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DESS: a versatile solution for preserving morphology and extractable DNA of nematodes

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Summary – A solution containing dimethyl sulphoxide, disodium EDTA, and saturated NaCl (abbreviated here as DESS) was tested for various applications in the preservation of nematodes for combined morphological and molecular analyses. The solution can be used to preserve individual nematodes, nematode extracts, or entire soil/sediment samples. Preserved material can be easily stored for months at room temperature, shipped by mail, or carried in luggage. Morphological features are usually well preserved; specimen quality being comparable to formalin-based fixatives and much better than ethanol fixation. Specimens can be transferred to glycerin with little or no modification of traditional protocols. Unlike formalin-preserved material, routine PCR can be performed on individual specimens after any of these procedures with success rates and amplification sizes comparable to PCR of fresh specimens. At this point we have no data on long-term preservation quality. Nevertheless, DESS solution clearly enhances and simplifies a wide range of nematological studies due to its combined suitability for morphological and molecular analyses, as well as its less hazardous chemical properties.

Keywords – dimethyl sulphoxide, EDTA, molecular, PCR, protocol, SEM.

With the discovery of the polymerase chain reaction (PCR) and the continual reduction of costs and time to amplify DNA, molecular studies have become widespread in all fields of biological research. Although DNA is a very informative molecule, it usually requires rapid intervention to avoid its degradation into small fragments by active nucleases. Specifically, at least one of three factors must be adjusted rapidly to inactivate these nucleases: temperature, pH, or salt concentration (Dixon & Webb, 1979). Dessauer *et al.* (1996) found cryopreservation to be the most effective method for long-term preservation of DNA. This is a useful method when working in a laboratory, but equipment needed to reach temperatures as low as -70°C is difficult to transport to and from the field. Samples must therefore be treated on-site with a preservative that will stop nuclease activity. In nematology, ethanol and formalin have been most frequently used or tested to preserve nematode DNA (Thomas *et al.*, 1997; Schander & Halanych, 2003; Roubtsova *et al.*, 2005). However, neither of these solutions combines the properties of an ideal

fixative, *i.e.*, adequate preservation of both DNA and morphology, straightforward handling in terms of transportation to and from the field, easy storage after collection of specimens, plus minimal hazard in terms of flammability or toxicity. As a result, a sample must often be split into subsamples for preservation of DNA in ethanol and morphology in formalin. This approach is less than ideal because it allows for discrepancies between subsamples in species composition and also because it precludes obtaining combined sequence and morphology data from single nematodes.

Several studies have compared and described the effects of different preservatives on DNA (Greer *et al.*, 1991; Seutin *et al.*, 1991; Dillon *et al.*, 1996; Holzmann & Pawlowski, 1996; Miller & Hook, 1996; Thomas *et al.*, 1997; Toe *et al.*, 1997; Dawson *et al.*, 1998; Kilpatrick, 2002). Seutin *et al.* (1991) suggested the use of a DMSO/EDTA/saturated NaCl solution for preserving DNA from avian tissues at room temperature. Dawson *et al.* (1998) subsequently showed that this solution allows

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preservation of both physical structures and high molecular weight DNA for up to 6 months without freezing. Kilpatrick (2002) tested long-term DNA preservation and determined that high molecular weight DNA was amplifiable for up to 2 years in this solution. We report here on the various uses of this solution (henceforth abbreviated as DESS solution) for combined preservation of nematode DNA and morphology.

Materials and methods

PRESERVATION OF EXTRACTS OF TERRESTRIAL AND MARINE NEMATODES

Soil samples were collected from the UCR Botanic Gardens and nematodes were extracted by modified Baermann technique (Schindler, 1961). In addition, a freshly extracted sample from Kern County, CA, USA (supplied by Dr Michael McKenry) was also used for initial testing. Individuals were picked for video capture and editing (VCE) (De Ley & Bert, 2002), PCR, and sequencing. Each specimen was mounted temporarily under a cover glass on ringed fluorescence slides in a drop of deionised water. The temporary mount was examined on an Olympus® BX51 microscope with differential interference contrast optics and the most important body parts were imaged via a Matrox RTMac on a Macintosh G4 PowerPC or Sony™ Handycam® HDR-HC1K Digital HD Video Camera Recorder using Apple iMovie HD version 5.0.2, as multifocal images. Multifocal vouchers, sample information, and DNA sequences (when available) were deposited in NemATOL (<http://nematol.unh.edu/>) for all specimens analysed.

The remaining nematodes were preserved in bulk by replacing water with DESS solution containing 20% dimethyl sulphoxide (DMSO) and 0.25 M disodium EDTA, saturated with NaCl, pH 8.0 (Seutin *et al.*, 1991) (http://nematol.unh.edu/Method-Protocol/DMSO_protocol.htm). This was done by pouring the extract over a 500 mesh sieve (25 µm opening) allowing most of the water to drain, then replacing with DESS solution as the contents of the sieve were collected into a vial. Preserved samples were stored at room temperature for 2 weeks, 7 weeks, and 7 months at which time individual nematodes were picked from the DESS solution, washed in deionised water, and video captured prior to PCR and sequencing.

Marine nematodes from Solana Beach, CA, USA, were obtained from the intertidal zone or from kelp holdfasts. Sediment was placed in a bucket with three times its

volume of 3% artificial seawater, stirred by hand, decanted over a 500 (25 µm) mesh sieve several times, and preserved in DESS solution as described above. Holdfasts were washed several times in a tray with 3% artificial seawater prior to decanting and preservation. Three days later, individual nematodes were picked from the DESS solution and video captured prior to PCR and sequencing.

SAFE TRANSPORTATION OF SAMPLES BY AIR OR BY MAIL

Soil, lichen, and moss samples were collected from various sites in Moldova during an exchange visit organised by the National Academy of Sciences International Research Experiences for Undergraduates (INTREU) programme between the University of California campuses at Riverside and Davis, Brigham Young University, and Moldova State University. Soil samples were processed using a series of 60 (250 µm), 200 (75 µm), and 500 (25 µm) mesh sieves and extracts placed on a modified Baermann for 3 days. Mosses and lichens were placed on a modified Baermann for 5 days to extract all nematodes. Each sample was split into subsamples for preservation in 5% formalin and DESS solution. All subsamples were stored at room temperature from a few days up to 2 weeks and then brought to UCR by air in checked luggage. After 24 h of travelling, samples were stored at room temperature for between 2 days and 6 months. Subsamples with DESS solution were then examined under the dissection microscope and nematodes were picked for VCE, PCR, and sequencing.

Several soil sample extracts preserved in DESS solution were also exchanged by mail between Dr Mark Blaxter's laboratory in Edinburgh, UK, and our laboratories at UCR. Samples took 3-4 weeks to arrive when nematodes were picked for VCE, PCR, and sequencing. Samples sent to the Blaxter laboratory were left at room temperature for up to 9 weeks, after which individuals were rinsed in sterile tap water before PCR and sequencing.

PREPARATION OF GLYCERIN MOUNTS

To test if DESS solution can preserve DNA and replace formalin in the preparation of glycerin mounts with the Seinhorst (1959) method as modified by De Grisse (1969), preserved nematodes from the UCR Botanic Gardens and Solana Beach, California, were first rinsed with purified water to remove any debris. A glass cavity block containing the nematode extract in purified water was then placed in an airtight jar containing 1.25 cm deep volume

of 96% ethanol and left overnight in an incubator set to 40°C. The glass block was removed from the jar the next morning, filled to the brim with five parts glycerol and 95 parts 96% ethanol solution, and left at 40°C with two-thirds of its cavity covered by a glass square. Gradual transition to glycerin was achieved by adding more of the glycerol:ethanol (5:95) solution every few hours. The next day, individual nematodes were mounted on glass slides which were set aside for between one week and five months. At the end of the allotted time period, one slide was broken open and the nematodes were washed three times in purified deionised water to remove excess glycerin. Individuals were then subjected to VCE, PCR, and sequencing.

BULK PRESERVATION OF SOIL SAMPLES BEFORE NEMATODE EXTRACTION

To investigate if the DESS solution could also replace formalin as a bulk preservative, DESS solution was used to preserve entire samples of soil or sediment obtained from the UCR campus and the Salton Sea, CA, USA. DESS solution was added at a ratio of 3:1 to each sample and mixed thoroughly. Samples were stored at room temperature for 1-2 weeks. Then 200 g of substrate and DESS solution were weighed from each sample, mixed with water, and decanted through a series of 80 (180 µm) and 500 (25 µm) mesh sieves for a minimum of three times per sample. The substrate remaining after decantation was centrifuged at 1450 g for 5 min and the supernatant recovered and set aside. Ludox® TM-50 Colloidal Silica (Grace Davison W.R. Grace & Co.-Conn., Columbia, MA, USA) at a 2:3 dilution in purified water was added and mixed thoroughly to each substrate. Samples were centrifuged again at 1450 g for 15 min and the supernatant recovered and set aside. Ludox® was added again, the sample was mixed and centrifuged as above a minimum of three times per sample. The supernatant set aside from each centrifugation was then rinsed with deionised water in a 500 (25 µm) mesh sieve and placed back into DESS solution for examination. Nematodes were picked from both extract fractions and recorded by VCE before PCR and sequencing.

PRESERVATION OF AN ARTHROPOD HOST BEFORE NEMATODE EXTRACTION

A 6 cm long polydesmid millipede (species and genus as yet unidentified) was captured in the Amazon forest of Peru, killed, stored in 30 ml of DESS solution,

and shipped at ambient temperature to Edinburgh, UK. It was stored at -20°C in DESS for 1 month before dissection. The specimen was thawed, head and tail were removed, and the body was sliced through every second diplosegment. Gut contents were washed into sterile tap water and examined for nematode parasites under a dissection microscope. A subset of the recovered nematodes, selected for morphological disparity, were picked to microscope slides and imaged on a Zeiss Axiovert 35 microscope with Openlab™ (Improvision®, Lexington, MA, USA) digital imaging system. Selections of images are available on the Internet for remote diagnosis (http://nemhelix.cap.ed.ac.uk/mpl/Peru_Nematodes/peruvian_millipede_nematod.html). All nematodes recovered from the millipede gut were used for PCR and sequencing.

PCR AND SEQUENCING OF TARGET LOCI

Each recorded specimen was recovered intact from the temporary mount, cut into two pieces in 20 µl of Worm Lysis Buffer (50 mM KCl, 10 mM Tris-Cl pH 8.3, 2.5 mM MgCl₂, 0.45% NP40, and 0.45% Tween 20, as described in Williams *et al.*, 1992), transferred to a microcentrifuge tube containing 2 µl of Proteinase K (60 µg ml⁻¹) for digestion, and stored at -80°C. PCR amplification was subsequently performed on either the D2D3 domain of the large subunit (LSU) or 18S (small subunit or SSU) rDNA gene. A 25 µl reaction contained 2.5 µl of genomic DNA as template, 2.5 µl of 10× reaction buffer with MgCl₂, dNTP-mix at 0.2 mM each, 0.4 µM each of primer (A (5'-AAA GAT TAA GCC ATG CAT G-3') and G18S4 or 18P (Blaxter *et al.*, 1998; Tandingan De Ley *et al.*, 2002) for SSU; D2Ab and D3b (De Ley *et al.*, 1999) for D2D3), and 1 unit of DyNAzyme EXT DNA polymerase (New England Biolabs®, Ipswich, MA, 01938, USA). PCR conditions were: denaturation at 94°C for 30 s, annealing at 55°C for 1 min, and extension at 72°C for 2 min, followed by polymerisation for 7 min at 72°C for 35 cycles. PCR products were separated on a 1% agarose gel stained with 0.0003% ethidium bromide with 1 kbp DNA ladder (Promega, Madison, WI, USA) as size markers. Positive products were cleaned with QIAquick® PCR Purification Kit (Qiagen, Valencia, CA, USA) following the manufacturer's protocol. Sequencing was performed using a 96-capillary ABI 3730xl at the UCR Core Instrumentation Facility. Sequences were assembled using GeneTool 2.0 (Biotoools, Edmonton, AB, Canada) and compared with published sequences in GenBank by means of BLAST search (Altschul *et al.*, 1997).

Specimens used for PCR amplification in Edinburgh were picked and digested in 20 µl (40 µl for large nematodes) of 0.25 M NaOH. Digests were incubated overnight at room temperature, heated to 95°C for 3 min, neutralised (4 µl 1 M HCl, 10 µl 0.5 M Tris-HCl (pH 8.0), 5 µl 2% Triton X-100 per digest) and then heated again at 95°C for 3 min (Floyd *et al.*, 2002). PCR amplification was performed on the SSU rDNA gene using 2–4 µl of the neutralised lysate, primer SSU_F_04 (sequence identical with G18S4) and SSU_R_26 (5'-CAT TCT TGG CAA ATG CTT TCG-3'). PCR conditions were: denaturation at 94°C for 5 min followed by 35 cycles of 94°C for 1 min, annealing at 55°C for 90 s and extension at 72°C for 2 min, followed by a final extension stage at 72°C for 10 min. PCR products were visualised by separation on a 1.5% agarose gel stained with 0.0002% ethidium bromide with 1 Kbp DNA ladder (Invitrogen™, Carlsbad, CA, USA) as size markers. Positive products were cleaned using exonuclease I (New England Biolabs®, Ipswich, MA, USA) and shrimp alkaline phosphatase (USB®, Cleveland, OH, USA) following the Wellcome Trust Sanger Institute's (<http://www.sanger.ac.uk/>) protocol and sequenced using the reverse primer SSU_R_09 (5'-AGC TGG AAT TAC CGC GGC TG-3'). Sequencing was performed in a 48-capillary ABI 3730, and assembled sequences were compared with published sequences in GenBank by means of BLAST search.

PREPARATION OF NEMATODES FOR SCANNING ELECTRON MICROSCOPY

Marine sediment samples were collected from the intertidal zone at Redondo Beach, CA, USA and Mazatlan, Sinaloa, Mexico. Nematodes were extracted from the marine sediments as described above, divided into two subsamples, one preserved in DESS solution while the other was fixed in 5% formalin. Preserved nematodes from Redondo Beach were left at room temperature for 24 h while those from the Mazatlan samples were left for 6 weeks before SEM preparation. Nematodes were rinsed with several changes of deionised water followed by 0.1 M phosphate buffer (pH 7.0), hand picked, and transferred to a

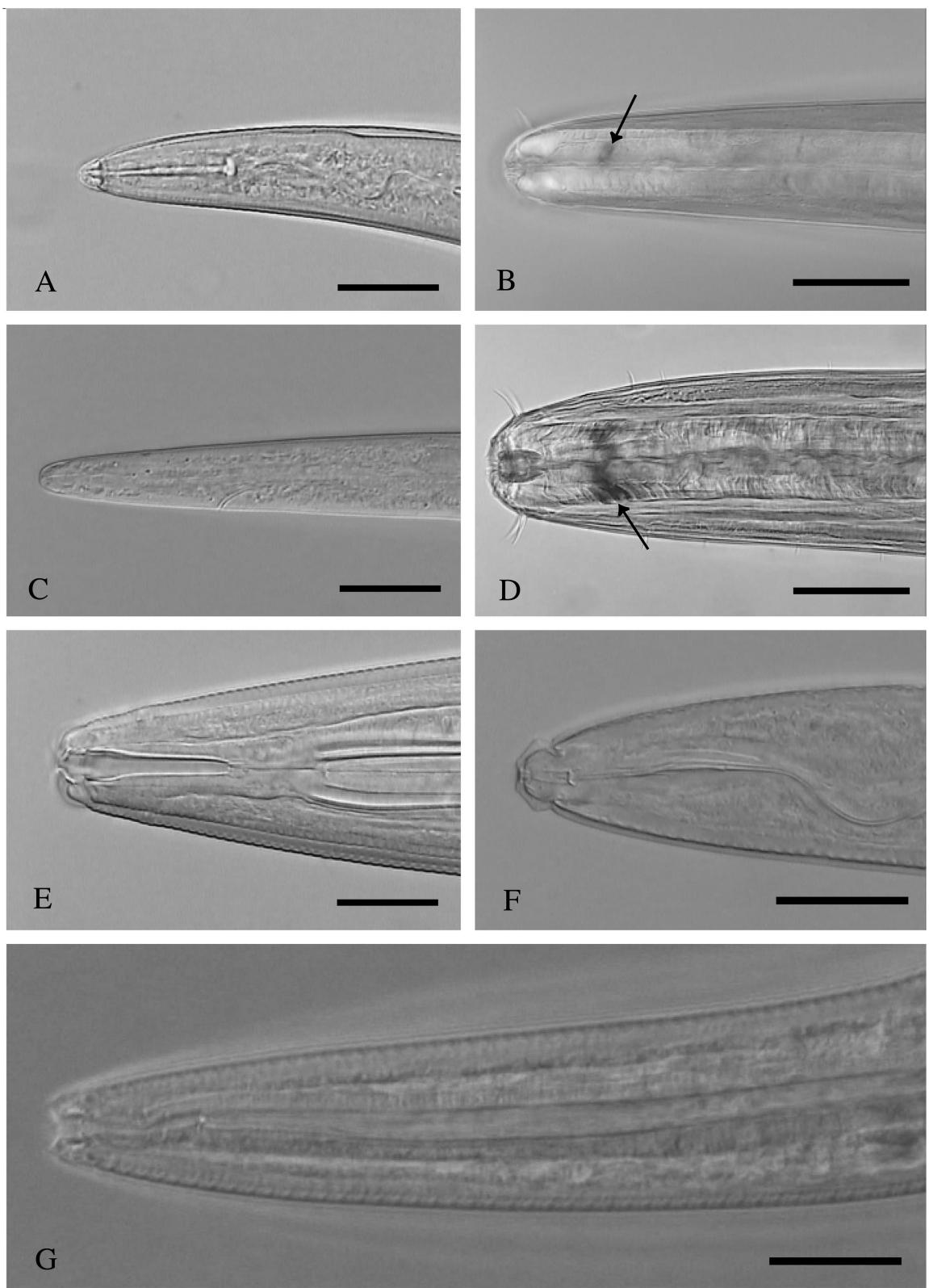
BEEM® capsule (Ted Pella, Redding, CA, USA). After post-fixation in 4% OsO₄, specimens were rinsed with three changes of cold (4°C) 0.1 M phosphate buffer (pH 7.2) within a 15 min period, dehydrated through a graded series of 100% absolute ethanol, and critical point dried using an Tousimis Autosamdry® -815 critical point drier. Dried nematodes were mounted on the surface of double-sided copper tape attached to aluminium stubs and sputter coated with a 25 nm layer of gold palladium in a Cressington 108 Auto. Specimens were observed using a Philips® XL30-FEG Scanning Electron Microscope (SEM) operated at 10 kV.

Results and discussion

All individuals video captured prior to PCR were identified to genus using the recorded multifocal images. Nematodes from 26 families consisting of 38 genera were isolated for VCE and PCR. Internal and external structures were usually well preserved in specimens left in DESS solution for periods between 3 days to 7 months (Fig. 1). Eyespot and muscle pigmentation in pharyngeal tissues of marine nematodes from kelp holdfasts were still visible after preservation in DESS solution (Fig. 1B, D, arrows). Samples that were bulk preserved in DESS solution and transported internationally also showed well-preserved morphology. Morphological preservation quality in glycerin mounts after 1 week was comparable overall to formalin-fixed specimens and, in the case of infective juveniles of *Steinerinema*, it was noticeably better, presumably due to the permeabilising properties of DMSO (Fig. 1C). After 5 months in glycerin mounts, specimens showed no signs of morphological deterioration and structures remained well preserved (Fig. 1G). Hence, DESS solution is a good short-term preservative for morphology.

Direct observation of specimens immediately after the addition of DESS solution revealed that nematodes became distorted due to collapse of the body, especially for species from freshwater environments. However, in the course of the next few minutes to hours, specimens

Fig. 1. Nematodes preserved in DESS solution used for VCE, PCR and sequencing. A: 7M5G5 – *Helicotylenchus* preserved in DESS solution for 7 weeks; B: 1117H5 – *Enoplus* with eyespot pigmentation still visible (arrow); C: 8M4H5 – *Steinerinema* juvenile preserved in DESS solution, transferred to glycerin, and mounted on a glass slide for 1 week; D: 2115H5 – *Enoplus* preserved in DESS solution, transferred to glycerin and mounted on a glass slide, showing pigmentation in pharyngeal tissues (arrow); E: 2M25G5 – *Plectus* transported back to the USA from Moldova via air; F: 13M17H5 – *Leptonchus* extracted by Ludox® TM-50 centrifugation; G: 19M9A6 – *Eucephalobus* preserved in DESS solution, transferred to glycerin, and mounted on a glass slide for 5 months, captured on a Sony™ Handycam Digital HD Video Camera Recorder. (Scale bars: A, C, E-G = 10 µm; B, D = 30 µm.)



slowly reinflated and eventually resumed their original turgor and appearance. Presumably this phenomenon is caused by the severe initial osmotic shock, followed by a slower return to osmotic balance under the permeabilising effects of the DMSO in the solution. Dimethyl sulphoxide is used to permeate the tissues and membranes while transporting non-ionised molecules of low molecular weight into tissues, membranes, and, eventually, the cells of an organism (Jacobs, 1971). Therefore, the disodium EDTA and sodium chloride in the DESS solution are transported quickly into the cells of the specimen with the help of DMSO, inactivating enzymes that degrade DNA.

DNA amplification and sequencing of individuals was successful with no need to change existing PCR protocols. Amplification of DNA fragments from 800 bp to 1800 bp was 80% successful and sequencing success from these amplicons was greater than 90% for all nematodes preserved in DESS solution for 3 days to 7 months. DNA amplification from D2D3 and 18S produced bright to moderate bands for all terrestrial and marine nematodes preserved in DESS solution. Samples transported internationally, samples bulk preserved in DESS solution, and samples transferred to glycerin for permanent mounts showed similar results for DNA amplification (Fig. 2) and sequencing as those above. Individuals used for PCR and sequencing from permanent mounts 5 months after mounting also showed bright to moderate bands for both D2D3 and 18S products. Average sequence read lengths for D2D3 and 18S PCR products were 750 bp and 1730 bp, respectively, which is comparable to sequences obtained from freshly lysed nematodes. Preserved samples used for DNA amplification and sequencing in Edinburgh yielded similar results with 73% and 97% success, respectively. Amplification produced bright to moderate bands *ca* 900 bp long and sequencing reads extended to the end of the amplified product (*ca* 500 bp). These results differ in several respects from those obtained from samples preserved in ethanol or formalin. Holzmann and Pawlowski (1996) concluded that 70% ethanol preserves DNA fragments up to 1300 bp, but amplification produced only weak bands. In our own experience, ethanol-preserved material performs quite inconsistently, perhaps due to problems with impurities or vapour substitution with air humidity. DNA amplification from formalin-preserved material is no better, resulting in DNA amplification of fragments 400 bp or less (Thomas *et al.*, 1997; Dorris *et al.*, 2002; Schander & Halanych, 2003; Roubtsova *et al.*, 2005). Formalin-preserved DNA is also prone to nucleotide substitutions and prob-

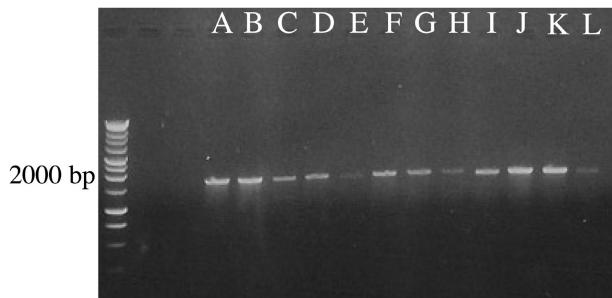


Fig. 2. SSU PCR products for nematodes preserved in DESS solution. A-E, G-J: Nematodes preserved in DESS solution, transferred to glycerin, and mounted on glass slides for 1 week. A: 1M4H5 – Steinernema juvenile; B: 2M4H5 – Steinernema juvenile; C: 3M4H5 – Steinernema juvenile; D: 4M4H5 – Steinernema juvenile; E: 5M4H5 – Aphelenchoïdes female; G: 6M4H5 – Steinernema juvenile; H: 7M4H5 – Steinernema juvenile; I: 8M4H5 – Steinernema juvenile; J: 9M4H5 – Aphelenchoïdes female. F, K-L: Preserved nematodes shipped from the Blaxter laboratory in Edinburgh, UK. F: 13M4H5 – Tylenchidae male; K: 11M4H5 – Plectus female; L: 12M4H5 – Plectus female.

lems with primer annealing caused by denaturing double-stranded DNA due to the use of hot formalin (Schander & Halanych, 2003). DMSO is known to perturb protein structure (Rammler, 1971) and can inhibit *Taq* polymerase activity during PCR (Gelfland, 1989). However, there is no evidence to suggest that nucleotide substitutions occur due to preservation in DESS solution. It is recommended that specimens preserved in DESS solution be washed thoroughly with purified deionised water before being used for DNA amplification and sequencing to avoid excess salts and residual DMSO in a PCR reaction. Consequently, the DESS solution is a better DNA preservative because it combines consistent PCR performance with the ability to amplify and sequence high molecular weight DNA.

DESS can also be used to preserve parasitic nematodes *in situ* inside hosts. From a single polydesmid millipede, 28 individual gut-parasitic nematodes were isolated, ranging from *ca* 0.5 mm to 4 mm in length. The specimens retained internal and external morphological characters (Fig. 3), and Dr David Hunt of CABI examined the digital images obtained. Notably, even fine details of decoration on the impressive spines (Fig. 3E) and other cuticular decoration of the specimens were visible. Five putative morphospecies were identified, belonging to the Hethidae, Rhigonematidae and Carnoyidae, all part of the Rhigonematomorpha (*sensu* De Ley & Blaxter, 2002). Rhigonematomorphs are a clade of nematodes related to

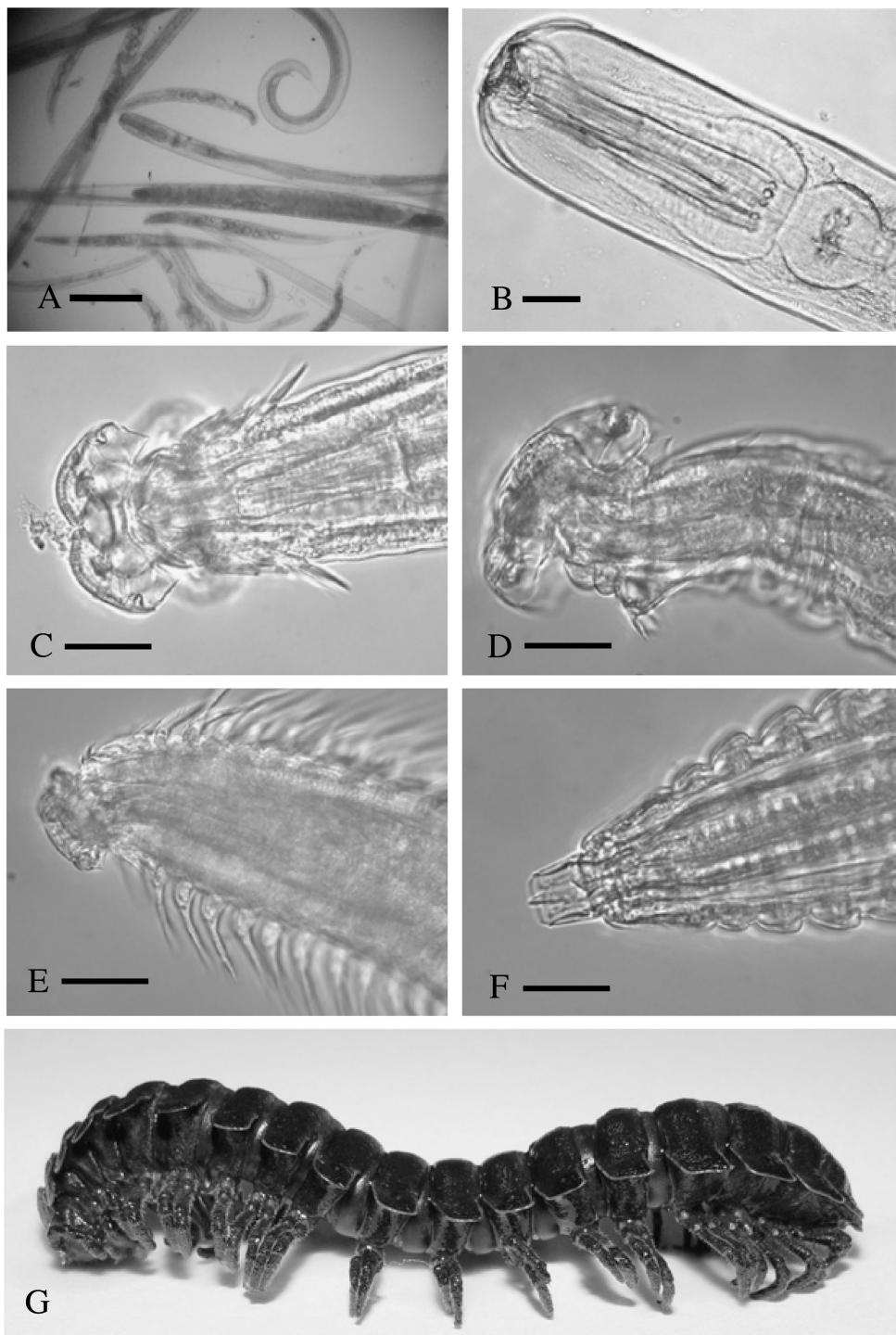


Fig. 3. Nematodes recovered from Peruvian millipede preserved in DESS solution. A: Low magnification digital image of nematodes extracted directly from gut of preserved millipede; B: Anterior end of *Rhigonema* sp.; C: Anterior end of female *Heth* sp. 1; D: Anterior end of female *Heth* sp. 1; E: Anterior end of female *Heth* sp. 2, showing longitudinal spine rows; F: Anterior end of male *Heth* sp. 2, showing anterior rows of cuticular tubercles; G: Peruvian millipede after preservation in DESS solution and before dissection. (Scale bars: A = 370 μm ; B = 50 μm ; C-F = 10 μm .)

the oxyurids, currently known only from the guts of large diplopods from tropical regions. PCR and sequencing of the specimens resulted in fungal rDNA sequences, presumably due to contamination with fungal amplicons that may derive from the millipede gut contents. It therefore remains unclear for the time being if DESS can be used to preserve and recover DNA of arthropod parasites inside host tissues. Presumably, better results can be obtained if the host is dissected immediately after killing, and the entire gut removed from the body and preserved separately in DESS. This would ensure much faster penetration of DESS through the gut and any nematodes inside it.

Scanning electron microscopy of nematodes preserved in both DESS solution and 5% formalin for 24 h show comparable results in terms of external morphology (Fig. 4). Slight differences in cuticular and amphidial condition were observed, most noticeably the lack of amphidial secretions in the DESS-preserved specimens allowing the fovea to be seen (Fig. 4D, F, arrowheads). When specimens were examined again 2 weeks after initial SEM preparation, collapse of structures was observed in some of the DESS-preserved nematodes (Fig. 4E). This is most likely due to the fact that DESS solution does not harden tissues as do fixatives such as formalin, thereby allowing structures to remain intact for long periods of time. Nematodes preserved in DESS or formalin for 6 weeks also showed comparable morphological results with some of the DESS-preserved specimens showing cuticular anomalies (Fig. 4F). Given the inconsistencies of SEM preparation, tests on a variety of parasitic and free-living nematodes from a wide range of terrestrial, freshwater, and marine habitats should be conducted and may give differing results. Also, the length of time exposed to DESS solution needs to be further examined in order to determine how long nematodes can remain in the solution and still be suitable for SEM.

Conclusions

DESS solution works quickly to inactivate enzymes that degrade DNA through the combined effects of a severe osmotic shock followed by rapid transportation of disodium EDTA and sodium chloride into tissues as enabled by DMSO. The solution preserves morphology with similar quality to formalin fixation and allows for a substantial reduction of chemical health hazards during the preservation and processing of samples. Although less volatile and less toxic than formalin, DESS can cause

mild skin irritations and its properties facilitate transportation of other substances into the body, including more hazardous toxins. Gloves are therefore a wise precaution when working with DMSO and DESS solution, especially in an environment where more dangerous chemicals are present.

Unlike ethanol, DESS solution is not flammable, allowing for the safe transportation of samples without requiring special precautions or permissions. Because of its high salt concentration, DESS spills are rather messy and it is recommended to place samples into plastic containers for transportation to avoid potential breakage (Seutin *et al.*, 1991). Safe and easy transportation of samples has become a major concern in recent years. The use of DESS solution greatly facilitates international shipment of important material while still allowing subsequent video recording and/or PCR analysis.

While it will take some years to establish the long-term effects on nematode morphology of preservation with DESS, we speculate that traditional refrigeration or freezing of specimens will substantially prolong the preservation of DNA compared to storage in DESS at room temperature. Because PCR amplifiable samples can be left in DESS at room temperature for months at a time, refrigerator and freezer space can be reserved for only the most important, temperature-sensitive material. Unlike fresh samples, DESS-preserved samples can be examined thoroughly and at length, without concern that interesting specimens will die before they can be picked out and prepared for PCR. Furthermore, DESS solution allows for the application of many traditional sample and specimen preparation methods for microscopy, in many cases retaining the ability to use these same nematodes for PCR. Based on this combination of features, we predict that during the next few years DESS solution will largely replace formalin for many nematological purposes, and especially where long-term preservation of morphology is not essential.

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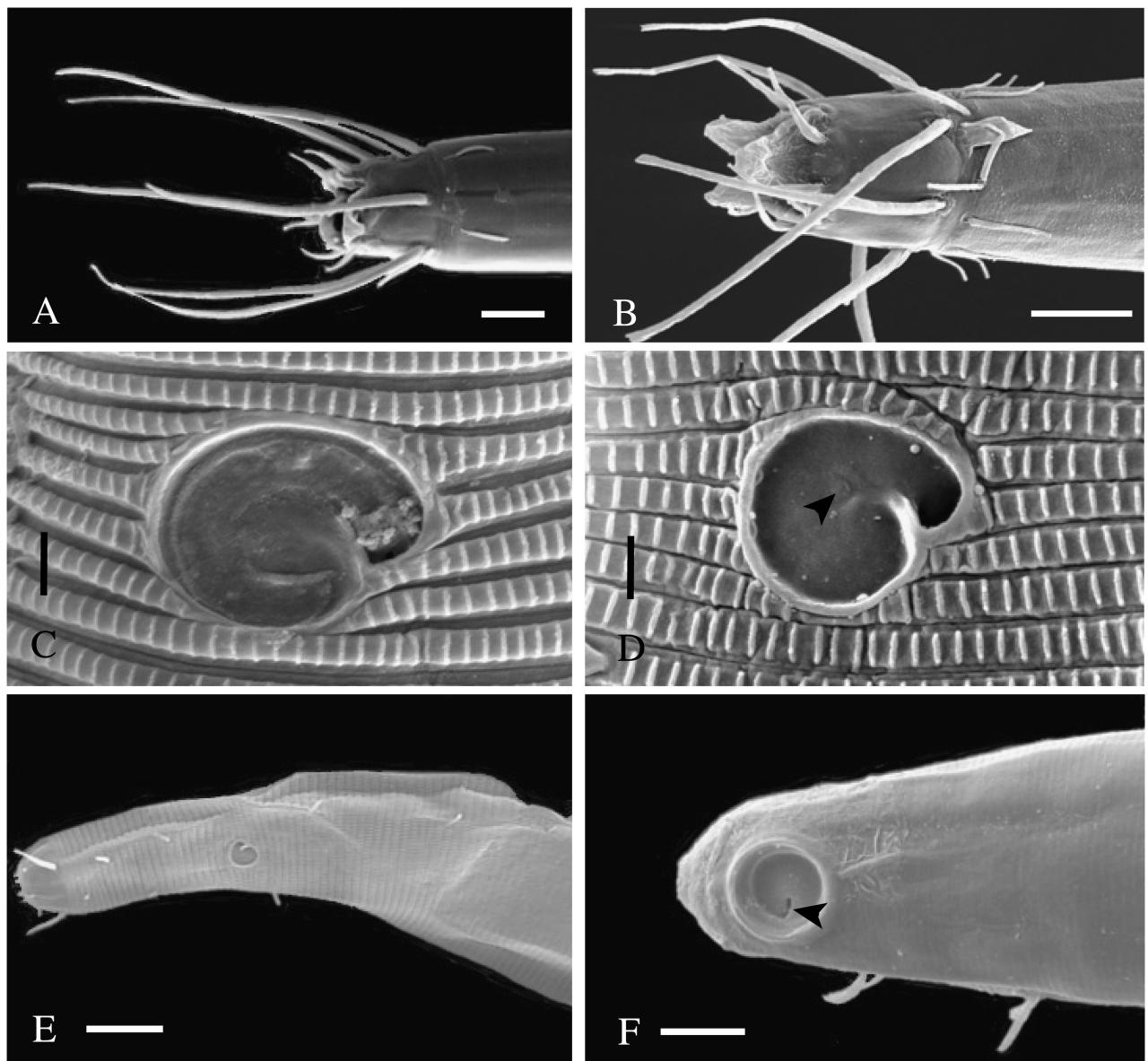


Fig. 4. Nematodes prepared for Scanning Electron Microscopy (SEM). A: Anterior end of *Enoplolaimus*, preserved in formalin for 24 h before SEM preparation; B: Anterior end of *Enoplolaimus*, preserved in DESS solution for 24 h before SEM preparation; C: *Calomicrolaimus*, amphid, preserved in formalin for 24 h prior to SEM preparation; D: *Calomicrolaimus*, amphid, preserved in DESS solution for 24 h prior to SEM preparation, showing the amphidial fovea (arrowhead); E: Anterior end of *Calomicrolaimus*, preserved in DESS solution for 24 h prior to SEM preparation and 2 weeks after being prepared for SEM; F: Anterior end of *Terschellingia*, preserved in DESS solution for 6 weeks before being prepared for SEM, showing amphidial fovea (arrowhead). (Scale bars: A, E = 10 µm; B, F = 5 µm; C, D = 1 µm.)

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