



## ***Knautia dinarica* (Caprifoliaceae): taxonomy, typification and update of the Italian distribution**

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### **Abstract**

New Italian populations of *Knautia dinarica*, formerly known only for Sila (Calabria, Southern Italy), were discovered in Abruzzo (Central Italy) and Basilicata (Southern Italy). In order to correctly classify these populations, a taxonomic study was carried out, including morphological and karyological analyses and the typification of the names *Knautia sylvatica* var. *dinarica* and *K. arvensis* f. *silana*. We conclude that in Italy only *K. dinarica* subsp. *silana* occurs, to be considered as endemic to central-southern Apennines and vicariant of *K. dinarica* subsp. *dinarica*, occurring in the Balkan peninsula. Both subspecies can be either diploid ( $2n = 20$ ) or tetraploid ( $2n = 40$ ), and are distinguished by the different mean shape of the basal leaves (the ratio length/width is higher in Italian plants).

**Key words:** Dipsacaceae, Dipsacales, karyology, *Knautia*, taxonomy, typification

### **Introduction**

The dipsacaceous clade within Caprifoliaceae (Dipsacales) contains ca. 300 herbaceous species, mostly distributed in the Mediterranean area, *Knautia* Linnaeus (1753: 101) representing a monophyletic genus with 40–60 species (Carlson *et al.* 2009). *Knautia dinarica* (Murbeck 1891: 111) Borbás (1894: 399) is currently known from Balkan Peninsula (in the former Yugoslavia, Albania, and Bulgaria countries) and Southern Italy (Calabria region) (Ehrendorfer 1976, Conti *et al.* 2005). Verlaque (1977) reported *K. dinarica* also from Greece, but this record was not confirmed in recent Checklists or Floras (Greuter *et al.* 1986, Strid & Tan 1991). *Knautia dinarica* subsp. *dinarica* occurs in the Balkans (mostly on limestones), while in Italy (on siliceous substrates, see Sarfatti 1965, Brullo *et al.* 2007) only the narrow endemic *K. dinarica* subsp. *silana* (Grande 1913: 107) Ehrendorfer (1975: 40) is reported. On the basis of the literature data, the subsp. *silana* differs from the nominal one in hair-type and petiole length of basal leaves (Ehrendorfer 1975, 1976, 1982a). During field research in Central and Southern Italy, we found two populations of *Knautia dinarica* s.l. well outside the historical range and growing on calcareous substrate: the first one in Abruzzo region (Central Italy), the second one in Basilicata region (Southern Italy). In order to correctly classify these populations, we carried out a taxonomic study, including morphological and karyological analyses and the typification of the names involved. This work is also carried out within the initiative "Italian Loci Classici Census" (Domina *et al.* 2012), launched in 2010 under the auspices of the Italian Botanic Society (see for instance Di Pietro *et al.* 2012, Gallo *et al.* 2012, Iamonico & Peruzzi 2012, Peruzzi & Carta, 2013, Peruzzi *et al.* 2012, 2013, Iamonico 2013, Bartolucci & Conti 2013, Bartolucci & Peruzzi 2013).

## Material and Methods

This study is based on the analysis of relevant literature, field surveys and revision of herbarium specimens (including the original material for the names studied) kept in APP, BEOU, CLU, FI, LD, NAP, PI and WU (acronyms follow Thiers 2012).

Despite a casual variation of the characters usually used to distinguish the two taxa was evident since our first observations, we noted on the contrary some variation in basal leaves size among the studied plants. For this reason, we decided to measure the following three characters: basal leaves length (cm), basal leaves width (cm), ratio basal leaves length / basal leaves width (L/W). Quantitative morphological investigation is performed on a total of 61 herbarium specimens of *K. dinarica*: 26 from Calabria (Southern Italy, fully corresponding to *K. dinarica* subsp. *silana*), 11 from the Balkans (fully corresponding to *K. dinarica* subsp. *dinarica*), 11 from Basilicata (Southern Italy, coded as BAS) and 13 from Abruzzo (Central Italy, coded as ABR). While ABR and BAS occur in single populations, the measurements of subsp. *dinarica* and subsp. *silana* were taken from samples coming from several localities (cfr. Appendix 1). The variables were processed singularly by means of ANOVA (after check of Levene statistics) with Tukey HSD test for comparing the same variable among pairs of Operational Taxonomic Units (OTU), using SPSS® 20 for Windows (SPSS, Chicago, IL, USA).

As regards the chromosome analysis, squash preparations were made from root-tips of plants cultivated in the Botanic Garden of Floristic Research Center of the Apennine [Polledrara, Secine (Palena), 430312N, 4638996E (UTM-ED50), 1730 m, 20 July 2006, *F. Conti* (acc. n. 236/06)], according to the following schedule: pretreatment in 0.4% colchicine solution for 4 hours; Carnoy fixing for 45 min; hydrolisis in HCl 1N for 6 minutes at 60°C; staining with leuco-basic fuchsin for 3 hours.

## Typification of the names

***Knautia dinarica*** (Murb.) Borbás (1894: 399)

Bas.:—*Knautia sylvatica* var. *dinarica* Murbeck (1891: 111). Type (lectotype, here designated):—BOSNIA-HERZEGOVINA. In pratis alpinis Matorac montis Vranica planina, 1800–1900 m, 9 September 1889, *Murbeck 1165781* (LD!; isolectotype WU! No. 0044885).

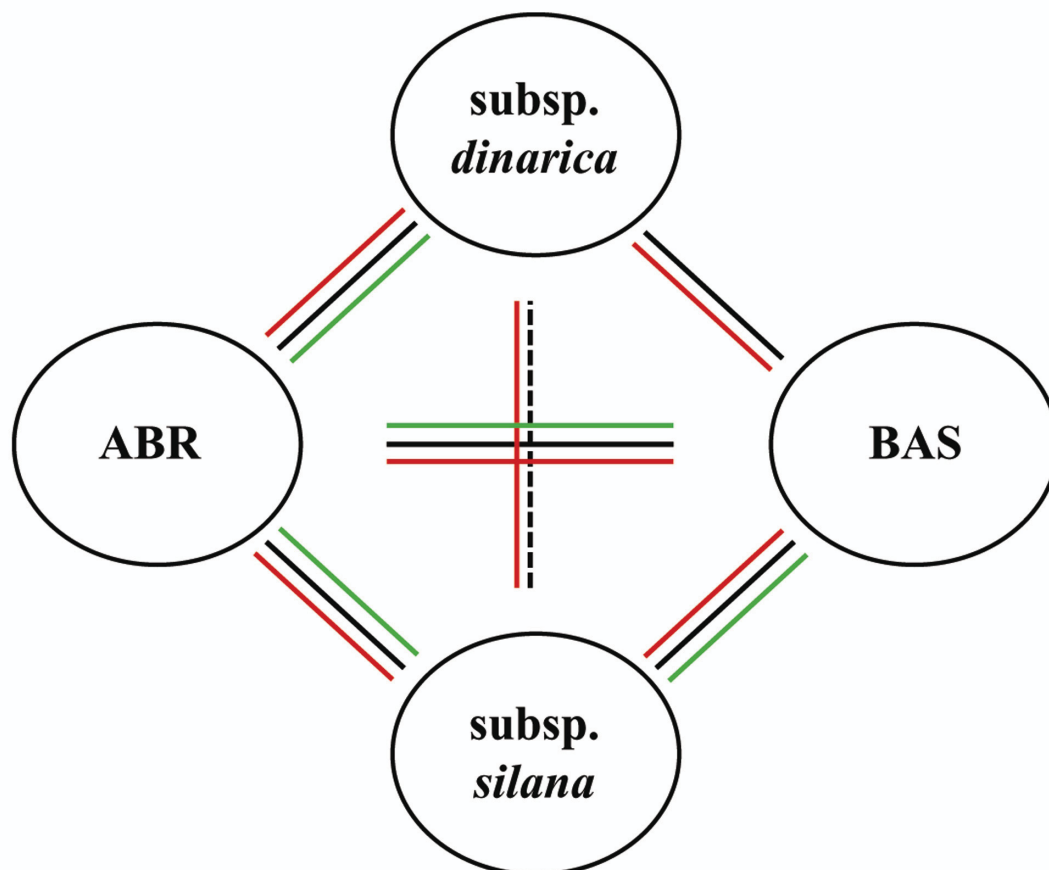
*Knautia sylvatica* var. *dinarica* was described from Bosnia-Herzegovina and Montenegro (Vranica and Bjelasnica Mountains) (Murbeck 1891). We traced eight herbarium sheets, kept in LD and WU, that were collected ante 1891 and match the protologue, so they can be considered as original material for the name. The best preserved and complete exsiccatum at LD is here selected as the lectotype. This specimen corresponds to the current concept of *K. dinarica*, but all the traced original material is showing only long hairs in the lower third of the individuals, in contrast with the description given by Ehrendorfer (1976), who refers to *K. dinarica* s.s. as a more or less pubescent plant showing also long hairs. According to the Art. 41.3 of the ICN (McNeill *et al.* 2012) the new combination *Knautia dinarica* (Murb.) Borbás was validly published (Borbás 1894), while the isonym proposed by Malý (1908: 233) has no nomenclatural status.

***Knautia dinarica*** subsp. *silana* (Grande) Ehrendorfer (1975: 40)

Bas.:—*Knautia arvensis* f. *silana* Grande (1913: 107). Type (neotype, here designated):—ITALY. Calabria: Loc. Calabria, in ditione Longobucco, loco Fossiata dicto, in herbosis humidis, alt. 1300 m, solo granitico, 7 August 1921, *A. Fiori s.n.* (FI! the specimen bearing also handwritings; isoneotypes in FI! and PI!).

Grande (1913: 107) refers to “*Scabiosa arvensis* B. *foliis lanceolatis subintegerrimis*” (Tenore 1831: 59) and to a number of localities with collections (e.g. syntypes) made by him together with F. Cavara (“*Nelle pinete, nei prati o lungo i rivoli a Botte Donato, alla caserma della Fossiata*”) and together with G. Lopez (“*ai Serriselli, al Pirillo, al Vallo d'Agnara, a Tacina*”). We were not able to trace in NAP (neither in Tenore nor in

Cavara-Grande collections) any of these specimens. Since no other original material were traced, we are forced to propose a neotype for this name. For this purpose, we selected a well representative, topotypical collection conserved in FI. According to the Art. 41.6 of the ICN (McNeill *et al.* 2012) the combination proposed by Ehrendorfer (1975) was validly published, despite the incorrect referring to basynim's rank (“*var.*”) and year of publication (“1911”).



**FIGURE 1.** Graphic showing the morphological relationships (based on the basal leaf features) between *Knautia dinarica* subsp. *dinarica* (N = 11), ABR (N = 13), BAS (N = 11) and *K. dinarica* subsp. *silana* (N = 26). The presence of a continuous line between two OTUs evidences no statistically significant difference (Tukey HSD test); the presence of a discontinuous line between two OTUs evidences a difference significant at 0.05 level (Tukey HSD test); the absence of a line between two OTUs evidences a difference significant at 0.01 level (Tukey HSD test). Black lines = length; red lines = width; green lines = ratio L/W.

## Morphological and Karyological Results

The relationships among the four considered OTUs are represented in Fig. 1 and Table 1. The width of basal leaves has not significance from the statistical point of view among the studied OTU (Tukey HSD test with p values ranging between 0.18 and 0.98). Concerning the length of basal leaves, there is no difference between subsp. *silana* / ABR / BAS and between subsp. *dinarica* / ABR / BAS (Tukey HSD test with p values ranging between 0.09 and 0.99), while moderately significant differences occur between subsp. *dinarica* / subsp. *silana* (p = 0.05). By considering the covariance of these two morphological parameters (the ratio L/W), there is no significant difference between subsp. *silana* / ABR / BAS (Tukey HSD test with p values between 0.96 and 0.99) and between subsp. *dinarica* / ABR (p = 0.06), while subsp. *silana* and BAS differ significantly from subsp. *dinarica* (p = 0.01).

The single studied population of *K. dinarica* from Abruzzo (ABR) turned out to be diploid with  $2n = 20$  chromosomes.

**TABLE 1.** Basal leaves morphological comparison in *Knautia dinarica* subsp. *dinarica* (N = 11), ABR (N = 13), BAS (N = 11) and *K. dinarica* subsp. *silana* (N = 26). Quantitative values are expressed as 10–90 percentile intervals, with minimum and/or maximum in brackets.

|                          | subsp. <i>dinarica</i> | ABR                   | BAS                 | subsp. <i>silana</i> |
|--------------------------|------------------------|-----------------------|---------------------|----------------------|
| basal leaves length (cm) | 6–12.5(–17.1)          | (10–)10.1–16.3(–20.5) | (4–)6.7–13.5(–17)   | (5–)6.9–19.7(–28)    |
| basal leaves width (cm)  | (1.3–)1.6–3.2(–3.8)    | (0.9–)1.3–2.7(–2.9)   | (0.5–)0.6–2.2(–3.7) | (0.7–)1–2.3(–3)      |
| ratio L/W                | (3–)3.3–7.0(–7.7)      | (4.7–)4.8–10.5(–11.5) | (4.6–)4.7–11.8(–14) | (4.5–)4.9–9.6(–14)   |

## Discussion

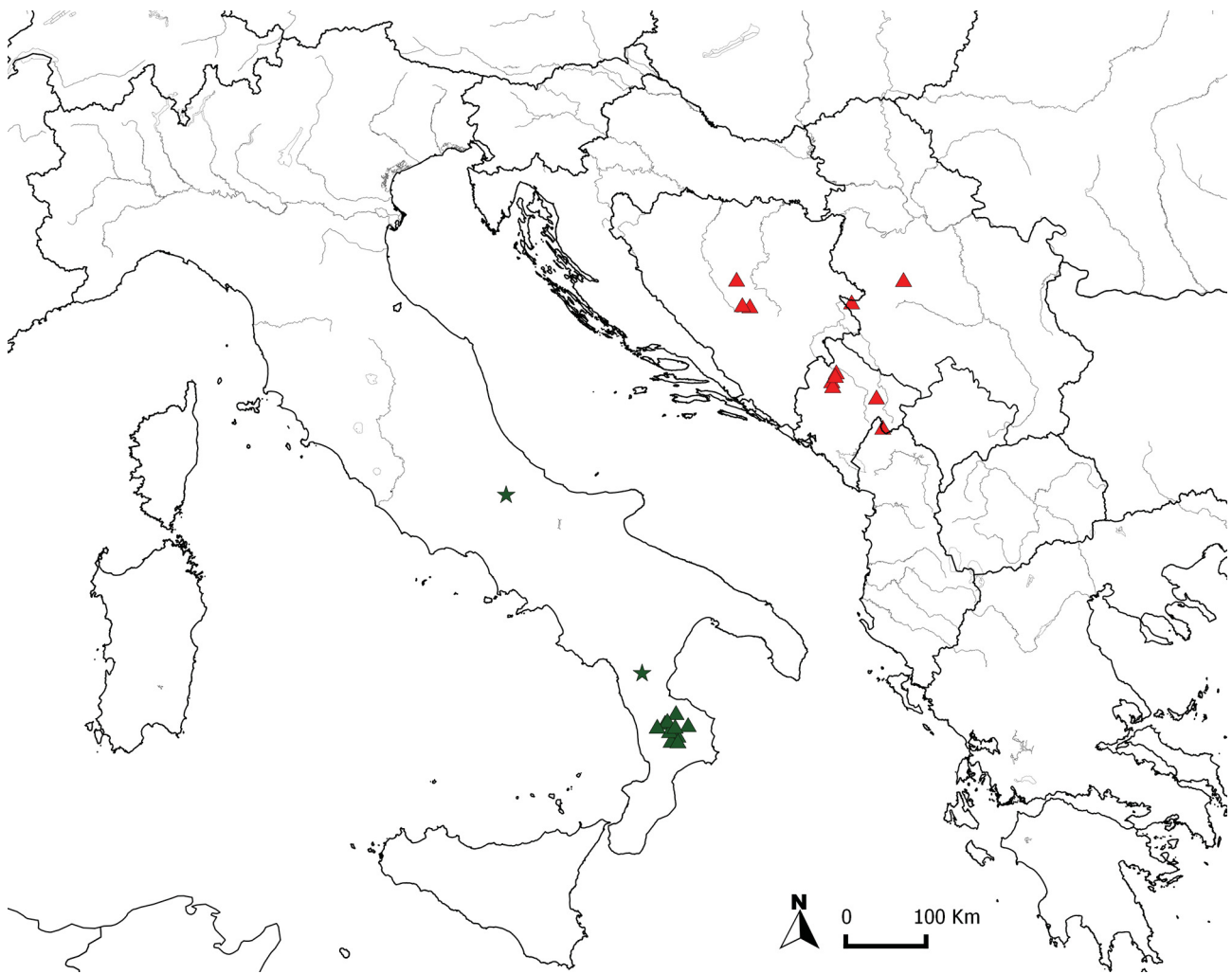
The traditional morphological features used to distinguish the two subspecies of *Knautia dinarica* (hair-type and petiole length in the basal leaves) are very inconstant, according to our own observations (data not shown) and in agreement with the study of the nomenclatural types. Our quantitative analysis of basal leaves dimensional features confirmed a substantial overlap in leaf length and width, among the two subspecies and the unassigned ABR and BAS OTUs. On the other hand, the ratio of these two morphological features (ratio L/W) revealed a statistically significant mean difference between subsp. *dinarica* and the most southern Italian populations, with ABR somehow linked to both subspecies. However, when performed between subsp. *silana* (circumscribed as including ABR and BAS) and subsp. *dinarica*, ANOVA evidences a difference in ratio L/R (df = 2, F = 12.338, p = 0.001). On the contrary, a narrower circumscription of subsp. *silana* (including only BAS) compared with a wider circumscription of the typical subspecies (including also ABR) showed only moderately significant differences (df = 2, F = 5.381, p = 0.02). Accordingly, and despite the weak and incomplete morphological differentiation, it seems actually opportune to keep the two subspecies distinct awaiting further morphological and molecular analyses: *K. dinarica* subsp. *dinarica* is confirmed as endemic to Balkans, *K. dinarica* subsp. *silana*, as here circumscribed, results endemic to central-southern Apennines, from Abruzzo to Calabria (irrespective of geological substrate, either calcareous or siliceous). The range of the latter subspecies is extended of about 350 km northwards (Fig. 2), and the plant could more common than expected in that area: for instance, a record of *Knautia dinarica* s.l. exists for a second locality in Basilicata, Mt. Volturino (Corbetta *et al.* 1986).

Previous chromosome counts have been reported for *Knautia dinarica* subsp. *dinarica* by Ehrendorfer (1962) from Bosnia ( $2n = 20$ ), Montenegro ( $2n = 40$ ), and Albania ( $2n = ca. 40$ ) and by Verlaque (1977, 1985) from Greece ( $2n = 20$ ). Ehrendorfer (1975, 1976, 1982b) reported also  $2n = 40$  for *K. dinarica* subsp. *silana* from Sila (Calabria, S. Italy). By adding our count from Abruzzo into this framework, it can be concluded that both subspecies can be either diploid or tetraploid. A (recurrent) polyploidy is common in many groups of *Knautia*, including *K. arvensis* (Linnaeus 1753: 99) Coulter (1823: 29), which is phylogenetically closely related to *K. dinarica* (Carlson *et al.* 2009).

## Identification key for the *Knautia dinarica* subspecies

With the aim to a correct identification, at least ten individuals per population are to be measured. We have verified that measurements taken on single individuals could lead to a wrong identification. For a general description of the species, refer to Ehrendorfer (1976).

1. Ratio basal leaves length / basal leaves width (3–)3.3–7(–7.7), endemic to Balkan peninsula ..... *K. dinarica* subsp. *dinarica*
- Ratio basal leaves length / basal leaves width (4.5–)4.7–11.1(–14), endemic to Italian peninsula ..... *K. dinarica* subsp. *silana*



**FIGURE 2.** Distribution map of *Knautia dinarica* subsp. *dinarica* (red triangles) and *K. dinarica* subsp. *silana* (green triangles: historical populations; green stars: new populations) according to the studied material.

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## APPENDIX 1. Specimens of *Knautia dinarica* studied.

***Knautia dinarica* subsp. *dinarica*:**—BOSNIA-HERZEGOVINA. Bosnia: in prat. alp. montis Vranica pl. (Matorac), 11 September 1889, *S. Murbeck 1165481* (LD!); Bosnia: in pratis alpinis Matorac montis Vranica planina, 1800–1900 m, 9 September 1889, *Murbeck 1165781* (LD!); Bosnia: in pasc. alp. montis Vranica pl. (Locike) - in HBL. culta, 18 July 1891–1889, *S. Murbeck 1165901* (LD!); *ibidem*, *S. Murbeck 1159481* (LD!); *ibidem*, *S. Murbeck 1159361* (LD!); in pascuis alpinis montis Vratnica pl., 9 September 1889, *S. Murbeck s.n.* (WU!); SERBIA: Suvobor, Planinica, serpentinit, 29 July 1994, *T. Zonjić 2892/96* (BEOU!); Tara planina, Zaovine - Vranjak *Euphorbietum glabriflorae*, serpentinit, 9 July 2000, *V. Stefanović, S. Jovanović, S. Vukojičić 14251* (BEOU!); Bosnia, Travnik [...], 1000 mts, June 1908, *E. Brandis s.n.* (PI-GUAD!); MONTENEGRO: in decliv. gram. montis Bjelasica pl., c. 1700 m., 19 August 1889, *S. Murbeck 1159661* (LD!); *ibidem*, *S. Murbeck 1165961* (LD!); Durmitor, Boljske grede, July 1983, *V. Stefanović 2450* (BEOU!); Durmitor, Žabljak livade, 1600 m, 7 July 1974, *V. Stefanović 1548/89* (BEOU!); Durmitor, Komarnica (Boljske grede), 7 July 1989, *V. Stefanović, S. Jovanović 1119/89* (BEOU!); Durmitor, uz obalu Modrog j., 9 August 1975, *V. Stefanović, B. Stefanović 37* (BEOU!); Durmitor, Virak Fagetum, krečnjak, 1400 m, 12 July 1997, *D. Lakušić, G. Tomović 5873* (BEOU!); Durmitor, Virak livade, krečnjak, 1400 m, 12 July 1997, *D. Lakušić, G. Tomović 5896* (BEOU!); Durmitor, Sevarita lokva, prati (*Festuco rubrae-Agrostetum capillaris*), 1400 m, 6 July 1996, *D. Lakušić, F. Conti, G. Tomović 9322* (APP!); *ibidem*, *D. Lakušić, F. Conti, G. Tomović s.n.* (BEOU!); Monte Prokletije, Popadija - Zuto prlo, 42°31'986"N, S 19°45'213"E (UTM-ED50), *Festuco-Seslerietea*, limestone, 1900 m, 18 July 2003, *D. Lakušić, F. Conti, Z. Bulić, M. Niketić, G. Ciaschetti, G. Tomović, S. Adžialahović 31611* (APP!); Štirni do, Gackove grede, 8 August 1984, *s. coll. 1825/89* (BEOU!).

***Knautia dinarica* subsp. *silana*:**—ITALY. Abruzzo: M. Secine presso Polledrara (Palena), prati umidi, 1700–1800 m, 19 June 1997, *F. Conti 13474* (APP!); Polledrara, Secine (Palena), 430312N, 4638996E (UTM-ED50), 1730 m, 20 July 2006, *F. Conti 33685* (APP!); *ibidem*, *F. Conti 33686* (APP!); *ibidem*, *F. Conti 33687* (APP!); *ibidem*, *F. Conti 33688* (APP!); *ibidem*, *F. Conti 33689* (APP!); *ibidem*, *F. Conti 33690* (APP!); *ibidem*, *F. Conti 33691* (APP!); *ibidem*, *F. Conti 33692* (APP!); *ibidem*, *F. Conti 33693* (APP!); *ibidem*, *F. Conti 33694* (APP!); M. Secine, Polledrara (Palena), 41°53.864'N, 14°09.174'E (UTM-ED50), 1700 m, 24 June 2006, *F. Conti 33871* (APP!); *ibidem*, *F. Conti 33908* (APP!); *ibidem*, *F. Conti 33909* (APP!); *ibidem*, *F. Conti 33910* (APP!); *ibidem*, *F. Conti 33911* (APP!); *ibidem*, *F. Conti 33912* (APP!); *ibidem*, *F. Conti 33913* (APP!); Basilicata: Piano Ruggio (Potenza), Pollino, Viggianello, 13 August 2009, *L. Bernardo, D. Gargano, L. Peruzzi 22002* (CLU!); *ibidem*, *L. Bernardo, D. Gargano, L. Peruzzi 22003* (CLU!); *ibidem*, *L. Bernardo, D. Gargano, L. Peruzzi 22004* (CLU!); Piano Ruggio (Potenza), lungo la strada, versante nord del Massiccio del Pollino, Viggianello, 1520-1550m, 27 June 2012, *L. Bernardo 21997* (CLU!); *ibidem*, *L. Bernardo 21998* (CLU!); *ibidem*, *L. Bernardo 21999* (CLU!); *ibidem*, *L. Bernardo 22000* (CLU!); *ibidem*, *L. Bernardo 22001* (CLU!); *ibidem*, *L. Bernardo 22011* (CLU!); Calabria: Loc. Calabria, in ditone Longobucco, loco Fossiatia dicto, in herbosis humidis, alt. 1300 m, solo granitico, 7 August 1921, *A. Fiori s.n.* (FI!, three specimens); *ibidem* (PI!); La Sila (Calabria), faggeta lungo l'Ampollino a S, m. 1300, 14 July 1951, *G. Negri, G. Sarfatti, A. Contardo s.n.* (FI!); La Sila (Calabria), Lorica, 8 Km. a W, prato su terreno discretamente profondo - m. 1300, 14 July 1951, *G. Negri, G. Sarfatti, A. Contardo s.n.* (FI!); La Sila (Calabria), Regione Ciricilla, zona piana umida - m. 1370, 27 June 1950, *G. Sarfatti, R. Corradi s.n.* (FI!); La Sila (Calabria), Molarotta - Stazione Alpeggio pascolo umido - m. 1130, 23 June 1950, *G. Sarfatti, R. Corradi s.n.* (FI!); Calabria - Sila, Fossiatia (Longobucco), 29 July–3 August 1918, alt. 1300 m, solo granitico, *A. Fiori s.n.* (FI!); Sila di Savelli, margine della pineta, 1300 m, 1–2 July 1917, *M. Guadagno 508* (PI-GUAD!); Fallistro, lungo la strada che porta ai "Giganti della Sila", Sila Grande, prov. Cosenza, Calabria, 1400 m, margine di strada e radure di bosco, 33S XD 26.53, 29 June 2005, *L. Bernardo 20009* (CLU!); *ibidem*, *L. Bernardo 20013* (CLU!); *ibidem*, *L. Bernardo 20014* (CLU!); *ibidem*, *L. Bernardo 20015* (CLU!); Ciricilla, nei pressi del centro abitato, Sila Piccola, Calabria, 1400 m, ambiente umido, 7 July 2005, *L. Bernardo 20011* (CLU!); Calabria: Sila Grande, nei pressi dello svincolo per Camigliatello (Cosenza), 1196 m, radura in pineta, 7 July 2006, *L. Peruzzi, D. Gargano, S. Carlson 21148* (CLU!); Sila Grande, Valle S. Bernardo, prov. Cosenza, Calabria, 1350 m, rimboschimento a *Pinus laricio*, 13 July 2011, *L. Bernardo 22006* (CLU!); Pisarello, lungo la strada per M.te Gariglione, Sila Piccola, Prov. di Catanzaro, Calabria, 1600 m, prato arido in estate, 28 July 2010, *L. Bernardo, D. Gargano, N.G. Passalacqua 22005* (CLU!).