#### NOTE



# Caudal papillae arrangement in *Tanqua tiara* (Nematoda: Gnathostomatidae)

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ABSTRACT: In order to solve discrepancies among the descriptions of male caudal papillae arrangement in *Tanqua tiara* (Nematoda: Gnathostomatidae), an observation was made using light and scanning electron microscopes. Seven pairs of caudal papillae, of which 5 pairs (I - V) with solid bases and 2 (VI and VII) with vesicular bases, and phasmidial orifices were confirmed. The termini of VI and VII were only weakly expressed on the cuticle and often hardly discernible by SEM. Careful re-examination should be made on *T. occlusa*, *T. gigantica*, and *T. herpestis*, in which the papillae corresponding to VI and VII have not been described.

KEY WORDS: Caudal papillae arrangement, Gnathostomatidae, SEM, Tanqua.

### INTRODUCTION

Adults of nematodes of the genus Tanqua Blanchard, 1904 (Gnathostomatidae) are mainly parasitic in the alimentary canal of reptiles. Baker (1987) listed eight species in this genus: T. tiara (von Linstow, 1879) (type species), T. anomala (von Linstow, 1904), T. bainae Ghadirian, 1968, T. diadema Baylis, 1916, T. geoclemydis Wang, 1979, T. gigantica Kung, 1948, T. herpestis Wang, Zhao et Chen, 1978, T. occlusa Schuurmans Stekhoven, 1943, T. ophidis Johnston et Mawson, 1948. Subsequently, T. ophidis was synonymized with T. anomala (Dewi et al., 2008). Although T. herpestis was described from a carnivorous mongoose, Herpestes urva, in China (Wang et al., 1978), it was suggested to be an accidental parasite acquired by ingesting a host reptile (Baker, 1987).

Tanqua tiara is parasitic in the semiaquatic lizards of Varanidae in the wide geographical region ranging from Africa to Australia through South Asia, Southeast Asia, and New Guinea (Baylis and Lane, 1920; Baker, 1987). The morphology of this nematode has been studied by various researchers using a light microscope and scanning electron microscope (SEM) (von Linstow, 1879; Baylis and Lane, 1920; Ichikawa, 1932; Gibbons and Keymer, 1991; Sou, 2020; Sou and Banerjee, 2021). However, there are some discrepancies in the explanation of the caudal papillae arrangement. Recently we had an opportunity to examine T. tiara specimens collected from varanids in Japan and Indonesia using a light microscope and SEM. Results are given herein with a discussion on the cause of the current confusion of the caudal papillae arrangement.

#### MATERIALS AND METHODS

**Specimens for Light Microscope observation:** Three males collected from *Varanus salvator* and fixed in 5% formalin solution on 8<sup>th</sup> November 1977 by Dr. Atsuo Ichihara of the Meguro Parasitological Museum, Tokyo, Japan (MPM Coll. No. 18599) were used to light microscope examination. The host was imported two months before from Southeast Asia to Japan by an animal dealer of Thailand, but died and necropsied. The worms were cleared in glycerol-ethanol solution by evaporation of ethanol and mounted on glass slides in 50% aqueous glycerol solution.

**Specimens for SEM observation:** Three males of *T. tiara* deposited in the Muzeum Zoologicum Bogoriense (MZBNa 322) collected from *V. salvator* from the Ahuloha River, Kendari, southeast Sulawesi, were processed for SEM observation. The host was collected on 21<sup>st</sup> August 1994 by Prof. (Em.) Dr. Sri Hartini of MZB. For the observation, the nematodes were prepared by fixing them in glutaraldehyde, dehydrated through an ethanol series, vacuum-dried using a TAITEC VC-96N vacuum dryer (TAITEC Co., Saitama, Japan), mounted on the stub by using double-sided carbon conductive tape and coated with gold at 5–8 mA for 5 min using an ion coater Eiko IB-2 (Eiko Co., Tokyo, Japan). Finally, the specimens were observed by a JEOL JSM-IT200 scanning electron microscope (JEOL Co., Tokyo, Japan).

### RESULTS

There are 7 pairs (I - VII) of caudal papillae (Figs. 1A–C). Two pairs precloacal (I and II) and 1 pair adcloacal (III) and 2 pairs postcloacal (IV and V), all with a solid base, located ventrolaterally. In addition, 2 pairs of papillae (VI and VII), each with vesicular base, are present



Fig. 1. Caudal papillae arrangement of male *Tanqua tiara*. A–C: Caudal portion in light microscopic image (A), line drawing (B) and SEM image (C), right lateral view, showing papillae I-V with solid base, VI and VII with vesicular base and phasmidial orifice (Ph); D: Magnified view of boxed area 1 in Fig. C, showing that termini of papillae VI and VII are not of usual form, but only wrinkles on cuticle; E: Magnified view of boxed area 2 in Fig. C showing slit-like phasmidial orifice (arrowed); F: Magnified view of phasmidial orifice of another male; G: Posterior end of one male showing papilla V and phasmidial orifice with duct, left lateral view; H: Magnified view of boxed part in Fig. G, showing two minute tubercles (arrowed) near apex.

just anteroventrally to papillae III and IV (Figs. 1A, B). Papilla II is positioned at an equal distance from I and III; the distance between III and IV is smaller than that between II and III; V is positioned dividing tail length 2: 1; III is the largest, and IV is the smallest among those with a solid base (Figs. 1A–C). External terminations of VI and VII are only wrinkles of the cuticle, especially indistinct in VII (Fig. 1D). Middle portion of the anterior cloacal lip is elevated but without a papilla-like terminus (Figs. 1A–C). The phasmidial orifice, pore- or slit-like, is opened postero-dorsally to V (Figs. 1E–G). A canal is directed anteriorly from the phasmidial orifice (Figs. 1B, G). In 1 male, 2 very minute tubercles, closely set with each other, were observed on the left lateral side near the tail tip (Fig. 1H).



#### DISCUSSION

In the original description of T. tiara, von Linstow (1879) noticed 7 pairs of caudal papillae: 2 precloacal and 3 postcloacal pairs in a row, along with 2 pairs standing diagonally side by side from the cloacal aperture. These features are in accordance with the present observation. Meanwhile, Baylis and Lane (1920) observed 8 pairs of caudal papillae: anterior 7 pairs are arranged as described by von Linstow (1879), but additional 1 pair of minute papillae were figured near tail end (papilla #1 in plate 3, Figs. 12 and 13 in Baylis and Lane, 1920). Ichikawa (1932) also observed 8 pairs of caudal papillae with the same arrangement pattern as Baylis and Lane (1920), giving a figure in which 2 pairs of papillae are present near the tail end (Fig. 2 in Ichikawa, 1932). As shown above, the papilla-like structures near the tail end were found to be phasmidial orifices by SEM observation. It is thus presumed that papilla #1 was not an actual papilla but a phasmidial orifice. In both Baylis and Lane (1920) and Ichikawa (1932), the papillae #4 and #6, that are corresponding to the papillae VI and VII of the present study, were found to be much smaller than the other papillae.

Gibbons and Keymer (1991) studied the morphology of T. tiara collected from Varanus niloticus in Nigeria with special attention to the pathology it caused using both a light microscope and SEM. They stated that there were 8 pairs of caudal papillae: 2 pairs precloacal, 2 pairs parallel adcloacal, and 4 pairs postcloacal. They also gave a line drawing of the ventral view of the male tail, showing the papillae arrangement (Fig. 38 in Gibbons and Keymer, 1991). According to this figure, papillae corresponding to VII of the present study (= #4 in Baylis and Lane, 1920) were not expressed. Because the terminus of VII is very faint, not of the usual form as shown in Fig. 4, it could be easily overlooked by SEM. Instead of VII, Gibbons and Keymer (1991) found 2 pairs of very minute papillae closely set at lateral fields near the tail tip (Fig. 10 in Gibbons and Keymer, 1991). These papillae resemble the 2 tubercles found in one of the present males (Fig. 1H). Because they seem to be so small as a papilla and their presence was confirmed in only 1 individual of the present worms, they are regarded as unstable structures. Ghadirian (1968) also wrote in the description of T. bainae that at the level of the caudal point, there are sometimes two small pulpy expansions that do not appear to be papillae (Fig. 20F in Ghadirian, 1968).

Recently, Sou (2020) also redescribed *T. tiara* based on the worms collected from *Varanus flavescens* of India, using a light microscope and SEM. He reported 2 pairs precloacal, 1 pair adcloacal, 2 pairs postcloacal papillae, and 1 unpaired median papilla on the anterior lip of the cloaca in males. The paired papillae seem to correspond to the papillae I – V of the present study. Sou (2020) considered that phasmids are located 96–117  $\mu$ m from tail tip in male, in agreement with the present observation. While the phasmids are located laterally in a line drawing of the tail in ventral view, the lateral view of the tail shows no such structure, and a phasmid is marked on the ventral side in SEM photo (Figs. e, f and h in Sou, 2020). The unpaired median papilla on the anterior lip of the cloaca seems to lack nerve termination and is seemingly absent (Fig. h in Sou, 2020) suggesting the unstable nature of this structure. Unfortunately, their SEM photos were of low resolution, preventing confirmation whether the papillae VI and VII were actually absent or not.

This study confirmed 7 pairs of caudal papillae and phasmidial orifices in the male tail of *T. tiara*. This pattern seems to be shared by other members of the genus, namely *T. anomala* and *T. diadema*, if papilla #1 by Baylis and Lane (1920) is regarded as the phasmidial orifice (see Figs. 14–16 in Baylis and Lane, 1920). The same feature was also described in *T. geoclemydis*, which has 7 pairs of papillae (3 pairs precloacal and 4 pairs postcloacal) and a pair of small structures corresponding to the phasmids midway between the posterior-most pair of papillae and tail tip (Fig. 4c in Wang *et al.*, 1979).

Meanwhile, other species of this genus have different arrangements of genital papillae: Schuurmans Stekhoven (1943) observed only 5 pairs of papillae in *T. occlusa* described from a Congolese snake, Kung (1948) also found 5 pairs of caudal papillae in *T. gigantica* from snakes of India and Southeast Asia, and Wang *et al.* (1978) recorded 6 pairs of papillae in *T. herpestis.* In these 3 species, the papillae corresponding to VI and VII were not shown in the figures. As shown above, the papillae VI and VII have unusual features and hence may be easily overlooked. Careful re-examination may be necessary to decide that the papillae VI and VII are actually absent in these species.

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