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First records of the bat bug *Cacodmus vicinus* (Heteroptera: Cimicidae) from Europe and further data on its distribution

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Abstract. First records of the bat bug *Cacodmus vicinus* from Europe and further data on its distribution in Asia and Africa are presented. The records from Cyprus and Syria represent an undoubtedly new evidence of the species in the respective countries. Furthermore, besides the implied presence of *Cimex lectularius* on humans, this is also the first record of the family in Spain. *Cacodmus vicinus* appears to be a host-specific parasite associated with *Pipistrellus kuhlii* and, therefore, its distribution fits inside the range of distribution of the bat host. Together with a record from Jordan, at least one of the collections from Spain represent the only records from another bat host species, *Pipistrellus pipistrellus* s.l. We confirmed the species determination using the barcoding fragment of cytochrome oxidase subunit I. The molecular data suggested population structuring due to geographic distances.

***Cacodmus vicinus*, distribution, new records. Chiroptera**

Introduction

Cacodmus vicinus Horváth, 1934 (Fig. 1) ranks among rather well-known members of the subfamily Cacodminae, perhaps due to its Palaearctic distribution range and a relative accessibility of the specimens. It belongs to the family Cimicidae (Heteroptera), a widespread family containing about 90 species belonging to 23 genera in six subfamilies (Péricart 1996). Cimicid bugs are obligatory ectoparasites with a unique parasitic strategy (Usinger 1966); both adults and larvae feed on the blood of endothermic vertebrates and stay on the body of their host solely for the period they need to feed. Their transmission between particular shelters is passive and rather occasional. About two thirds of the species of the family are associated with bats, and these mammals were suggested to be the original host group of the family (Horváth 1913). The rest of the species is associated with birds. Compared to other parasitic arthropods permanently attached to the host body, they are recorded more rarely. From most of the described species of this family, solely the type specimens are known or only a few records are available.

Cacodmus vicinus is a bat-associated species with close attachment to one of the most widespread and abundant Mediterranean bat species, *Pipistrellus kuhlii* (Kuhl, 1917) (Usinger 1966). Only one record is known to come from *Pipistrellus pipistrellus* (Shreber, 1774) (Jordan; Benda et al. 2010). The known distribution range of this bug comprises Algeria, Egypt, Chad, Israel, Jordan, Lebanon (reviewed by Péricart 1996) and Turkey (Aktaş & Kiyak 1990). The description of the locality name as "East bank of the Jordan river near Lake Galilee" mentioned by Usinger

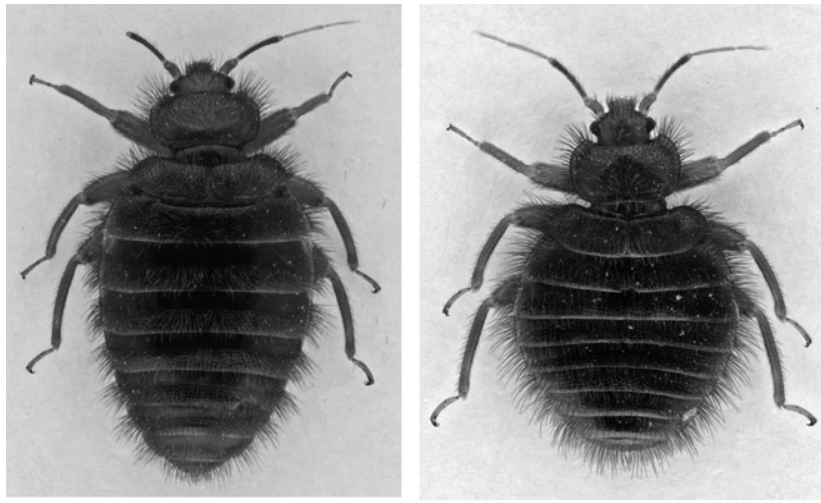

Fig. 1. *Cacodmus vicinus*: female (left, sample 242) and male (right, sample 239).

(1966) may mean several countries of the Levant, the present-day Syria, Israel or Jordan; however, for Syria it could represent the only available record.

Here we present several findings of *Cacodmus vicinus*, largely extending its known distribution range. Our experience with the taxonomic situation of the morphologically defined species within the genera *Cimex* Linnaeus, 1758 and *Oeciacus* Stål, 1873 (Balvín et al. 2012) impeaches the validity of the morpho-species concept in Cimicidae for valuable taxonomic conclusions. Therefore, we supply the data on distribution with mtDNA sequences from the available samples, namely the fragments broadly used for barcoding, in order to confirm the species identity. Moreover, although the sample size is rather limited, the sequences also bring information on the population structure of the species.

Material and methods

The collected specimens come from bat individuals examined during surveys focused on bat fauna, obtained both by nettings and roost prospections. The specimens were kept in 96% alcohol and are presently deposited in the collection by Ondřej Balvín.

The tissue for DNA extraction was obtained from a half of the thorax and legs. Extraction was performed using DNeasy® Blood & Tissue Kit (QIAGEN).

Amplification of the cytochrome oxidase subunit I (hereinafter COI) gene fragment was performed using modified DNA barcoding primers LepF (5'-ATT CAA CCA ATC ATA AAG ATA TNG G-3') and LepR (5'-TAW ACT TCW GGR TGT CCR AAR AAT CA-3') designed for Lepidoptera (e.g. Hajibabaei et al. 2006). The annealing temperature in the PCR was 48 °C. Where possible, we examined the sequences of COI of two individuals per each locality.

The sequences were aligned using MAFFT with default settings (Katoh et al. 2009). We constructed a median-joining network (for the algorithm and rationale for using this type of network, see Bandelt et al. (1999) and Huson et al. (2010)) in Network 4.516 (fluxus-engineering.com) using default parameters of the program to visualize the data.

We examined the genetic distances and components of genetic variability at hierarchical levels using the analysis of molecular variance (AMOVA) in Arlequin 3.1 (Excoffier et al. 2005). The components of diversity in the hierarchical model were comprised of within localities, among localities/within countries and among countries.

Table 1. List of the specimens recorded. Legend: IC – identification code of the sample (see Fig. 2); CC – country code (see Fig. 2); N – number of specimens collected; M – number of specimens examined for COI; * – specimen collected from the collector's body, bat species inferred from species mist-netted on that day. The collection of the specimen 240 was already published (Benda et al. 2010)

IC	CC	country	site	coordinates	coll. date	host	collector	N	M
78	SYR	Syria	Ras Al-Bassit, Al Lataquiyeh Prov.	35° 50' N, 35° 50' E	3 June 2001	*P. kuhlii*	R. Lučan	1♂	1
116	IL	Israel	Kibbutz Mash'abbim, Negev	30° 35' N, 34° 28' E	29 May 2007	*P. kuhlii*	C. Dietz	1♂, 1♀	2
121	E	Spain	Valencia de Alcántara, Caceres Prov.	39° 23' N, 07° 11' W	1 August 2005	*P. pygmaeus*	G. Schreur	2♀♀	2
123	E	Spain	Cristina, Badajoz Prov.	38° 50' N, 06° 05' W	1 Sept. 2006	*Pipistrellus* sp.	J. A. Díaz	2♂♂, 3♀♀	2
124	E	Spain	Plasencia, Caceres Prov.	40° 01' N, 06° 05' W	19 Sept. 2006	*Pipistrellus* sp.	J. A. Diaz	5 juvs.	2
237	SYR	Syria	Palmyra, Deir Ez Zor Prov.	34° 33' N, 38° 16' E	6 Oct. 2004	*P. kuhlii*	R. Lučan	3♀♀	2
239	ET	Egypt	Bashendi, Dakhla, Al Wadi Al Jadid	25° 30' N, 29° 05 'E	23 Jan. 2010	*P. kuhlii*	P. Benda	♀	1
240	JO	Jordan	Al Azraq Az Shimali, Zarqa' Prov.	31° 50' N, 36° 49' E	13 Oct. 2008	*P. kuhlii*	P. Benda	3♂♂, 2♀♀	2
241	JO	Jordan	As Salihiyyah, Karak Prov.	29° 42' N, 35° 22' E	2 July 2010	*P. kuhlii*	P. Benda	5♂♂, 4♀♀	2
242	JO	Jordan	As Salihiyyah, Karak Prov.	29° 42' N, 35° 22' E	2 July 2010	*P. pipistrellus*	P. Benda	2♀♀	2
245	CY	Cyprus	Afendrika, North Cyprus	35° 33' N, 34° 14' E	25 July 2006	*P. kuhlii*	P. Benda	3♀♀	2
246	ET	Egypt	Mushyie, Dachla Oasis	25° 30' N, 29° 05 'E	25 March 2011	*P. kuhlii**	R. Lučan	1♂	1
248	CY	Cyprus	Afendrika, North Cyprus	35° 01' N, 32° 39' E	17 Oct. 2005	*P. kuhlii*	R. Lučan	1♂	1

Results and Discussion

Fauna

Our study reports on 41 specimens of *Cacodmus vicinus* collected at 13 localities in five Middle Eastern countries and Spain (Table 1). One record from Jordan (cf. Benda et al. 2010) was made from *Pipistrellus pipistrellus* and one from Spain from *P. pygmaeus*. The rest of the records from Spain come from unidentified *Pipistrellus* species. All other here reported records come from *P. kuhlii*, as well as all other records already published (Péricart 1996).

Very few cimicid species are known well enough to evaluate their host specificity. Two of the best known species *Cimex lectularius* Linaneus, 1785 and *C. pipistrelli* Jenyns, 1839 readily parasitize many different species and genera of Vespertilionidae (Balvín 2010, Balvín et al. 2012). The host range of some species of the *Cimex pilosellus* Horváth, 1910 species group is also rather broad (Usinger 1966). In contrast, according to the data available, *C. vicinus* seems to be specialized and related to the genus *Pipistrellus*, particularly to *P. kuhlii*. Therefore the distribution of *C. vicinus* is likely to follow the range of distribution of this bat species.

The records (Table 1) represent the first evidence of *Cacodmus vicinus* from the European continent. This species is the only known European representative of the genus and subfamily for the time being. We report the species from Cyprus and from Syria s.str. for the first time as well. These records also extend the known distribution of *C. vicinus* in other countries. Furthermore, to our knowledge, no member of the family Cimicidae has been reported from Spain so far. We only did not search for reports of the implied presence of *C. lectularius*, a re-emerging pest (e.g. Reinhardt & Siva-Jothy 2007), on humans in non-scientific media. Thus we also present the first record of the family from a free-living host from Spain.

Phylogeny

Except the specimen 245 from Cyprus, in all cases when two individuals from one collection were examined for COI sequences, the individuals shared the same haplotype. Also, according to AMOVA (Table 1), much more genetic diversity is found among than within countries. This suggests that genetic distances among specimens are congruent with geographic distances, as also suggested by the haplotype network (Fig. 2).

Specimens from three Spanish localities shared identical haplotype which is considerably distant in genetic distance (pairwise FST=0.813) from the haplotypes from the Middle Eastern samples. Such a deep divergence in mtDNA may be caused by geographic means. Provided that all the records from Spain come from the *P. pipistrellus* complex, the other possible cause can be different host association.

The low diversity within the Spanish samples, though they are too few to be drawn much conclusions, could suggest that the first records from Europe point to either a recent colonization of the area by *Cacodmus vicinus* or a recent host-switch from *P. kuhlii* to the *P. pipistrellus*

Table 2. Hierarchical analysis of molecular variance (AMOVA)

variance component	variance	% total	p	Φ-statistics
among countries	4.093	71.40	0.004±0.002	Φ_{CT} = 0.714
within countries	1.577	27.51	<0.001	Φ_{SC} = 0.961
within localities	0.062	1.09	<0.001	Φ_{ST} = 0.989

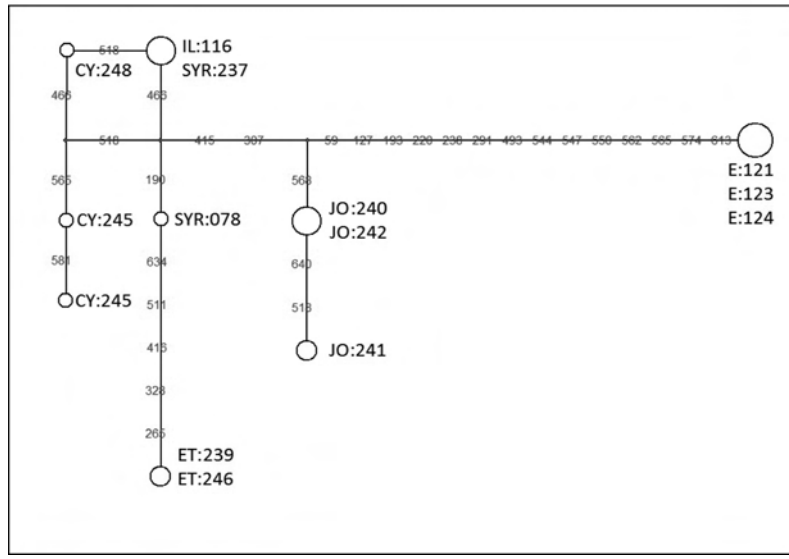

Fig. 2. Estimated haplotype network of populations of *Cacodmus vicinus* based on cytochrome oxidase subunit I. For abbreviations of countries and identification codes of samples see Table 1. Small numbers represent mutated positions.

complex. These two possibilities naturally do not exclude each other and both could be eased or preconditioned by the recent expansion of the distribution range of *P. kuhlii* (see Reiter et al. 2007 for a review). Therefore, further records from other European countries could then be expected. However, it is also likely that *C. vicinus* has been simply overlooked in Europe and these records are either coincidental or due to increased attention of bat specialists when mist-netting.

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