




# U-turn trajectories of magnetotactic cocci allow the study of the correlation between their magnetic moment, volume and velocity

Daniel Acosta-Avalos<sup>1</sup>  · Agnes Chacor de Figueiredo<sup>2</sup> · Cassia Picanço Conceição<sup>3</sup> · Jayane Julia Pereira da Silva<sup>4</sup> · Kaio José Monteiro São Paulo Aguiar<sup>5</sup> · Marciano de Lima Medeiros<sup>6</sup> · Moacyr do Nascimento<sup>1</sup> · Roger Duarte de Melo<sup>1,2</sup> · Saulo Machado Moreira Sousa<sup>1</sup> · Henrique Lins de Barros<sup>1</sup> · Odivaldo Cambraia Alves<sup>7</sup> · Fernanda Abreu<sup>8</sup>

Received: 19 January 2019 / Revised: 3 May 2019 / Accepted: 9 June 2019 / Published online: 15 June 2019  
© European Biophysical Societies' Association 2019

## Abstract

Magnetotactic bacteria are microorganisms that present intracellular chains of magnetic nanoparticles, the magnetosome chain. A challenge in the study of magnetotactic bacteria is the measurement of the magnetic moment associated with the magnetosome chain. Several techniques have been used to estimate the average magnetic moment of a population of magnetotactic bacteria, and others permit the measurement of the magnetic moment of individual bacteria. The U-turn technique allows the measurement of the individual magnetic moment and other parameters associated with the movement and magnetotaxis, such as the velocity and the orientation angle of the trajectory relative to the applied magnetic field. The aim of the present paper is to use the U-turn technique in a population of uncultured magnetotactic cocci to measure the magnetic moment, the volume, orientation angle and velocity for the same individuals. Our results showed that the magnetic moment is distributed in a log-normal distribution, with a mean value of  $8.2 \times 10^{-15} \text{ Am}^2$  and median of  $5.4 \times 10^{-15} \text{ Am}^2$ . An estimate of the average magnetic moment using the average value of the orientation cosine produces a value similar to the median of the distribution and to the average magnetic moment obtained using transmission electron microscopy. A strong positive correlation is observed between the magnetic moment and the volume. There is no correlation between the magnetic moment and the orientation cosine and between the magnetic moment and the velocity. Those null correlations can be explained by our current understanding of magnetotaxis.

**Keywords** Magnetotaxis · Magnetotactic bacteria · Magnetic moment · U-turn · Coccus · Bacterial velocity

**Electronic supplementary material** The online version of this article (<https://doi.org/10.1007/s00249-019-01375-2>) contains supplementary material, which is available to authorized users.

✉ Daniel Acosta-Avalos  
dacosta00@gmail.com

<sup>1</sup> Centro Brasileiro de Pesquisas Físicas (CBPF), Rua Xavier Sigaud 150, Urca, Rio de Janeiro, RJ 22290-180, Brazil

<sup>2</sup> Universidade Federal Do Rio de Janeiro (UFRJ), Av. Athos da Silveira Ramos, Cidade Universitária, Rio de Janeiro, RJ 21941-590, Brazil

<sup>3</sup> Universidade Federal Do Amapá (UNIFAP), Rod. Juscelino Kubitschek, KM-02, Jardim Marco Zero, Macapá, AP 68903-419, Brazil

<sup>4</sup> Universidade Federal Do Rio Grande Do Norte (UFRN), Av. Sen. Salgado Filho 3000, Campus Universitário, Lagoa Nova, Natal, RN 59078-970, Brazil

<sup>5</sup> Universidade Federal Do Amazonas (UFAM), Av. General Rodrigo Octavio 6200, Coroado I, Manaus, AM 69080-900, Brazil

<sup>6</sup> Universidade Regional Do Cariri (URCA), Av. Leão Sampaio 107, Triângulo, Juazeiro do Norte, CE 63041-082, Brazil

<sup>7</sup> Universidade Federal Fluminense (UFF), Outeiro de São João Batista, Campus do Valonguinho, Centro, Niterói, RJ 24020-141, Brazil

<sup>8</sup> Instituto de Microbiologia Paulo de Goes, Universidade Federal Do Rio de Janeiro (UFRJ), Rio de Janeiro, RJ 21941-902, Brazil

## Introduction

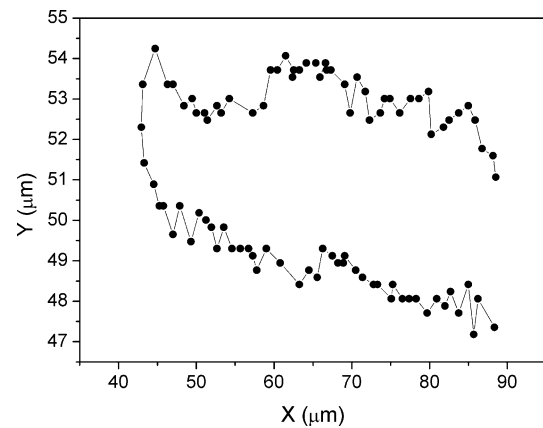
Magnetotactic bacteria (MTB) are microorganisms that contain magnetosomes that are magnetic nanoparticles of magnetite ( $\text{Fe}_3\text{O}_4$ ) or greigite ( $\text{Fe}_3\text{S}_4$ ) with a membrane and, in general, arranged in linear chains (Abreu and Acosta-Avalos 2018). The magnetosome chain confers a magnetic moment to MTB that permits them to interact passively with the geomagnetic field through the magnetic torque, which aligns the bacterial body and its swimming direction to the magnetic fields lines (Kalmijn 1981). That property is known as magnetotaxis. In optical microscopy MTB can be recognized because they start to swim when the local magnetic field direction is changed, following the new field direction. A challenge in the study of MTB is the measurement of the bacterial magnetic moment *in vivo*. Several techniques have been proposed to achieve this goal: estimate directly from the magnetosome chain by electron microscopy (Frankel and Blakemore 1980), the Langevin function (Kalmijn 1981), optical scattering and birefringence (Rosenblatt et al. 1982a, b), magnetic tweezers (Zahn et al. 2017), SQUID magnetometry (Wajnberg et al. 1986), analysis by U-turn trajectories (Esquivel and Lins de Barros 1986; De Melo and Acosta-Avalos 2017) and through rotating magnetic field trajectories (Petersen et al. 1989) among others. From those techniques, the U-turn analysis is one of the simplest techniques that permits the estimation of the magnetic moment for individual MTB. This technique is based on the analysis of the movement in the Low Reynolds number regime (Esquivel and Lins de Barros 1986). In this case, the total torque is null and the following must be satisfied:

$$T_{\text{mag}} + T_{\text{viscous}} = 0, \quad (1)$$

where  $T_{\text{mag}} = mB \sin(\gamma)$  is the magnetic torque and  $T_{\text{viscous}} = -8\pi\eta R^3(d\gamma/dt)$  is the viscous drag torque for a spherical microorganism.  $\gamma$  is the angle between the magnetic moment  $m$  and the magnetic field  $B$ , and is assumed that the magnetic moment follows the trajectory direction. In the above expressions,  $\eta$  is the water viscosity (about  $10^{-3}$  Pa s) and  $R$  is the microorganism radius.  $8\pi\eta R^3$  is known as the viscous coefficient and its expression is different for other body geometries. So, Eq. (1) can be rewritten as

$$d\gamma/dt = [mB/8\pi\eta R^3] \sin(\gamma). \quad (2)$$

Interestingly, Eqs. (1) and (2) do not include the effect of the flagellar movement. In some cases, to include the effect of the flagella, it has been proposed a correction to the viscous coefficient by a numerical factor (Chen et al. 2018) or the inclusion of an extra torque in Eq. (1) (Pichel et al. 2018). In the present study, we will ignore the flagellar effect. Figure 1 shows a U-turn observed in an optical microscope. Esquivel and Lins de Barros (1986) calculated two parameters from the U-turn curve using



**Fig. 1** U-turn trajectory example. Each point represents the position of the MTB coccus observed by videomicroscopy. The drop border is to the right and the trajectory starts in the point near to coordinates (90,47). Coordinates were obtained frame to frame and the units were converted from pixel to  $\mu\text{m}$  using a calibration ruler. Observe that in this example it is difficult to define a U-turn radius

Eq. (2): the U-turn radius and time  $\tau$ . The U-turn radius is difficult to be measured because it is necessary that the MTB swims in the focal plane during all the U-turn trajectory, and that is not the case in general. The U-turn time  $\tau$  is easier to measure from the U-turn trajectory (e.g., De Melo and Acosta-Avalos 2017). As the U-turn trajectory starts from a sudden inversion in the magnetic field direction and the trajectory is symmetrical, Eq. 2 can be rewritten as  $d\gamma/\sin(\gamma) = [mB/8\pi\eta R^3]dt$  and integrated from 0 to  $\tau/2$  in time and from  $\gamma_i \neq 0$  to  $\pi/2$  in the angle  $\gamma$  to avoid indeterminations found at  $\gamma=0$  and  $\gamma=\pi$ . Esquivel and Lins de Barros (1986) understand  $\gamma_i$  as a perturbation in the initial MTB orientation and calculate a value for it using the expression for the average MTB orientation to a magnetic field  $B$  through the Langevin equation (Kalmijn 1981):  $\langle \cos(\gamma) \rangle = L(mB/kT)$ , where  $L(x) = \coth x + 1/x$ . From that expression, it is possible to show that  $\gamma_i \approx (2kT/mB)^{1/2}$ . The U-turn time  $\tau$  becomes

$$\tau = [8\pi\eta R^3/mB] \ln(2mB/kT), \quad (3)$$

where  $k$  is the Boltzmann constant and  $T$  is the temperature (about 300 K). From Eq. (3) it is possible to estimate the value of  $m$  for each MTB observed in the microscope performing U-turn trajectories.

As far as we know, in the literature, there are no studies of simultaneous movement and magnetic measurements in individual MTB. Therefore, the aim of the present paper is to estimate the magnetic moment, the velocity and the body volume of natural MTB from a lagoon and observe their distribution and possible correlations among them.

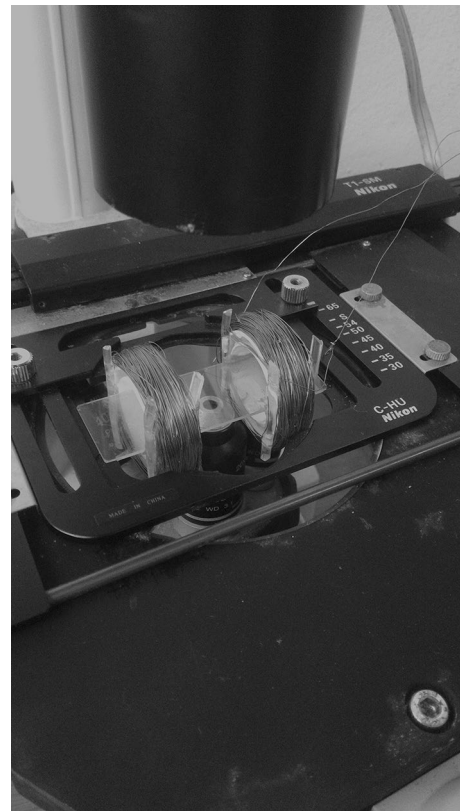
## Materials and methods

Uncultured magnetotactic cocci were collected in Rodrigo de Freitas lagoon (22°58'S, 43°12'W), an urban lagoon located in Rio de Janeiro city. The sediments were collected in 2007 and have been maintained in a glass aquarium since then, completing the aquarium water level from time to time using tap water. The local geo-magnetic parameters in Rio de Janeiro are horizontal component = 18  $\mu\text{T}$ , vertical component = - 15  $\mu\text{T}$ , and total intensity = 23  $\mu\text{T}$ .

To concentrate MTB for the experiments, a sub-sample was transferred to a specially designed flask containing a lateral capillary aperture and a small magnet generates a magnetic field aligned to the capillary aperture (Lins et al. 2003). The studied uncultured magnetotactic cocci were south-seeking and swam towards the capillary facing the north pole of a magnet. After 5 min, samples were collected with a micropipette.

Samples of MTB were prepared for scanning electron microscopy (SEM) as follows: MTB in a drop were concentrated in the border using a magnet, then MTB were fixed with 2.5% glutaraldehyde in 0.1 sodium cacodylate buffer, washed with fresh water and dehydrated in acetone series, later gold sputtered and observed in a JEOL JSM-6490LV. These magnetically enriched cells were transferred to formvar/carbon-coated copper grids and observed by transmission electron microscopy (Tecni Spirit, FEI Company).

To estimate the magnetic moment of MTB, the U-turn method was used to calculate the U-turn time  $\tau$ . On the stage of an inverted microscope (Nikon Eclipse TS100) was set a pair of coils connected to a DC power supply and fixed to a glass microscope slide where the collected drop with MTB was placed (Fig. 2). The used lens had magnification of 40 $\times$  and numerical aperture of 0.55 allowing the measurement of the MTB radius  $R$ . The magnetic field generated by the coils was of about 280  $\mu\text{T}$ . An electric circuit for changing the voltage polarity (reversal of current) was connected between the power supply and coils, leading to the inversion of the magnetic field direction when the button was turned on or off. After two magnetic field inversions, the MTB perform U-turn trajectories (Fig. 1). The magnetic moment  $m$  can be estimated using Eq. (3). To calculate  $\tau$ , the following procedure was performed: U-turn trajectories were recorded, at a rate of 82 fps, in the inverted microscope with a digital camera (Lumera Infinity 1). The coordinates of the U-turn trajectories were obtained using the software *ImageJ* (NIH—USA). The coordinates were in pixel units and the conversion to  $\mu\text{m}$  was done using a calibration ruler that consists of a 1 mm line divided into 100 parts. In the experimental



**Fig. 2** Experimental setup for observing MTB. A pair of coils was attached to microscope glass slide in such a way that the whole structure fits in the microscope stage over the objective lens in an inverted microscope Nikon TS100. The objective lens was 40 $\times$ . The wires to the right are connected to a DC power supply to generate a magnetic field between the coils

setup, the external magnetic field is applied in the  $x$ -direction, meaning that the  $x$ -coordinate of the U-turn in Fig. 1, as function of time, must be two straight lines with different slopes (Fig. 3a). The U-turn time  $\tau$  must be the time necessary for the change of slope and can be calculate through the derivative  $dx/dt$  (Fig. 3b). As  $\tau$  depends on the radius of the microorganism, a table of theoretical values for  $\tau/R^3$  using Eq. (3), as a function of  $m$  and maintaining  $\eta$ ,  $B$  and  $T$  constant, permits the determination of the value of  $m$  for every MTB through the comparison with the experimental values of  $\tau/R^3$ .

The axial velocity was measured analyzing the movement before the magnetic field inversion associated with the U-turn, assuming that the MTB trajectory is similar to a cylindrical helix. In this case, if the helix axis is aligned to the magnetic field direction then the coordinates must have the following parametrization:

$$x'(t) = x_0 + V_{ax} \times t, \quad (4a)$$

$$y'(t) = y_0 + r \times \cos(2\pi ft + \phi_0), \quad (4b)$$

**Fig. 3** **a** x-coordinate of the U-turn trajectory (Fig. 1) as function of time. MTB starts to move from the border of the drop when the magnetic field inverts its direction. Another sudden inversion in the magnetic field direction produce the U-turn curve observed in Fig. 1 and the x-coordinate shows that V shape. **b** Derivative of x. The transition time between the negative and positive velocities is identified as the U-turn time:  $\tau = t_f - t_i$ , where  $t_i$  is the initial time and  $t_f$  the final time of the turn. In this case,  $\tau = 0.036$  s. **c** x coordinate, corresponding to Fig. 1, before the second inversion in the magnetic field direction. The inclination of the straight line corresponds with  $V_x$ . In this case,  $V_x = -84.3$   $\mu\text{m/s}$ . **d** y-coordinate, corresponding to Fig. 1, before the second inversion in the magnetic field direction. The inclination of the straight line corresponds with  $V_y$ . In this case  $V_y = 4.9$   $\mu\text{m/s}$ . From  $V_x$  and  $V_y$  values, the axial velocity becomes  $V_{ax} = 84.5$   $\mu\text{m/s}$

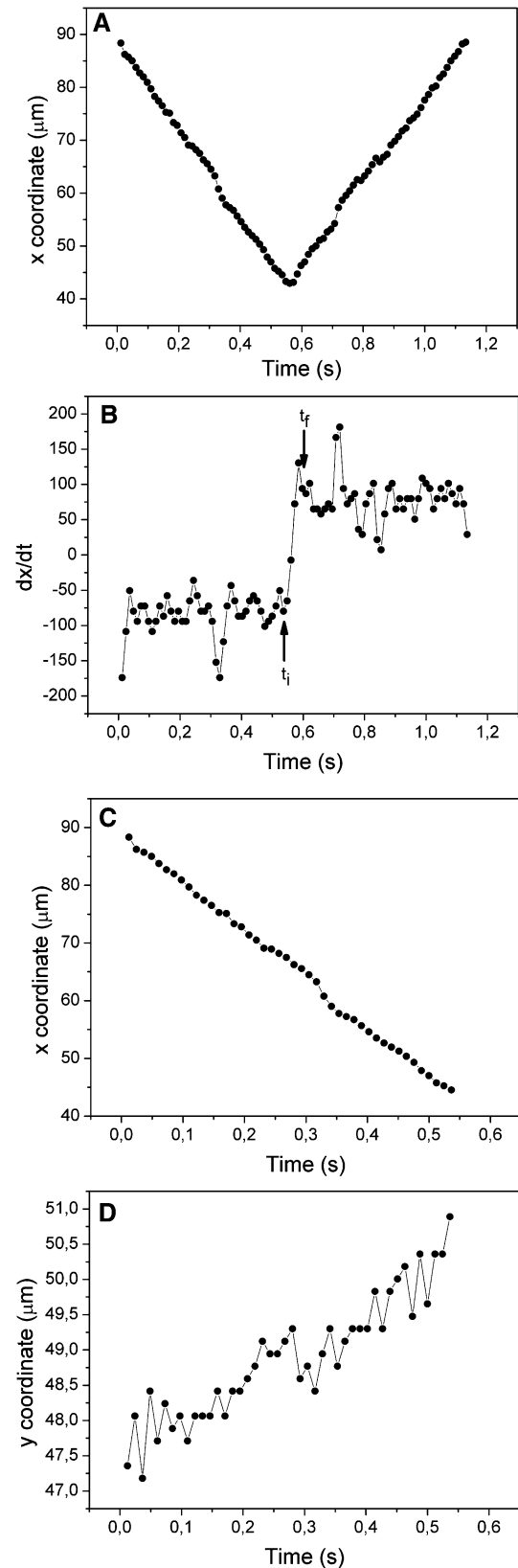
where  $x_0$  and  $y_0$  are initial values of the coordinates,  $V_{ax}$  is the axial velocity,  $r$  is helix radius,  $f$  is the helix frequency and  $\phi_0$  is the phase constant. However, the trajectory is not fully parallel to the magnetic field direction because of thermal perturbations that disorient the bacterial swimming (Kalmijn 1981). In the case that the trajectory is tilted relative to the magnetic field by an angle  $\theta$ , the coordinates must have the following expressions:

$$x(t) = x' \cos \theta + y' \sin \theta = r \sin \theta \cos(2\pi ft + \phi_0) + V_{ax} \times t \times \cos \theta, \quad (5a)$$

$$y(t) = -x' \sin \theta + y' \cos \theta = r \cos \theta \cos(2\pi ft + \phi_0) - V_{ax} \times t \times \sin \theta. \quad (5b)$$

The observed coordinates  $x$  and  $y$  as function of time must be oscillating functions with linear tendencies. If  $\theta$  is near  $0^\circ$  then  $x(t)$  must be similar to a straight line and  $y(t)$  must be an oscillating function with an inclination. An example of that can be observed in Fig. 3c, d. From that figures, the inclinations correspond to  $V_x = V_{ax} \times \cos \theta$  and  $V_y = V_{ax} \times \sin \theta$ , respectively, and  $V_{ax} = (V_x^2 + V_y^2)^{1/2}$ . The inclination for each coordinate is calculated through a linear fit as a function of time for the curve before the magnetic field inversion (Fig. 3c, d). In general, it is not easy to calculate  $r$  and  $f$  because the coordinate  $y(t)$  has a complex oscillating behavior, similar to the sum of two oscillating functions with two frequencies and radiuses. Even with that complex oscillating behavior in  $y(t)$ , the axial velocity can be calculated from the time derivatives shown in Eqs. 5a, 5b and in Fig. 3c, d.

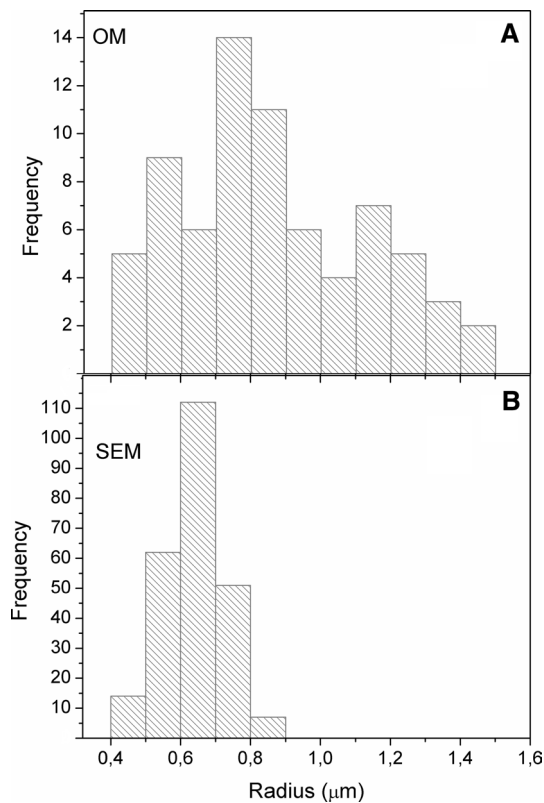
All graphics and analysis were done using the software *Microcal Origin* and the statistics with the software *Graph-Pad InStat* and *Oriana* for circular statistics.



**Table 1** Statistics for MTB radius, velocity, trajectory angle  $\theta$ ,  $\cos(\theta)$ , U-turn time and magnetic moment calculated using the U-turn time method

	Mean value	SD	<i>N</i>
Radius by SEM	0.64 $\mu\text{m}$	0.08 $\mu\text{m}$	247
Radius by OM	0.86 $\mu\text{m}$	0.26 $\mu\text{m}$	72
Velocity $V_{ax}$	85.6 $\mu\text{m/s}$	31.5 $\mu\text{m/s}$	72
Trajectory angle $\theta$	3.2°	5.7°	72
$\cos(\theta)$	0.993	0.0104	72
U-turn time $\tau$	0.07 s	0.04 s	72
Magnetic moment <i>m</i> (U-turn)	$8.2 \times 10^{-15} \text{ Am}^2$	$7.3 \times 10^{-15} \text{ Am}^2$	71
Magnetic moment (TEM)	$6.8 \times 10^{-15} \text{ Am}^2$	$4.3 \times 10^{-15} \text{ Am}^2$	7
Magnetic moment (paramagnetic model)	$4.2 \times 10^{-15} \text{ Am}^2$	–	–

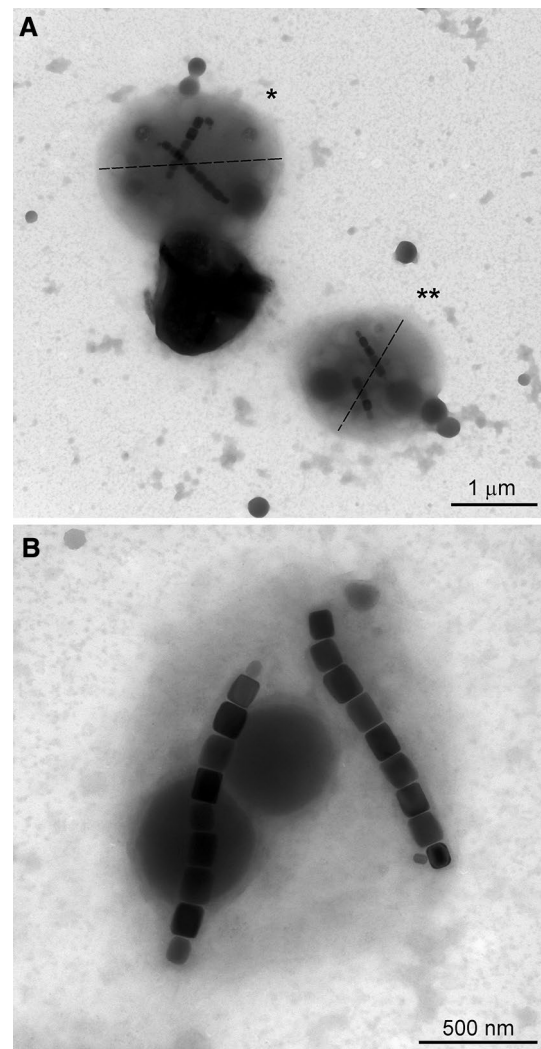
SEM scanning electron microscopy, OM optical microscopy, SD standard deviation, *N* sample size



**Fig. 4** **a** Histogram of MTB radii measured by optical microscopy (OM). Those values were obtained from the videos recorded for the U-turn analysis. As can be seen, two well-defined distributions are present, one with maximum at about 0.75  $\mu\text{m}$  and another maximum at about 1.15  $\mu\text{m}$ . **b** Histogram of MTB radii measured by scanning electron microscopy (SEM) images. In this case, only one distribution is observed with maximum at about 0.65  $\mu\text{m}$

### Results and discussion

Table 1 shows the results for the size of the uncultured magnetotactic cocci measured by SEM and by optical microscopy (OM). The mean values apparently are different (*t* test,

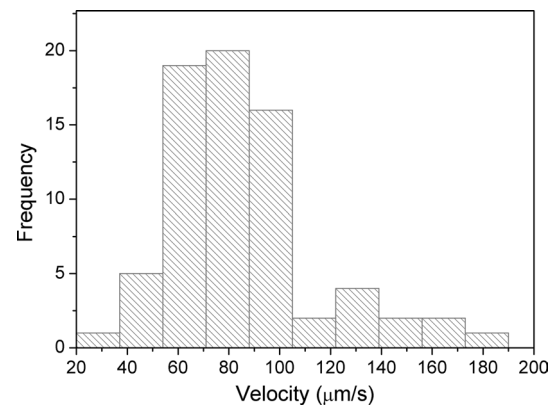


**Fig. 5** Transmission electron microscopy images of uncultured magnetotactic cocci. **a** Uncultured magnetotactic cocci showing different sizes. In the figure, the diameter is indicated by a dashed line. For the bacterium marked with asterisk, the diameter is 2  $\mu\text{m}$  and for the bacterium marked with double asterisk it is 1.5  $\mu\text{m}$ . **b** Magnetotactic coccus showing two non-parallel magnetosome chains

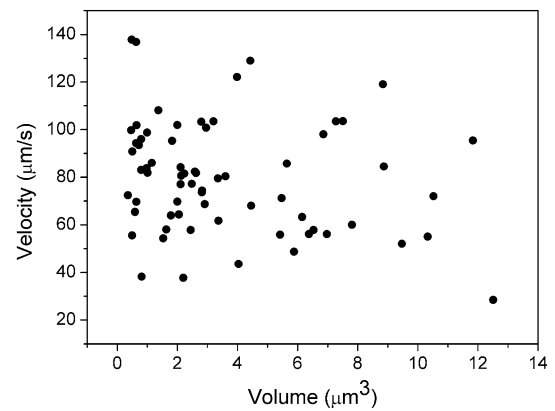
$p < 0.0001$ ) but observing the histograms for both distributions (Fig. 4), it is clear that by OM two size groups were measured, one with maximum at about  $0.75 \mu\text{m}$  and the other at about  $1.15 \mu\text{m}$ . The size distribution observed by SEM (Fig. 4b) has a peak at about  $0.65 \mu\text{m}$  and is similar to the first distribution observed by OM (Fig. 4a). Figure 5a shows TEM images of cocci with both sizes, being the majority in the first group. Video 1 (supplementary material) shows an example of swimming cocci where bacteria from both size groups can be observed. This shows that the size estimation using OM is trustable when compared with a well-established technique.

As magnetotaxis is related to the presence of the intracellular magnetosome chain, because it confers a magnetic moment to the magnetotactic microorganism, it is interesting to discuss their distribution in the uncultured magnetotactic cocci. Figure 5b shows magnetotactic cocci with two magnetosome chains. Each magnetosome chain has an average number of seven nanoparticles ( $SD = 2$ ,  $N = 16$ ). Assuming magnetite as the magnetic mineral, it is possible to estimate the average magnetic moment for the uncultured magnetotactic cocci, as done by Frankel and Blakemore (1980). Considering each nanoparticle as a parallelepiped, it is possible to calculate its magnetic moment as  $V \times M_V$ , where  $V$  is the volume and  $M_V$  is the magnetic moment per unit volume of magnetite ( $0.48 \text{ Am}^2/\text{cm}^3$ ). Each magnetosome has an average length of  $115 \text{ nm}$  ( $SD = 22 \text{ nm}$ ,  $N = 89$ ), width of  $93 \text{ nm}$  ( $SD = 23 \text{ nm}$ ,  $N = 89$ ) and width to length ratio of  $0.79$  ( $SD = 0.11$ ,  $N = 89$ ) which is characteristic for single-domain magnetite nanoparticles. Each magnetosome presents an average magnetic moment of  $5.3 \times 10^{-16} \text{ Am}^2$  ( $SD = 2.7 \times 10^{-16} \text{ Am}^2$ ,  $N = 89$ ). Each magnetotactic coccus has an average magnetic moment of  $6.8 \times 10^{-15} \text{ Am}^2$  ( $SD = 4.3 \times 10^{-15} \text{ Am}^2$ ,  $N = 7$ ) assuming that both magnetosome chains are parallel. Unfortunately, the flagella were not observed in TEM images. In other magnetotactic cocci, it is common to observe one tuft of flagella (Nogueira and de Barros 1995) or two parallel tufts of flagella as in *Magnetococcus marinus* strain MC-1 (Felfoul et al. 2016) or in *Magnetofaba australis* strain IT-1 (Araujo et al. 2016). As both magnetosome chains are not parallel in general, the resultant magnetic moment must present an inclination relative to the orientation of the tufts of flagella present in the magnetotactic coccus.

The statistics for the axial velocity  $V_{ax}$  are shown in Table 1 and the corresponding histogram in Fig. 6. The value of the mean velocity is in agreement with the velocity measured in other MTB (see, for example, Kalmijn 1981; Zhang et al. 2012). Removing the highest velocity outliers, Fig. 7 shows that there is no correlation between the MTB volume and velocity (Spearman rank correlation  $r = -0.21$ , significance of  $r$ :  $p = 0.08$ ).



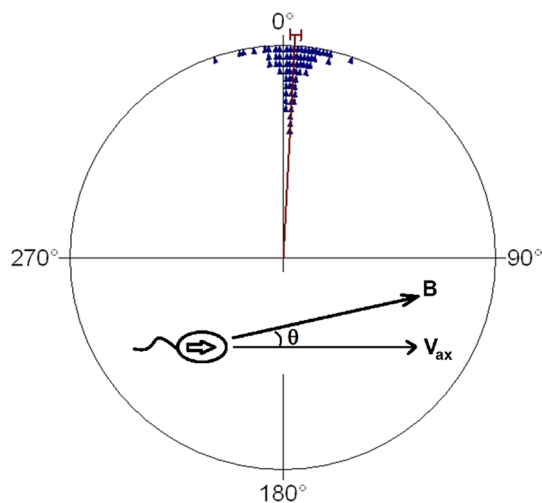
**Fig. 6** Histogram of MTB axial velocities  $V_{ax}$ . Two local maxima are observed, being the great majority of the individuals in the first distribution with maximum at about  $80 \mu\text{m/s}$ . The second distribution has less individuals and its maximum is at about  $130 \mu\text{m/s}$



**Fig. 7** Axial velocity  $V_{ax}$  as function of the MTB volume. There is no correlation between both variables (Spearman rank correlation  $r = -0.21$ , probability  $p = 0.08$  of  $r$  be significantly different from zero)

Through the axial velocity components,  $V_x$  and  $V_y$ , it is possible to calculate the angle  $\theta$  between the magnetic field and the MTB trajectory as discussed in Materials and Methods. Table 1 shows the mean angle and Fig. 8 shows their circular histogram. The applied magnetic field was directed in the  $0^\circ$  direction, and Fig. 8 shows that MTB swam following the magnetic field direction as expected for magnetotaxis. Also in Table 1 is shown the statistics for  $\cos(\theta)$ . Its mean value is  $0.993$  meaning a good MTB orientation related to the applied magnetic field.

Kalmijn (1981) analyzed the statistics for  $\cos(\theta)$  assuming that MTB behave as paramagnetic particles. In this way, a Boltzmann probability distribution can be applied to calculate the mean values of  $\cos(\theta)$ :  $\langle \cos(\theta) \rangle$  and its variance  $\sigma^2$ . The combination of  $\langle \cos(\theta) \rangle$  and  $\sigma^2$  permits the determination of the energy quotient  $X = mB/kT$ ,



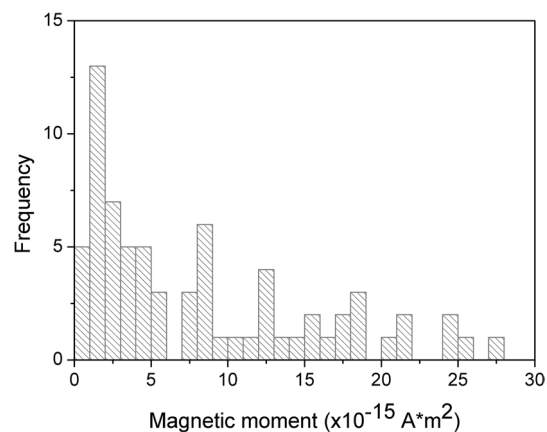
**Fig. 8** Circular histogram of the orientation angles  $\theta$ . Each value of  $\theta$  is represented by a triangle. The straight line represents the mean angle ( $3.2^\circ$ ) and the bar at the end of the line represents the standard deviation ( $5.7^\circ$ ). The magnetic field is directed in the  $0^\circ$  direction. The water drop border is in the line  $90^\circ$ – $270^\circ$ . The insert inside the circle represents the meaning of the angle  $\theta$ : it is the angle between the axial velocity  $V_{ax}$  and the magnetic field  $B$ . Both are not parallel because of thermal perturbations that disorient the bacterial swimming (Kalmijn 1981)

where the product  $mB$  is the magnetic energy and  $kT$  represents the thermal energy (being  $k$  the Boltzmann constant and  $T$  the absolute temperature). Assuming that the MTB magnetic moment is parallel to its motility axis, Kalmijn (1981) showed that  $\langle \cos(\theta) \rangle = \coth(X) - 1/X$  and  $\sigma^2 = 1 - \coth^2(X) + 1/X^2$ . Simple math permits to show that

$$X = (2 \langle \cos(\theta) \rangle) / (1 - \langle \cos(\theta) \rangle^2 - \sigma^2). \tag{6}$$

Using  $\langle \cos(\theta) \rangle = 0.993$  and  $\sigma^2 = 0.000107$ , we obtain  $X = 288.1$ . As  $kT \approx 4.1 \times 10^{-21}$  J for  $T = 300$  K and  $B = 2.8 \times 10^{-4}$  T,  $m = 4.2 \times 10^{-15}$  Am<sup>2</sup>, which is a good value for natural samples (e.g., Petersen et al. 1989). That value of  $m$  represents an average for all the analyzed sample and is in good agreement with the average value of the bacterial magnetic moment measured by TEM and to the median value of the magnetic moment distribution ( $5.4 \times 10^{-15}$  Am<sup>2</sup>).

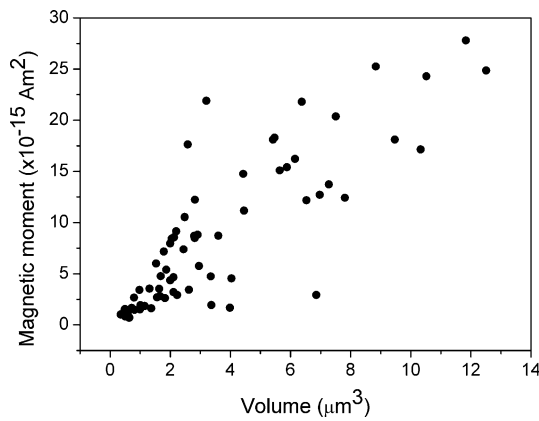
Table 1 shows the statistics for the U-turn time  $\tau$  that has a mean value of 0.07 s and a standard deviation of 0.04 s, meaning that the distribution has a large amplitude (minimum of 0.02 s and maximum of 0.3 s). As the experimental measurement of  $\tau$  does not depend on the measurement of the MTB volume it is interesting asking for the correlation between them. There is a positive correlation among both parameters (Spearman rank correlation  $r = 0.275$ , significance of  $r$ :  $p = 0.022$ ). The value of  $r$  is low, meaning a weak correlation between the U-turn time and the volume, and a tendency to bigger MTB have higher U-turn times.



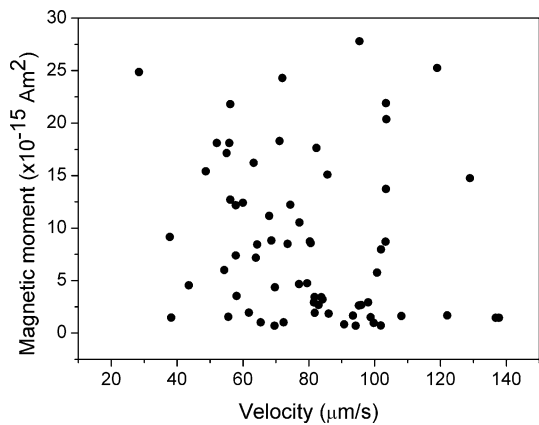
**Fig. 9** Histogram of MTB magnetic moments obtained using the U-turn technique. As can be seen, the distribution is complex and a first Gaussian-like distribution is observed until about  $6 \times 10^{-15}$  Am<sup>2</sup>, with maximum at about  $1.4 \times 10^{-15}$  Am<sup>2</sup>. Other maxima are observed, as one at  $8.5 \times 10^{-15}$  Am<sup>2</sup> and another at  $12.5 \times 10^{-15}$  Am<sup>2</sup>. This complex distribution produces a mean value at  $8.2 \times 10^{-15}$  Am<sup>2</sup> and median value at  $5.4 \times 10^{-15}$  Am<sup>2</sup>. Interestingly, the median value is similar to the average magnetic moment calculated using the expressions for  $\langle \cos(\theta) \rangle$  and  $\sigma^2$  (Eq. 6):  $4.2 \times 10^{-15}$  Am<sup>2</sup>

From that tendency, it is difficult to infer the tendency of the magnetic moment as function of the volume, because the U-turn time is a function of the magnetic torque (that must decrease the value of  $\tau$  when it increases) and of the viscous torque (that must increase the value of  $\tau$  when it increases). Using the U-turn technique, it is possible to measure the individual value of  $m$  for each MTB observed. Figure 9 shows the histogram for MTB’s magnetic moments. As is shown, the distribution has a large tail to the side of larger magnetic moments, similar to a log-normal distribution. The distribution shows a peak at about  $1.4 \times 10^{-15}$  Am<sup>2</sup>, which is a common value measured by several techniques (Abreu and Acosta-Avalos 2018). The higher values observed can be associated with the presence of bigger cells as shown in the size histogram (Fig. 4). In Table 1 is shown the statistics for the whole set of magnetic moment values. The standard deviation is high because of the non-symmetrical distribution observed in the histogram (Fig. 9).

Figure 10 shows the magnetic moment as a function of volume. There is a positive correlation between them (Spearman rank correlation  $r = 0.649$ , significance of  $r$ :  $p < 0.0001$ ). So, bigger MTB have bigger magnetic moments. This must be related to a continuous magnetosome production during the bacterium life cycle, in such a way that in the moment of cellular division it has enough magnetosomes to share between the new bacteria. A similar observation has been done in *Candidatus Magnetoglobus multicellularis* (Perantoni et al. 2009). Figure 11 shows the magnetic moment as function of velocity, and as happens with the velocity as function of the volume, there is no correlation between both



**Fig. 10** Magnetic moment  $m$  as function of MTB volume. It is observed a significant positive correlation (Spearman rank correlation  $r=0.649$ , significance of  $r: p<0.0001$ )



**Fig. 11** Magnetic moment  $m$  as a function of the axial velocity  $V_{ax}$ . In the same way as happens with  $V_{ax}$  and the volume (Fig. 7) there is no correlation between both parameters (Spearman rank correlation  $r=-0.17$ , significance of  $r: p=0.16$ )

variables (Spearman rank correlation  $r=-0.17$ , significance of  $r: p=0.16$ ). The correlation among the magnetic moment and  $\cos(\theta)$  is not significant (Spearman coefficient  $r=0.022$ , significance of  $r: p=0.85$ ).

The U-turn technique allows the measurement of the magnetic moment of individual MTB and also the measurement of parameters associated with the relative orientation (angle  $\theta$ ) and kinematics (the velocity  $V_{ax}$ ). The magnetic moment histogram (Fig. 9) shows that in natural samples its distribution is non-symmetrical and can be complex. The determination of an average magnetic moment for the studied sample, using the statistical method of Kalmijn (1981), hides that complexity. The value of  $4.2 \times 10^{-15} \text{ Am}^2$  is a good value compared with the average magnetic moment calculated from the magnetosome chains, but Fig. 9 shows a peak

at about  $1.4 \times 10^{-15} \text{ Am}^2$ , a value that has been measured as the MTB magnetic moment in natural samples and cultures [Abreu and Acosta Avalos 2018]. In our study, the three methods used to estimate the average magnetic moment of the magnetotactic cocci sample (U-turn, paramagnetic model and magnetosome observation) provided similar estimates of the average MTB magnetic moment.

The magnetic moment does not correlate with the velocity, in agreement with our understanding of magnetotaxis: the magnetic field only produces a torque to align the magnetosome chain and in consequence the bacterial body, and not produce any force to move the MTB through the water. Also, the magnetic moment shows a positive correlation with the volume. So, bigger MTB carry higher values of  $m$ . Also, the magnetic moment does not correlate with  $\cos(\theta)$ , considered to be a parameter related to the degree of orientation to the magnetic field. It means that MTB with higher magnetic moment are not better aligned to the magnetic field. This can mean that the magnetic moment in our natural samples is so high in each MTB that all of them are sufficiently aligned and the deviation from  $\cos(\theta) = 1$  (perfect alignment) can be due to thermal perturbations or (an) other kind(s) of perturbation(s).

## Conclusion

In conclusion, the U-turn technique was used for the measurement of the individual magnetic moments in a population of the uncultured magnetotactic cocci, together with their velocity and size. The use of the statistical technique based on the Langevin function produced a good value for the mean magnetic moment, similar to the average value obtained by TEM observations. With the measurement of the magnetic moment in individual MTB it was possible to analyze the correlation between the magnetic moment and the volume, the velocity and the coefficient of alignment  $\cos(\theta)$ . The magnetic moment distribution obtained is similar to a log-normal distribution which implies that the average value is not a good representative value for the magnetic moment when compared to the median value. The magnetic moment shows a strong positive correlation with the volume, and a null correlation with velocity and  $\cos(\theta)$ . The null correlations can be explained by the current understanding of magnetotaxis: the magnetic field only orients the bacterial body through the magnetic torque.

**Acknowledgements** Cassia Picanço Conceição, Jayane Julia Pereira da Silva, Kaio José Monteiro São Paulo Aguiar and Marciano de Lima Medeiros thank the financial support for the 3er EAFEX (Escola Avançada de Física Experimental do CBPF) done in February 2018. We thank also the support of LABNANO-CBPF for SEM. F. Abreu acknowledges support from FAPERJ, Conselho Nacional de



Desenvolvimento Científico e Tecnológico (CNPq) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and thanks the microscopy facilities CENABIO-UFRJ and UniMicro-UFRJ.

## References

- Abreu F, Acosta-Avalos D (2018) Biology and physics of magnetotactic bacteria. In: Barakat KM (ed) Bacteriology, 1st edn. IntechOpen, London. DOI: 10.5772/intechopen.79965.
- Araujo ACV, Morillo V, Cypriano J et al (2016) Combined genomic and structural analyses of a cultured magnetotactic bacterium reveals its niche adaptation to a dynamic environment. *BEM Genom* 17(Suppl 8):726
- Chen H, Zhang SD, Chen L, Cai Y, Zhang WJ, Song T, Wu LF (2018) Efficient genome editing of *Magnetospirillum magneticum* AMB-1 by CRISPR-Cas9 system for analyzing magnetotactic behavior. *Front Microbiol* 9:1569
- De Melo RD, Acosta-Avalos D (2017) The swimming polarity of multicellular magnetotactic prokaryotes can change during an isolation process employing magnets: evidence of a relation between swimming polarity and magnetic moment intensity. *Eur Biophys J* 46:533–539
- Esquivel DMS, Lins de Barros HGP (1986) Motion of magnetotactic microorganisms. *J Exp Biol* 121:153–163
- Felfoul O, Mohammadi M, Taherkhani S et al (2016) Magneto-aerobic bacteria deliver drug-containing nanoliposomes to tumour hypoxic regions. *Nat Nanotechnol* 11:941–947
- Frankel RB, Blakemore RP (1980) Navigational compass in magnetic bacteria. *J Magn Magn Mater* 15–18:1562–1564
- Kalmijn AJ (1981) Biophysics of geomagnetic field detection. *IEEE Trans Magn* 17:1113–1124
- Lins U, Freitas F, Keim CN, de Barros HL, Esquivel DMS, Farina M (2003) Simple homemade apparatus for harvesting uncultured magnetotactic microorganisms. *Br J Microbiol* 34:111–116
- Nogueira FS, de Barros HGPL (1995) Study of the motion of magnetotactic bacteria. *Eur Biophys J* 24:13–21
- Perantoni M, Esquivel DMS, Wajnberg E, Acosta-Avalos D, Cernichiaro G, de Barros HL (2009) Magnetic properties of the microorganism *Candidatus Magnetoglobus multicellularis*. *Naturwissenschaften* 96:685–690
- Petersen N, Weiss DG, Vali H (1989) Magnetic bacteria in lake sediments. In: Lowes F (ed) Geomagnetism and Paleomagnetism. Kluwer, Amsterdam-Berlin, pp 231–241. DOI: 10.1007/978-94-009-0905-2\_17
- Pichel MP, Hageman TAG, Khalil ISM, Manz A, Abelmann L (2018) Magnetic response of *Magnetospirillum gryphiswaldense* observed inside a microfluidic channel. *J Magn Magn Mater* 460:340–353
- Rosenblatt C, de Araujo FFT, Frankel RB (1982) Birefringence determination of magnetic moments of magnetotactic bacteria. *Biophys J* 40:83–85
- Rosenblatt C, de Araujo FFT, Frankel RB (1982) Light scattering determination of magnetic moments of magnetotactic bacteria. *J Appl Phys* 53:2727–2729
- Wajnberg E, de Souza LHS, de Barros HGPL, Esquivel DMS (1986) A study of magnetic properties of magnetotactic bacteria. *Biophys J* 50:451–455
- Zahn C, Keller S, Toro-Nahuelpan M, Dorscht P, Gross W, Laumann M, Gekle S, Zimmermann W, Schuler D, Kress H (2017) Measurement of the magnetic moment of single *Magnetospirillum gryphiswaldense* cells by magnetic tweezers. *Scientific Reports* 7:3558
- Zhang WY, Zhou K, Pan HM, Yue HD, Jiang M, Xiao T, Wu LF (2012) Two genera of magnetococci with bean-like morphology from intertidal sediments of the Yellow Sea, China. *Appl Environ Microbiol* 78:5606–5611

**Publisher's Note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.