SPECIMEN PREPARATION

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INTRODUCTION

- Organisms
- Tools
- Chemicals
- Safety & Health
- References, Sources, & Links
- Preparation of specimens
- Specimen storages
- Preparation & Mounting Techniques

ORGANISMS


Acarina
Tarsonemidae
Tetranychidae
Others
Homoptera
Aleyrodidae
Aphididae
Coccoidea
Coccoidea
Diaspididae
Pseudococcidae
Others
Thrips
Thripidae
Phlaeothrips
nymphs

TOOLS

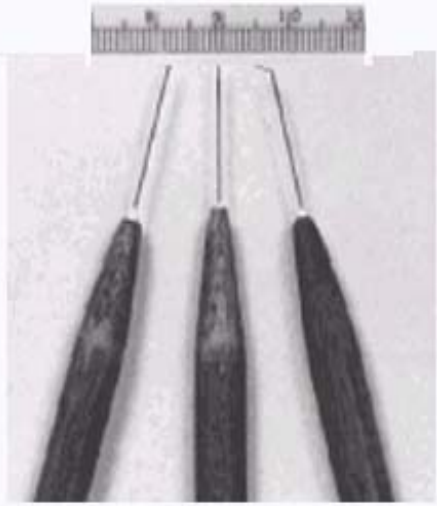
1. Wilkey micro tool set (from Bioquip)
2. slide warmer
3. hot Plate
4. spot plates or small specimen dishes or stender dishes (2 mm diameter)
5. crucibles
6. microscope slides & 18 mm cover slips
7. Funnel
8. Beaker

Tools for Slide Mounting
(Reference: Phil Johnson, SFO)

- Tools/Equipment:
  - Wilkey's Microtools (I also find you can make your own to suit your techniques/preferences out of insect pins and small wood dowels).
  - Small watch glasses
  - Coor's color plates (for dessication in alcohol/clove oil and for staining)
  - Coor's porcelain crucibles (for KOH)
  - Old 2 dram vials with caps (vinyl seal removed) for boiling mealybugs or placing specimens in cold KOH.
  - Alcohol lamp (for boiling specimens)
  - Hot plate (for heating specimens in KOH solution)
  - Test tube holder (for holding vials over alcohol flame)
  - Essig's, double stain, clove oil, alcohol.
  - Stereo zoom microscope (and older one you don't mind using for dissections).
  - Fume hood (a MUST).
  - Convection oven (for curing slides) or a slide warmer.

Microtools

Wilkey Microslide Tool Set



Robert Wilkey "invented" the microtool set that is needed to prepare microarthropods for mounting on slides. He used the tools to provide more than 100,000 (estimated) slides of Coccoidea organisms for USDA and CDFA. His slides are represented in collections around the world.

The tools consist of the following:

- a probe,
- an angled knife with a 1mm cutting blade, and
- a bent spatula with a 1 to 3 mm blade

The tools can be purchased from Bioquip at this url http://www.bioquip.com/singlepagepdfs/cat_page_22.pdf. It is catalogue # 4831.

Chemicals Required

1. AGA solution (Alcohol-Glycerin-Acetic Acid)
2. balsam and other Mounting media
3. Bleaching agent: Ammonia and Hydrogen Peroxide solution
4. Carbo-Histoclear (Histoclear with dissolved phenol)
5. Carbo-xylol (Xylene + 10% phenol)
6. chloral hydrate ***requires DEA permit
7. chloro-phenol
    - 50 grams chloral hydrate
    - 50 grams phenol
    - 5 ml water
8. clove oil
9. double stain
10. essig's aphid fluid
11. ETOH=ethanol (70 & 90%)
12. Histoclear (to thin Balsam) Manufactured by National Diagnostics, Atlanta GA 800-526-3867 (in a pinch citrus oil (orange or lemon) from a grocery store could be used)
13. hoyers medium (needs DEA permit for Chloral hydrate)
14. Maceration agent: Potassium or Sodium Hydroxide (10%)
15. Phenol
16. ROH=alcohol
17. Terpineol
18. Xylene

AGA SOLUTION

- ETOH — 8 parts by volume
- Water — 5 parts by volume
- Glycerin — 1 parts by volume
- Glacial acetic acid — 1 parts by volume

Mounting Media

- Balsam-permanent
  - Thin with Histoclear
- Euparal-permanent
  - Thin with Euparal Essence
- Hoyers-temporary
  - Needs DEA permit for Chlorohydrate
- Piccolyte
- Permount
- PVA-temporary

Hoyers medium

- chloral hydrate ** 20 parts by volume

****Needs DEA permit for Chloral hydrate**

- water 5 parts by volume
- gum Arabic granules 3 parts by volume
- glycerin 2 parts by volume

reference:

http://www.extento.hawaii.edu/kbase/resource/slide_resource.htm

Maceration Agents

- Maceration agents
  - KOH 14 pellets/50 ml
  - NaOH
- Bleaching agents
  - ammonia 1 part by volume
  - hydrogen peroxide 6 parts by Volume

Essig's Aphid Fluid

- lactic acid 20 parts by volume
- glacial acetic acid 4 parts by volume
- phenol 2 parts by volume
- water 1 part by volume

Nesbitt's Fluid
(for old mite specimens stored in ROH)

- Chloral hydrate** 40 grams
- Distilled Water 25 cc
- Concentrated hypochloric acid 2.5 cc

** Note this requires a DEA permit (controlled substance)

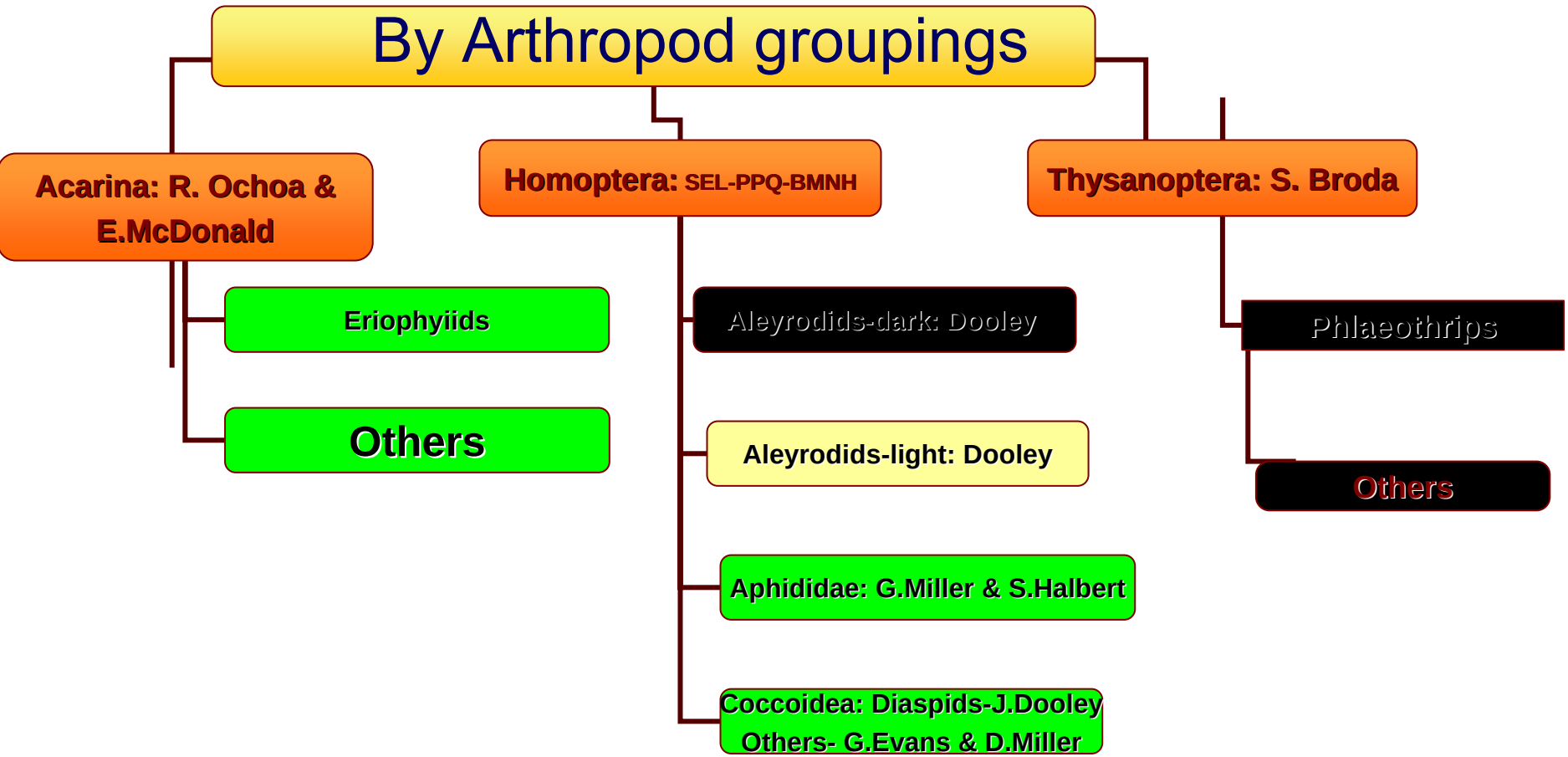
SAFETY & HEALTH

- Safety Precautions
  - Whenever heating specimens in vial or tube, always direct mouth of tube away from person and do not plug tube. Use appropriate tube holders.
  - Antidote for Chloro-phenol is plenty of ETOH as per Susan Halbert of DPI, Florida
- MSDS Sheets
- Many no longer use phenol, xylene, and other poisons because of health issues. Always consult the MSDS sheets before using chemicals.
- If using Chloral Hydrate, have a valid DEA permit on file.
- If using phenol or other dangerous chemicals, use a portable or fixed hood appropriate for the chemical.

Sources & Links

- Van Water Rogers: http://www.vwrsp.com/
- Bioquip (URL): http://www.bioquip.com
- COLLECTING AND PREPARING INSECTS AND MITES: TECHNIQUES AND TOOLS BY M. SCHAUFF (SEL NATIONAL MUSEUM NATURAL HISTORY):
- Collecting and Preserving Insects and Mites: Techniques and Tools
- Dr. Susan Halbert (DPI, Florida)
- Ray Gill (CDFA), retired
- Dr. Greg Evans (PPQ, Beltsville)
- Dr. Jon Martin (NHM, London)
- Eric McDonald (PPQ, Houston)
- Dr. Dug Miller (ARS, SEL)
- Dr. Ron Ochoa (ARS, SEL)
- Dr. Gary Miller (ARS, SEL)

VARIOUS SPECIMEN PREPARATION TECHNIQUES



PREPARATION OF ACARINA

Please refer to the

Acarina Powerpoint Presentation

Created by R. Ochoa, E. McDonald, & F. Salantri.

PREPARATION OF ACARINA

Mites other than Eriophyids.

- Source: Systematic Entomology Lab
  1. Submit specimen dried or in alcohol
  2. May clear heavily sclerotized mites or large ticks in lacto phenol or heat in KOH solution.
  3. Place a drop of Hoyers medium in center of slide.
  4. Use needle (wetted tip) to remove mite from tissue or spatula to remove from alcohol.
  5. Place single specimen in medium. Orient mite in the following way:
     - most dorsoventrally;
     - Acaridae & Tetranychidae laterally.
  6. Place cover slip on slide and heat on hotplate. Remove immediately when bubbling starts.
  7. Place in Oven at 45-50 degrees for 24 hours
  8. To prevent the slide from drying out, ring the slide with more hoyers medium after removing from oven.

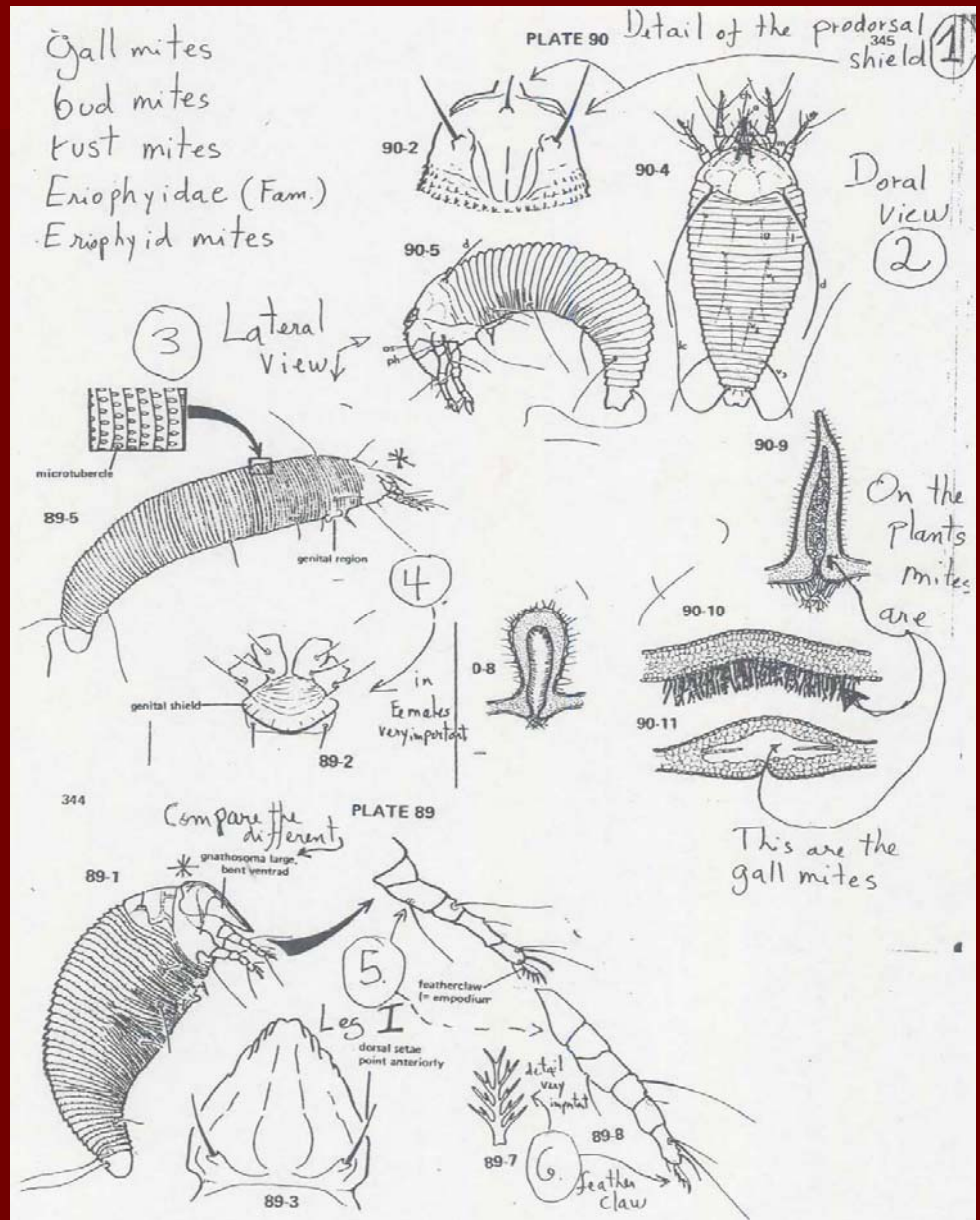
MOUNTING TECHNIQUES: MITES
(Reference-"Manual of Acaralogy" by Kranz (1971)

- Best Quick/Dirty technique is Hoyers.
  - If received in ROH, try to puncture lateral side with very small micro pin or cutter.
  - If time is no issue, place specimens in lacto-phenol and leave at room temperature 24-48 hours. Note that immersion more than 48 hours weakens the leg conjunctiva and shields of light colored mites.
  - If time is a factor, heat shortly in lacto-phenol.
  - Wash specimens 3 to 4 times in a porcelain spot plate until solution is clear.
  - Place a drop of hoyers on the center of the slide.
  - Transfer specimen with micro spatula to the drop of hoyers
  - Use a microprobe to tease the mite to the bottom of the drop (never leave on surface when applying the coverslip-it will roll to the edge).
  - Apply the cover slip.
  - heat (no more than 45^{o}C) from 4 to 7 days.
  - Hold the heat treated slides for one week to observe if the media bubbles or contracts. If so add more hoyers if the medium contracts.
  - Ring the slide with nonsoluable, such as zut or euparal. Apply a second coat when the slide is dried.

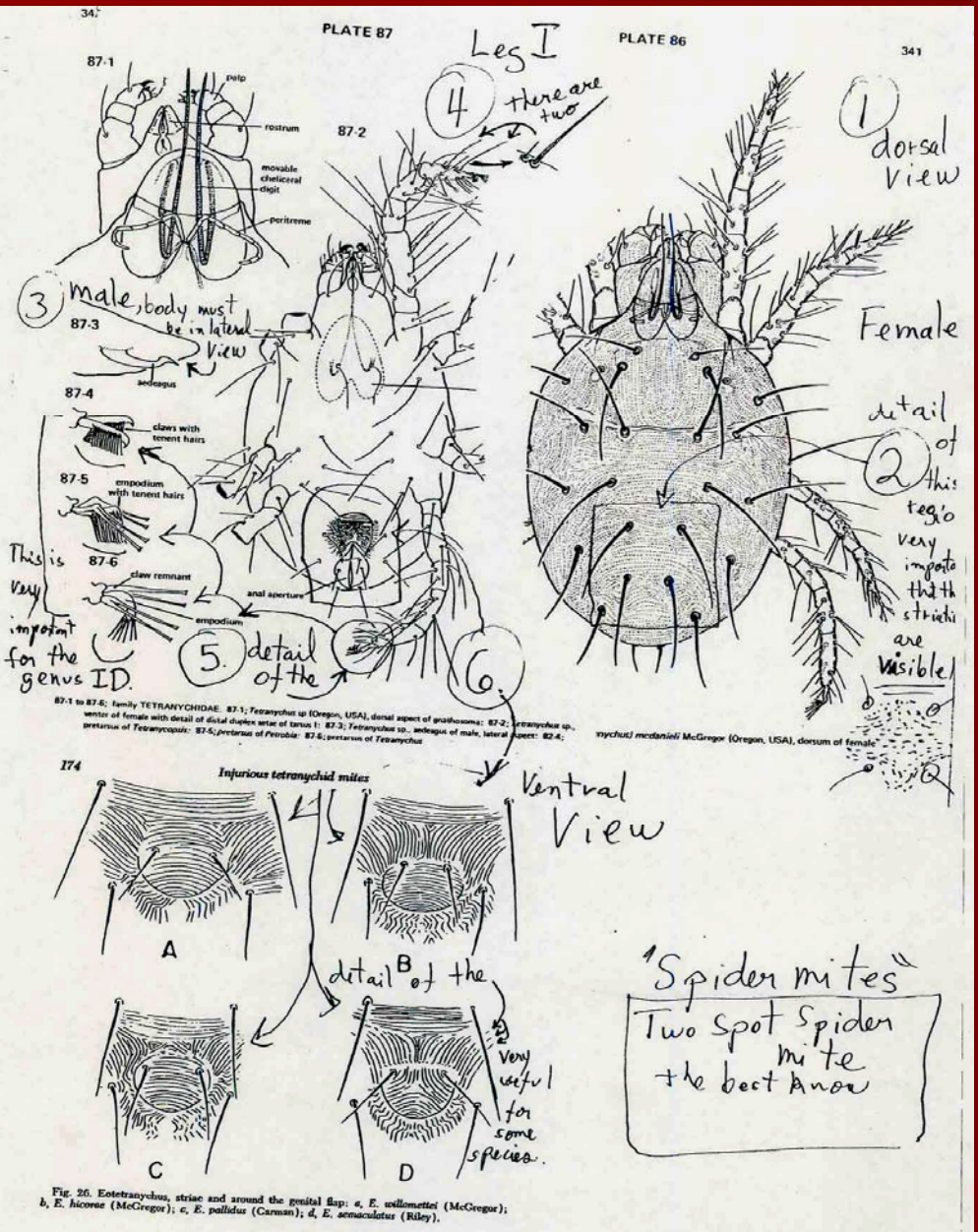
Mounting Procedures for Eriophyidae

- The drawing to the right was distributed by Dr. Ochoa. The following structure areas (numbered) are essential for ID determination:

1. prodorsal shield
2. dorsal View
3. lateral view
4. genital shield (importance with females
5. leg setae
6. Feather claw (detail very important)



Mounting Procedures for Tetranychids



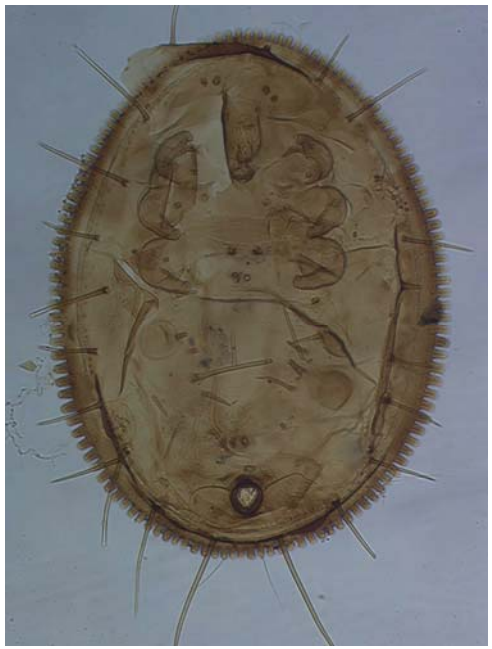
- The drawing to the right was distributed by Dr. Ochoa. The following structure areas (numbered) are essential for ID determination:

1. dorsal View
2. region must show striae- very important for ID
3. males must be mounted with a lateral view.
4. leg #1 must show setae
5. empodium area must be visible (detail very important)
6. Ventral anal aperture area must be visible

ALEYRODID PREPARATION (Pupae only) Tech Paper no. 38, June 1999 (CSIRO)

1. **Macerate body contents in tube or vial by warming pupa (~80 degrees C) in a 10% KOH for 5-10 minutes until contents become translucent.**
2. **Decant excess macerant.**
3. **"De-wax" the cuticle by gently warming specimens in any of the following media: carboxylol, carbo Histoclear, or Chloral-phenol.**
4. **Decant de-waxing fluid**
5. **Handling dark or pale puparia:**
    a) **Rinse dark puparia in strong alcohol and partially bleach cuticle cold in fresh mix of ammonia & hydrogen peroxide solution. Monitor the bleaching since it may be rapid. No need to stain. DO NOT USE HOUSEHOLD BLEACH.**
    b) **Stain pale puparia by adding acid fuchsin to solution of either glacial acetic acid, 100% isopropyl, or 95% ETOH.**
    c) **If the staining fails, excess wax still remains (repeat #3).**
6. **Decant bleach/stain and rinse in Glacial Acetic acid or 95% ETOH.**
7. **Complete final dehydration by soaking in glacial acetic acid, 100% isopropyl, or absolute ETOH.**
8. **Clear specimens by adding**
    a) **A few drops of clove oil or histoclear while using Balsam; or,**
    b) **Euparal essence while using Euparal.**
9. **Place specimen(s) in the mountant on a clean slide and orient both dorsally and ventrally if mounting several specimens per slide. Let the mountant partially dry; add fresh mountant to cover slip and lay cover slip on slide.**
10. **Adequately dry slides (balsam ay take up to two months). Recommend using only thick card labels, not paper labels.**

Dark Colored Aleyrodids-Correctly Bleached



- Note that the specimen needs to be light brown in color showing the legs, margin etc for Identification.

ALEYRODID PREPARATION (Adults & Pupae) per Ray Gill, CDFA.

- **Do not make incision as in other groups.**
- **Remove specimens dried with a pin or small spatula from 70% alcohol to a crucible or other container containing a10% KOH solution. Clear for several hours or overnight (preferred). NEVER HEAT SPECIMENS IN KOH!!!!!!!**
- **Transfer to alcohol to wash and neutralize KOH. May add a few drops of Essigs Aphid Fluid.**

If you need to make a quick slide (quick & dirty mount), transfer specimen to Hoyers on a slide from the ROH and heat from 1 to several hours on a slide warmer and then following "Mounting specimen…." section below. Be sure to ring slide with more hours.

- **Transfer to Essigs fluid (with stain for adults & light colored pupae) and heat at 50°C.**
  - **For adults with legs down/dorsum up.**
  - **Not necessary to submerge entire specimen.**
- **Transfer to Cellusolve and tease with fine point needle (0 or 00 insect pin).**
  - **Return to Essigs if necessary & reheat**
- **Place very thin, runny balsam on a slide (diluted with Xylene) to prevent the antennae & legs from collapsing.**
- **Mount specimens on a slide with a propped cover slip.**
  - **Props should be vinyl or short lengths of monofilament fishing leader (0.25 mm in diameter).**
  - **For adults orient the specimens either dorsum up or lateral.**
  - **For pupa orient the specimens both dorsal & ventral sides up.**
  - **Thicker balsam should be added before applying the cover slip**
- **Heat the slide on a slide warmer for several hours**
- **Label the slide appropriately**

Aphid Preparation as per Susan Halbert-DPI, Florida

Prepare and heat a water bath near but not boiling.
Make slide labels use pencil or indelible ink.
6 drops each terpineol & acetic acid in watch glass.
Poke a role in lateral abdominal area.

- **Heat specimen in 95% ethanol one minute or until boiling, whatever is first. Decant liquid from tube (not the aphid).**
- **Add 10% KOH (1/4th full) until aphid is translucent-do not boil to prevent forming bubbles. Do not leave overnight.**
- **Add 70% ETOH (3/4th full) and cap tube, invert to mix. Decant liquid.**
- **Cover aphid with chloro-phenol and heat 15-30 minutes until aphid is clear. Do not boil. May leave overnight but cold-not on heat.**
- **Pour into watch glass and gently pump abdomen to remove bubbles. If abdomen collapses, pumps thorax to reinflate it for better mount.**
- **Position aphid ventral side up with legs etc extended in a watch glass and add a 50-50 mixture of acetic acid and terpineol. Soak for 10 minutes. If legs collapse, leave in acid longer. Do not leave overnight.**
- **Reposition in pure terpineol right side up. Leave 10 minutes (overnight is OK).**
- **Place aphid on slide with balsam: orient right side up with legs, cornicles, antenna, and mouth parts (& wings) visible. Add cover slip and place on warmer for two weeks.**

Aphid Preparation as per Manya Stoetzl (SEL, USNM)

1. Place specimens in cold 10% solution of KOH (potassium hydroxide) for 24 to 48 hours to soften body contents. [Specimens should not be left in KOH any longer than is necessary to soften body contents because KOH destroys or lightens the natural body color that is frequently used in identifications.]
2. OR, Heat the KOH for 10-20 minutes (or until body contents have softened) on a hot plate set at 110 F. Make sure to cover specimen dish to prevent evaporation.
3. Using a sharp probe or needle, make a small incision on the side of the abdomen. If several specimens are being prepared, vary the location of the incision so that among the specimens there are some complete left and right abdominal margins.
4. With a small spatula or other tool, gently press down on abdomen and tease out body contents. If the body contents do not all come out after pumping, place specimens back onto hot plate for another 5 minutes or place into a solution of half dishwashing detergent-half distilled water for 3 minutes.
5. Pump specimens in distilled water and leave for 5 minutes.
6. If specimens are colorless, they can be stained with double stain. Leave in stain 5 minutes. If lighter stain is desired, mix stain with distilled water or 70% EtOH.
7. Pump in 70% EtOH and leave for 5-10 minutes.
8. Pump in 95% EtOH. If specimens are stained too dark, add water, a drop at a time, to remove some of the stain. Leave in 95% EtOH for a minimum of 5-10 minutes.Note: The presence of water in legs or antennae can cause them to collapse so make sure specimen is in EtOH long enough.
9. Transfer to clove oil for a minimum of 5 minutes. Gently pump specimens to remove excess EtOH. Specimens can be left overnight in clove oil if there is not time for mounting on a slide.
10. Place drop of thin Canada balsam in the center of a slide. [Thin balsam with histoclear instead of xylene.] Gently transfer specimens to slide. If specimens are small enough to allow for mounting 3-4 or more on a slide, mount at least one ventral side up. When mounting specimens, representatives of all stages (aptera, alate, alatoid nymph, smaller nymphs) should be included on each slide as space and material allows. Place specimens on slide head pointed down with younger stages to the left and older alate adults to the right. Do not mount aphid nymphs on slides without at least one adult.
11. Carefully place cover slip over balsam. Place slide on hot plate to spread balsam evenly.
12. Cure slides in a 110 F oven (40 C) for one month so the balsam is no longer soft. If no oven is available, slides can be left undisturbed for 2 months at room temperature.
13. Place a slide label to the left of the cover slip which contains the following information if available: (/ represents the end of a line)

Genus/ species Author/ Host/ date(7-IV-2000)/STATE or COUNTRY, City, County/Collector/ specimen ID number

To remount a balsam mount place slide in histoclear to dissolve old balsam then follow mounting procedure.

Mounting APHIDS
(Reference: Phil Johnson, SFO)

- Make a small incision in side of the abdomen.
- Place in cold KOH 24-48 hours.
- Gently manipulate while in KOH to remove body contents.
- Remove to water for several minute and continue to manipulate if necessary clear body contents.
- Place overnight or longer in Essig's for additional clearing. If mostly cleared, specimens can also go directly from KOH → Essig's. The "bubbling" that occurs as a result of the KOH mixing with the glacial acetic acid in the Essig's may "blow" out any material still in the body.
- Remove to 70% EtOH→ 100% EtOH → clove oil.
- Mount in Balsam (NO Euparal). Consistency should be slightly viscous, but not watery. "Bake" at 40-50◦C for 1-2 weeks.

Coccoidea Preparation as per Doug Miller (SEL, USNM)

1. Place specimens in cold 10% solution of KOH (potassium hydroxide) for 24 to 48 hours to soften body contents. [Specimens should not be left in KOH any longer than is necessary to soften body contents because KOH destroys or lightens the natural body color that is frequently used in identifications.]

2. OR, Heat the KOH for 10-20 minutes (or until body contents have softened) on a hot plate set at 110 F. Make sure to cover specimen dish to prevent evaporation.

3. Using a sharp probe or needle, make a small incision on the side of the abdomen. If several specimens are being prepared, vary the location of the incision so that among the specimens there are some complete left and right abdominal margins.

4. With a small spatula or other tool, gently press down on abdomen and tease out body contents. If the body contents do not all come out after pumping, place specimens back onto hot plate for another 5 minutes or place into a solution of half dishwashing detergent-half distilled water for 3 minutes.

5. Pump specimens in distilled water and leave for 5 minutes.

6. If specimens are colorless, they can be stained with double stain. Leave in stain 5 minutes. If lighter stain is desired, mix stain with distilled water or 70% EtOH.

7. Pump in 70% EtOH and leave for 5-10 minutes.

8. Pump in 95% EtOH. If specimens are stained too dark, add water, a drop at a time, to remove some of the stain. Leave in 95% EtOH for a minimum of 5-10 minutes.Note: The presence of water in legs or antennae can cause them to collapse so make sure specimen is in EtOH long enough.

9. Transfer to clove oil for a minimum of 5 minutes. Gently pump specimens to remove excess EtOH. Specimens can be left overnight in clove oil if there is not time for mounting on a slide.

10. Place drop of thin Canada balsam in the center of a slide. [Thin balsam with histoclear instead of xylene.] Gently transfer specimens to slide. If specimens are small enough to allow for mounting 3-4 or more on a slide, mount at least one ventral side up. When mounting specimens, representatives of all stages (adults, nymphs, and pupa males and females) should be included on each slide as space and material allows. Place specimens on slide head pointed down with younger stages to the left and older adults to the right.

11. Carefully place cover slip over balsam. Place slide on hot plate to spread balsam evenly.

12. Cure slides in a 110 F oven (40 C) for one month so the balsam is no longer soft. If no oven is available, slides can be left undisturbed for 2 months at room temperature.

13. Place a slide label to the left of the cover slip which contains the following information if available: (/ represents the end of a line)

Genus/ species Author/ Host/ date(7-IV-2000)/STATE or COUNTRY, City, County/Collector/ specimen ID number

To remount a balsam mount place slide in histoclear to dissolve old balsam then follow mounting procedure.

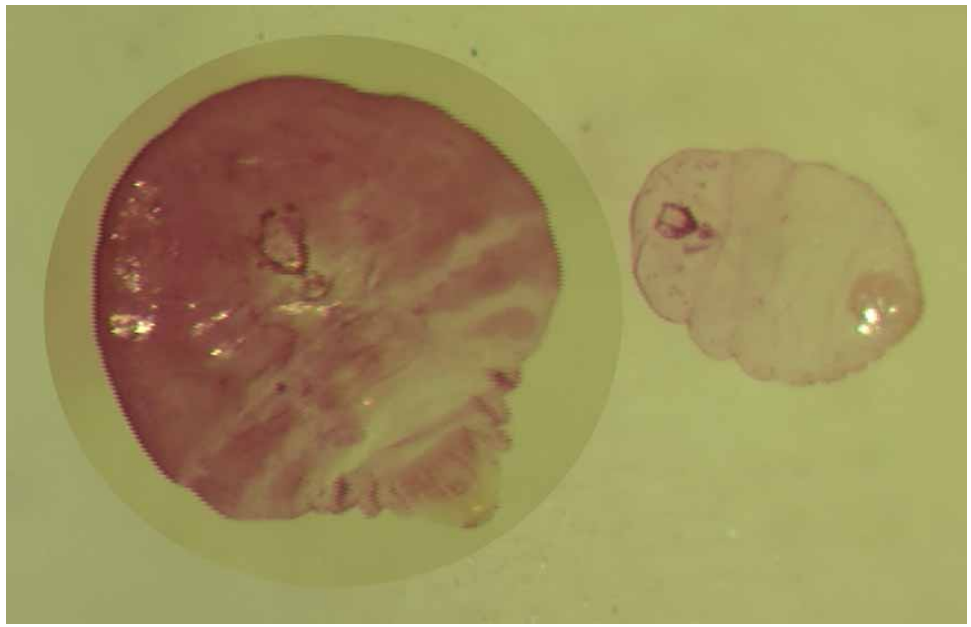
Mounting Aleyrodids & Coccoidea
(Reference: Phil Johnson, SFO)

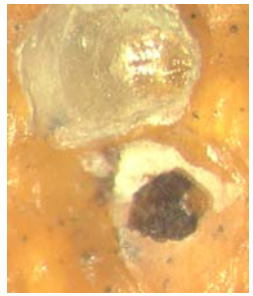
- **Pseudococcidae:**
  - Make a small cut in the side, between 2nd and 3rd leg.
  - Place in KOH and gently boil until body contents have cleared.
  - Remove to water and gently manipulate specimen to clear body contents.
  - Use 100% alcohol to remove any stubborn wax.
  - Place over night in dye.
  - Remove from dye to 70% EtOH→ 100% EtOH → clove oil.
  - Mount in Euparal or Balsam; “Bake” at 40-50◦C for 1-2 weeks.
- **Other Coccoidea and Aleyrodidae:**
  - Place in KOH over low heat.
  - Make an incision in the body wall if needed.
  - Remove to water and gently manipulate specimen to clear body contents.
  - Use 100% alcohol to remove any stubborn wax – be gentle as the alcohol will make the specimens fragile.
  - Place over night or longer in dye.
  - Remove from dye to 70% EtOH→ 100% EtOH → clove oil.

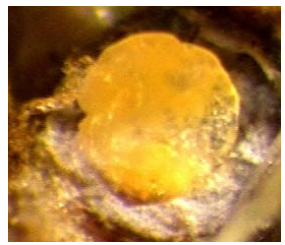
Mounting Mealy bugs and Scales

- While in isopropyl alcohol puncture it behind its last leg .
- Place into a 10% potassium hydroxide solution (14 pellets in 50 ml water).
- Gently pump and tease out the contents of the insect using a spatula. The scale exuvia should be removed at this time.
- Leave the insect in the solution at room temperature for 24 hours.
- Transfer the insect into water to rinse off the potassium hydroxide.
- Transfer into essig's aphid fluid
- Transfer the insect into 70% isopropyl alcohol to dehydrate.
- Transfer the insect into 70% isopropyl alcohol with a drop of stain and leave for 30 minutes.
- Transfer the insect to cellusolve for 5 minutes (skip if mounting in Hoyer's).
- Mount the insect ventral side up using Canada balsam or Hoyer's.
- Allow the slides to dry for 10 to 14 days on a slide warmer.
- Periodically check for air pockets. Remove them by adding Canada balsam or Hoyer's to the edge of the cover slip.
- Slight heat may be necessary to displace the air pockets.
- Ring the edge of the cover slip with Glyptal electric paint (skip if mounting in Canada balsam). Two coats are needed to
- keep out moisture.
- Scrape away excess Canada balsam or Hoyer's that seeped out of the cover slip.

Mounting Diaspids-Preparation



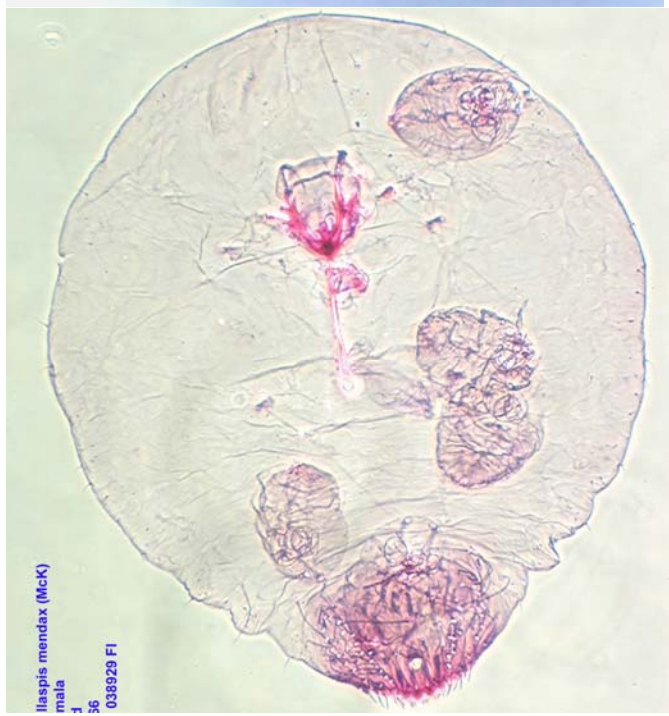




- The above left image shows the diaspids in clove oil. Need to be translucent to transparent. The above right two image show the live scale insects (exuvia top and female below the exuvia).

Mounting Diaspids-slides

- The pygidium for Diaspids must be “picture perfect for species ID (like image on the right). If with an internal parasite or blanketed with fungi- discard. If the stain does not hold on the permanent mount, OK to submit- use phase contrast.
- Note the internal eggs-try to remove but still sufficient to submit.



Mounting Thysanoptera
(Reference: CAB #2, BMNH by Dr. Mound et al)

- Let specimen stand in 5% NaOH (during this stage you may puncture the insect behind the hind coxae. Spread the legs and antennae. Only spread the wings when you are about to remove the specimen from NAOH.)
  - ½ hour for light colored thrips
  - Up to 4 hours for dark colored thrip

  [you may need to soak the dark thrips in H_2O_2/NH_4 soution-but monitor the bleaching to prevent overbleaching.]

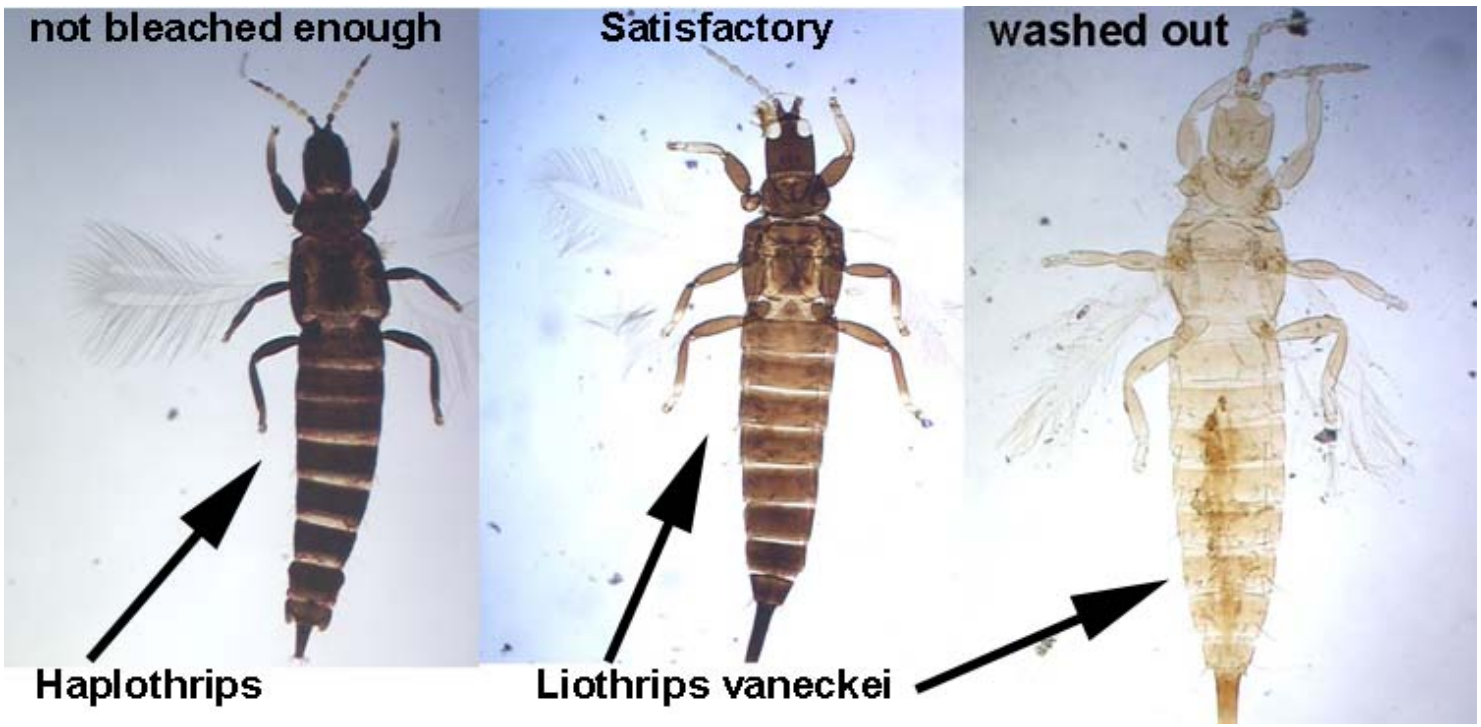
- Replace the NaOH with water. Slowly add 50% ROH.
- Replace effluent with 60% ROH and store for a minimum of 24 hours.
- Next dehydrate specimen to remove all water:
  - Replace 60% with 70% ROH and let stand for one hour.
  - Replace 70% with 80% ROH and let stand for 20 minutes
  - Replace 80% with 95% ROH and let stand for 10 minutes
  - Replace 95% with absolute ROH and let stand for 5 minutes
  - Refresh 95% ROH and let stand for 5 minutes
  - Transfer specimen to clove oil and let stand for one hour
- Apply drop of balsam on slide.
- Transfer one thrip-ventral side up to the balsam.
- Spread legs, antenna and wings
- Add cover slip
- Haet at 37°C until hard.
- Note: this publication also covers using a “mounting block” first before before placing on a slide. Please refer to the publication.

Mounting Thysanoptera
(Reference: Phil Johnson, SFO)

- **Thrips (routines):**
  - Make a small incision (a “poke with a fine needle) in side of the abdomen.
  - Place in cold KOH for 24-48 hours.
  - Gently manipulate while in KOH to remove body contents.
  - Remove to water for several minutes and continue to manipulate if necessary clear body contents.
  - Place overnight or longer in Essig’s (adults).
  - Place overnight or longer in 50% Essig’s/50% double stain (immatures).
  - Remove from Essig’s or dye to 70% EtOH→ 100% EtOH → Clove Oil.
  - Mount in a thin, almost watery consistency of Balsam directly from 100% EtOH or Clove Oil. Body parts can easily collapse in thicker Balsam. DO NOT USE EUPARAL. “Bake” at 40-50◦C for 1-2 weeks.
- **Thrips (urgents):**
  - Place directly in Hoyer’s and apply gentle heat to clear.
- OR
  - Make a small incision (a “poke with a fine needle) in side of the abdomen.
  - Place in KOH over low heat.
  - Gently manipulate while in KOH to remove body contents. Specimens need not be totally cleared – Hoyer’s will assist in an additional clearing.
  - Remove to water or 70% EtOH for several minutes for remove excess KOH.
  - Place directly in Hoyer’s and apply gentle heat.

MACERATION/BLEACHING OF DARK COLORED THRIPS

- Most Phlaeothrip adults need to be bleached-if they do not lighten after meaceration.



Thysanoptera: light colored & larvae

- Adult brown adult light larvae

- Remember: NEVER STAIN ADULT THRIPS. Larvae may be stained or not

LABELING TECHNIQUES

Please follow the following diagram for proper labeling of all slides

Family
Genus/species/author
Sex
Name (determined by)
Name (if confirmed by)
Mounting medium

specimen

Country
Host
Date collected
name of collector
acct/ID/PIN number

Tetranychidae [family]
Tetranychus urticae Koch
[genus species author]
Male [sex]
J.Dooley [determined by]
Conf: R. Ochoa (confirmed by)
Hoyers [mounting medium]

Japan
Malus sylvestris (fruit)
Date: II-01-2001
Coll: John Dooley
[collected by]
SFO 101250 CA
[PIN/acct number]

MOUNTING DEFICIENCIES

Slide Debris

CHEMICAL AGENTS

Bleaching agents

- Ammonia
- Hydrogen Peroxide
- Clorox

1. Water contamination

2. PVA etc medium cracking

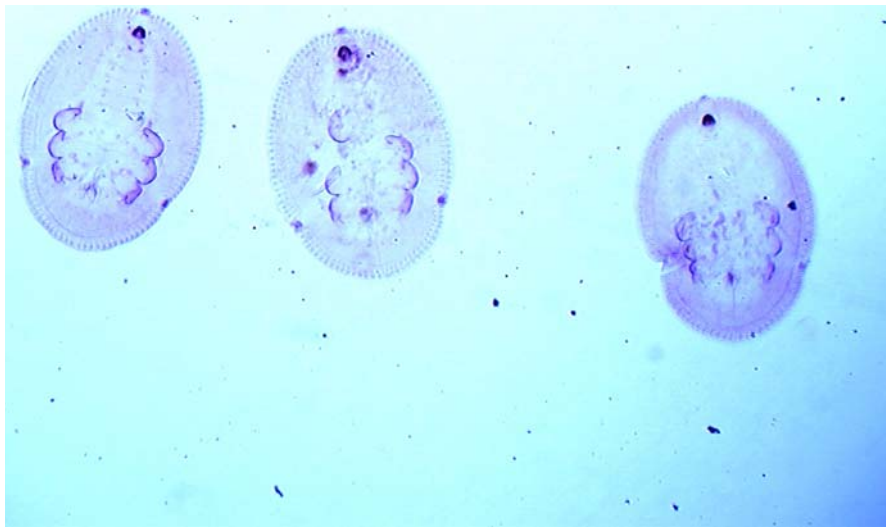
3. Wax Residue
   - Stain interference

BIOLOGICAL AGENTS

1. Disease agents
   - Aschersonia
   - Sooty Mold
   - Various fungi
2. Arthropod agents
   - Hymenoptera

Soiled Slides

Minor amount of debris-keep it to a minimum to avoid interfering with the view.



BLEACHING PROBLEMS

Mixing NH_4 (AMMONIA)/ H_2O_2 (PEROXIDE) vs. Clorox

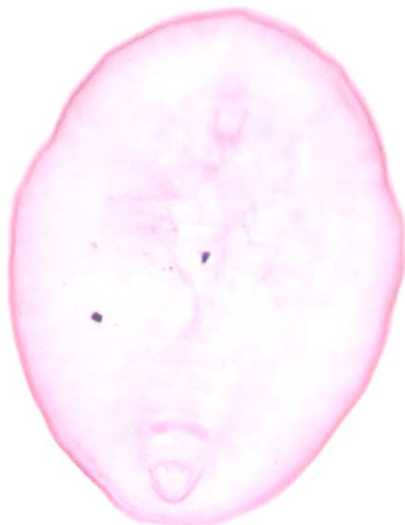
(DANGER!!!!!) -DO NOT MIX CLOROX WITH AMMONIA!)

- Upper right image shows *Aleurocanthus* that is over-bleached.
- Lower right shows *Aleurocanthus* when household bleach (Clorox) is used-no control over the bleach.
- Below shows *Aleurocerus* not bleached enough.

Medium cracking/Wax residue

- The image to the right shows the PVA mounting media cracking and shrinking from improper preparation. Also the spcimen was underbleached.

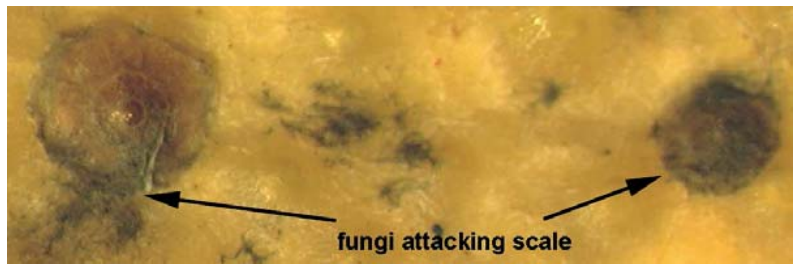
- The image to the right did pick up minimal stain but the wax was not completely removed blurring the structures and interfering with the staining process.



Biological Agents

- Note the fungi attacking the diaspid (Discard any pests attacked by fungi-make poor mounts):
  - Aschersonia
  - Sooty Mold
  - Other fungi

Aschersonia



- Hymenoptera internal parasites
  - Chalcicoidea larva internal parasite found inside the diaspid Pseuaulacaspis brimblecombei on Protea from Australia.
  - Most internal parasites interfere with mounting techniques-discard them.