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# The first African record of *Isothecium algarvicum* in Kroumiria (Tunisia), a relictual element of the Neogene flora?

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*Isothecium algarvicum* W.E.Nicholson & Dixon was discovered in Tunisia, which constitutes the first report of this Macaronesian-oceanic species in Africa. Three distinct populations were observed in ravines in the humid Meso-Mediterranean vegetation belt of Kroumiria. The Tunisian material is fully described, with particular attention to alar and supra-alar cells, prorate cells and other morphological characteristics. The density and size of the prorate cells appear to be a robust diagnostic characteristic for the separation of *Isothecium algarvicum*, *I. alopecuroides* and *I. myosuroides*. Detailed analysis of the forest habitat reveals a uniquely rich environment. The origin of a possibly relict species with Macaronesian-oceanic affinities is discussed.

Keywords: bryophytes, Maghreb, new records, North Africa, oceanicity, relict species, supra-alar cells

The studies addressing Tunisian bryoflora are both old and scattered. The checklists of bryophytes from North Africa (Ros et al. 1999), of hornworts and hepatics from the Mediterranean (Ros et al. 2007), and of mosses (Ros et al. 2013) incorporate all references to the Tunisian literature and have thus facilitated further studies. Major studies of hornworts and liverworts include those of Jovet-Ast and Bischler (1971), Jovet-Ast (1986) and Ben Osman et al. (2019, 2021c, 2022a, b). Further studies have addressed mosses, including Bescherelle (1897), Thériot (1900), Gillot (1904), Corbière and Pitard (1909), Bizot (1931), Potier de la Varde (1949), Labbe (1953), Jelenc (1954, 1955a, b, 1967), De Sloover (1965), Pócs (2007), Draper et al. (2008), Muller et al. (2010), Campisi et al. (2015), Ellis et al. (2017, 2018, 2019, 2021), Hugonnot et al. (2020) and Ben Osman et al. (2021a, b, c, 2022a). These works notwithstanding, much remains to be done to create an accurate picture of the Tunisian bryoflora.

In the Mediterranean region, the moss genus *Isothecium* Brid. (Lembophyllaceae) comprises seven species, some of which exhibit wide distributions. Although infrequent, *I. alopecuroides* (Lam. ex Dubois) Isov. and *I. myosuroides* Brid.

are the most widely distributed species of the genus, and are patchily recorded from Macaronesia to Iran and Azerbaijan (Blockeel et al. 2014). *Isothecium interludens* Stirt. and *I. holtii* Kindb. have comparable distributions: the former is a hyperoceanic taxon that has been raised recently to specific rank and is recorded in the Atlantic areas of Spain and France, Faroe Islands, Scotland, United Kingdom, Ireland, Norway (Hodgetts and Vanderpoorten 2018, Hodgetts and Lockhart 2020); the latter is known in France, Spain, Portugal, Belgium, Luxembourg and Germany (Hodgetts and Lockhart 2020), and in the United Kingdom. It has also been reported from Turkey (Blockeel et al. 2014). In this context, further verification of the Turkish records of *I. interludens* and *I. holtii* for their identity are warranted. *Isothecium prolixum* (Mitt.) M.Stech, Sim-Sim, Tangney & D.Quand and the recently described *I. montanum* Draper, Hedenäs, M.Stech, Lopes & Sim-Sim are endemic to Macaronesia and to Madeira, respectively (Draper et al. 2015).

Finally, *Isothecium algarvicum* W.E.Nicholson & Dixon is currently known in the Canary Islands (González-Mancebo et al. 2008), in Madeira (Sérgio et al. 2008) and in the southwest of the Iberian Peninsula (Sérgio and Carvalho 2003). Recent bryological surveys in northern Tunisia have led to the discovery of three small populations of this species, which constitutes a significant eastern extension to its range and a first record for North Africa. While *I. alopecuroides* and *I. myosuroides* are reported in Morocco (Jelenc 1955a, Jovet-Ast 1958, Sauvage 1958, Ahayoun et al. 2013) and in

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Algeria (Jelenc 1955a, 1967), no taxa of this genus has ever been reported in Tunisia.

*Isothecium algarvicum* belongs to a taxonomically difficult genus with a particularly high degree of morphological variation (Draper et al. 2007). This species has been described and illustrated previously in several publications (Hedenäs 1992, Elias and Guerra 2018), but we provide here a detailed morphological analysis of our gatherings. In particular, we carefully address the ecological setting of the population because of its relevance to both the phytosociological context and phytogeography.

## Nomenclature

The bryophyte and vascular plant nomenclature follows Ros et al. (2007, 2013) and Le Floch et al. (2010) respectively.

## Methods

During our field surveys we collected bryophytes at 108 different locations in Kroumiria and Mogods from 1–13 April 2019. One specimen of *Isothecium myosuroides*, collected in the Aïn Draham delegation, was found in the Jelenc herbarium at the Musée Lecoq (Clermont-Ferrand, France). Western European specimens of *I. myosuroides* and *I. alopecuroides* were also examined.

In each prospected location, the general conditions of the habitat were described. Bryophyte communities were surveyed and relevés made recording substrate type, aspect, slope, hydrological conditions, etc. Microscopic examination was performed using standard methods with a microscope. The morphological description is based on Tunisian material and includes all morphological aspects described in the literature. We paid particular attention to alar and supra-alar areolation as it is often referred to as the best diagnostic feature to distinguish *Isothecium algarvicum* from *I. alopecuroides* and *I. myosuroides*. To measure density and size of the prorate cells, we studied three species of *Isothecium*: *I. algarvicum*, *I. alopecuroides* and *I. myosuroides* (Table 1). Five leaves were dissected from each of five randomly chosen stems of each specimen, and measurements were made at 100 µm from the leaf apex in an area of ca 78 × 78 µm. For *I. myosuroides*, given the length of the leaf acumen, we took measurements where an area of 78 × 78 µm was reached.

The specimens that were collected are housed in the herbarium of the Faculté des Sciences de Tunis (TUN) and in the personal herbarium of Vincent Hugonnot.

Significance of differences between species were tested with ANOVA (Excel 2010).

## Results

The newly discovered populations of *Isothecium algarvicum* were found only in the Kroumirian region at three different sites: Aïn Jmel Waterfall, Oued Ellil and O. Zen (Fig. 1). Here we provide the complete description of this new species.

### Description of Tunisian material (Fig. 2)

#### *Isothecium algarvicum* W.E.Nicholson & Dixon

Grey-green, dull, medium-sized plants, moderately dendroid, irregularly branched; primary axes plagiotropic creeping, monopodial (sympodial if apex is damaged), pinnately or bipinnately branched laterally (on one plane, appearing flat), with relatively small leaves on branches; intermittent robust orthotropic secondary axes are acropetally arched and branched medially or distally and have larger leaves; second and third order branches are relatively short; the occasional flagelliform stolons are formed in the proximal sector of primary shoots and give rise to first order axes. Rhizoids form tufts on stolons. Proximal leaves suborbicular to shortly ovate, strongly dentate, forming a closed dome. Stem cortex of 3–4 layers of incrassate brownish cells; central cylinder composed of 8–15 cells. Leaves of primary axes measure 0.6 × 0.9 mm, distant, erecto-patent, triangular to ovate-triangular, weakly concave; costa 60 µm wide at base, reaching 2/3 of leaf length, occasionally forked, not ending in a spine; margin plane; very weakly denticulate below, slightly denticulate at apex; mid-leaf cells rhomboidal to shortly linear, slightly flexuose, 16–28 × 5–6 µm, incrassate, eporose, smooth; prorate cells absent dorsally; basal cells strongly incrassate, moderately porose; alar cells forming a large transversely rectangular to triangular, slightly opaque group, reaching midway from margin to costa, rarely bis-tratose, not decurrent; alar cells quadrate, short rectangular or oblate; supra-alar cells forming a triangular group reaching ¼ of the length of the leaf, composed of quadrate to short rectangular cells, intercalated with rows of rectangular cells in the upper part. Leaves of the second and third axes, 0.60–0.70 × 1.00–1.25 mm, short ovate, shortly acuminate, concave; costa reaching ¾ of the length of the leaf, 70 µm wide at base; not ending in a spine; margin plane or recurved below; strongly denticulate from apex to 1/3(–1/2) of the leaf length; mid-leaf cells rhomboidal to short linear, slightly flexuose, 20–30 × 5–6 µm, incrassate, eporose; numerous

Table 1. Provenance of the specimens used for the measurements of density and size of prorate cells.

Species	Country of origin	Localities
<i>Isothecium algarvicum</i>	Tunisia	Kroumiria: Aïn Jmel waterfall; Oued Ellil; O. Zen
	Canary Islands	La Palma, Barranco de San Juan
<i>Isothecium alopecuroides</i>	Spain	Málaga Benahavis; without location
	France	Pontgibaud, Puy de Dôme Auvergne: Haute Loire, Polignac Orne, Bellême forest
<i>Isothecium myosuroides</i>	France	Auvergne: Cantal, Vezels Roussy, les Bories Pays de Loire, Sarthe, Perseigne forest

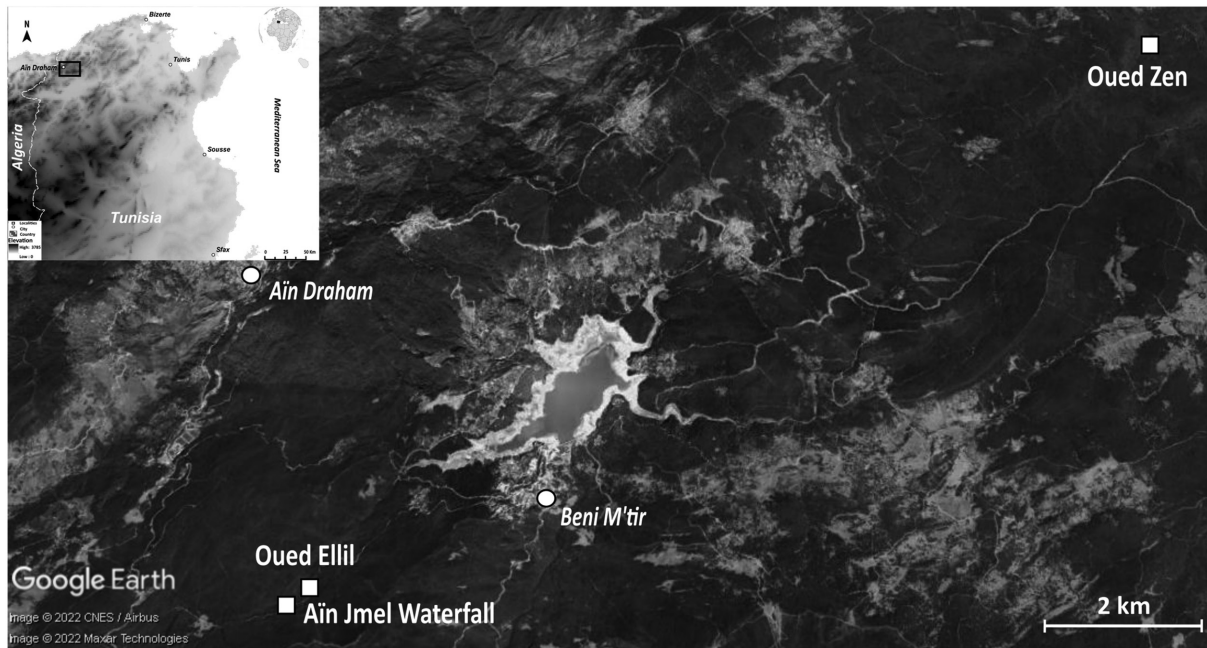


Figure 1. Localities of *Isotheicum algarvicum* in northern Tunisia.

cells prorate dorsally; basal cells strongly incrassate, moderately porose; alar cells forming a large quadrate opaque group, reaching midway from margin to costa, occasionally bistratose, not decurrent; alar cells quadrate, shortly rectangular or oblate; supra-alar cells quadrate to short rectangular, distinct from short linear limb cells, but intercalated with such cells in the upper part; alar cells and supra-alar cells together form a large group ascending the leaf margin (to 1/3–1/2 leaf length) as a very narrow band (Fig. 3). Small leaves of reduced axes more ovate-oblong, denticulate to 2/3 of leaf length, with prorate cells at back.

Dioicous. Perigonial buds not seen. Perichaetial buds consisting of ovate-oblong, abruptly acuminate-squarrose leaves, smooth; costa faint, single, ending at mid-leaf; numerous hyaline paraphyses to  $5\text{--}6 \times 12\text{--}15 \mu\text{m}$ , composed of 3–4 short brown cells at base and numerous relatively thick-walled cells; archegonia to  $500 \mu\text{m}$  long.

Sporophytes not seen.

The prorate cells are always numerous in *Isotheicum algarvicum* (averaging 13 on a surface of  $100 \mu\text{m}^2$  and more than 100 per leaf) and with tall projections (averaging  $8.2 \mu\text{m}$  above the leaf surface up to  $14 \mu\text{m}$ ). By contrast, they are less abundant in *I. alopecuroides* (about 5 cells on the same surface area) with a height of  $7.8 \mu\text{m}$  (max.  $13.3 \mu\text{m}$ ). Finally, they are almost absent or difficult to observe in *I. myosuroides*, and, when present, reach, on average,  $7.7 \mu\text{m}$  (max.  $12 \mu\text{m}$ ) (Fig. 2, 4 and 5).

#### Tunisian specimens examined

Tunisia, Kroumiria, Jendouba Governorate, Delegation of Ain Draham: Ain Jmel Waterfall ( $36^{\circ}43'33''\text{N}$ ,  $08^{\circ}42'09''\text{E}$ ; 594 m a.s.l.), 3 Apr 2019, leg. Imen Ben Osman, Vincent Hugonnot, T2019-612; Oued Ellil ( $36^{\circ}44'08.45''\text{N}$ ;  $08^{\circ}42'64.17''\text{E}$ ; 479 m a.s.l.), 3 Apr 2019, leg. Imen Ben Osman, Vincent Hugonnot, T2019-580; Delegation of Ferhana: Oued Zen ( $36^{\circ}48'71.80''\text{N}$ ,  $08^{\circ}50'68.88''\text{E}$ ; 368 m a.s.l.), 4 Apr 2019, leg. Imen Ben Osman, Vincent Hugonnot, T2019-393.

The specimens that were collected are housed in the herbarium of the Faculté des Sciences de Tunis (TUN) and in Vincent Hugonnot's personal herbarium.

#### Distribution

An updated distribution map is provided (Fig. 6). The Tunisian locality lies about 1250 km east of the nearest known locality (southern Spain). On the western Iberian Peninsula, it is recorded from northern Portugal to the Serra de Monchique in the south (Sérgio and Carvalho 2003, Sérgio et al. 2007, 2013), and from Andalucía in southern Spain (Guerra 1980). In Macaronesia, it is known on Madeira (Sérgio et al. 2008) and in the western Canary Islands (González-Mancebo et al. 2008).

#### Habitat in Tunisia

*Isotheicum algarvicum* was discovered in Kroumiria (NW Tunisia) on the banks of two permanent rivers, Oued Zen and Oued Ellil, and at the Ain Jmel Waterfall, on O. Ellil. In each location, it covered approximately  $1 \text{ m}^2$ . Kroumiria is a mountainous massif with alternating sandstone and clay from the Numidian flysch, reaching 1203 m a.s.l. at Jbel El Ghorra, on the Algerian-Tunisian border and descends eastwards to 800–900 m in the region of Ain Draham, and to around 500 m on the Nefza Plain. Located in a humid bioclimate (INRF 1975), within the Thermo- and Meso-Mediterranean vegetation belts, it is Tunisia's wettest region with rainfall ranging between 1000 and  $1500 \text{ mm year}^{-1}$ . It snows regularly in the mountains from December to February. Annual average temperatures range from 16 to  $20^{\circ}\text{C}$ , with minimums of  $2\text{--}7^{\circ}\text{C}$  (January), with relatively strong daily and seasonal fluctuations. The prevailing winds come from the northwest and preserve moisture in the region for much of the year (Debazac 1959). The region's vegetation is dominated by zeen oak *Quercus canariensis* Willd. forests in the Meso-Mediterranean vegetation belt and by cork oak *Q. suber* L. forests in the Thermo-Mediterranean belt.



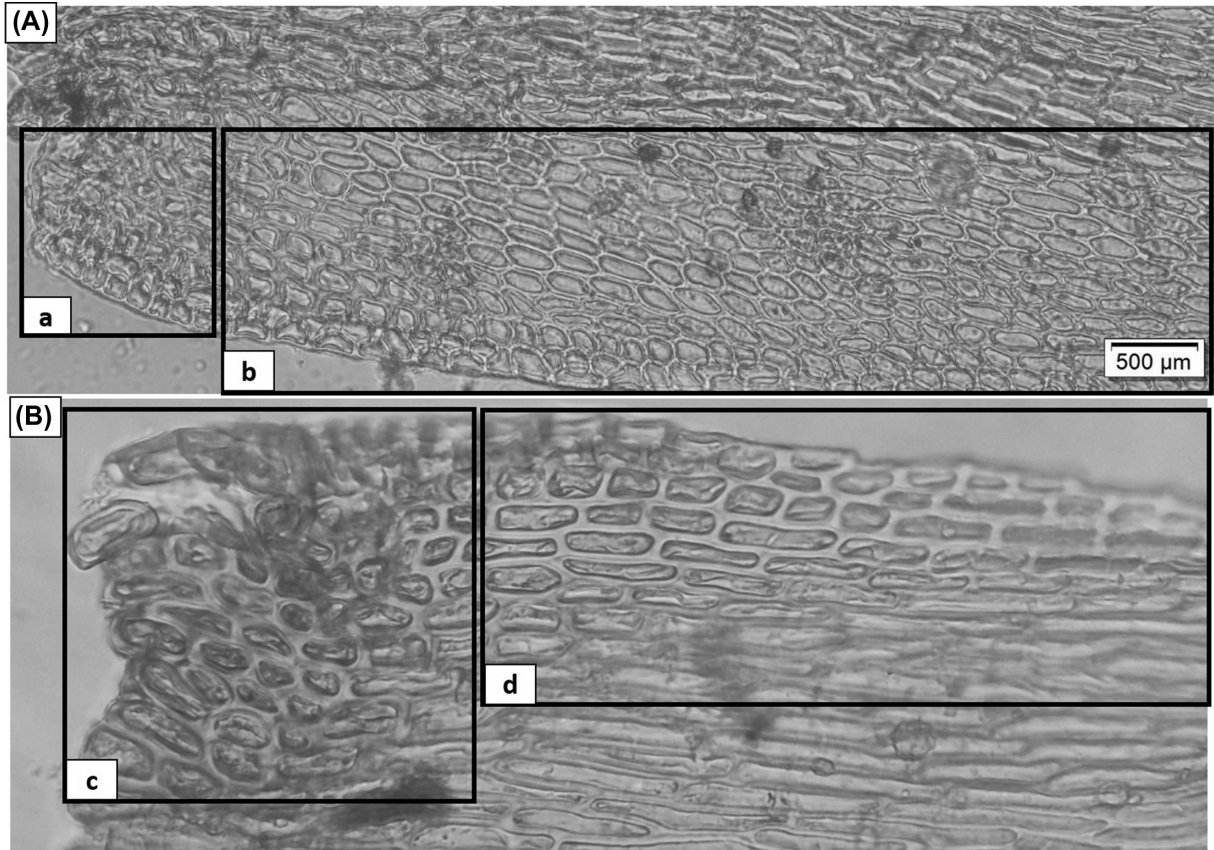


Figure 3. Alar and supra-alar zones of *Isoetecium algarvicum* and *I. alopecuroides*. (A) Leaf of *Isoetecium algarvicum*; (a) alar zone, (b) supra-alar zone. (B) Leaf of *I. alopecuroides*, (c) alar zone, (d) supra-alar zone.

Despite summer droughts in the Kroumiria, some permanent watercourses persist in its central part thanks to high annual rainfall. In Oued Zen and O. Ellil, *Alnus glutinosa* (L.) Gaertn. The forest is confined to valley bottoms and small flat areas. Individual alder trees extend narrowly and discontinuously along the main axis of minor

river beds and do not occur far from them. These riparian forests are characterized by the endemics *Campanula alata* Desf. and *Carex panormitana* Guss., associated with *Hypericum androsaemum* L. and more mesophilous woodland species such as *Luzula forsteri* (Sm.) DC. and *Prunella vulgaris* L. *Quercus canariensis* woodland comes into con-

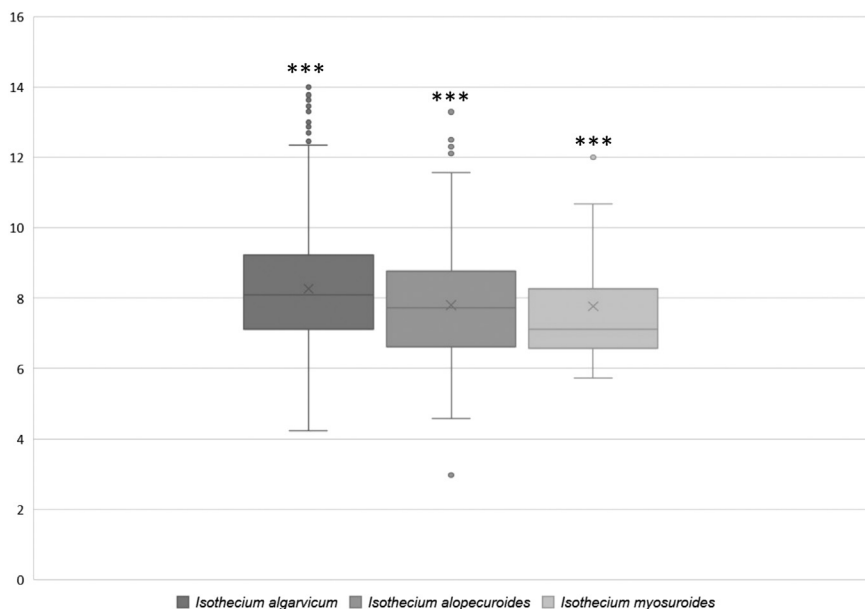


Figure 4. Size of prorate cells for *Isoetecium algarvicum*, *I. alopecuroides* and *I. myosuroides*.

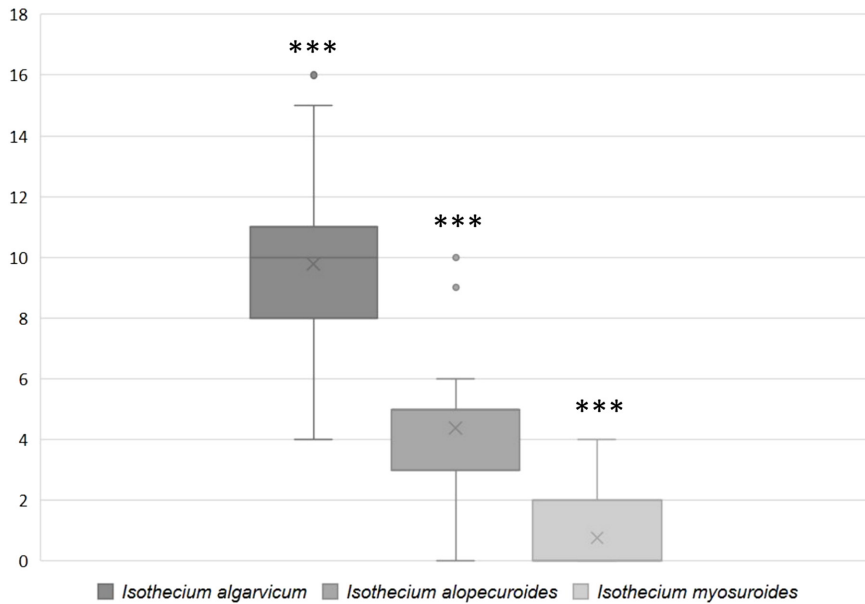


Figure 5. Prorate cell density of *Isothecium algarvicum*, *I. alopecuroides* and *I. myosuroides*.

tact with *Alnus glutinosa* stands towards the base of slopes. The understorey is well lit and often has a sparse cover of *Cytisus villosus* Pourr., *Crataegus monogyna* Jacquin, *Erica arborea* L., etc., locally mixed with thermophilous elements from *Quercetalia ilicis* Br.-Bl. ex Molinier 1934, such as *Arbutus unedo* L., *Phillyrea media* L., *Viburnum tinus* L., etc.

The remarkable zonation of the bryological communities ranges from the main riverbed to mesic slopes. The most significant populations of *Isothecium algarvicum* are found at the interface of *Alnus glutinosa* and *Quercus canariensis* woodlands, on the higher banks that have a certain instability, with small sandstone rock outcrops entangled with aerial roots and trunks. The vertical zonation of bryophytic communities of Oued Zen is briefly described (Table 2) and schematically represented (Fig. 7).

### Reproductive characters

No sporophytes were recorded in Tunisia, only female shoots with archegonia were seen. Sporophytes are apparently frequent in Macaronesia (Hedenäs 1992, 1993), and known from the Iberian Peninsula (sporophyte were found in Serra de Monchique) (Elias and Guerra 2018, R. D. Porley pers. comm).

### Discussion

Surprisingly, no species of the genus *Isothecium* were previously known in Tunisia. Under-recording may explain the poor knowledge of the Tunisian bryoflora. Here, we make the first record for *Isothecium algarvicum*. We also discovered *I. myosuroides*, which is treated elsewhere (Ben Osman et al. 2022a). The identification of *I. algarvicum* becomes more



Figure 6. Known global distribution of *Isothecium algarvicum* W.E.Nicholson & Dixon.

straightforward when the shape of the leaves, the size of the alar and supra-alar groups and the greater density and size of prorate dorsal cells are taken into consideration. Draper et al. (2015) previously described *I. algarvicum* leaves as being smooth or as having scattered prorate cells on its upper back, those of *I. alopecuroides* as distally prorate on back above and those of *I. mysuroides* as having occasional prorate cells, if presents scattered on back. Our results largely support this, in that they show *I. algarvicum* to be unambiguously the most prorate of the three species. We need to be careful however, because of the complex architecture of all *Isoetecium* being studied, where branch leaves obviously have more prorate cells than stem leaves (which may even be smooth): this could account for ambiguities. Molecular confirmation of the identity of the Tunisian plant would be useful since the genus is famous for being difficult to identify taxonomically.

*Isoetecium algarvicum* is a remarkable addition to North African flora. Its ecology appears to be fairly stable over its entire range. It grows on tree trunks and on acidic rocks in very humid environments in forested stands, in the Pinsapo forests of southern Spain (Guerra 1980) and mostly occurs in *Alnus glutinosa* riparian woodland, but also in *Quercus suber* and *Castanea sativa* woodland, and between boulders on rocky humid slopes in barrancos (steep sided valleys) and in the *Quercus robur*–*Q. pyrenaica* woodlands of Portugal (Sérgio et al. 2013). On the Iberian Peninsula, it is linked to a Montane-Mediterranean climate with more than 1000 mm year<sup>-1</sup> of precipitation and 5–6 dry summer months (Sérgio 1980–1981), which is consistent with the currently known locality in Kroumiria. In Madeira and on the Canary Islands, the species grows in many types of forests in sheltered, moist environments (Hedenäs 1992, Draper et al. 2015).

The occurrence of such a strong Macaronesian-oceanic element in North Africa allows for some phytogeographical speculation, which again, would benefit from molecular phylogeographic reconstruction. Some vascular plants dis-

play similar disjunct distributions centred in Macaronesia with peripheral isolated populations scattered in southern Europe and North Africa. This is particularly true for certain ferns, such as *Asplenium hemionitis* L., *Culcita macrocarpa* C.Presl., *Davallia canariensis* (L.) Sm., *Diplazium caudatum* (Cav.) Jermy, *Dryopteris guanchica* Gibby & Jermy and *Woodwardia radicans* (L.) Sm. (Prelli and Boudrie 2001). It is also true for some angiosperms, including *Dracaena draco* L. subsp. *ajgal* Benabid & Cuzin in S.W. Morocco (Benabid and Cuzin 1997), *Laurus azorica* (Seub.) Franco in southwest and central Morocco (Barbero et al. 1981) and *Prunus lusitanica* L. in northern Morocco, on the Iberian Peninsula and in southwestern France (Quézel and Médail 2003). Such disjunct distributions could result from long-distance bird-mediated dispersal (Brochet et al. 2009) or from the long-term fragmentation of what were previously continuous ranges (Cronk 1992). The concept of a metapopulation functioning through long-distance dispersal and implying a recent origin for the isolated Mediterranean populations is unlikely. Bird migration routes are north–south rather than west–east compared with the ranges of the concerned plants. On the other hand, the relict theory (Cronk 1992) stipulates that the Neogene Tethyan subtropical environments, reduced by Late Cenozoic glaciations (Rodríguez-Sánchez and Arroyo 2010), have partly persisted on Macaronesian archipelagos (Fernández-Palacios et al. 2011), and in favourable habitats scattered in southwestern Morocco (Médail and Quézel 1999), on the Atlantic Iberian coast (Postigo Mijarra et al. 2009) and around the Mediterranean (Quézel and Médail 2003). Patiño et al. (2015) suggests that oceanic and hyper-oceanic species, especially in bryophytes, reflect not only the extant flora but also possibly past ranges. Moreover, as these authors point out, their true insular populations and their isolated ‘insular-like’ continental populations, may constitute significant reservoirs of novel biodiversity for the assembly of continental flora during future humid periods.

Table 2. Bryophytic communities of Oued Ellil and O. Zen (underlined Latin names are only found in O. Ellil; names in bold type are only observed in O. Zen; other species are common to both rivers).

	Ecological characteristics	Main species	Bryosociology
1	Aquatic assemblage on rocks	<u>Chiloscyphus polyanthos</u> , <u>Fontinalis antipyretica</u>	<i>Fontinalion antipyreticae</i> W. Koch. 1936
2	Amphibious community of low topographical level	<b><i>Racomitrium aciculare</i></b> , <b><i>Rhynchostegium confertum</i></b> , <u><i>R. riparioides</i></u> , <i>Rhynchostegiella tubulosa</i> , <b><i>Riccardia chamedryfolia</i></b> , <i>Scapania undulata</i> , <u><i>Schistidium apocarpum</i></u> , <u><i>Scorpiurium deflexifolium</i></u>	<i>Platyhypnidion rusciiformis</i> Phil. 1956
3	Amphibious taxa on sand accumulation	<u><i>Cirriphyllum crassinervium</i></u> , <u><i>Lunularia cruciata</i></u> , <i>Rhynchostegiella tubulosa</i>	<i>Brachythecion rivularis</i> Hertel 1974
4	Ferruginous mud	<i>Calypogeia fissa</i> , <i>Cephalozia bicuspidata</i> , <b><i>Conocephalum conicum</i></b> , <b><i>Dicranella heteromalla</i></b> , <i>Epipterygium tozeri</i> , <i>Kindbergia praelonga</i> , <i>Scapania undulata</i> <i>Solenostoma hyalinum</i>	<i>Dicranellion heteromallae</i> Phil. 1963
5	Terricolous assemblages of wet silt	<i>Fissidens taxifolius</i> , <b><i>Homalia lusitanica</i></b> , <i>Lunularia cruciata</i> , <i>Microeurhynchium pumilum</i> , <b><i>Plagiomnium undulatum</i></b> , <i>Thamnobryum alopecurum</i>	<i>Fissidention taxifolii</i> Marst. in Marst. 2006
6	Terro-saxicolous mesic assemblages	<u><i>Cirriphyllum crassinervium</i></u> , <i>Cololejeunea rossettiana</i> , <b><i>Fissidens serrulatus</i></b> , <i>Fossombronina angulosa</i> , <i>Isoetecium algarvicum</i> , <u><i>I. mysuroides</i></u> , <i>Lejeunea cavifolia</i> , <b><i>Lophocolea fragrans</i></b> , <u><i>Plagiothecium nemorale</i></u> , <b><i>Saccogyna viticulosa</i></b> , <i>Thamnobryum alopecurum</i>	<i>Dicranellion heteromallae</i> Phil. 1963
7	Soil communities	<i>Fissidens taxifolius</i> , <i>Pleuroidium acuminatum</i> , <i>Trichostomum brachydontium</i>	<i>Fissidention taxifolii</i> Marst. in Marst. 2006
8	Dry rock community	<u><i>Grimmia lisae</i></u> , <u><i>Homalothecium sericeum</i></u> , <i>Nogopterium gracile</i>	<i>Grimmion commutatae</i> V. Krus. 1945
9	Bark-dwelling communities	<u><i>Hypnum cupressiforme</i></u> , <i>Leptodon smithii</i> , <i>Nogopterium gracile</i> , <i>Porella arboris-vitae</i> , <u><i>P. obtusata</i></u>	<i>Neckerion complanatae</i> Šm. & Had. ex Kl. 1948



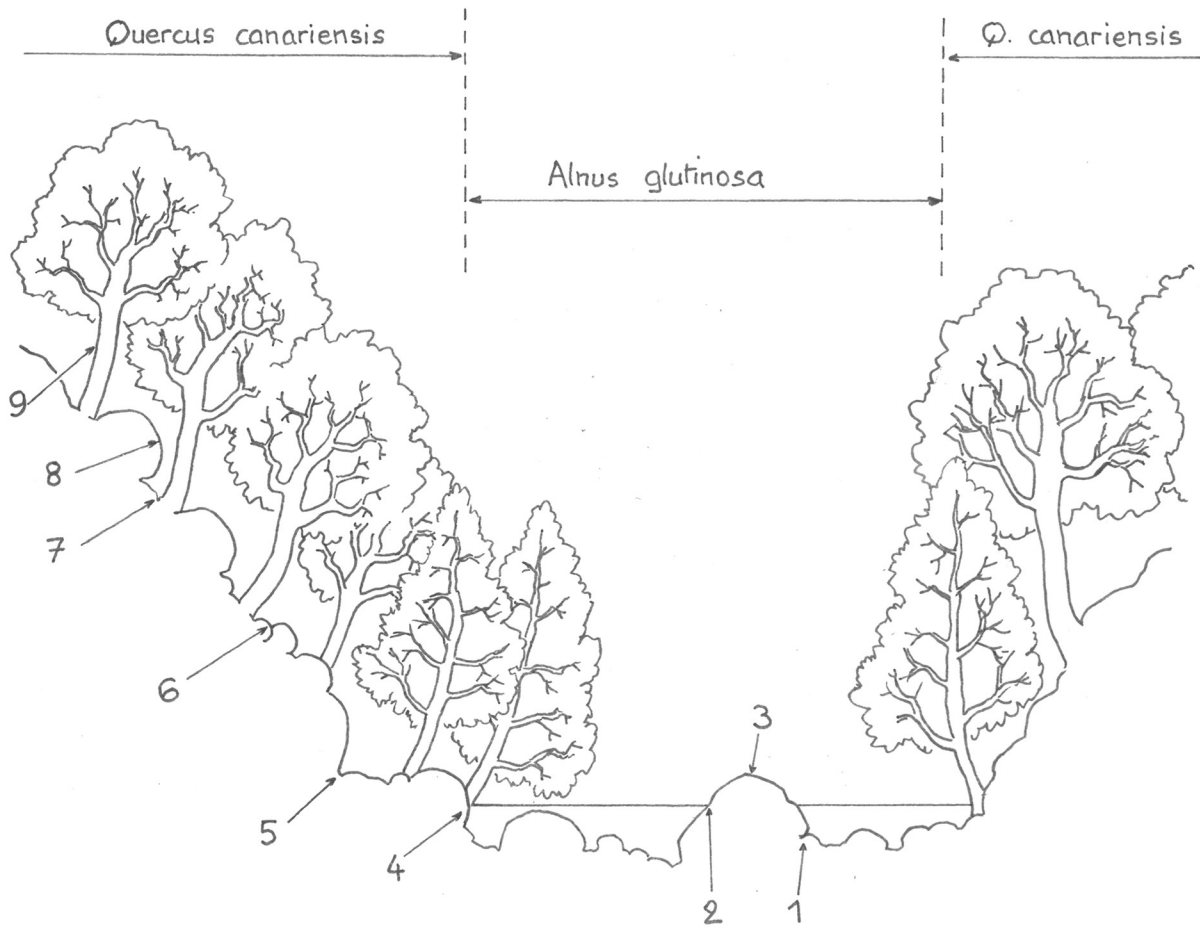


Figure 7. Topographical sequence of bryophytic communities in Oued Ellil (numbers refer to communities described in Table 2).

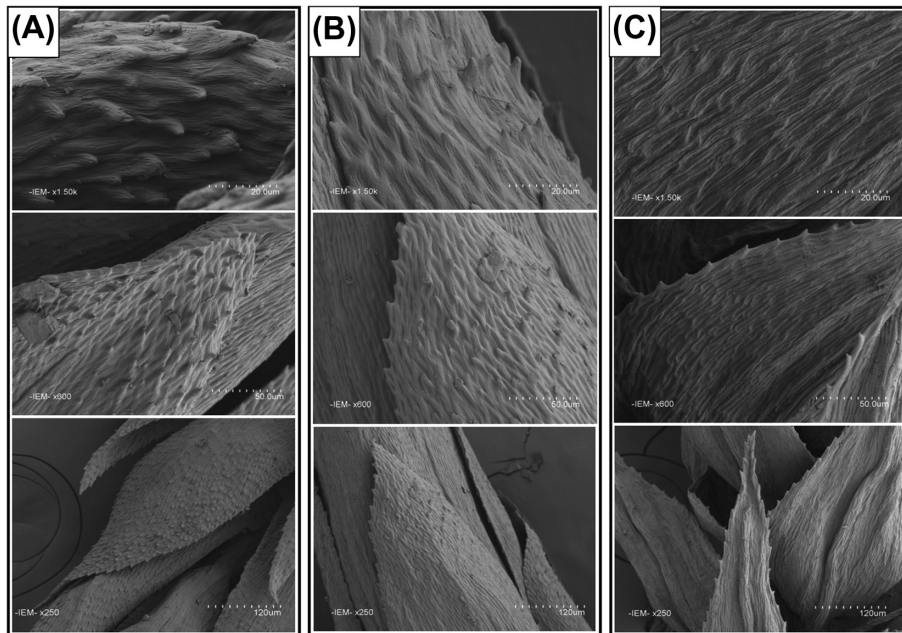


Figure 2. Dorsal side of leaves of *Isoetecium* species. Electron microscope scans: (A) *Isoetecium algarvicum*, (B) *I. alopecuroides*, (C) *I. myosuroides*.



*Isoetecium algarvicum* thus appears to be a remarkable element of this relict Neogene flora, making the riparian forests where it develops very valuable both historically and for conservation. The other species that are found in the wet riparian habitats of northwestern Tunisia can be considered comparable Tertiary relicts: e.g. *Hypericum androsaemum*, *Ilex aquifolium*, *Laurus nobilis* L. and *Osmunda regalis* L. (Quézel and Médail 2003). Although no phylogenetic data are available, the status of the liverworts *Lophocolea fragrans* (Moris & De Not.) Gottsche, Lindenb. & Nees, *Pallavicinia lyellii* (Hook.) Carruth., *Saccogyna viticulosa* (L.) Dumort. and the mosses *Heterocladium flaccidum* (Schimp.) A.J.E.Sm. and *Pseudotaxiphyllum elegans* (Brid.) Z. Iwats that were also recorded in Tunisia (Hugonnot et al. 2020, Ben Osman et al. 2021c, 2022b) is probably similar to that of *Isoetecium algarvicum*.

The reproductive status of *Isoetecium algarvicum* lends further support to the relictual hypothesis. The apparent absence of male individuals in North Africa may explain the inability of fertilisation occurring. This sterility drastically reduces the possibility of population expansion as no spores are available. Today, the multiplication of the species relies solely on vegetative expansion and is likely to be very slow. Given the unfavourable climatic characteristics of most of the adjacent landscape, the species appears confined along these three Kroumirian rivers.

## Conservation

While *Isoetecium algarvicum* is listed as being of least concern in Europe (Sérgio et al. 2019, Hodgetts and Lockhart 2020), it is relatively common in Macaronesia and thus classified as near threatened (Sim-Sim et al. 2014). Its restricted distribution on the Iberian Peninsula makes it vulnerable (Garilleti and Albertos 2012, Sérgio et al. 2013). To date, the Tunisian populations are the only ones known in Africa. The discovery of *I. algarvicum* in N. Tunisia raises the probability that it is present in other mountain ranges of North Africa, particularly in Morocco (Rif) and in Algeria (Numidia, Kabylia). Inadequate forest management (illegal cuts, overexploitation, fire) is certainly the main threat. The site should receive statutory protection given the high biological value of floristic assemblages occurring in it.

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## Data availability statement

Data are available from the Dryad Digital Repository: <<https://doi.org/10.5061/dryad.tb2rbp046>> (Ben Osman et al. 2022c).

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