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Integrative species delimitation in the morphologically conservative *Stegana* subgen. *Orthostegana* (Diptera, Drosophilidae)

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Abstract

With the increasing number of cryptic species discovered in major branches of the subfamily Steganinae, accurate species identification is becoming challenging. Possible evolutionary stasis often gives rise to the formation of morphological conservatism and cryptic species, as is the case for the members of the *Stegana* (subgen. *Orthostegana*). In this study, the species boundaries of all Asian *Orthostegana* were accessed based on two mitochondrial genes [cytochrome *c* oxidase subunit I (*COI*) and NADH dehydrogenase subunit 2 (*ND2*)] using different species delimitation methods and evolutionary models. By integrating morphological and molecular evidence, we clearly validated the evolutionary independence of all five known species and identified nine new species from Southwest China: *Stegana* (*Orthostegana*) *aini* Peng & Chen **sp. nov.**, *S.* (*O.*) *brevivittata* Peng & Chen **sp. nov.**, *S.* (*O.*) *cuodi* Peng & Chen **sp. nov.**, *S.* (*O.*) *fuscofemorata* Peng & Chen **sp. nov.**, *S.* (*O.*) *latipalpula* Peng & Chen **sp. nov.**, *S.* (*O.*) *macrostephana* Peng & Chen **sp. nov.**, *S.* (*O.*) *mohnihei* Peng & Chen **sp. nov.**, *S.* (*O.*) *obscurala* Peng & Chen **sp. nov.**, and *S.* (*O.*) *pinguitia* Peng & Chen **sp. nov.** Moreover, we discussed the relationship between the Asian *Orthostegana* lineage and the subgen. *Oxyphortica*, and the necessity of taxonomic re-evaluation for the Neotropical species with molecular data. The high morphological conservatism within *Orthostegana* is largely the outcome of *in situ* diversification for adapting to the ecological environment.

Keywords

Cryptic species, DNA barcode, new species, phylogeny, Steganinae, Yunnan

1. Introduction

The diversity and heterogeneity of biological organisms are the overall consequence of adaptations to the environment and its changes. However, diversifying selection alone does not give rise to new species, but requires the reinforcement of some factors, such as morphology, behavior, ecology, and genetics (Xie and Bu 2010). Hence, closely related species living in similar ecological environments tend to have convergent traits due to shared selective forces (Wiens et al. 2010).

Meigen (1830) established the genus *Stegana*, which is currently the most speciose genus in the subfamily Steganinae comprising six subgenera: *Ceratostylus* Enderlein (1922), *Crypsistegana* Wang and Chen (2022), *Orthostegana* Hendel (1913), *Oxyphortica* Duda (1923), *Stegana* Meigen (1830), and *Steganina* Wheeler (1960). A total of 374 *Stegana* species have been described (Brake and Bächli 2008; Wang et al. 2022, 2023; Bächli 2023) and the majority of them forage near streams in the tropical to subtropical broad-leaved forests of the Northern Hemisphere.

Currently, a total of 11 *Orthostegana* species have been reported and are mainly distributed in the Oriental and the Neotropical regions (Zhang et al. 2012; Vilela and Bächli 2020). Except for *S.* (*O.*) *singularis* (Sidorenko, 1990), which has also been recorded in the Palaearctic region. Compared with the *Oxyphortica* species, the *Orthostegana* species show higher morphological conservatism along with highly similar male genitalia (Zhang et al. 2012) and closer or overlapping geographical distribution in East Asia, which poses a huge challenge to the species delimitation in this subgenus.

Hendel (1913) originally established the genus *Orthostegana* based on the type species *O. acutangula* Hendel, 1913 with the examination of only two female adults from the Neotropical region (Mapiri, La Paz, Bolivia). Wheeler (1960) identified it as a subgenus of the genus *Stegana* based on its morphological similarity to the subgen. *Stegana* and subgen. *Steganina* in the profile view of heads. Sidorenko (2002) performed a cladistic analysis based on 78 adult morphological characters for 31 species representing six genera of Steganinae. He proposed to establish the subgen. *Anastega* for the only species, *S.* (*O.*) *singularis* (Sidorenko, 1990), which had been transferred from the subgen. *Stegana*. However, *S.* (*O.*) *singularis* was inserted into the subgen. *Orthostegana* and formed a robust branch (posterior probability, PP = 0.99) with *S.* (*O.*) *multicardua* Zhang and Chen, 2012 in a recent phylogenetic analysis for the genus *Stegana* (Li et al. 2013). After a thorough integration and comparison of morphological characters, the subgen. *Anastega* (*S. singularis*) was incorporated into the subgen. *Orthostegana* (Zhang et al. 2012).

Recently, the taxonomic status of the Asian *Orthostegana* lineage has been controversial. Vilela and Bächli (2020) redescribed *S.* (*O.*) *acutangula* (Hendel, 1913) and *S.* (*Steganina*) *triseta* (Duda, 1925) based on syntype male specimens, and incorporated *S.* (*S.*) *triseta* into the *Orthostegana* along with three identified new sibling species from the Neotropical region [i.e., *S.* (*O.*) *dudai* Vilela and Bächli, 2020, *S.* (*O.*) *turrialba* Vilela and Bächli, 2020, and *S.* (*O.*) *yasuni* Vilela and Bächli, 2020]. Through the comparison of some morphological characters including wing veins and male terminalia, they proposed to regard *Orthostegana* as an endemic American subgenus by transferring six Asian *Orthostegana* species to the subgen. *Oxyphortica*.

Orthostegana, however, is a very heterogenous lineage in the genus *Stegana* with specific genital structures and shows a clear molecular boundary with the *Oxyphortica*. The monophyly between the Asian *Orthostegana* lineage and the subgen. *Oxyphortica* has not been supported by partial mitochondrial and nuclear markers (Li et al. 2013; Wang et al. 2022). Species delimitation of *Orthostegana* was feasible using a minimum interspecific genetic pairwise genetic distances (*p*-distances) of 0.057 in both mitochondrial cytochrome *c* oxidase subunit I (*COI*) and NADH dehydrogenase subunit 2 (*ND2*) genes (Zhang et al. 2012). However, the interspecific boundaries of *Orthostegana* are becoming much more indistinct with the gradually increasing number of closely related new species. Therefore, the two major objectives of this study are to assess known and putative new species boundaries by integrating morphological and molecular evidence and to clarify the relationships among the Asian *Orthostegana* lineage, the Neotropical *Orthostegana* lineage, and the subgen. *Oxyphortica*.

2. Material and Methods

2.1. Sampling, morphological terminology, and abbreviations

The geographical distribution of all 11 known *Orthostegana* species was plotted on a map (Fig. 1) using the web-based SimpleMappr application (Shorthouse 2010). The *Orthostegana* specimens used in this study were mostly collected from trunks and bushes along streams in the montane forest in either Sichuan or Yunnan Province. They were collected and preserved in 75% ethanol immediately. The collection records of a total of 67 sampled *Stegana* specimens were listed in Table 1.

We observed and identified the specimens based on morphological characters under a stereoscope and microscope. Then, we sampled representative specimen(s) from each putative morphospecies, dissected their genitalia for further examination, and detached some abdominal tissues for further DNA extraction. Finally, we determined the new species from the putative morphospecies by integrating morphological and molecular data. Type specimens were deposited in the Department of Entomology, South China Agricultural University, Guangzhou, China (**SCAU**). The terminalia terminology followed Rice et al. (2019) and Toda et al. (2020).

The definitions of measurements, indices, and abbreviations followed Chen & Toda (2001): **BL** = straight distance from anterior edge of pedicel to tip of abdomen; **ThL** = medial distance from anterior notal margin to apex of scutellum; **WL** = distance from humeral cross vein to wing apex; **WW** = maximum wing width; **arb** = dorsal branches/ventral branches of arista; **avd** = longest ventral branch/longest dorsal branch of arista in length; **adf** = longest dorsal branch of arista/width of first flagellomere; **flw** = length/width of first flagellomere; **FW/HW** = frontal width/head width; **ch/o** = maximum width of gena/maximum diameter of eye; **prorb** = proclinate orbital/posterior reclinate orbital in length; **rcorb** = anterior

Table 1. List of the *Stegana* samples used in this study. All ingroup specimens were collected from China. Notes: the sequences obtained from [a] Zhang et al. 2012; [b] Li et al. 2013; [c] Zhang et al. 2015. NA, not applicable.

Subgenus	Species	Collection site	BOLD Process ID	GeneBank accession number	
				COI	*ND2*
Oxyphortica	*adentata* Toda and Peng, 1992	Nanling, Shaoguan, Guangdong	NA	HQ842774[b]	HQ842795[b]
Stegana	*quadrata* Cao and Chen, 2010	Kuankuoshui, Suiyang, Guizhou	NA	KP179318[c]	KP752416[c]
Steganina	*euryphylla* Chen and Chen, 2009	Wuliangshan, Jingdong, Yunnan	NA	MH373088	MH373156
Orthostegana	*curvinervis* Hendel, 1914 –1	Jianfengling, Ledong, Hainan	OSTZQ001-21	HQ842769[a]	HQ842790[a]
	curvinervis Hendel, 1914 –2	Diaoluoshan, Lingshui, Hainan	OSTZQ002-21	KU575097	ON600737
	flavicauda Zhang and Chen, 2012 –1	Baihualing, Baoshan, Yunnan	OSTZQ037-21	KU575103	JQ901408[b]
	flavicauda Zhang and Chen, 2012 –2	Hesong, Menghai, Yunnan	OSTZQ040-21	KU575101	ON600738
	flavicauda Zhang and Chen, 2012 –3	Baihualing, Baoshan, Yunnan	OSTZQ051-21	KU575100	ON600739
	flavicauda Zhang and Chen, 2012 –4	Hesong, Menghai, Yunnan	OSTZQ052-21	KU575102	ON600740
	flavicauda Zhang and Chen, 2012 –5	Botanic Garden, Ruili, Yunnan	OSTZQ053-21	ON553565	ON600741
	flavicauda Zhang and Chen, 2012 –6	Yixiang, Pu'er, Yunnan	OSTZQ059-21	KU575098	ON600742
	flavicauda Zhang and Chen, 2012 –7	Yixiang, Pu'er, Yunnan	OSTZQ080-21	KU575099	ON600743
	flavicauda Zhang and Chen, 2012 –8	Mengdong, Cangyuan, Yunnan	OSTZQ085-21	ON553566	ON600744
	flavicauda Zhang and Chen, 2012 –9	Mengdong, Cangyuan, Yunnan	OSTZQ086-21	ON553567	ON600745
	flavicauda Zhang and Chen, 2012 –10	Botanic Garden, Ruili, Yunnan	OSTZQ087-21	ON553563	ON600746
	flavicauda Zhang and Chen, 2012 –11	Botanic Garden, Ruili, Yunnan	OSTZQ091-21	ON553563	ON600747
	hirsutina Zhang and Chen, 2012 –1	Wangtianshu, Mengla, Yunnan	OSTZQ005-21	HQ842770[a]	HQ842791[a]
	hirsutina Zhang and Chen, 2012 –2	Menglun, Mengla, Yunnan	OSTZQ008-21	ON553568	ON600751
	hirsutina Zhang and Chen, 2012 –3	Yixiang, Pu'er, Yunnan	OSTZQ013-21	KU575104	ON600752
	hirsutina Zhang and Chen, 2012 –4	Longtan Park, Ximeng, Yunnan	OSTZQ017-21	KU575104	ON600753
	hirsutina Zhang and Chen, 2012 –5	Mengdong, Cangyuan, Yunnan	OSTZQ022-21	ON553570	ON600754
	hirsutina Zhang and Chen, 2012 –6	Muyiji Park, Ximeng, Yunnan	OSTZQ026-21	ON553571	ON600755
	hirsutina Zhang and Chen, 2012 –7	Mengdong, Cangyuan, Yunnan	OSTZQ036-21	ON553569	ON600756
	hirsutina Zhang and Chen, 2012 –8	Muyiji Park, Ximeng, Yunnan	NA	ON553572	ON600757
	hylecoeta Zhang and Chen, 2012 –1	Hesong, Menghai, Yunnan	OSTZQ103-21	KU575108	ON600751
	hylecoeta Zhang and Chen, 2012 –2	Wuliangshan, Jingdong, Yunnan	OSTZQ151-21	HQ842771[a]	HQ842792[a]
	hylecoeta Zhang and Chen, 2012 –3	Yixiang, Pu'er, Yunnan	OSTZQ154-21	KU575109	ON600758
	hylecoeta Zhang and Chen, 2012 –4	Hesong, Menghai, Yunnan	OSTZQ165-21	KU575106	ON600759
	hylecoeta Zhang and Chen, 2012 –5	Hesong, Menghai, Yunnan	OSTZQ170-21	KU575107	ON600760
	hylecoeta Zhang and Chen, 2012 –6	Mengdong, Cangyuan, Yunnan	OSTZQ195-21	ON553574	ON600761
	hylecoeta Zhang and Chen, 2012 –7	Mengma, Menglian, Yunnan	OSTZQ221-21	ON553575	ON600762
	hylecoeta Zhang and Chen, 2012 –8	Guanlei, Mengla, Yunnan	NA	ON553576	ON600763
	hylecoeta Zhang and Chen, 2012 –9	Mengma, Menglian, Yunnan	NA	ON553577	ON600764
	multicardua Zhang and Chen, 2012	Wuliangshan, Jingdong, Yunnan	OSTZQ222-21	HQ842772[a]	HQ842793[a]
	singularis Sidorenko, 1990 –1	Guanmenshan, Benxi, Liaoning	OSTZQ003-21	HQ842768[a]	HQ842789[a]
	singularis Sidorenko, 1990 –2	Guanmenshan, Benxi, Liaoning	OSTZQ004-21	ON553562	ON600779
	aini Zhang & Chen **sp. nov.** –1	Hesong, Menghai, Yunnan	STBX199-20	KU575113	ON600722
	aini Zhang & Chen **sp. nov.** –2	Hesong, Menghai, Yunnan	STBX209-20	KU575114	ON600723
	brevivittata Zhang & Chen **sp. nov.** –1	Muyiji Park, Ximeng, Yunnan	STBX216-20	ON553579	ON600724
	brevivittata Zhang & Chen **sp. nov.** –2	Likan, Ximeng, Yunnan	STBX220-20	ON553578	ON600725
	brevivittata Zhang & Chen **sp. nov.** –3	Mengdong, Cangyuan, Yunnan	STBX222-20	ON553583	ON600726
	brevivittata Zhang & Chen **sp. nov.** –4	Muyiji Park, Ximeng, Yunnan	STBX224-20	ON553584	ON600727
	brevivittata Zhang & Chen **sp. nov.** –5	Likan, Ximeng, Yunnan	NA	ON553580	ON600728
	brevivittata Zhang & Chen **sp. nov.** –6	Muyiji Park, Ximeng, Yunnan	NA	ON553581	ON600729
	brevivittata Zhang & Chen **sp. nov.** –7	Mengdong, Cangyuan, Yunnan	NA	ON553582	ON600730
	cuodi Peng & Chen **sp. nov.** –1	Guanlei, Mengla, Yunnan	STBX225-20	ON553586	ON600731
	cuodi Peng & Chen **sp. nov.** –2	Likan, Ximeng, Yunnan	STBX228-20	ON553587	ON600732
	cuodi Peng & Chen **sp. nov.** –3	Likan, Ximeng, Yunnan	STBX229-20	ON553588	ON600733
	cuodi Peng & Chen **sp. nov.** –4	Guanlei, Mengla, Yunnan	STBX230-20	ON553590	ON600734
	cuodi Peng & Chen **sp. nov.** –5	Guanlei, Mengla, Yunnan	STBX231-20	ON553589	ON600735
	cuodi Peng & Chen **sp. nov.** –6	Guanlei, Mengla, Yunnan	STBX232-20	ON553585	ON600736
	fuscofemorata Zhang & Chen **sp. nov.** –1	Muyiji Park, Ximeng, Yunnan	STBX133-20	ON553592	ON600748
	fuscofemorata Zhang & Chen **sp. nov.** –2	Baihualing, Baoshan, Yunnan	STBX139-20	KU575110	ON600749
	fuscofemorata Zhang & Chen **sp. nov.** –3	Wuliangshan, Jingdong, Yunnan	STBX140-20	ON553591	ON600750

Subgenus	Species	Collection site	BOLD Process ID	GeneBank accession number	
				COI	*ND2*
	latipalpula Peng & Chen **sp. nov.**	Wanba, Jiulong, Sichuan	STBX100-20	ON553593	ON600766
	macrostephana Peng & Chen **sp. nov.** –1	Mengdong, Cangyuan, Yunnan	STBX106-20	ON553594	ON600767
	macrostephana Peng & Chen **sp. nov.** –2	Botanic Garden, Ruili, Yunnan	STBX125-20	ON553595	ON600768
	macrostephana Peng & Chen **sp. nov.** –3	Mengdong, Cangyuan, Yunnan	STBX126-20	ON553596	ON600769
	macrostephana Peng & Chen **sp. nov.** –4	Mengdong, Cangyuan, Yunnan	STBX131-20	ON553597	ON600770
	mohnihei Peng & Chen **sp. nov.** –1	Mengdong, Cangyuan, Yunnan	STBX146-20	ON553598	ON600771
	mohnihei Peng & Chen **sp. nov.** –2	Muyiji Park, Ximeng, Yunnan	STBX187-20	ON553599	ON600772
	mohnihei Peng & Chen **sp. nov.** –3	Muyiji Park, Ximeng, Yunnan	STBX188-20	ON553600	ON600773
	mohnihei Peng & Chen **sp. nov.** –4	Baihualing, Baoshan, Yunnan	STBX197-20	ON553601	ON600774
	obscurala Zhang & Chen **sp. nov.** –1	Wangtianshu, Mengla, Yunnan	STBX141-20	KU575111	ON600775
	obscurala Zhang & Chen **sp. nov.** –2	Wangtianshu, Mengla, Yunnan	STBX144-20	KU575112	ON600776
	pinguitia Peng & Chen **sp. nov.** –1	Dafengding, Mabian, Sichuan	STBX101-20	ON553602	ON600777
	pinguitia Peng & Chen **sp. nov.** –2	Fengtongzhai, Baoxing, Sichuan	STBX105-20	ON553603	ON600778

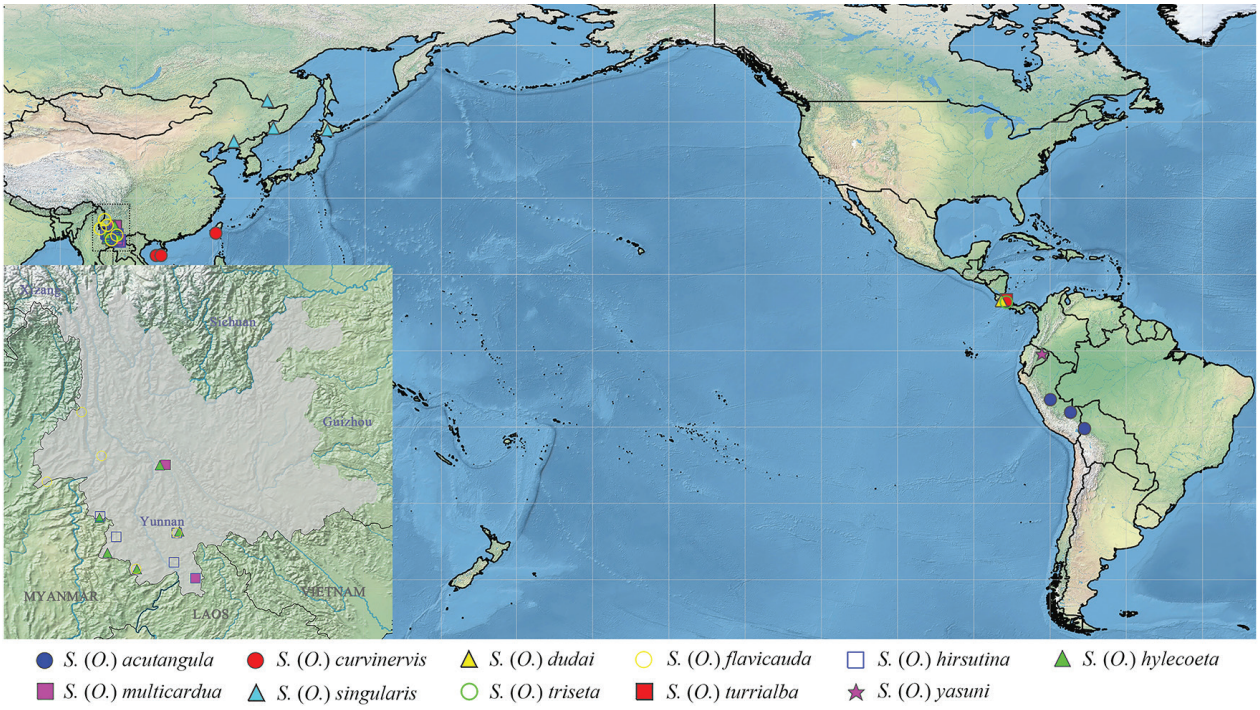

Figure 1. Geographical distribution of all 11 known species in the *Stegana* (subgen. *Orthostegana*)

reclinate orbital/posterior reclinate orbital in length; **vb** = subvibrissal/vibrissa in length; **dcl** = anterior dorsocentral/posterior dorsocentral in length; **presctl** = prescutellar/posterior dorsocentral in length; **sctl** = basal scutellar/apical scutellar in length; **sterno** = anterior katepisternal/posterior katepisternal in length; **orbito** = distance between proclinate and posterior reclinate orbitals/distance between inner vertical and posterior reclinate orbital; **dcp** = length distance between ipsilateral dorsocentrals/cross distance between anterior dorsocentrals; **sctlp** = distance between ipsilateral scutellars/cross distance between apical scutellars; **C** = second costal section between subcostal break and R_{2+3}/third costal section between R_{2+3} and R_{4+5}; **4c** = third costal section between R_{2+3} and R_{4+5}/M_1 between r-m and dm-cu; **4v** = M_1 between dm-cu and wing margin/M_1 between r-m and dm-cu; **5x** = CuA_1 between dm-cu and wing margin/dm-cu between M_1 and CuA_1; **ac** = third costal section between R_{2+3} and R_{4+5}/distance between distal ends of R_{4+5} and M_1; **M** = CuA_1 between dm-cu and wing margin/M_1 between r-m and dm-cu; **C3F** = length of heavy setation in third costal section/(length of heavy setation in third costal section + length of light setation in third costal section).

The abbreviations of male terminalia include: **aed s** (aedeagal sheath); **aed** (aedeagus); **cerc** (cercus); **epand** (epandrium); **hypd** (hypandrium); **phap** (phallapodeme); **pg** (pregonite); **sur** (surstylus).

The other abbreviations related to molecular analyses include: **ABGD** (Automatic Barcode Gap Discovery); **BI** (Bayesian inference); **BIC** (Bayesian information criterion); **BP&P** (Bayesian Phylogenetics and Phylogeography); **G** (gamma); **MCMC** (Markov chain Monte Carlo); **ML** (maximum likelihood); **MOTUs** (molecular operational taxonomic units); **mPTP** (multi-rate Poisson

tree processes); **USB** (ultrafast bootstrap); **X** (relative gap width); **θ** (ancestral population size); $\boldsymbol{\tau_0}$ (root age).

2.2. PCR amplification and sequencing

We extracted total DNA from the detached abdominal tissues from each sampled specimen (Table 1) using a Magen Hipure Insect DNA Kit (#D3129-02, Magen Biotech, Guangzhou, China). We then obtained partial *COI* and complete *ND2* sequences from them for further phylogenetic analyses and molecular species delimitation. We followed the methods of total DNA extraction, PCR amplification, and sequencing in Zhang et al. (2016). The primers, *COI*-F1: 5'–CGCCTAACTTCAGCACTT–3' (He et al. 2009), *COI*-HCO2198: 5'–TAAACTTCAGGGGTAAAACA–3' (Folmer et al. 1994), *ND2*: 5'–AAGCTACTGGGTTCATACC–3' (Wang et al. 2006), and *ND2*-T4: 5'–CTTTGAAGGCTATTAGTT–3' (Shao et al. 2014), were used in PCR amplification and sequencing.

2.3. Phylogenetic analyses

Three closely related subgenera (*Stegana*, *Steganina*, and *Oxyphortica*), each represented by one species, were selected as outgroup taxa (Table 1). The obtained *COI* and *ND2* sequences of a total of 67 samples including the three outgroup taxa, were aligned by Clustal W in MEGA v.7.0 (Kumar et al. 2016). The program PartitionFinder v.2.1.1 (Lanfear et al. 2017) was used to evaluate the best partitioning scheme and substitution models for phylogenetic analysis. Then GTR+I+G for COI^{1st} + COI^{2nd} + $ND2^{1st}$ + $ND2^{2nd}$ and TRN+I+G for COI^{3rd} + $ND2^{3rd}$ were selected and used for BI and ML analysis under a "greedy" algorithm and the BIC.

BI was performed using MrBayes v.3.2.6 (Ronquist and Huelsenbeck 2003) on the CIPRES science gateway (http://www.phylo.org). Two independent runs of MCMC (Huelsenbeck et al. 2004) with four chains (three heated and one cold) were conducted simultaneously for 20,000,000 generations. One thousand generations were set as the sampling frequency, and the first 25% of non-stationary trees were discarded as burn-in. The analysis was checked for convergence with an effective sample size > 200 in Tracer v1.7.1 (Rambaut et al. 2018) and an average standard deviation of split frequencies < 0.01 in MrBayes. The ML analysis was performed using IQ-TREE v.1.6.1 (Nguyen et al. 2015) with 1,000 replicates of UFBs and a thorough search for the best-scoring ML tree. The resulting trees were visualized using FigTree v.1.4 (http://tree.bio.ed.ac.uk/software/figtree).

2.4. Molecular species delimitation

To investigate the validity of the putative morphospecies and identify potential cryptic species within our sampled specimens, species boundaries were estimated based on the obtained *COI* and *ND2* sequences using two single-locus species delimitation methods: ABGD (Puillandre et al. 2012) and mPTP (Kapli et al. 2017), and a multi-locus species delimitation method: BP&P (Rannala and Yang 2003; Yang and Rannala 2010).

The initial MOTUs were assessed by the ABGD and mPTP analyses for the *COI* and *ND2* sequences, respectively. The ABGD analysis was performed on the ABGD web server (https://bioinfo.mnhn.fr/abi/public/abgd) using the Kimura 2-P model. The X was set as 0.5, and the *P*-values ranged from 0.001 to 0.1 in 10 steps. The mPTP analysis was conducted using the mPTP web server (https://mptp.h-its.org). Consensus MOTUs of the above four processing schemes were selected as candidate species for the subsequent BP&P analysis. The starting guide ultrametric tree was generated in BEAST v.1.10.4 (Suchard et al. 2018) using the following parameters: the Yule process of speciation, the GTR+G+I nucleotide substitution model, 10,000,000 generations of the MCMC chains sampling every 1,000 generations, and a 25% discard rate of the resultant trees as burn-in. The convergence was checked in Tracer. The BP&P analysis was performed by BP&P v.3.3 (Yang 2015). Given the θ and τ_0 may affect the posterior probabilities of the speciation modes (Yang and Rannala 2010), we set three different G prior combinations (Leaché and Fujita 2010): (1) θ ~ G (1, 10) and τ_0 ~ G (1, 10), large ancestral population sizes and deep divergences; (2) θ ~ G (2, 2000) and τ_0 ~ G (2, 2000), small ancestral population sizes and shallow divergences; (3) θ ~ G (1, 10) and τ_0 ~ G (2, 2000), large ancestral population sizes and shallow divergences. Finally, The intra- and interspecific *p*-distances of each putative valid species were calculated using MEGA.

3. Results

3.1. Phylogeny and species delimitation

The alignment analyzed in this study contained the concatenated 680 bp *COI* and 1034 bp *ND2* sequences from the 67 sampled *Stegana* specimens. The BI and ML analysis deduced identical tree topologies and well-supported the monophyly of the subgen. *Orthostegana* (PP = 1.00 and UFB = 92). In both analyses, the 64 sampled *Orthostegana* specimens formed three main clades and further were sorted into 15 taxa (Fig. 2). The clade containing *S.* (*O.*) *multicardua*, *S.* (*O.*) *singularis*, and *S.* (*O.*) *latipalpula* **sp. nov.** (PP = 1.00 and UFB = 75) located at the base of the phylogenetic trees, showed a high level of morphological differentiation [e.g., surstylus fused with epandrium and without prensiseta in *S.* (*O.*) *multicardua* (as fig. 7F in Zhang et al. 2012); gonopod distally strongly expanded and with numerous minute, serrated processes in *S.* (*O.*) *singularis* (as fig. 4C in Zhang et al. 2012); aedeagus distally with some minute, serrated processes in *S.* (*O.*) *latipalpula* **sp. nov.** (Fig. 16)]. Another clade

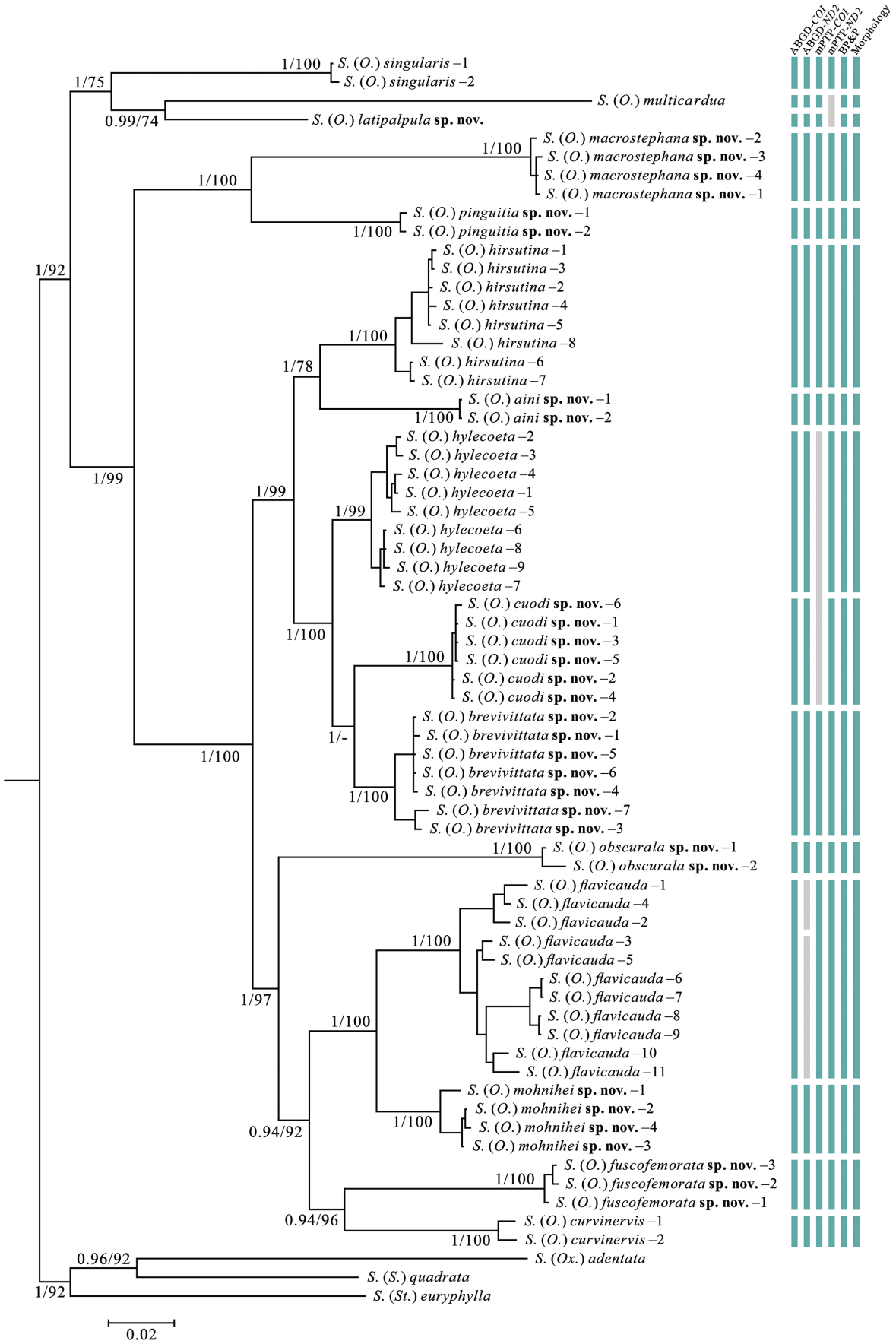

Figure 2. Phylogenetic tree constructed in this study. The tree constructed from the Bayesian inference (BI) based on the concatenated dataset of partial mitochondrial cytochrome *c* oxidase subunit I (*COI*) and complete NADH dehydrogenase subunit 2 (*ND2*) sequences of 18 Asian *Stegana* species from 67 sampled specimens. Numbers around the nodes indicate the posterior probabilities (PPs) in the BI and the ultrafast bootstrap (UFB) values in the maximum likelihood (ML) analysis. UFB lower than 50 is presented by a dash (-). The bar indicates the estimated number of substitutions per site.

Table 2. Divergences in the *COI* sequences. The divergences were calculated among and within all 15 Asian *Stegana* (*Orthostegana*) species from the 64 sampled specimens. Notes: Min. (Minimum), intra. (intraspecific), Max. (Maximum), Vari. (variability), inter. (interspecific), NA (not applicable).

Species	Number of sequence(s)	Min. intra./Max. intra./ Mean intra.±Vari.	Min. inter./Max. inter./ Mean inter.±Vari.	Species with Min. inter.
curvinervis	2	0.010	0.091/0.161/0.111±0.017	*hirsutina*, *mohnihei* **sp. nov.**
flavicauda	11	0.000/0.030/0.020±0.008	0.056/0.169/0.104±0.024	*mohnihei* **sp. nov.**
hirsutina	8	0.000/0.018/0.008±0.007	0.044/0.164/0.088±0.030	*brevivittata* **sp. nov.**
hylecoeta	9	0.000/0.019/0.011±0.007	0.036/0.164/0.088±0.033	*brevivittata* **sp. nov.**
multicardua	1	NA	0.147/0.182/0.160±0.006	*curvinervis*
singularis	2	0.001	0.121/0.164/0.137±0.011	*hylecoeta*, *brevivittata* **sp. nov.**
aini **sp. nov.**	2	0.000	0.065/0.160/0.097±0.025	*hirsutina*
brevivittata **sp. nov.**	7	0.000/0.012/0.005±0.004	0.036/0.156/0.082±0.034	*hylecoeta*
cuodi **sp. nov.**	6	0.000/0.001/0.001±0.001	0.055/0.166/0.100±0.033	*brevivittata* **sp. nov.**
fuscofemorata **sp. nov.**	3	0.001/0.006/0.004±0.002	0.105/0.157/0.120±0.012	*curvinervis*
latipalpula **sp. nov.**	1	NA	0.123/0.152/0.135±0.009	*hirsutina*, *singularis*, *brevivittata* **sp. nov.**
macrostephana **sp. nov.**	4	0.000/0.004/0.002±0.002	0.099/0.166/0.146±0.012	*pinguitia* **sp. nov.**
mohnihei **sp. nov.**	4	0.000/0.009/0.004±0.005	0.056/0.160/0.097±0.026	*flavicauda*
obscurala **sp. nov.**	2	0.001	0.089/0.182/0.113±0.016	*hirsutina*
pinguitia **sp. nov.**	2	0.003	0.099/0.155/0.131±0.011	*macrostephana* **sp. nov.**

Table 3. Divergences in the *ND2* sequences. The divergences were calculated among and within all 15 Asian *Stegana* (*Orthostegana*) species from the 64 sampled specimens. Notes: Min. (Minimum), intra. (intraspecific), Max. (Maximum), Vari. (variability), inter. (interspecific), NA (not applicable).

Species	Number of sequence(s)	Min. intra. / Max. intra. / Mean intra. ± Vari.	Min. inter. / Max. inter. / Mean inter. ± Vari.	Species with Min. inter.
curvinervis	2	0.009	0.084/0.238/0.115±0.036	*mohnihei* **sp. nov.**
flavicauda	11	0.000/0.038/0.021±0.011	0.049/0.232/0.111±0.038	*mohnihei* **sp. nov.**
hirsutina	8	0.001/0.017/0.010±0.006	0.054/0.229/0.101±0.044	*hylecoeta*
hylecoeta	9	0.000/0.008/0.004±0.002	0.031/0.216/0.086±0.045	*brevivittata* **sp. nov.**
multicardua	1	NA	0.176/0.289/0.226±0.020	*latipalpula* **sp. nov.**
singularis	2	0.002	0.104/0.207/0.165±0.018	*latipalpula* **sp. nov.**
aini **sp. nov.**	2	0.000	0.061/0.236/0.102±0.043	*brevivittata* **sp. nov.**
brevivittata **sp. nov.**	7	0.000/0.019/0.008±0.008	0.031/0.220/0.088±0.045	*hylecoeta*
cuodi **sp. nov.**	6	0.000/0.002/0.001±0.001	0.033/0.216/0.090±0.046	*hylecoeta*
fuscofemarata **sp. nov.**	3	0.002/0.004/0.003±0.001	0.093/0.240/0.125±0.031	*mohnihei* **sp. nov.**
latipalpula **sp. nov.**	1	NA	0.104/0.190/0.143±0.021	*singularis*
macrostephana **sp. nov.**	4	0.001/0.003/0.002±0.001	0.138/0.289/0.190±0.019	*pinguitia* **sp. nov.**
mohnihei **sp. nov.**	4	0.001/0.016/0.008±0.007	0.049/0.220/0.094±0.040	*flavicauda*
obscurala **sp. nov.**	2	0.011	0.116/0.257/0.144±0.032	*hylecoeta*
pinguitia **sp. nov.**	2	0.002	0.136/0.236/0.155±0.017	*hylecoeta*

corresponding to *S.* (*O.*) *macrostephana* **sp. nov.** + *S.* (*O.*) *pinguitia* **sp. nov.** (PP = 1.00 and UFB = 100) evolved extremely bushy, long hair on the terminal of aedeagus (Figs 17, 20), and was further sister to the largest clade including the remaining 10 *Orthostegana* species (PP = 1.00 and UFB = 100). Although they have high morphological similarity in male genitalia, each of them was strongly supported as a monophyletic clade (PPs = 1.00, UFBs ≥ 99) (Fig. 2).

The ABGD analyses based on the *COI* and *ND2* sequences from the 64 sampled *Orthostegana* specimens identified 15 and 16 MOTUs, respectively (Fig. 2). As the only inconsistency, *S.* (*O.*) *flavicauda* **sp. nov.** was split into two MOTUs in the *ND2* sequences. Both the mPTP analyses based on the same sequences identified 14 MOTUs (Fig. 2). *S.* (*O.*) *cuodi* **sp. nov.** and *S.* (*O.*) *hylecoeta* were inferred as a single MOTU in the *COI* mPTP analysis, whereas *S.* (*O.*) *multicardua* and *S.* (*O.*) *latipalpula* **sp. nov.** were inferred as a single MOTU in the *ND2* mPTP analysis (Fig. 2). The BP&P results using three different gamma (G) prior combinations all reached a consensus that the presence of 15 MOTUs in the 64 sampled *Orthostegana* specimens, was also consistent with our morphologically identified results (Fig. 2).

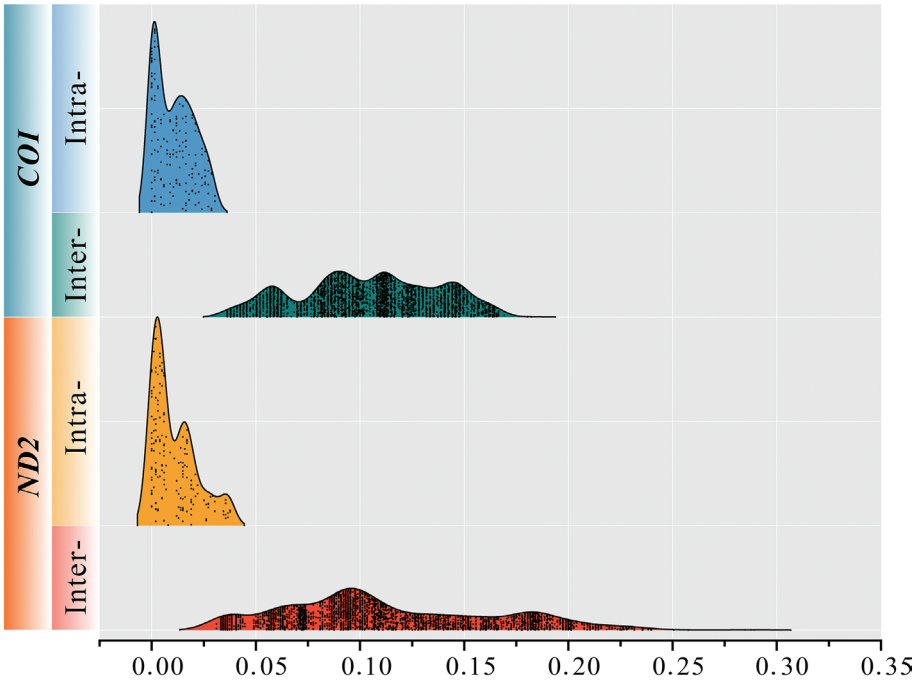

Figure 3. Distribution of intra- and interspecific pairwise genetic *p*-distances. The distances were calculated based on the *COI* and *ND2* sequences of all 15 Asian *Stegana* (*Orthostegana*) species from 64 sampled specimens, respectively.

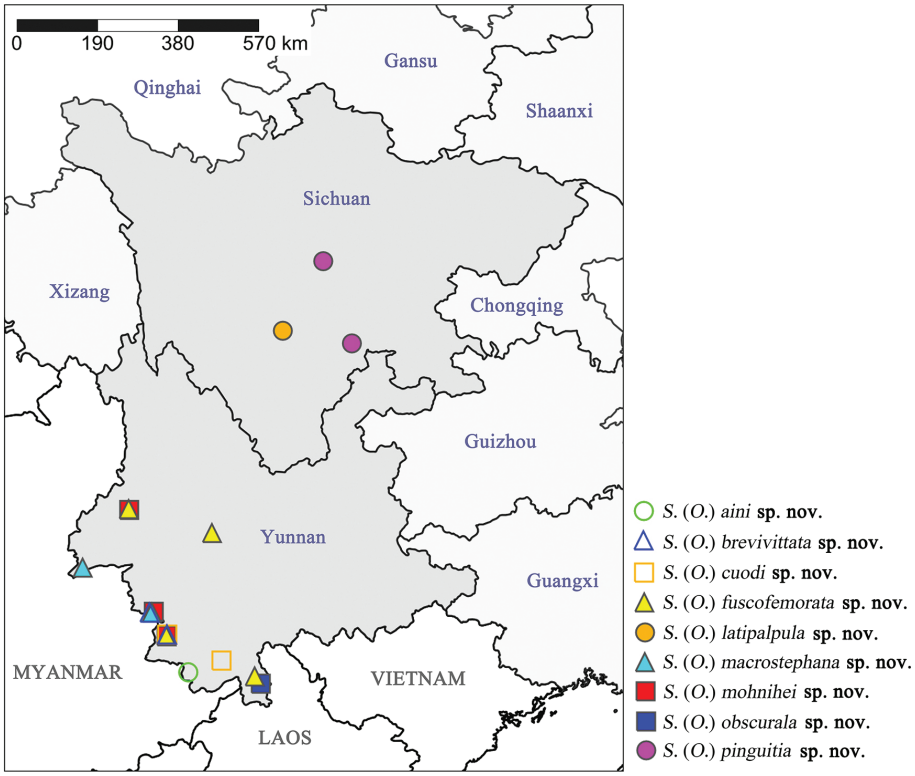

Figure 4. Geographical distribution of the nine *Stegana* (*Orthostegana*) new species.

Therefore, these 15 consensus MOTUs were confirmed as valid species in this study.

As a supplement to the results of molecular species delimitation, the distributions of pairwise intra- and interspecific distances of the 15 putative *Orthostegana* species were depicted in Fig. 3. Overall, the intraspecific distances ranged from 0.000 to 0.030 for *COI* and 0.000 to 0.038 for *ND2*; the interspecific distances ranged from 0.036 to 0.182 for *COI* and 0.031 to 0.298 for *ND2* (Tables 2, 3). Thus, the *COI* sequences presented an indistinct "barcoding gap", whereas no "barcoding gap" was found in the *ND2* sequences.

Collectively, a total of nine valid species from Southwest China (Fig. 4) were designated as valid new species based on the integrative results of molecular species delimitation and morphology, including *Stegana* (*Orthostegana*) *aini* Peng & Chen **sp. nov.**, *S.* (*O.*) *brevivittata* Peng & Chen **sp. nov.**, *S.* (*O.*) *cuodi* Peng & Chen **sp. nov.**, *S.* (*O.*) *fuscofemorata* Peng & Chen **sp. nov.**, *S.* (*O.*) *latipalpula* Peng & Chen **sp. nov.**, *S.* (*O.*) *macrostephana*

Peng & Chen **sp. nov.**, *S.* (*O.*) *mohnihei* Peng & Chen **sp. nov.**, *S.* (*O.*) *obscurala* Peng & Chen **sp. nov.**, and *S.* (*O.*) *pinguitia* Peng & Chen **sp. nov.**

3.2. Taxonomy

Most *Orthostegana* species possess similar morphological characters (Figs 5–8) and living habitats (Figs 1, 4). The morphological similarity in external genital structures mainly occurs in pregonites and aedeagus (Vilela and Bächli 2020; Zhang et al. 2012). In addition, some morphological characters, such as the numbers and layout patterns of the black, apically blunt, stout spines on the underside of mesotarsus are variable within this subgenus (Figs 9–11, Table 4), and may contribute to the rapid species identification in morphology.

Stegana (subgen. *Orthostegana*) Hendel

Orthostegana Hendel, 1913: 631. Type species: *Orthostegana acutangula* Hendel, 1913: 632.
Stegana (*Orthostegana*) Wheeler, 1960: 109; Zhang et al., 2012: 363; Vilela and Bächli, 2020.
Stegana (*Anastega*) Sidorenko, 2002: 14.

Diagnosis. Midleg tibia basally usually with 4 or 5 strong, erect setae (longer than width of corresponding tibia) on dorsal surface; abdominal tergites mostly dark brown to black except for yellow along lateral margins; aedeagus usually with hairs apically (Figs 12–15, 17–20) (modified from Zhang et al. 2012).

3.2.1. New species descriptions

3.2.1.1. *Stegana* (*Orthostegana*) *aini* Peng & Chen sp. nov.

https://zoobank.org/7A71D0C6-DF96-4442-999B-EC0AF4411096

Figs 5A–D, 9G, 12

Diagnosis. This species closely resembles *S.* (*O.*) *obscurala* **sp. nov.** in the shape of both male and female terminalia (Figs 12, 19), but can be distinguished from the latter by having the pleura mostly yellow, lacking distinct longitudinal stripe above (Fig. 5B, D); the mesotarsus ventrally with 2 rows of black, apically blunt, stout spines; metatarsus with 15/2+1 spines; 2nd to 4th segments with 5/1, 1/1, and 2/1 spines, respectively (Fig. 9G); pregonite with *ca*. 5 sensilla distally (Fig. 12C, D).

Description. Male and female. ***Head*****:** Ocellar triangle dark brown, with 2 small setae posterior to ocellar setae. Postocellar seta absent. Frons brown, with numerous minute interfrontal setulae. Pedicel and first flagellomere yellow, with black pubescence. Face yellow. Gena yellow, with brown stripes. Clypeus mostly yellow. Palpus yellow. ***Thorax*****:** Mesonotum mostly yellow to brownish yellow (Fig. 5A, C). Scutellum yellow in males (Fig. 5A), brownish in females (Fig. 5C). Halter mostly brownish, with a yellow patch at knob (Fig. 5A–D). Legs yellow (Fig. 9G). ***Abdominal tergites*****:** brown to dark brown. ***Male terminalia*****:** Epandrium mostly pubescent except for anterior and ventral margins, with *ca*. 30 setae on dorsal to posterior portions per side (Fig. 12A). Surstylus separated from epandrium, with *ca*. 4 long and 19 short peg-shaped prensisetae and some setae near posteroventral margin on inner surface (Fig. 12B). Pregonites separated and small, slightly expanded distally (Fig. 12C, D). Aedeagus pubescent distally, with some hairs apically (Fig. 12C, D). ***Female terminalia*****:** Eighth sternite with 3 rows of orderly and asymmetrical peg-shaped prensisetae subposteromedially and posterolaterally, and with 3 sensilla distally per side (Fig. 12E).

Measurements and indices. BL = 3.40 mm in holotype (range in 3♂ and 5♀ paratypes: 2.80–3.87 in ♂, 3.13–4.20 in ♀), **ThL** = 1.67 mm (1.60–1.80 in ♂, 1.60–1.80 in ♀), **WL** = 3.40 mm (2.80–4.13 in ♂, 3.13–4.00 in ♀), **WW** = 1.33 mm (1.20–1.53 in ♂, 1.20–1.40 in ♀), **arb** = 4/3 (4–5/2–3), **avd** = 0.71 (0.60–1.00), **adf** = 1.71 (1.33–2.25), **flw** = 1.50 (1.50–2.75), **FW/HW** = 0.37 (0.33–0.40), **ch/o** = 0.09 (0.07–0.13), **prorb** = 0.82 (0.63–0.89), **rcorb** = 0.59 (0.37–0.63), **vb** = 0.67 (0.67–0.88), **dcl** = 0.30 (0.32–0.40), **presctl** = 0.46 (0.36–0.50), **sctl** = 1.07 (0.86–1.35), **sterno** = 0.53 (0.43–1.00), **orbito** = 1.80 (2.00–2.50), **dcp** = 0.23 (0.25–0.36), **sctlp** = 1.10 (1.10–1.44), **C** = 2.40 (1.28–3.00), **4c** = 0.83 (0.75–1.64), **4v** = 1.67 (1.45–2.23), **5x** = 1.00 (0.60–1.25), **ac** = 5.40 (5.00–7.20), **M** = 0.42 (0.25–0.42), **C3F** = 0.60 (0.60–1.25).

Distribution. China (Yunnan).

Type material examined. Holotype: ♂ (SCAU, No. 112183), CHINA: Hesong, Menghai, Yunnan, 21°50′17″N, 100°05′48″E, altitude 1600 m, 11.v.2012, swept over tussock, H.W. Chen. — **Paratypes:** 3♂, 13♀ (SCAU, Nos. 112184–99), altitudes 1600–1900 m, 6–11.v.2012, other data same as the holotype.

Etymology. From the word "*aini*" of the Aini nationality living in Yunnan, China.

3.2.1.2. *Stegana* (*Orthostegana*) *brevivittata* Peng & Chen sp. nov.

https://zoobank.org/46EBFCC3-6F35-4679-92D5-3A9B8EBDD869

Figs 5E–H, 9H, 13

Diagnosis. This species closely resembles *S.* (*O.*) *cuodi* **sp. nov.** in the shape of male terminalia (Figs 13, 14), but can be distinguished from the latter by having the mesotarsus ventrally with 2 rows of black, apically blunt, stout spines; metatarsus with 14/3+1 spines; 2nd

Table 4. Diagnostic external morphological characters for all 20 *Stegana* (*Orthostegana*) species. Notes: slash (/), unknown from the original descriptions; Fig. 11B showed the expanded clypeus and Fig. 11C showed the expanded palpus.

Species	Postocellar seta(e)	Clypeus	Palpus	A longitudinal stripe on pleura	Number of posterodorsal seta(e) on midleg tibia	Number and layout pattern of black, apically blunt, stout spine(s) on underside of mesotarsus			
						Metatarsus	Segment 2nd	Segment 3rd	Segment 4th
acutangula	Present	Normal	Normal	Absent	2–4 (most 3)	/	/	/	/
curvinervis	Absent	Normal	Normal	Present	4–5	14/2+1	3/1	2/1	2/1
dudai	Present	Normal	Normal	Absent	3	/	/	/	/
flavicauda	Absent	Normal	Normal	Absent	4–5	14/4+1	3/1	1/1	2/1
hirsutina	Absent	Expanded	Normal	Present	3	12/1+1	6/1	2/1	2/1
hylecoeta	Absent	Normal	Normal	Present	3	16/4+1	4/1	2/1	2/1
multicardua	Absent	Normal	Normal	Present	3–4	15/6+1	5/1/1	4/1/1	4/1/1
singularis	Present	Normal	Normal	Present	5	7+1/15	1/1	1/1	1/1
triseta	Present	Normal	Normal	Present	3	/	/	/	/
turrialba	Present	Normal	Normal	/	/	/	/	/	/
yasuni	Present	Normal	Normal	/	/	/	/	/	/
aini **sp. nov.**	Absent	Normal	Normal	Absent	3	15/2+1	5/1	1/1	2/1
brevivittata **sp. nov.**	Absent	Normal	Normal	Present	3	14/3+1	5/1	2/1	2/1
cuodi **sp. nov.**	Absent	Normal	Normal	Present	3	12/1+1	2/1	2/1	2/1
fuscofemorata **sp. nov.**	Absent	Normal	Normal	Present	3	12/3+1	3/1	2/1	2/1
latipalpula **sp. nov.**	Present	Normal	Expanded	Present	3	19/6+1	1/1/1	2/1/1	1/1/1
macrostephana **sp. nov.**	Absent	Normal	Normal	Present	3	15/3+1	6/1	4/1	3/1
mohnihei **sp. nov.**	Absent	Normal	Normal	Present	3	14/1+1	2/1	1/1	2/1
obscurala **sp. nov.**	Absent	Normal	Normal	Present	3	12/6+1	6/1	3/1	2/1
pinguitia **sp. nov.**	Absent	Normal	Normal	Present	3	12/1/7+1	5/1	3/1	2/1

to 4th segments with 5/1, 2/1, and 2/1 spines, respectively (Fig. 9H); pregonite with *ca*. 3 sensilla distally (Fig. 13C, D).

Description. Male. ***Head*****:** Ocellar triangle brown, with 2 small setae posterior to ocellar setae. Postocellar seta absent. Frons brownish yellow, with numerous minute interfrontal setulae. Pedicel yellow; first flagellomere dark brown distally, with black pubescence. Face yellow. Gena brownish yellow. Clypeus dark brown medially and brownish yellow laterally. Palpus yellow. ***Thorax*****:** Mesonotum yellow (Fig. 5E, G). Pleura with a distinct brown to dark brown longitudinal stripe above (Fig. 5F, H). Scutellum mostly yellow (Fig. 5E, G). Halter mostly brownish, with a yellow patch at knob (Fig. 5E–H). Legs yellowish (Fig. 9H). ***Abdominal tergites*****:** brownish yellow to brownish. ***Male terminalia*****:** Epandrium mostly pubescent except for anterior margin, with *ca*. 19 setae on dorsal to posterior portions per side (Fig. 13A). Surstylus separated from epandrium, narrowed dorsally, with *ca*. 14 long and 8 short peg-shaped prensisetae near anterodorsal margin on inner surface and a few setae ventrally (Fig. 13B). Pregonites fused basally (Fig. 13C, D). Aedeagus pubescent distally, with some hairs medioventrally and apically (Fig. 13C, D).

Measurements and indices. BL = 2.77 mm in holotype (range in 5♂ paratypes: 2.53–3.07), **ThL** = 1.10 mm (1.07–1.20), **WL** = 2.03 mm (2.03–2.30), **WW** = 0.97 mm (0.87–1.17), **arb** = 5/4 (5–9/3–6), **avd** = 0.92 (0.75–0.92), **adf** = 2.00 (1.67–2.00), **flw** = 2.17 (1.83–2.00), **FW/HW** = 0.39 (0.38–0.49), **ch/o** = 0.12 (0.09–0.13), **prorb** = 1.00 (0.80–1.23), **rcorb** = 0.67 (0.55–0.79), **vb** = 0.67 (0.44–0.58), **dcl** = 0.46 (0.32–0.61), **presctl** = 0.42 (0.32–0.44), **sctl** = damaged (1.26–1.45), **sterno** = 063 (0.59–0.81), **orbito** = 1.00 (1.33–2.25), **dcp** = 0.25 (0.26–0.32), **sctlp** = 1.00 (0.79–1.20), **C** = 2.06 (2.19–2.86), **4c** = 0.94 (0.78–0.84), **4v** = 1.67 (1.53–1.76), **5x** = 1.33 (0.93–1.40), **ac** = 11.33 (7.00–13.33), **M** = 0.44 (0.33–0.44), **C3F** = 0.76 (0.76–0.82).

Distribution. China (Yunnan).

Type material examined. Holotype: ♂ (SCAU, No. 112200), China: Muyiji Park, Ximeng, Yunnan, 22°37′15″N, 99°35′42″E, altitude 1203 m, 29.iv.2016, swept from tree trunk, J. Huang. **Paratypes:** 3♂ (SCAU, Nos. 112201–03), Y.Q. Liu, Y.L. Wang, L. Zhu, other data same as the holotype; 2♂ (SCAU, Nos. 112204, 05), Likan, Ximeng, Yunnan, 22°39′21″N, 99°36′28″E, altitude 844 m, 1.v.2016, swept from tussocks, J. Huang; 2♀ (SCAU, Nos. 112206, 07), Mengdong, Cangyuan, Yunnan, 23°10′08″N, 99°13′52″E, altitude 1320 m, 6.v.2016, swept from tussocks, Y.Q. Liu, Y.L. Wang; 1♂ (SCAU, No. 112208), Muyiji Park, Ximeng, Yunnan, 22°37′15″N, 99°35′42″E, altitude 1100 m, 16.iv.2018, swept from tussock, Y.L. Wang.

Etymology. A combination of the Latin words "*brevis*" (= short) and "*vittatus*" (= stripe), referring to the pleura with a brown longitudinal stripe above.

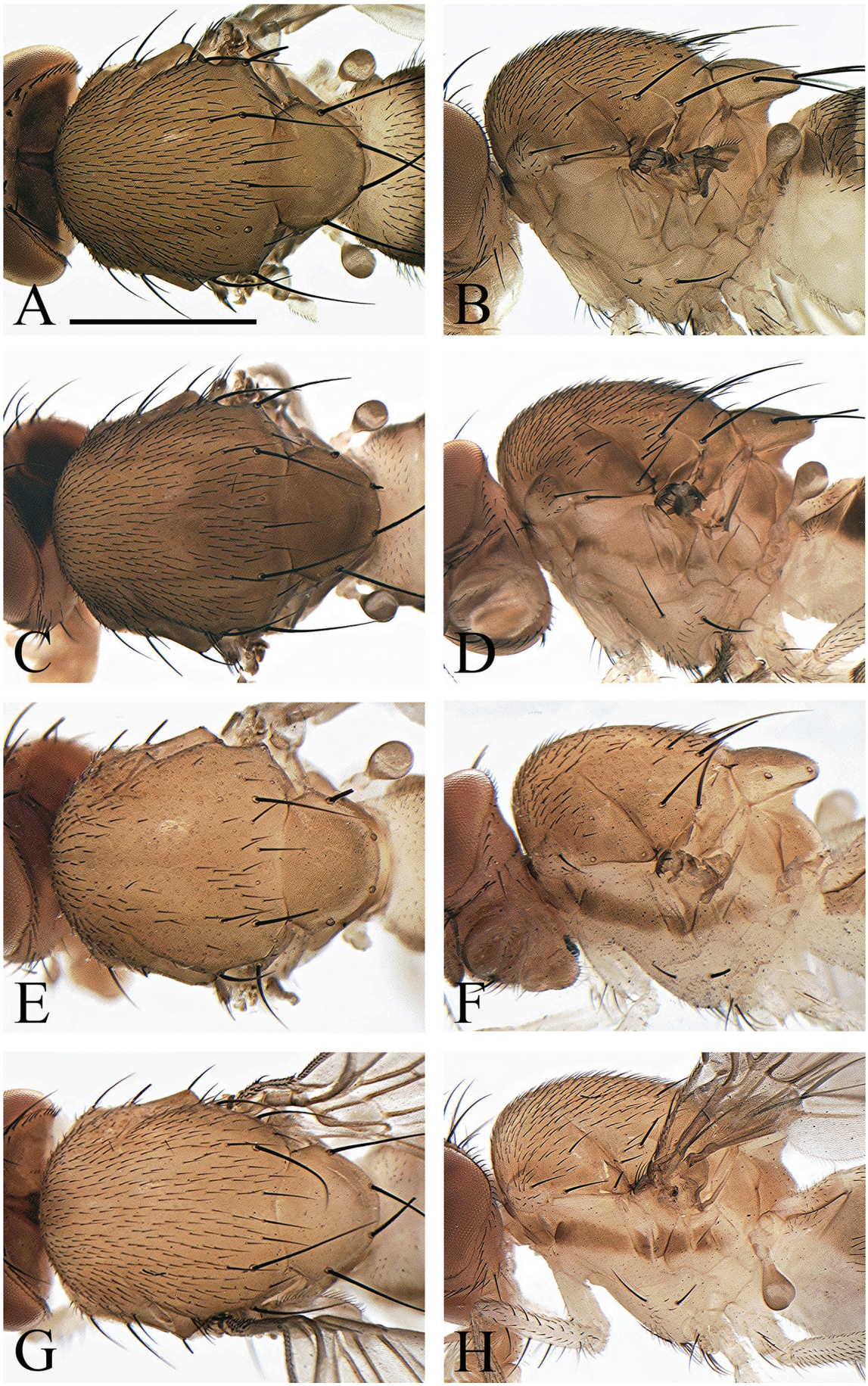

Figure 5. Head, thorax, and abdomen in dorsal and lateral views. **A**, **B** *Stegana* (*Orthostegana*) *aini* Peng & Chen **sp. nov.** (male); **C**, **D** *S.* (*O.*) *aini* Peng & Chen **sp. nov.** (female); **E**, **F** *S.* (*O.*) *brevivittata* Peng & Chen **sp. nov.** (male); **G**, **H** *S.* (*O.*) *brevivittata* Peng & Chen **sp. nov.** (female). Scale bar = 1 mm.

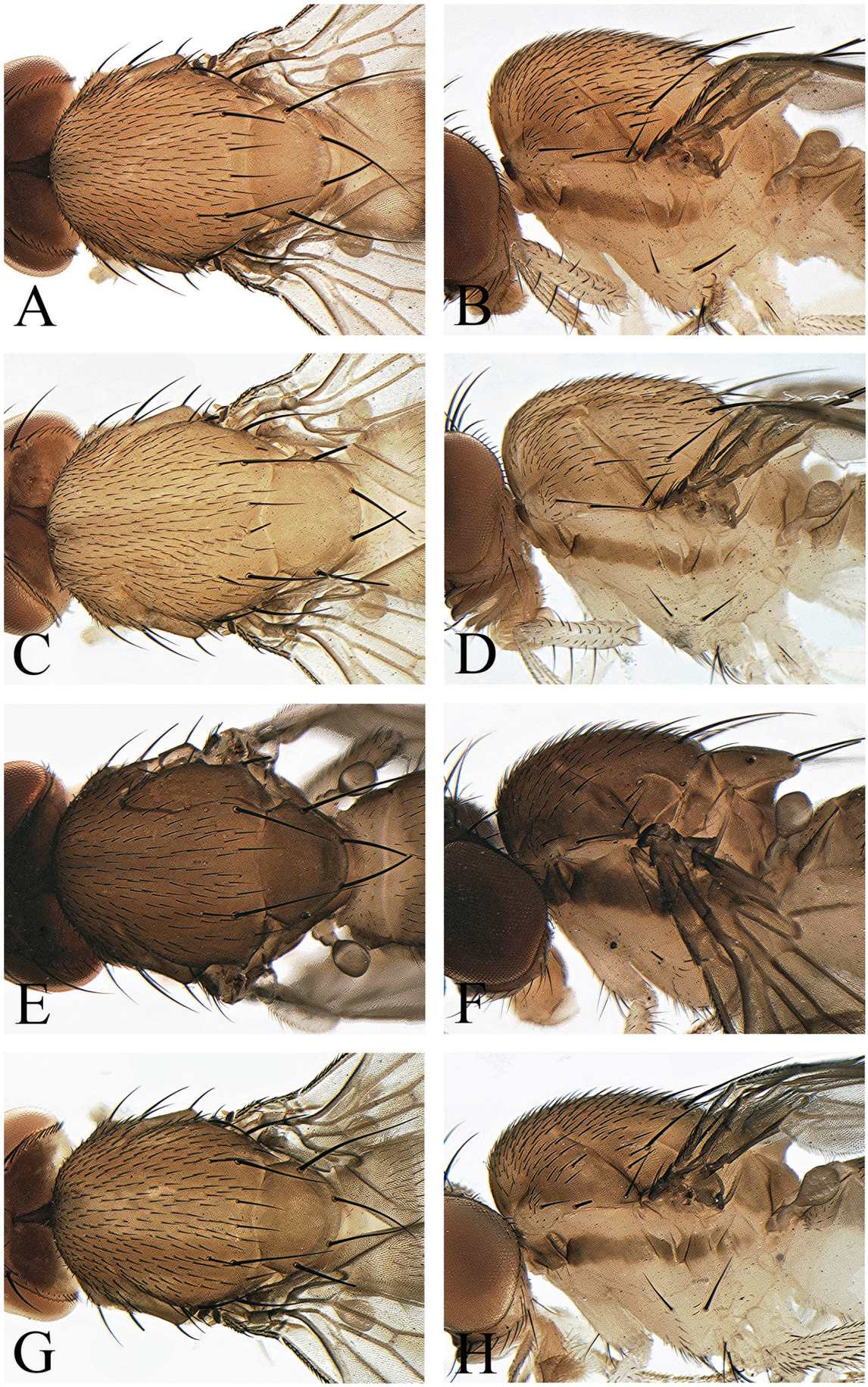

Figure 6. Head, thorax, and abdomen in dorsal and lateral views. **A**, **B** *Stegana* (*Orthostegana*) *cuodi* Peng & Chen **sp. nov.** (male); **C**, **D** *S.* (*O.*) *cuodi* Peng & Chen **sp. nov.** (female); **E**, **F** *S.* (*O.*) *fuscofemorata* Peng & Chen **sp. nov.** (male); **G**, **H** *S.* (*O.*) *fuscofemorata* Peng & Chen **sp. nov.** (female).

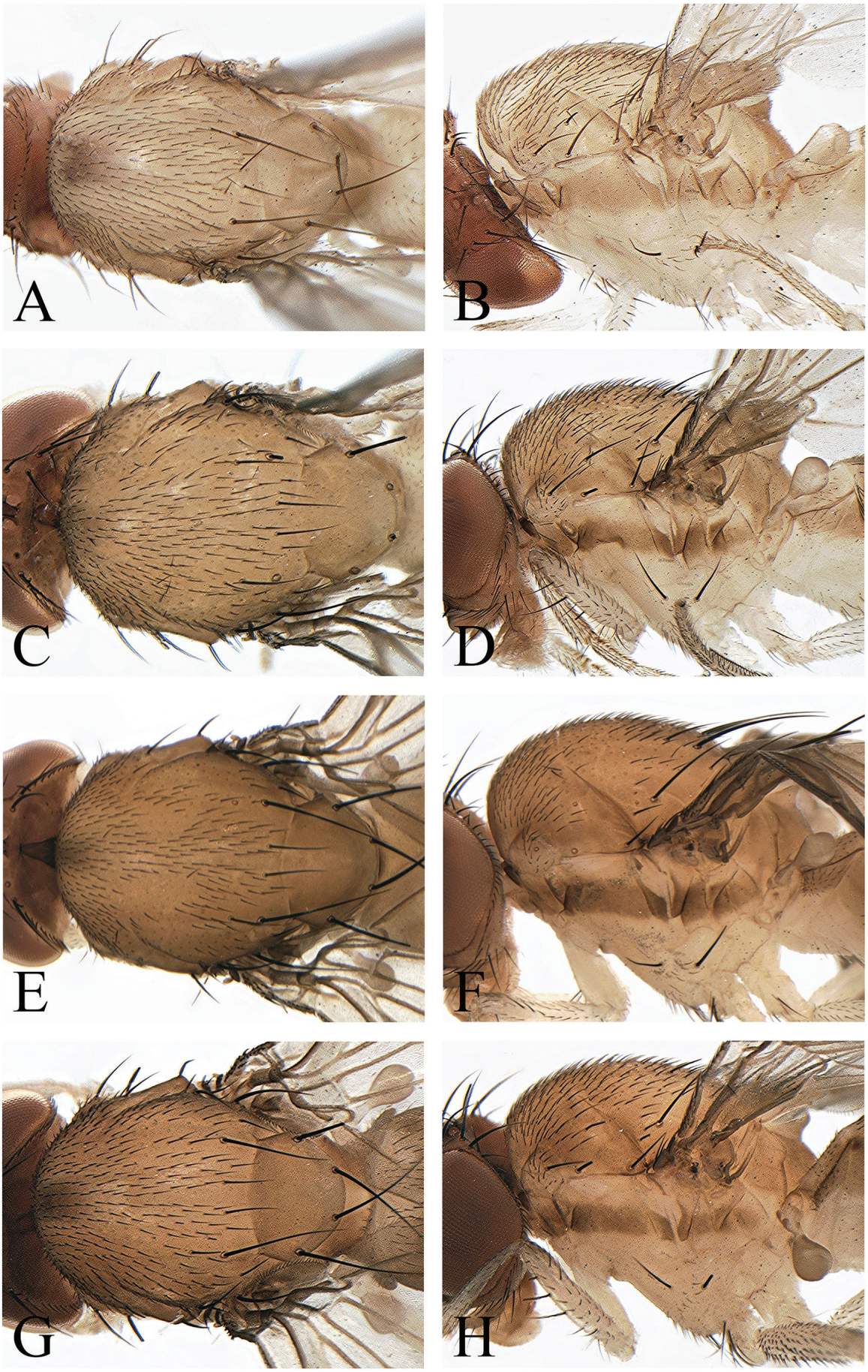

Figure 7. Head, thorax, and abdomen in dorsal and lateral views. **A**, **B** *Stegana* (*Orthostegana*) *latipalpula* Peng & Chen **sp. nov.** (male); **C**, **D** *S.* (*O.*) *macrostephana* Peng & Chen **sp. nov.** (male); **E**, **F** *S.* (*O.*) *macrostephana* Peng & Chen **sp. nov.** (female); **G**, **H** *S.* (*O.*) *mohnihei* Peng & Chen **sp. nov.** (male).

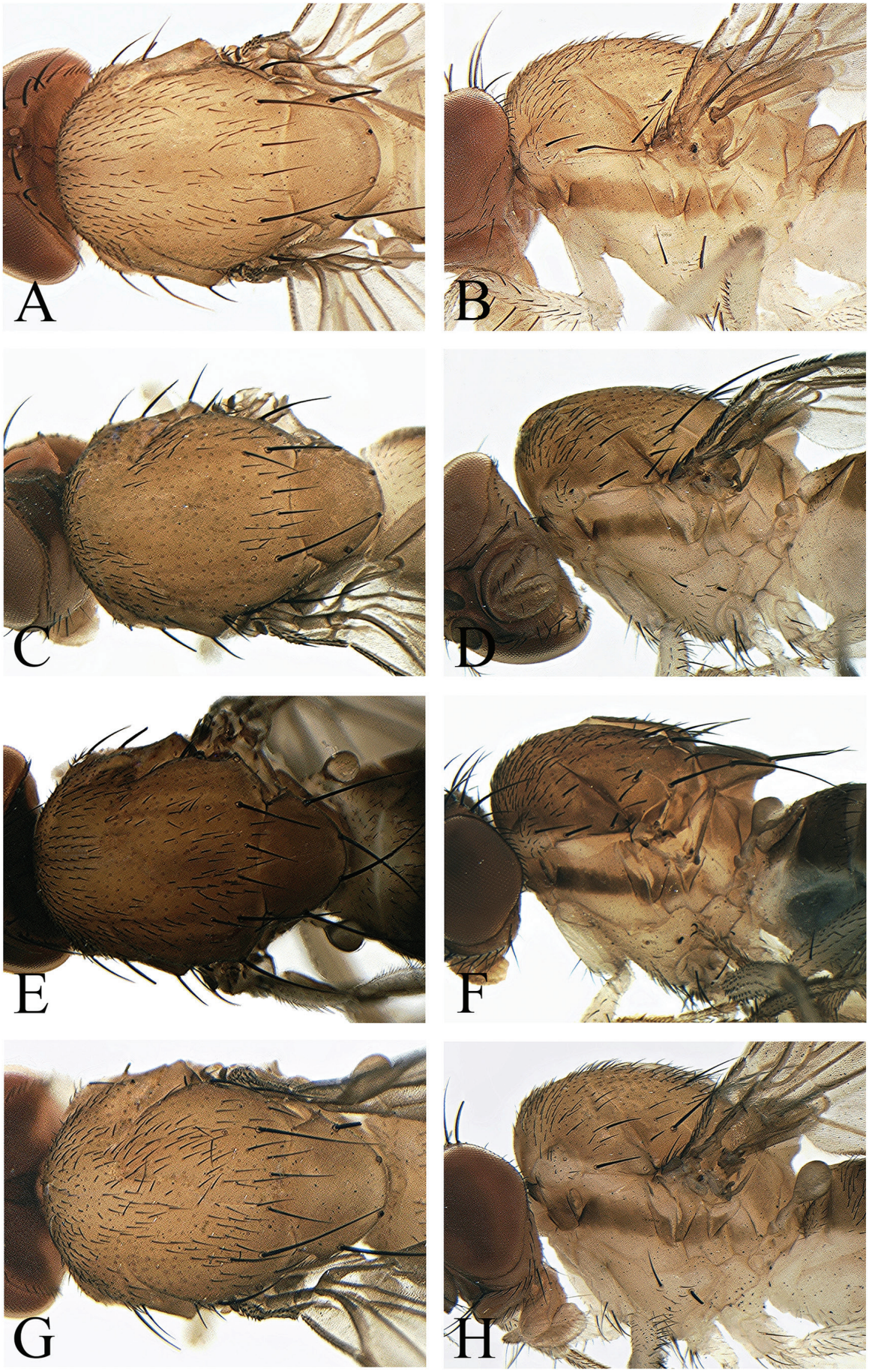

Figure 8. Head, thorax, and abdomen in dorsal and lateral views. **A**, **B** *Stegana* (*Orthostegana*) *mohnihei* Peng & Chen **sp. nov.** (female); **C**, **D** *S.* (*O.*) *obscurala* Peng & Chen **sp. nov.** (male); **E**, **F** *S.* (*O.*) *obscurala* Peng & Chen **sp. nov.** (female); **G**, **H** *S.* (*O.*) *pinguitia* Peng & Chen **sp. nov.** (male).

Figure 9. Mesotarsus of males in ventral view. **A** *Stegana* (*Orthostegana*) *curvinervis* (Hendle, 1914); **B** *S.* (*O.*) *singularis* (Sidorenko, 1990); **C** *S.* (*O.*) *flavicauda* Zhang and Chen, 2012; **D** *S.* (*O.*) *hirsutina* Zhang and Chen, 2012; **E** *S.* (*O.*) *hylecoeta* Zhang and Chen, 2012; **F** *S.* (*O.*) *multicardua* Zhang and Chen, 2012; **G** *S.* (*O.*) *aini* Peng & Chen **sp. nov.**; **H** *S.* (*O.*) *brevivittata* Peng & Chen **sp. nov.**; **I** *S.* (*O.*) *cuodi* Peng & Chen **sp. nov.**; **J** *S.* (*O.*) *fuscofemorata* Peng & Chen **sp. nov.** Scale bar = 0.2 mm.

Figure 10. Mesotarsus of males in ventral view. **A** *Stegana* (*Orthostegana*) *latipalpula* Peng & Chen **sp. nov.**; **B** *S.* (*O.*) *macrostephana* Peng & Chen **sp. nov.**; **C** *S.* (*O.*) *mohnihei* Peng & Chen **sp. nov.**; **D** *S.* (*O.*) *obscurala* Peng & Chen **sp. nov.**; **E** *S.* (*O.*) *pinguitia* Peng & Chen **sp. nov.**

3.2.1.3. *Stegana* (*Orthostegana*) *cuodi* Peng & Chen sp. nov.

https://zoobank.org/9C24B0AD-E0E9-4D9F-8394-2D56913037A6

Figs 6A–D, 9I, 14

Diagnosis. This species closely resembles *S.* (*O.*) *brevivittata* **sp. nov.** in the shape of male terminalia (Figs 13, 14), but can be distinguished from the latter by having the mesotarsus ventrally with 2 rows of black, apically blunt, stout spines; metatarsus with 12/1+1 spines; 2nd to 4th segments with 2/1, 2/1, and 2/1 spines, respectively (Fig. 9I); pregonite with *ca*. 4 sensilla distally (Fig. 14C, D).

Description. Male and female. ***Head*****:** Ocellar triangle dark brown, with 2 small setae posterior to ocellar setae. Postocellar seta absent. Frons brown, with numerous minute interfrontal setulae. Pedicel brownish yellow; first flagellomere dark brown distally, with black pubescence. Face yellow. Gena brownish yellow. Clypeus brownish to dark brown. Palpus brownish yellow. ***Thorax*****:** Mesonotum yellow (Fig. 6A, C). Pleura with a distinct brown longitudinal stripe above (Fig. 6B, D). Scutellum mostly yellow (Fig. 6A, C). Halter mostly brownish, with a yellow patch at knob (Fig. 6A–D). Legs yellowish (Fig. 9I). ***Abdominal tergites*****:** brownish. ***Male terminalia*****:** Epandrium mostly pubescent except for anterior margin, with *ca*. 21 setae on dorsal to posterior portions per side (Fig. 14A). Surstylus separated from epandrium, narrowed dorsally, with *ca*. 14 long and 9 short peg-shaped prensisetae near anterodorsal margin on inner surface, lacking seta (Fig. 14B). Pregonites fused basally (Fig. 14C, D). Aedeagus pubescent distally, with some hairs ventrally and apically (Fig. 14C, D).

Measurements and indices. BL = 3.47 mm in holotype (range in 5♂ and 2♀ paratypes: 2.67–3.43 in ♂, 3.02–3.51 in ♀), **ThL** = 1.44 mm (1.07–1.50 in ♂, 1.33–1.47 in ♀), **WL** = 2.60 mm (2.07–2.67 in ♂, 2.13–2.47 in ♀), **WW** = 1.17 mm (1.00–1.10 in ♂, 1.07 in ♀), **arb** = 8/5 (5–7/3–5), **avd** = 1.00 (0.57–1.11), **adf** = 1.88 (1.67–2.80), **flw** = 2.13 (1.67–2.60), **FW/HW** = 0.44 (0.47–0.54), **ch/o**

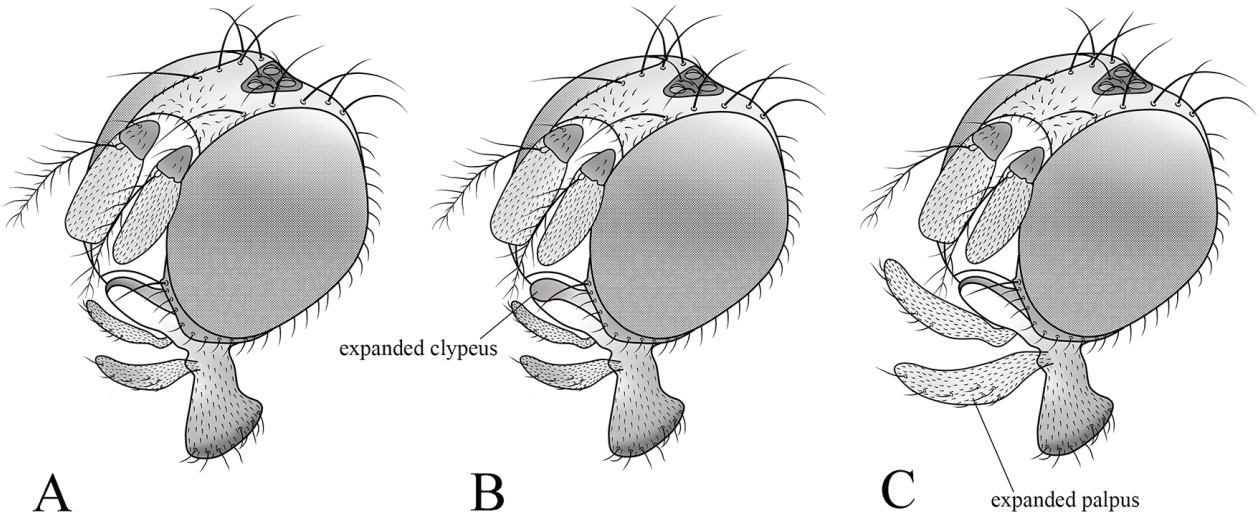

Figure 11. Heads of *Stegana* (*Orthostegana*) species in lateral view. **A** Normal; **B** Clypeus expanded in *S.* (*O.*) *hirsutina* Zhang and Chen, 2012; **C** Palpus expanded in *S.* (*O.*) *latipalpula* Peng & Chen **sp. nov.**

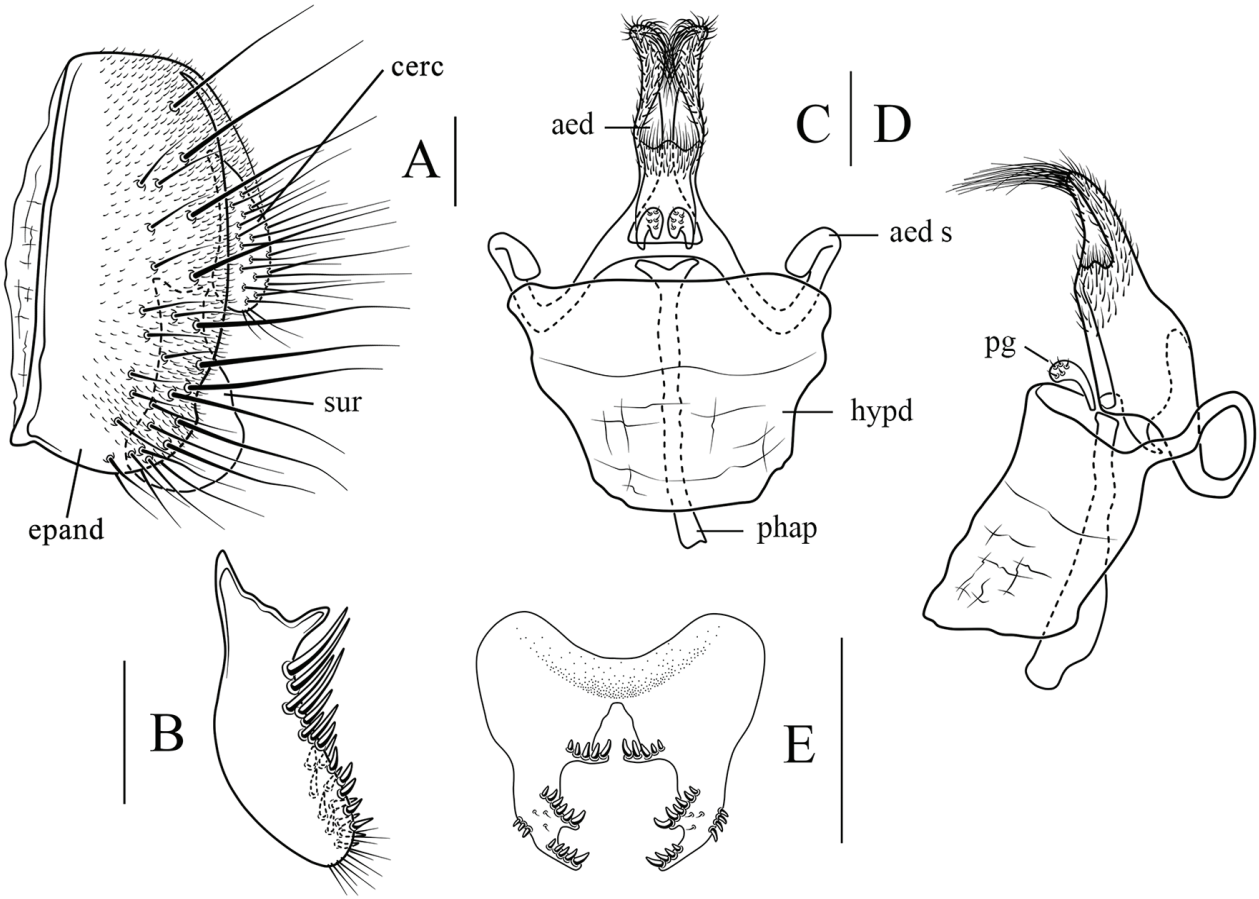

Figure 12. *Stegana* (*Orthostegana*) *aini* Peng & Chen **sp. nov.**, male (**A**–**D**) and female terminalia (**E**). **A** Epandrium, surstylus, and cercus in lateral view; **B** Surstylus in ventral view; **C**, **D** Hypandrium, phallapodeme, pregonite, aedeagus, and aedeagal sheath in ventral and lateral views; **E** Eighth sternite in ventral view. Scale bars = 0.1 mm.

= 0.15 (0.09–0.12), **prorb** = 1.00 (0.72–1.00), **rcorb** = 0.70 (0.56–0.81), **vb** = 0.62 (0.50–0.60), **dcl** = damaged (0.31–0.47), **presctl** = damaged (0.42–0.47), **sctl** = damaged (1.14–1.30), **sterno** = 0.81 (0.63–0.86), **orbito** = 2.00 (1.60–2.00), **dcp** = 0.29 (0.21–0.29), **sctlp** = 0.92 (0.80–1.20), **C** = 2.25 (2.16–3.40), **4c** = 0.91 (0.74–0.94), **4v** = 1.68 (1.45–1.89), **5x** = 1.13 (1.00–1.60), **ac** = 10.00 (8.00–11.33), **M** = 0.41 (0.35–0.44), **C3F** = 0.80 (0.56–0.82).

Distribution. China (Yunnan).

Type material examined. Holotype: ♂ (SCAU, No. 112209), China: Guanlei, Mengla, Yunnan, 21°38′39″N, 101°09′52″E, altitude 562 m,

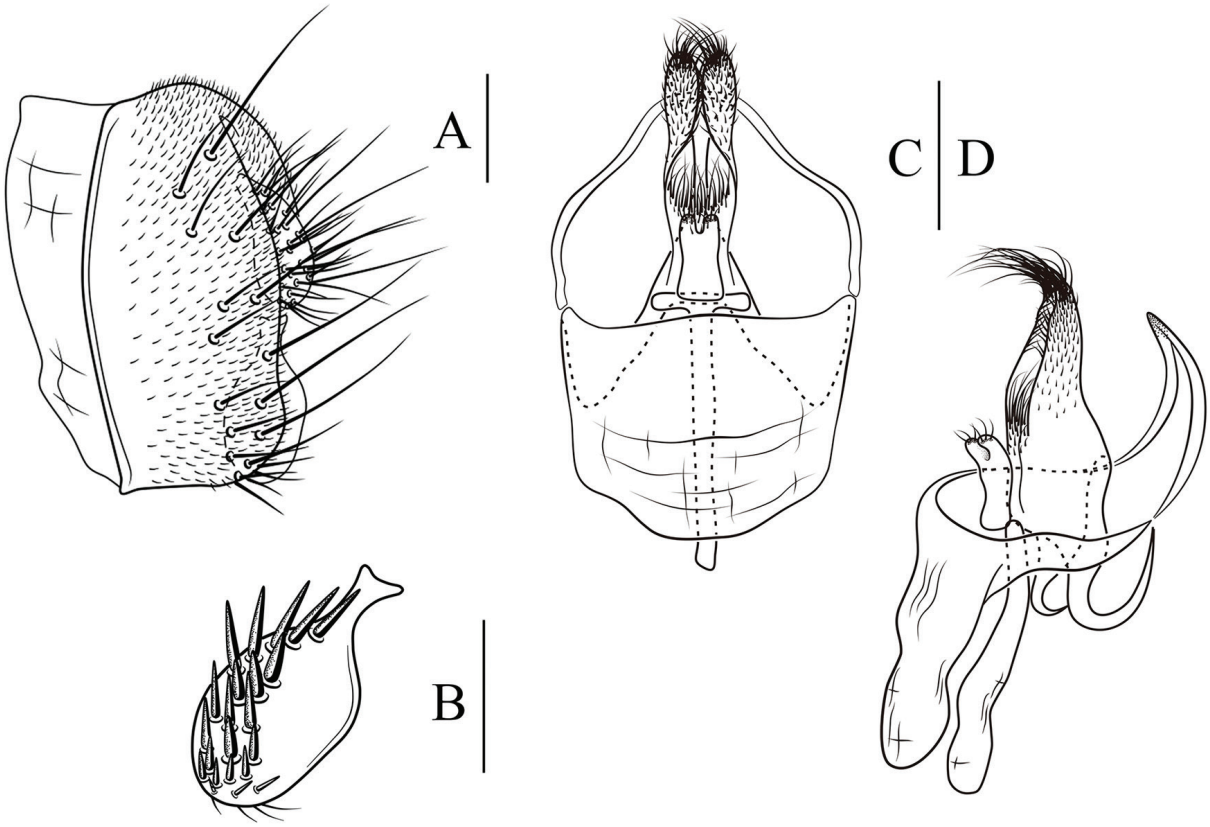

Figure 13. *Stegana* (*Orthostegana*) *brevivittata* Peng & Chen **sp. nov.**, male terminalia. **A** Epandrium, surstylus, and cercus in lateral view; **B** Surstylus in ventral view; **C**, **D** Hypandrium, phallapodeme, pregonite, aedeagus, and aedeagal sheath in ventral and lateral views. Scale bars = 0.1 mm.

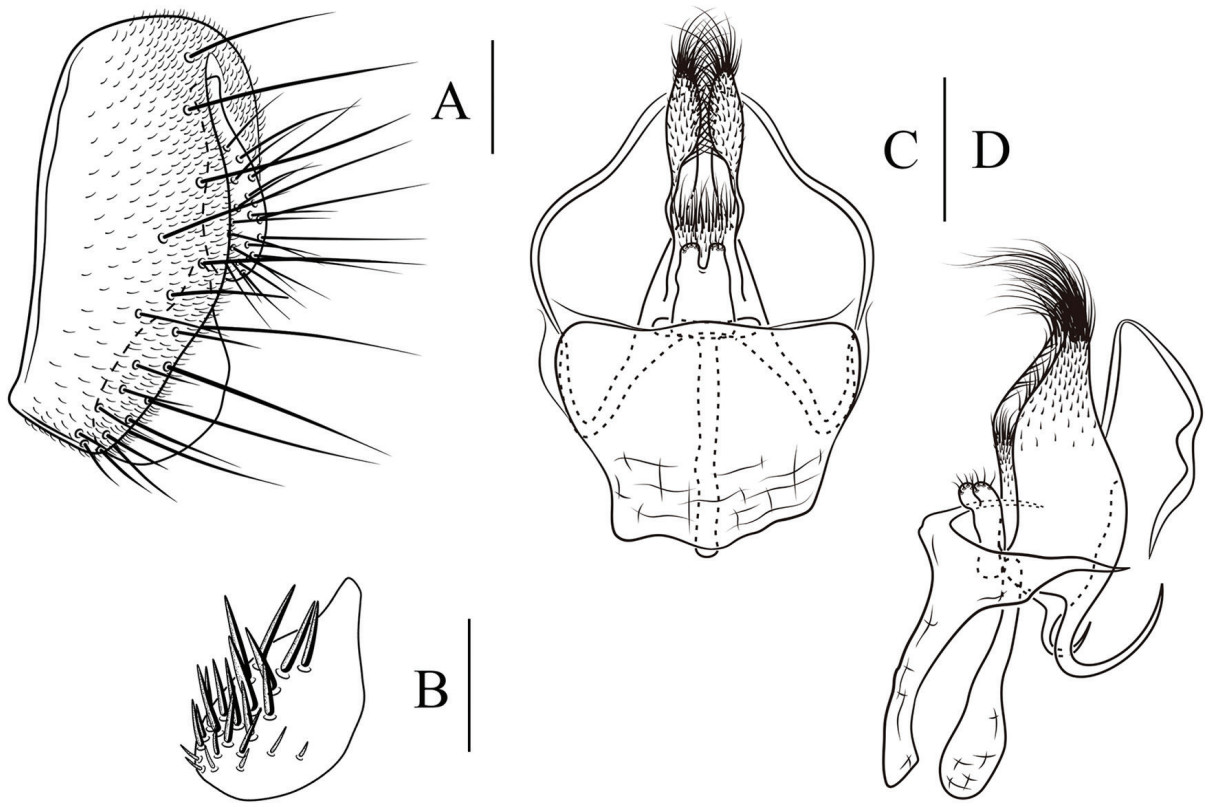

Figure 14. *Stegana* (*Orthostegana*) *cuodi* Peng & Chen **sp. nov.**, male terminalia. **A** Epandrium, surstylus, and cercus in lateral view; **B** Surstylus in ventral view; **C**, **D** Hypandrium, phallapodeme, pregonite, aedeagus, and aedeagal sheath in ventral and lateral views. Scale bars = 0.1 mm.

20.iv.2016, swept from tree trunk, Y.Q. Liu. **Paratypes***:* 2♂ (SCAU, Nos. 112210, 11), J. Huang, Y.Q. Liu, other data same as the holotype; 2♀ (SCAU, Nos. 112212, 13), Likan, Ximeng, Yunnan, 22°39′21″N, 99°36′28″E, altitude 840 m, 1.v.2016, swept from tussocks, J. Huang; 8♂ (SCAU, Nos. 112214–21), Guanlei, Mengla, Yunnan, 21°38′39″N, 101°09′52″E, altitude 560 m, 20.iv.2016, swept from tussocks, Y.Q. Liu, J. Huang.

Etymology. From the word "*cuodi*" in Dai language from the Dai nationality living in Yunnan, China, referring to the meaning "good luck".

3.2.1.4. *Stegana* (*Orthostegana*) *fuscofemorata* Peng & Chen sp. nov.

https://zoobank.org/D4FD7C40-8F9A-427A-8A08-4A8DDDCE2F3F

Figs 6E–H, 9J, 15

Diagnosis. This species closely resembles *S.* (*O.*) *brevivittata* **sp. nov.** in the shape of male terminalia (Figs 13, 15), but can be distinguished from the latter by having the mesotarsus ventrally with 2 rows of black, apically blunt, stout spines; metatarsus with 12/3+1 spines; 2nd to 4th segments with 3/1, 2/1, and 2/1 spines, respectively (Fig. 9J). Surstylus with some setae near ventral margin on inner surface (Fig. 15B).

Description. Male and female. ***Head*****:** Ocellar triangle brown to dark brown, with 2 small setae posterior to ocellar setae. Postocellar seta absent. Frons brownish yellow to brown, with numerous minute interfrontal setulae. Pedicel yellow; first flagellomere dark brown distally, with black pubescence. Face and gena brown to dark brown. Clypeus dark brown medially and brownish yellow laterally. Palpus brown. ***Thorax*****:** Mesonotum brownish yellow to brown (Fig. 6E, G). Pleura with a distinct brown to dark brown longitudinal stripe above (Fig. 6F, H). Scutellum mostly brownish yellow to brown (Fig. 6E, G). Halter mostly brownish, with a yellow patch at knob (Fig. 6E–H). Legs yellowish (Fig. 9J). ***Abdominal tergites*****:** brownish to brown. ***Male terminalia*****:** Epandrium mostly pubescent except for anterior margin, with *ca*. 16 setae on dorsal to posterior portions per side (Fig. 15A). Surstylus separated from epandrium, narrowed dorsally, with *ca*. 14 long peg-shaped prensisetae near anterodorsal margin on inner surface (Fig. 15B). Pregonites fused basally, with *ca*. 3 sensilla distally per side (Fig. 15C, D). Aedeagus pubescent distally, with some hairs ventrally and apically (Fig. 15C, D).

Measurements and indices. BL = 3.20 mm in holotype (range in 4♂ and 3♀ paratypes: 3.00–3.13 in ♂, 3.00–3.10 in ♀), **ThL** = 1.39 mm (1.27–1.30 in ♂, 1.20–1.27 in ♀), **WL** = 2.20 mm (2.17–2.30 in ♂, 2.30–2.53 in ♀), **WW** = 1.03 mm (1.03–1.07 in ♂, 1.03–1.23 in ♀), **arb** = 7/4 (6–7/4–5), **avd** = 0.79 (0.80–0.85), **adf** = 2.00 (1.71–2.17),

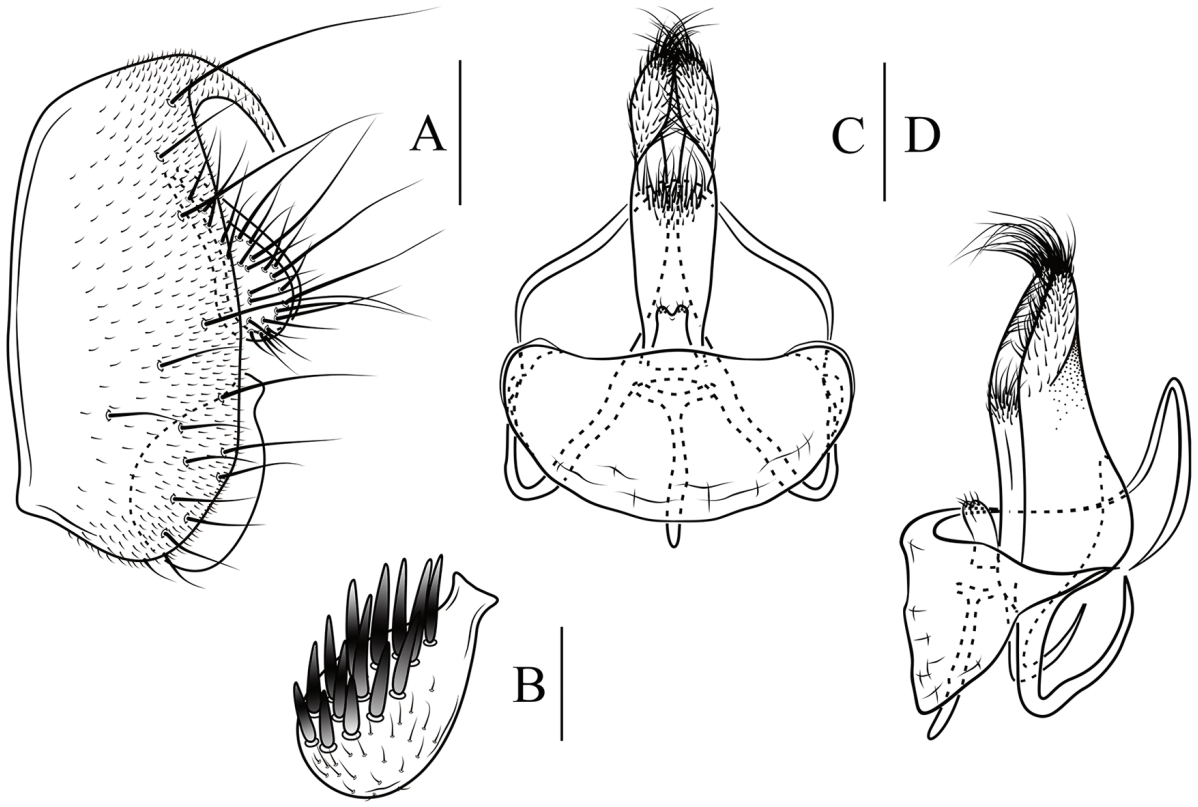

Figure 15. *Stegana* (*Orthostegana*) *fuscofemorata* Peng & Chen **sp. nov.**, male terminalia. **A** Epandrium, surstylus, and cercus in lateral view; **B** Surstylus in ventral view; **C**, **D** Hypandrium, phallapodeme, pregonite, aedeagus, and aedeagal sheath in ventral and lateral views. Scale bars = 0.1 mm.

flw = 2.14 (1.17–2.33), **FW/HW** = 0.40 (0.35–0.40), **ch/o** = 0.13 (0.13), **prorb** = 0.95 (0.81–1.05), **rcorb** = 0.71 (0.68–0.75), **vb** = 0.67 (0.40–0.67), **dcl** = 0.48 (0.40–0.50), **presctl** = 0.48 (0.44–0.53), **sctl** = 1.19 (1.31–1.48), **sterno** = 0.78 (0.62–0.81), **orbito** = 2.00 (2.00–2.25), **dcp** = 0.24 (0.23–0.26), **sctlp** = 0.92 (0.91–1.10), **C** = 2.22 (2.15–2.41), **4c** = 0.95 (0.81–0.95), **4v** = 1.68 (1.63–1.65), **5x** = 1.14 (1.13–1.14), **ac** = 12.00 (10.00–12.00), **M** = 0.42 (0.38–0.43), **C3F** = 0.72 (0.72–0.83).

Distribution. China (Yunnan).

Type material examined. Holotype: ♂ (SCAU, No. 112117), China: Muyiji Park, Ximeng, Yunnan, 22°37′15″N, 99°35′42″E, altitude 1203 m, 29.iv.2016, swept from tree trunk, J. Huang. **Paratypes:** 1♂ (SCAU, No. 112118), Y.L. Wang, other data same as the holotype; 3♂, 1♀ (SCAU, Nos. 112119–22), Menglun, Mengla, Yunnan, 21°55′11″N, 101°16′40″E, altitudes 800–900 m, 13.ix.2002, swept from tree trunks, H.W. Chen; 1♀ (SCAU, No. 112123), Baihualing, Baoshan, Yunnan, 25°17′53″N, 98°48′09″E, altitude 1370 m, 15.vi.2011, swept from tree trunk, J.J. Gao; 1♀ (SCAU, No. 112124), Wangtianshu, Mengla, Yunnan, 21°37′10″N, 101°35′17″E, altitude 570 m, 11.ix.2002, swept from tree trunk, H.W. Chen.

Etymology. A combination of the Latin words "*fuscus*" (= brown) and "*femoralis*" (= femora), referring to all femora distally brown in females.

3.2.1.5. *Stegana* (*Orthostegana*) *latipalpula* Peng & Chen sp. nov.

https://zoobank.org/6B3DAB9F-EB8C-432F-984FEB-6996BE0112

Figs 7A, 7B, 10A, 11C, 16

Diagnosis. This species differs from the other *Orthostegana* species in having the postocellar setae; palpus expanded (Fig. 11C); mesotarsus ventrally with 2 rows of black, apically blunt, stout spines; metatarsus with 19/6+1 spines; 2nd to 4th segments with 1/1/1, 2/1/1, and 1/1/1 spines, respectively (Fig. 10A); Pregonites large, with 1 sensillum submedially per side (Fig. 16C, D); aedeagus short, with some minute, serrated processes distally, lacking hair or pubescence (Fig. 16C, D); gonopod strongly protruded and with numerous minute, serrated processes distally (Fig. 16C, D).

Description. Male. ***Head*****:** Ocellar triangle brown, with 2 small setae posterior to ocellar setae. Frons brown, with numerous minute interfrontal setulae. Pedicel and first flagellomere brown distally, with black pubescence. Face and gena brownish yellow. Clypeus and palpus yellow. ***Thorax*****:** Mesonotum yellow (Fig. 7A). Pleura with a distinct brownish yellow longitudinal stripe above

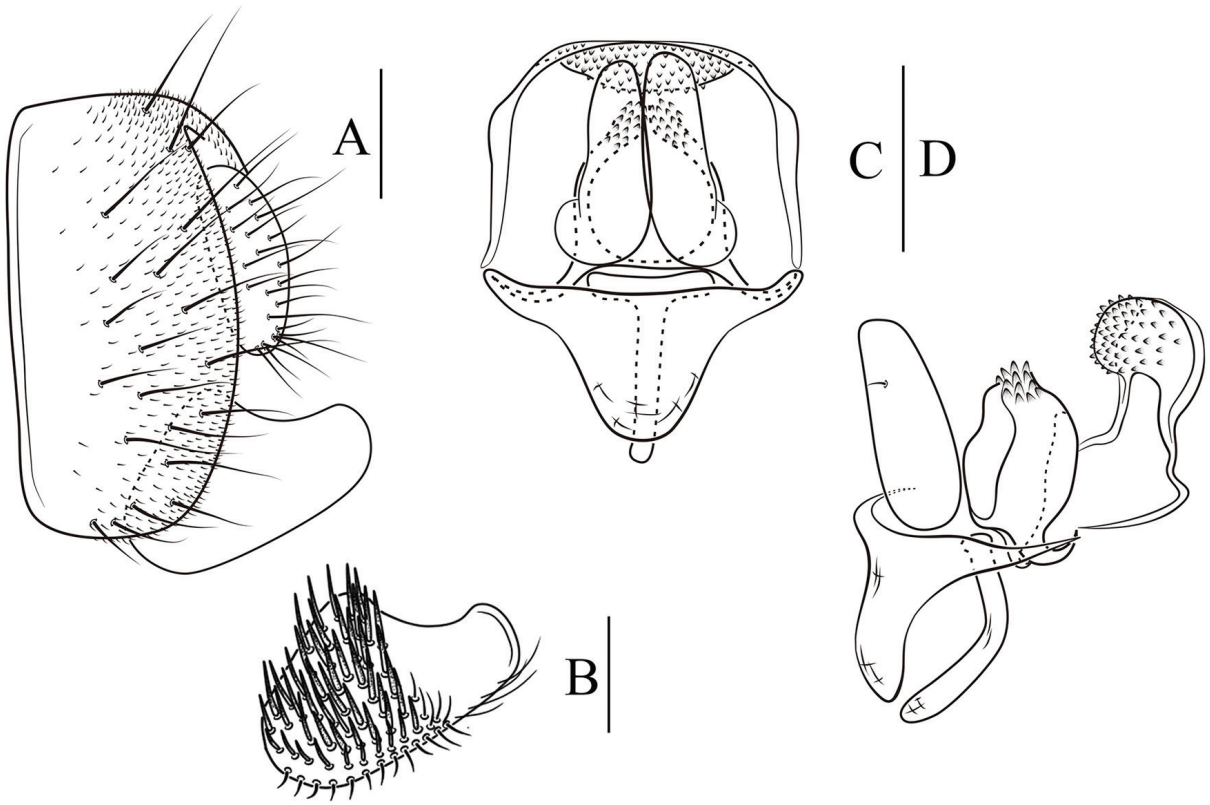

Figure 16. *Stegana* (*Orthostegana*) *latipalpula* Peng & Chen **sp. nov.**, male terminalia. **A** Epandrium, surstylus, and cercus in lateral view; **B** Surstylus in ventral view; **C**, **D** Hypandrium, phallapodeme, pregonite, aedeagus, and aedeagal sheath in ventral and lateral views. Scale bars = 0.1 mm.

(Fig. 7B). Scutellum yellow(Fig. 7A). Halter mostly yellow (Fig. 7A, B). Legs yellowish (Fig. 10A). ***Abdominal tergites*:** yellow. ***Male terminalia*:** Epandrium mostly pubescent except for anterior margin, with *ca*. 21 setae on dorsal to posterior portions per side (Fig. 16A). Surstylus separated from epandrium, with numerous peg-shaped prensisetae near anterior margin on inner surface and some setae ventrally (Fig. 16B). Pregonites separated (Fig. 16C, D).

Measurements and indices. BL = 3.60 mm in holotype, **ThL** = 1.60 mm, **WL** = 3.48 mm, **WW** = 1.48 mm, **arb** = 5/3, **avd** = 1.00, **adf** = 1.00, **flw** = 2.00, **FW/HW** = 0.45, **ch/o** = 0.14, **prorb** = 1.05, **rcorb** = 0.52, **vb** = 0.42, **dcl** = 0.39, **presctl** = 0.33, **sctl** = 1.14, **sterno** = 1.07, **orbito** = 1.29, **dcp** = 0.21, **sctlp** = 1.13, **C** = 2.25, **4c** = 1.05, **4v** = 2.11, **5x** = 1.50, **ac** = 10.00, **M** = 0.63, **C3F** = 0.50.

Distribution. China (Sichuan).

Type material examined. Holotype: ♂ (SCAU, No. 112084), China: Wanba, Jiulong, Sichuan, 29°03′08″N, 101°59′50″E, altitude 2470 m, 5.vii.2017, swept from tussock, N.N. Wang.

Etymology. A combination of the Latin words "*latus*" (= broad) and "*palpulus*" (= palpus), referring to the expanded palpus.

3.2.1.6. *Stegana* (*Orthostegana*) *macrostephana* Peng & Chen sp. nov.

https://zoobank.org/A88359E6-0151-4DD6-976C-8B008D-B7A153

Figs 7C–F, 10B, 17

Diagnosis. This species closely resembles *S.* (*O.*) *pinguitia* **sp. nov.** in the aedeagus medioventrally and distally with dense long hairs (Figs 17C, 17D, 20C, 20D), but can be distinguished from the latter by having the mesotarsus ventrally with 2 rows of black, apically blunt, stout spines; metatarsus with 15/3+1 spines; 2nd to 4th segments with 6/1, 4/1, and 3/1 spines, respectively (Fig. 10B); pregonites slender, with *ca*. 2 or 3 sensilla medioventrally and some setae distally per side (Fig. 17C, D).

Description. Male and female. ***Head*:** Ocellar triangle dark brown, with 2 small setae posterior to ocellar setae. Postocellar seta absent. Frons brown, with numerous minute interfrontal setulae. Pedicel yellow and first flagellomere brownish yellow to brown, with black pubescence. Face and gena brown. Clypeus dark brown medially and yellow laterally. Palpus brownish basally and dark brown distally. ***Thorax*:** Mesonotum yellow to brownish yellow (Fig. 7C, E). Pleura with a distinct

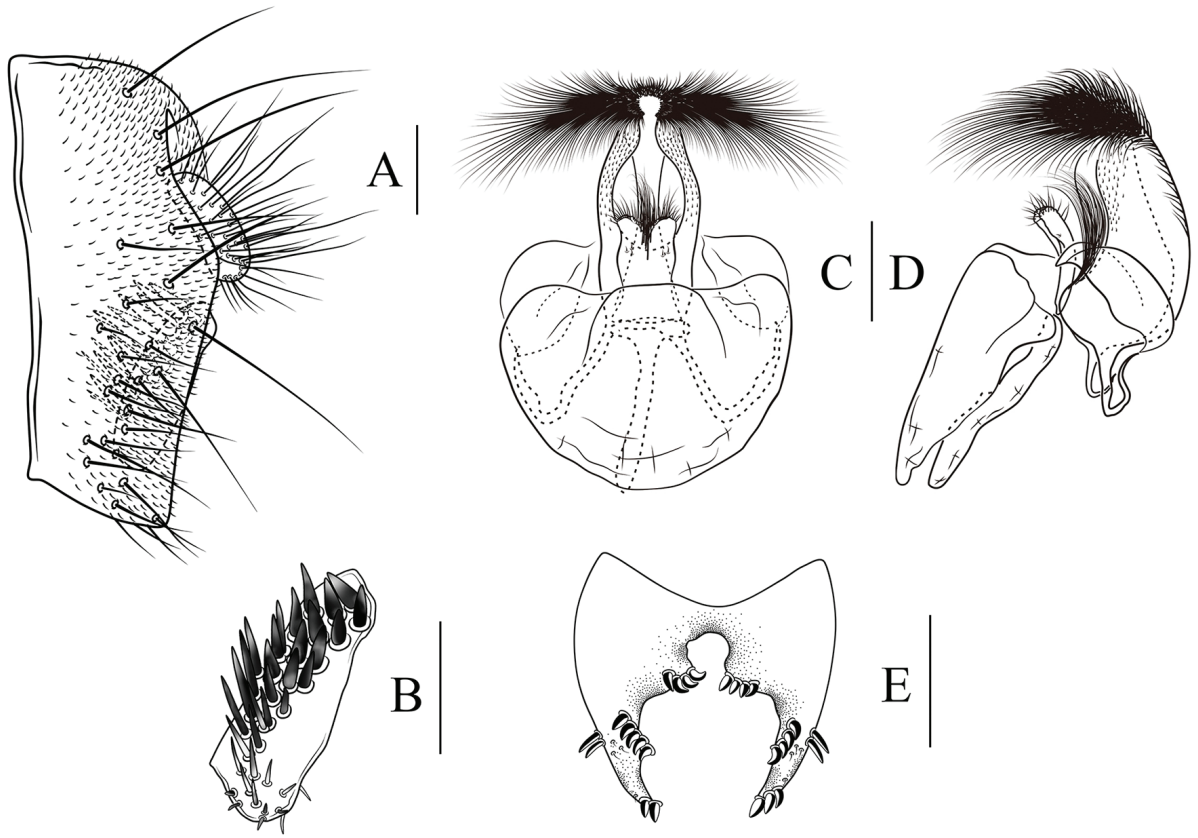

Figure 17. *Stegana* (*Orthostegana*) *macrostephana* Peng & Chen **sp. nov.**, male (**A**–**D**) and female terminalia (**E**). **A** Epandrium, surstylus, and cercus in lateral view; **B** Surstylus in ventral view; **C**, **D** Hypandrium, phallapodeme, pregonite, aedeagus, and aedeagal sheath in ventral and lateral views; **E** Eighth sternite in ventral view. Scale bars = 0.1 mm.

brownish to brown longitudinal stripe above (Fig. 7D, F). Scutellum mostly yellow to brownish yellow (Fig. 7C, E). Halter mostly brownish, with a yellow patch at knob (Fig. 7C–F). Legs yellowish (Fig. 10B). ***Abdominal tergites*:** brownish yellow to brownish. ***Male terminalia*:** Epandrium mostly pubescent except for anterior margin, with *ca*. 28 setae on dorsal to posterior portions per side (Fig. 17A). Surstylus separated from epandrium, with *ca*. 22 long and 15 short peg-shaped prensisetae near anterior margin on inner surface (Fig. 17B). Pregonites fused basally (Fig. 17C, D). Aedeagus pubescent distally (Fig. 17C, D). Aedeagal sheath expanded and lobe-shaped distally (Fig. 17D). ***Female terminalia*:** Eighth sternite with 3 rows of orderly and nearly symmetrical peg-shaped prensisetae subposteromedially and posterolaterally, and with 3 sensilla distally per side (Fig. 17E).

Measurements and indices. BL = 3.50 mm in holotype (range in 5♂ and 5♀ paratypes: 3.30–3.50 in ♂, 2.67–3.33 in ♀), **ThL** = 1.50 mm (1.40–1.50 in ♂, 1.00–1.53 in ♀), **WL** = 3.00 mm (2.77–2.95 in ♂, 2.27–3.23 in ♀), **WW** = 1.25 mm (1.20–1.30 in ♂, 1.16–1.50 in ♀), **arb** = 8/4 (5–9/4–5), **avd** = 0.63 (0.64–0.93), **adf** = 1.60 (1.20–2.50), **flw** = 1.70 (1.63–2.00), **FW/HW** = 0.41 (0.35–0.41), **ch/o** = 0.12 (0.08–0.14), **prorb** = 1.08 (0.90–1.39), **rcorb** = 0.80 (0.53–0.83), **vb** = 0.50 (0.24–0.56), **dcl** = 0.50 (0.33–0.67), **presctl** = 0.40 (0.33–0.57), **sctl** = 1.12 (1.06–1.29), **sterno** = 0.77 (0.68–0.97), **orbito** = 1.67 (1.60–2.20), **dcp** = 0.28 (0.23–0.41), **sctlp** = 1.00 (0.91–1.33), **C** = 2.33 (2.00–2.67), **4c** = 0.91 (0.85–1.00), **4v** = 1.70 (1.56–0.95), **5x** = 1.15 (1.20–2.11), **ac** = 10.00 (9.00–13.33), **M** = 0.45 (0.38–0.48), **C3F** = 0.74 (0.65–0.81).

Distribution. China (Yunnan).

Type material examined. Holotype: ♂ (SCAU, No. 112090), China: Mengdong, Cangyun, Yunnan, 23°10′08″N, 99°13′52″E, altitude 1323 m, 6.v.2016, swept from tussock, Y.Q. Liu. **Paratypes:** 10♂, 5♀ (SCAU, Nos. 112091–98, 112110–16), altitudes 1300–1320 m, J. Huang, Y.Q. Liu, Y.L. Wang, L. Zhu, other data same as the holotype; 1♂, 9♀ (SCAU, Nos. 112099–108), Muyiji Park, Ximeng, Yunnan, 22°37′15″N, 99°35′42″E, altitude 1203 m, 29.iv.2016, swept from tussocks, J. Huang, Y.Q. Liu, Y.L. Wang, L. Zhu; 1♂ (SCAU, No. 112109), Botanic Garden, Ruili, Yunnan, 24°01′12″N, 97°51′33″E, altitude 1174 m, 22.v.2016, swept from tussock, J. Huang.

Etymology. A combination of the Greek words "*macr-*" (= large) and "*stephan-*" (= crown), referring to the apically floriform aedeagus.

3.2.1.7. *Stegana* (*Orthostegana*) *mohnihei* Peng & Chen sp. nov.

https://zoobank.org/323D4AE6-A774-4022-842B-11B333E48EC5

Figs 7G, 7H, 8A, 8B, 10C, 18

Diagnosis. This species closely resembles *S.* (*O.*) *flavicauda* **sp. nov.** in the shape of both male and female terminalia (as fig. 5 in Zhang et al. 2012, Fig. 18), but can be distinguished from the latter by having the mesotarsus ventrally with 2 rows of black, apically blunt, stout spines; metatarsus with 14/1+1 spines; 2nd to 4th segments with 2/1, 1/1, and 2/1 spines, respectively (Fig. 10C); surstylus with *ca*. 20 long and 15 short peg-shaped prensisetae near anterior and ventral margins on inner surface (Fig. 18B).

Description. Male and female. *Head*: Ocellar triangle dark brown, with 2 small setae posterior to ocellar setae. Postocellar seta absent. Frons brown, with numerous minute interfrontal setulae. Pedicel brown, with 1 small seta; first flagellomere dark brown, with black pubescence. Face and gena brownish. Clypeus mostly brown. Palpus brownish yellow. ***Thorax*:** Mesonotum yellow to brownish yellow (Figs 7G, 8A). Pleura with a distinct brownish longitudinal stripe above (Figs 7H, 8B). Scutellum yellow to brownish yellow (Figs 7G, 8A). Halter mostly brownish, with a yellow patch at knob (Figs 7G, 7H, 8A, 8B). Legs yellowish (Fig. 10C). ***Abdominal tergites*:** brownish yellow to brownish. ***Male terminalia*:** Epandrium mostly pubescent except for anterior margin, with *ca*. 26 setae on dorsal to posterior portions per side (Fig. 18A). Surstylus separated from epandrium, with a few setae near ventral margin (Fig. 18B). Pregonites separated and small, with *ca*. 3 or 4 sensilla distally (Fig. 18C, D). Aedeagus pubescent distally and medioventrally, with some hairs medioventrally and apically (Fig. 18C, D). ***Female terminalia*:** Eighth sternite with 3 rows of orderly and asymmetrical peg-shaped prensisetae subposteromedially and posterolaterally, and with 3 sensilla distally per side (Fig. 18E).

Measurements and indices. BL = 3.24 mm in holotype (range in 5♂ and 5♀ paratypes: 3.11–3.42 in ♂, 3.02–3.78 in ♀), **ThL** = 1.33 mm (1.20–1.33 in ♂, 1.24–1.69 in ♀), **WL** = 2.67 mm (2.37–2.57 in ♂, 2.60–3.07 in ♀), **WW** = 1.23 mm (1.00–1.13 in ♂, 1.13–1.40 in ♀), **arb** = 6/4 (6/4–5), **avd** = 0.89 (0.89–1.00), **adf** = 1.50 (1.40–2.00), **flw** = 2.67 (2.67–3.25), **FW/HW** = 0.41 (0.38–0.42), **ch/o** = 0.11 (0.10–0.14), **prorb** = 0.80 (0.80–0.93), **rcorb** = 0.60 (0.67–0.76), **vb** = 0.42 (0.43–0.57), **dcl** = 0.38 (0.38–0.56), **presctl** = 0.42 (0.38–0.50), **sctl** = 1.20 (1.21–1.33), **sterno** = 0.75 (0.80–0.94), **orbito** = 2.00 (1.75–2.00), **dcp** = 0.30 (0.23–0.33), **sctlp** = 1.00 (0.88–1.13), **C** = 2.53 (2.19–2.47), **4c** = 0.79 (0.82–0.92), **4v** = 1.54 (1.55–1.81), **5x** = 1.13 (1.00–1.43), **ac** = 12.67 (10.00–12.67), **M** = 0.38 (0.30–0.46), **C3F** = 0.63 (0.63–0.74).

Distribution. China (Yunnan).

Type material examined. Holotype: ♂ (SCAU, No. 112130), China: Mengdong, Cangyuan, Yunnan, 23°10′08″N, 99°13′52″E, altitude 1320 m, 6.v.2016, swept from tussock, J. Huang. **Paratypes:** 21♂, 20♀ (SCAU, Nos. 112131–71), altitudes 1320–1323 m, J. Huang, Y.Q. Liu, Y.L. Wang, L. Zhu, other data same as the holotype; 6♀ (SCAU, Nos. 112172–77), Muyiji Park, Ximeng, Yunnan, 22°37′15″N, 99°35′42″E,

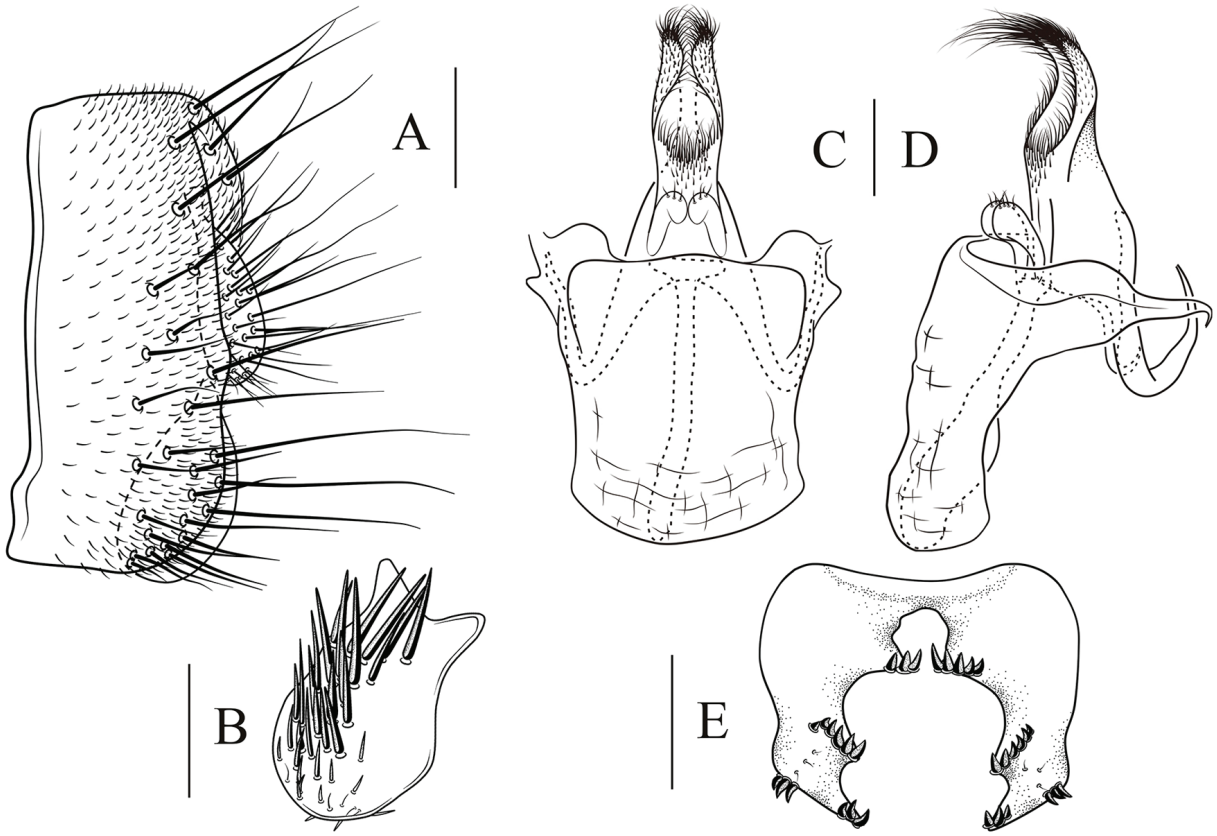

Figure 18. *Stegana* (*Orthostegana*) *mohnihei* Peng & Chen **sp. nov.**, male (**A**–**D**) and female terminalia (**E**). **A** Epandrium, surstylus, and cercus in lateral view; **B** Surstylus in ventral view; **C**, **D** Hypandrium, phallapodeme, pregonite, aedeagus, and aedeagal sheath in ventral and lateral views; **E** Eighth sternite in ventral view. Scale bars = 0.1 mm.

altitude 1203 m, 29.iv.2016, swept from tree trunks, J. Huang, Y.Q. Liu, Y.L. Wang, L. Zhu; 3♀ (SCAU, Nos. 112178–80), Muyiji Park, Ximeng, Yunnan, 22°37′15″N, 99°35′42″E, altitude 1100 m, 16.iv.2018, swept from tussocks, Y.L. Wang; 1♂, 1♀ (SCAU, Nos. 112181, 82), Baihualing, Baoshan, Yunnan, 25°17′53″N, 98°48′09″E, altitude 1370 m, 15.vi.2011, swept from tussocks, J.J. Gao.

Etymology. From the word "*mohnihei*" in Va language from the Va nationality living in Yunnan, China, referring to a carnival for them.

3.2.1.8. *Stegana* (*Orthostegana*) *obscurala* Peng & Chen sp. nov.

https://zoobank.org/89E07D8B-C1BF-42AF-AF97-26E1D-29ED9BF

Figs 8C–F, 10D, 19

Diagnosis. This species closely resembles *S.* (*O.*) *aini* **sp. nov.** in the shape of both male and female terminalia (Figs 12, 19), but can be distinguished from the latter by having the pleura with a distinct brown to dark brown longitudinal stripe above (Fig. 8D, F); the mesotarsus ventrally with 2 rows of black, apically blunt, stout spines; metatarsus with 12/6+1 spines; 2nd to 4th segments with 6/1, 3/1, and 2/1 spines, respectively (Fig. 10D); pregonite with *ca*. 3 sensilla distally (Fig. 19C, D).

Description. Male and female. ***Head*****:** Ocellar triangle dark brown, with 2 small setae posterior to ocellar setae. Postocellar seta absent. Frons brown, with numerous minute interfrontal setulae. Pedicel and first flagellomere yellow, with black pubescence. Face brown, with narrowed, black band on lower margin. Gena brownish. Clypeus mostly brownish. Palpus brownish. ***Thorax*****:** Mesonotum mostly yellow to brown (Fig. 8C, E). Scutellum yellow in males (Fig. 8C), dark brown in females (Fig. 8E). Halter mostly brownish, with a yellow patch at knob (Fig. 8D–F). Legs yellowish (Fig. 10D). ***Abdominal tergites*****:** brown to black. ***Male terminalia*****:** Epandrium mostly pubescent except for anterior and ventral margins, with *ca*. 20 setae on dorsal to posterior portions per side (Fig. 19A). Surstylus separated from epandrium, with *ca*. 2 long and 10 short peg-shaped prensisetae and a few setae near posteroventral margin (Fig. 19B). Pregonites separated and small, slightly expanded distally (Fig. 19C, D). Aedeagus pubescent distally, with some hairs apically (Fig. 19C, D). ***Female terminalia*****:** Eighth sternite with 4 rows of asymmetrical peg-shaped prensisetae subposteromedially and posterolaterally, and with 3 sensilla distally per side (Fig. 19E).

Measurements and indices. BL = 3.13 mm in holotype (range in 2♂ and 2♀ paratypes: 2.60–3.00 in ♂, 2.87–4.21 in ♀), **ThL** = 1.37 mm (1.00–1.13 in ♂, 1.07–1.60 in ♀), **WL** = 2.33 mm (1.90–2.17 in ♂, 2.20–2.77 in ♀), **WW** = 1.03 mm (0.93–0.97 in ♂, 1.00–1.27 in ♀), **arb** = 6/5 (6–

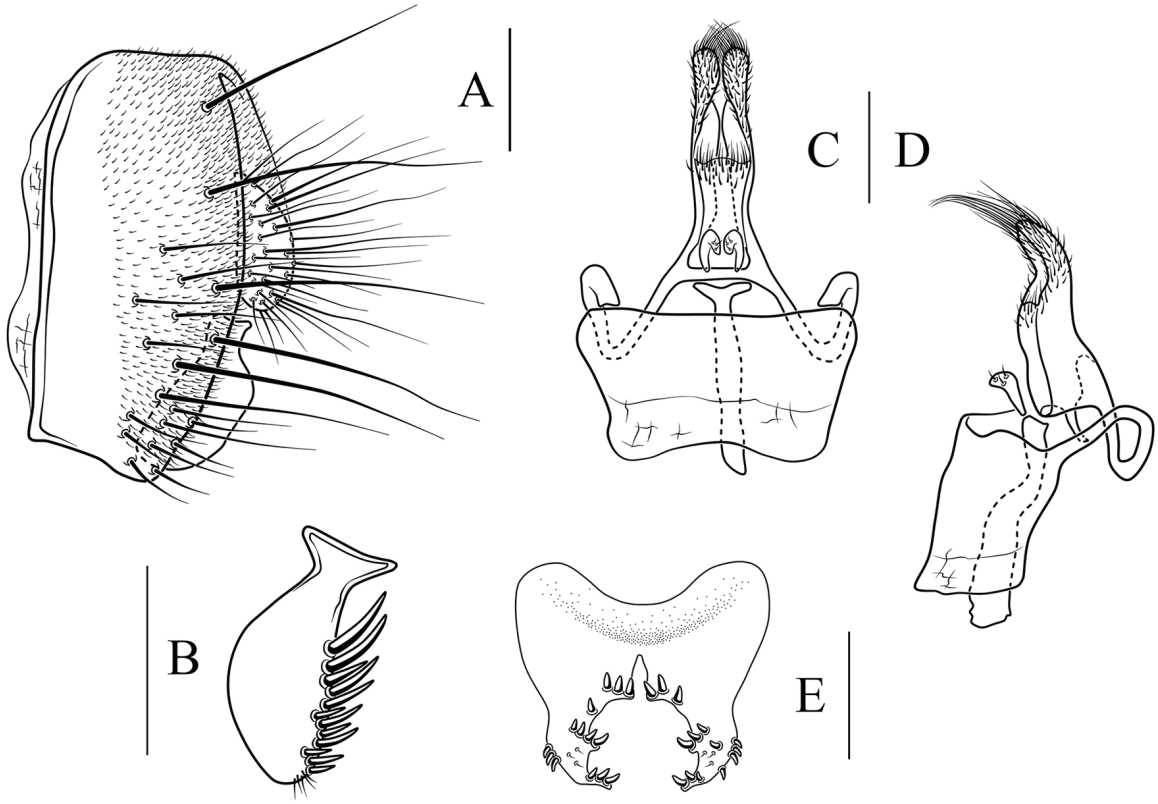

Figure 19. *Stegana* (*Orthostegana*) *obscurala* Peng & Chen **sp. nov.**, male (**A**–**D**) and female terminalia (**E**). **A** Epandrium, surstylus, and cercus in lateral view; **B** Surstylus in ventral view; **C**, **D** Hypandrium, phallapodeme, pregonite, aedeagus, and aedeagal sheath in ventral and lateral views; **E** Eighth sternite in ventral view. Scale bars = 0.1 mm.

7/4–6), **avd** = 0.92 (0.88–0.92), **adf** = 1.71 (1.71–2.13), **flw** = 1.86 (2.00–2.17), **FW/HW** = 0.38 (0.32–0.40), **ch/o** = 0.15 (0.13–0.16), **prorb** = 0.90 (0.82–0.90), **rcorb** = 0.50 (0.50–0.65), **vb** = 0.41 (0.40–0.75), **dcl** = 0.54 (0.38–0.50), **presctl** = 0.39 (0.40–0.50), **sctl** = 1.33 (1.33–1.50), **sterno** = 0.84 (0.77–0.84), **orbito** = 1.80 (1.67–2.00), **dcp** = 0.24 (0.22–0.29), **sctlp** = 0.90 (0.89–1.17), **C** = 2.75 (2.20–2.50), **4c** = 0.84 (0.84–1.00), **4v** = 1.79 (1.63–1.84), **5x** = 1.29 (1.25–1.50), **ac** = 16.00 (13.33–16.00), **M** = 0.47 (0.42–0.53), **C3F** = 0.75 (0.69–0.76).

Distribution. China (Yunnan).

Type material examined. Holotype*:* ♂ (SCAU, No. 112125), China: Wangtianshu, Mengla, Yunnan, 21°37′10″N, 101°35′17″E, altitude 570 m, 9.v.2012, swept from tussock, H.W. Chen. **Paratypes***:* 2♂, 2♀ (SCAU, Nos. 112126–29), H.W. Chen, J.J. Gao, other data same as the holotype.

Etymology. From the Latin word "*obscurus*" (= obscure), referring to the mostly dark-colored abdominal tergites.

3.2.1.9. *Stegana* (*Orthostegana*) *pinguitia* Peng & Chen sp. nov.

https://zoobank.org/562B4B37-F5EA-4D06-9CCF-533C85805C52

Figs 8G, 8H, 10E, 20

Diagnosis. This species closely resembles *S.* (*O.*) *macrostephana* **sp. nov.** in the aedeagus medioventrally and distally with dense long hairs (Figs 17C, 17D, 20C, 20D), but can be distinguished from the latter by having the mesotarsus ventrally with 2 rows of black, apically blunt, stout spines; metatarsus with 12/1/7+1 spines; 2nd to 4th segments with 5/1, 3/1, and 2/1 spines, respectively (Fig. 10E); pregonites large and expanded distally, lacking sensillum, with *ca*. 2 setae distally per side (Fig. 20C, D).

Description. Male. ***Head*****:** Ocellar triangle dark brown, with 2 small setae posterior to ocellar setae. Postocellar seta absent. Frons brown, with numerous minute interfrontal setulae. Pedicel yellow and first flagellomere dark brown, with black pubescence. Face, gena, and clypeus brown. Palpus brownish. ***Thorax*****:** Mesonotum brownish yellow (Fig. 8G). Pleura with a distinct brown longitudinal stripe above (Fig. 8H). Scutellum mostly brownish yellow (Fig. 8G). Halter mostly brownish, with a yellow patch at knob (Fig. 8H). Legs yellowish (Fig. 10E). ***Abdominal tergites*****:** brown. ***Male terminalia*****:** Epandrium mostly pubescent except for anterior margin, with *ca*. 8 setae on dorsal to posterior portions per side (Fig. 20A). Surstylus separated from epandrium, narrowed dorsally, with *ca*. 13 long and 3 short peg-shaped prensisetae near anterodorsal margin on inner surface (Fig. 20B). Pregonites fused basally (Fig. 20C, D). Aedeagus pubescent distally (Fig. 20C, D).

Measurements and indices. BL = 3.25 mm in holotype (range in 4♂ paratypes: 3.25–3.57), **ThL** = 1.49 mm

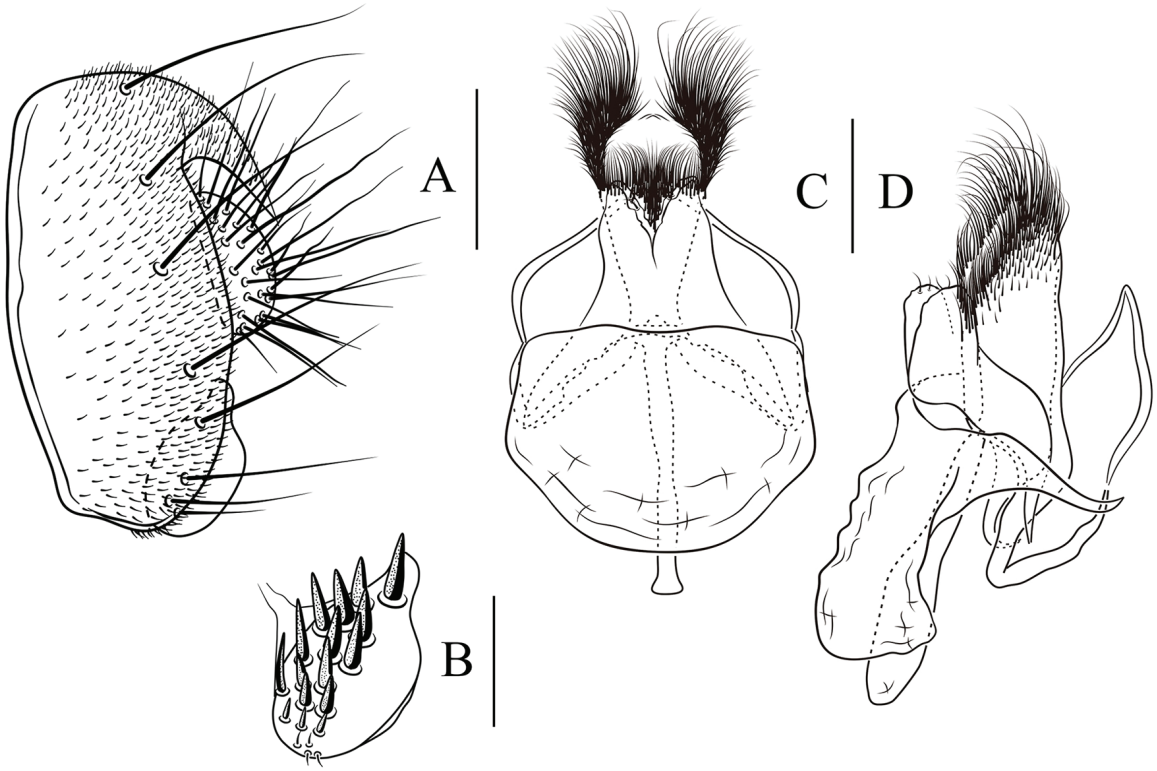

Figure 20. *Stegana* (*Orthostegana*) *pinguitia* Peng & Chen **sp. nov.**, male terminalia. **A** Epandrium, surstylus, and cercus in lateral view; **B** Surstylus in ventral view; **C**, **D** Hypandrium, phallapodeme, pregonite, aedeagus, and aedeagal sheath in ventral and lateral views. Scale bars = 0.1 mm.

(1.44–1.49), **WL** = 2.57 mm (2.60–2.87), **WW** = 1.27 mm (1.27–1.43), **arb** = 8/5 (6–8/3–5), **avd** = 0.91 (0.89–1.00), **adf** = 1.38 (1.00–1.29), **flw** = 1.88 (1.67–2.22), **FW/HW** = 0.40 (0.38–0.47), **ch/o** = 0.13 (0.13–0.14), **prorb** = 0.75 (0.74–0.87), **rcorb** = 0.67 (0.61–0.70), **vb** = 0.39 (0.39–0.55), **dcl** = 0.50 (damaged), **presctl** = 0.47 (0.56), **sctl** = damaged (1.08), **sterno** = 0.77 (0.76–0.82), **orbito** = 1.80 (1.67–2.00), **dcp** = 0.26 (0.19–0.23), **sct-lp** = 1.18 (0.85–1.00), **C** = 2.64 (2.14–2.37), **4c** = 1.10 (0.88–1.00), **4v** = 1.90 (1.68–1.82), **5x** = 1.50 (1.10–1.50), **ac** = 11.00 (11.00–11.50), **M** = 0.60 (0.48–0.55), **C3F** = 0.68 (0.65–0.68).

Distribution. China (Sichuan).

Type material examined. Holotype: ♂ (SCAU, No. 112085), China: Dafengding, Mabian, Sichuan, 28°34′42″N, 103°15′40″E, altitude 1270 m, 27.vi.2017, swept from tussock, N.N. Wang. **Paratypes:** 3♂ (SCAU, Nos. 112086–88), N.N. Wang, L. Gong, other data same as the holotype; 1♂ (SCAU, No. 112089), Fengtongzhai, Baoxing, Sichuan, 30°33′26″N, 102°57′09″E, altitude 1500 m, 18.vii.2017, swept from tussock, N.N. Wang.

Etymology. A combination of the Latin words "*pinguis*" (= thick) and "*-itia*" (= concept), referring to the aedeagus distally with dense long hairs.

3.2.2. Key to all 20 *Stegana* (*Orthostegana*) species based on morphological characters

1 Postocellar setae present **2**
– Postocellar seta absent **8**
2 Surstylus with peg-shaped prensisetae **3**
– Surstylus without peg-shaped prensiseta **4**
3 Palpus expanded (Fig. 11C) ..... ***S. latipalpula* sp. nov.**
– Palpus not expanded ..... ***S. singularis***
4 Cercus acute laterally and ventrally **5**
– Cercus not acute laterally or ventrally **7**
5 Surstylus distoposteriorly bearing a strong, acute projection, ventrally without strong, acute projection (as fig. 5 in Vilela and Bächli 2020) ..... ***S. acutangula***
– Surstylus distoposteriorly without strong, acute projection, ventrally with a strong, acute projection **6**
6 Surstylus nearly triangular (as fig. 40a–c in Vilela and Bächli 2020) ..... ***S. turrialba***
– Surstylus nearly rectangular (as fig. 45 in Vilela and Bächli 2020) ..... ***S. yasuni***

7 Cercus relatively large, extended and curved ventrally (as fig. 29 in Vilela and Bächli 2020); aedeagal sheath complex, three-sectioned (as fig. 30a–c in Vilela and Bächli 2020) ..... ***S. dudai***
– Cercus relatively small, ventral lobe fused with a large, rod-shaped structure (as fig. 21c, d in Vilela and Bächli 2020); aedeagal sheath relatively large, triangular (as fig. 22 in Vilela and Bächli 2020) ..... ***S. triseta***
8 Clypeus expanded (Fig. 11B) ..... ***S. hirsutina***
– Clypeus not expande **9**
9 Cercus fused ventrally; surstylus fused with epandrium (as fig. 7F in Zhang et al. 2012) ..... ***S. multicardua***
– Cercus entirely separated; surstylus separated from epandrium **10**
10 Pleura without distinct longitudinal stripe above **11**
– Pleura with a distinct brownish yellow to dark brown longitudinal stripe above **12**
11 Midleg tibia with 4 or 5 posterodorsal setae; mesotarsus ventrally with 2 rows of black, apically blunt, stout spines; metatarsus with 14/4+1 spines; 2nd to 4th segments with 3/1, 1/1, and 2/1 spines, respectively (Fig. 9C) ..... ***S. flavicauda***
– Midleg tibia with 3 posterodorsal setae; mesotarsus ventrally with two rows of black, apically blunt, stout spines; metatarsus with 15/2+1 spines; 2nd to 4th segments with 5/1, 1/1, and 2/1 spines, respectively (Fig. 9G) ..... ***S. aini* sp. nov.**
12 Pregonites large, similar to the size of aedeagus (Fig. 20C, D) ..... ***S. pinguitia* sp. nov.**
– Pregonites not large, much smaller than the size of aedeagus **13**
13 Aedeagus with dense long hairs medioventrally and distally (Fig. 17C, D) ..... ***S. macrostephana* sp. nov.**
– Aedeagus without dense long hairs medioventrally or distally **14**
14 Pregonites separated basally **15**
– Pregonites fused basally **17**
15 Pregonites narrowed distally (as fig. 3C, D in Zhang et al. 2012) ..... ***S. curvinervis***
– Pregonites expanded distally **16**
16 Mesotarsus ventrally with 2 rows of black, apically blunt, stout spines; metatarsus with 14/1+1 spines; 2nd to 4th segments with 2/1, 1/1, and 2/1 spines, respectively (Fig. 10C); surstylus *ca*. 20 long and 15 short peg-shaped prensisetae near anterior and ventral margins on inner surface (Fig. 18B) ..... ***S. mohnihei* sp. nov.**
– Mesotarsus ventrally with 2 rows of black, apically blunt, stout spines; metatarsus with 12/6+1 spines; 2nd to 4th segments with 6/1, 3/1, and 2/1 spines, respectively (Fig. 10D); surstylus with *ca*. 2 long and 10 short peg-shaped prensisetae and a few setae near posteroventral margin (Fig. 19B) ..... ***S. obscurala* sp. nov.**
17 Pregonites expanded distally in lateral view (as fig. 7D in Zhang et al. 2012) ..... ***S. hylecoeta***
– Pregonites not expanded distally in lateral view **18**
18 Surstylus with some setae near ventral margin on inner surface (Fig. 15B) ..... ***S. fuscofemorata* sp. nov.**
– Surstylus without seta on inner surface **19**
19 Mesotarsus ventrally with 2 rows of black, apically blunt, stout spines; metatarsus with 14/3+1 spines; 2nd to 4th segments with 5/1, 2/1, and 2/1 spines, respectively (Fig. 9H); pregonite with *ca*. 3 sensilla distally (Fig. 13C, D) ..... ***S. brevivittata* sp. nov.**
– Mesotarsus ventrally with 2 rows of black, apically blunt, stout spines; metatarsus with 12/1+1 spines; 2nd to 4th segments with 2/1, 2/1, and 2/1 spines, respectively (Fig. 9I); pregonite with *ca*. 4 sensilla distally (Fig. 14C, D) ..... ***S. cuodi* sp. nov.**

4. Discussion

4.1. Phylogeny and integrative species delimitation

Among the genus *Stegana*, the evolutionary relationship between the subgen. *Orthostegana* and subgen. *Oxyphortica* has long been controversial. Only a few morphological characters have been used for their subdivision: wing vein M_{1+2} strongly curved forward or not (Sidorenko 1998) and the number of strong, erect setae on the dorsal surface of midleg tibia (as fig. 2 in Zhang et al. 2012). With the increasing number of newly discovered *Orthostegana* and *Oxyphortica* cryptic species, the species boundaries between them are becoming increasingly blurred (Vilela and Bächli 2020; Wang et al. 2022). Vilela and Bächli (2020) proposed that the Asian *Orthostegana* lineage should be transferred to the subgen. *Oxyphortica*, due to the Neotropical species they examined and described were best included in the subgen. *Orthostegana* by having the possible presence of a completely membranous and mostly amorphous aedeagus. However, the distinct differences in genitalia structures (Zhang et al. 2012; Wang et al. 2021b, 2022) and a recent phylogenetic study revealed that the Asian *Orthostegana* lineage was sister to the subgen. *Stegana* + subgen. *Steganina* (see fig. 1 in Wang et al. 2022) rather than to the subgen. *Oxyphortica*. The absence of molecular data on the Neotropical species made this revision unconvincing to reflect the accurate affinities of the Asian *Orthostegana* lineage. Therefore, *Orthostegana* remains a taxonomically challenging group and we here recommend following the traditional classification system of the subgen. *Orthostegana* in this study.

We infer that the Asian *Orthostegana* species might be transferred to a newly-established subgenus of the *Stegana* with the support of molecular and other evidence of the Neotropical *Orthostegana* species.

As direct evidence of adaptation to reproductive isolation, male external genitalia is often used for rapid species identification by right of being the most rapidly evolving insect organs (Eberhard 1985). Even in a relatively stable intrapopulation with conservative genitalia structures, subtle differences in male genitalia often constitute the sole diagnostics of a type specimen that is required for naming a new species (Yassin 2016). However, compared with other taxa with distinct genitalia differentiation, the *Orthostegana* species exhibit higher conservatism in genitalia. Although a combination of geographic distribution and some external morphological characters is still useful for the identification of *Orthostegana* species (Table 4), it is easy to confuse and unsuitable for non-taxonomic scholars. To maintain the validity of species identification, this method needs to be continuously updated and revised for adapting to the discovery of more *Orthostegana* cryptic species. Searching for missing or intermediate characters, as well as new diagnostic characters with potential for species identification, remains an essential task for the *Orthostegana*.

In contrast to the lagging differentiation in morphology, the phylogenetic clustering showed clear genetic differentiation among the Asian *Orthostegana* species. In this study, the *COI* ABGD and BP&P analyses generated consistent species delimitation results as morphological identification, implying that these two methods were more suitable for the mitochondrial species delimitation in the Asian *Orthostegana* lineage. Although the results of ABGD and mPTP analyses usually vary among genetic markers, the multi-locus coalescent species delimitation method BP&P could counteract bias from individual genes (Gatesy et al. 1999) and provide reciprocally monophyletic species assignments. On the other hand, we found a weak "barcoding gap" between the intra- and interspecific *p*-distances in the *COI* rather than *ND2* sequences, indicating that *COI* has a stronger phylogenetic implication for the Asian *Orthostegana* lineage, in spite of the "barcoding gap" in *ND2* having been widely observed in other drosophilids taxa (Wang et al. 2020, 2021a, b). Finally, we conducted accurate species delimitation for the *Orthostegana* specimens and identified nine new species from Southwest China by integrating morphological and molecular evidence.

4.2. Ecological adaptability

The *Orthostegana* species are well adapted to a wider range of temperatures from temperate to tropical climate zones, with the highest species richness (approximately 80%) in Southwest China of the Oriental region (Figs 1, 4). The *Orthostegana* species from Southwest China generally exhibit high conservatism in external morphology and subtle differences in genitalia. This phenomenon is highly matched with the special ecological environment formed from the Qinghai-Tibet Plateau to Hengduan Mountains, where historically active orogenic movements and complex climatic conditions have frequently been demonstrated to favor speciation and rapid adaptive evolution (Xing and Ree 2017). Complex montane topography often forms a diversified ecological environment together with high species replacements and radiations, resulting in high morphological conservatism and possible evolutionary stasis, and consequently, driving this region into a Garden of Eden for cryptic species (e.g., Cao et al. 2011; Li et al. 2013; Lu et al. 2018; Wang et al. 2020, 2021b).

It is worth noting that the destruction of virgin forests may have led to the discontinuous distribution of *Orthostegana* species in East Asia, since 13 of the 20 *Orthostegana* species were collected from Southwest China, and 11 of them distributed in the virgin forests of southwest Yunnan. However, there has been no record of this subgenus in eastern and central mainland China so far. In addition, only *S.* (*O.*) *singularis* has been adapted to the cold climates of the Palaearctic region and evolved specific external genital structures: 10th sternite and aedeagal sheath bear numerous minute, serrated processes (as fig. 4 in Zhang et al. 2012). Morphological changes might be associated with adaptation to the environment and niches.

4.3. The current dilemma of Steganinae

Linnaeus (1767) described the first Steganinae species, *Stegana* (*Stegana*) *furta*, from Europe. To date, more than 1,150 Steganinae species have been known around the world (Brake and Bächli 2008; Bächli 2023; Wang et al. 2023), which remains far from sufficient investigation. Steganinae species are extremely sensitive to environmental changes and prefer the untraversed montane as habitats. In the past two decades, only very few scholars in the world have been dedicated to the taxonomy of Steganinae. Faced with the same dilemma as other insects, the shortage of taxonomists and financial investment hinders the discovery of biodiversity, especially in developing countries. In addition, the poor descriptions in early studies, undescribed species housed in natural history museums, as well as the lack of molecular data, have posed challenges to the species identification in Steganinae.

Morphological conservatism and possibly evolutionary stasis are frequently observed in Steganinae including the genera *Amiota* (Chen and Toda 2001; Zhang and Chen 2006), *Stegana* (Wang et al. 2017; Huang et al. 2018; Wang et al. 2021b), *Phortica* (Huang et al. 2019), and *Leucophenga* (Huang and Chen 2016; Huang et al. 2017), which often cause the emergence of the complexes of cryptic species and make species identification difficult. Moreover, the repetitive evolution of homoplastic characters brings challenges to the phylogenetic positioning between some early morphospecies and the non-monophyletic taxonomic assemblages (e.g., Xu et al. 2007; Shao et al. 2014; Lu et al. 2018; Wang 2019; Wang and Chen 2020).

Steganinae has not been fully used in other fields beyond taxonomic and phylogenetic studies. This is mainly attributed to the strict feeding habit of Steganinae, resulting in the difficulty of rearing in a laboratory. In order to have a deeper understanding and take full advantage of this large drosophilids group, an important subject is to develop a universal medium formula that is available for the rearing of Steganinae species. The successful breeding of *Phortica* (*Phortica*) *variegata* (Fallén, 1823) and *Phortica* (*Allophortica*) *oldenbergi* (Duda, 1924) under laboratory conditions (Otranto et al. 2012; Bernardini et al. 2022) may provide new ideas regarding this issue.

5. Conclusions

This study supported the initial *Orthostegana* lineage division and did not advocate transferring the Asian *Orthostegana* lineage to the subgen. *Oxyphortica*, due to the clear sisterhood between the Asian *Orthostegana* lineage and the subgen.*Stegana* + subgen. *Steganina* (Wang et al. 2022). We also call for the taxonomic re-evaluation of *Orthostegana* under the support of molecular data of the Neotropical species. This will not only solve parts of the taxonomic problems related to the *Orthostegana* and *Oxyphortica* but also facilitate a deeper understanding of the historical processes of Steganinae biodiversity.

Accurate species delimitation and cryptic species discovery of the *Orthostegana* could be assessed by integrating morphological and molecular evidence, paving the way for further ecological and evolutionary studies at regional scales. The current transpacific species distribution has aroused great enthusiasm for the historical biogeography and evolution of the genus *Stegana*, which highlights a great need for comprehensive sampling with molecular data in support of reliable species delimitation and biogeographic inference.

6. Competing interests

The authors have declared that no competing interests exist.

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8. References

Bächli G (2023) The database on Taxonomy of Drosophilidae. Available from: https://taxodros.uzh.ch (accessed 2 January 2023).

Brake I, Bächli G (2008) Drosophilidae (Diptera). World Catalogue of Insects, Vol. 9. Apollo Books, Stenstrup, 412 pp.

Bernardini I, Poggi C, Manzi S, Bezerra-Santos MA, Beugnet F, Fourie J, Otranto D, Pombi M (2022) Laboratory breeding of two *Phortica* species (Diptera: Drosophilidae), vectors of the zoonotic eyeworm *Thelazia callipaeda*. Parasites & Vectors 15: 200. https://doi.org/10.1186/s13071-022-05331-6

Cao H, Wang X, Gao J, Prigent SR, Watabe H, Zhang Y, Chen H (2011) Phylogeny of the African and Asian *Phortica* (Drosophilidae) deduced from nuclear and mitochondrial DNA sequences. Molecular Phylogenetics and Evolution 61: 677–685. https://doi.org/10.1016/j.ympev.2011.08.002

Chen HW, Toda MJ (2001) A revision of the Asian and European species in the subgenus *Amiota* Loew (Diptera, Drosophilidae) and the establishment of species-groups based on phylogenetic analysis. Journal of Natural History 35: 1517–1563. https://doi.org/10.1080/002229301317067665

Duda O (1923) Die orientalischen und australischen Drosophiliden-Arten (Dipteren) des Ungarischen National-Museums zu Budapest. Annales Historico-Naturales Musei Nationalis Hungarici 20: 24–59.

Duda O (1924) Beitrag zur Systematik der Drosophiliden unter besonderer Berücksichtigung der paläarktischen u. orientalischen Arten (Dipteren). Archiv für Naturgeschichte (A) 90: 172–234.

Duda O (1925) Die costaricanischen Drosophiliden des Ungarischen National-Museums zu Budapest. Annales historico-naturales Musei Nationalis Hungarici 22: 149–229.

Eberhard WG (1985) Sexual Selection and Animal Genitalia. Harvard University Press, Cambridge, 256 pp.

Enderlein G (1922) Einige neue Drosophiliden. Deutsche Entomologische Zeitschrift 1922: 295–296.

Fallén, JC (1823) Diptera, Sveciae, Geomyzides. Litteris Berlingianis, Lundae, 8 pp.

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome *c* oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology 3: 294–299.

Gatesy J, O'Grady P, Baker RH (1999) Corroboration among data sets in simultaneous analysis: hidden support for phylogenetic relationships among higher level artiodactyl taxa. Cladistics 15: 271–313. https://doi.org/10.1111/j.1096-0031.1999.tb00268.x

He XF, Gao JJ, Cao HZ, Zhang XL, Chen HW (2009) Taxonomy and molecular phylogeny of the *Phortica hani* species complex (Diptera: Drosophilidae). Zoological Journal of the Linnean Society 157: 359–372. https://doi.org/10.1111/j.1096-3642.2009.00516.x

Hendel F (1913) Neue amerikanische Dipteren. Deutsche Entomologische Zeitschrift 1913: 617–636.

Huang J, Chen HW (2016) The genus *Leucophenga* (Diptera, Drosophilidae), part VI: the *argentata* species group from the East Asia, with morphological and molecular evidence. Zootaxa 4161: 207–227. https://doi.org/10.11646/zootaxa.4161.2.4

Huang J, Su YR, Chen HW (2017) The genus *Leucophenga* (Diptera, Drosophilidae), part VII: the *subpollinosa* species group from China, with morphological and molecular evidence. Zootaxa 4247: 201–245. https://doi.org/10.11646/zootaxa.4247.3.1

Huang J, Wang NN, Zhang Y, Chen HW (2018) *Stegana* (*Oxyphortica*) *convergens* species group (Diptera: Drosophilidae) from the Oriental region, with morphological and molecular evidence. Journal of Natural History 52: 1473–1492. https://doi.org/10.1080/00222933.2018.1478010

Huang J, Gong L, Tsaur SC, Zhu L, An KY, Chen HW (2019) Revision of the subgenus *Phortica* (sensu stricto) (Diptera, Drosophilidae) from East Asia, with assessment of species delimitation using

DNA barcodes. Zootaxa 4678: 1–75. https://doi.org/10.11646/zootaxa.4678.1.1

Huelsenbeck JP, Larget B, Alfaro ME (2004) Bayesian phylogenetic model selection using reversible jump Markov chain Monte Carlo. Molecular Biology and Evolution 21: 1123–1133. https://doi.org/10.1093/molbev/msh123

Kapli P, Lutteropp S, Zhang J, Kobert K, Pavlidis P, Stamatakis A, Flouri T (2017) Multi-rate Poisson tree processes for single-locus species delimitation under maximum likelihood and Markov chain Monte Carlo. Bioinformatics 33: 1630–1638. https://doi.org/10.1093/bioinformatics/btx025

Kumar S, Stecher G, Tamura K (2016) MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Molecular Biology and Evolution 33: 1870–1874. https://doi.org/10.1093/molbev/msw054

Lanfear R, Frandsen PB, Wright AM, Senfeld T, Calcott B (2017) PartitionFinder 2: new methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. Molecular Biology and Evolution 34: 772–773. https://doi.org/10.1093/molbev/msw260

Leaché AD, Fujita MK (2010) Bayesian species delimitation in West African forest geckos (*Hemidactylus fasciatus*). Proceedings of the Royal Society B: Biological Sciences 277: 3071–3077. https://doi.org/10.1098/rspb.2010.0662

Li T, Gao JJ, Lu JM, Ji XL, Chen HW (2013) Phylogenetic relationship among East Asian species of the *Stegana* genus group (Diptera, Drosophilidae). Molecular Phylogenetics and Evolution 66: 412–416. https://doi.org/10.1016/j.ympev.2012.09.004

Linnaeus C (1767) Systema naturae, per regna tria naturae, secundum classes, ordines, genera, species, cum caracteribus, differentiis, synonymis, locis. Tomus I. Pars 2. Editio duodecima, reformata. Laurentius Salvius, Holmiae, pp. 533–1327.

Lu J, Zhang Y, Chen H (2018) Integrative taxonomy of the genus *Pseudostegana* (Diptera, Drosophilidae) from China, with descriptions of eleven new species. PeerJ 6: e5160. https://doi.org/10.7717/peerj.5160

Meigen JW (1830) Systematische Beschreibung der bekannten europäischen zweiflügeligen Insekten (Vol. 6). Schulze, 404 pp.

Nguyen LT, Schmidt HA, von Haeseler A, Minh BQ (2015) IQ-TREE: A fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Molecular Biology and Evolution 32: 268–274. https://doi.org/10.1093/molbev/msu300

Otranto D, Cantacessi C, Lia RP, Kadow ICG, Purayil SK, Dantas-Torres F, Máca J (2012) First laboratory culture of *Phortica variegata* (Diptera, Steganinae), a vector of *Thelazia callipaeda*. Journal of Vector Ecology 37: 458–461. https://doi.org/10.1111/j.1948-7134.2012.00251.x

Puillandre N, Lambert A, Brouillet S, Achaz G (2012) ABGD, automatic barcode gap discovery for primary species delimitation. Molecular Ecology 21: 1864–1877. https://doi.org/10.1111/j.1365-294X.2011.05239.x

Rambaut A, Drummond AJ, Xie D, Baele G, Suchard MA (2018) Posterior summarization in Bayesian phylogenetics using Tracer 1.7. Systematic Biology 67: 901–904. https://doi.org/10.1093/sysbio/syy032

Rannala B, Yang Z (2003) Bayes estimation of species divergence times and ancestral population sizes using DNA sequences from multiple loci. Genetics 164: 1645–1656. https://doi.org/10.1093/genetics/164.4.1645

Rice G, David JR, Kamimura Y, Masly JP, Mcgregor AP, Nagy O, Noselli S, Nunes MDS, O'Grady P, Sánchez-Herrero E, Siegal ML, Toda MJ, Rebeiz M, Courtier-Orgogozo V, Yassin A (2019) A standardized nomenclature and atlas of the male terminalia of *Drosophila melanogaster*. Fly 13: 51–64. https://doi.org/10.1080/19336934.2019.1653733

Ronquist F, Huelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19: 1572–1574. https://doi.org/10.1093/bioinformatics/btg180

Shao ZF, Li T, Jiang JJ, Lu JM, Chen HW (2014) Molecular phylogenetic analysis of the *Amiota taurusata* species group within the Chinese species, with descriptions of two new species. Journal of Insect Science 14: 33. https://doi.org/10.1093/jis/14.1.33

Shorthouse DP (2010) SimpleMappr, an online tool to produce publication-quality point maps. https://www.simplemappr.net (accessed October 9, 2022).

Sidorenko VS (1990) The review of the palearctic drosophilid flies of the subgenus *Stegana* Mg. (Diptera, Drosophilidae) with description of new species from Soviet Far East. News of Insects – Systematics of Soviet Far East 1990: 126–128 (In Russian).

Sidorenko VS (1998) New Asian species and new records of the genus *Stegana* Meigen (Diptera: Drosophilidae). III. Descriptions, taxonomic remarks and key to the Asian species. Annales de la Société Entomologique de France (N.S.) 34: 285–300.

Sidorenko VS (2002) Phylogeny of the tribe Steganini Hendel and some related taxa (Diptera, Drosophilidae). Far Eastern Entomologist 111: 1–20.

Suchard MA, Lemey P, Baele G, Ayres DL, Drummond AJ, Rambaut A (2018) Bayesian phylogenetic and phylodynamic data integration using BEAST 1.10. Virus Evolution 4: vey016. https://doi.org/10.1093/ve/vey016

Toda MJ, Bänziger H, Sati PC, Fartyal RS, Suwito A, Katoh T (2020) Taxonomy and evolution of asymmetric male genitalia in the subgenus *Ashima* Chen (Diptera: Drosophilidae: *Phortica* Schiner), with descriptions of seven new species. Zootaxa 4789: 1–54. https://doi.org/10.11646/zootaxa.4789.1.1

Vilela CR, Bächli G (2020) On the identities of Neotropical *Stegana* species (Diptera, Drosophilidae). II. *Stegana acutangular* (Hendel) and *Stegana triseta* (Duda), with descriptions of three new closely related species. Revista Brasileira de Entomologia 64: e20200097. https://doi.org/10.1590/1806-9665-RBENT-2020-0097

Wang BC, Park J, Watabe HA, Gao JJ, Xiangyu JG, Aotsuka T, Chen HW, Zhang YP (2006) Molecular phylogeny of the *Drosophila virilis* section (Diptera: Drosophilidae) based on mitochondrial and nuclear sequences. Molecular Phylogenetics and Evolution 40: 484–500. https://doi.org/10.1016/j.ympev.2006.03.026

Wang N, Zhang Y, Cheng Y, Chen H (2017) Nine new species of the subgenus *Stegana* (*Oxyphortica*) from the Oriental region, with morphological and molecular evidence based on Chinese species (Diptera: Drosophilidae). Journal of Natural History 51: 1943–1970. https://doi.org/10.1080/00222933.2017.1360960

Wang Y, Chen H (2020) Eleven new species in the genus *Amiota* (Diptera: Drosophilidae) from Southwest China. Entomotaxonomia 42: 206–226. https://doi.org/10.11680/entomotax.2020024

Wang YL (2019) Phylogenetics and evolution of morphological traits of *Amiota* (Diptera: Drosophilidae) from East Asia. PhD Thesis, South China Agricultural University, Guangzhou, China.

Wang YL, Cao HL, Chen HW (2020) Molecular phylogeny and species delimitation of *Amiota alboguttata* and *Amiota basdeni* species groups (Diptera: Drosophilidae) from East Asia. Zoological Journal of the Linnean Society 189: 1370–1397. https://doi.org/10.1093/zoolinnean/zlz132

Wang YL, Li Q, Toda MJ, Gao JJ (2021a) The genus *Dettopsomyia* Lamb, 1914 (Diptera, Drosophilidae) from Southern China. ZooKeys 1056: 73–94. https://doi.org/10.3897/zookeys.1056.56996

Wang YL, Wang NN, Zhang Y, Tsaur SC, Chen HW (2021b) Cryptic diversity in the subgenus *Oxyphortica* (Diptera, Drosophilidae, *Stegana*). PeerJ 9: e12347. https://doi.org/10.7717/peerj.12347

Wang YL, Lu JM, Gao JJ, Zhang Y, Chen HW (2022) Molecular phylogeny and systematics of the subgenus *Oxyphortica* Duda from the Oriental region (Diptera: Drosophilidae: *Stegana*). Systematic Entomology 47: 52–64. https://doi.org/10.1111/syen.12515

Wang YL, Li BX, Cui MD, Zhang Y, Wang L, Zhang CH, Tsaur SC, Chen HW, Huang J (2023) Revision of the subgenus *Stegana* (*Steganina*) from China, with assessment of species delimitation using DNA barcodes (Diptera, Drosophilidae). Zootaxa 5250: 1–109. https://doi.org/10.11646/zootaxa.5250.1.1

Wheeler MR (1960) A new subgenus and species of stegana meigen (Diptera: Drosophilidae). Proceedings of The Entomological Society of Washington 62: 109–111.

Wiens JJ, Ackerly DD, Allen AP, Anacker BL, Buckley LB, Cornell HV, Damschen EI, Davies TJ, Grytnes JA, Harrison SP, Hawkins BA, Holt RD, McCain CM, Stephens PR (2010) Niche conservatism as an emerging principle in ecology and conservation biology. Ecology Letters 13: 1310–1324. https://doi.org/10.1111/j.1461-0248.2010.01515.x

Xie Q, Bu WJ (2010) Evolutionary biology. Higher Education Press, Beijing, 449 pp.

Xing Y, Ree RH (2017) Uplift-driven diversification in the Hengduan Mountains, a temperate biodiversity hotspot. Proceedings of the National Academy of Sciences of the United States of America 114: E3444–E3451. https://doi.org/10.1073/pnas.1616063114

Xu M, Gao J, Chen H (2007) Genus *Amiota* Loew (Diptera: Drosophilidae) from the Qinling mountain system, central China. Entomological Science 10: 65–71. https://doi.org/10.1111/j.1479-8298.2006.00199.x

Yang Z (2015) The BPP program for species tree estimation and species delimitation. Current Zoology 61: 854–865. https://doi.org/10.1093/czoolo/61.5.854

Yang Z, Rannala B (2010) Bayesian species delimitation using multilocus sequence data. Proceedings of the National Academy of Sciences of the United States of America 107: 9264–9269. https://doi.org/10.1073/pnas.0913022107

Yassin A (2016) Unresolved questions in genitalia coevolution: bridging taxonomy, speciation, and developmental genetics. Organisms Diversity & Evolution 16: 681–688. https://doi.org/10.1007/s13127-016-0286-2

Zhang W, Chen H (2006) The genus *Amiota* (Diptera: Drosophilidae) from Hengduan Mountains, southwestern China. European Journal of Entomology 103: 483–495. https://doi.org/10.14411/eje.2006.062

Zhang Y, Xu M, Li T, Chen H (2012) Revision of the subgenus *Orthostegana* (Diptera: Drosophilidae: *Stegana*) from Eastern Asia. Entomotaxonomia 34: 361–374.

Zhang Y, Li T, Chen H (2016) The *Stegana* (sensu stricto) species from China, with morphological and molecular evidence (Diptera: Drosophilidae). Systematics and Biodiversity 14: 118–130. https://doi.org/10.1080/14772000.2015.1099574