Morphological Comparison of Seven Hypotriploid Populations of *Meloidogyne arenaria* with the Typical Triploid Populations

**ABDALLAH RAMMAH** and **HEDWIG HIRSCHMANN**

**Abstract:** A morphological comparison of seven hypotriploid populations of *Meloidogyne arenaria* was made to clarify their taxonomic status, using light and scanning electron microscopy. All populations differed from each other and from the typical triploid *M. arenaria* by certain features. Differences were not regarded as sufficient to justify recognition of the variants as distinct species. Morphological divergence of populations from the typical *M. arenaria* was gradual. The most useful characters were stylet and head morphology of males and stylet morphology of females. Perineal patterns and cephalic, stylet, and tail morphologies of second-stage juveniles were of little taxonomic value. Host races 1 and 2 could not be distinguished morphologically. Populations E445 and E551 with the atypical esterase phenotypes M3-F1 and S1-M1, respectively, were morphologically more similar to the typical *M. arenaria* than populations E255 and E467, which have the most common A2 esterase phenotype of *M. arenaria*.

**Key words:** biochemistry, cytology, host race, hypotriploid, light microscopy, *Meloidogyne arenaria*, morphology, nematode, root-knot nematode, scanning electron microscopy, taxonomy, triploid, variation.

The taxonomy of the genus *Meloidogyne* remains difficult and confusing due to extensive morphological and physiological variation within each species, especially the four most common ones, *M. incognita* (Kofoid & White) Chitwood, *M. arenaria* (Neal) Chitwood, *M. javanica* (Treub) Chitwood, and *M. hapla* Chitwood. Various morphological characters are used to distinguish the species of *Meloidogyne* (2–8,14,16–18). Detailed comparative morphological studies by light microscopy (LM) and scanning electron microscopy (SEM) of several populations of *M. hapla* (2–7), *M. incognita* (13), *M. arenaria* (1), and *M. javanica* (22) have shown that some characters are variable and unreliable, but others are of taxonomic value. For reliable and accurate identification of *Meloidogyne* species, several stable morphological characters of different life stages should be considered (14). Furthermore, the morphological data should be complemented by morphometric data and information on host response, cytology, and biochemistry (8).

*Meloidogyne arenaria*, one of the most variable species of the genus, has a complex morphology, host response, cytology, and biochemistry. Most morphological characters of *M. arenaria* have been found to be variable but still useful for species identification (1). Two physiological races can be recognized on the basis of host differentials: race 1 infects peanut, whereas race 2 does not (24). Cytologically, *M. arenaria* reproduces by mitotic parthenogenesis and includes populations varying in chromosome number from 2n = 30 to 2n = 56 (31). A triploid form (2n = 50–56) is the most common, and populations belonging to this form are considered to represent the typical *M. arenaria*. Diploid (2n = 30–38) and hypotriploid (2n = 40–48) populations, however, are also widely distributed and are included in the *M. arenaria* species complex. Enzymatically, most populations of *M. arenaria* exhibit the following three esterase phenotypes: A1, A2, and A3 (9). The A2 phenotype is the most common, whereas the A3 phenotype has been detected only in triploid populations. A few of the populations with lower chro-
mosome numbers have different esterase patterns (S1-M1, S2-M1, and M3-F1), which also are present in other Meloidogyne species.

The objectives of the present study were (i) to compare the morphology of some hypotriploid populations using LM and SEM and (ii) to clarify their taxonomic status in comparison with the typical triploid M. arenaria. All populations had been previously tested on differential hosts and characterized cytologically and enzymatically (9). Morphometric data for the same populations are presented in a separate paper (15).

**Materials and Methods**

Seven hypotriploid populations with a somatic chromosome number ranging from 40-48, which had been earlier identified as M. arenaria, or atypical populations of M. arenaria (31), were selected from the culture collection of the International Meloidogyne Project (25) (Table 1). They represented host races 1 and 2 and exhibited esterase phenotypes A2, S1-M1, M3-F1, and malate dehydrogenase phenotypes N1 and N3 (9).

All populations were maintained on tomato (Lycopersicon esculentum Mill. cv. Rutgers) under appropriate greenhouse conditions. Females and egg masses were hand-picked from infected roots. Males and second-stage juveniles (J2) were obtained after incubation of infected roots or egg masses in moist chambers at room temperature.

**Light microscopy (LM):** Males and J2 were killed and fixed in hot (70–80°C) TAF (7 ml 40% formaldehyde, 2 ml triethanolamine, 91 ml distilled water) and mounted in the same fixative. Females were fixed in 2% formalin and their anterior portions, including the esophageal region, were severed with an eye knife and mounted in 2% formalin. Perineal patterns were cut from live egg-laying females in 45% lactic acid and mounted in glycerin. At least 100 specimens of each life stage and population were assessed to obtain morphological data. Drawings were made with a Leitz drawing tube, and photographs were taken using a bright field microscope.

**Scanning electron microscopy (SEM):** Second-stage juveniles, males, excised stylets, and spicules were prepared for SEM according to previously described techniques (3–6,21). The specimens were viewed and photographed at different positions for comparison using a JEOL T200 scanning electron microscope operating at 25 kV accelerating voltage. At least 100 J2, 100 males, 20 stylets each of females and males, and 20 spicules were examined from each population.

**Results**

The morphology of the different populations examined was compared to that of the typical triploid M. arenaria. Only useful differentiating characters are considered in this comparative study. They include stylet and perineal pattern morphology of females, head shape, cephalic and spicule

<table>
<thead>
<tr>
<th>Population designation and origin</th>
<th>Chromosome number (2n)</th>
<th>Host race†</th>
<th>Enzyme phenotype‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>E255—Ecuador</td>
<td>40–42</td>
<td>1</td>
<td>A2, N1</td>
</tr>
<tr>
<td>E445—El Salvador</td>
<td>41–42</td>
<td>2</td>
<td>M3–F1, N1</td>
</tr>
<tr>
<td>E467—Korea</td>
<td>44–46</td>
<td>1</td>
<td>A2, N1</td>
</tr>
<tr>
<td>E551—Ivory Coast</td>
<td>42</td>
<td>1</td>
<td>S1–M1, N3</td>
</tr>
<tr>
<td>E553—Ivory Coast</td>
<td>40–44</td>
<td>1</td>
<td>A2, N1</td>
</tr>
<tr>
<td>E927—West Samoa</td>
<td>46–48</td>
<td>1</td>
<td>A2, N3</td>
</tr>
<tr>
<td>E1033—China</td>
<td>41</td>
<td>1</td>
<td>A2, N1</td>
</tr>
</tbody>
</table>

† 1 = reproduces on peanut; 2 = does not reproduce on peanut.
‡ Phenotype designation as in reference 9; Est = esterase; Mdh = malate dehydrogenase.

Table 1. Hypotriploid populations of the Meloidogyne arenaria species complex examined.
morphology of males, and cephalic morphology and tail shape of J2.

Females: Stylet morphology varies considerably among populations. The stylet cone is pointed, is generally curved dorsally, and widens posteriorly. The stylet opening is located close to the tip (Figs. 1, 2). The shaft is cylindrical and may broaden near its junction with the stylet knobs (Figs. 1, 2). The stylet knobs are variable in shape and size. Stylet knobs of populations E1033, E927, and E553 are trans-

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**Meloidogyne arenaria** Morphology: Rammah, Hirschmann 107

versely ovoid to tear-drop shaped (Figs. 1A–C; 2B–D), and may appear similar to those described for the typical *M. arenaria* (1). In contrast, the stylet knobs of populations E551, E445, E253, and E467 are markedly different from those of the typical *M. arenaria*. The knobs in E551 are smaller, rounded, set off from the shaft, and appear triangular in lateral view (Figs. 1D,2E), whereas the knobs of E255 are rounded, merging gradually with the shaft or may have a slight indentation anteriorly (Figs. 1F,2G). In population E445, the knobs are low and transversely elongated (Figs. 1E,2F), and in population E467 they are reniform (Figs. 1G,2H). The distance between stylet base and dorsal esophageal gland orifice (DGO) varies much within and among populations (2.2–8.2 μm).

Perineal pattern morphology is variable within and between populations and constitutes the least reliable character. Each population has perineal patterns that may be similar or markedly different from those of the typical *M. arenaria*. Aberrant patterns may occur, especially in population E551 (Fig. 3D,E). In addition, some patterns resemble those of *M. incognita* (Fig. 3B,F,G). Generally, the overall shape varies from rounded, sometimes with shoulders, to rectangular with a high, squared dorsal arch (Figs. 3,4). The dorsal striae may be discontinuous or may form a zigzag pattern (Figs. 3F,4D). The ventral striae are usually smooth and occasionally may have a wing near the lateral line area (Fig. 4B). The lateral lines are either faintly marked by the forking or interruption of the dorsal and ventral striae (Figs. 3D,4D), or very distinct with single or double incisures crossed by short vertical striae (Figs. 3A,B,H; 4B,C,F). The phasmids are small, without surface structure.

**Males:** Head shape is quite consistent among populations. Generally, all variants show the same basic head characters as those of the typical *M. arenaria* (Figs. 5–8). In LM, the head cap in most populations is rounded and low. Males of E551 differ from other variants by having a domed head cap (Figs. 5D,6E), whereas those of E255 have a more distinctly elevated head cap and a concave labial disc (Figs. 5F,6G,8E). The cephalic framework is well developed, and the vestibule and vestibule extension are distinct (Figs. 5A–G; 6). In SEM, the stoma is slit-like; the hexagonal prestoma is surrounded by six inner labial sensilla that open onto the labial disc. The labial disc is more or less rounded and slightly raised above but not set off the medial lips (Figs. 7,8). The medial lips are crescent shaped, extend for a short distance posteriorly, and generally have the same width as the labial disc. However, in E467, the labial disc may be narrower and has very pronounced indentations at its junction with the medial lips (Fig. 7F). Four cephalic sensilla are usually distinct on the medial lips. Lateral lips are absent. The amphidial openings are slit-like and located below the lateral edges of the labial disc. The head region is usually smooth, but incomplete annulations are often present in males of E551 (Figs. 7C,8C) and occasionally in males of E445 (Figs. 7D,8D) and E255.

Male stylet morphology is relatively stable and varies little within each population. The stylet cone is pointed and widens posteriorly at its junction with the shaft (Figs. 5A–G; 6; 9A–H). The shaft is cylindrical and broadens slightly at its base. The shape of the stylet knobs shows much variation among the variants and occasionally may appear considerably different from that of the typical *M. arenaria*. Knob shape varies from rounded to pyriform, and the knobs may be slightly set off from the shaft. The stylet knobs of E551 and E467 appear especially different: they are angular in E551, merge gradually with the shaft, and have a triangular profile (Figs. 5D,6E,9E), whereas the knobs of E467 are elongate to pyriform, are more amalgamated, and have a central longitudinal groove (Figs. 5G,6H,9H). The stylets of the few dwarf males of E553 were very short and were not considered in this comparison (Fig. 9D). The distance from stylet base to DGO averages 4.5 μm; in E551, however, the DGO mean is only 2.8 μm.

All populations examined are similar in spicule morphology (Figs. 5H; 9I–N). Each spicule consists of a cylindrical head with cytoplasmic core opening, a broad shaft, and an arcuate blade that terminates in a pointed tip bearing two small sensillar


pores. Two wing-like vela, one extending toward the dorsal, the other toward the ventral side, project from the spicule blade (Fig. 9N). Spicule size, however, is highly variable within and among populations. Spicules of dwarf males of E553 are short and aberrant (Fig. 9K).

Second-stage juveniles: Generally, J2 of all variants are similar in their morphology and cannot be distinguished from those of the typical *M. arenaria* (Fig. 10). In LM, differences were not detected in stylet morphology among the different variants of *M. arenaria* (Fig. 10A–G). The stylet is characterized by a pointed cone with very fine tip, cylindrical shaft, and pyriform
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knobs that merge gradually with the shaft. The DGO distance is usually long (2.6–5.3 μm) except in E551, which has a relatively short distance (2.8–3.6 μm). Tail shape is very variable and cannot be used as a distinguishing character. Generally, the tail is narrow, with irregular and constricting annules, and tapers to a fine, rounded tip (Fig. 10H–N). The hyaline tail terminus is distinct and variable in length. The rectum is dilated. In SEM, the head region is slightly set off from the body and is usually smooth, except in E551 and E255 (Figs. 11D,E; 12D,E), in which it has incomplete annulations. The labial disc is rounded to rectangular and slightly raised above the medial lips (Figs. 11,12). Six inner labial sensilla surround the oval prestoma. The medial lips are crescentic to rectangular with rounded corners. The cephalic sensilla are indistinct. Occasionally, the triangular lateral lips may fuse with the head region (Figs. 11B,C,F; 12B,F).

Characterization of variant populations of *M. arenaria*: Morphological divergence of the populations examined is gradual. E1033 is considered the variant closest to the typical *M. arenaria* and E467 the most distant. The populations are presented in sequence according to increasing degree of divergence from the typical *M. arenaria*.

**Variant E1033**: Most morphological features similar to those of typical *M. arenaria*. Stylet knobs of females transversely ovoid, not sloping posteriorly, slightly set off from shaft (Figs. 1A,2B). Perineal patterns
distinctive (Figs. 3A,4B). Dorsal arch moderately high, rounded to squarish. Striae coarse. Lateral lines distinct but not extending anteriorly, forming two widely spaced incisures crossed by irregular short vertical striae. Morphology of males (Figs. 5A,6B,7B,8B) and J2 (Figs. 10A,11B; 12B) similar to that of *M. arenaria*.

**Variant E927:** Female stylets and perineal patterns different from those of typical *M. arenaria*. Stylet knobs of females pyriform, markedly sloping posteriorly (Figs. 5A,6B,7B,8B) and J2 (Figs. 10A,11B; 12B) similar to that of *M. arenaria*. Scale bar: A = 2 μm; B–F = same scale as A.
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- A) Typical *M. arenaria*.
- B) E1033—China.
- C) E551—Ivory Coast.
- E) E255—Ecuador.
- F) E467—Korea. Scale bar: A = 2 μm; B–F = same scale as A.
Perineal patterns with high squarish dorsal arch and lateral lines around tail region (Fig. 3B). In SEM, stylet knobs of male rounded and set off from shaft (Fig. 9C). Generally, males (Figs. 5B, 6C) and J2 (Figs. 10B, I; 11C; 12C) resemble those of *M. arenaria*.

**Variant E553:** Perineal patterns highly
Meloidogyne arenaria Morphology: Rammah, Hirschmann 115


variable, rounded to squarish with coarse and wavy striae (Figs. 3C, 4C). Some aberrant females without vulva, and eggs retained within female body. Males dwarfed, and rare (Figs. 5C, 6D, 9D). J2 smaller than those of M. arenaria, or other variants (Figs. 10C, J) (15).

Variant E551: All life stages with features different from those of the typical M. arenaria. Stylet knobs of females small, rounded, set off from shaft and slightly sloping posteriorly (Figs. 1D, 2E). Knobs are triangular in LM. Perineal patterns highly variable, occasionally with aberrant
Fig. 11. SEM photographs of head regions (face view) of second-stage juveniles of Meloidogyne arenaria and its variants. A) Typical M. arenaria. B) E1933—China. C) E927—West Samoa. D) E551—Ivory Coast. E) E255—Ecuador. F) E467—Korea. Scale bar: A = 1 μm; B—F = same scale as A.
forms. Patterns rounded to rectangular (Fig. 3D,E) with high dorsal arch and sometimes shoulders or wings. Striae fine, continuous. Male head cap domed, head region annulated (Figs. 5D,6E). Stylet knobs of male merge gradually with shaft and appear triangular (Figs. 5D,6E,9E). Head region of J2 annulated (Figs. 10D, 11D,12D).

**Variant E445:** Stylet knobs of females low and transversely elongated (Figs. 1E,2F). Perineal patterns rounded with moderately high arch (Figs. 3F,4D). Dorsal and ventral striae may be wavy, discontinuously forming zig-zag arrangement on both sides of pattern. Stylet of male similar to that of *M. arenaria*. In SEM, male head with large labial disc and often incomplete annulations (Figs. 7D,8D).

**Variant E255:** Female stylet knobs rounded to pyriform with slight indentation anteriorly, merge gradually with shaft (Figs. 1F,2G). Perineal patterns rounded, occasionally similar to those of E445 (Figs. 3G,4E). Head region of male distinctly set off from body; head cap elevated; pre-stoma concave (Figs. 5F,6G,8E). Head regions of males and J2 with incomplete annulations (Figs. 5F,10F,11E,12E).

**Variant E467:** Stylets of males and females and perineal patterns distinctive. Stylet knobs of females set off, transversely elongated and reniform (Figs. 1G,2H). Perineal patterns usually rounded, may have shoulders and wavy striae (Figs. 3H,4F). Sometimes patterns very similar to those of E1033, with distinct double incisures at lateral lines. In SEM, pronounced indentations mark junction between labial disc and medial lips in males (Fig. 7F). Stylet knobs of males very characteristic, elongate to pyriform and amalgamated, with central longitudinal groove (Figs. 5G,9H).

**DISCUSSION**

All hypotriploid populations of *M. arenaria* studied differed from each other and from the typical *M. arenaria* by certain features. For each qualitative character, differences were exhibited in at least one life stage of these populations. Populations E551, E445, E255, and E467 were most different from the typical *M. arenaria* in various morphological features of each life stage. Although some of these differences were pronounced, they were not considered sufficient to justify recognition of these populations as different species. Consequently, it is suggested that these populations be regarded as intraspecific morphological variants of *M. arenaria*, and that the variability of the different morphological features revealed be included as inherent variation within this species. In a similar comparison of six triploid populations of *M. arenaria*, two populations were found somewhat different and were considered as variants (1).

In general, no correlation existed in the expression of morphological features across the three life stages. Within some populations, however, certain correlations were present between life stages with respect to stylet morphology and head region annulation.

Good differentiating characters of various life stages of *Meloidogyne* spp. are present either in the anterior or posterior body regions (14). Morphological characters recommended for distinguishing species include stylet morphology and perineal patterns of females, stylet morphology and head shape of males, and shape and tail length of J2 (2-8,14,16-18,22). Perineal patterns have been used as the primary differentiating character for species of *Meloidogyne* (11,26,27,32), but high intraspecific variability and the occurrence of aberrant or intermediate forms have lessened their utility for identification purposes (12,19,23).

We found that the most useful, specific characters of *M. arenaria* are stylet morphology and head shape of males, and stylet morphology of females. The cephalic and stylet morphologies of J2 were not useful as taxonomic characters in LM and had little value in SEM observations. The J2 tail shape of *M. arenaria* appears similar to that of the other common spe-
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Perineal patterns were highly variable within and among populations, and represent the least useful differentiating character. This character should be used with caution, and identification of *M. arenaria* based on perineal patterns alone is not recommended. A similarly high variability in perineal pattern morphology also has been observed among other populations of *M. arenaria* (1).

Differential host-range tests showed that all variants, except E445, had similar host responses and belonged to race 1 of *M. arenaria*. Only variant E445 reacted like race 2. Although E445 can be morphologically distinguished from other variants, its features do not differentiate it from host race 1. Previous SEM and LM comparisons of several populations of *M. arenaria* did not reveal any consistent morphological differences between the two host races (1). Similarly, a morphometric comparison showed that the two host races of *M. arenaria* are indistinguishable (2). In an extensive morphological study of *M. incognita* populations, no differentiating characters could be found to separate the four host races (13). The various populations exhibited the N1 and N3 phenotypes of malate dehydrogenase that are common to *M. arenaria* and have no taxonomic value. An extensive investigation of 27 enzymes of *Meloidogyne* species demonstrated that *M. arenaria* is the most variable species, and enzymatically no two populations of this species are identical (10).

In conclusion, our findings support the concept that morphologically, cytologically, and biochemically, *M. arenaria* has the most extensive intraspecific variation among the four most common *Meloidogyne* species.

**Literature Cited**

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