

Description of *Strashila daohugouensis* sp. nov.

Order Diptera

(= Order Nakridletia, syn. nov.)

Grade Nematocera

Family Strashilidae Rasnitsyn, 1992

(= Vosilidae, syn. nov.)

Genus *Strashila* Rasnitsyn, 1992***Strashila daohugouensis* sp. nov.**

Principle family characters. Head, thorax, fore and mid legs, and male genitalia typical for Diptera; males with lateral abdominal gills and hind legs with pincers and swollen femur and tibia; female without such structures; membranous male wings large, with veins reduced, fringed with marginal setae.

Material. All of the present strashilid specimens come from the Xiayingzi outcrop of the Daohugou beds, the same as those from which the giant fleas recently published originated¹, with the two exceptions being that of *Strashila daohugouensis* (NIGP155019) and *Vosila sinensis* (NIGP155021). NIGP155019 has been found in Donggou outcrop and NIGP155021 in Beigou outcrop. A distinctive feature of the Daohugou rock matrix is it laden with relatively small conchostracans of *Euestheria*, but these are absent in the present layers which instead contain abundant anostracans. The present strashilid fossils are compressed in fine, grayish, laminated, volcanic tuffs as brownish films with a slight three-dimensional preservation. The fossils are always exquisitely split by the median gap of the insect's body, thus the compression is of an internal view of structures permitting fine SEM imaging. All specimens are housed in the Nanjing Institute of Geology and Palaeontology (NIGP).

Holotype. NIGP155020, a complete male with details of head, associated with female allotype, without counterpart.

Allotype. A complete female NIGP156171, without counterpart.

Paratypes. NIGP155017, a large complete male, without counterpart; NIGP155018, a laterally preserved complete male mating with a small laterally preserved female (NIGP156170), both with counterparts; NIGP155019, a complete male with details of head, without counterpart; NIGP155022, a complete male with details of head, with counterpart; NIGP155023, an almost complete male with details of head, with counterpart; NIGP155024, a complete male, dorsally compressed, with right fore wing preserved, without counterpart;

NIGP155025, a complete male with well-preserved legs, slightly deformed, without counterpart; NIGP155027, a complete small male, without counterpart.

Detailed description. Small insects. Male: head, thorax, legs and genitalia strongly sclerotized, armed with numerous setiform sensilla of various lengths (Fig. 1b and Supplementary Figs 1a, b, 3b, c), head small, ovate, prognathous (Fig. 1a, b, h and Supplementary Fig. 3a, b, f), freely moveable, always retracted under pronotum (Fig. 1g and Supplementary Fig. 2a, d, f, h, j); a pair of large ovate compound eyes located anterior-dorsally on head, with nearly one hundred hexagonal facets visible (Fig. 1b, h and Supplementary Figs 1a–d, 2b); two dorsal ocelli present, located at inner section close to compound eyes (Figs 1d, 2b, c, e, i); antenna relatively short, three-segmented, inserted between compound eyes, scape and pedicel large, flagellum apparently composed of an elongate basal section and the rest annulated section subdivided into circa 10 subsegments, apical annulation small (Fig. 1h and Supplementary Figs 2b, c, e, i, 3b); dorsal ecdysial line visible, Y-shaped (Fig. 1b); mouthparts with a small labellum (Fig. 1b and Supplementary Figs 2e, 3b), armed with setiform sensilla pointed inner-anteriorly (Fig. 1b), a possible maxillary palps with four visible palpomeres, second longest, third small, fourth broad (Fig. 1b and Supplementary Fig. 3b); thorax always apterous, prothorax short and small, scutum (especially prescutum) large, long and broad, postscutum broad but shorter than prescutum, scutellum broad but also quite shorter than scutum, metathorax reduced (Fig. 1a, e, g and Supplementary Figs 1a, h, 2a, f); fore wing very large (7.0 mm long, 3.1 mm wide), membranous, armed with numerous long marginal setae, veins faint and strongly reduced, C thick anteriorly, Sc fused with R1 near wing base, Rs very close to C and parallel to it, extending to posterior part of wing (Fig. 1i, j and Supplementary Fig. 5a–c, e, f); halteres not discernable but possibly present; coxae large, cylindrical, separated; trochanter cylindrical, relatively elongate, fused with femur (Supplementary Fig. 4a–c, f); fore and mid legs homonomous, femur short and robust, tibia elongate, flattened and relatively broad, with a sub-basal zone (Supplementary Fig. 4f), tarsi five-segmented, slender, basitarsus long, second to fourth tarsomeres similar in length, fifth longest (Supplementary Fig. 1d, g), basitarsus bearing a row of small denticles on inner side and apex (Fig. 2f and Supplementary Figs 2g, 4d), second tarsomere obliquely inserted (Supplementary Fig. 2b, c, g, i); a pair of small pretarsal claws present, strongly curved, sharply pointed with a stout basal tooth, a setiform empodia between claws (Supplementary Fig. 4d, e); hind legs specialized as large chelas, trochanter elongate, femur and tibia huge, armed with sparse setae (Supplementary Fig. 1h), tibial process powerful (Supplementary Figs 1e, 2f–h, j), tarsomeres freely moveable, always

bent backwards (Fig. 1g and Supplementary Figs 2d, f, g), basitarsus largest, longer than tibial process, slightly tapered, armed with small denticles on inner edge (Supplementary Fig. 1e), forming a chela with tibial process, second and third tarsomere short, fourth longer, fifth shorter than first, pretarsal claws identical and as large as those of fore and mid legs (Fig. 2g); abdomen relatively poorly sclerotized, cylindrical, with nine visible sternites, sternites 1–7 associated with a pair of lateral appendages, interpreted as vestigial gill, simple tubular in form, enclosed in a membrane, quite similar to those of *Corydalus* (Megaloptera), last two pairs gradually shorter (Figs 1g, 3a and Supplementary Figs 5d, 6a–c), tracheal system formed by two main longitudinal latero-dorsal trunks on both sides, with transverse connectives between them forming segmentary loops (Fig. 1g and Supplementary Fig. 6a, b), and with spiracles branching on them, corresponding to the basic tracheal system of nematoceran larvae^{2,3}, dorsal anastomoses between two dorsal longitudinal trunks present (Supplementary Fig. 3d), spiracles small (Supplementary Fig. 6a–c); terminalia directed dorsally, tergite VIII armed with a rounded antero-lateral processes (Figs 1c, f, 2c and Supplementary Fig. 3i); male genitalia with a transverse tergite IX, a pair of separate elongate gonocoxites articulated on postero-lateral side of tergite IX, and bearing a distinct one-segmented gonostylus with apical relatively rectilinear hook (Fig. 1c, f); a rounded volsella is visible near the base of gonostylus (Fig. 1g), not articulated with the gonostylus, contra Rasnitsyn^{4–6}.

Female: head, thorax, and legs strongly sclerotized but not abdomen (as in the male); cephalic structure of female same as male; tibia much thinner than male (Supplementary Fig. 4g), basitarsus armed with a few small apical denticles, second tarsomere inserted on first in typical orientation; abdominal segmentation poorly visible, segment VIII prominent, as long as wide, tergite IX transverse (Fig. 1d); female genitalia small as a cerci-like structure (Fig. 1d, and Supplementary Fig. 3e, h).

Comments. The main differences between the male and female concern the absence of lateral abdominal gills and the hind legs of “typical” shape, not strongly broadened and without tibial processes (Fig. 4 and Supplementary Fig. 5).

The strashilids were previously considered as dorsoventrally flattened animals^{7,8}, but they have in fact a cylindrical body because laterally compressed specimens are not frequent (Figs 1f, h). Strashilids are always preserved in a dorso-ventral compression given that their massive and laterally extended hind legs prohibit lying on their sides. There are two exceptions among the present material and both are mating pairs. The holotype (NIGP155020)

is more or less dorsolaterally preserved and some of the legs were still grasping the female allotype (NIGP156171). A more unusual case is another pair, NIGP155018 and NIGP156170, in which the legs of the above male are tightly grasping the female for mating, together as a unit their centre of gravity is such that they could be uniquely preserved as a lateral compression (Fig. 1f, h and Supplementary 4).

Individuals of *Strashila daohugouensis* can range across very different sizes, from lengths of 4.57 to nearly 7 mm in males and 4.66 to 6.75 in females. In regard to the two mating pairs, the holotype (5.75) correlates with the allotype (6.75) and NIGP155018 (5.0) correlates with NIGP156170 (4.66), indicating that the mating individuals may be quite different in size. The small individuals exhibit the same morphology as the largest ones, which is compatible with intraspecific variation as in some Recent dipterans. Two new specimens of *V. sinensis* and its holotype are nearly 7 mm long (measurement data in Supplementary Table 1).

The wings are only clearly visible in one specimen (NIGP155024). This dorsally-displayed specimen exhibits only the right forewing. The matrix is concave along the wing margin. Thus the marginal setae are not clearly indicated in most sections, but they are still clearly visible in a posteriorly-folded section. Another specimen (NIGP155023) shows a strongly folded membranous structure overlapping the abdomen, associated with some long setae. It can tentatively be interpreted as a potential forewing of that individual.

Comparison of *Strashila*, *Vosila*, and *Parazila*

The two other strashilid forms described in Vršanský *et al.*⁸ from Daohugou, namely *Vosila sinensis* and *Parazila saurica*, are synonyms (newly established here). Their major differences concern differences in the structure of the gills resulting from differential preservation and therefore merely an artifact. The gills are apparently preserved laterally or dorso-ventrally, and can occur in the same specimen (Fig. 3b) as dorso-ventral compressed on the left side (Fig. 3d) and more or less laterally compression on the right side (Fig. 3e). Thus, the dorso-ventrally compressed gills in *V. sinensis* and laterally compressed gills in *P. saurica* are not a morphological difference between species but simply taphonomic distinctions. *Vosila* resembles *Strashila* in all critical characters and given this tight affinity we place them within a single family, Strashilidae, considering the Vosilidae as a junior synonym of the former (new synonymy). *Vosila sinensis* (= *P. saurica*) differs from *S. incredibilis* and *S. daohugouensis* in its much larger basal swollen section of the femur and particular gill filaments.

Hind legs of male *Strashila daohugouensis*

The hind leg is modified into a chela formed by a powerful tibial process, and a developed basitarsus with tapering shape and inner denticles. The tarsi of the hind legs are able to bend backwards. In addition, the tibial process of strashilids is comparable to the apex of the tibiae of mantises armed with a prominent spur. These structures clearly have a grasping and fighting function. Their presence in the male but not the female supports the hypothesis that they are linked to sexual activities, either aggression between males, for example as in male Lucanidae, and/or grasping the female during mating (Figs. 1a, e, 4 and Supplementary Figs 3a, f, g, 7a).

The pro- and mesobasitarsi have a possible large angle for locomotion (Supplementary Fig. 2b, i), and they are armed with a row of small denticles on the apical inner surface and apex. The second tarsomere is inserted obliquely on the outer section and thereby contributes to a wider space in which to use these denticles for compression and capture. Strashilids probably used their pro- and mesobasitarsi for grasping female.

Relationships of the Strashilidae

Only few Nymphomyiidae (the Recent *Nymphomyia walkeri* and Eocene amber *Nymphomyia succina*) share with the Strashilidae the presence of ventral abdominal extensions on some segments of males but not females (more precisely on segments V and VI)^{9,10}. A comparison with these flies is critical for understanding strashilid morphology and biology.

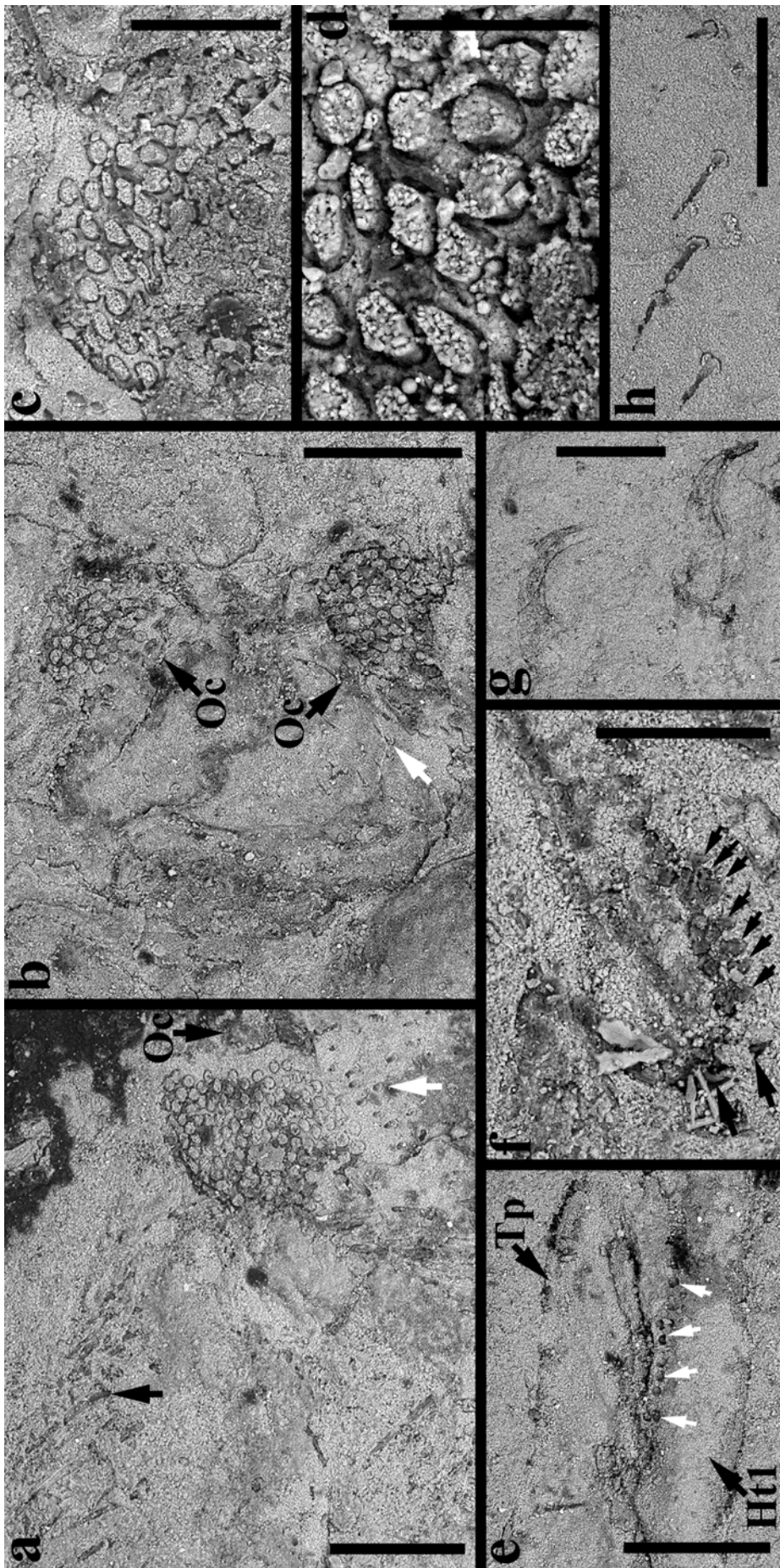
There are several potential synapomorphies of the Nymphomyiidae that are also present in the Strashilidae¹¹, viz. setiform empodia (a character present in Nymphomyiidae, Deuterophlebiidae, and Culicomorpha¹²); tergite VIII armed with rounded antero-lateral processes (an apomorphy of Nymphomyiidae); a membranous sub-basal zone on the tibiae (an apomorphy of Nymphomyiidae); wing probably deciduous, absent in many specimens, very broad at base, with marginal fringe of elongate macrotrichia, and venation reduced to a few veins (C, Sc, and R) along the costal margin; only two ocelli situated in lateral positions, near the compound eyes (the two ocelli are in a postero-lateral position in the Nymphomyiidae^{11,13,14}; they are closer to the compound eyes in *Strashila* than in modern Nymphomyiidae, but in a different position relative to other Diptera); tibial spurs absent (a structure different from the strong metatibial process of the male); pretarsal claws strongly curved, sharply pointed with a stout basal tooth (a structure that could correspond to an adaptation for anchoring the fly to some fixed object in the water^{9,15}).

These characters suggest that the Strashilidae are more closely related to the Nymphomyiidae than to any other fly family. The major difference between nymphomyiids and strashilids concerns the thoracic structures, but as strashilids show a plesiomorphic condition in that the scutellum is shorter than the scutum, this is not an argument against a close affinity between the two clades. Another distinct difference is the wing of *Strashila daohugouensis* is much broader than the Recent forms, but the venation of the former distinctly resembles that of *Nymphomyia walkeri*. The nymphomyiid flies are affected by neoteny on several characters, i.e. abdominal ganglia, occipital foramen and condyles, lateral ocelli, intersegmental antennal sensilla¹¹, but authors do not consider the presence of ventral abdominal extensions on segments V and VI of males of *Nymphomyia* as potential neotenic structures. These structures remain enigmatic. The discovery of neotenic abdominal gills in the Middle Jurassic Strashilidae, of very similar shape, suggests that these extensions in Nymphomyiidae could also be neotenic. Detailed developmental studies are now needed on modern species such as *N. walkeri*.

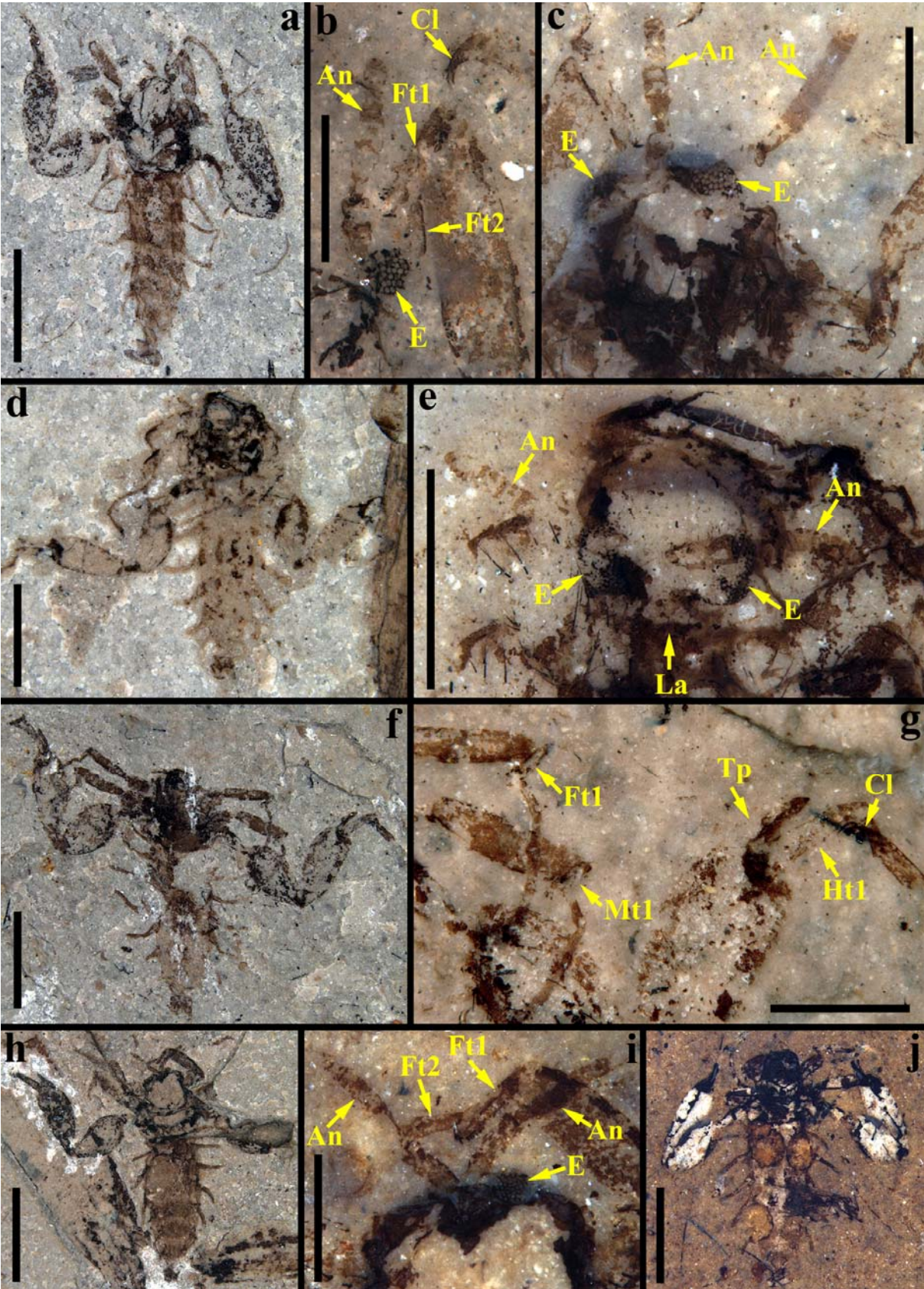
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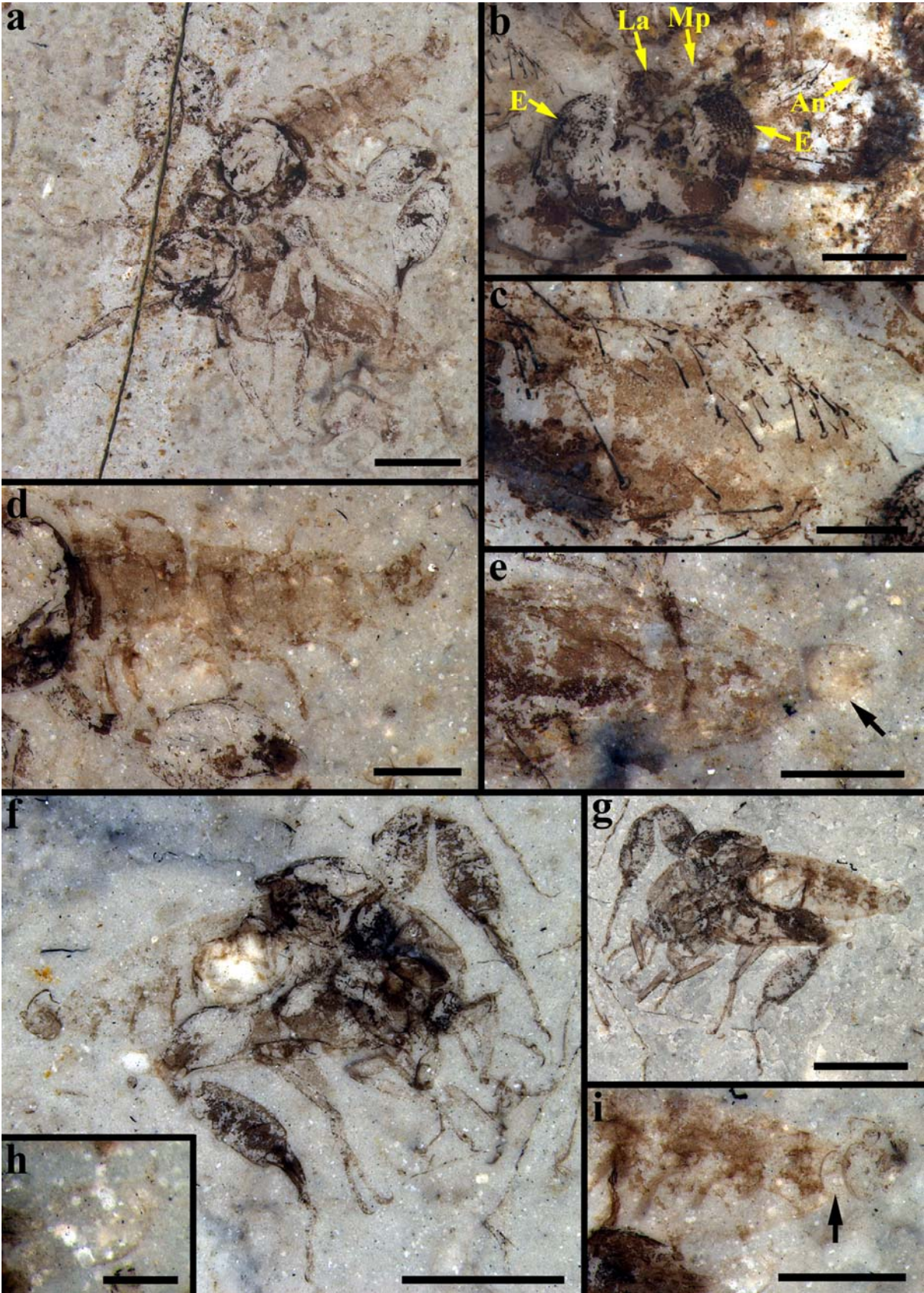
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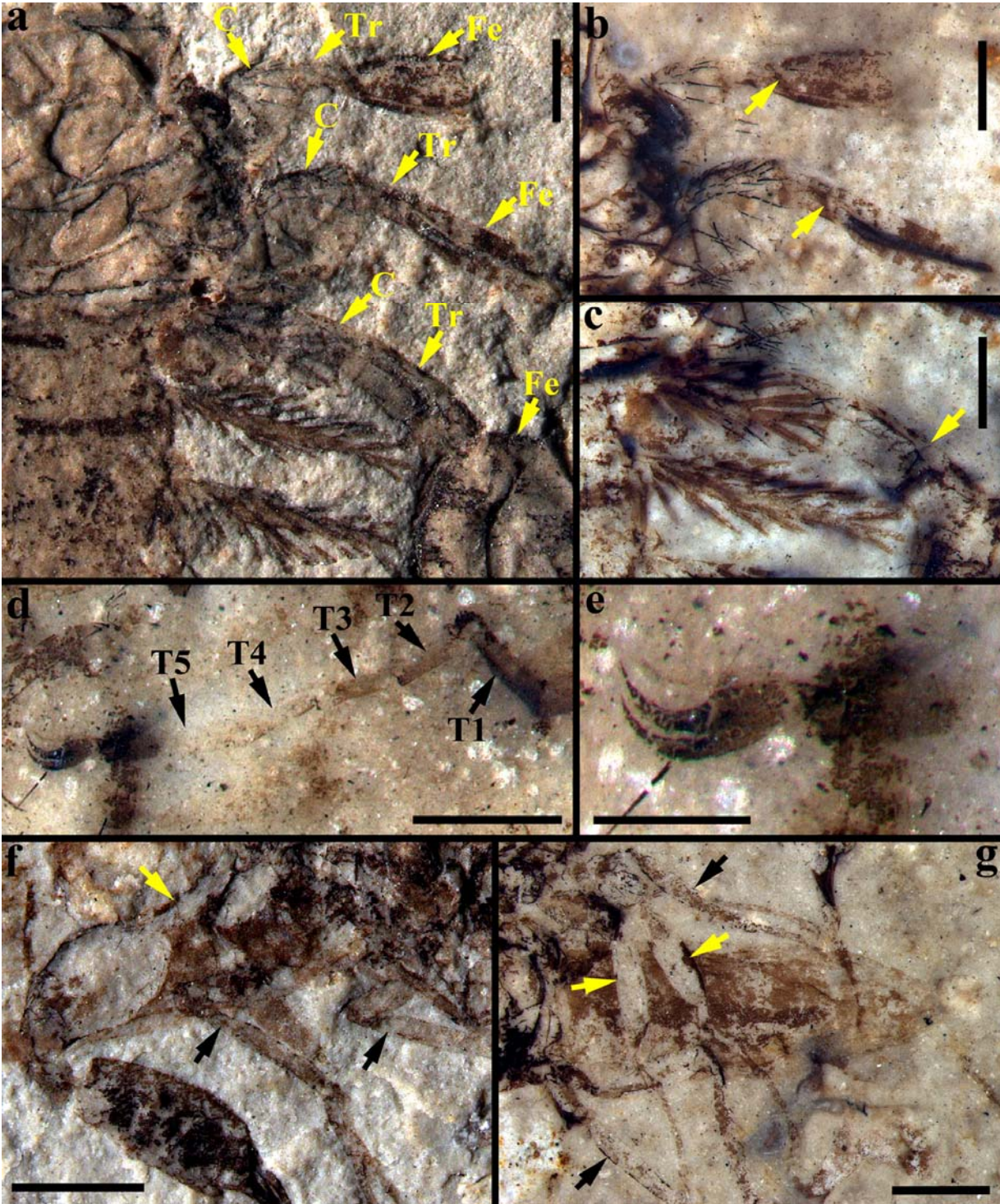
Supplementary Figure 1 | SEM photographs showing details, *Strashila daohugouensis* sp. nov. **a**, Part of head and fore legs of holotype (NIGP155020), white arrow indicating setiform sensilla on head, black arrow indicating setiform sensilla on leg, (Oc, Ocelli). **b**, Details of a female (NIGP156170b) head, white arrow indicating setiform sensilla on head. **c**, Details of male eyes (NIGP155018a). **d**, Enlargement from **c**, showing facets. **e**, Details of hind chela (NIGP155018a), white arrows indicating denticles, Ht1, first tarsomere of hind legs, Tp, metatibial process. **f**, Details on apical probasitarsus (NIGP155023a), large black arrow showing apical denticles, small black arrows showing marginal denticles. **g**, Middle (lower) and hind (upper) pretarsal claws (NIGP155018a). **h**, Setae of metafemur (NIGP155020). Scale bars, 200 μm in **a**, **b**, **e**, **g** and **h**, 100 μm in **c** and **f**, 50 μm in **d**.



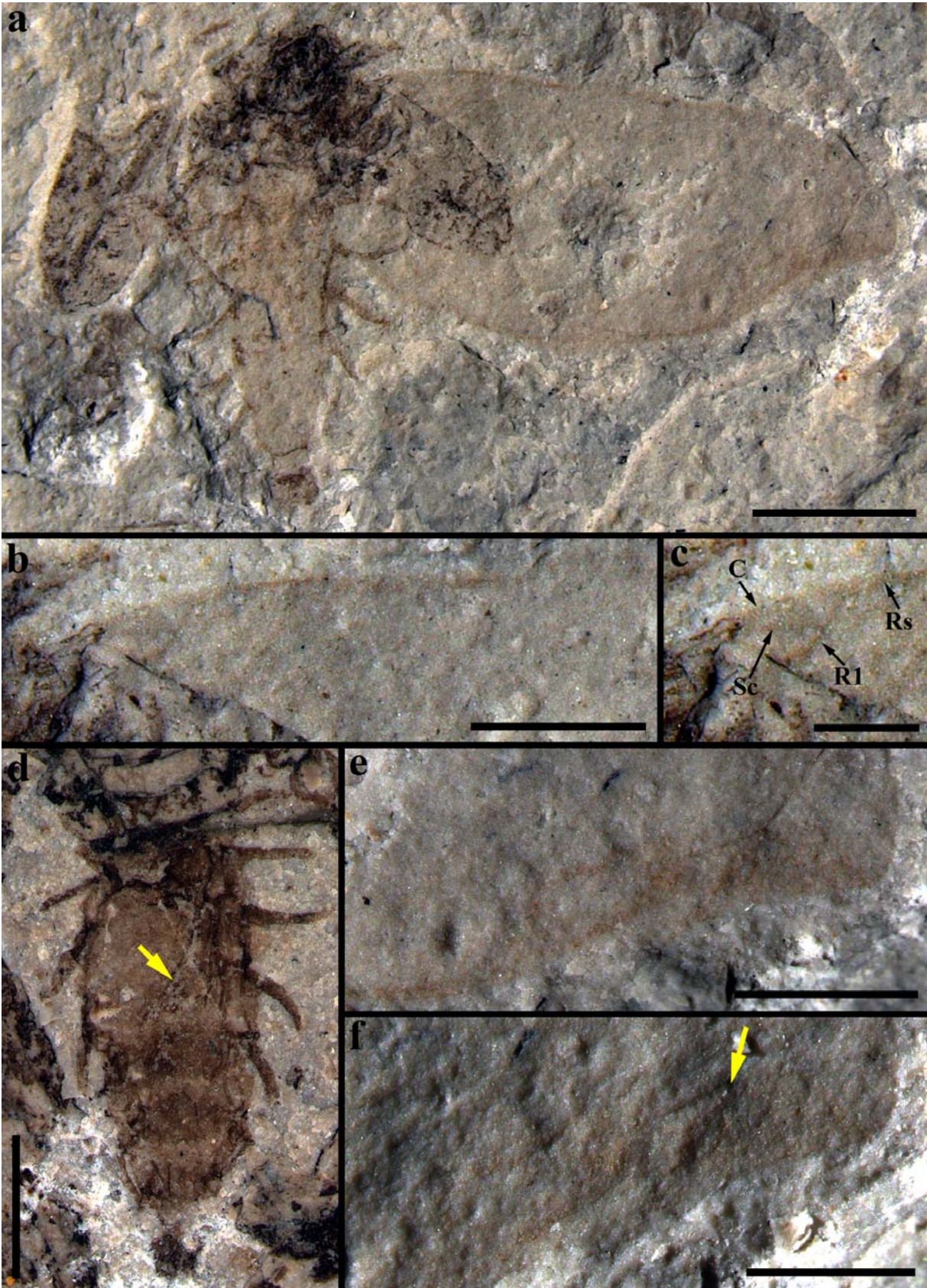
Supplementary Figure 2 | *Strashila daohugouensis* sp. nov. from the Middle Jurassic of Daohugou. **a**, Male (NIGP155022b), general habitus. **b**, Enlargement of **a**, showing eyes (E), antenna (An), probasitarsus (Ft1, first tarsomere, Ft2, second tarsomere), mid claws (Cl). **c**, Enlargement of NIGP155022a, showing head details. **d**, NIGP155019, general habitus. **e**, Enlargement of **d**, showing head details (La, labellum). **f**, NIGP155025, general habitus. **g**, Enlargement of **f**, showing details of legs (Mt1, first mesotarsomere, Ht1, first metatarsomere, Tp, metatibial process). **h**, NIGP155023a, general habitus. **i**, Enlargement of **h**, showing head details. **j**, NIGP155027, general habitus. Scale bars, 2 mm in **a**, **d**, **f**, **h**, and **j**, 1 mm in **g** and **i**, 500 μ m in **b**, **c**, and **e**.



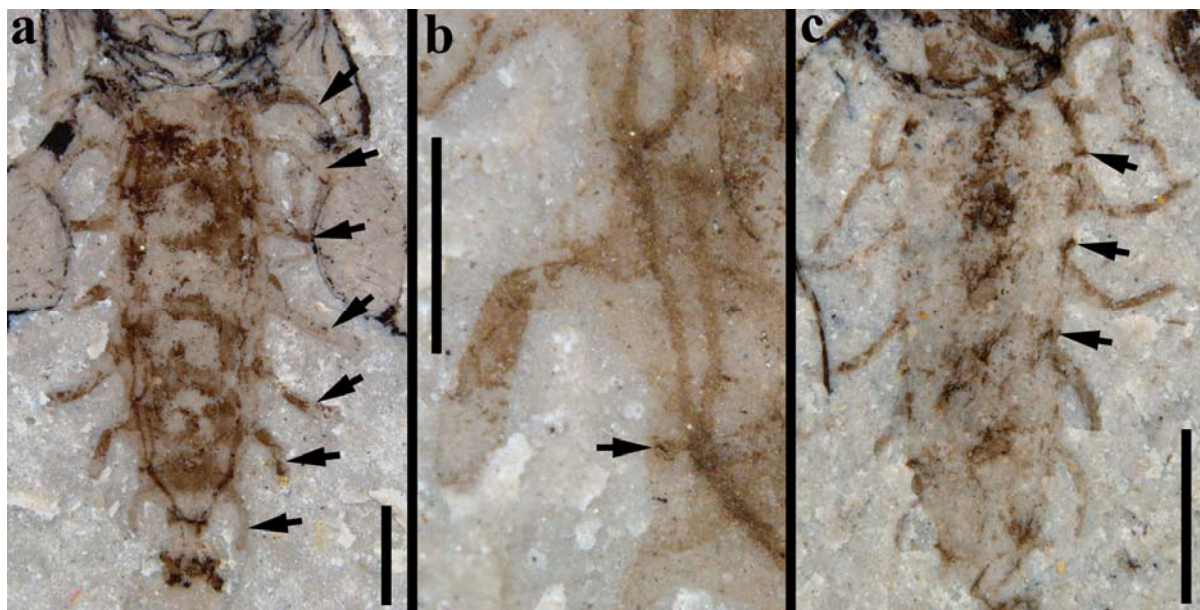
Supplementary Figure 3 | *Strashila daohugouensis* sp. nov. from the Middle Jurassic of Daohugou. **a**, Mating pair (NIGP155020 male, NIGP156170 female). **b**, Enlargement of **a**, details of male head (An, antenna, E, compound eye, La, labellum, Mp, possible maxillary palp). **c**, Enlargement of **a**, showing the setiform sensilla on leg. **d**, Enlargement of **a**, details of male abdomen. **e**, Enlargement of **a**, details of female abdomen, black indicating abdominal segment VIII. **f**, Mating pair (NIGP155018 male, NIGP156171 female). **g**, Mating pair, counterpart. **h**, Enlargement of **g**, female terminalia. **i**, Enlargement of **g**, male terminalia, black arrow indicating process on abdominal segment VIII. Scale bars, 2 mm in **a**, 1 mm in **d–g, i**, 500 μm in **b** and **c**, 200 μm in **h**.



Supplementary Figure 4 | *Vosila sinensis* and *Strashila daohugouensis* sp. nov. from the Middle Jurassic of Daohugou. **a**, Basal section of legs in *Vosila sinensis*, (C, coxae, Tr, trochanter, Fe, femur). **b**, Yellow arrow indicating the fusion of trochanter and femur in fore and mid legs. **c**, Yellow arrow indicating the fusion of trochanter and femur in hind leg. **d**, Details of tarsi (NIGP155019), T1–T1, tarsomere 1–5. **e**, Enlargement of **d**, showing the details of claws. **f**, Leg details of mating pair (NIGP155018 and NIGP156171), yellow arrow indicating the fusion of trochanter and femur in hind leg, black arrows indicating a membraneous sub-basal zone on the tibiae. **g**, Leg details of mating pair (NIGP155020 and NIGP156170), yellow arrows indicating the broader male tibiae, black arrows indicating the thinner female tibiae. Scale bars, 500 μ m in **a–d**, 200 μ m in **e**, 1 mm in **f** and **g**.

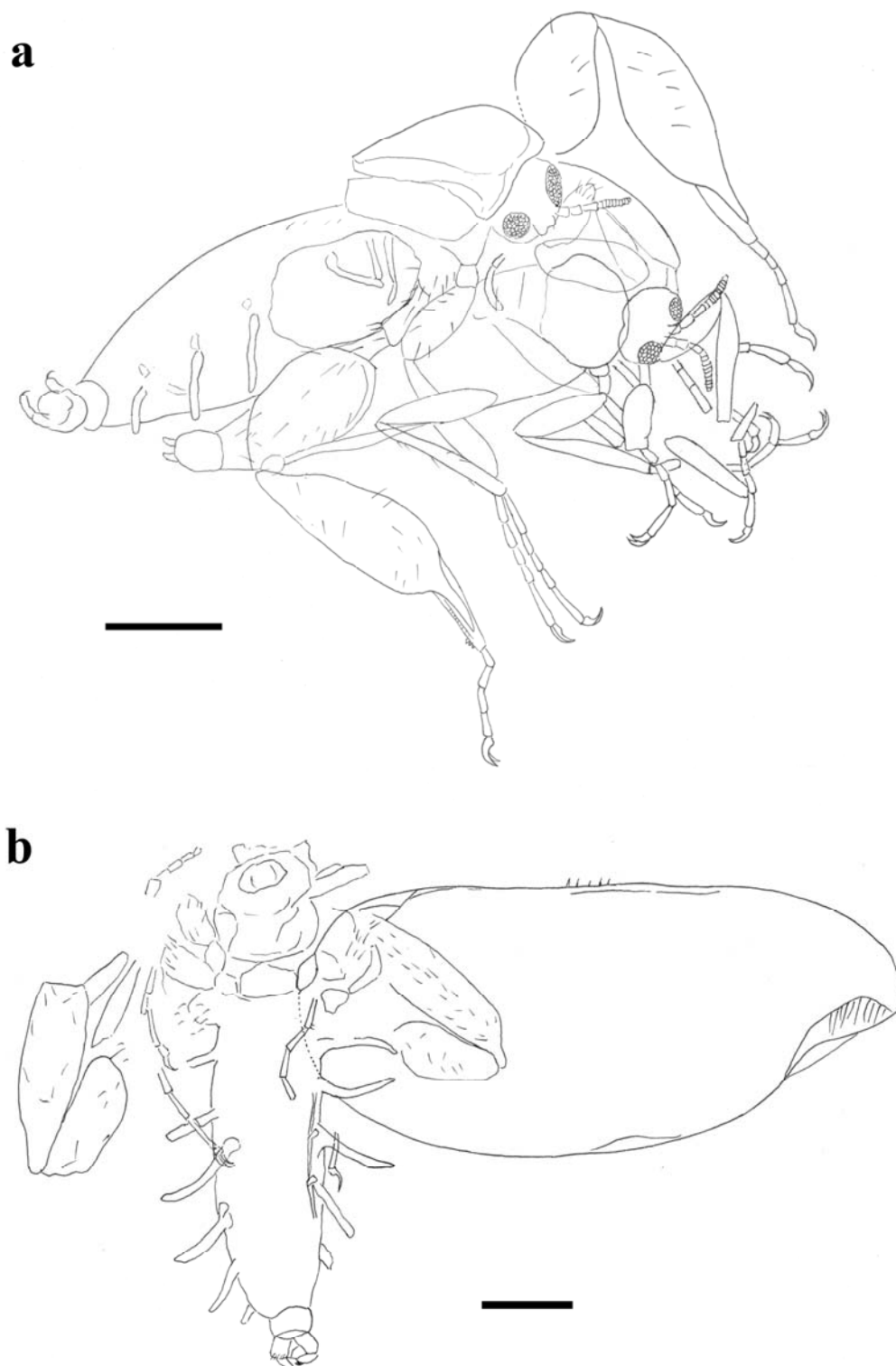


Supplementary Figure 5 | *Strashila daohugouensis* sp. nov. from the Middle Jurassic of Daohugou. **a**, A male (NIGP155024) with right fore wing. **b**, Enlargement of **a**, showing the vein section. **c**, Details of veins. **d**, Abdomen of NIGP155023, yellow arrow indicating a potential folded wing. **e**, Enlargement of **a**, showing the marginal setae at folded section of posterior wing, normal light. **f**, Enlargement of **a**, showing the marginal setae, yellow arrow indicating the margin of wing, low-angle light. Scale bars, 2 mm in **a**, 1 mm in **b**, **d–f**, 500 μm in **c**.

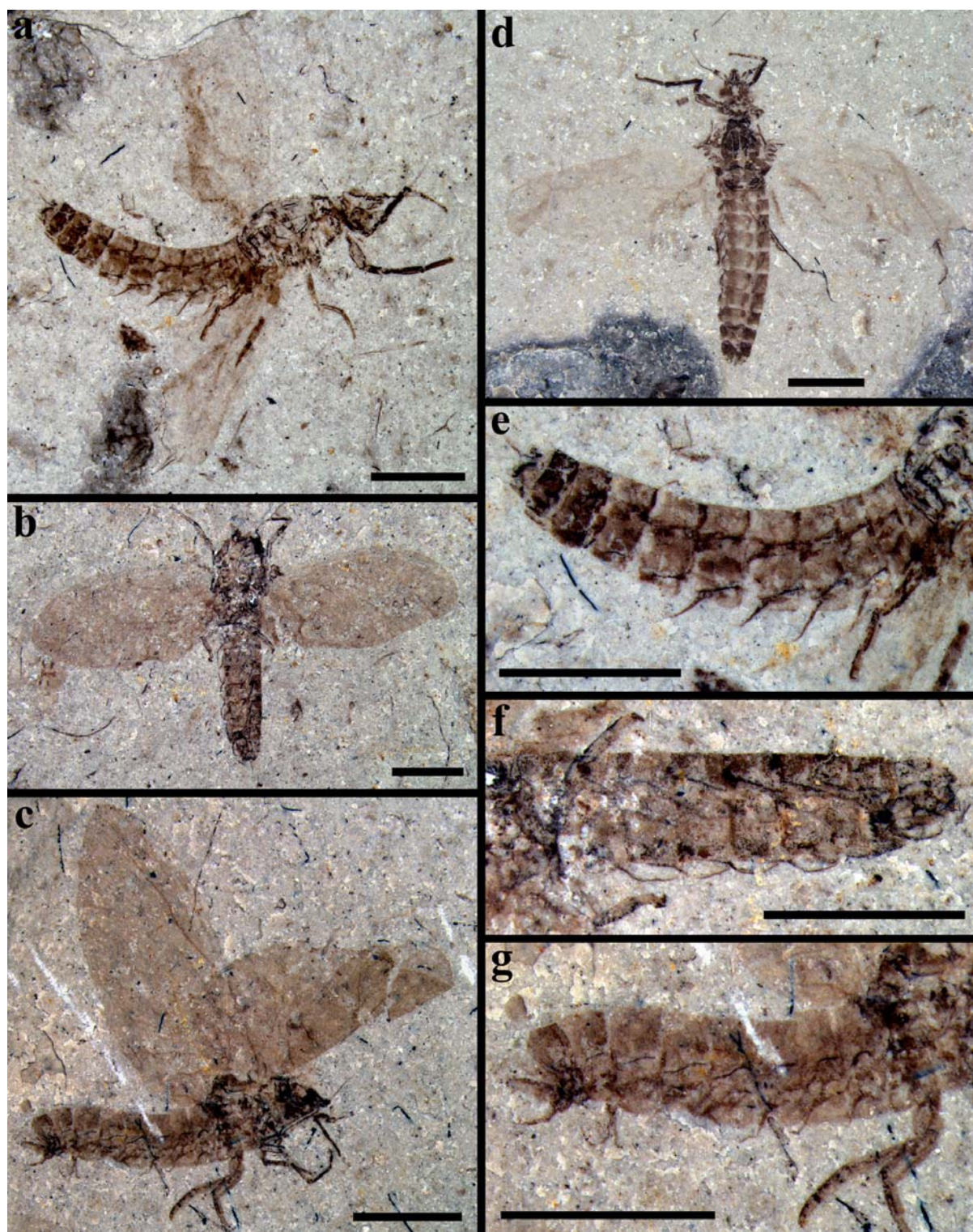


Supplementary Figure 6 | Details of tracheal system of *Strashila daohugouensis* sp. nov.

from Middle Jurassic Daohugou. **a**, Abdomen (NIGP155017), showing gills (black arrows). **b**, Enlargement of **a**, details of tracheal system, spiracle (black arrow). **c**, Abdomen (NIGP155022a), gills, spiracles (black arrows). Scale bars, 1 mm in **a** and **c**, 500 μm in **b**.



Supplementary Figure 7 | Line drawings of *Strashila daohugouensis* sp. nov. from the Middle Jurassic of Daohugou. **a**, A mating pair (NIGP155018 and NIGP156170). **b**, A male (NIGP155024) with fore wing. Scale bars, 1 mm.



Supplementary Figure 8 | Supplementary Figure 6 | Undescribed adult mayfly from the Middle Jurassic of Daohugou showing abdominal gills. **a–d** (NIGP156264–156267), General habitus. **e–g**, Enlargements of **a–c**, showing details of gills. Scale bars, 2 mm in **a–g**.

Supplementary Table 1 | Body sizes of strashilids.

Number	Species	Sex	Length (mm)
NIGP155017	<i>Strashila daohugouensis</i>	male	6.97
NIGP155018a, b	<i>S. daohugouensis</i>	male	5.0
NIGP155019	<i>S. daohugouensis</i>	male	5.46
NIGP155020	<i>S. daohugouensis</i>	male	5.75
NIGP155022a, b	<i>S. daohugouensis</i>	male	4.79
NIGP155023a, b	<i>S. daohugouensis</i>	male	4.57
NIGP155024	<i>S. daohugouensis</i>	male	5.70
NIGP155025	<i>S. daohugouensis</i>	male	5.03
NIGP155027	<i>S. daohugouensis</i>	male	4.79
NIGP156170a, b	<i>S. daohugouensis</i>	female	4.66
NIGP156171	<i>S. daohugouensis</i>	female	6.75
PIN 3084/60	<i>Strashila incredibilis</i>	male	7.0
NIGP155021a, b	<i>Vosila sinensis</i>	male	6.93
NIGP155026	<i>V. sinensis</i>	male	6.96
CNU-PARA-001	<i>V. sinensis</i>	male	ca. 6.8
CNU-NN-PARA-002	<i>V. sinensis</i>	male	5.7