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Survey on the Distribution, Diversity and Phyochemistry of Genus Thymus in Bulgaria

Ina Aneva[1*], Petar Zhelev[2], Stoyan Stoyanov[1], Yulian Marinov[3], Kristina Georgieva[4]

1 - Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, "Acad. G. Bonchev" Str., bl. 23, 1113 Sofia, BULGARIA.
2 - University of forestry, 10 "Kliment Ohridski" Blvd, 1797 Sofia, BULGARIA
3 - Regional Museum of Natural History, 34 "Hristo G. Danov" Str., 4000 Plovdiv, BULGARIA
4 - Institute of Organic Chemistry with Centre of Phytochemistry, „Acad. G. Bonchev" Str. bl. 9, 1113 Sofia, BULGARIA
[*]Corresponding author: ina.aneva@abv.bg

Abstract. The species of genus *Thymus* are important medicinal and aromatic plants. They provoke substantial interest worldwide from phytochemical point of view, due to their diverse biological activities with potential for application in pharmaceutical, cosmetic and food industries. The present study reports results of a survey on the distribution and diversity of the species in Bulgaria. Both field investigations and literature data were used in the study. The 20 species distributed naturally in Bulgaria differ in their natural range. The rarest species are *Thymus perinicus* (Bulgarian endemic) and *T. bracteosus*, each distributed in only one floristic region. *T. leucotrichus* and *T. stojanovii* occur in two floristic regions each, *T. longedentatus* – in four and the other species are distributed in five or more floristic regions. There are four Balkan endemics. Most widely distributed are *T. pannonicus*, *T. pulegioides* and *T. sibthorpii*, with natural localities in all 20 floristic regions in Bulgaria. Three species are of conservation importance. The richest in species floristic region was Rhodopes with 16 species followed by Pirin – 15, Stara plania – 13, and the least number of species was recorded in the Danubian plain (5). The information is being currently updated with new data and most probably, the species number will increase in most floristic regions. Still, there are many taxonomic uncertainties requiring further investigations that could result either in change of species number, or in change of status of some taxa. Field studies have shown that the species of genus *Thymus* occur in many different habitat types, ranging from sea shores, through forest and grassland habitats to the alpine zone and rock fissures and screes.

Key words: *Thymus*, medicinal plant, systematics, natural distribution.

Introduction

Genus *Thymus* includes about 215-220 species distributed across almost everywhere in Eurasia, including southern Greenland, and in part of Africa (MORALES, 2002). All species are highly valued medicinal and aromatic plants and are used by people since the ancient times. They are small shrubs, subshrubs or long-living perennial herbaceous

plants, with strong fragrance. The species of genus *Thymus* possess diverse biological activities and have, therefore, a significant potential for application in the pharmaceutical, cosmetic and food industries (JARIĆ *et al.*, 2015; LEAL *et al.*, 2017). They have been used traditionally to treat diseases of the respiratory and digestive system, as well as of colds (ZARZUELO & CRESPO, 2002), but also just as herbal tea and as spices.

Surprisingly, *Thymus* species in Bulgaria have received only occasional attention by experts. This concerns their distribution, systematics, and the aspects of their use as medicinal and aromatic plants.

Therefore, the objective of the present mini-review was to make a survey on the distribution, diversity, systematics, and some aspect of phytochemical properties of the genus *Thymus* in Bulgaria, and to identify the existing knowledge gaps and necessity of further studies.

Systematics and diversity

According to HARLLEY *et al.* (2004), genus Thymus belongs to the monophyletic group of subfamily *Nepetoideae* Kostel., tribe *Mentheae* Dumort., subtribe *Menthinae* Endl. JALAS (1971, 1972) classified the European species into eight sections based on their morphology. Of Bulgarian species, twelve belong to the section *Serpyllum* (Miller) Bentham, and eight – to the section Hyphodromi (A. Kerner) Halacsy.

The genus is characterized by high degree of hybridization, which was observed between species belonging to different sections, and also between species with different ploidy levels (MORALES, 2002; KARACA *et al.*, 2015), which causes additional difficulties to the taxonomists.

The taxonomic concept of the genus *Thymus* has undergone certain development in the course of years. Therefore, the species number and composition changed considerably and the issue still causes a lot of controversies and discussions among taxonomists.

The history of exploring the genus in Bulgaria reflects the complexities and controversies related to the status of different taxa. VELENOVSKY (1891) in his *Flora Bulgarica* listed 11 species for the territory of Bulgaria, and added five new species in the *Supplementum* (VELENOVSKY, 1898), thus increasing their number to 16. He described also four species new to science: *T. vandasii*, *T. thracicus*, *T. stribrnyi* and *T. carnosulus*, of which the first two species still keep their taxonomic rank, while the other two have lost taxonomic significance. Six other species listed by VELENOVSKY (1891, 1898) are not a part of the contemporary Bulgarian flora, being not confirmed later, or given status of synonyms, or lost their taxonomic significance. Velenovsky was especially interested in *Thymus* and wrote a prologue to a monographic essay defining and describing some sections and subsections, many of which still stand today. He introduced using the orientation of sterile and flowering stems as important trait in the species classification and identification (VELENOVSKY, 1903, 1906).

First Bulgarian floras (STOJANOFF & STEFANOFF, 1925, 1933, 1948; STOYANOV *et al.* 1967) illustrate the development of the taxonomic concept on the genus, with numerous species coming in and going out, and finally MARKOVA (1989) provided the (almost) final treatment in the multivolume edition of Flora of the Republic of Bulgaria. The opus of MARKOVA (1989) benefited by the appearance of Flora Europaea (JALAS, 1972), and by the expert advice of J. Jalas. Therefore, it is considered the most comprehensive essay on the genus in Bulgaria to date, with 19 "good" species and two interspecific hybrids included, and with critical reassessment of hundreds of other herbarium specimens, diagnoses and published taxa. Since then, one more species was added to the list (*T. bracteosus*; PAVLOVA *et al.*, 1998) thus rounding the species number to 20, which still stands today (ASSYOV & PETROVA, 2012).

However, the status of many taxa still cannot be considered the final one. Both MARKOVA (1989) and JALAS (1972) pointed

out that their taxonomic schemes represent open pictures, which can be improved with the accumulation of knowledge and information of further studies. The treatment of MARKOVA (1989) followed the classical approach and was based exclusively on morphological and anatomical traits, combined with chromosome numbers. Recent development and application of molecular techniques to taxonomic problems greatly enhanced the studies on systematics of difficult and complex genera, like *Thymus* (FEDERICI *et al.*, 2013). The results of the contemporary studies were incorporated in some taxonomic databases (e.g. EuroPlusMed (2018) and The Plant List, theplantlist.org) and therefore, the number of existing differences and mismatch between the classical floras and new databases increased. For example, there are five species presented in MARKOVA (1989) that are missing, or listed as synonyms, in the electronic database of "EuroPlusMed", and seven species listed in "EuroPlusMed" are missing or are without a species status in MARKOVA (1989). This fact indicates that there are still numerous open taxonomic questions and the application of modern molecular markers could help to better understanding of taxonomic mysteries. The existing scheme of MARKOVA (1989) can be critically interpreted only after detailed and extensive studies including a set of classical and modern approaches. FEDERICI *et al.* (2013) pointed out that neither class of markers should be neglected. They recommended to use different markers in the taxonomic studies of *Thymus*, both morphological and molecular, in order to delineate the natural units at species level. Our preliminary field studies revealed that some taxonomic and/or nomenclatural changes are inevitable.

Species diversity in Bulgaria is among the highest in Europe. A search in the "EuroPlusMed" database revealed that the richest country is Turkey with 40 species, but only 10 of them are in the European part of the country. Therefore, the country with highest *Thymus* species diversity is Spain with 35 species, followed by Greece with 26 species, Russia (European part) with 20 species, and Bulgaria with 18 species recognized by the database. A special case is former Yugoslavia with 25 species, but if the countries existing today are considered separately, their species diversity is lower than that of Bulgaria. Number of *Thymus* species in Bulgaria is higher than in countries with considerably larger territories, like, for example, France, Italy and Germany.

Within-species diversity is measured usually by intraspecific taxa and by genetic diversity. Due to the very dynamic nature of taxonomic concept, the number of intraspecific taxa cannot be used as reliable indicator of diversity, especially considering the fact that not all species have been studied to equal extent. Of Bulgarian species, one has two subspecies (*T. callieri*) and six are represented by two varieties (*T. moesiacus, T. pannonicus, T. sibthorpii, T. striatus, T. thracicus* and *T. zygioides*).

The studies on genetic diversity concern mostly species from Western Europe and occasionally, from other regions. Such studies could be classified conditionally into two groups: studies of genetic patterns and processes within the species and studies related to taxonomy of this complex and polymorphic genus (THOMPSON, 2002).

The first group includes diverse topics related to evolution of species, sex allocation and mating systems (BELHASSEN *et al.*, 1991, 1993), and also the distribution of genetic diversity (RUSTAIEE *et al.*, 2013). Recent studies addressed more specific questions related to the evolution under climate change (THOMPSON *et al.*, 2013).

The second direction of studies focuses mostly to the interspecific relationships within the genus and is not explicitly related to taxonomic decisions. In a study done in a region close to Bulgaria, SOSTARIĆ *et al.* (2012) established the genetic similarity among seven species growing naturally in Serbia by using AFLP genetic markers.

SONBOLI *et al.* (2013) verified the taxonomic status of *Thymus persicus* by means of molecular markers. MOLINS *et al.* (2011) performed a phylogeographic study of *Thymus herba-barona* in relation to the species evolution. More directly related to the taxonomy are the studies of KARACA *et al.* (2015), who succeeded to distinguish among the studied species and FEDERICI *et al.* (2013) whose results were controversial – only *Thymus capitatus* could be clearly distinguished from the remaining species.

In Bulgaria genetic studies on representatives of genus *Thymus* are practically lacking. Therefore, the studies planned in ANEVA *et al.* (2018) could elucidate many issues related to taxonomy and genetic diversity at species and population level.

Natural distribution and phytogeographic origin

Distribution of the species in Bulgaria was assessed following ASSYOV & PETROVA (2012), with considering the numerous floristic novelties published since (DIMITROV & VUTOV, 2014; PETROVA & VASSILEV, 2016; VASSILEV, 2013a, b; 2016a, b; FILIPOVA & VASSILEV, 2015). The species of genus *Thymus* occurring naturally in Bulgaria differ in their mode of distribution. The classification of floristic regions developed especially for the Flora of the Republic of Bulgaria (JORDANOV, 1963) was used as starting point. The analysis revealed that 3 species occur in all 20 floristic regions of Bulgaria, 3 species occur in 15 to 17 floristic regions and so on, while the opposite case is represented by two species, which can be found in one floristic region only (Fig. 1). There is one species endemic to Bulgaria and particularly – to Pirin Mts. (*T. perinicus*), and three species are endemics to Balkan Peninsula.

The richest floristic region– Rhodopes – contains 16 of all 20 species (80%), followed by Pirin (15 species, 75%), Western Frontier Mountains (12, 60%), and the Danubian plain is inhabited by only 5 species (25%) (Fig. 2). However, when commenting the species richness of the different floristic regions we should always consider the probability that higher richness could be due to higher number of studies performed in the respective regions.

The distribution is related to the phytogeographic origin of the species. Besides the four endemic species (one Bulgarian and three Balkan), there are also other three species with Balkan component of origin (Balkan-Anatolian, or Balkan-Dacian), and 9 species with Mediterranean component of origin – four Mediterranean, four sub-Mediterranean and one Euro-Mediterranean element. The other elements include three European and one Pontic element. The phytogeographic origin reflects a wide spectrum with predominance of the elements with Mediterranean component. Balkan biota is affected by different biogeographic influences – Mediterranean, oriental, continental, steppe – and this is one of the factors shaping the plant diversity here, including that of genus *Thymus*. The species occur in all altitudinal zones of Bulgaria. Four species occur up to 500 (700) m above sea level, 3 species – up to 1200 m, 5 species – up to 2000 m, and 8 species reach the zone above 2000 m a.s.l. (4 of them up to 2900 m). It means that genus *Thymus* occurs across a broad range of habitats from the sea level up to the alpine zone. Many species are part of the natural habitats of high conservation value (see BISERKOV, 2015).

Phytochemical studies and biological activity

The complexity of genus *Thymus* and difficult identification of the species result in lacking of phytochemical studies at species level. Usually samples of different species are bulked together for the purposes of the pharmaceutical industry, and they are designated as *Thymus* sp., which approach does not take into account the peculiarities in the phytochemical composition of the different species.

The medicinal activity of the species of genus *Thymus* is due to a large extent to the chemical constituents in the essential oils. Therefore, major part of the studies focused on the determining of chemical composition of the species (SÁEZ & STAHL-BISKUP, 2002; JUKIĆ & MILOŠ, 2005; CAVAR *et al.*, 2009; RĂDULESCU *et al.*, 2009; VIDIĆ *et al.*, 2010).

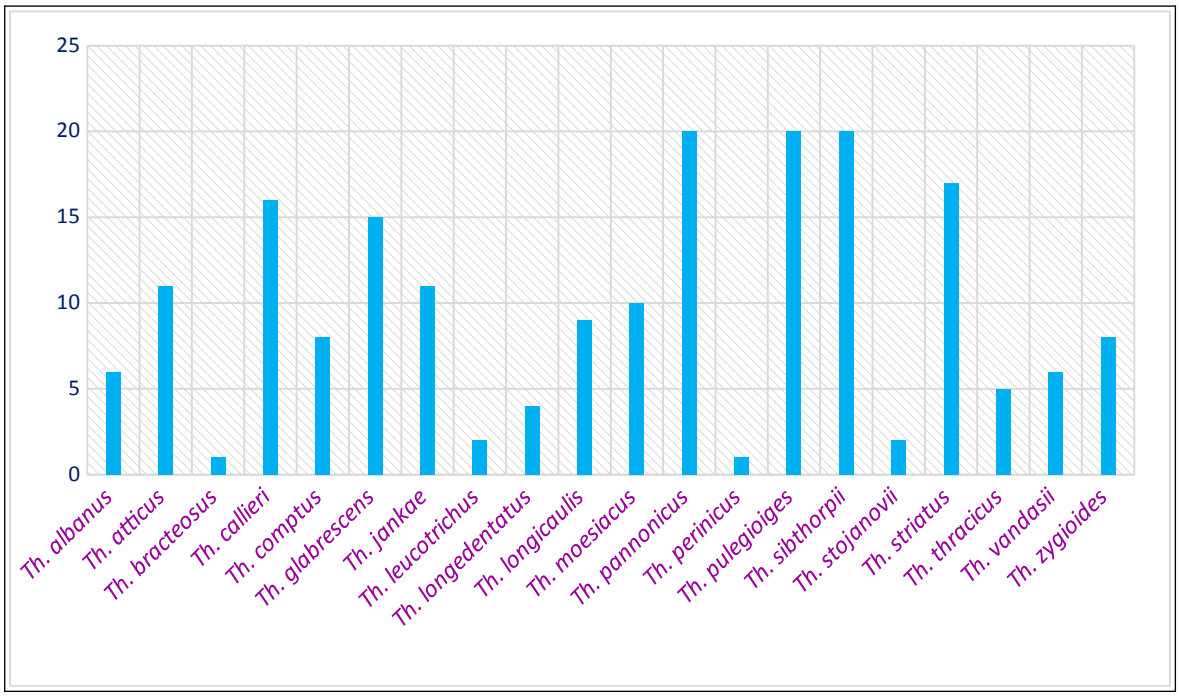

Fig. 1. Number of floristic regions in Bulgaria, where the different *Thymus* species occur.

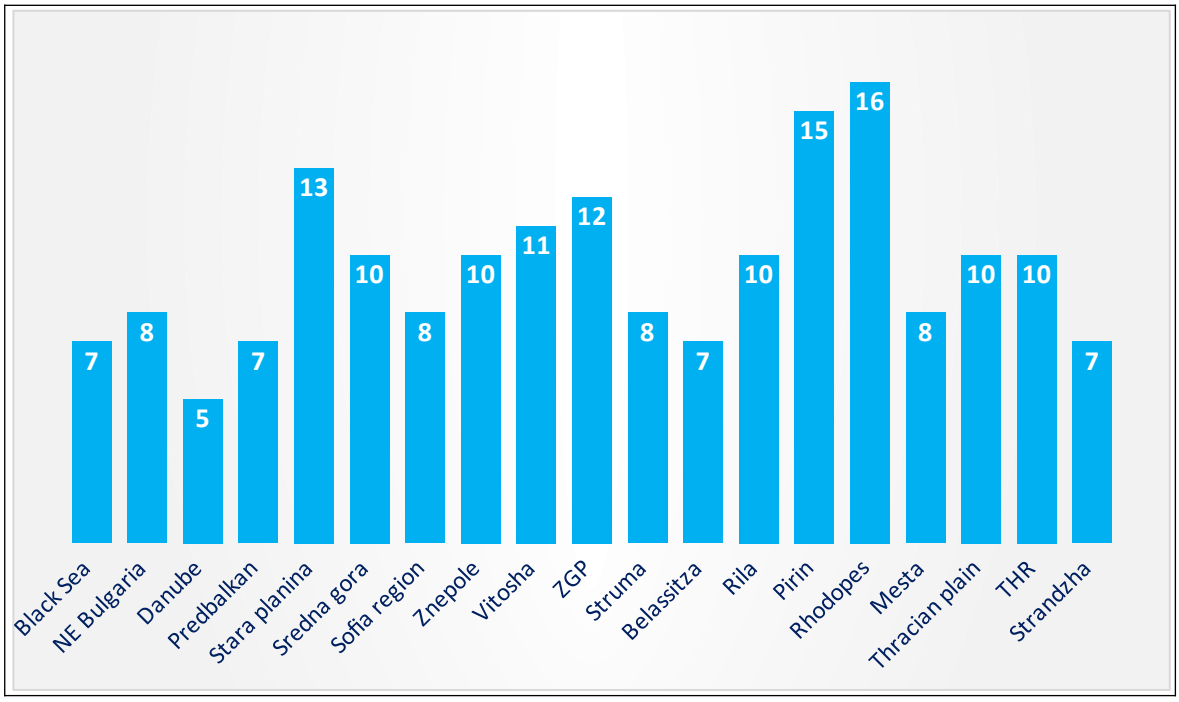

Fig. 2. Number of *Thymus* species recorded in each of the 20 floristic regions in Bulgaria.

A very typical phenomenon for the genus is the chemical polymorphism, which is due both to the ecological factors, and to genetic variation (LOŽIENĖ & VENSKUTONIS, 2005; RĂDULESCU *et al.*, 2009). Many of the species have more than 7 chemotypes (STAHL-BISKUP, 2002).

Different chemotypes were determined based on the dominant monoterpenes in the essential oils – geraniol, thymol, terpineol, linalool, carvacrol (THOMPSON *et al.*, 2003; CHIZZOLA *et al.*, 2008). As a rule, the sesquiterpenes are represented by low concentration in the species of the genus *Thymus* (STAHL-BISKUP, 2002), but several sesquiterpenoid chemotypes were detected in representatives of the genus, growing on the Balkan Peninsula (PAVEL *et al.*, 2009). Large part of the studies aims at determining of antioxidant activity of the species having different chemotypes (BOUNATIROU *et al.*, 2007; MIGUEL *et al.*, 2007; STOILOVA *et al.*, 2008; GRIGORE *et al.*, 2010; TEPE *et al.*, 2011). A correlation was established between the phenolic content and antioxidant activity of extracts with different polarity (MILIAUSKAS *et al.*, 2004; KISELOVA *et al.*, 2006).

As a whole, the essential oil composition of the species of genus *Thymus* in Bulgaria is poorly studied, or not studied at all. In the literature (IVANCHEVA & STANTCHEVA, 2000; NEDELCHEVA *et al.*, 2010) very frequently is used the very general name *Thymus* spp. diversa, and this fact confirms the necessity of a profound study and precise identification of the species. In the review paper on the species of genus *Thymus* on Balkans by ŽELJKOVIĆ & MAKSIMOVIĆ (2014) there are no data about the species from Bulgaria, with the exception of one note on *Thymus tosevii* and *Thymus macedonicus* (KULEVANOVA *et al.*, 2000). High bactericidal activity was recorded of different polarity extracts of *Thymus vulgaris* L., (DAMIANOVA *et al.*, 2008). A study on the phenol composition and the antioxidant activity of three *Thymus* cultivars, grown in Bulgaria, showed high potential for their use in the cosmetic and pharmaceutical industry (NIKOLOVA *et al.*, 2012).

The survey on genus *Thymus* in Bulgaria revealed that it poses a challenge to researchers of different, even though related domains, like plant systematics, nature conservation, phytochemisty, pharmacognosy. Bulgaria is among the countries with the highest species diversity and many issues related to *Thymus* species, are still not studied in detail. The cooperation among researchers from the above-mentioned domains, and from some other related disciplines could be of mutual interest and benefit.

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