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ARGE XANTHOGASTER (HYMENOPTERA: ARGIDAE): A NEW THREAT TO ROSE PLANTS IN MEGHALAYA, INDIA

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ABSTRACT

The sawfly, *Arge xanthogaster* (Cameron) (Hymenoptera: Argidae), has recently emerged as a major pest of roses (*Rosa* spp. L.; Rosales: Rosaceae) in Meghalaya and causes around 80% damage to wild and cultivated rose plants. This is a first report of *A. xanthogaster* as a pest of roses in India. Adults cause ovipositional injuries (split shoots) and larval feeding often results in complete defoliation. The species is multivoltine. Studies on its life history in new habitat are given. Since this species belongs to a species complex of *Arge* in which species are difficult to distinguish, an attempt was made to develop a DNA barcode based on standard barcoding gene cytochrome oxidase I (COI) of the mitochondrial DNA of this species.

Key Words: emerging pest, *Arge xanthogaster*, rose, gregarious and hymenopteran pest

RESUMEN

El árgido, *Arge xanthogaster* (Cameron) (Hymenoptera: Argidae), ha surgido recientemente como una de las principales plagas de rosas (*Rosa* spp L; Rosales: Rosaceae) en Meghalaya y causa alrededor del 80% de daño a las plantas silvestres y cultivadas de rosa. Esto es el primer informe de *A. xanthogaster* como una plaga de rosas en la India. Los adultos causan lesiones (brotes divididos) al poner los huevos y la alimentación de las larvas a menudo resulta en una defoliación total. La especie es multivoltina. Se presentan estudios sobre su historia de vida en el nuevo hábitat. Puesto que esta especie pertenece a un complejo de especies de *Arge* en que las especies son difíciles de distinguir, se hizo un intento para desarrollar un código de barras de ADN sobre la base de los códigos de barras estándar gen citocromo oxidasa I (COI) del ADN mitocondrial de esta especie.

Palabras Clave: plagas emergentes, *Arge xanthogaster*, rosa, plaga gregario e himenóptero

Roses (*Rosa* spp. L.; Rosales: Rosaceae) are one of the world's leading and most popular flower crops of commercial importance. In India, it is first in demand and popularity among cultivated flowers. A large number of insects attack rose plants at almost every stage, and sometimes they cause severe damage (Duraimurugan & Jagadish 2011). Recently, a sawfly, *Arge xanthogaster* (Cameron 1876) (Hymenoptera: Argidae) (Figs. 1-6) has emerged as a major defoliator of roses in Meghalaya causing about 80-85% damage to wild and cultivated rose plants. A significant increase in their population was observed during 2011-12.

The family Argidae includes a number of destructive, multivoltine species. *Arge* Schrank is a

large genus of Argidae with over 300 recognized world species (Taeger et al. 2010). A number of species of *Arge* are associated with roses e.g., *A. ochropus* (Gmelin 1790), *A. pagana* (Panzer, 1798), *A. nigrinodosa* Motschulsky (Kawasaki et al. 2012), *A. simlaensis* (Cameron) (Mahmood & Ullah 2011), *A. geei* Rohwer (Jiansheng et al. 1996), *A. przhewalskii* Gussakovskij (Ying & Yuan 1992) and *A. fumipennis* (F. Smith) (Chauhan et al. 2002). Seven species, including *A. xanthogaster*, are very closely related and are collectively considered as the "*xanthogaster* group" (Chen et al. 2009) or "*ochropa* group" (Smith 1989), viz., *A. paganiformis* Rohwer, *A. pagana* (Panzer, 1798), *A. luteiventris* Cameron, *A. brevigaster* Wei, *A.*

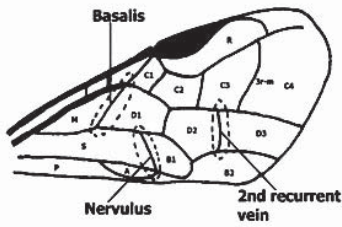


Figure 1

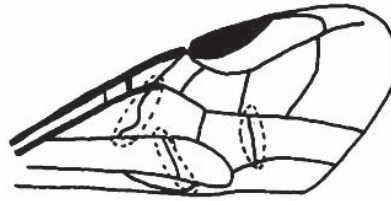
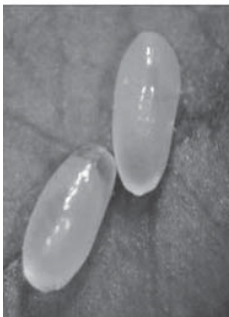


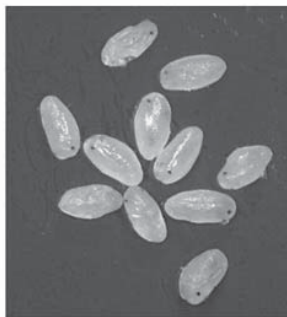
Figure 2



Figure 3



4a



4b

Figure 4

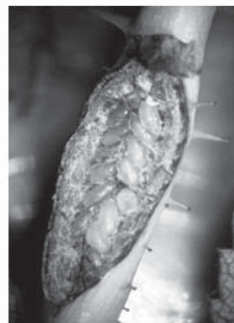


Figure 5



Figure 6

Figs. 1-6. *Arge xanthogaster* (Cameron, 1876). 1. Forewing of variant of Meghalaya; 2. Forewing of variant of Sikkim and Manipur. 3r-m = 3rd Cubital vein; A = Anal vein; B1-B2 = Brachial cells 1&2; C1-C4 = Cubital cells 1-4; D1-D3 = Discoidal cells 1-3; M = Medial cell; P = Postellian cell; R = Radial cell; S = Submedian cells; 3. Egg laying posture of female. Adult females possess a strong sawlike ovipositor, which is used to make parallel cuts in the fresh/tender shoots of the host plant; 4a and 4b. Eggs are oval shaped and yellow in color; 5. Eggs of in 2 longitudinal rows in a branch of a rose plant. Sawfly places eggs singly in 2 parallel longitudinal rows in slits in the young shoots; 6. First instar larvae feeding on rose leaf from the leaf edge. After hatching the neonates quickly start feeding and devour tender leaves on rose bushes.

albocaudalica Wei & Yan et He, *A. xanthogaster* Cameron and *A. geei* Rohwer.

Arge xanthogaster Cameron has been reported from Manipur, Sikkim and Uttar Pradesh states of India, but information on its hosts and its biology is lacking (Saini & Thind 1995). As per the existing primary literature and to our knowledge, the pest status of *A. xanthogaster* has not been reported, and therefore, this is the first report of *A. xanthogaster* as a pest of roses from India. Because of difficulties in identification of species of the *xanthogaster* group, we attempted to develop a DNA barcode based on standard barcoding of cytochrome oxidase I (COI) gene of the mitochondrial DNA of this species. Furthermore, proper management strategies of an emerging pest are largely based on better understanding of their life cycle and behavior. Here, we thus report on the biology and damage of *A. xanthogaster* in Meghalaya and on the development of a DNA barcode that may be helpful in future identification.

MATERIALS AND METHODS

Species identification was confirmed by an expert sawfly taxonomist, Dr. M. S. Saini (Punjab University, Patiala, Punjab state, India). Specimens were thoroughly examined for any kind of morphological variability among present specimens of sawfly of Meghalaya and those specimens previously collected from Manipur and Sikkim states of India. Wing venation was selected to explore variability among the present populations of Meghalaya and previous specimens of Manipur and Sikkim states. The illustrations were prepared with the aid of a zoom stereoscopic binocular (Kyowa Getner DVZ-555 with maximum magnification of 90X) fitted with an ocular grid in 1 eye piece. The inking of the final drawings was done with Rottering black ink. The illustrations were scanned at 600 dpi black and white, and mounted onto plates by Adobe©Photoshop©8.0.

For DNA extraction and PCR amplification, larvae were collected from infected rose plants from 2 different locations and reared to adults in the laboratory. Four adults (2 from each location) were used for DNA extraction. Genomic DNA was extracted from whole adults (minus wings and head) using a DNeasy® Blood and Tissue Kit (Qiagen, Cat. # 6950) and by following the supplier's instructions for animal tissue. PCR amplifications were performed using standard barcoding primers (LepF1 and LepR1) in a 50 µL reaction volume consisting of 25.0 µL of 2X Fermentas PCR Master Mix (Fermentas, Cat. # K0171); 2.5 µM of each of LepF1 (5'-ATTCAACCAATCATAAAGATATTGG-3') and LepR1 (5'-TAACCTCTGATGTCCAAAAATCA-3') primers and 40-60 ng of template DNA.

PCR conditions had following profile: initial denaturation at 94 °C for 2 min (1 cycle); 94 °C for 30 s, 50 °C for 40 s and 72 °C for 1 min (05 cycles); 94 °C for 30 s, 50 °C for 40 s and 72 °C for 1 min (35 cycles) followed by a final extension cycle at 72 °C for 10 min. The success of PCR amplification was confirmed by running out 10 µL of the post-PCR volume along with 100 bp ladder (Fermentas, Cat. # SM0241) on 1.5% agarose gels at 140 V for 30 min and stained with ethidium bromide and visualized over a UV light illuminator.

The successful PCR amplicons (40 µL post-PCR) were sent for direct sequencing to the Chromous Biotech Pvt. Ltd., Bangalore, India. For accuracy purpose, all the samples were sequenced from both the ends (5' and 3'). The DNA sequences obtained were analyzed using Pregap4 and Gap4 programs within the Staden Molecular Biology analysis software (Staden et al. 1998). Nucleotide sequences were aligned using the sequence alignment program Clustal X (Thompson et al. 1997) and were checked manually. Sequence identity was determined by BLASTN search (Altschul et al. 1997) against the nr DNA database deposited in GenBank.

Life history studies were undertaken at Umiam (N 25° 41' 01.91" and E 91° 54' 46.24") and adjoining areas of the Ri-Bhoi District (mid hills), Meghalaya State, India. This area receives annual rainfall of about 2,000 mm and its altitude varies from 1,000 to 1,100 m asl. In order to quantify the infestation level, a separate scale was made on the basis of plant damage, 0-25%, 26-50%, 51-75% and 76-100% defoliation/damaged plants were designated scale I, scale II, scale III and scale IV, respectively. A total of 432 rose plants (both wild and garden) were selected randomly in the locality and observations were taken at monthly intervals.

Rearing was carried out in the IPM laboratory, Division of Crop Improvement (Entomology), ICAR Research Complex of NEH Region, Umiam, Meghalaya, India (N 25.6532° E 91.8843°) during 2010-12. About 20 small pots (20 cm

diam × 20 cm height) containing medium sized rose plants (30 cm height) were kept in the natural environment (during Jun-Jul) and female *A. xanthogaster* were allowed to lay eggs inside the tender twigs. After 3 days, pots with rose plants were brought to the laboratory and kept in specially designed insect rearing cages (size: 45 × 45 × 45 cm, front and upper side covered with glass and the remaining 3 sides covered with fine insect proof nylon net) and photoperiod of 16:8 h L:D was maintained throughout the rearing. Newly emerged larvae (Figs. 7-12) were allowed to grow and develop to the prepupal stage on these rose plants. Morphological characters of each larval instar were recorded by a digital microscope and larval weight was measured by a digital electronic balance (Mettler Toledo® AB analytical balance, Model AB104-S). After pupal formation, cocoons were separated out and kept in large glass jars until adult emergence. Cotton soaked with water and honey solution (30%) was provided to adults for food.

After mating, 5 females were kept separately inside specially developed rearing cages (45 × 45 × 45 cm) and were allowed to lay eggs inside the tender twigs of rose plants. Fresh and medium-sized rose plants were provided regularly at 2-day intervals. The number of eggs laid per day was counted by a magnifying lens, and total life time fecundity was recorded. Rearing conditions during the experimental period were 23-25 °C, 85-90% RH and 16:8 L:D. Observations on incubation, larval period, pupal period, lifetime fecundity and adult longevity were recorded at appropriate stages and times.

RESULTS AND DISCUSSION

Identification of the Sawfly

Fresh specimens from Meghalaya were identified by comparing genitalia of sawflies with previously published reports of Saini & Thind (1995), and they were found to be *Arge xanthogaster* (Cameron) (Argidae: Hymenoptera).

Morphological Variation in *A. xanthogaster* of Northeastern India

Arge xanthogaster was previously reported from Manipur and Sikkim states of India by Saini & Thind (1995) without mentioning host plant records. Some external morphological variations were observed in *A. xanthogaster*. In some individuals, the bluish-black color of the abdominal terga extends to the last segment and usually the color of wings was dark brown or occasionally slightly lighter. In addition, a few differences were also observed in wing venation of *A. xanthogaster* obtained from Meghalaya (Figs. 1 and



Figure 7



Figure 8a

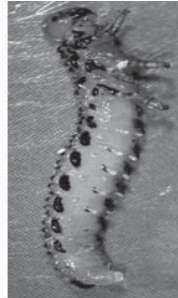


Figure 8b



Figure 9



Figure 10

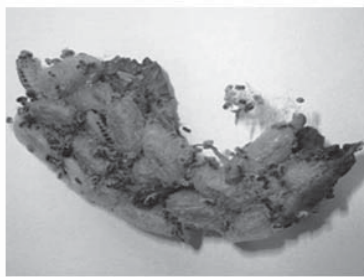


Figure 11



Figure 12

Figs. 7-12. *Arge xanthogaster*. 7. Larvae of feeding on rose leaves. Early larval instars are gregarious and feed heavily on leaves and flower buds. Slight disturbance of the larvae results in them curling the posterior of their bodies in a snake-like manner; 8a. Dorsal view of mature larva. Mature larvae turn yellow with black spots and feed individually. Full-grown larvae show aposematic body colors as well as pubescence; 8b. Ventral view of mature larva; 9. Cocoon. Mature larvae fall down to the ground and spin cocoons (dirty white in color) located on or in the soil; 10. Pupa of *Arge xanthogaster*; 11. Cocoons of *Arge xanthogaster* aggregated in a group; and 12. Dorsal view of an adult of *Arge xanthogaster*.

2). The nervulus of the forewing meets at the tip of the anal cell, whereas in specimens from Sikkim and Manipur (Fig. 2) the same joins the anal cell much before its tip. In specimens from Meghalaya, the 2nd recurrent vein joins the 3rd cubital cell in the middle, whereas it is joined to the first half of the 3rd cubital cell in case of the Sikkim and Manipur specimens. Furthermore, in specimens of Meghalaya, the basalis was almost straight whereas in specimens of Sikkim and Manipur it was distinctly curved in its basal half. These variations were found in almost all the available (collected) specimens of *A. xanthogaster* from the 3 above mentioned states. However, wing venation can vary in sawflies, but the populations exhibited no variation in genitalic morphology.

Development of a DNA Barcode

DNA from all individuals tested in this study was amplified successfully. The standard insect DNA barcoding primers (LepF1 and LepF2) used in this study amplified the target 790 bp fragment of COI gene of mtDNA. Direct se-

quencing gave good quality sequences for all the samples. A final 641 bp sequence were used for alignment after trimming the messy ends (5' and 3') of the sequences. Due to the absence of INDELS, alignment of all the sequences was straight forward. The SNPs (Single Nucleotide Polymorphisms) were not observed among all the 4 individual sequenced. BLASTN search of 641 bp sequence as a query in NCBI yielded many possible targets corresponding to the 5' end of COI gene; and *Arge nigripes* (Accession numbers EF032285 and AF146680) showed the closest homology (88%, E value 0.0) to the query (*A. xanthogaster*). Beside *A. nigripes*, other hits did not belong to the genus *Arge*. A representative sequence of *A. xanthogaster* has been deposited to the NCBI with accession number JX532103. Due to the absence of sequence information for this gene (COI) in genbanks, the correct identity of this species was not established at the molecular level; however, the taxonomic and molecular information generated and deposited in genbank from this study certainly should be helpful for future molecular identification and confirmation of this species.

Damage and Life History of *Arge xanthogaster*

During the peak infestation period (i.e., Jul and Aug), out of a total of 432 plant surveyed, 39.67%, 28%, 24% and 9.33% plants were found having 0-25% (Scale I), 26-50% (Scale II), 51-75% (Scale III) and 76- 100% (Scale IV) defoliation, respectively. Furthermore, about 22% of the rose plants were found completely damaged at the end of Nov (by both defoliation and ovipositional injury, i.e., split shoots)

Arge xanthogaster was active from May to Dec, and the maximum damage (80% larval damage) was found to occur from late Jul to Nov. Adult females possessed a strong sawlike ovipositor, which was used to make parallel cuts in the fresh/tender shoots of the host plant (Fig. 3) and in which they deposited yellow-colored oval eggs (Fig. 4). The eggs were placed singly in 2 longitudinal rows in slits (21.7 ± 2.83 mm long, $n = 386$) within the young shoots (Fig. 5). Each slit contained 27 ± 2.13 eggs ($n = 278$). The color of the eggs changed gradually, turning brown or black as the eggs matured.

Mean incubation period was found to be 7.60 ± 0.16 days ($n = 178$). After hatching the neonates quickly started to feed and began to devour tender leaves on rose bushes (Fig. 6). Early larval instars were gregarious and fed heavily on leaves and flower buds (Fig. 7). The young larvae were dark green and looked similar to lepidopteran caterpillars. Mature larvae (about 25 mm long) turned yellow with black spots and fed individually (Figs. 8a and 8b). In many cases, entire defoliation led to heavy plant damage especially to the flower bearing branches. Larval development took 16.30 ± 0.21 days and passed through about 5 or 6 larval instars ($n = 430$).

Full-grown larvae showed aposematic body colors as well as pubescence, which generally provide protection from other invertebrates and predatory birds (Guilford 1990; Costa 1997). Additionally, larvae curl and uplift their posterior portion in a snakelike manner, especially when threatened. When mature, larvae descended to the ground and spun cocoons (dirty white in color) in or on the soil (Fig. 9) and completion of pupation occurred inside the cocoon. The pupa was yellowish and exarate (Fig. 10). On occasion, full-grown larvae came together and formed grouped cocoons (Fig. 11). The pupal stage lasted for 10.10 ± 0.23 days ($n = 430$).

The adults were entirely black, except for the abdomen which was yellowish orange with black lines on the dorsal surface (Fig. 12). However, in the folded-wing condition, the sawfly appeared completely black. Female adults with a longevity of 6.40 ± 0.16 days, laid around 187 ± 3.5 eggs in 6 to 7 slits ($n = 382$). Overall, *A. xanthogaster* took about 40.40 ± 0.76 days to complete a full life cycle and around 6 to 7 overlapping generations

per yr have been found in the study region ($n = 298$). Females mate generally once during their life; however, parthenogenetic reproduction was also commonly observed. Pupal diapause has also been observed and overwintering occurred from the end of Dec to early May.

The overall results revealed that *A. xanthogaster* was mainly observed from May to Dec; and from Jan to Apr it was diapausing in the pupal stage. This schedule may be an adaptation to the severe winter (from mid-Dec to mid-Mar) in the region. The detailed biology of *A. pagana* (about N 48°), who found that late Jul to early Aug was its main flight period at this northern latitude.

Zenghe et al. (1991) studied the life cycle of a related sawfly, *A. pagana* and reported the following information on life cycle; incubation period (6-12 days), larval period (8.5-18 days), pupal period (8-13.5 days) and adult longevity (1.5-9.5 days); and this life cycle was more or less similar to that of *A. xanthogaster*. Early instar larvae of *A. xanthogaster* were gregarious and fed together. Subsequently during last mature stage, they became solitary. Bright coloration and gregariousness are generally linked traits in insects (Guilford 1990; Ruxton & Sherratt 2006), and *A. xanthogaster* had these traits. Similar characteristics have also been reported in several other sawfly species (Liston et al. 2010), and other common pest species, especially Lepidoptera (Fitzgerald 1995; Tullberg & Hunter 1996; Reader & Hochuli 2003; Lytan & Firake 2012). These characteristics could aid in avoiding increased risk of predation and parasitism, which, occurs comparatively more frequently under solitary conditions (Costa 1997; Firake et al. 2012). In addition, gregariousness also improves resource localization and assimilation (Bryant et al. 2000; Fordyce 2003), better survivorship and improved growth rate (Allen 2010). In addition to this, a slight disturbance to *A. xanthogaster* larvae results in them curling their posterior body (in a snake-like manner), which may reinforce the effectiveness of the pubescence by producing a 'hedghog effect'. Similar behaviors have been reported in *A. pagana*, and they are believed to be anti-predator defense activities (Petre et al. 2007).

Arge pagana produced 2 to 3 generations from May to Oct, whereas *A. ochropus* had 1 or 2 generations per yr (<http://uknature.co.uk/A.pagana-info.html>). However, in our study, *A. xanthogaster* produced 6 to 7 overlapping generations in a yr, which were facilitated by the prolonged favorable climate (mainly warm temperatures and ample rainfall) of the region. Parthenogenesis is quite widespread in sawflies (Ying 1992; Zhenghe et al. 1991; Sahragard & Heydari 2001; Mahmood & Ullah 2011), and was observed in our study. Unlike other rose sawflies, for example *A. rosae*

(Sahragard & Heydari 2001), female *A. xanthogaster* were found to be highly fecund and to lay about 3 times more eggs during their similar life span. Higher fecundity in *A. xanthogaster* may be caused by the favorable temperatures in their microhabitat. As the temperature of the microhabitat increases towards optimum, insects become more fecund (Sanders et al. 1978), develop faster (Lysyk 1989), and also have greater early instar survival (Lucuik 1984).

This study reports that *A. xanthogaster* has emerged as an additional pest of roses in the state of Meghalaya, India. Given the commercial importance of rose plants to the floriculture industry, timely management of this pest should be undertaken before it spreads to other parts of the country. This species lays eggs in groups inside the stem and early instar larvae are also gregarious; hence mechanical destruction of egg masses and larvae could be useful to control them effectively.

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