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Morphometrics, Distribution, and DNA Barcoding: An Integrative Identification Approach to the Genus *Odontotermes* (Termitidae: Blattodea) of Khyber Pakhtunkhwa, Pakistan

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Simple Summary: Termites are social insects that invade plantations, agricultural crops, and man-made structures throughout the world. The districts Buner, Haripur, and Swabi belong to different agro-ecological zones and a neglected area of Khyber Pakhtunkhwa (Pakistan: Oriental region) for work on the distribution and identity of termites. Termites were collected and identified morphologically and with DNA barcoding of the COII region. *Odontotermes assmuthi*, *Odontotermes obesus*, *Odontotermes parvidens*, and *Odontotermes horai* were identified, and a key to the genus *Odontotermes* of the area was made along with a distribution map. The identified species were found feeding on different forage substrates. Four novel COII sequences were submitted to GenBank.

Abstract: The neglected area of Khyber Pakhtunkhwa (Pakistan: Oriental region), consisting of Buner, Haripur, and Swabi districts, were surveyed for termites during the summer of 2016–2019 for identification and assessment of the distribution of colonies. Collections were made either directly from visible galleries or using traps with ethanol. Soldiers were used for morphometric identification and DNA extraction. Morphometric identification was carried out based on the available literature through measurements of 20 characters/indices and evaluating species differences statistically. Based on these characteristics, we generated a key and a distribution map of the genus *Odontotermes* for the study area. This is the first record of *Odontotermes assmuthi* and *Odontotermes obesus* in these three districts, the first record of *Odontotermes parvidens* for the Buner and Swabi districts, and the first record of *Odontotermes horai* for Haripur. We subsequently used barcoding of the mtDNA COII to verify species assignments of colonies and for phylogenetic analyses using Neighbor-Joining and Maximum Likelihood analyses.

Keywords: fungus-farming termites; Termitidae; morphometrics; distribution; COII barcoding; macrotermitinae; habitat; Pakistan; oriental region

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Citation: Zaman, M.; Khan, I.A.; Schmidt, S.; Murphy, R.; Poulsen, M. Morphometrics, Distribution, and DNA Barcoding: An Integrative Identification Approach to the Genus *Odontotermes* (Termitidae: Blattodea) of Khyber Pakhtunkhwa, Pakistan. *Forests* **2022**, *13*, 674. https://doi.org/10.3390/f13050674

Academic Editor: Bong-Kyu Byun

Received: 12 March 2022
Accepted: 21 April 2022
Published: 27 April 2022

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1. Introduction

Termites are social insects that belong to seven families (order Blattodea) [1,2] and they display division of labor between workers, soldiers, and reproductive castes within colonies [3]. There are 3106 known termite species, of which 363 are of significant pest status [4]. Termites contribute to various ecosystem engineering processes [5], including the breakdown and recycling of organic matter, removal of dung, soil loosening, formation, and fertility, greenhouse gas emission, pollination, and feed for wildlife, livestock, and humans, but they are also associated with huge losses caused to forestry, agriculture, and housing [6]. Worldwide, termite losses of 1.5, 11, 1, 0.5, 0.5, and 0.8–1 billion US

dollars' worth per annum have been reported for Australia, USA, France, Indonesia, Thailand, and Japan, respectively [7]. In forest plantations, older trees are at a higher risk of attack, but damages are also caused to seedling and saplings in the form of ring and root debarking [8], and range lands [9]. The extent of damage to standing trees in forests is poorly understood [10]. Percentage crop loss estimates in India amount to 15–25% of maize crop, with a total of 35.1 million US dollars per annum, while in southern Africa, 3–100% crop losses have been reported with unknown annual economic losses. Damages of 70%, 20%, and 10% have been reported in residential, industrial, and commercial buildings, respectively, in Malaysia [11]. In Pakistani agro-eco-systems, four pest genera of termites, including *Odontotermes* (Macrotermitinae: Termitidae), are responsible for damage to agricultural crops, fruits trees, ornamental plants, and grasses. Specifically, losses have been reported to be 90–100% for sugarcane, 2–43.5% for tobacco, 8–12% for wheat, and 1–13% to ground nuts [12]. Timber (6 to 8 months old *Acacia arabica* and *Pinus roxburghii*) in structures/buildings are severely damaged by termites, but relative resistance has been recorded for more than 100-year-old *Cedrus deodara* trees [12].

Considering the geological history, diverse latitude, and high-altitude ranges, Pakistan is a diverse ecological region for fauna and flora. Recently, [13] studied termites in Pakistan, focusing on some of the regions of Khyber Pakhtunkhwa (KP) (previously known as NWFP), and reported economic losses in some of the regions of the province [12]. However, the area with the districts Buner, Swabi, and Haripur (oriental region) unexplored. Iqbal et al. [14] reported that 11 out of 53 species are causing damages in Pakistan by species primarily belonging to the families Rhinotermitidae and Termitidae. Termitidae of the oriental region is well studied by [15], and the genus *Odontotermes* is one of the most abundant and diverse genera, responsible for forest, agricultural, and structural damage in various agro-ecological zones [12]. However, the abundance and diversity of the genus are unknown in Buner, Swabi, and Haripur.

Morphology is an important part of termite taxonomy, but it has several limitations, specifically in case of variation in phenotypic traits that leads to misidentification [16,17], and when taxa include unknown diversity [18]. Experts and skilled technicians familiar with species morphology are often needed to secure correct identification based on morphology alone [19]. In contrast, molecular methods do not require specialist knowledge, making them promising tools that have already improved insect systematics [20,21]. Specifically, DNA barcoding is currently helping to delineate and allocate unidentified specimens to species, to explore cryptic species, and to discover new species [22]. To improve our understanding of the species identity, abundance, and diversity of *Odontotermes* in Buner, Swabi, and Haripur, we took an integrative identification approach, combining morphometrics and DNA barcoding of the genus to generate a map of distributions across the region.

2. Materials and Methods

2.1. Survey of the Studied Areas

The districts Buner, Swabi, and Haripur were selected (Figure 1) from the agro-ecological zones of the Northern Mountainous (Malakand) Zone; Central Valley plains (Peshawar), and Eastern Wet Mountainous, Hazara Zone, respectively of the Khyber Pakhtunkhwa [23,24], and randomly surveyed from March to November, during 2016–2019 [25]. Sampling was performed from forests (standing trees, fallen logs, shrubs, and grasses), agricultural crops, and houses/structures, based on dividing the area into cell unit (10 × 10 km) for each district. Collection was made either on the spot from the visible galleries by following [26] or installation of modified NIFA Termaps having carton paper [27] and stored in vials containing ethanol for morphometrics (80%) and DNA extraction (99%). Sampling sites co-ordinates were recorded by using a GPS device (Garmin eTrex 10.0), and forage substrate was recorded (Figure 1C) for all sites.

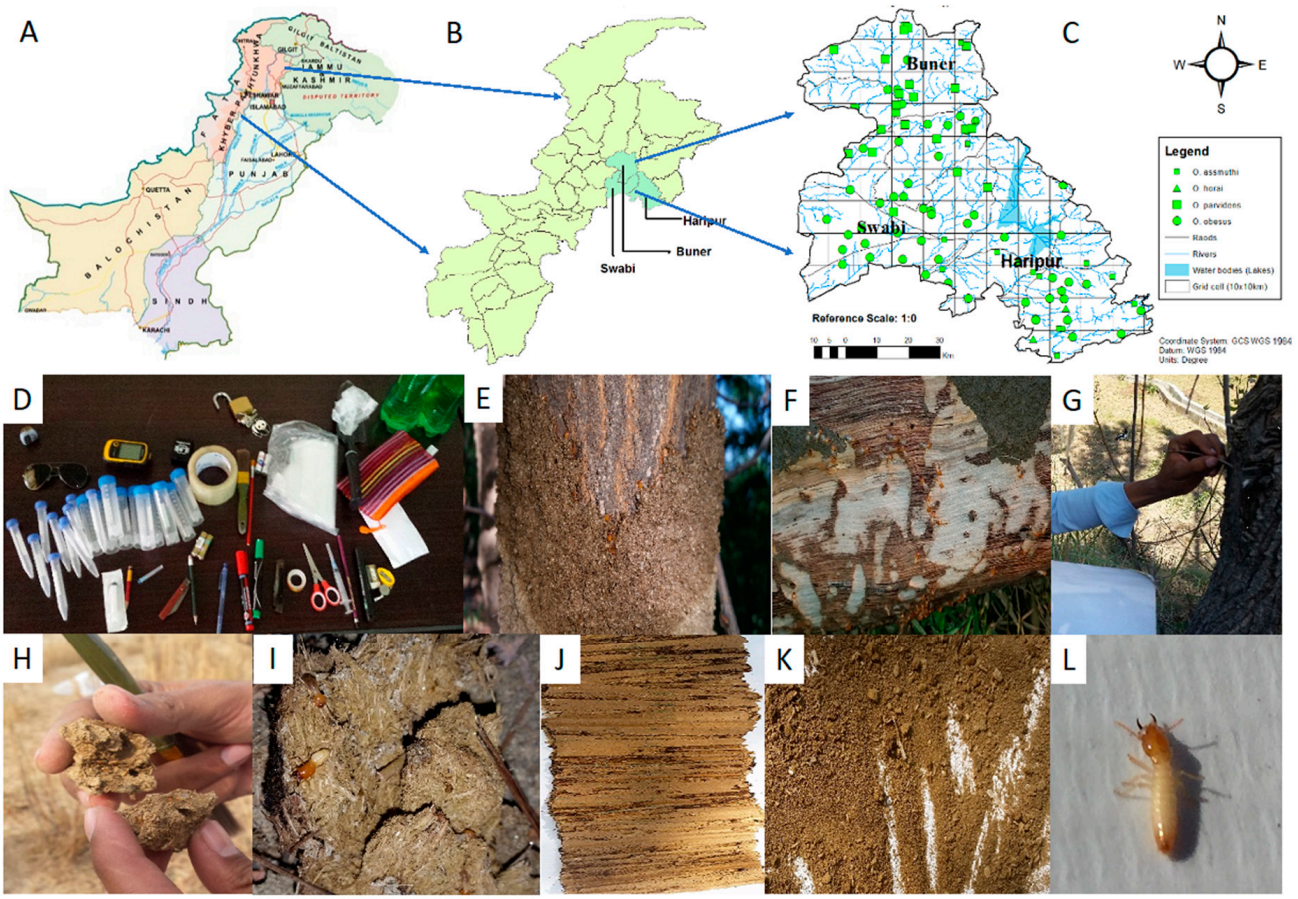


Figure 1. Collection of termites from the studied area during 2016–19. (**A** = Map of Pakistan*; **B** = Map of Khyber Pakhtunkhwa; **C** = Collection map of Genus *Odontotermes*; **D** = Material used; **E** = Termites feeding on *Acacia* spp. stem; **F** = On spot termites collection from fallen tree trunk; **G** = On spot termites collection by breaking galleries on standing tree; **H** = Breaking mud for on spot collection; **I** = On spot termites collection from animal dung; **J** = Carton used as a modified NIFA Termap; **K** = Separation of culture from installed NIFA Termap; **L** = Soldier from collection for storage (* = retrieved from http://surveyofpakistan.gov.pk/; accessed on 15 January 2020).

2.2. Morphometric Identification

Soldiers were identified morphometrically using the available literature [15,28] for keys, illustrations, pictures, characters, and indices. A binocular microscope was used for recording measurement in millimeters (mm) with built-in magnification, and means were calculated along with other statistical measures [15]. A Nikon 745T Stereo zoom trinocular microscope mounted with a Nikon FSi2 digital camera was used for photography, followed by processing on Helicon Focus 6.7.1 (Helicon Soft. Ltd, Ukraine, 2000-2022) [29] for stacking and Adobe Photoshop (Version CS6 13.1.2 San Jose, CA, USA, 2017) [30] for editing. Observations were recorded for a total of 20 characters/indices (max. length of left mandible from the base, length of left mandible from the base, length of tooth to apical tip, length of head to side base of mandible, max. length of head with mandible, min head width, max head width, width of pronotum, length of pronotum, postmentum max width, postmentum width, postmentum min length, postmentum length, total body length, length of labrum, width of labrum, head index (width/length), mandible head index (length of mandible/length of head), head-convergence index (min. width of head/max. width of head), tooth index (tooth distance to tip/mandible length). Representative specimens were deposited at the Insect Museum, Department of Entomology, The University of Agriculture, Peshawar, Pakistan.

2.3. Distribution and Mapping

Coordinates recorded for the sampling sites by GPS device (eTrex 10) were projected into map using ArcGIS 10.0 (ESRI, 10.4 Environmental Systems Research Institute: Redlands, CA, USA, 2010) [31], coloring green (new locality record) [32] and assigning different types of shapes for readability on map.

2.4. Molecular Analysis

2.4.1. DNA Extraction and Amplification

DNA was extracted by taking a piece (or whole) of (distilled water washed and air dried) a single representative soldier termite from already identified specimens (in 200 µL chelex (10%, *w*/*v*) of chelex-100 [33] in ddH_20 of 0.5 mL Eppendorf tube. The tissue was broken down with the end of a pipette tip. Tubes were vortexed and placed at 99.9 °C for 15 min (using PCR machine) and centrifuged for 2–3 min for induced phase separation. The 2 µL upper phase of extracted DNA was used for polymerase chain reaction (PCR). The PCR was used to amplify ~658 bp fragment of the COII gene with the primers A-tLeu CAGATAAGTGCATTGGATTT (forward); B-tLys GTTTAAGAGACCAGTACTTG (reverse) primers [34–37] by adding 2 µL of sample DNA to 23 µL master mix. PCR reaction was processed for initial denaturation for 5 min at 94 °C, followed by 35 cycles of 94 °C for 10 s, 50 °C for 20 s, 72 °C for 45 s and 72 °C for 7 min, and stored at 4 °C. The PCR products were checked with electrophoresis on a 2% agarose gel. For cleaning, 2 µL ExoSAP-IT^{TM} was added to 5 µL PCR product (stored in ice) and incubated for 15 min at 37 °C and 15 min at 80 °C.

2.4.2. DNA Sequencing and Phylogenetic Analysis

In total, 5 µL sterile distilled water was added, and then 15 µL of each sample was added to two separate Mix2Seq tubes. A total of 2 µL forward primer was added to one tube and 2 µL reverse primer was added to the other tube, and the samples were sent to Eurofins (Eurofins, Denmark) for Sanger sequencing. The resulting mtCOII sequences were trimmed to approximately 658 bp. The beginning and end fragment of some sequences were deleted to avoid interference and to obtain comparable sequence lengths. To check the nucleotide sequence similarity, only the top 50 GenBank sequences (BLASTn) based on % query covered, % identity, bit cover and relevant taxon matching were considered [38]. For understudied species, we included more than 50 sequences from NCBI and curated reference sequences (refseq) [39]. Moreover, to ensure that BLASTn top match result were uploaded for the correct species/taxa to GenBank, top match sequences of each sequence were aligned with the reference sequence. Only the top 10 GenBank matches per species were selected for neighbor-joining and maximum likelihood tree construction, duplicates were removed, and sequences were aligned using Clustal W [40] in MEGA 6.0 [41]. This resulted in a total of twenty sequences (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) of nine *Odontotermes* spp. (spanning both *Odontotermes* and *Hypotermes*) from GenBank. Resulting novel sequences were submitted to GenBank.

2.4.3. Data Deposition

Representative specimens have been deposited at the Insect Museum, Department of Entomology, The University of Agriculture, Peshawar, Pakistan. COII sequences were deposited in GenBank (accession numbers OM630470, OM630471, OM630472, and OM630473 for *O. obesus*, *O. parvidens*, *O. assmuthi*, and *O. horai*, respectively).

3. Results and Discussion

3.1. Key to the Soldier Caste of the Species of Genus Odontotermes in the Studied Area of Khyber Pakhtunkhwa, Pakistan

1. Larger size species: Head length to mandible base is more than 2.0 mm. Minute tooth on the left mandible and present at the mandible's basal third ... ... *parvidens*

- Smaller size species: Head length is lesser than or about 2.0 mm 2

2(1). Head sub-rectangularly oval to broadly oval, sides of head weakly to strongly convex, mandibles shorter, index mandible-length head-length 0.59–0.68 mm, labrum shorter and broadly rounded anteriorly ... ... *obesus*

- Sub rectangular shape of head and sides of head are subparallel 3

3(2) Minute tooth of left mandible and placed basally, larger in size, head-length 1.65–2.0 mm, max. width of head 1.28–1.65 mm, mandibles longer (length 1.05–1.23 mm), tooth of left mandible more prominent), antennae shorter ... ... *horai*

- Tooth of left mandible placed in front of middle, mandibles thicker and shorter, slightly longer than half of head length to base of mandibles (index mandible-length head-length 0.51–0.57 mm), tooth of left mandible placed at about distal third of mandible, tooth-index 0.33–0.37 mm) ... ... *assmuthi*

3.2. Identifying Characters of the Soldier Caste

3.2.1. *Odontotermes parvidens*

Odontotermes parvidens [42], head color varies from yellowish to dark reddish brown with creamy white to yellow straw antennae and body. Mandibles are brown and yellowish brown at the base. Head is scantily and body is moderately hairy, with the total length of the body at 5.30 to 7.45 mm. Head is of large size and sub-rectangularly oval, merging anteriorly (length to base of mandibles 1.89 to 2.70; max. width 1.45 to 2.15 mm). Antennae are of 16 to 17 segments; in the 16 segmented antennae, the 3rd segment is either sub-equal to 2nd or a little longer and sub-divided, while in 17 segmented antennae, the 3rd segment is shorter than the 2nd and sub-equal to the 4th and 5th, or smaller. Labrum is triangularly tongue shaped with a pointing tip. Mandibles are strong and half or a little longer than half length of the head (length 1.19 to 1.38 mm; index length of mandible/head length 0.50–0.60) and a bit dented at the tips. Left mandible has a minute, laterally directed tooth at the base of middle third (tooth-distance from the tip 0.70 to 0.90 mm; index tooth-distance/mandible length 0.58 to 0.65). Right mandible is either without or with a minute denticle near the base. Postmentum is sub-rectangular (length 1.15 to 1.70 mm; max. width 0.62 to 0.90 mm). Pronotum is saddle-shaped with anterior margins lightly notched and posterior margins strongly emarginate (length 0.60 to 0.93; width 1.05 to 1.65 mm) (Figure 2).

3.2.2. *Odontotermes obesus*

Odontotermes obesus [43], head capsule is pale yellow to castaneous brown with pale yellow to yellowish brown antennae that are darker distally. Mandibles are light brown to dark reddish-brown; body is pale yellow to pale brown. Head scantily and body fairly hairy. Total body length is 4.0 to 6.0 mm. Head-capsule is of oval shape, slightly bending anteriorly (length to the base of mandibles 1.03 to 1.67; max. width 0.95 to 1.37 mm; index width-length 0.82 to 0.95 and convergence-index i.e., width at the base of mandible/max. width 0.62 to 0.70). Antennae are of 16 or 17 segments, 2nd segment is subequal to 3rd and 4th in 16 segmented antennae, and 3rd is shortest in 17 segmented antennae. Labrum is of tongue shape with approximately rounded anterior end. Mandibles are long, slender, and sabre shaped of length 0.75 to 1.03 mm; index length of mandible/length head 0.59 to 0.68). Left mandible with a sharp and clear tooth at distal (distance of tooth from the tip 0.25 to 0.38; index tooth distance/length of mandible 0.31 to 0.40). Right mandible with a small tooth a bit below the level of the left mandible tooth. Postmentum is sub-rectangular (length 0.70 to 0.93; width 0.50 to 0.58 mm). Pronotum is saddle shaped and anterior lobe is semi-circular, anterior end is slightly to enormously notched, posterior end is also slightly emarginated to clearly notched (length 0.50 to 0.65; width 0.80 to 1.07 mm) (Figure 3).

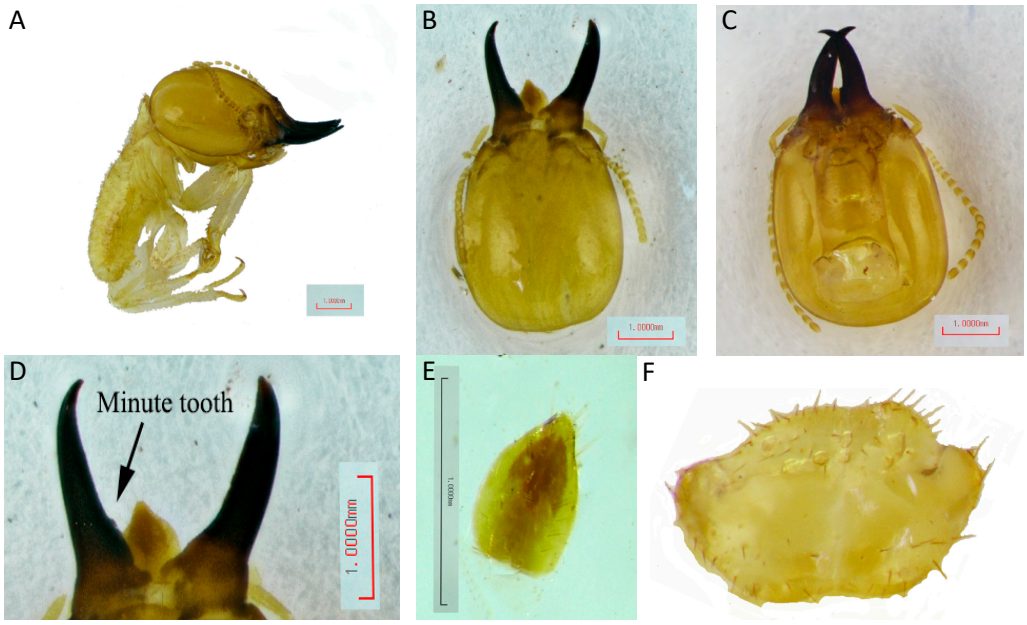


Figure 2. (**A**–**F**). Various identifying characters of the *O. parvidens* soldier caste. (**A** = Side view of the soldier, **B** = Dorsal view of head of the soldier, **C** = Postmentum, **D** = Dorsal view of mandibles, **E** = Dorsal view of labrum, **F** = Dorsal view of pronotum).

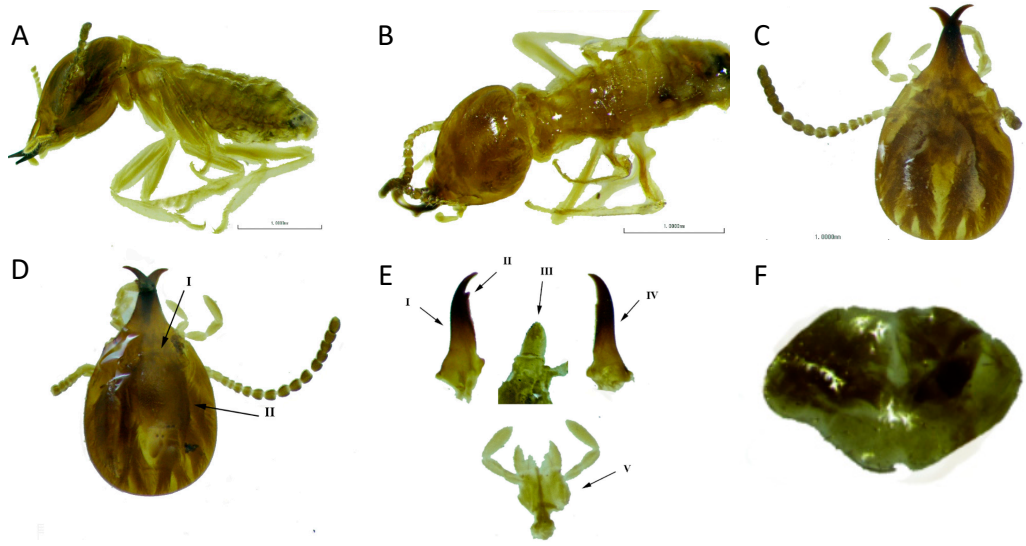


Figure 3. (**A**–**F**). Identifying characters of the *O. obesus* soldier caste. (**A**). Side view of the soldier, (**B**). Dorsal view of the soldier, (**C**). Dorsal view of head, (**D**). Postmentum of the soldier (**I** = width; **II** = length), (**E**). Mandibles (**I** and **IV**), tooth (**II**) Labrum (**III**) and Labium (**V**), (**F**). Dorsal view of pronotum.

3.2.3. *Odontotermes horai*

Odontotermes horai [44], head capsule is of brownish yellow color and antennae are equally yellow, mandibles are of dark brown color and yellowish brown at the base. Pronotum, legs, and body are of yellowish white color. Head capsule is scantily hairy, pronotum is medium hairy, and abdomen is very hairy. Total body length is 4.85 to 6.80 mm. Head capsule is sub-rectangular in shape with lightly tapered sides in the front, and longer than the width (length to the base of mandible 1.65 to 2.0; max. width 1.28 to 1.65 mm; index width/length 0.74 to 0.89). Antennae are of 15 to 16 segments, which are short, 3rd segment is smaller in size than the 2nd, and 4th is the shortest. Labrum is of sub-triangular shape with a keen tip, length is sub-equal to the width 0.26 to 0.33 mm. Mandibles are of sabre shape, lightly inward diverged at the tips, and are of roughly 2/3 of head length (length of mandible 1.05 to 1.23 mm; index length of mandible/length of head to the base of mandibles 0.55 to 0.65 mm). Left mandible with a minute tooth in the neighboring part of the middle 3rd; index tooth distance/length of mandible 0.60 to 0.66). There is no tooth or basal projection on the right mandible. Postmentum is of sub-rectangular shape (length 1.13 to 1.38; max. width 0.53 to 0.70 mm). Pronotum is of saddle shape; enough broader than the length (length 0.55 to 0.75; max. width 0.90 to 1.25 mm), anterior end is slightly notched in the medium, posterior end is visibly notched. Mesonotum is narrower than the pronotum, and the posterior end is slightly notched. Metanotum is wider, as much as the pronotum, with a sub straight posterior margin. Legs and abdomen are the same as discussed in genus characters (Figure 4).

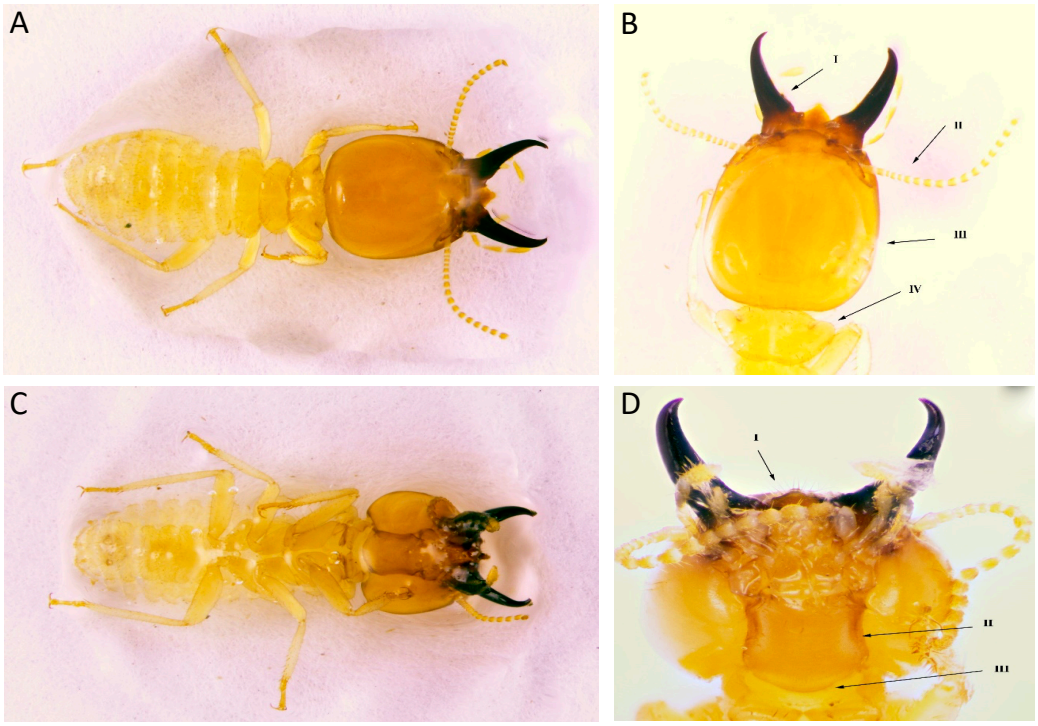


Figure 4. (**A**–**D**). Identifying characters of the *O. horai* soldier caste. (**A**). Dorsal view of the soldier, (**B**). Dorsal view of head portion of the soldier (**I** = mandibles with tooth; **II** = antenna; **III** = head capsule; **IV** = pronotum); (**C**). Ventral view of the soldier, (**D**). **I** = Labrum; **II** and **III** = Postmentum.

3.2.4. *Odontotermes assmuthi*

Odontotermes assmuthi [45], head capsule, antennae, and labrum are pale yellow to pale brown; antennae are equally colored, mandibles are brown to blackish-brown, and body is colored straw yellow to pale yellowish-brown. Head and pronotum are scantily hairy and abdomen is moderately hairy. Total length of body is 4.50 to 6.50 mm. Head capsule is sub-rectangular, sides are rather convex (length to base of mandibles 1.44–1.70,

max. width 1.07 to 1.35 mm); anteriorly moderately uniting at the base of the mandibles (width at the base of mandibles 0.78 to 0.98 mm, head convergence-index 0.68 to 0.78). Antennae are of 16 (sometimes 17) segments, and segment 4 is short. Labrum is of tongue shape (length 0.35; width 0.30 mm); anteriorly narrowly round at the tip. Mandibles are stout and short, apically slightly incurved (length 0.77 to 0.95 mm); moderately longer than 1/2 of the head length (index length of mandible/length of head 0.51 to 0.57). Left mandible with a clear cut, anteriorly directed tooth a bit later to apical 3rd, distance of tooth 0.28 to 0.35 mm, index distance of tooth/length of mandible 0.33 to 0.37. Right mandible with a weak denticle a bit below the position of the left mandible tooth. Postmentum is sub-rectangular (length 0.94 to 1.25; max. width 0.45 to 0.60 mm). Pronotum is of saddle shape (length 0.48 to 0.58; width 0.75 to 0.95 mm), the anterior end clearly and the posterior end delicately notched (Figure 5).

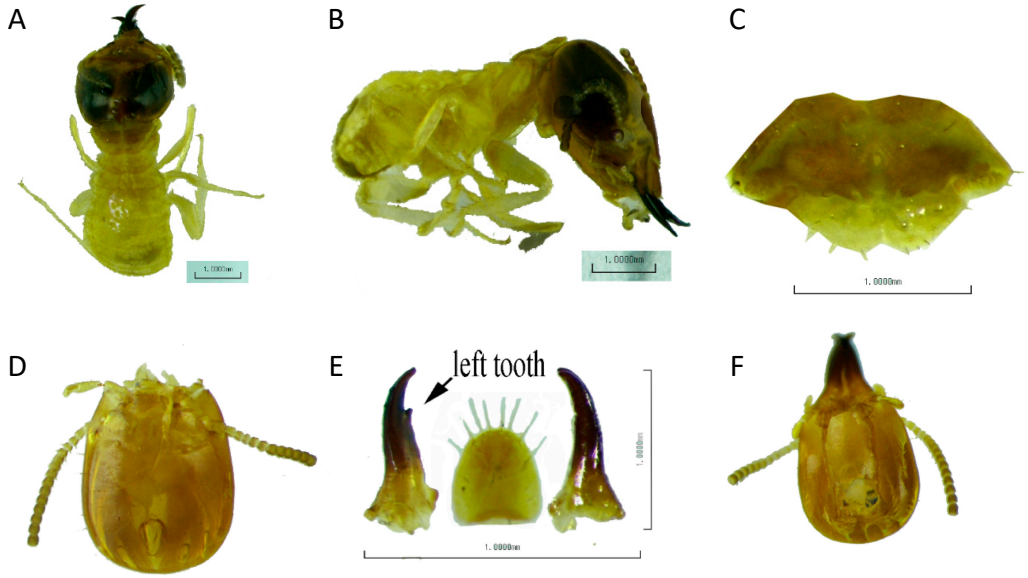


Figure 5. (**A**–**F**). Identifying characters of the *O. assmuthi* soldier caste. (**A**). Dorsal view of the soldier, (**B**). Side view of the soldier, (**C**). Dorsal view of the pronotum, (**D**). Dorsal view of the head without mandibles, (**E**). Dorsal view of mandibles and labrum, (**F**). Postmentum.

3.3. Morphological Measurements

Measurements for various characters and indices after measuring of all samples were analyzed by comparing their means with the ranges [12,35] for confirmation of morphometrics of the species *O. parvidens*, *O. obesus*, *O. horai*, and *O. assmuthi* (Table 1). Range in literature, observed range, mean, SD, SE, and CV are provided in Tables S1 and S2 for *O. parvidens*, Tables S3–S5 for *O. obesus*, Table S6 *O. horai* and Tables S7–S9 for *O. assmuthi*.

Table 1. Mean morphometric measurements for various characters of the identified species of genus *Odontotermes* in the study area.

S. No.	Characters/Indices *	*O. parvidens*		*O. obesus*			*O. horai*	*O. assmuthi*		
		Buner	Swabi	Buner	Haripur	Swabi	Haripur	Buner	Haripur	Swabi
1	Max. length of left mandible from the base	-	-	0.81	0.82	0.85	-	0.87	0.88	0.86
2	Length of left mandible from the base	1.29	1.30	-	-	-	1.17	-	-	-
3	Length of tooth to apical tip	0.83	0.83	0.26	0.30	0.31	0.72	0.30	0.31	0.30
4	Length of head to side base of mandible	2.54	2.50	1.36	1.33	1.38	1.83	1.65	1.64	1.66
5	Max. length of head with mandible	3.84	3.79	2.16	2.15	2.24	-	2.51	2.52	2.51
6	Min head width	-	-	0.76	0.80	0.83	-	0.90	0.88	0.87
7	Max head width	1.98	2.02	1.21	1.20	1.19	1.47	1.32	1.29	1.26
8	Width of pronotum	1.51	1.45	1.00	0.93	0.95	1.03	0.95	0.93	0.94
9	Length of pronotum	0.80	0.82	0.59	0.58	0.57	0.67	0.61	0.55	0.58
10	Postmentum max width	0.82	0.82	-	-	-	0.61	-	-	-
11	Postmentum width	-	-	0.57	0.58	0.56		0.53	0.55	0.55
12	Postmentum min length	1.57	1.50	-	-	-	-	-	-	-
13	Postmentum length	-	-	0.94	0.87	0.91	1.31	1.02	0.98	0.96
14	Total body length	-	-	-	-	-	5.52	-	-	-
15	Length of labrum	-	-	-	-	-	-	0.32	0.33	0.33
16	Width of labrum	-	-	-	-	-	0.28	0.33	0.31	0.32
17	Head index (Width/Length)	-	-	0.89	0.91	0.90	0.64	-	-	-
18	Mandible head index (Length of mandible/length of head)	0.51	0.52	0.59	0.62	0.66	0.64	0.53	0.54	0.52
19	Head-convergence index (min. width of head/max. width of head)	-	-	0.65	0.67	0.69	-	0.68	0.68	0.69
20	Tooth index (tooth distance to tip/mandible Length)	0.64	0.64	0.31	0.36	0.33	0.62	0.34	0.36	0.35

* All measurements are in millimeter.

3.4. Forage Substrate, Distribution and Mapping

3.4.1. Forage Substrate

As discussed earlier, termites are a major contributor to various processes in the ecosystem, but they also cause enormous damages to forestry, agriculture crops, and structures/houses. In this study, *Odontotermes* spp. were found feeding on different types of host substrates in different habitats. *O. parvidens* was found feeding on *Ficus carica, Morus alba*, *Populus* sp., *Acacia* sp., *Pinus*, *Triticum aestivum*, *Zea mays*, *Broussonetia papyrifera*, grasses, paper, and wood, while *Citrus*, *T. aestivum*, *Z. mays*, *Pinus*, grasses, shrubs, and wood were attacked by *O. assmuthi*. Similarly, *Z. mays*, *Ziziphus* sp., *Eucalyptus*, *Melia azedarach*, *Citrus* sp., *Acacia* sp., *T. aestivum*, *Rosa* sp., *Morus* sp., *M. alba*, *Olea* sp., *Pinus*,

Dodonaea sp., *Psidium guajava*, paper, dung, stubbles, wood, grasses, and shrubs were attacked by *O. obesus* while *O. horai* attack was observed only on *Punica* sp. and shrubs.

3.4.2. Distribution and Mapping

O. parvidens: Worldwide, it is distributed across India, Bhutan, Bangladesh [28], and Myanmar [4,46]. In Pakistan, it is found in sub-mountainous regions 5000 ft from sea level, including the Balakot, Parachinar, Rawalpindi [13], Abbottabad, Azad Kashmir [15], and Peshawar regions [12].

O. assmuthi: Worldwide, it is distributed across India and Bangladesh [4,15,28]. It is found in the Hungo [13] Nowshera, Charsadda, and Mardan regions of Pakistan [12].

O. obesus: Widely distributed in the Indian subcontinent [28], including Myanmar [46] and China [15]. It is one of the most widely distributed species of termite in Pakistan [12,13,15].

O. horai: Worldwide, it is distributed across India, Nepal [4,28] and Sri Lanka [15]. In Pakistan, it is one of the most abundant species found in the hard and stony soil of the sub-mountainous regions of Khyber Pakhtunkhwa, Punjab, and Baluchistan [12,13,15].

We found *O. parvidens* to be widely distributed in the sub-mountainous region of District Buner and adjacent areas of District Swabi, and we reported new locality records for both districts. *O. assmuthi* and *O. obesus* were locality recorded for all the studied districts while *O. horai* was only collected from the sub-mountainous regions of Haripur district (new locality record) (Table 2).

Table 2. *Odontotermes* spp. distribution in the various habitat of the studied area of Khyber Pakhtunkhwa, during 2016–2019. (+ = present; - = not found).

District	Habitat Type	Species			
		O. obesus	***O. parvidens***	***O. horai***	***O. assmuthi***
Haripur	Forest	+	-	+	+
	Structure	+	-	-	+
	Agriculture	+	-	+	+
Buner	Forest	+	+	-	+
	Structure	+	+	-	+
	Agriculture	+	-	-	+
Swabi	Forest	+	+	-	-
	Structure	+	+	-	+
	Agriculture	+	+	-	-

To gain insights into the *Odontotermes* species distribution in the studied area, coordinates of the collected and examined samples are projected on a map of the region (Figure 6). Locality name, X and Y co-ordinates, host feeding substrate, collector name with date of collection are provided in Tables S10–S13 for *O. parvidens*, *O. assmuthi*, *O. obesus*, and *O. horai*, respectively.

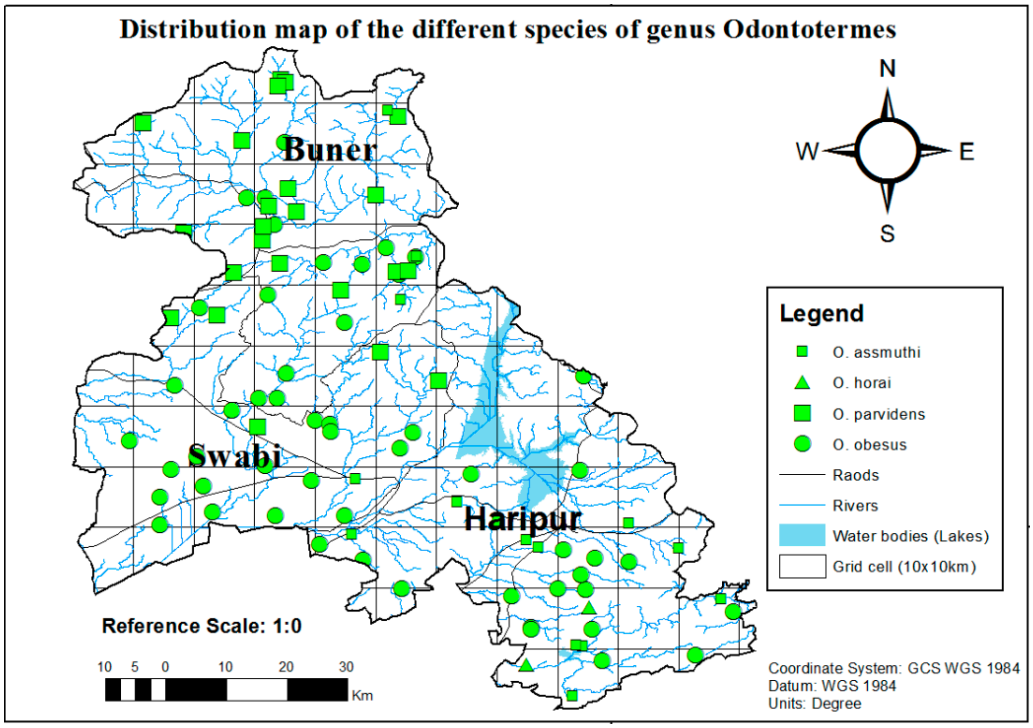


Figure 6. Distribution map of the species in the genus *Odontotermes* from the study area, during 2016–2019.

3.5. DNA Barcoding

3.5.1. Sequences Alignment and Similarity Validation

According to [47] and other researchers, there could be genetic diversity of 0.0 to 0.51% between individuals of the same species [48,49] while the expected divergence between different species could be greater than 3%. Although there are no strict criteria set for species identification through sequencing, matches < 98% can reasonably be considered novel [50]. Sequences of *O. parvidens*, *O. assmuthi* and *O. horai* did not match 100% with the sequences from the BLASTn search or refseq, except for *O. obesus* (for alignments, see Figures S1–S9. Thus, validating these three sequences as novel sequences, which is also supported by NJ and ML trees.

3.5.2. Neighbor-Joining Method Tree

Phylogenetic placement of specimens was inferred using the Neighbor-Joining (NJ) method [51]. The optimal tree with the sum of branch length = 0.33977302 is shown. The confidence probability (multiplied by 100) that the interior branch length is greater than 0, as estimated using the bootstrap test (500 replicates is shown next to the branches [52,53]. The tree is drawn to scale, with branch lengths (next to the branches) in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Kimura 2-parameter method [54] and are in the units of the number of base substitutions per site. The analysis involved 24 nucleotide sequences. Codon positions included were 1st + 2nd + 3rd + Noncoding. All positions containing gaps and missing data were eliminated. There was a total of 506 positions in the final dataset. Evolutionary analyses were conducted in MEGA 6.0 [41]. The phylogenetic tree of the *Odontotermes* sequence with the top 10 sequences from BLASTn searches successfully separated the clades of *O. parvidens* and its sister species from other *Odontotermes* and *Hypotermes* species (Figure 7). *O. horai* is the sister species, and the branch length suggests that it is genetically distinct from *O. obesus*. However, the *O. obesus* species is not monophyletic in the tree, with two additional clades containing proposed *O. obesus* species. Clade with

node 99 is the only true representative of the species while the other two are not (further discussed in the conclusion and remarks on this species status section below).

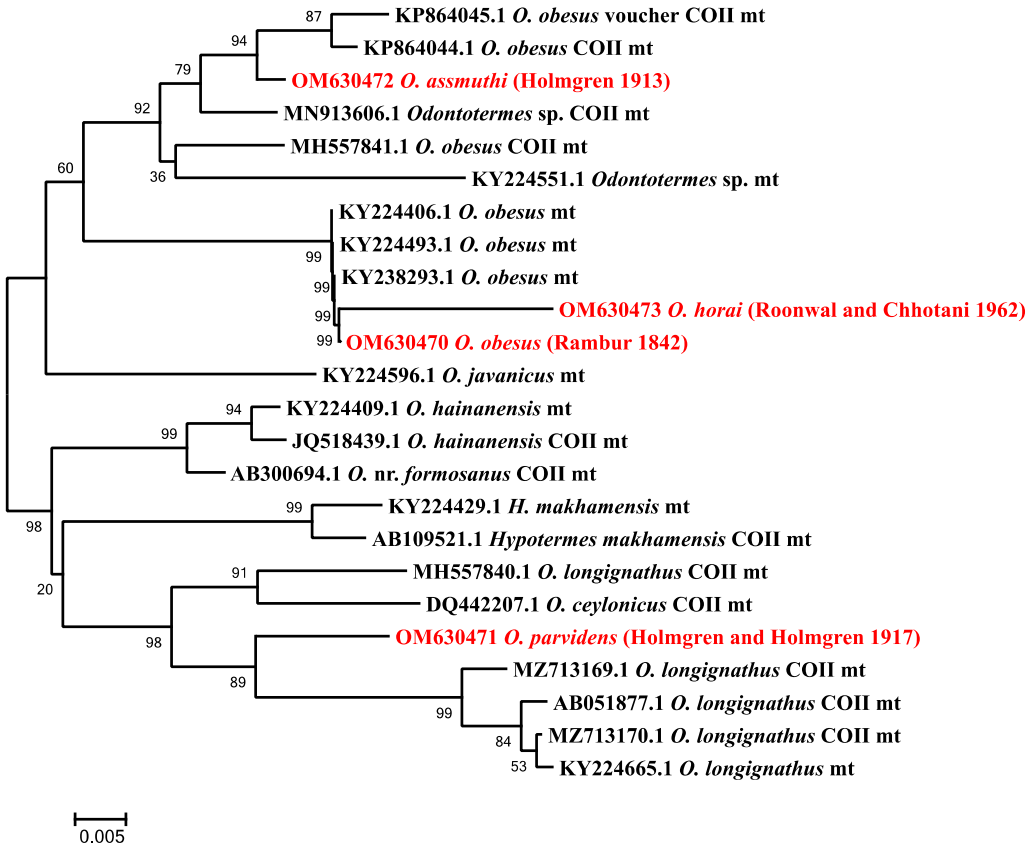


Figure 7. Unrooted Neighbor-Joining phylogeny of *Odontotermes* species collected from the various agro-ecological zones of Khyber Pakhtunkhwa, Pakistan. Type specimens in red are labeled with authority and year (nr. = near *O. formosanus*, as submitted by sequence uploader).

3.5.3. Maximum Likelihood Method Tree

The positioning of species was confirmed using the Maximum Likelihood (ML) method based on the Tamura–Nei model [55]. The tree with the highest log likelihood (−1713.6820) is shown. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Joining and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The analysis involved 24 nucleotide sequences. The codon positions included were 1st + 2nd + 3rd + Noncoding. All positions containing gaps and missing data were eliminated. There was a total of 506 positions in the final dataset. Evolutionary analyses were conducted in MEGA 6.0 [41]. The phylogenetic tree of the *Odontotermes* sequences with the top 10 sequences from BLASTn searches successfully divided the clades of *O. parvidens* and its sister species *O. longignathus* and *O. ceylonicus* species (Figure 8). The polyphyletic nature of the *O. obesus* designation from GenBank entries that was observed in the NJ tree was confirmed in the ML analysis.

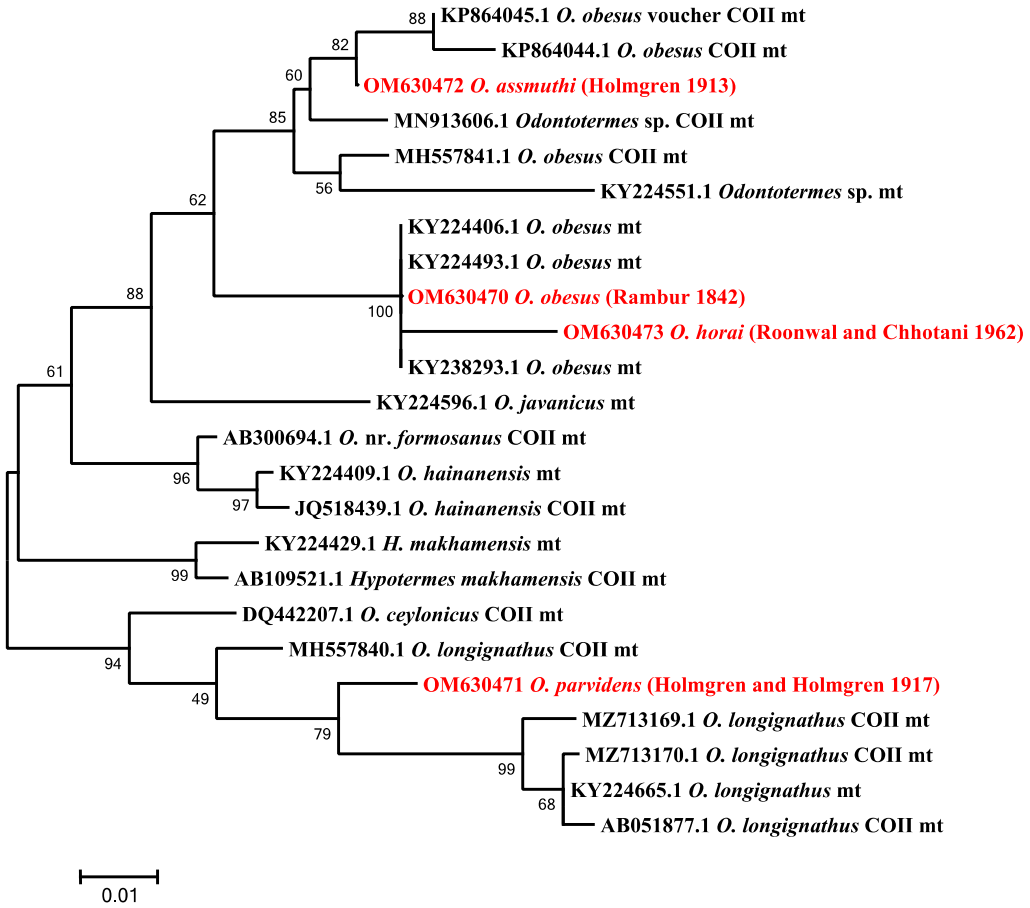


Figure 8. Unrooted ML phylogeny of *Odontotermes* species collected from the various agro-ecological zones of Khyber Pakhtunkhwa, Pakistan. Type specimens are indicated in red with authority and year (nr. = near *O. formosanus* as submitted by the sequence uploader).

3.5.4. Conclusion and Remarks on Species Status

O. parvidens

Ref. [56] suggested *parvidens* as a synonym of *distans* but, according to [57], it is considered as a separate species [28]. Morphologically, it was determined to be *O. parvidens* but due to the absence of its sequence and higher similarity, it matched up with *O. longignathus* in BLASTn search. The absence of its sequence in the GenBank and morphological variation reported in both species' descriptions [15] support that *O. parvidens* should be a valid species.

O. assmuthi

Morphologically, it was determined to be *O. assmuthi*, but in the absence of its sequence, it matched up with *O. obesus* in BLASTn search. Morphologically, there is variation noted in the head color, shape, length, hairs on the head region, body color, variation in labrum hairs, styli, and tooth size with mandible shape, which is discussed in detail by [28] in both the species' description. Because of the absence of its sequence data and morphological variation in both the species, it is determined that *O. assmuthi* should be a valid species.

O. obesus

Several sexual dimorphisms have been recorded in the various characters, although sexual dimorphism is mostly reported in workers and imagoes for several characters [58], but it is also observed in the soldiers of this study. A study on the variation of several morphological characters within and between colonies of *O. obesus*, with details of its synonyms and valid species along with the additional information from the previous authors,

is available in detail [59]. Addressing the issue of the three different clades formed by *O. obesus* in the NJ and ML phylogenies, the clade containing KY224493.1, KY224406.1, and KY238293.1 is most likely the correct taxon match with *O. obesus*, supported by morphometric and sequence alignments. The other two clades, having accession no. MH557841.1 [60] and KP864044.1; KP864045.1 [61], are morphologically similar (though no morphometric information is available on the soldier caste), so species assignments are based on the closest sequence match at the time they were deposited in GenBank. At that time, there were very few sequences available in GenBank, so they are likely to be incorrect names, suggesting that cryptic diversity in the genus exists and it is not at this point resolved.

O. horai

Considering the BLASTn search result of the examined species, it matched up with *O. obesus*, but there is variation noted in both the species' morphological characters. Thus, based on the variation in both the species' morphometrics and no sequence of it in GenBank, it is validated as *O. horai*.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/f13050674/s1, Figure S1: Alignment of the *O. parvidens* (sbjct) with the NC_034130.1 (Query) (NCBI curated refseq of *O. longignathus*) for checking the species identification validity, Figure S2: Alignment of the MH557840.1 (sbjct) with the NC_034130.1 (Query) (NCBI curated refseq of *O. longignathus*) for checking the species identification validity, Figure S3: Alignment of the *O. assmuthi* (sbjct) with the NC_034027.1 (Query) (NCBI curated refseq of *O. obesus*) for checking the species identification validity, Figure S4: Alignment of the KP864045.1 (*O. obesus*) (sbjct) with the NC_034027.1 (Query) (NCBI curated refseq of *O. obesus*) for checking the accession validity, Figure S5: Alignment of the KP864044.1 (*O. obesus*) (sbjct) with the NC_034027.1 (Query) (NCBI curated refseq of *O. obesus*) for checking the accession validity, Figure S6: Alignment of the MN913606.1 (*Odontotermes sp.*) (sbjct) with the NC_034027.1 (Query) (NCBI curated refseq of *O. obesus*) for checking the accession validity, Figure S7: Alignment of the MH557841.1 (*O. obesus*) (sbjct) with the NC_034027.1 (Query) (NCBI curated refseq of *O. obesus*) for checking the accession validity, Figure S8: Alignment of the *O. obesus* (sbjct) with the NC_034027.1 (Query) (NCBI curated refseq of *O. obesus*) for checking the species identification validity, Figure S9: Alignment of the *O. horai* (sbjct) with the NC_034027.1 (Query) (NCBI curated refseq of *O. obesus*) for checking the species identification validity, Table S1: Measurements of various taxonomic characters and indices for *O. parvidens* of district Buner during, 2016–19, Table S2: Measurements of various taxonomic characters and indices for *O. parvidens* of district Swabi during, 2016–19, Table S3: Measurements of various taxonomic characters and indices for *O. obesus* of district Buner during, 2016–19, Table S4: Measurements of various taxonomic characters and indices for *O. obesus* of district Haripur during, 2016–19, Table S5: Measurements of various taxonomic characters and indices for *O. obesus* of district Swabi during, 2016–19, Table S6: Measurements of various taxonomic characters and indices for *O. horai* of district Haripur during, 2016–19, Table S7: Measurements of various taxonomic characters and indices for *O. assmuthi* of district Buner during, 2016–19, Table S8: Measurements of various taxonomic characters and indices for *O. assmuthi* of district Haripur during, 2016–19, Table S9: Measurements of various taxonomic characters and indices for *O. assmuthi* of district Swabi during, 2016–19, Table S10: Material examined for the morphometric analysis of *O. parvidens* from the studied area during, 2016–19, Table S11: Material examined for the morphometric analysis of *O. assmuthi* from the studied area during, 2016–19, Table S12: Material examined for the morphometric analysis of *O. obesus* from the studied area during, 2016–19, Table S13: Material examined for the morphometric analysis of *O. horai* from the studied area during, 2016–19.

Author Contributions: Conceptualization, M.Z.; Methodology, I.A.K.; Software, M.Z.; Formal Analysis, M.Z.; Data Curation, S.S. and R.M.; Writing—Original Draft Preparation, M.Z.; Writing—Review & Editing, M.P. and R.M.; Supervision, I.A.K. and M.P.; Funding Acquisition, M.Z. and M.P. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by PhD-Indigenous Scholarship Phase-II, Higher Education Commission, Pakistan to M.Z. and an ERC Consolidator Grant: ERC-CoG 771349 to M.P.

Data Availability Statement: The authors confirm that the data supporting this study are available. Raw data and further details can be obtained from the corresponding authors.

Acknowledgments: Authors are thankful for the help of Israr Ali, Abdullah khan, Shahi Mulk, Shahid Ali, and Naeem Zada for conducting field trials and Misbahullah for help with morphometrics. Assistance of Abid Farid and Farukh Javed is highly acknowledged.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Hickey, C.D. Effect of Disodium Octaborate Tetrahydrate in Ethylene Glycol on Consumption and Mortality of Eastern Subterranean Termites. Master's Thesis, University of Florida, Gainesville, FL, USA, 2006; p. 57.
2. Khan, I.A.; Zaman, M.; Akbar, R.; Ali, I.; Alam, M.; Saeed, M.; Farid, A. Efficacy of single and mixed particles sand size as physical barrier against *Heterotermes indicola* under laboratory conditions. *J. Entomol. Zool. Stud.* **2015**, *3*, 106–109.
3. Ahmed, B.M.; French, J.R.J. An overview of termites control methods in Australia and their links to aspects of termite biology and ecology. *Pak. Entomol.* **2008**, *30*, 101–118.
4. Krishna, K.; Grimaldi, D.A.; Krishna, V.; Engel, M.S. Introduction: Neoisoptera excluding termitidae; Termitidae Part-I. Treatise on the Isoptera of the World. *Bull. Am. Mus. Nat. Hist.* **2013**, *377*, 1–200. [CrossRef]
5. Jouquet, P.; Traoré, S.; Choosai, C.; Hartmann, C.; Bignell, D. Influence of termites on ecosystem functioning. Ecosystem services provided by termites. *Eur. J. Soil Biol.* **2011**, *47*, 215–222. [CrossRef]
6. Govorushko, S. Economic and ecological importance of termites: A global review. *Entomol. Sci.* **2019**, *22*, 21–35. [CrossRef]
7. Ahmad, F.; Fouad, H.; Liang, S.Y.; Hu, Y.; Mo, J.-C. Termites and Chinese agricultural system: Applications and advances in integrated termite management and chemical control. *Insect Sci.* **2021**, *28*, 2–20. [CrossRef] [PubMed]
8. Mandal, B.K.; Bashar, K.; Howlader, A.J.; Rahman, K.M.Z. Incidence of termite infestation to tree species in Jahangirnagar University Campus, Bangladesh. *Bangladesh J. Life Sci.* **2010**, *22*, 7–15.
9. Mugerwa, S.; Nyangito, M.; Mpairwe, D.; Bakuneeta, C.; Nderitu, J.; Zziwa, E. Termite assemblage structure on Grazing lands in Semi-arid Nakasongola. *Agric. Biol. J. N. Am.* **2011**, *2*, 848–859. [CrossRef]
10. Rao, A.N.; Samatha, C.; Sammaiah, C. Biodiversity of Termites in Bhadrachalam Forest Region, Khammam District, Andhra Pradesh. *J. Biodivers.* **2012**, *3*, 55–59. [CrossRef]
11. Verma, M.; Sharma, S.; Prasad, R. Biological alternatives for termite control: A review. *Int. Biodeter. Biodegrad.* **2009**, *63*, 959–972. [CrossRef]
12. Salihah, Z.; Satar, A.; Farid, A.; Shakori, A.R. *Termites of Pakistan and Their Control*; Zoological Society of Pakistan: Lahore, Pakistan, 2012; pp. 11–32.
13. Chaudry, I.M.; Ahmad, M. *Termites of Pakistan, Identity, Distribution and Ecological Relationships*; Final Technical Report. Project No. A17-FS-12; Pakistan Forest Institute Peshawar-Pakistan: Peshawar, Pakistan, 1972; pp. 1–72.
14. Iqbal, N.; Saeed, S. Toxicity of six new chemical insecticides against the termite, *Microtermes mycophagus* D. (Isoptera: Termitidae: Macrotermitinae). *Pak. J. Zool.* **2013**, *45*, 709–713.
15. Manzoor, F. *Monograph on Morphometric Studies on the Termite genus Odontotermes*; Higher Education Commission: Islamabad, Pakistan, 2006; p. 154.
16. Bahder, B.W. Taxonomy of the Soldier Less Termites (Isoptera: Termitidae: Apicotermitinae) Of the Dominican Republic Based on Morphological Characters and Genetic Analysis of MtDNA and Nuclear DNA. Master's Thesis, University of Florida, Gainesville, FL, USA, 2009; pp. 1–95.
17. Jarman, S.N.; Elliott, N.G. DNA evidence for morphological and cryptic Cenozoic speciations in the Anaspididae, 'living fossils' from the Triassic. *J. Evol. Biol.* **2000**, *13*, 624–633. [CrossRef]
18. Blaxter, M.; Floyd, R. Molecular taxonomics for biodiversity surveys: Already a reality. *Trends Ecol. Evol.* **2003**, *18*, 268–269. [CrossRef]
19. Afzal, G. Phylogenetic Analysis and Prey Identification of Spiders from Wheat Fields Using COI as Molecular Marker. Ph.D. Thesis, University of Agriculture, Faisalabad, Pakistan, 2013; pp. 1–119.
20. Caterino, M.S.; Cho, S.; Sperling, F.A.H. The current state of insect molecular systematics: A thriving tower of Babel. *Ann. Rev. Entomol.* **2000**, *45*, 1–54. [CrossRef]
21. Sobti, R.C.; Kumari, M.; Sharma, V.L.; Sodhi, M.; Mukesh, M.; Shouche, Y. Sequence analysis of a few species of termites (Order: Isoptera) on the basis of partial characterization of COII gene. *Mol. Cell Biochem.* **2009**, *331*, 145–151. [CrossRef] [PubMed]
22. Liu, L.; Guo, Z.; Zhong, C.; Shi, S. DNA barcoding reveals insect diversity in the mangrove ecosystems of Hainan Island, China. *Genome* **2018**, *61*, 797–806. [CrossRef]
23. Inamullah; Khan, A.A. Agro-Ecological Zones of Pakistan. In *Agriculture the Basics*; Ikhwan Publisher: Peshawar, Pakistan, 2015; pp. 97–99.
24. PARC. *Agro-Ecological Zones of Pakistan*; Pakistan Agricultural Research Council: Islamabad, Pakistan, 1980.
25. Zaman, M.; Khan, I.A.; Usman, A.; Saljoqi, A.U.R. Species Diversity and damage incidence of Termites (Isoptera) in different agro-ecological zones of Khyber Pakhtunkhwa, Pakistan. *Sarhad J. Agric.* **2022**, *38*, 518–524. [CrossRef]
26. Saha, N.; Mazumdar, P.C.; Basak, J.; Raha, A.; Majumder, A.; Chandra, K. Subterranean termite genus *Odontotermes* (Blattaria: Isoptera: Termitidae) from Chhattisgarh, India with its annotated checklist and revised key. *J. Threat. Taxa.* **2016**, *8*, 8602–8610. [CrossRef]

27. Misbah-ul-Haq, M.; Khan, I.A.; Farid, A.; Ullah, M.; Gouge, D.H.; Baker, P.B. Efficacy of indoxacarb and chlorfenapyr against Subterranean termite *Heterotermes indicola* (Wasmann) (Isoptera: Rhinotermitidae) in the laboratory. *Turk. J. Entomol.* **2016**, *40*, 227–241. [CrossRef]
28. Chhotani, O.B. Fauna of India-Isoptera (Termites). *Zool. Surv. India* **1997**, *2*, 530.
29. Helicon Focus 6.0. Available online: https://www.heliconsoft.com/heliconsoft-products/helicon-focus/ (accessed on 20 August 2020).
30. Adobe Photoshop. Version CS6 13.1.2. Adobe: San Jose, CA, USA, 2017; Available online: https://www.adobe.com/products/photoshop.html (accessed on 10 June 2020).
31. ESRI. *ArcGIS Desktop: Release 10.4*; Environmental Systems Research Institute: Redlands, CA, USA, 2010.
32. Stephen, C.D.R. Alabama Rhinotermitidae: Nomenclature, Identification, Survey, and Phenology. Master's Thesis, Auburn University, Auburn, AL, USA, 2012; pp. 80–118.
33. Walsh, P.S.; Metzger, D.A.; Higuchi, R. Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. *Biotechniques* **1991**, *10*, 506–513. [CrossRef] [PubMed]
34. Fayle, T.M.; Scholtz, O.; Dumbrell, A.J.; Russell, S.; Segar, S.T.; Eggleton, P. Detection of mitochondrial COII DNA sequences in ant guts as a method for assessing termite predation by ants. *PLoS ONE* **2015**, *10*, e0122533. [CrossRef] [PubMed]
35. Inward, D.; Beccaloni, G.; Eggleton, P. Death of an order: A comprehensive molecular phylogenetic study confirms that termites are eusocial cockroaches. *Biol. Lett.* **2007**, *3*, 331–335. [CrossRef] [PubMed]
36. Liu, H.; Beckenbach, A.T. Evolution of the mitochondrial cytochrome oxidase II gene among 10 orders of insects. *Mol. Phylogenet. Evol.* **1992**, *1*, 41–52. [CrossRef]
37. Miura, T.; Maekawa, K.; Kitade, O.; Abe, T.; Matsumoto, T. Phylogenetic relationship among subfamilies in higher termites (Isoptera: Termitidae) based on mitochondrial COII gene sequences. *Ann. Entomol. Soc. Am.* **1998**, *91*, 515–525. [CrossRef]
38. Trinh, V.H.; Nguyen, T.H.; Nguyen, H.Y.; Huyen, T.T. Applying Molecular Technology to Identify Termite Species of the Genus Coptotermes in the Hanoi Old Quarter. Available online: http://www.prtrg.org/pdfs/S1%201%20Van%20Hanh.pdf (accessed on 23 December 2020).
39. Meiklejohn, K.A.; Damaso, N.; Robertson, J.M. Assessment of BOLD and GenBank—Their accuracy and reliability for the identification of biological materials. *PLoS ONE* **2019**, *14*, e0217084. [CrossRef]
40. Thompson, J.D.; Higgins, D.G.; Gibson, T.J. CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res.* **1994**, *22*, 4673–4680. [CrossRef]
41. Tamura, K.; Stecher, G.; Peterson, D.; Filipski, A.; Kumar, S. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Mol. Biol. Evol.* **2013**, *30*, 2725–2729. [CrossRef]
42. Holmgren, K.; Holmgrem, N. Report on a collection of termites from India. *Mem. Dep. Agric. India* **1917**, *5*, 138–171.
43. Rambur, J.P. *Histoire Naturelle des Insectes. Neuropteres*; Librairie Encyclopedique de Roret: Paris, France, 1842; p. 534.
44. Roonwal, M.L.; Chhotani, O.B. Termite fauna of Assam region, eastern India. *Proc. Natn. Inst. Sci. India.* **1962**, *28*, 181–406.
45. Holmgren, N. Termites from British India (near Bombay, in Gujarat and Bangalore) collected by Dr. J. Assmuth, S.J. Part II. *J. Bombay·Nat. Hist. Soc.* **1913**, *22*, 101–117.
46. Rathore, N.S.; Bhattacharyya, A.K. *Termite (Insecta: Isoptera) Fauna of Gujarat and Rajasthan-Present State of Knowledge*; Records of the Zoological Survey of India. Occasional Paper No. 223; Zoological Survey of India: Kolkata, India, 2004; pp. 1–77.
47. Hebert, P.D.N.; Cywinska, A.; Ball, S.L.; DeWaard, J.R. Biological identifications through DNA barcodes. *Proc. R. Soc. Lond. B. Biol. Sci.* **2003**, *270*, 313–321. [CrossRef] [PubMed]
48. Austen, J.W.; Szalanski Allen, L.; Solorzano, C.; Magnus, R.; Scheffrahn, R.H. Mitochondrial DNA Genetic Diversity of the Drywood Termites *Incisitermes minor* and *I. snyderi* (Isoptera: Kalotermitidae). *Fla. Entomol.* **2012**, *95*, 75–81. [CrossRef]
49. Firouzabadi, E.A.; Habibpour, B.; Galehdari, H.; Shishehbor, P. Genetic diversity and morphometric study of thirteen populations of *Microcerotermes diversus* (Silvestri) (Isoptera: Termitidae) in southern Iran. In Proceedings of the 43rd IRG Annual Meeting (ISSN 2000-8953), Stockholm Sweden, 8 May 2012.
50. Adetitun, D. Re: What Is the Acceptable Percentage Similarity of BLAST Nucleotide Sequence that Could Suggest a Novel Organism? 2016. Available online: https://www.researchgate.net/post/What_is_the_acceptable_percentage_similarity_of_BLAST_nucleotide_sequence_that_could_suggest_a_novel_organism/57070e6593553b217d3cfdf7/citation/download (accessed on 30 December 2020).
51. Saitou, N.; Nei, M. The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* **1987**, *4*, 406–425. [PubMed]
52. Dopazo, J. Estimating errors and confidence intervals for branch lengths in phylogenetic trees by a bootstrap approach. *J. Mol. Evol.* **1994**, *38*, 300–304. [CrossRef]
53. Rzhetsky, A.; Nei, M. A simple method for estimating and testing minimum evolution trees. *Mol. Biol. Evol.* **1992**, *9*, 945–967.
54. Kimura, M. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *J. Mol. Evol.* **1980**, *16*, 111–120. [CrossRef] [PubMed]
55. Tamura, K.; Nei, M. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Mol. Biol. Evol.* **1993**, *10*, 512–526.

56. Thakur, M.L. Revision of the termite genus *Odontotermes* Holmgren (Isoptera: Termitidae: Macrotermitinae) from India. *Indian For. Rec. (NS) Ent.* **1981**, *14*, 1–134.
57. Bose, G. Termite fauna of Southern India. *Occ. Pap. Rec. Zool. Surv. India* **1984**, *49*, 1–270.
58. Roonwal, M.L.; Chhotani, O.B. The fauna of India and the adjacent countries Isoptera (termites). *Zool. Surv. India* **1989**, *1*, 1–695.
59. Manzoor, F.; Akhtar, M.S. Morphometric analysis of population samples of soldier caste of *Odontotermes obesus* (Rambur) (Isoptera, Termitidae, Macrotermitinae). *Ani. Biod. Conser.* **2006**, *29*, 91–107.
60. Cibichakravarthy, B.; Prabagaran, S.R. Gut Bacterial Diversity of Fungus Growing *Odontotermes* sp. Available online: https://www.ncbi.nlm.nih.gov/nucleotide/MH557841.1?report=genbank&log$=nucltop&blast_rank=1&RID=5RWXKSBC01R (accessed on 17 April 2022).
61. Mahapatro, G.K. Identification of Alate of Subterranean Termite Odontotermes obesus by Molecular Characterization and Studying Its Swarming Pattern. Available online: https://www.krishisanskriti.org/vol_image/24Sep201507095616.pdf (accessed on 17 April 2022).