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Species-specific and environment-sensitive functional traits in six steppe plant species with different roles in community

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Abstract: Plant functional traits are often considered as indicators of plant-environment relationship; however, some plant features can be highly taxonomic-specific. The study of intraspecific trait variation is essential to understand what functional traits are influenced by the phylogeny and what traits are more dependent on environment. We studied six common steppe plant species in two natural vegetation plots near lake Baikal in Southern Siberia different in climate aridity and grazing degree: site 1 – native true grass steppe under lower climate aridity, site 2 – high disturbed sagebrush steppe under higher aridity. Plant functional traits showed different relevance to species and environment. Plant height, leaf thickness (LT), mesophyll cell volume (Vcell), and the chloroplast number per cell had the greatest contribution to differences between species and varied slightly within a species. Photosynthesis (Amax) and transpiration (E) rates, chlorophyll content, mesophyll surface area per leaf area unit (Ames/A) were more dependent on environment than on species. Amax and E decreased in all studied species in more adverse conditions of site 2, however plants differed in mechanisms of these changes. In Stipa krylovii, Artemisia frigida and Potentilla acaulis, most abundant in true steppe (site 1) mesophyll cell sizes, cell and chloroplast number per leaf area unit (Amax/A) were more dependent on environment than on species. Amax and E decreased in all studied species in more adverse conditions of site 2, however plants differed in mechanisms of these changes. In Stipa krylovii, Artemisia frigida and Potentilla acaulis, most abundant in true steppe (site 1) mesophyll cell sizes, cell and chloroplast number per leaf area decreased in site 2. Other species, Artemisia scoparia, Potentilla bifurca and Allium anisopodium which were more abundant in disturbed steppe (site 2), had larger cells and showed an increase in cell and chloroplast number per leaf area in site 2 and decrease in the photosynthetic capacity of a chloroplast. We concluded that the leaf thickness and cell size belong to species-specific features, whereas Amax, pigment content and integral mesophyll traits as Amax/A are more indicative for plant-environment relationships and their response to growth conditions depend on the ecological strategy of a species.

Keywords: cell volume, chlorophyll content, leaf structure, mesophyll, photosynthesis, plant height

Introduction

Plant functional traits can serve as indicators of plant adaptation to growth conditions (Niinemets, 2001; Poorter et al. 2009; Reich, 2014). These traits are measurable morphological and physiological parameters, closely related to the main functions of plants, but at the same time they should notably vary with environment (Violle et al., 2007; Garnier & Navas, 2012). To be able to survive in changing environment, plants will either shift their ranges or adapt to changing environment (Cornwell & Ackerly, 2009; Jung et
Therefore, environment impact not only causes changes in taxonomic diversity, but also can lead to functional shifts as species with given traits are replaced by other species with other traits (Grime, 2001; de Bello & Mudadk, 2013). Unlike taxonomic approaches, trait-based methods enable generalisations across regions (Diaz et al., 2004). The study of these mechanisms is of great importance for a deep and comprehensive understanding of environment effects on plants.

Nowadays many studies describe environmental variation in plant traits mostly in strongly controlled manipulative experiments, common gardens (transplant experiment), open-top chambers and rain-out shelters. Natural gradients have been used much less because of methodological reasons. However, it is often inherently difficult to extrapolate the results from controlled but rather artificial experiments to natural conditions in the real world (Poorter et al., 2016). There is certainly a pressing need for other approaches based on “natural” ecosystems (van der Plas, 2019). Thus, the natural variation in plant traits provides an excellent and unique opportunity to study the impact of many ecological factors on the structuring and functioning of plants. Different ecosystems / communities could be used as “natural laboratories” to assess response of terrestrial organisms to climate (de Frenne et al., 2013) or disturbance (Ivanov et al., 2018).

Climate change is one of the most important factors influencing the biodiversity and vegetation traits from individual species up to landscape-scale properties. On the other hand, the anthropogenic impact on ecosystems leads to a deterioration in the overall functional state of vegetation. Numerous studies in recent years have shown that the ecosystems of the Lake Baikal basin are changing under the influence of anthropogenic factors. The observed widespread overgrazing led to the formation of communities with low production and poor feeding quality phytomass (Miklyaeva et al., 2004; Bazha et al., 2012, 2015; Gunin et al., 2012, 2015). The range of functional traits is virtually infinite therefore we need to know what functional traits can be used to assess and monitor vegetation change in response to environmental change (Gillison, 2013). Leaf traits are of prime importance to specify the response of plant species to environment (Loranger & Shipley 2010; Gillison, 2019). Leaf mass per area (LMA) and leaf thickness (LT) were shown to be controlled by climate (Fonseca et al., 2000; Reich et al., 2003; Poorter et al., 2009). At the same time these parameters are linked with main physiological characteristics such as photosynthesis rate, relative growth rate, leaf lifespan and others (Poorter et al., 2009; Hassiotou et al., 2010; Sievert et al., 2015). Leaf thickness determines the amount of light absorbed by the leaf and the diffusion path of CO$_2$ through the tissues. Chlorophylls and carotenoids are responsible for light absorption and photochemical reactions in chloroplasts. Regulation of chlorophyll concentration and the ratio of pigments in the leaf is a function of photosynthesis adjustment to various environmental factors, first to light and water availability (Ivanov et al., 2004, 2022). Leaf mesophyll traits deserve special attention since they are particularly informative as functional parameters responsible for leaf gas exchange (Evans et al., 2009; Terashima et al., 2011). These parameters are useful for estimating the internal leaf conductance for CO$_2$ (Laish et al., 1970; Terashima et al., 2011), studying the adaptation mechanisms of plants to stress (Nobel & Walker 1985; Mokronosov 1981; Pyankov et al., 1999) and examining the response of different plant functional types (PFTs) to their environment (Poorter et al., 2009, 2019; Shipley et al., 2016). Therefore, traits combinations seem to be selected by local environment, disturbance and biotic interactions (Bruegelheide et al., 2018).

Research conducted in natural ecosystems often reveals challenges in discerning precise effects of various factors on the plant’s functioning. Typically, in steppe, plants experience the combined effects of multiple adverse factors, including water deficit, high insolation and fluctuating precipitation together known as climate aridity as well as livestock grazing. There is little information on the combined effects of different factors on plant functional traits (Havstad et al., 2008; Hunt, 2010; Xie et al., 2018). Both climate aridity and grazing have a significant impact on plant productivity, but they can act on different groups of functional traits (Ivanov et al., 2018). Trait variations often go beyond interspecific differences and are influenced by intraspecific phenotypic and genetic factors (Nicotra et al., 2010; Sievert et al., 2015). The combined study of intra- and interspecific variation makes it possible to find out what functional traits are determined by the phylogenetic nature of plants (species, taxonomy), and what traits are more dependent on changing environmental factors. The Trans-Baikal region is insufficiently studied in relation to...
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the structural and physiological mechanisms of plant response to environment. We studied six plant species of different taxa and life forms in two steppe sites differed by climate and grazing disturbance. The purpose of the work was to study the interspecific differences and intraspecific variation of plant functional traits. To reveal the degree of species-specificity and dependence on external conditions for biochemical, physiological, and structural traits at the leaf-, tissue- and cell-level we investigated the relationship between plant functional traits and environmental conditions.

Materials and methods

The study was conducted in 2018 in two steppe plant communities in Southern Siberia near Lake Baikal, Republic Buryatia, Russian Federation (Fig. 1). Table 1 presents the description of the studied communities. The material was collected in native true steppe and anthropogenically disturbed steppe during the period of active plant vegetation (budding-flowering) in early July. Data on average annual rainfall and temperature of three regions were taken from the site https://www.worldclim.org/data/worldclim21.html (Table 1). Climate of the study sites is characterised by mean annual precipitation (mm) (MAP) mean annual temperature (°C) (MAT) and the De Martonne aridity index (Ia) (Table 1), calculated using the equation: Ia = MAP/(MAT + 10). The minimum value of the index corresponds to the maximum aridity of the climate.

We studied 6 species of angiosperms of different taxa and growth forms (Table 2). *Artemisia frigida* is a widespread in Europe and Asia species, where grows in the steppes of the Urals, Southern Siberia, Kazakhstan, Mongolia, and China. *Artemisia scoparia* is an annual or biennial herb which is distributed across much of Eurasia from France to Japan, including China, India, Russia, Germany, Poland, central and southwest Asia. *Artemisia scoparia* grows in steppes, steppe meadows, pastures, fallow lands, roadsides, gravelly and sandy slopes, riverbanks, and sparse forests. The native range of *Potentilla acaulis* which is a perennial xerophytic herb is Central Asia, up to Siberia and Northern China. *Potentilla bifurca* is widespread in temperate and alpine zones of the Northern hemisphere, grows on crushed slopes, ramps, and rocks. *Stipa krylovii* is a grass that typically dominates in steppes of Central Asia. *Allium anisopodium* is a herbaceous perennial bulbous plant with succulent-like leaves. This species grows in steppes, dry slopes, and sands. Its natural wide range includes Kazakhstan, Siberia, the Far East (Russia), Mongolia, China, and the Korean Peninsula.

Fig. 1. Map of the locations of two study sites. 1 – Site 1, 2 – Site 2 (see Table 1).
We studied morphological, structural, physiological and biochemical parameters of plants (Table 3). To measure leaf traits, 10 to 20 leaves were harvested from the middle leaf tier of fully developed, healthy individuals per species. Ten to twenty fresh leaves per species were photographed directly in the field for leaf area and LMA measurements. Leaf area was determined using a digital camera and the Simagis Mesoplant™ image analyzer (SIAMS, Ekaterinburg, Russia). Collected fresh leaf material was stored in a...
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Table 1. Location, climatic and vegetation characteristics of the study sites. Sh – the share of grazing digression active plant species, P – precipitation, T – temperature, Ia – aridity index, SpN – species number, TotC – total coverage of community (%).

<table>
<thead>
<tr>
<th>Study site</th>
<th>Geographical location</th>
<th>Type of community</th>
<th>Dominant species</th>
<th>P</th>
<th>T</th>
<th>Ia</th>
<th>SpN</th>
<th>TotC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site 1</td>
<td>N53.34°E110.61°</td>
<td>Kurumkan, Russia</td>
<td>True grass steppe</td>
<td>345</td>
<td>-3.5</td>
<td>53</td>
<td>19</td>
<td>75</td>
</tr>
<tr>
<td>Site 2</td>
<td>N53.73°E110.27°</td>
<td>Barguzin, Russia</td>
<td>Highly disturbed sagebrush steppe</td>
<td>358</td>
<td>-2.8</td>
<td>50</td>
<td>17</td>
<td>55</td>
</tr>
</tbody>
</table>

Table 2. Studied plant species, their characteristics and relative abundance in studied communities (coverage, %): SN – short symbols of species used in figures, Site 1 – true grass steppe, Site 2 – highly disturbed sagebrush steppe (see Table 1). GF – growth form, DS – dwarf shrub, HA – herb annual, HP – herb perennial.

<table>
<thead>
<tr>
<th>Plant species</th>
<th>SN</th>
<th>Family</th>
<th>GF</th>
<th>Coverage, %</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Stipa krylovii</em> Roshev.</td>
<td>SK</td>
<td>Poaceae</td>
<td>HP</td>
<td>26</td>
</tr>
<tr>
<td><em>Artemisia frigida</em> Wild.</td>
<td>AF</td>
<td>Asteraceae</td>
<td>DS</td>
<td>13</td>
</tr>
<tr>
<td><em>Potentilla acaulis</em> L.</td>
<td>PA</td>
<td>Rosaceae</td>
<td>HP</td>
<td>9</td>
</tr>
<tr>
<td><em>Potentilla bifurca</em> L.</td>
<td>PB</td>
<td>Rosaceae</td>
<td>HP</td>
<td>1</td>
</tr>
<tr>
<td><em>Artemisia scoparia</em> Waldst. et Kit.</td>
<td>AS</td>
<td>Asteraceae</td>
<td>HA</td>
<td>7</td>
</tr>
<tr>
<td><em>Allium anisopodium</em> Ledeb.</td>
<td>AA</td>
<td>Amaryllidaceae</td>
<td>HP</td>
<td>3</td>
</tr>
</tbody>
</table>

refrigerator in wet filter paper for 2–3 h. Nine to twelve leaves were taken for measurements of chlorophyll and carotenoid contents and 20–30 leaves were fixed in tubes in a solution of glutaraldehyde (3.5% glutaraldehyde in 0.15M phosphate buffer, pH 7.4) for anatomical measurements.

Gas exchange was measured under field conditions on intact mature leaves of three individuals in each species per community. The maximal photosynthetic rate (A_{max}) and transpiration rate (E) were determined using a portable photosynthetic system Li-6400 XT (Li-COR, USA). Measurements were taken in the first half of the day from 9 to 12 hours. Water use efficiency (WUE) was calculated as the ratio of maximal rate of photosynthesis to transpiration rate, μmol mmol⁻¹: WUE = A_{max}/E. To determine the content of photosynthetic pigments, cuttings were made from 20–30 leaves of 10–15 plants of each species. Pigments were extracted with 80% acetone, then chlorophyll content (C_{a}/A, mg/dm²) and carotenoid content per leaf area unit (Car/A, mg/dm²) were measured using the Odyssey DR/2500 portable spectrophotometer (Hach, USA). C_{a}/A was calculated according to Lichtenthaler and Wellburn (1983): C_{a} (mg/l) = 11.63 • D665 – 2.39 • D649, Cb (mg/l) = 20.11 • D649 – 5.18 • D665, Car = 4.695 • D440.5 – 0.268(C_{a}/A), where C_{a} – chlorophyll a concentration, C_{b} – chlorophyll b concentration, D665, D649 and D440.5 – optical densities of the extract at the wavelengths 665, 649 и 440.5 nm, C_{a}/A – chlorophyll a+b content per unit leaf area. In addition, the ratio of chlorophylls a/b and the ratio of chlorophylls/carotenoids (Chl/Car) were calculated.

For anatomical and biochemical analyses, the central part of the leaf avoiding main veins was taken. The main leaf structural traits will be obtained according to Leaf Mesostructure Method of Ural School of Ecophysiology (Pyankov et al., 1999; Ivanova et al., 2016, 2018a). The number of cells per unit leaf area was determined in 20 replicates using a hemocytometer (Goryaev chamber; Minimed, Bryansk, Russia) in cell suspension obtained after heating to 90°C and after maceration of leaf pieces of known area (ca. 1 cm²) in 2–3 ml of 20% KOH (described in detail by Ivanova et al., 2016, 2018a). Chloroplast number per cell was determined in 30 replicates in the same cells.
that were measured for cell sizes. Cell sizes were determined in 30 replicates in cell suspension after maceration of leaf pieces in 1M HCl heated to 40–50°C. Cell projection area \(A_{cell}\) and perimeter \(P_{cell}\) are measured with a light microscope (Axiostar plus, Zeiss, Germany) using the Simagis Mesoplant\textsuperscript{TM} image analyzer (SIAMS, Ekaterinburg, Russia). The mesophyll cell volume \(V_{cell}\) and cell surface area \(S_{cell}\) were calculated separately for each type of photosynthetic tissue (palisade and spongy mesophyll cells, bundle sheath cells, and segmented mesophyll cells of grasses) using the projection method described in Ivanova et al. (2018a). Cell volume was calculated using the formula:

\[
V_{cell} = \frac{S_{cell}}{P_{cell}} \sqrt{\frac{h^3}{3bK_p}}
\]

where \(V_{cell}\) – cell volume (\(\mu m^3\)), \(S_{cell}\) – average cell projection area (\(\mu m^2\)), \(P_{cell}\) – average cell projection perimeter (\(\mu m\)); \(b\) and \(K_p\) – proportionality coefficient depending on the cell shape, for majority of mesophyll cells, \(b\) ranges from 3.2 to 4.0, \(K_p\) – ranges from 0.9 to 0.11 (described in Ivanova et al., 2018a).

Chloroplast number per unit leaf area \(N_{chl}/A\) was calculated by multiplying chloroplast number per cell and cell number per unit leaf area for palisade and spongy mesophyll, or for total mesophyll in the case of uniform or irregular cells. Total cell surface area per unit leaf area \(A_{mes}/A\) was determined by multiplying the average cell surface area and cell number per unit leaf area. Total chloroplast surface area per unit leaf area \(A_{chl}/A\) was determined by multiplying the average chloroplast surface area and the chloroplast number per unit leaf area. The chlorophyll a+b content per chloroplast \((C_{ab}/chl, 10^{-9} mg)\) was obtained by dividing the pigment content per unit leaf area \((C_{chl}/A)\) by the chloroplasts number per unit area \((N_{chl}/A)\). Photosynthetic activity of the chloroplast \(A_{max}/chl, 10^{-10} \mu mol CO_2 s^{-1}\) was calculated by divid-
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Other three species: *Artemisia scoparia*, *Potentilla bifurca* and *Allium anisopodium* were less abundant in true grass steppe and increased their abundance in disturbed steppe of site 2, moreover *Artemisia scoparia* became a dominant species at site 2.

Anatomical analysis revealed a high variation in leaf morphology among the studied species (Fig. 3). Two species of genus *Artemisia* – *A. frigida* and *A. scoparia* – had a typical for steppe dicotyledonous xerophytic herb isopalisade type of mesophyll with palisade tissue on both leaf side (Ivanova et al., 2018a, b). Two species of genus *Potentilla* – *P. acaulis* and *P. bifurca* – possessed a dorsoventral type of mesophyll with palisade tissue on the upper and spongy tissue on the lower side of a leaf. Grass *S. krylovii* characterised by long rolled leaves of graminoid type. Leaves of onion *A. anisopodium* were hemy-cylindrical with isopalisade-peripherical type of mesophyll and water-storing parenchyma in the centre of the leaf. Studied species also distinguished by values of gas exchange rate, pigment content and traits of a whole leaf and of a mesophyll (Figs 4, 5).

We analysed the influence of environmental conditions of site 1 and site 2 on different functional traits. Some functional traits were largely species-specific and hardly influenced by environment changes

Fig. 3. Pictures of the leaf cross-sections for studied plant species. Scale is 100 μm.
Thus, plant height, leaf thickness and density (LMA, LD) were determined mostly by plant species and did not differ between study sites in all variants of ANOVA analysis: both for all studied species and for two different groups of species divided on their roles in community. The first group – true steppe dominants (*S. krylovii*, *A. frigida* and *P. acaulis*) had thinner leaves (Fig. 4) and smaller mesophyll cells (Fig. 5) in comparison to other three species which were more abundant in site 2 (disturbed steppe abundant). Such traits as maximum photosynthesis rate ($A_{\text{max}}$) and transpiration ($E$) were more de-
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Fig. 5. Leaf mesophyll and cell-level functional traits of 6 studied species at two study sites: the first column (green) presents site 1, the second column (orange) presents site 2. Horizontal axis presents species names (full species names see Table 2). LogVcell – logarithmic values of cell volume (on the base of 2). The results of paired t-Test are presented in three variants: 1 – all species, 2 – the first three species (SK, AF, PA) which are the most abundant in native true steppe, 3 – the last three species (PB, AS, AA) which increase their abundance in disturbed community on site 2. n.s. – not significant. Species names see Table 2. Leaf traits and units on vertical axes see Table 3.

Pending on environment, but total mesophyll surface area per leaf area ($A_{mes}/A$) was dependent on environment in the case of dividing plant species in two groups: true steppe dominants and disturbed steppe abundant (Table 4). Mesophyll cell volume and cell number per leaf area unit were highly dependent on species in all three variants and influenced by both species and environment in the first group of species (true steppe dominants).

Despite high diversity in morphological and anatomical features among studied species, we found similar intra-specific trends in physiological changes...
in response to environment. Photosynthetic capacity and transpiration rate decreased in all studied plant species in highly disturbed and more arid steppe (site 2) compared to true steppe community (site 1) (Fig. 4). However, in true steppe dominants transpiration rate decreased in a greater degree than photosynthesis that is confirmed by an increase in water use efficiency (WUE) which is defined as the ratio of photosynthesis and transpiration. In the second group of species which were more abundant in disturbed steppe and did not change their WUE, the proportional changes of $A_{\text{max}}$ and $E$ were noted. $A_{\text{max}}$ did not relate to pigment content, leaf density and chloroplast number per leaf area, the last one, on the opposite, increased in the second group of species (variant 3 on Fig. 5). The main grounds of a diminishing in $A_{\text{max}}$ included microstructural changes of mesophyll as well a decrease in stomatal conductance evidenced by the data on transpiration. Changes in mesophyll structure differed between two groups of plant species. In the first group of true steppe dominants – S. krylovii, A. frigida and P. acaulis, a decrease in mesophyll cell number and size and as a result to a sharp decrease in $A_{\text{mes}}/A$ was caused by the influence of conditions of site 2. On the opposite, in A. scoparia, P. bifurca and Allium anisopodium, more abundant in disturbed steppe, increased cell number and $A_{\text{mes}}/A$ in site 2 were observed and they also decreased the CO$_2$ transport rate per mesophyll surface (TR$_{\text{mes}}$) and photosynthetic capacity of a chloroplast ($A_{\text{max}}$/chl) by 40–60% (Fig. 5).

**Discussion**

Our study is among a few studies which perform simultaneous analysis of morphological, structural, physiological, and biochemical changes in response to combine effect of climate aridity and grazing. Typically, only several traits or single factor are used to reveal the plant functional response due to laboriousness of such research in natural ecosystems. Detailed analysis of structural, chemical and physiological traits combined with ecological variables can reveal what plant functional traits from whole-plant to tissue- and cell-level are the best predictors of functional response of plants to environment. For example, the main adaptive features of xerophytes to a short period of growth are miniaturising of the leaf blade and

<table>
<thead>
<tr>
<th>Variable</th>
<th>Species 1</th>
<th>Environment</th>
<th>Species 2</th>
<th>Environment</th>
<th>Species 3</th>
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<td>PlantH</td>
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<td>LMA</td>
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<td>ns</td>
<td>44*</td>
<td>ns</td>
<td>21*</td>
<td>ns</td>
</tr>
<tr>
<td>LD</td>
<td>90***</td>
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<td>141**</td>
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<td>168**</td>
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<td>$V_{\text{cell}}$</td>
<td>62***</td>
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<td>112**</td>
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<tr>
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<td>44***</td>
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<tr>
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<td>103**</td>
<td>138***</td>
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<td>132**</td>
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<tr>
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<td>18**</td>
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<td>42*</td>
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<tr>
<td>$A_{\text{max}}$ per chl</td>
<td>10*</td>
<td>7*</td>
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<td>16*</td>
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</tr>
<tr>
<td>$C_{\text{ab}}$</td>
<td>12**</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>21*</td>
<td>ns</td>
</tr>
</tbody>
</table>

Table 4. Values of F-criterion in an analysis of variance (ANOVA) by the factors ‘Species (6 plant species)’, ‘Environment (two plots)’ and the statistical significance of these factors: *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$, ns – not significant. Variant 1 – all species were included in the analysis, Variant 2 – three species with the most abundance in true steppe at site 1 (Stipa krylovii, Artemisia frigida and Potentilla acaulis) were included in the analysis, Variant 3 – three species, which were rare at site 1 and increased their abundance in disturbed steppe (Artemisia scoparia, Potentilla bifurca and Allium anisopodium), were included in the analysis. See Table 3 for definitions of variables.
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higher leaf tissues density (Gamalei, 1984; Mokronosov, 1981; Galmes et al., 2012; Ivanova, 2014). The main leaf tissue – mesophyll is the material basis of photosynthesis and represents a system of structures providing photosynthesis. In this regard, plant adaptation to environment is closely related to the rearrangement of mesophyll cells and chloroplasts (Mokronosov, 1981; Nobel & Walker 1985; Pyankov et al., 1999; Terashima et al., 2011; Ivanova, 2014, 2016). Steppe xerophytes often possess isopalisade leaf structure which is an adaptation to high insolation. Isopalisade mesophyll type is characterised by the high concentration of cells and chloroplasts per unit leaf area (Pyankov et al., 1999; Zvereva, 2000; Ivanova, 2014). Xerophytes of the Karakum Desert had 60–90 million of chloroplasts per 1 cm² of leaf area (Mokronosov, 1981) that is two-three-fold higher than that in steppe plants (Zvereva, 2000; Ivanova et al., 2018a, 2019) and 6–8 times higher than in boreal mesophytes (Ivanova, 2014). Steppes often are dominated by xerophytic grasses as Stipa species which also have high concentration of photosynthetic cells and chloroplasts per leaf area (Ivanova et al., 2019). A special type of leaf adaptation to aridity is succulence. Succulent plants in addition to chlorenchyma, have a water-storing tissue or their mesophyll along with photosynthesis can perform a water-storage function and differ by large cell volume and low leaf density (Rozentsvet et al., 2016; Ivanova et al., 2019). Our species studied belong to different morphological leaf types – isopalisade (both Artemisia species), graminoid, dorsoventral (Potentilla species), succulent-like (Allium), however they showed similar functional response to environment.

Our study shows that plant functional traits have different relevance to interspecific variation and growing conditions. Such traits as leaf thickness, cell volume and chloroplast number per cell were highly species-specific and had minimal intraspecific variation. Indeed, a high species-specificity of these traits has been indicated earlier for other plant species including steppe plants (Mokronosov, 1981; Yudina et al., 2017, 2020). Previous studies of Artemisia frigida showed, that mesophyll cell volume in different steppe regions of Mongolia ranged from 2 to 6 thousand µm³ and LT for this species varied between 145 and 200 µm (Ivanova et al., 2019), which is fully consistent with our data for this species (Fig. 5). The data available in the literature for Artemisia scoparia and Allium anisopodium is also within the values we identified in this study: \( V_{cell} \) 4–5 thousand µm³ and 20–30 thousand µm³ accordingly. The same conclusion about species-specificity of cell volume and leaf thickness can be made for Potentilla species and Stipa krylovii, which we studied earlier in steppe communities of Northern Mongolia (Ivanova et al., 2019).

Mesophyll cell volume is a key parameter of leaf structure which is strongly coordinated to photosynthetic function. Cell volume can influence on gas exchange of the whole leaf due to a strong dependence of surface-volume ratio (S/V ratio) on cell size. It was shown that small cells with a volume of less than 10 thousand µm³ have higher S/V ratio and more dramatic change of S/V ratio with volume change (Ivanova et al., 2014; Migalina et al., 2014). Larger cells have lower S/V ratio, which negatively affects the metabolic transport rate, including the mesophyll diffusion of CO₂ from the intercellular space into the cell (Migalina et al., 2014). Besides, \( V_{cell} \) positively correlates with leaf thickness as it was shown for many plant species from steppe (Ivanova et al., 2018a) and high mountains (Pyankov et al., 1999). On the other hand, \( V_{cell} \) has inverse relationship to cell number per leaf area and partial share of intercellular air space within a leaf (Ivanova et al., 2014; Rosentsvet et al., 2016), that could also affect gas exchange within mesophyll (Flexas et al., 2012; Ivanova et al., 2018b). Further, there is a high positive correlation between \( V_{cell} \) and chloroplast number per cell that allows plants with larger cells inexpensively and quickly to enhance chloroplast number per leaf area and thereby to optimise leaf photosynthesis. Moreover, in previous studies of 193 plant species of Central Asia, we showed that leaf thickness, cell volume and chloroplast number per cell were closely linked with plant functional type (PFT), which combines species with similar functional traits (Ivanova et al., 2019). Thus, we suppose that LT and \( V_{cell} \) are not only species-specific as our data showed, but these traits are also highly indicative of functional features of a species, i.e. ecological or life strategy (Grime, 2001; Adler et al., 2014). Obviously, these three traits – mesophyll cell volume, chloroplast number per cell and leaf thickness – were the most informative traits which differentiated studied plant species into two groups according to their roles in the community.

Since plant distribution in the nature is also affected by multispecies interactions, we need to consider not only the presence of species but also their abundance in the community (Grime, 1998; Garnier

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et al., 2004). Many past studies (with only several exceptions) that assessed how traits vary across climatic gradients typically have not combined species trait values with species abundances (Wieczynski et al., 2018). Determining the functional traits of species while accounting for the species coverage or phytomass allows proceeding from the species to the ecosystem level (Garnier et al., 2004) as the effect of species on ecosystem properties will depend on their proportional abundance in the community (Grime, 1998). Studied plant species were divided into two groups on their abundance in studied communities. First three species Stipa krylovii, Artemisia scoparia and Potentilla acaulis which are among the most abundant species of true grass steppe had smaller cells and thinner leaves (Figs 4, 5). Other species with larger cells – Artemisia scoparia, Potentilla bifurca and Allium anisopodium – were less abundant in true grass steppe and had higher abundance in disturbed steppe of site 2. Larger V cell in species of the second group corresponded to higher LT and to a lower leaf tissue density (LD) (Fig. 4). Leaves with lower LD are less resistant to water deficit (Gamalei, 1984; Galmés et al., 2012). In this regard, lower LD reduces the resistance of these species to drought and unfavorable temperatures that inhibits their competitive advantage in steppe conditions and does not allow them to occupy a dominant position in true steppe communities. In the opposite, at grazing disturbed steppe at the site 2, where total vegetation coverage was less and intra-specific competition was minimal due to higher consumption of true steppe dominants by herbivores (Xie et al., 2018; Jäschke et al., 2019), species of the group 2 get an advantage. More favourable conditions at site 2 for plants of group 2 are confirmed by increasing coverage, an increase in cell number and A mes/A. An integral mesophyll trait A mes/A can serve as indicator of optimal conditions for given species because this characterises the size of exchange surface area for CO₂ uptake inside a leaf. We suppose that more favourable water regime, for instance, increased precipitation, should facilitate CO₂ diffusion into leaves, and species with higher A mes/A abundant in disturbed steppe (group 2) could improve their photosynthesis and productivity in areas highly disturbed by overgrazing.

The most variable indicators at the intraspecific level included physiological parameters as photosynthesis rate, transpiration and A mes/A. All species decreased their photosynthesis and transpiration in more adverse conditions at the site 2, however plants of different groups differed in changes A mes/A. Other sources reported a significant level of intraspecific variation in A mes/A (Yudina et al., 2017), chlorophyll content per unit leaf area (Ivanov et al., 2022) and per chloroplast (Yudina et al., 2017; Ivanova et al., 2018a). It has been shown that the number of cells and chloroplasts, A mes/A, the ratio of chlorophylls a/b and chl/car in steppe plants depend on climate aridity and were less influenced by taxonomic position (Yudina et al., 2017, 2020).

Plant adaptation to arid climate is aimed at reducing transpiration and maintaining plant water relations (Galmés et al., 2012). Both aridity and grazing affect can water use efficiency (WUE) (Ivanov et al., 2018). This parameter specifies the amount of absorbed carbon dioxide per unit of transpiration loss and is an indicator of plant resistance to drought (Lambers et al., 1998). In our study species of group 1 increased their WUE under higher aridity and disturbed conditions whereas species of the group 2 did not change WUE in different environment. Other study also revealed an increase in WUE with aridity in Artemisia frigida due to a twofold decrease in transpiration (Ivanov et al., 2018). We have not found any data on photosynthesis and WUE for other species studied in literature. We suppose that intraspecific changes of these physiological parameters could be highly coordinated with the response of integral mesophyll traits such as A mes/A that reflects the ecological properties of species. For example, in the study in West Siberia, the response of A mes/A to drought depended on the ecological properties of the species. Thus, A mes/A increased under drier conditions in leaves of steppe xerophyte, whereas it decreased in meadow mesophytes (Ivanova, 2014). In our study, most species decreased the CO₂ transfer rate through the mesophyll surface area unit, chlorophyll content per chloroplast and photosynthetic activity of chloroplast under increasing aridity and disturbance. Chlorophyll content per chloroplast varies among plant species depending on their ecological properties (Mokronosov, 1981). Desert sclerophytes typically contain low chlorophyll at the means of 0.2–0.3 10⁻⁹ mg per chloroplast (Mokronosov, 1981). For comparison, mesophytic herbaceous plants of deciduous forests possess 4–10 10⁻⁹ mg per chloroplast, and in steppe plants, the value of this trait varied between 0.4–3 mg per 10⁹ chloroplasts (Mokronosov, 1981; Zvereva, 2000). Thus, a decrease of chlorophyll content per single chloroplast
can serve as the adaptation of steppe plants to an increased insolation and water deficit. Declined photosynthetic activity of a single chloroplast in studied species is a consequence of a chlorophyll loss and delayed \( \text{CO}_2 \) diffusion into a leaf in drier and disturbed conditions. However, species with different roles in communities had different functional adaptation to aridity and grazing. The true steppe dominants decreased mesophyll cell size and number, which led to reduction of \( \text{A}_{\text{mes}}/\text{A} \), that allowed to economise for construction cost and enhanced WUE. In opposite, in abundant in disturbed steppe species increased cell and chloroplast number per leaf area caused an increase in \( \text{A}_{\text{mes}}/\text{A} \), that made it possible to optimise the photosynthetic performance of these species at disturbed areas. The results of this study will enable future research with similar plant functional types to differentiate the effects of phylogeny and environment and predict plant functional response to environmental change.

**Availability of data and material**

All raw data is available as a Supplementary material 01.

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Supplementary materials

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A small collection of terrestrial snails (Mollusca: Gastropoda) from Unguja Island (Zanzibar, Tanzania) revealed a species unknown for 67 years

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Abstract: The study presents findings from a survey of terrestrial snails conducted on Unguja Island (Zanzibar). The survey, conducted in March 2024, focused on the eastern coast of the island, specifically around Uroa Village and Kiwengwa Cave. A total of 12 species of terrestrial snails were recorded, including Gulella minutissima (Thiele, 1911), previously unknown for over six decades.

Keywords: distribution, habitats, tropical snails

Introduction

Unguja, the main island of the Zanzibar Archipelago, situated approximately 6° south of the equator and 40 km east of the mainland of Africa, boasts an area of approximately 1600 km² (Pakenham, 1984). The island’s diverse ecosystems, including high and low scrub forests characterised by coral limestone bedrock, provide habitats for a variety of flora and fauna (Siex, 2011). The vegetation cover on Unguja is classified as Eastern African Coastal Scrub Forest, according to Clarke’s scheme (Burgess & Clarke, 2000).

Despite its ecological significance, the land mollusc fauna of the East African coastal region, including Zanzibar, remains inadequately explored (Verdcourt, 2006). While the territory of the Zanzibar autonomous region has received attention in terms of species composition of land snails (Rowson, 2007; Rowson et al., 2010; Gittenberger & Bruggen, 2013), many species are reported without precise localities or are challenging to locate due to taxonomic uncertainties (Rowson, 2007). Recent studies have begun to address this gap in knowledge, with the author reporting 16 species of terrestrial and mangrove snails from Unguja Island in 2021, including the discovery of Thapsia insulsa (Preston, 1910), a new record for the island (Georgiev, 2021a).

Building on previous research, this study aims to contribute further to our understanding of the terrestrial snail fauna of Unguja Island. Specifically, it provides new information on the distribution and habitats of terrestrial snail species on the island. By conducting field surveys and documenting species occurrences, this study sheds light on the biodiversity of Unguja’s terrestrial ecosystems and highlights the importance of continued research and conservation efforts in the region.

Material and methods

The survey was conducted concurrently with an entomological study of Psocoptera (Georgiev, 2021b) between 2–8 March 2024, focusing on the eastern coast of Unguja Island, Zanzibar. The primary study area was the vicinity of Uroa Village, with additional sampling conducted at Kiwengwa Cave near the village.
of Upenja. Terrestrial snails were sampled using a combination of visual observations, shell collection, and active searching. Snail shells were collected from various habitats, including bush vegetation on coral limestone rocks and secondary broadleaf forests mixed with coconut palms (*Cocos nucifera*). Additionally, live specimens were occasionally observed on bush branches and cave floors (*Achatina, Opeas*). Detailed field notes, including GPS coordinates and habitat descriptions, were recorded for each specimen. All collected shells were deposited in the National Natural History Museum – Sofia, Bulgaria.

**Results and discussion**

The current study documented a total of 12 species of terrestrial snails from previously unexplored localities on Unguja Island, Zanzibar. Notably, among these species, *Gulella minutissima* (Thiele, 1911) was re-discovered after 67 years, with no photographic records of the shell available. To prevent redundancy with previous publications (Rowson, 2007; Georgiev, 2021a), the known distribution of species is not repeated in the following list.

**Species list**

**Maizaniidae**

*Maizania zanzibarica* Bequaert & Clench, 1936: 8.03.2024, Uroa Village, bush on coral limestone rocks, S06 05 02.9 E39 25 28.7, 5 m a.s.l., shells.

**Pomatiasidae**

*Tropidophora zanguebarica* (Petit, 1850): 2.03.2024, Uroa Village, bush on coral limestone rocks, S06 05 52.1 E39 25 21.3, 14 m a.s.l., shells; 7.03.2024, near the dirt road to Kiwengwa Cave, Upenja Village, bushes and scattered trees, S05 59 48.6 N39 21 32.5, 26 m a.s.l., living specimens on the bush branches.

**Cerastidae**

*Rachis punctata* (Anton, 1839): 4.03.2024, near Uroa Village, bush on coral limestone rocks, S06 05 59.6

**Ferussaciidae**

*Cecilioides kalawangaensis* Dartevelle & Venmans, 1951: 8.03.2024, Uroa Village, bush on coral limestone rocks, S06 05 02.9 E39 25 28.7, 5 m a.s.l., 1 shell.

**Subulinidae**

*Opeas lamoense* Melvill & Ponsonby, 1892: 7.03.2024, Kiwengwa Cave, near Upenja Village, S05 59 52.0 N39 21 35.0, 28 m a.s.l., many living specimens feeding on bat guano on the cave floor (Fig. 1).

*Pseudoglessula subolivacea* (E. A. Smith, 1890): 8.03.2024, Uroa Village, bush on coral limestone rocks, S06 05 02.9 E39 25 28.7, 5 m a.s.l., shells.

**Achatinidae**

*Achatina* (*Lissachatina*) *allisa* (L. Reeve, 1849): 8.03.2024, Uroa Village, bush on coral limestone rocks, S06 05 02.9 E39 25 28.7, 5 m a.s.l., shells.

*Achatina* (*Lissachatina*) *reticulata* (L. Pfeiffer, 1845): 5.03.2024, near Uroa Village, secondary broadleaf forest mixed with coconut palms (*Cocos nucifera*), shells; 7.03.2024, Kiwengwa Cave, near
A small collection of terrestrial snails from Unguja Island (Zanzibar, Tanzania) revealed a species unknown for 67 years.

Streptaxidae

Gonaxis gibbonsi Taylor, 1877: 8.03.2024, Uroa Village, bush on coral limestone rocks, S06 05 02.9 E39 25 28.7, 5 m a.s.l., shells.

Gulella minutissima (Thiele, 1911): 8.03.2024, Uroa Village, bush on coral limestone rocks, S06 05 02.9 E39 25 28.7, 5 m a.s.l., 1 shell (Fig. 2). Shell dimensions: shell height 3.0 mm, shell width 1.1 mm, aperture height 1.0 mm, aperture width 0.9 mm.

Remarkably, the exact type locality of this species remains uncertain, as it was simply recorded as “Sanzibar” by Thiele in 1911 (Thiele 1911), leaving ambiguity as to whether it refers to Unguja Island or the East African coast (Rowson, 2007). Verdcourt (1962) documented a recollection of this species in 1957, adding to its historical significance. This recent discovery, after a lapse of 67 years, provides concrete evidence of the species’ presence on Unguja Island, confirming its existence in the region. This species has not been found anywhere else, i.e., it is presumed to be endemic to Unguja. According to personal communication with Dr Ben Rowson (National Museum of Wales, UK), who checked the late Bernard Verdcourt’s unpublished notes, he provided the details as “Tumbatu I., Puopo (Ostheimer et al. in ANSP 214859)”. This locality, presumably the small island of Popu, is also in the northern half of Unguja and likely shares a similar habitat.

Ariophantidae

Sitala jenynsi (L. Pfeiffer, 1845): 8.03.2024, Uroa Village, bush on coral limestone rocks, S06 05 02.9 E39 25 28.7, 5 m a.s.l., shells; 8.03.2024, Uroa Village, bush on coral limestone rocks at the coast of the Indian Ocean, S06 05 10.0 E39 25 31.0, 9 m a.s.l., living specimens on the bush branches.

Succineidae

Quickia concisa (Morelet, 1848): 8.03.2024, Uroa Village, bush on coral limestone rocks, S06 05 02.9 E39 25 28.7, 5 m a.s.l., shells.
In conclusion, this study presents important findings regarding the terrestrial snail fauna of Unguja Island, Zanzibar, shedding light on previously unexplored localities and contributing to our understanding of regional biodiversity. The rediscovery of *Gulella minutissima* after 67 years underscores the significance of ongoing research efforts in documenting and conserving biodiversity. Moving forward, continued investigations are warranted to further elucidate the ecological roles and conservation status of terrestrial snails on Unguja Island and to support effective management and conservation strategies.

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References


Distribution, demography and conservation perspectives of *Retama raetam* subsp. *gussonei* (Fabaceae) in Calabria (S Italy)

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Abstract: The southernmost region of peninsular Italy, Calabria, hosts two well distinct sub-populations of *Retama raetam* (Forssk.) Webb. subsp. *gussonei* (Webb) Greuter (Fabaceae), a Sicilian-Calabrian endemic shrub. The population study model applied to this work on Calabrian sub-populations of this threatened taxon, demonstrated to be a powerful tool. A preliminary survey on a sample of the studied plants enabled the fast, easy and complete collection of biometric/anagraphic data. Present number of censussed plants (1,523; 82.5% by the Ionian Sea, 17.5% by the Tyrrhenian), the complex and heterogeneous composition of the population (divided in four biometric/age classes and spread on nine different sectors), its distribution within the study area, its evolution as a result of spontaneous population dynamic or future management actions, might be hopefully long term monitored through the study model here applied.

Keywords: biometry, Calabria, conservation, demography, endemism, *Retama raetam* subsp. *gussonei*

Introduction

Calabria (Fig. 1D), located at the centre of the Mediterranean basin, is the southernmost part of Italian Peninsula. It’s surrounded by about 740 km of coasts, most of which are sandy and altered by human settlement.

*Retama* Raf. is a genus belonging to Fabaceae family, close to *Spartium* L. and *Genista* L. (Webb, 1843; Zohary, 1959). *Retama raetam* (Forssk.) Webb subsp. *gussonei* (Webb) Greuter (Fabaceae), a Sicilian-Calabrian endemic shrub. The Calabrian population encompasses two geographic sub-populations, the first of which located by the Ionian Sea, the second by the Tyrrhenian Sea (Fig. 1A-B-C-D).

The Ionian sub-population, located in the northern part of the Cirò Municipality (Crotone Province), occurs close to seacoast (about 50–600 m inland from the coastline). The investigated area is encompassed SE by the final part of the Vota Ranna Stream (or Santa Venere Stream), NE by the Ionian Sea, NW by Carafuno di Cappellieri Stream and SW by the municipal road (Fig. 1A). The investigated area has altitude ranging 0–60 m a.s.l. and is crossed by the cited small streams as well as by Godano Stream, a small intermittent watercourse. Substrata are mainly fixed alluvia and dunes (Marchetti et al., 1968a-c). An inwards morphological terrace (silty clay, sands, and conglomerates) occurs, while around watercourses alluvial substrata prevail (Fig. 2A). The area is crossed by the national road S.S. 106 and by the railway following the coast line about 200–400 m inland (Fig. 1A). NE of the railway line, the landscape is dominated by exotic woody species plantations such as *Eucalyptus camaldulensis* Dehn. subsp. *camaldulensis*, *Pinus halepensis* Mill., *Pinus pinea* L., *Acacia saligna* (Labill.) H.L. Wendl. The inland slope of dunes is mostly dominated by *R. raetam* subsp. *gussonei*. W of the S.S. 106 a vegetation mosaic occurs, encompassing
both small Mediterranean steppe patches (especially close to S.S. 106), areas of Mediterranean maquis, and *R. raetam* subsp. *gussonei* vegetation. The inland landscape is dominated by cultivations (Gangale et al., 2008; Uzunov et al., 2009). Important threatening factors on Ionian stands are weeds such as *Acacia saligna* and *Carpobrotus edulis* (L.) N.E. Br. Both of them seem able to occupy wide areas of the dune system at the expenses of native flora, *Retama* included.

Tyrrenian sub-population occurs in a coastal area (around 400 m from coast line inwards) belonging to San Ferdinando (Reggio Calabria Province) and Nicotera municipalities (Vibo Valentia Province) (Caruso et al., 2010a). The area is crossed by water-courses, the most important of which is Mesima Stream. The dune system is altered and ecologically fragmented due to human activities (buildings, tourist facilities, roads, cultivations, exotic woody species plantation) and weeds such as *Cestrum parqui* L’Hér., which is probably the most hazardous. Anyway, the relict semi-natural coastal area hosts also the rare *Ephedra fragilis* Desf. (Caruso et al., 2010b; Caruso

Fig. 1. Calabrian sub-populations of *R. raetam* subsp. *gussonei* (black patches) (Ionian: I1-I2-I3-I4-I5; Tyrrenian: T1-T2-T3-T4) and thermo-pluviometric stations position (1 × 1 km grid).
et al., 2012; Caruso & Montepaone, 2020). From the geological point of view alluvia and dunes prevail (Marchetti et al., 1968b; Fig. 2B-C), although the whole district is currently undergoing an important erosion process (Chiaravalli et al., 1990; D’Alessandro et al., 1992).

*R. raetam* subsp. *gussonei*, according to IUCN criteria, was formerly considered Critically Endangered (CR) at regional and national level (Conti et al., 1997), but has been later redefined as Endangered EN B1ab(iii)+2ab(iii) (Caruso et al., 2010a).

Macrobioclimate, according to Rivas-Martínez & Rivas-Sáenz (1996–2009), in both localities is Mediterranean pluviseasonal-oceanic, upper thermomediterranean, upper dry. The low subhumid ubrotype registrated at the Rosarno thermo-pluviometric station depends on its altitude and inland location (Fig. 3), rather far from the Tyrrenian *Retama* stands.

Calabrian stands of *R. raetam* subsp. *gussonei* are threatened by anthropic activities (agriculture, woody plant cultivation, grazing, human settlement, alien in-
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vasive species, fire) (Caruso et al., 2010a), as well as illegal sand uptake and coastal erosion (Chiaravalli et al., 1990; D’Alessandro et al., 1992). Some exotic species found in the area do not apparently reproduce [e.g. *Eucalyptus camaldulensis* subsp. *camaldulensis*, *Opuntia subulata* (Mühlenpfordt) Engelm. in Gard] while other are rapidly expanding [e.g. *Cestrum parqui* L’Hér, *Carpobrotus edulis* (L.) N.E. Br., *Acacia saligna*, *Opuntia maxima* Miller, *Pinus pinea*, *Pinus halepensis*] (Caruso et al., 2007; Caruso et al., 2010a).

Aim of this work is to develop an easy-to-apply, low time-consuming, low-invasive scientific model to study shrub-like species population demography. Moreover, considering the present level of risk of extinction in the area of this rare endemic taxon, this work aims to provide detailed quantitative and qualitative data on Calabrian population of *R. raetam* subsp. *gussonei*, its demographic structure and geographical distribution. This may contribute to management policy needed to ensure population long term monitoring and conservation.

**Materials and methods**

The field work has been carried out mainly during 2007 and 2008 spring-summers, but additional information has been collected until the 2022 summer. The identification of the collected material has been done according to Flora d’Italia (Pignatti, 1982; Pignatti, 2017–2020) and Flora Europaea (Heywood, 1967).

**Study area**

Considering the physical (e.g. roads, railway, villages, camp areas, cultivated fields, disturbed areas, etc.) and biological barriers (e.g. exotic woody species forest) existing, inside the areas where *R. raetam* subsp. *gussonei* occurs, ecologically uniform sectors have been delimited (5 by the Ionian Sea, 4 by the Tyrrenian) as shown in Fig. 1.

**Biometric-anagrophic development model**

Before starting the census operation, a preliminary study has been necessary in order to define a simple, low time-consuming, low-invasive sampling technique suitable to know, with statistically acceptable approximation, the age of each single plant belonging to the studied population. *R. raetam* subsp. *gussonei* plants have, if observed from above, a circular shape. This depends on the uniform distribution in all directions of the branches from central stem axis (Fig. 4). A simple way to estimate the age (or age range) of this shrub, is to measure (or estimate) the diameter of the projection of the plant canopy on the ground. Except the infertile juvenile plants (class 0 = 0–0.5 m), the reproductive population of the surveyed species has been therefore arbitrarily divided into 4 biometric classes, based on the diameter of the projection of the canopy on the ground (class 1 = canopy diameter 0.5–1 m; class 2 = 1–2 m; class 3 = 2–3 m; class 4 = 3–6 m) (Fig. 4; Table 1). The next step has been to verify whether these biometric classes were in fact correspondent to different age groups. For this purpose the architectural/anagrophic structure of plants has been studied in detail. A first sample, made of 30 plants belonging to all morphological classes, have central stem axis and branches sampled by a Pressler gimlet. For each sample of wood annual rings have been counted, and so has been determined the age of each...
part of studied plants (Fig. 4). From these data it was possible to know, for each sampled plant, the individual age (= rings of central axis) and the age of the outer branch (= rings of branch). Consequently, has been possible to know the relationship among outer branch age, individual age and plant canopy diameter for each studied plant and all biometric classes (Fig. 4; Table 1). The biometric-anagraphic survey, as shown ahead (Table 1), demonstrated the existence of a close correlation among the average values of plant age, the outer branch age and the canopy diameter. This allowed, following the opposite path, on a second sample made of 120 plants, to know the age of a single plant just through the age of the outer branch easily sampled by Pressler gimlet. Moreover, once calculated the age range of each biometric class, it has been possible to trace the age range of each plant just measuring the canopy diameter. This demonstrates, for the surveyed taxon, the diameter of the canopy projection on the ground to be a reliable and easy-to-assess parameter for an effective biometric-anagraphic classification of plants.

Results and discussion

Biometric classes and plant development model

Biometric-anagraphic sampled measures provided data summarised in Table 1.

The study of populations of rare species and characterising of their development models are used in different study (Pogorzelec et al., 2023; Sakhraoui et al., 2024). Several plants belonging to the biometric class 0 (canopy diameter 0–0.5 m) have been found in each sector of the study area. This class, excluded by the current analysis for the reasons above explained, is composed by plants younger than 4 years (seedlings/not reproductive juveniles), usually infertile. Class 4 has not been split in different classes because very few plants with a canopy diameter wider

Population census and analysis

The reproductive Calabrian population of *R. raetam* subsp. *gussonei*, represented by the 4 biometric classes above described, has been censussed.
than 4 m have been found. Besides, these wider plants grow mainly isolated inside clearings. It has been believed that uncommon size reached by these plants was mainly due to the absence of competition, and consequently was poorly significant. Biometric-anatographic study showed that branches belonging to a single plant do not have the same age. Stem axis is the oldest part of the plant, while the outer branches are the youngest and inner branches are in between. Despite some overlapping values of plant age (e.g. classes 2, 3 and 4 in Table 1), probably due to micro-ecological factors locally affecting morphological development of plants, Table 1 shows high values of correlation (r) among canopy projection diameter and outer branch diameter (0.999), as well as canopy projection diameter and outer branches average age (0.991) and even canopy projection diameter and plant age (0.994). These make the canopy diameter, at least for the purpose of this work, a statistically reliable measure of individual age (or age range) for this taxon in the investigated area.

Population census and analysis

The features of Calabrian population R. raetam subsp. gussonei are summarised in Table 2, a double entry table where columns correspond to biometric classes and rows represent sectors (Ionian sectors: I1-I2-I3-I4-I5; Tyrrenian sectors: T1-T2-T3-T4). Each biometric class (with the only exception of class 0) is represented by two sub-columns. The sub-column on the left side of the table contains the number of plants belonging to that class occurring in that specific sector; the sub-column on the right contains the percentage value of that class on the total amount of plants occurring in that specific sector. Similarly, each sector is represented by two rows. The upper row corresponds to the above described left column value, so contains the number of plants for that class in that sector. The lower row contains the percentage value of that sector on the total amount of plants belonging to a specific class. For example, inside the class 1, in the first column there is 6, in the second 13.6, in the lower row 1.2. This means that inside the sector I1 6 plants belonging to class 1 occur; this class represents 13.6% of total individuals occurring in the sector I1; this means also that in the sector I1 1.2% of plants belonging to the whole class 1 occur. Grey column on the right summarise data per each sector, while the grey row at bottom summarise data per each class. The two columns at the extreme right host aggregate data (and %) of Ionian and Tyrrenian sub-populations.

The overall Calabrian population of R. raetam subsp. gussonei counts 1,523 fertile plants. Ionian subpopulation is clearly prevalent with 82.5% of the
Distribution, demography and conservation perspectives of *Retama raetam* subsp. *gussonei* in Calabria (S Italy)

Table 2. Calabrian population of *R. raetam* subsp. *gussonei* censussed within 9 sectors, 5 of which correspond to the Ionian sub-population (I), and 4 to the Tyrrhenian (T) one.

<table>
<thead>
<tr>
<th>biomec classes</th>
<th>total sectors</th>
<th>sub-populations</th>
</tr>
</thead>
<tbody>
<tr>
<td>class 0</td>
<td>1,523</td>
<td>1,256</td>
</tr>
<tr>
<td>class 1</td>
<td>28</td>
<td>267</td>
</tr>
<tr>
<td>class 2</td>
<td>533</td>
<td>234</td>
</tr>
<tr>
<td>class 3</td>
<td>69</td>
<td>457</td>
</tr>
<tr>
<td>class 4</td>
<td>498</td>
<td>423</td>
</tr>
<tr>
<td>class 5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>total</td>
<td>4,273</td>
<td>2,123</td>
</tr>
</tbody>
</table>

whole population (1,256 plants), while Tyrrhenian subpopulation counts just 267 plants (17.5%) (Table 2).

Considering the distribution of plants among biomec classes it can be noticed that class 1 (498 plants; 32.7% of total), class 2 (423; 27.8%) and class 3 (533; 35%) are rather balanced (around 1/3 each), while class 4 (69; 4.5%) is the less represented. The oldest component of the population (class 4) seems to be quantitatively less important, while the youngest components are dominant. A situation like this is expected in a healthy population with a good reproductive perspective.

Analysing the population by sectors it is evident that the most plant-rich sector is I3 (457 plants; 30% of the whole population); it follows sector I2 (322; 21.1%), sector I4 (239; 15.7%), sector T3 (234; 15.4%), sector I5 (194; 12.7%); quite far it follows sector I1 (44; 2.9%), T1 (28; 1.8%), T4 (4; 0.3%) and T2 (1; 0.1%).

The biometric classes are heterogeneously distributed among sectors. Class 1 prevails in sector I5 (124 plants; 63.9% of the sector) and in sector T3 (188; 80.3%). Class 2, conversely, prevails in sector I2 (122 plants; 37.9% of the sector) and T1 (18 plants; 64.3% of the sector). Class 3 prevails in sectors I1, I3 and I4 respectively with 25 plants (56.8% of the sector), 281 (61.5%) and 145 plants (60.7%). Class 4 is best represented in sector I2 (40 plants; 58%) and sector I3 (25 plants; 36.2%). The richness of young plants (class 1) into sector I5 is mainly due to a wildfire occurred during 2003 and responsible of the destruction of all adult plants of this sector (Table 2; Fig. 5). Because recently discovered (Caruso et al., 2010a) no histori-
cal distribution data are available about Tyrrhenian Calabrian stands of *R. raetam* subsp. *gussonei*. Randomly interviewed local elderly report a wider distribution of *R. raetam* subsp. *gussonei* by the Tyrrhenian coast of Calabria in the past. Human activities and coastal erosion reduced this area severely fragmenting it. Some weedy species (e.g. *Acacia saligna*) seems now to have a stable population, at least in some tracts of the coast where frequent are ruinous attempts of *Citrus* sp. div. cultivation. The recent formation of small patches of *R. raetam* subsp. *gussonei* in these abandoned orchards probably could explain the frequency of young plants (class 1) and, in the long term, could lead this rare taxon to recover part of the presumed former area of occurrence.

Geomorphological role

*R. raetam* subsp. *gussonei* plays an important geomorphological role contributing to create and stabilise the dune system. Sand moved by wind is stopped by plant branches, so tending to accumulate at the plant base mixed with litter. This determines the progressive uplift of the dune top and the partial burial of plant’s branches. Considering that number and age of branches increases with plant age and that in juveniles first branch is above the ground level, while in the adults is mostly covered by sand, the average dune uplift during the plant life (up to 20 years) is 50 (20–80) cm (Fig. 4).

Long term monitoring and conservation

As part of the European Natura 2000 network the Site of Communitary Importance (SCI) IT9320100 named “Dune di Marinella” has been created on the Ionian coast of Calabria (Regione Calabria, 2003) in order to protect an important nesting place of *Caretta caretta* L. (Cheloniidae) and rare and endemic plant species such as *Centaurea deusta* Ten. (Asteraceae), *Hypecoum imberbe* S. et S. (Papaveraceae), *Ephedra distachya* L. subsp. *distachya* (Ephedraceae), *Artemisia variabilis* Ten. (Asteraceae) etc. and habitats, also priority (*), listed in EEC Directive 92/43, such as 6220* (Pseudo-steppe with grasses and annuals of the Thero-Brachypodietea), 2240 (*Brachypodietalia* dune grasslands with annuals), 9320 (Olea and Ceratonia forests), 2210 (*Crucianellion maritimae* fixed beach dunes), 5330 (Thermo-Mediterranean and pre-desert scrub), 2120 (Shifting dunes along the

![Fig. 5. Absolute and percentage importance of biometric classes of *R. raetam* subsp. *gussonei* on the whole Calabrian population, on the Ionian sub-population and on the Tyrrhenian sub-population.](image-url)
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Fig. 6. Old (blue lines) and new (red lines) official perimeters of the “Dune di Marinella” SCI, the second one re-drawn in order to really include the Ionian *R. raetam* subsp. *gussonei* stands of Calabria (black).

Fig. 7. The official current perimeter of “Dune di Marinella” SCI (red lines), overlapping the here proposed new perimeter (green lines + green dots) re-drawn according to the criticalities emerged during the present survey.
shoreline with *Ammophila arenaria*, white dunes), 2230 (*Malcolmietalia* dune grasslands) (European Commission Environment, 2012; Biondi et al., 2012). Nonetheless, the former perimeter of the Dune di Marinella SCI did not really encompassed the Ionian *R. raetam* subsp. *gussonei* stands. That’s why a new perimeter has been drawn including the area where Ionian *R. raetam* subsp. *gussonei* stands occur (Fig. 6) while the old perimeter of the SCI has been deleted by the Rete Natura 2000 network. Nonetheless, a few criticalities emerged along with the new official perimeter. Firstly, the new SCI perimeter shows a deep inlet on the SW side, probably in order to avoid the inclusion of a cultivated area (Figs 6–7). This area is mainly characterised by low productive sandy soils (Fig. 2), constantly at risk of abandonment, mostly intended for herbaceous annual crops while the Cirò area is commonly renowned for high quality viticulture. On the other hand, the area could represent a future opportunity for *Retama* plants to colonise new habitats, therefore deserves to be included in the SCI perimeter, so providing also a more compact shape to the protected area. Second, the new official perimeter excluded some plants (Fig. 6) belonging to the I3 sector, in the NE of the Ionian distribution area. Third, in the SE corner of the new official perimeter some olive tree cultivations have been, maybe erroneously, included. Fourth, the name of this SCI can no longer be “Dune di Marinella”, because “Marinella” is a locality currently far from the SCI new official perimeter. Considering these observations, is here proposed, for the SCI area, the new name “Dune di Cirò” (meaning “Dunes of Cirò” in Italian), and a new perimeter (Fig. 7), virtually resolving all the recorded issues properly.

The lack of protection measures, the altered environment, as well as the Tyrrhenian *R. raetam* subsp. *gussonei* population size and structure, expose it to a high risk of extinction. Being the area also interested by the occurrence of one of the only two known stands of *Ephedra fragilis* Desf. of peninsular Italy (Caruso et al., 2009; Caruso et al., 2010b; Caruso et al., 2012; Caruso & Montepaone, 2020), it should be justified the establishment of a new protected area in this territory. The proposed protected area (as a floristic protected area, regional park or, better, as a SCI), possibly named “Dune di Nicotera Marina e S. Ferdinando”, would adopt measures against further anthropic pressure.

Long term conservation of this precious taxon largely depends on the environmental quality and on the possibility to maintain and even increase the present area of occurrence. The dangerous co-occurrence of invasive species such as *Carpobrotus edulis* (Ionian stands), *Cestrum parqui* (Tyrrhenian stands) or *Acacia saligna* (Ionian and Tyrrhenian stands) strongly affects the surveyed species actively competing for space and resources. In order to preserve the existing stands of *Retama raetam* subsp. *gussonei* the complete eradication of these alien taxa seems to be the only solution. The other woody exotic species widely planted in the surveyed area, although not invasive, occupy a large surface potentially useful to increase the present *R. raetam* subsp. *gussonei* area of occurrence. A progressive programme of eradication should be in this case a good solution.

Considering the IUCN category to which this taxon belongs and the persistence of threatening factors...
on studied sub-populations, a long term monitoring programme must be urgently planned. Considering biology and ecology of this taxon, applying the methodology developed as part of this work, the frequency of monitoring should be 3–10 years. This would allow comparable data to be easily collected. As the eradication of weeds proceeds a 2–3-year monitoring protocol should be scheduled in order to lead dynamic management.

Conclusions

The population study model applied to this survey on Calabrian sub-populations of *R. raetam* subsp. *gussonei* demonstrated to be a very powerful tool. A preliminary survey on a sample of investigated plants made it possible a fast, easy and complete collection of biometric/anagraphic data on the whole population.

The present number of plants, the complex and heterogeneous composition of the population, its distribution within the study area, its evolution as a result of spontaneous population dynamic or future management actions, as well as the possible establishment of specific protected areas needed for the protection of this rare and endemic taxon in Calabria, might be hopefully long term monitored through the study model here applied.

References


