

Blitum venetum (Chenopodiaceae), a new species from the north-eastern Dolomites (Italian Eastern Alps)

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Abstract – A new species, *Blitum venetum* Iamónico, Argenti, Sciuto & Wolf is described from the Dolomites Massif (North-Eastern Italy) on the basis of molecular analyses (nuclear ITS and plastid *trnL-F* regions) and morphological investigation. The new species is similar to *B. bonus-henricus* and *B. californicum*, but it differs from these taxa by characters of leaves (width, pubescence, and margins), inflorescence (presence of bracts), perianth segments (colour), and seeds (diameter, colour, and seed testa ornamentation). Moreover, in phylogenetic reconstructions *B. venetum* results as clearly separated from the other species of the genus; this finding was further strengthened by the nucleotide divergences calculated between the sequence of the type specimen and other *Blitum* taxa, which are comparable with the interspecific divergences calculated inside this genus.

Keywords: *Blitum*, *Blitum bonus-henricus*, *Blitum californicum*, ITS, taxonomy, *trnL-F*.

Introduction

The genus *Blitum* L. [tribe Anserineae Dumort., Chenopodiaceae Vent. *sensu* Hernández-Ledesma et al. 2015; some authors, e.g. APG IV (2016) consider Chenopodiaceae as included in Amaranthaceae Juss.], traditionally included in *Chenopodium* L. s.l. during the last centuries (with a few exceptions), was recently taxonomically resurrected by Fuentes-Bazan et al. (2012a, b) based on phylogenetic studies of Chenopodioideae Burnett, where a reclassification of the whole subfamily was proposed recognizing four tribes and ten genera. The generic classification outlined in Fuentes-Bazan et al. (2012b) has been updated in many recent publications (e.g., Iamónico 2012, Mosyakin 2013, Uotila 2011, 2017, Sukhorukov et al. 2013, Sukhorukov and Kushunina 2014) and generally accepted (e.g., Hernández-Ledesma et al. 2015; some online floras and databases, e.g., French flora; POWO 2021-onward).

Blitum (incl. *Agathophytum* Moq., *Monolepis* Schrad., *Scleroblitum* Ulbr., *Carocarpidium* S.C.Sand. & G.L.Chu) is currently known to include about 12 species and nothospecies (see Fuentes-Bazan et al. 2012b, Mosyakin 2013,

Hernández-Ledesma et al. 2015, Sukhorukov et al. 2018), ten of which occur in the Northern Hemisphere [*B. asiaticum* (Fisch. & C.A.Mey) S.Fuentes, Uotila & Borsch (= *Monolepis asiatica* Fisch. & C.A.Mey.), *B. bonus-henricus* (L.) Rchb., *B. californicum* S.Watson, *B. capitatum* L. s.l., *B. korshinskyi* Litv., *B. litwinowii* (Paulsen) S.Fuentes, Uotila & Borsch, *B. nuttalianum* Schult. (= *Monolepis nuttalliana* (Schult.) Greene), *B. petiolare* Link (= *Chenopodium exsuccum* (C.Loscos) Uotila), *B. xtkalcsicsii* (H.Melzer) Mosyakin, *B. virgatum* L. (= *Chenopodium foliosum* Asch.)], one in South America (*B. antarcticum* Hook. f.) and one [*B. atriplicinum* F.Muell. (= *Scleroblitum atriplicinum* (F.Muell.) Ulbr.)] in Australia.

As part of the ongoing study on *Chenopodium* s.l. (e.g., Iamónico 2010, 2011, 2012, 2013, 2014, Iamónico and Jarvis 2012, Mosyakin and Iamónico 2017), a population belonging to the genus *Blitum* was found in the Dolomites Massif (North-Eastern Italy). After a detailed analysis of pertinent literature, examination of specimens in herbaria and in virtual herbaria online (type material included), and molecular investigation, we reached the conclusion that the population

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found cannot be identified as belonging to any known species of *Blitum*. Consequently, we describe here a species new for science and compare it with the related taxa.

Materials and methods

Morphological and nomenclatural study

The work is based on field surveys, analysis of relevant literature (protologues of the names involved in the present study are included), and examination of specimens preserved in the following herbaria: BR, GH, K, LINN, NY, P, RO, SD, UC, and US (codes according to Thiers 2022). Photographs of inflorescence and fruits were made using an optical stereoscope Wild Heerbrugg M8.

Molecular study

Genomic DNA was extracted using the Genomic DNA purification kit (Thermo Scientific™, Waltham, MA, USA). The nuclear ITS region and the plastid *trnL*-F region were amplified following Fuentes-Bazan et al. (2012a). The obtained PCR products were cleaned using the HT ExoSAP-IT (Applied Biosystems™, Waltham, MA, USA) and sequencing was carried out at the Eurofins Genomics Sequencing Service (Germany), with the same primers employed in the amplification reactions. For sequencing of the *trnL*-F locus, two internal primers were also employed as reported in Fuentes-Bazan et al. (2012a). The GeneStudio sequence analysis software (<http://genestudio.com/>) was used to assemble the final consensus sequences. The new sequences were deposited in the International Nucleotide Sequence Database Collaboration (INSDC) repositories, through the European Nucleotide Archive (ENA) platform, with the following GenBank accession numbers: OU753550 (ITS region) and OU753551 (*trnL*-F region).

The obtained sequences were compared with those present in the INSDC archives using the BLAST program available at the USA National Center for Biotechnology Information (NCBI) web server (<http://www.ncbi.nlm.nih.gov>). Two distinct datasets of ITS and *trnL*-F sequences were created, including the newly obtained sequences and other suitable sequences available in the INSDC repositories, following the most recent classifications for the genus *Blitum* (Fuentes-Bazan et al. 2012b, Sukhorukov et al. 2018). For each analysis, a sequence of *Chenopodium pallidicaule* Aellen (INSDC accession number: HE577438 for the ITS locus; INSDC accession number: HE577573 for the *trnL*-F locus) was used as outgroup to orient the tree. Multiple sequence alignments were generated with MUSCLE (Edgar 2004) and used for phylogenetic analyses. The ITS multi-alignment included 15 sequences for a total of 507 aligned positions and the *trnL*-F multi-alignment included 13 sequences for a total of 987 aligned positions.

Phylogenetic analyses were performed with MEGA v. X program (Kumar et al. 2018) using the Maximum Parsimony (MP) and the Maximum Likelihood (ML) methods. For ML, the model that best fit the data, found with the “Find

best DNA Models” tool implemented in MEGA v. X under the BIC criterion (Schwarz 1978), was T92 + G for both the molecular markers. Nonparametric bootstrap re-sampling (Felsenstein 1985) was performed to test the robustness of the obtained topologies (1000 replicates). Bayesian Inference (BI) analyses were carried out with MrBayes version 3.1.2 (Ronquist and Huelsenbeck 2003). The analyses included two independent MCMC runs, each composed of four chains (three heated and one cold); each MCMC ran for 1×10^6 generations, sampling trees every 100 generations. The sampling of the posterior distribution was considered to be adequate if the average standard deviation of split frequencies was ≤ 0.01 . The first 2500 trees were discarded as burn-in, as determined by stationarity of log likelihood assessed using Tracer version 1.5 (Rambaut and Drummond 2007). The consensus topology and posterior probability values were then calculated from the remaining trees. The final tree pictures were created with Inkscape v. 0.92 (Figs. 1a, 1b).

Alignments of the ITS and *trnL*-F sequences of different *Blitum* species were also obtained with MUSCLE (Edgar 2004) to calculate the percent identities within the genus (Figs. 1c, 1d).

Results

Morphology

The genus *Blitum* is morphologically characterized by having stems often unbranched or nearly so, or mainly branched at or near the base, leaves often forming basal rosettes, stigma lobes 2–4, and seed vertical with the seed coat displaying a visible protoplast (Fuentes-Bazan et al. 2012b, Iamónico 2013, Sukhorukov et al. 2018). The genus is rather heterogeneous morphologically (Clemants and Mosyakin 2003, Zhu et al. 2003, Sukhorukov et al. 2018), the plants we found in the Dolomites Massif (North-Eastern Italy, Veneto region) appear to resemble both *B. bonus-henricus* L. and *B. californicum* S.Watson. In particular, they show the following combination of characters: perennial (other *Blitum* species, except perennial *B. bonus-henricus* and *B. californicum*, are usually annual), erect to ascending [*B. asiaticum* (Fisch. & C.A.Mey.) S.Fuentes, Uotila & Borsch, *B. litwinowii* (Paulsen) S.Fuentes, Uotila & Borsch, and *B. nuttalianum* Schult. have stems prostrate], basal rosette with many leaves [*B. asiaticum* and *B. nuttalianum* have just 1 or 2 leaves, that may wither away completely by anthesis], large and more or less triangular leaves with their base hastate or cordate, more than 5–6 cm long [the other species have leaves more or less rhomboid, with base cuneate, and blades usually up to 5–6 cm long (*B. virgatum* L. and *B. capitatum* L. have leaves triangular and up to 8 cm long, but they are annual species)], perianth with 5 segments (other species of the genus usually have 1–3 segments) not becoming red and succulent at the fruiting stage (in *B. virgatum* and *B. capitatum* perianth segments are fleshy and red at maturity, forming a berry-like structure).

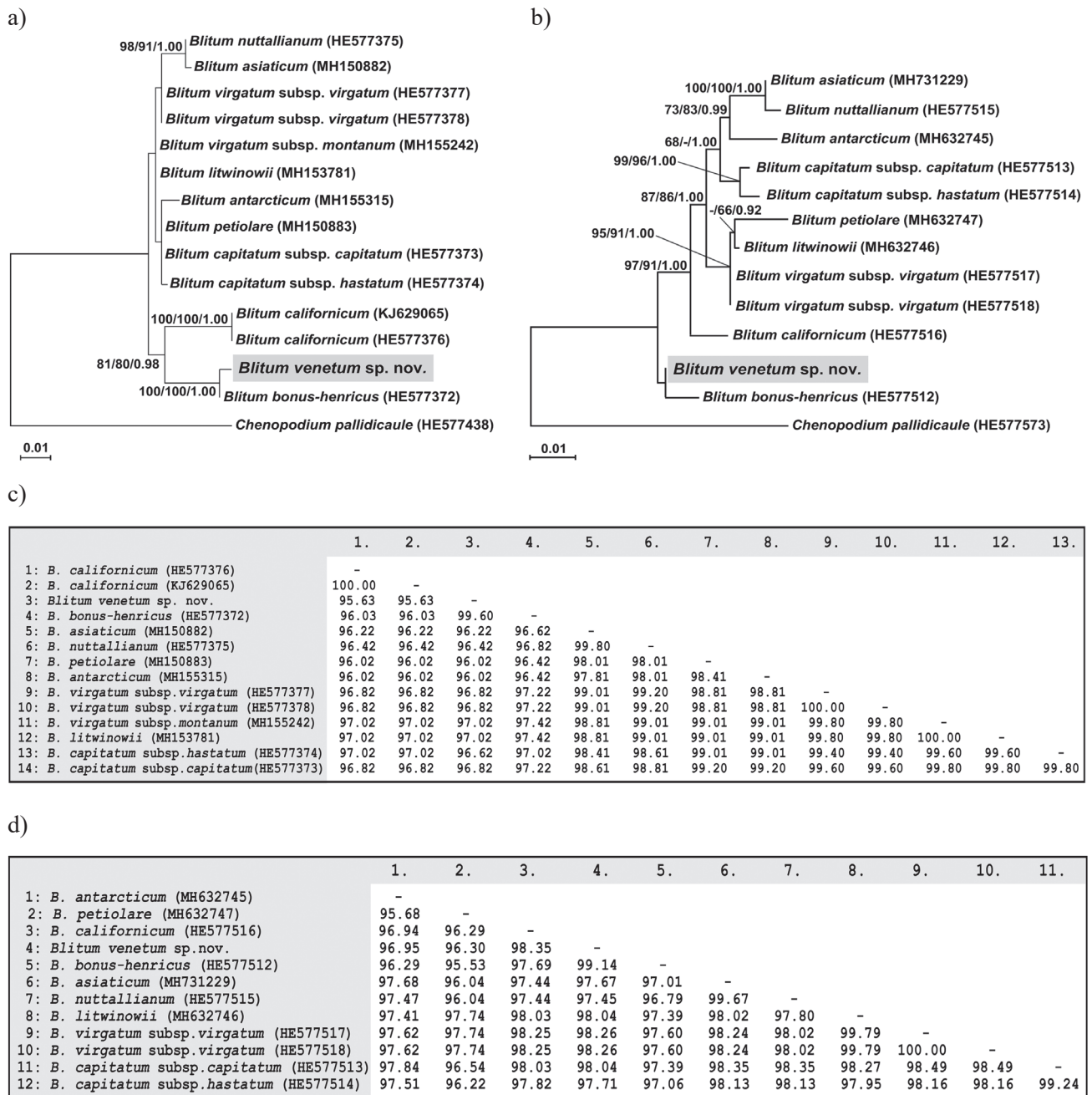


Fig. 1. Phylogenetic reconstructions obtained with ML method. Molecular analyses based on the ITS region (a) and molecular analyses based on the *trnL-F* region (b). For each node, the support values from ML bootstrap, MP bootstrap and BI posterior probabilities are reported, respectively. Only bootstrap supports $\geq 50\%$ and posterior probabilities ≥ 0.70 are shown. Values for nodes that obtained support in only one of the phylogenetic analyses were omitted. For each of the downloaded sequences, the species name, followed by the INSDC accession number between parentheses, is reported. The new species is highlighted with a grey box. Scale bar represents expected number of nucleotide substitutions per site. (c) Percent identity matrix (507 aligned positions) of the taxa belonging to the genus *Blitum* based on analysis of ITS region. (d) Percent identity matrix (987 aligned positions) of the taxa belonging to the genus *Blitum* based on analysis of *trnL-F* region.

In comparison with *Blitum bonus-henricus* (native in Europe, introduced in North America) and *B. californicum* [endemic to California and Mexico (Baja California)], the Italian population differs morphologically by both vegetative and generative characters, i.e. size, pubescence and leaf margins, colour of perianths segments, structure of the inflorescence, and size, colour, and surface of seeds (Tab. 1; see also the diagnosis under the taxonomic treatment).

Molecular data

In both phylogenetic reconstructions, those based on the ITS and *trnL-F* markers (Figs. 1a, 1b), the sequences obtained from the type specimen collected in the Belluno Dolomites (Italy) resulted clearly separated from the other species of *Blitum*. In the ITS tree (Fig. 1a) our sequence was included in a well-supported clade (100/100/1.00) with a se-

Tab. 1. Morphological comparison among *Blitum bonus-henricus*, *B. californicum*, and *B. venetum*. Differential characters are in bold.

	<i>Blitum bonus-henricus</i>	<i>Blitum californicum</i>	<i>Blitum venetum</i>
Stem	Erect to ascending, often unbranched, 20–70 cm, glabrous (sometimes farinose)	Erect to ascending, often unbranched, 25–80 cm, glabrous (sometimes farinose)	Erect to ascending, branched, 40–80(–90) cm, glabrous and sparsely pubescent in the inflorescence part
Leaves	Green, adaxially sparsely farinose , non-aromatic, petioled (petiole 5–20 cm), blade triangular, 5–15 × 3–10 cm (size decreasing along the stem upwards), base truncate to hastate or cordate; entire and undulate margins , apex usually acute	Green, adaxially sparsely farinose , non-aromatic, petioled (petiole 1–12 cm), blade triangular, 4–10 × 3–9 cm, (size decreasing along the stem upwards), base truncate to cordate; margins dentate (in larger leaves up to 10 teeth, teeth acute to acuminate , 0.3–1.0 cm long), apex acute to acuminate	Green, glabrous , non-aromatic, petioled (petiole 1–10 cm), blade triangular, 10–13 × 11–13 cm (size decreasing along the stem upwards), base truncate to hastate or cordate; margins dentate (in larger leaves 6–8 teeth, teeth acuminate , 1.0–2.5 cm long), apex acute to acuminate
Inflorescence	Terminal and lateral spiciform inflorescences ebracteate ; spikes 5–20 cm long, the terminal one usually longer than the lateral ones; glomerules about 3–5 mm in diameter	Terminal spiciform inflorescence ebracteate ; spikes 5–20 cm long; glomerules about 3–5 mm in diameter	Terminal and lateral spiciform structures; each glomerule subtended by a linear bract (1.0–2.5 mm long); some lower glomerules subtended by the upper cauline leaves; spikes 6–8 cm long, the terminal one longer than the lateral ones; glomerules about 2 mm in diameter
Flowers	Perianth segments connate up to the half, lobes 5, oblong to elliptic, 0.5–1.1 × 0.8–1.5 mm, scarcely keeled throughout, apex obtuse to rounded, glabrous, green throughout ; stamens 4–5; stigmas 2(–4)	Perianth segments connate up 1/2–2/3, lobes 5, oblong to elliptic, 0.6–1.0 × 0.4–1.2 mm, scarcely keeled throughout, apex obtuse to rounded, glabrous, green throughout ; stamens 4–5; stigmas 2	Perianth segments connate up to the 1/3 of the total length, lobes 4–5, ovate, about 0.5 × 1.0 mm, scarcely keeled throughout, apex rounded, glabrous, green with reddish apical part ; stamens 4–5; stigmas 2
Fruits	Achenes obovoid, 1.5–2.2 mm; pericarp adherent.	Achenes obovoid, 1.5–2.0 mm; pericarp adherent.	Achenes obovoid, 1.5–2.0 mm; pericarp adherent.
Seeds	Seeds vertical, obovoid, 1.5–2.0 mm in diameter , margins usually not rounded; seed coat red, smooth	Seeds vertical, obovoid, 1.5–2.0 mm in diameter , margins rounded; seed coat red	Seeds vertical, obovoid, about 1 mm in diameter , margins rounded; seed coat blackish, alveolate .

quence of *B. bonus-henricus* from Austria (HE577372). This phylogenetic relationship was not confirmed by the phylogenetic analysis based on the plastid spacer *trnL-F* (Fig. 1b), where the Belluno specimen and *B. bonus-henricus* were placed again as sister taxa, but without any statistical support.

On the basis of the percent identity matrices obtained with MUSCLE (Figs. 1c, 1d), the nucleotide divergences calculated between the *Blitum* sp. specimen from Belluno and each of the other recognized *Blitum* species ranged from 0.40% (*Blitum* sp. vs. *B. bonus-henricus*) to 4.37% (*Blitum* sp. vs. *B. californicum*) for the nucleotide ITS marker and from 0.86% (*Blitum* sp. vs. *B. bonus-henricus*) to 3.70% (*Blitum* sp. vs. *B. petiolare*) for the plastid *trnL-F* marker.

The interspecific divergences calculated between the ITS sequences of the currently taxonomically accepted species of this genus ranged from 0.20% [*Blitum nuttalianum* vs. *B. asiaticum*; *B. capitatum* subsp. *capitatum* vs. *B. capitatum* subsp. *hastatum* (Rydb.) Mosyakin; *B. capitatum* vs. *B. litwinowii*; *B. capitatum* vs. *B. virgatum* subsp. *montanum* (Uotila) S.Fuentes, Uotila & Borsch] to 3.98% (*B. californicum* vs. *B. petiolare*; *B. californicum* vs. *B. antarcticum*). The ITS sequence of *B. litwinowii* and that of the subspecies *B.*

virgatum subsp. *montanum* did not show divergences. For the plastid *trnL-F* spacer, the interspecific divergences ranged from 0.21% (*B. litwinowii* vs. *B. virgatum* subsp. *virgatum*) to 4.47% (*B. bonus-henricus* vs. *B. petiolare*).

Therefore, for both analysed markers, the nucleotide divergences calculated between the sequence of *Blitum* sp. from Belluno and other *Blitum* species are comparable with the interspecific divergences calculated inside this genus.

Discussion

The results obtained in the present study, both morphological and molecular data, support the description of the population from the Belluno Dolomites as a new species, *Blitum venetum*, which is formally described below.

Blitum venetum can be reliably distinguished from the closely related species *B. bonus-henricus* and *B. californicum* by various vegetative and generative morphological characters. All diagnostic characters found for *B. venetum* have high taxonomic value in the classification of both *Blitum* and the intrageneric taxa of *Chenopodium* s.l. (Mosyakin and Clemants 1996, Clemants and Mosyakin 2003, Zhu et al. 2003, Sukhorukov et al. 2018). Its recognition as a dis-



Fig. 2. Holotype of *Blitum venetum* (RO).

tinct species is further supported by its phylogenetic position in both nuclear ITS and plastid *trnL*-F trees, as well as considering, for both of the analysed markers, the nucleotide divergences among various *Blitum* species.

***Blitum venetum* Iamónico, Argenti, Sciuto & Wolf, sp. nov.**

Holotype. Italy, Veneto region, Belluno administrative Province, Vigo di Cadore Municipality, locality Casera Razzo, 46°28'41.8"N, 12°36'33.3"E, 1740 m a.s.l., nitrophilous grasslands, 11 August 2020, C. Argenti s.n.; holotype: RO! (Fig. 2).

Diagnosis (Fig. 3) – *Blitum venetum* differs from *B. bonus-henricus* and *B. californicum* in having the leaves wider (11–13 cm vs. 3–10 cm and 3–9 cm, respectively), glabrous (vs. adaxially sparsely farinose), with dentate and not undulate margins (vs. entire and/or undulate margins), the perianth segments with red or reddish terminal parts (vs. green throughout), the spike-like inflorescences bracteate, each floral glomerule subtended by a linear bract (vs. spiciform inflorescences ebracteate or nearly so), and the seed smaller (about 1 mm in diameter vs. usually 1.5–2.0 mm). In comparison with *B. bonus-henricus*, *B. venetum* also differs by the colour and surface of the seeds (blackish and al-



Fig. 3. *Blitum venetum*. A – habitat, B – young individual, C – details of the terminal inflorescence, D – seed. Scale bars: B – 3 cm, C – 3 mm, D – 0.5 mm. Photo: A-B – C. Argenti, C-D – D. Iamónico.

veolate vs. red and smooth; also *B. californicum* has reddish seeds).

Molecular vouchers. OU753550 (ITS region) and OU753551 (*trnL-F* region).

Description. *Stems* erect to ascending, branched, 40–80(–90) cm, glabrous; sparsely inflated hairs in the inflorescence part. *Leaves* green, non-aromatic, blades glabrous; petiole 1–10 cm; blade triangular, 10–13 × 11–13 cm (size decreasing along the stem upwards), base truncate to hastate or cordate; margins dentate (in larger leaves with 6–8 teeth, teeth acuminate, 1.0–2.5 cm long), apex acute to acuminate. *Inflorescences*: glomerules mostly densely arranged in terminal and lateral spiciform structures, each subtended by a glabrous leaf-like bract (0.5–3.0 × 2–5 cm); the uppermost bracts with attenuate bases (edges of the leaf base forming an angle of about 90°), with two basal teeth (triangular) or with entire margins (lanceolate); each glomerule subtended by a linear bract (1.0–2.5 mm long), with membranous hyaline borders; some lower glomerules solitary and sessile, subtended by the upper cauline leaves; spikes 6–8 cm long, the terminal one longer than the lateral ones; glomerules about 2 mm in diameter. *Flowers*: perianth segments connate up to the 1/3 of the total length, lobes 4 or 5, ovate, about 0.5 × 1.0 mm, scarcely keeled throughout, apex rounded, glabrous, green with reddish apical part; stamens 4 or 5; stigmas 2. *Achenes* obovoid, 1.5–2.0 mm; pericarp

adherent. *Seeds* vertical, obovoid, about 1 mm in diameter, margins rounded; seed coat blackish, alveolate.

Etymology. The specific epithet is dedicated to the Italian region (Veneto) in which the new species occurs.

Proposed vernacular name. Blito del Veneto (Italian), Veneto's Goosefoot (English).

Flowering time. August to September.

Distribution area and habitat. *Blitum venetum* is known only from *locus classicus*, i.e., locality Casera Razzo (Belluno administrative Province, Veneto region, North-Eastern Italy), where it grows on nitrophilous grasslands at 1740 m a.s.l. The *locus classicus* is part of the territory of the Dolomiti Bellunesi National Park (date of establishment: 1988) and of the UNESCO World Heritage Site “The Dolomites” (date of inscription: 2009; see <http://whc.unesco.org/en/list/1237>).

IUCN Conservation assessment. Only one population was found (*locus classicus*) and it occupies a small area (less than 50 m²). On the basis of criterion B3 of IUCN (2019), *Blitum venetum* is here assessed as Critically Endangered (CE).

Additional specimens examined

Blitum bonus-henricus. *Habitat in Europae ruderatis*, Herbarium Linnaeus no. 313.1 (LINN, lectotype). Belgium: Bastogne, 1884, A. Verheggen s.n. (P01089431); Theux, 18

June 1959, *J. Pelgrims* 753 (BR0000027710821V). France: Orcières, village Merlette, 1830 m a.s.l. 13 July 1980, *G. Sag* 986 (P00036524). Italy: Lazio region, Montagna d. Duchessa (Velino), June 1946, *B. Anzalone* 10739 (RO); Lazio region, Piani di Livata al M. Autore, 21 July 1966, *B. Anzalone* 10737 (RO); Lazio region, Monti della Laga (Amatrice – RI), Fosso di Selva Grande, 1350–1500 m a.s.l., 6 July 1997, *B. Anzalone* 10734 (RO!); Trentino-Alto Adige, San Martino di Castrozza, August 1958, *B. Anzalone s.n.* (RO). Netherlands: Epen, July 1954, *W. Voss s.n.* (BR0000026765815V). Slovenia, Jesenice, 1200 m a.s.l., 18 August 1976, *G. Sag* 986 (P00041309). Sweden: Uppland, Bondkyrka parish, about 1½ km southwest of Berthåga, 18 September 1956, *C. G. Alm* 2972 (US03654843). Switzerland: Wegkant to Davos Platz, July 1975, *W. Verbeke s.n.* (BR0000025468366V). U.S.A.: New York, Long Island, East Hampton, 10 June 1877, *E. S. Miller* 321 (US03652612).

Blitum californicum. Mexico: Baja California, Ensenada, Sierra San Pedro Martir. Colony under live oak, Los Encinos, 10 August 1969, *R. Moran* 16539 (UC1361814). U.S.A.: California, San Diego county, in rocks at base of cliff ½ mile a southeast of Eel Point, 28 January 1967, *R. M. Beauchamp* 134 (SD00017034); San Diego county, west side of San Felipe Wildlife Area, under shrubs and mesquite, 26 March 1997, *S. Bell* 248 (SD); San Buena Ventura, March 1861, *W. H. Brewer* 244 (GH00936570, syntype, image available at <https://plants.jstor.org/stable/10.5555/al.ap.specimen.gh00936570>); Fort Tejon, 1857–1859, *J. Xantus de Vesey* 104 (GH00936572, syntype, image available at <https://plants.jstor.org/stable/10.5555/al.ap.specimen.gh00936572>); *s.d.*, *J. C. Fremont s.n.* (NY00008484, syntype, image available at <http://sweetgum.nybg.org/science/vh/specimen-details/?irn=217758>); *s.d.*, *D. Douglas s.n.* (K000898461, syntype, image available at <http://apps.kew.org/herbcat/getImage.do?imageBarcode=K000898461>).

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