

Bats prey on amphipods of Lake Baikal: morphological and genetic evidence

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ABSTRACT. Study aims to assess the prevalence of endemic amphipods (Crustacea, Gammaroidea, Amphipoda) in the diet of insectivorous bats (Chiroptera) feeding over the surface of the unique freshwater Lake Baikal. In 2020–2023, 41 samples of bat faeces (mainly *Myotis petax*) were collected at 14 sampling sites in the water area, on the shore and islands of Lake Baikal. The body fragments of amphipods in faecal samples were identified using a light microscope. DNA was extracted from collected faecal samples and screened for presence of pelagic amphipod species *Macrohectopus branickii*. A fragment of the *COI* mitochondrial gene was amplified in three rounds of nested PCR. All newly sequenced fragments demonstrated 92–100% identity to sequences of the same species deposited in GenBank. DNA of *Ma. branickii* was detected in five of 41 samples (12.2%). Microscopic analysis revealed body fragments of *Ma. branickii* and of three benthic amphipod species (*Micrurus wohlii wohlii*, *Mi. wohlii platycercus*, and *Gmelinoides fasciatus*) as well as unidentified amphipod fragments in six of 18 samples (33.3%). Amphipod remains were detected in small amounts in many examined samples, but insect remains visually predominated at microscopy. Thus, the use of molecular and morphological approaches facilitated identification of remains of four amphipod species in samples from seven of 14 sampling sites. Direct evidence were received for new trophic interactions in Lake Baikal ecosystem: bat *My. petax* feeds on several species of endemic amphipods catching them during periodic nocturnal ascents to the water surface.

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Летучие мыши охотятся на амфипод озера Байкал: морфологические и генетические доказательства

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РЕЗЮМЕ. Цель исследования — оценить встречаемость эндемичных амфипод (Crustacea, Gammaroidea, Amphipoda) уникального пресноводного озера Байкал в рационе питания насекомоядных летучих мышей (Chiroptera). В 2020–2023 гг. была собрана 41 проба фекалий летучих мышей (в основном *Myotis petax*) в 14 точках над акваторией, на побережье и островах озера Байкал. Фрагменты амфипод в образцах фекалий идентифицировали с помощью светового микроскопа. Все пробы фекалий были исследованы молекулярно-генетическими методами на наличие ДНК пелагического вида амфипод *Macrohectopus branickii*. Фрагмент митохондриального гена *COI* амплифицировали в трех раундах вложенной ПЦР (nested PCR) со специфическими для данного вида праймерами. ДНК *Ma. branickii* обнаружена в пяти из 41 проб (12.2%). Полученные последовательности были на 92–100% идентичны последовательностям ДНК того же вида, депонированным ранее в GenBank. Микроскопический анализ выявил фрагменты хитинового скелета *Ma. branickii* и трех таксонов бентосных амфипод (*Micrurus wohlii wohlii*, *Mi. wohlii platycercus* и *Gmelinoides fasciatus*), а также фрагменты не идентифицированных видов амфипод в шести из 18 проб (33.3%). При микроскопии остатки амфипод обнаруживались в пробах в небольших ко-

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личествах на фоне преобладания остатков насекомых. Таким образом, использование морфологического и генетического подходов позволило обнаружить остатки амфипод четырех видов в копропробах в семи из 14 точек сбора. Получены прямые доказательства новых трофических связей в экосистеме Байкала: *My. petax* использует в пищу эндемичных амфипод нескольких видов, отлавливая их во время периодических ночных подъемов к поверхности воды в разных частях озера Байкал.

КЛЮЧЕВЫЕ СЛОВА: летучие мыши, *Myotis petax*, амфиподы, питание, трофические связи, озеро Байкал.

Introduction

Several species of bats (water-surface foragers or “trawling” bats) prefer to hunt over water bodies where they can grab their prey from the water surface with hind claws. In Palearctic region, the pond bat *Myotis dasycneme* (Boie, 1825), the Japanese large-footed bat *Myotis macrodactylus* (Temminck, 1840), the long-fingered bat *Myotis capaccinii* (Bonaparte, 1837), Daubenton’s bat *Myotis daubentonii* (Kuhl, 1818) and the eastern water bat *Myotis petax* (Hollister, 1912) belong to this specialized group. The echolocation systems of these bats enable them to detect water disturbances caused by surfacing aquatic organisms or falling flying insects (Kalko & Schnitzler 1989; Boonman *et al.*, 1998; Siemers *et al.*, 2001b; Sommer *et al.*, 2019). Insects from orders Diptera, Trichoptera, and Ephemeroptera are the most frequent food resources of water-surface foragers (Boonman *et al.*, 1998; Siemers *et al.*, 2001b; Shiveley *et al.*, 2018).

However, bats hunting over water may exploit more than just insect prey. Ahlén *et al.* (2009) proposed that bats *My. dasycneme* and *My. daubentonii* hunted crustaceans over the North Sea when these invertebrates were abundant near the water surface whereas flying insects were scarce (Ahlén *et al.*, 2009). Microscopic and genetic analysis of faecal samples revealed that *My. capaccinii* and *My. daubentonii* are capable of preying on fish in a similar way (Siemers *et al.*, 2001a; Aizpurua *et al.*, 2013; Sommer *et al.*, 2019). A similar data has been published on *Myotis adversus* (Horsfield, 1824) in Australia (Robson, 1984). The diet of the Rickett’s big-footed bat *Myotis pilosus* (Peters, 1869) in the eastern mainland of China consists for more than 60% of freshwater fish (Jiang *et al.*, 2019). This bat species inhabits the seashore mangrove forests in Vietnam (Thong *et al.*, 2022) and apparently uses the same feeding strategy. On the west coast of Mexico, the fish-eating bat *Myotis vivesi* (Menegaus, 1901) regularly preys on small fish and crustaceans, for instance the Californian anchovy *Engraulis mordax* (Girard, 1854) and a krill *Nyctiphanes simplex* (Hansen, 1911) (Drinkwater *et al.*, 2021). According to observations in the Gulf of California, the greater bulldog bat or fisherman bat *Noctilio leporinus* (Linnaeus, 1758) hunts predominately over the sea, catching fish. However, this species can change its dietary habits catching insects and marine crustaceans (Otalora-Ardila *et al.*, 2013). These observations suggest that bats use crustaceans as a food resource along with insects. The occurrence of crustaceans in bats diets seems to depend on prey avail-

ability in certain areas and seasons (Ahlén *et al.*, 2009; Otalora-Ardila *et al.*, 2013).

In our recent studies, we documented hunting events in which the eastern water bat (*My. petax*) preyed upon the pelagic amphipod *Macrohectopus branickii* (Dybowski, 1874) at the surface of Lake Baikal. However, these findings were based solely on visual observations and photographs from a single study site in the southwestern part of the lake (Didorenko *et al.*, 2021, 2022). Given that the coastline extends along approximately 2000 km, it would be valuable to investigate the prevalence of this phenomenon across different regions of the lake. Lake Baikal is the World deepest and one of the largest lakes, with an exceptionally high biodiversity and endemism among aquatic organisms (Rusinek *et al.*, 2012). Amphipods represent the morphologically diverse and species-rich group of invertebrates in Lake Baikal, occupying all depth zones of the lake. All 354 amphipod species and subspecies described in Baikal (Takhteev *et al.*, 2015), are endemic to the lake. Among these taxa, only a few dozen exhibit nocturnal vertical migrations to the water surface (Mekhanikova & Takhteev, 2001; Karnaukhov *et al.*, 2016; Takhteev *et al.*, 2019). Notably, just a limited subset of these migratory species forms dense surface aggregations (Takhteev *et al.*, 2019).

Studies of animal diets were historically based on morphological examination of faecal samples or gastrointestinal contents. Significant advances in detecting prey taxa from faecal pellets have been achieved through DNA barcoding and metabarcoding techniques. These methods allowed to reveal various taxa of insects (Vesterinen *et al.*, 2013; Clare *et al.*, 2014; Shiveley *et al.*, 2018), fish (Sommer *et al.*, 2019; Drinkwater *et al.*, 2022), and crustaceans (Drinkwater *et al.*, 2022) in bat diet. At present, no molecular data confirming the presence of freshwater amphipods in bat diet is currently available in public domain. This study aimed to evaluate the contribution of amphipods to the diet of bats foraging over Lake Baikal by combining morphological and genetic analyses of remains in faecal samples.

Materials and methods

Collection of bat faeces

In July–September 2020–2023, 41 samples of bat faeces were collected from captured bats and roosting sites at 14 sampling sites in the water area and the shore of Lake Baikal (Table 1, Fig. 1). Bats were captured using a mist net or a mobile trap (Borisenko, 1999) on the flyway or while foraging over water, and by hand in

daytime roosts. All captured bats were morphologically identified as the eastern water bats *My. petax* (Hollister, 1812) and were released near the capture site after collection of faeces. Capturing was complemented with visual and acoustic observations using Peterson D-100 bat detector from the shore and from the research vessel at night. Field observations of bats over Lake Baikal were described in detail earlier (Botvinkin *et al.*, 2023).

Most faecal samples ($n=32$) were collected from bats which were kept individually in cotton bags for 3–12 hours after capture. The pooled sample 41 contained faeces of six bat individuals held in one bag. Two samples (11 and 12) were collected during an observation of bat hunting on the amphipods *Ma. branickii* in 2020 (Didorenko *et al.*, 2022). These two faecal samples were shown to maintain amphipod body parts (Botvinkin *et al.* 2024) and were tested repeatedly in

this study after being stored dry at room temperature for about two years before DNA extraction. The remaining samples ($n=9$) were collected from walls and floor of roosting sites in human-made structures and caves. While these samples were confirmed to be of chiropteran origin, the exact bat species could not be determined.

Majority of newly collected faecal pellets were black or dark brown in color. Only few pellets from samples 27, 31, 40 were unusually light. About half of faecal pellets, collected during the mass elevation of *Ma. branickii* to the water surface in 2020 (including samples 11 and 12) were light brown or orange (Didorenko *et al.*, 2022).

Faecal samples collected in 2020–2022 were stored dry in paper bags or tubes at room temperature until laboratory analyses. In 2023, upon arrival to the labo-

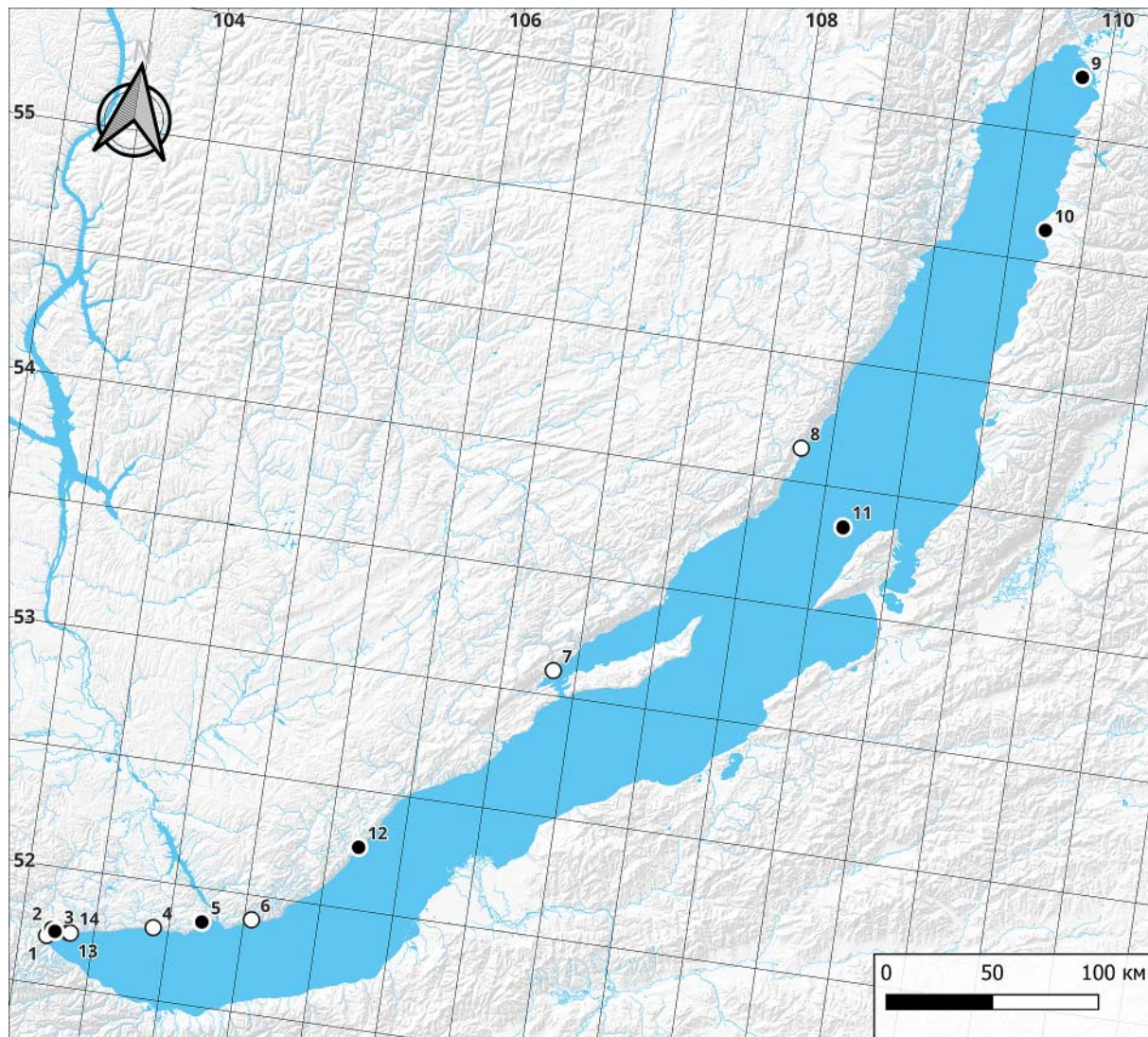


Fig. 1. Sampling sites of bat faeces around Lake Baikal in 2020–2023. Sampling sites where body fragments and/or DNA of amphipods were found in faeces are indicated in black.

Table 1. Collection of bats and bat faeces (Lake Baikal, 2020–2023).

Sampling sites	Bat species	Circumstances, location and years of collection	Coordinates	Samples numbers
1	<i>My. petax</i>	Capture over the Kultuchnaya River, 500 m from Lake Baikal, 2021–2023	51.4224°N 103.4101°E	1, 2, 7, 13, 17, 18, 19, 20, 22
2	<i>My. petax</i>	Capture in tunnel near Kultuk settlement, 1900 m from Lake Baikal, 2022–2023	51.4428°N 103.4219°E	8, 40
3	<i>My. petax</i>	Capture in the day roost (bridge) on Baikal, shore near Kultuk settlement, 2022–2023	51.4344°N 103.4407°E	9, 16, 41
4	<i>My. petax</i>	Capture over the River Ponomaryovka, 50 m from Lake Baikal, 2022	51.4815°N 104.2343°E	14, 15
5	<i>My. petax</i>	Capture over Lake Baikal from the pier, Serebryany Klyuch Railway Station, 2020–2021	51.5126°N 104.4304°E	10, 11, 12, 21
6	<i>My. petax</i>	Capture over Lake Baikal from the shore, Bolshiye Koty settlement, 2022	51.5348°N 105.0302°E	3, 4, 5
7	<i>My. petax</i>	Capture in the day roost, 400 m from Lake Baikal, Sarma Village, 2021	53.06 06°N 106.50 33°E	6
8	<i>My. petax</i>	Capture on board of a ship, 300 m from Baikal shore, near Cape Solontsovy, 2023	54.0805°N 108.1750°E	23
9	unidentified bat species	Collection of faeces at the day roost, island in the Verkhnyaya Angara River delta, 2023	55.4353°N 109.4947°E	24, 25
10	<i>My. petax</i>	Capture on board of a ship, 600 m from Baikal shore, the Tompuda River mouth, 2023	55.0690°N 10.4351°E	26
11	unidentified bat species	Collection of faeces in a cave on Bolshoy Ushkany Island, littoral zone, 2023	53.5004°N 108.3925°E	27, 28, 29
12	unidentified bat species	Collection of faeces in the day roost, 300 m from Lake Baikal, Peschanaya Bay, 2023	52.1544°N 105.4209°E	30, 31, 32
13	unidentified bat species	Collection of faeces in the day roost, 50 m from Lake Baikal, Staraya Angasolka Village, 2023	51.4353°N 103.0947°E	33
14	<i>My. petax</i>	Capture from a maternity colony, the shore of Lake Baikal, Staraya Angasolka Village, 2023	51.4352°N 105.5030°E	34, 35, 36, 37, 38, 39

Table 2. Primers used for nested PCR of mitochondrial *COI* gene fragment of *Ma. branickii*.

Amplification stages	Structure of the primers	Size of PCR product
1st round of nested PCR (DNA template is native DNA from fecal samples)	Mb_CO1_40F CGGCACCCTGTACTTTATTCTGG Mb_CO1_860R CCATACAATGAAACCCAGTAGACC	820 bp
2nd round of nested PCR (DNA template is the PCR product of the 1st round of amplification)	Mb_CO1_130F CCTGGTAGAATGATTAGAGACGACC Mb_CO1_740R GTAGGATCAGGATGTATACTTCAGG	610 bp
3rd round of nested PCR (DNA template is the PCR product of the 2nd round of amplification)	Mb_CO1_172F FACAGCCCACGCCTTCACAATAAT Mb_CO1_600R GACAGGTAGTGACAGAAGCAGTA	428 bp

ratory, samples designated for genetic analysis were preserved in 70% ethanol and stored at -20°C . For microscopic studies, a part of each sample was placed in 4% formalin, except for samples 27, 28, 40, which were used for genetic analysis only due to small amounts of material.

At sampling site 10, amphipods of the night migratory complex were captured from water surface by a net to compare their morphological features with amphi-

pod fragments in bat faeces. Collected amphipods were stored in 4% formalin.

Microscopic analysis

Microscopy was performed for 18 of 41 samples. Dry faecal samples were fixed in 4% formalin before microscopic sample preparation to prevent the destruction of fragile body parts of amphipods. Small portions of faeces (3–5 pellets) were taken and placed individually in a drop of water on a slide. Fecal pellets were dis-

tributed evenly on the slide using a needle and tweezers and investigated using a binocular microscope (MBS-10) and Ergaval light microscope. For samples containing amphipod fragments, microscopic slides were prepared using Faure-Berlese mounting medium to enable detailed morphological examination. The fragments of insects in the samples were also documented, without abundance counting and species identification. Photographs were taken using an Olympus CX21 light microscope with a Toupcam 5.1 camera adapter.

Genetic analysis

The molecular analysis included DNA identification of a pelagic amphipod species *Ma. branickii* as the most probable bat's prey. The total DNA was extracted from 10% suspensions of faeces in saline buffer using RIBO-prep kit (Center for Scientific Research and Expertise, Moscow) under manufacturer's instructions. To identify the amphipod *Ma. branickii* DNA, we designed specific primer sets for the cytochrome c oxidase subunit I gene (*COI*) fragment (Table 2) based on the complete mitochondrial genome sequence data (MT047459) of this species (Romanova *et al.*, 2021) using the Oligo Analysis Tool (Owczarzy *et al.*, 2008).

DNA fragments were amplified in three rounds of nested PCR using three primer pairs in turn (Table 2). In the first round of PCR, native DNA was used. For the

subsequent rounds, the PCR products obtained in the previous rounds of amplification were used as the DNA template. All three rounds of PCR were performed using the BioMaster HS-Taq PCR kit (2×) (Biolabmix, Novosibirsk) and the following program: 95°C for 5 min, followed by 33 cycles at 95°C for 10 s, 53°C for 15 s, and 72°C for 1 min. The PCR results were evaluated on agarose gel, and positive samples were purified for Sanger sequencing performed with Genetic Analyzer 3500 xL (Applied Biosystems) using Big Dye Terminator Cycle Sequencing Kit v 3.1 (Thermo).

Results

Microscopic analysis

Amphipod body fragments were found in six faecal samples (Table 3). These samples contained also numerous fragmented parts of insects. In most cases, amphipod remains in faeces were seen as shapeless chitin fragments (Fig. 2A) and setae of various shapes (simple, plumose, and others). These fragments did not allow identification of amphipod species but indicated their belonging to crustaceans. Even when examining undamaged fragments (antennae, legs, and mouthparts), we could rarely identify amphipods to genus or species level (Figs. 2B, 2C).

Table 3. Results of genetic and microscopic detection of amphipods in bat faeces.

Sampling site numbers	Number of examined samples /number of positive samples (total percentage of positive samples)		Amphipod taxa or unidentified amphipod remains found in morphological study	Numbers of samples contained amphipod' body fragments or DNA
	genetic detection of <i>Ma. branickii</i> <i>n</i> =41	morphological study of amphipod remains <i>n</i> =18		
1	9/0	NA	NA	–
2	2/1	NA	NA	40
3	3/1	1/1	<i>Ma. branickii</i> , gen. <i>Micruropus</i> , unidentified amphipods	41
4	2/0	NA	NA	–
5	4/2	2/2	<i>Ma. branickii</i> , unidentified amphipods	11, 12
6	2/0	NA	NA	–
7	1/0	NA	NA	–
8	1/0	1/0	Not found	–
9	2/0	2/1	<i>Mi. wohlii wohlii</i> , <i>Mi. wohlii platycercus</i> , <i>G. fasciatus</i> , unidentified amphipods	25
10	1/0	1/1	<i>Mi. wohlii wohlii</i> , <i>Mi. wohlii platycercus</i> , <i>G. fasciatus</i> , unidentified amphipods	26
11	3/1	NA	NA	27
12	3/0	3/1	<i>Mi. wohlii wohlii</i> , <i>Mi. wohlii platycercus</i> , <i>G. fasciatus</i> , unidentified amphipods	31
13	1/0	1/0	Not found	–
14	6/0	6/0	Not found	–
Total	5 (12.2%)	6 (33.3%)	=	8 (19.5%)

Note: NA — samples that have not been examined.

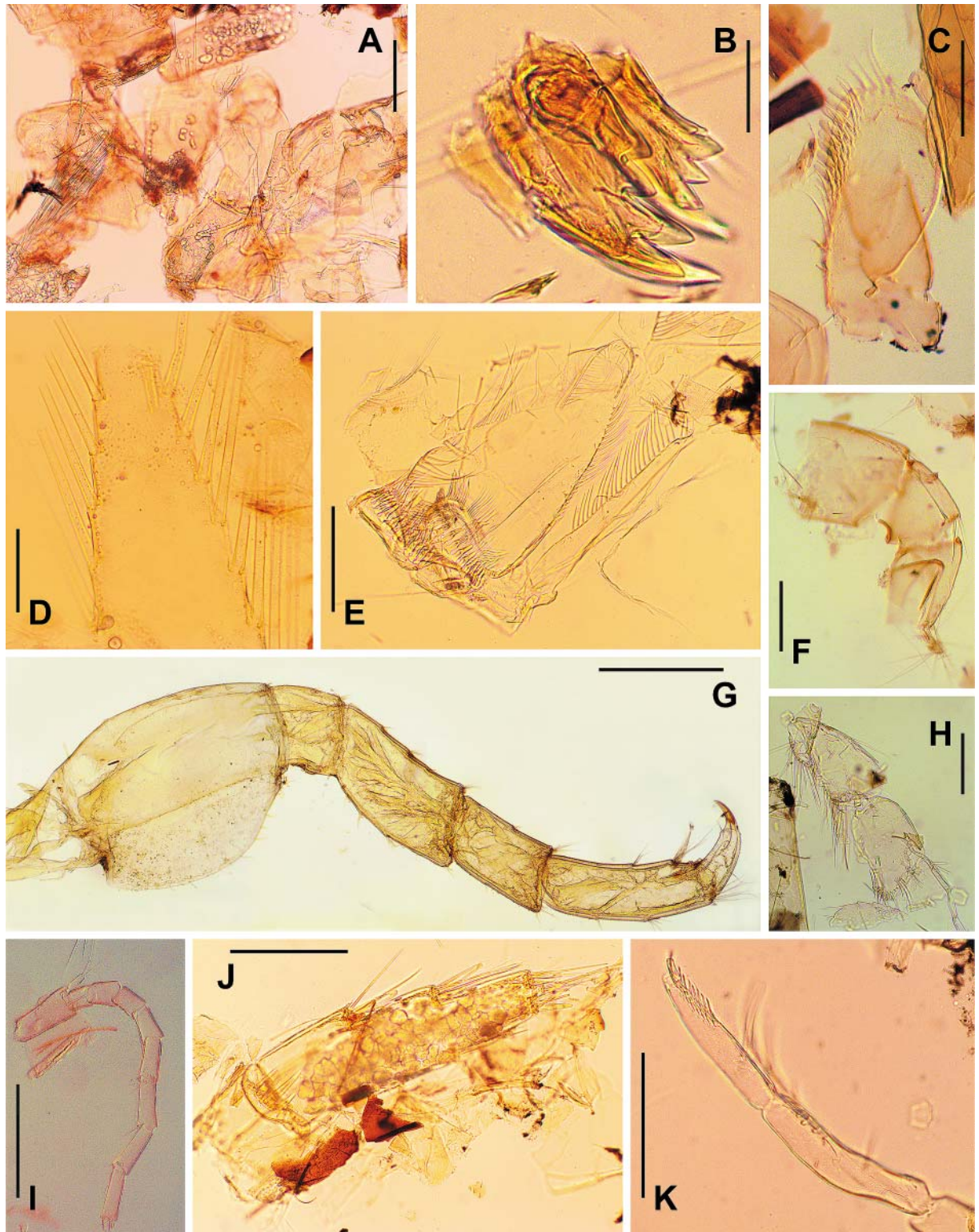


Fig. 2. Amphipod fragments in bat faeces. Scale bar: 200 μ m for A, E, F, H; 50 μ m for B; 150 μ m for C and K; 100 μ m for D; 1 mm for G; 250 μ m for I; 300 μ m for J. A, pieces of chitin; B, incisor and lacinia mobilis of left mandible; C, inner lobe of maxilla 2; D, fragment of uropod 3 of *Ma. branickii* (according to Botvinkin *et al.*, 2024); E, stomach; F, fragment of pereopod; G, pereopod 7 of *Mi. wohlii platycercus*; H, distal part of the gnathopod; I, fragment of antenna 1 with accessory flagellum; J, uropod 3 of *G. fasciatus*; K, mandibular palp.

Body fragments belonging to *Ma. branickii* were found in three samples (11, 12, and 41). The unique morphological features of the only Baikal pelagic amphipod allowed us to identify this species based on a few fragments. Branches of uropods 3 in *Ma. branickii* are of equal length, lanceolate, long, tapering towards the apex with dense plumose setae on both sides of the branches (Fig. 2D). Basipodites of pereopods 5–7 are short and almost cylindrical, pereopod 7 has a small pointed angle on posterior margin of basipodite. Terminal article of the mandibular palpus lacks the brush typical for other species, and accessory flagellum on antenna 1 and calceoli on antenna 2 are absent (Dybowski, 1874; Sowinsky, 1915; Timoshkin *et al.*, 1995). No Baikal other amphipod species has basipodites of pereopods 5–7 of similar shape, and the accessory flagellum (one-segmented or multi-segmented) is always present.

Sample 41 contained several small pieces of crustaceans (chitin, setae, pieces of pereopods, and pleiopod branches) among multiple insect fragments. Remains of *Ma. branickii* were identified by a large fragment of uropod 3 with long, well-preserved plumose setae. In one slide from sampling site 5, we found a part of a pereopod with a broad, rounded basipodite and a well-developed wing-like margin typical for species of genus *Micruropus*.

Samples 25, 26, and 31 contained fragments of at least three species of benthic amphipods identified as *Micruropus wohlii wohlii* (Dybowski, 1874), *Mi. wohlii platycercus* (Dybowski, 1874), and *Gmelinoides fasciatus* (Stebbing, 1899). In addition to the relatively large body parts used for species identification, the aforementioned samples also contained smaller amphipod fragments — such as branchiae, mouthparts, lateral alia, and gastric filters — that were unsuitable for species-level determination (Fig. 2E). Larger fragments of body parts of amphipods, well distinguished from fragments of *Ma. branickii*, were also found in aforementioned samples: basipodites of pereopods with rounded shapes, other parts of pereopods, distal part of gnathopod, antenna 1 with one-segmented accessory flagellum, intact uropod 3 (Fig. 2F–2K), fragment of flagellum of antenna 2 with antennal sensory organs (calceoli). The structure of different parts of pereopods found in microscopic analysis confirms that the eastern water bat feeds on representatives of the genus *Micruropus*. Among the Baikal amphipods of the genus *Micruropus*, nine species possess calceoli (Mekhanikova, 2021), but only five of them reach the water surface: *Mi. wohlii wohlii*, *Mi. wohlii platycercus*, *Mi. vortex* (Dybowski, 1874), *Mi. talitroides* (Dybowski, 1874), and occasionally *Mi. possolskii* (Sowinsky, 1915).

Sample 26 contained a distal part of a pereopod, which morphologically typical for the genus *Micruropus*. At sampling site 10, 38 specimens of *Mi. wohlii platycercus* (body length 5–6 to 9–10 mm) and one specimen of *Ma. branickii* (9 mm) were caught by net from water surface. In sample 25, one whole undamaged pereopod 7 was found among many fragments of insects (Fig. 2G). The morphological features let

us identify them as *Mi. wohlii platycercus*. Based on size of pereopod in the faeces (~5–6 mm long), it belonged to a large individual of about 10–11 mm long. In *Mi. wohlii platycercus* with a body length of about 9 mm (sampling of amphipods from the water surface in a bay near the Tompuda River), the length of pereopod 7 was four mm. Both subspecies of *Mi. wohlii* belong to a group of the largest forms among all representatives of the genus *Micruropus* with an average body length of 8–10 mm (Bazikalova, 1962).

In sample 31, collected from Peschanaya Bay, morphology of the terminal articles of mandibular palpus allowed us to identify two different species: *Mi. wohlii wohlii* which has palpus with setae of the same length (Fig. 2K), and *Mi. wohlii platycercus* which has setae of different length. *G. fasciatus* in this sample was identified by a well-preserved uropod 3 (Fig. 2J). The fragments of pereopod articles with harder cuticular coverings may also belong to this species.

Genetic analysis

Molecular analysis revealed the presence of *Ma. branickii* DNA in five of 41 samples tested (Table 3). The amplicons were sequenced and deposited in the GenBank database under the following numbers: OQ784044, OQ784045, PP536069–PP536071. The obtained *COI* gene sequences, which ranged from 381 to 429 bp in length, exhibited 100% identity to the corresponding *COI* gene sequence from the complete *Ma. branickii* mitochondrial genome (Romanova *et al.*, 2021). In addition, these sequences were 92–100% identical to other sequences of *COI* gene of the same amphipod species available in GenBank.

Comparison of results obtained via of microscopic and genetic analyses

Only 18 samples were analyzed using both morphological and molecular methods. DNA of *Ma. branickii* was detected in all three samples (11, 12, 41) in which body fragments of this species were found. Also, genetic analysis revealed DNA of *Ma. branickii* in samples 27 and 40, which were not studied morphologically (Table 3). Furthermore, morphological analysis revealed body fragments of other amphipod species and unidentified crustacean remains in six DNA-negative samples (11, 12, 25, 26, 31, and 41) (Table 3). Thus, DNA and (or) body fragments of the endemic Baikal amphipods were found in at least 33.3% of samples of bat faeces examined using two methods.

Circumstances of faecal samples collecting with amphipod remains

We identified amphipod DNA and/or body fragments in seven of 14 sampling sites. Despite the relatively small number of examined samples, we demonstrated that trophic interactions of *My. petax* with endemic crustaceans are widespread throughout Lake Baikal. In our study, samples containing amphipod remains were collected at different locations during several years.

Samples 11 and 12 were collected on the western coast of Lake Baikal near the springhead of the Angara River in late August 2020 when dense aggregations

of amphipods at the air-water interface were observed (Didorenko *et al.*, 2022). Throughout our 2021–2023 sampling periods, we never observed similar surface aggregations of *Ma. branickii*.

Sample 40 was obtained on September 2023, from an adult female *My. petax* captured near its roost about 2 km from the shore. The next morning, a group of *My. petax* (pooled sample 41) was captured from a gap of a bridge near Lake Baikal water area. Both these sampling sites are located near the southern extremity of Lake Baikal at a distance of about 2.5 km from each other. Thus, these bats were hunting on the same night in approximately the same place. Sample 26 was obtained from adult male *My. petax* captured from a vessel 600 m off the east shore. At this site, a group of bats (up to 10 individuals identified visually) was feeding near the vessel, flying low over water. At this sampling site, 38 specimens of *Mi. wohlii platycercus* (body length 5–6 to 9–10 mm) and one specimen of *Ma. branickii* (9 mm) were collected by a net from water surface.

Sample 27 was collected from the floor of a cave on Bolshoy Ushkany Island, located 11 km from the eastern shore of Lake Baikal. Samples 25 and 31 were also collected in roost sites (old wooden houses) on a small island in the Verkhnyaya Angara River delta and in a forest on the western shore of Lake Baikal. In these cases, it was impossible to define which bat species the samples belonged to. Furthermore, the deposition timeframe of these faecal samples in the roosts remains unknown.

Discussion

In this study, we have established that bats *My. petax* feed on amphipods at different points of Lake Baikal water area separated by distances ranging from tens to hundreds of kilometers (Fig. 1). Body fragments of at least four amphipod species endemic to Lake Baikal were identified in bat faeces. Chitin coverings of amphipods are more fragile than insect skeletons, so they crumbled up mainly into small fragments unsuitable for species identification. However, relatively large fragments of amphipods in our materials had a number of morphological features (presence of calceoli, presence of absence of accessory flagellum, shape of basipodites of pereopods, and branches of uropods), sufficient for species identification. In addition to previously published data (Didorenko *et al.*, 2020, 2022; Botvinkin *et al.*, 2024), we showed that the eastern water bat can consume not only relatively large pelagic amphipods *Ma. branickii* (females up to 38 mm in body length) but also small (10–15 mm) benthic littoral species.

The presence of *Ma. branickii* in *My. petax* prey was confirmed by the detection of DNA for the first time in faecal samples. In our genetic analysis, we used a custom set of primers to amplify fragment of mitochondrial DNA (gene *COI*) of the abundant pelagic Baikal amphipod *Ma. branickii* which was shown to be a food source for bats from previous observations (Didorenko *et al.*, 2022). Nested PCR allowed detec-

tion of *Ma. branickii* DNA in samples obtained from several new sampling sites. Dry faeces collected from bat roosts, were successfully investigated using this method.

The obtained *COI* sequences were identical to each other and matched the predominant sequence haplotype of this species reported in a previous population study by Zaidykov *et al.* (2023). Notably, these sequences were derived from faecal samples collected across different basins of Lake Baikal (samples 11, 12, 40, and 41 from the southwestern coast of the southern basin; sample 27 from the western coast of the middle basin). This genetic uniformity aligns with the population structure described by Zaidykov *et al.* (2023), suggesting widespread haplotype sharing across geographically distinct regions of the lake.

Although our study focused on molecular analysis of a single amphipod species, future investigations of bat feeding ecology over Lake Baikal should incorporate advanced genetic techniques, such as DNA barcoding and metabarcoding. These methods are now widely employed to study the dietary composition of different taxa (Compson *et al.*, 2020; Stenhouse *et al.*, 2023), including bats (Clare *et al.*, 2014; Vesterinen *et al.*, 2013; Shiveley *et al.*, 2018), and could provide a more comprehensive understanding of prey diversity and trophic interactions in this ecosystem.

Several insect taxa were identified in the diet of *My. petax* hunting near Lake Baikal (Botvinkin *et al.*, 2024). While microscopic analysis revealed that insect remains dominated the dietary samples, they could not be identified to lower taxonomic levels. In most samples (excluding 11, 12) amphipod remains were detected in small amounts. Thus, two aspects define the use of amphipods as a food source in bats: i) availability of amphipods for bats and ii) specificity of foraging behavior of *My. petax* over Lake Baikal.

Nocturnal vertical migrations are common in Lake Baikal amphipods. In different parts of the lake, there are various amphipod species in the nocturnal migration complex (Takhteev *et al.*, 2019; Batranin *et al.*, 2019), thus, the amphipod species diversity in bat diets may be greater than our study showed.

Massive appearance of the species *Ma. branickii* near the water surface at night has been described in many studies. This species lives in the pelagic zone of the lake mainly at depths of 200–700 m. Nocturnal migrations more often occur near steep underwater slopes at the western shore of Lake Baikal but have also been recorded in the open water of the lake. This species is the most abundant among endemic amphipods and is a key member of the trophic chain of the Baikal ecosystem (Bekman & Afanasyeva, 1977; Takhteev, 2000; Mekhanikova & Takhteev 2001; Karnaukhov *et al.*, 2016; Batranin *et al.*, 2019). Some species of benthic amphipods are also abundant in the nocturnal migration complex. The greatest migration activity is observed between midnight and 3 AM (Takhteev *et al.*, 2019). During the summertime, species from the genera *Micruropus*, *Gmelinoides*, *Eulimnogammarus*,

Echiurops, etc. are often found in the near-surface water layers. *Micruropus wohlii wohlii* and *Mi. wohlii platycercus* also form very dense nocturnal aggregations and were observed in the pelagic zone far from the shore (Bazikalova, 1962; Mekhanikova & Takhteev, 2001; Takhteev *et al.*, 2019). Three taxa of benthic amphipods (*Mi. wohlii wohlii*, *Mi. wohlii platycercus*, *G. fasciatus*) were identified in bat faeces.

Twelve species of bats are known to inhabit the Baikal region (Botvinkin *et al.*, 2023), but only the eastern water bat *My. petax* belongs to the group of “trawling” bats. We observed bats of this species above open water almost every night while collecting samples for the study. Usually, groups of several individuals hunt insects by flying low over water, and sometimes they pick up something from the water surface. Foraging activity of *My. petax* over the open water of Lake Baikal was detected at a distance of up to 8.5 km from nearest land and continued after total darkness until 2–3 AM. Therefore, any amphipod species that rise to the water surface at night could be a prey for the eastern water bat.

The presence of pelagic amphipods in bat faeces has been reliably confirmed by two different methods. The results of microscopy and genetic analysis confirm each other in terms of the detection of *Ma. branickii* remains. By microscopic examination, we were able to identify several other amphipod species additionally. However, the labor-intensive nature of morphological analysis, combined with the extensive fragmentation of arthropod remains by bats, significantly limits the effectiveness of this approach. To facilitate identification of amphipod remains in faeces, hydrobiological sampling should be made in the near-surface water layer in the areas of bat hunting.

DNA barcoding and other genetic methods offer new perspectives for rapid taxa identification (Clare *et al.*, 2014; Sommer *et al.*, 2019; Drinkwater *et al.*, 2022). However, identifying the exact amphipod species from faecal samples, that had been stored dry for extended periods in the laboratory, required a specialized approach, specifically a three-round nested PCR protocol using species-specific primers for *Ma. branickii*. Future studies investigating Lake Baikal amphipod taxa in bat faeces will require development of universal primer sets for this species group, followed by application of high-throughput sequencing methods (metabarcoding).

Further research on bat nutrition is essential also for understanding its influence on the composition of bat-associated microbiomes and viromes, particularly given their role as reservoirs for high-risk pathogens (Hornok *et al.*, 2015; Letko *et al.*, 2020; Corduneanu *et al.*, 2023). We suggest that an application of modern molecular methods will facilitate the revealing of new trophic interactions between bats and various aquatic organisms of Lake Baikal (aquatic insects, amphipods, fish, etc.) and contribute to a better understanding of complex ecological interactions in its ecosystem.

Conclusion

Several amphipod species (*Macrohectopus branickii*, *Micruropus wohlii wohlii*, *Mi. wohlii platycercus*, *Gmelinoides fasciatus*) serve as supplementary food source for *Myotis petax* across different regions of Lake Baikal. Among these amphipods, *Ma. branickii* is a pelagic species, whereas the other species belong to so-called the nocturnal migration complex. Apparently, bats consumed Baikal crustaceans during their periodic nocturnal ascents to the water surface. The results of the study provide new prospects for studying the ecological interactions of terrestrial vertebrates and aquatic invertebrates of Lake Baikal.

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