*Zootaxa* 5528 (1): 283–299
https://www.mapress.com/zt/

**Article**

ISSN 1175-5326 (print edition)
**ZOOTAXA**
ISSN 1175-5334 (online edition)

https://doi.org/10.11646/zootaxa.5528.1.20
http://zoobank.org/urn:lsid:zoobank.org:pub:725FC3C5-D1E5-4C35-83D1-BCC8F215C93A

# Exploring the *Anomala fuscosignata*-complex: discovery of two new species from southern China (Coleoptera: Scarabaeidae: Rutelinae)

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## Abstract

An investigation towards taxonomy of the *Anomala fuscosignata*-complex from southern China and northern Vietnam was conducted through direct morphological comparison and molecular phylogenetic analysis, which results in discovery of two new species: *A. nanlingensis* Zhao, Zorn & Liu, **sp. nov.** and *A. zhengyucheni* Zhao, Zorn & Liu, **sp. nov.** The morphological differences in female genitalia and low genetic divergence as well as their taxonomic implications within Anomalini are discussed.

**Key words:** taxonomy, phylogeny, species delimitation, female genitalia, Anomalini

## Introduction

The genus *Anomala* Samouelle, 1819 is amongst the most diverse genera in the tribe Anomalini and in the subfamily Rutelinae. In the past decade, the number of known *Anomala* species has increased most significantly in southern China and Indochina (e.g. Prokofiev 2013, 2015; Wang 2020; Zhao 2021; Zhao & Pham 2023; Zorn *et al.* 2017) as well as in Wallacea (Zorn 2007a, 2011b).

Despite limited systematic work, species groups in *Anomala* have consistently been proposed to accommodate species with common external traits (e.g. Filippini *et al.* 2015a; Zhao & Zorn 2022; Zorn 2000, 2007a, 2007b), thereby facilitating future research with phylogenetic implications. The *Anomala spiloptera*-group was first introduced by Zorn *et al.* (2017) and delimited by the following characters: parameres are usually very elongated and connected by a wide wrinkled membrane dorso-basally, the ventral plate usually is equipped with a long process resembling a narrow blade. However, a checklist of species with these characters was not included. In addition to the five species mentioned as related to the *A. spiloptera*-group by Zorn *et al.* (2017), we found such membrane also in the Vietnamese species *A. fuscosignata* Ohaus, 1905 and *A. luminosa* Benderitter, 1929. However, they differ from all other described members of the *A. spiloptera*-group by having rather simple punctation on pronotum and elytra (vs. densely transversely punctate in the other species) and by a simply pointed ventral plate (vs. flattened or blade-shaped). Together with some morphologically similar and so far unknown species collected in southern China, they constitute the *A. fuscosignata*-complex treated in the present study.

Molecular phylogenetic analyses using multiple gene fragments has been employed for species delimitation and classification across various pleurostict groups, including Cetoniinae (Vondráček *et al.* 2018), Melolonthinae (Ahrens *et al.* 2011; Eberle *et al.* 2019), and Sericinae (Eberle *et al.* 2015). Nevertheless, its application in the species-rich tribe Anomalini remains extensively underexplored. The phylogenetic studies of Costa Rican *Callistethus* (Filippini *et al.* 2015b) and the East Asian *Anomala albopilosa*-complex (Muraji *et al.* 2008) stand as few noteworthy examples within Anomalini. In this study, we take an initial step towards the phylogeny of the *A. fuscosignata*-complex by employing two mitochondrial markers, with the objective of clarifying its taxonomy.

*Accepted by Z.-W. Yin: 15 Jul. 2024; published: 23 Oct. 2024*

## Material and methods

### Examined specimens

The specimens were observed and dissected under a Motic SMZ-171 stereomicroscope. Images for habitus and male genitalia were taken using a Canon EOS 760D camera in conjunction with a Laowa 60 mm f/2.8 2X Ultra-Macro Lens and a Laowa 25 mm f/2.8 2.5-5X Ultra Macro Lens, respectively, or a Nikon D90 camera attached to a Zeiss Stemi 2000 stereo microscope was used. Zerene Stacker (version 1.04) and Helicon Focus (version 8.2.0) were used for stacking. All images were edited and arranged into plates in Adobe Photoshop CS5 or CS6. Acronyms of the depository are as following:

| | |
|---|---|
| CCCC | Chang-Chin Chen's personal collection, Tianjin, China; |
| CCZ | Carsten Zorn's personal collection, Gnoien, Germany; |
| CZMZ | Ming-Zhi Zhao's personal collection, Guangzhou, China; |
| IRSNB | Institut royal des Sciences naturelles de Belgique, Brussels, Belgique; |
| MFNB | Museum für Naturkunde, Berlin, Germany; |
| NMNS | National Museum of Nature Science, Tsukuba, Japan; |
| SCAU | South China Agricultural University, Guangzhou, China; |
| SYSU | The Museum of Biology, Sun Yat-sen University, Guangzhou, China. |

Type specimens of the new species are cited verbatim and each provided with one label "HOLOTYPE [or] PARATYPE [*taxon name*] des. Zhao, Zorn & Liu, 2024". Citation of individual labels were separated by a double slashes (//).

### DNA extraction and sequencing

Four *Anomala* species were used as outgroup of the *A. fuscosignata*-complex, including *A. rubripes rubripes* Lin, 1996 of the *A. cupripes*-group (Lin 1996) and three species of the *A. spiloptera*-group, *i.e. A. spiloptera* Burmeister, 1855, *A. viridicostata* Nonfried. 1892 and *A. triformis* Prokofiev, 2021. The last species is the closest sibling of *A. iridicollis* Ohaus, 1914, a member of the *A. spiloptera*-group mentioned by Zorn *et al.* (2017). Specimens were killed and preserved by 95% ethanol. All involved samples as well as Genbank accession numbers are listed in Table 1.

Genomic DNA was extracted from metathoracic tissue using a TIANamp Micro DNA Kit following the manufacturer's protocol. Two mitochondrial genes were amplified, including the barcoding region of the cytochrome oxidase subunit I (*COI*) gene and the 16S ribosomal RNA (*rrnL*), with the adjacent regions NAD dehydrogenase subunit 1 (*nad1*) and tRNA leucine (*trnLeu*). The latter three fragments (hereafter termed *rrnl*) were amplified using primer pair ND1A and 16Sar (Simon *et al.* 1994), while the barcoding region of *COI* was amplified by LCO1490-JJ and HCO2198-JJ (Folmer *et al.* 1994). Sanger sequencing results were assembled and edited using the SeqMan module of Lasergene, and identities were confirmed through BLAST searches.

### Phylogenetic analyses and species delimitations

All phylogenetic analyses were conducted on the PhyloSuite v.1.2.2 platform. Sequence alignments were generated using MAFFT. The best-fit models for the analyses were determined by PartitionFinder v.2.1.1. Bayesian inference (BI) was performed using MrBayes v.3.2.6 with the MCMC methods. For each reconstruction, two independent analyses were run for 20 million generations, with sampling every 100 generations and the first 20% of samples discarded as burn-in. Maximum likelihood (ML) analysis was implemented by IQ-TREE v.1.6.8 with 1000 bootstrap replicates. The resulting phylogenetic trees were visualized and edited in Figtree v.1.4.4.

The genetic distances, including overall mean distance and pairwise *p*-distances, were calculated using MEGA 7.0.26. Automatic Barcode Gap Discovery (ABGD) (https://bioinfo.mnhn.fr/abi/public/abgd/abgdweb.html) and

Bayesian implementation of Poisson Tree Processes (bPTP) (https://species.h-its.org/ptp/) were used to delimitate taxa for both genes separately. All the parameters follow default settings, while the relative gap width was set as 1.0 due to the low genetic divergence within *A. fuscosignata*-complex as calculated by MEGA.

**TABLE 1.** Chinese *Anomala* used for the phylogenetic study with Genbank accession numbers.

| Taxon | Voucher | Collecting data | *COI* | *rrnl* |
|---|---|---|---|---|
| *A. rubripes rubripes* | RU1 | (SCAU) Guangdong, Guangzhou, South China Agricultural University, 2021.IX.17, Ming-Zhi Zhao leg. | PQ008662 | PQ008697 |
| *A. spiloptera* | SPO | (SCAU) Hainan, Mt. Wuzhishan, Yatai Forest Hotel, 2023.IV.16, Zhi-Teng Chen leg. | PQ008663 | PQ008699 |
| *A. viridicostata* | VDC2 | (SCAU) Zhejiang, Linhai, Mt. Kuocangshan, 1400 m, 2022.VI.27, Mo-Lin Lu leg. | PQ008665 | PQ008700 |
| *A. triformis* | TFM1 | (CZMZ) Jiangsu, Liyang, Pingqiao, Shiba Sight, 31°12′30″N, 119°27′0″E, 2023.V.20, at light, Tian-Jiao Wang leg. | PQ008664 | PQ008698 |
| *A. fuscosignata* | FUCO1 | see material examined | PQ008666 | PQ008702 |
| *A. fuscosignata* | FUCO2 | see material examined | PQ008667 | PQ008703 |
| *A. luminosa* | LMIN | see material examined | PQ008672 | PQ008701 |
| *A. nanlingensis* | LG1 | see material examined | PQ008668 | PQ008704 |
| *A. nanlingensis* | LG2 | see material examined | PQ008669 | PQ008705 |
| *A. nanlingensis* | LG3 | see material examined | PQ008670 | PQ008706 |
| *A. nanlingensis* | LG4 | see material examined | PQ008671 | PQ008707 |
| *A. zhengyucheni* | ZYC1 | see material examined | PQ008673 | PQ008708 |
| *A. zhengyucheni* | ZYC2 | see material examined | PQ008674 | PQ008709 |

## Results

### Phylogeny

The trimmed dataset comprises nucleotides of 657 bp for *COI* and 805 bp for *rrnL*. Within the *A. spiloptera*-group, 89 substitutional polymorphic and 74 parsimony informative sites were detected for *COI* sequences. Those of the less polymorphic *rrnL* are 45 and 22, respectively.

Phylogenetic trees, inferred using both BI and ML methods with combined gene fragments yield identical topologies. Hence, only the BI tree is presented here (Fig. 8). Nodes were annotated with posterior probabilities (PP) for BI and bootstrap support values (BP) for ML. Rooting the tree with *A. rubripes*, the *A. fuscosignata*-complex is the sister group of another clade comprising the remaining three species of the *A. spiloptera*-group. The topology reveals that the *A. fuscosignata*-complex can be divided into two subclades: (1) *A. fuscosignata* + *A. luminosa* and (2) *A. nanlingensis* + *A. zhengyucheni*. The BI analysis received more significantly supported nodes than the ML analysis (PP≥95% and BP≥80%).

Furthermore, two additional phylogenetic trees (Fig. 9) were constructed exclusively based on the *COI* sequences to evaluate the efficacy of *COI*-based phylogeny within Anomalini. The BI tree displays an identical topology to those inferred from combined gene dataset. Conversely, the ML tree exhibits minor differences, with *A. zhengyucheni* emerging as the sister group to the remaining species in the *A. fuscosignata*-complex. Seven out of ten statistical nodes of the ML tree are weakly supported (BP<80%), including all more ancestral nodes.

### Species delimitations

We calculated the genetic distances within the *A. fuscosignata*-complex species based on the more polymorphic *COI* data. The overall mean distance is 0.018. The genetic *p*-distances within the complex are presented in Table 2. Notably, the *p*-distances between *A. fuscosignata* and *A. luminosa* are lower (0.003–0.005) than the intraspecific variations within *A. nanlingensis* (0.000–0.009). The distances between the morphologically similar *A. nanlingensis* and *A. zhengyucheni* range from 0.014 to 0.020.

The ABGD analyses based on both genes and bPTP analysis based on the *COI* dataset resulted in identical delimitation proposals (Fig. 8). The *A. fuscosignata*-complex comprises three operational taxonomic units (OTUs): *A. fuscosignata* and *A. luminosa* are treated as the same OTU, while *A. nanlingensis* and *A. zhengyucheni* are distinct. However, bPTP analysis based on the *rrnL* dataset suggested a different scenario, in which *A. fuscosignata* and *A. luminosa* are two OTUs, and the two populations of *A. nanlingensis* are also split.

**TABLE 2.** Genetic *p*-distances for the 657 bp barcoding region of the *COI* gene of *Anomala fuscosignata*-complex.

| Taxon and voucher name | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|
| 1. *A. luminosa* LMIN | | | | | | | | |
| 2. *A. fuscosignata* FUCO1 | 0.005 | | | | | | | |
| 3. *A. fuscosignata* FUCO2 | 0.003 | 0.002 | | | | | | |
| 4. *A. nanlingensis* LG1 | 0.026 | 0.027 | 0.026 | | | | | |
| 5. *A. nanlingensis* LG2 | 0.026 | 0.027 | 0.026 | 0.000 | | | | |
| 6. *A. nanlingensis* LG3 | 0.029 | 0.030 | 0.029 | 0.009 | 0.009 | | | |
| 7. *A. nanlingensis* LG4 | 0.029 | 0.027 | 0.029 | 0.009 | 0.009 | 0.003 | | |
| 8. *A. zhengyucheni* ZYC1 | 0.024 | 0.023 | 0.024 | 0.014 | 0.014 | 0.020 | 0.017 | |
| 9. *A. zhengyucheni* ZYC2 | 0.024 | 0.023 | 0.024 | 0.014 | 0.014 | 0.020 | 0.017 | 0.000 |

## Taxonomy

### *Anomala nanlingensis* Zhao, Zorn & Liu, sp. nov.

Chinese common name: 南岭异丽金龟
(Figs 1A–E, 3A–C, 4A–I, 6A–O, 7B, 7F, 7J)

**Type material examined. HOLOTYPE: CHINA:** ♂ (SCAU), "CHINA: Guangdong, Shaoguan, Nanling N. R., nr. Shikengkong, N 24°55′40″, E 112°59′2″, 1660 m, 30.V–4.VI.2022 (at light), lgt. Ye XH, Zhan BX & Ma YS". **PARATYPES: CHINA: Guangdong:** 22♂, 8♀ (CZMZ, SCAU), "CHINA: Guangdong, Shaoguan, Nanling N. R., nr. Shikengkong, N 24°55′40″, E 112°59′2″, 1660 m, 30.V–4.VI.2022 (at light), lgt. X.-H. Ye, B.-X. Zhan & Y.-S. Ma YS"; 1♂ (SCAU) CHINA: Guangdong, Shaoguan, Nanling N. R., nr. Shikengkong, N 24°55′40″, E 112°59′2″, 1660 m, 30.V–4.VI.2022 (at light), lgt. Ye XH, Zhan BX & Ma YS // LG1"; 13♂, 19♀ (CZMZ), "CHINA: Guangdong, Shaoguan, Ruyuan Co., Nanling N. R., N 24°55′32″, E 113°1′19″, 1027 m, 26–28.V.2022 (at light), lgt. Ming-Zhi Zhao *et al.*"; 20♂, 18♀ (CZMZ), "CHINA: Guangdong, Shaoguan City, Nanling N. R., Babaoshan Station N24°55′43.4″ E 113°0′57.0″, 1020m light trap 2017.V.5–6, Jing-Kun Zhang"; 6♂, 1♀ (IRSNB) "Coll. I.R.Sc.N.B. Chine - Nanling 9.V.2004 Leg. P. Grootaert"; 2♂ (IRSNB) "Coll. I.R.Sc.N.B. China, Nanling Ruyuan, Guangdong, forest, night catch, 7.V.2004 (24006) Leg. P. Grootaert"; 1♂ (NMNS) "Nanling Mts. (900-1.400m) Shaoguan, Guangdong [S-CHINA] 16-20.v.2009 Y. Kishida & M. Wang leg."; 7♂, 2♀ (ZMPC) "China: Guangdong, Nanling Nature Reserve, 892 m, 2019.IV.28, Bo-Yan Li leg."; 3♂ (SYSU), "Mts. Nanling, 700-1400m, Guangdong, 2005.v.11-16, Zhang C.T." **Guangxi:** 1♂ (SCAU) "Guangxi, Jinxiu, Dayaoshan, Pingbantun, 1200 m, 2020.IV.27, Leg. J.-T. Zhao // LG3"; 7♂, 1♀ (CCCC, CZMZ) "Guangxi, Jinxiu, Dayaoshan, Pingbantun, 1350 m, 2019.IV.24, leg. J.-T. Zhao"; 1♂ (SCAU) "Guangxi, Jinxiu, Dayaoshan, Pingbantun, 1350 m, 2019.IV.24, leg. J.-T. Zhao // LG4"; 2♂ (CCCC, CZMZ) "Guangxi, Jinxiu, Dayaoshan, Laoshan Forest Farm, 1400 m, 2019.VI.22, leg. J.-T. Zhao"; 1♂ (CZMZ) "Guangxi, Jinxiu, Dayaoshan, Laoshan Forest Farm, 1500 m, 2019.V.31, leg. J.-T. Zhao"; 3♂ (CCCC) "Guangxi, Jinxiu, Dayaoshan, Laoshan Forest Farm, 1580 m, 2018.VI.11, leg. J.-T. Zhao"; 8♂ (CCCC) "Guangxi, Jinxiu, Dayaoshan, Xinpingshan, 1200 m, 2018.V.17, leg. J.-T. Zhao"; 1♂ (CZMZ) "Guangxi, Jinxiu County, Dayaoshan, Yinshang Park, 2023.V.16, Xiao-Han Ye leg."; 1♂ (CCZ) "CHINA-Guangxi, Dayao Shan Jinxiu 100 km SE Liuzhou h~1200 m, June 2005, 23°45′ N 109°45′ E, lg. team of V. Siniaev".

**Other material examined.** 1♂ (SCAU) "CHINA: Guangdong, Shaoguan, Ruyuan Co., Nanling N. R., N 24°55′32″, E 113°1′19″, 1027 m, 26–28.V.2022 (at light), lgt. Ming-Zhi Zhao *et al.* // LG2".

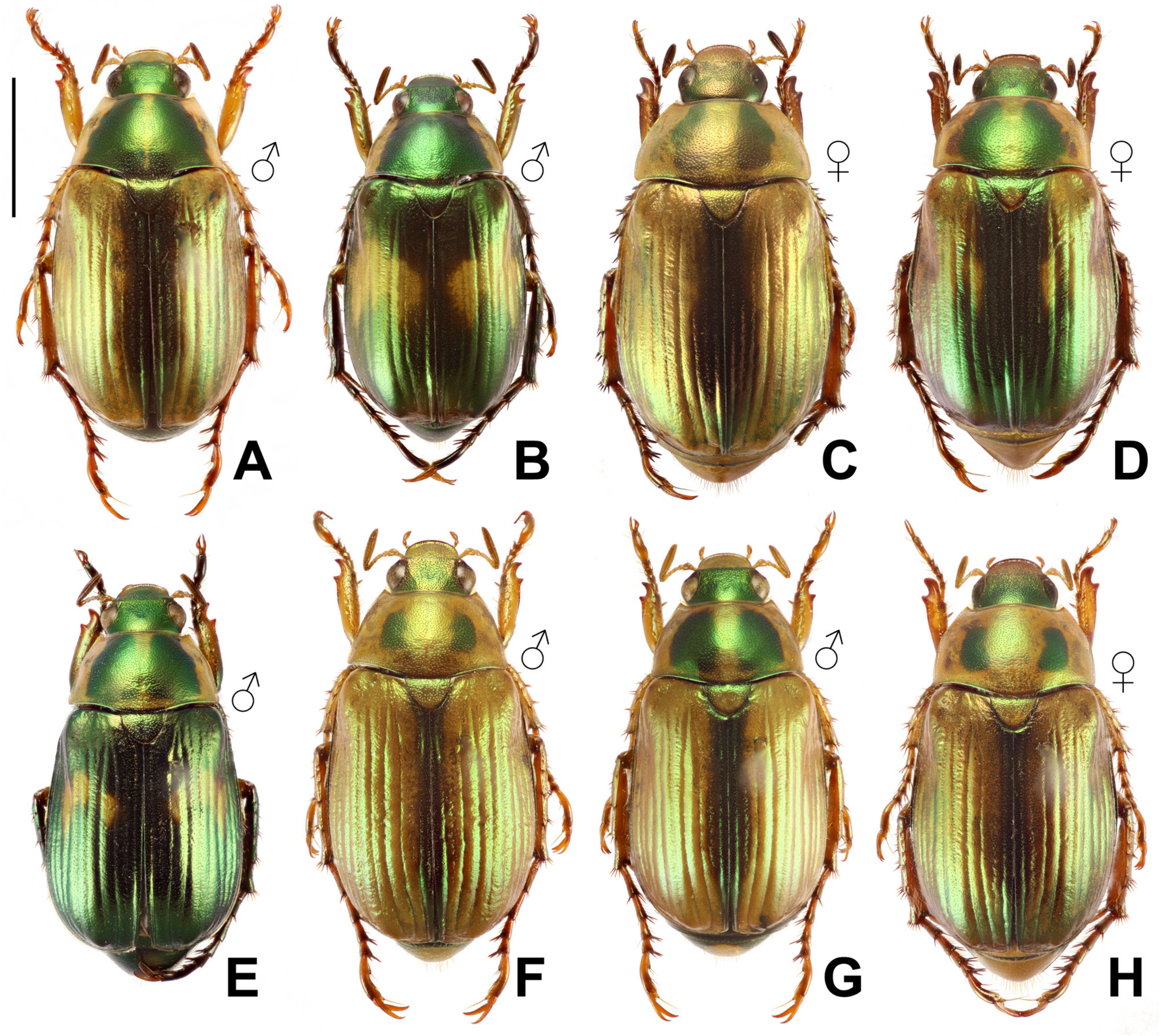

**FIGURE 1.** Habitus of *Anomala* spp. in dorsal view. **A–E.** *A. nanlingensis* Zhao, Zorn & Liu, **sp. nov. F–H.** *A. zhengyucheni* Zhao, Zorn & Liu, **sp. nov.** A, F. Holotypes. B–E, G–H. Paratypes (B–D. Guangdong. E. Guangxi). Scale bar = 5 mm.

**Description of holotype.** ***General.*** Body shape elongated ovoid, widest at apical third of elytra, strongly convex. ***Color*** generally pale yellow, darker at antennal club, metatibia and all tarsi; ventral surface of head (excluding mouthparts), epipleura, posterior half of mesosternum, metasternum and abdomen blackish brown, abdomen with greenish sheen, a broad longitudinal patch on pygidium pale yellow; frons and vertex, two large patches on pronotum extending from anterior to posterior margins, scutellum, as well as a broad stripe extending from scutellum to apex green; body with weak metallic luster; setae pale yellow, robust setae on legs reddish brown. ***Head.*** Greatest length/width of clypeus = 1/2.4, subtrapezoidal, anterior angle broadly rounded, anterior margin feebly convex, lateral margin strongly convergent anteriad; margins weakly reflexed; clypeal surface with dense and large punctures; frontoclypeal suture almost straight, fine; frons with dense and large punctures, gradually sparser to vertex; antennal club slightly shorter than combined length of antennomeres 1–6; greatest diameter of eye 0.55 times the minimal interocular width; inner margin of eyes with two long setae, apex of canthus without seta. ***Pronotum.*** Sides moderately convergent anteriad in basal half, strongly convergent anteriad in anterior half; anterior angle acute and protruding, posterior angle almost right-angled; anterior and lateral marginal lines complete, basal margin line interrupted anterior to scutellum; disc with moderately dense and large punctures; lateral margins with a few long setae. ***Scutellum*** subtriangular, lateral margins arched, with dense and large punctures. ***Elytra.*** Strial

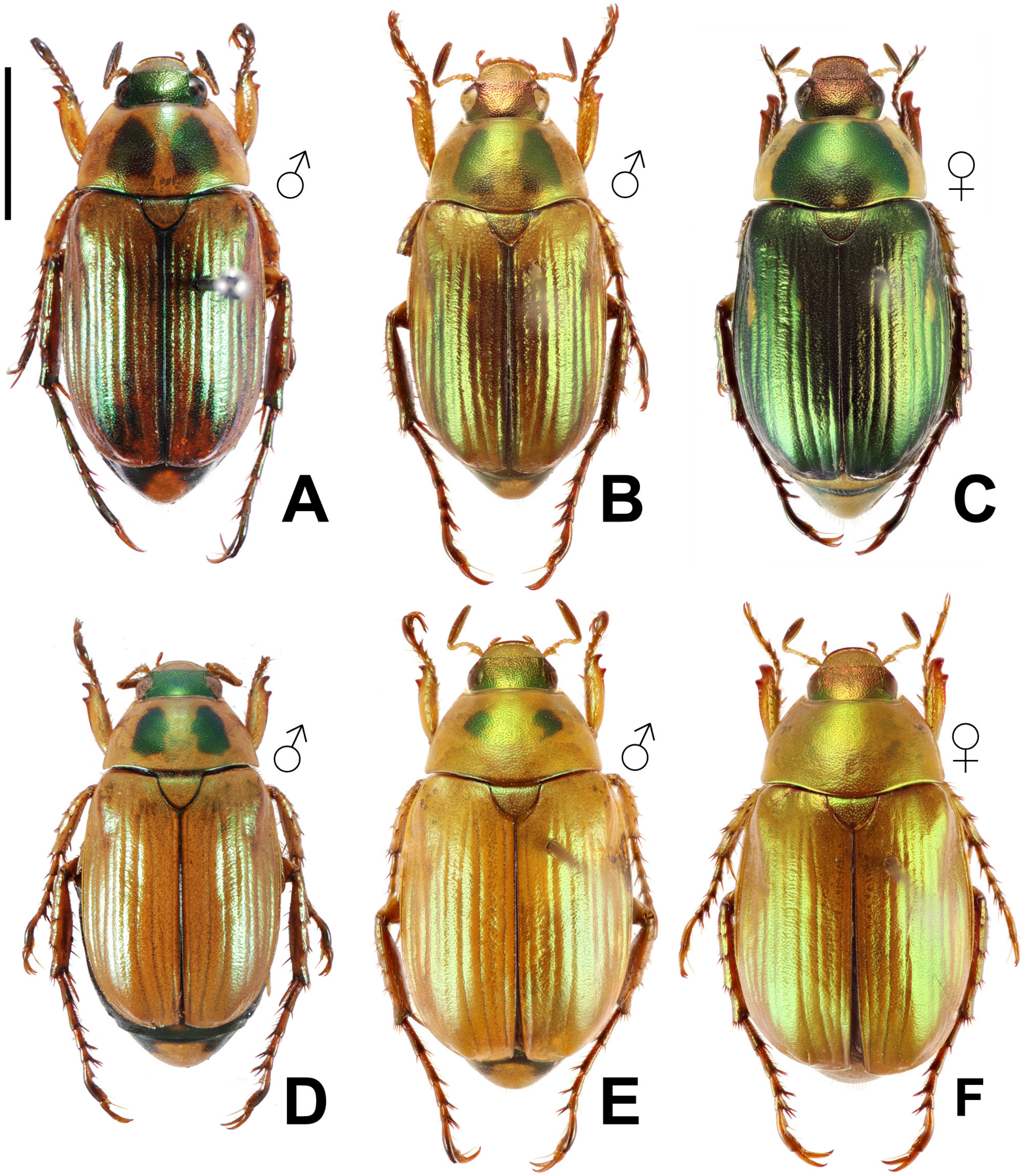

**FIGURE 2.** Habitus of *Anomala* spp. in dorsal view. **A–C.** *A. fuscosignata* Ohaus, 1905 (A, Lang Son; B–C, Guangxi). **D–F.** *A. luminosa* Benderitter, 1929 (D. Ha Giang. E–F. Yunnan). Scale bar = 5 mm.

punctures dense and large, interstice I widest, with a coarse and irregularly doubled secondary stria, interstice II with a similar secondary stria at basal third, other intervals with moderately dense and small punctures; humeral and apical protuberances moderately prominent; lateral carina narrow, epipleura wide at basal third, with a few setae basally. ***Ventral thoracic surface.*** Hypomeron sparsely setose, metathoracic surface with shallow and very large punctures, which usually merge into transverse striolation, with dense and long setae, middle part with short setae; mesosternum between mesocoxae very narrow, with dense and short setae. ***Abdomen.*** Ventrites with dense and transverse punctures, sides not carinate, each ventrite with a transverse row of long setae, which is denser and doubled in ventrites 1–2 and broadly interrupted in middle of ventrites 3–4; pygidium produced and depressed along lateral portions, with dense and transverse punctures, lateral and apical portion with long setae; propygidium punctate as in pygidium, glabrous. ***Legs*** slender; protibia bidentate, teeth acute, apical tooth extending to base of protarsomere 2, meso- and metatibiae each bearing two transverse groups of robust setae, basal portion with some randomly arranged and shorter robust setae; protarsomeres thickened, each 5th tarsomere with a protuberance mesoventrally; inner protarsal and outer mesotarsal claws incised apically, the lower and upper branches almost equal in length; inner protarsal claws strongly concave at base, its lower branch approximately 3 times wider than upper one. ***Male genitalia.*** Parameres slender, slightly divergent distally, ventral margin with a small tooth pre-apically, dorsal margin strongly concave baso-proximally; ventral plate spiniform, a short pre-apical carina bearing two small teeth.

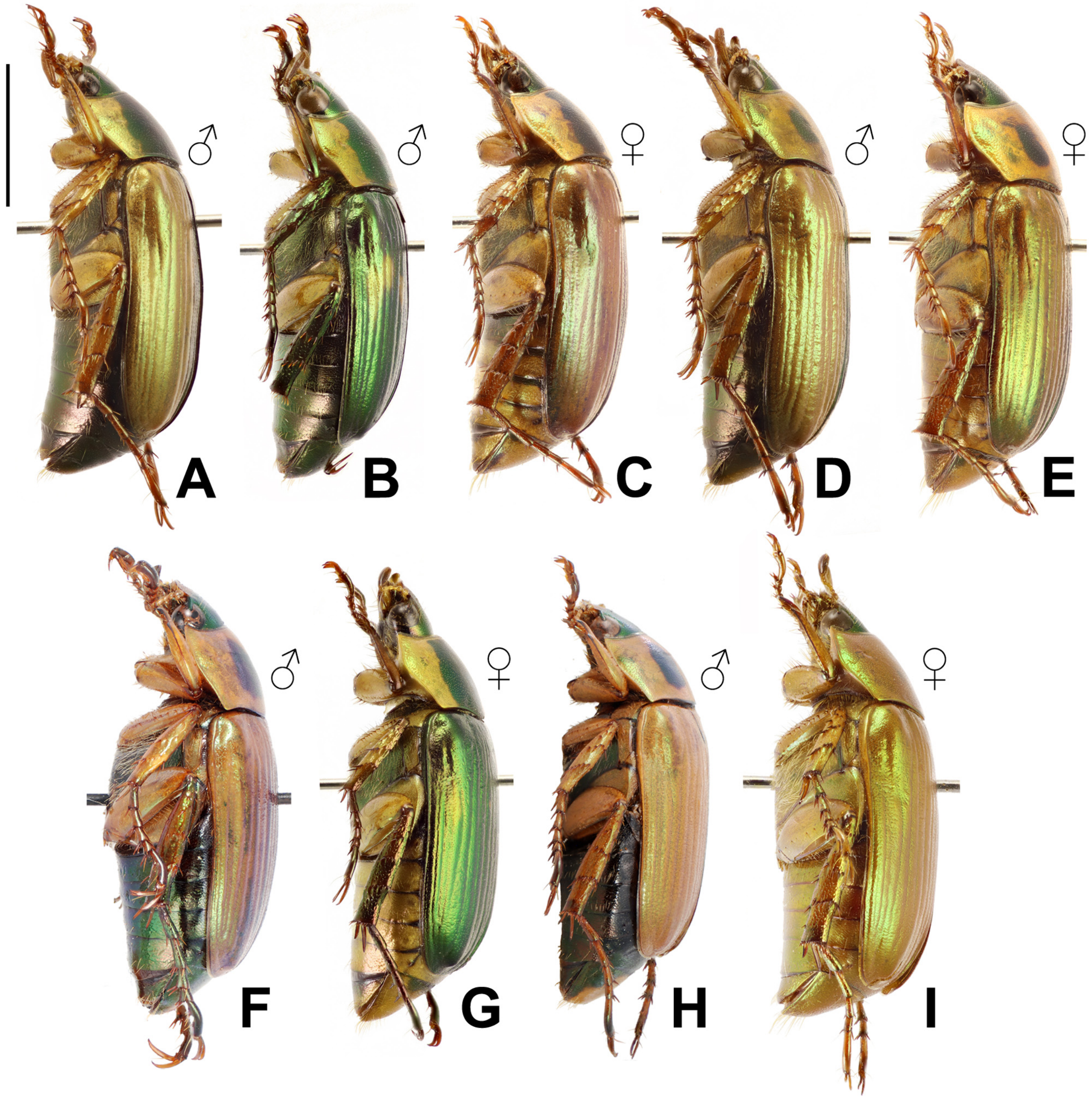

**FIGURE 3.** Habitus of *Anomala* spp. in lateral view. **A–C.** *A. nanlingensis* Zhao, Zorn & Liu, **sp. nov.** (A. Holotype. B. Paratype from Guangxi. C. Paratype from Guangdong). D–E. *A. zhengyucheni* Zhao, Zorn & Liu, **sp. nov.** (D. Holotype. E. Paratype). **F–G.** *A. fuscosignata* Ohaus, 1905 (F. Lang Son. G. Guangxi). **H–I.** *A. luminosa* Benderitter, 1929 (H. Ha Giang. I. Yunnan). Scale bar = 5 mm.

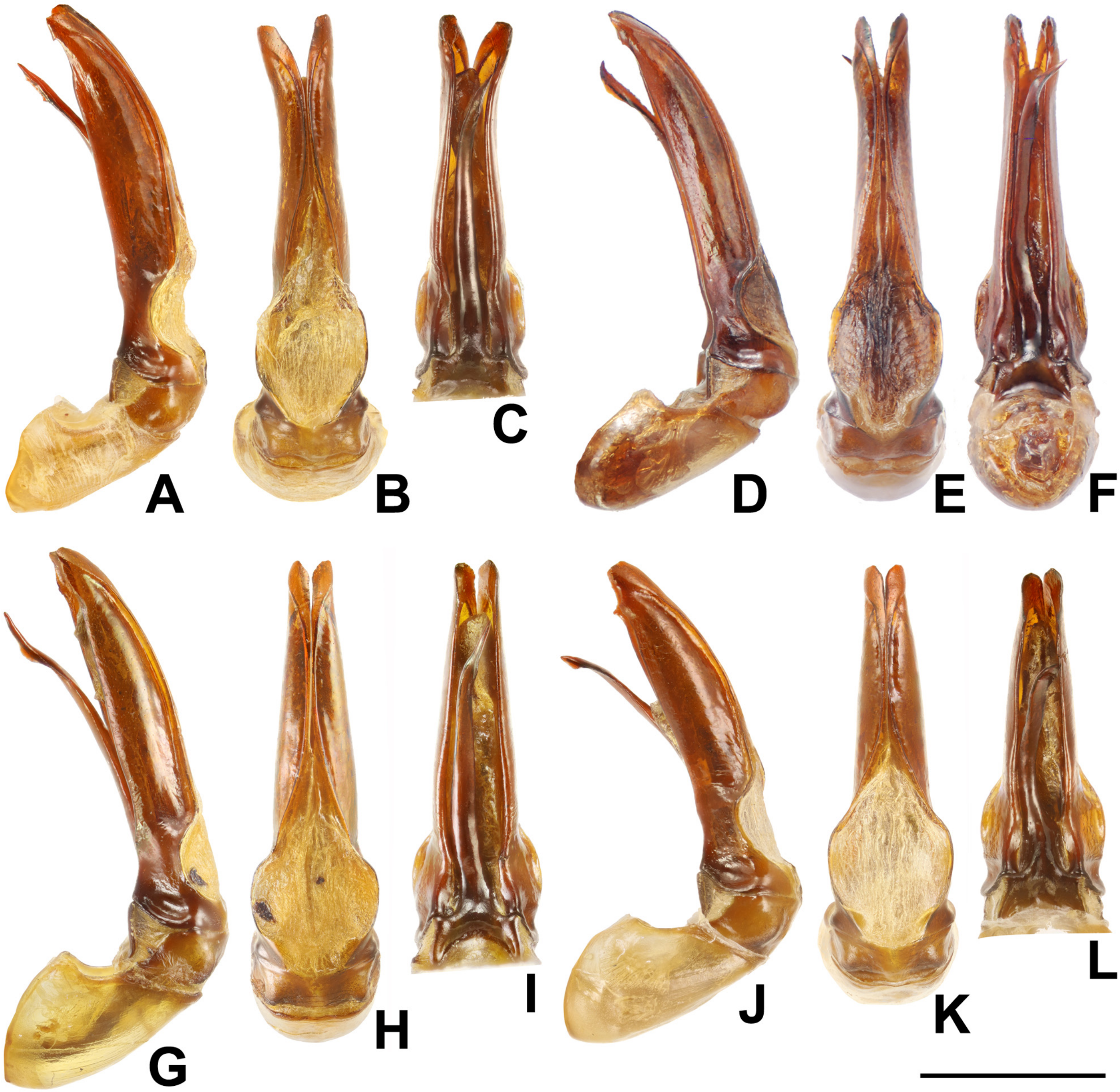

**FIGURE 4.** Male genitalia of *Anomala* spp. **A–I.** *A. nanlingensis* Zhao, Zorn & Liu, **sp. nov.** (A–C. Holotype. D–F. Paratype from Guangdong. G–I. Paratype from Guangxi). **J–L.** *A. zhengyucheni* Zhao, Zorn & Liu, **sp. nov.** (holotype). A, D, G, J. Right lateral view. B, E, H, K. Dorsal view. C, F, I, L. Ventral view. Scale bar = 2 mm.

**Variations.** Colors vary significantly: in yellowish forms, the size of the pronotal patches range from large, extending to anterior and posterior margins, to small, which do not reach either margin, scutellum varies from yellow to green, and dark stripes on elytra vary from touching the outer margins of interstice I to the outer margins of interstice IV (limited to the apical half), often accompanied by 1 or 2 elongated spots at middle in the latter case, the pygidium varies from green with yellowish longitudinal stripes; in greenish forms, the clypeus, scutellum, elytra and pygidium are all green, elytra typically exhibit a zigzag row of three yellow spots at midpoint, occasionally merged into a broad transverse patch, the pronotal patches merge at middle and always touch anterior and posterior margin of pronotum, lateral parts narrowly yellow, the meso- and metatibiae and tarsi dark green. Specimens of the greenish form from Guangxi typically have an incomplete longitudinal midline on the large pronotal patch, rarely divided into two isolated large patches. ***Female*** similar to males; in yellowish forms, the ventral surface generally yellow, a transverse band at middle and lateral margins of mesosternum, as well as basal third of each abdominal ventrite dark green, the darker portions sometimes extensive in ventrites 1–2, pygidium only bearing blackish basal margin; greenish forms identical to those in males; antennal club almost equally as long as the combined length of antennomeres 1–6, lateral carina of elytra slightly wider, apical protibial tooth extending to base of protarsomere 3 and rounded at apex, protarsomeres not thickened, lower branch of inner protarsal claw shorter and two times wider than upper branch. ***Female genitalia.*** Vagina well sclerotized, flattened, shortly widened into funnel shape distally, dorsal paraproct strongly emarginated at distal margin.

**Measurements.** body length: 11.6–14.0 mm in males (holotype 12.9 mm), 13.0–14.1 mm in females; greatest width: 5.5–7.0 mm in males (holotype 6.5 mm), 6.5–7.2 mm in females.

**Diagnosis.** *Anomala nanlingensis* **sp. nov.** and the following *A. zhengyucheni* **sp. nov.** differ from *A. fuscosignata* (Ohaus, 1905) and *A. luminosa* Benderitter, 1929 by the shape of their parameres, which are simply rounded or nearly truncate at apices. In contrast, *A. fuscosignata* and *A. luminosa* exhibit parameres with a strong apical constriction, forming a short and downward protrusion. The female genitalia of the two new species possess a longer and flatter vagina (Fig. 7A, B) compared to the shorter and thicker vagina found in *A. fuscosignata* and *A. luminosa* (Fig. 7C, D).

*A. nanlingensis* can be distinguished from the very similar *A. zhengyucheni* by its typically larger extend of greenish coloration, and the sharp and apically straight or moderately upward pointing ventral plate of the aedeagus (Fig. 6A–O), which is rather blunt and apically downward pointing in *A. zhengyucheni* (Fig. 6P–U). Additionally, the dorsal paraproct of the female genitalia differs significantly at the distal margin: it is emarginated in *A. nanlingensis* (Fig. 7F, J) and truncate in *A. zhengyucheni* (Fig. 7E, I).

**Etymology.** This new species is named after the Nanling Nature Reserve, its type locality and where it was first discovered.

**Distribution.** China (Guangdong, Guangxi).

### *Anomala zhengyucheni* Zhao, Zorn & Liu, sp. nov.

Chinese common name: 昱辰异丽金龟

(Figs 1F–H, 3D–E, 4J–L, 6P–U, 7A, 7E, 7I)

**Type material examined. HOLOTYPE: CHINA:** ♂ (SCAU), “CHINA: Fujian, Quanzhou, Dehua Co., Shuikou Town, Mt. Shiniushan, Bangshang Village, 1000 m, 2022.VI.2, Yu-Chen Zheng & Zheng Zhang leg.”; **PARATYPES: CHINA:** 44♂, 5♀ (CZMZ) “CHINA: Fujian, Quanzhou, Dehua Co., Shuikou Town, Mt. Shiniushan, Bangshang Village, 1000 m, 2022.VI.2, Yu-Chen Zheng & Zheng Zhang leg.”; 1♂ (SCAU) “CHINA: Fujian, Quanzhou, Dehua Co., Shuikou Town, Mt. Shiniushan, Bangshang Village, 1000 m, 2022.VI.2, Yu-Chen Zheng & Zheng Zhang leg. // ZYC1”; 1♂ (SCAU) “CHINA: Fujian, Quanzhou, Dehua Co., Shuikou Town, Mt. Shiniushan, Bangshang Village, 1000 m, 2022.VI.2, Yu-Chen Zheng & Zheng Zhang leg. // ZYC2”; 2♂, 1♀ (CZMZ) “Fujian, Quanzhou, Dehua County, Shuikou Town, Mt. Shiniushan, Geyao, 1200 m, 2020.V.14, Yu-Chen Zheng leg.”

**Description of holotype.** ***General.*** Body shape elongated ovoid, widest at apical third of elytra, strongly convex. ***Color*** generally pale yellowish brown, with slightly darker antennal club, metatibia and tarsi; ventral surface of head (excluding mouthparts), epipleura, a large central patch and a large, triangular patch on posterolateral part of mesosternum, metasternum and abdomen blackish brown, a broad longitudinal patch on pygidium yellow; frons and vertex, two large patches on approximately center of pronotum, as well as the costal interval 1 green; body with weak metallic luster; setae pale yellow, robust setae on legs reddish brown. ***Head.*** Greatest length/width of clypeus = 1/2.2, nearly semicircular, lateral margin strongly convergent anteriad; margins weakly reflexed; clypeal surface with dense and large punctures; frontoclypeal suture straight, fine; frons with dense and large punctures, gradually sparser to vertex; antennal club slightly shorter than combined length of antennomeres 1–6; greatest diameter of eye 0.56 times the minimal interocular width; inner margin of eyes with four or five long setae, apex of canthus without seta. ***Pronotum.*** Sides moderately convergent anteriad in basal half, strongly convergent anteriad in apical half; anterior angle acute and protruding, posterior angle almost right-angled; anterior and lateral marginal lines complete, basal margin line interrupted anterior to scutellum; disc with moderately dense and large punctures; lateral margins with a row of sparse long setae. ***Scutellum*** subtriangular, lateral margins arched, with dense and large punctures. ***Elytra.*** Strial punctures dense and large, interstice I widest, with a coarse and irregularly doubled secondary stria, interstice II with a similar secondary stria at basal fourth, other intervals with moderately dense and large punctures; humeral and apical protuberances moderately prominent; lateral carina narrow, epipleura wide at basal third, with a moderately dense row of short setae in the basal half, becoming sparser apically. ***Ventral thoracic surface.*** Hypomeron sparsely setose, metathoracic surface with shallow and very large punctures, which usually merge into transverse striolation, with dense and long setae, middle part with short setae; mesosternum between mesocoxae narrow, with dense and short setae. ***Abdomen.*** Ventrites with dense and transverse punctures, sides not carinate, each ventrite with a transverse row of long setae, which is denser and doubled in ventrites 1–2, sparse but not interrupted in middle of ventrites 3–4; pygidium produced and depressed along lateral portions, pygidium with dense and transverse punctures, lateral and apical portion with long setae; propygidium punctate as in pygidium, glabrous. ***Legs*** slender; protibia bidentate, teeth acute, apical tooth extending to base of protarsomere 2, meso- and metatibiae each bearing two transverse groups of robust setae, basal portion with some randomly arranged and shorter robust setae; protarsomeres thickened, each 5th tarsomere with a protuberance mesoventrally; inner protarsal and outer mesotarsal claws incised apically, the lower and upper branches almost equal in length; inner protarsal claws strongly concave at base, its lower branch approximately 3 times wider than upper one. ***Male genitalia.*** Parameres slender, slightly divergent distally, ventral margin with a small denticle subapically, dorsal margin strongly concave baso-proximally; ventral plate spiniform, with a short carina apically.

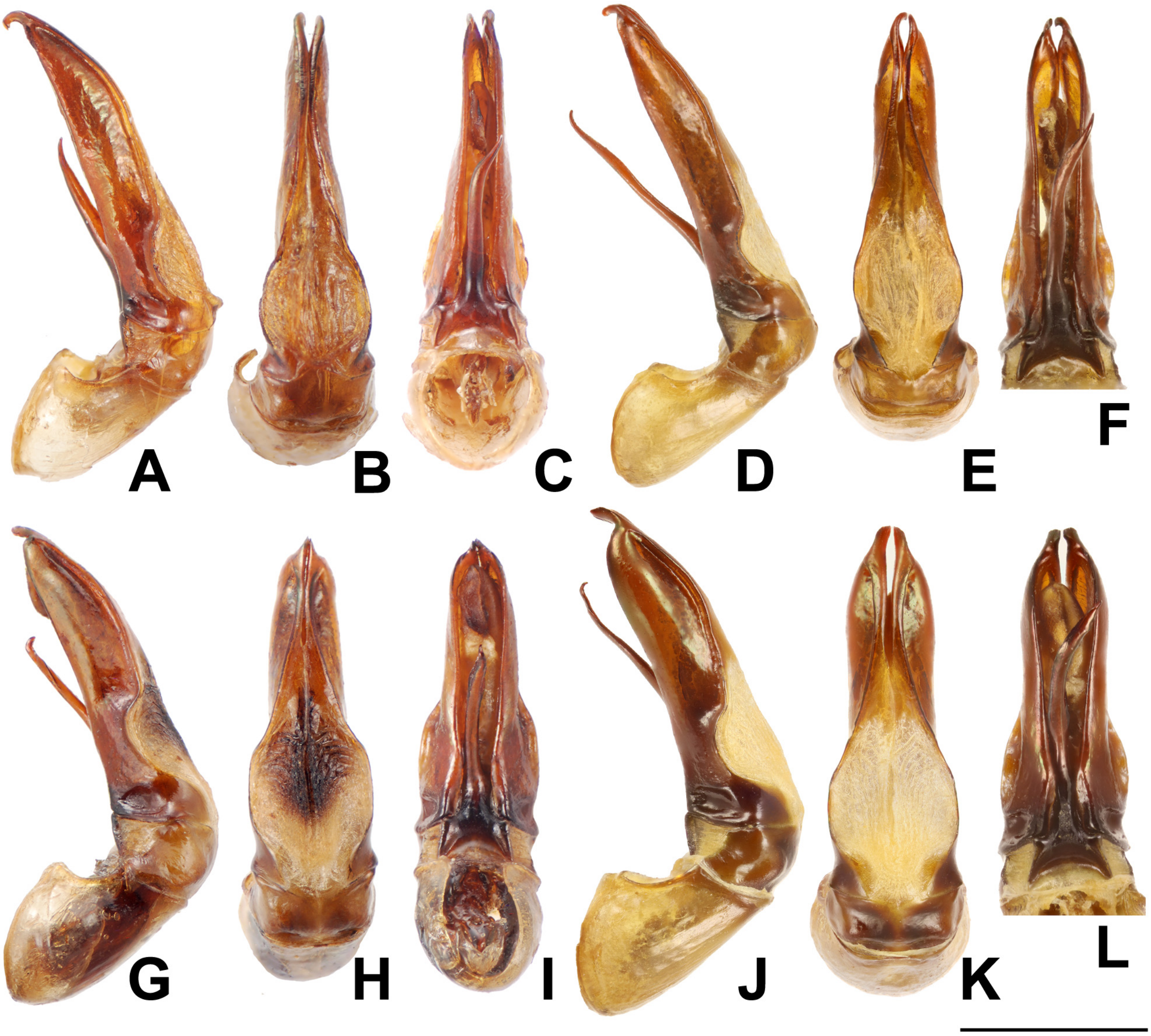

**FIGURE 5.** Male genitalia of *Anomala* spp. **A–F.** *A. fuscosignata* Ohaus, 1905 (A–C. Lang Son. D–F. Guangxi). **G–L.** *A. luminosa* Benderitter, 1929 (G–I. Ha Giang. J–L. Yunnan). A, D, G, J. Right lateral view. B, E, H, K. Dorsal view. C, F, I, L. Ventral view. Scale bar = 2 mm.

**Variations.** Pronotal patches rarely extending to near anterior and posterior margins, mesosternum sometimes almost completely blackish brown, dark stripes on elytra at most reach the outer margins of interstice I. ***Female*** similar to males; abdomen and metacoxae generally yellow, pygidium with narrow blackish margin; antennal club almost equally as long as the combined length of antennomeres 1–6, lateral carina of elytra slightly wider, apical protibial tooth extending to base of protarsomere 3 and rounded at apex, protarsomeres not thickened, lower branch of inner protarsal claw shorter and two times wider than upper branch. ***Female genitalia.*** Vagina well sclerotized, flattened, shortly widened into funnel shape distally, dorsal paraproct truncated at distal margin.

**Measurements.** body length: 12.3–13.8 mm in males (holotype 13.5 mm), 12.5–13.3 mm in females; greatest width: 6.2–7.0 mm in males (holotype 7.0 mm), 6.5–7.0 mm in females.

**Diagnosis.** See diagnosis of *Anomala nanlingensis* **sp. nov.**

**Etymology.** This new species is dedicated to Yu-Chen Zheng, a neuropterist who collected and kindly provided the type series to us.

**Distribution.** China (Fujian).

**FIGURE 6.** Apex of ventral plate of *Anomala* spp. in right lateral view. **A–O.** *A. nanlingensis* Zhao, Zorn & Liu, **sp. nov.** (A–H. Paratypes from Guangdong. I. Holotype. J–O. Paratypes from Guangxi). **P–U.** *A. zhengyucheni* Zhao, Zorn & Liu, **sp. nov.** (P. Holotype. Q–U. Paratypes). Scale bar = 1 mm.

## *Anomala fuscosignata* Ohaus, 1905

Chinese common name: 绿斑异丽金龟

(Figs 2A–C, 3F–G, 5A–F, 7C, 7G, 7K)

*Anomala fuscosignata* Ohaus, 1905: 86 [type locality: "Tonkin, Mauson-Berge, Than-moi"]; Zorn *et al.*, 2017: 341.

**Type material examined. Syntypes.** 1♂ (MFNB) "Tonkin Montes Mauson April,Mai 2-3000' H. Fruhstorfer // *Anomala fuscosignata* Type Ohs."; 3♂ (MFNB) "Tonkin Montes Mauson April.Mai 2-3000' H. Fruhstorfer // *Anomala fuscosignata* ♂ Cotype Ohs."; 1♀ (MFNB) "Tonkin Than-Moi Juni-Juli H. Fruhstorfer // *Anomala fuscosignata* ♀ Cotype Ohs."

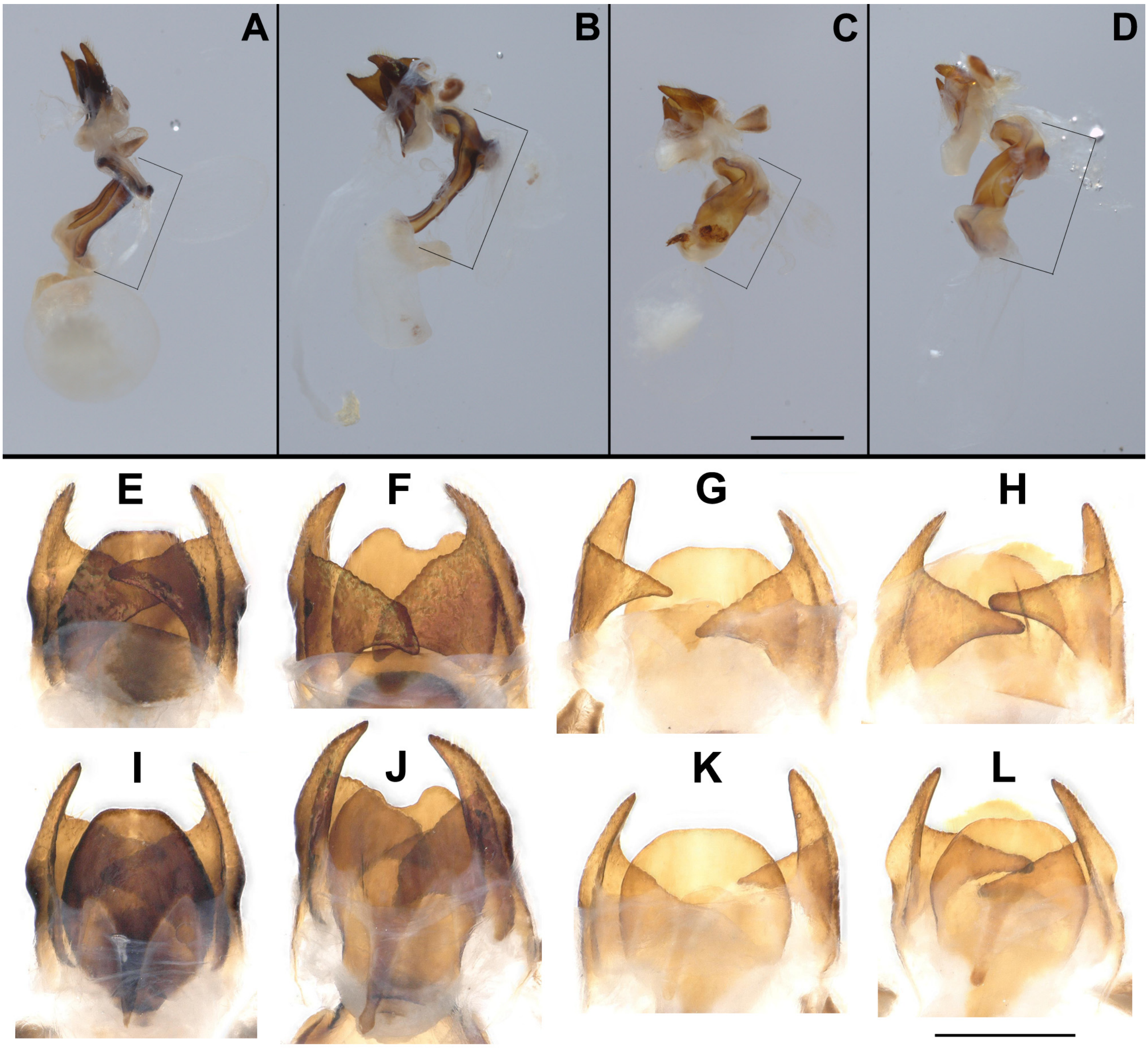

**FIGURE 7.** Female genitalia of *Anomala* spp. **A, E, I.** *A. zhengyucheni* Zhao, Zorn & Liu, **sp. nov.** (paratype). **B, F, J.** *A. nanlingensis* Zhao, Zorn & Liu, **sp. nov.** (paratype from Guangdong). **C, G, K.** *A. fuscosignata* Ohaus, 1905 (Guangxi). **D, H, L.** *A. luminosa* Benderitter, 1929 (Yunnan). A–D. Female genitalia in lateral view, with the sclerotized vagina indicated. E–H. Gonocoxite and dorsal paraproct in ventral view. I–L. Gonocoxite and dorsal paraproct in dorsal view. Scale bar for A–D = 2 mm, for E–L = 1 mm.

**Other material examined. CHINA:** 1♂ (CZMZ) Guangxi, Wuming, Mt. Damingshan, 2014.VI.2, Chao Li leg.; 1♀ (CZMZ) Guangxi, Shanglin, Mt. Damingshan, Tianping, 1200 m, 2022.V.29, Xiao-Yu Zhu leg. // FUCO1; 1♀ (CZMZ) Guangxi, Shanglin, Mt. Damingshan, Tianping, 1200 m, 2022.V.29, Xiao-Yu Zhu leg. // FUCO2; 1♀ (CZMZ) Guangxi, Shanglin, Mt. Damingshan, Tianping, 1200 m, 2022.V.29, Xiao-Yu Zhu leg. **VIETNAM:** 2♂, 4♀ (MFNB) Tonkin Montes Mauson April.Mai 2-3000' H. Fruhstorfer.

**Remarks.** Both genetic distances and species delimitations based on *COI* sequences demonstrate that *A. fuscosignata* and *A. luminosa* are conspecific from a molecular perspective. Morphologically, they are similar in the thicker and shorter vagina compared to *Anomala nanlingensis* **sp. nov.** and *A. zhengyucheni* **sp. nov.** as well as the presence of an apical constriction at the parameres. However, the parameres of *A. luminosa* are obviously wider than those of *A. fuscosignata* in profile, and the body shape appears to be slightly more robust. However, this observation is based on the few specimens available to us for examination. Additionally, our molecular analysis did not include any topotypical specimens of both taxa. In summary, our findings support to retain their status as distinct species.

The unique available male from Guangxi has a conspicuously longer ventral plate (Fig. 5D) compared to the Mauson specimens (Fig. 5A). Whether this has any taxonomic value remains unknown at present.

**Distribution.** China (Guangxi); Vietnam (Lang-Son).

### *Anomala luminosa* Benderitter, 1929

Chinese common name: 金光异丽金龟

(Figs 2D–F, 3H–I, 5G–L, 7D, 7H, 7L)

*Anomala luminosa* Benderitter, 1929: 105, fig. 4 [type locality: "Chapa"]; Zorn *et al.*, 2017: 344.

**Type material examined.** None.

**Other material examined. CHINA:** 1♂, 1♀ (CZMZ) Yunnan, Honghe, Pingbian, Mt. Daweishan, 2017. VI.21–27, Bill Yang leg.; 1♂ (CZMZ) Yunnan, Honghe, Pingbian, Mt. Daweishan, 2020.V.3, Zheng Zhou leg. // LMIN; 1♀ (CZMZ) Yunnan, Honghe, Pingbian, Mt. Daweishan, 2100 m, 2015.V.17, Jian-Yue Qiu leg.; 1♂ (CZMZ) Yunnan, Honghe, Jinping, Jinhe Town, Wujiazhai Village, 2019.VI; **VIETNAM:** 1♂ (CCZ) Ha Giang prov., 1500 m, 2011.VII, M. Peijcha leg.

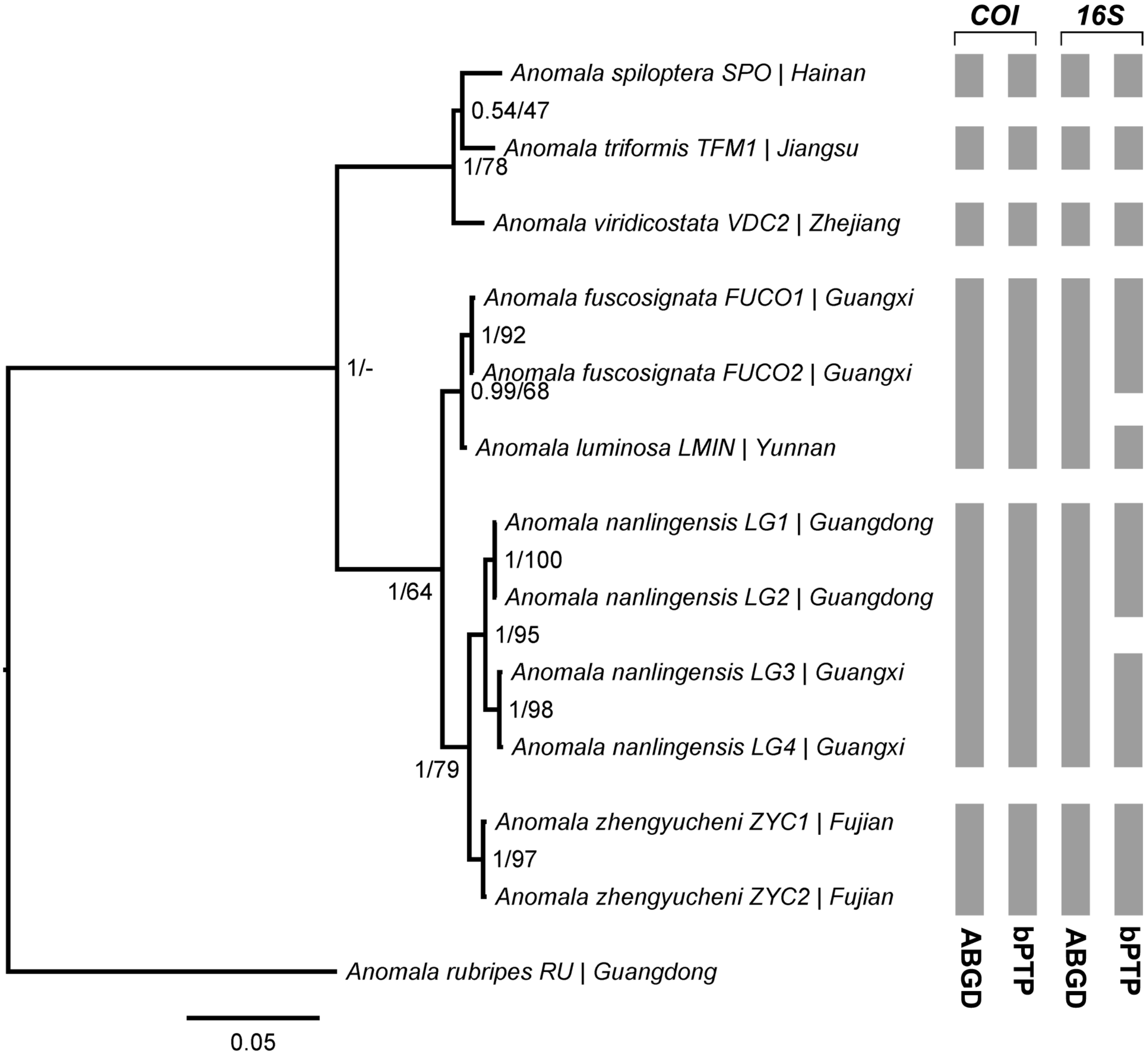

**FIGURE 8.** Bayesian phylogenetic tree based on concatenated partitioned dataset of *COI* and *rrnL* sequences. Numbers next to each node represent Bayesian posterior probabilities (left) and maximum likelihood bootstrap support values (right). Sidebars elucidate species delimitations proposed by ABGD and bPTP approaches.

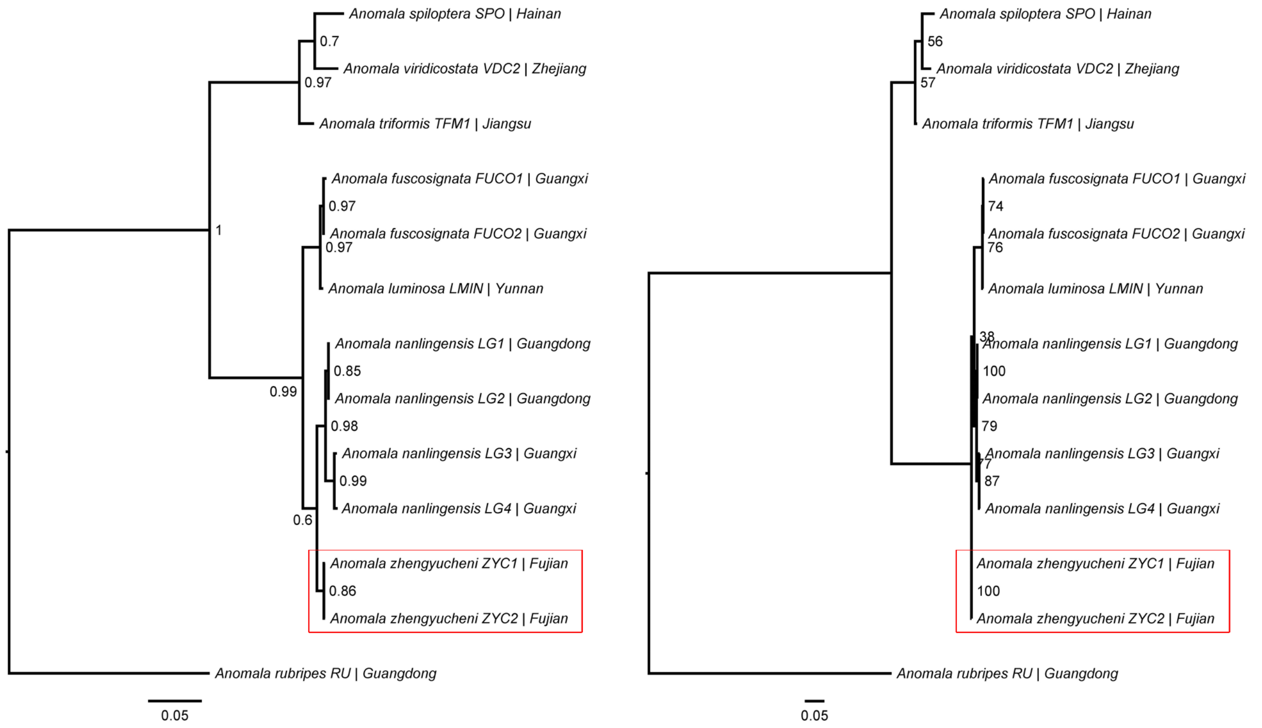

**FIGURE 9.** Phylogenetic trees based on *COI* dataset by Bayesian inference (left) and Maximum likelihood (right) analyses respectively.

**Remarks.** This species is reported from China for the first time. A re-examination of specimens from Vinh Phuc, reported as *A. luminosa* by Zorn *et al*. (2017), revealed a misidentification of these specimens, which appear to be related to *A. jeanvoinei* Benderitter, 1929. Therefore, the record of Vinh Phuc is deleted here.

The males from Yunnan have a conspicuously longer ventral plate (Fig. 5J) compared to the specimen from Ha Giang (Fig. 5G). The holotype, which has not yet been located, may have a similarly shorter ventral plate, judging from the drawings in the original description.

**Distribution.** China (Yunnan) **(new record)**; Vietnam (Ha Giang, Lao Cai).

## Discussion

In the present paper, we recognize two new species belonging to the *A. fuscosignata*-complex by carefully comparing the genital organs of both sexes and conducting a preliminary phylogenetic analysis. Although species of Anomalini typically show distinct interspecific variations in male genitalia, the female genitalia remain poorly studied. Most females are associated with certain males by external features or by localities. Previous works involving female genitalia have focused on the shape of the gonocoxite, the so-called vaginal palps (Huang & Wang 2019; Lin 1988, 1993, 1996; Zorn 1998, 2011a). The robust interspecific differences in dorsal paraproct and vagina observed within the *A. fuscosignata*-complex highlight the importance of examining the entire female genital organs of Anomalini for taxonomic purposes. This approach has proven effective also in other pleurostict scarabs, such as Sericini (Özgül-Siemund & Ahrens 2015).

The present case also shows incongruence between morphospecies and genospecies. The *p*-distances calculated based on the *COI* dataset indicate deeper genetic divergence between two populations of *A. nanlingensis* than between *A. fuscosignata* and *A. luminosa*. Consequently, this may have led to the merging of the latter two species in ABGD and bPTP species delimitations. Similar instances occur in flower chafers *Cetonia aurata* (L., 1761) and *Dicronocephalus wallichii* Hope, 1831, where mitochondrial DNA between subspecies diverge nearly as considerably as at interspecific levels between closely related congeners (Ahrens *et al.* 2013; Lee *et al.* 2015). However, these studies also emphasized the importance of integrating other evidence, such as geometric morphometry. Despite minimal genetic divergence, *A. fuscosignata* and *A. luminosa* can be easily distinguished morphologically. In contrast, we refrain from splitting the more genetically divergent populations of *A. nanlingensis* until further evidence becomes available.

Using complete and extensive samples (both individuals and localities) from a certain group is important for subdivision of taxa (Vondráček *et al.* 2018, 2023). All taxa in this complex inhabit forests in the elevation range of approximately 900 to 1700 meters (except for the three paratypes of *A. nanlingensis* in SYSU with broad altitude range). Currently, it remains uncertain whether *A. nanlingensis* and *A. zhengyucheni* are relict taxa, given their localized known distribution, especially when compared to the high population density and great efforts of fieldworks conducted in southern China. However, intermediate populations may still be found in the future.

The *COI* barcode is the most widely employed technique for distinguishing taxa due to its universality and high resolution in animals (Hebert *et al.* 2003). *COI*-based phylogeny has successfully resolved taxonomic problems in several genera within the tribe Rutelini, such as *Chrysina* and *Pelidnota* (Moore *et al.* 2017; Zubov *et al.* 2019), where the interspecific divergence is typically higher than 0.05 or 0.10. In the *A. fuscosignata*-complex, ML analysis based on *COI* sequences alone created a topology that renders the subclade 2 paraphyletic, which is discordant with the results of the combined dataset. The combined gene dataset yielded identical topologies between BI and ML analyses, in which subclades 1 and 2 of the *A. fuscosignata*-complex aligns with morphological similarities, particularly in the genital organs, and is therefore considered more reliable. This suggests that relying on a single marker may be insufficient for resolving taxonomic issues, even among closely related species with low genetic divergence. Future study regarding closely allied Anomalini taxa should aim to increase the sample size and incorporate additional molecular markers to resolve this discordance more effectively.

## Acknowledgements

Ming-Zhi Zhao is deeply indebted to Yu-Chen Zheng (郑昱辰), Chang-Chin Chen (陳常卿), Jing-Kun Zhang (张锦坤), Xiao-Han Ye (叶潇涵), Bao-Xiang Zhan (詹宝翔), Yun-Shu Ma (马云舒), Xiao-Yu Zhu (朱笑愚), Zheng Zhou (周正), Jian-Yue Qiu (邱见玥), Chao Li (李超), Tian-Jiao Wang (王天骄), Zhi-Teng Chen (陈志腾) and Mo-Lin Lu (鲁墨林) for collecting and kindly providing invaluable material for this study. Carsten Zorn wishes to express his sincere gratitude to Pol Limbourg (IRSNB) as well as to Bernd Jaeger and Joachim Willer (MFNB) for providing access to the collections under their care and for issuing generous loans of material. Pol Limbourg and an anonymous reviewer are acknowledged for improving the manuscript.

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# 绿斑异丽金龟复合群*Anomala fuscosignata*-complex研究暨中国南部二新种（鞘翅目：金龟科：丽金龟亚科）

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**摘要：**通过直接形态学比较和分子系统发育分析，对中国南部和越南北部绿斑异丽金龟复合群*Anomala fuscosignata*-complex的分类开展研究；发现二新种，即南岭异丽金龟*A. nanlingensis* **sp. nov.**和昱辰异丽金龟*A. zhengyucheni* **sp. nov.**；对雌外生殖器形态差异、低遗传差异及它们在异丽金龟族分类中的影响进行了讨论。

**关键词：**分类；系统发育；物种界定；雌外生殖器；异丽金龟族