On the kinematics-wave motion of living particles in suspension

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This work presents theoretical and experimental analyses on the kinematics-wave motion of suspended active particles in a biological fluid. The fluid is an active suspension of nematodes immersed in a gel-like biological structure, moving at a low Reynolds number. The nematode chosen for the study is *Caenorhabditis elegans*. Its motion is subjected to the time reversibility of creeping flows. We investigate how this worm reacts to this reversibility condition in order to break the flow symmetry and move in the surrounding fluid. We show that the relationship between the length of an individual nematode and the wavelength of its motion is linear and can be fitted by a theoretical prediction proposed in this work. We provide a deep discussion regarding the propulsion mechanics based on a scaling analysis that identifies three major forces acting on an individual nematode. These forces are a viscous force, a yield stress force due to gelification of agar molecules in the gel-like medium, and a bending force associated with the muscular tension imposed by the nematodes in the medium. By the scalings, we identify the most relevant physical parameters of the nematode's motion. In order to examine and quantify the motion, dynamical system tools such as FFT are used in the present analysis. The motion characterization is performed by examining (or studying) two different populations: (i) in the absence of food with starving nematodes and (ii) with well-fed nematodes. In addition, several kinematic quantities of the head, center of mass, and tail for a sample of nematodes are also investigated: their slip velocities, wavelengths, trajectories, frequency spectra, and mean curvatures. The main findings of this work are the confirmation of a linear relationship between the nematode's physical length and its motion wavelength, the identification of secondary movements in high frequencies that helps breaking the time-reversibility in which the worms are bonded, and the observation and interpretation of a systematic difference between the individual motion of well-fed and starving nematodes. *Published by AIP Publishing.* [<http://dx.doi.org/10.1063/1.4997715>]

I. INTRODUCTION

Complex fluids, such as intestinal fluid, human mucus, and even mud,^{1–5} were the basis of evolution of several microorganisms. The rheology of such fluids can substantially affect the swimming behaviour of a microorganism. Considering a viscoelastic medium, the regular

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beating pattern exhibited by a freely swimming spermatozoon is replaced with a high-amplitude and asymmetric bending of its flagellum.⁶

An interesting aspect on the mechanics of swimming is that the biomechanics of a living being must be adapted to its surroundings. For instance, small bodies seeking to swim in a viscous fluid are subjected to a principle known as kinematic reversibility in low Reynolds numbers.⁷ This principle is related to the linearity of the Stokes equation and forces microorganisms to produce highly nonlinear motions in order to break the time-reversibility to which they are bonded. Some rich examples of this nonlinear motion include flagellar^{8,9} and ciliary^{10,11} propulsion. Several works have focused on studying a specific nematode called *Caenorhabditis elegans* regarding its physical properties and aspects of low Reynolds number locomotion. Nematodes inhabit virtually all environments and are one of the most constantly found organisms on Earth. The reasons for using this nematode as a benchmark go from the easiness to manipulate them to the simplicity of its motion. We may say that 100 g of soil will typically house 300 individuals. Higher deformation speeds associated with lower internal viscous resistance are related to the shear thinning feature of an individual worm. The shape of an undulating crawler is defined by a dynamic balance between elastic, hydrodynamic, and muscular forces. As a result, the shear thinning property of the worm may influence the dynamics of motility, and shear thinning should be integrated into a full locomotory model. In a recent work,¹² researchers investigated the response of a single *C. elegans* worm to the application of tangential stresses and found out that the elastic feature of the nematode's body may be responsible for a shear thinning behavior of a suspension of *C. elegans*. Moreover, as pressure and shear strength between worm and agar increases, so does the friction, leading to the presence of greater yield-stresses. When nematodes are on the surface of this medium, they simply crawl. However, when they move inside the medium, the forces performed by their muscles end up breaking the bonding of the agar molecules, and water is released in a process called syneresis.

Berri *et al.*¹³ argue that forward locomotion of *C. elegans* in low Reynolds number flow is achieved through modulation of a single gait. They claim that its interaction with the surrounding media is highly complex, despite its biological simplicity (it has only 302 neurons), which allows it to be a very efficient swimmer even subjected to a kinematic reversibility condition due to its small size. As a collateral effect, the study of *C. elegans* has also produced important advances on image analysis techniques of moving bodies for biological purposes.¹⁴ Some of these techniques have shown us the efficiency of its undulatory motion, as shown in Fig. 1, which has inspired the production of moving micro- and nanorobots that use the same propelling principles.¹⁵

Considering that the Reynolds number related to the nematode's induced flow $Re \ll 1$, we shall refer this study as in the Stokesian realm, in contrast to the theories of inviscid flow, which might be termed as Eulerian realm. Most microorganisms move due to a periodic or near periodic motion of organelles such as cilia and flagella. Indeed time-reversal symmetry plays a key role in the selection of swimming strategies. Swimming cells, such as bacteria (prokaryotes) or spermatozoa (eukaryotes), represent the prototypical example of active soft matter.¹⁶ In addition, in the absence of inertia, the swimmer remains perpetually force and torque-free.¹⁷ In this condition, swimmers should change their shapes in a non-reciprocal fashion. In the case of *C. elegans*, flexibility or elasticity can lead to non-reciprocal shape change and thus to locomotion (Fig. 2).

Microorganisms, such as nematodes, respond to stimuli by swimming in particular directions. Such responses are called taxes. Taxes of importance are gravitaxis (or geotaxis), a response to gravity or acceleration; phototaxis, a response to light; and chemotaxis, a response to chemical gradients. Responses to shear in the ambient flow are sometimes called rheotaxis. Compensating torques due to shear and gravity produce gyrotaxis. According to Vidal-Gadea *et al.*,¹⁸ some bacteria contain magnetic particles (i.e., magnetosomes), which cause them to swim along the magnetic field lines (magnetotaxis).

Although several works have been done in the past exploring the propulsion of microorganisms immersed in liquids, specific information regarding *C. elegans* locomotion in high viscosity media (such as gels) with detailed information on the physical quantities, from the perspective of a kinematic study is still a poorly explored area. This is an important field that could apply

FIG. 1. Nematode sinusoidal like motion. The figures ($485\ \mu\text{m} \times 645\ \mu\text{m}$) indicate six different time steps during the recording. The time-step Δt between the images is $2.5 \times 10^{-3}\ \text{s}$, and the sequence illustrates the crawling motion of a single worm in the agar gel medium.

classical physical theories¹⁹ regarding propulsion and locomotion in low-Reynolds number to provide a deeper understanding on some fundamental questions of active matter. There are still several open questions on this field, mostly related to the characterization of this kind of material. Since the particles have now a metabolism and hence there is an input of energy within the

FIG. 2. Nematode's head motion from left to right and top to bottom, respectively. It is interesting to observe that even when the body maintains its sinusoidal motion in one frame, the head produces several movements in different directions when searching for food. This different motion induces secondary frequencies. The time-step Δt between figures ($485 \mu\text{m} \times 645 \mu\text{m}$) is $2.5 \times 10^{-3} \text{ s}$.

fluid in which they are immersed, the properties of this complex material may not be defined in a state of thermodynamic equilibrium.^{20,21} The present work seeks to enhance the amount of relevant data of a typical living suspension of *C. elegans* immersed in a high viscosity gel. This study aims to perform a statistical analysis on *C. elegans* size, wavelength, and velocity distribution when a population of nematodes crawls on agar gel. One of the most relevant features of this work is to highlight the difference, in terms of locomotion of well-fed and starving nematodes. Moreover, we intend to understand how these metabolic conditions change the collective behavior of a population of worms. The relative importance between the forces and time scales involved on the dynamics of the living particles (or nematodes) is also discussed and physically interpreted. Finally, we provide a spectral analysis of the motion of several individuals and show how secondary frequencies, as observed in Sec. IID, are able to break the time-reversibility to which these worms are subjected and produce a highly efficient motion in low Reynolds number flow.

A. Experimental methods

Experiments in a gel-like medium were performed using the nematode *C. elegans*. The high viscosity gel considered in this work is a mixture of water and agar molecules. During the nematode's crawling motion, fluid is released from the medium in a process called syneresis, which consists of the breaking of the agarose molecules bonds. The nematode then crawls in a thin water film that is formed around its body on a lubrication regime. *C. elegans* has 95 rhomboid-shaped muscle cells of the body wall, whose molecular makeup is very similar to the skeletal muscles of vertebrates.²²

These nematodes are allowed to swim, dig, and crawl through diverse environments due to several nerves that control their voluntary muscles and body undulations. Propulsion and bio-mechanics may be investigated owing to the abundance of biological knowledge accumulated till date.

The swimming behaviour of the nematode *C. elegans* (N2 wild type) immersed in a gel (NGM-agar plates with *Escherichia coli*) was investigated in a sealed acrylic chamber that is 2 cm in diameter and 1 mm in depth using a microscope and a high-speed camera. The *C. elegans* is a roundworm widely used for biological research that swims by generating travelling waves; organisms are approximately 400 μm in length and 80 μm in diameter.

The main part of the experiment was the nematode tracking, which is used for obtaining kinematic data such as swimming speed, beating frequency, and amplitude. The individuals were observed through an Olympus UC30 CCD camera coupled to an Olympus BX51

FIG. 3. Cumulative distribution function (CDF) of the standard normal distribution and frequency histogram of well-fed sample. In this case, 85% of individuals are smaller than 500 μm , and the length of most individuals varies between 300 μm and 400 μm .

microscope. Images were recorded with the focal plane at the centre of the chamber to avoid movies with nematode-wall interactions; out-of-plane recordings were discarded. Kinematics data consisted of an average of 20 individuals analyzed in 20 different recordings. The statistical distribution of the samples can be seen in Figs. 3 and 4. As a matter of fact, Figs. 3 and 4 illustrate the size distribution, in terms of length, of the samples of well-fed and starving nematodes. These figures illustrate that in average the nematodes size distribution of our well-fed and starving samples does not differ substantially. Thus, this geometrical difference is not the determinant factor that dictates their differences in terms of motion (dynamic behavior). Therefore, the metabolic feature can be isolated in terms of how it affects the worm's kinematics.

The nematodes swimming kinematics were obtained from the videos using an in-house software. Besides that, *WormLab*²³ was used to identify the speed of locomotion, bending angle, wavelength, and trajectories. The software extracts the nematode's centroid, and head and tail positions based on body shape and computes kinematic quantities. The nematode crawling produces the extraction of fluid from the gel. This fluid is mostly composed of a water-like buffer solution (M9 salt solution, $\mu = 1$ mPa s). Our initial experiments show that *C. elegans* exhibit a predominately two-dimensional sinusoidal beating pattern, producing a travelling wave that moves from head to tail. The head, tail, and centroid trajectories were analyzed using an in-house Fast Fourier Transform Algorithm.

II. RESULTS AND DISCUSSION

A. Preliminary characterization

The typical crawling motion of *C. elegans* was studied considering the geometry and variables defined in Fig. 5, where a is the worm's diameter, λ represents the nematode's motion

FIG. 4. Cumulative distribution function (CDF) of the standard normal distribution and frequency histogram of the starving sample. The CDF shows that 70% of individuals are smaller than 600 μm and the length of most nematodes varies between 300 μm and 400 μm .

FIG. 5. Geometrical sketch of variables a , δ , λ , θ , and v .

wavelength, δ is the *C. elegans*' motion amplitude, θ represents the bending angle, and v is the centroid velocity. The nematode's length is given by L .

A statistical analysis based on two populations of 20 individuals each was performed. In the first population, the nematodes were immersed in a gel medium with food (*Escherichia coli* bacterial suspension). For the second population of individuals, food was not available. The populations were evaluated at different days and in different growing stages. The idea of this preliminary analysis was to check whether different environmental conditions from a biological perspective could affect the nematode's motion in two very similar surrounding media.

The kinematic characteristics of the individuals were analyzed based on statistics over the population of 40 individuals analyzed (including the well-fed and the starving samples) in 40 different experiments. The relevant results of this analysis are presented in Tables I and II. The slip parameter, α , was defined as $\alpha = 1 - U_c/U_v$, where U_c is the nematode speed with respect to a fixed frame of reference and $U_v = \lambda f$ is the wave speed with f being the main frequency of the nematode's motion. Usually, when the trace left by the nematode in the base fluid is similar to a harmonic wave motion, α tends to zero. This means that nearly all bending force is converted into propulsion.

TABLE I. Nematodes motion characterization in the absence of food.

Kinematic variable	Range	Average	Standard deviation
δ (μm)	7.48–38.33	19.7875	10.65
L (μm)	193.14–1160.45	515.55	348.89
λ (μm)	101.92–613.07	276.87	183.95
v ($\mu\text{m/s}$)	25.51–170.5	76.89	42.10
a (μm)	26.36–74.49	43.20	17.31

TABLE II. Well-fed nematodes motion characterization.

Kinematic variable	Range	Average	Standard deviation
δ (μm)	5.7–158.2	22.02	33.82
L (μm)	185.49–1073.24	336.11	189.36
λ (μm)	98.14–640.38	179.18	115.20
v ($\mu\text{m/s}$)	24.56–88.94	47.79	18.02
a (μm)	17.29–68.71	33.742	11.16

B. Scalings arguments

Now, we shall provide a brief description of our living system. Let us consider a typical travelling wave $F = F(kx - \omega t)$ propagating from left to right with velocity $c = \omega/k$. When the nematode exerts a force on the agar plate, it causes syneresis or the extraction of water from a gel. The quantity of water released is inversely proportional to the agarose concentration squared. In order to move, the nematodes must bend so that the yield stress force $F_y \sim \tau_0 a^2 \alpha$ of the surrounding medium is exceeded. Here, τ_0 is the yield stress of the agar gel. This bending is directly related to the biological characteristics of the nematodes. The nematode body wall is composed of a cuticle and a single layer of longitudinal muscle cells. In nematodes, the cylindrical shape of the body tube is maintained when punctured and its diameter changes only slightly. However, nematodes are unique among worm-like organisms in lacking circumferential muscles; therefore, their motion is limited to what can be accomplished by applying longitudinal forces. In the case of bending, the net force due to increased muscle tension would shorten only one side of the local body tube, while the other side would stretch by the equilibrium of forces there.

A typical scale for this bending force is $F_b \sim M_f/\lambda$, where M_f represents the nematode's bending moment. We may write this bending moment as a function of the elastic modulus E , the moment of inertia, and the curvature of the nematode, scaling it with $M_f \sim E a^4 \delta/\lambda^2$. The viscous force, on the other hand, scales with the slip coefficient as $F_\mu \sim \mu \alpha f \lambda^2$, with μ being the carrier liquid viscosity.

The following calculation is based on the constitutive relation for the moment $M(s, t)$ in an inextensible filament of size s , representing the *C. elegans*. The total moment may be considered as $M = M_p + M_a$, where $M_p(s, t)$ is the passive moment and $M_a(s, t)$ is the active moment generated by the muscles of the nematode. The passive moment is given by the viscoelastic Voigt model.²⁴ This constitutive relation is given by

$$M_p = EIk + \mu I \frac{\partial k}{\partial t}, \quad (1)$$

where $k(s, t)$ is the curvature along the nematode and I is the nematode moment of inertia, considered to be a hollow cylindrical shell.⁶

Another force involved in the motion of the nematode is the inertial force on the liquid (which we will show to be negligible in this problem), $F_i \sim \rho \alpha f v \lambda^2 L$, where ρ is the fluid density. Based on statistics over 40 individuals, including starving and well-fed nematodes, the average slip for 40 analyzed individuals was 0.009. In this work, we have considered the elastic Young modulus proposed by Arratia *et al.*⁶ The average Young modulus used was 2659.7 Pa, and the calculated average Reynolds number was 0.035. Here, the Reynolds number is defined in the standard form as being $Re = \rho v L/\mu$, where μ is the fluid viscosity, V is the nematode's velocity, and L is its size. The bending number is defined as $Be = F_b/F_\mu$. The agarose yield stress is also important for the scaling analysis. When the nematode's bending tension is larger than the yield stress tension, the agarose gel behaves as a Newtonian fluid. In this condition, we may introduce the Bingham number as being $Bh = F_y/F_\mu$. We may notice that $F_b/F_y \sim 1$. Table III shows the main dimensional quantities, forces, and nondimensional physical parameters of our

TABLE III. Characterization of the main dimensional quantities, forces, and physical parameters averages of the nematode's locomotion.

Dimensional quantities	Forces (N)	Nondimensional quantities
$a \approx 3.80 \times 10^{-5}$ m		
$\delta \approx 2.10 \times 10^{-5}$ m		
$\lambda \approx 2.28 \times 10^{-4}$ m		
$L \approx 4.25 \times 10^{-4}$ m	$F_\mu \sim \mu \alpha f \lambda^2 \approx 1.4 \times 10^{-13}$	$\alpha \sim 10^{-3}$
$f \approx 3.00 \times 10^{-1}$ s ⁻¹	$F_b \sim M_f / \lambda \approx 8.8 \times 10^{-9}$	$F_b / F_\mu = Be \sim 10^4$
$v \approx 6.20 \times 10^{-5}$ m/s		
$E \approx 2.60 \times 10^3$ Pa	$F_i \sim \rho \alpha f v \lambda^2 L \approx 3.7 \times 10^{-15}$	$F_i / F_\mu = Re \sim 10^{-2}$
$\tau_0 \approx 5.00$ Pa	$F_y \sim \tau_0 a^2 \approx 7.22 \times 10^{-9}$	$F_y / F_\mu = Bh \sim 10^4$
$\mu \approx 1.00 \times 10^{-3}$ Pa s		
$M_f \approx 2.00 \times 10^{-12}$ N m		
$\rho \approx 1.00 \times 10^3$ kg/m ³		

living system. This scaling analysis represents every possible movement of the nematode. Moreover, a linear relationship is shown between λ and L .

Even though most of the analysis was done on worms crawling with no slip, it can be extremely important in several instances. Gray and Lissman¹⁹ conclude that $\frac{U_c}{U_v} = 0.9$ was an usual value for nematodes crawling on agar gel. On the other hand, this relation is much smaller when the nematodes are crawling rigid surfaces such as moist glass or swimming. Indeed, swimming is related to a C-shape movement and it is simply an extreme phase of an S-shaped travelling wave with wavelength longer than the worm's body.¹³

In our condition, the travelling wave is considered to be stationary relative to the substrate; thus, the slip tends to zero. Considering the works done by Parida,^{25,26} it is possible to still use the same scaling analysis. For example, according to the works by Parida,^{25,26} when the elastic modulus of the medium increases, the amplitude of the worms motion monotonically decreases. Our scale analysis estimates that the bending force $F_b = E a^4 \delta / \lambda^3$ is proportional to the worms amplitude δ . Thus, a stiffer medium demands a lower bending force for the nematode to move. However, the medium stiffness is inversely proportional to the thickness of the film after lubrication. In this condition, the lateral slip increases and the forward locomotion becomes less efficient. As shown in Fig. 6, our theoretical linear correlation given by Eq. (5) is also validated experimentally. It is possible to see that its characteristic motion remains the same regardless of the nematode size for a typical medium with $E \cong 2.6$ kPa. Consequently, the wavelength responds linearly to the nematode size. The agreement between our theory and the experimental measurements suggests that *C. elegans* uses indeed a sinusoidal locomotion. On the other hand, as mentioned by Parida,^{25,26} the stiffness of the medium may induce other characteristic shapes. Besides, during the animal reversal, for example, there is no sinusoidal pattern. As a matter of fact, a sharp turn (omega turn) results in approximately 180° change in locomotion. During the turning process, the animal suppresses its lateral head movements, and there is a deep ventral head bending.²⁷ In all these cases, the scaling analysis present in Table III represents the correct forces coupling considering the nematode and the agar dimensional quantities.

We postulate that locally, viscous effects may be extremely relevant. In the sharp edges of the nematode's tail and head, there is a concentration of viscous stresses. Although globally, in the nematode's wavelength scale, viscous and inertial effects are dominated by bending forces, there is a concentration of viscous stresses in a minor scale, related to the edges of the nematode's body. In this scale, the nematode is forced to move its parts in a highly non-linear and non-harmonic way in several higher frequencies. This motion emulates a flagellum-like motion and breaks the kinematic reversibility in which its solid boundaries are bonded.

This type of behavior is also explained anatomically. The head and body movements are controlled independently by distinct classes of motor neurons and muscles.²⁸ While the body bends are restricted to the dorsal-ventral plane, the animal can flex its head in three dimensions

FIG. 6. The average nematode size based on statistics over 15 starving individuals (black circles) and 15 well-fed nematodes (non-filled circles) as a function of the wavelength compared with the theoretical prediction (continuous line).

on specific conditions, which is called nictation. It is interesting to highlight that even though this is not common, nictation may be observed on old plates with contaminating fungi. In this scenario, the hyphal tips serve as the projections necessary for adherence and nictation.²⁹ However, on standard agar plates, *C. elegans* movement is limited to two dimensions, preventing the observation of a three-dimensional activity such as nictation, unless the nematode crawls inside the substrate. The head muscles are divided into eight radial symmetric sectors, and these are independently innervated by ten classes of motor neurons.^{22,30} As a consequence of this motor circuitry, worms can move their head through 360°.

From Table III, we may also conclude that the living suspension may be treated in the creeping flow regime, since the ratio $F_i/F_\mu = Re \ll 1$. It is also important to notice that the highest force involved in the problem is the nematode's bending force. This force is in the same order of magnitude as the yield stresses forces, and hence, the bending of the nematode's muscles is capable of breaking the agar molecules of the gel, releasing water (syneresis effect). It is also important to highlight that the bending forces are much higher than the viscous forces $F_b \gg F_\mu$. It is worth to emphasize that we do not consider elastic forces on the surrounding medium in this work. The syneresis effect is responsible for the nematode to crawl in a viscous fluid which is very similar to water, different from that used in the work of Keim *et al.*³¹

C. Theoretical prediction—Length and wavelength relation

Due to their small size, microorganisms such as bacteria, sperm cells, nematodes, and various kinds of protozoa move in the low Re number regime. In such a regime, linear viscous forces dominate the nonlinear inertial forces^{24,32,33} and locomotion must result from non-reciprocal motion in order to break time-reversal symmetry. This is the so-called “scallop theorem.” Taylor³⁴ demonstrated that a slender body could swim at low Reynolds number by generating traveling waves along its body. Nematodes, such as *C. elegans*, produce bending waves in order to move, which is an example of non-reciprocal motion. These bending waves consist of alternating phases of dorsal and ventral muscle contractions driven by the neuromuscular activity.²²

Lauga¹⁷ postulated that the scallop theorem does not hold for groups of more than one body. In this sense, a body undergoing reciprocal motion cannot swim; however, two bodies undergoing reciprocal motion with nontrivial phase differences are able to take advantage of the unsteady nature of the generated flow to move. These two bodies are able to create a collective dynamics.

This collective behavior is present in solutions with *C. elegans*. However, all analyses in this paper were made considering isolated individuals, disregarding hydrodynamic interactions.³⁵

Several observations by optical microscopy and video camera indicated that the nematode motion is harmonic, so the shape of an individual worm could be expressed, during its motion, by the following curve:

$$g(x) = C \sin\left(\frac{2\pi x}{\lambda}\right), \quad (2)$$

where $g(x)$ represents a smooth repetitive oscillation and x is the position on the horizontal direction on which the wave propagates. The wavelength λ describes the distance the wave propagates between two valleys of the sinusoidal curve and C is a calibration parameter. The total length of this curve is obtained through

$$L(\lambda) = \int_0^\lambda \left[1 + \left(\frac{dg}{dx} \right)^2 \right]^{1/2} dx, \quad (3)$$

with $C = \lambda/3$ calibrated by experimental data. Now, replacing Eq. (2) in (3) and performing the integration, we have

$$L(\lambda) = \left[\frac{2\sqrt{9 + 4\pi^2} E\left(\frac{4\pi^2}{9 + 4\pi^2}\right)}{3\pi} \right] \lambda, \quad (4)$$

where $E(x)$ represents the elliptical integral of the first kind so the expression can be simplified as

$$L(\lambda) \approx 1.72677\lambda. \quad (5)$$

As shown in Fig. 6, our theoretical linear correlation given in Eq. (5) is also validated experimentally. It is possible to see that its characteristic motion remains the same regardless of the nematode's size. Consequently, the wavelength responds linearly to the nematode's size even in its larval stage. The remarkable agreement between our theory and the experimental measurements suggests that *C. elegans* uses indeed a sinusoidal locomotion. It is interesting to observe that this prediction was proposed considering that $L \sim 2\lambda$. This condition was based on our previous experiments.

We may also compare the nematode's bending angle θ with the distance R between the head and tail. Considering a sinusoidal movement, the angle should reach its maximum at the smallest R and the minimum angle when R reaches its maximum. Figure 7 compares distance R and angle θ .

D. Non-harmonic motion analysis

Considering the scallop theorem, we may postulate that in order to move, a *C. elegans* has to conduct a non-harmonic motion. Analyzing the trajectory of the individuals, as presented in Figs. 8 and 9, we may observe a more complex non-harmonic oscillatory motion of the nematode. The head motion now is composed of different vibrational degrees of freedom in contrast with a simple harmonic motion. This condition breaks reversibility, and is necessary for the nematode to move and find food. However, the centroid trajectory is almost harmonic.

In the absence of food, the individuals tend to move faster and in different directions tracking the non-harmonic trajectories, as depicted in Fig. 8. Moreover, the worms increase their velocities and tend to crawl through higher distances. However, when a colony of bacteria is present, all individuals tend to crawl with similar velocities, i.e., proportional to the nematode's size. Under this condition, they also present fewer non-harmonic oscillations and do not explore the NGM (nematode growth medium) plate, reducing its crawling path. In the frequency domain, we may

FIG. 7. Nematode's bending angle θ and head to tail distance R (defined as $L/2$) as a function of time considering the same trajectory. It is possible to observe at points A , B , C and D how these two variables relate. At A , the bending angle is high, and the nematode's body is curved. The same behavior is seen at B and C . On the other hand, at D , the bending angle reaches its minimum and the nematode's body is fully stretched.

FIG. 8. Trajectory of 10 starving nematode's centroid taken randomly from the sample.

FIG. 9. Trajectory of 10 well-fed nematode's centroid. In this case, the individuals were taken randomly from the agar plate filled with *E. coli*.

understand how numerous modes of vibration are present in the complex nematode motion. The fast Fourier transforms were performed in *Scilab* using a script developed by the authors, and the results are shown in Figs. 10 and 11. There are typical differences among the tail, head, and centroid motions. The head presents a much more complex motion composed of several vibrational modes (degrees of freedom). Thus, there is a non-negligible energy in slightly higher frequencies. This is a direct consequence of the fact that the nematode's head must command the rest of the body, and so it responds at higher frequencies and develops a complex “bending” motion in the absence of food. The centroid follows an almost harmonic pattern showing only one mode corresponding to the fundamental frequency, i.e., having a minimum bending effort. The thinning of the tail in relation to the rest of the nematode's cylindrical body allows it to function similarly to a flagellum, presenting several motion modes and frequencies. Thus, the kinematic reversibility is overcome.

Considering that the starving nematodes tend to move faster and conduct a non-linear movement, we may expect some broadband spectrum in the frequency domain. Under this condition, the nematode is searching for food and is rapidly moving its head in different path lines. This motion transition of the nematode's head in the absence of food corresponds to a dynamically non-harmonic response of the nematode trajectories in contrast with the nearly harmonically sinusoidal periodic motion observed under favorable conditions, i.e., availability of food.

It is possible to remark that the starving nematodes present a dominant frequency, which represents the majority of its motion characteristics. However, there is some spectral spreading taking energy from this principal frequency. In this condition, the nematode starts to move its body differently from a harmonic pattern. Although this motion may enable the nematode to move in creeping flow conditions, it may also hinder its translational motion. Thus, the nematode must use some energy to bend its head in different directions.

Figures 10 and 11 show the difference from the head and centroid trajectory FFT from individuals taken from starving and colonized plates, respectively.

When food is available, all nematodes follow a very similar behavior following the *E. coli* colony. However, observing Fig. 11, we may conclude that the motion of the head is different

FIG. 10. Comparison of head (a) and centroid (b) motion of one specific individual of the starving sample with adjusted range. The dashed line shows the energy of the second harmonic.

from the centroid trajectory. This behavior is directly associated with the nematode's velocity and size, which can change significantly depending of its physical and chemical characteristics.

We may observe in Fig. 10 that the starving individuals present a spectrum with a more continuous distribution of modes of this complex oscillatory motion. Here, they show that the energy in each frequency is much higher than the energy shown in Fig. 11, for example. As shown before, when bacterial colonies are present, the nematodes tend to move slower and in well-defined harmonic sinusoidal trajectories. Under this condition, the trace left behind the nematode seems to be very similar to a sinusoidal wave and the slip coefficient should be close to zero. However, when crawling in the free surface (NGM without bacteria colonies), considering greater slip, individuals activate the “global search” mechanism, increasing their velocities, bending, and constantly changing their trajectories.

FIG. 11. Comparison of head (a) and centroid (b) motions of one specific individual of the well-fed sample with adjusted range. The dashed line shows the energy of the second harmonic.

It is also interesting to notice that the nematode's extremities excitation has its origins in viscous effects, where its head and tail describe a highly nonlinear motion with much more vibrational modes than its center of mass. On the other hand, when the nematode is hungry, seeking for food, its excitation is more influenced by biological–physiological aspects. In this regime, the development of different vibrational modes is more complex, since now its body presents a non-harmonic motion. This can be confirmed by the comparison of typical trajectories in the presence and absence of food.

III. CONCLUSIONS

In this work, we have presented a statistical analysis on the kinematics-wave motion of a suspension of *C. elegans* in a gel-like medium. We have studied two different populations from a biological perspective of the surrounding medium. These populations consist in a starving and

a well-fed population of nematodes. We have found experimentally a linear correlation between the length and the wavelength of the individuals for both populations. We proposed a theoretical correlation to justify this linear dependence. The results have indicated that *C. elegans* indeed uses sinusoidal propulsion to move in creeping flow.

We have also found that whereas the centroid of the individuals in both populations behaves nearly harmonic, their heads and tails evolve to highly non-harmonic motion. This non-linear motion is used to break the time reversibility in which they are trapped due to their small sizes, known as kinematic reversibility in low Reynolds number flows. Another important finding of the present work is the discrepancy observed in the collective motion of both populations. We observed that well-fed individuals tend to move in the direction of *E. coli* colonies with less spreading in the surrounding medium. On the other hand, a starving population collectively behaves differently, seeking for food in several possible directions and with a much more strong head motion.

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