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Molecular phylogenetic analysis of the *Amiota apodemata* and *Amiota sinuata* species groups (Diptera: Drosophilidae), with descriptions of four new species

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The phylogenetic relationships among the East Asian species of the *apodemata* and *sinuata* species groups of the genus *Amiota* were investigated based on DNA sequences of the mitochondrial NADH dehydrogenase subunit 2 (*ND2*) gene. A total of 23 samples of 12 species were employed as in-group taxa, and one sample for each of four other *Amiota* species were used as out-groups. The results suggested with strong confidence the monophyly of both the *apodemata* and the *sinuata* groups, whereas the monophyly of the '*apodemata* group + *sinuata* group' cluster was less supported. Based on its geographical distribution, the origin of the *sinuata* group is supposed to be southern China. Four new species were described from Guangxi and Yunnan, China: ***Amiota reikae*** Xu & Chen **sp. nov.**, ***Amiota guiensis*** Xu & Chen **sp. nov.**, ***Amiota hesongensis*** Xu & Chen **sp. nov.**, and ***Amiota polytreta*** Xu & Chen **sp. nov.**

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ADDITIONAL KEYWORDS: mtDNA – Oriental region – phylogeny – Steganinae – taxonomy.

INTRODUCTION

The *apodemata* and *sinuata* species groups of the genus *Amiota* were established by Chen & Toda (1998a, b). Species of these two groups are small, yellow-to-black flies, mostly collected from tree trunks by net sweeping in the tropical and subtropical rain forests from Australia to East Asia. The *apodemata* group includes only four known species from the Oriental region (China, India, Indonesia, and Myanmar), and bears the following characteristics: male fifth tergite with dark-coloured strips laterally; male sixth tergite very small, pointed laterally, and not reaching ventral margin of fifth tergite; aedeagus developed, basally fused to apodeme (Chen & Toda, 2001). All the species in the *sinuata* group, except for *Amiota aculeata* Chen & Aotsuka, 2005 from Yunnan, China (Chen *et al*., 2005), lack the prescutellar setae, which is present in all other lineages of the subfamily Steganinae. Until now, this group included 16 known species from China (Guangdong, Guangxi, Hainan, and Yunnan), Japan (Okinawa and Kyushu), Southeast Asia, Papua New Guinea, and Australia (Queensland) (Chen & Toda, 2001; Chen *et al*., 2004, 2005, 2007).

Chen & Toda (2001) performed a cladistic analysis of the Asian and European *Amiota* species based on 31 adult morphological characters. Their results show that the *apodemata* and *sinuata* species groups are sister groups, and are collectively most closely related to the *Amiota nagatai* species group. In the present study, the relationships among 12 eastern Asian species of the two groups (three from the *apodemata* group and nine from the *sinuata* group) have been investigated, based on the DNA sequences of the

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mitochondrial NADH dehydrogenase subunit 2 (*ND2*) gene, using four species as out-group taxa, i.e. *Amiota kimurai* Chen & Toda, 2001 and *Amiota okinawana* Okada, 1971 of the *nagatai* group, and two ungrouped species *Amiota furcata* Okada, 1971, and *Amiota kamui* Chen & Toda, 2001. Four new species: *Amiota reikae* Xu & Chen sp. nov., *Amiota guiensis* Xu & Chen sp. nov., *Amiota hesongensis* Xu & Chen sp. nov., and *Amiota polytreta* Xu & Chen sp. nov. are described from Guangxi and Yunnan, southern China.

MATERIAL AND METHODS

Materials and morphological treatment

Most of the specimens examined were collected from tree trunks by net sweeping and then preserved in 75% ethanol in the field. They were dried and pinned after morphological examination and identification in the Department of Entomology, South China Agricultural University, Guangzhou, China (SCAU). Detailed information about the samples used in our phylogenetic analyses is given in Table 1. The specimens were measured manually with a reticle in 75% ethanol. Male terminalia were detached and treated with 8% KOH, and then transferred into a drop of glycerin on a glass slide for observation and drawing under a stereomicroscope. The definitions of measurements and indices of Zhang & Toda (1992) and Chen & Toda (2001) are rehearsed and supplemented as follows. We followed McAlpine (1981) for morphological terminology and Zhang and Toda (1992) and Chen and Toda (2001) for the definitions of measurements and indices, with some new indices: 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 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;

Table 1. Taxon sampling and GenBank accession numbers of DNA sequences

Species group	Species	Material localites	Altitude (m a.s.l.)	Accession numbers
Ungrouped	*A. furcata* Okada, 1971	Yamanashi, Honshu, Japan	300	JF263570
	A. kamui Chen & Toda, 2001	Mt. Guanmen, Liaoning, China	400	EU431896
nagatai	*A. kimurai* Chen & Toda, 2001	Iriomota, Ryukyu, Japan	170	EU431897*
	A. okinawana Okada, 1971	Mt. Wuyi, Fujian, China	570	EU431902†
apodemata	*A. apodemata* Gupta & Panigrahy, 1987	Ledong, Hainan, China	220	JF263571
	A. planata Chen & Toda, 2001 –FS	Fushui, Guangxi, China	230	EU431849
	A. planata Chen & Toda, 2001 –CZ	Chongzuo, Guangxi, China	320	EU431840
	A. planata Chen & Toda, 2001 –GZ	Guangzhou, Guangdong, China	210	EU431875
	A. planata Chen & Toda, 2001 –IR	Iriomote, Ryukyu, Japan	170	EU431906
	A. reikae Xu & Chen **sp. nov.**	Mengla, Yunnan, China	570	EU431869
sinuata	*A. aculeata* Chen & Aotsuka, 2005 –ML1	Mengla, Yunnan, China	570	JF263572
	A. aculeata Chen & Aotsuka, 2005 –ML2	Mengla, Yunnan, China	570	JF263573
	A. aculeata Chen & Aotsuka, 2005 –LD	Ledong, Hainan, China	220	JF263574
	A. guiensis Xu & Chen **sp. nov.**	Fushui, Guangxi, China	230	JF263581
	A. hesongensis Xu & Chen **sp. nov.**	Menghai, Yunnan, China	1720	JN604575
	A. lambirensis Chen & Toda 2007 –LD	Ledong, Hainan, China	220	JF263575
	A. lambirensis Chen & Toda 2007–ML1	Mengla, Yunnan, China	600	JF263576
	A. lambirensis Chen & Toda 2007 –ML2	Mengla, Yunnan, China	600	JN604574
	A. pengi Chen & Toda, 1998b	Ledong, Hainan, China	220	EU431862
	A. polytreta Xu & Chen **sp. nov.**	Simao, Yunnan, China	900	JF263577
	A. sinuata Okada, 1968 –IR	Iriomote, Ryukyu, Japan	170	EU431907
	A. sinuata Okada, 1968 –LD1	Ledong, Hainan, China	220	EU431843‡
	A. sinuata Okada, 1968 –LD2	Ledong, Hainan, China	220	JF263580
	A. sinuata Okada, 1968 –ML	Mengla, Yunnan, China	570	JF263578
	A. sinuata Okada, 1968 –GD	Chebaling, Guangdong, China	550	JF263579
	A. subsinuata Chen & Aotsuka, 2005	Jinghong, Yunnan, China	580	JF263582
	A. xishuangbanna Chen & Aotsuka, 2005	Mengla, Yunnan, China	ditto	JF263583

Mistaken for *EU431851, †EU431847, and ‡EU431907 in He *et al*., 2009.

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).

DNA extraction and sequencing

Total DNA was extracted from the abdominal tissue of a single individual after the dissection of the genitalia, following the phenol/chloroform method of Gloor & Engels (1992), with slight modification. Before this, flies were treated in Tris-EDTA (TE) buffer for about 12 h to remove the alcohol. The *ND2* fragment was amplified by polymerase chain reaction (PCR) using the primers 5′-AAGCTACTGGGTTCATACC-3′ (Park, 1999) and 5′-ATATTTACAGCTTTGAAGG-3′ (Zhao, Gao & Chen, 2009), following the protocol described in Zhao *et al*. (2009). The PCR product was purified using the QIAquick PCR purification kit (Sangon Co., Ltd), and then subjected to sequencing reaction and bidirectional sequencing on the ABI 3730 DNA Analyzer. The GenBank accession numbers of all the newly collected sequences are presented in Table 1.

Phylogenetic analyses

The sequences were aligned by ClustalW (Thompson, Higgins & Gibson, 1994), implemented in MEGA 4.0 (Tamura *et al*., 2007), with default options, and because of the presence of alignment gaps, the alignment was then adjusted manually to make it conform with the codon assignments. The program DAMBE 5.0 (Xia & Xie, 2001, Xia *et al*., 2003) was used to assess the nucleotide substitution saturation of the sequences. A neighbour-joining (NJ) tree was constructed using MEGA 4.0 (Tamura *et al*., 2007) with the Tamura–Nei model and gamma-distributed rates. A maximum parsimony (MP) tree was constructed using PAUP* 4.0b10 (Swofford, 2001), with the branch-and-bound algorithm, and sequences of added taxa specified as 'furthest'. The confidence for each node in both the NJ and MP trees was assessed by the bootstrap analyses of 1000 replicates. In addition, a Bayesian phylogenetic inference was conducted using MrBayes 3.12 (Ronquist & Huelsenbeck, 2003) with the partitioned (site-specific) rate model, allowing each codon position to have its own rate. The classes of substitution models were selected by MODELTEST (Posada & Crandall, 1998), with the model parameters estimated by MrBayes. Two independent runs were implemented in parallel, with sampling of every 100 generations, and were stopped after 2 000 000 generations, when the average deviation of split frequencies fell well below 0.01. After a burn-in of 5000 early-phase samples, a majority-rule tree showing all compatible partitions was obtained.

RESULTS

The *apodemata* species group

Included species: Amiota apodemata Gupta & Panigrahy, 1987 (China, Hainan; India, Orissa); *Amiota parvipyga* Chen & Toda, 1998a (Indonesia, Java); *Amiota planata* Chen & Toda, 2001 (China, Guangdong, Guangxi; Japan, Ryukyu Islands); *Amiota yangonensis* Chen & Toda, 1998a (Myanmar, Yangon); ***Amiota reikae*** Xu & Chen **sp. nov.** (China, Yunnan).

Amiota reikae Xu & Chen sp. nov. (Fig. 1)

Specimens examined: Holotype male (SCAU 121071) and five male paratypes (SCAU 121072–121076), China, Mengla, Xishuangbanna, Yunnan, 600 m a.s.l., 17, 18 April 2007, HW Chen and JJ Gao.

Etymology: Patronym, in honor of Ms Lihua Wang (SCAU), who helped Hongwei Chen in the study of drosophlids.

Diagnosis: This species is similar to *A. apodemata* in the male terminalia, can be distinguished from the latter by having the paramere broad, slightly bifurcated dorsad, with a strongly sclerotized, arcuate process basally (pr; Fig. 1C, D).

Description: Only the important characters are listed here; see Chen & Toda (1998a) for the rest (common to the *apodemata* group). Male terminalia: epandrium not constricted mid-dorsally, with about nine setae near posterior to ventral margins on each side of the body (Fig. 1A). Surstylus lacking pubescence, with finger-like process at posteroventral corner, about seven prensisetae on distal margin, and a few stout, spine-like setae on inner surface (Fig. 1B). Hypandrium narrowly separated into two lateral arches at

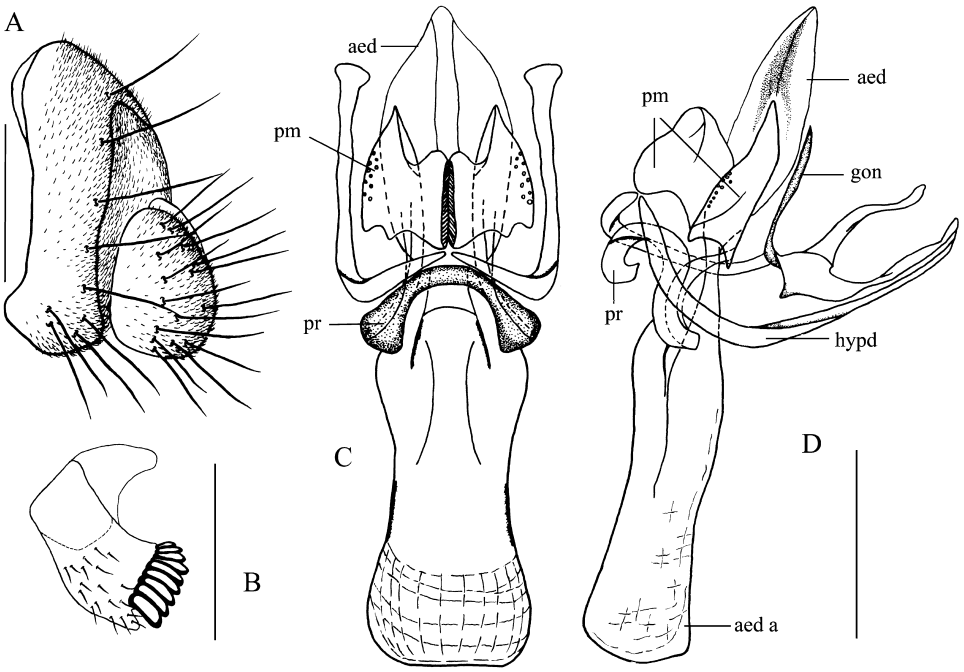

Figure 1. ***Amiota reikae*** Xu & Chen **sp. nov.**, male terminalia: A, epandrium and cercus; B, surstylus; C, D, hypandrium (hypd), parameres (pm), gonopods (gon; pr, arcuate basal process), aedeagus (aed), and aedeagal apodeme (aed a) (ventral and lateral view). Scale bars: 0.1 mm.

middle of anterior portion (Fig. 1C, D). Gonopods sclerotized and slender (Fig. 1D). Parameres subbasally fused to each other, with numerous pits along outer margins (Fig. 1C, D). Aedeagus single, somewhat sclerotized, spoon-shaped lobe, basally fused to apodeme (Fig. 1C, D). Aedeagal apodeme nearly straight (Fig. 1C, D). Female: unknown.

Measurements: Body length, BL = 2.46 mm in the holotype (range in five male paratypes: 2.44–2.60 mm), THL = 1.32 mm (1.20–1.36 mm), WL = 2.28 mm (2.00–2.32 mm), WW = 1.04 mm (0.92–1.08 mm), arb = 5/3 (4/3–5/3), avd = 0.89 (0.71–0.90), adf = 2.25 (1.40–2.20), flw = 2.75 (2.00–2.40), FW/HW = 0.44 (0.36–0.51), ch/o = 0.12 (0.07–0.15), prorb = 0.92 (0.69–0.91), rcorb = 0.83 (0.64–0.77), orbito = 1.60 (1.60–2.30), vb = 0.50 (0.38–0.43), dc1 = 0.41 (0.38–0.52), presct1 = 0.45 (0.38–0.50), sct1 = 1.28 (1.21–1.38), sterno = 0.79 (0.64–0.86), dcp = 0.25 (0.22–0.29), sct1p = 0.89 (0.88–1.33), C = 1.43 (1.32–1.77), 4c = 1.76 (1.61–1.76), 4v = 2.89 (2.35–2.67), 5x = 1.75 (1.38–1.88), ac = 5.00 (5.00–7.25), M = 0.82 (0.60–0.76), C3F = 0.81 (0.73–0.84).

Distribution: China (Yunnan).

THE *SINUATA* SPECIES GROUP

Included species: Amiota aculeata Chen & Aotsuka in Chen *et al*., 2005 (China, Hainan, Yunnan); *Amiota bicolorata* Bock, 1989 (Australia, Queensland); *Amiota bispinula* Chen & Toda in Chen *et al*., 2007 (Malaysia, Sabah); *Amiota cerata* Chen & Toda in Chen *et al*., 2007 (Malaysia, Sarawak); *Amiota curvibacula* Chen & Toda in Chen *et al*., 2007 (Malaysia, Sabah); *Amiota hernowoi* Chen & Toda, 1998b (Indonesia, West Kalimantan); *Amiota javaensis* Chen & Toda, 1998b (Indonesia, Java); *Amiota lambirensis* Chen & Toda in Chen *et al*., 2007 (China, Hainan, Yunnan; Malaysia, Sarawak); *Amiota parviserrata* Chen & Toda in Chen *et al*., 2007 (Malaysia, Sabah); *Amiota pengi* Chen & Toda, 1998b (China, Hainan); *Amiota pontianakensis* Chen & Toda, 1998b (Indonesia, West Kalimantan); *Amiota quadrifoliolata* Chen & Toda in Chen *et al.*, 2007 (Malaysia, Sabah); *Amiota ratnae* Chen & Toda, 1998b (Indonesia, Java); *Amiota sinuata* Okada, 1968 (Japan, Kyushu, Ryukyu Islands; China, Guangdong, Hainan, Yunnan; Myanmar; New Guinea); *Amiota subsinuata* Chen & Aotsuka in Chen *et al*., 2005 (China, Yunnan); *Amiota xishuangbanna* Chen & Aotsuka in Chen *et al*., 2005 (China, Yunnan); *Amiota guiensis* Xu & Chen sp. nov. (China, Guangxi); *Amiota hesongensis* Xu & Chen sp. nov. (China, Yunnan), and *Amiota polytreta* Xu & Chen sp. nov. (China, Yunnan).

Remarks: In the new species described, only characters that depart from the universal description (given by Chen & Toda, 1998b) are provided, for brevity.

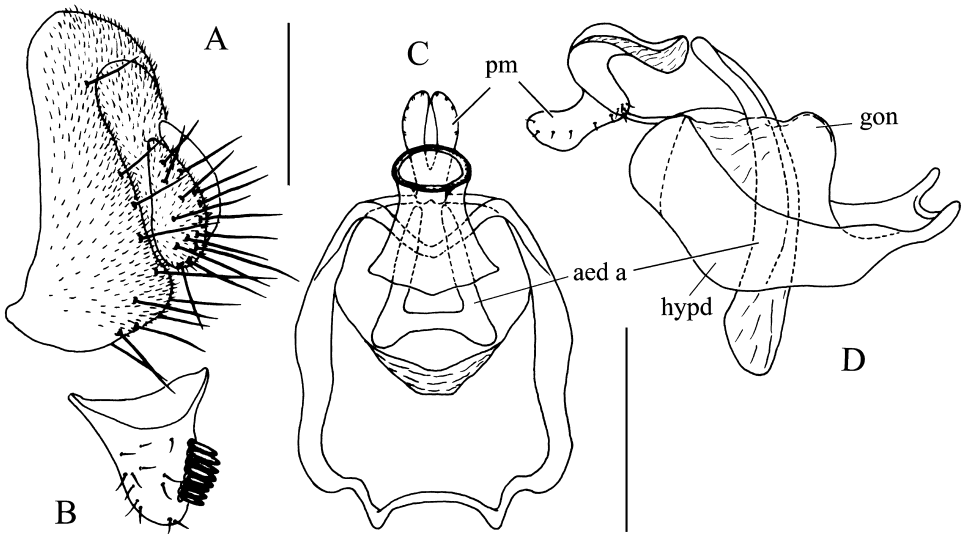

Figure 2. ***Amiota guiensis*** Xu & Chen **sp. nov.**, male terminalia: A, epandrium and cercus; B, surstylus; C, D, hypandrium (hypd), parameres (pm), gonopods (gon; pr, arcuate basal process), aedeagus (aed), and aedeagal apodeme (aed a) (ventral and lateral view). Scale bars: 0.1 mm.

AMIOTA GUIENSIS XU & CHEN **SP. NOV.** (FIG. 2)

Specimens examined: Holotype male (SCAU 121736) and six male paratypes (SCAU 121737–121742), China, Fushui, Chongzuo, Guangxi, 230 m a.s.l., 23 August 2004, H.W. Chen.

Etymology: Pertaining to shortened form Guangxi of the type locality.

Diagnosis: This species is slightly similar to *A. lambirensis* in the shape of the distal part of the paramere, but can be distinguished by the following characters: in *lambirensis* the distal part of the paramere is distinctly longer than the basal part in ventral view, and is basally triangularly expanded in lateral view (Chen *et al*., 2007: figs. 18, 19).

Description: Male. Thorax nearly entirely brown. Abdominal tergites dark brown, except for first to third yellow medially. Male terminalia: epandrium pubescent except for ventral margin, with ~11 setae near posterior margin on each side of the body (Fig. 2A). Surstylus with several setae and roughly eight prensisetae (Fig. 2B). Paramere distally with about five sensilla and slightly curved, submedially with about six sensilla arranged in a small patch, apparent in lateral view (Fig. 2D). Vertical lobe of gonopod broad, arched, slightly sclerotized (Fig. 2C, D). Aedeagal apodeme longer than wide (Fig. 2D). Female: unknown.

Measurements: BL = 2.64 mm in holotype (range in six male paratypes: 2.60–2.68 mm), THL = 1.16 mm (1.12–1.20 mm), WL = 2.04 mm (2.00–2.12 mm), WW = 1.00 mm (0.96–1.04 mm), arb = 5/4 (5/3–4), avb = 0.89 (0.78–0.90), adf = 2.00 (2.00–2.30), flw = 3.00 (2.60–3.25), FW/HW = 0.04 (0.34–0.44), ch/o = 0.09 (0.08–0.09), prorb = 0.91 (0.82–0.92), rcorb = 0.64 (0.64–0.73), orbito = 1.40 (1.40–1.80), vb = 0.50 (0.33–0.50), dcl = 0.53 (0.45–0.80), sctl = 1.38 (1.15–1.38), sterno = 0.87 (0.69–0.93), dcp = 0.25 (0.25–0.33), sctlp = 1.17 (1.17), C = 1.34 (1.18–1.65), 4v = 2.60 (2.21–2.60), 4c = 1.93 (1.75–2.00), M = 0.93 (0.68–0.93), 5x = 2.00 (1.44–2.00), C3F = 0.81 (0.79–0.86), ac = 4.83 (3.83–6.20).

Distribution: China (Guangxi).

AMIOTA HESONGENSIS XU & CHEN **SP. NOV.** (FIG. 3)

Specimens examined: Holotype male (SCAU 110042) and four male paratypes (SCAU 121256–121259), China, Hesong, Menghai, Xishuangbanna, Yunnan, 1900 m a.s.l, 6–9 April 2011, J.M. Lu, Z.F. Shao, Y.R. Su, and S.J. Yan.

Etymology: Pertaining to the type locality.

Diagnosis: This species is similar to *A. sinuata* in the shape of the distal part of the paramere, but can be distinguished by the following characters. In *A. sinuata*, the distal part of the paramere is slightly longer than the basal part, which is basally not narrowed in lateral view (Fig. 1C in Chen & Toda, 1998b).

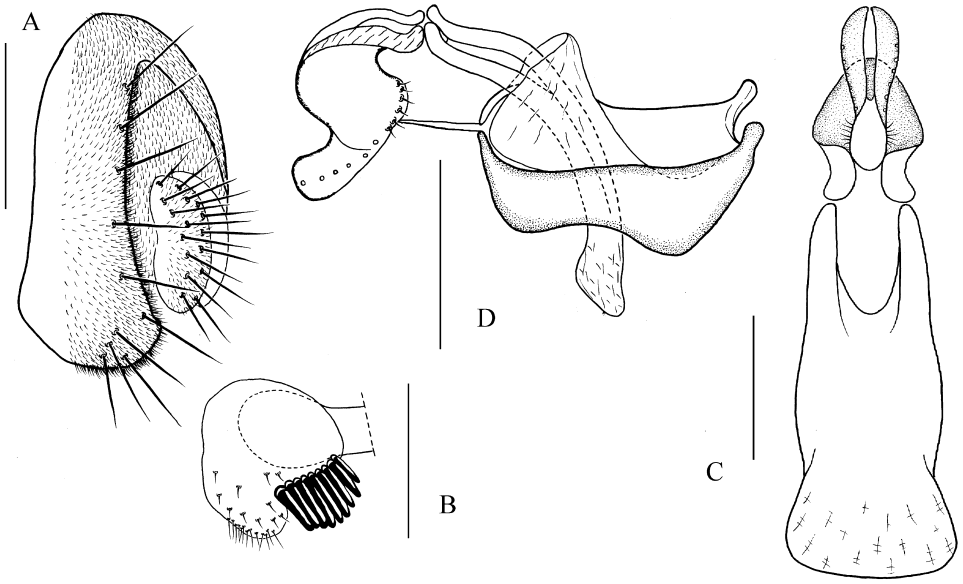

Figure 3. ***Amiota hesongensis*** Xu & Chen **sp. nov.**, male terminalia: A, epandrium and cercus; B, surstylus; C, D, hypandrium (hypd), parameres (pm), gonopods (gon; pr, arcuate basal process), aedeagus (aed), and aedeagal apodeme (aed a) (ventral and lateral view). Scale bars: 0.1 mm.

Description: Male. Thorax brownish yellow, with dark-brown patches and stripes; pleura brownish; scutellum yellow, brown on margin. Abdominal tergites black; first to third yellow, medially. Male terminalia: epandrium pubescent except for anterior margin, with about ten setae near posterior margin on each side of the body (Fig. 3A). Surstylus with several setae and about nine prensisetae (Fig. 3B). Vertical lobe of gonopod broad, arched, slightly sclerotized (Fig. 3C). Paramere with a row of ~12 sensilla submedially to apically (Fig. 3C). Aedeagal apodeme longer than wide (Fig. 3C). Female: unknown.

Measurements: BL = 2.80 mm in the holotype (range in four male paratypes: 2.78–2.85 mm), THL = 1.30 mm (1.20–1.35 mm), WL = 2.38 mm (2.22–2.40 mm), WW = 1.00 mm (0.90–1.00 mm), arb = 5/3 (5/3), avd = 0.85 (0.85–0.95), adf = 2.10 (2.00–2.10), flw = 2.00 (1.90–2.00), FW/HW = 0.35 (0.35), ch/o = 0.07 (0.07), prorb = 0.90 (0.90–1.00), rcorb = 0.65 (0.65–0.70), orbito = 2.30 (2.30–2.40), vb = 0.35 (0.35–0.40), dcl = 0.55 (0.50–0.60), sctl = 1.20 (1.20–1.30), sterno = 0.90 (0.85–0.95), dcp = 0.25 (0.25–0.28), sctlp = 1.00 (1.00), C = 1.23 (1.14–1.42), 4c = 2.10 (1.85–2.30), 4v = 2.85 (2.40–2.85), 5x = 1.50 (1.44–1.80), ac = 6.00 (4.11–5.75), M = 0.90 (0.64–0.83), C3F = 0.83 (0.76–0.81).

Distribution: China (Yunnan).

AMIOTA POLYTRETA XU & CHEN **SP. NOV.** (FIG. 4)

Specimens examined: Holotype male (SCAU 121077) and three male paratypes (SCAU 121278–121280), China, Yixiang, Puer, Yunnan, 1320 m a.s.l., 15 September 2002, 2 October 2011, H.W. Chen.

Etymology: From the Greek word: polys + tretos, referring to the paramere with dense pits.

Diagnosis: This species is very similar to *A. sinuata* in the male terminalia, but can be distinguished by the following characters: in *A. sinuata* the paramere is flat on the apical margin in lateral view, and medially has one row of pits on distal part (Fig. 1C in Chen & Toda, 1998b).

Description: Male. Thorax yellow, with dark-brown patches and stripes; pleura brown; scutellum yellow, brown on margin. Abdominal tergites black; first to third yellow, medially. Male terminalia: epandrium pubescent, except for anterior and ventral margins, with ~11 setae near posterior margin on each side of the body (Fig. 4A). Surstylus with several setae and about eight prensisetae (Fig. 4B). Paramere apically round, and distally with two groups of paramarginal pits in lateral view (Fig. 4C). Vertical lobe of gonopod broad, arched, slightly sclerotized (Fig. 4C). Aedeagal apodeme longer than wide (Fig. 4C). Female: unknown.

Measurements: BL = 2.60 mm in the holotype (range in three male paratypes: 2.48–2.65 mm), THL = 1.20 mm (1.00–1.15 mm), WL = 2.08 mm (1.90–2.00 mm), WW = 1.00 mm (0.88–0.90 mm), arb = 4/3

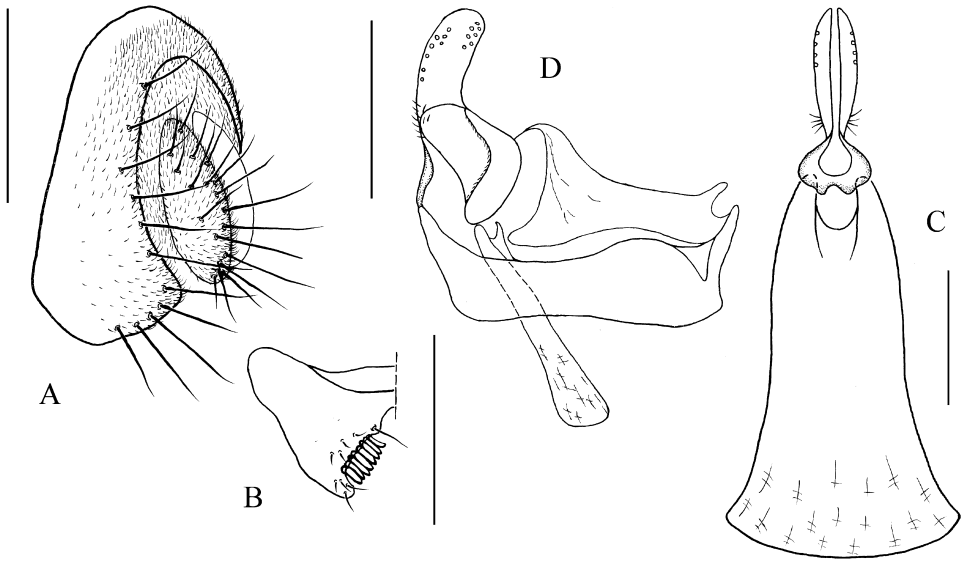

Figure 4. ***Amiota polytreta*** Xu & Chen **sp. nov.**, male terminalia: A, epandrium and cercus; B, surstylus; C, D, hypandrium (hypd), parameres (pm), gonopods (gon; pr, arcuate basal process), aedeagus (aed), and aedeagal apodeme (aed a) (ventral and lateral view). Scale bars: 0.1 mm.

(4–5/2–3), avd = 0.88 (0.85–1.00), adf = 2.00 (2.00–2.20), flw = 2.00 (1.80–2.00), FW/HW = 0.35 (0.30–0.35), ch/o = 0.06 (0.06), prorb = 1.10 (1.00–1.10), rcorb = 0.70 (0.70–0.80), orbito = 1.40 (1.40–1.60), vb = 0.43 (0.40–0.45), dc1 = 0.40 (0.40–0.60), sct1 = 1.28 (1.20–1.30), sterno = 0.87 (0.85–0.95), dcp = 0.28 (0.27–0.28), sct1p = 1.00 (1.00), C = 1.18 (1.14–1.22), 4c = 1.94 (1.85–2.20), 4v = 2.47 (2.40–2.65), 5x = 1.71 (1.44–1.80), ac = 5.50 (4.11–5.75), M = 0.71 (0.64–0.73), C3F = 0.83 (0.76–0.81).

Distribution: China (Yunnan).

PHYLOGENETIC RECONSTRUCTION

The alignment of the *ND2* sequences spans 1026 sites, codes 342 amino acid residues, and contains 281 parsimony-informative characters out of 400 variable sites, with consecutive alignment gaps and end gaps in some sequences. The average base frequencies across species (excluding out-groups) were 34.8, 47.5, 9.9, and 7.8% for A, T, C, and G respectively, showing a strong bias towards A and T.

The among-lineage base composition homogeneity is not rejected by the χ^2 test. The nucleotide frequencies were similar among species ($\chi^2 = 7$, d.f. = 66, $P = 1.00$). The DAMBE test yielded an index of substitution among species I_{ss} (= 0.1728) significantly lower than the critical values ($I_{ss.c} = 0.7614$, assuming a symmetrical tree; $I_{ss.c} = 0.5254$, assuming an asymmetrical tree), indicating that the sequences experienced little substitution saturation.

The Figures 5 and 6 show the Bayesian and NJ trees, respectively. The Bayesian tree ($-\ln L$ = 4842.26) shows similar topology to the NJ tree and the MP tree (not shown). In all of the trees, the monophyly of the *nagatai*, *apodemata*, and *sinuata* groups is strongly supported [MP bootstrap percentages (BPs) = 98; NJ BPs = 99; Bayesian posterior probability, PP = 1.00]. Both the *apodemata* and *sinuata* groups were supported as monophyletic with strong confidence (MP BPs = 100; NJ BPs = 99; Bayesian PP = 1.00), whereas the grouping between these two groups was not so strongly supported (MP BPs = 80; NJ BPs = 96; Bayesian PP = 0.94). In the *apodemata* group, *A. reikae* sp. nov. diverges first. In the *sinuata* group, *A. aculeata* splits first, although the remaining basal relationship within this group was only poorly resolved, but the closing relationship between *A. subsinuata* and *A. xishuangbanna* (MP BPs = 66; NJ BPs = 92; Bayesian PP = 0.96), and that between *A. lambriensis* and *A. sinuate*, were strongly supported. In the *apodemata* and *sinuate* groups, each of the four species, *A. planata*, *A. aculeata*, *A. lambriensis*, and *A. sinuata*, was suggested as monophyletic (MP BPs = 100; NJ BPs = 99; Bayesian PP = 1.00).

DISCUSSION

Here we analysed the phylogenetic relationships of the *apodemata* and the *sinuata* groups based on the DNA sequence of the *ND2* gene for twelve *Amiota* species. It was suggested by Chen & Toda's (2001)

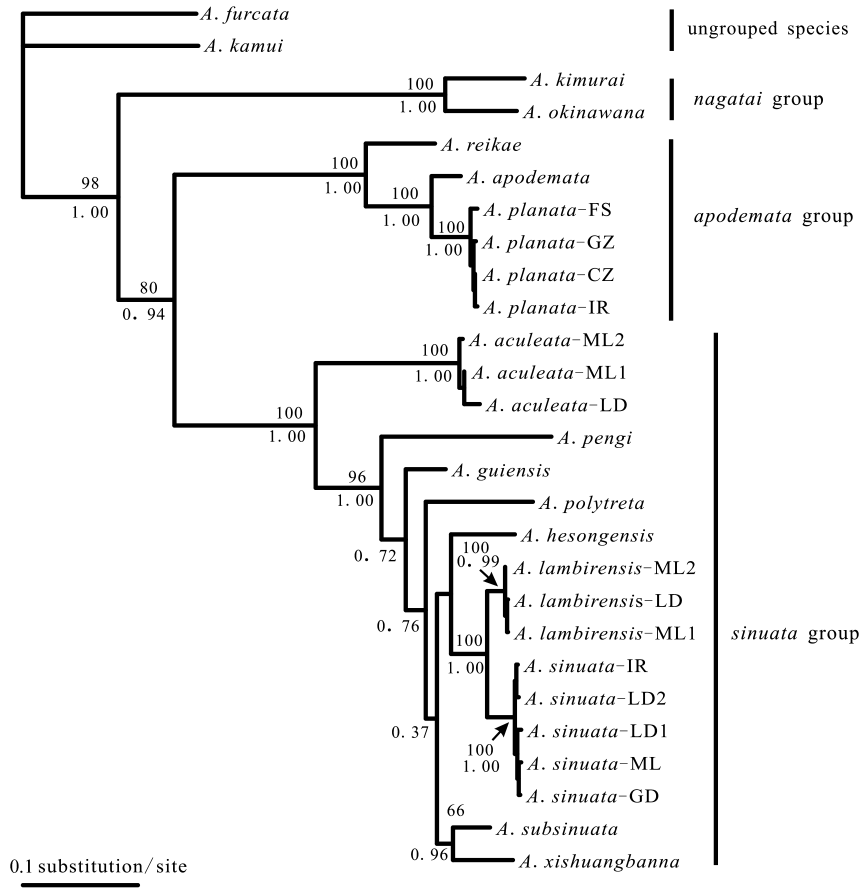

Figure 5. Bayesian tree deduced from the *ND2* sequences. Numbers above the branches show the bootstrap percentages of the nodes in the maximum parsimony analysis (tree length = 758 steps; consitency index, CI = 0.6609; retention index, RI = 0.8075), numbers below the branches indicate the posterior probabilities (PPs; –ln *L* = 4842.26).

cladistic analysis of adult morphological characters that the *apodemata* and *sinuata* groups are sibling taxa, and that these two groups collectively are closely related to the *nagatai* group. He *et al.*'s (2009) analysis based on DNA sequences of the *ND2* gene lent support to the monophyly of the *nagatai* group, with species from both the *apodemata* and *sinuata* groups used as out-group taxa. Our molecular phylogenetic analysis is consistent with Chen & Toda's (2001) morphological study with respect to the relationship among these species groups, indicating that the *apodemata*, *sinuate*, and *nagatai* groups form a monophyletic cluster, and that both the *apodemata* and the *sinuata* groups are monophyletic, and that these two species groups are closer to each other than either is to the *nagatai* species group.

Based on Cao *et al.*'s (2011) regression line of genetic distance (*D*) on divergence times (*T*) ($T = 2452.4D^{2.1048}$; $R^2 = 0.87$), the divergence time between the *apodemata* and the *sinuata* groups ($D = 0.166$ with the Tamura–Nei model and gamma-distributed rates) can be estimated as 56.0 Mya in the Palaeocene epoch, and the species *A. sinuata* is estimated to have arisen about 1.6 Mya, in the early Pleistocene. It is suggested that a land bridge existed between the Ryukyu Islands and mainland China during the early Pleistocene (Koshiro, 1986; Ota, 1998). So, presumably, during this peried *A. sinuata* dispersed from the continent to Ryukyu via this land bridge, and founded the current Ryukyu population of *A. sinuata*.

It was revealed by previous field survey (e.g. Chen & Toda, 1998b; Chen *et al.*, 2007) that the *sinuata* group has a major distribution in forests of the tropical region, ranging from Australia to Southeast Asia (e.g. Indonesia, Malaysia, and Myanmar) and East Asia (e.g. China and Japan). Our study suggested that within the *sinuata* group, *A. aculeata*, which was recorded only from South China, diverged first from the lineages of the remaining species of this group, most of which were also recorded from Southern China, indicating a high probability that the ancestral distribution of the *sinuata* group is in Southern China.

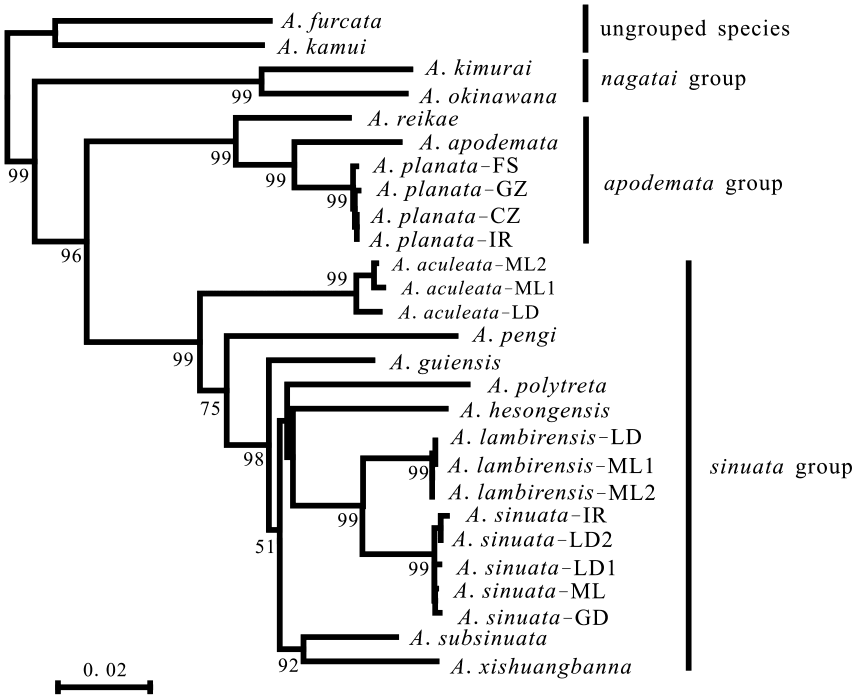

Figure 6. Neighbour-joining tree deduced from the *ND2* sequences. Numbers above the branches show the bootstrap percentages of the nodes with the Tamura–Nei model and gamma-distributed rates.

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