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Thymus paronychioides (Lamiaceae), a Neglected Species from Sicily Belonging to *Thymus* sect. *Hypodromi*

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Abstract The taxonomic position of *Thymus paronychioides* Čelak., a neglected species until recently treated as a synonym of *Th. striatus* or *Th. spinulosus*, is reviewed. Karyological and morphological characters were used for comparative analyses with closely related taxa belonging to *Th.* sect. *Hypodromi*, such as *Th. striatus* and *Th. spinulosus*, occurring in the Mediterranean area (Italy, Balkans and Turkey). Multivariate and univariate morphometric analyses showed that *Th. paronychioides* is clearly distinct from *Th. striatus* and *Th. spinulosus* in the unique features of leaves (elliptical-spathulate, fleshy, with ± curved and prominent lateral veins), in the inflorescence being always capitate with few flowers, and in the larger corolla with the tube usually exceeding the calyx. Furthermore, chromosome counts showed that *Th. paronychioides* is polyploid ($2n$ = ca. 60), whereas *Th. spinulosus* and *Th. striatus* are diploid ($2n = 2x = 26$). Hence, *Th. paronychioides* is shown to be an independent taxon, endemic to NW Sicily, placed within *Th.* sect. *Hypodromi* subsect. *Serpyllastrum*. Finally, an identification key to the Italian species of *Th.* sect. *Hypodromi* is provided.

Keywords Endemics · Italy · Karyology · Taxonomy · Typification

Introduction

Thymus L. (Lamiaceae) includes about 215 accepted species distributed in the Old World (Europe, northwest Africa and Ethiopia, Asia) and in Greenland. It was

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introduced to Canada, Chile and New Zealand (Morales 2002). The genus belongs to the monophyletic subfamily *Nepetoideae* Kostel., tribe *Mentheae* Dumort., subtribe *Menthinae* Endl. (Harley et al. 2004). According to the classification proposed by Jalas (1971), based solely on morphological characters, *Thymus* is subdivided into eight sections: *Th.* sect. *Micantes* Velen., *Th.* sect. *Mastichina* (Mill.) Benth., *Th.* sect. *Piperella* Willk., *Th.* sect. *Teucrioides* Jalas, *Th.* sect. *Pseudothymbra* Benth., *Th.* sect. *Thymus*, *Th.* sect. *Hypodromi* (A. Kern.) Halácsy and *Th.* sect. *Serpyllum* (Mill.) Benth. Five of them (*Micantes*, *Mastichina*, *Piperella*, *Teucrioides* and *Pseudothymbra*) are endemic to the western Mediterranean area (Iberian Peninsula and northwestern Africa). The center of diversity of the genus is in the Mediterranean region (Morales 2002).

Thymus sect. *Hypodromi* is distributed throughout the Mediterranean area and from the Caucasus to Central Asia, comprising about 60 species. Species belonging to this section are characterized by stems with holotrichous or amphitrichous indumentum, leaves flat or with revolute margins, usually glabrous, capitate or interrupted inflorescence, and bracts (floral leaves) different from the cauline leaves, rarely equal. Three subsections are currently recognized within this section. *Thymus* sect. *Hypodromi* subsect. *Subbracteati* (Klokov & Des.-Shost.) Jalas, distributed in central Spain, northern Africa, southeastern Europe and Western Asia, is characterized by leaves linear to narrowly elliptical, seldom narrowly spathulate showing ± prominent parallel venation, calyx cylindric-campanulate and bracts usually different from the leaves. Chromosome numbers known for the taxa of this subsection are $2n = 26, 28, 30, 42, 52, 54, 56$ and 84, corresponding to putative diploid, tetraploid and hexaploid levels. *Thymus* sect. *Hypodromi* subsect. *Serpyllastrum* Villar, widespread in Spain, the Balkans, Crimea, Turkey, the Caucasus and Central Asia, is characterized by prostrate stems, leaves spathulate to obovate or sometimes elliptical, with lateral veins not parallel, bracts more or less different from leaves, corolla tube clearly exceeding, or as long as, the cylindric calyx. The chromosome numbers $2n = 26, 28, 56, 58, 60, 62, 90$ are known for this subsection. *Thymus* sect. *Hypodromi* subsect. *Thymbropsis* Jalas ex R. Morales, distributed in northern Africa, Greece, Asia Minor, Saudi Arabia and Western Asia, is characterized by erect or ascending stems, lanceolate leaves, flat or with revolute margins and bracts equal to cauline leaves. The karyology of this subsection is poorly investigated, the only known chromosome number is $2n = 28$ for *Thymus holosericeus* Čelak., a species endemic to Greece.

Of the whole section *Hypodromi*, only two species of *Thymus* sect. *Hypodromi* subsect. *Subbracteati* were recently reported for the Italian Peninsula and Sicily (Conti et al. 2005; Giardina et al. 2007): *Th. striatus* Vahl and *Th. spinulosus* Ten. The former was considered to be a polymorphic species distributed in southeastern Europe, while the latter was supposed to be a taxon endemic to southern Italy and Sicily.

Thymus paronychioides was described by Čelakovský (1882) based on plants collected by Lojacono in the Madonie mountain range (Sicily). The populations referable to this species were earlier reported as *Th. acicularis* Waldst. & Kit. (Gussone 1828, "var. α") – a critical species belonging to the *Th. striatus* complex – or as *Th. zygis* L. (Gussone 1844, "var. a"; Arcangeli 1882), a taxon endemic to the Iberian Peninsula and Morocco belonging to *Th.* sect. *Thymus*. After the description,

Th. paronychioides has been included within *Th. striatus* (Parlatore 1884; Fiori 1926; Zangheri 1976; Conti et al. 2005; Giardina et al. 2007) or referred as a synonym of *Th. spinulosus* (Grande 1924; Jalas 1972; Greuter et al. 1986). Lojacono (1907: 198) described *Th. gussonei* from Rocca Busambra (Sicily) but in the Appendix to the same volume (1907: 407), he considered this species as a synonym of *Th. paronychioides*. For this reason, the nomenclatural validity of this name is at least questionable, under Art. 36.1 (a) of the ICN (McNeill et al. 2012).

Sicily is an island located in southern Italy. As a whole, it belongs to the Mediterranean phytogeographical region (Brullo et al. 1995) and to the Mediterranean ecological division (Blasi and Frondoni 2011). According to a recent estimate (Pierini et al. 2009), Sicily ranks just 11th out of the 20 Italian regions in terms of floristic richness, with 3,010 specific and subspecific taxa in a relatively large area (25,708 km^2). Despite this, the region has an exceptionally high rate of endemism, hence representing one of the biodiversity hotspots in the Mediterranean (Médail and Quézel 1997). This is further confirmed by more or less continuous discoveries of new taxa in that area (most recently, e.g., Castellano et al. 2012; Cataldo et al. 2012; Marino et al. 2012; Raimondo et al. 2012).

The present study, which is part of a biosystematic revision of the genus *Thymus* in Italy (Bartolucci 2010), aims to clarify the taxonomic value of *Th. paronychioides* and to examine its morphological and karyological variability in comparision with closely related taxa, such as the *Th. striatus* complex and *Th. spinulosus*, occurring in the Mediterranean area (Italian and Balkan Peninsulas).

Material and Methods

Plant Material

This study is based on an extensive analysis of relevant literature, field surveys and careful examination of herbarium specimens (including the original material), kept in APP, AQUI, BI, BM, CAME, CAT, CLU, FI, G, H, HBBS, IS, LEC, NAP, PAL, PI, PORUN, PR, RO, ROV, W, WU and ZA (acronyms follow Thiers 2012) and personal collections of R.P. Wagensommer (Rome) and A. Soldano (Torino). A total of 815 specimens from these herbaria were studied (see Appendix S1 in Electronic Supplementary Material). The morphometric analyses, based on measurements of both qualitative and quantitative characters, were carried out on 155 selected specimens (Appendix 1) including *Thymus paronychioides* (41 specimens), the *Th. striatus* complex (69 specimens, including *Th. striatus* var. *striatus*, *Th. acicularis* var. *acicularis*, *Th. acicularis* var. *ophioliticus* Lacaita and *Th. striatus* var. *interruptus* Jalas) and *Th. spinulosus* (45 specimens) coming from populations sampled in Sicily, the Italian Peninsula and the Balkan Peninsula (Fig. **1**). The individuals were measured or scored for 25 morphological characters (Table **1**), selected according to their common use for taxonomic identification in *Thymus* (i.e., Ronninger 1930; Jalas 1973, 1982; Baden 1991; Aytas 2003) and their variability among different taxa observed in a preliminary study of herbarium material. The micromorphological characters were examined under a stereomicroscope and parameters were measured with a grid 12 mm/120 or simply with a ruler.

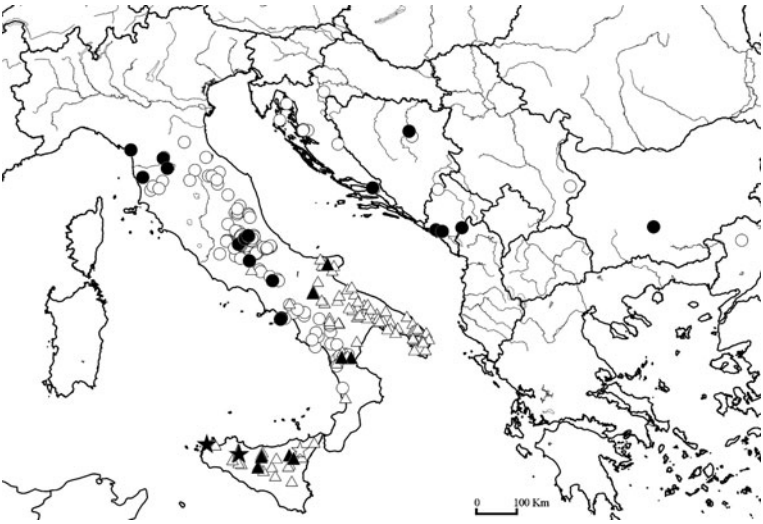

Fig. 1 Distribution map of *Thymus paronychioides* (*stars*), *Th. striatus* complex (*circles*) and *Th. spinulosus* (*triangles*) according to the material studied: black symbols indicate the populations included in the morphometric study and empty symbols refer to the specimens seen but not used for statistical analyses

Gynodioecy, i.e., the co-existence of hermaphrodite and female plants in natural populations, is very common in *Thymus* (Darwin 1877; Thompson 2002). In gynodioecious species, female individuals produce much-reduced anthers and little or no viable pollen, whereas in hermaphroditic individuals both sexes are functional. The study of populations in the field allowed us to confirm that female individuals have smaller calyx and corolla than hermaphrodite ones (Thompson et al. 2002). Thus, for morphological analyses, all measurements were performed on hermaphroditic plants only.

Morphometric Analyses

The data matrix (155 accessions × 25 characters) was processed using the statistical and multivariate analysis packages Data Desk 6.1 (Velleman 1997) and SYN-TAX 2000 (Podani 2001). Principal coordinate analysis (PCoA) and cluster analysis (UPGMA) were performed using Gower's coefficent for mixed data extended to ordinal characters (Podani 1999). We also explored variation ranges using boxplots and post hoc Tukey test for multiple comparison of characters among the three species recognized here (see Results) to identify which quantitative continuous characters most efficiently separated *Th. paronychioides* from the other taxa. To test the overall discrimination power of the continuous characters alone, classificatory discriminant analysis (DA) was carried out. DA is an identification optimization procedure based on the probability of identification (e.g., Geisser's classification probabilities) and was performed on the dataset of 20 continuous variables and 155 accessions. *A priori* classification (Henderson 2006) was used to test whether the circumscription of the three species recognized here (used as groups in the discriminant analysis), based solely on the four qualitative characters, also fits with the quantitative data. This model assumes that each taxon is a discrete unit. The discriminant analysis developed predictive discriminant functions for the groups, which

Table 1 Morphological characters employed in the morphometric analyses

Nr.	Character
1	Corolla color: 0=white; 1=pink to purplish
2	Eglandular hairs on the leaf surface: 0=absent; 1=present
3	Oil dots (leaves, calyx and flowers): 0=rare; 1=abundant
4	Lateral veins of leaves (best seen in dried specimens): 0=weak; 1=prominent, parallel; 2=prominent, curved
5	Inflorescence shape: 0=capitula; 1=elongated (basal verticillaster remote); 2=interrupted (2–3 verticillasters remote below oblong terminal head)
6	Flowering branches length (without inflorescence) (mm)
7	Middle cauline leaf width (LW) (mm)
8	Middle cauline leaf length (LL) (mm)
9	Inflorescence length (IL) (mm)
10	Corolla length (FL) (mm)
11	Pedicel length (mm)
12	Bracteole length (mm)
13	Calyx length (CL) (mm)
14	Calyx tube length (CTL) (mm)
15	Upper calyx teeth length (mm)
16	Lower calyx teeth length (LCT) (mm)
17	Upper limb length (UL) (mm)
18	Bract length (second verticillaster of the inflorescence) (BL) (mm)
19	Bract width (second verticillaster of the inflorescence) (BW) (mm)
20	Number of flowers
21	Number of verticillasters
22	Ratio LL/LW
23	Ratio CTL/CL
24	Ratio LCT/UL
25	Ratio BW/LW

were, in turn, subsequently applied to the accessions in each group. The model used *a priori* probability equal for each group, when classifying the accessions.

Furthermore, the variability of the analyzed morphological characters was described by standard statistical parameters (mean, standard deviation, minimum, maximum, 10th and 90th percentiles) (Table 2).

Chromosome Counts

Chromosomes were counted for each taxon involved in this study from one individual (ca. five metaphase plates) per locality (Table 3). Plants were collected in the field and then cultivated in the Botanical Gardens of Floristic Research Center of the

Table 2 Comparisons of morphological characters among *Thymus paronychioides*, *Th. spinulosus* and *Th. striatus*. Quantitative continuous characters are expressed in mm and are reported as mean ± standard deviation and 10−90 percentiles (extreme values in brackets). For quantitative discrete cardinal characters, 10−90 percentiles are given (extreme values in brackets)

	Th. paronychioides	*Th. spinulosus*	*Th. striatus*
Qualitative characters			
Corolla color	usually pink	white, rarely pink	pink to purplish
Eglandular hairs on the leaf surface	present	present	absent
Oil dots (leaves, calyx and flowers)	rare	abundant	rare
Lateral veins of leaves	prominent, curved	weak	prominent, parallel
Inflorescence shape	capitula	elongated to interrupted, rarely capitula	capitula, rarely elongated to interrupted
Quantitative continuous characters (mm)			
Flowering branches length (without inflorescence)	29.3±8.7	46.7±21.6	41.8±17.5
	(14)20−40(50)	(17.0)27.8−76.0(120.0)	(10.5)19.8−65(92)
Median cauline leaf width	2.1±0.4	1.8±0.4	1.5±0.4
	(1.3)1.6−2.6(3.2)	(1.1)1.2−2.2(2.7)	(0.7)1.0−2.0(2.6)
Median cauline leaf length	7.2±1.08	10.1±1.9	9.3±2.0
	(5.0)6.−8.(10.5)	(6.5)8.0−12.8(15)	(5.3)6.9−12.0(14.0)
Inflorescence length	11.1±3.5	26.5±13.5	11.9±6.5
	(7)9−13(30)	(10.0)14.4−41.2(70.0)	(5)7−17(46)
Corolla length	7.0±0.7	5.6±0.5	5.2±0.6
	(5.5)6.2−8.0(8.5)	(4.0)5.0−6.1(7.0)	(4.0)4.3−6.1(6.5)
Pedicel length	1.7±0.5	2.1±0.7	1.4±0.3
	(0.5)1.0−2.0 (3.0)	(0.5)1.3−3.0(4.0)	1.0−1.8(2.5)
Bracteole of pedicel length	1.6±0.4	1.4±0.4	1.3±0.3
	(0.7)1.0−2.0(3.0)	(0.8)1.0−2.0(2.2)	(0.5)0.9−1.7(2.1)
Calyx length	5.3±0.3	4.6±0.4	4.4±0.6
	(4.6)5.0−5.8(6)	(4.0)4.1−5.0(5.7)	(3.0)3.6−5.0 (5.1)
Calyx tube length	2.0±0.2	1.8±0.2	1.7±0.3

Table 2 (continued)

	Th. paronychioides	*Th. spinulosus*	*Th. striatus*
	(1.5)1.8−2.3(2.6)	(1.4)1.5−2.1(2.5)	(1.0)1.3−2.0(2.2)
Upper calyx teeth length	1.0±0.1	1.1±0.2	1.3±0.3
	(0.7)0.9−1.2(1.4)	(0.8)0.9−1.3(1.5)	(0.8)1.0−1.7(2.0)
Lower calyx teeth length	3.0±0.3	2.2±0.2	2.3±0.35
	(2.5)2.7−3.3(3.8)	(1.9)2.0−2.5(3)	(1.7)1.9−2.8(3.2)
Upper limb length	3.3±0.3	2.7±0.3	2.7±0.4
	(2.9)3.0−3.6(4.0)	(2.2)2.3−3.2(3.3)	(2.0)2.2−3.1(3.5)
Bract length (second verticillaster)	5.4±0.6	6.4±0.8	7.0±1.2
	(4.5)4.7−6.0 (7.0)	(5.0)5.4−7.1(9.0)	(5.3)5.5−8.8(9.7)
Bract width (second verticillaster)	2.4±0.4	2.2±0.4	3.1±0.8
	2.0−3.0(3.2)	(1.1)1.9−2.7(3.0)	(1.5)2−4(4.6)
Quantitative discrete cardinal characters			
Number of flowers	(7)10−20(36)	(20)24−58(70)	(17)22−50(55)
Number of verticillasters	(2)3−4(5)	4−8(9)	3−6
Ratios			
LL/LW	3.5±0.6	5.8±1.3	5.5±0.9
	(2.50)2.8−4.2(4.69)	4.0−7.6(9.0)	(3.8)4.4−6.9(7.7)
CTL/CL	0.4±0.1	0.5±0.1	0.4±0.4
	(0.3)0.3−0.5(0.6)	(0.3)0.3−0.7(0.9)	(0.3)0.3−0.4(0.5)
LCT/UL	0.9±0.1	0.8±0.08	0.8±0.1
	(0.7)0.8−1.0	(0.7)0.7−0.9(1.0)	(0.7)0.7−0.9(1.0)
BL/BW	2.3±0.3	2.9±0.6	2.1±0.5
	(1.6)1.8−2.6(3.0)	(2.0)2.3−3.5(5.0)	(1.5)1.6−2.7(4.5)
BW/LW	1.9±0.2	1.3±0.31	2.1±0.5
	(0.8)0.9−1.5(1.6)	(0.6)1.0−1.7(2.1)	(1.1)1.6−2.8(3.6)

Table 3 Chromosome number records for *Thymus paronychioides, Th. spinulosus* and *Th. striatus* determined in the present study and taken from literature

Species	Locality	2*n*	Source of data
Th. paronychioides	Italy: Sicily-Rocca Busambra	ca. 60	present study
Th. paronychioides	Italy: Sicily-Rocca Busambra	56	Colombo et al. 1981 (sub *Th. spinulosus*)
Th. spinulosus	Italy: Puglia-S.M. in Lamis	26	present study
Th. striatus	Italy: Abruzzo-Pizzoli	26	present study
Th. striatus	Italy: Tuscany-Monte Ferrato	26	present study; Arrigoni et al. 1976, 1981 (sub *Th. ophioliticus*)
Th. striatus	Italy: Marche-Gole di Prioraco	26	Hruška and Bellomaria 1982 (sub *Th. acicularis*)
Th. striatus	Macedonia: Sivec	54	Jalas and Kaleva 1966 (sub *Th. striatus* subsp. *neapolitanus*)
Th. striatus	Macedonia: Sivec	26	Jalas and Kaleva 1966 (sub *Th. pseudoatticus*)
Th. striatus	Bulgaria	28, 42, 56, 84	Markova and Goranova 1994 (sub *Th. striatus* var. *interruptus*)

Apennine and of University of Pisa (Appendix 2). Root tips were pretreated with a 0.4 % colchicine solution for 4 hours at room temperature and then fixed in Carnoy's solution for 45 minutes. Then they were hydrolyzed in 1 N HCl solution for 6 minutes at 60°C and stained with leucobasic fuchsine; finally, they were squashed in acetic orcein for chromosome counting.

Results

Lectotypification

Thymus paronychioides was described by Čelakovský (1882) on the basis of a single herbarium specimen collected by Lojacono on Madonie (Sicily) and distributed, under the name *Th. zygis*, in *Plantae Siculae Rariores*. One year later, Čelakovský (1883) pointed out the exact locality of this sample: "*in aridis montosis elatis, serre di Quacedda, Julio 1877*". In addition, he mentioned the existence of a duplicate of the allegedly same gathering stored in Tempsky's Herbarium (Prague) that, on the contrary, has to be referred to *Th. conspersus* Čelak. (currently a synonym of *Th. spinulosus* Ten.). This alleged duplicate contains flowering individuals, while the type material of *Th. paronychioides* is not in flower (Čelakovský 1883). Čelakovský (1883) quoted for *Th. paronychioides* another herbarium specimen (with flowering individuals), collected by Todaro on Mt. Busambra (Sicily), distributed in the *Flora Sicula exsiccata*, and stored in Tempsky's Herbarium (Prague). Despite that Tempsky's Herbarium is now in the Charles University Herbarium in Prague (PRC), we found the specimens cited by Čelakovský in the National Museum Herbarium in Prague (PR), where Čelakovský used to work.

We could study some duplicates (G!, PR!) of the same gathering made by Lojacono on Madonie in 1887 and we can confirm that Lojacono distributed two

different species under the name *Th. zygis* of his *Plantae Siculae Rariores: Th. spinulosus* and *Th. paronychioides*. However, the two species were never found admixed together on the same herbarium sheet.

In particular, in PR we have traced one herbarium specimen, identified by Weber as *Th. paronychioides*, which is in complete agreement with the protologue and with the additional details provided by Čelakovský (1883). On this sheet, three small branches of a non-flowering thyme bearing elliptic to subspathulate pubescent leaves, with prominent curved lateral veins and bracts different to cauline leaves are mounted. The branch on the right (Fig. **2**, arrow) is designated here as the lectotype of the name *Th. paronychioides*. Taking into account the taxonomic complexity of the genus *Thymus* and considering that the type material is lacking flowers (as already evidenced by Čelakovský 1883), we deem it necessary to select an interpretative type (epitype) to preserve the application of the name in Čelakovský's sense and avoid future misinterpretations. Therefore, the herbarium specimen collected by Todaro on Mt. Busambra, kept in PR, and identified by Čelakovský (1883) as *Th. paronychioides* is selected here as the epitype (Fig. **3**). The plants from Mt. Busambra were later described by Lojacono (1907) as *Th. gussonei* (typus at G!), a name that has to be considered as a taxonomic synonym of *Th. paronychioides*, albeit its validity is questionable (see Introduction).

Morphometric Analysis

The principal coordinate analysis (PCoA, Fig. **4**) shows on the first two axes (explained variance: 33.7 % and 21.5 %) a clear separation of the three taxa involved in this study. *Thymus spinulosus* is the best-separated unit (along the first coordinate axis), while *Th. paronychioides* and *Th. striatus* are less different from each other, being separated along the second axis. *Th. paronychioides* is a rather homogeneous unit, with scarcely dispersed individuals, while *Th. spinulosus* and *Th. striatus* show higher morphological variation. Concerning the intraspecific variation within *Th. striatus*, three somewhat separated groups of specimens can be recognized in the PCoA diagram. They can be assigned to *Th. striatus* from central and southern Italy (polygon a in Fig. **4**), *Th. acicularis* (including var. *ophioliticus*) from central Italy and Balkan Peninsula (polygon b in Fig. **4**) and *Th. striatus* var. *interruptus* from Bulgaria (c in Fig. **4**).

Cluster analysis (UPGMA, Fig. **5**) shows three main clusters ('a' and 'b', 'a' cluster further separated into clusters 1 and 2): cluster b is composed of the specimens of *Th. spinulosus* and cluster 1 includes only the specimens of *Th. paronychioides*. Cluster 2 corresponds to the *Th. striatus* complex; subcluster 2.1 includes all the specimens referable to *Th. striatus*, but also one individual originally identified as *Th. acicularis* (STR 69); subcluster 2.2 includes intermixed specimens referable to *Th. acicularis* var. *acicularis* and *Th. acicularis* var. *ophioliticus* (group 2.2c) and the only specimen studied of *Th. striatus* var. *interruptus* (2.2d − STR 40).

Figure **6** shows the results of univariate analysis performed on selected morphological quantitative characters. Morphological differences among the three analyzed taxa are summarized in Table **2**, showing the standard statistical parameters of the measured characters and the main differences for qualitative

Fig. 2 Lectotype (plant indicated by an arrow) of the name *Thymus paronychioides* conserved in PR (In aridis calcareis montosis elatis Madonie. Serre di Quacedda, Julio 1877, *M. Lojacono s.n.*)

Fig. 3 Epitype of the name *Thymus paronychioides* conserved in PR (in aridis montosis Siciliae, Busambra, Junio, *Todaro s.n.*)

features. Corolla color, the lateral veins of the cauline leaves, the density of oil dots, inflorescence length and number of flowers are the most useful to

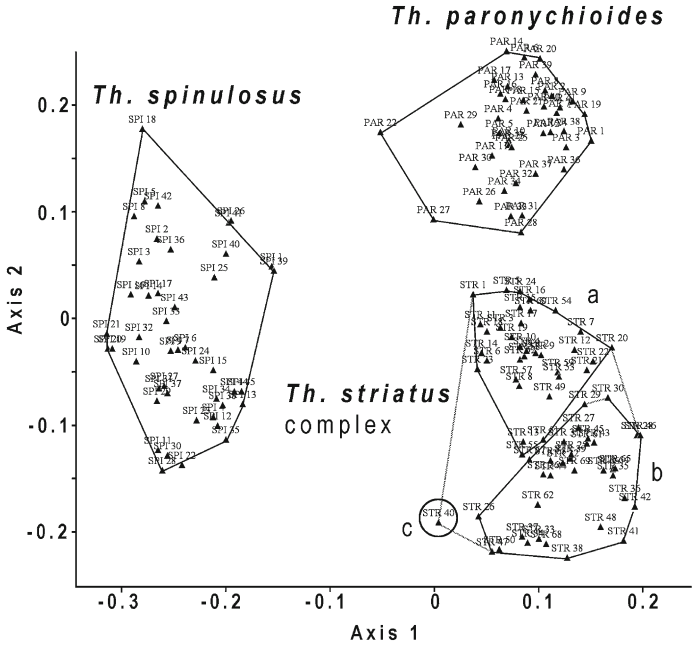

Fig. 4 Scatter plot of first two principal coordinate axes based on 25 morphological characters and 155 specimens. PAR − *Thymus paronychioides*, STR − *Th. striatus* complex, SPI − *Th. spinulosus*. **a**, **b** and **c** polygons/circle indicate three groupings within the *Th. striatus* complex: *Th. striatus* from central and southern Italy (**a**), *Th. acicularis* (including var. *ophioliticus*) from central Italy and Balkan Peninsula (**b**), *Th. striatus* var. *interruptus* from Bulgaria (**c**)

distinguish *Th. spinulosus* from the other taxa. Other characters, such as corolla length and calyx length contribute to the separation of *Th. paronychioides* from *Th. spinulosus* and *Th. striatus*, while glabrous leaves, the upper calyx teeth length and ratio of bract width/leaf width separate *Th. striatus* from *Th. paronychioides* and *Th. spinulosus*. However, *Th. paronychioides* seems to be clearly separated from *Th. striatus* in having eglandular hairs on the leaf surface, lateral veins of leaves curved, cauline leaves shorter, few flowers, corolla very large, up to 8.5 mm long, upper calyx teeth shorter and bracts 0.8−1.6 times wider than the cauline leaves. *Th. paronychioides* is also significantly different from *Th. spinulosus* in having pink flowers, capitate inflorescence, and prominent lateral leaf veins, few and pale oil dots and larger corolla and calyx.

In the classificatory discriminant analysis, restricted to the 20 continuous characters, *Th. paronychioides* was correctly recognized in 100 % of cases, *Th. spinulosus* in 88.9 % of cases and *Th. striatus* in 97.1 % of cases. From among misidentifications, *Th. spinulosus* was confused with *Th. striatus* and *vice versa*. A post hoc Tukey test comparing *Th. paronychoides* and *Th. spinulosus* showed significant difference in all characters ($P < 0.01$), with the exception of BPL, UCT, BW and BW/LW. Comparing *Th. paronychioides* and *Th. striatus*, values of all characters were significantly different ($P<0.01$), with the exception of IS, CC, IL, VN ($P<0.05$), PL ($P<0.05$) and CTL/CL. Finally, the post hoc Tukey test showed significant differences among *Th. spinulosus* and *Th. striatus* in all characters ($P<0.01$), with the exception of FBL, LL ($P<0.05$), LL/LW ($P<0.05$), BPL, CL, CTL ($P<0.05$), LCT, UL, LCT/UL ($P< 0.05$) and BL.

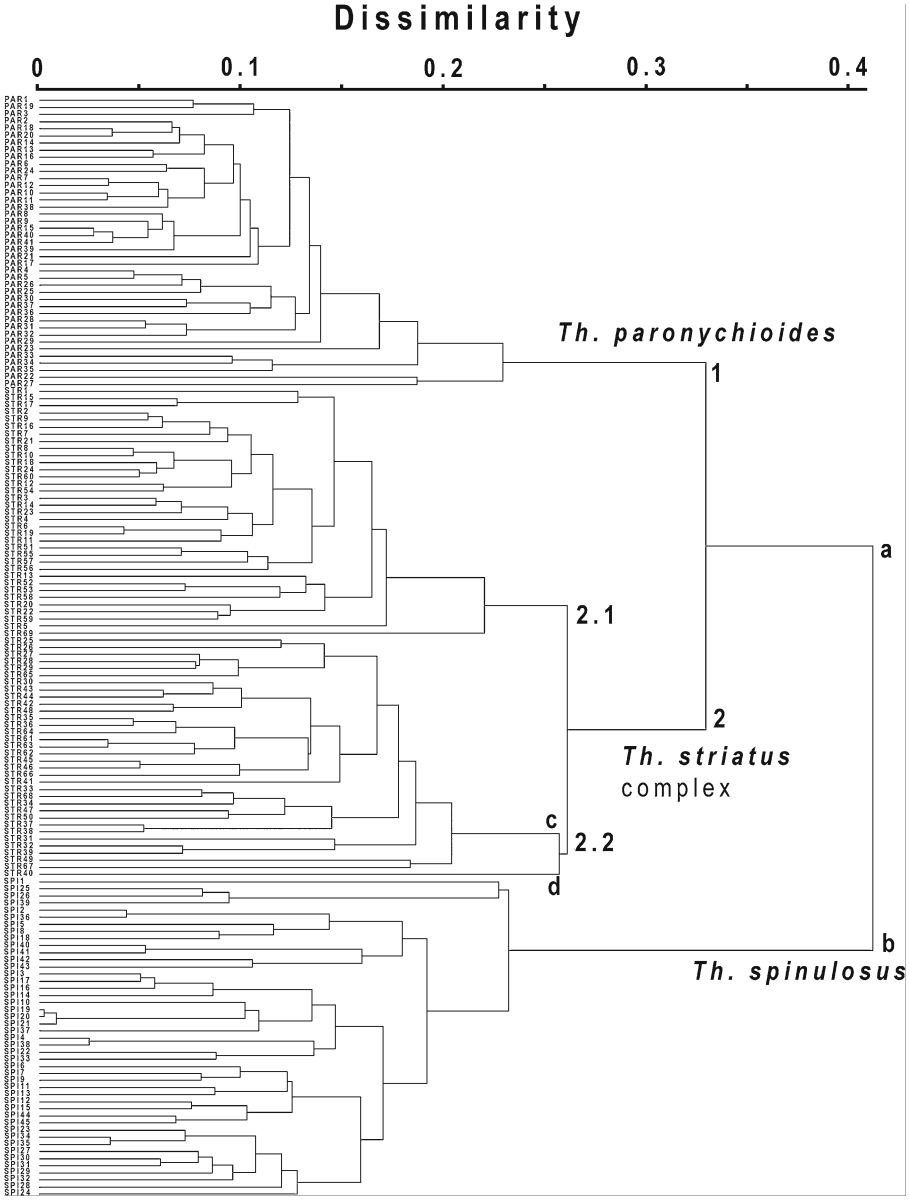

Fig. 5 UPGMA dendrogram, showing the phenetic relationships among the studied individuals of *Thymus paronychioides* (PAR), *Th. striatus* complex (STR) and *Th. spinulosus* (SPI)

Chromosome Counts

Chromosomes were counted in two populations of *Thymus striatus* and one population of *Th. spinulosus*: both species were diploid with $2n = 26$ chromosomes. The single studied population of *Th. paronychioides* turned out to be polyploid, with $2n =$ ca. 60 chromosomes (Table 3).

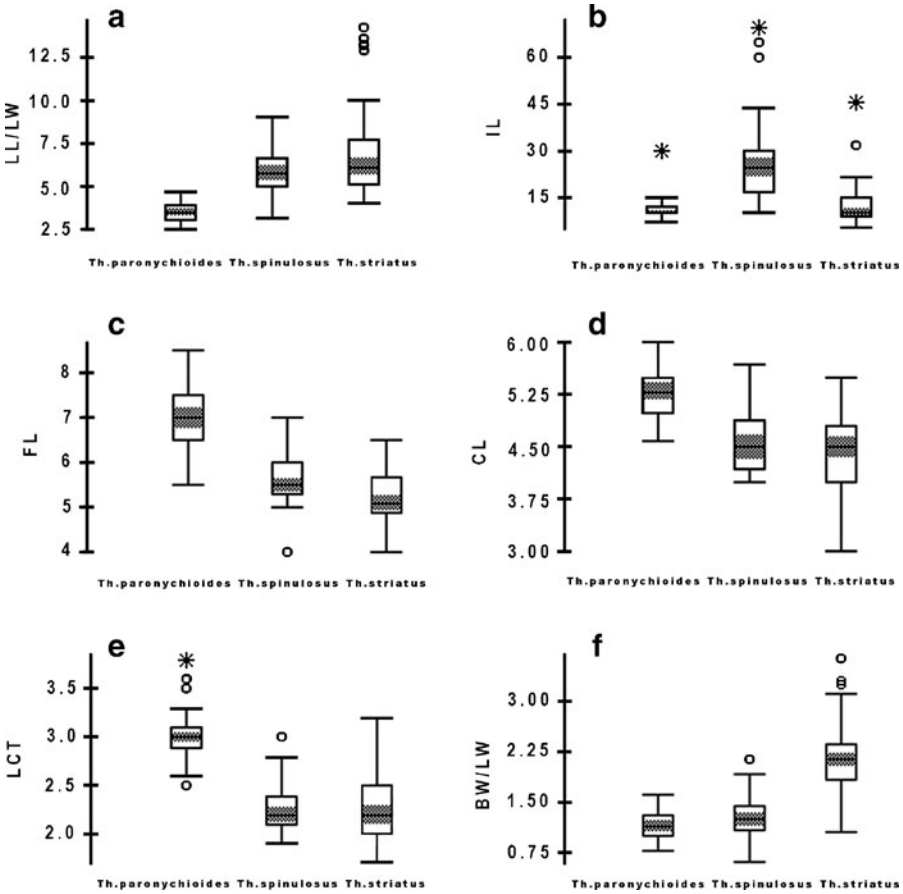

Fig. 6 Boxplots illustrating the variability among the three studied taxa. **a** leaf length/leaf width ratio (LL/LW); **b** inflorescence length (IL); **c** corolla length (FL); **d** calyx length (CL); **e** lower calyx teeth length (LCT); **f** bract width/leaf width ratio (BW/LW). The bottom and top of the boxes depict the 25th and 75th percentile, and the horizontal bar is the median. The ends of the whiskers indicate the minimum and maximum values, unless outliers are present, in which case the whiskers extend to a maximum of 1.5 times the interquartile range. Superimposed grey areas indicate confidence interval bounds around its median. Circles indicate outliers, unless extreme outliers are present, in which case the circles extend to a maximum of 3 times the interquartile range and the extreme outliers are indicated as asterisks

Discussion

The Taxonomic Status of Thymus paronychioides *and its Relationship with* Th. striatus *and* Th. spinulosus

The results of our morphological analyses (Table 2, Figs. 4, 5, 6) and chromosome counts support the species status of *Thymus paronychioides*, which is shown to be an endemic of northern Sicily. We also clarified the previous confusion with other taxa within the *Th.* sect. *Hypodromi* occurring in the Mediterranean area and reported for the Italian territory (Conti et al. 2005; Giardina et al. 2007), namely *Th. striatus* and *Th. spinulosus*. *Thymus paronychioides* is clearly differentiated from *Th. striatus* and

Th. spinulosus mainly by the unique characteristics of leaves that are elliptical-spathulate, fleshy, with curved and ± prominent lateral veins and by the larger corolla up to 8.5 mm long, with the tube usually exceeding the calyx. Chromosome counts provided evidence that *Th. paronychioides* is polyploid with $2n$ = ca. 60 chromosomes, whereas all studied populations of *Th. striatus* and *Th. spinulosus* are diploid with $2n$ = 26 chromosomes. There is a previous chromosome count for *Th. spinulosus* from Mt. Busambra ($2n$ = 56, Colombo et al. 1981) but unfortunately it has not been possible to trace the voucher specimen preserved in PAL. During our field investigations on Mt. Busambra in 2010 we found only *Th. paronychioides*, but there are specimens of *Th. spinulosus* collected by Lojacono and Gussone in PAL and NAP from this locality. However, based on our results, it is likely that the chromosome count published by Colombo et al. (Colombo et al. 1981) refers to *Th. paronychioides*.

Thymus striatus was described by Vahl (1794) from "*Regno Neapolitano*" (presumably Campania, Italy) and occurs throughout southeastern Europe (Italian and Balkan Peninsulas). According to the specimens studied and our field investigations, the previous records of *Th. striatus* for Sicily (Conti et al. 2005; Giardina et al. 2007) represent misidentifications and should refer to *Th. paronychioides*. Several authors studied the taxonomy of the *Th. striatus* complex (Lacaita 1911, 1925; Ronninger 1930; Moggi 1955; Jalas 1980; Bini Maleci et al. 1997, 1999), to understand its high morphological variability. The morphological variation of this group is also accompained by a high degree of karyological variation. Indeed, *Th. striatus* appears as a polyploid complex for which the following chromosome numbers were reported: $2n$ = 28, 42, 56, 84 for *Th. striatus* var. *interruptus* Jalas, Bulgaria (Markova and Goranova 1994); $2n$ = 54 for *Th. striatus* subsp. *neapolitanus* (Strobl) Ronninger, Macedonia (Jalas and Kaleva 1966); $2n$ = 26 for *Th. pseudoatticus* Ronniger ex Hayek, Macedonia (Jalas and Kaleva 1966). The latter species was described by Hayek from the formerly Serbian part of Macedonia (Hayek 1928) as being intermediate among *Th. striatus* Vahl and *Th. atticus* Čelak. It seems to be closely related to *Th. acicularis* from both a morphological (original material kept in W!) and karyological point of view. Our counts for *Th. striatus* ($2n$ = 26) perfectly agree with the previous ones reported for Italy (Arrigoni et al. 1976, 1981 for *Th. acicularis* var. *ophioliticus* Lacaita; Hruška and Bellomaria 1982 for *Th. striatus* var. *acicularis* (Waldst. & Kit.) Borbás). *Thymus striatus,* in recent floras and checklists (Jalas 1972; Baden 1991; Conti et al. 2005), is considered a single highly polymorphic species without infraspecific taxa, with the single exception of *Th. striatus* var. *interruptus*, reported for Bulgaria and Turkey (Jalas 1980, 1982; Markova 1989). A preliminary analysis of the herbarium specimens available, including the types, has shown that most of the taxa belonging to the *Th. striatus* complex have no taxonomic value (i.e., *Th. acicularis* var. *dinaricus* Heinr. Braun, *Th. acicularis* var. *stabianus* Lacaita, *Th. neapolitanus* Strobl, *Th. pseudoatticus* Ronniger ex Hayek, *Th. striatus* subsp. *calvanicensis* Ronninger, *Th. striatus* subsp. *orieni* Ronninger, *Th. striatus* subsp. *salernitanus* Ronninger, *Th. velenovskyi* Rohlena). On the contrary, other taxa usually included in this group appear to have distinctive morphological features, as shown in this study by the results of PCoA (Fig. **4**) and UPGMA (Fig. **5**). This is the case of *Th. acicularis* (polygon b, Fig. **4**; cluster 2.2c, Fig. **5**), described by Waldstein and Kitaibel (1805) on the basis of specimens collected in the Velebit Mts. in Croatia and characterized by slender habit, acicular, often falcate, leaves (0.7)0.8−1.4(1.6) mm wide, floral bracts generally tinged in red, calyx (3)3.5−4.5(4.9) mm long. Also *Thymus striatus* Vahl *sensu stricto* (polygon a, Fig. **4**;

cluster 2.1, Fig. 5) seems to be well-characterized in having a robust habit, leaves lanceolate to spathulate, (1.1)1.4−2.1(2.6) mm wide, and bracts usually greenish and calyx (4)4.4−5.2(5.5) mm long. *Th. striatus* var. *interruptus*, described by Jalas (1980) from Turkey, seems to be well differentiated, showing an inflorescence typically interrupted, with many flowers (polygon c, Fig. 4; cluster 2.2d − STR40, Fig. 5), and an isolated distribution confined to the Thracian plain (Europaean Turkey and southern Bulgaria), but further studies with more complete sampling are needed to understand its taxonomic position. Finally, *Th. acicularis* var. *ophioliticus,* a local endemic variant, described by Lacaita (1911) from the ophiolitic rocks of Tuscany (central Italy), is very close to *Th. acicularis* var. *acicularis* (cluster 2.2c, Fig. 5), but it differs in having a more slender habit, a smaller calyx and upper calyx teeth rarely exceeding 1 mm. In our opinion, based on the preliminary results shown in the PCoA and UPGMA regarding the *Th. striatus* complex in the Mediterranean area (Figs. 4 and 5), we can recognize at least two taxa. They have distinct geographical/ecological distribution, display sufficient morphological differentiation, and hence might deserve the subspecific rank within *Th. striatus* Vahl: *Th. striatus* subsp. *striatus* and *Th. striatus* subsp. *acicularis* (Waldst. & Kit.) Ronninger. *Th. striatus* subsp. *striatus* grows mainly on dry rocky slopes and xeric meadows on calcareous substrates, from mountain belt up to altitudes above 2,300 meters (i.e., Majella and Pollino massif, central-southern Apennine) and is distributed in central-southern Italy. Ronninger (1930) reported it also from Macedonia and Greece, but we have never seen specimens from these areas. The individual STR69 (cluster 2.1, Fig. 5), coming from Tijepsi in Albania (*A. Baldacci*, FI), shows intermediate morphological characters between *Th. striatus* subsp. *striatus* (big calyx) and subsp. *acicularis* (acicular leaf).

Thymus striatus subsp. *acicularis* grows on dry slopes, rocky and stony pastures on calcareous substrates and on arenaceous or serpentine outcrops, mainly from hill to mountain belt and is distributed in the central-northern Italy and in the Balkan Peninsula (Croatia, Bosnia and Herzegovina, Serbia, Montenegro, Albania, Macedonia, and Bulgaria).

Thymus spinulosus is a species endemic to southern Italy and Sicily, described by Tenore (1812) from Apulia and Basilicata (southern Italy). This species is well characterized from a morphological and karyological point of view. Rarely plants with pink flowers (SPI1, Figs. 4 and 5) can be found, but always within a typical population, characterized by white flowers. A single specimen from Mt. Quacella in Sicily (SPI18, Figs. 4 and 5) could have more relevance because it has flowers and calyx larger than others, but further detailed studies with larger sampling are needed. The previous records from Abruzzo, central Italy (i.e., Pignatti 1982; Conti et al. 2005), are based on misidentifications and should be actually referred to *Th. striatus* (Bartolucci and Conti 2011). The records for Molise (Lucchese 1995; Conti et al. 2005) are doubtful, because it was not possible to trace any herbarium specimen or population from this area. *Th. spinulosus* grows on xeric places on calcareous substrates, from coastal (i.e., Apulia) to hilly-mountainous habitats up to above 1,400 m a.s.l. (Sicily).

On the Subsectional Position of Thymus paronychioides

According to Jalas (1971), *Th. spinulosus* and *Th. striatus* both belong to *Th.* sect. *Hypodromi* subsect. *Subbracteati*. On the contrary, given its quite distinctive

features such as the elliptical-spathulate leaves, with curved lateral veins, and the corolla with the tube usually exceeding the calyx, *Th. paronychioides* seems to be better placed within *Th.* sect. *Hypodromi* subsect. *Serpyllastrum*. The latter subsection includes species that occur in Spain, the Balkans, Crimea, Turkey, the Caucasus and Central Asia. Hence, the presence of *Th. paronychioides* in Sicily fills a gap in the Mediterranean distribution of the subsection *Serpyllastrum*. Concerning karyology, for the subsection *Subbracteati* the chromosome numbers $2n$ = 26, 28, 30, 42, 52, 54, 56, 84 are known, while for the subsection *Serpyllastrum* the chromosome numbers $2n$ = 26, 28, 56, 58, 60, 62, 90 are reported (Morales 2002). Accordingly, also the chromosome number found for *Th. paronychioides* ($2n$ = ca. 60) is somehow supporting its inclusion within the subsection *Serpyllastrum*. Hence, to correctly categorize this Sicilian endemic according to cytogenetic criteria (Siljak-Yakovlev and Peruzzi 2012), the taxon relative of *Th. paronychioides* should be searched within the latter subsection. However, the phylogenetic value of all these infrageneric taxa should be first corroborated by further studies, especially considering that morphological and molecular variation in *Thymus* shows severe incongruencies (Federici et al. 2011).

Thymus paronychioides *in the Phytogeographic Context of Sicily*

By updating the nomenclature reported by Brullo et al. (1995) to the most recent checklists available (Conti et al. 2005; Giardina et al. 2007; Raimondo et al. 2010), it results that no other taxon belonging to Lamiaceae (Lamiales) is endemic to Deprano-Panormitano, Madonie and Nebrodi districts (Brullo et al. 1995), with the exception of the taxonomically doubtful *Clinopodium minae* (Lojac.) Peruzzi & Conti. The phylogenetically less distant species endemic to these districts are *Orobanche chironii* Lojac. (Orobanchaceae, Lamiales) and *Verbascum siculum* Tod. ex Lojac. (Scrophulariaceae, Lamiales), while the remaining endemics belong to other vascular plant lineages, prominently to Asteraceae (see also recent work on *Centaurea*: Raimondo and Spadaro 2008; Bancheva et al. 2011), Fabaceae (see also recent works on *Genista*: Bacchetta et al. 2011, 2012) and Brassicaceae (Brullo et al. 1995; Giardina et al. 2007). Sicily is once again confirmed as an extraordinarily rich biodiversity hotspot. Despite this, and despite the past and present efforts of botanists in biosystematic studies in this region (see for instance Bedini et al. 2012, for a recent survey on chromosome studies in endemics), much work has still to be done in this respect.

Taxonomic Treatment

Thymus sect. ***Hypodromi*** (A. Kern.) Halácsy, Denkschr. Kaiserl. Akad. Wiss., Wien Math.-Naturwiss. Kl. 61: 252. 1894.
Thymus sect. ***Hypodromi*** subsect. ***Serpyllastrum*** Villar, Cavanillesia 6: 124. 1934.
Thymus paronychioides Čelak., Flora 65: 564. 1882.
Ind. loc.: [Italy, Sicily] “Madonie auf Sicilien (Lojacono)”.

Lectotype (designated here): Plantae Siculae Rariores / *Thymus zygis* Lin. Sp. Pl. 826 / In aridis calcareis montosis / elatis / Madonie. Serre di Quacedda/ Julio 1877 / Legit. *M. Lojacono* (PR 746197).
Epitype (designated here): [the label is a copy *manu* Čelakovský] "Todaro Flora sicula exsiccata / in aridis montosis Siciliae - Busambra / Junio / *Todaro* (PR 224604; iso-: FI, PAL, RO).
- *Thymus gussonei* Lojac., Fl. Sicul. 2(2): 198. 1907, *nom. inval.?*
- *Thymus gussonei* var. *diminutus* Lojac., Fl. Sicul. 2(2): 198. 1907, *nom. inval.?*
- *Thymus acicularis* auct. p.p. non Waldst. & Kit.: Guss., Fl. Sic. Prodr. 2: 127. 1828.
- *Thymus siculus* Lojac., *nom. nud., in schedis*
- *Thymus spinulosus* auct. p.p. non Ten.: Grande, Nuovo Giorn. Bot. Ital. 31: 160. 1924; Jalas in Tutin et al., Fl. Eur. 3: 178. 1972; Pignatti, Flora d'Italia 2: 491. 1982; Greuter et al., Med-Checkl. 3: 393. 1986.
- *Thymus striatus* auct. p.p. non Vahl: Nyman, Consp. Fl. Eur.: 594. 1881; Parlatore, Fl. Ital. 6(1): 99. 1884; Arcang., Comp. Fl. Ital., ed. 2: 423. 1894; Fiori, Nuov. Fl. Italia 2(3): 454. 1926; Zang., Fl. Ital. 2: 575. 1976; Conti et al., Annot. Checkl. Italian Vasc. Fl.: 175. 2005; Giardina et al., Bocconea 20: 315. 2007.
- *Thymus zygis* auct. p.p. non L.: Guss., Fl. Sicul. Syn. 2(1): 95. 1844; Arcang., Comp. Fl. Ital.: 539. 1882; Tornab., Fl. Sicul.: 406. 1887.
Icon.: Lojacono, Fl. Sicul. 2(2): tab. XV, Fig. **1**. 1907 (sub *Thymus gussonei* Lojac.).

Morphological Description

Loosely mat-forming perennial herbs, with creeping branches. Flowering stems up to 5 cm, pubescent all around or rarely on two opposite sides on the lower internodes. Middle cauline leaves (5.0)6.0−8.5(10.5) mm long × (1.3)1.6−2.6(3.2) mm wide, elliptical-spathulate, ciliate at the base and more or less hairy on both sides, fleshy. Leaves 2.5−4.7 times longer than wide, with lateral veins ± prominent (on dried specimens), curved along the margins with few pale oil dots. Inflorescence capitate, also in fruit, with 7−36 flowers; bracts trullate, different from the cauline leaves (rarely similar), 0.8−1.6 times wider than cauline leaves. Corolla (5.5)6.2−8.0(8.5) mm long, pink or whitish-pink; corolla tube usually exceeding the calyx. Calyx (4.6)5.0−5.8(6) mm long, cylindrical, hairy all around, greenish to reddish; calyx tube 1.8−2.3(2.6) mm long, upper calyx teeth (0.7)0.9−1.2(1.4) mm long, lower ones (2.5)2.7−3.3(3.8) mm long (Fig. **7**).

Habitat, Distribution and Phenology

Thymus paronychioides grows on dry rocky slopes at altitudes from 600 to 1,400 m. It flowers from May to August. This species, endemic to northern Sicily, is distributed in Deprano-Panormitano, Madonie and Nebrodi districts.

Fig. 7 General view of the species studied here. **a** *Th. paronychioides* (Sicily: Rocca Busambra); **b** *Th. spinulosus* (Calabria: Morano Calabro); **c** *Th. striatus* (Campania: Monte Faito di Castellammare)

Identification Key to the Italian Species of Thymus *sect.* Hypodromi

(Note: measurements refer to hermaphroditic plants only!)

1a Plants densely covered with reddish oil dots (rarely pale), hairy leaves (at least on the upper surface), lateral veins weak, inflorescence usually interrupted, flowers usually white (rarely pale pink) ……..... ***Th. spinulosus***

1b Plants with rare, pale, oil dots, leaves with ± prominent lateral veins (visible on dried specimens), inflorescence capitate to interrupted, flowers pink to purplish…….....**2**

2a Leaves elliptic-spathulate, fleshy, with lateral veins curved, hairy; inflorescence capitate, bracts 0.8−1.6 times wider than the cauline leaves, greenish; corolla (5.5)6.2−8(8.5) mm long, pink; upper calyx teeth 0.9−1.2(1.3) mm long ……..*Th. paronychioides*

2b Leaves linear, falcate to subspathulate, glabrous (rarely with few eglandular hairs on the upper surface) with lateral veins parallel; inflorescence capitate, rarely elongated or interrupted, bracts (1.1)1.6−2.8(3.6) times wider than the cauline leaves, greenish to purplish; corolla (4)4.3−6.1(6.5) mm long, pink to purplish; upper calyx teeth (0.8)1−1.7(2) mm long.....**3**

3a Plants with robust habit, leaves lanceolate to subspathulate (1.1)1.4−2.1(2.6) mm wide; calyx (4)4.4−5.2(5.5) mm long, upper calyx teeth (1.1)1.2−1.7(2) mm long ……..…….. ***Th. striatus*** subsp. ***striatus***

3b Plants with slender habit, leaves acicular, usually falcate (0.7)0.8−1.4(1.6) mm wide; calyx (3)3.5−4.5(4.9) mm long, upper calyx teeth (0.8)0.9−1.4(1.8) mm long ……..……..…… ***Th. striatus*** subsp. ***acicularis***

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Appendix 1 Plant material studied and used for the morphometric analyses

Thymus paronychioides Čelak.

ITALY: Sicily: salendo verso la vetta di Rocca Busambra (Palermo) (ED50 33S 360268 4190974), pascoli aridi, 1,395 m, 5 Jun 2010, *F. Bartolucci, F. Conti, N. Ranalli* (APP, 20 sheets); lungo la strada sterrata tra Cima Cucco e Rocca Busambra (Palermo) (ED50 33S 360781 4191903), pendii rupestri, 900 m, 5 Jun 2010, *F. Bartolucci, F. Conti, N. Ranalli* (APP, 10 sheets); Rocca Busambra, Palermo, 16 Jun 1980, *S. Brullo* (CAT); *ibidem*, 4 Jun 1988, *Brullo, Minissale* (CAT, 2 sheets); Mt. San Giuliano near Erice (Erice, Trapani), 38°02′ N 12°35′ E, calcareus soil, 600–700 m, 13 Jun 1990, *Raimondo* et. al. (PAL); Busambra, […] aridis, Jul 1879, *Todaro* (FI); in aridis montosis, Siciliae, Busambra, VI, *Todaro* (PR, epitype of *Th. paronychioides*; FI, iso-epitype).

Thymus spinulosus Ten.

ITALY: Puglia: Valle Pezzente (tra S. Giovanni Rotondo e Cagnano Varano) (UTM 5683E 46246 N), pascolo roccioso (calcari), 570 m, 28 Jun 2009, *R.P. Wagensommer* (Herb. Wagensommer); Le Serre (Deliceto), 45627–45633 NE (UTM ED50), stipeto a *Stipa austroitalica*, 620–850 m, 26/05/2011, *F. Bartolucci*, *F. Conti* (APP, 2 sheets); versante sud di Monte Tre Titoli (Accadia), 45584-45589NE (UTM ED50), pascoli, 750–930 m, 27/05/2011, *F. Bartolucci*, *F. Conti* (APP, 11 sheets); – **Calabria:** Massiccio del Pollino, lungo la SS 19 nei pressi di Morano Calabro (Cosenza), 595415, 4411809, ED50 33S, pascoli aridi su calcare, 732 m, 8 Jun 2008, *F. Bartolucci* (APP, 12 sheets); Muraglione Cassano Jonio (Cosenza), 590 m, 15 May 1993, *A. Capparelli*, *L. Bernardo* (CLU); Massiccio del Pollino, ca. 9 km NNW of Castrovillari, Vallone Colloreto (Castrovillari, Cosenza), 900–1,000, 17 Jun 1997, *Partecipanti VIII Iter Mediterraneum* (CLU); Dirupata di Morano Calabro (Cosenza), 722 m, 29 May 1994, *P. Calvosa* (CLU); settore orientale del Massiccio del Pollino, lungo la strada che da Cerchiara porta a Monte Sparviere presso Pantano della Madonna (Cerchiaria di Calabria, Cosenza), flysh argillitico-calcareo a contatto con roccia calcarea, 1,200 m, 2 Jul 1994, *L. Bernardo* (CLU); – **Sicily:** base di Monte Quacella (ED50 33S 413202 4190744), pendii rupestri, 1,366 m, 4 Jun 2010, *F. Bartolucci, F. Conti, N. Ranalli* (APP); in aridis calcareis montosis elatis Modonie serre di Quacedda, Jul 1877, *M. Lojacono Pojero* (G, PR); Monte Acuto (Cesarò), 15 Jun 2004, *R. Galesi* (CAT); supram Bronte versus Silvam Bosco di Maletto, 3 Jul 1874, *P. Strobl* (PR, FI); Madonie, Piano della Canna, 5 Jul 1904, *Cavara* (FI); in asperis Montium Nebrodensium (alla Pietà presso Polizzi), 24 Jun 1840, *Heldreich* / Madonie sopra Polizzi Sicilia, s.d., *Parlatore* / […], s.d., *Minà* (FI); in aridis calcareis Marianopoli, VI, *M. Lojacono Pojero* (FI).

Thymus striatus Vahl

ITALY: Tuscany: copiosus in rupibus et saxis ophiolithicis montis Ferrato prope Prato, alt. 100–200 m, 11 Jun 1910 e 11 Jun 1911, *R. Pampanini*, *A. Fiori* (FI, 3 sheets); Montaione, in rupestribus et silvaticis prope pagum Montignoso, in rupestribus et silvaticis, solo ophiolitico (gabbro), 17 Jun 1911, *R. Pampanini* (FI); Monte Vaso (Santa Luce, Pisa), gariga su serpentino, 580 m, 23 May 2009, *F. Bartolucci* (APP); Monte Ferrato (Prato, Firenze), radura boschiva su serpentino, 204 m, 20 May 2009, *F. Bartolucci* (APP); M. Ferrato (Prato), 1 May 1934, *U. Losacco* (FI); Monte Ferrato presso Prato, 1 Jun 1866, s.coll. (FI); Impruneta ai Sassi Neri, suolo serpentinoso, 315 m, 4 Jun 1911, *A. Fiori* (FI); in rupibus ophiolithicis prope pagum Impruneta ad merid. Florentia, 12 Jun 1973, *E. Levier* (FI); – **Lazio:** Punta dell'Uccettu (Borgorose, Rieti), rupi, 1,972 m, 8 Jun 2009, *F. Bartolucci* (APP, 4 sheets); Montagna della Duchessa: M. Morrone vers. SW (Borgorose, Rieti), pascoli sassosi, su calcare, 2,000 m, 15 Jul 2008, *F. Bartolucci* (APP, 5 sheets); M. Duchessa presso Cartore (Borgorose, Rieti), pascoli aridi, 1,000 m, 5 Jun 2007, *F. Bartolucci* (APP); – **Abruzzo:** M. Breccioso (UTM-ED50 33 T 379516 4636948), pascoli aridi, 1,587 m, Jun 2010, *F. Bartolucci, N. Ranalli* (APP, 5 sheets)**;** lungo la strada tra Barisciano e S. Colombo (Barisciano, L'Aquila), pendii rupestri, 1,000 m, 4 Jun 2004, *F. Bartolucci* (APP, 3 sheets); *ibidem*, 16 Jun 2004, *F. Bartolucci* (APP); nei pressi di Fossa Raganesca (Ocre, L'Aquila), pascoli aridi, 830 m, 5 Jun 2007, *F. Bartolucci* (APP, 5 sheets); al Conveto di S. Spirito d'Ocre (L'Aquila), pascoli aridi, 700 m, 17 May 2006, *F. Bartolucci* (APP, 6 sheets); nei pressi di Terranera (L'Aquila), rupi, 1,250 m, Jun 2010, *F. Bartolucci* (APP, 3 sheets); loc. Costa Macere, tra Poggio Picenze e Barisciano (Barisciano, L'Aquila), pascoli aridi, 1,000 m, 17 May 2006, *F. Bartolucci* (APP, 6 sheets); S. Colombo (Barisciano, L'Aquila), pascoli aridi, 1,100 m, 28 Jun 2005, *F. Bartolucci* (APP); – **Campania:** M. S. Angelo di Castellammare (Salerno), s. calc., Jul 1911, *M. Guadagno* (FI, 3 sheets); M. S. Angelo di Castellammare in cacumine (Salerno), c. 4900′, 26 Jun 1883, *C. Lacaita* (FI); M. S. Angelo a Tre Pizzi (Costiera Amalfitana) (Castellammare di Stabia, Salerno), pascoli, 1,453 m, 21 Jun 2008, *L. Cancellieri*, *G. Salerno* (APP, 7 sheets); M. Faito di Castellammare, nei pressi del Convento di S. Michele (Castellammare di Stabia, Salerno), pascoli, 1,200 m,

3 Jun 2008, *F. Bartolucci, M. Iocchi* (APP, 15 sheets); in Monte S. Angelo prope Castellamare, solo calcareo, 1 Jul 1873, *P.G. Strobl* (WU, lectotype of *Th. neapolitanus*); S. Maria a Castello (Vico Equense, Salerno), s. calc., 700 m, 25 May 1913, *M. Guadagno* (FI); S. Angelo a Castellammare (Salerno), s.d., s.coll. (FI); **CROATIA:** In clivis. Montis Kiesovo, Jul 1881, *Pichler* (PAL); Postak […], s.d., *I. Horvat* (ZA); in Monte Crnopac (Velebit), 31 Jul 1896, *L. Rossi* (ZA); in monte Sladovaca […], 25 Jul 1909, *L. Rossi* (ZA); Alp. Badany, Debelo Berdo, s.d., *Kitaibel* (PR, Lectotype of *Th. acicularis*); in aridis fur m. Rumia, 11 Jul 1891, *A. Baldacci* (FI); Auf den Mt. Prologh bei […], Jul 1870, *Visiani* (FI); in apricis saxosis montis Biokovo, s.d., *Pichler* (FI, ZA); loc. rupestribus supra Cattaro in confinibus Montenegro, Jul 1885, *Pichler* (PAL); **MONTENEGRO:** Cetinje (Jugoslavia, Montenegro), in clivibus carsticis silvaticis in monte Lovćen supra viam publicam, situ meridionale, 1,350–1,400 m.s.m., 4 Jul 1977, *Černoch* 31664 (FI); **ALBANIA**: Cèpa Riskasit infra […], in saxosis, 20 Jul 1897, *A. Baldacci* (FI); ad Tijepsi […], in saxosis, 3 Jul 1898, *A. Baldacci* (FI); **BULGARIA:** Bulgaria australis, promontorius Rhodope, inter urb. Plovdiv et Pazardzhik, solo silicioso (semper!), in […] 250–400 m, 20 Jun 1973, *S. Staner* (H, 5 sheets).

Appendix 2 Source of living plant material investigated in the present study (locality, date, collectors and accession numbers). Voucher specimens are kept in APP

Thymus paronychioides Čelak.

ITALY: Sicily: salendo verso la vetta di Rocca Busambra (Palermo) (ED50 33S 360268 4190974), pascoli aridi, 1,395 m, 5 Jun 2010, *F. Bartolucci, F. Conti, N. Ranalli* (*cult.* Hort Bot CRFA, acc. n. 462/10).

Thymus striatus Vahl

ITALY: Tuscany: Monte Ferrato (Prato, Firenze), radura boschiva su serpentino, 204 m, 20 May 2009, *F. Bartolucci* (*cult.* Hort. Bot. University of Pisa, acc. n. 430/09; *cult.* Hort. Bot. CRFA, acc. n. 430/09, locus classicus of *Th. ophioliticus*); – **Abruzzo:** Pizzoli (L'Aquila), pascoli aridi, Mar 2007, *F. Bartolucci* (*cult.* Hort. Bot. University of Pisa, acc. n. 07)/07).

Thymus spinulosus Ten.

ITALY: Puglia: nei pressi di San Marco in Lamis (San Marco in Lamis, Foggia) (ED50 33 T 551525, 4618919), pascoli aridi su terra rossa, 507 m, 9 Jul 2007, *F. Bartolucci* (*cult.* Hort. Bot. CRFA, acc. n. 307/07); – **Sicily:** base di Monte Quacella (ED50 33S 413202 4190744), pendii rupestri, 1,366 m, 4 Jun 2010, *F. Bartolucci, F. Conti, N. Ranalli* (*cult.* Hort. Bot. CRFA, acc. n. 461/10).