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Research Note

Sodium dodecyl sulphate as a rapid clearing agent for studying the hard parts of monogeneans and nematodes

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Abstract

Hard structures of helminths have often been used for taxonomic identification but are usually not clearly defined when treated with conventional methods such as ammonium picrate-glycerin for monogeneans and glycerin for nematodes. The present study reports a rapid and simple technique to better resolve the hard parts of selected monogeneans and nematodes using 5–10% alkaline sodium dodecyl sulphate (SDS). In comparison with established methods, SDS-treated worms become more transparent. In monogeneans treated with SDS, clear details of the hooks, hook filaments, anchors, bars and the sclerotized copulatory organs could be observed. In SDS-treated nematodes, spicules and ornamentations of the buccal capsules could be clearly seen.

Identification of monogenean species is based mainly on the morphology of the hard parts of their haptors and copulatory organs. These sclerotized structures are usually surrounded with tissue such that their details are not clear if the tissues around them are not transparent. Ammonium picrate-glycerin has been widely used for fixing and clearing monogenean specimens in order to study the sclerotized parts using phase-contrast microscopy (Malmberg, 1956; Lim & Furtado, 1986). Selective staining methods have been developed to investigate the morphology of the sclerites of monogeneans (see Zdárska, 1976; Kritsky *et al.*, 1978; Richards & Chubb, 1995) but these techniques are time consuming and again the hard parts are not clearly defined. This problem prompted us to try other chemicals. We decided to use sodium dodecyl sulphate (SDS) because SDS is widely used in laboratories as an anionic detergent as it denatures proteins and solubilizes biological membranes.

The specimens used for this study includes the marine monogeneans, *Bravohollisia* and *Caballeria* species, the fresh-water monogeneans, *Dactylogyrus* species, and the nematodes, *Panagrellus redivivus* cultured in the laboratory at the School of Biology and Biochemistry, The Queen's University of Belfast. In the present study, different concentrations of SDS (5–10%) were used on fresh, frozen and alcohol-fixed parasites. For ethanol (95%) fixed worms, specimens were re-hydrated through a series of decreasing concentrations of ethanol to water prior to SDS-treatment. Some monogeneans and nematodes were fixed and cleared in ammonium picrate-glycerin as shown by Lim & Furtado (1986) and glycerin, respectively. Some worms were placed in a small drop of water on a slide and a drop of alkaline SDS containing 0.01M NaOH was added before flattening the parasites with a cover-slip. Five to 10% alkaline SDS was used depending on the thickness of the specimens. If too high a concentration of SDS is used, the specimens may shrink. Excess SDS solution on the edge of the cover-slip was drained off with filter paper. Nail varnish was applied to the four corners of the cover-slip in order to secure it in place. The edges of the cover-slip were next sealed with nail varnish to prevent the specimens from drying out. The SDS-treated specimens were left for 10 min before viewing under a phase-contrast microscope. Some SDS-treated monogeneans were washed with distilled water and stained with Gomori's trichrome (following the protocol of Kritsky *et al.*, 1978) (figs 1F, 1H and 3) in order to see if the hard parts of the SDS-treated materials could be stained. The treated specimens were examined under a light and phase-contrast microscope.

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Photomicrograph images of these differently treated specimens were taken and compared. The tissues of monogenean specimens fixed in ammonium picrate-glycerin appear opaque and granulated (fig. 1 A,C,E,G). On the other hand, monogeneans treated with SDS were almost transparent and the structures of the anchors, marginal hooks, filament loops of the marginal hooks, bars and sclerotized copulatory organs were very clear and better defined compared to those in the specimens fixed in ammonium picrate-glycerin (cf. fig. 1 B,D,F,H & fig. 1 A,C,E,G). The marginal hooks of the oncomiracidium of *Bravohollisia* sp. fixed in SDS were also clearly visible (fig. 2). The root of the anchor and bar of the SDS-treated *Caballeria liewi* were stained red in Gomori's

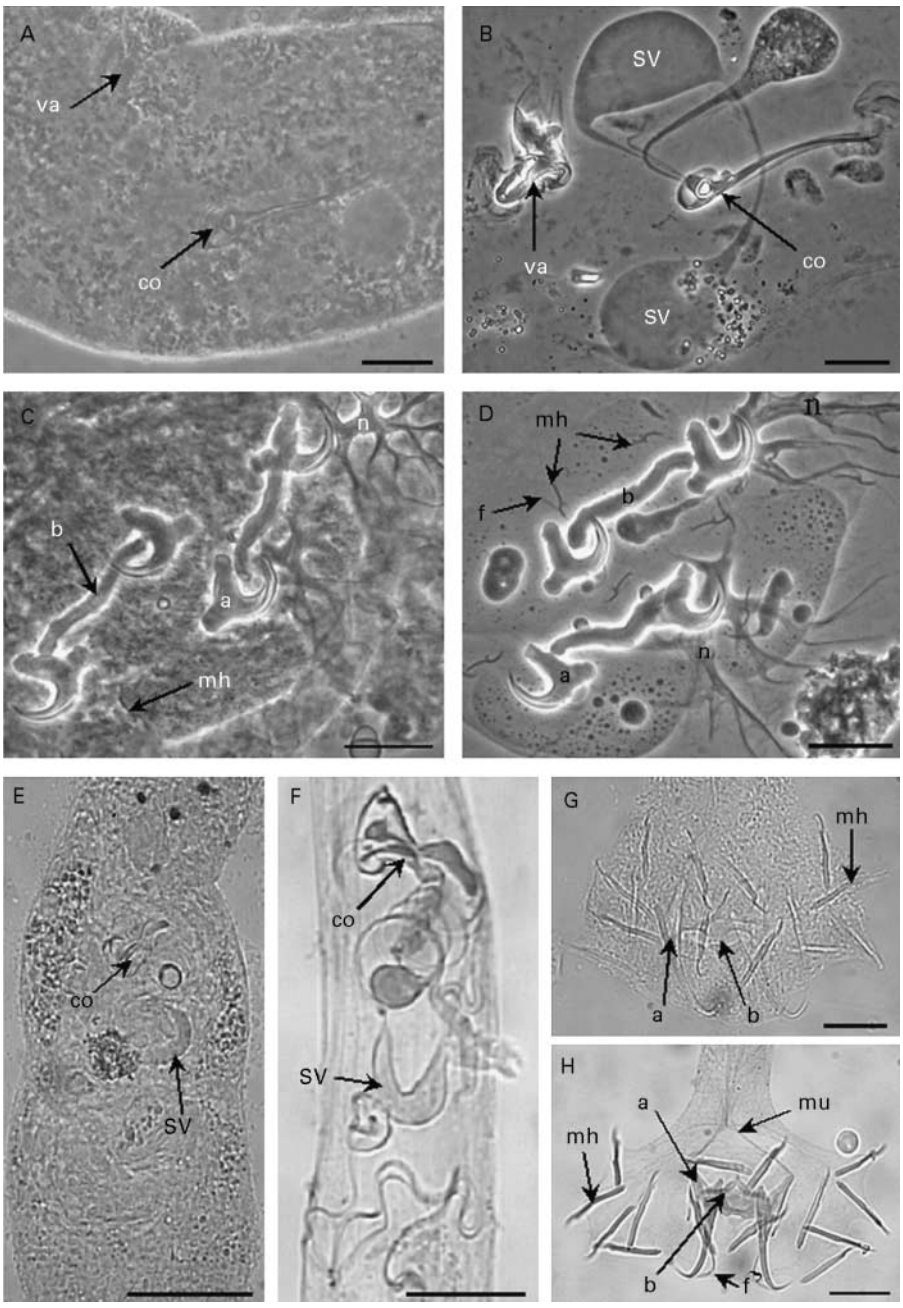

Fig. 1. Phase-contrast microscope images of ammonium picrate-glycerin mounted monogeneans (A, C, E, G) and SDS-treated monogeneans (B, D, F, H). A & B, copulatory organ of *Caballeria* sp.; C & D, haptor of *Bravohollisia reticulata*; E & F, copulatory organ of *Dactylogyrus* sp. (F is SDS-treated and stained with Gomori's trichrome); G & H, haptor of *Dactylogyrus* sp. (H is SDS-treated and stained with Gomori's trichrome). a, anchor; b, bar; co, copulatory organ; f, filament of marginal hook; f', filament of anchor; mh, marginal hook; mu, muscle; n, net-like structure; sv, seminal vesicle; va, vaginal apparatus. Scale bar = 20 μm.

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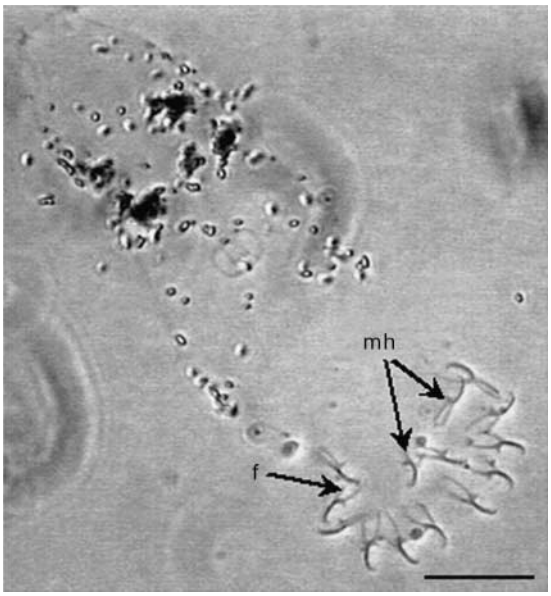

Fig. 2. SDS-treated oncomiracidium of *Bravohollisia* sp. showing marginal hooks (mh) with filaments (f). Scale bar = 20 μm.

trichrome while the copulatory organs were stained blue (fig. 3). Spicules and grinder (cuticular lining of the buccal capsule) of SDS-treated nematodes, *P. redivivus*, were more visible compared to the glycerin-cleared nematodes (cf. fig. 4A and 4B, 4C). The property of SDS as an anionic detergent probably helps to clear the soft tissues of the worms making them transparent and thus making the hard parts more visible. SDS-treated specimens could be kept for a minimum period of 6 months under laboratory conditions. If crystals of SDS appear around the specimens, a few drops of distilled water will dispel them readily.

The present study shows that SDS can be employed as a rapid clearing agent, making parasitic worms transparent

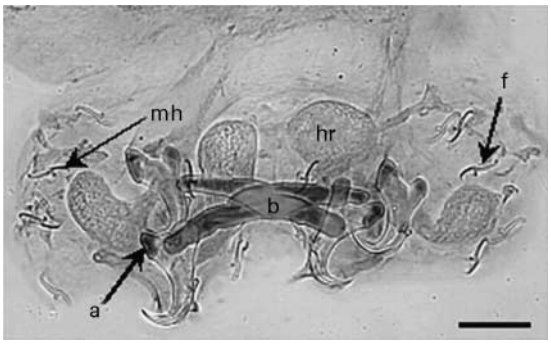

Fig. 3. *Caballeria liewi* is SDS-treated and stained with Gomori's trichrome. Bars (b) and roots of anchor (a) were stained red. f, filament of marginal hook; hr, haptoral reservoir; mh, marginal hook. Scale bar = 20 μm.

and thereby revealing clearly the hard sclerites of monogeneans and nematodes. This simple technique requires little time and technical skill and is easily performed under field conditions. The main advantage of using SDS-treatment is that it is rapid, easy to use and cost-effective, ensuring that the worms are transparent and the hard parts are clearly visible. The method may prove to be useful for taxonomic description and identification of other parasites with hard structures such as cestodes.

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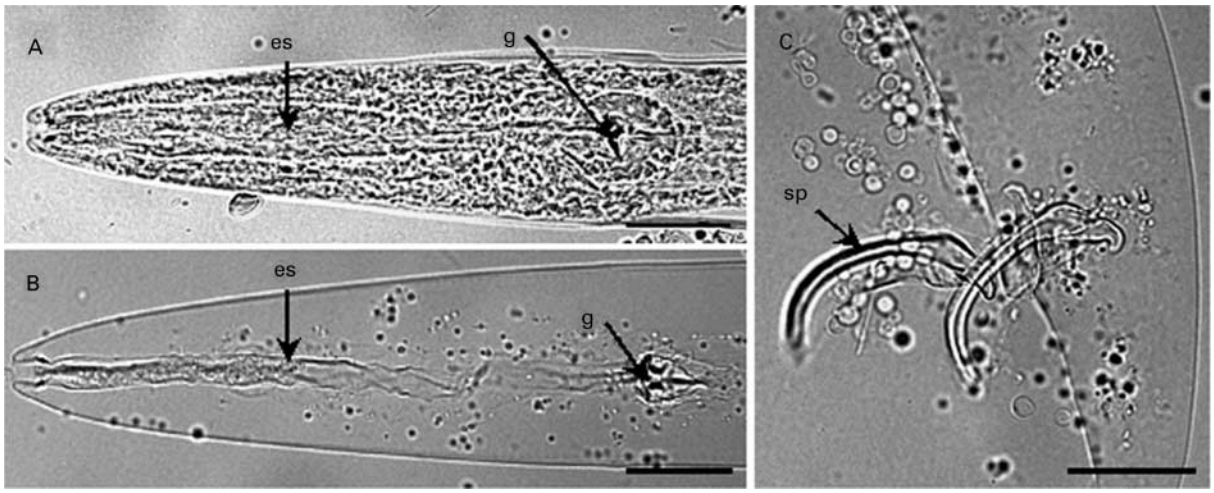

Fig. 4. Light microscope images of anterior region of glycerin-cleared *Panagrellus redivivus* (A), anterior region of SDS-treated *P. redivivus* (B) and posterior region of SDS-treated *P. redivivus* (C). es, oesophagus; g, grinder; sp, spicule. Scale bar = 30 μm.

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