Locomotion pattern and pace of free-living amoebae – a microscopic study

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Free-living amoebae are part of the microscopic environment and typically act as predators. They show a great variety of cell morphologies and can be differentiated into naked amoebae, testate amoebae (testacea), foraminifera, radiolaria and heliozoa. Amoebae are single-celled organisms, which can form pseudopodia as temporary cell extensions that are used for locomotion and as a means to feed. Like shape, size and tests, these pseudopodia vary greatly and are characteristic for the different amoebae and their locomotion. To assess the morphological diversity and locomotion characteristics of free-living amoebae, samples from different suburban habitats (Pietermaritzburg, South Africa) were analysed using light microscopy coupled with digital image analysis. Various naked and testate amoebae as well as heliozoa were detected, which represented the different types of known pseudopodia (lobopodia, reticulopodia, filopodia, axopodia). The heliozoa, only present in samples from permanent waterbodies, possessed stiff axopodia radiating from a rounded body. They typically floated in the waterbody without showing active locomotion. However, active movement was detected for naked as well as testate amoebae that were present in all habitats sampled. The use of lobopodia and reticulopodia - the latter only detected in naked amoebae - resulted in varying locomotion patterns from continuous, steady to more abrupt, eruptive locomotion, sometimes resulting in reorientation in space. The average speed established for individual cells of free-living amoebae varied usually between 0.5-4.5 μm per second.

Keywords: testate amoebae; naked amoebae; heliozoa; pseudopodia; locomotion; speed; pace; prey

1. Introduction

Amoebae, often referred to as amoeboid protozoa, are unicellular organisms which are sometimes referred to as the most primitive class of single-celled organisms [1]. The taxonomy of amoebae is quite complex and sometimes even uncertain [2-5]. The identification and taxonomic assignment of free-living amoebae by light microscopy is often difficult, usually more sophisticated methods are required [2-6]. However, based on their body shape and locomotion, they can be divided into five main types: naked amoebae, testate amoebae, heliozoa, foraminifera and radiolaria [7,8].

They are typically present in diverse habitats such as soil, moss or fresh and marine water environments with the exception of foraminifera and radiolaria, which are exclusively found in marine systems. A characteristic typical for all amoebae is the formation of pseudopodia, which are temporary cell extensions used for locomotion and feeding. These pseudopodia can be differentiated into four types: I) lobopodia, which are formed like a finger or tongue and consist of both ectoplasm and endoplasm. II) filopodia, which only contain ectoplasm and have a fine, almost filamentous usually pointed structure. III) rhizopodia or reticulopodia, which consist of ectoplasm and are filamentous but branched and often cross-connected. IV) axopodia, which are quite stiff and semi-permanent axial rods, usually protruding in a star-like fashion from the cell body. The use of these pseudopodia typically results in a floating, streaming or crawling movement, which sometimes resembles the movement of snails. Numerous studies analysing the mechanisms of amoeboid movement and locomotion were done in the past, mostly using pure cultures of amoebae [9-18]. These studies also addressed the measurement of cytoplasmic streaming, which, however, does not necessarily represent the locomotion speed of the whole organism. The measurement of speed is usually not the main focus of such studies and more recent studies analysing the locomotion speed of amoebae, especially of testate amoebae and heliozoa, appear to be sparse, rendering it difficult to find more recent data reporting speed values in the literature. As typical predators amoebae are known to feed on various microorganisms, including motile algae, bacteria, ciliates and even metazoa like rotifers [19-24] and one has to wonder if amoebae are fast enough to hunt moving prey.

To study amoebae in general and their locomotion pattern in vivo, light microscopy can be used. Ideally, this should be done using phase contrast, as especially the non-testate amoebae show limited contrast and can otherwise easily be overlooked by the non-experienced observer while screening samples. The focus of this study was to assess the variety of amoebae present in samples from different habitats collected in suburban gardens and to determine the pace and speed of amoebae using light microscopy.

2. Methods

Fresh samples were taken randomly during the year in suburban gardens in Pietermaritzburg, South Africa. Samples from locations harvesting rain and/or irrigation water (such as plant trunks and pots) were taken with a plastic Pasteur pipette and analysed directly, while larger samples from a fishpond were collected and kept in small glass vessels from which small samples were taken for analysis. Bark and moss samples were placed in glass containers with an overlay of
still mineral water (Aquelle, South Africa) prior to analysis and analysed over time. All environmental samples were analysed without prior cultivation at room temperature by light microscopy (Zeiss, AxioScope A1) using a glass slide covered with a cover slip.

Photographs and movies were taken using a digital image capturing system (AxioCam ERc5s, Zeiss) coupled with image analysis software (ZEN lite 2012, Blue edition, Zeiss). Locomotion pattern and speed of individual amoebae were established electronically by analysing picture sequences from movies, measuring the distance covered by amoeboid cells over time. Movies of individual amoebae were captured for at least 50 seconds, if possible several times.

The presumptive assignment of amoebae to taxonomical groups was done by using standard handbooks and publications [1-8, 25, 26].

3. Results and discussion

3.1 Morphological variety and locomotion

A wide variety of free-living amoebae was found in all habitats analysed, with naked and testate amoebae present in samples with and without permanent waterbodies while heliozoa were only present in permanent waterbodies. The frequency and variety of amoebae morphotypes present in samples seemed to follow a seasonal trend, with more active amoebae present in samples during summer time. This might be due to the lower average daily temperature in winter time as well as the fact that Pietermaritzburg usually experiences very low rainfall in that season resulting in lower moisture content or drying out of certain habitats. Similar seasonal trends were described for testate amoebae in fresh water with larger benthic populations present in the summer months [1].

Although some amoebae possess very unique characteristics that are clearly visible using light microscopy, assignment of free-living amoebae to specific genera or even species is quite difficult [6]. For example, for many testate amoebae the fine structure of the shell is a very important part of the identification [1, 3, 5]. However, this fine structure is often only visible at sufficient detail when analysing empty shells using electron microscopy. The need of more sophisticated microscopy applies also to naked amoebae, where the analysis of ultrastructural cell components might be necessary for the differentiation and reliable identification [2, 4]. Despite the development and use of more sophisticated methods, the taxonomy of certain amoebae is still uncertain and often a matter of scientific debate [2, 6].

The challenge of amoebae identification is not new and was already highlighted by King and Jahn in 1948 [27]. This was also highlighted by Page in 1969 who stated that “identification of any amoeba as the Amoeba limax of Dujardin must be made with much hesitation”, implying that many small to medium sized monopodial amoebae might be wrongly identified as that species [28]. The main intention of the present study was the examination of variability in amoeboid morphotypes and their locomotion patterns. Therefore, the assignment of amoebae in this study to genera is preliminary and their identification presumptive as only light microscopy was used without further purification or cultivation of specimens. Some morphotypes were even only found once, thereby limiting the possibility of a more detailed analysis for identification.

Representative amoebae found in this study are shown in Fig. 1. Some amoebae such as heliozoa were only present in habitats with permanent waterbodies. Figure 1A shows the heliozoan Actinophris sol with its typical round body from which many stiff and very fine axopodia protrude. A similar cell shape is represented by Astramoeba species (Fig. 1B), however these amoebae have long and thin pseudopodia which resemble the shape of axopodia but are thicker especially at the base. Both of these floating forms showed no visible locomotion but seemed to oscillate with the water movement. Floating states are also reported for some naked amoebae like Mayorella spp. [4, 29], which usually occur when these amoebae are disturbed. However, if left undisturbed, they reshape after a while to form their typical gliding state. Floating states with long slender pseudopodia radiating from an irregular body were detected for Mayorella-like amoebae from soil (Fig. 1C), which reshaped like described above and started to move by the use of several short, blunt lobopodia. Another group of naked amoebae reported to be frequently detected in soil and fresh water samples are Acanthamoebae [4], which show a gliding movement by several short, often spiny pseudopodia (subpseudopodia) frequently including changes of the cellular shape. Members of this group were frequently detected in sediment samples (Fig. 1D), showing the characteristic presence of clearly observable contractile vacuoles and hyaline zones. In contrast to the locomotion pattern of Acanthamoebae, the much larger Saccamoeba species found in this study (Fig. 1E) moved with a monopodial body with no distinct shape transition during locomotion and without formation of a clear hyaline zone. Typically, Saccamoeba species possessed a distinct nucleus and knobbed uroid. A clearly visible uroid was also detected for Amoeba-like specimens, which moved with few distinct fingerlike lobopodia (Fig. 1F). A different type of pseudopodium was detected for a Filamoeba-like organism (Fig. 1G). This amoeboid specimen had a fan-shaped body with many unbranched fine filopodia at the front pointing into the direction of movement, resulting in an uninterrupted “smooth” form of gliding. Lesser numbers of filamentous pseudopodia surrounding the whole cell body were present in beautiful Biomyxa-like specimens (Fig. 1H). As these filamentous pseudopodia were sometimes branching, they indicate the presence of reticulo- or rhizopodia.
Fig. 1 Representative morphological types of free-living amoebae from diverse suburban environmental samples. Floating forms: A) Actinophris sol (heliozoa); B) Astramoeba sp. and C) floating stadium of Mayorella-like specimen. Moving forms: naked amoebae D) Acanthamoeba-like specimen, E) Saccamoeba sp., F) Amoeba-like specimen, G) Filamoeba-like specimen and H) Biomyxa-like specimen; testate amoebae I) Euglypha-like specimen, J) Nebela-like specimen, K) Difflugia sp. and L) Arcella species. All scale bars = 50 μm.
In addition to naked amoebae, a wide variety of testate amoebae was found in the different samples analysed, often additionally evident by the presence of a great variety of empty shells. As for naked amoebae, different kinds of pseudopodia were detected in testate amoebae. Locomotion by the use of filopodia was evident for *Euglypha*-like amoebae (Fig. 1I), while *Nebela*-like organisms typically moved with several, fingerlike lobopodia (Fig. 1J). Much larger in size were individuals of *Diffugia* species (Fig. 1K), having a very distinct shell covered with small particles and a small peak on the top. These amoebae usually moved with one dominant, broad and fingerlike lobopodium. Only when the cells changed direction or were feeding, additional lobopodia were formed. For some testate amoebae, very slow movement was visible but the type of pseudopodia used could not be unambiguously determined as most of the time they were hidden underneath the shell, like for specimens of *Arcella* species (Fig. 1L).

### 3.2 Locomotion pattern and pace

The locomotion pattern of free-living amoebae detected in this study rarely followed a straight line (Fig. 2). This is not surprising as amoebae are predators and known to show typical animal behaviour, which includes searching for food or prey as well as resting or feeding phases [19].

Furthermore, as samples were taken without any treatment prior to microscopic examination, samples often contained particles. This resembles more likely native environmental conditions, with particles blocking the way of roaming free-living amoebae. Changes in direction as well as abrupt locomotion were frequently observed for individuals under these conditions. This was more obvious for testate amoebae while most of the naked amoebae examined in this study seemed to move more constantly. This more abrupt locomotion observed for testate amoebae might be a result of carrying an inflexible shell that cannot bend when the cell is changing direction or dodging obstacles. On the other hand, some naked amoebae such as *Mayorella*-like specimens showed great transitions in shape during locomotion. Variations in locomotion pace and pattern of single individual amoebae were already reported and discussed in 1924 by Schwitalla for cultured *Amoeba* species isolated from a spring water stream [30]. Schwitalla concluded that a uniform locomotion rate may occur more likely at lower than at higher temperatures and that the same rate of locomotion is seldom sustained for an extended period of time [30]. Additionally, the same author pointed out that the rate of locomotion may vary greatly even between single individuals of the same species (“fast” and “slow” individuals) [30].

![Fig. 2 Typical movement pattern of an Amoeba-like specimen. The locomotion path (black line) was established by observing the uroid of the cell over 55 s. Scale bar = 20 μm.](image)

When analysing the movement of individual amoebae by light microscopy on a slide, visible locomotion usually started only after a short time of adaptation as amoebae are highly sensitive to mechanical disturbance. To establish the average speed of the different types of amoebae detected in samples, a “fixed” point of the body (for example the uroid of some naked amoebae) or the shell of testate amoebae was selected to measure the distance covered over time using individual pictures of a movie sequence. However, this is sometimes difficult due to the above mentioned abrupt movements and shape transitions. Additionally, body and shell size as well as the type of pseudopodia used for locomotion were determined for individual amoebae cells. The results (Table 1) show that the average rates of locomotion for both naked and testate amoebae were in a similar range of 0.5 – 4.2 and 0.8 – 4.5 μm/s, respectively.

Similar values for locomotion rates of various naked amoebae were already reported several decades ago. In his description of three new species (one *Amoeba* sp. and two *Pelomyxa* sp.) in 1918, Schaeffer reported varying locomotion rates ranging from 0.02 μm/s and 1.6 μm/s (*Pelomyxa* sp.) up to 2.1 μm/s (*Amoeba* sp.) [21]. In the 1920s, Pantin reported maximum values of about 3.3 μm/s for marine species of *Amoeba* [9], while Schwitalla reported average speed rates between 0.3 to 2.8 μm/s for *Amoeba* sp. from fresh water [30], resembling the average speed established in this study for *Amoeba*-like and *Vahlkampfia*-like specimens. Mast and Prosser reported slightly higher average speed rates at ambient temperatures in 1932 for *Amoeba proteus* with values of about 4.2 μm/s [31], which match the average speed determined for *Saccamoeba* species in this study (Table 1). In contrast, *Acanthamoeba*-like and *Mayorella*-like individuals analysed in the present study moved much slower, generally not exceeding an average speed of 1 μm/s. Values of about 0.3 – 0.6 μm/s for the average locomotion rate were reported for *Mayorella cultura*. 

![Image](image)
and Naegleria gruberi [32]. However, the average speed of N. gruberi was increased by changes in electrolyte concentrations up to 2 μm/s using pure culture samples [32]. For trophozoites of an Acanthamoeba isolate, an average speed of about 0.8 μm/s was reported [33].

Table 1  Average speed, body per second value and type of pseudopodia of representative free-living amoebae from various suburban environmental samples.

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Size (length) in μm</th>
<th>Average speed in μm/s (min-max)*</th>
<th>Body per second (bps)</th>
<th>Type of pseudopodia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saccamoeba sp.</td>
<td>120</td>
<td>4.2 (2.2-6.3)</td>
<td>0.04</td>
<td>monopodial body</td>
</tr>
<tr>
<td>Vahlkampfia-like</td>
<td>43</td>
<td>2.9 (1.3-4.9)</td>
<td>0.07</td>
<td>monopodial body</td>
</tr>
<tr>
<td>Amoeba-like</td>
<td>70</td>
<td>2.1 (0.8-4.0)</td>
<td>0.03</td>
<td>lobopodia, fingerlike</td>
</tr>
<tr>
<td>Mayorella-like</td>
<td>60</td>
<td>1.0 (0.2-2.1)</td>
<td>0.02</td>
<td>short lobododia (subpseudopodia) polypodial</td>
</tr>
<tr>
<td>Acanthamoeba-like</td>
<td>25</td>
<td>0.6 (0.2-0.9)</td>
<td>0.02</td>
<td>very short, spiny lobopodia (subpseudopodia) polypodial</td>
</tr>
<tr>
<td>Filamoeba-like</td>
<td>25**</td>
<td>1.1 (0.4-1.8)</td>
<td>0.04</td>
<td>filopodia polypodial</td>
</tr>
<tr>
<td>Biomyxa-like</td>
<td>10**</td>
<td>0.5 (0.3-0.8)</td>
<td>0.05</td>
<td>filopodia, reticulopodia polypodial</td>
</tr>
<tr>
<td>Diffugia sp.</td>
<td>150 (shell)</td>
<td>4.5 (0.5-12.0)</td>
<td>0.03</td>
<td>lobopodia, mainly monopodial</td>
</tr>
<tr>
<td>Nebela-like</td>
<td>40 (shell)</td>
<td>1.4 (0.3-4.1)</td>
<td>0.04</td>
<td>lobopodia, fingerlike</td>
</tr>
<tr>
<td>Euglypha-like</td>
<td>45 (shell)</td>
<td>0.8 (0.2-1.7)</td>
<td>0.02</td>
<td>filopodia polypodial</td>
</tr>
<tr>
<td>Trinema-like</td>
<td>28 (shell)</td>
<td>1.5 (0.9-2.6)</td>
<td>0.05</td>
<td>filopodia polypodial</td>
</tr>
</tbody>
</table>

* minimal and maximal speed measured for individuals during locomotion
** body width (direction of movement)

Most of the above mentioned and discussed naked amoebae used lobopodia for their movement. However, two of the naked amoebae specimens detected in this study (presumptive Filamoeba sp. and Biomyxa sp.) used filopodia and/or reticulopodia instead. The speed of these two morphotypes was moderate to slow with an average of 0.6–1.1 μm/s and 0.5 μm/s, respectively, thereby clearly slower than the locomotion rates established for monopodial movement. Studies reporting the speed for similar filopodial species of naked amoebae were not found in the literature. Yet, Pennak mentioned that amoebae with lobopodia usually move at speed rates between 0.5 to 3.0 μm per second, while the use of other pseudopodia results in only slow if any motion [25]. However, no specific examples were given by Pennak.

Regarding the average speed of testate amoebae, it appears that only very few studies are available in the literature. For Diffugia corona, cytoplasmic streaming of 2 – 15 μm/s was reported [15]; however, this does not necessarily correspond to the locomotion speed of the whole cell. Lower values for locomotion rates were reported for the lobose testate amoeba Amphizonella violacea with 0.65 μm/s [34] and for Cochliopodium megatetrastylus with about 0.3 μm/s [35]. However, the latter species is a rather unique morphotype with a flexible surface coat instead of a solid test. Grospietsch stated for testate amoebae an average speed range of 1 – 1.5 μm/s, which can be exceeded by single individuals [7]. These values are in line with our results as most of the testate amoebae examined in this study showed speed averages of about 0.8 – 1.5 μm/s, independent of the type of pseudopodia present (Table 1). Only the larger individuals of Diffugia species, with a shell size of about 150 μm, showed higher average speed values up to 4.5 μm/s. No speed average was established for Arcella species.

For the free-floating forms of amoebae, no active locomotion was detected. Therefore, no speed average could be established for specimens of Astramoeba spp. or for Actinophris sol. However, it is reported that heliozoa can actively move by changing the length of the axopodia (mainly due to retraction) resulting in a rolling type of locomotion when attached to a surface [14]. Although typical speed rates of heliozoa are reported to be in the range of 0.1-1.7 μm/s, apparently even values of up to 4-5 μm/s are possible [7, 14]. As only free-floating forms of heliozoa without surface attachment were present in environmental samples, such locomotion was not observed in this study.
It is evident that both faster and slower specimens were observed among naked and testate amoebae (Figure 3). However, when the size of amoebae is considered to establish the relative speed as body per second values (bps), deviations in speed between the examined types of amoebae become less obvious (Figure 3). The values for the relative speed of the different types of amoebae analysed ranged from 0.02 – 0.07 bps (Table 1), but the largest individuals were not necessarily the fastest. The highest value of 0.07 bps was established for a smaller-sized monopodial Vahlkampfia-like specimen (about 40 μm), while a similarly shaped but three times larger monopodial Saccamoeba-like specimen moved at about half the relative speed with 0.04 bps. This result was not completely unexpected as Yates, when comparing different swimming microorganisms, established that larger organisms usually swim faster [36]. However, in relation to the body size, even the fastest human swimmer reaches just over one body length per second, while single-celled organisms can reach a relative speed of up to about 100 times their body size per second [36]. Herzog and Wirth even reported maximal relative swimming speeds under optimum conditions of close to 400 to 500 bps for certain Archaea, while strains of Escherichia coli usually reached a speed of about 20 bps, which is similar to the relative speed of one of the fastest warm-blooded animals, the cheetah [37]. For protozoa like Euglena gracilis and Chilomonas paramecium, swimming speeds at 20°C of about 40 and 100 μm/s, respectively, are reported [38], which translates to relative speed values of about 1 and 3 bps, respectively.

Fig. 3  Speed average and body per second values of different free-living amoebae in comparison with a typical prokaryotic prey organism. Specimens depicted from top to bottom: Saccamoeba sp., Diffugia sp., Acanthamoeba-like, Trinema-like, Filamoeba-like, Amoeba-like, Nebela-like and Escherichia coli.

Microorganisms like bacteria and diverse protists serve as prey organisms for free-living amoebae. However, the established locomotion data show that motile bacteria or non-amoeboid protists are usually much faster than the sluggish, slow moving amoebae. This makes it highly unlikely that amoebae could outrun and hunt down their prey like a cheetah but would rather graze on non-motile microorganisms, as it was reported for Acanthamoeba sp. [33], or
somehow trap motile prey cells. Although the type of food ingested by amoebae can in principal be identified by analysing residues present in food vacuoles, it does not explain how amoebae capture their prey. It is known that naked amoebae like *Vampyrella* spp. and testate amoebae like *Difflugia* spp. can penetrate the rigid cell wall of prey cells to suck out the content [7, 22]. Again, this would appear to be less likely for fast moving prey yet reports on amoebae feeding on motile microorganisms are long known. It was thus reported that feeding on mobile rotifers by free living amoebae takes place when the rotifer is attached to a substratum [20, 24]. The amoeboid predator flows around the foot of the prey rotifer and starts ingesting the foot first while preventing the prey from escaping [20, 24]. Gibbs and Dellinger [19] described in 1908 a form of pursuit for *Amoeba proteus* when feeding on ciliates such as *Paramecium* spp. After contact with the ciliate was established by collision, *Amoeba proteus* cells formed a chamber using pseudopodia to capture the prey by circumvallation. When the prey escaped, the amoeba often started a pursuit following the ciliate. The authors suggested that the amoebae somehow detected the prey in the distance and started its pursuit until it was captured or lost [19]. For *Chaos chaos*, feeding on *Paramecium* spp. involved the attachment of sticky granular material from the surface coat of the amoeba to the ciliate after first contact [23]. As a consequence the prey ciliate remains in the vicinity of the amoeba long enough to enable circumvallation. A similar strategy was described for heliozoa; with prey being immobilised after cell to cell contact had been established, involving adhesion to a mucus like substance and extrusomes [39]. These reports show that despite their apparent slowness amoebae are capable predators able to capture even mobile prey.

### 4. Conclusions

Data established in this study for the locomotion and speed of free-living amoebae in environmental samples indicate that these predators generally are slow moving organisms, which would not be able to hunt down mobile prey by outpacing it. The speed values determined for naked and testate amoebae are in a similar range. Monopodial movement seemed to result in higher speed averages in naked and testate amoebae. No difference in speed averages between a polypodial use of lobopodia and filopodia was apparent. However, as only a limited number of individuals was examined, this has to be confirmed by additional studies.

### References


