Systematic Entomology

Systematic Entomology (2015), DOI: 10.1111/syen.12127

Molecular evidence places the swallow bug genus *Oeciacus* Stål within the bat and bed bug genus *Cimex* Linnaeus (Heteroptera: Cimicidae)

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Abstract. The genera *Cimex* Linnaeus and *Oeciacus* Stål (Heteroptera: Cimicidae) are common haematophagous ectoparasites of bats or birds in the Holarctic region. Both their phylogenetic relationship and the systematics of the entire family previously were based on data from morphology and host relationships. Relationships among nine species of the genus *Cimex* and three species of the genus *Oeciacus* were analysed here using two mitochondrial and three nuclear genes. *Cimex* was shown to be paraphyletic with respect to *Oeciacus*. *Oeciacus* is thus proposed as a synonym of *Cimex*. The characteristic phenotype of *Oeciacus* results from the specific host association with different species of swallows (Hirundinidae). The morphological characters that have been used as diagnostic for the genera were shown to be valid and can be further used for determination at species level. The present analyses recovered the four traditional morphologically defined species groups of the genus *Cimex*. However, their relationships were poorly resolved – only the *C. hemipterus* group showed a well-supported relationship to the *C. pipistrelli* group. The molecular differentiation within the Palaearctic *C. pipistrelli* and the Nearctic *C. pilosellus* species groups correlates with their karyotype differentiation. Furthermore, the analyses suggest poly- or paraphyly of the former genus *Oeciacus.* Either way this indicates there is a large amount of host-associated phenotypic convergence in either bat- or bird-associated groups of species. The probability of host choice and subsequent switch in Cimicidae are discussed and possible scenarios of the evolution of host association in species of *Cimex* are suggested.

Introduction

In the context of evolutionary studies, parasitic organisms are extremely valuable models due to their diversity and complexity of life strategies. They exhibit diverse speciation modes, including sympatric speciation (Huyse *et al.*, 2005). Among these, the most common mechanism would likely be alloxenic speciation – the development of reproductive barriers between populations of a single parasitic species associated with different host organisms (Mehlhorn, 2008). Such an event can be mediated by different habitat preference or other characteristics of the host, but is likely associated with local adaptation of the parasite and shift in its host specificity (Poulin, 2007). Local adaptations can result in similar character combinations in different lineages (Poulin *et al.*, 2009). Convergent phenotypes often can be seen among different lineages of a higher taxon (Johnson *et al.*, 2012) or even within a single species (McCoy *et al.*, 2005). Interpretation of diversity or phylogeny of a group of ectoparasites based on morphology (e.g. Murrel & Barker, 2005; Light & Hafner, 2007; Perkins *et al.*, 2009; Roy *et al.*, 2009; Westram *et al.*, 2011) or host association (e.g. Johnson *et al.*, 2002) can therefore be misleading.

The family Cimicidae (Heteroptera) constitutes a group of specialized haematophagous ectoparasitic insects. Both adults and larvae stay on the body of their host only when feeding and the

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rest of the time they hide nearby in the shelter of the host (Hase, 1917). The transmission of cimicids between host shelters is passive and rather occasional. However, several studies report exclusively adult cimicids – mostly females – attached to the host body outside the shelter (Loye, 1985; Heise, 1988; Rupp *et al.*, 2004; Reinhardt & Jacobs, 2006; Balvín *et al.*, 2012a). As a mated female is able to found a new infestation on her own, such studies indicate that the attachment to the host body is intentional dispersal.

Cimicids exhibit traumatic insemination, which is associated with development of specialized organs in females, ectospermalege and mesospermalege, constituting the paragenital system. The male genitalia are also highly modified and asymmetric: the intromittent organ is represented by the left paramere. These structures are important characters in the phylogenetic reconstruction of the family based on morphological characters (Usinger, 1966).

Cimicidae contains about 110 species classified into 24 genera belonging to six subfamilies (Henry, 2009), which are distributed worldwide. About two-thirds of cimicids are associated primarily with bats, which have been suggested to be the ancestral hosts of the family (Horváth, 1913). The remaining species are associated with birds. Usinger (1966) suggested that cimicids have switched from bats to birds as hosts on four occasions: the entire subfamily Haematosiphoninae, the genera *Paracimex* Kiritshenko and *Oeciacus* Stål (Cimicinae), and the species *Cimex columbarius* (Jenyns) (Cimicinae). Several species of Cimicidae are able to use humans as occasional hosts. Three bat-associated species have developed continuous populations parasitizing man: *Cimex lectularius* (Linnaeus), *Cimex hemipterus* (Fabricius) and *Leptocimex boueti* (Brumpt) (Usinger, 1966). Cimicids require stable climatic conditions and blood sources, therefore they choose hosts that use shelters isolated from outside conditions, and are social or gregarious so that the parasites are not dependent on a single host individual or family to feed on.

Whereas the position of Cimicidae within Cimicomorpha is established based on both molecular and morphological characters (Schuh *et al.*, 2009), there is no recent analysis below the family level (Reinhardt & Siva-Jothy, 2007). The traditional hypothesis of phylogenetic relationships in the family (Usinger, 1966) is based on morphological characters, often related to reproductive organs, chromosome numbers and host associations. Chromosome numbers in Cimicidae are very diverse and definitely have high taxonomic relevance, but their phylogenetic implications seem to be limited (Poggio *et al.*, 2009; Kuznetsova *et al.*, 2011). Systematics at the species level is based mainly on continuous morphometric characters, which are often supported by data from hybridization experiments (Hase, 1938; Omori, 1939; Ueshima, 1964; Usinger, 1966). Within the subfamily Cimicinae, the genera *Oeciacus* and *Cimex* Linnaeus are considered sister groups. They are delimited by several distinctive but rather continuous characters that together with host association are considered useful enough to delimit genera (Usinger, 1966).

Within the genus *Cimex*, four groups are traditionally distinguished (Usinger, 1966). The *C. hemipterus* group is characterized by narrow lateral lobes of the pronotum, cleft and bristled paragenital sinus and consists of *C. hemipterus* (a pantropical species infesting man and bats) and *C. insuetus* Ueshima (a bat associated species from Thailand). The *C. lectularius* group is characterized by broad lateral lobes of the pronotum, and cleft and bristled paragenital sinus; it consists of *C. lectularius* – a cosmopolitan parasite of bats, man and domestic and synanthropic vertebrates – and *C. columbarius* – found on the domestic pigeon [*Columba livia* (Gmelin)] and the pied flycatcher (*Muscicapa atricapilla Linnaeus*) in Western and Central Europe. Based on morphology (Simov *et al.,* 2006), *C. emarginatus* Simov, a recently described species from a roost of Geoffroy's bat [*Myotis emarginatus* (Geoffroy)] in Bulgaria, was placed in the group as well. The *C. pipistrelli* group is characterized by narrow lateral lobes of the pronotum, cleft and naked paragenital sinus, and consists of ten described bat-associated Palaearctic species (Usinger, 1966; Ueshima, 1968; Bhat *et al.*, 1973; Bhat, 1974). The *C. pilosellus* group is characterized by narrow lateral lobes of the pronotum, and rounded and bristled paragenital sinus; it consists of six Nearctic species associated with bats. In the final group is the genus *Oeciacus*, with three species described. The Nearctic *Oeciacus vicarius* Horváth is a parasite of the cliff swallow [*Petrochelidon pyrrhonota* (Vieillot)], although it is also found rarely on the barn swallow (*Hirundo rustica erythrogaster* Boddaert) (Usinger, 1966) and the house sparrow [*Passer domesticus* (Linnaeus)] (Loye, 1985). The Palaearctic *Oeciacus hirundinis* (Lamarck) is common in nests of the house martin, [*Delichon urbica* (Linnaeus)] and is found in nests not only of several other birds, but also of the fat dormouse (*Glis glis* Linnaeus) (Országh *et al.*, 1990). *Oeciacus montandoni* Péricart was described from nests of the sand martin [*Riparia riparia* (Linnaeus)] from Romania. Later, Elov & Kerzhner (1977) reported this species from more localities in East Siberia, Kazakhstan and the European part of Russia in connection with *R. riparia, D. urbica* and the Pacific swift [*Apus pacificus* (Latham)].

Herein we test for the first time the traditional classification and taxonomic status of the genera *Cimex* and *Oeciacus* using a phylogeny based on DNA sequence data. Such an approach using algorithms modelling the evolution of protein coding or ribosomal gene sequences of mitochondrial or nuclear DNA, has repeatedly been useful for solving complicated taxonomic questions and has revealed surprising relationships not previously found based on traditional morphological methods (e.g. Marcilla *et al.,* 2002; Kim & Lee, 2008; Mas-Coma & Bargues, 2009; Johnson *et al.,* 2012).

We relate external morphological characters to our molecular phylogeny in order to assess their possible adaptive significance but also their validity for systematics. Aside from body size, most diagnostic characters used in delimiting the genera *Cimex* and *Oeciacus* have involved ratios of body dimensions (Jenyns, 1839; Horváth, 1912; Usinger, 1966; Péricart, 1972). Comparing such ratios, however, can be misleading as their differences can be due to shifts along a common allometric line (Kratochvíl *et al.,* 2003; Kratochvíl & Flegr, 2009). Using a modern statistical approach we tested the validity of the ratios of body dimensions for diagnostics. Moreover, we review the diagnostic characters for the bird-associated *Oeciacus* species including a

peculiar, possibly new species, sampled from birds from Japan. Comparing morphological and molecular results, we discuss the traditional classification at the generic level, assess the status of several species, and comment on hypotheses accounting for multiple parallel cimicid associations with birds.

Material and methods

Species

We studied material from the four *Cimex* species groups according to Usinger's (1966) classification (Table 1). We also included all three species originally classified in the genus *Oeciacus*, as well as bugs from martins from Japan, which are morphologically very similar to *Oeciacus hirundinis* and *O. vicarius,* but remain genetically distinct. Hereafter they are referred to as *Cimex* sp. Japan. For the genus *Oeciacus,* we were not able to obtain fresh material of *O. montandoni* Pericart. This species is rarely found in nests of sand martins and we are not aware of any record since 1976 (Elov & Kerzhner, 1977). Despite the recent date of collection of *Cimex emarginatus* Simov *et al.* type material, we were not able to extract DNA either from dry adults or larvae kept in ethanol.

As outgroups in the phylogenetic analyses we used the following taxa (Table 1): *Paracimex setosus* Ferris & Usinger (Cimicinae), *Aphrania elongata* Usinger*, Cacodmus vicinus* Horváth and an unidentified genus in the subfamily Cacodminae, *Orius niger* (Wolff) (Anthocoridae), *Lygus elisus* Van Duzee (Miridae) and *Rhodnius prolixus* Stal (Reduviidae).

Material of *O. montandoni*, *O. vicarius* and *C. emarginatus* used in the morphological study was loaned by the Zoological Museum in Saint Petersburg (Russia), Hungarian Natural History Museum in Budapest (Hungary), Charles R. Brown (University of Tulsa, USA) and Nikolay Simov (National Museum of Natural History, Sofia, Bulgaria). Specimens were identified based on morphological characters proposed by Usinger (1966) and Péricart (1972). Material was collected by the authors and other collectors, preserved in 96% ethanol, and deposited in the collection of Ondřej Balvín at Charles University in Prague.

DNA extraction, PCR and sequencing

The tissue for DNA extraction was obtained from half of the thorax and legs of each specimen. Extractions were performed using the DNAeasy Tissue kit (Qiagen, Hilden, Germany). The extracted DNA was kept in tris-EDTA and stored at −18°C. Amplification of cytochrome oxidase subunit I (*COI*, partial: nucleotides 42–699), large mitochondrial ribosomal subunit (*16S* rRNA, partial: 5′and central domains), small nuclear ribosomal subunit (*18S* rRNA, complete), internal transcribed spacer 2 (*ITS2*, complete) and elongation factor 1 subunit α (*EF1*α, partial: nucleotides corresponding to 322–892 in *Drosophila melanogaster* Meigen) was performed using primers specified in Table S1. The *18S* rDNA was amplified using two pairs of primers delimiting two partially overlapping regions. The annealing temperatures for polymerase chain reactions (PCR) and length of amplified fragments for each gene are given in Table S1. The sequencing was done in both directions using BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Waltham, MA, U.S.A.) and ABI PRISM® 3100-Avant Genetic Analyzer (Applied Biosystems) or using a commercial sequencing service (Macrogen Inc., Seoul, South Korea).

Alignments, saturation tests, and secondary and exon–intron structure

For species where multiple specimens shared the same sequences for all genes, only one was used in the phylogenetic analyses. The sequences were aligned using MAFFT (Katoh *et al.*, 2009). Protein-encoding genes were aligned using default settings. Other regions were aligned using the E-INS-i algorithm suitable for sequences with conserved domains and highly variable regions. The *ITS2* gene was aligned excluding all outgroup taxa, even the subfamily Cacodminae. Furthermore, even the variability in *ITS2* within Cimicinae was found to be very large and the alignment seemed to be partly ambiguous. Therefore, we analysed the concatenated datasets with and without *ITS2* and interpreted the results with caution. We were not able to amplify *ITS2* in *Paracimex setosus* Ferris & Usinger. In order to avoid misleading phylogenetic inference due to saturation of DNA, we plotted uncorrected pairwise distances against maximum-likelihood (ML) distances for all genes (Tian *et al.*, 2008). Furthermore, we plotted uncorrected pairwise distances for the coding genes against the number of transversions and transitions for each codon position (Maekawa & Matsumoto, 2000). We reconstructed the secondary structure of the *16S* and *18S* rRNA genes according to Buckley *et al.* (2000) and Ouvrard *et al.* (2000) with respect to matching of nucleotides actually present in stems. As the stems and loops have different evolutionary rates they were used as separate partitions in the phylogenetic analyses. The introns of *EF1*α were excluded from the analyses according to Djernaes & Damgaard (2006). The lengths of the aligned data partitions were 658 base pairs (bp) (*COI*), 397 bp (*16S*), 1906 bp (*18S*), 520 bp (*EF1*α) and 1127 (*ITS2*) (Table S4).

Phylogenetic analyses

The molecular matrix was assembled from 54 specimens taken from 33 localities belonging to 10–12 species of the nominal genera *Cimex* and *Oeciacus*. One species from the same subfamily, three from Cacodminae and three from other heteropteran families were used as outgroups. In the phylogenetic analyses we treated each of the genes as separate partitions and created the following concatenated matrices: mitochondrial, nuclear, nuclear excluding *ITS2*, coding, ribosomal, *18S* + *ITS2* (neighbouring regions), all genes and all genes excluding *ITS2*. The sequences of stems and loops of the *16S* and *18S* genes were also analysed as separate partitions. Each of the datasets

Table 1. List of material studied.

IC	Country	Locality	Host	Date	Legit	N	M	Genbank accession no.				
								COI	*16S*	*18S*	*EF1α*	*ITS2*
Cimicinae: *Cimex pipistrelli* group: Europe												
5	Czech Rep.	Točník castle	*Myotis myotis*	16.vii.2005	Ondřej Balvín	10	–	–	–	–	–	–
48	Czech Rep.	Blansko	*Myotis myotis*	27.vi.2006	Ondřej Balvín, Martin Pokorný	–	2	GU985531	GU985553	KC503546	KC503545	KC503542
52	Czech Rep.	Křtiny, Blansko district	*Myotis myotis*	27.vi.2006	Ondřej Balvín, Martin Pokorný	2	2	GU985527	GU985549	KC503547	KC503545	KC503543
57	Czech Rep.	Lipov, Hodonín district	*Myotis myotis*	29.vii.2006	Ondřej Balvín	2	2	GU985533	GU985555	KC503548	KC503545	KC503542
61	Czech Rep.	Luhačovice	*Myotis myotis*	29.vii.2006	Ondřej Balvín, Petr Wolf	2	2	GU985529	GU985551	KC503549	KC503545	KC503543
62	Czech Rep.	Veselí nad Lužnicí, Ruda	*Nyctalus noctula*	23.viii.2006	Radek Lučan	2	2	GU985531	GU985554	KC503550	KC503545	KC503542
73	Czech Rep.	Jeřišno - Heřmaň	*Myotis myotis*	18.ix.2007	Ondřej Balvín	6	1	GU985528	GU985550	KC503551	KC503545	KC503542
KR 83	U.K.	Rindleford, Bridgnorth, Shropshire	*Pipistrellus* sp.	14.xii.1999	John Mason	2	1	GU985534	GU985556	KC503552	KC503545	KC503543
103	Bulgaria	Červen, Ruse region	*Nyctalus noctula*	1.x.2005	Ivailo Borissov	2	2	GU985530	GU985552	KC503553	KC503545	KC503542
109	Switzerland	Flawil	*Myotis myotis*	4.vii.2007	René Güttinger	2	–	–	–	–	–	–
128	Germany	Schulzenhof bei Stechlin, Brandeburg	*Myotis naterreri*	23.iii.2007	D. Dolch	2	–	–	–	–	–	–
225	Czech Rep.	Veselí nad Lužnicí, Ruda	*Myotis daubentoni*	28.v.2004	Jitka Vilímová	6	–	–	–	–	–	–
Cimicinae: *Cimex pipistrelli* group: *Cimex japonicus*												
350	Japan	Akita prefecture, Daisen city, Omagari bridge	*Vespertilio superans*	10.v.2010	Mitsuru Mukohyama	8	2	KC503541	KF018727	KF018713	KF018744	KF018700
351	Japan	Aomori prefecture, Shichinohe town, Temmadate bathouse	*Vespertilio superans*	3.viii.2010	Mitsuru Mukohyama & Tomoya Kobayashi	8	2	KC503541	KF018727	KF018713	KF018744	KF018700
Cimicinae: *Cimex adjunctus* (*C. pilosellus* group)												
140	U.S.A.	Washington county, North Carolina	*Nycticeius humeralis*	7.vi.2005	Matina Kalcounis-Ruppell	–	1	GU985536	GU985558	KF018712	KF018742	KF018699
141	U.S.A.	Galesburg, Kalamazoo county, Michigan	*Eptesicus fuscus*	6.vii.2005	Lee Johnson	–	1	GU985535	GU985557	KF018712	KF018741	KF018698
142	U.S.A.	Fulton, Kalamazoo county, Michigan	*Eptesicus fuscus*	19.vi.2005	Lee Johnson	–	1	GU985537	GU985559	KF018712	KF018743	KF018699
Cimicinae: *Cimex* cf. *antennatus* (*C. pilosellus* group)												
TM C10	U.S.A.	Antelope Valley, California	–	15.viii.2002	A.C. Lohmann	–	1	KF018760	KF018732	KF018718	KF018749	KF018705
Cimicinae: *Cimex latipennis* (*C. pilosellus* group)												
KR C18	Canada	Hope, British Columbia	*Myotis lucifugus*	Unknown	T. Luszczell	–	1	KF018758	KF018734	KF018720	KF018750	KF018707
KR C19	Canada	Hope, British Columbia	*Myotis volans*	Unknown	T. Luszczell	–	1	KF018757	KF018733	KF018719	KF018750	KF018706
Cimicinae: *Cimex pilosellus*												
KR C20	U.S.A./Canada	Coal Banks area, Montana/British Columbia (?)	Bat	Unknown	C. Lausen	–	1	KF018759	KF018731	KF018717	KF018748	KF018704
Cimicinae: *Cimex hemipterus*												
91	India	Busline Bangaloren (Karnataka) - Ootyn (Tamil Nadu)	Human	27.ix.2005	Petr Šípek	1	–	–	–	–	–	–
92	India	Mannárakkát	Human	29.ix.2005	Petr Šípek	1	–	–	–	–	–	–
93	Indonesia	Kuala Lumpur	Human	Summer 2006	Magdalena Lučanová	1	–	–	–	–	–	–
145	Malaysia	Melaca	Human	15.xii.2011	Hana Šípková	1	1	KF018754	KF018724	KF018739	KF018710	KF018695
801	India	Tamil Nadu	Human	6.xii.2010	Robert Vlk	4	1	KF018755	KF018725	KF018739	KF018710	KF018696
Cimicinae: *Cimex lectularius*												
24	Czech Rep.	Držovice, Litoměřice district	*Myotis myotis*	10.ix.2005	Borek Franěk	5	–	–	–	–	–	–
26	Czech Rep.	Olomouc, stock	Human	Autumn 2005	Libor Mazánek	2	2	GU985524	GU985546	KF018711	KF018740	KF018697
39	Czech Rep.	Brandýs nad Orlicí	*Myotis myotis*	21.vi.2006	Ondřej Balvín	2	2	GU985526	GU985548	KF018711	KF018740	KF018697
46	Czech Rep.	Lysice, Blansko district	*Myotis myotis*	28.vi.2006	Ondřej Balvín, Martin Pokorný	2	2	GU985525	GU985547	KF018711	KF018740	KF018697
110	France	Marseille	Human	26.6.2007	Zuzana Tollerianová	2	2	GU985523	GU985545	KF018711	KF018740	KF018697
133	Serbia	Deliblatská Peščara, Rošiana	*Myotis emarginatus*	13.vii.2006	Milan Paunović	5	2	KF018756	KF018726	KF018711	KF018740	KF018697
165	Czech Rep.	Prague, student lodgings	Human	1.xii.2002	Václav Gvoždík	7	–	–	–	–	–	–
412	Hungary	Aggtelek, Vizsló	Diverse bat species	8.vii.2009	Ondřej Balvín	5	–	–	–	–	–	–

Table 1. Continued.

IC	Country	Locality	Host	Date	Legit	N	M	Genbank accession no.				
								COI	*16S*	*18S*	*EF1α*	*ITS2*
Cimicinae: *Cimex emarginatus*												
–	Bulgaria	Primorsko, Arkutino	*Myotis emarginatus*	19.viii.1999	Nikolay Simov, A. Gueorguieva	3	–	–	–	–	–	–
Cimicinae: *Oeciacus hirundinis*												
120	Czech Rep.	Žíteč, Jindřichův Hradec district	*Delichon urbica*	23.ix.2007	Jaroslav Cepák	4	2	GU985543	GU985565	KF148594	KF018736	KF018691
129	Germany	Postdam - Golm	*Delichon urbica*	17.viii.2007	Ingo Scheffler	4	1	GU985544	GU985566	KF148594	KF018736	KF018692
130	Germany	Postdam, Neu Fahrland	*Delichon urbica*	20.xii.2007	Ingo Scheffler	4	2	GU985544	GU985567	KF148594	KF018736	KF018692
893	Czech Rep.	Čížov	*Delichon urbica*	26.v.2010	Antonín Reiter	2	–	–	–	–	–	–
894	Czech Rep.	Jedovnice	*Delichon urbica*	22.v.2009	Robert Vlk	4	–	–	–	–	–	–
Cimicinae: *Oeciacus vicarius*												
SB04	U.S.A.	Sarben, Keith County, Nebraska	*Petrochelidon pyrrhonota*	2.10.2008	Charles R. Brown	–	3	GU985541	GU985563	KF018709	KF018738	KF018694
KR 88-10	U.S.A.	Keystone, Keith County, Nebraska	*Petrochelidon pyrrhonota*	3.x.2008	Charles R. Brown	16	2	KF018753	KF018723	KF018709	KF018738	KF018694
KR 88-10	U.S.A.	Keystone, Keith County, Nebraska	*Petrochelidon pyrrhonota*	3.x.2008	Charles R. Brown	–	1	KF018752	KF018722	KF018709	KF018738	KF018694
HNMN	U.S.A.	Los Angeles	*Petrochelidon pyrrhonota* (?)	Unknown	Unknown	1	–	–	–	–	–	–
HNMN	U.S.A.	Los Angeles	*Petrochelidon pyrrhonota* (?)	Unknown	C.V. Riley	1	–	–	–	–	–	–
HNMN-11423	U.S.A.	–	–	–	–	1	–	–	–	–	–	–
Cimicinae: *Oeciacus montandonii*												
ZMAS	Kazachstan	Turgaj	*Riparia riparia*	7.vi.1947	Formozov	4	–	–	–	–	–	–
ZMAS	Kazachstan	lake Čuškakul, 275 km south of Kustanaja	*Riparia riparia*	30.vi.1936	Formozov	3	–	–	–	–	–	–
ZMAS	Mongolia	Vostočnij Ajmak, Vost. ugol, lake Bujr-Nur	*Riparia riparia*	7.vi.1976	Kerzhner	6	–	–	–	–	–	–
Cimicinae: *Cimex* sp.												
895	Japan	Konda, Sasayama-City, Hyogo-pref.	*Delichon dasypus, Hirundo daurica japonica*	9.iii.2008	Nobuhiko Kataoka	8	2	GU985542	GU985564	KF018708	KF018737	KF018693
896	Japan	Kamiichi, Niimi-City, Okhama-pref.	*Delichon dasypus*	18.viii.2008	Nobuhiko Kataoka	2	1	GU985542	GU985564	KF018708	KF018737	KF018693
897	Japan	Hokodate, Nikaho-City, Akita-pref.	*Delichon dasypus*	31.viii.2007	Nobuhiko Kataoka	–	1	GU985542	GU985564	KF018708	KF018737	KF018693
898, 899	Japan	Konda, Sasayama-City, Hyogo-pref.	*Delichon dasypus*	9.i. + 3.vii.2007	Nobuhiko Kataoka	–	2	GU985542	GU985564	KF018708	KF018737	KF018693
Cimicinae: *Paracimex setosus*												
C9	–	–	–	–	–	–	1	KF018761	KF018735	KF018721	KF018751	–
Cacodminae: *Cacodmus vicinus*												
240	Jordan	Jordan, Azraq oasis	*Pipistrellus kuhlii*	13.x.2008	Petr Benda	–	1	KF018762	KF018728	KF018714	KF018745	KF018701
Cacodminae: gen. sp.												
244	Mauretania	Savanna (10 km SE of Bogué)	*Scotophilus leucogaster*	October 2010	Petr Benda	–	1	KF018764	KF018730	KF018716	KF018747	KF018702
Cacodminae: *Aphrania elongata*												
243	Mauretania	Savanna (10 km SE of Bogué)	*Nycticeinops schlieffenii*	October 2010	Petr Benda	–	2	KF018763	KF018729	KF018715	KF018746	KF018703
Anthocoridae: *Orius niger*			DNA sequences downloaded from GenBank					NC012429	NC012429	GQ258418.1	–	–
Miridae: *Lygus elisus*			DNA sequences downloaded from GenBank					HM215068.1	AY252785.1	AY252310.1	–	–
Reduviidae: *Rhodnius prolixus*			DNA sequences downloaded from GenBank					AF449138.1	AF324519.1, EU822954.1	AY345868.1	ACPB02032738.1	

IC, the identification code of the sample in the authors' or the original collection; museum loans: HNMN, Hungarian Natural History Museum in Budapest; ZMAS, Zoological Museum of the Zoological Institute of Russian Academy of Sciences; KR, Klaus Reinhardt; TM, Ted Morrow; M, number of individuals used in the molecular analysis; N, number of individuals used in the morphological analysis.

was analysed using two approaches. Bayesian analyses were run using MrBayes 3.0 (Ronquist & Huelsenbeck, 2003) and ML trees were constructed using RAxML (Stamatakis *et al.*, 2008). Each analysis was run three times using the same settings (see below) in order to assess congruence. In the Bayesian analyses the substitution models for each partition were set according to Modeltest (Posada & Crandall, 1998) using the Bayesian Information Criterion (BIC). The ML analysis was run using default model and settings. Bayesian analyses were run using four chains in two independent runs for 5 million generations. The consensus tree was created rejecting the first 5000 trees out of the 50 000 sampled as a burn-in as the subsequent 90% of trees always showed stabilized likelihood values.

The analyses described above were repeated for some or all datasets with the following alterations. For each dataset that included protein-encoding genes we performed extra analyses using the codon substitution model, as implemented in MrBayes 3.0 or RAxML. The saturation tests showed only the transitions at the third codon position of *COI* to be saturated, and therefore all analyses using *COI* were run twice – including and excluding the third codon position. As the position of *Paracimex setosus* was very unstable across different trees (Table S2) and rarely appeared sister to *Cimex* and *Oeciacus* as expected, we repeated each analysis excluding this taxon.

In order to discuss species limits and depth of divergence in particular lineages, we estimated the evolutionary divergence between sequences of species using Mega 5.1 (Tamura *et al.*, 2011). We chose the Kimura-2-parameter model (Kimura, 1980) to compare our results with reviews on barcoding in Heteroptera (Jung *et al.*, 2011; Park *et al.*, 2011). The distances were computed for *COI* but also for the concatenation of the four genes that we managed to unambiguously align (*COI*, *16S*, *18S* and *EF1α*). Ambiguously aligned positions were removed for each sequence pair.

Morphological analysis

Table 1 lists the specimens used in the morphological analysis. The specimens were photographed in a standardized manner and flattened by a smaller dish in a Petri dish with ethanol, using a stereoscopic microscope (Olympus SZX9) and a digital camera (Olympus C-5060) operated by Photo Micro 2.0. Measurements were taken using MeasureIT (Olympus). The museum specimens of *O. montandoni* were moistened and photographed in the same way in order to avoid errors in measurement. Only the museum specimens of *O. vicarius* were photographed dry. Important characters traditionally used to distinguish the genera *Cimex* and *Oeciacus* are summarized in Table 2. Because the samples named *Cimex* sp. Japan likely represent a new species similar to *Oeciacus*, we reviewed the diagnostic characters of the described species based on morphology as a putative new species. Characters measured were as follows: total body length (tl); length of antennal segments (al1-4); width of antennal segments (aw1-4); width of head (hw); eye diameter (ed); interocular space (is); width of the pronotum (pw); medial length of the pronotum (pm); total length of the pronotum (pl); maximum length of setae on the pronotum (sp); and maximum length of setae on the hemelytron (sh).

Statistical analyses were run using Statistica 8.0 (StatSoft Inc., 2009). We performed a principal component analysis (PCA) using the measured values. In order to test the difference between the genera or species in particular relative characters and disregard possible allometric effects, we performed analyses of covariance (ANCOVAs). The denominator of the fraction representing a relative character to test (Table 2) was used as the dependent variable, the taxon as the factor and the numerator as the covariate. First, we tested the effect of interaction of categorical factor and covariate. In case it did not show significance, we tested their effects separately. For ANCOVAs showing differences between *Oeciacus* species (including *Cimex* sp. Japan) we performed a Tukey HSD (honestly significant difference) posthoc test in order to test differences within particular pairs of samples.

Results

Molecular analysis

The tree based on four genes (Fig. 1) is regarded as the most reliable as it is based on all aligned DNA data. The topology of trees based on other datasets was often slightly different, however, almost all are congruent in the following results. The subfamily Cimicinae was shown to be monophyletic (Fig. 1; Table S2). *Paracimex setosus* was recovered at many different positions depending on the analysis. Three distinct clades were recovered: the *C. lectularius* and *C. pilosellus* species groups and a clade (Clade A) consisting of *C. hemipterus* and *C. pipistrelli* species groups and other specimens from birds. In all analyses, the genus *Cimex* was found to be paraphyletic with respect to the genus *Oeciacus.*

For each of the datasets, both Bayesian and ML analyses consistently produced trees with basically the same topology (Table S2). Use of a codon model for protein coding genes did not affect the topology of trees and did not considerably change support values for clades. Also, saturation of third codon positions of *COI* likely caused no bias in the topology of the trees. Exclusion of the third positions affected only the resolution of Clade A in analyses based only on *COI*. The saturated data thus did not contribute significantly to resolution of the major clades and species within the *C. pilosellus* group. We also plotted the genetic distance against number of transitions only for Clade A and found no indication of saturation.

Whereas the monophyly of the subfamily Cimicinae is supported by all datasets except for *18S*, the position of *Paracimex setosus* within the subfamily remained unclear. This taxon was usually recovered either within or sister to Clade A. It was also found sister to Cimicinae in a few analyses (Table S2). The exclusion of *P. setosus* from analyses, however, did not affect the topology of the trees.

Trees generated from concatenated matrices of nuclear genes, all five genes, or separate *18S* data, recovered a monophyletic clade containing all taxa collected from Hirundinidae. Other tree topologies (e.g. Fig. 1) recovered these taxa as paraphyletic with

Table 2. Review of characters used for distinction of genera and species in literature.

Character	Representation in this study	Difference between species or genera in literature	Reference
Total body size	tl	*Oeciacus* < *Cimex*	Jenyns (1839)
		O. hirundinis < *O. vicarius*	Horváth (1912)
Width of pronotum	pw	*O. hirundinis* = 0.83–0.9 mm	Usinger (1966)
		O. vicarius > 1 mm	
Ratio of the pronotum to the head width	pw/hw	*Oeciacus* < 1.5	Usinger (1966)
		Cimex > 1.5	
Depth of the anterior pronotal concavity	pl/pm	*Oeciacus* < *Cimex*	Jenyns (1839)
Shape of the anterior margin of the pronotum	Only discussed[a]	*O. vicarius:* sinuate	Horváth (1912)
		O. hirundinis: straight	
Ratio of the length of the third to the length of the fourth antennal segment	al3/al4	*Oeciacus* ≥ 1	Jenyns (1839)
		Cimex ≫ 1	
Ratios of the length of the third and fourth to the length the second antennal segment	al3/al2; al4/al2	*O. hirundinis*: third and fourth segments distinctly shorter than second	Péricart (1972)
		O. montandoni: third and fourth segments slightly shorter than second	
Ratio of the width of the last two to the first two antennal segments	aw3/aw2; aw4/aw2[b]	*Oeciacus*: slightly thinner	Horváth (1912)
		Cimex: distinctly thinner	
Ratio of the head width to the length of the third antennal segment	hw/al3	*O. vicarius* < 2.3	Usinger (1966)
		O. hirundinis > 2.3	
Ratio of the length of the second antennal segment to the width of the interocular space	al2/is	*Oeciacus* ≤ 2/3	Usinger (1966)
		Cimex ≤ 1	
		O. hirundinis = 2/3	Péricart (1972)
		O. montandoni = 0.8	
Length and character of the pubescence	sp/ed; sh/ed[c]	*Cimex*: lesser	Jenyns (1839)
		Oeciacus: larger	
		Cimex: thick and short	Usinger (1966)
		Oeciacus: fine and long	
		O. hirundinis: fine and long	Péricart (1972)
		O. montandoni: thick and short	

[a]We suppose Horváth meant the shape of that part of the margin that is in contact with the head, excluding the lateral lobes. Otherwise it would be a character similar to depth of the anterior pronotal concavity. Its consistency in the species is discussed only in the present study.
[b]In the present study the last two segments related only to the second.
[c]Only the length represented in the present study.
The representation of each character in the present study is expressed using the abbreviations of measured characters in column 2.

respect to the *C. pipistrelli* group. Almost all trees recovered three distinct clades: the *C. lectularius* and *C. pilosellus* species groups and Clade A. However, relationships among the three clades were seldom consistent across analyses based on different concatenated datasets, indicating the variable phylogenetic signal among the individual gene loci used here. Clade A was not monophyletic in trees based on solely on *18S*, or in combination with *ITS2*. Typically, the support values for the monophyly of Clade A increased with the number of genes used. *Cimex hemipterus* was recovered as sister to the rest of Clade A in the majority of trees. *Oeciacus vicarius* was sister to the rest of Clade A in trees based on *COI* (Fig. 1). A clade consisting of all samples collected from Hirundinidae was sister to the rest of Clade A in all trees based on *ITS2* and all five genes. In these cases, *C. hemipterus* appeared sister to the remaining samples from Clade A. However, in the trees based on all five genes it appeared to constitute a less supported clade together with *C. japonicus*. Monophyly of the West Palaearctic *C. pipistrelli* group was recovered only in trees based on datasets containing *ITS2*. In other cases, the group was paraphyletic with respect to either one or both of the samples from Japan (i.e. *C. japonicus* and *Cimex* sp. Japan).

The estimated evolutionary distances between sequences of species used in the study are given in Table 3. The sequence distances (K2P) for the 658-bp-long barcoding fragment of *COI* were 3.0–3.6% for the *C. pipistrelli* group including *Cimex* sp. Japan and *C. japonicus*. In comparison, the distances among other Cimicinae species were mostly >15% (range 6.3–23.8%). The differences between species of the subfamilies Cimicinae and Cacodminae were in the range of 27–31%.

Morphological analysis

The values of diagnostic characters in our specimens largely correspond to values given in the literature for each species and genus, except for the ratio of head and pronotum widths (Table S3). In the PCA (Fig. 2) using the measured values of morphological characters, the genera appeared to be well discriminated with the species also placed more or less in distinct clusters. All characters were strongly correlated with PC1 (72.26% of

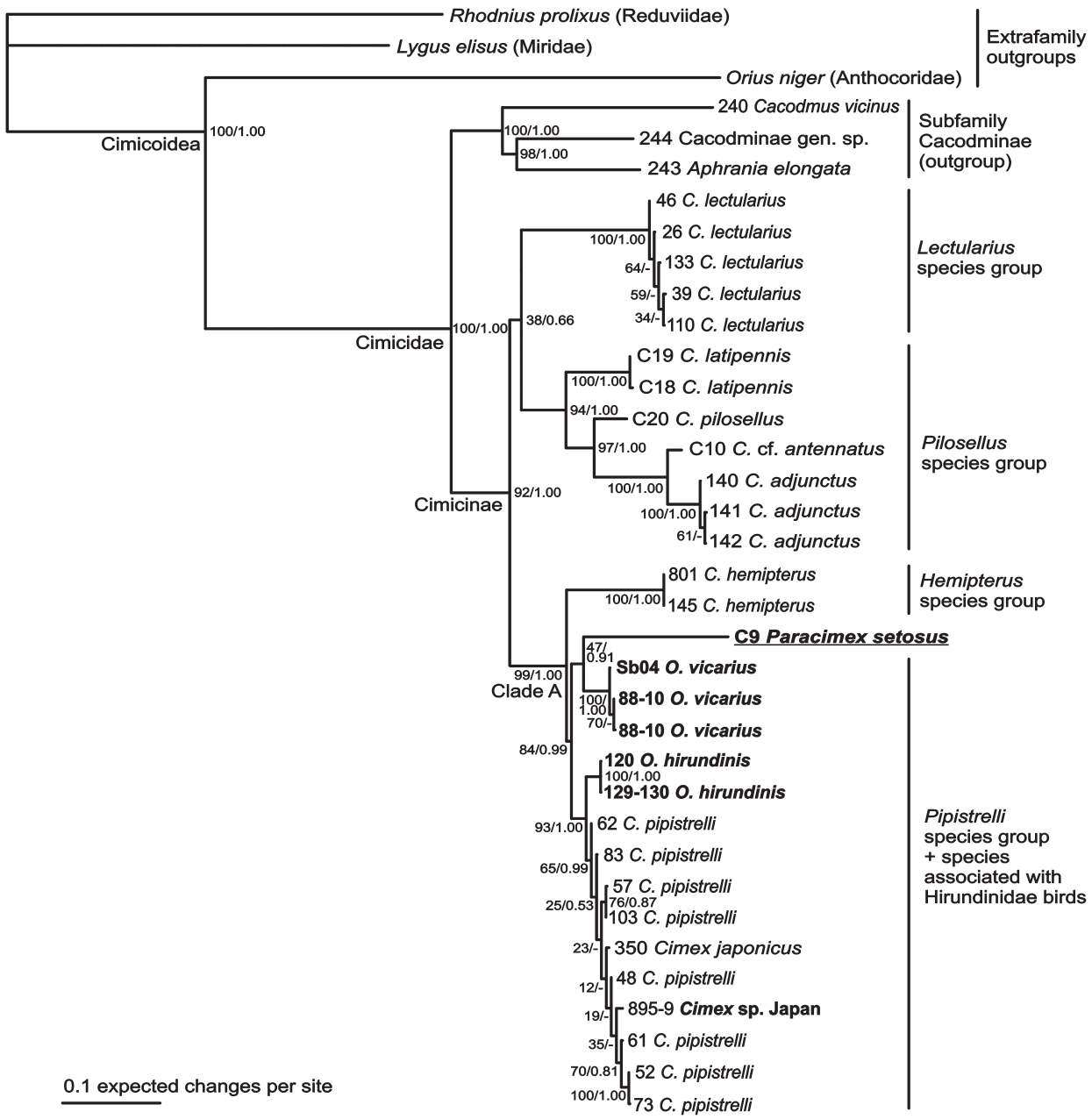

Fig. 1. Tree produced from RAxML analysis based on combined data of *16S*, *18S*, *COI* and *EF1α* and supplied with posterior probability values from Bayesian analysis (after slash); both analyses used the codon model. Bold names of taxa represent association with birds: swallows (Hirundinidae) (bold only), and swifts (Apodidae). (bold underlined). Numbers or signs in front of species names refer to the identification code of samples (IC, Table 1).

variability), which largely reflect the overall body size. Only the widths of third and fourth antennal segment and hair lengths were correlated with PC2 (9.28%). ANCOVAS (Table 4) showed that each diagnostic character which we reviewed represents a significant difference among the respective taxa, except for the ratio of head and pronotum widths between the genera. The differences in diagnostic characters for each pair of *Oeciacus* species suggested by literature were confirmed by Tukey HSD posthoc tests. *Cimex* sp. Japan was shown to be different in five out of the six characters from *O. vicarius* and *O. montandoni* but in none from *O. hirundinis*. The morphological analysis thus ruled out the possibility that the Japanese sample belongs to *O. montandoni*.

Discussion

The molecular phylogenetic analyses in our study basically support the division of the genus *Cimex* into the

Table 3. Estimates of evolutionary divergence between sequences of species using the Kimura-2-parameter model (Kimura, 1980).

	C. pip.	*C. jap.*	Sp.Jap.	*O. hir.*	*O. vic.*	*C.hem.*	*C. lec.*	*C. lat.*	*C. pil.*	*C.* sp.	*C. adj.*	*P. set.*	Cacod.
West Palaearctic *C. pipistrelli* group	***3***	**3**	**3.6**	**6.3**	**12.6**	17.8	19.4	21.2	19.2	20.3	21.4	17.1	26.3
C. japonicus	**0.1**	***0***	**3.3**	**6.3**	**12.6**	17.6	19.6	20.5	19	19.9	20.7	16.3	25.8
Cimex sp. Japan	**1.6**	**1.6**	***0***	**6.3**	**13.4**	16.6	18.2	21.6	18	20.3	21.4	17.1	26.1
O. hirundinis	**1.7**	**1.7**	**0.5**	***0.5***	**13.4**	16.8	18.7	21.8	18.8	19.7	20.6	15.8	27.4
O. vicarius	**1.6**	**1.6**	**0.6**	**0.9**	***0.6***	20.1	21.8	22.3	21.3	22.7	23.8	16.9	27.8
C. hemipterus	1.1	1.1	2.3	2.4	2.4	*0*	22.6	22.4	20.3	23.1	23.5	18.7	28.2
C. lectularius	6.2	6.1	5.9	6	5.8	6.4	*1.2*	23	20.5	20.8	22.9	21.3	29.6
C. latipennis	6.5	6.5	6.1	6.3	6.3	7	5.9	***1.4***	**16.2**	**17.6**	**20.7**	19.4	31.3
C. pilosellus	6.4	6.3	5.8	6	5.8	6.6	5.8	**2.6**	–	**16.2**	**18.8**	19.1	29.5
C. cf. *antennatus*	6.5	6.5	5.9	6.2	6	6.9	5.8	**2.7**	**1.6**	–	**11.3**	22.6	30.5
C. adjunctus	6.6	6.6	6.1	6.3	6.1	6.9	5.7	**3.1**	**2**	**1.9**	***0.9***	22.9	30
Paracimex setosus	4.4	4.4	4.6	4.5	4.5	4.4	4.6	4.5	5	4.9	4.9	–	27.5
Cacodminae[a]	3.5	3.5	3.7	3.6	3.7	3.4	3.4	4	3.9	3.8	3.6	6.7	*27.5*

[a]Nuclear distances for Cacodminae are based on *18S* and *EF1α* only.

All ambiguously analysed positions were removed for each sequence pair. Diagonal (italic): largest distance either within species or West Palaearctic *C. pipistrelli* species group (not shown in species represented by single individual) based on *COI*. Above diagonal: largest estimated divergence among species based on *COI*. Below diagonal: largest estimated divergence based on concatenate of all three nuclear genes. Bold underlined values represent diversity within clade A and the *C. pilosellus* group.

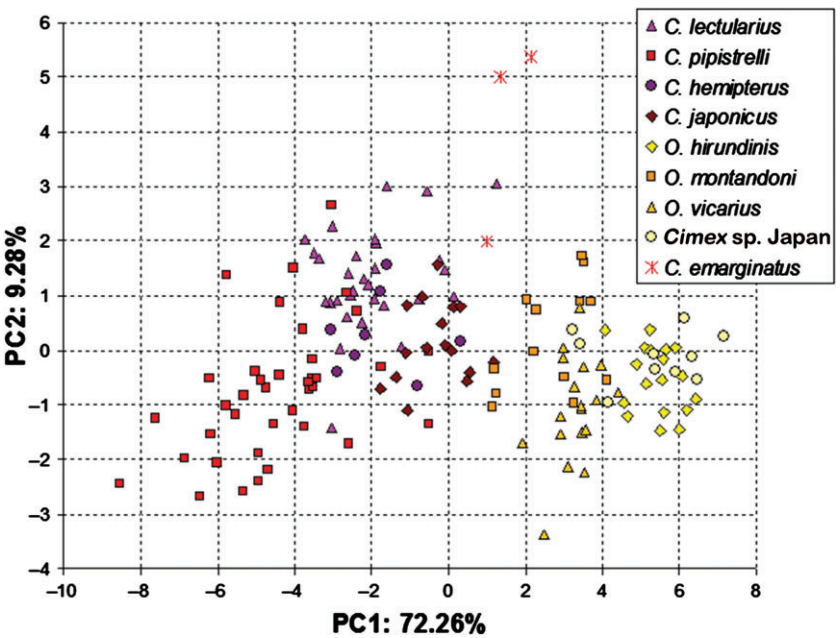

Fig. 2. Projection of the specimens on the first two axes of principal component analysis (PCA) based on measured values of morphological characters (Tables 2 and S3).

four morphologically distinct species groups according to Usinger (1966) – *C. lectularius, C pilosellus, C. pipistrelli* and *C. hemipterus*. The first three groups formed three distinct clades with high support in almost all analyses. *Cimex hemipterus* also remained distinct as the fourth group and sister to the *C. pipistrelli* group in most analyses. Only in trees based on all five genes did a clade consisting of *C. hemipterus* and *C. japonicus* appear, but with low support. Relationships between the first three clades (*C. lectularius* group; *C. pilosellus* group; *C. pipistrelli* + *C. hemipterus* groups + former *Oeciacus* species) remained unresolved in some analyses or resolved with very low support in others.

The most significant result of our phylogenetic analyses was the paraphyly of the genus *Cimex* with respect to the genera *Oeciacus* and *Paracimex*. The genera *Cimex* and *Oeciacus* have long been considered as sister taxa which are distinct enough to be maintained as separate genera (Usinger, 1966; Péricart, 1972). However, Usinger (1966) did question whether mostly continuous morphometric diagnostic characters are powerful enough to discriminate between the genera, concluding that

Table 4. F-values for ANCOVAs testing differences in relative characters between genera *Cimex* and *Oeciacus* and among the *Oeciacus* species including *Cimex* sp. Japan.

Dependent	Categorical factor	*F*-value	Covariate	*F*-value	*F*-value for interaction	*P*-values for Tukey HSD test					
						O. hirundinis × *O. vicarius*	*O. hirundinis* × *O. montandoni*	*O. hirundinis* × *Cimex* sp. Japan	*O. vicarius* × *O. montandoni*	*O. vicarius* × *Cimex* sp. Japan	*O. montandoni* × *Cimex* sp. Japan
pl	Genus	59.9***	pm	352***	n.s.						
al3	Genus	21***	al4	174***	4.9*						
aw3	Genus	7.3**	wa2	6.9*	4*						
aw4	Genus	9.9**	wa2	13.1***	n.s.						
pw	Genus	n.s.	hw	444.9***	n.s.						
sp	Genus	185.1***	ed	10.4**	n.s.						
sh	Genus	40.9***	ed	n.s.	n.s.						
al2	Genus	8.9**	is	195***	n.s.						
hw	Species	5.9**	la3	75.6***	5.8**	***	**	n.s.	n.s.	***	***
al2	Species	63.7***	is	51.4***	n.s.	***	***	n.s.	**	***	***
al3	Species	18.5***	la2	68.7***	n.s.	***	***	n.s.	***	***	***
al4	Species	10.3***	la2	23.2***	n.s.	***	***	n.s.	**	**	***
sp	Species	20.6***	ed	n.s.	n.s.	n.s.	***	n.s.	***	***	*
sh	Species	6.8**	ed	4.3*	n.s.	n.s.	*	n.s.	*	n.s.	n.s.

In cases where the interaction between categorical factor and covariate did not show a significant effect, the F-values for factor and covariate came from an additional analysis testing their effect independently. Significance levels: ***$P \leq 0.001$; **$P \leq 0.01$; *$P \leq 0.05$. For shorts of characters see the Material and methods.

they are when used together with the respective host association. Still, the results of our molecular analyses suggest that host association is a labile phenomenon that cannot discriminate between genera by itself. External morphology, although distinct in the two genera, is only correlated with host association. These two characters therefore cannot support each other, as suggested by Usinger (1966). Based on these results, species associated with hirundinine birds are clearly a derived group from bat-associated species; their status as a separate genus from *Cimex* cannot be justified at this time. Although the representation of *Cimex* species in our study may seem limited (<50% of the described species), it covers all major species groups, so it is unlikely that more complete sampling would result in a monophyletic *Cimex*. The genetic distances between species representing the *C. pipistrelli* group and *Oeciacus hirundinis* (K2P distance = 6.3%, Table 3) are more than three times lower than distances between the major *Cimex* species groups (K2P distance = 17.8–23.8%). The statistical support for the monophyly of Clade A (i.e. species associated with hirundinine birds and the *C. pipistrelli* group) was strong in all combined data trees (>99% BS and 1.0 PP). In conclusion, we suggest changes in taxonomy as follows: the name *Oeciacus* Stål is a junior synonym to *Cimex* Linnaeus. *Oeciacus hirundinis* = *Cimex hirundinis* Lamarck syn.n.; *Oeciacus vicarius* = *Cimex vicarius* (Horváth) comb.n.; *Oeciacus montandoni* = "*Cimex montandoni*" (Péricart) comb.n. The classification of *O. montandonii* newly in the genus *Cimex* is clear enough based on morphology, however, it is desirable to confirm it using molecular data in future.

The PCA based on morphological characters confirmed that *Oeciacus* species, along with *Cimex* sp. Japan, form a specific and distinct phenotype. Relatively smaller body size and other morphological characters have thus likely developed in response to host associations with birds. We dismiss the idea that the differences in relative diagnostic characters delimiting the two genera *Cimex* and *Oeciacus* are due to allometry and different body size alone. In congruence with another detailed study (Melzer, 2007), all characters used in the literature were shown to be fully valid. Furthermore, values of the characters in our specimens (Tables 2 and S3) largely corresponded to values given in literature. The only exception found both herein and by Melzer (2007) is the ratio of head:pronotum widths. According to ANCOVA the genera did not differ in this character and the values for all *Cimex* species except for *C. lectularius* extended below 1.5 in our analyses (Table S3). Remarkably, however, Melzer (2007) found a distinctive head:pronotum ratio for both genera by measuring the head widths without eyes. Nonetheless, even though the synonymy of *Oeciacus* with *Cimex* is clearly supported here based on our molecular data, we show that all characters – except for the ratio of head:pronotum widths – are powerful enough to delimit species.

The taxonomic identity of *Cimex* sp. Japan was problematic in our analyses. The relationships of this taxa to the remaining *C. pipistrelli* group is commented upon in detail in Balvín *et al.* (2013) based on mtDNA using a population genetic approach with a detailed morphological revision. Based on a representative sample we found two abundant haplogroups in the West Palaearctic population but no evidence that these groups represent separate species. Species limits within the *C. pipistrelli* group were found to be unclear. In the present study, one of the haplogroups is polyphyletic (samples 103 and 73; Table 1) within the other haplogroup (remaining samples). The haplogroups were recovered as separate clades only in trees based on mtDNA (Table 4; Figure S3). On one hand, in mtDNA *Cimex* sp. Japan and *C. japonicus* are not much more distant from each other (K2P distance for *COI* = 3.3%, Table 3) or from West Palaearctic samples (3.6%) than the two West Palaearctic haplogroups from each other (3%). On the other hand, there is almost no variability in nuclear genes in the West Palaearctic population (single variable site in *ITS2*), whereas a slight differentiation can be seen between these and *Cimex*

sp. Japan (K2P distance 1.6%). Indeed, in all trees based on datasets including ITS2 the West Palaearctic *C. pipistrelli* group was monophyletic (Table S1; Figures S1 and S2).

Genetic distances in *COI* sequences of all samples of the *C. pipistrelli* group, including *Cimex* sp. Japan, fall in the range of typical intraspecific variation found in heteropteran taxa (cf. Jung *et al*., 2011; Park *et al.,* 2011). In the bat-associated samples, the low mtDNA differentiation is in accordance with morphological studies (Wendt, 1941; Melzer, 2007; Balvín *et al.*, 2013) that failed to reproduce Usinger's (1966) species concept within the *C. pipistrelli* group. However, according to Usinger (1966), the West Palaearctic species of the *C. pipistrelli* group are intersterile at least with *C. japonicus,* suggesting the existence of several separate species (i.e. biological species concept), underpinning Usinger's classification of the species group. Considering the present state of knowledge and unclear species limits in the *C. pipistrelli* group, formally describing *Cimex* sp. Japan as a new species would be premature.

In contrast to the Palaearctic *C. pipistrelli* group, the Nearctic bat-associated *C. pilosellus* group appeared well supported and deeply differentiated in our molecular analyses (Fig. 1; Table S2). Almost every species of the group has its own unique karyotype (Ueshima, 1963, 1966). It is likely that the karyotype alterations built deeper reproduction barriers and allowed sympatric speciation of multiple species using the same hosts. According to Ueshima (1966), the karyotype of *C. latipennis* Usinger and Ueshima (14A + XY) is likely ancestral for the *C. pilosellus* group. Its single chromosome X is considered to be a precursor for fragmentation into multiple X chromosomes found in other species with 14 autosomes (*C. adjuctus* Barber*, C. brevis* Usinger & Ueshima, and *C. pilosellus*). The autosomes were supposed to have fused in the remaining species [*C. antennatus* Usinger & Ueshima (11A + XY) and *C. incrassatus* Usinger & Ueshima (10A + XY)]. The sister-group position of *C. latipennis* to the rest of the *C. pilosellus* group in our analyses is congruent with Ueshima (1966).

Our analyses suggest paraphyly of the genus *Cimex* with respect to the genus *Paracimex*. Unfortunately we were able to sample only a single species (*P. setosus*) of this genus of more than ten species (Usinger, 1966). Although analyses of single partitions failed to consistently place the species*,* it does fall within *Cimex* in the combined analyses. Usinger (1966) delimited *Paracimex* both from *Oeciacus* and *Cimex* by a different type of ectospermalege and other morphological characters. Furthermore, the chromosome number of *Paracimex* is different from any species of *Cimex* or *Oeciacus* (Ueshima, 1963, 1966). *Paracimex* is a rich complex of species that radiated among many particular host species of swiftlets (Apodiidae) and occurs on many different islands in East Asia. As the representation of species within the latter in our study is very limited, we leave conclusions regarding its taxonomic status to future investigations.

The analyses using all genes (Fig. 1) shows that the group of taxa associated with Hirundinidae are not monophyletic and that the genera *Cimex* and *Oeciacus* likely underwent several switches between birds and bats. We argue that the scenario of paraphyly of species associated with hirundinine birds and multiple host switches is likely. An eventual host switch has two basic constraints, probability of alternative host encounter and level of host specificity (Poulin, 2007). Experimental studies in other ectoparasitic insects often show the ability of long-term survival on nonspecific hosts (Bush, 2009), although usually with reduced fitness (Reed & Hafner, 1997; Tompkins & Clayton, 1999; Bush & Clayton, 2006). Even though both bat- and bird-associated cimicids are often known to use a wide range of species within their host category (i.e.,- birds or bats) (Usinger, 1966), there is definitely selection pressure in either direction. Species using either bats or birds are represented by the morphologically distinct genera *Cimex* and *Oeciacus.* At the population level, such adaptive differentiation has been suggested for *C. lectularius* on bats and people (Balvín *et al.*, 2012b). Moreover, the bat-bugs of the *C. pipistrelli* group were shown to morphologically differentiate among different bat host species (Balvín *et al.*, 2013) suggesting local adaptations and consequently lower fitness on non-native hosts. There are numerous data available that document the possibilities of encountering alternative hosts, or even possibly short-term survival on them. Bat bugs of the *C. pipistrelli* group have been found in nests of birds (Lis, 2001) and *Oeciacus vicarius* found on mist-netted bats (Rotschild, 1912; Ritzi *et al.*, 2001). We observed one adult and one juvenile *C. pipistrelli* in a nest of a pigeon apparently using the hatchlings as an alternative host when the bat colony moved to another corner of the same large attic (O. Balvín, personal observation). Among the bird species usually recorded as hosts for *Oeciacus* species (including *Cimex* sp. Japan), each has its own special requirements for a nest and it uses only one nest during a breeding season. Use of a nest built by different bird species is unlikely. In contrast, many bat species co-occur in the same breeding places as these birds, such as caves (Gaisler, 1966). Moreover, many species of bats use several different shelters during the year – breeding colonies of some species switch shelters during the season and many species use more than one shelter in a single night – and their requirements are not that strict in comparison to bird nests (e.g. Fleming & Eby, 2003; Bartonička & Gaisler, 2007). In addition, it is known that bats often use enclosed nests of birds of the family Hirundinidae (Buchanan, 1958; Jackson *et al.,* 1982; Loye, 1985; Pitts & Scharninghausen, 1986; Schulz, 1995; Ritzi *et al.*, 2001) or even breed there (K. Hirata, in lit.). Therefore, the switch between bird and bat hosts is more likely than between different bird species.

Conclusions

In this study the genus *Cimex* displays differentiated lineages that largely correspond to the traditional species groups according to Usinger (1966). The genus *Cimex* is shown to be paraphyletic with respect to *Oeciacus* and *Paracimex* Oeciacus is proposed to be a synonym to *Cimex*, however, the likely synonymy of *Paracimex* is uncertain at the moment and can be confirmed with more studies using expanded taxon sampling. The morphological differentiation of the taxa associated with bats and birds, which delimited higher groupings traditionally,

is apparently due to different host association rather than phylogenetic distance. The results of the molecular analyses suggest repeated host switches between bats and birds accompanied by morphological convergence among lineages of species on either bats or birds, or both. The phylogeny of the group deserves further investigation; more detailed sampling of Palaearctic populations of this group associated with both bats and birds is needed to examine in detail the biogeography, host-associated differentiation and speciation in these parasitic organisms.

Supporting Information

Additional Supporting Information may be found in the online version of this article under the DOI reference: 10.1111/syen.12127

Figure S1. Simplified trees based on datasets consisting of all five genes.

Figure S2. Simplified trees based on datasets consisting of all three nuclear genes.

Figure S3. Simplified trees based on datasets consisting of both mitochondrial genes.

Table S1. List of primers used.

Table S2. Summarized information on sets of trees based on different datasets and characteristics of particular genes.

Table S3. Values of diagnostic morphological characters for each genus and species.

Table S4. DNA sequence alignments.

Acknowledgements

We express our sincere thanks to those who collected or provided the material used: Petr Benda, Jaroslav Cepák, Václav Gvoždík (National Museum, Prague, Czech Republic), Borek Franěk (Protected Land Area České Středohoří, Czech Republic), Radek Lučan, Magdalena Lučanová, Petr Šípek (Charles University, Czech Republic), Libor Mazánek (Regional Hygiene Station Olomouc, Czech Republic), Martin Pokorný, Zuzana Tollerianová (Czech Republic), Antonín Reiter (Southern Moravia Museum, Znojmo, Czech Republic), Hana Šípková (Agricultural University, Czech Republic), Robert Vlk (Masaryk University, Czech Republic), Petr Wolf (Protected Land Area Beskydy, Czech Republic), Charles R. Brown (University of Tulsa, USA), Christian Dietz (Universität Tübingen, Germany), René Güttinger (Switzerland), Kazuhiko Hirata (Hokkaido University, Japan), Ted Morrow (University of Sussex, Great Britain), Milan Paunović (Natural History Museum, Belgrade, Serbia), Ingo Scheffler (Universität Potsdam, Germany), Tomi Trilar (Slovenian Museum of Natural History, Slovenia) and Marteen Vonhof (Western Michigan University, USA). Special thanks go to Nobuhiko Kataoka (Sasayama, Japan), who collected and later supplemented the samples of *Cimex* sp. Japan, and to Sándor Boldogh (Aggtelek National Park, Hungary) who let us collect in bat roosts in NE Hungary. We are obliged to Klaus Reinhardt (University of Sheffield, Great Britain), Nikolay Simov (National Museum of Natural History, Sofia, Bulgaria), Dávid Redei (Hungarian Natural History Museum, Budapest, Hungary) and Dimitry A. Gapon (Zoological Museum, St. Petersburg, Russia) for the material loans. We are very thankful for help with the lab work, analysing data and comments on the manuscript by Josef Bryja, Jan Zima (Academy of Sciences, Czech Republic), Petr Janšta, Lukáš Kratochvíl, Pavel Munclinger, Zuzana Starostová, Jakub Straka (Charles University, Czech Republic) and Martina Komárková (Institute of Animal Science, Prague, Czech Republic). We thank Bjarte Jordal (University Museum of Bergen, Norway) and Christiane Todt (University Museum of Bergen, Norway) for helpful comments, suggestions and linguistic correction of the text. The study was supported by the grant of Ministry of Education, Youth and Sports of the Czech Republic no. SVV-2013-267 201 and a grant from the Grant Agency of Charles University no. 122/2006 B/Bio.

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Accepted 11 February 2015