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**Research article**

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# *Phortica eparmata* species complex (Diptera, Drosophilidae) from the Oriental Region, with DNA barcoding information of Chinese species

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[1] urn:lsid:zoobank.org:author:5C60AC31-A76B-4072-8BD6-3BECEAB7BC54
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**Abstact.** A new species complex, the *eparmata* complex, is established within the subgenus *Phortica* s. str., based on eight known and five new species, all of which are endemic to the Oriental Region: *P. bipartita* (Toda & Peng, 1992), *P. eparmata* (Okada, 1977), *P. lanuginosa* Chen & Toda, 2007, *P. latipenis* Chen & Gao, 2005, *P. pangi* Chen & Wen, 2005, *P. setitabula* Chen & Gao, 2005, *P. unipetala* Chen & Wen, 2005 and *P. zeta* Chen & Toda, 2007; *P. jadete* sp. nov., *P. kava* sp. nov., *P. mengda* sp. nov., *P. wongding* sp. nov. and *P. yena* sp. nov. A key to all species of this complex is provided. Barcoding sequences (mitochondrial COI gene) were obtained for 22 specimens of five known and the five above-mentioned new species. The intra- and inter-specific pairwise K-2P (Kimura's two-parameter) distances of COI were determined. Phylogenetic analysis was performed using Bayesian inference based on COI sequences, confirming the monophyletic status of the *eparmata* complex, which is distinct from the species complexes of *magna*, *omega*, *variegata* and another two ungrouped species.

**Keywords.** DNA barcoding, drosophilid, East Asia, new species, systematics.

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## Introduction

The subgenus *Phortica* (sensu stricto) is the largest within the genus *Phortica* Schiner, 1862 and includes 81 worldwide species (Chen & Máca 2012). Up to the present, within the subgenus *Phortica*, 42 species have been classified into three species complexes (Chen & Máca 2012): the *magna* complex (Chen & Toda 1997), the *omega* complex (Chen & Toda 1998) and the *variegata* complex (Máca 1977). However, 39

species of the subgenus *Phortica* still cannot be assigned to any of the three aforementioned complexes. We discovered that eight of these unassigned species – *P. bipartita* (Toda & Peng, 1992), *P. eparmata* (Okada, 1977), *P. latipenis* Chen & Gao, 2005, *P. pangi* Chen & Wen, 2005, *P. setitabula* Chen & Gao, 2005 and *P. unipetala* Chen & Wen, 2005 from southern China, as well as *P. lanuginosa* Chen & Toda, 2007 and *P. zeta* Chen & Toda, 2007 from East Malaysia – share four morphological properties in males, distinct from the remaining species of the subgenus *Phortica*, i.e., anepisternum with a few setulae; arista lacking pubescence and branches on distal ½ (Figs 2A, 3A); aedeagal median rod expanded subapically (excluding *P. lanuginosa* as in Chen *et al*. 2007, fig. 29) (Fig. 2D, F); aedeagal basal bridge nearly tripartite (Figs 2D, 3D, 4C, 5C, 6C). Thus, based on these eight species, the *eparmata* species complex is established along with descriptions of five new species from Yunnan Province, southwestern China, which also share the above-mentioned morphological characters.

To further validate the monophyletic status of the *eparmata* complex, a total of 22 mitochondrial COI gene sequences from ten species affiliated with the subgenus *Phortica* collected in China are determined and supplied with a BOLD Process ID and GenBank accession number (Table 1). Species delimitations within the *eparmata* complex are supported by integrating barcodes with morphological information, in particular for the five new species which are considered to be cryptic. In addition, the phylogenetic relationship within the *eparmata* complex is constructed based on COI sequences. Six species belonging to the *magna*, *omega* and *variegata* species complexes and two ungrouped species of the subgenus *Phortica* are used as out-groups (Table 1).

## Material and methods

### Materials and morphological terminology

All adult specimens were collected while hovering around people's eyes in forests. Most of the specimens collected were identified as male, which could possibly be attributed to territorial behaviour. It is mainly males that are attracted to eyes of humans and some other mammals (e.g., cows, sheep, dogs, cats), black and shiny hair of humans, and reflective objects carried by humans (e.g., camera lenses, watches, black polyester accessories). When sucking sap on treetrunks, males are easily disturbed and attracted by human activity, leading to their capture. All of the photographs and line drawings were processed with the software Adobe Photoshop CS6 and SAI. The definitions of measurements, indices and abbreviations are explained in Chen & Toda (2001).

### Abbreviations

| | | |
|---|---|---|
| 4c | = | third costal section between R2+3 and R4+5/M1 between r-m and dm-cu |
| 4v | = | M1 between dm-cu and wing margin/M1 between r-m and dm-cu |
| 5x | = | CuA1 between dm-cu and wing margin/dm-cu between M1 and CuA1 |
| ac | = | third costal section between R2+3 and R4+5/distance between distal ends of R4+5 and M1 |
| adf | = | longest dorsal branch of arista/width of first flagellomere |
| arb | = | dorsal branches/ventral branches of arista |
| avd | = | longest ventral branch/longest dorsal branch of arista in length |
| BL | = | body length |
| C | = | second costal section between subcostal break and R2+3/third costal section between R2+3 and R4+5 |
| C3F | = | length of heavy setation in third costal section/(length of heavy setation in third costal section + length of light setation in third costal section). |
| ch/o | = | maximum width of gena/maximum diameter of eye |
| dcl | = | anterior dorsocentral/posterior dorsocentral in length |

**Table 1.** Specimens of *Phortica* used for sampling, including the DNA and accession numbers of the COI sequences.

| Species complexes | Species | Collection sites | BOLD process ID | GenBank accession numbers |
|---|---|---|---|---|
| ungrouped | *cardua* (Okada, 1977) | Diaoluoshan, Lingshui, Hainan | BDORK110–14 | KJ130757 |
| | *glabtabula* Chen & Gao, 2005 | Yixiang, Pu'er, Yunnan | BDORK111–14 | KJ130758 |
| *magna* | *antillaria* (Chen & Toda, 1997) | Dongyanshan, Taoyuan, Taiwan | BDORK018–14 | KJ130756 |
| | *foliata* (Chen & Toda, 1997) | Diaoluoshan, Lingshui, Hainan | BDORK127–16 | KY290274 |
| *omega* | *biprotrusa* (Chen & Toda, 1998) | Nankunshan, Longmen, Guangdong | BDORK026–14 | KJ130763 |
| | *omega* (Okada, 1977) | Dinghushan, Zhaoqing, Guangdong | BDORK033–14 | KJ130823 |
| *variegata* | *iota* (Toda & Sidorenko, 1996) | Yunmengshan, Huairou, Beijing | BDORK051–14 | KJ130800 |
| | *okadai* (Máca, 1977) | Kunlunshan, Yantai, Shandong | BDORK054–14 | KJ130816 |
| *eparmata* | *bipartita* (Toda & Peng, 1992) -1 | Jianfengling, Ledong, Hainan | BDORK112–14 | KJ130760 |
| (new) | *bipartita* (Toda & Peng, 1992) -2 | Yixiang, Pu'er, Yunnan | BDORK113–14 | KJ130759 |
| | *bipartita* (Toda & Peng, 1992) -3 | Chebaling, Shixing, Guangdong | BDORK128–16 | KY290275 |
| | *bipartita* (Toda & Peng, 1992) -4 | Muyiji, Ximeng, Yunnan | BDORK129–16 | KY290276 |
| | *eparmata* (Okada, 1977) | Dongyanshan, Taoyuan, Taiwan | BDORK006–14 | KJ130778 |
| | *pangi* Chen & Wen, 2005 -1 | Menglun, Mengla, Yunnan | BDORK115–14 | KJ130828 |
| | *pangi* Chen & Wen, 2005 -2 | Wulaoshan, Yingjiang, Yunnan | BDORK130–16 | KY290277 |
| | *setitabula* Chen & Gao, 2005 -1 | Muyiji Park, Ximeng, Yunnan | BDORK116–14 | KJ130858 |
| | *setitabula* Chen & Gao, 2005 -2 | Mengdong, Cangyuan, Yunnan | BDORK131–16 | KY290278 |
| | *unipetala* Chen & Wen, 2005 -1 | Guanlei, Menglan, Yunnan | BDORK132–16 | KY290279 |
| | *unipetala* Chen & Wen, 2005 -2 | Jinshan, Menglian, Yunnan | BDORK133–16 | KY290280 |
| | *jadete* sp. nov. -1 | Muyiji, Ximeng, Yunnan | BDORK134–16 | KY290281 |
| | *jadete* sp. nov. -2 | Botanic Garden, Ruili, Yunnan | BDORK136–16 | KY290282 |
| | *jadete* sp. nov. -3 | Likan, Ximeng, Yunnan | BDORK135–16 | KY290283 |
| | *kava* sp. nov. -1 | Muyiji, Ximeng, Yunnan | BDORK138–16 | KY290284 |
| | *kava* sp. nov. -2 | Muyiji, Ximeng, Yunnan | BDORK137–16 | KY290285 |
| | *mengda* sp. nov. | Wulaoshan, Lincang, Yunnan | BDORK139–16 | KY290286 |
| | *wongding* sp. nov. -1 | Muyiji, Ximeng, Yunnan | BDORK007–14 | KJ130860 |
| | *wongding* sp. nov. -2 | Muyiji, Ximeng, Yunnan | BDORK140–16 | KY290287 |
| | *wongding* sp. nov. -3 | Muyiji, Ximeng, Yunnan | BDORK141–16 | KY290288 |
| | *wongding* sp. nov. -4 | Muyiji, Ximeng, Yunnan | BDORK142–16 | KY290289 |
| | *yena* sp. nov. | Muyiji, Ximeng, Yunnan | BDORK143–16 | KY290290 |

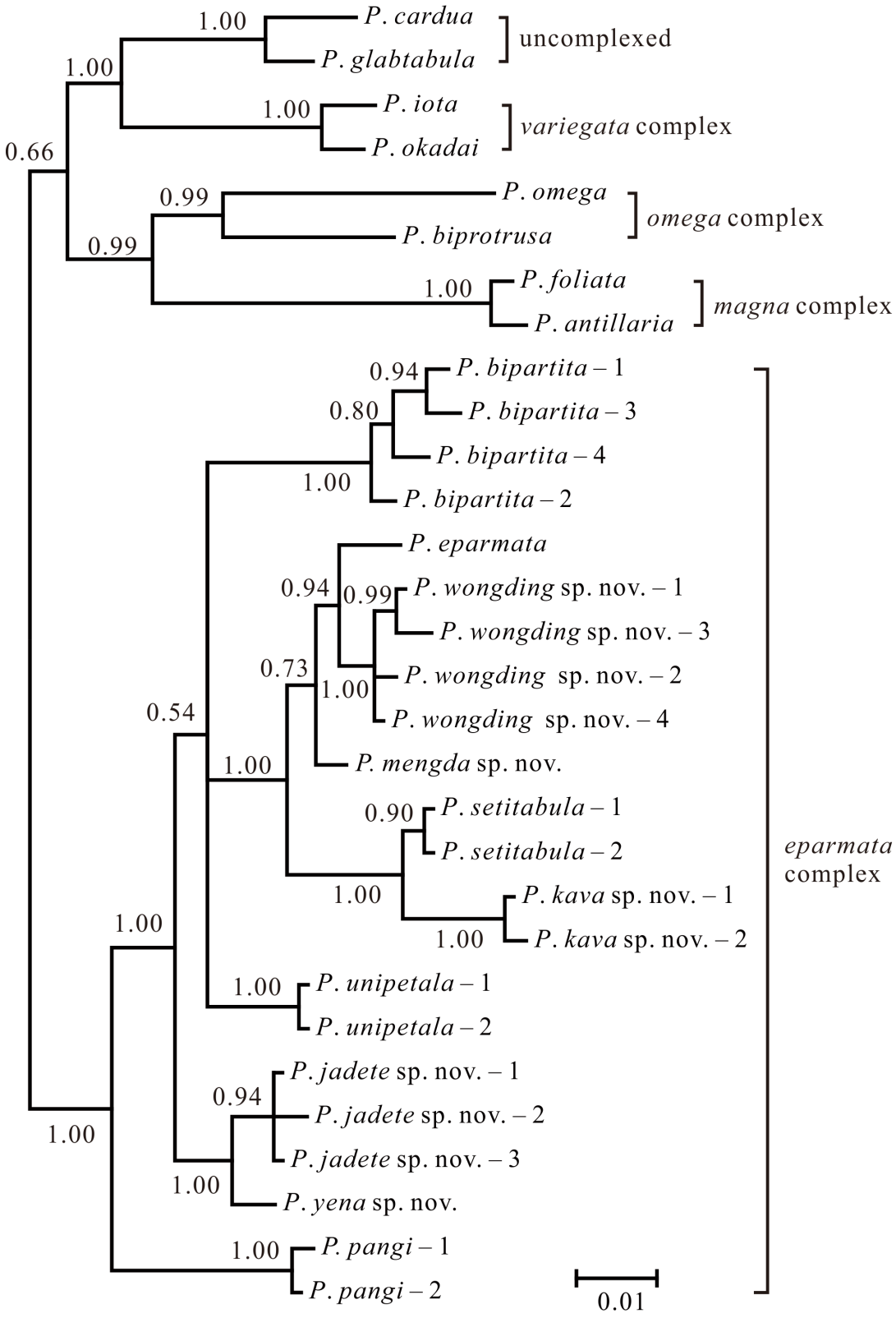


**Fig. 1.** Bayesian tree based on the combined data of *COI* gene. Numbers around the node indicate the Bayesian posterior probability; results lower than 0.5 are not shown.

**Table 2.** Summary of genetic distances. Intra. = intraspecific distance; Min. = minimum intraspecific distance; Max. = maximum intraspecific distance; SD = standard deviation; Min. inter. = minimum interspecific distance; Max. inter. = maximum interspecific distance; — = data not applicable.

| Species | Number of sequences | Intraspecific distance | | | | Interaspecific distance | | |
|---|---|---|---|---|---|---|---|---|
| | | Intra. | Min. | Max. | Mean (SD) | Min. | Max. | Mean (SD) |
| *P. bipartita* | 4 | — | 0.005 | 0.010 | 0.008 (0.002) | 0.031 | 0.055 | 0.039 (0.007) |
| *P. eparmata* | 1 | — | — | — | — | 0.010 | 0.043 | 0.027 (0.011) |
| *P. pangi* | 2 | 0.002 | — | — | — | 0.036 | 0.063 | 0.045 (0.008) |
| *P. setitabula* | 2 | 0.000 | — | — | — | 0.013 | 0.050 | 0.031 (0.011) |
| *P. unipetala* | 2 | 0.000 | — | — | — | 0.020 | 0.045 | 0.030 (0.009) |
| *P. jadete* | 3 | — | 0.000 | 0.003 | 0.002 (0.002) | 0.008 | 0.045 | 0.029 (0.009) |
| *P. kava* | 2 | 0.002 | — | — | — | 0.013 | 0.063 | 0.041 (0.013) |
| *P. mengda* | 1 | — | — | — | — | 0.008 | 0.040 | 0.024 (0.010) |
| *P. wongding* | 4 | — | 0.002 | 0.006 | 0.003 (0.002) | 0.008 | 0.042 | 0.028 (0.009) |
| *P. yena* | 1 | — | — | — | — | 0.008 | 0.047 | 0.029 (0.011) |

dcp = length distance between ipsilateral dorsocentrals/cross distance between anterior dorsocentrals
flw = length/width of first flagellomere
FW/HW = frontal width/head width
M = CuA1 between dm-cu and wing margin/M1 between r-m and dm-cu
orbito = distance between proclinate and posterior reclinate orbitals/distance between inner vertical and posterior reclinate orbital
presctl = prescutellar/posterior dorsocentral in length
prorb = proclinate orbital/posterior reclinate orbital in length
rcorb = anterior reclinate orbital/posterior reclinate orbital in length
sctl = basal scutellar/apical scutellar in length
sctlp = distance between ipsilateral scutellars/cross distance between apical scutellars
sterno = anterior katepisternal/posterior katepisternal in length
ThL = thorax length
vb = subvibrissal/vibrissa in length
WL = wing length
WW = wing width

Type specimens are deposited in the following institutions:

KIZ = Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming, China
SCAU = Department of Entomology, South China Agricultural University, Guangzhou, China

**DNA extraction and gene sequencing**

Total DNA was extracted from the abdominal tissue of a single individual after the dissection of the genitalia, using the Magen™ DNA extraction kit (Magen, China) and following the protocol provided by the manufacturer. The COI fragments were amplified using the cycle protocol described by Zhao *et al.*

(2009). The primer pair for PCR/sequencing is: 5'–CGCCTAAACTTCAGCCACTT–3' (Wang *et al.* 2006) and 5'–TAAACTTCAGGGTGACCAAAAAATCA–3' (Folmer *et al.* 1994).

### Sequence alignment and phylogenetic analysis

Sequences were aligned and edited using MEGA 5.05 (Tamura *et al.* 2011), resulting in a common frame length of 624 nucleotides which are shared by all of the samples. The intra- and inter-specific K–2P (Kimura's two-parameter; Kimura 1980) genetic distances were calculated in MEGA 5.05.

The molecular phylogeny was reconstructed using Bayesian Inference (BI) methods. Based on the Bayesian information criterion (BIC; Schwarz 1978), TrN+I, F81 and TIM2+G were selected as best-fit models for the 1st, 2nd and 3rd codon positions of the COI gene, respectively, using jModelTest 2.1.4 (Posada 2008). The BI analysis was carried out using MrBayes 3.2.6 (Ronquist & Huelsenbeck 2003), which was run on the CIPRES science gateway (http://www.phylo.org). Two independent Markov Chains Monte Carlo (MCMC) (Huelsenbeck *et al.* 2004) runs with 20 million generations were implemented in parallel, and sampling frequency of every 1000 generations was employed. After discarding the first 25% of MCMC generations as burn-in, tree topologies were summarized and the consensus tree was visualized using FigTree v1.4 (http://tree.bio.ed. ac.uk/software/figtree).

## Results

Based on the alignment (624 bp) of COI, 117 variable nucleotide positions are identified including 97 parsimony informative sites. The molecular phylogeny of COI based on Bayesian inference is shown in Fig. 1. Within the subgenus *Phortica*, four distinct clades corresponding to the *magna*, *omega*, *variegata* and *eparmata* complexes are identified on the basis of the Bayesian tree (Fig. 1). The 22 specimens of the *eparmata* complex, including the five new species described in the present study, are observed as a monophyletic clade supported by high posterior probability.

The range for intra- and inter-specific distances in the subgenus *Phortica* are 0.0%–1.0% and 0.8%–6.3%, respectively (Table 2), resulting in a slight overlap (0.8%–1.0%). In general, relatively small interspecific genetic distances are shown for the species within the *eparmata* complex. For instance, the minimum interspecific genetic distance between *P. jadete* sp. nov. and *P. yena* sp. nov. is only 0.8% (Table 2). *Phortica kava* sp. nov. is sister to *P. setitabula* (Fig. 1), and the minimum interspecific genetic distance between them is 1.3% (Table 2). Within a clade represented by *P. mengda* sp. nov., *P. wongding* sp. nov. and *P. eparmata* (Fig. 1), a low level of interspecific divergence was detected, ranging from 0.8%–1.5%.

### Taxonomy

Order Diptera Linnaeus, 1758
Family Drosophilidae Rondani, 1856
Subfamily Steganinae Duda, 1926
Tribe Gitonini Grimaldi, 1990
Genus *Phortica* Schiner, 1862

***Phortica eparmata*** species complex

### Diagnosis

Anepisternum with a few setulae; arista lacking pubescence and branches on distal ½ (Figs 2A, 3A); aedeagal median rod expanded subapically (excluding *P. lanuginosa* Chen & Toda, 2007; as in Chen *et al.* 2007, fig. 29) (Fig. 2D, F); aedeagal basal bridge nearly tripartite (Figs 2D, 3D, 4C, 5C, 6C).

**Description**

**Males and females**

Head. Eye brownish red. Ocellar triangle dark brown to black. Frons pollinose, grayish brown to black, with a few interfrontal setulae medially. Fronto-orbital plate often silvery white. Pedicel and first flagellomere grayish brown. Face grayish brown, with yellowish white patches on lower corners. Clypeus medially white to yellow, laterally dark brown to black. Gena grayish yellow to brown; postgena dark brown. Palpus somewhat triangular, grayish yellow distally, brown basally, with a few setae distally. Vibrissa prominent; other genal setae small.

Thorax. Mesoscutum and pleura grayish orange brown, with brownish to black patches and pollinose pattern. Postpronotal lobe pale yellow, with one long and a few short setae. Acrostichal setulae in ca. 6–8 irregular rows. Prescutellar setae usually one pair. Scutellum usually concolorous with thorax, with dark brown to black patch. Basal scutellar setae divergent; apicals cruciate.

Wing. Hyaline, sometimes smoky; veins grayish yellow. Basal medial-cubital crossvein present; $C_1$ setae 2, indistinctly differentiated. Costal vein with spinules on ventral surface between $R_{2+3}$ and $R_{4+5}$. $R_{2+3}$ slightly curved to costa at tip; $R_{4+5}$ distally convergent with $M_1$. Halteres white.

Legs. Yellow; femora usually brown to black except for apical portions; tibiae usually with three brown to black rings. Foreleg femur with 2–3 irregular rows of long setae on posterior surface. Preapical dorsal setae present on all tibiae. Midleg tarsus ventrally with two rows of minute cuneiform setulae on inner and outer sides; hindleg tarsus with one row of minute cuneiform setulae on underside; fore- and hindleg first tarsomeres each as long as three succeeding tarsomeres combined; midleg first tarsomere as long as other tarsomeres combined.

Abdomen. Tergites yellow to orange yellow; second to fifth tergites with broad brownish to black bands on posterior margins; sixth tergite nearly entirely dark, narrowed anterolaterally. Sternites usually grayish yellow.

Male terminalia. Epandrium almost not constricted mid-dorsally, with pubescence and setae; apodeme developed along anterior margins. Cercus almost oval, separated from epandrium, entirely pubescent and setigerous. Surstylus with numerous setae on outer surface. Membrane between epandrium and cercus pubescent. Hypandrium arched, usually with one pair of apodeme processes on anterior portion; posterior ends contiguous to lateral corners of gonopods and anteroventral corners of epandrium. Gonopods fused to each other, forming posteromedian plate, anteriorly forming vertical process. Parameres usually basally contiguous to anterior portion of hypandrium and tips of distally bifurcated ventral branch of aedeagal apodeme. Aedeagus composed of outer membranous tube and more or less sclerotized median rod; outer membrane posteriorly connected to vertical process of gonopod; median rod basally and/or medially connected to basal corners of gonopods by one or two pair(s) of bridges and basally contiguous to dorsal branch of aedeagal apodeme; basal bridge sometimes with sclerotized branch (Figs 2D, 3D, 4C, 5C, 6C); ventral bridge (termed inner paraphysis by Bächli *et al.* 2004) usually contiguous to medial process of aedeagus (as in Chen *et al.* 2007, figs 40, 44, 49, 53), sometimes elongated and dilated apically (as in Chen *et al.* 2007, fig. 57).

**Remarks**

For the new species described herein, only characters that depart from the above universal description are provided for brevity.

***Phortica* (*Phortica*) *jadete*** sp. nov.

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Fig. 2

**Diagnosis**

This species differs from the other species of this complex in having the paramere with one expanded, suberect lobe-like process submedially (Fig. 2D–E), and bifurcated submedially (Fig. 2D–E), the anterior branch with one strong, pointed tooth, the posterior branch with two acute processes.

**Etymology**

The name means "Emerald City", in reference to the type locality.

**Type material**

**Holotype**

CHINA: ♂, Yunnan, Ximeng, Muyiji Park, 22°37' N, 99°35' E, 1200 m a.s.l., 30 Apr. 2016, J. Huang leg. (SCAU, No. 124784).

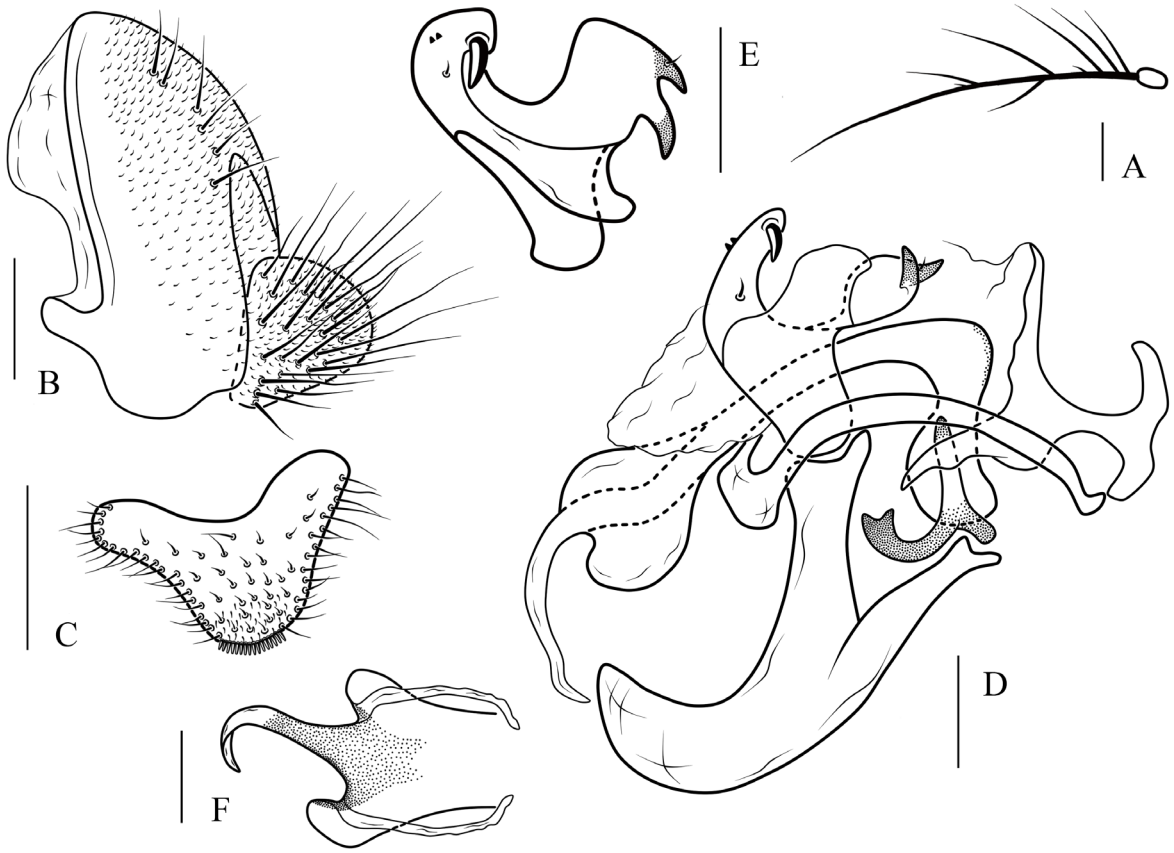


**Fig. 2.** *Phortica* (*Phortica*) *jadete* sp. nov., ♂. **A**. Arista. **B**. Epandrium and cercus (lateral view). **C**. Surstylus (frontal view). **D**. Hypandrium, paramere, aedeagus and aedeagal apodeme (lateral views). **E**. Paramere (frontal view). **F**. Tip of aedeagal median rod (ventral view). Scale bars = 0.1 mm.

**Paratypes**

CHINA: 1 ♂, Yunnan, Ximeng, Likan, 22°39’ N, 99°36’ E, 840 m a.s.l., 10 May 2016, Y.Q. Liu leg. (SCAU, No. 124785); 2 ♂♂, Yunnan, Ruili, Botanic garden, 24°1’ N, 97°51’ E, 1170 m a.s.l., 23 May 2016, Y.L. Wang and L. Zhu leg. (KIZ, Nos 0088085–86); 4 ♂♂, same locality, 22 Aug. 2016, H.W. Chen, L. Gong leg. (SCAU, Nos 124786–89); 3 ♂♂, Yunnan, Ruili, Bangdong, 24°6'9" N 97°50'55" E , 1370 m a.s.l. 6 Nov. 2017, H.W. Chen, L. Gong and B.X. Li leg. (SCAU, Nos 111455–57).

## Description

Male terminalia. Epandrium lacking pubescence anteroventrally, with six setae on dorsal portion per side (Fig. 2B). Surstylus with sparse pubescence and ca. 16 prensisetae on ventral margin (Fig. 2C). Aedeagal basal bridge tripartite: anterior lobe thick; median lobe slender; posterior lobe stout (Fig. 2D).

## Measurements

BL = 3.24 mm in holotype (range in 7 ♂♂ paratypes: 3.02–3.33), THL = 1.42 mm (1.38–1.60), WL = 2.40 mm (2.27–2.58), WW = 0.98 mm (0.93–1.11), arb = 5/1 (3–4/1), adv = 0.75 (0.70–0.73), adf = 1.85 (1.54–2.06), flw = 1.75 (1.57–2.06), FW/HW = 0.37 (0.43–0.47), ch/o = 0.11 (0.13–0.16), prorb = 0.86 (0.79–0.95), rcorb = 0.48 (0.41–0.58), vb = 0.56 (0.45–0.62), dcl = 0.52 (0.47–0.62), presctl = 0.66 (0.62–0.85), sctl = 1.09 (1.06–1.17), sterno = 0.73 (0.60–0.81), orbito = 1.29 (1.29–1.54), dcp = 0.30 (0.26–0.32), sctlp = 1.00 (0.86–1.08), C = 2.11 (1.86–2.25), 4c = 1.50 (1.50–1.74), 4v = 3.13 (2.71–3.36), 5x = 1.13 (1.00–1.29), ac = 3.00 (2.86–3.67), M = 0.75 (0.64–0.83), C3F = 0.75 (0.71–0.87).

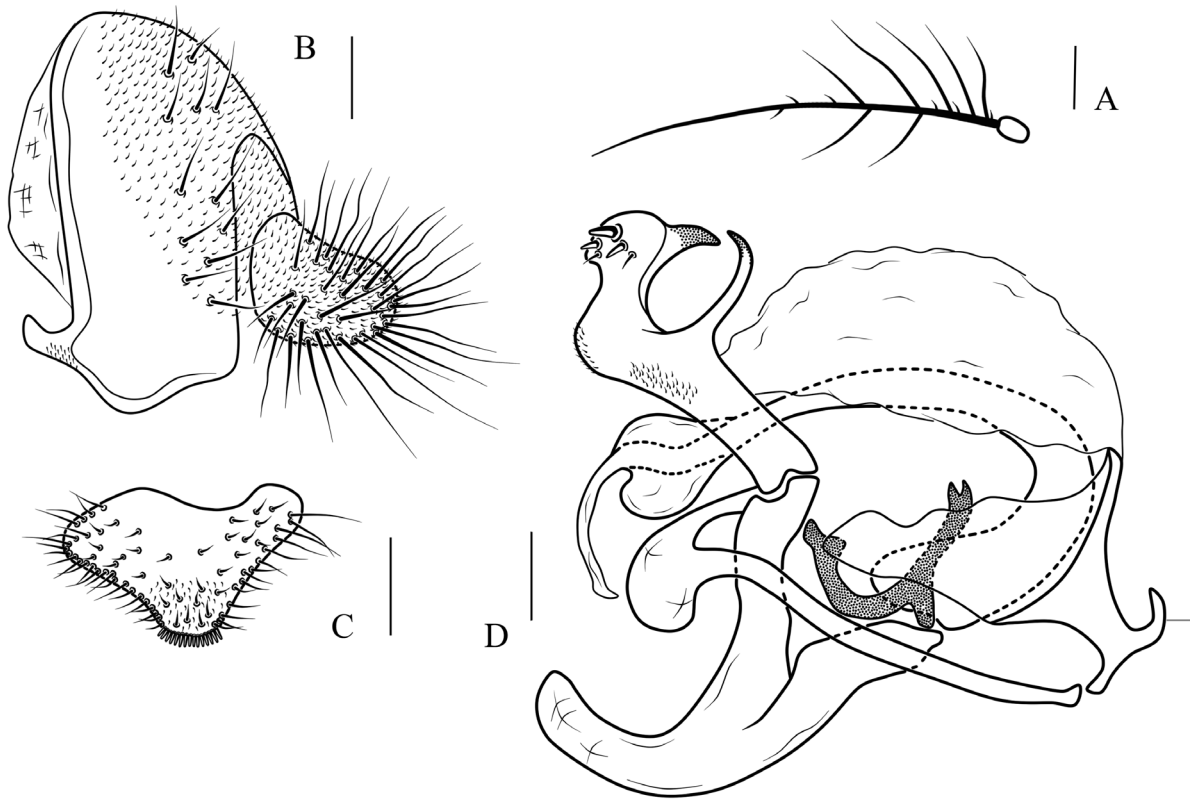


**Fig. 3.** *Phortica* (*Phortica*) *kava* sp. nov., ♂. **A**. Arista. **B**. Epandrium and cercus. **C**. Surstylus. **D**. Hypandrium, paramere, aedeagus and aedeagal apodeme. Scale bars = 0.1 mm.

***Phortica* (*Phortica*) *kava*** sp. nov.
urn:lsid:zoobank.org:act:23F4ACBD-767E-40ED-8A58-BF24E810B761
Fig. 3

**Diagnosis**

This species is very similar to *P. bipartita* in the male terminalia (Fig. 3B–D), but can be distinguished from the latter species by the paramere and body colour; in *P. kava* sp. nov., paramere mostly yellow, bifurcated from distal ⅓, the anterior branch with three pointed teeth apically (Fig. 3D); pleura mostly dark brown to black (in *P. bipartita*: paramere nearly black, bifurcated submedially, the anterior branch lacking teeth (as in Toda & Peng 1992, fig. 16); pleura mostly orange to orange brown).

**Etymology**

The name means "Live on hills", from the language of the Va community in Yunnan, China.

**Type material**

**Holotype**

CHINA: ♂, Yunnan, Ximeng, Muyiji Park, 22°37' N, 99°35' E, 1200 m a.s.l., 31 Mar. 2011, J.M. Lu leg. (SCAU, No. 124778).

**Paratypes**

CHINA: 7 ♂♂, Yunnan, Ximeng, Muyiji Park, 22°37' N, 99°35' E, 1200 m a.s.l., 30 Apr. 2016, J. Huang, Y.Q. Liu, Y.L. Wang and L. Zhu leg. (KIZ, Nos 0088083, 84; SCAU, Nos 124779–83).

**Description**

Male terminalia. Epandrium with sparse pubescence anteroventrally and ca 12 setae on dorsal to posterolateral portion per side (Fig. 3B). Surstylus with sparse pubescence and ca. 15 prensisetae on

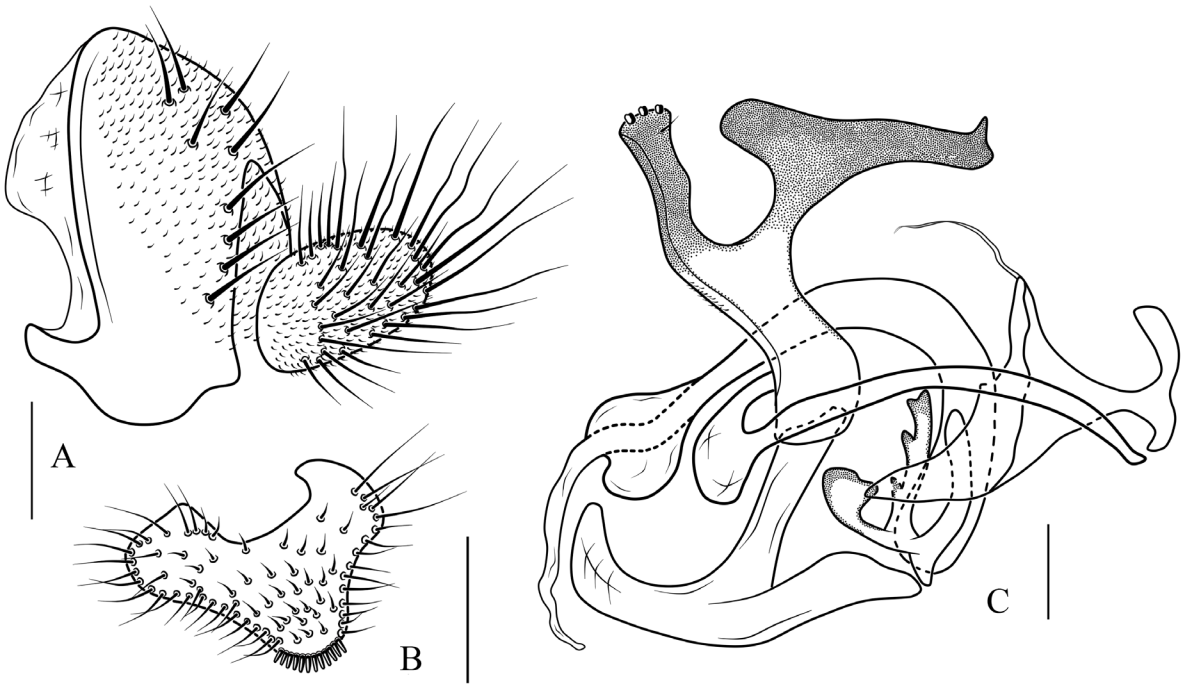


**Fig. 4.** *Phortica* (*Phortica*) *mengda* sp. nov., ♂. **A**. Epandrium and cercus. **B**. Surstylus. **C**. Hypandrium, paramere, aedeagus and aedeagal apodeme. Scale bars = 0.1 mm.

ventral margin (Fig. 3C). Aedeagal basal bridge tripartite: median lobe slender and bifurcated apically; posterior lobe stout (Fig. 3D).

**Measurements**

BL = 4.13 mm in holotype (range in 7 ♂♂ paratypes: 4.09–4.43), THL = 2.02 mm (2.07–2.22), WL = 3.11 mm (3.16–3.29), WW = 1.24 mm (1.24–1.44), arb = 4–5/1–2 (3–5/1–2), adv = 0.85 (0.64–0.83), adf = 1.71 (1.65–2.13), flw = 1.93 (1.65–2.13), FW/HW = 0.51 (0.47–0.51), ch/o = 0.17 (0.14–0.17), prorb = 0.88 (87–0.93), rcorb = 0.42 (0.38–0.42), vb = 0.49 (0.47–0.62), dcl = 0.58 (0.53–0.63), presctl = 0.81 (0.71–0.87), sctl = 0.95 (0.90–1.09), sterno = 0.68 (0.68–0.80), orbito = 1.36 (1.10–1.43), dcp = 0.27 (0.28–0.31), sctlp = 0.97 (1.00–1.11), C = 2.14 (2.00–2.33), 4c = 1.46 (1.27–1.48), 4v = 2.50 (2.20–2.56), 5x = 1.00 (0.88–1.06), ac = 2.40 (2.20–2.57), M = 0.68 (0.50–0.69), C3F = 0.61 (0.61–0.73).

***Phortica* (*Phortica*) *mengda*** sp. nov.

urn:lsid:zoobank.org:act:6A6EB859-A919-472D-A635-C0D4C947E171

Fig. 4

**Diagnosis**

This species is similar to *P. latipenis* in the male terminalia, it can be distinguished from the latter species by the paramere; in the new species, paramere black and bifurcated distally, the posterior branch strongly expanded submedially, with one acute projection apically (Fig. 4C) (in *P. latupenis*: paramere neither black nor expanded in posterior branch; as in Chen *et al.* 2005, fig. 7C).

**Etymology**

Toponym, according to the locality of type specimens in the language of the Dai community in Yunnan, China.

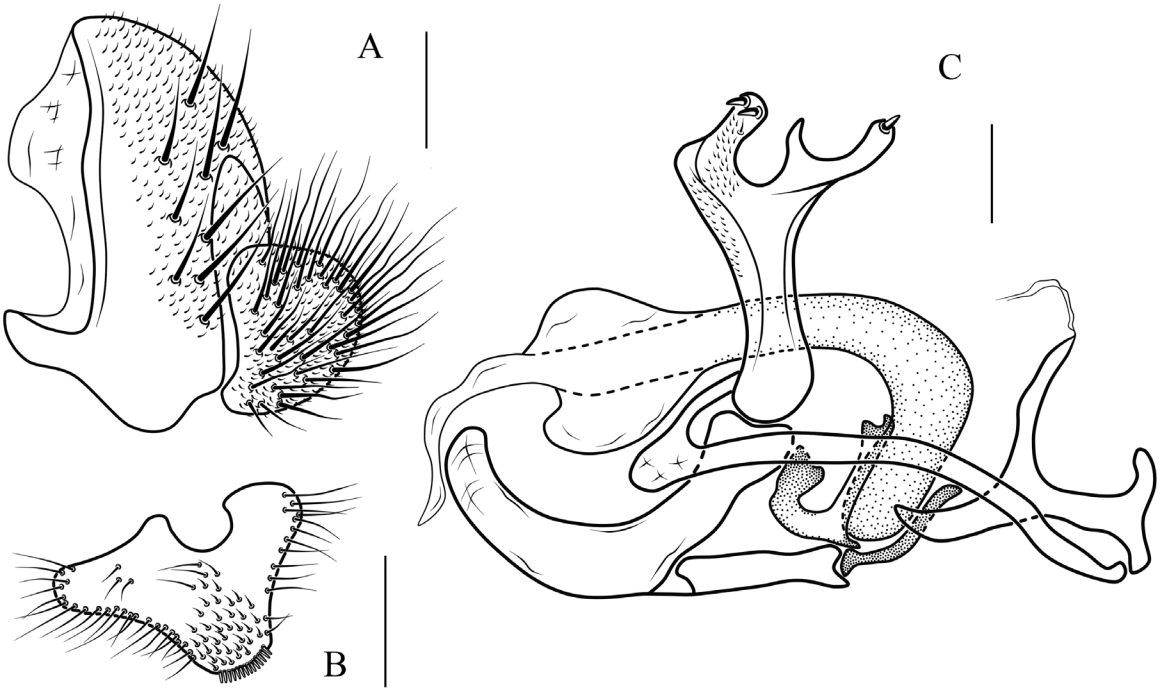


**Fig. 5.** *Phortica* (*Phortica*) *wongding* sp. nov., ♂. **A**. Epandrium and cercus; **B**. Surstylus; **C**. Hypandrium, paramere, aedeagus and aedeagal apodeme. Scale bars = 0.1 mm.

**Type material**

**Holotype**

CHINA: ♂, Wulaoshan, Lincang, Yunnan, 23°15' N, 100°11' E, 2150 m a.s.l., 14 May 2016, Y.Q. Liu leg. (SCAU, No. 124790).

**Description**

Male terminalia. Epandrium lacking pubescence anteroventrally, with ca. nine setae on dorsal to posterolateral portion per side (Fig. 4A). Surstylus lacking pubescence, with ca. 12 prensisetae on ventral margin (Fig. 4B). Paramere with three teeth and one sensillum apically in the anterior branch (Fig. 4C). Aedeagal basal bridge tripartite: anterior lobe expanded apically; median lobe with four projections; posterior lobe slender (Fig. 4C).

**Measurements**

BL = 3.64 mm in holotype, THL = 1.73 mm, WL = 2.93 mm, WW = 1.24 mm, arb = 5/2, adv = 0.75, adf = 1.88, flw = 1.76, FW/HW = 0.51, ch/o = 0.15, prorb = 0.88, rcorb = 0.42, vb = 0.47, dcl = 0.59, presctl = 0.76, sctl = 1.08, sterno = 0.64, orbito = 1.29, dcp = 0.28, sctlp = 1.13, C = 2.09, 4c = 1.54, 4v = 2.86, 5x = 1.06, ac = 2.53, M = 0.68, C3F = 0.71.

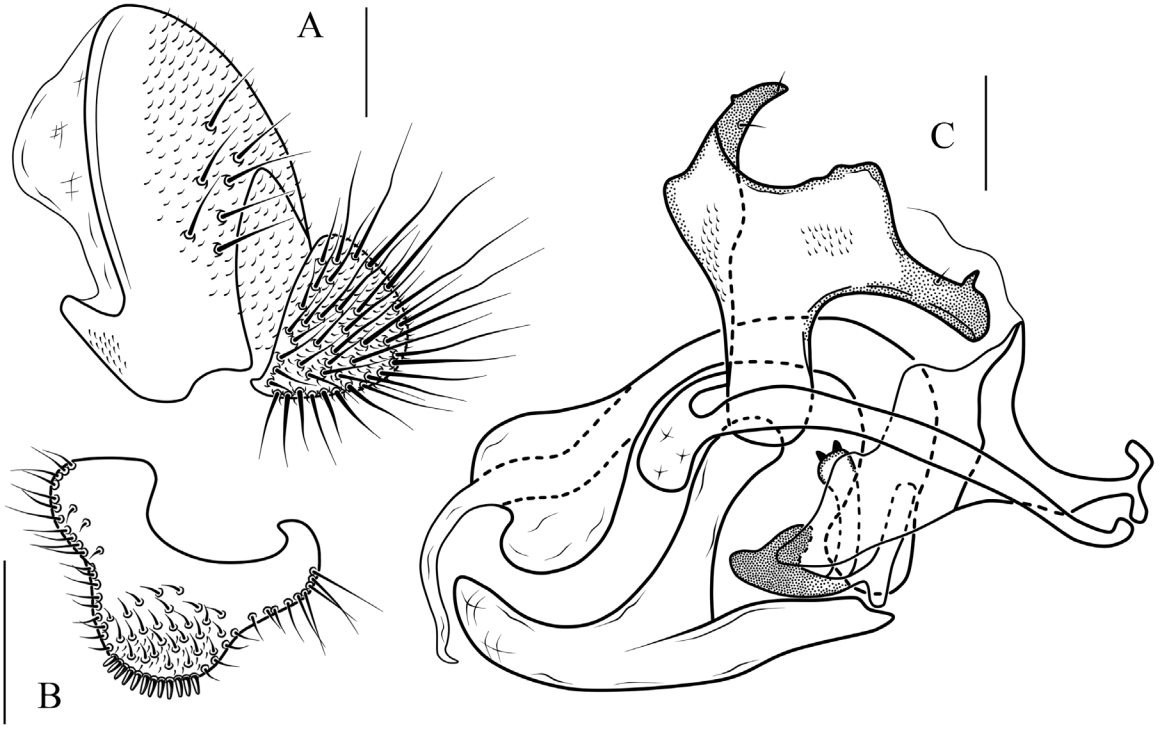


**Fig. 6.** *Phortica* (*Phortica*) *yena* sp. nov., ♂. **A**. Epandrium and cercus; **B**. Surstylus; **C**. Hypandrium, paramere, aedeagus and aedeagal apodeme. Scale bars = 0.1 mm.

***Phortica* (*Phortica*) *wongding*** sp. nov.

urn:lsid:zoobank.org:act:CB40EBE3-325B-45E0-B2D8-CB9A5C126A8D

Fig. 5

**Diagnosis**

This species resembles *P. eparmata* in male terminalia, but can be distinguished from it by the paramere and aedeagal basal bridge; in *P. wongding* sp. nov., posterior branch of paramere with one triangular process submedially, slender distally (Fig. 5C); while in *P. eparmata*, posterior branch of paramere with one quadrate process submedially, broadened distally (as in Okada 1977, fig. 3B).

**Etymology**

The name means "the place is surrounded by clouds", from the language of the Va community in Yunnan, China.

**Type material**

**Holotype**

CHINA: ♂, Muyiji Park, Ximeng, Yunnan, 22°37' N, 99°35' E, 1200 m a.s.l., 4 Apr. 2011,YR Su leg. (SCAU, No. 123274).

**Paratypes**

CHINA: 1 ♂ (SCAU, No. 123284), same data as holotype; 3 ♂♂, 29 Apr. 2016, J. Huang, Y.Q. Liu, Y.L. Wang and L. Zhu leg. (KIZ, Nos 0088087–89); 6 ♂♂, 11 Aug. 2016, same data as holotype, H.W. Chen, L. Gong and Y.Q. Liu leg. (SCAU, Nos 124791–96).

**Description**

Male terminalia. Epandrium lacking pubescence anteroventrally, with ca. nine setae on dorsal to posterolateral portion per side (Fig. 5A). Surstylus lacking pubescence, with ca. 14 prensisetae on ventral margin (Fig. 5B). Paramere pubescent, with two or three teeth and one sensillum apically in anterior branch, one tooth apically in posterior branch (Fig. 5C). Aedeagal basal bridge tripartite: anterior lobe thick; median lobe slender, concave apically; posterior lobe slender (Fig. 5C).

**Measurements**

BL = 3.56 mm in holotype (range in 10 ♂♂ paratypes: 3.47–3.87), THL = 1.69 mm (1.67–1.96), WL = 2.74 mm (2.62–3.02), WW = 1.17 mm (1.07–1.38), arb = 4–5/1–2 (4–5/1–2), adv = 0.67 (0.57–0.73), adf = 1.88 (1.75–2.14), flw = 1.66 (1.63–2.00), FW/HW = 0.45 (0.45–0.52), ch/o = 0.17 (0.14–0.18), prorb = 0.82 (0.80–0.92), rcorb = 0.45 (0.39–0.48), vb = 0.50 (0.50–0.60), dcl = 0.51 (0.44–0.58), presctl = 0.66 (0.58–0.71), sctl = 1.05 (1.00–1.07), sterno = 0.67 (0.67–0.83), orbito = 1.49 (1.29–1.50), dcp = 0.31 (0.25–0.31), sctlp = 1.07 (0.95–1.14), C = 2.05 (1.83–2.50), 4c = 1.57 (1.28–1.77), 4v = 2.86 (2.53–2.86), 5x = 1.00 (1.00–1.11), ac = 3.14 (2.86–3.29), M = 0.69 (0.56–0.69), C3F = 0.78 (0.67–0.80).

***Phortica* (*Phortica*) *yena*** sp. nov.

urn:lsid:zoobank.org:act:1F5BE800-3046-4980-AD96-9B8AA21C343B

Fig. 6

**Diagnosis**

This species is similar to *P. mengda* sp. nov. in the male terminalia but differs in paramere; in this species, anterior branch of paramere slightly pointed apically, lacking teeth, not bifurcated distally (Fig. 6C).

**Etymology**

From a girls name in the Va community of Yunnan, China.

**Type material**

**Holotype**

CHINA: ♂, Yunnan, Ximeng, Muyiji Park, 22°37' N, 99°35' E, 1200 m a.s.l., 11 Aug. 2016, H.W. Chen leg. (SCAU, No. 124797).

**Description**

Male terminalia. Epandrium with sparse pubescence anteroventrally and ca. seven setae on dorsal to posterolateral portion per side (Fig. 6A). Surstylus with pubescence and ca. 12 prensisetae on ventral margin (Fig. 6B). Paramere pubescent medially, secondary apically, each with one or two sensilla and one acute projection subapically in anterior and posterior branches (Fig. 6C). Aedeagal basal bridge tripartite: anterior lobe with strong projection subapically; median lobe with two small projections apically; posterior lobe thick (Fig. 6C).

**Measurements**

BL = 3.67 mm in holotype, THL = 1.71 mm, WL = 2.76 mm, WW = 1.20 mm, arb = 3/1, adv = 0.67, adf = 1.71, flw = 1.86, FW/HW = 0.48, ch/o = 0.14, prorb = 0.91, rcorb = 0.41, vb = 0.54, dcl = 0.61, presctl = 0.63, sctl = 1.07, sterno = 0.64, orbito = 1.25, dcp = 0.30, sctlp = 1.00, C = 2.11, 4c = 1.69, 4v = 3.05, 5x = 1.04, ac = 2.92, M = 0.77, C3F = 0.73.

## Key to all species of the Phortica eparmata species group

**Adults (males)**

1. Anepisternum lacking setulae ……other species of the subgenus ***Phortica***
– Anepisternum with setulae ……***eparmata* complex** …… 2

2. Aedeagal median rod not expanded subapically (as in Chen *et al.* 2007, fig. 29); anterior branch of aedeagal basal bridge with ca. 10 acute projections along margin (as in Chen *et al.* 2007, fig. 29) ……***P. lanuginosa*** Chen & Toda, 2007
– Aedeagal median rod expanded subapically (Fig. 2D, F); anterior branch of aedeagal basal bridge lacking acute projections ……3

3. Arista lacking ventral branch ……4
– Arista with ventral branch(es) ……5

4. Paramere submedially pubescent and expanded to two strong branches (as in Chen *et al.* 2005, fig. 7C) ……***P. latipenis*** Chen & Gao, 2005
– Paramere lacking pubescence, slightly bifurcated apically (as in Chen *et al.* 2005, fig. 8C) ……***P. setitabula*** Chen & Wen, 2005

5. Paramere with one expanded, suberect lobe-like process submedially (Fig. 2D, F) …… ……***P. jadete*** sp. nov.
– Paramere lacking expanded, suberect lobe-like process ……6

6. Paramere much expanded medially (as in Chen *et al.* 2005, fig. 9C; Chen *et al.* 2007, fig. 34) …7
– Paramere not expanded medially; aedeagal median rod lacking acute projections ……8

7. Paramere lacking process (as in Chen *et al.* 2007, fig. 34); aedeagal median rod with one pair of small acute projections submedially (as in Chen *et al.* 2007, fig. 33) …***P. zeta*** Chen & Toda, 2007
– Paramere with one small triangular process (as in Chen *et al.* 2005, fig. 9C); aedeagal median rod lacking projection submedially ……***P. unipetala*** Chen & Wen, 2005

8. Posterior branch of paramere slender, neither process nor projection (Fig. 3D) ……9
– Posterior branch of paramere thick, with process or projection ……10

9. Paramere mostly yellow, bifurcated from distal ⅓, the anterior branch with three pointed teeth apically (Fig. 3D); pleura mostly dark brown to black ……***P. kava*** sp. nov.
– Paramere nearly black, bifurcated submedially, the anterior branch lacking teeth (as in Toda & Peng 1992, fig. 16); pleura mostly orange to orange brown ……***P. bipartita*** (Toda & Peng, 1992)

10. Paramere strongly sclerotized distally or apically (Figs 4C, 6C) ……11
– Paramere not black ……12

11. Paramere deeply bifurcated distally, anterior branch truncate, with three teeth apically (Fig. 4C) ……..***P. mengda*** sp. nov.
Paramere not bifurcated distally, anterior branch pointed, lacking teeth apically (Fig. 6C) …… ……***P. yena*** sp. nov.

12. Paramere bifurcated distally (Fig. 5C); aedeagal median rod without processes subapically …13
– Paramere not bifurcated distally (as in Chen *et al.* 2005, fig. 10C); aedeagal median rod with a pair of triangular processes subapically (as in Chen *et al.* 2005, fig.10C) …***P. pangi*** Chen & Wen, 2005

13. Posterior branch of paramere with one triangular process submedially, slender distally (Fig. 5C) ……***P. wongding*** sp. nov.
– Posterior branch of paramere with one quadrate process submedially, broadened distally (as in Okada 1977, fig. 3B) ……***P. eparmata*** (Okada, 1977)

## Discussion

Systematics within the subgenus *Phortica* is complicated because the phylogenetic status of a batch of species is yet undetermined. Substantial work remains to be performed before we can understand phylogenetic relationships within the subgenus *Phortica* and conclude its relationships with other subgenera in the genus. In the present study, a new species complex, the *eparmata* complex, is established from previously unassigned species. The monophyly of the *eparmata* complex is credible and strongly supported by both molecular data and morphological characters. In particular, the *eparmata* complex clearly differs from the remaining species of the subgenus *Phortica* by the following morphological characters: anepisternum with several setulae; dorsal and ventral branches located at the sub-base of the arista; aedeagal median rod expanded subapically. Further evidence provided by the phylogenetic analysis (Fig. 1) validates the *eparmata* complex as monophyletic with robust support, distinct from the *magna*, *omega* and *variegata* species complexes and other ungrouped species. This will benefit systematic work within the subgenus *Phortica*. In addition, considering most species of the *eparmata* complex are found in Yunnan of China (Table 1), this again contributes support to the hypothesis that southern China, in particular Yunnan, could be the center of diversification for the subgenus *Phortica* (Cao *et al.* 2011).

Although the COI gene has been widely employed as a barcode to delineate species of insects, within the newly established *eparmata* complex, low levels of interspecific genetic divergences of mitochondrial COI gene were observed compared with other species in the present study (Table 2), for instance, 1.3%

between *P. setitabula* and *P. kava* sp. nov., 0.8% between *P. jadete* sp. nov. and *P. yena* sp. nov. However, obvious morphological differences were identified to distinguish species from each other, such as the shape of parameres for the two species pairs mentioned above, which also suggest potential reproductive isolation between them. Similar cases have been found among *P. epamata*, *P. wongding* sp. nov. and *P. mengda* sp. nov., and interspecific sequence divergences among these three species are below 1.5%, in contrast with the clear morphological differences between them, especially in parameres as described in the diagnoses of these species. Thus, in the present study, *P. jadete* sp. nov., *P. kava* sp. nov., *P. mengda* sp. nov., *P. wongding* sp. nov. and *P. yena* sp. nov. are considered to be new species. Similarly, some *Phortica* species distinct at the morphological level also show low inter-specific divergence in the mitochondrial COI gene, indicating a wide occurrence e.g., 1.6% between *P. variegata* (Fallén, 1823) and *P. semivirgo* Máca, 1977 (Otranto *et al.* 2008), 0.5% between *P. afoliolata* Chen & Toda, 2005 and *P. qingsongi* An & Chen, 2015, 0.2% between *P. hirtotibia* Cao & Chen, 2009 and *P. pinguiseta* Cao & Chen, 2009 (He *et al*. 2009), 0.1% between *P. panda* Cao & Chen, 2009 and *P. floccipes* Cao & Chen, 2009 (He *et al.* 2009). Although genetic divergence is low in these species of the genus *Phortica*, the differences in genital morphology are considered as the most reliable characters to delimit these species. The integration of morphological and DNA-based approaches has revealed an effective way to improve accuracy for species identification (An *et al.* 2015; Dayrat 2005; Lumley & Sperling 2010; Padial & Riva 2010). However, the present study raises concern as to the extent to which species delineation can be defined based on COI, which is a widely used gene for barcoding in *Drosophila*, and emphasizes the necessity of exploring potentially effective morphological diagnosis when lower resolution of molecular data is observed.

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