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Предговор

Со големо задоволство ви го претставуваме петтото издание на списанието „**Билтен на Истражувачкото друштво на студенти биолози“.** Иако со направена поголема пауза од 10 години, желбата за издавање на овој билтен никогаш не згасна. По многу успешно спроведени акции, во секоја измината година, секогаш се појавуваше на дневниот ред за дискусија. Впрочем, во него се наоѓаат научни трудови од повеќе изминати години и истражувачки акции. Самите автори исто така трпеливо го чекаа и беа лојални на изданието на овој билтен. Конечно, по долго тлеење на идејата за издавање, нашата споделена радост во моментов е неизмерна.

Во изминатите 25 години, **Истражувачкото друпштво на студенти биолози (ИДСБ)** прерасна во сериозно младинско здружение кое стана признаен и почитуван бренд во општеството. Сè повеќе растат амбициите и динамиката со која се спроведуваат едукација и истражувања. За постарите генерации ИДСБ симболизира гордост, а за своите членови прибежиште за нелимитирачки идеи и безбројни можности.

Како и сите други активности, издавањето билтен е извчлечена поука. Не сме разочарани, ниту се чувствуваме дека членовите или изминатите претседателства потфрлиле во својата мисија. Напротив, пресреќни сме што сè уште ја валоризираме исконската прогресивна мисла која го одржува ова здружение повеќе од 25 години. Самокритичноста нè води кон прогрес, но уште поважно е дека тимски остварените цели продолжуваат да ни докажуваат дека ништо не е невозможно. Бидејќи **ИДСБ** е местото каде заеднички учиме и напредуваме. Затоа што имаме обврска да ја пренесеме нашата љубов кон **ИДСБ** на следните генерации. Предизвиците секогаш ќе бидат големи, но никогаш поголеми од нашата заедничка визија.

Марија Тренчева

“I have called this principle, by which each slight variation,
if useful, is preserved,
by the term of Natural Selection”

\- Charles Darwin

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Preliminary contribution to the knowledge of two heterospecific tortoise populations from park-forest Gazi Baba in North Macedonia

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Herpetology section

Abstract: Skopje's Gazi Baba forest park is home to two tortoise species: Hermann's Tortoise (*Testudo hermanni*) and Spur-thighed Tortoise (*Testudo graeca*). During six unsystematic field trips, we caught and marked 99 Hermann's and 23 Spur-thighed Tortoises, suggesting a larger population of the former. Their respective adult sex ratios (males/females) are 0.77 and 1.63. Male Hermann's Tortoises were the only significantly different (smaller) cohort among both sexes of both species. Compared to 18 other studied Balkan populations of Hermann's Tortoises, Gazi Baba tortoise sizes were significantly different from only four, following a reverse Bergmann's rule previously elaborated in this species (regarding both altitude and latitude). Tick infestation propensity differed between species, Spur-thighed Tortoises expressing a 12-fold higher rate than Hermann's Tortoises (83% vs. 7%). There were no significant differences in body condition between species, as well as between infected and tick-free tortoises.

Key words*:* Gazi Baba forest park*, Testudo hermanni*, *Testudo graeca*, Ticks, Tortoises*.*

Извод: Скопската парк шума Гази Баба е дом на два вида копнени желки: ридската (*Testudo hermanni*) и полската желка (*Testudo graeca*). За време на шест несистематски теренски посети, фативме и маркиравме 99 ридски и 23 полски желки, што посочува кон веројатно поголема популација на ридска желка. Односот на адултни мажјаци спрема адултни женки изнесува 0,77, односно 1,63. Мажјаците ридски желки се издвоија како единствената значително различна (помала) група помеѓу двата пола од двата вида. Споредено со 18 други проучени балкански популации на ридска желка, големините на ридските желки од Гази Баба беа значително различни од само четири, следејќи го обратното правило на Бергман претходно забележано кај овој вид (и според надморска височина и според географска ширина). Полската желка имаше 12-кратно поголема процентуална зараженост со крлежи од ридската желка (83 наспроти 7 проценти). Немаше значителни разлики во индексот на телесна маса помеѓу видовите, како и помеѓу заразените и желките без крлежи.

Клучни зборови: Парк шума Гази Баба, *Testudo hermanni*, *Testudo graeca*, Копнени желки, Крлежи.

Introduction

Currently, there are 50 different extant species of tortoises in the world, three of which can be found in Europe: Hermann's Tortoise (*Testudo hermanni*), Spur-thighed Tortoise (*Testudo graeca*), and Marginated Tortoise (*Testudo marginata*). The Hermann's Tortoise is common in southern Europe (eastern Spain, southern France, central Italy, and the Balkan Peninsula, Bertolero et al. 2011), whereas the Spur-thighed Tortoise is more continuously distributed across southeastern Europe where it overlaps with the Hermann's Tortoise's distribution, but it is also patchily distributed across western Europe (Spain), and can also be found in the Caucasus, the Middle East, and North Africa.

The Marginated Tortoise can only be found in Greece and southern Albania and isolated (likely introduced) populations across Italy (Iverson and College 1992). Only the Hermann's and Spur-thighed Tortoises are found in North Macedonia, often overlapping in distribution, although the latter prefers drier and more open shrubland or degraded forest habitats (Iverson and College 1992; Sterijovski et al. 2014; Speybroeck et al. 2016). The Skopje Valley is close to the northern distribution edge of the Spur-thighed Tortoise (Ralev et al. 2013) and is one of the areas where it overlaps with the distribution of the more widespread Hermann's Tortoise. Both species are diurnal, but during summer they will seek cover under bushes or in burrows. External parasites are mainly represented by ticks, frequent on individuals in the Balkans (Hailey and Loubmourdis 1988; Kopstein and Wettstein 1921; Nöllert and Nöllert 1981; Široký et al. 2006; Zlatanova 1991). *Hyalomma aegyptium* is the most common tick on both species. On the Balkan Peninsula, the Spur-thighed and Marginated Tortoises represent the main hosts of *H. aegyptium*, whereas the Hermann's Tortoise serves only as an alternative host in areas within or nearby the range of either of the other two species (Široky et al. 2006). This tick inclination towards specific hosts is likely due to different habitat preferences and different activity levels of the three *Testudo* species (Hailey et al. 1988).

The best-studied tortoise species in the Balkan Peninsula is likely the Hermann's Tortoise, particularly life-history characteristics of many of its populations across Greece (Willemsen and Hailey 2002), whereas much less is known on the Spur-thighed Tortoise (e.g. Hailey and Loumbourdis 1987; Willemsen and Hailey 1999). Only one population of Hermann's Tortoises has been studied in detail in North Macedonia - an island population in Prespa Lake (Golem Grad Island) (e.g. Arsovski et al. 2018a, b; Bonnet et al. 2016; Golubović et al. 2013), whereas no data has been presented on the Spur-thighed Tortoise. Generally, sexual-size dimorphism is present in both species, i.e. females are larger and exhibit better body condition (Makridou et al. 2019). According to Willemsen and Hailey (1999), body size in Hermann's Tortoises increases with latitude and altitude, opposite to what is known in ectotherms (where the reverse Bergmann's rule applies), and the island population from North Macedonia (Arsovski et al. 2018a) seems to fit well in this pattern. Namely, this population is situated close to the border with Greece, and their male and female average sizes (171.4±0.41 and 173.6±1.68 cm, respectively) are seemingly larger than those from southern Greece (e.g. in Sparta: 135±10 and 153±11), and similar to those living close to the border in Greece (e.g. in Kastoria: 177±11 and 197±13). Females from Golem Grad seem smaller, but that is most likely a consequence of higher female mortality under a biased sex ratio (Arsovski 2018; Golubović et al. 2018). Namely, growth models from increments showed that females should still be the larger sex in this population if they lived long enough (Arsovski et al. 2018a), as was the case in all 17 studied populations in Greece (Willemsen and Hailey 1999). The same pattern was noticed in the mass-length relationship (i.e. body condition index [BCI]) of all three European *Testudo* species (Willemsen and Hailey 1992). Here we explore patterns of body size and BCI in a dataset from a one-year capture-recapture study of syntopic populations of Hermann's and Spur-thighed Tortoises inhabiting an urban park in the city of Skopje.

Materials and Methods

A total of six field trips were carried out unsystematically during the period of 21st April - 8th August, by three to eight people. Tortoises were located by walking through the habitat. All captured specimens were determined to species level, sexed and aged when possible (both tortoise species reach sexual maturity at 8-10 years of age, prior to which they are not sexually dimorphic; Arsovski et al. 2018a; Bertolero et al. 2011; Speybroeck et al. 2016), checked for damages and breaks, measured (straight carapace length [SCL] with calipers and body mass [BM] with an electronic scale). Sublocality and/or geographic coordinates, date and time of day, and behavior at capture were noted, and each individual was permanently marked using a unique code by notching the marginal scutes (Stubbs et al. 1984) with a hacksaw blade before releasing at the place of capture.

Study site

Park-forest Gazi Baba (Figure 1) is a man-made recreational park zone, located within a three-kilometer distance north-eastward from the center of Skopje. It was formed in the middle of the 20th century. The total area of the park is 102.44 hectares. The primary vegetation of this area used to be *Querco-Carpinetum orientalis macedonicum*, but today it is almost completely destroyed. This vegetation was replaced by anthropogenic plantations with autochthonous and allochthonous tree species, as well as ruderal vegetation of allochthonous, sometimes invasive grass species. The most commonly used species for afforestation were: *Pinus nigra, Robinia pseudoacacia*, *Maclura aurantiaca, Fraxinus americana*, *Gleditsia triacanthos, Ailanthus glandulosa* (Karadelev et al. 2019).

Data analyses

We calculated BCIs in Microsoft Excel 2016, and compared population and sex-specific mean SCLs and BCIs between species and sexes of our dataset using two-sample t-tests in the software R (R Core Team 2014). Two-sample t-tests were also used to check for differences between our studied populations' mean SCLs and those provided for Greek populations (Willemsen and Hailey 1999) and the Macedonian population from Golem Grad Island (Arsovski et al. 2018a).

RESULTS

Two species of *Testudo* are found in park-forest Gazi Baba: Spur-thighed Tortoise (*Testudo graeca ibera*, Mashkaryan et al. 2013) and Hermann's Tortoise (*Testudo hermanni boettgeri*, Bertolero et al. 2011). We caught and marked 99 Hermann's Tortoises and 23 Spur-thighed Tortoises.

The adult sex ratio (males/females) of our populations of Hermann's and Spur-thighed Tortoises are 0.77 and 1.63, respectively. A breakdown of body sizes and masses per species can be found in Table 1. Only the small area of low and sparse vegetation with degraded forest and thermophilic habitat surrounding the Faculty of Natural Sciences and Mathematics comprised 61% of all Spur-thighed Tortoises caught and measured. In contrast, Hermann's Tortoises seemed more equally spread throughout the entire park. Many individuals had breaks and cracks in their carapace, mostly used to ascertain individual recognition at points of recapture, but interestingly some individuals were also infested with ticks. Only 7% of Hermann's Tortoises were infested with ticks, compared to 83% of Spur-thighed Tortoises. *Hyalomma aegyptium* was the most commonly found tick in both species. All analyzed datasets and subsets were distributed normally, and between all compared subsets the variances were equal, except for male against female Spur-thighed Tortoise SCL and Hermann's against Spur-thighed female Tortoise SCL. This was taken into account in the analyses. There were no significant differences in SCL between male and female Spur-thighed Tortoises from Gazi Baba and females of both species.

Table 1. Basic body size information breakdown on Hermann's and Spur-thighed Tortoises from Gazi Baba park, North Macedonia. BM – body mass, SCL – straight carapace length, MCW – medium carapace width, MaxCW – maximum carapace width, SH – shell height.

	BM (g)	SCL (mm)	MCW (mm)	MAXCW (mm)	SH (mm)
SPUR-THIGHED TORTOISE					
Adult male mean	1152	173	130	137	/
Adult female mean	1502	186	130	136	/
Largest male	1593	191	143	151	/
Largest female	2161	218	162	170	/
Smallest individual	34	49	42	42	25
HERMANN'S TORTOISE					
Adult male mean	894	157	127	131	/
Adult female mean	1334	180	127	131	/
Largest male	1411	188	143	155	98
Largest female	2063	222	156	168	/
Smallest individual	43	54	51	50	28

On the other hand, male Hermann's Tortoises were significantly smaller than male Spur-thighed Tortoises and female Hermann's Tortoises ($t=-5.73$, $df=86$, $p<0.05$, and $t=-0.07$, $df=58$, $p=0.02$, respectively). Male and female Hermann's Tortoise SCLs from Gazi Baba did not differ significantly from most Greek populations except for the larger Deskati population ($p=0.03$ and $p=0.04$, respectively), the smaller Sparta and Kalamata (only males) populations, and from the only Macedonian population (Golem Grad) whose males were larger and females were smaller (Table 2).

There were no significant differences in BCIs between species, as well as between individuals infected with ticks versus those without ticks.

Table 2. Male and female Hermann's Tortoise mean straight carapace lengths (SCLs) ± 1SE per population from Greece (from Willemsen & Hailey 1999) and from Golem Grad Island, North Macedonia (from Arsovski et al. 2018a), and results from t-tests comparing these populations with mean SCLs from Gazi Baba park. Significantly different cohorts are bolded.

LOCALITY	male	t-value	p-value	female	t-value	p-value
Deskati	**195±12**	**-4.62**	**0.03**	**214±13**	**-4.13**	**0.04**
Kastoria	177±11	-2.36	0.11	197±13	-1.97	0.16
Agios Dimitrios	170±11	-1.34	0.28	191±11	-1.4	0.27
Keramoti	169±11	-1.19	0.33	185±15	-0.39	0.73
Mikra Volvi	165±9	-1.41	0.25	189±15	-0.84	0.48
Porto Lago	161±10	-0.03	0.98	181±10	0.08	0.94
Litochoron	155±11	0.85	0.47	175±11	0.95	0.42
Parga	155±11	0.85	0.47	177±12	0.61	0.59
Langadia	148±10	2.02	0.14	174±10	1.2	0.32
Meteora	151±13	1.23	0.32	165±17	1.63	0.23
Kilkis	154±8	1.28	0.28	165±9	2.88	0.07
Igoumenitsa	151±11	1.43	0.26	166±9	2.71	0.08
Alyki main heath	155±12	0.78	0.50	167±11	2.13	0.14
Alyki salt works	151±11	1.43	0.27	167±11	2.13	0.14
Olympia	144±10	2.65	0.08	163±12	2.52	0.10
Sparta	**135±10**	**4.07**	**0.03**	**153±11**	**4.2**	**0.03**
Kalamata	**132±9**	**4.95**	**0.01**	**156±13**	**3.23**	**0.06**
Golem Grad	**171.4±0**	**-4.04**	**<0.05**	**173.6±2**	**3.05**	**<0.04**

Discussion

Population and habitat preference

According to the above data, it can be summarized that the locality of Gazi Baba forest park likely holds a larger population of Hermann's Tortoises compared to Spur-thighed Tortoises. The populations should be continuously monitored for a few more years, in order to calculate species-specific capture-probabilities and estimate population sizes. Current knowledge indicates that Spur-thighed Tortoises' distribution was limited to the more open degraded habitats of the park close to, or in the immediate garden of an active university (61% of the whole sampled population of the species). On the other hand, Hermann's Tortoises seemed almost equally distributed throughout the study area. This likely reflects the Spur-thighed Tortoises' more thermophilic nature (Iverson and College 1992).

Regional body size variations in Hermann's Tortoises

Gazi Baba Hermann's Tortoise body size differed significantly from the high altitude Deskati and Golem Grad populations (~900 m) and the low latitude Kalamata (only males) and Sparta tortoises (Table 2). The rest of the populations that did not display significant differences in size were distributed at lower altitudes in northern or central Greece (at latitudes closer to North Macedonia). This corroborates the notion that body size in Hermann's Tortoises increases with latitude and altitude, likely due to longer continued growth (Willemsen and Hailey 1999). On the other hand, female tortoises from Golem Grad appear significantly smaller, whereas those from Kalamata did not differ significantly in size from Gazi Baba females. For the former, actual sizes do not reflect asymptotic size (Arsovski et al. 2018a), since they do not live long enough to express it due to sexual harassment (Arsovski 2018; Golubović et al. 2018), explaining their significantly smaller size. The lack of difference in size with Kalamata females might reflect a smaller potential for phenotypic plasticity in this sex. Namely, fecundity is correlated with size in the Hermann's Tortoise (Bertolero et al. 2011), thus a smaller size would lower the reproductive potential of the whole population. This should be further explored.

Sexual size dimorphism

While females are on average larger than males in both the Hermann's and Spur-thighed tortoise (Makridou et al. 2019), this was only evident in our sample of Hermann's Tortoises. This should be interpreted with care, since our sample of Spur-thighed Tortoises was much smaller and thus perhaps responsible for the lack of noticeable size difference. The analyses should be redone after a larger sample is collected in order to ascertain a possible population-specific effect.

Tick infestation

According to the current data, in Gazi Baba forest park Spur-thighed Tortoises seem more infested with ticks than Hermann's Tortoises, as was evident in other studies (Široky et al. 2006). Nevertheless, there were no significant differences in BCIs between individuals infected with ticks versus those without ticks. Future studies should concentrate on quantifying the tick infestation between species (mean numbers of ticks per infected individual) and delve into possible reasons behind tick tortoise-species-preference.

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Human desert turned herpeto-paradise: qualitative data on the reptile and amphibian fauna of the Macedonian Mariovo region

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Herpetology section

Abstract. Field research conducted from 2012 to 2020 in 19 broadly defined localities in the Mariovo region of North Macedonia, resulted with the observation of 123 individuals of 19 reptile and eight amphibian species. All eight amphibian species are new records for this region along with seven new reptile species. This new data places Mariovo firmly on the map as a national herpetological hotspot, with 59% and 57% of the reptile and amphibian national faunas represented, respectively. Future research involving trapping methods could possibly reveal the presence of even more species (particularly amphibians from the order Urodela).

Key words: amphibians, diversity, Mariovo, reptiles

Извод: Теренски истражувања изведени помеѓу 2012 и 2019 година во 19 широко дефинирани локалитети во Мариовскиот регион на Северна Македонија, резултираа со база на податоци од 123 единки на 19 вида влекачи и осум вида водоземци. Сите осум водоземци се први наоди за овој регион, заедно со седум нови влекачи. Овие нови податоци го издвојуваат Мариово како херпетолошки жешка точка, со 59% од националниот херпето-диверзитет и 57% од националниот батрахо-диверзитет. Дополнителни теренски истражувања со примена на замки најверојатно би откриле уште поголем диверзитет (особено на водоземци од редот Urodela).

Introduction

North Macedonia lies in the south western part of the Balkan Peninsula, and as such, particularly its southern ranges experience a modified dry Mediterranean climate, especially in the vicinity of rivers and lakes connected to the Aegean and Adriatic watersheds (Aschmann 1973; Lazarevski 1960, 1972). While not experiencing true Mediterranean climate (Aschmann 1973), these are nevertheless parts of the Mediterranean basin that has been recognized as a hot-spot for biodiversity (Džukić & Kalezić 2004; Médail & Quézel 1999).

Basic faunistic herpetological research has decently covered the country's surface area, demonstrating high diversity adding up to 32 reptile species, 66% of which are representative of Mediterranean chorotypes (Sterijovski et al. 2014). Nevertheless, substantial geographical gaps still exist. On the other hand, batrachological research has been very sporadic both geographically and temporally (Dimovski 1963, 1971; Džukić 1972, 2015; Fejervary 1921; Hristovski 1979; Karaman 1928; Radojćić et al. 2002; Sidorovska et al. 2006; Sterijovski et al. 2002, 2003; Uhrin et al. 2016) and no effort has recently tried to compile national knowledge into a comprehensive study allowing for a future systematic approach in line with current knowledge or lack thereof. Enriching national basic faunistic data will improve and possibly shift our knowledge of regional biodiversity hotspots, and additionally reveal potential new areas of conservation interest. This is of broad national interest, especially in light of North Macedonia's European Union aspirations and the designation of Natura 2000 sites that such an endeavour implies.

One region in the south of the country, Mariovo, has been recognized as a potential Natura 2000 site, is part of the national Emerald network, and holds at least two nationally protected areas (Monuments of nature "Gradeshnichka Reka" and "Manastir", Moepp 2018), yet it has seldom been the goal of herpetological research and is currently not recognized as a reptile diversity hotspot (Sterijovski et al. 2014). Currently, published reptilian fauna of Mariovo consists of 12 species with only individual records (species' taxonomic authorities given in Table 1): Sloworm *Anguis fragilis,* Smooth Snake *Coronella austriaca,* Caspian Whip Snake *Dolichophis caspius,* Balkan Green Lizard *Lacerta trilineata,* Green Lizard *L. viridis,* Eastern Montpellier Snake *Malpolon insignitus,* Dahl's Whip Snake *Platyceps najadum,* Erhard's Wall Lizard *Podarcis erhardii,* Common Wall Lizard *P. muralis,* Hermann's Tortoise *Testudo hermanni,* Spur-thighed Tortoise *T. graeca,* and Nose-horned Viper *Vipera ammodytes*; the region is therefore not among the four recognized national centres of highest reptile diversity (19-21 species) at a 10x10 km UTM grid. To the best of our knowledge, no published records of amphibians from Mariovo region exist.

Mariovo is largely covered in successive highland pastures (Andonoski 1987; Micevski et al. 1987). These open habitats are recognized as favourable for most reptile species inhabiting North Macedonia (e.g. for efficient thermoregulation, Speybroeck et al. 2016); such landscape along with the region's favourable climate and location are not mirrored onto its current surprisingly low reptile species count and the complete absence of amphibians. This hinted at a potential hotspot status of Mariovo if enough field attention was paid to the region, ultimately aiming at better understanding the national distribution of reptiles and amphibians.

Materials and methods

Field surveys were conducted in the spring and summer periods mostly in the dawn and dusk hours, when reptiles and amphibians are at peak activity in North Macedonia (Speybroeck et al. 2016; personal observations). Between 2012 and 2019 non-systematic field trips concerning the herpetofauna of this region were conducted, during which animals were searched for visually. Speybroeck et al. (2016) was regularly consulted to ascertain the taxonomy of observed and/or captured specimens.

Study area

The Mariovo region is situated in the southern part of North Macedonia, just about bordering the neighbouring country of Greece. There have been many misunderstandings concerning the exact borders of this region, but nonetheless its informal periphery runs somewhere along the slopes or ridges of Kozjak, Nidze, Selechka and Dren Mountains. According to the recent regional systematization of North Macedonia, Mariovo stretches over exactly 463,97 km^2 and its borders lie on, or around the foothills of the before mentioned surrounding mountains (Melovski et al. 2013). The relief of the Mariovo region is unusually complicated, owing to the presence of nearly all relief forms (Stojmilov 1984). Regardless, this clutter of relief forms is generally summarized as a hilly-mountainous region, with its lowest points running along the picturesque gorge of Crna Reka river at 300-550 m asl, and its highest point never surpassing the altitude of 1.300 m. Crna Reka river cuts through the whole region running from its southwestern border (near village Skochivir) towards the northeast where it flows into river Vardar meanwhile forming the Tikvesh accumulation while exiting Mariovo. Apart from the fact that the river's gorge along with the gorges of many of its smaller tributaries seem to naturally divide the region in two, and not that seldom are impossible to cross, they also play a huge part in forming Mariovo's specific climate. Two climatic zones are observed in Mariovo: modified Mediterranean zone along Crna Reka river up to 600 m asl, and modified temperate zone present throughout the surrounding hillsides (850-900 m asl). Due to the region's latitude and its relative proximity to the Aegean Sea (130 km), there is an occurrence of so-called stifling heat during the summer months (80% of which in July and August) with temperatures staying well above 30 °C during the day (Lazarevski 1969, 1971).

Although currently desolated and seemingly hard to reach, by 1961 Mariovo was a dynamic place with more than 13.000 inhabitants. This bustling region was home to more than 6.800 working residents, more than 6.000 of which were farmers and the rest were mostly loggers and miners (Stojmilov 1984). Such occupations have had an undeniable zooanthropogenic effect on Mariovo's landscapes today. It went from a dense Mediterranean oak forest to pastures, thickets, rarely meadows and abandoned fields whose looks were mostly tailored by farming and logging activities (Andonoski 1987; Micevski et al. 1987). Neverthe-

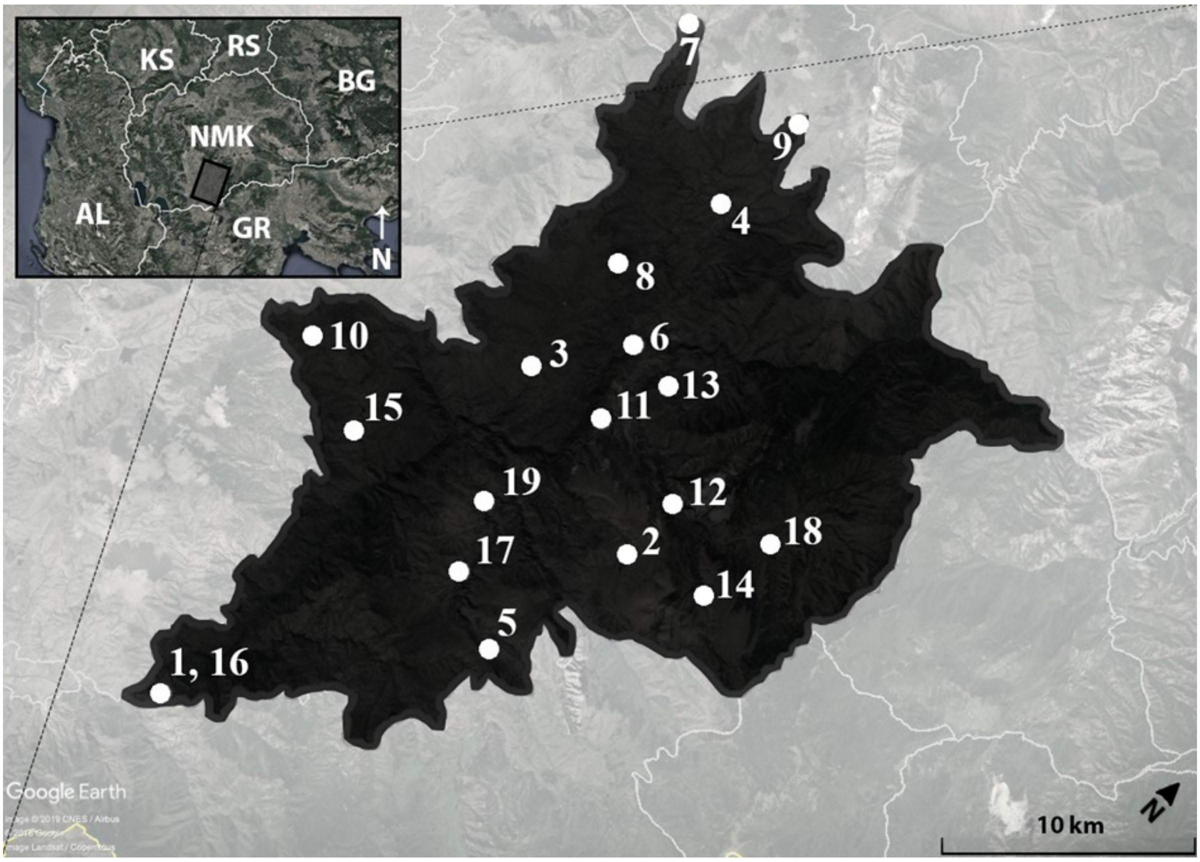

Figure 1. Mariovo region with numbered localities covered during field research and defined in Table 1. White lines represent national borders, NMK – North Macedonia, AL – Albania, GR – Greece, BG – Bulgaria, RS – Serbia, KS – Kosovo

less, due to external development the population has been dropping intensely, plummeting to 839 residents by 2002 (and likely down to only a fraction of that, today; Census of Population, Households and Dwellings in the Republic of Macedonia, 2002—Book XIII 2005). Such sudden standby of anthropogenic activities led to yet another transformation of the region and the occurrence of successive habitats – the highland pastures. These habitats occupy a dominant position in Mariovo today and thus predominantly contribute towards its landscapes (Matevski & Kostadinovski 1998).

Results

During field research with a total of 57 field days, the following species were observed and/or captured in 19 localities across Mariovo (Tab. 1 and Fig. 1):

Eight species of amphibians (scientific, English and Macedonian name provided): *Bombina variegata* (Yellow Bellied Toad, Жолт мукач), *Bufo bufo* (Common Toad, крастава жаба), *Hyla arborea* (Tree Frog, гаталинка), *Pelophylax ridibundus* (Marsh Frog, езерска жаба), *Bufotes viridis* (Green Toad, зелена крастава жаба), *Rana dalmatina* (Agile Frog, горска жаба), *Rana graeca* (Greek Stream Frog, поточна жаба) and *Salamandra salamandra* (Fire Salamander, дождовник).

Nineteen (seven of which new for the region in bold) species of reptiles, (scientific, English and Macedonian name provided): *Anguis fragilis* (Slowworm, слепче), *Coronella austriaca* (Smooth Snake, планински смок), *Dolichophis caspius* (Whip snake, жолт смок), ***Elaphe quatuorlineata*** (Four Lined Snake, ждрепка), ***Emys orbicularis*** (European Pond Turtle, блатна желка), *Lacerta trilineata* (Balkan Green Lizard, голем зелен гуштер), *Lacerta viridis* (Green Lizard, зелен гуштер), *Malpolon insignitus* (Eastern Montpellier Snake, длабокочелен смок), ***Natrix natrix*** (Grass Snake, белоушка), ***Natrix tessellata*** (Dice Snake, рибарка), *Platyceps najadum* (Dahl's Whip Snake, стрелец), *Podarcis erhardii* (Erhard's Wall Lizard, македонска гуштерица), *Podarcis muralis* (Wall Lizard, ѕидна гуштерица), ***Podarcis tauricus*** (Balkan Wall Lizard, степска гуштерица), *Testudo hermanni* (Hermann's Tortoise, шумска желка), *Vipera ammodytes* (Nose-horned Viper, поскок), ***Zamenis longissimus*** (Aesculapian Snake, шумски смок) and ***Zamenis situla*** (Leopard Snake, леопардов смок).

Discussion

Until the recent contribution to the reptile fauna of North Macedonia (Sterijovski et al. 2014), only one published observation referred to the herpetofauna of the Mariovo region in North Macedonia (Nose-horned Viper in Jelić et al. 2013). The current study confirms 12 of the observed reptilian species in Sterijovski et al. (2014), and adds seven to the current knowledge; eight are of national and/or European conservation importance (Table 1). We however, failed to encounter the Spur-thighed Tortoise (*Testudo graeca*, полска желка) in our observations. This might be a result of misidentification and confusion with the similar Hermann's tortoise in the previous publication, or simply very low densities of this species' populations in this region. Despite many published datapoints for the Spur-thighed Tortoise in western North Macedonia (Sterijovski et al. 2014), a significant portion of that area is covered with unsuitable habitat for the species - mountains and dense forests. Ample field work in the west of the country in 2019 (>60 days) and prior (unpublished personal observations) failed to reveal the presence of this conspicuous species. Its global distribution reveals that it is completely absent from north-western Greece (Sillero et al. 2014; Speybroeck et al. 2016; absent from Greek Prespa in Ioannidis & Bousbouras 1997), and a recent thorough contribution to the Albanian herpetofauna acknowledges that the Spur-thighed Tortoise is likely completely absent from Albania and past records are likely misidentifications with *T. hermanni* (Mizsei et al. 2017). Consequently, all observations in western North Macedonia along with Mariovo are considered uncertain (also likely misidentifications) and beyond the global distribution range of the Spur-thighed Tortoise. This approach was also implemented and accepted for the recent national red list assessment of the Spur-thighed Tortoise (please see the distribution map in Arsovski & Sterijovski 2020).

To the best of our knowledge, there are no contributions towards the amphibians of Mariovo, to date. Therefore, all eight species are new records for this region, four of which are of national and/or European conservation importance (Table 1). This is also most probably an underestimation, since no trapping methods were undertaken in some of the ponds in the region, which could potentially reveal the presence of newt species. Wielstra et al. (2014) identified *Triturus macedonicus* (Macedonian newt, Македонски мрморец) in water bodies around Mariovo, whereas *Triturus ivanbureschi* (Buresch's newt, Балкански мрморец) was observed all over the central and eastern parts of the country, and hybrids of the two species were identified in the vicinity of the area (at ~40 km distance on Kozhuf Mt.). Future research should employ trapping methods in some of the area's ponds (Vitolishte pond, Beshishte pond, Mariovo pond, Rapesh ponds, etc.), and tissue samples should be collected for genetic analyses that will reveal whether Mariovo is part of the newts' contact zone. Additionally, the smooth newt (*Lissotriton VUlgaris*, мал мрморец) and the elusive Eastern Spadefoot (*Pelobates syriacus,* лукова жаба) are present all over the country, and additional field work during suitable weather conditions, along with trapping methods (for the former) will likely reveal their presence in the region.

In summary, the herpeto- and batrachofauna of the Mariovo region represent 59% and 57% of the respective reptilian (Sterijovski et al. 2014) and amphibian (Dimovski 1963, 1971; Džukić 1972, 2015; Fejervary 1921; Hristovski 1979; Karaman 1928; Radojćić et al. 2002; Sidorovska et al. 2006; Sterijovski et al. 2002, 2003; Uhrin et al. 2016) faunas known for North Macedonia. So far, only four 10x10 km quadrates over the country revealed a reptilian diversity of ≥19 (Sterijovski et al. 2014). Mariovo does exhibit exceptional reptilian diversity, and is currently only the second region with 19 species west of Vardar (along with the Prespa region). Granted, Sterijovski et al. (2014) looked at a 10x10 km grids over the country in order to establish hotspots, rather than comparing regions, but Melovski et al. (2013) published a comprehensive regional division of the country based on geomorphology, traditional land use, ecology and biogeography, logically imposing it over such studies and making the grid system obsolete. Qualitative data puts Mariovo much more firmly on the map as a herpetofauna hot spot, but most of all – calls for further precise faunistic research across the country. Primarily, during general herpetological field research the exact distribution of the Spur-thighed Tortoise needs to be ascertained, and more batrachological field research needs to be conducted with a systematic approach according to current sporadic (temporal and geographical) knowledge.

Table 1. Qualitative and quantitative overview of observed reptiles (REP) and amphibians (AMP) in Mariovo, classified by locality (Agov Rid [1], Beshishte village [2], Chanishte village [3], Dunje village [4], Gradeshnica village [5], Hasin Bej Most [6], Kokre [7], Krushevica village [8], Ligurasa [9], Makovo [10], Manastir village [11], Melnica [12], Orlov Kamen [13], Polchishte village [14], Rapesh village [15], Skochivirska Klisura [16], Staravina village [17], Vitolishte village [18] and Zovich village [19]); threatened species according to the national red list (Sterijovski & Arsovski 2020) and species of conservation importance marked in superscript next to the species name: NT – Near Threatened, VU – Vulnerable, * - on Annex II and IV of the European species and habitats directive.

Табела 1. Квалитативен и квантитативен преглед на опсервирани влекачи (REP) и водоземци (AMP) во Мариово, класифицирани по локалитет (Агов Рид [1], Бешиште [2], Чаниште [3], Дуње [4], Градешница [5], Хасин Беј мост [6], Кокре [7], Крушевица [8], Лигураса [9], Маково [10], Манастир [11], Мелница [12], Орлов Камен [13], Полчиште [14], Рапеш [15], Скочивирска клисура [16], Старавина [17], Витолиште [18] и Зовиќ [19]); засегнати видови според националната црвена листа (Sterijovski & Arsovski 2020) и видови со конзервациско значење се означени во суперскрипт на видовото име: NT – Near Threatened, VU – Vulnerable, * - на анекс II и IV од Европската директива за видови и хабитати.

Class	Species	Authority	Locality	Number of specimens
REP	*A. fragilis*	Linnaeus 1758	11, 18, 5	2
REP	*C. austriaca*	Laurenti 1768	9	1
REP	*D. caspius*	Nagy et al. 2004	3, 4, 11, 14, 19	6
REP	*E. quatuorlineata*^{NT*}	Lacépède 1789	2, 3, 4, 11, 12, 13, 18, 19	10
REP	*E. orbicularis*^{VU*}	Linnaeus 1758	3, 15, 18, 19	5
REP	*L. trilineata*	Bedriaga 1886	2, 3, 4, 11, 14, 17, 18, 19	12
REP	*L. viridis*	Laurenti 1768	1, 2, 6, 11, 14, 16, 18, 19	12
REP	*M. insignitus*^{NT}	Geoffroy Saint-Hilaire 1827	4, 10	2
REP	*N. natrix*	Linnaeus 1758	4, 6, 14, 15, 18, 19	7
REP	*N. tessellata*^{NT}	Laurenti 1768	6, 18, 19, 19	3
REP	*P. najadum*^{NT}	Eichwald 1831	4, 11, 12, 14, 19	6
REP	*P. erhardii*	Bedriaga 1882	1, 2, 4, 11, 14, 15, 16, 17, 18, 19	18
REP	*P. muralis*	Laurenti 1768	5, 14, 18	3
REP	*P. tauricus*^{NT}	Pallas 1814	4	1
REP	*T. hermanni*^{VU*}	Gmelin 1789	1, 2, 4, 7, 14, 15, 17, 18, 19	20
REP	*V. ammodytes*	Linnaeus 1758	2, 4, 18, 19	7
REP	*Z. longissimus*	Laurenti 1768	12	1
REP	*Z. situla*^{NT*}	Linnaeus 1758	4, 6, 15, 17	4
AMP	*B. variegata**	Linnaeus 1758	4, 8, 11, 14, 17, 18, 19, 5	14
AMP	*B. bufo*	Linnaeus 1758	4, 11, 18, 19	5
AMP	*B. viridis*	Laurenti 1768	1, 18	3
AMP	*H. arborea*^{NT}	Linnaeus 1758	11, 18	2
AMP	*P. ridibundus*	Pallas 1771	4, 8, 11, 14, 15, 18, 19, 5, 17	13
AMP	*R. dalmatina*^{NT}	Bonaparte 1840	14, 17, 18, 19	4
AMP	*R. graeca*^{NT}	Boulenger 1891	2, 5, 14, 18, 19	8
AMP	*S. salamandra*	Linnaeus 1758	11, 14	2

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The cupric ion reducing antioxidant capacity and phenolic content in methanolic extracts of some Macedonian medicinal plants collected on Galichitsa mountain

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Plant physiology section

Abstract. In the present study, methanolic extracts from 30 plant species collected on Galichitsa Mt. were evaluated for their antioxidant properties using CUPRAC (CUPric Reducing Antioxidant Capacity) assay and total phenolic (TP) content according to the Folin-Ciocalteau method. Antioxidant properties and total phenolic content differed significantly among the selected plants. The total phenolic content of the tested plant species varied from 10.90 to 123.41 mg Galic Acid Equivalents (GAE)/g dry weight (DW). The CUPRAC assay ranged from 0.052 to 1.068 mmol Trolox Equivalents (TE)/g DW. Among the examined plant species, *Origanum vulgare* exhibited the strongest antioxidant potential (>1 mmol TE/g DW). In the Folin-Ciocalteau method, extracts of *O. vulgare, Epilobium dodonei* and *E. hirsutum* demonstrated remarkable contents of total phenolic compounds (>80 mg GAE/g DW). A positive linear correlation between antioxidant capacity and total phenolics indicates that phenolic compounds are the major contributors to the antioxidant properties of tested plants.

Key words: Antioxidant activity, CUPRAC assay, medicinal plants, *Origanum vulgare*, phenolic compounds.

INTRODUCTION

Free radicals are an integral part of normal physiological processes continuously formed as a consequence of aerobic metabolism in eukaryotic cells. Reactive oxygen species (ROS) are distributed in the plant cells as metabolic by-products by various intracellular systems: small cytoplasmic molecules, cytoplasmic proteins, membrane enzymes, peroxisomes and electron transport systems in mitochondria and chloroplasts (Mittler 2002). Namely, ROS, e.g. superoxide radical, hydrogen peroxide, hydroxyl radical and singlet oxygen are of the greatest biological significance (Schöneich 1999). Some of them at low-to-moderate concentrations play important roles in cell physiology, such as regulation of cell growth, cellular signal transduction pathways and defence against pathogens (Valko et al. 2007). In addition to their biological importance, overproduction of these extremely reactive and highly unstable oxygen species is considered to be the main contributor to various metabolic and cellular disturbances. These harmful by-products may induce some oxidative damage to functional biomolecules such as carbohydrates, proteins, lipids and DNA, giving rise to oxidative stress. Therefore, the oxidative stress has been suggested to play a cardinal role in the pathogenesis of many degenerative diseases as well in the ageing process (Halliwell & Aruoma 1991).

In modern western medicine, the maintaining of balance between antioxidant defence system and free radical formation is believed to be a critical concept for healthy biological system (Tiwari 2001). Plant cells possess efficient enzymatic and nonenzymatic antioxidant defence mechanisms.

The enzymatic antioxidants include superoxide dismutase, catalase, and glutathione peroxidase, while vitamins (ascorbic acid, tocopherols), carotenoids (β-carotene, lycopene, lutein) and mineral elements (selenium and zinc) have been proposed as nonenzymatic antioxidants (Atoui et al. 2005). Apart from these, plant secondary metabolites (phenolics, terpenoids and alkaloids) play an important role in free radical scavenging activity as excellent antioxidants (Govindarajan et al. 2005, Park & Pezzutto 2002). It is well known that phenolics are secondary metabolites synthesized by plants in order to protect themselves from biological and environmental stress factors. Recent studies have shown that phenolic compounds possess high antioxidant activity (Apostolidis et al. 2007). Polyphenols have an ideal structural chemistry for free radical scavenging activity, and have been shown to be more effective antioxidants *in vitro* than tocopherols and ascorbate (Rice-Evans et al. 1995). Antioxidant properties of polyphenols arise from their high reactivity as hydrogen or electron donors, the ability to stabilise and delocalise the unpaired electron (chain-breaking function), as well to chelate transition metal ions (Rice-Evans et al. 1997).

Over the past few years, medicinal and aromatic plants have been extensively studied for their antioxidant capacities, and a large number of reports have been published. Recent studies have been focussed on the plant species cultivated in the different regions of the world. For example, antioxidant capacity of numerous medicinal plants from different regions have been reported (Mantle et al. 2000; Cai et al. 2004; Ferreira et al. 2006; Wojdyło et al. 2007; Nićiforović et al. 2010). To our knowledge, there is no available information and systematical survey of the antioxidant activity and phenolic production of traditional Macedonian plants. This study is aimed at a preliminary screening of phenolic content and antioxidant activity of some wild-growing plants collected on Galichitsa Mt. Therefore, the main objectives of this study were: 1) to assay antioxidant activity of the 30 selected medicinal plants using the CUPRAC method; 2) to estimate their total phenolic content with Folin-Ciocalteu assay; and 3) to determine the relation between phenolic content and antioxidant activity in the plant extracts. Results from this study might be useful to select the collected medicinal plants as potential sources of natural antioxidants.

MATERIAL AND METHODS

Plant material

Thirty medicinal plant species from 14 families (Tab. 1) were collected in July 2010 from various locations of Galichitsa Mt, Republic of Macedonia. Voucher specimens of collected plants are deposited in the Herbarium Collection of Biology Students Research Society (BSRS), Institute of Biology, Faculty of Natural Sciences and Mathematics, Ss. Cyril and Methodius University, Skopje.

Extraction procedure

Plant material was air-dried, lyophilized and then grinded into a fine powder by laboratory mill. Antioxidant compounds were extracted from powdered plant material (0.02 g) with 80% methanol in ultrasonic bath for 30 min. The extracts were centrifuged (15 min. at 12000 rpm) and the supernatant was used for quantification of total phenolics and antioxidant activity (Gadzovska et al. 2007).

Total phenolic content (TP)

Total phenolic (TP) content in methanolic extracts was determined according to the Folin-Ciocalteu colorimetric method (Singleton & Rossi 1965) with following modifications. An aliquot of the diluted extract (100 µL) was mixed with 500 µL Folin–Ciocalteu reagent and 400 µL 0.7 M sodium carbonate. Samples were incubated for 5 min at 50ºC and then cooled 5 min at room temperature. Absorbance was measured spectrophotometrically at 765 nm. The concentration of TP was calculated using gallic acid (0-20 $mg \times mL^{-1}$) as a standard. The results were expressed as mg gallic acid equivalents (GAE) per g dry weight (mg GAE/g DW).

Cupric reducing antioxidant capacity (CUPRAC) assay

The cupric reducing antioxidant capacity (CUPRAC) of plant extracts was determined according to the method of Apak et al. (2004). To a test tube were added 1 mL 10 mM $CuCl_2$, 1 mL 7.5 mM neocuproine and 1 mL 1 M CH_3COONH_4 buffer (pH 7.0), followed by mixing; (x) mL plant extract followed by (1.1-x) mL water were then added (total

Table 1. Total phenolic (TP) content and total antioxidant capacity (CUPRAC values) of methanolic extracts from 30 Macedonian medicinal plants.
[a] Data are expressed as the mean of triplicate ± standard deviation.

Plant species (Family)	Plant parts	TP contents (mg GAE/g DW)	CUPRAC values (mmol TE/g DW)
Achillea holosericea Sibth. & Sm. (Asteraceae)	Aerial parts	24.52 ± 1.50	0.175 ± 0.014
Agrimonia eupatoria L. (Rosaceae)	Aerial parts	59.57 ± 0.08	0.419 ± 0.002
Alcea pallida Waldst. et Kit. (Malvaceae)	Flowers	10.90 ± 0.36	0.052 ± 0.001
Anthyllis vulneraria L. (Fabaceae)	Aerial parts	12.02 ± 0.42	0.089 ± 0.009
Astragalus glycyphyllos L. (Fabaceae)	Aerial parts	15.93 ± 0.47	0.063 ± 0.003
Centaurium erythraea Rafn. (Gentianaceae)	Aerial parts	22.28 ± 1.07	0.106 ± 0.006
Cichorium intybus L. (Asteraceae)	Aerial parts	33.36 ± 0.14	0.211 ± 0.012
Clinopodium vulgare L. (Lamiaceae)	Aerial parts	57.07 ± 4.15	0.526 ± 0.016
Digitalis ferruginea L. (Plantaginaceae)	Aerial parts	13.24 ± 1.28	0.063 ± 0.001
Digitalis lanata Ehrh. (Plantaginaceae)	Aerial parts	22.16 ± 1.02	0.153 ± 0.002
Epilobium dodonaei Hausskn. (Onagraceae)	Aerial parts	99.33 ± 2.04	0.630 ± 0.026
Epilobium hirsutum L. (Onagraceae)	Aerial parts	86.59 ± 1.02	0.604 ± 0.019
Galega officinalis L. (Fabaceae)	Aerial parts	32.53 ± 2.80	0.191 ± 0.013
Gratiola officinalis L. (Plantaginaceae)	Leaves	26.92 ± 0.95	0.169 ± 0.005
Helichrysum zivojinii Cernj. & Soska(Asteraceae)	Aerial parts	29.57 ± 0.34	0.140 ± 0.011
Inula britannica L. (Asteraceae)	Inflorescences	37.26 ± 5.78	0.253 ± 0.037
Linaria angustissima (Loisel.) Borbás (Scrophulariaceae)	Aerial parts	21.44 ± 1.36	0.081 ± 0.009
Linum hirsutum L. (Linaceae)	Aerial parts	18.99 ± 0.75	0.120 ± 0.012
Lythrum salicaria L. (Lythraceae)	Aerial parts	64.95 ± 2.38	0.475 ± 0.005
Melissa officinalis L. (Lamiaceae)	Leaves	70.86 ± 1.01	0.542 ± 0.001
Origanum vulgare L. (Lamiaceae)	Aerial parts	123.41 ± 8.77	1.068 ± 0.123
Salvia nemorosa L. (Lamiaceae)	Aerial parts	47.98 ± 1.63	0.378 ± 0.007
Salvia ringens Sibth. & Sm. (Lamiaceae)	Aerial parts	69.42 ± 1.36	0.631 ± 0.020
Salvia sclarea L. (Lamiaceae)	Aerial parts	48.75 ± 4.38	0.381 ± 0.052
Sambucus ebulus L. (Caprifoliaceae)	Flowers	44.09 ± 0.75	0.221 ± 0.010
Saponaria officinalis L. (Caryophyllaceae)	Aerial parts	12.72 ± 0.89	0.060 ± 0.007
Sideritis raeseri Boiss et Heldr. (Lamiaceae)	Aerial parts	52.60 ± 8.75	0.305 ± 0.049
Stachys thymphaea Hausskn. (Lamiaceae)	Aerial parts	21.11 ± 1.84	0.160 ± 0.005
Thymus tosevii Vel. (Lamiaceae)	Aerial parts	42.05 ± 1.94	0.415 ± 0.012
Verbena officinalis L. (Verbenaceae)	Aerial parts	31.01 ± 3.74	0.121 ± 0.008

volume, 4.1 mL) and mixed well. The mixture absorbance was recorded against a blank at 450 nm after 30 min incubation at room temperature. Total antioxidant capacity of plant samples was calculated using the molar extinction coefficient of trolox ($\varepsilon=1.67\times10^4$ $L\times mol^{-1}\times cm^{-1}$). CUPRAC values for total antioxidant capacity of plant extracts were expressed as mmol trolox equivalents (TE) per g dry weight (mmol TE/g DW).

Statistical analysis

Results were expressed as means ± standard deviation (SD) of three measurements. Correlations among data obtained were calculated using MS Excel software correlation coefficient statistical option. All statistical tests were considered significant at $p < 0.05$.

RESULTS

Total phenolic content

Results from this study showed a quite large variation in TP contents of the investigated plant species (Tab. 1). The TP values varied from 10.90 to 123.41 mg GAE/g DW, representing a variation of approximately 11-fold. Among the selected plants, only *O. vulgare* L. extracts showed a very high TP contents (>100 mg GAE/g DW). In addition, *E. dodonaei* Hausskn. and *E. hirsutum* L. extracts also demonstrated a high phenolic amounts of 99.33 and 86.59 mg GAE/g DW, respectively. *Melissa officinalis* L. and *Salvia ringens* Sibth. & Sm. were also selected as plants with high TP contents (70.86 and 69.42 mg GAE/g DW, respectively). On the other side, *Digitalis ferruginea* L., *Saponaria officinalis* L., *Anthyllis vulneraria* L. and *Alcea pallida* Waldst. et Kit. extracts showed a very low phenolic content (< 15 mg GAE/g DW).

Cupric reducing antioxidant capacity (CUPRAC) assay

The antioxidant activities of tested plant extracts using CUPRAC assay are shown in Tab. 1. Results from this study showed a wide range of antioxidant activity in plant extracts. Namely, CUPRAC values of total antioxidant capacity in plant extracts ranged between 0.052 and 1.068 mmol TE/g DW, which represents a variation of approximately 20-fold. Similar to the results obtained for TP assay, only *O. vulgare* L. extracts showed very strong antioxidant activity (>1.0 mmol TE/g DW). Relative high antioxidant capacity (>0.6 mmol TE/g DW) was noticed for *S. ringens* Sibth. & Sm., *E. dodonaei* Hausskn. and *E. hirsutum* L. extracts. In the opposite, *Astragalus glycyphyllos* L., *D. ferruginea* L., *S. officinalis* L. and *A. pallida* Waldst. et Kit. extracts possessed the lowest CUPRAC values (<0.063 mmol TE/g DW).

Correlation between total phenolic content and antioxidant capacity

The correlation coefficient between CUPRAC assay and TP content of the medicinal plants were calculated (Fig. 1). The overall relationship between antioxidant activity and TP content for all tested medicinal plants (30 species) was a positive and highly significant linear correlation (R^2=0.935) was achieved. Therefore, the higher TP content of the medicinal plants resulted in higher total antioxidant capacity.

DISCUSSION

Several comprehensive reviews on the natural antioxidants have recently appeared. Many published data highlight the antioxidant potential of the phenolic components of herbs, fruit, vegetables, grains, mushrooms and beverages (Zheng & Wang 2001, Soong & Barlow 2004, Ismail et al. 2004, Adom &

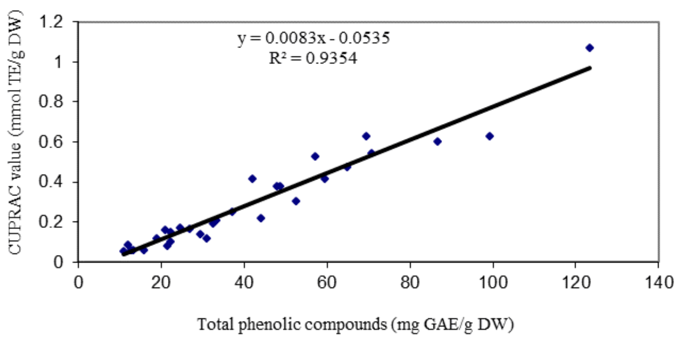

Figure 1.
Relationship between CUPRAC values for total antioxidant capacity (mmol TE/g DW) and TP content (mg GAE/g DW) of methanolic extracts from 30 Macedonian medicinal plants.

Liu 2002, Cheung et al. 2003, Seeram et al. 2008). Antioxidant activity of phenolic compounds is assumed to be mainly due to their redox properties and the ability for quenching and neutralizing free radicals (Louli et al. 2004). As plants produce significant amount of antioxidant metabolites to prevent the oxidative stress, they represent a potential source of new compounds with antioxidant activity. Thus, continued research is being undertaken all over the world on different plants for their antioxidant metabolites.

Total phenol assay by Folin-Ciocalteu reagent has been extensively used to measure the content of phenolics in plant materials for many years. This assay is based on electron transfer reaction and actually measures reducing capacity of samples (Huang et al. 2005). Therefore, it is accepted as a routine assay for rough estimation of the antioxidant capacity of food samples (Roginsky & Lissi 2005). In this study, the values for TP contents in the plant extracts varied from 10.90 to 123.41 mg GAE/g DW and represented a moderate variation of 11-fold. These results are within the range of the values reported by Shan et al. (2005) for 26 spices originated from Asian and Western countries. In discrepancy with our results, Surveswaran et al. (2007) have reported very high variation (695-fold) in TP contents for 133 Indian medicinal plant species. Nevertheless, it is not appropriate to directly compare these data with the results from our study owing to the differences in the number of investigated plant species belonging to different plant families and heterogeneity in the plant part tested. In the study of Surveswaran et al. (2007), whole plants, seeds, rhizomes, fruits, stems, leaves, flowers, tubers and spines were used as plant samples, compared with our study which generally include aerial parts of the tested plant species.

As shown in Tab. 1, the highest TP contents were observed for *O. vulgare* extracts (>100 mg GAE/g DW). Similarly, Kirca & Arslan (2008) have also reported very high content of TP for *O. vulgare* L. methanolic extract. Other selected plants with high phenolic contents were *E. dodonaei* Hausskn. and *E. hirsutum* L. (99.33 and 86.59 mg GAE/g DW, respectively). Similar results for TP contents in the extracts of *E. hirsutum* L. were reported by Ebrahimzadeh et al. (2008). Surprisingly high concentration of TP in *E. dodonaei* Hausskn. and *E. hirsutum* L. could be explained by possible interference of tocopherols previously identified in these species (Velasco & Goffman 1999). In the opposite, results from our study showed that *S. officinalis* L., *A. vulneraria* L. and *A. pallida* Waldst. et Kit. possess very low TP content. These results are in accordance with recent published studies for TP contents in methanolic extracts of *A. pallida* Waldst. et Kit. and *S. officinalis* L. (Kirca & Arslan 2008, Sengul et al. 2011). On the other hand, Godevac et al. (2008) reported relatively high content of TP in *A. vulneraria* L. However, significant differences between the results for TP contents are likely due to genotypic and environmental variations within species, plant part tested, harvesting time, extraction procedure and determination methods (Kim & Lee 2004, Shan et al. 2005).

Methods used for antioxidant activity determination differ in terms of their assay principles and experimental conditions. Consequently, in different methods particular antioxidants have varying contributions to total antioxidant potential (Huang et al. 2005). Therefore, an approach with multiple assays for evaluating the antioxidant potential of extracts would be more informative and even necessary. In this study, the antioxidant capacity of the selected plant extracts was determined using CUPRAC assay. It is worth to mention that CUPRAC assay is useful for estimation of wide variety of polyphenols, including phenolic acids, hydroxycinnamic acids, flavonoids, carotenoids, anthocyanins, as well as for determination of thiols, synthetic antioxidants, ascorbate and tocopherols (Apak et al. 2004). As seen in Tab. 1, CUPRAC values of plant extracts varied in a large scale between 0.052 and 1.068 mmol TE/g DW, represent marked variation of approximately 20-fold. However, our results are within the range of the CUPRAC values for aqueous herbal extracts reported by Apak et al. (2006). Among the plant species used in this study, the highest antioxidant capacity was obtained for *O. vulgare* L. extracts (1.068 mmol TE/g DW). This species, popularly known as Oregano, is an important Mediterranean herb rich in phenolic compounds with antioxidant and antimicrobial activity (Chun et al. 2004). Wojdyło et al. (2007) have reported that antioxidant activity of *O. vulgare* L. is due to presence of phenolic acids, especially caffeic acid and *p*-coumaric acid. Present results demonstrated strong antioxidant properties for *S. ringens* Sibth. & Sm., *E. dodonaei* Hausskn. and *E. hirsutum* L. extracts. In this view, several studies have confirmed the high antioxidant potential of different *Salvia* species (Bozan et al. 2002, Şenol et al. 2010). Our comparative study showed that among 3 tested *Salvia* species, *S. ringens* Sibth. & Sm. had strongest antioxidant activity (0.631 mmol TE/g DW), followed by moderate antioxidant activity of *S. sclarea* L. and *S. nemorosa* L. (0.381 and 0.378 mmol TE/g DW, respectively). Information regarding the antioxidant properties of *Epilobium* taxa in the scientific literature is limited to several species, such as *E. angustifolium* L. and *E. parviflorum* Schreb. (Štajner et al. 2007, Hevesi et al. 2009).However, analyzed species in this study e.g. *E. dodonaei* Hausskn and *E. hirsutum* L. showed very strong antioxidant properties. In accordance with our results, Wojdyło et al. (2007) have evaluated antioxidant capacity of 32 Polish spices and obtained strong antiradical activity for *E. hirsutum* L. extracts. These authors have reported that catechins and procyanidins are dominant class of phenolics in *E. hirsutum* L. extracts responsible for their strong antioxidant activities. Therefore, high antioxidant activity of *E. hirsutum* L. could be explained by the presence of procyanidins, which have the strongest radical-scavenging power among all natural phenolic compounds (Shan et al. 2005). However, it should be taken in account that large variations of reported data for the antioxidant activities may exist for the same plant collected from different sources, depending on the genotype and geographical origin. Thus, it is very hard to separate each antioxidant component and study it individually due to the chemical diversity of antioxidant compounds present in natural samples. In addition, concentrations of a single antioxidant do not necessarily reflect their total antioxidant capacity because of the possible synergistic interactions among the compounds in the plant extracts (Huang et al. 2005).

Results from regression analysis (Fig. 1) revealed significant positive correlation between antioxidant capacity and TP content (R^2=0.935). Namely, *O. vulgare* L., *E. dodonaei* Hausskn. and *E. hirsutum* L. extracts with the highest amounts of TP were the most potent in the CUPRAC assay. On the other side, *D. ferruginea* L., *S. officinalis* L. and *A. pallida* Waldst. et Kit. extracts showed low phenolic amounts and weakest antioxidant activity. In accordance with previous reports (Apak et al. 2006, Djeridane et al. 2006, Dudonné et al. 2009), these findings suggest that phenolic compounds significantly contribute to the antioxidant activity of selected medicinal plants.

CONCLUSIONS

The objective of this study was to evaluate the antioxidant capacity of phenolic compounds in 30 selected medicinal plants collected from Galitchitsa Mt, Republic of Macedonia. Results from this study revealed that plant species used in this study are rich in phenolic constituents and demonstrated strong antioxidant activity measured by CUPRAC method. The antioxidant properties and total phenolic content differed significantly among the tested plant extracts. Among the plants analysed, *O. vulgare* L., *S. ringens* Sibth. & Sm., *E. dodonaei* Hausskn. and *E. hirsutum* L. showed high TP content and strong antioxidant activity. A significant positive correlation between antioxidant capacity and TP contents indicated that phenolic compounds are the major contributor to the antioxidant properties of the plant extracts. This work reveals that Macedonian flora could be an interesting source of new antioxidant plant extracts, with a potential use in food, cosmetic and pharmaceutical industry.

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SUMMARY

Investigations have been made to study the production of total phenolic compounds and total antioxidant capacity in 30 Macedonian traditional medicinal plants collected on Galichitsa Mountain. Total phenolic compounds were estimated according to the Folin-Ciocalteu colorimetric method, while total antioxidant potential of plant extracts was analyzed by using cupric reducing antioxidant capacity (CUPRAC) assay. *Origanum vulgare* L. extract exhibited the highest content of total phenolic compounds and the strongest antioxidant capacity and can be proposed as a promising source of natural antioxidants. *Salvia ringens*, *Epilobium dodonaei* Hausskn. and *Epilobium hirsutum* L. were also identified as valuable sources of antioxidant compounds. A positive linear correlation between antioxidant activity and total phenolics indicates that these compounds are likely to be the main antioxidants contributing to the observed activities of evaluated plants. The investigation of interrelationship between phenolics and antioxidant activity will be a promising field to understand and elucidate possible mechanisms for utilization of selected medicinal plants as sources of bioactive compounds in food and pharmaceutical industry.

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Occurrence and intensity of hepatic capillariasis in the barbel (*Barbus rebeli*) from the river Crn Drim in the Republic of North Macedonia – Preliminary results

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Abstract. *Capillaria hepatica* is a parasitic nematode which infects the liver of mammals, as well as fish. As this parasite migrates through the host tissue, every phase of its life cycle can induce cellular response by the immune system. Due to the fact that very little is known about the prevalence of *Capillaria hepatica* as well as the cellular immune response to the parasitic infections in fish species *Barbus rebeli*, the aim of our study is to examine the changes in the amount of the pigmented macrophage aggregates as part of the non-specific immune system, the response to the parasitic infections in the liver and histopathological features of the liver in infected barbels. According to this, 104 barbels were collected from the river Crn Drim, their condition factor (CF) was calculated and the liver was investigated for presence of capillariasis eggs. The relative volume of parasitic eggs and pigmented macrophage aggregates as well as their mutual correlation were estimated using stereological techniques. The results from the research showed that the parasite slightly decreases CF, increases liver weight and induces cellular immune response by significantly increasing the relative volume of the macrophages in the liver of the barbels.

Key words: capillariasis, fish, barbel, pigmented macrophages, liver, stereology

Introduction

Capillaria hepatica is widely distributed parasite in the digestive system of higher classes of vertebrates (William et al. 2000). Although its primary hosts are rodents, it can be found in all mammal species including humans (Fuehrer et al. 2011). Their eggs have oval shape and double-layered membrane with many small-sized pores (Roberts and Janovy 2009), but the most important characteristic is the way they are being transmitted. The parasitic eggs stay incapsulated in the hepatic tissue of the host until released by his death or transferred in other host by consumption. In the first case, after the death of the host the eggs will pass in the soil where they are able to embryonate, infect other animals and start their life cycle again. In the second case, when the infected organism has been consumed by a predator, after ingestion the eggs pass through the intestines and in 24 hours the first-stage larvae are being developed. After that, they are translocated through the hepatic vein in the liver of the new host (Roberts and Janovy 2009).

Human population can be infected by *Capillaria hepatica* if the food is contaminated with debris from the rodents (William et al. 2000). Since some fish species which are part of the human diet can also be infected (Moravec 1994; Thilakaratne et al. 2003; Sattary et al.2005) it poses serious threats to human health, including mortality (Fuehrer et al. 2011; Dubey et al. 2017). Humans get infected by hepatic capillariasis if they consume the infected fish uncooked (Cross and Basaca-Sevilla 1984; Yong -Yill 2010).

In general, very little is known of capillariid nematodes in fish and often they remain unidentified. As far as we know, in our country this type of histological investigations in fish from our water bodies is very scarce. The only literature data for hepatic capillariasis is from Roganovic-Zafirova and co-workers, 2003. In this research, hepatic capillariasis has been found in barbel from the Lake Ohrid for which has been assumed that it is probably

Schulmanela petruschewskii species (Roganovic-Zafirova et al. 2003).

Among the first reactions to occur in fish infected by hepatic capillariasis is an immune response (Klinger and Floyd 1998). The pigmented macrophages are important part of the immune system in fish, which are involved in the defense of the organism in many different ways (Ellis 1977; Agius 1985). These cells are able to phagocytize, degrade, detoxify, recycle and/or destroy unnecessary endogenous and exogenous substances (Ferguson 1976; Ellis 1980; Herraez and Zapata 1986; Vogelbein et al. 1987; Wolke, 1992; Haaparanta et al. 1996). Although the pigmented macrophage aggregates react in the presence of parasites (Thilakarante et al. 2007), the information about their mutual correlation in barbell species is scarce. The aim of this paper is to demonstrate that the examined barbel species *Barbus rebeli* are frequently exposed to infection by capillariasis eggs, endangering the life of the people who use them in their diet. We will document their prevalence as a result of parasitic infections and hepatic pathology, as well as the association of the infection with the non-specific immune response. Particular attention was paid on investigating the correlation between the parasitic eggs and pigmented macrophages, which was observed for the first time on fish infected with hepatic capillariasis.

Materials and methods

A total of 104 specimens were captured with electrofishing method along the river Crn Drim. The fish were transported with an aerated container to the laboratory. Fish were visually examined for microscopically visible gross abnormalities. Total length (TL) and body weight (BW) were recorded for the estimation of condition factor (CF) according to the following formula: $CF = BW \times 100/TL^3$. Afterwards, fish were euthanized with Clove Oil (Sigma Aldrich, USA), the liver was isolated, weighted and the hepatosomatic index (HSI) was calculated by the formula: $HIS = LW \times 100/BW$. The collected organs were processed as whole or divided into several parts by the SRS method (Systematic Random Sampling), depending on their size, then placed in Bouin's fixative for 48h. After the fixation, the specimens were processed by routine histological techniques and finally embedded in paraffin blocks. 5 µm thick histological sections were made with rotation microtome and stained with hematoxylin and eosin. All histological slides were examined with light microscopy on 10 fold magnification for qualitative registration of liver lesions and presence of capillariasis eggs and according to their presence, fish were classified as infected (P) and uninfected (WP). For quantitative examination, microscopic evaluation was performed at 10 fold magnification on the light microscope, of in total 40-50 visual fields per fish selected using the SRS method. For stereological analyses, a square lattice grid with 180 points was inserted into the ocular of the microscope. This grid was used for estimating the amount (relative volume) of parasitic eggs and pigmented macrophages (MACs) within the hepatic parenchyma. For these analyses, standard point techniques were applied (Freere and Weibel 1967), according to the following formula:

$$Vv\,(\text{structure, reference}) = [P(s) \times 100] / P(r)$$

where Vv is the percentage of the total volume of a reference space occupied by particular given structure within that space, P(r) is the number of points falling over a chosen component (pigmented macrophages and parasitic eggs) and P(s) is the total number of points laying over the reference space of the liver.

In this study, the obtained quantitative data are given as average values, accompanied by standard deviation. Statistical data processing was performed using Statistica 12 for Windows software. The ANOVA method was used to study the effects of parasitic infection on the condition factor (CF) of the fish and their liver weight. Specimens with missing data for some of the observed or measured parameters were excluded from statistical analysis accordingly. With post-hoc Tukey test were revealed the differences between fish with and without parasites for every measured parameter. The correlation between macrophage aggregates and capillariasis eggs was determined by Spearman correlation coefficient. Differences were considered statistically significant when $p \leq 0.05$.

Research area

Crn Drim is a river in North Macedonia that flows from St. Naum, passes through the Ohrid Lake and exits from it at the city of Struga, at an altitude of 695 meters. Near the city of Debar, at 476 meters above sea level this river leaves the country. After the confluence of Crn Drim with the river Bel Drim near the city of Kukës in Albania, they flow into the Adriatic Sea, under one name - Drim. On the Macedonian territory, Crn Drim is 56 km long. In its waters there is a variety of fish species, such as carp, chub, eel, barbel and others. The river's name Crn Drim comes from the presence of the silicate, endemic algae with a black color. It represents an important hydropower potential for the Republic of North Macedonia.

RESULTS

Morphological inspection of the collected individuals performed prior to histological assessment showed that all specimens were without any visual abnormalities in regard to external features. The body weight (BW) of the infected fish (Tab. 1, Chart 1) was slightly decreased in comparison to the uninfected fish as well as their condition factor (CF) (Tab. 1, Chart 2), although these changes were not statistically significant. On the other hand, infected fish had slightly increased liver weight (LW) (Tab. 1, Chart 3) and insignificantly decreased hepatosomatic index (HSI) (Tab. 1, Chart 4). Liver analysis showed presence of capillariasis eggs (Fig. 1) in 80.68% of the examined barbels, which in their appearance corresponded to the eggs recorded by Roganovic-Zafirova and co-workers (2003) in barbels of Lake Ohrid. From histopathological examination, several lesions were observed, as necrosis (Fig. 2), lymphocyte infiltration and fibrosis around the stromal pathways. The investigation showed that the pathological disorders in the liver parenchyma are poorly associated with infection. An immune response was also observed from pigmented macrophages with a relative volume ranging from 0.027% to 5.077% in the infected barbels while in the uninfected the range was from 0% to 1.53% (Tab. 1, Chart 5). ANOVA showed that infected fish have significantly higher values (p=0,011) for MACs in the liver of barbels. The intensity of infection varied from 1 egg to 150 eggs per tissue. Stereological analysis showed that eggs occupy a volume of 0.014% to 5.89% of the liver parenchyma. In most cases, macrophage aggregates were randomly distributed throughout the tissue or rarely associated with the eggs' capsule (Fig. 3). Investigation of the amount of pigmented macrophages and parasitic eggs showed that there is a positive correlation between this two parameters ($r = 0.16$; $P < 0.05$).

Table 1. BW, CF, LW, HSI and Vv (MACs, liver) % in the barbel with (P) and without (WP) hepatic capillariasis infection

Lesion status	N	BW (g)		CF (%)		LW (g)		HSI (%)		Vv (MACs, liver) %*	
P	71	93.27	(53.96)	1.36	(0.22)	1.34	(1.21)	1.05	(0.54)	1,32	$(0.93)^{a}$
WP	17	108.12	(57.85)	1.40	(0.16)	1.06	(0.89)	1.08	(0.88)	0,52	$(0.42)^{b}$

Values are expressed as mean (standard deviation).
*different lowercase superscript letters represent differences between fish with and without parasites; values are significantly different ($p<0.05$), according to the Tukey test.

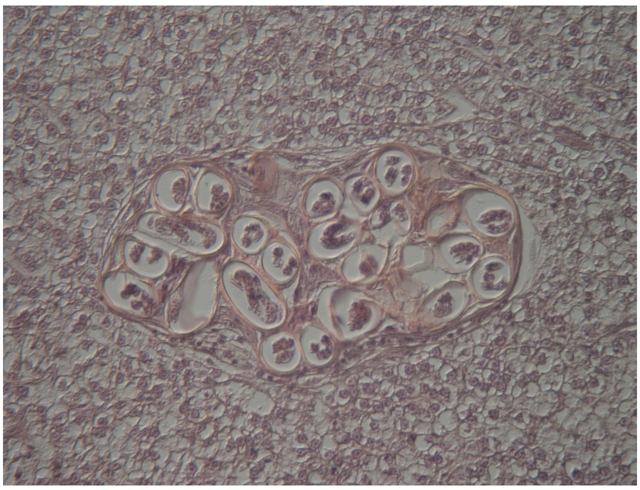

Figure 1: A tissue section showing grouped capillariasis eggs in the liver parenchyma of *Barbus rebeli*

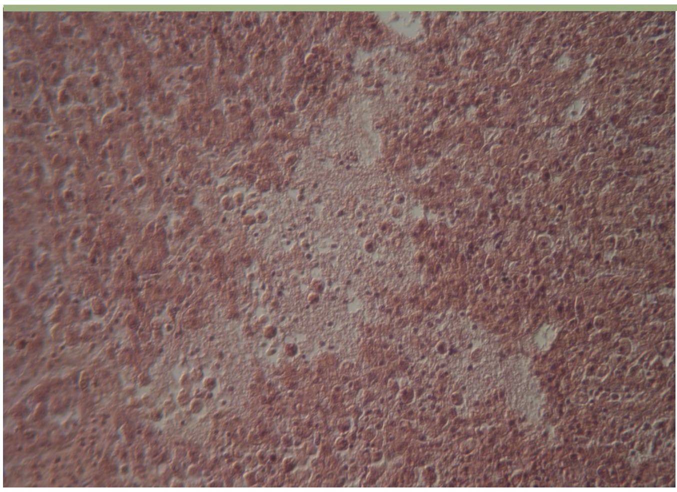

Figure 2: A tissue section showing necrosis in the liver of *Barbus rebeli*

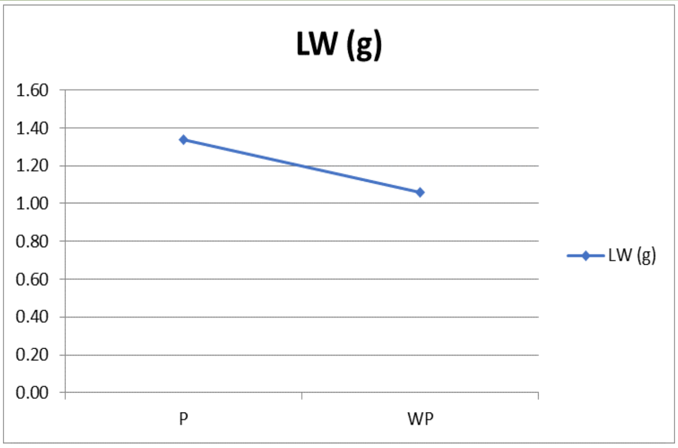

Chart 3. Liver weight (LW) in barbels with (P) and without parasites (WP).

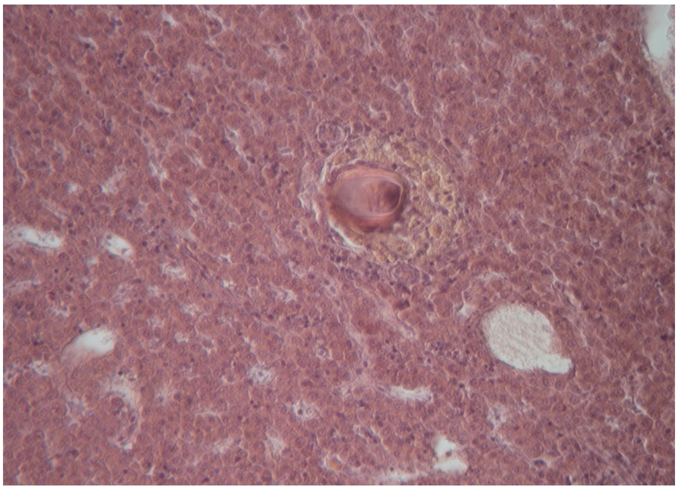

Figure 3: Pigmented macrophage in association with capillariasis egg in the liver parenchyma of *Barbus rebeli*

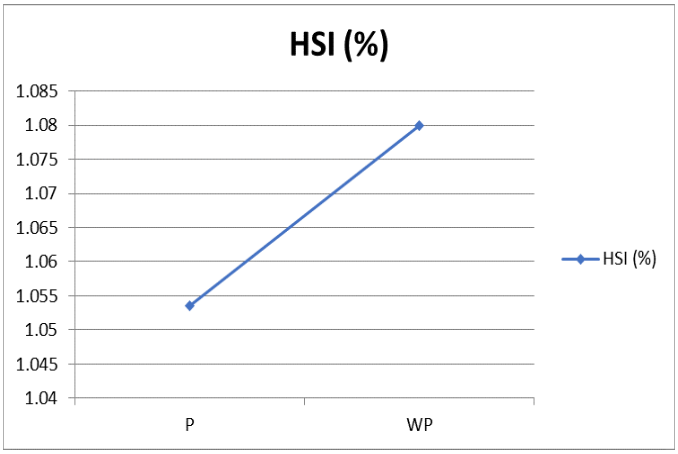

Chart 4. Hepatosomatic index (HSI) in barbels with (P) and without parasites (WP)

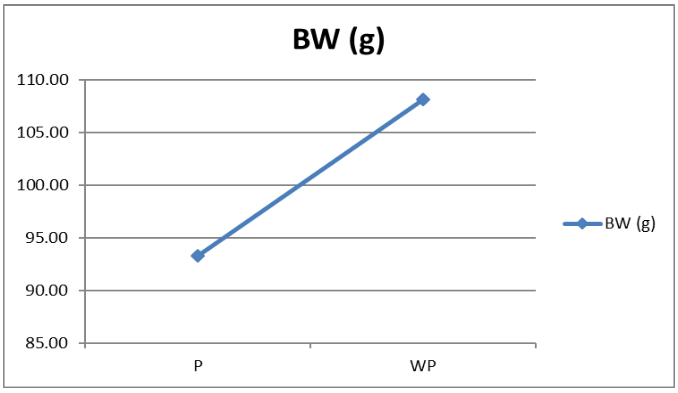

Chart 1. Body weight (BW) in barbels with (P) and without parasites (WP).

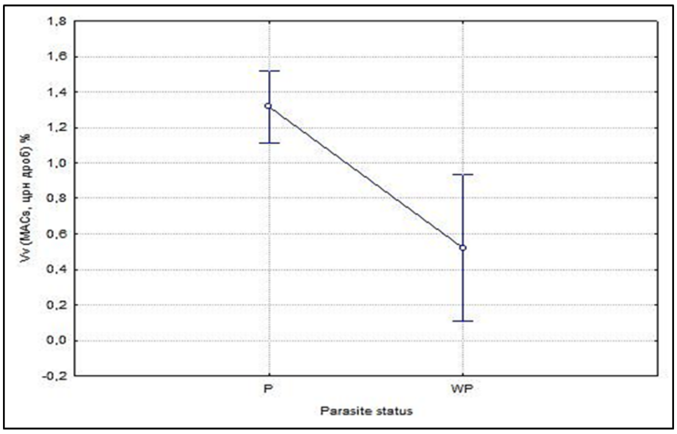

Chart 5. Relative volume of the pigmented macrophages in the liver [Vv (MACs, liver)] of barbels with (P) and without parasites

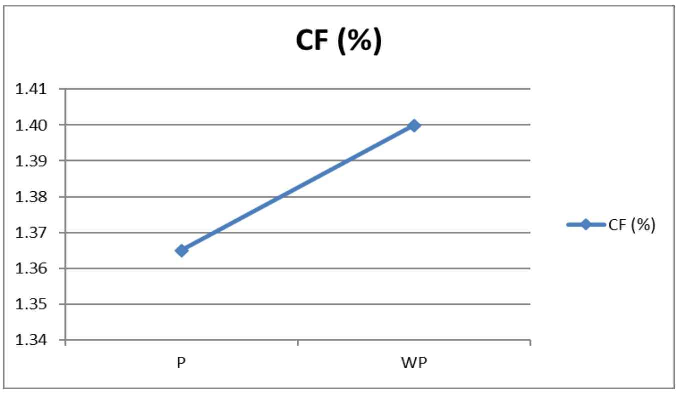

Chart 2. Condition factor (CF) in barbels with (P) and without parasites (WP).

Discussion

The liver is the central, metabolic organ of the fish that has numerous anabolic and catabolic functions and therefore its damage can be lethal to the body. Our research showed that 80.68% of the examined barbels were infected with hepatic capillariasis. The female parasites produce their eggs in the liver tissue which remain there until the death of the animals (Fuehrer et al. 2011). In order to determine the impact of capillariasis eggs on fish health, CF was compared between the infected and uninfected barbels. CF is a useful index for monitoring the feeding intensity, age and growth rate of fish. It is strongly influenced by biotic and abiotic environmental conditions and can be used as an index to assess the status of the aquatic ecosystem in which fish live (Anene et al. 2005). Nutrition factor, the presence of pathogenic organisms or exposure to toxic substances can cause its increase or decrease (Schmitt and Dethloff 2000).

According to the results of this study, the reduction in body weight (BW) of the infected fish resulted in decrease of their condition factor (1.36%). Although we found intense parasitic infection, this decrease in CF was not statistically significant. Despite the fact that infected fish had slightly increased liver weight (LW) in comparison with the uninfected, their HSI decreased. Since HSI represents the ratio between organ size and body weight, its decrease was not influenced as much by changes in LW, as it was by the decrease in BW.

The liver plays a key role in the metabolism and biochemical transformations of environmental pollutants, therefore organ changes will inevitably affect its integrity by producing lesions and other histopathological changes in the liver or bile duct parenchyma (Robert 1978). Necrosis, inflammatory response and fibrosis in individuals infected with parasites were observed by Roganovic-Zafirova and coworkers (2003) and Simá and coworkers (1996). Similar findings, fibrosis, necrosis and sometimes granulomatous infections, have also been found in rats (Oliviera and Andrade, 2001). In this study, hepatic parenchymal reactions to capillariasis were minimal. In addition, cholangiopathic changes were extremely rare compared with data from Roganovic-Zafirova and co-workers (2003), where such changes are very often associated with hepatic capillariasis disease. On the other hand, an immune response was observed, i.e. a significant increase of the relative volume of pigmented macrophages. According to some authors, the increase in the number of pigmented macrophages depends on both the type of parasite and the presence of contaminants in the water, which may further impair fish health (Thilakarante et al. 2007). Since adult form of the parasite was not found, we were not able to identify the capillariid species. Although in our study there is a positive correlation between pigmented macrophages and the presence of capillariasis eggs, these data are not sufficient to draw conclusions about the functional status of the liver infected with this parasite. More detailed research is needed to determine the effect of the infection not only on the immune system but also on the overall health status of the barbels.

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First data on the spider fauna (Araneae) of Maleshevski Mountains in the Republic of Macedonia

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Arachnology section

Abstract. The first study of the spider fauna of Maleshevski Mountains in Macedonia conducted on 21 sites (1020-1820 m a.s.l.) in the period between 2018-2019 resulted in a total number of 107 species belonging to 17 families (Dysderidae – 4, Theridiidae – 6, Linyphiidae – 43, Tetragnathidae – 1, Lycosidae – 23, Pisauridae – 1, Agelenidae – 3, Cybaeidae – 1, Miturgidae – 2, Liocranidae – 1, Phrurolithidae – 1, Clubionidae – 1, Zodariidae – 1, Gnaphosidae – 9, Philodromidae – 2, Thomisidae – 5 and Salticidae – 3). Nine species represent new records for the spider fauna of Macedonia: *Harpactea mentor*, *Micrargus apertus*, *Ozyptila trux*, *Paidiscura pallens*, *Poecilochroa variana*, *Saaristoa firma*, *Styloctetor compar*, *Tenuiphantes alacris* and *T. cristatus*. Finding of the species *Saaristoa firma* on Maleshevski Mts represent one of the most interesting records. So far, this species has been mainly recorded from Northern and Western Europe, and the record from Maleshevski Mts represents second record from the Balkan Peninsula. According to their current distribution, the spiders have been classified into 14 zoogeographic categories, combined into 4 chorological complexes (widely distributed, European, Mediterranean and endemics). In the complex of widely distributed species consisting of 54 species (50.4 %), Euro-Siberian species (19.6 %) dominate, followed by Holarctic (9.3 %) and Euro-Asian species (6.5 %). European species complex comprises 32 species and it is consisting of European (28 %) and S-European (1.8 %) chorotypes. In Endemic species complex consisting of 13 species, Subendemic chorotype dominate (4.6 %), followed by Balkanic (3.7%), Carpatho-Balkanic (2.8%) and Alpine-Balkanic chorotype (0.9 %).

Key words: Araneae, spiders, Maleshevski Mountains, taxonomy, faunistic, zoogeography.

Извод. Овој труд, кој претставува прва студија за фауната на пајаците на Малешевските Планини во Македонија спроведена на 21 локалитет (1020-1820 м н.в.) во период меѓу 2018-2019 година, резултираше со вкупно 107 видови кои припаѓаат на 17 семејства (Dysderidae – 4, Theridiidae – 6, Linyphiidae – 43, Tetragnathidae – 1, Lycosidae – 23, Pisauridae – 1, Agelenidae – 3, Cybaeidae – 1, Miturgidae – 2, Liocranidae – 1, Phrurolithidae – 1, Clubionidae – 1, Zodariidae – 1, Gnaphosidae – 9, Philodromidae – 2, Thomisidae – 5 and Salticidae – 3). Девет видови претставуваат нови наоди за фауната на пајаците на Македонија: *Harpactea mentor*, *Micrargus apertus*, *Ozyptila trux*, *Paidiscura pallens*, *Poecilochroa variana*, *Saaristoa firma*, *Styloctetor compar*, *Tenuiphantes alacris* и *T. cristatus*. Пронаоѓањето на видот *Saaristoa firma* на Малешевските Планини претставува еден од најзначајните наоди. Досега, овој вид главно е регистриран во Северна и Западна Европа, и наодот на Малешевски Планини претставува втор податок за Балканскиот Полуостров. Според нивната тековна дистрибуција, пајаците се класифицирани во 14 зоогеографски категории, комбинирани во 4 хоролошки комплекси (широко распространети, европски, медитерански и ендемични). Во комплексот на широко распространети, кој се состои од 54 видови (50,4%), доминираат евро-сибирските видови (19,6%), а потоа следуваат холарктичките (9,3%) и евроазиските видови (6,5%). Комплексот на Европски видови опфаќа 32 вида и се состои од европскиот (28%) и јужно-европскиот (1,8%) хоротип. Во комплексот на ендемични видови, кој се состои од 13 видови, доминира субендемичниот хоротип (4,6%), проследен со балканскиот (3,7%), карпато-балканскиот (2,8%) и алпско-балканскиот хоротип (0,9%).

Клучни зборови: Araneae, пајаци, Малешевски Планини, тасономија, фаунистика, зоогеографија

Introduction

In terms of faunistic research for many groups, eastern part of the Republic of Macedonia is one of least studied areas in the country. There is no existing data about the spider fauna of the Macedonian part of the Maleshevski Mts. The only data can be found for the Bulgarian part of the mountain (Lazarov, 2007a).

The aim of this study is to present first data about the spider fauna of the Macedonian part of the Maleshevski Mts.

Study area

Maleshevski Mts. are located in the eastern part of the Republic of Macedonia (subject to the present study) and the southwestern part of the Republic of Bulgaria. Its highest peaks are Dzhami Tepe (1803 m) and Čengino [=Chengino] Kale (1748 m). On the north-east side, the mountain gradually merges with Vlaina Mt. and there is no clearly marked fold between them, except for the saddle Ajdučki [=Ajduchki] Premin (1612 m). Geologically, the Macedonian part of the mountain is mainly composed of gneisses and mica schists, and in the lower parts of chlorite schists and Pliocene sediments. The climate is continental-mountainous with cool summers (maximum temperatures up to 35 °C) and cold winters (minimum temperatures up to -31 °C). The mountain is known for its mixed coniferous-beech forests.

Materials and methods

The present study is a result of the first investigation of the spider fauna on Maleshevski Mts (including Vlaina Mt.) conducted in the period of 13.06-15.08.2018 and 26-28.08.2019 in the frame of the project "Nature Conservation Program in Macedonia - Phase 2", organized by the Macedonian Ecological Society. A total of 21 sites (1020-1820 m a.s.l.) (Tables 1 and 2) were visited where spiders were collected by hand, pitfall traps, sieving leaf litter and suction sampling. Hand collection was conducted at eight sites in the period of 28-29.06.2018 and 26-28.08.2019 at altitudes from 1020 to 1750 m a.s.l. The pitfall traps consisted of a plastic cup (9 cm in diameter and 11 cm height) filled with 8% vinegar. A set of 50 pitfall traps were placed at ten sites at different altitudes (from 1300 to 1820 m). The pitfall traps were active in the period of 13.06-15.08.2018. Sieving leaf litter was done on 28.06.2018 in beech forest at one locality only (Bukovik, Orlovec) at altitude of 1675 m. Suction sampling was performed at five sites in the period of 26-27.08.2019 at altitudes from 1198-1760 m.

Specimens were examined and photographed using a WILD M5 stereomicroscope equipped with a DigiRetina 16M digital camera.

The terminology for species distributional patterns follows Komnenov (2014) except for the chorotype subendemic (SUBE) which is introduced here. This chorotype contains species with narrow distribution in Macedonia and some of the border regions with the neighbouring countries (Serbia, Bulgaria, Greece, Albania). Example: *Harpactea srednagora* – distributed in NE-Macedonia and SW-Bulgaria.

Spider nomenclature is after World Spider Catalog (2020). List of species is given in taxonomic order. The materials are kept in the private collections of the author.

Results and disccusion

In total 107 species from 17 families have been registered from 21 sites (1020-1820 m a.s.l.) on Maleshevski Mts in Macedonia (Dysderidae – 4, Theridiidae – 6, Linyphiidae – 43, Tetragnathidae – 1, Lycosidae – 23, Pisauridae – 1, Agelenidae – 3, Cybaeidae – 1, Miturgidae – 2, Liocranidae – 1, Phrurolithidae – 1, Clubionidae – 1, Zodariidae – 1, Gnaphosidae – 9, Philodromidae – 2, Thomisidae – 5 and Salticidae – 3) (Table 3).

Nine species represent new records for the spider fauna of Macedonia: *Harpactea mentor* Lazarov & Naumova, 2010, *Micrargus apertus* (O. P. -Cambridge, 1871), *Ozyptila trux* (Blackwall, 1846), *Paidiscura pallens* (Blackwall, 1834), *Poecilochroa variana* (C. L. Koch, 1839), *Saaristoa firma* (O. P.-Cambridge, 1906), *Styloctetor compar* (Westring, 1861), *Tenuiphantes alacris* (Blackwall, 1853) and *T. cristatus* (Menge, 1866).

The most characteristic is the family Linyphiidae with 43 species, followed by Lycosidae (23), Gnaphosidae (9) and Theridiidae (6). The genera with the largest number of species are *Pardosa* (12), *Tenuiphantes* (5) and *Alopecosa* (5).

One of the most interesting records is the finding of the species *Saaristoa firma.* It was found in beech forest at altitude of 1675 m on the locality Bukovik. So far, this species has been mainly rec-

Table 1. List of localities on Maleshevski Mt.	
1	Bukovik, Elensko Blato, 1470 m a.s.l., bog, pitfall traps, 28.06-15.08.2018, leg. S. Hristovski, A C. Gjorgievska & M. Komnenov
2	Bukovik, Elensko Blato, 1470 m a.s.l., mixed forest, hand collection, 28.06.2018, leg. M. Komnenov
3a	Bukovik, Orlovec, 1675 m a.s.l., beech forest, pitfall traps, 28.06-15.08.2018, S. Hristovski, A. C. Gjorgievska & M. Komnenov
3b	Bukovik, Orlovec, 1675 m a.s.l., beech forest, hand collection, 28.06.2018, leg. M. Komnenov
3c	Bukovik, Orlovec, 1675 m a.s.l., beech forest, sieving leaf litter, 28.06.2018, leg. M. Komnenov
4	Čengino [=Chengino] Kale, 1740 m a.s.l., bog, pitfall traps, 29.06-28.07.2018, S. Hristovski, A. C. Gjorgievska, M. Komnenov & D. Chobanov
5	Čengino [=Chengino] Kale, 1750 m a.s.l., shrubs, pitfall traps, 29.06-28.07.2018, S. Hristovski, A. C. Gjorgievska, M. Komnenov & D. Chobanov
6	Čengino [=Chengino] Kale, 1750 m a.s.l., heaths, hand collection, 29.06.2018, leg. M. Komnenov
7	Mlečna [=Mlechna], 1590 m a.s.l., beech forest, pitfall traps, 29.06-28.07.2018, S. Hristovski, A. C. Gjorgievska, M. Komnenov & D. Chobanov
8	Mlečna [=Mlechna], 1380 m a.s.l., mixed forest, pitfall traps, 29.06-28.07.2018, S. Hristovski, A. C. Gjorgievska, M. Komnenov & D. Chobanov
9	Pehčevo [=Pehchevo], Debeli Rid, 1300 m a.s.l., wet meadow, hand collection, 29.06.2018, leg. M. Komnenov
10	Strednačka [=Strednachka] river, 1300 m a.s.l., wet meadow, pitfall traps, 29.06-28.07.2018, S. Hristovski, A. C. Gjorgievska, M. Komnenov & D. Chobanov
15	Trebomirska river, 1198 m a.s.l., wet vegetation, suction sampling, 26.08.2019, leg. M. Komnenov
16	Klepalo, 1306 m a.s.l., under logs in beech forest, hand collection, 26.08.2019, leg. M. Komnenov
17	Bukva, 1478 m a.s.l., wet meadow, suction sampling, 26.08.2019, leg. M. Komnenov
18	Čengino [=Chengino] Kale, 1760 m a.s.l., subalpine pasture, suction sampling, 26.08.2019, leg. M. Komnenov
19	Vrtena Skala, Pehčevska [=Pehcevska] river, 1390 m a.s.l., wet meadow, suction sampling, 27.08.2019, leg. M. Komnenov
20	Bukovik, Elensko Blato, 1480 m a.s.l., wet vegetation, suction sampling, 27.08.2019, leg. M. Komnenov
21	Berovo lake, Zamenica, near Zamenička [=Zamenichka] river, 1020 m a.s.l., under stones in mixed forest, hand collection, 28.08.2019, leg. M. Komnenov

Table 2. List of localities on Vlaina Mt.	
11	Kadiica, 1810 m a.s.l., subalpine pasture, pitfall traps, 28.06-15.08.2018, S. Hristovski, A. C. Gjorgievska & M. Komnenov
12	Kadiica, 1820 m a.s.l., shrubs, pitfall traps, 13.06-15.08.2018, leg. S. Hristovski, A. C. Gjorgievska & M. Komnenov
13a	Treščen [=Treshchen] Kamen, 1620 m a.s.l., subalpine pasture, hand collection, 13.06.2018, leg. M. Komnenov
13b	Treščen [=Treshchen] Kamen, 1620 m a.s.l., subalpine pasture, hand collection, 28.06.2018, leg. M. Komnenov
14	Treščen [=Treshchen] Kamen, 1625 m a.s.l., bog, pitfall traps, 28.06-15.08.2018, leg. S. Hristovski, A. C. Gjorgievska & M. Komnenov

orded from Northern and Western Europe (Blick, 2009). In the Balkan Peninsula it was previously known from Serbia only (Ćurčić et al., 2003). Male palp and female epigyne are shown on Figures 1-2.

Zoogeographical analysis

In the complex of **widely distributed species** Euro-Siberian species (19.6 %) dominate, followed by Holarctic (9.3 %) and Euro-Asian species (6.5 %), distributed on high altitudes from 1000-1900 m. The majority of the species from this complex belong to the family Linyphiidae (13) (*Agyneta rurestris*, *Bathyphantes nigrinus*, *Bolephthyphantes index*, *Gonatium rubens*, *Lepthyphantes leprosus*, *Linyphia hortensis*, *Linyphia triangularis*, *Minyriolus pusillus*, *Oedothorax agrestis*, *Tenuiphantes tenebricola*, *Troxochrus scabriculus*, *Walckenaeria alticeps* and *Walckenaeria furcillata*).

European species complex (European and S-European chorotypes) comprises 32 species (29.9 %). **European species** are dominant (29.9 %) (*Alopecosa farinosa* (1760-1820 m), *A. trabalis* (1740-1820 m), *Apostenus fuscus* (1020 m), *Arctosa maculata* (1198 m), *Centromerus cavernarum* (1675 m), *Dicymbium tibiale* (1480 m), *Dipoena braccata* (1470 m), *Gonatium paradoxum* (1198-1480 m), *Gongylidiellum latebricola* (1478-1480 m), *Man-*

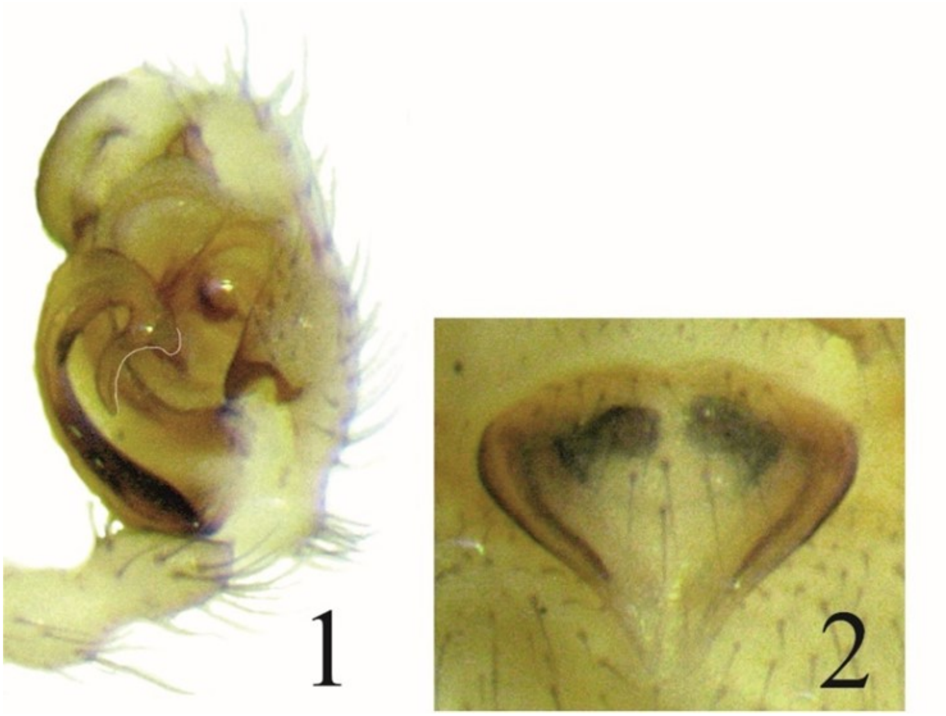
suphantes mansuetus (1390-1478 m), *Metellina segmentata* (1478 m), *Micrargus apertus* (1675 m), *Palliduphantes pallidus* (1470 m), *Pardosa agrestis* (1300 m), *P. alacris* (1306-1478 m), *P. amentata* (1300-1740 m), *P. lugubris* (1470 m), *P. monticola* (1760-1820 m), *P. prativaga* (1300-1620 m), *Philodromus collinus* (1300 m), *Phylloneta sisyphia* (1620 m), *Piratula latitans* (1470 m), *Poecilochroa variana* (1740 m), *Saaristoa firma* (1675 m), *Tenuiphantes alacris* (1675 m), *T. cristatus* (1198-1390 m), *Walckenaeria mitrata* (1478 m), *Xysticus erraticus* (1740 m), *Zelotes apricorum* (1300-1620 m) and *Zora silvestris* (1300 m)) while **S-European species** (1.8 %) are represented by two species only (*Megalepthyphantes collinus* (1306 m) and *Pardosa blanda* (1740-1820 m).

Mediterranean species complex comprised by Ponto-E-Mediterranean chorotype includes one species only: *Dysdera longirostris* (1306-1470 m). The taxonomy of this species in the E-Mediterranean is not clear. The poor representation of Mediterranean species is not too surprising since only higher parts of the mountain were investigated (1020-1820 m a.s.l).

Endemic species complex consisting of Alpine-Balkanic, Carpatho-Balkanic, Balkanic and Subendemic chorotypes contains 13 species (12.1 %). This complex is dominated by the **Subendemic species** (4.6 %): *Harpactea mentor* (1020 m), *H. srednagora* (1020 m), *Inermocoelotes kulczynskii* (1380-1675 m), *Pardosa tasevi* (1300-1820 m) and *Tegenaria rilaensis* (1590 m).

So far, *Harpactea mentor* was known only from Alibotush Mt. in NW-Bulgaria, where it occurs between 1000 and 2000 m a.s.l (Lazarov & Naumova, 2010). The finding of this species in mixed forest at 1020 m altitude on Maleshevski Mts in Macedonia, extends its distribution for about 50 km to the NW.

The distribution of *H. srednagora* in Bulgaria is restricted to south-western parts, in the mountainous area of Sushtinska Sredna Gora, Vitosha and Western Rhodopes, where it inhabits scree and dry stony areas covered with bushes and young trees between 584 and 992 m altitude (Lazarov, 2007b). In Macedonia, this species is known from



Figures 1-2. *Saaristoa firma* (O. Pickard-Cambridge, 1906). **1**. palp, retrolateral view; **2**. epigyne, ventral view.

Table 3. List of species, habitats and zoogeographical classification of spiders registered on Maleshevski Mts. New records for the country are marked with an asterisk: *.

Family Genus/Species/Author	Localities	Zoogeogr. categories
DYSDERIDAE - 4		
Dysdera longirostris Doblika, 1853	1 (1 ♀), 16 (1 ♀)	PEM
**Harpactea mentor* Lazarov & Naumova, 2010	21 (5 ♂♂ 4 ♀♀)	SUBE
Harpactea saeva (Herman, 1879)	7 (1 ♂)	KBA
Harpactea srednagora Dimitrov & Lazarov, 1999	21 (2 ♂♂ 2 ♀♀)	SUBE
THERIDIIDAE - 6		
Dipoena braccata (C. L. Koch, 1841)	1 (1 ♂)	EUR
Episinus truncatus Latreille, 1809	13b (1 ♀)	WPA
Neottiura bimaculata (Linnaeus, 1767)	20 (1 ♀)	PAL
**Paidiscura pallens* (Blackwall, 1834)	17 (1 ♀)	WPA
Phylloneta sisyphia (Clerck, 1757)	13b (1 ♀)	EUR
Robertus arundineti (O. P.-Cambridge, 1871)	13a (1 ♀)	EMM
LINYPHIIDAE - 43		
Agyneta rurestris (C. L. Koch, 1836)	2 (1 ♀), 12 (1 ♂), 13b (1 ♀), 17 (1 ♂ 1 ♀), 18 (7 ♂♂ 1 ♀), 20 (1 ♂)	EUS
Bathyphantes nigrinus (Westring, 1851)	15 (5 ♂♂ 9 ♀♀), 19 (9 ♂♂ 3 ♀♀), 20 (1 ♂)	EUS
Bolephthyphantes index (Thorell, 1856)	18 (2 ♂♂)	EUS
Bolyphantes kolosvaryi (Caporiacco, 1936)	17 (6 ♂♂ 4 ♀♀), 18 (6 ♂♂ 4 ♀♀), 19 (1 ♀)	ABA
Centromerus cavernarum (L. Koch, 1872)	3b (1 ♀)	EUR
Dicymbium tibiale (Blackwall, 1836)	20 (4 ♂♂ 1 ♀)	EUR
Diplostyla concolor (Wider, 1834)	15 (1 ♂), 16 (1 ♂), 17 (3 ♂♂ 3 ♀♀)	HOL
Gonatium paradoxum (L. Koch, 1869)	1 (1 ♀), 15 (2 ♂♂), 19 (1 ♂), 20 (2 ♂♂)	EUR
Gonatium rubens (Blackwall, 1833)	17 (3 ♂♂ 9 ♀♀), 18 (1 ♂ 1 ♀), 19 (1 ♀), 20 (2 ♂♂ 3 ♀♀)	EUS
Gongylidiellum latebricola (O. P.-Cambridge, 1871)	17 (1 ♀), 20 (1 ♀)	EUR
Improphantes decolor (Westring, 1861)	17 (2 ♂♂), 18 (1 ♂)	WPA
Lepthyphantes centromeroides Kulczyński, 1914	7 (1 ♂), 2 (1 ♀), 3b (♀)	KBA
Lepthyphantes leprosus (Ohlert, 1865)	21 (1 ♀)	EUS
Linyphia hortensis Sundevall, 1830	20 (1 ♀)	EUS
Linyphia triangularis (Clerck, 1757)	15 (1 ♀), 19 (1 ♀), 20 (2 ♂♂ 2 ♀♀)	EUS
Mansuphantes mansuetus (Thorell, 1875)	17 (1 ♀), 19 (3 ♀♀)	EUR
Maso sundevalli (Westring, 1851)	18 (1 ♀)	HOL
Megalepthyphantes collinus (L. Koch, 1872)	16 (1 ♀)	SEU
**Micrargus apertus* (O. P.-Cambridge, 1871)	3b (1 ♂ 2 ♀♀)	EUR
Micrargus herbigradus (Blackwall, 1854)	19 (♀♀), 20 (4 ♀♀)	PAL
Microneta viaria (Blackwall, 1841)	3a (1 ♂), 3c (1 ♀), 15 (1 ♀)	HOL
Minyriolus pusillus (Wider, 1834)	17 (1 ♂)	EUS
Neriene clathrata (Sundevall, 1830)	17 (2 ♀♀)	HOL

Family Genus/Species/Author	Localities	Zoogeogr. categories
Neriene peltata (Wider, 1834)	9 (1 ♀)	EKA
Oedothorax agrestis (Blackwall, 1853)	19 (2 ♂♂), 20 (6 ♂♂ 3 ♀♀)	EUS
Oedothorax apicatus (Blackwall, 1850)	1 (1 ♀), 10 (1 ♀)	EMA
Palliduphantes byzantinus (Fage, 1931)	5 (1 ♀)	BALK
Palliduphantes pallidus (O. Pickard-Cambridge, 1871)	2 (1 ♀)	EUR
Pocadicnemis juncea Locket & Millidge, 1953	1 (1 ♀), 13b (1 ♂), 17 (2 ♀♀), 18 (1 ♀), 20 (2 ♀♀)	EKA
Prinerigone vagans (Audouin, 1826)	13b (1 ♀)	EMM
**Saaristoa firma* (O. P.-Cambridge, 1906)	3b (1 ♂ 2 ♀♀)	EUR
Sintula corniger (Blackwall, 1856)	20 (4 ♀♀)	EKA
Stemonyphantes lineatus (Linnaeus, 1758)	18 (1 ♀)	EMA
**Styloctetor compar* (Westring, 1861)	10 (1 ♂)	HOL
**Tenuiphantes alacris* (Blackwall, 1853)	3b (1 ♂ 1 ♀)	EUR
**Tenuiphantes cristatus* (Menge, 1866)	15 (1 ♀), 19 (2 ♀♀)	EUR
Tenuiphantes mengei (Kulczyński, 1887)	10 (1 ♀), 15 (1 ♂), 19 (2 ♂♂)	EKA
Tenuiphantes tenebricola (Wider, 1834)	3c (1 ♀), 7 (1 ♀)	EUS
Tenuiphantes tenuis (Blackwall, 1852)	3b (1 ♀), 9 (1 ♂), 14 (1 ♂ 1 ♀), 17 (12 ♂♂ 1 ♀), 18 (6 ♂♂ 4 ♀♀)	EMM
Troxochrus scabriculus (Westring, 1851)	15 (1 ♀)	EUS
Walckenaeria alticeps (Denis, 1952)	7 (1 ♀)	EUS
Walckenaeria furcillata (Menge, 1869)	1 (1 ♀)	EUS
Walckenaeria mitrata (Menge, 1868)	17 (1 ♀)	EUR
TETRAGNATHIDAE - 1		
Metellina segmentata (Clerck, 1757)	17 (1 ♀)	EUR
LYCOSIDAE - 23		
Alopecosa cuneata (Clerck, 1757)	10 (6 ♀♀)	EUS
Alopecosa farinosa (Herman, 1879)	6 (1 ♀), 11 (1 ♂ 2 ♀♀), 12 (1 ♀)	EUR
Alopecosa inquilina (Clerck, 1757)	18 (1 ♀)	EUS
Alopecosa pulverulenta (Clerck, 1757)	12 (1 ♀)	PAL
Alopecosa trabalis (Clerck, 1757)	4 (1 ♀), 12 (4 ♂♂)	EUR
Arctosa maculata (Hahn, 1822)	15 (1 ♀)	EUR
Aulonia albimana (Walckenaer, 1805)	1 (1 ♀), 10 (1 ♂), 12 (1 ♂), 13a (1 ♀), 13b (1 ♂ 1 ♀)	WPA
Pardosa agrestis (Westring, 1861)	10 (1 ♀)	EUR
Pardosa alacris (C. L. Koch, 1833)	16 (1 ♀), 17 (1 ♀)	EUR
Pardosa albatula (Roewer, 1951)	1 (2 ♂♂), 6 (2 ♀♀), 10 (1 ♂ 1 ♀), 12 (1 ♂), 14 (2 ♂♂)	KBA
Pardosa amentata (Clerck, 1757)	1 (2 ♀♀), 2 (1 ♂ 1 ♀), 4 (1 ♂), 9 (1 ♀), 10 (1 ♂ 15 ♀♀)	EUR
Pardosa bifasciata (C. L. Koch, 1834)	10 (2 ♂♂), 12 (7 ♂♂)	EUS
Pardosa blanda (C.L. Koch, 1834)	4 (2 ♂♂ 1 ♀), 5 (29 ♂♂ 9 ♀♀), 6 (6 ♂♂ 6 ♀♀), 11 (38 ♂♂ 3 ♀♀), 12 (188 ♂♂ 13 ♀♀), 13a (3 ♂♂ 3 ♀♀), 18 (1 ♀)	SEU
Pardosa hortensis (Thorell, 1872)	1 (1 ♀)	EKA
Pardosa lugubris (Walckenaer, 1802)	1 (1 ♀)	EUR
Pardosa monticola (Clerck, 1757)	6 (2 ♀♀), 11 (15 ♂♂), 12 (1 ♀)	EUR
Pardosa palustris (Linnaeus, 1758)	6 (1 ♂), 10 (79 ♂♂ 58 ♀♀)	HOL
Pardosa lugubris (Walckenaer, 1802)	1 (1 ♀)	EUR

Family Genus/Species/Author	Localities	Zoogeogr. categories
Pardosa monticola (Clerck, 1757)	6 (2 ♀♀), 11 (15 ♂♂), 12 (1 ♀)	EUR
Pardosa palustris (Linnaeus, 1758)	6 (1 ♂), 10 (79 ♂♂ 58 ♀♀)	HOL
Pardosa prativaga (L. Koch, 1870)	9 (2 ♀♀), 13a (1 ♂), 17 (1 ♀), 20 (1 ♀)	EUR
Pardosa tasevi Buchar, 1968	1 (7 ♂♂ 4 ♀♀), 4 (1 ♂ 2 ♀♀), 5 (14 ♂♂ 13 ♀♀), 6 (5 ♂♂ 8 ♀♀), 10 (5 ♂♂ 20 ♀♀), 11 (6 ♂♂), 12 (12 ♂♂ 1 ♀), 13b (4 ♂♂), 14 (3 ♂♂ 3 ♀♀)	SUBE
Piratula hygrophila (Thorell, 1872)	1 (16 ♂♂ 10 ♀♀), 2 (1 ♀), 9 (2 ♀♀), 10 (1 ♀), 14 (3 ♂♂ 7 ♀♀)	EUS
Piratula latitans (Blackwall, 1841)	1 (1 ♂)	EUR
Trochosa terricola Thorell, 1856	5 (1 ♀), 10 (1 ♂), 12 (1 ♂)	HOL
Xerolycosa nemoralis (Westring, 1861)	1 (7 ♂♂), 2 (2 ♂♂ 1 ♀), 10 (3 ♂♂)	EUA
PISAURIDAE - 1		
Pisaura mirabilis (Clerck, 1757)	2 (1 ♂)	EMM
AGELENIDAE - 3		
Inermocoelotes karlinskii (Kulczyński, 1906)	2 (4 ♀♀)	BALK
Inermocoelotes kulczynskii (Drensky, 1915)	2 (1 ♀), 3a (1 ♂), 3c (1 ♀), 7 (1 ♂ 1 ♀), 8 (1 ♂ 1 ♀)	SUBE
Tegenaria rilaensis Deltshev, 1993	7 (1 ♀)	SUBE
CYBAEIDAE - 1		
Cybaeus balkanus Deltshev, 1997	16 (1 ♀)	BALK
MITURGIDAE - 2		
Zora silvestris Kulczyński, 1897	10 (1 ♂)	EUR
Zora spinimana (Sundevall, 1833)	1 (1 ♂), 15 (3 ♂♂)	EUA
LIOCRANIDAE - 1		
Apostenus fuscus Westring, 1851	21 (1 ♂ 1 ♀)	EUR
PHRUROLITHIDAE - 1		
Phrurolithus festivus (C. L. Koch, 1835)	10 (1 ♂ 1 ♀)	PAL
CLUBIONIDAE - 1		
Clubiona neglecta O. P.-Cambridge, 1862	13b (1 ♂), 17 (1 ♂)	EKA
ZODARIIDAE - 1		
Zodarion ohridense Wunderlich, 1973	13b (1 ♀)	BALK
GNAPHOSIDAE - 9		
Drassodes pubescens (Thorell, 1856)	1 (2 ♂♂), 12 (4 ♂♂ 1 ♀)	EUA
Drassyllus praeficus (L. Koch, 1866)	13b (1 ♀)	EMA
Drassyllus pusillus (C. L. Koch, 1833)	11 (3 ♂♂), 12 (2 ♂♂)	EUA
Haplodrassus signifier (C. L. Koch, 1839)	11 (2 ♂♂ 4 ♀♀), 12 (9 ♂♂ 5 ♀♀)	HOL
Micaria albovittata (Lucas, 1846)	1 (1 ♀)	WPA
Micaria pulicaria (Sundevall, 1831)	1 (1 ♀), 12 (1 ♀)	HOL
**Poecilochroa variana* (C. L. Koch, 1839)	4 (1 ♂)	EUR

Family Genus/Species/Author	Localities	Zoogeogr. categories
Micaria pulicaria (Sundevall, 1831)	1 (1 ♀), 12 (1 ♀)	HOL
**Poecilochroa variana* (C. L. Koch, 1839)	4 (1 ♂)	EUR
Zelotes apricorum (L. Koch, 1876)	10 (1 ♀), 13a (1 ♀)	EUR
Zelotes latreillei (Simon, 1878)	1 (3 ♀♀), 5 (2 ♀♀), 10 (2 ♂♂ 3 ♀♀)	EUS
PHILODROMIDAE - 2		
Philodromus collinus C. L. Koch, 1835	9 (1 ♂)	EUR
Thanatus formicinus (Clerck, 1757)	10 (1 ♀), 13a (1 ♀)	HOL
THOMISIDAE - 5		
**Ozyptila trux* (Blackwall, 1846)	5 (1 ♂)	EUA
Xysticus bifasciatus C. L. Koch, 1837	12 (1 ♂)	EUS
Xysticus erraticus (Blackwall, 1834)	4 (1 ♂)	EUR
Xysticus gallicus Simon, 1875	12 (1 ♂)	EKA
Xysticus robustus (Hahn, 1832)	1 (1 ♂)	EUS
SALTICIDAE - 3		
Evarcha arcuata (Clerck, 1757)	16 (1 ♂), 17 (2 ♂♂ 2 ♀♀), 18 (1 ♀)	EUA
Evarcha falcata (Clerck, 1757)	15 (1 ♂)	EUS
Synageles venator (Lucas, 1836)	17 (1 ♀), 20 (2 ♂♂)	EUA

the eastern part of the country only (Osogovo and Maleshevski Mts) at altitudes from 531-1247 m (Komnenov, 2014; present data). It has been recorded mainly from forest and xerothermic hill pastures: xerophytic hill pastures with low vegetation, white pine forest, oak forest (*Quercetum frainetto-cerris*), oak and white hornbeam forests (*Querco-Carpinetum orientalis*), sessile oak forest (*Orno-Quercetum petraeae*) and beech forest (*Festuco heterophyllae-Fagetum*).

Inermocoelotes kulczynskii was described from Bulgaria on a basis of female only. The type locality was not designated in the original publication. Despite revision works by Deltshev (1990) and Wang et al. (2010), the questions about type locality and existence of holotype were not solved. Even the fact that Deltshev (1990) claimed that he revised Drensky's collection, it remains unclear whether he examined the holotype or not. He gave redescription of the species, including description of the missing male, providing no information about the origin of the specimens on which he based the redescription. Also, it remains doubtful if female holotype is conspecific with the newly described male.

Pardosa tasevi has been described from Vitosha Mt. in Bulgaria at altitude of 1350 m. In Macedonia this species is known only from Shar Mt, Osogovo Mt. and Maleshevski Mts at altitudes from 1200 to 1900 m (Komnenov, 2014, 2017; present data). Distribution given by World Spider Catalog (2020) as "Eastern Europe, Turkey, Caucasus (Russia, Azerbaijan)" does not correspond with the real distribution of the species. According to my knowledge, reliable data for this species exist only for N-Greece, Macedonia, Bulgaria and Romania. As taxonomically insufficiently known species, *P. tasevi* can be easily mixed with *P. prativaga*, especially when identification is based on females only. Accordingly, the records from Turkey (Buchar & Dolanský, 2011) and the Caucasus (all records) should be revised. Therefore, the chorotype subendemic used here should be treated temporary.

The distribution of *Tegenaria rilaensis* in Macedonia is restricted to its north-eastern part, on the Osogovo and Maleshevski Mts, at altitudes of 2038 m and 1590 m respectively (Komnenov, 2014; present data). In Bulgaria this species is known only from the eastern part of the country, from the Rila Mt. at altitudes from 1800 to 2550 m and Central Stara Planina Mt. at altitudes from 1700-2000 m (Deltshev, 1993, 1998).

Balkanic chorotype contains four species:

Table 4. Zoogeographical composition of the spider fauna of Maleshevski Mts.

Complexes	Chorotypes	Code	N_0 of species	%
Widely distributed	Holarctic	HOL	10	9.3
	Palearctic	PAL	4	3.7
	Euro-Asian	EUA	7	6.5
	Euro-Siberian	EUS	21	19.6
	Euro-Middle Asian	EMA	3	2.8
	Euro-Mediterranean-Middle Asiatic	EMM	4	3.7
	W-Palearctic	WPA	5	4.6
	Total	**7**	**54**	**50.4**
European	European	EUR	30	28
	S-European	SEU	2	1.8
	Total	**2**	**32**	**29.9**
Mediterranean	Ponto-E-Mediterranean	PEM	1	0.9
	Total	**1**	**1**	**0.9**
Endemic	Alpine-Balkanic	ABA	1	0.9
	Carpatho-Balkanic	KBA	3	2.8
	Balkanic	BALK	4	3.7
	Subendemic	SUBE	5	4.6
	Total	**4**	**13**	**12.1**

Pallidiphantes byzantinus, *Inermocoelotes karlinskii*, *Cybaeus balkanus* and *Zodarion ohridense*.

Palliduphantes byzantinus has been described from the European part of Turkey, from Yarimburgaz Cave near Istanbul. It is troglophile species, widespread in the Balkan Peninsula: Romania - Dobrudzha, Bulgaria, Macedonia and Greece (Komnenov, 2014). In Macedonia, it is registered on several localities at different habitats such as caves, degraded oak forests and subalpine pastures, showing wider vertical distribution from 280-2028 m a.s.l (unpublished data). Recent record from Italy is doubtful from zoogeographic and taxonomic reasons. According to my knowledge, there are no examples of spider species distributed in the Balkan Peninsula and S-Italy. Photo of lamella characteristica (figure 10) of male specimen from Basilicata in S-Italy provided by IJland & van Helsdingen (2016), differ from lamella characteristica of specimens from the Balkan Peninsula. Most likely the record from S-Italy is based on misidentification.

Inermocoelotes karlinskii so far is known from Bosnia & Herzegovina (type locality), Montenegro, Albania, Macedonia, Bulgaria and European part of Turkey (Komnenov, 2014; Demircan & Topçu, 2015). The record from Romania by Vasiliu (1971) is dubious and most likely based on misidentification. The photos of the male bulb (fig. 1) in Vasiliu (1971) clearly shows that the shape of the conductor in Romanian specimen is different from those from the Balkan Peninsula.

Cybaeus balkanus hitherto known from Serbia, Bulgaria, Macedonia and Greece. In Macedonia it is known from Shar Mt. (type locality), Osogovo Mt. and Maleshevski Mts at altitudes from 1005-1850 m (Komnenov, 2014, 2017; present data).

Zodarion ohridense until recently was known only from the Balkan Peninsula: Greece, Albania, Macedonia, Bulgaria and Croatia (Komnenov, 2014). The recent record from Czech Republic, far away from its native area, extends its range outside Balkans, which can be explained due human introduction (Krejčí et al., 2017). In Macedonia it is the most common and the most numerous *Zodarion* species with the largest vertical distribution of all other species, ranging from 280 to 2497 m a.s.l. It can be found in different habitats such as beech and oak forests, subalpine and alpine pastures, riverbanks, meadows, urban areas etc (unpublished data).

Carpatho-Balkanic chorotype contains tree species: *Harpactea saeva*, *Lepthyphantes centromeroides* and *Pardosa albatula*.

Harpactea saeva has been registered from the Carpathian parts of Slovakia, Hungary, Romania, southern Ukraine and Moldova. In the Balkan Peninsula it is known from Serbia, Bulgaria, Macedonia, Albania and Greece (Komnenov, 2014). In Macedonia it is the the commonest species from genus *Harpactea* which can be found on different habitats at altitudes from 150-1333 m (unpublished data).

Lepthyphantes centromeroides on the Balkan Peninsula is distributed in Macedonia, Montenegro, Bosnia & Herzegovina, Serbia and Bulgaria (Komnenov, 2009, 2017; Pavićević et al., 2012). Outside this area, it is registered on Carpathian parts of Romania (Dumitrescu & Georgescu, 1970) and SW-Ukraine (Prokopenko, 2019). In Macedonia it can be found in caves but also in leaf litter and under stones in beech forest, on altitude from 850-1590 m (unpublished data).

Pardosa albatula has been described from the High Tatras, the area between N-Slovakia and S-Poland. Despite numerous records in the Balkan Peninsula (Serbia, Bulgaria, Macedonia, Albania, Greece) (Komnenov, 2014) it is a poorly known species with problematic taxonomy. Affiliation in Carpatho-Balkanic chorotype should be considered provisionally.

Alpine-Balkanic species complex is represent only by one species *Bolyphantes kolosvaryi*. In the Alps it is registered in Slovenia, Northern Italy, Switzerland, with small extension into mountainous areas in S-France. In the Balkan Peninsula it has been recorded from Serbia, Macedonia and Bulgaria (Helsdingen et al., 2001). So far, in Macedonia *B. kolosvaryi* was known from Shar Mt. in NW-Macedonia only, at altitudes from 1479-1822 m (Komnenov, 2017). Finding of this species on Maleshevski Mts comes from the same altitudes: 1390-1760 m.

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Резиме

Фауната на пајаците на македонскиот дел од Малешевските Планини е досега комплетно непозната. Првите истражувања беа спроведени во периодот од 13.06-15.08.2018 и 26-28.08.2019 година. Беа посетени вкупно 21 локалитет (1020-1820 м н.в.) (Табели 1 и 2) каде пајаците беа собирани рачно, со помош на почвени клопки, просејување на листена простирка и со помош на машина за усисување. Вкупно 107 видови од 17 семејства беа регистрирани (Dysderidae – 4, Theridiidae – 6, Linyphiidae – 43, Tetragnathidae – 1, Lycosidae – 23, Pisauridae – 1, Agelenidae – 3, Cybaeidae – 1, Miturgidae – 2, Liocranidae – 1, Phrurolithidae – 1, Clubionidae – 1, Zodariidae – 1, Gnaphosidae – 9, Philodromidae – 2, Thomisidae – 5 and Salticidae – 3) (Табела 3). Девет видови претставуваат нови наоди за фауната на пајаците на Македонија: *Harpactea mentor*, *Micrargus apertus*, *Ozyptila trux*, *Paidiscura pallens*, *Poecilochroa variana*, *Saaristoa firma*, *Styloctetor compar*, *Tenuiphantes alacris* и *T. cristatus*. Еден од најинтересните резултати на овој труд претставува пронаоѓањето на видот *Saaristoa firma* во букова шума на надморска височина од 1675 м на локалитетот Буковик. Овој вид воглавно е распространен во Северна и Западна Европа, додека од Балканскиот полуостров досега беше познат само на база еден податок од Србија. Според моментално познатата дистрибуција, идентификуваните видови се класифицирани во 14 зогеографски категории, групирани во 4 хоролошки комплекси: широко распространети, европски, медитерански и ендемични видови (Табела 4).

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Diversity and distribution of diatoms (Heterocontophyta, Bacilariophyceae) on National Park Pelister

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Algology section

Abstract. Results from the field and laboratory research on mountain Baba (National Park Pelister) organized by the Biology Students Research Society (BSRS) in the summer of 2019 are presented. A total of 211 taxa from five different habitat groups (rivers, streams, lakes, ponds and wetlands) have been identified. Data for distribution in North Macedonia and Europe of each species are provided. Diatom assemblages are dominated by widely distributed genera as *Pinnularia*, *Gomphonema*, *Eunotia*, *Navicula*, *Sellaphora*, and *Nitzschia*. In total, 38 of observed species are recorded only from a single locality in North Macedonia, on mountain Pelister, that is almost 18% from the total number of species. Such results suggest that diatom assemblages, although not very diverse, contain specific taxa with limited distribution in North Macedonia. Additionally, the conservation status of the diatoms in National Park Pelister according to the red list of diatoms for Central Europe is also provided. Several species from different genera are not completely identified since they possess different morphological features than know species and additional observations are warranted for their proper identification or description as new species.

Key words: Diatoms, mountain, Pelister, glacial lakes, alpine ponds, bogs .

Introduction

Diatoms (Heterocontophyta, Bacilariophycease) are a major group of microscopic eukaryotic algae, unicellular, but often forming colonies. The cell wall is highly differentiated, impregnated with silica (SiO_2) and is composed of two valves connected by girdle bands, forming a cell wall that is termed as frustule (Round et al. 1990). The frustule is ornamented by many openings which can be simple pores or extremely complex structures. These structures in combination with the size and shape of the frustule are taxonomically diagnostic. Due to their structure as well as the number and distribution, these algae are very suitable for assessing environmental conditions in lakes, rivers and streams (Collins et al. 2012; Bennion et al. 2014). The diatoms also give excellent results as indicators of surface-water acidity and lake eutrophication. Moreover, because of their siliceous composition, they are often very well preserved in fossil deposits and can be used in paleoenvironmental reconstructions (Smol & Stoermer 2010; Snyder et al. 2013; Cvetkoska et al. 2015).

Diatoms can be found in various habitats such as springs, streams, rivers, ponds, marshes, lakes and marine ecosystems. Additionally, they can be found at wet rocks, mosses, soil and even caves. There are different estimates of diatom diversity. Mann & Dropp (1996) suggest that there are approximately 200,000 species. Based on the modern diatom concept of species determination, as well as the use of fine ultrastructural characteristics for species separation, and molecular analyses, maybe a more realistic estimate have been made by Mann & Vanormelingen (2013), who claim that there are approximately 30–100,000 species.

Previous studies

For the diatom flora of Mountain Baba (National Park Pelister) so far there is a little data of composition from only one paper by Stojanov

(1982) in which he presents a list with a total of 91 diatom taxa. Further research has been made later but there is still relatively small data. The first data on a new species is given by Pavlov et al. (2009) describing the species *Luticola grupcei* Pavlov, Nakov & Levkov. Later Lange-Bertalot et al. (2011) and Pavlov & Levkov (2013a) described two new species from the genus *Eunotia* Ehrenberg: *Eunotia macedonica* Lange-Bertalot, Pavlov & Levkov, *Eunotia pseudominor* Pavlov & Levkov. Additionally several rare species such as *Eunotia cantonatii* Lange-Bertalot & Tagliaventi, *E. crista-galii* Cleve, *E. meisteroides* Lange-Bertalot, *E. mihoi* Lange-Bertalot, Pavlov & Levkov, *E. nymanniana* Grunow, *E. suecica* A. Cleve and two potentially new species such as *Eunotia* aff. *curtagrunowii* Nörpel-Schempp & Lange-Bertalot and *Eunotia* cf. *pseudogroenlandica* Lange-Bertalot & Tagliaventi are listed. In the paper by Pavlov & Levkov (2013b) for the genus *Pinnularia*, from Pelister, is described the species *Pinnularia idsbensis* Pavlov & Levkov. Further, from a study of the genus *Luticola* D.G. Mann several species are described such us: *Luticola aequalis* Levkov, Pavlov & Metzeltin, *Luticola pseudoimbricata* Levkov, Pavlov & Metzeltin, *L. subaequalis* Levkov, Pavlov & Metzeltin, *L. triundulata* Levkov, Pavlov & Metzeltin *L. vesnae* Levkov, Pavlov & Metzeltin. The most recent study on the taxonomy of the genus *Gomphonema* Ehrenberg in the Pelister Mountains describes the species *Gomphonema pelisteriense* Levkov, Mitic-Kopanja & E. Reichardt.

Having in mind that mountain Baba (National Park Pelister) is so far unexplored, the aim in this study is to document the diatom composition from several different habitats. The main focus of this research is glacial lakes, considering that there are no published data on the composition of diatoms in the Great Pelister Lake. Other aquatic ecosystems as peat bogs, rivers and streams are also poorly studied.

Material and methods

Samples from Mt. Baba (National Park Pelister) were collected in July 2019. A total number of 50 samples from different water habitats (rivers, streams, bogs, springs, wetlands and lakes) were collected and fixed with 4% formalin. More than 100 permanent slides were prepared appropriately treated with $KMnO_4$ and 37% HCl to remove organic material and using Naphrax® as a medium for permanent slides. The permanent slides were analysed by an oleimersion x1500 magnification technique with a Nikon Eclipse 80i light microscope and Nikon Eclipse Ni, and diatom species were recorded on a Nikon Coolpix P6000 and Canon EOS 600D camera at the Institute of Biology in Skopje. Some of the samples containing larger diversity or interesting species were analysed on SEM. Light microscope (LM) and electron microscope (SEM) preparations are preserved at the Macedonian National Diatom Collection (MKNDC) at the Institute of Biology at the Faculty of Natural Sciences and Mathematics in Skopje. Diatom identification was carried out following Kramer & Lange-Bertalot (1986-1991), Krammer (2000, 2002, 2003), Lange-Bertalot (2001), Werum & Lange-Bertalot & Metzeltin (2004), Lange-Bertalot et al. (2003) and Levkov et al. (2007).

Results and discussion

The diatom taxa found on mountain Baba (National Park Pelister) are presented in Appendix 1 with their distribution of the species in North Macedonia (presented in the first column in Appendix 1) and conservation status (CS) according to the red list of diatom for Central Europe (Lange-Bertalot & Stainford 1996) presented in the second column of Appendic 1. Samples were grouped into 5 habitat groups (Table 1) corresponding to the habitat type (Rivers, Streams, Lakes, Ponds and Wetlands).

In this study, a total of 211 taxa (Appendix 1) are documented from mountain Baba (National Park Pelister). Most of the species that have been recorded on Pelister so far have been confirmed within this study. The most species-rich genera are *Pinnularia* and *Gomphonema* with 14 species, *Eunotia* with 13, then *Naviculla* with 12 species and *Sellaphora* and *Nitzschia* with 10 species. In the frame of this research, a presence of several acidophilic species, such as *Aulacoseira nivalis, Frustulia saxonica* and *Brachysira brebissonii* have been identified as good indicators of acidification.

Generally, Pelister diatoms can be divided into four categories according to their distribution in Macedonia (Table 3):

- First record – found only on one locality in North Macedonia on mountain Baba.
- rare species - found only on few locations in

Macedonia with low abundance (2-5 localities)

- common species - are often found on certain habitat types (e.g. oligotrophic to dystrophic ponds, bogs, springs or rivers)

- very common species - registered in over 20 localities in Macedonia and often found in different habitats.

Based on the observations in this study can be noticed that most of the species found only on one locality in R. Macedonia are recorded in alpine ponds and glacial lakes and peat bogs in the high-mountain regions of Pelister. These habitats until now were completely unexplored - especially the glacial lakes. In this study, 38 taxa are presented as First record for North Macedonia and most of them are observed in the glacial lakes (Small and Big Pelister Lake) and ponds located near the lakes. For some of them, further investigations and SEM analyses are necessary and they can be probably described as new species for the science (e.g. *Cocconeis* sp., *Craticula* sp., etc). Also most of the species designated as rare for Macedonia are found in the lakes and ponds. The common and very common

Table 1. Diatom diversity per habitat group. Shown are: samples included in habitat groups and the total number of taxa found in the group

Habitat group	Samples included	Total taxa	Total taxa (%)
Habitat group 1	Rivers	58	27,5
Habitat group 2	Streams	63	29,8
Habitat group 3	Lakes	61	28,9
Habitat group 4	Ponds	80	37,9
Habitat group 5	Wetlands	95	45,1

Table 2. Clasification of taxa by representation in North Macedonia

Symbol	Symbol Explanation	Total taxa	Total taxa (%)
A	First record	38	18,0
B	Rare	33	16,6
C	Common	23	10,9
D	Very common	109	51,6

Table 3. Clasification of taxa according to Lange-Bertalot & Steindorf 1996

Symbol	Symbol Explanation	Total taxa	Total taxa (%)
1	extremely endangered	0	0
2	very endangered	3	1,4
3	endangered	8	3,8
G	slightly endangered	7	3,3
R	extremely rare	5	2,4
V	population reducig	18	8,5
*	common	26	12,3
**	very common	36	17
D	data insufficient	4	1,9

Appendix 1. List of determinated diatom taxa with their Representation in North Macedonia and endangerment status (ES) in investigated area.

MK	EU	Species/Habitat Group	1	2	3	4	5
B		*Achnanthidium lineare* W.Smith	+		+	+	
A	3	*Achnantidium didyma* Hustedt			+		
C	V	*Achnanthidium subatomus* (Hustedt) Lange Bertalot	+	+	+	+	+
A	R	*Adlafia* sp.	+		+	+	
A	R	*Adlafia suchlandtii* (Hustedt) Monnier & Ector	+	+			
D	*	*Adlafia minuscula* (Grunow) Lange-Bertalot					+
D	3	*Amphora inariensis* Krammer		+			
D	**	*Amphora pediculus* (Kützing) Grunow	+	+			+
D		*Aneumastus minor* Lange-Bertalot					+
B		*Aneumastus subapiculatus* Z.Levkov & T.Nakov					+
D	G	*Aulacoseira alpigena* (Grunow) Krammer	+		+	+	
		Aulacoseira aff. *granulata* (Ehrenberg) Simonsen			+		
C	*	*Aulacoseira nivalis* (W.Smith) J.English & Potapova				+	
A	2	*Aulacoseira valida* (Grunow) Krammer			+	+	
D	*	*Brachysira brebissonii* R.Ross				+	
D		*Caloneis fontinalis* (Grunow) Cleve-Euler	+	+		+	
D		*Caloneis lancettula* (Schulz) Lange-Bertalot & Witkowski			+	+	
D	*	*Caloneis silicula* (Ehrenberg) Cleve					+
D	G	*Caloneis tenuis* (W.Gregory) Krammer	+				
B	G	*Cavinula cocconeiformis* f. elliptica (Hustedt) Lange-Bertalot	+				
D	3	*Cavinula pseudoscutiformis* (Hustedt) D.G.Mann & A.J.Stickle		+	+		
D	V	*Cavinula scutelloides* (W. Smith) Lange - Bertalot					+
B	*	*Chamaepinnularia mediocris* (Krasske) Lange-Bertalot			+		
		Chamaepinnularia sp.				+	
D	**	*Cocconeis euglypta* Ehrenberg					+
D	D	*Cocconeis pseudolineata* (Geitler) Lange-Bertalot	+	+			+
B		*Cocconeis* sp. 1 nov	+				+
D	**	*Craticula cuspidata* (Kutzing) D.G.Mann					+
A		*Craticula* sp. 1 nov			+	+	
D	**	*Cyclotella meneghiniana* Kützing					+
D	*	*Cyclotella ocellata* Pantocsek					+
A		*Cyclotella paraocellata* A.Cvetkoska, P.B.Hamilton, N.Ognjanova-Rumenova & Z.Levkov		+			
	G	*Cyclotella* aff. *iris* Brun & Héribaud-Joseph			+		
D	**	*Cymatopleura solea* (Brébisson) W.Smith					+
D	V	*Cymbella neocistula* Krammer					+
D	*	*Cymbopleura naviculiformis* (Auerswald ex Heiberg) Krammer	+		+	+	+
D		*Diploneis fontanella* Lange-Bertalot in Werum & Lange-Bertalot	+				
D	3	*Diploneis petersenii* Hustedt		+			
D	*	*Encyonema minutum* (Hilse) D.G.Mann				+	+
C		*Encyonema hebridicum* (Gregory) Grunow			+	+	
		Encyonema aff. *minutiforme* Krammer			+	+	

MK	EU	Species/Habitat Group	1	2	3	4	5
B		*Encyonema hebridiforme* Krammer			+	+	
C	3	*Encyonema neogracile* Krammer			+	+	
A		*Encyonema rostratum* Krammer			+	+	
D		*Encyonema silesiacum* (Bleisch) D.G.Mann	+	+			+
D		*Cumbella tumida* (Brébisson) Van Heurck					+
D	**	*Epithemia sorex* Kützing					+
C		*Eucocconeis flexella* (Kützing) Meister			+		
D	**	*Eunotia bilunaris* (Ehrenberg) Schaarschmidt			+	+	+
D		*Eunotia boreoalpina* Lange-Bertalot & Nörpel-Schempp	+			+	
B		*Eunotia cantonatii* Lange - Bertalot & Tagliaventi		+			
A		*Eunotia crasiminor* Cantonati & Lange Bertalot	+	+			+
A		*Eunotia arctica var. simplex* Hustedt		+			
B		*Eunotia curtagrunowii* Nörpel-Schempp & Lange-Bertalot	+		+		
B		*Eunotia palatina* Lange-Bertalot & W.Krüger		+			
A		*Eunotia pseudominor* A.Pavlov & Z.Levkov C				+	
C	.	*Eunotia groenlandica* Nörpel-Schempp & Lange-Bertalot		+	+	+	
D	D	*Eunotia islandica* Østrup		+	+	+	
C		*Eunotia macedonica* Lange - Bertalot, Pavlov et Levkov				+	
C		*Eunotia soleirolii*(Kützing) Rabenhorst				+	
D	2	*Eunotia tetraodon* Ehrenberg	+			+	
A	*	*Fragilaria* aff. *gracilis* Østrup	+				+
A	V	*Fragilaria* aff. *tenera* (W.Smith) Lange-Bertalot			+	+	
D		*Fragilaria vaucheriae* (Kützing) Petersen					+
C	**	*Fragilaria capucina* Desmazières				+	
D		*Fragilaria pararumpens* Lange-Bertalot, G.Hofmann & Werum			+		
A		*Fragilaria* sp.					+
D	V	*Fragilariforma virescens* (Ralfs) D.M.Williams & Round			+	+	
D	V	*Frustulia crassinervia* (Brébisson ex W.Smith)				+	
D	V	*Frustulia saxonica* Rabenhorst	+		+	+	
A		*Frustulia* sp. 1		+			
D	**	*Frustulia vulgaris* (Thwaites) De Toni	+	+		+	+
C		*Geissleria acceptata* (Hustedt) Lange-Bertalot & Metzeltin	+	+			+
D		*Geissleria decussis* (Østrup) Lange-Bertalot & Metzeltin					+
A		*Geissleria* sp.			+	+	
D	**	*Gomphonema acuminatum* Ehrenberg					+
D	V	*Gomphonema angustatum* (Kützing) Rabenhorst				+	
C		*Gomphonema calcifugum* Lange-Bertalot & E.Reichardt	+	+		+	+
C		*Gomphonema drutelingense* E.Reichardt				+	
D	V	*Gomphonema exilisimum* (Grunow) Lange-Bertalot & E. Reichardt	+	+	+		
B	V	*Gomphonema hebridense* W. Gregory				+	
D	*	*Gomphonema italicum* Kützing					+
D	*	*Gomphonema micropus* Kützing	+	+			+
D	**	*Gomphonema olivaceum* (Horneman) Brébisson					+
D	**	*Gomphonema parvulum* (Kützing) Kützing					+
B		*Gomphonema pelisteriense* Levkov, Mitic-Kopanja & E.Reichardt	+				

MK	EU	Species/Habitat Group	1	2	3	4	5
D	*	*Gomphonema pumilum* (Grunow) E. Reichardt & Lange-Bertalot	+	+			+
D	V	*Gomphonema sarcophagus* W. Gregory	+	+			
D	*	*Gomphonema subclavatum* (Grunow) Grunow	+	+	+	+	+
B		*Gomphonema variscohercynicum* Lange-Bertalot & E. Reichardt		+			
A		*Gomphosphenia tackei* (Hustedt) Lange-Bertalot	+	+			
D	**	*Hannaea arcus* (Ehrenberg) R.M. Patrick	+	+	+	+	+
D	**	*Hantzschia amphioxys* (Ehrenberg) Grunow			+		+
D	R	*Hygropetra balfouriana* (Grunow) Krammer & Lange-Bertalot	+	+			
C	*	*Hippodonta capitata* (Ehrenberg) Lange-Bertalot, Meltzeltin & Witkowski			+		
B		*Hippodonta rostrata* (Hustedt) Lange-Bertalot, Meltzeltin & Witkowski			+		
D		*Humidophila contenta* (Grunow) Lowe, Kociolek, J.R.Johansen, Van de Vijver, Lange-Bertalot & Kopalová	+	+			+
D	**	*Sellaphora pupula* (Kützing) Mereschkovsky					+
D		*Humidophila perpusilla* Lowe, Kociolek, Johansen, Van de Vijver, Lange-Bertalot & Kopalová	+	+	+		+
D	*	*Karayevia clevei* (Grunow) F.E. Round & Bukhtiyarova					+
B	3	*Karayevia laterostrata* (Hustedt) F.E. Round & Bukhtiyarova	+			+	
A		*Kobayasiella* sp.				+	
C	V	*Kobayasiella subtilissima* (Cleve) H. Lange-Bertalot				+	
B		*Luticola aequalis* Levkov, Metzeltin & A. Pavlov	+				
B		*Luticola subaequalis* Levkov, Metzeltin & A. Pavlov			+	+	
D		*Luticola acidoclinata* Lange-Bertalot		+			+
D	**	*Melosira varians* C.Agardh					+
D	**	*Meridion circulare* (Greville) C.Agardh	+	+			+
D	**	*Meridion constrictum* Ralfs	+	+	+	+	
A		*Microcostatus krasskei* (Hustedt) J.R.Johansen & Sray			+		
D		*Navicula antonii* Lange-Bertalot					+
B		*Navicula cantonatii* Lange-Bertalot	+				
D	**	*Navicula cryptocephala* Kützing	+	+			
D	**	*Navicula gregaria* Donkin					+
B	R	*Navicula medioconvexa* Hustedt		+			
D	**	*Navicula reichardtiana* Lange-Bertalot	+				
D		*Navicula rostellata* Kützing					+
C		*Navicula wendlingii* Lange-Bertalot, G. Hofmann & Van de Vijver				+	
A		*Navicula* sp 4.					+
D		*Navicula tenelloides* Hustedt					+
D	**	*Navicula lanceolata* (C. Agardh) Kützing	+	+			+
D	**	*Navicula rhynchocephala* Kützing					+
A	3	*Neidium* aff. *alpinum* Hustedt			+		
B		*Neidium alpinum* Hustedt			+	+	
B		*Neidium alpinum* var. *quadripunctatum* (Hustedt) Hamilton		+	+		
D	3	*Neidium bisulcatum* (Lagerstedt) Cleve				+	
A		*Neidium* sp 19					+
A		*Neidium* sp. 17			+	+	

MK	EU	Species/Habitat Group	1	2	3	4	5
A	D	*Nitzschia acicularoides*(Kützing) W.Smith			+	+	+
D	*	*Nitzschia acidoclinata* Lange-Bertalot				+	
D	G	*Nitzschia alpina* Hustedt	+	+	+		
D	**	*Nitzschia dissipata* (Kützing) Grunow					+
D	**	*Nitzschia fonticola* Grunow					+
D	**	*Nitzschia linearis* (C. Agardh) W.Smith		+			+
D	**	*Nitzschia palea* var. debilis (Kützing) Grunow		+			+
D	**	*Nitzschia recta* Hantzsch					+
D	**	*Nitzschia sigmoidea* (Nitzsch) W. Smith					+
B	D	*Nitzschia suchlandtii* Hustedt				+	
C	V	*Nupela lapidosa* (Krasske) Lange-Bertalot	+	+			+
D	*	*Odontidium hyemale* (Roth) Kützing	+	+	+	+	+
D		*Odontidium rostratum* Levkov & Jüttner	+				
D	V	*Orthoseira roseana* (Rabenhorst) O'Meara		+			
D	**	*Pinnularia borealis* Ehrenberg		+	+	+	
B	G	*Pinnularia eifeleana* (Krammer) Krammer				+	
D	**	*Pinnularia gibba* Ehrenberg					+
D		*Pinnularia grunowii* Krammer					+
A		*Pinnularia idsbensis* Pavlov & Levkov				+	
B		*Pinnularia microstauron* var. angusta Krammer			+	+	
B		*Pinnularia microstauron* var. nonfasciata Krammer			+	+	
B		*Pinnularia microstauron* var. rostrata Krammer		+	+	+	
C		*Pinnularia rhombarea* Krammer				+	
C	*	*Pinnularia subcapitata* var. subrostrata Krammer				+	
D	G	*Pinnularia viridiformis* Krammer	+		+	+	
C	*	*Pinnularia viridis* (Nitzsch) Ehrenberg				+	
C	V	*Pinnularia microstauron* (Ehrenberg) Cleve			+	+	+
A		*Pinnularia* aff. *divergentisima* Grunow	+				
B		*Placoneis anglica* (Ralfs) E.J. Cox					+
D	E	*Placoneis balcanica* (Hustedt) Lange-Bertalot, Metzeltin & Levkov				+	
D		*Placoneis ignorata* (Schimanski) Lange-Bertalot	+				
D		*Placoneis paraelginensis* Lange-Bertalot	+	+			
D		*Planothidium alekseevae* Gogorev & Lange	+	+			+
D	**	*Planothidium lanceolatum* (Brébisson) Lange-Bertalot	+	+			+
A		*Planothidium* sp 1	+				
A		*Planothidium* sp. 2		+			
B		*Planothidium* sp. 5					+
D		*Planothidium victorii* P.M.Novis, J.Braidwood & C.Kilroy	+	+			
D		*Platessa saxonica* (Krasske ex Hustedt) C.E.Wetzel, Lange-Bertalot & Ector	+	+	+		+
D	*	*Prestauroneis protracta* (Grunow) I.W.Bishop, Minerovic, Q.Liu and Kociolek			+		
D	V	*Psammothidium bioretii* (Germain) Bukhtiyarova & F.E. Round	+		+	+	+
A		*Psammothidium* sp. 2			+	+	
A		*Psammothidium* sp.1			+	+	

MK	EU	Species/Habitat Group	1	2	3	4	5
D	*	*Psammothidium* helveticum (Hustedt) Bukhtiyarova & F.E. Round	+		+	+	
B	2	*Psammothidium rossii* (Hustedt) Bukhtiyarova & F.E. Round	+				
D	**	*Pseudostaurosira subconstricta* (Grunow) Kulikovskiy & Genkal				+	
A		*Punctastriata* sp.		+			
D	**	*Reimeria sinuata* (W. Gregory) Kociolek & Stoermer	+	+			+
B		*Sellaphora* sp. 8					+
A		*Sellaphora* sp. 18					+
A		*Sellaphora* sp. 19					+
D		*Sellaphora atomoides* (Grunow) C.E. Wetzel & Van de Vijver	+	+			+
A		*Sellaphora bisexualis* D.G.Mann & K.M.Evans					+
C	**	*Sellaphora lanceolata* D.G.Mann & S.Droop					+
D		*Sellaphora nigrii* (De Notaris) C.E.Wetzel & L.Ector in C.E	+	+		+	+
B		*Sellaphora parapupula* Lange-Bertalot					+
C		*Sellaphora rotunda* (Hustedt) Wetzel, Ector, Van de Vijver, Compère & D.G.Mann		+			
D		*Sellaphora saugerresii* (Desmazières) C.E.Wetzel & D.G. Mann	+			+	
B		*Stauroneis gracilior* E. Reichardt				+	+
D		*Stauroneis jarensis* Lange-Bertalot, Cavacini, Tagliaventi & Alfinito		+	+		
D		*Stauroneis parathermicola* Lange-Bertalot		+			
D	V	*Stauroneis phoenicenteron* (Nitzsch) Ehrenberg				+	
B	R	*Stauroneis prominula* (Grunow) Hustedt	+				
D	*	*Stauroneis smithii* Grunow		+			
A		*Stauroneis* sp. aff intrincans	+	+			
A		*Staurosira* sp. 3			+		
C	*	*Stauroneis thermicolla* (Petersen) Lund		+			
D		*Staurosira venter* (Ehrenberg) Cleve & J.D.Möller		+	+	+	+
B		*Stenopterobia delicatissima* (F.W. Lewis) Brébisson ex Van Heurck		+	+		
A		*Stenopterobia* sp. nov			+	+	
B	V	*Stephanodiscus alpinus* Hustedt			+	+	
D	*	*Surirella angusta* Kützing					+
D	*	*Surirella linearis* W.Smith				+	
D		*Surirella minuta* Brébisson					+
A		*Surirella* sp. 1			+	+	
A		*Surirella* sp. 2			+		
D	**	*Tabellaria flocculosa* (Roth) Kützing			+	+	
		Tabellaria flocculosa var. *geniculata* (A. Cleve) B. M. Knudson		+			
		Tertiarius mariovensis Ognjanova–Rumenova Jovanovska, Cvetkoska & Levkov	+		+		
D		*Tryblionella angustata* W. Smith					+
D	G	*Ulnaria biceps* (Kützing) Compère		+			
D	*	*Ulnaria ulna* (Nitzsch) Compère					+

Symbol explanation:	* - common
1 - extremely endangered	** - very common
2 - very endangered	D - data insufficient
3 - endangered	A - First record
G - slightly endangered	B - Rare
R - extremely rare	C - Common
V - population reducing	D - Very common

Appendix 1– symbol explanation

species are most represented in rivers, streams and wetlands.

According to the species endangerment status according to the red list of diatoms for Central Europe (Lange-Bertalot & Steindorf 1996), (Table 3), 3 species are listed as very endangered including *Aulacoseira valida* (Grunow) Krammer found in lakes and ponds, *Eunotia tetraodon* Ehrenberg in rivers and ponds and *Psammothidium rossii* (Hustedt) Bukhtiyarova & F.E. Round found in streams. As an endangered are classified 8 species (*Achnantidium didyma* Hustedt, *Amphora inariensis* Krammer*, Cavinula pseudoscutiformis (Hustedt) D.G.Mann & A.J.Stickle, Diploneis petersenii* Hustedt*, Encyonema neogracile* Krammer*, Karayevia laterostrata(Hustedt) F.E. Round & Bukhtiyarova, Neidium alpinum* Hustedt and *Neidium bisulcatum* (Lagerstedt) Cleve) and most of them are found in lakes and pond. As presented in Table 3 seven species are described as slightly endangered and 5 species as extremely rare. The other species are common or very common species for the waters in Central Europe.

Although most of the species that are labelled as "first record" are found in the glacial lakes and ponds, the importance of other habitats as rivers, streams and wetlands should not be excluded. The analysis conducted on rivers (River Brajcinska) has shown the presence of 8 taxa, presented as First record for North Macedonia. For 5 of these taxa (*Adlafia* sp., *Fragilaria* aff. *gracilis*, *Pinnularia* aff *divergentisima*, *Planothidium* sp. 1 and *Stauroneis* sp. aff *intricans*), further investigations are necessary. Similar results are obtained in streams. Although most of the taxa from this habitat are presented as "Common" or "Very common" species, the results have shown that there are 7 species labelled as "First recorded" for North Macedonia. Most of the species found in wetlands are also common and eutrophic species including *Amphora pediculus* (Kützing) Grunow, *Cocconeis euglypta* Ehrenberg, *Epithemia sorex* Kützing, etc. The importance of this habitat, except the presence of 9 taxa labelled as "First" recorded, is that there are determined 9 different taxa from the genus *Sellaphora* Mereschowsky among which 3 show different morphological characteristics and will require further research in future and can be probably described as new species for the science.

Conclusions

According to our research on mountain Baba (National Park Pelister), a total of 211 taxa from five different habitat groups (Rivers, Streams, Lakes, Ponds and Wetlands) were identified. The most species-rich genera are *Pinnularia* and *Gomphonema* with 14 species, *Eunotia* with 13, then *Naviculla* with 12 species and *Sellaphora* and *Nitzschia* with 10 species.

Based on the species conservation status according to the red list of diatoms for Central Europe, 3 species are listed as very endangered, 8 species as endangered and 5 species as extremely rare. According to the distribution in North Macedonia, 38 taxa are presented as First recorded taxa for North Macedonia. The largest number of potentially new species for science has been recorded in ponds and glacial lakes and peat bogs in the high-mountain regions of Pelister. The most important sites are the glacial lakes (Small and Big Pelister Lake) and the ponds below the glacial lakes. These habitats also contain the largest number of rare and extremely rare species.

In the frame of this research, a presence of several acidophilic species, such as *Aulacoseira nivalis*, *Frustulia saxonica* and *Brachysira brebissonii*, has been identified. These species indicate the danger of acidification caused by acid rain. The reasons for this occurrence need to be further analyzed in the future.

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Plate 1 (x1500)

Figs 1–10. Aulacoseira *nivalis* (W.Smith) J.English & Potapova
Figs 11–18. Aulacoseira valida (Grunow) Krammer
Figs 19–37. *Aulacoseira alpigena* (Grunow) Krammer
Figs 38–40. *Aulacoseira* aff. *granulata* (Ehrenberg) Simonsen
Figs 41–42. *Tertiarius mariovensis* Ognjanova–Rumenova Jovanovska, Cvetkoska & Levkov
Figs 43–45. *Stephanodiscus alpinus* Hustedt
Fig. 46. *Cyclotella* aff. *iris* Brun & Héribaud-Joseph

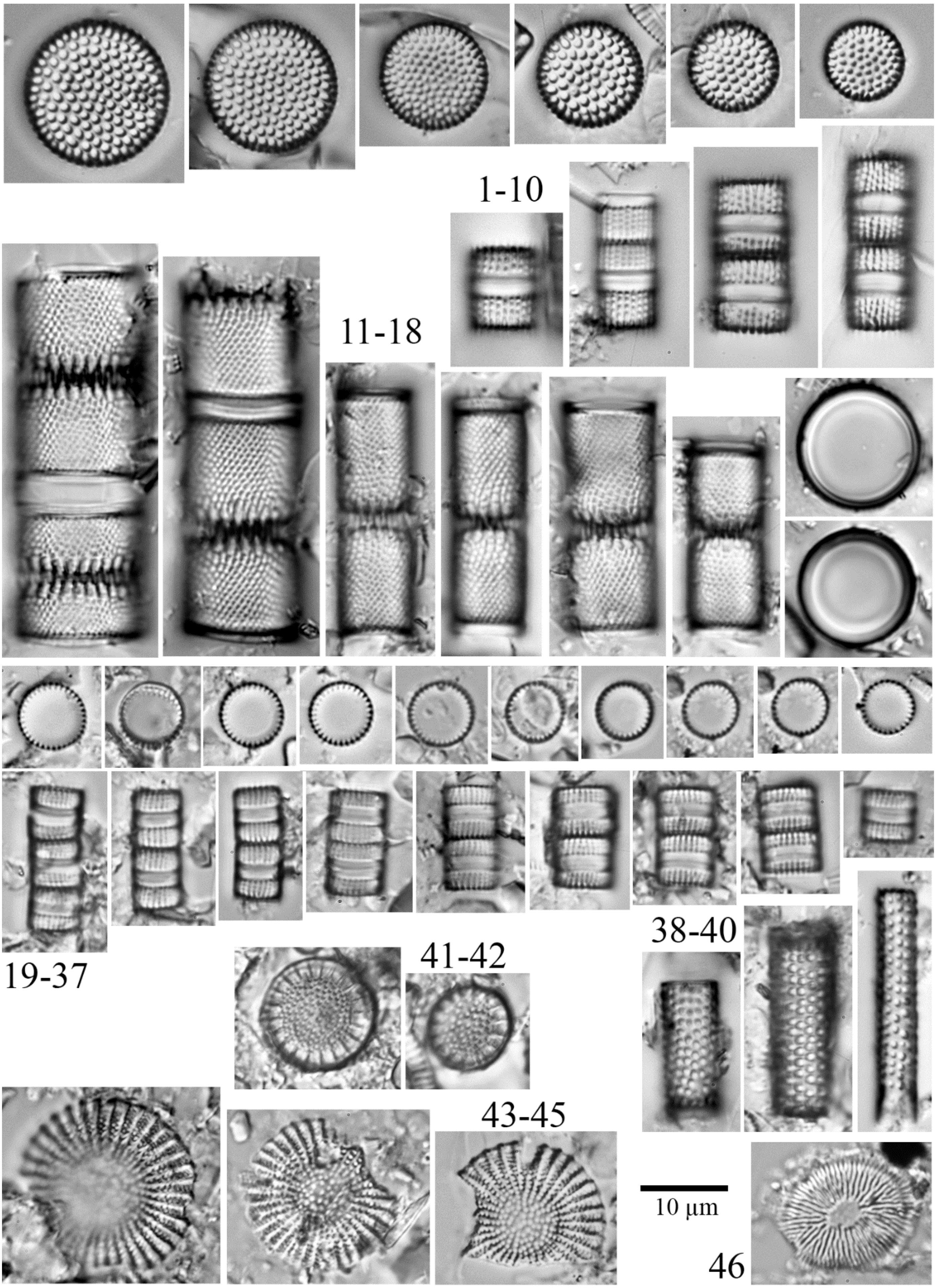

1-10
11-18
19-37
41-42
38-40
43-45
10 µm
46

Plate 2 (x1500)

Figs 1–15.	*Tabellaria flocculosa* (Roth) Kützing
Figs 16, 17.	*Fragilariforma virescens* (Ralfs) D.M.Williams & Round
Fig. 18.	*Meridion constrictum* Ralfs
Figs 19, 20.	*Meridion circulare* (Greville) C.Agardh
Figs 21–24.	*Odontidium hyemale* (Roth) Kützing
Figs 25–28.	*Odontidium rostratum* Levkov & Jüttner
Fig. 29.	*Fragilaria vaucheriae* (Kützing) Petersen
Figs 30, 31.	*Fragilaria* sp.
Fig. 32.	*Hannaea arcus* (Ehrenberg) R.M. Patrick
Fig. 33.	*Ulnaria biceps* (Kützing) Compère

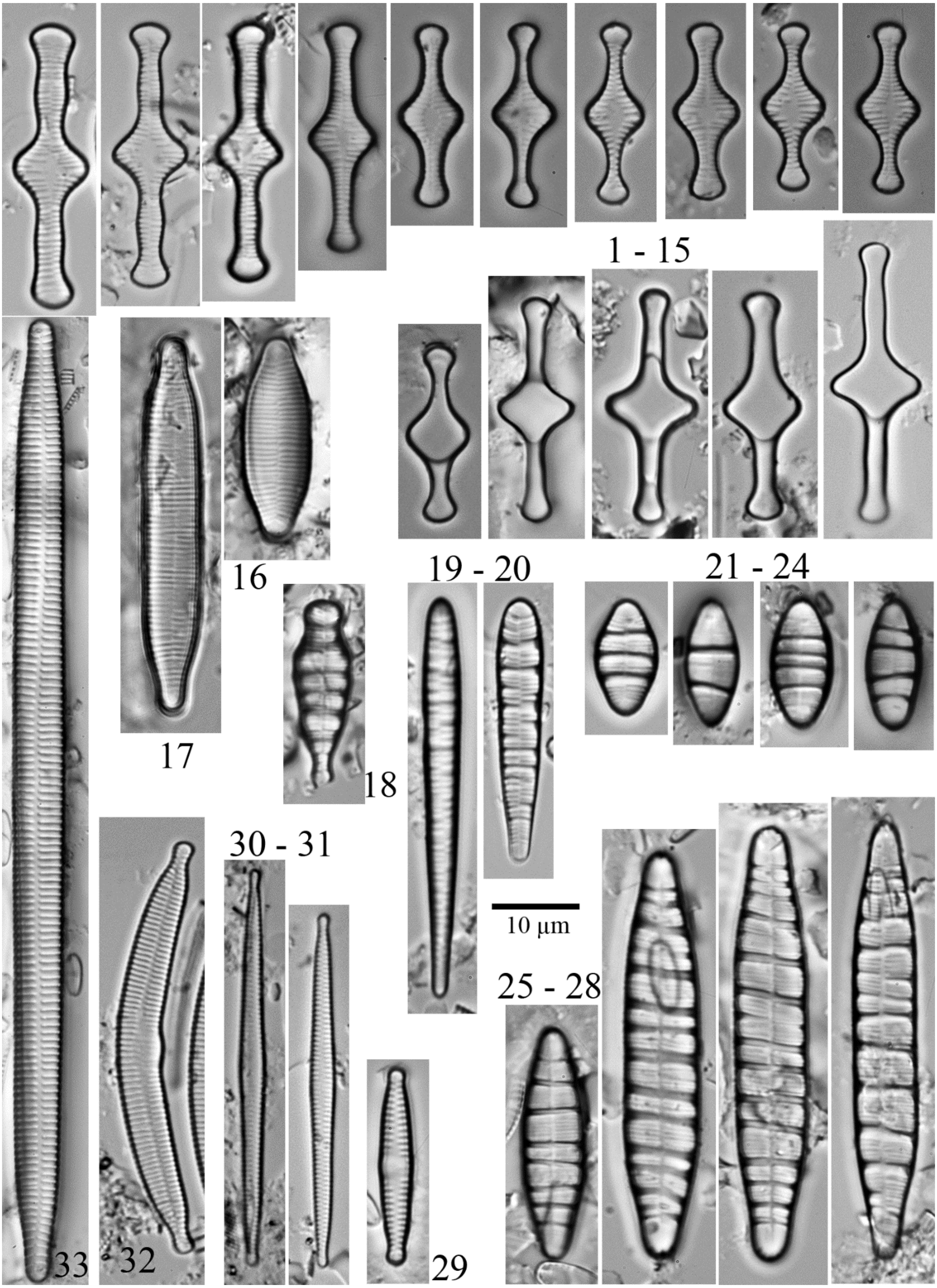

1 - 15
16
17
18
19 - 20
21 - 24
10 µm
25 - 28
30 - 31
33
32
29

Plate 3 (x1500)

Figs 1, 2. *Eunotia macedonica* Lange - Bertalot, Pavlov & Levkov
Figs 3, 4. *Eunotia bilunaris* (Ehrenberg) Schaarschmidt
Figs 5, 6. *Eunotia soleirolii* (Kützing) Rabenhorst
Figs 7–12. *Eunotia crasiminor* Cantonati & Lange Bertalot

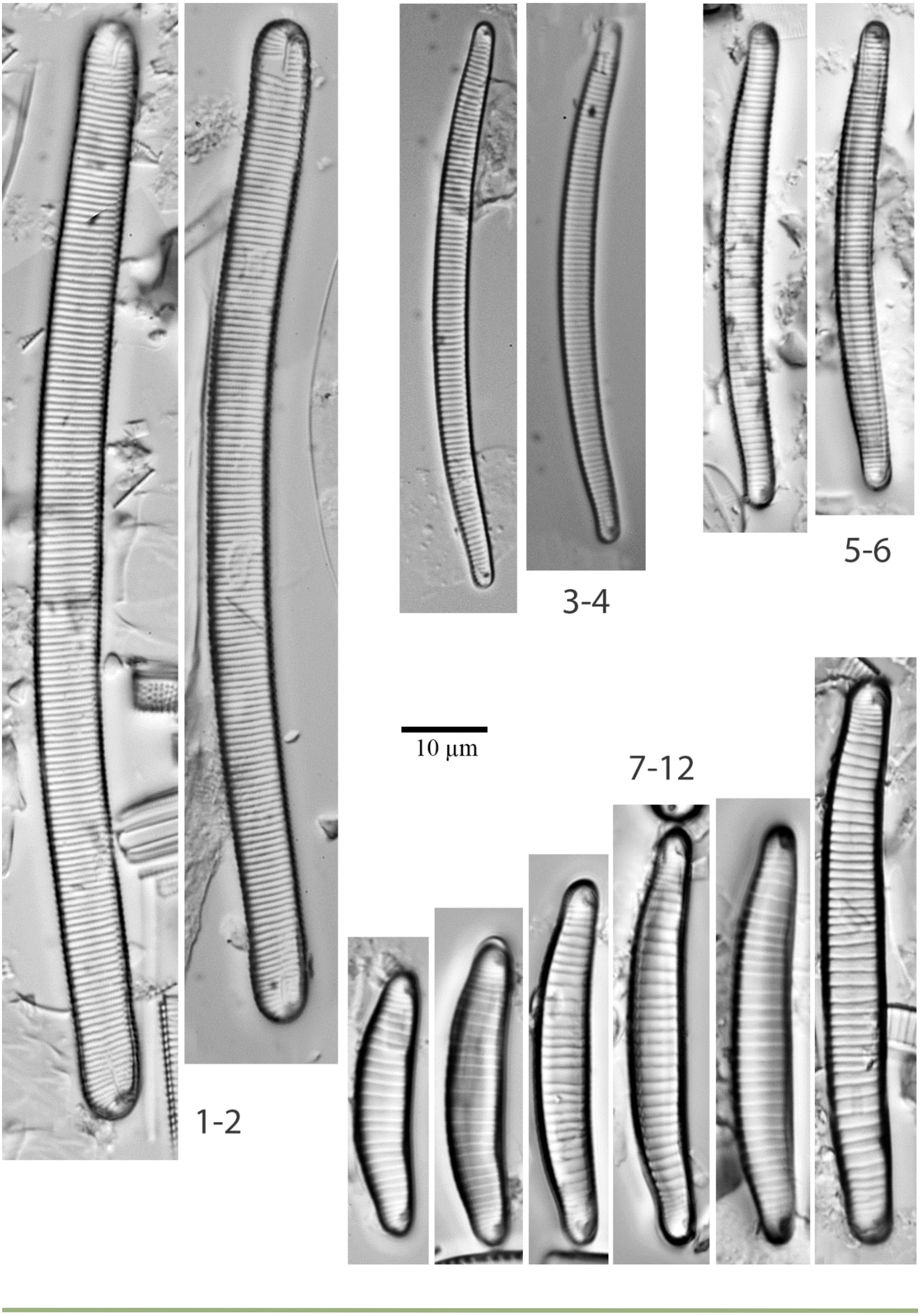

1-2
3-4
5-6
10 μm
7-12

Plate 4 (x1500)

Figs 1–4. *Eunotia tetraodon* Ehrenberg
Fig. 5. *Eunotia palatina* Lange-Bertalot & W.Krüger
Figs 6, 7. *Eunotia pseudominor* A.Pavlov & Z.Levkov C
Figs 8–10. *Eunotia boreoalpina*
Figs 11–13. *Eunotia curtagrunowii* Nörpel-Schempp & Lange-Bertalot
Figs 14–16. *Eunotia groenlandica* Nörpel-Schempp & Lange-Bertalot
Figs 17–24. *Eunotia islandica*

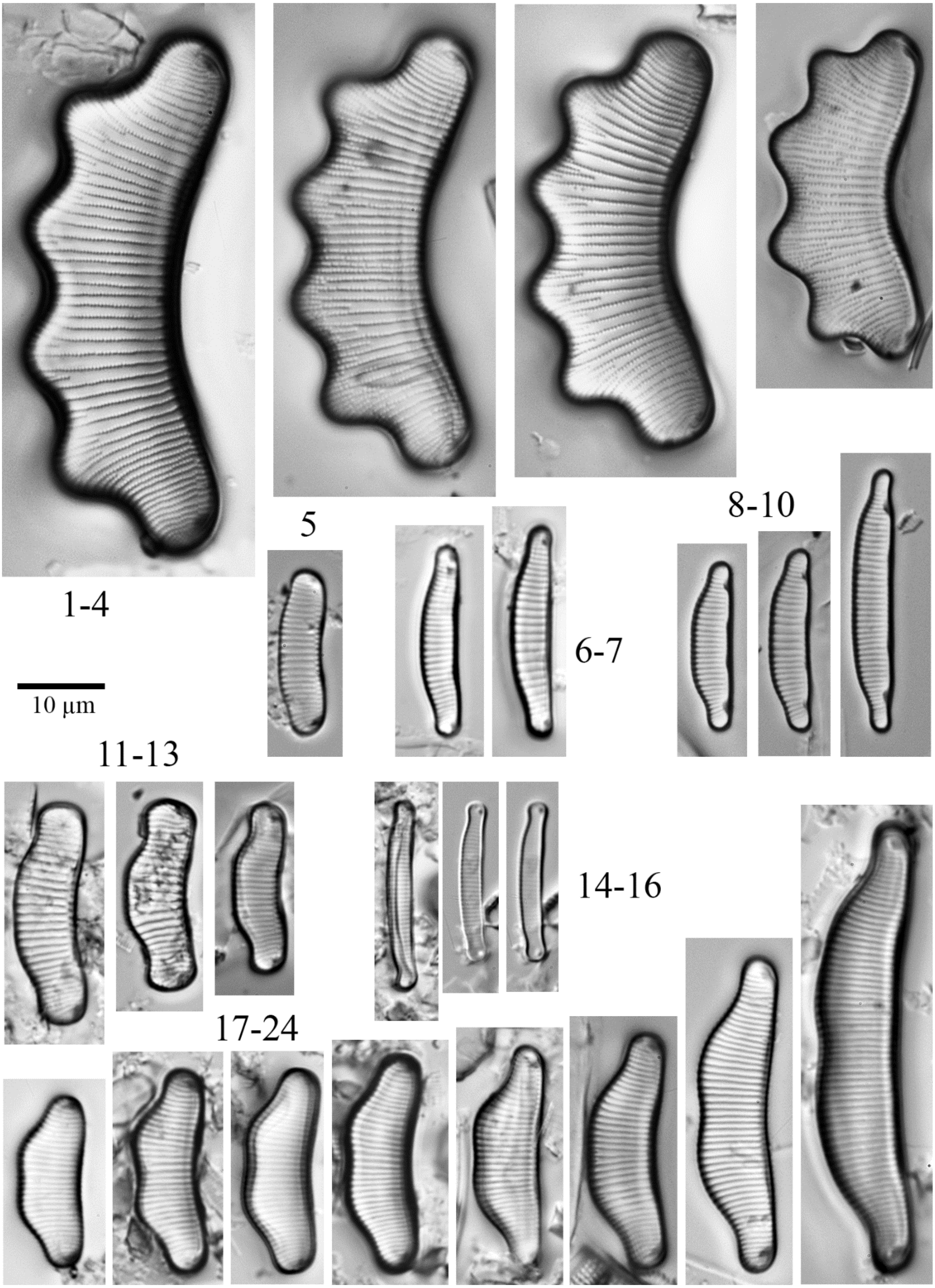

1-4
5
6-7
8-10
10 µm
11-13
14-16
17-24

Plate 5 (x1500)

Figs 1-6. Cocconeis sp. 1 nov
Fig. 7. *Eucocconeis flexella* (Kützing) Meister
Figs 8–11. *Nupela lapidosa* (Krasske) Lange-Bertalot
Figs 12-15. *Psammothidium* sp.1
Figs 16-19. *Psammothidium helveticum* (Hustedt) Bukhtiyarova & F.E. Round
Fig 20-27 *Psammothidium* sp.2
Fig 28-29 *Planothidium alekseevae* Gogorev & Lange
Fig 30-33 *Planothidium sp*. 1
Fig 34-36 *Planothidium* victorii P.M.Novis, J.Braidwood & C.Kilroy
Fig 37-39 *Planothidium lanceolatum* (Brébisson) Lange-Bertalot
Figs 40-45. *Planothidium* sp. 2
Figs 46, 47. *Achnanthidium lineare* W.Smith
Figs 48, 49 *Achnantidium didyma* Hustedt
Figs 50-61 *Platessa saxonica* (Krasske) C.E.Wetzel, Lange-Bertalot & Ector

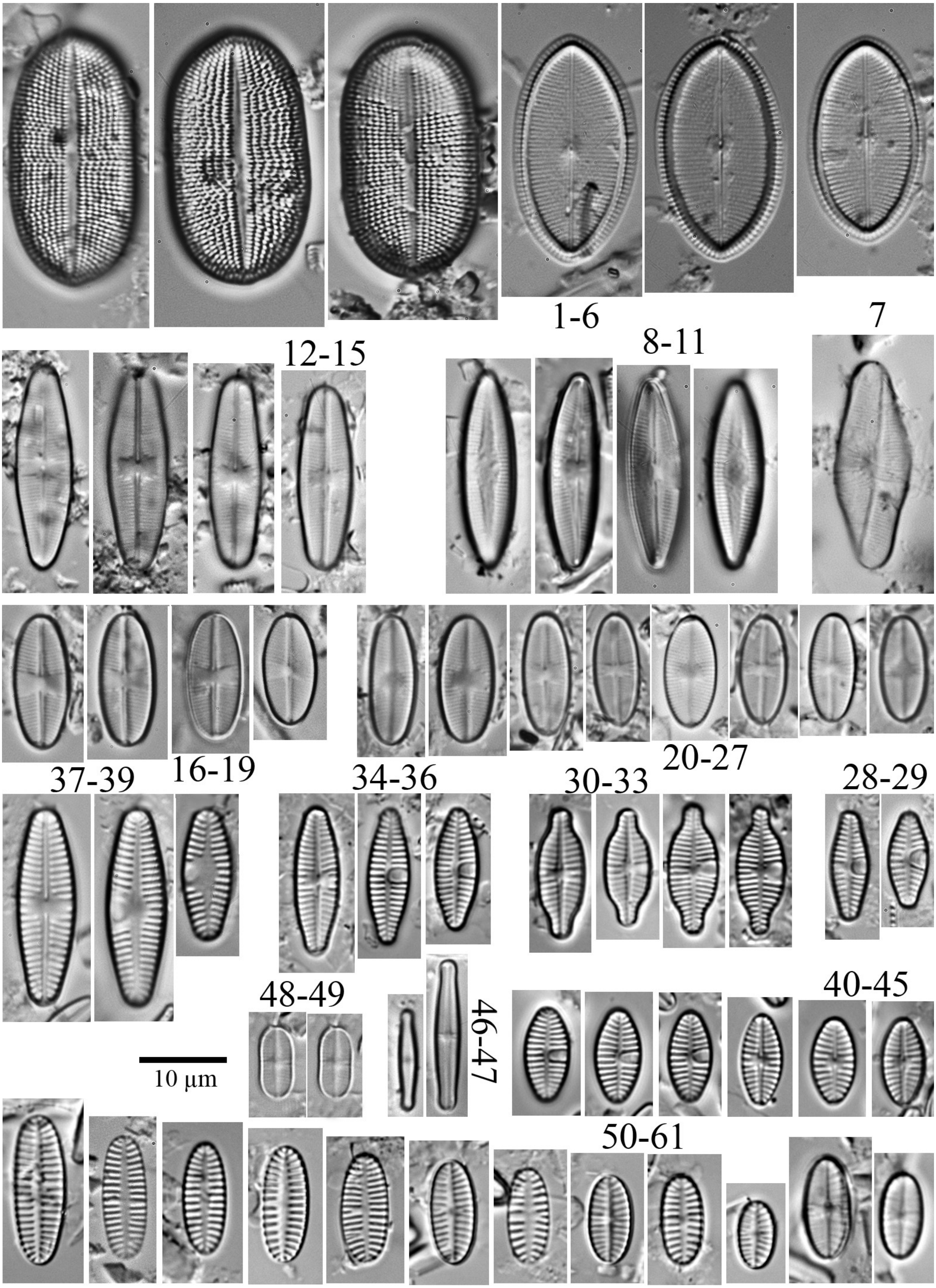

1-6
7
12-15
8-11
37-39
16-19
34-36
30-33
20-27
28-29
40-45
48-49
46-47
10 µm
50-61

Plate 6 (x1500)

Figs 1–7. *Encyonema neogracile* Krammer
Figs 8-15 *Encyonema hebridicum* (Gregory) Grunow
Figs 16–23. *Encyonema rostratum* Krammer
Figs 24–35. *Encyonema minutiforme* Krammer

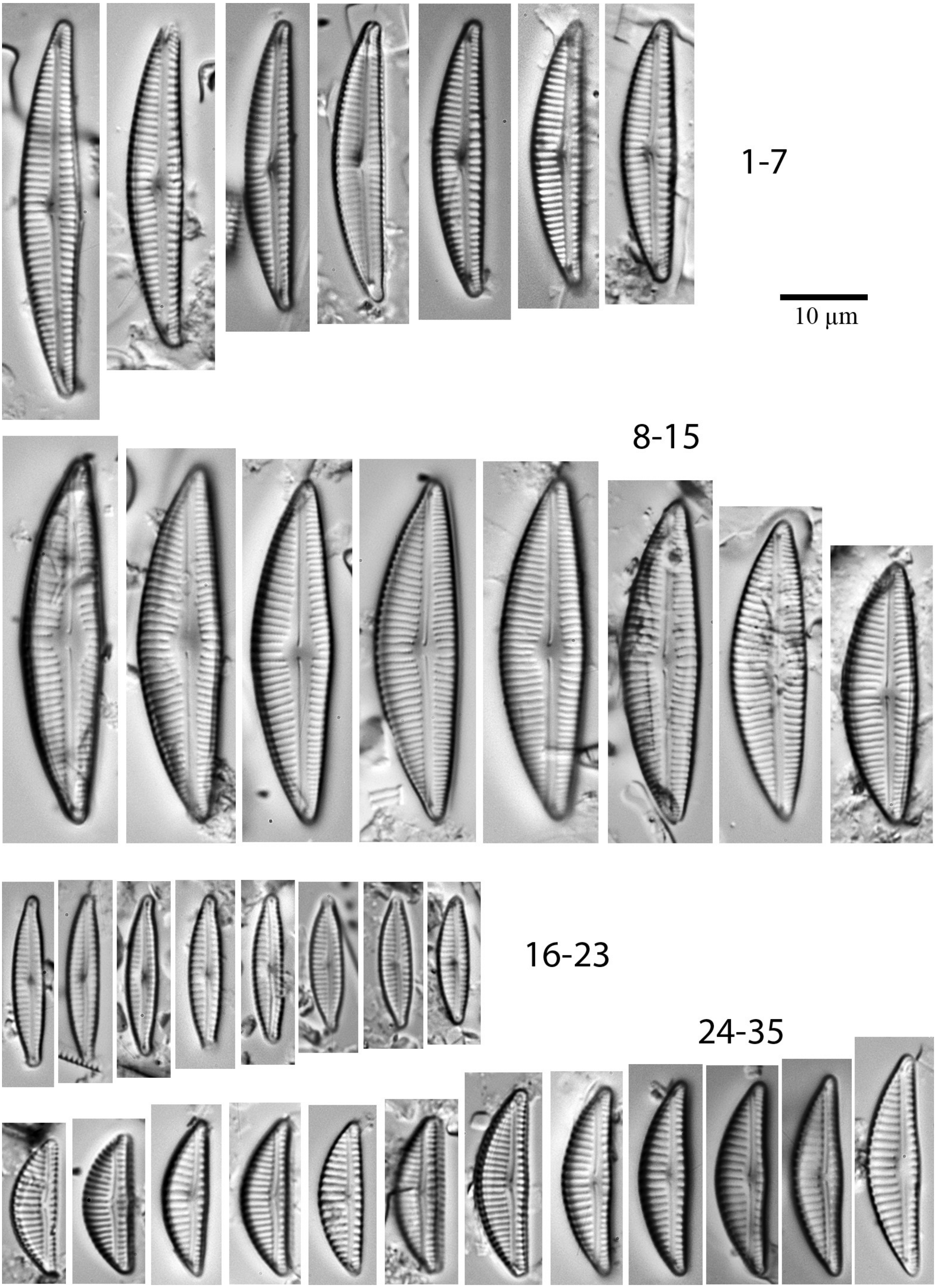

1-7
10 µm
8-15
16-23
24-35

Plate 7 (x1500)

Figs 1–4. *Gomphonema hebridense* W. Gregory
Figs 5–7. *Gomphonema subclavatum* (Grunow) Grunow
Figs 8–10. *Gomphonema pelisteriense* Levkov, Mitic-Kopanja & E.Reichardt
Figs 11–13. *Gomphonema exilisimum* (Grunow) Lange-Bertalot & E. Reichardt
Figs 14–16. *Navicula wendlingii* Lange-Bertalot, G. Hofmann & Van de Vijver
Figs 17–20. *Placoneis anglica* (Ralfs) E.J. Cox
Figs 21–23. *Placoneis ignorata* (Schimanski) Lange-Bertalot
Figs 24–27. *Brachysira brebissonii* R.Ross

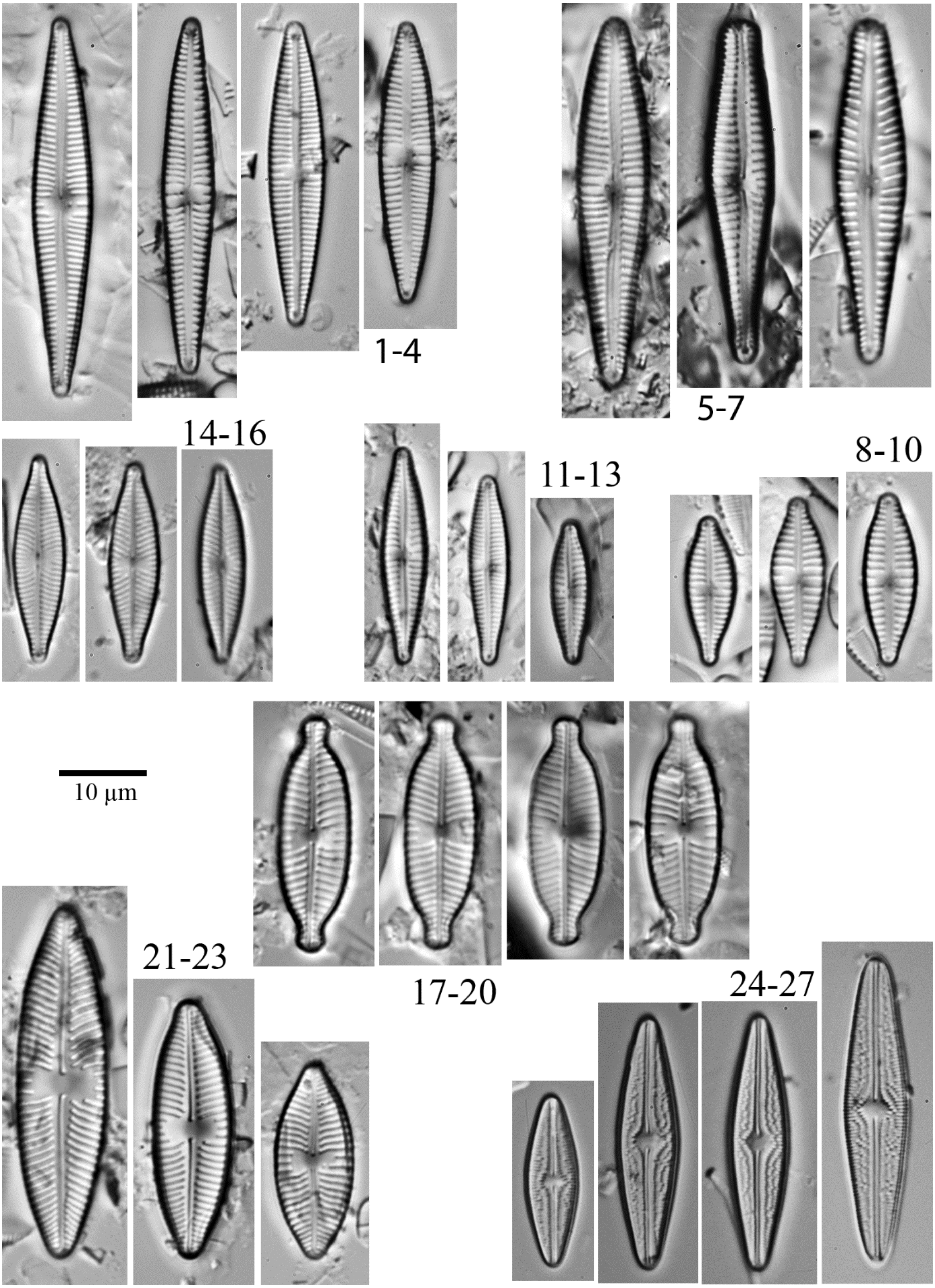

1-4
5-7
14-16
11-13
8-10
10 µm
21-23
17-20
24-27

Plate 8 (x1500)

Figs 1–9. *Sellaphora* sp. 8
Figs 10–12. *Sellaphora* sp. 18.
Figs 13–15. *Sellaphora pseudopupula* (Krasske) Lange-Bertalot
Figs 16–18. *Sellaphora* sp. 19
Figs 19, 20. *Sellaphora saugerresii* (Desmazières) C.E.Wetzel & D.G. Mann
Figs 21–25. *Craticula* sp.1 nov.
Figs 26–29. *Adlafia* sp.
Figs 30–32. *Chamaepinnularia mediocris* (Krasske) Lange-Bertalot
Figs 33–35. *Chamaepinnularia* sp.
Figs 36, 37. *Geissleria* sp.
Figs 38–41. *Geissleria acceptata* (Hustedt) Lange-Bertalot & Metzeltin
Fig. 42. *Diploneis fontanella* Lange-Bertalot in Werum & Lange-Bertalot
Fig. 43. *Luticola acidoclinata* Lange-Bertalot
Fig. 44. *Luticola subaequalis* Levkov, Metzeltin & A. Pavlov
Figs 45, 46. *Cavinula pseudoscutiformis* (Hustedt) D.G.Mann & A.J.Stickle
Fig. 47. *Humidophila perpusilla* Lowe, Kociolek, Johansen, Van de Vijver, Lange-Bertalot & Kopalová
Fig. 48. *Humidophila contenta* (Grunow) Lowe, Kociolek, J.R.Johansen, Van de Vijver, Lange-Bertalot & Kopalová
Fig. 49. *Microcostatus krasskei* (Hustedt) J.R.Johansen & Sray
Fig. 50. *Kobayasiella* sp.
Fig. 51. *K obayasiella subtilissima* (Cleve) Lange-Bertalot

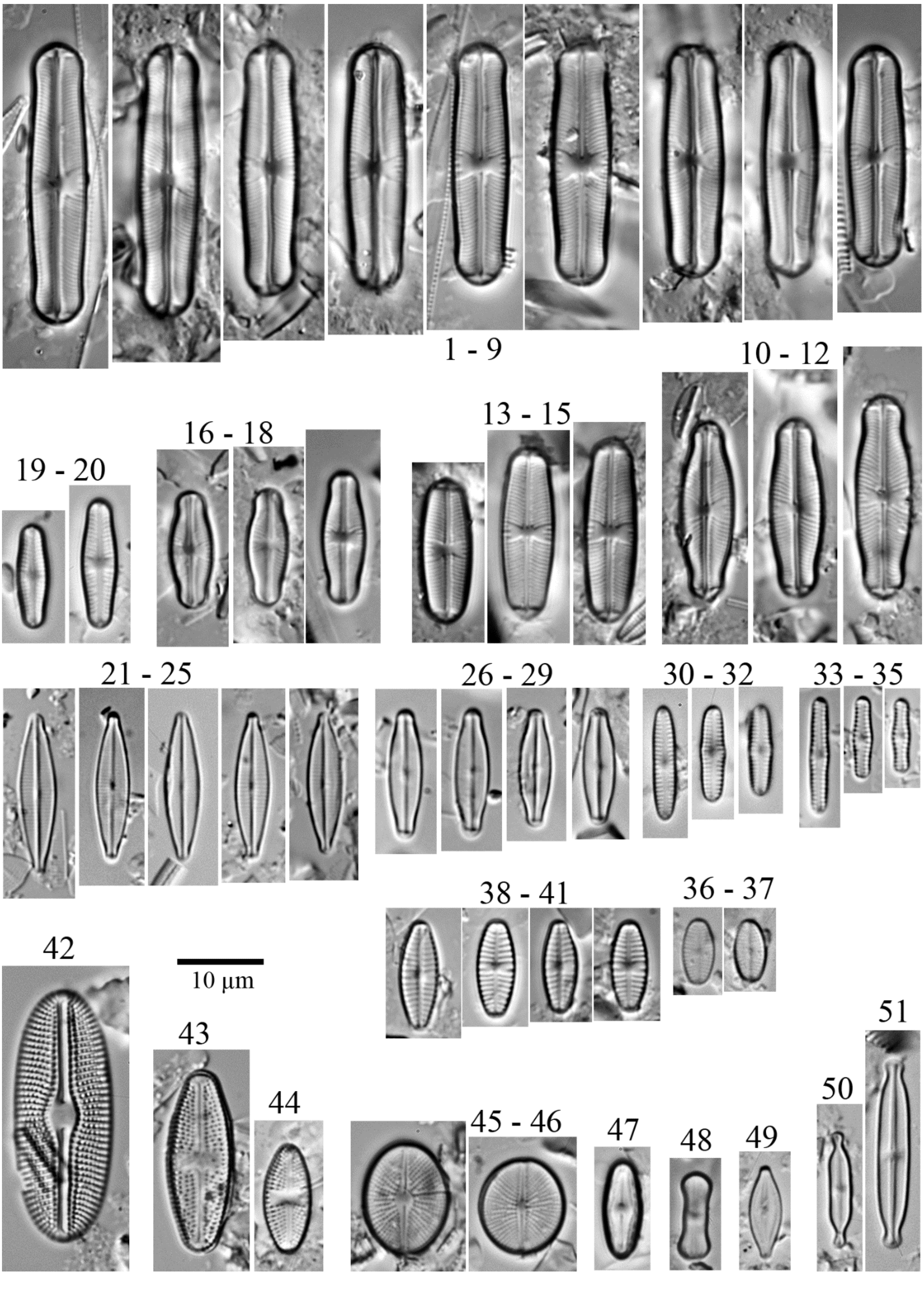

1 - 9
10 - 12
13 - 15
16 - 18
19 - 20
21 - 25
26 - 29
30 - 32
33 - 35
38 - 41
36 - 37
42
10 µm
43
44
45 - 46
47
48
49
50
51

Plate 9 (x1500)

Fig. 1. *Pinnularia viridiformis* Krammer
Fig. 2. *Pinnularia microstauron* (Ehrenberg) Cleve
Fig. 3, 4. *Pinnularia eifeleana* (Krammer) Krammer
Fig. 5, 6. *Pinnularia subcapitata* var. *subrostrata* Krammer
Fig. 7, 8. *Pinnularia microstauron* var. *rostrata* Krammer
Fig. 9, 10. *Pinnularia idsbensis* Pavlov & Levkov
Fig. 11, 12. *Pinnularia borealis* Ehrenberg
Fig. 13–15. *Pinnularia microstauron* var. *nonfasciata* Krammer

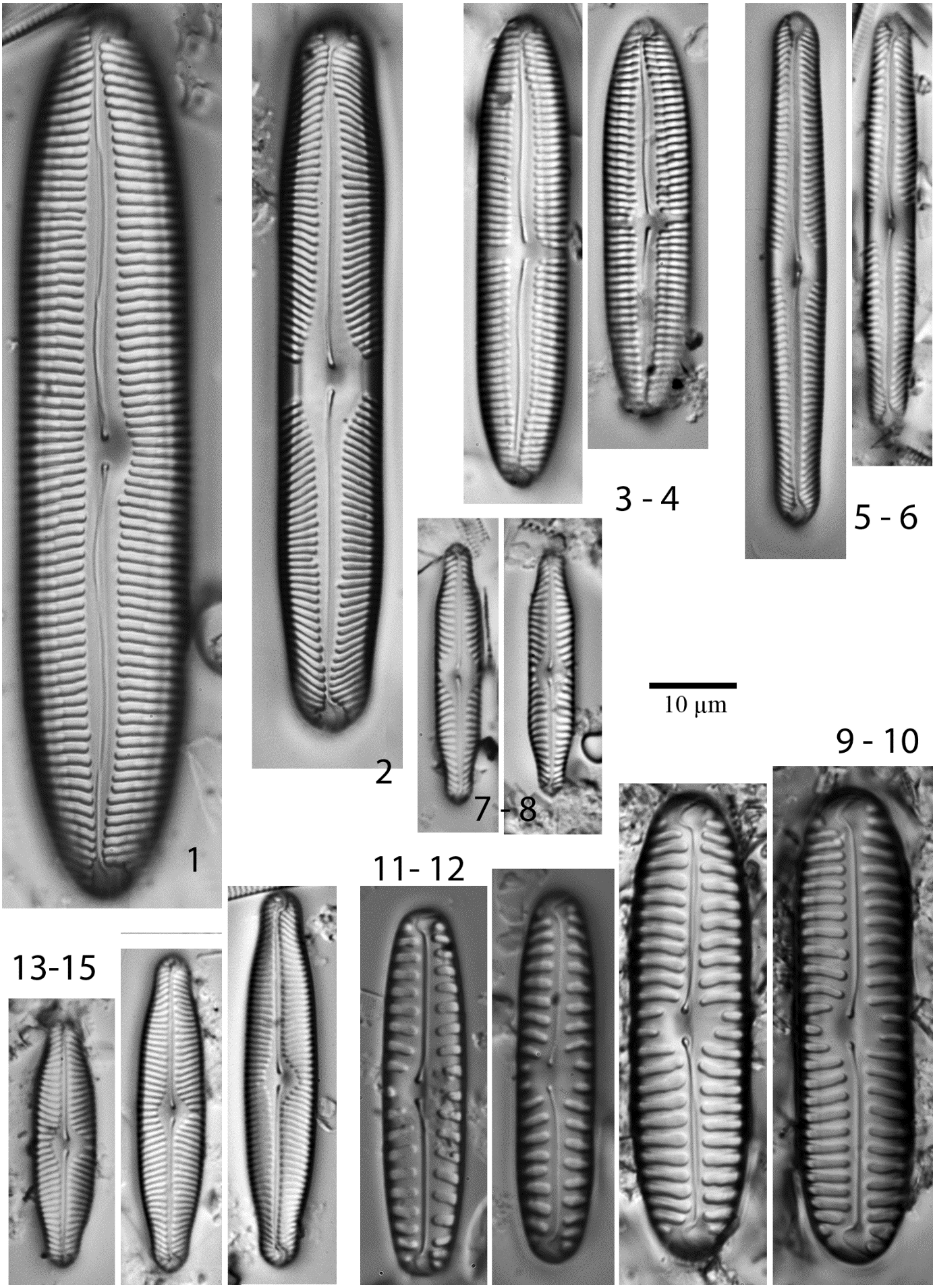

1
2
3 - 4
5 - 6
7 - 8
10 μm
9 - 10
11- 12
13-15

Plate 10 (x1500)

Fig. 1. *Stauroneis phoenicenteron* (Nitzsch) Ehrenberg
Figs 2–5. *Stauroneis jarensis* Lange-Bertalot, Cavacini, Tagliaventi & Alfinito
Figs 6. *Frustulia saxonica* Rabenhorst
Figs 7–9. *Frustulia crassinervia* (Brébisson ex W.Smith)
Figs 10–12. *Frustulia* sp. 1. nov.

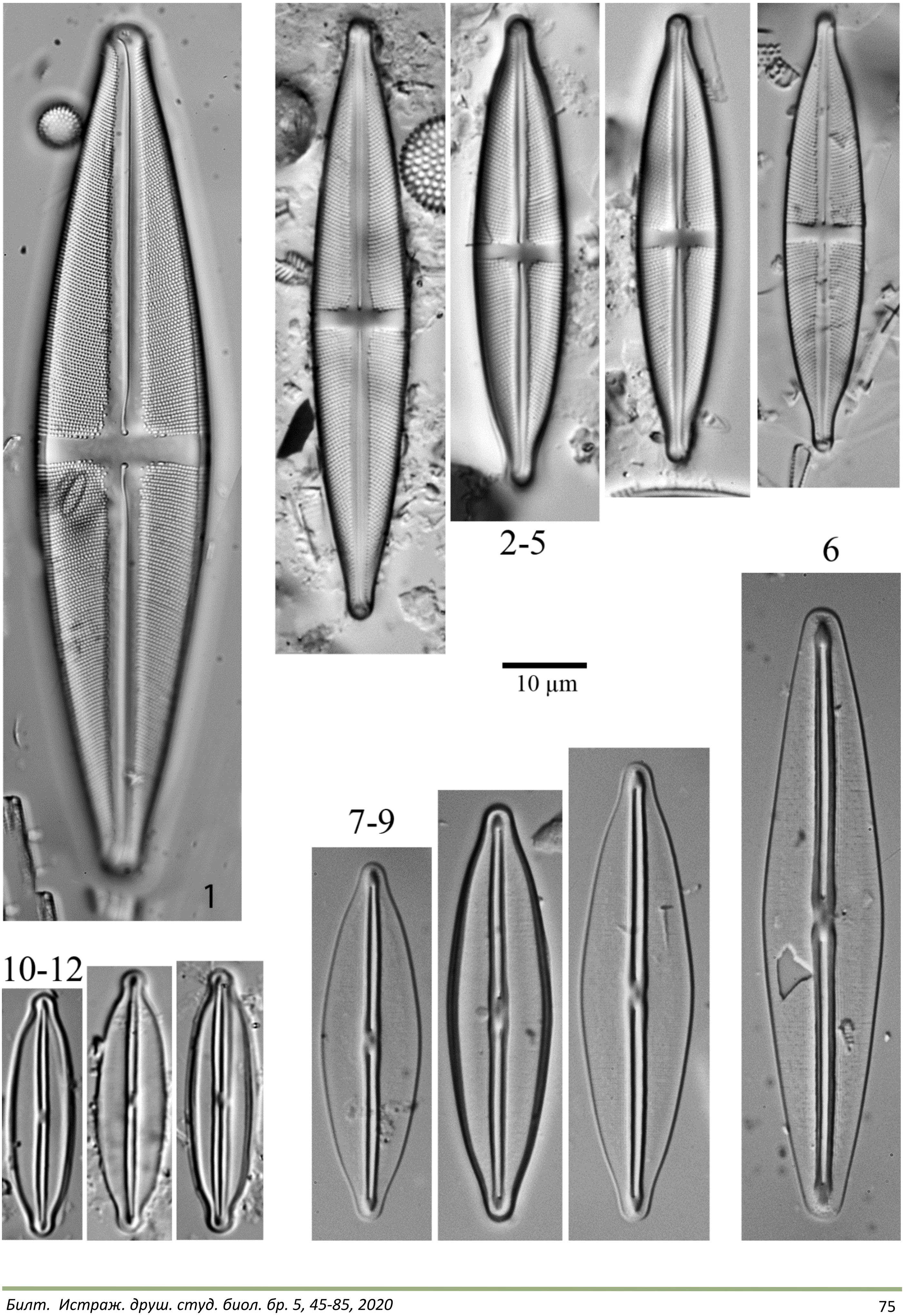

1
2-5
6
10 µm
7-9
10-12

Plate 11 (x1500)

Figs 1-3. *Neidium bisulcatum* (Lagerstedt) Cleve
Figs 4-6. *Neidium* sp. 19.
Figs 7, 8. *Neidium* sp. aff. *alpinum* Hustedt
Figs 9-12. *Neidium alpinum* var. *quadripunctatum* (Hustedt) Hamilton
Figs 13, 14. *Neidium* sp. 17.
Figs 15, 16. *Neidium* sp 19.
Figs 17-19. *Neidium alpinum* Hustedt

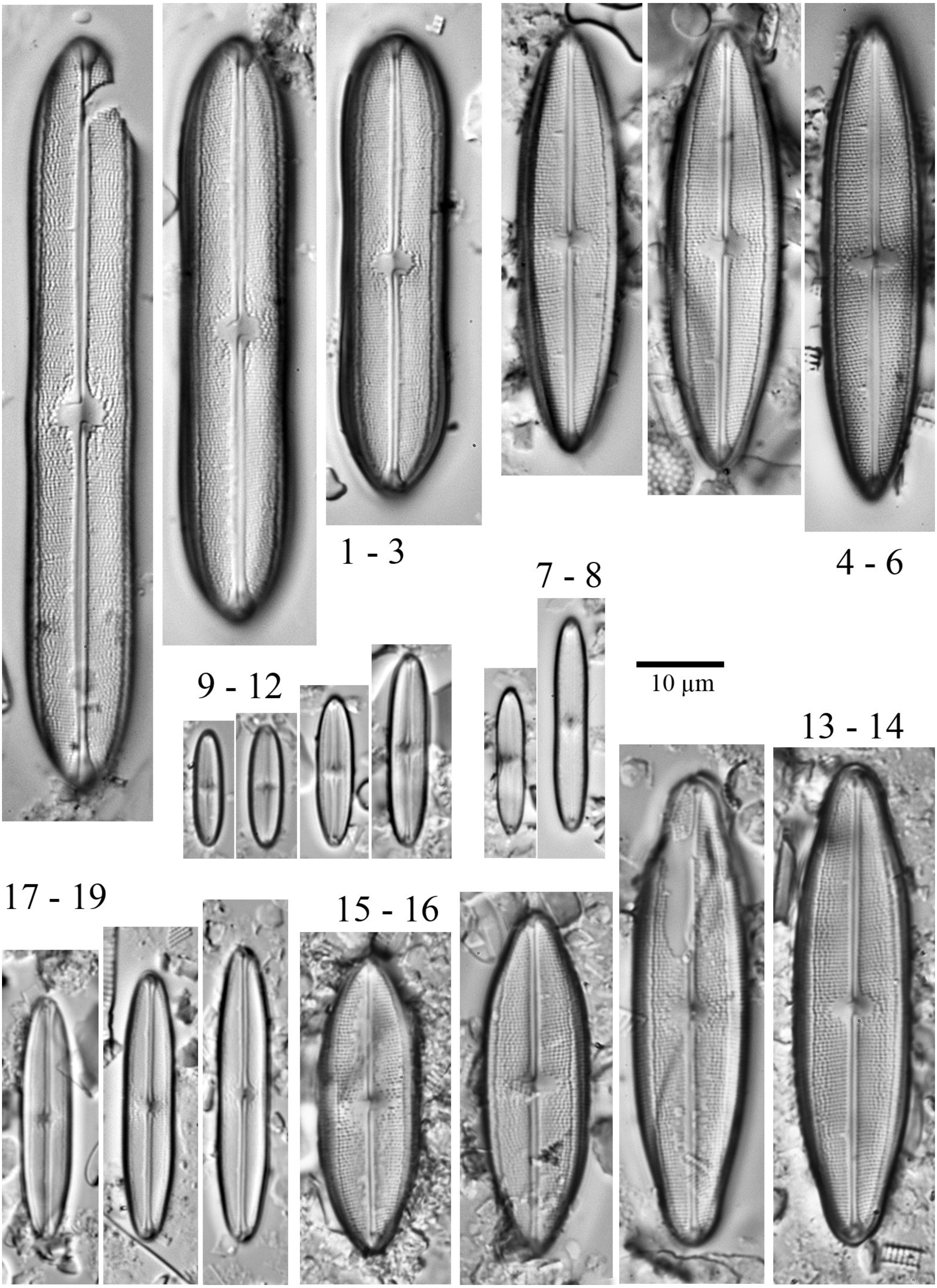

1 - 3
4 - 6
7 - 8
9 - 12
10 µm
13 - 14
17 - 19
15 - 16

Plate 12 (x1500)

Figs 1, 2. *Surirella* sp. 1
Figs 3, 4. *Surirella* sp. 2
Figs 5–7. *Nitzschia alpina* Hustedt
Figs 8–10. *Nitzschia acicularoides* (Kützing) W.Smith
Fig. 11. *Hantzschia amphioxys* (Ehrenberg) Grunow
Figs 12–14. *Stenopterobia* sp. nov
Figs 15–17. *Stenopterobia delicatissima* (F.W. Lewis) Brébisson ex Van Heurck

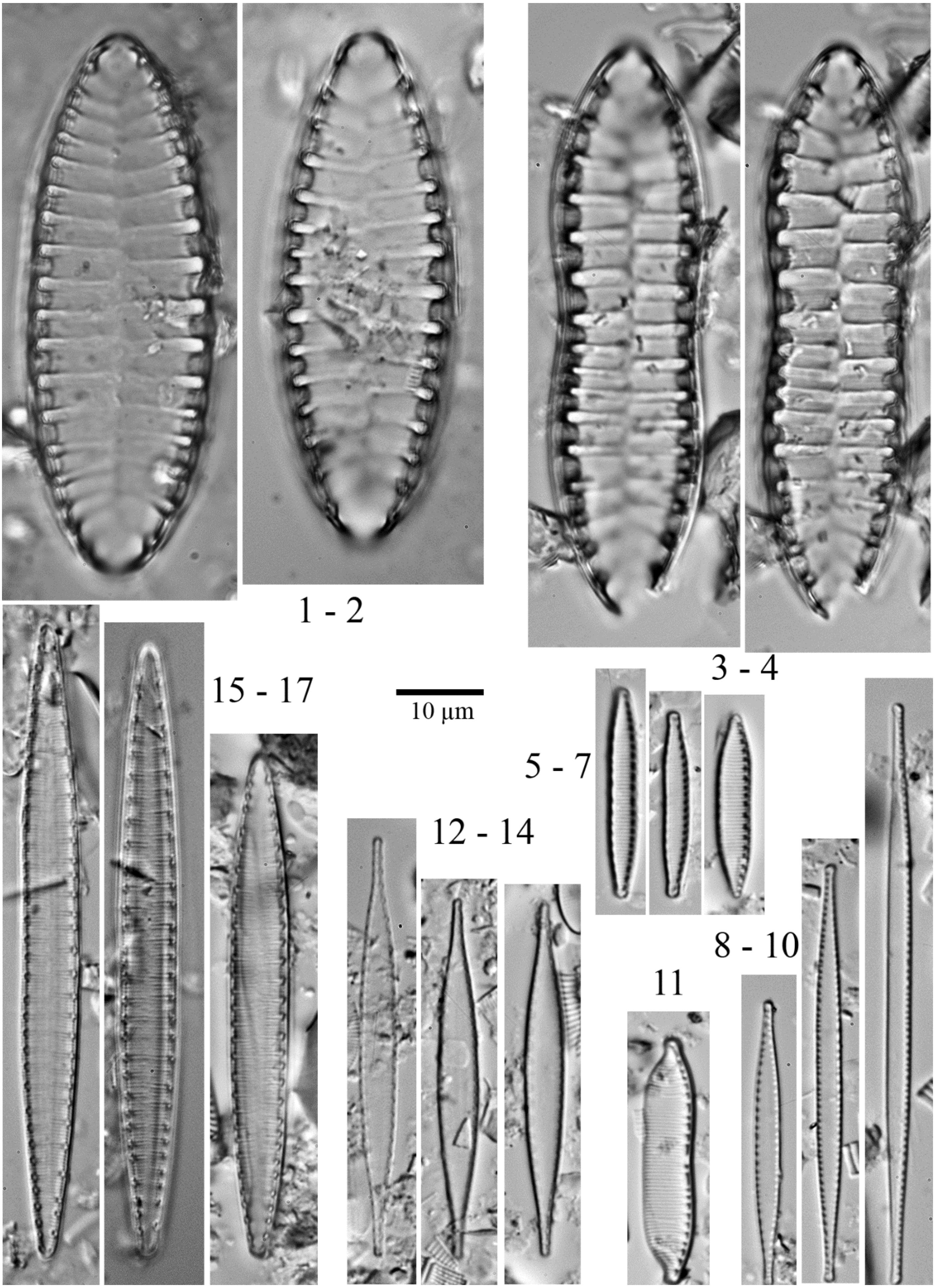

1 - 2
3 - 4
15 - 17
10 µm
5 - 7
12 - 14
8 - 10
11

Plate 13 (SEM)

Fig. 1 *Aulacoseira nivalis* (W.Smith) J.English & Potapova, valve external view, scale bar 2 µm.
Fig. 2 *Aulacoseira nivalis* (W.Smith) J.English & Potapova, valve internal view, scale bar 2 µm.
Fig. 3 *Aulacoseira alpigena* (Grunow) Krammer, valve external view, scale bar 1 µm
Fig. 4 *Aulacoseira alpigena* (Grunow) Krammer, valve internal view, scale bar 1 µm
Fig. 5 *Aulacoseira nivalis* (W.Smith) J.English & Potapova, girdle view, scale bar 2 µm.
Fig. 6 *Aulacoseira alpigena* (Grunow) Krammer, girdle view, scale bar 1 µm
Fig 7. *Aulacoseira nivalis* (W.Smith) J.English & Potapova, girdle view, scale bar 2 µm.

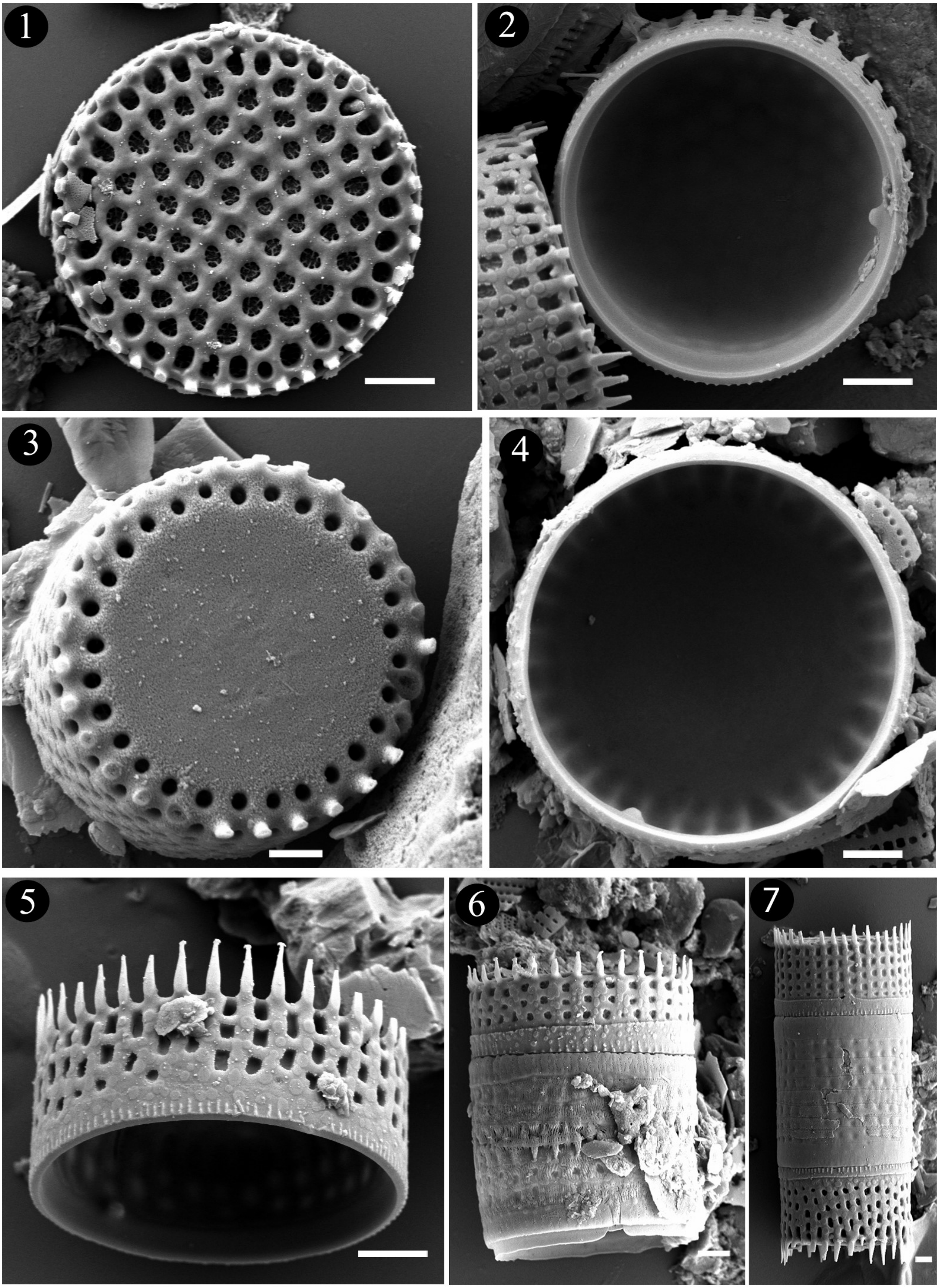

1
2
3
4
5
6
7

Plate 14 (SEM)

Fig. 1 *Cocconeis* sp. 1 nov, valve external view, scale bar 2 µm
Fig. 2 *Cocconeis* sp. 1 nov, valve internal view, scale bar 2 µm
Fig. 3 *Platessa saxonica* (Krasske) C.E.Wetzel, Lange-Bertalot & Ector, valve external view, scale bar 2 µm
Fig. 4 *Nupela lapidosa* (Krasske) Lange-Bertalot valve internal view, scale bar 2 µm
Fig. 5 *Platessa saxonica* (Krasske) C.E.Wetzel, Lange-Bertalot & Ector, valve external view, scale bar 2 µm
Fig. 6 *Platessa saxonica* (Krasske) C.E.Wetzel, Lange-Bertalot & Ector, valve external view, scale bar 2 µm
Fig. 7 *Nupela lapidosa* (Krasske) Lange-Bertalot valve external view, scale bar 2 µm
Fig. 8 *Psamotidium* sp.**,** valve external view, scale bar 2 µm
Fig. 9 *Psamotidium* sp., valve external view, scale bar 1 µm
Fig. 10 *Psamotidium* sp., valve external view, scale bar 1 µm

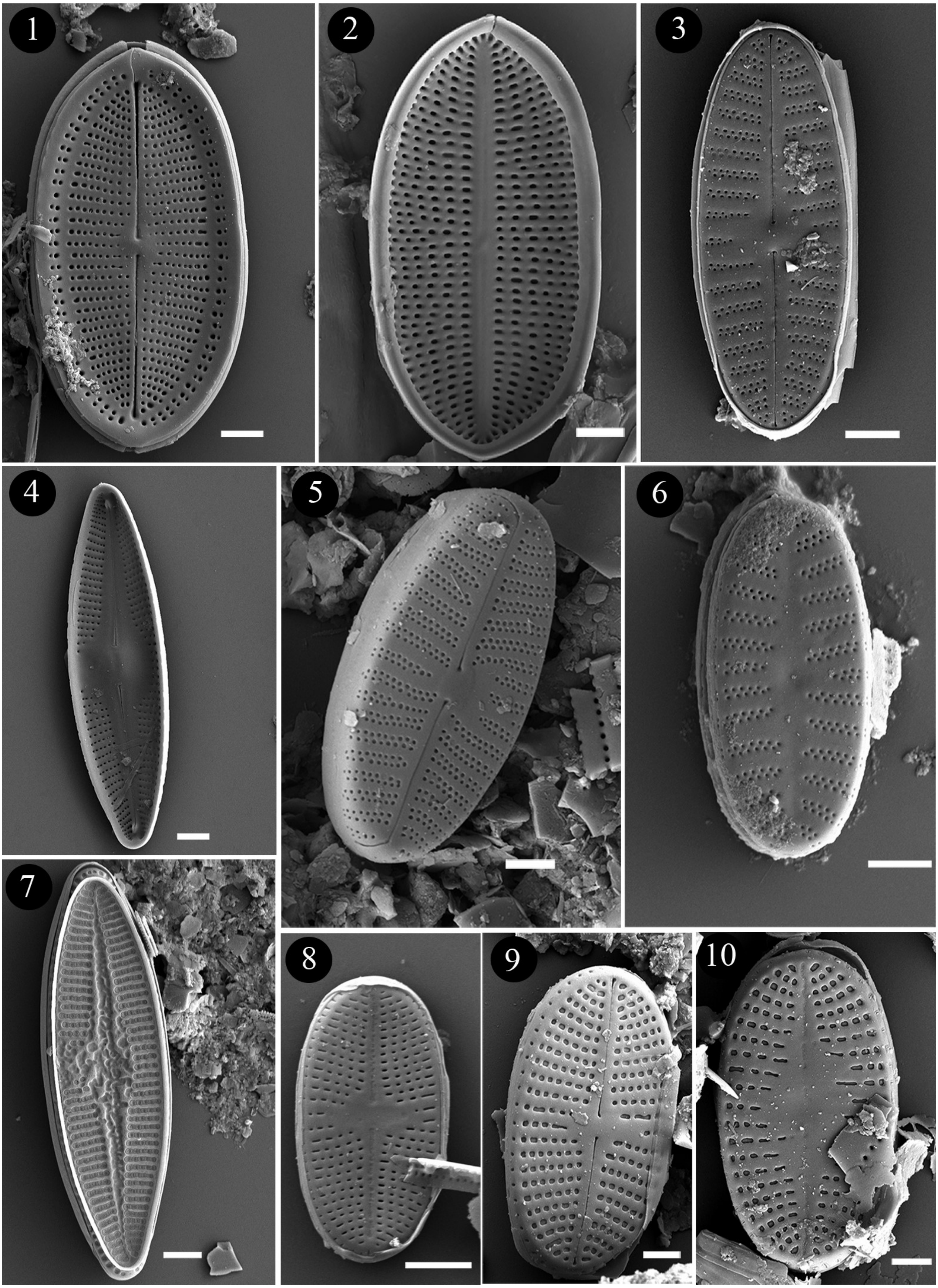
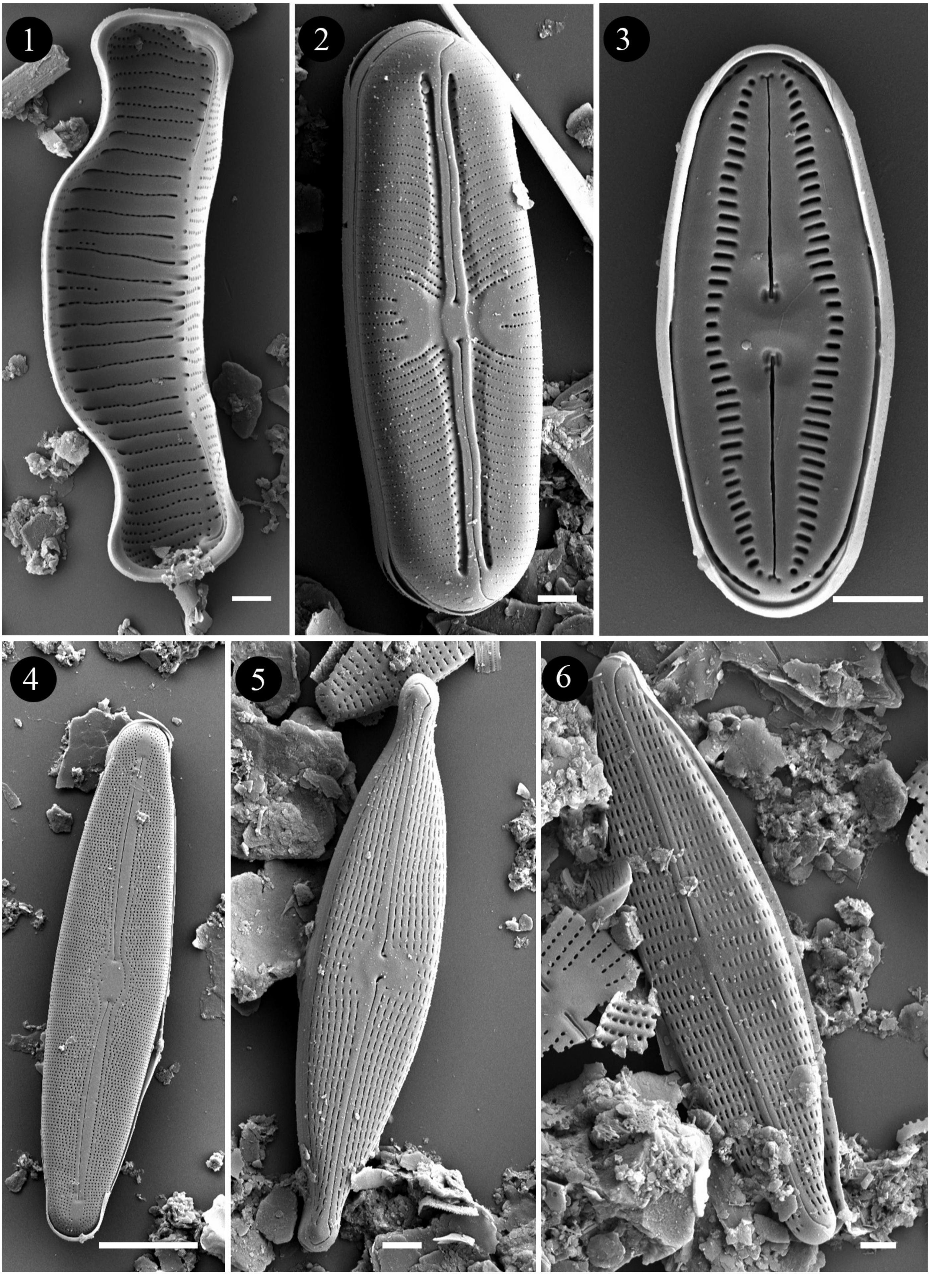

1
2
3
4
5
6
7
8
9
10

Plate 14 (SEM)

Fig.1 *Eunotia curtagrunowii* Nörpel-Schempp & Lange-Bertalot, valve internal view, scale bar 2 µm
Fig.2 *Sellaphora parapupula* Lange-Bertalot, valve external view, scale bar 2 µm
Fig.3 *Humidophila perpusilla* Lowe, Kociolek, Johansen, Van de Vijver, Lange-Bertalot & Kopalová, valve external view, scale bar 2 µm
Fig.4 *Frustulia saxonica* Rabenhorst, valve external view, scale bar 10 µm
Fig. 5 *Navicula gregaria,* Donkin, valve external view, scale bar 2 µm
Fig.6 *Craticula* sp.1 nov. valve external view, scale bar 1 µm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Conservation-important butterflies in an under-studied part of Macedonia: Plachkovica, Vlaina and Maleshevski Mountains

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Entomology section

Abstract. Being of high conservation interest, butterflies are one of the most studied groups of insects in Europe. With nearly 10% of endangered species on European level, each small contribution of the distribution status of butterflies represents a significant input in further conservation efforts. Although the butterflies are a well-studied insect group in the Republic of Macedonia, more knowledge is needed on the presence and distribution of the species of conservation concern. Our study focuses on faunistic research of butterflies on Maleshevo, Plachkovica and Vlaina Mountains, located in the eastern part of the country. Each of these mountain ranges offers diverse habitats that could support healthy populations and rich butterfly diversity. Therefore, we conducted a field research on these mountains using an unsystematic sampling. A wide array of localities and habitats has been studied in which 84 species were registered. The three mountains support 41% of the total butterfly fauna in Macedonia. During our research, we have recorded eleven butterflies of high conservation interest, which demonstrates the conservation importance of this part of the country. The most interesting finding was the syntopic occurrence of *Polyommatus (Polyommatus) eros eros* and *Polyommatus (Polyommatus) eros eroides*. Future studies should focus on abundance estimates and habitat availability for targeted species that are of high conservation priorities.

Key words: Maleshevski Mountains, Vlaina Mountain, Plachkovica Mountain, butterfly diversity, field research, butterfly conservation.

Introduction

Butterflies constitute an important model group used in diverse areas of biological research, including biodiversity conservation, pest control, ecology, physiology, evolution, genetics, and population dynamics, the impact of habitat loss and fragmentation and climate change (Wiemers et al., 2018). Because of their complex life cycle, they react extremely quickly to minor changes in the environment, making them a good biodiversity indicator and therefore provide an early warning system for biodiversity loss. As a result, butterflies are now one of the best-monitored insects in the world (Wiemers et al., 2018). According to Van Swaay et al. (2010), nearly 9% of European butterflies are in the threatened category of the IUCN and an additional 10% are Near Threatened. Furthermore, only some of them receive some protection and conservation actions (Van Swaay et al., 2010), while others are either understudied or receive no conservation attention despite obvious threats to their survival (for example the Macedonian Grayling (Verovnik et al., 2013)).

Starting already a century ago (Rebel, 1913), Macedonian butterfly fauna received a good amount of faunistic attention, which is a commodity that not so many insect groups have. In the recent times, but also taking the global commotion for the biodiversity worldwide, the need for conservation approach towards butterflies in Macedonia was evident. In this regard, several publications emphasized the need for priority areas for conservation (for ex. Krpač et al., 2013, Micevski & Micevski, 2015, Bozhinovska & Melovski in press.), as well as contributed directly towards the endangered status of certain species (for ex. Micevski et al., 2009; Verovnik et al., 2013; Melovski & Bozhinovska, 2014). We conducted a study on Plachkovica, Vlaina and Maleshevo Mountains, in the eastern part of Macedonia. So far, precise data for the butterfly fauna are lacking. The maps provided by Schaider & Jakšić (1989) are presented on fairly rough scale of 100 km^2. In comparison with the western part of the country, only fragmented studies with sporadic and opportunistic research in eastern Macedonia had been conducted (Micevski & Micevski, 2008; Verovnik et al., 2010; Verovnik, 2012).

Thus, the aim of this study is to show the butterfly diversity of these mountains, as well as to present their habitat preferences. Considering the importance of butterfly conservation, we highlight the presence of some flagship species, which shows the importance of protecting the mountains in this part of the country.

STUDY AREA

All three mountains are located in the eastern part of Macedonia (Fig. 1). Plachkovica is large and medium-high mountain with moderate continental climate which is modified by the Mediterranean climate influence from Strumica Valley on the southern slopes. Vlaina Planina is medium-high mountain as well, where the whole borderline with Bulgaria presents one mountain range. The highest peak is Kadiica peak (1932 m a.s.l.). On the other hand, Maleshevski Planini Mts. is vast mountainous range shared between Macedonia and Bulgaria. The most prominent peak is Chengino Kale (1745 m a.s.l.). The climate is continental and mountainous on higher elevations (Melovski et al., 2013). . Altitudinal belts of Italian and Turkey oak forests, submontane and montane beech forests, areas of Black and Scots pine forests as well as open grasslands and heatlhlands in the highest treeless zone represent the main vegetation. The zonal vegetation is intersected by other habitat types that cover smaller surfaces such as pseudo-steppe with grasses, oro-Moesian acidophilous grasslands, hydrophilous tall herb fringe communities, riparian belts of willows, poplars or Black alder, mountain hay meadows, submediterranean meadows, transition mires and quaking bogs, siliceous rocky slopes and bog woodlands (Matevski & Ćušterevska unpublished report).

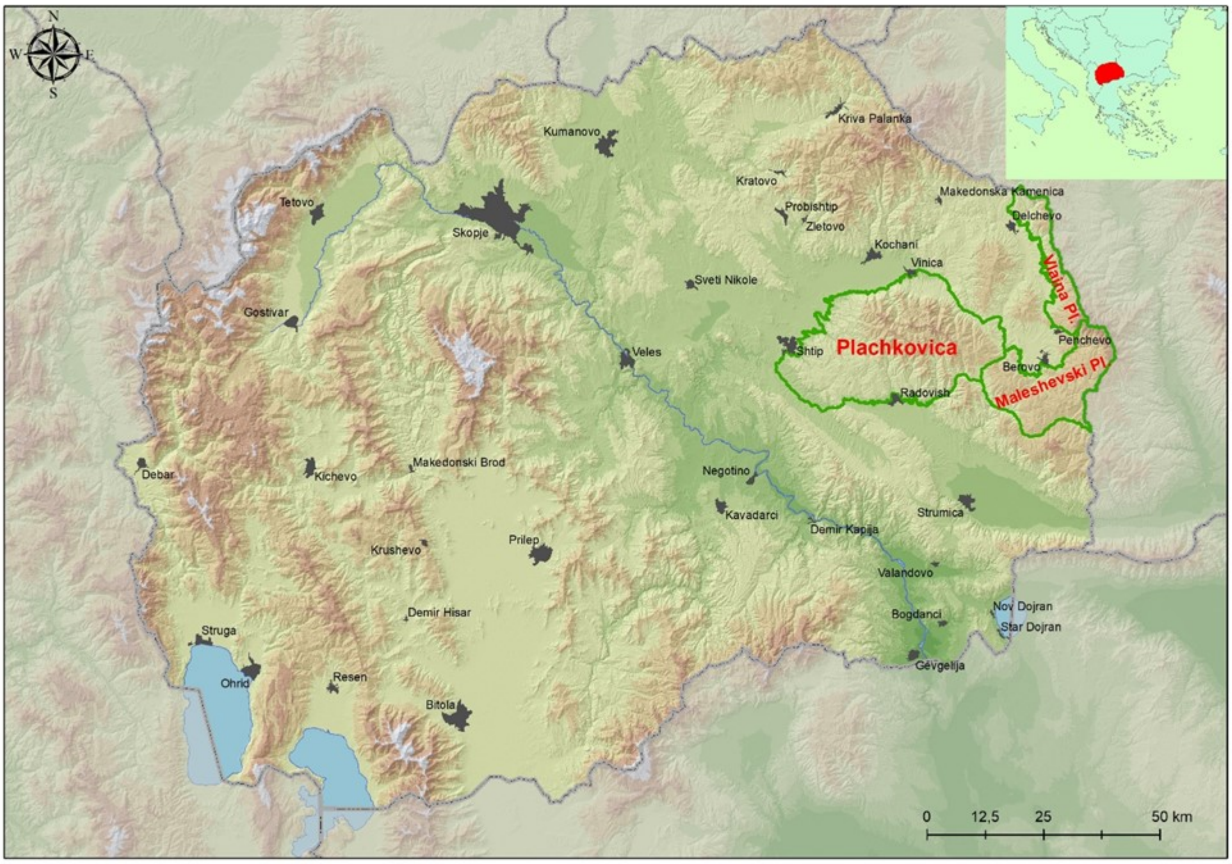

Figure 1. Map of the studied area outlined in green polygons

Materials and Methods

Collected material refers to field trips conducted in 2002, 2003, 2010, 2012, 2014, 2015, 2018 and 2019. Material was intensively sampled in July 2014 and July 2015 when field trips were part of the annual Biology Students' Research Society's field research, and in July and September 2019, when biodiversity evaluation of Maleshevski Mountains was conducted in the frame of the project "Nature Conservation Programme, phase 2". Irregular samples refer to the rest of the period where few individual field trips were conducted on limited number of localities. The working methods consisted of photographing, collection, preparation and determination of butterflies. We used unsystematic sampling and caught butterflies using entomological nets. Some of the specimens, which were identified on site, were released right away, while others were kept in paper envelopes for further examination. All specimens were labelled with date and place of their collection (coordinates), name of collector, habitat type and elevation. The collected butterflies were prepared on spreading boards and kept in entomological boxes. Specimens were determined according to Tolman (1997, 2008), Pamperis (1997), Lafranchis (2004) and Popović (2011). The nomenclature is according to de Jong (2014). Data are stored into a Microsoft Excel data base.

Regarding the conservation status, Appendix II of the Bern Convention, Annex IV of the Habitats Directive, restricted distribution in Europe and the national legislation were taken into consideration. Additionally, the threatened status of butterflies was presented for the assessed species according to the IUCN Red List categorization (Van Swaay et al., 2010) and the national Red List (Krpac & Darcemont, 2012).

Results

During our research, we have visited 36 localities. The most diverse one was Berovsko Ezero locality, with 37 registered species (Tab. 1). The study area supports diverse habitats including: wetlands, meadows, mountain pastures and heaths, riparian habitats, forests, clearing in forests, edge of forests and ruderal settlements. All specimens registered in this paper are found in the altitude between 550 and 1750m a.s.l. Localities, together with habitat types, elevation, date and coordinates are presented in Table 1.

Table 1. List of visited localities on Plachkovica, Vlaina and Maleshevski Mountains with given habitat type, elevation, coordinates and date. List of localities had been prepared for presenting the main results using code names for each unique record.

MALESHEVSKI MTS.					
Code name	**Locality**	**Habitat type**	**Elevation (m)**	**Coordinates (decimal degrees)**	**Date**
M01	Ablanica	riparian habitat	900	41.69766, 22.87877	13.07.2015
M02	Berovsko Ezero	edge of forest	1000	41.66293, 22.91769	25.07.2019
M03	Berovsko Ezero	meadow	1000	41.67301, 22.91190	25.07.2019
M04	Berovsko Ezero	riparian habitat	1000	41.66181, 22.91395	10.07.2015
M05	Berovsko Ezero	riparian habitat	1000	41.66810, 22.91729	25.07.2019
M06	Berovsko Ezero	forest clearing	1050	41.67523, 22.93057	20.07.2015
M07	Berovsko Ezero	riparian habitat	1100	41.65790, 22.94873	20.07.2015
M08	Berovsko Ezero	rural settelments	1100	41.65790, 22.94873	20.07.2015
M09	Berovsko Ezero	forest clearing	1200	41.67523, 22.93057	10.07.2015
M10	Berovsko Ezero	mountain pastures and heaths	1300	41.67989, 22.93748	25.07.2019
M11	Charshija	mountain pastures and heaths	1500-1600	41.69596, 23.00289	16.07.2015
M12	Chaushica	riparian habitat	1200	41.64633, 22.93180	09.07.2015
M13	Chengino Kale	mountain pastures and heaths	1450	41.72362, 22.99725	21.09.2019
M14	Dabovec	forest clearing	1100-1200	41.67144, 22.97208	12.07.2015
M15	Dabovec	riparian habitat	1100-1200	41.67144, 22.97208	12.07.2015
M16	Dabovec	riparian habitat	1100-1200	41.67144, 22.97208	13.07.2015
M17	Divna	meadow	1320	41.64733, 22.95598	09.07.2015

Code name	Locality	Habitat type	Elevation (m)	Coordinates (decimal degrees)	Date
M18	Golo Brdo	wetland	1520	41.70427, 23.01627	15.07.2015
M19	Guril Cheshma	wetland	1750	41.72139, 23.02806	16.07.2015
M20	Judovi Livadi	wetland	1200	41.75946, 22.91045	09.06.2012
M21	Keramizhdzan	forest clearing	1300	41.67173, 22.96457	13.07.2015
M22	Klepalo	forest clearing	1230	41.65952, 22.98560	11.07.2015
M23	Klepalo	meadow	1250	41.65952, 22.98560	12.07.2015
M24	Klepalo	meadow	1250-1300	41.65496, 22.97333	11.07.2015
M25	Klepalo	meadow	1260	41.65952, 22.98560	13.07.2015
M26	Klepalo	meadow	1300	41.65952, 22.98560	23.06.2015
M27	Klepalo	forest clearing	1300-1400	41.65952, 22.98560	15.07.2015
M28	Klepalo	forest clearing	1380	41.65952, 22.98560	09.07.2015
M29	Nebojsha	forest clearing	1520	41.730311, 23.01875	10.08.2018
M30	Nebojsha	forest clearing	1520	41.73031, 23.01875	16.07.2015
M31	Pesok	meadow	1300	41.65901, 22.95070	09.07.2015
M32	Ramna river	riparian habitat	1040	41.72921, 22.92955	29.07.2019
M33	Ramna river	forest	1100	41.72712, 22.92120	21.09.2019
M34	Ramna river	forest clearing	1260	41.72819, 22.98839	21.09.2019
M35	Ramni Rid	meadow	1230	41.65314, 22.92568	09.07.2015
M36	Shirinata	meadow	1300	41.65985, 22.94560	09.07.2015
M37	Srbnica	rural settelments	1300	41.63653, 22.93907	19.07.2015
PLACHKOVICA MT.					
Code name	Locality	Habitat type	Elevation (m)	Coordinates (decimal degrees)	Date
P01	Boshchalija	rural settelments	1350-1450	41.75068, 22.56107	18.07.2014
P02	Crkvishte	mountain pastures and heaths	1580	41.78707, 22.44255	14.07.2014
P03	Crkvishte	meadow	1600	41.78707, 22.44255	14.07.2014
P04	Dzumaja	forest clearing	1200	41.74635, 22.50581	11.07.2014
P05	Dzumaja	riparian habitat	1200	41.75433, 22.51466	11.07.2014
P06	Dzumaja	rural settelments	1400	41.74010, 22.50192	17.07.2014
P07	Edendere river	riparian habitat	1200-1300	41.74672, 22.50539	17.07.2014
P08	Gradeshka Reka	riparian habitat	550	41.84469, 22.50676	30.07.2010
P09	Gradeshka Reka	edge of forest	680	41.84495, 22.50781	30.07.2010
P10	Gradeshka Reka	forest	900	41.82523, 22.50366	30.07.2010
P11	Gradeshka Reka	forest clearing	900	41.82523, 22.50366	30.07.2010
P12	Gradeshka Reka	riparian habitat	900	41.82523, 22.50366	30.07.2010
P13	Lomija river	riparian habitat	1150	41.76709, 22.50274	11.07.2014
P14	Lomija river	mountain pastures and heaths	1200	41.76572, 22.51616	17.07.2014
P15	Meshishka river	riparian habitat	1200	41.76186, 22.52853	11.07.2014
P16	Momin Preslap	forest clearing	1500	41.76773, 22.57613	18.07.2014
P17	Odmaralishte	meadow	700-800	41.77702, 22.35034	01.05.2003
P18	Turtel	forest	1200-1300	41.78684, 22.42784	19.07.2014
P19	Turtel	edge of forest	1400	41.79803, 22.42341	19.07.2014

Code name	Locality	Habitat type	Elevation (m)	Coordinates (decimal degrees)	Date
P20	Turtel	meadow	1400	41.79803, 22.423412	15.07.2014
P21	vill. Ebeplija	meadow	800-900	41.760515, 22.37304	01.05.2003
P22	vill. Kozbunar	forest	1100-1200	41.72856, 22.47129	19.07.2014
P23	vill. Vidovishte	meadow	400-500	41.85951, 22.33757	06.05.2002
P24	Vlashka Koliba	rural settelments	1450	41.80420, 22.41680	14.07.2014
P25	Vlashka Koliba	forest clearing	1500	41.80420, 22.41680	14.07.2014
VLAINA MT.					
Code name	Locality	Habitat type	Elevation (m)	Coordinates (decimal degrees)	Date
V01	Kadiica	meadow	1600	41.73788, 22.81900	27.07.2019
V02	Pancharevo	meadow	1050	41.82639, 22.85772	26.07.2019
V03	vill. Crnik	meadow	1100	41.80565, 22.91700	28.07.2019
V04	vill. Crnik	meadow	1280	41.80957, 22.92801	20.09.2019

The most diverse habitats on different localities were meadows and riparian habitats with 78 and 77 species found respectively (Fig. 2).

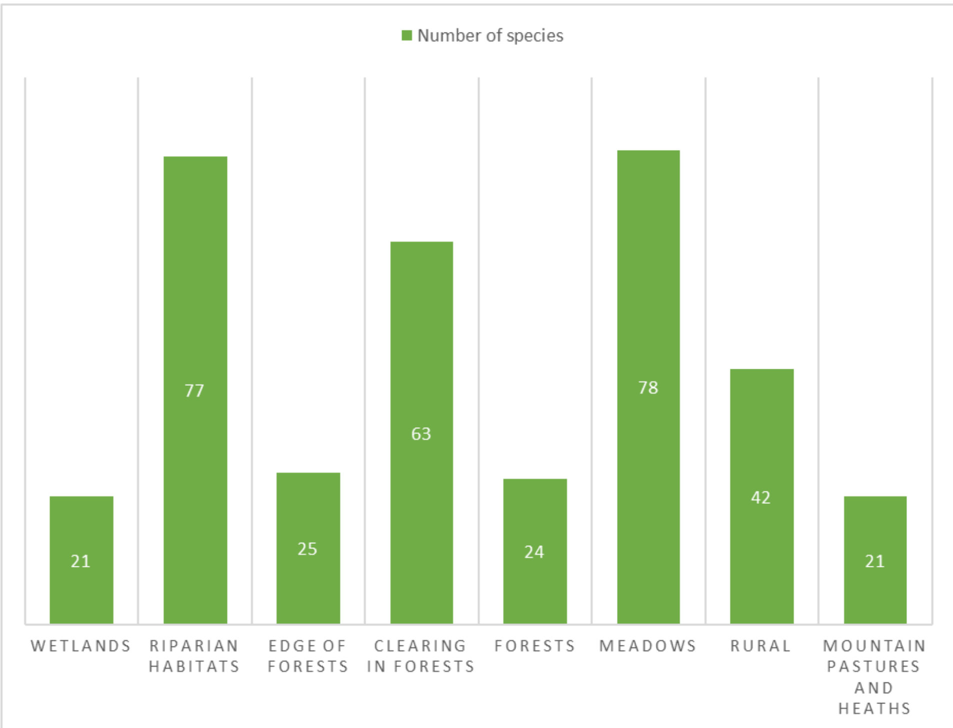

Figure 2. Number of species found in different habitat type

In total, 84 species (85 taxa) belonging to five families were registered in the study area (Tab.2).

Table 2. List of butterflies recorded on Plachkovica, Vlaina and Maleshevski Mountainswith collecting data cross-referencing table 1. Species already reported by Schaider & Jakšićare marked with an asterisk .

	Fam. Papilionidae	
	Species	**Code name**
1.	*Iphiclides podalirius* (Linnaeus 1758)	M01, M03, M20, M32, P12, P17, P18, V03
2.	*Parnassius mnemosyne* (Linnaeus 1758) *	M16, P15
3.	*Zerynthia (Zerynthia) polyxena* (Denis & Schiffermüller 1775) *	P13

	Fam. Pieridae	
4.	*Aporia crategi* (Linnaeus 1758) *	M14, M20
5.	*Colias caucasica subsp. balcanica* Staudinger 1871	M11, M30
6.	*Colias croceus* (Fourcroy 1785)	M02, M03, M05, M08, M10, M24, M33, M34, P03, P09, P13, P19, P21, P25, V02, V03, V04
7.	*Gonepteryx rhamni* (Linnaeus 1758)	M15, M26, P09, P21
8.	*Leptidea duponcheli* (Staudinger 1871)	M08, M15, M17, M24, M26, M35, M37, P01, P06, P08, P22
9.	*Leptidea sinapis* (Linnaeus 1758)	M03, M05, M07, M24, M30, M32, M37, P05, P09, P21, P22, V01, V02, V03
10.	*Pieris balcana* Lorkovic 1970	M09, M15, M21, M24, M26, M36, P17
11.	*Pieris brassicae* (Linnaeus 1758)	M19, P01, P09, P15
12.	*Pieris mannii* (Mayer 1851)	M32, P05, P15
13.	*Pieris napi* (Linnaeus 1758)	M08, M14, M24, M28, M34
14.	*Pieris rapae* (Linnaeus 1758)	M23, P08, P17, V02
	Fam. Lycaenidae	
15.	*Aricia agestis* (Denis & Schiffermüller 1775)	M05, M24, M26, M27, M31, M33, P01, P10, P19, P22, V03
16.	*Callophrys rubi* (Linnaeus 1758)	M14
17.	*Celastrina argiolus* (Linnaeus 1758)	M03, M08, M15, M21, P06, P12
18.	*Cupido (Cupido) minimus* (Fuessly 1775)	P12
19.	*Cyaniris semiargus* (Rottemburg 1775)	M09, M20, M24, M26, P03
20.	*Lycaena alciphron* (Rottenburg 1775)	M24, M27, M28, M35, M37, P03, P22, P24, P25
21.	*Lycaena candens* (Herrich-Schäffer 1884)	M12, M21, M24, V01
22.	*Lycaena phleas* (Linnaeus 1761)	M08, M11, M13, M19, M33, M34, P08, P12, P16,P21
23.	*Lycaena tityrus* (Poda 1761)	M02, M03, M20, M34, P03, P08, P14, P16, P25, V02, V03, V04
24.	*Lycaena vigaureae* (Linnaeus 1758)	M03, M05, M07, M08, M09, M19, M24, M37, V01, V03
25.	*Phengaris arion* (Linnaeus 1758)	M21, M35, P14, P20
26.	*Plebejus (Plebejus) argus* (Linnaeus 1758)	M02, M03, M08, M10, M20, M29, M32, M35, P23, V02, V03
27.	*Plebejus (Plebejus) idas* (Linnaeus 1761)	M02, M18, P23, V03
28.	*Polyommatus (Polyommatus) eros subsp. eros* (Ochsenheimer, 1808)	V01
29.	*Polyommatus (Polyommatus) eros subsp. eroides* (Frivaldszky 1835)	V01, M11, M18, M24, M27, P25
30.	*Polyommatus (Polyommatus) icarus* (Rottemburg 1775) *	M02, M03, M04, M05, M11, M17, M20, M24, M26, M27, M32, M33, M37, P08, P13, P16, P18, P19, P23, P24, P25, V01, V02, V03, V04
31.	*Polyommatus (Polyommatus) dorylas* (Denis & Schiffermüller 1775)	P18
32.	*Polyommatus (Polyommatus) thersites* (Cantener 1835)	M02, M24
33.	*Pseudophilotes vicrama* (Moore 1865)	M32
	Fam. Hesperidae	
34.	*Carcharodus alceae* (Esper 1780) *	P25
35.	*Erynnis tages* (Linnaeus 1758) *	M14, V02
36.	*Ochlodes sylvanus* (Esper 1777) *	M02, M08, M19, M24, M35, M37, P08, P16, V01
37.	*Pyrgus alveus* (Hübner 1803)	M15
38.	*Pyrgus armoricanus* (Oberthür 1910)	M20
39.	Pyrguis *malvae* (Linnaeus 1758) *	P12
40.	*Pyrgus* serriatulae (Rambur 1839)	M27, P03, P18
41.	*Thymelicus acteon* (Rottemburg 1775)	V03
42.	*Thymelicus lineola* (Ochsenheimer 1808)	P14, P25
43.	*Thymelicus sylvestris* (Poda 1761) *	P08, P18
	Fam. Nymphalidae	
44.	*Aglais io* (Linnaeus1758) *	M22, M24, M32, P04, P07, P12, P22, V03, V04
45.	*Aglais urticae* (Linnaeus1758)	M14, M26, V01
46.	*Apatura ilia* (Denis & Schiffermüller 1775)	M32

47.	*Apatura iris* (Linnaeus1758)	M07, M09, M25
48.	*Aphantopus hyperantus* (Linnaeus1758)	M03, M05, M08, M15, M17, M24, M37, P05, P07, P08, V01, V02, V03
49.	*Araschnia levana* (Linnaeus1758)	M15, M24, M32
50.	*Arethusana arethusa* (Denis & Schiffermüller 1775)	M29, M33, P10
51.	*Argynnis* (Argynnis) *paphia* (Linnaeus1758)	M05, M06, M32, P08, V01
52.	*Argynnis* (Fabriciana) *adippe* (Denis & Schiffermüller 1775)	M02, M08, M15, M24, M27, M32, M36, M37
53.	*Argynnis* (Fabriciana) *niobe* (Linnaeus1758)	M03, M08, M30, P02, P12, V03
54.	*Argynnis* (Mesoacidalia) *aglaja* (Linnaeus1758)	M11, M14, M15, M24, P04
55.	Argynnis (Pandoriana) *pandora* (Denis & Schiffermüller 1775)	P12
56.	*Boloria* (Clossiana) *dia* (Linnaeus 1767) *	M29, M37, V01, V02
57.	*Boloria* (Clossiana) *euphrosyne* (Linnaeus 1758) *	M15, M20, M24
58.	*Brenthis daphne* (Bergsträsser 1780)	M05, M07, M08, M15, M31, M32, P05, P25
59.	*Brenthis hecate* (Denis & Schiffermüller 1775)	M35
60.	*Brintesia circe* (Fabricius 1775)	M02, M08, M27, P12, P14, P19, V02
61.	*Coenonympha arcania* (Linnaeus 1761)	M08, M17, M21, M31, M37, P04, P10
62.	*Coenonympha leander* (Esper 1784)	M19, M20
63.	*Coenonympha pamphilus* (Linnaeus 1758)	M01, M03, M20, M26, M33, M34, P15, V02, V03
64.	*Erebia medusa* (Denis & Schiffermüller 1775) *	M20, M26, P16
65.	*Hipparchia* (Hipparchia) *fagi* (Scopoli 1763)	M32, P12, V03
66.	*Hipparchia* (Neohipparchia) *statilinus* (Hufnagel 1766)	V04
67.	*Hyponephele lupinus* (O. Costa 1836)	P18
68.	*Issoria* (Issoria) *lathonia* (Linnaeus 1758)	M11, M15, M20, M29, M34, P12, P13, P14, P21, V01, V03, V04
69.	*Lasiommata maera* (Linnaeus 1758)	M11, M15, M24, M26, M37, P06, P19, P20, V01
70.	*Lasiommata petropolitana* (Fabricius 1787)	M19
71.	*Libythea celtis* (Laicharting 1782)	M06, P16
72.	*Limenitis camilla* (Linnaeus 1764)	M05, M07, M32
73.	*Limenitis reducta* Staudinger 1901 *	P12
74	*Maniola jurtina* (Linnaeus 1758) *	M03, M05, M08, M24, M36, P12, P13, P14, P18, V01, V02, V03
75.	*Melanargia galathea* (Linnaeus 1758)	M02, M03, M05, M08, M10, M24, M27, M37, P13, P18, P19, P25, V01, V02, V03
76.	*Melitaea athalia* (Rottemburg 1775) *	M02, M24, M31, M36, P24
77.	*Melitaea didyma* (Esper 1778)	M02, M08, M10, P19, V03
78.	*Melitaea phoebe* (Denis & Schiffermüller 1775) *	M08, M31, V02, V03
79.	*Melitaea trivia* (Denis & Schiffermüller 1775)	P13
80.	*Nymphali polychloros* (Linnaeus 1758) *	P06
81.	*Pararge aegeria* (Linnaeus 1758)	P11, V03
82.	*Polygonia c-album* (Linnaeus 1758) *	M02, M05, M07, M13, M15, M21, M24, M32, M34, M37, P04, P12, P16, P22, V01, V03
83.	*Pyronia (Pyronia) tithonus* (Linnaeus 1767)	M05, M08, V02, V03
84.	*Vanessa atalanta* (Linnaeus 1758)	M15, M26, M30, P06, P15, P21, V04
85.	*Vanessa cardui* (Linnaeus 1758)	M02, M03, M10, M11, M15, M24, M26, M31, P13, P14, P18, V01, V02, V03

We consider 11 out of 84 registered species of higher importance. Three of them are listed on the Appendix II of the Bern Convention and Annex IV of the Habitat Directive (*Phengaris arion*, *Parnassius mnemosyne* and *Zerynthia polyxena*). According to national legislation *Phengaris arion* and *Hipparchia fagi* are regarded of conservation concern. Additionally, according to the Red List of European butterflies (2010), *Phengaris arion* is categorized as Endangered, *Parnassius mnemosyne*, *Polyommatus (Polyommatus) dorylas*, *Polyommatus (Polyommatus) eros*, *Pseudophilotes vicrama, Hipparchia (Hipparchia) fagi*, *Hipparchia (Neohipparchia) statilinus*and *Thymelicus acteon* are categorized as Near Threatened. *Pieris balcana* and *Colias caucasica balcanica* have not only restricted distribution to Europe, but are also endemic for the Balkans (Tab. 3).

Table 3. Butterfly species of conservation concern recorded on Plachkovica, Vlaina and Maleshevski Mountains

Species	Bern Convention Appendix II	Habitat Directive Annex IV	Red List status of European butterflies 2010	National Red List of butterflies 2012 Krpac & Darcemont	Distribution restricted to Europe	National legislation
Fam. PAPILIONIDAE						
Parnassius mnemosyne	•	•	NT	NT		
Zerynthia polyxena	•	•	LC	NT		
Fam. PIERIDAE						
Pieris balcana			LC		•	
Colias caucasica balcanica			LC		•	
Fam. LYCAENIDAE						
Phengaris arion	•	•	EN	NT		•
Polyommatus dorylas			NT			
Polyommatus eros			NT			
Pseudophilotes vicrama			NT			
Fam. NYMPHALIDAE						
Hipparchia fagi			NT			•
Hipparchia statilinus			NT			
Fam. HESPERIDAE						
Thymelicus acteon			NT			

Discussion

The Macedonian butterfly fauna numbers 205 species (Melovski & Bozhinovska, 2014). These results indicate that at least 41% of the total butterfly fauna in Macedonia is present on Plachkovica, Vlaina and Maleshevski Mountains. Even though Macedonian butterfly diversity is well studied, knowledge on the distribution and threats to butterflies are far from being well known (Verovnik et al., 2010). Therefore, it is important to emphasize the need of gaining data in under-researched areas in Macedonia. Combining the faunistic checklist together with the species of conservation concern gives an overview of how much this region is important regarding butterfly diversity. This information is a prerequisite for designation of further protected areas, as well as Prime Butterfly Areas. Additional research in spring months will most likely expand the total species number. The study area is bordering an already designated Prime Butterfly Area in its southern part – Ograzhden (van Swaay & Warren, 2003).

As compared with the previous data for the region, we have recorded 19 species that match with the results by Schaider & Jakšić (1989) (Tab. 2). Only four species mentioned by the latter authors were not detected in our research (*Anthocharis cardamines* Staudinger 1869, *Lycaena thersamon* (Esper 1784), *Pontia daplidice* (Linnaeus 1758) and *Spialia orbifer* (Hübner 1823)), while the other 61 recorded species were not listed in their research. The book of Schaider & Jakšić (1989) provide only 10x10 km grid presence data which is of low precision. Taking into consideration the very limited research done in the eastern part of the country in the recent years, (see introduction), we can conclude that there is a need for further research, especially now, when we have an overview of the habitat condition and localities' richness.

The most interesting finding was the syntopic occurrence of *Polyommatus (Polyommatus) eros* subsp. *eros* (Ochsenheimer 1808) and *Polyommatus (Polyommatus) eros* subsp. *eroides* (Frivaldszky 1835) at Kadiica locality (Vlaina Mt). Considering the recent taxonomical rearrangements of the latter taxa (Wiemers et al., 2010), this finding provides clues for revisiting their taxonomy. According to the latest update on Fauna Europaea, both subspecies appear in Macedonia, Serbia and Montenegro even though no recent data from *Polyommatus (Polyommatus) eros* subsp. *eros* is available for Macedonia.

Summary

Having in consideration that the Macedonian butterfly fauna numbers 205 species (Melovski & Bozhinovska, 2014), our results show that 41% (84 species) of the total number, are present on Maleshevo, Vlaina and Plachkovica Mountains. Overall, the specimens were collected from 37 lo-

calities and 8 habitat types. The richest locality with species is Berovsko Ezero (37) while the most diverse habitat types were meadows with 78 species recorded. Schaider & Jakšić (1989) reported 23 species in this study area from which 18 matched with our results, 61 species are newly recorded and we did not record 4 species given by their research.

We have recorded eleven species that have conservation importance (*Phengaris arion*, *Parnassius mnemosyne*, *Hipparchia fagi*, *Pieris balcana*, *Zerynthia polyxena*, *Colias caucasica balcanica, Polyommatus (Polyommatus) dorylas*, *Polyommatus (Polyommatus) eros*, *Pseudophilotes vicrama, Hipparchia (Neohipparchia) statilinus* and *Thymelicus acteon*). We believe that further research will enrich this checklist in order to enlarge the public attention about the urge of conservation of the whole area. The most interesting finding was the syntopic occurrence of *Polyommatus (Polyommatus) eros eros* (Ochsenheimer, 1808) and *Polyommatus (Polyommatus) eros eroides* (Frivaldszky, 1835) at Kadiica locality.

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Diversity and distribution of butterflies Lepidoptera, Papilonidea & Hesperoidea in the Prespa region, North Macedonia, Albania and Greece

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Entomology section

Abstract. Diversity and comparative distribution study of butterflies was conducted in Prespa region on Macedonian, Albanian and Greek side in 2010, 2013 and 2016 respectively. The main aim of this study was to ascertain the realistic state of the butterfly fauna in the Prespa Region. We have registered 119 butterfly species in variety of habitats, from lacustrine emergent wetlands through thermophilous forests to orchards. Detection of the Large Copper (*Lycaena dispar*), a wetland butterfly that is classified as Near Threatened (NT) according to the IUCN Red List is of [articular importance. The Apollo and the Marsh Fritillary are target species for proclamation of Prime Butterfly Areas across Europe. Significant finding is the recording of *Gonepteryx cleopatra* at Galichica Mt., for the first time since 1983 (Krpač & Mihajlova, 1997). We compared our butterfly species list with previous studies and discuss the possible negative impacts on butterflies caused by agricultural management and habitat loss

Key words: butterflies, checklist, Prespa region, wetland habitats, agriculture, conservation.

Introduction

The Prespa-Ohrid region of the south-west Balkans is one of the most significant biodiversity hotspots of Europe (Schwaderer & Spangenberg, 2009). It receives a significant amount of conservation attention over the last couple of decades which resulted in many protected areas being established. The agricultural intensification along the lake shore of Prespa however pose a great threat to the creatures occupying these habitats. Constant monitoring and research need to be in place in order to detect and mitigate serious threats to the area. Butterflies are group of insects that can well be used as a biological indicator and are relatively well studied in N. Macedonia (Melovski & Bozhinovska, 2014). Although few of the already listed species haven't been registered for decades, taking into consideration the new findings (Kolev & Van der Porten, 1997; Krpač and Mihajlova, 1997; Melovski, 2004; Verovnik & Micevski, 2008; Micevski et al., 2009a; Verovnik et al., 2010), the total number of butterfly species in N. Macedonia almost definitely exceeds 200 species. While in Europe a total of 482 butterfly species can be found (Van Sway, 2010), N. Macedonia's 40% of the total European fauna is largely assigned to its relief, diverse habitats and climate (Melovski & Bozhinovska, 2014). The last comprehensive butterfly study for N. Macedonia taking into account their distribution was conducted by Shaider and Jakšić (1989). According to the approximated 10x10 km grid cell maps, the Prespa region in the Macedonian side should number 124 butterfly species, which is 60% of the total number of butterflies of N. Macedonia, and 25% of the European species. The Prespa region accounts for a small share of the Macedonian territory, and yet it is home to a high proportion of butterflies. This makes Prespa region an important area for butterflies. However, this list of butterfly species for the Prespa region is outdated and does not take into account the changes in the habitats close to the shore of Prespa Lake. Intensification of farming around the shore and with that the usage of herbicides and pesticides and drainage of wetlands for arable land could inevitably cause decline in species richness in the region (Bozhinovska, 2010).
Primarily, with this research we are aiming at determining the current number of butterfly species and

their distribution in the Macedonian part of Prespa by comparing it to the checklist from 1989 (Shaider and Jakšić, 1989); and, discuss possible changes of the butterfly composition. Target habitat types in this research were the agricultural areas as well as wetland habitats assuming that they are mostly affected by intensive agriculture. These were the habitats where most of our surveys were conducted.

In addition, this research incorporates butterfly fauna of the Albanian and Greek Prespa National Parks, complementing the list of butterflies for this region.

Study area

Location, climate and protected areas

The Prespa region is located in the Balkan Peninsula, Southeast Europe (Fig. 1). This region is a high-altitude basin, which includes two inter-linked lakes, Macro Prespa and Micro Prespa, situated at approximately 850 m a.s.l. and surrounded by mountains exceeding 2000 m. The south part of the Prespa region is shared between N. Macedonia, Albania and Greece. All countries of the Prespa Lakes Basin have designated protected areas: The National Park of Prespa (Albania), the Galichica and Pelister National Parks (N. Macedonia), and the National Prespa Park (Greece) (Prespa Lakes Basin Strategic Action Programme, 2012) (Fig. 1). Furthermore, the lake of Prespa in the Macedonian side is a Monument of Nature (IUCN category III), while the area of Ezerani is designated Nature Park (IUCN category IV) (Fig. 1). This region is one the largest transboundary protected area system in the Balkans and receives a great deal of conservation attention. In recognition of the ecological, historical and cultural significance of the transboundary Prespa Lakes region, in 2000 the Prime Ministers of the three neighboring countries (Albania, Greece, and N. Macedonia) issued a Declaration for creation of the "Prespa Park" as the first transboundary protected area in Southeast Europe. The national Prespa Park region is considered an ecological entity of global significance (Integrated Ecosystem Management in the Transboundary Prespa Park Region). In 2014, the Ohrid-Prespa Transboundary Biosphere Reserve between Albania and N. Macedonia was added to UNESCO's World Network of Biosphere Reserves. Continental climate across the high Prespa valley and foothill of Galichica Mt. is with notable Mediterranean climate influence. At higher altitudes of Galichica Mt., the mountainous climate is modified by the impact

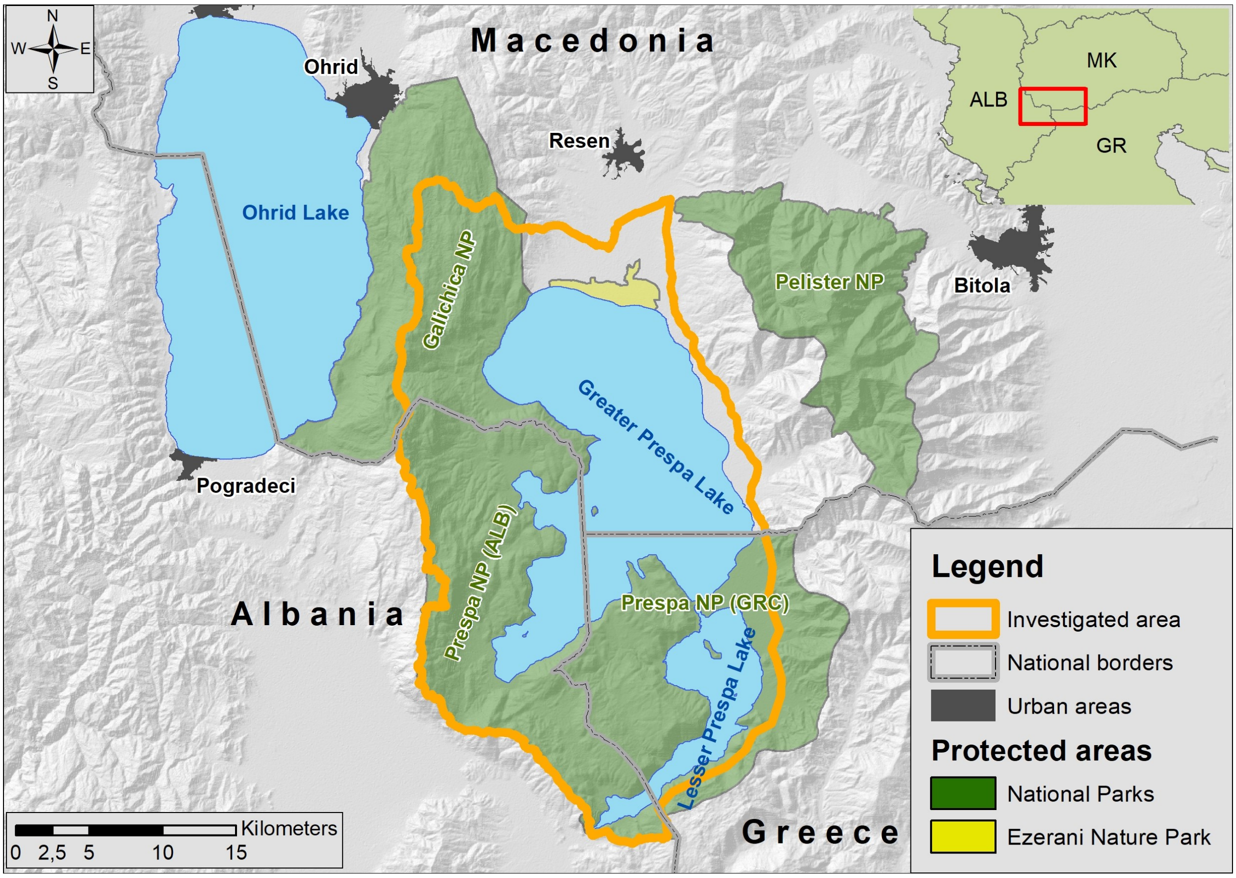

Fig 1. Prespa region and the investigated area.

of water masses of Ohrid and Prespa lakes (Melovski et al., 2013).

According to the regional division of the Republic of N. Macedonia (Melovski et al., 2013), the study area spans across the Ohrid-Prespa area, particularly in the regions of:

- Galichica – eastern slopes;
- Prespa – the study area includes the area between the shoreline of Prespa Lake and the foothill of Galichica Mt. at an altitude of about 950-1000 m, excluding the foothills of Bigla and Pelister Mt.; and Prespansko Ezero – Stenje and Ezerani marches, island Golem Grad, excluding the water basin of Prespa Lake, Zverinjec and Metileec marshes.

Our study area also extended across the Albanian and Greek Prespa National Park (Fig. 1). The surveyed habitats were diverse, ranging from lacustrine wetlands, through meadows, forests, and agricultural fields, to mountain pastures. The study area in all three countries, excluding the water surface, covers 516 km^2 (Fig. 1).

Surveyed localities

***Prespa region*:**

Island of Golem Grad (MKD)

The Island of Golem Grad is located in the Macro Lake Prespa. It is mainly covered by Greek juniper (*Juniperus excelsa*). The periphery of the island is a rocky shore.

Prechna (MKD)

The Prechna locality is a hill situated between v. Stenje and v. Konjsko. The locality is predominantly comprised of deciduous mixed forest, but also includes a few lake shore habitats.

Oteshevo (MKD)

The lake shore from v. Stenje to v. Sirhan. Main habitat types are wetlands, meadows and riparian habitats.

***Prespansko Ezero region*:**

Ezerani (MKD)

The locality Ezerani was recognized as globally important wetland area and was designated a Ramsar site in the 1995, because of its internationally valuable wetland habitats and high biological diversity. Subsequently, in 1996 it was declared a Strict Nature Reserve (IUCN Category Ia), especially for its ornithological importance. In 2012, the Strict Nature Reserve Ezerani was demoted to Nature Park Ezerani (IUCN Category IV) and reduced its protected territory, as a result of long-term inappropriate land-use practices, which lead to biodiversity depletion and degradation of its habitats (Management Plan for the Nature Park "Ezerani" 2012-2021", 2012). From all localities presented in this paper, Ezerani was the largest, the most frequently visited, and the most intensely researched locality. The Ezerani locality was the focal point of the project "Diversity of butterflies in the Nature Reserve Ezerani". It included diverse habitats ranging from wetlands, lake shore, meadows, to extremely modified habitats such as orchards and other arable lands.

v. Stenje (MKD)

The v. Stenje locality borders with Prechna locality on its south and Carina locality on its north side. It mostly consists of wet meadows and lake shore habitats, but also agricultural lands.

Carina (MKD)

Carina is located between Oteshevo and v. Stenje localities. It represents a relatively small territory in our study area with lake shore habitats and a few bushy areas.

Shtrbsko Blato (MKD)

The locality Shtrbsko Blato is a wetland area along the east Prespa Lake shore, situated south of v. Asamati, the eastern border of Ezerani locality.

***Galichica region*:**

v. Leskoec (MKD)

The v. Leskoec is positioned north-west of v. Stenje, in the foothills of Galichica Mt. On its west and south side, it borders with Stara Galichica, and on the north, with Oteshevo and Mala Galichica locality. It consists mainly of forest and riparian habitats.

Mala Galichica (MKD)

The part of Galichica Mt. stretching from Preslap locality towards north to Mt. Istok, is called Mala Galichica. During our study, only the eastern part of Mala Galichica was visited. It is characterized with diverse habitats, such as hill pastures, forests, clearings in a forest, meadows, and high mountain pastures.

Stara Galichica (MKD & ALB)

Stara Galichica stretches from Preslap across the Macedonian-Albanian border, the largest part being in Albania. Both Macedonian and Albanian part of Stara Galichica were studied, visiting localities only in its eastern side. It is characterized with diverse habitats, such as hill pastures, forests, clearings in forest, meadows, and high-mountain pastures.

Albanian Prespa National Park:

Dlaboko, St. Marena (ALB)

This locality is situated on the shore of Prespa Lake, south-east of the Tuminec village. It was researched along the lake shore habitats.

Mal Grad (ALB)

Mal Grad is a small island in Prespa Lake positioned on the very southern edge of Macro Lake Prespa. Habitats along the lake shore were studied.

Small Prespa Lake (ALB)

The small lake of Prespa is divided between Albania and Greece. Only a minor part of the lake belongs to Albania and most of this part is covered with thick reed vegetation.

v. Globochani (ALB)

Globochani is a village on the shore of Macro Prespa Lake stretched between Pustec and Dolna Gorica. It was researched along the lake shore and in ruderal habitats.

v. Dolna Gorica (ALB)

Dolna Gorica is a village located near the shore of Macro Prespa Lake and is very close to the Macedonian border. It mainly consists of termophilous oak forest.

v. Zrnovsko (ALB)

Zrnovsko is a village near the south rim of Macro Prespa Lake. Habitats along the lake shore and shrublands were studied.

v. Tuminec (ALB)

Tuminec is a village near the north rim of Macro Prespa. Wetland habitats were researched.

***Greek Prespa National Park*:**

Kale (GRC)

Hill on the eastern shore of Great Prespa Lake. Agricultural habitats were visited.

Psarades (Nivici) (GRC)

A village on the south shore of Great Prespa Lake. Termophilous forests and their clearings were studied.

Island of St. Achilleios, (GRC)

Island on Little Prespa Lake with mostly termophilous vegetation.

Isthmus between Great and Little Prespa Lake (GRC)

The riparian habitats between the two lakes were visited.

Materials and methods

The study area was surveyed from June to September 2010 on the Macedonian side, and from May to September 2013 on the Albanian side of Prespa. Greek side was visited for four days only in June 2016. We used entomological nets for collecting butterfly specimens, which we conserved inside paper envelopes. Date, location, habitat and altitude were noted for each specimen. Easily recognizable species were released after identification. The rest of the specimens were conserved and were subjected to later determination. Determination was conducted according to various publications (Thurner, 1964; Schaider & Jakšić, 1989; Popović & Đurić, 2011; Pamperis, 1997; Tolman & Lewington 1997; Lafranchis, 2004). The nomenclature in this paper is in accordance with Fauna Europaea (http://www.faunaeur.org). The results from the Macedonian side of Prespa region (excluding Galichica Mt.) were compared to the findings from two 1x1 km grid cells of the area from Schaider & Jaksic (1989).

Results and discussion

A total of 119 butterfly species (Tab. 1) were recorded in the Prespa region, within 11 habitat types (Fig. 2). In the Macedonian Prespa region (excluding Galichica Mt.) we identified 87 butterfly species. We mostly focused our fieldwork on the lake shore around Prespa Lake with a total effort of 13 days. Meadows were second most researched habitats, with 10 days of fieldwork and are followed by: wetlands 9 days, clearings in forests 8 days, forests and agricultural areas 7 days each, riparian habitats and shrublands 4 days each and the least visited were hill pastures with 2 days and ruderal habitats with only one day visit.

The most widespread butterfly species across localities is *Polyommatus icarus*, which occurred in 13 localities (Fig. 3). *Colias croceus* and *Maniola jurtina* have also high occurrence rate, present in ten localities. All of these species are common and widespread in N. Macedonia. The locality with most species detected is Starta Galichica (54 species) (Fig. 4) and this comes with no surprise since Krpač et al. (2010) detected staggering 166 species. *Coenonympha pamphilus* and *Polyommatus icarus* are species that are occurring in as many as nine habitat types, whereas *Maniola jurtina*, *Colias croceus* and *Plebejus argus* were found in eight (Fig. 3). The family Nymphalidae (50 species) has highest butterfly diversity, and the family Papilionidae lowest (4 species).

In the Albanian part of Prespa region we recorded 73 species of which 14 were not registered in the Macedonian Prespa Region: *Erynnis marloyi*, *Spialia orbifer*, *Spialia phlomidis*, *Thymelicus acteon*, *Aricia anteros*, *Lycaena phlaeas*, *Polyommatus bellargus*, *Polyommatus thersites*, *Plebejus argyrognomon*, *Satyrium spini*, *Scolitantides orion*, *Apatura iris*, *Coenonympha leander* and *Polygonia egea*. One species, *Thymelicus acteon*, is considered as Near Threatened on the IUCN Butterfly Red List of Species. An interesting find are *Erynnis marloyi* and *Polygonia egea*, which are characteristic species for Southeast Europe. One male individual of *Gonepteryx cleopatra* was recorded in Galichica NP (Fig. 1) in July 2014. In the Greek part of Prespa we recorded 38 species of which only *Lampides boeticus* was exclusively found there.

In the Macedonian Prespa region we identified 87 butterfly species, which is 37 species less than Shaider and Jakšić (1989). Verovnik et al. (2010) have done a butterfly survey which partly coincided with our study area. Within that survey, they recorded 10 butterfly species that were not present in our study (*Aricia anteros*, *Aricia eumedon*, *Boloria euphrosyne*, *Carcharodus flocciferus*, *Colas caucasica*, *Erynnis tages*, *Hamearis lucina*, *Lasiommata petropolitana*, *Polyommatus belargus*, and *Polyommatus thersites*). Taking into account this data, we note a decline of 22% in butterfly species richness in the study area.

It is important to be stated that we recorded the Large Copper, which is a flagship species of wetlands (Boggs, 2008). The Large Copper is also listed under the 1979 Convention on the Conservation of European Wildlife and Natural Habitats and is included in Annexes II and IV of the European Community Habitats Directive requiring strict protection in its own right and designation of Special Areas of Conservation. This species was recorded in the small Stenje wetland (170 ha) and the isthmus between the two lakes in Greece, however, it was absent from the Ezerani wetlands in which its larval host plant *Rumex hydrolapathum* can also be found. This may be as a result of the decline of ground water levels in the Prespa region and the conversion of wetlands into arable land and orchards, which leads to lack of habitats of adequate quality. The Apollo *Parnassius apollo* and the Marsh Fritillary *Euphydryas aurinia*, used for designation of Prime Butterfly Areas (PBA) in Europe, are also present in the area of interest and this is one of the reasons why Galichica NP is one of the eight PBAs in N. Macedonia (Jakšić, 2003).

The highest butterfly diversity was recorded along the Lake Prespa shore (60 species), and clearings in a forest at higher altitudes of the Galichica Mountain (46) (Fig. 2). The lowest butterfly diversity was

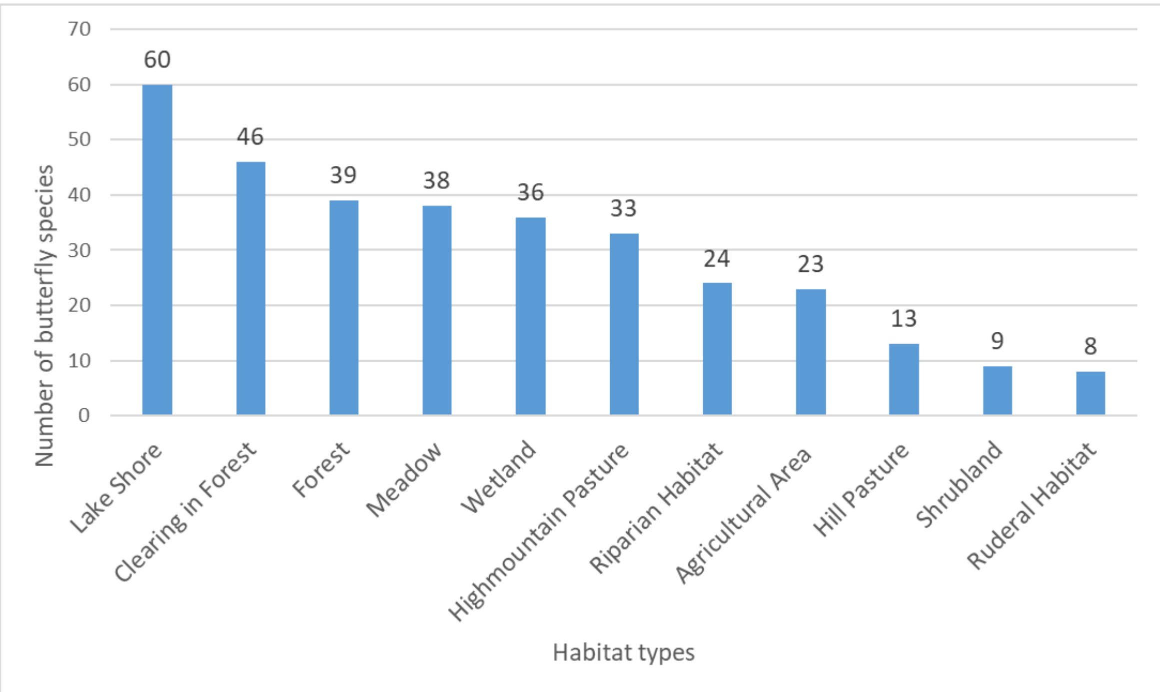

Figure 2. Number of species found in each habitat type in the investigated area

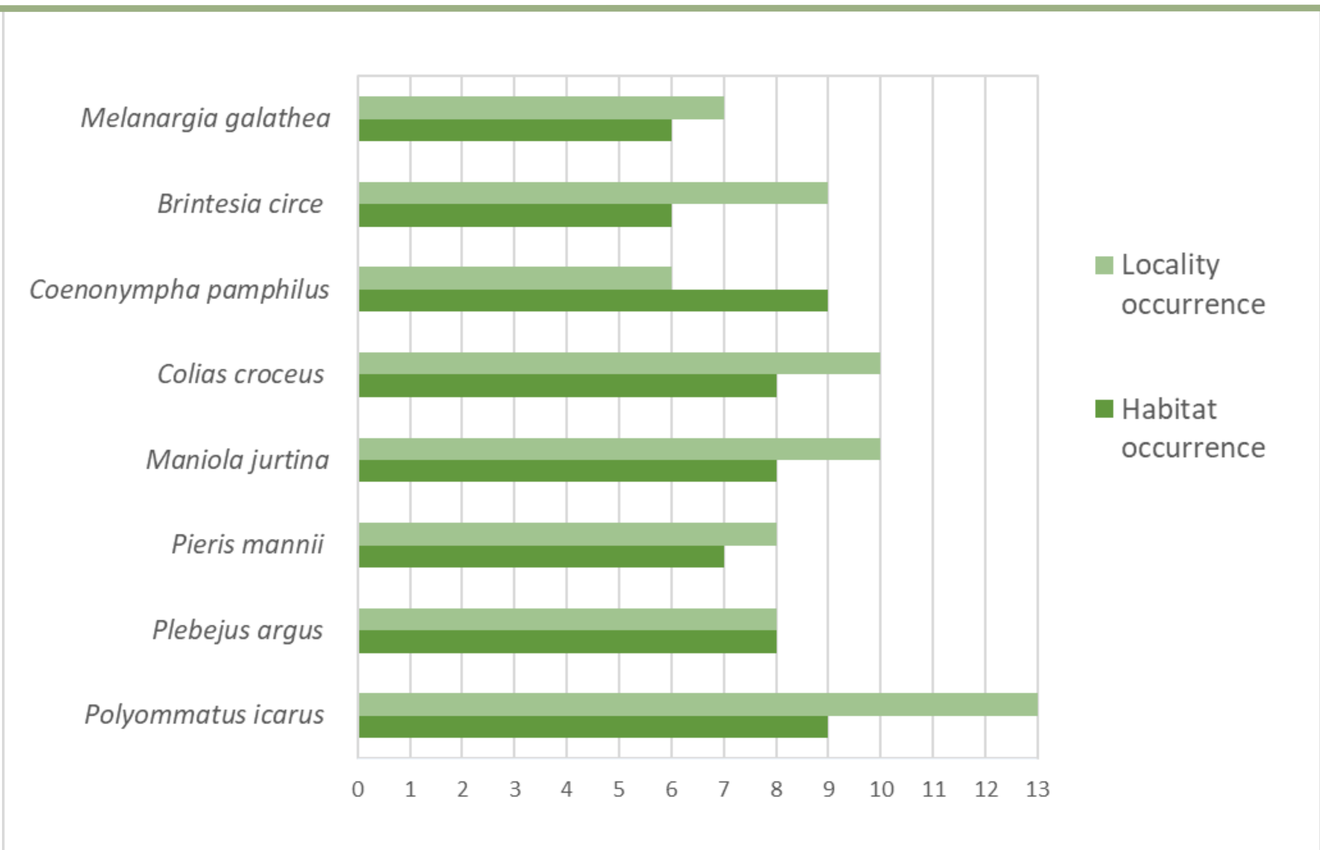

Fig. 3. Number of occurrences per locality and habitat of the most widespread species in the investigated area

documented in ruderal habitats (8 species), shrublands (9) and hill pastures (13), which were also scarcely represented in the region (Fig. 2). Low butterfly diversity was recorded in the agricultural areas (23 species) and riparian habitats (24). In the agricultural areas we noted overuse of pesticides in the orchards, which might be the main reason for the low number of butterfly species. The majority of *Pieris* species (*Pieris napi*, *P.mannii, P.rapae*) were recorded in the agricultural habitats, except for *P. brassicae*. *P. napi* is found to be about half as sensitive as *Pieris brassicae* in their response to insecticides (Davis et al., 1991). Correspondingly, *P. brassicae* could be used as an indicator species for bioassay studies of insecticide spray drift (Davis et al., 1991). This may be explanation why *P. brassicae* in our study is only present in Golem Grad locality and in the higher parts of Galichica Mt, which are further off the agricultural lands.

In our results, the riparian habitats showed low butterfly diversity (24) (Fig. 2), which are intensively used for irrigation purposes in the region. The high-mountain pastures (33 species), wetlands (36), meadows (38), and forests (39) have moderate butterfly diversity (Fig. 2).

The localities Stara and Mala Galichica host almost 80% of the total butterfly diversity (94 species) which we recorded in this study (Fig. 4). The locality Ezerani showed moderate butterfly diversity (48 species), partially as a result of its big size compared to the other localities in our study. According to previous studies (Schaider & Jaksic, 1989), 99 butterfly species were expected to be found in Ezerani, along with occurrence of rare and threatened species. which implies a failure to detect 53% of the butterfly species recorded then. One of these species is the endangered Large Blue (*Phengaris arion*) (Van Swaay et al., 2010). This butterfly has a steep decline trend in Europe (European Environmental Agency, 2013) and because of its complex life cycle, use of fertilizers and pesticides have significant negative impact (Orbicon et al., 2009).

The localities Prechna, v. Stenje and Oteshevo show moderate butterfly diversity, with 21, 26 and 31 butterfly species, respectively (Fig. 4). The localities v. Tuminec (5 species) and Dlaboko, St. Marena (7) showed very low number of species mostly due to the one-day visits to these localities and short period of research. The island of Golem Grad was visited only once, where 2 species were encountered, one of which is *P. brassicae*, a rare species for this study. The remaining localities showed moderate to low butterfly diversity.

The only record of *Gonepteryx cleopatra* for the Macedonian butterfly fauna so far comes from the locality of Gorica, near the town of Ohrid (Krpač &

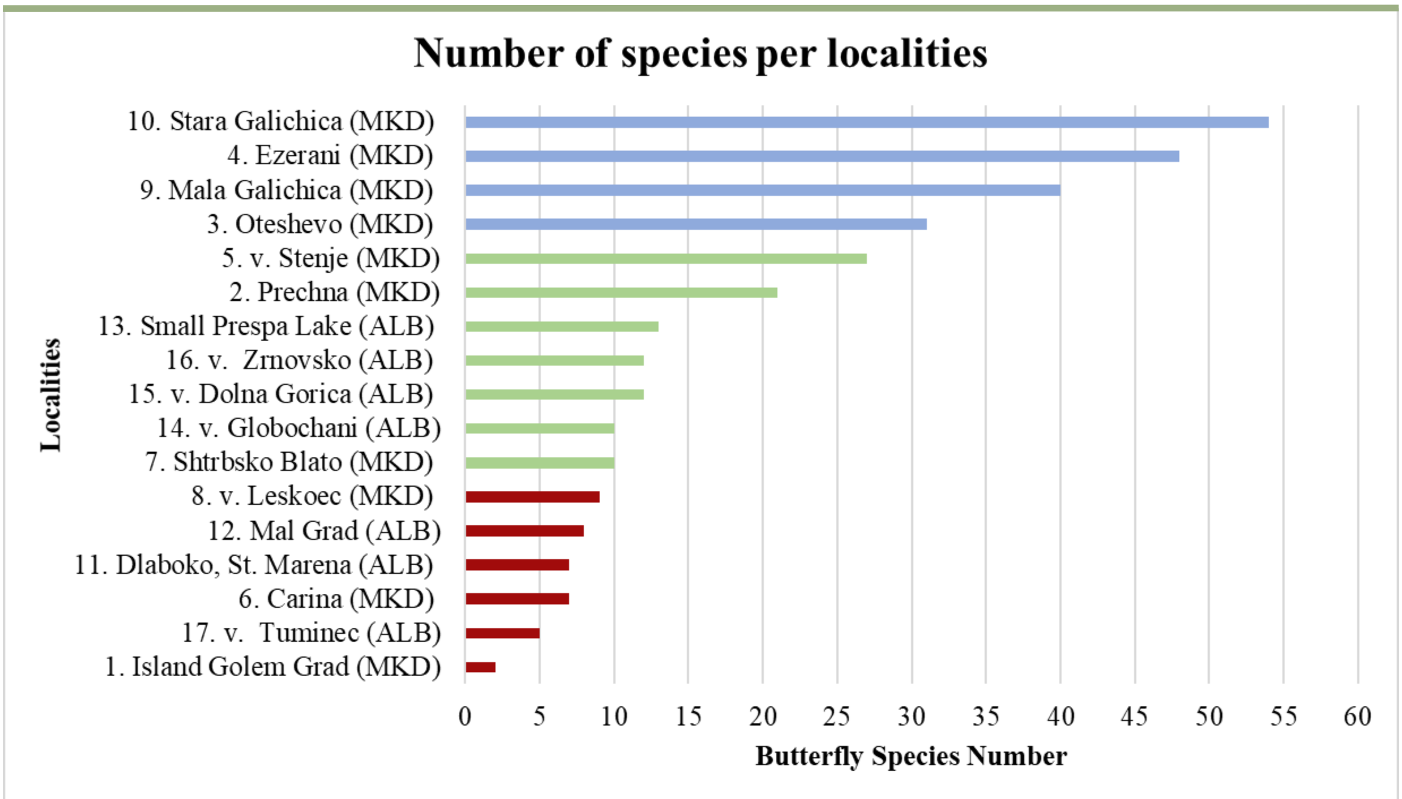

Figure 4. Number of species registered per localities: red colour – localities with less than 10 species; green colour – localities with less than 30 species; blue colour – more than 30 species.

Mihajlova, 1997), some 15 km north-west of the locality Gola Buka. According to the authors, 56 specimens of *Gonepteryx cleopatra* were recorded in April 1983, but not in the following years, which implies that it could be a case of vagrancy (Krpač & Mihajlova, 1997). Our recent discovery, the single observation on Sechena Skala locality, Mt. Belasica on the triangle between N. Macedonia, Greece and Bulgaria in 2002 (Ljupčo Melovski pers. comm., 2012), as well as Dojran, Kozjak and Prisad (Micevski & Micevski, 2014), could mean that this species has already established a viable colony in Macedonia, and it is spreading its distribution towards the Sub-Mediterranean regions of Europe.

Taking into account that the research was done mostly in the summer months (June, July and August), several spring butterflies which are mentioned in Shaider & Jakšić (1989) are likely to have been missed out in our study: *Anthocharis damone, Iolana iolas, Zerynthia polyxena* and *Erynnis marloyi* (recorded only in Albania in our study). The above-mentioned species usually fly in only one generation that starts either March or April and ends beginning of June each year.

Conclusion

We recorded 119 butterfly species in 17 localities and 11 habitat types in the Prespa region (N. Macedonia, Albania and Greece). We note a decline of 22% of the previously recorded species which might be an indicator for poor conditions of the habitat due to intensified agricultural activities. The absence of the Large Copper in the wetland of Ezerani indicates a negative trend of this specialist species. Habitat loss and fragmentation, driven by conversion of wild to arable lands, is one of the main causes for biodiversity depletion in the region. The overuse of pesticides is an additional source of pressure on biodiversity, especially on butterflies, which has long-term and far-reaching negative effects. We stress the need of conservation actions in order to improve the status of butterflies in the Prespa region, such as: warranting legislation protection for butterflies, ensuring sustainable management of key butterfly habitats, creating a national red list of threatened butterfly species, implementing monitoring and indicator schemes, and improving agricultural policies.

Acknowledgments

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Table 1. List of butterfly species found in the Macedonian, Albanian and Greek side of Prespa by habitats. Numbers in the table correspond with the localities where species were found (see chapter Study Area).

Species	Lake Shore	Meadow	Forest	Wetland	Riparian Habitat	Agricultural area	Hill Pasture	High mountain Pasture	Clearing in forest	Ruderal Habitat	Shrubland
Hesperiidae (15)											
***Carcharodus alceae* (Esper 1780)**	4			15	3	4; 5			19	13	
***Erynnis marloyi* (Boisduval 1834)**								10			
***Hesperia comma* (Linnaeus 1758)**								10			
***Ochlodes sylvanus* (Esper 1777)**			2; 20			4					
***Pyrgus alveus* (Hübner 1803)**						4					
***Pyrgus armoricanus* (Oberthür 1910)**	5		19	5; 17		4					
***Pyrgus cinarae* (Rambur 1839)**	3			15	3				19		
Pyrgus *malvae* (Linnaeus 1758)	11			5			9		19		
***Pyrgus serratulae* (Rambur 1839)**		9									
***Pyrgus sidae* (Esper 1784)**				10					10	3	
***Spialia orbifer* (Hübner 1823)**			15								
***Spialia phlomidis* (Herrich-Schäffer 1845)**	14										
***Thymelicus acteon* (Rottemburg 1775)**	14								19		
***Thymelicus lineola* (Ochsenheimer 1808)**	4					4			3; 9		
Thymelicus sylvestris (Poda 1761)	5			15			9		19		
Lycaenidae (35)											
Aricia agestis (Denis & Schiffermüller 1775)	3		10; 19		21		13				
***Aricia anteros* (Freyer 1838)**		10							10		
***Celastrina argiolus* (Linnaeus 1758)**	14				4						
Cupido decolorata (Staudinger 1886)			2		4	4					
Cupido minimus (Fuessly 1775)									3		
Cupido osiris (Meigen 1829)					3						
Cyaniris semiargus (Rottemburg 1775)		10		10					10		

Species	Lake Shore	Meadow	Forest	Wetland	Riparian Habitat	Agricultural area	Hill Pasture	High mountain Pasture	Clearing in forest	Ruderal Habitat	Shrubland
Glaucopsyche alexis (Poda 1761)	**3**										
Hyponephele lycaon (Rottemburg 1775)								10			
Lycaena phlaeas (Linnaeus 1761)	16		15						10	13	
Leptotes pirithous (Linnaeus 1767)	4	4		4; 7	4	4					
Lycaena alciphron (Rottemburg 1775)		4									
Lycaena candens (Herrich-Schäffer 1844)				10				10			
Lycaena dispar (Haworth 1803)				5							
Lycaena thersamon (Esper 1784)	4										
Lycaena tityrus (Poda 1761)	4	4		4; 7		4			10		
Lycaena virgaureae (Linnaeus 1758)			10					9; 10			
Plebejus argus (Linnaeus 1758)	3; 6	4; 9		5	3		9; 13	9	3		16
Polyommatus admetus (Esper 1783)	3			4; 7	3		13	9	10		6
Polyommatus amandus (Schneider 1792)				10				9; 10			
Polyommatus bellargus (Rottemburg 1775)									10		
Polyommatus coridon (Poda 1761)								9			
Polyommatus damon (Denis & Schiffermüller 1775)		9							10		
Polyommatus daphnis (Denis & Schiffermüller 1775)	2; 4; 16		2								10
Polyommatus dorylas (Denis & Schiffermüller 1775)	3	4; 9; 10		10	4; 8	4		9	9		
Polyommatus eros (Ochsenheimer 1808)	5		13								
Polyommatus icarus (Rottemburg 1775)	1; 3; 4; 5; 11	4	15	4; 5; 7; 17	3; 4	4			10	13	16
Polyommatus ripartii (Freyer 1830)	3				3	4		9	10		
Polyommatus thersites (Cantener 1835)	11										
Plebejus argyrognomon (Bergsträsser 1779)			15								
Plebejus sephirus (Frivaldzky 1835)								9			
Satyrium acaciae (Fabricius 1787)								10			
Satyrium ilicis (Esper 1779)	2		15								
Satyrium spini (Denis & Schiffermüller 1775)			15								

Species	Lake Shore	Meadow	Forest	Wetland	Riparian Habitat	Agricultural area	Hill Pasture	High mountain Pasture	Clearing in forest	Ruderal Habitat	Shrubland
Scolitantides orion (Pallas 1771)		10									
Nymphalidae (50)											
Aglais io (Linnaeus 1758)	3			4		4		9	8		
Aglais urticae (Linnaeus 1758)			10		4			10			
Apatura iris (Linnaeus 1758)									10		
Anthocharis cardamines (Linnaeus 1758)					4						
Anthocharis gruneri (Herrich-Schäffer 1851)								9			
Aphantopus hyperantus (Linnaeus 1758)				7					8		
Arethusana arethusa (Denis & Schiffermüller 1775)			4		4						
Argynnis adippe (Denis & Schiffermüller 1775)	5; 14		2	5; 15					10		
Argynnis aglaja (Linnaeus 1758)		9; 10							8		
Argynnis niobe (Linnaeus 1758)									9		
Argynnis pandora (Denis & Schiffermüller 1775)	3; 6								9		
Argynnis paphia (Linnaeus 1758)	2; 3; 14; 16		10		3				4		
Boloria graeca (Staudinger 1870)		10									
Brenthis daphne (Bergsträsser 1780)	5		10	17					8; 9		
Brenthis hecate (Denis & Schiffermüller 1775)											3
Brintesia circe (Fabricius 1775)	3; 4; 5; 6; 16	4; 9	2; 10	5		4			8		
Coenonympha arcania (Linnaeus 1761)		4									
Coenonympha leander (Esper (1784)				10				10			
Coenonympha pamphilus (Linnaeus 1758)	4	4	10	4; 5	3; 4	4			10	13	16
Coenonympha rhodopensis Elwes 1900	6							10			
Erebia medusa (Denis & Schiffermüller 1775)								10	10		
Erebia melas (Herbst 1796)								10	10		
Euphydryas aurinia (Rottemburg 1775)								10			
Hipparchia fagi (Scopoli 1763)			9						9		
Hipparchia statilinus (Hufnagel 1766)	4	4									
Hipparchia syriaca (Staudinger 1871)	2; 3; 5		2; 15						10		
Hipparchia volgensis (Mazochin-Porshnjakov 1952)	2; 3		2; 15								

Species	Lake Shore	Meadow	Forest	Wetland	Riparian Habitat	Agricultural area	Hill Pasture	High mountain Pasture	Clearing in forest	Ruderal Habitat	Shrub-land
Issoria lathonia (Linnaeus 1758)		9			3						
Kirinia roxelana (Cramer 1777)	6		2; 4		4						
Lasiommata megera (Linnaeus 1767)	11; 12	4		10			13	10	10		
Limenitis reducta (Staudinger 1901)			2; 4					9			
Maniola jurtina (Linnaeus 1758)	3; 4; 5; 12	4; 9	2	4; 7	4	4			8; 9		
Melanargia galathea (Linnaeus 1758)	3; 4; 5	4	2; 10	4; 7		4; 5					
Melanargia larissa (Geyer 1828)	5		2	4					9		
Melanargia russiae (Esper 1783)		9; 10					9				
Melitaea arduinna (Esper 1783)							9		9		
Melitaea athalia (Rottemburg 1775)		4; 10					9; 10		9		
Melitaea didyma (Esper 1778)	3; 4	4; 10		5; 7	3	4	9		9		
Melitaea phoebe (Denis & Schiffermüller 1775)	5	4	4				9				
Melitaea trivia (Denis & Schiffermüller 1775)	5	4	9	5				9			
Nymphalis antiopa (Linnaeus 1758)					4						
Nymphalis polychloros (Linnaeus 1758)		4									
Pararge aegeria (Linnaeus 1758)	4		4; 9								
Polygonia *c-album* (Linnaeus 1758)			2								
Polygonia egea (Cramer 1775)			10								
Pseudochazara geyeri (Herrich-Schäffer 1846)								9; 10			
Pyronia tithonus (Linnaeus 1767)		4									
Satyrus ferula (Fabricius 1793)	11							9	10		
Vanessa atalanta (Linnaeus 1758)	3; 12; 14	10						10			
Vanessa cardui (Linnaeus 1758)	3; 4	4	2; 10	5		4		10			
Papilionidae (4)											
Iphiclides podalirius (Linnaeus 1758)	3	16									
Papilio machaon (Linnaeus 1758)	4	11; 12						10			
Parnassius apollo (Linnaeus 1758)								9	10		
Parnassius mnemosyne (Linnaeus 1758)								9			
Pieridae (12)											
Aporia crataegi (Linnaeus 1758)	3; 5; 14	10		5		4		10	8; 9		
Colias alfacariensis Ribbe 1905	16	5; 10		10					10		
Colias croceus (Fourcroy 1785)	3; 4; 5	4; 10	2	4; 5; 7	3; 4	4			10	13	
Gonepteryx *cleopatra* (Linnaeus 1767)									10		

Species	Lake Shore	Meadow	Forest	Wetland	Riparian Habitat	Agricul-tural area	Hill Pas-ture	High mountain Pasture	Clearing in forest	Ruderal Habitat	Shrub- land
Gonepteryx rhamni (Linnaeus 1758)		10	2					10	8		
Leptidea duponcheli (Staudinger 1871)			2				13		2		
Leptidea sinapis (Linnaeus 1758)	3; 4; 5; 14		4; 9	7					9		
Pieris balcana (Lorkovic 1970)	12; 16		2						10		
Pieris brassicae (Linnaeus 1758)	1; 12		1							14	
Pieris napi (Linnaeus 1758)	12				4	4					16
Pieris mannii (Mayer 1851)	2; 4; 5; 11; 12	4		4; 17	4	4			9	13	
Pieris rapae (Linnaeus 1758)	14					4					
Pontia edusa (Fabricius 1777)	3; 4; 5	4		17			13				6

Park on the basis of selected invertebrate groups". We want to express gratitude to Dragan Arsovski and Makedonka Makarovska and acknowledge their contribution in all aspects of the project “Diversity of butterflies in the Nature Reserve Ezerani” realization. Special thanks to Vasko Avukatov, who created the map of our study area.

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РЕЗИМЕ

Од крајот на јуни до почетокот на септември во 2010, 2013 и 2016 година беа спроведени истражувања за разновидноста и распространувањето на дневните пеперутки во регионот Преспа (македонска, албанска и грчка територија) и на преспанската страна на планината Галичица. Главната цел на ова истражување беше да се одреди вистинската состојба на дневните пеперутки во регионот на Преспа. Беа регистрирани 119 видови на дневни пеперутки на 17 локалитети и 11 различни типови на станишта, од езерски емерзни влажни станишта, преку топлољубиви шуми до овоштарници и други обработливи површини. На планината Галичица во локалитетот Гола Бука беше регистриран видот *Gonepteryx cleopatra*, за првпат по 1983 година (Krpač & Mihajlova, 1997). Во албанскиот дел на преспанскиот регион регистриравме 14 видови на дневни пеперутки кои не беа претходно регистрирани во нашето истражување (*Erynnis marloyi*, *Spialia orbifer*, *Spialia phlomidis*, *Thymelicus acteon*, *Aricia anteros*, *Lycaena phlaeas*, *Polyommatus bellargus*, *Polyommatus thersites*, *Plebejus argyrognomon*, *Satyrium spini*, *Scolitantides orion*, *Apatura iris*, *Coenonympha leander* и *Polygonia egea*). Еден вид, *Thymelicus acteon*, е близу загрозен (NT) според Црвената листа на дневни пеперутки на Меѓународната унија за заштита на природата. Интересен наод се пеперутките *Erynnis marloyi* и *Polygonia egea*, кои се карактеристични видови за Југоисточна Европа. Големиот бакарец *Lycaena dispar* беше забележан на два локалитети: Стењско Блато на македонска страна и истмусот помеѓу двете езера на грчка страна.

Споредбената анализа на нашата листа на дневни пеперутки со таа на Shaider и Jakšić (1989) покажа пад за 22% во бројноста на видови на дневни пеперутки во македонскиот дел на Паркот Преспа. Во главниот локалитет на ова истражување, Езерани, увидовме опаѓање од 53% на бројноста на видови на дневни пеперутки. Ова е силен показател за потребата од подобра заштита на биолошката разновидност и одржливо користење на ресурсите и земјиштата во преспанскиот регион. Една од главните причини за намалувањето на разновидноста на дневните пеперутки е загубата на погодни хабитати и/или нивна фрагментација проследена со пренамена на природните површини во обработливи. Дополнителен притисок врз биолошката разновидност е прекумерната употреба на пестициди, која има долгорочен и далекосежен негативен ефект. Потребно е да се воспостават следните мерки за заштита на статусот на пеперутките во Преспа: воведување законска заштита на пеперутките во Македонија, воспоставување одржливо користење и управување на клучните живеалишта на пеперутките, изработка на национална црвена листа на загрозени пеперутки, спроведување програми за мониторинг со воспоставување показатели, како и подобрување на земјоделските закони.

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Contribution to the knowledge on the genus *Quedius* STEPHENS, 1829 (Coleoptera: Staphylinidae: Staphylinini: Quediina) on the Macedonian side of Shar Mountain

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Entomology section

Abstract. The aim of this study is to expand the knowledge of the *Quedius* fauna (Coleoptera: Staphylinidae: Staphylinini: Quediina) on the Macedonian side of Shar Planina. A total of 70 specimens belonging to 12 species are reported. Ten species are recorded from Shar Planina for the first time. Three species, *Q. boops* (GRAVENHORST, 1802), *Q. persimilis* Mulsant & Rey, 1876 and *Q. fuliginosus* Gravenhorst, 1802 are reported from Macedonia for the first time. Omitted data in the latest edition of Catalogue of Palaearctic (Smetana & Schülke, 2015) are noted. A list of all *Quedius* species recorded from Macedonia and Shar Planina is given. The intraspecific variation of *Q. nemoralis* Baudi, 1848 and *Q. illyricus* Wendeler, 1928 is discussed.

Key words: Coleoptera, Staphylinidae, Staphylininae, Quediina, *Quedius*, new data, Shar Planina, Macedonia.

Introduction

With more than 700 species (Herman, 2001; Smetana & Schülke, 2015) the mainly Holarctic genus *Quedius* Stephens, 1829 is one of the largest among rove beetles (family Staphylinidae) (Salnitska & Solodovnikov, 2019). The majority of *Quedius* species are inhabitants of the forest leaf and log litter, but they can also be found in other ground-based debris of open landscapes. Some species occur in mammal and bird burrows and nests, in the nests of ants and wasps, or they are highly adapted to hypogean microhabitats (Salnitska & Solodovnikov, 2019). The genus *Quedius* and Staphylinidae in general are poorly studied in Macedonia. The *Quedius* fauna in Macedonia included 23 species (Table 1), according to Smetana & Schülke (2015) and Assing (2016, 2019). The first data on the genus *Quedius* of Shar Planina were published by Korge (1964), who reported four species: *Q. paganettii* (Bernhauer, 1936) (now a junior synonym of *Q. illyricus* Wendeler, 1928, *Q. incensus* (Smetana, 1959), *Q. paradisianus* (Heer, 1839), *and Q. collaris italicus* (Gridelli, 1925) (not included in the Catalogue of Palaearctic Coleoptera (Smetana & Schülke, 2015.)) Assing (2016) reported *Q. rodopianus* Coiffait, 1971 for the first time from Kobilica (Shar Planina) while revising material collected by Korge. Subsequently, Štourač (1998) described *Q. pavelmoraveci* from the Pelister mountain range; in the same paper he states the presence of this species on Shar Planina. Thus, six species were previously known from Shar Planina.

Research area

Shar Planina is a mountain situated in the north-westernmost part of the Republic of Macedonia. It is one of the highest (Titov Vrv peak at 2748 m) and largest mountains by area in the Balkans. It has been widely recognized that the mountain of Shar Planina possesses exceptionally rich biodiversity. The specific biological diversity has great scientific and conservation importance. The high richness and diversity of Shar Mountain ecosystems can be compared to the largest and highest mountain ranges on the Balkan Peninsula (Prokletije, Rila, and

Pirin). (Melovski et al., 2010). In this paper the research was mainly focused on exploring the beech forests of Shar Planina in the vicinity of Ljuboten and Leshnica, as well as in the area of the village Gorno Jelovce. Spruce forests in the area of Popova Shapka were also covered. As much as the field conditions allowed, opportunistically, some material was collected from oak forests in the area of Gorno Jelovce and sweet chestnut forests surrounding Staro Selo.

Materials and methods

All specimens treated in this study were collected unsystematically by the author with the help of several colleagues in the period of 2016-2019 using different techniques such as hand collecting, sifting leaf litter, and sifting moss. All specimens were dissected and mounted. The morphological studies were conducted using a Nikon SMZ 10 stereo microscope and a Carl Zeiss Stemi 305 stereomicroscope. Female specimens were identified only when collected together with the males. Distribution and currently accepted taxonomic data follow Smetana & Schülke (2015), as well as Assing (2016, 2018, 2019). All the specimens are deposited in the author's private collection.

Results and discussion

Quedius (Distichalius) *cinctus* (Paykull, 1790)

Material examined: 2♂, 2♀, Gorno Jelovce, Oko, 1290 m. a. s. l., beech forest, sifted from leaf litter, 04.06.2017, 20.826276, 41.799213, leg: A. Anovski and S. Hristovski, Det. A. Anovski. 1♂, Ljuboten, 1415m, beech forest, sifted from leaf litter, 17.07.2016, 21.135719, 42.16773, Leg: A. Anovski, S. Hristovski, M. Komnenov, S. Nakev, det: A. Anovski.

Comment: Common and widespread Palearctic species found in leaf litter. This is a new record for Shar Planina mt.

*Quedius (*Quedius*) fuliginosus* (Gravenhorst, 1802)

Material examined: 1♂, Ljuboten, Rogachevo, Gradishta, 1250 m a.s.l., beech forest, collected by hand, 04.07.2017, 21.142609, 42.163753, leg: A. Anovski and S. Hristovski, det: A. Anovski & S. Hristovski.

Comment: Common and widespread Palaearctic species. This species is reported from Shar Planina and Macedonia for the first time.

Quedius (Raphirus) *ochropterus ochropterus* Erichson, 1840

Material examined: 1♂, Popova Shapka, 1800 m a.s.l., spruce forest, sifted from moss, 18.10.2019, 20.87415, 42.011694, leg: A. Anovski, det: A. Anovski. 1♂, Jelak, Studena Reka, 1750 m a.s.l, riparian, collected by hand, 10.07.2017, 20.857659, 42.030992, leg et det: A. Anovski.

Comment: The distribution of *Q. ochropterus ochropterus* ranges from the United Kingdom and Central Europe to Southeast Europe, including Turkey. This species is reported from Shar Planina for the first time.

Quedius (Raphirus) *nemoralis* Baudi, 1848

Material examined: 3♂ 1♀, Ljuboten, Staro Selo, 960 m a.s.l., sweet chestnut forest, sifted in leaf litter, 19.07.2016, 21.1270425, 42.1545601, leg: A. Anovski, S. Hristovski, M. Komnenov, S. Nakev, Det: A. Anovski; 6♂ 2♀, Ljuboten, 1415 m.a.sl., beech forest, sifted in leaf litter, 17.07.2016, 21.135719, 42.16773, leg: A. Anovski, S. Hristovski, M. Komnenov, S. Nakev. 1♂ Ljuboten, 1351 m a.s.l, beech forest, Sifted in leaf litter, 17.07.2016, 21.135719, 42.16773, leg: A. Anovski, S. Hristovski, M. Komnenov, S. Nakev, det: A. Anovski. 1♂ 1♀, Jelovce, 1200 m a.s.l., oak forest, sifted in leaf litter, 02.06.2017, 20.824648, 41.798255, leg: A. Anovski, S. Hristovski, M. Komnenov, det: A. Anovski.

Comment: *Quedius nemoralis* is reported from Shar Planina for the first time. Examination of the specimen series from Staro Selo (960 m a.s.l, sweet chestnut forest), Ljuboten (1351 m a.s.l, 1415 m a.s.l, beech forest) and Jelovce (1200 m a.s.l, beech forest) revealed the intraspecific variation both within populations and within different populations, exactly as described by Assing (2018). Specimen series from Staro Selo have a lighter body color and a slightly more expressed ventral tooth of the median lobe (in the range of intraspecific variation) in comparison to those from Ljuboten and Jelovce. Very common and widespread species, with a distribution extending across the territory of Europe and only a small part of Asia (Turkey, Cyprus and India).

Quedius (Raphirus) *paradisianus* (Heer, 1839)=

Material examined: 1♂ 4♀, Popova Shapka, 1800 m a.s.l., spruce forest, sifted in moss, 18.10.2019, 20.87415, 42.011694, leg et det: A. Anovski.

Comment: *Quedius paradisianus* is distributed in the region of the Alps, Apennines, the mountain ranges of southern and south-eastern Central Europe, the Carpathians, and the Balkans southwards to North Greece (Assing, 2016). This species was previously reported from Shar Planina by Korge (1964).

Quedius (Raphirus) *cohaesus* Eppelsheim, 1888

Material examined: 1♂, Ljuboten, 1415 m a.s.l, Shar Planina, Beech forest, Sifted in leaf litter, 17.07.2016, 21.135719, 42.16773, Leg: A. Anovski, S. Hristovski, M. Komnenov, S. Nakev, Det: A. Anovski. 1♂, 4 ♀ Leshnica, Kadite, 1450 m a.s.l., beech forest, sifted in leaf litter, 10.08.2019, 20.777559, 42.015343, leg et det: A. Anovski**.** 1⍰ Rudoka, 2270 m a.s.l, along a snow patch, 28.07.2019, 41.923766 20.781328.

Comment: After checking the distribution data in the Catalogue of Palaearctic Coleoptera (Smetana & Schülke, 2015), I noted that there are no data about the presence of *Q. cohaesus* in Macedonia. This indicated that the series examined in this paper represent the first data for Macedonia, although according to the distribution of this species, provided in some published articles, it is obvious that this species occupies the territory of Macedonia. However, Solodovnikov (2012) placed *Q. noricus*, BERNHAUER, 1927 in synonymy with *Q. pseudonigriceps* REITTER, 1909. In the same study, Solodovnikov examined material of *Q. pseudonigriceps* from Macedonia (Pelister mt. and Galichica mt.), collected by Rambousek. These data were omitted in the latest edition of Catalogue of Palaearctic Coleoptera (Smetana & Schülke, 2015). Later on, Assing (2019) with clear argumentation designated *Q. pseudonigriceps* as a junior synonym of *Q. cohaesus*. Therefore, the presence of this species in Macedonia is already known and data on its distribution in the Catalogue of Palaearctic Coleoptera (Smetana & Schülke, 2015) are omitted. The distribution of *Q. cohaesus* ranges from Austria, southeast Europe, and Turkey to the Caucasus region and West Asia eastwards to Middle Asia [Kyrgyzstan, Kazakhstan] (Schülke & Smetana 2015; Salnitska & Solodovnikov, 2018;). The material listed above represents the first records from Shar Planina.

Quedius (Raphirus) *lateralis* (Gravenhorst, 1802)=

Material examined: 1♀, Jelovce, 1290 m a.s.l., oak forest, sifted in leaf litter, 02.06.2017, 20.824648, 41.798255, leg: A. Anovski, S. Hristovski, M. Komnenov, det: A. Anovski. 1♂ 2♀, Ljuboten, Rogachevo, Gradishta, 1250 m a.s.l., beech forest, collected by hand, 04.07.2017, 21.142609, 42.163753, leg: A. Anovski, S. Hristovski, det: S. Hristovski.

Comment: Very common species on the Balkan Peninsula and Central Europe in general. *Quedius lateralis* represents a new record from Shar Planina.

Quedius (Raphirus) *humeralis* Stephens, 1832

Material examined: 7♂ 4♀, Ljuboten, 1600 m a.s.l., beech forest, sifted in leaf litter, 19.07.2016, 21.131207, 42.186938, leg: A. Anovski, S. Hristovski, M. Komnenov, S. Nakev, det: A. Anovski. 3♂ 3♀, Ljuboten, 1415 m a.s.l., beech forest, sifted in leaf litter, 17.07.2016, 21.135719, 42.16773, leg: A. Anovski, S. Hristovski, M. Komnenov, S. Nakev, det: A. Anovski. 1♂ 3♀, Ljuboten, Rogachevo, Gradishta, 1250 m a.s.l., beech forest, collected by hand, 04.07.2017, 21.142609, 42.163753, leg: A. Anovski, S. Hristovski, det: A. Anovski.

Comment: Widespread throughout Europe, its distribution extends from Great Britain, Denmark, and Estonia in the north, to Greece, Cyprus, and Turkey in the south. This species is recorded from Shar Planina for the first time.

Quedius (Raphirus) *illyricus* Wendeler, 1928

Material examined: 3♂ 5♀, Leshnica, Kadite, 1450 m a.s.l., beech forest, sifted in leaf litter, 10.08.2019, 20.777559, 42.015343, leg et det: A. Anovski. 1♂, Dolna Leshnica, 1420 m a.s.l, beech and spruce forest, sifted in leaf litter, 10.08.2019, 20.785535, 42.024293, leg: A. Anovski, det: A. Anovski. 1♂, Ljuboten, 1600 m a.s.l, beech forest, sifted in leaf litter, 19.07.2016, 21.131207, 42.186938, leg: A. Anovski, S. Hristovski, M. Komnenov, S. Nakev, det: A. Anovski.

Comment: *Quedius illyricus* represents an endemic species on the Balkan Peninsula. It has been reported from Albania, Bulgaria, Macedonia, Bosnia & Herzegovina, Croatia, Greece and Serbia by Smetana & Schülke (2015). Korge (1964) reported *Q. paganettii* (now a junior synonym of *Q. illyricus*) for the first time on Shar Planina. The coloration of the head, pronotum and elytra between the populations are ranging from brown, dark brown to pitch black. Among specimens collected from Leshnica,

Table 1. List of all *Quedius* species reported from Macedonia and Shar Planina (Korge (1964), Smetana & Schulke (2015), Assing (2016, 2018, 2019) + present study) *- names in bold represent new records for Macedonia

Species	Presence on Shar Planina	Comment
1. *Quedius cinctus* (Paykull, 1790)	+	New record for Shar Planina
2. *Quedius abietum* Kiesenwetter, 1858b	-	
3. *Quedius bernhaueri* Rambousek, 1915	-	
4. *Quedius mesomelinus mesomelinus* (Marsham, 1802)	-	
5. *Quedius ochripennis* (Ménétriés, 1832)	-	
6. *Quedius levicollis* (Brullé, 1832)=	-	
7. *Quedius collaris* Erichson, 1840	-	
8. *Quedius italicus* Gridelli, 1925	-	
9. *Quedius humeralis* Stephens, 1832	+	New record for Shar Planina
10. *Quedius incensus* Smetana, 1959	+	
11. *Quedius lateralis* (Gravenhorst, 1802)	+	New record for Shar Planina
12. *Quedius nemoralis nemoralis* Baudi di Selve, 1848	+	New record for Shar Planina
13. *Quedius nitipennis* (Stephens, 1833)=	-	
14. *Quedius ochropterus ochropterus* Erichson, 1840	+	New record for Shar Planina
15. Quedius obscuripennis obscuripennis Bernhauer, 1901	-	
16. *Quedius illyricus* Wendeler, 1928	+	
17. *Quedius paradisianus* (Heer, 1839)	+	
18. *Quedius rodopianus* Coiffait, 1971	+	
19. *Quedius pavelmoraveci* Štourač, 1998	+	
20. *Quedius picipes* (Mannerheim, 1830)	+	New record for Shar Planina
21. *Quedius umbrinus* Erichson, 1839b		
22. *Quedius semiruber* Fauvel, 1875b		
23. *Quedius fuliginosus* (Gravenhorst, 1802)	+	New record for Shar Planina
24. *Quedius cohaesus* Eppelsheim, 1888	+	New record for Shar Planina
25. *Quedius persimilis* Mulsant & Rey, 1876	+	New record for Shar Planina
26. *Quedius boops* (Gravenhorst, 1802)	+	New record for Shar Planina

there is a clear variation in the color of the elytra. The margin of the elytra is yellow and is more expressed in the female specimens. There is no notable variation (difference) in the aedeagi.

Quedius (Raphirus) *persimilis* Mulsant & Rey, 1876

Material examined: 1♂ 1♀, Ljuboten, 1600 m a.s.l, beech forest, sifted in leaf litter, 19.07.2016 21.131207, 42.186938, leg: A. Anovski, S. Hristovski, M. Komnenov, S. Nakev, det: A. Anovski.

Comment: This species is reported from Macedonia and Shar Planina for the first time. The distribution of *Q. persimilis* extends from West Europe (Portugal), Scandinavia, and Central Europe to Southeast Europe.

Quedius (Raphirus) *boops* (Gravenhorst, 1802)=

Material examined: 2♂, Ljuboten, 1600 m a.s.l., beech forest, sifted in leaf litter, 19.07.2016, 21.131207, 42.186938, leg: A. Anovski, S. Hristovski, M. Komnenov, S. Nakev, det: A. Anovski.

Comment: Very common and widespread species throughout the whole Palaearctic region. This species is reported from Macedonia and Shar Planina for the first time.

Quedius (Raphirus) *picipes* (Mannerheim, 1830)

Material examined: 2♂, Ljuboten, 1415 m a.s.l, beech forest, sifted from leaf litter, 17.07.2016, 21.135719, 42.16773, leg: A. Anovski, S. Hristovski, M. Komnenov, S. Nakev, det: A. Anovski.

Comment: Very common and widespread Palaearctic species. Reported from Shar Planina for the first time.

Conclusion

In total, 12 species of *Quedius* are reported from Shar Planina Mt. All of them are new records for this mountain, except for *Q. illyricus* and *Q. paradisianus*. Three species, *Q. fuliginosus*, *Q. boops*, and *Q. persimilis* are reported from Macedonia for the first time, despite their commonness and widespread distribution. A total of 26 species of the genus *Quedius* are present in Macedonia, 16 of them are present on Shar Planina. From this study it can be concluded that: **a)** the knowledge of the genus *Quedius* from Shar Planina and Macedonia in general is poor and further research will undoubtedly reveal more new species both for Macedonia and for Shar Planina mountain; **b)** there are at least 2 and 3 different populations of *Q. illyricus and Q. nemoralis on* Shar Planina, respectively; **c)** there is some omitted data in the Catalogue of Palaearctic Coleoptera, and **d)** this study will represent a basis for further related research on the genus *Quedius* on Shar Planina and in Macedonia.

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In memoriam

Професорот Љупчо Меловски беше основоположник и еден од најголемите поддржувачи на Истражувачкото друштво на студенти биолози, а воедно и член на првото претседателство. Уште од самите почетоци, безрезевно беше вложен во охрабрување и помагање на работата на Друштвото. Изминатата година, а и сите следни, нема повеќе да бидат исти. Во сите нас остана голема празнина, чувство на неправда и неверување.

Без ниедна пропуштена истражувачка акција, годишно Собрание, терен или дружење, низ годините неуморно и несебично продолжи да го пренесува не само неговото знаење и искуство, туку и неисцрпниот ентузијазам и љубовта за природата, кон членовите на ИДСБ. Покрај менторството на флористичката секција, студентите од него учеа и како се пишуваат научни трудови кои во најголем дел се објавуваа во Билтенот на Друштвото.

Како најстар член, не заборавaше да истакне колку е важна тимската работа во Друштвото и неизбежното теренско дружење. Децениски собираната збирка со растенија од неговата флористичка секција од терените на ИДСБ, за секогаш ќе остане национално богатство.

Петтото издрание на Билтенот на Истражувачкото друштво на студенти биолози го посветуваме на нашиот водач и најголем поддржувач. За членовите ќе остане запаметен како најголема инспирација, но и мотивација за продолжување на неговото дело.

Ви благодариме за целиот ваш несебичен придонес за биологијата во нашата држава, за ИДСБ, за целото знаење, терени, дружби, совети и моменти кои ги споделивте со нас!

Вечна Ви слава Професоре!

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