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Authors: Wang, Yan, Ma, Ji, and Mao, Xinfang

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RESEARCH

Development of a Nondestructive Method for Sexing Live Adult *Sternoplax souvorowiana* (Coleoptera: Tenebrionidae)

Yan Wang,[1] Ji Ma,[1,2] and Xinfang Mao[1]

[1]Xinjiang Key Laboratory of Biological Resources and Genetic Engineering, College of Life Science and Technology, Xinjiang University, 14 Shengli Rd., Urumqi 830046, China
[2]Corresponding author, e-mail: majiuci@xju.edu.cn

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ABSTRACT. The darkling beetle, *Sternoplax souvorowiana* (Reitter) (Coleoptera: Tenebrionidae), is flightless and lives in the Guerbantonggut desert in northwestern China. Its special eggshell structure, day-active habit, large body size, short life cycle, and ease of rearing under laboratory conditions make it an excellent model for advanced studies on desert adaptation. Determining the sex of this beetle is usually complicated by the lack of a discreet, externally visible gender-specific character. To date, dissection has been used for sex identification in this species, whereas a nondestructive means is needed for further studies of sexual dimorphism. Here, a new method based on the difference of the pigmentation pattern on the eighth tergite of each sex is described and illustrated. This method can be quickly learned, is nondestructive, is 100% accurate, and is fast enough for most applications in both the field and the laboratory. Experienced users in our laboratory routinely sex 8–10 beetles per minute.

Key Words: sexual dimorphism, sex determination, eighth tergite, pigmentation pattern

Beetles, especially of the family Tenebrionidae, are among the most successful animals in desert habitats. The study of desert beetles is important, because it illustrates many of the evolutionary solutions of arthropods to the problems engendered, in an extreme form, by life in all terrestrial environments (Cloudsley-Thompson 2001). *Sternoplax souvorowiana* Reitter (Coleoptera: Tenebrionidae), belonging to the tribe Pimeliini, is a day-active, flightless beetle living in the Guerbantonggut desert (Ren and Yu 1999, Huang et al. 2005), the second largest desert in the northwest of China. The maximum air temperature in the desert is above 40°C, and the lowest is below −40°C (Wei and Liu 2000, Qian et al. 2004). It is interesting that the egg of *S. souvorowiana* has a sponge-like exochorion (Fig. 1). This characteristic is not found in other studied desert darkling beetles in the same region, such as *Microdera punctipennis*, *Anatolica polita borealis*, *Colposcelis microderoides microderoides*, and *Oodescelis chinensis*, which have homogeneous and compact eggshells when observed under scanning electronic microscope (LEO1430VP SEM, LEO Corp., Oberkochen, Germany). It remains largely unknown whether the *S. souvorowiana* eggshell plays a role in water conservation. The special eggshell, the population abundance, and the ease of rearing the species under laboratory conditions (Wang et al. 2010, 2011) make *S. souvorowiana* an excellent model for studying the adaptive mechanisms that enable beetles to live in the desert.

It was reported that tenebrionid beetles had sexual differences in supercooling point temperature (Salin et al. 2000) and in life span under dry conditions (Renault and Coray 2004). It thus appears that desert darkling beetles have sexual differences in adaptive mechanisms to desert environments. To conduct studies for further investigation of sex-related differences in desert adaptation of *S. souvorowiana*, one needs to know the sex of live individuals. Usually, sex can be determined by watching insects copulate (Bailez et al. 2003, Wang et al. 2011); however, the copulate sometimes is not a real couple of two sexes; male–male mountings can be found occasionally. In nonmating seasons, this method is not practical. Sex can also be determined by squeezing the abdomen to see the genitalia (Pszczolkowski et al. 2008) or withdrawing the eighth sternite from the abdomen with forceps (Vinod et al. 2008). Unfortunately, these methods usually result in injury or death of the examined beetles (Bhattacharya et al. 1970), especially when the eighth sternite is bent to the seventh sternite, and the genitalia are still difficult to see in *S. souvorowiana*. Besides, their two elytra are fused and difficult to separate from each other (Draney 1993), because of their locking devices (Gorb 1998), which are very common in desert tenebrionid beetles. Because it was not possible to visually assess the sex of living beetles, beetles were usually sexed by dissection after measurements (Salin et al. 2000). For *S. souvorowiana*, many of the reported external visible sex-specific characteristics for sexing adults in Coleopteran insects (Halstead 1963, Schat et al. 2007, Öhrn et al. 2008, Mora and Tuchina 2011) are absent, and it is necessary to develop a reliable method to distinguish sexes without harming the beetles.

To our knowledge, no methods have been reported to differentiate the sex of live adults of the darkling beetle. In this study, a new method, which we called the "tergal pigmentation pattern probing method," is described and illustrated with photographs. This method permits sex determination of the living *S. souvorowiana* adult with 100% accuracy and without hurting the insects.

Materials and Methods

S. souvorowiana, *Anatolica pseudiduma* Frivaldszky, and *Adesmia anomala dejeani* Gebler adults were collected in 2011 from Fukang (44° 24′ N, 087° 51′ E, 444 m), which is about 100 km northeast of the geological center of Asia. Insects were maintained at 30 ± 0.5°C, 30 ± 6% relative humidity (RH), and 16:8 (L:D) h photoperiod conditions and supplied with cabbage daily. Adult rearing, egg collection, larval rearing, and pupal collection were conducted following the methods of Wang et al. (2011). All adults used in these experiments were obtained from both field collections and colonies maintained under laboratory conditions.

To determine sexual dimorphism in external morphology, body parameters of adults (including head width, pronotum width and length, elytra width, and body length) of each sex were measured under stereomicroscope equipped with Elements 3.0 software (Nikon SMZ-800, Nikon Corporation, Tokyo, Japan) and calibrated using an objective micrometer. Statistical significance for adult body parameters was

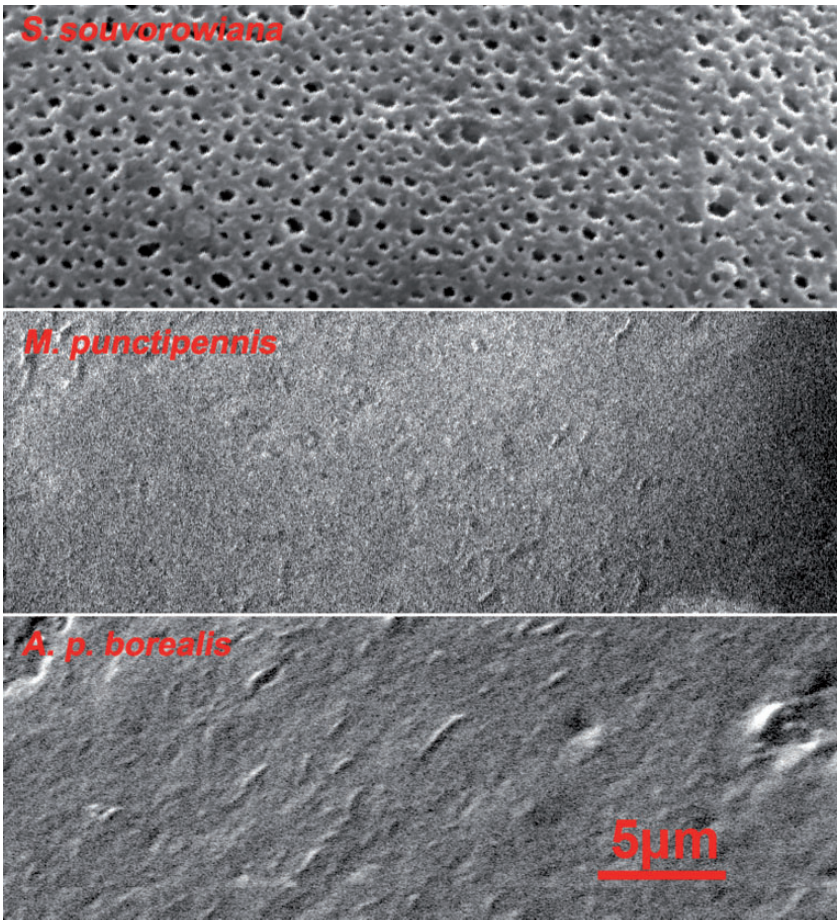

Fig. 1. Scanning electron micrographs of the egg shell in *S. souvorowiana*, *M. punctipennis*, and *A. polita borealis*. The exochorion of *S. souvorowiana* has a sponge-like structure, whereas the exochorion is smooth in both *M. punctipennis* and *A. polita borealis*.

determined using a Student's two-tailed *t*-test with a significance level of $P < 0.05$. Data were subjected to log transformation prior to analysis. The analysis was conducted by GraphPad Prism 4 software.

When sexing by the tergal pigmentation pattern probing method, it was most effective to use the thumb and forefinger to hold each beetle with its head downward and against the microscope stage (with its ventral side up), while using the other hand to manipulate the probe. A magnification of 10× is adequate. The probe, such as a separated arm of stainless tweezers (134-mm length), with the point blunted and polished, was inserted between the elytra and the abdomen, about 1–2 mm from the tip of the abdomen. By sliding the probe slightly beyond the apex of the abdomen, the edges of the last outer sternite and last outer tergite separate (Sappington and Spurgeon 2000). Pressure from the probe on the dorsum of the abdomen exposes the eighth abdominal tergite (Fig. 2).

In the field, this observation can be conducted with the assistance of an eye loupe (10×). Sexed beetles were segregated, and confirmation of sex was carried out by dissecting the genitalia. Dissection was also carried out in the field.

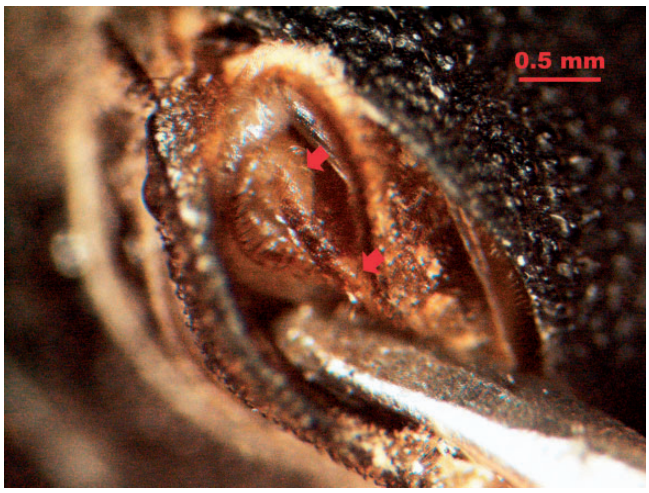

Fig. 2. Female *S. souvorowiana* adult being sexed by observing the posterior margin of the eighth tergite via probing. Pressure from a probe on the dorsum of the abdomen exposes the posterior edge of the eighth tergite. The regions lacking pigmentation are indicated by arrows.

Results and Discussion

Differences between male and female adults of *S. souvorowiana* are difficult to distinguish as this species does not have obvious sex-related external markings (Fig. 3). There were no significant differences (Student's *t*-test, two tailed) between males ($n = 14$) and females

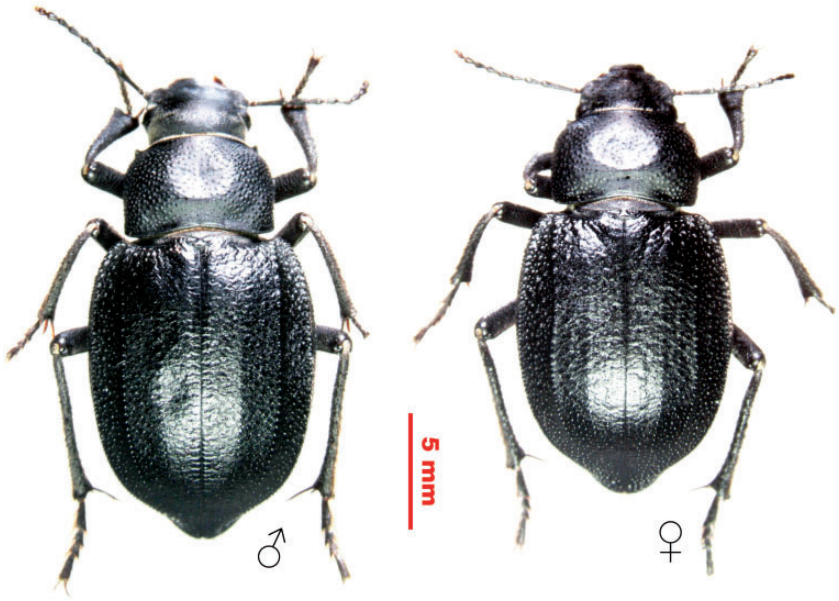

Fig. 3. Dorsal view of male (left) and female (right) adults of *S. souvorowiana*.

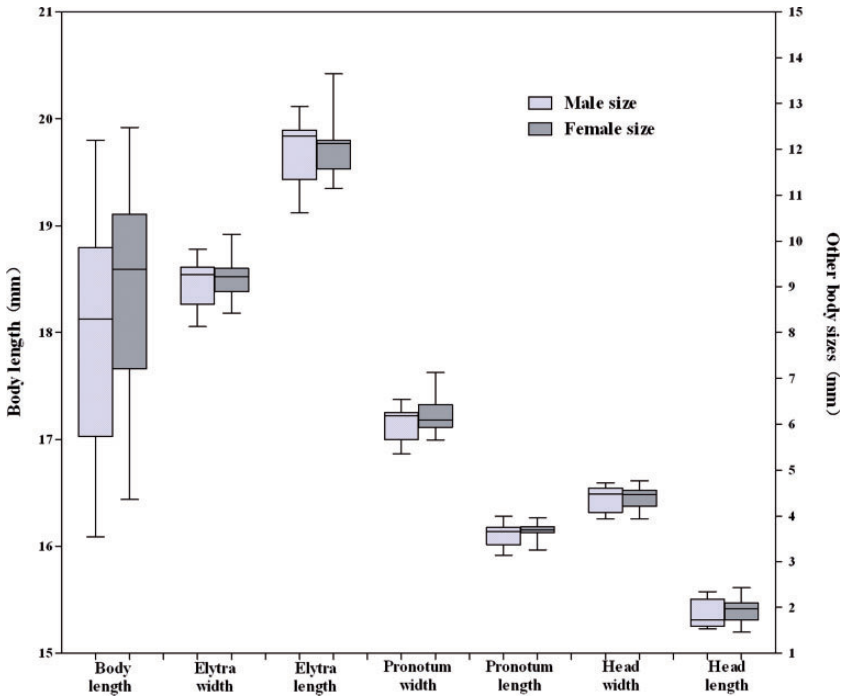

Fig. 4. Variation in morphological characters of male ($n = 14$) and female ($n = 12$) *S. souvorowiana* adults. Box and whiskers show the extreme minimum, lower quartile, median, upper quartile, and extreme maximum. Differences in means for body length and other body sizes are not statistically significant between sexes (Student's *t*-test, two tailed). The ranges show broad overlap.

($n = 12$) in the head width (df = 24, $t = 0.19$, $P = 0.85$), head length (df = 24, $t = 0.87$, $P = 0.39$), pronotum width (df = 24, $t = 1.11$, $P = 0.28$), pronotum length (df = 24, $t = 0.96$, $P = 0.35$), elytra width (df = 24, $t = 0.59$, $P = 0.56$), elytra length (df = 24, $t = 0.44$, $P = 0.66$), or body length (df = 24, $t = 0.94$, $P = 0.35$) (Fig. 4). Similar results were found in other insects (Sappington and Spurgeon 2000, Fairn et al. 2007a,b).

After dissection of hundreds of the insects, we recognized the morphological differences in *S. souvorowiana* between the male and the female in pigmentation and the hairs on the eighth tergite (Table 1). The pigmentation occurred everywhere on the eighth abdominal tergite in males, whereas the pigmentation in females was absent (white regions) at the laterocaudal angles (Fig. 5). We speculate that the white regions (lacking pigmentation, unsclerotic) on female tergite may help to accurately perceive and accept the male aedeagus. This gender-specific characteristic could be revealed by gently probing (Fig. 2), using a polished probe (a separated arm of the stainless tweezers, 134-mm length). Based on this sexual-specific characteristic, a new sexing method was established, which we termed the tergal pigmentation pattern probing method.

At the beginning of this study, we attempted to expose the eighth tergite as fully as possible to gain a complete view. With our experience

Table 1. Keys for sexing adults of *S. souvorowiana*

Character	Male	Female
Pigmentation on the eighth tergite	Present all over	Absent at the laterocaudal angles
Hairs on the eighth tergite	Dense	Sparse

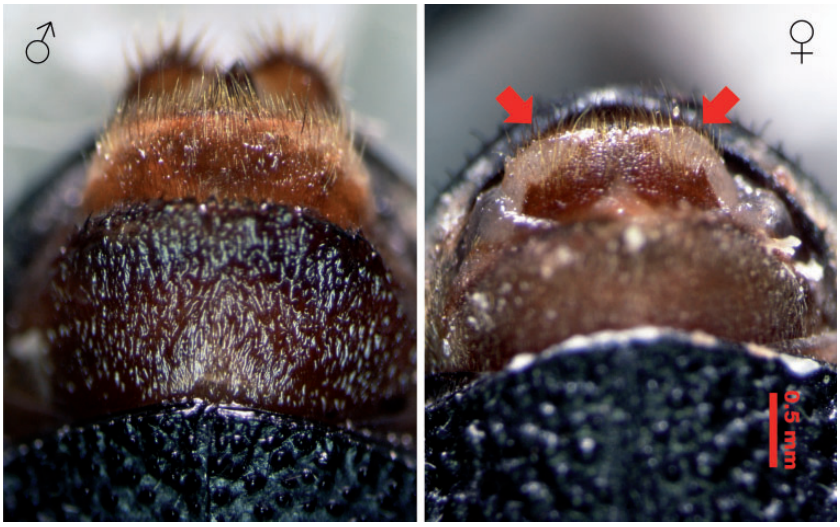

Fig. 5. Dorsal view of the eight tergites in *S. souvorowiana* adults. These beetles were killed to facilitate full exposure of the gender-specific characteristics. The eighth tergite of female has two white regions lacking pigmentation at its laterocaudal angles (arrows); however, the eighth tergite of male is covered with dark pigment all over. Meanwhile, hairs on the eighth tergite of the male are denser than those of the female.

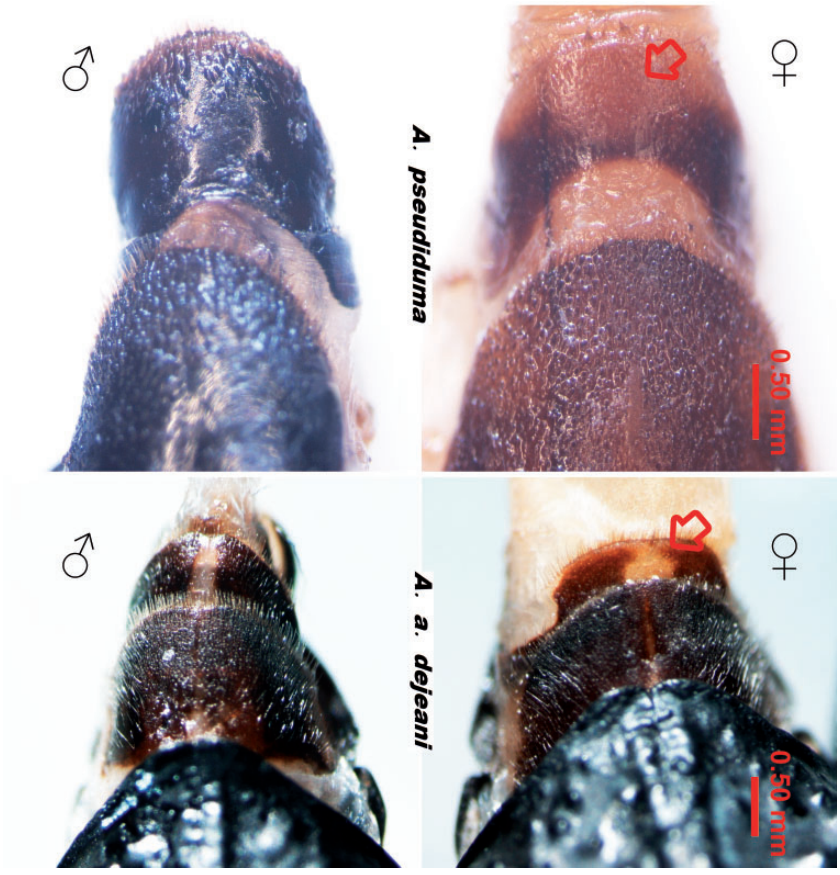

Fig. 6. Dorsal view of the eighth tergites in *A. pseudiduma* and *A. anomala dejeani* adults. These beetles are killed to facilitate full exposure of the gender-specific character. Pigmentation patterns on the eighth tergites are different between the males and the females. Arrows indicate the white regions. Moreover, the colors on the eighth tergites of the females are lighter than those of the males.

obtained, it became unnecessary to completely separate the seventh sternite and tergite to see the whole eighth tergite. The pigmentation pattern was clearly shown on the posterior part of the tergite. Moreover, hairs on the eighth tergite of male were denser than that of female (Fig. 5).

Without exception, live male and female adults of *S. souvorowiana* ($n = 200$ total) were nondestructively distinguished by the tergal pigmentation pattern probing method, as was true with a sample of 100 dead individuals as well. This method was not as slow as generally perceived. The experienced personnel in our laboratory can routinely discriminate live beetles about 8–10 per minute. In addition to the pigmentation patterns, the density of hairs on the eighth tergite was helpful for sex determination. Also, the sexing experiment could be conducted with 100% accuracy using an eye loupe (10×) in the field ($n = 30$). For students with good eyesight, beetles could be sexed with naked eyes.

With the tergal pigmentation pattern probing method, there was no need to squeeze the abdomen of insect or withdraw the sternite from the abdomen with forceps. Together, this method minimized the possibility of hurting insects. In addition, the eighth sternite usually bent into the last external visible sternite and was difficult to be observed. It is easier to check the eighth tergite. Therefore, the tergal pigmentation pattern probing method is more effective.

The nondestructive method we developed for sex determination of the adult darkling beetle *S. souvorowiana* is as accurate as dissection methods. This method is also applicable to other species of tenebrionid beetles, such as *A. pseudiduma* and *A. anomala dejeani* (Fig. 6). It is possible that this method would be useful for identification of more species in tenebrionids. Further work is required to determine the effectiveness of this method for predicting sex in other tenebrionid beetles.

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References Cited

Bailez, O. E., A. M. Viana-Bailez, J.O.G. de Lima, and D.D.O. Moreira. 2003. Life-history of the guava weevil, *Conotrachelus psidii* Marshall (Coleoptera: Curculionidae), under laboratory conditions. Neotrop. Entomol. 32: 203–207.

Bhattacharya, A. K., J. J. Ameel, and G. P. Waldbauer. 1970. A method for sexing living pupal and adult yellow mealworms. Ann. Entomol. Soc. Am. 63: 1783.

Cloudsley-Thompson, J. L. 2001. Thermal and water relations of desert beetles. Naturwissenschaften 88: 447–460.

Draney, M. L. 1993. The subelytral cavity of desert tenebrionids. Fla. Entomol. 76: 539–549.

Fairn, E., Y. Alarie, and A. Schulte-Hostedde. 2007a. Sexual size dimorphism in the diving beetle *Laccophilus maculosus* Say (Coleoptera: Dytiscidae). Coleopt. Bull. 61: 409–418.

Fairn, E. R., Y. Alarie, and A. I. Schulte-Hostedde. 2007b. Sexual size and shape dimorphism in *Dineutus nigrior* Roberts (Coleoptera: Gyrinidae). Coleopt. Bull. 61: 113–120.

Gorb, S. N. 1998. Frictional surfaces of the elytra-to-body arresting mechanism in tenebrionid beetles (Coleoptera: Tenebrionidae): design of co-opted fields of microtrichia and cuticle ultrastructure. Int. J. Insect Morphol. Embryol. 27: 205–225.

Halstead, D.G.H. 1963. External sex differences in stored-products Coleoptera. Bull. Entomol. Res. 54: 119–134.

Huang, R. X., W. Wu, X. F. Mao, H. Y. Hu, Z. T. Fan, Y. J. Hou, X. P. Li, C. H. Du, H. G. Shao, X. Huang, et al. 2005. The fauna of the desert insects of Xinjiang and its formation and evolution. Xinjiang Science and Technology Publishing House, Wurumqi, China (in Chinese with English abstract).

Mora, A.D.R., and O. Tuchina. 2011. Sexing of adults for three Mexican pine cone beetle species, *Conophthorus conicolens*, *C. teocotum*, and C. michoacanae. J. Insect Sci. 11: 170.

Öhrn, P., M. Klingenberg, G. Hopkins, and N. Björklund. 2008. Two nondestructive techniques for determining the sex of live adult *Hylobius warreni*. Can. Entomol. 140: 617–620.

Pszczolkowski, M. A., K. Hampton, and D. Johnson. 2008. Sexual characteristics in a midwestern USA population of *Cotinis nitida* Linnaeus (Coleoptera: Scarabaeidae) and consequences for determining gender. Coleopt. Bull. 62: 527–534.

Qian, Y. B., X. W. Xu, J. Q. Lei, and Z. N. Wu. 2004. Physical environmental comparison between two biological sand-control system constructions along the linear project areas of the two large deserts in Xinjiang. J. Arid Land Resour. Environ. 18: 33–37 (in Chinese with English abstract).

Ren, G. D., and Y. Z. Yu. 1999. The desert and semi-desert tenebrionids of China. Hebei University Publishing House, Baoding, China (in Chinese with English abstract).

Renault, D., and Y. Coray. 2004. Water loss of male and female *Alphitobius diaperinus* (Coleoptera: Tenebrionidae) maintained under dry conditions. Eur. J. Entomol. 101: 491–494.

Salin, C., D. Renault, G. Vannier, and P. Vernon. 2000. A sexually dimorphic response in supercooling temperature, enhanced by starvation, in the lesser mealworm *Alphitobius diaperinus* (Coleoptera: Tenebrionidae). J. Therm. Biol. 25: 411–418.

Sappington, T. W., and D. W. Spurgeon. 2000. Preferred technique for adult sex determination of the boll weevil (Coleoptera: Curculionidae). Ann. Entomol. Soc. Am. 93: 10–615.

Schat, M., S. E. Sing, and R.K.D. Peterson. 2007. External rostral characters for differentiation of sexes in biological control agent *Mecinus janthinus* (Coleoptera: Curculionidae). Can. Entomol. 139: 354–357.

Vinod, K. V., T. K. Sabu, and T. M. Benny. 2008. Sex determination of the live rubber plantation litter beetle, *Luprops tristis*: a novel method. J. Insect Sci. 8: 12.

Wang, Y., J. Ma, X. N. Liu, and J. M. Luo. 2010. Egg morphology and chorionic ultrastructures of the desert insect *Sternoplax souvorowiana* (Coleoptera: Tenebrionidae). Xinjiang Agric. Sci. 47: 1703–1708.

Wang, Y., X. Liu, J. Zhao, K. Rexili, and J. Ma. 2011. The rearing and biology of the desert beetle, *Microdera punctipennis*, under laboratory conditions. J. Insect Sci. 11: 39.

Wei, W. S., and M. Z. Liu. 2000. Modern desert environment and climate change—a case study in Gurbantonggut desert. J. Desert Res. 20: 178–184 (in Chinese with English abstract).

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