ISSN: 0583-4988 (printed version)
ISSN: 2545-4587 (on-line version)

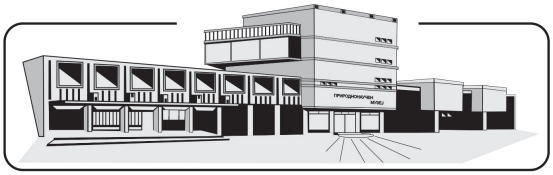
ИЗДАНИЕ НА ПРИРОДОНАУЧНИОТ МУЗЕЈ НА РЕПУБЛИКА СЕВЕРНА МАКЕДОНИЈА

BOTIM I MUZEUT TË SHKENCAVE DE NATYRËS E REPUBLIKËS SE MAQEDONISE SË VERIUT

ACTA

MUSEI MACEDONICI SCIENTIARUM NATURALIUM

Volume 26 2023 Issue 1



Скопје - Shkup - Skopje

Издавач:

Национална установа „Природонаучен музеј на Република Северна Македонија“

Ni “Muzeu i shkencave natyrore i republikes se Maqedonisë së veriut”

Издавањето на ова списание е целосно финансирано од Министерството за култура на Република Северна Македонија.

Botimi i kësaj reviste është tërësisht i finsnsuar nga Ministria e cultures së Maqedonisë së veruit.

Фотографии на насловната страна: *Centaurea rufidula* Bornm. (Фот.: З. Николов).

Печати: Европа 92, Кочани.

Тираж: 100 примероци.

Интернет страница на електронската верзија: acta.musmacscinat.mk

Адреса на издавачот:
Природонаучен музеј на
Република Северна Македонија
Булевар „Илинден“, 86
1000 Скопје

Adresa e botueist:
Muzeu i shkencave natyrore i
Republikes se Maqedonisë së veriut
Bulevardi “Ilinden”, 86
1000, Shkup

Publisher:
National Institution "Natural History Museum of North Macedonia"

The publication of this volume was fully supported by the Ministry of Culture of the Republic of North Macedonia.

Photos on the cover page: *Centaurea rufidula* Bornm. (Photo: Z. Nikolov).

Print: Evropa 92, Kočani

Copies: 100.

Web site of the electronic version: acta.musmacscinat.mk

Publisher's address:
Natural History Museum of the Republic of North Macedonia
Boulevard Ilinden, 86
1000 Skopje, Republic of North Macedonia

CONTENT

UDK: 582.998.1.063.6(497.7)
Received: 01.11.2023
Accepted: 02.11.2023
Original scientific paper

Acta Musei Macedonici Scientiarum Naturalium, 2023, Vol. 26, pp: 7-14
ISSN: 0583-4988 (printed version)
ISSN: 2545-4587 (on-line version)
Available on-line at: www.acta.musmacscinat.mk

Centaurea rufidula Bornm. (*Asteraceae*) in the flora of the Republic of North Macedonia

Zoran Nikolov

Natural History Museum of the Republic of North Macedonia, Blvd. Ilinden, 86, 1000, Skopje, Republic of North Macedonia.
E-mail: znikolov61@yahoo.com.mk

Abstract

The presence of *Centaurea rufidula* (*Asteraceae*), in the flora of North Macedonia, is confirmed. *C. rufidula* is a Macedonian endemic species described by Bornmüller in 1923, from the surrounding of Bogdanci (southern part of North Macedonia). Since then, no data of its occurrence in Macedonia was reported. In spite of Bornmuller's allegation (south of Bogdanci), the new locality is on the way, eastern of Bogdanci, toward the city Dojran. The specimens match the description of Bornmüller in all characteristics except the length of the pappus. However, in this case, this variation known also by other species is not of a bigger taxonomical significance. The only population of *C. rufidula,* that we have found, grows on arable or on abandoned arable land, in habitat with dominance of *Paliurus spina-christi*.

Key words: *Centaurea rufidula*, endemic species, confirmation, century-old data, North Macedonia.

Introduction

Centaurea rufidula Bornm. is a Macedonian endemic species, described by Bornmüller (1923), from the surrounding of the city Bogdanci. It belongs to the subsect. Cylindracea (Hayek) Dostal of the subgenus Acrolophus (Cass.) Dobrocz [Sect. Acrolophus (Cass.) DC] (Dostal, 1976). This subsect. includes species with small ivolucre and capitula like *C. tymphaea* Hausskn., *C. orphanidea* Heldr. & Sart., *C. diffusa* Lam., *C. bovina* Velen. and *C. aemulans* Klokov (Dostal, 1976). According to the literature data, the following species from this subsection, among the above-mentioned, are present in the flora of the Republic of North Macedonia: *C. tymphaea* (Dimitrov, 1908; Bornmüller, 1928), *C. orphanidea* (Matevski et al., 2008) and, *C. diffusa* (Dimitrov, 1908; Jurišić, 1923; Bornmüller, J., 1928; Soška, 1938-1939a,b, 1941; Rudski, 1943).

In Bornmüller's protolog, there are no habitat's characteristics but, in the works of Hayek (1930) and Dostal (1976), *C. rufidula* is associated with rocky places.

Materials and methods

During the field-researches (2019-2021), six individuals of one very small and restricted population, were collected. The samples were dried up and labeled, according to the commonly accepted procedures. The classification is made according to Dostal (1976). The main source, for the determination of the material, was Bornmüller's protolog (1923), then the works of Hayek (1930) and Dostal (1976). Voucher specimens are stored in the Herbarium of Natural History Museum under the following numbers: 14 581, 14 582, 14 583, 14 653, 14 654 and 15 164. Photos of habitat, general appearance of the plants in the locality as well as herbarized specimen, and illustration of the upper part of the plant are also given. Species distribution, including literature and new data from this study, is shown on a map of North Macedonia.

Results and discussion

Centaurea rufidula Bornm., Feddes Repert. 19: 103 (1923).

Macedonia centralis: "Doiransee-Gebiet, südlich von Bogdanci" (15-30.07.1916, Leg. cl. Burgeff; nr. 2158, 2160).

New locality

Bogdanci, the surroundings of the city, toward Doiran, 105 m a.s.l., 12.07.2019; Leg.: Z. Nikolov; Det.: Z. Nikolov/A. Teofilovski; 41°12'18.18"N, 22°35'58.15"E.

Description of the collected specimens

Biennial. Stem erect, 26-50 cm, in the lower and

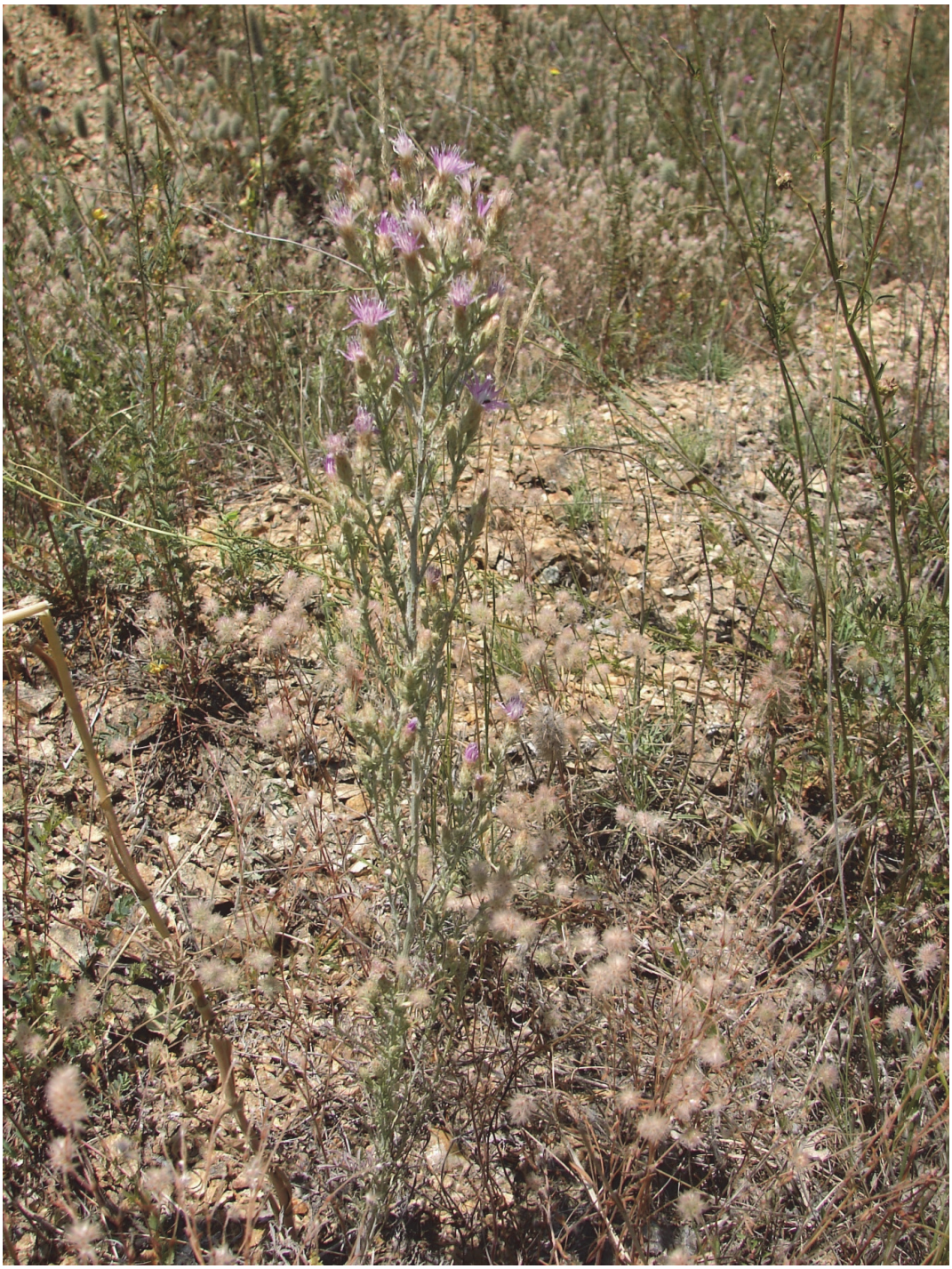


Fig. 1. *Centaurea rufidula* Bornm.
- Habitus

upper part fastigiated ("*fastigiato-ramoso"*), indumentum, including the leaves, arachnoid, giving gray appearance of the whole plant. Leaves, except the highest and on the branches, pinatisect, the basal (lower) petiolate, the upper (stem leaves) sessile. Capitula solitary, small, oblong-cylindrical, (10)11-14 mm long (involucre and flowers), 3-4(5) mm wide. The bracts pale, both with appendages glandular-puberulent, appendages 4-6 pairs, reddish-brown ("*rufidulo-coloratis*"), the apical spine longer than the last laterals, erect or a slightly patent. Flowers pale-pink, indistinctly radiant. Achenes small, c. 2 mm, covered with dense glands, the pappus variable in length, usually much shorter, c. 0.5 mm, rarely up to 1 mm or only exceptionally longer than 1 mm.

Distribution

During the field researches, in the surrounding of the city Bogdanci, a very small and restricted population of *C. rufidula* was found (Fig. 1; 3, a; 4, b; 8, 9). It was the first finding, after Burgeff's, in 1916. Later Bornmüller (1923), on the base of this Burgeff's material, described the new species. Bornmüller pointed that the plants were collected "südlich von Bogdanci" (south of Bogdanci). However, the new locality is situated eastern of Bogdanci, towards the city of Dojran. No other population, despite thorough research of the entire area of Bogdanci, has been found and for now, it is the only one of this species.

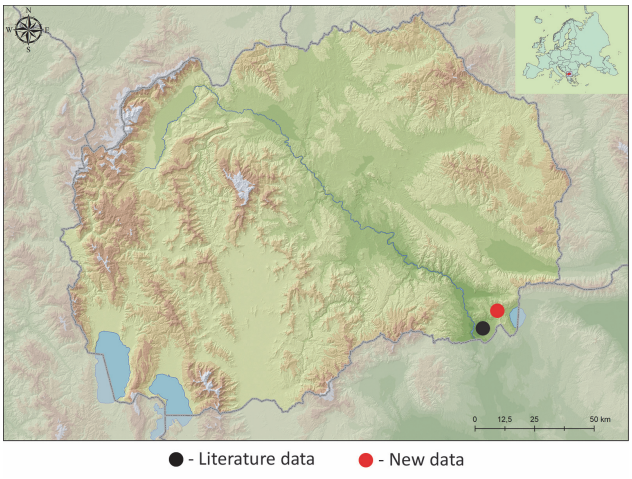


Fig. 2 Distribution of *Centaurea rufidula* Bornm. in North Macedonia

Habitat

C. rufidula grows on active (Fig. 3, a) and abandoned arable lands (Fig. 3 b), in an area with dominance of *Paliurus spina-christi* Mill, but also *Quercus pubescens* Willd., *Pistacia terebinthus* L. and *Carpinus orientalis* Mill. Bornmueller (1923) also reported *Centaurea diffusa* L. β *brevispina* Boiss. from the same location (südlich von Bogdanci), together with *C. rufidula*. We were not able to confirm the presence of *C. diffusa* but, we encountered another *Centaurea* species that unfortunately was not identified (Fig. 4, a).

Main characteristics of *Centaurea rufidula*

The main characteristics of *C. rufidula* that make a clear morphological distinction from the closest *Centaurea* species (subsect. Cylindracea) are, first of all the "fastigato-ramosus" appearance and gray indumentum as a result of the arachnoid stem and leaves pubescence (Fig. 1, 5, a,b; 9), then the solitary, small and ovoid-cylindrical capitula (Fig. 6, a,b), the reddish-brown (rufidulo-coloratis) appendices (Fig. 6, a,b) and

Fig. 3. Habitats of *Centaurea rufidula* Bornm.:
a) Active arable land with *Diospyros kaki* b) Abandoned arable land

Fig. 4. a) *Centaurea* sp. b) *Centaurea rufidula* Bornm., withered

the small, dense-glandular achenaes with, preferably, short pappus, up to 0.5 mm, rarely longer, up to 1 mm (Fig. 7, a,b). Exceptionally, the achenes can be longer than 1 mm (Fig. 7, b). In addition, the flowers are pale-pink, not or sometimes partially radial (Fig. 6, a,b).

In compare to the Bornmüller's description (1923), the variable length of the achene's pappus is the only difference. But, considering the fact that Bornmüller had only two samples (No. 2158, 2160) collected by Burgeff and, consequently, the limited amount of achenes, allowed us to reveal this pappus length variability (known also in other species like *C. deusta* Ten., *C. deustiformis* Adamović, *C. leucomala* Bornm.) as usual and acceptable, in the frame of the species, with no major taxonomical significance.

Conclusion:

- This is the first confirmation of the presence of *C. rufidula*, on the territory of North Macedonia, after Bornmüller's publication in 1923;

- The recent population, found on only one location in the surrounding of Bogdanci, is very small and restricted to an area of no more than 1000 m^2;

- The plants completely match the description of Bornmüller (1923), except for the variation in the pappus length. But, this difference, noticed also in other *Centaurea* species, has no large taxonomical significance in this case.

Dedication

This paper is dedicated to the passed professor and great botanist Ljupčo Melovski who initiated but, regrettably, didn't finish the project "Working together for Conservation of National Endemic Plants in Macedonia" (2019-2023, phase I), focusing on *C. rufidula* and four other species: Astragalus *physocallyx* Fisch., *Campanula debarensis* Rech. f., *Crocus jablanicensis* Randj. & V. Randj. and *Aesculus hippocastanum* L.

Acknowledgement

I am deeply in debt to the botanist Aco Teofilovski for his immense contribution, in every phase of the realization of the Project "Working together for Conservation of National Endemic Plants in Macedonia" (2019-2023, phase I), implemented by the Macedonian Ecological Society and financially supported by CEPF. Also, I am grateful to the reviewers

Fig. 5. *Centaurea rufidula* Bornm.
a) Inflorescence b) Middle part of the stem, with leaves

Fig. 6. *Centaurea rufidula* Bornm. - capitulum
a) No radial flowers b) Partially radial flowers

Fig. 7. *Centaurea rufidula* Bornm.
a, b) Different lengths of the achene's pappus

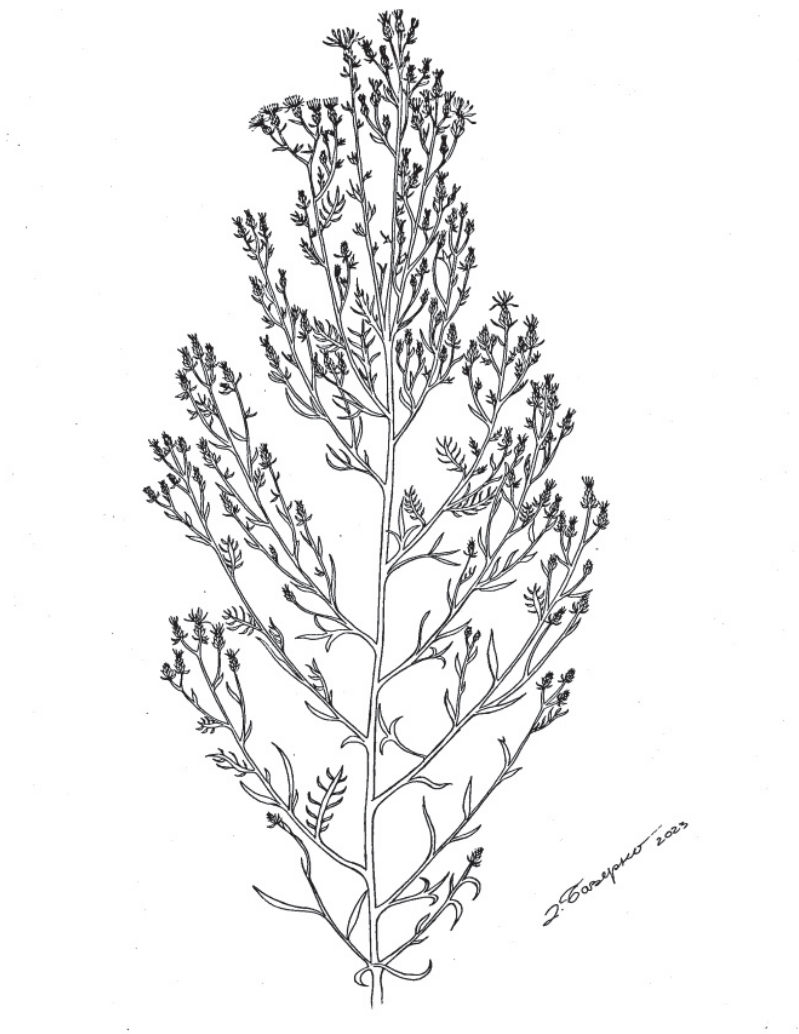


Fig. 8. *Centaurea rufidula* Bornm.
- The upper, branched part of the plant, with inflorescences
(Ilustration, Darko Bazerko)

Vlado Matevski and Bojan Zlatković for all their remarks and suggestions in order to improve the manuscript. Many thanks to Natalija Melovska and Svetlana Pejović (Macedonian Ecological Society), for all their help during the work on this project, than to Darko Bazerko for the remarkable illustration of *C. rufidula* and Nataša Stefanovska, for the scan of the achenes.

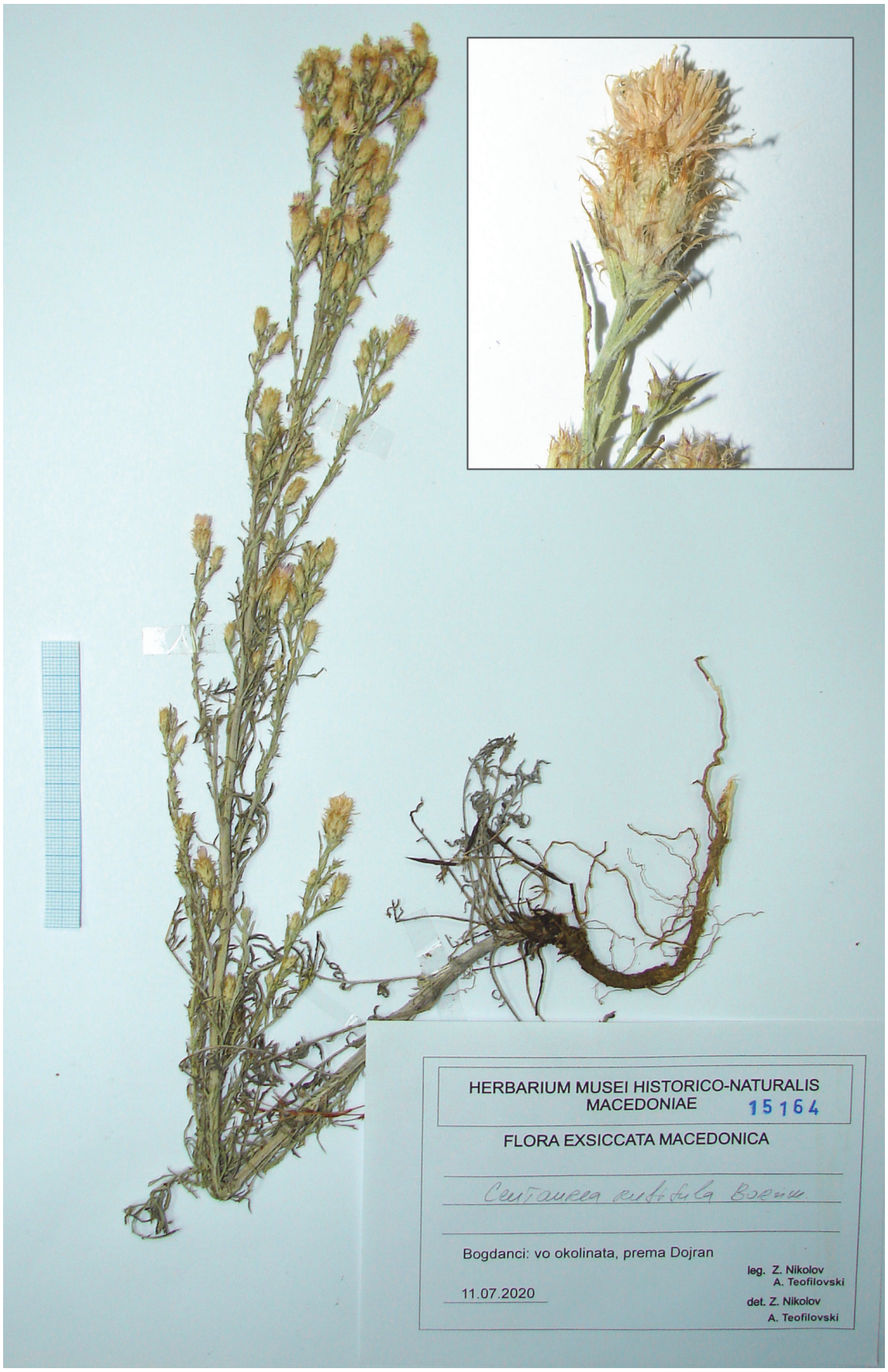


Fig. 9. *Centaurea rufidula* Bornm.
- The herbarium's specimen (photo)

References

Bornmüller, J., 1923: Zur Gattung *Centaurea* der mazedonischen und kleinasiatischen Flora. Repertorium Specierum Novarum Regni Vegetabilis, 19:103. Berlin-Dahlem.

Bornmüller, J., 1926: Beiträge zur Flora Mazedoniens, II. Engl.Bot.Jahrb., 60:110-11. Leipzig.

Bornmüller, J., 1928: Bearbeitung der von H. Burgeff und Th. Herzog in den Kriegsjahren 1916/18 in Macedonien gesammelten Pflanzen, II. – Allgemeine Botanische Zeitschrift für Systematik, Floristik, Pflanzengeographie etc. 33:37.

Bornmüller, J., 1937: Zur Flora Mazedonien. Fedde Rep., 42:137 (49).

Dimitrov, A., 1908: Materijali po florata na Bitolskata okolnost. Periodičesko spisanie na Bugarskoto kniževno društvo, 69(1-2):693-748. Bugarsko kniževno društvo, Sofija. Plovdiv.

Dostal, J, 1976. *Centaurea*. In: Tutin, T.G. et al. Flora Europaea, IV (Eds.). Cambridge Universaty Press.

Hayek, A., (1930): Prodromus florae peninsulae Balcanicae. Vol. 2: Dicotyledoneae Sympetalae. Repertorium Specierum Novarum Regni Vegetabilis. Beihefte 30(2): 97–336.

Matevski, V. & al., 2008: Flora and Vegetation of the Macedonian Steppe. Ljubljana.

Micevski, K., 1971: “Steppenvegetazion" in Mazedonien. Jahresausgabe, 23:151-150. Botanisches Institut, Naturwiss. Mathem. Fakultät, Skopje.

Rudski, I., 1943: Prilog za poznavanje flore okoline Strumice. Ohridski Zbornik, 2(86):205-238. Srpska Kraljevska Akademija, Posebna izdanja. Beograd.

Soška, Th., 1938-1939a: Beitrag zur Kenntnis der Schluchten-floren von Südserbien, II Teil. BSS Sk., 20 (7):35-58.

Soška, Th., 1938-1939b: Beitrag zur Kenntnis der Schluchten-floren von Südserbien, III Teil. BSS Sk., 20(7):167-191.

Soška, Th., 1941: Beitrag zur Kenntnis der Schluchten-floren von Südserbien, IV Teil. BSS Sk., 22(8):169-184.

UDK: 595.76-19(497.734:23)
Received: 23.03.2023
Accepted: 20.09.2023
Original scientific paper

Acta Musei Macedonici Scientiarum Naturalium, 2023, Vol. 26, pp: 15-20
ISSN: 0583-4988 (printed version)
ISSN: 2545-4587 (on-line version)
Available on-line at: www.acta.musmacscinat.mk

Ground-beetles (Coleoptera) diversity on Maleševo mountains (North Macedonia)

Slavčo Hristovski*, Aleksandra Cvetkovska-Gjorgjievska

Institute of Biology, Faculty of Natural Sciences and Mathematics, Ss. Cyril and Methodius University, Arhimedova 5, 1000 Skopje, Republic of North Macedonia
* slavco_h@pmf.ukim.mk

Abstract
The paper provides first data on the ground-beetle fauna of the Macedonian part of Maleševo Mts. (eastern parts of North Macedonia) collected as a result of a long-term intensive research. The material was collected by hand searching and by pitfall traps in the period of 2010-2019, in different habitat types.
In total 685 specimens belonging to 74 species from 32 genera were registered. Among them, 7 Balkan endemic, 4 subendemic, 7 rare and 1 Corine species were registered, emphasizing conservational importance of Maleševo Mts. in North Macedonia.
The obtained results supplement the knowledge about the distribution of ground-beetles on the Macedonian side of Maleševo Mountains and emphasize the conservational importance of the mountain as well.

Key words: Carabidae, faunistic data, country records, Republic of North Macedonia.

INTRODUCTION

The recognition of the importance of ground-beetles in the biodiversity protection contributed to increased faunistic research. But, despite the significant role that beetles play in ecosystems, they were poorly studied in the past, especially this is the case for the beetle diversity of Maleševo Mts. (Vlaina, Bukovik and Maleševski Planini) which are recognized as protected area on a national and international level.

The review of published literature revealed only 8 species for the Macedonian part of Maleševo Mountains (Hieke and Wrase 1988; Brajković et al. 2004; Chehlarov et al. 2016; Hristovski and Guéorguiev 2015).

Fortunately, the authors' research on Maleševo Mts. over the past years yielded a sizable amount of previously unreported faunistic data of ground-beetles which are summarized in this paper and give more comprehensive review of the present level of faunistic knowledge in this region.

STUDY AREA

The study area is situated in the eastern border mountains with Bulgaria, and includes three mountains: parts of Vlaina mountain (Kadiica, 1932 m) in the north, parts of Maleševski Planini (Džami Tepe, 1803 m) in the southeast, and the small (volcanic) mountain Bukovik (Orlovec, 1723 m) in the west which is usually considered as a part of Vlaina (Melovski et al. 2013). The area to the west is confined by Berovo valley and to the north by Delčevo valley. Maleševski Planini are medium-high mountains occupying the southeastern part of the Maleševo Region, while Bukovik is a small mountain elevation on the western side of Vlaina Mts. The study area (Fig. 1) covers surface of 10.14 km^2 on Bukovik and 5.01 km^2 on Vlaina – the whole area of Vlaina is actually 163.11 km^2 (Melovski et al. 2013). The study area on Maleševski Planini covers surface of 158.16 km^2 out its total surface of 356.43 km^2 (Melovski et al. 2013).

The most widespread soils in the region are the rankers that occur on the higher parts of Vlaina and Maleševo Mountains, while brown forest soils are mainly distributed in dense forested mountain areas (Filipovski et al. 1996).

Lower parts of the mountain (at about 900-1100 m above sea level) are under cold continental climate, with an average annual temperature range of about 9-10^oC and the annual precipitation range of 800-850

mm. Higher parts are influenced by mountain climate with the average annual temperatures between 3.5-8.5°C and the annual precipitation range of 800-1000 mm (Lazarevski 1993).

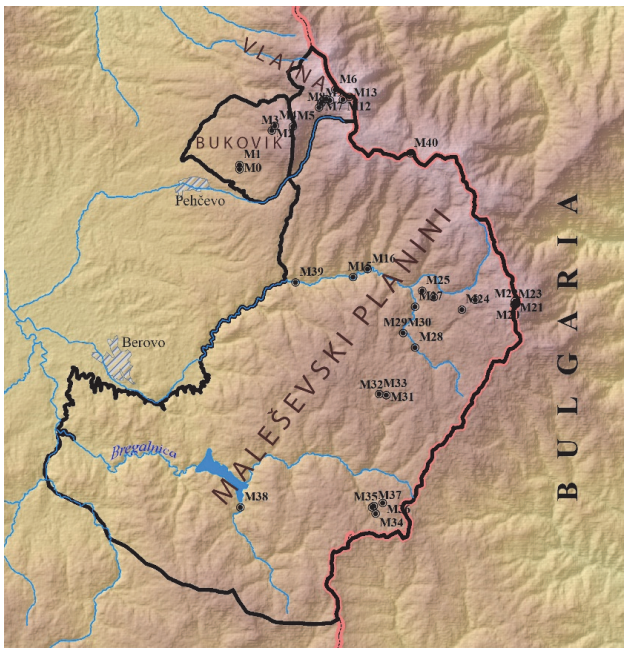


Fig. 1 Map of the studied area

MATERIAL AND METHODS

The material was collected manually and by pitfall traps during field trips on three mountains (Bukovik, Vlaina and Maleševo) in the Republic of North Macedonia, in the period 2010–2019. The research was conducted in different habitats and habitat types: wet meadows and riparian habitats (Strednjačka Reka), gravel banks of Kriva Reka, Strednjačka Reka and Zamenička Reka, wetlands (Ajdučki Kladenec, Tresčen Kamen, Čengino Kale peak), peatbogs, marshes (Tresčen Kamen - Kadiica, Elensko Blato), meadows (Mlečna, Klepalo and along Ravna Reka), cattle breeding farm (Tresčen Kamen), habitats with *Chamaecytisus* (Čengino Kale and Kadiica), hill pastures (Judovi Livadi), pastures (Mlečna, Kadiica, Tresčen Kamen and Čengino Kale peak), beech forests (Orlovec, Čengino Kale, Klepalo, Ravna Reka), *Salix caprea* stand (Čengino Kale peak), beech-fir-spruce forests (Murite), white pine-beech forest (Mlečna) and stands of *Betula pendula* (Čengino Kale).

The material is deposited in the personal collection of S. Hristovski. Sampled sites, habitats and sampling dates are presented below:

M0: **Bukovik**, Judovi Livadi, 1190 m a.s.l., peat bog, 24.06.2015, leg. S. Hristovski

M1: **Bukovik**, Judovi Livadi, hill pasture, 1210 m a.s.l., 22.91513 N 41.768136 E, 28.06.2018, leg. S. Hristovski

M2: **Bukovik**, Elensko Blato, peatbog, 1450 m a.s.l., 22.929399 N 41.779013 E, 28.06-15.08.2018, leg. S. Hristovski

M3: **Bukovik**, Elensko Blato, peatbog, 1450 m a.s.l., 22.929369 N 41.778965 E, 27.08.2019, leg. S. Hristovski

M4: **Bukovik**, Elensko Blato, peatbog, 1470 m a.s.l., 41.780519 N 22.930677 E, 28.06.2018, leg. S. Hristovski

M5: **Bukovik**, Orlovec, beech forest, 1690 m a.s.l., 41.780203 N 22.938527 E, 28.06–15.08.2018, leg. S. Hristovski

M6: **Vlaina**, Kadiica, Chamaecytisus, 1820 m a.s.l., 41.791748 N 22.957085 E, 28.06.-15.08.2018, leg. S. Hristovski

M7: **Vlaina**, Kadiica, pasture, 1820 m a.s.l., 41.787382 N 22.950874 E, 28.06.-15.08.2018, leg. S. Hristovski

M8: **Vlaina**, Tresčen Kamen, mandra, 1620 m a.s.l., 41.786072 N 22.949923 E, 13.07.2018, leg. S. Hristovski

M9: **Vlaina**, Tresčen Kamen, peatbog, 1630 m a.s.l., 41.787382 N 22.950874 E, 28.06-15.08.2018, leg. S. Hristovski

M10: **Vlaina**, Tresčen Kamen, peatbog, 1650 m a.s.l., 41.787974 N 22.951413 E, 28.06.2018, leg. S. Hristovski

M11: **Vlaina**, Tresčen Kamen, pasture, 1700 m a.s.l., 41.788832 N 22.953101 E, 28.06.2018, leg. S. Hristovski

M12: **Vlaina**, Tresčen Kamen, pasture, 1850 m a.s.l., 41.788451 N 22.960388 E, 24.06.2015, leg. S. Hristovski

M13: **Vlaina**, Tresčen Kamen, pasture, 1930 m a.s.l., 41.789329 N 22.963156 E, 24.06.2015, leg. S. Hristovski

M14: **Vlaina**, Tresčen Kamen, wetland, 1730 m a.s.l., 41.78829 N 22.954767 E, 24.06.2015, leg. S. Hristovski

M15: **Maleševski Planini**, Ravna Reka, h. Idila, beech forest, 1080 m a.s.l., 41.731107 N 22.962995 E, 29.07.2018, leg. S. Hristovski

M16: **Maleševski Planini**, Ravna Reka, meadow, 1100 m a.s.l., 41.733537 N 22.969311 E, 27.10.2014, leg. S. Hristovski

M17: **Maleševski Planini**, Čengino Kale, beech forest, 1575 m a.s.l., 41.722816 N 23.0152 E, 29.06.-28.07.2018, leg. S. Hristovski

M18: **Maleševski Planini**, Čengino Kale, Betula pendula stand, 1740 m a.s.l., 41.722134 N 23,032275 E,

28.08.-21.09.2019, leg. S. Hristovski

M19: Maleševo Mts., Čengino Kale peak, wetland, 1748 m a.s.l., 41.721288 N 23,032901 E, 29.06.-28.07.2018, leg. S. Hristovski

M20: **Maleševski Planini**, Čengino Kale peak, wetland, 1748 m a.s.l., 41.721326 N 23,032867 E, 29.06.2018, leg. S. Hristovski

M21: **Maleševski Planini**, Čengino Kale peak, pasture, 1748 m a.s.l., 41.721154 N 23,032588 E, 29.06.2018, leg. S. Hristovski

M22: **Maleševski Planini**, Čengino Kale, *Chamaecytisus* shrubland, 1750 m a.s.l., 29.06.-28.07.2018, 41.721083 N 23,032199 E, leg. S. Hristovski

M23: **Maleševski Planini**, Čengino Kale peak, *Salix caprea* stand, 1750 m a.s.l., 41.72099 N 23,032037 E, 29.06.2018, leg. S. Hristovski

M24: **Maleševski Planini**, Čengino Kale, Mlečna, pasture, 1510 m a.s.l., 41.719735 N 23,009416 E, 29.06.2018, leg. S. Hristovski & A. Cvetkovska-Gjorgjievska

M25: **Maleševski Planini**, Mlečna, *Fago-Pinetum sylvestris*, 1385 m a.s.l., 41.726058 N, 22.992221 E, 28.06.-15.08.2018, leg. S. Hristovski

M26: **Maleševski Planini**, Mlečna, meadow, 1450 m a.s.l., 41.724009 N 22.997397 E, 29.06.2018, leg. S. Hristovski

M27: **Maleševski Planini**, Kriva Reka - grip, beech forest, 1240 m a.s.l., 41.72103 N, 22.989257 E, 29.06.2018, leg. S. Hristovski

M28: **Maleševski Planini**, Kriva Reka, gravel bank, 1320 m a.s.l., 41.707865 N 22.988946 E, 21.08.2010, leg. S. Hristovski

M29: **Maleševski Planini**, Strednjačka Reka, gravel bank, 1298 m a.s.l., 41.712641 N 22.983953 E, 29.06.2018, leg. S. Hristovski

M30:**Maleševski Planini**, Strednjačka Reka, wet meadow, 1300 m a.s.l., 41.712651 N 22.983951 E, 28.06.-15.08.2018, leg. S. Hristovski

M31: **Maleševski Planini**, Murite, beech-fir-spruce forest, 1350 m a.s.l., 41.692676 N 22.976071 E, 26.10.2014, leg. S. Hristovski

M32: **Maleševski Planini**, Murite, beech-fir-spruce forest, 1350 m a.s.l., 41.692676 N 22.976071 E, 12.07.2015, leg. S. Hristovski

M33: **Maleševski Planini**, Murite, near road, 1350 m a.s.l., 41.693148 N 22.973046 E, 12.07.2015, leg. S. Hristovski

M34: **Maleševski Planini**, Klepalo, beech forest, 1250 m a.s.l., 41.654588 N 22.970262 E, 12.06.2015, leg. S. Hristovski

M35: **Maleševski Planini**, Klepalo, meadow, 1250 m a.s.l., 41.65665 N 22.968773 E, 11.07.2015, leg. S. Hristovski

M36: **Maleševski Planini**, Klepalo, meadow, 1270-1350 m a.s.l., 41.657963 N 22.973427 E, 11.07.2015, leg. S. Hristovski

M37: **Maleševski Planini**, Klepalo, meadow, 1270 m a.s.l., 41.657027 N 22.969582 E, 16.08.2017, leg. S. Hristovski

M38: **Maleševski Planini**, Berovsko Ezero, Zamenička River, gravel bank, 990 m a.s.l., 41.657551 N 22.9122 E, 28.08.2019, leg. S. Hristovski

M39: **Maleševski Planini**, meadow, 1020 m a.s.l., 41.729816 N 22.938199 E, 13.06.2018, leg. S. Hristovski

M40: **Maleševski Planini**, Ajdučki Kladenec, wetland, 1660 m a.s.l., 41.770572 N 22.988737 E, 15.08.2018, leg. S. Hristovski

RESULTS AND DISCUSSION

In total 685 specimens belonging to 74 species from 32 genera were registered (Tab. 1). *Amara* (10), *Bembidion* (8), *Carabus* (6), *Harpalus* (6) and *Pterostichus* (6) had the highest species richness, while other genera were registered with 3 to 1 species. The most abundant species was *Tapinopterus balcanicus belasicensis* (118 specimens), followed by *Calathus melanocephalus melanocephalus* (82), *Poecilus versicolor* (68) and *Carabus violaceus azurescens* (45), representing about 45.75% of total abundance.

Among species with conservation importance, the endemics are represented with the highest share (11 species), while only the species *Carabus convexus dilatatus* is noted on the CORINE list.

Pterostichus vecors, Pterostichus bruckii, Tapinopterus balcanicus belasicensis, Carabus violaceus azurescens, Carabus gigas gigas, Cyhrus semigranosus balcanicus, Zabrus balcanicus rhodopensis are Balkan endemics**,** while four species: *Xenion ignitum, Molops rufipes denteletus, Molops piceus osogovensis, Platynus scrobiculatus bulgaricus* are recognized as subendemics, with populations outside the boundaries of the researched area. Until recently we considered *Platynus scrobiculatus bulgaricus* and *Molops rufipes denteletus* as local endemics for the Osogovo Mountains, but with their discovery on Maleševo Mts, their known range of distribution has been expanded. *Amara lunicollis, Notiophilus laticollis, Pterostichus apfelbecki*,

Pterostichus diligens, Bembidion stephensii, Bembidion brunnicorne, Bradycellus caucasicus have restricted distribution and narrower habitat tolerances, therefore are considered as rare. Of these, special attention deserve *Pterostichus apfelbecki* a species with a disjunct distribution (which indicates its relictness), registered only at the locality Judovi Livadi (Chehlarov et al. 2016) and *Pterostichus diligens*, a rare species in North Macedonia, associated with peatlands and other wet habitats.

All above presented species mostly belong to Palearctic or European/Eurosiberian complexes. All of the records are from different habitat types harboring rich carabid diversity, with highest species diversity registered in wet meadows (22), habitats with *Chamaecytisus* (17), peatbogs (16), meadows (15) and wetlands (14). The refugial sites provide presence of endemic and rare species as well, highlighting the well

Table 1. A list of recorded ground-beetle species on the Maleševo Mts. [abbreviations of localities M1-M40 are presented in the Materials and Methods chapter].

Species/subspecies	Localities (number of specimens)	Specimens
1. Abax ovalis (Duftschmid, 1812)*	M25 (2), M31 (1)	3
2. Agonum sexpunctatum (Linnaeus, 1758)**	M19 (2)	2
3. Agonum viduum Panzer, 1796**	M19 (7)	7
4. Amara aenea (DeGeer, 1774)*	M1 (1)	1
5. Amara aulica (Panzer, 1796)**	M30 (1)	1
6. Amara communis (Panzer, 1797)**	M30 (13), M35 (1)	14
7. Amara convexior Stephens, 1828*	M30 (3), M39 (1)	4
8. Amara curta Dejean, 1828*	M22 (1), M30 (1), M35 (1)	3
9. Amara equestris (Duftschmid, 1812)*	M22 (2), M30 (7), M6 (4), M7 (6)	19
10. Amara littorea C. G. Thomson, 1857*	M30 (2), M9 (1)	3
11. Amara lunicollis Schiødte, 1837**	M22 (1), M6 (1)	2
12. Amara nitida Sturm, 1825**	M19 (1), M22 (1), M30 (26), M6 (1)	29
13. Amara similata (Gyllenhal, 1810)**	M19 (2)	2
14. Anchomenus dorsalis (Pontoppidan, 1763)*	M16 (2)	2
15. Anisodactylus binotatus (Fabricius, 1787)*	M30 (1)	1
16. Bembidion brunnicorne Dejean, 1831**	M3 (3)	3
17. Bembidion dalmatinum Dejean, 1831*	M14 (1), M2 (1)	2
18. Bembidion deletum Audinet-Serville, 1821*	M14 (1), M25 (1)	2
19. Bembidion geniculatum geniculatum Heer, 1837**	M3 (4), M31 (1), M4 (1)	6
20. Bembidion lampros (Herbst, 1784)*	M14 (1), M25 (7), M29 (1), M37 (1), M4 (5)	15
21. Bembidion properans (Stephens, 1828)*	M35 (2)	2
22. Bembidion stephensi Crotch, 1866**	M2 (1), M28 (2), M3 (1), M4 (2)	6
23. Bembidion tibiale (Duftschmid, 1812)*	M16 (1), M28 (7), M29 (1), M38 (5)	14
24. Brachinus explodens Duftschmid, 1812*	M16 (1)	1
25. Bradycellus caucasicus (Chaudoir, 1846)**	M20 (1)	1
26. Calathus distinguendus distinguendus Chaudoir, 1846**	M18 (1)	1
27. Calathus fuscipes (Goeze, 1777)*	M19 (1), M24 (1), M30 (2), M36 (4), M8 (1)	9
28. Calathus melanocephalus melanocephalus (Linnaeus, 1758)*	M16 (1), M18 (28), M22 (27), M24 (6), M30 (13), M36 (2), M40 (1), M6 (2), M7 (1)	81
29. Carabus convexus dilatatus Dejean, 1826*	M30 (2)	2

Species/subspecies	Localities (number of specimens)	Specimens
30. Carabus coriaceus cerisyi Dejean, 1826*	M23 (1)	1
31. Carabus gigas gigas Creutzer, 1799*	M15 (1)	1
32. Carabus hortensis Linnaeus, 1758*	M17 (1), M18 (28), M22 (1), M23 (1), M25 (5)	36
33. Carabus montivagus Palliardi, 1825*	M18 (1)	1
34. Carabus violaceus azurescens Dejean, 1826	M17 (2), M2 (1), M22 (7), M25 (5), M30 (17), M34 (2), M6 (6), M7 (3), M9 (2)	45
35. Cicindela campestris campestris Linnaeus, 1758*	M33 (1)	1
36. Cychrus semigranosus balcanicus Hopffgarten, 1881*	M17 (1), M5 (2)	3
37. Cymindis humeralis (Geoffroy, 1785)**	M36 (1)	1
38. Harpalus affinis (Schrank, 1781)	M8 (5)	5
39.Harpalus latus (Linnaeus, 1758)**	M30 (13), M6 (1)	14
40. Harpalus rufipalpis Sturm, 1818*	M2 (1), M6 (1)	2
41. Harpalus rufipes (DeGeer, 1774)*	M8 (2)	2
42. Harpalus subcylindricus Dejean, 1829*	M1 (2)	2
43. Harpalus tenebrosus Dejean, 1829**	M17 (1), M22 (1), M6 (1)	3
44. Laemostenus terricola punctatus (Dejean, 1828)**	M22 (1), M6 (3)	4
45.Leistus spinibarbis rufipes Chaudoir, 1843**	M13 (1)	1
46. Molops piceus osogovensis B. V. Guéorguiev, 1997*	M38 (2)	2
47. Molops rufipes denteletus B. V. Guéorguiev, 1997*	M17 (2), M23 (1), M6 (1)	4
48. Nebria brevicollis (Fabricius, 1792)*	M10 (1), M23 (1)	2
49. Notiophilus laticollis Chaudoir, 1850**	M6 (1)	1
50. Notiophilus substriatus C. R. Waterhouse, 1833**	M17 (1), M18 (1), M25 (5)	7
51.Ophonus azureus (Fabricius, 1775)**	M35 (2)	2
52. Panagaeus bipustulatus (Fabricius, 1775)**	M30 (2)	2
53. Paranchus albipes (Fabricius, 1796)*	M38 (1)	1
54. Platynus scrobiculatus bulgaricus Schmidt, 2009*	M32 (1), M38 (2)	3
55. Poecilus lepidus (Leske, 1785)*	M30 (3)	3
53. Poecilus versicolor (Sturm, 1824)*	M12 (1), M21 (1), M30 (61), M36 (5)	68
57. Pterostichus apfelbecki Csiki, 1908	M0 (1)	1
58. Pterostichus bruckii Schaum, 1859*	M27 (1)	1
59. Pterostichus diligens (Sturm, 1824)**	M19 (2), M2 (1), M9 (3)	6
60. Pterostichus niger (Schaller, 1783)*	M2 (16), M30 (1), M4 (1)	18
61. Pterostichus nigrita (Paykull, 1790)*	M19 (5), M9 (2)	7
62. Pterostichus strenuus (Panzer, 1796)*	M29 (1), M30 (2), M40 (1)	4
63. Pterostichus vecors (Tschitschérine, 1897)*	M17 (4), M2 (2), M25 (4), M4 (1), M5 (1), M6 (2), M9 (1)	15
64. Sinechostictus millerianus Heyden, 1883*	M28 (1), M38 (3)	4
65. Syntomus obscuroguttatus (Duftschmid, 1812)**	M30 (1)	1
66. Syntomus truncatellus (Linnaeus, 1761)**	M22 (2), M30 (2)	4
67. Synuchus vivalis (Illiger, 1798)*	M30 (4)	4

Species/subspecies	Localities (number of specimens)	Specimens
68. Tapinopterus balcanicus belasicensis Mařan, 1933*	M17 (43), M22 (5), M23 (3), M25 (48), M26 (1), M5 (2), M6 (7), M7 (8), M9 (1)	118
69. Trechus obtusus obtusus Erichson, 1837*	M30 (1)	1
70. Trechus quadristriatus (Schrank, 1781)*	M16 (1)	1
71. Trechus gr. subnotatus Dejean, 1831*	M13 (1), M40 (5)	6
72. Xenion ignitum (Kraatz, 1875)*	M17 (9), M2 (1), M22 (17), M25 (6), M27 (1), M32 (1), M6 (1)	36
73. Zabrus balcanicus rhodopensis Apfelbeck, 1904*	M11 (1)	1
74. Zabrus tenebrioides (Goeze, 1777)**	M2 (1)	1

* First records for the Macedonian part of Maleševo mountains; ** First records for Maleševo mountains (both Macedonian and Bulgarian parts)

preserved nature and conservation importance of the mountain massif.

Overall, 71 species were recorded for the first time for the Macedonian part of Maleševo mountains. The following five species known from the literature were not confirmed: *Acinopus ammophilus* Dejean, 1829, *Amara kulti* Fassati, 1947, *Lebia cyanocephala* (Linnaeus, 1758), *Pedius longicollis* (Duftschmid, 1812) and *Stenolophus teutonus* (Schrank, 1781). Thus, the total number of species for the Macedonian part of Maleševo mountains is 79.

For comparison, during a faunistic study of the ground-beetle fauna on the Bulgarian side of the Maleševo Mts, relatively high species diversity of 125 species was registered, with 11 species of conservation importance among which the above-mentioned Balkan endemics dominate (Guéorguiev and Ljubomirov 2009). Our research contributed with 25 first species records for the whole Maleševo mountains in both Macedonian and Bulgarian parts. As a result of the studies in Bulgarian and Macedonian part of Maleševo Mt. the total number of ground beetle species is 153.

ACKNOWLEDGEMENT

I am grateful to the reviewers Rumyana Kostova and Stanislava Lazarevska for their suggestions to improve the manuscript.

REFERENCES

Brajković, M. M., Ćurčić, S. B., Mihajlova, B. (2004). On some ground beetles (Carabidae, Coleoptera) from the Republic of Macedonia. *Archives of Biological Sciences* **56**(3–4): 25–26.

Chehlarov, E., Guéorguiev, B., Hristovski, S., Fancello, L., Cvetkovska-Gorgievska, A., Preliḱ, D. (2016). New Country Records and Rare and Interesting Species of Coleoptera from the Balkan Peninsula. *Acta zoologica bulgarica* **68**(3): 331–338.

Filipovski, G., Rizovski, R., Ristevski, P. (1996). *The characteristics of the climate-vegetation-soil zones (regions) in the Republic of Macedonia*. Macedonian Academy of Sciences and Arts, Skopje, 177 p.

Guéorguiev, B. V., Ljubomirov, T. (2009). Coleoptera and Hymenoptera (Insecta) from Bulgarian Section of Maleshevska Planina Mountain: Study of an Until Recently Unknown Biodiversity. *Acta zoologica bulgarica* **61**(3): 235–276.

Hieke, F., Wrase, D. W. (1988). Faunistik der Laufkäfer Bulgariens.(Coleoptera, Carabidae). *Deutsche Entomologische Zeitschrift* **35**(1–3): 1–171.

Hristovski, S., Guéorguiev, B. (2015). Annotated catalogue of the carabid beetles of the Republic of Macedonia (Coleoptera: Carabidae). *Zootaxa* **4002** (1): 1–190.

Lazarevski, A. (1993). *Climate in Macedonia*. Kultura, Skopje, 253 p.

Melovski, L., Markovski, B., Hristovski, S., Jovanovska, D., Anastasovski, V., Klincharov, S., Velevski, M., Velkovski, N., Trendafilov, A., Matevski, V., Kostadinovski, M., Karadelev, M., Levkov, Z., Kolchakovski, D. (2013). Regional division of the Republic of Macedonia for the needs of biological databases. *Macedonian Journal of Ecology and Environment* **15**(2): 31.

UDK: 597.552.51-146.31.086
Received: 29.10.2023
Accepted: 03.11.2023
Original scientific paper

Acta Musei Macedonici Scientiarum Naturalium, 2023, Vol. 26, pp: 21-25
ISSN: 0583-4988 (printed version)
ISSN: 2545-4587 (on-line version)
Available on-line at: www.acta.musmacscinat.mk

Ultrastructural analysis of degenerative changes of spermatogonia of Salmonidae from Ohrid lake during spermatogenesis

Irena Tavchiovska-Vasileva

Institute of Biology, Faculty of Natural Sciences and Mathematics, Gazi Baba b.b., P. O. 162, 1000 Skopje, Republic of Macedonia
irena@pmf.ukim.mk

Abstract

The purpose of these article is to represent the cytomorphological aspect of the spermatogonia in Ohrid trout (*Salmo letnica* (Karaman, 1924) and Ohrid belvica (*Salmo ohridanus* Steindachner, 1892) on ultrastructural level, which degenerate in the postspawning period. At certain sites in the wall of the seminiferous lobules of Ohrid trout and Ohrid belvica, in addition to normal spermatogonia, there are some that show clear signs of degeneration and are characterized by asymmetric shape, granular or vacuolated cytoplasm, condensed chromatin, and karyopicnotic features.

On a level with certain lobules spermatogonia which separate from the walls of the lobules can be observed. We can notice presence of completely homogenous mass which originates from spermatogonia with delaminated cell membrane, as well as mitochondria with disintegrated crusts, vacuoles and poor cytoplasm or lysed parts of the cytoplasm. It has to be noted that spermatogonia whivh are situated next to the Sertoli cells are degenerated, as well as the Sertoli cells are. Our results which concern the cytomorphological characteristics of the spermatogonia which degenerate during the postspawning period correspond with findings of many other autors, as well as our previous results which concern the same or other teleost species. Our results show that in the period of rebuilding of the testicular parenchyma (postspawning period) the degenerative changes which are visible on a level with the spermatogonial population, should be explained as normal accompanying phenomenon which follows the initial phase of the future reproductive cycle whose intensive development happens in the later period, period of regeneration.

Key words: Ohrid trout, Ohrid belvica, degenerated spermatogonia, ultrastructural analysis, annual reproductive cycle.

INTRODUCTION

In this paper the degenerative changes on the level of spermatogonia have been analysed, as a normal accompanuing phenomenon of spermatogenesis during the beginning phase of the annual reproductive cycle. In the literature there are relatively a small number of data which point out the degenerative changes of spermatogonia with Teleostei during the annual reproductive cycle (Almeida et al., 2008; Leal et al., 2009; Print & Loveland, 2000). The degeneration of some spermatogonia with *Perca fluviatilis* Linneus, 1758 was noticed by Kulaev (1927). Also, in the testes perch from Dojran Lake the presence of spermatogonia which show visible signs of degeneration was noticed (Tavciovska-Vasileva, 1992, 1994). In the postspawning period with *Perca fluviatilis from Dojran Lake* the presence of necrotic formations of spermatogonial origin was noticed (Tavciovska-Vasileva, 1992, 1994). Similar formations, i. e. necrotic (lipophuscine) groups with *Fundulus heteroclitus* (Linnaeus, 1766) were noticed by Matthews (1938) and Lofts et al. (1966). Necrotic formations rich with lipids and PAS positive cells in the testes of *Salaria pavo* (Risso, 1810) were noticed (Patzner & seiwald, 1987). The spermatogonial degeneration in the testes of Salmonidae, i.e. Ohrid trout and Ohrid belvica was noticed by Dimovska & Tavciovska-Vasileva (1998), Tavciovska-Vasileva (1999, 2001, 2002, 2004) and Tavciovska-Vasileva & Rebok (2009).

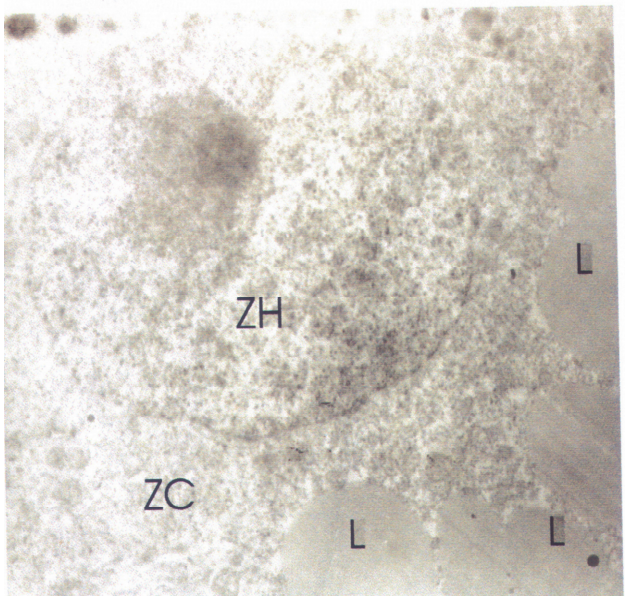


Fig. 1 Spermatogonium in beginning phase of degeneration with granular cytoplasm (ZC) and granular chromatin (ZH) in Ohrid trout. Presence of lipids (L) with low electron density. Ultrathin section, 7.000x

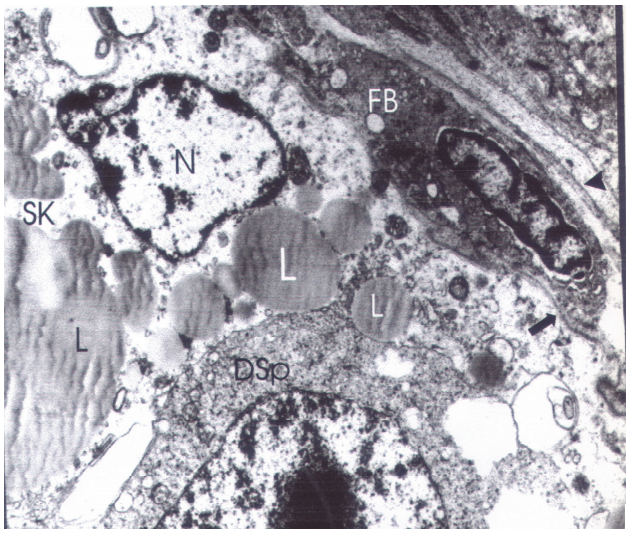


Fig. 2 A part of cytoplasm of Sertoli cell (SK) in degeneration in Ohrid trout. Presence of lipids (L) with different dimensions, prominentnucleus (N). Spermatogonium in degeneration (DSp). Visible interstitium with fibroblast (FB) in direct closeness of basal lamina (arrow), presence of colagenous fibrils (small arrow). Ultrathin section, 4.000x.

MATERIAL AND METHODS

Testes of sexually mature Ohrid trout and Ohrid belvica males caught in Ohrid Lake have been analysed. Analyses have been done with electronic microscope. Smal parts of testes 1-2 mm big have been used for electronic microscopy. The material has been fixed according to following procedure: Immediately after the tissue sections have been taken, they are fixed in 3% glutaraldehyde and then conserved in 0.1 M phosphate buffer. After adequate fixation the material has been subunitted to postfixation in 1% osmium tetraoxid (OsO_4). In the further treatment the material has been washed in phosphate buffer, dehydrated in series of acetone and uranil acetate. The tissue parts have been infiltrated with Durcopan ACM mixture, mixture of acetone-Durcopan, Durco-pan No.1, Durcopan No. 2, fit in Durcopan No. 2 and polymerised. For the ultrastructural analysis, ultrathin sections of 40-60 hm tickness have been prepared, with the help of glass knives, on Reichert-Yung “Ultracut” ultramicrotome, installed on copper nets, contrasted with uranil acetate and lead cytrate. The sections have been observed on Tesla BS 500 and OPTON (Zeis) EM 109 electronic microscope. The micro-photographs for electronic microscope were obtained on Agfa Scientia EM Film 23056/6,5 x 9 cm, ORWO NP 20 panchromatic 120, Kodak 120 and made on Agfa papirtone Paper P1-3.

RESULTS

On a level of seminiferous lobules of Ohrid trout in dependence of annual cycle, cysts with different germinal generations can be present. In some spermatogonia, directly before the spawning, beginning degenerative changes can be noticed, i. e. on a level of some spermatogonia granular cytoplasm and granular chromatin, as well as a presence of lipids with low electron density can be noticed (Fig. 1). In the postspawning period, as the process goes on, i. e. final phase, except normal spermatogonia, there are some which shows signs of degeneration and are characterized by unsymmetrical form and thick cytoplasm (Fig. 2, 3, 4). The nucleus of these cells doesn`t have clear contours. It has an unsymetrical form, condensed chromatin and karyopycnotic characteristics. On a level with certain seminiferous lobules spermatogonia which separate from the walls of the lobules can be observed. It has to be noted that spermatogonia which are situated next to Sertoli cells are degenerated, as well as the Sertoli cells are (Fig. 2, 3). Also, in the period of regeneration which follows the postspawning period, is characterized by the presence of spermatogonial generation for the following reproductive cycle. On a level with some seminiferous lobules spermatogonia in degeneration can be observed, which are manifested hyperchromatic characteristics of the nuclei or karyopycnosis has al-

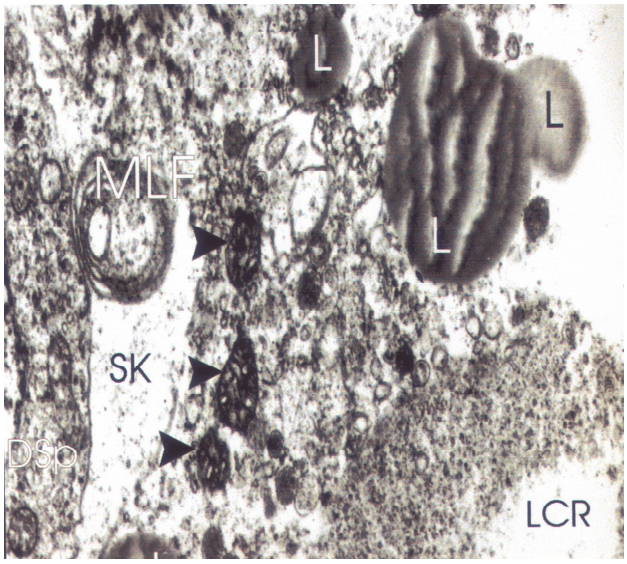


Fig. 3 A part of cytoplasm of Sertoli cell in degeneration, lysosomes with "myelin like" figures (MLF), lysed cytoplasmatic regions (LCR), mitochondria in degeneration (small arrows), lipids (L) with different dimensions in Ohrid trout. A part of one spermatogonium in degeneration (DSp). Ultrathin section, 8.000x

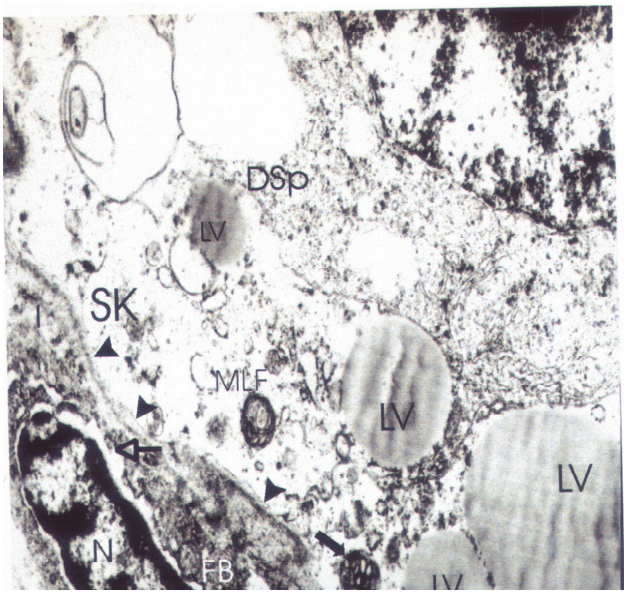


Fig. 4 A part of spermatogonium in degeneration (DSp) in direct closeness with Sertoli cell in degeneration in Ohrid trout. In the cytoplasm of Sertoli cell (SK) presence of lysosomes with "myelin like" figures (MLF), mitochondrium in degeneration (black arrow), lipid vacuoles (LV) with different dimensions. Visible basal lamina (small arrows). In the interstitium (I) fibroblast (FB) with prominent nucleus (n) with widened perinuclear space (arrow). Ultrathin section, 8.000x.

ready happened with them. In Ohrid belvica the degeneration of spermatoginia are clearly noticed on ultrathin sections. The beginning degenerative changes of spermatogonia are manifested with cytoplasm with granular structure in which single vacuoles, disintegrated nuclear membrane and heterochromatic nucleus and nucleolus have been noticed (Fig. 5). Also, on ultrastructural level in the cytoplasm of degenerated spermatogonia, mitochondria with disintegrated crusts and lysed parts of the cytoplasm can be noticed (Fig. 6). Our results have pointed out that during the period of rebuilding of testicular parenchyma, i. e. the postspawning period, the degenerative changes which have been seen on a level of spermatogonial population, they can be explained as a normal following phenomenon which follows the initial phase of the future reproductive (spermatogenetic) cycle, whose intensive development happenes in the later period, i. e. period of regeneration.

DISCUSSION

The studies of ultrastructural features of teleost spermatogenesis are scarce and they focused mostly on common carp (*Cyprinus carpio*), silver pomfret (*Pampus argenteus*), burbot (*Lota lota*), rock flounder (*Kareius bicoloratus*) and grey armored catfish (*Liposarcus anisitsi*) (Dadras et al. 2023) with other species receiving less attention.

In the seminiferous lobules of Ohrid trout and Ohrid belvica except normal spermatogonia, we found out a presence of spermatogonia which show evident signs of degeneration, which are manifested by karyopycnosis, granular or vacuolated cytoplasm, completely homogenous mass with delaminated cell membrane, delamination of spermatogonia from the walls of the seminiferous lobules. In our preliminary research spermatogonial degeneration of the testes of Salmonidae, i. e. Ohrid trout and Ohrid belvica was noticed by Dimovska & Tavciovska-Vasileva (1998), Tavciovska-Vasileva (1999, 2001, 2002, 2004) and Tavciovska-Vasileva & Rebok (2009). The degeneration of certain spermatogonia with *Perca fluviatilis* was noticed too by Kulaev (1927). Also, in the testes of Dojran perch presence of degenerated spermatogonia was stated (Tavciovska-Vasileva, 1992, 1994). Similar formations, i. e. necrotic (lipophuscine) groups with *Fundulus heteroclitus* were noticed by Matthews (1938) and Lofts et al. (1966). Necrotic formations rich with lipids and PAS positive cells in the testes of *Salaria pavo* were noticed (Patzner & Seiwald, 1987). In the literature there are

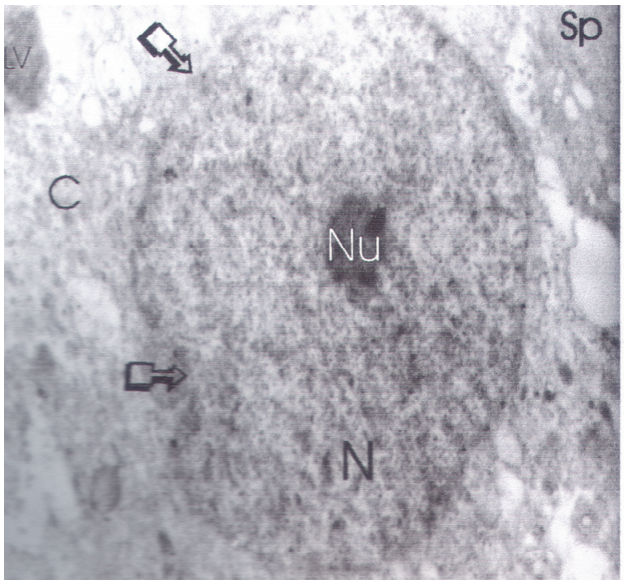


Fig. 5 Spermatogonium (Sp) in degeneration, cytoplasm with granular structure, single lipid vacuole (LV), disintegrated nuclear membrane (arrows), heterochromatic nucleus (N), homogenous nucleolus (Nu) in Ohrid belvica. Ultrathin section, 7.000x.

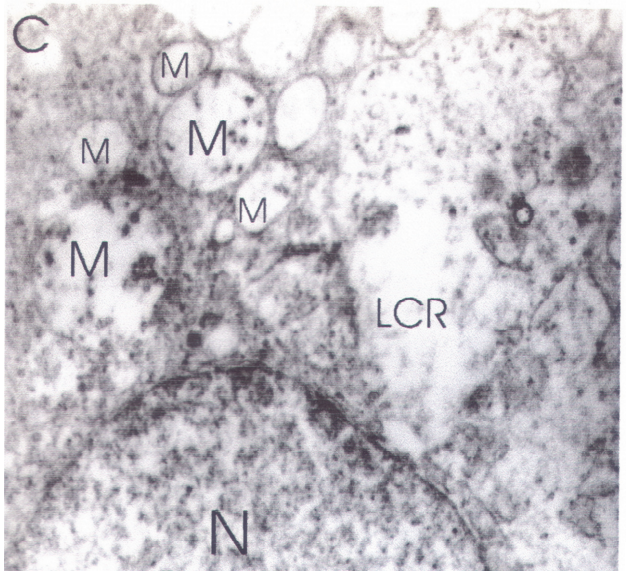


Fig. 6 A part of cytoplasm (C) and nucleus (N) of one spermatogonium in degeneration. In the cytoplasm presence of mitochondria (M) with disintegrated crusts and lysed parts of cytoplasm (LCR) in Ohrid belvica. Ultrathin section, 12.000x.

relatively a small number of data which point out the degenerative changes of spermatogonia with Teleostei during the annual reproductive cycle (Almeida et al., 2008; Leal et al., 2009; Print & Loveland, 2000). The degenerative processes which are visible on a level with the spermatogonial generation should be explained as a normal accompanuing phenomenon which follows the initial phase of the future reproductive cucle, i. e. the proliferation and the differentiation of spermatogonia. This intensive development happenes in the later period (period of regeneration), in contrast to a large number of data which concern other Vertebrata, especially for Mammalia (Roosen-Runge & Leik, 1968; Collins, 1987) which point to an enormous number of gonocytes which are exposed to degeneration to degeneration during the fetal and early postnatal period.

CONCLUSION

On the basis of our research concerning the testes of Ohrid trout and Ohrid belvica in the postspawning period and spermatogonial population, we can conclude the following: in certainseminiferous lobules, besides spermatogonia with normal cytomorphological aspect, they can be noticed in degeneration, which is manifested with karyopycnosis, mitochondria with disintegrated crusts, vacuoles and poor cytoplasm, as well as delamination from the walls of the lobules.

REFERENCES

Almeida, F. F. L., Kristoffersen, C., Taranger, G. L., Schulz, R. W. (2008): Spermatogenesis in Atlantic Cod (*Gadus morhua*): A novel model of cystic germ cell development. Biology of Reproduction, 78 (1): 27-34.

Collins, P. M., Pudney, J., Tsang, W. N. (1987): Postnatal differentiation of the gametogenic and endocrine functions of the testis of the tree-shrew (*Tupaia belangeri*). Cell Tiss. Res., 25:681-687.

Dadras, H., Dadras, F., Imentai, A., Malinovskyi, O., Pěnka, T., Kolářová, J., & Policar, T. (2023). Fine ultrastructural features of germ cells and spermatozoa during spermatogenesis in the European grayling, *Thymallus thymallus* (Teleostei, Salmoniformes, Salmonidae). Frontiers in Veterinary Science, 10, 1188479.

Dimovska, A., Tavciovska-Vasileva, I. (1998): Citoloski alteracii na spermatogonijalnata populacija na Ohridskata belvica (*Salmothymus ochridanus* Steind.) vo postmrestitelniot period. 2 Kongres na biolozite na Makedonija. Zbornik na apstrakti. Ohrid, Republika Makedonija.

Kulaev, S. U. (1927): Nablodenija nad izmeneniem semenikov recnogo okunja (*Perca fluviatilis* L.) v te-

cenie godovogo cikla. Russk. Zool. Zurn., 7: 15-54.

Leal, M. C., Cardoso, E. R., Nobrega, R. H., Batlouni, S. R., Bogerd, J., Franca, L. R., Schulz, W. (2009): Histological and stereological evaluation of Zebrafish (Danio rerio) spermatogenesis with an emphasis on spermatogonial generations. Biology of Reproduction, 81 (1): 177-187.

Lofts, B., Pickford, G. E., Atz, J. W. (1966): Effects of Methyltestosterone on the testes of a hypophysectomized cyprinodont fish, *Fundulus heteroclitus*. Gen. Comp. Endocrinol., 6: 74-88.

Matthews, S. A. (1938): The seasonal cycle in the testes of *Fundulus*. Biol. Bull., 75: 66-74.

Patzner, R. A., Seiwald, M. (1987): Reproduction of Blenius pavo (Teleostei, Blenniidae). VI. Testicular Cycle. Zool. Anz., 219 (5/6): 265-273.

Print, C. G., Loveland, K. L. (2000): Germ cell suicide: new insights into apoptosis during spermatogenesis. BioEssays: news and reviews in molecular, cellular and developmental biology, 22 (5): 423-430.

Roosen-Runge, E. C., Leik, J. (1968): Gonocite degeneration in the postnatal male ret. Am. J. Anat., 122: 275 -300.

Tavciovska-Vasileva, I. (1992): Histoloska struktura na semenikot na Dojranskata perkija (*Perca fluviatilis macedonica* Kar.) vo periodot po mrestenjeto. Magisterski trud. Skopje, Republika Makedonija.

Tavciovska-Vasileva, I. (1994): Spermatogonijalna degeneracija kako propraten fenomen na spermatogenezata vo postmrestitelniot period kaj Dojranskata perkija (*Perca fluviatilis macedonica* Kar.). God. Zb. Biol., Skopje, 47: 189-198.

Tavciovska-Vasileva, I. (1999): Komparativni strukturni i ultrastrukturni karakteristiki na semenicite kaj Salmonidae (Pisces: Teleostei) od Ohridskoto Ezero vo postmrestitelniot period. Doktorska disertacija. Skopje, Republika Makedonija.

Tavciovska-Vasileva, I. (2001): Degeneration of spermatogonial population as a phenomenon which follows the proliferation and differentiation of spermatogonia of Ohrid trout (*Salmo letnica* Kar.) in the period after the spawning. Veterinary Medicine, VII (3-4): 89-92.

Tavciovska-Vasileva, I. (2002): Spermatogonial degeneration as a normal accompanying phenomenon of the reproductive cycle of Ohrid belvica (*Acantholingua ohridana*) during the postspawning period. XVII International Symposium on Morphological Sciences. Abstract book. Timisoara, Romania.

Tavciovska-Vasileva, I. (2004): Degeneration of spermatogonia-a normal phenomenon of the spermatogenesis of the Ohrid belvica (*Acantholingua ohridana*) during the period after the spawning. Veterinary Medicine, VIII(1-2): 58-61.

Tavciovska-Vasileva, I., Rebok, K. (2009): Ultrastructural features of degenerated spermatogonia of Ohrid trout (*Salmo letnica* Kar.) during the reproductive cycle. Annual of Konstantin Preslavky University, Faculty of Natural Sciences, Shumen, Bulgaria, Vol. XIX B6: 110-119.

UDK: 582.998.1-19(497.7)
Received: 08.09.2023
Accepted: 15.09.2023
Original scientific paper

Acta Musei Macedonici Scientiarum Naturalium, 2023, Vol. 26, pp: 27-34
ISSN: 0583-4988 (printed version)
ISSN: 2545-4587 (on-line version)
Available on-line at: www.acta.musmacscinat.mk

Study on *Hieracium* s.str. (*Asteraceae*) in North Macedonia, I.

Aco Teofilovski

Public Enterprise Nacionalni šumi, Sv. Kliment Ohridski, 68, MK-1000 Skopje, North Macedonia
Corresponding author e-mail: acoteofilovski@hotmail.com

Abstract

The author's further study of *Hieracium* s.str. (Asteraceae) revealed some additional new data and confirmations regarding the diversity and chorology of this genus in North Macedonia. This paper deals with six species of which two are reported for the first time in the country - *Hieracium macropannosum*, found near Delčevo (Dzvegor village), and *Hieracium oxyodon*, found in Šar Mountains (Plat, Brezno village). The present occurrence of the following three species in the country, reported in the literature from a single locality 85 years ago or earlier, is confirmed by their records in one or more additional locations: *Hieracium lachenalii*, *Hieracium prenanthoides*, and *Hieracium pseudofastigiatum*. *Hieracium glabratum*, collected in the country only once in 1918, in Šar Mountains (Ljuboten), is confirmed in the same locality. A photograph of a herbarium or live specimen, along with a map of its distribution in the country, is given for each presented taxa.

Key words: confirmation, first record, *Hieracium*, species, Macedonia.

Introduction

The diversity and distribution of the taxonomically problematic genus *Hieracium* s.str. (Asteraceae) in North Macedonia have not been comprehensively studied so far. Data regarding the presence of more than 65 broadly defined species are reported in the floristic literature to date. They do not satisfactorily depict the real diversity and chorology of the genus in the country, with part of them appearing to be unreliable. Additionally, the presence of many taxa, known only from a single or few localities published in the first half of the 20th century, lacks recent confirmations in the field. This, apparently insufficient knowledge regarding this genus, suggests the necessity for its further taxonomic and chorological studies in the country.

During the author's floristic fieldwork in various mountain regions of North Macedonia over the past two decades, special attention has been paid to the genus *Hieracium* s.str. Thereby, an abundant collection of herbarium material and photographs of live specimens has been provided, part of which have already been studied and published. As the most noteworthy published data could be mentioned the first record of six species in the country [*H. jurassicum* Griseb., *H. jankae* R. Uechtr., *H. maculatum* Schrank, *H. camkorijense* Zahn, *H. paniculatissimum* (Zahn) Teofilovski and *H. pellense* Gottschl. & Dunkel.] and the description of one species new to science (*H. micevskii* Teofilovski) (Teofilovski 2011, 2019, 2021, 2022). The more recent author's studies have revealed some additional *Hieracium* species that were previously unknown in the country or were little known in the literature, often by a single, very old record. Aiming to update the present knowledge regarding the diversity and chorology of this genus in the country and in general, a part of such new data is presented in this article.

Material and methods

The fieldwork was conducted in the period 1998 – 2023, mainly in the mountains of western, southern, and eastern parts of North Macedonia. It was carried out in the frame of enthusiastic floristic studies, some professional engagements, and engagements in some applicative projects. Appropriate, mostly abundant, plant material was herbarized and photographed. Herbarium material is stored in the herbarium of the

author. Relevant treatments of the *Hieracim* s.str. were used for identification of the collections and providing data for their distribution in North Macedonia (when present) and their general distribution: Boissier 1875, Frain 1895, Zhan 1921-1923, 1931-1935, 1936-1938, Hayek 1928-1931, and Nyárády 1965. Scans of the type or other specimens belonging to the studied or some other relevant taxa, from various European public herbaria, are used as comparative material. All the presented herbarium collections are collected and identified by the author of this article (A.T.).

Results and discussions

Hieracium glabratum subsp. nudum Nägeli & Peter (Figures 1, 2)

= *H. glabratum* subsp. *glabrescens* (F. W. Schultz) Dalla Torre & Sarnth.

Šar Mountains: Ljuboten, grassy place, limestone, 2180 m, 42.204252°N, 21.114246°E, 28.7.2014, leg. & det. A.T.

This is the first confirmation of the presence of this species and subspecies in North Macedonia. The previous reports were based on a single collection gathered by J. Bornmüller in 1918, also in Ljuboten (Šar Mountains) (Bornmüller 1926, Zhan 1931-1935, as *H. glabratum* subsp. *glabrescns*). In Euro+Med Plantbase the presence of *H. glabratum* in North Macedonia is considered questionable (Greuter 2006+). *H. glabratum* subsp. *nudum* is distributed in the southern Alps, Dinarides, and Romanian Carpatians, extending southward to the Ljuboten peak, in the Šar Mountains. The nearest localities to the one in the Šar Mountains are situated in North Albania (Prokletije Mts.) (Zhan 1931-1935), ca. 110 km northwest.

Figure 1. *Hieracium glabratum* subsp. *nudum* - herbarium specimen from Ljuboten peak, Šar Mountains, scale bar 2 cm.

Figure 2. *Hieracium glabratum* subsp. *nudum* - distribution in North Macedonia (confirmed locality from literature).

Hieracium lachenalii Suter s.l. (Figures 3, 4)

Jakupica Mt.: road to Begovo Pole, shrubby place, 1796 m, 41.737802°N, 21.433354°E, 27.7.2023. leg. & det. A.T.

This is the first confirmation of the presence of this species in the country, previously known only by a single and doubtful data from the vicinity of Valandovo (Kajali) (Stojanoff 1928, as *H. vulgatum* Fr.). This old literature data requires confirmation, particularly as the xerothermic habitats, widely prevailing in the locality, seem to be not well appropriate for this species.

H. lachenalii s.l. is among the most polymorphic complexes of *Hieracium* s. str., with more than 150 recognized subspecies so far (Zhan 1921-1923, as *H. vulgatum*, 1931-1935; Greuter 2006+). It is distributed throughout much of Europe (northward up to the subarctic parts while becoming rare in the Mediterra-

nean) and central and temperate parts of W Asia. It inhabits forest clearings and margins, shrubby places, and sparse forests (Zhan 1921-1923). It is known to occur, although with a scattered distribution, in all countries neighboring North Macedonia (Stojanov & al. 1966, Gajić 1975, Josifović 1986, Gottschlich & al. 2011, Gottschlich & Barina 2017).

Figure 3. *Hieracium lachenalii* s.l. - herbarium specimen from Jakupica Mt., scale bar 2 cm.

Figure 4. *Hieracium lachenalii* s.l. - distribution in North Macedonia

(●- new locality, ○ - doubtful locality from literature).

Hieracium macropannosum (Rech. f. & Zahn) Greuter (Figures 5, 6)

≡ *H. pilosissimum* subsp. *macropannosum* Rech. f. & Zahn, ≡ *H. chalcidicum* subsp. *macropannosum* (Rech. f. & Zahn) Greuter.

Delčevo: NE of Dzvegor vill., black pine forests, limestone, 930-1110 m, 41.980224°N, 22.838173°E, 24 & 25.6.2021, 14.7.2021, 30.7.2022, leg. & det. A.T.

This is the first record of this species in North Macedonia. It was described from Leila Mt. in Northern Greece (Rechinger 1939, as *H. pilosissimum* subsp. *macropannosum*), and has not been found elsewhere since then. The data regarding its distribution in the northeastern floristic region of Greece (Dimopoulos & al. 2013, as *H. chalcidicum* subsp. *macropannosum*) is most likely based on the protologue.

The rank of a separate species of *H. macropanosum* was initially proposed by Greuter (2005), who, however, two years later accepted its original rank of subspecies, proposing a new combination - *H. chalcidicum* subsp. *macropannosum* (Rech. f. & Zahn) Greuter (Greuter 2007). Szeląg (2018), included *H. macropannosum* in the newly extended *H. heldreichii* agg., which, includes all species intermediating between *H. pannosum* s.l. and *H. racemosum* s.l. Such intermediate species occur within the range of *H. pannosum* s.l., which includes the Balkan Peninsula, Turkey, and Armenia (Greuter 2006+). In North Macedonia, only two additional species of *H. heldreichii* agg. are known to be present - *H. chalcidicum* Boiss. & Heldr. and *H. heldreichii* Boiss., both reported from the locality Markovi Kuli near Prilep [see Zhan 1936-1938, as *H. pilosissimum* ssp. *chalcidicum* (Boiss. & Heldr.) Zahn and *H. heldreichii* Boiss. subsp. *heldreichii*].

H. macropannosum is a common species throughout the *Pinus nigra* forests in the vicinity of Zvegor village, which covers a limestone area of nearly 200 ha. This area is the only enclave of limestone geological substrate in the eastern parts of North Macedonia which is situated c. 110 km northeast of the type locality, Leila Mt. in N Greece. According to the labels of the specimens in the type collection [100001170 (B); 1046709 (LD), 000648180 (BM), 2138 (Herb. Mus. Hist. Natur. Vindob.)], in Leila Mt. this species grows in pine forests on a siliceous substrate.

Figure 5. *Hieracium macropannosum* - specimen from Dzvegor village, Delčevo (Photo A. Teofilovski).

Figure 6. *Hieracium macropannosum* - distribution (first record) in North Macedonia.

Hieracium oxyodon Fr. subsp. oxyodon (Figures 7, 8)

Šar Mountains: Brezjanski Kuli, stony places, limestone, 1800-1850 m, 42.101134°N, 20.993418°E, 19.6.2018, 12.7.2018, leg. & det. A.T.; Šar Mountains: Plat, limestone rocks, 2060 m, 42.028600°N, 20.814596°E, 21.7.2012, leg. & det. A.T.; Šar Mountains: Plat, limestone rocks, 1850 m, 42.030188°N, 20.828876°E, 7.6.2018, leg. & det. A.T.

These are the first records of this species and subspecies in North Macedonia. *H. oxyodon* is distributed in the mountains of C Europe (Alps, Jura) and the Balkan Peninsula (Dinarides, Šar Mountains). It is considered a hybrid species, presumably originating from *H. glaucum* (or *H. bubpleuroides*) and *H. bifidum* (*H. glaucum* > *H. bifidum* or *H. bubpleuroides* > *H. bifidum*) (Zhan 1931-1935). The cited author accepted 22 subspecies in its frame. *H. oxyodon* subsp. *oxyodon* has a similar range of distribution with *H. oxyodon* s.l. but missing in Germany and Croatia (Zhan 1931-1935, Greuter 2006+, Niketič & al. 2021). In the Balkan Peninsula, it is known for Monte Negro (Durmitor Mt., Komovi Mt.), Serbia (Mučanj Mt.), and Kosovo (Prokletije Mt., Šar Mountains) (Niketić 1999, Niketić &

Figure 7. *Hieracium oxyodon* subsp. *oxyodon* - herbarium specimen from Brezjanski Kuli, Šar Mountains, scale bar 2 cm.

Figure 8. *Hieracium oxyodon* subsp. *oxyodon* - distribution (first records) in North Macedonia.

al. 2021).

Hieracium prenanthoides Vill. s.l. (Figures 9, 10)

Bistra Mt.: Careva Češma, limestone, 1700-1720 m, 41.64639°N, 20.70913°E, 17.7.2022, leg. & det. A.T.

Until now, information on this striking species in North Macedonia was limited to a single report from the locality Vojtina in Galičica Mt., which was based on material collected more than a century ago by K. Vandas [Zhan 1936-1938, as *H. prenanthoides* subsp. *bupleurifolium* (Tausch) Zahn]. In the new locality in Bistra Mt. only a small population of c. 20 specimens were observed, growing just near the road Mavrovo-Galičnik, on shallow soil with abundant herbaceous plant cover (*Anthyllis vulneraria*, *Calamagrostis* sp., *Chamaenerion dodonaei*, *Lathyrus pratensis*, *Helianthemum nummularium*, *Onobrychis scardica*, *Sedum ochroleucum*, *Achillea holosericea*, *Leontodon hispidus*, *Lactuca muralis*, etc.).

H. prenanthoides s.l. is a polymorphic complex, in which Zhan (1921-1923) recognized 40 subspecies many of them originally described as separate species. It has a wide distribution in much of Europe and N & W Asia but is rare in the Balkan Penisula. In the neighboring countries to North Macedonia, it has only been reported from Korab Mt. (Albania) (Gottschlich & Barina 2017), Kruševac (Serbia) (Zhan 1936-1938, Gajić 1975), W & C Stara Planina Mt., Rila Mt. (Bulgaria) (Stojanov & al. 1966), and Chelmos Mt. (Greece) (Gottschlich & Raabe 2011).

Hieracium pseudofastigiatum Degen & Zahn (Figures 11, 12)

≡ *H. umbrosum* subsp. *pseudofastigiatum* (Degen &

Figure 9. *Hieracium prenanthoides* s.l. - herbarium specimen from Careva Češma, Bistra Mt., scale bar 3 cm.

Figure 10. *Hieracium prenanthoides* - distribution in North Macedonia
(●- new locality, ○ - locality from literature).

Zahn) Zahn.

Šar Mountains: Selce Keč vill., beech forest, 1010 m, 41.933263°N, 20.866613°E, 24.9.2015, leg. & det. A.T. (Teofilovski 2016, as *H. umbrsum* s.l.); Šar Mountains: Novo Selo vill., forest treeline, 1640 m, 41.942244°N, 20.837925°E, 5.9.2015, leg. & det. A.T. (Teofilovski 2016, as *H. umbrsum* s.l.); Suva Gora Mt.: Trnovo vill., deciduous forest, limestone, 1360 m, 41.768333°N, 21.004730°E, 28.6.2023, leg. & det. A.T.; Bistra Mt.: NW -W of Straža, roadside, limestone, 1418 m, 41.673187° N, 20.844388°E, 14.6.2022, leg. & det. A.T.; Ilinska Mt.: above Prostranje vill., roadside in beech forest, 7.2020, leg. & det. A.T.; Jablanica Mt.: Gorna Belica vill., beech forest, 41.223712°N, 20.544648°E, 1540 m, 3.7.2017, leg. & det. A.T.; Galičica Mt.: Evla vill., sparse mixed *Fagus* and *Ostrya* forest, 1400 m, 15.7.2010, leg. & det. A.T. (Teofilovski 2011, as *H. umbrosum* s.l.).

The only previously reported locality of this species in the country was Korab Mt. (Zahn 1938, as *H. umbrosum* subsp. *pseudofastigiatum*). Besides North Macedonia, this species is also present in Albania, Serbia, Bulgaria, and Romania (Zahn 1938, Greuter 2006+, Niketić & al. 2020). The locality near Evla village in Galičica Mt. marks the new southernmost point of its known distribution, with the previously known one being situated c. 85 km NW-N, in Korab Mt.

H. pseudofastigiatum belongs to the hybridogenic complex *H. umbrosum* whose origin is interpreted by Zhan (1936-1938) as "*H. murorum* > *H. prenanthoides*". In this complex, which is distributed throughout much of Europe, the cited author included 20 subspecies, some of them originally being described as distinct species. In North Macedonia, there is a literature report also for *H. umbrosum* Jord. subsp. *umbrosum* (Jakupica Mt. - Pepelak, Behr & al. 1938).

Conclusions

1. Two *Hieracium* s.str. species are reported for the first time in North Macedonia:

- *H. macropannosum*, previously known only from the type locality in NE Greece (Leila Mt.), is recorded near Delčevo (Dzvegor village), 110 km northwest. Here it grows abundantly, in black pine forests, on limestone.

- The mainly central European species *H. oxyodon* is recorded in the subalpine zone of Šar Mountains (Plat, Brezno village). The collected specimens belong to subsp. *oxyodon*, known also from the Kosovo part of this mountain.

Figure 11. *Hieracium pseudofastigiatum* - herbarium specimen from Gorna Belica vill., Jablanica Mt., scale bar 4 cm.

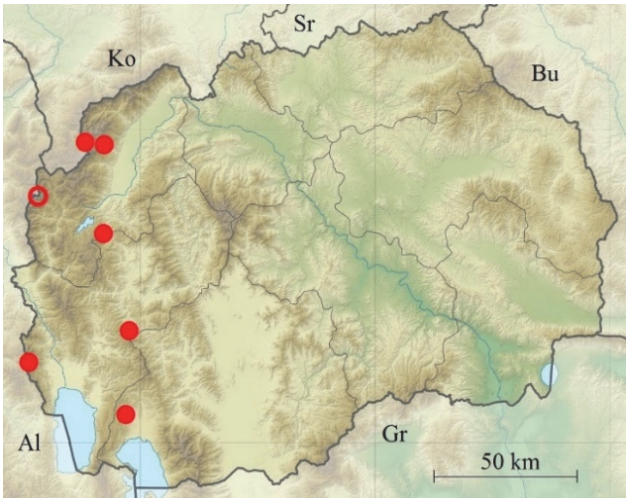


Figure 12. *Hieracium pseudofastigiatum* - distribution in North Macedonia,
(●- new locality, ○ - locality from literature).

2. The presence of three *Hieracium* s.str. species in North Macedonia, in literature known only from a single, old report, is confirmed with its findings in one or more additional locations:

- *H. lachenalii* s.l. is recorded in the upper montane zone of Jakupica Mt. (road to Begovo Pole). The only, almost a century-old literature report in the country, from the vicinity of Valandovo is doubtful, requiring confirmation.

- *H. prenanthoides* s.l. is recorded in Bistra Mt. (Careva Češma). The only previous literature report is based on a single, more than a century-old collection from Galičica Mt.

- The Balkan-Romanian species *H. pseudofastigiatum*, previously reported only from Korab Mt. 85 years ago, has now been confirmed to be more widespread in the country. It is recorded in Šar Mountains, Suva Gora Mt., Bistra Mt., Ilinska Mt., Jablanica Mt., and Galičica Mt. The southernmost point of its distributional range is shifted from Korab Mt. to Galičica Mt., c. 85 km SE-E.

3. The record of *H. glabratum* subsp. *nudum* in Šar Mountains (Ljuboten) confirms the current occurrence of the species and subspecies in the locality and the country, 105 years after its initial finding.

Acknowledgments

I am grateful to Marjan Niketić and Zbigniew Szeląg for reviewing the manuscript and providing valuable suggestions for its improvement and to Zoran Nikolov for organizing the joint field trip to Ljuboten and Jakupica Mt.

References

Behr, O., Behr, E. & Zahn, K. H. (1937). Beiträge zur Kenntnis der Hieracien der Balkanhalbinsel. Glasnik skopskog naučnog društva, Odeljenje prirodnih nauka, 18: 51-67.

Behr, O., Behr, E. & Zahn, K.H. (1938). Beitrage zur Kenntnis der Hieracien der Balkanhalbinsel. – Glasn. Skopsk. Naučn. Društva, Odeljenje Prir. Nauka, 18 (6): 51-67.

Boissier, E. (1875). Flora Orientalis, vol. 3. H. Georg, Geneva & Basilea [Basel], 1033 pp.

Bornmüller, J. (1926). Beiträge zur flora Macedoniens. Engl. Bot. Jahrb., 2. 60: 51-125.

Bornmüller, J. (1932). Bearbeitung der von H. Burgeff und Th. Herzog in den Kriegsjahren 1916-1918 in Mazedonien gesammelten Pflanzen., 3. Feddes Repertorium, 30: 337-362.

Dimopoulos, P., Raus, Th., Bergmeier, E., Constantinidis, Th., Iatrou, G., Kokkini, S., Strid, A. & Tzanoudakis, D. (2013). Vascular plants of Greece: An annotated checklist. Englera, 31: 1-372.

Dunkel, F. G. & Gottschlich, G. (2021). Notes on some taxa of Hieracium and Pilosella (Asteraceae), new for the Greek flora. Phytologia balcanica 24(2): 217–224.

Dunkel, F. G. & Gottschlich, G. (2021). Notes on some taxa of *Hieracium* and *Pilosella* (Asteraceae), new for the Greek flora, II and a nomenclatural comment on *Hieracium* "chalasinense". Phytologia balcanica 27(1): 71–83.

Frain, J. (1895). Ueber neue and bemerkewerthe orientalishe Phlancenarten (fortsetzung). Bulletin de l'Herbier Boissier. 3(10): 36-671.

Gottschlich & Raabe, U. (2011). *Hieracium prenanthoides* Vill., In: Greuter, W. & Raus, T. (eds.), Med-Checklist Notulae, Willdenowia, 30, 41: 311-328.

Gottschlich, G., Bergmeier, E., Minoglou, D. Petermann, J. & Tsiripidis, I. (2011). *Hieracium lachenalii* Suter, In: Greuter, W. & Raus, T. (eds.), Med-Checklist Notulae, Willdenowia, 30, 41: 311-328.

Gajić, M. (1975). Asteraceae Dumortier In: Josifović, M. (ed.). Flora SR Srbije, 7. Srpska akademijanauka i umetnosti, Beograd, 1-465.

Gottschlich, G. & Barina, Z. (2017). *Hieracium lachenalii* Suter. In: Barina, Z. (ed.) Distribution atlas of vascular plants in Albania. Hungarian Natural History Museum, Budapest, 1-445.

Greuter, W. (2005). *Hieracium macropannosum* (Rech. f. & Zahn) Greuter, comb. & stat. nov. In Greuter, W. & Raab-StraubE, E. V. (eds.). Euro+Med Notulae, 1. Willdenowia 35: 223-239.

Greuter, W. (2006+). Compositae (pro parte majore). – In: Greuter, W. & Raab-Straube, E. von (ed.), Compositae. Euro+Med Plantbase - the information resource for Euro-Mediterranean plant diversity. Accessed 7.4.2023.

Greuter, W. (2007). *Hieracium chalcidicum* subsp. *macropannosum* (Rech. f. & Zahn) Greuter, comb. nov. In Greuter, W. & Raab-StraubE, E. V. (eds.). Euro+Med Notulae, 3. Willdenowia 37: 139-189.

Josifović, M. (1986). Flora SR Srbije, 10. Srpska akademijanauka i umetnosti, Beograd, 1-400.

Niketić, M. (1999). *Hieracium oxyodon* Fries subsp. *oxyodon*. In: Stevanović, V. (ed.): The Red Data Book of Flora of Serbia 1. Extinct and critically endan-

gered taxa: 344–345, 511–512. – Ministry of Environment of the Republic of Serbia, Faculty of Biology, University of Belgrade, Institution for protection of nature of the Republic of Serbia, Belgrade. [in Serbian with English Summary]

Niketić, M., Tomović, G., Anačkov, G., Bartula, M., Gjorgjević, V., Gjorgjević-Milošsvić, S., Duraki, Š., gavrilović, M., Jannačković, P., Kabaš, E., Nevena, K., Lakušić, D., Lazarević, P., Perić, R., Randjelović, V., Savić, D., Stanković, M., Stevanovski, I., Stanojević, V., Vasić, O., Vukojićić, S., Zlatković, B., Stevanović, V. (2020). Material on the annotated checklist of vascular flora of Serbia. Nomenclatural, taxonomic and floristic notes III. Bulletin of the Natural History Museum, 13: 87-169. doi:10.5937/bnhmb2013087N

Niketić, M., Tomović, G., Bokić, B., Buzurović, U., Duraki, Š., Djordjević, V., Djurović, S., Krivošej, Z., Lazarević, P., Perić, R., Prodanović, D., Radak, B., Rat, M., Raninirović, M., Stevanović, V. (2021). Material on the annotated checklist of vascular flora of Serbia. Nomenclatural, taxonomic and floristic notes III. Bulletin of the Natural History Museum, 14: 77-132. doi:10.5937/bnhmb2114077N

Nyárády, E. I. (1965). *Hieracium*. – In: Săvulescu, T. (ed.): Flora Reipublicae Popularis Romanicae. Tom. X: 1-746. Editura Academiei Republicii Populare Romîne, Bucuresti.

Rechinger, K.H. (1939). Zur Flora von Ostmazedonien und Westthrazien. – Bot. Jahrb. Syst., 69(4): 419-552. Dimopoulos, P., Raus, Th., Bergmeier, E., Constantinidis, Th., Iatrou, G., Kokkini, S., Strid, A. & Tzanoudakis, D. 2013. Vascular plants of Greece: An annotated checklist. Englera, 31: 1-372.

Stojanoff, N. (1928). Thracische und Mazedonische Herbarmaterialen des Verstorbenen Prof. Dr. Theodor Nikoloff. Zeitschr. Bulg. Acad. Wissensch., 18: 49-209.

Stojanov, N., Stefanov, B. & Kitanov, B. (1966). Flora of Bulgaria. Ed. 4, vol. 1. Naouka & Izkoustvo, Sofia (in Bulgarian).

Szeląg, Z. (2018). Hieracia balcanica XV. Taxonomic and nomenclatural notes on *Hieracium pilosissimum* and *H. divaricatum*, with remarks on the *H. heldreichii* aggregate (Asteraceae). Phytotaxa 356 (1): 81-90. https://doi.org/10.11646/phytotaxa.356.1.7

Teofilovski A. (2016). Reports 85-96, In Vladimirov, V., Dane, F., Matevski, V. & Tan, Kit, New floristic records in the Balkans, 29. Phytol. Balcan. 22(1): 108-112.

Teofilovski A. (2019). New chorological data on *Hieracium* (Asteraceae) in North Macedonia. Glasnik Hrvatskog Botaničkog Društva 7(2): 71–75.

Teofilovski A. (2021). Novelties of *Hieracium* s. str. (Asteraceae) in the flora of North Macedonia, Acta Musei Macedonici Scientarum Naturalium, 24: 45-50.

Teofilovski A. (2022). *Hieracium micevskii* (Asteraceae), a new species from North Macedonia. Botanica Serbica 46(1): 121-124. DOI: https://doi.org/10.2298/BOTSERB2201121T

Teofilovski, A. (2011). Contributions to the flora of the Republic of Macedonia. Private edition, Skopje.

Zahn, K. H. (1921-1923). *Hieracium* L. In: Engler,A. (ed.): Das Pflanzenreich (75, 76, 77, 79, 82). Engelmann, Leipzig, 1-1705.

Zahn, K. H. (1922-1930). *Hieracium* L. In: Ascherson, P., Graebner, P.: Synopsis der mitteleuropäischen Flora 12(1). Borntraeger, Leipzig, 1-492.

Zahn, K. H. (1931-1935). *Hieracium* L. In: Ascherson, P., Graebner, P.: Synopsis der mitteleuropäischen Flora 12(2). Borntraeger, Leipzig, 1-790.

Zahn, K. H. (1936-1938). *Hieracium* L. In: Ascherson, P., Graebner, P.: Synopsis der mitteleuropäischen Flora 12(3). Borntraeger, Leipzig, 1-708.

UDK: 582.929.4(497.7)
Received: 10.10.2023
Accepted: 12.10.2023
Original scientific paper

Acta Musei Macedonici Scientiarum Naturalium, 2023, Vol. 26, pp: 35-44
ISSN: 0583-4988 (printed version)
ISSN: 2545-4587 (on-line version)
Available on-line at: www.acta.musmacscinat.mk

Orobanche pancicii Beck (*Orobanchaceae*) in the flora of the Republic of North Macedonia

Zoran Nikolov

Natural History Museum of the Republic of North Macedonia, Blvd. Ilinden, 86, 1000, Skopje, Republic of North Macedonia.
E-mail: znikolov61@yahoo.com.mk

Abstract

The confirmation of the presence of *Orobanche pancicii* Beck, in the flora of North Macedonia, is presented in this paper. The only data for the occurrence of this species is from 1921 by Stojanov, for the mountain Belasica (southeastern part of North Macedonia). The new findings includes the mountains in the central (Karadjica, Kozjak) and western part of North Macedonia (Korab, Jablanica). In general, the distribution area of *O. pancici* includes Balkan Peninsula and countries from Central Europe (Austria, Hungary). In Euro+Med PlantBase, North Macedonia is not included in the distribution area of this species. *O. pancicii* belongs to the Subsect. Glandulosae that includes species in which corolla, especially the upper lip is covered with more or less dense dark-purple glandular hairs. *Knautia*- and *Scabiosa*-species appear to be the most frequent host plants for *O. pancicii*, but also some others are reported: *Valeriana montana*, *Ligustrum vulgare*, *Wulfenia carinthiaca, Centaurea scabiosa, Cephalaria sp., Euonimus europaeus, Ailanthus altissima*. In our case, *Stachys tymphaea* and *Valeriana montana* are determined as certain host plants while the host plants for the population from the mountain Kozjak (Prilep) stay unknown. As it is mentioned above, *V. montana* is already known, but *S. tymphaea* is so far unknown host plant for *O. pancicii*. Species of the genus *Hieracium*, alleged for the mountain Belasica, are also unknown host plants, for this species.

Key words: rare species, host plants, North Macedonia.

Introduction

The work on the genus *Orobanche*, for more than a decade, revealed both new and rare species for the flora of North Macedonia (Nikolov, 2019, 2020, 2022). A total of 25 species for the territory of North Macedonia was reported, based on literature and new data (Nikolov, 2022). Meanwhile, another new species, *Orobanche laserpitii-sileris* Reut. ex Jord. for our flora, was recorded by Dimitrov (2022). Consequently, the number of species now reaches 26.

The presence of *Orobanche pancicii* Beck (Fig. 1) in the flora of North Macedonia, was based on only one and very old record by Stojanov (1921) for the mountain Belasica (southeastern part of North Macedonia). The new findings are the first confirmation after more than a century, for its occurrence in our flora and significantly extends the distribution area on the territory of North Macedonia.

General distribution

Up to now, the presence of *Orobanche pancicii* is reported from Austria, Hungary, Slovenia, Bosnia and Herzegovina, Serbia, Montenegro, Albania, Bulgaria and Greece (Rohlena, 1942; Parabućski, 1974; Hartvig, 1991; Pusch, 2000; Pusch & Günter 2009; Kiraly, Barina, Horvath & Mesterhazy, 2005; Dakskobler, Vreš & Pusch, 2010; Domina & von Raab-Straube (2010+); Stojanov, 2020).

Taxonomy position of *Orobanche pancicii*

Orobanche pancicii belongs to the subsect. Glandulosae (Beck) Teryokhin which comprises species with dark purple glandular hairs on the corolla. There are, in this subsection, 11 worldwide species, six of them in Europe (Pusch, 2000). Among the latter, *Orobanche alba* Stephan ex Willd., *Orobanche reticulata* Wallr. and *Orobanche serbica* Beck & Petrović are recorded for the territory of North Macedonia (Grecescu, 1899; Vandas, 1909: Stojanov, 1921; Jurišić, 1923; Urumov, 1923; Bornmüller, 1928; Grebenščikov, 1937(1938); Soška,

Fig. 1. *Orobanche pancicii* Beck
Kozjak (Prilep)

1937(1938), 1938-1939a, 1941; Petrovič, 1941: Černjavski, 1943; Rudski, 1943; Grupče, 1958; Drenkovski, 1969; Leute, 1978; Teofilovski, 2011; Nikolov, 2020).

Host plants

O. pancicii parasitizes mostly on *Dipsacaceae*-species: *Scabiosa leucophylla* Borbás, *Knautia fleischmanni* (Rchb.) Pacher, *K. magnifica* Boiss., *K. arvensis* (L.) Coult., *K. drymeia* Heuff., *K. dipsacifolia* Kreuzer, but also on *Valeriana montana* L., *Ligustrum vulgare* L., *Wulfenia carinthiaca* Jacq., *Centaurea scabiosa* L., *Hieracium sp., Cephalaria* sp., *Euonymus europaeus* L., *Ailanthus altissima* (Mill.) Swingle, *Teucrium chamaedrys* L. (Hartvig, 1991; Uhlich, Pusch & Barthel, 1995; Pusch, 2000; Pusch & Barthel, 2009; Stojanov, 2020).

Materials and methods

An abundant material of this species was collected during the field research between 2010 and 2023, especially in the period 2011–2013 when the project "Taxonomy and chorology of the genus *Orobanche* in the Republic of Macedonia" was carried out. According to the standard procedures, the plants were herbarised, labelled and numbered. Different literature sources were used for the identification and distribution of *Orobanche* species: Beck (1890, 1930), Hayek (1929), Chater & Webb (1972), Delipavlov (1995), Hartvig (1991), Pusch (2000), Pusch & Günter (2009). Photos of the whole plants of *O. pancicii* as well as photos with the host plants are presented in the paper. A distribution map with literature records and new data is also presented. The voucher specimens are deposited in the herbarium of the Natural History Museum of the Republic of North Macedonia.

Results and discussion

Orobanche pancicii Beck in Ann. K. K. Naturhist. Hofmus. 2: 148. 1887.

Description of the collected plants from North Macedonia

Stem (11)15-40 (55) cm, erect, simple, yellowish to yellowish-purple, rarely dark purple, with glandular hairs. Scales up to 20 mm, at the base up to 8 mm, lanceolate, covered with dense glandular hairs. Inflorescence (3)4-17(22) cm, mostly dense, many-flowered, with dense glandular hairs. Calyx with two free, mostly unequally bidentate segments, rarely entire. Corolla (16)19-23(26) mm, yellowish, rarely yellow-whitish with less to intense purple to sometimes purple-brown nuisance, the veins often dark purple; the whole corolla especially the upper lip, even from the inner side, covered with more or less dense dark purple glandular hairs; the upper lip emarginated to 2-lobed, the lower 3 -lobed. The filaments inserted at 2-2.5 (3) mm from the corolla base, ciliate in the lower part, near the anthers with few to many glandular hairs; the anthers with rare glandular hairs. The style in the lower part with rare, nearby the stigma with many glandular hairs. The stigma 2-lobed, intense-yellow, with red aureole.

Distribution in North Macedonia

In the following text, a detailed distribution of *O. pancicii* is presented based on the literature and new data for the area of North Macedonia, as well as a distribution map (Fig. 2).

Literature data: Belasica: maedows in beech forest above the villages Gabrovo and Kolešino, on *Hieracium sp.* (Stojanov, 1921).

New data:

Korab mountain

Korab (Štirovica): on the trail to "Golema Korabska Vrata", at the foothill of "Ziberova kula", 2028 m a.s.l., 31.07.2013, No. 9539, N: 41°49'00.1"; E: 020°34'22.3", Leg./Det.: Z. Nikolov; Korab (Štirovica): on the trail to "Golema Korabska Vrata", locality "Pesok", 1850 m a.s.l., 31.07.2013, No. 9540, Leg./Det: Z. Nikolov; Korab (Štirovica): on the trail to "Golema Korabska Vrata", alongside the r. Štirovica", 1900 m a.s.l., 31.07.2013, No. 9541, Leg./Det: Z. Nikolov; Korab (Štirovica): on the trail to "Golema Korabska Vrata", 1900 m a.s.l., 31.07.2013, No. 9542, Leg./Det: Z. Nikolov; Korab (Štirovica): on the trail to "Golema Korabska Vrata", 1900 m a.s.l., 31.07.2013, No. 9543, Leg./Det.: Z. Nikolov; Korab (Štirovica): on the trail to "Golema Korabska Vrata", 1900 m a.s.l., 31.07.2013, No. 9544, Leg./Det.: Z. Nikolov; Korab (Štirovica): on the trail to "Golema Korabska Vrata", 1900 m a.s.l., 31.07.2013, No. 9545, Leg./Det.: Z. Nikolov; Korab (Štirovica): on the trail to "Golema Korabska Vrata", 1900 m a.s.l., 31.07.2013, No. 9546, Leg./Det.: Z. Nikolov; Korab (Štirovica): on the trail to "Golema Korabska Vrata", locality "Pesok", 1700-1800 m a.s.l., 31.07.2013, No. 9547, Leg./Det.: Z. Nikolov; Korab (Štirovica): on the trail to "Golema Korabska Vrata", locality "Pesok", 1700 -1800 m a.s.l., 31.07.2013, No. 9548, Leg./Det.: Z. Nikolov; Korab (Štirovica): on the trail to "Golema Korabska Vrata" (Ziberova kula), locality "Pesok", 1860 m a.s.l., 31.07.2013, No. 9549, N: 41° 49' 27.4"; E: 020° 34' 44.3", Leg./Det.: Z. Nikolov; Korab (Štirovica): on

the trail to "Golema Korabska Vrata" (Ziberova kula), locality "Pesok", 1860 m a.s.l., 31.07.2013, No. 9550. N: 41° 49' 27.4"; E: 020° 34' 44.3", Leg./Det.: Z. Nikolov; Korab (Štirovica): on the trail to "Golema Korabska Vrata" (Ziberova kula), locality "Pesok", 1900 m a.s.l., 31.07.2013, No. 9567. Leg./Det.: Z. Nikolov; Korab (Štirovica): on the trail to "Golema Korabska Vrata" (Ziberova kula), locality "Pesok", 1860 m.n.v.; 31.07.2013; br. 9568. N: 41° 49' 27.4"; E: 020° 34' 44.3". Leg./Det.: Z. Nikolov; Korab (Štirovica): on the trail to "Golema Korabska Vrata" (Ziberova kula), locality "Pesok", 1860 m a.s.l., 31.07.2013, No. 9569. N: 41° 49' 27.4"; E: 020° 34' 44.3", Leg./Det.: Z. Nikolov; Korab (Štirovica): on the trail to "Golema Korabska Vrata" (Ziberova kula), locality "Pesok", 1900 m a.s.l., 31.07.2013, No. 9570, Leg./Det.: Z. Nikolov; Korab (Štirovica): on the trail to "Golema Korabska Vrata" (Ziberova kula), locality "Pesok", 1900 m a.s.l., 31.07.2013, No. 9571 Leg./Det.: Z. Nikolov; Korab (Štirovica): on the trail to "Golema Korabska Vrata" (Ziberova kula), locality "Pesok", 1850 m a.s.l., 31.07.2013, No. 9572, Leg./Det.: Z. Nikolov; Korab (Štirovica): on the trail to "Golema Korabska Vrata" (Ziberova kula), locality "Pesok", 1700-1800 m a.s.l., 31.07.2013, No. 9573, Leg./Det.: Z. Nikolov; Korab (Štirovica): on the trail to "Golema Korabska Vrata" (Ziberova kula), locality "Pesok", 1700-1800 m a.s.l., 31.07.2013, No. 9574, Leg./Det.: Z. Nikolov; Korab (Štirovica): "Kobilino Pole", 2157 m a.s.l., 08.08.2014, No. 11359, N: 41º46'51.3"; E: 020º24'44.0", Leg./Det.: Z. Nikolov; Korab (Štirovica): "Kobilino Pole", 2157 m a.s.l., 08.08.2014, No. 11360, N: 41º46'51.3"; E: 020º24'44.0", Leg./Det.: Z. Nikolov; Korab (Štirovica): "Kobilino Pole", 2160 m a.s.l., 08.08.2014, No. 11423, N: 41º46'51.9"; E: 020º34'44.3", Leg./Det.: Z. Nikolov; Korab (Štirovica): "Kobilino Pole", 2160 m a,s,l., 08.08.2014, No. 11424, N: 41º46'51.9"; E: 020º34'44.3", Leg./Det.: Z. Nikolov; Korab (Štirovica): Kobilino Pole, 2160 m a.s.l., 08.08.2014, No. 11425, N: 41º46'51.9"; E: 020º34'44.3", Leg./Det.: Z. Nikolov; Korab (Štirovica): Kobilino Pole, 2160 m a.s.l., 08.08.2014, No. 11426; N: 41º46'51.9"; E: 020º34'44.3", Leg./Det.: Z. Nikolov; Korab (Štirovica): Kobilino Pole, 2170 m a.s.l., 08.08.2014, No. 11403, Leg./Det.: Z. Nikolov; Korab (Štirovica): Kobilino Pole, 2170 m a.s.l., 08.08.2014, No. 11404, Leg./Det.: Z. Nikolov; Korab (Štirovica): Kobilino Pole, 2170 m a.s.l., 08.08.2014, No. 11405, Leg./Det.: Z. Nikolov; Korab (Štirovica): Kobilino Pole, 2170 m a.s.l., 08.08.2014, No. 11406, Leg./Det.: Z. Nikolov; Korab (Štirovica): Kobilino Pole, 2170 m a.s.l., 08.08.2014, No. 11407, Leg./Det.: Z. Nikolov; Korab (Strezimir): above the watchtower "Štirovica", 1700 m a.s.l., 23.07.2011, No. 8405, Leg./Det.: Z. Nikolov; Korab (Štirovica): Kobilino Pole, 2000-2100 m a.s.l., 04.08.2011, No. 8469, Leg./Det.: Z. Nikolov; Korab (Štirovica): Kobilino Pole, 2000-2100 m a.s.l., 04.08.2011, No. 8471, Leg./Det.: Z. Nikolov; Korab (Štirovica): Kobilino Pole, 2000-2100 m a,s.l., 04.08.2011, No. 8472, Leg./Det.: Z. Nikolov; Korab: in the vicinity of the sheep yard "Korab", 1700 m a.s.l., 22.07.2011, No. 8417, Leg./Det.: Z. Nikolov; Korab: in the vicinity of the sheep yard "Korab", 1750 m a.s.l., 22.07.2011, No. 8495, Leg./Det.: Z. Nikolov; Korab (Strezimir): on the trail to the peak „Golem Korab", 2200 m a.s.l., No. 8496, 22.07.2011, Leg./Det.: Z. Nikolov; Korab: Kobilino Pole, 2000-2100 m.n.v., 04.08.2011; No. 8394, Leg./Det.: Z. Nikolov; Korab: Kobilino Pole, 2000-2100 m a.s.l., 04.08.2011, No. 8395; Leg./Det.: Z. Nikolov; Korab: Kobilino Pole, 2000-2100 m a.s.l., 04.08.2011, No. 8396, Leg./Det.: Z. Nikolov; Korab: Kobilino Pole, 2000-2100 m a.sl., 04.08.2011, No. 8397; Leg./Det.: Z. Nikolov; Korab: Kobilino Pole, 2000-2100 m a.s.l., 04.08.2011, No. 8398, Leg./Det.: Z. Nikolov; Korab: Kobilino Pole, 2000-2100 m a.s.l., 04.08.2011; No. 8399, Leg./Det.: Z. Nikolov; Korab (Strezimir): on the trail to the peak "Golem Korab", 2200 m a.s.l., 22.07.2011, No. 8496, Leg./Det.: Z. Nikolov; Korab: Kobilino pole, 2000 m a.s.l., 04.08.2011, No. 8389, Leg./Det.: Z. Nikolov; Korab (Strezimir): Kobilino pole, 2000-2100 m a.s.l., 21.07.2012, No. 8873, Leg./Det.: Z. Nikolov; Korab (Strezimir): Kobilino pole, 2000-2100 m a.s.l., 21.07.2012, No. 8874, Leg./Det.: Z. Nikolov; Korab (Strezimir): Kobilino pole, 2000-2100 m a.s.l., 21.07.2012, No. 8875, Leg./Det.: Z. Nikolov; Korab (Strezimir): Kobilino pole, 2018 m a.s.l., 04.08.2011, No. 8468, Leg./Det.: Z. Nikolov; Korab (Strezimir): on the trail to the peak "Golem Korab", 1900-2750 m a.s.l., 22.07.2011, No 8351, Leg./Det.: Z. Nikolov; Korab: from „Kobilino pole" to the waterfalls of „Dlaboka Reka", maedows, 1800-2015 m a.s.l., No. 8567, Leg./Det.: Z. Nikolov; Korab: from „Kobilino pole" to the waterfalls of „Dlaboka Reka", maedows, 1800-2015 m a.s.l., No. 856,; Leg./Det.: Z. Nikolov; Korab (Štirovica): on the trail to the peak "Golem Korab", 1900-2200 m a.s.l., 22.07.2011, No. 8410, Leg./Det.: Z. Nikolov; Korab (Štirovica): on the trail to the peak "Golem Korab", 1900-2200 m a.s.l., 22.07.2011, No. 8411, Leg./Det.: Z.

Nikolov; Korab (Štirovica): on the trail to the peak "Golem Korab", 1900-2200 m a.s.l., 22.07.2011, No. 8412, Leg./Det.: Z. Nikolov; Korab (Štirovica): on the trail to the peak "Golem Korab", 1900-2200 m a.s.l., 22.07.2011, No. 8413, Leg./Det.: Z. Nikolov; Korab (Štirovica): on the trail to the the peak "Golem Korab", 1900-2200 m a.s.l., 22.07.2011, br. 8414, Leg./Det.: Z. Nikolov; Korab (Štirovica): on the trail to the peak "Golem Korab", 1900-2200 m a.s.l., 22.07.2011, No. 8415, Leg./Det.: Z. Nikolov; Korab (Štirovica): on the trail to the peak "Golem Korab", 1900-2200 m a.s.l., 22.07.2011, No. 8416, Leg./Det.: Z. Nikolov; Korab: Kobilino Pole, 2300 m a.s.l., No. 8669, 19.07.2003, Leg./Det.: Z. Nikolov; Korab: Kobilino Pole, 2300 m a.s.l., No. 8670, 19.07.2003, Leg./Det.: Z. Nikolov; Korab: Kobilino Pole, 2300 m a.s.l., No. 8671, 19.07.2003, Leg./Det.: Z. Nikolov; Korab: above the v. Tanuše, 1616 m a.s.l., 05.08.2011, No. 8474, Leg./Det.: Z. Nikolov; Korab: obove the v. Tanuše, 1616 m a.s.l., 05.08.2011, No. 8475, Leg./Det.: Z. Nikolov; Korab: above the v. Tanuše, 1616 m a.s.l., 05.08.2011, No. 8476, Leg./Det.: Z. Nikolov; Korab: above the v. Tanuše, 1616 m a.s.l., 05.08.2011, No. 8477, Leg./Det.: Z. Nikolov.

Jablanica mountain

Jablanica (v. Labunište): Upper (small) Labuniško lake, 1933 m a.s.l., 25.07.2013, No. 9698, Leg./Det.: Z. Nikolov; Jablanica (v. Labunište): Upper (small) Labuniško lake, 1933 m a.s.l., 25.07.2013, No. 9699; Leg./Det.: Z. Nikolov; Jablanica (v. Labunište): Upper (small) Labuniško lake, 1933 m a.s.l., 25.07.2013, No. 9700, Leg./Det.: Z. Nikolov; Jablanica (v. Labunište): Upper (small) Labuniško lake, 1933 m a.s.l., 25.07.2013, No. 9701, Leg./Det.: Z. Nikolov; Jablanica (v. Labunište): Upper (small) Labuniško lake, 1933 m a.s.l., 25.07.2013, No. 9702; Leg./Det.: Z. Nikolov; Jablanica (v. Labunište): Upper (small) Labuniško lake, 1933 m a.s.l., 25.07.2013, No. 9703; Leg./Det.: Z. Nikolov; Jablanica: Krstec, mountain pastures, 1800 m a.s.l,, 18.07.2006, No. 8555, Leg./Det.: Z. Nikolov; Jablanica: Krstec, mountain pastures, 1800 m a.s.l., 18.07.2006, No. 8556, Leg./Det.: Z. Nikolov; Jablanica: Krstec, mountain pastures, 1800 m a.s.l., 18.07.2006, No. 8557; Leg./Det.: Z. Nikolov; Jablanica: Krstec, mountain pastures, 1800 m a.s.l., 18.07.2006, No. 8757, Leg./Det.: Z. Nikolov; Jablanica (v. G. Belica): "Krstec", mountain pastures, 1800 m a.s.l., 18.07.2006, br. 8560, Leg./Det.: Z. Nikolov; Jablanica (v. G. Belica): "Krstec", mountain pastures, 1800 m a.s.l., 18.07.2006, No. 856,; Leg./Det.: Z. Nikolov; Jablanica (v. G. Belica): "Krstec", mountain pastures, 1800 m a.s.l., 18.07.2006, No. 8562, Leg./Det.: Z. Nikolov; Jablanica (v. G. Belica): "Krstec", mountain pastures, 1800 m a.s.l., 18.07.2006, No. 8563, Leg./Det.: Z. Nikolov; Jablanica (v. G. Belica): "Krstec", mountain pastures, 1800 m a.s.l., 18.07.2006, No. 8564, Leg./Det.: Z. Nikolov; Jablanica (v. Labuništa): Podgorečko lake, pastures, 1902 m a.s.l., 15.07.2012, No. 8866, Leg./Det.: Z. Nikolov; Jablanica (v. Labuništa): Podgorečko lake, 1900 m a.s.l., 26.07.2012, No. 8885, Leg./Det.: Z. Nikolov; Jablanica (v. Labuništa): Podgorečko ezero, pastures, 1902 m a.s.l., 15.07.2012, No. 8867, Leg./Det.: Z. Nikolov; Jablanica (v. Labuništa): Podgorečko lake, pastures, 1902 m a.s.l., 15.07.201,; No. 8868, Leg./Det.: Z. Nikolov; Jablanica (v. G. Belica): Čuma, 1669 m a.s.l., 11.07.2021, No. 14850, Leg./Det.: Z. Nikolov; Jablanica (v. Labunište): in the vicinity of the upper (small) Labuniško lake, 1900 m a.s.l., 16.07.2021, No. 14854, Leg./Det.: Z. Nikolov

Kozjak mountain (Prilep)

Prilep: Kozjak, 1200-1400 m a.s.l., 26.06.2010, No. 8685, Leg./Det: Z. Nikolov; Prilep: Kozjak, 1300 m a.s.l., 26.06.2010, No. 8679, Leg./Det.: Z. Nikolov; Prilep: Kozjak, 1300 m a.s.l., 26.06.2010, No. 8680, Leg./Det.: Z. Nikolov; Prilep: Kozjak, 1300 m a.s.l., 26.06.2010, No. 8681; Leg./Det.: Z. Nikolov; Prilep: Kozjak, 1300 m a.s.l., 26.06.201, No. 8682, Leg./Det.: Z. Nikolov; Prilep: Kozjak, 1300 m a.s.l., 26.06.2010, No. 8683; Leg./Det.: Z. Nikolov; Prilep: Kozjak, 1300 m a.s.l., 26.06.2010, No. 8684, Leg./Det.: Z. Nikolov; Prilep (v. Pletvar): Kozjak, 1242 m a.s.l., 19.06.2012, No. 8833, Leg./Det.: Z. Nikolov

Karadjica mountain

Karadjica (v. Patiška Reka): in the forest belt, above the village, 1607 m a.s.l., 26.07.2022, No. 14828, N: 41º46.817'; E: 021º19.390', Leg./Det.: Z. Nikolov; Karadjica (v. Patiška Reka): in the forest belt, above the village, 1607 m a.s.l., 26.07.202, No. 14829, N: 41º46.817'; E: 021º19.390', Leg./Det.: Z. Nikolov; Karadjica (v. Patiška Reka): in the forest belt, above the village, 1607 m a.s.l., 26.07.2022, No. 14830, N: 41º46.817'; E: 021º19.390', Leg./Det.: Z. Nikolov; Karadjica (v. Patiška Reka): in the forest belt, above the village, 1607 m a.s.l., 26.07.2022, No. 1483,; N: 41º46.817'; E: 021º19.390', Leg./Det.: Z. Nikolov; Karadjica (v. Patiška Reka): in the forest belt, above the village, 1607 m a.s.l., 26.07.2022, No. 14833, N: 41º46.817'; E: 021º19.390', Leg./Det.: Z. Nikolov; Karadjica (v. Patiška Reka): in the forest belt, above the belt, 1607 m a.s.l., 26.07.2022, No. 14834, N:

41º46.817'; E: 021º19.390', Leg./Det.: Z. Nikolov; Karadjica (v. Patiška Reka): above the village, in the forest belt, 1512 m a.s.l., 15.07.2023, No. 15 158, N: 41º46.859'; E: 021º19.216', Leg./Det.: Z. Nikolov; Karadjica (v. Patiška Reka): above the village, in the forest belt, 1512 m a.s.l., 15.07.2023, No. 15 159, N: 41º46.859'; E: 021º19.216', Leg./Det.: Z. Nikolov; Karadjica (v. Patiška Reka): above the village, in the forest belt, 1512 m a.s.l., 15.07.2023, No. 15 160, N: 41º46.859'; E: 021º19.216', Leg./Det.: Z. Nikolov; Karadjica (v. Patiška Reka): above the village, In the forest belt, 1512 m a.s.l., 15.07.2023, No. 15 161, N: 41º46.859'; E: 021º19.216', Leg./Det.: Z. Nikolov; Karadjica (v. Patiška Reka): above the village, in the forest belt, 1512 m a.s.l., 15.07.2023, No. 15 162, N: 41º46.859'; E: 021º19.216', Leg./Det.: Z. Nikolov; Karadjica (v. Patiška Reka): above the village, in the forest belt, 1512 m a.s.l., 15.07.2023, No. 15 163, N: 41º46.859'; E: 021º19.216', Leg./Det.: Z. Nikolov;

Host plants for *O. pancicii* in North Macedonia

Two species are evident host plants for *O. pancicii* in North Macedonia: *Stachys tymphaea* Hausskn. for the populations from the mountains Korab (Fig. 3, a) and Jablanica and, *Valeriana montana* L. for the plants on Karadjica mountain (Fig. 3, b). While *V. montana* is already known as a host plant (Uhlich, Pusch & Barthel, 1995), *S. tymphaea*, in general, was recorded for first time, as a host plant of *O. pancicii*. Unfortunately, the host plants from the mountain Kozjak (Prilep), are unknown. Although the population of *O. pancicii,* from

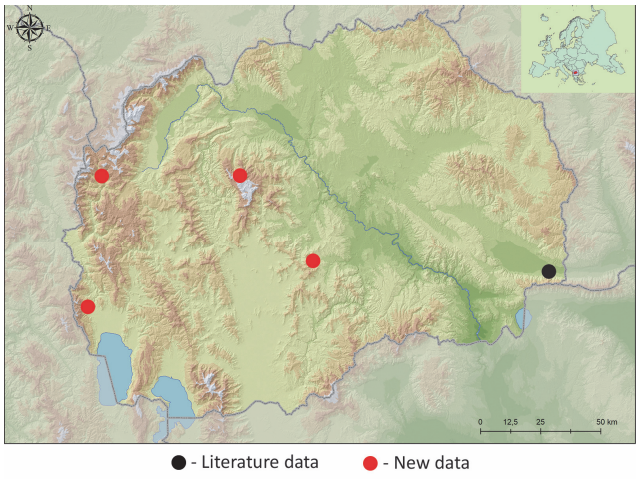


Fig. 2. The distribution of *Orobanche pancicii* in North Macedonia

this locality (Kozjak), was found in the middle of well-developed population of *Trifolium pignantii* Fauché & Chaub. (Fig. 4, a), still, we can speak only for "probable or supposed" host because of lack of a solid evidence for the connection parasite – host plant.

Conclusion

- The plants of *O. pancicii*, found in North Macedonia, completely match the description given by Beck (1890, 1930), Hartvig (1991), Delipavlov (1995), Pusch (2000, 2009), Stojanov (2020). The variabilities of the stem- and corolla color, noticed by plants from all localities (Kozjak, Karadjica, Korab, Jablanica), give them different appearance (Fig. 4–7) but the color has no taxonomical importance in this species (Pusch, 2009),

Fig. 3. *Orobanche pancicii* Beck with the hosts
a) *Stachys tymphaea* Hausskn. b) *Valeriana montana* L.

Fig. 4. *Orobanche pancicii* Beck
a, b) Kozjak mountain (Prilep)

Fig. 5. *Orobanche pancicii* Beck
a) Karadjica mountain b) Korab mountain

Fig. 6. *Orobanche pancicii* Beck
Korab: a) Kobilino pole, with the host *Stachys tymphaea* Ten. b) above the watchtower "Štirovica"

Fig. 7. *Orobanche pancicii* Beck
Jablanica: a) Lower (Big) Labuniško lake b) Podgorečko lake

- *O. pancicii* grows on meadows and mountain pastures in altitudinal range from 1300 (Kozjak mountain) to 2400 (Korab mountain) m a.s.l., on limestone. The flowering time is from mid. of June to mid. of August,

- The new findings extend the distribution area from eastern to central and western part of North Macedonia,

- Host plants, determined so far, are *Stachys tymphaea* and *Valeriana montana*. Also, another host plant species are probable.

Acknowledgement

I would like to express my sincerest thanks to Aco Teofilovski, Dejan Mandjukovski, Marko Naumovski and Mitko Sovkovski for all their help during the field researches. Also, my gratitude to the reviewers Simona Strgulc Krajšek and Kiril Stoyanov for their immense help in improving the manuscript. And, I am grateful to our Ministry of culture of North Macedonia for the financial support of the project “Taxonomy and chorology of the genus *Orobanche* in the Republic of Macedonia” (2011-2013).

References

Beck, G., 1890: Monographie der Gattung *Orobanche*. Bibl. Bot., 19:1-275. Theodor Fischer, Cassel.

Beck, G., 1930: *Orobanchaceae*. In: Engler, A. (Eds.). Das Pflanzenreich Regni vegetabilis conspectus, 96 (IV/261):1–348. Verlag von Wilhelm Engelmann, Leipzig.

Bornmüller, J., 1928: Beiträge zur Flora Mazedoniens. III. Sammlungen in den Kriegsjahren 1916 – 1918. Bot. Jahrb. Syst. 61. Beibl. 140: 1 – 195.

Chater, A. O. & Webb, D. A., 1972: *Orobanche*. In: Tutin, T. G., Heywood, V. H., Burges, N. A., Moore, D. M., Valentine, D. H., Walters, S. M. & Webb, D. A. Flora Europaea, 3:286-293. Cambridge.

Černjavski, P., 1943: Prilog za florističko poznavanje šire okoline Ohridskog jezera. Ohridski zbornik, 35(2):11 -88. Srpska Kraljevska Akademija, Posebna izdanja. Beograd.

Dakskobler, I., Vreš, B. & Pusch, J., 2010: *Orobanche pancicii* Beck, a new species to the flora of Slovenia. Hacquetia 9/2:171–176.

Delipavlov, D., 1995: *Orobanche*. In: Kožuharov, S.I. & Kuzmanov, B.A. (Eds.). Flora Republicae Bulgaricae, 10:291-325. BAN. Sofia.

Dimitrov, D., 2022: *Orobanche laserpitii-sileris*. In: Raab -Straube, E. von & Raus, Th. (ed.) 2022: Euro+Med-Checklist Notulae, 15. – Willdenowia 52: 273-299.

Drenkovski, R., 1969: Ein Überblick über die flora des Vodnogebirges. GzPMF, Skopje, 21, 163 - 184.

Euro+Med PlantBase - Preview of the new data portal. Source: Domina, G. & Raab-Straube, E. von (2010+): *Orobanche*. – In: Euro+Med Plantbase - the information resource for Euro-Mediterranean plant diversity.

Grecescu, D., 1899: Plantes de la Macedoine, apartenant Au vilayet de Monastir. Faculte de Medicine de Bucarest, Labaratoire de Botanique, 01-52 pp. Imprimerie de "L'independence Roumaine". Bucarest.

Grebenščikov, O., 1937(1938): Biljno-geografski pregled šuma u slivu gornjr Radike. Glasnik SND, 18(6):107-124. Skopje.

Grupče, Lj., 1958: Vrz rastitelnosta na Skopska Crna Gora. FFU - Skopje, 9:3-80.

Hartvig, P, 1991: *Orobanche*. In: Strid, A. & Tan, K. Mountain flora of Greece, 2. Edinburgh University Press.

Hayek, A., 1929: Prodromus Florae Peninsulae Balcanicae, 2:224-225. Verlag des Repertoriums. Dahlem bei Berlin.

Jurišić, Ž., 1923: Prilog flori Južne Srbije. Spom. SKA, 60:1-45.

Kiraly, G., Barina, Z., Horvath, T. & Mesterhazy, A., 2005: Occurrence of *Orobanche pancicii* Beck in Hungary. Flora Pannonica, 3:3-15.

Leute, H.G., 1978: Ergebnisse einer botanischen Sammelreise durch das südliche Jugoslawien im Jahre 1969. Ann. Naturhistor. Mus. Wien, 81:94.

Nikolov, Z., 2019: *Orobanche elator* Suton and *Orobanche centaurina* Bertol. (*Orobanchaceae*) in the locality “Kozjak” (Skopje) in North Macedonia. ACTA Musei Macedonici Scientarum Naturalium, 22/1:13-20. Skopje.

Nikolov, Z., 2020: New and rare species of the genus *Orobanche* (Broomrapes) in the flora of the Republic of North Macedonia. Acta Musei Macedonici Scientarum Naturalium, 23(1): 19-30. Skopje.

Nikolov, Z., 2022: *Orobanche alsatica* Kirschl. (*Orobanchaceae*) – another new species of the genus *Orobanche* in the flora of the Republic of North Macedonia. Acta Musei Macedonici Scientarum Naturalium, 25(1):67-70. Skopje.

Parabućski, S., 1974: *Orobanche*. In Josifović: Flora SR Srbije (ed.), 6:286-308. SANU, Beograd.

Petrović, J., 1941: Prilog flori Skopske Kotline. Glasnik SND, 22(8):79-89. Skopje.

Pusch, J., 2000: *Orobanche pančićii* – neu für Österreich und für ganz Mitelleuropa. Floristische Rundbriefe (Flor. Rundbr.), 34(1), 29-42. Bochum.

Pusch, J. & Günther, K.F., 2009: *Orobanchaceae* (Sommerwurzgewächse). In: J. Pusch & K. F. Günther (Eds.). G. Hegi. Illustrierte Flora von Mitteleuropa, Bd. 6/1A, Lieferung 1; p. 1-99. Weissdorn-Verlag, Jena.

Rohlena, J., 1942: Conspectus Florae Montenegrinae. Preslia. Vest.Češ.Bot.Spol., 21-22:1- 506. Praha.

Rudski, I., 1943: Prilog za poznavanje flore okoline Strumice. Ohridski zbornik, 2(86):205-238.

Soška, Th., 1937(1938): Beitrag zur Kenntnis der Schluchtenfloren von Südserbien, I Teil. BSS Sk., t. XVIII, fasc. 6, p. 223 - 238.

Soška, Th., 1938-1939a: Beitrag zur Kenntnis der Schluchtenfloren von Südserbien, II Teil. BSS Sk., t. XX, fasc. 7, p. 35-58.

Soška, Th., 1941: Beitrag zur Kenntnis der Schluchtenfloren von Südserbien, IV Teil, Östliche Grenzschluchten. 2) Die Schlucht der Bregalnica bei Štip und deren Umgebung. Glasnik Skopskog Naučnog Društva, 22(8): 169-184. Skopje.

Stojanov, K., 2020: Holoparasitic plants of *Orobanchaceae* family in Bulgaria. Agricultural University – Plovdiv, Department of Botany and Agrometeorology.

Stojanov, N. 1921: Floristični materijali od Belasica. Godišnik: Su (FMF), 15 – 16: 123.

Teofilovski, A., 2011: Prilozi za florata na Republika Makedonija. Privatno izdanie, 1-142. Skopje.

Uhlich, H., Pusch, J. & Barthel, K. J., 1995: Die Sommerwurzarten Europas: Gattung *Orobanche.* 235 pp. Westarp-Wiss., Magdeburg.

Urumov, I. K., 1923: Prinosi za florata na Belomorska Trakija. Spisanie BAN, 28(13):1-107. Sofija.

Vandas, C., 1909: Reliquiae Formanekianae. Enumeratio critica plantarum vascularum iquas itineribus in Haemo peninsula et Asia Minore (Bithynia) factis collegit Dr. E. Formanek, Professor gymnasii Brunensis Bohemici. Brunae.

UDK: 582.284-15(497.7)"2020/2022"
Received: 25.10.2023
Accepted: 26.10.2023
Original scientific paper

Acta Musei Macedonici Scientiarum Naturalium, 2023, Vol. 26, pp: 45-53
ISSN: 0583-4988 (printed version)
ISSN: 2545-4587 (on-line version)
Available on-line at: www.acta.musmacscinat.mk

New data on the distribution of hypogeous species *Leucogaster nudus* (Basidiomycota) in the Republic of North Macedonia with note on its taxonomy and morphology

Slavica Tofilovska[1,2*], Mitko Karadelev[1,2], Tome Jovanovski[2] & Katerina Rusevska[1,2]

[1]Ss Cyril and Methodius University, Faculty of Natural Sciences and Mathematics, Institute of Biology, Arhimedova 5, Skopje, North Macedonia
[2]Macedonian Mycological Society, Arhimedova 5, Skopje, North Macedonia
*Corresponding author: stofilovska@pmf.ukim.mk

Abstract

A field work organized in 2020 and 2022 revealed new data on distribution and habitat types for *Leucogaster nudus* in North Macedonia. While examining the newly collected materials and comparing it with the original description of *Hydnangium nudum* a suspicion on the taxonomic position of *L. nudus* arose, since in the original description the species is defined by having warty ornamentation of the spores instead of reticulate. Therefore, we made an extensive literature review on the characteristics of *L. nudus* and its proposed synonyms and provide an in-detail morphological examination of the observed materials with illustrations. In order to clarify the taxonomic position of *L. nudus* and the diversity of this genus in Europe along with morphological, phylogenetic approach is needed.

Key words: hypogeous fungi, diversity, ecology, *Leucogaster fragrans, L. tozzianus, L. badius, L. floccosus, Leucophleps*.

Introduction

The interest on hypogeous fungi is continuously increasing with an aim to get acquainted with the biodiversity of this ecological group of organisms. Until now, a total of 22 hypogeous species within the phylum Basidiomycota are known from North Macedonia (Pilat & Lindthner, 1938; Chavdarova et al., 2011; Karadelev et al., 2018; Tofilovska et al., 2019). The latest article containing data on this topic was a contribution to the checklist of Basidiomycota for North Macedonia where 10 hypogeous species belonging to the genera *Gautieria*, *Hymenogaster*, *Leucogaster*, *Melanogaster* and *Russula* were reported for the first time for the country (Tofilovska et al., 2019).

Genus *Leucogaster* R. Hesse was described based on the characteristics of *L. liosporus* R. Hesse (1882) as the type species and at the moment it comprises 24 valid species according to Index Fungorum database (Index Fungorum, 2023). Species within this genus produce hypogeous to emergent false-truffles and have gleba with characteristic cavities filled with gelatinous mass. They are mostly distributed in the Northern Hemisphere (Zeller & Dodge, 1924; Fogel, 1975) and only one is reported from Australia (Beaton et al., 1985). Most of the species are described from Noth America where this genus has been under revision by Zeller & Dodge (1924) and Fogel (1975) while the European taxa have not been subject of revision and are not clearly delimitated (Fogel, 1975; Montecchi and Sarasini, 2000). By most authors it is regarded that in Europe only the species *L. nudus* (Hazsl.) Hollós is present while other *Leucogaster* taxa as *L. badius* Mattir*., L. fragrans* Mattir*., L. tozzianus* (Cavara & Sacc.) Mattir. ex Zeller & C.W. Dodge*, L. floccosus* R. Hesse are regarded as synonyms (Szemere, 1965; Montecchi and Sarasini, 2000) and here in we follow this concept.

L. nudus is widespread species throughout Europe, known from at least 20 countries. In GBIF 55 occurrences are listed from 12 countries, Austria, Belgium, France, Georgia, Germany, Hungary, Italy, Poland, Slovakia, Spain, Switzerland and United Kingdom, mostly from preserved specimens (GBIF, 2023). It is included in the check list of gasteroid and secotioid fungi of Europe where additionally the Czech Republic, Lithuania, Sweden, Turkey and Ukraine are listed (Kreisel, 2001). Concerning the type of habitat, it is mainly found in decidu-

ous forest of *Fagus* and *Quercus*, in mixed woods with conifers, as well as in *Abies* forests on calcareous and sandy soils, at altitude between 400-1000 m.a.s.l. where the sporocarps are produced from late spring to autumn (Montecchi & Sarasini, 2000; Kreisel, 2001). In Turkey it has been found in mixed forest of *Abies nordmanniana* var. *bornmulleriana* (Mattf.) Coode & Cullen and *Fagus orientalis* Lipsky (Türkoğlu at al., 2015), as well as in mixed forest of *F. orientalis*, *Castanea sativa* Mill., *Rhododendron ponticum* L. and *Alnus* sp., and in mixed forest of *F. orientalis* with *Picea orientalis* (L.) Link and *R. ponticum* (Kaya and Uzun, 2020). In Poland, one of the localities is in *Abies* forest with scattered trees of *Fagus*, *Quercus* and *Salix* on clay soil (Snowarski, 2023). It has also been confirmed on Pieniny Mountains, a limestone mountain range in the Western Carpathians of Poland, where it was proved to be part of the diet of small mammals (Komur et al., 2021). From the neighboring countries it has been recorded in Bulgaria in *Fagus* forest*, Picea* forest and *Abies* forest (Nedelin et al., 2018; Nedelev, 2019), as well as in Serbia (Ivančević, 2016) and Greece (Kauonas, 2015).

Methodology

The examined material was collected in autumn seasons of 2020 and 2022 during fieldwork organized with truffle hunter with trained dogs. Material was collected in the biogeographical region Skopska Crna Gora, a middle altitude mountain range, mostly covered by grasslands, oak and beech forests (Melovski et al., 2013). During the fieldwork data on locality, altitude and type of habitat were noted. The collected basidiocarps were photographed in the field or in the lab with Canon EOS 2000D and Samsung A7. After examination the specimens were dried on air dehydrator at 50°C and deposited in the Macedonian Collection of Fungi (MCF), Mycological laboratory, Institute of Biology, Faculty of Natural Sciences and Mathematics, Skopje. The microscopic analyses were performed on fresh and dried sporocarps, the slides were prepared by cross-section of the peridium and gleba mounted in Meltzer's reagent and 3% KOH. LW Scientific i4 microscope was used for observation of the material prepared in Meltzer's reagent while the photos were taken with MiniVID USB 1MP camera. For the material observed in 3% KOH, microscope ZEISS Primostar 3 was used, photos were taken on ZEISS Axiocam 208 color microscope camera with the Software ZEN 3.0 blue edition for obtaining of the measurements. The photos presented on the figures were arranged in Photoshop CC 2018. Randomly selected mature basidiospores (nu. 60) were measured with included perisporium. The minimum and maximum values of length, width and quotient (Q), along with the average values are presented. Average thickness of the peridium was analyzed by cross section of five basidiocarps and a total number of 25 measurements of the peridium were taken. SEM images were taken using a Phenom G2-Pro desktop scanning electron microscope with accelerator voltage 5 kV and emission current 1.2 nA at the Bialystok University of Technology, Institute of Forest Sciences, Scientific Research Centre in Hajnowka, Poland. The spores were mounted on the SEM sample tip by pressing onto the carbon disk. All samples were coated with gold using a vacuum sputtering machine.

Results and discussion

Leucogaster nudus (Hazslinsky) Hollós (Russulales, Albatrellaceae) was reported for the first time in North Macedonia, as part of a contribution to the check list of Basidiomycota, on only one locality in mixed forest of *Fagus* and *Abies* (Tofilovska et al., 2019). Here in, we report a new data on the distribution of this species in North Macedonia with detailed morphological examination of its characters and critical note on its taxonomy.

Taxonomy

Leucogaster nudus (Hazsl.) Hollós, Annls hist.-nat. Mus. natn. hung. 6: 319 (1908)

Basionym: Hydnangium nudum Hazsl., Verh. Kaiserl. -Königl. zool.-bot. Ges. Wien 25: 64 (1875). Synonym: Leucogaster floccosus R. Hesse, Botan. Centralbl. 40: 1 (1889); Octaviania pityophila L. Becker, Schlesiens unterirdische pilz-flora 35: 356 (1886); Octaviania silesiaca L. Becker, Schlesiens unterirdische pilz-flora 35: 356 (1886).

Morphology

Macroscopic features. Basidiocarp hypogeous, gasteroid type, subglobose, ovoid to irregularly lobbed, 5 – 18 mm long and 3 – 13 mm wide (Fig. 1), solitary to gregarious. Peridium yellow-ochre (Fig. 1a) soon becoming ocher, later when drying is darkening to brown-reddish color, not separable from the gleba, when completely dried at some spots it looks like having warts. Gleba compact, white, gelatinous and very bright, when drying becoming dark yellow-olive, denser and

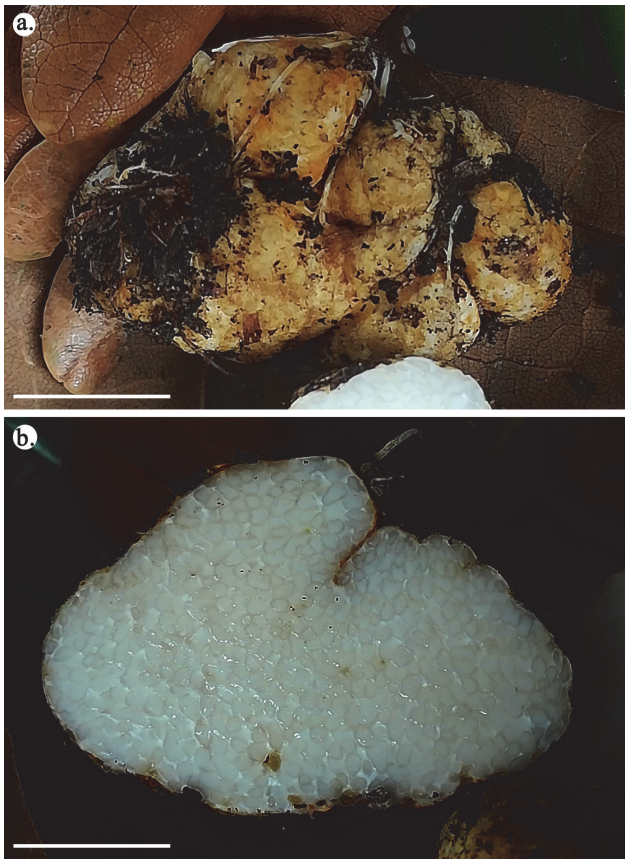


Fig. 1. *Leucogaster nudus,* fresh basidiocarp. a. yellow color of peridium. b. gleba, gelatinous and very bright, chambers can be noticed. Scale bar: a-b. 5 mm. (MCF17818)

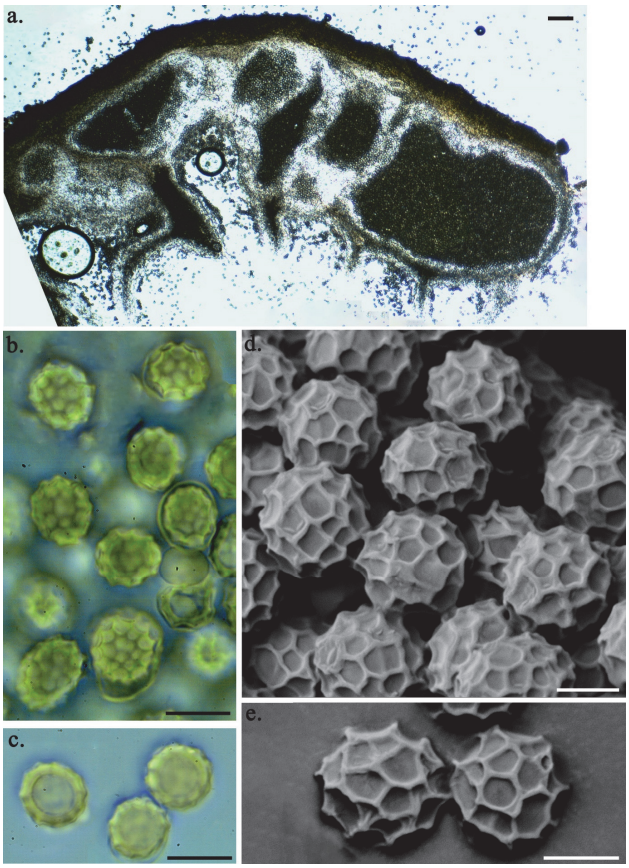


Fig. 2. *Leucogaster nudus.* a. cross section of basidiocarp, peridium and gleba with chambers filled with spores. b-c. basidiospores, focus on reticulate ornamentation (b) and focus on shape (c), also the perisporium can be observed. d-e. basidiospores viewed with SEM, reticulate ornamentation at the conjunction places is taller, spine-like. Scale bar: a. 100 µm, b-c. 10 µm, d-e. 5 µm. Light microscope slides prepared in Meltser's reagent. (a, d-e. MCF17818, b-c MCF17051)

powdery (Fig. 1b), it is composed of round to polygonal chambers 0.3–1.2 mm broad (Fig. 2a), when the fruitbody is cut and fresh the chambers exudates liquid. Odor very pleasant, intensive, fruity, cherry like. Taste is very sweet and also pleasant.

Microscopic features. Peridium is composed of two layers (Figs 3a-b, 4a), external and internal, total thickness varies between 90 µm and 350 µm, on the average is 215 µm (measured on 5 basidiocarps at randomly chosen 25 places in total). External peridium is brown, composed of hyphae that are hard to be observed since they are covered with brown crystal mass, width varies between 50 µm and 215 µm, on average 110 µm, hyphae are ±5 µm wide and cell wall is ±0.5 µm thick. Sometimes in one basidiocarp the color of external peridium can be red-brown or yellow at places in 3% KOH. Internal peridium width varies between 40 µm and 175 µm, on average 105 µm, hyphae are parallel, 2.5 - 5 µm wide and cell wall is ±1 µm thick, at some places hyphae are swollen and look like pseudoparenhymatic tissue, forming more elongated or rounded elements (5.2 × 7; 10 × 12; 5.7 × 15; 9 × 14 µm). Sometimes, especially if the cross-section is thicker the color of internal peridium might become pink at some places in 3% KOH. Tramal plate (Figs. 3a, 4b) composed of elongated, thin hyphae 2.5 – 4 µm wide, at places swollen, somewhere swollen elements look dominant (inflated cells), found in different sizes, smaller or larger, cell wall is ±0.5 µm thick. Basidiospores (Fig. 2b-e, 4c-d) mainly globose, some subglobose, sometimes variable in form due to perisporium formation, size (10) 11–14(15) × (10)10.5–13.5(14) µm, 12.3 × 11.6 µm on average, Q = 1.00–1.20, Qav = 1.05 (n = 60), including the perisporium which might be more adherent to the cell wall or more loosely attached (1 – 1.7 µm), reticulate ornamentation with 3-4 (5) openings, 1 µm tall.

Specimens examined:

Mountain Bistra (10309), vicinity of Mavrovo Lake, 41°41'58.16"N 20°44'7.31"E, hypogeous, mixed forest of *Fagus sylvatica* L. and *Abies borisii-regis* Mattf., classified as Balkano-Pontic Abies forests (G3.17) according to EUNIS (European Nature Information System) Habitat Classification 2012, at altitude 1300 m.a.s.l., leg. Toni Mitrov, Mitko Karadelev, Tine Grebenc, MCF 17051 (01.11.2014). Specimen reported in Tofilovska et al. (2019).

New data

Mountain Skopska Crna Gora (62078), near village Pobozje, 42°07'13.2"N 21°25'39.7"E, hypogeous*, Quercus pubescens* forest, classified as Thermophilous deciduous woodland (G1.7) by EUNIS Habitat Classification 2012, at altitude 650 m.a.s.l., leg. Tome Jovanovski, MCF 17818 (07.11.2020).

Mountain Skopska Crna Gora (62078), near village Brodec, 42°08'59.6"N 21°27'11.0"E, hypogeous, monodominant forest stand of *Fagus sylvatica*, classified as Moesian beech forests (G1.69) by EUNIS Habitat Classification 2012, at altitude 1200 m.a.s.l., leg. Tome Jovanovski, Mitko Karadelev and Slavica Tofilovska, MCF 19486 (12.11.2022).

Habitat

Leucogaster nudus has been found in three types of habitats at an altitude range between 650-1300 m.a.s.l. (Fig. 5). One of the habitats is Balkano-Pontic *Abies* forests which is defined mainly as mixed forest of *Fagus sylvatica* and *Abies borisii-regis*, at the precise locality the edificatory species of the habitat *A. borisii-regis* occurs as a small group of stands in the beech forest. In this type of habitat, usually the shrub layer is not developed or it has a small vegetation cover, while the layer of herbaceous plants and half-shrubs could cover 50% to 90%, however sometimes only 15% of vegetation cover (Matevski et al., 2021). The geological substrate of this locality is diabase (Pendzerkovski & Hadzimitrova, 1977) while the soil type is chromic leptic luvisol on hard limestones according to World Reference Base for Soil Resources Classification System (MaSIS, 2015). The second type of habitat, Moesian beech forests, is characterized by the presence of *Fagus sylvatica* as edificatory species, it comprises pure or mixed broadleaf deciduous forests. This habitat is mainly distributed between 1,100-1,650 m.a.s.l., however sometimes it is found bellow 1,000 m or it may spread up to 2,200 m which is the reason for its high variation of the ecological and flora features (Matevski et al., 2021). This locality is found on geological substrate of biotite-muscovite schist (Pendzerkovski & Hadzimitrova, 1977) while the soil is complex of cambisol, humic eutric and umbric regosol (umbrisol) (MaSIS, 2015). The last type of habitat, the Thermophilous deciduous woodland is a forest stand of *Quercus pubescens* Willd., that is located on a substrate of flysch formations (Pendzerkovski & Hadzimitrova, 1977) and the soil is classified as a complex of rendzic leptosol and chromic leptic luvisol on hard limestones (MaSIS, 2015).

Taxonomic note

The basidiocarp dimensions reported in this study (5-18 mm) corresponds to description of other authors, however the size of 5 mm is reported for the first time, in available publications up until now, the minimum size is 10 mm (Zeller & Dodge, 1924) and the maximum size is 50 mm (Pegler et al., 1993). Montecchi and Sarasini (2000) report size between 10-30 mm, Türkoğlu et al. (2015) between 15-30 mm and Kaya and Uzun (2020) between 20-45 mm.

According to our observations, the total thickness of the peridium is variable when compared between different collections, basidiocarps, as well as in different places in one basidiocarp. For example, in the collection MCF17818 which is consisted of five basidiocarps, in one the measurements varied between 90-240 µm (MCF17818B) and in other basidiocarp between 130-350 µm (MCF17818D). In the other two collections which ave only one basidiocarp the dimensions are 110-270 µm (MCF19486) and 155-330 µm (MCF17051). However, the total thickness of the peridium never reached 500 µm as reported by Montecchi and Sarasini (2000) and Pegler et al. (1993) who report thickness between 200-500 µm. Different size of peridium thickness is reported by other authors as well, 220-520 µm (Türkoğlu et al., 2015), 200-400 µm (Kaya & Uzun, 2020). Interestingly, Fogel (1975) reports smaller dimensions between 85-100 µm while Zeller and Dodge (1924) report only that the peridium is very thin. In the original description in one place is noted that it does not possess a proper peridium, however the author later describes composition of parallel hyphae that form almost pseudoparenchymatic-looking layer and

secrete a brown mucous-like mass which replaces the peridium (Hazslinsky, 1875). Compared with some of the synonymized species, for *Leucogaster badius* is reported size between 200-340 µm (Zeller & Dodge, 1924) and 120-190 µm (Fogel, 1975), for *L. fragrans* (syn. *L. tozzianus*) 140-220 µm (Zeller & Dodge, 1924) and 100-170 µm (35-70 µm after drying) (Fogel, 1975). Türkoğlu et al. (2015) reports *L. tozzianus* as separate species and provides measurements of total peridium thickness of 150-220 µm.

Concerning the spore size in our observations they vary between 10-14 µm and that fits in the reported dimensions by almost all authors, 10-14 µm (Montecchi & Sarasini, 2000), 11-18 µm (Pegler et al., 1993), 13-16 (Fogel, 1975). Though, in the original description a narrower range is given, 16-18 µm and a different ornamentation of the spores which are described as having warty ornamentation (Hazslinsky, 1875). Sizes of the spores, of *L. badius* are 12-16 µm (Zeller & Dodge, 1924), 11-14 µm (Fogel, 1975) and 12-15 µm in the original description (Mattirolo, 1903). For *L. fragrans* (syn. *L. tozzianus*) the size of spores is 12 µm (Zeller & Dodge, 1924), 10-12 µm (Fogel, 1975) and 12 µm in the original description (Mattirolo, 1900). Türkoğlu et al. (2015) reports spore size of 9.7-11.4 µm for *L. tozzianus* while in the original description is 10-12 µm (Saccardo & Cavara, 1900).

At the moment is accepted that in Europe only one species of *Leucogaster* genus is present, *L. nudus*, while the species *L. floccosus, L. badius, L. fragrans* and *L. tozzianus* are regarded as synonyms due to lack of differential characters that could justify the distinction (Szemere, 1965; Montecchi & Sarasini 2000). The materials that are examined in this study correspond to the description of *L. nudus* provided by Montecchi and Sarasini (2000), Pegler et al. (1993), Fogel (1975) but when looking at the original description of *Hydnangium nudum* (Hazslinsky, 1875) the differences rose a suspicion whether the material of *H. nudum* belongs to *Leucogaster*.

Leucogaster nudus (Hazsl.) Hollós was first described by Hazslinsky in 1875 as *Hydnangium nudum* Hazsl. and it was later transferred to *Leucogaster* by Hollós (1908). Hollós compared the type material with *L. fragrans* Mattir., *L. badius* Mattir. and *L. bucholtzii* Mattir. therefore, concluded that *H. nudum* Hazsl. with no doubt belongs to *Leucogaster* genus. Also, Hollós (1908) based only on the drawings and description of *L. liosporus* R. Hesse proposed *L. liosporus* as synonym to *H. nudum*. However, since he did not make comparison with the original material this was not accepted (Zeller & Dodge, 1924). Two of the species which he compared *H. nudum* with to make the transfer, *L. fragrans* and *L. badius* are species with reticulate spore ornamentation which is noted in the original description and is confirmed after examination of other authors (Zeller & Dodge 1924, Fogel 1975). Contradictory, another of the species which Hollós compared *H. nudum* with, *L. bucholtzii* has aculeate ornamentation as noted in the original description (Mattirolo, 1900) and confirmed by Zeller and Dodge (1924) who suggest that this might be only due to phases in spore development. In their publication this is explained as *Leucophleps* stage (page 402), though they provide description of species that have spores with echinate ornamentation as a stable character, as the species *Leucophleps magnata* Harkn. For the type species of *Leucogaster, L. liosporus* in the original description the ornamentation of the spores is not mentioned, just the presence of characteristic exo- and endo-sporium that develops depending on the stage of development of the spores and that the protoplasm is finely grained (Hesse, 1882). Zeller and Dodge (1924) did not have the chance to observe the original material, but as it is stated in the publication, they examined material they believed it corresponded to the description of *L. liosporus* (exsiccate 2605a von Hoehnel Herbarium). Afterwards, Fogel (1979) examined the same material (exsiccate 2605a von Hoehnel Herbarium) and described new species *Leucophleps aculeatispora* Fogel that have aculeate ornamentation. Also, Fogel (1975) gives description of *L. liosporus* based on literature and materials he was able to obtain from Europe (2705a von Hoehnel Herbarium and PR 485777) and described spores with aculate ornamentation in those specimens. Therefore, when Hollós made the transfer of *H. nudum* to *Leucogaster* he compared the type material with species that have aculeate (*L. bucholtzii* and *L. liosporus*) and reticulate ornamentation (*L. fragrans* and *L. badius*). Additionally, the accepted synonym *L. floccosus* R. Hesse in the work of Zeller and Dodge (1924) is described by having echinulate spores with total diameter of 7-10 µm and the authors note that is quite distinct from *L. fragrans* (page 390) expressing surprise why it is regarded as synonym by some authors. Interestingly, Pilát (1937) for *L. floccosus* explains that the mature spores possess a reticulum, while in a paper by Honrubia and Llimona

(1981) they present *L.* cf. *floccosus* with smooth spores expressing suspicion that the material might be too young. It should be examined whether it is really a development stage, or it is maybe a species of *Leucophleps*.

In the original description of *Hydnangium nudum* Hazsl. (Hazslinsky 1875) concerning the spores, their size is between 16-18 µm and the ornamentation is described as warty (Latin description, page 64: "… Sporis globosis in apicibus hypharum evolutis, demum cum articulo hypharum coniformi deciduis; maturis ochraceis episporio pellucido **verrucoso tectis**, diametro 0·016—0·018 Mm."; German description, page 65: "Die vollkommen entwickelten Sporen hingegen besitzen alle ein durchsichtiges **grosswarziges** Episporiurn, welches mich am meisten bewog diesen Pilz als selbstständige Species anzuführen."). Also, according to the drawing (Taf. III, Figs 12-14) it looks like the spores have warty ornamentation and the author specifies that this character is the main reason why he described this as a new species.

Fogel (1975) compared the illustrations for *Leucogaster nudus* made by Hollos (1911), Hazslinsky (1875), Pilát (1937), Svrček (1958) and also examined material from Europe. He reviewed two collections from Hungary, one of Hazlinsky and one of Bresadola, one collection from then Czechoslovakia from Pilát (PR 37908) which had been labeled as *Hydnangium virescens* Quél. and two collections from Germany probably borrowed from Soehner [1147 (M) and 1193 (M)]. Then he presents a description with different spore size than the one of *H. nudum* and ornamentation that he states is aleveolate with prominent reticulations, but also mentions "ornamentation of spines" where he presents the high of the spines, obviously meaning on the high of ornamentation, not mentioning warts anywhere as in concordance with the rest of the description (page 72: "Basidiospores 13-16(-17) x (12-)13-14(-15) µm, including alveolate ornamentation, globose, enclosed in a hyaline perisporal sac; ornamentation of spines 1 µm high, 3 µm apart, alveoli 5-6 sided, reticulations prominent, spore wall 1.5-2 µm thick, excluding ornamentation"). He proposes synonymy of *L. tozzianus* and *L. fragrans*, as previously proposed by Zeller and Dodge (1924). However, Fogel (1975) is not proposing synonymy of *L. nudus* with *L. badius* and *L. tozzianus* noting that the size of the spores of *L. nudus* are larger, though with the description he provides, he sets bigger spore range, therefore the sizes of spores of these species are overlapping. There is also overlapping between these species in another important characters as the peridium width. It is worth to mention that in the original description, Hazslinsky (1875) reports that the basidiocarp does not possess proper peridium which is probably the reason for the name of the species "nudum – naked", though in the same description later he describes peridium of parallel hyphae, as mentioned above.

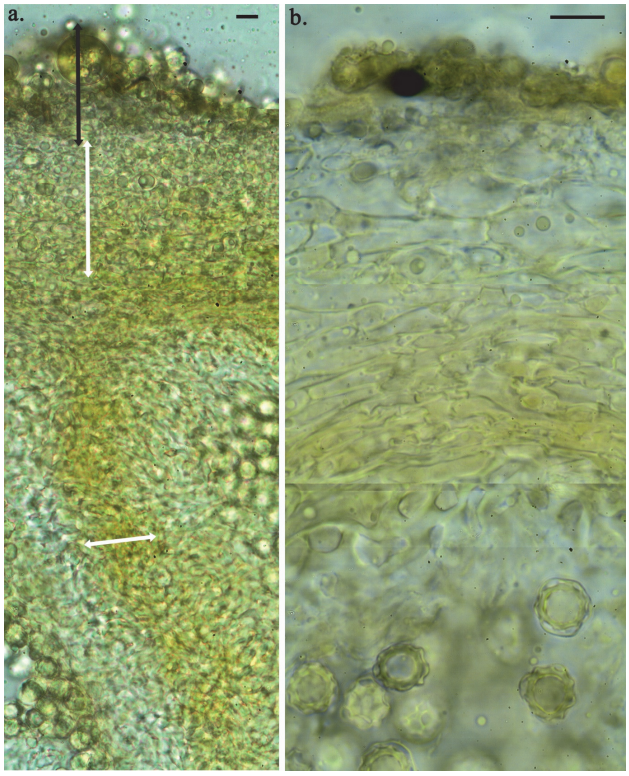


Fig. 3. *Leucogaster nudus*. a. cross-section, external peridium (black arrow), internal peridium (white arrow vertical), tramal plate between chambers (white arrow horizontal). b. details of the structure of peridium, composed of parallel hyphae, swollen and elongated at places resembling to pseudoparenchymatous tissue. Scale bar: a-b. 10 µm. Slides are prepared in Meltser's reagent. (MCF17818)

Based on the comparison of the descriptions of *L. nudus* and the taxa regarded as its synonyms from the available literature, we suspect that it is possible the collection Hazslinsky (1875) described under the name *Hydnangium nudum* has different characteristics compared to the collections that have been examined by subsequent researchers. It is necessary to make a revision of the holotype of *H. nudum* and to compare it with *L. fragrans*, *L. tozzianus* and *L. badius* which, according to the authors mentioned above, have reticu-

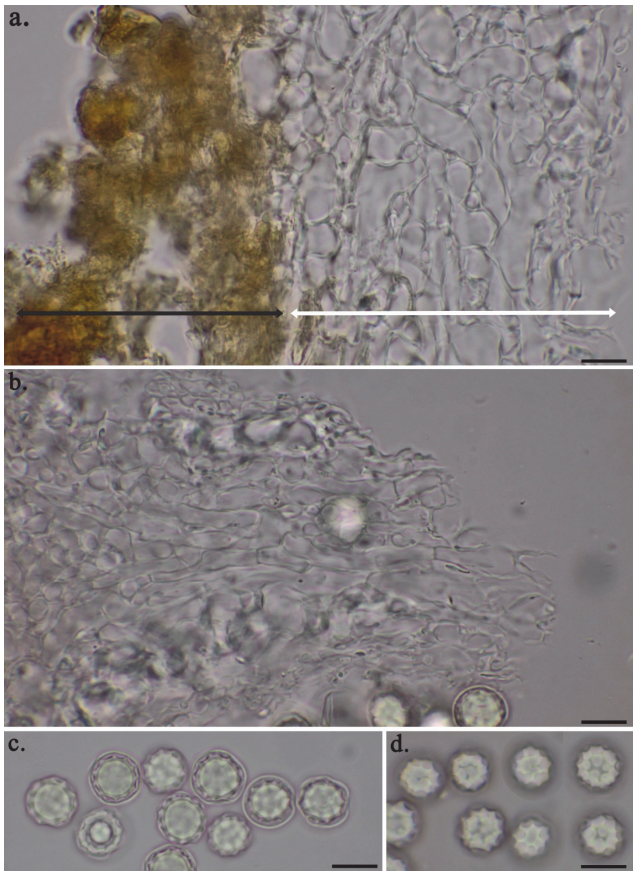


Fig. 4. *Leucogaster nudus*. a. external peridium brown, hyphae covered with crystal mass (black arrow), internal peridium of parallel hyphae looking like pseudoparenchym (white arrow). b. hyphae of tramal plate, elongated and swollen at places. c-d. basidiospores, focus on shape where the perisporium is easily observed (c), focus on reticulate ornamentation (d). Scale bar: a-d. 10 µm. Slides prepared in 3% KOH. (a-b. MCF19486, c-d. MCF17818)

late ornamentation. Comparison is also necessary with *L. floccossus* that might have warty ornamentation, as well as with *L. bucholtzii*. An in-depth revision on genus *Leucogaster* along with *Leucophles* in Europe is needed with an aim to precisely determine the morphological characters and their phylogeny. In the NCBI data base only 44 sequences are annotated as *Leucogaster* (Accessed date: 18 September 2023), most of them determined only to genus level, while for *L. nudus* only one sequence from Italy is available.

With this detail overview of the characteristics of *Leucogaster nudus* form literature, and the presented results from the observed material we hope to contribute and encourage a further in detail research on this species and the genus.

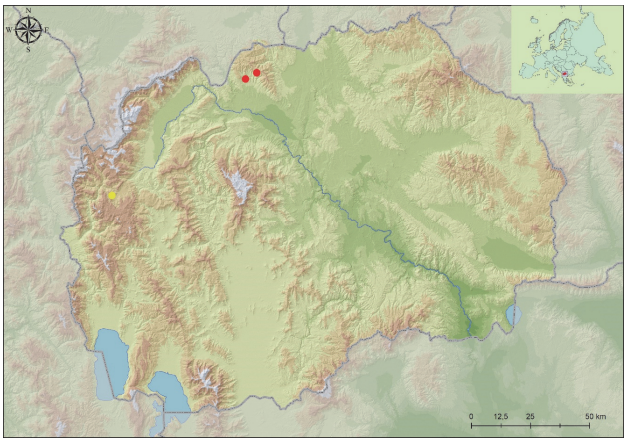


Fig. 5. Distribution of *Leucogaster nudus* (Hazsl.) Hollós. ● - New location ● - Previously known location.

Acknowledgement

We would like to express our deep gratitude to Konrad Wilamowski and Marek Wolkowycki from the Bialystok University of Technology, Institute of Forest Sciences, Scientific research Centre in Hajnowka, Poland, for their kindness and effort in providing the images of SEM. As well, we highly appreciate, and we would like to thank the reviewers for the valuable suggestions that improved the manuscript.

References

Beaton, G., Pegler, D.N. & Young, T.W.K. 1985. Gasteroid Basidiomycota of Victoria State, Australia: 8–9. Kew Bulletin 40: 827–842.

Chavdarova, S., Kajevska, I., Rusevska, K., Grebenc, T. & Karadelev, M. 2011. Distribution and ecology of hypogeous fungi (excluding *Tuber*) in the Republic of Macedonia. Biologia Macedonica, 62: 37–48.

Fogel, R. 1975. The genus *Leucogaster* (Basidiomycetes, Leucogastraceae). Thesis for Doctor of Philosophy. Oregon State University. 114 pp.

Fogel, R. 1979. The genus *Leucophlebs* (Basidiomycotina, Leucogastrales). Can. J. Bot. 57: 1718-1728.

GBIF, 2023. GBIF.org (20 September 2023) GBIF Occurrence Download. https://doi.org/10.15468/dl.fvx9ss

Hazslinsky, A.F. 1875. Beiträge zur Kenntniss der ungarischen Pilzflora. III. Fungi hypogaei. In Verhandlungen der kaiserlich-königlichen zoologisch-botanischen Gesellschaft in Wien. Herausgegeben von der Gesellschaft. Band 25: 63-68.

Hesse, R. 1882. *Leucogaster* eine neue Hymenogastreengattung. Jahrbücher für Wissenschaftliche Botanik 13 (2): 189–194.

Hollós, L. 1908. Új adatok földalatti gombáink ismeretéhez. (Analecta nova ad cognitionem fungorum hypogaeorum nostrorum). Annls hist.-nat. Mus. natn. hung. 6: 317-319.

Hollós, L. 1911. Fungi hypogaei Hungariae. Franklin-Tarulat Nyomdaja, Budapest. 248 pp.

Honrubia, M. & Llimona, X. 1981. Aportación al conocimiento de los hongos del S.E. de España. IV. Tres citas nuevas para la micoflora española: *Pustularia insignis, Tuber borchii, Leucogaster* cf. *floccosus.* An. Univ. Murcia Cienc. 37(1-4): 81-90.

Index Fungorum 2023. Index Fungorum. Accessed date: 16 September 2023. http://indexfungorum.org/Names/Names.asp

Ivančević. B. 2016. Prostorna distribucija i ekološke varijacije staništa hipogeičnih makromiceta (Mycota) u Srbiji. Doktorska disertacija. 325 pp.

Karadelev, M., Rusevska, K., Kost, G. & Mitic-Kopanja, D. 2018. Checklist of macrofungal species from the phylum Basidiomycota of the Republic of Macedonia. Acta Musei Macedonici Scientiarum Naturalium, 21(1): 23–112.

Kauonas, V. 2015. *Leucogaster nudus.* (accessed 28.08.2023) https://www.mycohellas.gr/mch/index.asp

Kaya, A. & Uzun, Y. 2020. New locality records for two truffle taxa in Turkey. Türler ve Habitatlar 1(2): 58–65. www.turvehab.com

Komur, P., Chachuła, P., Kapusta, J., Wierzbowska, I. A., Rola, K., Olejniczak, P. & Mleczko, P. 2021. What determines species composition and diversity of hypogeous fungi in the diet of small mammals? A comparison across mammal species, habitat types and seasons in Central European mountains. Fungal Ecology, 50, 101021. doi:10.1016/j.funeco.2020.101021

Kreisel, H. 2001. Checklist of the gasteral and secotioid Basidiomycetes of Europe, Africa, and the Middle East. Österr. Z. Pilzk., 10: 213-313.

Matevski, V., Kostadinovski, M., Kjusterevska, R., Mandzukovski, D. 2021. Catalog of habitat types of EU importance in the Republic of North Macedonia.

MaSIS. 2015. Macedonian Soils Information System. http://www.maksoil.ukim.mk/masis/

Mattirolo, O. 1900. Elenco Dei Fungi Hypogaei, raceolti nelle Foreste di Vallombrosa negli anni 1899-1900. Malpighia, Rassegna Mensuale Di Botanica. Ed. Penzig, O. & Pirotta, R., Genova, 266 pp. Malpighia 14: 247-270.

Mattirolo, O. 1903. I funghi ipogei italiani. Mém. R. Accad. Sci. Torino, Ser. 2 (53): 331-366.

Melovski, L., Markovski, B., Hristovski, S., Jovanovska, D., Anastasovski, V., Klincharov, S., Velevski, M., Velkovski, N., Trendafilov, A., Matevski, V., Kostadinovski, M., Karadelev, M., Levkov, Z. & Kolchakovski, D. 2013. Regional division of the Republic

of Macedonia for the needs of biological databases. Macedonian Journal of Ecology and Environment 15 (2): 81-111.

Montecchi, A. & Sarasini, M. 2000. Funghi Ipogei d'Europa. Trento-Vicenza, Italy: Associazione Micologica Bresadola, Fondazione Centro Studi Micologici.

Nedelin, T., Gyosheva, M. & Savev, S. 2018. A contribution to the species diversity of hypogeous fungi of Bulgaria. 11th Seminar of Ecology, 26–27 April 2018, Sofia, Bulgaria, Book of Abstracts, IBER-BAS.

Nedelev, P. 2019. *Leucogaster nudus*. (Access date 28.08.2023) https://manatarka.org/leucogaster-nudus/

Pegler, D.N., Spooner, B.M. & Young, T.W.K. 1993. British truffles. A revision of British hypogeous fungi. Royal Botanic Garden, Kew.

Pendzerkovski, J. & Hadzimitrova, S. 1997. Geological Map of Republic Macedonia. Council for Research in Mining, Geological Institute of Skopje. Department of Cartography "Geokarta" Belgrade.

Pilát, A. & Lindtner, V. 1939. Ein Beitrag zur Kenntnis der Basidiomiceten von Sudserbien II. Glasnik skopskog naucnog drustva, 20: 1–11.

Pilát, A. 1937. Additamenta ad floram Asiae Minoris Hyrnenomyceturn et Gasteromycettun. Bull. Soc. Mycol. France 53:253-264.

Saccardo, P.A. & Cavara, F. 1900. Funghi di Vallombrosa. Nuovo giornale botanico Italiano, nuova serie. Firenze. Vol. 7: 272-296.

Snowarski, M. 2023. Atlas of Polish mushrooms. (Access date 28.08.2023) https://grzyby.pl/gatunki/Leucogaster_nudus.htm

Svrček, M. 1958. Gasteromycetes. In: Pilát, A. (ed.) Flora CSR. Ceskolovenske Akad. Ved., Praha. Vol. 1, pp. 547-557, 800-802.

Szemere, L. 1965. Die unterirdischen Pilze des Karpatenbeckens. Fungi hypogaei territorii Carpato-Pannonici. Akadémiai Kiadó, Budapest.

Tofilovska, S., Rusevska, K., Grebenc, T., Kost, G. & Karadelev, M. 2019. Contribution to the Checklist of Basidiomycota for the Republic of North Macedo-

nia. Acta Musei Macedonici Scientiarum Naturalium, 22: 27–33. ISSN: 0583-4988 (printed version) ISSN: 2545-4587 (on-line version) www.acta.musmacscinat.mk

Türkoğlu, A., Castellano, M.A., Trappe, J.M. & Yaratanakul Güngör, M. 2015. Turkish truffles I: 18 new records for Turkey. Turk J Bot 39(2): 359–376. DOI: 10.3906/bot-1406-42.

Zeller, S.M. & Dodge, C.W. 1924. *Leucogaster* and *Leucophlebs* in North America. Annals of the Missouri Botanical Garden 11: 389–410.

UDK: 582.284-192(497.7)
Received: 15.11.2023
Accepted: 16.11.2023
Original scientific paper

Acta Musei Macedonici Scientiarum Naturalium, 2023, Vol. 26, pp: 55-58
ISSN: 0583-4988 (printed version)
ISSN: 2545-4587 (on-line version)
Available on-line at: www.acta.musmacscinat.mk

First records of *Galerina paludosa* (Basidiomycota) in North Macedonia

Kristijan Jakimovski[1], Slavica Tofilovska[1,2], Mitko Karadelev[1,2], Katerina Rusevska[1,2]

[1]Macedonian Mycological Society, Arhimedova 5, Skopje, NORTH MACEDONIA
[2]Ss Cyril and Methodius University, Faculty of Natural Sciences and Mathematics, Institute of Biology, Arhimedova 5, Skopje, NORTH MACEDONIA
*Corresponding author: kristijanjakim@gmail.com

Abstract
This study documents the first occurrence of *Galerina paludosa*, a species of agaric fungus, in North Macedonia. The identification of this species was confirmed through a combination of macroscopic and microscopic morphological analysis. The discovery of this species in North Macedonia enriches the knowledge on fungal biodiversity of the country and expands the knowledge on the distribution of this species globally.

Key words: fungal biodiversity, morphology, bogs.

Introduction

The genus *Galerina* Earle consists of ca. 300 species of small brown-spored agarics predominately found in the Northern Hemisphere. Most of the species are associated with bryophytes, while others are living on other wood or plant remains and their ecology is important for identification of the species. Also, different macroscopic features are important for identification of *Galerina* species, as basidiocarp size, surface features on the pileus, type of lamellae connection to the stipe, color, flavor and odor. The basidiocarps are usually mycenoid, although some species are larger in size and occasionally an anulus on the stipe is present. In most species, the spore surface is usually weakly verrucose, however in some species near the apex, a smooth area called “plage” with callus is present. The spores which have this plage structure have an inner wall layer forming an endospore which inflates after exposure to diluted base or acid solution (Gulden et al., 2005).

Galerina paludosa (Fr.) Kühner is a sphagnicolous species that is fairly common throughout most of Europe and North America where it grows in association with *Sphagnum* mosses in bogs and wet mossy woodlands (Redhead, 1981; Kirk et al., 2008). This type of habitats is present in the zone immediately around Lake Lokuv, the glacial lake that covers an area of 4000 m^2 where waters from the surrounding slopes converge. It is one of the five glacial lakes located on Mount Deshat near the village of Trebishte. Situated at an altitude of 1565 m.a.s.l. it is the lowest glacial lake in North Macedonia (Vasilevski, 2008). Summer temperatures of the water in the lake reach 25℃, while in winter the lake completely freezes over (Micevski et al., 2008). The area around the lake is mostly covered by Sphagnum mosses after which a transition zone of grassy forest edge is present followed by beech forest. This habitat is suitable for sphagnicolous species of genus *Galerina*, such as *G. paludosa.*

Methodology

The material was collected on a student fieldtrip organized by BSRS (Biological Students` Research Society) in the summer season of 2023 on Mount Deshat near the glacial lake Lokuv. Photographs of the fresh specimens were made on site using a Samsung A52. The samples were dried in an air dehydrator at a temperature of 50℃. Identification of the basidiocarps was done on dried samples in the Mycological laboratory, Institute of Biology, Faculty of Natural Sciences and Mathematics in Skopje. Preparations were made from the hymenium and pileipellis in Meltzer’s reagent and water. The slides were observed with “ZEISS Primostar 3” microscope while the photos were

taken using "ZEISS Axiocam 208 color" camera with the software "ZEN 3.0 blue edition". Multiple photos were taken from the spores, hyphae and cheilocystidia on which measurements were conducted. Measurements were taken from 30 randomly selected basidiospores, as well as from hyphae and cheilocystidia. Length and width of the spores were measured, average values and quotient were calculated. The presented photos were edited in "Photoshop CC 2018".

Results and discussion

A morphological analysis of basidiocarps collected along the edge of Lake Lokuv among *Sphagnum* mosses revealed new species for the mycobiota of North Macedonia. The species *G. paludosa* was for the first time identified in its typical type of habitat.

Taxonomy

Galerina paludosa (Fr.) Kühner, Encyclop. Mycol. 7: 184 (1935).

*Basionym: Agaricus paludosus Fr., Epicr. syst. mycol. (Upsaliae): 209 (1838). Synonym: Agaricus paludosus var. stygius Fr., Epicr. syst. mycol. (Upsaliae): 209 (1838); Galera paludosa (Fr.) P. Kumm., Führ. Pilzk. (Zerbst): 75 (1871); Galerula paludosa (Fr.) A.H. Sm., Pap. Mich. Acad. Sci. 20: 175 (1935); Hydrocybe paludosa (Fr.) M.M. Moser, in Gams, Kl. Krypt.-Fl. Mitteleuropa - Die Blätter- und Bauchpilze (Agaricales und Gastromycetes) (Stuttgart) 2: 181 (1953); Naucoria paludosa (Fr.) Henn., in Engler & Prantl, Nat. Pflanzenfam., Teil. I (Leipzig) 1(1**): 250 (1898); Pholiota paludosa (Fr.) Pat., Hyménomyc. Eur. (Paris): 116 (1887); Tubaria paludosa (Fr.) P. Karst., Bidr. Känn. Finl. Nat. Folk 32: 445 (1879); Tubaria paludosa f. limosa Sacc., Syll. fung. (Abellini) 5: 873 (1887).*

Morphology

Macroscopic features (Fig. 1). Pileus is 10-20 mm across, conical, campanulate when young, convex to bell-shaped when mature, a distinct umbo present, cap finely felty to scruffy, non-striate, hygrophanous with white border from velar remains. Flesh color is brownish with a faintly farinaceous to mild taste and odor. Gills are rusty brown, brighter brown towards the edges, adnate or with a small decurrent tooth. Stipe is 40-80 mm long and 2-3 mm wide, cylindrical, solid when young, fragile and hollow when old, rusty brown, paler than the cap, white pruinose, white veil fibrils on the entire length of the stipe.

Fig. 1. *Galerina paludosa* fruitbody (MCF19622) . a. stipe with white veil fibrils. b. brown cap with velar remains. c. ochre brown gills with decurrent tooth. Scale bar: a-c. 5 mm.

Microscopic features (Fig. 2). Spores are ovoid to amygdaliform, tapering at apex, weakly verrucose, plage just above the apiculus with callus is present and color is yellow-brown to tawny. Size of spores is 7.6–10.6 × 5.3–6.8 µm and Q = 1.4–1.8, spore print is red-brown. Cheilocystidia is lageniform to lageniform capitate 23,3–26,5 × 6.6–9.5 µm. Pileipelis is composed of periclinal hyphae 5 – 9.5 µm wide, light yellow to brown and encrusted, septa is present with clamp.

Specimen examined

North Macedonia, Mount Deshat, Lokuv, 41° 38'7.008"N, 20°33'38.088"E (Fig. 3), in association with *Sphagnum* moss, at an altitude of 1580 m.a.s.l., leg. Kristijan Jakimovski, , MCF19622 (24.7.2023).

G. paludosa is easily distinguished from other sphagnicolous *Galerina* species. From the similar G. sphagnorum (Pers.) Kühner and G. tibiicystis (G.F. Atk.) Kühner is separated by the white velar remnants on the stipe. Concerning the odor and taste of *G. paludosa* it is farinaceous which is the same in *G. sphagnorum* while *G. tibiicystis* lacks any specific odor or taste (Breitenbach et al., 2000). *G. tibiicystis* has a tybiiform cheilocystidia with a narrow neck and often capitate

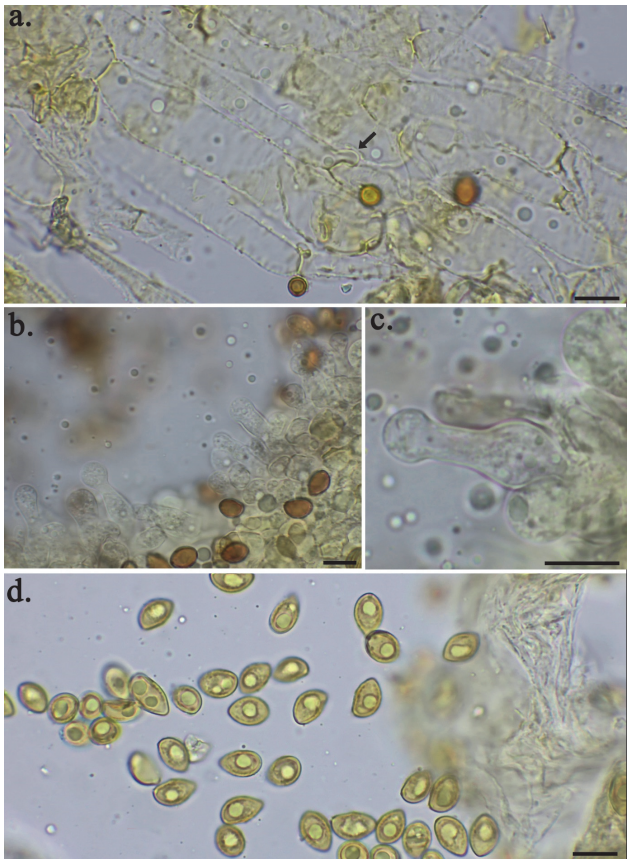


Fig. 2. *Galerina paludosa* microscopic characteristics. a. hyphae with clamp connections. b. lageniform cheilocystidia and dextrinoid spores in Meltzer reagent. c. close up of cheilocystidia. d. spores prepared in water. Scale bar: a-d. 10 µm.

apex, while the other two species have cheilocystidia with a broader neck (Pegler et al., 1972).

This species is distributed in both Europe and North America. In Europe it is quite common in bogs, fen and wet parts of the forest overgrown with mosses. It is reported in many different countries such as Poland (Grzesiak & Wolski, 2015), Bulgaria (Gyosheva & Dimitrova, 2011), Ukraine (Prylutsky, 2014), Romania (Chinan, 2010), Hungary (Pál-Fám & Benedek, 2017), Turkey (Acar et al., 2021), Faroe Islands (Gulden & Vesterholt, 1999), Russia (Filippova, 2008), Canada (Noordeloos & Gulden, 1992), with 4521 occurrences on GBIF (GBIF, 2023).

The frequency of finds of this species increases the further on the north which correlates with the increase in the abundance of bogs and other wet habitats with *Sphagnum* mosses. This type of habitats is not as common in southern countries like North Macedonia. Peat bogs are among the priority habitats for conservation listed in Annex I of the Habitats Directive in the EC project Natura2000 (Eur-lex, 2013).

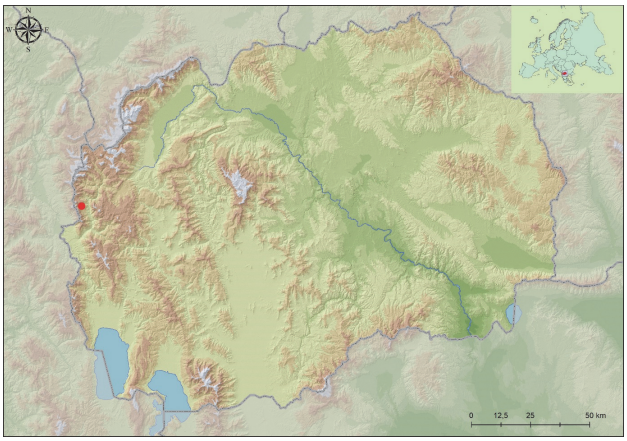


Fig. 3. Distribution map of *Galerina paludosa.*

Acknowledgement

We would like to thank the reviewers for the valuable suggestions that improved the manuscript.

References

Acar, I., Uzun, Y., Kalmer, A., Dizkirici, A.T., & Öğün, Y. 2021. A new record for Turkish mycobiota from Selim (Kars) district. Mantar Dergisi, 12(1), 65-70. DOI: 10.30708.mantar.820871

Breitenbach, J. & Kränzlin, F. 2000. Fungi of Switzerland, Vol. 5. Agarics 3rd part, Cortinariaceae. Mycologia Luzern, Lucerne.

Chinan, V. 2010. Macrofungi identified in the peat bogs from the Natura 2000 site Gaina Lucina (Eastern Carpathians, Romania). Analele Stiintifice ale Universitatii Al. I. Cuza din Iasi, 56(1), 61.

Eur-lex. 2013. eur-lex.europa.eu (Access date 15 November 2023) https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A01992L0043-20130701

Filippova, N.V. & Thormann, M.N. 2014. Communities of larger fungi of ombrotrophic bogs in West Siberia. Mires and Peat, 14, 1-22.

GBIF. 2023. GBIF.org (Access date 31 October 2023) GBIF Occurrence Download. https:// doi.org/10.15468/39omei

Grzesiak, B. & Wolski, G.J. 2015. Bryophilous species of the genus Galerina in peat bogs of central Poland. Herzogia, 28(2), 607-623.

Gulden, G., Stensrud, Ø., Shalchian-Tabrizi, K. & Kauserud, H. 2005. Galerina Earle: A polyphyletic genus in the consortium of dark-spored agarics, Mycologia, 97:4, 823-837, DOI: 10.1080/15572536.2006.11832774

Gyosheva, M.M. & Dimitrova, G.E. 2011. New records of larger fungi established in habitats of glacial relict plants in Bulgaria. Phytol. Balcan, 17(2),

165-167.

Kirk, P.M., Cannon, P.F., Minter, D.W. & Stalpers, J.A. 2008. Dictionary of the Fungi. 10th Edition, Wallingford, CABI, 22.

Micevski, N., Micevski, B. & Bedjanič, M. 2008. Aeshna cyanea and A. juncea, new for the fauna of Macedonia (Odonata: Aeshnidae). Libellula 27 (3/4) 2008: 267-274 p. 269

Noordeloos, M.E. & Gulden, G. 1992. Studies in the genus Galerina from the Shefferville area on the Quebec-Labrador Peninsula, Canada. Persoonia-Molecular Phylogeny and Evolution of Fungi, 14 (4), 625-639.

Pál-Fám, F. & Benedek, L. 2017. Oligotrophic peat bogs as macrofungal refuges in the Eastern Carpathians. Acta Biologica Plantarum Agriensis, 5(1), 41-41.

Pegler, D.N. & Young, T.W.K. 1972. Basidiospore form in the British species of Galerina and Kuehneromyces. Kew Bulletin, 27(3), 483.

Prylutsky, O.V. 2014. Agarics and Boleti (Agaricomycetes, Basidiomycota) of Sphagnum peatbogs and swampy forests of "Slobozhansky" National Natural Park. Studia Biologica, 8(3–4), 179-186.

Redhead, S.A. 1981. Parasitism of bryophytes by agarics. Can J Bot 59:63–67.

Vasilleski, D. 2008. Planinski ezera na Deshat - Lakes on mountain Desat. AD Napredok, Tetovo;

Vesterholt, J. 2012. Funga Nordica. Agaricoid, Boletoid, Cyphelloid and Gasteroid Genera, 9811021. p.897