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Survey of the genus *Stegana* Meigen (Diptera, Drosophilidae) from Taiwan, with DNA barcodes and descriptions of three new species

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Abstract

Background: Twelve *Stegana* species have been reported from Taiwan, yet only four were also recorded from Mainland China. This may not reflect the actual fauna between both sides of the strait. This report mainly deals with a fly collection of the genus *Stegana* during a short visit to Taiwan in 2012. It represents the most recent drosophilid faunal survey of Taiwan associating with bleeding trees.

Results: In this study, 17 species were recognized including three new ones and eight new records. They are *Stegana (Oxyphortica) convergens* (de Meijere, 1911); *Stegana (Oxyphortica) nigripennis* (Hendel, 1914); *Stegana (Stegana) taiwana* Okada, 1991; *Stegana (Steganina) bacilla* Chen and Aotsuka, 2004; *Stegana (Steganina) chitouensis* Sidorenko, 1998; *Stegana (Steganina) ctenaria* Nishiharu, 1979; *Stegana (Steganina) euryphylla* Chen and Chen, 2009; *Stegana (Steganina) langufoliacea* Wu, Gao and Chen, 2010; *Stegana (Steganina) melanostoma* Chen and Chen, 2009; *Stegana (Steganina) nigrolimbata* Duda, 1924; *Stegana (Steganina) ornatipes* Wheeler and Takada, 1964; *Stegana (Steganina) reni* Wang, Gao and Chen, 2010; *Stegana (Steganina) tongi* Sp. nov.; *Stegana (Steganina) wulai* sp. nov. Six recorded species are redescribed based on new materials. The key to all species of the genus *Stegana* in Taiwan is presented. The DNA barcoding fragments of the mitochondrial *COI* gene are sequenced and used to delineate species.

Conclusions: Among the 23 recorded species, two most widely distributed species range across two zoogeographic regions, and one occurs in both Taiwan and India. Five species are insular species recorded from Taiwan and Japan. The remaining 16 species are distributed in both Taiwan and southern Mainland China. One montane species was collected at an elevation of 1,500 m. As a whole, this implies that Taiwanese *Stegana* fauna should be largely of a Mainland China origin, probably as a consequence of the east- and/or southward dispersals of the ancestral species during the glacial epoch. The 20% (5/23) endemism at the genus level is comparable to that of the family level at 63/320. It is notable that the Fujian province, which is bordered by Guangdong to the south but isolated from Taiwan by the 180-km-wide strait, has no species in common with Guangdong and Taiwan. This may be due to insufficient drosophilid faunal survey in these areas, especially for Fujian.

Keywords: Barcoding; COI gene; Drosophilid; East Asia; Fauna; Taxonomy

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Background

This study is concerned with the genus Stegana Meigen (1830) mostly collected from Taiwan. Conspecific specimens from Mainland China were also included for comparison whenever possible. Before this study, twelve Stegana species have been reported from Taiwan (Brake and Bächli 2008), namely Stegana (Orthostegana) curvinervis (Hendel, 1914); Stegana (Oxyphortica) convergens (de Meijere, 1911); Stegana (Oxyphortica) nigripennis (Hendel, 1914); Stegana (Stegana) antlia Okada, 1991; Stegana (Stegana) taiwana Okada, 1991; Stegana (Steganina) chitouensis Sidorenko, 1998; Stegana (Steganina) izu Sidorenko, 1997; Stegana (Steganina) kanmiyai Okada and Sidorenko, 1992; Stegana (Steganina) nigrithorax Strobl, 1898; Stegana (Steganina) nigrolimbata Duda, 1924; Stegana (Steganina) ornatipes Wheeler and Takada, 1964; and Stegana (Steganina) shirozui Okada, 1971. However, the taxonomic descriptions of these species were largely inadequate, especially in illustrations of male terminalia and the diagnoses. Here, we reported 17 species including three new ones and eight new records; six known species are redescribed, with emphases given to diagnostic drawings as supplement. The DNA barcoding fragments of the mitochondrial COI gene are sequenced for 28 representative individuals of the aforementioned 17 species (Table 1). This brings the total number of Taiwanese Stegana species to 23.

Methods

Materials and morphological terminology

The *Stegana* flies have been collected mostly from tree trunks and tussocks nearby streams in forests. All the specimens examined here were preserved in 75% ethanol, then dried and pinned after morphological examination and identification in the laboratory, and deposited in the Department of Entomology, South China Agricultural University, Guangzhou, China (SCAU). We followed Zhang and Toda (1992) and Chen and Toda (2001) for the definitions of measurements, indices, and abbreviations.

DNA extraction, sequencing, and sequence alignment

A total of 28 representative individuals of 17 species were employed for DNA sequencing of the mitochondrial *COI* gene (Table 1). For this, total DNA was extracted from each fly using the TIANGEN[™] DNA extraction kit following the manufacturer's instructions. The *COI* fragments were amplified using the cycle protocol as in Zhao et al. (2009). The polymerase chain reaction (PCR)/sequencing primer pair were 5'-CGCCTAAACTTCAGCCACTT-3' (Wang et al. 2006) and 5'-TAAACTTCAGGGTGAC CAAAAAATCA-3' (Folmer et al. 1994). The PCR products were purified and then directly sequenced on Takara sequencer (Kyoto, Japan). The obtained nucleotide sequences were translated into amino acid sequences to ensure their integrity and accuracy and then were aligned with the ClustalW as implemented in MEGA 5.05 (Tamura et al. 2011) to rectify the nucleotide level alignment.

Calculation of genetic distances

Our *COI* sequences were 568 to 708 base pairs in length; the obtained sequences were submitted to the BOLD and the GenBank (Table 1). Using these sequences, Kimura's two-parameter (K-2P; Kimura 1980) genetic distances among the 17 species were calculated also in MEGA.

Taxonomic account

Stegana (Oxyphortica) convergens (de Meijere)
Drosophila convergens de Meijere, 1911: 400
Orthostegana convergens: Hendel, 1914: 115
Stegana convergens: Sturtevant, 1921: 135
Oxyphortica convergens: Duda, 1923: 34; Duda, 1924: 182
Stegana (Oxyphortica) convergens: Okada, 1971: 90;
Cheng et al., 2010: 58

The diagnosis, measurements, specimens examined, and distribution are as follows:

- *Diagnosis*. Surstylus with two strong prensisetae, one each basally and submedially (Figure 1B); dorsal lobe of gonopods slender on distal half, nearly as long as aedeagus (Figure 1C,D).
- Measurements. Body length (BL) = 2.75 to 3.85 mm in five \checkmark and 3.10 to 3.60 mm in five $\stackrel{\bigcirc}{\rightarrow}$, thorax length (THL) = 1.25 to 1.75 mm in $\overline{\triangleleft}$ and 1.25 to 1.48 mm in $\stackrel{\circ}{\rightarrow}$, wing length (WL) = 2.30 to 2.95 mm in \bigcirc and 2.50 to 2.95 mm in $\stackrel{\bigcirc}{\rightarrow}$, wing width (WW) = 1.00 to 1.35 in \checkmark and 1.15 to 1.35 mm in $\stackrel{\circ}{\rightarrow}$, dorsal branches/ventral branches of arista (arb) = 4 to 6/3 to 4, longest ventral branch/longest dorsal branch of arista in length (avd) = 0.63 to 0.86, longest dorsal branch of arista/width of the first flagellomere (adf) = 1.29 to 2.67, length/width of the first flagellomere (flw) = 1.50 to 2.33, frontal width/head width (FW/HW) = 0.46 to 0.62, maximum width of gena/maximum diameter of the eye (ch/o) = 0.14to 0.20, proclinate orbital/posterior reclinate orbital in length (prorb) = 0.88 to 1.29, anterior reclinate orbital/posterior reclinate orbital in length (rcorb) = 0.33 to 0.88, distance between proclinate and posterior reclinate orbitals/distance between inner vertical and posterior reclinate orbital (orbito) = 1.67 to 1.80, subvibrissal/vibrissa in length (vb) = 0.33 to 0.57, anterior dorsocentral/posterior dorsocentral in length (dcl) = 0.41 to 0.60, prescutellar/posterior dorsocentral in length (presctl) = 0.47 to 0.58, basal scutellar/apical scutellar in length (sctl) = 1.10 to 1.33, anterior katepisternal/

Table	1 D	Petails	of	the	samp	les	using	in	COI	sequencing	and	accession	numbers
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Species	Collection sites	BOLD process ID	GenBank accession numbers
S. (O.) convergens Okada, 1971	Bahsienshan, Taichung, Taiwan	BDORB005-13	KF642615
S. (O.) convergens Okada, 1971	Guanshan, Taitung, Taiwan	BDORB006-13	KF642616
S. (O.) convergens Okada, 1971	Wulai, Hsinpei, Taiwan	BDORB007-13	KF642617
S. (O.) nigripennis Hendel, 1914	Guanghua, Chiayi, Taiwan	BDORB014-13	KF642624
S. (O.) nigripennis Hendel, 1914	Wulai, Hsinpei, Taiwan	BDORB013-13	KF642623
S. (S.) <i>taiwana</i> Okada, 1991	Wulu, Taitung, Taiwan	BDORG014-13	KF670987
S. (S.) <i>yangi</i> sp. nov.	Renai, Nantou, Taiwan	BDORG005-13	KC861380
S. (S.) <i>yangi</i> sp. nov.	Wulai, Hsinpei, Taiwan	BDORG006-13	KC861379
S. (Sti.) bacilla Chen and Aotsuka, 2004	Guanshan, Taitung, Taiwan	BDORB001-13	KF642611
S. (Sti.) chitouensis Sidorenko, 1998	Shuili, Nantou, Taiwan	BDORB002-13	KF642612
S. (Sti.) chitouensis Sidorenko, 1998	Wulai, Hsinpei, Taiwan	BDORB003-13	KF642613
S. (Sti.) chitouensis Sidorenko, 1998	Guanshan, Taitung, Taiwan	BDORB004-13	KF642614
S. (Sti.) ornatipes Wheeler and Takada, 1964	Mingchih, Ilan, Taiwan	BDORB019-13	KF642628
S. (Sti.) ornatipes Wheeler and Takada, 1964	Guanshan, Taitung, Taiwan	BDORB021-13	KF642629
S. (Sti.) langufoliacea Wu, Gao and Chen, 2010	Guanshan, Taitung, Taiwan	BDORB011-13	KF642621
S. (Sti.) ctenaria Nishiharu, 1979	Guanghua, Chiayi, Taiwan	BDORB008-13	KF642618
S. (Sti.) ctenaria Nishiharu, 1979	Guanshan, Taitung, Taiwan	BDORB009-13	KF642619
S. (Sti.) euryphylla Chen and Chen, 2009	Chipen, Taitung, Taiwan	BDORB010-13	KF642620
S. (Sti.) melanostoma Chen and Chen, 2009	Shuili, Nantou, Taiwan	BDORB012-13	KF642622
S. (Sti.) nigrolimbata Duda, 1924	Shuili, Nantou, Taiwan	BDORB016-13	KF642625
S. (Sti.) nigrolimbata Duda, 1924	Wulai, Hsinpei, Taiwan	BDORB017-13	KF642626
S. (Sti.) nigrolimbata Duda, 1924	Guanghua, Chiayi, Taiwan	BDORB018-13	KF642627
S. (Sti.) reni Wang, Gao and Chen, 2011	Chipen, Taitung, Taiwan	BDORB022-13	KF642630
S. (Sti.) tongi Wang, Gao and Chen, 2011	Shuili, Nantou, Taiwan	BDORB024-13	KF642632
S. (Sti.) xui Wang, Gao and Chen, 2011	Chipen, Taitung, Taiwan	BDORB025-13	KF642633
S. (Sti.) jianqinae sp. nov.	Wugongshan, Kaohsiung, Taiwan	BDORG018-13	KF670983
S. (Sti.) jianqinae sp. nov.	Nonggang, Guangxi, China	BDORG017-13	KF670984
S. (Sti.) wulai sp. nov.	Wulai, Hsinpei, Taiwan	BDORB023-13	KF642631

O., subgenus Oxyphortica; S., Stegana; Sti., subgenus Steganina.

posterior katepisternal in length (sterno) = 0.69 to 0.93, length distance between ipsilateral dorsocentrals/cross distance between anterior dorsocentrals (dcp) = 0.77 to 0.92, distance between ipsilateral scutellars/cross distance between apical scutellars (sctlp) = 1.00 to 1.33, second costal section between subcostal break and R₂₊₃/third costal section between R_{2+3} and R_{4+5} (C) = 2.92 to 3.33, M_1 between dm-cu and wing margin/M₁ between r-m and dm-cu (4v) = 1.64 to 2.33, third costal section between R_{2+3} and R_{4+5}/M_1 between r-m and dm-cu (4c) = 0.71 to 0.82, CuA₁ between dm-cu and wing margin/ M_1 between r-m and dm-cu (M) = 0.44 to 0.55, CuA₁ between dm-cu and wing margin/dm-cu between M_1 and CuA_1 (5x) = 1.17 to 1.50, length of heavy setation in the third costal section/length of the third costal section (C3F) = 0.75 to 0.84, and third costal section between R_{2+3} and R_{4+5} /fourth costal section (ac) = 3.00 to 4.00.

- *Specimens examined.* Taiwan: 7 ♂, 5 ♀ (SCAU, no. 123408–19), Taichung, Bahsienshan, 5.vi.2011, swept from tussock, XY Liu; 9 ♂, 7 ♀ (SCAU, no. 124020–35), Taitung, Chipen, 23°10' N, 121°03' E, altitude 500 m, 29,30.x.2012, swept from tree trunks, HW Chen and JJ Gao; 2 ♂, 1 ♀ (SCAU, no. 124036–38), Hsinpei, Wulai, 24°46' N, 121°35' E, altitude 400 m, 19.x.2012, HW Chen and JJ Gao.
- *Distribution.* Taiwan, Vietnam, Indonesia (Java), and Papua New Guinea.

Stegana (Oxyphortica) nigripennis (Hendel) Orthostegana nigripennis Hendel, 1914: 115



Chaetocnema (Oxyphortica) poeciloptera Duda, 1926: 243; synonymized by Okada, 1971: 89

Protostegana kanoi Okada, 1956: 14; synonymized by Okada, 1968: 304

Stegana (Stegana) nigripennis: Okada, 1968: 304 Stegana (Orhtostegana) nigripennis: Okada, 1971: 89 Stegana (Oxyphortica) nigripennis: Wheeler, 1981: 30; Chen and Aotsuka, 2004: 2780

The specimens examined and distribution are as follows:

- *Specimens examined.* Taiwan: 1 ♂, 1 ♀ (SCAU, no. 123301–02), Chiayi, Guanghua, 23°28′47′′ N, 120° 39′11′′ E, altitude 630 m, 16.x.2012, swept from tree trunk, HW Chen; 1 ♂, 1 ♀ (SCAU, no. 123303–04), Hsinpei, Wulai, altitude 400 m, 19.×.2012, swept from tree trunk, HW Chen.
- *Distribution*. Mainland China (Guangxi, Hainan), Taiwan, and Japan (Ryukyu Islands).

Stegana (Stegana) taiwana Okada

Stegana (Stegana) taiwana Okada in Sidorenko and Okada, 1991: 657

The diagnosis, description, measurements, specimen examined, and distribution are as follows:

- *Diagnosis.* Gonopods sharply curved dorsad, nearly triangular distally (Figure 2C); aedeagus laterally expanded ventrad, lacking spine-like processes (Figure 2D,E).
- Description. Only the characters that depart from the universal description, as given by Li et al. (2010) for this subgenus, are provided for brevity. Male: frons, yellow. Lunule, black. First flagellomere, yellow on basal one third, black on distal two third. Face, yellow above, brown on middle and along lower margin. Clypeus, brown. Scutum, brown, with three thin, yellow longitudinal stripes medially and sublaterally. Katepisternum, dark brown above, white below. Scutellum, brown. Legs, white, brown on knee of foreleg, distal half of femur of midleg, and femur of hind legs. Fore femur with four setae on distal part of ventral surface. Abdominal, all tergites and sternites brown. Male terminalia: surstylus rounded, lacking prensiseta (Figure 2B). Parameres fused basally, ipsilaterally with a long seta and several minute sensilla (Figure 2D,E). Aedeagus with numerous tentacle-like setae and some serrations along the peristome (Figure 2D,E).
- *Measurements.* BL = 2.13 mm, THL = 1.00 mm, WL = 1.70 mm, WW = 0.97 mm, arb = 5/4, avd = 0.82, adf = 1.57, flw = 2.00, FW/HW = 0.33, ch/o = 0.14, prorb = 1.15, rcorb = 0.77, vb = 0.46, dcl = 0.35, presctl = 0.55, sctl = damaged, sterno = 0.88, orbito = 1.67, dcp = 0.27, sctlp = 1.50, C = 1.83, 4c = 1.36, 4v = 2.09, 5x = 1.17, ac = 7.50, M = 0.64, and C3F = 0.76
- *Specimen examined.* Taiwan: 1 ♂ (SCAU, no. 121743), Taitung, Guanshan, 23°09′54′′ N, 121°02′



 $49^{\prime\prime}$ E, altitude 350 m, 28.x.2012, swept from tree trunk, HW Chen

• Distribution. Taiwan

Stegana (Stegana) yangi Zhang, Tsaur and Chen, sp. nov. The diagnosis, description, measurements, type of materials, etymology, and distribution are as follows:

- *Diagnosis.* Aedeagus with one rod-like process basoventrally (pr; Figure 3E); gonopods laterally, triangularly expanded ventrad (Figure 3D).
- *Description*. Only the characters that depart from the universal description, as given by Li et al. (2010) for this subgenus, are provided for brevity. Male and female: frons, yellow. Lunule, slightly brown. Pedicel, yellow brown; first flagellomere, nearly black. Face, yellow, slightly brown on lower margin. Clypeus, brown. Mesonotum, brown in male, dark brown in female, in both sexes with one indistinct, thin, yellow longitudinal stripe medially. Katepisternum, yellow, with brown longitudinal stripe above. Basisternum, white. Scutellum, brown in male, dark brown in female. Costal vein with *ca.* six minute spinules on ventral surface between R₂₊₃ and R₄₊₅.

Halters, almost gray. Legs, yellowish white; foreleg, brown on knee; midleg and hind legs, dark brown on distal two third of femora and basal one third of hind tibiae. Fore femur with four setae on distal part of ventral surface. Abdominal, all tergites brown in male, dark brown in female, with yellow parches near lateral margins of the second to fourth tergites. Sternites, brown. Male terminalia: surstylus with one prensiseta on the outer surface (Figure 3A). Parameres fused basally to a U-shaped formation, each with one long seta apically and several minute sensilla submedially (Figure 3D). Aedeagus with numerous tentacle-like setae along the gonopore (Figure 3D).

Measurements. BL = 2.80 mm in holotype (range in 2 ♂ and 1 ♀ paratypes: 2.53 to 2.87 mm in ♂, 2.80 mm in ♀), THL = 1.33 mm (1.27 to 1.40 mm in ♂, 1.40 mm in ♀), WL = 2.00 mm (1.93 to 2.00 mm in ♂, 2.13 mm in ♀), WW = 1.07 mm (1.13 to 1.17 mm in ♂, 1.23 mm in ♀), arb = 7/5 (6 to 7/5 to 6), avd = 0.83 (0.79 to 0.83), adf = 2.00 (2.00 to 2.40), flw = 2.67 (2.33 to 3.00), FW/HW = 0.35 (0.36 to 0.37), ch/o = 0.11 (0.10 to 0.11), prorb = 1.13 (1.12 to 1.25), rcorb = 0.80 (0.82 to 0.88), vb = 0.41 (0.40 to



0.50), dcl = 0.56 (0.48 to 0.50), presctl = 0.72 (0.66 to 0.69), sctl = 1.85 (1.83 to 2.00), sterno = 0.90 (0.88 to 0.92), orbito = 1.63 (1.50 to 1.71), dcp = 0.24 (0.19 to 0.23), sctlp = 1.20 (1.13 to 1.25), C = 1.72 (1.80 to 1.95), 4c = 1.44 (1.38 to 1.40), 4v = 2.07 (1.96 to 2.10), 5x = 1.07 (1.08 to 1.25), ac = 7.80 (7.00 to 8.20), M = 0.56 (0.56 to 0.67), and C3F = 0.71 (0.63 to 0.68).

- *Type of materials*. Holotype ♂ (SCAU, no. 121744), Taiwan: Nantou, Renai, altitude 675 m, 11.vi.2010, D Yang. Paratypes, Taiwan: 1 ♂ (SCAU, no. 122944), Hsinpei, Wulai, altitude 400 m, 19.×.2012, swept from tussock, JJ Gao; 1 ♂, 1 ♀ (SCAU, nos. 121260, 61), Taitung, Guanshan, altitude 350 m, 28.×.2012, swept from tree trunks, HW Chen.
- *Etymology*. Patronym of the collector, Prof. Ding Yang (China Agricultural University).
- Distribution. Taiwan

Stegana (Steganina) bacilla Chen and Aotsuka, new record

Stegana (Steganina) bacilla Chen and Aotsuka, 2004: 2785 The specimens examined and distribution are as follows:

- *Specimens examined*. Taiwan: 7 ♂ (SCAU, no. 123305–11), Taitung, Guanshan, altitude 770 m, 30.×.2012, swept from tree trunks, HW Chen and JJ Gao.
- Distribution. Taiwan and Japan (Ryukyu Islands).

Stegana (Steganina) chitouensis Sidorenko

Stegana (Steganina) chitouensis Sidorenko, 1998: 292; Cheng et al., 2009: 39 The diagnosis, measurements, specimens examined, and distribution are as follows:

- *Diagnosis*. Paramere rounded and weekly sclerotized (Figure 4B,C); aedeagus trilobed: dorsal rod single, strongly sclerotized, ventral rod coupled, with dense pubescence and four finger-like processes per side (Figure 4B,C) (modified from Cheng et al. 2009).
- *Measurements.* BL = 1.60 to 2.93 mm in 5 ♂, THL = 1.27 to 1.47 mm, WL = 1.97 to 2.23 mm, WW = 1.03 to 1.27 mm, arb = 5 to 6/4 to 5, avd = 0.64 to 0.82, adf = 1.11 to 2.00, flw = 1.78 to 2.17, FW/HW = 0.32 to 0.43, ch/o = 0.11 to 0.16, prorb = 1.07 to 1.25, rcorb = 0.69 to 0.82, vb = 0.35 to 0.50, dcl = 0.31 to 0.34, presctl = 0.46 to 0.56, sctl = 1.48 to 1.73, sterno = 0.85 to 0.90, orbito = 1.43 to 1.67, dcp = 0.21 to 0.23, sctlp = 1.13 to 1.25, C = 1.75 to 1.88, 4c = 1.30 to 1.44, 4v = 1.73 to 1.96, 5x = 1.07 to 1.18, ac = 7.20 to 10.75, M = 0.43 to 0.56, and C3F = 0.58 to 0.69
- Specimens examined. Taiwan: 1 ♂ (SCAU, no. 123312), Nantou, Lugu, 23°28′47′′ N, 120°39′10′′ E, altitude 1,150 m, 16.x.2012, swept from tussock, JJ Gao; 3 ♂ (SCAU, no. 123313–15), Hsinpei, Wulai, altitude 400 m, 19.x.2012, swept from tussock, HW Chen and JJ Gao; 1 ♂ (SCAU, no. 123316), Taitung, Guanshan, altitude 770 m, 28.x.2012, swept from tree trunk, HW Chen.
- Distribution. Taiwan

Stegana (Steganina) ctenaria Nishiharu, new record

Stegana (Steganina) ctenaria Nishiharu, 1979: 38; Chen et al., 2009: 1912



The diagnosis, measurements, specimens examined, and distribution are as follows:

• *Diagnosis.* This species resembles *Stegana* (*Steganina*) *multidentata* Chen et al., 2009 from Hubei, China in the gonopods rounded apically and aedeagus with one pair of processes bearing

minute serrations but can be distinguished from the latter by epandrium not broadened on posterior margins, approximately twice as high as wide (Figure 5A); aedeagal opening oval in ventral view (Figure 5C).

• *Measurements*. BL = 3.13 to 3.40 mm in 2 ♂ and 3.33 mm in 1 ♀, THL = 1.53 to 1.73 mm in ♂,



1.87 mm in \bigcirc , WL = 2.50 to 2.63 mm in \bigtriangledown , 2.60 mm in \bigcirc , WW = 1.33 to 1.50 mm in \bigtriangledown , 1.37 mm in \bigcirc , arb = 6 to 7/5, avd = 0.67 to 0.73, adf = 2.29 to 3.00, flw = 2.43 to 3.00, FW/HW = 0.40 to 0.44, ch/o = 0.10 to 0.11, prorb = 1.05 to 1.15, rcorb = 0.72 to 0.75, vb = 0.31 to 0.44, dcl = 0.43 to 0.44, presctl = 0.50 to 0.61, sctl = 1.70 to 1.80, sterno = 0.63 to 0.93, orbito = 1.30 to 1.56, dcp = 0.20 to 0.26, sctlp = 1.47 to 1.69, C = 1.84 to 2.09, 4c = 1.05 to 1.14, 4v = 1.71 to 1.73, 5x = 1.53 to 1.83, ac = 9.20 to 12.50, M = 0.48 to 0.55, and C3F = 0.67 to 0.74

- Specimens examined. Taiwan: 1 ♂ (SCAU, no. 123405), Chiayi, Guanghua, altitude 620 m, 16.×.2012, swept from tree trunk, HW Chen; 1 ♂; 1 ♀ (SCAU, no. 123406–07), Taitung, Guanshan, altitude 740 m, 28.×.2012, swept from tree trunk, HW Chen.
- *Distribution*. Mainland China (Jilin and Liaoning), Taiwan, Russia (Far East), Japan (Hokkaido, Honshu, and Kyushu), and Korea.

Stegana (Steganina) euryphylla Chen and Chen, new record

Stegana (Steganina) euryphylla Chen and Chen, 2009: 498 The specimens examined and distribution are as follows:

- *Specimens examined*. Taiwan: 4 ♂ (SCAU, no. 123317–20), Taitung, Chipen, altitude 500 m, 30.×.2012, swept from tree trunks, HW Chen.
- Distribution. Mainland China (Yunnan) and Taiwan.

Stegana (Steganina) langufoliacea Wu, Gao and Chen, new record

Stegana (*Steganina*) *langufoliacea* Wu et al., 2010: 51 The specimens examined and distribution are as follows:

- *Specimens examined*. Taiwan: 4 ♂ (SCAU, no. 123321–24), Chiayi, Guanghua, altitude 620 m, 16.×.2012, swept from tree trunk, HW Chen.
- Distribution. Mainland China (Guangxi) and Taiwan

Stegana (Steganina) melanostoma Chen and Chen, new record Stegana (Steganina) melanostoma Chen and Chen, 2008: 57

The specimens examined and distribution are as follows:

- *Specimen examined.* Taiwan: 1 ♂ (SCAU, no. 123325), Nantou, Renai, altitude 675 m, 11.vi.2010, D Yang.
- Distribution. Mainland China (Hubei) and Taiwan.

Stegana (Steganina) nigrolimbata Duda

Stegana nigrolimbata Duda, 1924: 181

Stegana (*Steganina*) *nigrolimbata*: Okada, 1971: 83; Cao and Chen, 2008: 29 The diagnosis, description, measurements, specimens examined, and distribution are as follows:

- *Diagnosis*. Katepisternum almost brown, slightly pale ventrally; aedeagal filiform-like processes broadly separated (Figure 6C).
- Description. Male and female: Frons, brown, with indistinct, narrow, yellow band submedially. Palpus, yellow basally, black distally. Gena, brownish. Mesonotum, dark brown medially, brown laterally. Postpronotal lobe, yellowish white on anterior one half, brown on posterior one half. Katepisternum, dark brown above, brownish yellow below. Basisternum, brown. Scutellum, dark brown medially, yellow along margin. Legs, brown to dark brown, white on basal part of fore femur, apical part of mid tibia, and on greater part of mid and hind tarsomeres except for basal part of the first tarsomeres. Fore femur with three setae on distal part of ventral surface. Abdominal tergites, brown. Sternites, dark brown. Male terminalia: aedeagus distally with six pairs of rod-like and one pair of filiform-like processes (Figure 6C); aedeagal ventral lobe with sparse pubescence (Figure 6C).
- *Measurements.* BL = 3.00 to 3.07 mm in 4 ♂, 2.93 to 3.27 mm in 5 ♀, THL = 1.33 to 1.47 mm in ♂, 1.33 to 1.53 mm in ♀, WL = 2.00 to 2.10 mm in ♂, 2.10 to 2.37 mm in ♀, WW = 1.17 to 1.20 mm in ♂, 1.07 to 1.30 mm in ♀, arb = 6 to 7/4 to 5, avd = 0.62 to 0.82, adf = 1.71 to 2.17, flw = 2.29 to 2.80, FW/HW = 0.34 to 0.38, ch/o = 0.10 to 0.14, prorb = 1.06 to 1.31, rcorb = 0.77 to 0.88, vb = 0.36 to 0.44, dcl = 0.37 to 0.48, presctl = 0.52 to 0.64, sctl = 1.56 to 1.75, sterno = 0.84 to 0.89, orbito = 1.38 to 1.57, dcp = 0.21 to 0.22, sctlp = 1.11 to 1.36, C = 1.72 to 2.06, 4c = 1.13 to 1.33, 4v = 1.53 to 1.90, 5x = 1.14 to 1.46, ac = 8.20 to 10.25, M = 0.47 to 0.53, and C3F = 0.66 to 0.73.
- Specimens examined. Taiwan: 1 ♂ (SCAU, no. 123353), Nantou, Renai, altitude 675 m, 11.vi.2010, D Yang; 1 ♀ (SCAU, no. 123352), Kaohsiung, Wugongshan, 22°54′53′′ N, 120°39′00′′ E, altitude 640 m, 15.v.2011, XY Liu; 3 ♂ (SCAU, no. 123326–28), Nantou, Shuili, 23°45′42′′ N, 120°48′26′′ E, altitude 620 m, 18.×.2012, HW Chen; 5 ♂, 4 ♀ (SCAU, no. 123329–37), Nantou, Xiushan, 23°46′04′′ N, 120°45′21′′ E, altitude 350 m, 18.×.2012, swept from tree trunks and tussock, HW Chen and JJ Gao; 1 ♂, 1 ♀ (SCAU, no. 12338–39), Hsinpei, Wulai, altitude 400 m, 19.×.2012, swept from tussock, JJ Gao; 6 ♂, 6 ♀ (SCAU, no. 123340–51), Taitung, Guanshan, altitude 340 m, 28,29.×.2012, swept from tree trunk and tussock, HW Chen and JJ Gao.
- *Distribution*. Mainland China (Guangdong, Guangxi, Hainan, and Yunnan) and Taiwan.



Stegana (Steganina) ornatipes Wheeler and Takada

Stegana (*Steganina*) *ornatipes* Wheeler and Takada, 1964: 233; Cheng et al., 2009: 39

The diagnosis, measurements, specimens examined, and distribution are as follows:

- *Diagnosis*. Aedeagus composed of one curved process apically heaving pubescence and one membranous process (Figure 7C,D); projection of gonopods with one lobe-like and three vertical, spine-like processes on each side (Figure 7C,D) (following Cheng et al. 2009)
- *Measurements.* BL = 2.53 to 2.67 mm in 5 ♂ and 2.53 to 2.67 mm in 5 ♀, THL = 1.07 to 1.13 mm in ♂, 1.27 to 1.33 mm in ♀, WL = 1.70 to 1.77 mm in ♂, 1.90 to 2.00 mm in ♀, WW = 0.90 to 1.00 mm in ♂, 1.00 to 1.07 mm in ♀, arb = 5 to 6/4 to 5, avd = 0.73 to 0.90, adf = 1.57 to 2.20, flw = 2.14 to 3.20, FW/HW = 0.31 to 0.34, ch/o = 0.14 to 0.16, prorb = 1.07 to 1.21, rcorb = 0.80 to 0.87, vb = 0.24 to 0.33, dcl = 0.38 to 0.46, presctl = 0.50 to 0.57, sctl = 1.52 to 1.70, sterno = 0.74 to 0.83, orbito = 1.38 to 1.57, dcp = 0.19 to 0.23, sctlp = 1.08 to 1.17, C = 1.97 to 2.06, 4c = 1.17 to 1.29, 4v = 1.61 to 1.92, 5x = 1.00 to 1.42, ac = 6.60 to 10.33, M = 0.50 to 0.63, and C3F = 0.61 to 0.70.
- Specimens examined. Taiwan: 1 ♂ (SCAU, no. 123354), Ilan, Mingchih, 13.vi2011, swept from tussock, XY Liu; 4 ♂ (SCAU, no. 123355–58), Nantou, Shuili, altitude 620 m, 18.×.2012, swept from tussock, HW Chen and JJ Gao; 7 ♂, 5 ♀ (SCAU, no. 123359–70), Nantou, Xiushan, altitude 350 m, 18.×.2012, swept from tree trunks and tussock, HW Chen and JJ Gao; 4 ♂, 6 ♀ (SCAU, no. 123371–80), Hsinpei, Wulai, altitude 400 m, 19.×.2012, swept from tree trunks and tussock, HW Chen and JJ Gao; 7 ♂, 7 ♀ (SCAU, no. 123381–94), Taitung, Guanshan, altitude 340 to 770 m, 28,29.×.2012, swept from tree trunks and tussock, HW Chen and JJ Gao.
- *Distribution*. Taiwan, Japan (Ryukyu Islands), and Micronesia (Caroline Islands)

Stegana (Steganina) reni Wang, Gao and Chen, new record Stegana (Steganina) reni Wang et al., 2011: 507

The specimens examined and distribution are as follows:

- *Specimens examined*. Taiwan: 3 ♂ (SCAU, no. 123395), Taitung, Chipen, altitude 500 m, 30.×.2012, swept from tree trunks, HW Chen.
- Distribution. Mainland China (Yunnan) and Taiwan



Stegana (Steganina) tongi Wang, Gao and Chen, new record Stegana (Steganina) tongi Wang et al., 2011: 510

The specimens examined and distribution are as follows:

- Specimens examined. Taiwan: 4 ♂, 2 ♀ (SCAU, no. 123396–3401), Nantou, Shuili, altitude 620 m, 18.×.2012, swept from tree trunks, HW Chen and JJ Gao
- *Distribution*. Mainland China (Guangdong, Guangxi, and Hainan) and Taiwan.

Stegana (Steganina) xui Wang, Gao and Chen, new record

Stegana (Steganina) xui Wang et al., 2011: 516 The specimen examined and distribution are as follows:

- *Specimen examined*. Taiwan: 1 ♂ (SCAU, no. 123402), Taitung, Chipen, altitude 500 m, 30.×.2012, swept from tree trunk, HW Chen.
- Distribution. Mainland China (Yunnan) and Taiwan.

Stegana (Steganina) jianqinae Zhang, Tsaur and Chen, sp. nov. Stegana (Steganina) sp. in Li et al., 2013: 414

The diagnosis, description, measurements, type of materials, etymology, distribution, and remarks are as follows:

- *Diagnosis*. Surstylus with dense pubescence (Figure 8B); paramere strong, with dense pubescence (Figure 8D,E); aedeagus submedially with a pair of sclerotized, round processes (Figure 8D,E).
- *Description*. Only the characters that depart from the universal description, as given by Chen and Chen, 2009, for this subgenus are provided for brevity. Male: frons, shiny, brown. Pedicel, yellow; first flagellomere, yellow on basal one third, black on distal two third. Face, yellow above, black below; facial carina, absent. Clypeus, gena, and palpus, yellow. Mesonotum and scutellum, brown Katepisternum, white-yellow in male, grayish black in female. Wing, dark brown anteriorly, paler posteriorly. Costal vein with ca. ten minute spinules on ventral surface between R_{2+3} and R_{4+5} . Halters almost gray. Fore femur with three suberect setae on distal part of ventral surface. Legs, mostly yellow, brown on distal one third of fore femur, distal two third of mid and hind femora, and basal half of mid tibia. All abdominal tergites, brown. Sternites, yellow, broadened, nearly as wide as double length. Male terminalia: epandrium with approximately eight setae near posterior to ventral margin on each body side (Figure 8A). Cercus with dense pubescence (Figure 8A). Surstylus, broadly crescent, with numerous setae, lacking prensiseta (Figure 8B). Tenth sternite nearly quadrate (Figure 8C). Paramere accretes to hypandrium (Figure 8D,E). Gonopods indented medially (Figure 8F). Aedeagal apodeme, slender, curved in lateral view, and fused with base of aedeagus (Figure 8D,E).
- *Measurements*. BL = 2.88 mm in holotype (range in 2 ♂ paratypes: 2.86 to 2.92), THL = 1.32 mm (1.28



to 1.40), WL = 2.00 mm (2.00 to 2.04), WW = 1.08 mm (1.06 to 1.14 mm), arb = 8/5 (7 to 9/4 to 6), avd = 0.64 (0.73 to 0.80), adf = 1.57 (1.57 to 1.67), flw = 1.71 (1.86 to 2.00), FW/HW = 0.36 (0.36 to 0.39), ch/o = 0.13 (0.12 to 0.15), prorb = 1.18 (1.20 to 1.27), rcorb = 0.82 (0.90 to 1.00), vb = 2.17 (2.50 to 2.60), dc1 = 0.39 (0.35 to 0.42), presct1 = 0.61 (0.52 to 0.53), sct1 = 2.08 (1.58), sterno = damaged, orbito = 2.25 (1.80 to 2.50), dcp = 0.23 (0.24 to 0.32), sct1p = 2.67 (1.75 to 2.00), C = 1.81 (1.97 to 2.03), 4c = 1.07 (0.91 to 1.04), 4v = 1.45 (1.24 to 1.50), 5x = 1.09 (1.08 to 1.18), ac = 12.4 (9.67 to 12.00), M = 0.41 (0.39 to 0.46), and C3F = 0.67 (0.58 to 0.72).

- *Type of materials*. Holotype ♂ (SCAU, no. 120915; accession number of the mtDNA *COI* sequence in the [GenBank:HQ842779]), China: Hainan, Ledong, Jianfengling, altitude 700 m, 16.v.2005, swept from fallen logs, MF Xu. Paratypes, China: 1 ♂ (SCAU, no. 120917), same data as holotype; 1 ♂ (SCAU, no. 124040), Guangxi, Chongzuo, Nonggang, altitude 250 m, 24.iiiv.2004, swept from tree trunk, HW Chen; Taiwan: 3 ♀ (SCAU, nos. 123353, 124041, 42), Kaohsiung, Wugongshan, altitude 640 m, 15. vi.2011, XY Liu.
- *Etymology*. Patronym, in gratitude of Ms. Jian-Qin Wang (SCAU) who drew the figures of this new species.
- *Distribution*. Mainland China (Hainan and Guangxi) and Taiwan.
- *Remarks.* The three female samples from Taiwan are thought same species as the holotype, because of their similarity in body color and the uncorrected pairwise *p* distance being 0.014 (with holotype) and

0.005 (with paratypes from Guangxi) based on the *COI* sequences, which suggests their conspecificity.

Stegana (Steganina) wulai Zhang, Tsaur and Chen, sp. nov. The diagnosis, description, measurements, type of materials, etymology, and distribution are as follows:

- *Diagnosis*. This species resembles *Stegana* (*Steganina*) *setivena* Wang et al., 2013 from Yunnan, China in some characters of male terminalia but can be distinguished from the latter by the aedeagus nearly round apically in ventral view (Figure 9C,D); the gonopods protruded dorsad in lateral view (Figure 9C,D). In *setivena*, the aedeagus nearly square distally in ventral view (Figure nine D in Wang et al. 2013; the dorsal projection of gonopods slightly roundly expanded (Figure nine F, G in Wang et al. 2013).
- Description. Only the characters that depart from the universal description, as given by Chen and Chen, 2009 for this subgenus, are provided for brevity. Male and female: frons, entirely black. Pedicel, yellowish brown; first flagellomere, brown. Face, black on upper two third, yellow on lower margin; facial carina, narrow and developed above. Clypeus, gena, and palpus, yellow. Mesonotum, broadly brown medially, brownish yellow laterally. Katepisternum and basisternum, white. Scutellum, entirely black. Costal vein with *ca*. ten minute spinules on ventral surface between R₂₊₃ and R₄₊₅. Halters, almost gray. Fore femur with four suberect setae on distal part of ventral surface; apical seta, present on fore tibia. Mid tibia with three strong,



aedeagus, aedeagal apodeme, and gonopods.

suberect setae on basal part of dorsal surface. Abdominal tergites, entirely black; sternites, dark brown, broadened, wider than long. Male terminalia: epandrium pubescent except for anterior margin, with *ca*. nine setae on dorsal to posterolateral portion per side (Figure 9A). Surstylus with numerous serrations and one strong prensiseta, lacking pubescence (Figure 9B). Hypandrium roundly protruded anteromedially (Figure 9C,D). Paramere small, with two sensilla (Figure 9C,D). Aedeagus with several serrations subapically (Figure 9C,D). Gonopods with scale-like, minute processes distally (Figure 9C,D).

- *Measurements.* BL = 2.80 mm in holotype (2.67 mm in ♀ paratype), THL = 1.33 mm (1.33 mm), WL = 1.83 mm (1.93 mm), WW = 1.00 mm (0.90 mm), arb = 6/5 (5/4), avd = 1.00 (0.91), adf = 1.43 (1.38), flw = 2.14 (1.88), FW/HW = 0.39 (0.40), ch/o = 0.14 (0.16), prorb = 1.05 (1.05), rcorb = 0.79 (0.70), vb = 0.41 (0.40), dcl = 0.44 (0.44), presctl = 0.56 (0.60), sctl = 1.74 (1.75), sterno = 0.95 (0.90), orbito = 1.57 (1.71), dcp = 0.20 (0.24), sctlp = 2.00 (2.00), C = 1.80 (1.83), 4c = 1.17 (1.13), 4v = 1.67 (1.66), 5x = 1.80 (1.58), ac = 17.50 (18.00), M = 0.60 (0.59), and C3F = 0.71 (0.69).
- *Type of materials.* Holotype ♂ (SCAU, no. 123403), Taiwan: Hsinpei, Wulai, altitude 400 m, 19.×.2012, swept from tree trunk, HW Chen. Paratype, 1 ♀ (SCAU, no. 124039), same data as holotype
- *Etymology*. The name means 'reeky water' in the language of the Taiyal one of the aboriginal tribes in Taiwan.
- *Distribution*. Taiwan.

Key to the Stegana species in Taiwan

The key to the *Stegana* species in Taiwan is as follows:

4. Eye, oblong, longest axis rectangular to body length; face, entirely yellow; palpus, large and entirely black, with numerous small setae; surstylus anterodorsally fused with epandrium (Figures 2B and 3B). subgenus Stegana 5 - Eye, roundish, longest axis oblique to body length; face, with black band(s); palpus, mostly slender and yellow, sometimes black distally; surstylus anterodorsally separated from epandrium (Figures 8B and 9B). subgenus Steganina7 5. Parameres only fused each other on basal one fifth (Figure 3E); gonopods expanded ventrad laterally (Figure 3D). S. yangi sp. nov. 6. Surstylus lacking strong prensiseta (Figure 2B); paramere with one long seta and several minute sensilla (Figure 2D,E); aedeagus expanded ventrad laterally (Figure 2D,E); gonopods distally nearly triangular (Figure 2C). S. taiwana Okada - Surstylus with one strong prensiseta (Figure ten in Sidorenko and Okada 1991); parameres with one to three sensilla (µm); aedeagus sclerotized basally, with several spine-like processes along the median portion (Figure eleven in Sidorenko and Okada 1991). S. antlia Okada - Facial carina present above 11 8. Surstylus with dense pubescence (Figure 8B); paramere strong, with dense pubescence (Figure 8D,E);

 each side (Figure 7C,D).

- Aedeagus convex apicomedially. 18 18. Scutellum yellow medially; hypandrium with dense pubescence posterolaterally (Figure six C and D in Wang et al. 2011); aedeagus with two furcated, slightly sclerotized apophyses each on ventral and dorsal portions (Figure six C and D in Wang et al. 2011).

...... *S. xui* Wang, Gao and Chen - Scutellum yellow at tip at most; hypandrium lacking pubescence (Figures one D, E and three D, E in Wang et al. 2011); aedeagus lacking sclerotized apophysis (Figures one D, E and three D, E in Wang et al. 2011).

20. Paramere absent or undistinguishable (Figures four, five, and six in Sidorenko 1997). S. izu Sidorenko

22 22. Katepisternum and basisternum, white; surstylus with

Results and discussion

Twelve species of the genus Stegana were recognized from Taiwan before this study. Here, we bring the total number to 23 by reporting eight new records and three new species from the local fauna. In terms of the species richness and distribution, the Taiwanese Stagana species show mostly characteristics of the Oriental region. Among the 23 recorded species, S. convergens and S. ctenaria are two of the most widely distributed species ranging across two zoogeographic regions, and S. shirozui occurs in both Taiwan and India (Sidorenko 1998). The five species, namely S. bailla, S. izu, S. kanmiyai, S. nigrithorax, and S. ornatipes are known as insular species recorded from Taiwan and Japan. The remaining 16 species are distributed in both Taiwan and southern part of Mainland China (e.g., Hubei, Guangdong, Guangxi, Hainan, and Yunnan). One montane species, S. ornatipes was collected from Mingchih, Ilan at an elevation of 1,500 m. Taken together, this implies that Taiwanese Stegana fauna should be largely of a Mainland China origin, probably as a consequence of the east- and/or southward dispersals of the ancestral species during the glacial epoch. The 20% (5/23) endemism at the genus level conforms to that of the family level at 63/320. It is notable that the Fujian province, which is bordered by Guangdong to the south but isolated from Taiwan by the 180-km-wide strait, has no species in common with Guangdong and Taiwan. This may due to insufficient drosophilid faunal survey in these areas, especially for Fujian.

Conclusions

Among the 23 recorded species, two most widely distributed species range across two zoogeographic regions, and one occurs in both Taiwan and India. Five species are insular species recorded from Taiwan and Japan. The remaining 16 species are distributed in both Taiwan and southern Mainland China. One montane species was collected at an elevation of 1,500 m. As a whole, this implies that Taiwanese Stegana fauna should be largely of a Mainland China origin, probably as a consequence of the east- and/or southward dispersals of the ancestral species during the glacial epoch. The 20% (5/23) endemism at the genus level is comparable to that of the family level at 63/320. It is notable that the Fujian province, which is bordered by Guangdong to the south but isolated from Taiwan by the 180-km-wide strait, has no species in common with Guangdong and Taiwan. This may be due to insufficient drosophilid faunal survey in these areas, especially for Fujian.

Abbreviations

ac: third costal section between R₂₊₃ and R₄₊₅/fourth costal section; adf: longest dorsal branch of arista/width of the first flagellomere; arb: dorsal branches/ventral branches of arista; avd: longest ventral branch/longest dorsal branch of arista in length; BL: body length; THL: thorax length; C: second costal section between subcostal break and R₂₊₂/third costal section between R_{2+3} and $R_{4+5};\,ch/o:$ maximum width of gena/maximum diameter of the eye; C3F: length of heavy setation in the third costal section/ length of the third costal section; dcl: anterior dorsocentral/posterior dorsocentral in length; dcp: length distance between ipsilateral dorsocentrals/cross distance between anterior dorsocentrals: flw: length/ width of the first flagellomere; FW/HW: frontal width/head width; M: CuA1 between dm-cu and wing margin/M1 between r-m and dm-cu; orbito: distance between proclinate and posterior reclinate orbitals/distance between inner vertical and posterior reclinate orbital; prorb: proclinate orbital/posterior reclinate orbital in length; rcorb: anterior reclinate orbital/ posterior reclinate orbital in length; sctl: basal scutellar/apical scutellar in length; sctlp: distance between ipsilateral scutellars/cross distance between apical scutellars; sterno: anterior katepisternal/posterior katepisternal in length; vb: subvibrissal/vibrissa in length; presctl: prescutellar/posterior dorsocentral in length; WL: wing length; WW: wing width; 4c: third costal section between R₂₊₃ and R₄₊₅/M₁ between r-m and dm-cu; 4v: M₁ between dm-cu and wing margin/M₁ between r-m and dm-cu; 5x: CuA₁ between dm-cu and wing margin/dm-cu between M1 and CuA1.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

The work presented here was carried out in collaboration among all authors. YZ and H-WC defined the research theme. YZ prepared all the figures and took the measurements. H-WC designed the methods and experiments, carried out most of the laboratory experiments, analyzed the data, and interpreted the results. H-WC and S-CT collected most of the samples in Taiwan and wrote the paper. All authors read and approved the final manuscript.

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