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Mitochondrial COI and morphological evidence for host specificity of the black cherry aphids *Myzus cerasi* (Fabricius, 1775) collected from different cherry tree species in Europe (Hemiptera, Aphididae)

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Abstract

Partial sequences of the mitochondrial COI gene of forty eight European and two Turkish population samples of *Myzus cerasi* from different winter hosts (*Prunus* spp.) were subjected to phylogenetic analyses. The analysed *M. cerasi* samples emerged as paraphyletic relative to a *Myzus borealis* sample used as an outgroup, and formed two major clades in neighbor joining, maximum parsimony, maximum likelihood and Bayesian inference trees, corresponding to subspecies living specifically on *Prunus avium* and *P. cerasus*. Multivariate discriminant analysis (method of canonical variates) was applied to find out if morphological variation of samples correlated with mitochondrial COI and host plant information. Mean scores on the first two canonical variables clustered samples fully in accordance with their COI haplotypes and host plants confirming the existence of two morphologically similar winter host - specific subspecies of *M. cerasi* in Europe. No single morphological character enabled satisfactory discrimination between apterous viviparous females of the two subspecies. A three-character linear discriminant function enabled 92.37% correct identification of apterous viviparous females of *M. cerasi cerasi* (n=118) and 93.64% of *M. cerasi pruniavium* (n=110). A key for the morphological identification of the two subspecies is presented and their taxonomic status is discussed.

Keywords

Molecular systematics, cherry aphids, morphological key to subspecies

Introduction

Black cherry aphid *Myzus cerasi* (Fabricius, 1775) is reported to be a serious pest of cherries all over the world (Barbagallo et al. 1997, Blackman and Eastop 2000, Holman 2009) and its morphology, life cycle, host specificity and potential harmfulness have therefore been the subject of intensive studies (Ross 1918, Wimshurst 1925, Pokrovskyj 1932, Vereshchagina 1966, Fomicheva 1967, Karczewska 1970, Rakauskas 1984, Gruppe 1990, Cichocka and Goszczynski 2004). Nevertheless, the species level classification of black cherry aphids has not been satisfactorily resolved. The black shiny aphids inhabiting cherry trees were originally described as a single species, *Myzus cerasi* (Fabricius, 1775), but European populations inhabiting sweet cherry (*Prunus avium*) were later separated as *Myzus pruniavium* Börner, 1926. Börner's species has been accepted by some (Börner 1943, 1952, Heinze 1961), but synonymised with *cerasi* by others (Miyazaki 1971, Eastop and Hille Ris Lambers 1976, Remaudière and Remaudière 1997), while others have treated it as a subspecies of *cerasi* (Shaposhnikov 1972, Favret 2014). Differences in host specificity of the two taxa have been documented in experimental transfer studies (Karczewska 1970, Dahl 1968, Vereshchagina 1966), showing *M. cerasi cerasi* as heteroecious, alternating between cherries (both *Prunus cerasus* and *P. avium*) and herbaceous hosts (*Galium*, *Euphrasia*, *Odontites*, *Veronica*). *M. cerasi pruniavium* Börner, 1926 differs from the nominative subspecies in having *P. avium* as the only winter host (it is incapable of living on *P. cerasus*), and also has a somewhat different summer host list. Enzyme electrophoretic studies indicated a reduced gene exchange between the subspecies (Gruppe 1988a), perhaps due to differences in the phloem sap of sour and sweet cherry causing divergent selection (Gruppe 1989). Morphological characters for discrimination between the two taxa have been suggested (Heinze 1961), but none of these enabled satisfactory separation between viviparous females of *M. c. cerasi* and *M. c. pruniavium* when applied to independent aphid material (Dahl 1968, Gruppe 1988b). The taxonomic status of the black cherry aphid in the western Palaearctic thus remains unclear (Blackman and Eastop 2000, 2006, Lampel and Meier 2007, Holman 2009, Osiadacz and Halaj 2010). They are possibly members of a complex of cryptic aphid species that includes *M. cerasi umefoliae* (Shinji, 1924), which overwinters on *P. mume* in Japan with *Artemisia* as its summer host (Miyazaki 1971). In northern Europe there is also another potentially distinct taxon that lives all year without host alternation on herbaceous plants (Dahl 1968), and to which the name *M. cerasi veronicae* (Walker, 1848) may be applicable.

A similar species complex is found in the mealy plum aphid, *Hyalopterus* spp. (Lozier et al. 2008). Separation of three *Hyalopterus* species was eventually justified by their distinctness at molecular level (Lozier et al. 2008), but they still remain difficult to separate by their morphological characters (Basky and Szalay-Marszó 1987, Blackman and Eastop 1994, 2000, 2006), including those used in the most recent identification keys (Lozier et al. 2008, Rakauskas et al. 2013). A similar recent case is that of birch- and oak-inhabiting aphid species of the genus *Stomaphis* Walker, 1870 (Depa et al. 2012).

The *M. cerasi* complex has not yet been the subject of detailed molecular study, although certain DNA sequences have been isolated (Foottit et al. 2008, Valenzuela et al. 2007, Clements et al. 2000, 2000a). Preliminary data on the partial sequences of the mitochondrial COI gene support the existence of subspecies of *M. cerasi* (Voronova et al. 2011). The aim of this study was to clarify the taxonomic status of the host-alternating taxa in the *M. cerasi* complex by a combined study of partial sequences of the mitochondrial COI gene and morphological characters of the European samples collected from different species of cherries.

Material and methods

Material studied

Fifty population samples of apterous viviparae of black cherry aphids from nine European countries and Turkey were collected in 2004–2013, mostly from *P. cerasus*, *P. avium*, but also from three other *Prunus* species (Table 1). Twenty samples were used for morphology - based stepwise discriminant analysis. The remaining 30 samples were used for subsequent evaluation of the derived discrimination functions. Samples of *Myzus borealis* Ossiannilsson, 1959 and *Myzus persicae* (Sulzer, 1776) (GenBank Accession No AB506741) were used as out-groups for the phylogenetic analyses.

DNA extraction, PCR amplification and sequencing

For molecular analysis, a single aphid individual from one sampled plant was considered as a unique sample. Total genomic DNA was extracted from a single aphid using the DNeasy Blood & Tissue kit (Qiagen), which involved at least a 2 h digestion of tissue with proteinase K. Partial sequences of mitochondrial COI gene were PCR-amplified using earlier published primers (Turčinavičienė et al. 2006). PCR amplification was carried out in a thermal cycler (Eppendorf) in 50 µl volumes containing 2 µl genomic DNA, 5 µl of each primer (10 µM), 5 µl of PCR-reaction buffer, 5 µl of dNTP mix (2mM each), 4–8 µl of 25mM $MgCl_2$ and 1.25 U of AmpliTaq Gold 360 polymerase (5U/µl) and ddH_2O to 50 µl. The cycling parameters were as follows: denaturizing at 95 °C for 10 min (1 cycle), denaturizing at 95 °C for 30", annealing at 49 °C for 30" and extension at 72 °C for 30" (37 cycles in total), and a final extension for 5 min (1 cycle). PCR products were subjected to electrophoresis on 2% TopVision agarose (Fermentas, Lithuania), stained with GelRed and sized against a MassRuler Low Range DNA ladder (Fermentas, Lithuania) under UV light. PCR products were purified and sequenced at Macrogen Europe (Amsterdam, the Netherlands) and Institute of Biotechnology of the Vilnius University (Vilnius, Lithuania). The amplification primers were also used as sequencing primers. DNA sequences for each specimen were confirmed with both sense and anti-sense strands and aligned in

Table 1. Aphid material used in the present study. Samples used for morphology-based discriminant analysis are given in bold.

Place, date, collection number; (number of individual apterae per sample)	GenBank Accession No
***Myzus cerasi* on *Prunus cerasus* L.**	
Jieznas, Prienai distr., Lithuania, 2012.05.30, 12-25; (8)	KF754311
Alytus, Lithuania, 2012.05.30, 12-30; (8)	KF754310
Daugai, Alytus distr., Lithuania, 2012.05.30, 12-32; (8)	KF754303
Skirgiškės, Vilnius distr., Lithuania, 2012.06.05, 12-37; (8)	KF754304
Eišiškės, Šalčininkai distr., Lithuania, 2012.06.13, 12-43; (7)	KF754329
Labanoras, Švenčionys distr., Lithuania, 2012.06.19, 12-70; (8)	KF754306
Molėtai, Lithuania, 2012.06.19, 12-72; (8)	KF754314
Kraujaliai, Molėtai distr., Lithuania, 2012.07.10, 12-120; (8)	KF754305
Žičkai, Molėtai distr., Lithuania, 2012.07.13, 12-132; (8)	KF754321
Nida, Neringa, Lithuania, 2012.08.09, 12-176; (8)	KF754322
Smiltynė, Klaipėda, Lithuania, 2012.08.12, 12-191; (8)	KF754309
Preila, Neringa, Lithuania, 2012.08.13, 12-203; (8)	KF754330
Bagnolo Mella, Brescia prov., Italy, 2013.05.01, 13-27; (8)	KF754338
Poncarale, Brescia prov., Italy, 2013.05.02, 13-33; (8)	KF754337
Suginčiai, Molėtai distr., Lithuania, 2013.06.15, 13-83; (8)	KF754345
Akmeniai, Lazdijai distr., Lithuania, 2013.05.30, 13-57; (8)	KF754341
Karsava, Ludza distr., Latvia, 2013.07.17, 13-133; (8)	KF754344
Gorodok, Vitebsk distr., Belarus, 2008.06.17, 08-6; (6)	KF754302
Zadrachje, Vitebsk distr., Belarus, 2008.06.18, 08-18; (8)	KF754325
Riga, Latvia, 2008.07.03, 08-73; (8)	KF754328
Skirgiškės, Vilnius distr., Lithuania, 2011.06.15, 11-46; (8)	KF754316
Cluj Gilau, Romania, 2012.06.19, Z12-112; (8)	KF754327
Cluj Gilau, Romania, 2012.06.19, Z12-116; (8)	KF754319
Poncarale, Brescia prov., Italy, 2013.05.02, 13-30; (8)	KF754346
Mezöpeterd, Hajdu-Bihar distr., Hungary, 2012.06.20, Z12-122; (8)	KF754333
***Myzus cerasi* on *Prunus avium* L.**	
Skirgiškės, Vilnius distr., Lithuania, 2012.06.05, 12-39; (7)	KF754332
Šalčininkai, Lithuania, 2012.06.13, 12-48; (6)	KF754317
Bratoniškės, Vilnius distr., Lithuania, 2012.06.14, 12-56; (7)	KF754313
Blagoevgrad, Bulgaria, 2012.06.26, 12-83; (7)	KF754318
Frankfurt/Main, Germany, 2012.06.30, 12-104; (8)	KF754307
Kraujaliai, Molėtai distr., Lithuania, 2012.07.10, 12-111; (8)	KF754308
Stirniai, Molėtai distr., Lithuania, 2012.07.12, 12-128; (8)	KF754320
Juodkrantė, Neringa, Lithuania, 2012.08.10, 12-182; (8)	KF754323
Pervalka, Neringa, Lithuania, 2012.08.11, 12-188; (7)	KF754336
Preila, Neringa, Lithuania, 2012.08.13, 12-199; (7)	KF754335
Rondo, Katowice, Poland, 2011.05.13, 11-10; (8)	KF754349
Tekir, Karamanmarash distr., Turkey, 2011.05.21, 11-25; (5)	KF754312
Göksun, Karamanmarash distr., Turkey, 2011.05.21, 11-27; (8)	KF754315
Kairėnai, Vilnius, Lithuania, 2010.07.01, 10-3; (7)	KF754324
Zafferana, Catania, Italy, 2004.06.28, 04-49; (5)	KF754339
Costinesti, Romania, 2012.06.15, Z12-90; (8)	KF754326
Galata, Varna, Bulgaria, 2012.06.18, Z12-102; (7)	KF754351
Burgas, Bulgaria, 2012.06.19, Z12-110; (8)	KF754331
Cluj Gilau, Romania, 2012.06.19, Z12-117; (7)	KF747679

Place, date, collection number; (number of individual apterae per sample)	**GenBank Accession No**
Carpendolo, Brescia prov., Italy, 2013.04.27, 13-12; (7)	KF754340
Akmeniai, Lazdijai distr., Lithuania, 2013.05.30, 13-60; (8)	KF754347
Wojslawice, Lower Silesia, Poland, 2013.06.20, 13-98; (8)	KF754342
***Myzus cerasi* on *Prunus serrulata* Lindl.**	
Wojslawice, Lower Silesia, Poland, 2013.06.20, 13-97; (8)	KF754348
***Myzus cerasi* on *Prunus maackii* Rupr.**	
Dobele, Latvia, 2013.07.03, 13-119; (8)	KF754343
***Myzus cerasi* on *Prunus mahaleb* L.**	
Medias, Sibiu distr., Romania, 2012.06.19, Z12-113; (8)	KF754334
***Myzus borealis* on *Galium rubioides* L.**	
Zmejinyje ostrova, Kanev distr., Cherkasy reg., Ukraine, 2006.06.16, 06-74	KF754350

the BioEdit Sequence Alignment Editor (Hall 1999). Partial sequences were tested for stop codons and none were found. The sequence data have been submitted to the GenBank, accession numbers are given in Table 1.

Analysis of DNA sequences

In addition to the sequences from 50 samples of *M. cerasi*, COI sequences of *M. borealis* from subgenus *Myzus* sensu stricto (the same subgenus as *M. cerasi*) and *M. persicae* from subgenus *Nectarosiphon* Schouteden, 1901 were selected as out-groups for the phylogenetic analyses, which included neighbor joining (NJ), maximum parsimony (MP), maximum likelihood (ML) and Bayesian inference in phylogeny (BI). NJ, MP and ML analyses were performed using MEGA 5 (Tamura et al. 2011). For NJ analysis Kimura 2-parameter (K2P) model of base substitution was used. Bootstrap values for NJ, MP and ML trees were generated from 1000 replicates. For ML analysis Tamura 3-parameter model with Gamma distribution (T92+G) was selected by MEGA 5 model selection option (Tamura et al. 2011). Bayesian analysis was conducted in MrBayes 3.2.1 (Ronquist and Huelsenbeck 2003) using Hasegawa-Kishino-Yano model with Invariable sites and Gamma distribution (HKY+I+G), which was selected by jModeltest (Posada 2008). Four simultaneous chains, 3 heated and 1 "cold", were run for 3 000 000 generations with tree sampling every 1000 generations. The topologies obtained by NJ, MP, ML and BI were similar, so only ML tree is shown with values of NJ/MP and ML/BI bootstrap support and posterior probabilities over 50% indicated above and below branches respectively. Statistical parsimony haplotype networks were constructed for samples of *M. cerasi* and *M. borealis* using TCS v 1.21 (Clement et al. 2000).

Morphological study and discrimination analysis

Samples representing different clades in the molecular tree and haplotype network were used for stepwise discriminant analysis followed by canonical analysis: 10 sam-

ples from sour cherry, *P. cerasus*, and 10 samples from sweet cherry, *P. avium* (shown in bold in Table 1).

Based on earlier taxonomic work (Heinze 1961, Dahl 1968), 19 metric (in mm) characters were studied: A2L – length of antennal segment 2; A3L – length of antennal segment 3; A4L – length of antennal segment 4; A5L – length of antennal segment 5; A6BL – length of basal part of antennal segment 6; A6TPL – length of terminal process of antennal segment 6; BL – body length (excluding cauda); Bwant3 – basal width of antennal segment 3; CL – length of cauda; CW – basal width of cauda; DT3L – length of the second segment of hind tarsus; F3L – length of hind femur; FF – depth of the frontal furrow; SL – length of siphunculus; T3L – length of hind tibia; URL – length of ultimate rostral segment; URW – basal width of ultimate rostral segment; VBSLmax – maximal length of the ventral body hairs; VBSLmin – minimal length of the ventral body hairs.

Measurements of the slide-mounted apterous viviparous females were performed by means of interactive measurement system Micro-Image (Olympus Optical Co. GmbH). STATISTICA 8 version software (Statsoft 2007) was exploited for data analysis. Pearson's correlation coefficients were calculated to evaluate the correlation of morphometric characters with body length. Characters with strong ($| r | \geq 0.70$) statistically significant ($p<0.05$) correlation with body length were removed from the further analysis: BL (r=1.00), F3L (r=0.83), T3L (r=0.82), A2L (r=0.7), A3L (r=0.75), A4L (r=0.71), A5L (r=0.7). The remaining 12 characters were used for the forward stepwise discriminant analysis followed by canonical analysis, with sample collection number as the grouping variable, thus excluding information about the host plant from the analysis. Mean canonical scores of the first two canonical variables were represented as bivariate scatter plots, in order to show any clustering of samples.

Morphological characters that contributed most to canonical discrimination functions were evaluated as having potential for separation of taxa. An identification key was constructed based on these discrimination functions. The key was then tested on the 30 aphid samples that had not been used in its construction (listed in normal font in Table 1).

Results

Partial sequences of mitochondrial COI gene

Fifty partial COI sequences of *M. cerasi* and one of *M. borealis* from 11 countries were included in analysis. The alignment contained 616 bases in the final set with three variable sites, all of which appeared parsimony informative. The average base composition was A = 34.0%, C = 12.7%, G = 12.3% and T = 41.0%. The overall transition/transversion ratio R = 1.221 for all sites.

Five COI haplotypes were detected (Fig. 1): one for *M. borealis*, two for samples from *P. cerasus* and two for samples from *P. avium* (Table 2). Aphids collected from *P. mahaleb*, *P. maackii* and *P. serrulata* had the same haplotype (No. 3) as the majority

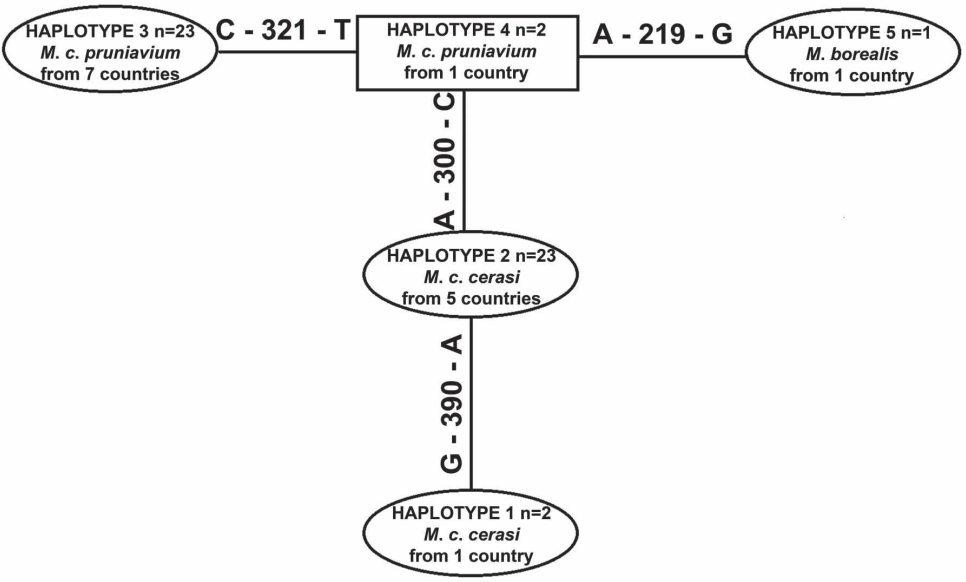

Figure 1. Haplotype network (TCS 1.21 software: Clement et al. 2000) for COI fragment (616 positions in final set) haplotypes of *Myzus cerasi* and *Myzus borealis*. The haplotype with the highest outgroup probability is displayed as a square, while others are displayed as ovals. For sample information, see Table 2.

Table 2. COI haplotypes of three *Myzus* taxa revealed by construction of haplotype network. Sample numbers are the same as given in Table 1.

Haplotype number	Number of sequences	Sequence length (bp)	Sample numbers
M. cerasi cerasi (collected from *Prunus cerasus*)			
1	2	616	13-33; 13-27.
2	23	616	08-6;12-32; 12-176; 12-30; 08-18; z12-122; 12-120; z12-112; 12-132; 12-25; 12-43; 12-191; 12-203; 12-70; 12-72; 08-73; 13-83; 13-133; 11-46; z12-116; 12-37; 13-30; 13-57
M. cerasi pruniavium (collected from *Prunus avium*, except where otherwise noted)			
3	23	616	11-10; 12-39; z12-110; 12-182; 12-104; 12-56; 12-83; 12-111; 12-199; z12-90; z12-102; 12-48; 12-188; z12-113 (*P. maackii*); 04-49; 12-128; 10-03; 13-60; 13-98; 13-97 (*P. serrulata*); 13-12; 13-119 (*P. mahaleb*); z12-117
4	2	616	11-27; 11-25
M. borealis (collected from *Galium rubioides*)			
5	1	616	06-74

of samples from *P. avium*. COI haplotypes detected among samples from *P. cerasus* (No. 1 and 2) and the remaining *Prunus* species (No. 3 and 4) differed in the following nucleotide positions of 616 bp alignment: 300 (between No. 1–2 and No. 3–4), 321 (between No. 3 and No. 4) and 390 (between No. 1 and No. 2). The range of the intraspecific pairwise sample divergences (K2P model) was 0.0 – 0.5% (average 0.2%),

Table 3. Range of pairwise interspecific sample divergences of mitochondrial COI gene fragment (K2P model) for three species of *Myzus* (number of samples used is in parentheses).

Species 1	Species 2	Range of divergence, %
M. cerasi (50)	*M. borealis* (1)	0.2 – 0.5
M. cerasi (50)	*M. persicae* (1)	6.6 – 6.8
M. borealis (1)	*M. persicae* (1)	6.8

whilst interspecific pairwise sample divergences between three species of *Myzus* ranged from 0.2 to 6.8% (Table 3).

The maximum parsimony (MP) analysis of partial COI sequences resulted in 930 equally parsimonious trees (length = 43, CI=1.00, RI=1.00). The ML tree (T92 model) showed similar topology, as did NJ (K2P distances) and BI (HKY+I+G model) analyses. NJ, MP and ML bootstrap values over 50% together with BI posterior probabilities over 0.50 are given at respective nodes of the same tree in Fig. 2. Thus the *M. cerasi* samples form two major clades corresponding to two host-specific black cherry aphid taxa. One clade consists of all but two of the samples from *P. avium*, plus aphids collected from *P. mahaleb*, *P. maackii* and *P. serrulata*. The other clade contains all samples from *P. cerasus* and also includes the sample of *M. borealis* collected from *Galium rubioides*.

Morphology

When morphometric data of apterous viviparous females from 20 different geographical localities were subjected to discriminant analysis with sample collection number as the grouping variable, the first two canonical variates (Fig. 3) clearly separated sour cherry samples (COI haplotype No. 2) from those collected from sweet cherry (COI haplotype No. 3). Length of terminal process of antennal segment 6 (A6TPL), length of siphunculus (SL) and maximal length of the ventral body hairs (VBSLmax) appeared to be important predictors for separation of the two taxa (Table 4).

To discriminate between apterous viviparous females of host-specific black cherry aphid samples representing different clades in the haplotype network and the phylogenetic tree (Figs 1–2), the following linear discriminant function (LDF) was obtained: 3.924682×SL - 5.6667×A6TPL - 32.5504×VBSLmax + 1. Using this LDF, 97.37% individuals from the whole dataset were reclassified correctly into their *a priori* specified groups with host plant species as grouping variable, including 96.2% of apterous viviparous females from *P. avium* (n=79) and 98.6% from *P. cerasus* (n=73). The *post hoc* classification of the remaining thirty samples gave 92.37% correct specimen identification of *M. cerasi cerasi* (n=118) and 93.64% of *M. cerasi pruniavium* (n=110). The scatterplot of the mean LDF and body length values calculated for each of 30 samples representing different host specific subspecies of *M. cerasi* is shown in Fig. 4. The following key is therefore suggested for the identification of apterous viviparous females of the two subspecies of *Myzus cerasi* when sampled from winter hosts.

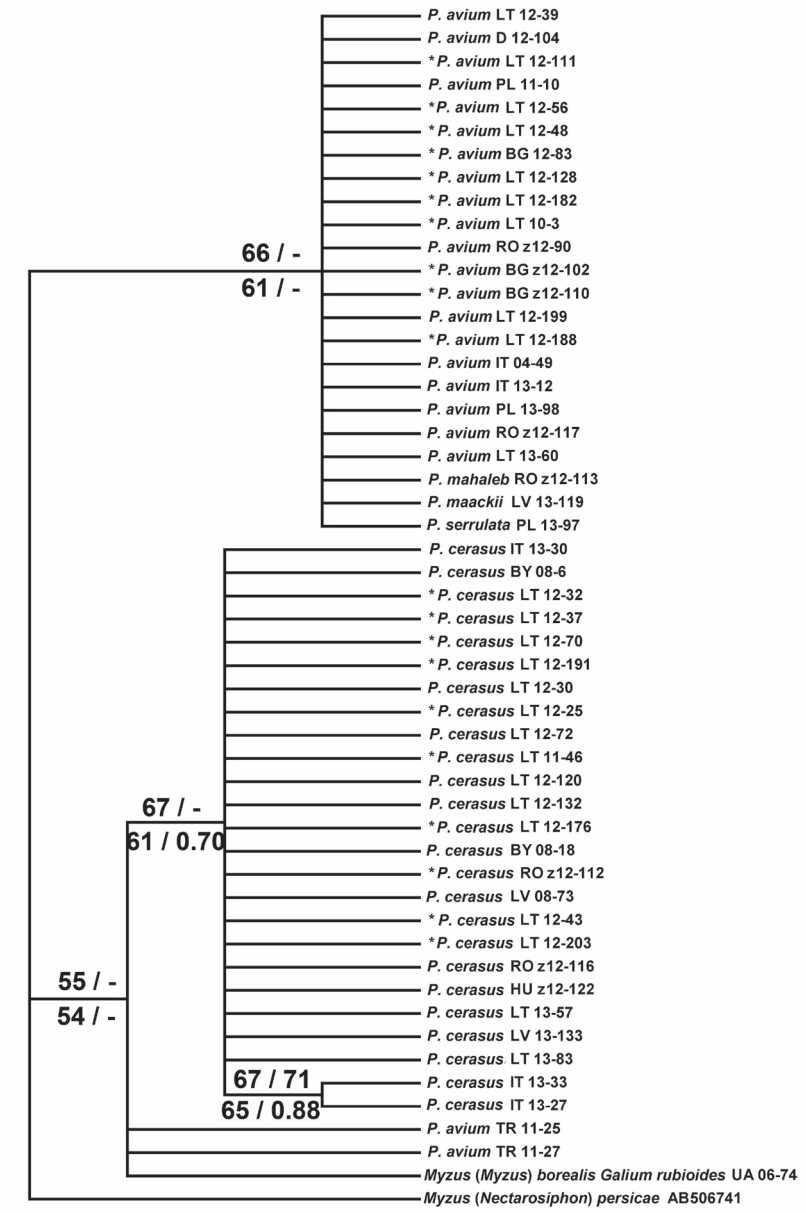

Figure 2. Maximum likelihood (ML) tree showing phylogenetic relationships among *Myzus cerasi* based on partial sequences of mitochondrial COI (616 positions in final set). Numbers above branches indicate support of NJ (left, > 50%) and MP (right, > 50%) bootstrap test with 1000 replicates, and numbers below branches indicate support of ML (left, > 50%) bootstrap test with 1000 replicates and posterior probabilities of BI analysis (right, > 0.50). Samples used for the discriminant analysis with *a priori* specified group membership followed by the construction of identification key are asterisked (*). The remaining samples were used for the *post hoc* classification. Sample numbers are the same as given in Table 1, together with the abbreviated symbol of respective country BG – Bulgaria, BY – Belarus, D – Germany, HU – Hungary, IT – Italy, LV – Latvia, LT – Lithuania, PL – Poland, RO – Romania, TR – Turkey, UA – Ukraine.

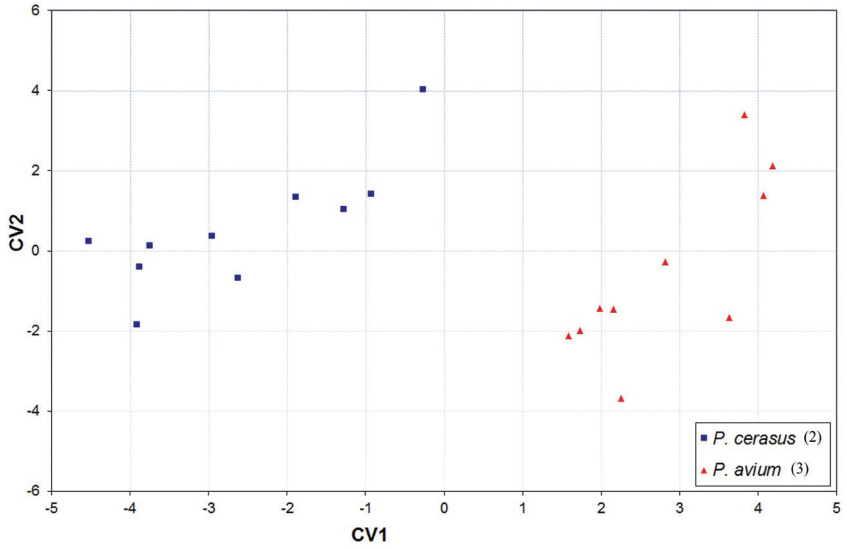

Figure 3. Plot of the mean scores of the first two canonical variates for 20 samples of *Myzus cerasi* (for specimen numbers per sample see Table 1). Samples cluster in accordance with winter host plant and COI haplotype (haplotype number is given in parentheses, see Table 2 for other haplotypes).

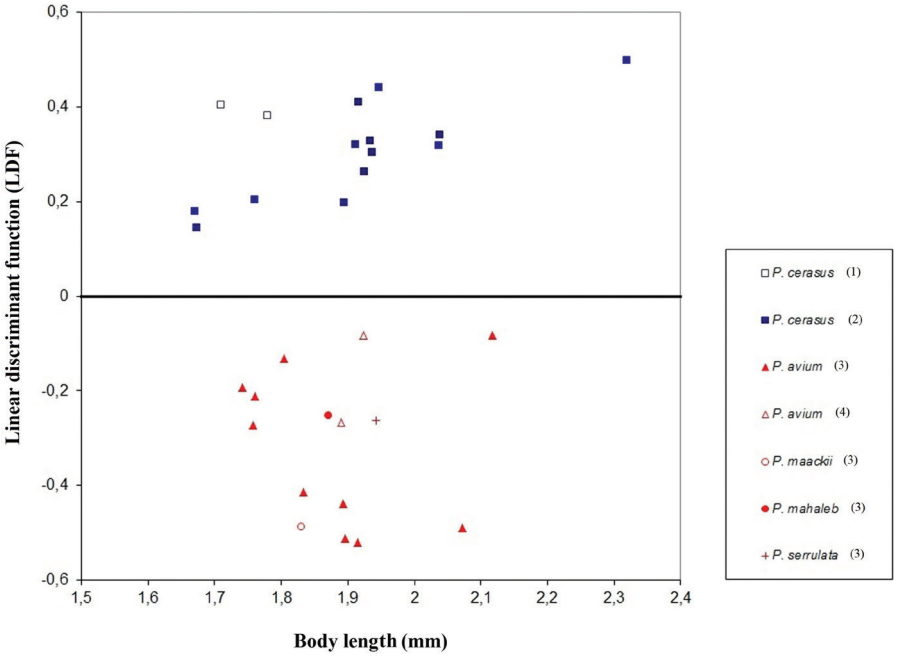

Figure 4. Plot of the mean scores of the individual LDF values (number of specimens per sample is given in Table 1) plotted against the mean body length for 30 samples of *Myzus cerasi* (normal font in Table 1) used to evaluate effectiveness of the eventual identification key. The icons are color-coded to match the COI haplotypes. Samples cluster in accordance with winter host plant and COI haplotype (haplotype number is given in parentheses, see Table 2 for haplotype information).

Table 4. Contributions of 11 morphological characters to the canonical function discriminating 20 samples of *M. cerasi*. Character abbreviations are the same as in the text (Material and methods).

	Wilks' Lambda	Partial Wilks' Lambda	F-remove	p-level	Toler.	1-Toler. (R-Sqr.)
A6TPL	0.34	0.65	72.00	0.00	0.46	0.54
SL	0.30	0.73	49.26	0.00	0.31	0.69
VBSLmax	0.27	0.83	28.31	0.00	0.79	0.21
Bwant3	0.24	0.91	12.99	0.00	0.71	0.29
URL	0.23	0.93	9.51	0.00	0.65	0.35
A6BL	0.23	0.94	8.69	0.00	0.47	0.53
DT3L	0.23	0.97	4.62	0.01	0.49	0.51
FF	0.23	0.97	4.23	0.02	0.86	0.14
VBSLmin	0.23	0.97	3.70	0.03	0.85	0.15
CL	0.22	0.98	2.38	0.10	0.57	0.43
CW	0.22	0.99	1.75	0.18	0.56	0.44

Key to European subspecies of *Myzus cerasi* on *Prunus* (apterous viviparous females)

1 Value of LDF [3.92×(length of siphunculus) - 5. 67×(length of terminal process of antennal segment 6) - 32.55×(maximal length of the ventral body hairs) +1] greater than zero. On *P. cerasus* (and sometimes on *P. avium*) .....***M. c. cerasi***

– Value of LDF less than zero. On *P. avium*, *P. maackii*, *P. mahaleb*, *P. serrulata*... .....***M. c. pruniavium***

Discussion and conclusions

The combination of genetic distance evaluation with phylogenetic tree-building methods and multivariate analyses of morphometric data has been successfully applied to solve taxonomic problems in aphids, particularly in the genera *Hyalopterus* (Lozier et al. 2008), *Pentalonia* (Foottit et al. 2010), *Aulacorthum* and *Neoaulacorthum* (=*Pseudomegoura*) (Lee et al. 2011a). Based on the global data set, the average genetic divergence of COI barcode sequences between aphid species within the same genus was reported to be 5.84% (range 0.46 – 11.3%), and that within species 0.05% (0.00–1.00%) (Foottit et al. 2008, Lee et al. 2011b). Interspecific divergence of six species representing three subgenera of *Myzus* calculated for COI barcode sequences was reported as ranging from 5.55 to 11.3% (Foottit et al. 2008). In comparison, partial COI sequences (GenBank, 1145 bp) of three *Aphis fabae* subspecies (*A. f. fabae*, GenBank accession numbers FJ965713, FJ965717–FJ965718; *A. f. cirsiiacanthoidis*, FJ965698–FJ965709; *A. f. mordvilkoi*, FJ965710–FJ965712) show values of genetic divergence (K2P model) ranging from 0.00 to 1.42% (Table 5). Therefore, the range of genetic divergence between the two clades of *M. cerasi* emerging in phylogenetic trees presented in this paper (0.0 to 0.5%) appears to be of intraspecific level. Based on the available COI data, black cherry

Table 5. Pairwise sample divergences of 1145 bp mitochondrial COI gene fragment (K2P model) between three subspecies of *Aphis fabae* (number of sequences used is in parentheses).

Subspecies 1	Subspecies 2	Mean and range of divergence, %
A. f. fabae (3)	*A. f. cirsiiacanthoidis* (12)	1.2 (0.97–1.42)
A. f. fabae (3)	*A. f. mordvilkoi* (3)	0.15 (0.00–0.26)
A. f. cirsiiacanthoidis (12)	*A. f. mordvilkoi* (3)	1.05 (0.97–1.15)

aphids inhabiting sour and sweet cherries should therefore still be regarded as a single species.

M. borealis is clearly closely related to *M. cerasi* and differs by only 0.2–0.5% of the COI sequences involved in the analysis. This suggests that it may also belong to the same species level taxon. More samples of *M. borealis* are needed to confirm this hypothesis. However, it should be noted that partial COI sequences of two biologically distinct *Macrosiphum* species, *M. rosae* (Linnaeus, 1758) and *M. knautiae* Holman, 1972 are very similar (Turčinavičienė and Rakauskas 2009), and low divergence levels have also been reported for *Bursaphis* species (Rakauskas et al. 2011) and adelgids (Žurovcová et al. 2010). It seems probable that in rapidly speciating aphid groups one may expect to find low levels of COI sequence divergence between taxa that are nevertheless functioning as distinct species.

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RESEARCH ARTICLE

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A review of *Rheocricotopus* (*Psilocricotopus*) *chalybeatus* species group from China, with the description of three new species (Diptera, Chironomidae)

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Abstract

The *Rheocricotopus* (*Psilocricotopus*) *chalybeatus* species group from China is reviewed. Three new species, *R.* (*P.*) *brochus* **sp. n.**, *R.* (*P.*) *rotundus* **sp. n.** and *R.* (*P.*) *serratus* **sp. n.** are described as adult males. *R.* (*P.*) *imperfectus* Makarchenko & Makarchenko, 2005, *R.* (*P.*) *robacki* (Beck & Beck, 1964) and *R.* (*P.*) *valgus* Chaudhuri & Sinharay, 1983 are recorded from China for the first time and annotated. The diagnosis for the species group is emended and a key to adult males of the species group in China is presented.

Keywords

Chironomidae, *Rheocricotopus*, *chalybeatus* species group, new species, key, China

Introduction

The genus *Rheocricotopus* was erected by Thienemann and Harnisch (1932). Originally, Edwards (1929) treated it as a group of the genus *Spaniotoma* Philippi, 1865. Brundin (1956) reaffirmed the valid generic status of *Rheocricotopus* to be followed by Lehmann (1969) and other workers. It can be separated from other orthoclad genera by the following combination of characters: hairy eyes; without dorsomedial extension;

developed pulvilli; acrostichals beginning near antepronotum; plate-like superior volsella and pointed anal point with posterolaterally directed setae. The immature stages of *Rheocricotopus* can be collected in streams and rivers, rarely in the littoral zone of lakes (Cranston et al. 1989). So far, 70 species (Ashe and O'Connor 2012) were recorded in all zoogeographic regions in the world.

Sæther (1985) reviewed the genus *Rheocricotopus* Thienemann & Harnisch, 1932 in the world and divided the genus into two subgenera (*Rheocricotopus sensu stricto* and *Psilocricotopus* Sæther) including six species groups (*atripes* species group, *chalybeatus* species group, *godavarius* species group, *tuberculatus* species group, *fuscipes* species group and *effusus* species group). Wang and Sæther (2001) erected *orientalis*, a new species group. The *Rheocricotopus chalybeatus* species group can be distinguished from other species groups by the following combination of characters: gonostylus either with pronounced, preapical, triangular crista dorsalis or with apically sharp upward bend fused with apparent crista dorsalis; humeral pit moderately large, ovoid or circular, if large and somewhat rectangular gonostylus bent sharply upwards distally; superior volsella rounded, relatively small, never with projection. To date, 22 species were recorded in the *chalybeatus* species group (Sæther 1985; Caspers 1987; Chaudhuri and Sinharay 1983; Hazra and Chaudhuri 2004; Johannsen 1932; Makarchenko and Makarchenko 2005; Sasa 1990, 1991; Sasa and Suzuki 2000; Wang and Zheng 1989, 1991; Wang et al. 2004).

In China, 6 species of *chalybeatus* species group [*R.* (*P.*) *emeiensis* Wang & Zheng, 1989, *R.* (*P.*) *nigrus* Wang & Zheng, 1989, *R.* (*P.*) *bifasciatus* Wang & Zheng, 1991, *R.* (*P.*) *brachypus* Wang & Zheng, 1991, *R.* (*P.*) *chalybeatus* (Edwards, 1929) and *R.* (*P.*) *taiwanensis* Wang et al., 2004] had been recorded (Wang 2000, Wang et al. 2004).

Based on specimens from China, in this paper, three new species are described, and a key to the Chinese species of *chalybeatus* group is presented.

Materials and methods

The morphological nomenclature follows Sæther (1980). The material examined was mounted on slides following the procedures outlined by Sæther (1969). The specimens examined in this study are deposited in the College of Life Sciences, Nankai University, China.

Taxonomy

Rheocricotopus (*Psilocricotopus*) *bifasciatus* Wang & Zheng, 1991

http://species-id.net/wiki/Rheocricotopus_bifasciatus

Rheocricotopus bifasciatus Wang & Zheng, 1991: 100.
Rheocricotopus (*Psilocricotopus*) *bifasciatus* Wang, 2000: 639, Ashe and O'Connor 2012: 560.

Specimens examined. Type material: Holotype, ♂, Chongqing City, Jinfo Mountain, 29°01'90"N, 107°16'20"E, 9.v.1986, sweeping, Wang XH. Paratype (1): 1♂, as holotype.

Additional material. 1♂, Sichuan Province, Yajiang County, 30°15'00"N, 101°02'00"E, 14.vii.1997, sweeping, Wang XH; 1♂, Hunan Province, Taoyuan County, 28°63'72"N, 111°13'79"E, 17.vii.2004, sweeping, Yan CC; 3♂♂, Ningxia Hui Autonomous Region, Jingyuan County, 35°66'33"N, 106°29'08"E, 7.viii.1987, sweeping, Wang XH; 1♂, Ningxia Hui Autonomous Region, Jingyuan County, 35°66'33"N, 106°29'08"E, 8.viii.1987, sweeping, Wang XH; 1♂, Gansu Province, Yuzhong County, 35°90'00"N, 104°11'00"E, 4.viii.1993, sweeping, Bu WJ.

Diagnosis. This species can be separated from other members of the group by the following combination of characters: tergites I, II and IV pale brown, others dark brown; AR 0.90; wing anal lobe reduced; humeral pit large, ovoid; Costal extension 83 μm long.

Remarks. The additional specimens are similar to the description of Wang and Zheng (1991). The species is recorded from Palearctic Region for the first time.

Distribution. China (Chongqing Municipality, Sichuan, Hunan and Gansu Provinces, Ningxia Hui Autonomous Region).

***Rheocricotopus* (*Psilocricotopus*) *brachypus* Wang & Zheng, 1991**

http://species-id.net/wiki/Rheocricotopus_brachypus

Rheocricotopus brachypus Wang & Zheng, 1991: 101.
Rheocricotopus (*Psilocricotopus*) *brachypus* Wang, 2000: 639, Ashe and O'Connor 2012: 560.

Specimens examined. Type material: Holotype, ♂, Guangdong Province, Fengkai County, Heishiding National Nature Reserve, 23°30'02"N, 111°55'01"E, 12.iv.1985, sweeping, Wang XH.

Additional material. 1♂, Hubei Province, Hefeng County, 29°91'00"N, 110°03'00"E, 16.vii.1999, light trap, Ji BC; 1♂, Sichuan Province, Yajiang County, 30°15'00"N, 101°02'00"E, 14.vii.1997, sweeping, Wang XH; 1♂, Xizang Autonomous Region, Bayi County, Shergmla Mountain, 29°64'07"N, 94°36'01"E, 28–30. ix.1997, yellow trap, Solhøy T & Skartveit J.

Diagnosis. This species can be separated from other members of the group by the following combination of characters: AR 0.43; anal lobe developed; anal point robust.

Remarks. Wang and Zheng (1991) described this species without humeral pit which as diagnostic characteristic. However, after examining the holotype, we find a medium, relatively shallow, ovoid humeral pit existing.

Distribution. Oriental China (Guangdong, Hubei and Sichuan Provinces, Xizang Autonomous Region).

***Rheocricotopus* (*Psilocricotopus*) *brochus* sp. n.**
http://zoobank.org/74D7F9E8-E9E8-4BE0-A21B-DDEB23C88D8B
http://species-id.net/wiki/Rheocricotopus_brochus
Figs 1–6

Diagnosis. The adult male can be distinguished from known species of the species group and the genus by the following combination of characters: crista dorsalis tooth-liked; tergites I, II and IV yellow, tergite III mainly yellow with a brown circular area, other tergites brown.

Description. Male imago (n = 12)

Total length 1.75–2.70, 2.18 mm. Wing length 1.25–1.60, 1.33 mm. Total length/wing length 1.35–1.78, 1.68. Wing length/length of profemur 1.76–2.31, 2.04.

Coloration. Head and thorax brown. Tergites (Fig. 1) I, II and IV yellow, tergite III mainly yellow but having a brown circular area, other tergites brown.

Head. AR 0.63–0.89, 0.72. Ultimate flagellomere 245–360, 284 μm long. Temporal setae 4–7, 5, including 1–3, 2 inner verticals; 1–3, 2 outer verticals and 0–2, 1 postorbital. Clypeus with 7–10, 8 setae. Cibarial pump, tentorium and stipes as in Fig. 2. Tentorium 75–140, 123 μm long, 13–25, 19 μm wide. Stipes 100–125, 118 μm long, 8–10, 8 μm wide. Palpomere lengths (in μm): 20–25, 23; 38–55, 47; 90–130, 105; 110–138, 120; 205–250, 232. L: $5^{th}/3^{rd}$ 1.92–2.67, 2.23. Third palpal segment with 2 sensilla clavata.

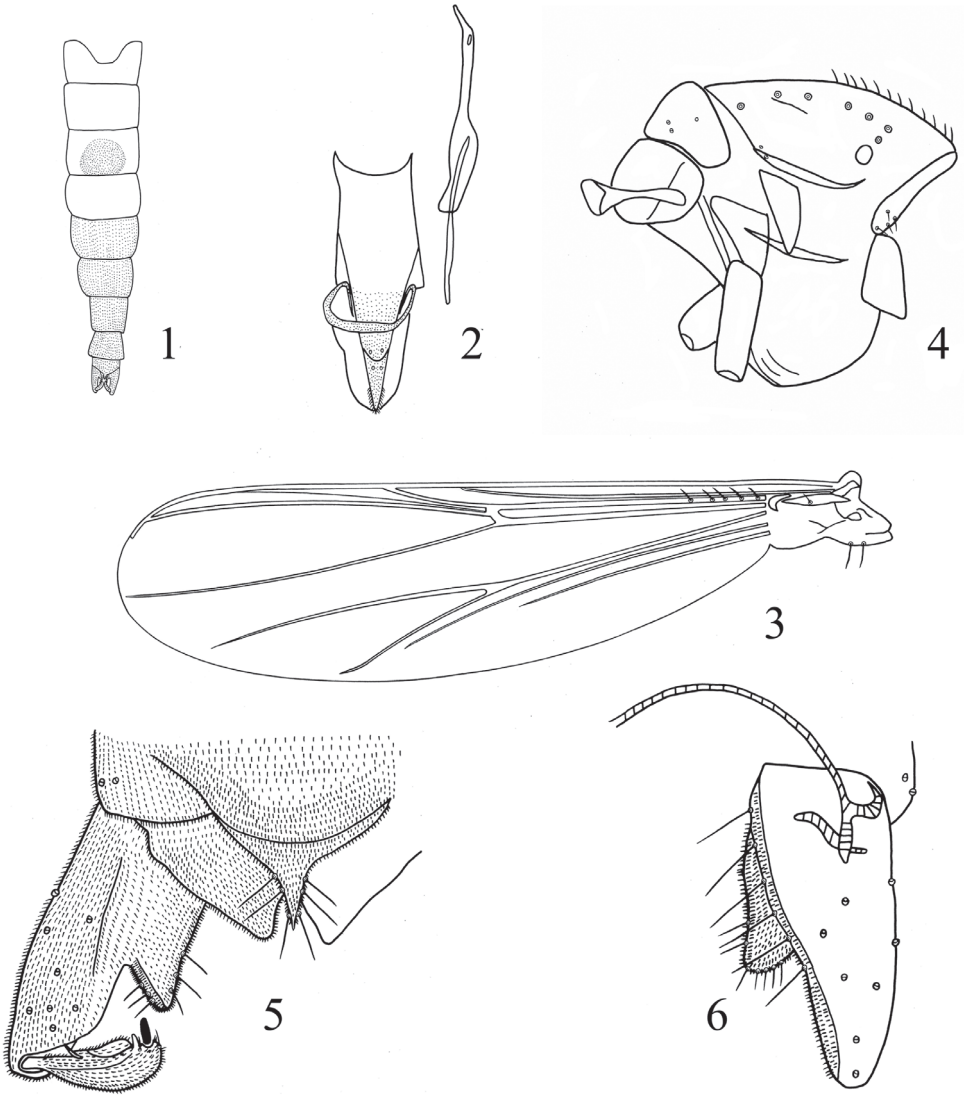
Wing (Fig. 3). Anal lobe reduced. VR 1.06–1.13, 1.07. Costal extension 45–65, 51 μm long. Brachiolum with 1 seta. R with 3–6, 5 setae. Remaining veins bare. Squama with 1–2, 2 setae.

Thorax (Fig. 4). Antepronotum with 2–4, 4 lateral setae. Dorsocentrals 5–7, 6; acrostichals 9–13, 11; prealars 2–3, 3. Scutellum with 2–4, 4 setae. Humeral pit moderately large, ovoid.

Legs. Spur of fore tibia 23–40, 35 μm long; spurs of mid tibia 13–25, 15 μm and 10–18, 12 μm long; spurs of hind tibia 38–50, 41 μm and 10–20, 15 μm long. Hind tibial comb with 9–12, 10 spines, 20–48, 31μm long. Width at apex of fore tibia

Table 1. Lengths (in μm) and proportions of legs of *R.* (*P.*) *brochus* sp. n.

	P_1	P_2	P_3
fe	530–924, 662	530–800, 713	570–820, 629
ti	640–860, 713	530–690, 670	660–870, 724
ta_1	570–770, 618	330–450, 362	410–550, 446
ta_2	310–420, 343	150–200, 161	180–260, 205
ta_3	210–300, 240	100–130, 110	150–210, 169
ta_4	150–200, 163	50–120, 63	70–100, 83
ta_5	70–95, 81	55–70, 57	60–80, 69
LR	0.83–0.90, 0.88	0.61–0.65, 0.63	0.60–0.63, 0.61
BV	2.32–2.52, 2.41	3.94–4.62, 4.10	3.30–3.49, 3.42
SV	2.07–2.32, 2.20	3.11–3.48, 3.26	2.95–3.07, 3.02
BR	2.00–4.00, 2.98	1.57–3.00, 2.51	2.25–3.25, 2.67



Figures 1–6. *Rheocricotopus* (*Psilocricotopus*) *brochus* sp. n., male. **1** abdomen tergites coloration **2** cibarial pump, tentorium and stipes **3** wing **4** thorax **5** hypopygium (dorsal view) **6** hypopygium (ventral view).

35–45, 41 mm, of mid tibia 33–45, 41 mm, of hind tibia 30–45, 38 mm. Lengths (in μm) and proportions of legs as in Table 1.

Hypopygium (Figs 5–6). Anal point tapering to apex, 33–41, 37 μm long, 25–50, 37 μm wide in base, with 3–4, 4 lateral setae in each side. Laterosernite IX with 1–3, 2 setae. Phallapodeme 35–68, 55 μm long. Transverse sternapodeme 35–88, 59 μm long. Gonocoxite 138–191, 157 μm long. Superior volsella triangular, 20–40, 31 μm long, with 5–8, 7 setae. Gonostylus 60–75, 66 μm long. Megaseta 9–13, 11 μm long. Crista dorsalis tooth-shaped. HR 2.11–2.60, 2.38. HV 2.59–3.60, 3.31.

Table 2. Main differences between *R.* (*P.*) *brochus* sp. n., *R.* (*P.*) *bifasciatus* and *R.* (*P.*) *insularis*.

	***R. (P.) brochus* sp. n.**	***R. (P.) bifasciatus***	***R. (P.) insularis***
AR	0.63–0.89, 0.72	0.90	0.71–0.74
Length of costal extension	45–65, 51 μm	83 μm	96 μm
Squama	1–2, 2 setae	2 setae	8 setae
Shape of humeral pit	medium, oviod	large, rounded	oviod
Tergite coloration	TI, II, IV yellow, TIII with a brown circular area, others brown	TI, II, IV yellow, others brown	all tergites brown

Type material. Holotype: ♂ (BDN. C11A32), China, Zhejiang Province, Yueqing City, Lingdi County, Jiulong Village, 28°31'00"N, 120°96'00"E, 18.iv.2011, sweeping, Lin XL. Paratypes (11): 4♂♂, as holotype; 6♂♂, Hubei Province, Hefeng County, 29°91'00"N, 110°03'00"E, 16.vii.1999, light trap, Ji BC; 1♂, Jiangxi Province, Wuyi Mountain National Nature Reserve, 27°48'11"N, 117°39'30"E, 13.vi.2004, light trap, Yan CC.

Etymology. The specific name is an adjective, from Latin *brochus*, meaning tooth, referring to tooth-shaped crista dorsalis.

Remarks. The new species resembles *R.* (*P.*) *bifasciatus* Wang & Zheng, 1991 and *R.* (*P.*) *insularis* Makarchenko & Makarchenko, 2005 in the following combination of characters: anal point long, pointed distally; wing anal lobe reduced; crista dorsalis tooth-shaped. But the new species can be separated from the latter species in the basis following combination of characters in Table 2.

Female and immature stages unknown.

***Rheocricotopus* (*Psilocricotopus*) *chalybeatus* (Edwards, 1929)**
http://species-id.net/wiki/Rheocricotopus_chalybeatus

Spaniotoma chalybeatus Edwards, 1929: 331.
Eukiefferiella urbanus Goetghebuer, 1932: 101.
Trichocladius lerutbi Goetghebuer, 1939: 2.
Rheocricotopus chalybeatus Lehmann, 1969: 354; Hirvenoja 1973: 340; Langton 1984: 98.
Rheocricotopus (*Psilocricotopus*) *chalybeatus* Sæther, 1985: 82; Wang 2000: 639, Ashe and O'Connor 2012: 561.

Specimens examined. 2♂♂, Liaoning Province, Dandong City, Fengcheng City, Cao River, 40°62'50"N, 124°06'96"E, 1.iv.1993, sweeping, Wang JC; 4♂♂, Gansu Province, Longnan City, Gankang County, 33°33'10"N, 105°90'31"E, 2.viii.1982, sweeping, Bu WJ; 1♂, Shandong Province, Zaozhuang City, Shanting District, Beizhuang Town, 34°99'20"N, 102°52'32"E, 28.v.1994, sweeping, Wang XH; 1♂, Shandong Province, Yantai City, Muping District, 37°38'62"N, 121°59'57"E, 28.viii.1988, sweeping, Li HY; 1♂, Shaanxi Province, Baoji City, Feng County, Tsinling Moun-

tains, 34°23'44"N, 106°90'01"E, 27.vii.1994, sweeping, Ji BC; 3♂♂, Zhejiang Province, Wenzhou City, Yueqing City, Furong Town, 33°64'03"N, 121°02'73"E, 2.viii.2010, light trap, Lin XL.

Diagnosis. This species can be separated from its congeners by the following combination of characters: AR 0.89–1.15; R with 2–4 setae; squama with 8–14 setae; Costa not produced or scarcely produced.

Remarks. The additional specimens mainly agree with the description of Lehmann and other workers. But costal extension of specimens from Oriental Region (35–40 μm long) longer than from Palearctic Region (0–15 μm long).

Distribution. China (Liaoning, Gansu, Shandong, Shaanxi and Zhejiang Provinces), Algeria, Balearic Islands, Belarus, Belgium, Corsica, Czech Republic, Denmark, Finland, France, Germany, Great Britain, Hungary, Ireland, Italy, Japan, Lebanon, Luxembourg, Mongolia, Morocco, Netherlands, Poland, Portugal, Romania, Russia, Slovakia, Spain, Switzerland, Syria, Tunisia, Turkey, Ukraine.

***Rheocricotopus* (*Psilocricotopus*) *emeiensis* Wang & Zheng, 1989**

http://species-id.net/wiki/Rheocricotopus_emeiensis

Rheocricotopus emeiensis Wang & Zheng, 1989: 311.
Rheocricotopus (*Psilocricotopus*) *emeiensis* Wang, 2000: 639, Ashe and O'Connor 2012: 563.

Specimens examined. Type material: Holotype, ♂, Sichuan Province, Leshan City, Emei Mountain, 29°58'18"N, 103°29'15"E, 17.v.1986, sweeping, Wang XH.

Additional material. 1♂, Xinjiang Uygur Autonomous Region, Haba County, 48°17'00"N, 86°42'00"E, 15.vii.2002, sweeping, Tang HQ; 2♂♂, Shaanxi Province, Hanzhong City, Liuba County, Xiaoliuba Village, 33°64'03"N, 106°90'31"E, 4.viii.1994, sweeping, Ji BC; 2♂♂, Shaanxi Province, Xi'an City, Zhouzhi County, Banfangzi, 33°81'84"N, 107°99'64"E, 7.viii.1994, light trap, Ji BC; 2♂♂, Shaanxi Province, Xi'an City, Zhouzhi County, Banfangzi, 33°81'84"N, 107°99'64"E, 10.viii.1994, light trap, Ji BC; 1♂, Yunnan Province, Kunming City, Fumin County, 25°22'61"N, 102°52'32"E, 1.vi.1996, sweeping, Wang XH; 1♂, Yunnan Province, Kunming City, Yiliang County, 24°92'24"N, 103°13'95"E, 2.vi.1996, sweeping, Wang XH; 1♂, Fujian Province, Xiamen City, 24°48'24"N, 118°08'44"E, 15.v.1993, sweeping, Bu WJ; 1♂, Guizhou Province, Guiyang City, 26°60'17"N, 106°70'36"E, 10.vii.1995, light trap, Bu WJ; 1♂, Guizhou Province, Guiyang City, Huaxi District, 26°41'34"N, 106°66'66"E, 23.vii.1995, sweeping, Bu WJ.

Diagnosis. This species can be separated from other members of the group by the following combination of characters: R bare; anal point short, pointed distally.

Remarks. The additional specimens are similar to the description of Wang and Zheng (1989). The species is recorded from Palearctic Region for the first time.

Distribution. China (Fujian, Guizhou, Sichuan, Shaanxi and Yunnan Provinces, Xinjiang Uygur Autonomous Region).

***Rheocricotopus* (*Psilocricotopus*) *imperfectus* Makarchenko & Makarchenko, 2005**
http://species-id.net/wiki/Rheocricotopus_imperfectus

Rheocricotopus (*Psilocricotopus*) *imperfectus* Makarchenko & Makarchenko, 2005: 126; Makarchenko and Makarchenko 2011: 120, Ashe and O'Connor 2012: 564.

Specimens examined. 1♂, Hubei Province, Shennongjia Forest Region, 31°74'56"N, 110°67'53"E, 19.vii.1997, sweeping, Du YZ; 1♂, Hubei Province, Lichuan City, 30°29'37"N, 108°93'20"E, 30.vii.1999, sweeping, Ji BC; 12♂♂, Shaanxi, Baoji City, Feng County, Tsinling Mountains, 34°23'44"N, 106°90'01"E, 28–30.vii.1994, sweeping, Bu WJ; 1♂, Shaanxi, Ankang City, Ningshan County, Huoditang Town, 33°43'38"N, 108°44'81"E, 12.viii.1994, sweeping, Bu WJ; 1♂, Shaanxi, Ankang City, Ningshan County, Xunyangba Town, 33°54'82"N, 108°54'77"E, 17.viii.1994, sweeping, Bu WJ; 10♂♂, Ningxia Hui Autonomous Region, Guyuan City, Jingyuan County, Liupan Mountain, 35°78'97"N, 106°28'93"E, 6–7.viii.1987, sweeping, Wang XH.

Diagnosis. This species can be separated from other members of the group by the following combination of characters: AR 0.47; humeral pit large and rounded; acrostichal absent; anal point of hypopygium sharply triangular, with 10 setae along the edges; gonostylus slightly curved, with roundish triangular crista dorsalis.

Remarks. Chinese specimens mainly agree with the description of Makarchenko and Makarchenko (2005), but Chinese specimens with more setae in R (4–8) than the specimens in Russia (R with 3 setae).

Distribution. China (Hubei and Shaanxi Provinces, Ningxia Hui Autonomous Region), Russia (Far East).

***Rheocricotopus* (*Psilocricotopus*) *nigrus* Wang & Zheng, 1989**
http://species-id.net/wiki/Rheocricotopus_nigrus

Rheocricotopus nigrus Wang & Zheng, 1989: 311.
Rheocricotopus (*Psilocricotopus*) *nigrus* Wang, 2000: 639; Makarchenko and Makarchenko 2011: 120, Ashe and O'Connor 2012: 566.

Specimens examined. Type material: Holotype, ♂, Hubei Province, Xiangyang City, Gucheng County, 32°29'00"N, 111°64'00"E, 5.v.1986, sweeping, Wang XH; Paratype (1): 1♂, as holotype.

Additional material. 2♂♂, Zhejiang Province, Qingyuan County, Baishanzu National Nature Reserve, 27°73'23"N, 119°19'06"E, 18.iv.1995, sweeping, Ji BC; 11♂♂, Xinjiang Uygur Autonomous Region, Mongolian Autonomous Prefecture of Bayingolin, Kuerleying Town, 41°74'10"N, 86°10'82"E, 26.viii.2002, light trap, Tang HQ; 1♂, Xinjiang Uygur Autonomous Region, Mongolian Autonomous Prefecture of Bayingolin, Yuli County, 41°57'00"N, 86°30'00"E, 25.v.2002, sweeping, Tang HQ; 1♂, Xinjiang Uygur Autonomous Region, Haba County, 48°17'00"N,

86°42'00"E, 15.vii.2002, sweeping, Tang HQ; 1♂, Fujian Province, Nanping City, Mangdang Mountain, 26°69'52"N, 118°12'55"E, 22.ix.2002, sweeping, Liu Z; 1♂, Fujian Province, Nanping City, Mangdang Mountain, 26°69'52"N, 118°12'55"E, 23.ix.2002, sweeping, Liu Z.

Diagnosis. The adult male can be separated from other members of the group by the following combination of characters: body totally dark brown; AR 1.30; dorsocentrals 20; humeral pit large, similar to the square.

Remarks. The species distributed in both Oriental and Palearctic Region. The specimens from Palearctic Region have fewer dorsocentrals (13–17) than those from Oriental Region (20).

Distribution. China (Hubei, Zhejiang and Fujian Provinces, Xinjiang Uygur Autonomous Region), Russia (Far East).

***Rheocricotopus* (*Psilocricotopus*) *robacki* (Beck & Beck, 1964)**
http://species-id.net/wiki/Rheocricotopus_robacki

Tricocladius robacki Beck & Beck, 1964: 204.
Rheocricotopus kenorensis Sæther, 1969: 88.
Rheocricotopus (*Psilocricotopus*) *robacki* Sæther, 1985: 79, Ashe and O'Connor 2012: 567.

Specimens examined. 1♂, Shaanxi Province, Xi'an City, Zhouzhi County, Banfangzi, 33°81'84"N, 107°99'64"E, 10.viii.1994, light trap, Ji BC; 8♂♂, Jiangxi Province, Yichun City, Yifeng County, 28°39'69"N, 114°67'55"E, sweeping, Yan CC; 1♂, Fujian Province, Longyan City, Shanghang County, 25°05'21"N, 116°41'52"E, 6.v.1993, sweeping, Wang XH; 2♂♂, Xinjiang Uygur Autonomous Region, Boertala Mongol Autonomous Prefecture, Sailimu lake, 44°62'32"N, 81°20'48"E,30.vii.2002, sweeping, Tang HQ; 7♂♂, Guizhou Province, Zunhua City, Daozhen County, Dasha River, 28°86'58"N, 107°60'73"E, 25.v.2004, light trap, Tang HQ; 2♂♂, Guizhou Province, Zunhua City, Daozhen County, Dasha River, 28°86'58"N, 107°60'73"E, 24.viii.2004, sweeping, Yu X; 3♂♂, Yunnan Province, Dali Bai Autonomous Prefecture, Eryuan County, Niujie Town, 26°25'55"N, 99°98'90"E, light trap, Wang BX; 1♂, Tibet, Xigaze, Nielamu County, 27°98'73"N, 85°98'32"E, 15.8.1987, light trap, Deng CY; 1♂, Tibet, Xigaze, Nielamu County, 27°98'73"N, 85°98'32"E, 21.9.1987, light trap, Deng CY.

Diagnosis. The species is characterized by having a relatively high AR (1.14–1.24), very weak and short acrostichals, 8–14 dorsocentrals, reduced number of bristles on squama, anal tergite extending beyond tip of anal point, superior volsella triangular, crista dorsalis triangular, apex pointed.

Remarks. Chinese specimens mainly agree with the description of Sæther (1969, 1985). It is recorded in Palearctic Region for the first time. Chinese specimens have lower body length (2.55–3.13 mm) and lower AR 1.07 than species from Nearctic Region (total length 3.10–3.30 mm, AR 1.18).

Distribution. China (Fujian, Guizhou, Jiangxi, Shaanxi and Yunnan Provinces, Xinjiang Uygur Autonomous Region, Tibet), Canada, U.S.A.

***Rheocricotopus* (*Psilocricotopus*) *rotundus* sp. n.**
http://zoobank.org/5796E5A3-6914-462C-97E2-9CE1709E522E
http://species-id.net/wiki/Rheocricotopus_rotundus
Figs 7–11

Diagnosis. The adult male of the new species can be distinguished from known species of the genus by the following combination of characters: low AR 0.25–0.29; superior volsella rounded.

Description. Male (n = 2).

Total length 1.58–1.98 mm. Wing length 0.86–1.20 mm. Total length/wing length 1.66–1.84. Wing length/length of profemur 1.74–2.61.

Coloration. Head and abdomen yellow brown, thorax without distinct pattern.

Head. Antenna as in Fig. 7. AR 0.25–0.29. Ultimate flagellomere 88–118 μm long. Temporal setae 3–4, including 1–2 inner verticals and 2 outer verticals. Clypeus with 6–12 setae. Tentorium 115–130 μm long, 23–25 μm wide. Stipes 115–118 μm long, 4–5 μm wide. Palpomere lengths (in μm): 48–53, 30–45, 48–60, 68–90, 123–163. L: $5^{th}/3^{rd}$ 2.56–2.71.

Wing (Fig. 8). Anal lobe normally developed. VR 1.17–1.19. Costal extension 30–38 μm long. Brachiolum with 1 seta. R with 1–3 setae. Remaining veins bare. Squama with 2 setae.

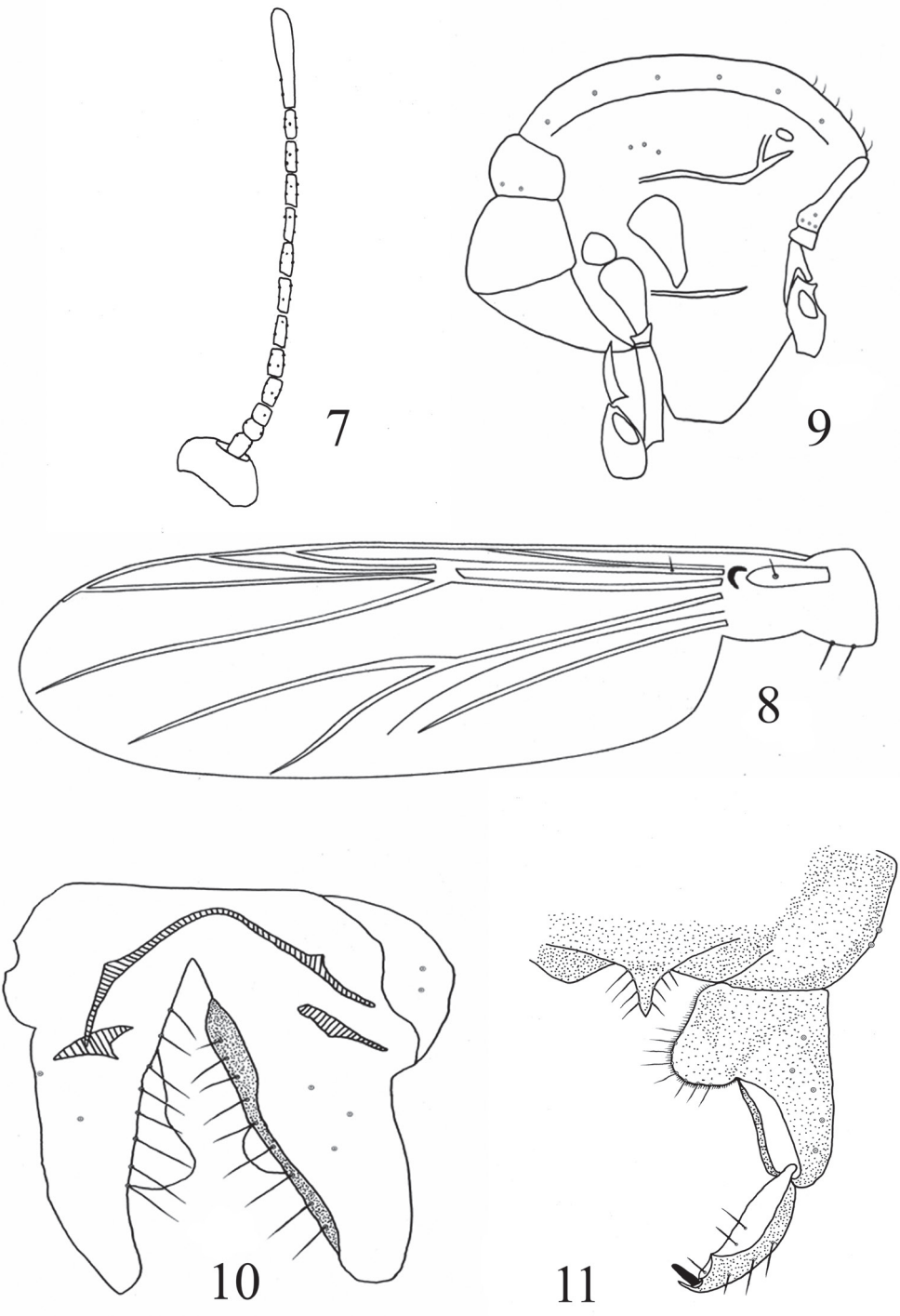
Thorax (Fig. 9). Antepronotum with 4 lateral setae. Dorsocentrals 6–11; acrostichals 6–8, prealars 3. Scutellum with 2–6 setae. Humeral pit moderately large, ovoid.

Legs. Spur of fore tibia 23–40 μm long; spurs of mid tibia 13–18 μm long and 12–15 μm long; spurs of hind tibia 27–38 μm and 13–15 μm long. Hind tibial comb with 8–16 spines, 13–27 μm long. Width at apex of fore tibia 25–40 mm, of mid tibia 25–38 mm, of hind tibia 23–40 mm. Lengths and proportions of legs as in Table 3.

Hypopygium (Figs 10–11). Anal point triangular, 20–28 μm long, with 4 lateral setae each side. Laterosernite IX with 2 setae. Phallapodeme 48–50 μm long. Transverse

Table 3. Lengths (in μm) and proportions of legs of *R.* (*P.*) *rotundus* sp. n.

	P_1	P_2	P_3
fe	330–490	350–475	310–445
ti	380–500	310–485	350–520
ta_1	218	135–240	188–300
ta_2	153	80–135	108–165
ta_3	120	65–110	90–145
ta_4	75	35–50	35–65
ta_5	50	40–50	50–65
LR	0.57	0.44–0.49	0.54–0.58
BV	2.33	3.48–3.61	2.88–3.00
SV	3.26	4.00–4.89	3.22–3.51
BR	2.67	2.00–2.80	3.06–3.83



Figures 7–11. *Rheocricotopus* (*Psilocricotopus*) *rotundus* sp. n., male. **7** antenna **8** wing **9** thorax **10** hypopygium (ventral view) **11** hypopygium (dorsal view).

sternapodeme arcuate with normally oral projection, 33–40 μm long. Gonocoxite 125–135 μm long. Superior volsella rounded, plate-shaped and sclerotized, 28–38 μm long and 20–27 μm wide, covered with 8 short setae and a few microtrichia. Gonostylus 55–70 μm long, with distinct, triangular crista dorsalis. Megaseta 8–10 μm long. HR 1.93–2.27. HV 2.82–2.87.

Type material. Holotype: ♂ (BDN. G5A42), China, Zhejiang Province, Jinhua City, Pan'an County, Dapanshan National Nature Reserve, 28°98'02"N, 120°52'63"E, 18.vii.2012, sweeping, Lin XL. Paratype (1): 1♂, Yunnan Province, Dali Bai Autonomous Prefecture, Eryuan County, Niujie Town, 26°25'55"N, 99°98'90"E, sweeping, Wang BX.

Etymology. The specific name is an adjective, from Latin *rotundus*, meaning rounded, referring to rounded superior volsella.

Remarks. The new species resembles *R.* (*P.*) *notabilits* Caspers, 1987 in the following combination of characters: low AR; humeral pit medium, ovoid; the shape of anal point. But the new species can be separated from latter species on the basis of following characters: (1) costal extension of the new species (30–38 μm) much shorter than *R.* (*P.*) *notabilits* Caspers (72 μm); (2) anal lobe of the new species developed, which reduced in *R.* (*P.*) *notabilits* Caspers; and (3) crista dorsalis of the new species distinct, triangular subapical, which pronounced, rounded in *R.* (*P.*) *notabilits* Caspers.

Female and immature stages unknown.

***Rheocricotopus* (*Psilocricotopus*) *serratus* sp. n.**
http://zoobank.org/D6FD7A2F-87EB-4513-A6D4-F122B14548DA
http://species-id.net/wiki/Rheocricotopus_serratus
Figs 12–15

Diagnosis. The adult male of the new species can be distinguished from known species of the species group and the genus by the following combination of characters: crista dorsalis sawtooth-shaped, hyaline, high as megaseta; high HR (2.61–3.42) and HV (3.40–5.00).

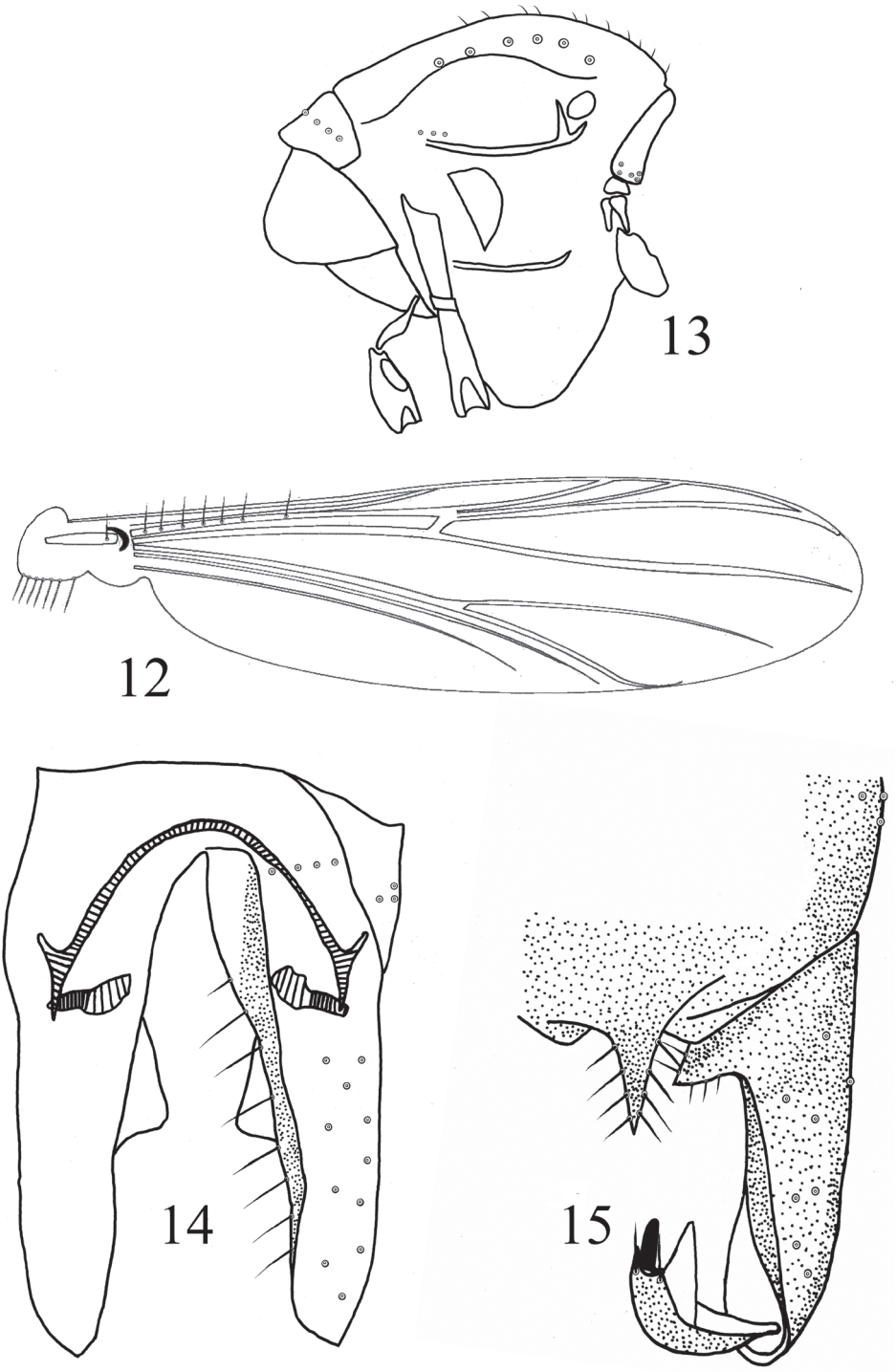
Description. Male imago (n = 4)

Total length 2.55–3.00, 2.73 mm. Wing length 1.33–1.95, 1.69 mm. Total length/wing length 1.44–1.94, 1.64. Wing length/length of profemur 2.21–2.35, 2.27.

Coloration. Head and abdomen yellow, thorax dark brown.

Head. AR 0.71–0.78, 0.74. Ultimate flagellomere 285–355, 323 μm long. Temporal setae 0–3, 1, including 0–1, 1 inner vertical; 0–2, 1 outer vertical and 0–1, 1 postorbital. Clypeus with 9–12, 10 setae. Tentorium 140–153, 148 μm long, 33–40, 38 μm wide. Stipes 65–75, 68 μm long, 3–7, 4 μm wide. Palpomere lengths (in μm): 35–70, 47; 48–58, 53; 103–123, 114; 118–155, 141; 205–238, 219. L: $5^{th}/3^{rd}$ 1.67–2.02, 1.92.

Wing (Fig. 12). Anal lobe slightly developed. VR 1.13–1.20, 1.17. Costal extension 50–88, 71 μm long. Brachiolum with 1 seta. R with 4–11, 7 setae. Remaining veins bare. Squama with 6–13, 9 setae.



Figures 12–15. *Rheocricotopus* (*Psilocricotopus*) *serratus* sp. n., male. **12** wing **13** thorax **14** hypopygium (ventral view) **15** hypopygium (dorsal view).

Table 4. Lengths (in μm) and proportions of legs of *R.* (*P.*) *serratus* sp. n.

	P_1	P_2	P_3
fe	600–830, 743	570–800, 700	600–780, 715
ti	650–810, 760	660–720, 698	660–850, 783
ta_1	520–780, 640	310–410, 380	380–530, 475
ta_2	290–390, 350	150–200, 183	200–270, 253
ta_3	205–270, 250	100–140, 125	160–220, 198
ta_4	155–200, 181	55–80, 71	90–120, 105
ta_5	90–100, 95	60–80, 73	70–90, 83
LR	0.78–0.96, 0.84	0.47–0.58, 0.54	0.58–0.63, 0.61
BV	2.39–2.55, 2.44	3.71–4.22, 3.96	2.17–3.04, 2.81
SV	1.95–2.61, 2.37	3.56–3.97, 3.69	2.92–3.32, 3.17
BR	1.75–2.11, 1.91	2.10–2.22, 2.19	2.40–3.55, 2.99

Thorax (Fig. 13). Antepronotum with 1–7, 4 lateral setae. Dorsocentrals 6–9, 7; acrostichals 9–15, 12; prealars 3. Scutellum with 4–6, 5 setae. Humeral pit moderately large, egg-shaped.

Legs. Spur of fore tibia 40–48, 44 μm long; spurs of mid tibia 15–25, 19 μm and 15–20, 17 μm long; spurs of hind tibia 43–55, 48 μm and 18–23, 19 μm long. Hind tibial comb with 11–13, 12 spines, 30–50, 40 μm long. Width at apex of fore tibia 35–45, 41 mm, of mid tibia 36–45, 41 mm, of hind tibia 38–45, 43 mm. Lengths (in μm) and proportions of legs as in Table 4.

Hypopygium (Figs 14–15). Anal point triangular, pointed distally, 33–50, 41 μm long, 20–30, 24 μm wide, with 4–5, 5 lateral setae each side. Laterosernite IX with 2–3, 3 setae. Phallapodeme 23–28, 25 μm long. Transverse sternapodeme 18–33, 26 μm long. Gonocoxite 180–205, 195 μm long. Superior volsella triangular, 35–43, 40 μm long, with 7–8, 8 setae. Gonostylus 60–75, 67 μm long. Megaseta 13–15, 14 μm long. Crista dorsalis sawtooth-shaped, hyaline, high as megaseta. HR 2.61–3.42, 2.91. HV 3.40–5.00, 4.09.

Type material. Holotype: ♂ (BDN. 10058), China, Yunnan Province, Dali Bai Autonomous Prefecture, Eryuan County, Niujie Town, 26°25'55"N, 99°98'90"E, light trap, Zhou CF. Paratypes (3): 1♂, Zhejiang Province, Qingyuan County, Baishanzu National Nature Reserve, 27°73'23"N, 119°19'06"E, 15.vii.1994, Ji BC; 1♂, Tibet, Xigaze, Nielamu County, 27°98'73"N, 85°98'32"E, 21.9.1987, light trap, Deng CY; 1♂, Sichuan Province, Xiangcheng County, 28°93'44"N, 99°79'72"E, 12.vi.1996, light trap, Wang XH.

Etymology. The specific name is an adjective, from Latin *serratus*, meaning sawtooth, referring to the sawtooth-shaped crista dorsalis.

Remarks. The new species resembles *R.* (*P.*) *himalayensis* Chaudhuri & Sinharay, 1983 in the triangular anal point, but it can be separated from the latter species on the basis of following characters: (1) costal extension of the new species much longer (50–88 μm), than *R.* (*P.*) *himalayensis* Chaudhuri & Sinharay (25 μm); (2) humeral

pit in the new species medium, ovoid, which rounded in the latter species; (3) crista dorsalis sawtooth-shaped, hyaline in the new species, which moderately pronounced in the latter species.

Female and immature stages unknown.

Rheocricotopus (*Psilocricotopus*) *taiwanensis* Wang, Yan & Maa, 2004

http://species-id.net/wiki/Rheocricotopus_taiwanensis

Rheocricotopus (*Psilocricotopus*) *taiwanensis* Wang, Yan & Maa, 2004: 239, Ashe and O'Connor 2012: 567.

Specimens examined. Type material: Holotype, ♂, Taiwan Province, Taipei City, Guandu, Wetland, 25°11'56"N, 121°47'14"E, 20.x.1988, sweeping, Maa CJ.

Diagnosis. The adult male can be separated from other members of the group by the following combination of characters: all veins of wing bare; low AR (0.71); squama with 3 setae; very pronounced crista dorsalis.

Distribution. China (Taiwan Province).

Rheocricotopus (*Psilocricotopus*) *valgus* Chaudhuri & Sinharay, 1983

http://species-id.net/wiki/Rheocricotopus_valgus

Rheocricotopus valgus Chaudhuri & Sinharay, 1983: 402.
Rheocricotopus (*Psilocricotopus*) *valgus* Ashe & O'Connor, 2012: 568.

Specimens examined. 1♂, Guangdong Province, Fengkai County, Heishiding, 23°30'02"N, 111°55'01"E, 20.iv.1988, sweeping, Wang XH; 1♂, Guangxi Province, Longsheng County, 25°89'26"N, 110°21'21"E, 16.v.1990, sweeping, Wang XH; 1♂, Guangxi Province, Jinxiu County, 24°14'00"N, 110°19'00"E, 1.vi.1990, light trap, Wang XH; 2♂♂, Hubei Province, Hefeng County, 29°91'00"N, 110°03'00"E, 16.vii.1999, light trap, Ji BC; 1♂, Hubei Province, Xianfeng Mountain, 29°70'00"N, 119°14'00"E, 25.vii.1999, sweeping, Ji BC; 1♂, Zhejiang Province, Lishui City, Qingyuan County, Baishanzu, 27°73'23"N, 119°19'06"E, 13.vii.1995, light trap, Ji BC; 4♂♂, Zhejiang Province, Lishui City, Qingyuan County, Baishanzu National Nature Reserve, 27°73'23"N, 119°19'06"E, 24.vii.2012, light trap, Lin XL; 1♂, Zhejiang Province, Wenzhou City, Taishun County, Wuyanling National Nature Reserve, 27°71'15"N, 119°64'64"E, 3.viii.2005, light trap, Ji BC; 1♂, Zhejiang Province, Lishui City, Jingning County, 27°97'67"N, 119°63'12"E, 27.vii.2012, light trap, Lin XL.

Diagnosis. This species can be separated from other members of the group by the following combination of characters: R without seta; tergites I, II and anterior part of tergite V pale brown, tergites IV and VIII brown, anal point with 4–5 setae on each

Table 5. Differences in the Chinese and Indian specimens of *R.* (*P.*) *valgus*

	Chinese specimens	Indian specimens
Squama	5–8, 7 setae	9 setae (average)
HR	1.98–2.15, 2.08	2.20 (average)
HV	2.88–3.20, 3.03	3.40 (average)

side and 1 seta at the base; gonocoxite with a prominent triangular basal lobe bearing 3–4 setae.

Remarks. The Chinese specimens generally agree with the original description by Chaudhuri and Sinharay (1983), though some measured differences between the Chinese specimens and those of Chaudhuri and Sinharay (1983) are shown in Table 5.

Distribution. China (Guangdong, Guangxi, Hubei and Zhejiang Provinces), India.

Key to adult males of *Rheocricotopus chalybeatus* species group in China

1 Anal lobe reduced **2**
– Anal lobe moderately or very developed **5**
2 Costa not beyond R_{4+5} ..... ***R.* (*P.*) *robacki* (Beck & Beck)**
– Costa beyond R_{4+5} 3
3 Squama with 9 setae ..... ***R.* (*P.*) *bifasciatus* Wang & Zheng**
– Squama bare, or with 1 seta **4**
4 Tergites I, II, IV yellow, tergite III with a brown circular area, others brown .. .. ***R.* (*P.*) *brochus* sp. n.**
– All tergites I-IX dark brown .... ***R.* (*P.*) *imperfectus* Makarchenko & Makarchenko**
5 AR 0.25–0.29; superior volsella rounded ..... ***R.* (*P.*) *rotundus* sp. n.**
- AR 0.45–1.30; superior volsella triangular **6**
6 Humeral pit large, similar to the square **7**
– Humeral pit rounded or ellipsoid **8**
7 Dorsocentrals 20; R_1 with 1 seta ..... ***R.* (*P.*) *nigrus* Wang & Zheng**
– Dorsocentrals 10–14; R_1 bare ..... ***R.* (*P.*) *chalybeatus* (Edwards)**
8 Crista dorsalis sawtooth-shaped, transparent ..... ***R.* (*P.*) *serratus* sp. n.**
– Crista dorsalis triangular or rectangular **9**
9 Costal extension 120 μm long; AR 1.20 ... ***R.* (*P.*) *valgus* Chaudhuri & Sinharay**
– Costal extension 30–45 μm long; AR 0.43–1.00 ..... **10**
10 Acrostichals absent **11**
– Acrostichals 7–9 ..... ***R.* (*P.*) *brachypus* Wang & Zheng**
11 Supraalars present; crista dorsalis rectangular ... ***R.* (*P.*) *emeiensis* Wang & Zheng**
– Supraalars absent; crista dorsalis triangular .... ***R.* (*P.*) *taiwanensis* Wang, Yan & Ma**

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RESEARCH ARTICLE

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New cave-dwelling armored spiders (Araneae, Tetrablemmidae) from Southwest China

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http://zoobank.org/9EEB8A81-1E58-46D7-9A16-1A2271B67709

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Abstract

A new genus and five new species belonging to the family Tetrablemmidae are described from caves in Southwest China, i.e., *Sinamma oxycera* **gen. & sp. n.**, *Singaporemma banxiaoensis* **sp. n.**, *Singaporemma wulongensis* **sp. n.**, *Tetrablemma ziyaoensis* **sp. n.** and *Tetrablemma menglaensis* **sp. n.** The following new combination is proposed: *Sinamma sanya* (Lin & Li, 2010), **comb. n.** ex. *Shearella* Lehtinen, 1981. The relationships of the *Sinamma* **gen. n.** with other genera are discussed. Diagnoses and illustrations for all new taxa are given.

Keywords

Taxonomy, haplogyne spiders, new species, diagnosis, distribution

Introduction

Tetrablemmids are medium-sized (Pacullinae) to small (Tetrablemminae) haplogyne spiders, characterized by a complex pattern of abdominal scuta (Shear 1978; Lehtinen 1981; Jocqué and Dippenaar-Schoeman 2006). They are mainly distributed in the tropical and subtropical regions where they are found in leaf litter, soil, and in caves (Burger et al. 2010). This family has been revised by Lehtinen (1981). Burger et al. (2006) and Burger (2008) studied the functional morphology of the genitalia of the family Tetrablemmidae. A total of 144 species belonging to 30 genera of the family Tetrablemmidae have been described (Platnick 2014).

Chinese tetrablemmids have been studied recently. Five species belonging to four genera from Hainan Province were reported by Tong and Li (2008). Lian (2009) reported one species of the genus *Perania* Thorell, 1890 from Yunnan Province. Lin and Li (2010) described five more species (two from rainforest habitats in Hainan and three from caves in the Yunnan-Guizhou Plateau). In total, 11 species belonging to eight genera have been reported from China (cf. Platnick 2014).

In the period of October 2010 to August 2011 we found several new and interesting species of the family Tetrablemmidae from Southwest China and in this paper we now describe five new tetrablemmid taxa.

Material and methods

Specimens were examined and measured under an Olympus SZX7 stereomicroscope. Further details were studied under an Olympus BX43 compound microscope. All drawings were made using a drawing tube attached to an Olympus BX43 compound microscope, and then hand inked on ink jet plotter paper. Photographs were taken with a Canon EOS 60D wide zoom digital camera (8.5 megapixels). The images were combined using Helicon Focus 3.10 software. Male palp and female genitalia were examined and illustrated after they were dissected and detached from the spiders' bodies. Vulvae were removed and treated in KOH solution before examination and illustration. To reveal the course of the sperm duct, the bulbs were treated in lactic acid and mounted in Hoyer's Solution before examination and illustration. Left palp is illustrated unless missing, in which case the right palp is illustrated.

All measurements are provided in millimeters. Height of carapace is measured with tubercle. Leg measurements are given as total length (femur, patella, tibia, metatarsus, tarsus). The terminology mostly follows Lehtinen (1981) and Burger (2008). The abbreviations used in figures as follows: CP – central process; EP – epigynal pit; EF – epigynal fold; IVP – inner vulval plate; LH – lateral horn; PA – preanal scutum; PEG – perigenital scutum; POG – postepigastral scutum; SR – seminal receptaculum; VD – vulval duct; VS – vulval stem.

All specimens are acquired from caves by manual collection and preserved in 85% ethanol solution. All specimens are deposited in the Sichuan University Museum (SCUM) in Chengdu and in the Institute of Zoology, Chinese Academy of Sciences (IZCAS) in Beijing.

Taxonomy

***Sinamma* Lin & Li, gen. n.**
http://zoobank.org/01376E2E-D204-4B55-8597-BD18F5AF0223
http://species-id.net/wiki/Sinamma

Type species. *Sinamma oxycera* sp. n. from cave of Guangxi, China.

Etymology. The generic name derives from the Latin word "*sina*" and "*-mma*" as a suffix of the genus *Tetrablemma*. The gender is feminine, with *sina* meaning China.

Diagnosis. *Sinamma* gen. n. differs from all known genera of Tetrablemminae by the presence of a tubercle on the male carapace (Figs 1G, 1E; Lin and Li 2010: 24, figs 19, 20) and sometimes in females (Figs 1H, 1F; Lin and Li 2010: 25, figs 25, 26), the strongly modified male leg I (Figs 2C–E; Lin and Li 2010: 24, fig. 22), and by the exceptionally narrow postepigastral scutum in the both sexes (Figs 1B, 1D, 3A–C, 19B; Lin and Li 2010: 25, figs 27–28).

Description. Small (1.2–1.6), six eyes compact in a group, male carapace usually with cephalic tubercle (present or absent in female). Male leg I robust, with tubercles at tibia and metatarsus. Cheliceral horn present (absent in female), much longer than in *Brignoliella* Shear, 1978 or *Shearella* Lehtinen, 1981, but shorter than in *Tetrablemma* O. P.-Cambridge, 1873 and *Gunasekara* Lehtinen, 1981. Abdomen oval, lateral scuta II–IV wide in both sexes.

Bulb long pyriform, embolus simple, needle-shaped; epigynal fold narrow; postepigastral scutum exceptionally narrow; central process absent, inner vulval plate well developed, vulval stem transverse.

Remarks. The new genus *Sinamma* gen. n. contains two species and belongs to the subfamily Tetrablemminae. *Sinamma* gen. n. is similar to *Shearella* by the conical cheliceral horn and the pyriform bulb in the males, and is also similar to *Gunasekara* in having strongly modified leg I in the males. However, it can be distinguished from both *Shearella* and *Gunasekara* by the carapace having cephalic tubercle in male (sometimes in female also, e.g. *Sinamma oxycera* sp. n. and *Shearella sanya* Lin & Li, 2010), the presence of a distinct inner vulval plate in the female, and by the exceptionally narrow postepigastral scutum. The only oriental genus, *Singalangia* Lehtinen, 1981, for which the male is not yet known, has entirely different ocular pattern and vulval structures compared with those of *Sinamma* gen. n. In summary, *Sinamma oxycera* sp. n. and *Shearella sanya* Lin & Li share the following synapomorphies: an obvious cephalic tubercle, a strongly modified leg I in the male, and an exceptionally narrow postepigastral scutum in the female. These features distinguish them from other tetrablemmids. The new genus *Sinamma* is therefore proposed to accommodate these two oriental species, *Sinamma oxycera* sp. n. and *Sinamma sanya* (Lin & Li, 2010), comb. n., previously considered in *Shearella*.

Composition. *Sinamma oxycera* sp. n. and *Sinamma sanya* (Lin & Li, 2010).

Distribution. China (Guangxi, Hainan).

***Sinamma oxycera* sp. n.**
http://zoobank.org/104113CB-D708-415B-BD6F-1BBE847F6AFA
http://species-id.net/wiki/Sinamma_oxycera
Figs 1–3, 16A–B, 19B, 22

Material. Holotype ♂ and paratypes 2♀ (IZCAS), CHINA, Guangxi: Chongzuo City, Longzhou County, Shanglong Town, Xinlian Village, Gengyitun, Longmolai Cave, 22°29.809'N, 106°54.103'E, elevation ca. 224 m, 24 July 2011, Xiaoxiao Wang leg.

Etymology. The specific name derives from the Greek word "*oxycerus*" = sharp horn, and refers to the sharp cephalic tubercle in the male; noun.

Diagnosis. Males of *S. oxycera* sp. n. can be distinguished from *S. sanya* (see Lin and Li 2010: 23, figs 19–28) by a long cephalic tubercle (Figs 1G, E), the strongly modified leg I (Figs 2C–E), and the long pyriform palpal bulb (Figs 2A–B). Females can be recognized by a pair of cephalic tubercles (Fig. 1H), a wide, translucent vulval dorsal plate (Fig. 19B), a straight, long, inner vulval plate (Figs 3C, 19B), and the anteriorly wrinkled preanal scutum (Figs 3B, 19B).

Description. Male (holotype). Coloration: body reddish-brown; legs yellowish-brown. Measurements: total length 1.48; carapace 0.68 long, 0.57 wide, 0.57 high; abdomen 0.95 long, 0.71 wide, 0.69 high; clypeus 0.48 high; sternum 0.41 long, 0.41 wide. Length of legs: I 1.89 (0.62, 0.20, 0.42, 0.33, 0.33); II 1.79 (0.57, 0.18, 0.41, 0.30, 0.32); III 1.61 (0.47, 0.16, 0.37, 0.29, 0.32); IV 2.15 (0.66, 0.18, 0.55, 0.40, 0.36).

Carapace (Figs 1A, E, G) finely reticulated, margin rugose; ocular area raised, cephalic tubercle long, sharp (Figs 1G, E); clypeus very high, anterior margin rounded (Figs 1A, G); clypeal area slightly convex; cheliceral horn long, basally wide, distally crooked (Figs 1G, E); sternum with sparse setae, margins rugose (Fig. 1B). Legs: femur I swollen (Fig. 2C); tibiae I–III with 3 trichobothria, tibia IV with 4 trichobothria, and metatarsi I-IV with a thichobothrium; tibia I medially wide, with two small lateral tubercles; metatarsus I with a proximal and a distal tubercle (Figs 2C–E).

Abdomen (Figs 1A–B, E) dorsal scutum oval, finely granulated; ventral scutum reticulated, margin striated; lateral scutum I short; postepigastral scutum exceptionally narrow, subequal in width to preanal scutum (Fig. 1B).

Palp (Figs 2A–B, 16A–B): femur slightly swollen, ventrally granulated; patella approximately 1/2 femur in length; tibia smooth, swollen; bulb long, pyriform, smooth; embolus long, curved slightly, strongly sclerotized; sperm duct extending, visible through the bulbal integument.

Female (paratype). Coloration: same as in male.

Measurements: total length 1.59; carapace 0.68 long, 0.53 wide, 0.41 high; abdomen 1.02 long, 0.84 wide, 0.88 high; clypeus 0.23 high; sternum 0.40 long, 0.39 wide. Length of legs: I 1.89 (0.61, 0.18, 0.46, 0.31, 0.34); II 1.73 (0.55, 0.16, 0.41, 0.29, 0.32); III 1.61 (0.46, 0.15, 0.39, 0.29, 0.31); IV 2.13 (0.64, 0.17, 0.55, 0.39, 0.37).

Carapace (Figs 1C, H and F) with a pair of cephalic tubercles; cephalic part slightly elevated, clypeus lower than in male; cheliceral horn absent. Legs as in male, except for leg I undecorated.

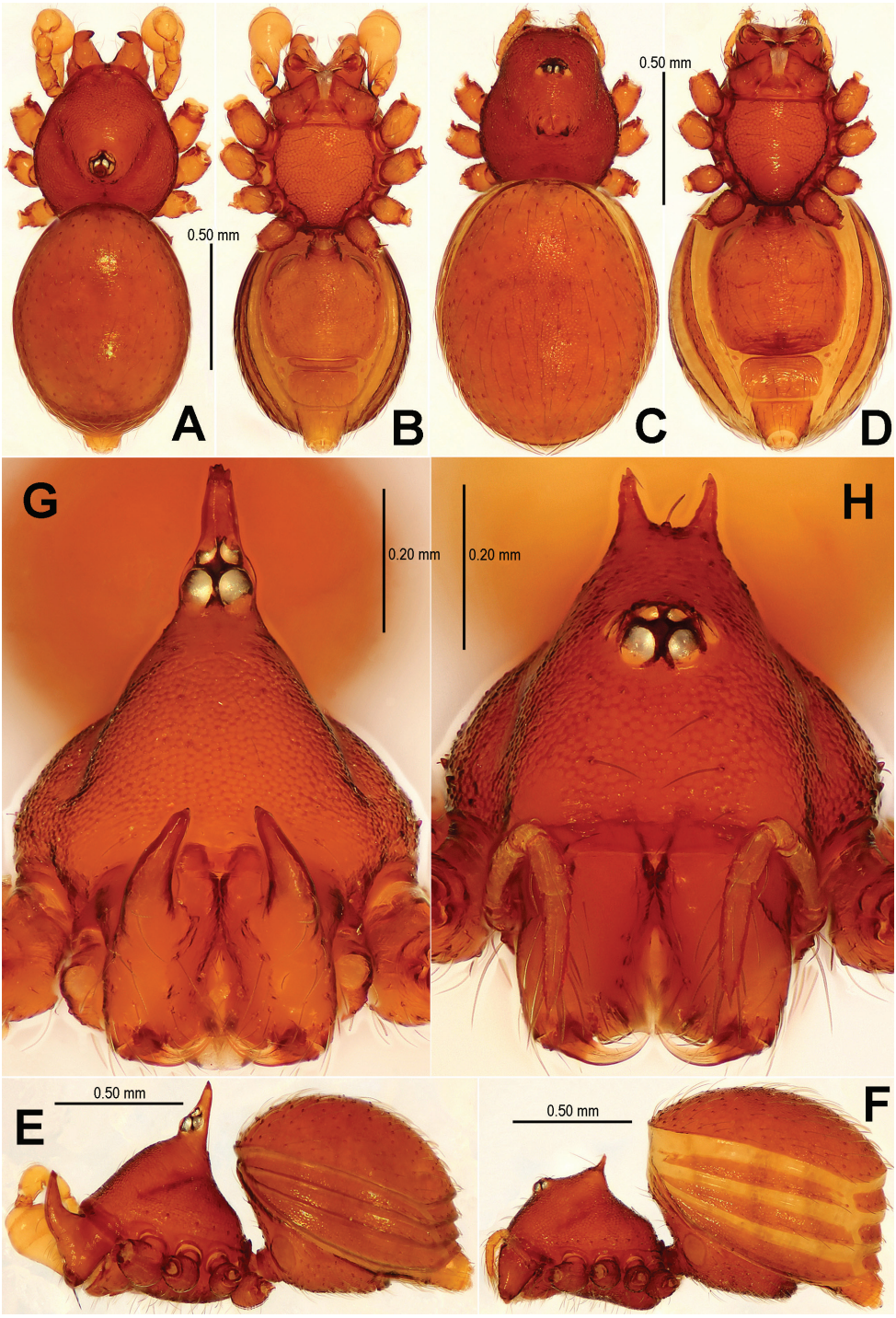

Figure 1. *Sinamma oxycera* gen. n. & sp. n., male holotype (**A–B, E, G**) and female paratype (**C–D, F, H**). **A–F** Habitus **G, H** Prosoma. **A, C** dorsal view **B, D** ventral view **E, F** lateral view **G, H** anterior view.

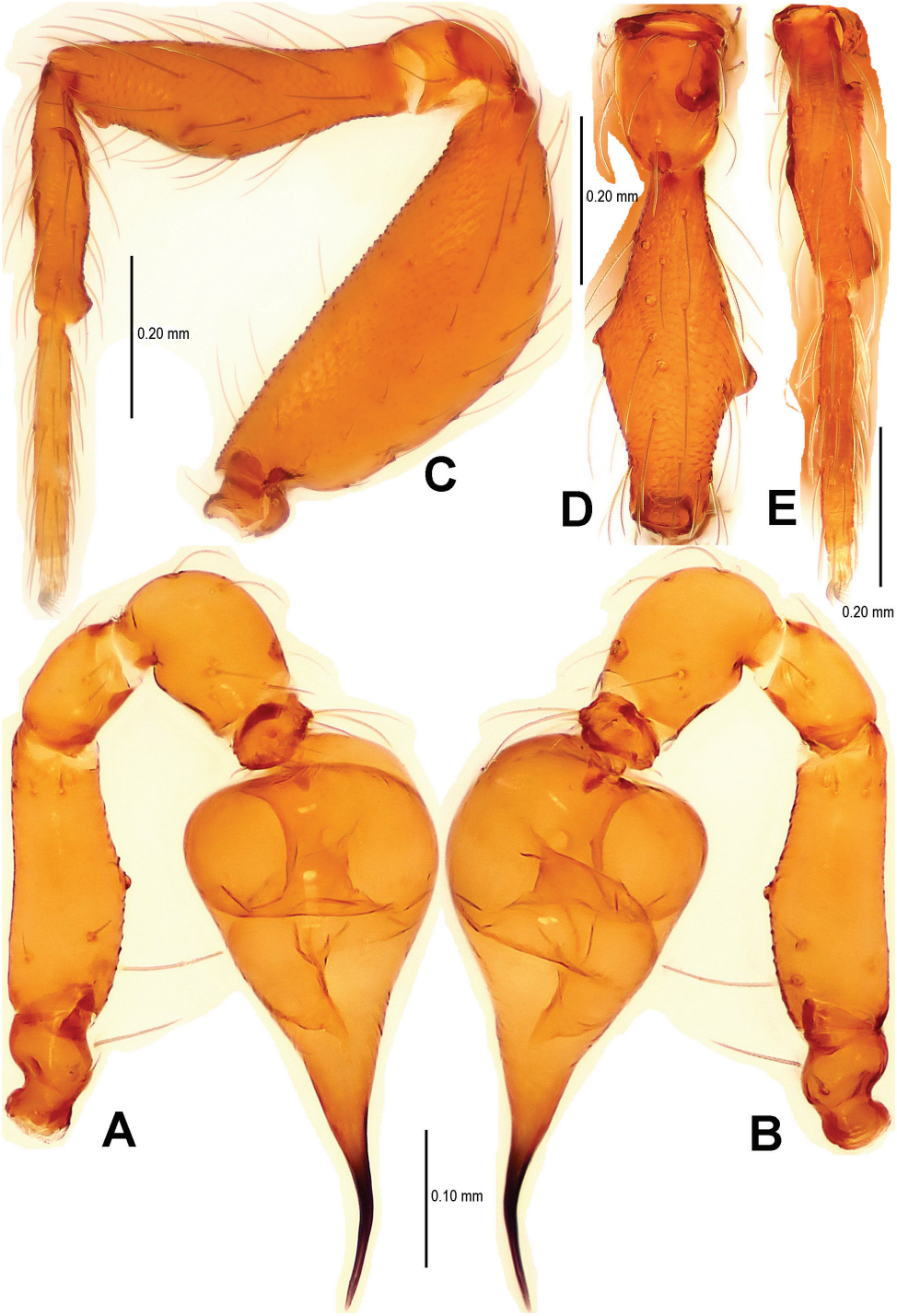

Figure 2. *Sinamma oxycera* gen. n. & sp. n., male holotype. **A, B** Left palp **C** Left leg I **D** Left tibia I **E** Left metatarsus I and tarsus I. **A** prolateral view **B, C** retrolateral view **D, E** anterior view.

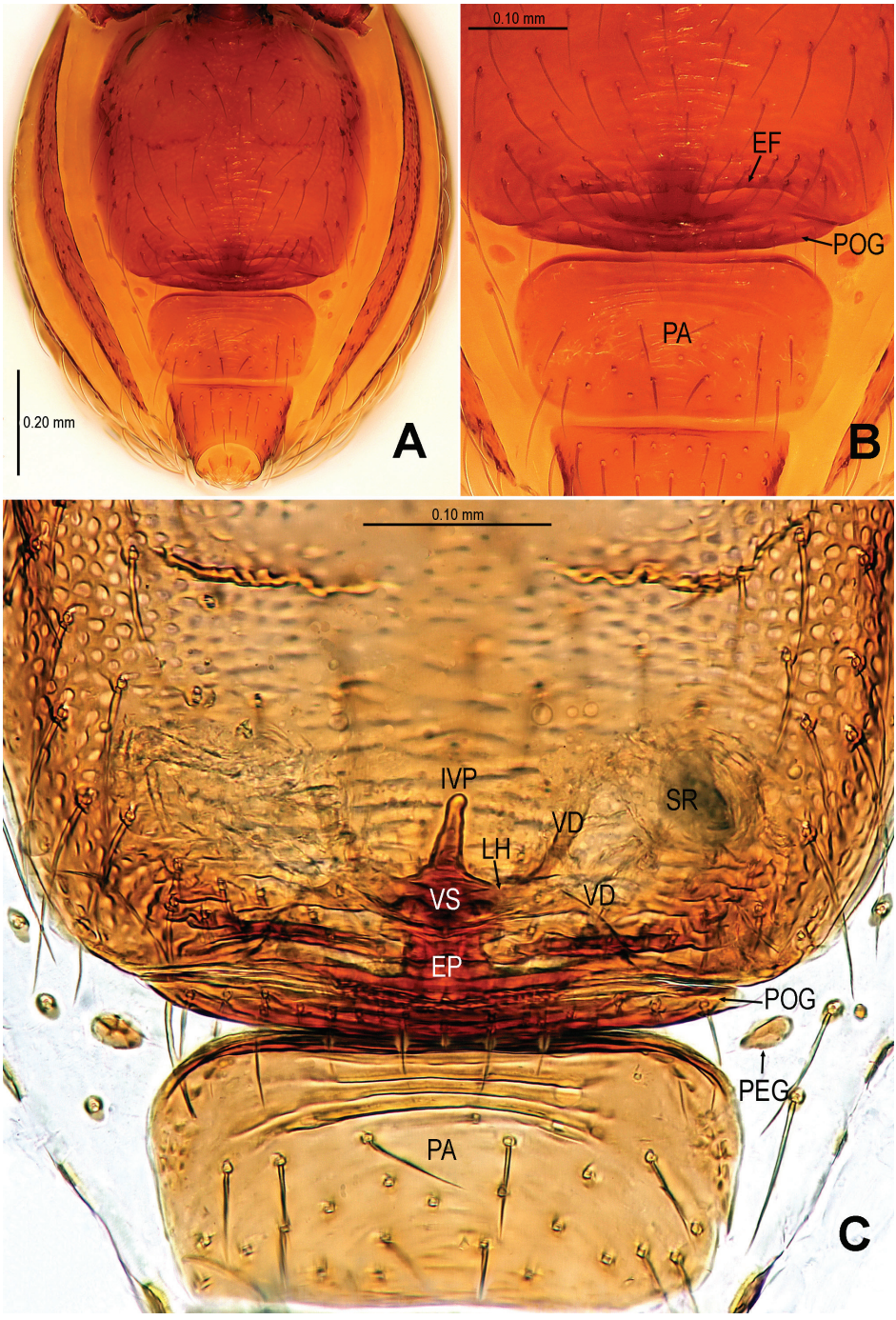

Figure 3. *Sinamma oxycera* gen. n. & sp. n., female paratype. **A** Opisthosoma **B** Genital area (untreated) **C** Cleared vulva (KOH-treated). **A, B** ventral view **C** dorsal view. Abbrs.: **EF** epigynal fold; **EP** epigynal pit; **IVP** inner vulval plate; **LH** lateral horn; **PA** preanal plate; **POG** postgenital plate; **SR** seminal receptaculum; **VD** vulval duct; **VS** vulval stem.

Abdomen (Figs 1C–D, F; 3A): lateral scutum I anteriorly extending beyond the anterior rim of operculum; preanal scutum anteriorly rugose, covered with sparse serrated setae.

Genitalia (Figs 3B–C; 19B): vulval stem transverse, sclerotized; lateral horns robust, supporting the base of vulval ducts; vulval duct narrow, weakly sclerotized; spermathecae translucent, rugose, membranous; vulval dorsal plate wide, fused to lateral horn and base of vulval ducts; inner vulval plate finger-shaped, sclerotized, basally wide; central process absent.

Distribution. Known only from the type locality (Fig. 22).

Singaporemma Shear, 1978

http://species-id.net/wiki/Singaporemma

Type species. *Singaporemma singularis* Shear, 1978 from Singapore.

Diagnosis. Distinguished from other tetrablemmids by the swollen palpal tibia, the egg-shaped bulb, the originating position of embolus in male, and by the oval epigynal pit, the central process larger than inner vulval plate in female.

Distribution. Four species are known from China, Singapore and Vietnam before the current study.

Singaporemma banxiaoensis sp. n.

http://zoobank.org/EB434E0D-E8B9-4D2D-879A-C8C39DB4AF70
http://species-id.net/wiki/Singaporemma_banxiaoensis
Figs 4–6, 16C–D, 20A, 22

Material. Holotype ♂, paratypes 5♂ and 10♀ (IZCAS), CHINA, Guangxi: Pingxiang City, Xiashi Town, Xinming Village, Banxiaotun, Banxiao Cave, 22°5.542'N, 106°52.148'E, elevation ca. 175 m, 26 July 2011, Xiaoxiao Wang leg.

Etymology. The specific name refers to the type locality; adjective.

Diagnosis. This new species is similar to *S. halongense* Lehtinen, 1981 (see Lehtinen 1981: 31, figs. 43, 49, 54, 58, 62), but can be distinguished by the white vestigial eyespots in both sexes (Figs 4A, C, G–H), the shape of bulb and psembolus in the male (Figs 5A–D, 16C–D), the inverted triangular inner vulval plate and the wide, robust central process (Figs 6C, 20A), and the presence of a rhombic vulval dorsal plate (Figs 6C, 20A) in the female.

Description. Male (holotype). Coloration: body brownish-yellow; legs yellowish-orange.

Measurements: total length 1.02; carapace 0.45 long, 0.36 wide, 0.21 high; abdomen 0.64 long, 0.55 wide, 0.55 high; clypeus 0.16 high; sternum 0.28 long, 0.29 wide. Length of legs: I 1.15 (0.38, 0.12, 0.27, 0.20, 0.20); II 1.13 (0.36, 0.11, 0.27, 0.20, 0.20); III 1.04 (0.30, 0.11, 0.23, 0.20, 0.20); IV 1.35 (0.43, 0.12, 0.34, 0.23, 0.23).

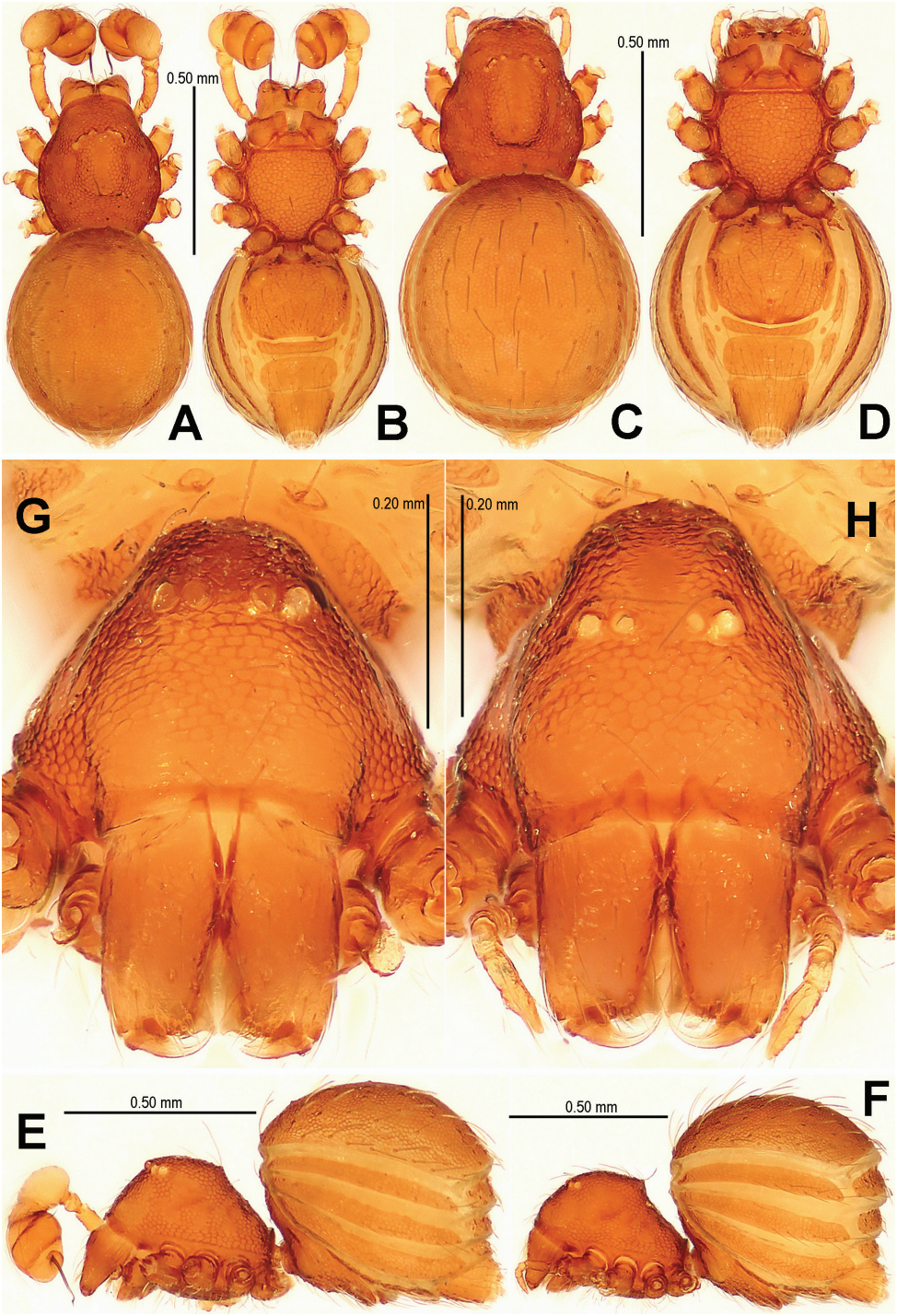

Figure 4. *Singaporemma banxiaoensis* sp. n., male holotype (**A–B, E, G**) and female paratype (**C–D, F, H**). **A–F** Habitus **G, H** Prosoma. **A, C** dorsal view **B, D** ventral view **E, F** lateral view **G, H** anterior view.

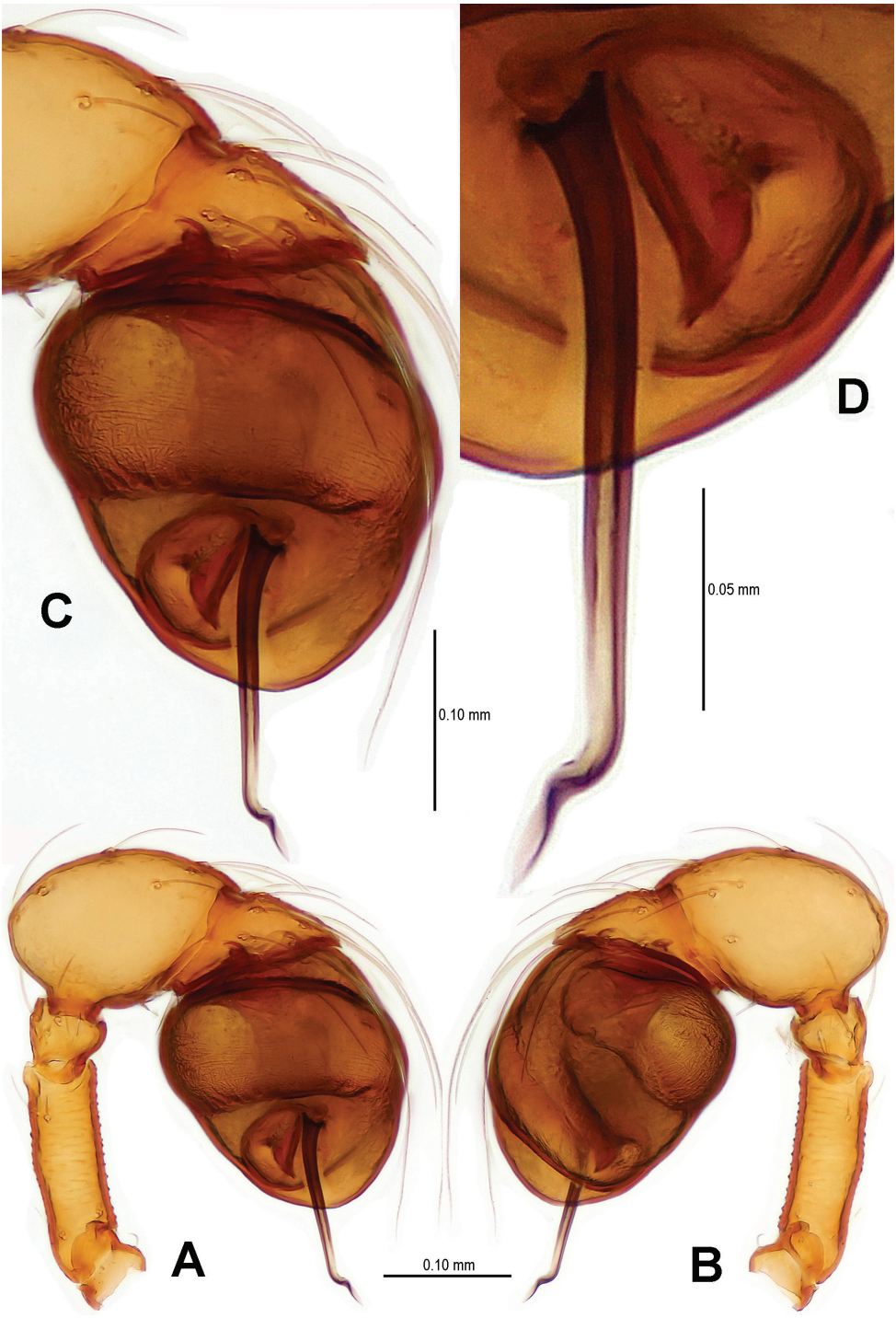

Figure 5. *Singaporemma banxiaoensis* sp. n., male holotype. **A, B** Left palp **C** Bulb **D** Embolus. **A, C–D** prolateral view **B** retrolateral view.

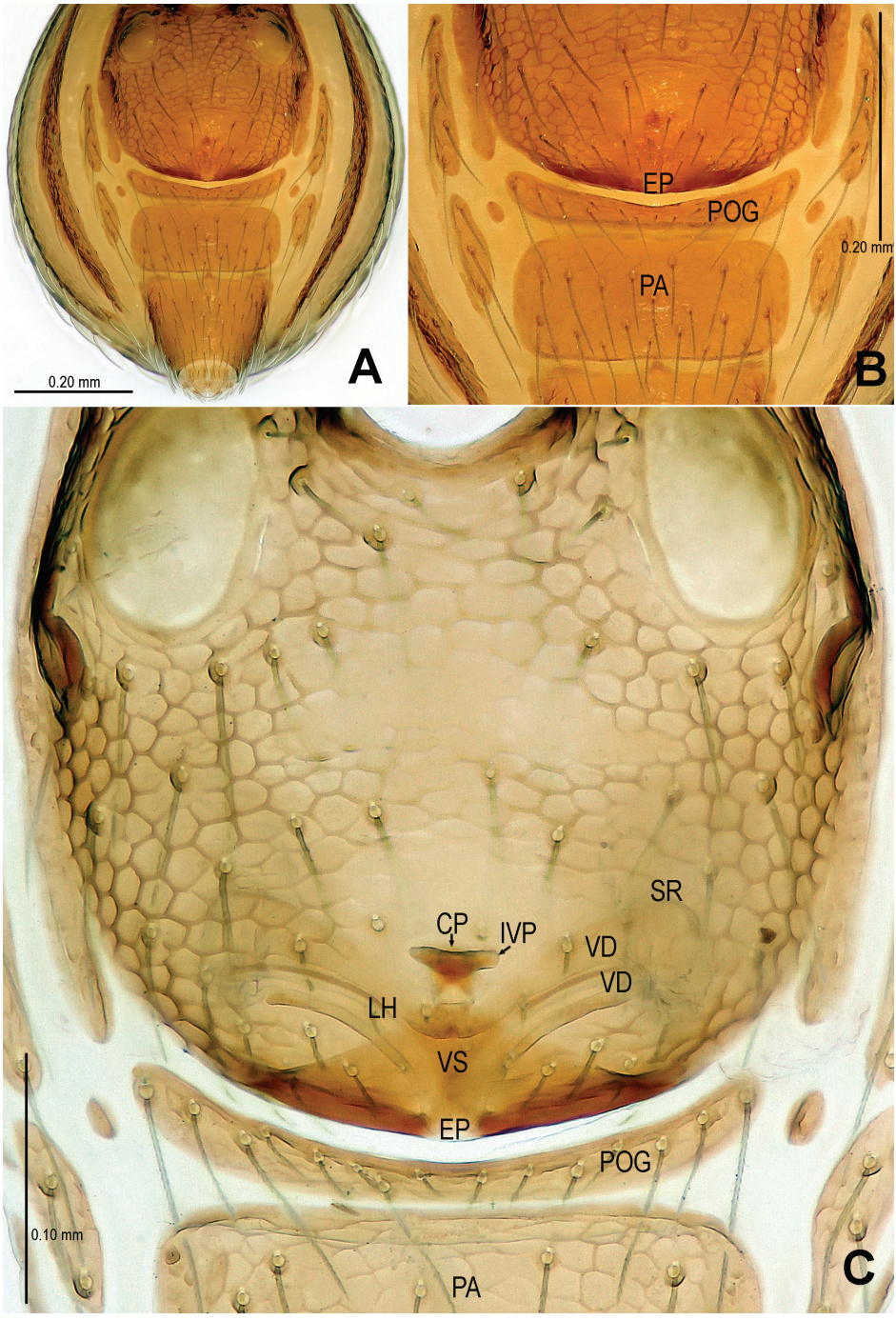

Figure 6. *Singaporemma banxiaoensis* sp. n., female paratype. **A** Opisthosoma **B** Genital area (untreated) **C** Cleared vulva (KOH-treated). **A, B** ventral view **C** dorsal view. Abbrs.: **CP** central process; **EP** epigynal pit; **IVP** inner vulval plate; **LH** lateral horn; **PA** preanal plate; **POG** postgenital plate; **SR** seminal receptaculum; **VD** vulval duct; **VS** vulval stem.

Carapace (Figs 4A–B, G, E) reticulated, margin rugose; eyes white, vestigial; clypeus sloping forward, marginally rounded; cephalic part flat; sternum finely reticulated, margin rugose, with sparse setae. Legs: cuticle striated; all tibiae with 2 trichobothria, and one on metatarsi I–IV.

Abdomen (Figs 4A–B, E): dorsal scutum oval, finely reticulated, covered with sparse setae; ventral scutum reticulated; perigenital scutum present; postepigastral scutum slightly recurved; preanal scutum rectangular.

Palp (Figs 5A–D; 16C, D): femoral cuticle sculptured and granulated, approximate 3 times patella in length; tibia strongly swollen, approx. 2.2 times femur in width (Figs 5A, B); cymbium posteriorly narrow and anteriorly wide (Fig. 5C); bulb egg-shaped, its surface with irregular lines (Fig. 5C); embolus long, proximally straight but distally flexible (Figs 16C–D).

Female (paratype). Coloration: same as in male.

Measurements: total length 1.11; carapace 0.47 long, 0.38 wide, 0.22 high; abdomen 0.68 long, 0.64 wide, 0.64 high; clypeus 0.14 high; sternum 0.29 long, 0.29 wide. Lengths of legs: I 1.22 (0.41, 0.13, 0.28, 0.20, 0.21); II 1.15 (0.38, 0.12, 0.27, 0.20, 0.20); III 1.06 (0.32, 0.12, 0.24, 0.20, 0.19); IV 1.41 (0.45, 0.13, 0.36, 0.25, 0.23).

Carapace (Figs 4C–D, H and F) as in male; clypeus slightly lower than in male. Legs: the chaetotaxy as in male.

Abdomen (Figs 4C–D, F; 6A): dorsal scutum reticulated, covered with sparse setae; lateral scutum I anteriorly short, not extending beyond posterior rim of operculum; operculum centrally smooth, laterally rugose; postepigastral scutum slightly curved, medially narrow and laterally wide; perigenital plates long; preanal scutum reticulated, rectangular, with sparse serrated setae.

Genitalia (Figs 6C; 20A): epigynal pit distinct, oval; vulval posterior margin strongly sclerotized (Fig. 6B); vulval dorsal plate rhombic, fused to vulval posterior margin (Figs 6C; 20A); vulval stem triangular (Fig. 20A); vulval ducts proximally narrow, distally wide; lateral horns and vulval ducts forming a "V"-shape; spermathecae rugose, translucent, and membranous; inner vulval plate inverted triangle, distinctly sclerotized, wider than central process (Figs 6C; 20A); central process contracted basally, medially wide (Fig. 20A).

Variation. Total length from 1.00 to 1.07 in males (n = 6) and from 1.05 to 1.14 in females (n = 10).

Distribution. Known only from the type locality (Fig. 22).

***Singaporemma wulongensis* sp. n.**

http://zoobank.org/6FF00E16-1A9B-4497-BFC7-317B0B9A403A
http://species-id.net/wiki/Singaporemma_wulongensis
Figs 7–9, 17, 20B, 22

Material. Holotype ♂, paratypes 8♂ and 20♀ (SCUM), CHINA, Chongqing: Wulong County, Tudi Town, Tiansheng Village, Xiaodong Cave, 29°31.853'N, 107°50.817'E, elevation ca. 1050 m, 17 October 2010, Liang Dou and Yucheng Lin leg.

Etymology. The specific name refers to the type locality; adjective.

Diagnosis. This new species is similar to *S. bifurcata* Lin & Li, 2010 (see Lin & Li 2010: figs 29–37) but the male can be distinguished by the base of embolus (Figs 8A–C, 17 A–C), the flexible embolic end (Figs 8D–F, 17 D–E), and the long oval palpal bulb (Figs 8A–B, 17A–B). The females are distinguished by the "Ω"-shaped inner vulval plate, and the long central process (Figs 9C, 20B).

Description. Male (holotype). Coloration: body reddish-brown; legs yellowish-brown.

Measurements: total length 1.21; carapace 0.53 long, 0.45 wide, 0.44 high; abdomen 0.79 long, 0.60 wide, 0.54 high; clypeus 0.18 high; sternum 0.32 long, 0.32 wide. Length of legs: I 1.29 (0.41, 0.13, 0.30, 0.21, 0.23); II 1.16 (0.36, 0.13, 0.27, 0.20, 0.21); III 1.07 (0.32, 0.13, 0.23, 0.19, 0.21); IV 1.43 (0.43, 0.13, 0.36, 0.25, 0.26).

Carapace (Figs 7A–B, G and E) finely reticulated, margin rugose; eyes with black base; cephalic part flat, covered with long setae; clypeus sharply sloping anteriorly; sternum reticulated, marginally rugose. Legs: cuticle striated; all tibiae with 3 trichobothria and all metatarsi with a trichobothrium.

Abdomen (Figs 7A–B, E): dorsal scutum long, oval, covered with long setae, margin reticulated, center granulated; ventral scutum reticulated, margin rugose; lateral scutum I short, perigenital plate broad.

Palp (Figs 8A–F; 17A–E): femoral cuticle granular, striated, approx. 3 times as long as patella; patella proximally narrow, distally wide; tibia short, swollen, 1.6 times as wide as femur; bulb egg-shaped, surface smooth; embolus long, strongly sclerotized, starting from subproximal-ventral 1/3 position of bulbous surface, and curved downwards (Figs 8C; 17A, C); embolic tip flexuous, forked (Figs 8D–F; 17D–E).

Female (paratype). Coloration: body slightly lighter than in male; legs yellowish-brown.

Measurement: total length 1.23; carapace 0.54 long, 0.43 wide, 0.41 high; abdomen 0.80 long, 0.63 wide, 0.59 high; clypeus 0.17 high; sternum 0.30 long, 0.31 wide. Length of legs: I 1.21 (0.38, 0.13, 0.29, 0.20, 0.21); II 1.11 (0.34, 0.13, 0.25, 0.18, 0.21); III 1.03 (0.30, 0.12, 0.22, 0.18, 0.21); IV 1.38 (0.42, 0.13, 0.34, 0.23, 0.25).

Carapace (Figs 7C–D, F and H) as in male, except for clypeal area smooth. Legs: chaetotaxy as in male.

Abdomen (Figs 7C–D, F; 9A): as in male, except for lighter coloration; lateral scutum I anteriorly short, not extending beyond the posterior rim of operculum; perigenital plate broad; postepigastral scutum curved, its posterior margin overlapped with anterior margin of preanal scutum; preanal scutum regularly rectangular, width equal to 2 times length, surface covered with serrated setae.

Genitalia (Figs 9B–C; 20B): genital area smooth; epigynal folds absent, genital basal margin sclerotized (Fig. 9B); epigynal pit small, oval, transverse; vulval stem absent; lateral horns contracted, supporting the base of vulval ducts; inner vulval plate "Ω"-shaped, slightly sclerotized, shorter and narrower than central process (Figs 9C; 20B); central process large, translucent; vulval duct wide; spermathecae large, rugose. (Fig 20B).

Variation. Total length from 1.14 to 1.25 in males (n = 9) and from 1.16 to 1.30 in females (n = 20).

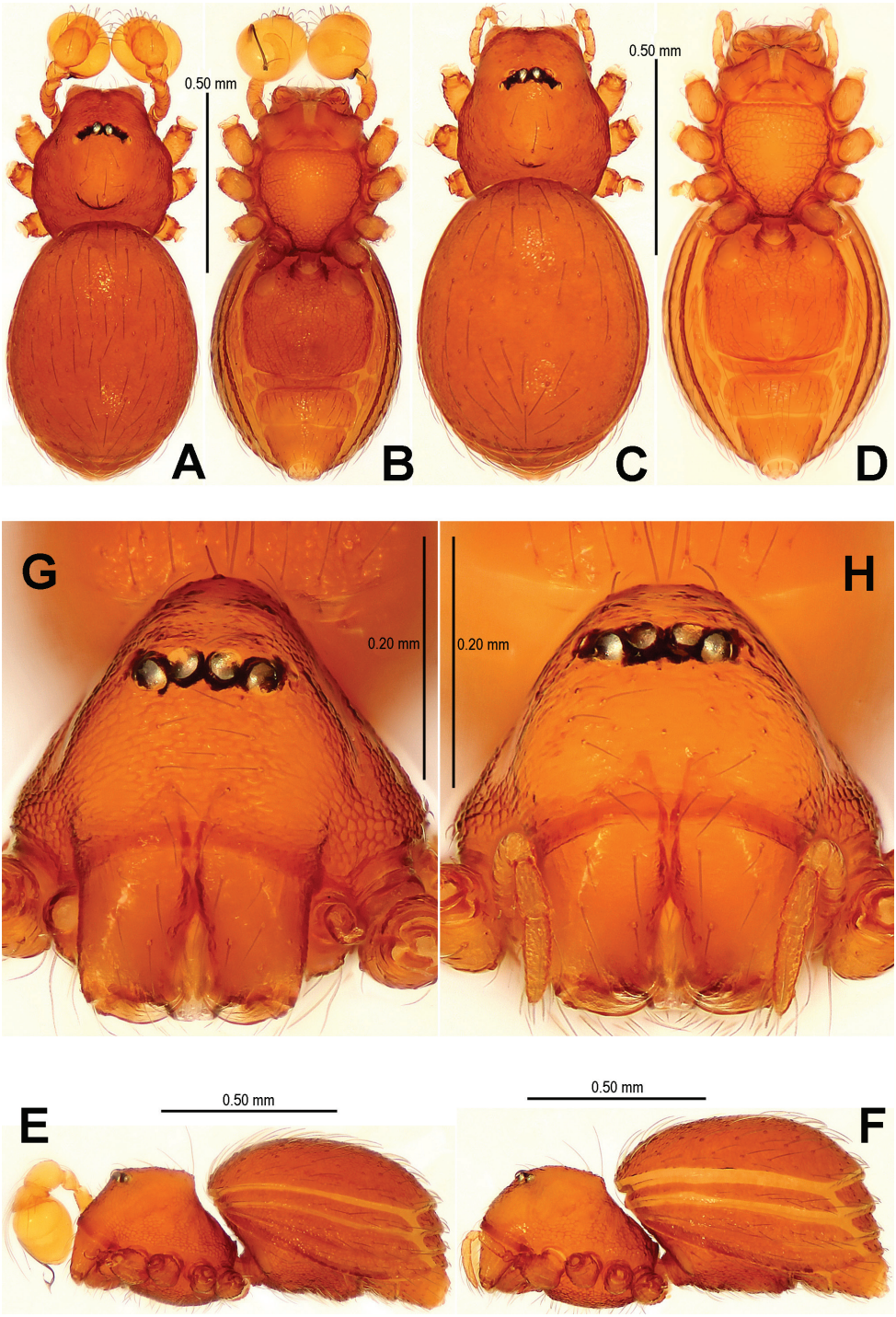

Figure 7. *Singaporemma wulongensis* sp. n., male holotype (**A–B, E, G**) and female paratype (**C–D, F, H**). **A–F** Habitus **G–H** Prosoma. **A, C** dorsal view **B, D** ventral view **E, F** lateral view **G, H** anterior view.

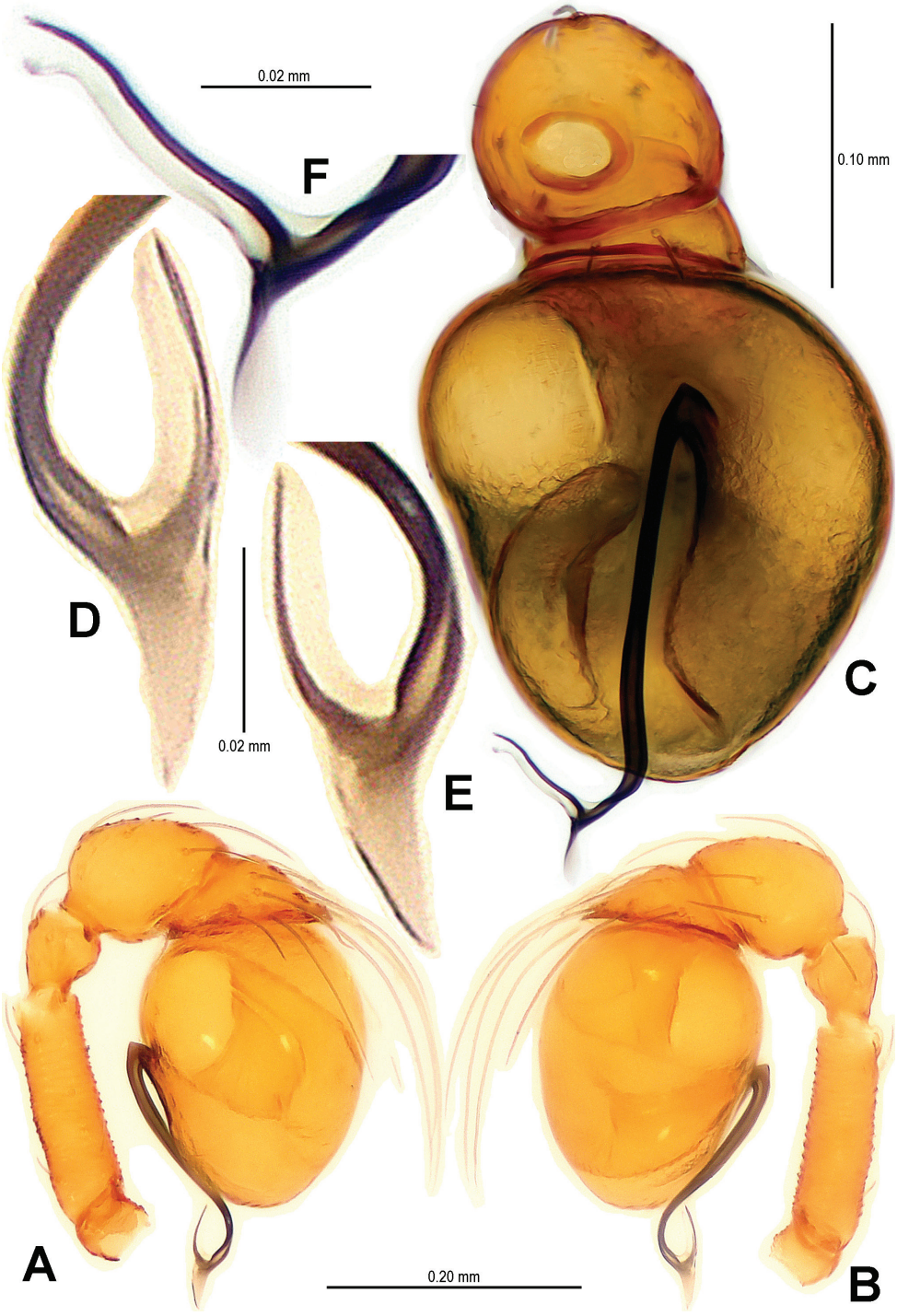

Figure 8. *Singaporemma wulongensis* sp. n., male holotype. **A, B** Left palp **C** Bulb (KOH-treated) **D–F** Distal embolus. **A, E** prolateral view **B, D** retrolateral view **C, F** posterior view.

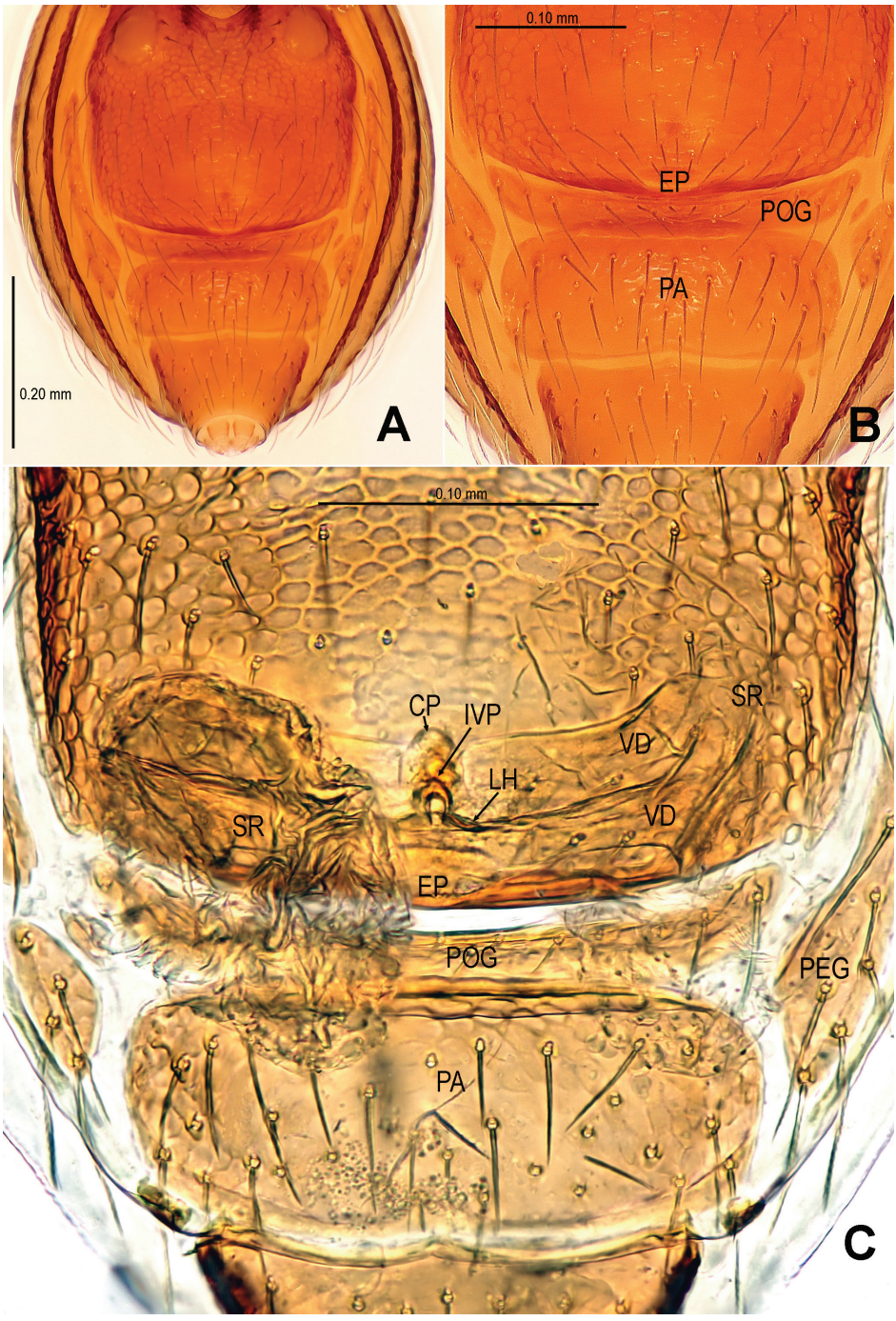

Figure 9. *Singaporemma wulongensis* sp. n., female paratype. **A** Opisthosoma **B** Genital area (untreated) **C** Cleared vulva (KOH-treated). **A, B** ventral view **C** dorsal view. Abbrs.: **CP** central process; **EP** epigynal pit; **IVP** inner vulval plate; **LH** lateral horn; **PA** preanal plate; **POG** postgenital plate; **SR** seminal receptaculum; **VD** vulval duct; **VS** vulval stem.

Distribution. Known only from the type locality (Fig. 22).

Tetrablemma O. P.-Cambridge, 1873

http://species-id.net/wiki/Tetrablemma

Type species. *Tetrablemma medioculatum* O. P.-Cambridge, 1873 from Sri Lanka.

Diagnosis. *Tetrablemma* is close to *Singalangia* Lehtinen, 1981 and *Rhinoblemma* Lehtinen, 1981. *Tetrablemma* is separated from *Singalangia* by largely different pattern of abdominal plates, by well developed lateral horns of vulva, and by lack of apomorphic modifications of the same type in sternum and epigynal area. It is separated from *Rhinoblemma* by different types of sexual dimorphism in the male clypeal area and chelicerae.

Distribution. Twenty five species have previously been described from Angola, Western Australia, China, Flores, India, Laos, Micronesia, Myanmar, Nepal, St. Helena, Queensland, Samoa, Seychelles, Sri Lanka, Sulawesi, Sumatra, Trinidad, Victoria, and Vietnam, collected mainly from forest litter or caves.

Tetrablemma menglaensis sp. n.

http://zoobank.org/22DDEE3E-1084-45A3-9BE2-484281B16A9B
http://species-id.net/wiki/Tetrablemma_menglaensis
Figs 10–12, 18C–D, 21, 22

Material. Holotype ♂, paratypes 2♀ (SCUM), CHINA, Yunnan: Mengla County, Mengyuan Town, Chengzi Village, Yeniudong Scenery Spot, Yeniu 2# Cave, 21°43.208'N, 101°23.294'E, elevation ca. 760 m, 16 August 2011, Yucheng Lin and Guo Zheng leg.

Etymology. The specific name refers to the type locality; adjective.

Diagnosis. The male of this new species is similar to *T. namkhan* Lin & Li, 2012 (see Lin and Li 2012: figs 4A–G, 5A–C, 6A–C), *T. loebli* Bourne, 1980 (see Lehtinen 1981: figs 219, 221, 223–224), *T. marawula* Lehtinen, 1981 (see Lehtinen 1981: figs 255, 266), and *T. brevidens* Tong & Li, 2008 (see Tong and Li 2008: figs 5A, C, F–I), but can be distinguished by a forked cephalic tubercle (Figs 10E, G), a crooked cheliceral horn (Figs 10A–B, E), the swollen palpal tibia (Figs 11B–C, 18C), the course of sperm duct, and the long-tongue shaped embolus (Figs 11A, 18D). The female is similar to *T. nandan* Lin & Li, 2010 (see Lin and Li 2010: figs 46–49) and *T. marawula* Lehtinen, 1981 (see Lehtinen 1981: figs 256, 283), but can be recognized by the narrow postepigastral scutum (Figs 12A–B), the long S-shaped inner vulval plate (Figs 12C–D, 21A–B), the absence of vulval dorsal plate, and the wide central process (Figs 12D, 21B).

Description. Male (holotype). Coloration: body reddish-brown; legs yellowish-brown.

Measurements: total length 1.18; carapace 0.54 long, 0.46 wide, 0.36 high; abdomen 0.98 long, 0.63 wide, 0.52 high; clypeus 0.27 high. Sternum 0.31 long, 0.34 wide.

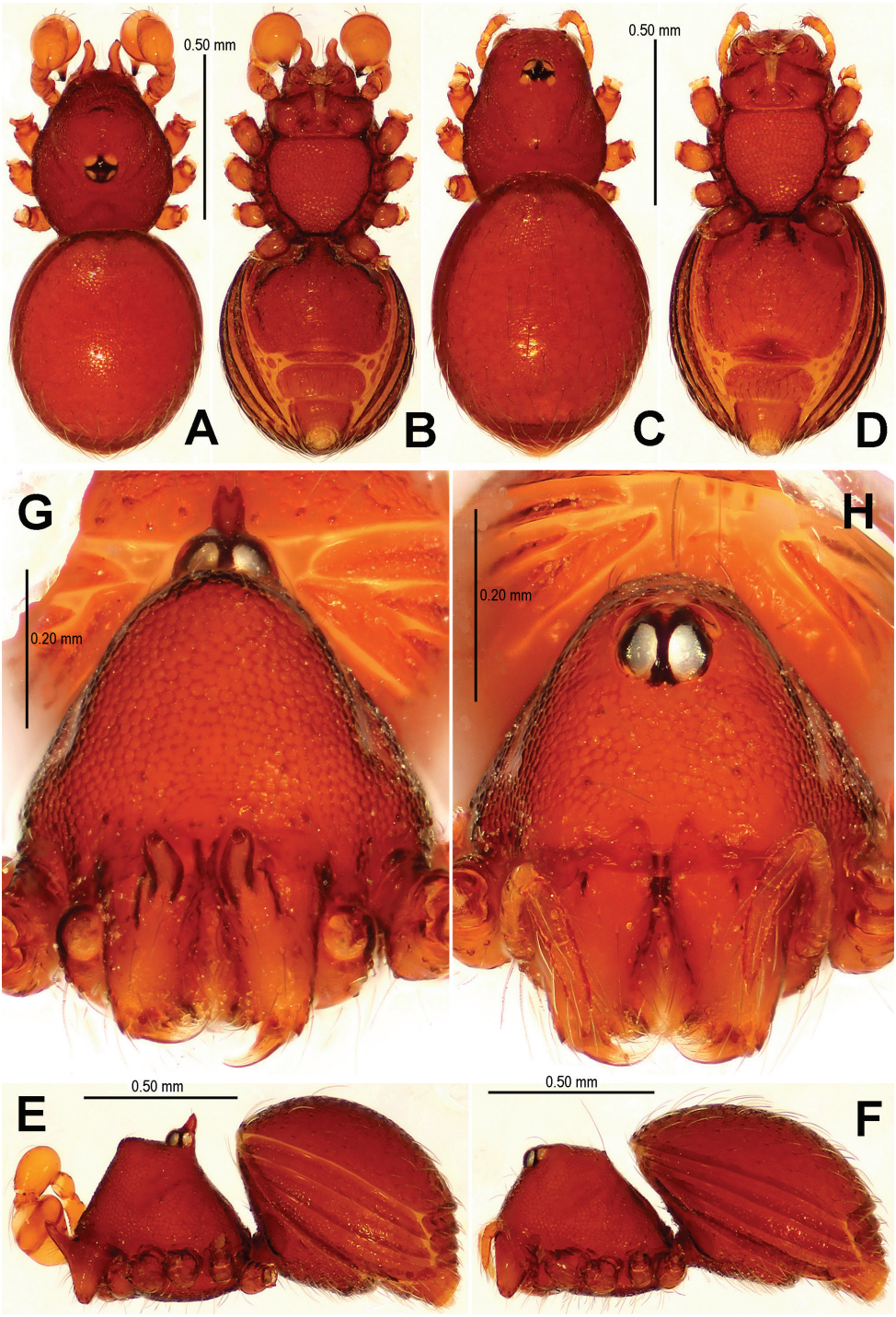

Figure 10. *Tetrablemma menglaensis* sp. n., male holotype (**A–B, E, G**) and female paratype (**C–D, F, H**). **A–F** Habitus **G, H** Prosoma. **A, C** dorsal view **B, D** ventral view **E, F** lateral view **G, H** anterior view.

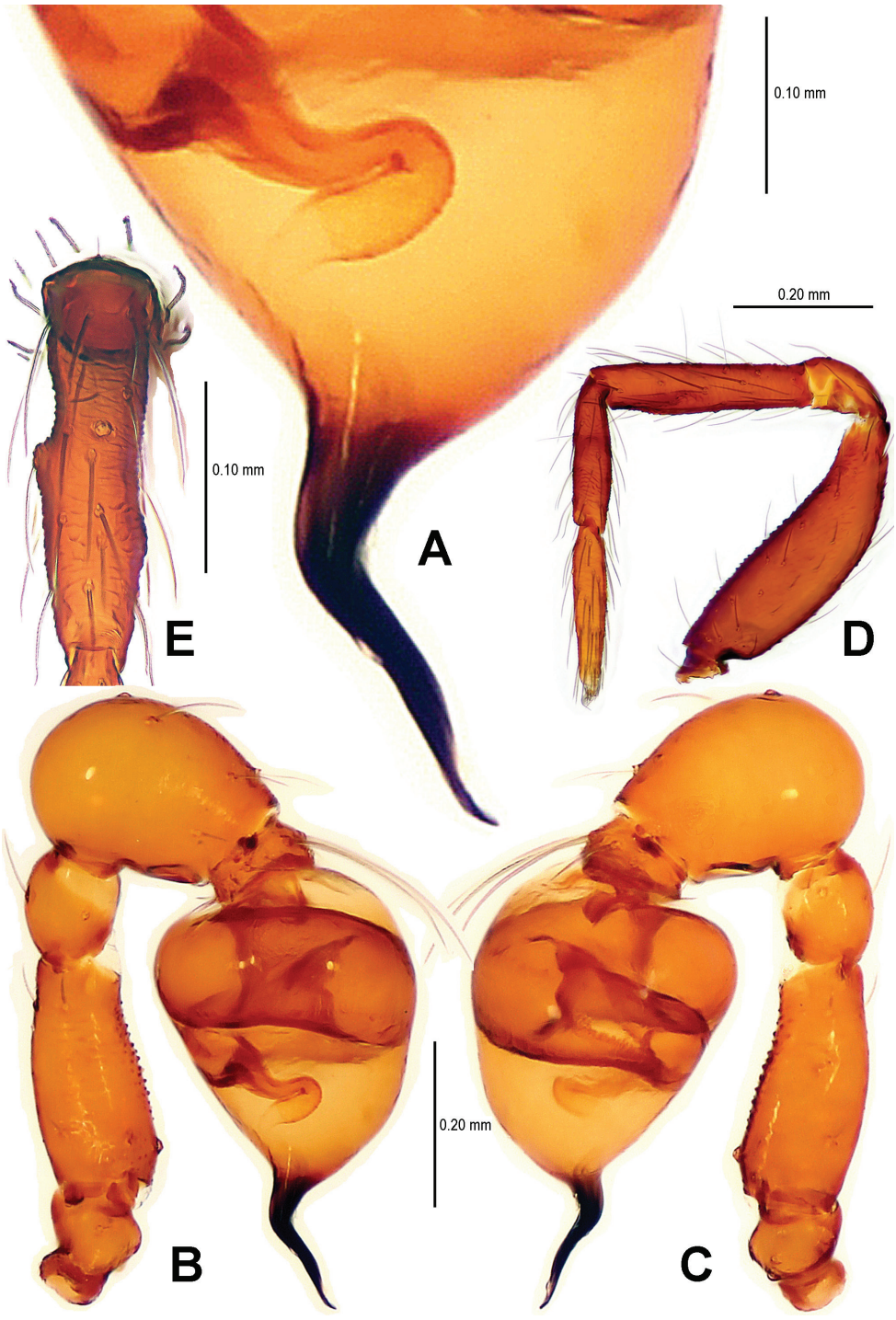

Figure 11. *Tetrablemma menglaensis* sp. n., male holotype. **A** Embolus and sperm duct **B, C** Left palp **D** Left leg I **E** Left metatarsus I. **A, B** prolateral view **C, D** retrolateral view **E** anterior view.

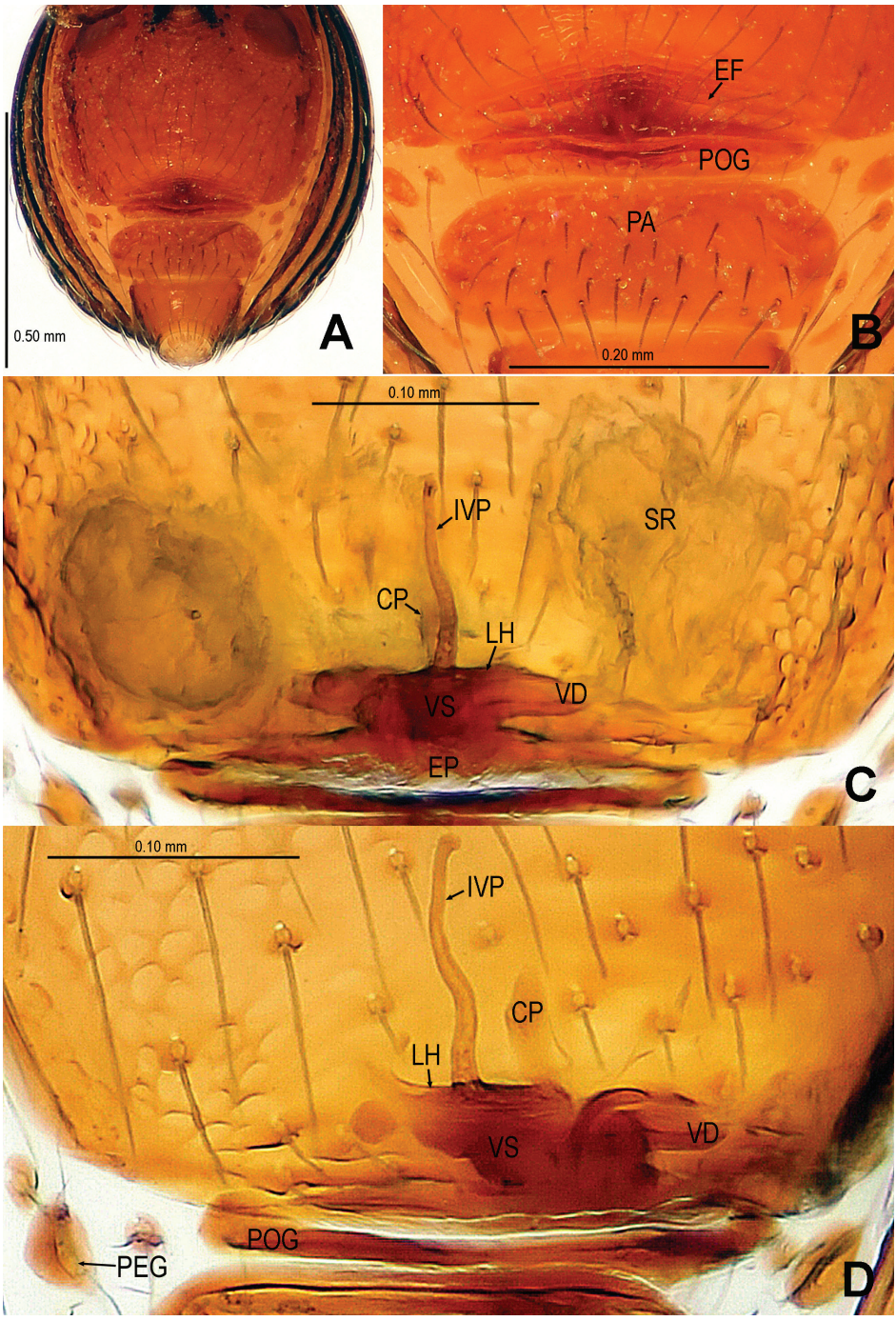

Figure 12. *Tetrablemma menglaensis* sp. n., female paratype. **A** Opisthosoma **B** Genital area (untreated) **C, D** Cleared vulva (KOH-treated). **A, B** ventral view **C** dorsal view **D** dorsal-lateral view. Abbrs.: **CP** central process; **EF** epigynal fold; **EP** epigynal pit; **IVP** inner vulval plate; **LH** lateral horn; **PA** preanal plate; **POG** postgenital plate; **SR** seminal receptaculum; **VD** vulval duct; **VS** vulval stem.

Length of legs: I 1.27 (0.38, 0.14, 0.30, 0.21, 0.23); II 1.21 (0.38, 0.13, 0.29, 0.21, 0.22); III 1.11 (0.34, 0.11, 0.25, 0.20, 0.21); IV 1.40 (0.43, 0.11, 0.36, 0.26, 0.25).

Carapace (Figs 10A, E and G) completely reticulate, margin rugose; ocular area with a short, bifurcate tubercle; clypeal area distinctly convex, margin rounded; cheliceral horn narrow, medially curved in dorsal view; sternum centrally reticulated, marginally sclerotized and rugose. Legs: cuticle sculptured; femur I slightly swollen; all tibiae with 2 trichobothria, and one on metatarsi I–IV; metatarsus I with two small lateral tubercles (Figs 11D, E).

Abdomen (Figs 10A–B, E): dorsal scutum short, oval, finely granulated; ventral scuta reticulated and striated; lateral scutum I short; postepigastral scutum short, narrower than preanal scutum (Fig. 10B).

Palp (Figs 11A–C; 18C–D): femur slightly swollen, ventrally granulated; patella short, approx. as 1/2 long as femur; tibia smooth, swollen, approx. 2 times as wide as patella; bulb pyriform, smooth; embolus long, bent, strongly sclerotized; sperm duct visible through bulbal integument (Figs 11A–C; 18C, D).

Female (paratype). Coloration and modifications as in male, but cephalic tubercle and cheliceral horn absent.

Measurements: total length 1.27; carapace 0.58 long, 0.45 wide, 0.28 high; clypeus 0.19 high; sternum 0.32 long, 0.34 wide; abdomen 0.91 long, 0.68 wide, 0.50 high. Length of legs: I 1.29 (0.41, 0.13, 0.31, 0.21, 0.23); II 1.21 (0.38, 0.13, 0.29, 0.21, 0.22); III 1.12 (0.34, 0.12, 0.25, 0.20, 0.21); IV 1.47 (0.46, 0.13, 0.38, 0.27, 0.24).

Carapace (Figs 10C, F and H): reticulation as in male; clypeal area nearly vertical anteriorly; cephalic part flat; cheliceral frontal surface with a small basal tubercle; sternum as in male. Legs: chaetotaxy and number of trichobothria as in male.

Abdomen (Figs 10C–D, F; 12A): lateral scutum I anteriorly short, not extending beyond the posterior rim of operculum; postgenital plate straight, narrower than preanal scutum; preanal scutum wider than long, anterior margin rugose (Fig. 21A), covered with serrated setae (Fig. 12B).

Genitalia (Figs 12B–D; 21A–B): epigynal folds laterally narrow, medially wide (Fig.12B); epigynal pit narrow, indistinct, separated with vulval stem and lateral horns; vulval stem strongly sclerotized (Figs 12C; 21A); spermathecae rugose, membranous; lateral horns wide, strong, supporting the base of vulval ducts of seminal receptacle; inner vulval plate S-shaped, very long, at least 2 times longer than central process (Figs 12D; 21B); central process wide, basally contracted (Fig 21B); vulval duct narrow, connected with lateral horn and spermathecae.

Distribution. Known only from the type locality (Fig. 22).

***Tetrablemma ziyaoensis* sp. n.**

http://zoobank.org/38AA57F1-7F11-46A4-A2AA-6A259B2A52D5
http://species-id.net/wiki/Tetrablemma_ziyaoensis
Figs 13–15, 18A–B, 19A, 22

Material. Holotype ♂, paratypes 1♂ and 1♀ (IZCAS), CHINA, Guangxi: Chongzuo City, Fusui County, Dongmen Town, Ziyao Village, Yinhe Cave, 22°19.763'N, 107°47.526'E, elevation ca. 154 m, 13 July 2011, Xiaoxiao Wang leg.

Etymology. The specific name refers to the type locality; adjective.

Diagnosis. This new species is similar to *T. thamin* Labarque & Grismado, 2009 (see Labarque and Grismado 2009: figs 1–4, 6–11), *T. marawula* (see Lehtinen 1981: 61, figs 255–256, 266, 281), and *T. manggarai* Lehtinen, 1981 (see Lehtinen 1981: 61, figs 259–260, 269, 282a, 287), but males can be distinguished by the tapering cheliceral horn, the no swollen palpal tibia, the deflective pear-shape bulb (Figs 14C, D) and the medial forked embolus (Figs 14A, B, 18B vs. Labarque and Grismado 2009: figs 1, 3, but not forked in *T. marawula* and *T. manggarai*). Females differ by the presence of a triangular dorsal plate (absent in *T. thamin*, *T. marawula* and *T. manggarai*), the basally contracted central plate, the wide postepigastral scutum, and the presence of a long and distinct anterior fold on the preanal scutum (Figs 15B, C; 19A) (absent in *T. thamin* and *T. marawula*, unknown in *T. manggarai*).

Description. Male (holotype). Coloration: body yellowish-brown; legs light brown.

Measurements: total length 1.13; carapace 0.50 long, 0.39 wide, 0.26 high; abdomen 0.75 long, 0.55 wide, 0.44 high; clypeus 0.26 high; sternum 0.29 long, 0.30 wide. Length of legs: I 1.29 (0.41, 0.13, 0.30, 0.22, 0.23); II 1.18 (0.38, 0.11, 0.27, 0.21, 0.22); III 1.07 (0.32, 0.11, 0.23, 0.20, 0.21); IV 1.47 (0.45, 0.13, 0.38, 0.29, 0.24).

Carapace (Figs 13A–B, E and G): most of surface reticulated, marginally rugose; ocular area slightly raised, located at center, with two long setae between PLEs; clypeal area distinctly convex, margin rounded; cheliceral horn thin, sharp, distally curved; sternum finely reticulated, strongly sclerotized at margin. Legs: cuticle striated and granular; femur I not swollen; tibiae I–III with 3 trichobothria, 4 on tibia IV, and metatarsi I–IV with one trichobothrium.

Abdomen (Figs 13A–B, E): dorsal scutum short, oval, slightly granulated; ventral episgastric scutum centrally reticulated, laterally striated; lateral scutum I short; postepigastral scutum narrow, approximate 1/2 times width of preanal scutum, but same length.

Palp (Figs 14A–D; 18A–B): femur ventrally granulated (Figs 14C, D); tibia smooth, not swollen, approx. as long as 2/3 times femur; bulb deflective pear-shape, smooth; embolus long, needle-like, slightly sclerotized, medially forked (Figs 14A, B; 18B).

Female (paratype). Coloration as in male.

Measurements: total length 1.21; carapace 0.54 long, 0.41 wide, 0.23 high; abdomen 0.84 long, 0.63 wide, 0.45 high; clypeus 0.16 high; sternum 0.30 long, 0.31 wide. Length of legs: I 1.36 (0.45, 0.13, 0.30, 0.23, 0.25); II 1.25 (0.39, 0.12, 0.28, 0.22, 0.24); III 1.13 (0.32, 0.13, 0.23, 0.21, 0.23); IV 1.54 (0.47, 0.13, 0.39, 0.29, 0.25).

Carapace (Figs 13C–D, F and H): cephalic part flat, smooth; ocular area anterior; clypeal area smooth, anteriorly upright; thoracic area reticulated; chelicerae with a small baso-lateral tubercle; sternum reticulated, covered with sparse setae. Legs: chaetotaxy and number of trichobothria as in male.

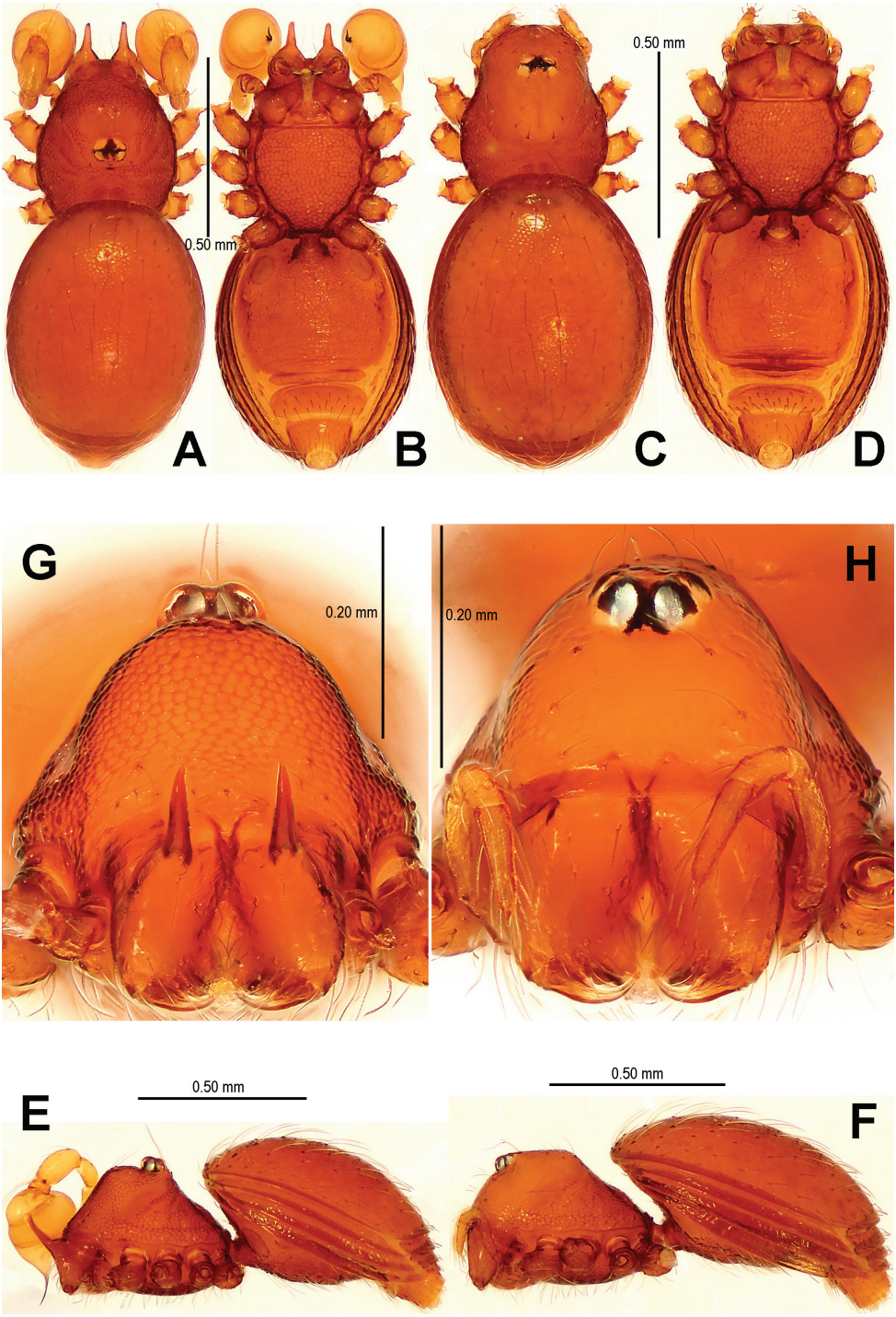

Figure 13. *Tetrablemma ziyaoensis* sp. n., male holotype (**A–B, E, G**) and female paratype (**C–D, F, H**). **A–F** Habitus **G, H** Prosoma. **A, C** dorsal view **B, D** ventral view **E, F** lateral view **G, H** anterior view.

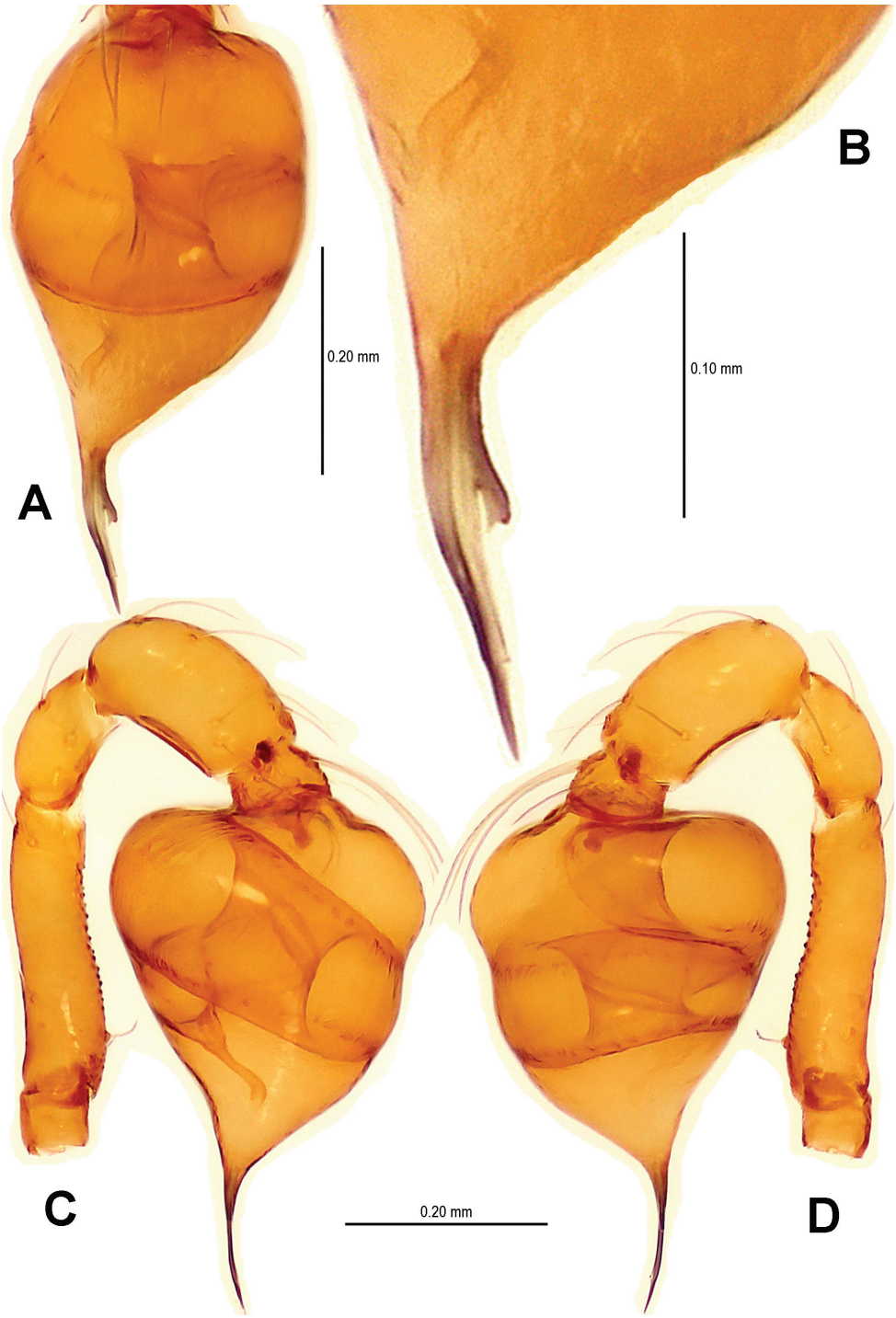

Figure 14. *Tetrablemma ziyaoensis* sp. n., male holotype. **A** Bulb **B** Embolus and spermic duct **C, D** Left palp. **A, B** anterior view **C** prolateral view **D** retrolateral view.

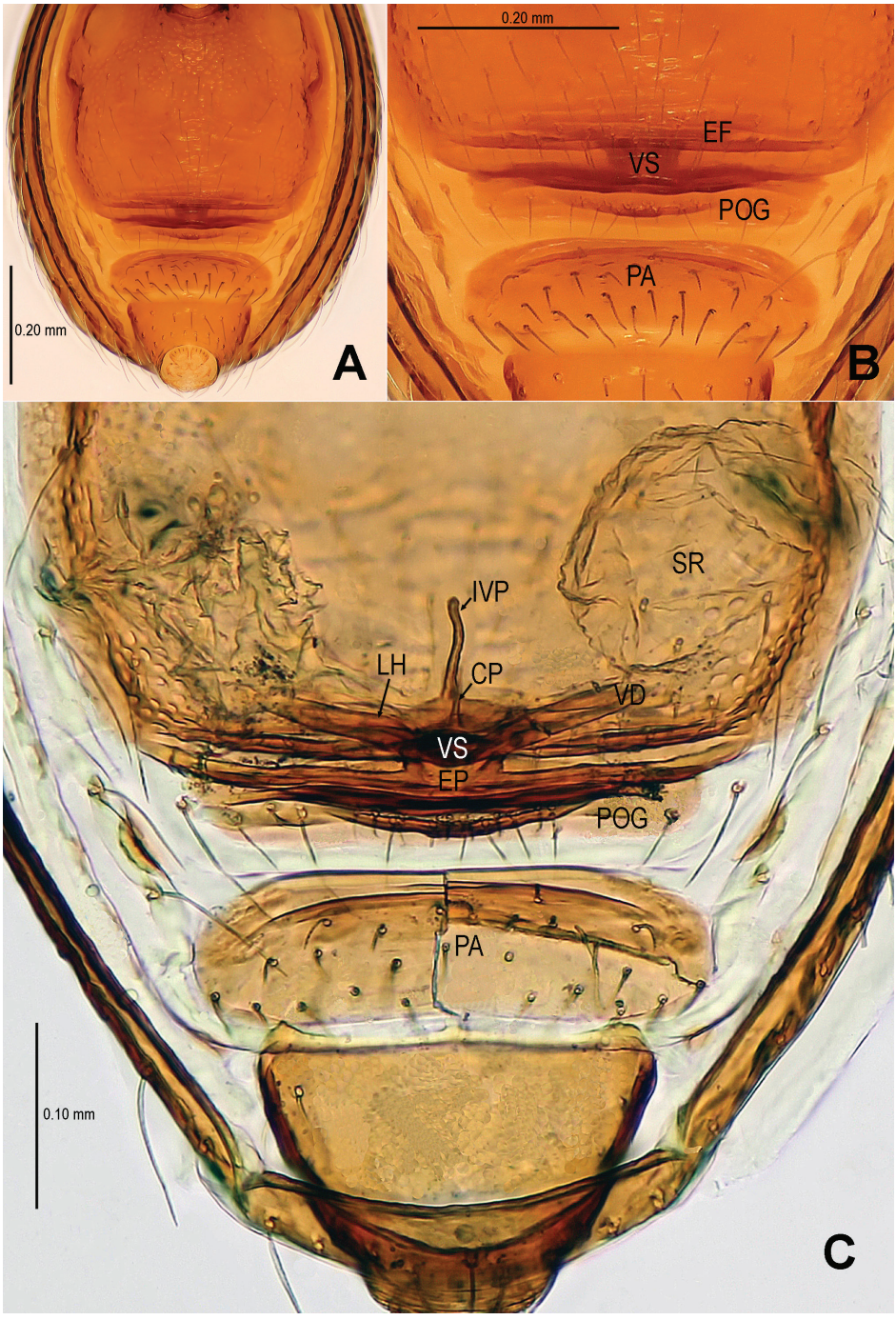

Figure 15. *Tetrablemma ziyaoensis* sp. n., female paratype. **A** Opisthosoma **B** Genital area (untreated) **C** Cleared vulva (KOH-treated). **A, B** ventral view **C** dorsal view. Abbrs.: **CP** central process; **EF** epigynal fold; **EP** epigynal pit; **IVP** inner vulval plate; **LH** lateral horn; **PA** preanal plate; **PEG** perigenital plate; **POG** postgenital plate; **SR** seminal receptaculum; **VD** vulval duct; **VS** vulval stem.

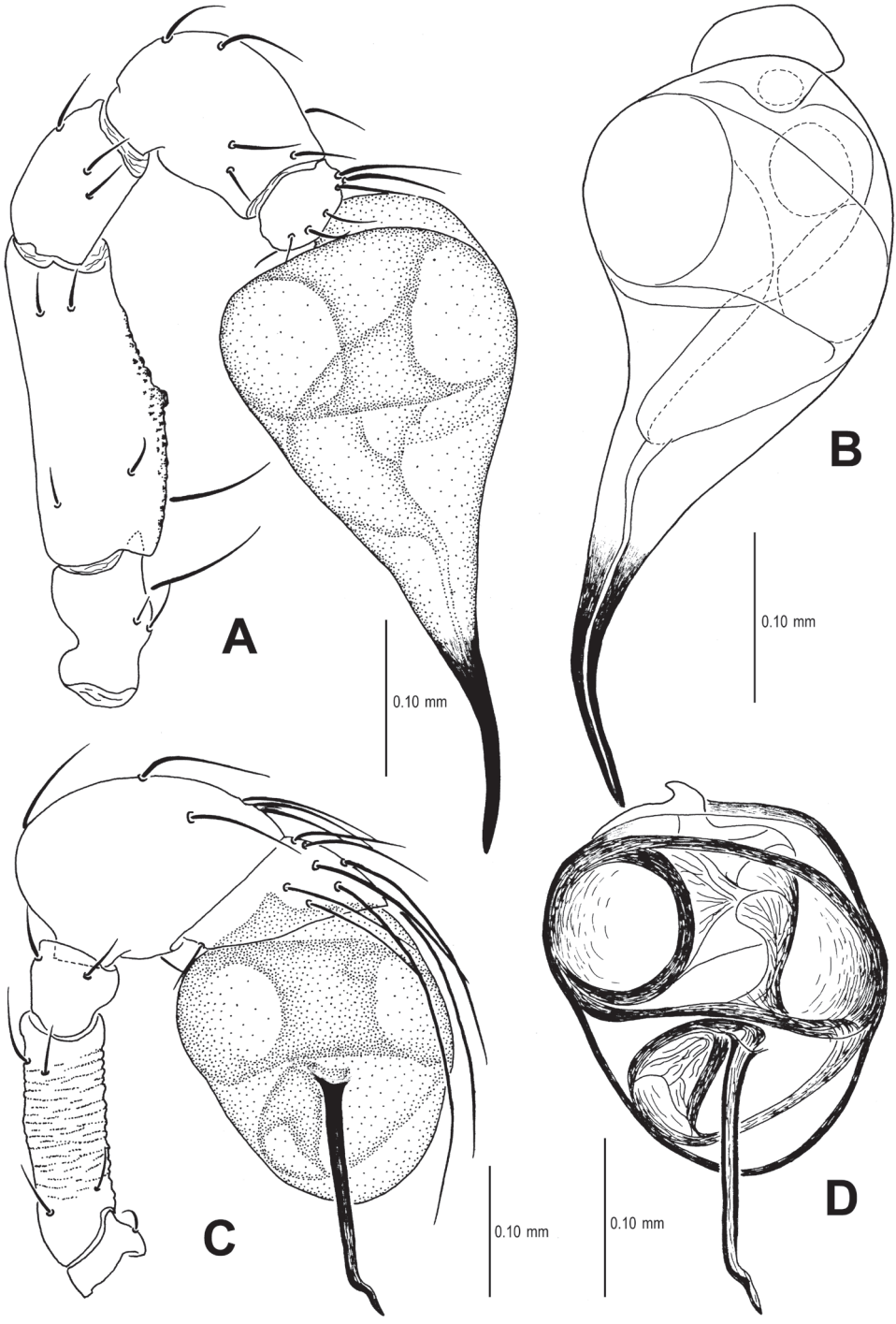

Figure 16. *Sinamma oxycera* gen. n. & sp. n., male holotype (**A, B**) *Singaporemma banxiaoensis* sp. n., male holotype (**C, D**). **A, C** Left palp **B, D** Bulb and spermic duct (lactic acid-treated). **A, C–D** prolateral view **B** anterior view.

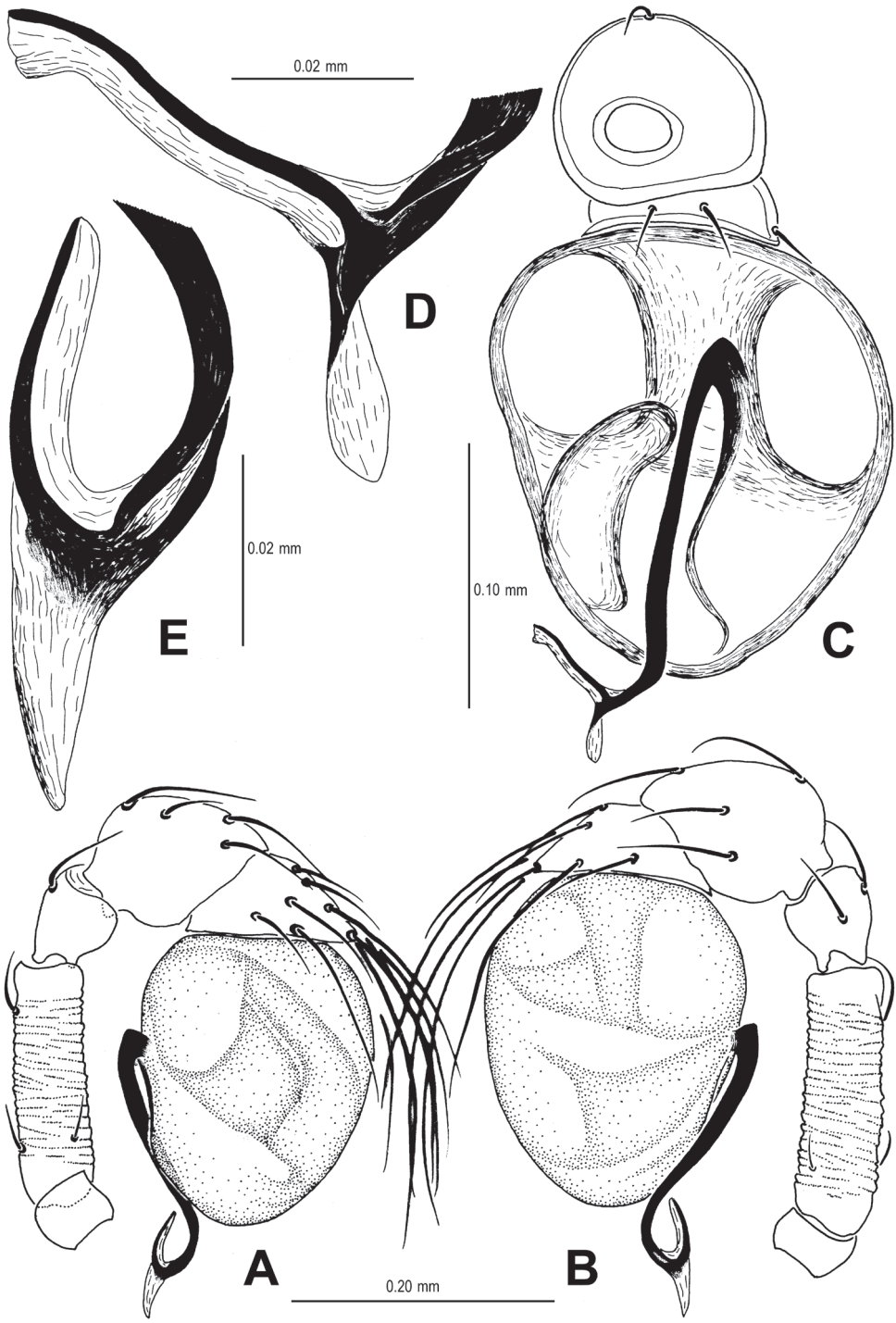

Figure 17. *Singaporemma wulongensis* sp. n., male holotype. **A, B** Left palp **C** Bulb and spermic duct (lactic acid-treated) **D, E** Tip of embolus. **A, E** prolateral view **B** retrolateral view **C, D** posterior view.

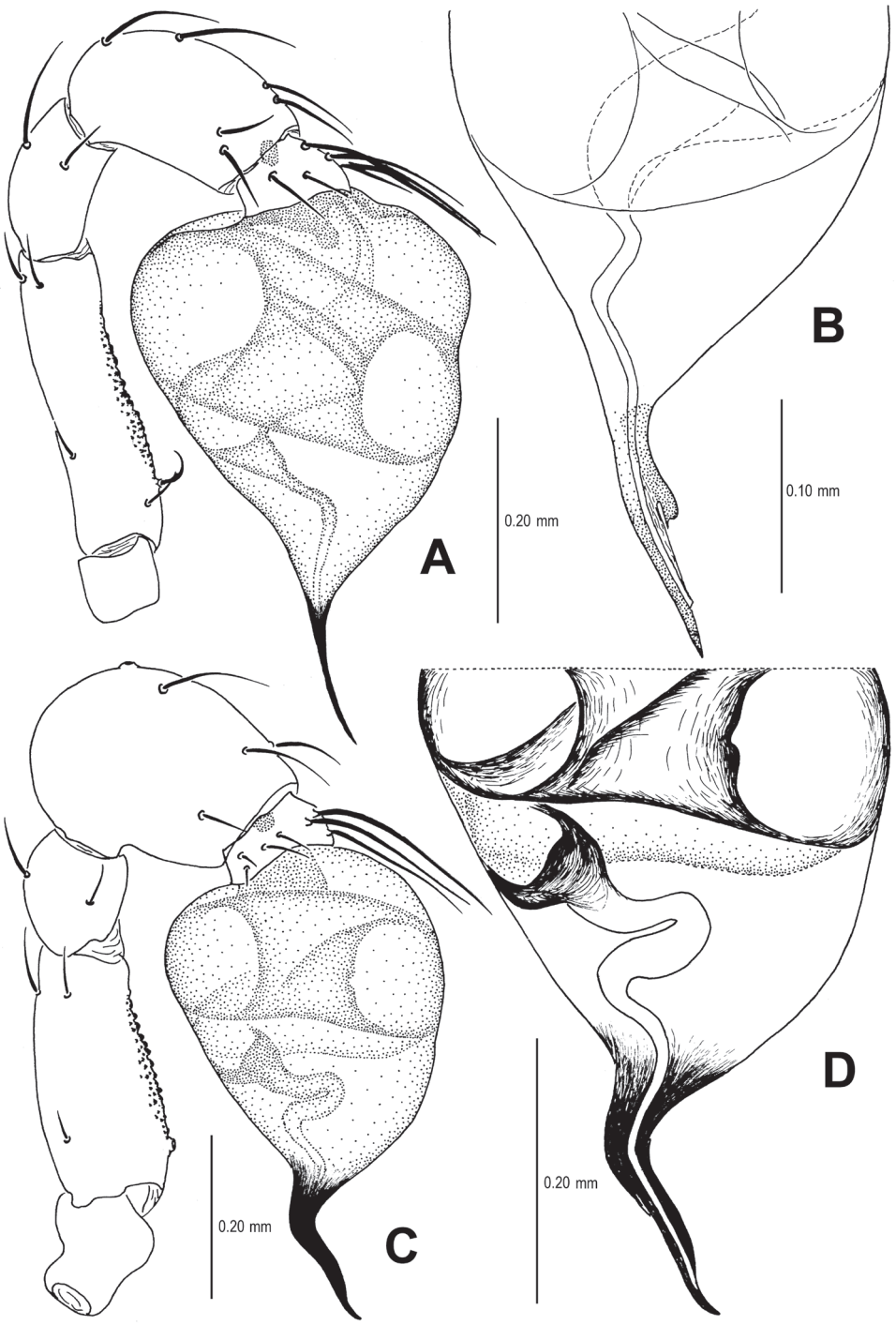

Figure 18. *Tetrablemma ziyaoensis* sp. n., male holotype (**A, B**) *Tetrablemma menglaensis* sp. n., male holotype (**C, D**). **A, C** Left palp **B, D** Bulb and spermic duct (lactic acid-treated). **A, C–D** prolateral view **B** anterior view.

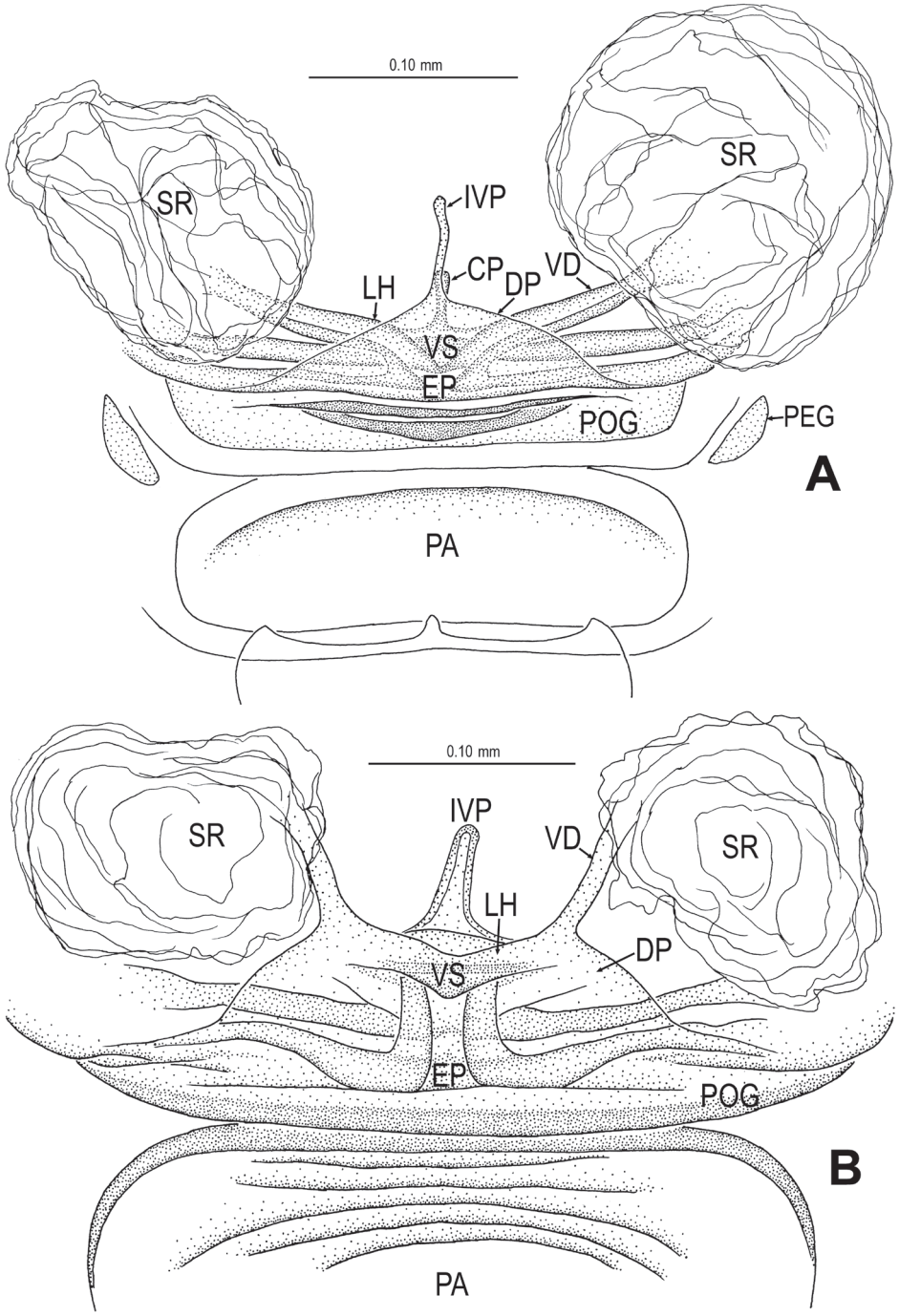

Figure 19. *Tetrablemma ziyaoensis* sp. n., female paratype (**A**) *Sinamma oxycera* gen. n. & sp. n., female paratype (**B**). **A, B** Cleared vulva (KOH-treated), dorsal view. Abbrs.: **CP** central process; **DP** dorsal plate; **EP** epigynal pit; **IVP** inner vulval plate; **LH** lateral horn; **PA** preanal plate; **PEG** perigenital plate; **POG** postgenital plate; **SR** seminal receptaculum; **VD** vulval duct; **VS** vulval stem.

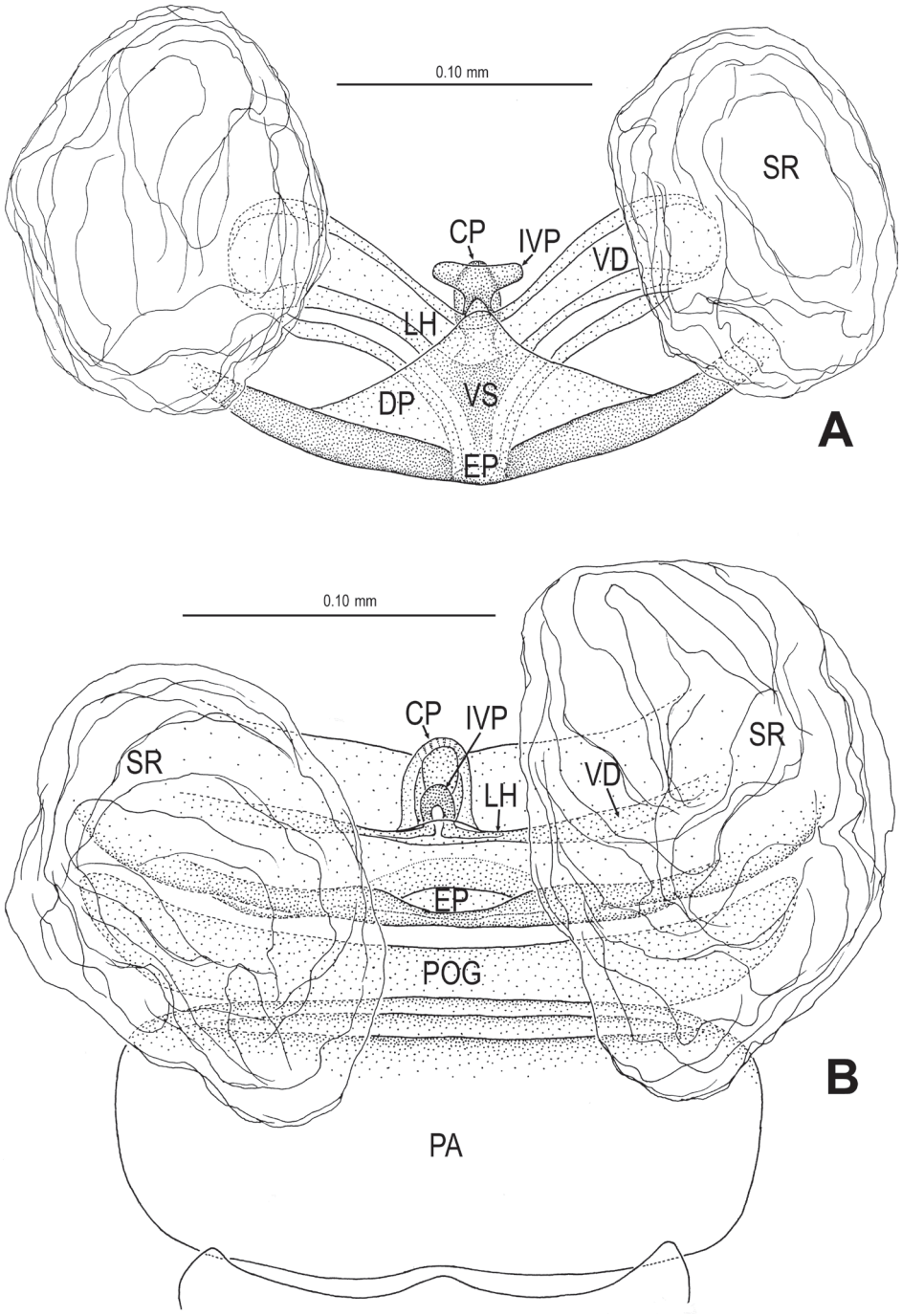

Figure 20. *Singaporemma banxiaoensis* sp. n., female paratype (**A**) *Singaporemma wulongensis* sp. n., female paratype (**B**). **A, B** Cleared vulva (KOH-treated), dorsal view. Abbrs.: **CP** central process; **DP** dorsal plate; **EP** epigynal pit; **IVP** inner vulval plate; **LH** lateral horn; **PA** preanal plate; **POG** postgenital plate; **SR** seminal receptaculum; **VD** vulval duct; **VS** vulval stem.

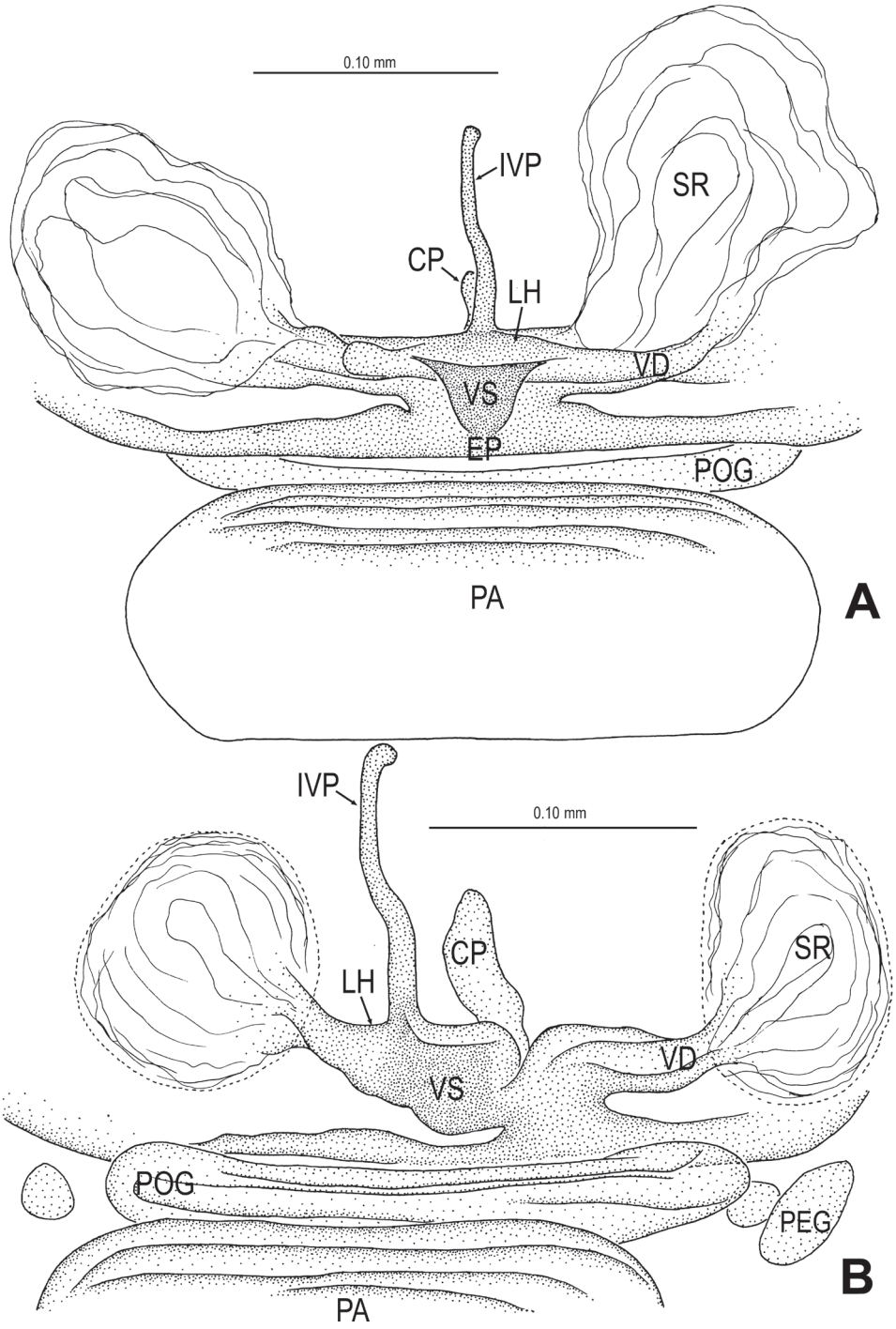

Figure 21. *Tetrablemma menglaensis* sp. n., female paratype. **A, B** Cleared vulva (KOH-treated) **A** dorsal view **B** dorsal-lateral view. Abbrs.: **CP** central process; **EP** epigynal pit; **IVP** inner vulval plate; **LH** lateral horn; **PA** preanal plate; **POG** postgenital plate; **SR** seminal receptaculum; **VD** vulval duct; **VS** vulval stem.

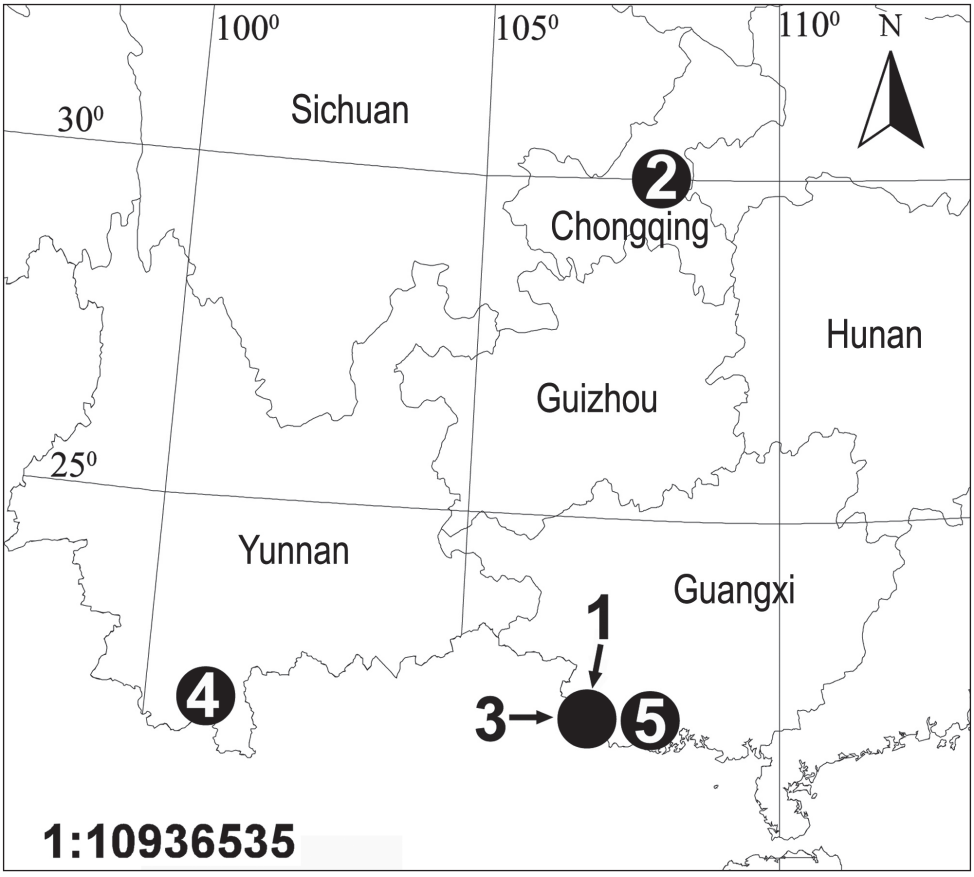

Figure 22. Distribution records of five new tetrablemmid species from China. **1** *Singaporemma banxiaoensis* sp. n. **2** *Singaporemma wulongensis* sp. n. **3** *Sinamma oxycera* gen. n. & sp. n. **4** *Tetrablemma menglaensis* sp. n. **5** *Tetrablemma ziyaoensis* sp. n.

Abdomen (Figs 13C–D, F; 15A): lateral scutum I anteriorly long, extending beyond the anterior rim of operculum; ventral episgastric scutum reticulated, laterally rugose; postepigastral scutum straight, medially narrow, laterally wide, as wide as preanal scutum; preanal scutum long oval, covered with serrated setae, a distinct furrow near anterior margin (Fig 15B).

Genitalia (Figs 15 B–C; 19A): epigynal fold wide (Fig. 15B), epigynal pit small, rift-shaped; vulval stem wide "V"-shaped, strongly sclerotized; lateral horns slightly sclerotized, supporting the base of vulval ducts of spermathecae; dorsal plate broad, triangular, distal margin extending to form a long inner vulval plate; inner vulval plate narrow, slightly sclerotized, longer than central process (Figs 15C; 19A); central process contracted at base (Fig. 19A).

Distribution. Known only from the type locality (Fig. 22).

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New synonymy of *Proceratium williamsi* Tiwari (Hymenoptera, Formicidae)

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Abstract

Proceratium bhutanense De Andrade, 2003, **syn. n.** is here found to be conspecific with *Proceratium williamsi* Tiwari, 2000 and accordingly treated as a junior synonym.

Keywords

Ants, *Proceratium bhutanense*, *Proceratium williamsi*, synonymy, India

Introduction

At present 79 extant and 5 fossil species are listed in the ant genus *Proceratium* across the globe (Bolton et al. 2007; Bolton 2012). In India, this genus is represented by two species (Bharti 2011). *Proceratium williamsi* was described by Tiwari (2000) as the first record of the genus from India; shortly afterwards De Andrade (2003) added *P. bhutanense* to the Indian *Proceratium.*

Unfortunately, Baroni Urbani and De Andrade (2003) left out *P. williamsi* from their global taxonomic revision of *Proceratium,* possibly due to lack of access to an obscure paper published locally. However, re-examination of both Indian species finds them conspecific. The specimens of *P. williamsi* collected by R. Mathew from the type

locality Meghalaya, Khasi hills, Shillong also form part of the material examined for *P. bhutanense*. Descriptions, morphometrics, line drawings, images and collection localities of two are also akin. Therefore, *P. bhutanense* is considered here as a junior synonym of *P. williamsi*.

Material and methods

The morphological observation was conducted on a Nikon SMZ 1500 stereo zoom microscope. For digital images, MP evolution digital camera was used on the same microscope with Auto-Montage (Syncroscopy, Division of Synoptics, Ltd.) software. Later, images were cleaned as required with Adobe Photoshop CS5.

Abbreviations of the specimen depositories are:

BMNH The Natural History Museum, London, England, U.K.
MRSN Museo Regionale di Scienze Naturali, Torino, Italy.
NHMB Naturhistorisches Museum Basel, Switzerland.
PUPAC Punjabi University Patiala, Ant Collection, Patiala, India.
ZSIK Zoological Survey of India, Kolkata, India.

Results and discussion

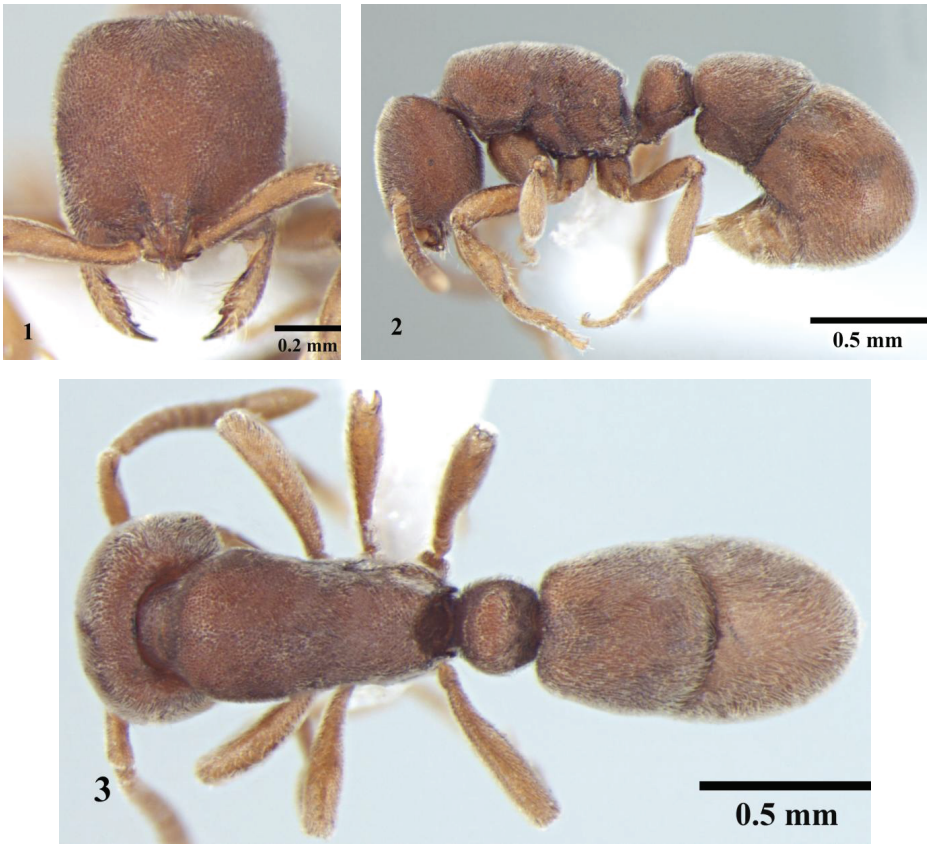
***Proceratium williamsi* Tiwari, 2000**
http://species-id.net/wiki/Proceratium_williamsi
Figs 1–3

Proceratium williamsi Tiwari, in Mathew and Tiwari 2000: 272, Figs 14-15 (w.). Holotype and paratype workers: Meghalaya, Khasi hills, Shillong, India [ZSIK].

Proceratium bhutanense De Andrade, in Baroni Urbani and De Andrade 2003: 278, Figs 116–117 (w.). Holoype and paratype workers: Phuntsholing, Bhutan [NHMB]; one paratype worker Phuntsholing, Bhutan [MRSN]. **Syn. n.**

Material examined. Paratype, worker, Meghalaya, Khasi hills, Shillong, India [ZSIK]; Worker [BMNH] (coll. R. Mathew, Det. De Andrade); worker photographs also examined on AntWeb (www.antweb.org): CASENT0281860. *Other Material: Uttarakhand:* Dakpathar, 750m, 4 (w.), 20.viii.2009; Rajaji Forest Area, 660m, 3(w.), 11.viii.2009, 1(w.), 12.viii.2009 (coll. Aijaz A. Wachkoo) [PUPAC]. *West Bengal:* Darjeeling, 1850m, 4(w.), 20.vi.2009 (coll. Irfan Gul) [PUPAC]. *Meghalaya:* Cherapunji, 1200m, 3(w.), 2.iv.2009 (coll. Irfan Gul); Khasi hills, Shillong, 1496m, 3(w.), 1(q.), 10.iv.2009 (coll. Irfan Gul) [PUPAC].

Ecology. This species was found mainly in leaf litter of primary, subtropical forests of Himalaya and occasionally in soil samples of secondary forests collected in cool



Figures 1–3. Worker; *Proceratium williamsi* Tiwari, 2000. **1** Head in full-face view **2** Body, lateral view **3** Body, dorsal view.

shady places. Although infrequent in collections, this species seems to be widely distributed throughout the Himalayan ranges.

Remarks. Examination of the specimens coupled with the images and descriptions reveal that there are no characters which could delimit *P. bhutanense* and *P. williamsi.* Moreover, the studied material does not exhibit any marked variation throughout the collection range, thereby enabling us to confidently treat *P. bhutanense* as a junior synonym of *P. williamsi*.

Acknowledgments

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