Typological characters of adults

General

Body cylindrical. Cuticle with fine annulation. Lateral field inconspicuous, only weakly separated from the other part of body surface by lack of annules, ridges (alae) absent. Lip region not clearly offset, with six equal-sized sectors, two dorsal sectors, right lateral and subventral sectors and left lateral and subventral sectors are close to each other and form a somewhat triangular stomatal opening. Each lip with a setiform labial sensillum. Four setiform cephalic sensilla present. Ampids very small pores at the level of the posterior end of cheilostom. Stoma cylindrical, separated into three elements, cheilostom, gymnostom and stegostom. Cheilostom simple cylinder, not clearly cuticularized. Gymnostom simple cylinder, clearly cuticularized, margin of arcade syncytia is faintly visible at the middle of gymnostom. Pro- and mesostegostom simple cylinder, comprising a little more than half of the stomatal tube (form pharyngeal sleeve); metastegostom slightly anisotopic and isomorphic with two small denticles on each sector. Procorpus cylindrical; metacorpus forming a well-developed median bulb, isthmus slender, basal bulb rounded (not polygonal) with weak duplex haustrulum posterior to valves. Procorpus plus metacorpus slightly longer than isthmus plus basal bulb. Cardia (pharynx-intestine junction) conspicuous. Nerve ring surrounding the posterior part of isthmus. Excretory pore conspicuous in ventral and lateral views, variable in position among individuals, but around the level of basal bulb. Excretory tube extends anteriorly, and then reflexes to continue posterior. An excretory cell is observed a little posterior to the excretory pore opening. Deirid at the same level with excretory pore (**Figure S3A-G**).

Female and hermaphrodite

Females and hermaphrodites are morphologically indistinguishable. Body weakly, smoothly and ventrally arcuate when heat-relaxed. Vulva located at mid body, forming horizontal slit 1/3 of vulval body diam. in length; cuticle around the vulva lacks annulations. Gonads didelphic and dorsally reflexed, anterior and posterior gonads on the right and left of intestine respectively. The gonadal system in young individuals is observable, but because juveniles are present in mature female/hermaphrodite bodies, the detailed structure of the gonadal system becomes unclear. Thus each organ is described based on young individuals. The germ cells are arranged in two to three rows in distal half, well-developed oocytes arranged in a single row in the other part. Oviduct connecting ovary (ovotestis) and spermatheca, formed by small rounded cells. Spermatheca and uterus not clearly distinguished. However, the sperm mass is sometimes present at the distal part (neighboring to oviduct), suggesting the part works as spermatheca. Dorsal wall of the junction of anterior/posterior uterus thickened. Vagina perpendicular to body surface, possessing thick wall, constricted by sphincter muscle at the vagina-uterus junction. Young females/hermaphrodites usually carry a few embryos in various sizes in each uterus (**Figure S3H-K)**. Two subventral and one dorsal rectal glands observed surrounding intestine-rectum junction and anterior part of rectum. Rectum as long or slightly longer than anal body diam. (ABD). Anal opening (AO) a horizontal slit; posterior anal lip expands slightly in lateral view. Phasmid small, opening laterally at ca. two ABD posterior to AO. Tail smoothly tapered to finely elongated conical tip but not filiform.

Male

Tail region clearly ventrally curved when killed by heat. Testis single, on the right of intestine; anterior part ventrally reflexed. Distal part of gonad is usually empty or contains small sperm cells; this part is interpreted as vas deferens. Two subventral and one dorsal cloacal (anal) glands visible at the level of anterior end of retracted spicules. Spicules paired, forming a “V” shape in ventral view; smoothly arcuate in lateral view, often protracted in heat-killed specimens. Gubernaculum, straight stick-like in lateral view, a little more than the half of spicule in length. Bursa anteriorly open, short, leptoderan, covering the posterior part of tail. Eight pairs of genital papillae (GP) and a ventral single papilla (hook) present. Ventral single papilla on the anterior cloacal lip. Within eight pairs of GP, GP5 and GP7 directed laterally and subdorsally, respectively, and others directed subventrally. However, direction of GP8 is slightly inconsistent among individuals, i.e., it directed laterally in some individuals. GP1 and GP2 close to each other, far (ca. three cloacal body diam.: CBD), anterior to cloacal opening (CO); GP3 and GP4 close to each other, slightly (ca. 0.5 CBD) anterior to CO; GP5 adcloacal or slightly posterior; GP6 on the midway between CO and the root of tail spike; GP7 and GP8 close to each other at the root of the tail spike. Papilliform phasmids with ventral openings at the level of (overlapping with) GP8. Tail tip forming spike with ca. two CBD in length (**Figure S3L-Q**).

Biological characters

Genders

The species has three sexes, male, female and hermaphrodite. When dauer juveniles were reared individually, all juveniles developed to adult hermaphrodites (n = 20). Then the offspring derived from the hermaphrodites were examined for their sex by removing male juveniles. There, some part of female-form adults produced offspring (= hermaphrodites), but the others did not produce offspring and had many undeveloped oocytes in their uterus (= unmated females). Although the detailed sex ratio, number of offspring and the sex determination pattern were not examined, male and female offspring appeared in the early stage of reproduction.

Reproduction

The reproductive trait is hypothesized to be viviparous, because the embryos in young hermaphrodite/female individuals are varying in size, and the early stage embryo is obviously smaller than well-developed embryo (**Figure S3I)**. This suggests that the parent provides nutrient to its embryo, and the embryos of ovoviviparous species, e.g., embryo in the entomoparasitic female of *Deladenus* spp., are uniform in size [S1]. The similar viviparous embryonic development is reported in a diplogastrid nematode genus, *Sudhausia* [S2, S3].

Dauer juveniles

Hermaphrodites in crowded (old) culture often lay dauer juveniles. The juveniles crawl around the media, and do not show nictation behavior.

Taxonomic status

Based upon its phylogenetic status and three-gendered reproductive mode, the new species is clearly close to *Auanema* spp. The general typological characters, e.g., stomatal structure and eight pairs of male genital papillae with laterally and subdorsally directed GP5 and GP7, support the closeness between the new species and *Auanema* [S4]. However, the arrangement of male genital papillae, i.e., four precloacal pairs forming two doublets, does not fit the present generic character of *Auanema*. Further, the new species does not show characteristic tube waving (nictation) behavior. In addition, the new species has a characteristic reproductive trait, viviparity, which is quite unique for the species. Thus, the new species would be suitable to be described as a member of a new genus. Nevertheless, we tentatively refer to this species as “*Auanema* sp.”, because the number of nominal and molecularly analyzed *Auanema* species is not sufficient to characterize these unique traits of the new species in evolutionary context. In other words, several more species are necessary to determine these characters as the clade (= genus) specific apomorphy or derived characters that occurred in the *Auanema* clade.

Materials and methods

The strain PS8402 was used for all observations. Twenty dauer juveniles were individually transferred to NGM agar plates (ø = 40 mm) previously inoculated with *E. coli* OP50, and kept at 20˚C. The development of the dauers was examined twice a day, or more, and the first batch of juveniles (ca. 10 individuals) was examined closely for their gender. Male and non-male juveniles were separately transferred to new NGM plates and kept at 20˚C. After developing to adults, male tail characters (number and arrangement of genital papillae and phasmid) and reproductive mode of non-male individuals (presence/absence of sperm and developing embryo) were examined under light microscope. Light micrographs were taken with a digital camera system, MC 170 HD (Leica) connected to the light microscope, Eclipse 80i (Nikon) facilitated with DIC optics. The micrographs were edited using a computer program PhotoShop Element 2017 (Adobe) to construct the figures.