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Rapid Communication

Morphological and molecular data confirm the first record of *Dysphania cristata* (Dysphanieae, Chenopodioideae, Amaranthaceae) in the Mediterranean basin

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Abstract

Both morphological and molecular data confirmed the presence of the Australian *Dysphania cristata* in the Mediterranean basin. This is the first record in this region (made in Mahdia city, Tunisia). A brief morphological description of this species is provided along with a subsequent comparison with related and similarly looking *Dysphania* species. The status of *D. cristata* in Tunisia is also discussed.

Key words: alien plant, Chenopodiaceae, new record, North Africa, Tunisia, weed

Introduction

The genus *Dysphania* R.Br. in its recent circumscription encompasses more than 50 species with predominant occurrence in the tropics and subtropics around the world (Uotila et al. 2021). In Africa, Sukhorukov et al. (2016) counted five native species [*Dysphania botrys* (L.) Mosyakin & Clemants, *D. congolana* (Hauman) Mosyakin & Clemants, *D. procera* (Hochst. ex Moq.) Mosyakin & Clemants, *D. pseudomultiflora* (Murr) Verloove & Lambinon, and *D. schraderiana* (Schult.) Mosyakin & Clemants] and four alien species [*D. ambrosioides* (L.) Mosyakin & Clemants, *D. carinata* (R.Br.) Mosyakin & Clemants, *D. multifida* (L.) Mosyakin & Clemants, and *D. pumilio* (R.Br.) Mosyakin & Clemants]. In Tunisia, the genus is represented only by the naturalized American species *D. ambrosioides* (Pottier-Alapetite 1979; Dobignard and Chatelain 2011, in both references as *Chenopodium ambrosioides* L.). Recent floristic studies revealed another species of *Dysphania* in Tunisia: *D. cristata* (F.Muell.) Mosyakin & Clemants.

Materials and methods

Sampling and morphological identification

In the spring (March–April) of 2021, during botanical field surveys in Mahdia governorate (northeast of Tunisia), the first author (REM) collected an unknown aromatic species of *Dysphania*. The specimens were identified as *Dysphania cristata*, but we decided to verify this identity by means of molecular phylogeny owing to a striking resemblance of this species to *D. carinata* (R.Br.) Mosyakin & Clemants. Voucher herbarium specimens are preserved in the personal herbarium of R. El Mokni at the Faculty of Pharmacy of Monastir (Herb. Univ. Monastir, not listed in Index Herbariorum), and duplicates were deposited at MHA [multiaccess center Herbarium MBG RAS (supported by program no. 075-15-2021-678)] and LE herbaria (herbarium abbreviations according to Thiers 2023+).

DNA extraction and sequencing

The total DNA was extracted from dried leaves by the CTAB procedure according to the manufacturer's protocol (Doyle and Doyle 1987). Following Uotila et al. (2021), for the molecular phylogenetic analysis, we used two nuclear ribosomal markers (ITS and ETS) and two plastid DNA markers (*rpl16* intron and *atp*B-*rbc*L). All PCR protocols and primer sequences were exactly the same as in ref. (Uotila et al. 2021). The PCR mix (20 µL) contained 1.5–2.0 ng of the total DNA, 5 pmol of each primer, 4 µL of Ready-to-Use PCR Master mix 5× MasDDTaqMIX-2025 containing a "hot-start" SmarTaq DNA polymerase (Dialat Ltd., Moscow, Russia, http://en.dialat.ru), and 13 µL of deionized water. PCR was carried out on an MJ Research PTC220 DNA Engine Dyad Thermal Cycler (Bio-Rad, Foster City, CA, USA, https://www.bio-rad.com). The PCR products were purified by precipitation in a 0.125 M ammonium acetate solution in 70% ethanol and visualized by electrophoresis in a 1% agarose gel in 0.5× TBE buffer containing ethidium bromide. All PCR products were sequenced on a 3730 DNA Analyzer (Applied Biosystems, Foster City, CA, USA, https://www.thermofisher.com) at Syntol LLC (Moscow, Russia, https://www.syntol.ru) with the same primers that were used to amplify each locus in both directions (MBG RAS state assignments nº. 122042700002-6). All sequences were deposited in the GenBank database; accession numbers of the newly obtained sequences of *D. cristata* (TUN1) are: OR584244 (ITS), OR593985 (ETS), OR593983 (*atp*B-*rbc*L), and OR593984 (*rpl16* intron).

Molecular alignment and phylogenetic analysis

The core dataset was reconstructed utilizing the GenBank accession numbers first published by Uotila et al. (2021); the new sequences of the loci ITS, ETS, *atp*B-*rbc*L, and *rpl16* intron of *D. cristata* were added to the

analyses. The concatenated alignment consisted of 58 nucleotide sequences. All the sequences were aligned in MAFFT v.7 with the L-INS-I strategy (Katoh et al. 2002; Katoh and Standley 2013) and manually corrected and concatenated in BioEdit v.7.0 (Hall 1999).

Maximum likelihood (ML) analyses of all molecular alignments (Felsenstein 1981) were conducted in RAxML v.8.2.10 using raxmlGUI beta version 2.0 (Stamatakis 2014; Kozlov et al. 2019; Edler et al. 2021) under the assumptions of the GTR + GAMMA model (Stamatakis 2014). ML bootstrap values (Sauermann 1989) were based on 1000 fast replicates (Stamatakis 2014). We visualized the resulting trees in FigTree v.1.4.3 (Rambaut and Drummond 2012) and finally prepared all the figures by means of InkScape v.0.48.2 (https://inkscape.org/release/inkscape-0.48/). The ML tree was rooted by *Axyris prostrata* L. (isolate Chen3003) and *Axyris prostrata* (isolate Chen0118) (Uotila et al. 2021).

Results

Only one locality of ca. eight individuals was observed on roadsides in ruderal habitats.

Dysphania cristata (F.Muell.) Mosyakin & Clemants, Ukr. Bot. Zhurn. 59: 382 (2002)

≡ *Blitum cristatum* F.Muell., Trans. Phil. Inst. Viet. 2: 73 (1858) ≡ *Chenopodium cristatum* (F.Muell.) F.Muell., Fragm. 7: 11 (1864) ≡ *Neobotrydium cristatum* (F.Muell.) M.L.Zhang & G.L.Chu, Pl. Diversity 38: 327 (2016).

Lectotype (Wilson 1983: 176). Raro in planitiis sterilioribus subsalsis prope montis Flindersianos, Oct. 1851, F. Mueller (MEL-19998; isolectotype: PERTH).

Morphology. (Figure 1) A description of this Australian species is available, e.g., in refs. Wilson (1983, 1984; as *Chenopodium cristatum* (F.Muell.) F.Muell.).

The principal distinguishing characteristics of *D. cristata* are the annual life form; stems prostrate (Figure 1A); leaves short-petiolate, blades ca. 1 cm long, ovate, lobed; inflorescences axillary (Figure 1B); flowers with five free crested perianth segments carrying multicellular hairs (Figure 2A). Morphologically, *D. cristata* is very similar to another Australian member of *Dysphania*: *D. carinata* (R.Br.) Mosyakin & Clemants; both species are well distinguished by the perianth shape (Figure 2A, B).

Molecular phylogeny. The total number of characters in the final concatenated alignment was 2,611, consisting of 541 parsimony informative characters and 836 variable characters. An ML analysis of the combined dataset resulted in a tree with –InL = 11442.776242. The molecular phylogenetic analysis confirmed that the specimens collected in Tunisia belong to *D. cristata* (Figure 3).

Examined specimen. (new record). **TUNISIA**: Mahdia, Mahdia city (Mahdia governorate), roadsides in human-made habitats, alt. 3 m a.s.l., 30 March 2021, R. El Mokni s.n. (MHA, LE, Herb. Univ. Monastir).

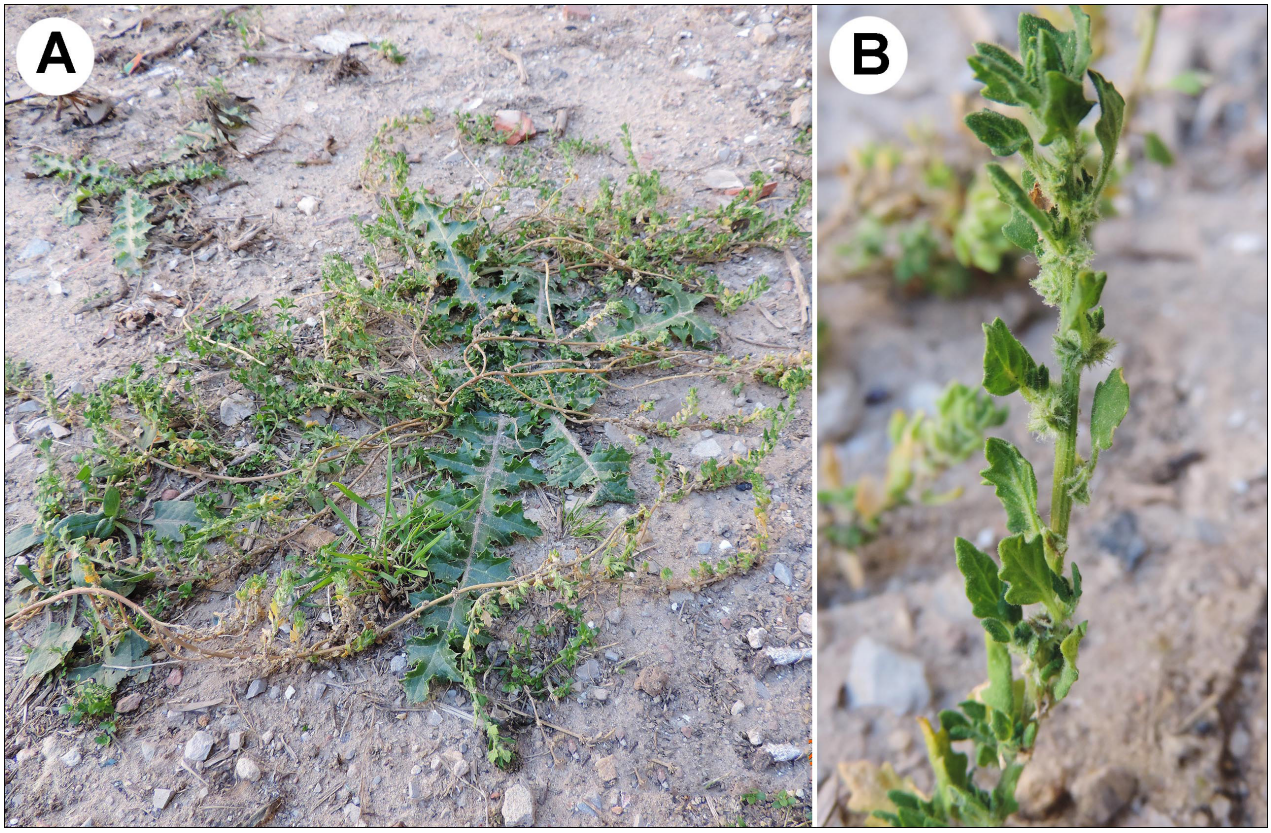

Figure 1. *Dysphania cristata*. A: General view of the plant (together with rosulate leaves of *Scolymus grandiflorus*), B: close-up of a shoot. Photographs by R. El Mokni (Tunisia, Mahdia city, 27 March 2021).

Phenology in Tunisia. Flowering and fruiting from March to April.

Habitat and ecology. The single small population was observed in roadsides in human-made habitats and near flowerbeds on sandy-loam coastal soils. The plants of *D. cristata* were accompanied mainly by *Chenopodiastrum murale* (L.) Fuentes, Uotila & Borsch; *Cynodon dactylon* (L.) Pers.; *Rumex spinosus* L. [formerly known as *Emex spinosa* (L.) Campd.]; *Euphorbia peplus* L.; *Hordeum murinum* L.; *Oxalis corniculata* L.; *Polycarpon tetraphyllum* (L.) L.; and *Scolymus grandiflorus* Desf.

Pathways of introduction and current naturalization status in Tunisia. The exact introduction mode of *D. cristata* remains unknown but most likely has resulted from the use of imported seed mixtures for gardening purposes or for feeding of domesticated birds. However, other pathways of introduction are also possible (see e.g. Sirbu et al. 2022). The species seems to be a recent introduction, only known from a single locality. According to the terminology of Pyšek et al. (2004), this species can be now considered only a casual alien. This status may change in the future if further observations are available.

General distribution. Outside its native range (Australia: see Wilson 1983, 1984; POWO 2023), the species is reported as a casual alien from Great Britain (Uotila 2011+), Belgium (F. Verloove *pers. comm.*), the Netherlands and Sweden (GBIF 2023), South Africa and the southeastern USA (POWO 2023; WFO 2023). Our report here is the first record of this species in the Mediterranean area including North Africa (Figure 4).

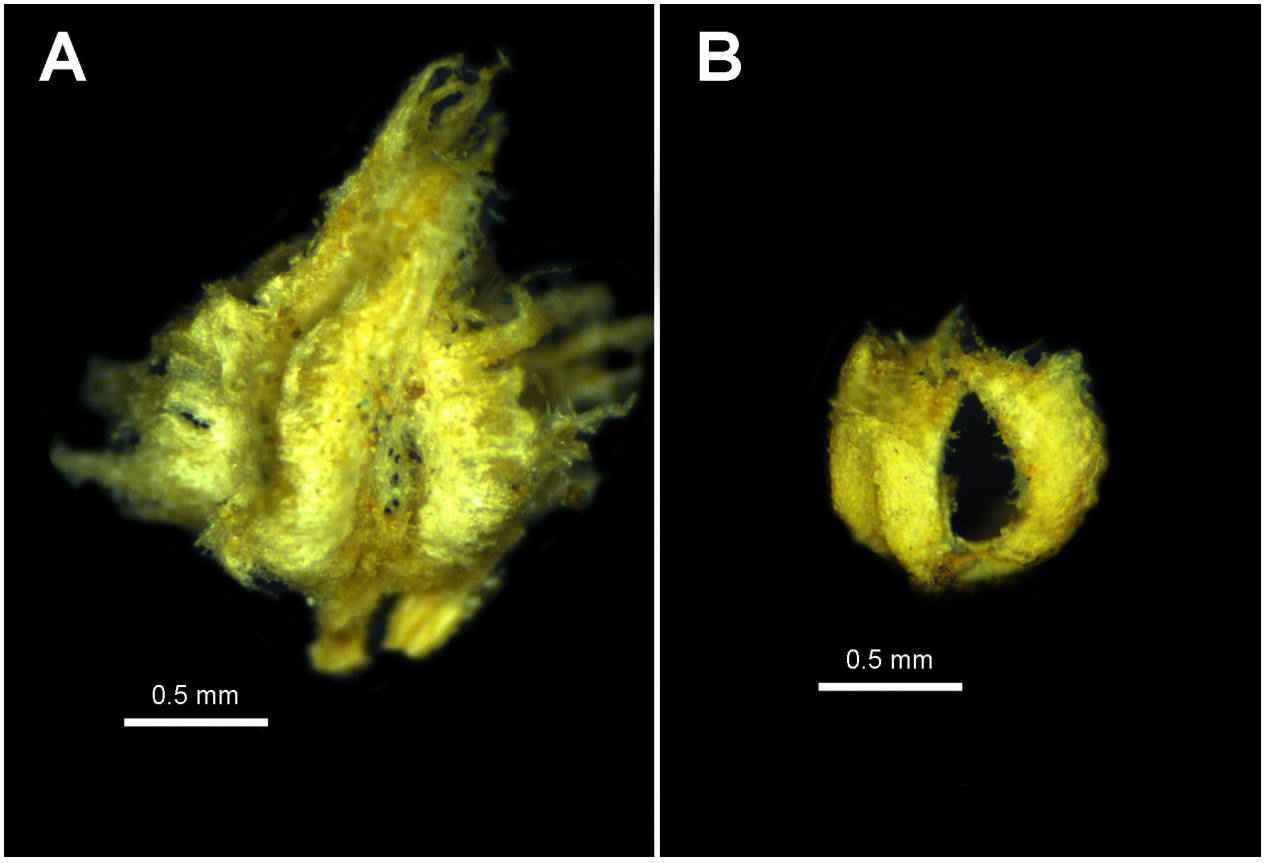

Figure 2. Perianth segments at the fruiting stage. A: *D. cristata*, B: *D. carinata.* Photographs by M. Kushunina.

Discussion

In the past, some *Dysphania* species have often been confused with one another, especially alien Australian members; for more details, see refs. (Lhotská and Hejný 1979, as *Chenopodium*) and (Sukhorukov et al. 2019, 2021). The taxonomic composition of native African *Dysphania* has been clarified lately (Sukhorukov et al. 2016, 2018, 2019). *Dysphania cristata* was reported in southern Africa and as well as its putative hybrid with *D. cristata* [*D.* × *bontei* Aellen] (Germishuizen and Meyer 2003, all species as *Chenopodium*). In Mediterranean North Africa, alien *Dysphania* taxa are represented by only two species: the widely naturalized Central American *D. ambrosioides* (Maire 1962; Quézel and Santa 1962; Boulos 1995; Uotila 2011; Dobignard and Chatelain 2011) and the locally occurring coastal *D. multifida* from South America that is found in the western part of the region (Morocco and Algeria: Uotila 2011). The number of alien *Dysphania* species in North Africa is clearly underestimated in comparison with southern Europe, where four alien species of the genus (*D. ambrosioides*, *D. multifida*, *D. pumilio*, and *D. schraderiana*) are known (Uotila 2011). The presence of a fifth alien species from America—*D. anthelmintica* (L.) Mosyakin & Clemants, a close relative of *D. ambrosioides* claimed from Italy (Iamonico 2011a)—is questionable due to only weak morphological differences between these species. On the basis of field and herbarium studies and the literature,

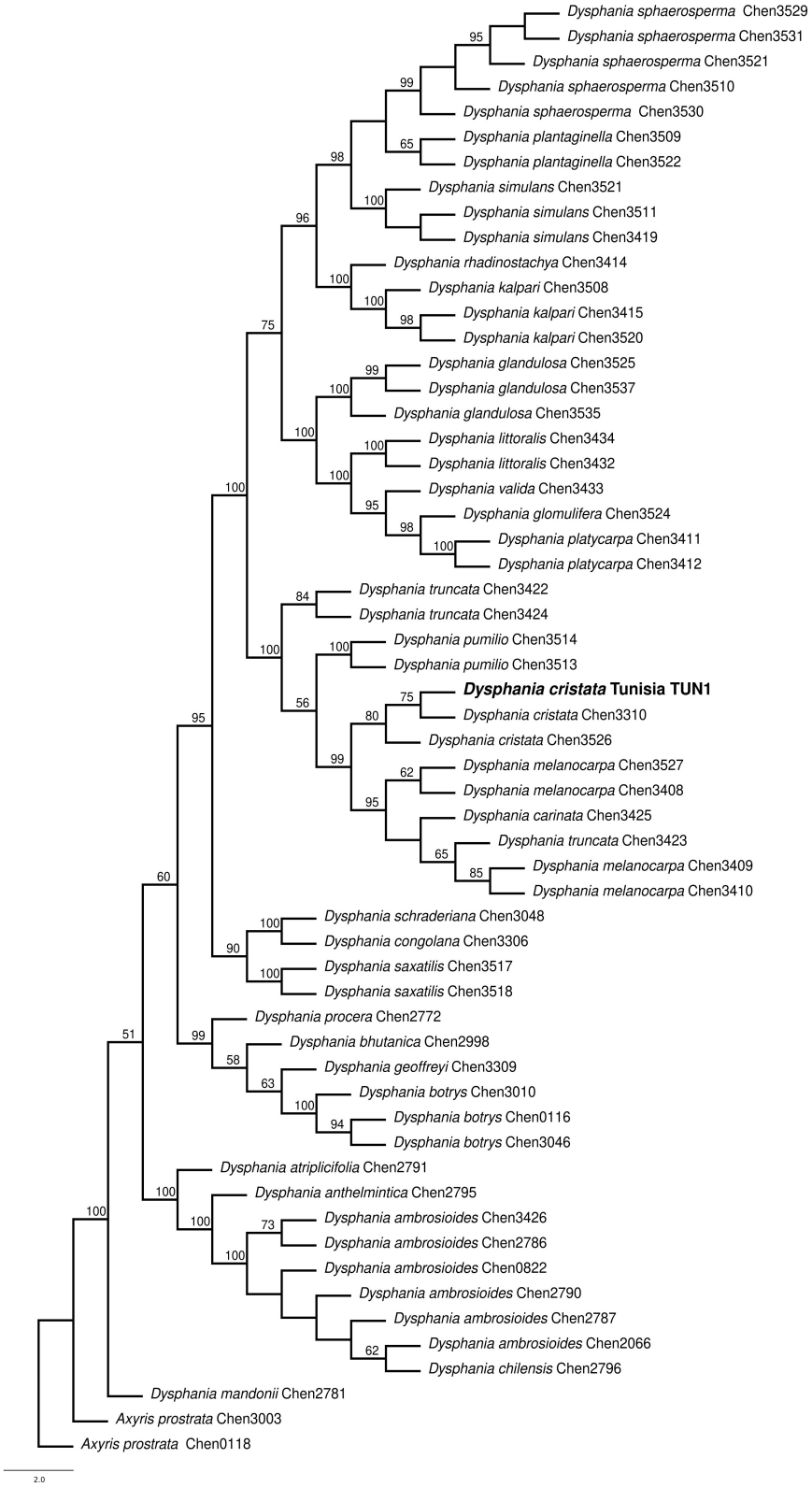

Figure 3. The phylogenetic tree resulting from an ML analysis (–InL = 11442.776242) of combined plastid and nuclear data from 58 Dysphanieae taxa. Representatives of Axyrideae serve as an outgroup. Bootstrap support values > 50 are given above branches.

Sukhorukov et al. (2021, with references therein) stated that *D. pumilio* is the most successful invader of the genus in southern Europe. Nevertheless, in Mediterranean North Africa, it still has not been reported. As to *D. schraderiana*

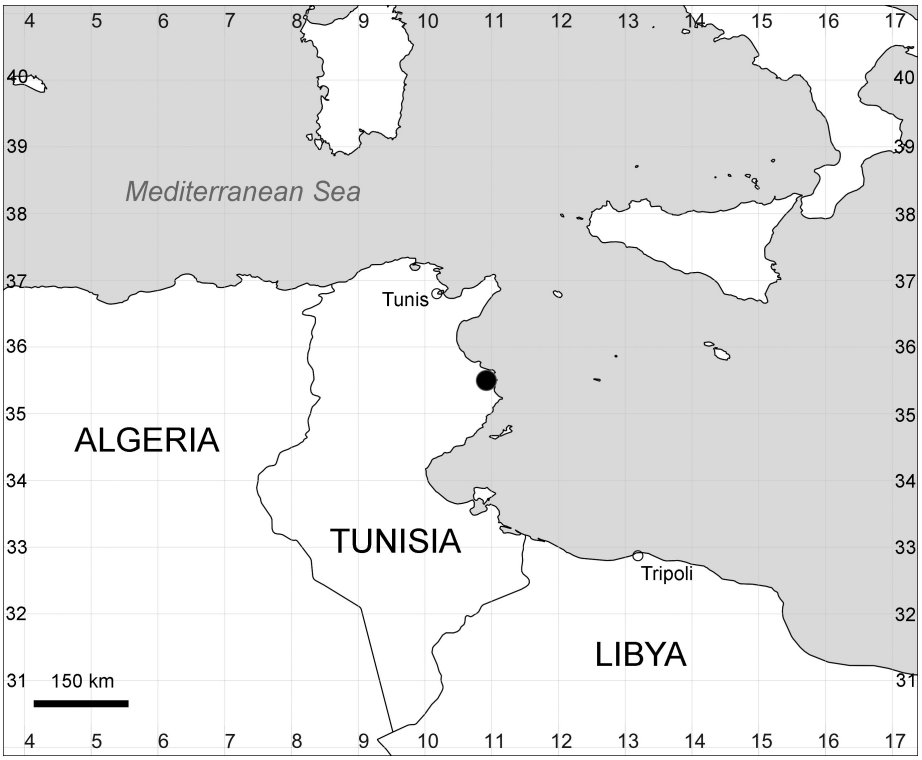

Figure 4. The map with the new record of *D. cristata* in the Mediterranean basin.

(a species native to East Africa and South Arabia), its sporadic presence in Europe is probably connected with past cultivation as a medicinal plant (Kaplan et al. 2017). Besides, field studies of the last author (APS) in some regions of Africa suggested that the Australian *D. carinata* occurs widely in river beds and ruderal places in East Tropical Africa and Namibia, while it is still rare and ephemeral in temperate Europe and absent in the Mediterranean region (Uotila 2011; Sukhorukov et al. 2021; Verloove 2023).

Key to the *Dysphania* species registered in the Mediterranean basin

A simplified key includes both native (*D. botrys*) and all alien *Dysphania* species known in the Mediterranean basin. *Dysphania carinata* is also included because of its presence in West and Central Europe and some regions of Africa. *Dysphania anthelmintica* claimed from Italy (Iamonico 2011a) is not included in the key (see the note above).

1. Leaves entire to sinuate; perianth segments connate to half-way..... .....*D. ambrosioides*
–. Leaves lobate, pinnatifid to pinnatisect; perianth segments almost free or completely fused.....2
2. Perennial coastal herb; perianth elliptic, hardened, with fused segments.... .....*D. multifida*
–. Annuals; perianth ± rounded or ovate when closed, its segments (almost) free.....3

3. Plants erect; leaves (including petioles) usually more than 4.0 cm long; seed embryo horizontal 4
–. Plants prostrate or ascending; leaves up to 2.5(3) cm long; seed embryo vertical 5
4. Inflorescence leafy in lower part or completely aphyllous; perianth segments not cristate, with stalked glandular hairs ..... *D. botrys*
–. Inflorescence leafy at least in lower and middle parts; perianth segments cristate at the back, with sessile glands..... *D. schraderiana*
5. Perianth segments slightly thickened but not clearly keeled or crested, glabrous or with scattered hairs ..... *D. pumilio*
–. Perianth segments keeled or crested, hairy..... 6
6. Perianth segments crested (Figure 2A) ..... *D. cristata*
–. Perianth segments keeled (at least in their upper part; Figure 2B)..... ..... *D. carinata*

For the distribution pattern of each species in the Mediterranean basin see Uotila (2011) with an addition by Iamonico (2011b) for Italy (*D. pumilio*). The records of *D. botrys* from Egypt (Boulos 1995), Algeria, and Libya (Uotila 2011; APD 2023), if correctly identified, are remarkably isolated from the main range in Eurasia and may be of alien origin. The alien status of *D. botrys* can be even stated for Yaffa port city, where it was collected once in the 19th century (G!) but later has not been included in any checklists. The native status of this species in North Africa should be confirmed by further research.

Conclusion

Our report rigorously confirmed an unexpected first record of *D. cristata* in the Mediterranean area (Tunisia). Records of other alien species of the genus in North Africa are highly probable.

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Author's contribution

Research conceptualization: Ridha El Mokni, Alexander P. Sukhorukov. Sample design and methodology: Ridha El Mokni, Alina V. Fedorova, Maria Kushunina, Alexander P. Sukhorukov. Investigation and data collection: Ridha El Mokni, Alina V. Fedorova, Alexander P. Sukhorukov. Data analysis and interpretation: Alina V. Fedorova, Alexander P. Sukhorukov. Funding provision: Alexander P. Sukhorukov. Writing: Ridha El Mokni, Alina V. Fedorova, Alexander P. Sukhorukov.

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