

## Feature Extraction and Classification for Multiple Species of Gyrodactylus Ectoparasite

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### Abstract

Active Shape Models (ASM) are applied to the attachment hooks of several species of *Gyrodactylus*, including the notifiable pathogen *G. salaris*, to assign each species to its true species type. ASM is used as a feature extraction tool to select information from hook images that can be used as input data into trained classifiers. Linear (i.e. LDA and K-NN) and non-linear (i.e. MLP and SVM) models are used to classify *Gyrodactylus* species. Species of *Gyrodactylus*, ectoparasitic monogenetic flukes of fish, are difficult to discriminate and identify according to morphology alone and their speciation currently requires taxonomic expertise. The current exercise sets out to confidently classify species, which in this example includes a species which is a notifiable pathogen of Atlantic salmon, to their true class with a high degree of accuracy. The findings from the current exercise demonstrates that import of ASM data into a MLP classifier, outperforms several other methods of classification (i.e. LDA, K-NN and SVM) that were assessed, with an average classification accuracy of 98.72%.

**Keywords:** marginal hooks, feature extraction, gyrodactylus, machine learning

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### 1. Introduction

There are over 440 described species of *Gyrodactylus*, which are typically small (<1mm), ectoparasitic worms principally infecting fish [1]. Most species are imperfectly known, with many descriptions limited to an incomplete morphological description of their attachment hooks. While molecular techniques have, in recent years, made a vast contribution to the discrimination of one species from another, species definitions often continue to rely on morphological characteristics (i.e. attachment hook morphology and in particular the shape of the sickle of the 16 small peripheral marginal hooks which are regarded as the key taxonomic feature) [2]. While most species of *Gyrodactylus* are non-pathogenic, causing little harm to their hosts, other species like *Gyrodactylus salaris* Malmberg, 1957, which is an OIE (Office International des Epizooties) - listed pathogen of Atlantic salmon can major effects on wild and cultured fish. *Gyrodactylus salaris* has decimated the juvenile salmon population in over 40 Norwegian rivers [3] is an uncontrolled increases in the size of the parasite population on resident salmon populations have necessitated extreme measures such as the use of the biocide rotenone to strategically kill-out stretches of river systems, in order to remove the entire fish and *G. salaris* population within a river [3]. Given the impact that *G. salaris* has had in Norway and elsewhere in Scandinavia and Russia [4-6], many European states including the UK now have mandatory surveillance programmes screening wild salmonid populations (e.g. brown trout, charr, grayling, Atlantic salmon etc) for the presence of notifiable pathogens including *G. salaris*. Current OIE methodologies for the discrimination of *G. salaris* from other species of *Gyrodactylus* that occur on salmonids require confirmation of identity by both morphological and molecular approaches, which can be time consuming. In the case of a type I error, where *G. salaris* is misidentified as another species and goes undetected resulting in the death of fish, or a type II error, where a non-pathogenic species is misclassified as *G. salaris* causing fish to be treated unnecessarily, the environmental and economic implications can be considerable [7]. For this reason, and because of the widely varying pathogenicity seen between closely related species, accurate pathogen identification is of paramount importance. The difficulty of discriminating pathogenic species from similar congeners, is compounded by the very limited number of morphologically discrete characteristics in these species. Owing to

these difficulties, the task of morphological identification currently relies upon a very limited number of domain experts capable of analysing and determining species. Time taken to identify species can be dramatically reduced if the initial identification of a specimen as being *G. salaris* or *G. salaris*-like according to its morphology can be achieved both more quickly and more accurately. In the event of a suspected outbreak, the demand for identification may exceed the available supply of suitable expertise and facilities. There is, therefore, a real need for the development of rapid, accurate, semi-automatic / automatic diagnostic tools that are able to confidently identify *G. salaris* in any given population of specimens.

The aims of the current study were therefore, to explore the potential use of an Active Shape Model (ASM) to extract feature information from the attachment hooks of each species of *Gyrodactylus*. Given the subtle differences in the hook shape of each species, it is hoped that this approach moves towards the rapid automated classification of species with improved rates of correct classification over existing methods and negates the current laborious process of taking manual measurements which are used to assist experts in identifying species. In this work, it not only contributes to the image processing area, also to the agriculture area, where the systematic and accurate system is provided in predicting the ectoparasite of *Gyrodactylus* species.

## **2. Methodological Approach**

### **2.1. Specimen Preparation**

Specimens of *Gyrodactylus* (*G. colemani* n = 10; *G. derjavinoidei* n = 25; *G. salaris* n = 34; *G. truttae* n = 9) were removed from their respective salmonid hosts and fixed in 80% ethanol. Subsequently specimens were prepared for scanning electron microscopy (SEM) by transferring individual, distilled water rinsed, specimens onto 13 mm diameter round glass coverslips, where they had their posterior attachment organ excised using a scalpel and the attachment hooks released using a proteinase-K based digestion fluid (i.e. 100 µg/ml proteinase K, 75 mM Tris-HCl, pH 8, 10 mM EDTA, 5% SDS). Once the hooks were freed from enclosing tissue, the preparations were flushed with distilled water, air-dried, sputter-coated with gold and then examined and photographed using a JEOL JSM5200 scanning electron microscope operating at an accelerating voltage of 10kV.

### **2.2. Current Approach**

A number of statistical classification based approaches applied to morphological data [8-10], and molecular-based techniques targeting specific genomic regions [11, 12], have been developed to discriminate the pathogenic species, *G. salaris*, from other non-pathogenic species of *Gyrodactylus* that co-occur on salmonid hosts. While each technique is able to detect *G. salaris* within a population of specimens and to discriminate it from its congeners with high levels of correct classification, the techniques can be time consuming [7]. If image recognition software could be developed to extract key discriminatory features from the attachment hooks of each species, then it is anticipated that the identification process could be accelerated with equivalent or better rates of correct identification.

Several efforts have been devoted to the recognition of digital images, especially microscope images, but so far it is still an unresolved problem [13, 14], due to distortion, noise, segmentation errors, overlap and occlusion in colour images. Recognition and classification techniques have gained a lot of attention in recent years due to many scientists utilising these techniques in order to enhance their own problem domains.

To provide a potential solution to the problem described above, image analysis is explored. Image analysis is a field of science which allows scientists to explore a complex assortment of images and effectively predict structure from the images autonomously. According to Kasturi [15], image analysis refers to algorithms and techniques that are applied to images to obtain a computer readable description from pixel data. Instead of image analysis, image processing techniques have also been developed. In contrast with image analysis, image processing involves the use of electronic tools which allow the user to define changes within the parameters of the electronic signal [16]. This approach is needed to be applied to increase the pictorial information for human interpretation. One of the examples of image processing is removing the illumination from images.

Recognising species group from the hook features makes the species recognition process more accurate and effective. Feature extraction is the key to both object segmentation and recognition, as it is to any pattern classification task. Examples of the features that might be of interest to extract include length, width, shape and angle. In the manual measurement of features, these tasks heavily depend on the concentration of the person taking the measurement; otherwise, the result of morphometric analysis will be false. And of course, the temporal duration of the manual measurement process is substantial. With state-of-the-art computer processing techniques, these processes are possible to be done efficiently and effectively, and thus, will provide the prediction in a shorter time and more accurately.

Feature extraction is essential in many vision and biometric applications. The performance of feature-based face recognition algorithms relies heavily on the quality of the feature extraction. Selection of a feature extraction method is probably the single most important factor in achieving high recognition performance [17, 18]. In this study, accuracy in the feature extraction is a must, since, the majority of the Gyrodactylus species have a similar shape to each other, especially *G. salaris* and *G. thymalli* [7].

In human communication, shape description (features) have been used. It is one of the most important visual attributes of an object and the first used to perform object classification and identification [19, 20]. Specifically, in classification and identification of multiple species of Gyrodactylus, shape information has been used, although different methods of identification has been applied [8-10], [21].

One of the objective of this research is to identify and utilise an image processing technology that has ability to extract the noisy images with similar pattern representation. For these reasons, the Active Shape Model (ASM) technique has been explored to evaluate the suitability of using it for extracting informative features of multiple species of Gyrodactylus. In the case of SEM images of the fish parasite, only the shape features are considered, since, it found that the texture information does not increase the accuracy of predicting the species. Shape or contour refers to the boundary of the object, and that represents the shape of the object.

### 2.3. Potential Solutions

ASMs method have been successfully utilised for understanding of factors underlying morphologic and pitch related functional variations affecting vocal structures and the airway in health and disease [22]. In addition, the ASM method was found to be the best method that can account for the varieties in variation [23].

Another successful application of ASM for face recognition [24]. In this study, ASM was applied to the alignment of the face, with four major improvements. These are: (1) a model combining a Sobel filter [25] and the 2D profile in searching for a face in an image; (2) application of the Canny [26] method for edge enhancement; (3) use of a SVM to classify the landmark points; and (4) automatic adjustment of the 2D profile according to the size of the input image. With the introduction of this improvement, it has improved the process of finding landmarks and thus will save time during the training and testing of images.

ASM was also implemented for extracting features for plant recognition based on the leaf shape [23]. In this study, ASM was applied for recognising weed species, and due to using the ASM, it was found to be possible to not only take leaf shapes into account, but also the overall geometry of the seedlings.

With the statistical shape models, shape can be characterised in terms of independent modes of variation. Variation in the image presentation is a key point that needs to be focused. This is because a single species may come in a variation, yet still be part of the same species. For example, location and water temperature can contribute to these differences. Although despite this variation, the overall shape of the hook remains the same.

The ASM technique permits users to construct a general shape model which is subsequently applied to all images in order to landmark the image area for every given image, providing a pattern that encapsulates the variation seen across the range of shape images. The subsequent ability (classification rate) of the developed model to separate "image classes" is in part based on the number of images used in the training set - in theory, the greater the number of images that are used in training and constructing the models, the better the classification ability of the resultant model. Given the success of ASM in resolving image-based, shape

recognition problems within the biomedical sphere, the research presented in this chapter aims to determine its utility when applied to SEM images of Gyrodactylus hooks.

The application of the ASM method to the analysis of Gyrodactylus attachment hooks is presented in Figure 1. The input for the classification system is the specimen images, where a pre-processing step is applied to the required images. Once hooks have been processed to a common orientation, the ASM approach is then applied to extract informative features. These features are then reduced by a subsequent PCA step to select key features to be used as input features for each of the machine learning classification techniques. Four machine learning classifiers have been used to evaluate the ASM performance.

### 2.3.1. ASM Construction

ASM were originally developed for the recognition of landmarks on medical x-rays. Landmark points can be acquired by applying a sample template to a "problem area", which appears to represent a better strategy over edgebased detection approaches [27], as any noise or unwanted objects within the image can be ignored in the selection of the shape contour. In the current study, the shape of each attachment hook image is presented by a vector of the position of each landmark,  $D = (d_1; e_1; \dots; d_n; e_n)$ , where  $(d_i, e_i)$  denotes the 2D image coordinate of the  $i$ th landmark point. The shape vector of the hook is then normalised into a common coordinate system. Procrustes analysis is then applied in aligning the training set of images. This aligns each shape so that the sum of distances of each shape to the mean  $F = \sum |D_i - D|^2$  is minimised. For this purpose, one hook image is selected as an example initial estimate of the mean shape and scaled so that  $|D| = 1$ , which minimises the F.

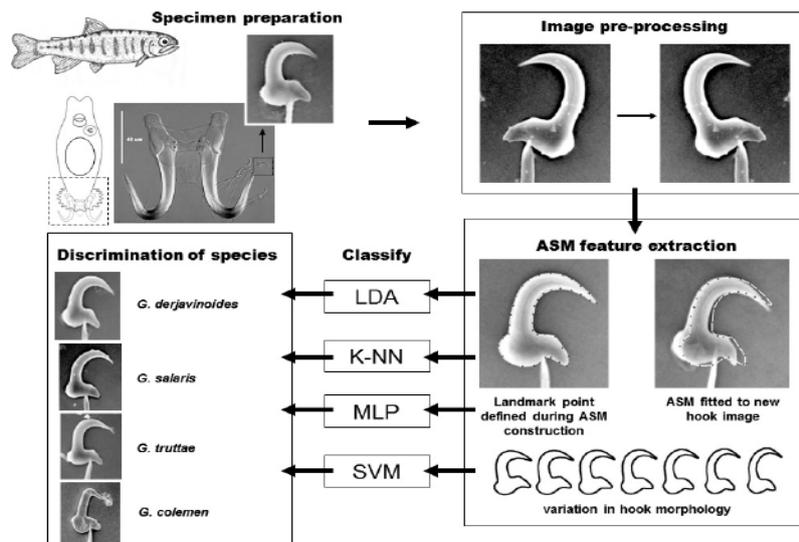


Figure 1. The methodological approach used in the current study

Specimens of Gyrodactylus were picked from the skin and fins of salmonids and their attachment hooks released by proteolytic digestion. Images of the smallest hook structures, the marginal hook sickles which are the key to separating species and typically measure less than 0.007mm in length, were captured using a scanning electron microscope. The images were pre-processed before being subjected to an Active Shape Model feature extraction step to define 110 landmarks and to fit the model to the training set of hook images. Then, this information were used to train 4 classifiers (K-NN, LDA, MLP, SVM) and separate the four species of Gyrodactylus which includes the notifiable pathogen, *G. salaris*. Abbreviations: K-NN, K Nearest Neighbors; LDA, Linear Discriminant Analysis; MLP, Multi-Layer Perceptron; SVM, Support Vector Machine.

Assuming  $s$  sets of landmark points  $D_i$  which are aligned into a common shape pattern for each species, if this distribution can be modelled, then new examples can be generated similar to those in the original training sets, and then these new shapes can be examined to

decide whether they represent reasonable examples. In particular,  $D = M(b)$  is used to generate new vectors, where  $b$  is a vector of parameters of the model. If the distribution parameters can be modelled,  $p(b)$ , these can then be limited such that the generated  $D$ 's are similar to those in the training set. Similarly it should be possible to estimate  $p(D)$  using the model. To simplify the problem, Principle Component Analysis (PCA) is applied, to reduce the dimensionality of the data. PCA summarizes the variation seen across the data, allowing one to approximate any of the original points using a model. The model constructed here was based on 68 SEM hook images, each with 45 points determined as the optimal number of landmark points to effectively characterise the shape of each hook. The subsequent PCA step reduced the number of extracted shape features, removing redundant features and retaining those that best characterise morphological differences between the true species of *Gyrodactylus*.

### 2.3.2. ASM Fitting

Once the ASM model has been constructed, it is important to fit the defined model to a series of new input images to determine the parameters of the model that are the best descriptors of hook shape. ASM finds the most accurate parameters of the defined model for the new hook images. The ASM fitting attempts to "best fit" the defined model parameter to each image. Cootes et al. [28] explained that by adjusting each model parameter from the defined model will permit an extraction pattern of the image series to be created. During the model fitting, it measures newly introduced images and uses this model to correct the values of current parameters, leading to a better fit.

### 2.4. Machine Learning Classifiers

Following ASM and PCA, the data were assessed using four methods of machine learning classifiers. These are briefly described below.

**Linear Discriminant Analysis (LDA):** LDA classification is performed by finding a linear combination of features which best characterise two or more classes of objects [29]. The purpose of LDA is to find a linear function of  $\hat{N}_i = W_0 + W_{i1}D_1 + W_{i2}D_2 + \dots + W_{in}D_n$ .

**K-Nearest Neighbors (K-NN):** The K-NN finds the K nearest neighbor and uses a majority vote to determine the class label [30]. The objective is to classify an unknown example  $R$ , where the equation is  $\hat{N}(R, X_i) = \sum_D W_D \delta(R_D, X_{iD})$ .

**Multi-Layer Perceptron (MLP):** Several layers of neurons are designed in MLP or feed forward neural network. Each layer is completely connected to the next and each neuron calculates a transformed weighted linear combination of its inputs, with the vector of output activations from the preceding layer, the transposed column vector of weights and a bounded non-decreasing non-linear function (sigmoid), with one of the weights acting as a trainable bias connected to a constant input [31]. The algorithm is expressed as  $\hat{N}_k(M_D) = \varphi_k(\sum_{b=0}^u w_{kb} \varphi_b(\sum_{c=0}^e w_{bc} M_{cd}))$ .

**Support Vector Machine (SVM):** The goal of SVM is to produce a model which predicts a species class of data instances in the testing set which are given only the features. SVM uses a kernel to map the feature space (hyperplane) into a high-dimensional transformed space [32]. It is expressed as  $\hat{N} = \sum_i w_i g(v_i D) + a$ .

For each approach, a 10-fold cross validation was used i.e. the data were divided into  $k$  (10) subsets, where  $k-1$  subsets were used for training and the remaining subset used as the test set. This process was repeated 10 times using a different test set on each run and the average classification performance computed.

## 3. Experimental Results

Although the attachment apparatus of *Gyrodactylus* consists of three main elements (i.e. two larger centrally positioned anchors or hamuli; two connecting bars between the hamuli; and, 16 peripherally distributed marginal hooks), this study sets out to classify species based on features extracted from the sickles of the marginal hooks only. As the study is based on the analysis of biological structures, these require processing subsequent to capture in order to standardised the position and format of the image. Processing to standardise the orientation of the image is applied to reduce processing time and complexity during the training and

construction of the ASM model. Table 1 presents detailed accuracy results for selected classifier.

Table 1. Classification rate for multiple species of *Gyrodactylus* using ASM approach

Classifier	Accuracy (%)
LDA	97.14 $\pm$ 6.02
K-NN	93.71 $\pm$ 8.90
MLP	98.72 $\pm$ 3.83
SVM	91.03 $\pm$ 7.96

Table 2. A confusion matrix showing the classification of *Gyrodactylus* specimen using an LDA classifier. Each of *G. derjavinoideis* (*G. der*) and *G. salaris* (*G. sal*) have an individual misclassification

	<i>G. col</i>	<i>G. der</i>	<i>G. sal</i>	<i>G. tru</i>	Precision (%)
<i>G. col</i>	10	0	0	0	100
<i>G. der</i>	0	24	0	1	96
<i>G. sal</i>	0	0	33	1	97.06
<i>G. tru</i>	0	0	0	9	100
Recall (%)	100	100	100	81.82	

Table 3. Using the K-NN classifier, *G. coleman* (*G. col*) is manage to have full classification, while other species (*G. derjavinoideis* (*G. der*), *G. salaris* (*G. sal*) and *G. truttae* (*G. tru*) remain misclassified

	<i>G. col</i>	<i>G. der</i>	<i>G. sal</i>	<i>G. tru</i>	Precision (%)
<i>G. col</i>	10	0	0	0	100
<i>G. der</i>	2	22	0	1	88
<i>G. sal</i>	0	0	33	1	97.06
<i>G. tru</i>	0	1	0	8	88.89
Recall (%)	83.33	95.65	100	80	

Table 4. MLP classifier performs well with the correct classification *G. coleman* (*G. col*), *G. derjavinoideis* (*G. der*) and *G. truttae* (*G. tru*).

	<i>G. col</i>	<i>G. der</i>	<i>G. sal</i>	<i>G. tru</i>	Precision (%)
<i>G. col</i>	10	0	0	0	100
<i>G. der</i>	0	25	0	0	100
<i>G. sal</i>	0	0	33	1	97.06
<i>G. tru</i>	0	0	0	9	100
Recall (%)	100	100	100	90	

Table 5. Using SVM classifier, only one species (*G. coleman* (*G. col*)) has managed to get full classification, while the worst misclassification is *G. truttae* (*G. tru*).

	<i>G. col</i>	<i>G. der</i>	<i>G. sal</i>	<i>G. tru</i>	Precision (%)
<i>G. col</i>	10	0	0	0	100
<i>G. der</i>	0	24	1	0	96
<i>G. sal</i>	0	1	32	1	94.12
<i>G. tru</i>	0	2	2	5	55.56
Recall (%)	100	88.89	91.43	83.33	

The LDA classifier, using 110 of landmark points was able to correctly classify all specimens of *Gyrodactylus* to their true class, except for one specimen of *G. derjavinoideis* which were classified as *G. truttae* and one specimen of *G. salaris* has been identified as *G. truttae* (Table 2). The K-NN classifier also has similar true classification as LDA classifier (Table 3), with addition of one of the nine *G. truttae* specimens was misallocated as *G. salaris*. The two non-linear approaches MLP (Table 4) and SVM (Table 5) were also able to achieve high rates of correct classification, both with 98.72% and 91.03%. Comparing the four classifiers, MLP classifier is the leading for classifier in achieving the highest classification of the four species of *Gyrodactylus*. This is not surprising, since MLP is a well performance classifier in many field [33, 34].

The current study is based on a smaller set of higher quality SEM images and although the average correct classification is higher (i.e. 98.53%) than that achieved using the LDA method applied to 25 point-to-point measurements manually extracted from light micrographs of 557 specimens (i.e. 92.59% and 98.53%) [35, 36], this approach appears promising and now will be applied to hooks prepared for light microscopy hopefully with equal or better rates of correct classification. The ASM-PCA-MLP based approach applied to SEM images of the hook sickles of *Gyrodactylus* appears to out perform other methods that have been tested to identify and discriminate this species with confidence.

With these successful results for extraction and classification, the difficulties faced by domain expert can be minimised. These difficulties include manual classification, a tedious and time consuming process. Another challenge in the manual approach, inaccurate point to point measurements, which result in inaccurate species identification, can also be overcome. Now, with this newly applied combination of techniques, domain experts use these methods for feature measurements and species identification.

#### 4. Conclusion

The current study set out to explore the utility of a novel ASM-PCA-machine learning classifier based approach in classifying species of *Gyrodactylus* which are ectoparasites of fish. ASM applied to 68 SEM images of the marginal hook sickle was able to overcome the limitation and difficulties in extracting feature information from the hooks. The best approach, which used a MLP method of classification, was able to improve upon the performance of previous approaches (i.e. 98.72% cf. 92.59% using an LDA-based classifier applied to manually extracted morphometric data). Future work will assess the performance of this method on larger datasets and will explore new methods based on an ensemble of classifiers, which have shown promising results, with the aims of providing a reliable model for the identification of species, including the pathogen *G. salaris*, by non-experts and fish health researchers.

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