Identification and Description of Odonate Larvae in Lentic Habitats of Singapore

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ABSTRACT

This project aims to identify odonate larvae from still freshwaters of Singapore. This is done firstly through morphological comparison using identification keys, genetic barcoding using Polymerase Chain Reaction and sequencing, and lastly matching larval forms with adult forms by rearing larvae in the laboratory. The ecology and biology of Singapore odonates are also reviewed. Morphological identification keys for Asian odonate larvae are currently useful, though only to family level. DNA barcoding, while feasible, is not yet effective. It would require much more extensive sampling and sequencing to complete a database currently deficient in sequences of Singaporean species. Rearing of larvae was successful for what was probably one species of damselfly. At present, rearing of larvae would probably confer the greatest certainty in species identification, as adult Odonata are far better described than larvae. This, though time-consuming, is unavoidable for acquiring morphological descriptions of larvae. The study of odonate larva can not only further understanding of Singapore’s biodiversity, but can also have useful applications in biomonitoring.

INTRODUCTION

In Singapore, there are 117 species from the order Odonata (Norma-Rashid et al., 2008). Although it may be possible to identify odonate larvae elsewhere to species (Sahlén and Ekestubbe, 2001), there is inadequate knowledge on the taxonomy of Singaporean odonate larvae. Thus the objective of this project is to evaluate the methods for identification and description of Singaporean odonate larvae of families Coenagrionidae, Platycnemididae, Libellulidae and Gomphidae in lentic habitats. These methods are firstly morphological comparison of odonate larvae, including drawing and describing, with reference to identification keys. The second objective is to carry out DNA barcoding of larvae with the NADH-dehydrogenase subunit 1 (NADH1) gene. The final aim is to rear of odonate larvae in the laboratory to match larval specimens with adult forms.

MATERIALS AND METHODS

Odonate larvae were collected from three localities in Singapore using submerged weighted traps with coconut brush, a substrate grab, sweep nets and scoop nets. The odonates were transported cooled back to the laboratory alive. Odonate larvae were examined, identified and selected for either drawing using a camera Lucida, or photographed using a Leica camera and

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microscope. Odonate larvae were identified with Asian genus keys by Dudgeon (1999), Yule and Yong (2004) and Mekong (2006). Adult damselflies were identified with reference to the odonate guidebook by Orr (2005). Using Lucid3 software, a Lucid key was constructed using key features of the four odonate families.

DNA extraction was carried out using the Qiagen DNeasy Blood and Tissue Kit. Polymerase chain reaction (PCR) was conducted using NADH1 gene primers. Bioline ‘Sure Clean’ solution was used for purification for cycle sequencing, and the gene fragments amplified using BigDye Terminator cycle sequencing kit. Agencourt CleanSeq was used to purify the DNA. Sequencing was carried out using a ABI PRISM 3100 Genetic Analyzer machine. The sequences obtained were then compared with each other, and with sequences in the NCBI nucleotide BLAST database.

The odonate larvae were reared in plastic containers in dechlorinated aerated water at room temperature, with appropriate substrates, and fed chironomid larvae (Rice, 2008) until they emerged as adults. Adults were preserved in 100% ethanol.

RESULTS

The zygopteran families Coenagrionidae (Fig. 1) and Platycnemididae can be distinguished by the relative length, apex shape and presence of tracheal branching of the caudal gills (Figs. 1b &1c), and the relative length of the second and third antennal segments. (Dudgeon, 1999).

The anisopteran families Gomphidae (Fig. 2a) and Libellulidae (Fig. 2b) have different distinguishing features.

The family Gomphidae are characterised by their third of 4 antennal segments greatly enlarged and the fourth segment vestigial. The libellulid are recognised by a bowl-shaped labium with setae on the papal lobes and usually on the mentum. The distal margins of the labial papal lobes are smooth or evenly toothed.

The barcoding results were inconclusive, with the differences in base pairs of percentages from 16.2% to 22.6%.

Nine of 17 individuals reared survived to adulthood (Fig. 3), six of which were identified as likely to be Pseudagrion microcephalum (Rambur, 1842), with 4 male and 2 females.

Figure 1a. Coenagrionidae larva. (dorsal view) 1b. Coenagrionidae larva caudal gill. 1c. Platycnemididae larva caudal gill.
Table 1. Genetic distances between four odonate individuals.

<table>
<thead>
<tr>
<th>% difference in base pairs between individuals</th>
<th>C1</th>
<th>C2</th>
<th>L1</th>
<th>L 2</th>
</tr>
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<td>-</td>
<td>18.8</td>
<td>22.6</td>
<td>21.5</td>
</tr>
<tr>
<td>Coenagrionidae 2</td>
<td>18.8</td>
<td>-</td>
<td>17.8</td>
<td>16.2</td>
</tr>
<tr>
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<td>-</td>
<td>18.1</td>
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<tr>
<td>Libellulidae 2</td>
<td>21.5</td>
<td>16.2</td>
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DISCUSSION

While drawing and description was relatively simple for smaller individuals, it was not possible for larger, opaque anisopterans and photographs were taken instead. Due to preservation in ethanol, some of the colour of specimens was lost, and in some cases key features were not visible. Identification beyond the family level was hindered by lack of Asian keys beyond that.

There was a lack of local species in the NCBI sequence database so sequenced individuals could not be identified with certainty and attempts to sequence adult DNA were unsuccessful, preventing the matching of larvae to known adults.
During collection, several individuals died during transportation even though they were cooled. Predation among odonates occurred although the larvae were kept at low densities, probably due to insufficient prey (Rice, 2008). This could be eliminated by using one container per larva, but this requires more resources. The teneral adults that emerged might be morphologically different from mature adults, so there is uncertainty in identification (Orr, 2005). Also, likely only one species of damselfly was successfully reared, so results might differ for others.

Descriptions of the larval and adult stages of the same individual could not be obtained as the odonates did not survive cooling for facilitate photography. No anisopterans that were collected survived to adulthood also because those collected were immature. For a more comprehensive understanding of matching larval forms to adult forms, more extensive sampling and rearing must be carried out. It would be ideal but complex to rear adults to mature forms. Two adult damselflies remain unidentified as they had begun to disintegrate, avoidable by using containers with a greater perching surface out of the water, but more resource-intensive.

Time was the major limiting factor on the scope of the project, restricting sampling to a few sites and barcoding to four successful sequences. Also, the rearing of early instars of odonate larvae to adulthood was not possible.

At present, of the three methods of larval identification, neither sufficiently specific keys nor a complete genetic database is available to identify Singaporean odonate larvae to species or even genus. However, as the adult forms are well known, rearing of adults is probably the most certain method of identification, even though this is resource-demanding and slow. Further work is necessary to complete a genetic database and morphological key for Singaporean odonates.

REFERENCES


Orr, A. G. (2005), *Dragonflies of Peninsular Malaysia and Singapore*, Natural History Publications (Borneo), Kota Kinabalu.


