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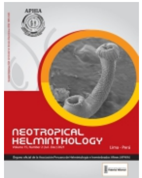

ORIGINAL ARTICLE / ARTÍCULO ORIGINAL

INVARIANT CORRELATION WITH SPECIES-SPECIFIC COMPOSITE FILTERS FOR THE RECOGNITION OF TRICHODINIDS (CILIOPHORA: PERITRICHIDA) PARASITIZING *OREOCHROMIS NILOTICUS* (LINNAEUS, 1758) BASED ON MORPHOLOGICAL METHODS

CORRELACIÓN INVARIANTE CON FILTROS COMPUESTOS ESPECÍFICOS PARA EL RECONOCIMIENTO DE TRICODÍNIDOS (CILIOPHORA: PERITRICHIDA) PARASITIZANDO *OREOCHROMIS NILOTICUS* (LINNAEUS, 1758) BASADO EN MÉTODOS MORFOLÓGICOS

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ABSTRACT

Trichodinids are the most common ectoparasites of both freshwater and marine fishes. Their generic identification is relatively easy, while the specific diagnosis is laborious due to the high intraspecific variability of some species. Four species of the genus *Trichodina* from the skin and fins of *Oreochromis niloticus* (Linnaeus, 1758) Egyptian black variety were identified by means of morphological methods and the use of digital correlation invariant to position, rotation and scale by species-specific composite filters. *Trichodina magna* Van As Bassson, 1989 and *Trichodina nigra* Lom, 1961 represent new records of host and geographic location, while *Trichodina centrostrigata* Van As, Basson & Van As, 1998 and *Trichodina heterodentata* Duncan, 1977, have already been reported for *O. niloticus* in Mexico. The automatic identification of the four species studied was done through the development and application of a mathematical algorithm within a recognition process of objects (invariant digital correlation), based on the frequency contents of the parasite species. This algorithm essential characteristic is its use to recognize the object in spite of the fact that its location level has changes of position, rotation and scale.

Keywords: *Trichodina* – *Oreochromis niloticus* – taxonomy – automatic systems – invariant correlation

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RESUMEN

Los tricodínidos son los ectoparásitos más comunes en los peces marinos y de agua dulce. Su identificación genérica es relativamente fácil, mientras que el diagnóstico específico es laborioso debido a la alta variabilidad intraespecífica de algunas especies. Se identificaron cuatro especies del género *Trichodina* de la piel y aletas de *Oreochromis niloticus* (Linnaeus, 1758) variedad negra egipcia mediante métodos morfológicos y el uso de correlación digital invariante a la posición, rotación y escala mediante filtros compuestos específicos de la especie. *Trichodina magna* Van As y Bassson, 1989 y *Trichodina nigra* Lom, 1961 representan nuevos registros de hospedante y ubicación geográfica, mientras que *Trichodina centrostrigata* Van As, Basson & Van As, 1998 y *Trichodina heterodentata* Duncan 1977, ya hayan sido reportados para *O. niloticus* en México. La identificación automática de las cuatro especies estudiadas se realizó mediante el desarrollo y aplicación de un algoritmo matemático dentro de un proceso de reconocimiento de objetos (correlación digital invariante), basado en los contenidos de frecuencia de la especie del parásito. Esta característica esencial del algoritmo se usa para reconocer el objeto a pesar de que su nivel de ubicación tenga cambios de posición, rotación y escala.

Palabras clave: Trichodina – *Oreochromis niloticus* – taxonomía – sistemas automáticos – correlación invariante

INTRODUCTION

Ciliated protozoa of the genus *Trichodina* Ehrenberg, 1830 are the most common ectoparasites of both freshwater and marine fishes (Basson *et al.*, 1983; Dobberstein & Palm, 2000) capable in some cases of inflicting heavy damage to their hosts with resultant mortalities (Lom, 1999). Particular species of *Trichodina* may be highly susceptible to organic pollutans (Marcogliense *et al.*, 1998), in contrast to most, *Trichodina* species where intensity of infections actually increases in polluted conditions (Khan, 1991; Khan *et al.*, 1994; Wang *et al.*, 2020) Yeomans *et al*., 1997; Khallaf *et al*., 2020; I Tayel *et al*., 2020). The genus *Trichodina* comprises more than 170 species, but despite their economical importance and high frequency in the aquatic environment, the exact identification of trichodinid ciliates often remains unclear (Arthur & Lom, 1984; Islas-Ortega & Aguilar-Aguilar, 2014; Khallaf *et al*., 2020).

Parasites identification is considered to be of great importance, even though it is complicated, particularly in organisms that are closely related. The specific diagnosis is slow, laborious and because although the stain used to identify is quick and simple, different specimens impregnate differently and can be also in different stages of growth, so consultations with experts on taxonomic group will be require (Rodriguez-Santiago *et al*., 2019).

The use of automated methods may be the answer to this problem. If performing an automated analysis of the slides were possible, researchers would be relieved from the tedious activity of identifying and measuring the organisms, which will result in a greater efficiency (I Tayel *et al*., 2020). In this sense, the optical and digital systems for target recognition start being interesting for taxonomists and biologists, since it allows better observations on the presence of specific microorganisms in the sample (García-Magaña *et al*., 2019).

The digital correlation methods, based on the shape of the objects, have been used for patterns recognition that require different types of filters, which have been developed looking forward to recognize diverse objects with great success (Álvarez-Borrego *et al.,* 2002; Pech-Pacheco *et al.,* 2003; Marcotegui *et al.*, 2018; Pacheco-Venegas *et al*., 2021). Castro-Longoria *et al.* (2003) comparing three different methodologies (taxonomic, genetic probe and invariant correlations) to determine the identification of three cryptic harpacticoid copepod species, which are difficult to identify. Pech-Pacheco *et al*. (2003) showed, through a rigorous theory, that the problem of a lack of recognition in some images is basically due to their resolution and not to a failure of the mathematical algorithm to identify (Rodriguez-Santiago, 2002; Pacheco-Venegas *et al*., 2021).

An algorithm has been developed, in this study, inside a process of an image color correlation with species-specific composite phase filters (which contain the information of the organisms to be identified) for the recognition of four species of the genus *Trichodina*, previously studied by morphological methods. The invariant correlation is performed using the phase information obtained after all the mathematical steps associated with the image of the organisms were recognized, which has been previously carried out as well, using the phase information of the species-specific composite filter (Castro-Valdez & Álvarez-Borrego, 2018).

MATERIALS AND METHODS

Morphological Taxonomy

Fish were captured at the fish farm from the Secretaria de Agricultura, Ganaderia, Desarrollo Rural, Pesca y Alimentación (SAGARPA), located in the state of Sinaloa, Mexico (Chametla: 22° 50'N-106° 01'W). Five hundred and fifty *Oreochromis niloticus* (Linnaeus, 1758) fingerlings Egyptian black variety 20 days old were used. Scrapings from the skin of the fish were examined under a dissecting microscope for the presence of trichodinids; smears were prepared from heavily infected tissues and air-dried. The adhesive disc morphology was examined by Klein dry silver impregnation technique, and additional preparations were stained with Harris haematoxylin for the studying of the nuclear device. Terminology and measurement method of the components from the adhesive disc and denticles given by Lom (1958). The taxonomic criteria used during this study is essentially the same as those reported by Lom & Dyková (1992), and from recent publications for species determination. (Rodriguez-Santiago *et al*., 2019). The range followed in parentheses by the arithmetic mean, standard deviation and number of specimen measured were given for morph metric measurements expressed in micrometers. Radial pins and centre ridges only the range and number of specimens measured were given in the case of the number of denticles.

Trichodinids recognition in color images with species-specific composite phase filters with invariants.

A numeric simulation was performed to correlate the square modulus of the Fourier transform (frequency contents) of the parasite species, only with phase filters (Horner & Gianino, 1984).

In Figure 1, the method for obtaining the species-specific composite filters is presented in blocks. It is necessary to include this information in the filters, since parasites have different morphologies. In the first step (Step 1), all different morphologies for a single species $f_1(x,y)$, $f_2(x,y)$, $f_n(x,y)$ were chosen to obtain the square modulus of the Fourier transform (FFT) of each one $|F_1(w_x,w_y)|^2$, $|F_2(w_x,w_y)|^2$, $|F_n(w_x,w_y)|^2$ (Step 2). Subsequently, all the square modulus was added to produce a single image (Step 3). This provides, in a single matrix, all the frequency information related to the morphology of the species to be recognized. A parabolic filter is applied in step 3, the low frequencies are attenuated and the high frequencies are then enhanced. This weighing towards high frequencies will help to improve the identification of the “unknown” species to be recognized. A uniform scale and orientation for the images becomes unnecessary for steps 1 to 3, since the final product is scale and orientation is invariant.

A scale factor $\sqrt{r}$ is applied. This process differentiates the scale transform from the Mellin transform. After these steps, the Cartesian coordinates are mapped to polar coordinates to obtain invariance for rotation (Step 4). A bilinear interpolation of the first coordinate conversion data is introduced in step 5 (Pech-Pacheco *et al.*, 2003), willing to minimize sampling errors, which affect “unknown” species identification. The Fourier transform was again applied in order to obtain the scale transform. The phase information will be used only for this result. Thus, the resulting image (Step 6) is the species-specific composite filter, which will be used in the invariant correlation with data arising from images of individual trichodinid specimens. S_{POF} is the name used for the species-specific composite phase filter only obtained in Step 6.

Steps 3, 4, 5 and 6 from Fig. 1 are necessary to assure that the information contained in the square modulus of the Fourier transformation is invariant for rotation, scale and position. Due color images were utilized, one species-specific composite filter was used for each RGB channel (a color image correlation is carried out in each Red, Green and Blue channel). The explanation for Fig. 2 is exactly the same as for Fig. 1.

Images of each individual to be recognized were transformed as indicated in Fig. 2 to discriminate among parasite species, which mirrors Fig. 1, except that in Step 1 only a single image provides the input. SI_{POF} is the name used for the "unknown" species to be recognized (phase information only) in Step 5. The result obtained using the same steps as Fig. 1 is observed in step 6. The invariant correlation is made between the parasite to be recognized and the individual species-specific composite filters in the Step7 from Figure 2. The recognition of a particular species of parasite is complex. Color can be an important discriminative feature, which needs to be included for successful identification. Since our analysis was digital, it was possible to separate the color image in 3 channels (RGB) using a pixel view. Thus, the process shown in Fig. 2 was repeated for each channel (R, G and B). The composite filter to be used is matched with the corresponding component of the target in each color channel. In general, objects, which have a determined component $A_{\lambda_i}(x,y)$ similar to the respective component of the target, $P_{\lambda_i}(x,y)$ will give a maximum correlation in this channel (λ_i).

Only the target will give a maximum correlation in all channels. An object is detected as the target if it produces a correlation peak in the 3 channels simultaneously, thus.

The final result will be the product of the correlation for each of the multiplied RGB channels. The digital correlations were performed by an algorithm specifically created by us, using MATLAB software (Copyright 1984-2000, The Mathworks, Inc.). The algorithm allowed the development of the different steps showed in Figs. 1 and 2.

Selecting the morphologies observed in each species made the species-specific composite filters. Ten different images were selected to make each filter. Figure 3 shows the frequency information content. It was observed that the frequency content covered all the possible angles. For this reason, it was concluded that ten images were sufficient for the purpose of this study.

Ethic aspects: The authors indicate that all the ethical requirements of the country and international were met.

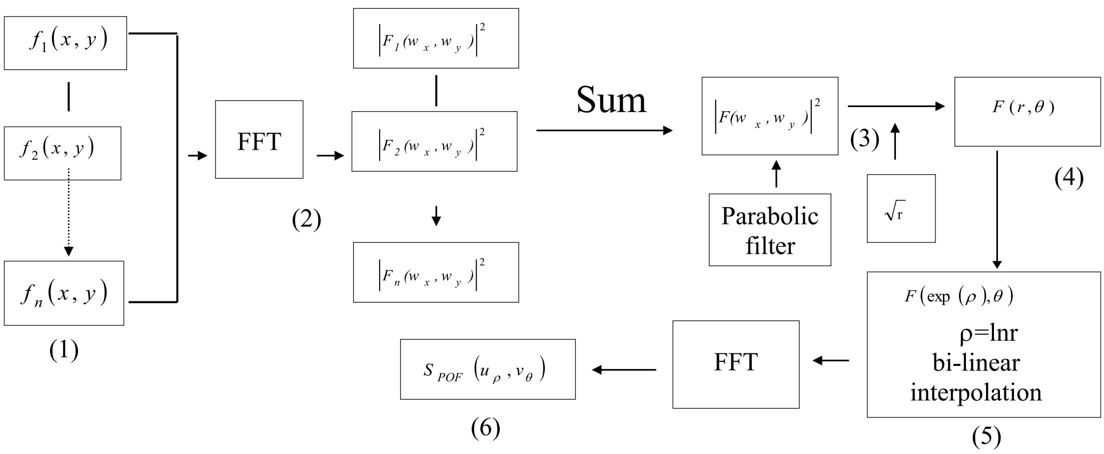


Figure 1. Method for obtaining the species-specific composite filters. $f_1, f_2, \ldots, f_n$ are the morphologies of the parasites images; FFT is the Fourier transform of each image; $\left|F_1(w_x, w_y)\right|^2, \left|F_2(w_x, w_y)\right|^2, \ldots, \left|F_n(w_x, w_y)\right|^2$ are the square modulus of the Fourier transform and w_x, w_y are the coordinates in the Fourier plane (frequencies content of the image); $\left|F(w_x, w_y)\right|^2$ is the sum of all square modulus; $F(r, \theta)$ is the polar plane and $S_{POF}(u_\rho, v_\theta)$ is the species-specific composite phase only filter where u_ρ and v_θ are the new coordinates.

DISCUSIÓN

Studies of the trichodinids in Mexico are scarce and not well documented (Wellborn, 1967). The information on species discrimination of the genus *Trichodina* in Mexico is limited to the report of *T. wellborni* Lom, 1970 in *Cyprinus carpio* Linnaeus, 1758 cultured in Morelos, Michoacan state (Herroz-Zamorano 1998; Islas-Ortega & Aguilar-Aguilar, 2014; Rodríguez-Santiago *et al*., 2019).

Trichodina magna and *T. nigra* found in *O. niloticus* Egyptian black variety in this study represent new host records and geographic location while *T. centrostrigata* and *T. heterodentata* have already been reported by *O. niloticus*; although not in Mexico (Rodríguez-Santiago *et al*., 2019).

The specimens of *T. heterodentata* found in *O. niloticus* Egyptian black variety revealed a considerable intrapopulation variation, similar to Van As & Basson records (1986) for *O. mossambicus* (Peters, 1852) from Taiwan revised by Van As & Basson (1989) and Bondad-Reantaso & Arthur (1989) from *O. niloticus* in the Philippines. After the original description of *T. heterodentata* (Duncan, 1977) from cultured cichlids was carried out, it has been recorded from a large number of fish species (El Tantawi & Kazubski, 1986) Dove & O'Donoghue, 2005; Tantry *et al*., 2016; Rodriguez-Santiago *et al*., 2019; Sousa-Filho *et al*., 2021).

T. centrostrigata and *T. magna* have been recorded mainly in African cichlids and other indigenous fish from several locations that have not been influenced by the introduction of any fish outside Africa (Van As & Basson, 1989). So, the *T. centrostrigata,* and *T. magna* incident in *O. niloticus* from Northwest coast of Mexico could have been introduced with their host or by different tilapia species from different locations. Three tilapia species (*T. rendalli (Boulenger, 1897), O. mossambicus,* and *O. aureus*) were introduced from Auburn, Alabama University in 1964 (Morales-Diaz, 1991; Attia *et al*., 2021); one species from Panama (*O. niloticus*) in 1978, two species (*O. mossambicus* and *O. urolepis hornorum* (Norman, 1922)) from Florida in 1981 (Secretaría de Pesca, 1974) and *O. niloticus* red variety from Stirling University, Scotland in 1986

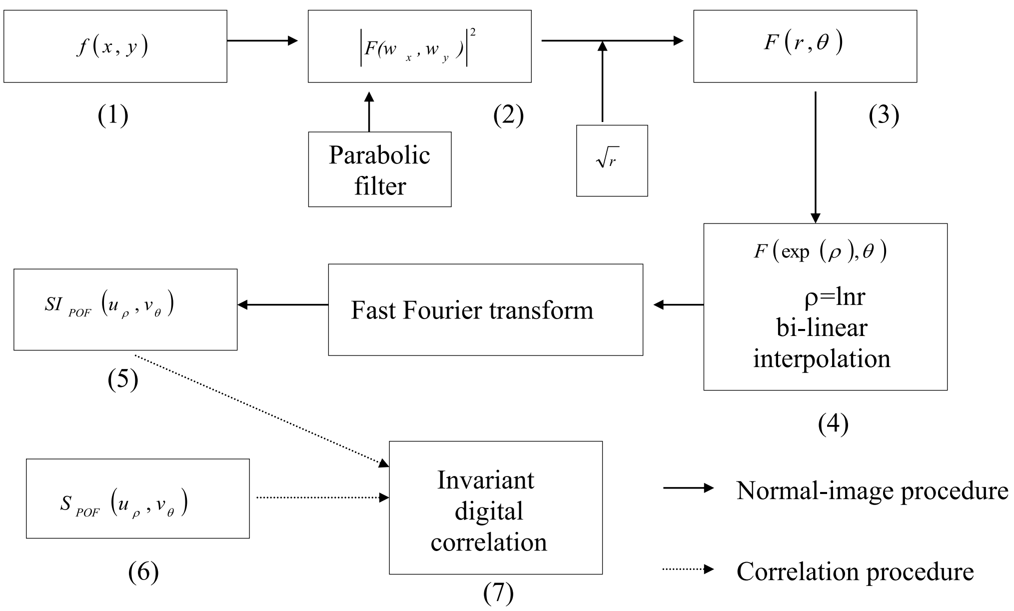


Figure 2. Invariant correlation to position, rotation and scale. $f(x,y)$ is the unknown species to be recognized; $|F(w_x, w_y)|^2$ is the square modulus of the Fourier transform (square modulus of the frequencies content) of the unknown image; $F(r,\theta)$ is the polar plane like Fig. 1 and $SI_{POF}(u_\rho, v_\theta)$ is the phase information of the unknown species with the position, rotation and scale information.

(Sosa-Lima *et al.*, 2000; García-Magaña *et al.*, 2019; Rodríguez-Santiago, *et al.*, 2019).

Trichodina nigra is probably the most reported trichodinid, with at least 36 records in literature and there has also been considerable confusion surrounding the specific identification of some populations (Basson & Van As, 1994). The morphology of *T. nigra* observed in this study showed a high gradient of morphological variability which coincides with Lom (1961) and Gaze & Wootten (1998) descriptions for cyprinids and *Oncorhynchus mykiss* (Walbaum, 1792) respectively, detecting fine rays in some organisms, and thicker in others, a characteristic appearance of older individuals according to the Kazubski & Migala (1968) and Islas-Ortega & Aguilar-Aguilar (2014) criteria.

The differences of the algorithm proposed in this work, with those carried out earlier (Pech-Pacheco *et al.*, 2003; Lopez-Leyva *et al.*, 2021), are due to

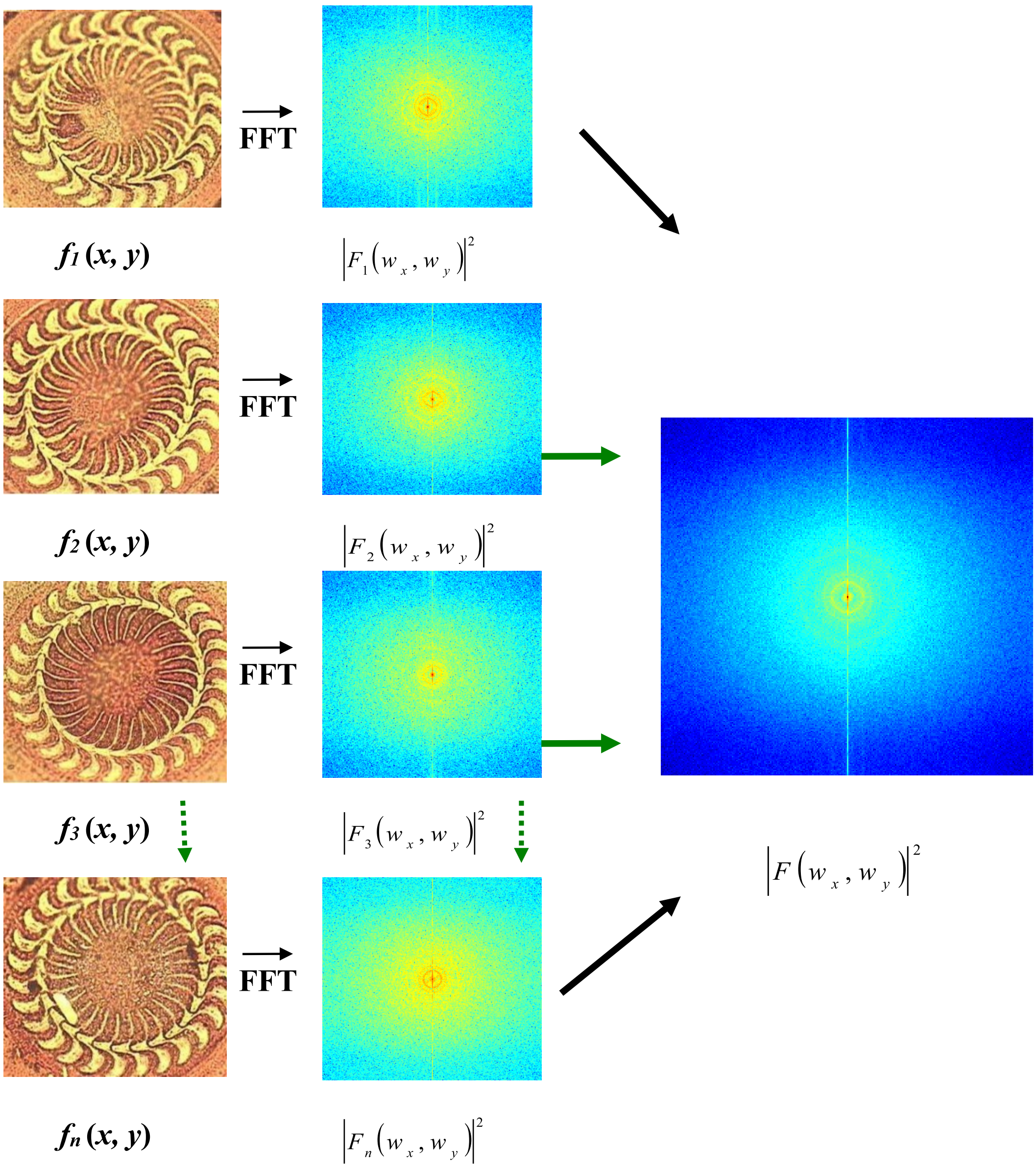
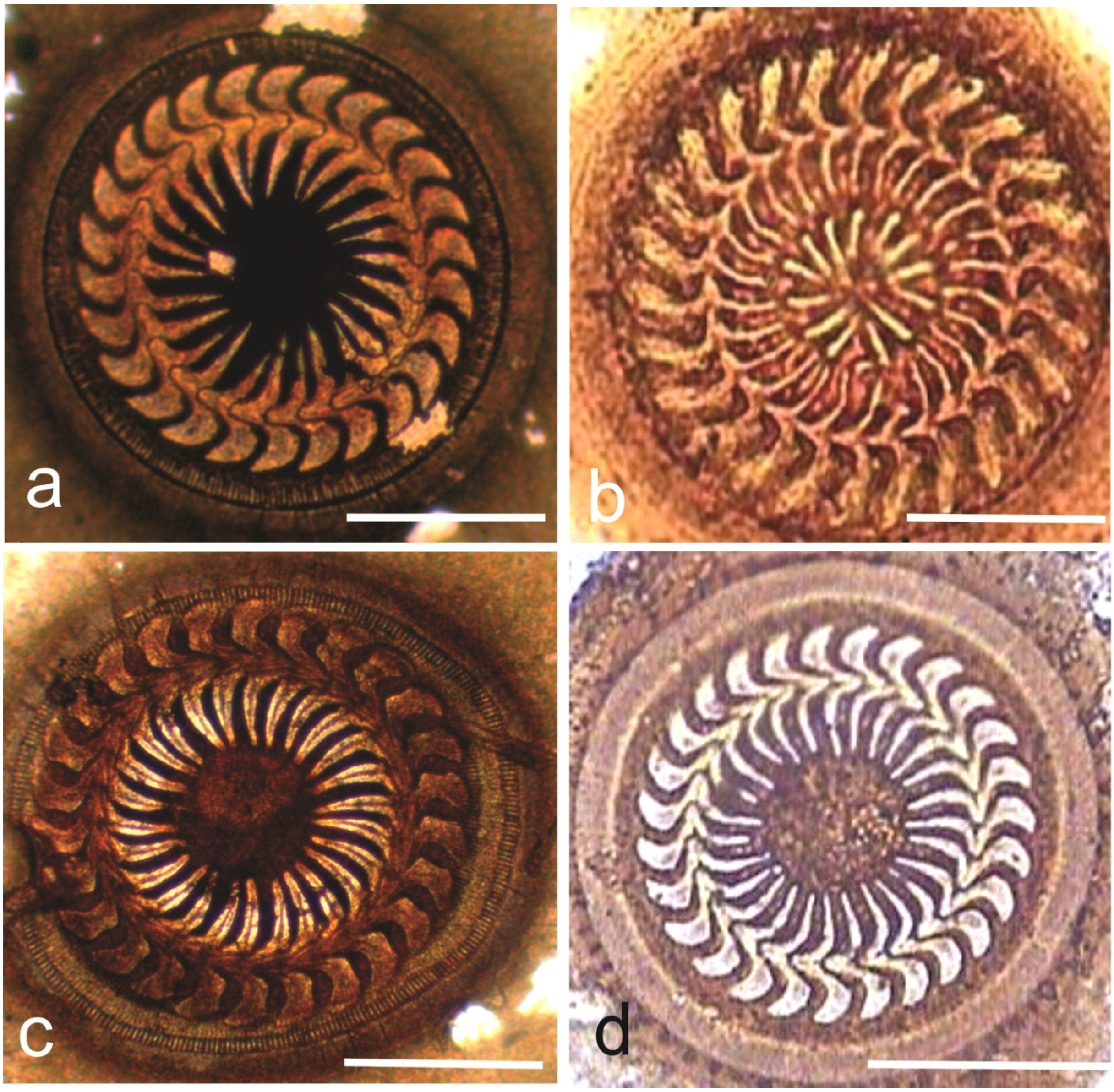


Figure 3. Flow diagram representing the steps followed to obtain the sum of the square modulus of the frequency contents of the different morphologies for the species-specific composite filter.

the fact that the invariant correlation was done with species-specific composite filters. In this algorithm an extreme phase invariant correlation is perfomed (Castro-Valdez & Álvarez-Borrego, 2018).

The recognition of an object depends on the analysis of each one of the monochromatic components in the three RGB channels, multiplying the information of the monochromatic components in the three channels and obtaining one single result. Therefore, in order to establish the ideal algorithm, an analysis of the content of the channels of the object to identify, must be done first. The three channels provided information, in this study, for the discrimination between objects (Barajas-Garcia *et al*., 2016).

Thus, the efficiency of the system of correlation through species-specific composite filters for the recognition of trichodinids is based on the multiplication of the result of the invariant correlation of the three channels, since the values



Figures 4. Photomicrographs of silver impregnated specimens of trichodinid species from *Oreochromis niloticus* Egyptian black variety. 4 and 5. *Trichodina heterodentata* Duncan, 1977 and *T.magna* Van As and Basson, 1989 (scale bar=30 µm). 6 and 7. *T. centrostrigata* Basson, Van As & Paperna, 1983 and *T. nigra* Lom, 1960 (scale bar=20 µm).

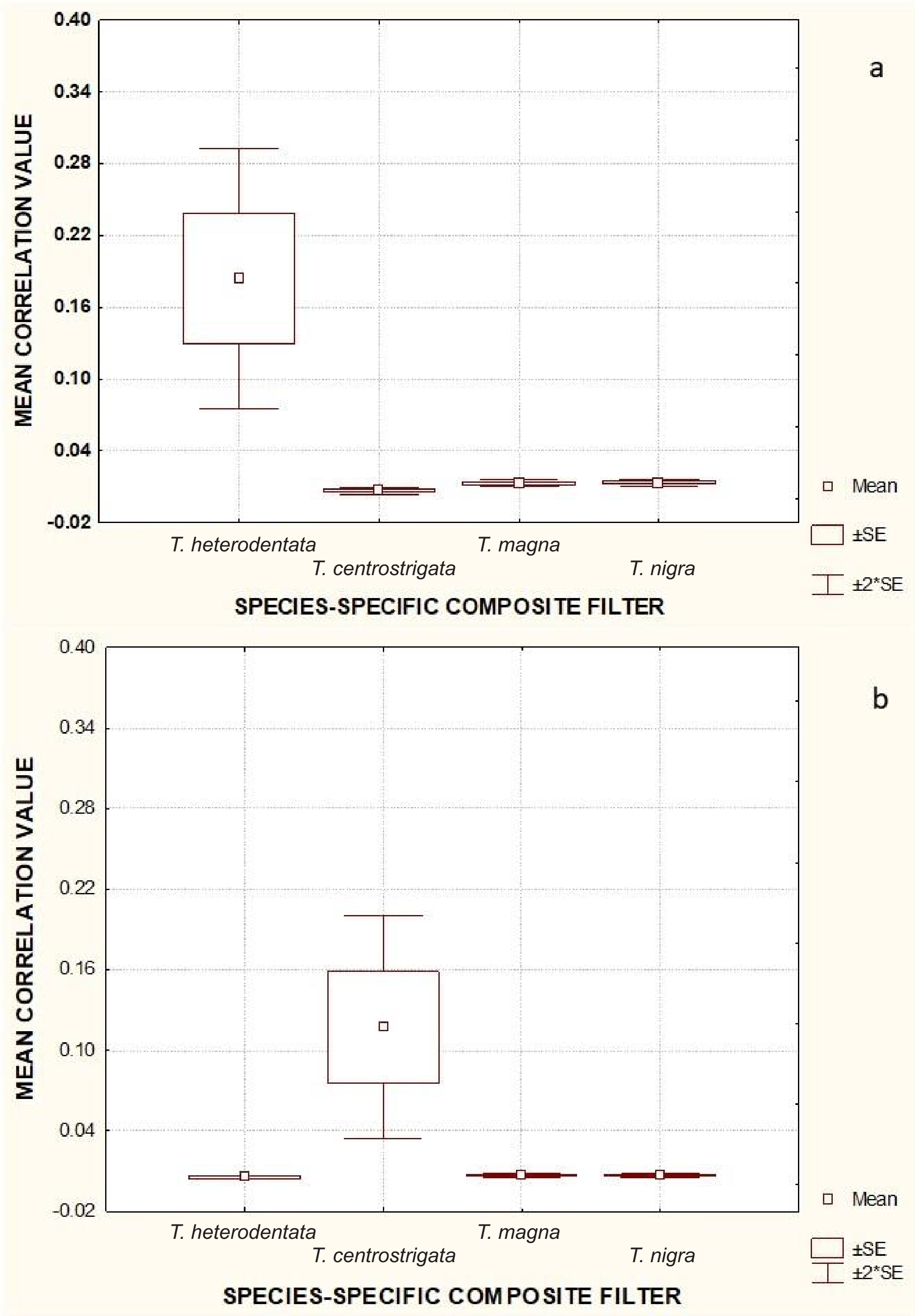


Figure 5. Correlation results for. a) *Trichodina heterodentata* (Th) and b) *Trichodina centrostrigata* (Tc). Boxplots show mean correlation for product of 3 colour channels (RGB).

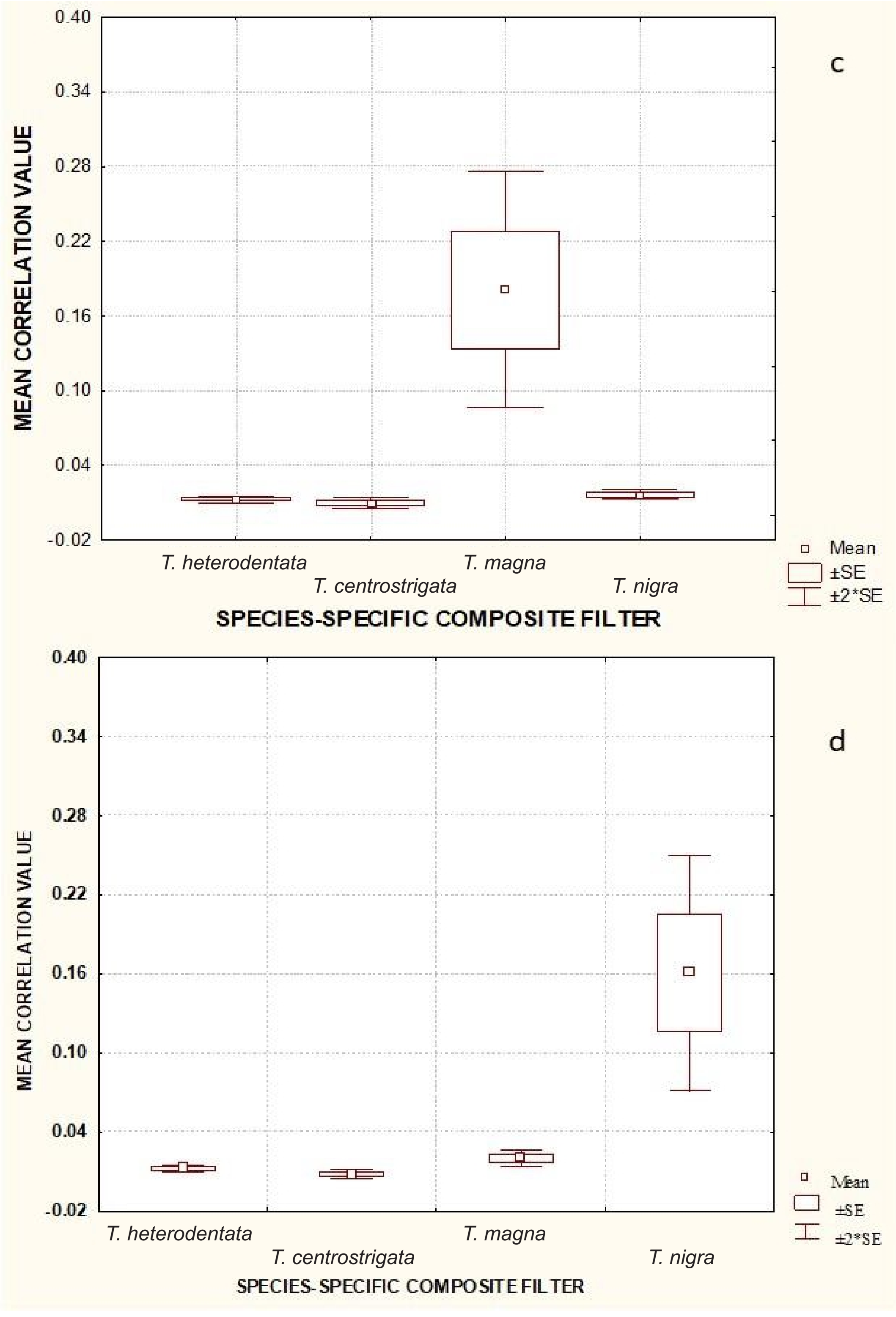


Figure 6. Correlation results for. a) *Trichodina magna* (Tm) and b) *Trichodina nigra* (Tn). Boxplots show mean correlation for product of 3 colour channels (RGB).

that coincide in the three channels (which give rise to the recognition of a species) when multiplying, will be values higher than the rest; the smaller values when being multiplied will give even smaller values (Solorza & Álvarez-Borrego, 2015).

An important aspect is that the system is reliable, if this is considered as the capacity of a test or method for repeating a measurement under similar conditions with the minimum variation. It may be stated that any observer, who may be an expert, will probably obtain different results when faced after some time to an equal scene, this may be the result of fatigue or any other cause of human nature. The advantage of the correlation with composite filters is that for a minimum set of images, the same result will always be found, and this will not vary independently of the number of images evaluated (Solorza & Álvarez-Borrego, 2014; Pacheco-Venegas *et al*., 2021).

When using the correlation invariant to position, scale and rotation in the identification of trichodinids, the result will always be the same, although some organisms of trichodinids vary in size or may be rotated in the plane in which the image is taken. Due to the morphological variability that these organisms represent, it was necessary to use species-specific composite filters, which have the capacity of using the information of the morphological differences among them. The recognition of an image is done in about 8 seconds using a Pentium III model 1700. The species-specific composite filter was obtained through ten different images. These ten images are only a sample, and the total frequency content resulting from the sum of the frequency contents of each one of them shows that the information of frequencies covers all possible angles (Fig. 3). Based on these observations, it was concluded that ten images are good enough to differentiate the species (Castro-Valdez *et al*., 2020). The morphology of the species varies intra-specifically; therefore, it is very difficult to differentiate them, specifically with respect to the denticles, since even the same species there present high variability, due to the geographical distribution or to the host they parasite. Some denticles are thinner than others, but these morphological differences are included in the composite filters when the ten images were considered. However, a parabolic filter is applied in the algorithm, which allows enhancing the fine details of the image to identify by attenuating the low frequencies and enhancing the high frequencies. The use of this system is simple and has minimum requirements of computer equipment and of a color CCD camera. The system of species-specific composite filters is a useful tool for the identification of protozoans infecting a single host species regardless their shape, size and position. Once the system is established, the identification takes seconds (Castro-Valdez *et al*., 2018; Castro-Valdez & Álvarez-Borrego, 2018).

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