Discovery of the nest of the yellow jacket *Vespula structor* (Smith) (Hymenoptera, Vespidae) from China with description of its immature stages

Qing-Qing Tan¹, Jiang-Li Tan², Ruo-Nan Zhang¹, Xiao-Xia Tian¹, Jie-Ke Jian¹

¹ Shaanxi Key Laboratory for Animal Conservation / Key Laboratory of Resource Biology and Biotechnology in Western China, College of Life Sciences, Northwest University, Xi’an, Shaanxi, China
² College of Life Sciences, Northwest University, Xi’an, Shaanxi, China

Corresponding author: Jiang-Li Tan (tanjl@nwu.edu.cn)

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Abstract

Data on the nest structure, morphology of all stages and behaviour have contributed to the phylogenetic and taxonomic studies of social vespid. Two underground nests of *Vespula structor* (Hymenoptera, Vespidae, Vespinae) were discovered in China. For the first time, the morphology of all the stages, and the nests are described in detail and illustrated. It is additional evidence supporting the recent conclusion that synonymized *Vespula gongshanensis* with *V. structor*. The status of *Vespula structor* within both the genus *Vespula* and the *vulgaris-group* are briefly discussed.

Keywords

larval instars, nest structure, morphology, Vespinae

Introduction

The yellow jacket genus *Vespula* Thomson (Hymenoptera: Vespidae: Vespinae) consists of 25 valid species, of which 12 species occur in China (Carpenter and Kojima 1997, Archer 2012, Kimsey and Carpenter 2012, Tan et al. 2015, Kumar and
Carpenter 2018). As a highly-developed eusocial wasp group, most species are widely distributed, and probably as a result, most species are subject to considerable colour variation (Archer 1989; Carpenter and Kojima 1997; Carpenter et al. 2011; Tan et al. 2015). The three castes (queen, drone and worker) of these eusocial wasps enlarge the spectrum of variation found within a species. In addition, its three castes, as well as the nest architectural and the larval characteristics, are important for phylogenetics and taxonomy of the genus (Yamane 1976, Kojima 1998, Wenzel 1998, Tan et al. 2013, 2014, 2015, 2017, Tan et al. 2018). Unfortunately, seven species (i.e., *Vespula arisana*, *V. austriaca*, *V. kingdonwardi*, *V. koreensis*, *V. nursei*, *V. orbata*, *V. structor*) are reported from China based on limited material. Among them, *Vespula structor* was originally described (only the female) as *Vespa structor* (Smith). Bingham (1897) briefly described the three castes. Yamane et al. (1980) provided the facial and gastral colour patterns of the female. The label data indicate that *V. structor* occurs in mountainous regions between 900–3700m (Archer 2008). Since then, no more information (i.e., immature stages, nest structure, or a clear illustration of genitalia which is the most important identification characteristic) has been added (Das and Gupta 1989, Archer 1989, 2012, Tan et al. 2015, Dorji et al. 2017).

In 2005, *Vespula gongshanensis* Dong, 2005 was described from Yunnan and collected at 2950 m altitude. Carpenter et al. (2011) considered it a synonym of *Vespula rufa* (Linnaeus) based on the original description. Tan et al. (2015) synonymized it with *V. structor* after studying the type series. Unfortunately, the available material is too limited to draw clear conclusions, considering the variation observed, it is expected that additional data will corroborate our provisional results.

During our field work in the Qinling (Shaanxi, China), two underground nests of *V. structor* were discovered in secondary scrubland. The envelope was complete and the combs were still undergoing enlargement. All castes and larval stages were collected in two nests, and they provided the additional information on nest biology, castes and all larval stages of *V. structor* documented in this paper.

**Material and methods**

Two underground nests of *V. structor* were discovered in Ankang (Xunyangba, 33°18′N; 108°19′E, altitude 1481 m) and Baoji (Jialing River source, 34°.08′N 106°33′E, altitude 1558 m). The specimens were collected either together with the nest or by hand net. The nest was excavated carefully, wrapped in a woven bag and placed in a plastic case to carry it back to the laboratory. Adults were picked off the nests after the bag had been 15 minutes in a freezer to avoid stinging. Samples were kept in 70% ethanol before preparation. Mature larvae and pupae were preserved in 2.5% glutaraldehyde and then transferred to 70% ethanol for later observation. The nests were kept in plastic boxes in the refrigerator at 4 °C.

For the descriptions and measurements, an Olympus SZX11 stereomicroscope was used and the photographs were taken with a Keyence VHX-5000 digital microscope.
The photographs of the nest and combs were taken using a Canon SX60 HS. For scanning electron microscopy, the larvae were fixed for 8 hours in a 2.5% glutaraldehyde solution buffered with phosphate (pH 7.2). They were then dehydrated in a graded ethanol series (30%, 50%, 70%, 90%, 100%, 100%), placed in isoamylacetate twice for 30 minutes each time, dried in a CO$_2$ critical-point dryer, sputter-coated with gold and observed with a Hitachi S3400N scanning electron microscope at 15 kV.

Morphological terminology follows Archer (1989, 2012), Wenzel (1998) and Tan et al. (2015). The following main abbreviations are used in the text: APOL = antero-postocellar line; HW = head width; OMS = oculo-malar space; OOL = postocellar-ocular line; PBHL = postocellar back of head line; POL = postocellar line. Other abbreviations used in the figures are explained in the legends. The length of the body is measured from the anterior margin of the head to the apex of the second metasomal tergum. The specimens examined are preserved in the Hymenoptera Collection at the College of Life Sciences, Northwest University, Xi’an, China (NWUX).

**Results**

**Nesting sites and nest structure**

Both nests of *Vespula structor* were found buried underground in the secondary scrubland at an altitude of about 1500 m. The nest was connected to the surface by a narrow channel of about 10 cm long. The envelope of the nest is tan and scaly with wavy parallel lines (Figure 1A, C). The combs were attached to each other by a large buttressed sheet centrally with many narrow auxiliary pedicels. The distance between the combs was about 4.5 mm (Figure 1D, E).

The nest from Xunyangba was a developing colony with many workers, uncertain number of males and one queen. Due to the nest’s having been burned by locals before it was excavated, it was less developed and had only three layers of combs with about 1100 cells in total. Each comb is almost circular and surrounds the central mainstay, where a slight swelling is apparent. The combs are nearly flat but the uppermost one bends upwards rather steeply toward its edge (Figure 1B). Diameters of the combs (measured from top down) were 7.6 cm, 9.1 cm and 8.2 cm, respectively (Figure 2A-C). Unfortunately, the envelope was mostly broken; therefore, it is unknown how the uppermost comb was connected to the envelope. The second comb was attached to the one above by a buttressed sheet about 2.1 cm wide centrally. The lowest comb had only a narrow central pedicel and most cells were devoid of immature stages. The distance between two layers was approx. 4.5 mm (Figure 1B). Cells were hexagonal and mostly regularly arranged with the average diameter (between opposite sides) of 4–5 mm, usually 4.5 mm ($n = 30$). Cells were variable in length and in the degree to which cocoons extended outside, 6–8 mm ($n = 30$) high at the periphery without cocoon and increased as the stage advanced. Cells had been capped by silken cocoons 9–11 mm high, usually 10 mm (measured without cocoon, $n = 30$).
The nest from Baoji housed a developing colony with many workers, males and one queen. The nest consisted of six combs with about 220 large cells and 3500 small cells (Figure 2L). Diameters of the combs (measured from top down) were 12 cm, 15 cm, 17 cm, 15 cm, 8.5 cm and 6.5 cm, respectively (Figure 2D–I). Each layer was similar to those of the other nest described previously except for differences in cell distribution. The upper comb was connected to the inner top of the envelope broadly by a central buttressed sheet with diameter of 2.5 cm and many narrow-marginal pedicels completely connected with the inner combs of envelope (Figure 2J). The second through fifth layers were attached to the respective one above by many thinner buttressed peduncles, which were widened centrally (Figure 2K). The lowest comb was
devoid of immature stages; obviously only large cells with the diameter (between opposite sides) about 7.0 mm were present (Figure 2L). The other combs having a diameter (between opposite sides) of approx. 4.5 mm. Three stages (egg, larvae, pupa) were visible according to the cyclic arrangement of the successive cocoons of the preliminary five layers (Figure 1F). Most cells were capped by silken cocoons and these capped cells were 1.0–1.3 cm high. Cocoon caps of the third and fourth combs are slightly larger than those of the upper combs, protruding about 0.5 cm beyond the cell mouth.
Adults

Diagnosis. This species can be identified by the combination of the following characteristics: ocular sinus yellow or brown with a ventral black marking; clypeus yellow or brown without a black central mark; apical lateral angles of the clypeus semicircular; gena with a continuous yellow band not interrupted by black markings; oculo-malar space short; pronotum punctate and without rugae; first metasomal tergum (T I) yellow with long yellow setae and without a median black mark; T II–V black produced angularly medio-posteriorly; the last tergum almost entirely black; propodeum smooth with fine punctures; male genitalia: shaft of aedeagus narrow and its apex spoon-shaped with a backwardly directed pointed barb on each side below apical spoon-shaped region.

Queen (Figure 3A–E). ♀q (n = 2), length of body (measured from the anterior margin of the head to the end of the second metasomal tergum (T II)) about 13.4 mm; fore wing length about 15.6 mm; width of metanotum (including tegula) about 4.0 mm; hind wing about 10.0 mm long and about 2.0 mm wide; width of mesoscutum (including tegulae) about 5.6 mm; vertex brown or yellow with erect black setae (Figure 3E); HW/OMS ratio about 15.6; head, mesosoma and legs generally yellowish brown with few black markings; anterior and posterior margins of pronotum, tegulae, lateral angles of mesoscutellum and postscutellum reddish yellow. T I–IV black basally with an vague brown transverse band subapically and narrowly yellowish brown apically, respectively; the subapical band of T I and T II irregularly expand laterally; T III and T IV produced angularly medially; T V–VI entire black with terminal brown marking (Figure 3A–C).

Worker (Figure 4A–E). ♀w, length of body (measured in same way as the queen, n= 10) about 8.9 mm; fore wing length about 9.3 mm; width of metanotum (including tegula) about 3.4 mm; HW/OMS ratio 15; POL/APOL (postocellar line/antero postocellar line) = 1.1. POL/PBHL (postocellar back of headline) = 0.8. Head extensively yellow or orange; vertex black with two yellow spots or narrow stripes posteriorly; temple black or mostly so (Figure 4D, E); mesosoma black except metanotum with two small yellow spots anteriorly; declivous part of metasomal T I entirely black or black with orange; horizontal part entirely orange; basal at most 1/3 of T II–V black produced angularly medio-posteriorly while apical 2/3 orange; one shallow spot on each side of T II–V (sometimes invisible); T VI entirely black (Figure 4A–C). The colour of metasomal sternum paler than of tergum, black basally with two black spots laterally and terminal sternum nearly entirely orange yellow.

Male (Figures 4F–H, 5A–H). ♂, length of body (measured in the same way as the queen, n = 5) about 10.0 mm, fore wing length about 12.4 mm; width of metanotum (including tegula) about 3.8 mm; mesosoma entirely intense black except for bright yellow posterior margin of pronotum; T I-VI black area produced angularly medio-posteriorly while apical 2/3 orange; one shallow spot on each side of T II–V (sometimes invisible); T VI entirely black (Figure 4A–C). The colour of metasomal sternum paler than of tergum, black basally with two black spots laterally and terminal sternum nearly entirely orange yellow.
Figure 3. *Vespula structor* (Smith). **A–E** Queen (♀): **A** Habitus (from Ningxia), dorsal view **B** habitus (from Baoji, Shaanxi), dorsal view **C** habitus (from Xunyangba, Shaanxi) **D** head and mesosoma, lateral view **E** head, anterior view. **F–H** *Vespula gongshanensis* (type series from Yunnan) **F** Queen (♀), habitus, dorsal view **G** worker (♀w), habitus, dorsal view **H** male (♂), habitus, dorsal view.
Figure 4. *Vespula structor* (Smith). **A–E** Worker (♀): **A–C** Dorsal view, showing variation of colour pattern **D** habitus, lateral view **E** head, anterior view. **F–H** Male (♂), dorsal view, showing variation of colour pattern.
Figure 5. *Vespula structor* (Smith), male (♂). A Body, lateral view B habitus, anterior view C right antenna and terminal seven segments of antenna D metasomal apex of paratype of *Vespula gongshanensis* (from Yunnan), showing part of aedeagus E genitalia, lateral view F paramere, inner view G genitalia, ventral view H ibid, dorsal view. Abbreviations: ae, aedeagus; di, digitus; pa, paramere; DMP, dorsal margin of paramere; PP, parameral process; PS, parameral spine.
Genitalia. Parameres (pa) in dorsal view smoothly curved inwards; dorsal margin of paramere (DMP) inwards projecting and with long setae; ventral terminal process columnar apically; in ventral view paramere distinctly straight projecting inwards at their one fourth of length basally; dorsal terminal process (parameral process, PP) flattened, pointed apically, shortly forwards and inwards without setae; in lateral view ventral margin depressed medially (Figure 5E); parameral spine (PS) ends in round structure with long setae dorsally, extending beyond parameral processes (PP); volsella arises from inner wall of each paramere, not visible in ventral view of gonostipes (Figure 5F); shaft of aedeagus narrow and its apex spoon-shaped with a backwardly directed pointed barb (elongate and triangularly shaped) on each side subapically (Figure 5G, H).

Distribution

China (Henan, Shaanxi, Gansu, Ningxia, Sichuan, Yunnan, Tibet); India; Nepal; Burma; Laos; Bhutan.

Immature stages

Egg. The egg is white, elongate oval, approx. 2.1 mm long, its maximum width 0.9 mm, a little wider at one end, slightly curved, and is generally attached by its smaller end to the inner side wall of cell basally, adhering to the wall by a gummy substance on its surface. The egg chorion is membranous; its surface is soft and smooth without any sculpture (Figure 6A).

Larvae. The larval body is milky-white and soft except for the chitinized head; it consists of three thoracic and ten abdominal segments, with no constriction between thorax and abdomen (Figure 6B–F).

The structure of the head in the younger larvae is generally the same as that in the mature larvae. Distinct differences in the shape of the mandible are present in each instar (Figure 7A–H). Observations of the changes in mandibular shape and body size of V. structor larvae showed that there are five larval instars. In the first instar larvae, the average body length is approx. 1.75 mm and the cranial width is approx. 0.42 mm; the clypeus, labrum and mandibular teeth show a dim transparent outline; the mandibles are apically pointed and have only one tooth; the abdominal segments have shallow lines and the terminal segment is connected to the cell (Figures 6B, 7A). In the second larval instar, the average body length is approx. 2.33 mm and the cranial width approx. 0.85 mm; the mandibular tooth has three teeth; the two smaller teeth are slightly pointed and the middle tooth is chitinized and strongly pointed apically; the outline of the clypeus, labrum are clearer (Figures 6C, 7B). In the third larval instar, the average body length is approx. 2.48 mm and the cranial width approx. 0.98 mm; the inner and outer teeth are chitinized and strongly pointed apically, and are smaller than the middle tooth (Figure 7C–F); the abdominal segments show a distinct curved
Figure 7. *Vespula structor* (Smith), development of mandible. A Fifth instar B second instar C–F third instar G fourth instar H fifth instar.
surface (Figure 6D). These stages still have the egg shell attached to the body. The cement by which the queen attaches the egg to the side of the cell thus acts as the point of attachment for the first to third instars. The larvae hold themselves onto the egg shell by means of a viscid secretion. After the ecdysis to the fourth larval instar, the average body length is approx. 5.99 mm and the cranial width approx. 1.95 mm; the larvae can hold their position in the cells by means of their pleural lobes, dorsal ridges and attachment and can easily move in their cells (Figure 6E). In this instar, the larvae now re-orientate themselves, and they rotate through 180°, from the fixed, outward facing position of the earlier instars, to face inwards towards the axis of the comb. The fourth larval instar is not free from the last ecdysis and remains connected to the cell by skin of the ecdysis (Figure 6E). The fourth instar is similar to the early fifth instar, except that the mandibular teeth are of almost the same size and less chitinized (Figure 7G). The final larval instar grows very rapidly and fills the cell completely, the average body length is approx. 10.03 mm and the cranial width approx. 2.14 mm; the dorsal ridges and pleural lobes become less obvious and the larvae become more rounded and less annulated in appearance (Figures 6F, 7H).

*Mature larvae.* Cranium nearly yellowish and some parts brown. Margin of parietal band, apical part of mandible, margins of maxillary and labial palpi and galea brown; mandibular teeth dark brown; margins of spiracles pale orange brown (Figure 8A–F). Cranium. Widest part of cranium in frontal view above level of line joining antennae, about 1.1 times as wide as high, laterally slightly sinuate near ventral margin (Figure 8A); in profile posterior margin nearly straight; integument with scattered minute punctures, each bearing minute setae (Figure 8G); part of gena (or temple) along its latero-posterior margin with sparse minute setae and remainder smooth. Ecdysial sulcus distinct. Outer one-third of parietal band reticulate (Figure 8C). Antenna small, separated from anterior tentorial pit by distance about 2.2 diameter of antenna, with three minute campaniform sensilla arranged in a triangle; frontoclypeal suture well sclerotized. Gena ventrally with sparse spicules; clypeus nearly half as wide as maximum width of cranium with few punctures and about 65 short setae (Figure 8A). Labrum narrowed at junction with clypeus, with many small punctures bearing 32 minute setae on average and 9 small conical papillae along two lateral margins (Figure 8B). Each side of palate with three small yellowish brown patches bearing more than 15 relatively large sensilla; the medio-ventral margin with about 11 conical papillae; dense minute spicules present in median part and ventro-lateral area (Figure 9A). Mandible well chitinized, especially in its concave apical part, with three apical teeth (I, II and III, counted from below) of which tooth III nearly truncate apically; tooth I markedly projecting as compared with tooth II and tooth III (Figure 8B). Maxilla with scattered short setae; upper surface and apex with spicules; four apical sensilla (sometimes three) on maxillary palpus and two apical sensilla on galea were observed, respectively (Figures 8E, 9D), area between labial palpi and spinneret with spicules; spinneret similar or slightly longer than the distance between the two labial palpi and surrounded by dense spicules; each labial palp with four apical sensilla and a single seta behind palpus (Figures 8D, 9C).
Thorax and abdomen. The cuticle integument with scattered, short setae and minute spicules (Figure 8G); terminal segment with two projections dorsally. Setae on venter of second to ninth abdominal segments confined to anterior half of each segments; setae on dorsum arranged in a transverse, median band on each segment; spicules on third to ninth abdominal segments becoming sparser towards posterior segment; The abdomen has 10 pairs of spiracles and first, second, ninth and tenth spiracle nearly equal in size, third a little smaller than first one; fourth to eight spiracles larger than third one; atrial wall of spiracle with sparse minute spicules; processes at perimeter of primary tracheal opening simple. Collar process of spiracle sort, even rudimentary (Figures 8F, 9B).
**Pupa.** Average length of body approx. 16.0 mm and 5.5 mm wide (n = 5); pupa exarate, creamy-white; compound eye colour changes gradually from translucent pinkish-brown to black brown; mandible tips yellowish brown. Similar to adults in appearance; wing pads extending approximately to the posterior margin of the first metasomal segment (Figure 6G–I).

**Discussion**

The genus *Vespula* consists of 26 species which are divided into four groups, i.e. *rufa*-*, squamosa*-*, vulgaris*- and *koreensis*-group (Carpenter 1991). *Vespula structor* belongs to the *vulgaris*-group (comprised of 10 species) which are characterized in the male by having the shaft of aedeagus narrow and its apex spoon-shaped, which differs from the strap found in the in the *V. rufa*, *V. squamosa*, *V. koreensis* (Yamane et al. 1980, Carpenter and Perera 2006, Archer 2008, 2012, 2016, Tan et al. 2015). Although the three castes of *Vespula structor* has been briefly described, detailed illustration of the genitalia and the colour pattern were still lacking (Yamane et al. 1980, Archer 1989, 2012, Das and Gupta 1989, Dorji et al. 2017). This study is the first to show the clear
characteristics of all the life stages and its various. Compared within *vulgaris-* groups, *Vespula structor* subtly differs from others on the shape of the small backwardly directed pointed barb on each side below the apical spoon-shaped region of the aedeagus which is consistent with the male specimens of *Vespula gongshanensis* (checked by TJL) and strongly supports the result of Tan et al. (2015).

The male is similar to the worker, but show more black marking on metasomal tergum and the light-colour parts of the body. There are some distinct differences in the colouration of the queen. According to Bingham (1897) the head and metasoma are yellow; the mesosoma is black and the femora anteriorly with a broad black streak basally. Our result is agreement with the pictured queen in Tan et al. (2015): the mesosoma is reddish yellow and the metasoma is more extensively black. The colour variation maybe due to the altitude. Our nests were collected about 1500 m. The queen of Bingham (1897) was collected at 8000 ft (2438 m) in Sikkim (India). The same colour pattern is shown in *V. gongshanensis* collected at 2950 m. The colour pattern of *V. gongshanensis* (both of male and female) agrees with the colour pattern we observed (Figure 3G correspond Figure 4A; Figure 3H correspond Figure 4F). The shape of the aedeagus agrees with that of our sample (Figures 5D, H). The shaft of the aedeagus is narrow and its apex spoon-shaped with the subapical barb elongate and triangularly shaped. The digitus is large, extending nearly as far as the level of the parameral spine (Figures 5D, H). Obviously, it supports the synonymy of *V. gongshanensis* with *V. structor*.

Before this study, there were only ten species (*Vespula atropilosa*, *V. consobrina*, *V. flaviceps*, *V. flavopilosa*, *V. germanica*, *V. maculifrons*, *V. pensylvanica*, *V. rufa*, *V. shidai*, *V. vulgaris*) known from their immature stages (Wheeler and Wheeler 1976, Yamane 1976, Matsuura and Yamane 1990, Kojima 1998, Archer 2012). For the first time, the immature stages of *V. structor* are reported here. Compared with its known congeners, there are subtle differences in the palate and in the processes at the perimeter of the primary tracheal opening of the spiracles. The developmental process of the mandible fits well with other known species within the *vulgaris-* groups (Potter 1964, Yamane 1976). Additionally, the colour pictures which show the development of mandible are the first illustrated firstly in this genus.

Potter (1964) and Edwards (1980) reported that the abdomen got free from egg shell in the third instar larvae, but the tip of abdomen is not separated from the skin of the previous instar. This is also found in *Vespa bicolor* Fabricius, and *Vespa mocsaryana* du Buysson (Tan et al. 2015). But this is uncertain according to Matsuura & Yamane (1990). They rarely found the fourth instar larvae dropping from the cell as sometimes happens with the fifth instar larvae. The fourth instar larvae may be connected within the cell. In our study, the fourth instar sometimes is not free from the skin of the previous instar (Figure 6E). This result corroborates Matsuura & Yamane’s (1990) conclusion.

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