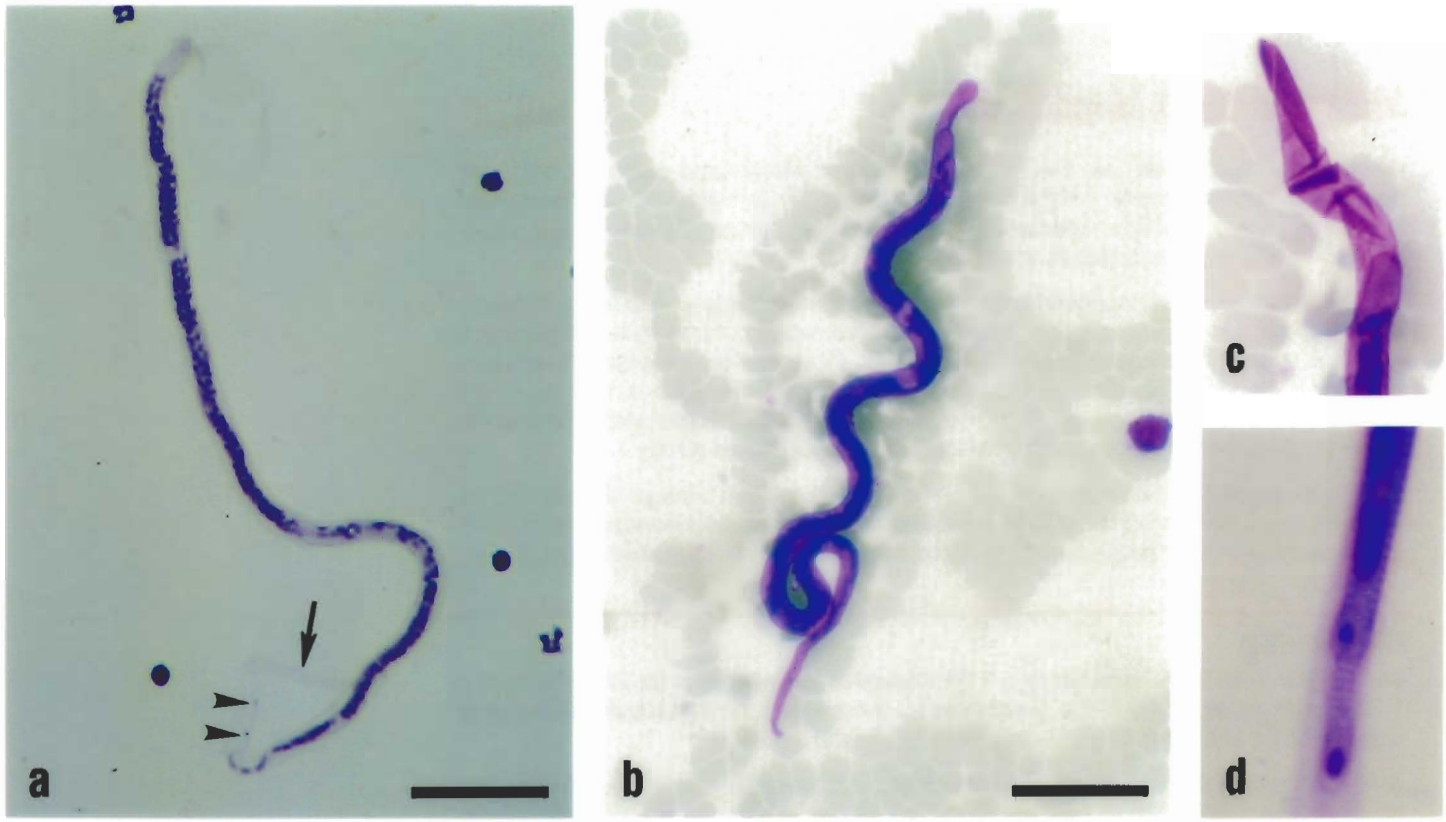
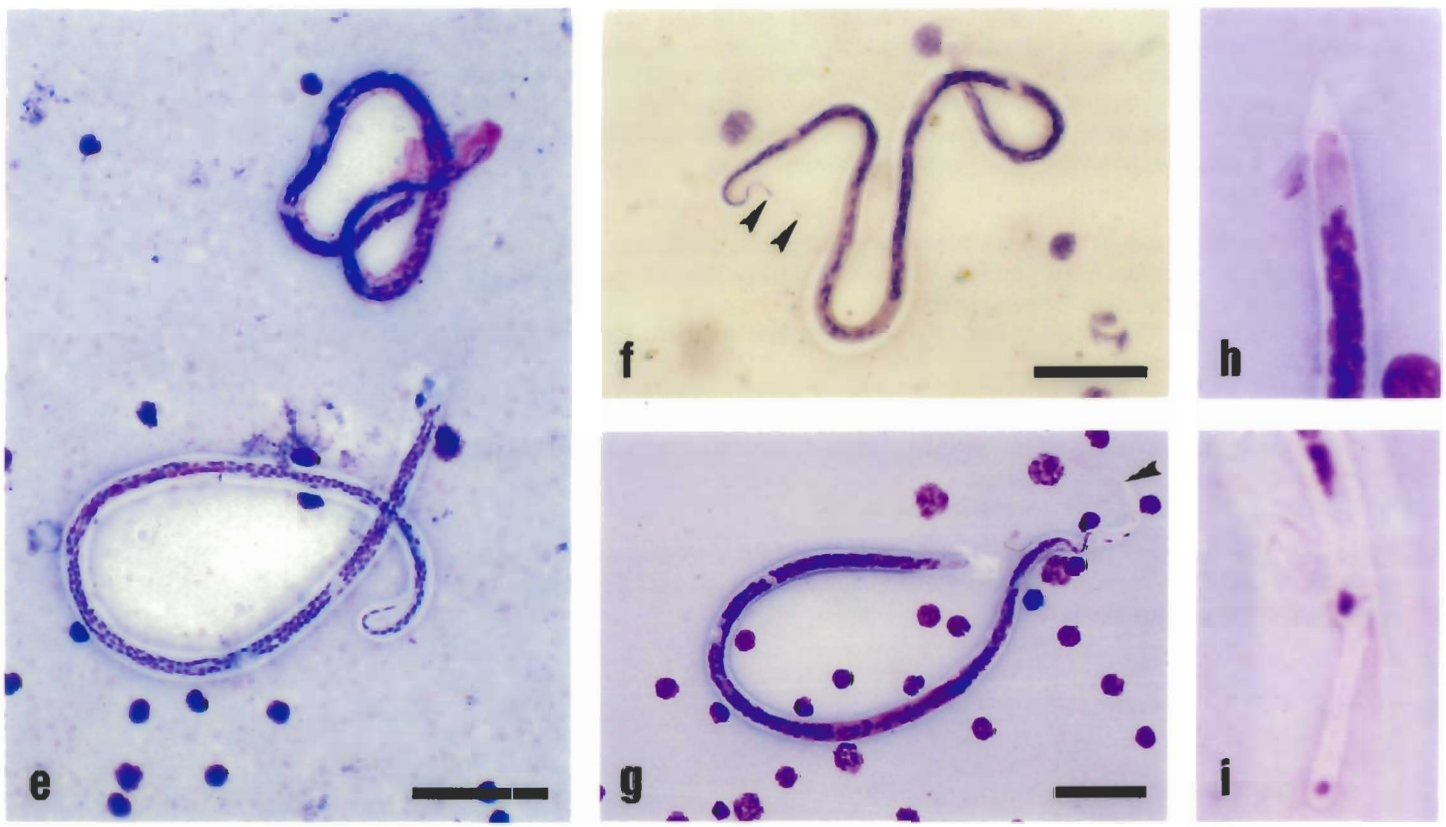
Note: All measuring bars = 30 µm



Brugia malayi microfilariae in haematoxylin (a) and Giemsa (b–d) stains. In haematoxylin, the sheath does not stain but may be faintly visible (a, *arrow*). This contrasts with the pink-stained sheath seen in Giemsa preparations (b, c). The column of nuclei is compact, and the widely separated subterminal and terminal nuclei in the tail are key diagnostic features (a, *arrowheads*; d). Nuclei are sparse in the region of the innerbody (a).



B. malayi (upper) and ***W. bancrofti*** (lower) microfilariae in the same field of a Giemsa-stained blood film (e). The pink-stained sheath and the darkly stained, compact column of nuclei identify ***B. malayi*** and distinguish it from ***W. bancrofti***

Brugia timori microfilariae in haematoxylin (f) and Giemsa (g–i) stains. ***B. timori*** is larger than ***B. malayi*** and the sheath does not stain pink (g, *arrowhead*) with Giemsa stain. The long head space and the subterminal and terminal nuclei are conspicuous features (f–i).

Staining of thick blood films

Giemsa and haematoxylin are the preferred and most widely used stains for preparing permanently stained blood films. Each has its advantages, but Giemsa stain is used most often. Slides can be processed in either small or large numbers using stainless steel, glass, or plastic staining racks and dishes.

Before staining, thoroughly dried films must be dehaemoglobinized and fixed. Immerse slides in tap or distilled water until the haemoglobin leaches out of the film, which becomes whitish in colour; this requires about 3–5 minutes. Films that are prepared from blood containing an anticoagulant and that have dried for more than a few days will dehaemoglobinize slowly, usually in 8–10 minutes. Allow dehaemoglobinized films to air-dry thoroughly. Fix the films in methanol for 30–60 seconds and air-dry.

Note: In the event that the same films are being used for malaria surveys, they should be stained without dehaemoglobinization or fixation. Microfilariae found in these preparations usually appear slightly swollen, and the nuclei are not sharply demarcated (Plate 3b).

Giemsa stain

Stain blood films for 45 minutes in a 1:50 dilution of Giemsa stain (or 20 minutes in a 1:20 dilution) at a pH of 6.8–7.2; wash films for 3–5 minutes in neutral buffered water or under running tap water. Dry films in a vertical position.

Note: The staining dilution and procedure used for processing malaria films can be used here with the expectation of acceptable results. Nuclei of microfilariae will stain blue to purple in colour. A sheath, if present, will stain pink (*B. malayi*) or not at all. The innerbody of *W. bancrofti* will stain a bright pink colour, but that of most other species does not stain.

Haematoxylin stain

Various haematoxylin stains are used as alternatives to Giemsa stain; Delafield's haematoxylin is recommended and is widely used. It enhances nuclear detail in the microfilaria and stains the sheath, when present, a greyish-blue colour. For preparation of Delafield's haematoxylin and details of another useful staining procedure, consult the WHO publication *Basic laboratory methods in medical parasitology* (1991). It is also acceptable to use other available stains and procedures.

Procedure

1. Thick blood films should be dried thoroughly, dehaemoglobinized, and fixed as described above. If films are prepared from sedimented Knott concentration material, dehaemoglobinization and fixation are omitted.
2. Stain slides for 10–15 minutes in Delafield's haematoxylin solution. Rinse in distilled water to remove excess stain.
3. Destain in 0.1% (1 g/l) aqueous hydrochloric or acetic acid for approximately 1 minute. Rinse slides in distilled water for 1 minute.
4. Place the slides in tap water containing several drops of ammonia for 3–5 minutes. The films will become dark blue in colour.
5. Rinse in tap water for 2–5 minutes and allow to dry.

Note: Films may be made permanent by adding a synthetic mounting medium and a coverslip. Alternatively, simply clarify with a drop of immersion oil, add a coverslip, and examine under low magnification.