Functional morphology of *Trichadenotecnum* male and female genitalia analyzed using μCT (Insecta: Psocodea: Psocomorpha)

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Abstract

Although the great genital diversity of the barklouse genus *Trichadenotecnum* has been described in previous studies, the specific function of the genital structures during the copulation process has received less investigative attention. We reconstructed the 3D models of each structure and muscle of the male and female genitalia of *T. incognitum* in copula and those of uncopulated male and female of *T. pseudomedium*. By comparing the changes in male and female genital structures and related muscles in copulated and uncopulated states, the function of each genital structure can be described. During the *Trichadenotecnum* copulation process, we found that the female subgenital plate was hooked into the male body by the distal process on the male paraproct and was fixed by the male epiproct, hypandrium and phallosome. In addition, the presence of sexually antagonistic coevolution was suggested by tightly contacting structures, i.e., thorny male hypandrium and female gonopore plate. These results not only give us a new understanding of the copulating process of *Trichadenotecnum* but also explain the reasons why each genital structure is extremely diversified in the genus.

Keywords

genital morphology, genital function, muscles, μCT, *Trichadenotecnum,* copulating process

1. Introduction

The barklouse genus *Trichadenotecnum* Enderlein, 1909 is one of the largest genera among the free-living members of the order Psocodea (formerly known as an independent order "Psocoptera": Yoshizawa et al., 2006) (Yoshizawa et al., 2016). The genus consists of more than 200 species distributed in all zoogeographical regions (summarized in Lienhard & Smithers, 2002, Lienhard, 2011, Lienhard, 2015, Yoshizawa et al., 2016) except for the Australian Region (Yoshizawa & Smithers, 2006). Although the species of *Trichadenotecnum* are superficially very similar to each other, their male genital structures are highly variable (Yoshizawa, 2004; Yoshizawa et al., 2016). The species of *Trichadenotecnum* are subdivided into several species-groups mainly based on the male genital characters (Yoshizawa, 2001, 2003; Yoshizawa et al., 2016).

However, the molecular phylogeny of the genus revealed that convergences and reversals exist for the male genitalia of *Trichadenotecnum*. For example, a reversal occurred in the *sexpunctatum* + *medium* clade. A close relationship between the *sexpunctatum* and *medium* groups has been suggested from synapomorphies in male genital characters (Yoshizawa, 2001). Species of the *sexpunctatum* group have a more developed hypandrial process than those in the *medium* group. However, molecular phylogeny demonstrated that the *medium* group is embedded within the *sexpunctatum* group, suggesting secondary simplification of the genital conditions in the *medium* group from the more developed *sexpunctatum*-like condition (Yoshizawa et al., 2016). To elucidate the evolutionary process of the male genital diversity of *Trichadenotecnum*, it is essential to understand the function of each genital structure. Although the great diversity of the genital morphology in *Trichadenotecnum* has been described in many taxonomic studies, the specific function of the genital structures of *Trichadenotecnum* during the copulating process has not been thoroughly investigated. The only research in this area has been conducted by Betz (1983), but this study is superficial and very preliminary.

Recently, microcomputed tomography (μCT) has been widely used to study the 3D structure of insect morphology. Using μCT, we can reconstruct the detailed relationships of the genital structures during copulation. Furthermore, this technology allows us to reconstruct the muscles of the genitalia more clearly than ever before, which is valuable for estimating the movements of genital structures.

In this study, we reconstructed the 3D model of each structure and muscle of the male and female genitalia of *Trichadenotecnum incognitum* (the *sexpunctatum* group) in copula and describe their copulating process. For comparison, uncopulated male and female of *T. pseudomedium* of the *medium* group were also examined. These two groups have a close affinity as evidenced by high support values from molecular phylogenies (Yoshizawa et al., 2016) and as also suggested on the basis of the similarities in genital character (Yoshizawa, 2001, 2004). We can characterize the function of the genital structures and the process of copulation by comparing the changes in the states of the structures and muscles before and after copulation of the two species.

2. Materials and Methods

Two species of *Trichadenotecnum*, *T. incognitum* and *T. pseudomedium*, were examined. The basic morphology of the genital structures and associated muscles were shared between these two species.

A female of *T. pseudomedium* in an uncopulated state (Figures 5f, g and 7a, b), a male of *T. pseudomedium* in an uncopulated state (Figures 4a, b and 6l) and a copulating pair of *T. incognitum* (Figures 7c, d, 9 and 11) were used for µCT examination. Samples were fixed with FAA solution (*T. pseudomedium*) or hot water (*T. incognitum*) and then preserved in 80% ethanol. Dehydration was conducted in ascending order with 80 – 100% ethanol before drying them at the critical point (EM CPD300, Leica, Wetzlar, Germany) to remove water without serious organ shrinkage. Samples were then scanned using synchrotron µCT at the BL47XU (Uesugi et al., 2012) beamline of the Super Photon ring-8 GeV (SPring-8; Hyogo, Japan) using a stable beam energy of 8 keV in absorption-contrast mode. The tomography system consists of a full-field X-ray microscope with Fresnel zone plate optics (Uesugi et al., 2017). We used semiautomatic segmentation algorithms based on gray-value differences in the software ITK-SNAP (Yushkevich et al., 2006) to obtain 3D representations of the postabdomen of *Trichadenotecnum*.

For light microscopy photographs, we used BABB (1:2 benzyl alcohol: benzyl benzoate) to make muscles and sclerites transparent. Separated abdomens in copula were dehydrated with 100% ethanol and placed in BABB for seven days at room temperature, and observations were conducted in BABB. Photographs were taken with OM-D E-M5 digital camera (Olympus, Tokyo, Japan) attached to a Axiophot compound light microscope (Olympus, Oberkochen, Germany).

3. Results

First, we describe the basic structure of the male and female postabdomen and muscles related to them. We grouped those muscles according to their origin as follows: muscles of the epiproct [ep]; paraproct [pa]; hypandrium [hy]; phallosome [p]; subgenital plate[sg]; gonapophyses [go].

Abbreviations: O – origin; I – insertion; F – assumed function (based on morphological conditions).

3.1. Structures and movements of the male postabdomen.

3.1.1. Clunium

The clunium is composed of the fused tergies of segments IX and X (Yoshizawa, 2005) and houses many muscle attachments originating from the epiproct, paraproct, hypandrium and phallosome (see below). In *Trichadenotecnum*, including *T. incognitum* and *T. pseudomedium*, the clunium usually has a pair of posterior arms extending from its posterolateral margin (Figure 3a).

3.1.2. Epiproct

The epiproct is a structure placed dorsal to the anus. The epiproct of the species in the *medium* and *sexpunctatum* groups is chair-shaped and has a plate-like projection along the anterior margin called the epiproct lobe (Yoshizawa, 2001) (Figure 1a). During copulation, the basal region of the epiproct is turned into the male body, and the epiproct lobe is directed posteriorly (Figure 2a).

01 m-epX-01 (Figure 6b); O: posterior end of the epiproct; I: anterodorsal margin of the tergum X; F: related to the flipping of the epiproct.

3.1.3. Paraproct

The paraproct is a paired structure placed ventrolateral to the epiproct: these three structures together surround the anus. The paraproct consists of a sclerotized dorsal to dorsolateral region and a membrane on the ventral to internal region. The sclerotized part has a distal process directed upwards (Figure 1b). There are three groups of muscles connected to the paraproct. The male paraproct is completely flipped into the male body during copulating (Figure 2b).

02 m-paX-02 (Figure 6c); O: anterodorsal end of the paraproct, composed of six bundles; I: anterodorsal region of the tergum X; F: related to the flipping of the paraproct.

03 m-paX-03 (Figure 6c); O: internal margin of the paraproct near the anal opening; I: anterolateral region of the segment X; F: related to the flipping of the paraproct.

04 m-paX-04 (Figure 6c); O: ventral margin of the paraproct membrane; I: anteroventral end of the clunium, near the junction with the hypandrium; F: related to the flipping of the paraproct.

3.1.4. Hypandrium

The hypandrium represents the sternum IX and articulates laterally with the clunium (Figure 1c). The hypandrium of *Trichadenotecnum* has a tongue-like lobe surrounded by a membrane at the middle (median tongue: Figure 3b) and some characteristic processes on the posterior margin. There is no muscle connected to the median tongue. The right arm is a long process characteristic of the *medium* and *sexpunctatum* groups that arises from near the right posterolateral corner and that is directed posteriorly (Figure 3b). The left process is a conical process that arises from near the distal margin of the left side of the hypandrium (Figure 3b). Only a group of muscles is associated with the hypandrium.

05 m-hyIX-05 (Figure 6i); O: posterolateral edge of the hypandrium; I: anterolateral margin of the tergum IX; F: related to the movement of the hypandrium.

3.1.5. Phallosome

The phallosome is a ring-like structure and closely fits into the hypandrium via a closed genital chamber in an uncopulated state. Posteriorly, it has a pair of projections called pseudoparameres (Figure 1d). There are four groups of muscles connected to the phallosome.

06 m-pIX-06 (Figure 6j); O: anterior apodeme of the phallosome; I: lateral edge of the hypandrium; F: related to the protrusion of the phallosome.

07 m-pIX-07 (Figure 6k); O: middle of the lateral margin of the phallosome; I: small sclerite on sternum VIII; F: related to the restoration of the phallosome.

08 m-pIX-08 (Figure 6k); O: middle of the lateral margin of the phallosome; I: posterolateral margin of the hypandrium; F: related to the restoration of the position of the phallosome.

09 m-pIX-09 (Figure 6o); O: apex of the anterior apodeme of the phallosome; I: middle of the posterior margin of the hypandrium; F: related to the restoration of the phallosome (not found in *T. pseudomedium*).

3.2. Structures and movements of the female postabdomen.

3.2.1. Clunium

As in males, the female clunium houses many muscle attachments originating from the epiproct, paraproct, and gonapophyses (see below) but lacks the clunial arms (Figure 5a).

3.2.2. Epiproct and Paraproct

The basic structure, including musculature (**01-04**; Figure 6d, h), of the female epiproct and paraproct is almost the same as that found in males; however, the female epiproct and paraproct have a much simpler external structure (lacking lobes and spines). Since there was no significant change in the female epiproct and paraproct during copulation, they apparently did not participate in the process of copulation.

3.2.3. Gonapophyses

The female gonapophyses are composed of the external, dorsal and ventral valves.

The ventral valve arises from sternum VIII near the anteroventral corner of the clunium. It is needle-like in structure.

The dorsal valve arises from the sternum IX near the ventral margin of the clunium. It is swollen basally and bears a slender posterior projection (Figure 5b). The gonopore is located at the middle of the base of the dorsal valves, and the bases of the paired dorsal valves are tightly attached to each other in the uncopulated state, maintaining closure of the gonopore.

The external valve arises laterally from the base of the dorsal valve where both are basally fused (Figure 5d). The external valve is closely associated with the subgenital plate and buckles its posterior extension (egg guide) in the uncopulated state (Figure 5f).

There are four groups of muscles related to the gonapophyses. No muscle connected with the ventral valve was detected.

05 f-goIX-05 (Figure 7a); O: base of the dorsal valve; I: on the membrane connected to the gonopore plate, near the posterior tip the gonopore plate; F: relating to the restoration of the gonopore plate.

06 f-goIX-06 (Figure 7a); O: base of the external valve; I: on the membrane connected to the gonopore plate, near the posterior tip of the gonopore plate, partial overlap with muscle 01; F: relating to the restoration of the gonopore plate.

07 f-goIX-07 (Figure 7a); O: anterior end of the dorsal valve; I: posterolateral margin of the segment IX; F: related to the opening of the dorsal valve.

08 f-goIX-08 (Figure 7a); O: lateral margin of the external valve; I: posterolateral margin of the segment IX, partial overlap with muscle 03; F: related to the opening of the external valve.

3.2.4. Subgenital Plate

The subgenital plate, including its posterior extension (egg guide), is formed by sternum VIII. In the uncopulated state, the subgenital plate, including the egg guide, covers the base of the dorsal valve of the gonapophyses, and the external valve buckles the base of the egg guide (Figure 5c). In the copulated state, the subgenital plate is fully opened and pulls into the male body (Figure 5h).

There are two groups of muscles connected to the subgenital plate.

09 f-sgVIII-09 (Figure 7b); O: middle of the dorsal surface of the subgenital plate; I: middle of the ventral surface of the egg guide; F: related to the closure of the subgenital plate

10 f-sgVIII-10 (Figure 7b); O: middle of the dorsal surface of the subgenital plate; I: anterior margin of the sternum VIII, partial overlap with muscle f-05; F: related to the opening of the subgenital plate.

3.2.5. Gonopore plate

The gonopore plate is a sclerite bearing the spermapore and represents the sternum IX. Its lateral margins loosely articulate via membrane with surrounding structures (such as the paraproct, gonapophyses, and subgenital plate) (Figure 5e). The muscles f-05 and 06, both originating from gonapophyses, are associated with the membranous part of the gonopore plate. During copulation, the dorsal valve is in an open state exposing the gonopore plate (Figure 5h), and the membrane connected to the gonopore plate is expanded.

3.3. Male-female genital interaction

In the copulated state, the egg guide of the subgenital plate is firmly grasped by the distal process on the male paraproct, which makes the subgenital plate open fully and protrude into the male body (Figures 5h, I, 8a and 9b).

The male epiproct lobe exerts pressure from the ventral side of the female subgenital plate to push it into the male body and then to fix it in place. The conical projection on the center of the epiproct also acts to fix the female subgenital plate (Figures 8b, d and 9a). The male clunial arms support the posterolateral region of the dorsal surface of the subgenital plate (Figure 8e).

The hypandrium is contracted in an anterodorsal direction, and the right arm is placed onto the ventrolateral region of the subgenital plate (Figure 8b, d). The phallosome moves from its position close to the hypandrium and protrudes posterodorsally, with its pseudoparameres supporting the dorsal surface of the egg guide and exerting pressure from the inside (Figures 8b, d and 9a).

Associated with the fixation of the subgenital plate, the hypandrial median tongue is inserted between the female gonapophyses to keep them open and to maintain the exposure of the gonopore plate (Figure 4c). The membranous part of the gonopore plate is expanded during copulation, and the left process on the hypandrium is deeply inserted into the membranous pouch of the gonopore plate (Figures 8c and 9c). The membranes of the pouch are greatly thickened.

The female gonopore plate and the male phallosome are separated by the male hypandrium so that the spermatophore cannot be transferred to females in this condition.

4. Discussion

4.1. Copulation process

Genital coupling in 'Psocoptera' mostly occurs in a symmetric female-above position (Klier, 1956; Huber et al., 2007). In *Trichadenotecnum*, when the copulation begins, the female pushes up her body by her legs to accept the approach from a male (Yoshizawa, 1999). Contraction of the muscle f-10 and the positional relationship between the external valve and subgenital plate (Figure 5f, h) strongly suggested that the unlocking of the subgenital plate by opening the external valve and opening of the egg guide of the subgenital plate are actively controlled by females.

In contrast, judging from the observed states of structures and muscles, it is obvious that most subsequent coupling and holding processes are actively controlled by the male, and the female is generally passive. Both dorsal and external valves, including related muscles f-07 and 08: Figure 7c), exhibit a relaxed state during copulation (Figure 5h). It seems that the external valve (except the release of the subgenital plate) and dorsal valve do not participate in copulatory action.

In contrast, the muscles f-05 and 06 are in a contracted state when they are uncopulated but are stretched with the expansion of the membranous part of the gonopore plate during copulation (Figure 7c), which indicates that those female muscles are mostly associated with the restoration of the gonopore plate membrane after copulation. Although muscle f-09 (on the egg guide) exhibits no obvious change before and during mating (Figure 7b, d), this muscle probably has the same restorative function. Because the external valve buckles the egg guide in the uncopulated state, it is unnecessary for muscle f-09 to contract continuously after returning to its original position and buckled state.

In *Trichadenotecnum*, fixation of the coupling condition is apparently controlled actively by males by holding the female subgenital plate with the male epiproct, paraproct, clunial arms, phallosome, and hypandrium. Tight association between the female subgenital plate and male paraproct and phallosome was also reported from a trogiomorphan psocid (genus *Neotrogla*: Yoshizawa et al., 2014). In contrast, in *Lepinotus* (Trogiomorpha: Trogiidae), it is the male parameres that anchor the female and, in *Lachesilla* (Psocomorpha: Lachesillidae), the male hooks the female's gonopore plate by using the epiproct spine (Klier, 1956).

In *Trichadenotecnum*, under the action of muscles m-02, 03, and 04, the distal process of the male paraproct hooks the female subgenital plate and pulls it into the male body (Figure 6c, e, g). The contraction of the muscle m-01 makes the epiproct invert into the male body, exerting pressure on the ventral side of the female subgenital plate by the epiproct lobe and median projection (Figures 6b, f, 8b and d). The clunial arms support the dorsolateral part of the subgenital plate (Figure 8e). The posterior part of the phallosome (pseudoparamere) also exerts pressure on the egg guide by contraction of m-06 (Figures 8b, d and 9a). The combination of the above structures is principally responsible for holding the female during copulation. The contraction of muscle m-05 causes anterodorsal movement of the hypandrium (Figure 6i and m), and with this movement, the right arm also supports the subgenital plate (Figure 8d). However, although the subgenital plate and coupling position are both symmetrical, the right arm only provides support for one side. Therefore, it is questionable whether arm placement provides an important locking function.

Through contraction of the muscle m-05, the hypandrial median tongue is inserted between the female gonapophyses, maintaining the exposure of the gonopore plate (Figure 4c) where the opening of the sperm storage organ (spermatheca) is located. At this moment, female muscles f-05, 06, 07 and 08 relax, releasing the membrane attached to the gonopore plate and causing the membrane to expand and spread (Figures

5h and 7c). The left process of the hypandrium is deeply inserted into the membranous pouch of the gonopore plate when muscle m-05 contracts (Figure 8c). By observing the copulating state, it can be found that both the female gonopore plate and the male phallosome move to a position close to each other but are blocked by the male hypandrium, which interrupts spermatophore delivery to the female. Therefore, further action of male and female genitalia, including the release of the hypandrium from the gonopore plate, must be associated with the stage of spermatophore transfer.

Judging from the musculature and holding system, the release from the copulatory condition likely proceeds as follows. The relaxation of the male genital muscles causes unlocking of the coupling condition, and contraction of m-07 and 08 muscles restores the phallosome to the original position. On the female side, the membrane of the gonopore plate gathers under the action of muscles f-05 and 06 so that the gonopore plate, external valve and dorsal valve are restored to the original position. Muscle f-08 contracts to make the external valve open, the female subgenital plate closes under the action of muscle f-10, and then muscle f-08 is relieved in order to restore the external valve to its original position.

4.2. Genital morphology and sexual selection

Although little is known about genital evolution in *Trichadenotecnum*, understanding the basic structures and copulatory state provide several insights into the evolution of the highly diversified genitalia in this genus.

Usually, males and females maintain the stability of the copulating position, e.g., by using the intromittent organ inserted into the female body. For *Trichadenotecnum*, it is the female subgenital plate that is pulled into the male body and the plate is used to stabilize coupling. In both cases, the copulating process is more actively controlled by males, although the mechanisms of pushing (intromittent organ) and pulling (subgenital plate) are different. The male epiproct, paraproct, clunial arms, and distal part of the phallosome play principal roles in holding the female subgenital plate during copulation. All of these male structures are known to be highly variable between species, and several convergences and reversals have been detected (Yoshizawa, 2004; Yoshizawa et al., 2016). These findings suggest that sexual selection is acting on these structures, and sexual conflict between sexes (House, 2007) or cryptic female choice (McPeek et al. 2008; Simmons, 2014) may explain the divergence of these grasping organs.

In addition to the abovementioned grasping organs, the male hypandrial left process deeply penetrates into the membranous pocket of the female gonopore plate, which may also have a female grasping function. Interestingly, the gonopore pocket membrane that accepts the process is greatly thickened (Figure 8c). This condition is analogous to the spiny penis and thickened vagina observed in the genitalia of seed beetles (Rönn et al., 2007), which is generally regarded as an example of sexually antagonistic coevolution resulting from sexual conflict. Insertion of the hypandrial distal process into the membranous pocket of the gonopore plate was also observed in *T. alexanderae* (Betz, 1983), and in this species, both the hypandrium and gonopore plate are symmetrical. The morphological correlation between the hypandrium and gonopore plate seems to be a general trend in *Trichadenotecnum* (e.g., Betz, 1983; Yoshizawa, 2001). The thickening of the female's thorn-accepting structure and morphological correlation between the hypandrium and gonopore plate may also be the product of sexually antagonistic coevolution (e.g., Mcpeek et al. 2008; Rönn et al, 2007).

However, the exact function of the hypandrium is still questionable. As mentioned above, the hypandrial left process deeply penetrates the gonopore plate, and during copulation, males continue moving the hypandrium (Yoshizawa, 1999). Judging from the large contracting muscle bundles connecting to the hypandrium (muscle m-05), strong pushing movement of the hypandrium against the female body should have an important function in *Trichadenotecnum*. However, when the left process is inserted into the membranous pocket of the gonopore plate, the hypandrium is placed between the phallosome (spermatophore delivery organ) and the gonopore plate (the receiver of the spermatophore). Therefore, to deliver a spermatophore to females, the grasping of the gonopore plate by the hypandrium must be released. This indicates that the close interaction between the hypandrium and gonopore plate may be important only before the spermatophore-transferring stage. Further studies are needed to elucidate the function and cause of the morphological diversity of the hypandrium and other genital structures in *Trichadenotecnum*.

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Figure legends

Figure 1

Male terminalia of *T. pseudomedium*, highlighting different structures: (a) epiproct (left: dorsal view; right: lateral view); (b) paraproct (left: posterior view; right: lateral view); (c) hypandrium (left: posterior view; right: lateral view); (d) phallosome (left: internal view; right: lateral view).

Figure 2

Male terminalia of *T. incognitum* in a copulated state, highlighting different structures: (a) epiproct (top: dorsal view; below: lateral view); (b) paraproct (top: internal view; below: lateral view); (c) phallosome (top: internal view; below: lateral view).

Figure 3

Male terminalia of *T. incognitum* in a copulated state: (a) clunium (left: posterior view; right: lateral view); (b) hypandrium (left: dorsal view; middle: posterior view; right: lateral view).

Figure 4

Male terminalia of *T. pseudomedium* (a, b) and *T. incognitum* (c, d). (a, c, d) lateral view; (b) posterior view.

Figure 5

Female terminalia of *T. pseudomedium* (a-g) and *T. incognitum* (h, i), highlighting different structures: (a) clunium (left: posterior view; right: lateral view); (b) dorsal valve (ventral view); (c) subgenital plate (ventral view); (d) external valve (ventral view); (e) gonopore plate (internal view); (f) ventral view; (g) lateral view; (h) ventral view; (i) lateral view.

Figure 6

Male (a-c, i-l; internal view) and female (d, h; internal view) terminalia of *T. pseudomedium* and terminalia of *T. incognitum* (e-g, m-p; internal view), highlighting terminal muscles; (1) muscle m-01; (2) muscle m-02; (3) muscle m-03; (4) muscle m-04; (5) muscle m-05; (6) muscle m-06; (7) muscle m-07; (8) muscle m-08; (9) muscle m-09; (10) muscle f-01; (11) muscle f-02; (12) muscle f-03; (13) muscle f-04.

Figure 7

Female terminalia of *T. pseudomedium* (a, b) and *T. incognitum* (c, d), highlighting terminal muscles; (a) internal view; (b) left: ventral view; right: lateral view; (c) internal view (red oval: muscle f-05 and f-06); (d) internal view (red oval: muscle f-09); (1) muscle f-05; (2) muscle f-06; (3) muscle f-07; (4) muscle f-08; (5) muscle f-09; (6) muscle f-10.

Figure 8

Male terminalia of *T. incognitum* in copula; (a) internal view; (b) internal view; (c) hypandrium (left: internal view; right: lateral view); (d) lateral view; (e) left: lateral view; right: posterior view.

Figure 9

Sections of *T. incognitum* during copulation. (a, c) longitudinal section; (b) cross-section. A. epiproct (male); B. paraproct (male); B_1. membrane of the paraproct (male); C. subgenital plate (female); D. phallosome (male); E_1. gonopore plate (female); E_2. membrane of gonopore plate (female); F. hypandrium (male); F_1. Left process of hypandrium (male); G. muscle m-03 (male); H. muscle f-05 (female).

Figure 10

Light microscopy photograph of the postabdomen of *T. incognitum* in copula (lateral view). (a) focused on the median plane; (b) focused on the external structures. Male: A. epiproct; B. paraproct; C. hypandrium; D. hypandrial median tongue; E. right arm; F. phallosoma; J. clunial arm; Female: G. gonopore plate; H. external valvae; I. subgenital plate.

Figure 11

Postabdomen of *T. incognitum* in a copulated state. Female structures are marked with red lines, and male structures are marked with white lines. The intersection of red and white lines is the point at which males exert pressure on females.

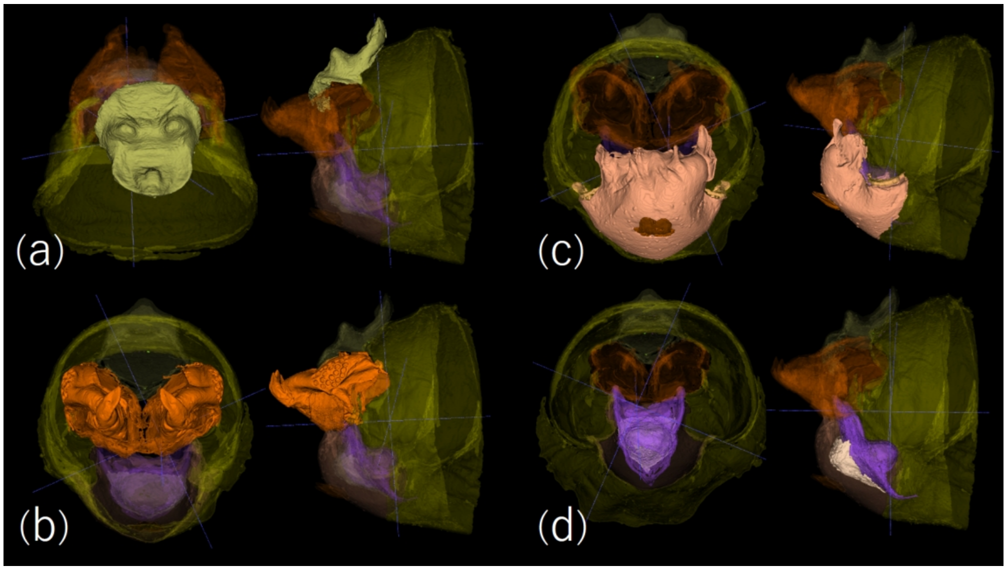

Figure 1
Male terminalia of T. pseudomedium, highlighting different structures: (a) epiproct (left: dorsal view; right: lateral view); (b) paraproct (left: posterior view; right: lateral view); (c) hypandrium (left: posterior view; right: lateral view); (d) phallosome (left: internal view; right: lateral view).

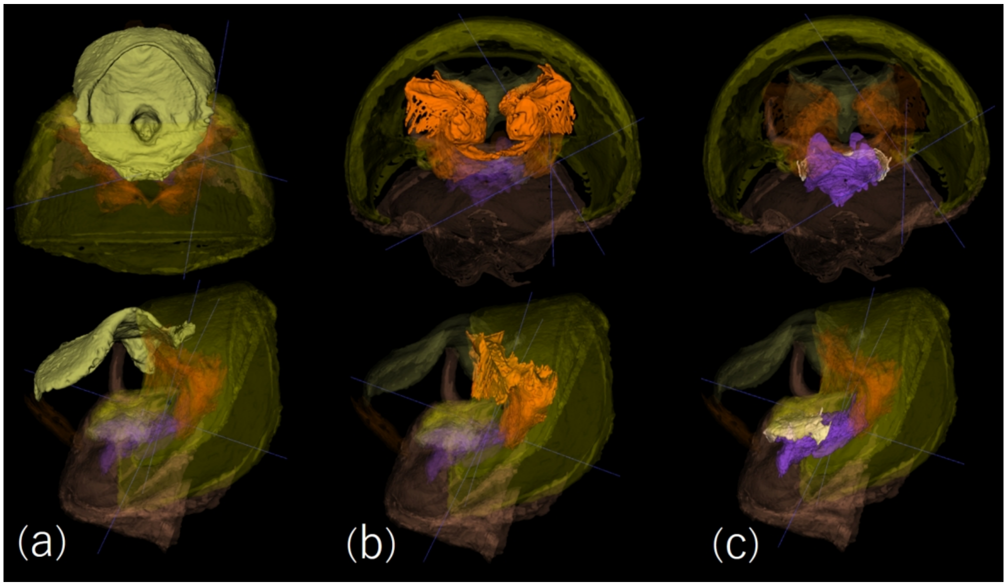

Figure 2
Male terminalia of T. incognitum in a copulated state, highlighting different structures: (a) epiproct (top: dorsal view; below: lateral view); (b) paraproct (top: internal view; below: lateral view); (c) phallosome (top: internal view; below: lateral view).

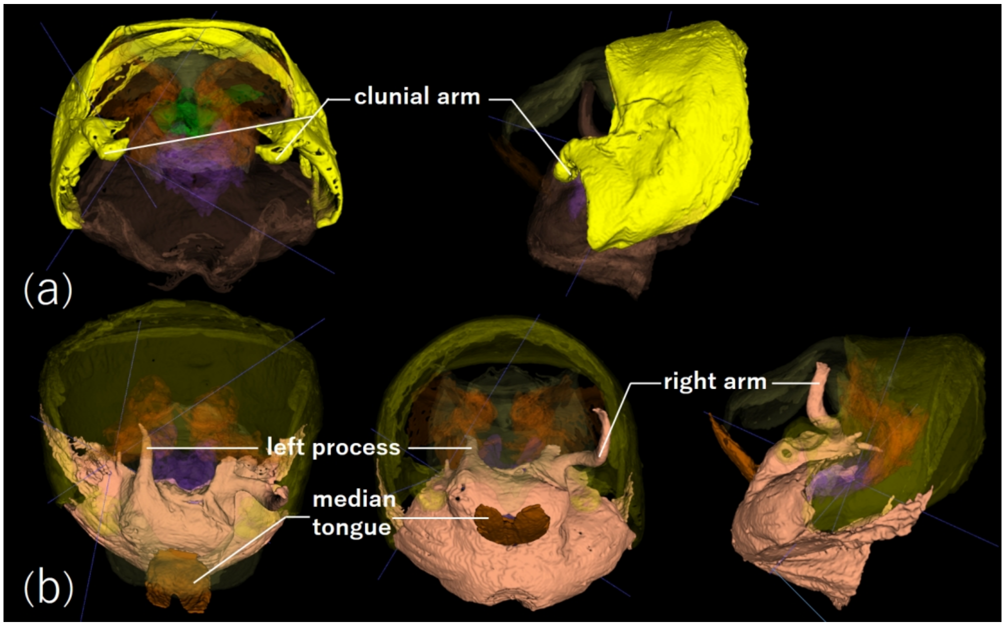

Figure 3
Male terminalia of T. incognitum in a copulated state: (a) clunium (left: posterior view; right: lateral view); (b) hypandrium (left: dorsal view; middle: posterior view; right: lateral view).

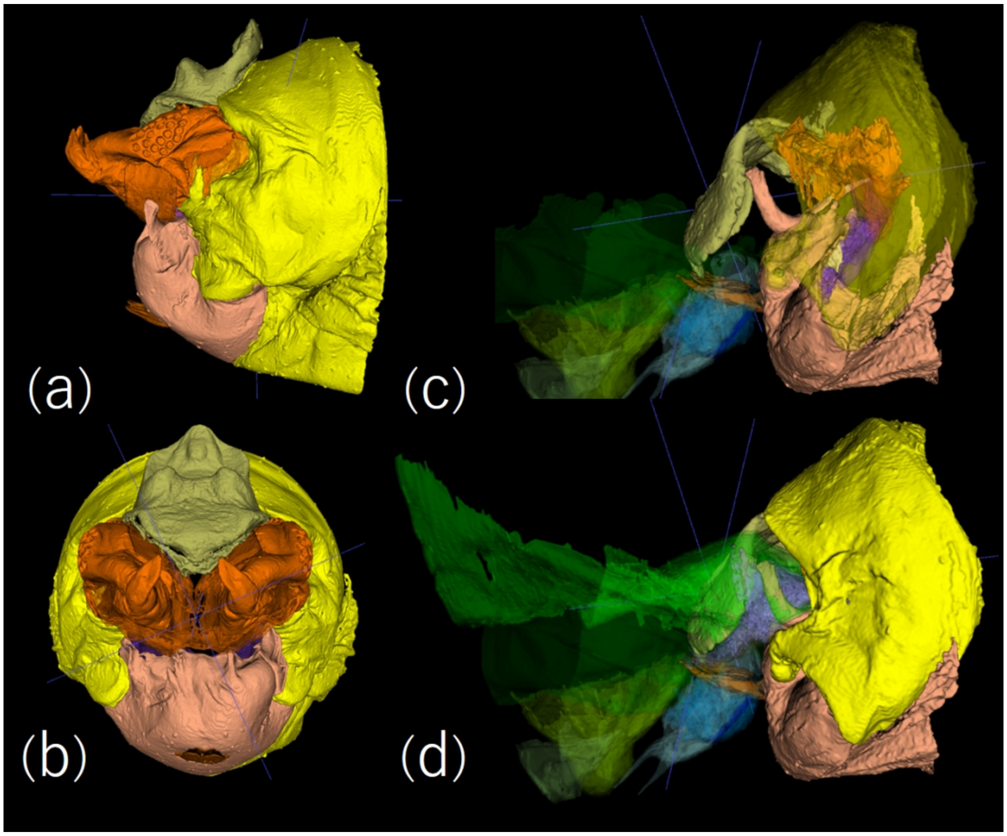

Figure 4
Male terminalia of T. pseudomedium (a, b) and T. incognitum (c, d). (a, c, d) lateral view; (b) posterior view.

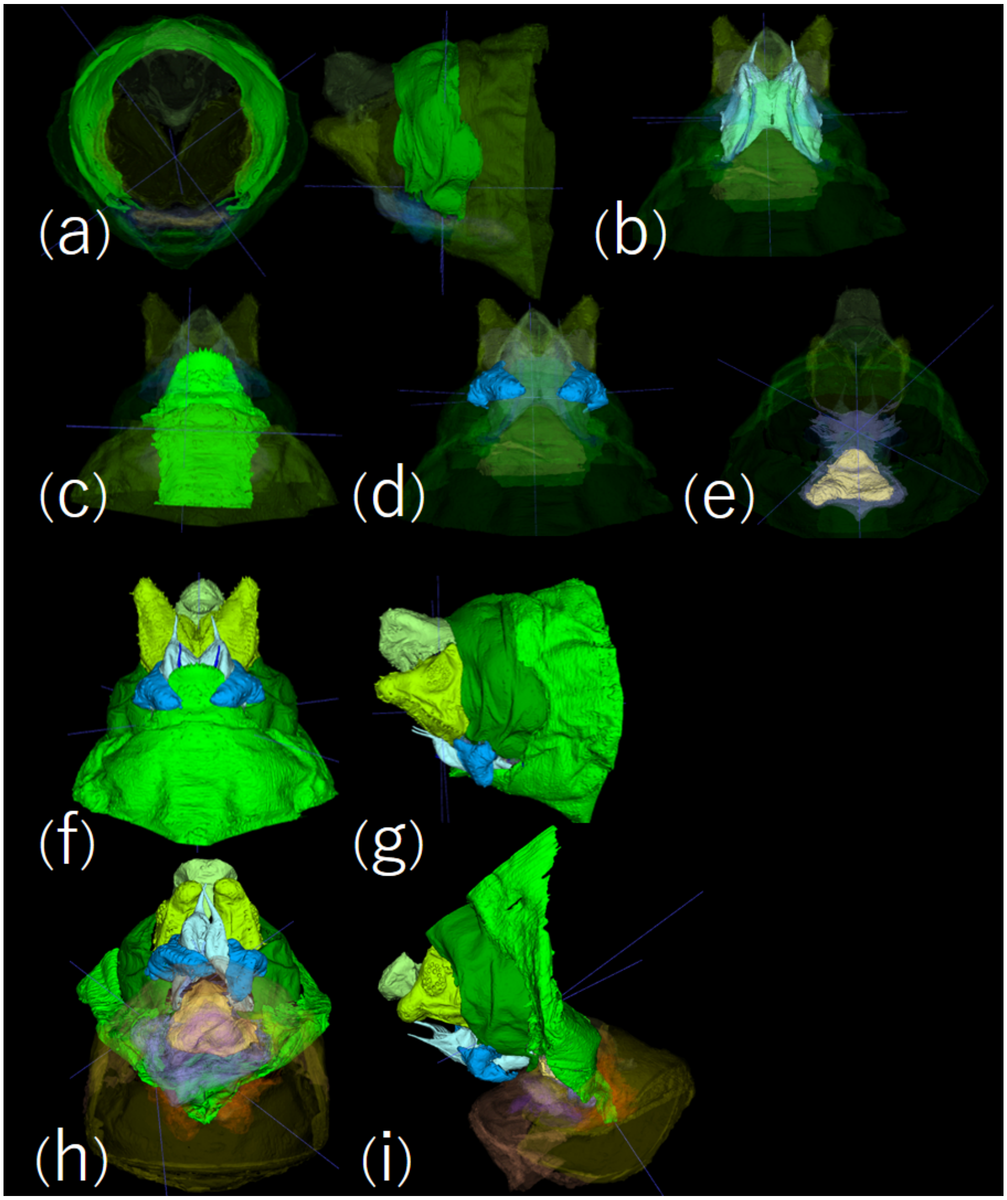

Figure 5
Female terminalia of T. pseudomedium (a-g) and T. incognitum (h, i), highlighting different structures: (a) clunium (left: posterior view; right: lateral view); (b) dorsal valve (ventral view); (c) subgenital plate (ventral view); (d) external valve (ventral view); (e) gonopore plate (internal view); (f) ventral view; (g) lateral view; (h) ventral view; (i) lateral view.

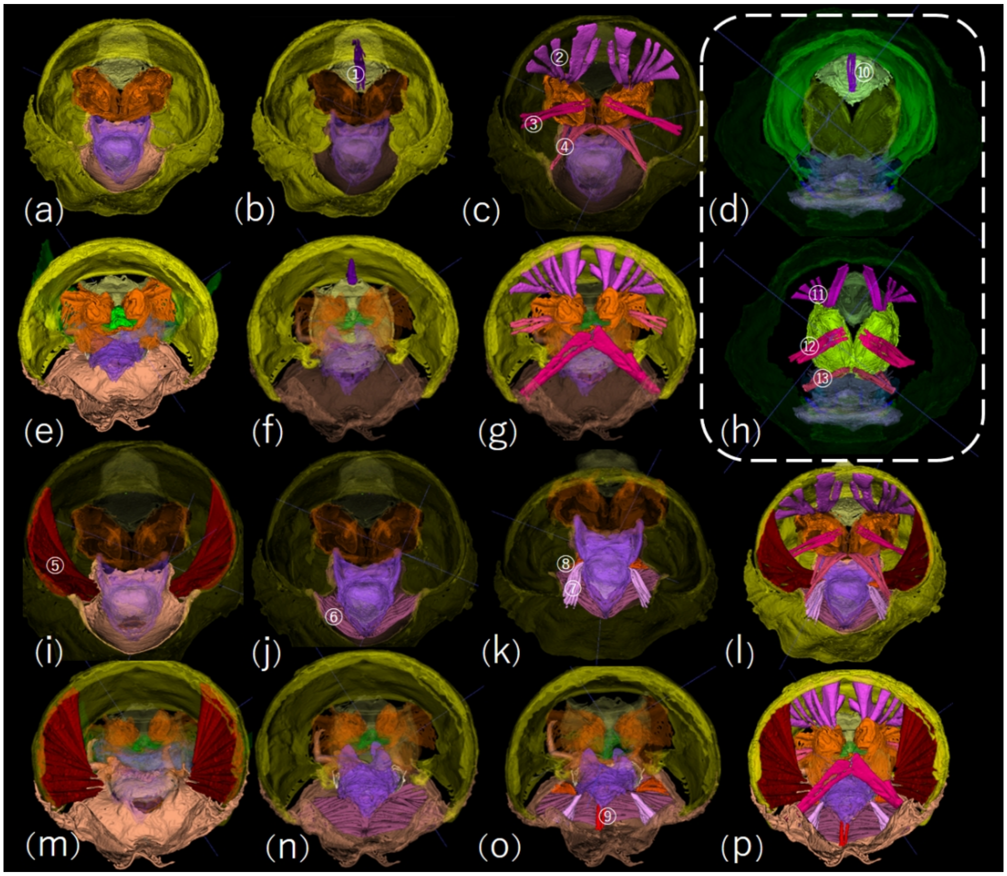

Figure 6
Male (a-c, i-l; internal view) and female (d, h; internal view) terminalia of T. pseudomedium and terminalia of T. incognitum (e-g, m-p; internal view), highlighting terminal muscles; (1) muscle m-01; (2) muscle m-02; (3) muscle m-03; (4) muscle m-04; (5) muscle m-05; (6) muscle m-06; (7) muscle m-07; (8) muscle m-08; (9) muscle m-09; (10) muscle f-01; (11) muscle f-02; (12) muscle f-03; (13) muscle f-04.

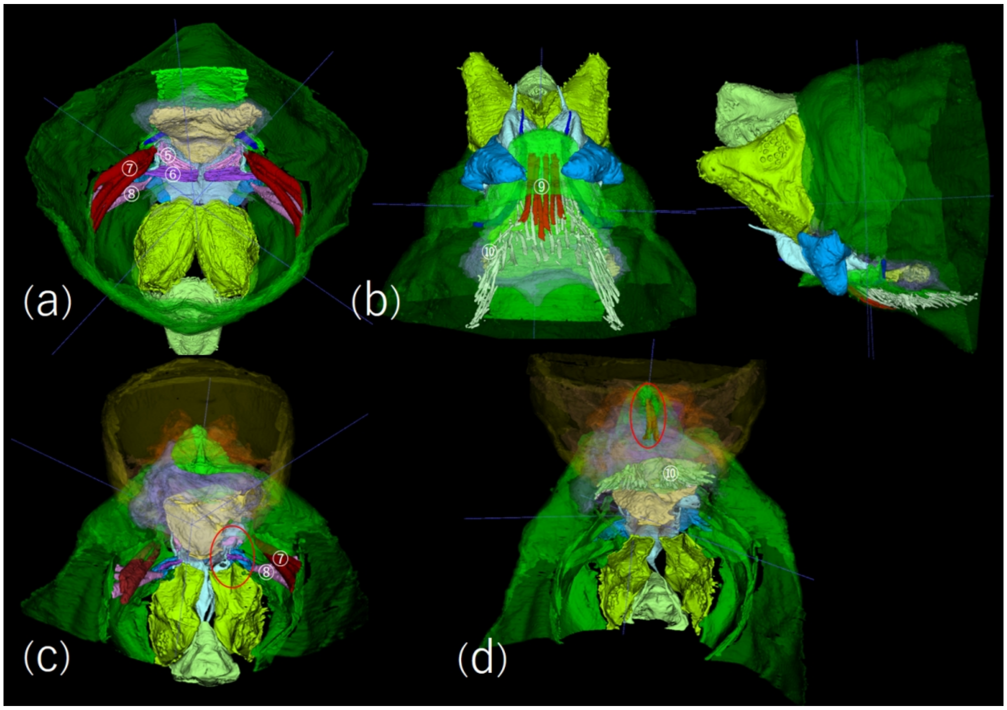

Figure 7
Female terminalia of T. pseudomedium (a, b) and T. incognitum (c, d), highlighting terminal muscles; (a) internal view; (b) left: ventral view; right: lateral view; (c) internal view (red oval: muscle f-05 and f-06); (d) internal view (red oval: muscle f-09); (1) muscle f-05; (2) muscle f-06; (3) muscle f-07; (4) muscle f-08; (5) muscle f-09; (6) muscle f-10.

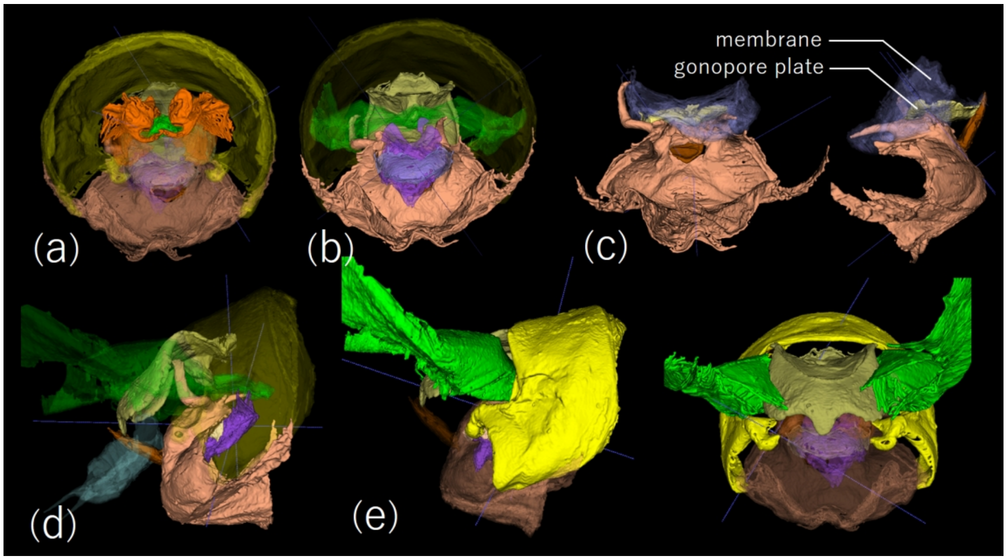

Figure 8
Male terminalia of T. incognitum in copula; (a) internal view; (b) internal view; (c) hypandrium (left: internal view; right: lateral view); (d) lateral view; (e) left: lateral view; right: posterior view.

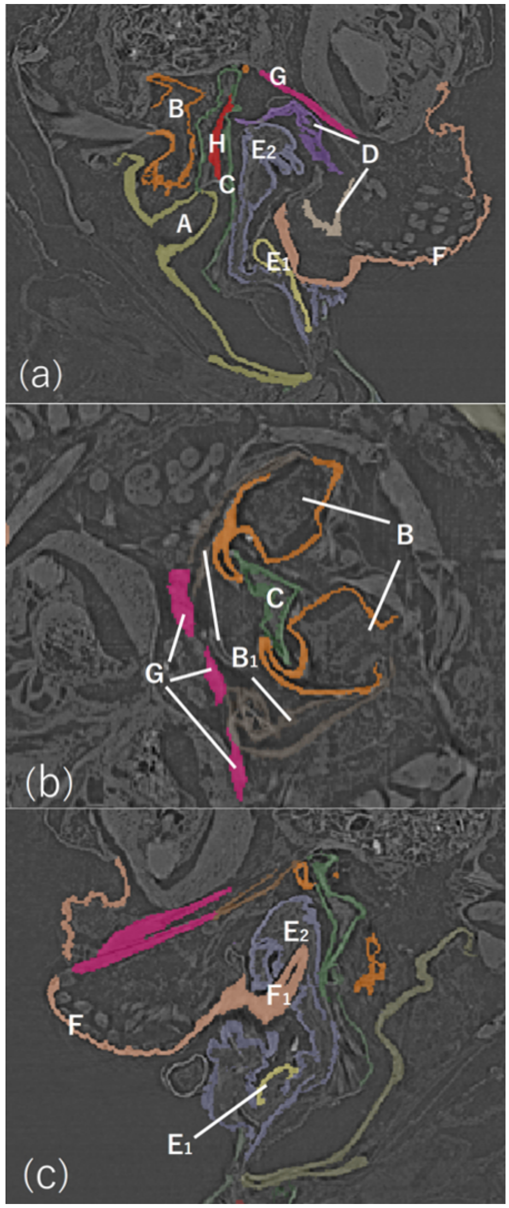

Figure 9
Sections of T. incognitum during copulation. (a, c) longitudinal section; (b) cross-section. A. epiproct (male); B. paraproct (male); B1. membrane of the paraproct (male); C. subgenital plate (female); D. phallosome (male); E1. gonopore plate (female); E2. membrane of gonopore plate (female); F. hypandrium (male); F1. Left process of hypandrium (male); G. muscle m-03 (male); H. muscle f-05 (female).

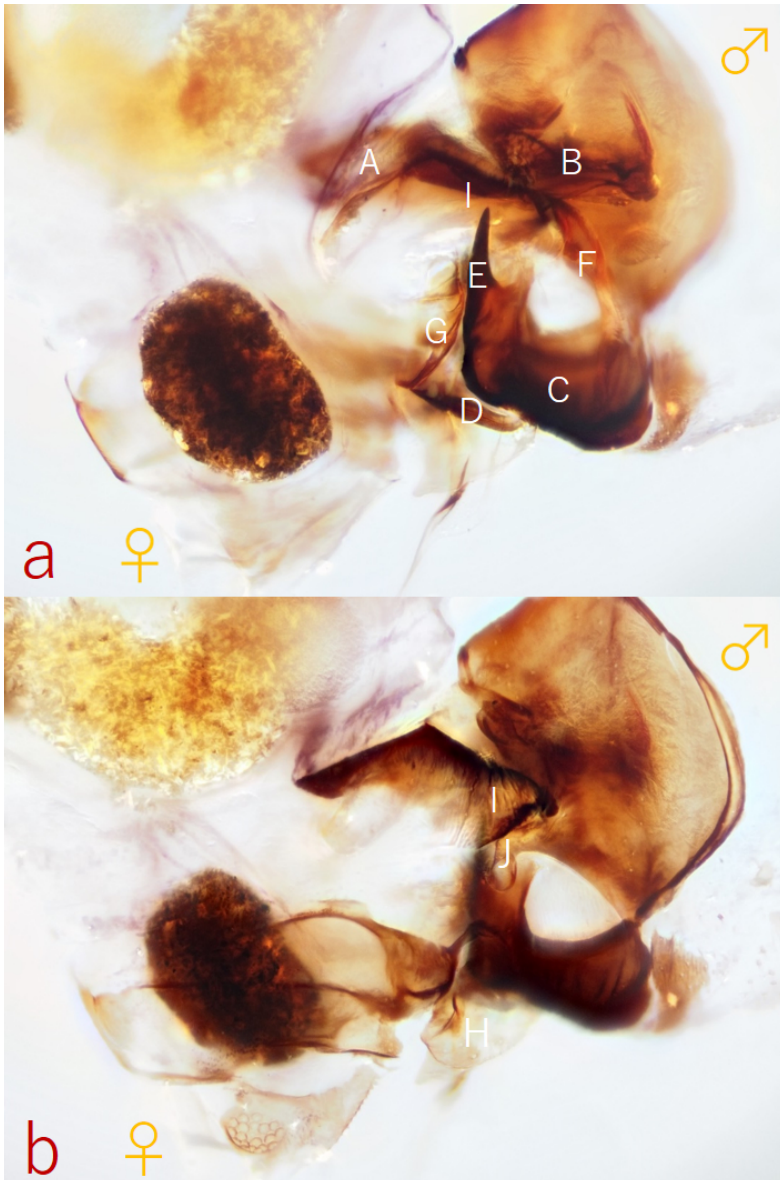

Figure 10
Light microscopy photograph of the postabdomen of T. incognitum in copula (lateral view). (a) focused on the median plane; (b) focused on the external structures. Male: A. epiproct; B. paraproct; C. hypandrium; D. hypandrial median tongue; E. right arm; F. phallosoma; J. clunial arm; Female: G. gonopore plate; H. external valvae; I. subgenital plate.

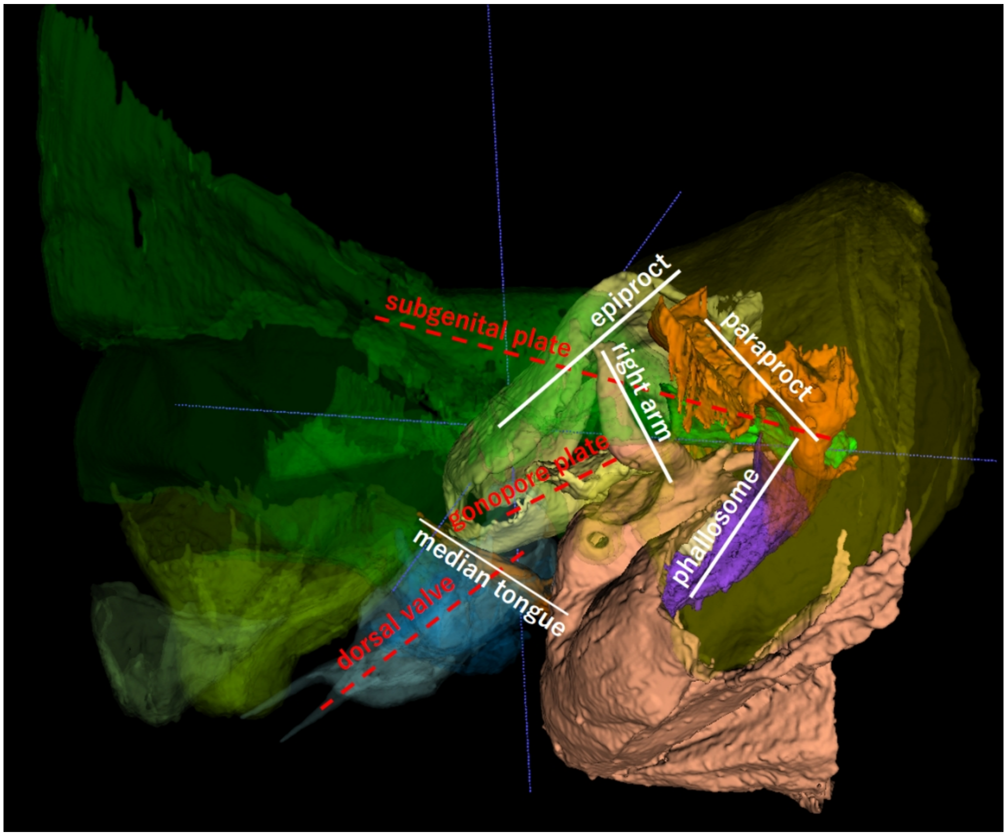

Figure 11
Postabdomen of T. incognitum in a copulated state. Female structures are marked with red lines, and male structures are marked with white lines. The intersection of red and white lines is the point at which males exert pressure on females.