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Winter diet analysis in *Rhinolophus euryale* (Chiroptera)

Research Article

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Abstract: We investigated the winter food of Mediterranean horseshoe bats (*Rhinolophus euryale*) in four winter cave roosts in southern Slovakia and northern Hungary and investigated the relationship between food and ambient temperature. The bats were active during the whole winter period and they produced excrement throughout the entire hibernation period, even when outside temperatures dropped below zero. The guano was in two forms, containing (1) prey items and (2) non-prey items. The identifiable items belonged to lepidopteran species, but only one was identified, on the basis of the genital fragments, the moth *Colotois pennaria*, which was the main prey species in autumn and early winter. Our results shed light on the extraordinarily high level of activity in this bat species during winter hibernation, which in temperate regions is a strategy that enables bats to survive when prey is reduced or absent. In *R. euryale*, the torpor in the course of hibernation is not continuous and our results help to explain how energy losses caused by bat movements are covered.

Keywords: *Hibernation • Bats • Moths • Slime-like guano • Winter activity*

1. Introduction

In temperate regions bats typically hibernate, using this strategy to survive the long periods when the numbers of arthropod prey are reduced or totally absent. The torpor during hibernation is not continuous, and there is evidence of relatively frequent breaks [1]. The frequency of such breaks varies among species and individuals [1-4]. However, the causes of these arousals appear varied and uncertain, though temperature in the roost has been suggested to play an important role [5]. Breaks in hibernation can lead to changing the hibernation site, drinking or to foraging activity. When temperatures are sufficiently high, and other climatic conditions prove suitable, breaks can be rather frequent [1,4,6-10].

The Mediterranean horseshoe bat, *Rhinolophus euryale* Blasius, 1853, is a typical bat species of the thermo-Mediterranean zone of the Mediterranean region – the southern limits of its distribution range are in the Levant and Iran, while the northern limits extend to southern Slovakia and northern Hungary [11]. *R. euryale* has been reported feeding close to vegetation, with moths as the main prey [12]. However, the nematocerans, beetles and lacewings could also play an important role in some habitats or in certain seasons [13-15]. In the Slovakian/Hungarian border zone an isolated population of *Rhinolophus euryale* occurs – at the very north of its range [16-18]. The population is small with a total of 10–12,000 individuals estimated in this area [18-21]. It has become clear that this population favours underground sites as

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hibernacula, and that individuals move between roosts during winter – although the reasons for changing roosts remain unclear. The bats' foraging activity in caves has also been poorly researched, although there was an assumption that bats prey on insects which withdraw into caves in winter [22]. We hypothesise that individuals can forage during their flights between roosts and during breaks in hibernation, and thus renew their energy losses. The aim of this study is to clarify the winter food composition of *Rhinolophus euryale* and to relate the type of food detected to ambient temperatures outside the roosting caves. We believe the results of this study may have implications in regard to the conservation of the species.

2. Experimental Procedures

The study was conducted in the Slovak Karst region (SE Slovakia) and in the Aggtelek Karst (NE Hungary). Guano samples were collected from a foil lying under clusters of hibernating individuals in four localities where *Rhinolophus euryale* hibernated during winter in 2011/2012 (Drienovská jaskyňa cave: min. 693 individuals (hereinafter as inds.) (8. Nov. 2011), min. 600 inds. (30. Nov. 2011); Ardovská jaskyňa cave: min. 40 inds. (3. Nov. 2011), min. 270 inds. (10. Apr. 2012); Baradla cave: min. 3746 inds. (28. Nov. 2011; Figure 1); min. 3500 inds. (16. Dec. 2011), min. 3644 inds. (3. Jan. 2012); min. 3241 inds. (3. Febr. 2012); min. 1670 inds. (27. Febr. 2012); 30 inds. (19. March 2012), and from one site used in the pre-hibernation period (Domica cave: min. 1500 inds. (3. Nov. 2011), min. 1500 inds. (17. Nov. 2011), min. 40 inds. (30. Nov. 2011)). A long term monitoring of bats has been carried out in all study sites and aggregations of *R. euryale* were always confirmed as a mono-specific (for detail see [21]). Clusters were always checked for the species composition directly at the roost or later in photographs. Samples were collected roughly twice per month, between November 2011 and April 2012 (19 batches in total). Each sample comprises 60–120 guano pellets collected randomly from the foil surface. 544 faecal droppings were further analysed. The foil was changed after each pellet sampling. Samples were stored in 96% alcohol in microtubes and later examined with a binocular magnifier. Prey categories were identified using comparative slides, methodological works and entomological keys [23-25]. Expressing the proportion of items identified in analysed droppings, percentage volume (vol%) was used [24]. Samples from all sites were pooled and divided into three winter periods, November, December – February and March – April. The male lepidopteran chitine genital fragments (uncus, juxta, valva) were fixed in Swann solution and photographed (OLYMPUS C-5060 Widezoom) using software M.I.S. Quick PHOTO MICRO. Genital preparations enabled us to identify the species of lepidopterans [26]. Temperature readings which were used for the analysis were taken in two official meteorological stations (Jósvafő, Hungary and Domica, Slovakia). We tried to minimise disturbance to the bats during sample collection.

Figure 1. Aggregation of hibernating *Rhinolophus euryale* in the Baradla cave, Hungary (28 November 2011).

3. Results

Bats produced guano pellets throughout the entire hibernation period, including periods when outside temperatures were below zero (Figure 2). The guano pellets were in general in two forms: (1) containing prey items (200 pellets) and (2) containing non-prey items (344 pellets) (Figure 3). Identifiable prey items in the guano samples belonged only to lepidopteran species. The second pellet type included unidentifiable organic material resembling dissolved jelly tissue. A small number (10.5%) of pellets of both guano types contained hairs and mites. The proportion of moths was very high (69.6 vol%, Figure 2) at the end of the active season (November), but gel-like guano (29.1 vol%) and hairs-containing faeces (1.3 vol%) were also present in this period. When outside temperatures dropped below zero (December – February), bats produced gel-like excrement (79.0 vol%), mostly with hairs (11.0 vol%) and the proportion of Lepidoptera was 10.0 vol%. In the beginning of the active season (March – April), the rate of lepidopteran fragments rose in the droppings again (87.6 vol%). Gel-like guano (11.0 vol%) and hairs (1.4 vol%) were still present, but in smaller proportions. In all pellets analysed, 99 moth genitalia fragments were found, 42 being clearly identified as *Colotois pennaria*

Linnaeus, 1761 (Figure 4). Other fragments were not suitable for analysis because of their fragmentation level. However, we identified the moth *C. pennaria* as the main prey species in the autumn and early winter on the basis of the genital fragments found. Species identity was confirmed also by moth wing fragments found under the bat aggregations.

4. Discussion

When we analysed the main prey category for *Rhinolophus euryale* during the winter season, we observed only the order Lepidoptera. We therefore concluded that moths play a crucial role in foraging during the winter activity of this species. This is consistent with data from the active season, as this horseshoe bat is characterized as a moth-eater [12,14]. The diversity of foraged insect species changes during the year; it is more limited at the end of the season and during the more inhospitable climatic conditions, when the proportion of the main foraged group increases [12,14,27]. In early winter it is understandable that active bats foraged mainly on the one lepidopteran species, *Colotois pennaria*. This is a mid-sized monovoltinous moth (wingspan 35–45 mm), with adults active from September to November. The males fly actively, the females are rather slow and are usually found sitting on the twigs, branches and trunks of trees. The moth larval period lasts from April to July and overwinters as an egg [28,29]. Based on long-term light trapping studies, sufficient availability of many nocturnal lepidopteran species were observed in winter periods in the surrounding of the Domica-Baradla cave system [30], including *Colotois pennaria* (Varga, unpublished data). This moth species has even been observed during winter conditions on snow [31]. The wingspan of this moth species is, according to some authors [12,27], almost the maximum prey wingspan foraged by *Rhinolophus euryale*. *C. pennaria* has even larger body length (13.2–15.1 mm, own data) comparing to average prey sizes found in *R. euryale* diet in the

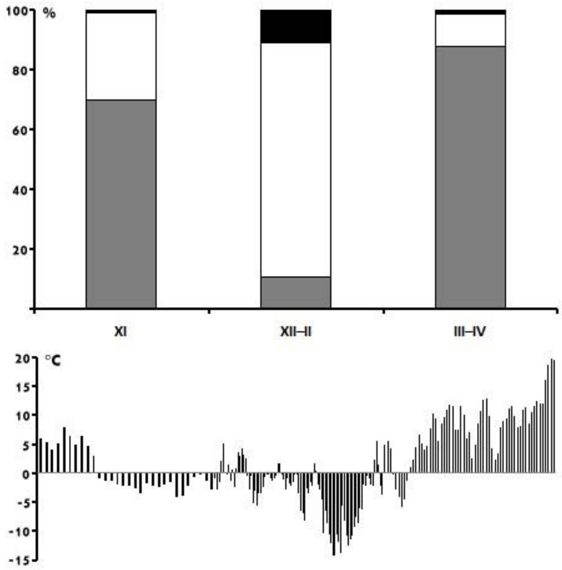

Figure 2. Proportion of prey items in faecal composition (above) and course of outer temperature (below). Grey – Lepidoptera, white – gel-like and tissue structures, black – hairs. Sample sizes: November, n=293 pellets, December – February, n=191 pellets, March – April, n=60 pellets.

Figure 3. Examples of droppings containing prey items (left) and containing non-prey items (right).

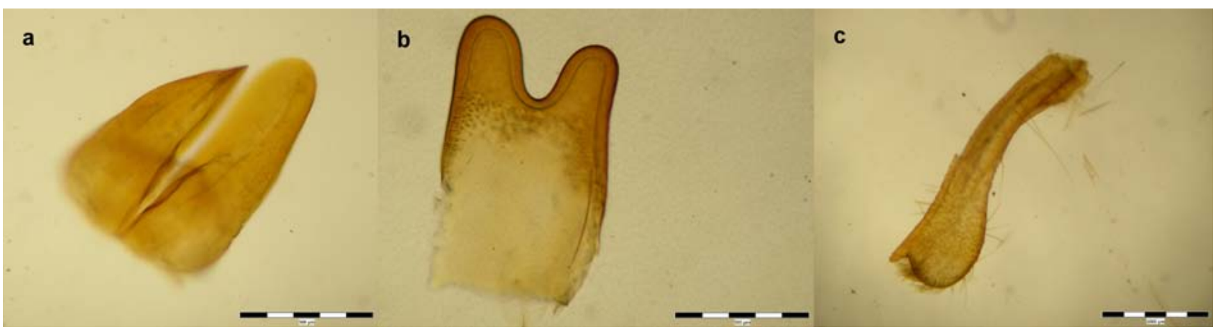

Figure 4. Genital fragments of *Colotois pennaria* identified in guano pellets. a – uncus, b – juxta, c – valva.

western Mediterranean region [32]. Foraging on bigger prey is more energy efficient, and is followed by digestion on the perch. This could explain the collection of moth wings bellow the cluster of individuals. In Britain, the congeneric species *Rhinolophus hipposideros* foraged mostly on Diptera during the winter, and the presence of Lepidoptera increases from February [33]. We conclude that the different behaviour patterns are caused by outside temperatures. Britain, where temperatures fall below freezing relatively infrequently, has a milder climate than the Hungarian-Slovak border region [34]. There is also little doubt that some of the bats leave the hibernaculum [1] to drink, feed and to change hibernation sites. Several studies have confirmed winter activity in other species [35-37], but such activity still depends on air temperatures and other climatic conditions. There is also evidence, based on ringing data, of movements between hibernacula within this sub-population of *R. euryale* [21,38]. These data document the relationship between several roosts located close to each other and this pattern corroborates with observations from parallel counts between the Domica and Baradla caves. In the Domica cave, pre-hibernation clusters of *R. euryale* (*ca*. 1,500 inds.) usually disband at the end of November, which is immediately mirrored by the increasing number of the species in the hibernation aggregation (*ca*. 4,000 inds.) in the Baradla cave. Both sites are at a distance of *ca.* 3 kilometres within this cave system. A high level of movements between, or within, the hibernacula and the continuous production of droppings showed that at least part of the aggregation is active in winter. Ransome [6] showed that *Rhinolophus ferrumequinum* select sites within a hibernaculum on the basis of ambient temperature and season. Warmer sites were chosen after warm days, presumably to trigger arousal more frequently so that the bats can feed during such warm spells. Avery [7] showed that winter flights in pipistrelles, *Pipistrellus pipistrellus*, were more frequent on warm nights, when insects were more abundant. Their behaviour could be explained by a model based on emergence for food. However, laboratory studies on the same species [35] give an alternative explanation that the primary function of breaks in hibernation is to find water to drink. All this movement raises the question of what energy resources are being used. According to Whitaker *et al*. [39], the enzyme chitinase may break down remnants of chitin that remain from summer foraging to provide both an energy and nutrient source. We suppose that gel-like guano is the result of intensive grooming, continuous digesting processes, changing of intestinal tissues and/or endobacteria activity. Kaňuch *et al*. [40] explained that the presence of such secondary components (e.g. hairs and slime) in the winter diet of noctule bats (*Nyctalus noctula*) is due to insufficient food supply. This type of winter bat guano is a novelty, and the origin of this material is unclear. Non-prey items, hairs and acarines found in our *Rhinolophus euryale* samples was taken as evidence of intensive grooming [41]. However, the presence of droppings containing Lepidoptera from frosty winter periods remains unexplained; it is possible that these moths had been hunting inside the cave. Sano [42] discovered that *Rhinolophus ferrumequinum* is able to prey on the diapausing noctuid moths. Such foraging is an important energy source at the end of the hibernation period. Dudich [22] also assumed that bats might prey on different insects, e.g. mosquitos, which withdraw to caves during winter. We hypothesized that *Rhinolophus euryale* may also be able to forage on wintering moths inside the hibernacula at times when the weather outside is completely unfavourable for such activity. A similar situation was described for *Nyctalus noctula* [40], which consumed invertebrates inside shelters in prefab houses.

The identification of moth species based on genitalia fragments facilitated the recognition of the main winter prey species, however, some samples were indeterminate. Methods based on molecular techniques of prey identification [43-45] could further illuminate the issue. Our results show that *Rhinolophus euryale* can compensate for energy losses and survive during the low-energy hibernation season, even when the species is subject to frequent breaks in its torpor, or to movements between the hibernacula. This bat is both physiologically and ethologically adapted to reuse older foraged prey, or to forage anew, even in sub-zero temperatures, or to forage inside the cave itself. It is also clear that the species hunts opportunistically before hibernation, although more research into these questions is needed.

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References

[1] Daan S., Activity during natural hibernation in three species of vespertilionid bats, Neth. J. Zool., 1973, 23, 1-71

[2] Menaker M., The free running period of the bat clock; seasonal variations at low body temperature, J. Cell. Comp. Physiol., 1964, 57, 81-86

[3] Funakoshi K., Uchida T.A., Studies on the physiological and ecological adaptation of temperate insectivorous bats. Hibernation and winter activity in some cave-dwelling bats, Jpn. J. Ecol., 1978, 28, 237-261

[4] Ransome R.D., The Natural History of Hibernating Bats, Christopher Helm, Kent, 1990

[5] Park K.J., Jones G., Ransome R.D., Torpor, arousal and activity in hibernating bats, a telemetric study of free living greater horseshoe bats (Rhinolophus ferrumequinum), Funct. Ecol., 2000, 14, 580-588

[6] Ransome R.D., The effect of ambient temperature on the arousal frequency of the hibernating greater horseshoe bat, Rhinolophus ferrumequinum, in relation to site selection and the hibernation state, J. Zool., 1971, 164, 353-371

[7] Avery M.I., Winter activity of pipistrelle bats, J. Anim. Ecol., 1985, 54, 721-738

[8] Brack V., Twente J.W., The duration of the period of hibernation of three species of vespertilionid bats. I. Field studies, Can. J. Zool., 1985, 63, 2952-2954

[9] Duvergé P.L., Jones G., Greater horseshoe bats – activity, foraging behaviour and habitat use, Br. Wildl., 1994, 6, 69-77

[10] Zukal J., Berková H., Řehák Z., Activity and shelter selection by Myotis myotis in the Kateřinská cave (Czech Republic), Mamm. Biol., 2005, 70, 271-281

[11] Horáček I., Hanák V., Gaisler J., Bats of the Palearctic region, a taxonomic and biogeographic review, In: Wołoszyn B.W. (Ed.), Proceedings of the VIIIth EBRS Vol. 1, Approaches to biogeography and ecology of bats, Chiropterological Information Center & Institute of Systematics and Evolution of Animals PAS, Cracow, 2000

[12] Goiti U., Aihartza J.R., Garin I., Diet and prey selection in the Mediterranean horseshoe bat Rhinolophus euryale (Chiroptera, Rhinolophidae) during the pre-breeding season, Mammalia, 2004, 68, 397-402

[13] Koselj K., Krystufek B., Diet of the Mediterranean horseshoe bat Rhinolophus euryale in southeastern Slovenia, In: Cruz M., Kozakiewicz K. (Eds.), VIIIth European Bat Research Symposium. Abstracts (23-27 August 1999, Kraków - Poland), Chiropterological Information Center, Institute of Animal Systematics and Evolution PAS, Kraków, 1999,32

[14] Goiti U., Garin I., Almenar D., Salsamendi E., Aihartza J., Foraging by Mediterranean horseshoe bats (Rhinolophus euryale) in relation to prey distribution and edge habitat, J. Mammal, 2008, 89, 493-502

[15] Andreas M., Reiter A., Cepáková E., Uhrin M., Body size as an important factor determining trophic niche partitioning in three syntopic rhinolophid bat species, Biologia, 2013, 68, 170-175

[16] Uhrin M., Danko Š., Obuch J., Horáček I., Pačenovský S., Pjenčák P., et al., Distributional patterns of bats (Mammalia, Chiroptera) in Slovakia. Part 1, Horseshoe bats (Rhinolophidae), Acta Soc. Zool. Bohem., 1996, 60, 247-279

[17] Gaisler J., Rhinolophus euryale Blasius, 1853 – Mediterranean horseshoe bat, In: Krapp F. (Ed.), Handbook of the mammals of Europe, Volume 4, bats. Part I, Chiroptera I. Rhinolophidae, Vespertilionidae 1, AULA-Verlag, Wiebelsheim, 2001

[18] Boldogh S., Mediteranean horseshoe bat Rhinolophus euryale Blasius, 1853 [Kereknyergű patkósdenevér Rhinolophus euryale Blasius, 1853], In: Bihari Z., Csorba G., Heltai M. (Eds.), Atlas of mammals in Hungary [Magyarország emlőseinek atlasza], Kossuth Kiadó, Budapest, 2007 (in Hungarian)

[19] Paulovics P., Juhász M., One possible way to save the remaining Transdanubian population of the Mediterranean horseshoe bat (Rhinolophus euryale, 1853) from extinction: strengthening with resettlement (action plan) [Egy lehetséges módszer a kereknyergű patkósdenevér (Rhinolophus euryale, 1853) dunántúli maradványállományának megmentésére: megerősítés áttelepítéssel (protokoll tervezet)], Denevérkutatás, 2008, 4, 18-37 (in Hungarian)

[20] Miková E., Andreas M., Boldogh S., Dobrý M., Jehličková V., Löbbová D., et al., Rhinolophus euryale (Chiroptera, Rhinolophidae), the summary results of ecological research of cave dwelling bat, In: Kováč Ľ., Uhrin M., Mock A., Ľuptáčik P. (Eds.), 21st international conference on subterranean biology. Abstract book (2-7 September, 2012, Košice, Slovakia), Pavol Jozef Šafárik University, Košice, 2012, 71-72

[21] Uhrin M., Boldogh S., Bücs S., Paunović M., Miková E., Juhász M., et al., Revision of the occurrence of Rhinolophus euryale in the Carpathian region, Central Europe, Vespertilio, 2012, 16, 289-328

[22] Dudich E., Wildlife food sources in the Aggtelek cave [Az Aggteleki barlang állatvilágának élelemforrásai (Nahrungsquellen der Tierwelt in der Aggteleker Tropfsteinhöhle)], Állattani Közlemények,1930, 27, 62-85 (in Hungarian, with a summary in German)

[23] McAney C.M., Shiel C., Sullivan C., Fairley J., The analysis of bat droppings, The Mammal Society, London, 1991

[24] Whitaker Jr. J.O., McMracken G.F., Siemers B.M., Food habits analysis of insectivorous species, In: Kunz T.H., Parsons S. (Eds.),Ecological and behavioural methods for the study of bats, The Johns Hopkins University Press, Baltimore, MD, USA, 2009

[25] Chinery M., A field guide to the insects of Britain and northern Europe, Collins, London, 1977

[26] von Hausmann A., Systematics of some eastern Mediterranean geometrid species [Zur Systematik einiger ostmediterraner Geometridenarten (Lepidoptera, Geometridae)], Mitt. Münch. Ent. Ges., 1995, 85, 73-78

[27] Koselj K., Diet and ecology of Mediterranean horseshoe bat (Rhinolophus euryale, 1853; Mammalia, Chiroptera) in south-eastern Slovenia [Prehrana in ekologija južnega podkovnjaka (Rhinolophus euryale, 1853; Mammalia, Chiroptera) v jugovzhodni Sloveniji], Thesis, Department of Biology, Biotechnical faculty, University of Ljubljana, 2002 (in Slovenian)

[28] Redondo V.M., Gastón F.J., Gimeno R., Geometridae Ibericae, Apollo Books, Denmark, 2009

[29] Macek J., Procházka J., Traxler L., Moths III. Butterflies and caterpillars of Central Europe –Geometrid moths [Noční motýli III. Motýli a housenky střední Evropy – Píďalkovití], Academia, Prague, 2012 (in Czech)

[30] Szabó S., Árnyas E., Tóthmérész B., Varga Z.S., Long term light-trap study on the macro-moth (Lepidoptera: Macroheterocera) fauna at the Aggtelek National Park, Acta Zool. Acad. Sci. Hung., 2007, 53, 257-269

[31] Soszynska-Maj A., Buszko J., Lepidoptera recorded on snow in Central Poland, Ent. Fenn., 2011, 22, 21-28

[32] Salsamendi E., Garin I., Arostegui I., Goiti U., Aihartza J., What mechanism of niche segregation allows the coexistence of sympatric sibling rhinolophid bats? Front Zool, 2012, 9, 30

[33] Williams C., Salter L., Jones G., The winter diet of the lesser horseshoe bat (Rhinolophus hipposideros) Britain and Ireland, Hystrix, It. J. Mamm., 2011, 22, 159-166

[34] Klein Tank A.M.G., Wijngaard J.B., Können G.P., Böhm R., Demarée G., Gocheva A., et al., Daily dataset of 20th-century surface air temperature and precipitation series for the European Climate Assessment, Int. J. Climat., 2002, 22, 1441-1453

[35] Speakman J.R., Racey P.A., Hibernal ecology of the pipistrelle bat, energy expenditure, water requirements and mass loss, implications for survival and the function of winter emergence flights, J. Anim. Ecol., 1989, 58, 797-813

[36] Park K.J., Jones G., Ransome R.D., Winter activity of a population of greater horseshoe bats (Rhinolophus ferrumequinum), J. Zool., 1999, 248, 419-427

[37] Turbill C., Winter activity of Australian tree-roosting bats, influence of temperature and climatic patterns, J. Zool., 2008, 276, 285-290

[38] Gaisler J., Hanák V., Hanzal V., Jarský V., Results of bat banding in the Czech and Slovak Republics, 1948-2000 [Výsledky kroužkování netopýrů v České republice a na Slovensku, 1948-2000], Vespertilio, 2003, 7, 3-61 [in Czech with English abstract and summary]

[39] Whitaker Jr. J.O., Dannely H.K., Prentice D.A., Chitinase in insectivorous bats, J. Mammal., 2004, 85, 15-18

[40] Kaňuch P., Janečková K., Krištín A., Winter diet of the noctule bat Nyctalus noctula, Folia Zool., 2005, 54, 53-60

[41] Dietz C., von Helversen O., Nill D., Bats of Britain, Europe & Northwest Africa, A&C Black, London, 2009

[42] Sano A., Impact of predation by a cave-dwelling bat, Rhinolophus ferrumequinum, on the diapausing population of a troglophilic moth, Goniocraspidum preyeri, Ecol. Res., 2006, 21, 321-324

[43] Clare E.L., Fraser E.E., Braid H.E., Fenton M.B., Hebert P.D.N., Species on the menu of a generalist predator, the eastern red bat (Lasiurus borealis): using a molecular approach to detect arthropod prey, Mol. Ecol., 2009, 18, 2532-2542

[44] Bohmann K., Monadjem A., Lehmkuhl Noer C., Rasmussen M., Zeale M.R.K., Clare E., et al., Molecular diet analysis of two African free-tailed bats (Molossidae) using high through put sequencing, PLoS ONE, 2011, 6, e21441

[45] Alberdi A., Garin I., Aizpurua O., Aihartza J., The foraging ecology of the mountain long-eared bat revealed with DNA mini-barcodes, PLoS ONE, 2012, 7, e35692