Insects found in birds’ nests from Argentina: cytogenetic studies in Cimicidae (Hemiptera) and its taxonomical and phylogenetic implications

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Abstract

The Cimicidae (Hemiptera) are known to be blood ectoparasites primarily on birds and bats. Three species of the subfamily Haematosiphoninae are known from Argentina: Acanthocrios furnarii, Ornithocoris toledoi, and Psitticimex uritui; all feed on diverse avian hosts. The chromosome number and male meiosis of A. furnarii, and P. uritui from new Argentinean samples are analyzed and compared with previous data. The sample of A. furnarii described by Ueshima (1966) with 2n = 32 + XY (male), strikingly differs from the present results (2n = 10 + XY, male). The diploid number of P. uritui agree with the previously reported by Ueshima (1966), 2n = 28 + X,X,Y (male). Taxonomical implications about the identity of A. furnarii are discussed and the mechanisms of the karyotype evolution of species belonging to Haematosiphoninae are proposed.

Key words: Acanthocrios, Psitticimex, Hemiptera, Cimicidae, birds’ nests, achiasmatic meiosis, karyotype evolution

Introduction

The Cimicidae (Hemiptera) are known to be blood ectoparasites primarily on birds and bats, with man as a secondary host (Usinger 1966). Five of the seven genera in the subfamily Haematosiphoninae are monotypic, an unusual feature in the Cimicidae: four genera are distributed in North America [Haematosiphon Champion, 1900; Cimexopsis List, 1925; Synxenoderus List, 1925; Hesperocimex List, 1925], and three genera in South America [Ornithocoris Pinto, 1927; Acanthocrios Del Ponte & Riesel, 1945 (= Caminicimex Usinger, 1966: Di Iorio & Turienzo 2008); Psitticimex Usinger, 1966] (Usinger 1966). Later, Alayocimex Hernandez Triana & De la Cruz, 1994, also monotypic, was described from Cuba. Three species of Haematosiphoninae are known from Argentina: Acanthocrios furnarii (Cordero & Vogelsang, 1928), Ornithocoris toledoi Pinto, 1927, and Psitticimex uritui (Lent & Abalos, 1946). They all feed on diverse avian hosts in their nests (Usinger 1966, Turienzo & Di Iorio 2007, Carpintero & Aramburú 2007, Di Iorio et al. 2008, Di Iorio & Turienzo 2009, Santillán et al. 2009a, 2009b).

Cytenogenetic studies in Cimicidae

Forty-five species in this family show a diploid chromosome number that ranges from 10 to 42 with a peak at 31. Cimicid bugs have simple (XY/XX, male/female) and multiple sex chromosome systems, and some species possess supernumerary Xs and Ys (Ueshima 1979; Manna 1984; Grozeva & Nokkala 2002).
Within the subfamily Haematosiphoninae, only nine species of six genera have been cytogenetically analyzed. According to their morphological and cytogenetic characteristics, they were divided into three groups (Ueshima 1966, Ueshima 1979). The genus *Ornithocoris*, with the least specialized spermalege, has a male diploid number of 10 (8 + XY), and it is the only genus in the first group. The second group, which has a more specialized spermalege, and includes the genera *Haematosiphon, Acanthocrios, Psitticimex*, and *Synxenoderus*, has 2n = 31 (28 + X,X,Y, male) and 2n = 34 (32 + XY, male). The third group, which has a peculiar latero-ventral spermalege and contains only the genus *Hesperocimex*, carries a high chromosome number (2n = 40 – 42), with simple and multiple sex chromosome systems (Table 1).

### TABLE 1. Species of Haematosiphoninae (Hemiptera: Cimicidae) cytogenetically analyzed.

<table>
<thead>
<tr>
<th>Species</th>
<th>2n♂</th>
<th>2n♀</th>
<th>n♂</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Neotropical region</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td><em>Acanthocrios furnarii</em> (Cordero &amp; Vogelsang, 1928)</td>
<td>34</td>
<td>-</td>
<td>16+XY</td>
<td>Ueshima, 1966</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>12</td>
<td>5+XY</td>
<td>Present work</td>
</tr>
<tr>
<td><em>Ornithocoris toledoi</em> Pinto, 1927</td>
<td>-</td>
<td>-</td>
<td>4+XY</td>
<td>Ueshima, 1966</td>
</tr>
<tr>
<td><em>O. pallidus</em> Usinger, 1959</td>
<td>10</td>
<td>-</td>
<td>4+XY</td>
<td>Ueshima, 1966</td>
</tr>
<tr>
<td><em>Psitticimex uritui</em> (Lent &amp; Abalos, 1946)</td>
<td>31</td>
<td>-</td>
<td>14+X,X,Y</td>
<td>Ueshima, 1966</td>
</tr>
<tr>
<td></td>
<td>31</td>
<td>-</td>
<td>14+X,X,Y</td>
<td>Present work</td>
</tr>
<tr>
<td><strong>Nearctic region</strong></td>
<td></td>
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</tr>
<tr>
<td><em>Haematosiphon inodorus</em> (Duges, 1892)</td>
<td>31</td>
<td>32</td>
<td>14+X,X,Y</td>
<td>Ueshima, 1966</td>
</tr>
<tr>
<td><em>Hesperocimex coloradensis</em> List, 1925</td>
<td>42</td>
<td>44</td>
<td>19+X,X,X,Y</td>
<td>Ryckman &amp; Ueshima 1964</td>
</tr>
<tr>
<td><em>H. cochimensis</em> Ryckman &amp; Ueshima 1963</td>
<td>40</td>
<td>40</td>
<td>19+XY</td>
<td>Ryckman &amp; Ueshima 1964</td>
</tr>
<tr>
<td><em>H. sonorensis</em> Ryckman, 1958</td>
<td>42</td>
<td>42</td>
<td>20+XY</td>
<td>Ryckman &amp; Ueshima 1964</td>
</tr>
<tr>
<td><em>Synxenoderus comosus</em> List 1925</td>
<td>31</td>
<td>32</td>
<td>14+X,X,Y</td>
<td>Ueshima, 1966</td>
</tr>
</tbody>
</table>

Specimens of *A. furnarii* analyzed by Ueshima (1966) came “from a laboratory culture first collected near Tucumán, Argentina, in August, 1957 (R.L. Usinger and P. Wygodzinsky)” (Usinger 1966: 469), and were found in nests of *Furnarius rufus* (Gmelin, 1788) [Aves: Furnariidae]. *Psitticimex uritui* was redescribed from a male and a female collected in nests of *Myiopsitta monachus cotorra* (Vieillot, 1817) [Aves: Psittacidae], “near Tucumán, Argentina, in August, 1957 (R.L. Usinger and P. Wygodzinsky)” (Usinger 1966: 472), although a laboratory culture was not mentioned.

In the present contribution, the chromosome number and male meiosis of these two species from new Argentinian population samples are analyzed, and some striking differences with respect to those presented by Ueshima (1966) are encountered.

### Materials and methods

The total number of specimens collected, and dissected specimens for cytogenetic studies between parentheses, came from the following localities and birds’ nests:

**Acanthocrios furnarii**

*Furnarius rufus rufus* (Gmelin, 1788) [Aves: Furnariidae]

leg., 108 exx. (1 male), in a nest later inhabited by *Sicalis flaveola pelzelni*.

**Psitticimex uritui**  
*Myiopsitta uritui* (Jardine & Selby, 1830) [Aves: Psittacidae]  
ARGENTINA: Córdoba: Ea. El Sauce, 6 km W La Falda, 13-VI-2009, Di Iorio leg., 538 exx. (27 males, 2 females); La Pampa: Toay, 23-IV-2009, Turienzo & Di Iorio leg., 8 exx. (2 males, 4 females), in an old nest of *Pseudoseisura lophotes argentina* Parker, 1960 [Aves: Furnariidae], remodelled and inhabited by a breeding pair of *M. m. catita*.

**Myiopsitta monachus monachus** (Boddaert, 1783) [Aves: Psittacidae]  

All specimens of *A. furnarii* from each population sample collected during autumn and winter were included in the meiotic analysis, because male meiotic cells were found in the testes. However, only the specimens of *P. uritui* from a sample collected in autumn (June) were cytogenetically analysed, because other samples that were collected in winter (August) and late winter (September), only had spermatozoa in testes. Gonads were dissected out in a saline solution, fixed for 15 min in freshly prepared Carnoy fixative (ethanol: chloroform: acetic acid, 6:3:1), and kept at 4°C in 70 % ethanol. Slides were done by the squash method and the chromosomes were stained in 2 % iron acetic haematoxylin following conventional procedures. Chromosome preparations were observed in a Leica DMLB microscope. Black-and-white images of chromosomes were recorded with a CCD camera (Leica DFC350 FX, Leica IM50 Version 4.0, Leica Microsystems Imaging Solutions Ltd. Cambridge, UK).

*Acanthocrios furnarii* and *P. uritui* were identified by O.R. Di Iorio following the key and descriptions of Usinger (1966). Voucher specimens were deposited in the collection ODI. This work is part of the research project “Insects found in birds’ nests from Argentina”, and part of the doctoral thesis of P. Turienzo (both under direction of O.R. Di Iorio).

**Results**

**Acanthocrios furnarii**

As is characteristic of Hemiptera, chromosomes are holokinetic, i.e. without a localized centromere. In all *A. furnarii* population samples, the diploid chromosome number of males and females is 2n = 12 (10 + XY/XX).

In oogonial prometaphases, two chromosomes are much smaller than the others, and correspond to the smallest autosomal pair (Fig. 1a); in spermatogonial prometaphases three smaller chromosomes are detected: two corresponding to the smallest autosomal pair and the other one to the Y chromosome (Fig. 1b).

During male meiosis chromosomes condense from leptotene to pachytene, but no diplotene or diakinesis stages are observed, i.e., meiosis is achiasmatic. At metaphase I, the five autosomal bivalents are completely condensed; homologues are connected with each other through the medial region, but the telomeric regions are separated (Fig. 1c). At this stage both the autosomal bivalents and the sex univalents dispose at the equatorial plate with their long axes perpendicular to the spindle pole. At anaphase I, the sex chromosomes X and Y segregate sister chromatids (equational division) whereas autosomal bivalents segregate homologous chromosomes (reductional division). Both the sex chromatids and the homologous autosomes migrate parallel to the equatorial plane. At metaphase II, the sex chromatids associate forming an XY pseudobivalent, which lies at the centre of the ring formed by the autosomes (Fig. 1d). At anaphase II, the autosomes segregate equationally and the X and Y chromatids divide reductionally. At this stage the chromosomes segregate also with their long axes parallel to the equator. Telophase II nuclei have 6 chromosomes (5A + X or 5A + Y).
Psitticimex uritui

This species possesses \(2n = 31 / 32 = 28 + X_1X_2Y / 28 + X_1X_2X_2\) (male/female), with all the chromosomes of similar size (Fig. 2a). The course of meiosis is similar to that described for A. furnarii. During early male prophase I the three sex chromosomes are positively heteropycnotic. Autosomes gradually condense during leptotene, zygotene, and pachytene, but no diplotene or diakinesis is observed, i.e., meiosis is achiasmatic. At metaphase I, homologous chromosomes lie side by side and are connected with each other through their medial region by non-chiasmatic associations. The 14 autosomal bivalents and the three sex univalents are arranged with their long axes perpendicular to the spindle pole (Fig. 2b). At anaphase I, autosomes segregate reductionally whereas the sex chromosomes segregate chromatids (equational division). At metaphase II, autosomes dispose in a ring configuration and the \(X_1, X_2,\) and \(Y\) chromatids form a pseudotriivalent (Fig. 2c). At anaphase II, autosomes segregate sister chromatids; and the \(X_1\) and \(X_2\) chromatids segregate from the \(Y\). Telophase II nuclei have 16 (14 + \(X_1X_2\)) or 15 (14 + \(Y\)) chromosomes.

**FIGURE 1.** Mitotic and meiotic chromosomes of *Acanthocriocis furnarii*: a, oogonial prometaphase; b, spermatogonial prometaphase; c, metaphase I; d, metaphase II. Arrows: sex chromosomes. Arrowheads: smallest autosomal pair. Chromosomes are stained with 2\% iron acetic haematoxylin. Bar = 10 µm.

**FIGURE 2.** Male meiotic chromosomes of *Psitticimex uritui*: a, prometaphase I; b, metaphase I; c, metaphase II. Arrows: sex chromosomes. N: nucleolus. Chromosomes are stained with 2\% iron acetic haematoxylin. Bar = 10 µm.
FIGURE 3. Phylogeny on the origin and evolution of the species of the subfamily Haematosiphoninae (modified from Usinger 1966, and Di Iorio & Turienzo 2009) based on their morphological and cytogenetic characteristics, and habitats (diploid autosomal number, sex chromosome system, chromosomal rearrangements, bird’s hosts). Primary birds’ hosts: Apodidae (A), Hirundinidae (H), Psittacidae (P), and raptor birds (R). Asterisks: taxa with unknown chromosome numbers.

Discussion

Cytogenetic studies in the family Cimicidae are relatively scarce, and most of them refer to the genera *Cimex* and *Paracimex*, representing 44% of the 45 species cytogenetically analyzed (Ueshima 1979, Manna 1984, Nokkala & Grozeva 2000, Grozeva & Nokkala 2002). Furthermore, although the karyotype has been extensively examined in different aspects, there is only little information on the pattern of male meiosis. The present results in *A. furnarii* and *P. uritui* clearly show that male meiosis is achiasmatic. In the infraorder Cimicomorpha, achiasmatic meiosis has been so far described in a few species belonging to the four phylogenetically closed families (Schuh & Štys 1991, Tian et al. 2008, Schuh et al. 2009): Nabidae (Nokkala & Nokkala 1984, Kuznetsova & Maryanska-Nadachowska 2000, Kuznetsova et al. 2004, Kuznetsova et al. 2007, Kuznetsova & Grozeva 2008), Miridae (Nokkala & Nokkala 1986a, Grozeva 2003, Grozeva et al. 2006, Grozeva et al. 2007), Anthocoridae (Nokkala & Nokkala 1986b), and Microphysidae (Nokkala & Grozeva 2000). According also to previous results, achiasmatic meiosis should be extended to all members of Cimicidae (Grozeva & Nokkala 2002). The absence of recombination, at least in the heterogametic sex, brings about a reduction in the generation of variability, and then the maintenance of linkage groups with coadapted allelic variants (Ituarte & Papeschi 2004). Nevertheless, the presence of achiasmatic meiosis could not be related to an adaptive strategy in the species here analyzed.

The cytogenetic observations in *P. uritui* agree with the diploid number previously reported by Ueshima (1966), 2n = 28 + X1X2Y (male). However, the sample of *A. furnarii* described by Ueshima (1966) (2n = 32 + XY) strikingly differs from the present results (2n = 10 + XY). This marked difference in the diploid number
could not be explained as a polytypism because many chromosome rearrangements in a very short evolutionary time should have to be proposed, and all population samples examined here have shown not only a similar morphology but also the same karyotype. Taking into account that all specimens here analyzed were determined as belonging to A. furnarii by the characters given in the generic description of Caminicimex (synonymized to Acanthocrios), and following the keys of Usinger (1966), it can be suggested that two different taxonomic entities are involved. The morphological comparison of the material analyzed by Ueshima and ours would shed light on this subject.

In most discussions of the karyotype evolution of bugs, it is generally accepted that the modal chromosome number of a particular taxonomic group, i.e., the commonest number present in a family, a subfamily, a tribe, or a genus, is considered the ancestral one for the group under study (Ueshima 1979). When analyzing karyotype evolution at the family level, some karyotypes are highly homogeneous, but others show intensive processes of karyotype alterations. Cimicidae shows a very heterogeneous chromosome constitution, with a diploid chromosome number between 10 and 42, and simple and multiple sex chromosome systems. However, the modal diploid chromosome number of 28 + XY / XX has been suggested as the ancestral one (Ueshima 1979, Manna 1984).

Among the principal mechanisms of karyotype evolution in Hemiptera, autosomal fusions and both autosomal and sex chromosome fragmentations can be included (Ueshima 1979, Manna 1984, Thomas 1987, Papeschi 1994, 1996, Papeschi & Bressa 2006). If the chromosome size and number of P. uritui (2n = 31 = 28 + X,X,Y) and A. furnarii (2n = 10 + XY) are compared, it can be seen that the high chromosome number of P. uritui is associated with a small chromosome size, and that the low chromosome number of A. furnarii is correlated with chromosomes of large size. For this reason, it is probable that the karyotype of A. furnarii originated through a series of multiple autosomal fusions from the ancestral karyotype, whereas the karyotype of P. uritui arose by a fragmentation of the original X chromosome, causing a derived multiple sex chromosome system (Fig. 3).

Considering the most common mechanisms of karyotype evolution in Hemiptera, the ancestral diploid number proposed for the family Cimicidae (28 + XY / XX) (Ueshima 1979, Manna 1984), and the results here obtained, the following scenario for karyotype evolution in the nine species of Haematosiphoninae could be suggested (Fig. 3):

- autosomal fusions brought about a reduction in the modal autosomal number (Neotropical Region: Ornithocoris toledoi, O. pallidus, and Acanthocrios furnarii);
- fragmentation of the ancestral X chromosome originated a derived multiple sex chromosome system X1X2Y (Neotropical Region: Psitticimex uritui, and Nearctic Region: Synxenoderus comosus and Haematosiphon inodorus);
- autosomal fragmentation resulted in an increase in the number of autosomes (Nearctic Region: Hesperocimex cochimienensis and H. sonorensis), and also the fragmentation of the X chromosome originated a derived multiple sex chromosome system (Nearctic Region: H. coloradensis).

According to the three groups of species within the subfamily Haematosiphoninae proposed by Ueshima (1966), based on morphological and cytological characteristics, A. furnarii and P. uritui belong to the same group. However, considering our results, it is suggested that A. furnarii should belong to the first group which includes the species of the genus Ornithocoris, because both genera possess a low autosomal number and the ancestral simple system of sex chromosomes (Fig. 3).

Finally, if the hypothesis of two different taxonomic entities is confirmed, the synonymy of Cimex passerinus Cordero & Vogelsang, 1928 with A. furnarii made by Usinger (1966) needs to be reevaluated (although the single type specimen of C. passerinus is lost). Furthermore, the low chromosome number found now in the specimens identified as A. furnarii approaches this entity to the genus Ornithocoris, where A. furnarii was previously located (Carvalho 1939).
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