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Morphology, ecology, and co-occurrence of *Iris polystictica* and *Iris oratoria* (Insecta: Mantodea) in the Western Black Sea Region

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Abstract. The distribution of *Iris polystictica* Fischer-Waldheim, 1846, and *Iris oratoria* Linne, 1758, in the Western Black Sea region is discussed, based on new data, citizen science records, and literature. *Iris polystictica* is reported for the first time in Romania, and the status of *Iris oratoria* in the country is reassessed. New methods for distinguishing between these two species are presented, based on ratios of different body parts, which can also be applied to photographs. Finally, the habitat preferences of *Iris polystictica* in Ukraine are discussed, with implications for the distribution of these two *Iris* species in the Balkans and Black Sea regions.

Keywords: Balkans, Dictyoptera, praying mantis, Romania, Ukraine.

Introduction

The genus *Iris* Saussure, 1869, belongs to the family Eremiaphilidae within the order Mantodea and comprises 14 species (Otte et al. 2020). The general distribution of the genus extends from the Atlantic coast of the Mediterranean and North Africa to the Ordos Desert in eastern Central Asia. In addition, *Iris oratoria* Linne, 1758 has been introduced to the southwestern United States, where it has established a stable, extensive population (Battiston et al. 2010, 2021). In Europe, two species of the genus *Iris* are found: *Iris oratoria* and *Iris polystictica* Fischer-Waldheim, 1846. At the beginning of the last century, *I. polystictica* was regarded as a synonym or subspecies of *I. oratoria*. It was only in 1935 that M. Beier recognized it as a distinct species, a distinction later supported by significant differences in the structure of the male copulatory organs (Kaltenbach 1963).

The natural distribution of *I. oratoria* spans almost the entire Mediterranean and includes Iran, but its presence in Pakistan and India has yet to be confirmed (Battiston 2020, Battiston et al. 2021). The general distribution of *I. polystictica* is situated to the northeast of *I. oratoria*'s range, extending through steppes, deserts, and semi-deserts from the Northern Black Sea coast in the west to Inner Mongolia in the east (Shcherbakov et al. 2013, Shcherbakov & Battiston 2020). The ranges of these two species are either approaching each other or may overlap in the Western Black Sea, as well as south of the Caucasus and the Caspian Sea (Battiston 2020, Shcherbakov & Battiston 2020). However, the distributional limits of both species in the Balkan Peninsula and the Western Black Sea region remain unknown. The range of *I. oratoria* does not extend beyond the Mediterranean climate (Battiston et al. 2021). On the Balkan Peninsula, the northernmost distribution of the species extends to Croatia (Kment 2012), Montenegro (Vujić & Zlatić 2021), North Macedonia (Chobanov & Mihajlova 2010), and southern Bulgaria (Popov & Chobanov 2004), with a recorded presence of *I. oratoria* in Romania (Cazacu 2019). At the western edge of *I. polystictica*'s distribution, several localities are known from the Danube and Dniester interfluve in southwestern Ukraine, with two records from Moldova (Popa & Tishenkov 1996, Derjanschi et al. 2016, Bronskov 2024, Bronskov et al. 2024).

It is important to note that mantises, including species of the genus *Iris*, often spread artificially beyond their established ranges, primarily due to their ability to deposit oothecae on hard surfaces. However, due to significant climatic differences in new areas, they often fail to form viable populations there (Schwarz & Ehrmann 2018). Therefore, to distinguish between accidental introductions and established populations, it is crucial to confirm their presence at the same site across multiple years.

Despite its seemingly rare occurrence in the wild, *I. oratoria* is quite frequently represented in photographs on social media, thanks to its interesting appearance and coloration (Battiston et al. 2021), which applies to *I. polystictica*, too. The development of digital photography on smartphones and global electronic biodiversity databases has enabled the collection of vast amounts of information on species distribution, with the added advantage of confirming this information through photographs, in addition to scientific data. However, the characteristics used for insect identification are not always reliable for identifying species from photographs.

The genus *Iris* is relatively easy to distinguish from other mantis genera through photographs, but identifying species within the genus can be more challenging. *I. polystictica* is similar to *I. oratoria*, but slightly smaller. Its forewings are narrower, and the discoidal area of the hindwings is greenish-yellow, without transparent windows, and spotted with brownish marks. The female wings are ochre-colored with a zigzag dark spot, and the edge is yellowish-lemon. The male pseudophallus is long and pointed, with a lateral spike (Kaltenbach 1976, Battiston & Massa 2008). In photographs, the two species can be easily distinguished by the presence or absence of transparent windows in the disc-shaped area of the hindwings. However, photos of *Iris* with open wings are rare on social media. The color of the internal spines on the anterior femur is highly variable, making this characteristic

unreliable for identifying *Iris* species. Additionally, the other differences mentioned above are not visible in photographs, so other traits are needed for accurate species identification based on pictures.

Material and methods

Various surveys were conducted in Romania and Ukraine to gather new data on the distribution and habitats of *Iris oratoria* and *Iris polystictica*.

In Romania, surveys were conducted between 2022 and 2024, in the Dunele Marine de la Agigea Nature Reserve. The surveys were primarily carried out by visual inspection of vegetation with a sweeping net, and occasionally with a UV light trap. The collected specimens are stored in the personal collection of A.-M. Pintilioaie.

In Ukraine, surveys were conducted between 2018 and 2024, in 34 habitats of *I. polystitica*. These include the southwestern part of the Donetsk region (2018-2020; Bronskov & Filchakova 2022) and the southwest part of the Odesa region (2022-2023; Bronskov et al. 2024). The surveys involved visual inspection and sweeping in both natural and anthropogenically altered habitats, including areas within human settlements. *I. polystictica* is included in the Red Book of Ukraine, and its collection is legally prohibited except in exceptional cases with a special permit. As a result, no specimens were collected during the research; instead, photographs of the captured individuals were taken, and the individuals were released in the same place afterwards.

Alternatively, data on the distribution of *Iris* genus was critically analyzed using scientific (212 records) sources (Popa & Tishenkov 1996, Popov & Chobanov 2004, Chobanov & Mihajlova 2010, Derjanschi et al. 2016, Kavurka et al. 2018, 2019, Krainyk et al. 2018, Suchkov 2018, Viter 2018, Yepishin 2018, Cazacu 2019, Nitochko 2019, 2020, Shekhovtsov & Polstyanoy 2019, Havrylenko et al. 2020, Horbenko 2020, 2022, Battiston et al. 2021, Vujić & Zlatić 2021, Bronskov & Filchakova 2022, Bronskov et al. 2024), as well as citizen science data (iNaturalist (2024) – 521 records, UkrBIN (2024) – 19 records) from the Balkan Peninsula, the Black Sea region and Anatolia (Figure 1). The citizen science data were verified using available photographs to assess both the feasibility and accuracy of species identification.

Using original photographs as well as images from public scientific sources, we measured the relative wing length of females *I. polystictica* (Figure 2a) (75 individuals) and *I. oratoria* (Figure 2b) (105 individuals) in relation to their tergites. The number of fully closed tergites and the degree of overlap of partially closed tergites by the hindwings were measured in tenths. In addition, the same photographs were used to measure and compare the ratio of the length of the pronotum, wings, and abdomen in females. It should be noted that absolute measurements cannot be made from photographs; however, the ability to enlarge images enhances the accuracy of measuring and comparing different parts. Wings were measured from the posterior edge of the pronotum to the edge of the hindwings when folded, while the abdomen was measured from the posterior edge of the pronotum to the end of tergite 10. When selecting photographs for measuring the ratios, special attention was given to the angle of the insects in the images. Any position other than perpendicular to the camera lens axis could result in distortion of the length ratios due to the perspective effect in the photographs. For the measurements, we selected photographs of *Iris* from the European regions within the species' ranges, excluding areas where their ranges might intersect. The average and standard deviation (SD) were calculated for all measurements. To compare the variation of specific quantitative traits across species, we used probability density diagrams. Statistical analyses were conducted using Microsoft Excel 2021. Geographic information was processed using the free software QGIS. Plant names are provided according to the Plants of the World Online website (POWO 2024).

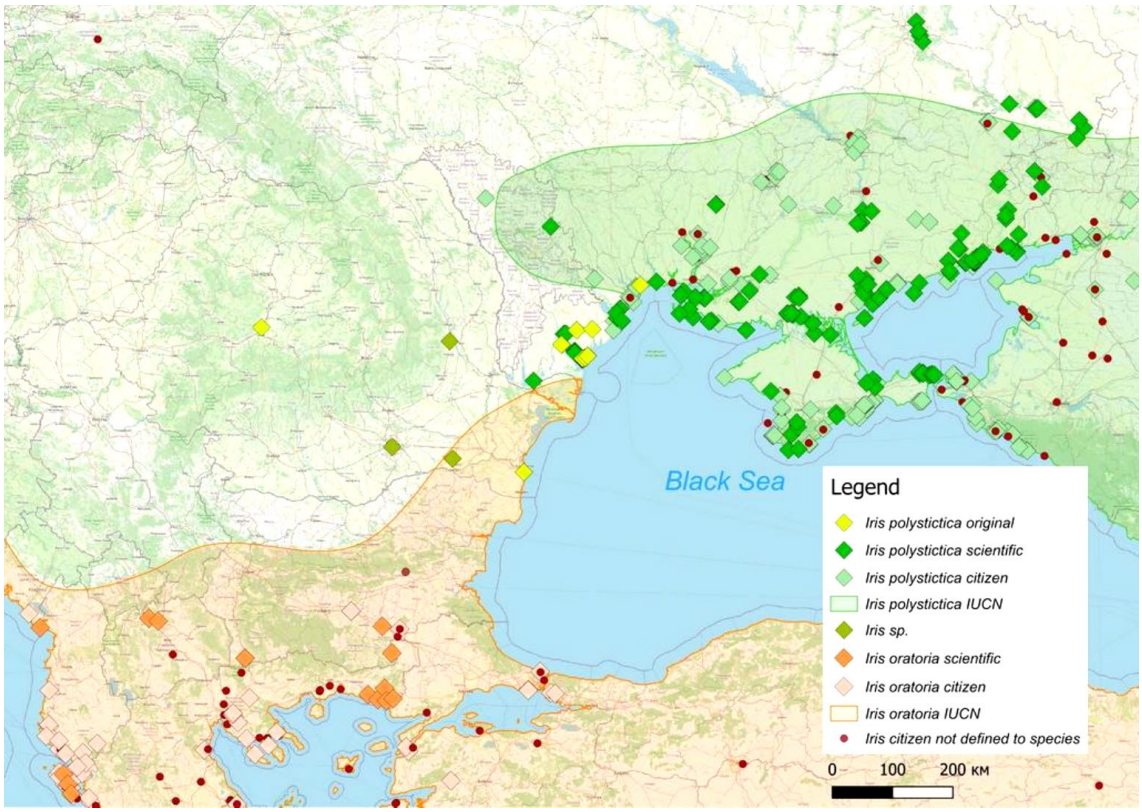

Figure 1. Presence records of *Iris* in the Black Sea region, the northern Balkans, and north Anatolia. Distribution of *Iris polystictica* according to Shcherbakov & Battiston (2020) and *Iris oratoria* according to Battiston (2020). Base map: OpenStreetMap.

Figure 2. a. Female of *Iris polystictica* from Bilosarayska Spit, Ukraine, photographed on 15.08.2018; b. Female of *Iris oratoria* from Festus, Crete, Greece, photographed on 07.09.2021 (photo: O. Bronskov)

Results

Material examined

Iris polystictica. ROMANIA: Constanţa county: Agigea, Dunele Marine de la Agigea Nature Reserve, 44.08710°N/28.64217°E, 26.08.2022, 1 male, Leg. Pintilioaie Alexandru-Mihai (Figure 3); same locality, 16.08.2023, 1 female, Leg. Pintilioaie Alexandru-Mihai (Figure 4a, b). UKRAINE: Odesa Oblast: Lymanskyi district, coast of the Velykyi Adzhalytskyi Lyman, 46.61983°N/30.88622°E, 26.08.2011, 1 male, Obs. Shchvets Oleksandr; Bilhorod-Dnistrovskyi district, coast of the Dzhantshei Lyman: 45.63536°N/29.78547°E, 22.07.2024, 1 nymph; same location: 45.63626°N/29.784463°E, 22.07.2024, 1 nymph; same location: 45.63629°N/29.78541°E, 22.07.2024, 1 nymph; same location: 45.63692°N/29.77573°E, 22.07.2024, 2 males; same location: 45.63766°N/29.77548°E, 22.07.2024, 2 nymphs; same location: 45.63824°N/29.77549°E, 22.07.2024, 1 nymph; same location: 45.63881°N/29.77520°E, 22.07.2024, 1 male, Obs. Bronskov Oleksandr; coast of the Malyi Sasyk Lyman, 45.67560°N 29.86909°E, 22.07.2024, 1 male, Obs. Khalaim Yevhen; Sarata, 46.01909°N/29.65834°E, 11.08.2024, 1 female; same location, 46.01832°N/29.65803°E, 11.08.2024, 1 female; coast of the Dzhantshei Lyman, 45.64901°N/29.82699°E, 13.08.2024, 1 female; coast of the Malyi Sasyk Lyman: 45.67524°N/29.86902°E, 20.08.2024, 1 female; same location, 45.67519°N/29.86982°E, 20.08.2024, 1 male; valley of the Khadzhyder river, 46.03194°N/29.95887°E, 02.09.2024, 1 female; Bolhradskyi district, Vynohradivka, 45.82688°N/29.37629°E, 24.09.2024, 1 female; surroundings of Vynohradivka, 45.82651°N/29.36308°E, 24.09.2024, 1 female, Obs. Bronskov Oleksandr.

Iris ssp. ROMANIA: Ilfov county, Bucharest, no exact coordinates, 29.07.2024, 1 adult male, observed inside a train, obs. Andreea Popa. Since the specimen was not collected but only photographed, and given the circumstances of the observation, it is impossible to identify it with certainty.

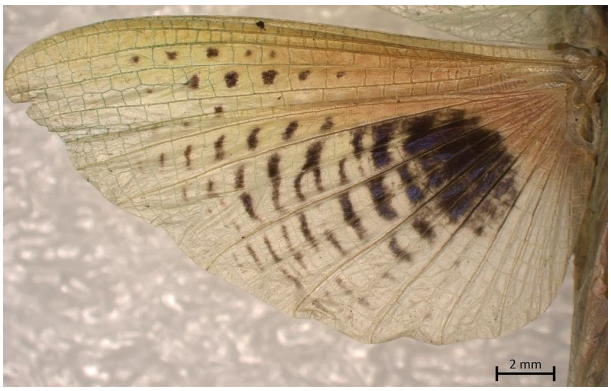

Figure 3. Hindwing of the male of *Iris polystictica* from Agigea, Romania (photo: A. Pintilioaie)

Figure 4.a. Habitus of the female of *Iris polystictica* from Agigea, Romania; b. hindwing of the same female (photo: A. Pintilioaie).

We also tried to examine the specimens of *Iris oratoria* (all males) reported before from Romania (Cazacu 2019), in order to clarify their identification. Only 2 of the 4 specimens have open wings (the ones from Alba County), allowing for clear identification, and it turns out these specimens were misidentified as *I. oratoria*, when in fact they belong to *I. polystictica*. These two specimens are housed at the Brukenthal Museum of Natural History, Sibiu, Romania, and the Grigore Antipa National Museum of Natural History, Bucharest, Romania (Cazacu, pers. comm. 2024). The male from Vrancea County is housed in the personal collection of Cazacu, but unfortunately, the wings are not open, making it impossible to assign the specimen to either *Iris* species. As for the specimen from Călăraşi County, only a few pictures are available, none of which show the wings spread, and the specimen was not collected. However, based on the geographical distribution of *Iris polystictica* and *Iris oratoria*, the records from Vrancea and Calarasi counties most probably belong to *Iris polystictica*.

The female from Agigea was kept in laboratory conditions and fed mealworms when necessary, once every 2-3 days. It laid one ootheca (Figure 5) on a stick placed inside the terrarium on 28 August 2023, after 10 days. After more than one week (exact data unavailable), it laid one more on the same stick. The two oothecae were kept under laboratory conditions to evaluate their fertility and the number of viable nymphs that will eventually emerge from them. During the following months, no nymphs were present in the terrarium, so we can assume that the female was infertile at the time of collection or that the conditions necessary for egg development were not met.

Figure 5. Ootheca of *Iris polystictica* from Agigea, Romania (photo: A. Pintilioaie)

Morphometric analyses of the specimens

Based on the results of the photo analysis, it was found that the wings (Figure 6a) of *I. polystictica* females, on average, cover 3.6 tergites (SD = 0.22; range: 3.2–4.1). At the same time, the wings of *I. oratoria* females cover an average of 4.97 tergites (SD = 0.33; range: 4.3–5.9) (Figure 6a). Measurements of the ratios of wing, pronotum, and abdominal lengths in females revealed significant differences between the two species (Figure 6b, c). The ratio of wing to pronotum length in *I. polystictica* is 1.35 (SD = 0.07; range: 1.23–1.49), while the ratio of wing to abdomen length is 0.58 (SD = 0.04; range: 0.48–0.70). In contrast, the corresponding ratios for *I. oratoria* are 1.54 (SD = 0.09; range: 1.35–1.81) for wing to pronotum length, and 0.72 (SD = 0.04; range: 0.62–0.81) for wing to abdomen length. The ratio of pronotum length to abdomen length in *I. polystictica* and *I. oratoria* is strikingly similar, measuring 0.43 (SD = 0.03; range: 0.36–0.54) and 0.47 (SD = 0.04; range: 0.37–0.57), respectively (Figure 6d). It is important to note that when using these characteristics for species identification, special attention must be given to the angle of the insect in the photograph, and it works only for females.

We evaluated the accuracy of species identification and the feasibility of species determination for 521 records of *Iris* nymphs and adults, using photos from iNaturalist (2024) (Figure 1). Six records were misidentified at the genus level, and 11 additional records could not be identified due to poor photo quality, representing 3.26% of all records. Thirty-seven adults (9.37%) were photographed with their wings open, enabling confident species identification. Out of the 241 records of female specimens from both species, 19 individuals (7.88%) could be confidently identified to species based on photographs with open wings. Additionally, 205 females (85.06%) were identifiable to species using the relative wing length or the ratio of wing length to pronotum length.

Discussion

The relative wing length of females in *I. polystictica* and *I. oratoria* has proven to be a consistent and reliable trait for distinguishing between the two species (Figure 6a). In *I. oratoria*, this characteristic remains stable throughout its European range, with females exhibiting both longer and shorter wings in different parts of the range, but always staying within the defined length limits. Similarly, for *I. polystictica*, the consistency of this trait can be observed along the Northwestern Black Sea coast and into the foothills of the Altai Mountains (Shcherbakov et al. 2013), further supporting its utility for species identification. This trait enables confident identification of *I. polystictica* and *I. oratoria* from photographs available in public science sources, where a substantial number of female images can be used to distinguish these species based on wing length. This is particularly valuable in areas where the two species may overlap and where scientific data is limited. However, it is important to note that other *Iris* species are found in North Africa and Western Asia, which limits the applicability of this character for identifying *Iris* specimens from these regions, at least until similar studies are conducted on the local species.

The use of the wing-to-pronotum and wing-to-abdomen length ratios for species identification in *Iris* is somewhat limited due to the overlap in the ratios of these two species. However, these ratios remain useful for identification outside the overlapping zones (Fig. 6b, c). In cases where a body part is missing from a photographed individual, such as in the sole photograph from Moldova (Stanislavschi 2022), this character may be the only reliable one available for identification. On the other hand, the ratios of pronotum to abdomen lengths in *I. polystictica* and *I. oratoria* almost completely overlap, making them unsuitable for species determination (Fig. 6d).

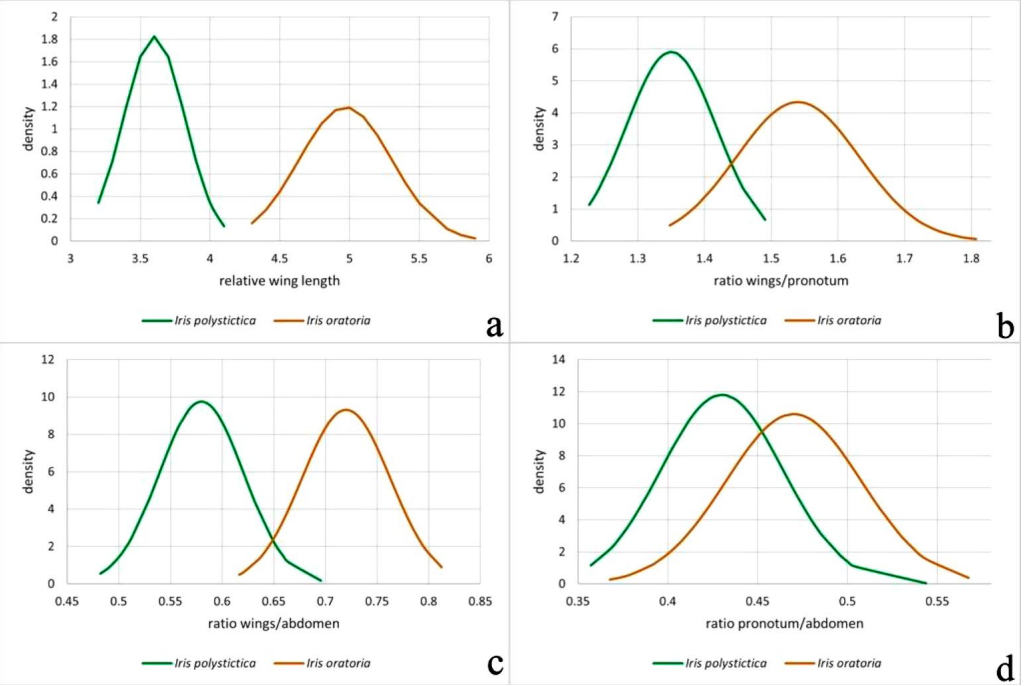

Figure 6.a. Probability density of the relative wing length of *Iris* females in relation to their tergites; b. Probability density of the ratio of wing and pronotum lengths in *Iris* females; c. Probability density of the ratio of wing and abdomen lengths in *Iris* females; d. Probability density of the ratio of pronotum and abdomen lengths in *Iris* females.

In 2024, 18 individuals of *I. polystictica* were recorded in Ukraine, 13 of which were found within the previously known natural habitat in Tuzlivski Lymany National Park (Bronskov et al. 2024). Additionally, one female was observed in a degraded pasture near Kulevcha village, dominated by *Elymus repens* (L.) Gould, *Bromus squarrosus* L., and *Xeranthemum annuum* L. Another individual was found in a plot with 100% projective cover of *Bothriochloa ischaemum* (L.) Keng, likely a fallow land near Vynohradivka village. Three females were recorded in old cemeteries with secondary steppe vegetation (*Agropyron cristatum* (L.) Gaertn., *E. repens*, *Glycyrrhiza glabra* L.) in the villages of Sarata and Vynohradivka. All individuals were collected using a sweeping net.

Of the 341 *Iris polystictica* records available for Ukraine, 331 were found in natural, open herbaceous communities. This species is not particularly moisture-demanding and can be found in psammophytic, petrophytic, bunchgrass, and forb-bunchgrass steppes, as well as in halophytic meadows. The highest relative abundance of *I. polystictica* was observed in areas with a mix of grasses (e.g., *Agropyron cristatum*, *Bothriochloa ischaemum*, *Elymus repens*) and herbaceous plants (e.g., *Artemisia* spp., *Limonium* spp., *Marrubium peregrinum* L.), with vegetation heights ranging from 50-80 cm and a total cover exceeding 80%. *I. polystictica* frequently selects flowering plants such as *Cynanchum acutum* L., *Echinops ritro* subsp. *ruthenicus* (M.Bieb.), *Limonium* spp., and *Glycyrrhiza glabra* for hunting. In areas with less herb-layer cover, *I. polystictica* tends to prefer locations with as dense a grass stand as possible. Its relative abundance decreases significantly in areas with monotonous vegetation, such as sites dominated by *Artemisia* spp. In regions with a monotonous cover of grasses (different species of the family Poaceae), only a few individuals are typically found, and it is entirely absent from areas with low and sparse vegetation, such as overgrazed pastures. Additionally, we did not observe this species in the retrodunal biocenoses along the Black Sea coast, where well-developed shrub stands of *Artemisia trautvetteriana* Besser are present (Bronskov et al. 2024). In herbaceous habitats with shrubs, *I. polystictica* can also utilize these shrubs, as noted by Pushkar & Yermolenko (2009), and numerous photographs from citizen science sources further support this behavior. We have also observed *I. polystictica* in settlements in areas with preserved or slightly modified natural vegetation, with 10 records, including 3 in old cemeteries.

In our opinion, the greatest threat to mantises in general and the genus *Iris* in particular after the destruction of natural complexes is fires in the remnants of natural habitats, which mainly occur due to human activity. As a result, mantises may be completely absent or have low numbers in habitats that meet their ecological needs. We have repeatedly noted the absence of mantises in large, monotonous habitats where fires spread unimpeded and very quickly, and at the same time, they were present in small areas along their perimeters, separated from each other by roads, clearings, etc., which were obstacles to fire spread. *I. polystictica* is also threatened by afforestation in steppe areas, the impact of which increases as tree crowns close, and excessive grazing pressure. At the same time, some populations may exist in low numbers in isolated, small areas that have not been exposed to catastrophic anthropogenic impacts. Against this background, human settlements with their relative protection from fire and a mosaic of artificial and remnant natural vegetation can serve as a refugium for *Iris* species.

In Romania, Dunele Marine de la Agigea Nature Reserve,

is represented by an 11.8-hectare protected area, the last remaining piece of land in the region that has largely remained in its natural state, bordered on one side by Constanța harbor and on the other by residential and commercial buildings. As such, the reserve functions as a green oasis amidst concrete, supporting a rich biodiversity, including a high variety of insect species. The habitats within the reserve include sand dunes, predominantly covered with psammophytic and steppe vegetation, along with some isolated shrubs and a small, denser cluster of deciduous trees. The habitat diversity in the Dunele Marine de la Agigea nature reserve supports at least four species of Mantodea within this small area, out of the five species present in Romania. These species include *Mantis religiosa* (Linnaeus, 1758), *Ameles heldreichi* Brunner von Wattenwyl, 1882, *Hierodula tenuidentata* Saussure, 1869, and *Iris polystictica*. The presence of a viable population of *Empusa fasciata* Brulle, 1832 remains uncertain. While *E. fasciata* is known to inhabit steppe-like habitats in Dobrogea, and a single nymph was found at Agigea (Georgescu 2022), no additional specimens have been recorded despite numerous studies in the area. This suggests that the nymph may have been accidentally introduced or that the population is very small, making the species difficult to locate in the region.

The discovery of one male and one female of *I. polystictica* one year apart at the same location may indicate the presence of a viable population, albeit likely small. This is suggested by the fact that only two specimens were observed over two years of comprehensive entomological surveys in the area, pointing to a limited number of individuals in that population. It is also worth noting that five out of the seven specimens recorded in Romania were observed in areas with significant human infrastructure, such as railway stations (the males from Alba Iulia), inside trains (the specimen from Bucharest), national roads (the male from Vrancea county), and in close proximity to the international seaport (the male and female from Agigea). These findings may also suggest that the origin of at least some of the specimens could be anthropogenic, as it is well known that unintentional human introduction plays a significant role in the colonization of new areas by various mantid species (Schwarz & Ehrmann 2018). It is worth noting that some of the males were attracted to artificial lights during the night (those from Agigea and Alba Iulia) or were found in close proximity to artificial light sources (the male from Vrancea) (Cazacu 2019).

I. oratoria is strongly associated with Mediterranean-type vegetation, even outside the Mediterranean basin, such as in Central Asia. Its distribution aligns with the boundaries of the Mediterranean climate region (Battiston et al. 2021) and extends into an ecotonal band adjacent to this area. In contrast, *I. polystictica* has adapted to survive in continental climates characterized by sub-zero winter temperatures and hot summers, making it a typical representative of steppe-zone fauna. These ecological preferences, along with the available evidence of occurrences, enable us to confidently delineate the ranges of these two species in the Western Black Sea region (Figure 7). The repeated occurrences of *I. polystictica* across Romania further support this, as the Pontic Steppe Grasslands bioregion extends southwest into northern Bulgaria, where this species is also expected to occur. In contrast, *I. oratoria* in the Western Black Sea region is distributed within the Balkan mixed forests ecoregion, where it is most likely confined to the northern reaches of the Balkan Mountains.

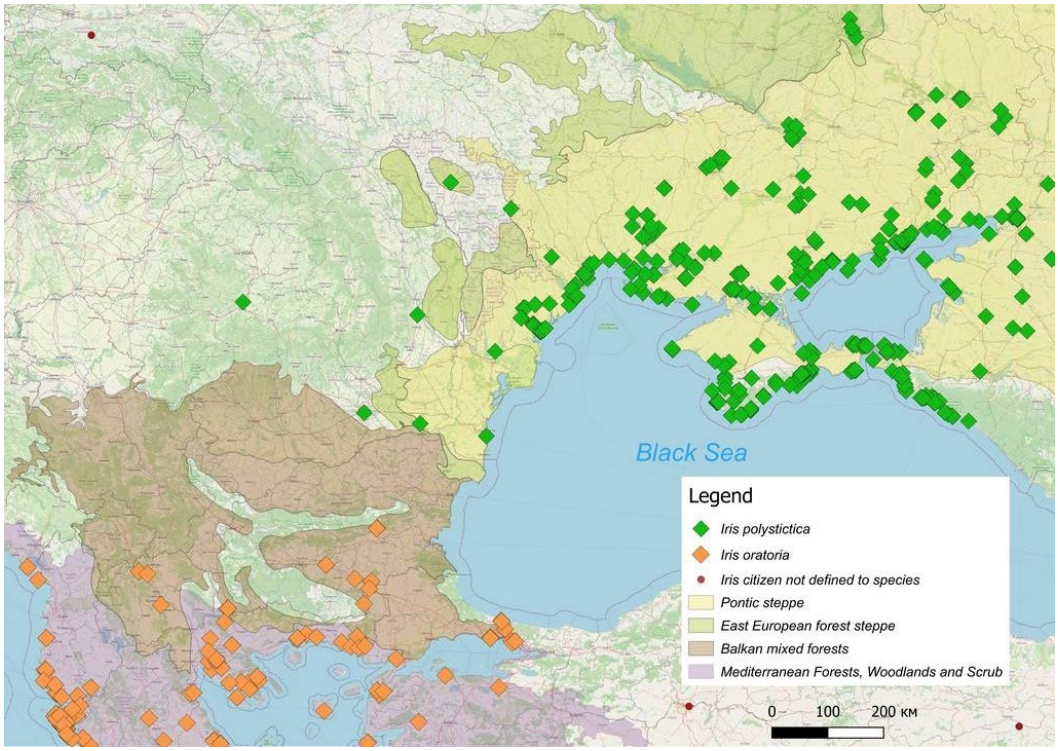

Figure 7. Distribution of *Iris polystictica* and *Iris oratoria* in the Black Sea region, the northern Balkans, and the north Anatolia relative to the bioregions of the Pontic steppe and Mediterranean Forests, woodlands, and scrubs (Olson et al. 2001). Base map: OpenStreetMap.

To fully understand the current distribution of mantis species, it is essential to consider not only their ecological requirements but also the historical economic development of the regions they inhabit. Today, probably no truly untouched natural environments remain in Europe. Even habitats within nature reserves, which may appear to be largely natural, have often been influenced by past human activities to some extent. Thus, when discussing the habitat preferences of mantises, we refer to areas that have consistently provided the conditions necessary for the species' survival, both historically and currently. Some habitats, such as the placoric steppes, have been severely degraded, leaving only scattered remnants, making the current distribution patterns of mantises in these areas poorly understood.

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