Some quick and easy techniques for nematode morphological observations are provided. Silicone grease or petroleum jelly can be applied to adjust the nematode direction to observe lateral or ventral aspects. Small spots of the grease are placed between the slide glass and coverslip to fix the coverslip and adjust sample thickness. Nematodes can be turned by moving the coverslip, which can be pushed to observe the complicated stomatal morphology. Squashing of the nematode is handy to evert the buccal cavity. Furthermore, diluted detergents (0.1-0.5% Tween 20 or 80) can be used to remove bacterial particles stuck on the nematode surface and observe the surface structure of the nematode. Nematol. Res. 43(1), 15-17 (2013)

Key words: light microscope, methodology, morphology, taxonomy.

Despite their small body size, nematode morphology is very complicated. Partly because they are so small, some characters are difficult to interpret correctly. For example, the stomata of some diplogastrid nematodes are asymmetric and anisotopic, and close examination is required to understand its structure correctly. Baldwin et al. (1997) and Giblin-Davis et al. (2006) employed transmission electron microscopy (TEM) to interpret the stomatal structure of Acrostichus halicti and Parasitodiplogaster larvigata. Light microscopy and a video-capture system were used to understand muscular movements and reconstruct complicated three-dimensional morphology of diplogastrids (Fürst von Lieven and Sudhaus, 2000). Fürst von Lieven (2003) used 96% lactic acid to evert the buccal cavity of Oigolaimella spp., which made it easier to observe the various structures within it. To confirm the presence/absence and shape of the bursal flap, and vulval and anal morphology, it is important to observe the ventral aspect of nematodes. Given these needs, Hooper (1986) developed several methods based upon fixed materials and glycerin-mounted materials. However, some of these methodologies require long times, special chemicals, and/or specific facilities, e.g., TEM and scanning electron microscopy. New simple, quick, and easy methods are needed for routine observation. Here, some methodologies are outlined for the observation of stomatal morphology and ventral aspects of nematodes using silicone grease or petroleum jelly.

The method is as follows. First, put three to four small spots of silicone grease and a drop of water, buffer, or fixative (depending on the nematode species and sample condition) on a glass slide (Fig. 1). Second, pick up and transfer the nematode material(s) to the drop of fluid, put a coverslip on it, and push it gently to adjust the thickness of the fluid layer to the body diameter of the nematode. Third, examine the orientation of the nematode under a dissecting microscope and move the coverslip using forceps or a finger to turn the nematode so that it is ventral-side up. The silicone grease holds the coverslip like an agar pad, and the material can be observed using an oil lens without sealing. Silicon grease is most suitable for this observation technique because it is neutral (does not affect pH) and insoluble in water, but petroleum jelly is also available as an alternative to silicon grease.

The nematodes can be turned in different directions, and thus, a single individual nematode can be observed from different orientations, e.g., right lateral, left lateral and ventral views can be obtained from a single individual (Figs. 2A-G).

If necessary, the nematode can be squashed to evert the mouthparts by pressing the coverslip (Fig. 2H-K). To evert the mouthparts (and pharynx), a spot a quarter to one third of the body length from the anterior, or just behind the cardium, should be pressed quickly. In diplogastrids, the chelostom sometimes catches on the lip part and gymnostom, and posteriorly everts from the body (Fig. 2H-K).

When examining cultured material contaminated by bacteria (e.g., new cultures established without sterilization), the nematode surface is often covered by bacteria which obscure the observation of surface structures, e.g., genital papillae arrangement and surface annulation and/or striation. Such nematodes can be washed with 0.1-0.5%
Tween 20 or Tween 80 to remove bacterial particles. Place a drop of Tween solution on a small dish or glass slide, transfer the nematodes to the drop, and stir the solution for ca 30 sec to 1 min. Using this procedure, bacterial particles will be removed or reduced, and surface observation becomes easier. However, some bacterial species are sticky and not easily removed, meaning that this washing method is not applicable to all bacteria.

This methodology can also be used to observe the ventral aspect and stylet morphology of plant parasitic and fungal feeding species (e.g., Bursaphelenchus spp.). However, it is more difficult to use this methodology with strongly coiled species (e.g., Helicotylenchus spp.), because the nematode may be damaged while extending or turning it.
The biggest disadvantage of this method is the difficulty of removing the coverslip from the slide glass. In addition, fixed nematodes (e.g., TAF-fixed material) are less flexible than live materials and it is sometimes difficult to turn them smoothly. More flexible fixed materials (e.g., DESS-fixed and rehydrated materials: see Yoder et al., 2006) could be handled. Therefore, this silicone grease method may be most suitable for living cultured materials. Further verification of applicability of the method is necessary using additional different nematodes in various conditions.

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