Contents

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Research article

First record of bat flea *Thaumapsylla breviceps* Rothschild, 1907 (Siphonaptera: Ischnopsyllidae) on Bicol Region, Luzon Island, Philippines

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Abstract: *Thaumapsylla* Rothschild is a small genus of Old World ischnopsyllid fleas parasitic only on the family Pteropodidae (fruit bats). Despite its widespread record in the Philippines, it has never been reported in the Bicol Region, southern Luzon. In this account, we report the first record of *Thaumapsylla breviceps* Rothschild in the Bicol Region. It was collected from *Eonycteris spelaea* (Dobson), a nectarivorous fruit bat native to Southeast Asia. Also, this paper represents the first record of the genus *Thaumapsylla* on Bicol Region, Luzon Island.

Keywords: Albay, Chiroptera, ectoparasite, flea

Introduction

Bicol Region is the southernmost region of Luzon Island and well known for its high volcanic activity (Andal et al., 2005). This region is composed of four provinces situated within the Bicol Peninsula (Camarines Norte, Camarines Sur, Albay, and Sorsogon) and two island provinces (Catanduanes and Masbate). The mountainous landscapes and remaining forest cover of Bicol Region is home to many Philippine native vertebrates (Goodman & Gonzales, 1990; Heaney et al., 2016; Binaday et al., 2017) and invertebrate fauna (Zolotuhin et al., 1997; Hong & James, 2009; Amarga & Mercado, 2022). In addition, this region is also home to several Bicol endemics, many of which are forest-dwelling species (Balete et al., 2015; Siler et al., 2017; Bollino et al., 2019).

Published records of bat ectoparasites in the Bicol Region are relatively limited. Cuy (1980) reported *Eucampsipoda inermis* Theodor, 1955 from Catanduanes Island. Subsequently, Amarga & Yap (2017) reported the rare bat bug *Eoctenes spasmae* (Waterhouse, 1879) from specimens collected in Batan Island. In this paper, we present the first record of the bat flea *Thaumapsylla breviceps* Rothschild, 1907 in Bicol Region, Luzon Island, as well as the first documentation of the genus *Thaumapsylla* Rothschild in the area.

Material and methods

During a rapid biodiversity survey conducted on Batan Island, Albay (Fig. 1) in December 2015, *Thaumapsylla breviceps* specimens were obtained from an unnamed cave near the island's coastline. Flea specimens were collected from *Eonycteris spelaea* (Dobson, 1871) using fine-tipped forceps and were preserved in 95% ethanol prior to identification and mounting. Identification of flea samples follow from the key of Amarga & Hastriter (2022), and host species were identified using Ingle & Heaney (1992).

Fig. 1. Location of Batan Island in southern Luzon, Philippines (inset, black box).

Result and discussion

Class Insecta Linnaeus, 1758
Order Siphonaptera Latreille, 1825
Infraorder Ceratophyllomorpha in Medvedev, 1998
Superfamily Ceratophylloidea Dampf, 1908
Family Ischnopsyllidae Wahlgren, 1907
Subfamily Thaumapsyllinae Jordan, 1947
Genus *Thaumapsylla* Rothschild, 1907
Thaumapsylla breviceps Rothschild, 1907

Thaumapsylla breviceps Rothschild, 1907: 329. Type locality: South Africa (Cape Colony). Type host: *Rousettus collaris* [now *R. aegyptiacus* (Geoffroy, 1810)].

Material examined: Philippines: Luzon: on *Eonycteris spelaea*: 2 ♀♀, 2 ♂♂, Albay Province, Ba-

tan Island, Barangay Lagundi, XII.2015, coll. A.K. Amarga.

Thaumapsylla breviceps is one of the four recorded species bat fleas (Ischnopsyllidae) occurring in the Philippines (Amarga & Hastriter, 2022). This is a widely distributed species occurring in Afrotropical, Australasian, and Oriental faunal region (Hastriter & Bush, 2013). It has been reported in different areas including the Afrotropics, India, mainland Southeast Asia, Philippines, and Indonesia (Beaucournu & Kock, 1994; Amarga & Hastriter, 2022).

In terms of host associations, *T. breviceps* is primarily parasitic on pteropodid bats and in the Philippines, it has been reported on *Eonycteris spelaea* (Dobson) (cave nectar bat), *E. robusta* Miller, 1913 (Philippine dawn bat), and *Rousettus amplexicaudatus* Geoffroy, 1810 (Geoffroy's rousette) (Beaucournu &

Fig. 2. *Thaumapsylla breviceps* (female) collected from *Eonycteris spelaea* on Batan Island.

Kock, 1994; Hastriter & Bush, 2013; Amarga et al., 2017; Amarga & Hastriter, 2022). Host species such as *E. spelaea* and *R. amplexicaudatus* have been known to co-exist in caves and roost in large numbers (Heaney et al., 2010). Furthermore, due to its strict host associations, presence of *T. breviceps* on microbats can be regarded as a facultative occurrence and/or accidental host record.

Acknowledgments

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Research article

Faunal remains from the Early Iron Age rock-cut complex Gluhite Kamani (Eastern Rhodopes, Bulgaria)

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Abstract: The faunal remains recovered from the Gluhite Kamani site offer the unique opportunity to explore the consumption patterns of the Early Iron Age communities inhabiting the Rhodope Mountains. While a lot high-altitude and rock-cut sanctuaries have been registered within the Rhodope Mountains, this is the only site yielding a larger assemblage (n = 3160) from a stratigraphic sequence dating to the Late Bronze Age – Early Iron Age transition and the Early Iron Age. Domestic animals dominate, with sheep/goat being the main exploited species through all stratigraphic layers. The results show that the percentage of represented domestic animals is relatively continuous, while the number of wild animals seems to vary throughout the different periods., As the site is regarded as a sanctuary during the Iron age, the extent to which we can identify any ritual activity from the osteological assemblage is commented on in the discussion regarding the cull patterns observed, and taphonomy.

Keywords: archaeology, Early Iron Age, faunal analysis, Mediterranean, taphonomy, zooarchaeology

The site

Gluhite Kamani is an archaeological site located on the north-eastern ridge of the Rhodope Mountains chain. The site is situated on the south-eastern slope of the Sveta Marina Peak. Archaeological excavations – which started in 2008 – have shown that the site was occupied from the final phase of the Late Chalcolithic (First half of the 4th mil. BCE) to the medieval period, with the densest stratigraphic layers belonging to the Early Iron Age.

Current understanding of the site's range, plan, stratigraphic sequence and chronology has been garnered through fifteen years of on-going archaeological research, which yielded marvellous results. Through this it can be stated that the site functioned as a cult complex with a rich history throughout the centuries. The centre of cult activity seems to be the most elevated point, on which the ruins of a medieval church can be seen to this very day. It is most likely that an earlier pagan sanctuary functioned on the same spot, which would explain the accumulation of dense debris layers of the central, southern, and northern sectors, which contain the remains of sacrificial feasts and offering. The sanctuary continued to function during the Roman period, proof of which is the fragment of a marble votive plaque, several coins and pottery sherds. However, the deity which was worshiped is still unknown. Sometime during the 4th – 5th century a Christian church was constructed on top of the ancient ruins, which shows the sought continuity. The church, a basilica, continued its existence into the medieval period, with some small reconstructions. During this period a second, larger
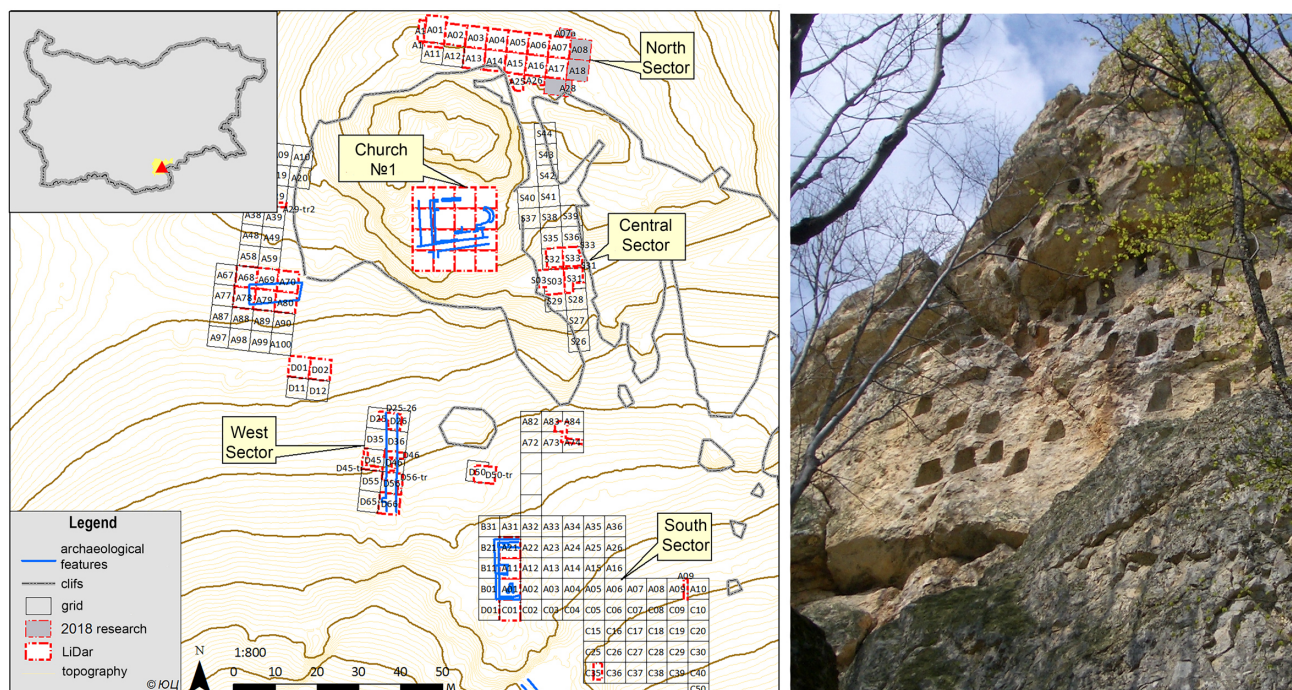
Fig. 1. Plan and topography of the archaeologically researched area with labelled sectors (left), view of the carved niches in the rock formation (right).

basilica was established on the southern slope. A large stone wall was constructed to protect this very important complex.

The site stopped functioning in the beginning of the 13th century and its demise can be linked to the knights of the 4th Crusade, who passed through these lands.

This study focuses on the recovered faunal remains during the excavation campaigns of 2015–2021 (Fig. 1).

Geographic setting

The site is located in South-eastern Bulgaria, on the north-eastern ridge of the Rhodope Mountains, laying beneath the Sveta Marina (St. Marina) Peak (708.6 m), one of the highest reaches of the ridge "Gorata". The site's name translates to "the Deaf Stones", referring to the lack of echo among the rhyolite rock formations surrounding the site. The southern slope of the Sveta Marina Peak consists of several groups of rocks, divided by geological faults. The "Gorata" Ridge is the watershed of the Martisa and Arda rivers. The northern slopes descend into the right tributaries of the Martisa,

while the southern merge with the Arda. The terrain has a dense vegetation cover consisting of oak forests.

Chronology

Archaeological research on the site has been ongoing since 2008. The excavations have been carried out in five distinct sectors by two teams. The Central sector has provided the best opportunity to explore the site's stratigraphy, as the cultural layer is well preserved to a maximum height of 3 metres (Nekhrizov & Tsvetkova, 2018). Seven phases have been distinguished based on the stratigraphic sequence. The earliest (GK I) dates to the final Chalcolithic period (around the second quarter of the 4 mil. BCE). GK II belongs to the transition period from the Late Bronze (LBA) to the Early Iron Age (EIA) (12th cent. BCE). GK III encompasses the first phase of the Early Iron Age (11-10th up until the beginning of the 9th cent. BCE). The next phase, GK IV is attributed to the second period of the Early Iron Age (9 – 6th cent. BCE). Based on the presence of grey monochromic ware and coins, the GK V is dated to the Late Classical – Early Hellenistic period (Late 5th – 4th cent. BCE) (Table 1). The next chronological phases

Table 1. Chronology of rock-cut complex Gluhite Kamani by archaeological periods.

Stratigraphic layer name	Chronology
Gluhite Kamani II (GK II)	Late Bronze Age – Early Iron Age transition
Gluhite Kamani III (GK III)	Early Iron Age first phase
Gluhite Kamani IV (GK IV)	Early Iron Age second phase

GK VI and GK VII (Late Antiquity and medieval period), cannot be accurately distinguished within the available stratigraphic layers and contain no archaeological features in this sector. However, materials from the Early Byzantine and medieval periods are abundant among in the other sectors (Nekhrizov & Tsvetkova, 2018). None of the other sectors – Church 1, Northern, Western and Southern contains all seven phases, and the intensity of their occupation seems to have varied considerably. Therefore, the subject of this article is the osteological material from the Central sector and phases GK II – GK IV, from the LBA – EIA transition, till the end of the EIA.

Materials and methods

In this study, the faunal remains from the Central sector were examined, with the most significant and richest remains in the cultural layers. The material is from three periods – LBA/EIA (GKII), EIA (GK III) and EIA second period (GKIV). The animal remains from the site were first examined by Assoc. Prof. L. Ninov (NAIM – BAS) who worked on the campaigns from 2010 to 2014. He recorded 1800 faunal remains from the same sector, and the faunal ratios from his unpublished reports overlap with our results.

A total of 3160 faunal remains were registered in these layers and features. About 99.6% of the osteological material is mammalian, with a meagre quantity of reptiles, fish and birds (Table 2). From the examined material, 1828 remains are identifiable to species and families. Due to the very high fragmentation of the bones, the unidentifiable splinters are separated into three groups: large sized mammals (cattle/deer/horse sized), medium sized mammals (pig, sheep/goat, and roe deer) and small sized mammals (dog, fox, hare, and cat) (Supplementary material 02 [.xlsx]).

Faunal material was analysed using the reference collection in the Laboratory of Archaeozoology in the National Museum of Natural History – BAS. Measurements of the bones were taken (in mm) by the method of von den Driesch (1976). Due to the high fragmentation rates, 169 elements could be measured from the whole assemblage. Metapodials, phalanges and tali are the only bones that yielded a preserved greatest length. Since the quantity of measurable elements is low, the metric data are given as raw values in the appendix and cannot be used to conduct any statistical comparisons (Supplementary material 01 [.xlsx]). The fragmentation obstructed the identification of most of the elements from sheep and goat, therefore, in this paper, the term "sheep/goat" will be used for sheep/goat unidentified bones. The material was gathered from only one sector of the site, which gives a statistically significant sample, but it must be kept in mind that it may not be representative of the whole site.

The number of identified specimens (NISP) is used for quantitative techniques in species identification. Using the diagnostic zone recording for long bones (Dobney & Rielly, 1988), the percentage of completeness of each bone element was calculated. This calculation was made using the method developed by Morlan (1994) with the following formula: Total diagnostic zone counts per element/total number of defined zones in the element = percentage of fragmentation. The percentage of completeness is a good unit to apply when measuring the general fragmentation of the assemblage – the lower the percentage, the higher the fragmentation. For analysis of the traces on the bone's surfaces – butchering marks and cooking/burning the methods follow Binford (1981) and Nicholson (1993).

The cattle breeds are reconstructed based on the method of Iliev (1994). In his study, he defines three main types of breeds according to the dimensions of the bones and withers' height. These conditional breeds are the primigenious (big size cattle), the crossbreed

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Table 2. Count of faunal remains found on the site by classes.

Class	NISP	NISP%
Mammalia	3146	99.63%
Aves	2	0.05%
Reptilia	10	0.26%
Actinopterygii	2	0.05%
Total	3160	100.00%

(medium size cattle) and the short horn breed (small size cattle).

Cull patterns (age of death) of the sheep/goat are calculated using the summarised information from the data of teeth eruption/wearing stages following Payne (1973) and epiphysis fusion stages by Moran & O'Connor (1994). The studies of Schmid (1972) and Grant (1975) are used for cattle and pigs. They are combined because the teeth and mandibles from this material alone do not make a statistically significant sample. The age classes are grouped into four categories: infant, juvenile, subadult and adult. The age in months of these categories depends on the domestic animals (Forest, 1997).

Results

A total of 1828 faunal remains were identified on the site. Domestic animals dominate all taxa, representing about 90% of the material (Supplementary material 03 [.xlsx]). From them, the sheep/goat (47.64%) are most abundant, followed by the domestic pig (24.50%) and cattle (16.44%). There are only a few bones from dog, horse and donkey. Wild animals make up 11% of all material. Although their percentage is low, there is a high species diversity. Most remains are from red deer, but there are also small amounts of bones from fallow deer, roe deer, wild boar, red fox, European hare, Eurasian beaver, partridge, tortoise and fish (Fig. 2). The species composition ratio of domestic animals is stable throughout all chronological phases, with very little variance (Fig. 3).

Sheep/goat (*Ovis aries*/*Capra hircus* L.)

Sheep/goat are the site's most abundant animal from all periods, with 884 remains. The high fragmentation of the bones and young age does not allow correct identification of most bones. Only 63 elements are identified as goats and 39 – as sheep. Almost all elements from the skeleton of the animals are present.
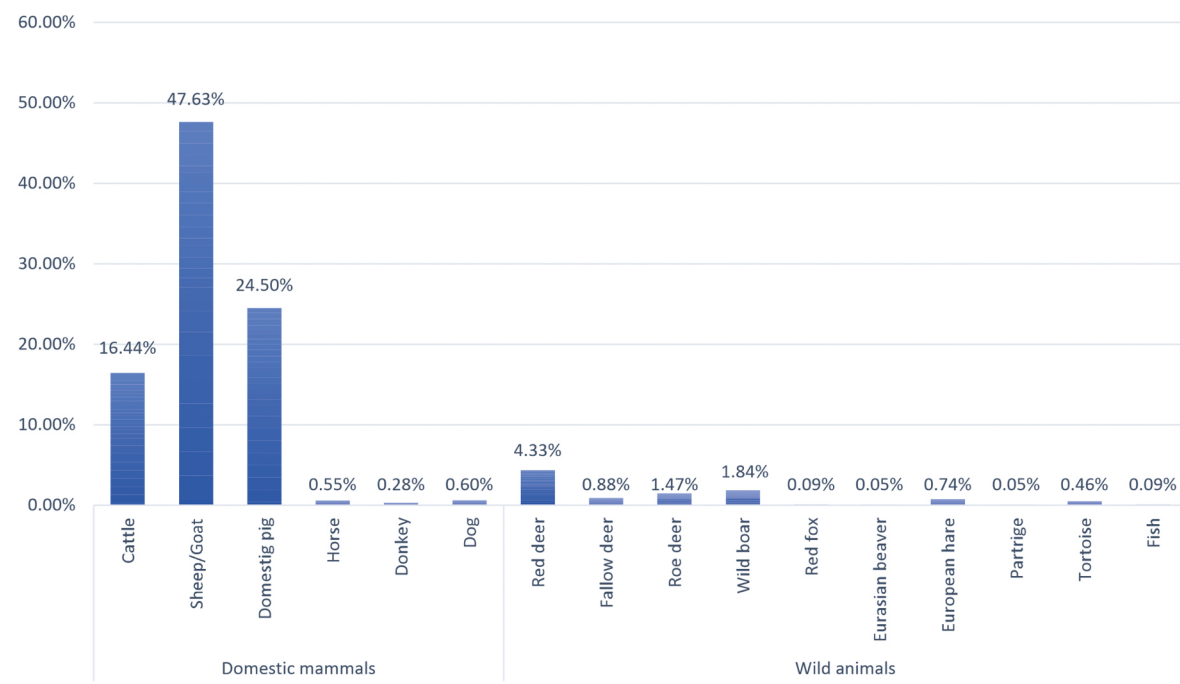

Fig. 2. Quotative ratio (in percentage) of the wild and domestic animals found in Gluhite Kamani.
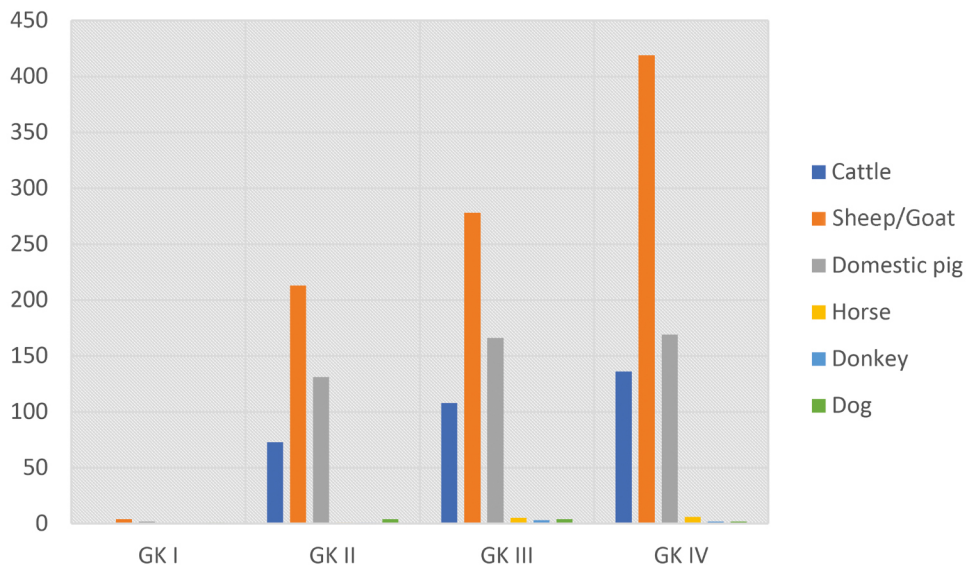
Fig. 3. Ratio representation of domestic mammals from different layers in the site.
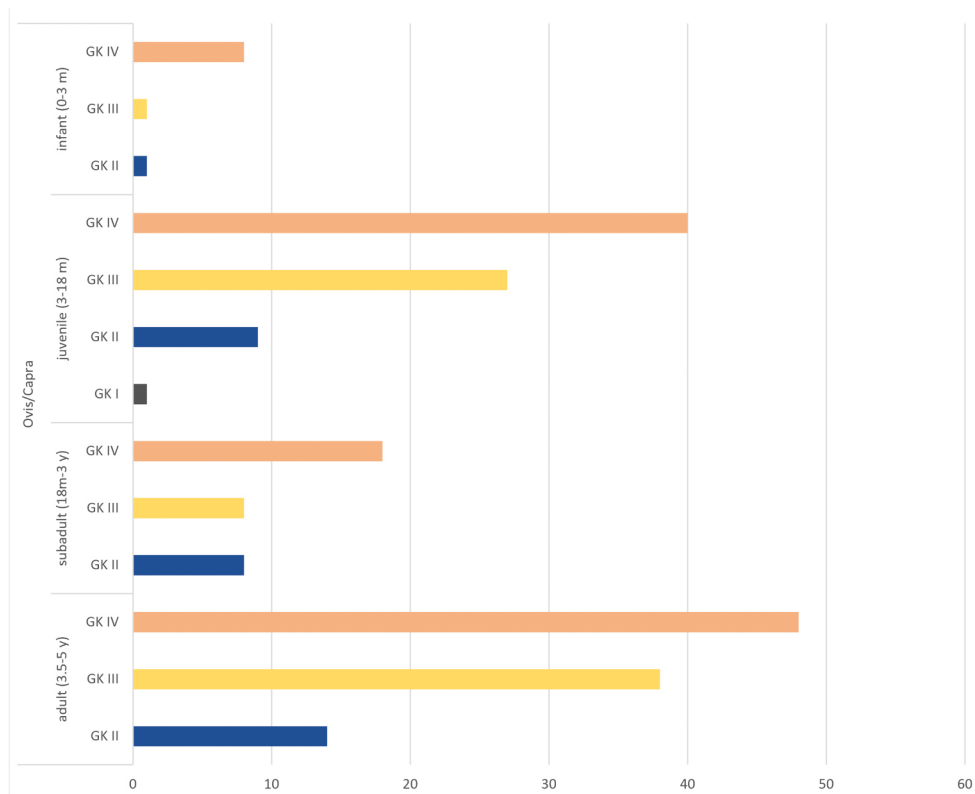
Fig. 4. Age of death in sheep/goat according to the identified elements.

The fragments from horn cores belong to goats; only one is from a sheep. The possible explanation of the small amount of horn cores is the presence of hornless sheep in the populations.

Cull patterns on the site according to the age of death exhibit a bimodal distribution of adult and juvenile individuals in all stratigraphic layers. This profile corresponds to the milk production profile. Still,

Table 3. Size comparison of bovine metapodia.

Cattle breed	n	Bone	GL	Bp	Bd
Gluhite Kamani	3	ossa metacarpalia	–	47.69–55.70	58.57–64.90
	5	ossa metatarsalia	–	44.85	48.80–51.89
Short-horn cattle	1	ossa metacarpalia	152	43.44	43.61
	1	ossa metatarsalia	173	38.54	38.24
Ezero (Early Bronze Age)	–	ossa metacarpalia	182–245	51–71	53–77
	–	ossa metatarsalia	208–267	41–58	50–72

the small quantity means that restraint is necessary regarding making general conclusions (Fig. 4). However, the site's ritual function may suggest the consumption of the animals in specific periods. Typically, sheep/goat give birth from the end of February until the end of March. The presence of juveniles between 3 and 12 months and infants from 0 to 3 months suggests at least two consumption events took place on the site. One in the spring, May/June, and one in September/October. The large amount of animal remains from adult individuals shows that they were also consumed alongside the young animals. The small number of infant and subadult animals suggest that they were not preferred for consumption and probably are exceptions (as young animals do not yield much meat, but must also consider that their remains are highly prone to deterioration and may not have preserved).

Cattle (*Bos taurus* L.)

Cattle make up a total of 16.44% (n=314) of all material. The percentage is almost constant in the analysed stratigraphic sequences. They come in third in the category of husbandry animals, suggesting they were not the preferred animals for consumption. The small number of bones and high fragmentation rate do not allow for statistical and comprehensive metric analysis of the cattle remains.

There are only a few metapodials which allow measurements. Their size in proximal and distal end breaths show individuals larger than brachiceros breed characteristic for the region in Rhodope Mountains – Rhodopean shorthorn cattle (Table 3). Their

dimensions fall in the ranges of the primigenius type of breeds after Iliev (1994).

The age of death of the animals shows a preference towards adult individuals, but juveniles are also present in all strata. Only a few bones belonging to subadults and infants are reported in layers GK III and GK IV (Fig. 5).

Domestic pig (*Sus domesticus* L.)

The domestic pig is the second most abundant animal after the sheep/goat. A total of 449 remains were identified, which make up about 25% of the fauna. They maintain a stable percentage throughout all layers. The material is highly fragmented; the only preserved complete elements are the phalanges and tarsal bones.

The age of death shows that juvenile (between 6–15 months) and subadult animals (between 15 months and 2.5 years) were preferred for consumption. The infants and adults are present in all layers; but they are scares. (Fig. 6).

Horse and donkey
(*Equus caballus* L.), (*Equus asinus* L.)

A total of 12 fragments from horses were identified on the site. Almost all remains come from the GK III and GK IV layers and are distributed equally. The fragments belong to the legs of the animals, mandibles and teeth. In GK III, there are parts of the metapodia, tarsal bones, and some loose teeth, while in GK IV, they are mainly from the head and pelvic bones. The
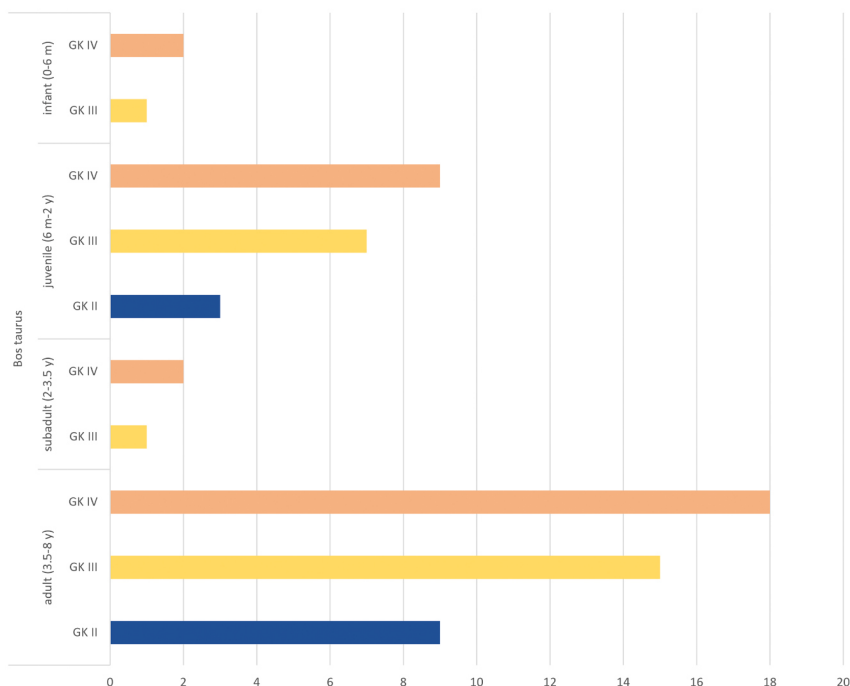
Fig. 5. Age of death in cattle according to the identified elements.
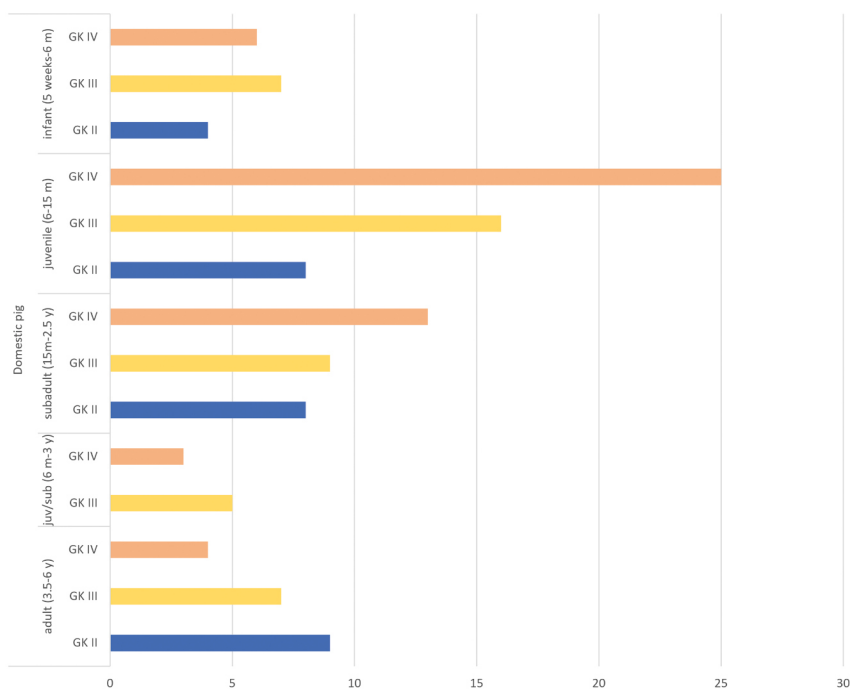
Fig. 6. Age of death in domestic pig according to the identified elements.

only bone from GK II is a shaft fragment from a tibia, which was burnt at a very high temperature (cremated). The other remains were also exposed to the fire according to the black spots and grey colour.

Six fragments were identified as belonging to a donkey based on their small size. They consist of mainly metacarpals and metatarsal bone fragments, except one part of a humerus from GK II and an upper

molar from GK IV. Only two metapodials have traces of burning.

The humerus fragment from GK II is among the earliest identified domestic donkey bones from Bulgaria. The only other reported donkey bone comes from a Late Bronze-Early Iron age transitional context, from the site of Vratitsa (Burgas Region), but it was mentioned briefly without the exact date (Ribarov & Ribarova, 2015: 260). The earliest donkey remains in the Mediterranean were discovered at the site of Lerna, Peloponnese, where they first appear in the Early Helladic II layers (22700–2450 BCE) (Gejvall, 1969: 35). Closer to the Balkans they are rare in Late Bronze assemblages, but non the less present. There is a small third phalanx, determined to be of a donkey dating to the Late Bronze Age layer (1750–1300 BCE), from the site of Troy, located at the Gallipoli Peninsula (Gejvall, 1939) and in Kastanas, Macedonia, where they appear in the Middle Bronze Age layers (Becker, 1986: 87). While, we have only one specimen that can be attributed to the Late bronze age layers, and the bone has not been carbon dated, it is possible to expect that these animals did spread into the southern parts of the Balkans. It is interesting to note that while assemblages from the Bronze age have been examined closely in Romania, donkeys seem to appear in the Late Iron age (6th century BCE) for the first time (Balasescu et al., 2003), but the lack of published Late Bronze – Early Iron age faunal assemblages from Bulgaria, restricts us to trace their spread.

Dog (*Canis familiaris* L.)

There are only ten remains from dogs found on the site. From GK I, we have a fragment from the calcaneus. Four vertebrae, a femur and a tibia come from the GK II layer. In GK III, we have fragments from the skull, humerus, ulna and a complete metatarsal bone. The humerus belongs to a young individual under six months. GK IV has only two fragments – from an axis and tibia. Almost all remains have black spots indicating burning or some fire treatment.

Wild animals

The wild taxa encompass mainly mammals: red (*Cervus elaphus* L.), fallow (*Dama dama* L.) and roe deer (*Capreolus capreolus* L.), wild boar (*Sus scrofa* L.), red fox (*Vulpes vulpes* L.), Eurasian beaver (*Castor fiber* L.) and European hare (*Lepus europaeus* Pallas, 1778). There are also some remains from partridge, fish and tortoise. Wild animals make up 11% of all material (NISP 145) which is a relatively high percentage (see the discussion) (Supplementary material 03 [.xlsx]). There is a significant diversity in species, and it can be attributed to the specific region. Unlike domestic animals, where the percentage is almost the same in every layer, the ratio of wild animals tends to vary in the different stratigraphic layers. The highest percentage of wild animals is in layers – GK II and GK III, where the red deer dominates. The remains from fallow, roe deer and wild boar are almost the same quantity. A few remains from red fox, hare, fish and tortoise are also present in layer GK II. The red deer and wild boar are the most abundant in GK III. Fallow, roe deer, hare and tortoise maintain the same percentage as in the earlier layer. In GK IV, wild taxa decline in number; the most abundant animal is the roe deer. Remains from red deer, wild boar and hare are almost equal. There are a few bones from a red fox and one from a partridge (Fig.7).

This ratio suggests some changes in the hunting preference. LBA/EIA period (GK II and III), the most preferred wild animal is the red deer, but in the second phase of the EIA period, roe deer and hares become the most abundant game animals. These can be caused by local climate changes or some cultural change.

These animals are typical for this habitat. A total of 55 remains from red deer were identified. Most consist of parts of the legs, with only four fragments belonging to antlers. The most numerous are the remains in GK II. Only ten bones belong to fallow deer in GK II/III. Roe deer is in second place as quantity (NISP 29), with the highest number of elements in GK IV. Wild boar is one of the main game animals with 25 fragments from bones and teeth. From the red fox, only one mandibula was found close to the fireplaces and metacarpal bones over the stone cluster. There is one mandible from the Eurasian beaver, which was found in GK II. Probably the fox and beaver were not consumed but used as pelts, as there are no traces of butchering. There are 16 remains from European hares, primarily parts of the pelvic and long bones. Most of the bones were found in GK IV, where the hare is the second most abundant wild animal. From the tortoise, only fragments from the shell were present. We cannot be certain if the tortoises are part of the archaeological material or if they were a recent intrusion, as the hibernation of these
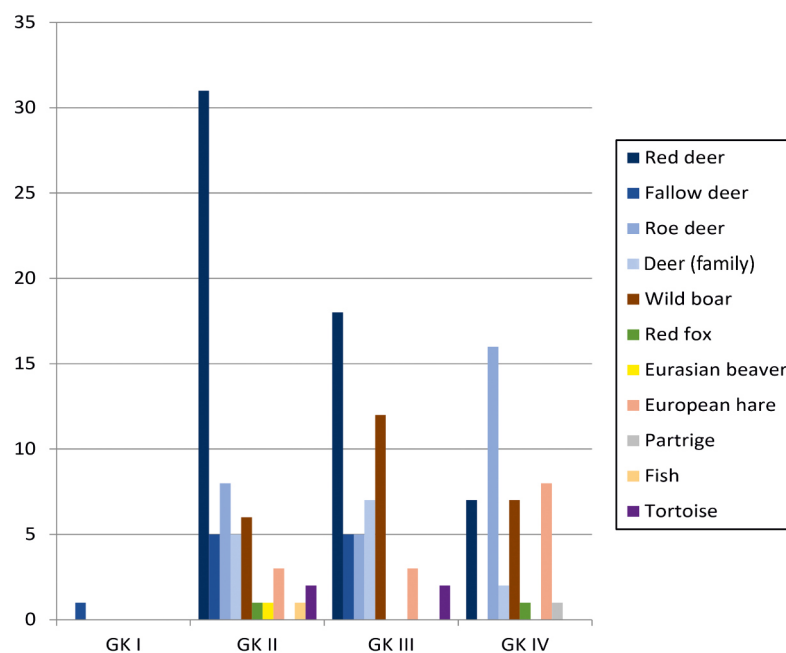
Fig. 7. Ratio representation of wild animals from different layers in the site.
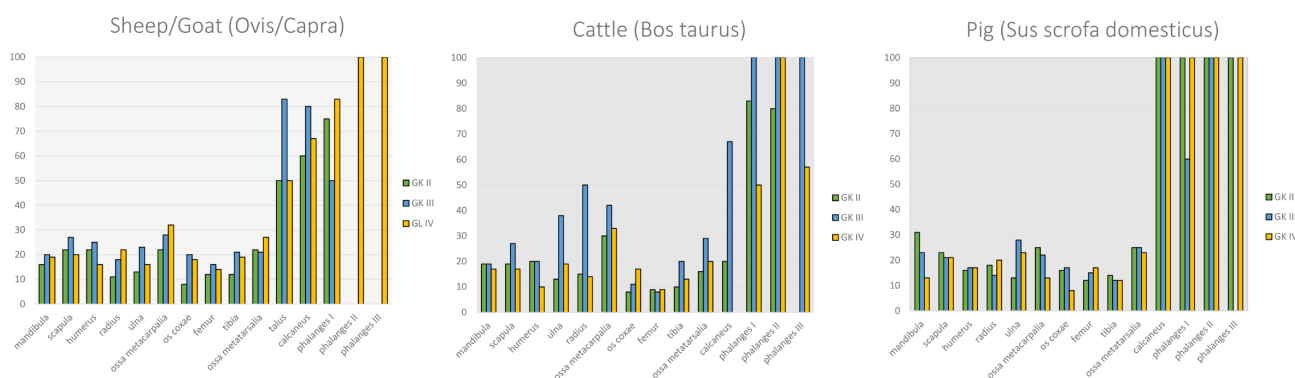

Fig. 8. Calculated percentage of long bones' completeness for each main domestic animal. Preservation rates are low, with very little difference between species. Colours reflect the different stratigraphic layers (after Morlan, 1994).

animals requires digging into the ground. Only one bone from fish, a premaxilla of a Cyprinidae sp., was found in layer GK IV. The bone is large, and the fish was most probably a catch from one of the big rivers nearby (the Arda or Maritsa), which shows that at least some of the meat types were brought from the wider geographical surroundings.

Bone fragmentation and preservation

The material is highly fragmented throughout all stratigraphic layers, with long bones having an average of 20% preservation. This contrasts smaller and more compact bone element (phalanges (PH), carpal and tarsal bones), mainly discovered intact. There is slight variation in the preservation rates between stratigraphic layers and individual species, except for a higher preservation rate of ulna, radius and metatarsals of bovines in stratigraphic layer GK III. The highly fragmented long bone and the mandibular pattern are consistent with marrow extraction, as some elements bear the typical breakage ridges (Fig. 8). Secondary fragmentation seems to have occurred through exposure to the physical surroundings, as all layers seem to have accumulated over a period of time.
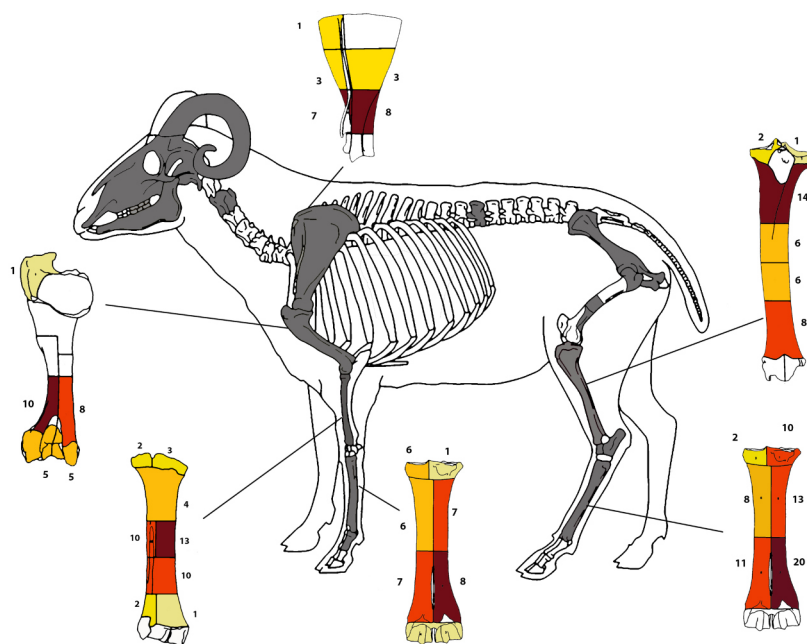
Fig. 9. Schematic representation of the skeleton of a sheep illustrating the anatomical element representation in the body and by diagnostic zone (Dobney & Rielly, 1988). The darker the colour, the more abundant the fragment.

Skeletal element representation

All body elements are represented by the groups of the leading domestic animals (sheep/goat, cattle, pigs, dogs). Equid remains are scarce, but they come from different skeletal elements. At this point, one cannot state whether or not they were consumed, as no cut marks were observed on equine bones. An inter-species difference is observed when examining the NISP values and DZ counts of body element representation). The radius and metatarsal fragments are the most abundant for sheep/goat through all stratigraphic periods, while the tibia is underrepresented in layer GK III. Very few fragments from ulnae, pelvises and femora are present in layers GK II. III, each forming under 2% of the total caprine element count per stratigraphic layer (Fig. 9). Femora and ulnae are slightly more abundant in layer GK IV, each representing 5% of the NISP. In comparison, pelvises continue to make up 2% of the assemblage. Phalanges are registered mainly in GK IV. Suid remains exhibit slight variation in element representation through the stratigraphic layers. The only difference is the higher number of the tibia in GK IV (n=32 (21% of all pig

elements of the layer)). Metapodia are slightly underrepresented for suids. The mandibula dominates in GK III and IV in bovines, while metatarsals are more abundant in GK II; all other elements do not exhibit any specific pattern. Notably, hyoids from 5 different large ruminants (probably bovine) were discovered in GK II. Apart from them, the only fragment from a single large hyoid was found in strata GK IV.

Like the domestic animals, deer and hare carcasses seem to have been brought whole to the site and butchered there, based on the presence of metapodia, phalanges and cranial elements. Their remains are too few to tell if a particular element dominated the assemblage. Interestingly, we only have a mandibula and a metacarpal from a fox and a single mandibula from a beaver, and this could mean they were attached to a pelt.

Traces

About 40% of the bones have traces of burning, from which the most abundant are black spots caused by tanning due to cooking on fire (Nicholson, 1993). A
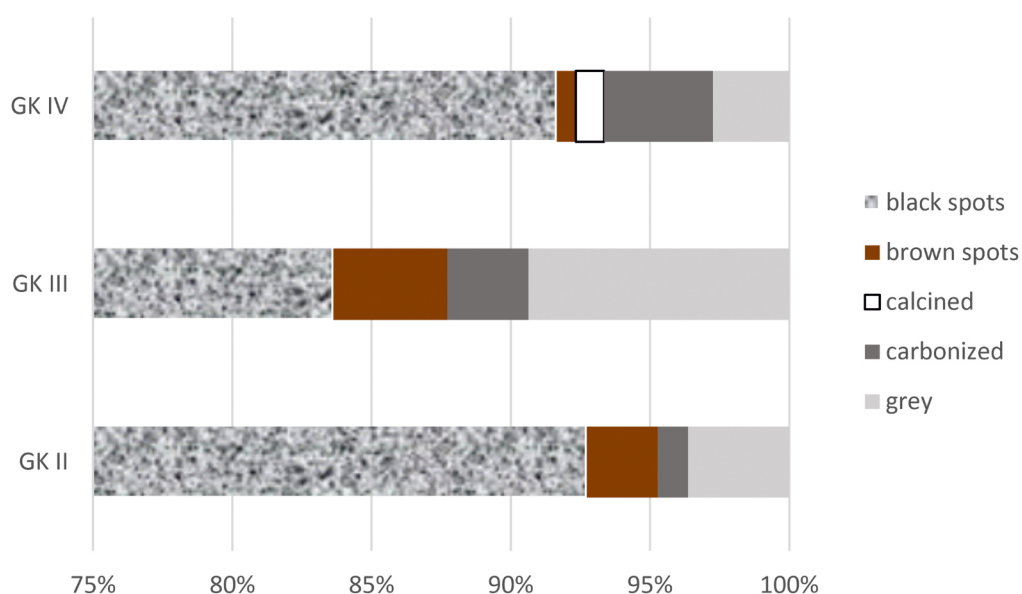
Fig. 10. Burning traces on bones from the layers of the site.

significantly smaller percentage is made up of bones with a brown and grey surface. The higher percentage of burned bones are from layer GK IV, where all burned bones have black spots. From GK II, again there are many bones with black spots and a brownish surface. The smaller quantity of burned bones is from GK III (Fig. 10). Most of the black spots are situated on the surface of the epiphyses of the bones. These traces can be caused by exposure of the bone to fire or high temperature (300–400°C), which usually happens when roasting the meat (Fig. 12). The most numerous bones with traces of burning are from sheep/goat, domestic pig, cattle and red deer. A few bones from dog, horse and donkey are burned. The mandible from the beaver and the bird bone also has traces from high temperature/burning.

Very few bones were burned in high temperatures over 500°C (cremated bones). A single unidentified rib, flat and long bone splinters from GK IV represent calcined bone.

Only 80 bones have traces of butchering. Most traces are from dismemberment and filleting of the meat and were caused by a knife. They are about 83% of the all-butchered bones. The knife was the preferred tool for butchering, but the axe and flints were used for dismemberment and filleting of the meat. Interestingly, flint was primarily used in the late layers GK III and VI.

Skinning traces are observed on the skull of sheep/goat, cattle and red deer. An axe was used to remove the horn cores and antlers from the skull. Portioning marks were found only on pig bones made by axe and knife. Filleting as well as dismemberment cut marks were noted on the ribs, pelvis and long bones of cattle, sheep/goat, deer and suids (Fig. 11). The meat was probably filleted and portioned into small pieces to fit into the pots for cooking. This cooking preference is also attested in the pottery assemblage from the site, which is dominated by cooking pots (Nekhrizov & Tsvetkova, 2012).

A total of 189 bone fragments has traces of carnivore and rodent gnawing, but carnivore marks prevail. The traces suggests that part of the food remains was exposed on the surface before accumulating in the stratigraphic layers.

Discussion

While over 200 hundred high-altitude sites have been registered in the Rhodope Mountains (Tsvetkova, 2016), most are single layered and few were subjected to a systematic archaeological excavation. The osteological material is of low quantity and quality (high fragmentation). The function of these sites is widely disputed, ranging between cult sites occupying
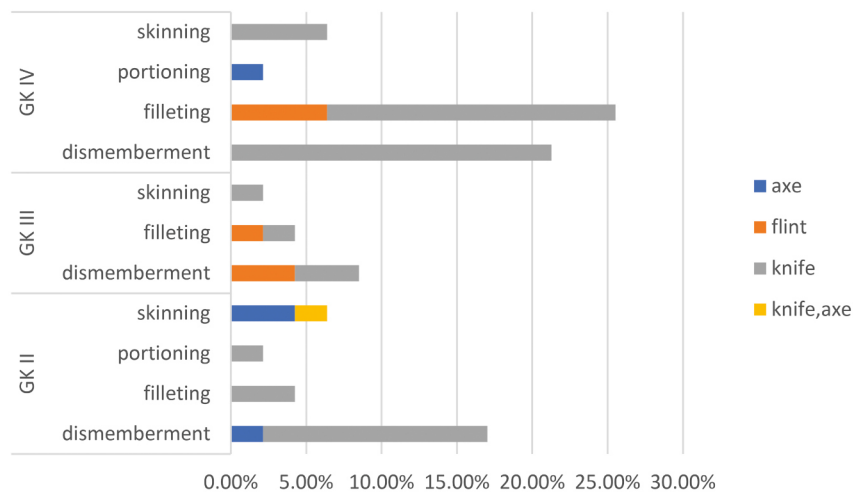
Fig. 11. Traces of butchering on the bones from Gluhite Kamani (after Binford, 1981).
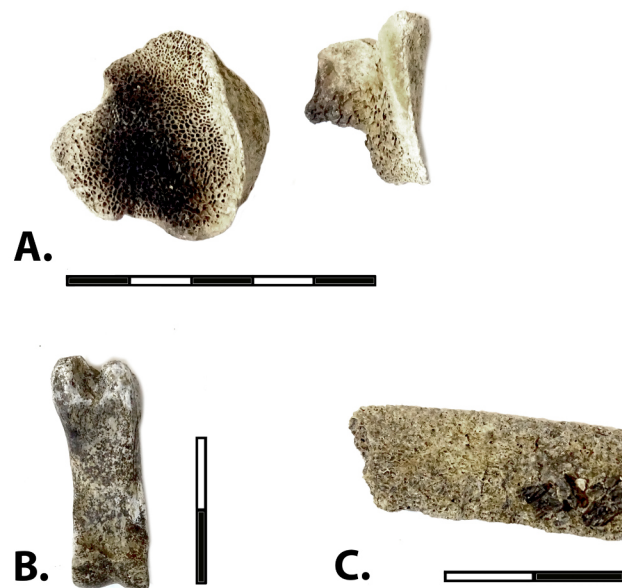

Fig. 12. Examples of secondary burning (A. The shaft fragment of the cattle first phalanx exhibits a secondary burning of the spongiosa (left), and the radial shaft fragment of caprine indicating secondary burning (right); B. A first posterior phalanx of a sheep showing a high degree of burning, intense grey colour with calcined spots; C. Part of a caprine sternum with fused-on fragments of charcoal).

natural rock formations (Tsvetkova, 2016; Kiotsekoglou, 2015), seasonal sites linked to a pastoral economy model (Efstratiou, 1993), to permanent settlements for practising some activity utilising natural resources, like mining (Popov, 2009). The only nearby site yielding a more substantial faunal assemblage is the mining centre Ada Tepe, near Krumovgrad (South Bulgaria, Eastern Rhodopes), inhabited during the LBA and EIA. In Ada Tepe, the species composition differs from the Gluhite Kamani, with cattle being the main species, closely followed by sheep/goat. Similar to the site discussed here, the Ada

Tepe assemblage demonstrates slight variation between the NISP values of the LBA and EIA layers, characterised by a slight growth in cattle numbers during the EIA. These results must be taken cautiously, as the EIA assemblage is much smaller (Nikov et al., 2018). While the presence of caprine remains has been used in favour of the interpretation of some high-altitude sites, like the site of Tsouka (Efstratiou, 1993) as seasonal shepherd stands, the consistent number of pigs at this site prevents the assumption that the Gluhite Kamani site was occupied by a community practicing a specialised type of pastoralism. The debate on whether or not the ancient Mediterranean and Rhodope Mountains' population practiced an advanced transhumance model before the Middle Ages (Arnold & Greenfield, 2006) falls out of the scope of this study. Still, our data exhibit characteristics of a mixed farming model (Halstead, 1996). Comparing the Gluhite Kamani assemblage to other archaeological sites from the same period faces obstruction from the published data of Early Iron Age and Late Bronze Age Mediterranean sites, because when assessing only the NISP, there is no coherent patterning between the different sites (Fig. 13).

Food or offering or both?

Stratigraphic layers containing abundant animal bones and pottery can be linked to settlement and sacrificial debris. From an archaeological point of view, the two can be hard to distinguish. In recent years the growing body of literature on faunal remains from religious sites has helped establish a series of criteria which characterise cultic deposits (Morris, 2011; Grant, 1984; Peters, 1993). In the Mediterranean, it is generally believed that layers of sacrificial debris must contain charred animal remains, either deposited in pits or re-used in paving the flooring levels (Ekroth, 2017). The Gluhite Kamani site contains a series of hearths. However, based on the recovered assemblage, we currently do not have any data on the ritual burning of specific body parts similar to the "thysia sacrifice" described in ancient written sources (Ekroth, 2009). Few bones display prolonged contact with fire, some exhibiting secondary burning, as it seems they were sporadically thrown into the hearths (Fig. 12). While the preference for a specific bone element and side has been attested in sanctuaries in Greece (Pöllath & Peters, 2011), our assemblage does not account for this

as all elements from the commercial/domestic species and deer are represented within the stratigraphic layers. This also means that the animals were brought whole and processed on-site. Vertebrae, pelvises and femorae are underrepresented among both the identified and unidentified faunal remains, especially for sheep/goat. Still, this patterning could be attributed to preservation rates as these bones have a thinner, have a more porous matrix, and are prone to weathering. Assemblages in known Greek sanctuaries seem variable regarding topography (Velarde, 2001). A medieval church is present on the highest point of the Gluhite Kamani site, which is located just above the central sector yielding the discussed materials (Nezhrizov, 2012). Underneath the church, a small layer containing EIA materials was recovered. It contained only a few individual bones, which do not allow the tracing of any patterning (unpublished report by Lazar Ninov from 2009). Generally, in known Greek sanctuaries, the faunal assemblages are not very diverse, showing a preference towards a specific domestic species (Tab.5). Sometimes, these preferences match the animal of the deity listed in written sources (Stroud, 1965). Adult caprine and cattle were preferred as the sacrificial victims in Greek sanctuaries from the last phases of the Early Iron Age and Archaic period, as seen from the sites of Dydyma (Boessneck & Driesch, 1983) and the Heraion of Samos (Boessneck & Driesch, 1988). In the Gluhite Kamani site a preference towards a specific age group of animals is absent as the consumption of animals seems to be highly dependent on their overall economic value. Cattle, valued for secondary products and traction, were slaughtered predominantly as adults. In sheep/goat, both meat and secondary products seems to have been taken into consideration, as the numbers of juveniles and adults are almost equal. Pigs were utilised only for their meat, as they were slaughtered at an age where they reached their maximum weight. This patterning is similar to that found in settlements, and the presence of animals of different age stages – infants and juveniles show that they were slaughtered at different times of the year. The age of slaughter does not show any significant variation between the different phases, unlike some settlements like Kastanas, where the cull patterns do vary between the different chronological periods, as it seems the utilisation of the animals in the Early Iron Age shifted from the use of secondary product towards a more meat dependent model (Becker, 1986). While our assemblage does not have any concrete patterning that
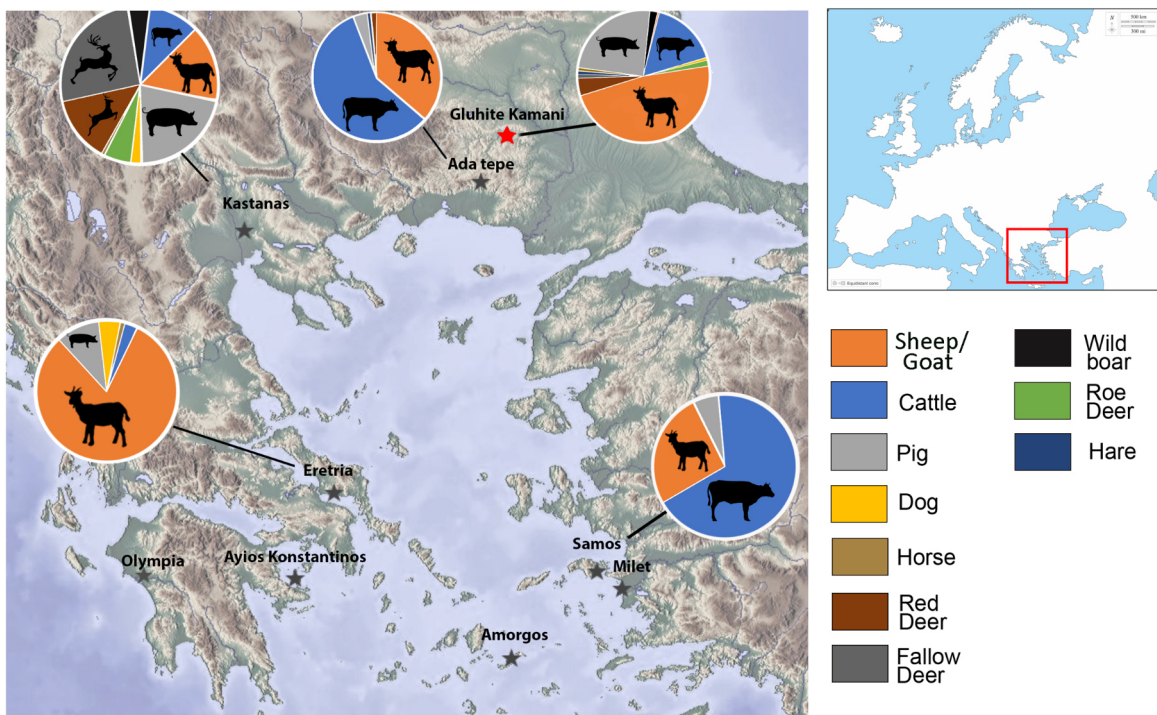
Fig. 13. Map showing some of the main sites with published zooarchaeological data, used for comparison and five pie charts illustrating the diversity in the species NISP counts. NB. For Kastanas, the pie chart shows the LBA/EIA transition layer, and the Gluhite Kamani and Ada Tepe pie chart shows the EIA data.

Fig. 14. Zoomorphic and anthropomorphic figures discovered on the site.

can be interpreted as part of a deliberate deposition ritual, we must consider that not all sanctuaries carry out rituals that leave a specific archaeological assemblage. The lack of patterning alone cannot be used to argue that religious activity with banqueting did not occur on the site (Gaastra, 2018). Almost all current knowledge on religious assemblages is based on examples from the Late Iron Age and the

Mediterranean, so it is not possible to say if this population did not have a different practice. When examining the archaeological contexts, there are some implications on the religious function of parts of the site. First, the central sector occupies the foot of one of the rocks bearing a series of artificially cut niches. In the strata of the GK II, a collective find of anthropomorphic and zoomorphic clay figurines was discovered near a hearth. The zoomorphic figurines from the hoard are fascinating, and most seem to portray cattle, with a single figurine resembling sheep (Fig. 13). The interpretation is based both on their horn style and the presence of a yoke. Cattle are underrepresented as taxa on this site and were not the main consumption choice, yet the figurines depicting them were deposited. This phenomenon is widely spread, as it seems that we get depictions of more expensive and valuable animals which would rarely be sacrificed (Russell, 2011). A similar case was present in the Late Bronze Mycenaean sanctuary at Ayios Konstantinos at Methana, north-east Peloponnese, where zoomorphic figurines of bovines dominated, but pigs were the main slaughter victims (Hamilakis & Konsolaki, 2004) (Fig. 14).

What can we tell about the environment?

It is interesting that no major variation exists between the main domesticates NISP counts in the Late Bronze and Early Iron Age strata within this assemblage. While the Late Bronze Age collapse in 1200 BC led to significant changes in the Mediterranean economy and religion (Marakas, 2007), the data do not reflect any significant change in the region's population. We cannot accept the hypothesis of Dibble & Finné (2021) that the presumable growing dominance of goats in the EIA may be linked to a drought in the Late Bronze – Early Iron Age transition, as sheep/goat are a constant species for this site, and seem to be the main exploited species overall in the prehistoric societies of Anatolia and the Aegean (Popkin, 2014; Trantalidou, 2017). On this site, while sheep/goat domestic animals dominate, the faunal assemblage is quite diverse, showing the presence of both domestic and wild taxa, meaning that consumption rates mainly depended on the available species. Interestingly, unlike the synchronic settlement sites of Troy and Kastanas, where wild taxa dominated the transition phase between the Early Iron and Late Bronze Age (Becker, 1986; Gejvall, 1939), the data

from this site show that wild animals were more common in the Early Iron Age phases (Supplementary material 04 [.xlsx] ☑).

Conclusion

At Gluhite Kamani, remains from wild and domestic animals were found from which the domestic prevail, but there is great diversity of fauna. These remains are mainly food residues of feasts or offerings at the site. The great diversity of wild and domestic species suggests that all available animals for consumption (wild and domestic) were presented in the layers, but sheep/goat and domestic pig predominates as the preferred animals.

The age of death of the animals shows that sheep/goat were killed and consumed at a young age as well as the adults. The same is true with cattle, but there are also remains from infant animals that were probably killed for special occasions. The sheep/goat cull patterns show that at least two events are happening in spring (May) and autumn (September).

The high fragmentation and the traces of burning suggest that probably the meat was cooked – roasted or boiled on the site, which the presence of cooking pots and other pottery and hearths can attest.

Acknowledgements

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Supplementary materials

01
Document title: Metric data from the mammalian remains of the site
Kind of document: Microsoft Excel (OpenXML)
MIME type: application/vnd.openxmlformats-officedocument.spreadsheetml.sheet
Document name: 000512000452023-01.xlsx

02
Document title: Number of unidentifiable bone splinters by size groups
Kind of document: Microsoft Excel (OpenXML)
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03
Document title: Domestic species skeletal elements (quantified by diagnostic zone counts and NISP); Wild species skeletal elements (quantified by diagnostic zone counts and NISP)
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Research article

Snacking during hibernation? Winter bat diet and prey availabilities, a case study from Iskar Gorge, Bulgaria

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Abstract: Better empirical knowledge of how bat and insect phenology are influenced by seasonal environmental conditions and how this may affect fitness is essential in the face of changing climatic conditions. We examined the winter diet of the Schreibers' bent-winged bat *Miniopterus schreibersii* (Kuhl, 1817) from Razhishkata Cave, Balkan Mountains, Bulgaria during four sampling periods in the winter of 2021 (end of January – end of March). We used a combination of DNA metabarcoding and a microscope-based morphological analyses of bats droppings. Additionally, we tracked prey availability in the studied area using insect flight interception traps (FIT). The species was actively feeding outside the cave and did not use the cave fauna. Our samples indicate a shift in the winter diet of the bats throughout the studied periods. Even though Diptera was the most abundant order of insects during two of the study periods, bats were preying predominantly on Lepidoptera and Hymenoptera. The metabarcoding also supported this with data for the presence of insects with diurnal activity in the samples. The temperature loggers showed a strong correlation between the outside temperature and the temperature in the cave where the colony was located, probably impacting the activity patterns of the bats. To our knowledge, this is the first study of winter bat activity and diet in Bulgaria. Our research can serve as a potential framework for studying winter bat activity and insect activity during this sensitive period.

Keywords: bat guano, DNA metabarcoding, insects, *Miniopterus schreibersii,* winter activity, winter diet

Introduction

With winter's onset and the lowering of ambient temperatures, mammals face serious energetic challenges. Small insectivorous mammals like bats need to cope with two key challenges. First, during the winter, there is a reduction in insect abundance, and second, they need to minimise heat loss due to their high surface-to-volume ratio (Speakman & Thomas, 2003). Some species of bats migrate to warmer regions, while others use hibernation to survive. Hibernation is a condition in which the body temperature drops, leading to a decrease in metabolic rate and increased energy savings for an extended period. It is crucial for survival during periods of limited resources (Buck & Barnes, 2000; Geiser, 2004). The hibernation period consists of frequently interrupted torpor bouts. These periodic arousals can cost bats up to 80% of their energy reserves (Thomas et al., 1990). Rising temperatures in the hibernacula (Humphries et al., 2006) and changes in airflow are expected to play a significant role in the arousals. Additionally, frequent extreme temperature changes during the winter can cause premature arousal (Thomas & Geiser, 1997), which can deplete critical energy reserves to the extent that the bat cannot survive the winter (Jones et al., 2009). This emphasises the need to increase our understanding of bat activity and the need for remote monitoring systems that cause less disturbance in the roosts and are low-cost and efficient (Revilla-Martín et al., 2020). The importance of remote monitoring systems capable of continuous data collection on the abundance and activity of bats has been even more pronounced since the COVID-19 pandemic. Additionally, non-invasive studies on bat populations are essential for understanding the natural activity patterns and can be used to set a baseline against which aberrant behaviour can be detected.

Water demands have been considered the main reasons for activity (Speakman & Racey, 1989), but research has been proposing multiple explanations for the winter activity, like activation of the immune system, sleep, and foraging (Hays et al., 1992; Boyles et al., 2006; Zahn & Kriner, 2016). Bat winter activity has been frequently observed in Bulgaria, due to milder winters. However, their feeding activity and dietary preference during hibernation has been poorly studied (Kanuch et al., 2005; Sano, 2006; Willams et al., 2011; Miková et al., 2013, Hope et al., 2014; Lino et al., 2014). Disturbance related to more frequent extreme temperature changes during winter can cause premature arousal (Thomas & Geiser, 1997), which can deplete critical energy reserves to the extent that the bat cannot survive the winter (Jones et al., 2009).

A special complex of invertebrates is active during the winter (Taylor, 1963). In Europe, the most abundant invertebrate order during the winter is Diptera. Flies (Diptera), springtails (Collembola), snow scorpionflies (Mecoptera: Boreidae), spiders (Araneae), beetles (Coleoptera), psyllids (Psylloidea), bugs (Heteroptera), earwigs (Dermaptera) and many species of moths (Lepidoptera) belong to the assemblage of snow-active invertebrates (Aitchison, 2001; Hågvar, 2001, 2007, 2010; Hågvar & Greeve, 2003; Hågvar & Aakra, 2006; Hågvar & Krzeminska, 2007; Soszyńska-Maj, 2008; Jaskuła & Soszyńska-Maj, 2011; Soszyńska-Maj & Buszko, 2011; Langourov, 2004; Pavlova & Stoyanova, 2020). Some aquatic insects like stoneflies (Plecoptera), caddisflies (Trichoptera) and non-biting midges (Chironomidae) (Bouchard & Ferrington, 2009; Hågvar, 2010) have also been observed during the winter and on snow. This complex of invertebrates active during the winter can provide hunting opportunities for bats (Park et al., 1999, 2000).

The winter activity and diet of bats in Bulgaria have not been studied yet and little is known about the winter insect activity (Langourov, 2004; Pavlova & Stojanova, 2020). Considering that the overwintering bat populations in Bulgaria are some of the largest European bat aggregations, more research and conservation efforts are needed. The aim of our study was to investigate the winter activity patterns and trophic interactions of Schreiber's bent-winged bat in the Lakatnik region, using two techniques: a microscope-based morphological analysis and DNA metabarcoding analysis. We studied the insect abundance and how insect availability will shape the dietary preference of the studied bat species. In addition, we investigated if there is a correlation between the outside temperature and the cave temperature at the location of the studied bat colony.

Materials and methods

Study site and species

The study was conducted in the middle of the Iskar Gorge, Balkan Range, Bulgaria. The canyon walls of

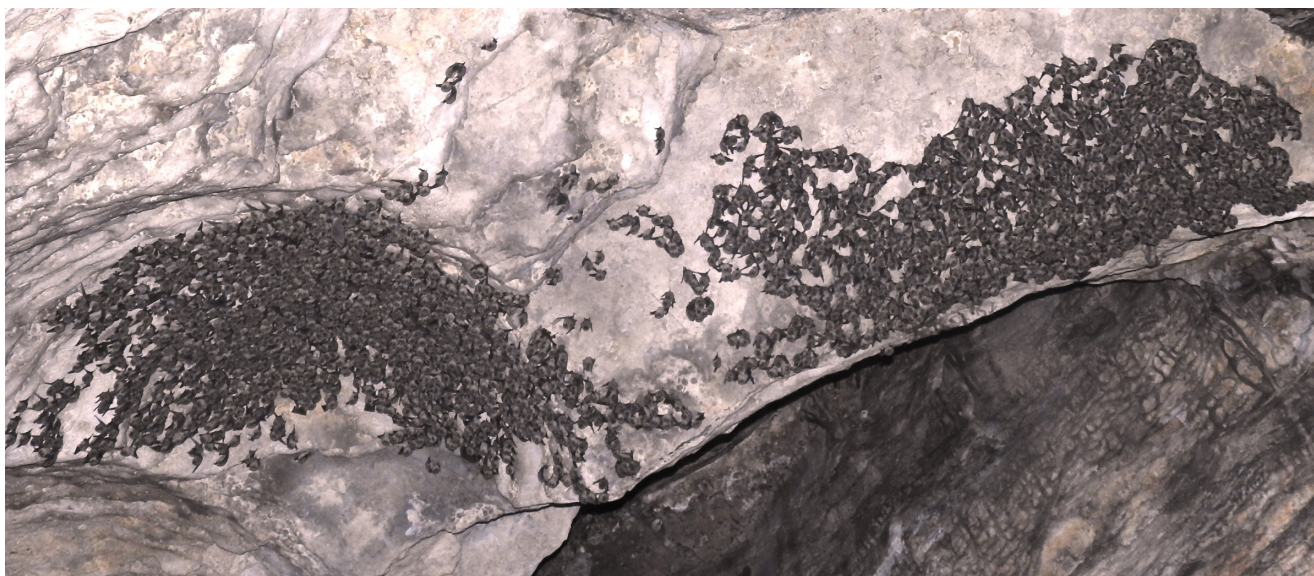Fig. 1. The study area with rocky complex above the entrance of Razhishkata Cave.

Fig. 2. The winter colony of Schreiber's bent-winged bat (*Miniopterus schreibersii*) from Razhishkata Cave.

limestone were carved down by the Iskar River over thousands of years, resulting in rugged crags, caves and other typical karst reliefs. The studied region is included in several protected territories: Vrachanski Balkan Natural Park, Lakatnishki Skali Protected Area and four Natura 2000 zones (BG0002053; BG0001042; BG0002005; BG0001040). Razhishkata Cave (43.09008°N; 23.38507°E; 575 m a.s.l.) (Fig. 1) hosts one large winter colony of Schreiber's bent-winged bat (*Miniopterus schreibersii* (Kuhl, 1817)) and several smaller clusters of five different bat species (*Rhinolophus ferrumequinum* (Schreber, 1774), *Rhinolophus hipposideros* (Bechstein, 1800), *Nyctalus noctula* (Schreber, 1774), *Pipistrellus pipistrellus* (Schreber, 1774) and *Pipistrellus pygmaeus* (Leach, 1825)). We focused our research only on *M. schreibersii* as it is the main resident species in the cave. The species is included as Vulnerable A2c in the Red Data Book of the Republic of Bulgaria and in the International Union for Conservation of Nature (IUCN) Red List (Popov & Ivanova, 2015; Gazaryan et al., 2020). Recent unexplained mass mortality events have been documented in Europe (Roué & Némoz, 2002). It is a specialist predator, feeding mainly on moths, but can opportunistically switch to other prey that are more seasonably abundant (Preselnik & Aulagnier, 2013). Forage near the vegetation and over woodland habitats (Dietz et al, 2009; Vincent et al., 2011). The size of the Schreibers' bent-winged bat winter colony in Razhishkata Cave (Fig. 2) varies from about 600 individuals at the beginning of the winter (January) to a maximum of 2000 individuals towards the end of the winter (March). The cave is among the important hibernacula for bats in Bulgaria (Ivanova, 2005).

Sample collection

Bat guano samples were collected during the following periods: 8.i–12.ii.2021 (period I); 12–23.ii.2021 (period II); 23.ii–5.iii.2021 (period III); 5–26.iii.2021 (period IV). White polythene sheeting (3 x 4 m) was laid down under the Schreiber's bent-winged bat cluster, which is located approximately 300 m from the cave entrance. Each cluster was checked, and we documented the species composition and density during the roost survey. During collection, the guano samples were placed on a paper sheet in a plastic box. After sample collection, the sheeting was changed to avoid mixing material between survey periods. Samples were stored at room temperature. Due to heavy rain, water dripped from the cave ceiling where the sheet was placed, and the guano collected on 12.ii.2021 was liquefied. Therefore, it was collected in a zip-lock bag and stored in the freezer. After heavy snowfalls during the last sampling period and flooding in the cave, the guano was practically washed from the plastic sheet. As a result, the colony changed its location to a drier place, and no guano was collected from this period (5–26.iii.2021). We followed all ethical requirements for working with bats. The research was carried out under permit by the Bulgarian Biodiversity Act (No 830/19.09.2020).

Microscope-based morphological analysis

Often, insectivorous bats do not chew their prey entirely. Because of their fast digestion, parts of insects can be traced in the guano, which can be used for further identification (Kovtun & Zhukova 1994). Insect fragments were identified with the help of an entomological key (Shiel et al., 1997) and identification guidelines (Whitaker et al., 2009; Whitaker & Castor, 2009) to the lowest possible taxonomic level. We selected only whole guano pellets with still preserved cylindrical shapes for the analysis. In order to avoid biased choice by picking only pellets that look more interesting than others, the suitable pellets were selected randomly. In total, 254 faecal samples were analysed. Of all morphologically analysed droppings, 126 (49.6%) were collected in February and 128 (50.4%) in March 2021.

Each pellet was pre-soaked in a small Eppendorf tube with ethyl alcohol (70%) for at least one hour to soften. Once a pellet had softened, it was placed on a microscope slide, and two drops of glycerol were added. The slide was then put under a low-power binocular microscope (x20–40), with graph paper sellotaped to the bottom of the microscope's stage. Using two fine dissecting needles, each sample was separated and examined. After tearing apart, more glycerol was added if needed. The remains were compared with whole insects caught in the area with the interception traps. For the liquified sample, the procedure was the same as stated above. The only exception was that after the sample was taken from the fridge, it was left for around 15 min at room temperature to unfreeze itself. After that, almost equal

proportions (31 in number) were taken from the sample using a small teaspoon until everything was analysed. The relative importance of different prey orders in the diet was quantified by calculating the percentage frequency (%F), defined as the number of occurrences of an order divided by the total occurrences of all orders x 100.

Molecular analysis

From each sample period, we created pools, each containing 100 pellets selected randomly, and for the liquified sample, a pool containing a similar volume of sample material to the other one. Species identification from the guano samples was performed using DNA metabarcoding following the protocol published in Hausmann et al., 2020. The samples (n=3) were dried in a 60°C oven for at least eight hours. Then we homogenised them in a FastPrep96 machine (MP Biomedicals) using sterile steel beads to generate a homogeneous mixture of guano and submitted them for subsequent metabarcoding (conducted by AIM GmbH). Before the DNA extraction, 1 mg of each homogenate was weighed into sample vials. The samples were then processed using adapted volumes of lysis buffer and the Zymo faeces kit (Zymo), following the manufacturer's instructions. We performed a 2-step PCR using mlCOIintF with jgHCO2198 primers to amplify the CO1-5P target region and prepare the MiSeq libraries. First, a 313 bp long mini-barcode region was amplified by PCR (Leray et al., 2013; Morinière et al., 2016), using forward and reverse HTS primers equipped with complementary sites for the Illumina sequencing tails. In a subsequent PCR reaction, index primers with unique i5 and i7 inline tags and sequencing tails were used to amplify the indexed amplicons. Afterwards, equimolar amplicon pools were created, and size was selected using preparative gel electrophoresis. The pooled DNA was purified using 92 MagSi-NGSprep Plus beads (Steinbrenner Laborsysteme GmbH, Wiesenbach, Germany). A bioanalyser (High Sensitivity DNA Kit, Agilent Technologies) was used for a final check of the bp distribution and concentration of the amplicons before creating the final library. High-throughput sequencing (HTS) was performed on an Illumina MiSeq using v2 (2*250 bp, 500 cycles, maximum of 20 min reads) chemistry (Illumina).

Metabarcoding library preparation

The bioinformatics processing of raw FASTQ files from Illumina was carried out using the VSEARCH suite v2.9.1 (Rognes et al., 2016) and Cutadapt v1.18 (Martin, 2011). Forward and reverse reads in each sample were merged using the VSEARCH program "fastq_mergepairs" with a minimum overlap of 10 bp, yielding approximately 313 bp sequences. Forward and reverse primers were removed with Cutadapt, using the "discard_untrimmed" option to discard sequences for which primers were not reliably detected at ≥90% identity. Quality filtering was done with the "fastq_filter" in VSEARCH, keeping sequences with zero expected errors ("fastq_maxee" 1). Sequences were dereplicated with "derep_fulllength", first at the sample level and then concatenated into one FASTA file, which was subsequently dereplicated. Chimeric sequences were filtered out from the FASTA file using the VSEARCH program "uchime_denovo." The remaining sequences were then clustered into OTUs at 97% identity with "cluster_size," a greedy centroid-based clustering program. OTUs were blasted against a custom Animalia database downloaded from BOLD, including taxonomy and BIN information, through Geneious (v.10.2.5 – Biomatters, Auckland – New Zealand) and following methods described in Morinière et al. (2016). The resulting CSV file included the OTU ID, BOLD Process ID, BIN, Hit-%-ID value (percentage of overlap similarity (identical base pairs) of an OTU query sequence with its closest counterpart in the database), length of the top BLAST hit sequence, phylum, class, order, family, genus, and species information for each detected OTU was exported from Geneious and combined with the OTU table generated by the bioinformatics pipeline. The combined results table was then filtered by Hit-%-ID value and total read numbers per OTU. All entries with identifications below 97% and total read numbers below 0.01% of the summed reads per sample were removed from the analysis. OTUs were then assigned to the respective BIN. Additionally, the API provided by BOLD was used to retrieve BIN species and BIN countries for every OTU, and the Hit-%-IDs were aggregated over OTUs that found a hit in the same BIN and shown in the corresponding column as a % range. To validate the BOLD BLAST results, a separate BLAST search was carried out in Geneious (using the same parameters) against a local copy of the NCBI nucleotide database downloaded from ftp://ftp.ncbi.nlm.nih.gov/blast/db/ .
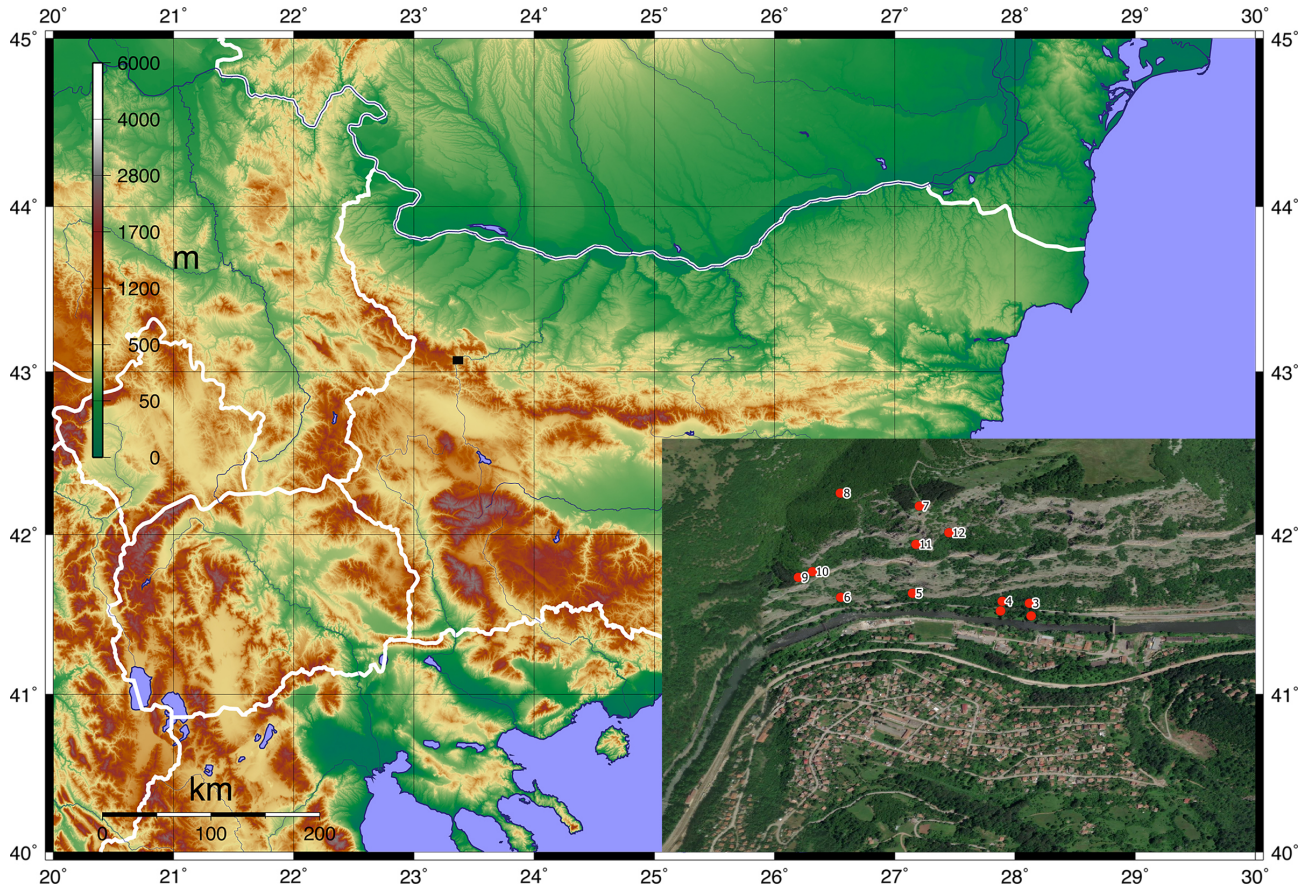
Fig. 3. Map of the locations of the 12 flight interception traps in the area of Iskar Gorge.

← Fig. 4. Photo of one of the flight interception traps in the area of Iskar Gorge.

Species identification in bulk samples was based on high-throughput sequencing (HTS) data grouped to genetic clusters (OTUs), blasted, and assigned to barcode index numbers ("BINs": Ratnasingham & Hebert, 2013) which are considered to be a good proxy for species numbers (Hausmann et al., 2013; Ratnasingham & Hebert, 2013).

Insect survey

To determine the winter activity of the insects in potential feeding places in the area of Iskar Gorge (Lakatnishki Skali Protected Area), 12 flight interception traps (FIT) were placed in different habitat types and at various altitudes at a distance of no more than 1 km from the cave (Fig. 3). We selected this type

Table 1. Flight interception traps in the studied area in Iskar Gorge, Lakatnishki Skali Protected Area.

Trap number	Decimal geographic coordinates		alt.	EUNIS Habitat types	Tree species
	N	E			
1	43.08805	23.38756	384 m	T1-2116: Dacio-Moesian ash-alder forests T1-1112: Eastern European poplar-willow forests	*Juglans regia*
2	43.08790	23.38845	384 m	T1-2116: Dacio-Moesian ash-alder forests T1-1112: Eastern European poplar-willow forests	*Alnus glutinosa*
3	43.08828	23.38840	390 m	T1-2116: Dacio-Moesian ash-alder forests	*Fraxinus excelsior*
4	43.08834	23.38761	392 m	T1-9B8: Sub-Mediterranean and Pannonic mixed forests H3.2A13: Balkan Range calcicolous chasmophyte communities	*Quercus* sp.
5	43.08858	23.38500	406 m	H3.2A13: Balkan Range calcicolous chasmophyte communities H3.2E: Bare limestone inland cliffs	*Prunus domestica*
6	43.08846	23.38291	408 m	H3.2A13: Balkan Range calcicolous chasmophyte communities H3.2E: Bare limestone inland cliffs T1-9B8: Sub-Mediterranean and Pannonic mixed forests T1-1112: Eastern European poplar-willow forests	*Juglans regia*
7	43.09120	23.38520	671 m	T3-M2: Native pine plantations	*Pinus sylvestris*
8	43.09159	23.38290	633 m	T1-9B222: Moesian oriental hornbeam forests	*Carpinus orientalis*
9	43.08906	23.38168	530 m	H3.2A13: Balkan Range calcicolous chasmophyte communities T1-9B8: Sub-Mediterranean and Pannonic mixed forests	*Quercus* sp.
10	43.08923	23.38209	530 m	H3.2A13: Balkan Range calcicolous chasmophyte communities T1-9B8: Sub-Mediterranean and Pannonic mixed forests	*Quercus* sp.
11	43.09005	23.38510	570 m	H3.2A13: Balkan Range calcicolous chasmophyte communities H3.2E: Bare limestone inland cliffs	*Robinia pseudoacacia*
12	43.09040	23.38606	608 m	H3.2A13: Balkan Range calcicolous chasmophyte communities H3.2E: Bare limestone inland cliffs T1-9B8: Sub-Mediterranean and Pannonic mixed forests	*Fraxinus excelsior*

of trap because it does not use any attractants, making it ideal for our research purposes. Each FIT (our modification of Wilkening et al., (1981) model) with a collecting surface of 0.5 m^2 was filled with a collecting fluid (propylene glycol ($C_3H_8O_2$) (Fig. 4). They were deployed on mid-section of tree crowns, at a 7–9 m above the ground, in order to collect as many ecologically different invertebrates as possible. In Table 1, we have presented the exact locations, main habitat types, and data of the tree species around each

trap. The trap collection period was synchronised with the guano collection periods II and III . After each sampling period, the caught insects from each trap were identified to order and stored for future reference and species identification.

Air temperature data

The temperature and precipitation data were extracted from the European Center for Medium-Range Weather Forecast (ECMWF) fifth-generation Reanalysis (ERA5). The ERA5 is a global reanalysis of weather data, spanning from 1901 to the present, with assimilated data from the surface, upper-air and satellite observations with a spatial resolution of 0.1 degrees (10 km) and temporal resolution of 1 hour (Muñoz-Sabater et al., 2021). The data is extracted as 2 m temperature and daily accumulated precipitation from the surface ERA5-L and datasets with coordinates (N 43.1, E 23.4) and 814 m above sea level altitude of the selected nod. The temperature data is not interpolated to the altitude of 575 m above sea level, which is the altitude of the Razhiskata Cave entrance. Additionally, we recorded the cave temperature with temperature and humidity logger iButtons (model DS1923-F5, Maxim Integrated Products, Inc., Sunnyvale, California) situated close to the colony. They were set to record air temperature and relative humidity twice a day (at 0000 and 1200 hrs) for the whole study period.

Results

Microscope-based morphological analysis

The microscope-based morphological analysis of the guano revealed fragments from 6 taxonomic orders of arthropods (Araneae, Lepidoptera, Diptera, Trichoptera, Coleoptera, and Hemiptera). Lepidoptera was the most frequently occurring order measured by % frequency (%F) (Fig. 5), though many of these remains were attributed to lepidopteran scales. Diptera was the second most frequently encountered order in the pellets, followed by Trichoptera. Coleoptera and Araneae were the least abundant food items identified in the diet during the winter season (<5 %F). Parts from Hymenoptera were traced from the February guano samples but were less than 1 %F. The percentage

frequency of Lepidoptera was higher during February compared to March. Moreover, the percentage of frequency of Trichoptera was almost doubled during March (15 %F in March, 8 %F in February).

The liquid guano sample revealed Lepidoptera was the most frequently occurring order, followed by Diptera, Coleoptera, and Trichoptera. Class Arachnida was the least frequently occurring of invertebrates. The data was skewed because the sample was in liquid form. Thereafter, the interpretation is not as accurate. All raw data is available in Supplementary material 01 [*.xlsx] ➦.

Molecular analysis

From the molecular analyses of the three pooled samples of bat faecal pellets, we identified a total of 215 Operational Taxonomic Units (OTU). The data was further approved by taxonomists and only possible prey items were used for further analyses. The criteria for inclusions were: 1) the taxon is possibly prey for bats; 2) The insect species have been recorded in Bulgaria/Europe. We excluded: fungal taxa, which are potentially growing on the guano in the cave; Amoebozoa or other unicellular organisms which are probably part of the cave fauna; slugs, earthworms, nematodes which are taxa that are not potential prey for bats; skin and internal parasites; taxa with strict extra Palaearctic distribution which are probably contamination or mistake in the reference database, some examples of this are: *Bertmainius tingle* (Main, 1991), *Celatoblatta laevispinata* Johns, 1966, *Declivitata hamata* (Thunberg, 1808) and *Essigella eastopi* Sorensen, 1994. After this initial data elimination, the number of OTUs accepted as potential bat prey was reduced to 66 taxa for the three sampling periods together. Even though some species were amplified with a few reads (<10 repeats), we included them because they were detected in the outside traps, are common in the region, and could potentially be food sources for the bat species. The molecular results show that in the bat guano samples from Razhishka Cave, 5 orders of insects can be distinguished (Fig. 6).

The most abundant taxa revealed by the metabarcoding analyses were *Ypsolopha ustella* (Clerck, 1759), which was recorded during all periods, but the number of reads were 3 times greater during period III; *Emmelina monodactyla* (Linnaeus, 1758) – during all periods, but the number of reads were around
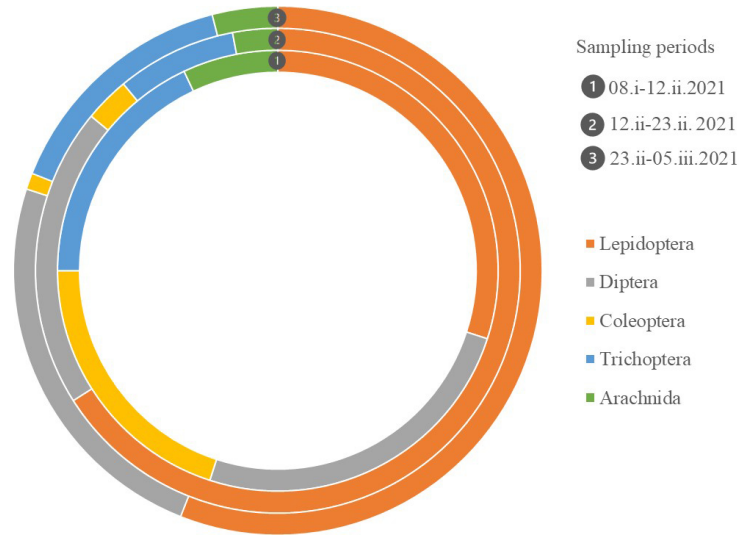
Fig. 5. Percentage frequency of prey orders encountered within bat guano pellets collected from three sample periods in 2021 from the Razhishka Cave – microscope-based morphological analysis.
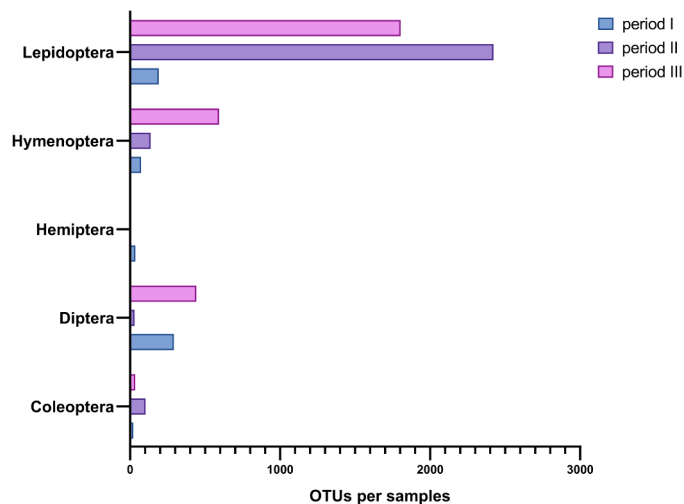

Fig. 6. Summarised results from the metabarcoding sequencing analysis of bat guano samples collected in 2021 from three time periods from the Razhishkata Cave.

200 times greater during period II; *Conistra vaccinii* (Linnaeus, 1761) – during all periods, but the number of reads were 100 times greater during period III; *Eupsilia transversa* (Hufnagel, 1766) – during periods II and III; followed by chalcid wasps and hyperparasitoids of the family Perilampidae – during all periods, but the number of reads were greater during period III; flies *Limnophora tigrina* (Am Stein, 1857) recorded in periods I and III; *Heteromyza rotundicornis* (Zetterstedt, 1846) recorded only in period III; and two

species of the genus *Poecilus* recorded in periods II and III. Raw data is available in Supplementary material 02 [*.xlsx] .

Insect activity

A total number of 2921 arthropod specimens were collected by FIT during the study. Diptera was the most abundant order recorded during the winter, followed by
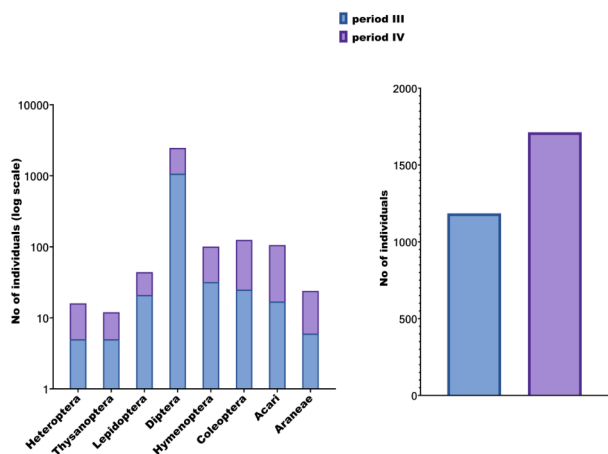
Fig. 7. Summarised results of the insect abundance, collected with FIT during the two sampling periods in 2021 in the studied area of Iskar Gorge.
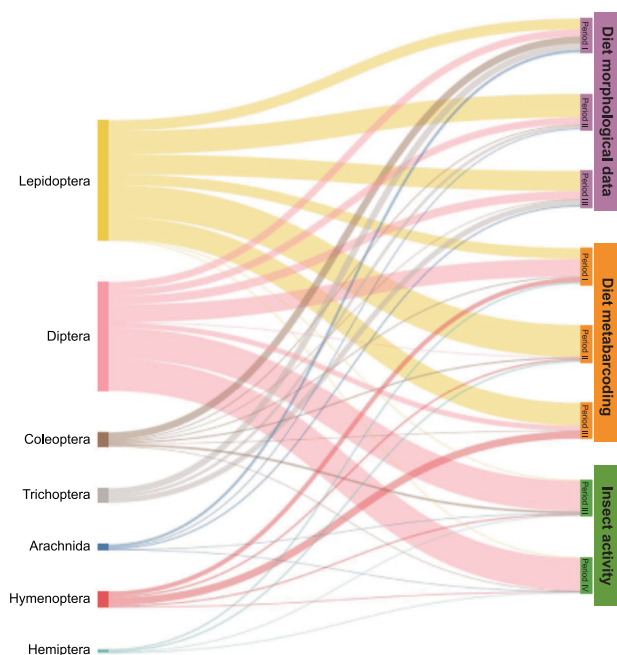

Fig. 8. Summarised results from the relative abundance of prey items in the metabarcoding sequencing analysis and the morphological analyses of the bat guano samples collected in 2021 from the three time periods from the Razhishkata Cave, together with the data for insect activity in the study region.

Hymenoptera and Coleoptera (Figs 7, 8; raw data is available in Supplementary material 03 [*.xlsx]). During our study, we observed temporal changes in insect activity. More Diptera and Lepidoptera were found in February compared to March. Blattodea was recorded only in February, while Neuroptera was recorded in March. For the whole study period, only 44 individuals from the order Lepidoptera were collected in the traps, compared to 2323 individuals from Diptera. The majority of the collected Lepidoptera species are nocturnal, and only 3 of them are known to be active during the day. Thus, almost all of the recorded moths are potential prey for the bats in the region. The activity pattern of beetles is unclear, but some might be active around dusk. Most Coleoptera species are rarely recorded in flight.

The insect orders Lepidoptera, Hemiptera, Coleoptera, Hymenoptera, Diptera (partly) were identified to the lowest possible taxonomic level (Supplementary material 04 [*.xlsx]). During the first sampling period (23.ii–5.iii.2021) Lepidoptera are presented with 11 species of 9 genera. During the second sampling period (5–26.iii.2021), we collected 9 species of 8 genera. Regarding Coleoptera, for the first period, we recorded 18 species of 17 genera; for the second period – 8 species from 8 genera respectively. Diptera: first period 20 species from 7 genera and second period – 21 species from 8 genera. Hymenoptera: first period 21 species from 12 genera and second period – 13 species from 7 genera. Hemiptera: first period 9 species from 8 genera and second period – 5 species from 5 genera. As all specimens of order Araneae collected in the traps were juveniles, their species identification based on morphology was not feasible.

Air temperature data

We performed a Pearson r correlation analysis to compare the effect of the outside temperature on the cave temperature at the colony's location. For this purpose, we used the daily average temperature from the surface and the daily average from the cave. The r value was 0.53, and the R squared value was 0.28. There is a significant positive correlation between the outside temperature and the cave temperature, with a P-value of 0.0001. These values suggest that the temperature inside the cave is influenced by the air temperature outside the cave. Only 30% of the temperature variance inside the cave can be attributed to internal cave factors. Since the temperature data from outside the cave is representative of 814 metres above sea level and the entrance of the cave is at 575
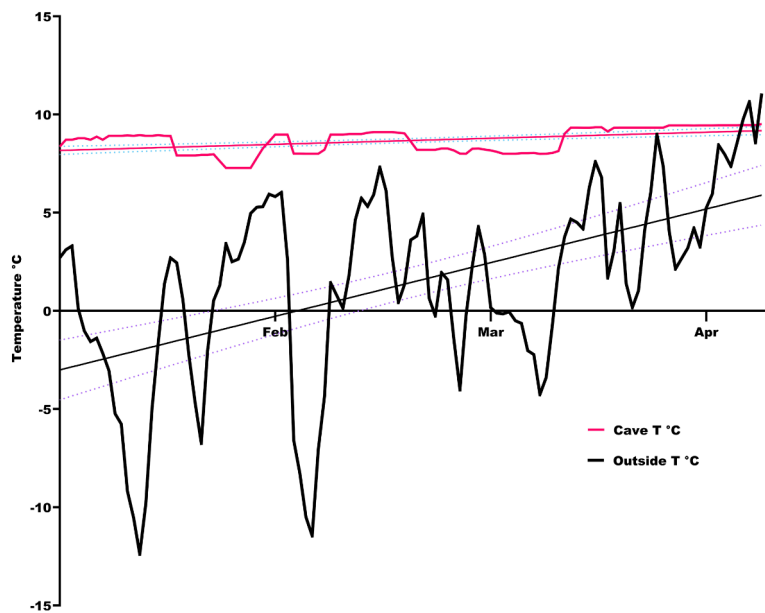
Fig. 9. Average daily temperature fluctuations in Razhishkata Cave and outside the roost.

Discussion

Our study is the first regarding the winter activity and dietary preference of bat diet of Schreiber's bent-winged bat. The winter diet of only four European bat species has been studied so far: the Mediterranean horseshoe bats (*Rhinolophus euryale* Blasius, 1853) from Slovakia and Hungary (Miková et al., 2013), the lesser horseshoe bat (*Rhinolophus hipposideros* (Bechstein, 1800)) from Great Britain and Ireland (Willams et al., 2011), the Natterer's bat (*Myotis nattereri* (Kuhl, 1817) from Great Britain (Hope et al., 2014) and noctule bat (*Nyctalus noctula* (Schreber, 1774)) from Slovakia and the Czech Republic (Kanuch et al., 2005). Three of the studies used only morphological diet identification. In the study from 2014, Hope et al. used molecular and morphological diet approaches to determine the winter diet of *Myotis nattereri*. Our study was the first to combine both approaches with an intensive insect survey in the

metres above sea level, the altitude difference of 239 metres introduces a negative bias of between 1.4–1.8 K. This is one of the contributing factors to the temperature bias between the temperature outside and inside the cave. The graphic representation of the data is presented in Fig. 9. All raw data is available in Supplementary material 05 [*.xlsx] ☑.

studied area and the first to look at the winter diet of the Schreiber's bent-winged bat (*Miniopterus schreibersii*). In addition, we also collected data for correlation of the temperature fluctuation in and outside the roost with an ultimate goal to see how this is affecting the bat activity patterns. Even though we could not measure the total guano accumulated in the guano traps during our study, we could observe differences between the studied periods. The coldest winter period was also followed by the least droppings gathered on the guano sheet. This also corroborates other European studies showing that outside temperatures and wind speed strongly affected bat foraging behaviour during the winter (Avery, 1986; Barros et al., 2017; Mas et al., 2022). Thus, we argue that the accumulation of guano can be used to measure Schreibers' bent-winged bat activity during the winter. This gives information on the activity outside the roost and samples for their trophic interactions.

Given the limitations of our study, we could only assess the most frequently occurring (FO) prey item in the diet. Metabarcoding on pooled samples leads to high error rates in estimating the FO rate of prey items (Mata et al., 2018). Furthermore, common prey species will become much more abundant but less common prey will be in an even smaller proportion in the pool samples (Mata et al., 2018). The analysis of a single pellet is as likely to detect abundant species as is the analysis of a pooled sample (Mata et al., 2018). Thus,

pooled and small sample-size metabarcoding research can be used for low-cost and preliminary research, especially during periods when prey availability and overall prey diversity are scarce. Additionally, the molecular data used in our study was under rigid validation by experienced taxonomists. This validation was crucial as a fraction (5%) of the assessed prey items were species and genera that occur neither in Bulgaria nor in the Palaearctic. Their presence in the studied species' diet is probably a mistake from the taxonomic assignment or initial lab contamination. Hence, we argue that combining metabarcoding with specialist checks for the taxa and insect surveys in the region which provide a reference "menu" of the available food resources, is essential for higher levels of confidence in the results and better interpretation of the bat diet.

With the metabarcoding, we could identify insects that were not detected in the traps, like the common moth species *Emmelina monodactyla* (Linnaeus, 1758), which is indeed active in this region and has been frequently observed during the winter (our data with UV traps). All of the most abundant moth species detected via barcoding are nocturnal. According to our observations in the region, they are active all winter if the temperature is above 0°C in the evening. A lot of the detected in metabarcoding bees (*Andrena helvola* (Linnaeus, 1758), *Bombus* spp.), wasps (*Vespa crabro* Linnaeus, 1758, *Cerceris rybyensis* (Linnaeus, 1771), *Sphex funerarius* Gussakovskij, 1934)), flies (*Acemya* sp., *Atherigona soccata* Rondani, 1871, *Chrysomya* sp. , *Cheilosia soror* (Zetterstedt, 1843), *Eristalis pertinax* (Scopoli, 1763), *Hebia flavipes* Robineau-Desvoidy, 1830, *Heteromyza rotundicornis* (Zetterstedt, 1846), *Hydrotaea basdeni* Collin, 1939, *Limnophora tigrina* (Am Stein, 1860), *Phaonia valida* (Harris, 1780), *Silba adipata* McAlpine, 1956), butterflies (*Maniola jurtina* (Linnaeus, 1758)) have a diurnal activity. Rarely *Bombus* spp. or some of the above-mentioned flies are active at dusk, but only in warmer periods of the year (our observations). Presence of prey with obligatory diurnal activity in the identified diet items, suggests that the bats might also be feeding during the day. We could only speculate that bats have a diurnal activity or an activity shifted towards twilight during the winter period (our previous observations of flying bats at noon in similar habitats in the winter). This confirms some observations for other areas and periods of the year (Russo et al., 2011; Mikula et al., 2016; Malmqvis et al. 2018). Some studies have claimed that bats might feed

on the cave fauna during the winter (Kanuch et al., 2005; Sano, 2006). Nine non-parasitic insect species (6 Lepidoptera, 1 Hymenoptera and 2 Diptera) are recorded in the well-studied Razhishkata Cave (Beron, 2015). In the metabarcoding analysis, none of the detected prey items were cave dwellers and the above-mentioned species were also missed. We argue that the densities of their populations are very low and cannot be considered a substantial food resource for bats during any period of their life. Our results showed that winter insect activity in the region near the roost provides the bats with enough food opportunities.

The flight interception traps give a good and attractant-independent picture of the number of flying insects. This together with the easy maintenance were the reasons for choosing them for this study. On the other hand, our results indicate that the FITs are not the best alternative for estimating the species diversity of potential Schreibers' bent-winged bat prey. A lot of common noctuid moth species in the Lakatnik region, collected by UV light (our observations) were missed in our material. The reasons could be the smaller population density of the winter active moths in comparison to other insect groups. The light traps with UV-rich spectra are the best methods for collecting nocturnal moths, but relying on attraction, their use can produce unrealistic assessment of the population density compared to other insects that are not attracted to artificial light. In general, for future studies, the combination of different trap techniques (light traps with UV-rich spectra, Malaise traps, pitfall traps, Langourov type tree traps) would be a good solution.

Many factors affect the temperature inside caves and the underground temperature is strictly connected with the external climate. Consequently, the global climatic changes will somewhat influence the cave climate (Badino, 2004). Our data shows that the temperature in Razhishkata Cave, at the location of the bat colony, positively correlates with the temperature fluctuation outside the cave. This might have a strong influence on the activity patterns of the bats. Many of the winter colonies of the Schreibers' bent-winged bat in the important bat underground habitats in Bulgaria (Ivanova, 2005; Deleva et al., 2022) are situated at a similar distance from the roost entrance as in the Rezhishkata Cave (own observations). It could be speculated that bats choose sites for hibernation in the winter roosts that are relatively close to entrances and temperature fluctuations could be felt. This may give them an advantage and opportunity to be active in

winter with good weather and presumed winter activity of the insects. *Miniopterus schreibersii* selects warmer caves to hibernate (Barbu, 1958; Pandurska, 1993). During the first sampling period, strong snow melting led to significant water dripping from the ceiling of the studied cave. As a result, the bat cluster was more spread out which could lead to decreased advantages from the social thermoregulation (Willis & Brigham, 2007). After the heavy snowfalls, during the last sampling period and flooding in the cave, the colony changed its location to a drier place close to the entrance. More data regarding temperature fluctuation in caves at the locations of the bat colonies, and how this is affecting winter bat activity is needed. Additionally, more information on the flooding vulnerability in the important winter underground bat roosts will be key for identifying sites under risk and ultimately can lead to better conservation planning.

Lastly, we want to elaborate on some practical tips for future research. The selection of the study cave should consciously consider easy to access and survey winter roost; a flat floor under the colony is preferable and the size of the guano trap should be larger than the colony's size. Considering that winter is sensitive period of the life cycle of the bats and frequent visits to the roost could lead to disturbance following all the rules and recommendations is essential. Good knowledge of the potential spatial positions of the colonies will help to predict other locations for traps in the event of in-roost activity of the colony. Samples should be measured and collected weekly for better resolution. Including acoustic data for the bats exiting the roost, together with data on the temperature and humidity fluctuations could provide a good picture for understanding the winter activity patterns of bats of the Balkan Peninsula and Europe. Our main goal was to establish a low-cost and easy-to-replicate research framework which we plan to repeat once every few years. Ideally in the future, a long-term dataset could potentially shed light on the effect of climate change on hibernating bats in temperate regions. Hence, publishing this first season of data collection was a way to propose this strategy on a broader scale and inspire similar studies in different locations.

Availability of data and material

All datasets generated and analysed during the current study are available as supplementary materials.

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Conflicts of interest

The authors have no conflicts of interest to declare. All co-authors have seen and agree with the contents of the manuscript and there is no financial interest to report. We certify that the submission is original work and is not under review at any other publication.

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Supplementary materials

01
Document title: Raw data collected during the microscope-based morphological analysis used in the study
Kind of document: Microsoft Excel (OpenXML)
MIME type: application/vnd.openxmlformats-officedocument.spreadsheetml.sheet
Document name: 000513000452023-01.xlsx

02
Document title: Validated and raw metabarcoding data used in the study
Kind of document: Microsoft Excel (OpenXML)
MIME type: application/vnd.openxmlformats-officedocument.spreadsheetml.sheet
Document name: 000513000452023-02.xlsx

03
Document title: Flight interception traps data (February-March) presenting all the invertebrate orders recorded in the study
Kind of document: Microsoft Excel (OpenXML)
MIME type: application/vnd.openxmlformats-officedocument.spreadsheetml.sheet
Document name: 000513000452023-03.xlsx

04
Document title: Flight interception traps data (February-March) listing the model groups recorded in the study
Kind of document: Microsoft Excel (OpenXML)
MIME type: application/vnd.openxmlformats-officedocument.spreadsheetml.sheet
Document name: 000513000452023-04.xlsx

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Research article

Extraordinary occurrence of Pallas's leaf warbler (*Phylloscopus proregulus* Pallas, 1811) in Bulgaria and the Balkan Peninsula in the autumn and winter of 2022/2023

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Abstract: Four Pallas's leaf warblers (*Phylloscopus proregulus*) were captured at Durankulak ringing camp, NE Bulgaria, in frames of four days between 12 and 16 October 2022. The species is a rare vagrant in Bulgaria and neighbouring Balkan countries. It has not been captured and ringed neither at Durankulak nor elsewhere in Bulgaria until now. Annual population fluctuations and weather conditions are discussed as possible explanations for that influx.

Keywords: Durankulak, migration, Pallas's leaf warbler, vagrancy, weather

Introduction

During recent decades the number of records of vagrants of Siberian origin in Europe increased substantially (De Juana, 2008; Dufour et al., 2022). Vagrancy can have intrinsic or/and extrinsic causes (Dufour et al., 2022). Among the extrinsic causes weather conditions or magnetic anomalies are often involved (Alestam, 1991; Zawadski et al., 2019). Mutations of the genes coding migratory orientation are pointed as the main intrinsic cause of vagrancy although the wrong use of inherited cues for orientation during the ontogeny of a bird could also have a similar effect (Thorup, 1998; Akesson & Helm, 2020; Dufour et al., 2022). The results in some cases are mirror-image navigational errors of migrants which were first described in North America (Diamond, 1982; De Sante, 1983). Even the creation of new migration routes of these birds, or the "pseudo-vagrancy" and "reverse migration" hypotheses has been discussed (Gilroy & Lees, 2003). Reverse migration itself can be the reason for vagrancy in some birds (Thorup, 2004). In some studies, the vagrancy of these birds was linked to climate change through predicted changes in the climatic suitability of their breeding ranges (Jiguet et al., 2013).

In the present article, we discuss an extraordinary influx of a Siberian vagrant − Pallas's leaf warbler

(*Phyllloscopus proregulus* Pallas, 1811) in Bulgaria and neighbouring Balkan countries.

Pallas's leaf warbler is a rare vagrant in Bulgaria and neighbouring Balkan countries (Sciban et al., 2015; Ivanov et al., 2019; Marton, 2020; AOS, 2020). Its breeding range lies in Central and Eastern Siberia, Mongolia, Central and Northeastern China, and Sakhalin Island. Normally the species spends the winter in Southern China, Thailand, Laos, India, and Vietnam (Cramp, 1992). As a vagrant Pallas's leaf warbler is much more common and numerous in NW and W Europe – in Britain, Ireland, Denmark, Netherlands, Finland, Sweden, etc. and the number of records there increased notably after the 1960s (Cramp, 1992). After the mid-2000s the number of observations decreased in the UK and Germany (White & Kehoe 2015).

The species has not been captured and ringed neither at Durankulak nor elsewhere in Bulgaria until now. Up to December 2019, the species has been recorded 7 times in the country (Ivanov et al., 2019). Five of the records were supported by photographs. Six of these seven sightings came from the coastal Dobrudzha, NE Bulgaria – four at Cape Kaliakra (15.10.1996 – P. Oliver, P. Redman; 14.10.2013 – A.Williams; 28.04.2018 – George and Paul Gay; 19.10.2018 – S. Fischer), one at Shabla Lake (18–20.10.2016 – Steve & Pauline Fischer), and one at Durankulak (18.10.2015 – Pavel Simeonov). Only one observation was reported from Sofia, W Bulgaria (Nankinov & Dalakchieva, 1999). These observations were made during the period 1996–2018, all of them except one in autumn, in October and November.

Material and methods

Durankulak ringing camp operates every autumn since 2019. It is situated at the SE part of the Durankulak

Lake, Dobrich Province, NE Bulgaria, at coordinates N43°39'38.49 and E28°33'56.8. In 2022 ringing activity started on 14 August and continued until 31 October. Vertical nets with a total length of approximately 220 m were placed at two habitats – reed massif and low broad-leaved forest with bushes. In 2022 a total of 13703 birds of 92 species were captured. Sound lures for attracting some species of migratory birds were used. Pallas's leaf warbler was included in the list of bird species attracted using lures in October 2022.

Results

Four Pallas's leaf warblers were captured at Durankulak ringing camp, NE Bulgaria, on 12, 14 and 16 October 2022 (Figs 1–2). On 16 October two individuals were captured. All these birds were captured in the forest nets. Measurements of the Pallas's leaf warblers are presented in Table 1.

Description of the Pallas's leaf warblers captured

Very small warblers with distinct yellowish supercilium and head stripe. Clear black eye stripe. The general colour of the upperparts is olive-greenish. Two distinct lemon-colored wing bars are clearly visible. The rump is lemon-coloured, the tail is greenish. Underparts greyish-white. The sex is determined by the wing and tail measurements (Cramp, 1992; Bozo & Heim 2016). Wing formulas are checked and determination is confirmed according to the wing formula presented by Svensson (1992).

During the period 12–16 October the weather was dynamic, partially cloudy, without rainfalls, with moderate to strong N-NE winds (8–30 km/h during the day) and temperatures between 7 and 20 °C.

Table 1. Measurements of captured Pallas's leaf warblers.

No. of individual	Date	Sex	Wing, mm	Tail, mm	Weight, g	Fat score
1	12.10.2022	male	52,5	39	5,4	3
2	14.10.2022	male	53,5	37	4,7	1
3	16.10.2022	female	48	34	4,7	3
4	16.10.2022	female	49,5	34	4,9	2

Fig. 1. Pallas's leaf warbler (*Phylloscopus proregulus*) captured on 12.10.2022, Durankulak, NE-Bulgaria, photo: Ivaylo Tonev.

Fig. 2. Pallas's leaf warbler (*Phylloscopus proregulus*), captured on 16.10.2022, Durankulak, NE Bulgaria, photo: Pavlina Taseva.

Table 2. Records of Pallas's leaf warbler in Balkan region during 2022/2023 and in previous years.

Country	Records during 2022/2023 autumn/winter	Records in previous years
Bulgaria	4 ind. captured at Durankulak, present study 9.11.2022, Sofia (Georgi Kamov, ebird.org 🔗) 8.10.2022, Bolata, Dobrich District (Chris Day and Teodor Trifonov, pers. comm.)	7 records until 2019 (Ivanov et al., 2019)
Romania	October 2022, 3 ind. captured, Chituc ringing camp (https://www.facebook.com/chitucringingcamp/ 🔗) 16.10.2022, Agigea ringing station (https://www.facebook.com/profile.php?id=100064458441581 🔗)	At least 13 records: Chituc ringing camp – 6 captured and 5 observed there and in Danube Delta (Droz, 2014; Marton, 2020). Agigea ringing station – 2 captured, 2018 and 2021 (https://www.facebook.com/profile.php?id=100064458441581 🔗)
Serbia	24–30.03.2023, Belgrade (https://macaulaylibrary.org/asset/551392801 🔗)	28 October 2008, Stanisic, Sombor (Sciban et al., 2015)
Greece	14.01–11.02.2023, Kerkini Lake, Northern Greece (ebird.org 🔗)	3–12.04.2011, Athens (ebird.org 🔗)
Turkey	1–3.11.2022, Istanbul (Cagan Abasoglu, https://www.facebook.com/photo/?fbid=429859789303749&set=pcb.909103303796111 🔗) 7.01–1.03.2023, Istanbul (ebird.org 🔗) 13.11.2022, Kocacay Delta (Alper Teydes, ebird.org 🔗) 2.11.2022, Aras ringing station (ebird.org 🔗)	3 records in 2010–2011 (Kirwan et al., 2014) 3 records in Kizilirmak River Delta – on 6.11.2014, 17.10.2017, and 26.10.2021 (ebird.org 🔗)
Albania	—	14.01.2020 (AOS, 2020)

High number of observations of Pallas's leaf warbler in 2022/2023 autumn–winter season was reported not only from Durankulak ringing station but also from other locations in the Balkan region (Table 2). We may speculate that there is a moderate influx of the species in that region with much higher number of records of the species than normally.

Discussion

Mass occurrence of Siberian vagrants and especially Pallas's leaf warblers has been explained by the westward movement in anticyclonic conditions (Baker 1977; Howey & Bell 1985). In another study, it was found that inter-annual variability in the westwards displacement of yellow-browed warblers (*Phylloscopus inornatus* (Blyth, 1842)) and Pallas's leaf warblers to Europe is not correlated with predominant easterly airflow (Van Impe & Derasse, 1994). Other explanations include the breeding population growth in the particular year combined with favourable weather conditions, including winds (Zawadski et al., 2019). In the same study, it was shown that population growth and indices have prevailing importance in the explanation of vagrancy compared to weather conditions. Thus in the case of Pallas's leaf warbler, presented by us, we consider that the dynamic weather with prevailing NE winds created good conditions but did not explain alone the observed influx. Obviously these the factors causing the observed phenomenon are much more complex – they include annual fluctuations in population offspring production and particular weather conditions.

Taking in account the numbers of existing records normally the main wave of Siberian vagrants, including Pallas's leaf warblers, cross Europe during autumn to the north from the Balkan Peninsula (Van den Berg & Bosman, 1999; De Juana, 2008). The observations of these vagrants made in N and NW Europe are much more numerous compared to SE Europe and that fact probably cannot be explained only by the much higher number of observers in the first region. Hopefully when we have a good long-term dataset of observations also in SE Europe we will see the real large-scale situation of eastern vagrancy in Europe. We can conclude that only in favourable years with good population growth and appropriate weather conditions in the period October–November we can expect a more evident presence of Pallas's leaf

warblers in the Balkan region. The background of the observed phenomenon is not known exactly and further studies are needed.

Acknowledgments

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Research article

First record of *Clogmia albipunctata* and *Atrichobrunettia* (*Mirousiella*) *graeca* (Diptera: Psychodidae) from Romania, with the updated checklist of non-Phlebotominae species

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Abstract: The rare European species *Atrichobrunettia* (*Mirousiella*) *graeca* Ježek et Goutner, 1993 and the invasive species *Clogmia albipunctata* (Williston, 1893) (both Diptera: Psychodidae) are newly recorded from "Dunele Marine de la Agigea" Nature Reserve from Romania. The updated checklist of non-Phlebotomine species of the family Psychodidae for Romania with 53 species is given.

Keywords: biodiversity, checklist, Europe, Maruinini, Mormiini, Palaearctic Region, Paramormiini, Pericomaini, Psychodinae, Psychodini, Sycoracinae, Trichomyiinae

Introduction

Psychodidae (Diptera: Nematocera) is a family of small hairy midges, known as "moth flies" and "sand flies", with approximately 3000 described species worldwide (e.g. Pate et al., 2011; Curler & Moulton, 2012; Morelli & Biscaccianti, 2021). The sand flies (subfamily Phlebotominae) with nine species are well studied in Romania (e.g. Lupaşco et al., 1965; Dancesco, 2008; Cazan et al., 2019a, b, 2021a, b.; Dvorak et al., 2020). The non-phlebotomine moth flies (subfamilies: Bruchomyiinae, Psychodinae, Sycoracinae, and Trichomyiinae) are not sufficiently researched in Romania.

Therefore, the main goal of this work is to publish new faunistic records and to summarise and re-evaluate the checklist of non-Phlebotominae species of the family Psychodidae of Romania.

Material and methods

The material presented in this work was caught using a Malaise trap in Agigea (Constanţa County, "Dunele Marine de la Agigea" Nature Reserve, 44°05'16.4"N 28°38'32.3"E, 13 m a.s.l. (locality – Fig. 1); Romania) by A.-M.P. The samples were stored in 96% ethanol and subsequently determined by J.O. using Ježek & Goutner (1993) and Ježek & van Harten (2009). The material is deposited in the ethanol collection in the Department of Ecology, Faculty of Humanities and Natural Sciences, University of Prešov, Slovakia.

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Fig. 1. The "Dunele Marine de la Agigea" Nature Reserve – the location of Malaise trap used in the summer season in 2022 (recent photo: March 2023).

Results and discussion

Faunistic results

Family Psychodidae
Subfamily Psychodinae
Tribus Mormiini
Subtribus Brunettiina

Atrichobrunettia (*Mirousiella*) *graeca* Ježek et Goutner, 1993

Material examined: 1 ♂ (Fig. 2), 25.viii–6.ix.2022, Malaise trap, A.-M.P. leg, J.O. det.

   Rare species known only from Greece, Albania, Czech Republic, Great Britain, Ireland (*angustipennis* sensu Withers, 1989) (Ježek & Goutner, 1993; Ježek, 2003; Ježek & Omelková, 2012). First record for Romania.

Tribus Paramormiini
Subtribus Paramormiina

Clogmia albipunctata (Williston, 1893)

Material examined: 1 ♂ (Fig. 3), 6–13.ix.2022, Malaise trap, A.-M.P. leg, J.O. det. Expansive, often synanthropic, circumtropical and circumsubtropical species (e.g. Ježek & Goutner, 1995; Oboňa & Ježek, 2012; Humala & Polevoi, 2015; Afzan & Belqat, 2016; Bejarano & Estrada, 2016; Cazorla-Perfetti & Moreno, 2017; Ježek et al., 2018; Cazorla-Perfetti, 2019; Oboňa et al., 2019, 2021; Salmela et al., 2019; Zittra et al., 2020; Morelli & Biscaccianti, 2021; Jaume-Schinkel et al., 2022; Martynov et al., 2022). Its occurrence in Romania has not yet been published. First record for Romania.

   With the exception of epidemiologically interest-ing species from the subfamily Phlebotominae (e.g.
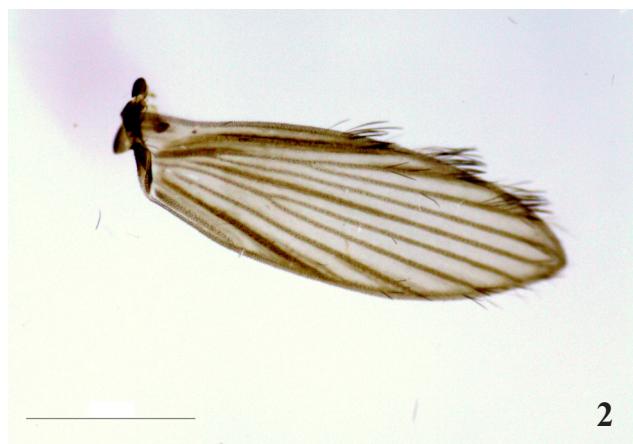
Figs 2, 3. The wings of *Atrichobrunettia* (*Mirousiella*) *graeca* Ježek et Goutner, 1993 (2) and *Clogmia albipunctata* (Williston, 1893) (3); scales: 0.5 mm.

Cazan et al., 2019b), the family Psychodidae is under-researched in Romania. Firstly, Vaillant & Botoşăneanu (1966) published 23 species from Romania. These records are complemented mainly based on records by Botoşăneanu & Vaillant (1965), as well as Vaillant (1963), (1971–1983), etc. Summarisation was also added in Fauna Europaea by Wagner (2013) with 38 records of non Phlebotominae species of this family. However not all species mentioned in previous papers are summarised here (13 species miss).

Altogether, 53 species are listed in the present checklist of non-Phlebotominae species of family Psychodidae in Romania (Sycoracinae: 2 spp., Trichomyiinae: 1 sp., Psychodinae: 50 spp. (in detail: Mormiini: 2 spp., Paramormiini: 12 spp. Pericomaini: 23 spp., Psychodini: 12 spp., Maruinini: 1 sp.)).

The updated checklist of non-Phlebotominae species of the family Psychodidae of Romania
(sensu Vaillant, 1963; Botoşăneanu & Vaillant, 1965; Vaillant & Botoşăneanu, 1966; Vaillant, 1971–1983; Wagner, 2013)

Family Psychodidae

Subfamily Sycoracinae
Sycorax silacea Haliday in Curtis, 1839
Sycorax similis (Müller, 1927)

Subfamily Trichomyiinae
Trichomyia urbica Haliday in Curtis, 1839

Subfamily Psychodinae
Tribus Mormiini
Subtribus Brunettiina
Atrichobrunettia (*Mirousiella*) *graeca* Ježek et Goutner, 1993 *

Subtribus Mormiina
Yomormia banatica (Vaillant, 1974)

Tribus Paramormiini
Subtribus Paramormiina
Clogmia albipunctata (Williston, 1893) *
Jungiella (*Jungiella*) *botosaneanui* (Vaillant, 1963) (larva only)
Jungiella (*Jungiella*) *soleata* (Walker, 1856)
Jungiella (*Jungiella*) *valachica* (Vaillant, 1963)
Panimerus albifacies (Tonnoir, 1919)
Panimerus notabilis (Eaton, 1893)
Paramormia (*Duckhousiella*) *ustulata* (Walker, 1856)
Paramormia (*Phyllotelmatoscopus*) *decipiens* (Eaton, 1893)
Peripsychoda auriculata (Haliday in Curtis, 1839)

Subtribus Trichopsychodina
Philosepedon (*Philosepedon*) *carpaticum* Vaillant, 1974
Philosepedon (*Philosepedon*) *kalehnum* Vaillant, 1974
Philosepedon scutigerum Vaillant, 1963 ? (larva only)

Tribus Pericomaini
Berdeniella bucegica Vaillant, 1976
Berdeniella unispinosa (Tonnoir, 1919)
Clytocerus (*Boreoclytocerus*) *ocellaris* (Meigen, 1818)

Parabazarella subneglecta (Tonnoir, 1922)
Pericoma (*Botosaneanuiella*) *attenuata* Vaillant, 1978
Pericoma (*Pachypericoma*) *blandula* Eaton, 1893
Pericoma (*Pericoma*) *calcilega* Feuerborn, 1923
Pericoma (*Pericoma*) *incrustans* Vailant, 1978 (larva only)
Pericoma (*Pericoma*) *motasi* Vaillant, 1978
Pericoma (*Pericoma*) *pingarestica* Vaillant, 1978
Pneumia bucegiana (Vaillant, 1981)
Pneumia canescens (Meigen, 1804)
Pneumia nubila (Meigen, 1818)
Pneumia palustris (Meigen, 1818)
Pneumia vittata (Tonnoir, 1919)
Saraiella austriana (Vaillant, 1963)
Saraiella carpatica Vaillant, 1981
Saraiella crypta (Vaillant, 1955)
Saraiella parva (Vaillant, 1963)
Tonnoiriella pulchra (Eaton, 1893)
Ulomyia fuliginosa (Meigen, 1818)
Ulomyia ophicornis Vaillant, 1983
Ulomyia rostrata Vaillant, 1983

Tribus Psychodini
Chodopsycha lobata (Tonnoir, 1940)
Feuerborniella obscura (Tonnoir, 1919)
Logima albipennis (Zetterstedt, 1850)
Logima erminea (Eaton, 1893)
Psychoda phalaenoides Linnaeus, 1758
Psychodocha cinerea (Banks, 1894)
Psychodocha gemina (Eaton, 1904)
Psychodula minuta (Banks, 1894)
Psychomora trinodulosa (Tonnoir, 1922)
Tinearia alternata (Say, 1824)
Tinearia lativentris (Berden, 1952)
Ypsydocha setigera (Tonnoir, 1922)

Tribus Maruinini
Lobulosa transsylvanica (Szabó, 1960)

The new country records are marked by an asterisk. *P. scutigerum* Vaillant, 1963 is known only from the larva. According to Vaillant (who doubts the correctness of the taxon), it could (see the Vaillant's comments in the Lindner's monography 9d, 305 (1974), p. 119) also be a synonym of the species *P. balcanicum* Krek, 1971, *P. mayeri* (Satchell, 1955) or *P. soljani* Krek, 1971, or, according to today's view it could be provisionally included in one of the subgenera

Trichosepedon Krek, 1999 or *Philothreticus* Krek, 1999. Therefore, the species is not even mentioned by Omelková & Ježek (2012).

From 53 species recorded, three are known only based on larvae. One recorded species, *C. albipunctata* appears to be as a dangerous species. This invasive, often synanthropic species may pose a risk for native synanthropic species, rare tree holes species, and also is epidemiological significant as a possible causal agent of various myiasis (e.g. Oboňa & Ježek, 2012; Cazorla-Perfetti, 2019; Martynov et al., 2022; Jaume-Schinkel et al., 2022). From the ecosozological point of view, *A.* (*M.*) *graeca,* together with *T. urbica, Y. banatica, P.* (*P.*) *decipiens, P.* (*P.*) *carpaticum, P.* (*P.*) *kalehnum, B. bucegica, P.* (*B.*) *attenuata, P.* (*P.*) *motasi, P. bucegiana, P. austriana, S. carpatica, S. crypta, S. parva, U. ophicornis, U. rostrata,* and *L. transsylvanica* are considered to be rare species and may be included in the Romanian red list in the future.

The investigation of non-Phlebotominae Psychodidae in Romania is still far from finished. This argument can by supported by Ježek et al. (2020), who examined Psychodidae fauna in a neighbouring country, Bulgaria, with 99 species.

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