The Phortica (Phortica) hani species complex is established, with descriptions of six new species discovered from the Hengduan to the Qinling mountains, China: *Phortica floccipes* Cao & Chen, *Phortica hirtotibia* Cao & Chen, *Phortica longicauda* Cao & Chen, *Phortica longiseta* Cao & Chen, *Phortica panda* Cao & Chen, and *Phortica pinguiseta* Cao & Chen spp. nov. A key to all the species of the *hani* complex is provided based on morphological data. In addition, a ‘molecular’ key to seven species of this complex was also elaborated based on DNA sequences of the mitochondrial ND2 and COI genes. The relationships amongst these seven species, and their relationships to the *Phortica magna* and *Phortica variegata* species complexes, are analysed based on the ND2 and COI sequence data. The results suggest that the *P. hani* complex is monophyletic; the *P. magna* and *P. variegata* complexes are closer to each other than either is to the *P. hani* complex, and both are closer to the *P. hani* complex than the *Phortica foliiseta* species complex or the *Phortica varipes* species group.


**ADDITIONAL KEYWORDS:** China – mtDNA – new species – oriental region – Steganiniae.

**INTRODUCTION**

The species *Phortica (Phortica) hani* was first described from Yunnan, south-west China (Zhang & Shi, 1997). This species was thought to morphologically resemble *Phortica (Phortica) varipes* Duda, 1926 from Sumatra, Indonesia and *Phortica (Phortica) sobodo* Burla, 1954 from Cameroon by Máca (2003). However, Máca (2003) also indicated that *P. hani* is remotely related to the *varipes* group, as the surstylus of *P. hani* is not stick-like, and its aedeagal apodeme is not desclerotized apically. Recently, six new species of the subgenus *Phortica*, all strongly resembling *P. hani*, were discovered from high-elevation (c. 1600–2800 m) forest habitats in the Shennongjia Mountains in central China, and the Hengduan Mountains in south-west China. It is notable that these Chinese

---

*Corresponding author. E-mail: hongweic@scau.edu.cn
†These authors contributed equally to the present study.
(Fig. 13); (2) wing R₄₋₅ and M₁ veins nearly parallel; (3) fifth sternite of male with dense setae, notched posteromedially (Figs 7–12); (4) paramere with one spike-like process basally (Figs 15, 17, 19, 21, 23, 25); (5) aedeagus nearly membranous, basally fused to gonopods, distally with sensilla (Figs 15, 17, 19, 21, 23, 25).

MATERIAL AND METHODS

TYPE MATERIALS FOR NEW SPECIES

All specimen examined were captured whilst hovering in front of collectors’ eyes in the forest, and preserved in 70% ethanol. The type specimens were dried, pinned, and deposited in the following institutions: Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming, China (KIZ); Department of Entomology, South China Agricultural University, Guangzhou, China (SCAU) and Systematic Entomology, the Hokkaido University Museum, Hokkaido University, Sapporo, Japan (SEHU).

SAMPLES FOR MOLECULAR PHYLOGENETIC ANALYSES

Samples used for the molecular phylogenetic study are listed in Table 1. Each of the seven species of the hani complex is represented by one to seven individuals collected from the same or different localities. Máca (2003), after examining the type specimens Phortica helva [described afterwards by Chen & Gao in Cheng et al. (2008)], considered that this species should be included in the P. varipes group. Therefore, P. helva and one species from the foliiseta complex [i.e. Phortica speculum (Máca & Lin, 1993)], were selected as outgroups in our phylogenetic analyses. To test the monophyly of the hani complex, two species, Phortica magna (Okada, 1960) and Phortica okadai (Máca, 1977) from the magna and variegata complexes, respectively, were also included as ingroup taxa. With respect to some morphological characters, both the latter two complexes are closer to the hani complex than the above outgroup taxa.

DNA EXTRACTION, AMPLIFICATION, AND SEQUENCING

Total DNA was extracted from a single fly by the phenol-chloroform method, or from a tiny amount of tissue sampled from preserved specimens following the method of Wen & He (2003). The PCR cycle programme comprised an initial predenaturation at

### Table 1. Samples for molecular phylogenetic analyses, and data of collection sites and accession numbers of sequences

<table>
<thead>
<tr>
<th>Complex/group</th>
<th>Taxon sampled</th>
<th>Collection site</th>
<th>Longitude and latitude</th>
<th>Altitude (m)</th>
<th>GenBank accession nos.</th>
</tr>
</thead>
<tbody>
<tr>
<td>hani</td>
<td>floccipes-SH1 (♂)</td>
<td>Shennongjia, Hubei</td>
<td>31°49'N, 109°41'E</td>
<td>1900</td>
<td>EU431951 EU431925</td>
</tr>
<tr>
<td></td>
<td>floccipes-SH2 (♂)</td>
<td>&quot;</td>
<td>&quot;</td>
<td>1900</td>
<td>EU431955 EU431930</td>
</tr>
<tr>
<td></td>
<td>floccipes-SH3 (♀)</td>
<td>&quot;</td>
<td>&quot;</td>
<td>1900</td>
<td>EU431957 EU431933</td>
</tr>
<tr>
<td></td>
<td>floccipes-SH4 (♀)</td>
<td>&quot;</td>
<td>&quot;</td>
<td>1900</td>
<td>EU431954 EU431938</td>
</tr>
<tr>
<td></td>
<td>floccipes-SH5 (♀)</td>
<td>&quot;</td>
<td>&quot;</td>
<td>1900</td>
<td>EU431956 EU431940</td>
</tr>
<tr>
<td></td>
<td>floccipes-MS1 (♂)</td>
<td>Miyaluo, Sichuan</td>
<td>31°39'N, 102°49'E</td>
<td>2815</td>
<td>EU431952 EU431932</td>
</tr>
<tr>
<td></td>
<td>floccipes-MS2 (♀)</td>
<td>&quot;</td>
<td>&quot;</td>
<td>2185</td>
<td>EU431953 EU431929</td>
</tr>
<tr>
<td>hani-1 (♂)</td>
<td>Danba, Sichuan</td>
<td>30°41'N, 101°45'E</td>
<td>2670</td>
<td>EU431943 EU431926</td>
<td></td>
</tr>
<tr>
<td>hani-2 (♂)</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>2670</td>
<td>EU431944 EU431936</td>
</tr>
<tr>
<td>hirtotibia-1 (♂)</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>2670</td>
<td>EU431959 EU431922</td>
</tr>
<tr>
<td>hirtotibia-2 (♂)</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>2670</td>
<td>EU431960 EU431934</td>
</tr>
<tr>
<td>hirtotibia-3 (♀)</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>2815</td>
<td>EU431958 EU431923</td>
</tr>
<tr>
<td>hirtotibia-4 (♀)</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>2670</td>
<td>EU431961 EU431935</td>
</tr>
<tr>
<td>longicauda-1 (♂)</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>2670</td>
<td>EU431947 EU431927</td>
</tr>
<tr>
<td>longicauda-2 (♂)</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>2670</td>
<td>EU431948 EU431937</td>
</tr>
<tr>
<td>longicauda-3 (♀)</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>2670</td>
<td>EU431949 EU431939</td>
</tr>
<tr>
<td>longiseta (♂)</td>
<td>Shennongjia, Hubei</td>
<td>31°49'N, 109°41'E</td>
<td>1600</td>
<td>EU431950 EU431921</td>
<td></td>
</tr>
<tr>
<td>panda (♂)</td>
<td>Fengtongzhai, Sichuan</td>
<td>30°34'N, 102°53'E</td>
<td>1578</td>
<td>EU431945 EU431928</td>
<td></td>
</tr>
<tr>
<td>pinguiseta (♂)</td>
<td>Weixi, Yunnan</td>
<td>26°56'N, 99°23'E</td>
<td>1900</td>
<td>EU431946 EU431924</td>
<td></td>
</tr>
<tr>
<td>magna</td>
<td>magna (♂)</td>
<td>Tokyo, Japan</td>
<td>35°37'N, 139°20'E</td>
<td>50</td>
<td>EU431941 EU431919</td>
</tr>
<tr>
<td>variegata</td>
<td>okadai (♂)</td>
<td>&quot;</td>
<td>&quot;</td>
<td>50</td>
<td>EU431942 EU431920</td>
</tr>
<tr>
<td>foliiseta</td>
<td>speculum (♂)</td>
<td>Guanshan, Jiangxi</td>
<td>28°33'N, 114°57'E</td>
<td>00</td>
<td>EU500838 EU500839</td>
</tr>
<tr>
<td>varipes</td>
<td>helva (♂)</td>
<td>Xishuangbanna, Yunnan</td>
<td>21°41'N, 101°25'E</td>
<td>700</td>
<td>EU500837 EU500840</td>
</tr>
</tbody>
</table>
94 °C for 3 min; 35 cycles of amplification (denaturation at 94 °C for 45 s, annealing at 55 °C for 45 s, 1 min extension at 72 °C), and 5 min of sequence postextension at 72 °C. The primers for PCR and sequencing are listed in Table 2. PCR products were purified using Wizard PCR preps and then sequenced directly from two directions using an ABI-3730 DNA sequencer with an ABI Prism Big Dye cycle sequencing kit.

## Table 2. Primers used for PCR and sequencing

<table>
<thead>
<tr>
<th>Target gene</th>
<th>Primer</th>
<th>Primer sequence (5′–3′)</th>
<th>References</th>
<th>Utilities</th>
</tr>
</thead>
<tbody>
<tr>
<td>COI</td>
<td>COI-F1</td>
<td>CGCCTAACTTCAGCCACCTT</td>
<td>Present study</td>
<td>PCR/sequencing</td>
</tr>
<tr>
<td></td>
<td>COI-F2</td>
<td>ATCGCCTAACTTCAGCCAC</td>
<td>Wang et al., 2006</td>
<td>&quot;&quot;</td>
</tr>
<tr>
<td></td>
<td>COI-R1</td>
<td>CTTAATGCGTCATGAGAC</td>
<td>Present study</td>
<td>&quot;&quot;</td>
</tr>
<tr>
<td></td>
<td>COI-R2</td>
<td>TCCATTGCACTAATCAGCCA</td>
<td>Wang et al., 2006</td>
<td>&quot;&quot;</td>
</tr>
<tr>
<td>ND2</td>
<td>ND2-H1</td>
<td>AAGCTCTGGGTCCTATCC</td>
<td>Park, 1999</td>
<td>&quot;&quot;</td>
</tr>
<tr>
<td></td>
<td>ND2-T1</td>
<td>ATATTTACAGATTTGGAAGG</td>
<td>&quot;&quot;</td>
<td>&quot;&quot;</td>
</tr>
<tr>
<td></td>
<td>ND2-T2</td>
<td>GTTGGAAAGCTTATAGTT</td>
<td>Present study</td>
<td>&quot;&quot;</td>
</tr>
<tr>
<td></td>
<td>ND2-T3</td>
<td>AGGCATAGTGGTAGAAC</td>
<td>&quot;&quot;</td>
<td>&quot;&quot;</td>
</tr>
<tr>
<td></td>
<td>ND2-T5</td>
<td>CTGCAATTCTAAAGGAG</td>
<td>&quot;&quot;</td>
<td>&quot;&quot;</td>
</tr>
<tr>
<td></td>
<td>ND2-T6</td>
<td>GCTTTGAAAGCTTATAG</td>
<td>&quot;&quot;</td>
<td>&quot;&quot;</td>
</tr>
<tr>
<td></td>
<td>ND2-MF</td>
<td>TTTCAATTTTGGATT</td>
<td>&quot;&quot;</td>
<td>Sequencing</td>
</tr>
</tbody>
</table>

### Diagnosing species of the P. hani complex using DNA sequences

The 23 concatenated sequences of the ND2 and COI genes were aligned using the ClustalW (Thompson, Higgins & Gibson, 1994) method using the MegAlign 1.02 module in the DNAStar package (DNAStar Inc.). The alignment was 2565 (1026 for ND2 and 1539 for COI, respectively) nucleotide positions in length. The concatenated sequences of the two local samples of Phortica floccipes (i.e., P. floccipes SH3, SH4) show no variation across the whole alignment, and neither do the two sequences of P. hani (hani 1, 2), nor the three sequences of Phortica hirtotibia (hirtotibia 1, 2, and 3), whereas only one sequence for each group (floccipes SH4, hani 1, and hirtotibia 3, respectively) was selected, reducing the total sequence number for phylogenetic analyses to 19.

The above alignment was used for diagnostic character (nucleotide position) selection and the DNA substitution model test, as well for phylogenetic reconstruction. Nucleotide positions in either the ND2 or the COI partitions of the alignment were selected as characters to diagnose different taxa in the P. hani species complex, and a ‘molecular’ key elaborated according to the method of Zhao, Gao & Chen (2008).

### Phylogenetic reconstruction

The nucleotide compositions in the sequences of the ND2 and COI data sets were calculated in MEGA4 (Tamura et al., 2007). For all the following analyses, P. helva and P. speculum were used as outgroups. A Chi-square test of homogeneity of base frequencies across taxa was performed for ingroup + outgroup sequences, and also for ingroup sequences only. A partition homogeneity test (PHT; Farris et al., 1995) between the ND2 and COI sequences was performed with PAUP*4.0b10 (Swofford, 2001). The relevant reference about this test is supplied: Farris JS, Kallersjo M, Kluge AG, Bult C. 1995. Testing significance of incongruence. Cladistics 10: 315–319.

Nucleotide substitution models were selected for the whole data matrix, as well as for the character sets partitioned by gene loci or by codon positions, using MODELTEST3.7 (Posada & Crandall, 1998). Phylogenetic trees were constructed using the neighbor-joining (NJ), maximum parsimony (MP), and maximum likelihood (ML) methods in PAUP*4.0b10 (Swofford, 2001), or using the Bayesian inferring (BI) method in MrBayes3.12 (Ronquist & Huelsenbeck, 2003). In the NJ analysis, a ML estimator of distance was specified. In the MP analysis, the optimal tree was searched using the branch-and-bound algorithm, with the sequence of adding taxa specified as ‘furthest’. The ML tree was searched by the heuristic method, with initial trees obtained by randomly adding taxa, and the Tree Bisection and Reconnection (TBR) algorithm was used in branch swapping. The confidence for each node in the NJ, MP, and ML trees was accessed by bootstrap analyses (1000 replicates for the NJ and MP analyses, but only 500 replicates for the ML analysis) with a heuristic search. To access the confidence of ML tree selection, multiple optimal constraint trees were inferred and site-wise likelihoods calculated in
PAUP*4.0b10 (Swofford, 2001) for each constraint tree. The resulting scores of likelihoods were then subjected to an approximately unbiased (AU) test (Shimodaira, 2002) performed with the program CONSEL0.1 (Shimodaira & Hasegawa, 2001). In the Bayesian analysis, characters were partitioned into six sets in light of their affiliations to gene loci and codon positions, and therefore specific models were assigned for each set; a starting tree was randomly selected and four chains were run; two independent runs were implemented in parallel. The sample frequency was specified as every 100 generations and the number of chains as four. Both runs were stopped after 1 000 000 generations, when the average deviation of split frequencies fell well below 0.01. Based on the changing of the likelihood values, 1000 early-phase samples were discarded, and a majority rule tree showing all the compatible partitions was obtained.

SYSTEMATIC ACCOUNT
PHORTICA HANI SPECIES COMPLEX

Diagnosis: Arista with short dorsal branches (adf < 1.0) and sparse micropubesce, lacking ventral branches (Fig. 13); wing R_{4+5} and M_{1} veins nearly parallel; fifth sternite with dense setae, notched posteroomediately (Figs 6–12); cercus with two to three long, strong setae ventrally (Figs 14, 16, 18, 20, 22, 24); paramere with one spike-like process basally (Figs 15, 17, 19, 21, 23); aedeagus nearly membranous, basally fused to gonopods, distally with sensilla (Figs 15, 17, 19, 21, 23, 25).


Male terminalia: Epandrium not constricted mid-dorsally, with pubescence and setae (Figs 14, 16, 18, 20, 22, 24). Surstylus elongated, with several small setae distally. Cercus separated from epandrium, elongated ventrally, entirely pubescent and setigerous. Membrane between epandrium and cercus pubescent. Tenth sternite separated into lateral lobes. Hypantrium arched, apically slightly sunken, with one pair of apodeme processes on anterior portion; posterior ends contiguous to posterolateral corners of gonopods and anteroventral corners of epandrium (Figs 15, 17, 19, 21, 23, 25). Paramere apically with two small, acute processes, subbasally broadened and pointed, basally contiguous to anterior portion of hypantrium and ventral branch of aedeagal apodeme Gonopods fused to each other, forming posteromedial plate in T-shape. Aedeagus developed, curved ventrad, with numerous spinules. Aedeagal apodeme thick.

PHORTICA HANI (Zhang & Shi, 1997) (Figs 1, 7)

Material examined: CHINA: Holotype male, Pianma, Lushui, 26°01′N, 98°37′E, altitude (alt.) 2200 m, 28.vi.1993, L. X. Han (KIZ). one male, Ninglang, Yunnan, 27°38′N, 100°47′E, alt. 2200 m, 25.vi.2001, J. J. Gao (KIZ); nine males, Maoniuh, Danba, Sichuan, 14,15.ix.2005, M. F. Xu, H. L. Cao, and H. W. Chen (SCAU, No. 120010-18).

Distribution: China (Sichuan, Yunnan).

PHORTICA FLOCCIPES CAO & CHEN SP. NOV.
(Figs 1, 8, 13–15)

Description: Male and female. Head: frons black, with grey pollinose. Thorax: scutum almost black, with grey pollinose pattern. Scutellum black, with a pair of yellow patches submedially. Legs: hind tibiae with three black rings, three irregular rows of suberect setae on ventral surface, which are slightly shorter than tibia width (Fig. 2A), apically with one long curved seta (Fig. 2B). All fourth and fifth tarsomeres black, the rest grey-yellow. Male terminalia: cercus with two strong setae (Fig. 14). Surstylus medially
A KEY TO SPECIES OF THE Phortica hani SPECIES COMPLEX (MALES)

1. Arista with only short dorsal branches; wing R 4.5 and M 1 veins nearly parallel; fifth sternite notched postero- medi ally; cercus ventrally with two to three long, strong setae; paramere with one spike-like process basally; aedeagus nearly membranous (Phortica hani complex) .......................................................... 2

2. Hindleg tibia lacking a long seta apically (males only known); surstylus with one presnisseta apically; basal spike-like process of paramere lacking fine sawteeth ................................................................. 3

– Hindleg tibia with a long seta apically; surstylus apically with two presnissetae at least; basal process of paramere with fine sawteeth ......................................................... 4

3. Surstylus apically truncate; cercus ventrally with three long setae ........................................ Phortica longicauda Cao & Chen sp. nov.

– Surstylus apically pointed; cercus ventrally with two long setae. ........................................ Phortica longiseta Cao & Chen sp. nov.

4. Hindleg tibia apically with one row of short, scopiform setae on ventral surfaces (male only known); surstylus with two presnissetae apically ........................................................ Phortica panda Cao & Chen sp. nov.

– Hindleg tibia lacking scopiform setae apically; surstylus with three presnissetae at least .................. 5

5. Hindleg tibiae with three black rings and two to three rows of suberect setae on ventral surface, lacking long setae on posteroventral surface; surstylus not covered by epandrium ................................................................. 6

– Hindleg tibiae with two rings, lacking fringe-like setae on ventral surface, with one row of long setae on posteroventral surface, surstylus covered by epandrium .................................................. 7

6. Fringe-like setae of hindleg tibiae as long as 1.5x of tibia width; surstylus medially not protrudent, with two presnissetae ................................................................. Phortica hani (Zhang & Shi)

– Fringe-like setae of hindleg tibiae shorter than tibia width; surstylus medially protrudent, with seven to eight presnissetae arranged in two rows ........................................ Phortica floccipes Cao & Chen sp. nov.

7. Scutellum orange-brown, black along margin; hindleg tibiae basally to apically with one row of about 11 long setae on anteroventral surface which are about as long as the tibia width ................................................................................................................................. Phortica hirtotibia Cao & Chen sp. nov.

– Scutellum entirely blackish; hind tibiae distally with one row of about eight strong setae on anteroventral surface, most of which are shorter than the tibia width .... Phortica pinguiseta Cao & Chen sp. nov.

A KEY TO Phortica hani SPECIES COMPLEX BASED ON ND2 AND COI SEQUENCES

In the following key to seven species of the P. hani complex, character states at diagnostic sites were compared between a given pair of taxa. Each status depicts starts with a letter denoting its affiliated gene (N: ND2; C: COI), followed by a number (in subscript) indicating the site positions in the sequences of the affiliated gene, and then by the codon position of the site (in parentheses and in subscript), the nucleotide type (after a colon), and the corresponding amino acid type (after a slash, all shown by standard three-letter abbreviations). No character was found to diagnose between P. floccipes and P. panda based on their ND2 or COI sequences.

1. N 330 (3rd): A/Leu; N 627 (3rd): A/Leu; N 919 (1st): A/Met; C 204 (3rd): A/Val; C 534 (3rd): T/Arg; C 574 (1st): C/Leu; C 627 (3rd): T/Thr; C 705 (3rd): ...

2. N 292 (1st): A/Met; N 605 (2nd): T/Ile; N 996 (3rd): T/Ile; C 72 (3rd): T/Ala; C 279 (3rd): C/Pro; C 402 (3rd): T/Ile; C 573 (3rd): C/Phe; C 756 (3rd): C/Ile; ...

3. N 144 (3rd): T/Thr; N 333 (3rd): C/His; C 1023 (3rd): G/Gly; C 1089 (3rd): C/Ile; ...

4. N 441 (3rd): A/Lys; N 444 (3rd): T/Pro; N 795 (3rd): A/Gln; N 810 (3rd): C/Asn; C 201 (3rd): A/Met; C 456 (3rd): T/Gly; C 474 (3rd): A/Gly; C 537 (3rd): A/Met; C 645 (3rd): C/Ser; C 747 (3rd): T/Pro; C 855 (3rd): C/Asn; C 915 (3rd): A/Pro; C 957 (3rd): C/Val; C 1201 (3rd): T/Leu; C 1315 (3rd): T/Tyr; ...

5. N 405 (3rd): T/Ile; N 603 (3rd): C/Pro; N 651 (3rd): T/Thr; N 724 (3rd): T/Pro; N 915 (3rd): T/Thr; N 996 (3rd): T/Pro; C 504 (3rd): C/Val; C 568 (3rd): G/Val; C 712 (3rd): A/Leu; C 766 (3rd): T/Phe; C 819 (3rd): T/Tyr; C 894 (3rd): T/Asp; C 1002 (3rd): G/Pro; C 1030 (3rd): A/Asn; C 1089 (3rd): C/Ile; ...

6. N 441 (3rd): A/Lys; N 444 (3rd): T/Pro; N 795 (3rd): A/Gln; N 810 (3rd): C/Asn; C 201 (3rd): A/Met; C 456 (3rd): T/Gly; C 474 (3rd): A/Gly; C 537 (3rd): A/Met; C 645 (3rd): C/Ser; C 747 (3rd): T/Pro; C 855 (3rd): C/Asn; C 915 (3rd): A/Pro; C 957 (3rd): C/Val; C 1201 (3rd): T/Leu; C 1315 (3rd): T/Tyr; ...

7. N 405 (3rd): T/Ile; N 603 (3rd): C/Pro; N 651 (3rd): T/Thr; N 724 (3rd): T/Pro; N 915 (3rd): T/Thr; N 996 (3rd): T/Pro; C 504 (3rd): C/Val; C 568 (3rd): G/Val; C 712 (3rd): A/Leu; C 766 (3rd): T/Phe; C 819 (3rd): T/Tyr; C 894 (3rd): T/Asp; C 1002 (3rd): G/Pro; C 1030 (3rd): A/Asn; C 1089 (3rd): C/Ile; ...


Downloaded from https://academic.oup.com/zoolinnean/article-abstract/157/2/359/2623028 by guest on 11 March 2019
protrudent, with two rows of about seven to eight prensisetae, apically round, with four prensisetae (Fig. 14). Paramere submedially with three small processes, two of them with sensillum, apically with one sensillum; basal process with fine sawteeth (Fig. 15).

**Measurements:**  
BL = 3.70 mm in holotype (range in four male and five female paratypes: 3.50–4.20); ThL = 1.75 mm (1.60–2.00); WL = 3.30 mm (3.05–3.60); WW = 1.35 mm (1.30–1.75).

**Indices:**  
arb = 3/0 (3–6/0), adf = 0.79 (0.53–0.79), flw = 1.64 (1.54–2.00), FW/HW = 0.50 (0.49–0.53), ch/o = 0.13 (0.12–0.14), prorb = 1.21 (1.00–1.33), rcorb = 0.52 (0.38–0.53), vb = 0.36 (0.32–0.59), dcl = 0.52 (0.49–0.62), presctl = 0.44 (0.44–0.57), sctl = 1.05 (1.03–1.07), sterno = 0.81 (0.65–0.94), orbito = 1.70 (1.46–1.67), dcp = 0.26 (0.19–0.30), sc1tp = 0.87 (0.72–0.96), C = 2.54 (2.57–3.24), 4c = 1.37 (1.07–1.26), 4v = 2.50 (2.25–2.65), 5x = 0.88 (0.68–1.10), ac = 2.25 (1.69–2.08), M = 0.65 (0.49–0.76), C3F = 0.56 (0.43–0.60).

**Etymology:** A combination of the Latin words floccus and pes, referring to the hindleg tibia with suberect setae.

**Distribution:** China (Hubei, Sichuan).

**PHORTICA HIRTOTIBIA CAO & CHEN SP. NOV.**
(FIGS 3, 9, 16, 17)

**Material examined:** Holotype male, CHINA: Maoniuhe, Danba, Sichuan, 15.ix.2005, HW Chen (SCAU, No. 120031). Paratypes: 23 males, five females, same data as holotype except 15–16.ix.2005, H. L. Cao, H. W. Chen, and M. F. Xu (three males, two females in KIZ; the rest in SCAU, Nos 120032-54).

**Description:** Male and female. Head: frons almost black. Face black on upper part, orange-brown on lower part. Thorax: blackish, with silver-grey pollinose pattern. Scutellum orange-brown, black along margin, with silver-grey pollinosis. Legs: hind tibia with two black rings, one row of about 11 long setae on anteroventral surface which are about as long as the tibia width (Fig. 3C), basally with six thin, long

setae on posterolateral surface that are 1.5 times longer than the tibia width (Fig. 3D); apically with one long seta (Fig. 3B). All fifth tarsomeres black, the rest grey-yellow. Male terminalia: epandrium with much elongated ventral margin, covering surstylus (Fig. 16). Cercus with three strong setae (Fig. 16). Surstylus with four prensisetae apically (Fig. 16). Paramere submedially with one small process bearing
one sensillum, apically with one sensillum; basal process with fine sawteeth (Fig. 17).

**Measurements:** BL = 3.85 mm in holotype (range in four male paratypes: 3.60–4.50); ThL = 1.70 mm (1.80–2.00); WL = 3.50 mm (3.45–3.80); WW = 1.50 mm (1.45–1.55).

**Indices:** arb = 4/0 (4–7/0), adf = 0.67 (0.50–0.78), flw = 1.83 (1.57–1.92), FW/HW = 0.51 (0.50–0.52), ch/o = 0.12 (0.11–0.15), prorb = 1.32 (1.17–1.55), rcorb = 0.43 (0.39–0.55), vb = 0.50 (0.39–0.55), dcl = 0.55 (0.45–0.54), presctl = 0.55 (0.46–0.56), sctl = 1.23 (1.03–1.10), sterno = 0.78 (0.80–0.90), orbito = 1.73 (1.50–1.80), dcp = 0.32 (0.25–0.35), sctlp = 0.95 (0.91–1.05), C = 2.70 (2.33–2.65), 4c = 1.11 (1.19–1.50), 4v = 2.13 (2.20–3.00), 5x = 0.79 (0.55–0.97), ac = 1.90 (1.80–2.00), M = 0.50 (0.37–0.77), C3F = 0.51 (0.47–0.58).

**Etymology:** A combination of the Latin words hirtus and tibia, referring to the hindleg tibia with long setae.

**Distribution:** China (Sichuan).

**Phortica pinguiseta** Cao & Chen sp. nov.

(Figs 4, 18, 19)

**Material examined:** Holotype male, CHINA: Weixi, Shangrila, Yunnan, 27.vii.2004, HW Chen (SCAU, No. 120003).

**Measurements:** BL = 3.36 mm in holotype; ThL = 1.90 mm; WL = 3.25 mm; WW = 1.35 mm.

**Indices:** arb = 3/0, adf = 0.58, flw = 1.83, FW/HW = 0.50, ch/o = 0.14, prorb = 1.14, rcorb = 0.39, vb = 0.24, dcl = 0.54, presctl = 0.51, sctl = 1.08, sterno = 0.85, orbito = 1.73, dcp = 0.33, sctlp = 1.04, C = 2.63, 4c = 1.46, 4v = 2.80, 5x = 0.94, ac = 2.07, M = 0.68, C3F = 0.54.

**Etymology:** A combination of the Latin words pinguis and setae meaning strong setae, referring to the cercus with setae ventrally.
Distribution: China (Yunnan).

**Phortica panda** Cao & Chen sp. nov.  
(Figs 5, 10, 20, 21)


*Description:* Male. Head: frons black on upper part, brown on lower part. Face black on upper part, orange-brown on lower part. Thorax: scutum with black patches and silver-grey pollinose pattern. Scutellum orange-brown, black along margin. Legs: hind tibiae with three black rings; subapically with two to three rows of short, scopiform setae on ventral surfaces (Fig. 5E) and one very long apical seta (Fig. 5B), lacking any long setae on ventral and posteroverteral surface (Fig. 5). All fifth tarsomeres black, the rest grey-yellow. Male terminalia: ventral margin of epandrium not elongated (Fig. 20). Cercus with three strong setae (Fig. 20). Surstylus with two prensisetae apically (Fig. 20). Paramere mediobasally with a triangular projection, submedially with one small process, which bears one sensillum; basal process with fine sawteeth (Fig. 21).

Measurements: BL = 4.40 mm in holotype; ThL = 1.95 mm; WL = 3.50 mm; WW = 1.50 mm.

Indices: arb = 4/0, adf = 0.69, flw = 1.92, FW/HW = 0.56, ch/o = 0.12, prob = 0.97, rcorb = 0.41, vb = 0.33, dcl = 0.59, presctl = 0.63, sctl = 1.05, sterno = 0.77, orbito = 2.00, dcp = 0.30, sctlp = 0.89, C = 2.70, 4c = 1.42, 4v = 2.83, 5x = 0.77, ac = 2.02, M = 0.64, C3F = 0.59.

Etymology: Pertaining to the giant panda, which was first discovered by P.D. Armand in 1869 in Baoxing, the type locality of the new fly species.

Distribution: China (Sichuan).

**Phortica longicauda Cao & Chen sp. nov.**

(Figs 6, 11, 22, 23)


Description: Male. Head: frons almost black. Face almost brown. Thorax: black with silver-grey pollinose pattern. Scutellum orange-yellow medially, black along margin, yellow at tip. Legs: hind tibia with three black rings, lacking any long setae (Fig. 6). All fifth tarsomeres black, the rest grey yellow. Male terminalia: epandrium elongated ventrally (Fig. 22). Cercus with three long setae (Fig. 22). Surstylus with one prensiseta apically (Fig. 22). Paramere submedially with two small processes and two sensilla; basal process lacking fine sawteeth (Fig. 23).

Measurements: BL = 3.55 mm (range in four male paratypes: 3.55–3.70); ThL = 1.75 mm (1.75–1.80); WL = 3.40 mm (3.30–3.45); WW = 1.50 mm (1.45–1.50).

Indices: arb = 5/0 (4/0–6/0), adf = 0.67 (0.46–0.75), flw = 1.75 (1.47–1.92), FW/HW = 0.54 (0.51–0.52), ch/o = 0.12 (0.11–0.14), prob = 1.16 (1.07–1.29), rcorb = 0.45 (0.40–0.50), vb = 0.36 (0.33–0.50), dcl = 0.55 (0.48–0.62), presctl = 0.48 (0.45–0.55), sctl = 1.05 (1.05–1.09), sterno = 0.86 (0.82–0.88), orbito = 1.80 (1.46–1.55), dcp = 0.233 (0.25–0.31), sctlp = 1.14 (0.83–1.04), C = 2.81 (2.77–3.04), 4c = 1.22 (1.17–1.28), 4v = 2.34 (2.31–2.78), 5x = 0.82 (0.69–1.00), ac = 1.91 (1.80–2.02), M = 0.59 (0.49–0.67), C3F = 0.45 (0.51–0.56).

Etymology: A combination of the Latin words: longus and cauda, referring to the elongated cercus.

Distribution: China (Sichuan, Yunnan).

**Phortica longiseta Cao & Chen sp. nov.**

(Figs 12, 24, 25)


Description: Male. Head: frons almost black. Thorax: Scutellum black, with dense silver-grey pollinosity, yellow at tip. Legs: hind tibia with three 3 black rings, lacking any long setae. All fifth tarsomeres
Figure 24, 25. Phortica longiseta Cao & Chen sp. nov., male. 24, epandrium, surstylus, and cercus; 25, hypandrium, gonopods, paramere, aedeagus, and aedeagal apodeme. For abbreviations see Figs 14 and 15. Scale bars = 0.1 mm.

Table 3. Nucleotide composition in the sequences of the ND2 and COI data sets

<table>
<thead>
<tr>
<th>Data set</th>
<th>ND2</th>
<th>COI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1st</td>
<td>2nd</td>
</tr>
<tr>
<td>T</td>
<td>43.7</td>
<td>50.4</td>
</tr>
<tr>
<td>C</td>
<td>7.4</td>
<td>20.3</td>
</tr>
<tr>
<td>A</td>
<td>38.1</td>
<td>20.3</td>
</tr>
<tr>
<td>G</td>
<td>10.8</td>
<td>9.1</td>
</tr>
<tr>
<td>A + T</td>
<td>81.8</td>
<td>70.7</td>
</tr>
<tr>
<td>G + C</td>
<td>18.2</td>
<td>29.4</td>
</tr>
</tbody>
</table>

Measurements: BL = 3.25 mm in holotype; ThL = 1.60 mm; WL = 2.90 mm; WW = 1.25 mm.

Indices: arb = 5/0, adf = 0.75, flw = 1.67, FW/HW = 0.52, ch/o = 0.14, prob = 1.12, rcorb = 0.32, vb = 0.50, dcl = 0.35, presctl = 0.44, sclp = 1.15, sterno = 0.92, orbi = 1.36, dep = 0.21, sctlp = 0.90, C = 2.90, 4c = 1.24, 4v = 2.66, 5x = 1.03, ac = 1.8, M = 0.71, C3F = 0.44.

Etymology: A combination of the Latin words longus and seta, referring to the cercus with long setae.

Distribution: China (Hubei).

RESULTS OF PHYLOGENETIC ANALYSES

SUMMARY OF THE DNA SEQUENCES

The nucleotide representations of the ND2 and COI sequences are shown in Table 3. Either the ND2 or the COI sequences have much higher AT (81.9 and 70.1%, respectively) than GC contents, especially at the third codon positions (95.3 and 93.3%, respectively). The chi-square test of homogeneity of base frequencies revealed no heterogeneity across the 17 ingroup taxa (χ² = 3.476; P = 1.000), and also across all the 19 taxa (χ² = 6.167; P = 1.000).

PHT AND MODEL SELECTION

The PHT resulted in a P value of 0.869, indicating that no significant incongruence was found between
the ND2 and COI data sets. According to the results of model selection by MODELTEST3.7 (Posada & Crandall, 1998), the maximum likelihood settings in the NJ and ML analyses were: base frequencies (A, C, G, and T) = 0.3255, 0.1138, 0.1188, and 0.4419, respectively; substitution rates (A–C, A–G, A–T, C–G, C–T, and G–T) = 0.4044, 17.3743, 11.6821, 1.5227, 59.7368, and 1.0000, respectively; proportion of invariable sites (I) = 0.6545; gamma distribution shape parameter = 1.7021. The selected models for the six data partitions (nd2-1, nd2-2, nd2-3, coi-1, coi-2, coi-3; corresponding to the first, second, and third codon positions of the ND2 and COI loci, respectively) in the BI analysis were HKY + G, F81 + G, HKY + I, TrN + G, F81, and HKY + G, respectively. Accordingly, the parameters were set as: nst (number of substitution types) = 1 for nd2-2 and coi-2; 2 for nd2-1, nd2-3, and coi-3; 6 for coi-1; rates (rate variation across sites, and proportion of invariable sites) = gamma except for nd2-3 (propinv) and coi-2 (equal). The remaining parameters were estimated by the program.

PHyLOGENETIC RECONSTRUCTION

The phylogenetic trees constructed are shown in Figures 26–29. All of them strongly support the closer relationship of the hani complex to the P. magna/P. okadai lineage (which is also strongly supported) than to either P. speculum or P. helva [bootstrap percentage (BP) = 94–100; posterior probability (PP) = 1.00], as well the monophyly of the P. hani species complex (BP = 100; PP = 1.00). The NJ, ML, and BI trees subdivide the hani complex into two species clusters, one consisting of P. longiseta, P. hirtotibia, and P. pinguiseta (BP = 66–77; PP = 0.88), the other consisting of P. flocipes, P. panda, P. hani, and P. longicauda (BP = 97–100; PP = 0.98). The MP tree differs from the other trees in the placement of P. longiseta, as it is clustered in the latter cluster, but the support is rather low (BP = 45). The four trees show a consistent relationship between P. hirtotibia and P. pinguiseta, as well as between P. hani and P. longicauda. The sequences of P. flocipes do not form a monophyletic assemblage: four of them (MS1, MS2, SH2, and SH3/SH4) form a cluster with P. panda (BP = 92–96; PP = 1.00), whereas the remaining two (SH1 and SH5) form a tight pair (BP = 98–100; PP = 1.00), which groups with the above cluster of four other P.
DISCUSSION

PHYLOGENETIC RELATIONSHIPS

Our phylogenetic analyses lend strong support to the monophyly of the hani complex, an assemblage of species characterized by a variety of morphological traits, especially in the arista, wing R4, and M1 veins, fifth sternite, cercus, paramere, and aedeagus. Máca (2003) placed P. hani and P. sobodo as closer to the varipes group than to the other Phortica (s.s.), whereas our phylogenetic analyses strongly suggest that the magna and variegate complexes are closer to the hani complex than the foliiesta complex or the varipes group. This is in agreement with some morphological data from the present study. In addition, the closer relationship between the magna and variegate complexes than either is to the hani complex is also consistent with the morphological affinity between the former two – arista with long dorsal branches and numerous micropubescescences, mostly with ventral branches; wing vein R4 distally convergent with M1 vein; fifth sternite neither dense setae, nor notched posteromedially; cercus lacking long, strong setae ventrally; additional plate between cerci and tenth sternite present; paramere without spike-like process basally; aedeagus with more or less sclerotized median rod.

The MP tree (Fig. 27) clusters P. longiseta with P. floccipes, P. panda, and P. hani longicauda. This relationship is only weakly supported (BP = 45), and is favoured only by the parsimony criterion, for which the evolutionary model of nucleotide substitution is implicit. Compared to this, the clustering of P. longiseta with P. pinguiseta and P. hirtotibia in the trees built with model-based methods, i.e. the NJ, ML, and BI trees, is more likely to reflect the true relationship of P. longiseta.

Small genetic divergences were found between P. pinguiseta and P. hirtotibia (p-distance = 0.002–0.005), between P. panda and P. floccipes (p-distance = 0.002–0.010), as well between P. hani and P. longicauda (p-distance = 0.005–0.006). However, diagnostic morphological characters were found to distinguish species from each other for each species pairs: P. pinguiseta differs from P. hirtotibia in the number, length, size and distribution of setae on the male hindleg tibia, and scuteillum colour; P. panda differs from P. floccipes in the presence or absence of scopiform setae on the male hindleg tibiae, as well as in the number of prensisetae on surstylus; in contrast to P. hani, P. longicauda lacks long seta on the male hind tibiae, sawteeth on the basiphallus and the caudoventral group of two prensiseta on the surstylius. Therefore, the new species P. longicauda, P. panda, P. floccipes, P. hirtotibia, and P. pinguiseta seem to be morphologically well recognizable. Although the four haplotypes of P. floccipes, i.e., MS1, MS2, SH2, SH3/SH4, and P. panda, as well the branch leading to P. floccipes SH1 and SH5 in the ML tree) (Fig. 28).

Taking into account the strong supports in the molecular phylogenetic trees, it is reasonable to consider these morphological similarities to be homoplasy.

**Biogeography**
The subgenus *Phortica* is distributed mainly in the Oriental region, with its centre of species diversity in the south-west part of China (from the Xishuangbanna to Hengduan Mountains) (Chen, Gao & Wen, 2005; Chen et al., 2007; Cheng et al., 2008), and only a few species recorded from the Palaearctic or the Afrotropical regions (Prigent & Chen, 2008). The members of the *hani* complex are notable in that all were found from a zone of high elevation (c. 1600–2800 m) ranges from the Hengduan Mountain in south-west China to the Shennongjia Mountains in central China. This may indicate that the founder of this complex arose in the Hengduan mountainous area, presumably somewhere in the uplifting Qinghai-Tibet Plateau, and became adapted to the high elevation habitat there. The largely overlapping nature of the distributions of the two major clades within the *hani* complex may indicate that the founder underwent some differentiation before the expansion into adjacent areas.

**Acknowledgements**
We thank Mrs H. L. Cao, J. J. Jiang, T. Li, M. F. Xu, and F. Zhao (SCAU) for help in field collection. This work was supported by the National Natural Science Foundation of China (Nos. 30470212, 30670248), Scientific Research Foundation for the Returned Overseas Chinese Scholars and Science Foundation of Doctor Subjects, State Education Ministry of China (No. 20050564016).

**References**