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PRELIMINARY EXPERIMENTATIONS ON THE IN VITRO REARING OF HEMATOPHAGOUS PIGEON LOUSE *HOHORSTIELLA LATA* (AMBLYCERA: PHTHIRAPTERA: INSECTA)

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KEY WORDS

Amblycera
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ABSTRACT

Rearing hematophagous amblyceran lice in vitro is a challenging task. The hematophagous nature and active habits of amblycerans are distinct hurdles to in vitro rearing. The literature indicates only limited success in rearing the hematophagous amblyceran avian louse. Herein we report on the results of in vitro experimentation on an amblyceran pigeon louse, *Hohorstiella lata*. The incubation period of eggs was 5.47 ± 0.52 days. The durations of first, second, and third nymphal instars were 5.14 ± 0.55, 5.65 ± 0.83, and 6.35 ± 0.82 days, respectively. The average lifespan of adult females (7.45 ± 5.88 days) was higher than adult males (4.61 ± 3.57 days). Adult females laid a lifetime average of 3.73 eggs at a rate of 0.45 eggs/female/day under in vitro conditions (35 ± 1 C, 75–82% relative humidity, feather diet).

The study of in vitro bionomics of parasitic insects is useful in understanding the insect's adaptation to the host. During the last 20 yr, selected ischnoceran lice have been reared off the host and the data obtained through in vitro experimentation have been used to compute their life tables (Beg et al., 2005; Gupta et al., 2007; Arya et al., 2009; Saxena et al., 2009; Agarwal et al., 2011; Singh et al., 2012; Kumar and Hasan, 2016). Maturano and Daemon (2014) reported the in vitro biology of a large turkey louse, *Chelopistes meleagridis*, and noted that the inclusion of skin in diet improved the survival of lice.

As far as amblyceran phthirapterans are concerned, there has been limited success rearing them off of their hosts. A survey of the literature shows that certain aspects of bionomics (i.e., incubation period of eggs, duration of 3 nymphal instars, and the egg rate) of a hematophagous amblyceran poultry louse, *Menacanthus stramineus*, was studied by Stockdale and Raun (1965). Scanty information on the in vitro biology of another hematophagous amblyceran poultry louse, *Menopon gallinae*, has been furnished by Surman et al. (1998). Nelson (1971) performed long-term in vitro colonization of a nonhematophagous amblyceran pigeon louse, *Colpocephalum turbinatum*, and detailed information on the bionomics of the louse has been furnished by Rana et al. (2018). The present report provides preliminary information on the in vitro biology of a hematophagous amblyceran pigeon louse, *Hohorstiella lata*.

Hohorstiella lata is a light-brown colored louse, with slightly dark margins on the head, thorax, and abdomen. It is a typical menacanthid type louse. The head is broadly convex and broadest at the temples, which are rounded and small. The preocular slit is straight and narrow and the occipital and ocular nodii are well developed. The mandibles are sharp and the gular plate is rounded. The maxillary palps are prominent with a spine-like postpalpal process near the base of the maxillary palps. The hypopharynx is reduced. Antennae are concealed in antennal fossa and the pedicel is laterally expanded into a thumb-like projection. The prothorax is slightly trapezoidal with a roughly triangular metasternal plate and typically short legs. The abdomen is large and rounded with 9 distinct segments. It is broadest at the fourth and fifth segments with the tergal plates completely undivided and weakly sclerotized. The pleurites are slightly expanded medio-posteriorly and sternites 8–10 are fused. Female terminalia comprise segments 9 and 10 and the posterior margin of the terminal segment is more or less crescent-shaped. Males are considerably smaller than females with a slightly conical frons. In male genitalia, the parameres are slender and basal apodeme is thick and broad. The genital sclerite is wedge-shaped and the terminal segment is rounded.

Price et al. (2003) listed the genus *Hohorstiella* (occurring on pigeons and doves) among intrinsically rare lice. However, its prevalence was quite low on pigeons of Cuba (1.5%, n = 65, 2008–2017; Garcia et al., 2018), moderate in Iran (20.0%, n = 5, 2008–2010; Dik and Halajian, 2013) and India (29.0%, n = 205, 2003–2004; Khan et al., 2009) but quite high in Pakistan (51.4%, n = 68, 2004–2007; Naz et al., 2010) as well as Ukraine (72.7%, n = 55, 2016–2017; Kolomak and Kruchynenko, 2017). However, the mean intensity of infestation of the louse on Indian pigeons was

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lower (6.2 lice per bird, range of infestation 1–14; Khan et al., 2009) than that of Pakistan (230.1 lice per bird, range of infestation 87–740; Naz et al., 2010).

Hohorstiella lata is a fast-running active louse, occurring more frequently near the skin and on down parts of small feathers of the vent and abdominal regions of pigeons. It prefers to lay its eggs on the feathers belonging to the foreparts of the body (nape, neck, and head) as done by *Menacanthus eurysternus* infesting *Pycnonotus jacossus* (Saxena et al., 2012). *Hohorstiella lata* is partly a hematophagous louse: the crop contents of 57% males, 73% females, 20% third instars, 17% second instars, and 10% first instars contained red content compatible with host blood, along with feather barbules (Kumar et al., 2018). The hematophagous amblyceran species deserve special attention, as 2 poultry lice (*Menacanthus stramineus* and *Menopon gallinae*) have been convicted to act as reservoir and transmitter of bacterial strains (*Pasteurella multocida*, *Salmonella gallinarum*, *Streptococcus equinus*, *Escherichia coli*, and *Ornithosis bedsoniae*) and few other species (*Dennyus hirundinis*, *Pseudomenopon pilosum*, *Actornithophilus limosae*, *Trinoton anserinum*, *Pseudomenopon* species, and *Austromenopon phaeopodis*) infesting African swift, American coot, marbled godwit, mute swan, red-necked grebe, and Whimbrel reportedly act as intermediate host of filarial worms, *Filaria cypseli*, *Pelecitus fulicaeatrae*, *Eulimdana wongae*, *Sarconema eurycera*, and *Eulimdana bainae* (Clayton et al., 2016). However, *H. lata* has not been investigated as a disease vector.

MATERIALS AND METHODS

For in vitro experimentation on *H. lata*, the adult lice, eggs (glued to neck/nape feathers), and nymphal instars (Fig. 1A–F) were obtained from lousy pigeons. Most of the experiments were performed in glass beakers (without spout) lined with filter paper and suitably chopped feathers. Feathers from preferred egg-laying sites (nape and neck) were placed to facilitate oviposition. Freshly plucked feathers (shafts of which were filled with pulpy material consisting of blood and lymph) were supplied twice per day, as done by Stockdale and Raun (1965) to rear *M. stramineus*. After transferring the lice, the mouth of the culture vial was covered with a plate. Culture vials were then placed into desiccators containing 100 ml saturated solution of salts for maintaining 75–82% relative humidity (RH) (Winston and Bates, 1960). The desiccators were then transferred to a bio-oxygen demand (B.O.D.) incubator (Fig. 2A–C) maintained at 35 ± 1 C (most suitable temperature to rear the lice). Culture vials were examined daily to record the number of survivors and the number of eggs laid and mean values were computed. For scanning electron microscopy lice were fixed in 2.5% glutaraldehyde (12 hr), postfixed in 0.2-M phosphate buffer (24 hr), critically dried, arranged on aluminum stubs (covered with double-sided cello tape), gold coated, and examined under SEM.

RESULTS

Out of 38 eggs (Fig. 1F) of *H. lata* incubated in vitro (35 ± 1 C, 75–82% RH, in batches) 30 eggs hatched successfully. Two, 14, 12, and 2 eggs were hatched on the fourth, fifth, sixth, and seventh day, respectively. The mean duration of the incubation period of the eggs of *H. lata* was 5.47 ± 0.52 days (range 4–7 days, n = 30, Fig. 3; Table I). The percentage of mortality (unhatched eggs) was 21.1% at the egg stage (Fig. 4).

Out of 18 first-instar nymphs (Fig. 1A), 14 molted. Two, 9, 2, and 1 egg were hatched on the fourth, fifth, sixth, and the seventh day postincubation, respectively. The mean duration of first-instar nymphs was 5.14 ± 0.55 days (range 4–7 days, n = 14, Fig. 3; Table I). The percentage of mortality remained high (22.2%) at the first-instar stage (Fig. 4). Likewise, out of 23 second-instar nymphs (Fig. 1B) reared similarly, 12, 4, 3, and 1 second instars molted on the fifth, sixth, seventh, and the eighth day, respectively. The mean duration of second-instar nymphs was 5.65 ± 0.83 days (range 5–8 days, n = 20, Fig. 3; Table I). The percentage of mortality was 13% at the second-instar stage (Fig. 4). Likewise, 20 third-instar nymphs (Fig. 1C) were reared in aforesaid conditions to determine the duration of the stage. Out of 20 third instars 1, 12, 2, 1, and 1 molted on the fifth, sixth, seventh, eighth, and the ninth day, respectively. The mean duration of third instars was 6.35 ± 0.82 days (range 5–9 days, n = 17, Fig. 3; Table I). The percentage of mortality was 15% at the third-instar stage (Fig. 4).

To determine the average lifespan of adults, a total of 18 males and 22 females (Fig. 1D, E) were reared in vitro (in 2 colonies) in glass beakers (without spout), until the survival of the last adult (at 35 ± 1 C, 75–82% RH, freshly plucked feather diet). The average duration of lifespan of adult males was 4.61 ± 3.57 days (range 1–9 days, n = 18, Fig. 3; Table I). The maximum survival of adult males was 9 days. Adult females exhibited a slightly longer lifespan (7.45 ± 5.88 days, range 1–12 days, n = 22, Fig. 3; Table I). The maximum survival of adult females was 12 days. A total of 82 eggs were laid by the females. Thus, a female laid an average of 3.73 eggs/day during her lifespan, at a rate of 0.45 eggs/female/day.

DISCUSSION

The literature indicates that only limited success has been obtained in rearing the amblyceran lice in vitro condition. Amblyceran phthirapterans are very active and fast-running lice and create difficulties in handling during in vitro culture. However, amblyceran lice are partly or exclusively hematophagous (Clayton et al., 2016) and can be successfully reared by fulfilling their dietary requirements. Amblycerans are "telmophages" (Lavoipierre, 1965) and obtain the host blood possibly by puncturing the quills of growing feathers/epidermis or imbibing the blood from wounds formed by excessive preening/grooming by host birds. On the other hand, ischnoceran lice can be easily cultured, as they are sluggish and feed on feather derivatives or skin scrapings (nonhematophagous). Hence, most of the workers have performed in vitro experimentations on ischnoceran lice.

Stockdale and Raun (1965) have performed in vitro experimentation on an amblyceran poultry louse, *M. stramineus* (at 35 ± 1 C, 95% RH, feather diet) and determined an incubation period of eggs (4.5 days), the duration of 3 nymphal instars (3.0 days each) and the egg rate (1.6 eggs/female/day). Rana et al. (2018) reared a nonhematophagous amblyceran pigeon louse, *C. turbinatum* in vitro (35 ± 1 C, 75–82% RH, feather diet), which exhibited a slightly longer incubation period of the egg (5.37 ± 0.67 days) and duration of nymphal instars (5.04 ± 0.65, 5.12 ± 0.89, and 5.0 ± 0.57 days, respectively) but exhibited a lower egg production rate (0.63 eggs/female/day). The maximum survival of *C. turbinatum* is reported as 18 days. In contrast to aforesaid lice, *H. lata* exhibits a slightly longer incubation period of eggs (5.47 ±

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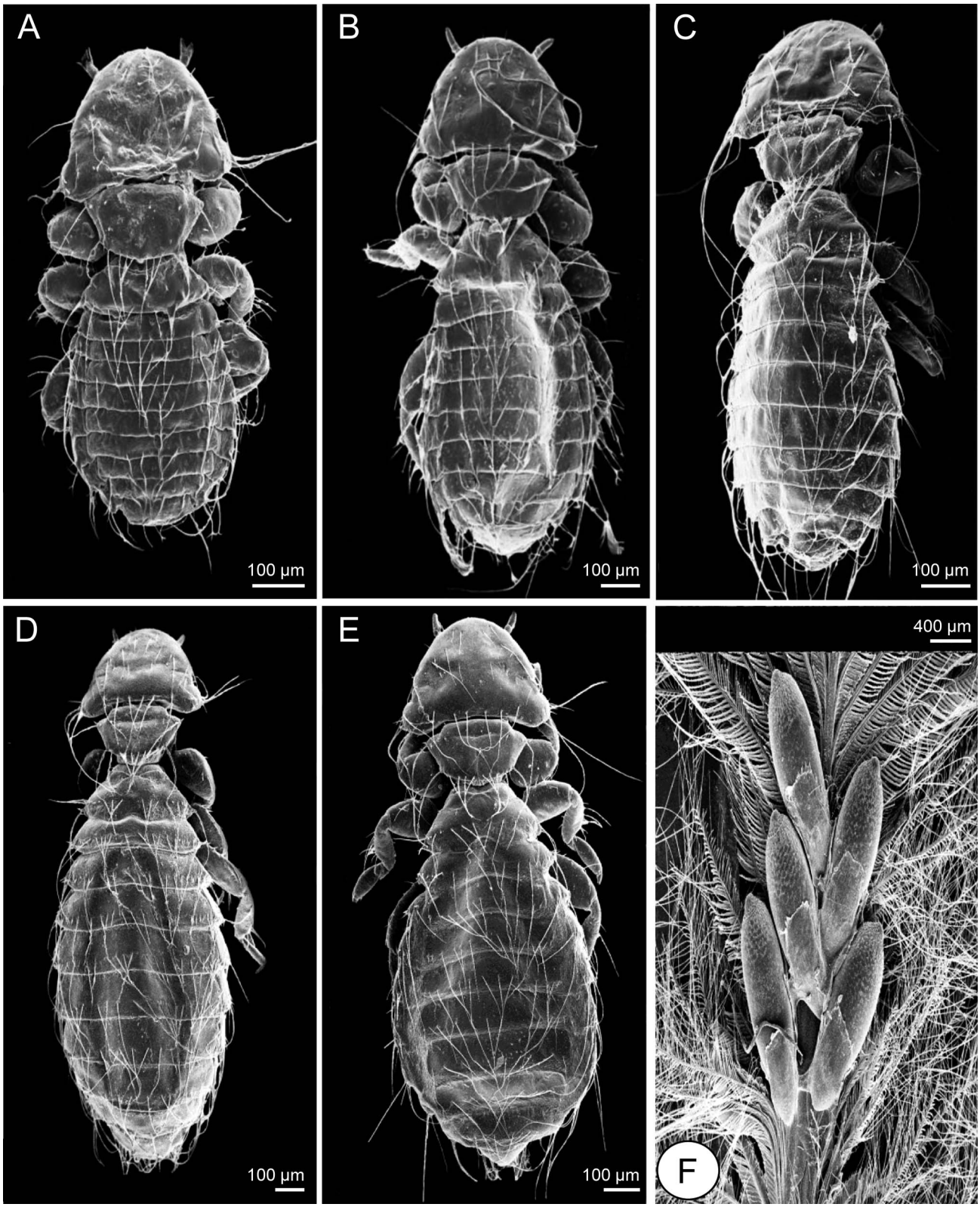

Figure 1. Scanning electron micrographs of life-cycle stages of pigeon louse *Hohorstiella lata*. (**A**) First instar nymph. (**B**) Second instar nymph. (**C**) Third instar nymph. (**D**) Adult female. (**E**) Adult male. (**F**) Eggs.

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Figure 2. (**A**) Photo showing the placement of feathers in the rearing container. (**B**) Photo showing the placement of rearing container in the desiccators. (**C**) Photo showing the placement of desiccators in the B.O.D. incubator.

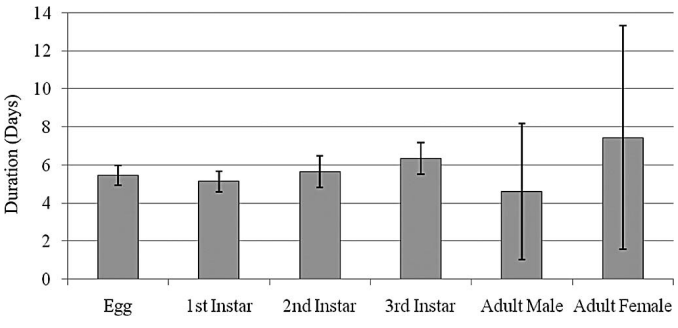

Figure 3. Duration of the egg stage, 3 nymphal instars and adults (male and female) of *Hohorstiella lata* (reared at 35 ± 1 C, 75–82% relative humidity, feather diet). Mean duration (days) bars are represented by SD (standard deviation).

Table I. In vitro biology of *Hohorstiella lata* (reared at 35 ± 1 C, 75–82% relative humidity, feather diet).

Incubation period of eggs	5.47 ± 0.52 days (range 4–7 days, n = 30)
Duration of first nymphal instar	5.14 ± 0.55 days (range 4–7 days, n = 14)
Duration of second nymphal instar	5.65 ± 0.83 days (range 5–8 days, n = 20)
Duration of third nymphal instar	6.35 ± 0.82 days (range 5–9 days, n = 17)
Adult lifespan (male)	4.61 ± 3.57 days (range 1–9 days, n = 18)
Adult lifespan (female)	7.45 ± 5.88 days (range 1–12 days, n = 22)
Egg rate/female during lifespan	3.73
Egg rate/female/day	0.45

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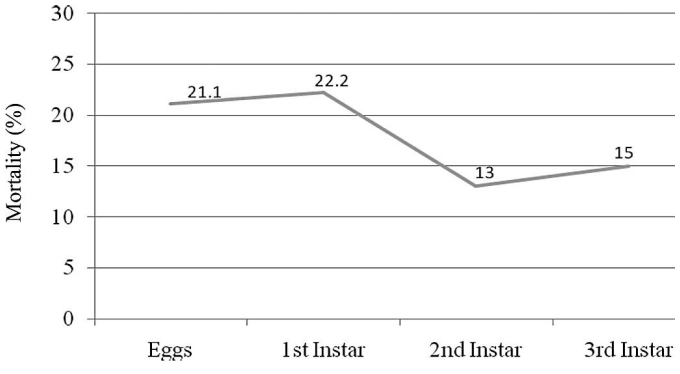

Figure 4. Mortality (%) of eggs and 3 nymphal stages of *Hohorstiella lata* (reared at 35 ± 1 C, 75–82% relative humidity, feather diet).

0.52 days) and duration of nymphal instars (5.14 ± 0.55, 5.65 ± 0.83, and 6.35 ± 0.82 days, respectively) but lower egg rate (0.45 eggs/female/day). Nelson (1971) successfully performed in vitro culture of *C. turbinatum* for several months, but he did not determine the adult lifespan and reproductive potential of the louse. Nelson cautioned that frequent examination of culture stock causes lice mortality. However, examination of culture stock is necessary when the goal is to determine the adult lifespan and egg rate. During the present study, partial success has been obtained in rearing the hematophagous amblyceran pigeon louse, *H. lata*. However, the reproductive potential and lifespan of this louse might be higher under in vivo conditions, but low prevalence and intensity of infestation and their hematophagous nature create hurdles to such experimentation. Future efforts involving larger sample sizes may produce better results.

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