Latrocimicinae completes the phylogeny of Cimicidae: meeting old morphologic data rather than modern host phylogeny

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Abstract

The family Cimicidae includes obligate hematophagous ectoparasites (bed bugs and their relatives) with high veterinary/medical importance. The evolutionary relationships of Cimicidae and their hosts have recently been reported in a phylogenetic context, but in the relevant study, one of the six subfamilies, the bat-specific Latrocimicinae, was not represented. In this study the only known species of Latrocimicinae, i.e., Latrocimex spectans, was analyzed with molecular and phylogenetic methods based on four (two nuclear and two mitochondrial) genetic markers. The completed subfamily-level phylogeny of Cimicidae showed that Latrocimicinae is most closely related to Haematosiphoninae (ectoparasites of birds and humans), with which it shares systematically important morphologic characters, but not hosts. Moreover, in the phylogenetic analyses, cimicid bugs that are known to infest phylogenetically distant bat hosts clustered together (e.g., Leptocimex and Stricticimex within Cacodminae), while cimicid subfamilies (Latrocimicinae, Primicimicinae) that are known to infest bat hosts from closely related superfamilies clustered distantly. In conclusion, adding Latrocimicinae significantly contributed to the resolution of the phylogeny of Cimicidae. The close phylogenetic relationship between Latrocimicinae and Haematosiphoninae is consistent with long-known morphologic data. At the same time, phylogenetic relationships of genera within subfamilies are inconsistent with the phylogeny of relevant hosts.

Keywords: Latrocimex spectans, Heteroptera, Bug, Ectoparasite

The family Cimicidae (Insecta: Hemiptera: Heteroptera) includes obligate hematophagous ectoparasites, with more than 110 described species in 24 genera and six subfamilies [1–3]. While the majority of extant cimicid species rely on bat hosts [4], several groups are adapted to other warm-blooded vertebrates, including birds and humans. In this respect, a few cimicids are generalists [3], although they have most likely evolved from host-specialist insects [5].

Even strictly host-specific permanent ectoparasites (such as blood-sucking lice) tend to show incongruent phylogenies in comparison with their hosts, probably as the result of a complex evolutionary history of host-switching events [6]. Cimicidae includes temporary ectoparasites which, in the course of their evolution, colonized bat hosts several times independently [5]. Thus, it can be expected that cimicid bugs and their bat hosts show independent phylogenetic clustering. Recently, when the host relationships of Cimicidae were analyzed in an evolutionary/phylogenetic context, one of the six subfamilies, Latrocimicinae, was not represented [5]. Here we complete the subfamily-level phylogeny of Cimicidae by adding Latrocimex spectans Lent, 1941, the only
known species of Latrocimicinae, with Noctilio sp. bats as its specific hosts [1].

In this study, two male bugs were used, which were collected near the resting places of greater bulldog bats (Noctilio leporinus) in Belize (latitude 18.25677 and longitude −88.267594) in Central America on July 19, 2017. Both specimens were identified as L. spectans according to Usinger [1], and one of them was molecularly analyzed here.

The DNA was extracted from one leg of the bug with the QIAamp DNA Mini Kit (QIAGEN, Hilden, Germany) according to the manufacturer’s instruction, including an overnight digestion in tissue lysis buffer and proteinase K at 56 °C.

Part of the cytochrome c oxidase subunit I (cox1) gene was amplified with a conventional PCR using the primers Lep1F (5′-ATT CAA CCA ATC ATA AAG ATA TTG G-3′), Lep1Fdeg (5′-ATT CAA CCA ATC ATA AAG ATA TNG G-3′), and Lep3R (5′-TAT ACT TCA GGG TGT CCG AAA AAT CA-3′) [7] as reported [8]. For the amplification of part of the 16S rRNA gene, the primers 16S LR-J (5′-TTA CGC TGT TAT CCC TAA-3′) and 16S LR-N (5′-CGC CTG TTT ATC AAA AAC AT-3′) were used [9, 10]. Two conventional PCRs were carried out with the primer pairs 18S-1 (5′-CTG GTT GAT CCT GCC AGT AGT-3′), and 18S-3 (5′-GGT TAG AAC TAG GGC GGT ATC T-3′), and 18S-2 (5′-AGA TAC CGC CCT AGT TCT AAC C-3′) and 18S-4 (5′-GAT CCT TCT GCA GGT TCA CC-3′) [11] to amplify approx. 1200 bp and 800 bp long fragments, respectively, of the 18S rRNA gene. In addition, the primers 1274 (5′-GAC CGG TCT TGA AAC ACG GA-3′) and 1275 (5′-TCG GAA GGA ACC AGC TAC TA-3′) [12] were used to amplify part of the 28S rRNA gene. In summary, amplified parts of the cox1, 16S, 18S, and 28S rRNA genes correspond to those reported in Roth et al. [5], where sequence lengths are also listed.

All above PCRs were performed in a reaction volume of 25 μl, which included 5 μl of extracted DNA and 20 μl of reaction mixture containing 1 unit HotStarTaq Plus DNA polymerase (5 U/μl) (QIAGEN, Hilden, Germany), 200 μM PCR nucleotide mix, 1 μM each primer, and 2.5 μl of 10× Coral Load PCR buffer (15 mM MgCl₂ included). For amplification, an initial denaturation step at 95 °C for 5 min was followed by 40 cycles of denaturation at 94 °C for 40 s, annealing at 48 °C (in case of the cox1 PCR at 53 °C) for 1 min, and extension at 72 °C for 1 min. Final extension was performed at 72 °C for 10 min.

Purification and sequencing of the PCR products were performed by Biomi Ltd. (Gödöllő, Hungary). Representative sequences were submitted to GenBank (cox1: MW269881, 16S rRNA: MW270938, 18S rRNA: MZ378786, 28S rRNA: MW270939). All those species for which sequences of all four of the above genetic markers were retrievable from GenBank according to accession numbers in Roth et al. [5] were included in the phylogenetic analyses (Additional file 1: Fig. S1). The out-group contained all taxa from Roth et al. [5], for which all four genetic markers analyzed here were available in GenBank.

The sequences were concatenated with Geneious Prime 2019.2.3 [13] and aligned with the MAFFT algorithm [14]. A Bayesian consensus tree was created from the previously concatenated, aligned sequences using the MrBayes [15, 16] Geneious plugin, GTR (General Time Reversible) model with gamma distribution and invariant sites (GTR + G + I). The chain length was set to 5,000,000, sampling frequency to 500, and burn-in length to 100,000. The gene partitions were treated as unlinked. The random seed was set to 3967. The final phylogenetic tree was loaded into MEGAX 10.0.5 [17] for analysis. An additional phylogenetic analysis was conducted with the maximum likelihood method and Tamura–Nei model with gamma distribution and invariant sites (bootstrap: 1000) in MEGAX (10.0.5.).

The Bayesian consensus tree (Fig. 1) showed that Latrocimicinae (represented by L. spectans) was most closely related to Haematosiphoninae (ectoparasites of birds and humans), with strong support. These two subfamilies...
formed a sister group to Cacodminae and Cimicinae (Fig. 1). The topology of the maximum likelihood tree was slightly different: while the close relationship between Latrocimicinae and Haematosiphoninae was confirmed, these two belonged to a sister group of Cimicinae (Fig. 2). In comparison with previous findings [5], the above results imply that by adding Latrocimicinae, the sister group position of Cacodminae and Haematosiphoninae changed.

Considering the hitherto enigmatic position of Latrocimicinae, these results confirm the morphologic tree [18] which was based on the relationships proposed by Usinger [1]. In particular, the genus *Latrocimex* belonged to the same cluster with (and next to)
Haematosphinae, forming a sister group to Cimici- 
nae together with Cacodminae [18]. Usinger [1] already 
noted that Latrocimicinae and Haematosphinae 
share systematically important morphologic characters, 
such as the prominent bristles on the hind angles of the 
pronotum, or the stout, spine-like bristles on the tibiae, 
as well as the right-ventral position of the paragenital 
sinus.

By contrast, when comparing Latrocimex with Afro-
cimex, it was mentioned that the number of autosomes 
is the same, but the two groups are completely unlike in 
appearance and therefore probably have no close connection 
[1]. This is also well reflected by the concatenated 
phylogenetic tree (Fig. 1), on which the two subfamilies, 
Afrocinicinae and Latrocimicinae, are only distantly 
related. Thus, the above morphologic characters are in 
line with the results of phylogenetic analyses performed 
here when including Latrocimicinae.

On the other hand, completing the phylogenetic tree 
with Latrocimicinae also highlights inconsistencies 
between host and parasite phylogenies. For instance, 
regarding bug genera with bats as typical hosts, the two 
distantly related cimicid subfamilies, Latrocimicinae and 
Primicimicinae, have hosts from two bat superfamilies 
(Noctilionoidea and Vespertilionoidea, respectively) [1] 
which are closely related (sister groups: [19]). At the same 
time, closely related cimicid genera such as Leptocimex 
and Stricticimex (Fig. 2) have bat hosts [1] from the most 
distantly related bat superfamilies Vespertilionoidea, 
Emballonuroidea, and Rhinolophoidea [19].

Regarding geographical ranges on the subfamily level, 
the ancestral Primicimicinae is Nearctic-Neotropical, 
whereas the core of the phylogenetic tree is occupied by 
Old World groups, as well as by Cimicinae showing global 
distribution (Fig. 1). The sister groups Latrocimicinae and 
Haematosphinae both occur in the New World. This 
geographically consistent pattern might reflect that the 
temporary parasitism by cimicid bugs prevented them from 
frequent natural dispersal events, especially on a 
transcontinental scale [3]. Accordingly, while their hosts 
are highly mobile, cimicid populations tend to be geneti-

cally more isolated [20] (except for the common bed bug, 
C. lectularius, and the tropical bed bug, C. hemipterus, 
which became cosmopolitan in distribution as the result 
of artificial dispersal events, owing to their association 
with humans and man-made objects). 

In summary, the complete phylogeny of Cimicinae 
reflects morphologic relationships, rather than host asso-
ciations. However, the latter might be more relevant if 
approached in a geographical context.

Abbreviation 
cor1: Cytochrome c oxidase subunit 1.
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