Xylotrechus smeii (Cerambycidae: Cerambycinae: Clytini): A potential threat to Red Sanders cultivation

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Abstract

Red sanders (*Pterocarpus santalinus* L.f.) is an economically important, endangered timber species endemic to the restricted forest tracts of the Eastern Ghats, India. Here, we report the occurrence of long horned beetle *Xylotrechus smeii* infestation on red sanders for the first time from Telangana, India. Infestation was characterized by complete drying of standing trees, presence of galleries and bore holes in cut stems. In addition, supplementary description of *X. smeii*, including the terminal abdominal segments and genitalia of both the sexes, immatures and its illustrations are provided for more authentic identification of this important pest. Further, the identity of the insect as *X. smeii* was confirmed through *mtCOI* based DNA barcode. Phylogenetic and sequence divergence analyses based on partial *mtCOI* gene sequence showed that *X. smeii* isolate obtained in the current study was closely related to *X. smeii* isolate WA13 10.01r that was intercepted in wood packaging material in one of the ports of entry of United States (U.S.).

1. Introduction

Pterocarpus santalinus L.f., commonly known as red sanders, is an endemic tree species confined to the southern pockets of the Eastern Ghats, India (Pullaiah, 2019). The tree is valued for its blood red coloured hard heartwood for which the demand in global market is increasing in a faster pace similar to gold. The wood is used in making high value furniture, musical instruments and the santalins extracted from wood is put to use in textile, food and pharmaceutical industries. Besides, the heartwood is also used for medicinal purposes in the treatment of coughs, digestive tract problems and fluid retention (Pullaiah & Anuradha, 2019). In its recent threat assessment, International Union for Conservation of Nature and Natural Resources has placed the species in 'Endangered' category, up listing from its earlier 'Near Threatened' category (Ahmedullah, 2021). The species is listed in Appendix II of CITES which restricts international trade of its wood and wood products from natural sources (Pattanaik, 2019). In general, this species is inherently resistant to insects, fungi and marine borers. Even in nurseries and plantations, red sanders will not encounter any serious effects of pests. Insects that were reported to infest red sanders prior to harvest include *Eotetranychus sexmaculatus*, species of *Planococcus*, leaf-eating caterpillars, Pyralid caterpillars and white grubs. However, their effects on red sanders were subtle (Umalatha & Anuradha, 2019).

Cerambycidae is one of the economically and ecologically important species-rich families of beetles (Coleoptera) with over 35,000 described species. The number of wood-boring cerambycid beetles in the tropics is very large. (Kariyanna et al., 2017a; Švácha & Lawrence, 2014). *Xylotrechus smeii* (Castelnau & Gory, 1841) belongs to the subfamily Cerambycinae and tribe Clytini that contains small to large, elongate beetles with reniform eyes, pronotum without lateral margin, notosternal suture rarely complete, usually indistinct and empodium usually small or indistinct (Monné et al., 2017). Several well established alien species belong to the tribe Clytini and the genus *Xylotrechus* with majority of them originating from Asia (Hănceanu et al., 2021).

In India, *Xylotrechus* is represented by 19 species (Kariyanna et al., 2017a). Among these, *X. stebbingi* Gahan, 1906 that originated in Northern India, is regarded as notorious pest for invading several Mediterranean and European countries through imported wood. (Braud et al., 2002; Sama & Cocquempo, 1995). Similar to *X. stebbingi*, yet another polyphagous species of this genus is *X. smei*. *X. smei* was originally described from India and it morphologically resembles *X. stebbingi* (Laporte & Gory, 1841; Sama, 2006; Vitali, 2004).

Recently, *X. smei* is reported in several ports of entry in France and US (Roques et al., 2017; Wu et al., 2017). Though widely distributed in India and reported so far from Himachal Pradesh, Kashmir, Uttar Pradesh, Madhya Pradesh and West Bengal, the major hot spots of *X. smei* are the Deccan and the North Eastern regions of India. More than 40 trees were reported as hosts of this pest among which *Morus alba* and *Morus indica* are the most preferred ones in the hot spot areas (Kariyanna et al., 2017b; Kariyanna et al., 2019).

Discrepancies that revolved around identification of *X. stebbingi* that was introduced into the western Palaearctic as *X. smei* was recently clarified by Sama (2006). However, it was based on the characters found in prothorax and elytra. Furthermore, information about this pest that appeared as part of faunistic studies from India (Mitra et al., 2015; Mitra et al., 2017) in the last decade concentrated mainly on the patterns and characters of the surface. However, no attempt was made to describe the details of terminal abdominal segments and genitalia, including their measurements that are inevitable for the authentic identification of this pest as well as in differentiating the male and female.

The current study was undertaken to investigate the possible cause of dying red sander trees in a red sander plantation in Telangana, India and reports the association of *X. smei* with the dying trees. This also provided us an opportunity to report the first detailed description of terminal abdominal segments and genitalia of both sexes of *X. smei* adults along with additional supplementary characters and measurements of grubs and pupae with illustrations for authentic identification of this polyphagous pest.

2. Material And Methods

2.1. Field visit

In March, 2021, dying of few red sanders trees in a plantation near Kothagudem, Telangana, India (referred hereafter as location 1) (Figure 1) was brought to the notice of Institute of Forest Biodiversity (IFB), Hyderabad. A field visit was made in the last week of March, 2021 and data on spacing, tree age and other tree species cultivated alongwith red sanders were recorded. Few of the infested trees were uprooted and split open to observe the presence of grubs, galleries and bore holes. Field photography was done using Canon SX60 with 65x zoom. Besides, intact wood from infested trees were collected from the field, brought to the IFB laboratory and maintained in insect rearing chamber for adult emergence. Similar tree drying in a red sander plantation near Khammam, Telanagana, India (referred

hereafter as location 2) (Figure 1) was brought to the notice of IFB in December, 2021. Investigation on the presence of grubs in infested trees was carried out and the field data was recorded.

2.2. Morphological identification

The adults that emerged from the collected wood were sent for identification to Coleoptera Identification Service, National Pusa Collection, Division of Entomology, Indian Agricultural Research Institute, New Delhi.

The identification was done based on comparison of specimens with the photographs of the type, key as well as description by Sama (2006). For genitalia dissection, specimens were relaxed overnight, and the abdomen was detached and boiled in 10% KOH. The genitalia and terminal abdominal segments were dissected, rinsed with water, dehydrated in alcohol and then preserved in genitalia vials after imaging. General morphological characters were studied using a Leica stereo zoom microscope M205C and imaging was done using a MC190HD digital camera. Measurements were made using Leica software LAS V4.13.0. After taxonomic studies the Voucher specimens were deposited in the National Pusa Collection, Division of Entomology, Indian Agricultural Research Institute, New Delhi, India.

2.3. Molecular characterization

Total DNA was isolated from the collected grubs using Cetyl Trimethyl Ammonium Bromide method (Doyle, 1990). Amplification of *mtCOI* gene from three sets of isolated DNA was performed in Bio-Rad C1000 thermal cycler (Bio-Rad Laboratories Inc, Berkeley, CA, USA) using LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') primer pair (Folmer et al., 1994). The 25 µL final reaction mixture contained 500 ng of isolated DNA, 0.5 µM of each forward and reverse primers and 1× DreamTaq Green PCR Master Mix (Thermo Fisher Scientific, USA). Thermal cycling conditions were as follows: initial denaturation at 94 °C for 5 min, 35 cycles of 94 °C for 1 min, 52 °C for 1 min and 72 °C for 1 min 40 s followed by final extension for 10 min at 72 °C. The amplicons were run on 1.2% agarose gel along with a non-template control and visualized using a gel documentation system. Amplicons were purified and subjected to Sanger sequencing bidirectionally (at Green Genome India Pvt Ltd.). Obtained sequences in triplicates were aligned using the CLUSTALW tool available in MEGA7 v7.0.26 (Kumar et al., 2016) and the consensus sequence was derived from the alignment using the online tool EMBOSS cons available at <https://www.bioinformatics.nl/cgi-bin/emboss/cons>. For species level identification, the consensus sequences were subjected to BLAST analysis in National Centre Biotechnology Information (NCBI) 'non-redundant'(nr) database (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>; accessed on 19 January 2022) and Barcode Of Life Data System (BOLD) database v4 (<http://www.boldsystems.org/index.php>; accessed on 19 January 2022). The obtained consensus sequence alongwith the *mtCOI* sequences of *X. smeii* and other *Xylotrechus* spp. retrieved from NCBI were aligned using the CLUSTALW tool in MEGA7. Further, the aligned sequences were subjected to phylogenetic tree construction using Maximum Likelihood (ML) method and the best-fit GTR+G+I model with 1000 bootstrap replicates. *Nortia carinicolis mtCOI* sequence was taken as

outgroup during phylogenetic tree construction. Divergence values between the aligned sequences were obtained using MEGA7.

3. Results

3.1. Infestation of *X. smei* on red sanders

The 12 acres field (location 1) contained 4000 red sander trees (5-6 years old) planted at 2.4 x 2.4 m and 3.0 x 3.0 m spacing. Besides, 2000 sandalwood trees were grown along with other tree species in small numbers. Out of 4000 red sander trees in location 1, 25 trees displayed complete drying of crowns (Figure 2(a),(b),(c)). Based on our discussion with the farmer, it was learnt that tree drying was gradual starting from two years of age. Initially, leaves in the lower crown showed symptoms of chlorosis followed by gradual drying of the crown while the bark got loosened from the main stem. A dead tree was uprooted and the roots and stems were examined. The roots were intact and no internal discoloration could be observed within the root tissues and at the base of the stem excluding the possibility of root rot and wilt. However, when the stem (up to 1m height from the ground level) was split open, creamy white, apodous, eucephalous grubs (Figure 2(d)) were seen feeding the internal sapwood. The feeding galleries (Figure 2(e),(f)) were covered with frass. Obvious bore holes could not be observed in the wood exterior probably due to the rough nature of the bark. Splitting of wood of dead trees revealed the presence of galleries and bore holes. Subsequently, six other dead trees were uprooted and examined for internal discoloration and presence of grubs (Figure 2(d),(e),(f),(g)). Only in one of the examined trees, pinkish discoloration was observed in the internal tissue while all the observed trees housed the grubs. From one of the trees, a pupa (Figure 2(h)) and a cerambycid adult were collected from the collar region of the cut stem. Adult emergence was observed from the collected samples during the months of May, June and July and the emerged adults resembled that of the one collected from the field. Two types of adults emerged from the collected samples. The predominant one (more than 40 in number) was identified as *Xylotrechus smei* while the other (8 in number) was a species of *Chrysobothris*. The 1.5 acres field (location 2) contained 200 red sander trees (20 years old) planted at 2.0 x 2.0 m spacing along with teak. A total of 5 trees out of 200 red sander trees showed symptoms of complete drying while 4 additional trees showed partial drying of crown. Splitting of stems of infested trees revealed the presence of grubs of *X. smei*.

3.2. Morphological descriptions

Xylotrechus smei (Laporte de Castelnau & Gory, 1841)

Clytus smei Laporte de Castelnau & Gory, 1841: 37, pl. VIII, Figure 46; White, 1855: 262 (m. s.).

Type locality: India: India Orientalis (HT ☒)

The major borer found infesting and emerged from the trees were *X. smei* along with few *Chrysobothris* sp.

3.2.1. Supplementary description

Male: Length: 10.4–11.9 mm, humeral width: 2.5–2.9 mm. Female (Figure 3(a),(b),(c),(d)): Length: 11.9–16.6 mm, humeral width: 2.8–3.9 mm. Prothorax (Figure 3(e)) 1.3 times wider than head in both sexes. Mandible black, broader in basal half with broad, flat shallow depression covered with pale white setae, few golden setae in middle, narrow toward blunt glabrous apex. Antennomere 1–4 covered with pale white setae; rest with minute golden pubescence, last segment pre apical lateral margin slightly constricted; lateral pre apical margin of antennomere 3–6 with few long, golden setae; antennomere 2 smallest, antennomere 3, 2.3 times longer than antennomere 2. Vertex and sides irregularly alveolate. Scutellar shield (Figure 3(f)) semicircular, 1.5 times broader than long; covered with dense, pale white, pubescence. Procoxae round, slightly convex, middle of procoxae with shallow, short longitudinal depression, margin bicarinate, from base to middle; prosternal process narrow. Mesocoxae flat as compared to procoxae; mesosternal process broader than prosternal process, covered with dense, pale white pubescence. Metasternum with fine sparser punctures covered with pale white setae, margin with denser setae; shallow longitudinal discripen medially, from base to apex. Elytra 2.4 times as long as broad in both sexes; base of elytra laterally beyond middle with shallow depression, surface with minute punctures separated by a distance little more than their own diameter, apex obliquely truncate, margin weakly projected in middle, outer spine prominent than feeble sutural spine. Ventrites covered with pale white pubescence, denser form patches on lateral side, mostly continued as thin band in first two, sparser towards last; punctuations on ventrite similar to elytra but placed wider compared to elytra.

3.2.2. Description of terminal abdominal segments and genitalia.

Males and females can be separated based on the appearance of 5th visible sternite or sternite VII both in shape and size. Sternite VII (Figure 3(h)) of female is 1.8 and 1.5 times longer and wider than male; nearly broad triangular, gradually narrowing towards apex, middle of apical margin weakly projected and truncate. Sternite VII (Figure 4(b)) of male broadly rounded at apex, with few hairs on surface, margin fringed with hairs.

Tergite VII (Figure 2(g)) of female 1.5 and 1.2 times longer and wider than male; 1.0 time longer than its own width; distal half and margin fringe with hairs; surface finely punctulate covered with moderately long setae, apico lateral and posterior margin covered with short setae intermixed with few long setae; posterior margin is distinctly emarginated with weak projection in middle. Tergite VII (Figure 4(a)) in male 1.4 times longer than wide, posterior margin very weakly emarginated. Tergite VIII (Figure 4(c)) of male 1.1 times as wide as long, widest at base triangular, middle membranous, gradually narrowing towards apex, apical middle margin truncate, margin fringed with hairs. Sternite VIII (Figure 4(d)) is as long as wide till median strut; transverse, anterior edge with median strut, apico-lateral margin round and middle of apical margin shallowly or weakly emarginated, margin covered with short setae and apico; lateral margin with few long setae. Spiculum ventrale (Figure 4(g)) total length 1.4 mm, forked and median strut

is curved at tip. Ratio of length of tegmen to parameres (Figure 4(e)) is 2.0; ringed part of tegmen widest near middle and converge towards base; tegmen abruptly curved towards middle in lateral view (Figure 4(f)), with two elongate nearly parallel lateral lobes with apex covered with short hairs and few elongate longer hairs at tip and longer than length of lateral lobes. Median lobe and median struts (Figure 4(h),(i)) moderately curved in lateral view and apical part of median lobe strongly projected in dorsal view and side nearly parallel including median struts. **Female genitalia:** Eighth abdominal segment (Figure 3(i)) 1.2 times longer than wide, apico-lateral margin covered with intermix of few short and long setae. Spiculum gastrale is 3.43 mm long; ovipositor (Figure 3(j)) with distinct paraproct, gonocoxite and gonostylus. Surface of gonostylus with few setae.

Material Examined. INDIA: Telangana: Kothagudem District: (11♀♀; 19♂♂), adults emerged from infested *Pterocarpus santalinus* logs, 29.iii.2021, Kavi Sidharthan and Avula Kishore. (Figure 1)

3.3. Molecular characterization

BLAST analysis of *mtCOI* consensus sequence derived from the *X. smei* isolate in the present study against BOLD database revealed its greatest sequence similarity of 99.39% with isolates of *X. smei*. Similar BLAST searches in NCBI 'nr' database showed the maximum nucleotide sequence similarity (99.39% at 100% query coverage) of *X. smei* isolate of the current study with an U.S. isolate WA13_10.01r (KY357552). Phylogenetic analysis grouped the *X. smei* isolate of the present study with an *X. smei* isolate WA13_10.01r (KY357552) and a voucher specimen (MN315205) from U.S. All the *X. smei* sequences, except the one submitted from India (KU321042), fell in a distinct sister clade to the *X. stebbingi* sequences (Figure 5). Excepting for the Indian *X. smei* isolate, sequence divergence of the isolate under study with other *X. smei* isolates (KY357552, MN315205, MN315204, MN315224) ranged from 0.7–4.0% while the *X. smei* Indian isolate (KU321042) showed 4.4% nucleotide sequence divergence from the *X. smei* isolate obtained from red sanders. On the other hand, *X. smei* isolate of current study showed 4.2% nucleotide sequence divergence from the *X. stebbingi* isolates (MN182949, MN182963) used for analysis.

4. Discussion

Red sanders is an economically important, endangered and endemic tree species that is relatively resistant to pests and diseases (Umalatha & Anuradha, 2019). Because of the continual harvest since sixteenth century, population structure of red sanders is skewed that mature and harvestable trees make up less than five percent of total trees in the wild (Ahmedullah, 2021). Despite its listing in CITES and prevalence of protective laws at national and state level, red sanders is illegally harvested and traded globally that is evident from the quantum and frequency of seizures in international market (Ahmedullah, 2021). Currently, a tonne of red sanders timber has a market value of approximately 58.040USD. It is presumed that commercial cultivation of red sanders would reduce the pressure on wild population of red sanders (Ahmedullah, 2021).

In the current study, severe infestation of *X.smei* is reported from red sanders plantations in two locations of Telangana, India. Earlier, the occurrence of *X. smei* on *P. marsupium* was reported. However, to our knowledge, this is the first report of *X. smei* on red sanders. Both the locations fall under different forest divisions of Telangana and the distance between the locations is more than 60 km. As a result of damage caused by the grubs, complete drying of 5-6 year old trees in location 1 and 20 year old trees in location 2 was observed. It is worthy of note that location 1 was situated close to a natural forest and there exists possibility of host shift due to reduction in populations of preferred hosts in the natural forest range. Interestingly, in location 2, red sanders were co-cultivated with teak that is reported as one of the hosts of *X. smei* (Kariyanna et al., 2017b). This pest poses a serious threat not only to the red sanders cultivation but also for its conservation. Since the crop is highly valued for its wood in international market, there exists ample scope for the introduction of this pest into newer areas through timber trade. In addition, in the light of its recent interceptions in various ports of entry (Roques et al., 2017; Wu et al., 2017), it may be concluded that *X. smei* has the potential to establish in new areas in the context of timber export. Undoubtedly, the supplementary description of *X. smei* provided in this report will help in the authentic identification of this pest globally. Further, phylogenetic and sequence analyses confirmed the identity of the Cerambycid obtained in the current study as *X. smei*. To our surprise, a sequence designated as *X. smei* from India (KU321042) that diverged largely from the isolate obtained in the current study, grouped along with *X. stebbingi* in phylogenetic analysis. Thus, it is speculated that the sequence designated as *X. smei* (KU321042) could possibly be *X. stebbingi*.

In conclusion, the present study reports the infestation of red sanders by *X. smei* for the first time from Telangana, India. Considering the importance of red sanders and severity of *X. smei* on red sanders, control measures are needed for effective management of this pest from both economic and conservation point of view. Morphological descriptions provided in this report will help in authentic identification and differentiation of sexes of *X. smei*.

Declarations

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6. Author Contributions

Conceptualization, N.C. and V.K.S.; methodology, N.C.; formal analysis, N.C. V.K.S. B.P and S.P; resources, N.C. and S.P; writing-original draft preparation, N.C. and V.K.S.; writing-review and editing, B.P. and S.P. All authors have read and agreed to the published version of the manuscript.

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8. Data Availability Statement

The nucleotide sequence generated in this study was deposited in NCBI with accession number OM368356.

9. Conflict of interest

There is no conflict of interest.

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Figures

Figure 1

Map showing the locations of red sander plantations infested with *Xylotrechus smeii*.

Figure 2

Xylotrechus smeii damage on red sanders. (a) Completely dried crown, (b), (c) completely dried trees, (d) Larvae among frass, (e), (f) Bore holes and galleries of *X. smeii*, (g) Larva and (h) Pupa of *X. smeii*

Figure 3

Xylotrechus smei female. Habitus (a) Dorsal, (b) Ventral, (c) Lateral, (d) Head, Anterior view (e) Pronotum dorsal view, (f) Scutellar Shield, (g) Tergite VII, (h) Sternite VII, (i) Eighth abdominal segment along with Spiculum gastrale and (j) Ovipositor

Figure 4

Xylotrechus smei male. (a) Tergite VII, (b) Sternite VII, (c) Tergite VIII, (d) Sternite VIII, (e) Tegmen along with parameres dorsal view, (f) Tegmen along with paramere lateral view, (g) Spiculum ventral, (h) Median lobe dorsal view and (i) Median lobe lateral view

Figure 5

Maximum Likelihood tree showing the relationship of *Xylotrechus smeii* red sanders isolate obtained in the current study with global isolates based on *mtCOI* sequence. Phylogenetic tree was constructed using the best-fit GTR+G+I model with 1000 bootstrap replicates. Only bootstrap values more than 50 are shown.