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Two new species of genus *Rhopalopsole* (Insecta, Plecoptera, Leuctridae) from China

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Abstract

Two new species of *Rhopalopsole* Klapálek from China are described: *R. exiguspina* Du & Qian, **sp. n.** and *R. ampulla* Du & Qian, **sp. n.**, which were collected in Guizhou province, China.

Keywords

Rhopalopsole, Leuctridae, Plecoptera, new species, China

Introduction

The genus *Rhopalopsole* belongs to the family Leuctridae and is distributed throughout the Oriental and Palaearctic Regions. The abdominal segments of *Rhopalopsole* species are unmodified, but the last segment bears on both lateral sides a chitinous process, the shape of which is an important character for distinguishing species. Cerci are one-segmented and slightly modified in males, and cerci shape varies according to species (Kawai 1967). The genus *Rhopalopsole* first was described by Klapálek from a Taiwanese species, *R. dentata* Klapálek (1912). Contributions to and revisions of *Rhopalopsole* were made by Okamoto (1922), Wu (1949, 1973), Illies (1966), Kawai (1967, 1968, 1969), Jewett (1958, 1975a, b), Harper (1977), Zwick (1977), Yang and Yang (1991a,

b1994, 1995a, b), Yang et al. (2004) and Yang and Li (2006). Harrison and Stark (2008) provided a checklist of the genus that included 29 species, and an additional 43 species were subsequently described by Sivec et al. (2008), Stark and Sivec (2008), Yang et al. (2009), Li and Yang (2010), Li et al. (2010, 2011).

Here we describe two new *Rhopalopsole* species collected in Guizhou province, China. All type specimens were preserved in 75% ethanol and deposited in the Insect Collection of Yangzhou University, Jiangsu, China.

Taxonomy

***Rhopalopsole exiguspina* Du & Qian, sp. n.**
urn:lsid:zoobank.org:act:B26AB2A6-972F-4A18-9D1D-486A980CF80F
http://species-id.net/wiki/Rhopalopsole_exiguspina
Figs 1–4

Material examined. Holotype ♂ from China, Guizhou, Yanhe County, Shaba Village, 903m, 5 Oct. 2007, Leg. Xue Hai-Yang. Paratypes 18♂♂, the same details as holotype.

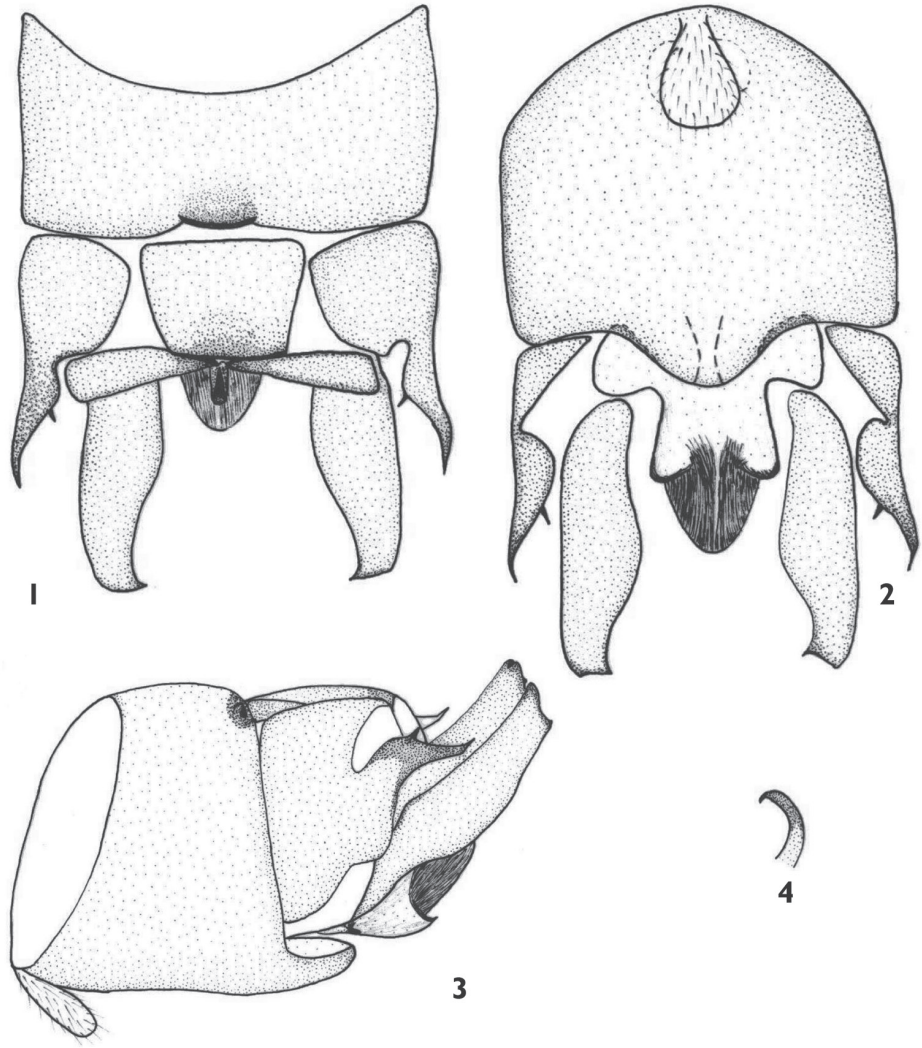
Adult habitus. General color: Light brown. Head brown or light brown, wider than prothorax, hind ocelli much closer to the eyes than to each other, antennae and palpi yellowish brown. Prothorax light brown, subquadrate, all angles somewhat rounded and some black irregular stripes on it. Legs light brown. Wings hyaline and veins light brown.

Male. Approximate measurement: forewing length 6.0 mm, body length 6.5 mm. Mid-posterior margins of tergite 9 sclerotized, slightly emarginated (Fig. 1). Sternite 9 basally with a tongue-like vesicle bears dense hairs, apically with a subgenital plate wider than long and rounded apically (Fig. 2). Tergite 10 with strongly sclerotized lateral process beak-like somewhat acute and curving inward apically and a small spine at the middle of lateral process in dorsal view, thick basally and slightly curved upward apically in lateral view. Mid-anterior sclerite sclerotized, posterior margin more sclerotized; one pair of transverse triangle sclerite weakly sclerotized (Fig. 1). Epiproct a simple curved process, erect hook-like apical portion curved inward (Fig. 4). Subanal lobe sinuate in lateral aspect, rounded and strongly sclerotized apically, apex membranous in ventral aspect; subanal lobe clearly with a pair of little lobes at middle of subanal lobe and each little lobes rounded apically in ventral aspect. Cerci long and cylindrical, thick basally and thin apically, distinctly upturned in lateral aspect, apex with a tiny spine.

Female. Unknown.

Etymology. The species name refers to the small spine at the middle of lateral process of tergite 10.

Diagnosis. This new species resembles *Rhopalopsole aculeata* Harper (1977) from Nepal and *R. xui* Yang, Zhu & Li (2004) from Guangdong in having an epiproct with a thin spine-like apical portion and a strongly sclerotized lateral process without



Figures 1–4. *Rhopalopsole exiguspina* male structures **1** Male terminal, dorsal aspect **2** Male terminal, ventral aspect **3** Male terminal, lateral aspect **4** Epiproct, lateral aspect.

bifurcation of tergite 10. The new species can be distinguished from *R. aculeata* by the presence of spines on the lateral processes and cerci. *R. aculeate* has no spines on the middle of lateral process or on the cerci. The new species can be distinguished from *R. xui* by the shapes of the mid-anterior sclerite, lateral process, subanal lobe and cerci. In *R. xui*, the mid-anterior sclerite is wider than long and has two short obtuse lateral processes. *R. xui* lacks spines at the middle of the lateral processes and on the cerci and has a wide and apically rounded subanal lobe without a pair of small lobes. The characteristics of the subanal lobe and lateral process distinguish this new species from other

Rhopalopsole species, which possess an epiproct with thin spine-like apical portions and no bifurcated lateral processes on tergite 10.

Rhopalopsole ampulla **Du & Qian, sp. n.**
urn:lsid:zoobank.org:act:B6B94919-A1E4-48D2-9CE6-6AA7A1DF63C9
http://species-id.net/wiki/Rhopalopsole_ampulla
Figs 5–7

Material examined. Holotype ♂ from China, Guizhou, Yanhe County, Shaba Village, 903m, 5 Oct. 2007, Leg. Xue Hai-Yang. Paratypes 6♂♂, the same details as holotype.

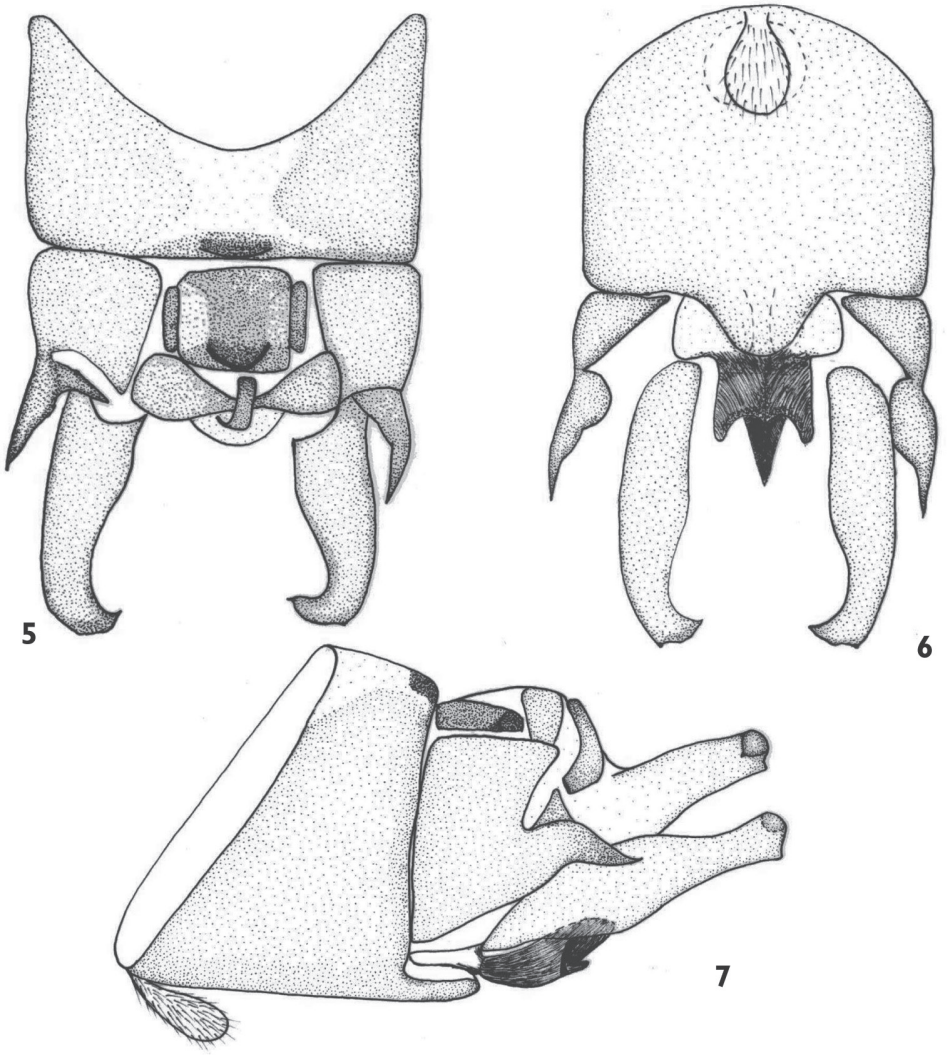
Adult habitus. General color: Brown and dark brown. Head brown or dark brown, wider than prothorax, hind ocelli much closer to the eyes than to each other, antennae and palpi brown. Prothorax dark brown, quadrate, longer than wide, all angles rounded and some black irregular stripes on it. Legs light brown. Wings hyaline and veins light brown.

Male. Approximate measurement: forewing length 8 mm, body length 8.5 mm. Tergite 9 sclerotized, with a large central membranous area, the mid-posterior margin strongly sclerotized (Fig. 5). Sternite 9 with a subgenital plate wider than long and rounded apically, basally with a tongue-like vesicle bears dense hairs (Fig. 6). Tergite 10 with two small narrow lateral mid-anterior sclerites and one large broad median mid-anterior sclerite; mid-posterior more sclerotized and protrusive; one pair of transverse sclerite weakly sclerotized (Fig. 5). Lateral processes each strongly sclerotized, spine-like rather than thick basally, narrowed apically and downward in lateral aspect (Fig. 7). Epiproct curved forward, thick and blunt apically (Fig. 5, 7). Subanal lobe strongly sclerotized at base, trident-like apically in ventral aspect and membranous at its apex (Fig. 6). Cerci long and cylindrical, ampulla-like, thick basally and thin apically, each with a tiny spine.

Female. Unknown.

Etymology. The species name refers to the shape of cerci on segment 10.

Diagnosis. This new species is similar to other species in the *Rhopalopsole assamensis* group (Sivec et al. 2008) in having a sclerotized area on the mid-posterior margin of tergite 9, thick epiproct, lateral sclerites at each side of the central sclerite and cerci with tiny spines. It can be diagnosed by the shape of the subanal lobes, which are trident-like apically. Other species in the *R. assamensis* group possess subanal lobes that are flat and narrow at the base but expand into a wide rectangular apical portion. The lateral processes of species in the *R. assamensis* group typically end in a forked process on tergite 10, but those of this new species lack bifurcation. *R. ampulla* is similar to *R. exiguspina*, but *R. ampulla* can be distinguished by the shapes of the subanal lobes and the lateral processes on tergite 10. The subanal lobes of *R. exiguspina* are rounded apically and each posses a small spine at the middle of



Figures 5–7. *Rhopalopsole ampulla* male structures **5** Male terminal, dorsal aspect **6** Male terminal, ventral aspect **7** Male terminal, lateral aspect.

lateral process, but those of *R. ampulla* are strongly sclerotized and trident-like apically in ventral aspect.

Acknowledgments

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Species recognition through wing interference patterns (WIPs) in *Achrysocharoides* Girault (Hymenoptera, Eulophidae) including two new species

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Abstract

Wing interference patterns (WIPs) are shown to be an important tool for species recognition in the genus *Achrysocharoides* Girault (Hymenoptera: Eulophidae). This is demonstrated by combining information from two previously published papers, comprising two cases of cryptic species, and by new material including the description of two new species, *A. maieri* and *A. serotinae* from North America. The cryptic species were initially separated through their distinct male WIPs. Subsequent analyses of the external morphology uncovered additional morphological differences supporting the original findings through WIPs, and biological data further strengthened the identity of these species. The new species described here also differ in their WIPs but the WIPs are similar in both sexes. Thus they provide a strong link between male and female and demonstrate that WIPs can also be useful for species recognition when the sexes are otherwise difficult to associate. Both new species are from Connecticut, USA, and were reared from *Phyllonorycter propinquinella* (Braun) (Lepidoptera: Gracillariidae) on black cherry (*Prunus serotina*); *A. maieri* has also been reared from *Ph. nr crataegella* on pin cherry (*P. pensylvanica*). To facilitate the identification of the new species they are included in a previously published key to North American species of *Achrysocharoides*. As a supplement to colourful WIPs we also demonstrate that grey scale images of uncoated wings from scanning electron microscopy can be used for visualization of the thickness distribution pattern in wing membranes.

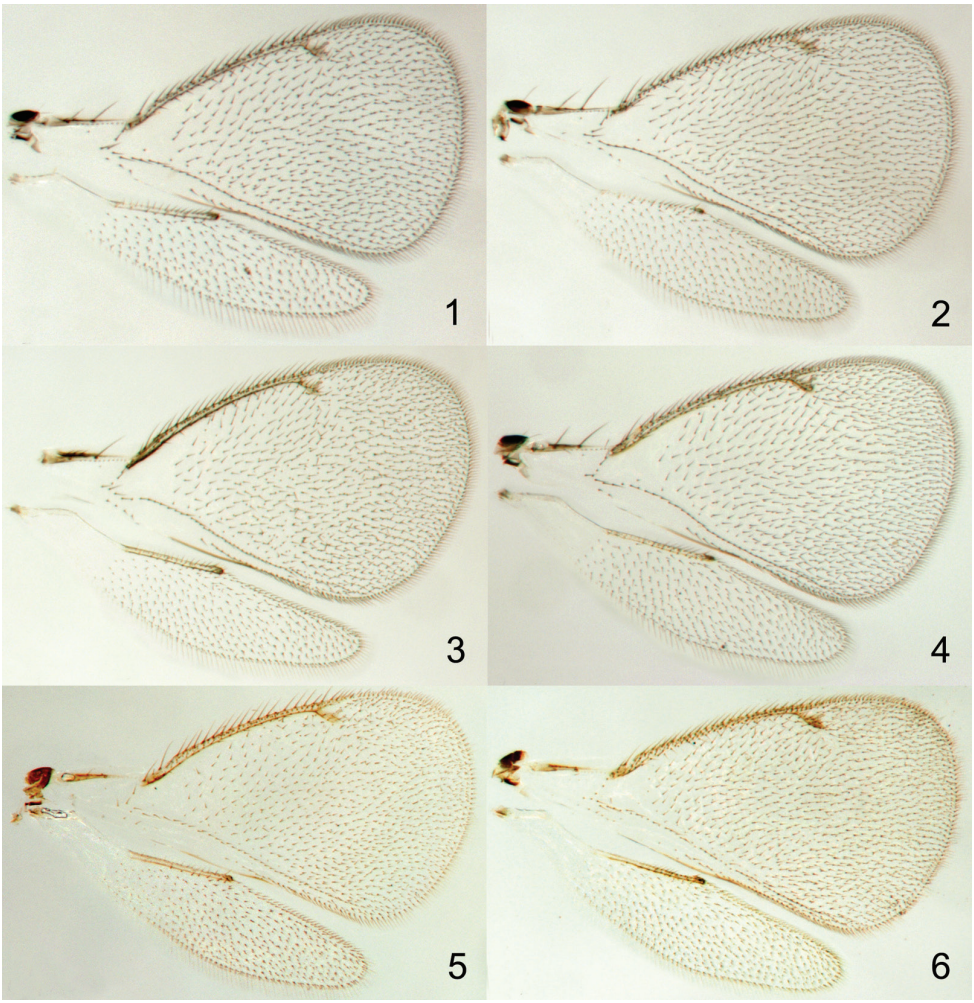
Keywords
taxonomy, cryptic species, structural colours, sexual dimorphism, wing membrane thickness, Chalcidoidea, Entedoninae, leafminer parasitoids, *Achrysocharoides acerianus, Achrysocharoides platanoidae, Achrysocharoides robiniae, Achrysocharoides robinicolus, Achrysocharoides butus, Achrysocharoides latreilleii, Achrysocharoides albiscapus, Achrysocharoides maieri*, *Achrysocharoides serotinae*, *Phyllonorycter propinquinella*, *Phyllonorycter nr crataegella*, *Prunus serotina*, *Prunus pensylvanica*

Introduction

Species of *Achrysocharoides* Girault (Hymenoptera: Eulophidae) are small parasitic wasps with transparent non-pigmented wings (Figs 1–6, 9). The short postmarginal vein in the fore wing is characteristic for the genus and the shape of the fore wing can be used to distinguish males of some species, but otherwise wings have been disregarded as non-informative neutral entities in this genus (e.g. Askew and Ruse 1974; Kamijo 1991). Recently wings in this group were discovered to display patterns with stable structural colours (Fig. 7), comparable to other insect groups with colourful wings such as butterflies (Shevtsova et al. 2011). These wing interference patterns (WIPs) become visible when transparent insect wings are seen against a dark background, and are most distinctive in small species with exceptionally thin wing membranes.

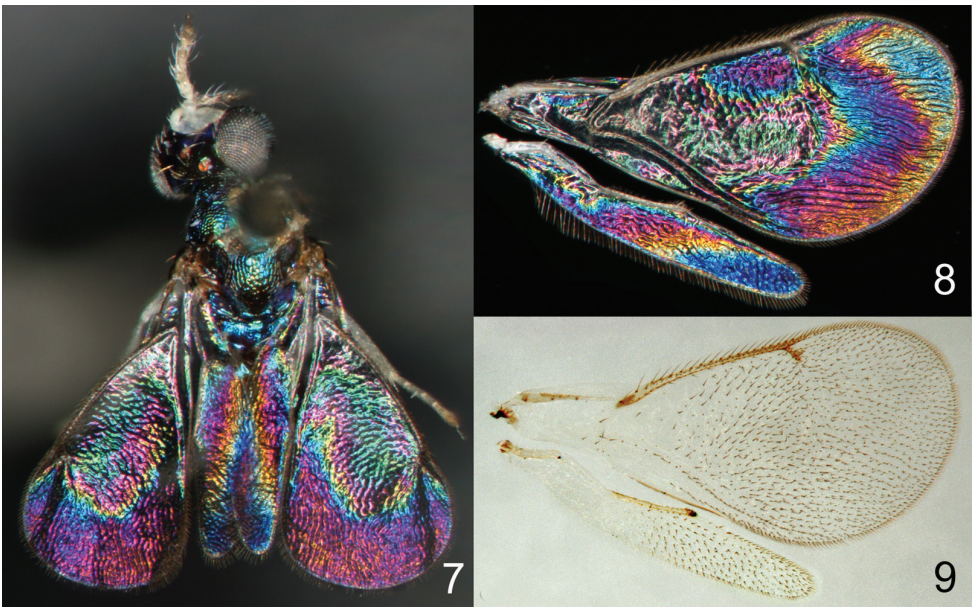
WIPs as a morphological character are so new that very little is known about the significance of these patterns for their bearers or for entomologists studying them, although they have already proven useful for generic-level classification in Eulophidae (Hansson 2011). The application of WIPs as a species character was first used in a study including two cases of cryptic species in *Achrysocharoides* (Hansson and Shevtsova 2010), where the initial species separation was based solely on male WIPs. However, data showing the usefulness of WIPs were withheld pending the publication of Shevtsova et al. (2011) where a general background to these patterns was outlined. In order to expand the knowledge of WIP diversity and to prove the usefulness of these patterns for studies at the species level it is important to link the information from these two publications. To further enhance this knowledge we also describe two new *Achrysocharoides* species with distinct WIPs.

The two new species of *Achrysocharoides* described here are from North America and the genus was initially recorded from this region by Miller (1962), as the genus *Enaysma* Delucchi, including six new species from Canada which were placed in the same subgenus (*Pentenaysma* Graham). Yoshimoto (1977) synonymized *Enaysma* with *Achrysocharoides*, and added nine species (six newly described) to the six described by Miller. He also separated the 15 species into two newly created species groups, thus abandoning the division into subgenera. The latest comprehensive treatment of North American *Achrysocharoides* is by Kamijo (1991), who treated 18 species, including four new species and one new synonym, separated into five



Figures 1–6. *Achrysocharoides* spp., transparent wings: **1** *A. acerianus* (Askew), male **2** Ditto, female **3** *A. platanoidae* Hansson & Shevtsova, male **4** Ditto, female **5** *A. butus* (Walker), male **6** Ditto, female. Wings on Figs **1–4** from Sweden, Skåne, 2010 **5–6** from Wales, 1976.

species groups, two of which were newly created. Hansson and Shevtsova (2010) added two new species to the North American fauna, increasing the total to 20 species. With the two new species described here this total is now 22, equal to the number of species in Europe. Worldwide, including the two new species described here, 56 species of *Achrysocharoides* are known. The majority (ten) of the remaining species are from Japan (Kamijo 1990a, b), thus establishing the main distribution of *Achrysocharoides* as the northern hemisphere.

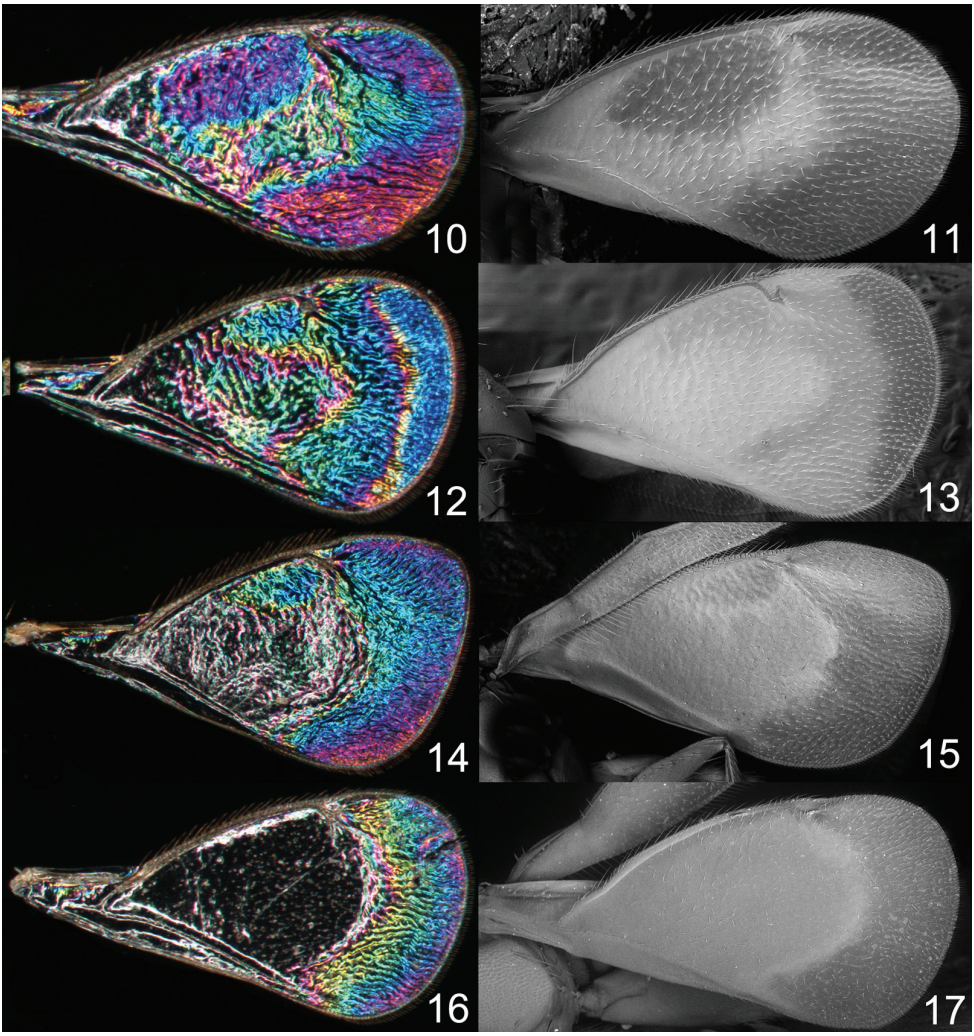


Figures 7–9. *Achrysocharoides* spp.: **7** *A. zwoelferi* (Delucchi), male, from Sweden, Blekinge, 1956 **8** Undescribed species from USA, Arizona, 1982, male, wing interference pattern (WIP) **9** The same wings as in Fig. **8** in transparent mode.

Material and methods

The observation and documentation of WIPs do not require a special light source and can be done on any dry specimen with intact wings arranged against a dark background. However, to make the illustrations comparable all photos in this paper as well as in Shevtsova et al. (2011) are of wings removed from the specimens and horizontally arranged, and with the same magnification (6×). To achieve this, the wings are flattened between a glass slide and a glass cover slip on top of the wings. The underside of the glass slide is stained with a drop of black ink to make the background pitch black and homogeneous (this was proposed by J. Kjærandsen). In a few cases where the wings could not be properly flattened the slide was slightly tilted so that the pattern in a non-flattened area, e.g. in a wrinkle, became visible and could be documented. This area was then manually combined in Adobe Photoshop with the initial horizontal photo of the wing, thus showing the complete pattern. A Nikon SMZ1000 stereomicroscope and 5MP Nikon DS-L1 camera were used to take photos of the wings at different focus levels, and Helicon Focus Pro version 4.75 software was used to merge them into a single image. WIPs are usually too shiny for the camera to balance brightness automatically and therefore the brightness was individually adjusted in Adobe Photoshop. Subsequent editing included cleaning and cropping of the photo. After the fore and hind wings were documented they were glued back to the card with the original specimen, which retained the second pair of wings for future observations – structural colours disappear on glued or slide mounted wings (Figs 1–6). The images of transparent wings in this paper are from temporary slide

preparations with wings mounted in a water-soluble clear gel. The scanning electron microscopy (SEM) images (Figs 11, 13, 15, 17, 66–71, 82–87) are from uncoated specimens on their original card mountings. The photos were taken in low vacuum mode via a backscattered electron detector on a JEOL® JSM 5600LV microscope.



Figures 10–17. *Achrysocharoides* spp., males, wing interference patterns (WIPs) to the left, scanning electron micrographs from uncoated wings to the right: **10–11** *A. robiniae* Hansson & Shevtsova **12–13** *A. butus* (Walker) **14–15** *A. latreilleii* (Curtis) **16–17** *A. albiscapus* (Delucchi).

Morphological abbreviations and acronyms

HE = height of eye; HW = height of fore wing; LG = length of gaster; LM = length of marginal vein; LW = length of fore wing, measured from base of marginal vein to apex of wing; MM = length of mesosoma; MS = malar space; OOL = distance between one

posterior ocellus and eye; PM = length of postmarginal vein; POL = distance between posterior ocelli; POO = distance between posterior ocelli and occipital margin; ST = length of stigmal vein; WH = width of head; WM = width of mouth; WT = width of thorax. For illustrations of the morphological terms see http://www.neotropicaleulophidae.com/.

Collection acronyms, for the deposition of type material: BMNH = Natural History Museum, London, England; CAES = Connecticut Agricultural Experiment Station, New Haven, U.S.A; CNC = Canadian National Collection of Insects, Ottawa, Canada.

Results and discussion

The paper by Hansson and Shevtsova (2010) included two cryptic *Achrysocharoides* species from *Acer, A. platanoidae* Hansson & Shevtsova from *Acer platanoides* and *A. acerianus* (Askew) from *A. pseudoplatanus,* and two cryptic species from *Robinia pseudoacacia, A. robiniae* and *A. robinicolus*, both described in that paper. The transparent wings in these four species are very similar and identical between males and females (Figs 1–4). Nevertheless the initial differences distinguishing these cryptic species were found in the wing morphology through distinct WIPs, which visualize uneven thickness of the wing membrane through different interference colours (Shevtsova et al. 2011).

In both cryptic cases only one of the species displays a distinct species specific WIP, and in males only, while conspecific females and both sexes of the other cryptic species have similar WIPs. In the two *Achrysocharoides* species associated with *Acer* only males of *A. platanoidae* have a distinctive WIP with an eye-catching blue spot in the upper-apical corner of the fore wing (Figs 18–21). The female WIP of *A. platanoidae* displays no such spot (Figs 22–23) and is very similar to *A. acerianus*, which has the same WIP in both sexes (Figs 24–27). In the two other cryptic species, associated with *Robinia pseudoacacia,* only males of *A. robiniae* display a very characteristic WIP with a large ovate spot below the marginal vein. The male WIP also has an extended and usually green triangular area in the medio-apical part of the fore wing (Figs 28–33). In the female WIP the triangular area is usually less pronounced than in males and the submarginal ovate spot is significantly smaller (Figs 34–35). As the female does not display the characteristic features in these patterns as distinctly as the male, it can be confused with the female of *A. robinicolus*, which has the same WIP in both sexes (Figs 36, 37).

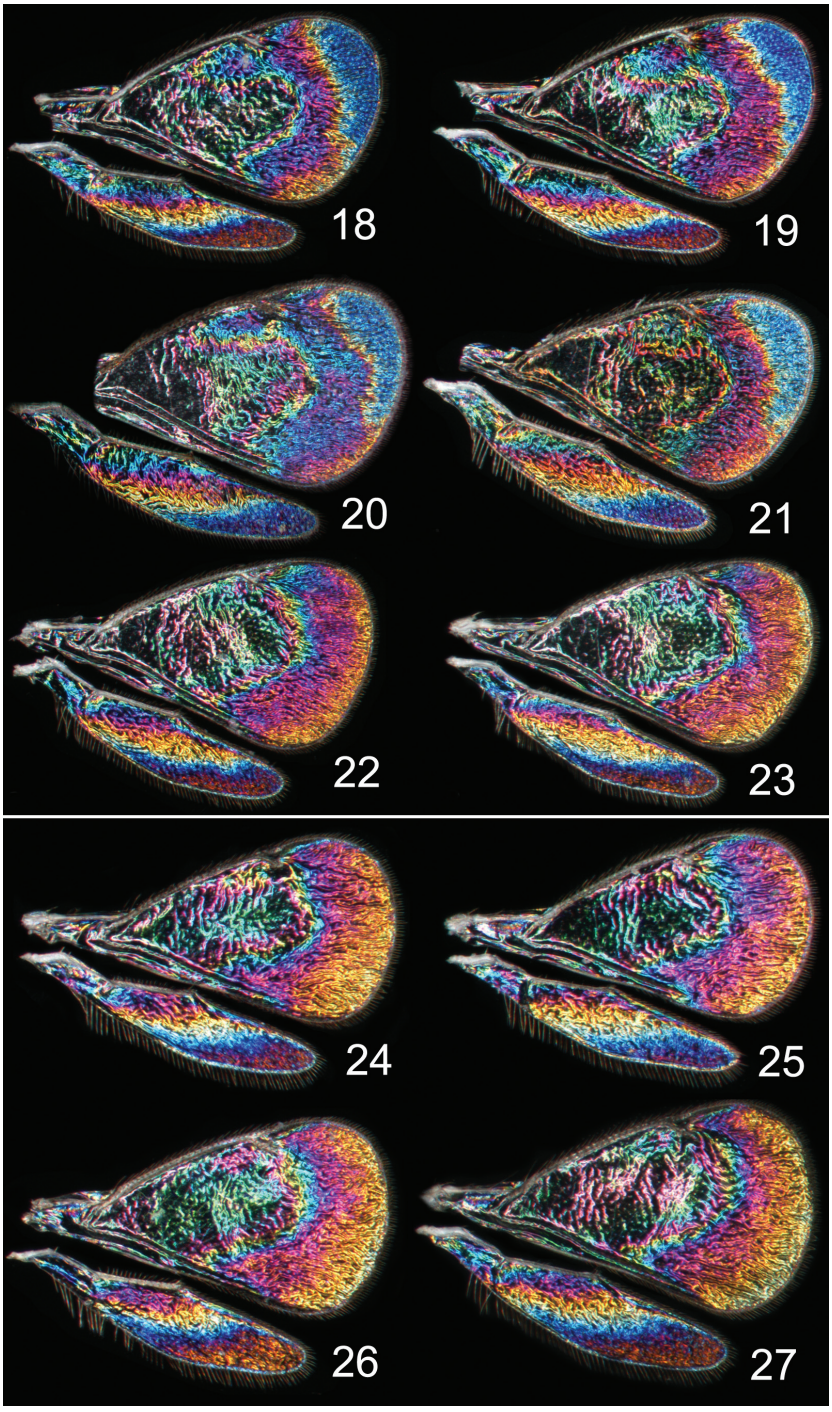
The two North American species described here, *A. maieri* and *A. serotinae*, are known only from, and are probably confined to, *Phyllonorycter* species on *Prunus*. Males can be distinguished through easy-to-see differences in the external morphology, e.g. the shape of the head (Figs 57, 67, 73, 83) but females are not so distinct and display less divergent characters (Figs 56, 66, 72, 82). Even though the wings of *A. maieri* and *A. serotinae* appear very similar in transparent mode (similar to Figs 1–6) the WIPs in these species are distinct and specific. Apart from being useful in the discrimination of the females, in this case WIPs are also useful for the association of otherwise

dimorphic males and females of the same species. The external morphology in these species exhibits a pronounced sexual dimorphism and as they share the same host it is not obvious which females and males are conspecific. However, there is one important character they have in common – WIPs, which are identical in both sexes but different between the species. *Achrysocharoides maieri* has a WIP with wide coloured cross bands on the fore wing (Figs 64–65), and *A. serotinae* has a quite featureless almost unicoloured WIP (Figs 80–81).

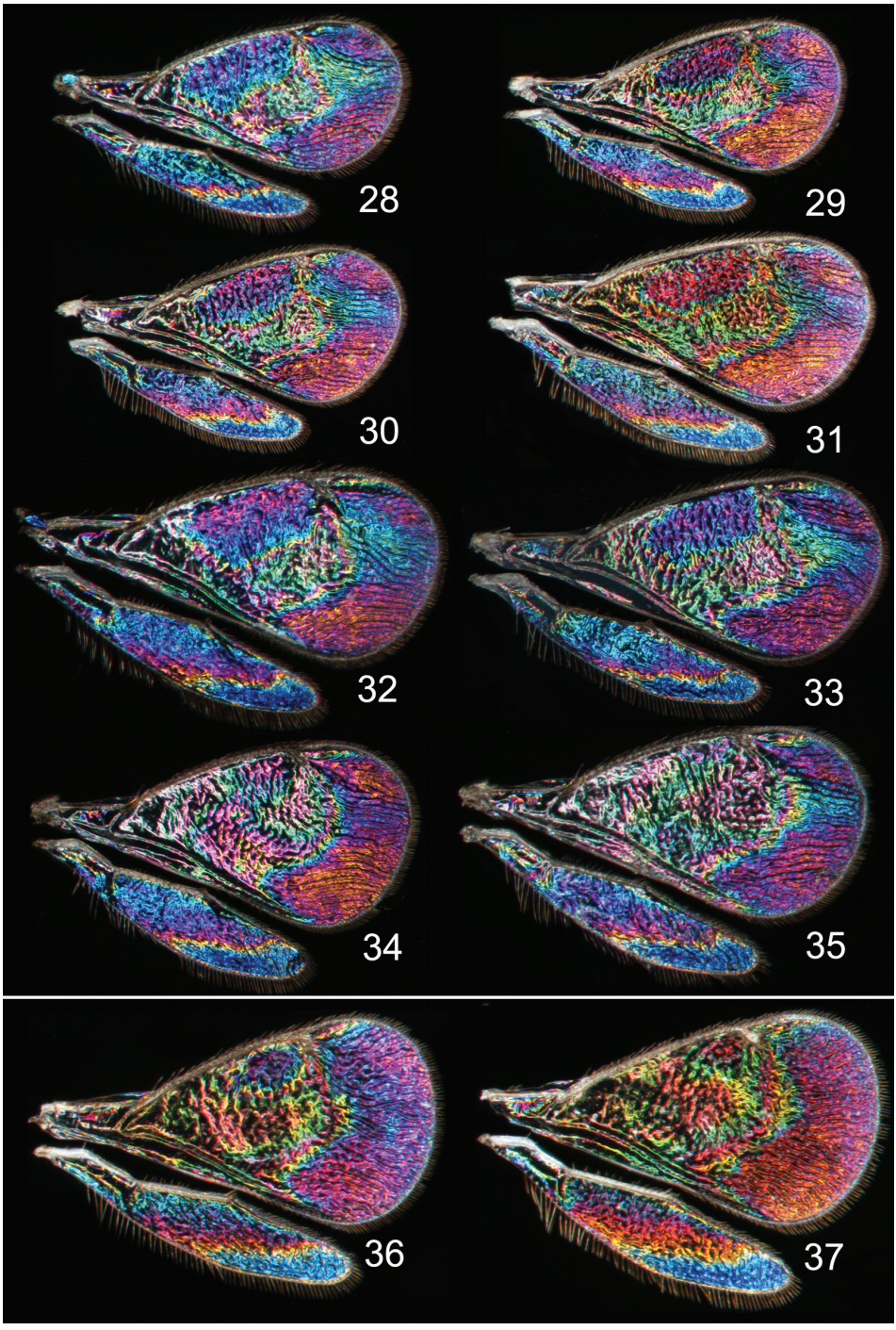
Additional examples of *Achrysocharoides* species with distinct and sexually dimorphic WIPs are *A. butus* (Walker) (Figs 38-43) and *A. latreilleii* (Curtis) (Figs 46-49) where characteristic and specific WIPs, again, are confined to males. Female WIPs of these two species are similar (Figs 44–45, 50–51), and as in females of *A. platanoidae* and *A. acerianus* (Figs 22–23, 26–27), and *A. robiniae* and *A. robinicolus* (Figs 34–35, 37), WIPs are not useful for species recognition. The WIP of male *A. butus* is similar to that of male *A. platanoidae* because the apical margin of the fore wing has a blue spot in both species. However, in *A. butus* this spot is prolonged and reaches along a major part of the apical margin (Figs 38–43) whereas in *A. platanoidae* the spot is short and confined to the upper-apical corner of the fore wing (Figs 18–21). The male of *A. latreilleii* is distinct not only in the truncate shape of the fore wing but also in its WIP (Figs 46–49). The basal 2/3 of the fore wing is the thickest part of the wing membrane and due to its micromorphology reflects very weak interference colours (Shevtsova et al. 2011). The apical part of the fore wing, and a small submarginal spot located in the corner between marginal and stigmal veins, are brightly coloured. The potential of WIPs as a character for separating species can be further demonstrated through two species where only male WIPs are known. *Achrysocharoides albiscapus* (Delucchi) has a WIP similar to that of *A. latreilleii*, but differs in having the basal 2/3 of the fore wing completely transparent without colour reflections and no submarginal colour spot (Figs 52–55). The shape of the fore wing is also different between males of these two species. The other species is undescribed, from Arizona, USA (specimen in CNC), and we have only seen a single male. This specimen has a distinctive WIP which emphasizes very unusual shapes of both fore and hind wings. The WIP includes a blue spot in the upper-apical corner of the fore wing (Fig. 8), comparable to *A. platanoidae* (Figs 18–21) but with the blue spot smaller and differently shaped.

Similar to other morphological characters there is a certain intraspecific variation in WIPs (Figs 18–55), but the species specific traits nevertheless remain clearly recognizable and are reliable for species separation. The intraspecific variation in WIPs can be divided into two types, variation in colour and in shape of patterns. Variation in colour is basically size-dependent – the thickness of the wing membrane usually varies with the size of the specimen and there is a general shift of the hues in WIPs from larger to smaller specimens. Variation in the shapes of pattern outlines of conspecific WIPs is not apparently size dependent but reflects individual differences between specimens - the overall pattern nevertheless remains the same.

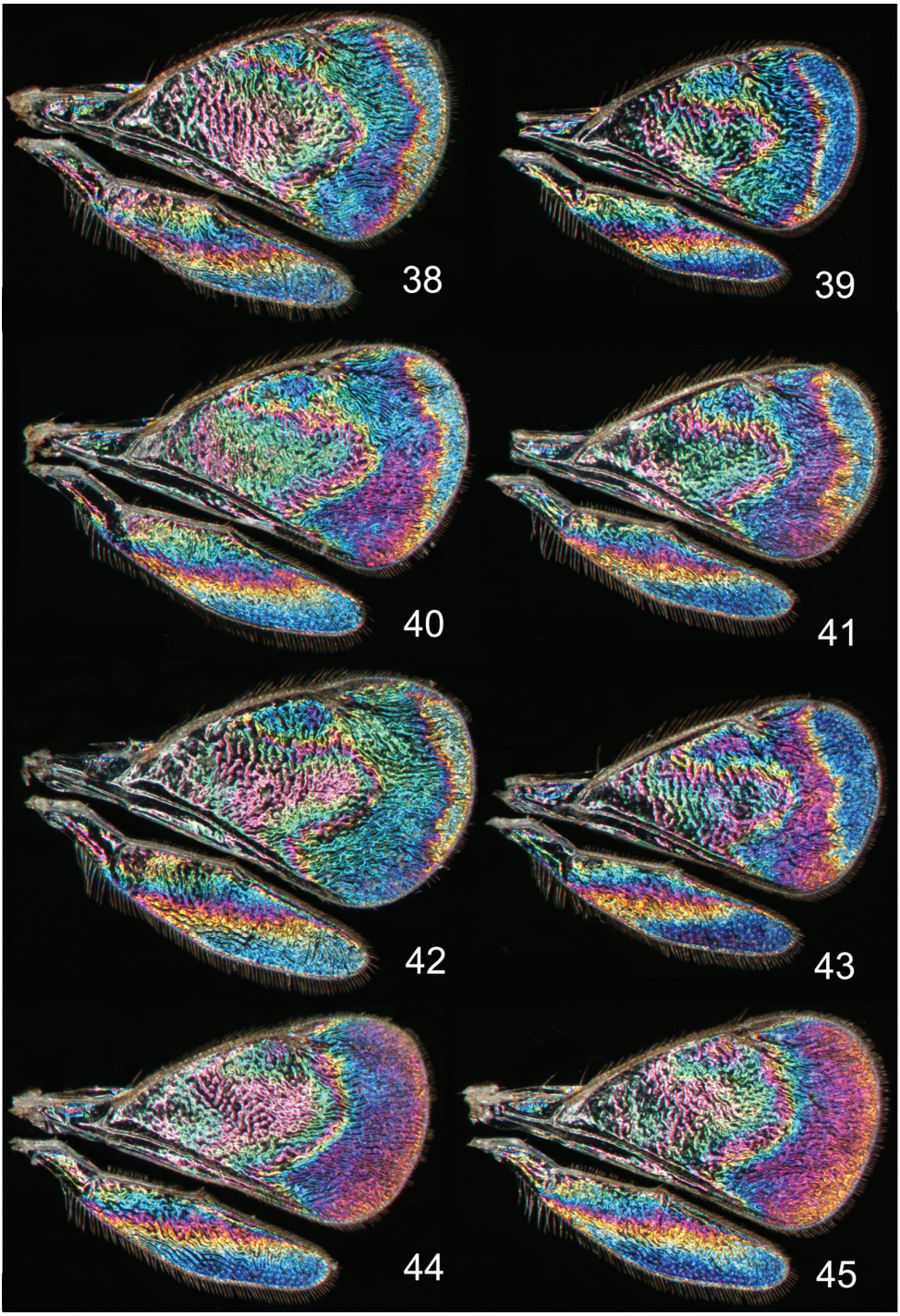
Wing interference patterns are due to structural organization patterns of the wing membrane where areas of different thickness reflect certain interference colours (Shevt-



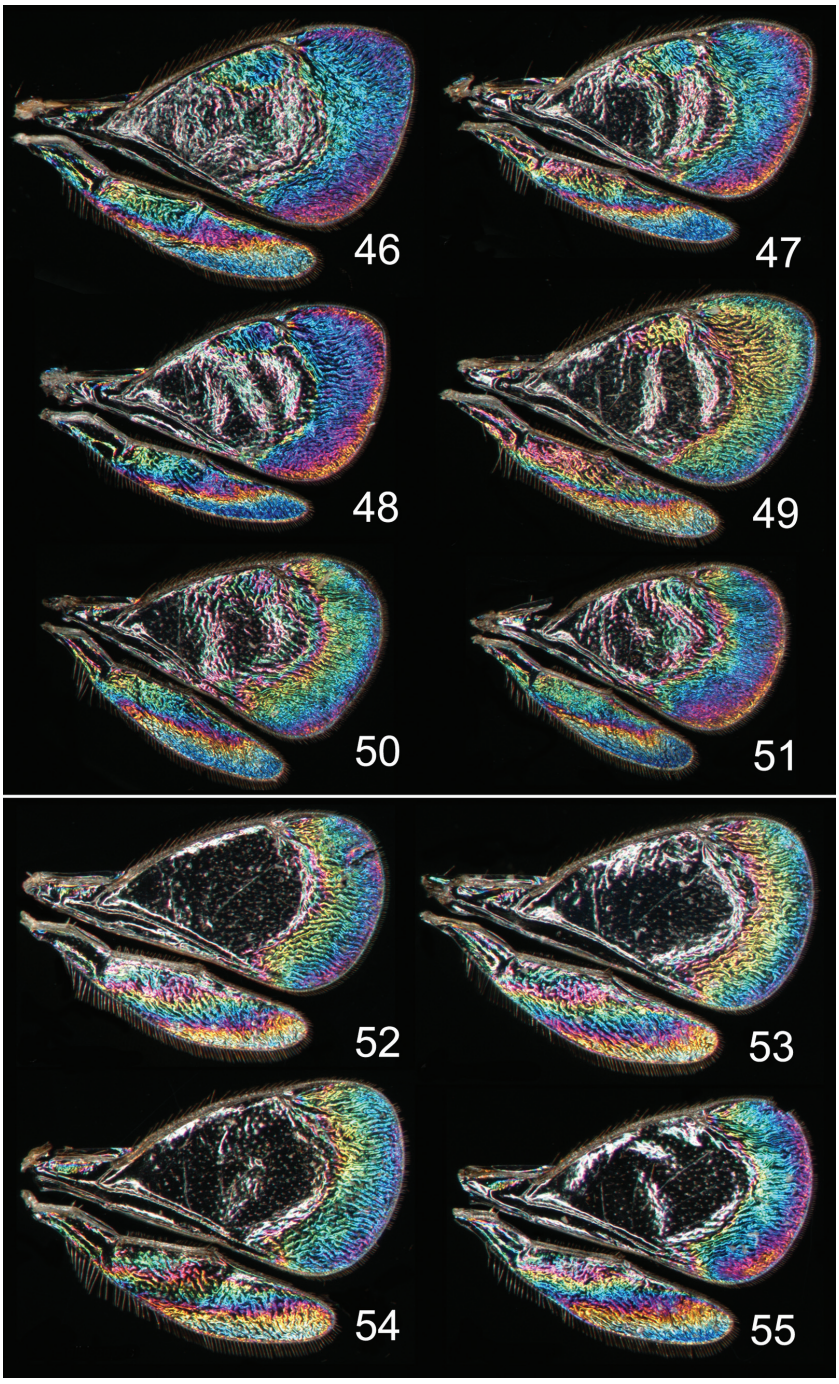
Figures 18–27. *Achrysocharoides* spp., wing interference patterns (WIPs): **18–23** *A. platanoidae* Hansson & Shevtsova **18–21** Males **22–23** Females **24–27** *A. acerianus* (Askew) **24–25** Males **26–27** Females. All wings from specimens from Sweden, Skåne, 2010.



Figures 28–37. *Achrysocharoides* spp., wing interference patterns (WIPs): **28–35** *A. robiniae* Hansson & Shevtsova **28–33** Males **34–35** Females **36–37** *A. robinicolus* Hansson & Shevtsova **36** Male **37** Female. Wings on Figs **28–31, 34–37** from USA, Connecticut, 2002 **32, 33**, from Hungary, Vas Co., 2002.



Figures 38–45. *Achrysocharoides butus* (Walker), wing interference patterns (WIPs): **38–43** Males **44–45** Females. Wings on Figs **38, 40–45** from Wales, 1976 **39** from Sweden, Skåne, 2010.



Figures 46–55. *Achrysocharoides* spp., wing interference patterns (WIPs): **46–51** *A. latreilleii* (Curtis) **46–49** Males **50–51** Females **52–55** *A. albiscapus* (Delucchi), males. Wings on Figs **46, 47, 49–51** from England, Surrey 1986–2004 **48** from Sweden, Skåne, 2010; **52, 53, 55** from Greece, Crete, 1997 **54** from France, 1984.

sova et al. 2011). We have found that the uneven thickness of the wing membrane also can be demonstrated and authenticated through the contrast in grey scale SEM images of uncoated wings. The SEM images created through back-scattered electrons (BSEs) visualize specific patterns on wings (Figs 11, 13, 15, 17). These patterns fully correspond to the approximate mapping of the wing thickness based on WIPs where the thickness of the wing membrane at any point can be estimated by the reflected interference colour (Shevtsova et al. 2011). The thickness gradient as seen through grey scale gradients in SEM images is due to specific properties of BSEs which have the escape depth of up to hundreds of nanometers (Egerton 2005). This means that the signal comes from a sample depth in the range comparable to membrane thickness in wings producing bright WIPs, i.e. 100–600 nm. In uncoated wings the primary (incident) electrons are scattered inside the membrane and reflected as BSEs to the back-scatter detector. In thick areas of the membrane the amount of BSEs is large, resulting in a strong signal, while thin areas of the membrane produce fewer BSEs and a weaker signal, thus displaying light and dark grey hues respectively. If the wings are coated with platinum or gold, the resulting picture is completely different due to secondary electrons (SEs) which are generated only within a very small distance below the surface as the escape depth of SEs is less than two nanometers (Egerton 2005), thus displaying the surface of the specimen rather than the underlying structure. In Shevtsova *et al*. (2011) secondary electron images were used to illustrate the microstructures of the wing surface, such as the ridges of membrane corrugations with rows of setae.

The clarification of the two cryptic species on *Acer* spp. (Hansson and Shevtsova 2010, Shevtsova et al. 2011) requires a correction of the molecular information deposited in Genbank. At the time of the publication of Lopez-Vaamonde et al. (2005) the identity of the *Achrysocharoides* species associated with *Acer* spp. was not clear, and "Achrysocharoides acerianus ex Acer platanoides" and "Achrysocharoides sp. ex Acer pseudoplatanus" in Lopez-Vaamonde et al. (2005) are *A. platanoidae* and *A. acerianus* respectively, which is confirmed here with new molecular analyses compared to data of "Achrysocharoides sp." and "A. acerianus" in Genbank. Our new sequences include CO1, 18S, 28S and will be deposited in Genbank.

Species descriptions

***Achrysocharoides maieri* sp. n.**
urn:lsid:zoobank.org:act:2E20B2E3-557F-413E-8729-D9ADD646FE3C
http://species-id.net/wiki/Achrysocharoides_maieri
Figures 56–71

Material. HOLOTYPE male (CNC) glued to a card, labeled "U.S.A.: Connecticut, New Haven Co., New Hamden, Lockwood Farm, 1.viii.1980, C.T. Maier", "Tentiform mine of Phyllonorycter propinquinella on Prunus serotina, emerged in laboratory within 3 weeks". PARATYPES: 1 female 3 males with same label data as holotype

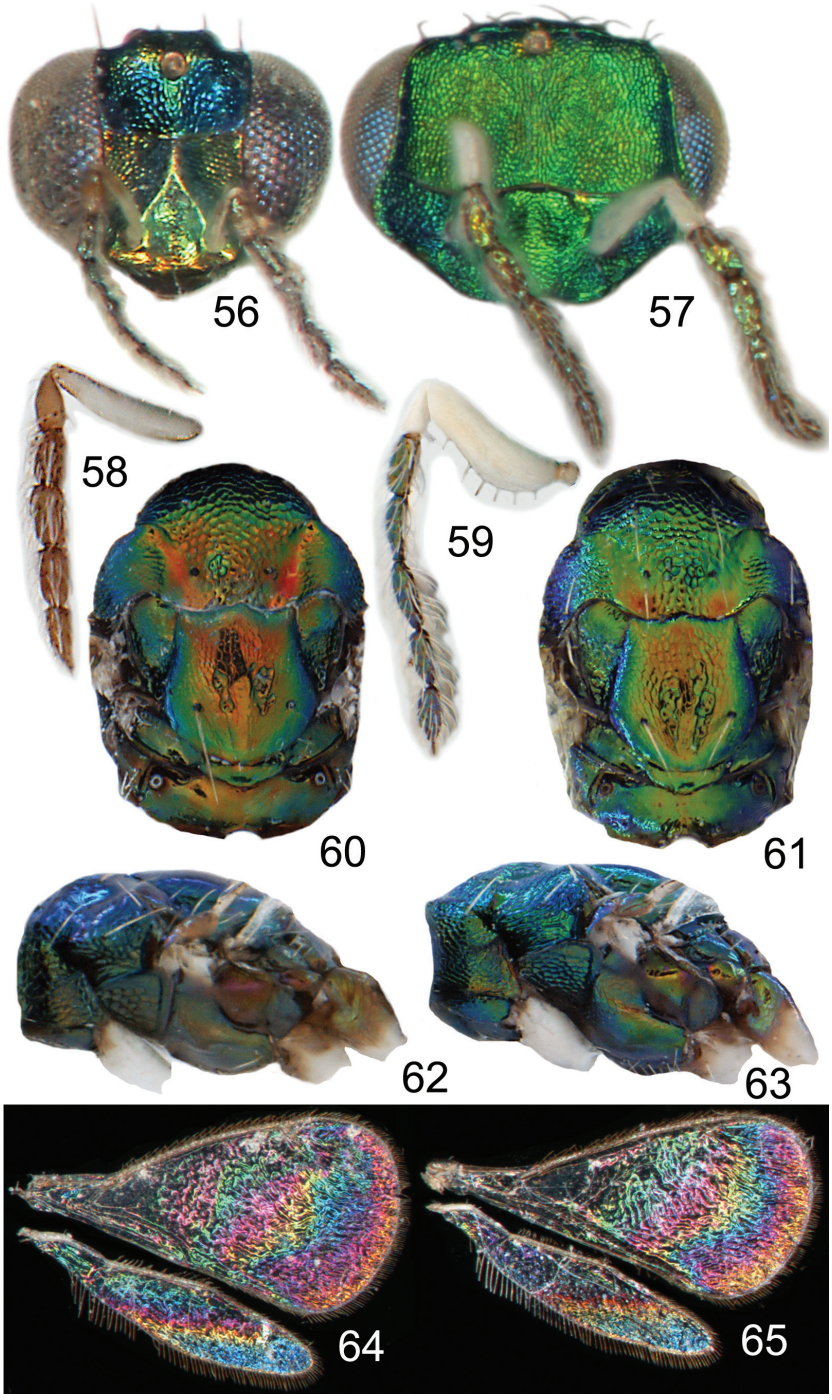
(BMNH, CAES, CNC)); 1 male labeled "U.S.A.: Connecticut, Tolland Co., Willington, 21.x.1981, Chris T. Maier", "Mines of Phyllonorycter propinquinella on black cherry, Prunus serotina, on 21.x.1981, chilled outdoors, parasitoid emerged in laboratory in April 1982" (CNC); 3 females "U.S.A.: Connecticut, New Haven Co., North Haven, 1.vii.1981, C.T. Maier", "Tentiform mine of Phyllonorycter nr crataegella on Prunus pensylvanica, emerged in laboratory within 3 weeks" (BMNH, CNC); 2 females 1 male from same locality and same host as previous but collected 2.vi.1986 (BMNH).

Diagnosis. Both sexes: fore wing WIP with several distinct wide colourful cross-bands traversing the wing (Figs 64, 65), fore coxa white, hind coxa except apex golden green (Figs 62, 63); male: scape widest just below median part, with a single sparse row of setae along ventral margin (Fig. 59), antennal scrobes join frontal suture wide apart (Figs 67), vertex with long forward pointing setae (Fig. 69) – setae about as long as distance between posterior ocelli, upper frons without setae (Fig. 67), frons very large and wide (Fig. 67) - at its widest part 0.8X as wide as width of head; female: scape predominantly white and widest medially, with a single row of setae along ventral margin, propodeum smooth (Fig. 70), frons above frontal suture with raised and strong reticulation (Fig. 66).

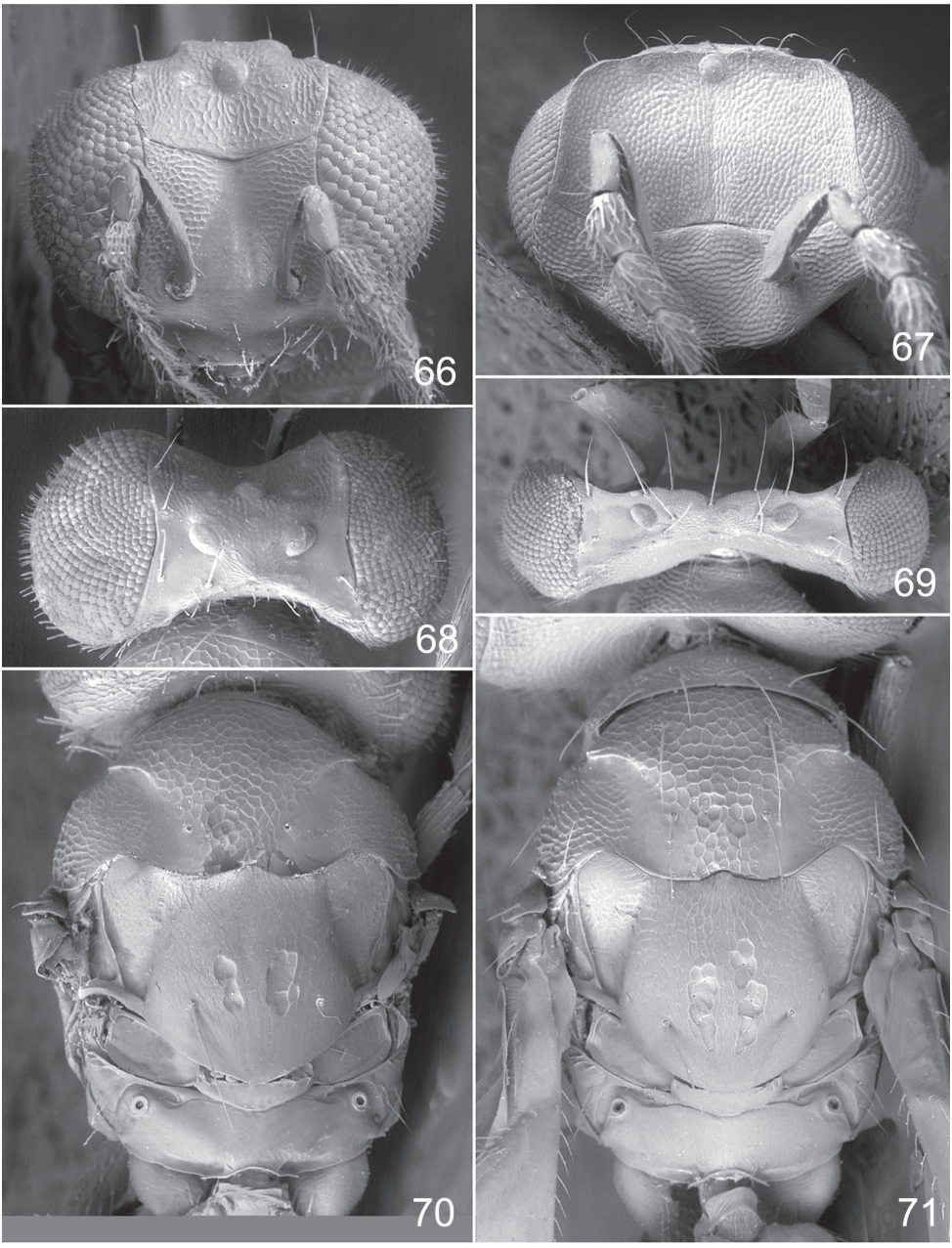
Description. *Female*. Length 1.1–1.5 mm. Scape white with inner apical tip infuscate; pedicel pale brown; flagellum dark brown (Fig. 58). Frons below frontal suture golden green to golden red, above frontal suture bluish green metallic (Fig. 56). Vertex inside ocellar triangle golden red, outside ocellar triangle golden green. Mesoscutum golden green with golden red areas – especially so in smooth posterior notaular depressions, to completely golden green (Fig. 60). Scutellum golden red with sides and posterior margin bluish green metallic, to completely golden green (Fig. 60). Propodeum golden red to golden green (Fig. 60). Fore coxa white, mid coxa dark brown with apical 1/3 white to completely dark brown, hind coxa golden green (Fig. 62); femora, tibiae and tarsi on all legs white. Wings without pigmented areas; WIP in fore wing with several distinct wide colourful cross-bands traversing the wing (Fig. 64). Gaster with first two tergites golden green, remaining tergites golden purple with green metallic tinges.

Antenna as in Fig. 58. Frons below level of toruli smooth and shiny (Fig. 66), between level of toruli and frontal suture with raised and strong reticulation lateral to antennal scrobes, between antennal scrobes with very weak reticulation, above frontal suture with raised and strong reticulation. Vertex inside ocellar triangle with engraved and weak reticulation, outside ocellar triangle smooth and shiny (Fig. 68). Occipital margin rounded.

Pronotal collar without transverse carina (Fig. 70). Mesoscutum with raised and strong reticulation (Fig. 70), meshes of reticulation smaller on sidelobes than on midlobe, midlobe with pits (i.e. with very strong reticulation) posteromedially; notauli as smooth impressions in posterior 2/3. Scutellum with very weak reticulation and shiny, smooth along posterior margin, with 3–4 pits medially on either side of imaginary median longitudinal line (Fig. 70). Dorsellum flat and smooth, anterolaterally with two foveae. Propodeum smooth and shiny (Fig. 70); propodeal callus with three setae. Fore wing speculum closed below. Petiole conical without shoulders.



Figures 56–65. *Achrysocharoides maieri* sp. nov.: **56** Head frontal, female **57** Ditto, male **58** Antenna lateral, female **59** Ditto, male **60** Mesosoma dorsal, female **61** Ditto, male **62** Mesosoma lateral, female **63** Ditto, male **64** Wing interference pattern (WIP), female **65** Ditto, male.



Figures 66–71. *Achrysocharoides maieri* sp. n.: **66** Head frontal, female **67** Ditto, male **68** Vertex, female **69** Ditto, male **70** Mesosoma dorsal, female. **71** Ditto, male.

Ratios. HE/MS/WM = 5.0/1.0/2.3; POL/OOL/POO = 2.6/1.1/1.0; WH/WT = 1.2; LW/LM/HW = 1.6/1.0/1.0; PM/ST = 1.0; MM/LG = 0.8–0.9.

Male. Length 1.4–1.5 mm. Scape and pedicel white; flagellum dark brown with golden green tinges (Fig. 59). Frons green metallic (Fig. 57). Vertex inside ocellar tri-

angle golden red, outside ocellar triangle golden green. Mesoscutum golden green with posterior 1/3 of notaular depressions golden red (Fig. 61). Scutellum golden red with sides bluish green metallic (Fig. 61). Propodeum golden green (Fig. 61). Fore coxa white, mid coxa dark brown with apical 1/3 white to completely dark brown, hind coxa golden green with apical half white (Fig. 63); femora, tibiae and tarsi on all legs white. Wings without pigmented areas; WIP very similar to that of the female (Fig. 65). Gaster with tergites 1–2 golden green with a large white spot medially, remaining tergites dark brown with purple metallic tinges.

Antenna as in Fig. 59, i.e. scape widest just below middle. Frons with engraved and strong reticulation (Fig. 67); antennal scrobes reaching frontal suture wide apart; transverse ridge straight medially. Vertex inside ocellar triangle with engraved and very weak reticulation, outside ocellar triangle smooth and shiny (Fig. 69); anterior part with a row of seven long and proclinate setae. Occipital margin rounded.

Pronotal collar without transverse carina (Fig. 71). Mesoscutum with raised and strong reticulation (Fig. 71), meshes of reticulation smaller on sidelobes than on midlobe, midlobe with pits (i.e. with very strong reticulation) posteromedially; notauli as smooth impressions in posterior 2/3. Scutellum very weak reticulation and shiny, smooth along posterior margin, with 3–4 pits medially on either side of imaginary median longitudinal line (Fig. 71). Dorsellum flat and smooth, anterolaterally with two foveae. Propodeum smooth and shiny (Fig. 71); propodeal callus with three setae. Fore wing speculum closed below. Petiole conical without shoulders.

Ratios. HE/MS/WM = 2.3/1.0/1.3; POL/OOL/POO = 14.4/6.4/1.0; WH/WT = 1.4; LW/LM/HW = 1.5/1.0/1.0; PM/ST = 1.0; MM/LG = 1.0.

Etymology. Named after Dr. Chris T. Maier, Entomologist at the Connecticut Agricultural Experiment Station, who collected all material of the two new species described here.

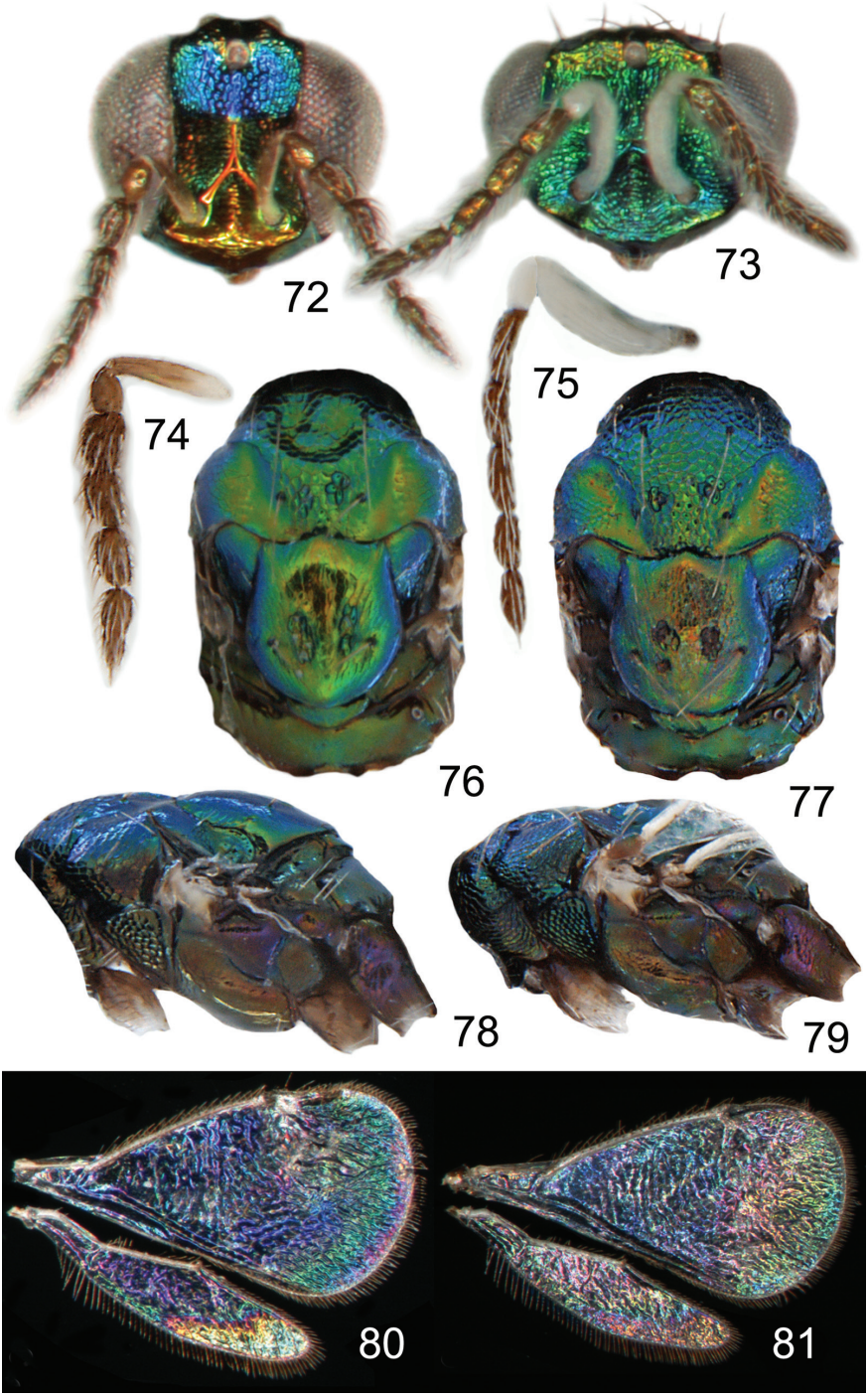
Distribution. U.S.A. (Connecticut).

Hosts. *Phyllonorycter propinquinella* (Braun) (Lepidoptera: Gracillariidae) on black cherry (*Prunus serotina*), and *Phyllonorycter nr crataegella* on pin cherry (*Prunus pensylvanica*).

Achrysocharoides serotinae sp.n.

urn:lsid:zoobank.org:act:C0EA95FF-793E-46BF-AF38-E300F345AB48
http://species-id.net/wiki/Achrysocharoides_serotinae
Figures 72–87

Material. HOLOTYPE male (CNC) glued to a card, labelled "U.S.A.: Connecticut, New Haven Co., North Haven, 30.ix.1981, Chris T. Maier", "Adult parasitoid lab-reared from tentiform mine of *Phyllonorycter propinquinella* collected on black cherry, *Prunus serotina* on 30.ix.1981". PARATYPES: 1 male with same label data as holotype (CNC); 2 females labeled "U.S.A.: Connecticut, Tolland Co., Union, 23.vi.1981, Chris T. Maier", "Adult parasitoid lab-reared from tentiform mine of *Phyllonorycter propinquinella* collected on black cherry, *Prunus serotina* on 23.vi.1981" (CNC).



Figures 72–81. *Achrysocharoides serotinae* sp. n.: **72** Head frontal, female **73** Ditto, male **74** Antenna lateral, female **75** Ditto, male **76** Mesosoma dorsal, female **77** Ditto, male **78** Mesosoma lateral, female **79** Ditto, male **80** Wing interference pattern (WIP), female **81** Ditto, male.

Diagnosis. Both sexes: fore wing WIP almost unicoloured, gradually changing hue from purple to green towards the margin, without any distinct details such as lines or spots (Figs 80, 81), fore coxa predominantly dark brown, hind coxa golden green (Figs 78, 79); male: scape with about same width throughout, with a single sparse row of setae along ventral margin, antennal scrobes joining on frontal suture (Fig. 73, 83), vertex with long forward pointing setae (Fig. 85) – setae at most as long as distance between posterior ocelli, upper frons without setae (Fig. 83); female: scape pale brown and widest medially, with a single row of setae along ventral margin (Fig. 74), propodeum smooth (Fig. 86), frons above frontal suture with raised and strong reticulation (Fig. 82).

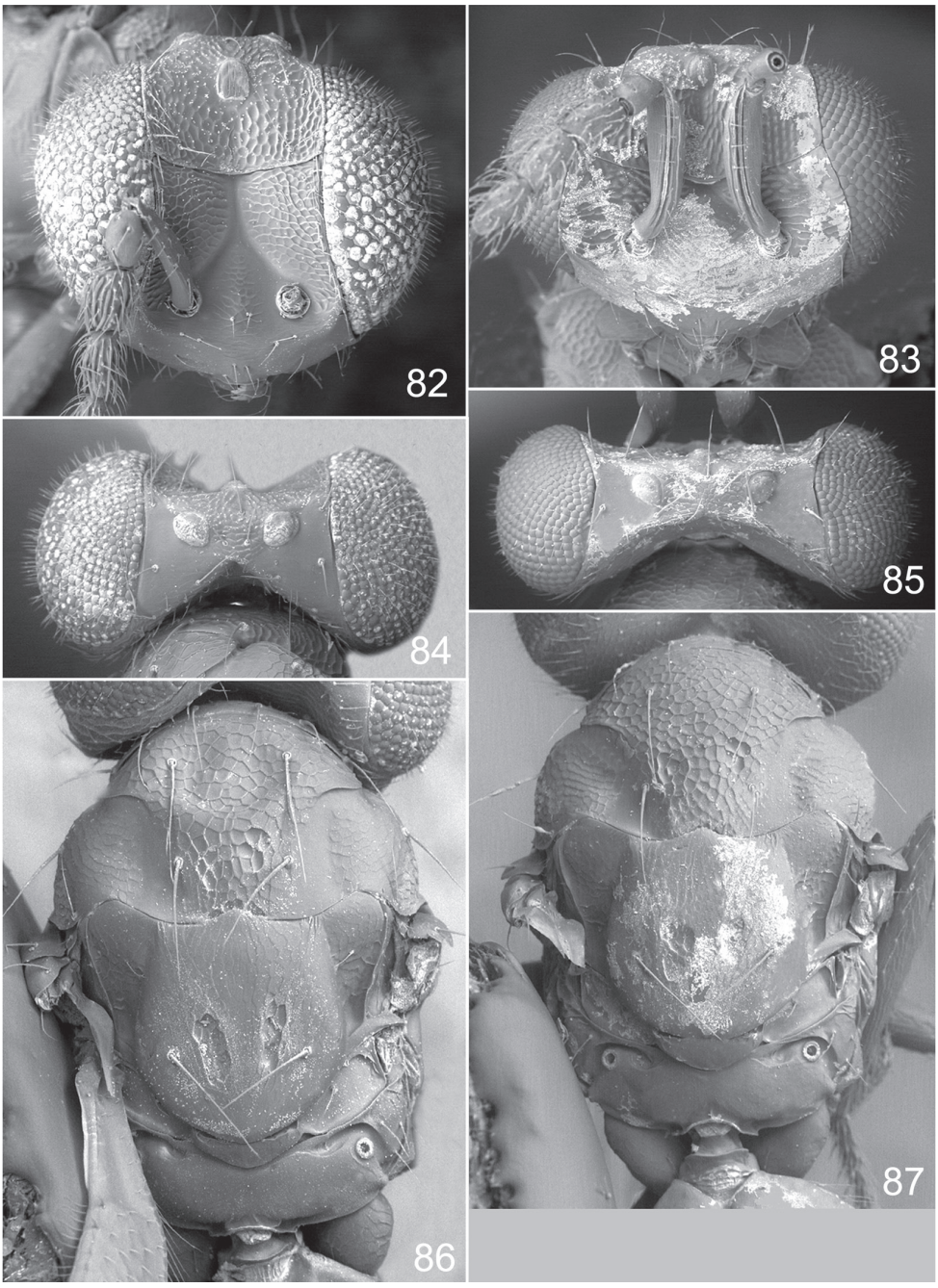
Description. *Female*. Length 1.2–1.3 mm. Scape and pedicel pale brown; flagellum dark brown (Fig. 74). Frons below frontal suture golden red, above frontal suture bluish green metallic (Fig. 72). Vertex inside ocellar triangle golden red, outside ocellar triangle golden green. Mesoscutum green metallic with blue metallic tinges, smooth parts of notaular depression golden green (Fig. 74). Scutellum golden green with sides and posterior margin bluish green metallic (Fig. 74). Propodeum golden green with blue metallic tinges (Fig. 74). Fore coxa dark brown with apical 1/3 white, mid coxa dark brown, hind coxa purple metallic (Fig. 78); femora, tibiae and tarsi on all legs white. Wings without pigmented areas; WIP in fore wing almost unicoloured, gradually changing hue from blue to green towards the margin when the membrane becomes gradually thinner (Fig. 80). Gaster with first two tergites golden green, remaining tergites golden purple with green metallic tinges.

Antenna as in Fig. 74. Frons below level of toruli smooth and shiny (Fig. 82), between level of toruli and frontal suture with raised and strong reticulation with antennal scrobes smooth, above frontal suture with raised and strong reticulation. Vertex inside ocellar triangle with engraved and weak reticulation, outside ocellar triangle smooth and shiny (Fig. 84). Occipital margin rounded.

Pronotal collar without transverse carina (Fig. 86). Mesoscutum with raised and strong reticulation (Fig. 86), meshes of reticulation smaller on sidelobes than on midlobe, midlobe with singular pits (i.e. with very strong reticulation) posteromedially; notauli as smooth impressions in posterior 2/3. Scutellum with very weak reticulation and shiny, smooth along posterior margin, with 2–4 pits medially on either side of imaginary median longitudinal line (Fig. 86). Dorsellum flat and smooth, anterolaterally with two foveae. Propodeum smooth and shiny (Fig. 86); propodeal callus with three setae. Fore wing speculum closed below. Petiole conical without shoulders.

Ratios. HE/MS/WM = 3.7/1.0/1.6; POL/OOL/POO = 1.7/1.0/1.0; WH/WT = 1.2; LW/LM/HW = 1.6/1.0/1.0; PM/ST = 1.0; MM/LG = 0.8–0.9.

Male. Length 1.4 mm. Scape and pedicel white; flagellum dark brown (Fig. 75). Frons green metallic (Fig. 73B). Vertex inside ocellar triangle golden red, outside ocellar triangle golden green. Mesoscutum golden green with anterior part blue (Fig. 77). Scutellum golden green with golden red tinges and with lateral parts blue (Fig. 77).



Figures 82–87. *Achrysocharoides serotinae* sp. n.: **82** Head frontal, female **83** Ditto, male **84** Vertex, female **85** Ditto, male **86** Mesosoma dorsal, female **87** Ditto, male.

Propodeum golden green with golden red tinges (Fig. 77). Fore coxa dark brown with apical 1/3 white, mid coxa dark brown, hind coxa purple metallic (Fig. 79); femora, tibiae and tarsi on all legs white. Wings without pigmented areas; WIP very similar to that of the female (Fig. 81). Gaster with tergites 1–2 dark brown with golden green tinges, remaining tergites dark brown with weak metallic tinges, over tergites 1–3 with a large median white spot.

Antenna as in Fig. 75, i.e. scape with about same width throughout. Frons with raised and strong reticulation, some parts with transverse striation (Fig. 83); antennal scrobes joining on frontal suture; transverse ridge evenly curved. Vertex inside ocellar triangle with engraved and very weak reticulation (Fig. 85), outside ocellar triangle smooth and shiny; anterior part with a row of 3–5 long and forward directed setae. Occipital margin rounded.

Pronotal collar without transverse carina (Fig. 87). Mesoscutum with raised and strong reticulation (Fig. 87), meshes of reticulation smaller on sidelobes than on midlobe, midlobe with pits (i.e. with very strong reticulation) posteromedially; notauli as smooth impressions in posterior 2/3. Scutellum with weak reticulation, smooth along posterior and lateral margins, with 2–5 pits medially on either side of imaginary median longitudinal line (Fig. 87). Dorsellum flat and smooth, anterolaterally with two foveae. Propodeum smooth and shiny (Fig. 87); propodeal callus with three setae. Fore wing speculum closed below. Petiole conical without shoulders.

Ratios. HE/MS/WM = 2.5/1.0/1.3; POL/OOL/POO = 4.6/1.8/1.0; WH/WT = 1.1; LW/LM/HW = 1.6/1.0/1.0; PM/ST = 1.0; MM/LG = 0.9.

Etymology. Named after black cherry (*Prunus serotina*), the host plant.

Distribution. U.S.A. (Connecticut).

Host. *Phyllonorycter propinquinella* (Braun) (Lepidoptera: Gracillariidae) on black cherry (*Prunus serotina*).

Identification of the new species

In the most recent key to North American *Achrysocharoides* by Kamijo (1991) the two newly described species both key out to the *clypeatus* group. To include them in the key to species of this group the following changes can be made:

Females of both species run to couplet 3, alternative 2 (where *A. arienascapus* falls out). The second alternative is changed to lead to 3a instead of *A. arienascapus* and then:

3a Fore coxa and scape predominantly brown (Figs 74, 78) ... ***A. serotinae* sp. n.**

– Fore coxa and scape white **3b**

3b Entire frons above frontal suture with raised and strong reticulation (Fig. 66); scutellum with very weak and superficial reticulation (Fig. 70) ... ***A. maieri* sp. n.**

– Frons strongly reticulate medially and weakly reticulate laterally; scutellum with strong reticulation ..... ***A. arienascapus* (Miller)**

Males run to couplet 4:

4 Frons above frontal suture with many short and scattered setae (see fig. 5 in Kamijo (1991)); scape with long dense setae ventrally (see fig. 5 in Kamijo (1991)) ..... ***A. hirtiscapus*** **(Miller)**

– Frons above frontal suture bare (Figs 67, 83); scape with a few short setae along ventral edge (Figs 59, 75) **5a**

5a Vertex with long setae about as long as distance between posterior ocelli (Figs 69, 85) **5b**

– Vertex with long setae at least as long as width of vertex (see fig. 7 in Kamijo (1991)) **5**

5b Scape widest close to base (Fig. 59); fore coxa white (Fig. 63) .... ***A. maieri*** **sp. n.**

– Scape with about same width throughout (Fig. 75); fore coxa predominantly brown (Fig. 79) ..... ***A. serotinae*** **sp. n.**

Acknowledgements

Our foremost thanks are due to ArtDatabanken who through the Swedish Taxonomy Initiative funds the PhD project of Ekaterina Shevtsova. Thanks also to Chris T. Maier (at CAES) who generously sent us his material of *Achrysocharoides*, and to Gary A.P. Gibson and John T. Huber (both at CNC) for the loan of material. The help of Andrew Polaszek (BMNH) with obtaining molecular data for *Achrysocharoides acerianus* and *A. platanoidae* is highly appreciated. We thank the microscopy unit at the Biology Department, Lund University, for the use of their SEM. We also thank two anonymous reviewers for their useful suggestions.

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Cytogenetics of the true bug infraorder Cimicomorpha (Hemiptera, Heteroptera): a review

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Abstract

The Cimicomorpha is one of the largest and highly diversified infraorders of the Heteroptera. This group is also highly diversified cytogenetically and demonstrates a number of unusual cytogenetic characters such as holokinetic chromosomes; m-chromosomes; multiple sex chromosome systems; post-reduction of sex chromosomes in meiosis; variation in the presence/absence of chiasmata in spermatogenesis; different types of achiasmate meiosis. We present here a review of essential cytogenetic characters of the Cimicomorpha and outline the chief objectives and goals of future investigations in the field.

Keywords

Hemiptera, Heteroptera, Cimicomorpha, holokinetic chromosomes, telomeres, NOR, chromosome number, m-chromosomes, sex chromosomes, B-chromosomes, meiosis

Introduction

The Heteroptera, or true bugs, are a diversified group of insects displaying a number of unusual and sometimes unique cytogenetic characters such as holokinetic chromosomes, m-chromosomes, multiple sex chromosome systems, sex chromosome post-reduction and occasionally pre-reduction in male meiosis, variation in the presence/absence of chiasmata in spermatogenesis, different types of achiasmate meiosis and oth-

ers. The pioneer investigators of true bug cytogenetics were Henking (1891), McClung (1902) and Wilson (1905, 1909). It should be noticed that Hermann Henking (1891) and his object, the firebug *Pyrrhocoris apterus* Linnaeus, 1758 (Pentatomomorpha: Pyrrhocoridae), deserve the credit for the discovery of a relation between chromosomes and sex determination in animals. Since that time chromosomal sex determination has become more and more widely accepted among biologists.

The cytogenetics of the Heteroptera has been firstly comprehensively reviewed by Ueshima (1979) and shortly afterwards by Manna (1984). Ueshima's (1979) superior monograph covers characteristics of all but one (Enicocephalomorpha, for which information is lacking to this day) heteropteran infraorders. However, the infraorders are cytogenetically unequally explored.

Since Ueshima's publication a large body of new cytogenetic data on the Heteroptera has been obtained, including those on the cimicomorphan families Tingidae (Nokkala and Nokkala 1984a, Grozeva and Nokkala 2001), Anthocoridae s.str. (Nokkala and Nokkala 1986a, Wang et al. 2003), Microphysidae (Nokkala and Grozeva 2000), Cimicidae (Grozeva and Nokkala 2002, Poggio et al. 2009, Grozeva et al. 2010, 2011), Reduviidae (Pérez et al. 2004, Severi-Aguiar et al. 2006, Poggio et al. 2007, 2011, Panzera et al. 2010, Bardella et al. 2010), Nabidae s.str. (Nokkala and Nokkala 1984b, Kuznetsova and Maryańska-Nadahowska 2000, Kuznetsova et al. 2004, 2007, Kuznetsova and Grozeva 2008, Angus et al. 2008), and Miridae (Nokkala 1986a, Nokkala and Nokkala 1986b, Grozeva et al. 2006, 2007, 2011, Grozeva and Simov 2008a, b, Grozeva and Simov 2009). At present, the families Miridae and Reduviidae are the most extensively studied (data are available for 196 species in 83 genera and for 148 species in 45 genera, respectively), whereas the families Anthocoridae s.str. (5 species, 3 genera), Polyctenidae (3 species, 2 genera), Microphysidae (2 species, 2 genera), and the monospecific family Joppeicidae, are the least studied. In the three remaining families, data are available for 53 species (20 genera) in Cimicidae; 29 species (7 genera) in Nabidae s.str.; and 28 species (17 genera) in Tingidae (Table 1). At present, no cytogenetic data are available for the families Pachynomidae, Vianaididae (often included in the Tingidae), Velocipedidae and Medocostidae (both sometimes included in the Nabidae s.l.), Thaumastocoridae (possibly partly belonging to the Pentatomomorpha), Plokiophilidae, and Lasiochilidae and Lyctocoridae (prior to Schuh and Štys (1991), classified within Anthocoridae s.l.).

The Cimicomorpha is one of the largest and highly diversified heteropteran infraorders. Although this group has attracted considerable interest for several reasons (disease transmission in the Triatominae, evolution of host-plant relationships in the Miridae, maternal care in the Tingidae and so on; Schuh et al. 2009), cimicomorphan higher-level relationships are complex both at the family and tribal levels and subjected to several recent analyses (Schuh and Štys 1991, Schuh 1995, Schuh et al. 2009). Cytogenetically considered, Cimicomorpha appear likewise sufficiently heterogeneous. The aim of the present paper is to synthesize main data available concerning cytogenetic characteristics of cimicomorphan true bugs and to gain a better insight into the

cytogenetic evolution within different families and the Cimicomorpha as a whole. A further aim is to outline the chief objectives and goals of future investigations in the field. The principle cytogenetic features of Cimicomorpha are summarized in Table 1 and in Figures 1 and 2.

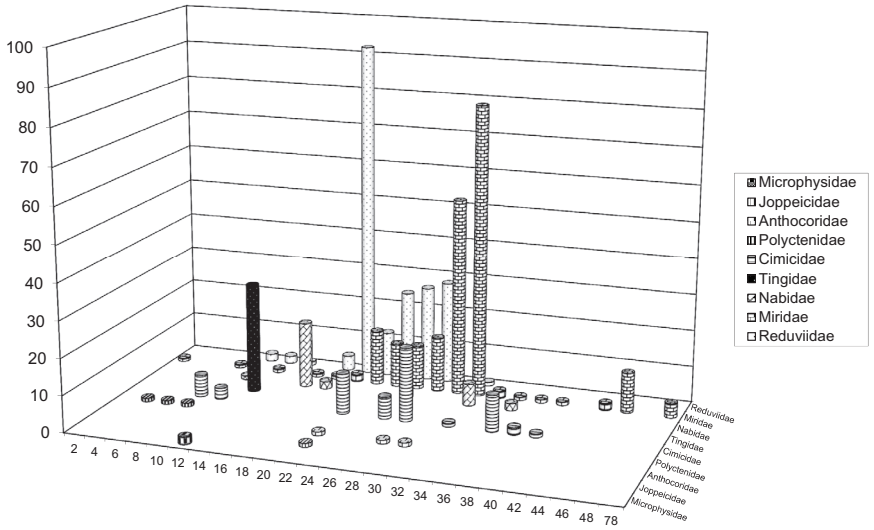

Figure 1. Autosome numbers' range in Cimicomorpha. X-axis denotes the diploid number of autosomes, Y-axis shows the number of species

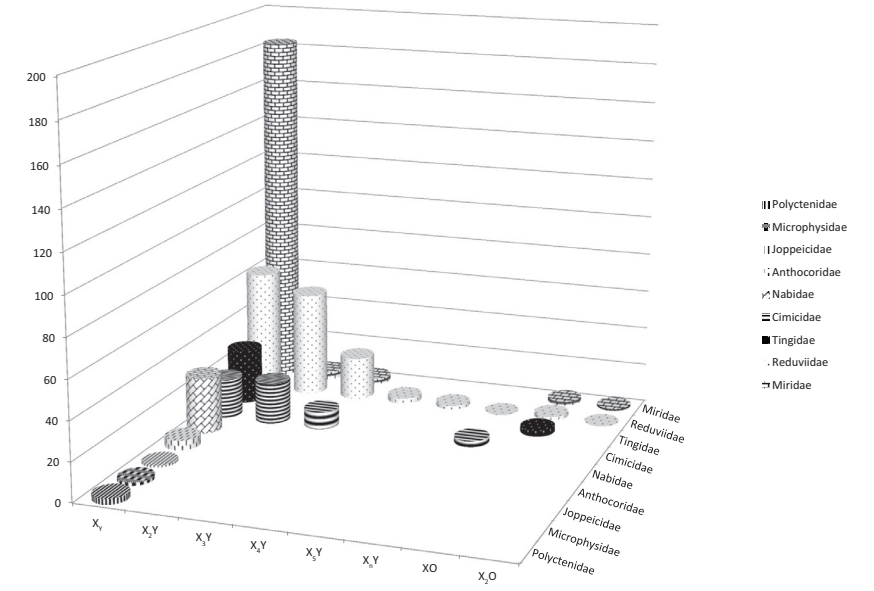

Figure 2. Distribution of sex chromosome systems in Cimicomorpha. Different sex chromosome systems are plotted on the X-axis. Y-axis shows the number of species. X_n - the number of X-chromosomes exceeds 5.

Table 1. Chromosome numbers and sex chromosome systems in Cimicomorpha (The systematics at superfamily and family level is after Schuh and Štys (1991); the systematics at subfamily level generally follows Maldonado (1990), Putshkov and Putshkov (1986–1989) and Weirauch (2008) for the Reduviidae; Schuh (1995) and Schuh (2011) for the Miridae; Kerzhner (1996) for the Nabidae s.str.; Schuh and Štys (1991) and Schuh and Slater (1995) for the rest of families)

Taxa			2n (number of species)	References
Family	**Subfamily**	**Genus (number of species studied)**		
Superfamily Reduvioidea				
Reduviidae Latreille, 1807 (45/148) (genera/species studied)	Bactrodinae Stål, 1866 (1/1)	*Bactrodes* Stål, 1860 (1)	24+XY	Ueshima 1979
	Ectrichodiinae Amyot and Serville, 1843 (1/2)	*Ectrychotes* Burmeister, 1835 (2)	28+X0	Manna 1951, Manna and Deb-Mallick 1981
	Emesinae Amyot and Serville, 1843 (3/3)	*Bagauda* Bergroth, 1903 (1)	32+XY	Ueshima 1979
		Barce Stål, 1866 (1)	18+XY	Ueshima 1963b
		Empicoris Wolf, 1881 (1)	14+XY	Ueshima 1963b
	Hammacerinae Stål, 1859 (1/2)	*Microtomus* Illiger, 1807 (2)	26+2m+XY	Piza 1957, Poggio et al. 2011
	Harpactorinae Amyot and Serville, 1843 (18/35)	*Acholla* Stål, 1862 (2)	20+$X_1X_2X_3X_4X_5$Y (1) n=16 (1)	Payne 1909, 1910, Troedsson 1944 Payne 1909, Montgomery 1901a
		Apiomeris Hahn, 1831 (5)	22+XY	Payne 1912, Ueshima 1979, Poggio et al. 2007
		Arilus Hahn, 1831 (1)	22+$X_1X_2X_3$Y	*A. cristatus* (Linnaeus, 1763) (Montgomery 1901a, Payne 1909, Troedsson 1944: as *Prionidus* Uhler, 1886)
		Cosmoclopius Stål, 1866 (2)	24+$X_1X_2X_3$Y	Poggio et al. 2007
		Cydnocoris Stål, 1866 (1)	24+X_1X_2Y	Dey and Wangdi 1988
		Coranaus Curtis, 1833 (1)	24+X_1X_2Y	Jande 1959a
		Fitchia Stål, 1859 (1)	24+X_1X_2Y	Payne 1909
		Harpactor Laporte, 1833 (2)	24+$X_1X_2X_3$Y	Manna 1951, Banerjee 1958, Jande 1959b

Taxa			2n (number of species)	References
Family	**Subfamily**	**Genus (number of species studied)**		
		Heniartes Spinola, 1840 (1)	22+XY	Ueshima 1979
		Lophocephala Laporte, 1833 (1)	24+X_1X_2Y	Satapathy and Patnaik 1989
		Polididus Stål, 1858 (2)	10+XY (1)	Manna and Deb-Mallick 1981
			10+XY	*P. armatissimus* Stål, 1859 (Toshioka 1936, Banerjee 1958)
			12+XY	*P. armatissimus* (Jande 1960)
		Pselliopus Bergroth, 1905 (1)	24+$X_1X_2X_3Y$	Payne 1912, Goldsmith 1916
		Rhynocoris Hahn, 1834 (3)	24+$X_1X_2X_3Y$	Manna 1951, Banerjee 1958, Jande 1959b, Dey and Wangdi 1988, Satapathy and Patnaik 1989
		Rocconota Stål, 1859 (1)	24+X_1X_2Y	Payne 1909
		Sinea Amyot and Serville, 1843 (6)	24+$X_1X_2X_3Y$ (5) 20+$X_1X_2X_3X_4X_5Y$ (1)	Montgomery 1901a*, Payne 1909, 1912, Troedsson 1944, Manna 1951
		Sycanus Amyot and Serville, 1843 (2)	24+$X_1X_2X_3Y$	Manna 1951, Jande 1959a
		Velinus Stål, 1865 (1)	24+$X_1X_2X_3Y$	Toshioka 1933, Yoshida 1947
		Zelus Fabricius, 1802 (2)	24+XY	*Zelus exsanguis* Stål, 1862 (Payne 1909: as *Diplacodus* Kirkaldy, 1900)
			24+XY	Poggio et al. 2007
	Peiratinae Stål, 1859 (4/6)	*Androclus* Stål, 1863 (1)	20+XY	Jande 1959b
		Ectomocoris Mayr, 1865 (3)	20+XY (1) 20+X_1X_2Y (2)	Jande 1959a
		Rasahus Amyot and Serville, 1843 (1)	20+X_1X_2Y	Ueshima 1979
		Sirthenea Spinola, 1837 (1)	26+XY	Jande 1959b

Taxa			2n (number of species)	References
Family	**Subfamily**	**Genus (number of species studied)**		
	Phymatinae Laporte, 1832 (2/2)	*Macrocephalus* Swederus, 1787 (1)	26+XY	Ueshima 1979
		Phymata Latreille, 1802 (1)	26+XY	Montgomery 1901a*
	Reduviinae (3/4)	*Pasiropsis* Reuter, 1881 (1)	$24+X_1X_2X_3X_4Y$	Jande 1959b
		Reduvius Fabricius, 1775 (2)	20+XY (1) 26+XY (1)	Payne 1912, Ueshima 1979 Ueshima 1979
		Staliastes Kirkaldi, 1900 (1)	24+XY	Jande 1959a
	Stenopodinae Amyot and Servielle, 1843 (4/11)	*Oncocephalus* Klug, 1830 (7)	$20+X_1X_2Y$ (4) $20+X_1X_2X_3Y$ (1) $22+X_1X_2X_3Y$ (2)	Jande 1959a, Ueshima 1979, Manna and Deb-Mallick 1981, Satapathy and Patnaik 1989
		Pnirontis Stål, 1859 (1)	$20+X_1X_2X_3X_4Y$	Payne 1912
		Pygolampis Germar, 1817 (2)	22+XY (1) $22+X_1X_2Y$ (1)	Banerjee 1958 Jande 1959a
		Stenopoda Laporte, 1833 (1)	$20+X_1X_2X_3X_4Y$	Poggio et al. 2007
	Triatominae Jeannel, 1919 (8/84)	*Belminus* Stäl, 1859 (2)	$20+X_1X_2Y$	Panzera et al. 2010
		Dipetalogaster Usinger, 1939 (1)	20+XY	Ueshima 1966a
		Eratyrus Stäl, 1859 (2)	$20+X_1X_2Y$	Panzera et al. 2010
		Mepraia Mazza, Gajardo and Jörg, 1940 (3)	$20+X_1X_2Y$	Pérez et al. 2004, Frìas-Lasserre 2010
		Panstrongylus Berg, 1879 (9)	$18+X_1X_2Y$ (1) $20+X_1X_2Y$ (7)	Ueshima 1966a, Schreiber et al. 1972, Panzera et al. 2010
			$18+X_1X_2Y$ (1)	*P. megistus* (Burmeister, 1835) (Schreiber and Pellegrino 1950, Barth 1956: as *Mestor* Kirkaldi, 1904)
		Paratriatoma Barber, 1938 (1)	20+XY (1)	Ueshima 1966a

Taxa			2n (number of species)	References
Family	**Subfamily**	**Genus (number of species studied)**		
		Rhodnius Stål, 1859 (15)	20+XY	*R. coreodes* (Bergroth 1911) (Schreiber and Pellegrino 1950: *as Psammolestes* Bergroth, 1911), Barth 1956, Ueshima 1966a, Petitpierre 1996, Panzera et al. 2010
		Triatoma Laporte, 1832 (51)	18+X_1X_2Y (1) 20+XY (25) 20+X_1X_2Y (21) 20+$X_1X_2X_3Y$ (2)	Schreiber and Pellegrino 1950, Barth 1956, Ueshima 1966a, Bargues et al. 2006; Panzera et al. 1995, 2004, 2006, 2010
			20+X_1X_2Y	*T. sanguisuga* (LeConte, 1855) (Payne 1909, Panzera et al. 2010: as *Conorhinus* Laporte, 1832)
			22+X_1X_2Y	*T. rubrofasciatus* (De Geer, 1773) (Manna 1950, 1951, Panzera et al. 2010: as *Conorhinus*)
Superfamily Microphysoidea				
Microphysidae Dohrn, 1859 (2/2)		*Myrmedobia* Bärensprung, 1857 (1)	12+XY	Nokkala and Grozeva 2000
		Loricula Curtis, 1833 (1)	12+XY	*L. pselapfiformis* Curtis, 1833 (Grozeva, unpublished)
Superfamily Joppeicoidea				
Joppeicidae Reuter, 1910 (1/1)		*Joppeicus* Putton, 1881 (1)	22+XY	Ueshima 1979
Superfamily Miroidea				
Miridae Hahn, 1833 (83/196)	Bryocorinae Baerensprung, 1860 (9/27)	*Bryocoropsis* Schumacher, 1917 (1)	32+XY	Kumar 1971
		Bryocoris Fallén, 1829 (1)	32+XY	Grozeva and Simov 2008b

Taxa			2n (number of species)	References
Family	**Subfamily**	**Genus (number of species studied)**		
		Campyloneura Fieber, 1858 (1)	32+XX (♀♀)	Grozeva and Simov 2008b
		Dicyphus Fieber, 1858 (15)	36+XY (1) 40+XY (1) 44+XY(1) 44+X_1X_2Y (1) 46+XY (8) 46+2m+$X_1X_2X_3Y$ (1) 46+X_1X_2Y (1) 46+$X_1X_2X_3Y$ (1)	Slack 1938b*, Leston 1957, Grozeva 2003, Grozeva and Simov 2008b
		Distantiella China, 1944 (1)	26+XY	Kumar 1971
		Helopeltis Signoret, 1858 (2)	16+XY (1) 18+XY (1)	Kumar 1971
		Macrolophus Fieber, 1858 (3)	24+XY (1) 24+$X_1X_2X_3Y$ (1) 26+XY (1)	Grozeva et al. 2006, 2007
		Molanocoris Dahlbom, 1851(2)	32+XY	Slack 1938b, Leston 1957, Kumar 1971, Akingbohungbe 1974*, Grozeva and Simov 2008b
		Sahlbergella Haglund, 1895 (1)	26+XY	Kumar 1971
	Deraeocorinae Douglas and Scott, 1865 (2/17)	*Deraeocoris* Kirschbaum, 1856 (15)	32+XY (11) 34+XY (1) 30+2m+XY (2)	Southwood and Leston 1959*, Takenouchi and Muramoto 1968, Akingbohungbe 1974*, Grozeva et al. 2011
			32+XY	*D. lutescens* Schilling, 1837 (Leston 1957: as *Camptobrochis* (Schilling, 1837))
		Hyaliodes Reuter, 1876 (2)	32+XY (1) 34+XY (1)	Akingbohungbe 1974*
	Mirinae Hahn, 1833 (34/73)	*Adelphocoris* Reuter, 1896 (7)	20+XY (1) 22+XY (2) 24+XY (1) 26+XY (1)	Takenouchi and Muramoto 1967, 1972a, b, Muramoto 1973a, Akingbohungbe 1974*

Taxa			2n (number of species)	References
Family	**Subfamily**	**Genus (number of species studied)**		
			12+2m+XO	*A. lineolatus (Goeze, 1778) (Schachow 1932*)*
			30+XY	*A. lineolatus* (Ekblom 1941: as /Calocoris chenopodii /Westhoff, 1881, Leston 1957, Akingbohungbe 1974*)
			2n=28	*A. rapidus* (Say, 1832) (Akingbohungbe 1974*: as *Calocoris rapidus* Say, 1832)
			$26+X_1X_20$	*A. rapidus* (Mongomery 1901a, 1906: as *Calocoris rapidus*)
		Apolygus China, 1941 (3)	32+XY	Leston 1957, Muramoto 1973a
		Camptozygum Reuter, 1896 (1)	32+XY	Southwood and Leston 1959*
		Capsus Fabricius, 1803 (1)	32+XY	*C. ater* (Linnaeus, 1758) (Slack 1938b*, Akingbohungbe 1974*)
			30+2m+XY	*C. ater* (Nokkala and Nokkala 1986b)
		Charagochilus Fieber, 1858 (1)	32+XY	Muramoto 1973a
		Closterotomus Fieber, 1858 (2)	32+XY (1)	*C. fulvomaculatus* De Geer, 1773 (Slack 1938b*: as *Calocoris* Fieber, 1858)
			30+XY	*C. norvegicus* Gmelin, 1790 Slack 1938b*: as *Calocoris*)
			32+XY	*C. norvegicus* (Leston 1957: as *Calocoris*)
		Collaria Provancher, 1872 (1)	12+XY	Akingbohungbe 1974*
		Creontiades Distant, 1883 (1)	30+XY	Takenouchi and Muramoto 1970
		Cyphodemidea Reuter, 1903 (1)	32+XY	*C. saundersi* Reuter, 1896 (Takenouchi and Muramoto 1967: as *Orthops*)
		Dichrooscytus Fieber, 1858 (2)	32+XY (1)	Akingbohungbe 1974*
			28+2m+XY (1)	Grozeva 2003
		Eurystylus Stål, 1871 (1)	30+XY	Takenouchi and Muramoto 1968
		Garganus Stål, 1862 (1)	32+XY	Akingbohungbe 1974*

Taxa			2n (number of species)	References
Family	Subfamily	Genus (number of species studied)		
		Grypocoris Douglas and Scott, 1868 (1)	30+XY	*G. sexguttatus* (Fabricius, 1777) (Slack 1938b*: as *Calocoris*)
		Horcias Distant, 1884 (1)	32+XY	Montgomery 1901a, 1906, Akingbohungbe 1974*
		Leptopterna Fieber, 1858 (1)	32+XY	Montgomery 1901a, Slack 1938b*, Leston 1957, Akingbohungbe 1974*
		Liocoris Fieber, 1858 (1)	32+XY	*L. tripustulatus* (Fabricius, 1781) (Leston 1957: as *Lygus*)
		Litomiris Slater, 1956 (1)	32+XY	Akingbohungbe 1974*
		Lygocoris Reuter, 1875 (11)	32+XY (10)	Geitler 1939*, Leston 1957, Southwood and Leston 1959*, Muramoto 1973a, Akingbohungbe 1974*
			32+XY	*L. rubripes* Jakovlev, 1876 (Takenouchi and Muramoto 1970: as *Adelphocoris*)
		Lygus Hahn, 1833 (5)	32+XY (4)	Leston 1957, Southwood and Leston 1959*, Akingbohungbe 1974*, Ueshima 1979
			n=19?	*L. pratensis* (Linnaeus, 1758) (Montgomery 1901a, 1906)
			30+2m+X0	*L. pratensis* (Schachow 1932*)
			32+XY	*L. pratensis* (Geitler 1939*)
		Megacoelum Fieber, 1858 (1)	30+XY	Leston 1957
		Megaloceroea Fieber, 1858 (1)	32+XY	*M. recticornis* (Geoffroy, 1785) (Leston 1957)
			30+XY	*M. recticornis* (Grozeva et al. 2011)
		Neurocolpus Reuter, 1876 (3)	32+XY	Akingbohungbe 1974*
		Notostira Fieber, 1858 (1)	12+2m+X0	Schachow 1932
		Onomaus Distant, 1904 (1)	30+XY	Takenouchi and Muramoto 1968
		Orthops Fieber, 1858 (1)	22+XY	*O. campestris* (Linnaeus, 1758) (Muramoto 1973a)

Taxa			2n (number of species)	References
Family	**Subfamily**	**Genus (number of species studied)**		
			32+XY	*O. campestris* (Akingbohungbe 1974*)
		Phytocoris Fallén, 1814 (9)	32+XY (8) 30+XY (1)	Slack 1938b, Leston 1957, Southwood and Leston 1959*, Akingbohungbe 1974*
		Poecilocapsus Reuter, 1876 (1)	32+XY	Montgomery 1901a, Akingbohungbe 1974*
		Polymerus Hahn, 1831 (3)	32+XY	Leston 1957, Akingbohungbe 1974*
		Rhabdomiris Wagner, 1968	30+XY	Nokkala and Nokkala 1986b: as *Calocoris quadripunctatus* (Villers, 1789)
		Stenodema Laporte, 1833 (2)	30+XY (1) 32+XY (1)	Leston 1957 Slack 1938b*, Leston 1957
		Stenotus Jakovlev, 1877 (2)	40+XY (1)	Takenouchi and Muramoto 1967
			30+XY	*S. binotatus* (Fabricius 1794) (Slack 1938b*, Takenouchi and Muramoto 1967)
			32+XY	*S. binotatus* (Leston 1957)
			2n=32-34	*S. binotatus* (Akingbohungbe 1974*)
		Taedia Distant, 1883 (1)	32+XY	Akingbohungbe 1974*
		Trigonotylus Fieber, 1858 (2)	32+XY	Takenouchi and Muramoto 1967, Akingbohungbe 1974*
		Tropidosteptes Uhler, 1878 (1)	32+XY	Akingbohungbe 1974*
	Orthotylinae Van Duzee, 1916 (16/33)	*Brepharidopterus* Kolenati, 1845 (1)	22+XY	Leston 1957
		Ceratocapsus Reuter, 1876 (4)	18+XY (1) 22+XY (3)	Akingbohungbe 1974*
		Cyllecoris Hahn, 1834 (1)	21+ X_1X_2Y	Southwood and Leston 1959*
		Dryophilocoris Reuter, 1875 (2)	32+XY (1)	Grozeva and Simov 2011
			32+XY	*D. flavoquadrimaculatus* (De Geer, 1773) (Grozeva and Simov 2011)

Taxa			2n (number of species)	References
Family	**Subfamily**	**Genus (number of species studied)**		
			34+X_1X_2Y	*D. flavoquadrimaculatus* (Leston 1957)
		Halticus Hahn, 1832 (1)	28+XY	Akingbohungbe 1974*
		Heterotoma Lepeletier and Serville, 1825 (1)	32+XY	Southwood and Leston 1959*
		Ilnacora Reuter, 1876 (2)	24+XY	Akingbohungbe 1974*
		Labops Burmeister, 1835 (1)	38+XY	Akingbohungbe 1974*
		Lopidea Uhler, 1872 (4)	78+XY	Akingbohungbe 1974*
		Malacocoris Fieber, 1858 (1)	20+XY (1)	Leston 1957
		Orthocephalus Fieber, 1858 (2)	26+XY	Takenouchi and Muramoto 1967
			28+XY	Takenouchi and Muramoto 1972b, Muramoto 1973b, 1974
		Orthotylus Fieber, 1858 (7)	22+XY (2) 24+XY (1) 26+XY (2) 28+XY (1)	Slack 1938b*, Leston 1957, Southwood and Leston 1959*, Akingbohungbe 1974*
			24+XY	*O. flavosparsus* (C Sahlberg, 1841) (Leston 1957)
			26+XY	*O. flavosparsus* (Akingbohungbe 1974*)
		Parthenicus Reuter, 1876 (1)	22+XY	Akingbohungbe 1974*
		Pseudoxenetus Reuter, 1909 (1)	28+XY	Akingbohungbe 1974*
		Reuteria Puton, 1875 (1)	24+XY	Akingbohungbe 1974*
		Slaterocoris Wagner, 1956 (3)	24+XY	Akingbohungbe 1974*
	Phylinae Douglas and Scott, 1865 (22/46)	*Amblytylus* Fieber, 1858 (1)	30+XY	Southwood and Leston 1959*, Akingbohungbe 1974*
		Atractotomus Fieber, 1858 (1)	30+XY	Southwood and Leston 1959*
		Campylomma Reuter, 1878 (1)	30+XY	Akingbohungbe 1974*
		Chlamydatus Curtis, 1833 (4)	30+XY	Slack 1938b*, Leston 1957, Southwood and Leston 1959*, Akingbohungbe 1974*

Taxa			2n (number of species)	References
Family	**Subfamily**	**Genus (number of species studied)**		
		Compsidolon Reuter, 1899 (1)	30+XY	*C. beckeri* Reuter, 1904 (Akingbohungbe 1974*: as *Psallus*)
		Conostethus Fieber, 1858 (1)	30+XY	Slack 1938b*
		Cremnocephalus Fieber, 1860 (2)	26+XY (1) 28+XY (1)	Grozeva and Simov 2008a
		Criocoris Fieber, 1858 (1)	30+XY	Akingbohungbe 1974*
		Europiella Reuter, 1909 (1)	30+XY	*E. albipennis* (Fallén, 1829) (Leston 1957: as *Plagyognathus*)
		Harpocera Curtis, 1838 (1)	32+XY	Grozeva 2003
		Hallodapus Fieber, 1858 (2)	24+XY (1) 2n=4 (1)**	Muramoto 1973a Cobben 1986
		Lopus Hahn, 1831(1)	30+XY	Slack 1938b*
		Macrotylus Fieber, 1858 (2)	32+XY	Geitler 1939*, Southwood and Leston 1959*
		Megalocoleus Reuter, 1890 (2)	30+XY	Leston 1957
		Oncotylus Fieber, 1858 (1)	30+XY	Leston 1957
		Orectoderus Uhler, 1890 (1)	32+XY	Akingbohungbe 1974*
		Phoenicocoris Reuter, 1875 (1)	30+XY	*P. rostratus* Knight, 1923 (Akingbohungbe 1974*: as *Lepidopsallus* Knight, 1923)
		Phylus Hahn, 1831(1)	30+X0	Leston 1957
		Pilophorus Hahn, 1826 (4)	26+XY (1) 28+XY (3)	Muramoto 1973a, Akingbohungbe 1974*
		Plagiognathus Fieber, 1858 (9)	30+X0 (1) 30+XY (4) 32+XY (1)	Slack 1938b*, Leston 1957, Takenouchi and Muramoto 1967, 1968
			32+XY (1)	*P. longirostris* (Knight, 1923) (Akingbohungbe 1974*: as *Microphylellus* Reuter, 1909)
			28+XY	*P. chrysanthemi* (Wolf, 1804) (Slack 1938b*, Akingbohungbe 1974*)

Taxa			2n (number of species)	References
Family	**Subfamily**	**Genus (number of species studied)**		
			30+XY	*P. chrysanthemi* (Leston, 1957)
			32+XY	*P. arbustorum* (Fabricius, 1794) (Slack 1938b*)
			30+XY	*P. arbustorum* (Leston 1957)
		Psallus Fieber, 1858 (7)	28+XY (3) 30+XY (4)	Slack 1938b*, Southwood and Leston 1959*, Takenouchi and Muramoto 1968, Muramoto 1973a
		Systellonotus Fieber, 1958 (1)	2n=8**	Cobben 1986
Tingidae Laporte, 1832 (17/28)	Tinginae Laporte, 1832 (17/28)	*Acalypta* Westwood, 1840 (3)	12+X0 (2)	Grozeva and Nokkala 2001
			10+XY	*A. parvula* (Fallén, 1807) (Southwood and Leston 1959*)
			12+X0	*A. parvula* (Grozeva and Nokkala 2001)
		Agramma Stephens, 1829 (1)	12+XY	Muramoto 1973c
		Cochlochila Stål, 1873 (1)	12+XY	Takenouchi and Muramoto 1967
		Copium Thunberg, 1822 (1)	12+XY	Grozeva and Nokkala 2001
		Corythucha Stål, 1873 (1)	12+XY	Grozeva and Nokkala 2001
		Cysteochila Stål, 1873 (1)	12+XY	Jande 1960 (as *Bredenbachius* Distant, 1903)
		Dasytingis Drake and Poor, 1936 (1)	12+XY	*D. bengalana* Drake, 1956 (Jande 1960: as *Tingis* Fabricius, 1803)
		Dictyla Stål, 1874 (2)	12+XY	*D. humuli* (Fabricius, 1794) (Southwood and Leston 1959*: as *Monanthia* Lepeletier and Serville, 1828), Grozeva and Nokkala 2001
		Dictyonota Curtis, 1827 (1)	12+XY	Southwood and Leston 1959*
		Elasmotropis Stål, 1874 (1)	12+XY	Grozeva and Nokkala 2001*
		Kalama Puton, 1876 (1)	12+XY	Grozeva and Nokkala 2001
		Lasiacantha Stål, 1873 (1)	12+XY	Grozeva and Nokkala 2001
		Leptobyrsa Stål, 1873 (1)	12+XY	Harley and Kassulke 1971
		Physatocheila Fieber, 1844 (1)	12+XY	Grozeva and Nokkala 2001

Taxa			2n (number of species)	References
Family	**Subfamily**	**Genus (number of species studied)**		
		Stephanitis Stål, 1873 (3)	12+XY	*S. takeyai* Drake and Maa, 1955 (Toshioka 1934, Jande 1960: as *Monathia globulifera* Matsumura, 1905), Grozeva and Nokkala 2001
		Teleonemia Costa, 1864 (2)	12+XY	Harley and Kassulke 1971
		Tingis Fabricius, 1803 (6)	12+XY	Montgomery 1901a, 1906, Southwood and Leston 1959*, Muramoto 1973c*, Grozeva and Nokkala 2001
Superfamily Naboidea				
Nabidae Costa A, 1853 (7/29)	Nabinae (5/27)	*Arachnocoris* Scott, 1881 (1)	10+XY	Kuznetsova et al. 2007, Kuznetsova and Grozeva 2008
		Himacerus s. str. Wolff, 1811 (1)	36+XY	*H. apterus* (Fabricius, 1798) (Yoshida 1950, Kuznetsova et al. 2004, Angus et al. 2008)
			16+XY	*H. apterus* (De Meijere 1930*, Leston 1957)
			38+XY	*H. apterus* (Takenouchi and Muramoto 1968)
		H. (*Aptus* Hahn, 1831) (2)	32-36+XY (1)	*H. maracandicus* (Reuter, 1890) (Kuznetsova and Maryańska-Nadachowska 2000)
			32+XY (1)	*H. mirmicoides* (O. Costa, 1834) (Leston 1957 Kuznetsova et al. 2004, Angus et al. 2008)
		H. (*Stalia*) Reiter, 1872 (1)	30+XY	*H. major* (A. Costa, 1841) (Angus et al. 2008: as *Stalia* Reuter, 1872)
		Hoplistoscelis Reuter, 1890 (1)	16+XY	Kuznetsova and Maryańska-Nadachowska 2000
		Lasiomerus Reuter, 1890 (1)	16+XY	Montgomery 1901b
		Nabis s.l. Latreille, 1802 (20)		
		Nabis s.str. (8)	16+XY (6)	Schachow 1932, Mikolajski 1964, 1965, 1967, Takenouchi and Muramoto 1967, 1968, 1969, Muramoto 1978, 1979, Nokkala and Nokkala 1984b, Kuznetsova and Maryańska-Nadachowska 2000, Grozeva and Nokkala 2003

Taxa			2n (number of species)	References
Family	Subfamily	Genus (number of species studied)		
			16+XY	*Nabis ericetorum* Scholtz, 1947 (Mikolajski 1965, 1967) *Nabis rugosus* (Linnaeus, 1758) (Schachow 1932, Leston 1957, Mikolajski 1965, 1967, Kuznetsova and Maryańska-Nadachowska 2000)
			18+XY	*Nabis ericetorum* (Leston 1957) *Nabis rugosus* (Leston 1957)
		N. (*Aspilaspis*) Stål, 1873 (3)	32+XY	Montgomery 1901a, Schachow 1932, Leston 1957, Mikolajski 1964, 1965, 1967, Takenouchi and Muramoto 1967, 1968, 1969, Muramoto 1979, Nokkala and Nokkala 1984b, Kuznetsova and Maryańska-Nadachowska 2000, Kuznetsova et al. 2004
		N. (*Dolichonabis*) Reuter, 1908 (2)	16+XY	
		N. (*Halonabis*) Reuter, 1890 (1)	32+XY	
		N. (*Limnonabis*) Kerzhner, 1968 (1)	16+XY	
		N. (*Milu*) Kirkaldy, 1907 (1)	16+XY	
		N. (*Nabicula*) Kirby, 1837 (2)	16+XY	
		N. (*Reduviolus*) Kirby, 1837 (1)	16+XY	
		N. (*Tropiconabis*) Kerzhner, 1968 (1)	16+XY	
	Prostemmatinae Reuter, 1890 (2/2)	*Pagasa* Stål, 1862 (1)	26+XY	Kuznetsova and Maryańska-Nadachowska 2000
		Prostemma Laporte, 1832 (1)	26+XY	Kuznetsova et al. 2004

Taxa			2n (number of species)	References
Family	**Subfamily**	**Genus (number of species studied)**		
Superfamily Cimicoidea				
Anthocoridae Fieber, 1836 (3/5)	Anthocorinae s.str. Fieber, 1836 (2/4)	*Anthocoris* Fallén, 1814 (3)	28+XY	Southwood and Leston 1959*, Nokkala and Nokkala 1984b
		Orius Wolff, 1811 (1)	22+XY	Takenouchi and Muramoto 1971
	Xylocorinae Reuter, 1884 (1/1)	*Amphiareus* Distant, 1904 (1)	30+XY	Takenouchi and Muramoto 1971
Cimicidae Latreille, 1802 (20/53)	Afrocimicinae Usinger, 1966 (1/1)	*Afrocimex* Schoutedon, 1951 (1)	22+X_1X_2Y	Ueshima 1966b
	Cacodminae Kirkaldy, 1899 (6/9)	*Aphrania* Ferris and Usinger, 1957 (1)	8+XY	Ueshima 1966b
		Cacodmus Stål, 1873 (2)	8+XY 10+XY	Ueshima 1966b Ueshima 1979
		Crassicimex Ferris and Usinger, 1957 (1)	36+X_1X_2Y	Ueshima 1966b
		Leptocimex Roubaud, 1913 (2)	22+XY	Ueshima 1966b, 1979
		Loxaspis Rothschild, 1912 (1)	8+XY	Ueshima 1966b
		Stricticimex Ferris and Usinger, 1957 (2)	22+XY (1) 36+X_1X_2Y (1)	Ueshima 1966b Ueshima 1979
	Cimicinae Latreille, 1802 (3/29)	*Cimex* Linnaeus, 1758 (19)	22+XY (1) 24+XY (1) 28+XY (1) 26+X_1X_2Y (3) 28+X_1X_2Y (6) 28+$X_1X_2X_3Y$ (6) 28+$X_1X_2X_3X_4Y$ (1)	Slack 1938a, 1939a,b, Darlington 1939*, Ueshima 1963a, 1966b, 1968a, Grozeva and Nokkala 2002, Grozeva et al. 2010
		Oeciacus Stål, 1873 (2)	28+X_1X_2Y	Ueshima 1966b
		Paracimex Kiritschenko, 1914 (8)	36+X_1X_2Y (4) 36+$X_1X_2X_3Y$ (2) 36+4-9XY (2)	Ueshima 1966b, 1968b

Taxa			2n (number of species)	References
Family	**Subfamily**	**Genus (number of species studied)**		
	Haematosiphoninae Jordan and Rothschild, 1912 (6/9)	*Acanthocrios* Del Ponte and Riesel, 1945 (1)	32+XY	*A. furnarii* (Cordero and Vogelsang, 1928) (Ueshima 1966b: as *Caminicimex* Usinger, 1966)
			10+XY	*A. furnarii* (Poggio et al. 2009)
		Haematosiphon Champion, 1900 (1)	$28+X_1X_2Y$	Ueshima 1966b
		Hesperocimex List, 1925 (3)	$38+X_1X_2X_3Y$ (1) 38+XY (1) 40+XY (1)	Ryckman and Ueshima 1964
		Ornithocoris Pinto, 1927 (2)	8+XY	Ueshima 1966b
		Psitticimex Usinger, 1966 (1)	$28+X_1X_2Y$	Ueshima 1966b, Poggio et al. 2009
		Synxenoderus List, 1925 (1)	$28+X_1X_2Y$	Ueshima 1966b
	Latrocimicinae Usinger, 1966 (2/3)	*Latrocimex* Lent, 1941(1)	22+XY	Ueshima 1966b
		Leptocimex Roubaud, 1913 (2)	22+XY	Ueshima 1966b, 1979
	Primicimicinae Ferris and Usinger, 1955 (2/2)	*Bucimex* Usinger, 1963 (1)	26+XY	Ueshima 1966b
		Primicimex Barber, 1941 (1)	28+XY	Ueshima 1968a
Polyctenidae Westwood, 1874 (2/3)	Hesperocteninae Maa, 1964 (1/2)	*Hesperoctenes* Kirkaldi, 1906 (2)	4+XY (1) 10+XY (1)	Ueshima 1979
	Polycteninae Westwood, 1874 (1/1)	*Eoctenes* Kirkaldy, 1906 (1)	6+XY	Ueshima 1979

* In the paper, only the number of chromosomes (2n/n) is provided, then, the karyotype formula for the species is deduced here from 2n/n,

** But see the text

Holokinetic chromosomes and mechanisms of their evolution

Holokinetic chromosomes (sometimes designated as holocentric) occur in certain scattered groups of plants and animals, being particularly widespread in insects, including the Heteroptera (Kuznetsova et al. 2002, Lukhtanov and Kuznetsova 2009). These chromosomes have no primary constriction, the centromere, which is considered non-localized, or diffuse, formed by a large kinetochore plate extending all or most of the length of a chromosome (Schrader 1947, Wolf 1996).

Despite an important role of chromosomal change in the evolution and diversification of many groups of organisms (White 1978, King 1993, Coyne and Orr 2004, Ayala and Coluzzi 2005), the mechanisms behind this process are still little known, and this is especially true for groups with holokinetic chromosomes. Theoretically, the large kinetochore plate facilities karyotype evolution via occasional fusion/fission events. First, fused holokinetic chromosomes can not give rise to dicentric chromosomes. Second, any chromosome fragment exhibits a part of the kinetochore plate and can attach to spindle fibers at cell divisions. As a result, chromosome fragments that would be acentric (lacking a centromere) and hence lost in organisms with monocentric chromosomes (with localized centromeres) may be inherited in Mendelian fashion in holokinetic organisms, and gametes harbouring chromosome fragments are consequently expected to be viable (Hipp et al. 2010). Fusion/fission rearrangements are therefore conventionally accepted as the commonest mechanisms of chromosomal evolution in holokinetic groups. This assumption seems to receive support from the fact that the greatest range of within-genus karyotype variation related to the fusion/fission rearrangements is just described in organisms with holokinetic chromosomes. In metazoan animals, these are the blue butterfly genus *Agrodiaetus* Übner, 1822 and the gall inducing coccomorphan genus *Apiomorpha* Rübsaamen, 1894 in which diploid chromosome number ranges from 20 to 268 (Lukhtanov et al. 2005) and from 4 to ca. 192 (Cook 2000), respectively, whereas in plants – the angiosperm genus *Carex* Linnaeus, 1753 and the grass genus *Bromus* Linnaeus, 1753 in which it varies from 12 to 132 (Hipp 2007) and from 14 to 105 (Joachimiak et al. 2001), respectively. Although variations in chromosome number of related species are probably due to both fissions and fusions of holokinetic chromosomes, fusions are suggested to be more common. The point is that a chromosome, be it holokinetic or monocentric, has to display two functional telomeres in order to survive a mitotic cycle. The fusion chromosome always displays functional telomeres originated from the ancestral chromosomes, whereas a fission chromosome has to be able to develop a functional telomere *de novo* (Nokkala et al. 2007).

Chromosome numbers and modes of their transformation in Cimicomorpha

Chromosome numbers have been published for approximately 465 species (180 genera) of cimicomorphan true bugs, including many of the higher taxonomic cat-

egories within the infraorder (Table 1). In these species chromosome numbers range from 2n=6 in *Hesperoctenes fumarius* (Westwood, 1874) from the family Polyctenidae (Ueshima 1979) to 2n=80 in four species of the genus *Lopidea* Uhler, 1872 from the family Miridae (Akingbohungbe 1974). Cobben (1986) claimed to have found 2n=4 in *Hallodapus albofasciatus* (Motschulsky, 1863), Miridae, but this interpretation is not necessarily correct (this refers equally *Systellonotus alpinus* Frey-Gessner, 1871, 2n=8, in the same paper), since bivalents in the figures provided are clearly organized in a chain giving the false impression that the number of chromosomes is less than it is in reality (similarly as observed in *Piesma kochiae* (Becker, 1867); Grozeva 1991). It is worth noting that this range in chromosome number is larger than that reported for any other true bug infraorder, the number of 80 representing the highest one currently known in the Heteroptera as a whole. A number of cimicomorphan families demonstrate a considerably wide range of diploid chromosome numbers, the widest being in the families Miridae (from 14 to 80) and Cimicidae (from 10 to 50) (Table 1). These facts seemingly reinforce the fusion/fission hypothesis. However quite many cimicomorphan taxa show apparent karyotype conservation, with all or almost all species sharing the same chromosome number. This suggests that chromosomal fusions/fissions have played a minor role in the karyotype evolution and species diversification within these groups. By far the best example is the lace bug family Tingidae where all of 28 species studied (from 17 genera) have 12 autosomes in diploid complements differing only in sex chromosome system, which is XY or occasionally X0 in males (Nokkala and Nokkala 1984a, Grozeva and Nokkala 2001, Table 1). In other families chromosome number is more variable (Table 1, Fig. 1), and there is currently no obvious explanation of why karyotypes are less variable in the Tingidae than in the other cimicomorphan families. However in some within-family groups, for which a considerable body of information is amassed, chromosome number likewise appears remarkably stable, and modal (the commonest) chromosome numbers (at least autosome numbers) become obvious. The subfamilies Mirinae (Miridae) and Triatominae (Reduviidae) are a good case in point. In the Mirinae, the great majority of species have 2n=32+XY. In the Triatominae, which includes over 140 recognized species (in 15–19 genera), karyotypes are currently known for 84 (in 8 genera), and 80 of these species have 20 autosomes (Panzera et al. 2010, Table 1). Ueshima (1979) has suggested that this autosome number is plesiomorphic in the Triatominae and that fission and fusion rearrangements have resulted in the complements with 22 autosomes, as in *Triatoma rubrofasciata* (De Geer, 1773), and with 18 autosomes, as in *T. nitida* Usinger, 1939 and *Panstrongylus megistus* (Burmeister, 1835).

However, the commonest chromosome number needs not to be plesiomorphic in a taxon. A good example comes from the family Nabidae s.str. In this relatively small family (20 genera and approximately 400 species), the number of autosomes reported for 29 species in 7 genera varies between 10 and 38 (Table 1). In addition to these values, there are also species with 16, 26, 30, and 32 autosomes. The predomination of the karyotype 2n=18(16+XY) discovered in 11 species and 4 genera has led to the hypothesis that it is the plesiomorphic condition in the family, and other

chromosome numbers represent apomorphic characters (Leston 1957; Ueshima 1979, Thomas 1996, Kuznetsova and Maryańska-Nadachowska 2000). However, combining cytogenetic and karyosystematic knowledge (Kuznetsova et al. 2004, 2007) with a molecular phylogeny of the family based on 18S rDNA (Nokkala et al. 2007) provided conclusive evidence that the karyotype 2n=32+XY is plesiomorphic and the karyotype 2n=16+XY is apomorphic in the Nabidae s.str., and hence the evolution of karyotypes has been accompanied mainly by fusions of autosomes (Kuznetsova et al. 2004, 2007, Nokkala et al. 2007). In support of this conjecture one can argue that the high-number karyotypes, 2n=32+XY or close to it, appear also characteristic of the closely related families Miridae, Anthocoridae s.str., and Cimicidae (Table 1).

Considering the lack of a centromere, holokinetic chromosomes exhibit a very limited number of characters that can be used as markers. That is why, in spite of recent progress in developing of different staining techniques, chromosomal rearrangements not changing the number of chromosomes, such as inversions and reciprocal translocations, have been very rarely reported in the Heteroptera (Papeschi and Mola 1990, Bressa et al. 1998). Amongst Cimicomorpha, the triatomine species *Mepraia gajardoi* Frias, Henry and Gonzalez, 1998 provides an occasional example of a spontaneous translocation (Pérez et al. 2004). In a natural population of *M. gajardoi*, a fusion between two non-homologous chromosomes was found in one of the eleven studied individuals. This autosomal translocation resulted in chromosomal irregularities such as an autosomal trivalent, autosomal univalents and chromosomal fragments, which altered the normal segregation of both autosomes and sex chromosomes. The extremely rare occurrence of translocations in the Triatominae led Pérez et al. (2004) to suggest that these structural rearrangements are strongly negatively selected, at least in this group.

The m-chromosomes

The term "m-chromosomes" has been introduced by Wilson (1905) for a pair of very minute autosomes, which were first discovered in the coreid species *Anasa tristis* De Geer, 1773, in which these peculiar chromosomes behaved differently from both autosomes and sex chromosomes during male meiosis (Paulmier 1899). Thereafter m-chromosomes have been described in the karyotypes of many bug species (Ueshima, 1979); however their origin and significance in genomes remain still obscure. As a rule, m-chromosomes are extremely small while in some species they might be of approximately the same size as the autosomes (Grozeva et al. 2009). Typically, m-chromosomes show negative heteropycnosis during meiotic divisions in males; they are unpaired during early meiotic prophase and hence form no chiasmata; they associate in a co-orientating pseudo-bivalent (the so-called a "touch-and-go" pairing) at metaphase I and segregate pre-reductionally at anaphase I (Ueshima 1979). However there are several observations suggesting that meiotic behavior of m-chromosomes is more complicated than has been understood earlier (cf. White 1973, Ueshima 1979). In

Coreus marginatus Linnaeus, 1758 (Pentatomomorpha: Coreidae) the synapsis of m-chromosomes is shown to be quite normal at pachytene, suggesting that m-bivalents observed in a part of prophase cells are based on chiasma formation. Still m-chromosomes appear in a substantial part of prophase cells as univalents. In female meiosis, m-chromosomes form a chiasmate bivalent (Nokkala 1986b)

In Ueshima's review (1979, p. 12) altogether 14 bug families are mentioned as having m-chromosomes, no cimicomorphan family being among them. Although in the recent reviews of Papeschi and Bressa (2006a, b) the Cimicomorpha is also referred as lacking m-chromosomes, they are however encountered sporadically among species in the families Miridae, namely, in *Adelphocoris lineolatus* (Goeze, 1778), *Dicyphus digitalidis* Josifov, 1958, *Deraeocoris rubber* Linnaeus, 1758, and *D. rutilus* (Herrich-Schaeffer, 1838), *Capsus ater* (Linnaeus, 1758), *Dichrooscytus bureschi* Josifov 1969, *Lygus pratensis* (Linnaeus, 1758), *Notostira erratica* (Linnaeus, 1758) (Schachow 1932, Nokkala and Nokkala 1986b, Grozeva 2003, Grozeva and Simov 2008b, Grozeva et al. 2011), and Reduviidae, namely, in *Microtomus conspicillaris* Drury, 1782 and *M. lunifer* (Berg, 1900) (Piza 1957, Poggio et al. 2011). The identification of m-chromosomes in the families with achiasmate type of male meiosis (see below) may be difficult, because in those meioses m-chromosomes always appear as a bivalent and not as univalents during meiotic prophase. Consequently, identification is based on the tiny size of a bivalent and the negative heteropycnosis it shows (Nokkala and Nokkala 1986b).

The currently available data suggest that the presence or absence of m-chromosomes represents a quite stable character at higher taxonomic levels in the Heteroptera, but only a few instances of the presence/absence of m-chromosomes in closely related true bug species have been reported (Ueshima 1979). In the Cimicomorpha, such examples are two mirid species, *Dicyphus albonasutus* Wagner, 1951 and *D. digitalidis* Josifov, 1958, the former lacking and the latter possessing m-chromosomes (Grozeva and Simov 2008b). However, the possibility can not be ruled out that in some cases m-chromosomes were not revealed because of their too small size and negative heteropycnosis in meiosis. The discovery of m-chromosomes in the basal infraorder Dipsocoromorpha (in the families Dipsocoridae and Schizopteridae) allowed the suggestion that m-chromosomes were present in the plesiomorphic karyotype of the Heteroptera (Grozeva and Nokkala 1996).

Sex chromosome systems

Genetic sex determination is predominant in insects and is often accompanied by the presence of a heteromorphic chromosome pair in one sex. The true bugs share male heterogamety with the great majority of other insects. Within the Heteroptera, the XX/XY sex determination is of commonest occurrence, although XX/X0 and multiple sex chromosome systems (X_n0, X_nY, and XY_n) as well as rare neo-XY systems do occur (Ueshima 1979, Fig. 2).

The question as to whether the common ancestor of all Heteroptera was X0 or XY is still open. Ueshima (1979) has proposed that the XY system, despite its widespread occurrence in this group, is derived from the plesiomorphic X0 condition. The fact that sex determination in non-heteropteran Hemiptera groups is predominantly X0, the system being also considered plesiomorphic in Insecta as a whole (Blackman, 1995), seems to support this hypothesis.

On the other hand, Nokkala and Nokkala (1983, 1984a) formulated an alternative hypothesis assuming that the XY mechanism is plesiomorphic in the Heteroptera, and the existence of X0 species is due to repeated loss of the Y chromosome, i.e. the result of convergent evolution (homoplasy). Their arguments are based on the discovery of an XY species, *Saldula orthochila* (Fieber, 1859), among X0 species in the genus *Saldula* Van Duzee, 1914 (Leptopodomorpha, Saldidae) and the sporadic occurrence of similar intrageneric X0-XY variation within the infraorders Gerromorpha, Cimicomorpha and Pentatomomorpha, indicating that the Y-chromosome has a tendency to get lost during evolution.

The most basal heteropteran infraorders are considered to be Enicocephalomorpha and Dipsocoromorpha (Wheeler et al. 1993, Schuh et al. 2009, Cassis and Schuh 2010). Unfortunately, in Enicocephalomorpha chromosomal data are still absent. In Dipsocoromorpha, such data are available for 2 species of the family Schizopteridae and for 4 species of the family Dipsocoridae, these species showing different sex chromosome systems, X0, XY and XY_1Y_2 (Ueshima 1979, Grozeva and Nokkala 1996). Moreover, within the genus *Pachycoleus* Fieber, 1860 both X0 species (*P. rufescens* Sahlberg, 1875; Ueshima, 1979) and XY_1Y_2 species (*P. pusillimus* (J. Sahlberg, 1870); Grozeva and Nokkala 1996: as *Cryptostemma* Herrich-Schaeffer, 1835) occur.

The existence of Y-chromosome in the Dipsocoromorpha seems to support the view that XY system evolved early in the evolution of the Heteroptera. Since the overwhelming majority of the true bug species possess Y chromosomes, the question arises about the origin of Y-chromosome in the Heteroptera. There is a variety of ways in which a Y-chromosome can evolve from an autosome (White 1973, Blackman 1995). One of those is a fusion between the X chromosome and an autosome (in an initially X0 species) resulting in a neo-XY system. In a recently formed neo-XY system, autosomally derived Y chromosome (a neo-Y) is still homologous with the autosome part of the neo-X and therefore synapses with it in meiosis. However the X and Y chromosomes in Heteroptera generally show little or no evidence of homology expected of a neo-XY system (Blackman 1995). Recently, a mechanism is revealed by which a heteropteran-like achiasmate Y-chromosome can evolve from a B-chromosome (supernumerary, or accessory, or extra chromosome; see below) (Nokkala et al. 2003, Nokkala and Nokkala 2004, Carvalho et al. 2009).

In the Cimicomorpha, the whole range of sex chromosome systems occurs. Within this infraorder, different sex chromosomes have evolved among closely related species or even intraspecific populations (Ueshima 1979, Panzera et al. 2010, Grozeva et al. 2010, Table 1, Fig. 2). However, species with the XY system clearly predominate. Amongst those families, in which the information is available on many species, the

family Nabidae s.str. is the single one being exclusively XX/XY (Nokkala and Nokkala 1984b, Kuznetsova and Maryańska-Nadahowska 2000, Kuznetsova et al. 2004, 2007, Angus et al. 2008). Of the three most extensively studied families, X0 species are limited in Reduviidae and Miridae, and have never been reported in Cimicidae (Fig. 2, Table 1).

Compared to other Heteroptera, the Cimicomorpha is unique in that the majority of species posses multiple X chromosomes. Also, in this group the greatest number of X chromosomes in a species – up to 5 in the Reduviidae and up to 15 in the Cimicidae (Ueshima 1966a, b, 1979, Poggio et al. 2007, Grozeva et al. 2010, Table 1) is found. Within the Reduviidae, multiple sex chromosome systems are the most frequent in the subfamilies Harpactorinae and Stenopodinae (Poggio et al. 2007, 2011). In the Cimicidae, they are quite frequent in the subfamilies Cimicinae and Haematosiphoninae (Ueshima 1979, Poggio et al. 2009). One of the most intriguing examples is the bed bug *Cimex lectularius* Linnaeus, 1758 (Cimicidae), in which X chromosomes vary in number from two (X_1X_2Y) to 15 (X_1X_2Y+13 extra Xs) in different populations while sometimes between males of a population and even between different cells of a male (Ueshima 1966a, b, for other references see Grozeva et al. 2010). The origin of multiple systems in the Heteroptera is usually ascribed to simple transverse fissions of an original X chromosome, the process which is suggested to be facilitated by the holokinetic nature of true bug chromosomes (Schrader 1947, Ueshima 1966a, b, 1979). It is worth noting however that the application of C-banding to study the chromosomes of several Triatominae (Reduviidae) species led Panzera et al. (2010) to the conclusion that chromosomal rearrangements other than fissions might have been involved in the formation of the multiple sex chromosome systems in Heteroptera. However this problem clearly calls for further investigation.

B-chromosomes

B-chromosomes, also known as supernumerary, accessory, or extra chromosomes, are dispensable elements which do not recombine with other chromosomes (the A-chromosomes) of the standard complement and follow their own evolutionary pathway (Beukeboom 1994). B-chromosomes are present in a part of individuals from some populations of a species resulting in intraspecific variation in chromosome number. The evolutionary significance of B-chromosomes seems to be evidenced by their widespread occurrence in very many plant and animal groups; however the origin, structure and evolution of these enigmatic chromosomes are still the subject of much controversy (Jones and Rees 1982, Camacho et al. 2000, Jones and Houben 2003, Camacho 2004). Within Cimicomorpha, B-chromosomes were described in 12 species, namely, *Triatoma longipennis* Usinger, 1939, *Mepraia gajardoi* Frias, Henry and Gonzalez, 1998, and *M. spinolai* Porter, 1934 from the family Reduviidae (Pérez et al. 2004, Panzera et al. 2010); *Orthocephalus funestus* Jakovlev 1881 from the Miridae (Takenouchi and Muramoto 1972b), *Acalypta parvula* (Fallén, 1807) and *Stephanitis*

oberti (Kolenati, 1857) from the Tingidae (Grozeva and Nokkala 2001); *Nabis rugosus* (Linnaeus, 1758), *N. brevis* Scholtz, 1847, *N. ericetorum* Scholtz, 1847, and *N. pseudoferus* Remane, 1949 from the Nabidae s.str. (Grozeva and Nokkala 2003); *Paracimex borneensis* Usinger, 1959 and *P. capitatus* Usinger, 1966 from the Cimicidae (Ueshima 1966b). The data obtained point to a sufficient variability of these supernumeraries in terms of their size, C-heterochromatin amount and distribution, meiotic behavior and impact on segregation of A-chromosomes in the species. By this is meant that B chromosomes in Cimicomorpha are of polyphyletic origin that correlates well with the modern concept of polyphyletic origin of B-chromosomes in different groups of animals and plants.

Male meiosis

It is common knowledge that in meiosis, chiasmata (the points of genetic crossing-over) are formed uniting homologous chromosomes together until their separation in the reductional division. However in some animal groups chiasma formation is replaced by other, achiasmate means. When meiosis is achiasmate, at early prophase I one can see the conventional sequence of leptotene, zygotene and pachytene stages. However, no chiasmata are formed and hence no diplotene or diakinesis stages can be recognized. Typically, achiasmate meiosis is restricted to the heterogametic sex of a species. In most heteropteran males, autosomal bivalents are chiasmate whereas sex chromosomes have no chiasmata, however in a number of families male meiosis is completely achiasmate (Kuznetsova and Grozeva 2010). The first paper to describe the achiasmate meiosis within the Heteroptera was that of Nokkala and Nokkala (1983) dealing with the family Saldidae (Leptopodomorpha). Since that time, this meiotic pattern has been documented in six further heteropteran families, such as Micronectidae from Nepomorpha (Ituarte and Papeschi 2004, Grozeva et al. 2008) as well as Microphysidae, Nabidae s.str., Anthocoridae s.str., Cimicidae, and Miridae from Cimicomorpha (Nokkala and Nokkala 1984b, 1986a, b, Kuznetsova and Maryańska-Nadahowska 2000, Nokkala and Grozeva 2000, Grozeva and Nokkala 2002, Kuznetsova et al. 2004, 2007, Poggio et al. 2009, Grozeva et al. 2010, 2011). In Tingidae and Reduviidae, the remaining cimicomorphan families for which such evidence is available, males show the orthodox chiasmate meiosis. Nokkala and Nokkala (1984b) argued for a monophyletic origin of achiasmate meiosis in the Heteroptera. However, when more observations of achiasmate meiosis in Cimicomorpha and Nepomorpha became available, the polyphyletic origin of this type of meiosis in Heteroptera was suggested (Ituarte and Papeschi 2004, Grozeva et al. 2008).

Multiple origins of achiasmate meiosis in Heteroptera is substantiated by the placement of families with achiasmate meiosis in the cladogram based on combined analysis of 16S, 18S, 28S and COI sequence data and 73 morphological characters by Schuh et al. (2009, fig. 10). The existence of achiasmate meiosis in one family (Micronectidae) within Nepomorpha, in one family (Saldidae) within

Leptopodomorpha, and in several families within Cimicomorpha is undoubtedly the result of independent events. Within the Cimicomorpha, the change from chiasmate to achiasmate meiosis could trace back to the separation of the clades Cimiciformes and Miroidea from the rest of the Geocorisae (node 12 in Schuh et al. 2009). All the families cytologically studied in the clade Cimiciformes show achiasmate male meiosis (Microphysidae, Nabidae s.str., Anthocoridae s.str., Cimicidae). In the sister clade Miroidea, the family Miridae shows achiasmate male meiosis, but male meiosis in the family Tingidae is chiasmate. According to this interpretation, achiasmate male meiosis in the Cimicomorpha is of monophyletic origin, and chiasmate meiosis in Tingidae represents reversal from achiasmate to chiasmate meiosis. An alternative explanation is that achiasmate meiosis has appeared coupled with the emergence of Cimiciformes and independently when the family Miridae was separated from their common ancestor with the Tingidae. In this alternative, achiasmate meiosis in the Cimicomorpha is of multiple origins and chiasmate meiosis in Tingidae is not of reversal type. As the latter alternative includes no reversal from achiasmate to chiasmate meiosis it seems more probable.

The multiple origin of achiasmate meiosis is well in accordance with the observations on the divergence in its cytological properties. The most common type of achiasmate meiosis is the so-called *alignment* type. In this type of meiosis, homologous chromosomes in a bivalent are held together along all their length during whole prophase up to metaphase I (Nokkala and Nokkala 1983). Within Cimicomorpha, the *alignment* type of meiosis has been described in the families Nabidae s.str. (Nokkala and Nokkala 1984b, Kuznetsova and Maryańska-Nadachowska 2000), Anthocoridae s.str. (Nokkala and Nokkala 1986a), and Microphysidae (Nokkala and Grozeva 2000). Beyond Cimicomorpha, this meiotic pattern is observed in both Saldidae (Nokkala and Nokkala 1983) and Micronectidae (Ituarte and Papeschi 2004, Grozeva et al. 2008).

In the *collochore* type, as it is called, one or occasionally two tenacious threads, the collochores, are formed to hold homologous chromosomes together in the absence of chiasmata. This pattern was described in the families Miridae and Cimicidae, in all the species studied in this respect (Nokkala and Nokkala 1986b, Grozeva and Nokkala 2002, Poggio et al. 2009, Grozeva and Simov 2008a, b, Grozeva et al. 2010, 2011, Grozeva and Simov 2009). Within the Miridae, the collochore meiosis is inherent in the genera *Bryocoris* Fallén, 1829 (1 species studied), *Dicyphus* Fieber, 1858 (10), and *Campyloneura* Fieber, 1858 (1), all of the subfamily Bryocorinae Baerensprung, 1860; *Deraeocoris* Kirschbaum, 1856 (2) from Deraeocorinae Douglas and Scott, 1865; *Capsus* Fabricius, 1803 (1) and *Megaloceroea* Fieber, 1858 (1) from Mirinae Hahn, 1833; *Driophylocoris* Reuter, 1875 (2) from Orthotylinae Van Duzee, 1916); *Rhabdomiris* Wagner, 1968 (1), *Pilophorus* Hahn, 1826 (1), *Plagiognathus* Fieber, 1858 (1), and *Cremnocephalus* Fieber, 1860 (2) from Phylinae Douglas and Scott, 1865). Within the Cimicidae, this meiotic pattern in inherent in the genera *Cimex* Linnaeus, 1758 (3 species studied) from the subfamily Cimicinae Latreille, 1802, and in *Acanthocrios* Del Ponte and Riesel, 1945 (1), *Ornithocoris* Pinto, 1927 (1), and *Psitticimex* Usinger, 1966 (1) from the subfamily Haematosiphoninae Jordan and Rothschild, 1912.

Additionally, in the Nabidae s.str., where the tribes Nabini (Nabinae) and Prostemmatini (Prostematinae) are characterized by meiosis of the *alignment* type, a pattern intermediate between *alignment* and *collochore* meioses, has been described in *Arachnocoris trinitatus* Bergroth, 1916, the only representative of the tribe Arachnocorini Reuter, 1890 studied so far (Kuznetsova et al. 2007, Kuznetsova and Grozeva 2008).

In general, during the first division of meiosis the chromosomes reduce in number (reductional division), whereas during the second division the chromatids separate (equational division), and this pattern is named "pre-reduction" (White 1973). One of the unique cytogenetic characters of the Heteroptera, also presented in most taxa of Cimicomorpha, is the sex chromosome "post-reduction", with sex chromosomes undergoing equational separation during first division and reductional segregation during second division. Autosomes always show the orthodox sequence of meiotic divisions in male meiosis. On occasion, individual bug species demonstrate sex chromosome pre-reduction, the Tingidae being the only heteropteran family showing pre-reductional behavior of sex chromosomes in spermatogenesis of all the species studied (Ueshima 1979, Grozeva and Nokkala 2003). The Tingidae are thus unique in having, besides sex chromosome pre-reduction, also unusually stable karyotype and chiasmate meiosis in males. It is interesting that all of these characters distinguish Tingidae from Miridae, the families considered to form a monophyletic group within Cimicomorpha (Schuh et al. 2009). In this infraorder, sex chromosome pre-reduction occurs likewise in all the three studied species of the genus *Macrolophus* Fieber, 1858 from Miridae (Grozeva et al. 2006, 2007) as well as in both studied species of the genus *Ectrychotes* Burmeister, 1835 from Reduviidae (Ueshima 1979, Manna and Deb-Mallick 1981), all other species of these families sharing sex chromosome post-reduction.

In most Cimicomorpha, as common in Heteroptera, sex chromosomes demonstrate the "*touch-and-go*" pairing at metaphase II of male meiosis, i.e. they come together forming a characteristic co-orientating pseudo-pair in the spindle and segregate polewards at anaphase II. The mechanism involved in this "*touch-and-go*" process (the term has been introduced by Wilson in 1925 for m-chromosomes demonstrating a similar behavior at metaphase I of meiosis) is a very puzzling one (Schrader 1940, Nokkala 1986a). The only exception presently known in Heteroptera is the subfamily Nabinae (Nabidae s.str.) where a kind of "*distance pairing"* of sex chromosomes at metaphase II is observed (Nokkala and Nokkala 1984b, Kuznetsova and Maryańska-Nadachowska 2000, Kuznetsova et al. 2004). Typical of *distance pairing* is that the sex chromosomes do not associate at metaphase II; they orientate towards opposite poles forming a kind of "*distance pseudo-pair*" and then segregate. It should be recorded that the other nabid subfamily, Prostemmatinae, shows the orthodox "*touch-and-go*" process (Kuznetsova et al. 2007).

Another characteristic feature is the configuration of metaphase I and metaphase II plates, which pattern seems to show species-specific variation in the Heteroptera (see Ueshima 1979). Meiotic metaphase plates in males are very often organized in such a way that both autosomal bivalents at MI and autosomes at MII form a circle in the

center of which univalent chromosomes (X and Y chromosomes, m-chromosomes, B chromosomes) are placed. This configuration of the metaphase plate is referred to as radial as opposed to the other configuration, a nonradial one, where univalent chromosomes and autosomal bivalents are randomly distributed within the metaphase plate. The formation of radial metaphase plate is based on the congressional movements of bivalents and univalents that occur exceptionally along the nuclear envelope towards spindle equator during prometaphase I, resulting in both bivalents and univalents lying in a single ring at late prometaphase. Congression is followed by stabilization phase during which m-chromosome or sex chromosome univalents move along the equator to the center of the plate and form a co-oriented pseudo-bivalent at metaphase I or a pseudo-pair at metaphase II ("*touch-and-go*") (Nokkala 1986a). Metaphases I and II or occasionally only one of them may be radial, closely related species sometimes differing in this pattern. In the families Nabidae s.str., Miridae, Microphysidae, and Anthocoridae s.str. the first metaphase plate is shown to be nonradial and the second metaphase plate radial (Nokkala and Nokkala 1984b, 1986a, b, Nokkala 1986a, Nokkala and Grozeva 2000, Kuznetsova et al. 2004). In the Cimicidae, *Cimex lectularius* demonstrates the same pattern (Grozeva et al. 2010), whereas in *Psiticimex uritui* Lent and Abalos, 1946, both MI and MII plates seem to be radial (see Figs 2b, c in Poggio et al. 2009). Typically, the stage between two meiotic divisions, interkinesis, is absent in spermatogenesis in the Heteroptera, and the first anaphase spindle is transformed directly into the second division spindle (Ueshima 1979). However, interkinesis stage is present in those taxa, where, as in Nabidae s.str. and Miridae, the first metaphase is nonradial and the second metaphase is radial. This stage is necessary for the formation of a radial metaphase II after a nonradial metaphase I (Nokkala and Nokkala 1984b, Nokkala 1986b).

Female meiosis

For technical reasons, most research on heteropteran chromosomes has used males and as a consequence, there is very little evidence on meiosis in females. Helenius (1952; see also Piza 1957) was first to point out different orientation of autosomal metaphase I bivalents in male and female meiosis of the lygaeid bugs (Pentatomomorpha, Lygaeidae s.l.): in males parallel and in females perpendicular to the spindle axis. Based on this he claimed that meiosis in females was of the inverted type or post-reductional. On similar basis post-reduction was also suggested by Nokkala (1986a) in female meiosis of *Coreus marginatus* Linnaeus, 1758 (Pentatomomorpha, Coreidae). Later, however, it has been established that chiasma terminalization is absent in holokinetic chromosomes as evidenced by observations in *Triatoma infestans* (Klug, 1834) (Cimicomorpha, Reduviidae) (Pérez et. al.1997) and *Myrmus miriformis* (Fallen, 1807) (Pentatomomorpha, Rhopalidae) (Nokkala and Nokkala 1997). Consequently, the part of a half-bivalent extending from the chiasma point to the kinetic end separates pre-reductionally. Hence**,** chiasmate bivalents, irrespective of their orientation at metaphase I,

always undergo pre-reduction (Nokkala and Nokkala 1997, Pérez et al. 1997, Nokkala et al. 2006, Viera et al. 2009) both in males and females.

One of the mirid species, *Campyloneura virgula* (Herrich-Schaeffer, 1835), is known to be mainly parthenogenetic, and males are extremely rare over the species distribution range (Wheeler 2001). A cytogenetic study of a parthenogenetic population from Samothraki (Northern Greece) has shown females to be diploid with the karyotype most characteristic of the family Miridae, i.e. 2n=32+XX. In these females, normal meiosis is suggested to be substituted by a modified mitotic division, and the oogenesis is hence of the apomictic type (Grozeva and Simov 2008b).

Challenges and perspectives

In general, cytogenetic studies of the Heteroptera use standard techniques providing evidence on chromosome number, sex chromosome mechanisms and, in outline, the behavior of chromosomes during meiosis. For an investigator of true bug cytogenetics the basic challenge is the identification of individual chromosomes and chromosomal regions in a karyotype. This is just a condition under which the evolutionary rearrangements, both interchromosomal and intrachromosomal, could be detected in holokinetic chromosomes that would result in considerable progress in the field. With differential cytogenetic techniques, only C-banding and DNA specific fluorochrome staining to reveal C-heterochromatin amount, distribution and composition, and NOR-staining to detect the number and location of nucleolus organizer regions (NORs) have been generally applied in the Heteroptera. However these approaches made possible only a few markers to be revealed in karyotypes. Nevertheless, they made it clear that taxonomically closely related species, even though they have the same chromosome number, do not in fact display identical karyotypes due to accumulation of many rearrangements since divergence from the common ancestor (Grozeva and Nokkala 2001, Angus et al. 2004, Grozeva et al. 2004, Kuznetsova et al. 2007). For example, the tribes in the family Nabidae s.str. were shown to differ in the location of NORs which are situated on sex chromosomes in Nabini (Nabinae) and Prostemmatini (Prostemmatinae) (Grozeva et al. 2004) and on a pair of large autosomes in Arachnocorini (Nabinae) (Kuznetsova et al. 2007).

In the last few decades, the ability to identify chromosomes has been markedly improved by the development of molecular cytogenetic technologies such as fluorescence *in situ* hybridization (FISH) for the mapping of genes and sequences, comparative genomic hybridization (CGH) for comparative analyses of genome homology, and others. Unfortunately, these useful approaches are not yet developed in the Heteroptera, with the sole exception of FISH with ribosomal probes to determine where ribosomal genes (18S, 28S or 45S) are located on the chromosomes of a species (Severi-Aguiar et al. 2006, Papeschi and Bressa 2006b, Grozeva et al. 2010, 2011, Panzera et al. 2010, Bardella et al. 2010, Poggio et al. 2011). Based on the very first data obtained we safely assume that molecular cytogenetic techniques will be beneficial for revealing additional

chromosome markers and providing useful insight into the understanding of genome constitution and the mechanisms of karyotype evolution in true bugs. For example, in the family Reduviidae, FISH experiments using a 45S rDNA probe revealed differences in the number and location of hybridization sites between triatomine species sharing the same chromosome number, 2n=20+XY. In *Triatoma brasiliensis* Neiva, 1911 and *T. rubrovaria* Blanchard, 1834, a single 45S rDNA cluster was found on a pair of autosomes, whereas in *T. infestans melanosoma* Lent, Jurberg, Galvão and Carcavallo, 1994 on the X chromosome, while in *T. matogrossensis* hybridization signals were located on both X and Y chromosomes (Bardella et al. 2010).

A potential field of interest concerns the molecular composition of telomeres, which is totally unknown in the true bugs. Telomeres are terminal regions of chromosomes that protect chromosomes from destruction and stabilize their structure (Zakian 1995). DNA of the telomeric regions consists of short nucleotide motifs repeated thousands and millions of times. Comparative analysis of these motifs in various groups of organisms showed that they were evolutionarily stable, and mark taxa and phylogenetic branches of higher ranks (Traut et al. 1999). A pentanucleotide repetitive sequence, $(TTAGG)_n$, is the commonest and most likely an ancestral telomeric motif of Insecta that supports their origin from a common ancestor. Heteroptera belong to a very few higher taxa of Insecta in which $(TTAGG)_n$ telomeric sequence is absent as evidenced by FISH and/or Southern and/or dot-blot hybridization with a TTAGG probe (Sahara et al. 1999, Frydrychová et al. 2004, Grozeva et al. 2011). It is worthy of note that non-heteropteran Hemiptera, the Auchenorrhyncha included, retain this telomeric sequence (Frydrychová et al. 2004), however at present, data are not available for Colleorrhyncha, or moss bugs, widely considered to be the sister-group to Heteroptera. The $(TTAGG)_n$ motif was suggested to be lost in the early evolution of the true bugs being secondarily replaced by another motif or an alternative telomerase-independent mechanism of telomere maintenance (Frydrychová et al. 2004, Lukhtanov and Kuznetsova 2010). Importantly, dot-blot hybridization of the genomic DNA from the true bug species with telomeric probes of different groups of animals and plants, namely, ciliate $(TTTTGGGG)_n$ and $(TTGGGG)_n$, nematode $(TTAGGC)_n$, shrimp $(TAACC)_n$, vertebrate $(TTAGGG)_n$, and plant $(TTTAGGG)_n$, yielded likewise negative results (Grozeva et al. 2011). On the basis of present knowledge, it may be inferred that telomere elongation is telomerase-independent in true bugs.

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At the lower size limit for tetrapods, two new species of the miniaturized frog genus *Paedophryne* (Anura, Microhylidae)

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Abstract

I describe two new species in the miniaturized microhylid frog genus *Paedophryne* from forests in south-eastern Papua New Guinea. The first species is described on the basis of two specimens and exhibits female snout-vent length of 8.5–9.0 mm (no males known), whereas that of the second species, described on the basis of 12 specimens, is 8.8–9.3 mm, with males 8.1–8.9 mm. These frogs are smaller than the other two diminutive species described when the genus was recently erected, and they represent what are currently the smallest known species of tetrapods. The two species replace each other elevationally on the same mountain massif and occur in relative geographic proximity to the other named species of the genus. Females of both species contain only two enlarged ova, suggesting that they also possess clutch sizes at the extreme lower end of variation in frogs. All species of *Paedophryne* inhabit leaf litter, as seen for most other miniaturized anurans.

Keywords

clutch size, diminutive, ecomorph, Milne Bay Province, Mt. Dayman, Mt. Suckling

Introduction

Asterophryine frogs represent a large subfamily of the Microhylidae that contains 22 named genera, more than 240 named species, and scores of unnamed forms. The subfamily is monophyletic (Savage 1973; van Bocxlaer et al. 2006; Frost et al. 2006;

Roelants et al. 2007; van der Meijden et al. 2007) and centered in the Papuan region of New Guinea and surrounding islands, although one genus (*Oreophryne*) extends as far north as the southern Philippines and another genus (*Cophixalus*) has several species in Queensland, Australia. Member genera of the Asterophryinae vary considerably in morphological and ecological attributes, with burrowing, terrestrial, semiaquatic, scansorial, and arboreal ecotypes represented (Burton 1986; Menzies 2006; FK, unpubl. data). Sizes vary from 10 to 100 mm snout-vent length (Zweifel 1972; Kraus 2010).

Among those asterophryines of the "terrestrial" ecomorph, which solely inhabit the forest floor, I recently described from southeastern New Guinea a new genus, *Paedophryne*, that included two new species comprising some of the smallest frogs in the world (Kraus 2010). *Paedophryne* was characterized by its small adult size (10.1–11.3 mm in snout-vent length), reduced phalangeal formula, prepollex and prehallux each consisting of a single element, reduced number of presacral vertebrae, *Musculus depressor mandibulae* overlying posterior margin of tympanum, *M. adductor mandibularis anterior longus* small and inserting only on lateral portions of frontoparietals, *M. submentalis* hypertrophied, and tongue long and straplike. The reduced phalangeal formula imparts a unique appearance to the hands and feet among Papuan frogs, leaving the hands with only three functional digits and the toes with only four. Relationships of the new genus to other asterophryines are the subject of current research, but I earlier gave reasons to expect that *Paedophryne* might be one of the most ancient lineages of asterophryines. The two described species (*P. kathismaphlox* Kraus, *P. oyatabu* Kraus) are included among the smallest four or five species of frogs in the world; however, sample sizes of each were limited, making a clearer assessment of size ranking relative to other species uncertain. This is because size differs between sexes in most frog species. Size can be measured relative to a variety of different standards (average or maximum male size, female size, or some combination of them), and many of the world's smallest frogs have been poorly sampled such that size information is lacking for one sex or another (Lehr and Coloma 2008, table 3).

On a recent survey of an isolated mountain massif in southeastern Papua New Guinea I discovered two more species of *Paedophryne*. Each is of a smaller size than the two species already known in the genus and stands at the lower size limit currently known for anurans (Lehr and Coloma 2008; Kraus 2010) and, therefore, tetrapods (Estrada and Hedges 1996), although both are larger than the world's smallest fish (Kottelat et al. 2006).

Materials and methods

All measurements were made with an optical micrometer to the nearest 0.1 mm, except for toe disc width, measured to the nearest 0.03 mm; measurements, terminology, and abbreviations follow Zweifel (1985) and Kraus and Allison (2006): body length from snout–vent (SV); tibia length from heel to outer surface of flexed knee (TL); horizontal diameter of eye (EY); distance from anterior corner of eye to center of

naris (EN); internarial distance, between centers of external nares (IN); distance from anterior corner of eye to tip of snout (SN); head width at widest point, typically at the level of the tympana (HW); head length, from tip of snout to posterior margin of tympanum (HL); horizontal tympanum diameter (TY); hand length, from proximal edge of palm to tip of 3rd finger (HandL); foot length, from proximal edge of sole to tip of 4th toe (FootL); width of the fourth toe disc (4thT). I determined sex by presence of vocal slits (males) or examination of gonads (females and males for which the vocal slits were not clearly discernible). Frogs were identified to genus based on diminutive size; presence of eleutherognathine maxillae and a long, strap-like tongue; and the reduced phalangeal pattern that reduces their first digits to mere nubs. The last is unique among Papuan frogs and immediately diagnostic.

I recorded calls in the field using a Sennheiser K6 microphone and a Marantz 660 digital audio recorder; I analyzed call structure using the computer program Avisoft-SASLab Pro(v4.34) available from Avisoft Bioacoustics (http://www.avisoft.com/).

Type specimens are deposited in the Bernice P. Bishop Museum, Honolulu (BPBM) and the Papua New Guinea National Museum and Art Gallery, Port Moresby (PNGNM). All latitude and longitude coordinates use the World Geodetic System, 1984 (WGS 84) and were taken from a hand-held GPS unit.

***Paedophryne dekot* Kraus, sp. n.**
urn:lsid:zoobank.org:act:3A6D9296-93A9-45C6-96F4-92FBAE0D428D
http://species-id.net/wiki/Paedophryne_dekot
Figs 1, 2A, B

Holotype. BPBM 37753 (field tag FK 15615), alcohol specimen, adult female, collected by F. Kraus and local villagers at Binigun, W slope Mt. Dayman, 9.7071°S, 149.2498°E, 900 m, Milne Bay Province, Papua New Guinea, 1 April 2011.

Paratype (n = 1). BPBM 37754, same data as holotype, except collected 4 April 2011.

Diagnosis. A minute microhylid (female SV = 8.5–9.0 mm) with smooth dorsal skin; a relatively long leg (TL/SV = 0.45–0.46); short and broad snout (EN/SV = 0.067–0.071, IN/SV = 0.106–0.111, EN/IN = 0.60–0.67); relatively large discs on third and fourth toes (4thT/SV = 0.044–0.052, 3rdF/4thT = 0.43–0.58, Fig. 1E); a uniform brown or red-brown dorsum with two large dorsolateral, downward-pointing, black triangular blotches on each side; and a pale gray venter with brown flecks.

Comparisons with other species. *Paedophryne dekot* differs from *P. kathismaphlox* and *P. oyatabu*, the only two other species currently described in this genus, in its smaller size (female SV = 8.5–9.0 in *P. dekot*, 10.4–10.9 mm in *P. kathismaphlox*, 11.3 mm in *P. oyatabu*), longer leg (TL/SV = 0.45–0.46 in *P. dekot*, 0.35–0.39 in *P. kathismaphlox*, 0.40 in *P. oyatabu*), shorter snout (IN/SV = 0.106–0.111, EN/IN = 0.60–0.67, EN/SV = 0.067–0.071 in *P. dekot*; IN/SV = 0.087–0.099, EN/IN = 0.78–0.80 in *P. kathismaphlox*; EN/SV = 0.062, IN/SV = 0.097 in *P. oyatabu*), larger discs on third and fourth toes (4thT/SV = 0.044–0.052 in *P. dekot*, 0.032–0.037 in P. *kathismaphlox*,

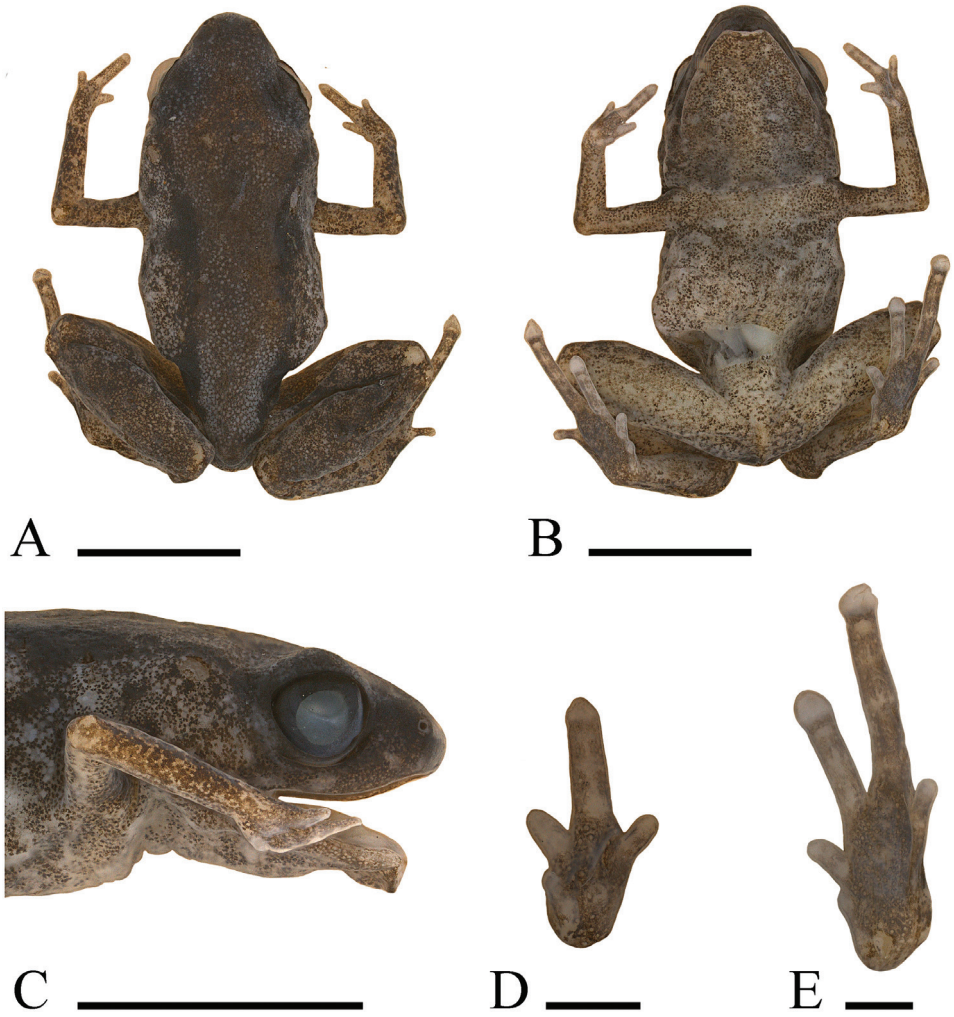

Figure 1. A Dorsum, **B** ventrum, **C** side of head, **D** palmar view of left hand, and **E** plantar view of left foot of holotype of *Paedophryne dekot* (BPBM 37753). Scale bars = 5 mm **A–C** and 1 mm **D, E**.

0.031 in *P. oyatabu*; 3rdF/4thT = 0.43–0.58 in *P. dekot*, 0.66–0.86 in *P. kathismaphlox*, 0.80 in *P. oyatabu*), dorsum with two large dorsolateral triangular blotches on each side (dorsum brown vaguely mottled with black or dark brown in *P. kathismaphlox*, dorsum brown with two dark mid-dorsal chevrons in *P. oyatabu*), venter pale gray with brown flecks (venter dark brown with scattered light straw-brown or gray flecks in *P. kathismaphlox* and *P. oyatabu*), and no brightly colored patch below anus (a burnt-orange patch below anus in *P. kathismaphlox*).

Description of holotype. An adult female with an incision on right side and across rear of abdomen; liver removed and stored separately for DNA analysis. Head moderately wide (HW/SV = 0.37, Fig. 1A), with steeply oblique loreal region; canthus

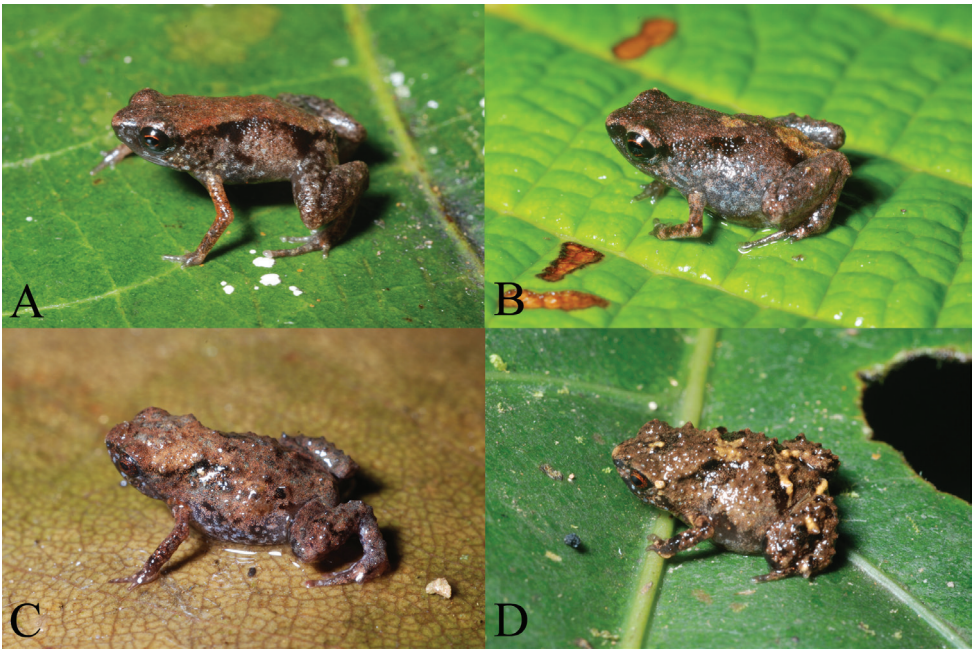

Figure 2. Portraits in life of **A** holotype of *Paedophryne dekot* (BPBM 37753), **B** paratype of *P. dekot* (BPBM 37754), **C** paratype of *P. verrucosa* (BPBM 37743), and **D** paratype of *P. verrucosa* (BPBM 37745).

rostralis rounded, slightly convex when viewed from above; nostrils directed anterolaterally, closer to tip of snout than to eyes; internarial distance much larger than distance from naris to eye (EN/IN = 0.60, IN/SV = 0.111, EN/SV = 0.067); snout rounded when viewed from the side or from above (Fig. 1A, C); eyes moderately large (EY/SV = 0.13; EY/SN = 1.0, Fig. 1C), pupil horizontal; eyelid approximately two-thirds width of interorbital distance; tympanum indistinct and small (TY/SV = 0.044), visible only when skin dries slightly, hidden posterodorsally. Skin smooth; supratympanic fold absent. Fingers unwebbed, flattened; F1 reduced to a vestigial nub; relative lengths 3>2=4>1 (Fig. 1D); discs absent. Subarticular and metacarpal tubercles absent. Toes unwebbed; T3 and T4 with flattened discs and terminal grooves; disc of T4 not wider than penultimate phalanx. Second and fifth toes with round tips and no discs; T1 a vestigial nub; relative lengths of toes 4>3>2=5>1 (Fig. 1E). Subarticular and metatarsal tubercles absent. Plantar and palmar surfaces smooth. Hind legs rather long (TL/SV = 0.46, Fig. 1A). Tongue elongate, straplike, anterior third attached to floor of mouth.

In preservative, dorsum brown with a more-or-less continuous dorsolateral row of black blotchs and flecks; sides and front and rear of thighs pale gray heavily flecked with dark brown. Face dark brown. Ventral surfaces pale gray flecked with dark brown. Iris black.

In life, the holotype was noted as: "Dorsum red brown, sides gray, dorsolateral series of black flecks. Rear of thighs light red brown with black punctations. Face black, posterior to eye spotted with light gray. Venter dark gray with small pale-gray flecks."

The iris was black with a red rim around the pupil, and scattered pale blue-gray flecks are apparent on the lower sides and limbs (Fig. 2A).

Measurements (in mm).—SV = 9.0, TL = 4.1, HW = 3.3, HL = 2.9, IN = 1.0, EN = 0.6, SN = 1.2, EY = 1.2, TY = 0.4, 4th T = 0.40.

Variation. There is little mensural difference between the paratype and holotype, except that the former has a somewhat larger tympanum (TY/SV = 0.059) and a slightly longer snout (EN/IN = 0.67). In coloration, the paratype is similar to the holotype but the black dorsal blotching is not concentrated into dorsolateral lines; there is a pale tan blotch over the rump; and the dark brown on the sides, limbs, and ventral surfaces is reduced to even stippling instead of flecking. Liver was also removed from the paratype for DNA analysis. Measurements for the paratype are: SV = 8.5, TL = 3.8, HW = 3.2, HL = 2.7, IN = 0.9, EN = 0.6, SN = 1.1, EY = 1.3, TY = 0.5, 4th T = 0.44.

Etymology. The species name "dekot" is the word for "very small" in Daga, the language spoken in the area from which this species was collected.

Range. Known only from the western slope of Mt. Dayman in the saddle where it joins Mt. Suckling to the northwest, Milne Bay Province, Papua New Guinea (Fig. 3, square).

Ecological notes. *Paedophryne dekot* inhabits leaf litter on the floor of steeply sloping primary foothill rainforest. Canopy at the type locality was approximately 35 m high; understory was dense, with some moss on trees and ground. This forest type terminates at approximately 1200 m elevation in this area, so *P. dekot* seems unlikely to occur higher than that.

This species was heard calling from the forest floor in mid- to late afternoon and at dusk but could not be recorded by me.

Both females contained two enlarged, well-yolked, cream-colored eggs and approximately a dozen small white oocytes.

***Paedophryne verrucosa* Kraus, sp. n.**
urn:lsid:zoobank.org:act:8DEA8ED4-D3D2-49DC-AFF2-8B8A21CF0044
http://species-id.net/wiki/Paedophryne_verrucosa
Figs 2C, D, 4

Holotype. BPBM 37747 (field tag FK 15516), alcohol specimen, adult male, collected by F. Kraus and local villagers at Sota, SE slope Mt. Dayman, 9.7580°S, 149.1822°E, 1860 m, Milne Bay Province, Papua New Guinea, 27 March 2011.

Paratypes (n = 11). BPBM 37745–46, same data as holotype; BPBM 37743, same data as holotype, except female collected 23 March 2011; BPBM 37744, same data as holotype, except collected 26 March 2011; BPBM 37748–50, PNGNM 24121–22, same data as holotype, except collected 28 March 2011; BPBM 37751–52, same data as holotype, except females collected 29 March 2011.

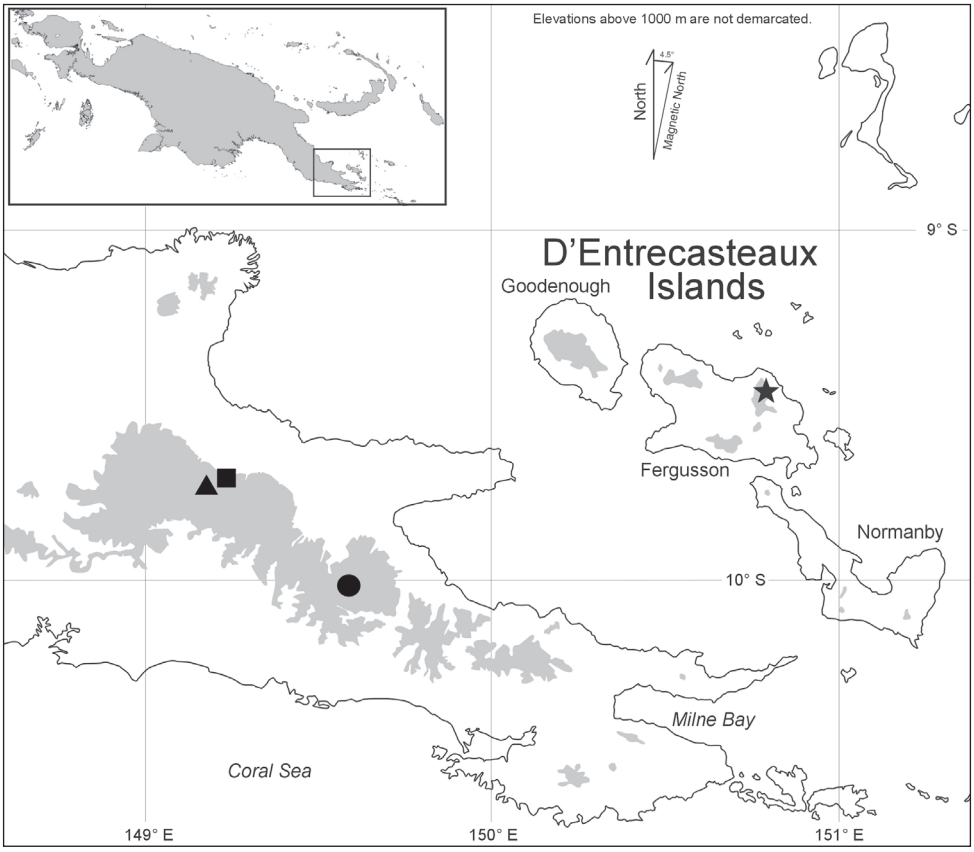

Figure 3. Map of southeastern Papua New Guinea, showing type localities for *Paedophryne dekot* (square) and *P. verrucosa* (triangle). Only known localities for the related and geographically proximate *P. kathismaphlox* (filled circle) and *P. oyatabu* (star) are shown for comparison.

Diagnosis. A minute microhylid (male SV = 8.1–8.9 mm, female SV = 8.8–9.3 mm) with highly pustulose dorsal skin and plantar surfaces; a relatively short leg (TL/SV = 0.37–0.42); short and broad snout (EN/SV = 0.067–0.080, IN/SV = 0.108–0.123, EN/IN = 0.60–0.70); wide head (HW/SV = 0.38–0.44), fifth toe distinctly shorter than second; relatively large discs on third and fourth toes (4thT/SV = 0.044–0.055); and a light-brown dorsum and sides flecked with black.

Comparisons with other species. *Paedophryne verrucosa* differs from all other members of the genus in its warty plantar surfaces and in having the fifth toe distinctly shorter than the second; it further differs from *P. kathismaphlox* in its smaller size (male SV = 8.1–8.9 mm in *P. verrucosa*, 10.1 mm in *P. kathismaphlox*; female SV = 8.8–9.3 mm in *P. verrucosa*, 10.4–10.9 mm in *P. kathismaphlox*), more heavily warty dorsal skin, shorter snout (IN/SV = 0.108–0.123 in *P. verrucosa*, 0.087–0.099 in *P. kathismaphlox*; EN/SV = 0.067–0.080 in *P. verrucosa*, 0.78–0.80 in *P. kathismaphlox*), larger discs on third and fourth toes (4thT/SV = 0.044–0.055 in *P. ver-*

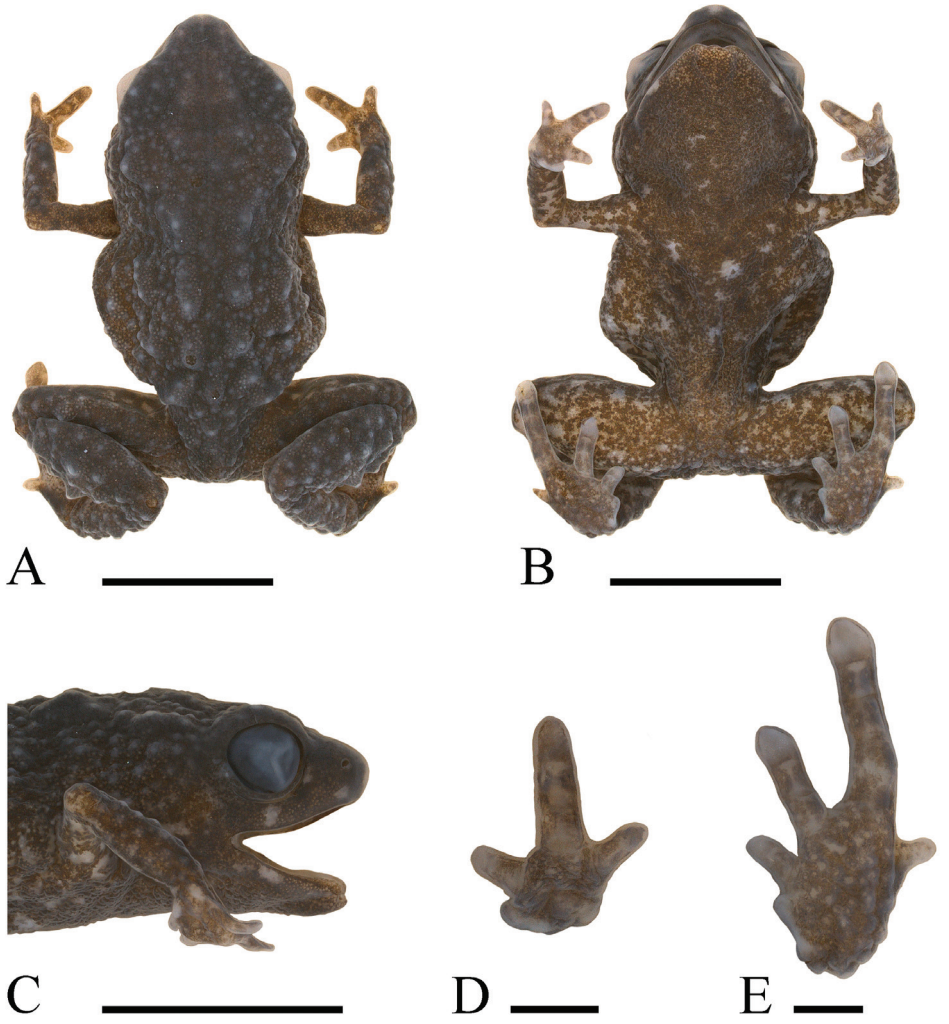

Figure 4. A Dorsum, **B** ventrum, **C** side of head, **D** palmar view of left hand, and **E** plantar view of left foot of holotype of *Paedophryne verrucosa* (BPBM 37747). Scale bars = 5 mm **A–C** and 1 mm **D, E**.

rucosa, 0.032–0.037 in *P. kathismaphlox*), in having the lateral surfaces the same color as the dorsum (lateral surfaces sharply darker than dorsum and punctated with pale gray in *P. kathismaphlox*), and in generally lacking a colored patch below anus (tan in one specimen of *P. verrucosa*, burnt-orange patch below anus in all *P. kathismaphlox*). The new species further differs from *P. oyatabu* in its smaller size (female SV = 8.8–9.3 mm in *P. verrucosa*, 11.3 mm in *P. oyatabu*), heavily warty dorsal skin (smooth in *P. oyatabu*), shorter snout (EN/SV = 0.067–0.080 in *P. verrucosa*, 0.062 in *P. oyatabu*, IN/SV = 0.108–0.123 in *P. verrucosa*, 0.097 in *P. oyatabu*), larger discs on third and fourth toes (4thT/SV = 0.044–0.055 in *P. verrucosa*, 0.031 in *P. oyatabu*), dorsum brown with black flecks (brown with two darker scapular chevrons

in *P. oyatabu*); it further differs from *P. dekot* in its heavily warty dorsal skin (smooth in *P. dekot*), shorter leg (TL/SV = 0.37–0.42 in *P. verrucosa*, 0.45–0.46 in *P. dekot*), wider head (HW/SV = 0.38–0.44 in *P. verrucosa*, 0.37–0.38 in *P. dekot*), and light-brown dorsum flecked with black (dorsum brown or red-brown with dorsolateral black triangular blotches in *P. dekot*).

Description of holotype. An adult male with vocal slits. Head wide (HW/SV = 0.43, Fig. 3A), with steeply oblique loreal region; canthus rostralis rounded, straight when viewed from above; nostrils directed anterolaterally, closer to tip of snout than to eyes; internarial distance much larger than distance from naris to eye (EN/IN = 0.60, IN/SV = 0.112, EN/SV = 0.067); snout somewhat pointed, sharply rounded when viewed from the side or from above (Fig. 4A, C); eyes moderately large (EY/SV = 0.13; EY/SN = 1.0, Fig. 4C), pupil horizontal; eyelid more than half width of inter-orbital distance; tympanum indistinct and small (TY/SV = 0.056), visible only when skin dries slightly, hidden posterodorsally. Skin granular and highly pustulose dorsally, granular to slightly pustulose ventrally; supratympanic fold absent. Fingers unwebbed, flattened; F1 very reduced in size; relative lengths 3>2=4>1 (Fig. 4D); discs absent. Subarticular and metacarpal tubercles absent; plantar surfaces granular to slightly pustulose. Toes unwebbed; T3 and T4 with flattened discs and terminal grooves; disc of T4 not wider than penultimate phalanx. Second and fifth toes reduced in size, with round tip and no disc; T1 a vestigial nub; relative lengths of toes 4>3>2>5>1 (Fig. 4E). Subarticular and metatarsal tubercles absent, but plantar surfaces heavily pustulose. Hind legs rather short (TL/SV = 0.38, Fig. 4A). Tongue elongate, straplike, anterior one-third attached to floor of mouth.

In preservative, uniform dark brown above, lighter on sides, where many granules are light brown. Face dark brown with few pale-brown spots. Ventral surfaces and front and rear of thighs pale brown heavily flecked with dark brown, the latter most heavily concentrated on chin and throat. Iris black.

Measurements (in mm).—SV = 8.9, TL = 3.4, HW = 3.8, HL = 2.9, IN = 1.0, EN = 0.6, SN = 1.2, EY = 1.2, TY = 0.5, 4^{th} T = 0.49.

Variation. Females attain larger size and have smaller tympana than males (Table 1). Mensural variation in the sample is slight, and important differences are not obvious between the sexes.

In preservative, most specimens are somewhat lighter than the holotype, varying to medium brown dorsally, in which case a few small black blotches are evident. Three specimens directly fixed in alcohol each exhibit a pale gray-brown dorsum with a pair of black scapular blotches and a pair of black lumbar blotches, with a smaller mid-dorsal black blotch between the lumbar blotches. This pattern is evident in life in some specimens (Fig. 2D). Ventral coloration can be lighter in overall tone than seen in the holotype due to presence of fewer dark-brown flecks. Both tan and black spots may also occur sparsely in the ventral pattern. Three specimens have a pale-gray patch below the anus.

Color in life. Field notes for paratype BPBM 37743 (Fig. 2C): "Brown with black flecks; warty. Face and venter black with light-gray flecks, posterior of abdomen

brown. Rear of thighs brown, each with one large black spot." For paratype BPBM 37745 (Fig. 2D): "Dark brown with black markings, some tan flecks posteriorly and on legs, tan patch around anus. Venter charcoal gray and light gray." BPBM 37746 was light brown above with two black scapular triangles and no light anal patch. Its chin, throat, and chest were charcoal gray and its abdomen brown with light-gray punctations and dark-gray flecks. BPBM 37747 was dark brown above and also lacked a light-colored anal patch. Its venter was charcoal gray with light-gray flecks. BPBM 37748 was dorsally as for BPBM 37745 and ventrally as for BPBM 37746 and also lacked an anal patch. PNGNM 24121 was brown and black above and gray flecked with light brown below; PNGNM 24122 tan with black flecks above and gray with light-brown flecks below; BPBM 37749 also brown and black above and light and dark gray below.

Call. The advertisement call of the holotype was recorded. Each call is a single drawn-out pulsed note given in a long train, with call trains varying from 67–102 s in the two series recorded by me. To the human ear, each call sounds like a quick drag of a finger over a comb. Calls are brief, with average duration varying from 1.210–1.652 s between animals and ranging from 0.712–1.942 s overall (Table 2, Fig. 5). Calls are given at a rate of 0.19–0.46 notes/s, with faster rates at higher temperatures (Table 2). Intervals between calls were longer than the calls themselves and were shorter at higher temperatures, averaging 1.093 s (range 0.897–3.876 s) at 14.9 °C, and longer at colder temperatures, averaging 3.694 s (range 1.824–11.655 s) at 19.4 °C (Table 2). Calls are highly pulsed, with 15–36 pulses/call, each pulse lasting 0.032–0.066 s (Table 2), and they increase and decrease in maximum amplitude gradually, with maximum amplitude sustained over most of the call duration (Fig. 5A). The power spectrum was rather broad, with a dominant frequency varying from 6510–7890 Hz (Fig. 5B).

Etymology. The species name "verrucosa" is a Latin adjective meaning "full of warts".

Range. Known only from the southeastern slope of Mt. Suckling near the saddle where it joins Mt. Dayman to the southeast, Milne Bay Province, Papua New Guinea.

Ecological notes. *Paedophryne verrucosa* inhabits leaf litter on the floor of primary mid-montane rainforest, seeming to prefer the lower slopes of steep hillsides. Canopy at the type locality was approximately 35 m high; understory was dense, with fallen trees, *Nastus*, and melastomes common. This mid-montane forest begins at approximately 1200 m elevation, so *P. verrucosa* seems likely to inhabit forest down to that elevation.

This species typically called at dusk, even continuing through the deafening period of cicada calling at approximately 1800–1830 h, but calling ceased soon after dark. It also frequently called before dawn, and occasional individuals were heard to call briefly in mid-morning. It was not heard by me to call on days lacking rain.

All three females contained two enlarged, well-yolked, cream-colored eggs and approximately a dozen small white oocytes.

Table 1. Mensural data for type series of *Paedophryne verrucosa*.

Character	Males (n = 9)		Females (n = 3)	
	mean	range	mean	range
SV (mm)	8.5	8.1–8.9	9.0	8.8–9.3
TL/SV	0.40	0.37–0.42	0.39	0.39–0.40
EN/SV	0.070	0.067–0.074	0.074	0.068–0.080
IN/SV	0.12	0.11–0.12	0.11	0.11–0.11
SN/SV	0.13	0.12–0.14	0.13	0.13–0.14
TY/SV	0.057	0.048–0.062	0.044	0.043–0.045
EY/SV	0.14	0.12–0.14	0.14	0.13–0.15
HW/SV	0.41	0.38–0.44	0.39	0.38–0.40
HL/SV	0.33	0.32–0.35	0.32	0.30–0.33
4thT/SV	0.049	0.045–0.055	0.044	0.044–0.044
EN/IN	0.61	0.60–0.67	0.67	0.60–0.70
HL/HW	0.79	0.76–0.85	0.81	0.80–0.83

Table 2. Call data for two specimens of *Paedophryne verrucosa* from Mt. Suckling. Numbers for call parameters are mean (range).

Specimen	Temperature (°C)	Number of calls	Calling duration (s)	Call rate (calls/s)	Call duration (s)	Interval between calls (s)	Number of pulses/ call	Number of pulses/ call	Pulse length (s)	Dominant frequency (kHz)
BPBM 37747	14.9	13	66.7	0.19	1.652 (0.985–1.942)	3.694 (1.824–11.655)	30.3 (15–36)	30.3 (15–36)	0.055 (0.051–0.066)	7.27 (6.51–7.60)
uncap-tured	19.4	47	102.1	0.46	1.210 (0.712–1.315)	1.093 (0.897–3.876)	29.8 (22–32)	29.8 (22–32)	0.040 (0.032–0.043)	7.65 (7.35–7.89)

Discussion

These two new frog species are at the lower size limit known for tetrapods and appear to marginally extend that limit. The smallest known tetrapods are all frogs (Lehr and Coloma 2008, Kraus 2010), with the smallest known amniotes being the lizards *Sphaerodactylus ariasae* Hedges and Thomas and *S. parthenopion* Thomas. These lizards attain snout-vent lengths of 18 mm in both sexes (*S. ariasae*, Hedges and Thomas 2001) and 16 mm in males and 18 mm in females (*S. parthenopion*, Schwartz and Henderson 1991). These are approximately the same size as the smallest known salamander, *Thorius arboreus* Hanken and Wake, with average adult size of 17 mm snout-vent length (Hanken and Wake 1994). The only other frog comparable in size to the two new species of *Paedophryne* described herein is *Brachycephalus didactylus* Izecksohn, for which maximum body sizes are slightly

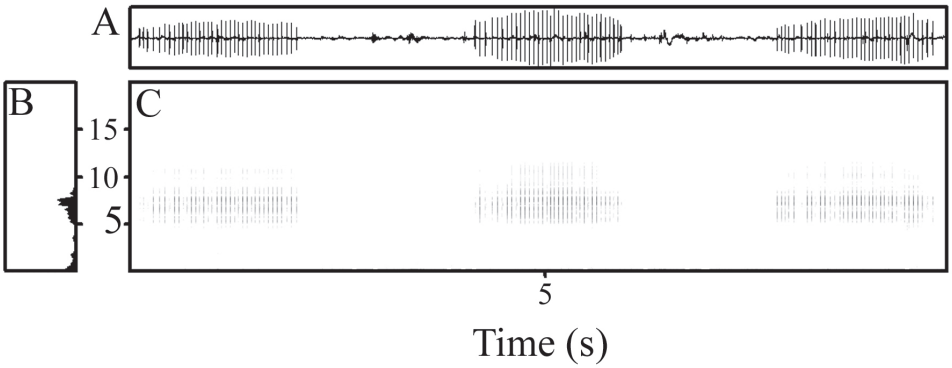

Figure 5. A Waveform, **B** power spectrum, and **C** spectrogram (frequency axis in kHz) of three calls of holotype of *Paedophryne verrucosa* (BPBM 37747) recorded on southeastern slope of Mt. Suckling, 27 March 2011, air temperature 14.9 °C.

larger (9.5 mm in males, 10.7 mm in females, Almeida-Santos et al. 2011). Almeida-Santos et al. (2011) present a considerable range of body sizes for *B. didactylus* (6.6–10.7 mm), with mean male sizes of 8.2 and 6.7 mm in two populations and mean female sizes of 10.1 and 8.8 mm in the same populations. However, these authors apparently included juveniles and adults together when deriving their averages, so it is uncertain how well those numbers approximate mean adult body sizes or the range of adult sizes. Furthermore, it is unclear from their study whether body measurements were taken before or after fixation – the former would be expected to introduce greater measurement error because of the malleability of flaccid frogs. In any event, the two new *Paedophryne* species appear to be slightly smaller than *B. didactylus*, as measured by maximum body sizes, although the range of male body sizes seen in *B. didactylus* appears to widely overlap that seen in the two *Paedophryne* species.

As discussed earlier (Kraus 2010), the useful tabulation of the world's smallest frogs provided by Lehr and Coloma (2008) overlooked several species from New Guinea, and at least two more species from Borneo also fall in the size range covered by their table (Das and Haas 2010; Matsui 2011). To better reflect body-size information on minute frogs globally, I present in Table 3 these missing records formatted in accord with the presentation provided by Lehr and Coloma (2008). Nineteen species are involved, which represent an increase of almost 50% in the numbers of minute species tabulated by Lehr and Coloma (2008). And I have at hand at least four other minute species of Papuan asterophryines that remain to be described but are omitted from Table 3.

It is uncertain whether the presence of so many minute frogs in the Papuan region represents a biological oddity of that region or whether similar frogs have simply been overlooked or underappreciated elsewhere. Given the difficulty of locating miniaturized frogs in the field and the rate at which they've been discovered during the past 15 years, additional miniaturized species no doubt await discovery or description in other poorly surveyed areas of the tropics. For example, further species are known in the diminutive Mada-

gascan *Stumpffia* but have been awaiting description for years (Wollenberg et al. 2008; S.-H. Wu, pers. comm.), and Lehr and Catenazzi (2009) discuss the likelihood that additional miniaturized frogs will be discovered in the Andean cloud forests. Yet, because much attention has been focused over the years on the minute frogs in the genera *Brachycephalus*, *Eleutherodactylus*, and *Sooglossus* (with less attention given to the Madagascan *Stumpffia*), and far more herpetofaunal survey work has been focused on the New World and Asian tropics than on the Papuan and African regions, it seems likely that a disproportionately large complement of overlooked miniaturized frog species is not awaiting discovery in the former regions. However, that remark must be tempered by recognition that diminutive frogs are unlikely to disperse widely, are thus liable to diversification over relatively small areas of mountainous terrain (Lehr and Catenazzi 2009), and, hence, may have hidden diversity in poorly surveyed upland areas of the Neotropics and Southeast Asia.

Given these observations, I tentatively suggest that the remarkable diversity of miniaturized frogs may represent a biological oddity of the Papuan region. Not only are a surprisingly large number of species involved, but the taxonomic diversity is also large: extremely diminutized frogs occur in seven genera of Papuan asterophryines (one genus omitted from Table 3 because its representative species is not yet described), whereas only 12 genera are involved across the remainder of the globe (Lehr and Coloma 2008; Das and Haas 2010). The prevalence of miniaturization in New Guinea may perhaps reflect that open niches were widely available for multiple colonization by early asterophryines ca. 30 MY (van Bocxlaer et al. 2006; Roelants et al. 2007), or the accretionary geological history of New Guinea from formerly isolated island-arc systems (Pigram and Davies 1987; Davies et al. 1996, 1997) may have provided several independent geographic loci for origin of minaturized lineages. Clearer assessment of these options awaits better clarification of asterophryine phylogenetic relationships.

The connection between anuran miniaturization and exploitation of leaf-litter and moss habitats has been briefly discussed previously (Lehr and Coloma 2008; Lehr and Catenazzi 2009; Kraus 2010). The two species under present consideration also fit this pattern, with frogs of both species found active or heard calling only from leaf litter accumulated on the ground. It is worth noting, however, that not all miniaturized frogs are leaf-litter inhabitants: the Papuan *Cophixalus sisyphus* Kraus and Allison and an undescribed *Cophixalus* are arboreal or semi-arboreal (Kraus and Allison 2006, Kraus unpubl. data). Nonetheless, the strong connection with leaf-litter or moss habitats is suggestive that miniaturization is a frequently evolved means to exploit these habitats. Given that anurans with a large range of body sizes reside in leaf litter, it seems likely that the impetus for miniaturization is exploitation of new, minute food resources (e.g., mites) not available to larger frogs (Lehr and Coloma 2008). Given the high surface-area-to-volume ratios of these small frogs, it is hardly surprising that they are restricted to very wet tropical forests.

Also consistent with other diminutive frogs, the new species of *Paedophryne* exhibit clutch sizes at the lower end of those known for anurans. All five females of the two species (2 *P. dekot*, 3 *P. verrucosa*) each contained two enlarged ova and a complement of approximately one dozen tiny oocytes. This strongly suggests that clutch size in these species is one

Table 3. Body sizes of minute frog species omitted from the survey of Lehr and Coloma (2008) or described since that time. Included are species with body-size tendencies < 16 mm SV. All species are from New Guinea except for *Microhyla* (from Borneo).

Species	Max SV male	Max SV female	Mean SV males	Mean SV females	Mean SV males and females	Range males	Range females	Sample size males	Sample size females	References
Paedophryne dekot	–	9.0	–	8.8	–	–	8.5–9.0	–	2	this study
Paedophryne verrucosa	8.9	9.3	8.5	9.0	8.7	8.1–8.9	8.8–9.3	9	3	this study
Paedophryne kathismaphlox	10.1	10.9	–	10.6	10.5	–	10.4–10.9	1	3	Kraus 2010
Paedophryne oyatabu	–	11.3	–	–	–	–	–	–	1	Kraus 2010
Microhyla perparva	10.5	12.4	–	–	–	10.1–10.5	11.4–12.4	–	–	Matsui 2011
Oreophryne minuta	11.5	–	–	–	–	9.2–11.5	–	4	–	Richards and Iskandar 2000
Choerophryne allisoni	11.6	–	11.6	–	–	11.5–11.6	–	2	–	Richards and Burton 2003
Aphantophryne minuta	–	11.8	–	–	–	–	–	–	1	Zweifel and Parker 1989
Choerophryne burtoni	12.4	–	12.3	–	–	12.1–12.4	–	3	–	Richards et al. 2007
Microhyla borneensis	12.8	18.8	11.7	18.4	–	10.6–12.8	17.9–18.8	8	2	Das and Haas 2010
Cophixalus kethuk	13.5	15.0	12.9	13.9	13.4	12.4–13.5	13.2–15.0	6	5	Kraus and Allison 2009
Cophixalus sisyphus	14.1	13.6	13.3	13.6	13.4	12.0–14.1	13.5–13.6	24	2	Kraus and Allison 2006
Cophixalus linnaeus	14.7	16.7	14.1	15.6	14.7	13.4–14.7	14.9–16.7	4	3	Kraus and Allison 2009
Choerophryne arndtorum	14.8	–	13.8	–	–	11.2–14.8	–	13	–	Günther 2008

Species	Max SV male	Max SV female	Mean SV males	Mean SV females	Mean SV males and females	Range males	Range females	Sample size males	Sample size females	References
Choerophryne amomani	15.1	–	13.8	–	–	11.8–15.1	–	9	–	Günther 2008
Austrochaperina minutissima	15.8	16.6	15.5	–	15.7	15.0–15.8	–	4	1	Günther 2009
Cophixalus iovaorum	16.0	17.2	14.5	16.9	14.6	13.2–16.0	16.6–17.2	29	2	Kraus and Allison 2009
Cophixalus tomaiodactylus	16.1	16.6	14.2	15.7	14.7	13.2–16.1	14.2–16.6	11	5	Kraus and Allison 2009
Cophixalus desticans	16.2	19.1	14.6	18.4	14.8	13.1–16.2	17.6–19.1	32	2	Kraus and Allison 2009

or two, although it remains to be determined how frequently females deposit clutches. Other species of minute frogs (e.g., *Brachycephalus didactylus*, *Eleutherodactylus iberia* Estrada and Hedges, *E. limbatus* Cope, *E. orientalis* Barbour and Shreve) have even smaller clutches, with females producing only a single egg at a time (Estrada and Hedges 1996). *Brachycephalus didactylus* carries 2–6 mature ova and is surmised to perhaps lay single eggs on different days (Almeida-Santos et al. 2011). Small clutch size in these minute frogs is unsurprising inasmuch as each of these species has non-aquatic oviposition with direct development from eggs into froglets, such direct-developing frog species produce large eggs heavily endowed with yolk, and the minute size of such frogs will constrain the numbers of well-yolked eggs that a female can produce at a single time (Wells 2007). Even if female *Paedophryne* produce several clutches of eggs each year (known for other tropical direct developers, e.g., Townsend and Stewart 1994), these species would still appear to have intrinsically low demographic growth parameters, suggesting that should populations become seriously depleted they would have long recovery times. Nonetheless, from my observations, *P. verrucosa* at least is very common where it occurs, suggesting that the species may enjoy relatively high survivorship despite the seeming vulnerability conferred by its small body and clutch sizes.

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BOOK REVIEW

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Book review: A long-awaited book about spiders from the Asian part of Russia

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The authors of the book are well known scientists in Russian arachnology. Yuri Marusik has written more than 250 articles and several monographs. As a result of his work the number of known species from this region has increased from 500 at the end of the 1970s to approximately 2000 at present. He has described more than 400 new species and genera as well as having compiled check-lists for the majority of sub-regions of the Asian part of Russia. Yuri Marusik has reported many new records for the region and sometimes for the entire country. For example, many of the following families he recorded were previously unknown: Leptonetidae, Theridiosomatidae, Mysmenidae, Ctenidae, Cybaeidae, Oonopidae and Nesticidae.

The second author – Nikolay Kovblyuk – works primarily in the Crimea (Ukraine) but has written a number of articles with Yuri Marusik or by himself concerning many taxa from Siberia and the Russian Far East. The book was edited by Professor B.R. Striganova of the Russian Academy of Sciences and is dedicated to Dr Kirill Eskov who was the first Russian researcher to make significant contributions to our knowledge on the spider fauna of this region.

Researchers beginning the study of spiders in Russia, especially in its Asian region immediately encountered the problem of an absence of identification guides and a dearth of other literature containing summarized data known to date. Sometimes even assigning collected spiders to family level was problematic (when the genus was known this task was easily accomplished using Platnick's catalogue). Only one identification guide for spiders (covering a large area: the European part of the former USSR, and incorporating more than 1000 species) has been written in Russian, but this was published more than 40 years ago (Tyshchenko 1971). Since then spider classification has undergone major changes. The limits of many families have been revised, their number has almost doubled, many new taxa have been found and/or described. Moreover, Tyshchenko's guide covered only the European part of the country, so it is not particularly useful for the identification of spiders in families which do not occur to the east of the Ural Mountains. Therefore, the publication of a book with identification keys and descriptions of families for this previously uncovered region was a necessary and long-awaited event.

The book consists of an abstract, the contents, 4 main chapters, 5 appendices and the index. The abstract and contents are provided both in Russian and English, but the remaining text is in Russian only. In the introduction (Chapter 1) the authors give basic information about the order, the region covered, some historical data, and comment on the aim of the book. The detailed historical review is subdivided into two parts. The first includes information about the study of spiders in the whole of Russia and the second is restricted to the Asian part of the country. This section discusses the contributions from all arachnologists (past and present) which have researched the spider fauna of this region.

Beyond doubt, chapter 2, which explains the methods pertaining to research on spiders, will be extremely useful for all beginners and even professional arachnologists. At the beginning of this chapter the authors give a list of the equipment necessary for collecting spiders. This is followed by descriptions of all the main collecting methods: by hand in different habitats, sifting the litter, pitfall trapping, sweep net sampling and so on. Information about the best time to collect spiders, with regard to different habitats and seasons is also provided. Most of this chapter is devoted to methods of processing collected spiders: how to examine them using a microscope, how to extract the epigyne and so forth. The procedures for drawing and photographing the copulatory organs are discussed over several pages. At the end the authors give recommendations as two which literature should be used and how to recognize the spiders.

This chapter also includes a number of pictures illustrating different types of equipment for collecting and studying material, examples of drawings and so on. It is known to the first author of this review, that most of these images were supposed to have been printed in colour, but in the final version of the book they are black-and-white. However, this does not affect the merits of this chapter. There are descriptions of some original methods for collecting spiders which have never been described in the literature before. For example, the authors write that some interesting spiders can be collected from water while standing within the water body itself (surface of the water

should be at neck level). Also in this chapter the authors offer advice on the practicalities of studying spiders. They suggest the use of dishes with paraffin on the bottom in order to place a specimen or its copulatory organs in the proper position for taking pictures. Tiny glass beads (used in chromatography) are often used for this purpose in other countries, but they are not easy to obtain in Russia.

Chapter 3 covers the morphology and classification of spiders. One important aspect of this chapter are the keys for recognizing spider families that occur in Siberia and the Russian Far East. The authors provide five keys and a pictorial identification guide. Three of the keys are for all spider families (pictorial, dichotomous and multi-entry keys), one key is intended for araneoid families only and the last one is for families which are poorly recognized and utilizes easy characters such as eyes, spinnerets, legs and so forth. These keys certainly represent the most important part of the chapter and maybe even of the book as a whole, at least for non-arachnologists. Thanks to these keys anyone (student, arachnologist, amateur or entomologist) will be able to identify a spider to family level. Unfortunately there are no keys for genera. In our opinion it would be easy to make such keys at least for some of the families that are not so diverse in this region.

Chapter 4 is the most voluminous and occupies about half of the book. Here, descriptions of all families occurring in the Asian part of Russia are given as well as three additional families (Oecobiidae, Segestriidae and Zodariidae) that are currently unknown from this territory but may be expected to be found there in the future. All descriptions are made in a standard way similar to those in the books by Dippenaar-Schoeman and Jocqué (1997) and Jocqué and Dippenaar-Schoeman (2006). Each subsection includes diagnostic characters of a particular family, a list of taxa within a family, description, genera known, distribution, details on biology, collecting methods, taxonomy, ways of differentiation of species and opportunities for further investigations. The description of each family is more detailed than in the two books mentioned above and comprise a lot of drawings and photos (up to nearly 30 per family) to illustrate the copulatory organs, details of external morphology as well as living spiders in their natural habitat. The big merit is that all the photos are printed in colour. Unfortunately, the high quality of the original photos has been lost in part while printing, therefore some images have superfluous contrast or look irregular (e.g. figs on pages 121, 125, 177, 259 and some more), and many of the black-and-white drawings have a greenish tint. In several cases the arrangement of some pictures seems strange. For example, the photo of *Cheiracanthium* sp. (Cheiracanthiidae) follows the description of Leptonetidae (p. 167), photos of two crab-spiders (Thomisidae) are located after Mysmenidae, a photo of *Marpissa pulla* (Salticidae) occurs on p. 249 in the Theridiosomatidae section, and so on. According to information from the senior author of the book this arrangement was made in order to occupy empty spaces, in cases where photos of spiders of a particular family were absent. However, this is going to be misleading because, taking the last example, the specific epithet *pulla* is not listed in the index and *Marpissa* is listed, but only as appearing on pages 224 and 316; thus the index provides no reference to the photograph which occurs out of systematic context within this

chapter. One genus (*Diphya*, Tetragnathidae) and one species (*Callobius hokkaidensis*, Amaurobiidae) are reported from Russia for the first time. Each description ends with the section "Prospects for further investigations". Here, useful information about how many described species can be found in the region, which scientist is studying this particular family in Russia and so on is given.

In spite of these small flaws the chapter is rather interesting and provides a wealth of information. In addition to recent families, the authors provide brief information about fossil spiders, including the two extinct families Juraraneidae and Lagonomegopidae, and the extant Mecicobothriidae, which have been found in the Asian part of Russia. For these families no illustrations are provided, which is a shame because this would have made this chapter a little more substantial, rather than appearing as a single, incomplete page tagged on at the end. The authors also overlooked the publication of Selden (2010) which described several well preserved specimens of Theridiosomatidae from Transbaikalia.

There are several appendices in the book. The first concerns the etymology of spider genus names and is rather interesting. Some of these etymologies are derived from Cameron (2005), but many of the names of taxa that do not occur in the Nearctic are original. In addition, there are lists of Russian-speaking arachnologists (including address and group of interest), web sites useful for recognizing spiders, books and a glossary of terms. The book ends with a comprehensive index to both genera and species.

In terms of the technical production, we have critiqued the quality of some of the images above. The paper has a nice, glossy finish, but unfortunately is a little too thin, resulting in show through from the opposite side of the page. This is not a big problem, but it does generate an upleasing finish to what was obviously a lot of hard work. The contents do not always match up with the pages on which the sections supposedly start. For example, the fossil families are indicated to start on page 278, but they actually occur on page 279. For some reason in the design process the decision was made to omit page numbers from pages which start a new section. This is rather bizarre because the contents should direct the reader to a numbered page at the start of a specific section. This has resulted in 52 pages without numbers, which equates to around 15% of all pages.

Despite the few small drawbacks mentioned above, publication of this book is an extremely important event for Russian arachnology, and it will doubtless form a benchmark reference work for the foreseeable future. In our opinion the book will be of great interest among Russian-speaking readers. It is possible that publication of this book will stimulate investigations in Russia and adjacent countries. For specialists who cannot read Russian text, the book will be useful because of the numerous, clear pictures (there are more than 600 illustrations, both in colour and black-and-white), the majority of which have not been published before. Thus, we recommend this book for all Russian-based zoological libraries, but also to other libraries of individuals and institutions that may have an arachnological research interest in this region. The book will also be of use to the Russian-speaking layperson with an interest in the natural history of spiders.

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