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DNA BARCODING, MORPHOLOGICAL DESCRIPTION AND FIELD DIAGNOSTICS OF *EUBLEMMA AMABILIS* (LEPIDOPTERA: EREBIDAE)

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ABSTRACT

Lac is an important commodity because of its versatile usage, biodegradability, and potentiality to sustain the livelihood of tribal community of India. Lepidopteran pest of Indian lac insect, *Eublemma amabilis* Moore [1884] (Lepidoptera: Erebidae) causes significant loss than any other lepidopteran pest thereof, both in wild and cultivated crop. Even though it's a regular pest recorded for decades, its taxonomic studies are lacking. In the present study, we have redescribed *E. amabilis* added photographic illustrations of male and female genitalia and which is also supported by a novel mitochondrial cytochrome oxidase I DNA barcode.

Key words: *Kerria* sp., lac, Tachardiidae, COI, lepidopteran predator, *Eublemma amabilis*, biotic stress, lac cultivation

Kerria lacca (Kerr) (Tachardiidae), Indian lac insect is commercially most used for the production of lac both in wild and cultivated regions. Biotic stress is one of the important constrain in lac cultivation. There are about nine species of Lepidopteran pests associated with lac insects in India *viz., Eublemma amabilis* Moore (Erebidae), *Pseudohypatopa* (=*Holcocera*) *pulverea* (Meyrick) (Blastobasidae), *Pyroderces falcatella* Staint (Cosmopterygidae), *Oedematopoda* sp., (Stathmopodidae), *Stathmopoda auriferella* (Walker, 1864) (Stathmopodidae), *Lacciferophaga yunnanea* Zagulajev (Momphidae), *Berginus maindroni* Grouvelle (Mycetophagidae), *Mataeomera sumbavensis* Hampson (Erebidae), *Autoba coccidiphaga* (Hampson, 1896), *E. roseonivea* (Walker), *E. scitula* (Rambur) (Erebidae), *Cryptoblabes ephestialis* Hampson (Pyralidae) and *Ephestia* sp. (Pyralidae) (Mohanasundaram et al., 2016). Among them two species cause major economic loss viz., *Eublemma amabilis* Moore (Erebidae) and *Pseudohypatopa pulverea* (Meyrick) (Blastobasidae). The use of insecticides in lac cultivation is challenging because you are dealing with both living systems (host and pest). The other alternatives for management of these pests are pheromones (monitoring and management) and biological control. The taxonomy of any pest is the basic foundation for pest management. Appropriate identification of these pests with distinguishing characters is very important. Confusions are prevailing in the nomenclature and identity of associated lepidopteran pests in the lac ecosystem. Especially for a major predator such as *E. amabilis*, there are no clear morphological descriptions with photographic illustrations. Further, molecular marker mtCOI is regarded as useful tool to diagnose and discourse important biological aspects like solving cryptic species complexes (Valade et al., 2009; Hafiz and Samreen 2016; Shashank et al., 2018). Till date, there are no DNA barcodes are available on the public platforms for this species. There is need to ascertain the identity of *E. amabilis* using voucher-based DNA barcode. By keeping this, in present study we have barcoded the species by using mtCOI gene and also provided morphological redescriptions for *E. amabilis* with photographic illustrations.

MATERIALS AND METHODS

The specimens included in this study were collected

from different localities of India viz., Mundgod (14° 58' 25.32'' N, 75° 2' 26.52'' E), Karnataka; Ranchi (23° 20' 38.7636'' N, 85° 18' 34.4268'' E), Jharkhand; Purulia (23° 20' 38.7636'' N, 85° 18' 34.4268'' E), West Bengal and Udaipur (24° 34' 16.5720'' N, 73° 41' 29.5584'' E), Rajasthan. Observations were taken to distinguish peculiar damage done by *E. amabilis* on standing crop. The material includes specimens collected from eight localities and deposited at the National Pusa Collection, Division of Entomology, Indian Agricultural Research Institute (IARI), New Delhi, India (NPC). Male and female genitalia terminology is after Klots (1970). The abdomen was treated in 10% KOH for 10 to 20 min at 90 °C using a Dry Block Heizgerat 2800. Subsequently, genitalia were cleaned and stored in glycerol. For photographs, genitalia were placed on a slide in glycerol with a cover slip. For wing venation studies, a right pair wings were detached from the body and kept in absolute ethanol for a few seconds. The wings were placed in 20% ethanol and cleaned with a Camlin brush (size 000) in cavity block and stained with 2% Eosin overnight. Remaining scales were removed in 70% ethanol after staining; dehydrated in absolute ethanol before slide mounting. Slide mounting was done with Euparal for venation studies. Photographs of an adult specimen were taken using a Canon EOS70D digital camera. Wing venation and genitalia photographs were taken using a Leica DFC 425 camera mounted on a Leica M205C stereozoom microscope with automontage.

Freshly emerged specimens from Ranchi were used for DNA barcoding. DNA was extracted from the specimens using Qiagen DNeasy® kit, following the manufacturer's protocols. The extracts were subjected to PCR amplification of a 658 bp region near the 5′ terminus of the cox1 gene following standard protocols (Hebert et al., 2003). Primers used were forward primer: (LCO 1490 5'-GGTCAACAAATCAT AAAGATATTGG-3'), and reverse primer: (HCO 2198 5' TAAACTTCAGGGTGACCAAAAAATCA-3') (Folmer et al., 1994). PCR reactions were carried out in 50 µL reaction volumes containing: GeNeiTM Taq buffer 5 µL, 1µL of GeNeiTM 10 mM dNTP mix, 2.5 µL of (20 pmol/µL) forward primer, 2.5 µL of (20 pmol/ µL) reverse primer, 1µL of GeNeiTM Taq DNA polymerase (1U/µL), DNA (50 ng/µL) 2 µL and sterile water 36 µL. Thermo-cycling consisted of an initial denaturation of 94 °C for 5 min, followed by 30 cycles of denaturation at 94 °C or 1 min, annealing at 55 °C for 1 min, extension at 72 °C for 1 min. PCR was performed using C1000™ thermal cycler. The amplified products were electrophoresed on a 1.5% agarose gel and were sequenced. The sequences were submitted to NCBI for GenBank accessions.

RESULTS AND DISCUSSION

Field identification of infested lac materials

Eublemma amabilis infested lac materials showed presence of pink coloured disc shaped excreta deposited on the tunnels, as well as larvae constructed exit tubes made from excreta for adult emergence. Larvae of *E. amabilis* are white in colour and sluggish before pupation. Infested lac possesses holes which are visible externally (Fig. 4). We observed that lac encrustation thickness is directly related to incidence of *E. amabilis*.

Taxonomic studies on *Eublemma amabilis*

The morphological redescriptions of distinguishing characters are carried for *E. amabilis* are presented herewith.

Eublemma amabilis Moore (1884) (Family: Erebidae; Subfamily: Boletobiinae; Tribe: Eublemmini) (Fig. 1-3).

Diagnosis: *Eublemma barlowi* has white fore wing with prominent discal tooth more or less central position similar to *E. amabilis*. Both species can be distinguished by female genitalia *E. barlowi* has larger ductus and corpus bursae than *E. amabilis*. Also, another related species, *E. roseonivea* can be distinguished from *E. amabilis* based on the presence of pair of spines on corpus bursae.

Head**:** Head covered from above with short white scales, vertex light brown in colour clothed with erect hairs mixed with white scales; Antennae scape and pedicel enlarged, clothed with white scales; flagellum pinkish yellow; 48-52 segmented in male and hairy, 43-56 segmented in female. Labial palpi curved upward and 3 segmented, 1st segment of labial palpi shorter 0.19-0.22 mm than 2nd one and clothed with elongated light yellowish scales, 2nd segment largest 0.45-0.55 mm covered with white scales. Proboscis yellowish in more or less flattened, and without scales at base. Compound eyes large and dark brown, more or less dorsally placed, large facets; ocelli dark colored. Maxillary palpi inconspicuous.

Thorax: Silvery white, slightly pink. Fore legs: coxa broader than femur basally, femur longer than coxa and tibia; tibia with one spur like process at proximal portion; 5 segmented tarsi; Mid legs: femur length 1.86-2.05 mm, tibia with two spur at distal end tibial length

1.40-1.80 mm, femur longer than tibia, tarsus with 5 segmented having 1.90-2.20 mm length and longer than tibia but equal or sub equal to femur, mid legs longer than hind legs; Hind legs: femur length 1.30-1.60mm, tibia with two pairs of spur at distal end 1.30-1.50mm, hind tarsi 1.50-1.90 mm tarsus with 5 segments (Fig. 1).

Wings: Wing span female 20-22 mm and male 18-21 mm; Fore-wing somewhat triangular in shape, basally silver white in color up to 1/4th of wing, having Violet to pink broad discal band pale, the inner margin of the band darkest and indented to the discal cell end, the darker outer border, angled outward at the middle. median vein, upper radial, and at the costal end, white serpentine line borders the angled margin from apical side, which bears intermingled black scales. Cilia with light pinkish to violet color, Rs2 and Rs3 stalked, bifurcating from single point, Rs3 ends in apical angle; M1 and M2 sub-parallel, prominent anal vein present (Fig. 3).

Hind wing smaller, single frenulum in male, group 4-5 frenulum in female. Roundish anal margin, hindwing basal 1/4th silver white colored, presence of pinkish-violet discal band similar to forewing but differs from later in being angled only at lower end of wing, pale pink outer border; distinctly black speckled scales along its entire length; the outer margin of hindwing as well as the cilia also suffused with pale pinkish-violet. Prominent single radial vein, M1, M2, M3 runs subparallel arising from discal cell, discal cell irregularly trapezoidal (Fig. 3).

Abdomen: Pinkish to white in color anteriorly whereas posterior half greyish pink to blackish brown in color; it tapers in posterior portion in male however, in female it is swollen in middle and then tapers gradually in terminal tufts of white hair scales present at terminal of abdomen; Length of entire body in male about 6.50-7.50 mm, in female 7.50-8.00 mm.

Male genitalia: Uncus elongated, slender and spine like pointed, 0.45-0.50mm; Juxta heavily fused at base of valve to saccular base' valve narrow, measuring

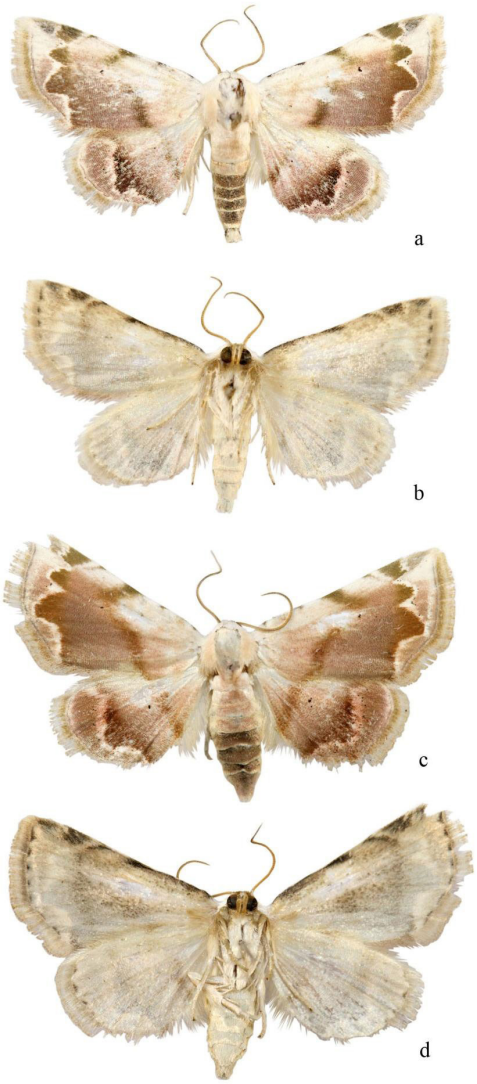

Fig. 1. *Eublemma amabilis* Moore a-d;
a & b. male adult (a. dorsal, b. ventral view), c & d female adult (c. dorsal, d. ventral view)

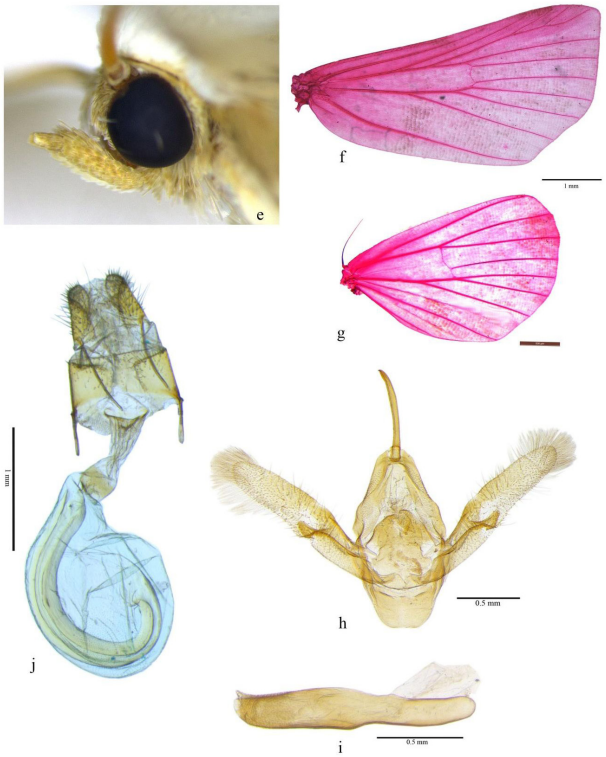

Fig. 2. *Eublemma amabilis* Moore, e-j; e. labial palp;
f. fore wing; g. hind wing; h. male genitalia; i. aedeagus;
j. female genitalia

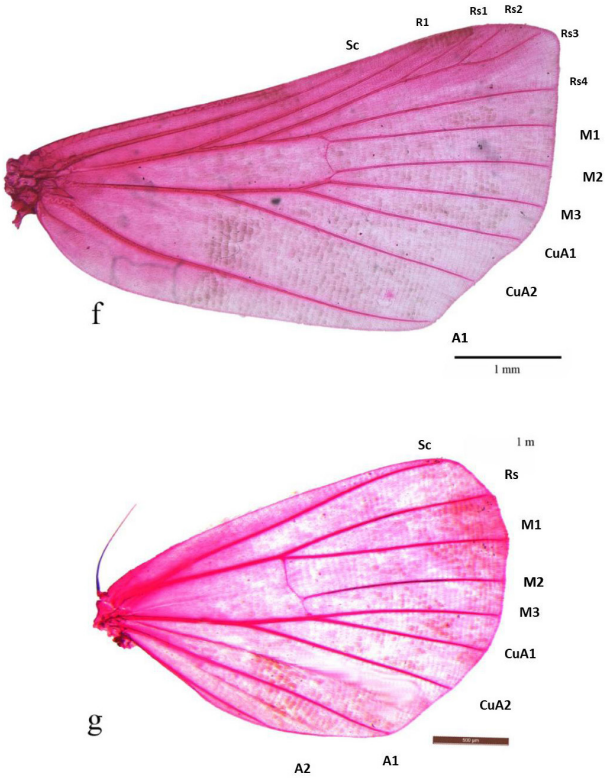

Fig. 3. Wing venation of *Eublemma amabilis*.
f. forewing; g. hindwing

0.80mm; Saccus length 0.18-0.20mm; Aedeagus: aedeagus straight with long coecum, length 0.80-0.90 mm (Fig. 2).

Female genitalia: Relatively shorter ductus and corpus bursae, corpus bursae devoid of spines, absence of signum; ovipositor longer than sub genital plates; Posterior apophysis longer than anterior apophysis (Fig. 2).

Material examined: INDIA: Karnataka: Mundgod (14° 58'25.32" N, 75° 2' 26.52" E), 21♀♀ and 20♂♂, 17.vi.2018, Host plant: Kusum and *Flemingia semialata,* Lac Strain: Kusmi, Coll. J. Mahesh.; INDIA: Rajasthan: Udaipur (24° 34' 16.5720" N, 73° 41' 29.5584" E), 22♀♀ and 21♂♂ 15.xi.2018, Host plant: Ber, Lac Strain: Kusmi, Coll. K. Vikram; INDIA: Jharkhand: Ranchi (23° 20' 38.7636" N, 85° 18' 34.4268" E), 23♀♀ and 21♂♂, 17.vi.2018, Host plant: Palas and *Flemingia semialata*, Lac strain: Rangeeni and Kusmi, Coll. A. Mohansundaram; INDIA: Jharkhand: Ranchi (23° 20' 38.7636" N, 85° 18' 34.4268" E), 20♀♀ and 22♂♂, 15.xi.2018, Host plant: Palas, Lac strain: Rangeeni, Coll. J. Mahesh; INDIA: West Bengal: Purulia (23° 20' 38.7636" N, 85° 18' 34.4268" E), 21♀♀ and 20♂♂, 25.ii.2019, Host plant: Palas, Lac strain: Rangeeni, Coll. N. N. Rajgopal.

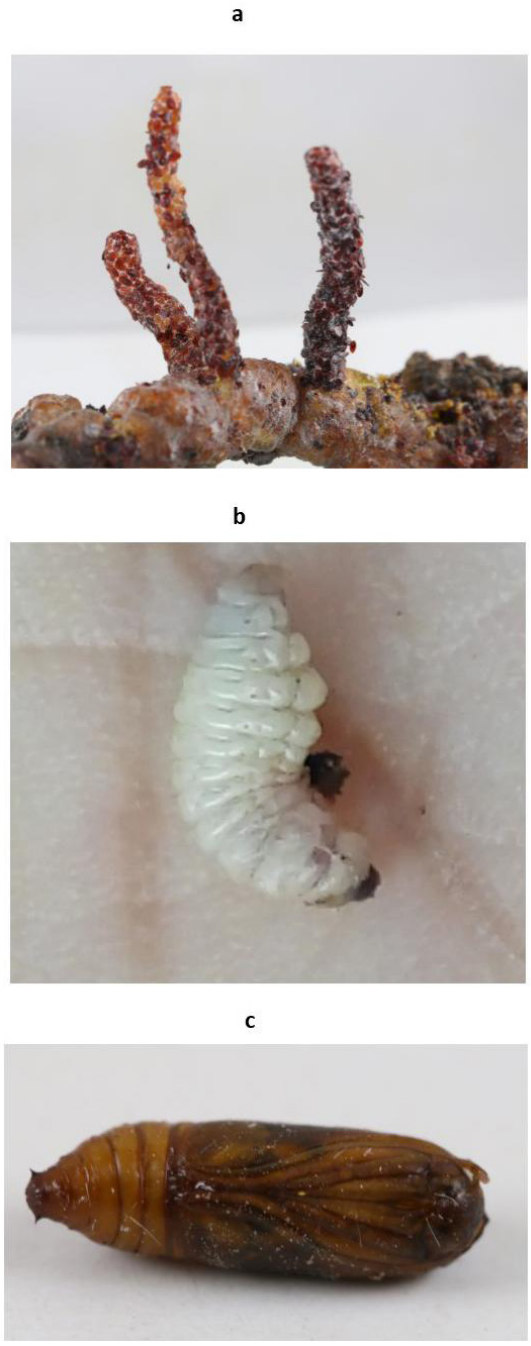

Fig. 4. a. Tubes with exit holes for *E. amabilis* adult emergence made by larva; b. larva; c. pupa

DNA barcoding

Mitochondrial cytochrome oxidase I DNA barcode gene was sequenced. The obtained sequences were checked for quality and >600bp sequences are submitted to NCBI GenBank database and obtained the accession numbers (MW881783 and MW881784). The specimens from which DNA barcodes were generated are kept as voucher specimens and deposited in National Pusa Collection, Division of Entomology, ICAR-IARI,

New Delhi. The BLAST analysis in NCBI resulted the sequence match with the taxa of family Erebidae, Lepidoptera. Till now, there are no *E. amabilis* specimen-based DNA barcodes in public database (BOLD and GenBank). This makes present sequences submitted in the study as novel barcodes.

In present study, *E. amabilis* has been redescribed with respect of different morphological traits like head, vertex, frons, labial palpi, thorax, legs, wing pattern, venation, abdomen and genitalia. Moore (1884) described and illustrated *E. amabilis* from the type locality Ceylon, Sri Lanka without any host information. He had described only wing pattern. Hampson (1894) mentioned *E. amabilis* from Ceylon along with 43 other species of *Eublemma* Hubner found in India and Sri Lanka. Holloway (2009) studied 48 species of *Eublemma* from Borneo along with diagnosis of *E. barlowi* and *E. plagirosea* in comparison to *E. amabilis*. In this publication we provide with diagnosis of genitalia of *E. amabilis*. Thus, based on female genitalia *E. amabilis* can be distinguished from *E. barlowi* by length of ductus and corpus bursae; from *E. roseonivea* by pair of spines on corpus bursae with respect to other species. In present study, four different lac growing areas were surveyed for infestation of lepidopteron pests. Infested lac possesses holes which are visible externally. During the field surveys, *Eublemma amabilis* infested lac materials showed presence of pink colored disc shaped excreta, as well as larvae constructed exit tubes using excreta before pupation for adults to escape. When such lac encrustations were scrapped for confirmation; it showed presence of white colored larva of *E. amabilis*, tapered at both end but swollen in middle and sluggish before pupation. The DNA sequences are used as genetic 'barcodes' that may potentially be used as a bio-identification system for all animals and has proven to be useful identification tool for hexapod orders including Lepidoptera (Hajibabaei et al., 2006). In this view we have barcoded mtCOI gene of the species and submitted to GenBank of NCBI which is of its first kind.

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REFERENCES

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R. 1994. DNA primers for amplification of mitochondrial Cytochrome Coxidase subunit I from diverse metazoan invertebrates.Molecular Marine Biology and Biotechnology3(5): 294-299.

Hafiz M T, Samreen A. 2016. Services of DNA barcoding in different fields. Mitochondrial DNA. Part A 27: 4463-4474.

Hajibabaei M, Janzen D H, Burns J M, Hallwachs W, Hebert P D N. DNA barcodes distinguish species of tropical Lepidoptera. Proceedings of the National Academy of Sciences of the United States of America 103: 968.

Hampson G F. 1894. Fauna of British India, Moths, including Ceylon and Burma, Vol. II. Taylor and Francis Ltd., London. 338-344pp.

Hebert P D, Cywinska A, Ball S L, De Waard J R. 2003. Biological identifications through DNA barcodes. Proceedings of the Royal Society of London. Series B: Biological Sciences 270 **(**1512): 313-321.

Holloway J D. 2009. The Moths of Borneo (part 13): family Noctuidae, subfamilies Pantheinae (part), Bagisarinae, Acontiinae, Aediinae, Eustrotiinae, Bryophilinae, Araeopteroninae, Aventiinae, Eublemminae and further miscellaneous genera. Malayan Nature Journal 62: 1–240.

Klots A B. 1970. Lepidoptera, pp 115–139. In: Tuxen S L (ed.). Taxonomist's Glossary of Genitalia in Insects. Munksgaard, Copenhagen 359pp.

Misra M P, Negi P S, Gupta S N. 1930. The noctuid moth (*Eublemma amabilis* Moore): a predator of the lac insect, and its control. Journal of Bombay Natural History Society 34: 431-446.

Mohanasundaram A, Monobrullah Md., Sharma K K, Meena S C, Ramani R. 2016. Lac insect and associated fauna - A practical manual. ICAR-Indian Institute of Natural Resins and Gums, Ranchi (Jharkhand), India. Bulletin (Technical) No. 14/2016 01-42 pp.

Moore F. 1884. Lepidoptera of Ceylon Vol. III, L. Reeve and Co. London, 54pp.

Rahman M M, Ahmed K, Karim K S, Ali M S. 2009. Bionomics of *Eublemma amabilis* Moore (Lepidoptera: Noctuidae), a Major Predator of Lac insect and Its Control Measure. Bangladesh Journal of Scientific and Industrial Research 44(1): 57-64.

Shashank P R, Twinkle S, Chandrashehar K, Meshram N M, Suroshe S S, Bajracharya A S R. 2018. Genetic homogeneity in South American tomato pinworm, *Tuta absoluta*: a new invasive pest to oriental region. 3 Biotech 8: 350.

Valade R, Kenis M, Hernandez-lopez A, Augustin S, Mari Mena N, Magnoux E, Rougerie R, Lakatos F, Roques A, Lopez-Vaamonde C. 2009. Mitochondrial and microsatellite DNA markers reveal a Balkan origin for the highly invasive horse-chestnut leaf miner *Cameraria ohridella* (Lepidoptera, Gracillariidae). Molecular Ecology 18: 3458-3470.

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