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Life cycle and biology of *Pityogenes scitus* Blandford, 1893 (Coleoptera: Curculionidae: Scolytinae), a pest of *Pinus wallichiana* in Kashmir, India

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Abstract: The bark beetle, *Pityogenes scitus* Blandford, 1893 (Coleoptera: Curculionidae: Scolytinae) is one of the main pests in *Pinus wallichiana* A.B. Jackson, 1938 (Pinaceae) stands, and it has also been found on other Oriental Pinaceae species. This pest is aggressive and has caused significant loss of host trees, but little is known of its biology and ecology. Based on the field and laboratory observations, this study describes the detailed bioecology of *P. scitus.* This beetle pest overwinters in larval stage on blue pine (*P. wallichiana*) trees in Kashmir. After emergence, the adults fly to suitable trees and undergo maturation feeding for 4–6 days. Reproduction is polygamous type. After mating, each of the females makes one gallery with an average length 2.30 (± 0.41 SD) cm. The female lays 26.53 (± 6.32 SD) eggs on an average. The eggs hatch in 5 to 10 days. The larvae have 5 instars and complete their development in 18 to 28 days constructing larval galleries 1.76 (± 0.25 SD) cm in length. The larvae pupate for 13-22 days and finally the adults emerge to attack new suitable trees. The adults live for 28–40 days and the total life-span of this species ranges from 66 to 92 days. The seasonal distribution of various developmental stages and the number of generations were also recorded (5 generations (the last a partial one) per year in Kashmir). In general, the life cycle of *P. scitus* is similar to those described for other *Pityogenes* Bedel, 1 888 species.

Keywords: bark beetle; morphometry; seasonal history; Scolytinae

Blue pine (*Pinus wallichiana* A. B. Jackson, 1938) forests extend throughout the length of Himalayan ranges. They are concerned to the certain pockets in Eastern Himalaya and become more widespread in the Western Himalaya and distributed between 1 950–3 350 m a.s.l. elevations (Bargali et al. 1989). The ring-studies of this species (*P. wallichiana*) over the several past decades (Shah and Bhattacharyya 2012; Gaire et al. 2019) indicate its responsiveness to global climate change, which affects its susceptibility to various pathogens and pests. For example, large scale infestation and mortality of *P. wallichiana* associated with Himalayan dwarf mistletoe (*Arceuthobium minutissimum* Hook. F.) infestation has been reported in the Gangotri National Park, Western Himalaya (Dorji et al. 2012). The infestation was never recorded in this landscape (Kashmir Himalaya). Long term climate data indicate trends of change in minimum and average temperature during last two decades which may serve as suitable conditions for wide dieback of this tree species in future scenarios (Rai et al. 2018).

Among numerous pests of woody plants, the first signs of attenuation by various biotic and abiotic

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factors are perceived by bark beetles (Coleoptera: Curculionidae; Scolytinae) as a signal that the weakened tree can be colonized and eliminated from the stand (Yan et al. 2005; Buhroo, Lakatos 2007; Khanday, Buhroo 2015; Raffa et al. 2015). The colonised trees by beetle pests become hosts to dozens of arthropod, nematode and vertebrate species (Raffa et al. 2008). In addition to causing widespread tree mortality, scolytine beetles serve as some of the prominent model systems for studies of coevolution, chemical ecology, symbiosis, sexual selection, and population dynamics (Raffa et al. 2015).

According to faunistic estimates, the genus *Pityogenes* Bedel, 1888 (Coleoptera: Curculionidae: Scolytinae), including economically significant pests, inhabits only the northern hemisphere. Among the twenty four known *Pityogenes* species, only two, that is, *Pityogenes scitus* Blandford, 1893 and *P. spessivtsevi* Lebedev, 1926 were recorded from India (Maiti, Saha 2009; Knížek 2011). Both species are phloeophagous and oligophagous (Wood, Bright 1992; Maiti, Saha 2009) and like other conspecifics have evolved adaptations to exploit Pinaceae despite formidable defenses that these tree species can mount (Franceschi et al. 2005).

The bark beetle, *P. scitus* is a typical representative of the Oriental (India, China (Tibet), Nepal and Pakistan) zoogeographic region (Wood 2007, Yu et al. 2015). Despite of its aggressive habit of utilizing various conifer hosts (Stebbing 1914; Maiti, Saha 2009), no investigation on the various aspects of *P. scitus* has been undertaken, which is a significant obstacle to success in terms of the development and application of pest management in the field conditions (Yu et al. 2015; Khanday et al. 2018).

Here, we report the life cycle and biology of *P. scitus* on blue pine, describe the gallery pattern and confirm the seasonal incidence.

MATERIAL AND METHODS

Study site. Experimentation on the various bio-ecological aspects of *Pityogenes scitus* associated with *Pinus wallichiana* (Pinaceae) were conducted over a period of two years (from March to November 2017 and from March to November 2018) at Nowpora village (33°61.078'N, 075°18.700'E and elevation, 1 804 m) in Anantnag District, Jammu and Kashmir, India, situated within a temperate climatic zone (Figure 1). In the study area, inhabited by 30–40 year old blue pine trees, some felling had been done in the previous year (December 2016) to initiate infestation by *P. scitus.*

Beetle sampling. The various bioecological aspects of the bark beetle *P. scitus* such as the beetle's pioneer flight to the host tree (*P. wallichiana*), initiation of the entrance hole, excavation of the gallery and seasonal incidence was observed both in the field and in the laboratory conditions.

In the study area, the sampling procedure for monitoring the *P. scitus* activity was similar to that adopted by Khanday and Buhroo (2015). By us-

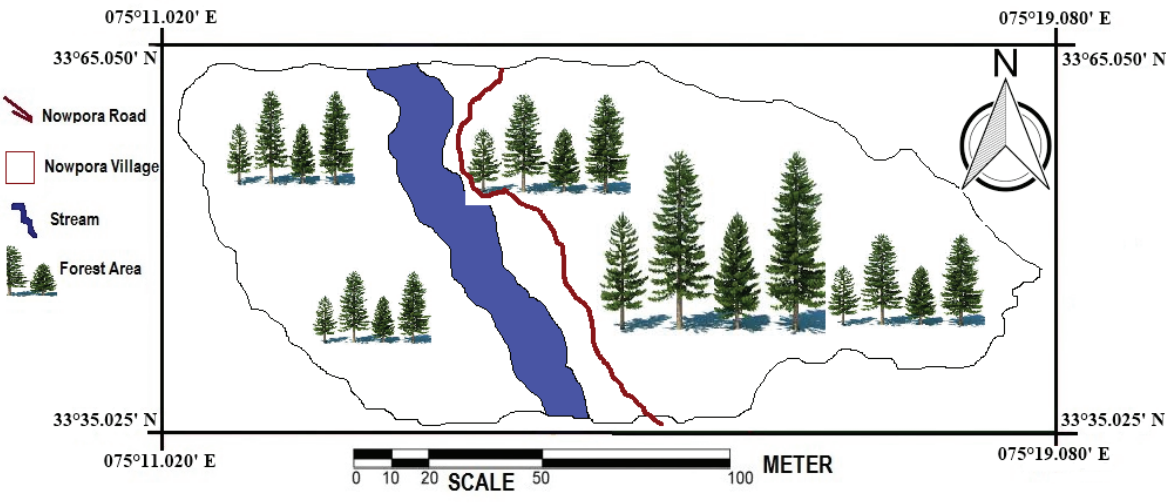

Figure 1. Map showing the location of study area (Nowpora village)

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ing trap logs and other infested branches of standing trees (Beaver 1967; Buhroo, Lakatos 2007; Khanday, Buhroo 2015), observations of the various life stages were made once or twice weekly throughout the experimental period. These trap logs were cut periodically from April to October each year from 2017 to 2018 (Buhroo, Lakatos 2007; Khanday, Buhroo 2015). Further information was obtained by careful removal of bark sections both in the field and in the laboratory. The eggs found in maternal galleries were exposed carefully and counted in their individual chambers. For measurement of galleries (both maternal and larval) a digital caliper scale was used (Khanday, Buhroo 2015). The correlation between the length of maternal galleries and the number of eggs deposited by beetle pests was worked out using the method of Zhang et al (1992).

At the experimental site, the entrance holes, that the newly emerged beetles started grooving, were marked on one main branch and some twigs of a declining pine tree during May–June. After the marking dates, 15 beetle entries were dissected each day and examined for eggs to determine female maturation feeding period as per the method by Buhroo, Lakatos (2007).

The laboratory rearing of bark beetle species was accomplished by placing infested logs (25–45 cm long and 2–10 cm in diameter) in three rearing boxes of similar design made up of glass with dimensions of 75 × 35 × 40 cm, 45 × 35 × 35 cm, 45 × 35 × 35 cm and 55 × 35 × 35 cm. The top face of each box was fitted with white muslin cloth. Each box could be opened from the top to facilitate exchange of logs. After every month, cut branches (20–40 cm long and 2–8 cm in diameter) from the host tree (*P. wallichiana*) were placed in the rearing boxes to induce fresh attack, 5–10 days prior to the emergence of adults in every generation. This enabled continuous rearing and examination of beetle development. A few infested logs were also debarked at regular intervals (10 days) to study various stages of the beetle under bark. Measurements of various developmental stages including egg, larva, pupa and adult were recorded. The development process and duration of beetle life stages were recorded and compared with the field results using the methods of Buhroo, Lakatos (2007) and Khanday, Buhroo (2015).

Photographs during the field study were taken by using Canon PowerShot SX60 camera fitted with macro lens (Raynox MSN-505, 37mm, Yoshida Industry Co., Ltd. Tokyo). Analysis of digital images was done by using ImageJ analysis software (Version 2006. 02.01). For morphometric description of developmental stages, images were taken using an M205A Leica Stereomicroscope (Leica Microsystems GmbH, Germany) with a DFC295 camera (Leica Microsystems GmbH, Germany) and Leica Application Suit software (Version 4.10, 2017) and focused using the same software. Spatial information regarding sample site was recorded in the form of latitude and longitude with the help of handheld GPS (Garmin eTrex 10, India).

Statistical analyses. Statistical analyses were performed using Origin Pro software (Version 2015). The gallery pattern and developmental stages were analyzed using descriptive statistics (mean and standard deviation). The larval instars were separated from each other by head capsule measurements (Dyar 1890; Khanday, Buhroo 2015).

RESULTS

Mating behavior

After host tree selection, the male of *P. scitus* was observed to form an initial gallery known as mating–chamber (Figure 2). Many female beetles (2–4)

Figure 2. Initial gallery (pairing–chamber)

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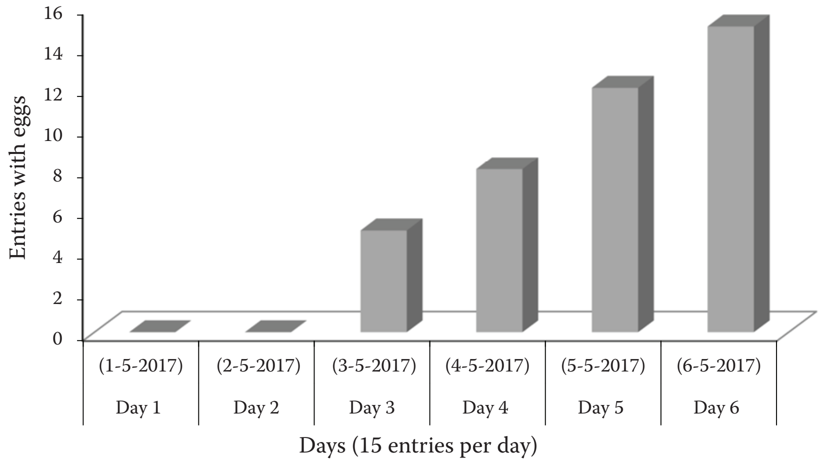

Figure 3. Female maturation period of *P. scitus*

successively enter the mating–chamber through the tunnel made by the male and after copulation, proceed to excavate their galleries (Figure 2).

Maturation feeding

Bark dissections for female maturation feeding (Figure 3) showed that no eggs were collected from beetle entries on 1st and 2nd day; only 5/15 from 3-day-old entries yielded eggs; 8/15 from 4-day-old entries contained eggs; while 12/15 from 5-day-old entries contained eggs; but all the 15 of 6-day-old beetle entries contained eggs. This indicated that newly emerged females mostly fed and oviposited in 4 to 6 days in the field. The feeding sites were then continued into the maternal galleries.

Gallery patterns

The gallery patterns are shown in Figs 4A–B. After mating, each of the females makes one gallery and each takes a different direction to that taken by other conspecifics. The average length of maternal galleries is 2.30 (± 0.41 SD) cm (Figure 4A,

Figure 4. A typical gallery pattern of *P. scitus* excavated in *P. wallichiana*: maternal galleries (A), larval galleries (B)

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Table 1. Measurement of gallery length

Variable	*N*	Mean	Standard Deviation
Maternal gallery length	30	2.30	0.41
Larval gallery length	30	1.76	0.25

N – Number of observations

Table 1). Larval galleries radiate out from the maternal galleries. The average length of larval galleries measures 1.76 (± 0.25 SD) cm (Figure 4B, Table 1). The larval tunnels are initially perpendicular to the maternal gallery, then radiate in different directions and can intersect (Figure 4B). These tunnels are closely packed with the wood excreta passed out by the larvae in feeding.

Data based on the length of the maternal gallery and the numbers of eggs deposited indicate that there was a positive correlation ($y = 1.8904x + 7.2156$, $r = 0.75$) between the two variables (Figure 5). The number of eggs increases linearly with maternal gallery length.

Developmental Stages

Egg. After copulation, each of the female bores out her tunnel, gnaws out little notches at the side and places an egg in each (Figure 6A). These notches are not made so symmetrically as in the case of the monogamous bark beetle species, there being usually more on one side than on the other. On an average 26.53 (± 6.32 SD) eggs were laid per female (Table 2). The eggs are about 0.43 (± 0.04 SD) mm in length and 0.29 (± 0.01 SD) mm in width (Table 2). The eggs hatched after an incubation period of 5–10 days (Table 3).

Larva. The larva on hatching was minute, wrinkled, cylindrical and creamy white in color (Figure 6B). The mature larvae reached to an average length of 1.82 (± 0.08 SD) mm and width of 0.50 (± 0.07 SD) mm (Table 2). On the basis of the data recorded in the field, five larval instars were observed (Table 4). The expected head capsule width of each instar was also determined by Dyar's ratio (Dyar 1890) which states that the growth ratio remains constant between the molts. Each instar was progressively longer (length) than preceding instar. Development from hatching to the prepupal larvae took 18–28 days (Table 3).

Pupa. Pupation took place at the end of the larval galleries in pupal chambers. The pupae were soft and creamy white with 1.41 (± 0.04 SD) mm in length and 0.45 (± 0.05 SD) mm in width (Figure 6C, Table 2). The pupal stage lasted for 13–22 days (Table 3). The adults emerged from the pupal chamber by tunneling straight through the bark over it. After emergence, adults flew to the suitable trees to produce the next generation.

Adult. The adult females (Figure 6D) have an average body length of 1.70 (± 0.05 SD) mm and 0.55 (± 0.08 SD) mm width and male (Figure 6E) body length is 1.63 (± 0.04 SD) mm and width is 0.46 (± 0.03SD) mm (Table 2). Females (Figure 6D) are very similar to male (Figure 6E) except three deep cavities on frons in triangular positon, bigger one at central vertex and small two at either side at lower level, elytral declivity less broadly impressed, three

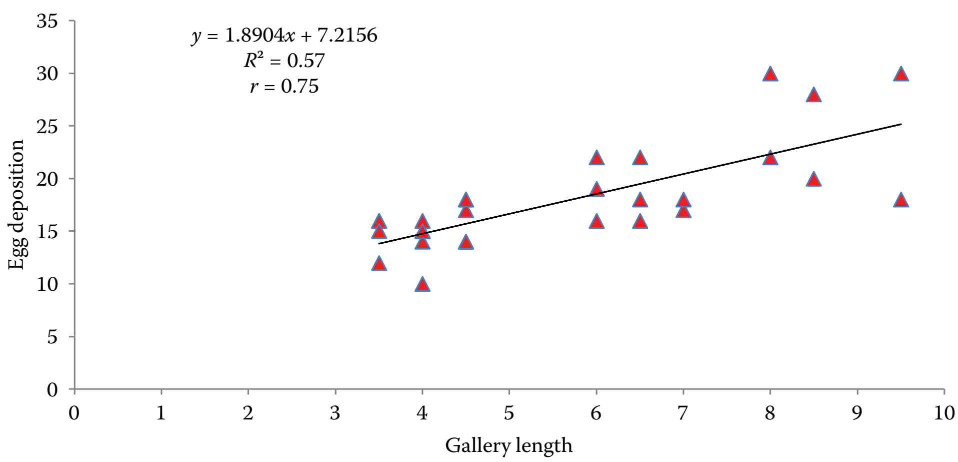

Figure 5. Relationship between the length of maternal galleries (cm) and eggs per gallery in *P. scitus*

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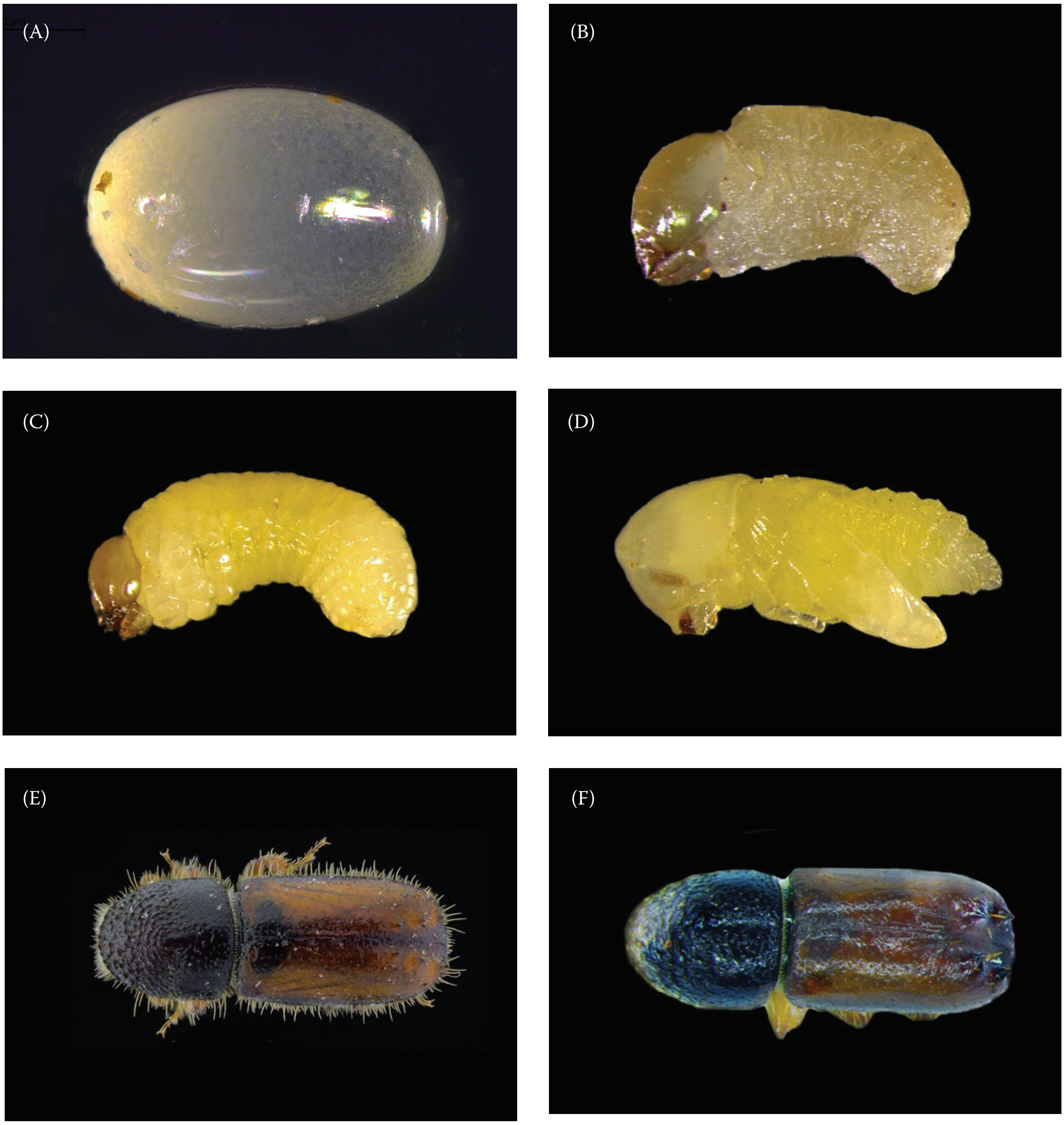

Figure 6. Developmental stages of *P. scitus*: egg (A), 1st and 5th larval instars (B,C), pupa (D) adult female (E), adult male (F)

pairs of lateral tubercules almost of equal size and smaller. The adults lived for 28–40 days (Table 3).

Seasonal incidence

In the present study, we are reporting that *P. scitus* produced five generations (the last a partial one) per year in Kashmir (Figure 7). The generations were also found to overlap to some extent; however, further studies are warranted on this aspect especially under field conditions. The adults lived for 28–40 days (Table 3). The first generation developed from the second week of April to June having a total life span of 72–92 days (Table 3). The second generation extended from the first week of June to August having a total life span of 66–87 days, third generation extended from the last week of July to October having a total life span of 68–85 days, fourth generation extended from the last week of September to November having a total life span of 70–91 days while the overwin-

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Table 2. Egg deposition and measurement of developmental stages of *P. scitus*

Variable	*N*	Mean	Standard Deviation
Egg deposition	30	26.53	6.32
Egg length	30	0.43	0.04
Egg width	30	0.29	0.01
Larval length	30	1.82	0.08
Larval width	30	0.50	0.07
Pupal length	30	1.41	0.04
Pupal width	30	0.45	0.05
Female length	30	1.70	0.05
Female width	30	0.55	0.08
Male length	30	1.63	0.04
Male width	30	0.46	0.03

N – Number of observations

tering fifth generation (the last a partial one) took 167–195 days and was extended from November to April of the following year (Table 3). Laboratory observations also confirmed 5 generations (the last a partial one) with little difference in development process and duration of insect stages.

DISCUSSION

Bark beetle species within the scolytid genera *Dendroctonus* Erichson 1836, *Ips* DeGeer 1775, *Pityogenes* Bedel 1888, and *Tomicus* Latreille 1802 are known to cause extensive ecological and economical damage in spruce and pine forests during epidemic outbreaks all around the world (Avtzis et al. 2012). The findings of the present study clearly show the high similarity of the bioecological features of *P. scitus* with the well-studied economically significant and widespread in the Palaearctic species *P. chalcographus* L. For example, they have a

Table 3. Developmental duration of *P. scitus*

Duration of generations (days)					
Stage	*P. scitus*				
	1st generation	2nd generation	3rd generation	4th generation	5th generation
Egg	6–9	5–7	5–8	6–8	7–10
Larva	20–28	18–26	18–25	19–28	115–125
Pupa	14–20	13–17	15-18	15–20	17–22
Adult	32–40	30–37	30–34	30–35	28–38
Total	72–97	66–87	68–85	70–91	167–195

Table 4. Comparison of observed (mean) and expected values of head capsule widths (mm) of the larvae of *P. scitus*

Larval instars	Head capsule width (mm)			Difference (mm)
	observed (Mean ± SD)	Range	Expected[a]	
I	0.20 ± 0.03	0.09	0.20	0.00
II	0.27 ± 0.02	0.07	0.27	0.00
III	0.35 ± 0.01	0.07	0.36	–0.01
IV	0.44 ± 0.02	0.07	0.47	–0.03
V	0.53 ± 0.02	0.08	0.59	–0.06

mean observed head capsule width of 1st instar larva (N = 30) = 0.20; mean observed head capsule width of 2nd instar larva (N = 30) = 0.27; Growth ratio (Dyar's ratio*) = Head capsule width of 2nd instar larva/ Head capsule width of 1st instar larva = 0.27/0.20 = 1.35; mean observed head capsule width of 5th instar (mature larvae) (N = 30) = 0.53

*expected head capsule width established by Dyar's ratio (1.35). Multiplying Dyar's ratio with the observed head capsule width of 1st instar larva gives the expected head capsule width of 2nd instar which when multiplied again with Dyar's ratio gives expected head capsule width of 3rd instar and so on

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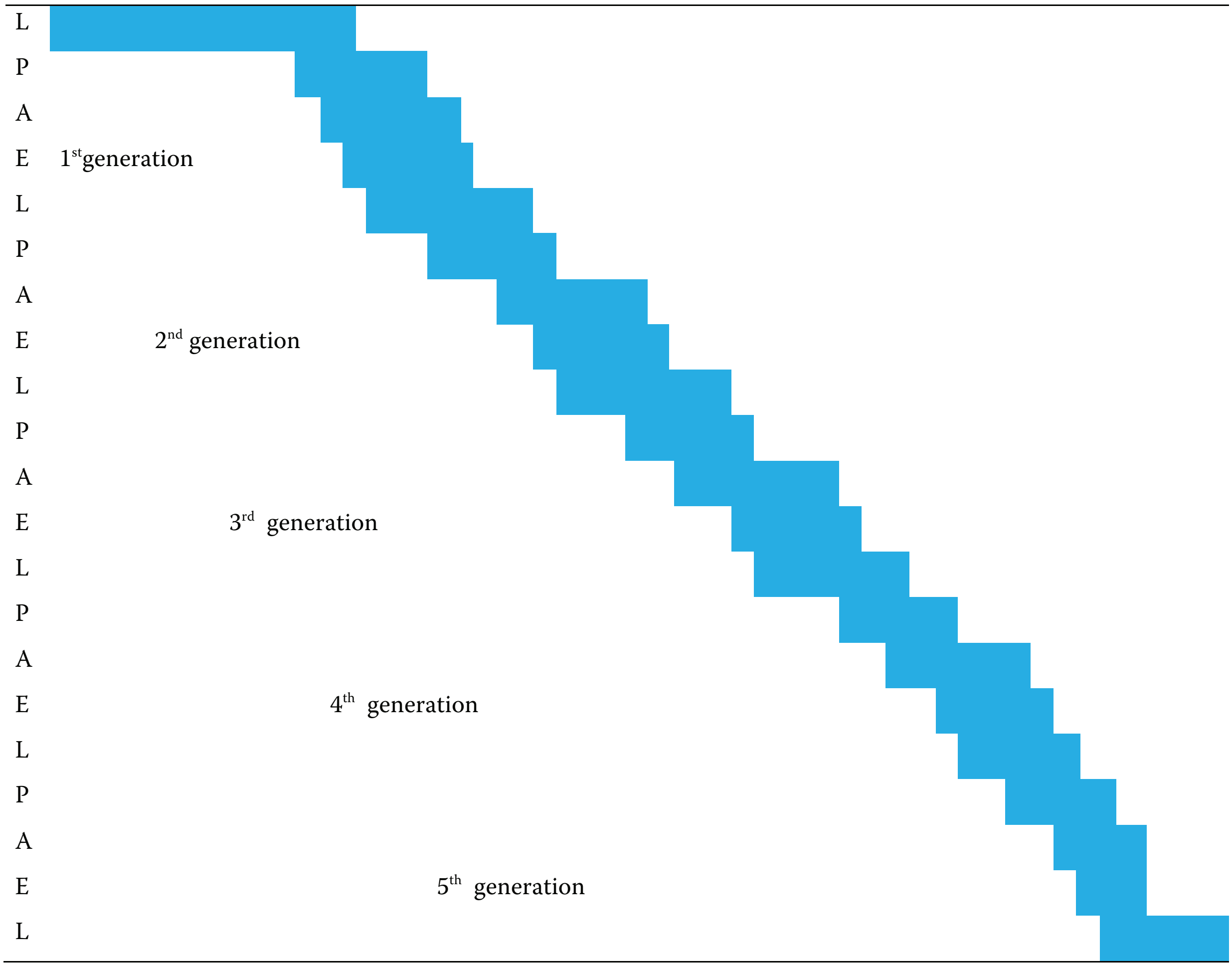

Figure 7. Seasonal distribution of *P. scitus* (A – adult, E – egg, L – larva, P – pupa)

similar harem-polygyny type of sexual behavior in contrast to monogamous reproduction (females select brood material, begin galley construction, and are subsequently joined by males).

Prior to colonizing new host trees, bark beetles may engage in maturation feeding, often in their galleries prior to dispersal. It has been reported that some species disperse to a specific maturation feeding site, usually a live or stressed tree, prior to seeking a suitable host for reproduction (Stoszek, Rudinsky 1967; McNee et al. 2000). In the present study, we reported that the maturation feeding of *P. scitus* lasted for 4 to 6 days in the field, enabling the imago to undergo sexual development. However, according to the laboratory observations in the artificial medium, the adult females of the bark beetle *Xyleborus affinis* Eichhoff, 1868 are fully capable of breeding independently when they are experimentally removed from their natal nest (Biedermann et al. 2011), which suggests that maturation feeding is not essential for egg laying.

The pattern of maternal galleries was found to be irregular with species-specific modifications (Lieutier et al. 2004). In *P. scitus,* the gallery has star-like appearance with long maternal galleries and relatively long larval galleries, similarly as in other *Pityogenes* species (e.g. *P. chalcographus*). Data based on the length of the maternal gallery and the numbers of eggs deposited indicate that there was a positive correlation between the two variables. The number of eggs increases linearly with maternal gallery length. In trees with thick phloem, beetles possibly lay more eggs per centimeter of the maternal gallery, experience less intraspecific competition among larval stages and thus produce larger brood beetles compared to bark beetles in trees with thin phloem (Amman, Cole 1983). The decline of maternal gallery length and oviposition with in-

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creasing attack density is partially due to intraspecific competition. There are reports of intraspecific competition at high breeding densities which results in shorter maternal galleries and thus reduced oviposition (Schroeder and Weslien 1994). It has been shown that offspring number per female decreases at higher attack densities (Anderbrant et al. 1985).

In studies of the chemoreception of *P. chalcographus* it is attracted to host species by a mix of monoterpenes (Chararas 1962; Kangas 1968; Byers et al. 1988), transformed in the bowels into an aggregation pheromone with synergic effect (Byers et al. 1988, 1990), often allowing a massive attack on a tree. Most likely, *P. scitus* has similar mechanisms of pheromone production with the presence of a species-specific component or monoterpene ratio, but these issues remain unexplored. In case of the bark beetle *Ips pini* (Say, 1826), if mass attack is not elicited relatively quickly, the ratio of monoterpenes to pheromones rises to such high levels that the likelihood of a tunneling beetle being joined by conspecifics becomes very low (Erbilgin et al. 2006).

The galleries of *P. scitus* contain all developmental stages from egg to adult, whose general qualitative characteristics are similar to those described for other scolytines (Wood 2007; Jordal 2014). This study for the first time described the duration of developmental passage through individual stages and determined the number of instars using the distribution of head capsule widths, a method that has been used to define instars of other insects (Caltagirone et al. 1983; Weber, McPherson 1983) and applying geometric progression which have also been shown to be effective in determining the number of scolytine instars (Dallara et al. 2012).

The major responses of climate change to insect are an earlier spring flight, enhanced winter survival, acceleration of development rate and more generations per season. Beetles complete development in 6–8 weeks given summer temperatures and thus one to five generations per year can occur depending on the climate (Furniss, Carolin, 1992). In contrast to *P. chalcographus* having 3 generations per year (Schwerdtfeger 1929; Postner 1974), we reported 5 generations (the last a partial one) per year in Kashmir. The differences in the seasonal generations of scolytine beetles around the world can be explained by both species-specific variation and different environmental factors as well the variation of the biochemical composition of host trees. For these species, generations are often not synchronized and exhibit considerable overlap (Cibrian et al. 1995). Multiple life stages often overwinter and in some cases, development continues year round. It is well known that periods of mass swarming among bark beetles occur during periods of active growth of a tree, when it is facing a dilemma of spending its nutrient reserves on protection or growth or during periods of fluctuation in moisture (Allen et al. 2010; McDowell et al. 2008; Sala et al. 2010).

According to dendrochronological studies of *P. wallichiana* presented in the literature, correlation analysis revealed significant positive relationships for annual ring growth with February–August precipitation, but negative correlations with temperature. High positive correlations with the self-calibrated Palmer drought severity index (*scPDSI*) configured that moisture availability during the early growing season and full growing season is the primary limiting factor for blue pine tree growth in the trans–Himalayan region (Gaire et al. 2018).

CONCLUSION

Scolytine beetles (Coleoptera: Curculionidae: Scolytinae) are key players in forest ecosystems, and some species can have huge economic and ecological impacts on their environment (Grégoire et al. 2015; Raffa et al. 2015). In the present study, we reported detailed bioecological field and laboratory observations of *P. scitus* including mating behavior, maturation feeding, gallery pattern, life cycle and seasonal history. These findings are important for pest management in forests and health and phytosanitary measures in the timber trade, especially for risk assessment in blue pine forests (Himalayan region).

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