

# Morphological, Molecular Characterization, Life Cycle And Age Specific Life Table Parameters of Coconut Rugose Spiralling Whitefly, *Aleurodicus Rugioperculatus* Martin: An Exotic Pest of India

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## Research Article

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# Abstract

The present study aimed at reporting the incidence of exotic pest on Coconut rugose spiralling whitefly, *Aleurodicus rugioperculatus* Martin in Tamil Nadu, India. The taxonomical, morphometric, molecular characterization, life cycle and age specific life table parameters of the exotic pest were studied on coconut during 2017-19. The results revealed that rugose spiralling whitefly (RSW) is an exotic and polyphagous in nature and had total life period of  $56.33 \pm 1.01$  days which includes  $6.9 \pm 0.88$ ,  $19.57 \pm 2.17$ ,  $10.9 \pm 0.78$  and  $26.00 \pm 2.34$  days of egg, nymphal, pupal and adult period, respectively. The rugose spiralling whitefly male adult was  $2.6300 \pm 0.0596$  mm in length and  $2.1220 \pm 0.0576$  mm width and female adult were  $3.1460 \pm 0.0329$  mm in length and  $2.1654 \pm 0.0246$  mm width. *A. rugioperculatus* was bigger than other previously reported whiteflies in India. The operculum was wrinkled and having compound pores with dagger shaped axial pores. Ligula of *A. rugioperculatus* extended beyond vasiform orifice. The identity of the species was further confirmed through amplification of mitochondrial COI gene 249 bp was obtained for all the isolates which were sequenced. The sequenced DNA product was submitted to NCBI- Gen Bank (Accession No. MT540964, MT679537, MT679538, MT679539, MT679540 and MT682638). Since, it is a first study on the age specific life table parameters of *A. rugioperculatus* and the results revealed that the net reproductive rates ( $R_0$ ) 24.08 females/female/generation, intrinsic rate of increase 0.0646/day, finite rate of Increase ( $\lambda$ ) 1.066/ day, mean fecundity (F) 41.28 eggs/female and mean generation time (T) 49.25 days, respectively

# Introduction

Coconut (*Cocos nucifera* L) is one of the most important crops in tropical areas and referred as 'tree of heaven' or 'kalpavriksha' because it provides more useful and diverse products to the people's utility. In India, Kerala, Tamil Nadu, Goa, Karnataka, Maharashtra, Orissa, West Bengal and Assam are the major coconut producing states. India consumes 50% of annual production for their culinary and religious purpose, 35% used as copra, 2% for manufacturing of value added products, 11% for tender uses and 2% for seed purpose (Nadanasabapathy and Kumar, 2013). In Tamil Nadu, the coconut is cultivated in various districts viz., Coimbatore, Tiruppur, Erode, Theni, Pudukkottai and Kanyakumari. Tamil Nadu stands third position in India having 461.06 thousand hectares of cultivable land with production of 6570.63 million nuts. More than 900 species of pests are associated with coconut palm. This includes both invertebrates and vertebrates (Kumara et al., 2015). Rugose spiralling whitefly (RSW) is an exotic pest of coconut and first reported by Shanas et al. (2016) from Kerala, India. Further, it has been reported from peninsular India viz., Tamil Nadu, Karnataka and Andhra Pradesh (Sundararaj and Selvaraj, 2017). RSW is a polyphagous which is likely to expand the host range and mainly infests the coconut palms and other broad-leaved hosts in its native range (Elango and Nelson, 2020). RSW feeding causes stress to the host plant by removing water and nutrients and secrete honeydew, which favors the growth of sooty mold on the lower leaves which reduces the photosynthesis (Taravati and Mannion, 2016). Presently it is spread all over Tamil Nadu and southern states of India and the farmers were facing hard ships due to this infestation of RSW in coconut ecosystem. Any change in population structure or unexpected

dynamics may lead high damage and yield losses of coconut. As there are nil systematic studies and research on the life table parameters of *A. rugioperculatus* are scanty in India. .Therefore, the aim of this study on the morphology, molecular characters, life cycle and construct the life table of *A. rugioperculatus* fed on coconut to determine the survival rate and rate of increase of this exotic pest for effective pest management in India.

## Materials And Methods

### Insect sample collection

Surveys were conducted in different agro ecological zones of Tamil Nadu were coconut growing districts of Tamil Nadu viz., Thondamuthur (Coimbatore), Udumalpet (Tiruppur), Chennimalai (Erode), Periyakulam (Theni), Veppangudi (Pudukkottai) and Thatkalai Kanyakumari) during August 2017 to February 2019. As the occurrence of RSW was confirmed with the typical characteristic like eggs on the underside of leaves in a concentric circular or spiral pattern and cover it with white waxy matter on leaves and the different stages of whitefly viz., eggs, nymphs, pupa and adult were collected from coconut. Adult insects were collected using aspirator and other stages were collected with help of camel brush. The collected RSW samples was kept in 70% alcohol and brought to the bio control laboratory, Department of Entomology, Tamil Nadu Agricultural University, Coimbatore for detailed study about morphological, molecular characterization and age specific life table parameters.

### Taxonomic Characterization

Field collected samples of *A. rugioperculatus* (pupal stage) were mounted on slides as per the protocol of Martin (2004), maceration of body contents is carried out by warming to around 80°C in a 10 % Potassium Hydroxide solution for 5–10 minutes or longer, until visible contents have become translucent. A small puncture may be made in the ventral surface of each specimen in order to speed up processes, and to help prevent osmotic collapse. De-waxing of the cuticle was carried out by gently warming the specimens in carbol-xylol (Xylene with 10% dissolved Phenol). Pale puparia staining was be carried out by adding an excess of Glacial Acetic Acid and a few drops of Acid fuchsin stain solution. Di staining was done using 95% Ethanol. Finally dehydration of specimens was carried out by soaking in absolute Ethanol for a few minutes. Clove Oil was dropped on the specimen and placed on slide for observed under microscope.

#### Morphometrics of *Aleurodicus rugioperculatus*

The morphometric studies were made on life stages of *A. rugioperculatus*. The sample drawn from the survey collections were used for the study. Measurements on eggs, nymphal instars (I to IV), pseudo puparium and adults were made using phase contrast research microscope at 40x for studying the essential characters. Adult characters (male and female) studied include wing span, Length and width of the antenna, measurement on left and right claspers of male. Measurements and photographs were

taken in Leica image analyser (Leica M205C) at biosystematics laboratory, Department of Agricultural Entomology, Tamil Nadu Agricultural University, Coimbatore.

## Dna Isolation

To extract the DNA from a single whitefly, lysis method of DNA extraction was followed as detailed below (Zeidan and Czosnek, 1991). A petri dish lid was covered with aluminum foil and parafilm one on another. Then 5µl of lysis buffer (5µl of Tris Hcl, 1µl of EDTA, 5µl of (Triton X 100), 50µl of proteinase K 20 mg/ ml and 939µl of ddH<sub>2</sub>O) was spotted on the surface of the lid covered with parafilm and single whitefly was placed on the buffer spot using brush and crushed with the edge of PCR tube. After crushing, the entire content was transferred into fresh PCR collection tube. In addition to this, edge of the PCR tube which was used to crush the whitefly was washed with 35µl of lysis buffer. The entire content was kept in ice for 5 minutes and incubated at 65°C for 15 minutes followed by 95°C for 10 minutes and placed immediately on ice. The contents were spun for five seconds and supernatant was processed for PCR.

## Mitochondrial Cytochrome Oxidase (Mtcoi) Subunit I Amplification:

The mtCOI gene sequence was analyzed for whitefly samples collected from six locations for genetic identification. Approximately 249 bp of the mtCOI gene fragment was amplified using forward primer (1852) 5' GGGGGAAGTGGTTGAACAAT 3' and reverse primer (2100) 5' AAAAGTTCTATTTAAATTTTCGATCT3' (Dickey et al.,2015). The 25µl reaction volume containing 12.5 µl of 2 X smART master mix, Cat. No. 280311 (readymade mix of taq polymerase, dNTPs and PCR buffer), 5µl of template DNA, (approximately 50 ng) 3.5µl of sterile distilled water and 2µl of each forward and reverse primer (15pg each). The PCR was performed with initial denaturation at 94°C for 3 minutes, followed by 40 cycles each consists of denaturation for 30 seconds at 94°C, annealing for 40 seconds at 53°C with final extension for one minute at 72°C followed by final extension for 20 minutes at 72°C. The PCR products were gel purified and sequenced availing the commercial facility.

## Sequence Analysis

All mtCOI sequences of different group of whiteflies were downloaded from the National Center for Biotechnology Information (NCBI) GenBank. [<https://www.ncbi.nlm.nih.gov/Blast.cgi>]. Sequence alignment was performed employing MUSCLE implemented in Seaview (Thomp-son et al. 1994). Genetic divergence was calculated employing MEGA 7 using ClustalW (Tamura et al. 2011). The mtCOI DNA sequences generated in this study were submitted to the NCBI database.

**Life table parameters of *A. rugioperculatus***

Life table of *A. rugioperculatus* was studied on dwarf coconut trees (Chowghat orange variety) at 25 to 30°C with relative humidity of 70 to 85 per cent at coconut farm, Tamil Nadu Agricultural University (11.01° N, 76.93° E). The leaves with egg spirals were collected and kept in a plastic container for the emergence of nymphs from the eggs. The leaves were examined every 24 hours interval for the nymphal emergence and assessing the incubation period of eggs. After the emergence, the nymphs were examined under hand magnifier (15x) on every day to observe the period of each instar from first to fourth instar nymphs and the total developmental time was recorded. The fourth instar nymph of RSW was considering as pseudo puparial stage and it was covered with small leaf clip-cage (10 X 10 X 5 cm) to trap the emerging RSW adults from pseudo puparium and five freshly emerged males and females were collected with the help of aspirator and confined within in a small leaf clip-cage. The caged adults were observed daily for survival rate, mortality and fecundity. The period between the release of adults and the adult mortality was recorded as the adult longevity. Life stage parameters of *A. rugioperculatus* from egg to adults were collected from thirty individuals. The data of all thirty individuals of *A. rugioperculatus* were pooled and analyzed through age specific-two sex life table software of TWOSEX-MS chart programme described by Chi (2018).

## Results

### Life cycle and morphometrics of *A. rugioperculatus*

Eggs are smooth, elliptical, whitish to yellow, translucent and laid mainly on under surface of the leaves of coconut in characteristic spiral manner covered with waxy coating. Eggs were  $0.3426 \pm 0.0028$  mm length and  $0.1720 \pm 0.0034$  mm width in characteristic spiral manner covered with waxy coating. There were four distinct nymphal instars. The first instar nymphs were translucent, yellowish green, elliptical with a convex dorsum, functional walking legs and antennae. The first instar nymphs were  $0.4098 \pm 0.0068$  mm in length and  $0.2556 \pm 0.0450$  mm in width. First instar nymphal period was  $5.80 \pm 0.78$  days (Fig. 2). The second instar nymphs were oval, translucent and had many marginal fringes of wax covering the body of dorsum. The second instar nymphs were  $0.9540 \pm 0.0340$  mm in length and  $0.7300 \pm 0.029$  mm in width. Puparium is oval shaped, size  $1.5940 \pm 0.0385$  mm. Operculum was wrinkled and having compound pores with dagger shaped axial pores. Ligula extended beyond vasiform orifice and elongate, triangular at proximal end almost sharply pointed (Fig. 1). Pupa period was  $10.9 \pm 0.78$  days (Table.2). Adults emerged through a 'T' shape exit slit on the dorsal surface of the pupae. The wings of newly emerged adults were clear after unfurling, later was covered with a coat of white waxy powder. Eyes dark reddish brown, forewings with three irregular brown bands on the upper side near costal and hind margin and one towards ternal margin. Male adult with  $2.6300 \pm 0.0596$  mm length and  $2.1220 \pm 0.0576$  mm width and having longevity  $23.00 \pm 3.48$  days. Female adult was  $3.1460 \pm 0.0329$  mm length and  $2.1654 \pm 0.0246$  mm width and having longevity  $26.00 \pm 2.34$  days, respectively (Table.1).

### Molecular confirmation and phylogenetic tree analysis of rugose spiralling whitefly

Genomic DNA was isolated from individual adults of *A. rugioeperculatus*, which were subjected to PCR analysis for confirmation of rugose spiralling whitefly using specific primers (Dickey et al., 2015). The expected amplicon size was found to be 249 bp in all the samples were collected from six locations in Tamil Nadu (Fig. 3). The nucleotides were aligned and edited using Bioedit software. After aligning the sequences, these were subjected to BLAST analysis and compared with previously deposited sequences of *A. rugioeperculatus* in the NCBI database. Based on the nucleotide sequence analysis of *A. rugioeperculatus* isolates (CO1, TI1, ER1, PK1 and TH1) shared 99 per cent similarity with previously reported isolate (MK883218). Whereas, KK1 isolate of *A. rugioeperculatus* exhibit 97 per cent similarity with *A. rugioeperculatus* (MK883215). The nucleotides sequences were deposited in the NCBI database (Accession No. MT540964, MT679537, MT679538, MT679539, MT679540 and MT682638) (Table.3). Similar reports were given by Dickey et al., (2015) and Selvaraj et al., (2017) for molecular identification of *A. rugioeperculatus* through COI gene. Our study isolates also share 99 per cent identity with KY209909 and KP032219 isolates of *A. rugioeperculatus*. A phylogenetic tree was constructed on the basis of mtCOI nucleotide sequence (Fig. 4.) clearly showed that the populations CO1, TI1, ER1, PK1, and TH1 were clustered with *A. rugioeperculatus* group. Though the population KK1 belonged to *A. rugioeperculatus* group, it branched off from *A. rugioeperculatus* due to some substitutions in nucleotide sequence. The phylogenetic tree was divided three main branches; in that first main branch was sub divided into two clades and each clade contain single group of whitefly population from different countries were placed together viz., *Paraleyrodes bondari* and *Aleurothrixus floccosus*, whereas the second main branch contain *Paraleyrodes minei* population. Third main branch was divided into two sub clades which contain *A. dispersus* and *A. rugioeperculatus* populations were well separated and clustered together.

### Life table parameters of *A. rugioeperculatus*

Age-specific survival rate ( $l_x$ ) and fecundity ( $m_x$ ) of *A. rugioeperculatus* shown in Fig. 5. Survivorship curves shown in the figure were based on the mortality rate from egg to adult stage of *A. rugioeperculatus*. The survivorship study of *A. rugioeperculatus* revealed that most of the mortality happened at later instar of life cycle and less mortality in the earlier life stages of an organism. The life table parameters of *A. rugioeperculatus* revealed that the net reproductive rates ( $R_0$ ) 24.08 females/female/generation, intrinsic rate of increase  $r$  0.0646/day, finite rate of Increase ( $\lambda$ ) 1.066/ day, mean fecundity ( $F$ ) 41.28 eggs/female and mean generation time ( $T$ ) 49.25 days, respectively (Table.4).

## Discussion

Rugose spiralling whitefly, *A. rugioeperculatus* had total life period of  $56.33 \pm 1.01$  days which includes  $6.9 \pm 0.88$ ,  $19.57 \pm 2.17$ ,  $10.9 \pm 0.78$  and  $26.00 \pm 2.34$  days of egg, nymphal, pupal and adult period, respectively. Similarly, Kumar et al. (2013) also documented that RSW females lay eggs on underside of the leaves in concentric circular or spiral pattern covered with waxy matter and also reported eggs are elliptical and creamy yellow in colour. Egg period of *A. rugioeperculatus* was  $6.90 \pm 0.88$  days. Boughton et al. (2015) also reported that the egg period of RSW was  $8.3 \pm 0.1$  days on bird of paradise plants. Mannion (2010) documented that *A. rugioeperculatus* crawlers molt into immature stages that are

immobile, oval and flat initially but become more convex with progression of its life cycle. Stocks and Hodges (2012) reported that the nymphs are light to golden yellow in colour and nymphs will produce a dense cottony wax as well as long, thin waxy filaments reported by Boughton et al. (2015) stated that life durations of first instar, second instar, third instar and fourth instar nymphs of *A. rugioferulatus* were  $6.7 \pm 1.2$  days,  $3.8 \pm 0.6$  days,  $5.5 \pm 0.8$  days and  $8.2 \pm 0.2$  days respectively. The same observations were also reported by Stocks and Hodges (2012) who stated that puparia of *A. rugioferulatus* are characterized by an apically acute lingula that is exerted and slightly short of the posterior margin of the pupa. Shanas et al. (2016) reported that the RSW puparial cuticle are reticulated and has compound pores on 7th and 8th abdominal segments and acute lingual Stocks and Hodges (2012) also documented that adult whiteflies are larger in size with a pair of irregular light brown pars across the wing. RSW adults can be distinguished by their large size and the presence of a pair of irregular light brown bands across the wings. Males have long pincer like structures at the end of their abdomen. The rugose spiralling whitefly male adult with  $2.6300 \pm 0.0596$  mm length and  $2.1220 \pm 0.0576$  mm width and female adult were  $3.1460 \pm 0.0329$  mm length and  $2.1654 \pm 0.0246$  mm width. The length of male RSW is  $0.8680 \pm 0.0497$  mm in male and  $0.9220 \pm 0.0268$  mm in female. The present study was compared with *B. tabaci* morphometric studies reported by Harish et al. (2016) they observed that in the case of adult female and adult male, antennal length was (0.376 mm and 0.339 mm) and body width (0.288 mm and 0.253 mm), respectively. It is clearly shows that the size of the *A. rugioferulatus* was bigger than *B. tabaci*. The identity of the species was further confirmed through amplification of mitochondrial COI gene 249 bp was obtained for all the isolates which were sequenced. The sequenced DNA product was submitted to NCBI- GenBank (Accession No. MT540964, MT679537, MT679538, MT679539, MT679540 and MT682638). A phylogenetic relationship of spiralling whitefly *A. rugioferulatus* is closely related to giant whitefly, *A. dugesii* and spiralling whitefly, *A. dispersus* were reported by (Stocks and Hogges, 2012). Dickey et al., (2015) studied the phylogenetic relationship of whitefly species in Florida and their phylogenetic analysis showed that the genus of *Aleurodicus*, *Paraleyrodes*, *Aleurocanthus*, *Dialeurodes* and *Trialeurodes* were clustered in single group, whereas *Bemisia* genus alone clustered in another group. The life table parameters of *A. rugioferulatus* revealed that the net reproductive rates ( $R_0$ ) 24.08 females/female/generation, intrinsic rate of increase  $r$  0.0646/day, finite rate of Increase ( $\lambda$ ) 1.066/ day, mean fecundity (F) 41.28 eggs/female and mean generation time (T) 49.25 days, respectively Since, it is a first study on the age specific life table parameters of *A. rugioferulatus*. Hence, the results of present study was discussed with other whitefly species. The results are in agreement with the reports of Ahmad et al. (2014) and they have recorded similar findings on *B. tabaci* on pulses and reported that maximum mortality of whitefly was recorded at late instars highest survival percentage at all the immature stages was observed on black gram.

## Declarations

### Authors' contributions

SJN and KE performed the idea of this article, and SJN and KE wrote the manuscript. KE and TT participated in writing the manuscript and image capturing. The authors read and approved the final

manuscript.

### **Availability of data and materials**

Not applicable

### **Competing interests**

The authors declare that they have no competing interests

### **Funding**

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### **Conflict of Interest**

None declared

### **Ethical approval**

This article does not contain any studies with human participants or animals performed by any of the authors

### **Consent for publication**

I agree to publish this paper in the *Biologia*

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## Tables

Table 1  
Morphometric parameters of different stages of *A. rugioperculatus*

<b>Stages</b>	<b>Length (mm) (Mean ± SD)</b>	<b>Width (mm) (Mean ± SD)</b>
Egg	0.3426 ± 0.0028	0.1720 ± 0.0034
I instar	0.4098 ± 0.0068	0.2556 ± 0.0450
II instar	0.9540 ± 0.0340	0.7300 ± 0.029
III instar	1.3680 ± 0.0400	0.8660 ± 0.0428
IV instar	1.5940 ± 0.0385	1.1640 ± 0.0391
Male adult	2.6300 ± 0.0596	2.1220 ± 0.0576
Female adult	3.1460 ± 0.0329	2.1654 ± 0.0246
Left wing of male	2.1860 ± 0.0483	0.9480 ± 0.0277
Right wing of male	2.2940 ± 0.0385	0.9000 ± 0.0316
Left wing of female	2.6220 ± 0.0390	0.8220 ± 0.0390
Right wing of female	2.445 ± 0.0695	0.8300 ± 0.0316
Left antenna of male	0.8680 ± 0.0497	
Right antenna of male	0.7740 ± 0.0288	
Left antenna of female	0.9220 ± 0.0268	
Right antenna of female	0.9620 ± 0.0192	
Left clasper	0.8012 ± 0.0254	
Right clasper	0.7810 ± 0.0620	
SD: Standard Deviation ; *Mean of fifty replication		

Table 2  
Developmental duration of rugose spiralling  
whitefly on coconut

Life stages of rugose spiralling whitefly	Duration (days) (Mean $\pm$ SD*)
Egg	6.90 $\pm$ 0.88
I Instar	5.80 $\pm$ 0.78
II Instar	5.40 $\pm$ 0.50
III Instar	8.37 $\pm$ 0.89
IV Instar	10.90 $\pm$ 0.78
Total development period	37.30 $\pm$ 3.66
Male adult longevity	23.00 $\pm$ 3.48
Female adult longevity	26.00 $\pm$ 2.34
Total longevity	56.33 $\pm$ 1.01
*Mean of thirty individuals	

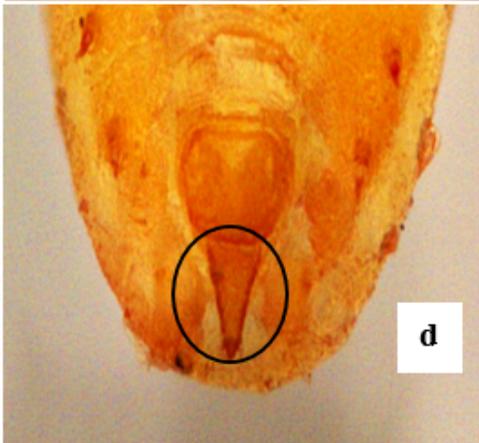
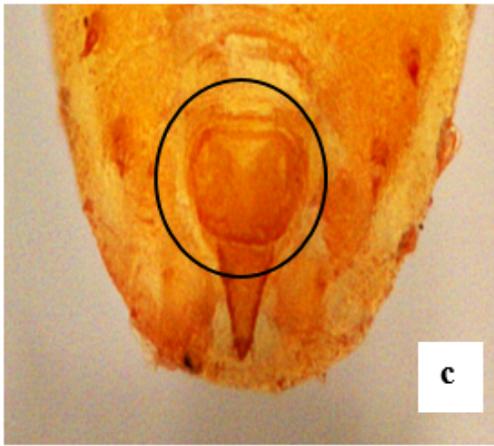
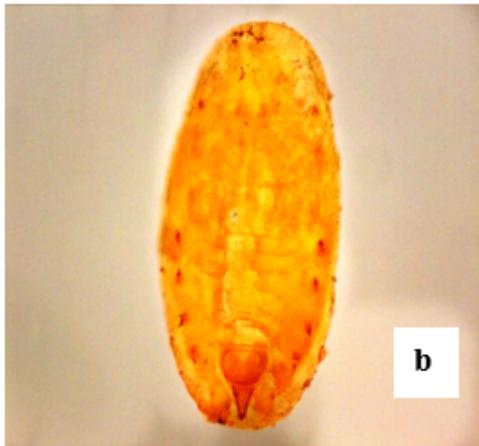
Table 3  
Survey and molecular identification of *A. rugioperculatus* in Tamil Nadu

Districts	Places	Latitude	Longitude	Code	Host	GenBank accession
Coimbatore	Thondamuthur	11.0152° N	76.9326° E	CO1	<i>Cocos nucifera</i>	MT540964
Tiruppur	Udumalpet	11.1085° N	77.3411° E	TI1	<i>Cocos nucifera</i>	MT682638
Erode	Chennimalai	11.1676° N	77.6042° E	ER1	<i>Cocos nucifera</i>	MT679537
Pudukottai	Veppangudi	10.3833° N	78.8001° E	PK1	<i>Cocos nucifera</i>	MT679539
Theni	Periyakulam	10.0104° N	77.4768° E	TH1	<i>Cocos nucifera</i>	MT679538
Kanyakumari	Thatkalai	8.2456° N	77.3156° E	KK1	<i>Cocos nucifera</i>	MT679540

Table 4  
Life table parameters of *A. rugioferculatus*

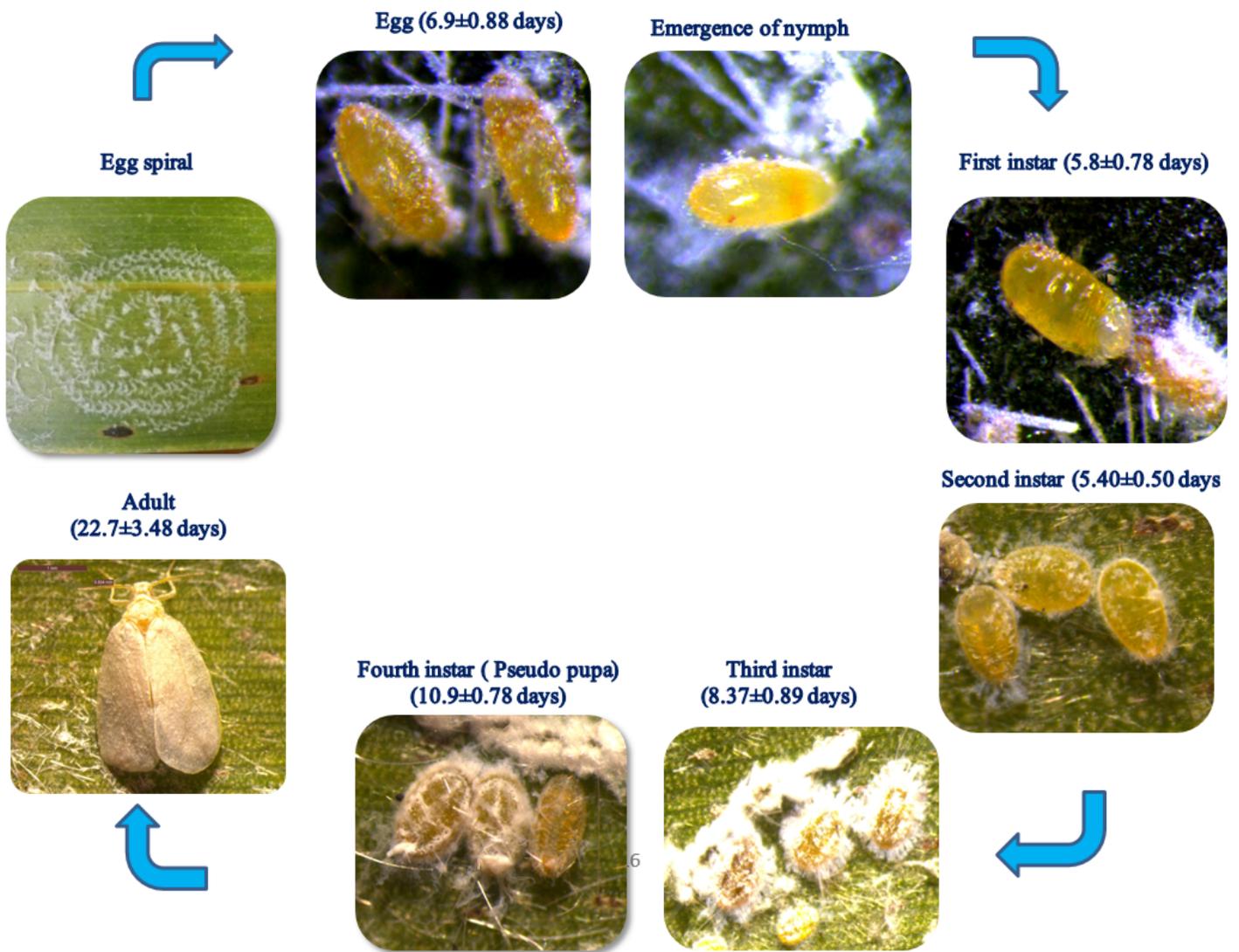
<b>Life table parameters</b>	<b>Mean / Duration(days)*</b>
Net reproductive rate (R <sub>0</sub> )	24.08 females/female/generation
Intrinsic rate of increase (r)	0.0646/day
Finite rate of increase ( $\lambda$ )	1.0667/day
Mean generation time (T)	49.25 days
Mean fecundity (F)	41.28 eggs/female
*Mean of thirty individuals	

## Figures



**Figure 1**

Taxonomical identification of *A. rugioперculatus* a) Pseudo puparium of RSW, b) Mounting of Pseudopupe, c) Rugoseness/ wrinkled nature of operculum, d) Triangular shape of lingula, e) Compound pores with dagger-shaped axial process



**Figure 2**

Life cycle of rugose spiralling whitefly, *Aleurodicus rugioperculatus*

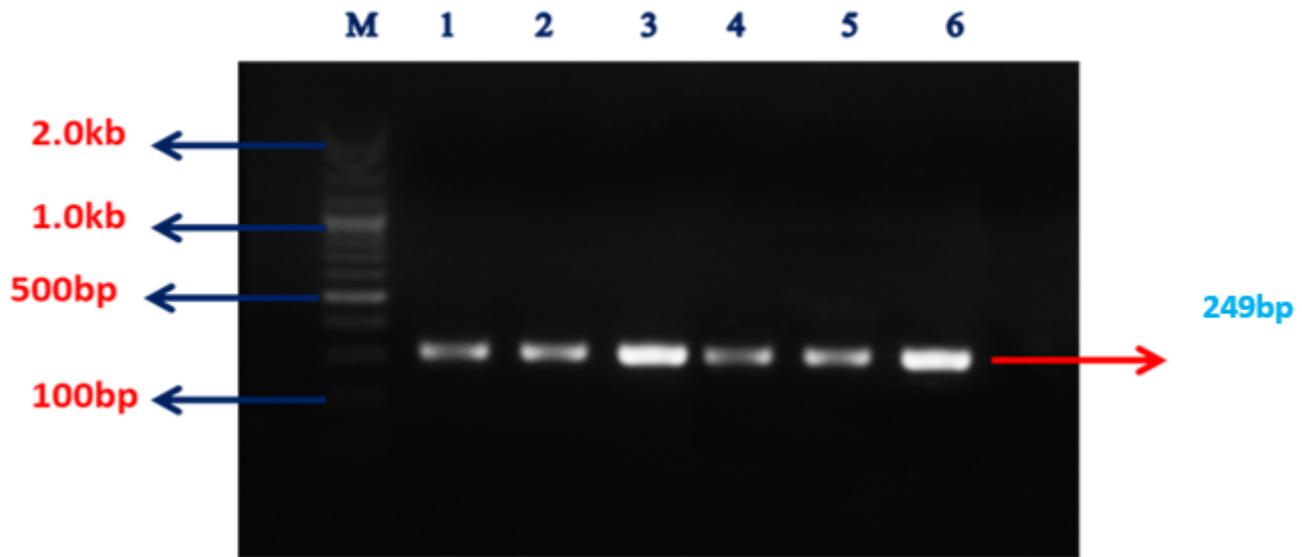


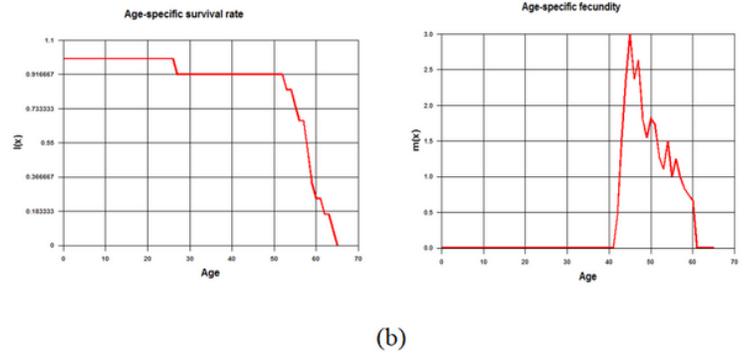
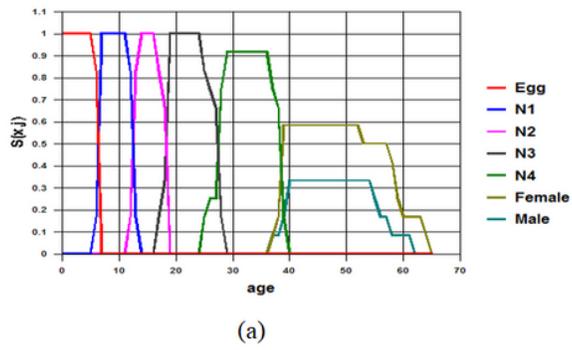
Figure 3

Molecular Characterization of rugose spiralling whitefly, *Aleurodicus rugioperculatus* L – 100 bp DNA ladder, 1- Coimbatore, 2- Tiruppur, 3- Erode, 4 – Theni, 5- Pudukkottai, 6 - Kanyakumari



**Figure 4**

Phylogenetic dendrogram based on mtCOI partial nucleotide sequences of *Aleurodicus rugiosperculatus* Phylogenetic tree generated from aligned partial mtCOI nucleotide sequences of *Aleurodicus rugiosperculatus* genotypes with other selected whiteflies. Tree was generated by neighbour joining method by aligning the sequences in MEGA 7 using ClustalW. Vertical branches are arbitrary; horizontal branches are proportional to calculated mutation distances; values at nodes indicate percentage boot straps values (1000 replicates).



**Figure 5**

Life table parameters of *A. rugioeperculatus* a. Survival rate to each age stage of *A. rugioeperculatus* N1- First instar nymph, N2 – Second instar nymph, N3- Third instar nymph, N4 – Fourth instar nymph b. Age specific survival rate and fecundity of *A. rugioeperculatus*