

Morphological diversity of
Chara oedophylla Feldmann and description
of *C. acortiphylla* S.D. Muller & A. Daoud-Bouattour,
sp. nov., a new related taxon from Tunisia

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Morphological diversity of *Chara oedophylla* Feldmann and description of *C. acortiphylla* S.D. Muller & A. Daoud-Bouattour, sp. nov., a new related taxon from Tunisia

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MOTS CLÉS

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ABSTRACT

Chara oedophylla Feldmann, previously known to exist in only a few sites around the Mediterranean and in southern Russia, has been identified in 17 new localities in Tunisia. Plants from 13 of these new populations and from four co-occurring populations of *Chara vulgaris* L. were collected and used for a detailed morphological study so as to define the diagnostic characteristics of *C. oedophylla* with respect to *C. vulgaris*. We also discovered a different plant form in southern Tunisia displaying the characteristic gametangia of *C. oedophylla* but with ecorticated branchlets. We describe that plant as *C. acortiphylla* S.D. Muller & A. Daoud-Bouattour, sp. nov.

RÉSUMÉ

Diversité morphologique de Chara oedophylla Feldmann et description de C. acortiphylla S.D. Muller & A. Daoud-Bouattour, sp. nov., un nouveau taxon apparenté de Tunisie.

Chara oedophylla Feldmann, seulement connue à ce jour de quelques localités du pourtour méditerranéen et du sud de la Russie, a été trouvée dans 17 nouvelles localités à travers la Tunisie. Des plantes, collectées dans 13 de ces nouvelles populations ainsi que dans quatre populations en mélange avec *Chara vulgaris* L., ont été utilisées pour une étude morphologique détaillée afin de préciser les caractères diagnostiques de *C. oedophylla* par rapport à *C. vulgaris*. Par ailleurs, nous avons découvert, dans le sud de la Tunisie, une forme différente présentant la disposition caractéristique des gamétanges de *C. oedophylla*, mais avec des phylloïdes non cortiqués. Cette forme est décrite ici sous *C. acortiphylla* S.D. Muller & A. Daoud-Bouattour, sp. nov.

INTRODUCTION

Chara oedophylla Feldmann is a rare species that has only been found until now in northwestern Morocco, Portugal, Spain, southeastern France, Corsica, Sicily, northern Tunisia and southern Russia (Soulié-Märsche *et al.* 2024). It was first described by Feldmann (1945) from herbarium specimens collected in 1926 by L. Gauthier-Lièvre at Oued Tinja in northern Tunisia. Wood (1962) subsequently reevaluated its taxonomical rank, considering it to be a variety of *Chara vulgaris* L. Later, Soulié-Märsche & Muller (2016) reinstated its status as a species. These authors considered the main characteristic and most striking diagnostic feature of *C. oedophylla* to be the mostly sejoined gametangia (antheridia and oogonia on different branchlet nodes): the species generally has nodes with either solitary antheridia or geminate, vertically-arranged oogonia. Its most recent and complete description (Soulié-Märsche *et al.* 2024) is based on earlier publications (Feldmann 1945; Wood 1962; Guerlesquin 1960, 1961; Comelles 1981, 1982) as well as on our observations (Soulié-Märsche 2003; Soulié-Märsche & Muller 2016; Muller *et al.* 2017).

Since 2018, intensive fieldwork conducted in Tunisia has led to the discovery of 19 new populations present from the north to the south of the country, with the species' diagnostic arrangement of gametangia. These populations display a broad and unexpected range of variability, implying the need to revisit the diagnostic characteristics of the species. Our discussion of the distinctive features of *Chara oedophylla* compared to *C. vulgaris* (understood here in a strict sense, according to Schubert *et al.* [2024]) is based on an in-depth morphological and morphometric study of new Tunisian populations. Over the course of that study, we identified a new taxon close to *C. oedophylla* that we describe as *Chara acortiphylla* S.D. Muller & A. Daoud-Bouattour, sp. nov.

MATERIAL AND METHODS

STUDIED SITES AND EX-SITU CULTURES

Prior to 2018, *Chara oedophylla* was known in Tunisia from five sites in the Mogods region that include a former pond near Oued Tinja (Feldmann 1945) and four sites in and near Garâa Sejenane (Muller *et al.* 2017; A. Daoud-Bouattour & S.D. Muller, personal observations). Since 2018, 19 other populations presenting the characteristic arrangement of gametangia of *Chara oedophylla* were found in sites throughout Tunisia (Table 1) ranging from temporary to permanent bodies of water and fresh to slightly brackish waters, confirming its ecological amplitude (Muller *et al.* 2017; Soulié-Märsche *et al.* 2024). *C. oedophylla* was also observed in both natural and artificial habitats, in running bodies of waters (springs, wadis, ditches) and isolated waterholes (marshes, ponds). Populations generally cover small surfaces, but can in some cases – in Ghezala Marsch (ICH8) and Utique Nouvelle (UTIN) – be larger (Table 1).

The populations from Enfidha (ENF) and Ghomrassen (GHO) have been cultivated outside their natural habitat for some years from sediment containing oospores. Installed in transparent trays in Tunis, these cultures were placed in 10 cm of water from November-December until May-June and kept dry for the rest of the year.

MEASUREMENTS

We made morphometric measurements of individual plants from the 13 populations of *Chara oedophylla*, the four co-occurring populations of *C. vulgaris*, and the population of Chbika (CHB), attributed here to the new species *C. acortiphylla* S.D. Muller & A. Daoud-Bouattour, sp. nov. (see below). We collected these populations between 16 March and 15 May 2018 and identified them according to the keys

TABLE 1. — Studied sites with either *Chara acortiphylla* S.D. Muller & A. Daoud-Bouattour, sp. nov. (Chbika, Ghomrassen) or *C. oedophylla* Feldmann (all other sites). **Abbreviations:** CB, Cap Bon; CT, Central Tunisia; K, Kroumiria; M, Mogods; NE, North-east; ST, Southern Tunisia; *, type population of the species. The “Morpho” column identifies the populations used to make morphometric measurements: A, *Chara acortiphylla*; O, *C. oedophylla*; and V, *C. vulgaris*.

Code	Site	Region	Alt. (m)	Latitude N/ Longitude E	Nature	Population surface (m ²)	Year of observation	Morpho
<i>Chara acortiphylla</i> S.D. Muller & A. Daoud-Bouattour, sp. nov.								
CHB	Chbika	ST	160	34°19'23"N, 07°56'28"E	Spring	1	2018	A
GHO	Ghomrassen *	ST	290	33°03'33"N, 10°19'17"E	Wadi	5	2022, 2023	
<i>Chara oedophylla</i> Feldmann								
ABI1	Oued el Abid	CB	31	36°52'15"N, 10°42'57"E	Spring	3	2018	O
ABI2	Oued el Abid	CB	24	36°52'22"N, 10°42'54"E	Spring	5	2018	O
AHM	Oued el Ahmar	NE	62	36°38'51"N, 09°42'39"E	Wadi	15	2018	O
CRIA	Cité Riadh	NE	20	36°49'59"N, 09°58'43"E	Ditch	5	2018	O
ENF	Enfidha airport	CT	5	36°04'09"N, 10°24'24"E	Wadi	1	2018, 2019	O
GUE	Guetma	NE	94	37°07'29"N, 09°16'25"E	Flooded crop	100	2007	
ICH4	Joumine (Ichkeul)	NE	0	37°06'40"N, 09°42'31"E	Channel	15	2018, 2019	O/V
ICH5	Joumine (Ichkeul)	NE	2	37°06'37"N, 09°41'55"E	Temporary pond	300	2018	O
ICH6	Joumine (Ichkeul)	NE	0	37°06'37"N, 09°40'59"E	Temporary pond	5	2018, 2019	O
ICH8	Ghezala (Ichkeul)	NE	11	37°06'41"N, 09°37'41"E	Salt marsh	2000	2018, 2019	O/V
JEB	Jebbès Lake	NE	109	36°53'15"N, 10°03'52"E	Artificial lake	30	2018	O
MHA	Mharza	CT	68	35°15'40"N, 10°15'40"E	Ditch	50	2018	O
MEJ	Oued Mejerda	NE	8	36°59'36"N, 10°03'28"E	Backwater	1	2018, 2022	
SEJE	Garâa Sejenane Euca	M	106	37°04'43"N, 09°12'38"E	Artificial pond	50	2007, 2014	
SEJO	Oued Sejenane	M	93	37°07'26"N, 09°16'31"E	Wadi	10	2007	
SEJ1	Garâa Sejenane GG1	M	99	37°05'11"N, 09°11'57"E	Flooded crop	10	2007	
SEJ51	Garâa Sejenane GG51	M	106	37°04'37"N, 09°12'41"E	Ditch	10	2018	O/V
TAM	Tamerza	ST	242	34°22'45"N, 07°54'56"E	Spring	1	2018	O
TINJ	Oued Tinja	NE	2	37°10'12"N, 09°45'36"E	Wadi	n.a.	1926	
UTIN	Utique Nouvelle	NE	1	37°07'20"N, 10°05'03"E	Salt marsh	1000	2022	V
UTIZ	Utique-Zone industrielle	NE	6	37°03'24"N, 10°01'15"E	Ditch	10	2018	
ZHA	Zhana-Mabtouha	NE	7	37°01'21"N, 09°59'55"E	Temporary pond	500	2018	

and descriptions by Soulié-Märsche & Muller (2016) and Muller *et al.* (2017). The protocol of gametangia study follows Comelles (1982). Measurements were made using a Wild binocular ($\times 40$) with a reticulum.

Measurements were made on three axes of three different individuals of each population, with the exception of the *Chara oedophylla* from Cité Riadh (CRIA), Jebbès Lake (JEB), Oued el Abid (ABI1), and *C. vulgaris* from Joumine Marsch (ICH4), which were in poor condition when the measurements were being made. The bract-cells are not distinguished from the bracteoles, i.e. the two upper cells of the cell crown developed at each node, nor from the bractlets, i.e. the lower cells developed beneath oogonia on unisexual female nodes (Schubert *et al.* 2024). We noted the following parameters (with the number of measurements carried out in parenthesis): 1) distribution of gametangia per node (*C. acortiphylla*: 423; *C. oedophylla*: 7369; *C. vulgaris*: 2905); 2) diameter of the three largest wet ripe antheridia per individual, without considering the organization of the gametangia of the nodes in question (*C. acortiphylla*: 9; *C. oedophylla*: 117; *C. vulgaris*: 36); 3) length of the two longest bract-cells/bracteoles/bractlets per branchlet (*C. acortiphylla*: 379; *C. oedophylla*: 4872; *C. vulgaris*: 1598); 4) number of bract-cells/bracteoles/bractlets per node (*C. acortiphylla*: 423; *C. oedophylla*: 7361; *C. vulgaris*: 2894); and 5) number of corticated segments per branchlet (*C. acortiphylla*: 191; *C. oedophylla*: 2354; *C. vulgaris*: 783).

Distribution data on the gametangia and number of bract-cells/bracteoles/bractlets in *Chara acortiphylla* S.D. Muller & A. Daoud-Bouattour, sp. nov., *C. oedophylla* and *C. vulgaris*

were used to construct ascendant hierarchical classifications (AHC) based on Euclidian distances. Because the distribution of antheridia diameter, the length and number of bract-cells/bracteoles/bractlets, and number of corticated segments were not normally distributed (Shapiro-Wilk test; Shapiro & Wilk 1965), distributions were compared using the non-parametric Mann-Whitney test (Mann & Whitney 1947). Numerical analyses and graphs were performed using PAST (Hammer *et al.* 2001).

RESULTS

The biometrical data are summarized in Table 2.

ARRANGEMENT OF GAMETANGIA

The ascendant hierarchical classification (Fig. 1) distinguishes *Chara acortiphylla* S.D. Muller & A. Daoud-Bouattour, sp. nov. and *C. oedophylla* from *C. vulgaris*. Figure 2 highlights the striking differences in their gametangia arrangements. In *C. vulgaris*, bisexual nodes (mainly, MF, 75.5%; MMFF, 2.2%) dominate whereas in *C. acortiphylla* S.D. Muller & A. Daoud-Bouattour, sp. nov. and *C. oedophylla*, they are less abundant (*c.* 20%) with a clear separation of sexes on different nodes: male nodes (M, 18.2% and 24.5%, respectively) and female nodes (61.9% and 55.1%, respectively). The female nodes mainly bear geminate (31.4% and 29.6%, respectively) and triplicate (15.6% and 7.9%, respectively) oogonia that are always arranged vertically. By contrast, in *C. vulgaris*, only

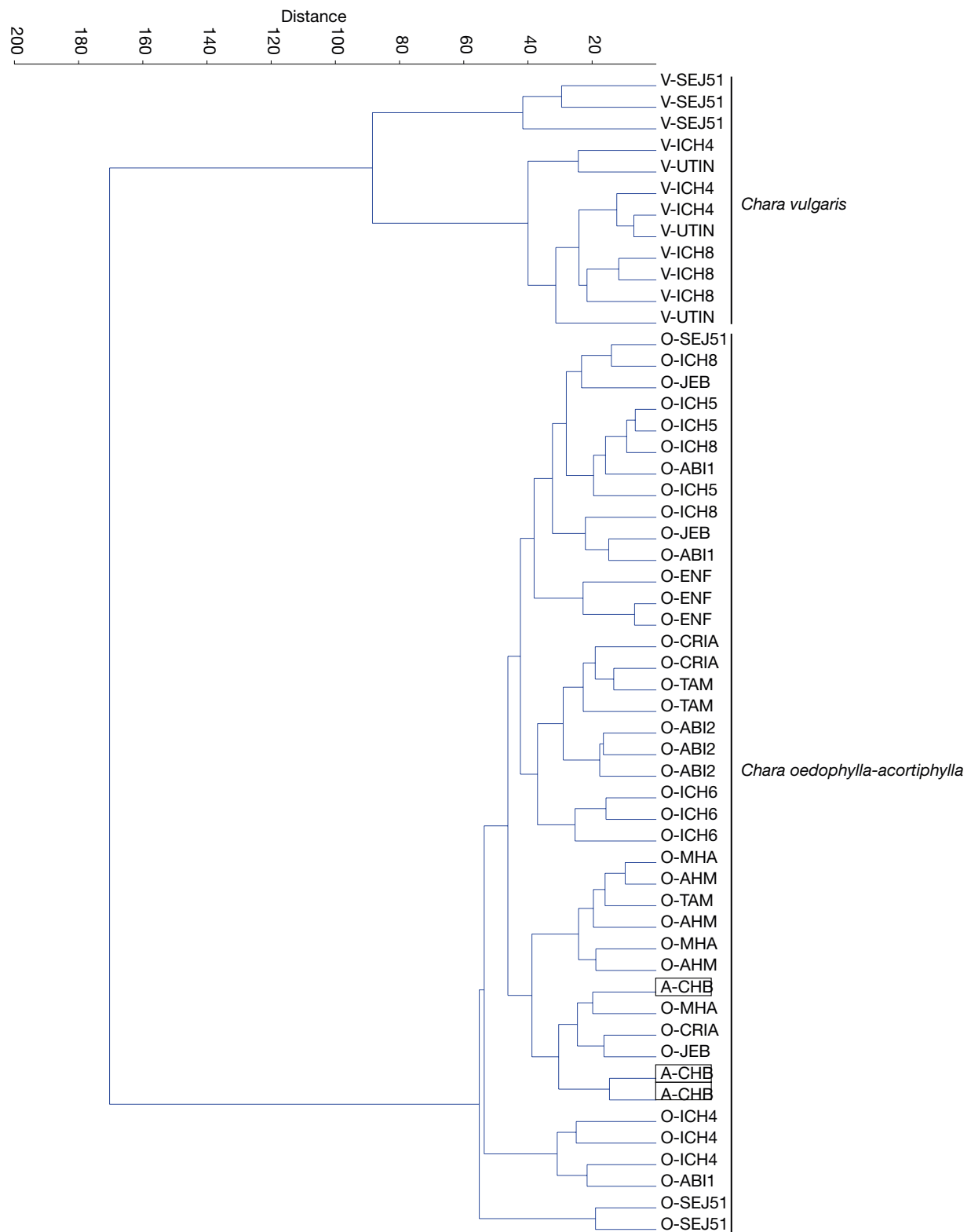


FIG. 1. — Dendrogram obtained from an ascendant hierarchical classification (Euclidian distance) performed on the number of nodes of the 11 different modes of gametangia arrangement, counted on three individuals per population in 18 populations studied. The individual code is composed of the site code (see Table 1) preceded by A- (*Chara acortiphylla* S.D. Muller & A. Daoud-Bouattour, sp. nov.), O- (*C. oedophylla* Feldmann) or V- (*C. vulgaris* L.).

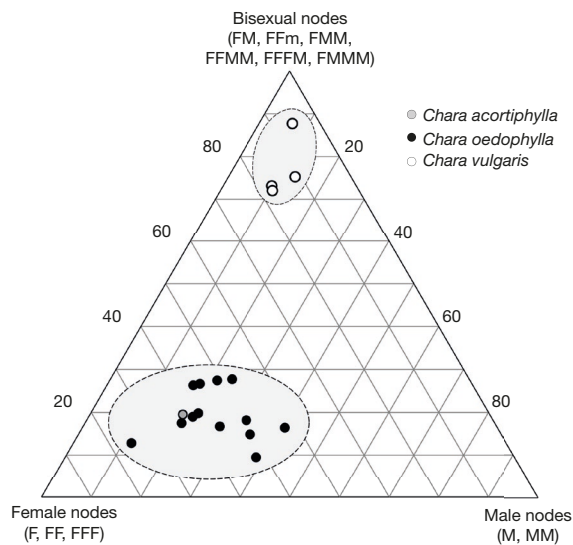


FIG. 2. — Distribution of gametangia arrangement in the 18 populations studied linked to *Chara acortiphylla* S.D. Muller & A. Daoud-Bouattour, sp. nov., *C. oedophylla* Feldmann and *C. vulgaris* L. Female nodes include solitary (F), geminate (FF) and three-arranged oogonia (FFF); male nodes include solitary (M) and geminate antheridia (MM); and bisexual nodes include diverse arrangements of oogonia (F) and antheridia (M).

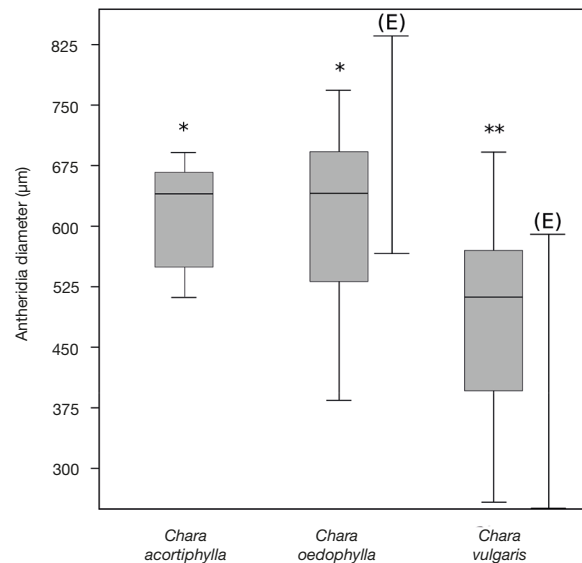


FIG. 3. — Box plot of antheridia diameter of *Chara acortiphylla* S.D. Muller & A. Daoud-Bouattour, sp. nov., *C. oedophylla* Feldmann and *C. vulgaris* L. The range of antheridia diameter in Europe (E) is represented for *C. oedophylla* and *C. vulgaris* on the right-hand side of boxes (after Denys *et al.* [2024] and Soulié-Märsche *et al.* [2024]). Different asterisk number denotes significant differences ($p < 0.001$; Mann-Whitney test).

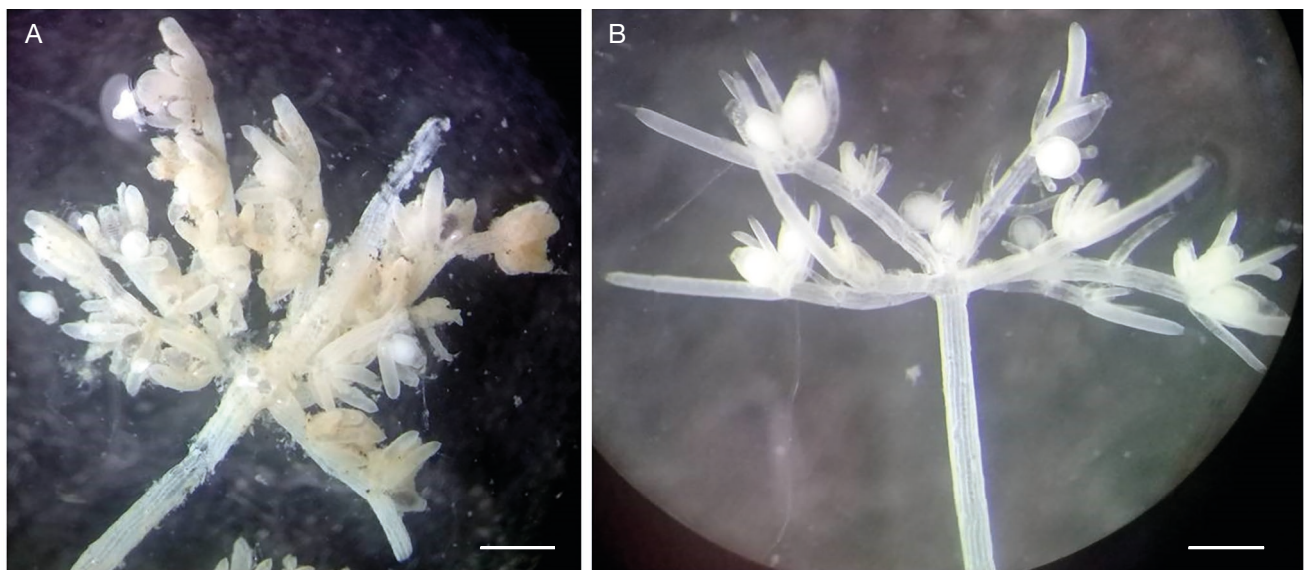


FIG. 4. — *Chara oedophylla* Feldmann from Enfidha (ENF): **A**, individual with swollen bract-cells, taken in nature (01.IV.2018); **B**, individual with thin bract-cells, from laboratory culture. Scale bars: 1 mm. Photo credits: I. Hammami & A. Daoud-Bouattour.

9.5% of the nodes are male, 12.1% are purely female with solitary oogonia, and only 0.4% have geminate oogonia that are always arranged laterally.

DIAMETER OF ANTERIDIA

We measured the diameter of the three largest wet ripe antheridia of each individual for *Chara acortiphylla* (9), *C. oedophylla*

(117) and *C. vulgaris* (36). These vary from 513 to 692 μm in *C. acortiphylla*, from 385 to 769 μm (88% $> 500 \mu\text{m}$) in *C. oedophylla* and from 256 to 692 μm in *C. vulgaris* (Fig. 3). The average antheridia diameter (Table 2) differs significantly between *C. vulgaris* and both *C. acortiphylla* (Mann-Whitney test: $U = 49.5$; $p < 0.001$) and *C. oedophylla* (Mann-Whitney test: $U = 848.0$; $p < 0.001$).

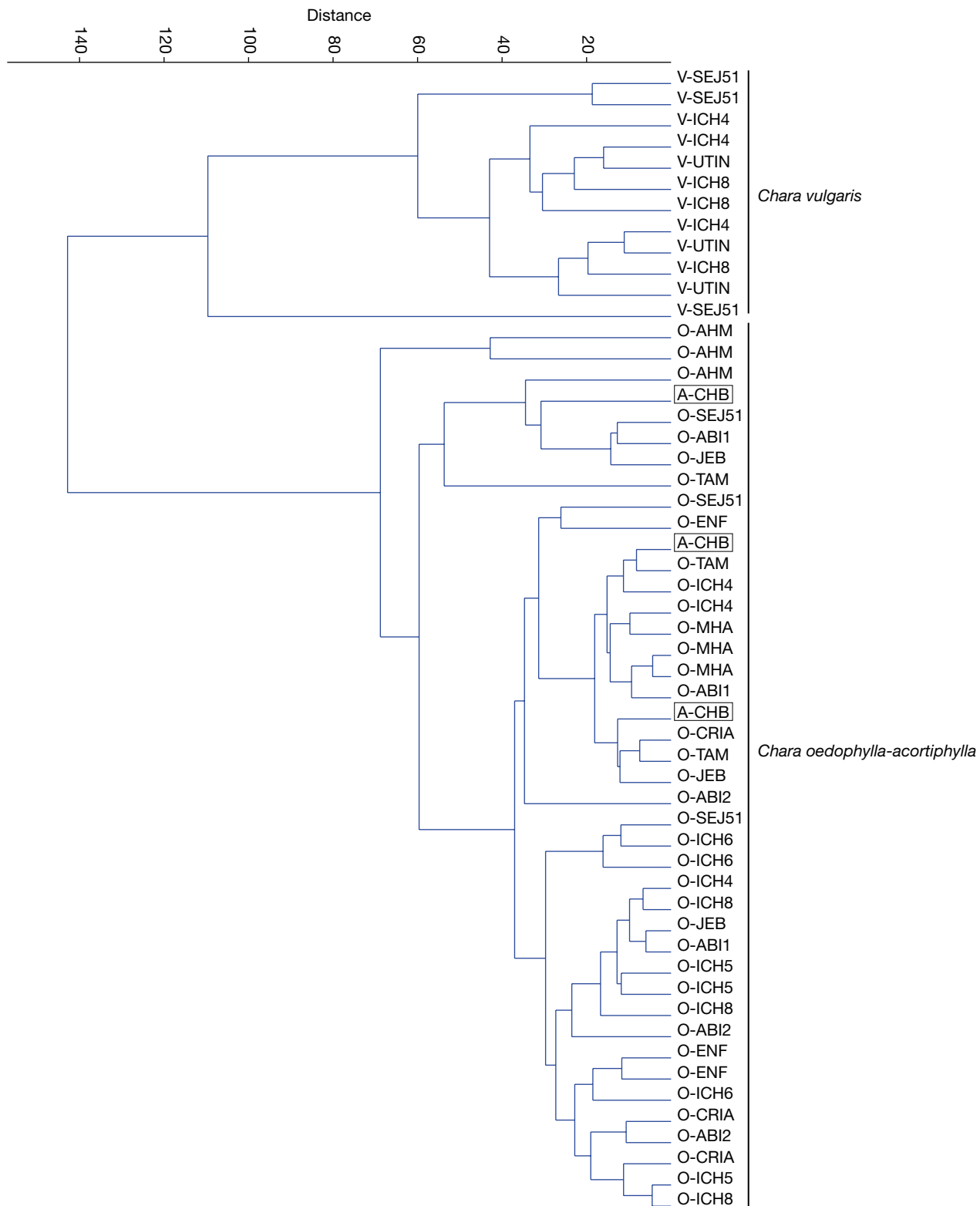
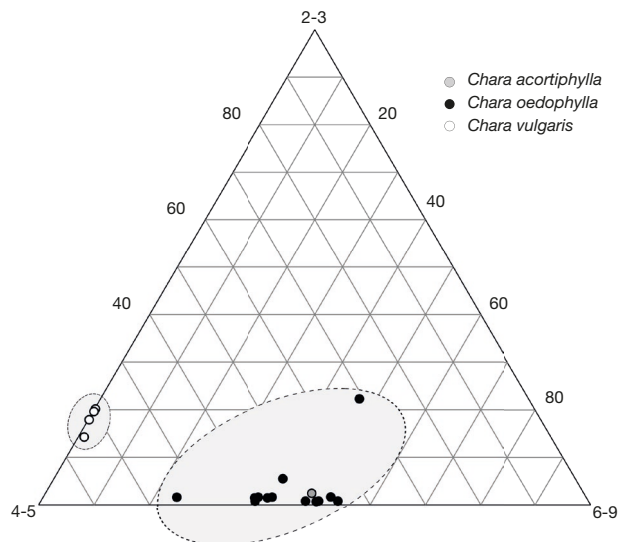


FIG. 5. — Dendrogram obtained from an ascendant hierarchical classification (Euclidian distance) performed, per the three individuals of each of the 18 populations, on the number of nodes of each modality of bract-cell. For the significance of site codes, refer to Figure 1.

TABLE 2. — Main characteristics distinguishing *Chara acortiphylla* S.D. Muller & A. Daoud-Bouattour, sp. nov. and *C. oedophylla* Feldmann from *C. vulgaris* L.

Character	<i>C. acortiphylla</i> S.D. Muller & A. Daoud- Bouattour, sp. nov.	<i>C. oedophylla</i> Feldmann	<i>C. vulgaris</i> L.
Proportion of unisexual nodes	80.1%	79.6%	21.9%
Proportion of 2-3 oogonia on female nodes	47.0%	37.5%	0.4%
Arrangement of oogonia on female nodes	Vertical	Vertical	Lateral
Mean diameter of antheridia (µm)	612.5 ± 42.3	610.6 ± 17.1	488.6 ± 34.8
Mean length of bract-cells (mm)	1.396 ± 0.104	1.265 ± 0.025	1.465 ± 0.046
Proportion of nodes with 4-7 bract-cells	50.2%	43.3%	0.2%

FIG. 6. — Distribution of populations according to the proportion of nodes with 0-1, 2-3 and 4-7 bract-cells, respectively. Male nodes of *Chara acortiphylla* S.D. Muller & A. Daoud-Bouattour, sp. nov. and *C. oedophylla* Feldmann, which only harbor two bracteoles, are not considered.

LENGTH OF BRACT-CELLS/BRACTEOLES/BRACTLETS

The length of the two longest bract-cells/bracteoles/bractlets of each branchlet was measured on three individuals of all studied populations of *Chara acortiphylla* (1), *C. oedophylla* (13) and *C. vulgaris* (4). The average length (Table 2) differs significantly between *C. vulgaris* and *C. oedophylla* (Mann-Whitney test; $U = 79379$; $p < 0.001$). The bract-cells/bracteoles/bractlets in nearly all of the populations of *C. acortiphylla* S.D. Muller & A. Daoud-Bouattour, sp. nov. and *C. oedophylla* are “oedemized”. Only the *in-situ* population of *C. oedophylla* from Enfidha (ENF) shows swollen bract-cells/bracteoles/bractlets (observations made in 2018 and 2019; Fig. 4A) whereas those of individuals obtained from an *in-vitro* culture of oospores from the same population are “non-oedemized” (Fig. 4B). The swollen bract-cells/bracteoles/bractlets – the basis of the species’ name – based on population type, do not appear to be consistent and might be a morphological adaptation to growing conditions.

NUMBER OF BRACT-CELLS/BRACTEOLES/BRACTLETS

In order to focus on the number of bract-cells/bracteoles/bractlets, a recognized diagnostic criterion of *Chara oedophylla*

(Feldmann 1945; Soulié-Märsche & Muller 2016), the statistical study does not take into consideration the male nodes that generally have two short bracteoles accompanied by punctiform rudimentary bract-cells. The ascending hierarchical classification based on the number of bract-cells/bracteoles/bractlets (Fig. 5) clearly distinguishes the populations of *Chara acortiphylla* S.D. Muller & A. Daoud-Bouattour, sp. nov. and *C. oedophylla* from those of *C. vulgaris*. In *C. acortiphylla* S.D. Muller & A. Daoud-Bouattour, sp. nov. and *C. oedophylla*, the two bracteoles are always accompanied by two to six well-developed bract-cells on unisexual female nodes. The difference is highly significant (Mann-Whitney test; $U = 0$; $p < 0.001$). Figure 6 shows the dominance of nodes with four-five bract-cells/bracteoles/bractlets in *C. vulgaris* (bisexual nodes; 81.4%) and, to a lesser extent, in *C. acortiphylla* (43.8%) and *C. oedophylla* (48.9%). Finally, while there are practically no nodes with four to seven bract-cells/bracteoles/bractlets in *C. vulgaris* (0.2%), these are found in all populations of *C. acortiphylla* S.D. Muller & A. Daoud-Bouattour, sp. nov. and *C. oedophylla*, where they represent more than 40% of nodes (Table 2).

NUMBER OF CORTICATED SEGMENTS OF THE BRANCHLET'S

The average number of corticated segments per whorl differs significantly between *Chara oedophylla* (2.4 ± 1.0) and *C. vulgaris* (3.8 ± 0.5) (Mann-Whitney test; $U = 4538$; $p < 0.001$) (Fig. 7), ranging from two to five per branchlet in *C. vulgaris*, with a majority of branchlets (55%) showing four corticated segments, and between zero and five in *C. oedophylla*, with a majority of branchlets having one-three corticated segments (18%, 40% and 27% respectively). Only 1% of branchlets is fully corticated in this species.

The plants collected at Chbika Falls (CHB) in southwest Tunisia are very different from the studied populations of *Chara oedophylla*. They showed 50% ecorticated branchlets and 45% corticated only on the basal segment (Fig. 7). Similar plants, with completely ecorticated branchlets, were found at a different site in southern Tunisia, Ghomrassen (GHO). While the arrangement of gametangia (mainly sejoined and vertically arranged, geminate oogonia) undoubtedly links this form to *C. oedophylla*, its ecorticated branchlets and the presence of gametangia on the ecorticated nodes distinguishes it.

Because these features recall those currently used to distinguish *Chara gymnophylla* A. Br. from *C. vulgaris* (Schubert

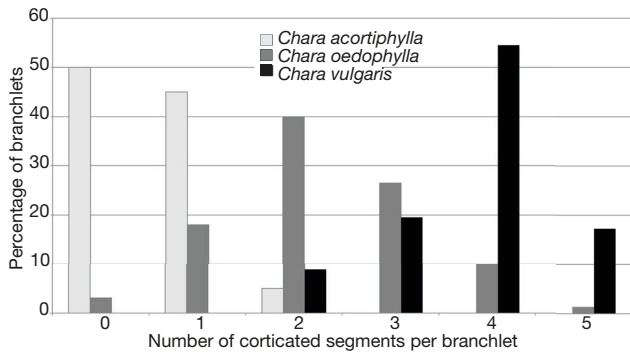


FIG. 7. — Percentage of branchlets for each modality of the number of corticated segments in *Chara acortiphylla* S.D. Muller & A. Daoud-Bouattour, sp. nov., *C. oedophylla* Feldmann and *C. vulgaris* L..

et al. 2024), we propose defining these poorly corticated plants as a separate species.

TAXONOMY

Family CHARACEAE Gray
Gender *Chara* L.

Chara acortiphylla S.D. Muller & A. Daoud-Bouattour,
sp. nov. (Figs 8; 9)

DIAGNOSIS. — Plants with the morphology characteristic of *Chara oedophylla* (sejoined gametangia) with ecorticated or poorly corticated branchlets.

MATERIAL EXAMINED. — **Tunisia** • Ghomrassen; 33°03'33"N, 10°19'17"E; 290 m alt.; 26.XII.2023; *Z. Ghrabi-Gammar*; dried after cultivation at Montpellier, 27.VI.2024; holotype deposited at the Montpellier Herbarium, Université de Montpellier, no. MPU896993; isotypes INAT and personal herbarium of S.D. Muller.

LOCALITY. — *Chara acortiphylla* S.D. Muller & A. Daoud-Bouattour, sp. nov. is currently known in two localities in southern Tunisia from 160 to 290 m a.s.l.: the type locality (Ghomrassen), where it develops as patches along the wadi traversing the city, and the mountain oasis of Chbika, where it occurs in a spring. To date, *C. acortiphylla* S.D. Muller & A. Daoud-Bouattour, sp. nov. is considered endemic to southern Tunisia, but can also occur in similar habitats in continuous countries.

ETYMOLOGY. — The specific epithet is composed of the two terms “acorti”, from the Latin cortex preceded by the privative prefix a, and “phylla”, from the Greek *phyllon* (leaf). It refers to the ecorticated branchlets that distinguish this species from *Chara oedophylla*. Although it is generally recommended not to mix Greek and Latin roots in a scientific name, we preferred the ending “phylla” to “folia” in order to recall *C. gymnophylla* (from the Greek *gymnós*, naked, and *phyllon*, leaf), which exhibits the same feature.

DESCRIPTION

Axes corticated, isostichous, more or less spiraled. Secondary axes with ecorticated basal segments. Stipulodes short. Spine cells rare, short to punctiform. Eight-nine branchlets per node. From 50-100% of the branchlets are completely ecorticated while the balance are corticated only on the basal

segment (very rarely on the two basal segments). Branchlets composed of six segments: the nodes of the two basal segments generally bear bracteoles, bract-cells and gametangia, and the upper segments form a long, thin, acute termination. Lateral bract-cells and bracteoles are more or less long and thin, longer than four-eight times the length of gametangia, not swollen as is sometimes seen in *Chara oedophylla*. Dorsal bract-cells very short to punctiform.

Taxon monoecious with antheridia and oogonia mostly sejoined, rarely together at the same node. Antheridia generally solitary (diameter of wet ripe antheridia: 500-700 µm), accompanied by two bracteoles and zero-two punctiform bract-cells. Oogonia solitary or geminate, aligned vertically on the branchlet axis with a basipetal development. In addition to the ever present two bracteoles, solitary oogonia are accompanied by zero-two bract-cells/branchlets and geminate oogonia by zero-six bract-cells/branchlets. We observed no groups of three oogonia as are often found in *Chara oedophylla*. The distribution mode of the gametangia arrangement seems random, but basal whorls often harbor only female nodes, while the proportion of male nodes seems to increase towards the upper part of the plant (Fig. 8). Oospores and gyrogonites (calcified oospores) present 12-13 striae. Oospores measure 400-500 × 270-300 µm, and gyrogonites, 500-550 × 330-350 µm (Fig. 9).

NOTES

The species rank of this taxon follows the microspecies conception of Schubert *et al.* (2024).

DISCUSSION

VARIABILITY OF *CHARA OEDOPHYLLA* AND DIFFERENCES WITH *C. VULGARIS*

Our observations confirm the unchanging morphology of *Chara oedophylla* (detailed by Feldmann [1945]; Wood [1962] and Mouronval *et al.* [2015], and synthesized by Soulié-Märsche & Muller [2016] and Soulié-Märsche *et al.* [2024]), both in nature (sometimes in the presence of *C. vulgaris*) and under different environmental conditions. Such morphological constancy, also documented in southern France (Soulié-Märsche 2003), argues strongly for the status of the taxon as a species (Soulié-Märsche & Muller 2016). Moreover, our measurements confirm the morphological differences of *Chara oedophylla* and *C. vulgaris* (Table 2), which are based mainly on the following.

Distribution of gametangia

Majority of unisexual nodes in *C. oedophylla* vs bisexual nodes in *C. vulgaris*.

Arrangement of oogonia on female nodes

Significant occurrence of two-three oogonia per node, always vertically arranged in *C. oedophylla* vs rare geminate oogonia in *C. vulgaris*, always arranged side by side.

Significantly larger antheridia in *C. oedophylla* than in *C. vulgaris*.



FIG. 8. — *Chara acortiphylla* S.D. Muller & A. Daoud-Bouattour sp. nov.: **A**, axis portion showing the four upper whorls, the ecorticated branchlets and the heterogeneous arrangement of gametangia; **B**, the detail shows the typical geminated vertically arranged oogonia surrounded by two bracteoles and two elongated bract-cells. Scale bars: A, 1 cm; B, 1 mm. Drawing: S.D. Muller

REMARKS

Nevertheless, two points should be emphasized. The first concerns the swollen aspect of bract-cells, bracteoles and bractlets from which the species takes its name, and which appear to be a constant feature in populations outside Tunisia. Here, on

the contrary, it appears infrequently, and was observed only at Oued Tinja (Feldmann 1945), Garâa Sejenane (Soulié-Märsche & Muller 2016; Muller *et al.* 2017) and Enfidha (Fig. 4A). Almost all populations observed in 2018 had long, thin bracts. The Enfidha population has swollen bract-cells

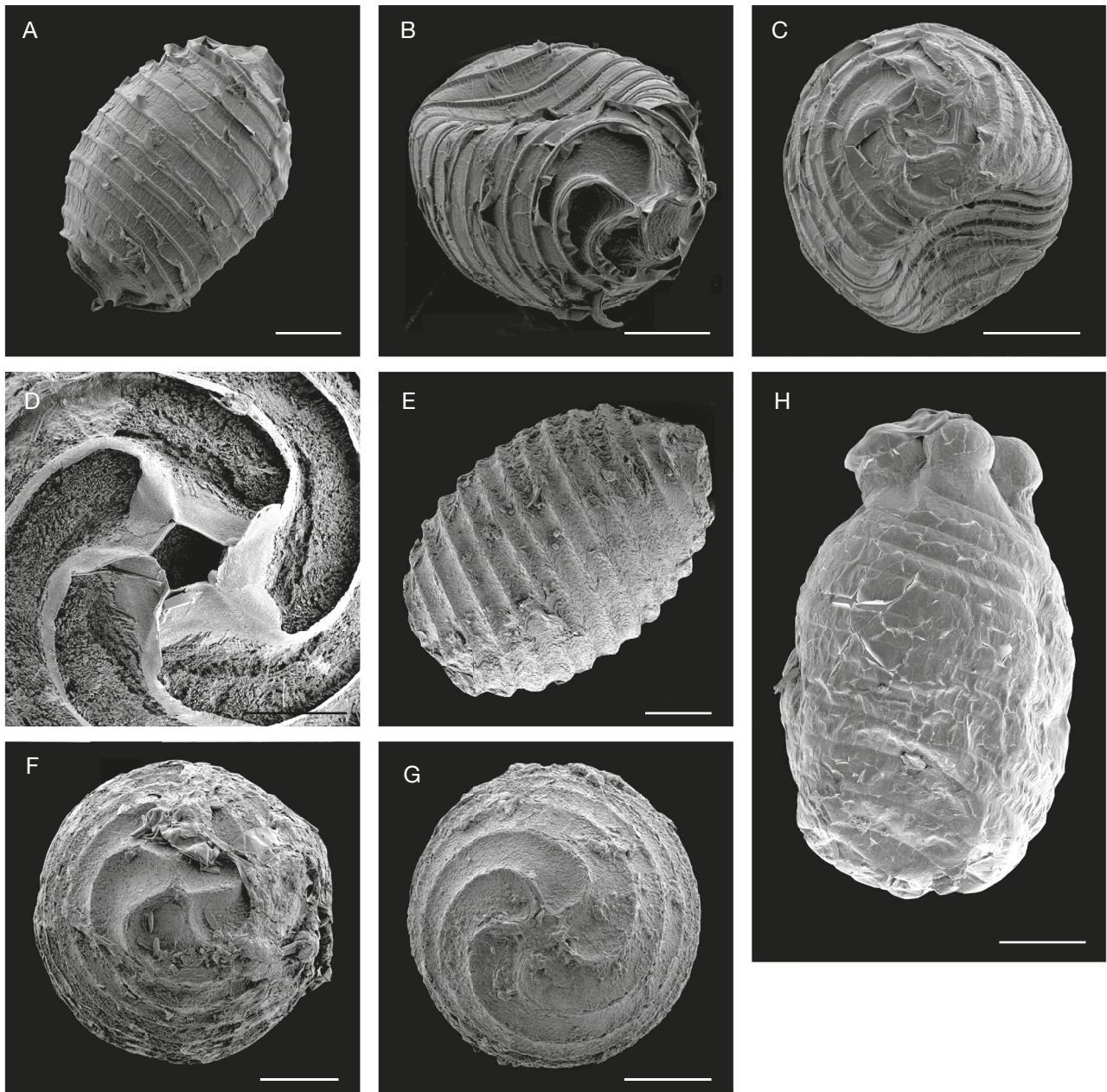


FIG. 9. — *Chara acortiphylla* S.D. Muller & A. Daoud-Bouattour sp. nov. Oospore (A-D): **A**, lateral view; **B**, apex; **C**, impression of the basal plate; **D**, basal cage detail. Gyrogonite (E-G): **E**, lateral view; **F**, apex; **G**, basal view; **H**, oogonium showing the spherical cells of coronula. Scale bars: A-C, E-H, 100 μ m; D, 50 μ m. Photocredits: B. Rebière, Université de Montpellier.

in the field (observed in 2018 and 2019) but not in culture (Fig. 4), suggesting that this characteristic should be considered an ecomorphosis, probably related to environmental factors such as low water depth or relatively warm waters. The fact that *Chara oedophylla* frequently develops this swollen or “oedemized” habit is nevertheless a supplementary distinctive characteristic from *C. vulgaris*.

The second point concerns our measurements of the antheridia diameter. These do not fit completely with the

measurements given for both species by Soulié-Märsche *et al.* (2024) and Denys *et al.* (2024), and are shifted towards lower values for *Chara oedophylla* whereas the average values for *C. vulgaris* are higher overall than those for Europe (Fig. 3). The discrepancy between our measurements and those reported by Denys *et al.* (2024) for *C. vulgaris* could be due to the fact that our ones were uniquely made on wet material, while the others could have been at least partly made on dried material.

COMPARISON OF *CHARA ACORTIPHYLLA* S.D. MULLER & A. DAOUD-BOUATTOUR, SP. NOV. AND *C. GYMNOPHYLLA/SQUAMOSA*

As noted, the features of *Chara acortiphylla* S.D. Muller & A. Daoud-Bouattour, sp. nov. recall those of *C. gymnophylla* and *C. squamosa* Desf. (Mouronval *et al.* 2015; Muller *et al.* 2017; Schubert *et al.* 2024). However, the arrangement of gametangia is distinctly different – unisexual in *C. acortiphylla* vs bisexual in *C. gymnophylla* and *C. squamosa* – as is the lower number of fructified nodes per branchlet (two vs three-four) (Romanov & Becker 2024; Romanov *et al.* 2024).

The characteristics of *Chara acortiphylla* S.D. Muller & A. Daoud-Bouattour, sp. nov. proved to be quite consistent as shown by the population of Ghomrassen (GHO), which includes only individuals with completely ecorticated branchlets. Those plants were first found and collected on March 15, 2022. When site sediment was cultivated in Tunis over a period of two years, populations presenting exactly the same morphology as the field population developed. After the December 26, 2023 collection was cultivated for four months in Tunis and for a further four months in Montpellier, the consistent morphology in both the field and in culture was confirmed.

In comparison to *Chara oedophylla*, the plants from Ghomrassen and Chbika present the same differences as *C. gymnophylla* compared to *C. vulgaris* and as *C. squamosa* compared to *C. contraria* A. Br. ex Kütz.: either there is no branchlet cortication or it is restricted to one or two segments, and gametangia are borne on ecorticated nodes.

CONCLUSION

The discovery in Tunisia of 17 new populations of *Chara oedophylla* makes it possible to better circumscribe the morphological diversity of this rare and poorly known species. The additional discovery of a partly ecorticated taxon, described here as *C. acortiphylla* S.D. Muller & A. Daoud-Bouattour, sp. nov., clearly links the complex *Chara oedophylla-acortiphylla* to the similar pairs of taxa *C. vulgaris-gymnophylla* and *C. contraria-squamosa*. In each of these pairs, the first species bear gametangia on corticated nodes while the second bear them on ecorticated nodes. These morphological differences could result from recent or even ongoing speciation, as suggested for *Chara oedophylla*, which probably represents an evolutionary stage from an initially monoecious state towards dioecy (Soulié-Märsche & Muller 2016). The question of the taxonomical rank of such related taxa remains unresolved. While *Chara squamosa* has long been considered a synonym for *C. gymnophylla*, considered in turn to be a variety of *C. vulgaris* (Wood 1962), the most recent reference for Europe (Schubert *et al.* 2024) returns to the microspecies concept established by Braun (1883) and Migula (1900): these taxa are all ranked as species. However, molecular phylogenetic analyses (Nowak *et al.* 2024) do not reflect the morphological differences between these microspecies, especially in the genus *Chara*, which reveals the group's high polymorphism (Schneider *et al.* 2016). Concerning our own observations

of *Chara oedophylla* and *C. vulgaris*, the constancy of most of the characteristics over time and in space, in the field and in culture, and in mixed populations, supports the argument for related but distinct species. That said, the overlap of the antheridia diameter values in both species and the occurrence of non-swollen populations of *C. oedophylla* suggest the possibility of introgression. Unfortunately, no molecular data are available for the rarer taxa including *C. oedophylla*, *C. squamosa* (Nowak *et al.* 2024), and *C. acortiphylla* S.D. Muller & A. Daoud-Bouattour, sp. nov., making it impossible to determine their actual status.

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