

Article

Herzog Vindicated: Integrative Taxonomy Reveals That *Trichostomum brachydontium* (Pottiaceae, Bryophyta) Comprises Several Species

Rosa M. Ros ¹, Olaf Werner ¹ and Ron D. Porley ^{2,*}

¹ Departamento de Biología Vegetal, Facultad de Biología, Campus de Espinardo, Universidad de Murcia, 30100 Murcia, Spain; rmmros@um.es (R.M.R.); werner@um.es (O.W.)

² Cerca dos Pomares, CxP 409M, 8670-052 Aljezur, Portugal

* Correspondence: ron.porley@sapo.pt

Abstract: The morphologically variable moss *Trichostomum brachydontium* is very common in south and west Europe, particularly under Mediterranean and Atlantic climates. A morphological study was conducted alongside a molecular phylogenetic study based on nr ITS and cp-*rbcl* regions in order to assess if *T. brachydontium* is an exceptionally polymorphic species as evidenced by the number of described infraspecific taxa in the last century or, alternatively, if it includes more than one species, and if so, to find the valid name for them. Phylogenetic analyses of both nuclear and chloroplast datasets show that there are four well-supported clades. While the ITS-based tree is in good agreement with the morphological data, there are a few inconsistencies with reference to the *rbcl* tree; this may be explained by incomplete lineage sorting by hybridization or by the persistence of isolated ancestral molecular races. The morphological survey revealed well-defined discriminate differences between the four phylogenetic lineages. The taxonomic conclusions include the recognition of four species: *T. brachydontium* s.s., *T. herzogii* (a new name proposed for var. *cuspidatum*), *T. littorale*, and *T. meridionale* (a new name proposed for var. *densum*). Lectotypes are designated for *T. brachydontium* and *T. littorale*. Our results underline the ongoing need of integrative studies to examine further the underestimated diversity of the *T. brachydontium* complex in other regions.

Keywords: cp-*rbcl*; nr-ITS region; biodiversity; distribution; Mediterranean-Atlantic Europe; mosses; nomenclature; taxonomy; *Trichostomum herzogii* nom. nov.; *Trichostomum meridionale* sp. nov.



Citation: Ros, R.M.; Werner, O.; Porley, R.D. Herzog Vindicated: Integrative Taxonomy Reveals That *Trichostomum brachydontium* (Pottiaceae, Bryophyta) Comprises Several Species. *Taxonomy* **2022**, *2*, 57–88. <https://doi.org/10.3390/taxonomy2010005>

Academic Editor: Francisco Lara

Received: 29 December 2021

Accepted: 17 January 2022

Published: 21 January 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

The morphologically variable moss *Trichostomum brachydontium* Bruch is a very common plant of arid, exposed sites in the Mediterranean macrobioclimate, but it occurs equally in moist, sheltered biomes in oceanic and hyperoceanic (Atlantic) zones; furthermore, this Submediterranean-Subatlantic moss [1] occupies a wide range of niches. It has a worldwide cosmopolitan distribution, absent only in Antarctica. It occurs in North, Central, and South America, Eurasia, Africa, Atlantic Islands, Indian Ocean Islands, Pacific Islands (New Zealand, New Caledonia), and Australia [2,3]. In the Iberian Peninsula, it is present in the majority of provinces [4], though it is most characteristic of coastal and lowland districts (generally lacking on the Iberian Central Range), whereas in Britain and Ireland, it has a western or Atlantic tendency [1].

Since the publication of *T. brachydontium* Bruch [5], a large number of subspecies, varieties, and forms have been described. Herzog [6] took a special interest in the forms of *T. brachydontium* (*T. mutabile* Bruch nom. illeg.) during a trip he made to Sardinia in 1904. As well as his own extensive collections of *T. brachydontium*, he had access to collections from other contemporary bryologists to study the various forms known in Europe and Macaronesia (and beyond). He established four ideal types (*densum*, *littorale*, *mutabile*, and *cuspidatum*), with four subtypes within *mutabile* (*normale*, *cylindricum*, *cophocarpum*,

and *longirostre*), and a further five forms that he considered as intermediate between the four ideal types. The work of Herzog is still relevant to this day and indeed to our study, and is an early example exploring phylogenetic relationships.

The four Herzog types have been considered in later floras and taxonomical works worldwide mostly as subspecies or other taxa of *T. brachydontium*, notwithstanding that Herzog very clearly characterized them morphologically. In Guerra [4], four varieties of *T. brachydontium* are briefly mentioned for the Iberian Peninsula (var. *cophocarpum* (Schimp.) Cout.; var. *cuspidatum* (Braithw.) L.I. Savicz); var. *cylindricum* (Schimp.) Cout.; and var. *littorale* (Mitt.) C.E.O. Jensen), while a further variety (var. *unguiculatum* (H. Philib.) Corb. & Jahand.) is more or less dismissed as falling within the range of variation of the type. Rungby [7] and Koppe [8] reported the var. *densum* (Bruch & Schimp.) Düll for Spanish areas and the last author proposed the subsp. *pusillum* F. Koppe based on material collected in the Balearic Islands. In earlier years additional varieties were described, for example var. *robustum* (Renauld & Cardot) During and var. *nigroviride* (Renauld & Cardot) Luisier, both from the Canary Islands [9], and from Madeira a form of *T. brachydontium* was reported as a new species, *T. contortum* (Kunze) Sérgio [10,11], though this remains uncertain and has not been accepted in later checklists of European and Mediterranean mosses [12–14]. In other Mediterranean areas, var. *angustifolium* (Lindb.) Wijk & Margad. and *T. mutabile* var. *brevifolium* Schiffn. were also reported [13]. In Britain, bryological floras [15–19] state that two infraspecific taxa have been recorded (var. *littorale* (Mitt.) C. Jens. (considered at species level by Braithwaite [15], and var. *cophocarpum* (Schimp.) P. Cout.). Nevertheless, Smith [19] stated that they intergrade with the type to such an extent that they cannot be maintained. As a result, most of these forms of *T. brachydontium* are now overlooked in most worldwide floras. This tendency towards taxonomic lumping may have the unintentional and indeed undesirable consequence of dismissing morphologically distinct taxa and ensuing recognition and reinstatement may require a revision of the species circumscription [20].

The first two authors of the present study have been using both morphometric and molecular techniques to study the Pottiaceae family and more specifically the subfamily Trichostomoideae [21–26] for many years, and most recently investigating forms of *T. brachydontium* from SE Spain. Moreover, the third author made many collections of *T. brachydontium*, mostly from Serra de Monchique in Algarve, Portugal, and recognized the wide variation therein. The present study does not attempt to address the full degree of variation shown by *T. brachydontium* across its entire distributional range—this would require looking at many more collections as well as from different geographical regions—but to analyze the morphological variation of the species from a fresh perspective, using mostly Mediterranean and Atlantic samples and employing an integrative methodology. In this work, a morphological and molecular phylogenetic study was performed with the following objectives: (1) to assess if *T. brachydontium* is an exceptionally polymorphic species as evidenced by the number of described forms from the last century or if it includes more than one species, (2) to establish whether the morphotypes detected in *T. brachydontium* s.l. are genetically supported, and (3) in the case that molecular data supports the existence of more than one species within *T. brachydontium* s.l., to find the valid name for them.

2. Materials and Methods

2.1. Plant Material

A total of 37 *Trichostomum* samples were used for the molecular study. One sequence was taken from GenBank and the other sequences were generated for this work from recent specimens mainly collected by the authors. Other bryologists (mentioned in the Acknowledgements section) also contributed material from Mediterranean and Atlantic zones. A group of species of the genera *Oxystegus* (Limpr.) Hilp. and *Tortella* (Müll. Hal.) Limpr. was used as outgroup for the ITS tree, and *Barbula unguiculata* Hedw. and *Tortella flavovirens* (Bruch) Broth. for the *rbcL* tree, the sequences of which were also generated for this work. In addition, 58 samples were used for the morphological study only, as they could not

be sequenced (Appendix A). The type specimens of most of the taxa considered in this work were studied (*T. brachydontium*, *T. brachydontium* var. *cophocarpum*, *T. brachydontium* var. *cuspidatum*, *T. brachydontium* var. *cylindricum*, *T. brachydontium* var. *unguiculatum*, *T. littorale* Mitt.) obtained from GLM, GOET, H, JE, and NY herbaria.

2.2. DNA Sequencing

DNA was extracted according to the NaOH protocol of Werner et al. [27]. The nr-ITS region was amplified with the primers ITS1 and ITS4 [28], and a fragment of the cp-*rbcL* gene was amplified with the primers *rbcLa-F* [29] and *rbcLa-rev* [30]. All primers were used at a final concentration of 0.4 μ M and 2 μ L of stock DNA was used as a template in 25 μ L reaction volume with the Phire Plant Direct PCR Master Mix (Thermo Fisher, Spain). The amplification conditions were as follows: 3 min at 98 °C, 35 cycles with 5 s at 98 °C, 30 s at 55 °C and 30 s at 72 °C, and a final 5-min extension step at 72 °C. Amplification products were controlled on 1% agarose gels and successful reactions were cleaned by adding 1u FastAP (Thermo Fisher) and 10u ExoI (Thermo Fisher) for one hour at 37 °C followed by a thermal inactivation of 15 min at 85 °C. Cycle sequencing was performed by the central labs of the University of Murcia using the amplification primers.

2.3. Data Analysis

The sequences were edited using Bioedit 5.0.9 [31] and aligned manually. Between taxa mean distances and within taxa mean distances were calculated with MEGA X [32]. We used the program gappy [33] in order to identify reliable indels and recodify the indels in the data matrix as 0/1 character states. The TCS module of T-Coffee [34] was used to identify regions in the ITS alignment with low local reliability which were excluded from further analyses. The data were analyzed by Bayesian inference implemented with MrBayes 3.2 [35–37]. Gaps were treated as standard data. Trees were sampled across the substitution model space in Bayesian MCMC analysis itself [38] using the option *nst* = mixed. Therefore, a priori model testing was not necessary. Indels were treated as separate unlinked partitions, using the restriction site model (F81). Two runs with four chains were conducted with 15,000,000 generations in the case of ITS, and 5,000,000 in the case of *rbcL*. Trees were sampled every 1000th generation and the first 1,000,000 generations were discarded (burn-in) in order to exclude the trees before the chain reached the stationary phase. We checked for stationarity of the log likelihood values, that the Potential Scale Reduction Factor (PSRF) was close to “1” ($0.99 < \text{PSRF} < 1.01$), and that the estimated sample size was above 200. The final trees were edited with TreeGraph2 [39].

2.4. Morphological Study

Standard microscopic observations were applied to study the plants of the clades revealed in the molecular analysis. Material was photographed using a light microscope (Leica DM1000 LED) with a micrometer inserted in an ocular and in a stereomicroscope (Leica A8APO) with a video camera connected (Leica camera: DFC295) in order to transfer the images to a computer. The Leica Application Suite Version 4.1.0 was used for image analysis.

The plant material was critically studied, mindful of the four ideal types proposed by Herzog [6] and based mainly on the following character traits: size and color of plants, growth form, stems comose or not, position of leaves when dry and moist, shape and size of leaves, apex of leaves, leaf lamina, margins incurved or not, teeth development in the basal part of lamina, color and wall thickness of basal cells, length of the excurrent costa. If sporophytes were present, length of seta, size, shape and color of the capsule, operculum beak length, peristome development, and spores were also scored.

3. Results

3.1. Molecular Data

We first used an ITS tree with several outgroup species belonging to the genera *Oxystegus* and *Tortella* to establish that *Trichostomum crispulum* Bruch can be considered an outgroup with respect to *T. brachydontium* s.l. (Figure 1a). Once this was confirmed, we used a second alignment excluding the non-*Trichostomum* specimens to study with more detail the relationships within *Trichostomum*. This way, in the final alignment, fewer characters had to be excluded due to unreliable positions. The final ITS tree (Figure 1b) first separates an isolated sample from Austria. The remaining samples are divided in two sister clades. The first (0.92 pp) comprises *T. brachydontium* s.s. (1.0 pp) and *T. meridionale* (1.0 pp), the second *T. littorale* (1.0 pp), *T. herzogii* (1.0 pp), and an isolated sample from Greece close to *T. herzogii* (0.99 pp). The names given to the samples are in accordance with the combination of the molecular and morphological results (see taxonomic treatment).

A look at the genetic distances based on ITS sequences (Table 1) shows that the within species mean distances are much lower than the between species values. The maximum within species distance at individual level was 4 in the case of *T. littorale*, and within *T. brachydontium* s.s. and *T. herzogii* all sequences were identical. The minimum between species distance was 6 between several samples of *T. brachydontium* s.s. and *T. meridionale*.

In the case of the *rbcL* sequences, the samples of *T. crispulum* shared a haplotype with three samples of *T. brachydontium* s.l. and the basal part of the *rbcL* tree is therefore not resolved (Figure 2). One of these samples, morphologically and based on the ITS sequences, belongs to *T. brachydontium* s.s., another to *T. meridionale*, and the third is the isolated sample from Greece. The remaining samples are situated in well-supported clades, but the relationship among these clades remains unresolved. These clades largely conform to the species based on morphological a priori observations and the nuclear ITS sequences, except for one sample (*T. brachydontium* 3234 from Portugal) that according to ITS belongs to *T. brachydontium* s.s. and which here is placed with the *T. littorale* samples. The isolated sample from Austria is situated close to *T. herzogii*.

The genetic distances calculated for the *rbcL* gene fragment are generally much lower than those calculated for the ITS region (Table 2). The within species distances were 0 in the case of *T. crispulum*, *T. herzogii*, and *T. littorale*, and low (0.15) in the case of *T. meridionale*. In the case of *T. brachydontium*, the within species distance (1.67) was higher than the between species distance of this species compared with most of the remaining species. This is due to the samples 3228 and 3234, which are clearly different from the two remaining *rbcL* sequences we obtained for this species.

3.2. Morphological Study

Plants from the four main clades correspond well to the four main morphotypes, based on characters used by Herzog [6] which were checked by the study of the type specimens: *T. meridionale* (*densum* type), *T. littorale* (*littorale* type), *T. brachydontium* s.s. (*mutabile* type), and *T. herzogii* (*cuspidatum* type).

The Austrian *Trichostomum* sp. sample 2178 shows morphological similarities with *T. herzogii*, and the Greek *Trichostomum* sp. sample 3228 with *T. meridionale*.

The main diagnostic characters that enable the separation of the four species are summarized in Table 3.

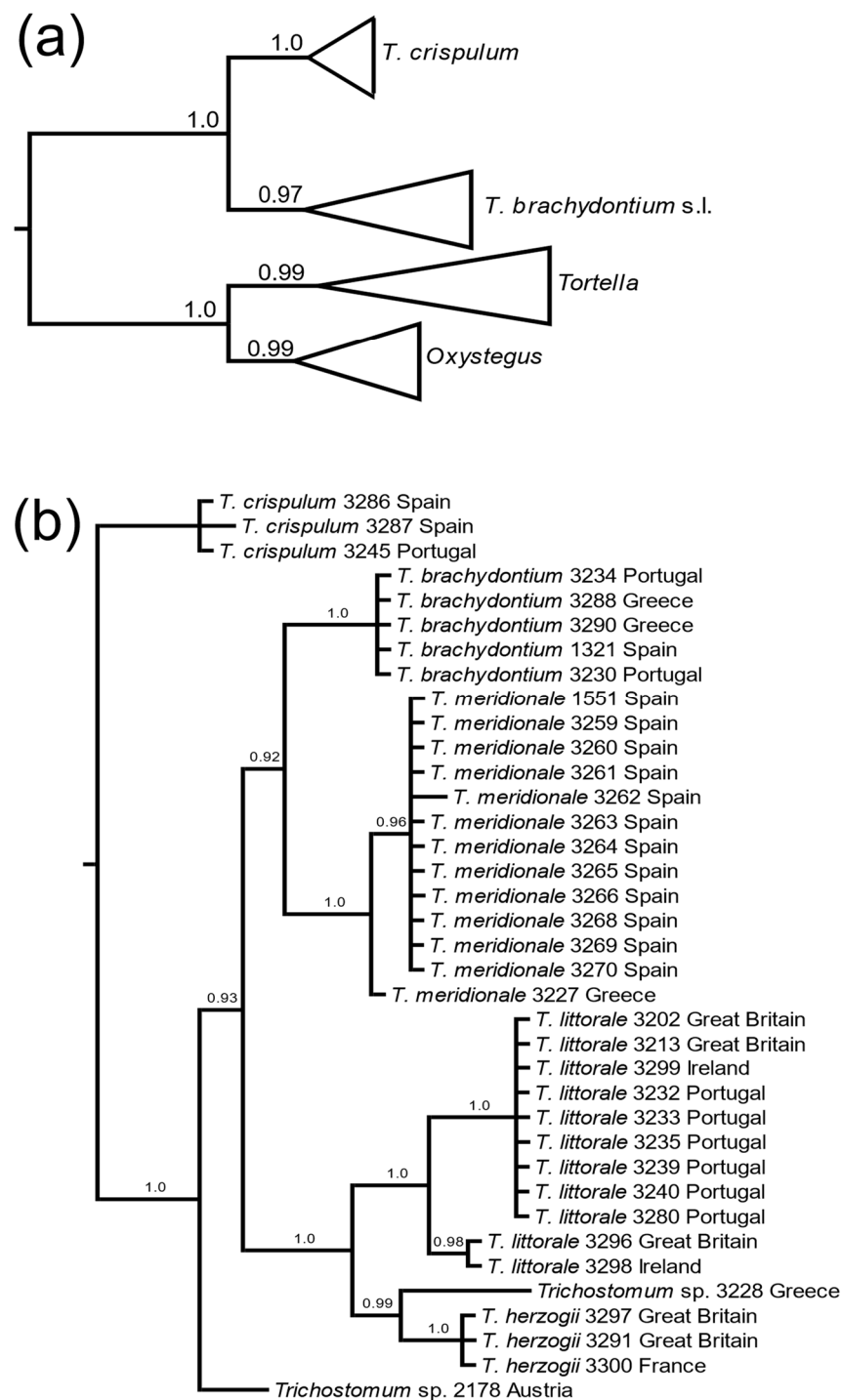


Figure 1. Phylogenetic relationships of *Trichostomum* based on ITS sequences. (a) Schematic overview of the phylogenetic relationships including outgroup. A group of species of the genera *Oxystegus* and *Tortella* was used as outgroup. *Trichostomum brachydontium* s.l. forms a monophyletic clade sister to *T. crispulum*; (b) detailed view of the *Trichostomum* sequences reveals several well-supported clades within *T. brachydontium* s.l. which correspond to morphological descriptions (*T. brachydontium* s.s., *T. meridionale*, *T. littorale* and *T. herzogii*). Two samples within the *T. littorale* clade are slightly different from the majority of this clade.

Table 1. Mean genetic distances between and within species based on ITS sequences. The within species differences are much lower than the between species distances.

	1	2	3	4	5	6	7
1 <i>T. crispulum</i>	0.67						
2 <i>T. brachydontium</i>	12.3	0.0					
3 <i>T. littorale</i>	17.0	13.6	1.3				
4 <i>T. herzogii</i>	16.3	11.0	8.6	0.0			
5 <i>T. meridionale</i>	11.3	7.0	13.6	12.0	0.3		
6 <i>Trichostomum</i> sp. 2178 Austria	10.3	8.0	11.6	11.0	7.0	-	
7 <i>Trichostomum</i> sp. 3228 Greece	17.3	12.0	11.6	7.0	8.2	14.0	-

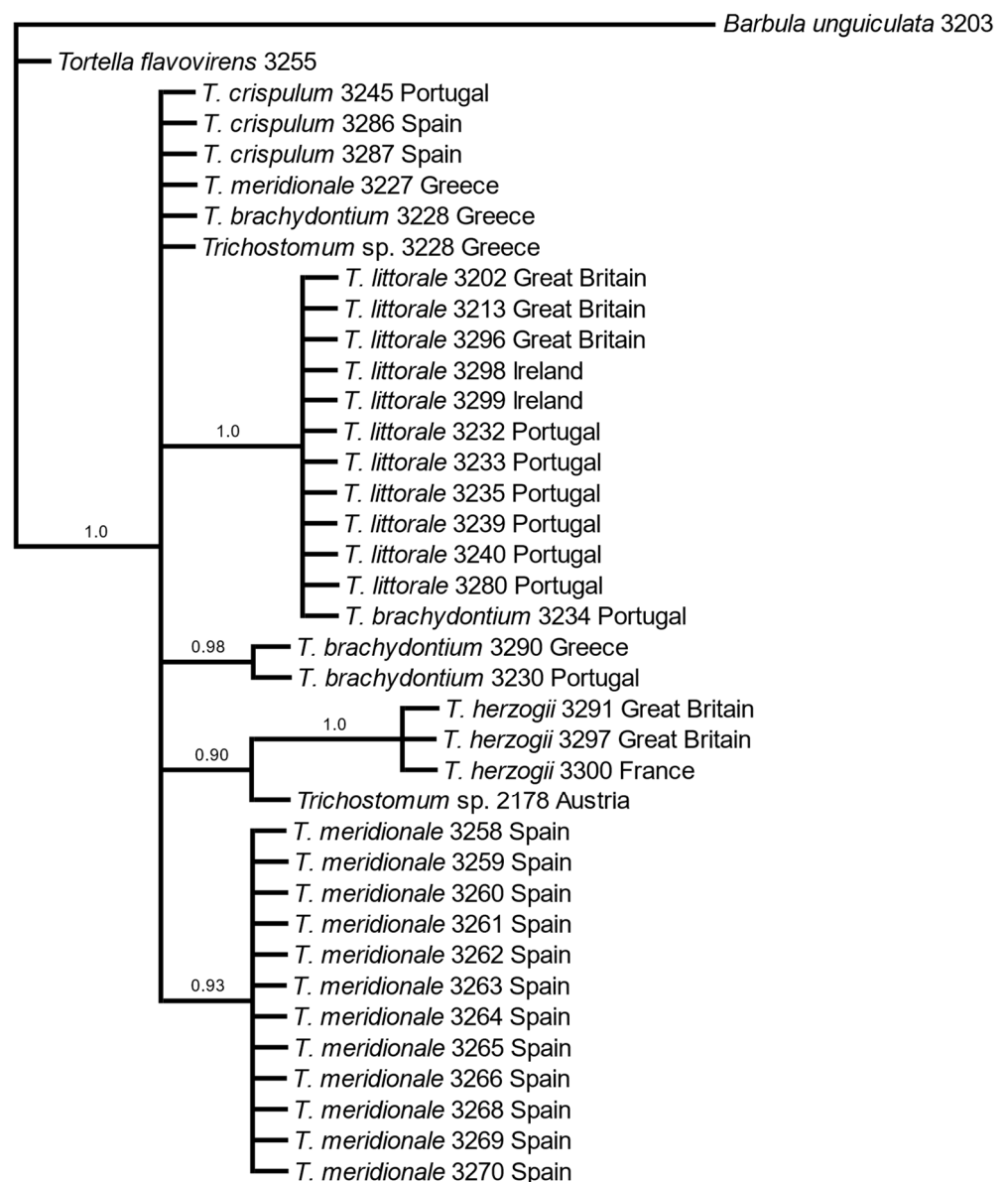


Figure 2. Phylogram based on *rbcL* sequences. Three of the *T. brachydontium* s.l samples share their *rbcL* haplotype with the *T. crispulum* samples. The four resolved clades correspond mainly with the species defined based on morphology, except sample 3234, which shows *T. brachydontium* morphology but a *T. littorale* *rbcL* haplotype. The sample from Austria (*Trichostomum* sp. 2178) is placed near *T. herzogii*.

Table 2. Mean genetic distances between and within species based on *rbcL* sequences. The within species differences are relatively high in *T. brachydontium*.

	1	2	3	4	5	6	7
1 <i>T. crispulum</i>	0.00						
2 <i>T. brachydontium</i>	0.17	1.67					
3 <i>T. littorale</i>	2.00	1.17	0.00				
4 <i>T. herzogii</i>	3.00	3.17	5.00	0.0			
5 <i>T. meridionale</i>	0.85	1.01	2.85	3.85	0.15		
6 <i>Trichostomum</i> sp. 2178 Austria	1.0	1.17	3.00	2.00	1.85	-	
7 <i>Trichostomum</i> sp. 3228 Greece	0.00	0.17	2.00	3.00	0.85	1.00	-

Within the *T. brachydontium* complex, the most notable feature of *T. meridionale* is its small size, consistent across Spanish, Sardinian, and Greek specimens available for study. The non-tomentose stems are no more than 1 cm tall, and form very short and dense turfs, not cushions, and are easily detached from the substrate. The shoots are green above, grading to yellow to brown below, and superficially resemble a small *Weissia* Hedw. with incurved leaf margins. In spite of their small size, stems can be distinctly interrupted and are distinctly comose. When dry, the leaves are incurved; when moist, they are erect to erect-patent, only the comal leaves are spreading, and the leaves are small, no more than 2.30 mm long (with a low length/width ratio not exceeding 4.0). The leaf lamina is usually flat, often with incurved margins in the upper leaf, with an obtuse apex ending in a small mucro, 25–60 µm long. Leaf shape on the microscope slide is variable, but is typically lingulate, narrowly elliptical, spatulate to panduriform (constricted at the point of transition between the smooth basal laminal cells and the papillose cells above). Marginal teeth in the lower third of the leaf are lacking or weakly developed. A useful character is the strong costa, remaining strong to about mid-leaf from where it gradually narrows to the apex. Sporophytes are usually always present, the ellipsoidal to cylindrical urn is up to 1.5 mm long, and when mature is yellow-reddish to brownish, and held on a yellow seta to 10 mm long. The peristome is rudimentary or absent.

Microscopically, *T. littorale* is easily separated from *T. meridionale* and other *Trichostomum* in the present study by possession of a few to several distinct teeth on the lower third margin at about the point where the smooth basal laminal cells merge into the papillose upper cells (transitional zone). The teeth are formed from an outgrowth of the transverse cell wall and may be slightly recurved or weakly bifurcate. In some specimens, there may be leaves in which the teeth are weakly developed, but normally there will be some leaves that present distinct teeth; moreover, the basal scale-like leaves are dentate from base to apex. In the field, *T. littorale* is larger than *T. meridionale*, with stems up to 3 cm, several times branched, with innovations at each point of branching, sometimes with flagelliform shoots, and distinctly comose, forming olive-green cushions or tufts. The leaves are small, reaching a maximal length of 1.90(–2.30) mm, and as in *T. meridionale*, they present a very short length/width ratio, typically 3.0–4.6, although in some samples the ratio can attain 6.6. The lamina is usually canaliculate and bistratose in small patches below, both features shared only by *T. herzogii*. Some forms are recognizable in the field due to the comal leaves spreading, sometimes with the leaf tips recurved, and the mid-lower stem leaves erect to erect-patent when moist with a slightly sheathing base, often undulate and strongly incurved when dry. Other useful characters include the leaf shape, lingulate to spatulate, occasionally panduriform, rarely lanceolate, often slightly narrowed at the base, and an obtuse and mucronate apex, occasionally unguiculate, with a short excurrent costa to 20–85 µm. Sporophytes were not seen, but only perichaetia, having the perichaetial leaves, in contrast to the vegetative leaves, acute apices.

Table 3. Comparison of morphological traits among species of *Trichostomum brachydontium* complex. Differential characters are written in bold.

Traits	<i>T. brachydontium</i>	<i>T. herzogii</i>	<i>T. littorale</i>	<i>T. meridionale</i>
Plants size	(0.7-)1.0–2.0 cm	(1.0-)2.0–3.0(-5.0) cm	(0.5-)1.5–3.0 cm	0.3–0.5(-1.0) cm
Color of plants	bright green above, brown below	yellowish green above, reddish below	olive-green above, brown below	bright green above, yellow to brown below
Life-form	tuft	tuft	tuft/cushion	dense and short turf
Stem tomentum	present	present	absent	absent
Stem ramification	distinctly interrupted	distinctly interrupted or not	distinctly interrupted	distinctly interrupted or not
Stem apex	sometimes distinctly comose	not distinctly comose	distinctly comose	distinctly comose
Flagelliform shoots	not seen	occasional	frequent	not seen
Leaves when dry	crispate	incurved or crispate	incurved or slightly crispate	incurved
Leaves when moist	patent to spreading , apical leaves with the tips recurved	erect to erect-patent, apical leaves spreading	erect to erect-patent, comal leaves spreading or with the tips recurved	erect to erect-patent, comal leaves spreading
Median and upper leaf shape	narrowly lingulate to narrowly lanceolate or ensiform	linear-lanceolate	lingulate, oblong-lingulate sometimes slightly lanceolate or spatulate	lingulate to spatulate, occasionally panduriform or lanceolate
Median and upper leaves size	(1.35-)1.80–3.00(-3.80) × 0.30–0.50(-0.60) mm	(1.22-)2.00–3.45(-3.61) × (0.21-)0.35–0.50(-0.57) mm	(0.60-)1.00–1.90(-2.30) × 0.20–0.40(-0.50) mm	0.90–1.90(-2.30) × (0.20-)0.30–0.50(-0.60) mm
Length/width ratio	5.0–8.0:1	5.0–7.6(–8.9):1	3.0–4.6(–6.6):1	3.4–4.0:1
Lamina	flat to slightly canaliculate, twisted, undulate, unistratose, fragile	strongly canaliculate , not twisted, undulate or not, rarely bistratose in small patches below , not fragile	canaliculate , rarely flat, not twisted, slightly undulate, rarely bistratose in small patches below , not fragile	flat to canaliculate , not twisted, slightly undulate, unistratose, not fragile
Basal lamina color	yellowish or hyaline, contrasting with upper lamina	reddish, concolorous with upper lamina	yellowish or hyaline, contrasting with upper lamina	yellowish or hyaline, contrasting with upper lamina
Leaf apex	short-acuminate or more rarely acute	long-acuminate to subulate	obtuse, mucronate	obtuse, mucronate
Basal margin teeth	absent or weakly developed	absent or weakly developed	usually prominent	absent or weakly developed
Costa	gradually narrowing from base to apex	gradually narrowing from base to apex	gradually narrowing from base to apex	gradually narrowing from mid-leaf to apex
Length of excurrent costa	(50-)90–150 μm	70–150 μm	20–85 μm	25–60 μm
Basal lamina costa cross-section	plane-convex	biconvex	plane-convex	plane-convex
Mid-basal and paracostal basal cells	thin-walled	usually thick-walled, obscurely nodulose	sometimes thick-walled	thin-walled
Sporophyte	often present, seta (5-)10–20 mm long, urn 1.2–2.2 mm long, peristome up to 250 μm or rudimentary	not seen	not seen	often present, seta 5–10 mm long, urn 1.0–1.5 mm long, peristome absent or rudimentary up to 55 μm

Trichostomum brachydontium s.s. and *T. herzogii* are most easily differentiated from the former taxa by leaf shape, size, and apex. Both species present longer leaves, reaching about 3 mm, with length/width ratio greater than 5.0, an acuminate apex and a long excurrent costa. In *T. brachydontium* s.s., plants are medium-sized, not exceeding 2 cm tall, bright green above, brown below, with tomentose stems, clearly interrupted in ramifications and sometimes distinctly comose. Leaves are crispate when dry, erect-patent to spreading when moist, sometimes with recurved tips to the apical leaves. Other distinguishing characters of the leaves include the narrowly lingulate to narrowly lanceolate or ensiform shape, with a short-acuminate apex, rarely acute, and flat or slightly canaliculate, twisted, undulate and fragile lamina. The excurrent costa typically reaches 90–150 μm , and the basal margin is entire or obscurely denticulate causing possible confusion with *T. littorale*, but it is never as pronounced as in that species. Sporophytes are frequent and larger than in *T. meridionale*, with an ellipsoidal to cylindrical orange capsule up to 2.2 mm long on a straw-colored seta up to 20 mm long, a rudimentary or well-developed peristome ($\leq 250 \mu\text{m}$) usually present, and spores 13–20 μm diameter.

Trichostomum herzogii presents the largest plants observed in the complex, being typically 2–3 cm high, but reaching up to 5 cm, and is the only species with a reddish color below the stem apex. The stems are clearly tomentose, as in *T. brachydontium* s.s., ramified but not distinctly interrupted, nor comose, and sometimes with flagelliform shoots. The orientation of the leaves is particularly distinctive, being incurved to slightly crispate when dry, strongly crispate at the apex, whereas when moist, they are erect to erect-patent and stiff, sometimes with the apical leaves tending to spread. The leaves may also be longer than in *T. brachydontium* s.s. reaching up to 3.61 mm long, with a length/width ratio up to 7.6(–8.9). In accordance with its linear-lanceolate shape, the apex is sharply acuminate to subulate and the excurrent costa is approximately as long as in *T. brachydontium* s.s., reaching 70–140 μm , and the basal margin is entire or obscurely denticulate. Distinguishing characters of the species include the clearly canaliculate lamina, the leaf base concolorous with the upper lamina, the bistratose lower lamina in small patches, the biconvex costa in cross-section in the lower leaf, and the usually thick-walled, obscurely nodulose mid-basal and paracostal cells. Sporophytes were not observed.

4. Discussion

Morphologically, *T. brachydontium* s.l. presents a very variable complex regarding most of its character states, an issue that has been an impediment to providing a satisfactory circumscription of the taxon. Leaf morphology including size, basal and laminal cell dimensions, and margin attributes, taken in isolation, provide few unambiguous quantitative characters, and furthermore character states overlap within the group. The present molecular study has however elucidated the relationships within the *T. brachydontium* complex in the Mediterranean-Atlantic region of Europe, and we have shown that morphological characters, considered in combination, allow confident recognition of the four clades that signify distinct taxa at the species level. Therefore, our results support the original hypothesis that there is more than one species within the protean taxon *T. brachydontium* and indicates the necessity of searching for their valid names.

The molecular results show that there are four well-supported clades in both chloroplast and nuclear-based trees. While the ITS-based tree is in good agreement with the morphological data, there are a few inconsistencies with reference to the *rbcL* tree. These especially affect samples assigned to *T. brachydontium* s.s., one of which has a haplotype shared with *T. crispulum* and the other shared with *T. littorale*. Gene trees and species trees are not necessarily congruent; these results can be explained by incomplete lineage sorting, by which “ancestral polymorphisms persist through several speciation events” [40]. Large effective population sizes and short phylogenetic branches (expressed in generations) favor incomplete lineage sorting [41]. Another possible explanation for incongruencies of gene trees and species trees is hybridization [40]. Alternatively, these results may be viewed as the persistence of isolated ancestral molecular races that form their own molecular lin-

eages in evolutionary stasis [42] and have their own phylogenetically distinct descendant morphospecies. At this point, we cannot decide which of the mechanisms may explain the inconsistencies of the *rbcL* tree with the ITS tree and the morphological data, but in any case, the ITS tree is in good accordance with the morphological data and only a minor fraction of the samples shows these problems.

Regarding the ITS data, the within species variation is clearly lower than the between species variation. In two of the species (*T. brachydontium* and *T. herzogii*), the within species variation of the ITS sequences is even 0 and in the case of *T. littorale*, the two subclades are morphologically slightly different and the within subclade variability is 0. Consequently, it is plausible to propose a different taxonomic status for the two subclades. Herzog [6] also detected intermediate forms between *T. mutabile* and *T. littorale* ideal types, that he named *littorale* > *mutabile* and *littorale* < *mutabile* depending on the greater proximity to one or another type. The subclade of *T. littorale* with samples coded 3296 from Great Britain and 3298 from Ireland can be assigned to these intermediate forms, both having narrowly lingulate to lanceolate leaves up to 2.3 mm long, an undulate and fragile lamina and an acuminate to acute leaf apex as in *T. brachydontium* s.s. The presence in sample 3296 of teeth moderately developed in the basal leaf margin and upper leaves patent position it closer to *T. littorale*, whereas the teeth weakly developed or absent and the upper leaves spreading in sample 3298 place it closer to *T. brachydontium* s.s. Therefore, the morphology of both these intermediate specimens raises the question of whether they are deserving of taxonomic rank. Furthermore, during our study, we observed other ostensibly intermediate forms, an issue Herzog was all too conscious of. These forms were not amenable to sequencing so we were unable to assign them to any particular clade or taxonomic rank, and the possibility of hybridization remains. That is the case of a Sardinian specimen in CAG herbarium, having very small plants as in *T. meridionale* but with basal margin clearly dentate as in *T. littorale*. A particular issue is regarding material named *T. contortum* from Madeira [11]. We examined four specimens (LISU 149232, 162400, 162405, and 162406) and although we concluded that they most likely represented *T. brachydontium* s.s., collection 162406 exhibited characters reminiscent of *T. herzogii*, particularly in the long linear-lanceolate, canaliculate erect leaves with an acuminate apex, but the basal areolation and lack of any red coloration suggests *T. brachydontium*. Unfortunately, the material is too old to be sequenced.

Two isolated samples, one from Austria and the other from Greece, could not be matched with any of the four species recognized in the present study. The Austrian sample is morphologically similar to *T. herzogii* and comparison with *T. herzogii* types and the sequenced specimens reveals that the Austrian plant has shorter and less canaliculate leaves. However, being a single sample means it is not possible to draw any taxonomic conclusion. The Greek sample, though lacking sporophytes, shows a close gametophytic similarity to *T. meridionale*. At this point, it is not possible to assess which processes are causing this incongruence between genetic and morphological data or indeed if these two specimens potentially represent distinct species. Very likely, the taxonomy of the *T. brachydontium* complex is much more complicated than we have been able to detect in the present study, as the data certainly suggest by the discovery of different genotypes in non-European specimens (unpubl.).

Based on the data presented in this work, the reports of *T. brachydontium* and its synonyms in Mediterranean-Atlantic Europe should be re-evaluated, allowing a more accurate picture of the present distribution of the four species recognized here. The records of var. *densum* and var. *cuspidatum* can be fairly confidently related to *T. meridionale* and *T. herzogii*, respectively. Of particular concern is the need for a critical revision of the reports of var. *cophocarpum* in Britain, due to the confusion with var. *cuspidatum*. Herzog [6] (pp. 475, 476) was already aware that both Braithwaite and Dixon made erroneous statements regarding var. *cophocarpum*. It is clear from the description of var. *cophocarpum* in Braithwaite [15] and in Dixon [17] that the morphology corresponds to var. *cuspidatum*. This error has been perpetuated in the British floras, where besides

var. *littorale*, only var. *cophocarpum* is said to be present, even though Herzog [6] and Mönkemeyer [43] both reported the occurrence of var. *cuspidatum* in England.

Some members of the *T. brachydontium* complex can be confused with *Weissia*. However, the latter genus is autoecious and in a moist state, the leaf margins are more strongly incurved or involute above than typically seen in *Trichostomum*. Some morphs of *Tortella nitida* (Bruch) Broth. may also be mistaken for *Trichostomum*, as this species is genetically and morphologically very variable [26,44]. In some specimens of *Tortella nitida*, little indication is given of belonging to this genus because the characteristic hyaline V-shape basal area is not clearly delimited, but it can usually be recognized by two to several rows of elongate, thin-walled, smooth hyaline cells ascending the leaf margins [44], albeit sometimes indistinct. Such a cell pattern can also be seen in the *T. brachydontium* complex, although it is usually not so distinctive. Husnot [45] states in his key *T. brachydontium* (sub *T. mutabile*) peristome teeth are smooth and in *T. nitida* papillose, but in JE 04008874 syntype of *T. brachydontium* s.s., as well as in other specimens studied in this work, we have observed papillose peristome teeth, and also in *T. meridionale*. Herzog [6] was especially concerned about the distinction between *T. brachydontium* s.s. and *T. nitida* and concluded that the position of the papillae was critical (however, we were unable to corroborate his interpretation regarding position of papillae and suggest that it is not a satisfactory character to differentiate between them). Schimper [46] maintained that *T. nitida* differs from *T. brachydontium* in the more robust stature, with longer and slightly wider leaves, more arched-involute when dry, with a thicker costa that presents a smooth shiny dorsal surface (hence the name), and a basal areolation that is less thin and shorter. Eckel [44] also compared both taxa, but as it is possible that *T. brachydontium* s.s. was not used for the comparison, the results are difficult to apply. In our opinion, *T. nitida* presents a more extensive zone of smooth hyaline, rectangular, thick-walled cells extending further up the leaf than in *T. brachydontium* s.s., and older leaves are often fragile and broken. When dry, the upper leaves of the *T. nitida* complex are oriented in a characteristic way, either regularly incurved and facing towards the stem axis or, in some forms, the leaves are very strongly crisped to circinate. The presence of mature sporophytes, however, allows a clear separation, *T. nitida* having capsules erect to somewhat inclined, elongate peristome teeth ca. 0.5 mm long, and spores 9–10 µm in diameter [44].

5. Taxonomic Treatment

In the present study, by combining molecular and morphological studies, we confirm that *T. brachydontium* includes at least four clearly distinct taxa. Based on the present work, we consider species rank to be the most suitable option.

5.1. The List of Taxa

An account of the four species within *T. brachydontium* s.l. accepted in the present work is given below. Information is provided regarding types, nomenclature, taxonomy and etymology, morphological descriptions, diagnostic characters for infra-specific taxa, pertinent observations on morphology and distribution and habitat.

(1) *Trichostomum brachydontium* Bruch in F.A. Müll., Flora 12: 393, pl. 3. 1829 (Figures 3 and 4). Type citation: “An einer feuchten Erdwand bei Spezzia und auf Hügeln unter Gebüsch bei Cagliari. März und April”. Lectotype (designated here): (Italy, Sardinia) *Trichostomum brachydontium* Bruch, In umbrosis ad terram prope Cagliari. Febr. U. I. Müller, JE 04008873!; Isolectotype: *Trichostomum brachydontium* Bruch, In umbrosis ad terram prope Cagliari. Febr. U. I. Müller, PC 0703925. Syntypes: (Italy, Sardinia) *Trichostomum brachydontium* Br, In umbrosis ad terram pr. Cagliari, Febr. F. Müller, JE 04008874!; (Italy) *Trichostomum mutabile* Br. Eur., Golfo di Spezzia, Muller 1828, BM000666185.

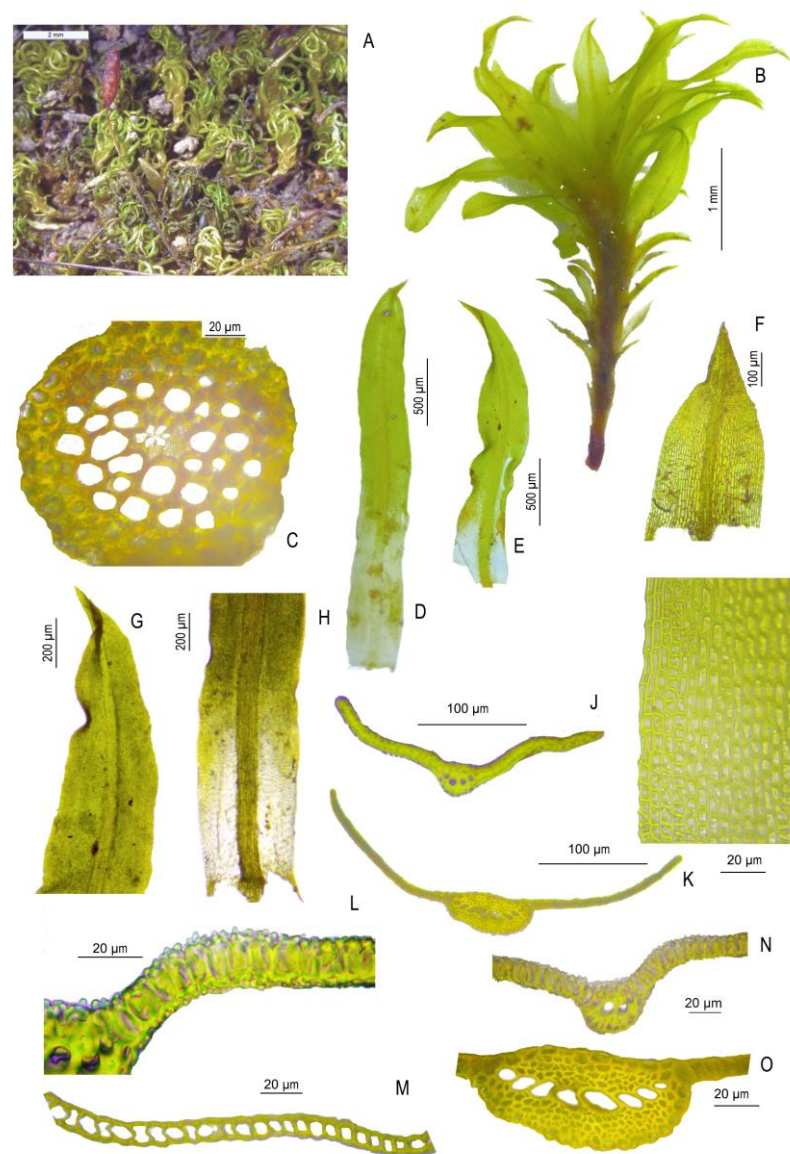


Figure 3. Macro- and microphotographs of gametophyte of *Trichostomum brachydontium*. (A) habit of fertile plants, (B) comose innovation, (C) stem cross-section, (D,E) apical and median leaves, (F) scale-like basal leaf, (G) upper part of leaf showing acuminate apex, (H) hyaline basal part of leaf not expanded, contrasting with upper part, (I) margin near base of lamina with weakly developed teeth, (J) leaf cross-section in upper lamina, (K) leaf cross-section in basal lamina, (L) upper lamina cross-section, (M) basal lamina cross-section, (N) detail of costa in upper lamina cross-section, (O) detail of costa in basal lamina cross-section. Photos (A,B,D–G,I) from sample coded 3230 (MUB 60458), (C,H,J–O) from sample coded 1321 (MUB 12650).

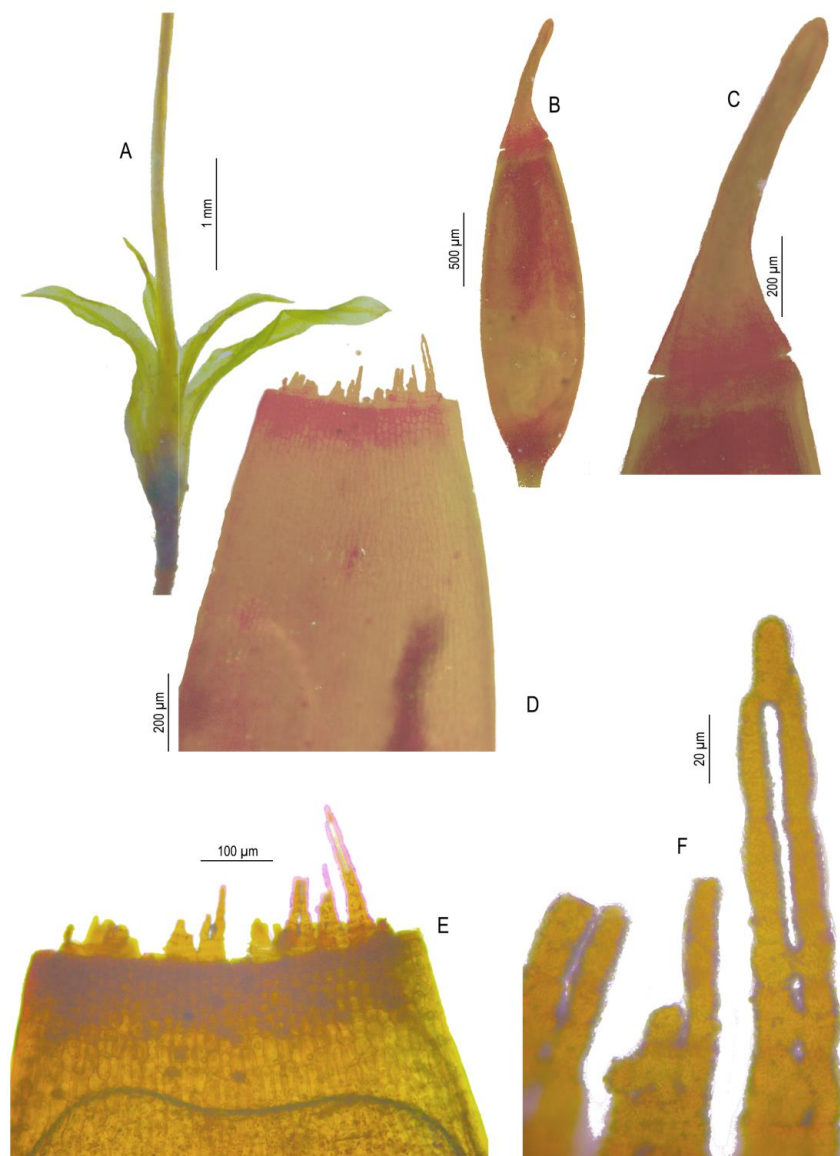


Figure 4. Macro- and microphotographs of perichaetium and sporophyte of *Trichostomum brachydontium*. (A) perichaetium, (B) capsule, (C) operculum, (D) upper part of capsule showing peristome, (E) mouth of capsule showing annulus and peristome, (F) detail view of peristome teeth. All photos from sample coded 3230 (MUB 60458).

Other names for this basionym: *Didymodon brachydontius* (Bruch in F.A. Müll.) Wilson, Brit. Fl. (ed. 4) 2: 30. 1833 \equiv *Tortula brachydontia* (Bruch in F.A. Müll.) Mitt., J. Linn. Soc., Bot. 12: 148. 1869 \equiv *Mollia brachydontia* (Bruch in F.A. Müll.) Lindb., Musci Scand. 21. 1879 \equiv *Tortella brachydontia* (Bruch in F.A. Müll.) C.E.O. Jensen, Danmarks Mosser 2: 320. 1923.

Synonyms:

Trichostomum mutabile Bruch ex De Not., illegitimate, superfluous, Syllab. Musc. 192. 1838 \equiv *Trichostomum brachydontium* subsp. *mutabile* (Bruch ex De Not.) Giacom. illegitimate, type of species included, Ist. Bot. Reale Univ. Reale Lab. Crittog. Pavia, Atti 4: 204. 1947.

Trichostomum mutabile var. *cophocarpum* Schimp., Syn. Musc. Eur. (ed. 2) 171. 1876. Type citation: "In Algarvia (H. Solms)". Lectotype (designated by Herzog [6]): (Portugal) Algarve, San Marcos da Serra inter cisteta c. frct!, leg. Solms, 66 (as *T. cophocarpum* Solms), GOET. Isotype: 237 *cophocarpum* Original JE04008872! \equiv *Mollia brachydontia*

var. *cophocarpa* (Schimp.) Braithw., Brit. Moss Fl. 1: 246. 1887 \equiv *Trichostomum brachydontium* var. *cophocarpum* (Schimp.) Cout., Musci Lusit. 36. 1917.

Hymenostomum unguiculatum H. Philib. in Schimp., Syn. Musc. Eur. (ed. 2) 37. 1876. Type citation: “in rupium calcarearum fissuris et in terra lapidosa prope Aix Gallo-Provinciae ubi clar. Prof. PHILIBERT anno 1871 detexit et ipse, eo duce, anno 1872 legi; provenit sociis *Weisia viridula*, *Trichostomo crispulo*, *Bryo torquescens* etc. Fruct. matur. Aprili”. Isotype: *Trichostomum gymnostomum* sp. nov., Aix en Provence, vere 1872, HA.H3400240!; topotype: *Hymenostomum unguiculatum* Philb., in rupibus calcareis umbrosis prope Aix, 29 mai 1869, HA.H3400239! \equiv *Trichostomum mutabile* var. *unguiculatum* (H. Philib. in Schimp.) Husn., Muscol. Gall. 87. 1885 \equiv *Trichostomum brachydontium* var. *unguiculatum* (H. Philib. in Schimp.) Corb. & Jahand., Ann. Soc. Hist. Nat. Toulon 4 (Suppl.): 1–63. 1920 \equiv *Trichostomum viridulum* (unranked) *unguiculatum* (H. Philib. in Schimp.) Gams & C. Cortés, invalid, rank not clearly indicated (mutsp.), Anales Inst. Bot. Cavanilles 13: 123. 1956.

Nomenclature—Bruch mentioned two syntypes in the protologue of *Trichostomum brachydontium*, one in continental Italy, “bei Spezzia”, and the other in the Italian island of Sardinia, “bei Cagliari”. Both syntypes were collected by F.A. Müller. More specifically, the Sardinian syntype was collected for “Unio Itineraria” (U.I.), also called “Botanischer Reiseverein” in Bruch’s travels to the southeastern Alps, Istria and Sardinia [47], and to Algeria and Austro-Hungary between 1826 and 1828 [48]. Therefore, the Sardinian syntype of *T. brachydontium* is spread between many herbaria. In JE herbarium there is one exiccata of the Unio Itineraria of *T. brachydontium* from Sardinia collected by F.A. Müller, in which the number of the exiccata (229a) has been added later in pencil to the label. It was studied by the authors of this work and has been selected as the lectotype. The PC sample considered an isolectotype has the same printed label as the JE specimen but was not examined by the authors of this work. In both specimens, only February is indicated as the date of collection, not the year. According to Wörz [47], F.A. Müller traveled to Sardinia for the U.I. in 1827, and therefore that should be the year of collection. Although in the protologue Bruch indicates “März und April”, the date of the collection in the field is unknown, therefore, the non-coincidence of the months mentioned in the protologue with that indicated in the lectotype label should not be considered a reason to reject our lectotypification. In JE herbarium, there are another two specimens (JE 04008874 and JE 04005034) that could belong to the original material. In the first one, the same data as in the lectotype are handwritten and without mention of U.I., therefore it is here considered a syntype. In the JE 04005034 sample both original localities mentioned in the protologue are indicated on the label (“Sardinia in fruticetis prope Cagliari & in aggere limoso prope Golfo di Spezzia”), but as the true origin of the plants is unknown, we do not consider it a syntype.

In relation to the type of *T. b.* var. *cophocarpum*, Herzog [6] (pp. 456 and 475) considered the GOET specimen to be the original material used by Schimper to describe the taxon; therefore, though not expressly mentioned, it should be considered the lectotype. Unfortunately, the specimen in GOET seems to be lost, or at least it was not found when the loan was requested. Fortunately, in the Herzog herbarium, preserved at JE, there is a small envelope without collection data, indicating only that it is the “*cophocarpum* original” material. It contains two complete fructified plants. They were probably detached from the original ex GOET that Herzog took as a duplicate and kept in his herbarium, and therefore the material should be considered an isotype. In the protologue, Schimper [46] only mentioned some characteristics in which this variety apparently differs from typical *T. brachydontium*: the acute to acuminate lanceolate leaves and the ovate, more solid and brown, capsule on a short seta, and a mostly rudimentary peristome. In fact, the plants have similar leaves to those of *T. brachydontium* s.s., being about 1 cm tall, greenish above and brown below, with narrowly lanceolate leaves, crispate when dry and erect-patent when moist, $2.00\text{--}2.65 \times 0.33\text{--}0.55$ mm (4.7 to 6.0 times as long as wide), with a short acuminate apex but not cuspidate, costa excurrent in a mucro up to 126 μ m long and basal margin of leaves entire, and a sporophyte with an orange seta 7.8–8.5 mm long, ellipsoidal urn $1.10\text{--}1.20 \times 0.60\text{--}0.72$ mm, rudimentary peristome and spores very similar to those exemplified by *T. brachydontium* s.s., and therefore it should be considered a synonym of this

species. Braithwaite [15] proposed the combination *Mollia brachydontia* var. *cophocarpa* (Schimp.) Braithw., but the description and comparison he made with the type species (“Plants more slender, tall, bright green above, rufescent below; leaves lanceolate below, longer, acutely acuminate; caps. on a shorter pedicel, oval, brown, peristome very rudimentary”) do not correspond to the characteristics of the original material of *T. brachydontium* var. *cophocarpum*, as was highlighted by Herzog [6] (p. 475), but to *T. brachydontium* var. *cuspidatum*.

Concerning the type of *Hymenostomum unguiculatum* H. Philib. in Schimp., the study of an isotype and a topotype kept at H herbarium revealed that these specimens correspond to *T. brachydontium* s.s. A third specimen at H (HA.H3400241) coming from the same locality and collected in 1874 was identified by the authors of this work as *T. brachydontium* var. *cuspidatum*, therefore it must be rejected as original material. Other original specimens of Philibert kept at AUT herbarium could not be studied but were analyzed by means of a high resolution photograph of the herbarium plate kindly sent by the AUT curator to the authors of this work. In this photograph, the size of plants, greater than 1 cm in length and the crispate median and upper leaves when dry, led us to conclude that these collections also correspond to *T. brachydontium* s.s. Consequently, we propose that *T. brachydontium* var. *unguiculatum* is a synonym of *T. brachydontium* s.s.

Morphological Description—Plants medium, (0.7-)1.0–2.0 cm tall, bright green above, brown below, in tufts; stems tomentose, several times branched by the growth of innovations, distinctly interrupted, each innovation with smaller scale-like lower leaves becoming progressively larger up stem and sometimes distinctly comose; flagelliform shoots not seen; stem cross-section rounded, 150–300 μm wide, hyalodermis irregularly present, central strand present, sometimes poorly developed; axillary hairs hyaline, up to 10 cells long, basal cell shorter; basal leaves short-triangular to ovate, apex obtuse, sharply narrowed to acute, margins entire or slightly toothed from base to apex, costa excurrent in a short mucro; median and upper leaves crispate when dry, patent to spreading when moist, the apical with the tips recurved, narrowly lingulate to narrowly lanceolate to ensiform, sometimes narrowed at base, (1.35-)1.80–3.00(-3.80) \times 0.30–0.50(-0.60) mm (5.0 to 8.0 times as long as wide), lamina flat to slightly canaliculate, frequently twisted, with a more or less erect basal part, slightly sheathing the stem, but not or scarcely expanded, yellowish or hyaline, contrasting with upper part, usually markedly undulate above the basal part, sometimes slightly undulate, straight to curved, unistratose, fragile, breaking easily when dissecting; apex short-acuminate or more rarely acute; margins plane or incurved below, plane in the upper part, entire or slightly toothed in the third basal part, papillose-crenulate above; costa greenish, gradually narrowing from base to apex, 63–120 μm wide at base, excurrent in an arista, (50-)90–150 μm long, plane-convex in cross-section with two well developed stereid bands, up to 9 guide cells in the basal part of leaf and 3–4 in the upper part, one row of ventral cells and one row of dorsal cells, dorsal side of costa smooth or slightly papillose; laminal cells mid-basal and paracostals long rectangular and thin-walled, marginal basal cells longer and narrower, with 1–2 rows ascending the lamina and exceeding the transitional papillose cell area, upper cells quadrate to shortly rectangular, sometimes oblate, 5.0–6.5 \times 5.0–8.0 μm , opaque, with several bifurcate, blunt papillae, frequently more developed in the ventral side, marginal cells in the median and upper part not differentiated, sometimes less papillose; dioicous?; perigonia not seen; perichaetia apical; perichaetial leaves usually similar to vegetative leaves, but internal ones narrower with longer acuminate apex. Seta (5) 10–20 mm long, yellowish; capsules ellipsoidal to cylindrical, orange, 1.20–2.20 \times 0.40–0.65 mm; operculum longly rostrate, erect or inclined, 0.70–1.00 mm long; annulus persistent, formed by 3–4 rows of quadrate, thick-walled, reddish cells; peristome rudimentary to well developed, when rudimentary formed by a short smooth basal membrane, apparently absent, or by small papillose segments arising from a basal membrane, not exceeding the mouth of the urn, when well developed, formed by bifurcate, perforate, orange, papillose teeth, up to 250 μm long; spores 13–20 μm , densely and regularly warty.

Distribution and habitat—Herzog [6] remarked on the wide and fragmented distribution of this species, centred on Macaronesia and the western Mediterranean, with occurrences from the British Isles east to the Caucasus. He listed Macaronesia (Azores, Canary Islands, Madeira), Balearic Islands, Channel Islands, Corsica, France, Great Britain (England, Scotland), Italy, Portugal, Sardinia, Spain, as well as Japan, New Zealand, and Réunion. From our study, we can confirm Macaronesia (Canary Islands), Balearic Islands, Greece, Italy, Portugal, Sardinia, Spain, and also Morocco. Its true distribution elsewhere, including North, Central, and South America, Africa, Indian Ocean Islands, and Australia [2,3] is uncertain because of taxonomic issues and indeed the present study has suggested further undescribed taxa (unpubl.) exist. Based on occurrences in European countries, *T. brachydontium* s.l. appears to be preferential for warmer regions [49], though surprisingly it is not reported for Gibraltar, Malta, or Netherlands. Further east, it is apparently rare or absent. It is Red Listed for Bulgaria, Estonia, Germany, Luxembourg, and Slovenia [49]. From the present study, *T. brachydontium* s.s. appears to be tolerant of a wide range of conditions, recorded from insolated situations where it is subject to periodic desiccation such as semi-fixed dunes and exposed rocks, to humid habitats including barrancos (ravines), autochthonous woodland, and on stream banks. It is apparently indifferent to substrate, from base-rich to acid, occurring on soils, protosoils, soil-filled rock crevices, on organic material (humus) and even epiphytic (on *Rhododendron* in humid ravines), predominately at low to moderate elevations; material studied ranged from near sea-level to 670 m (Greece).

(1.1) *Trichostomum brachydontium* var. *cylindricum* (Schimp.) Cout., Musci Lusit. 36. 1917.

Basionym: *Trichostomum mutabile* var. *cylindricum* Schimp., Syn. Musc. Eur. (ed. 2) 171. 1876. Type citation: “In Algarvia (H. Solms)”. Lectotype (designated by Herzog [6]): (Portugal) Flora Lusitanica Algarve, 110 *Trichostomum mutabile* Schimper . . . Monchique, Serra da Picota, Barranco do Banho, colleg. 1866, H. CR.Z. Solms, GOET!.

Nomenclature—Herzog [6] (pp. 456 and 474) considered the GOET specimen to be the original material used by Schimper to describe the taxon, and therefore we accept it as the lectotype.

Diagnostic characters—Schimper described this taxon in the protologue as having leaves slightly different from type form in leaf shape, apex, costa, and mucro and capsule oblong-cylindrical on a long seta and peristome teeth fairly regular, scarcely articulated and slightly papillose. Study of the type specimen allowed the authors of this work to ascertain that the only reliable characters of var. *cylindricum* that separates it from other specimens examined is the long cylindrical urn of the capsule measuring 2.0×0.4 mm. The other characteristics have been observed in other specimens of *T. brachydontium* s.s., including a long seta up to 1.5 cm and the well-developed peristome teeth, formed by bifurcate, yellowish teeth, up to 160 μ m long. For the moment, we can only hypothesize that the variation in capsule size and shape may be due to environmentally induced variation, and that it should be studied further by the sequencing of other specimens with this morphology. Therefore, pending further work, we retain the taxon at varietal level.

(2) *Trichostomum herzogii* Ros, O.Werner and R.D.Porley *nom. nov.* (Figure 5).

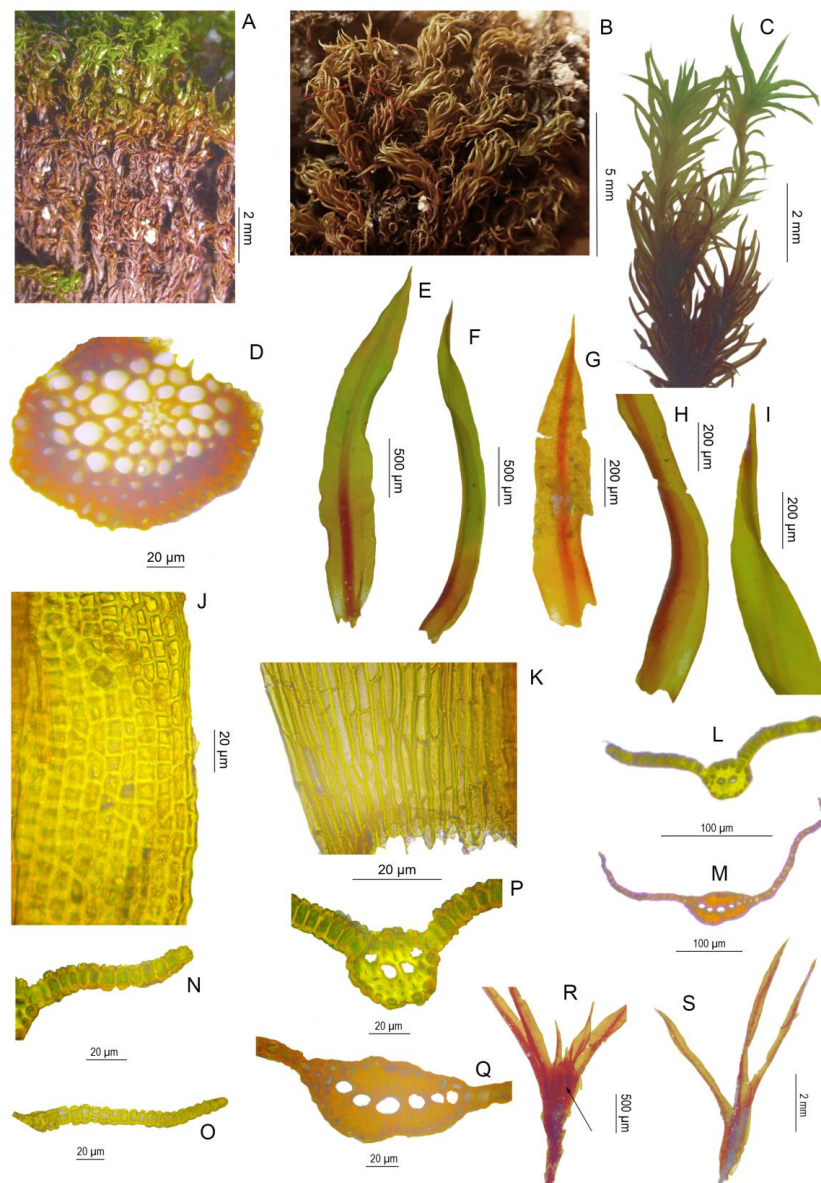


Figure 5. Macro- and microphotographs of gametophyte of *Trichostomum herzogii*. (A) plants showing yellowish green color above, reddish below, (B) tuft showing flagelliform shoots (red arrows), (C) individual plant showing non-comose stems, (D) stem cross-section, (E–G) apical and median leaves, (H) concolorous and canaliculate basal part of leaf, not contrasting with upper part, (I) upper part of leaf showing long-acuminate apex, (J) margin near base of lamina with weakly developed teeth, (K) basal hemilimb of leaf showing narrow-rectangular, thick-walled paracostal cells, (L) leaf cross-section in upper lamina, (M) leaf cross-section in basal lamina, (N) upper lamina cross-section, (O) basal lamina cross-section, (P) detail of costa in upper lamina cross-section, (Q) detail of costa in basal lamina cross-section, (R) axillary perigonium (black arrow), (S) perichaetium. Photos (A,D,H,K–Q,S) from sample coded 3291 (MUB 60473), (B) from sample *R.D. Porley 1470*, Herb. Porley, (C,E,F,I,J) from sample coded 3300 (MUB 60457), (G) from isotype JE 04008878, (R) from isotype JE 04008877.

Trichostomum cuspidatum Schimp., *nom. illeg.*, Syn. Musc. Eur. (ed. 2) 181. 1876. Type citation: “Ad rupes calcareas Hohenstein pr. Warstein Westphaliae socia *Solorina saccata* (Dr. H. Müller)”. Lectotype (designated by Herzog [6]): (Germany) *Trichostomum cuspidatum* = *T. mutabile* var. *cuspidatum* Schimper, Limpr. (Synopsis ed. II), Flora von Westfalen, auf Kalkfelsen des Hohensteins bei Warburg, *leg. et mihit 111*, steril (Collector unknown s.n.,

s.d.) (Herb. T. Herzog ex Herb. A. Geheeb) JE 04008876!. Isotypes: Westfalens Laubmoose, 205. *Trichostomum mutabile* Brch., unfruchtbar, Veräntlicher Haartmund. Am Massenkalkfels des Hohenstein bei Warstein, nur unfruchtbar und mit männlichen Blüten. Dr. H. Müller, JE 04008877!; *Trichostomum mutabile*, Warstein, HM. 267 (Herb. Hermann Winter, Gotha), JE 04008878!.

Other names for this basionym: *Mollia cuspidata* Braithw., Brit. Moss Fl. 1: 230. 1887 \equiv *Trichostomum mutabile* var. *cuspidatum* (Braithw.) Limpr., Laubm. Deutschl. 1: 50. 1888 \equiv *Trichostomum brachydontium* subsp. *cuspidatum* (Braithw.) Giacom., Ist. Bot. Reale Univ. Reale Lab. Crittog. Pavia, Atti 4: 204. 1947 \equiv *Trichostomum mutabile* subsp. *cuspidatum* (Braithw.) Podp., Consp. Musc. Eur. 196. 1954 \equiv *Trichostomum brachydontium* var. *cuspidatum* (Braithw.) L.I. Savicz, Bot. Mater. Otd. Sporov. Rast. Bot. Inst. Komarova Akad. Nauk S.S.S.R. 11: 208. 1956.

Nomenclature—Herzog [6] (pp. 477) mentioned the label data of JE 04008876 exemplar as being Schimper's original material of *T. cuspidatum*, and therefore it should be considered as the lectotype. Herzog also studied another specimen in Geheeb Herbarium (*Trichostomum mutabile* Bruch, Nur steril in Klüften der Massenkalkfelsen im Lürmekethal [Lörmecketal] u. am Hohen Stein bei Warstein im Sauerland u. bei Höxter, Dr. H. Müller ex Wenck (Herb. E. Wenck), JE 04008875!), but as three localities are given on the label (prompting Herzog to annotate in pencil on the label "Woher stammt nun dieses Expl?"), the specimen should not be considered an isotype, in spite of its morphology corresponding to *T. herzogii*.

Etymology—Because *T. cuspidatum* Schimp. is an illegitimate name and later homonym, we propose here a new name for this species in honor of the German bryologist Theodor Karl Julius Herzog (1880–1961), for his pioneering study of the *T. brachydontium* complex at the beginning of the 20th century. He correctly interpreted the morphological traits of this difficult and variable complex. Regrettably, his results were not taken seriously or simply ignored by his contemporaries and later workers, resulting in only one accepted species in modern bryological floras. However, some 100 years later, his approach has been vindicated by employing molecular methods.

Morphological description—Plants medium to large, (1.0-)2.0–3.0(-5.0) cm tall, yellowish green above, reddish below, in tufts; stems tomentose, several times branched, innovations not clearly differentiated, distinctly interrupted or not, each branch with lower leaves shorter than those above, becoming progressively larger up stem but not distinctly comose; flagelliform shoots sometimes present; stem cross-section rounded, 150–180 μ m wide, hyalodermis irregularly present, central strand present, sometimes poorly developed; axillary hairs hyaline, up to 10 cells long, basal cell shorter; basal leaves scarcely differentiated; median and upper leaves incurved or crispate when dry (depending on the length, the longer the leaves the more crispate), erect to erect-patent when moist, the apical sometimes spreading, linear-lanceolate, (1.22-)2.00–3.45(-3.61) \times (0.21-)0.35–0.50(-0.57) mm (5.0 to 7.6(-8.9) times as long as wide), lamina strongly canaliculate, not twisted, with a more or less erect basal part, sheathing the stem and expanded, concolorous with upper part, not or undulate above the basal part, straight to sigmoid, unistratose, rarely bistratose patches in the lower lamina, not fragile; apex long-acuminate to subulate; margins incurved from below to the upper part, entire or obscurely toothed in the third basal part, papillose-crenulate above; costa reddish, gradually narrowing from base to apex, 50–150 μ m wide at base, excurrent in an arista, 70–150 μ m long, biconvex in basal lamina cross-section, plano-convex in the rest of lamina, with two well-developed stereid bands, up to eight guide cells in the leaf base and two in the upper part, one row of ventral cells and one row of dorsal cells, dorsal side of costa smooth or slightly papillose; laminal cells mid-basal and paracostals rectangular, sometimes very narrow-rectangular, usually thick-walled, obscurely nodulose, marginal basal cells longer, narrower and thin-walled, with 2–3 rows ascending the lamina and exceeding the transitional papillose cell area, upper cells quadrate to shortly rectangular, sometimes oblate, 5.0–6.5(-8.0) \times 5.0–6.5(-8.0) μ m, opaque, with several bifurcate, blunt papillae, frequently more developed on the ventral

side, marginal cells in the median and upper part not differentiated, sometimes less papillose and oblate; dioicous; perigonia in branch axils; perigonial leaves similar to vegetative leaves but smaller; perichaetia in branch axils; perichaetial leaves similar to vegetative leaves, sometimes with longer acuminate apex; sporophytes not seen.

Observations—The specimens sequenced in the present work are generally larger than the types studied. In particular, the British plants from Water-Cum-Jolly Dale (DNA code 3291) attain 5 cm in length and the French plants (DNA code 3300) 3 cm, the latter specimen also having longer leaves with a higher L/W ratio, up to 8.9, whereas the German type specimens studied are 2–3 cm in length and the leaves are shorter, with a maximal length/width ratio of 7.6. The three German type specimens and a British specimen (Alberbury, *R.D. Porley 1391*) include male plants, while the British specimen (DNA code 3291) includes female plants. Fully developed sporophytes have not been observed in material studied for this work, though in a French specimen from Aix en Provence (HA.H3400241), the vestige of a seta is present. Other authors (e.g., [15]) described the sporophyte as having an oval brown capsule on a shorter pedicel, and a very rudimentary peristome, but if it corresponds to this species or *T. brachydontium* s.s. is doubtful as this author confused var. *cuspidatum* with var. *cophocarpum*.

Distribution and habitat—In contrast to the other species, Herzog [6] noted that this taxon is absent from the Mediterranean basin, occurring in the southern foothills of the Alps and in the mountains of Central Europe, extending east to the British Isles and to southern Sweden. He reports it is widespread within this area and gave Austria, France, Germany, Great Britain (England, Wales), Italy, Sweden, and Switzerland. The present study has confirmed it for France, Germany, Great Britain (England, Wales), and Italy. As this species has been confused with *T. brachydontium* var. *cophocarpum*, the distribution of *T. herzogii* is incompletely known, at least in Britain. *Trichostomum brachydontium* var. *cuspidatum* is reported for Madeira [11] with the caveat that all material needs revising, but its occurrence here is most unlikely. Our data regarding habitat agree with Herzog [6] that it is characteristic of limestone cliffs, but it also occurs on damp base-rich montane rock ledges, in gorges and on calcareous rock outcrops in upland grassland, in open or partially shaded situations. Data from the few specimens we have seen indicate *T. herzogii* occurs at moderate to high elevations, from c. 100 m to 950 m in Britain, but throughout its central European range it is likely to occur at higher altitudes.

(3) *Trichostomum littorale* Mitt., J. Bot. 6: 99. pl. 77: f. 7–9. 1868 (Figure 6).

Type citation: “Ireland (Drummond); Whitsand Bay, Cornwall (Mr. Brent, communicated by Mr. Holmes), below the cliffs, east of Hastings, in sandy ground”. Lectotype (designated here): *Trichostomum mutabile* Botsch (Wilson), Ireland, Div. 28, *Gymnostomum tortile* Schwaegr, NY 01449062!. Syntypes: (Great Britain) *Trichostomum l.*, Whitstand Bay Cornwall, Mr. F. Brent, 3.2.68, NY 01449063!; (Great Britain) *Trichostomum littorale* Mitt., in large yellow patches a little East of Fairlight Down near Hastings, Sussex, W.M., July 1847, NY 01449064!.

Other names for this basionym: *Mollia littoralis* (Mitt.) Braithw., Brit. Moss Fl. 1: 244. 35 E. 1887 \equiv *Trichostomum mutabile* var. *littorale* (Mitt.) Dixon & Jameson, Stud. Handb. Brit. Mosses 216. 1896 \equiv *Didymodon littoralis* (Mitt.) Kindb., Eur. N. Amer. Bryin. 2: 275. 1897 \equiv *Tortella brachydontia* var. *littoralis* (Mitt.) C.E.O. Jensen, Danmarks Mosser 2: 321. 1923 \equiv *Trichostomum brachydontium* var. *littorale* (Mitt.) C.E.O. Jensen, Förteckn. Skand. Växt., Moss. (ed. 2). Mossor (Andra Upplagan) 25. 1937 \equiv *Trichostomum mutabile* subsp. *littorale* (Mitt.) Podp., Consp. Musc. Eur. 195. 1954.

Nomenclature—Mitten [50] mentioned three syntypes in the protologue of *T. littorale*, one from Ireland and two from the United Kingdom. The three syntypes are kept in the Mitten herbarium at NY. The syntype from Ireland (NY 01449062) is the more substantial collection and contains the largest plants, up to 3 cm high. The British syntype from Cornwall (NY 01449063) is sparse and contains smaller plants, about 1 cm high. The other British syntype, from East Sussex (NY 01449064), is also ample and of a similar size to the former. Mitten [50] (plate 77) illustrated both plant sizes in the original publication of the

species. The syntype from Ireland, although containing no information regarding collector or date, has been selected as the lectotype due to the better development of the plants. Mitten did not mention in the protologue the denticulate margins a short distance above the leaf base, possibly because the British syntypes are rather poorly developed. Nevertheless, it was later correctly highlighted in later works such as Husnot [45], Dixon & Jameson [16] and Herzog [6].

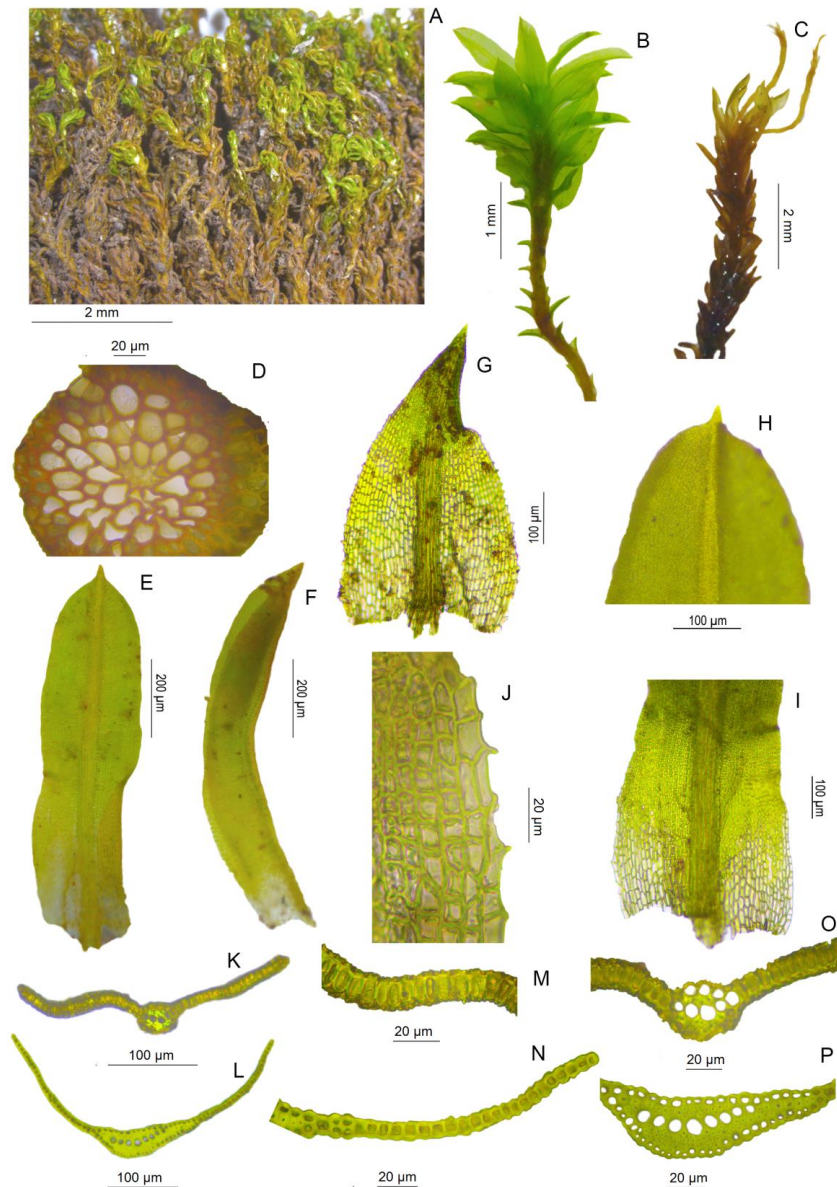


Figure 6. Macro- and microphotographs of gametophyte of *Trichostomum littorale*. (A) Plants showing bright green color above, brown below, (B) comose innovation, (C) flagelliform shoots arising from apical branch, (D) stem cross-section, (E,F) apical and median leaves, (G) scale-like basal leaf with dentate margins from base to apex, (H) upper part of leaf showing obtuse mucronate apex, (I) hyaline basal part of leaf scarcely expanded, contrasting with upper part, (J) margin near base of lamina with strongly irregular teeth, (K) leaf cross-section in upper lamina, (L) leaf cross-section in basal lamina, (M) upper lamina cross-section, (N) basal lamina cross-section, (O) detail of costa in upper lamina cross-section, (P) detail of costa in basal lamina cross-section. Photos (A,E,F) from sample coded 3233 (MUB 60478), (B,G,I) from sample coded 3299 (MUB 60485), C from sample coded 3202 (MUB 60475), (D,K–P) from sample coded 3240 (MUB 60481), (H) from sample coded 3235 (MUB 60479), (J) from lectotype NY 01449062.

Morphological description—Plants medium to large, (0.5-)1.5–3.0 cm tall, bright green above, brown below, in cushions or tufts; stems not tomentose, several times branched by the growth of innovations, distinctly interrupted and comose, each innovation with smaller scale-like lower leaves becoming progressively larger up stem, apical leaves crowded, forming a comal tuft; flagelliform shoots sometimes present, up to fascicles of 5, 2–3 mm long; stem cross-section rounded, 160–180 μm wide, hyalodermis irregularly present, central strand present, sometimes poorly developed; axillary hairs hyaline, up to 10 cells long, basal cell shorter; basal leaves short-triangular to ovate, apex obtuse, margins dentate from base to apex, costa usually not excurrent; median and upper leaves incurved or slightly crispate when dry, erect to erect-patent when moist, comal leaves spreading or with the tips recurved, lingulate or oblong-lingulate, sometimes slightly lanceolate or spatulate, slightly narrowed at base, sometimes constricted at transitional zone, (0.60-)1.00–1.90(-2.30) \times 0.20–0.40(-0.50) mm (3.0 to 4.6(-6.6) times as long as wide), lamina canaliculate, not twisted, with a more or less erect basal part, slightly sheathing the stem, but not or scarcely expanded, yellowish or hyaline in an oval zone of basal part, sharply differentiated from other basal cells and contrasting with the upper part, slightly undulate above the basal part, curved to sigmoid, unistratose, rarely bistratose patches in the lower lamina, not fragile, does not break easily when dissecting; apex obtuse, mucronate, sometimes weakly cucullate; margins plane or incurved below, incurved in the upper part, usually strongly irregularly toothed in the third basal part, teeth sometimes recurved, rarely bifurcate, sometimes scarcely developed, papillose-crenulate above; costa mostly greenish, rarely red, gradually narrowing from base to apex, (40-)50–90(-125) μm wide at the base, excurrent in a mucro, 20–85 μm long, occasionally unguiculate, plane-convex in cross-section with two well developed stereid bands, up to 8 guide cells in the basal part of leaf and 3–4 in the upper part, one row of ventral cells and one row of dorsal cells, dorsal side of costa slightly to coarsely papillose, smooth at apex; laminal paracostal basal cells long rectangular, sometimes thick-walled, mid-basal cells shorter and marginal basal cells longer, narrower and thin-walled, with 1–2 rows ascending the lamina and exceeding the transitional papillose cell area, upper cells quadrate to shortly-rectangular, 5.0–8.0(-9.5) \times 5.0–8.0 μm , opaque, with several bifurcate, blunt or rarely sharp papillae, more developed on the ventral side, marginal cells in the median and upper part not differentiated, sometimes less papillose; dioicous; perigonia not seen; perichaetia in leaf axils and at stem apex; perichaetial leaves acute, inner most lanceolate with acuminate apex; sporophytes not seen.

Distribution and habitat—This species is characteristic of oceanic climates, with an Atlantic distribution, on the western seaboard of Europe [51], an observation supported by our own studies. Herzog [6] listed Belgium, Channel Islands, France, Great Britain (England, Scotland, Wales), Ireland, Italy, and Sardinia. Mönkemeyer [43] reported to be present also in southern and central Europe, Norway, and on Bornholm Island (Denmark). It is also reported for Madeira [11] and Faroes [52]. During the present study, we have seen specimens from Great Britain (England, Scotland, Wales), Ireland, Portugal, and Spain (Cádiz). Data from the present study reveal that it has a preference for sheltered niches, mostly on rock (schists, granite, syenite) in block-fields, or on soil-filled fissures on rocks and occasionally on soil, usually in shade but sometimes insolated. Typical habitats include rocky ravines, north-facing rocky mountain slopes, terrace walls, by cascades, spring-heads, and cliffs above streams, in woodlands (*Quercus* spp., *Castanea sativa*) and, in Ireland, on lake-side boulders. It is also reported from coastal rocks in Faroes, Great Britain, and Norway [52]. The material studied exhibited an elevational range from near sea-level to 815 m (Serra de Monchique, Portugal).

(4) *Trichostomum meridionale* Ros, O. Werner, R.D. Porley *sp. nov.* (Figures 7 and 8).

Holotype—SPAIN. Murcia Region, Murcia, El Valle Perdido, proximidades a Santuario de la Fuensanta, 37°56'7.0" N, 1°7'11.9" W, 143 m a.s.l., R.M. Ros & O. Werner *s.n.*, 14.03.2020, DNA code 3268 (MUB 60295). Isotypes: (MA, Herb. Porley).

Diagnosis—*Trichostomum meridionale* can be distinguished by plants 0.3–0.5(–1.0) cm tall, in dense and short turfs; stems comose; leaves incurved when dry and erect to erect-patent, or comal leaves spreading when moist, lingulate to spatulate, occasionally panduriform or lanceolate, 0.90–1.90(–2.30) × (0.20–)0.30–0.50(–0.60) mm (3.4 to 4.0 times as long as wide); lamina flat to canaliculate, slightly undulate above the basal part, straight to curved, unistratose, not fragile; apex obtuse and mucronate; margins plane or incurved below, more incurved in the upper part, entire or slightly denticulate in the third basal part; costa gradually narrowing from mid-leaf to apex, excurrent in a mucro 25–60 µm long; mid-basal and paracostal cells long rectangular and thin-walled, marginal basal cells longer and narrower; upper cells quadrate to shortly rectangular, 5.0–8.0 × 5.0–8.0 µm, opaque, with several bifurcate blunt papillae, more developed in the ventral side; dioicous; sporophyte usually present, with seta 5–10 mm long, urn ellipsoidal to cylindrical, yellow-red, 1.00–1.50 × 0.35–0.60 mm; peristome absent or rudimentary, formed by a short membrane or by small papillose segments arising from basal membrane, not exceeding the mouth of the urn, with bifurcate, perforate, hyaline to pale orange, papillose teeth, up to 55 µm long; spores 14–20 µm, densely and irregularly warty.

Paratypes—GREECE. Eastern Aegean Islands, Rhodes, ca 1.2 km SE of Moni Ar-tamiti, ca. 240 m, T.L. Blockeel 47/027, 08.03.2018, DNA code 3227 (Herb. Blockeel, duplicate MUB 60472).

SPAIN. Murcia Region: Cartagena, Sierra Minera de Cartagena-La Unión, Rambla del Gorguel, 37°35′40.1″ N, 0°53′3.8″ W, 04.03.2020, 131 m. a.s.l., R.M. Ros, O. Werner & P. Egea Benavente s.n., DNA code 3259 (MUB 60287, Herb. Porley). Cartagena, Atamaría, camino que sube al monte de las Cenizas, en la carretera RM-514, 37°35′37.58″ N, 0°49′21.94″ W, 04.03.2020, 151 m. a.s.l., R.M. Ros, O. Werner & P. Egea Benavente s.n., DNA code 3261 (MUB 60289, Herb. Porley). *Ibidem*, DNA code 3260 (MUB 60288, Herb. Porley). Cartagena, Calarreona, 37°37′7.6″ N, 0°42′50″ W, 04.03.2020, 0 m. a.s.l., R.M. Ros, O. Werner & P. Egea Benavente s.n., DNA code 3262 (MUB 60290, Herb. Porley); *Ibidem*, DNA code 3263 (MUB 60291, Herb. Porley); Murcia, Rambla del Puerto de La Cadena, 37°54′31.46″ N, 1°9′43.75″ W, 260 m a.s.l., R.M. Ros & M. Farag s.n., 26.03.2019, DNA code 3266 (MUB 60292, Herb. Porley); Murcia, El Valle Perdido, proximidades a Santuario de la Fuensanta, 37°56′7.0″ N, 1°7′11.9″ W, 143 m a.s.l., R.M. Ros & O. Wener s.n., 14.03.2020, DNA code 3269 (MUB 60294, Herb. Porley); Las Torres de Cotillas, urbanización Los Romeros, calle I, parcela en la zona más alta, 38°00′35.76″ N, 1°14′36.77″ W, 144 m a.s.l., R.M. Ros s.n., 07.03.2020, DNA code 3264 (MUB 60293, Herb. Porley); *Ibidem*, 02.05.2020, DNA code 3265 (MUB 60296, Herb. Porley).

Synonyms—*Trichostomum mutabile* var. *densum* Bruch & Schimp., Bryol. Eur. 2: 122. pl. 174: B (fasc. 18–20. Mon. 16. pl. 5: B). 1843 ≡ *Trichostomum mutabile* subsp. *densum* (Bruch & Schimp.) J.J. Amann, Fl. Mouss. Suisse 2: 96. 1918 ≡ *Trichostomum brachydontium* subsp. *densum* (Bruch & Schimp.) Giacom., Ist. Bot. Reale Univ. Reale Lab. Crittog. Pavia, Atti 4: 204. 1947 ≡ *Trichostomum brachydontium* var. *densum* (Bruch & Schimp.) Düll, Bryol. Beitr. 4: 90. 1984. Type not seen.

Nomenclature—Cortes Latorre [53] and Esteve Chueca and Cortes Latorre [54] reported *Trichostomum brachydontium* var. *unguiculatum* (H. Philib. in Schimp.) Corb. & Jahand. from the Murcian littoral mountains in the neighborhood of Cartagena (SE Spain), a taxon described in Schimper [46] from Aix en Provence (France) as *Hymenostomum unguiculatum* H. Philib. in Schimp. The latter, as explained in the nomenclature section of *T. brachydontium* s.s., is here considered a synonym of the former species.

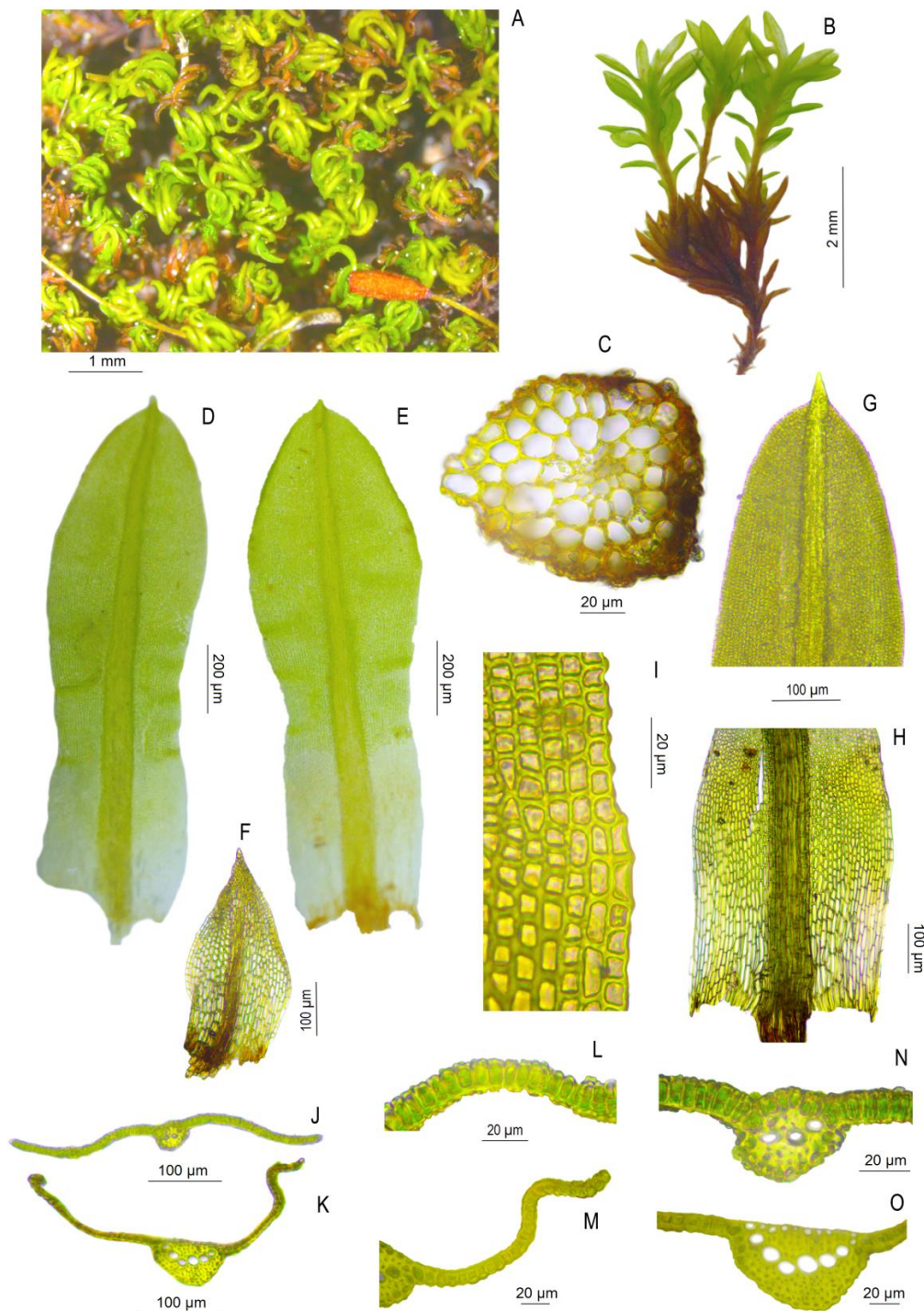


Figure 7. Macro- and microphotographs of gametophyte of *Trichostomum meridionale*. (A) Habit of fertile plants, (B) comose stem ramification, (C) stem cross-section, (D,E) apical and median leaves, (F) scale-like basal leaf, (G) upper part of leaf showing obtuse mucronate apex, (H) hyaline basal part of leaf not expanded, (I) margin near base of leaf lamina with weakly developed teeth, (J) leaf cross-section in upper lamina, (K) leaf cross-section in basal lamina, (L) upper lamina cross-section, (M) basal lamina cross-section, (N) detail of costa in upper lamina cross-section, (O) detail of costa in basal lamina cross-section. Photos (A) from sample coded 3264 (MUB 60293), (B–E,K,M,N) from sample coded 3259 (MUB 60287), (F–J,L,O) from holotype, sample coded 3268 (MUB 60295).

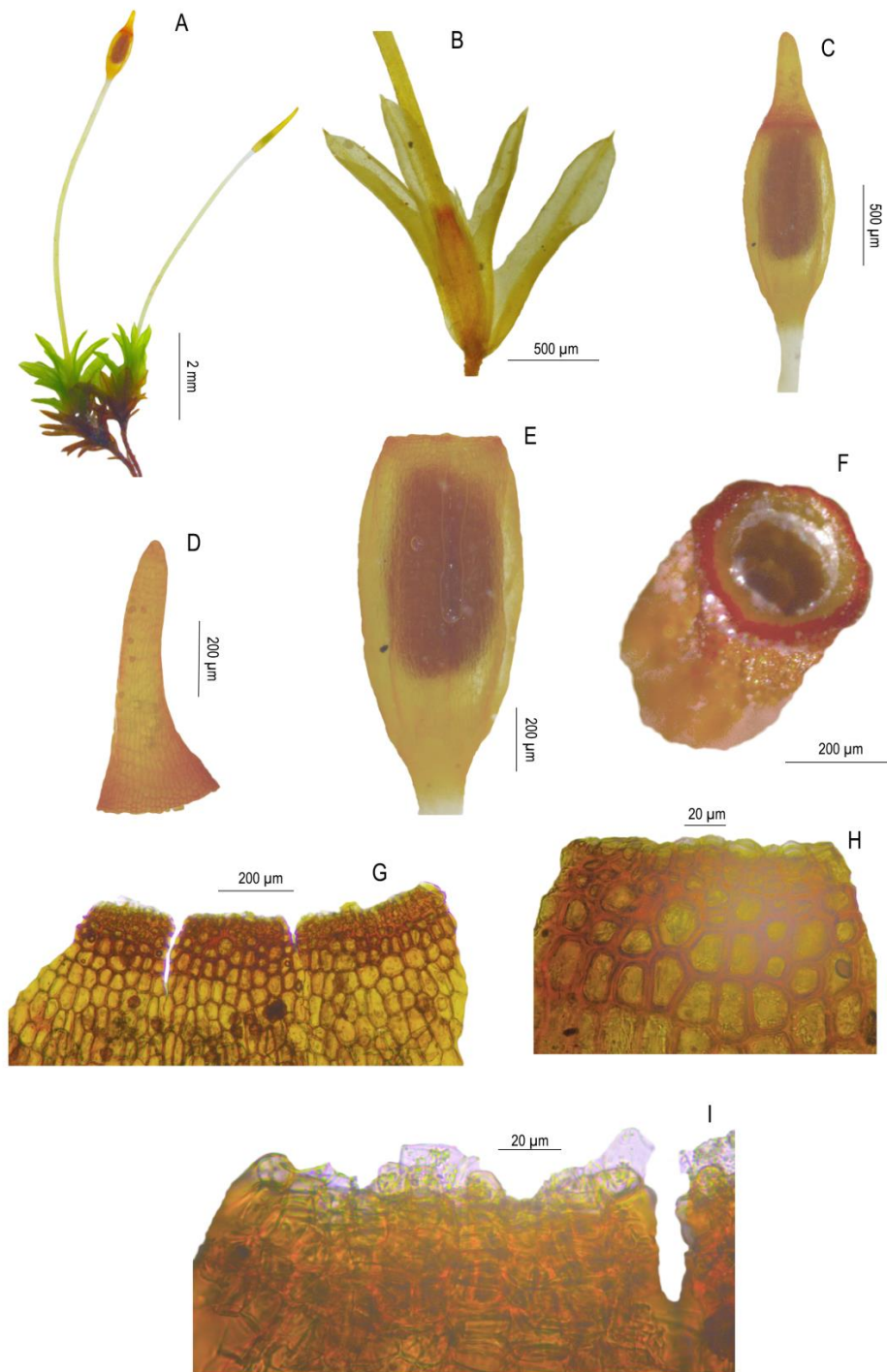


Figure 8. Macro- and microphotographs of perichaetium and sporophyte of *Trichostomum meridionale*. (A) fructified plants, (B) perichaetium, (C) capsule, (D) operculum, (E) deoperculated capsule, (F) overhead view of a capsule showing a rudimentary hyaline peristome not exceeding the mouth of the urn, (G) detail view of the mouth of the urn, (H) external detail view of urn mouth showing annulus cells, (I) internal detail view of urn mouth showing a rudimentary peristome formed by short, hyaline, papillose small segments arising from basal membrane. Photos (A,C,E,G–I) from sample coded 3259 (MUB 60287), (B) from sample MUB 5565, (D) from sample coded 3264 (MUB 60293), (F) from sample coded 3261 (MUB 60289).

Morphological description—Plants small, 0.3–0.5(-1.0) cm tall, bright green above, yellow to brown below, in dense and short turfs; stems not tomentose, not or scarcely

branched by the growth of innovations, distinctly interrupted or not (depending on the size of plants, when very short than not distinctly interrupted) and comose, each innovation with smaller lower leaves becoming progressively larger up stem, the apical leaves crowded forming a comal tuft; flagelliform shoots not seen; stem cross-section rounded, up to 140 μm wide, hyalodermis irregularly present, central strand well developed; axillary hairs hyaline, up to 10 cells long, basal cells shorter; basal leaves short-triangular, apex acute, margins entire or slightly toothed from base to apex, costa ending below apex or excurrent in a short mucro; median and upper leaves incurved when dry, erect-patent to patent, comal leaves more rarely spreading when moist, lingulate to spatulate, occasionally panduriform or lanceolate, $0.90\text{--}1.90(-2.30) \times (0.20\text{--}0.30\text{--}0.50(-0.60))$ mm (3.4 to 4.0 times as long as wide), lamina flat to canaliculate, not twisted, with basal part not or slightly sheathing the stem, not or scarcely expanded, yellowish or hyaline, contrasting with upper part, slightly undulate above the basal part, straight to curved, unistratose, not fragile, does not break easily when dissecting; apex obtuse, mucronate; margins plane or incurved below, more incurved in the upper part, entire or slightly toothed in the third basal part, papillose-crenulate above; costa mostly greenish, rarely red, gradually narrowing from mid-leaf to apex, $(65\text{--})80\text{--}100(-140)$ μm wide at base, excurrent in a mucro, 25–60 μm long, plane-convex in cross-section with two well developed stereid bands, up to 6 guide cells in the basal part and 3–4 in the upper part, one row of ventral cells and one row of dorsal cells, dorsal side of costa slightly to coarsely papillose to the apex; laminal cells mid-basal and paracostals long-rectangular and thin-walled, marginal basal cells longer and narrower, with 1–2 rows ascending the lamina and exceeding the transitional papillose cell area, upper cells quadrate to shortly rectangular, $5.0\text{--}8.0 \times 5.0\text{--}8.0$ μm , opaque, with several bifurcate blunt papillae, more developed on the ventral side, marginal cells in the median and upper part not differentiated; dioicous; perigonia subapical; perigonial leaves similar to vegetative leaves but smaller; perichaetia apical; perichaetial leaves usually similar to vegetative leaves, occasionally heteromorphic, with one leaf narrower and longer than the other, or sometimes smaller; seta 5–10 mm long, yellowish; capsule ellipsoidal to cylindrical, yellow-reddish to brownish, $1.00\text{--}1.50 \times 0.35\text{--}0.60$ mm; operculum long-conical to rostrate, erect or inclined, 0.60–0.70 mm long; annulus persistent, formed by 3–5 rows of quadrate, thick-walled, reddish cells; peristome absent to rudimentary formed by a short papillose basal membrane or by small segments arising from basal membrane, not exceeding the mouth of the urn, bifurcate, perforate, hyaline to pale orange, papillose, up to 55 μm long; spores 14–20 μm , densely and irregularly warty.

Distribution and habitat—Of the four species, *T. meridionale* clearly occupies the narrowest distribution and ecological niche. Its known range includes Macaronesia (Canary Islands) and on the shores of the Mediterranean including Corsica, Italy (Tuscany), and Sardinia [6]. *Trichostomum brachyodontium* var. *densum* is reported in southern Europe [43], and more precisely Balearic Islands and Spain [7,8]. The present study has confirmed its presence in southern Spain (Alicante, Almería, and Murcia provinces), Greece (Eastern Aegean Islands), and Sardinia. It is more or less confined to open coastal scrubland, including phrygana and garigue, on relatively alkaline and calcareous soils in the Mediterranean bioclimatic zone. The material studied shows an elevational range from sea-level to 650 m (Spain).

5.2. Key to Species

1a. Leaves lingulate, oblong-lingulate, elliptical, slightly lanceolate, spatulate or panduriform, 0.60–2.30 mm long, length/width ratio 3.0–4.6(–6.6):1, with an obtuse apex ending in a short mucro, 20–85 μm long formed by the excurrent costa

..... 2

1b. Leaves narrowly lingulate, narrowly lanceolate, ensiform or linear-lanceolate, 1.22–3.80 mm long, length/width ratio 5.0–8.0(–8.9):1, with a short- or long-acuminate, more rarely acute apex ending in an arista, $(50\text{--})70\text{--}150$ μm long formed by the excurrent

- costa 3
- 2a. Plants very small, 0.3–0.5(-1.0) cm tall; margin near base of leaf lamina (at transitional zone to papillose upper cells) entire or with weakly developed teeth; leaf lamina usually flat, unistratose; costa gradually narrowing from mid-leaf to apex; flagelliform shoots absent; sporophyte often present, seta 5–10 mm long, urn 1.0–1.5 mm long, peristome absent or rudimentary up to 55 µm long *T. meridionale*
- 2b. Plants larger, (0.5-)1.5–3.0 cm tall; margin near base of leaf lamina usually with prominent teeth; leaf lamina usually canaliculate, sometimes bistratose in small patches below; costa gradually narrowing from base to apex; flagelliform shoots frequent; sporophyte not seen *T. littorale*
- 3a. Plants (1.0-)2.0–3.0(-5.0) cm tall, yellowish green above, reddish below; stems not distinctly comose; leaves linear-lanceolate, strongly canaliculate, not twisted, undulate or not, not fragile, sometimes bistratose in small patches below, basal lamina reddish, concolorous with upper lamina; costa biconvex in cross-section in the lower leaf; mid-basal and paracostal basal cells usually thick-walled, obscurely nodulose; sporophyte rarely present *T. herzogii*.
- 3b. Plants (0.7-)1.0–2.0 cm tall, bright green above, brown below; stems distinctly comose or not; leaves narrowly lingulate to narrowly lanceolate or ensiform, flat to slightly canaliculate, twisted, undulate, fragile, unistratose, basal lamina yellowish or hyaline, contrasting with upper lamina; costa plano-convex in cross-section in the lower leaf; mid-basal and paracostal basal cells thin-walled; sporophyte often present, seta (5-)10–20 mm long, urn 1.2–2.2 mm long, peristome rudimentary or well developed up to 250 µm long *T. brachydontium*

Author Contributions: Conceptualization, R.D.P., R.M.R. and O.W.; methodology, R.M.R., O.W. and R.D.P.; field research, R.D.P., R.M.R. and O.W.; molecular analysis, O.W.; morphological study, R.D.P. and R.M.R., writing—original draft preparation, R.M.R., R.D.P. and O.W.; writing—review and editing, R.D.P., R.M.R. and O.W.; funding acquisition, R.M.R. and O.W. All authors have read and agreed to the published version of the manuscript.

Funding: Field sampling in SE Spain was funded by Fundación Séneca—Agencia de Ciencia y Tecnología de la Región de Murcia, grant number 20785/PI/18.

Data Availability Statement: DNA sequences are available on the GenBank database and all authors agree with MDPI Research Data Policies.

Acknowledgments: We are especially grateful to T.L. Blockeel, N.G. Hodgetts, D. Callaghan, H. Köckinger, M. Aleffi, V. Hugonnot, C. Sérgio, and A. Cogoni for undertaking field collecting and providing material for the study. We thank J. Muñoz for his invaluable help in relation to nomenclature and study of types and providing literature and V. Mazimpaka for his help with botanical Latin. Many thanks also to S. Fernández and A. Werner for their help in preparing the photographic plates. Curators of the following herbaria gave their time in searching for types in AUT, BM, BR, DR G, LY, and LYJB, and we wish to acknowledge GLM, GOET, H, JE, and NY for the loan of type specimens. We are also grateful to R. Zander, to an anonymous reviewer, as well as M. Philippe and J. Muñoz, as they have contributed to improving a previous version of the manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

Sampled taxa with voucher information and GenBank accession numbers for both or any of the sequenced markers. If sequence is already published, literature reference is given. Non-sequenced specimens are also indicated.

Code (Haplotype)	Locality (Voucher)	GenBank Accession Numbers ITS/rbcL
<i>Trichostomum brachydontium</i> s.s.		
1321	SPAIN, Andalusia, Cádiz, Los Barrios, Sierra de Ojén, Canuto del Cebrillo, 28.12.1999, J. Guerra & R.M. Ros s.n., MUB 12650	AY796252 [22]/-
3230	PORTUGAL, Algarve, Serra de Monchique, Vale de Cova da Serra, 17.05.2020, R.D. Porley 01, Herb. Porley, duplicate MUB 60458.	OM234698/OM260133
3234	PORTUGAL, Algarve, Serra de Monchique, Barranco Parral North, 06.04.2014, R.D. Porley 05, Herb. Porley, duplicate MUB 60459	OM234695/OM260134
3288	GREECE, Cyclades, Andros, Frousei, near Palestou, ca. 240 m, 19.03.2016, T.L. Blockeel 45/085, Herb. Blockeel, duplicate MUB 60460	OM234696/OM260097
3290	GREECE, Cyclades, Andros, above Arnim near Vourkoti, ca. 670 m, 19.03.2016, T.L. Blockeel 45/129, Herb. Blockeel, duplicate MUB 60461.	OM234697/OM260099
-	ITALY, Sardinia, prope Cagliari, Feb. 1827, Müller, JE 04008873 (Lectotype of <i>T. brachydontium</i>).	-
-	ITALY, Sardinia, prope Cagliari, Feb. 1827, Müller, JE 04008874 (Syntype of <i>T. brachydontium</i>).	-
-	ITALY, Toscana, Firenze, Giardino di Boboli, 29.03.1988, C. Cortini s.n., CAME	-
-	ITALY, Toscana, Alpi Apuane, lungo il torrente Renara fra Guadine e Casania (Massa), 20.11.1988, C. Cortini & M. Aleffi s.n., CAME	-
-	ITALY, Toscana, Alpi Apuane, Desiate (Riomagno), 19.11.1988, C. Cortini & M. Aleffi s.n., CAME	-
-	ITALY, Sardinia, Chia, Domus de Maria, 07.04.2006, A. Cogoni s.n., CAG	-
-	ITALY, Sardinia, Rio Santa Lucia, Assemini, 01.10.2020, A. Cogoni s.n., CAG	-
-	ITALY, Sardinia, Is Pauceris, Assemini, 25.05.2021, A. Cogoni s.n., CAG (var. <i>cylindricum</i>)	-
-	MOROCCO, Bab Taza, ascensión al Jbel Bouhalla, El Maounzil, 35°04'45'' N, 5°10'05'' W, 1100 m, 17.03.1997, M.J. Cano, M.T. Gallego & R.M. Ros s.n. MUB 10961	-
-	PORTUGAL, Algarve, Serra de Monchique, Ribeira de Seixoso, 07.01.2010, R.D. Porley s.n., Herb. Porley, duplicate MUB 60462	-
-	PORTUGAL, Algarve, Carrapateira, Costa Vicentina PN, 27.21.2007, R.D. Porley 14, Herb. Porley, duplicate MUB 60463	-
-	PORTUGAL, Algarve, Serra de Monchique, Ribeira do Seixoso, 07.01.2010, R.D. Porley 31, Herb. Porley, duplicate MUB 60464	-
-	PORTUGAL, Algarve, Serra de Monchique, Ribeira do Seixoso, 07.01.2010, R.D. Porley 32, Herb. Porley, duplicate MUB 60465	-
-	PORTUGAL, Algarve, Serra de Monchique, Ribeira do Seixoso, 05.02.2010, R.D. Porley 33, Herb. Porley, duplicate MUB 60466	-
-	PORTUGAL, Algarve, Serra de Monchique, Ribeira do Seixoso, 07.01.2010, R.D. Porley 34, Herb. Porley, duplicate MUB 60467	-
-	PORTUGAL, Algarve, Serra de Monchique, Ribeira do Seixoso, 05.02.2010, R.D. Porley 35, Herb. Porley, duplicate MUB 60468	-
-	PORTUGAL, Algarve, Serra de Monchique, Ribeira do Seixoso, 07.01.2010, R.D. Porley 36, Herb. Porley, duplicate MUB 60469	-
-	PORTUGAL, Algarve, Serra de Monchique, Ribeira de Boina, S. of Brejão, 22.12.2016, R.D. Porley 37, Herb. Porley, duplicate MUB 60470	-
-	PORTUGAL, Algarve, Serra de Monchique, Ribeira do Seixoso, 18.01.2010, R.D. Porley 45, Herb. Porley, duplicate MUB 60471	-
-	PORTUGAL, Algarve, Monte do Galo, near Cerca dos Pomares, 12.11.2021, R.D. Porley s.n., Herb. Porley	-
-	PORTUGAL, Madeira, S. Vicente, Folhadal, entre o 1° e 2° Tunel a partir da Encumeada, 29.02.1982, M. Pita 5, LISU1492332	-
-	PORTUGAL, Madeira, Ribeiro Frio, 12.05.1979, C. Sérgio 2365, LISU162400	-
-	PORTUGAL, Madeira, Faldas do Pico Jorge, Levada para a Ribeiro Bonito, 12.04.1988, C. Sérgio & M. Nóbrega 6132, LISU162405	-
-	PORTUGAL, Madeira, Fanal de Baixo, Riberia Funda descendo para a Ribeira, 28.06.1988, C. Sérgio & M. Nóbrega 6132, LISU162406	-

Code (Haplotype)	Locality (Voucher)	GenBank Accession Numbers ITS/rbcL
-	SPAIN, Balearic Islands, Mallorca, cruce de Carretera Sacalobra y carretera a Pollensa, cerca del chiringuito, 39°49'05" N, 2°49'05" E, 580 m, 16.04.1999, M.J. Cano, M.T. Gallego & M.C Sánchez-Moya, MUB 11128	-
-	SPAIN, Canary Islands, Tenerife, Anaga, Junto a Puente, 29.10.2003. R.M. Ros, A. Losada & J.M. González-Mancebo, MUB 16625	-
-	GREAT BRITAIN, England, N. Somerset, Walton-in-Gordano, 31.01.1989, R.D. Porley 1392, Herb. Porley	-
-	GREAT BRITAIN, Wales, Montgomeryshire, Guilsfield, 02.07.1978, M.J. Wigginton s.n., Herb. Porley	-
<i>Trichostomum herzogii</i>		
3291	GREAT BRITAIN, England, Derbyshire, Water-cum-Jolly Dale, near Cressbrook, 30.04.2021, T.L. Blockeel 50/064, Herb. Blockeel, duplicate MUB 60473	OM234712/OM260100
3297	GREAT BRITAIN, England, Miller's Dale, T.L. Blockeel 50/123, DNA 3297, Herb. Blockeel, duplicate MUB 60474	OM234711/OM260102
3300	FRANCE, Ain, Lélex, Pont du Rouffes, 01.07.2020, V. Hugonnot s.n., Herb. Hugonnot, duplicates Herb. Porley, MUB 60457	OM234726/OM260122
-	FRANCE, prope Aix en Provence, 30.06.1874, Philibert, HA.H3400241 (sub <i>Hymenostomum unguiculatum</i>)	-
-	GERMANY, auf Kalkfelsen des Hohensteins bei Warburg (collector unknown s.n., s.d.) Lectotype, JE04008876 (Lectotype of <i>Trichostomum cuspidatum</i>)	-
-	GERMANY, am Massenkalkfels des Hohenstein bei Warstein (s.d.) H. Müller, JE04008877 (Isotype of <i>Trichostomum cuspidatum</i>)	-
-	GERMANY, Warstein, H. Müller (s.d.) JE04008878 (Isotype of <i>Trichostomum cuspidatum</i>)	-
-	GERMANY, in Klüften der Massenkalkfelsen im Lürmekethal [Lörmecketal] u. am Hohen Stein bei Warstein im Sauerland u. bei Höxter, H. Müller au Wenck, JE04008875	-
-	GREAT BRITAIN, Wales, Breconshire, Craig Cerrig Gleisand NNR, 950 m, R.D. Porley 1470, Herb. Porley	-
-	GREAT BRITAIN, England, Shropshire, Alberbury, R.D. Porley 1391, Herb. Porley	-
-	ITALY, Toscana, Afuane, Can. del Rio, 680 m, 27.07.1990. M. Aleffi, CAME	-
<i>Trichostomum littorale</i>		
3202	GREAT BRITAIN, Cornwall, Kynance Cove, Lizard, 13.02.2011, J.A. Norton 130211, Herb. D. Callaghan, duplicate MUB 60475	OM 234703/OM260112
3213	GREAT BRITAIN, Shetland Islands, Eshaness, 31.08.2014, S.V.O' Leary s.n., Herb. D. Callaghan 310814, duplicate MUB 60476	OM234704/OM260094
3232	PORTUGAL, Algarve, Serra de Monchique, Barbelote amphitheatre, 527 m, 27.10.2016, R.D. Porley 03, Herb. R. Porley, duplicate MUB 60477	OM234705/OM260107
3233	PORTUGAL, Algarve, Serra de Monchique, Picota, 737 n, 02.03.2017, R.D. Porley 04, Herb. Porley, duplicate MUB 60478	OM234706/OM260108
3235	PORTUGAL, Algarve, Serra de Monchique, Barranco do Lajeado, N of Caldas, 227 m, 20.05.2016, R.D. Porley 06, Herb. Porley, duplicate MUB 60479	OM234707/OM260109
3239	PORTUGAL, Algarve, Serra de Monchique, Chilrão, 480 m, 30.07.2015, R. Porley 10, Herb. Porley, duplicate MUB 60480	OM234708/OM260110
3240	PORTUGAL, Algarve, Serra de Monchique, Fóia, Penedo do Buraco, 740 m, 06.02.2015, R.D. Porley 11, Herb. Porley, duplicate MUB 60481	OM234709/OM260111
3280	PORTUGAL, Algarve, Serra de Monchique, Ribeira da Cerca, W. of Marmelete, 19.08.2015, R.D. Porley 41, Herb. Porley, duplicate MUB 60482	OM234699/OM260095
3296	GREAT BRITAIN, Wales, Upper part of Cwm yr Allt-Lwyd, 17.09.2021, T.L. Blockeel 50/152, Herb. Blockeel, duplicates Herb. Porley, MUB 60483	OM234700/OM260101
3298	IRELAND, County Kerry, Torc Cascade, ca. 140 m, N.G. Hodgetts 11187, Herb. N.G. Hodgetts, duplicates Herb. Porley, MUB 60484	OM234701/OM260103

Code (Haplotype)	Locality (Voucher)	GenBank Accession Numbers ITS/rbcL
3299	IRELAND, County Kerry, Torc, Muckcross Lake, N.G. <i>Hodgetts 11181</i> , Herb. Hodgetts, duplicates Herb. Porley, MUB 60485	OM234702/OM260104
-	IRELAND, NY 01449062 (Lectotype)	-
-	GREAT BRITAIN, Whitsand Bay Cornwall, <i>F. Brent</i> , 0 3.02.1868, NY 01449063 (Syntype)	-
-	GREAT BRITAIN, a little East of Fairlight Down near Hastings, Sussex, July 1847, <i>W.M.</i> , NY 01449064 (Syntype)	-
-	GREAT BRITAIN, Scotland, Orkney, Egilsay, 6 m, 12.07.2006, <i>R.D. Porley 2917</i> , Herb. Porley	-
-	GREAT BRITAIN, England, North Devon, Bideford Bay, Saunton, 8 m, 05.08.2003, <i>R.D. Porley 2644</i> , Herb. Porley	-
-	ITALY, Prov. La Spezia, Bachschlucht ca. 1 km vor Pignone an der Str. nach La Spezia im <i>Castanea</i> -Wald, 25.03.1965, <i>R. Diüll s.n.</i> , CAME.	-
-	PORTUGAL, Algarve, Serra de Monchique, Barbelote amphitheatre, 600 m, 02.04.2015, <i>R.D. Porley 02</i> , Herb. Porley, duplicate MUB 60486	-
-	PORTUGAL, Algarve, Serra de Monchique, Ribeira de Seixoso, 125 m, 05.02.2010, <i>R.D. Porley 07</i> , Herb. Porley, duplicate MUB 60487	-
-	PORTUGAL, Algarve, Serra de Monchique, Ribeira de Seixoso, 125 m, 05.02.2010, <i>R.D. Porley 09</i> , Herb. Porley, duplicate MUB 60488	-
-	PORTUGAL, Algarve, Serra de Monchique, Fóia, north side, 815 m, 03.12.2015, <i>R.D. Porley 12</i> , Herb. Porley, duplicate MUB 60489	-
-	PORTUGAL, Algarve, Serra de Monchique, Fóia, Penado do Buraco, 05.02.2015, <i>R.D. Porley 24</i> , Herb. Porley	-
-	PORTUGAL, Algarve, Serra de Monchique, Chilhão, 500 m, 28.02.2014, <i>R.D. Porley 29</i> , Herb. Porley	-
-	PORTUGAL, Algarve, Serra de Monchique, Ribeira do Seixoso, 125 m, 05.02.2010, <i>R.D. Porley 30</i> , Herb. Porley	-
-	PORTUGAL, Algarve, Ribeira da Cerca, Cerca dos Pomares, 50 m, 26.06.2013, <i>R.D. Porley 39</i> , Herb. Porley	-
-	PORTUGAL, Algarve, Serra de Monchique, Ribeira da Cerca, W. of Marmelete, 180 m, 19.08.2020, <i>R.D. Porley 40</i> , Herb. Porley	-
-	PORTUGAL, Algarve, Serra de Monchique, Ribeira da Cerca, W. of Marmelete, 163 m, 05.08.2020, <i>R.D. Porley 43</i> , Herb. Porley	-
-	SPAIN, Andalusia, Cádiz, Medina-Sidonia, Sierra Blanquilla, arroyo del Alisoso, 16.04.2000, <i>J. Guerra s.n.</i> , MUB 11257	-
-	GREAT BRITAIN, Scotland, Outer Hebrides, Clash na Bearnaich, Hirta, St. Kilda, 100 m, 09.07.2013, <i>R.D. Porley s.n.</i> , Herb. Porley	-
<i>Trichostomum meridionale</i>		
1551	SPAIN, Murcia Region, Bullas, Salto Lucero, 650 m, 20.11.2002, <i>R.M. Ros s.n.</i> , MUB 14240 (Paratype)	AY796255/-
3227	GREECE, Eastern Aegean Islands, Rhodes, ca 1.2 km SE of Moni Artamiti, ca, 240 m, 08.03.2018, <i>T.L. Blockeel 47/027</i> , Herb. Blockeel, duplicate MUB 60472 (Paratype)	OM234718/OM260105
3259	SPAIN, Murcia Region, Sierra Minera de Cartagena-La Unión, Rambla del Gorguel, 131 m, 04.03.2020, <i>R.M. Ros, O. Werner & P. Egea Benavente s.n.</i> , MUB 60287, duplicate Herb. Porley (Paratype)	OM234713/OM260119
3260	SPAIN, Murcia Region, Cartagena, Atamaría, camino que sube al Monte de las Cenizas, en la carretera RM-514, 151 m, 04.03.2020, DNA 3260, <i>R.M. Ros, O. Werner & P. Egea Benavente s.n.</i> , MUB 60288, duplicate Herb. Porley (Paratype)	OM234714/OM260120
3261	SPAIN, Murcia Region, Cartagena, Atamaría, camino que sube al Monte de las Cenizas, en la carretera RM-514, 151 m, 04.03.2020, DNA 3261, <i>R.M. Ros, O. Werner & P. Egea Benavente s.n.</i> , MUB 60289, duplicate Herb. Porley (Paratype)	OM234715/OM260123
3262	SPAIN, Murcia Region, Cartagena, Calarreona, 0 m, 04.03.2020, DNA 3262, <i>R.M. Ros, O. Werner & P. Egea Benavente s.n.</i> , MUB 60290, duplicate Herb. Porley (Paratype)	OM234716/OM260124

Code (Haplotype)	Locality (Voucher)	GenBank Accession Numbers ITS/rbcL
3263	SPAIN, Murcia Region, Cartagena, Calarreona, 0 m, 04.03.2020, DNA 3263, R.M. Ros, O. Werner & P. Egea Benavente s.n., MUB 60291, duplicate Herb. Porley (Paratype)	OM234717/OM260125
3264	SPAIN, Murcia Region, Las Torres de Cotillas, Urbanización Los Romeros, calle I, parcela en la zona más alta, 144 m, 07.03.2020, R.M. Ros s.n., MUB 60293 (Paratype)	OM234719/OM260126
3265	SPAIN, Murcia Region, Las Torres de Cotillas, Urbanización Los Romeros, calle I, parcela en la zona más alta, 144 m, 02.05.2020, R.M. Ros s.n., MUB 60296, duplicate Herb. Porley (Paratype)	OM234720/OM260127
3266	SPAIN, Murcia Region, Murcia, Rambla del Puerto de La Cadena, 260 m, 26.03.2019, R.M. Ros & M. Farag s.n., MUB 60292, duplicate Herb. Porley (Paratype)	OM234721/OM260128
3268	SPAIN, Murcia Region, Murcia, El Valle Perdido, proximidades a Santuario de la Fuensanta, 143 m, 14.03.2020, R.M. Ros & O. Werner s.n., MUB 60295, duplicate Herb. Porley (Holotype)	OM234722/OM260130
3269	SPAIN, Murcia Region, Murcia, El Valle Perdido, proximidades a Santuario de la Fuensanta, 143 n, 14.03.2020, R.M. Ros & O. Werner s.n., MUB 60294, duplicate Herb. Porley (Paratype)	OM234723/OM260131
3270	SPAIN, Murcia Region, Cartagena, Punta de la Azohía, 50 m, 06.03.2009, R.M. Ros s.n., MUB 29467, duplicate Herb. Porley (Paratype)	OM234724/OM260132
-	ITALY, Sardinia, S. Elia, Cagliari, pianoro per Cala Fighera, 17.05.2001, A. Cogoni s.n., CAG	-
-	ITALY, Sardinia, Casa Laura, Giara di Gesturi, Genoni, 08.03.2002, A. Cogoni s.n., CAG (sample with intermediate characters with <i>T. littorale</i>)	-
-	SPAIN, Alicante, Cabo de la Nao, (Jávea), M.J. Cano s.n., MUB 5565	-
-	SPAIN, Almería, camino a Río de Aguas desde El Cerrón (Sorbas), 18.03.1988, J.J. Martínez-Sánchez, R.M. Ros & J. Guerra s.n., MUB 6101	-
<i>Trichostomum</i> sp.		
2178	AUSTRIA, Styria, Hochschwab massif, Pfarrlerlacke W of Tragöss, ca 930 m, 06.10.2006, H. Köckinger 12338, Herb. Köckinger, duplicates Herb. Porley, MUB 60490	OM234727/OM260121
3228	GREECE, Kithira Island, ca 1.5 km east of Limnionas, 140 m, 27.03.2019, T.L. Blockeel 48/098, Herb. Blockeel, duplicates Herb. Porley, MUB 60491	OM234710/OM260106
<i>Trichostomum crispulum</i>		
3245	PORTUGAL, Algarve, Senhora da Rocha, Lagoa, 17.03.2013, R.D. Porley 16, Herb. Porley, duplicate MUB 60492	OM234693/OM260091
3286	SPAIN, Galicia, Orense, Monasterio de San Estevo de Ribas de Sil, 20.08.2021, R.M. Ros & A. Werner Ros s.n., MUB 60493	OM234691/OM260092
3287	SPAIN, Andalusia, Jaén, Sierras del Sur de Jaén, carretera hacia Monumento Natural Quejigo del Carbón o del Amo, 04.05.2021, R.M. Ros & O. Werner s.n., MUB 60494	OM234692/OM260093
Outgroup specimens		
<i>Barbula unguiculata</i> 3203	GREAT BRITAIN, Dursston Head, Dorset, A. Norton 81213, Herb. Callaghan, duplicate MUB 60495	-/OM260089
<i>Oxystegus</i> sp.	MALAWI, Zomba plateau, peak road below Malumbe Peak, D. Long 12608, BM	AY854390 [22,25]/-
<i>Oxystegus tenuirostris</i>	AUSTRIA, Styria, Stubalpe, Granitzgraben SE of Weisskirchen, H. Köckinger 14245a, GZU, MUB 31054	HM049797 [25]/-
<i>Tortella alpicola</i>	SPAIN, Sierra Nevada, Collado de La Mosca, S. Rams s.n., MUB 21926	HM049809 [25]/-
<i>Tortella flavovirens</i>	GREECE, Pelopónnisos, M.J. Cano et al. s.n., MUB 11940	AY796262 [22]/-
<i>Tortella flavovirens</i> 3255	PORTUGAL, Algarve, Serra de Monchique, Gralhos, Via Alagarviana, 545 m, 26.01.2018, R.D. Porley 26, Herb. Porley	-/OM260117
<i>Tortella humilis</i>	SPAIN, Albacete, R.M. Ros & O. Werner s.n., MUB 17767	AY796260 [22]/-
<i>Tortella squarrosa</i>	CZECH REPUBLIC, Tmaň-Kotýz, J. Košnar 1266, CBFS 562	JX679950 [55]/-
<i>Tortella tortuosa</i>	MOROCCO, Rif, M.J. Cano & R.M. Ros, s.n., MUB 10397	AY796266 [22]/-

References

1. Blockeel, T.L. *Trichostomum brachydontium*. In *Atlas of British & Irish Bryophytes*; Blockeel, T.L., Bosanquet, S.D.S., Hill, M.O., Preston, C.D., Eds.; Pisces Publications: Newbury, UK, 2014; p. 141, ISBN 978-1-874357-62-9.

2. Zander, R.H. *Trichostomum*. In *Flora of North America North of Mexico*; Flora of North America Editorial Committee, Ed.; Oxford University Press: New York, NY, USA, 2007; Volume 27, pp. 488–494.
3. Thouvenot, L.; Bardat, J. Contribution to the bryophyte flora of New Caledonia. I. New taxa and amendments. *Cryptogam. Bryol.* **2013**, *34*, 37–47. [[CrossRef](#)]
4. Guerra, J. *Trichostomum* Bruch. In *Flora Briofítica Ibérica*; Guerra, J., Cano, M.J., Ros, R.M., Eds.; Universidad de Murcia y Sociedad Española de Briología: Murcia, Spain, 2006; Volume 3, pp. 76–83.
5. Müller, F.A. Erstes Verzeichnis sardinischer Laubmoose, wie auch derjenigen welche von meinen Freunde Herrn Fleischer bei Smyrna aufgefunden worden sind, nebst Beschreibungen und Abbildungen einiger neuen Arten. *Flora* **1829**, *12*, 385–399.
6. Herzog, T. Studien über den Formenkreis des *Trichostomum mutabile* Br. *Abh. Kais. Leopold.-Carol. Dtsch. Akad. Nat. Nova Acta* **1907**, *73*, 453–481.
7. Rungby, S. A contribution to the bryophytic flora of Spain and Morocco, especially the area between Gandía and Alcoy. *Bot. Not.* **1962**, *115*, 61–64.
8. Koppe, F. Bryologische Beobachtungen auf der Insel Mallorca. *Bot. Not.* **1965**, *118*, 25–48.
9. Renauld, F.; Cardot, J. Mousses des Canaries récoltées par M. Albert Tullgren et coup d’œil sur la flore bryologique des îles atlantiques. *Bull. L’herbier Boissier Sér. 2* **1902**, *2*, 433–453.
10. Sérgio, C. Acerca da identidade de *Hyophila contorta* (Kunze) Jaeg. Pottiaceae da Ilha da Madeira. *Port. Acta Biol. Série B Sist. Ecol. Biogeogr. E Paleontol.* **1985**, *14*, 168–172.
11. Sérgio, C.; Sim-Sim, M.; Carvalho, M. Annotated catalogue of Madeiran bryophytes. *Bol. do Mus. Munic. do Funchal (Hist. Nat.) Supl.* **2006**, *10*, 5–163.
12. Hill, M.O.; Bell, N.; Bruggeman-Nannenga, M.A.; Brugués, M.; Cano, M.J.; Enroth, J.; Flatberg, K.; Frahm, J.-P.; Gallego, M.; Garilleti, R.; et al. An annotated checklist of the mosses of Europe and Macaronesia. *J. Bryol.* **2006**, *28*, 198–267. [[CrossRef](#)]
13. Ros, R.M.; Mazimpaka, V.; Abou-salama, U.; Aleffi, M.; Blockeel, T.L.; Brugués, M.; Cros, R.M.; Dia, M.G.; Dirkse, G.M.; Draper, I.; et al. Mosses of the Mediterranean, an annotated checklist. *Cryptogam. Bryol.* **2013**, *34*, 99–283. [[CrossRef](#)]
14. Hodgetts, N.G.; Söderström, L.; Blockeel, T.L.; Caspari, S.; Ignatov, M.S.; Konstantinova, N.A.; Lockhart, N.; Papp, B.; Schröck, C.; Sim-Sim, M.; et al. An annotated checklist of bryophytes of Europe, Macaronesia and Cyprus. *J. Bryol.* **2020**, *42*, 1–116. [[CrossRef](#)]
15. Braithwaite, R. *The British Moss-Flora*; L. Reeve & Co.: London, UK, 1887; Volume 1, pp. 1–315.
16. Dixon, H.N.; Jameson, H.G. *The Student’s Handbook of British Mosses*, 1st ed.; V.T. Sumfield: Eastbourne, UK, 1896; pp. 1–582.
17. Dixon, H.N. *The Student’s Handbook of British Mosses*, reprint 3rd ed.; Sumfield & Day: Eastbourne, UK, 1954; p. XLVI + 1–582.
18. Smith, A.J.E. *The Moss Flora of Britain and Ireland*; Cambridge University Press: Cambridge, UK, 1978; p. VIII + 1–706.
19. Smith, A.J.E. *The Moss Flora of Britain and Ireland*, 2nd ed.; Cambridge University Press: Cambridge, UK, 2004; p. XII + 1–1012.
20. Renner, M.A.M. Opportunities and challenges presented by cryptic bryophyte species. *Telopea* **2020**, *23*, 41–60. [[CrossRef](#)]
21. Werner, O.; Ros, R.M.; Cano, M.J.; Guerra, J. Molecular phylogeny of Pottiaceae (Musci) based on chloroplast *rps4* sequence data. *Plant Syst. Evol.* **2004**, *243*, 147–164. [[CrossRef](#)]
22. Werner, O.; Ros, R.M.; Grundmann, M. 2005. Molecular phylogeny of Trichostomoideae (Pottiaceae, Bryophyta) based on nr ITS sequence data. *Taxon* **2005**, *54*, 361–368. [[CrossRef](#)]
23. Werner, O.; Köckinger, H.; Magdy, M.; Ros, R.M. On the systematic position of *Tortella arctica* and *Trichostomum arcticum* (Bryophyta, Pottiaceae). *Nova Hedwig.* **2014**, *98*, 273–293. [[CrossRef](#)]
24. Ros, R.M.; Werner, O. The circumscription of the genus *Pottiopsis* (Pottiaceae, Bryophyta) based on morphology and molecular sequence data. *Nova Hedwig. Beih.* **2007**, *131*, 65–79.
25. Köckinger, H.; Werner, O.; Ros, R.M. A new taxonomic approach to the genus *Oxystegus* (Pottiaceae, Bryophyta) in Europe based on molecular data. *Nova Hedwig.* **2010**, *138*, 31–49.
26. Köckinger, H.; Lüth, M.; Werner, O.; Ros, R.M. *Tortella mediterranea* (Pottiaceae), a new species from southern Europe, its molecular affinities, and taxonomic notes on *T. nitida*. *Bryologist* **2018**, *121*, 560–570. [[CrossRef](#)]
27. Werner, O.; Ros, R.M.; Guerra, J. Direct amplification and NaOH extraction: Two rapid and simple methods for preparing bryophyte DNA for polymerase chain reaction (PCR). *J. Bryol.* **2002**, *24*, 127–131. [[CrossRef](#)]
28. White, T.J.; Bruns, T.; Lee, S.; Taylor, J. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In *PCR Protocols a Guide to Methods and Applications*; Innis, M.A., Gelfand, D.H., Sninsky, J.J., White, T.J., Eds.; Academic Press: New York, NY, USA, 1990; pp. 315–322.
29. Kress, W.J.; Erickson, D.L. A two-locus global DNA barcode for land plants: The coding *rbcL* gene complements the non-coding *trnH-psbA* spacer region. *PLoS ONE* **2007**, *2*, e508. [[CrossRef](#)]
30. Kress, W.J.; Erickson, D.L.; Jones, F.A.; Swenson, N.G.; Perez, R.; Sanjur, O.; Bermingham, E. Plant DNA barcodes and a community phylogeny of a tropical forest dynamics plot in Panama. *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 18621–18626. [[CrossRef](#)]
31. Hall, T.A. BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp. Ser.* **1999**, *41*, 95–98.
32. Kumar, S.; Stecher, G.; Li, M.; Nnyaz, C.; Tamura, K. MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Mol. Biol. Evol.* **2018**, *35*, 1547–1549. [[CrossRef](#)]
33. Donath, A.; Stadler, P.F. Split-inducing indels in phylogenomic analysis. *Algorithms Mol. Biol.* **2018**, *13*, 12. [[CrossRef](#)] [[PubMed](#)]
34. Notredame, C.; Higgins, D.G.; Heringa, J. T-Coffee. A novel method for fast and accurate multiple sequence alignment. *J. Mol. Biol.* **2000**, *302*, 205–217. [[CrossRef](#)] [[PubMed](#)]

35. Huelsenbeck, J.P.; Ronquist, F. MrBayes: Bayesian inference of phylogeny. *Bioinformatics* **2001**, *17*, 754–755. [[CrossRef](#)]
36. Ronquist, F.; Huelsenbeck, J.P. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **2003**, *19*, 1572–1574. [[CrossRef](#)] [[PubMed](#)]
37. Ronquist, F.; Teslenko, M.; van der Mark, P.; Ayres, D.L.; Darling, A.; Höhna, S.; Larget, B.; Liu, L.; Suchard, M.A.; Huelsenbeck, J.P. MrBayes 3.2: Efficient bayesian phylogenetic inference and model choice across a large model space. *Syst. Biol.* **2012**, *61*, 539–542. [[CrossRef](#)] [[PubMed](#)]
38. Huelsenbeck, J.P.; Larget, B.; Alfaro, M.E. Bayesian phylogenetic model selection using reversible jump Markov chain Monte Carlo. *Mol. Biol. Evol.* **2004**, *21*, 1123–1133. [[CrossRef](#)]
39. Stöver, B.C.; Müller, K.F. TreeGraph 2: Combining and visualizing evidence from different phylogenetic analyses. *BMC Bioinformatics* **2010**, *11*, 7. [[CrossRef](#)]
40. Maddison, W.P. Gene trees in species trees. *Syst. Biol.* **1997**, *46*, 523–536. [[CrossRef](#)]
41. Pampilo, P.; Nei, M. Relationships between gene trees and species trees. *Mol. Biol. Evol.* **1988**, *5*, 568–583.
42. Zander, R.H. Evolutionary analysis of five bryophyte families using virtual fossils. *An. Jardín Botánico Madr.* **2009**, *66*, 263–277. [[CrossRef](#)]
43. Mönkemeyer, W. *Die Laubmoose Europas, Andreales-Bryales*; Akademische Verlagsgesellschaft: Leipzig, Germany, 1927; pp. 1–960.
44. Eckel, P.M. Re-evaluation of *Tortella* (Musci, Pottiaceae) in conterminous U.S.A. and Canada with a treatment of the European species *Tortella nitida*. *Bull. Buffalo Soc. Nat. Sci.* **1998**, *36*, 117–191.
45. Husnot, P.T. *Muscologia Gallica*; F. Savy, Libraire: Paris, France, 1884–1890; pp. 1–458.
46. Schimper, W.P. *Synopsis Muscorum Europaeorum, Praemissa Introductione de Elementis Bryologicis Tractante*, 2nd ed.; Schweizerbart: Stuttgart, Germany, 1876; p. CXXX 1-866.
47. Wörz, A. The “Botanische Reiseverein” A 19th-century joint stock company for the collecting of herbarium specimens. *Huntia* **2007**, *13*, 121–141.
48. Stafleu, F.A.; Cowan, R.S. *Taxonomic Literature*, 2nd ed.; Bohn, Scheltema & Holkema Utrecht/Dr. W. Junk b.v.: The Hague, The Netherlands, 1981; Volume 3, pp. 1–980.
49. Hodgetts, N.; Lockhart, N. *Checklist and Country Status of European Bryophytes—Update 2020*; Irish Wildlife Manuals, 123; National Parks and Wildlife Service: Dublin, Ireland, 2020; pp. 1–214.
50. Mitten, W. New or rare British mosses. *J. Bot. Br. Foreign* **1868**, *6*, 97–99.
51. Herzog, T. *Geographie der Moose*; Gustav Fischer: Jena, Germany, 1926; p. XI + 1-439.
52. Frey, W.; Frahm, J.-P.; Fischer, E.; Lobin, W. *The Liverworts, Mosses and Ferns of Europe, English Edition*; Blockeel, T.L., Ed.; Harley Books: Colchester, UK, 2006; p. XV + 1-512.
53. Cortes Latorre, C. Aportaciones a la Briología española (dos musgos nuevos para la Flora española). *An. Inst. Botánico A. J. Cavanilles* **1956**, *13*, 135–147.
54. Esteve Chueca, F.; Cortes Latorre, C. El estrato liquénico-muscinal de la durilignosa de la Sierra de Cartagena. *An. Inst. Botánico A. J. Cavanilles* **1956**, *13*, 121–128.
55. Kučera, J.; Košnar, J.; Werner, O. Partial generic revision of *Barbula* (Musci: Pottiaceae): Re-establishment of *Hydrogonium* and *Streblotrichum*, and the new genus *Gymnobarbula*. *Taxon* **2013**, *62*, 21–39. [[CrossRef](#)]