

## Morphometric and chemical evaluation of *Solenopsora carpatica* (Catillariaceae)

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Guttová A., Bačkor M., Marhold K. & Slezáková V. 2006. Morphometric and chemical evaluation of *Solenopsora carpatica* (Catillariaceae). In: Lackovičová A., Guttová A., Lisická E. & Lizoň P. (eds.), Central European lichens - diversity and threat, p. 85–96. Mycotaxon Ltd., Ithaca.

**Abstract** – The position of *Solenopsora carpatica* is discussed in terms of its relationships to *S. candicans* and *S. cesatii* var. *cesatii*. Specimens collected in the Western Carpathians and Mediterranean region were analyzed. Morphological/anatomical and chemical characters were measured or scored. For the Western Carpathian populations, climatic data were also used. Data matrices were analysed by multivariate methods (UPGMA, PCA). Based on the morphology/anatomy and chemistry, the three species studied are well differentiated. Differences in the climatic requirements of the Western Carpathian populations of *S. carpatica* and *S. candicans* were also elucidated.

**Key words** – the Western Carpathians, the Alps, lichens, taxonomy, nomenclature

### Introduction

The name *Solenopsora carpatica* Pišút et Vězda accommodates placodioid lichens segregated from the *S. cesatii* group on account of their medullary reaction (Pd+ yellow to orange), smaller spores and non-squamulose central part of the thallus (Vězda 1970). Previously it was known exclusively from its *locus classicus* and a remote site in Lombardy, Italy (Nimis 1993, 2003). Apart from that, a collection of a poorly developed thallus is known from the Jeseníky Mts., Czech Republic (Vězda & Liška 1999). Over the past years, however, several new localities were discovered in the Western Carpathians (Guttová 2000, Guttová & Palice 2004). Simultaneously, several localities of *S. candicans*, frequent in the submediterranean-subatlantic, yet noteworthy in a Central European context, were discovered in Slovakia (Guttová 2000, Lackovičová & Guttová 2005). An isotype of *S. carpatica* (no L490, Vězda: Lich. Sel. Exs. No 896) was revised by Kotlov in 2003 and annotated as *S. cesatii*

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([www.binran.spb.ru/infys/li\\_types/s.htm](http://www.binran.spb.ru/infys/li_types/s.htm)). This information was published subsequently in a preliminary checklist of *Catillariaceae* (Kotlov 2004), but without supporting arguments.

All these facts challenged us to study the Central European representatives of *Solenopsora*, in order to investigate the status of *S. carpatica* with respect to *S. cesatii* var. *cesatii*, and to explore the potential distributional patterns of the species in the Western Carpathians.

### Materials and methods

Forty one thalli of the genus *Solenopsora*, collected in Bulgaria, Croatia, Italy, Slovakia and Turkey (BRA, SAV, GZU, herb. I. Pišút, herb. A. Vězda), were subjected to identification of their chemical constituents by both standard spot tests (Pd, K) and TLC (Culberson & Kristinsson 1970, Culberson 1972, Culberson & Johnson 1982). Each sample was analyzed in triplicate on TLC plates, using a minimum of three different solvent systems. Pure substances (e.g. atranorin, usnic acid) and microextracts of herbarium specimens with TLC-determined chemistry (didymic acid, fumarprotocetraric acid, lobaric acid, norstictic acid, pannarin, stictic acid and zeorin) were used as standards.

Morphometric analysis of five anatomical and morphological characters (mature ascus and mature spore sizes, hymenium height, diameter of mature apothecium and character of thallus surface) was performed. Hand-cut sections mounted in water and 10% KOH were examined. For the Western Carpathian specimens, climatic, drought and moisture indices according to the Atlas of Landscape of the Slovak Republic (Zaťko 2002) were used as well. In addition, hypothecium pigmentation, colour of the epihymenium and size of the apical cell of the paraphyses were recorded. Because they did not display significant differences, these characters were not included in the analysis. The number of observations was limited by the restricted number of specimens of *S. carpatica* accessible in collections. The thalli frequently bear juvenile apothecia, so 7-10 observations on asci, ascospores, hymenium and apothecium were made.

Two datasets were analyzed. The first comprised all 41 specimens using characters 1-7. The second dataset covered the 36 specimens of *S. carpatica* and *S. candicans* from the Western Carpathians and all the 10 characters, including additional region-specific climate characters 7-10 in order to find out potential climatic preferences. To ascertain the hierarchical structure within the datasets, a cluster analysis was carried out using UPGMA (unweighted pair-group method using arithmetic averages) as a clustering algorithm and Euclidean distance as a resemblance measure (SYN-TAX 2000, Podani 2001). The characters (represented by mean values

where applicable) were standardized to zero mean and unit standard deviation. To explore the variation among the species, a principal component analysis (PCA) was computed (SAS 8.2 package, SAS Institute 2000).

The species *Solenopsora olivacea* (Fr.) Kilias, reported from Hungary (Verseghy 1994, Bielczyk et al. 1994) was not included in the analyses, because the voucher specimen, kept in EGR, is not available (Kiss, in litt.).

### List of characters

1. Length to width ratio of a mature ascus; mean value of 7-10 measurements
2. Length to width ratio of a mature spore; mean value of 7-10 measurements
3. Hymenium height [µm]
4. Diameter of a mature apothecium [mm]
5. Thallus surface [0 – undulate, 1 – plain]
6. TLC  
[0 – main pannarin, zeorin, one unidentified spot (probably fatty acid), 1 – main atranorin, three minor unidentified substances]
7. Macrochemistry [0 – Pd+ yellow-orange red, K+ yellow, 1 – P+ yellow to orange or P-, K-]
8. Climate [0 – warm region, 1 moderately warm or cool region]
9. Index of drought [0 – 1.00–1.25; 1 – 0–0.75]
10. Moisture (surplus of precipitations in both the groups) [0 – 100 to 0.1; 1 – -200 to -800]

### List of specimens

#### *Solenopsora carpatica*

- [1] Slovakia, Muránska planina plateau: Muráň, massif of Cigánka Mt., SE slopes above the quarry, open limestone outcrops („Homôlky“), on limestone rock, alt. ca 700 m a. s. l., leg. A. Guttová, Z. Palice, 29. 10. 2001 (SAV)
- [2] Slovacia, Veľká Fatra Mts: ad saxa calcarea in monte Veľká skala, partes cacumin. (regio montis Sidorovo) supra opp. Ružomberok, loco apricoso, alt. ca 600 m a. s. l., leg. A. Guttová, I. Pišút 8. 1996 (SAV)
- [3] Slovacia, Veľká Fatra Mts: ad saxa calcarea in monte Veľká skala, partes cacumin. (regio montis Sidorovo) supra opp. Ružomberok, loco apricoso, alt. ca 600 m a. s. l., leg. A. Guttová, I. Pišút 8. 1996 (SAV)
- [4] Slovacia, Veľká Fatra Mts: ad saxa calcarea in monte Veľká skala, partes cacumin. (regio montis Sidorovo) supra opp. Ružomberok, loco apricoso, alt. ca 600 m a. s. l., leg. A. Guttová, I. Pišút 8. 1996 (SAV)
- [5] Slovacia, Veľká Fatra Mts: ad saxa calcarea in monte Veľká skala, partes cacumin. (regio

- montis Sidorovo) supra opp. Ružomberok, loco apricoso, alt. ca 600 m a. s. l., leg. A. Guttová, I. Pišút 8. 1996 (SAV)
- [13] Slovakia, Muránska planina plateau: Muráň, Cigánka Mt., S slope, a well-lit deciduous forest with open limestone outcrops, on vertical to overhanging side of a limestone boulder, alt. 605 m a. s. l., 29. 10. 2001 leg. A. Guttová, Z. Palice N 48°45'05", E 20°03'09" (herb. Z. Palice no 5708)
- [14] Slovakia, Muránska planina plateau: Muráň, Cigánka Mt., S slope, a well-lit deciduous forest with open limestone outcrops, on vertical to overhanging side of a limestone boulder, alt. 605 m a. s. l., 29. 10. 2001 leg. A. Guttová, Z. Palice N 48°45'05", E 20°03'09" (herb. Z. Palice no 5708)
- [15] Slovakia, Muránska planina plateau: Muráň, Cigánka Mt., S slope, a well-lit deciduous forest with open limestone outcrops, on vertical to overhanging side of a limestone boulder, alt. 605 m a. s. l., 29. 10. 2001 leg. A. Guttová, Z. Palice N 48°45'05", E 20°03'09" (herb. Z. Palice no 5708)
- [16] Slovakia, Muránska planina plateau: Muráň, Cigánka Mt., S slope, a well-lit deciduous forest with open limestone outcrops, on vertical to overhanging side of a limestone boulder, alt. 605 m a. s. l., 29. 10. 2001 leg. A. Guttová, Z. Palice N 48°45'05", E 20°03'09" (herb. Z. Palice no 5708)
- [17] Slovakia, Muránska planina plateau: Muráň, Cigánka Mt., S slope, a well-lit deciduous forest with open limestone outcrops, on vertical to overhanging side of a limestone boulder, alt. 605 m a. s. l., 29. 10. 2001 leg. A. Guttová, Z. Palice N 48°45'05", E 20°03'09" (SAV)
- [18] Slovakia, Muránska planina plateau: Muráň, Cigánka Mt., S slope, a well-lit deciduous forest with open limestone outcrops, on vertical to overhanging side of a limestone boulder, alt. 605 m a. s. l., 29. 10. 2001 leg. A. Guttová, Z. Palice N 48°45'05", E 20°03'09" (herb. Z. Palice no 5708)
- [19] Slovakia, Muránska planina plateau: Muráň, Cigánka Mt., S slope, a well-lit deciduous forest with open limestone outcrops, on vertical to overhanging side of a limestone boulder, alt. 605 m a. s. l., 29. 10. 2001 leg. A. Guttová, Z. Palice N 48°45'05", E 20°03'09" (herb. Z. Palice no 5725)
- [25] Slovacia, Veľká Fatra Mts: ad saxa calcarea in monte Veľká skala, partes cacumin. (regio montis Sidorovo) supra opp. Ružomberok, loco apricoso, alt. ca 600 m s. m., 8. 1996 leg. A. Guttová (SAV)
- [26] Slovacia, Veľká Fatra Mts: ad saxa calcarea in monte Veľká skala, partes cacumin. (regio montis Sidorovo) supra opp. Ružomberok, loco apricoso, alt. ca 600 m s. m., 8. 1996 leg. A. Guttová (SAV)
- [27] Slovacia, Veľká Fatra Mts: ad saxa calcarea in monte Veľká skala, partes cacumin. (regio montis Sidorovo) supra opp. Ružomberok, loco apricoso, alt. ca 600 m s. m., 8. 1996 leg. A. Guttová (SAV)
- [28] Slovacia, Veľká Fatra Mts: ad saxa calcarea in monte Veľká skala, partes cacumin. (regio montis Sidorovo) supra opp. Ružomberok, loco apricoso, alt. ca 600 m s. m., 8. 1996 leg. A. Guttová (SAV)
- [29] Slovacia, Veľká Fatra Mts: ad saxa calcarea in monte Veľká skala, partes cacumin. (regio montis Sidorovo) supra opp. Ružomberok, loco apricoso, alt. ca 600 m s. m., 8. 1996 leg. A. Guttová (SAV)

- [30] Isotypus – A. Vězda: Lich. Sel. Exs. no 896 – Bohemoslovacia – Carpati, montes Fatra Magna: in rupibus Veľká skala dictis, ad latera montis Sidorovo prope urbem Ružomberok, ad parietes rupium calcarearum, alt. 600 m s. m., 3. 10. 1968 leg. I. Pišút, A. Vězda (BM)
- [31] Isotypus – A. Vězda: Lich. Sel. Exs. no 896 – Bohemoslovacia – Carpati, montes Fatra Magna: in rupibus Veľká skala dictis, ad latera montis Sidorovo prope urbem Ružomberok, ad parietes rupium calcarearum, alt. 600 m s. m., 3. 10. 1968 leg. I. Pišút, A. Vězda (BM)
- [32] Italien, Bergamasker Alpen, Prov. Bergamo: Osthänge des Presolana – Massivs über dem Valle di Scalve bei La Sponda, Felsabbrüche, um 900–920 m, 31. 5. 1975 leg. J. Poelt (GZU)
- [33] Italien, Bergamasker Alpen, Prov. Bergamo: Osthänge des Presolana – Massivs über dem Valle di Scalve bei La Sponda, Felsabbrüche, um 900–920 m, 31. 5. 1975 leg. J. Poelt (GZU)
- [34] Italien, Bergamasker Alpen, Prov. Bergamo: Osthänge des Presolana – Massivs über dem Valle di Scalve bei La Sponda, Felsabbrüche, um 900–920 m, 31. 5. 1975 leg. J. Poelt (GZU)
- [41] Flora Slovaciae: Veľká Fatra: in decl. orient. montis Veľká skala (reg. M. Sidorovo) supra opp. Ružomberok, alt. 600 m, 31. 10. 1967 leg. I. Pišút (BRA, BRACR5557)

#### *Solenopsora candidans*

- [6] Italia, Insula Elba: ad lapid. Arenosas muri in pago S. Ilario (in vicinitate pag. Campo nell'Elba), alt. 200 m s. m., 12. 6. 1995 leg. I. Pišút (herb. I. Pišút)
- [7] Italia, Insula Elba: ad lapid. Arenosas muri in pago S. Ilario (in vicinitate pag. Campo nell'Elba), alt. 200 m s. m., 12. 6. 1995 leg. I. Pišút (herb. I. Pišút)
- [8] Italia, Insula Elba: ad lapid. Arenosas muri in pago S. Ilario (in vicinitate pag. Campo nell'Elba), alt. 200 m s. m., 12. 6. 1995 leg. I. Pišút (herb. I. Pišút)
- [9] Krypt. Exs. no 1364: Istria: ad saxa dolomitica murorum ad viam publicam inter Hosti et Sv. Matje, alt. 200-250 m s. m. (herb. I. Pišút)
- [10] Krypt. Exs. no 1364: Istria: ad saxa dolomitica murorum ad viam publicam inter Hosti et Sv. Matje, alt. 200-250 m s. m. (herb. I. Pišút)
- [11] Krypt. Exs. no 1364: Istria: ad saxa dolomitica murorum ad viam publicam inter Hosti et Sv. Matje, alt. 200-250 m s. m. (herb. I. Pišút)
- [12] Krypt. Exs. no 1364: Istria: ad saxa dolomitica murorum ad viam publicam inter Hosti et Sv. Matje, alt. 200-250 m s. m. (herb. I. Pišút)
- [20] Lichenes Bulgariae, distr. Burgas: ad saxa silice in litore maris inter pag. Mičurin et Varvara, alt. 10 m s. m., 24. 8. 1977 leg. I. Pišút (BRA)
- [21] Lichenes Bulgariae, distr. Burgas: ad saxa silice in litore maris inter pag. Mičurin et Varvara, alt. 10 m s. m., 24. 8. 1977 leg. I. Pišút (BRA)
- [22] Slovacia, Malé Karpaty Mts: pagus Sološnica, reservatum naturae Roštún, ad saxa conglomerata calcarea in summo collis Malá Vápenná (Malý Roštún) merid. orient. spectantia loco apricoso, alt. 573 m s. m., 22. 10. 1999 leg. A. Guttová (SAV)

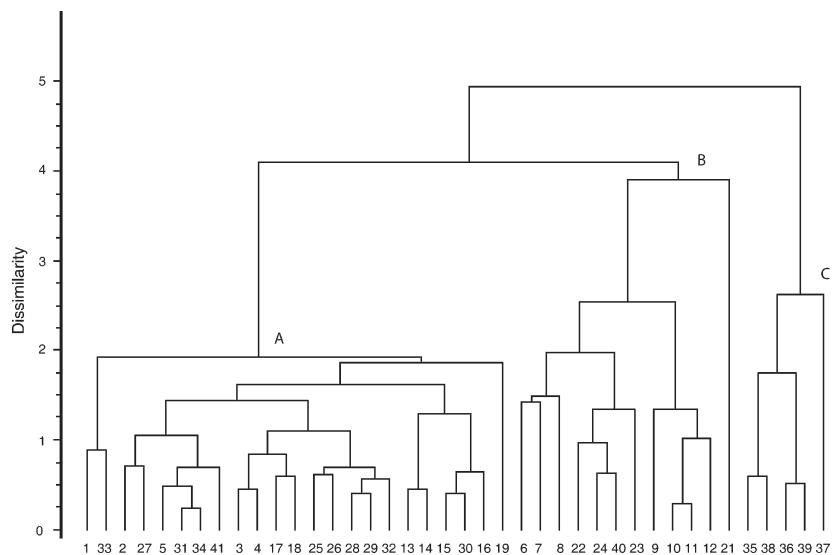
- [23] Slovacia, Malé Karpaty Mts: pagus Sološnica, reservatum naturae Roštún, ad saxa conglomerata calcarea in summo collis Malá Vápenná (Malý Roštún) merid. orient. spectantia loco apricoso, alt. 573 m s. m., 22. 10. 1999 leg. A. Guttová (SAV)
- [24] Slovacia, Malé Karpaty Mts: pagus Sološnica, reservatum naturae Roštún, ad saxa conglomerata calcarea in summo collis Malá Vápenná (Malý Roštún) merid. orient. spectantia loco apricoso, alt. 573 m s. m., 22. 10. 1999 leg. A. Guttová (SAV)
- [40] Slovakia, Strážovské vrchy, Trebichava, Kňaží stôl Mt. (elevation point 637.1), the summit, on dolomite rocks, alt. 630–637 m a.s.l., 20. 6. 2005 leg. A. Lackovičová & A. Guttová; N 48°48'26.9" E 18°17'57.5" (7175d) (SAV)

*Solenopsora cesatii* var. *cesatii*

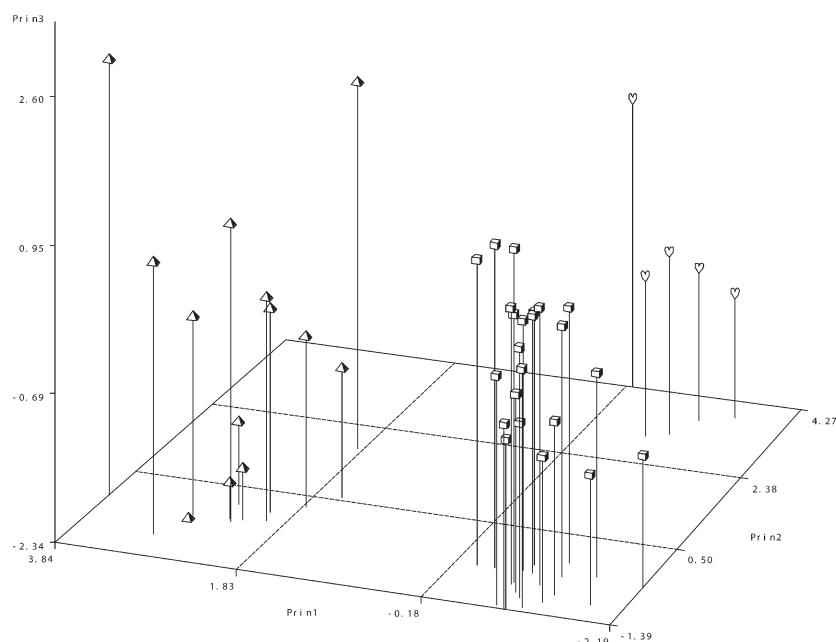
- [35] Lichenes Graeciae, Insula Hydra: ad saxa calcarea supra opp. Hydra, alt. ca 30 m s. m., 14. 9. 1994 leg. I. Pišút (SAV)
- [36] Dalmatia, ad saxa calcarea supra pag. Tučepi in vicinitate opp. Makarska, alt. ca 50 m s. m., 2.9.2002, leg. I. Pišút (BRA)
- [37] Turciae, Anatolia austrooccid., in decl. Merid. collis prope opp. Kusadasi, loco sub-umbroso, ad parietes rupium calcarearum, 100 m, 17.6.1969, leg. I. Pišút (BRA)
- [38] Turciae, Anatolia austrooccid., in decl. Merid. collis prope opp. Kusadasi, loco sub-umbroso, ad parietes rupium calcarearum, 100 m, 17.6.1969, leg. I. Pišút (BRA)
- [39] Dalmatia. In valleculo prope pagum Senj, alt. 50 m s. m. Ad saxa calcarea., 7.8.1969, leg. et det. A. Vězda (BRA)

## Results and discussion

Both cluster and ordination procedures clearly arranged the first dataset (41 specimens, 7 characters) into the same three groups, as is clear in UPGMA diagram. The first division level splits the samples into two clusters (Fig. 1). Cluster C represents the specimens of *S. cesatii* var. *cesatii*. The second one consists of two subclusters equivalent to the species *S. carpatica* (A) and *S. candicans* (B). PCA results also distinctly feature the same data structure, the three non-overlapping groups each representing the three species: *S. candicans*, *S. carpatica* and *S. cesatii* var. *cesatii* (Fig. 2, Tab. 2). The first axis accounts for 43% of variation. The characters significantly contributing to differentiation of the groups are: thallus surface, length to width ratio of a mature spore, macrochemistry and length to width ratio of a mature ascus. On this basis two groups can be distinguished – *S. candicans* and the group comprising *S. carpatica* and *S. cesatii* var. *cesatii*. The second axis, explaining 31% of variation is delimited by chemistry defined by TLC, diameter of mature apothecium, hymenium height and macrochemistry. Two groups are discriminated by the second axis too – the specimens of *S. cesatii* var. *cesatii* and a cluster covering *S. candicans* and *S. cesatii*. The third axis accounts for 14% of variation and is significantly correlated with length to width ratio of a mature ascus, diameter of mature apothecium, hymenium height and length to width ratio of a mature spore.



**Fig. 1.** Dendrogram of the unweighted pair-group method using arithmetic averages of *Solenopsora carpatica* (cluster A), *S. candicans* (cluster B) and *S. cesatii* var. *cesatii* (cluster C). For species codes see Material and methods.



**Fig. 2.** Ordination diagram of the principal component analysis of the three *Solenopsora* species. Cube – *S. carpatica*, pyramid – *S. candicans*, heart – *S. cesatii* var. *cesatii*. The first three components account for 43, 31 and 14 % of variation respectively.

**Tab. 1.** Eigenvectors of the principal component analysis (Prin 1, Prin 2, Prin 3) representing correlation of the characters of all the 41 specimens with the axes. Number of characters correspond to those given in Material and methods.

Character	Prin 1	Prin 2	Prin 3
1	0.408	0.063	0.506
2	0.445	-0.098	-0.565
3	0.320	-0.430	0.420
4	0.117	0.503	0.437
5	0.557	-0.003	-0.131
6	-0.128	0.620	-0.087
7	0.438	0.406	-0.180

**Tab. 2.** Descriptive quantitative data analysis of all the 41 specimens of *Solenopsora carpatica*, *S. candicans* and *S. cesatii* var. *cesatii*. For ascus and spore a length to width ratio is used.

Character	Species	Mean	Median	SD	Percentiles	
					10 (1)	90 (99)
Ascus	<i>carpatica</i>	3.91	3.9	0.59	3.16 (2.9)	4.8 (4.9)
	<i>candicans</i>	4.95	4.7	0.95	4.32 (4.3)	5.1 (7.6)
	<i>cesatii</i>	4.16	4.5	0.66	3.4 (3.3)	4.7 (4.8)
Spore	<i>carpatica</i>	2.15	2.1	0.29	1.9 (1.6)	2.5 (2.7)
	<i>candicans</i>	3.8	3.4	1.15	2.7 (2.1)	5.1 (5.9)
	<i>cesatii</i>	1.94	2	0.21	1.7 (1.6)	2.1 (2.1)
Hymenium height	<i>carpatica</i>	61.8	61.9	5.4	56.2 (49.2)	66.7 (71.4)
	<i>candicans</i>	70	64.3	12	59.5 (55.3)	83.3 (93.8)
	<i>cesatii</i>	39.86	39.1	4.7	35.7 (33.6)	44.4 (46.3)
Apothecium diameter	<i>carpatica</i>	0.86	0.8	0.13	0.7 (0.6)	1 (1)
	<i>candicans</i>	0.98	1	0.78	0.8 (0.8)	1 (1.4)
	<i>cesatii</i>	1.18	1.2	0.2	1 (1)	1.4 (1.5)

Specimens representing *S. carpatica* are always grouped within an isolated cluster. This species is defined by a thallus with undulate surface, spores (7.1–)7.7 – 10.7 (–10.7) × (3.1–)3.6 – 4.8 µm, asci (37.7–)38.4 – 49.5(–52.0) × (9.3–)9.5 – 13.2 (–14.1) µm (Tab. 2). It produces pannarin and zeorin as the main secondary metabolites plus one unidentified substance, confirmed by the K+ yellow and P+yellow-orange spot test reactions. The analysis of the second dataset (36 exclusively Western Carpathian collections, 10 characters) discriminated the specimens of *S. carpatica* well from those of *S. candicans* by climatic, drought and moisture indices. *S. carpatica* grows in moderately warm or cool regions with a drought index 0–0.75 and moisture -200 to -800.

Specimens of *S. candicans* are similarly well-delimited. This species is characterized by a thallus with more or less plane surface in comparison to that of *S. carpatica*), spores (9.8–)12.2 – 14.8 × (2.4–)2.4 – 4.8 µm, asci (41.1–)45.5 – 53.8–(56.2) × (7.4–)9.5 – 10.9(–11.2) µm (Tab. 2). The main secondary metabolites are pannarin and zeorin, and one unidentified substance, confirmed by the K- and P+orange spot test reaction. Western Carpathian populations are well discriminated by climatic, drought and moisture indices as was clear from the analysis of the second dataset. They grow in warm regions with a drought index 1.00–1.25 and moisture 100–0.1.

Specimens of *S. cesatii* var. *cesatii* are likewise well delimited. This species is defined by a thallus with an undulate surface, spores 6.2 – 10.3 × (2.1–)4.1 µm, asci (26.7) 28.5 – 37.3(–44.3) × 6.2 – 10.3 µm (Tab. 2). It produces atranorin as the main substance and 3 unidentified minor substances; spot test reactions with K and P are negative. As this taxon is not known from the Western Carpathians, climatic, drought and moisture indices were not applied here.

The characters studied clearly delimit the three taxa as independent entities, thus contradicting Kotlov's concept (Kotlov 2004). Morphological, anatomical, chemical and ecological characters are widely used and accepted in current taxonomical studies in the genus *Solenopsora* (e.g. Verdon & Rambold 1998, Kantvilas 2004). Future investigations are needed to acquire more comprehensive data over a greater geographical scale within the Western Carpathians, where the species are obviously very rare and at the periphery of their distributional optimum.

### Acknowledgements

We dedicate this paper to Ivan Pišút, whose work is a giant leap for lichenology mainly in Slovakia and the Czech Republic. AG and VS thank him for first insights into lichenology and AG also for the excursion to *locus classicus* of *S. carpatica*. The curators of relevant lichenological collections are thanked for their assistance: Walter Obermayer (Graz, GZU), Gabriella Kis (Eger, EGR); Ivan Pišút and Antonín

Vězda (Brno) for putting their private collections into our disposal. We are indebted to Tomáš Dražil (Spišská Nová Ves) and Drahoš Blanár (Revúca) for information on local climatic conditions. Zdeněk Palice (Praha), Josef P. Halda (Rychnov nad Kněžnou), Štěpánka Bayerová-Slavíková (Praha), David Svoboda (Praha) and Ondřej Peksa (Praha) participated in several collecting trips. Gintaras Kantvilas (Hobart) delivered invaluable comments and language improvements. AG is obliged to the support by VEGA grant agency, project no. 2/4035/26.

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