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A combined morphological and molecular approach in identifying barnacle cyprids from the Matang Mangrove Forest Reserve in Malaysia: essentials for larval ecology studies

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Abstract. Identification of larval mesoplankton is essential to the study of the supply-side ecology of marine benthic or sessile organisms, such as barnacles. Combined morphological and molecular identifications of wild-caught barnacle cyprids from Matang Mangrove Forest Reserve (MMFR), Malaysia were studied based on mitochondrial 12S-rRNA gene sequences of the unidentified larvae and identified adults. Six species of barnacle adults and cyprids had matched DNA sequences. These included *Fistulobalanus pattellaris*, *Fistulobalanus* sp., *Amphibalanus reticulatus*, *Amphibalanus variegatus*, *Amphibalanus amphitrite*, and *Euraphia withersi*. Morphological characters of the identified cyprids were described, and used to develop a morphology-based classification tree. Carapace sculpturing pattern on the cyprids was the most important morphological discriminator. Preliminary analysis of the diversity of barnacle cyprids in MMFR showed that the dominant species could be morphologically classified with high accuracy.

Key words. larval ecology, barnacle cyprids, classification, DNA barcoding, mangrove

INTRODUCTION

Thoracican barnacles are important filter feeders in the mangrove food web (Fry & Smith, 2002) and can play a role in the filtration function of mangroves (Soares-Gomes et al., 2010). They are common on the surface of roots, trunks, and leaves of mangrove plants, fallen propagules and plant debris, and shells of crustaceans and molluscs. In replanted mangrove system, barnacles are considered as pests because their settlement on the stems and leaves can result in mortality or reduced fitness of mangrove seedlings (Perry, 1988; Li & Chan, 2008; Li et al., 2009). In fact, barnacle infestation on newly replanted mangrove seedlings is recognised as one of the important problems in mangrove rehabilitation (Angsupanich & Havanona, 1996; Primavera & Esteban, 2008).

The life cycle of thoracican barnacles is composed of both planktonic larval and sessile adult stages. The planktonic larvae include six naupliar stages and a final cyprid stage prior to settlement. The distribution of the barnacle cyprids in the water column is patchy on spatial and temporal scales (Pineda, 2000) which can affect the subsequent recruitment dynamics of adults (Grosberg, 1982; Pineda et al., 2002), including those that inhabit the mangrove ecosystem (Ross & Underwood, 1997; Satumanatpan et al., 1999; Ross, 2001; Satumanatpan & Keough, 2001). In replanted mangroves at Ban Don Bay, Thailand (Angsupanich & Havanona, 1996), and Haji Dorani, Malaysia (Tan, 2013), the pulse recruitment of barnacle cyprids is often intense, resulting in rapid cover by barnacles on the replanted mangrove. The supply-side ecology of barnacle cyprids is, therefore, important to understanding the distribution and larval settlement processes of barnacles in mangroves. However, the remarkable similarity of cyprid morphology among species (Elfimov, 1995) and lack of detailed morphological descriptions of larvae of many species make identification difficult and pose a major obstacle to the study of barnacle supply-side ecology.

At present, descriptions of barnacle cyprids are mostly dependent upon laboratory-reared larvae. There are very few morphological keys for the identification of wild caught barnacle cyprids. Such keys are often limited in their usefulness. For instance, the guide developed by Standing (1981) pertains to only the cyprids of Oregon waters in U.S.A. A guide has yet to be developed for barnacle cyprids for any particular region in the tropics. Moreover, larval culture itself poses several challenges in terms of suitability of larval feed and rearing conditions to ensure sufficient larval survival. Molecular techniques which enable accurate species identifications could dispense with the need for larval culture. For example, DNA barcoding has been extensively used for species identification in recent years. By matching a chosen region of DNA fragments from the specimen with known reference specimens, identification can be achieved (Hebert et al., 2003). The method is very useful for the identification of species with different life stages, if the adult

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