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Ultra- and microstructure of the female reproductive system of *Matsucoccus matsumurae*

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ABSTRACT

The ultra- and microstructure of the female reproductive system of *Matsucoccus matsumurae* was studied using light microscopy, scanning and transmission electron microscopy. The results revealed that the female reproductive system of *M. matsumurae* is composed of a pair of ovaries, a common oviduct, a pair of lateral oviducts, a spermatheca and two pairs of accessory glands. Each ovary is composed of approximately 50 telotrophic ovarioles that are devoid of terminal filaments. Each ovariole is subdivided into an apical tropharium, a vitellarium and a short pedicel connected to a lateral oviduct. The tropharium contains 8–10 trophocytes and two early previtellogenic oocytes termed arrested oocytes. The trophocytes degenerate after egg maturation, and the arrested oocytes are capable of further development. The vitellarium contains 3–6 oocytes of different developmental stages: previtellogenesis, vitellogenesis and choriogenesis. The surface of the vitellarium is rough and composed of a pattern of polygonal reticular formations with a center protuberance. The oocyte possesses numerous yolk spheres and lipid droplets, and is surrounded by a mono-layered follicular epithelium that becomes binucleate at the beginning of vitellogenesis. Accessory nuclei are observed in the peripheral ooplasm during vitellogenesis.

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1. Introduction

Scale insects (Hemiptera: Coccoidea) contain nearly 8000 species and many are economically important as pests of agriculture, horticulture and forestry. The superfamily Coccoidea is currently divided into 49 families (http://www.sel.barc.usda.gov/scalenet/ scalenet.htm). These families are divided into two informal groups, archaeococcoids and neococcoids (Koteja, 1974, 1996; Miller, 1984).

The neococcoids are composed of advanced scale insects, including Coccidae, Pseudococcidae, Diaspididae, Eriococcidae, Kermesidae, Asterolecaniidae and several other small families. Morphological and genetic data indicate that the neococcoids form a monophyletic group (Koteja, 1974; Cook et al., 2002). In contrast, the archaeococcoids contain two families, Margarodidae *sensu lato* and Ortheziidae, with plesiomorphy in the previous classification system (Morrison, 1928). The monophyly of Margarodidae *sensu lato* and Ortheziidae is unproven (Koteja, 1974; Foldi, 1997; Gullan and

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Siaarda. 2001: Cook et al., 2002: Cook and Gullan, 2004: Gullan and Cook, 2007). Koteja (1974) raised the subfamilies or tribes in Margarodidae to family status, based primarily on a detailed study of the morphology of female mouthparts. Since then, a series of studies have been conducted on their phylogeny. Gullan and Sjaarda (2001) undertook a cladistic analysis using morphological characters of adult females and first-instar nymphs as well as 2 life-history characters of 25 margarodid genera, Cook et al., (2002) published a preliminary phylogeny of scale insects based on nuclear small subunit ribosomal DNA, and Hodgson and Foldi (2005, 2006) studied the morphology of macropterous adult males. These results support the recognition of up to 15 extant families of archaeococcoids, including 11 families of the Margarodidae sensu Morrison (Coelostomidiidae, Marchalinidae, Pityococcidae, Callipappidae, Kuwaniidae, Margarodidae, Monophlebidae, Steingeliidae, Matsucoccidae, Stigmacoccidae and Xylococcidae) as well as the Ortheziidae, Carayonemidae, Phenacoleachiidae and Putoidae. However, these morphological and molecular phylogenetic studies have failed to demonstrate monophyly of the archaeococcoids.

Matsucoccidae was suggested as one of the earliest and most specialized families among the Archaeococcoids by Koteja (1974, 1984 and 1990), who studied fossils of scale insects. The family







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Fig. 1. Schematic representation of the female reproductive system of *Matsucoccus matsumurae*. Note: tropharium (t), vitellarium (v), lateral oviduct (lo), spermatheca (sp), accessory gland (ag), common oviduct (co).

Matsucoccidae currently includes two genera with 44 species: the extant genus *Matsucoccus* with 34 extant and six fossil species and the extinct genus *Eomatsucoccus* with four fossil species (Foldi, 2001). External morphological characteristics (Foldi, 2004) and molecular data (Booth and Gullan, 2006) were used for phylogenetic studies of *Matsucoccus*; however, it was discovered that the variety of internal organs, such as digestive system and female reproductive system, was significant to the phylogeny of the insects (Heming, 2003). Previous studies on the structure of the reproductive systems of *Orthezia urticae* L, *Newsteadia floccosa* Degeer,

Steingelia gorodetskia Nassonov, Nipaecoccus nipae Maskell, Cryptococcus fagisuga Lindinger, Palaeococcus fuscipennis (Burmeister), Dactylopius coccus Costa, Puto albicans and Crypticerya morrilli, showed that some ovary features (number of germ cells per cluster, number of trophocytes per ovariole, presence or absence of arrested oocytes) can be employed for elucidation of evolutionary trends within this insect group (Szklarzewicz, 1997, 1998a, 1998b, 2002; Vogelgesang and Szklarzewicz, 2001; Szklarzewicz et al., 2002; Koteja et al., 2003; Szklarzewicz et al., 2005, 2006; Ramirez-Cruz et al., 2008; Michalik, 2010; Szklarzewicz et al., 2010). However, the female reproductive organs of matsucoccids have not been thoroughly examined, except for the characteristics of the ovary in Matsucoccus pini and Matsucoccus acalyptus reported by Buchner (1966).

In this study, we studied the female reproductive system of the Japanese pine bast scale, *Matsucoccus matsumurae*, which is the type species of the genus. This species was first recorded in 1905 by Kuwana, in Tokyo, Japan, and the original host for the species was *Pinus thunbergii* Parlatore. Since then, it has spread to the Korean Peninsula and China and has become a highly destructive pest of pine forests (*Pinus tabulaeformis* and *Pinus massoniana*) throughout eastern China.

The objective of this study was to determine the structure of the reproductive organs of *M. matsumurae* to better understand the evolutionary trends within Arechaeococcoidea.

2. Materials and methods

2.1. Insects

Approximately 100 live adult females of *M. matsumurae* were collected with forceps from the twigs and branches of *P. massoniana* in April 2011 and 2012 in Jinhua (N 28°32', E 119°14'), Zhejiang Province (China).



Fig. 2. Anatomy of the female reproductive system of *Matsucoccus matsumurae*. A: showing a pair of ovaries composed of about 100 synchronously developing ovarioles that radially extend from the lateral oviduct (lo). common oviduct (co), accessory gland (ag), tropharium (t) and vitellarium (v). B: showing the ovarioles composed of an anterior tropharium (t) and a vitellarium (v), which house one small egg chamber (ec₁) and five large egg chambers (ec₂) of similar size.

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