Research on the Classification of Fish Parasitoids Based on Digital Image Processing Technology

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Abstract. The traditional classification method of morphology and the combination of morphology and molecular biology have low efficiency and accuracy in the classification of fish parasitoids. In this paper, Four kinds of blepharoplasma, such as ugly round Myxobolus, Myxobolus shantungensis, Myxobolus Koi and Myxobolus dispar, were selected as the research objects. A new method based on digital image processing technology is proposed to extract the edge of a spore and pouches, and calculates various morphological parameters of the Myxobolus. Based on the obtained parameters, a classification standard is established to classify four different Myxobolus and a new classification method based on morphological criteria was proposed.

1. Introduction

There are many species and wide distribution of myxosporidium [1], which is a very common and main parasite of fish. Because of the parasitism of myxosporidium to fish, there are a lot of losses in fish breeding every year. As one of the myxosporidium, the taxonomic study of Myxobolus will help to eliminate and prevent many kinds of fish diseases caused by Myxobolus.

At present, the taxonomic research on the sporidium is mainly focused on the morphological characteristics, such as calculating various morphological parameters of the sporidium: Spore shape, pouches shape, sporophyte shape, intercystal process shape. Spore size, pouch size, sporophyte size and intercystal process size. The position of pouches in spores, the relative position between pouches, the position of sporophyte in spores, the relative position of polar processes, etc. The traditional morphological classification method needs to measure all kinds of parameters manually as the basis of classification [2], which has great limitations and subjectivity. Many scholars use morphological classification and molecular biological classification [3] together to improve the accuracy of classification. However, when a lot of discrimination work is needed, the efficiency of classification is low because of the limitation of DNA extraction speed.

In this paper, the edge extraction and pouch extraction of spores are realized by using digital image processing technology [4], and various morphological parameters [5] of Myxobolus are calculated. From the obtained parameters, a classification standard is established to classify four different kinds of Myxobolus, and a new classification method based on morphological standard is proposed.
2. Materials and Methods

2.1 Material

Four kinds of microscopic image samples were selected, and spore images [6] were selected from the samples. The boundary between foreground and background was obvious, and the noise was small. In the next calculation, pixel is used as the unit. As shown in Figure 1, there are ugly round Myxobolus, Myxobolus shantungensis, Myxobolus Koi and Myxobolus dispar samples from left to right.

Figure 1. Four kinds of Myxobolus samples

2.2 Methods and models

2.2.1 Canny algorithm and edge extraction. Canny edge detection operator is a multilevel edge detection algorithm developed by John F. canny in 1986. Canny algorithm has three advantages in edge extraction [7-11], which are: (1) the algorithm can identify as many actual edges as possible; (2) the edge identified by the algorithm is very close to the edge in the actual image; (3) the algorithm is not easy to be interfered by noise, and can detect the real weak edge. In this paper, Canny algorithm is used to extract the edge of spore image. The high and low thresholds of Canny algorithm are 180 and 120, respectively.

2.2.2 Ellipse fitting and spore position correction. The fitting ellipse of the outermost edge of spore can be obtained by using the ellipse fitting method based on the least square method [12-14].

Figure 2. Fitting results of three species of Myxobolus

Figure 2 shows the Canny algorithm processing results (left) and ellipse fitting results (right) of ugly round Myxobolus, Myxobolus shantungensis and Myxobolus dispar, respectively. After the fitting ellipse that meets the conditions is obtained, the offset angle of the ellipse can be obtained to correct the spore position. The statistical operation of the maximum length width was carried out in each rotation, and the angle of the maximum length obtained was the migration angle of the spore. Here, take Myxobolus shantungensis as an example, and the rotation result is as shown in Figure 3.

Figure 3. Results of two rotations of Myxobolus shantungensis

Figure 4. Results of circular expansion of Myxobolus shantungensis in the rotating picture

Figure 3 shows the effect after rotation is not ideal, and two images need to be judged separately. In the spore image region (width / 2, height / 4) and (width / 2, height * 3 / 4), the circular expansion operation is carried out. The results of circular expansion of Figure 3 are shown in Figure 4.

According to the maximum radius of the circular extension, the image that is 0 degree or 180 degree from the correct position is determined, and then the angle is corrected twice.
according to the size of the upper and lower circular radius of the image. Finally, the rotated image is as shown in Figure 5.

Figure 5. Spore correction results

2.2.3 Statistical spore aspect ratio and area ellipse fitting and spore position correction. The length, width and area of spores were counted after the final correction pictures were obtained. Scan the spore image from top to bottom, starting from the left and right sides of the image, and stop scanning the first highlight pixel. When calculating the width, add the width value of each time, and the sum is the area. Next, the symbols width, height and area are used to represent the length, width and area of spores respectively.

2.2.4 Statistical spore aspect ratio and area Ellipse fitting and spore position correction. In order to locate the relative position of pouch in spore more accurately and calculate the parameters of spore, a method is designed in this paper. Firstly, the position P (width / 2, height * 3 / 4) of spore is located, and the two sides of spore are expanded from the point P to the height of spore. The expansion mode is as follows: in the process of P point swimming upward, it expands to both sides at the same time. When the left and right sides touch the boundary point of pouch for the first time, they stop swimming upward. At this time, the vertical coordinate y_divide as shown in Figure 6 is obtained.

Figure 6. Search for seed points of pouch

The search of pouch seed point is to pave the way for the next regional growth algorithm. First, position P1 (width / 4, y_divide) and P2 (width * 3 / 4, y_divide) in Figure 6, let them swim upward and expand left and right at the same time. Each time when the expansion touches the boundary point and stops, define the middle point of the maximum extended length as the seed point of the pouch. The results after processing are shown in Figure 7.

Figure 7. Boundary and seed point of pouch

2.2.5 Secondary extraction of spore boundary and acquisition of pouch. It can be seen from Figure 7 that the image of spore will lose frame to some extent after rotation, which is generated by angle function calculation and cannot be avoided. In addition, the gap of pouch in the figure is large, so it is easy to expand the pouch to the whole spore in the region growing algorithm, which leads to the failure of pouch acquisition. In order to solve this problem, we first rotate the original spore image, and then use Canny algorithm to extract the edge. In this operation, the low threshold value of Canny algorithm should be reduced to 40, that is, the standard of weak boundary should be reduced, so as to generate more boundary contours to fill the gap of pouch.

As shown in Figure 8, it can be seen that the image which is rotated by the original image and edge extracted again is clearer.
After getting a more complete pouch boundary, we extract the pouch from the boundary map in Figure 8. In order to prevent boundary overflow during region growing, the traditional region growing algorithm [5] is improved in this paper: the traditional point growing is changed to matrix growing. Here, the choice of matrix needs to take a middle way. Because the matrix is too large, the edge of the growing pouch will generate sawtooth, the boundary is not accurate, and the matrix is too small and overflows, so the matrix of 5 * 5 is chosen here, and the matrix is placed in a diamond way for regional growth.

It can be seen from Figure 9 that although there are some deviations in pouch extraction results, the general outline is still obtained. Although there are errors in the area, length and width of the final pouch, the error is within the acceptable range.

2.2.6 Extraction of pouch boundary and acquisition of morphological parameters of pouch. The Canny algorithm was used to extract the pouch boundary, and then the ellipse fitting was carried out on the pouch boundary. According to the angle of fitting ellipse of pouch, the left and right position can be judged. The ellipse with an angle less than 90 degrees is the left pouch, and the other pouch is the right pouch. The difference between the angles of fitting ellipse of two pouches is the included angle between pouches. The image of pouch can be corrected by the left and right position of pouch and the angle of fitting ellipse. After correction, the length, width and area of pouch were calculated in the same way as spores.

2.2.7 Establish classification standards. After the above parameters are obtained, the four species can be distinguished according to the morphological parameters.

3. Results
3.1 processing objects and results of expected results
In this paper, we select 50 clear images from many spore microscopic images of each Myxobolus as the processing data of the experiment, and then process these images through the above steps. Data are consolidated in the table below. The parameter units in the table are pixel. When the number of decimal places is involved, it takes two places after the decimal point. The following table shows the average data of the morphological parameters of the four kinds of Myxobolus:
Table 1. Morphological parameters of four kinds of Myxobolus extracted

| Myxobolus              | width/height | cell area | left/right pouch width | left/right pouch height | left/right pouch width/height | left/right pouch area | Left and right pouch angle |
|------------------------|--------------|-----------|------------------------|-------------------------|-------------------------------|----------------------|---------------------------|
| Ugly round Myxobolus   | 0.95         | 3370.93   | 19.70/17.60            | 27.10/24.70             | 0.73/0.71                     | 368.30 / 305.20      | 45.03                     |
| Myxobolus shantungensis| 1.11         | 3625.68   | 18.10/21.20            | 21.30/29.30             | 0.85/0.72                     | 382.30 / 478.70      | 74.84                     |
| Myxobolus Koi          | 0.66         | 14401.30  | 38.00/35.10            | 86.70/102.10            | 0.44/0.34                     | 1970.30 / 1780.10    | 21.87                     |
| Myxobolus dispar       | 0.84         | 17321.30  | 31.70/44.10            | 42.30/65.70             | 0.75/0.67                     | 912.70 / 2122.10     | 47.60                     |

3.2 Summaries of morphological parameters and establishment of classification criteria

It can be found that there is a relatively large gap between the morphological parameter data of the four kinds of Myxobolus through the analysis of the data in Table 1. First, establish a first-order classification based on spore area. Then, the second-order classification is established based on the length width ratio and the left and right pouch area ratios. The specific classification standards of the four species are as follows:

1. Ugly round Myxobolus: The area is less than 10000 pixel and width/height is less than or equal to one.
2. Myxobolus shantungensis: The area is less than 10000 pixels and width/height is more than one.
3. Myxobolus Koi: The area is more than or equal to 10000 pixel and right pouch area / left pouch area is less than or equal to two.
4. Myxobolus dispar: The area is more than or equal to 10000 pixel, and right pouch area / left pouch area is more than two.

So far, the four kinds of Myxobolus have been separated successfully. It can be seen that the effect of classification by morphological characteristic parameters is very good and rapid.

4. Discussion and Conclusions

This paper proposes a new method to extract the morphological parameters of Myxobolus, and realizes the classification operation of four kinds of Myxobolus combined with Canny algorithm, ellipse fitting improved region growing algorithm and so on in the digital image processing technology. The main conclusions are as follows:

1) For the first time, this paper proposes the application method of digital image processing technology in the field of spore classification. Compared with the traditional manual measurement method for morphological parameter extraction, this method is more rapid; compared with the human eye recognition method for spore classification, the cost of this method is lower and more feasible.
2) The accuracy of this method mainly depends on the image quality of the sporidium to be classified. The clearer the image is, the higher the accuracy of classification is, and the sporidium which is not clear enough is not recognizable. The accuracy and applicability of this method will be greatly improved when a new edge extraction algorithm is proposed in the future.
3) Although this paper only realizes the classification operation of four kinds of Myxobolus, there are many kinds of Myxobolus. When a large number of different kinds of Myxobolus were classified, the classification standard needs to be more complex and accurate. There are still many morphological parameters not used. As long as there are enough kinds of morphological parameters extracted and classification standards established carefully, it is easy to achieve the classification operation of a large number of different kinds of Myxobolus.
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References

[1] Qiliu Chen, Chenglun Ma, Fauna of China, phylum myxozoon, myxosporidia, Beijing: Science Press, 1998: 332-528.
[2] Rongqian Du, Introductory Biostatistics, Beijing: Higher Education Press, 2003: 67-68.
[3] Yishan Lu, Ping Nie, PHYLOGENETIC ANALYSES OF THE MYXOSPOREANS PARASITIC IN FRESHWATER FISH OF CHINA BASED ON 18S RIBOSOMAL DNA, Acta Hydrobiologica Sinica, 2004, 28
[4] Dongjian He, Hailiang Zhang, Jifeng Ning, et al, Application of Computer Vision Technique to Automatic Production in Agriculture, Transactions of the Chinese Society of Agricultural Engineering, 2002, 18(2): 171-175.
[5] Kun Bi, Pan Jiang, Lei Li, et al, Non-destructive measurement of wheat spike characteristics based on morphological image processing, Transactions of the CSAE, 2010, 26(12): 212-216.
[6] Baijing Qiu, Tianbo Wang, Juanjuan Li, et al, Image Recognition and Counting for Glasshouse Aphids gossypii, Transactions of the Chinese Society for Agricultural Machinery, 2010, 41(8): 151-155.
[7] Min Li, Hui Sun, Liyang Wu, et al, Multi-scale edge detection method based on synthesized morphological transform, Computer Engineering and Applications, 2010, 46(5): 160-161.
[8] Zhiyu Zhou, Yingchun Liu, Jianxin Zhang, Orange edge detection based on adaptive Canny operator, Transactions of the Chinese Society of Agricultural Engineering, 2008, 24(3): 21-24.
[9] Fengcai Ling, Mu Kang, Xiaohua Li, Improved Canny Edge Detection Algorithm, Computer Science, 2016, 43(8): 309-312.
[10] Chao Liu, Jiliu Zhou, Kun He, Adaptive edge-detection method based on Canny algorithm, Computer Engineering and Design, 2010, 31(18): 4306-4039.
[11] Fan Zhang, Zhongwei Peng, Shuijin Meng, et al, Improved Canny edge detection method based on adaptive threshold, Journal of Computer Applications, 2012, 32(8): 2296-2298.
[12] Zhibin Wang, Kaiyi Wang, Shuifa Zhang, et al, Whiteflies counting with K-means clustering and ellipse fitting, Transactions of the Chinese Society of Agricultural Engineering, 2014, 30(1): 105-112.
[13] Bei Yan, Bin Wang, Yuan Li, An improved ellipse fitting algorithm based on the least square method, Journal of Beijing University of Aeronautics and Astronautics, 2008, 34(3): 295-298.
[14] Yimin Zou, Bo Wang, Fragmental ellipse fitting based on least square algorithm, Chinese Journal of Scientific Instrument, 2006, 27(7): 808-812.
[15] Yuehua Chen, Xiaoguang Hu, Changli Zhang, Algorithm for segmentation of insect pest images from wheat leaves based on machine vision, Transactions of the Chinese Society of Agricultural Engineering, 2007, 23(12): 187-191.