2J). Stipulodes are mostly haplostephanous (Fig. 2C), but can be both haplo-and diplostephanous on the same plant (Fig. 2D). The upper stipulodes are two per branchlet, conical and pointed to cylindrical with conical pointed ends, mostly two-four-times shorter of basal segment of branchlets, rarely equal to them at upper, less developed, whorls, densely arranged and appressed to the branchlet base, 330-880 µm length. Their whorl in outline looks like a dentate cap at lower magnification (Figs 1C, E-G; 2B). The lower row of stipulodes is incomplete and consists of cells of variable length (Fig. 2D), but always shorter of upper stipulodes, with obtuse to narrowed ends, 130-220 µm length.

Branchlets are 10 (11?) in a whorl, straight, 180-220 µm in diameter, completely differentiated into nodes and internodes, consisting of four or rarely five segments, shorter to slightly longer than the internodes at upper parts, shorter than the internodes at lower parts, 3.2-10.1 mm length (Figs 1C-H; 2A, B). The branchlets with five nodes (in other words, consisting of five segments) can occur singly in a whorl or the whole whorl can consist of them (Fig. 2A, B). The basal segment of the branchlet is always ecorticate (Figs 1G, H; 2A, B), (424)655-1940 × 153-300 µm, 2.7-8.6 times longer of width. Mostly two, rarely one or three, and even four corticate segments above the basal segment are perfectly covered with diplostichous stem cortex (Figs 1E-H; 2A, B). Three and four corticate segments are formed at branchlets with five nodes (Fig. 2A, B). In the case of four corticate segments the last branchlet segment is corticate (Fig. 2B). The length of corticate segments is usually slightly shorter than ecorticate ones but can be nearly equal to them. The branchlets have two-four fructifying nodes, mostly two or three, and end with a tassel consisting of three-six pointed cells (Figs 1C, E-G; 2B). They are not shortened, incl. the longer and distinctly recognizable end cell being at ⅓ longer than bract-cells, or its length is nearly the same in comparison with three bract-cells at the last node more or less equal each other in length (Figs 1D, G, H; 2A, B). The end cell of the branchlet is 560-2080 µm in length.

Bract-cells are pointed, two-three times shorter than the neighboring branchlet segment up to mostly equal and somewhat exceeding their length, longest and verticillate at sterile and fertile nodes, but adaxial cells are frequently slightly or distinctly shorter, very rarely slightly longer than the abaxial ones (Figs 1D-H; 2A, B). Bract-cells are unilateral at many fertile nodes, i.e., abaxial cells are distinctly shorter than adaxial ones. Adaxial bract-cells can be up to five times shorter than abaxial ones. Adaxial bract-cells are slightly longer to mostly significantly longer than oogonia, rarely equal to slightly shorter than oogonia, (181)510-2040(2076) µm length. Abaxial bract-cells are not rudimentary, (78)103-2110 µm length. Bracteoles are slightly shorter to evidently shorter of adaxial bract cells but could be slightly longer of adaxial bract-cells as an exception, 203-1674(2140) µm length. Gametangia are conjoined (Fig. 2K), almost only at the base and top of corticate segments and between them, solitary, with vertically geminate oogonia in a single case only. They can be at the last node of the branchlet nearly replacing the end cell and making the end tassel of cells not obvious (Fig. 1D). Oogonia are 340-504 × 240-330 µm, and coronulae are 89-106 × 134-179 µm. Ripe oospores are brown to black, with low spiral ribs, 290-370 × 172-226 µm. Antheridia are octoscutate, (180)208-310 µm in diameter.

TAXONOMIC NOTES

The holotype of *C. pseudohydropithys* was not indicated by the author (Imahori 1950). All three syntypes were lost in fire from the atomic bombing of Hiroshima at the end of the Second World War in 1945 (Imahori 1950, 1954; Wood & Imahori 1965; Proctor 1980). Only excellent drawings from the paper by Imahori (1953) could be used for the typification implemented here. These drawings were based on syntypes as can be concluded from a comparison of the lists of specimens mentioned in the protologue and subsequent article (cf. Imahori 1950: 262; Imahori 1953: 125). These drawings were unpublished illustrations at the moment of protologue

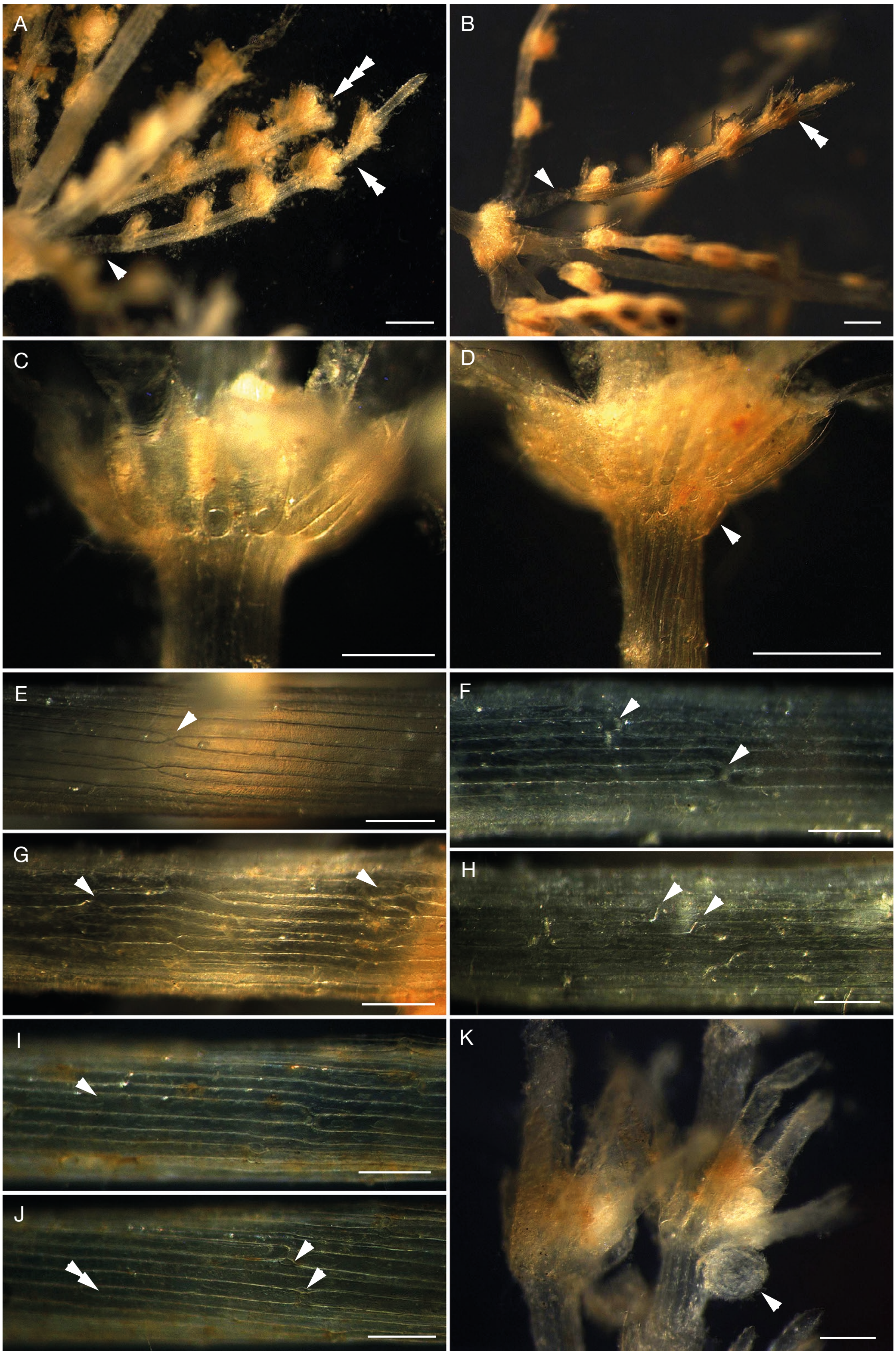

Fig. 2. — Morphological characters of *Chara pseudohydropithys* Imahori from Laos (LE): **A**, branchlet with ecorticate basal segment (**arrowhead**), three corticate segments, ecorticate last segment (**double arrowhead**), long end cell of branchlet, **triple arrowhead** indicates broken end of branchlet; **B**, branchlet with basal ecorticate segment (**arrowhead**), four corticate segments, and short end cell, **double arrowhead** indicates fourth corticate segment; **C**, acute haplostephanous stipulodes; **D**, diplostephanous stipulodes, low row in incomplete and consisted of stipulodes of unequal length (**arrowhead**); **E**-**J**, details of arrangement of axial cortex; **E**, diplostichous slightly tylacanthous cortex with non-joining secondary tubes (**arrowhead**); **F**, triplostichous isostichous cortex with barely visible short solitary spine-cells (**arrowheads**); **G**, irregular triplostichous cortex, most irregular parts indicated with **arrowheads**; **H**, oblique joining of secondary tubes (**arrowheads**) in triplostichous isostichous cortex; **I**, slightly tylacanthous triplostichous cortex, **arrowhead** indicates more prominent primary tube; **J**, small lateral accessory cells (**arrowheads**) near small solitary spine-cells; **K**, conjoined gametangia, **arrowhead** indicates antheridium. Scale bars: A, B, D, 0.5 mm, C, E-K, 0.2 mm. Photo credits: R. E. Romanov.

publication because the specimens upon which they were based were already lost. These drawings were undoubtedly associated with this taxon by its author and were available to the author at the time of the description preparation with the analysis validating the name. Therefore, it seems to be possible to recognize these drawings as a part of original material suitable for designation as a lectotype according to the Art. 9.11 and 9.12 (Turland *et al.* 2025). The search for plants or specimens and selection of an epitype from Taiwan, placed as close as possible to three localities of its syntypes, is preferable and should be done in the future. It will allow the application of the newly selected epitype for barcoding. *Chara pseudohydropithys* was described under the name '*C. pseudohydropitys*' as opposite to a very similar *C. hydropithys* Reichenbach according to the protologue (Imahori 1950) and is corrected to '*C. pseudohydropithys*' according to the Art. ICN 60 (Turland *et al.* 2025) and suggestion by Guiry & Guiry (2024).

It was already noted that *C. hydropithys* can form both haplostephanous and diplostephanous stipulodes in the same plant (Proctor 1980; Krishnan 2006). The haplostephanous stipulodes could be recognized as a result of the suppressed development of initials of stipulodes forming the lower row in the case of this species (Sundaralingam 1966). A similar result of the developmental pattern was reported for *C. strigosa* A.Braun, *C. locuples* Hollerb. and *C. uzbekistanica* Hollerb. (Hollerbach 1960; Romanov *et al.* 2014; Romanov 2021; Romanov & Zhakova 2024). Therefore, *C. pseudohydropithys* seems to be a morphotype of *C. hydropithys* producing stipulodes in the lower row and as such could be recognized as its synonym as it was suggested by Proctor (1980). This decision can be expected in future after integrative taxonomic studies across the species distribution range covering Africa: Ethiopia and Madagascar (Braun & Nordstedt 1882; Groves 1928), Asia (Zaneveld 1940; Imahori 1953; Gupta 2012), North and South Americas (Braun & Nordstedt 1882; Tindall 1966; Proctor 1980; Mann *et al.* 1999; Borges & Necchi 2017; Bueno & Bicudo 2021).

Chara pseudohydropithys was described as having a regularly diplostichous axial cortex, but the original drawing shows the secondary tubes slightly overlapping (Imahori 1953). Moreover, the transverse section of the axis could be interpreted as evidence of a diplo-triplostichous stem cortex. Our specimens show a wider variability of axial cortex arrangement than known before this study. Short bract-cells are typical for plants from Laos having smaller oospores but without evident hiatus in this trait with the original material of *C. pseudohydropithys* (cf. Imahori 1953: 370-390 × 220-240 µm).

Despite many morphological similarities with species of the subsection *Agardhia* R.D.Wood, *C. hydropithys* is distant from this group according to molecular genetics (Meiers *et al.* 1999). Considering its diplostephanous stipulodes and constantly ecorticate basal segment of branchlet its placement in the subsection *Willdenowia* R.D.Wood, whose similarity to *C. pseudohydropithys* as was noted by Imahori (1953), looks more preferable from a morphological perspective than placement in the subsection *Agardhia* R.D.Wood implemented by Wood & Imahori (1965). The assignment of *C. hydropithys* to the subsection *Willdenowia* was suggested by Meiers *et al.* (1999), confirmed by Borges & Necchi (2017), Becker *et al.* (2021), Mjelde *et al.* (2021) and Romanov *et al.* (2022a, c) from a genetic perspective and that assignment is followed here. The same decision is suggested here for *C. pseudohydropithys*. The section *Imahoria* J.van Raam *nom. inval.* (van Raam 2010) described for accommodation of *C. hydropithys* and some related taxa appears to be a redundant taxonomic solution.

Genus *Nitella* C.Agardh

REMARK
Nitella specimens collected in Laos have been described here as a new species.

Nitella laotica R.E.Romanov, V.Yu.Nikulin, K.-G.Bernhardt & A.A.Gontcharov, sp. nov.

DIAGNOSIS. — *Nitella laotica* sp. nov. differs from the somewhat similar habit of *N. mucronata* (A.Braun) Miq. by the presence of a central secondary ray in some branchlets, the ability to form clear spike-like apical parts of plants, as well as ornamentation of the oospores including meshes with a sophisticated outline on the fossae, more sculptured and unflanged striae, an absence of meshes with shallower smaller meshes inside them on the oospore fossae (Figs 3; 4; 5; 6; 7A-E; 8), and *rbc*L sequence (Fig. 10). *Nitella laotica* sp. nov. differs from *N. oligospira* A.Braun, a species showing affinity in *rbc*L and ITS-based phylogenies (Figs 10; 11), with mainly two × furcated branchlets, an absence of four- and five-furcated branchlets, the presence of gametangia at the first furcations of branchlets, unabbreviated dactyls, and the presence of a central secondary ray at some branchlets as well as a well-developed reticulate surface of the oospores. *Nitella laotica* sp. nov. shares features with *N. flagellifera* J.Groves & G.O.Allen, which is also related phylogenetically, but is distinguished by the absence of an accessory branch arising from the top of the central secondary ray. The same trait allows easy separation of the new species from *N. graciliformis* J.Groves, a taxon close to *N. flagellifera*. The second morphotype of *N. laotica* sp. nov. resembles *N. confervacea* (Bréb.) A.Braun ex Leonh. by branchlet arrangement, but differs distinctly by the presence of central secondary ray and reticulate oospore surface, as well as *rbc*L sequence. *Nitella laotica* sp. nov. differs from *N. tenuissima* (Desv.) Kütz., a species having a similar arrangement of branchlets, by non-shortened secondary rays at lax branchlets, the presence of gametangia at the first furcation of branchlets, absence of a densely beaded ornamentation, and denser reticulation of oospore surface, and *rbc*L sequence.

MATERIAL EXAMINED. — **Laos** • Northern Laos, Provinz Xieng Khouang, Phonsavan, Ebene der Steinkrüge, Site 1, in wassergefülltem Steinkrug; 19°25'49.2"N, 103°09'19.0"E; 1109 m alt.; pressed specimens; DNA voucher specimen; 11.VIII.2018; *K.-G. Bernhardt & N. Stoeckl*; deposited in the Herbarium of the University of Natural Resources and Life Sciences, Vienna (holotype: WHB[WHB72405]); (Fig. 3) • same data as for holotype; deposited in the Herbarium of the Komarov Botanical Institute of the Russian Academy of Sciences, Saint Petersburg, Russian Federation (isotype: LE[LEA0006487]). GenBank accession: (*rbc*L PP470899) and (ITS PP476817) • Xiangkhouang Province, Plain of Jars, Site 2, in an inundated stone jar; *c.* 19°19'11.2"N, 103°09'14.8"E; pressed specimens; DNA voucher specimen; 06.XII.2012; *A. M. Glushchenko, E. L. Konstantinov & other biologists of the Tsiolkovsky Kaluga State University* (paratype: LE[LEA0006486]; GenBank accession: *rbc*L[PP470898]); together with *C. braunii*.

Fig. 3. — Holotype of *Nitella laotica* R.E.Romanov, V.Yu.Nikulin, K.-G.Bernhardt & A.A.Gontcharov, sp. nov., moderately lax morphotype, pressed together with *Chara braunii* C.C.Gmelin (WHB [72405]). Note: **Arrowheads** indicate specimens of *N. laotica* sp. nov.; **double arrowhead** indicate most complete specimen of *N. laotica*, sp. nov., designated as a holotype. Scale bar: 2 cm. Photo credits: R. E. Romanov.

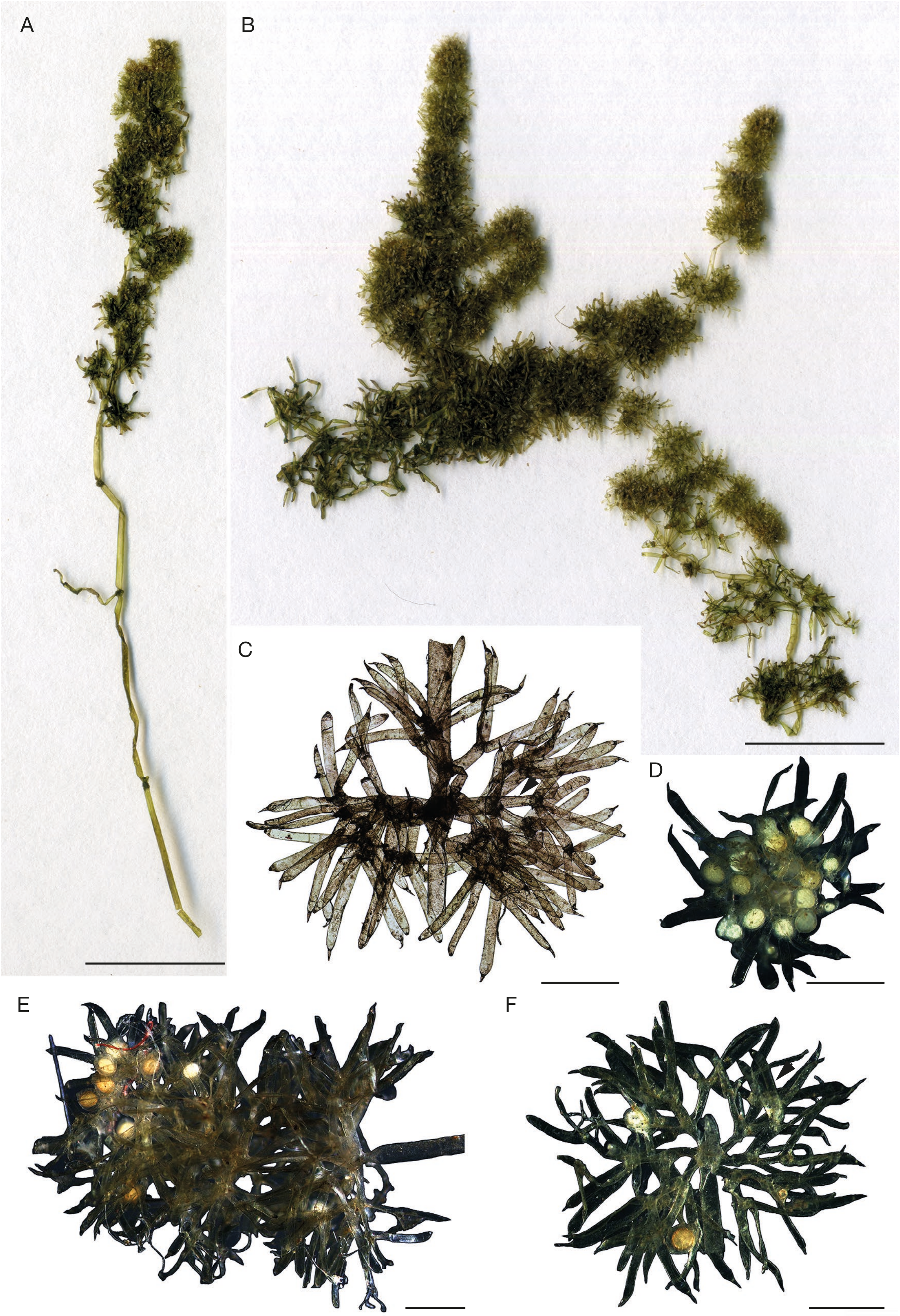

Fig. 4. — General appearance, branchlet whorls of the second morphotype of *Nitella laotica* R.E.Romanov, V.Yu.Nikulin, K.-G.Bernhardt & A.A.Gontcharov, sp. nov. with compacted moniliform whorls forming spike-like upper parts of the plant (LE[A0006486]): **A**, **B**, general appearance of pressed plants; **C**-**F**, whorls of abbreviated branchlets, rewetted; **C**, a whorl of 2-furcated sterile branchlets, bicellulate dactyls with mucronate end cells are visible, **arrowhead** indicates central secondary ray; **D**, a whorl of richly fertile compacted branchlets; **E**, apical part with compacted branchlets; **F**, a whorl of compacted branchlets with oogonia at second furcations, **arrowhead** indicates branchlet with clearly visible central secondary ray. Scale bars: A, B, 1 cm; C-F, 1 mm. Photo credits: R. E. Romanov.

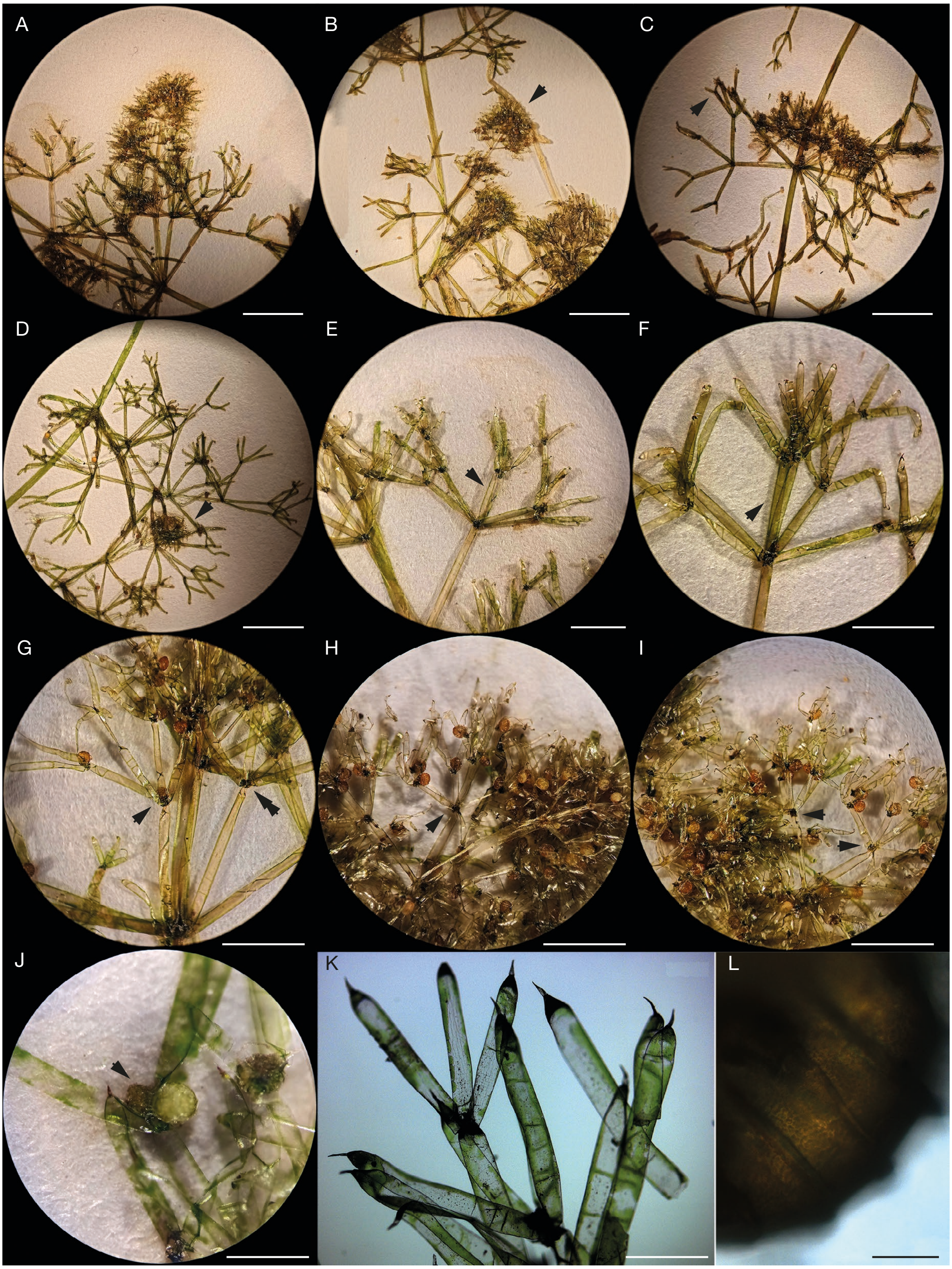

Fig. 5. — Morphological traits of the holotype of *Nitella laotica* R.E.Romanov, V.Yu.Nikulin, K.-G.Bernhardt & A.A.Gontcharov, sp. nov. (WHB [72405]), dry plants: **A**, **B**, lax heads with compacted branchlets closely resembling second morphotype; **C**, indistinct lateral head and whorl of lax branchlets with abbreviated dactyls (**arrowhead**); **D**, small apical head (**arrowhead**) and whorl of lax branchlets; **E**, **F**, branchlets with central secondary rays (**arrowheads**) pressed together with lateral secondary rays of branchlets; **G**, branchlet without central secondary ray having fertile first node and branchlet with central secondary ray without gametangia, **arrowhead** indicates first node of branchlet with oogonium, **double arrowhead** indicates sterile basal node of branchlet with central secondary ray; **H**, **I**, richly fertile compacted branchlets with gametangia mostly at second nodes, **arrowheads** indicate sterile first nodes of branchlets with central secondary rays; **J**, conjoined gametangia, **arrowhead** indicates antheridium; **K**, bicellular dactyls with pointed conical mucronate end cells; **L**, reticulate oospore surface recognizable through collapsed transparent spiral cells. Scale bars: A-D, 5 mm; E-I, 2 mm; J, K, 0.5 mm; L, 50 µm. Photo credits: R. E. Romanov.

Type locality. — **Laos** • Northern Laos, Xieng Khouang Province, Phonsavan, Plain of Jars, Site 1; 1109 m alt.; 19°25’49.2”N, 103°09’19.0”E.

Etymology. — This species was named to highlight its country of origin.

Distribution. — *Nitella laotica* sp. nov. is known from two localities in Laos only.

Habitat. — The plants were growing inside inundated artificial rock, mostly sandstone, jars with internal depths mostly not exceeding 50 cm and volumes up to 254 L (Colani 1935; Genovese 2015; Devantier-Thomas 2021; Käse *et al.* 2025). The jars harbored rainwater, which was nutrient-poor and brownish-colored by humic acids. Few submerged and floating water plants were spotted there. Species of *Lemna* L. and dense stands of charophytes consisting of *Chara braunii* and *N. laotica* sp. nov. were notable among them. In both cases, *N. laotica* sp. nov. was associated with *C. braunii*. This species could be associated with small water bodies only.

Variability. — The compacted to lax branchlets, overlapping at the apical parts of plants and resulting in the spike-like appearance of plants or more or less regularly distributed result in the different general appearance of *N. laotica* sp. nov. represented with two intergrading morphotypes illustrated above. The type specimen is not moniliform, but with regularly spaced whorls, moderately lax plants without apical spikes but able to form heads. The second morphotype is represented by densely moniliform plants with clasping whorls, forming spikes at apices. Compacted branchlets of both morphotypes differ with dactyls being mostly not so different in length in comparison to branchlet secondary rays in the case of type material and distinctly longer than branchlet secondary rays in the case of the second morphotype. The variable appearance of dactyls from not mucronate to mucronate is notable too.

Description

Green transparent plants are mostly without traces of lime incrustation, sometimes with subtle to evident lime incrustation at dactyls, moderately branched, not compacted, usually without overlapping of upper whorls, able to form diffuse heads at apex and branches (Fig. 3), with branchlets of moderately lax appearance, without mucilage, at least 13 cm length with whorl spans 12-25 mm. The other morphotype is moderately branched, having a moniliform appearance because of overlapping dense whorls spacing becoming gradually denser towards the apex, forming terminal spike-like upper parts (Fig. 4), without mucilage, at least 7 cm high (the longest part of the incomplete plant is 6.5 cm length) with whorls spans 3.2-12.5 mm. Axes are moderately slender, 230-290 µm in diameter at shortened parts, and 420-580 µm in diameter at lax parts in a collapsed pressed state. Well-developed branchlets are 9-12 mm and in the case of the second morphotype are 2.0-2.8 mm long in heads and 3-7.5 mm long, six-seven in a whorl, nearly equal to internode length and longer of internodes at upper parts of plants (Figs 3; 5A-D) and in case of the second morphotype somewhat shorter to nearly equal to internode length at basal parts of plants (Fig. 4A, B), and distinctly longer of internodes at upper parts of plants, two-three-furcated, mostly two-furcated, with and without central secondary rays at the same plant and even at the same whorl (Figs 4D, F; 5C-I). Sterile branchlets can be one-furcated and one-two-furcated. The lowermost available branchlets are distinctly to moderately lax (Figs 3; 4A, B).

Primary rays are 1.4–3-4.8 mm length, at compacted branchlets (CB) – 360-892 µm, secondary rays – 0.8–1.8-4 mm length, at CB – lateral secondary rays – 358-783 µm and central secondary rays – 440-777 µm, tertiary rays – *c.* 1.4 mm length, at CB – 373-823 µm. The rays of each order at lax branchlets do not appear compacted or shortened (Figs 3; 5C-F). Primary rays are longest in comparison to rays of other orders, more or less equal to half of the branchlet length or slightly shorter. Lateral secondary rays are shorter of the primary ones at *c.* one-third or half a length, but mostly longer of dactyls at one-fourth at lax branchlets and distinctly shorter of them at the second morphotype and compacted branchlets, (3) 4-5. The central secondary ray surrounded with four lateral secondary rays is not formed at every branchlet (Figs 4C, F; 5C-I; 6A-E). Dactyls are strictly two-celled (Figs 4C; 5F, K; 6A-F), not abbreviated, nearly equal to secondary rays, slightly longer or shorter than last (secondary) rays, usually somewhat shorter than secondary rays at lax branchlets (Fig. 5C-F), but longer to nearly equal to secondary rays at compacted branchlets (Figs 4C-F; 5H, I; 6A-E), at third furcation shorter than dactyls at second furcation (Fig. 5C), 2-4, 1.05-2.6 mm length, at abbreviated branchlets (AB) – (0.7) 0.85-1.7 mm length, at third furcation of AB – 0.87-1.15 mm length. Dactyls which are distinctly longer than secondary rays are typical for the second morphotype. The end cells of dactyls are conical mucronate through submucronate to nearly confluent with apex of penultimate cell (Figs 4C; 5F, J, K; 6A-F), pointed, can be fallen at old branchlets, 100-194(226-287) × 36-80 µm. Non-mucronate or submucronate end cells are frequent at most compacted branchlets, whereas mucronate end cells are common at lax branchlets. The plants are macrodactylous, but moderately abbreviated dactyls as rare cases only at third furcation of two-three-furcated branchlets, *c.* 0.48-0.68 mm length, are notable.

Gametangia are sessile, conjoined (Figs 5J; 6F), at first and second furcations of branchlets (Figs 5G, 6E), but usually missing at first furcation because of central secondary ray presence (Fig. 5E, F, H, I), absent at the whorl base. They are conjoined at upper whorls only because of probably early falling of antheridia. Both oogonia and antheridia are solitary only. Oogonia are widely ellipsoid with seven-eight convolutions of spiral cell, not swollen at the apical part (Fig. 6G), *c.* 330-355 × *c.* 289 × *c.* 214 µm in dry state, 431-484 × 351-425 µm × *c.* 322 µm in the rewetted state. Coronulae are consisted of cells nearly equal to each other in both rows (Fig. 6G), 30-50 × 54-77 µm in the rewetted state.

Oospores are widely ellipsoid to nearly circular in lateral view, with seven-eight spiral low to moderately flanged striae, without a collar at the top (Figs 6G; 7; 8), 282-314 × 252-295 µm in the rewetted state. Ripe oospores are light yellow-reddish in reflecting light and yellow-brown to golden-brown in transmitting light. The oospore surface is distinctly reticulate in light and scanning electron microscopy (Figs 6 G, H; 7A-E; 8). The fossa surface is reticulate, with mostly eight-ten meshes across the fossa, without or with few small irregular granules

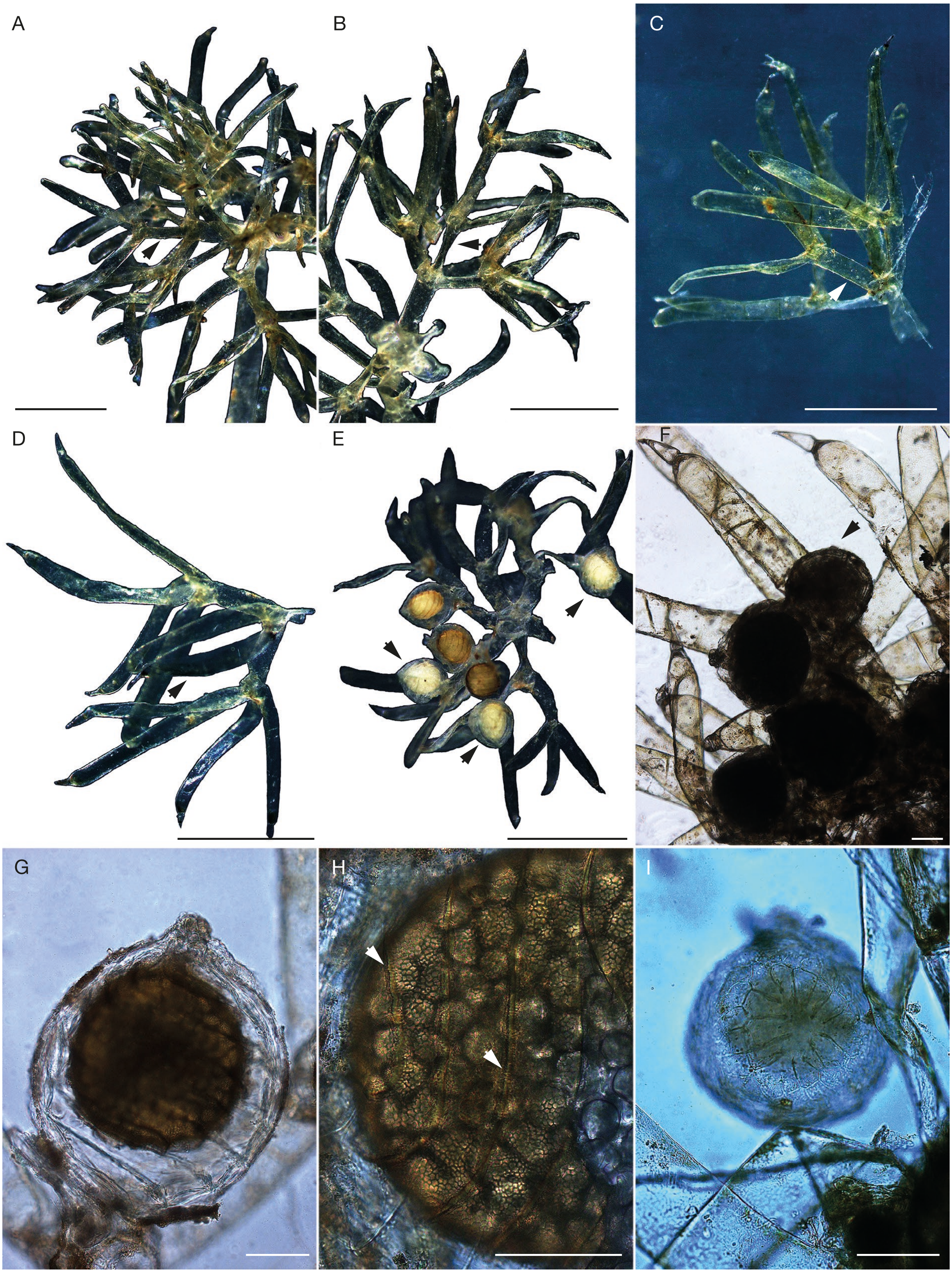

Fig. 6. — Morphological traits of the second morphotype of *Nitella laotica* R.E.Romanov, V.Yu.Nikulin, K.-G.Bernhardt & A.A.Gontcharov, sp. nov. (LE[A0006486]), rewetted plants: **A**-**E**, sterile compacted branchlets with central secondary ray (**arrowheads**); **C**, closest lateral secondary ray is lost; **D**, sterile compacted one-two-furcated branchlet without central secondary ray, **arrowhead** indicates one of two simple lateral secondary rays (actually dactyls); **E**, fertile compacted branchlet with oogonia at second and third furcations, **arrowheads** indicate oogonia at third furcations; **F**, conjoined gametangia at last furcation of branchlet, **arrowhead** indicates antheridium; **G**, oogonium with ripe oospore inside; **H**, squashed oogonium with ripe oospore showing reticulated surface of oospore and low reticulated spiral ribs (**arrowheads**); **I**, antheridium, triangular shield is visible. Scale bars: A-E, 1 mm; F-I, 100 μm. Photo credits: R. E. Romanov.

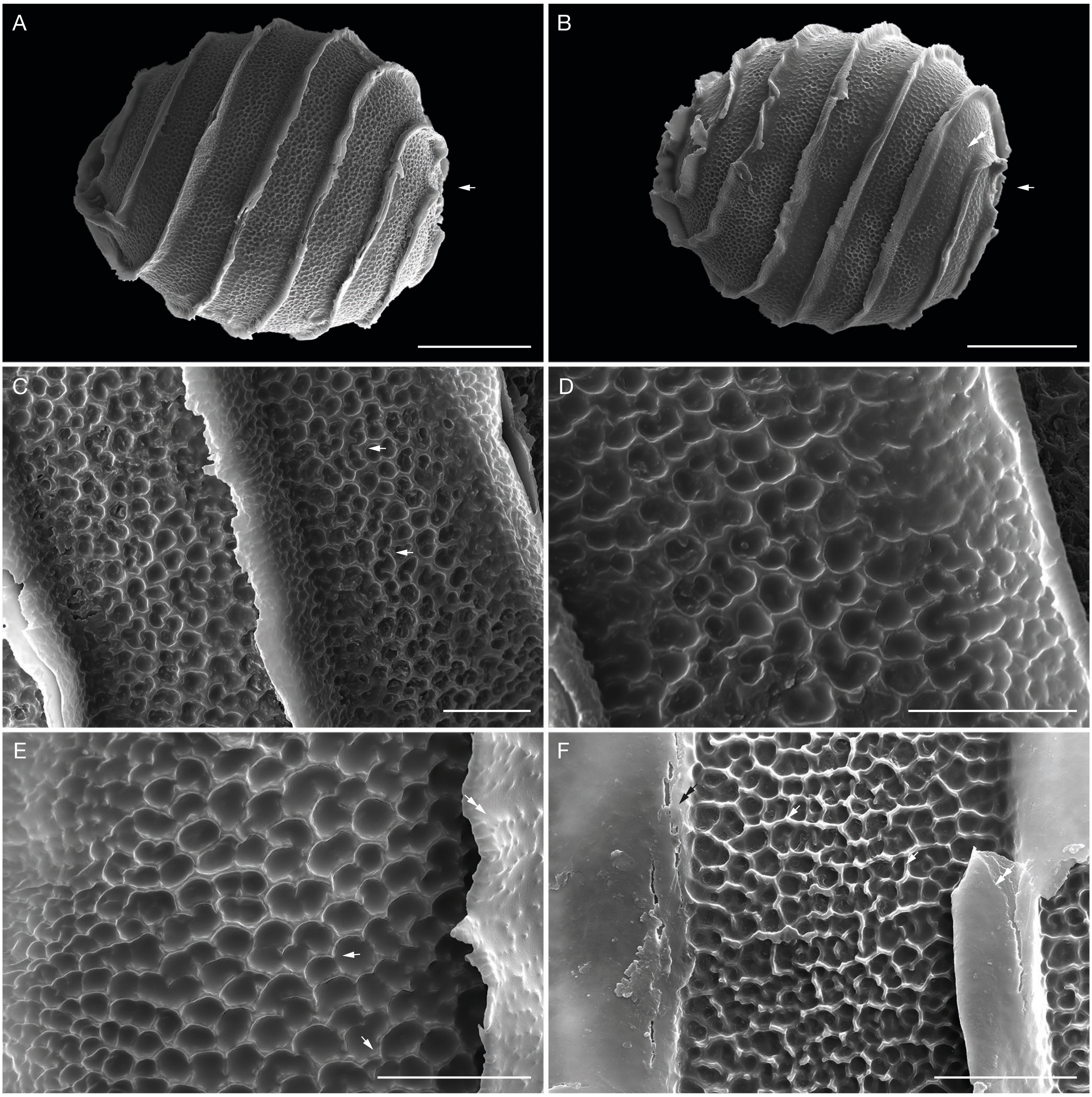

Fig. 7. — Oospores of *Nitella laotica* R.E.Romanov, V.Yu.Nikulin, K.-G.Bernhardt & A.A.Gontcharov, sp. nov. from Laos, holotype in WHB[72405] (**A**-**E**) and *N. mucronata* from the south of West Siberia, a plant from the Novosibirsk Reservoir stored in LE (**F**), SEM: **A**, **B**, general appearance in lateral view, **arrowheads** indicate basal poles; **A**, oospore with completely developed reticulated sculpture of surface; **B**, oospore with incompletely developed reticulated sculpture of surface, **double arrowhead** indicates area with intermediate stage of surface ripening having projected pyramid-like appearance of initials of mesh vertices; **C**, two fossae showing reticulated surface of fossae and spiral ribs, **arrowheads** indicate meshes with sophisticated outline; **D**, **E**, fossae and basal ribs with less deep reticulation (**D**) or with pyramid-looking initials of mesh vertices (**E**), **arrowheads** indicate projected mesh vertices, **double arrowhead** indicate initials of mesh vertices at rib; **F,** fossa with reticulate surface and smooth ribbon-like spiral ribs, **arrowheads** indicate meshes with less developed mesh ridges inside, **double arrowheads** indicate smooth ribs. Scale bars: A, B, 100 µm; C-F, 20 µm. Photo credits: R. E. Romanov.

inside meshes (Figs 7D, E; 8C, D), with slight conical projected appearance of mesh vertices and more or less concave mesh edges (Figs 7D, E; 8E, F). Rarely mesh edges bear mesh vertices-looking projections (tubercules or beads) in their middle parts. Meshes are more or less isodiametric to somewhat elongated, sometimes with unfinished separation of individual mesh cells resulting in their constricted to even more sophisticated outline (Fig. 7C, D). The second morphotype has many sophisticated meshes (Fig. 8C, D). Mesh size measured as the distance between most remote mesh vertices of the same mesh is 2.58-9.06 µm. The reticulate surface of the oospore seems to be developed as mesh vertices appearing

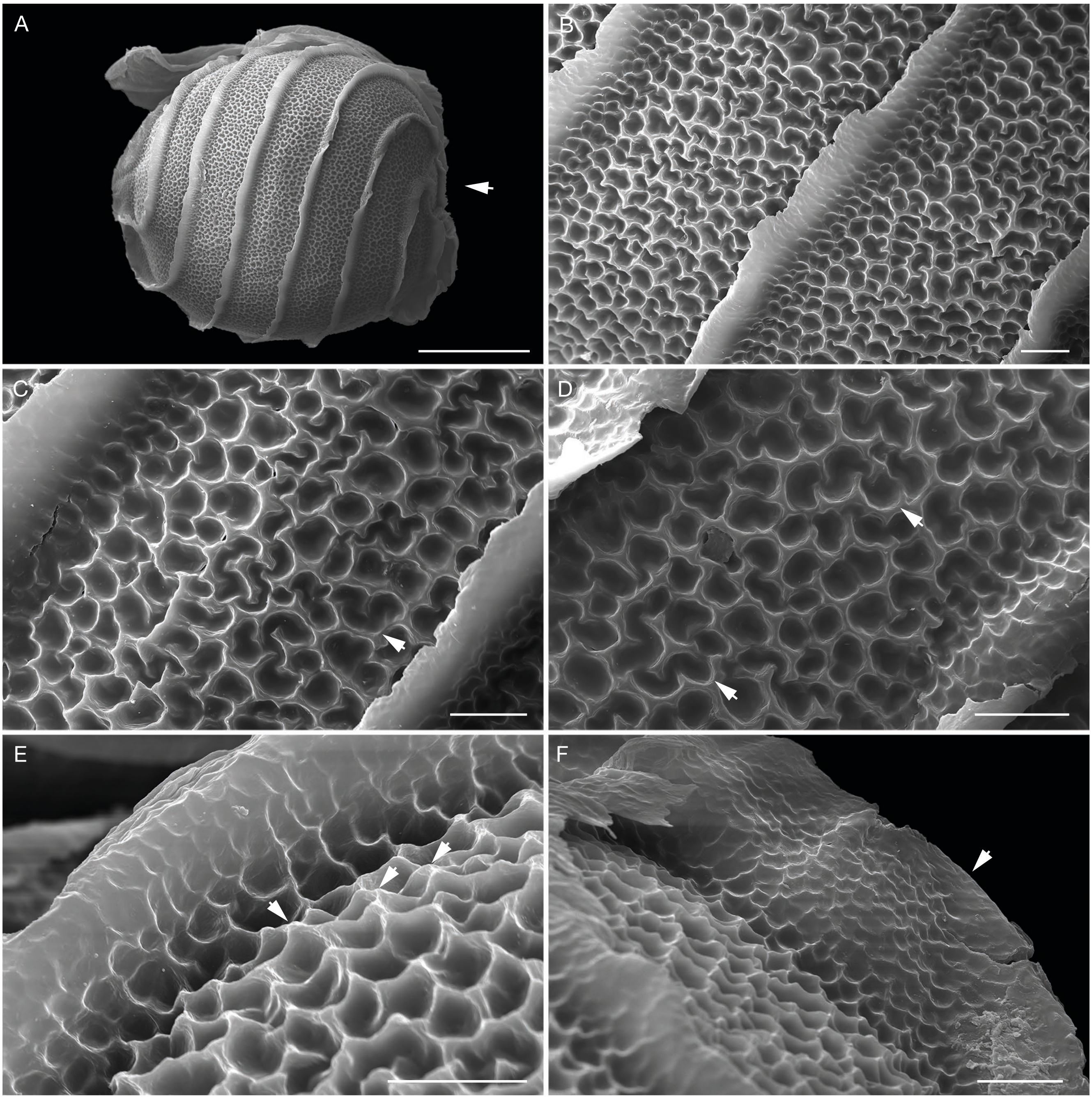

Fig. 8. — Oospores of the second morphotype of *Nitella laotica* R.E.Romanov, V.Yu.Nikulin, K.-G.Bernhardt & A.A.Gontcharov, sp. nov. from Laos (LE[A0006486]), SEM: **A**, general appearance in lateral view, **arrowhead** indicate basal poles; **B**, two fossae showing reticulated surface with many meshes with sophisticated outline; **C**, **D**, fossae with reticulated surface and many meshes with sophisticated outline indicated with **arrowheads**; **E**, **F**, tangential view of parts of fossae and spiral ribs with less deep reticulation, **arrowheads** indicate projected mesh vertices (**E**) and narrow ribbon-like spiral rib (**F**). Scale bars: **A**, 100 µm, **B**-**F**, 10 µm. Photo credits: R. E. Romanov.

first as granules forming a mesh pattern without mesh edges in the middle of fossae (Fig. 7B). Then these granules increase in size because of the growth of mesh edges from the granules giving granules short trihedral pyramid-like appearance. The appearance of concave surfaces inside meshes is developing because of the development of mesh edges. The patches of more or less developed meshes originate at the middle parts of fossae expanding toward ribs and neighboring patches. The same pattern could be suggested for rib surfaces but with some delay and culminating in less deep inside of meshes. Hence well-developed mesh pattern is traceable at the parts of ribs proximal to the oospore surface (Figs 6H; 7C, D; 8B, D-F). The unripe sculpture of rib flanges is not well-developed, being nearly smooth to net-like granulated (Figs 7E; 8E).

The antheridia are octoscutate (Fig. 6I). The diameter of antheridia is much less than the oogonia size in the dry state

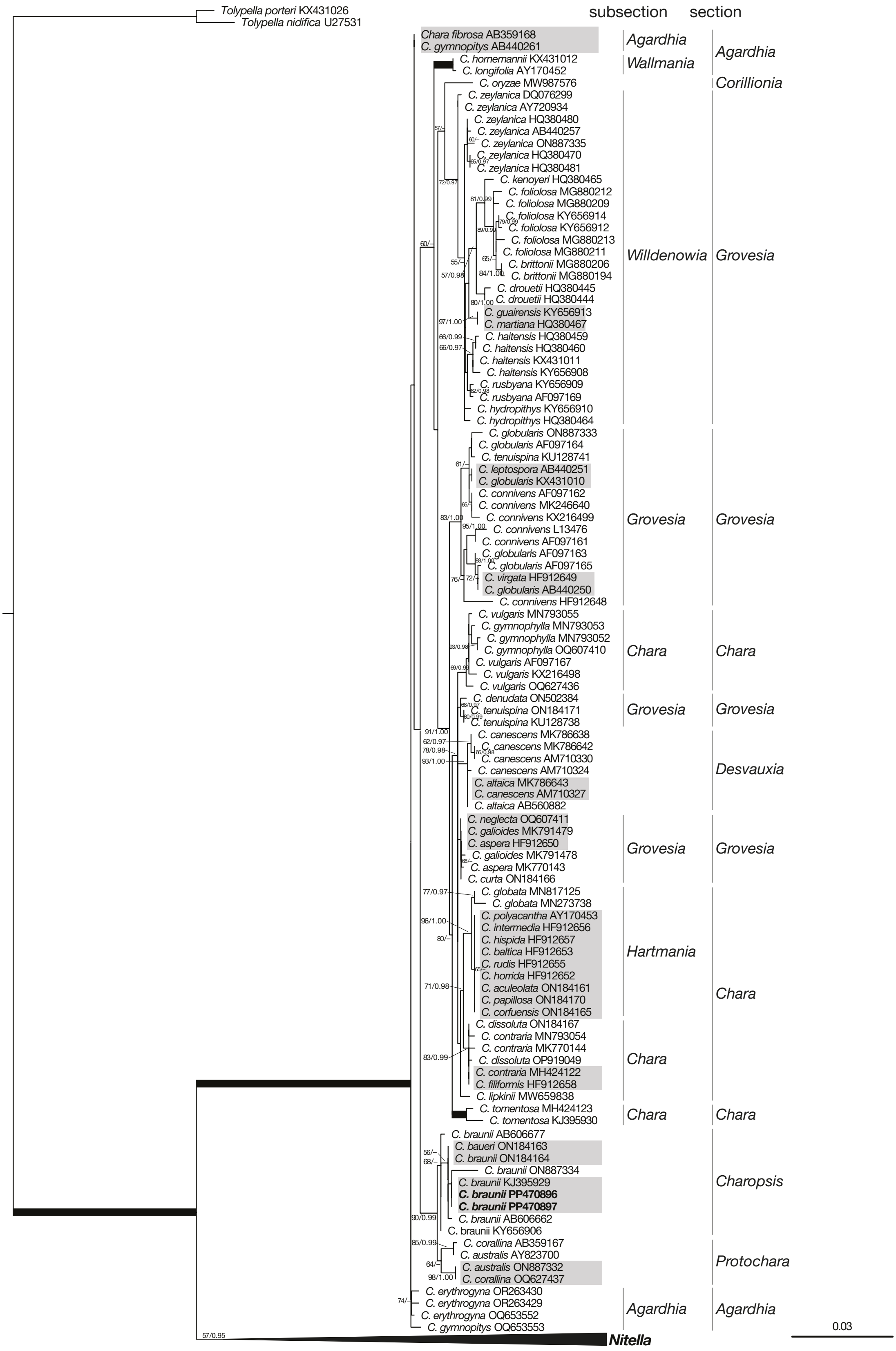

FIG. 9. — Maximum likelihood phylogenetic tree inferred in PAUP with GTR+I+G nucleotide substitution model from 216 *rbc*L sequences of *Chara* L. and *Nitella* C.Agardh genotypes. *Nitella* clade is collapsed. ML BP (>50%) and BI PP (>0.95) are shown. Branches received 100% BP and 1.00 PP support and the newly obtained sequence of *C. braunii* C.C.Gmelin is shown in **bold**. Sequences of different species carrying one genotype are marked with **grey**. The sequences obtained in this study are shown in **boldface**. *Chara* sections and subsections are according to Wood & Imahori (1965) with updates (Casanova & Karol 2014; Romanov *et al.* 2022b).

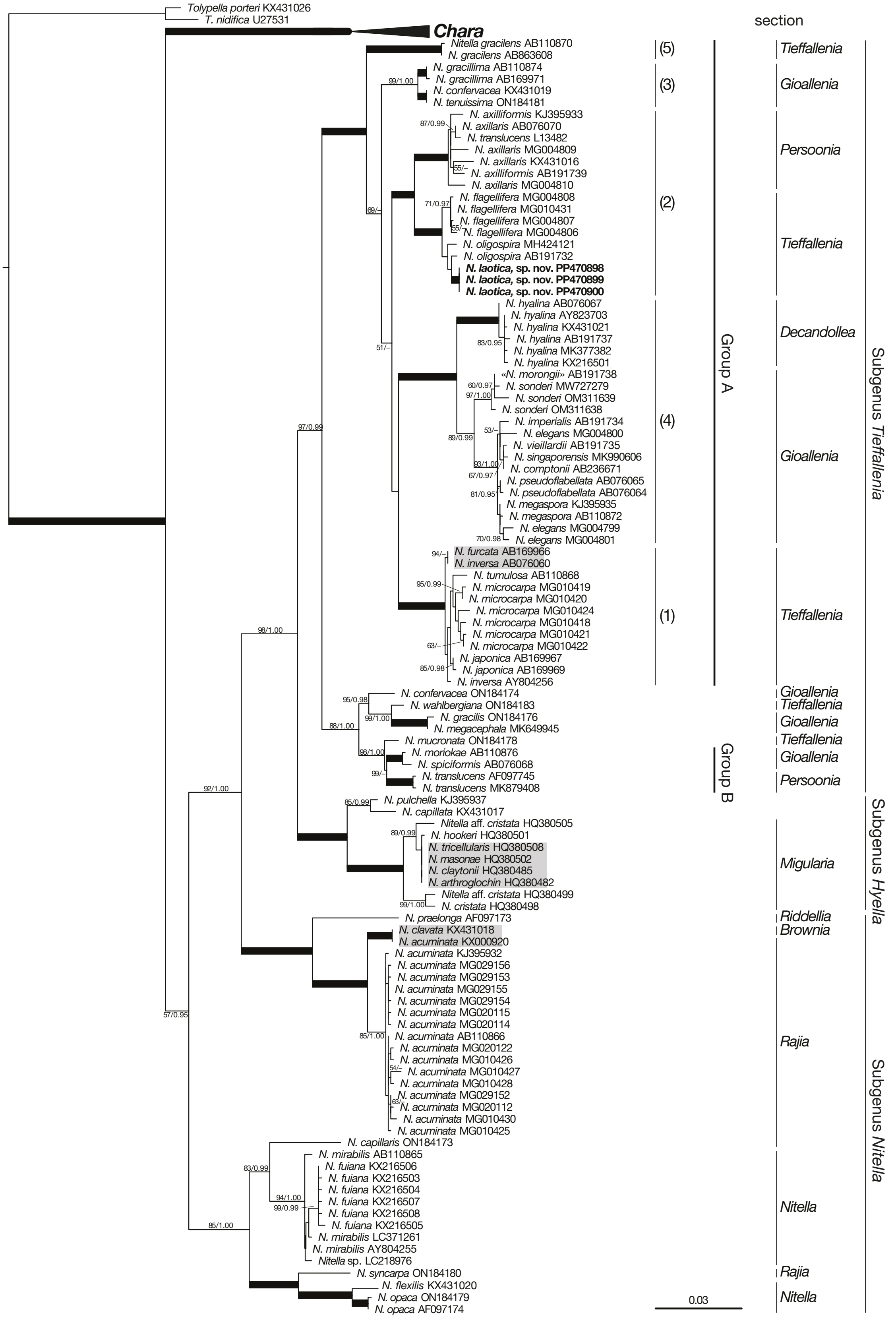

Fig. 10. — Maximum likelihood phylogenetic tree inferred in PAUP with GTR+I+G nucleotide substitution model from 216 *rbc*L sequences of *Chara* L. and *Nitella* C.Agardh members. *Chara* clade is collapsed. See the Fig. 9 legend for details. The sequences obtained in this study are shown in **boldface**. *Nitella* subgenera and sections and subsections are according to Wood & Imahori (1965) with updates (Casanova 2009). Clade designation is according to Sakayama (2008). The numbered groups by Sakayama (2008) were expanded to include most similar sequences. The sequence AB191738 does not belong to *N. morongii* Allen (Romanov *et al.* 2022c).

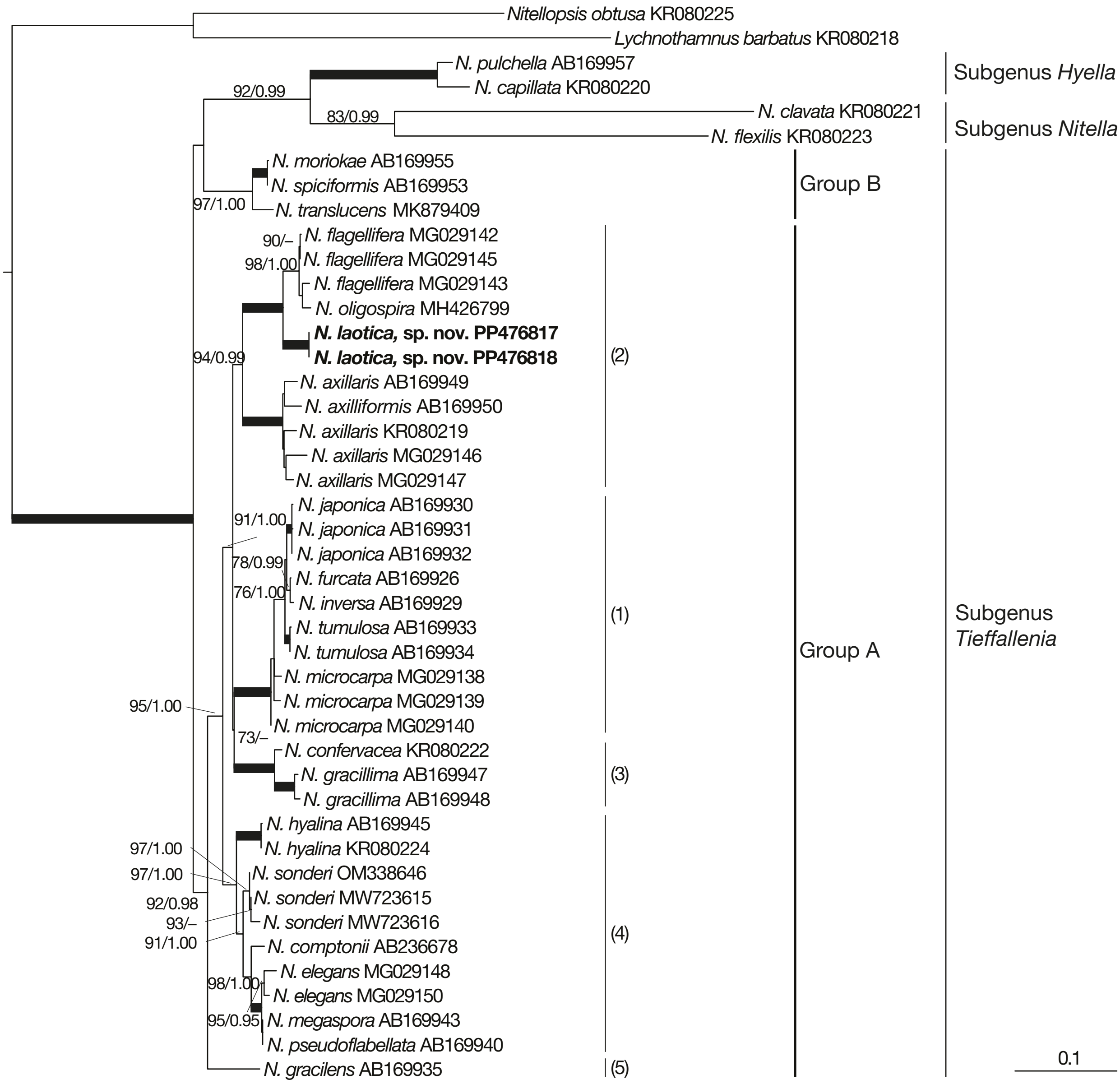

FIG. 11. — Maximum likelihood phylogenetic tree inferred in PAUP with GTR+I+G nucleotide substitution model from 42 ITS rDNA sequences of *Nitella* C.Agardh. See Figures 9 and 10 legends for details. The sequences obtained in this study are shown in **boldface**.

(Fig. 5J). An isolated antheridium was spotted between four dactyls in a single case only. Dry antheridia are up to 230 µm in diameter, rewetted ones are 269-310 µm in diameter, and unripe ones are *c.* 245-250 µm in diameter.

TAXONOMIC NOTES

The monoicy/dioicy, 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 & Imahori 1965; Sakayama 2008; Casanova 2009). The oospore surface pattern changes during its ripening (Casanova 1991), further complicating the use of this key trait. For example, oospores of plants from Australia erroneously referred to *N. morongii* Allen (cf. Sakayama *et al.* 2005; Romanov *et al.* 2022c) could be completely ripe oospores of *N. sonderi* A.Braun having difference in background surface of oospore with probably not fully ripe oospores (cf. Casanova 2007, 2009). The development of oospore ornamentation was described for three Australian species of *Nitella*, illustrating greater difference between different developmental stages in comparison to mature oospores (Casanova 1991), which according to our tentative description of this pattern can be suggested for *N. laotica* sp. nov. as well.

According to morphological traits, *N. laotica* sp. nov. belongs to the subgenus *Tieffallenia* R.D.Wood, section *Tieffallenia* R.D.Wood (Wood & Imahori 1965), or to the section *Gioallenia* R.D.Wood of the same subgenus according to the key by Casanova (2009) only, if variable shape of end cells of branchlets is not considered. Variability of the end cells of branchlets fits both sections, although, in the case of well-developed branchlets, it seems more suitable for *Tieffallenia*, agreeing with molecular data (Figs 10; 11). Delineation of these sections appears to be not far from senseless because they are polyphyletic if their concepts by Wood are used (Karol 2004; Sakayama 2008; this study). Despite the prominent morphological variability described above, further study of numerous well-developed specimens is desirable in the future for better knowledge of the variability of tropical charophytes. More attention to a local expression of morphological traits can overcome the artificial assignment of distant populations to the same species despite their non-identical morphology, few common traits, and origin from different continents. E.g. *N. oligospira* was already recognized by Wood & Imahori (1965: 500, 505) as one of the examples of this practice.

PHYLOGENETIC ANALYSES

The phylogenetic tree including 216 genotypes representing genera *Nitella* and *Chara* was divided into two figures for convenience (Figs 9; 10). Species of the genus *Tolypella* (A.Braun) A.Braun were taken as an outgroup. Our samples of *C. braunii* were placed within the species clade (68/–; Fig. 9) and showed identity to one of six known genotypes (Kato *et al.* 2008; Fig. 9; Appendix 1). The sequence of *C. baueri* A.Braun ON184163 was also a part of this clade sharing the same genotype with *C. braunii* ON184164.

Nitella laotica sp. nov. (three accessions, 100/1.00) was a member of the robust clade (100/1.00; Fig. 10) formed by representatives of *N. flagellifera* (four genotypes) and *N. oligospira* (two genotypes). Two morphotypes of new *Nitella* species have identical *rbc*L sequences, despite differences in general appearance. They differed from the most closely related *N. oligospira* AB191732 by five substitutions. Phylogeny based on the ITS rDNA sequence comparisons revealed similar affinity of the new species (Fig. 11). *Nitella laotica* sp. nov. (two identical sequences) again was resolved as a sister (100/1.00) to *N. flagellifera* (three genotypes) and *N. oligospira* (one genotype).

CONCLUSION

The charophyte records from Laos seem to be restricted to a report of *Chara* sp. from rice fields (Moody 1989). The morphological variability is still insufficiently known for tropical charophytes. It complicates both the identification of species from tropical regions and the harmonization of charophyte species concepts at different scales, from national through continental to worldwide. The detailed study of local material implemented here is an essential step towards the improvement of this perspective in Southeast Asia. *Chara pseudohydropithys* was described in detail here, giving a base for further research on testing of very probable conspecifity of *C. hydropithys* and *C. pseudohydropithys*. A new species of *Nitella* from small water bodies was described here on the basis of integrative taxonomy. Further studies on the morphological variability of tropical charophytes in its framework would allow testing whether huge species richness known for some regions is a reflection of actual species diversity or a result of still insufficient and perhaps sometimes too sophisticated taxonomic treatment of charophytes in some particular cases.

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APPENDIX

Appendix 1. — Species name, GenBank accession number, and the haplotypes for the taxa used in our analyses. The sequences obtained in this study are shown in **boldface**, available at: https://doi.org/10.5252/cryptogamie-algologie2026v47a1.s1