

HANDLING TECHNIQUES FOR SOIL INVERTEBRATES

Valerie M. Behan-Pelletier
Biosystematics Research Institute
Agriculture Canada
Ottawa K1A 0C6

While sampling soil and litter for oribatid mites, I often encounter representatives of other groups of soil invertebrates for which I am unsure of the correct preservation method. This prompted me to assemble the following tables on handling techniques for soil invertebrates, other than Pterygota (which are dealt with in Martin (1977)), and materials for slide preparation. I must stress that these are generalized tables; every specialist will have many modifications, and techniques, especially those of slide mounting, are constantly improving. I welcome comments on new, improved or more correct techniques for any group.

My thanks to invertebrate zoologists at the National Museum of Natural Sciences in Ottawa for their helpful comments.

HANDLING TECHNIQUES FOR SOIL INVERTEBRATES

GROUP	KILLING SOLN.	STORING/MOUNTING	COMMENTS	REFERENCES
PROTOZOA		Consult textbooks for mounting and staining techniques	Examine while alive. Add 2% methyl cellulose to slow movement of ciliates. 0.01% nickel sulphate is anaesthetic for ciliates.	Mackinnon and Hawes 1961; Sleigh 1973
ROTIFERA		Glycerine jelly mounts	Examine and identify while alive in drop of H ₂ O. Maintain coverslip at desired distance with band of vaseline. If necessary slow movement of rotifers with strychnine <u>or</u> pronephrine <u>or</u> 2% soln. of benzamine.	Donner 1966
GASTROTRICHA				
NEMATODA	Hot water (slowly heated) <u>or</u> hot 2% formalin <u>or</u> hot water and formalin combined (Formalin or H ₂ O must be hot enough or nematodes will not die)	2-4% formalin	Slide mount in glycerine. Ring with Zut. A trace of copper sulphate or thymol may be added to glycerine to prevent growth of molds.	Bird 1971; Ayoub 1980
NEMATOMORPHA	Hot water <u>or</u> 75% ETOH	2-4% formalin <u>or</u> 85% ETOH <u>or</u> 2-4% formalin for 24-48 hours, then 75% ETOH		Pimentel 1967

GROUP	KILLING SOLN.	STORING/MOUNTING	COMMENTS	REFERENCES
ENCHYTRAEIDAE	10% formalin	75% ETOH <u>or</u> 10% formalin	Examine and identify while alive in drop of water on slide. Maintain coverslip at desired distance with vaseline band.	
LUMBRICIDAE	Kill by dipping in Bouin's for 1-20 secs. Fix in FAA in V-shaped container. (10% formalin can be used)	3-5% formalin for 24-48 hours (to harden), then 70-80% ETOH	Narcotize with 1 part sat. MgSO ₄ to 4 parts H ₂ O or <u>slowly</u> with ETOH until relaxed, then kill by dipping into Bouin's for 1-20 secs (depending on size).	Fender 1982
MOLLUSCA - SLUGS	Narcotize and drown in water (10x vol./ spec.) with 1-2 drops nembutal added slowly (time 1-48 hrs). <u>or</u> drown in jar of deoxygenated water allowing no air space.	70-75% ETOH for relaxed, killed and fixd specimens	Fix in 4% formalin for 2-48 hrs., when animal is fully relaxed.	Hubricht 1951, Runham and Hunter 1970
MOLLUSCA - SNAILS	Submerge in boiling water for approx. 2 mins., cool rapidly for approx. 2 mins. Extract body from shell, fix and preserve	70-75% ETOH	Usually only shell needed for identification. If snail is <u>very</u> small allow body to desiccate in shell.	Pimentel 1967

GROUP	KILLING SOLN.	STORING/MOUNTING	COMMENTS	REFERENCES
CRUSTACEA	70-75% ETOH	70-75% ETOH		Sutton 1972
MILLIPEDES	Ethylene glycol <u>or</u> 75% ETOH	75% ETOH	For identification may have to mount gonopods: clear in lactic acid or lactophenol; mount in André's medium or P.V.A. and ring.	Demange 1981
CENTIPEDES	Ethylene glycol <u>or</u> 75% ETOH	75% ETOH	For identification may have to mount mouthparts: clear in 10% KOH; mount in André's medium or P.V.A. and ring.	Demange 1981
PAUROPODA SYMPHYLA	75-90% ETOH	75-90% ETOH	For identification will need to mount; clear in lactophenol; mount in Swan's <u>or</u> Gisin's medium <u>or</u> polyvinyl lactophenol and ring.	Demange 1981
TARDIGRADA	75% ETOH	Slide-mount. (Substrate with specimens may be air dried. Tardigrades will subsequently revive when placed in H ₂ O).	Examine while alive; can use 10% MgCl ₂ as anaesthetic. Mount in glycerine-gum arabic.	Morgan and King 1976

GROUP	KILLING SOLN.	STORING/MOUNTING	COMMENTS	REFERENCES
PROTURA	95% ETOH	95% ETOH or slide mount.	Slide mounts are necessary for identification. Clear in Essig's Aphid Fluid. Mount in Swan's or Gisin's Medium or polyvinyl lactophenol or Hoyers and ring.	Nosek 1973
DIPLURA	75% ETOH	75% ETOH	If it is necessary to slide mount for identification - follow procedures as for Protura.	Nosek 1973
THYSANURA	75% ETOH	75% ETOH		
COLLEMBOLA	75-80% ETOH	75% ETOH or slide mount	Heat freshly killed specimens in 75-80% ETOH on bath to ensure specimens sink in alcohol and become fixed. Temporarily mount in cavity slide in lactic acid. Clear in Nesbitt's soln. or warm lactic acid or 10% KOH. Mount in Gisin's or Hoyer's medium and ring.	Christensen and Bellinger 1980, 1981 Rusek 1974, 1975

GROUP	KILLING SOLN.	STORING/MOUNTING	COMMENTS	REFERENCES
PSEUDO- SCORPIONS SPIDERS	70-75% ETOH	70-75% ETOH	5% glycerine may be added to the alcohol.	Weygoldt 1969
ACARI- ORIBATEI	75% ETOH	75-80% ETOH or slide mount.	Temporarily mount in cavity slide in lactic acid for identification.	Krantz 1978
ACARI-WATER MITES	75% ETOH <u>or</u> Koenike's fluid	Koenike's Fluid	Slide mount in glycerine jelly	Krantz 1978
ACARI (IN GENERAL)	75% ETOH	75% ETOH or slide mount.	Specimens may need clearing prior to mounting on slides. Clear in lactophenol <u>or</u> André's fluid <u>or</u> Vitzhum's fluid <u>or</u> Nesbitt's fluid. Mount in modified Berlese medium e.g., Hoyer's <u>or</u> Fauré's <u>or</u> King, Bradley and McNeel and ring.	Krantz 1978

MATERIALS FOR SLIDE PREPARATION

CLEARING AGENTS

Alkalis: 5% to 15% KOH or NaOH
Acids: 60% to 85% lactic acid

Lactophenol: 85% lactic acid - phenol crystals
- dist. H₂O; 50: 25: 25.

Essig's Aphid Fluid: 85% lactic acid - phenol (sat H₂O) -
glacial acetic acid - dist. H₂O;
20:2:4:1. (Heat at 56-60°C for
30-60 mins.). (Store in BROWN BOTTLE)

Nesbitt's Soln.: chloral hydrate 40 gms. + dist. H₂O;
25 ml + conc. HCl 2.5 ml.

Andrés Fluid: Dist H₂O - chloral hydrate -
glacial acetic acid; 3:4:3

Vitzhum's Fluid: chloral hydrate - phenol - dist. H₂O;
10:9:1. (Store in BROWN BOTTLE)

STAINING AGENTS

Collembola and Protura: Aqueous or ethanol soln. of chlorazol black E.

RINGING AGENT

- Glyptal No. G1202 Red Enamel Insulating Paint (General Electric) - BEST
- nail varnish
- Zut

MOUNTANTS

André's Medium: Dist. H₂O - 50 cc, chloral hydrate - 200 g,
glycerine - 20 g, gum arabic - 30 g.

P.V.A.: polyvinyl alc. (sat. soln. in H₂O) - 3 ml,
phenol - 1 ml, lactic acid - 1 ml.

Swan's Medium: Dist. H₂O - chloral hydrate - gum arabic -
glucose² - lactic acid; 20:60:15:3:2

Gisin's Medium: lactic acid - glycerol - glycerol (sat. with
solid picric acid) - 4% formaldehyde;
179:36:28:7

- Polyvinyl Lactophenol: (Lipowski) 15% sat. soln. of polyvinyl - phenol - lactic acid; 56: 22:22
- Hoyer's Medium: Dist. H₂O - 5 ml, gum arabic - 3 g, chloral hydrate - 20 g, glycerol - 2 ml.
- Fauré's Medium: gum arabic - 3 g, chloral hydrate - 5 g, glycerol - 2 ml.
- King, Bradley, McNeel: Dist. H₂O - 8 ml, gum arabic - 8 g, glacial acetic acid - 3 ml, chloral hydrate - 70 g, glycerol - 5 ml.
- Berlese Medium: Dist. H₂O - 4 ml, gum arabic - 3 g, glacial acetic acid - 0.1 ml, chloral hydrate - 32 g, glucose syrup - 2 ml.
- Glycerine Jelly: Infiltrate specimen in glycerol by putting in a bath of 50/50 glycerine and alcohol. Evaporate alcohol and mount specimen in liquified glycerine jelly. Allow jelly to solidify.

KILLING AGENTS AND FIXANTS

- Bouin's Picro-Formol: *Picric Acid, saturated aqueous soln. - commercial formalin - glacial acetic acid; 75:25:5.

*Picric acid is explosive when perfectly dry, but is safe when stored as a saturated solution.

- FAA (Lavdowsky's Fluid): Formalin - 95% ETOH - glacial acetic acid - H₂O; 10:50:1:40.

REFERENCES

- Ayoub, S.M. 1980. Plant Nematology. An Agricultural Training Aid. Nema Aid Publication, California. 195 pp.
- Bird, A.F. 1971. The structure of Nematodes. Academic Press, New York. 318 pp.
- Demange, J.M. 1981. Les milles-pattes. Myriapodes. Société Nouvelle des Editions Boubée. 284 pp.

- Donner, J. 1966. Rotifers. Frederick Warne & Co., Ltd., Lond. 80 pp.
- Fender, W.M. 1982. Notes on earthworm preservation.
- Hubricht, L. 1951. The preservation of slugs. Nautilus 64(3): 90-91.
- Krantz, G.W. 1978. A Manual of Acarology. Oregon State Univ., Corvallis, Oregon. 509 pp. 2nd Ed.
- Mackinnon, D.L. and R.S.J. Hawes. 1961. An Introduction to the study of Protozoa. University Press, Oxford.
- Martin, J.E.H. 1977. Collecting, Preparing and Preserving Insects, Mites, and Spiders. The Insects and Arachnids of Canada. Part I. Agriculture Canada 182 pp.
- Morgan, C.I. and P.E. King. 1976. British tardigrades, Tardigrada: keys and notes for the identification of the species. Synopsis of the British Fauna No. 9. Academic Press, London. 133 pp.
- Nosek, J. 1973. The European Protura. Muséum d'Histoire Naturelle, Genève. 345 pp.
- Pimentel, R.A. 1967. Invertebrate identification manual. Van Nostrand Reinhold Co., New York. 151 pp.
- Runham, N.W. and P.J. Hunter. 1970. Terrestrial slugs. Hutchinson Univ. Library, Lond. 185 pp.
- Rusek, J. 1974. Die Präparation von Kleininsekten. - Mikrokosmos 64(1): 10-12.
- Rusek, J. 1975. Ein Präparationstechnik für Springschwänze ähnliche Gliederfüsser. - Mikrokosmos 65(12): 378-380.
- Sleigh, M.A. 1973. The biology of Protozoa. Edward Arnold. 315 pp.
- Sutton, S.L. 1980. Woodlice. Ginn & Co., Ltd., London.
- Weygoldt, P. 1969. The Biology of Pseudoscorpions. (Harvard Books in Biology, 6). Harvard Univ. Press, Cambridge, Mass.

* * * * *

HANDLING TECHNIQUES FOR SOIL INVERTEBRATES

GROUP	KILLING SOLN.	STORING/MOUNTING	COMMENTS	REFERENCES
PROTOZOA		Consult textbooks for mounting and staining techniques	Examine while alive. Add 2% methyl cellulose to slow movement of ciliates. 0.01% nickel sulphate is anaesthetic for ciliates.	Mackinnon and Hawes 1961; Sleigh 1973
ROTIFERA		Glycerine jelly mounts	Examine and identify while alive in drop of H ₂ O. Maintain coverslip at desired distance with band of vaseline. If necessary slow movement of rotifers with strychnine <u>or</u> pronephrine <u>or</u> 2% soln. of benzamine.	Donner 1966
GASTROTRICHA				
NEMATODA	Hot water (slowly heated) <u>or</u> hot 2% formalin <u>or</u> hot water and formalin combined (Formalin or H ₂ O must be hot enough or nematodes will not die)	2-4% formalin	Slide mount in glycerine. Ring with Zut. A trace of copper sulphate or thymol may be added to glycerine to prevent growth of molds.	Bird 1971; Ayoub 1980
NEMATOMORPHA	Hot water <u>or</u> 75% ETOH	2-4% formalin <u>or</u> 85% ETOH <u>or</u> 2-4% formalin for 24-48 hours, then 75% ETOH		Pimentel 1967

GROUP	KILLING SOLN.	STORING/MOUNTING	COMMENTS	REFERENCES
ENCHYTRAEIDAE	10% formalin	75% ETOH <u>or</u> 10% formalin	Examine and identify while alive in drop of water on slide. Maintain coverslip at desired distance with vaseline band.	
LUMBRICIDAE	Kill by dipping in Bouin's for 1-20 secs. Fix in FAA in V-shaped container. (10% formalin can be used)	3-5% formalin for 24-48 hours (to harden), then 70-80% ETOH	Narcotize with 1 part sat. MgSO ₄ to 4 parts H ₂ O or <u>slowly</u> with ETOH until relaxed, then kill by dipping into Bouin's for 1-20 secs (depending on size).	Fender 1982
MOLLUSCA - SLUGS	Narcotize and drown in water (10x vol./ spec.) with 1-2 drops nembutal added slowly (time 1-48 hrs). <u>or</u> drown in jar of deoxygenated water allowing no air space.	70-75% ETOH for relaxed, killed and fixd specimens	Fix in 4% formalin for 2-48 hrs., when animal is fully relaxed.	Hubricht 1951, Runham and Hunter 1970
MOLLUSCA - SNAILS	Submerge in boiling water for approx. 2 mins., cool rapidly for approx. 2 mins. Extract body from shell, fix and preserve	70-75% ETOH	Usually only shell needed for identification. If snail is <u>very</u> small allow body to desiccate in shell.	Pimentel 1967

GROUP	KILLING SOLN.	STORING/MOUNTING	COMMENTS	REFERENCES
CRUSTACEA	70-75% ETOH	70-75% ETOH		Sutton 1972
MILLIPEDES	Ethylene glycol <u>or</u> 75% ETOH	75% ETOH	For identification may have to mount gonopods: clear in lactic acid or lactophenol; mount in André's medium or P.V.A. and ring.	Demange 1981
CENTIPEDES	Ethylene glycol <u>or</u> 75% ETOH	75% ETOH	For identification may have to mount mouthparts: clear in 10% KOH; mount in André's medium or P.V.A. and ring.	Demange 1981
PAUROPODA SYMPHYLA	75-90% ETOH	75-90% ETOH	For identification will need to mount; clear in lactophenol; mount in Swan's <u>or</u> Gisin's medium <u>or</u> polyvinyl lactophenol and ring.	Demange 1981
TARDIGRADA	75% ETOH	Slide-mount. (Substrate with specimens may be air dried. Tardigrades will subsequently revive when placed in H ₂ O).	Examine while alive; can use 10% MgCl ₂ as anaesthetic. Mount in glycerine-gum arabic.	Morgan and King 1976

GROUP	KILLING SOLN.	STORING/MOUNTING	COMMENTS	REFERENCES
PROTURA	95% ETOH	95% ETOH or slide mount.	Slide mounts are necessary for identification. Clear in Essig's Aphid Fluid. Mount in Swan's or Gisin's Medium or polyvinyl lactophenol or Hoyers and ring.	Nosek 1973
DIPLURA	75% ETOH	75% ETOH	If it is necessary to slide mount for identification - follow procedures as for Protura.	Nosek 1973
THYSANURA	75% ETOH	75% ETOH		
COLLEMBOLA	75-80% ETOH	75% ETOH or slide mount	Heat freshly killed specimens in 75-80% ETOH on bath to ensure specimens sink in alcohol and become fixed. Temporarily mount in cavity slide in lactic acid. Clear in Nesbitt's soln. or warm lactic acid or 10% KOH. Mount in Gisin's or Hoyer's medium and ring.	Christensen and Bellinger 1980, 1981 Rusek 1974, 1975

GROUP	KILLING SOLN.	STORING/MOUNTING	COMMENTS	REFERENCES
PSEUDO- SCORPIONS SPIDERS	70-75% ETOH	70-75% ETOH	5% glycerine may be added to the alcohol.	Weygoldt 1969
ACARI- ORIBATEI	75% ETOH	75-80% ETOH or slide mount.	Temporarily mount in cavity slide in lactic acid for identification.	Krantz 1978
ACARI-WATER MITES	75% ETOH <u>or</u> Koenike's fluid	Koenike's Fluid	Slide mount in glycerine jelly	Krantz 1978
ACARI (IN GENERAL)	75% ETOH	75% ETOH or slide mount.	Specimens may need clearing prior to mounting on slides. Clear in lactophenol <u>or</u> André's fluid <u>or</u> Vitzhum's fluid <u>or</u> Nesbitt's fluid. Mount in modified Berlese medium e.g., Hoyer's <u>or</u> Fauré's <u>or</u> King, Bradley and McNeel and ring.	Krantz 1978

MATERIALS FOR SLIDE PREPARATION

CLEARING AGENTS

Alkalis: 5% to 15% KOH or NaOH
Acids: 60% to 85% lactic acid

Lactophenol: 85% lactic acid - phenol crystals
- dist. H₂O; 50: 25: 25.

Essig's Aphid Fluid: 85% lactic acid - phenol (sat H₂O) -
glacial acetic acid - dist. H₂O;
20:2:4:1. (Heat at 56-60°C for
30-60 mins.). (Store in BROWN BOTTLE)

Nesbitt's Soln.: chloral hydrate 40 gms. + dist. H₂O;
25 ml + conc. HCl 2.5 ml.

Andrés Fluid: Dist H₂O - chloral hydrate -
glacial acetic acid; 3:4:3

Vitzhum's Fluid: chloral hydrate - phenol - dist. H₂O;
10:9:1. (Store in BROWN BOTTLE)

STAINING AGENTS

Collembola and Protura: Aqueous or ethanol soln. of chlorazol black E.

RINGING AGENT

- Glyptal No. G1202 Red Enamel Insulating Paint (General Electric) - BEST
- nail varnish
- Zut

MOUNTANTS

André's Medium: Dist. H₂O - 50 cc, chloral hydrate - 200 g,
glycerine - 20 g, gum arabic - 30 g.

P.V.A.: polyvinyl alc. (sat. soln. in H₂O) - 3 ml,
phenol - 1 ml, lactic acid - 1 ml.

Swan's Medium: Dist. H₂O - chloral hydrate - gum arabic -
glucose₂ - lactic acid; 20:60:15:3:2

Gisin's Medium: lactic acid - glycerol - glycerol (sat. with
solid picric acid) - 4% formaldehyde;
179:36:28:7

- Polyvinyl Lactophenol: (Lipowski) 15% sat. soln. of polyvinyl - phenol - lactic acid; 56: 22:22
- Hoyer's Medium: Dist. H₂O - 5 ml, gum arabic - 3 g, chloral hydrate - 20 g, glycerol - 2 ml.
- Fauré's Medium: gum arabic - 3 g, chloral hydrate - 5 g, glycerol - 2 ml.
- King, Bradley, McNeel: Dist. H₂O - 8 ml, gum arabic - 8 g, glacial acetic acid - 3 ml, chloral hydrate - 70 g, glycerol - 5 ml.
- Berlese Medium: Dist. H₂O - 4 ml, gum arabic - 3 g, glacial acetic acid - 0.1 ml, chloral hydrate - 32 g, glucose syrup - 2 ml.
- Glycerine Jelly: Infiltrate specimen in glycerol by putting in a bath of 50/50 glycerine and alcohol. Evaporate alcohol and mount specimen in liquified glycerine jelly. Allow jelly to solidify.

KILLING AGENTS AND FIXANTS

- Bouin's Picro-Formol: *Picric Acid, saturated aqueous soln. - commercial formalin - glacial acetic acid; 75:25:5.

*Picric acid is explosive when perfectly dry, but is safe when stored as a saturated solution.

- FAA (Lavdowsky's Fluid): Formalin - 95% ETOH - glacial acetic acid - H₂O; 10:50:1:40.

REFERENCES

- Ayoub, S.M. 1980. Plant Nematology. An Agricultural Training Aid. Nema Aid Publication, California. 195 pp.
- Bird, A.F. 1971. The structure of Nematodes. Academic Press, New York. 318 pp.
- Demange, J.M. 1981. Les milles-pattes. Myriapodes. Société Nouvelle des Editions Boubée. 284 pp.

- Donner, J. 1966. Rotifers. Frederick Warne & Co., Ltd., Lond. 80 pp.
- Fender, W.M. 1982. Notes on earthworm preservation.
- Hubricht, L. 1951. The preservation of slugs. Nautilus 64(3): 90-91.
- Krantz, G.W. 1978. A Manual of Acarology. Oregon State Univ., Corvallis, Oregon. 509 pp. 2nd Ed.
- Mackinnon, D.L. and R.S.J. Hawes. 1961. An Introduction to the study of Protozoa. University Press, Oxford.
- Martin, J.E.H. 1977. Collecting, Preparing and Preserving Insects, Mites, and Spiders. The Insects and Arachnids of Canada. Part I. Agriculture Canada 182 pp.
- Morgan, C.I. and P.E. King. 1976. British tardigrades, Tardigrada: keys and notes for the identification of the species. Synopsis of the British Fauna No. 9. Academic Press, London. 133 pp.
- Nosek, J. 1973. The European Protura. Muséum d'Histoire Naturelle, Genève. 345 pp.
- Pimentel, R.A. 1967. Invertebrate identification manual. Van Nostrand Reinhold Co., New York. 151 pp.
- Runham, N.W. and P.J. Hunter. 1970. Terrestrial slugs. Hutchinson Univ. Library, Lond. 185 pp.
- Rusek, J. 1974. Die Präparation von Kleininsekten. - Mikrokosmos 64(1): 10-12.
- Rusek, J. 1975. Ein Präparationstechnik für Springschwänze ähnliche Gliederfüsser. - Mikrokosmos 65(12): 378-380.
- Sleigh, M.A. 1973. The biology of Protozoa. Edward Arnold. 315 pp.
- Sutton, S.L. 1980. Woodlice. Ginn & Co., Ltd., London.
- Weygoldt, P. 1969. The Biology of Pseudoscorpions. (Harvard Books in Biology, 6). Harvard Univ. Press, Cambridge, Mass.

* * * * *