

Termite Colonies' Distribution by Morphologically, at Different Ecological Zone via Bar Coding Patterns

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Abstracts

In summer, termites are collected for Identify and assess colony distribution. Collections are direct from a visible gallery or use a trap with ethanol. Soldier for pattern recognition and DNA extraction. Morphological identification according to existing literature. Species differences were assessed by measuring 20 characters/index and statistics. Based on these features, we generated a keyword and distribution map of the genus Termites. Research area. This is the first record of *Odontotermesasmuthi* and *Odontotermesobesus* of the three Regions, the first record of *Odontotermes parvidens* in the Buner and Swabi regions, and the first Records of *Odontotermeshorai* of Haripur. We then used the barcode of mtDNA COII to Validation of species assignment and phylogenetic analysis of colonies using Neighbor-Joining maximum likelihood analysis.

Introduction

Termites (Isoptera) are divided into seven families and is known for its social behavior. They are social insect. They lived in the colonies as workers, soldiers and reproductive castes. There are 80% to 90%. Termite workers in ant colony foraging Gallery Activities and Construction and clean. Soldiers play key role in identification Species and Colony Defense. They can't eat and fed by workers via trophallaxis, which is also a source of communication. Third One is the reproductive caste (king, queen, primary and secondary reproduction) are responsible for reproduction. Subterranean termites, wet wood termites, and dry wood termites are types of termites that differ from each other based on changes in characteristics including nesting behavior and ecology naturally, termites spread over vast lands Especially in tropical environments, as the main invertebrate decomposers though Some subtropical species are of low ecological importance, but their economic importance cannot If they are damaged in an urban environment, they are ignored. In the tropics, termites are the main pest in agriculture, forestry and housing Some species contribute to ecosystem processes, carbon and nitrogen cycles, but they are well known Because of its economic importance In the case of subterranean termites, moist structures are the source of providing shelter. There are 2650 known species in the world, of which Only 300 species are considered pests the same only 3% of species are reported to be responsible.

Damage to buildings and crops. Calculate percentage Crop losses in India, i.e. 15-25% of the maize crop. A total of \$35.12 million per year. Although In Southern Africa, 03-100% crop losses have been reported, and the actual annual economic losses are unknown. Similarly, Brazil is 42.7%, China is 80-90%, 53.2% and 70% of residential, 20% industrial and 10% commercial buildings in Spain have been damaged Reporting for Malaysia. Economic loss 248.68- \$292.79, 8-10, 95.24, 1000, 313 and 800 million China, Malaysia, Australia, USA, Europe and Japan Considering the diverse ecological regions, geological History, diverse latitudinal distribution, high altitude

Scope of Pakistan, here is evidence of wealth biodiversity in the area. Termite diversity in Pakistan fails to get Considerable attraction to surveys since 1972. Certain termite species are good for forests Recycle dead wood material, but some are destructive, as reported there Damage to structures/buildings, crops and forest plantations, excluding Swabi, Buner, Haripur districts in Khyber Pakhtunkhwa (KP) province. There is a long list of management practices to control termites, which have several environmental concerns.

The type of habitat attacked may also contribute to Control Strategy. Due to existence Termites, farmers, foresters and the public has an unknown annual loss without any available comprehensive knowledge Types of species present, host food and habitat. Therefore, this study aims to report the diversity, genus intensity, percentage of termite species Hosts and habitats attacked in each region 2016-19 for subsequent use in management Policy makers' approach Morphology is an important part of termite taxonomy, but it has several limitations, especially in the case of phenotypic trait variants that lead to misidentification when taxa include unknown diversity.

Familiar experts and skilled technicians Species morphology is often required to ensure correct identification based on morphology alone. In contrast, molecular methods do not require specialized knowledge, making them a promising tool for already improving insect systematics. Specifically, DNA barcoding is currently helping to delineate and assign unidentified specimens species, explore mysterious species, discover new species. To improve our Learn about the species identity, abundance and diversity of the burnell tooth termite, Swabi and Haripur, we employed an integrative identification approach combining morphometric and DNA barcoding of the genus to generate a distribution map area.

Material and Method

Survey of the research area

During Survey different Research Areas Buner, Swabi and Haripur districts selected from agro ecological zones in the northern mountains (Malakand); central valley plains (Peshawar) and the eastern wet mountains, Hazara region and a random survey from March to November 2016-2019. From the forest (standing trees, fallen logs, shrubs and grass), crops, and houses/structures, based on dividing the area into unit cells (5 x 5 km) for each district. Collect from visible gallery site by following or installing modified NIFA Termaps with carton paper and stored in vials containing ethanol for morphometry (80%) and DNA extraction (99%). Use a GPS device (Garmin eTrex 10.0) to record sample point coordinates, and Record forage substrates at all locations.



1 = material used; 2 = termites feeding on acacia plants stem; 3 = field collection of termites from fallen tree trunks; 4 = On-site termite collection by breaking galleries on standing trees; 5 = On-site mud breaking Collection; 6 = Field collection of termites from animal droppings; 7 = Carton used as an improved NIFA Terminal map; 8 = culture isolated from installed NIFA Termap; 9 = soldier collected from collection for storage.

Morphometric Identification

Morphometric identification of soldiers using existing literature keys, illustrations, pictures, characters and indexes. Binocular microscope for measurements are recorded in millimeters (mm), with built-in magnification, the unit is calculated along with other statistical measures. Zoom in with high resolution Observe the record for a total of 20 characters/index (maximum length is Left mandible from base, length of left mandible from base, length of tooth to root tip.

The tip, the length from the head to the base of the mandible, the largest. Long head with lower jaw, smallest head width, maximum head width, front back panel width, front back panel length, back panel maximum width, rear width, rear minimum length, rear length, full length, length Labral width, labral width, head index (width/length), mandibular head index (length Mandible/Head Length), Head Convergence Index (Min Head Width/Max Width head), tooth index (distance from tooth to tip/length of mandible). Representative specimens are Stored in the Insect Museum of the Department of Entomology, Agricultural University, Peshawar, Pakistan.

Distribution and Mapping

The coordinates recorded for the sampling points were projected by a GPS device (eTrex 10) Use ArcGIS 10.0 (ESRI, 10.4 Environmental Systems Research Institute: Redlands, CA, USA, 2010) to color the map green (new location records) and assign different the type of shape for readability on the map.

Molecular Analysis

DNA Extraction and Amplification

By taking a piece (or whole) (washed with distilled water and air Dried) single representative termite termites (200 μ L) extracted from the identified specimens Chelex (10%, w/v) of chelex-100 in ddH₂O in a 0.5 mL Eppendorf tube. The organization is Disintegrate with the end of a pipette tip. Vortex tubes and place at 99.9°C 15 min (using a PCR machine) and centrifugation for 2-3 min to induce phase separation. The extracted 2 μ L of upper DNA was used for polymerase chain reaction (PCR).

PCR was used to amplify the COII gene fragment of approximately 658 bp with primer A-tLeuCAGATAAGTGCATTGGATTT (forward); B-tLys GTTTAAGAGACCAGTACTTG (reverse) Prime by adding 2 μ L of sample DNA to 23 μ L of master mix. PCR reaction is Initial denaturation treatment at 94°C for 5 min, followed by 35 cycles of 10 s at 94°C, 50°C for 20 seconds, 72°C for 45 seconds and 72°C for 7 minutes, and store at 4°C. The PCR product is Check by 2% agarose gel electrophoresis. For washing, use 2 μ L of ExoSAP-IT™. Add to 5 μ L PCR product (stored on ice), incubate at 37°C for 15 minutes, and incubate at 37°C for 15 minutes 80°C.

DNA sequencing and phylogenetic analysis

Add a total of 5 μ L of sterile distilled water, then 15 μ L of each sample. Add to two separate Mix2Seq tubes. Add a total of 2 μ L of forward primer to aAdd 2 μ L of reverse primer to another tube and send the sample to Eurofins (Eurofins, Denmark) was used for Sanger sequencing. The resulting mtCOII sequence is Trimmed to ~658 bp. Beginning and ending fragments of some sequences was removed to avoid interference and to obtain comparable sequence lengths. to check Nucleotide sequence similarity, based only on the top 50 GenBank sequences (BLASTn) % query coverage, % identity, bit coverage and related class matches are considered for understudied species, we have included and curated over 50 sequences from NCBI Reference sequences (refseq).

In addition, to ensure that the top matching results of BLASTn are Upload the correct species/taxa to GenBank, with the top matching sequences for each sequence Align with reference sequence. Only top 10 GenBank matches per species was chosen for adjacency and maximum likelihood tree construction, repeated were removed and sequences were aligned using Clustal W in MEGA 6.0. this A total of twenty sequences were generated (accession numbers: MZ713169.1; MZ713170.1; MN913606.1; MH557841.1; MH557840.1; KY238293.1; KY224665.1; KY224596.1; KY224551.1; KY224493.1; KY224429.1; KY224409.1; KY224406.1; KP864045.1; KP864044.1; JQ518439.1; DQ442207.1; AB300694.1; AB095521.1; AB051877.1) Termites. (acrossOdontotermes and Hypotermes from GenBank. Submit the resulting new sequence to GenBank)

Data deposition

Representative specimens have been deposited in the Entomological Museum, Department of Entomology, Agricultural University, Peshawar, Pakistan. COII sequences are stored in GenBank.

Result and Discussion

Key to Soldier Caste of Termites in Khyber Study Area KPK Pakistan.

Identifying Characters of the Soldier Caste

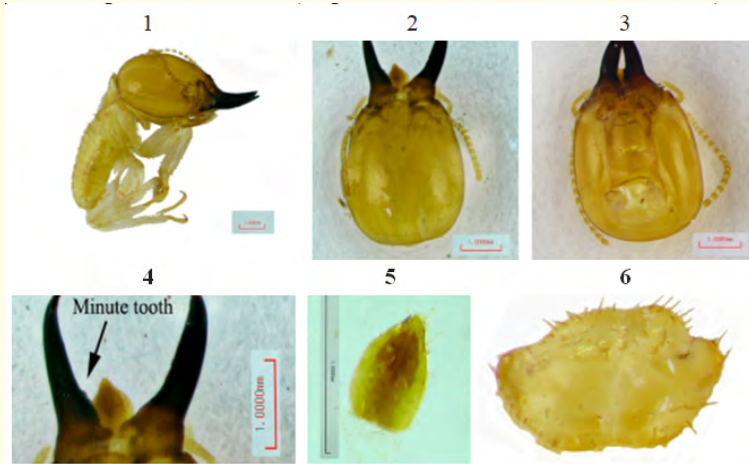
Odontotermesparvidens have Head color varies from pale yellow to dark reddish-brown Has creamy to yellow straw tentacles and body. The lower jaw is brown and yellowish The bottom is brown. Sparse head, moderate body hair, overall length Body at 5.30 to 7.45 mm. The head is large, sub-rectangular oval, merged Anterior (1.89 to 2.70 in length to base of mandible; 1.45 to 2.15 mm in maximum width).

Antenna There are 16 to 17 segments; in a 16-segment antenna, segment 3 is not equal to section 2 or slightly longer and subdivided, while out of 17 segmented antennas, section 3 Shorter than the 2nd, less than or equal to the 4th and 5th, or less. The labrum is triangular Tongue-shaped, pointed. Jaw strong, half or slightly longer Head Length (Length 1.19 to 1.38 mm; Mandible Index Length/Head Length 0.50–0.60) and it's a little recessed at the tip. A tiny lateral tooth at the base of the left mandible Middle third (0.70 to 0.90 mm pitch from tip; index pitch/mandible length 0.58 to 0.65). With or without dentition near the right mandible according to. Postmentum is sub-rectangular (length 1.15 to 1.70 mm; maximum width 0.62 to 0.90 mm). The front back panel is saddle-shaped with a slight notch in the front edge and a rear edge Noticeably notch (length 0.60 to 0.93; width 1.05 to 1.65 mm).

Odontotermesobesus

Capsule is pale yellow to chestnut brown with pale Yellow to yellow-brown antennae with darker ends. The lower jaw is light brown To dark reddish-brown; body pale yellow to pale brown. Weak head, quite body hairy. Overall length is 4.0 to 6.0 mm. The head capsule is oval, slightly curved Anterior (length to base of mandible 1.03 to 1.67; maximum width 0.95 to 1.37 mm; index Width - Length 0.82 to 0.95 and convergence index, which is the width/maximum of the base of the mandible. width 0.62 to 0.70).

Antennas have 16 or 17 sections, with section 2 less than or equal to section 3 and the 4th position among the 16 segment antennas, and the 3rd position is the shortest among the 17 segment antennas. labrum is Tongue-shaped, the front end is approximately round. The mandible is long and thin, Sword Length 0.75 to 1.03 mm; Mandibular Index Length/Head Length 0.59 to 1.03 mm 0.68). Sharp and clear teeth on the distal end of the left mandible (the distance from the teeth to the tip) 0.25 to 0.38; index pitch/mandibular length 0.31 to 0.40). right mandible The small teeth are slightly below the level of the left mandibular teeth. Postmentum is sub-rectangular (Length 0.70 to 0.93; Width 0.50 to 0.58 mm). The front back panel is saddle-shaped, and the front leaf is saddle-shaped Semi-circular with a slight to huge notch in the front and a slight rear Edge to distinct notch (length 0.50 to 0.65; width 0.80 to 1.07 mm).



Various identifying characteristics of the *O. parvidens* soldier's caste. (1 = side view Soldier, 2 = dorsal view of soldier's head, 3 = posterior teeth, 4 = dorsal view of mandible, 5 =Dorsal view of labrum, 6 = dorsal view of anterior dorsal plate).Tooth termites.



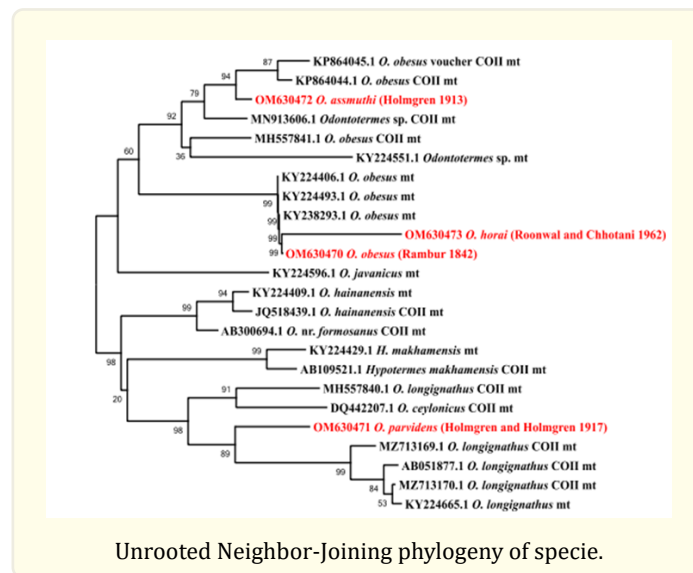
Identify the characteristics of the *O. obesus* soldier caste. (1). side view of soldier, (2). Dorsal view of soldier, (3). Back of the head, (4). soldier's back (5 = width; II = length), (6). Mandible (I and IV), teeth (II), lips (III) and labia (V), (6). dorsal view Pronotum.

Sequences Alignment and Similarity Validation

According to and other researchers, genetic diversity can range from 0.0 to The difference between individuals of the same species is 0.51%, while the expected difference between different species may be greater than 3%. Although no strict standards are set For species identification by sequencing, it is reasonable to consider that new sequences matching <98% of *O. parvidens*, *O. assmuthi* and *O. horai* do not match 100% Use sequences from BLASTn searches or refseq, except *O. obesus* (for alignments, See Figures S1-S9. Therefore, verifying these three sequences as new sequences, which is also Backed by NJ and ML trees.

Neighbor-Joining Method Tree

Using Neighbor-Joining (NJ) to infer the phylogenetic position of specimens method. The optimal tree with sum of branch lengths = 0.33977302 is shown. This the confidence probability that the inner branch length is greater than 0 (multiplied by 100), As estimated using bootstrap tests (500 replicates are shown next to the branch. The tree is drawn to scale and the unit of branch length (next to the branch) is the same as Evolutionary distance for inferring phylogenetic trees. Evolution Distances are calculated using the Kimura 2-parameter method in units of the number of base substitutions at each site. The analysis involved 24 nucleotide sequences. Included codon positions are 1st + 2nd + 3rd + noncoding. All positions include Blank and missing data were eliminated. A total of 506 places in the final data set. Evolutionary analysis was performed in MEGA 6.0. phylogenetic tree of The top 10 sequences from BLASTn were successfully searched for the dentate termite sequences.



Conclusion

O. parvidens

Morphologically, it was identified as *O. parvidens* But it matched *O. longignathus* in BLASTn searches due to lack of its sequence and high similarity. Its sequences are lacking in GenBank and Morphology the variants reported in both species descriptions support that *O. parvidens* should be effective species

O. assmuthi

Morphologically, it was identified as *O. assmuthi*, but in the absence of its sequence, It matched *O. obesus* in a BLASTn search. From a morphological point of view, there are change in head color, shape, length, hair in head area, body color, labrum jaw shape hair, stylus and tooth size, which are discussed in detail in both Description of species. Due to the lack of its sequence data and morphology Variation in two species, determining that *O. assmuthi* should be a valid species Several sexual dimorphisms have been documented in different characters, although Gender dimorphism is reported primarily in workers and a few characters in the image, But it was also observed in the soldiers in this study. Several Variation Studies Morphological characteristics within and between colonies of *O. obesus*, details of its synonyms and effective species and additional information from previous authors, Forest 2022, 13, 674 14 of 17.

Details can be found problem of three different clades formed by *O. obesus* in NJ and ML phylogeny, including KY224493.1, KY224406.1 and KY238293.1 is likely the correct taxon match to *O. obesus*, supported by morphometric and sequence alignments.

The other two clades, accession no. MH557841.1 and KP864044.1; KP864045.1, morphologically similar (though no morphometric information about soldier caste), so species allocation is based on the closest sequence match when they were deposited into GenBank. At that time, there were Very few sequences are available in GenBank, so they are most likely incorrect names, indicates the existence of a mysterious diversity in the genus, which is currently unresolved.

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