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Cytogenetic comparison between *Terrobittacus implicatus* and *Bittacus planus* (Mecoptera: Bittacidae) with some phylogenetic implications

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Abstract

Cytogenetic data contribute greatly to taxonomic and phylogenetic analyses in many insect groups. However, such data have been largely neglected in Mecoptera to date. In the present investigation, we compared the meiotic progresses and karyotypes of the hangingflies *Terrobittacus implicatus* (Huang & Hua, 2006) and *Bittacus planus* Cheng, 1949 (Bittacidae) using C-banding technique and DAPI (4'-6-diamino-2-phenylindole) and Giemsa staining. The karyotypical analyses show that *T. implicatus* possesses the highest chromosome number (2n = 41) ever observed in Bittacidae and an asymmetric karyotype with metacentric, submetacentric, subtelocentric and telocentric chromosomes. *B. planus* has a high diploid number (2n = 35), but a nearly symmetric karyotype with mainly metacentric and submetacentric chromosomes and a subtelocentric pair. Chiasmate meiosis and X0 sex determination mechanism are likely plesiomorphic in Bittacidae. The pronounced variations in cytogenetic traits within and between the genera *Terrobittacus* Tan & Hua, 2009 and *Bittacus* Latreille, 1805 suggest that they are substantial traits both for species delimitation and systematic analysis. The potential utilization of cytogenetic data for understanding the phylogeny of Bittacidae is briefly discussed.

Key words

Karyotype, chromosome number, meiosis, *Terrobittacus*, *Bittacus*, taxonomy, evolution.

1. Introduction

The chromosomes of eukaryotic organisms can provide uniquely important information for taxonomic and phylogenetic analyses due to their evolutionary conservation (Dyer 1979; Dobigny et al. 2004; Gokhman & Kuznetsova 2006). Chromosomal analyses may help reveal the evolutionary relationships of species or higher taxa (White 1974; Faria & Navarro 2010), and play an important role in differentiating sibling species (Bickmore 2001; Gokhman & Kuznetsova 2006). Cytogenetic data have been well documented in many holometabolous insect groups, including Coleoptera (Cabral-de-Mello et al. 2008), Hymenoptera (Lorite & Palomeque 2010), Lepidoptera (Lukhtanov et al. 2011), and Diptera (Vicoso & Bachtrog 2013). In Mecoptera, however, the cytogenetics was studied mainly before the 1970s, and many families including Bittacidae had been neglected for decades (Naville & Beaumont 1934; Cooper 1951, 1974; Ullerich 1961; Bush 1967; Atchley & Jackson 1970; Xu et al. 2013).

The family Bittacidae is commonly known as hangingflies with a cosmopolitan distribution, and consists of 18 extant genera (Chen et al. 2013). *Bittacus* Latreille,

1805 is the most speciose genus, and was regarded as a repository for many "non-outstanding" species in Bittacidae (Lambkin 1988). *Terrobittacus* Tan & Hua, 2009 comprises four species endemic to China (Tan & Hua 2009). The phylogenetic position of Bittacidae in Mecoptera is still a controversial problem. Bittacidae was treated as a basal taxon based on morphological characters (Willmann 1987), but was regarded as a sister group to Panorpodidae based on molecular data (Whiting 2002).

The cytogenetic data of Bittacidae were considerably scarce, with only three species of *Bittacus* having been reported so far. Matthey (1950) was the first to discover that the males of *B. italicus* (Müller, 1766) had low chromosome number (2n = 25), chiasmate meiosis, and an X0 sex determination mechanism. Atchley & Jackson (1970) provided the cytological observations of *B. pilicornis* Westwood, 1846 (2n = 29) and *B. stigmaterus* Say, 1823 (2n = 31), and proposed that the differences in chromosome number were crucial characters for differentiating Bittacidae from Panorpidae.

In this paper, we present information on karyotypic details and male meiosis of the hangingflies *Terrobittacus implicatus* (Huang & Hua, 2006) and *Bittacus planus* Cheng, 1949 for the first time, in an attempt to contribute the cytogenetic data of Bittacidae for future systematic analysis.

2. Materials and methods

2.1. Biological materials

Adults of *Terrobittacus implicatus* and *Bittacus planus* were collected from the Huoditang Forest Farm (33°26′N 108°26′E, elev. 1570–1600 m) in the Qinling Mountains, Shaanxi Province, China from late July to August in 2015.

2.2. Insect Rearing

Live adults were reared in screen-wired cages (40 cm × 60 cm × 60 cm) furnished with fresh plant twigs for resting and moist absorbent cotton for drinking and keeping humidity (Gao & Hua 2013; Jiang et al. 2015). Eggs, larvae, and pupae were incubated or reared in plastic containers with humid humus. Live *Musca domestica* adults were provided as food items for the adults, and freshly-killed *Tenebrio molitor* larvae were given to the larvae. The eggs, larvae and pupae were reared in the laboratory from October 2015 to May 2016. The temperature was kept at 16 ± 2°C for the larvae, 21 ± 2°C for the pupae and 23 ± 2°C for the adults. The relative humidity was 75 ± 10%.

2.3. Cytogenetic analysis

Chromosome spreads were prepared from the male testes of the fourth-instar larvae, pupae and newly-emerged adults following Imai et al. (1988). Testes were dissected and submerged in fresh hypotonic KCl solution (0.045 M) for 20 min at room temperature. After a short fixation of 30–40 s in acetic-ethanol (1 : 3, v/v), the testes were transferred to a drop of 45% acetic acid on slides and torn into small pieces. The slides were air-dried for 24 h and then treated with Sørensen's phosphate buffer (pH 7.0) at 60°C for 30 min before staining (Pijnacker & Ferwerda 1984).

C-banding was performed using the technique of King (1980). Air-dried slides were placed in HCl solution (0.2 M) for 30 min at room temperature, rinsed in distilled water and dried. The slides were then placed in saturated $Ba(OH)_2$ solution at 60°C for 3 min, dipped briefly in HCl and rinsed in distilled water. Afterwards, the slides were placed in Sørensen's phosphate buffer (pH 7.0) at 65°C for 30 min, rinsed in distilled water and stained in 5% Giemsa for 15 min. Slides were then rinsed in distilled water and dried. Some air-dried slides were subjected to fluorescent staining with DAPI (4'-6-diamino-2-phenylindole) for 3−5 min at room temperature (Rebagliati et al. 2003). Photographs were taken with a Nikon DS-Fil digital camera mounted on a Nikon Eclipse 80i microscope (Nikon, Tokyo, Japan). The fluorescent signals were observed with UA filter (330–385 nm) for the fluorochrome DAPI.

2.4. Statistical analyses

At least five spermatogonial cells with well-spread chromosomes at metaphase were used to measure the statistics of chromosomes for each species. The captured images were quantified using the NIS-Element D 3.22 software (Nikon, Tokyo, Japan). For each chromosome, centrometric index (i = the length of short arm/the length of chromosome), arm ratio (r = the length of long arm × 100 / the length of short arm), and means and standard deviations (SD) of absolute length (AL = actual length of chromosomes) and relative length (RL = absolute length of chromosome × 100 / total length of the haploid complement) were calculated using a Microsoft Excel spreadsheet (Table 1). The centromeric nomenclature system adopted Levan et al. (1964). Stebbins' type and asymmetry index methods were used to assess the degree of karyotype asymmetry (Table 2). Stebbins' type belonged to a qualitative category, and was established by recognizing three degrees of difference (A–C) between the largest and smallest chromosome of the complement, and four degrees of proportion (1–4) of chromosomes that are metacentric with an arm ratio of less than 2 : 1 (Stebbins 1971). Asymmetry index (AI) was a quantitative parameter, refering to the coefficient of variation of centromeric index (CV_{CI}) × coefficient of variation of chromosome length (CV_{CL})/100 (Paszko 2006).

3. Results

3.1. Karyology

Terrobittacus implicatus (Fig. 1A) and *Bittacus planus* (Fig. 2A) exhibit different chromosome numbers and karyotype morphologies (Figs. 1B,C, 2B,C; Tables 1, 2).

T. implicatus possesses 2n = 41, with a karyotypic formula of 5m + 6sm + 2st + 28T, and a fundamental number of total chromosome arms (FN) of 54 (Fig. 1C, Table 1). *T. implicatus* has an asymmetric karyotype with AI of 32.93 (Table 2). The proportion of chromosomes with arm ratio (r) less than 2 : 1 is 0.14 (Table 1), belonging to the third degree (1.50 – 0.01). The ratio between the largest and smallest chromosome of the complement is 2.09, corresponding to type B (2 : 1 – 4 : 1). Therefore, *T. implicatus* accords with Stebbins' type 3B.

B. planus exhibits 2n = 35, with a karyotypic formula of 27m + 6sm + 2st and a FN of 70 (Fig. 2C, Table 1). *B. planus* has a nearly symmetric karyotype with AI of 2.12 and Stebbins' type 2A (Table 2). The proportion of chromosomes with r less than 2 : 1 is 0.89 (Table 1), belonging to the second degree (0.99 – 0.51). The ratio between the largest and smallest chromosome of the complement is 1.72, corresponding to type A (< 2 : 1).

Both *T. implicatus* and *B. planus* show several AT-rich regions (positive signals) on some chromosomes, although the signals are weak (Figs. 1C, 2C).

3.2. Chiasmate male meiosis

In *B. planus*, the homologues are closely appressed to form bivalents, which are paired parallelly at the pachytene stage. After C-banding treatment, the majority of bivalents are reacted to show a heterochromatic region at one terminal. The heterochromatic terminals cluster together and form one or several large chromomeres (Fig. 3A). The pachytene is followed by a diffuse diplotene, which can be interpreted as uncondensed bivalents connected by chiasmata (Fig. 3B). During this stage, one terminal region of the bivalents is heavily stained and arranged dispersedly, while the rest of the bivalents are weakly stained and often overlooked as a consequence. Chiasmata can be clearly seen after some condensation of the chromosomes probably at diplotene (Fig. 3C,D) and at diakinesis. Bivalents of large metacentric (submetacentric) chromosomes may exhibit two interstitial chiasmata, whereas those of subtelocentric and telocentric chromosomes contain only one chiasma at the euchromatic side. Our observations of *T. implicatus* at these stages are similar to those of *B. planus*. Therefore, we shall not describe this aspect in detail for *T. implicatus*.

In *B. planus* chiasmata range from 17 to 21 in 50 nuclei counted, with a mean chiasma frequency of 19.5 per nucleus and a mean chiasma frequency of 1.1 per autosomal bivalent, and 77% of nuclei have ring-shaped bivalents in which each chromosomal arm possesses a single chiasma. In *T. implicatus* chiasmata range from 11 to 20 in 50 nuclei counted, with a mean chiasma frequency of 15.3 per nucleus and a mean chiasma frequency of 0.8 per autosomal bivalent. None of the nuclei observed exhibits ring-shaped bivalents.

Bivalents assemble at the equatorial plate and become oriented with their centromeres poleward at metaphase I (Fig. 4A). The two centromeres are oriented in the long axis of the spindle equidistant from the equator, and the chiasmata are located in the equatorial plate. Three kinds of bivalents are frequently observed at the metaphase and anaphase I: M-shaped or half-ring (metacentric bivalent with one terminal chiasma, or with two chiasmata but one releasing first; arrowheads in Fig. 4A,B), cross-shaped (autosomal bivalent with one interstitial chiasma; open arrows in Fig. 5A,B), and rod-shaped (autosomal bivalent with one terminal chiasma; arrowheads in Fig. 5A,B).

Anaphase disjunction is observed in both species (Figs. 4B,C, 5A,B). The spindle fibers are attached to the centromere, giving rise to all chromosomes of a typical V-shape (Figs. 4B, 5A,B). Metacentric chromosomes usually exhibit a four-armed structure as observed in *B. planus* (Fig. 4C), whereas acrocentric and telocentric chromosomes commonly exhibit two-armed structure as observed in *T. implicatus*, where each arm apparently consists of only a single chromatid (Fig. 5A). During this disjunction, several terminal chiasmata resulted from terminalization are observed. These chiasmata occupy an interstitial position at early stages, but their position moves to the tip of the chromosome arm where it occurs as the cell division progresses (Figs. 4B, 5A,B). Chromosomes reach their respective poles with the anaphase progressing, and polar groups of chromosomes become compact during the telophase (Fig. 5C). The X univalent moves undividedly to one pole (arrow, Fig. 5C). This unequal division of the sex chromosome indicates the meiosis of these two species is initial-reductional (pre-reductional).

The second division of meiosis resembles the corresponding stages of mitotic divisions. Prophase II differs in appearance from the first prophase in that the sister chromatids of each dyad chromosome show a very striking repulsion so that the chromatid arms are widely separated from each other (Fig. 5D). The centromeres of the dyad chromosomes are situated on the equatorial plate as in an ordinary somatic division during metaphase II (Fig. 4D). At this stage the chromosomes are at their highest level of coiling and therefore appear shorter and thicker than at any other stage.

4. Discussion

As far as we know, the present study may represent the first attempt to investigate the karyotype and meiosis of *T. implicatus* and *B. planus*. *T. implicatus* exhibits the highest number of chromosomes known in Bittacidae,

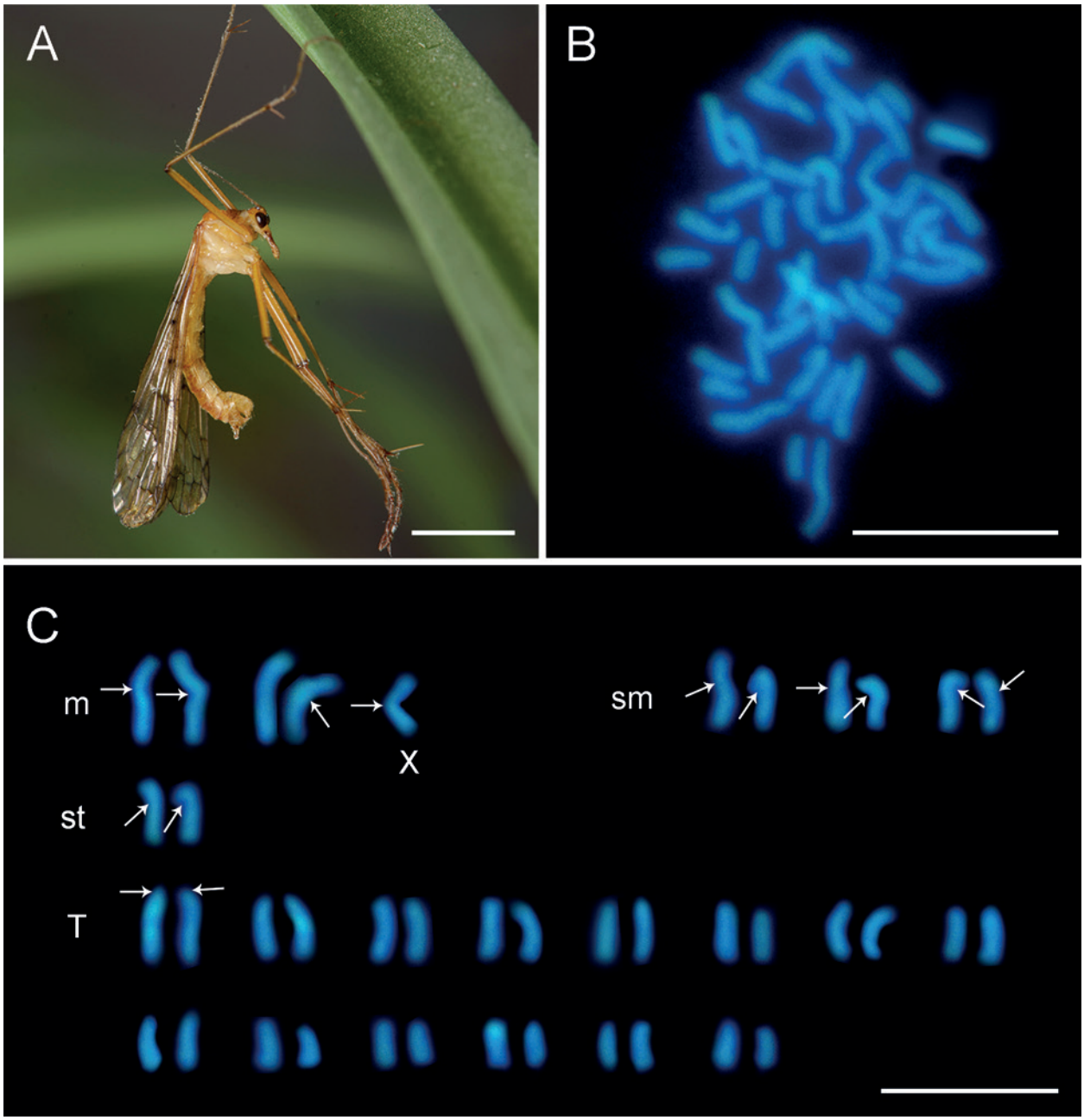

Fig. 1. DAPI staining of spermatogonial cells of *Terrobittacus implicatus*. **A**: A male adult (photo by Ji-Shen Wang). **B**: Spermatogonial metaphase. **C**: Karyogram 2n = 41. — ***Abbreviations & arrows***: m = metacentric, sm = submetacentric, st = subtelocentric, T = telocentric, and X = sex chromosome. Arrows show the primary constriction on the chromosome. (Scale bars: A: 5 mm; B,C: 10 µm)

2n = 41, an asymmetric karyotype, low frequencies of chiasmata, and peculiar shapes of metaphase bivalents, whereas *B. planus* has the highest number of chromosomes in the genus *Bittacus*, 2n = 35, a nearly symmetric karyotype, relatively high frequencies of chiasmata, and common shape of metaphase bivalents. The differences of karyotypes and meiosis between *Bittacus* and *Terrobittacus* may provide cytogenetic supports for the separation of these two genera. The two species studied herein have an X0 sex determination mechanism, as in almost all the species studied previously in Mecoptera (Naville & Beaumont 1934; Matthey 1950; Ullerich 1961; Bush 1967; Atchley & Jackson 1970; Cooper 1974; Xu et al. 2013), except *Boreus brumalis* Fitch, 1847, which has an X_1X_2Y (Cooper 1951). The diversities of the cytogenetic characters at specific and higher levels suggest that cytogenetic data can bring useful information for taxonomic analyses in Bittacidae.

Our observations at pachytene show a high frequency of end-to-end association in the bittacids studied, and are different from the speculation of Atchley & Jackson (1970) that the frequency of tandem associations in Bittacidae was lower than that in Panorpidae. In *Bittacus* and *Terrobittacus*, almost all bivalents are associated by the heavily stained terminal, forming one or several large chromomeres. In *Panorpa* Linnaeus, 1758, however, there may be as many as five or six bivalents associated in a chain, and in some instances the bivalents became

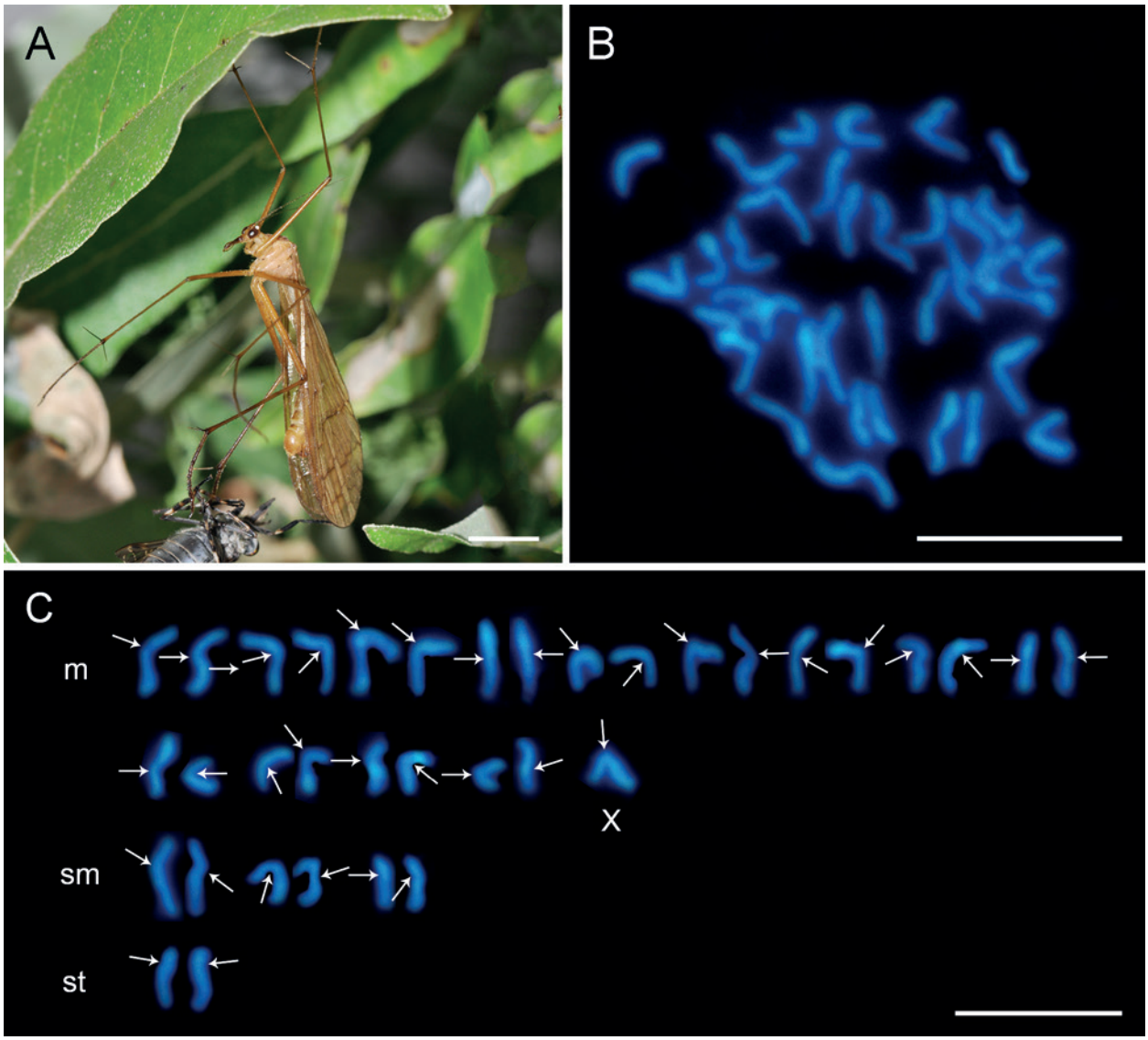

Fig. 2. DAPI staining of spermatogonial cells of *Bittacus planus*. **A**: A male adult (photo by Lu Jiang). **B**: Spermatogonial metaphase. **C**: Karyogram 2n = 35. — ***Abbreviations & arrows***: m = metacentric, sm = submetacentric, st = subtelocentric, and X = sex chromosome. Arrows show the primary constriction on the chromosome. (Scale bars: A: 5 mm; B,C: 10 µm)

associated at right angles with the main chain (Ullerich 1961; Atchley & Jackson 1970).

Chromosome numbers are the most commonly used cytotaxonomic character in organisms (Gokhman & Kuznetsova 2006; White 1956; Jackson 1971; Guerra 2008). Based on the present study, *T. implicatus* displays 2n = 41 and *B. planus* possesses 2n = 35. Previous records showed that 2n = 25 in the European *B. italicus* (Müller, 1766) (Matthey 1950), and 2n = 29 and 31 in the North American *B. pilicornis* Westwood, 1846 and *B. stigmaterus* Say, 1823, respectively (Atchley & Jackson 1970). Therefore, each species examined shows a distinctive chromosome number, suggesting its potential utilization in species delimitation. The considerable variation of chromosome number in Bittacidae is in contrast to the previous point of view that the species of Bittacidae possessed low chromosome numbers as in Boreidae, Choristidae and Meropeidae and differed greatly from Panorpidae, the species of which show relatively high chromosome numbers (Atchley & Jackson 1970; Cooper 1974; Xu et al. 2013).

Extensive variation in chromosome numbers results from polyploidy in many insect groups, such as Coleoptera, Diptera, Hemiptera and Hymenoptera (Lokki & Saura 1980). In Bittacidae, however, the highly variable numbers of chromosomes from 2n = 25 to 41 unlikely resulted from polyploidy. Firstly, the chromosome sizes of *B. planus* with 2n = 35 are prominently larger than those of *T. implicatus* with 2n = 41, suggesting a negative correlation between the chromosome sizes and numbers. Similar findings were reported in Hemiptera (Blackman 1980; Cook 2000) and Lepidoptera (Suomalainen & Brown 1984). In these studies, authors suggested that chromosome number variation resulted from chromosome fissions and/or fusions rather than polyploidy when chromosome size covaries inversely with number. Secondly, the progressive changes of chromosome number also support our argument that polyploidy seems unlikely

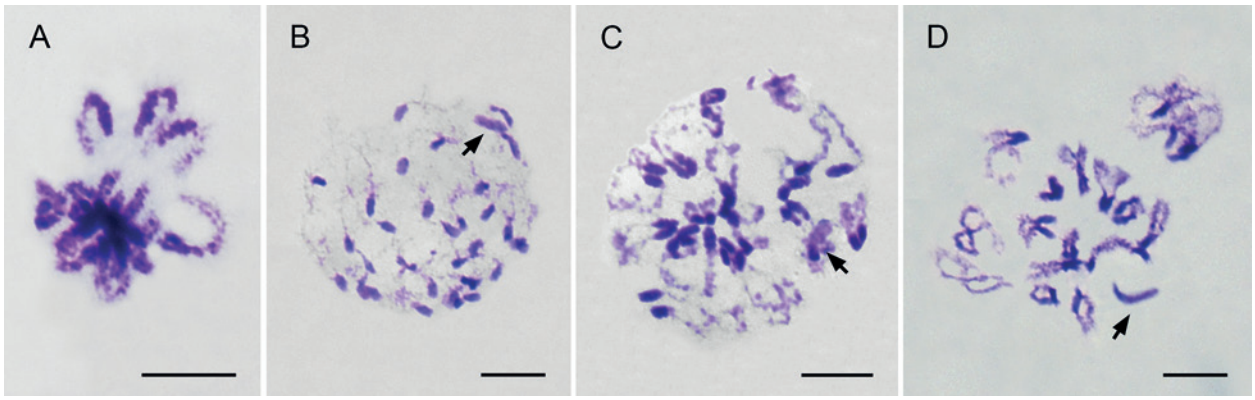

Fig. 3. Meiotic prophase of spermatogenesis stained with C-banding in *Bittacus planus*. **A**: Pachytene. **B**: Diffuse diplotene. **C**: Mid-diplotene. **D**: Early diakinesis. — ***Arrows***: Arrows show the sex chromosome. (Scale bars: 10 µm)

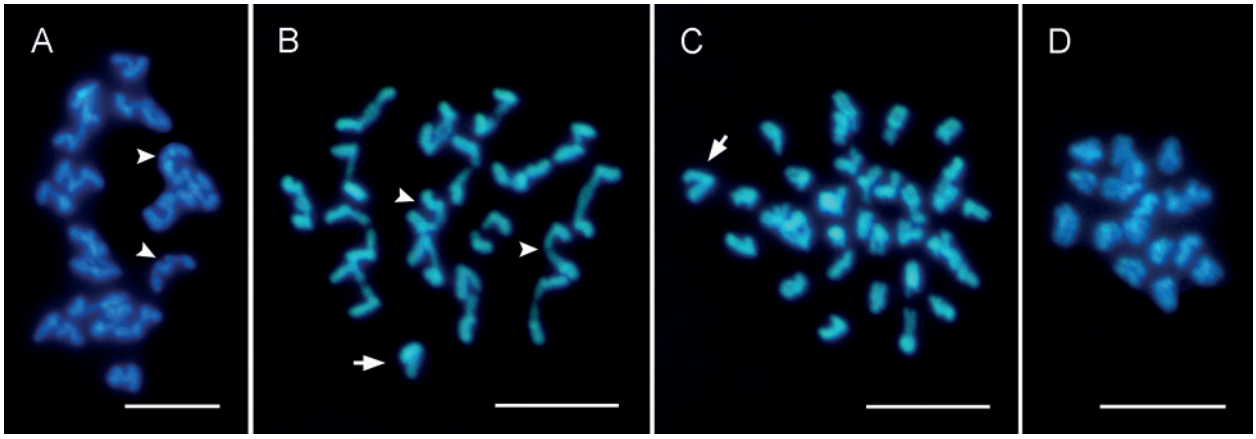

Fig. 4. Chiasmate meiosis of *Bittacus planus* stained with DAPI. **A**: Metaphase I. **B**: Early anaphase I (side view). **C**: Anaphase I (polar view). **D**: Metaphase II. — ***Arrows***: Arrows show the sex chromosome; arrowheads show the M-shaped or half-ring bivalents. (Scale bars: 10 µm)

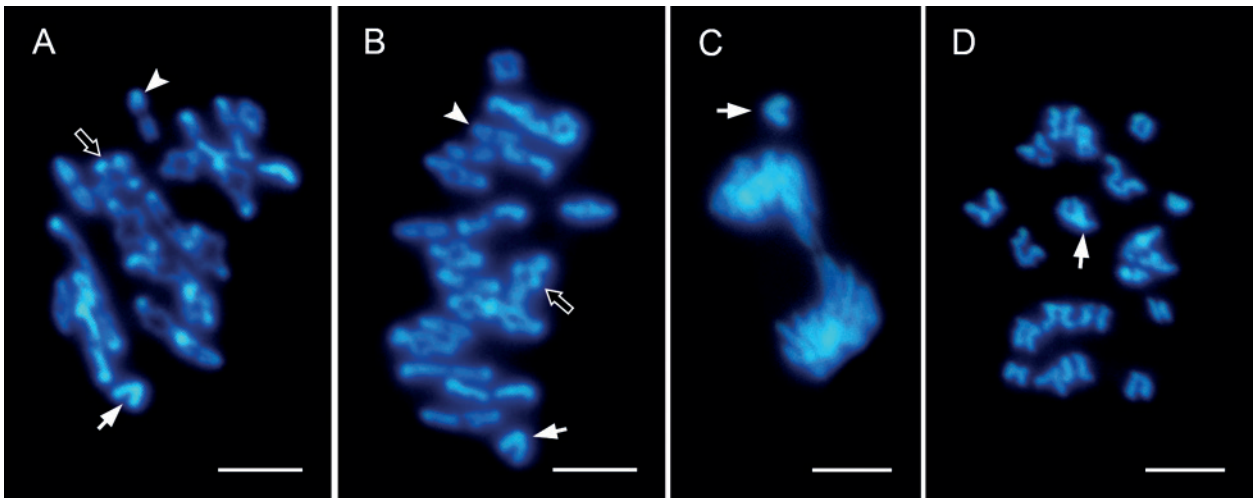

Fig. 5. Chiasmate meiosis of *Terrobittacus implicatus* stained with DAPI. **A**: Early anaphase I. **B**: Mid-anaphase I. **C**: Telophase I. **D**: Prophase II. — ***Arrows***: Arrows show the sex chromosome; arrowheads show the rod-shaped bivalents; open arrows show the cross-shaped bivalents. (Scale bars: 5 µm)

to contribute to *Bittacus* evolution. It is difficult to present an exact basic chromosome number for this genus. Alternatively, the gradual variation in chromosome numbers may be explained with a step-by-step mechanism of karyotype evolution (Lukhtanov et al. 2011). This hypothesis suggested that extreme differences in chromosome number evolved gradually through multiple events, each being involved in the fixation of a single (or a few) chromosomal rearrangement(s), and may be followed by extinction of intermediate karyotypes.

Table 1. Morphometric data on chromosomes of *Terrobittacus implicatus* and *Bittacus planus*. — ***Abbreviations***: AL (absolute chromosome length) = actual length of chromosomes (μm); i (centromeric index) = the length of short arm × 100 / the length of chromosome; r (arm ratio) = the length of long arm × 100 / the length of short arm; RL (relative chromosome length) = absolute length of chromosome × 100 / total length of the haploid complement; SD = standard deviation; m = metacentric, sm = submetacentric, st = subtelocentric, T = telocentric.

Pair number	*Terrobittacus implicatus*					*Bittacus planus*				
	AL ± SD (μm)	**RL ± SD**	**i**	**Type**	**r**	**AL ± SD** (μm)	**RL ± SD**	**i**	**Type**	**r**
1	5.24 ± 0.29	7.06 ± 0.39	41.61	m	1.40	4.82 ± 0.07	6.37 ± 0.09	42.35	m	1.36
2	5.21 ± 0.28	7.02 ± 0.37	38.97	m	1.57	4.90 ± 0.03	6.47 ± 0.03	34.82	m	1.87
3	3.96 ± 0.06	5.34 ± 0.09	43.95	m	1.28	5.42 ± 0.56	7.09 ± 0.67	41.95	m	1.38
4	4.20 ± 0.18	5.66 ± 0.24	27.39	sm	2.65	4.79 ± 0.22	6.30 ± 0.26	43.23	m	1.31
5	3.75 ± 0.30	5.06 ± 0.41	29.01	sm	2.45	3.92 ± 0.09	5.16 ± 0.11	44.65	m	1.23
6	3.69 ± 0.13	4.98 ± 0.17	26.41	sm	2.79	4.56 ± 0.11	6.00 ± 0.12	41.40	m	1.42
7	3.69 ± 0.07	4.97 ± 0.09	19.47	st	4.14	4.15 ± 0.07	5.47 ± 0.09	47.35	m	1.11
8	4.19 ± 0.06	5.65 ± 0.08	0	T	∞	4.24 ± 0.31	5.57 ± 0.38	41.82	m	1.39
9	3.66 ± 0.13	4.93 ± 0.17	0	T	∞	3.64 ± 0.13	4.77 ± 0.08	44.59	m	1.24
10	3.67 ± 0.07	4.95 ± 0.09	0	T	∞	3.83 ± 0.24	5.08 ± 0.28	41.71	m	1.40
11	3.49 ± 0.03	4.71 ± 0.04	0	T	∞	3.51 ± 0.05	4.64 ± 0.06	38.70	m	1.58
12	3.48 ± 0.08	4.69 ± 0.10	0	T	∞	3.51 ± 0.28	4.57 ± 0.29	38.41	m	1.60
13	3.15 ± 0.27	4.25 ± 0.36	0	T	∞	3.16 ± 0.52	4.01 ± 0.65	40.03	m	1.49
14	3.47 ± 0.04	4.68 ± 0.06	0	T	∞	4.11 ± 0.31	5.48 ± 0.16	40.98	m	1.44
15	3.16 ± 0.22	4.25 ± 0.30	0	T	∞	4.86 ± 0.25	6.43 ± 0.30	34.82	sm	1.87
16	3.21 ± 0.09	4.33 ± 0.12	0	T	∞	4.02 ± 0.80	5.47 ± 0.34	34.14	sm	1.92
17	2.63 ± 0.26	3.54 ± 0.12	0	T	∞	3.34 ± 0.09	4.38 ± 0.09	31.56	sm	2.16
18	2.61 ± 0.19	3.52 ± 0.25	0	T	∞	3.74 ± 0.21	4.88 ± 0.23	22.39	st	3.10
19	2.60 ± 0.11	3.50 ± 0.14	0	T	∞					
20	2.63 ± 0.13	3.55 ± 0.17	0	T	∞					
21	2.51 ± 0.22	3.38 ± 0.30	0	T	∞					

Table 2. Karyotype statistics of *Terrobittacus implicatus* and *Bittacus planus*. — ***Abbreviations***: AI (asymmetry index) = $CV_{CI} \times CV_{CL}$ / 100; CV_{CI} (coefficient of variation of centromeric index) = $S_{CI} \times 100 / X_{CI}$; CV_{CL} (coefficient of variation of chromosome length) = $S_{CL} \times 100 / X_{CL}$; S_{CI} = the standard deviation of mean chromosome index; S_{CL} = the standard deviation of mean chromosome length; X_{CI} = mean centromeric index; X_{CL} = mean chromosome length (μm).

Species	$X_{CL} \pm S_{CL}$ (μm)	$X_{CI} \pm S_{CI}$	CV_{CI}	CV_{CL}	AI	Stebbins' types
T. implicatus	3.53 ± 0.77	0.11 ± 0.16	152.14	21.65	32.93	3B
B. planus	4.14 ± 0.64	0.40 ± 0.05	13.75	15.45	2.12	2A

T. implicatus possesses an asymmetric karyotype and low frequency of chiasmata, whereas *B. planus* has a nearly symmetric karyotype and normal chiasmate frequency as in previous records (Matthey 1950; Atchley & Jackson 1970). Ullerich (1961) speculated that the reduction or even disappearance of chiasmata was a derived trait in Mecoptera, based on the fact that males of the derived family Panorpidae lacked diplotene and diakinesis and thus did not have chiasmata during meiotic prophase, whereas the females had usual chiasmate meiosis as observed in other mecopteran species. With summarizing the cytogenetic data in Insecta, White (1956) proposed that a moderate number of monocentric chromosomes with relatively large size are primitive characters in Pterygota. Therefore, the species of *Bittacus* investigated may have a primitive position corresponding to the cytogenetic characters with a number of large metacentric chromosomes and relatively high frequency of chiasmata, while *T. implicatus* is probably derived with reduced frequencies of chiasmata and a small number of large chromosomes. *T. implicatus* may have experienced an early radiation with a high number of chromosomal rearrangements, which led to its modern karyotype structure.

According to the non-outstanding morphological features and complicated evolutionary history, the genus *Bittacus* was hypothesized to be a paraphyletic grade that may need to be further divided (Penny 1975; Penny & Byers 1979; Lambkin 1988). Later, molecular data also confirmed that *Bittacus* was paraphyletic (Whiting 2002). Although only four species of *Bittacus* have been examined, the pronounced variation in chromosome numbers ranges from 2n = 25 in *B. italicus* to 2n = 35 in *B. planus*, providing additional cytological information for the paraphyly of *Bittacus*.

The phylogenetic position of Bittacidae has not been resolved satisfactorily in Mecoptera. Based on chromosomal data, Bittacidae was regarded as a closely related group to Boreidae (Matthey 1950; Cooper 1951; Atchley & Jackson 1970). The phylogeny reconstructed from the head structures showed that Bittacidae had a distant relationship with Meropeidae and Boreidae, but was closer to the group consisting of Panorpidae, Panorpodidae, Eomeropidae, Choristidae and Apteropanorpidae

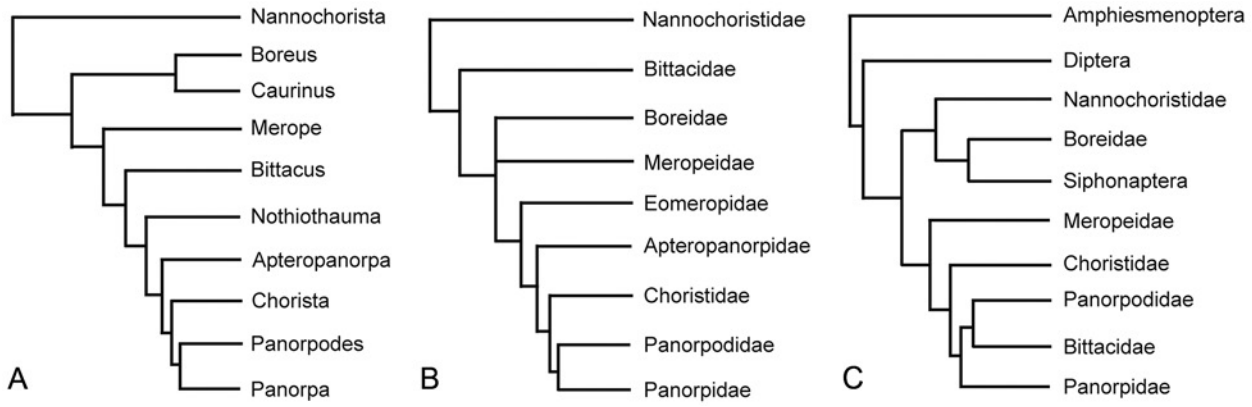

Fig. 6. Phylogeny of extant Mecoptera. **A**: Modified from Friedrich et al. (2013) based on head structures. **B**: Modified from Willmann (1987) based on morphological characters. **C**: Modified from Whiting (2002) based on molecular data.

(Beutel & Baum 2008; Friedrich et al. 2013: Fig. 6A). Willmann (1987) analyzed morphological phylogeny of Mecoptera and concluded that Bittacidae was a basalmost taxon in extant Pistillifera next to Nannochoristidae (Fig. 6B). The molecular evidence, however, indicated that Bittacidae and Panorpodidae formed a clade as the sister group to Panorpidae (Whiting 2002: Fig. 6C).

Combined with our present data, Bittacidae differs evidently from Boreidae in the variation of chromosome numbers with bittacids from 2n = 25 to 41 and boreids from 2n = 18 to 31. The distant relationship of Bittacidae from Nannochoristidae may be partially supported by the low chromosome number and doubtful achiasmate meiosis of Nannochoristidae, although achiasmate pattern lacks convincing evidence (Bush 1967). On the other hand, the so-called close relationship of Bittacidae and Panorpidae lacks a cytogenetic support. On the contrary, these two families differ by several cytological features, including the meiosis type, the morphology of bivalents and variation range of chromosome numbers (Naville & Beaumont 1934; Matthey 1950; Ullerich 1961; Atchley & Jackson 1970; Xu et al. 2013). We conclude that the phylogeny proposed by Beutel & Baum (2008) and Friedrich et al. (2013) receives more support from our current cytogenetic data.

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