Philippine Journal of Science
153 No. 6A: 1979-2001, December 2024
ISSN 0031 - 7683
Date Received: 25 Jan 2024

Taxonomic Review and Delimitation of Species Boundaries of *Leucopholis* Dejean (Coleoptera: Scarabaeidae: Melolonthinae: Leucopholini) Associated with *Areca catechu* L. (Areca Nut) in South India

Kunthanahalli V. Prakash[1], Hesaraghatta M. Yeshwanth[2], Shayamal Lakshminarayanan[3], John Vincent B. Cabusas[4], Barbara L. Caoili[4,5], and Orlando A. Calcetas[6]*

[1]All India Network Project on Soil Arthropod Pests, Department of Entomology, University of Agricultural Sciences, G.K.V.K., Bengaluru, India
[2]Department of Entomology, University of Agricultural Sciences, G.K.V.K., Bengaluru, India
[3]Tatanagar, Bengaluru, India
[4]Institute of Weed Science, Entomology, and Plant Pathology, College of Agriculture and Food Science, University of the Philippines Los Baños, College, Laguna, the Philippines
[5]National Crop Protection Center, College of Agriculture and Food Science, University of the Philippines Los Baños, College, Laguna, the Philippines
[6]Regional Crop Protection Center, Regional Field Office-IVA-CALABARZON, Department of Agriculture, Marawoy, Lipa City, Batangas, the Philippines

The white grubs of the genus *Leucopholis* Dejean are the most devastating insect pests of areca nut and coconut palms in south India. To date, three species, *L. lepidophora* Blanchard, 1851, *L. coneophora* Burmeister, 1855, and *Leucopholis burmeisteri* Brenske, 1894 have been reported from Karnataka. *Leucopholis lepidophora* is distinct from the two species based on their genitalia male and molecular markers. An average of 3.43–15.4% genetic distances were observed among *L. coneophora* and *L. burmeisteri* species based on the mitochondrial *COI* gene DNA barcode region. These results imply that they belong to distinct species. A key to the species of *Leucopholis* in south India is also provided.

Keywords: integrative taxonomy, *Leucopholis*, monophyly, scarabs, synonymy

INTRODUCTION

Leucopholis Dejean, 1833 is a predominantly oriental genus with many pestiferous species. It is the type genus of the tribe Leucopholini (eventually subtribe Leucopholina) of the subfamily Melolonthinae [*e.g.* Bezděk (2016), Smith (2006)]. However, the taxonomic status of Leucopholini is still under discussion and is not fully clear (Britton 1978; Allsopp 2022).

Currently, there are four *Leucopholis* species in India subcontinent – namely *L. lepidophora* Blanchard, 1851, *L. coneophora* Burmeister, 1855, *L. burmeisteri* Brenske, 1894, and *L. crassa* Brenske, 1892 – which was described from Sylhet (Bangladesh) and recorded from

*Corresponding author: orly.calcetasgis@gmail.com

India by Barlow (1899) (Appendix Figure I). However, no specimen of *L. crassa* was examined in this paper. *Leucopholis lepidophora* can be easily distinguished from *L. coneophora* and *L. burmeisteri*, whereas the latter two species are hard to separate based on their external morphological characters alone (Veeresh *et al.* 1982). Calcetas (2019, 2023) observed these limitations in the genus *Leucopholis* and *Carlschoenherria* Bezděk 2016 (Melolonthinae: Melolonthini), wherein genital characters are helpful in separating very distinct species but difficult to distinguish solely on their external morphology.

Blanchard (1851) described *L. lepidophora* from Bombay (Mumbai), which is located in the central-western part of India. Later on, Burmeister (1855) added *L. coneophora* and also recorded *L. lepidophora* from Mangalore in southwestern India. Finally, Brenske (1894) re-examined specimens that Burmeister (1855) identified as *L. lepidophora* and pointed out, that they represented an undescribed species. Thus, Brenske (1894) proposed the name *L. burmeisteri* in honor of Burmeister for the species from Mangalore.

A comprehensive literature on the systematics and biogeography of *Leucopholis* is only available for some regions, including the review of the genus and three newly described species of *Leucopholis* from the Philippines (Calcetas and Adorada 2017; Calcetas 2023). Recently, Kumar and Pandey (2024) reported the occurrence of *L. lepidophora* in the western Himalayas region of India and provided a brief morphological redescription of the species.

In the past, there have been many attempts to differentiate the adult forms of *L. coneophora* and *L. burmeisteri.* The shape of the scales on *L. lepidophora* were distinct, as mentioned by Veeresh *et al.* (1982). For instance, Veeresh *et al*. (1982) concluded that the adults of these two species differ in life history and are isolated geographically. It was also observed that *L. lepidophora* and *L. burmeisteri* take two years to complete a generation, whereas *L. coneophora* takes only a year (Veeresh *et al*. 1982). However, *L. lepidophora* and *L. burmeisteri* exhibit limited spatial isolation in the Western Ghats. *Leucopholis lepidophora* is mostly found at 100–930 m ASL, *L. burmeisteri* at 300–730 m ASL, whereas *L. coneophora* is limited to the hot, humid coastal areas of elevations lower than 100 m ASL (Veeresh *et al*. 1990). The white grubs are patchily distributed with very rare overlapping of any two species (Prakash *et al*. 2011) (Appendix Figure I). Mahadeva Swamy *et al*. (2019) tried to distinguish the two using the characters of the male genitalia and molecular data. The adult and third instar larva of these white grubs have been previously described (Patil and Veeresh 1981; Veeresh *et al*. 1982; Kumar 1997; Jacob 2010); however, their morphological descriptions alone are inadequate to distinguish species. Recently, the chaetotaxy of third larval instars of the three species exhibited morphological differences, and their ethological differences with respect to flight behavior and congregation patterns were also examined (Pratibha *et al.* 2023).

Furthermore, Burmeister (1855) distinguished *L. coneophora* and *L. burmeisteri* based on three morphometric characters. First, the body length of adult male specimens of *L. burmeisteri* and *L. coneophora* measured 33.9 and 29.3 mm, respectively. Second, the coloration of the elytal scales of *L. burmeisteri* is covered with reddish-brown, pointed scales, whereas the elytra of *L. coneophora* is covered with grayish, pointed, scaly hairs. Brenske (1894) added additional characters such as "*zwischen den grauen kurzen Schuppen auf den Flügeldecken, breiteren weißen Schuppen, besonders an der Naht, auf den schwachen Rippen und der Rückenfläche der Flügeldecken*" [= "*between the gray short scales on the elytra, wider white scales especially at the suture, on the weak costae and dorsal surface of elytra"*] in *L. coneophora*. Third, the length and sculpture of the metaventral process are large and strong in *L. burmeisteri*, whereas it is very short, pointed wart-shaped in *L. coneophora*. However, Veeresh *et al.* (1982) distinguished the three species based on adult body size (*L. lepidophora* is larger and *L. coneophora* is smaller), degree of clypeus sinuation (strongly sinuate in *L. lepidophora* and *L. burmeisteri* compared to *L. coneophora*), the shape of the scales (*L. lepidophora*: elongate, oval to round with acute tips; *L. burmeisteri*: slightly elongate yellowish with acute tips, interspersed with large white scales; *L. coneophora*: minute, brown acute with tips bent), the orientation of the parameres (*L. lepidophora*: closely separated; *L. burmeisteri*: narrowly separated; *L. coneophora*: widely separated), and the presence of epizygum and proplegmatia in the larva of *L. coneophora* and absence in *L. lepidophora* and *L. burmeisteri*.

The larval taxonomy is sparsely known for the majority of the species in the tropics. It is difficult because larvae are less distinctive in their morphology. However, this problem can be overcome with an integrative taxonomy approach (Šípek and Ahrens 2011). In the field, a white grub is usually identified by examining the raster architecture (Jepson 1956). Lefort *et al*. (2013) accurately differentiated the grass grub *Costelytra zealandica* (White, 1846) and the closely related non-economically important pest species *C. brunneum* (Broun, 1880) based on the septula of raster as a key larval morphological character in the field, as larval morphology often provides better information than adult characters for establishing phylogenetic relationships (Mico *et al*. 2008); however, the Central American *Phyllophaga* Harris, 1827 (Coleoptera: Scarabaeidae: Melolonthinae: Melolonthini) of the

rorulenta group could not be distinguished by this manner (King 1984). Therefore, in the current study, we used larval molecular data separate from adult morphological and molecular traits to differentiate *L. burmeisteri* and *L. coneophora.*

ECONOMIC IMPORTANCE

The three species in this study cause significant damage to areca nut (*Areca catechu* L.) and coconut palms (*Cocos nucifera* L.) in southern India (Appendix Figure IIA). The larvae or white grubs of these scarabs feed on and destroy the roots of palms of all age groups (Veeresh *et al*. 1982) (Appendix Figure IIB). *Leucopholis lepidophora* is considered an important pest of areca nut from Karnataka (Puttarudraiah and Channabasavanna 1957) and *L. burmeisteri* in Dakshina Kannada district of Karnataka (Nair and Daniel 1982). On the other hand, *L. coneophora* was considered the only species affecting coconut palms in the coastal areas of Kerala (Nirula *et al*. 1952; Nair and Devasahayam 1982).

Karnataka is the major producer of areca nut in India, followed by Kerala (Mitra and Devi 2018). Although areca nut is grown widely in Karnataka, the areca nut white grubs are ecologically limited to high rainfall habitats (receiving an annual rainfall of more than 2,500 mm) in the Sahyadri mountain range and coastal areas of Karnataka. The pest intensity is higher in paddy fields converted to areca nut plantations (Prakash *et al*. 2011). Of the three larval instars, the third is the most prolonged, highly destructive, and active feeding stage, with a preference for tender roots. Affected palms exhibited symptoms of yellowing of leaves, reduced numbers of inflorescences, reduced number and size of leaves, shortening of internodes, and the reduction in the girth of the stem at the crown region (tapering of the stem). With persistent larval feeding, the roots at the base are completely consumed causing the palms to topple down when disturbed, thus opening up the canopy (Appendix Appendix Figures IIIA–C). Early symptoms are often neglected by the farmers and are noticed only when yield losses are significant (Kumar 1997; Prakash *et al*. 2011).

Accurate species identification and knowledge of their life history are prerequisites for effective pest management (Ahrens *et al*. 2007). Precise identification of field-collected larvae leads to a valid interpretation of data if those are used for ecological or behavioral studies. Hence, reliable characters must be identified for accurate and quick differentiation of the target pests (Lefort *et al*. 2013). Early detection is often crucial in pest management (Britton *et al*. 2010). One of the main limitations in the management of areca nut white grubs is the diversity of species. The long life cycle, differential life history, subterranean habits, and perennial nature of areca palms (availability of continuous supply of food for larvae) make it difficult to study and manage this pest. Management of areca nut white grubs in ecologically fragile ecosystems is not only tricky but also very tedious. Due to inadequate knowledge of the species diversity of white grubs, farmers believe that all species can be managed in the same manner. The absence of adequate information about their life cycle resulted in the adoption of several irrational control methods among farmers (Prakash *et al*. 2011).

Delimitation of species based on only morphological traits fails to clearly differentiate species in instances of closely related taxa, very neoteric radiations, or in taxonomic groups that show extreme morphological similarity. In such cases, the integrative taxonomic approach improves the precision of species delimitation (Wiens 2007). The discovery of new, cryptic species from existing, morphologically indistinguishable groups using DNA is neither controversial nor novel (Knowlton 1993), but rather, this further facilitates taxonomists to resolve the species complex on the basis of morphology, behavior, and other features (Hebert *et al.* 2004). Delineation of species boundaries in recent radiations of closely related species remains a significant challenge (Hey and Machado 2003). Hence, we used an integrative approach to develop suitable identification characters for areca nut white grubs, and these results are presented in this paper.

MATERIALS AND METHODS

Morphological Study

Specimens of third instar larvae and adult beetles were collected from affected areca nut fields. The third instar larvae of *L. lepidophora* and *L. burmeisteri* were collected at less than 15-cm depth from the soil surface by digging around the base of affected palms during August–September, whereas larvae of *L. coneophora* were collected during November–December. All larvae were labeled and divided into two groups. One group of larvae was stored at –20 ˚C in absolute ethanol to extract DNA. The second group of larvae was used for rearing in the laboratory. Adult beetles were also collected from the same locations during their emergence period with the help of flashlights. In addition, beetles were also collected from other locations, where the life cycle of the white grubs was previously known.

A set of morphological characters such as shape, coloration, and sculpture of body; clypeal emargination; punctation on pronotum, elytra, and scutellum; characters of protibia, meso- and metasternal process, and structure

of male genitalia of all the three species were recorded using a LEICA S9D stereo binocular microscope. Body measurements were recorded for 15 randomly selected specimens of each sex and expressed in millimeters (mm).

The methodology for the preparation of male genitalia, eversion of endophallus, and the terminology used for the description of the endophallic structures were those given by D'Hotman and Scholtz (1990a, b) and Zorn (2007).

Principal component analysis (PCA) was performed using R (prcomp with centering and scaling) on 16 morphological characters of 15 females and 15 males of presumptive *L. burmeisteri* and *L. coneophora.* The measurements taken were as follows: LEN (body length), WID (body width), CLY (width of clypeus), PRL (pronotum length), PRW (pronotum width), PYL (pygidium length), PYW (pygidium width), ANL (first antennal segment length), ACL (antennal club length), PSL (prosternal process length), MSL (mesosternal process length), TIL (metatibial length), ISL (inner metatibial spur length), ISW (inner metatibial spur width), OSL (outer metatibial spur length), OSW (outer metatibial spur width), and NUM (number of chitinized spines on endophallus). Besides using PCA the elevation (altitude) of the specimens' location, the number of chitinized spines on the endophallus along with other morphometrics of males was also examined.

Imaging

Photographs of the male genitalia were made using a Leica M205 C stereo-microscope with an attached DFC 425 camera. Images of the habitus of adult beetles were made using a Canon 7D camera with a 100-mm lens. The photos were captured at various depths and stacked using Zerene software. Photographs were appropriately edited using Adobe Photoshop CS6 for publication. The SEM images for scales on elytra and chitinized spines on endophallus were also prepared.

The mitochondrial *cytochrome c oxidase subunit I* (*COI*) was used to test for the reciprocal monophyly of *L. burmeisteri* and *L. coneophora*.

MOLECULAR ANALYSIS

The DNA of adult *L. burmeisteri* and *L. coneophora* were obtained from the legs and processed using a Promega Wizard® genomic DNA purification kit (Promega, Madison, WI), following the developer's protocol. The DNAs were eluted at 100 µL and stored at –20 °C. Amplification of the mitochondrial *COI* was done by mixing 12.5 µL of 2X Taq Master Mix (Vivantis Technologies Sdn Bhd, Malaysia), 0.5 µM of $MgCl_2$, 1 µL each of 10 mM primer pair LCO 1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO 2198 (5′-TAAACTTCAGGGTGACCAAAAAATCA-3′), 8 µL of nuclease-free water, and 2 µL of DNA template. PCR amplification of the Folmer region was done using this PCR program: initial denaturation at 94 °C for 3 min in 5 cycles of denaturation at 94 °C for 30 s, annealing at 45 °C for 30 s, extension at 72 °C for 1 min; 35 cycles of denaturation at 94 °C for 30 s, 51 °C for 1 min, extension at 72 °C for 1 min; and final extension step of 72 °C for 10 min (Becker *et al*. 2021). Amplicons were run under 1.5% agarose gel stained with GelRed® (www.biotium.com) and visualized under 302-nm UV light using AlphaImager® MINI (ProteinSimple, San Jose, CA, USA). Samples with the expected band size were sent for sequencing at Apical Scientific Sdn Bhd (Taman Serdang Perdana, 43300 Seri Kembangan, Selangor, Malaysia). Sequences were pre-processed using BioEdit software version 7.0.5.3 (Hall 1999) and were subjected to the Basic Local Alignment Search Tool (NCBI) to compare with other sequences in the GenBank. Genetic distances from the *COI* region were determined using MEGA X software (Kumar *et al.* 2018).

The voucher specimens examined are deposited in the All-India Network Project on Soil Arthropod Pests, Department of Entomology, University of Agricultural Sciences, G.K.V.K., Bengaluru, India. Digital photographs of some type specimens were requested from other museums, namely:

MLU Martin-Luther-Universität, Halle-Wittenberg, Germany
MNHN Muséum National d'Histoire Naturelle, Paris, France
RBINS Royal Belgian Institute of Natural Sciences, Brussels, Belgium
ZMHB Berlin Museum für Naturkunde Berlin, Germany

RESULTS AND DISCUSSION

***Leucopholis lepidophora* Blanchard, 1851**
(Appendix Appendix Figures IV and V)
Leucopholis lepidophora Blanchard, 1851: 158.

Adult
Type locality. Bombay

Type material examined. Syntype, 1♂, Pontanier, 301-39 (MNHN)

Additional material examined. India: 2♂, 1♀, India, 13.iv.1895, von Oberthur, Ex. Museo Sharp, 1890, Coll. Brenske (RBINS); 1♂, Goalpara (MLU); 1 unsexed, India (MLU); 1 unsexed, Assam (MLU); 1♂, no

locality (MLU); 1 unsexed, no locality (MLU); 5♂, 3♀, Karnataka: Sirsi, Mundagesara, 572 m ASL, 14˚.39'N; 74˚.75'E, 8.vii.2002; 12♂, 9♀, Thirthahalli, Biluve, 650 m ASL, 13˚.48'N; 75˚.24'E, 13.viii.2015; 5♂, 7♀, Sringeri, Kalige, 672 m ASL, 13˚.41'N; 75˚.25'E, 1.viii.2016; 5♂, 2♀, Thirthahalli, Kannangi, 660 m ASL, 13˚.47'N; 75˚.21'E, 16.viii.2018; 2♂, Sringeri, Gandagatta, 703 m ASL, 13˚.42'N; 75˚.21'E, 2.viii.2016;

Distribution. India: Mumbai (earlier Bombay), Karnataka, Uttarakhand, Assam.

Description, males. Body length 32.40 ± 1.76 mm, width 22.21 ± 1.29 mm (n = 15). Body monochromatic to dichromatic, black, blackish brown to black reddish-brown (Appendix Figure IVA); body covered with relatively large, nearly uniform, elongate ovoid or lanceolate, apically rounded or tapered, off white, yellowish to brownish adpressed, sparsely spaced scales; head and pronotum covered mostly with moderate-sized spindle ovoid to lanceolate ovoid scales; elytron mostly covered with nearly uniform size ovoid or lanceolate scales with mesal depression; scales nearly uniform in size, with rounded base (Appendix Figure IVB); abdomen covered mostly with large, ovoid or lanceolate scales; densely spaced on both sides, sparsely spaced mesally; metasternum covered mostly with narrow lanceolate scales and yellowish setae; each scale attached to a minute, rounded puncture; dorsal surface with large, rounded punctures except below scutellum minutely punctate; metacoxal scales nearly the same scale as abdominal scales.

Head. Clypeus width 2.35 ± 0.19 mm (n = 15), distinctly narrow anteriorly, nearly as wide as funicle apex, convex mesally, surface deeply punctate on both sides, less punctate mesally; dorsal anterior margin broadly emarginate, rounded, concave mesally; both sides convex; lateral margin slightly wedge-shaped; anterior and antero-lateral margins bent upwards, inclined backward at approximately 45º, with median cleft laterally; frons rugosely punctate anteriorly, deeply and thickly punctate posteriorly; frontal suture arcuate, slightly concave mesally; vertex with sparse, large punctures; lamellae length 2.2 mm, nearly same length as funicle.

Pronotum. Length 9.43 ± 0.43 mm, width 15.44 ± 0.65 mm (n = 15), anterior margin deeply concave, rounded mesally, margin smooth; anterior angle obtuse, blunt apically; lateral margin distinctly convex mesally, crenulate, carinate; posterior angle angulate, nearly at 90º, blunt apically; posterior margin deeply concave, rounded mesally, margin smooth.

Scutellum. Anterior margin length 3.5–3.6 mm, rounded; posterior margin deeply concave, rounded apically; surface with large, moderate, and small-sized ovoid to elongate ovoid scales.

Elytron. Humeral margin slightly wedge-shaped mesally toward anterior angle; anterior angle rounded; lateral margin slightly convex mesally, upper lateral margin narrowly explanate; upper margin thickly carinate, disappearing toward posterior angle; posterior angle widely rounded; posterior margin explanate; sutural angle angulate, obtuse; sutural margin carinate near sutural angle and mesally, disappearing toward scutellum; surface with four indistinct costae – namely costae I, II, and III mesally and IV adjacent lateral margin.

Legs. Foretibiae tridentate, teeth rounded apically, posterior tooth prominent, base distinctly wider; surface of coxae and tibiae covered with elongate, lanceolate scales; inner margin of meso- and metatibiae with rows of long, stiff brownish setae; covered with narrowly to broadly lanceolate, apically tapered scales; posterior metatibiae with 19–24 spicules; small and large metatibial spurs, narrow, tapered apically, either straight or curve.

Ventral side of thorax. Prosternal process anterior margin isosceles triangle-shaped, rounded apically; posterior margin equilateral triangle-shaped, rounded apically. Metaventral process length 2.2 mm, equilateral or isosceles triangle-shaped, wide mesally, rounded apically; prolonged, little above procoxae, sub-apically attached to prosternal process (Appendix Figure IVC). Meteepimera anterior angle distinctly acute.

Abdominal ventrites. Uniformly covered with moderate-sized ovoid scales except mesal, with sparsely covered with scales or with fewer scales and with impunctate portion; abdominal margin II slightly convex, III and IV distinctly convex.

Pygidium. Length 7.31 ± 0.49 mm, width 10.10 ± 0.70 mm (n = 15). Broad anteriorly and arched posteriorly, 1.3 times wider than long; uniformly covered with small, narrow, spindle-shaped scales; with few, 2–3 rows of lanceolate scales and very few stiff setae along apical margin.

Male genitalia. Parameres apical process fist-like, margin rounded to nearly flat or straight mesally; lateral margins broad, nearly parallel; posterior margin of parameres inverted funnel-shaped, rounded protuberance, margin distinctly wedge-shaped toward lateral margin; parameres and phallobase combined length 6.8–7.2 mm (Appendix Figure IVD).

Female. Body length 33.85 ± 1.00 mm, width 22.54 ± 0.95 mm (n = 15). Clypeal width 2.48 ± 0.14 mm (n = 15). Lamellae length 1.7–1.8 mm. Pronotal length 9.68 ± 0.22 mm, width 15.62 ± 0.33 mm (n = 15). Anterior margin of scutellum 3.8–4.0 mm. Pygidium length 7.63 ± 0.34 mm, width 10.10 ± 0.43 mm (n = 15). Posterior metatibiae with 33–41 spicules. Small and large metatibial spurs broad, spatulate apically; either straight or curve.

DIFFERENTIAL DIAGNOSIS

A digital habitus photo of a male syntype of *L. lepidophora* was provided by Dr. Olivier Montreuil from MNHN (Appendix Figure VA). Body scales of *L. lepidophora are* distinctly large and less numerous, whereas they are mostly minute and less numerous in *L. coneophora.* The approximate count of the number of elytral scales at 500 µm using a scanning electron microscope (SEM) was estimated at 10 x 9 or 90 for *L. lepidophora* (Appendix Appendix Figures VB and C). The scales of *L. lepidophora* are adpressed to the integument, its surface is smooth, and its lanceolate-shaped scales are apically blunt, whereas in *L. coneophora* is upright and slanted to the integument, its surface is coarse, and its lanceolate-shaped scales are apically tapered and acicular-shaped (Appendix Appendix Figures VB and C). Furthermore, the shape and arrangement of scales of *L. burmeisteri* and *L. coneophora* resembled each other.

Distinct sexual dimorphism was observed. Females are larger than males. Lamellae are 1.3 times longer in males compared to females. The anterior margin of the scutellum is much wider in females compared to males. Pygidium anterior margin of females is 0.05 times much broader in length than in males. Small (ventral) and large (dorsal) metatibial spurs are broad and spatulate apically in females, whereas it is narrow and apically tapered or pointed in males. Both spurs are either straight or curved. Females with more metatibial spicules than males.

Leucopholis lepidophora specimens reflected and appeared gray when the white scales blended with the black integument. Missing scales and covered with dirt change the look to the original color of the integument. However, specimens appeared reddish or brownish when the brown scales blended with black or red integument. The geographical and ecological distribution of areca nut white grubs indicated that *L. lepidophora* was found at the relatively higher elevation of 572–703 m ASL, *L. coneophora* seemed to occur on a much wider range of elevations of 120–654 m ASL, whereas *L. burmeisteri* can be found at 300 to 730 m ASL. Recently, Kumar and Pandey (2024) added the northern region of Uttarakhand, India, as a new distribution record of *L. lepidophora*.

Leucopholis coneophora Burmeister, 1855

(Appendix Appendix Figures VI, VII, VIII, IX, X, and XI)

Leucopholis coneophora Burmeister, 1855: 303.

Adult

Type locality. "Indien."

Type material examined. Syntype**,** 1♂, *coneophora* Nob. (MLU).

Additional material examined. India: 1♂, 9852, M.R. Belg.; 1350, Coll. J. Thomson (RBINS); 5♂, 7♀, Karnataka: Dakshina Kannada, Mantrady, 80 m ASL, 13˚.10'N; 75˚.10'E, 12.vi.2012, same location [3♂, 4.vi.2013]; 10♂, 5♀, Dakshina Kannada, Iruvail, 64 m ASL, 13˚N; 75˚E, 3-vi-2013; 10♂, 5♀, Kerala: (CPCRI); Kasaragod, 10.7 m ASL, 12˚.53'N; 75˚E, 10.vi.2015. **Bangladesh:** 1♂, Assam, Sylhet, Coll. Brenske, ZMHB.

Distribution. India: Karnataka, Kerala and Assam; Bangladesh: Sylhet.

Description, males. Body length 31.73 ± 1.86 mm, width 20.20 ± 1.30 mm (n = 15). Body dichromatic, head black, pronotum black, sometimes reddish brown, elytra reddish-brown (Appendix Figure VIA); body mostly covered with distinctly minute, densely spaced, lanceolate, apically tapered to acicular-like, off-white, brownish to yellowish scales; surface of clypeus with distinctly large, elongate, lanceolate scales; vertex and pronotum covered with distinctly narrower, lanceolate scales; surface with minute round punctures; scales distinctly wider than base of scales; elytron covered with numerous distinctly minute, densely spaced, lanceolate, wider basally, tapered apically, off white scales and very few distinctly large apically tapered, off white scales; abdominal scales tapered apically (Appendix Figure VIB); metasternum covered with long, numerous yellowish setae, especially on both sides and less mesally.

Head. Clypeus width 1.88 ± 0.18 mm (n = 15), distinctly narrow anteriorly, nearly as wide as funicle apex, convex mesally; dorsal anterior margin broadly emarginate, rounded, concave mesally; both sides convex; lateral margin slightly wedge-shaped (Appendix Appendix Figures VIA, VIIA, and VIIIA); anterior and antero-lateral margins bent upwards, inclined backward at approximately 45º, with mesal cleft laterally; deeply and finely punctate anteriorly, deeply, thickly punctate posteriorly; frontal suture arcuate; frons deeply, thickly punctate; vertex deeply, closely punctate; labium deeply notched; lamellae length 2.8–3.5 mm, distinctly longer than funicle.

Pronotum. Length 8.70 ± 0.41 mm (n = 15), width 14.39 ± 0.70 mm (n = 15), with anterior margin distinctly concave, slightly raised mesally; margin smooth; anterior angle obtuse, blunt apically; lateral margin distinctly convex mesally, crenulate, carinate; posterior angle angulate, obtuse, blunt apically; posterior margin distinctly concave mesally, rounded mesally, smooth.

Scutellum. Anterior margin length 3.0 mm, rounded; posterior margin deeply concave, rounded apically; surface finely punctate, covered with same scales as vertex and pronotum.

Elytron. Anterior angle rounded; lateral margin slightly convex mesally, upper to mesal margin narrowly explanate; upper and mesal margin thickly carinate, narrowing toward posterior angle; posterior angle widely rounded; posterior margin explanate; sutural angle angulate, obtuse; sutural margin carinate near sutural angle and mesally, less prominent toward scutellum; with four distinct to indistinct costae, costae I, II, and III mesally and IV adjacent lateral margin; costae mostly not covered with scales.

Legs. Foretibiae tridentate, first two anterior teeth distinctly large, widely rounded, apically; posterior tooth less prominent, base distinctly narrower; surface of coxae and tibiae covered with more elongate, larger, lanceolate scales; inner margin of meso and metatibiae with rows of long, stiff yellowish to brownish setae; posterior metatibiae with 13–20 spicules; small and large metatibial spurs, narrow, tapered apically, either straight or curve.

Ventral side of thorax. Prosternal process anterior margin equilateral triangle-shaped, rounded apically; posterior margin rounded. Metaventral process length 2.0 mm, isosceles triangle-shaped, narrow mesally, rounded apically; prolonged, little above procoxae, sub-apically attached to prosternal process (Appendix Figure VIC). Meteepimera anterior angle nearly at a right angle.

Abdominal ventrites. Uniformly covered with minute-sized lanceolate, apically tapered scales except mesal, with fewer scales and impunctate portion; posterior abdominal margin II slightly convex, III and IV distinctly convex.

Pygidium. Length 6.59 ± 0.58 mm, width 10.17 ± 0.42 mm (n = 15). Broad anteriorly and arched posteriorly, 1.4–1.5 times wider than long; uniformly covered with lanceolate scales, tapered to acicular apically and with very few, long, stiff setae along the apical margin.

Male genitalia. Posterior margin of parameres bell-shaped, mesal margin rounded, protuberance rounded, margin distinctly wedge-shaped toward the lateral margin; apical process knob-like, margin widely rounded mesally. Parameres and phallobase combined length 5.5–7.3 mm (Appendix Figure VID).

Female. Body length 33.13 ± 2.53 mm, width 21.06 ± 1.81 mm (n = 15). Clypeal width 2.04 ± 0.26 mm (n = 15). Lamellae length 1.6–1.9 mm. Pronotal length 8.95 ± 0.81 mm, width 15.23 ± 1.11 mm (n = 15). Anterior margin of scutellum 3.8–4.0 mm. Pygidium length 6.97 ± 0.68 mm, width 10.46 ± 0.92 mm (n = 15). Posterior metatibiae with 21–36 spicules. Small and large metatibial spurs broad but spatulate apically; either straight or curved.

DIFFERENTIAL DIAGNOSIS

A digital photo of a male syntype of *Leucopholis coneophora* was provided by Dr. Hendrik Mueller of MLU, Halle, Germany (Appendix Appendix Figures VIIA and B). It perfectly matched the specimens collected in India (Appendix Appendix Figures VIA–D).

There are also the habitus and male genitalia digital photos of the non-type male *Leucopholis coneophora* specimen examined by Brenske (1894) and deposited in the collection of J. Thomson (now in RBINS) provided by Dr. Alain Drumont and Ms. Julien Lalanne of RBINS, Brussels, Belgium (Appendix Appendix Figures VIIIA–C). In addition, digital photos of the habitus of another non-type *Leucopholis coneophora* specimen – including the lateral view photo of the male genitalia – were provided by Dr. Bernd Jaeger from ZMHB (Appendix Appendix Figures XA–C). The specimen reflected and appeared yellowish when the white scales blended with the red integument. Missing scales and covered with dirt and change the appearance from the original red to brownish red color of the integument. However, specimens appeared brownish when the brown scales blended with red integument. The approximate count of the number of elytral scales at 500 µm using an SEM was estimated at 40 x 32 or 1,280 for *L. coneophora* (Appendix Appendix Figures XIA and B).

The posterior tooth of *L. lepidophora* is much more prominent and its base is distinctly wider while it is less prominent and its base distinctly narrower in *L. coneophora*. The prosternal process of *L. lepidophora* is isosceles triangle-shaped, whereas it is equilateral triangle-shaped in *L. coneophora.* However, the metaventral process of *L. lepidophora* is equilateral or isosceles triangle-shaped and is wider mesally and rounded apically, whereas it is isosceles triangle-shaped, narrow mesally, and rounded apically in *L. coneophora.* The anterior angle of metepimera of *L. lepidophora* is distinctly acute while it's nearly at 90º angle in *L. coneophora* and *L. burmeisteri*. The lamellae of male *L. lepidophora* are nearly the same length as the funicle, whereas it is distinctly longer than the funicle in male *L. coneophora*.

***Leucopholis burmeisteri* Brenske, 1894**
(Appendix Appendix Figures XII and XIII)

Leucopholis lepidophora Blanchard: sensu Burmeister, 1855: 302 (specimens from Mangalore) (misidentification)

Leucopholis burmeisteri Brenske, 1894: 30

Type locality. Mangalore, India

Type material examined. None. Type material was not located.

Additional material examined. India: Karnataka: 10♂, 3♀, Chikkamagalur, Begane, 672 m ASL, 13˚.11'N; 76˚.63'E, 4-vi-2009; 1♂, Dakshina Kannada, Markanja, 123 m ASL, 12˚.56'N; 75˚.51'E, 9-vi-2010; same location 2♂, 1♀, 10-vi-2011; 5♂, 4♀, 28-v-2012; 2♂, 3♀, Dakshina Kannada, Balnadpet, 125 m ASL, 12˚.30'N; 75˚.30'E, 10-vi-2010; 10♂, 5♀, Thirthahalli, Hulagar, 654 m ASL, 13˚.36'N; 75˚.16'E, 12-vi-2020; 2♂, 1♀, Shivamogga, Hosanagara, 569 m ASL, 13˚.55'N; 75˚.04'E, 20-ix-2016; 5♂, 1♀, Thirthahalli, Shuntikatte, 650 m ASL, 13˚.41'N; 75˚.14'E, 30-v-2018; 10♂, 5♀, Thirthahalli, Araga, 656 m ASL, 13˚.45'N; 75˚.11'E, 15-vi-2020.

Distribution. Mangalore, India.

Description, males. Body length: 33.02 ± 1.63 mm, width: 20.40 ± 0.71 mm. Body elongate ovoid, slightly convex; dichromatic, head and pronotum black, elytra light to dark reddish-brown (Appendix Figure XIIA); body mostly covered with distinctly minute, densely spaced, lanceolate, apically tapered to acicular-like, off-white, brownish to yellowish scales; surface of clypeus with distinctly large, elongate, lanceolate scales; vertex and pronotum covered with distinctly narrower, lanceolate scales; surface with distinctly minute, point size, round punctures; scales distinctly narrower than base of scales; elytron covered with numerous distinctly minute, densely spaced, lanceolate, triangulate-shaped base, tapered apically, off white scales and very few distinctly large apically tapered, off white scales; abdominal scales blunt apically; metasternum covered with long, numerous yellowish setae especially on both sides and less mesally (Appendix Figure XIIB).

Head. Clypeal width 1.90 ± 0.16 mm. Clypeus anterior and antero-lateral margins bent upwards, broadly emarginate; deeply and finely punctate anteriorly, deeply and thickly punctate posteriorly; frontal suture arcuate; frons deeply and thickly punctate; vertex deeply and closely punctate; labium deeply notched; terminal maxillary palpomere; antennal club 1.7 times longer in males compared to females, the first segment with fringes of hair ventrally, scattered hair on other segments; lamellae length mm, distinctly longer than funicle.

Pronotum. Length 8.89 ± 0.41 mm width 14.75 ± 0.56 mm (n = 15); with anterior margin distinctly concave, slightly raised medially; margin distinctly raised toward the anterior angle and thickly margined, emarginate medially; anterior angle obtuse, rounded; lateral margin distinctly crenulate; posterior angle obtuse, rounded; posterior margin distinctly concave medially, emarginated medially; surface covered mostly with minute, ovoid, elongate, lanceolate, yellowish white scales, tapered apically.

Scutellum. Anterior margin length 3.5 mm, rounded; posterior margin moderately concave, rounded apically; surface finely punctate, covered with same scales as elytra.

Elytron. Anterior angle rounded; lateral margin slightly convex mesally, thinly explanate near anterior angle, disappearing mesally; mesal margin and before posterior angle not explanate; apical margin widely rounded, widely explanate toward sutural angle; sutural angle angulate, nearly at a right angle; sutural margin carinate near sutural angle, disappearing mesally toward scutellum; surface with faint costae, with four distinct to indistinct costae, costae I, II, and III mesally and IV adjacent lateral margin; costae mostly not covered with scales.

Legs. Foretibiae tridentate, first two anterior teeth distinctly large, widely rounded, apically; posterior tooth distinctly small, blunt apically. Metatibiae with 14–20 spicules.

Ventral side of thorax. Prosternal process widely triangulate, rounded apically. Metaventral process moderately prolonged, not reaching procoxae; flat laterally; widely rounded apically; metaventral process length 2.0 mm (Appendix Figure XIIC). Each side of mesosterna densely covered with fine, elongate setae and scales; sparsely covered with short yellowish-white setae and scales mesally. Metepimera anterior angle nearly at a right angle.

Abdominal ventrites. Posterior abdominal margin II slightly convex, III–IV distinctly convex; covered mostly with basally ovoid spindle-shaped, apically rounded scales.

Pygidium. Length 7.16 ± 0.39 mm, width 10.71 ± 0.38 mm (n = 15). Broad anteriorly and arched posteriorly, 1.4–1.5 times wider than long, bristly along margins, covered with pointed scales, deeply and finely punctate, slightly bent upwards laterally.

Sternum. Prosternal process triangular, sparsely covered with scales and relatively long hairs; metaventral process short and smooth.

Male genitalia. Basal piece 5.5–7.3 mm long, concave dorsally and convex ventrally, paramere 5.1–6.4 mm long; paramere darker than basal piece, symmetrical, both arms separated, concave in lateral view, connected basally by a membranous region and ventrally connected by the extensions of apically pointed arms, apex rounded with a carina on lateral surface; spiculum gastrale Y-shaped with arms approximated medially forming a stem and fused in basal half, arms as long as stem, and connected by a membranous region (Appendix Figure XIID).

Endophallus. Endophallus is like a tubular sac with two elongated, longitudinal sclerotized areas on each side ventrally. Sclerotized areas densely packed with stout, relatively short, heavily sclerotized, black spines directed

toward the base, spines more prominent on basal sclerite. Apical sclerite either connected to basal sclerite by a chain of small spines directed cephalad. Dorsal surface with a number of pointed and unevenly distributed chitinized spines of variable lengths. The number of chitinized spines varied from 2–74. Surface of sac covered with minute, thorn-like projections pointing cephalad.

Female. Body length 35.30 ± 1.20 mm, width 23.05 ± 1.15 mm. (n = 15). Clypeus width: 2.14 ± 0.15 mm. Pronotum length 9.43 ± 0.31 mm, width: 15.89 ± 0.74 mm (n = 15). Anterior margin of scutellum length 4.0 mm. Pygidium length: 7.59 ± 0.53 mm, width: 11.61 ± 0.86 mm (n = 15). Metatibial spurs broad. Posterior metatibiae with 24–31 spicules.

DIFFERENTIAL DIAGNOSIS

The lateral margins of the parameres of *L. coneophora* has a subapical constriction, whereas *L. burmeisteri* has none. Also, the pronotal scales are distinctly thin and different from elytral scales in *L. burmeisteri*, whereas they are nearly the same in *L. coneophora*. Unfortunately, the type specimen of *L. burmeisteri* has not been found even with numerous requests to different European museums, including MLU.

Additionally, the diagnostic character used by the earlier authors to identify these species such as the distribution of chitinized spines on the endophallus of *L. burmeisteri* with a single row of short chitinized spines running from ventral anterior to the mid-region, whereas in *L. coneophora*, the endophallus has a mass of short chitinized spines in the ventral mid anterior region was found to be highly variable in our study. In the current study, examination of the endophallus of *L. burmeisteri* revealed that the dorsal surface was decorated with a number of pointed and unevenly distributed chitinized spines of variable lengths, and the number of spines varied from 2–74. In the case of *L. coneophora* the dorsal surface of endophallus is arranged with a similar type of chitinized spines and the number of spines varied from 5–91.

However, the approximate count of the number of elytral scales at 500 μm using an SEM was estimated at 31 x 18 or 558 for *L. burmeisteri* (Appendix Appendix Figures XIIIA and B). A summary of the morphological character differences among the three species of *Leucopholis* is presented (Appendix Table I).

Key to the Males of *Leucopholis* from Southern India

1. Parameres with lateral margins nearly parallel, apical processes fist-like; metepimera anterior angle distinctly acute; prosternal process narrow isosceles triangle-shaped, with narrow triangle-shaped base, rounded apex; metaventral process mound-shaped, with wider base, rounded apex; body scales nearly uniform in size; distinctly large, elongate ovoid, rounded to spindle-shaped, apically rounded or tapered, adpressed to integument scales; lamellae nearly same length as funicle; blackish to dark brown (Appendix Appendix Figures IVA–D and VB and C). …… *Leucopholis lepidophora* Blanchard

   Parameres with lateral margins not parallel, apical processes not fist-like; metepimera anterior angle nearly at right angle; prosternal process lanceolate-shaped, with narrow base; metaventral process mound-shaped, with narrow base, rounded apex; body scales with numerous distinctly minute and very few distinctly large spindle-shaped, tapered apically, distinctly appressed to integument scales; lamellae distinctly longer than funicle; brownish or reddish brown. ……..…... 2

2. Parameres with lateral margins with subapical constriction; pronotal scales the same as elytral scales (Appendix Appendix Figures VIA–D, IXB, XC, and XIA and B) …… *Leucopholis coneophora* Burmeister

   Parameres with lateral margins without subapical constriction; pronotal scales distinctly thin, different from elytral scales (Appendix Appendix Figures XIIA–D and XIIIA and B) …..... .....*Leucopholis burmeisteri* Brenske

PCA Analysis

PCA analysis showed that 54 and 17% of the variability by the first two principal components. There is considerable overlap between the two presumptive species in the projected morphological measurement space and the only distinction arises between males and females, principally accounted for the differences in length of the antennal club (Appendix Figure XIV).

When only male specimens were examined by the altitude of the location, the number of spines on the endophallus along with other morphometrics using PCA, the first two PCs account for 64.5 and 19.3% variability, and we noticed two clusters that separated chiefly based on altitude, and the number of spines declined with elevation. We found that the presumptive *L. burmeisteri* specimens from Markanja fell well within the morphospace defined by our presumptive *L. coneophora* (Appendix Figure XV).

***COI*-based Analysis of Three *Leucopholis* Species**

Our presumptive *Leucopholis* species – namely *L. burmeisteri* and *L. coneophora* – revealed a 96.02%

similarity with *L. burmeisteri* MZ414222.1 and 97.72–98.18% similarity with *L. coneophora* MK121983 (Appendix Table II). The genetic distances were also compared between our specimens and voucher specimens from GenBank that show close nucleotide similarity without samples (Appendix Table III). On average, the following distances between *L. burmeisteri–L. coneophora*, *L. burmeisteri–L. lepidophora*, and *L. coneophora–L. lepidophora* are 0.0343 ± 0.0054, 0.1471 ± 0.0165, and 0.154 ± 0.017, respectively (Appendix Table IV). Within species, the estimated distance in *L. burmeisteri*, *L. coneophora*, and *L. lepidophora* are 0.04 ± 0.01, 0.02, and 0.03 ± 0.01.

Mitochondrial DNA (mtDNA) Analysis

The mtDNA can clarify genetic variation and species limits in different insect species (Sperling *et al*. 1993; Langor and Sperling 1995; Sperling and Hickey 1995; Armstrong *et al*. 1997). A pair of adult cane larva species – *Lepidiota frenchi* Blackburn, 1912 and *L. negatoria* Blackburn, 1912 (Coleoptera: Scarabaeidae: Melolonthinae: Leucopholini) – are morphologically identical than being compared to *L. noxia* Britton, 1985. However, the molecular diagnostic technique using mtDNA revealed that *L. frenchi* and *L. noxia* are more closely related to one another than being compared to *L. negatoria* (Miller *et al*. 1999). Molecular approach with traditional taxonomy successfully identified the larva of *Costelytra zealandica* (White) (Coleoptera: Scarabaeidae: Melolonthinae: Liparetini) and *C. brunneum* (Broun) (Lefort *et al*. 2013) (Coleoptera: Scarabaeidae: Melolonthinae: Liparetini). Pentinsaari *et al*. (2014) also utilized the *COI* region in more than 1800 beetle species and revealed > 2% interspecific divergence in more than 95% of species tested. In a study involving ground beetles from Central Europe (Raupach *et al*. 2010), at least 3.6% interspecific divergence from the *COI* sequences has been observed. In our study, the 3.43–15.4% average genetic distance among the three *Leucopholis* species indicates that the congeners belong to distinct species. However, within the pool of the *Leucopholis burmeisteri* individuals, a 4% divergence could signal a species complex present and should warrant more in-depth biological, morphological, ecological, and molecular investigations to confirm this assumption.

CONCLUSION

Although a number of morphological as well as molecular characters were investigated in the present study, *L. coneophora* and *L. burmeisteri* can only be distinguished with certainty based on two morphological characters. The parameres of the male genitalia lateral margins with subapical constriction on *L. coneophora*, whereas there is none in *L. burmeisteri*. Also, the pronotal scales and the elytral scales are the same in terms of size and dimension in *L. coneophora*, whereas the pronotal scales are distinctly thin and different from the elytral scales of *L. burmeisteri.*

Although our study on the *Leucopholis* species complex remains incomplete, the strategy taken here contributes a framework that will direct research emphasis on the fine-scale study of their niches that might help to resolve this issue. The *COI* genetic distances among the three beetle species suggest that they belong to distinct species. Moreover, our results also imply that *L. burmeisteri* and *L. coneophora* represent ecotypes that may require examination of type specimens for confirmation and treating both as a single species. At this point, we do not propose synonymization as both species differ in their life histories and possibly occupy different ecological zones. However, we emphasize the need for further detailed studies to develop precise identification techniques to distinguish these two ecotypes.

The present study also underlines the need for more understanding of *L. burmeisteri* and *L. coneophora*, which are ecologically isolated and biologically distinct but harvest similar kinds of resources in the high-rainfall areas of south India. The re-description of adult and larvae of *L. burmeisteri*, *L. coneophora*, and *L. lepidophora* presented here represents an important addition to the knowledge of the systematics of areca nut white grubs of southern India.

ACKNOWLEDGMENTS

We would like to convey our deepest gratitude and esteemed appreciation to the following individuals and institutions who contributed to the completion of this collaborative project between India and the Philippines. Special gratitude to the Indian Council of Agricultural Research's All-India Network Project on Soil Arthropod Pests. Sincere thanks to Dr. C.A. Viraktamath (Emeritus Professor; Department of Entomology, University of Agricultural Sciences, G.K.V.K., Bengaluru, India) for insightful suggestions. Special thanks to P. Nirmala for the support rendered during the study. To Reynaldo V. Ebora (Executive Director; DOST-PCAARRD Los Baños, Laguna) and the Accelerated Science and Technology Human Resource Development Program for supporting Dr. Orlando A. Calcetas's Philippine June beetle MS thesis and other off-shoot projects, including several genera of scarabs in the country. To the leadership and support of Fidel L. Libao (Regional Executive Director, DA-RFO-IVA-CALABARZON) and especially to the hardworking staff of the Regional Crop Protection Center IV-A headed by Agricultural

Center Chief Pamela F. Marasigan for the numerous support and assistance in the conduct of the study. We are grateful to Dr. Olivier Montreuil (MNHN, Paris, France) for the syntype specimen of *L. lepidophora* Blanchard, Dr. Hendrik Mueller (Martin Luther University, Halle, Germany) for the syntype specimen of *L. coneophora* Burmeister, Dr. Alain Drumont and Dr. Julien Lalanne (Royal Belgian Institute of Natural Sciences, Brussels, Belgium) for the non-type specimen of *L. coneophora* Burmeister examined by Brenske, and Dr. Bernd Jaeger (Museum für Naturkunde Berlin, Berlin, Germany) for the non-type specimen of *L. coneophora* Burmeister. To Dr. Charles Staines (Smithsonian Environmental Research Center, Maryland, United States) for proofreading the manuscript. To Dr. Ales Bezděk (Czech Republic) for his expertise in ensuring the accuracy and quality of the paper to meet the highest standard possible and providing some literature. Special thanks also to all anonymous reviewers of this paper.

REFERENCES

AHRENS D, ZORN C, YUBAK DHOJ GC, KELLER S, NAGEL P. 2007. Illustrated key of phytophagous scarabs of Nepal – a guide to white grubs and chafers of the lower central regions (Coleoptera, Scarabaeidae). Opuscula Biogeographica Basileensia 5: 1–44.

ALLSOPP P. 2022. Australian Melolonthini (Coleoptera: Scarabaeidae: Melolonthinae): reclassification of eight species to *Antitrogus* Burmeister, 1855 and *Barryfilius* new genus. Zootaxa 5213(5): 513–545.

ARMSTRONG KF, CAMERON CM, FRAMPTON ER. 1997. Fruit fly (Diptera: Tephritidae) species identification: a rapid molecular diagnostic technique for quarantine application. Bulletin of Entomological Research 87: 111–118.

BARLOW E. 1899. List of the Melolonthini contained in the collection of the Indian Museum. Indian Museum Notes 4: 236–273.

BECKER M, KÖNIG S, HOPPE B. 2021. A simple PCR-based approach for rapid detection of *Ips typographus* and *Ips duplicatus* in the presence of (associated) symbionts and parasites. Journal of Plant Diseases and Protection 128: 527–534.

BEZDĚK A. 2016. Tribe Leucopholini Burmeister, 1855. In: Catalogue of Palaearctic Coleoptera, 3; Revised and Updated Edition. Löbl I, Löbl D eds. Brill, Leiden. p. 224–226.

BLANCHARD CE. 1851. Ordre des Coléoptères. In: Museum d'Histoire Naturelle de Paris: catalogue de la collection entomologique; classe des insectes. Milne-Edwards H, Blanchard CE, Lucas H eds. Tome 1, deuxième livraison. Paris: Gide & Baudry. p. 129–240.

BRENSKE E. 1894. Die Melolonthiden der palaearctischen und orientalischen region im koniglichen naturhistorischen meusum zu Brussel. Mémoires de la Société Entomologique de Belgique, Vol. 2. 187p.

BRITTON EB. 1978. A revision of the Australian chafers (Coleoptera: Scarabaeidae: Melolonthinae), Vol. 2; Tribe Melolonthini. Australian Journal of Zoology Supplementary Series 60: 1–150.

BRITTON EB. 1985. *Lepidiota noxia* sp.n. (Coleoptera: Scarabaeidae: Melolonthinae), a pest of sugarcane in Queensland. Journal of the Australian Entomological Society 24(2): 117–119.

BRITTON KO, WHITE P, KRAMER A, HUDLER G. 2010. A new approach to stopping the spread of invasive insects and pathogens: early detection and rapid response *via* a global network of sentinel plantings. New Zealand Journal of Forestry Science 40: 109–114.

BURMEISTER H. 1855. Handbuch der entomologie; vierter band, zweite abtheilung. Coleoptera Lamellicornia Phyllophaga Chaenochela. T.C.F. Berlin: Enslin. x+ 570p.

CALCETAS OA. 2019. Review of the genus *Carlschoenherria* (Coleoptera: Scarabaeidae: Melolonthinae) of the Philippines. Zootaxa 4619(2): 330–346.

CALCETAS OA. 2023. Three new species of the genus *Leucopholis* Dejean, 1833 (Coleoptera, Scarabaeidae, Melolonthinae, Leucopholini) from the Philippines and designation of a neotype for *L. semperi* Brenske, 1896. European Journal of Taxonomy 890: 184–203.

CALCETAS OA, ADORADA JR. 2017. Taxonomic review of the genus *Leucopholis* Dejean, 1833 (Coleoptera: Scarabaeidae: Melolonthinae: Leucopholini) in the Philippines. Zootaxa 4232(1): 085–103.

DEJEAN PFMA. 1833. Catalogue des Coléoptères de la collection de M. le Comte Dejean [Livraisons 1-2]. Paris: Méquignon-Marvis. 176p.

D'HOTMAN D, SCHOLTZ CH. 1990a. Comparative morphology of the male genitala of derived groups of Scarabaeoidea (Coleoptera). Elytron 4: 3–39.

D'HOTMAN D, SCHOLTZ CH. 1990b. Phylogenetic significance of the structure of the external male genitalia in the Scarabaeoidea (Coleoptera). Entomology Memoir 77: 1–51.

HALL TA. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41: 95–98.

HEBERT PDN, STOECKLE MY, ZEMLAK TS, FRANCIS CM. 2004. Identification of birds through DNA barcodes. PLOS Biology 2: 1657–1663.

HEY J, MACHADO CA. 2003. The study of structured populations—new hope for a difficult and divided science. Nature Reviews Genetics 4: 535–543.

JACOB J. 2010. Taxonomic revision of *Holotrichia* Hope and *Leucopholis* Dejean (Scarabaeidae: Melolonthinae) of Karnataka [M.Sc. thesis]. UAS, GKVK, Bangalore, India. 107p.

JEPSON WF. 1956. The biology and control of the sugarcane chafer beetles in Tanganyika. Bulletin of Entomological Research 47: 377–397.

KING ABS. 1984. Biology and identification of white grubs (*Phyllophaga*) of economic importance in Central America. Tropical Pest Management 30(1): 36–50.

KNOWLTON N. 1993. Sibling species in the sea. Trends in Ecology and Evolution 24: 189–216.

KUMAR ARV. 1997. Bio-ecology and management of arecanut white grubs *Leucopholis* spp. (Coleoptera: Scarabaeidae) in Karnataka [Ph.D. thesis]. UAS, GKVK, Bangalore, India. 218p.

KUMAR M, PANDEY AK. 2024. First Record of *Leucopholis lepidophora* Blanchard, 1850 (Scarabaeidae; Melolonthinae) from western Himalayas along with a checklist of the genus *Leucopholis* from India. Journal of Entomological Research Society 26(1): 129–136.

KUMAR S, STECHER G, LI M, KNYAZ C, TAMURA K. 2018. MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. Molecular Biology and Evolution 35: 1547–1549.

LANGOR DW, SPERLING FAH. 1995. Mitochondrial DNA variation and identification of bark weevils in the *Pissodes strobi* species group in western Canada (Coleoptera: Curculionidae). Canadian Entomologist 127: 895–911.

LEFORT MC, BARRATT BIP, MARRIS JWM, BOYER S. 2013. Combining molecular and morphological approaches to differentiate the pest *Costelytra zealandica* (White) (Coleoptera: Scarabaeidae: Melolonthinae) from the non-pest *Costelytra brunneum* (Broun) at the larval stage. New Zealand Entomologist 36(1): 15–21.

MAHADEVA SWAMY HM, RAMASAMY A, KALLESHWARASWAMY CM, ADARSHA SK. 2019. Arecanut white grubs *Leucopholis* species (Melolonthinae: Scarabaeidae: Coleoptera) morphological, molecular identification, and phylogenetic analysis. Journal of Asia-Pacific Entomology 22: 880–888.

MICO E, MORON MA, SIPEK P, GALANTE E. 2008. Larval morphology enhances phylogenetic reconstruction in Cetoniidae (Coleoptera: Scarabaeoidea) and allows the interpretation of the evolution of larval feeding habits. Systematic Entomology 33: 128–144.

MILLER LJ, ALLSOPP PG, GRAHAM GC, YEATES DK. 1999. Identification of morphologically similar canegrubs (Coleoptera: Scarabaeidae: Melolonthini) using a molecular diagnostic technique. Australian Journal of Entomology 38: 189–196.

MITRA SK, DEVI H. 2018. Arecanut in India-present situation and future prospects. Acta Horticulturae 1205: 789–794.

NAIR CPR, DANIEL M. 1982. Pests of arecanut. In: The arecanut palm (*Areca catechu* Linn.). Bavappa KVA, Nair MK, Premkumar T eds. CPCRI, Kasaragod, India. p. 162–184.

NAIR CPR, DEVASAHAYAM S. 1982. Pests of coconut and their control. Indian Cocoa Arecanut Spices Journal 6: 35–36.

NIRULA KK, ANTHONY J, MENON KPV. 1952. A new pest of coconut palm in India. Indian Coconut Journal 5: 57–70.

PATIL SP, VEERESH GK. 1981. Description of larvae (*Leucopholis burmeisteri* and *L. lepidophora* (Scarabaeidae: Coleoptera). In: Progress in soil biology and ecology in India. Veeresh GK ed. UAS Technical Series No. 37. p. 172–178.

PENTINSAARI M, HEBERT PD, MUTANEN M. 2014. Barcoding beetles: a regional survey of 1872 species reveals high identification success and unusually deep interspecific divergences. PLOS One 9(9): 108–651.

PRAKASH KV, KUMAR ARV, RAVIKUMAR B. 2011. Pest management practices of areca nut farmers against *Leucopholis* spp. (Coleoptera: Scarabaeidae) in Karnataka. In: Insect pest management, a current scenario. Ambrose DP ed. Entomology Research Unit, St. Xavier's College, Palayamkottai, India. p. 558–563.

PRATHIBHA PS, RAJESH MK, SABANA AA, SUBAHARAN K, VENUGOPAL V, JILU VS. 2023. Distinguishing palm white grub complex, *Leucopholis* spp. (Coleoptera: Scarabaeidae: Melolonthinae) from India using high-resolution melting (HRM) analyses. International Journal of Tropical Insect Science. p. 1–12.

PUTTARUDRAIAH M, CHANNABASAVANNA GP. 1957. Some new insects and mites on areca palm in Mysore-II. Indian Arecanut Journal 7(1): 9–10.

RAUPACH MJ, ASTRIN JJ, HANNIG K, PETERS MK, STOECKLE MY, WÄGELE JW. 2010. Molecular species identification of Central European ground beetles (Coleoptera: Carabidae) using nuclear rDNA expansion segments and DNA barcodes. Frontiers in Zoology 7(1): 1–15.

SIPEK P, AHRENS D. 2011. Inferring larval taxonomy and morphology in *Maladera* species (Coleoptera: Scarabaeidae: Sericini) using DNA taxonomy tools. Systematic Entomology 36: 628–643.

SMITH ABT. 2006. A review of the family-group names for the superfamily Scarabaeoidea (Coleoptera) with corrections to nomenclature and a current classification. Coleopterists Society Monographs 5: 144–204.

SPERLING FAH, ANDERSON GS, HICKEY DA. 1993. A DNA-based approach to the identification of insect species used for postmortem interval estimation. Journal of Forensic Sciences 39: 418–427.

SPERLING FAH, HICKEY DA. 1995. Amplified mitochondrial DNA as a diagnostic marker for species of conifer-feeding Choristoneura (Lepidoptera: Tortricidae). Canadian Entomologist 127: 277–288.

VEERESH GK, KUMAR ARV, ALI TMM. 1990. Biogeography of pest species of white grubs of Karnataka. In: Advances in management and conservation of soil fauna. Veeresh GK, Rajagopal D, Viraktamath CA eds. New Delhi: Oxford and IBH Publishing Co. p. 191–198.

VEERESH GK, VIJAYENDRA M, REDDY NVM, RAJANNA C. 1982. Bioecology and management of areca white grubs (*Leucopholis* spp.) (Coleoptera: Scarabaeidae: Melolonthinae). Journal of Soil Biology and Ecology 2(2): 78–86.

WIENS JJ. 2007. Species delimitation: new approaches for discovering diversity. Systematic Biology 56: 875–878.

ZORN C. 2007. Taxonomic revision of the *Anomala cuprascens*–species group of Sulawesi and the Papuan region: the species with unidentate protibiae (*A. chlorotica*–subgroup) (Coleoptera: Scarabaeidae: Rutelinae). Arthropod Systematics Phylogeny 65(1): 25–71.

APPENDICES

Table I. Summary of the major morphological character differences of *L. lepidophora*, *L. coneophora*, and *L. burmeisteri*.

Species	Morphological characters				
	Body scales		Pronotal and elytral scales	Length of lamellae to funicle	Lateral margin of parameres (male genitalia)
Leucopholis lepidophora	• Distinctly large • Elongate ovoid, rounded to spindle-shaped	• Adpressed to the integument	—	• Lamellae nearly the same length as funicle	—
Leucopholis coneophora	• Distinctly minute -Spindle-shaped	• Appressed to the integument	• Nearly the same	• Lamellae distinctly longer than funicle	• With subapical constriction
Leucopholis burmeisteri	• Distinctly minute -Spindle-shaped	• Appressed to the integument	• Pronotal scales distinctly thin, different from elytral scales	• Lamellae distinctly longer than funicle	• Without subapical constriction

Table II. BLASTn results from the *COI* nucleotide sequences of presumptive *Leucopholis burmeisteri* and *L. coneophora* specimens from India using the GenBank database from NCBI.

	Specimen	Nucleotide length (bp)	Top hit/origin	Query cover (%)	E-value	Nucleotide similarity (%)	GenBank accession no.	Reference
Leucopholis burmeisteri	Lb1F	680	*L. burmeisteri* / India	100%	0.0	96.02%	MZ414222.1	Shivaji *et al.* (2021)
	Lb2F	680	*L. burmeisteri* / India	100%	0.0	96.02%	MZ414222.1	Shivaji *et al.* (2021)
	Lb3F	680	*L. burmeisteri* / India	100%	0.0	96.02%	MZ414222.1	Shivaji *et al.* (2021)
Leucopholis coneophora	Lc10M	680	*L. coneophora* / India	96%	0.0	98.18%	MK121983.1	Mahadeva Swamy *et al.* (2018)
	Lc12M	680	*L. coneophora* / India	96%	0.0	97.72%	MK121983.1	Mahadeva Swamy *et al.* (2018)

Table III. Estimated genetic distance between our *Leucopholis* samples and voucher specimens obtained from the GenBank.

	Specimen	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	**Lb1F OQ891241.1**	–															
2	**Lb2F OQ891242.1**	0.00															
3	**Lb3F OQ891243.1**	0.00	0.00														
4	**Lc10M OQ891244.1**	0.06	0.06	0.06													
5	**Lc12M OQ891245.1**	0.05	0.05	0.05	0.03												
6	***Leucopholis burmeisteri* MZ414222.1**	0.05	0.05	0.05	0.03	0.03											
7	***Leucopholis burmeisteri* JN126323.1**	0.06	0.06	0.06	0.02	0.03	0.03										
8	***Leucopholis burmeisteri* KX009511.1**	0.05	0.05	0.05	0.03	0.02	0.03	0.02									

Table III. Cont.

	Specimen	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
9	***Leucopholis burmeisteri* MH712759.1**	0.06	0.06	0.06	0.02	0.03	0.03	0.00	0.02								
10	***Leucopholis burmeisteri* MG717658.1**	0.05	0.05	0.05	0.03	0.02	0.03	0.02	0.02	0.02							
11	***Leucopholis burmeisteri* KX009512.1**	0.06	0.06	0.06	0.02	0.03	0.03	0.00	0.03	0.00	0.02						
12	***Leucopholis coneophora* MK121983.1**	0.05	0.05	0.05	0.02	0.02	0.03	0.02	0.02	0.02	0.02	0.02					
13	***Leucopholis coneophora* MG717664.1**	0.06	0.06	0.06	0.01	0.03	0.03	0.01	0.02	0.01	0.02	0.02	0.02				
14	***Leucopholis coneophora* MG717668.1**	0.06	0.06	0.06	0.01	0.03	0.03	0.02	0.03	0.02	0.03	0.02	0.02	0.00			
15	***Leucopholis lepidophora* KX351390.1**	0.15	0.15	0.15	0.15	0.15	0.14	0.14	0.15	0.14	0.13	0.14	0.15	0.15	0.15		
16	***Leucopholis lepidophora* MG717671.1**	0.16	0.16	0.16	0.16	0.15	0.15	0.14	0.15	0.14	0.15	0.14	0.15	0.15	0.16	0.03	

Table IV. Genetic distance estimates between groups of *Leucopholis* species. Above the diagonal line are standard error estimates.

	Specimen	1	2	3
1	***L. burmeisteri***	–	0.0054	0.0165
2	***L. coneophora***	0.0343	–	0.017
3	***L. lepidophora***	0.1471	0.154	–