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Authors: Thulin, Mats, Rydberg, Anders, and Thiede, Joachim

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MATS THULIN[1*], ANDERS RYDBERG[1] & JOACHIM THIEDE[2]

Identity of *Tetragonia pentandra* and taxonomy and distribution of *Patellifolia (Chenopodiaceae)*

Abstract

Thulin M., Rydberg A. & Thiede J.: Identity of *Tetragonia pentandra* and taxonomy and distribution of *Patellifolia (Chenopodiaceae)*. – Willdenowia 40: 5–11. – Online ISSN 1868-6397; © 2010 BGBM Berlin-Dahlem.
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The little-known *Tetragonia pentandra,* long regarded as a member of *Aizoaceae* in the Horn of Africa region, is included in a phylogenetic analysis using ITS sequence data of members of *Betoideae (Chenopodiaceae). Patellifolia,* including *Tetragonia pentandra,* is strongly supported as monophyletic and weakly supported as sister to a clade with *Aphanisma, Oreobliton* and *Beta*. Within *Patellifolia* relationships are largely unresolved and, as the characters that have been used to distinguish taxa in the group are found to be unreliable, we propose that all members of the *Patellifolia* clade are treated as a single variable species, *P. procumbens*. Synonymies of *Patellifolia* and of *P. procumbens* are provided and two names are lectotypified. The distribution of *P. procumbens* is mapped and shown to be widely disjunct between the W Mediterranean region/Macaronesia and the Horn of Africa region, with isolated occurrences also in the Saharan mountains.

Additional key words: *Beta,* disjunct distribution, Horn of Africa, Macaronesia, molecular phylogeny, nomenclature, typification, ITS.

Introduction

Tetragonia pentandra Balf. f. was described as a member of *"Ficoideae"* (currently *Aizoaceae*) from Socotra (Yemen) by Balfour (1884), who regarded it as a "quite distinct species of this genus" characterised "by the small number of stamens, and by its small, obconoid, smooth, almost sessile fruits" (Balfour 1888). It was stated to have its nearest affinity with the South African species *T. microptera* Fenzl ex Harv. & Sond. and *T. echinata* Aiton.

For a long time *Tetragonia pentandra* was known only from the type specimen from 1880, but a second collection from Socotra was made in 1953 (*Popov So/197,* BM) and Gilbert (1993) reported it from neighbouring northern Somalia based on the collection *Thulin & Warfa 6185* (K, UPS) made in 1986. Thus, by today, the name *T. pentandra* has only been used for three collections, all from the Horn of Africa region.

In 2003 a leaf sample of the Somali material of *Tetragonia pentandra* was sent by M. Thulin to J. Thiede, who was involved in a molecular phylogenetic study of *Aizoaceae* (Thiede 2004) that included several species of *Tetragonia* L. Thiede reported back that an ITS sequence of the plant did not agree with *Tetragonia* or *Aizoaceae,* but strongly indicated a position of the plant near *Beta* L. in *Chenopodiaceae-Betoideae*. Thulin (2006) subsequently, on morphological grounds, identified *T. pentandra* as apparently conspecific with *Beta patellaris* Moq. (≡ *Pa-*

1 Systematic Biology, IEGS, Uppsala University, Norbyvägen 18D, SE-752 36 Uppsala, Sweden; *e-mail: mats.thulin@ebc.uu.se (author for correspondence).
2 Schenefelder Holt 3, 22589 Hamburg, Germany.

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tellifolia patellaris (Moq.) A. J. Scott & al.), a species otherwise found in Macaronesia and the W Mediterranean region.

Beta patellaris was placed by Scott & al. (1977) in the segregate genus *Patellifolia* A. J. Scott & al. (= *Beta* sect. *Procumbentes* Ulbr.) with three species. The other two, *P. procumbens* (C. Sm.) J. T. Williams & al. and *P. webbiana* (Moq.) J. T. Williams & al., are endemic in Macaronesia.

The recognition of *Patellifolia* as distinct from *Beta* was supported in a molecular study by Hohmann & al. (2006). However, the number of species of *Patellifolia* is uncertain and Hohmann & al. (2006), as well as Kadereit & al. (2006), talk about two or three species in the genus. Other students of the group, such as Santoni & Bervillé (1992), have even questioned if more than one species can be recognised.

In the present study *Tetragonia pentandra* is included in a molecular analysis of members of *Patellifolia* and other members of subfam. *Betoideae* with the aims (1) to find out if the synonymisation of *T. pentandra* with *Beta patellaris* (≡ *Patellifolia patellaris*) by Thulin (2006) is supported, and (2) to throw new light on the taxonomy and distribution of *Patellifolia*.

Material and methods

Taxon sampling. — For the molecular study we used all ITS sequences of members of *Betoideae* (i.e., the genera *Aphanisma* Nutt. ex Moq., *Beta, Hablitzia* M. Bieb., *Oreobliton* Durieau and *Patellifolia*) from the study by Hohmann & al. (2006). *Acroglochin chenopodioides* Schrad., with an uncertain position, was not included. To this matrix, which comprised sequences from two members of *Patellifolia, P. patellaris* from Gran Canaria and *P. procumbens* from Tenerife, we added new sequences of (1) *Tetragonia pentandra* from Somalia, (2) *Patellifolia patellaris* from Tenerife, and (3) *P. webbiana* from Tenerife. *Chenopodium botrys* L. and *Salsola kali* L. from outside the *Betoideae* were also included in the analysis. As most distant outgroup we used *Illecebrum verticillatum* L. Information on GenBank accession numbers for all sequences used are provided in the Appendix.

DNA extraction, amplification and sequencing. — Leaves preserved in silica gel or from herbarium material were ground using a Mini-Beadbeater (BioSpec Products) and genomic DNA was isolated using a DNeasy Plant Mini kit (Qiagen) following the manufacturer's protocol. The ITS region was subsequently amplified with primers ITS5 (GGAAGGAGAAGTCGTAACAAGG) and ITS4 (TCCTCCGCTTATTGATATGC) using the following PCR protocol: an initial denaturation phase at 95° for 10 minutes was followed by 35 cycles of 95° for 15 seconds, 55° for 1 minute and 72° for 2 minutes, and a final extension phase at 72° for 10 minutes. PCR reactions were contained in 200 µl reaction tubes, each tube containing a mixture of 5 µl reaction buffer (ABgene, 10×), 3 µl $MgCl_2$ (25mM), 1 µl dNTP's (10 µM), 0.25 µl Taq-polymerase (ABgene; 5U/µl), 0.25 µl BSA (Roche Diagnostics), 12.5 µl of each primer (2mM) and 1 µl template DNA.

Sequencing was performed by Macrogen Inc. (Seoul, South Korea) on an ABI3730XL automated sequencer (Applied Biosystems). For the sequencing reactions, internal primers ITS2 (GCTGCGTTCTTCATCGATGC) and ITS3 (GCATCGATGAAGAACGCAGC) were used in addition to the primers used in the PCR reactions.

Sequence assembly, alignment and phylogenetic analysis. — Readings were assembled automatically and manually into contigs using the Staden package (Staden & al. 1998). The sequences were aligned with ClustalW v. 2.0 (Larkin & al. 2007) at default settings and manually inspected for errors. Longer sequences were cropped so as to avoid gaps at both ends of the matrix.

The parsimony analysis was performed heuristically in PAUP* (Swofford 2002), employing 100 random additions of taxa and TBR branch swapping. Gaps were treated as missing data and all characters were given equal weight. Bootstrap support values were generated from 1000 replicate datasets.

Results

The aligned matrix comprised 637 characters, of which 176 (27.6 %) were parsimony-informative.

The consensus tree resulting from the parsimony analysis (Fig. 1) shows *Hablitzia* as sister to the rest of *Betoideae*. *Beta* is strongly supported as monophyletic and weakly supported as sister to a clade with *Oreobliton* and *Aphanisma* as strongly supported sisters. Finally, *Patellifolia,* including *Tetragonia pentandra,* is strongly supported as monophyletic and weakly supported as sister to the clade with *Aphanisma, Oreobliton* and *Beta*. Within *Patellifolia* there is moderate support for a sister group relationship between *P. webbiana* and *P. patellaris* from Tenerife, whereas the relationships between *P. patellaris* from Gran Canaria, *P. procumbens* and *Tetragonia pentandra* are unresolved.

Overall variation for ITS within the *Patellifolia/Tetragonia* clade is low, e.g. the *P. procumbens* and *P. patellaris* (Gran Canaria) sequences are identical, whereas *T. pentandra* differs from these at three nucleotide positions. *P. patellaris* (Tenerife) and *P. webbiana* differ from the former two at four and six positions respectively.

Discussion

In the study by Hohmann & al. (2006) a monophyletic *Betoideae* (except possibly *Acroglochin*) was identified, but both its position within *Chenopodiaceae* and its internal topology varied according to the molecular marker used.

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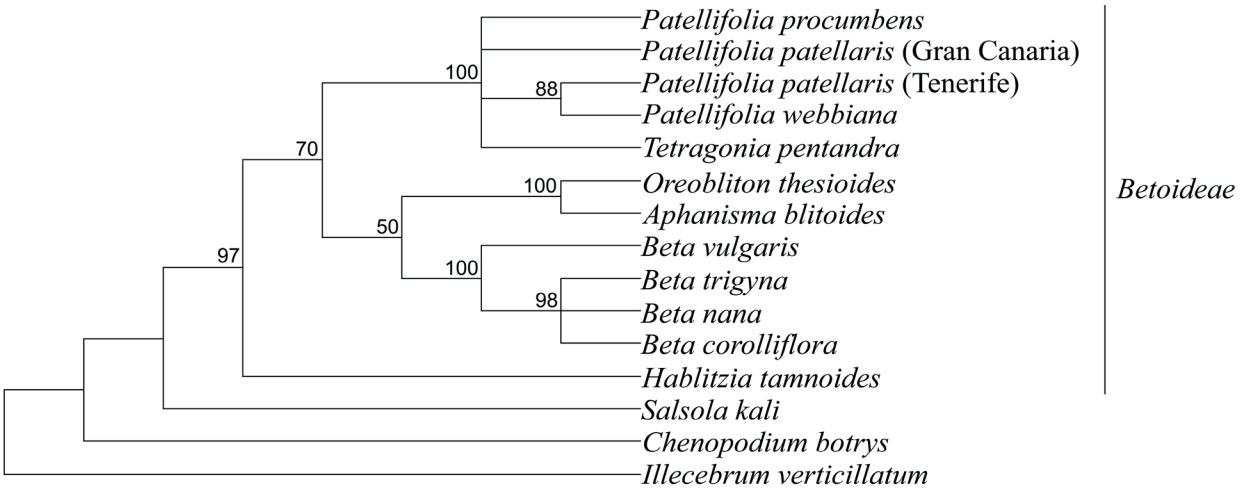


Fig. 1. Majority-rule consensus of eight equally parsimonious trees from the analysis of ITS data from *Betoideae*. Bootstrap support values (≥ 50) are indicated above branches.

Within *Betoideae,* a ML analysis based on *ndhF* sequences showed *Beta* (except *B.* sect. *Procumbentes* = *Patellifolia*) as sister to a clade with *Hablitzia, Oreobliton, Aphanisma* and *Patellifolia*. Within the latter clade, called tribe *Hablitzieae, Oreobliton* and *Aphanisma* formed a clade that is sister to *Patellifolia,* while *Hablitzia* is sister to all these three genera together. However, in the *trnL* intron and *matK* analyses *Hablitzia/Patellifolia* are sister to *Aphanisma/Oreobliton*. An ITS analysis was said to give the same result as the *ndhF* analysis, but the result of such an analysis depends on the rooting. Hohmann & al. (2006) used an ingroup rooting for their ITS analysis, with *Beta* (excl. *Patellifolia*) as sister to the rest of *Betoideae*. If, instead, *Salsola* and *Chenopodium* are used as outgroups to *Betoideae, Hablitzia* becomes the sister to the rest of the subfamily, just as in our analysis (Fig. 1). However, the main point here is that all molecular analyses made so far, including our own, agree in placing *Patellifolia* outside *Beta*.

Tetragonia pentandra clearly is a member of *Patellifolia,* but does not group with *P. patellaris* or with any of the other taxa included in the analysis. The relationships of *P. procumbens* from Tenerife, *P. patellaris* from Gran Canaria and *Tetragonia pentandra* from Somalia are unresolved, whereas there is moderate support for a sister group relationship between *P. patellaris* from Tenerife and *P. webbiana* from Tenerife.

The taxonomy of the three species currently included in *Patellifolia* has long been controversial. An experimental study by Curtis (1968), based on material of unspecified origin, indicated that *P. patellaris* (as *Beta patellaris*) is tetraploid ($2n = 36$) and self-compatible, whereas *P. procumbens* and *P. webbiana* (as *B. procumbens* and *B. webbiana*) are diploid and self-incompatible. *P. procumbens* and *P. webbiana* could also be hybridised easily, whereas attempts of hybridisation between these two species and *P. patellaris* failed. However, Larsen (1962) reported $2n = 18$ in *P. patellaris* (as *B. patellaris*) from Tenerife, whereas Dalgaard (1986) reported $2n = 36$ in material of the same species from Gran Canaria.

Early molecular studies, summarised by Shen & al. (1998), indicate that *Patellifolia* is distinct from *Beta,* but give little information on the internal relationships within this group. Santoni & Bervillé (1992), on the basis of a study of rDNA unit types, concluded that "the three *Procumbentes* species are closely related and could correspond to one species only". Shen & al. (1998) stated that the subgroupings obtained in their RAPD study "do not correlate with the original classification into species, which might result from natural gene flow and could support the single-species hypothesis".

In recent floristic literature, such as Bramwell & Bramwell (2001), *P. patellaris* (as *Beta patellaris*) is keyed out as an annual with cordate leaves, whereas *P. procumbens* and *P. webbiana* (as *B. procumbens* and *B. webbiana*) are perennials with hastate or sagittate leaves. *P. webbiana* is further differentiated by having "leaves more or less linear" versus "ovate or deltoid" in *P. procumbens*. Schönfelder & Schönfelder (1997) also classify into three species, but state about *P. webbiana:* "der systematische Rang dieser Sippe ist zweifelhaft, unterscheidbar scheinen nur die Extremformen".

In our experience of *Patellifolia* from the field on the Canary Islands, where the variation is greatest, and from the study of numerous herbarium collections, the identification of these three species is notoriously difficult. The primary distinction between annuals and perennials is often hard to make in practice, and plants having cordate leaves on some branches (corresponding to *P. patellaris*) and hastate on others (corresponding to *P. procumbens* and *P. webbiana*) may also be encountered. Also, in the protologue of *B. patellaris* (Moquin-Tandon 1849) it was described as annual or biennial and in Flora iberica (Gutiérrez Bustillo 1990) *P. patellaris* is described as annual or biennial with the chromosome numbers 18 or 36. Another complication is that *B. procumbens,* the first name

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to be published for a member of *Patellifolia* (Smith 1819), was said in the protologue to be an annual and it was said to be annual also by, e.g., Moquin-Tandon (1849). Further, Curtis (1968) stated about members of the group that "such physiological distinctions as annual and perennial habit … have not been found to apply in the material we have observed here. Individual plants live from one to many years in the glasshouse, independent of the specific origin."

The few plants known of *"Tetragonia pentandra"* from Socotra and Somalia appear to be annuals and have cordate leaves, and accordingly would be referred to *Patellifolia patellaris* using the conventional taxonomy of the group. This is also how they were identified by Thulin (2006). However, we think it is time now to formally propose a reduction of the species number in *Patellifolia*. Neither our molecular study, nor any previous one, has shown support for more than a single species in the genus and the characters that have been used to distinguish taxa in the group are found to be unreliable. We have therefore come to support the "single-species hypothesis" (Shen & al. 1998). Until it can be disproved by other data, we propose that all members of *Patellifolia* are included within a variable *P. procumbens* with two ploidy levels. A synopsis of the nomenclature and synonymy is given below.

Taxonomy

Patellifolia A. J. Scott & al. in Taxon 26: 284. 1977 ≡ *Patellaria* J. T. Williams & Ford-Lloyd ex J. T. Williams & al. in Feddes Repert. 87: 289. 1976, nom. illeg. – Type: *Patellifolia webbiana* (Moq.) A. J. Scott & al. (≡ *Beta webbiana* Moq.)

= *Beta* [unranked] *Patellares* Tranzschel in Trudy Prikl. Bot. Selekts. 17: 205. 1927. – Type: *Beta patellaris* Moq.

= *Beta* sect. *Procumbentes* Ulbr. in Engler & Prantl, Nat. Pflanzenfam., ed. 2, 16c: 463. 1964. – Type: *Beta procumbens* C. Sm.

Genus of a single variable species.

Patellifolia procumbens (C. Sm.) A. J. Scott & al. in Taxon 26: 284. 1977 ≡ *Beta procumbens* C. Sm. in Hornemann, Suppl. Horti Bot. Hafn.: 31. 1819 ≡ *Patellaria procumbens* (C. Sm.) J. T. Williams & al. in Feddes Repert. 87: 290. 1976. – Holotype: Spain, Canary Islands, *Christen Smith s.n.* (C, seen on microfiche).

= *Beta hastata* Desf., Tabl. Ėcol. Bot., ed. 3: 389. 1829. – Holotype: "Ex Herb. Desfontaines", Herb. Webbianum n. 155905 (FI-W, seen as digital image).

= *Beta webbiana* Moq., Chenop. Monogr. Enum.: 16. 1840 ≡ *Patellaria webbiana* (Moq.) J. T. Williams & al. in Feddes Repert. 87: 290. 1976 ≡ *Patellifolia webbiana* (Moq.) A. J. Scott & al. in Taxon 26: 284. 1977. – Holotype: Spain, Canary Islands, Gran Canaria, "Canaria ad Isletam" *Webb & Berthelot s.n.,* Herb. Webbianum n. 155961 (FI-W, seen as digital image).

= *Beta patellaris* Moq. in Candolle, Prodr. 13(2): 57. 1849 ≡ *Patellaria cordata* J. T. Williams & al. in Feddes Repert. 87: 290. 1976, nom. illeg. ≡ *Patellifolia patellaris* (Moq.) A. J. Scott & al. in Taxon 26: 284. 1977. – Holotype: Spain, Canary Islands, *Bourgeau s.n.* (P, seen as digital image).

= *Beta diffusa* Coss., Notes Pl. Crit.: 178. 1852 ≡ *Beta patellaris* var. *diffusa* (Coss.) Maire in Bull. Soc. Hist. Nat. Afrique N. 22: 312. 1931. – Lectotype (designated here): Spain, "infra rupes maritimas prope Roqueta in ditione Almeriensi", *Bourgeau 1457* (P, in herb. E. Cosson, seen as digital image; isolectotypes: K!, P, seen as digital image).

= *Tetragonia pentandra* Balf. f. in Proc. Roy. Soc. Edinburgh 12: 404. 1884. – Holotype: Yemen, Socotra, "prope Galonsir", Feb.-Mar. 1880, *Balfour, Cockburn & Scott 37* (K).

= *Beta monodiana* Maire in Bull. Mus. Hist. Nat. (Paris), ser. 2, 3: 526. 1931 ≡ *Beta patellaris* var. *monodiana* (Maire) Maire in Bull. Soc. Hist. Nat. Afrique N. 22: 312. 1931 ≡ *Beta patellaris* f. *monodiana* (Maire) Maire in Fl. Afrique N. 8: 19. 1962. – Lectotype (designated here): Algeria, Ahaggar, Atakor-n-Ahaggar, 30.10.1927, *Monod 114* (MPU, seen as digital image; isolectotype: P, seen as digital image).

= *Beta patellaris* var. *luthereaui* Maire in Bull. Soc. Hist. Nat. Afrique N. 26: 151. 1935 ≡ *Beta patellaris* f. *luthereaui* (Maire) Maire in Fl. Afrique N. 8: 18. 1962. – Holotype: Mauritania, Zemmour, 1933–34, *Luthereau s.n.* (MPU, seen as digital image).

Nomenclatural remarks. — The often used infrageneric name *Patellares* was unranked when published and therefore *Beta* sect. *Procumbentes* has priority when the group is treated as a section.

Beta pumila Link in Buch, Phys. Beschr. Canar. Ins.: 141. 1825 was cited as a synonym of *B. webbiana* by Moquin-Tandon (1840) and of *Patellifolia webbiana* by Williams & al. (1976 under *Patellaria*) and Scott & al. (1977). If validly published this would be an earlier name for *P. webbiana,* but *B. pumila* is a nomen nudum. No material was cited by Link, but the locality "Martianez. Orotava" is given. There is a sterile narrow-leaved specimen in BM with the annotation "*Beta pumila* Sp. n, Isletia ins Canaria". The collector may have been Christen Smith. There is also a similar specimen of "*Beta pumila* Link" in herb. Moquin-Tandon in P annotated "Iles Canaries (Chr. Sm.) Ex herb. DC."

Beta hastata Link in Buch, Phys. Beschr. Canar. Ins.: 141. 1825 was cited as another synonym of *Patellifolia webbiana* by Ulbrich (1934 under *Beta*), Williams & al. (1976 under *Patellaria*) and Scott & al. (1977). If validly published this would also be an earlier name for *P. webbiana*. However, Link only mentioned *B. hastata* in the synonymy of *B. patula* Aiton. As the name therefore was not accepted by the author, it cannot be regarded as validly published by Link. However, the name was validly

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published by Desfontaine (see above), and *B. hastata* Desf. has been regarded as a synonym of *B. procumbens* since Moquin-Tandon (1840).

Beta diffusa Coss., Notes Pl. Crit.: 178. 1852 is here lectotypified by *Bourgeau 1457* in herb. E. Cosson (P), a specimen with a handwritten label. A duplicate with a printed label in herb. E. Drake (P) is regarded as an isolectotype.

Beta campanulata Coss. in Bull. Soc. Bot. France 22: 66. 1875, based on a specimen from Morocco, is sometimes cited as a synonym of *B. patellaris*. However, the name is a nomen nudum, and the combinations *B. patellaris* var. *campanulata* and f. *campanulata* are therefore also not validly published.

Beta patellaris f. *vilmoriniana* Maire & Weiller, proposed in Fl. Afrique N. 8: 18. 1962, was not validly published as a description or diagnosis in Latin is lacking.

Beta patellaris f. *diffusa* (Coss.) Maire, proposed in Fl. Afrique N. 8: 19. 1962, was not validly published as a reference to the basionym is lacking.

Distribution and habitat. — The distribution of *Patellifolia,* which was provisionally mapped by Hohmann & al. (2006) and Kadereit & al. (2006), is considerably extended by the inclusion of *Tetragonia pentandra* in the synonymy. A new and more detailed map based on herbarium material in BM, K and UPS, supplemented with literature records, is presented in Fig. 2. This also includes records from the Saharan mountains and from the Cape Verde Islands, as well as a new country record for Libya based on *Park 214* (K).

The disjunction between the W Mediterranean region/Macaronesia and the Horn of Africa region shown by *Patellifolia procumbens* is fairly closely matched by, for example, *Bowlesia glandulosa* (Poir.) Kuntze (≡ *Drusa glandulosa* (Poir.) Bornm.), distributed in the Canary Islands, Morocco and northern Somalia. The only known locality for *P. procumbens* in Somalia is also very close to the only known, few localities of *B. glandulosa*. Other examples of similar disjunctions are provided by Thiede (1994) and Thulin (1994).

According to the age estimates by Hohmann & al. (2006), *Patellifolia* is 30.9–15.3 my depending on the marker used, but its diversification took place only in the late Pliocene or early Quaternary. Kadereit & al. (2006) stated that "the geographical distribution of the three (or two) species, with *P. procumbens* and *P. webbiana* growing only in the Canary Islands, and *P. patellaris* in the Canary Islands and the W Mediterranean area, in combination with ploidy level, raises the interesting and unusual possibility that the Mediterranean area was colonised from the Canary Islands by the tetraploid selfer *P. patellaris*". However, the extension of the distribution of *Patellifolia* to the Horn of Africa region reported here, as well as its occurrences in the Saharan mountains, a fact not considered by Kadereit & al. (2006), rather suggest a previously widespread taxon that has got its present distribution through fragmentation and extensive extinctions.

In Macaronesia and the W Mediterranean region *Patellifolia procumbens* occurs in more or less coastal habitats at altitudes from close to sea level up to about 250 m. However, in the localities in the interior parts of North Africa (Ahaggar and Tassili des Ajjer), the altitudes are obviously higher, perhaps up to about 2000 m, although there are no specific figures available. In Socotra the species also occurs at a low altitude, whereas the single Somali collection was made in limestone gravel below an escarpment at 1150 m.

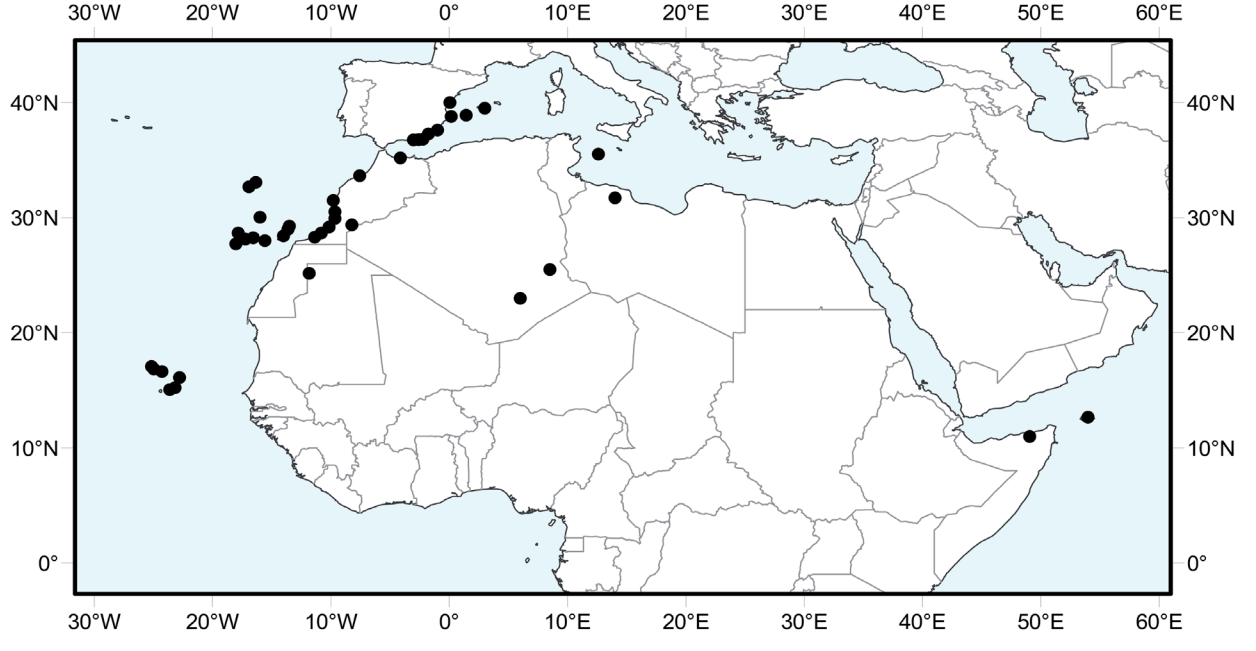


Fig. 2. Distribution of *Patellifolia procumbens*.

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Conclusions

Patellifolia is confirmed as a genus distinct from *Beta,* and we propose that all its members are treated as a single variable species, *P. procumbens*. *Tetragonia pentandra,* previously long regarded as a member of *Aizoaceae* in the Horn of Africa region, is included in the synonymy of *P. procumbens*. The distribution of *P. procumbens* is widely disjunct between the W Mediterranean region/ Macaronesia and the Horn of Africa region.

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Appendix

Taxa sampled, origin when known, and GenBank accession numbers. For the new sequences voucher information is given.

Chenopodiaceae, Betoideae: Aphanisma blitoides Nutt. ex Moq., U.S.A., California.: AY858591. ***Beta corolliflora*** Zosimovic ex Buttler, Italy: AY858598. ***B. nana*** Boiss. & Heldr., Greece: AY858596. ***B. trigyna*** Waldst. & Kit., Turkey: AY 858595. ***B. vulgaris*** L., Romania: AY858597. ***Hablitzia tamnoides*** M. Bieb., Bot. Gard. Bonn: AY858590. ***Oreobliton thesioides*** Durieu & Moq., Tunisia: AY858592. ***Patellifolia patellaris*** (Moq.) A. J. Scott, Ford-Lloyd & J. T. Williams, Spain, Gran Canaria: AY858593. ***P. patellaris***, Spain, Tenerife, *Thulin 11083* (UPS): HM367684. ***P. procumbens*** (C. Sm.) A. J. Scott, Ford-Lloyd & J. T. Williams, Spain, Tenerife: AY858594. ***P. webbiana*** (Moq.) A. J. Scott, Ford-Lloyd & J. T. Williams, Spain, Tenerife, *Lundqvist 9216* (UPS): HM367683. ***"Tetragonia pentandra"*** Balf. f., Somalia, *Thulin & Warfa 6185* (UPS): HM367682.

Chenopodiaceae, Chenopodioideae: Chenopodium botrys L.: DQ499336.

Chenopodiaceae, Salsoloideae: Salsola kali L.: EU643789.

Outgroup: ***Caryophyllaceae: Illecebrum verticillatum*** L.: AJ310977.

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