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Tetrigidae of Ethiopia: First Species Delimitation via DNA Barcoding and Description of Three New Species †

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Abstract

Tetrigidae is a caeliferan family of Orthoptera constituting a diverse and relatively ancient lineage of small Orthopterans, which has its greatest diversity in tropical and subtropical areas. However, to date, few studies have been conducted on the identification and description of Tetrigidae species in Ethiopia, and even fewer molecular data are available. Hence, we performed the first species delimitation study via DNA barcoding of species belonging to the genera *Paratettix*, *Leptacrydium*, *Dasyleurotettix*, and *Morphopoides* from Ethiopia. We provide 35 new sequences of the COI gene belonging to six species of these genera. We show that Ethiopian Tetrigidae can be successfully delineated using DNA barcodes, even in cryptic genera such as *Paratettix*: species delimitation on the basis of this gene was strongly congruent with the phylogenetic tree and morphological assignments. We report three species: *Dasyleurotettix infaustus* (Walker, 1871), *Morphopoides tessmanni* (Günther, 1939), and *M. folipes* (Hancock, 1908) from Ethiopia for the first time. In addition, we describe three new species, which were confirmed with morphological, phylogenetic, and species delimitation methods: *Paratettix tanai* sp. nov., *Paratettix geminus* sp. nov., and *Leptacrydium naqamteensis* sp. nov. Further, we studied *Paratettix macrostenus*, which is considered a new synonym of *P. subpustulatus*. Future integrative taxonomic studies, including more material from diverse regions, additional genetic loci and more comprehensive taxon sampling, need to be performed to understand the diversity of Tetrigidae across Africa.

Keywords: afrotropical; grasshopper; biodiversity; African; new records; *Paratettix*

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1. Introduction

Tetrigidae is a caeliferan family of Orthoptera constituting a diverse and relatively ancient lineage of small Orthopterans that are characterized by their stunning, often cryptic, morphological appearance [1–4]. The family is highly diverse; more than 2000 species have been described and reported worldwide, mostly living in tropical and subtropical areas [5]. Since they inhabit a variety of tropical and subtropical habitats and exhibit a wide range of behaviors, including specialized plant feeding and distinct ecologies, far greater diversity than currently known is expected [6]; therefore, a more thorough study of Tetrigidae diversity, especially in unexplored areas, is needed.

To date, Tetrigidae research has been mostly based on morphology [3,7–11]. However, morphological studies alone often cannot detect cryptic species and evaluate phenotypic plasticity, leading to difficulties in the development of consistent taxonomy and systematics [12–14]. Systematic molecular research on Tetrigidae has gradually emerged in recent years, with few studies, largely with narrow taxonomic or geographic scopes [15–17]. More specifically, only a small number of species of the subfamily Tetriginae have been examined with molecular methods [18–21], despite being among the most diverse groups of Tetrigidae and being taxonomically most challenging. However, molecular data have the potential to resolve the taxonomic impediment in this group.

Despite the power of DNA barcoding for species identification and delimitation, some problems are well accepted, especially in Orthoptera, because of its large genome size and high frequency of pseudogenes [22–24]. Hence, barcode data should always be enriched with morphological information to understand complex groups [25].

Currently, species delimitation is regarded as a vital component of taxonomic research across different clades to successfully and precisely identify cryptic and closely related species, thus offering a robust scientific basis for elucidating species boundaries and improving taxonomic frameworks [26–28]. Owing to the challenges linked to DNA barcoding in orthopterans, a diverse approach utilizing different theoretical frameworks has been employed for the identification and delineation of molecularly defined operational taxonomic units mOTUs). COI barcoding is a method frequently used in conjunction with species delimitation algorithms. Species delimitation utilizing DNA was conducted through various analytical methods, including distance-based models like Automatic Barcode Gap Discovery (ABGD) [29] and Assemble Species by Automatic Partitioning (ASAP) [30], as well as an alternative clustering-based approach, Bayesian Poisson tree process (bPTP) [31]. Furthermore, DNA barcoding strongly relies on well-curated databases, for which comprehensive and high-quality barcode reference libraries are missing, particularly in the most biodiverse regions. One such region with no data is tropical East Africa, specifically the country of Ethiopia.

Ethiopia is a biodiversity hotspot with diverse but strongly threatened flora and fauna [32,33]. Baseline data on the occurrence and identification of Tetrigidae are unavailable for Ethiopia, and only one species of Tetrigidae is currently listed in the Orthoptera Species File [5]: the widespread *Paratettix meridionalis* (Rambur 1838). The database lists eight species for northeastern tropical Africa, whereas more than 200 species are known for Africa [5]. However, these numbers are likely underestimates, as little systematic research has been performed recently on the African and, specifically, the eastern African Tetrigid fauna. Here, we attempt to improve the situation and aim to identify the Tetrigidae fauna of Ethiopia. We describe three new species and provide the first species delimitation via DNA barcodes for the region to lay a foundation for the future identification of Tetrigidae in Africa.

2. Materials and Methods

2.1. Sample Collection

Field sampling was conducted at ten different sampling localities in Ethiopia by visiting lakes, ponds, rivers, and wetlands, including the Kaffa Biosphere Reservoir (Kaffa BR), from February to October 2023 (sampling permission by Ethiopian Biodiversity Institute (EBI)). The Kaffa BR is located in Ethiopia's southwest highlands in the Kaffa zone, which is a part of the Eastern Afromontane Biodiversity Hotspots [34]. The Kaffa BR is mostly a highland area between 500 m and approximately 3500 m above sea level (m.a.s.l.), with an evergreen montane cloud forest covering the ground, three conventional climatic zones [35], and various habitat types (forest–crop land matrix, Afromontane evergreen forest, wetland, grassland complex, plantation, and coffee forest) [36]. Except for the Kaffa BR biodiversity hotspot, the sampling localities were selected on the basis of road accessibility. A detailed description of the sampling locations and distribution is provided in Table 1 and Figure 1.

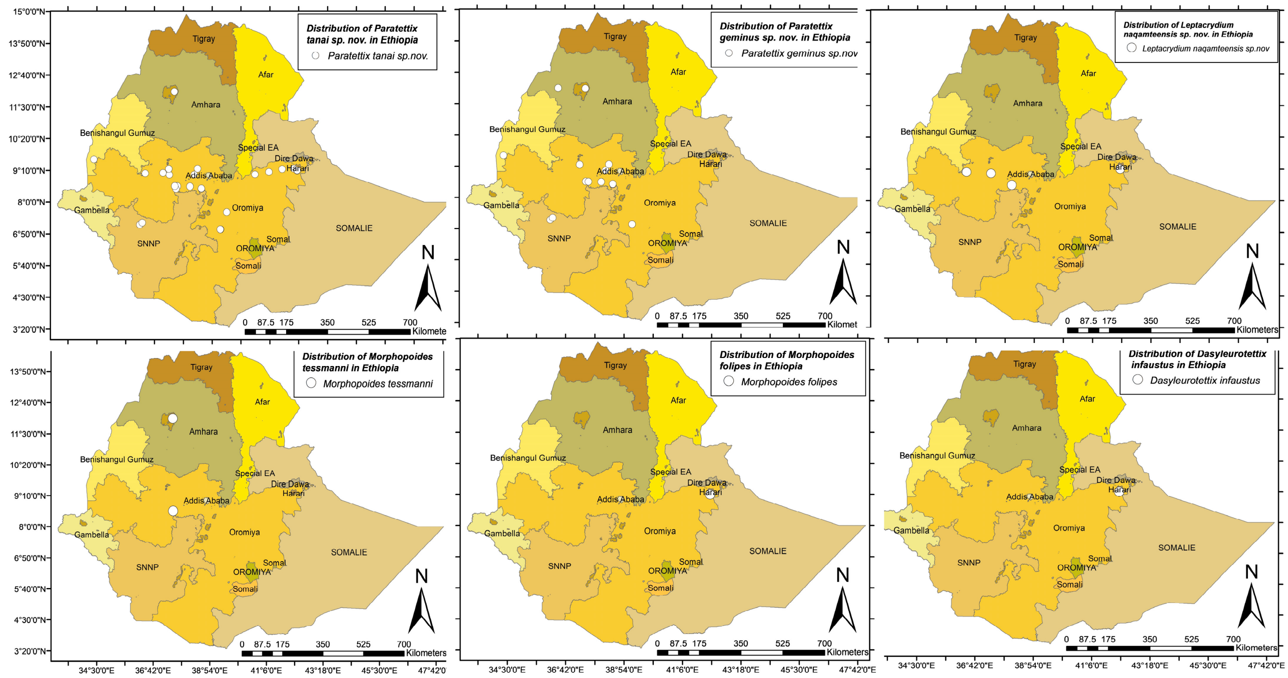

Figure 1. Distribution and sampling sites of Tetrigidae in Ethiopia. The map was drawn using ArcGIS 10.3.1.

The samples were collected by hand and with sweep nets on sunny days. Specimens were stored in airtight plastic vials containing absolute ethanol and labeled with available metadata (altitudes, longitude, and latitude). The specimens were subsequently pinned and dry preserved; one of the hind legs (middle legs whenever the hind legs were not present) was preserved in absolute ethanol and kept in a deep freezer at −23 °C until DNA extraction at the Staatliches Museum für Naturkunde Karlsruhe (SMNK), Molecular Laboratory, Karlsruhe, Germany (ABS Permit: 2024-03_ETH_MTA4_Tarekegn).

Morphological identification of the samples was performed with the keys of Devriese et al. [9] and Devriese [37] by Tarekegn Fite and verified by Hendrik Devriese. Key specimens were imaged dorsally, laterally, and anteriorly using a Keyence VHX-7100 (Keyence Corporation, Osaka, Japan) and then edited in Adobe Photoshop ver. 25.11. Vouchers are stored at the SMNK (Karlsruhe, Germany).

Table 1. Description of the Tetrigidae samples from Ethiopia used for DNA barcoding.

Region	Zones	District/Town	Specific Places	Habitat Type	Species	Number of Samples	Collector	Collection Date	Altitude (m)	Longitude	Latitude
Oromiya	West Showa	Tokkee Kutaye	Boji River	River bench and shore	*Morphopoides tessmanni* Günther, 1939	2	Tarekegn F. and Baritu T.	10 March 2023	1945	37.4753	8.5918
Amhara	South Gonder	Addis Zemen	River crossing the town	River bench and shore	*M. tessmanni*	2	Tarekegn F.	25 February 2025	1988	37.4639	12.0719
Amhara	South Gonder	Addis Zemen	River crossing the town	River bench and shore	*Paratettix geminus* sp. nov.	1	Tarekegn F.	25 February 2023	1988	37.4639	12.0719
Amhara	South Gonder	Addis Zemen	River crossing the town	River bench and shore	*P. geminus* sp. nov.	9	M. Husemann	1 March 2023	1988	37.7736	12.1136
Amhara	South Gonder	Addis Zemen	Tara Gedam	Church forest, near pond	*P. tanai* sp. nov.	1	M. Husemann	28 March 2023	2291	37.7473	12.1259
Amhara	Bahir Dar	Lake Tana	Zege Peninsula	Wetland	*P. geminus* sp. nov.	6	M. Husemann	1 March 2023	1988	37.7736	12.1136
Oromiya	E/Wallaga	Guto Gida	Calalaqi	Wetland	*Leptacrydium naqamteensis* sp. nov.	1	Tarekegn F.	7 August 2023	2097	36.3455	9.6051
Oromiya	W/Showa	Caliya	Gedo	River bench and shore	*L. naqamteensis* sp. nov.	1	Tarekegn F.	8 August 2023	2502	37.2738	9.0470
Harari	Harari	Dherer Walda	Dherer River	River bench and shore	*M. folipes* Hancock, 1908	4	Tarekegn F.	7 October 2023	1394	42.1424	9.1711
Harari	Harari	Dherer Walda	Dherer River	River bench and shore	*Dasyleurotettix infaustus* Walker, 1871	1	Tarekegn F.	7 October 2023	1394	42.1424	9.1711
South Ethiopia	Bonga	Bonga	Sheka River	Wetland	*L. naqamteensis* sp. nov.	3	Tarekegn F.	14 October 2023	1581	36.1410	7.1713
South Ethiopia	Bonga	Bonga	Sheka River	Wetland	*P. tanai* sp. nov.	1	Tarekegn F.	14 October 2023	1581	36.1410	7.1713
Oromiya	Jimma	Amiyo	Gojeb River	Wetland near river	*L. naqamteensis* sp. nov.	3	Tarekegn F.	15 October 2023	1298	36.2322	7.2439

For the description of morphological characteristics, we follow Devriese [37] and Tumbrinck [3], but we introduce a new measure: the ratio of fastigium width/width of eyes + fastigium (Figure 2). This characteristic is easier to measure than the traditionally used ratio of fastigium width/eye width, which makes it difficult for the eyes to be implanted obliquely on the head, as seen in the dorsal view.

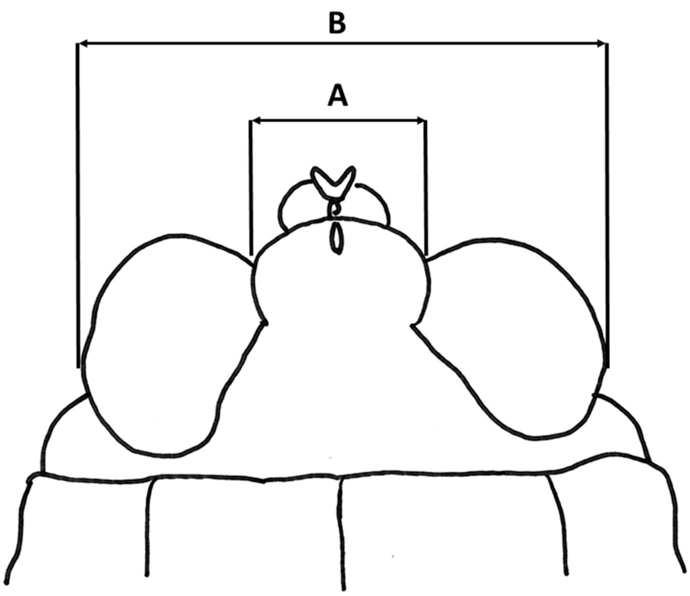

Figure 2. The ratio of fastigium (A)/eyes + fastigium (B) is a key characteristic used for the differentiation of *P. geminus* and *P. tanai*.

2.2. DNA Extraction

One to nine samples from each morphologically identified species were randomly selected for DNA barcoding. Whole-genome DNA was extracted from muscle tissue derived from one hind leg using a MagnaExtract kit with sera-mag SpeedBeads (Merck, Darmstadt, Germany) and a magnetic bead protocol [38] or a DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) following the respective manufacturer's protocol.

2.3. PCR Amplification

Polymerase chain reaction (PCR) amplification of the barcode fragment of the COI gene was performed with the primers LCO1490 and HCO2198 [39]. PCRs were performed in a 15 µL volume containing 7.5 µL of Qiagen MasterMix, 1 µL of each primer, 1 µL of template DNA, and 4.5 µL of ddH_2O. PCR amplification was performed under the following conditions: initial denaturation at 95 °C for 15 min; 35 cycles at 72 °C for 1 min and 30 s, followed by 94 °C for 30 s; annealing at 49 °C for 1 min and 30 s; and a final extension at 72 °C for 10 min. Then, 2 µL of the PCR product was loaded on a 1% agarose gel for electrophoresis, and after the PCR products were purified via the Exo-CIP™ Rapid PCR Cleanup Kit (New England Biolabs, MA, USA) following the manufacturer's instructions, the amplified gene PCR products were sequenced in both directions by Macrogen, Inc. (Amsterdam, The Netherlands).

2.4. Phylogenetic Analysis

Forward and reverse sequences of each sample were assembled with Geneious Prime v. 2025.0.3 (Biomatters Ltd., Auckland, New Zealand) https://www.geneious.com/ (accessed on 20 September 2024) to generate consensus sequences. The sequences were aligned using MUSCLE v. 5.1 [40] and manually corrected. Additionally, we used six sequences from two countries obtained from NCBI GenBank: three from Portugal for *Paratettix* *meridionalis* (accessions OR974775, OR974720, and OR974717) and three from Germany for *Tetrix subulata* (accessions GU706096, GU706097, and HQ955725). A sequence of *Xya iberica* (OR974754) was used as an outgroup for phylogenetic tree reconstruction.

The best-fit substitution model was determined as general time reversible (GTR) + I using MEGA ver. 11.0.13 [41]. Phylogenetic trees were constructed on the basis of maximum likelihood (ML) and Bayesian inference (BI). The ML tree was reconstructed with PhyML v. 3.3.20180621 [42] with 2000 bootstrap replicates and otherwise preset parameters. BI analysis was carried out using MrBayes v. 3.2.6 [43], with 10 million generations sampled every 1000 generations. Trees were visualized using FigTree v. 1.4.4.

Sequence divergence for intraspecific and interspecific pairwise distances was computed based on the Kimura-2 Parameter (K2P) model in MEGA ver. 12.0.11 [44].

- **Species delimitation**

Species delimitation based on COI sequence was conducted using three methods: two distance-based models, Automatic Barcode Gap Discovery (ABGD) [29] and Assemble Species by Automatic Partitioning (ASAP) analysis [30], and one clustering-based method, Bayesian Poisson tree process (bPTP) [31].

The ABGD technique is an automated approach for species delimitation that utilizes pairwise genetic distance to identify the barcode gap and classifies sequences into potential species without requiring a predetermined threshold. The ABGD analysis was performed utilizing the default parameters and a standard Kimura (K80) distance model to classify species according to genetic distance, utilizing the ABGD webserver (http://galaxy.itaxotoolsweb.org/?tool_id=ABGD&version=latest, accessed on 12 August 2025).

The ASAP forms new partitions by combining sequences at identical pairwise distances into increasingly larger clusters until the ultimate partition contains all records [30]. The ASAP analysis was performed utilizing the ASAP webserver (http://galaxy.itaxotoolsweb.org/?tool_id=ASAP&version=latest, accessed on 12 April 2025) with the default setting and a Kimura (K80) model.

Thirdly, the PTP approach outlines theoretical species groups by examining branch length distributions in a rooted non-ultrametric gene tree. The PTP model, improved by the bPTP variant with Bayesian backing for defined species on the input tree [31], was analyzed using an online server for bPTP versions (https://species.h-its.org/ptp/, accessed on 12 August 2025). The analysis parameters comprised 100,000 MCMC generations, with default parameters utilized for the remainder as suggested by [31]. The result from prior phylogenetic analysis, using ML trees, served as the input for the PTP analyses.

3. Results

In this study, we collected a total of 1490 Tetrigidae samples from 29 sampling areas in Ethiopia in 2023. We identified a total of six species of Tetrigidae from the collection in Ethiopia: *Paratettix tanai* sp. nov., *P. geminus* sp. nov., *Leptacrydium naqamteensis* sp. nov., *Dasyleurotettix infaustus* (Walker, 1871), *Morphopoides tessmanni* (Günther, 1939) and *M. folipes* (Hancock, 1908). All are new country records. The only species reported before, *P. meridionalis*, was not found in our samples.

Systematic Entomology

Family Tetrigidae (Rambur, 1838)

Subfamily Cladonotinae (Bolívar, 1887)

Tribe Xerophyllini (Günther, 1979)

The genera of this tribe, which is restricted to Africa, have previously been placed in Cladonotinae and Metrodorinae [45,46]. Günther [47] created the tribes Xerophyllini and

Acmophyllini without connecting them to a subfamily. Devriese [37] assigned both within a single tribe, Xerophyllini. Cladonotinae is clearly a polyphyletic taxon [5,48].

Genus *Morphopoides* (Rehn, 1930)

General characteristics of the genus *Morphopoides* (Rehn, 1930)

This genus is newly recorded in Ethiopia, with two species new to the country: *Morphopoides tessmanni* (Günther, 1939) and *Morphopoides folipes* (Hancock, 1908). This genus is characterized by a set of characters: pronotum almost flat or with two low bumps; small projection next to the eyes; pronotum sometimes/usually presenting small shiny tubercles in the shape of ocelli; median keel more or less protruding; dorsal keels are not very prominent, rounded, narrow, or toothed; lateral carinae beginning after the scapular area; fastigium quite narrow or narrow; median carinule not very prominent; without tubercles; transverse carina of the fastigium very short; frontal rib narrow, widened toward the upper eyespot, slightly projecting; antennal grooves situated below the lower margin of the compound eyes; anterior and middle shins enlarged and with tubercles or small spines, enlarged fore and mid femora with pointed lobes; posterior femora with slightly toothed edges; and macropronotal pronotum with a right anterior edge.

Key to species of *Morphopoides* (Rehn, 1930) **in Ethiopia**

1. Robust species: from the dorsal view, the pronotum is highly and broadly elevated at the anterior part; it mimics small stones; it is very broad (Figure 3A,B), convex pronotum; lower and upper edges of the fore and middle femora have irregular notched teeth (upper and lower edge) (Figure 3B), fastigium is very narrow, equal to a quarter of the width of an eye; and eyes are not very prominent; rounded (Figure 3C).. *Morphopoides tessmanni* (Günther, 1939).
2. Medium-sized pronotum from dorsal view, pronotum with small shiny tubercles in the shape of ocelli (Figure 4A,B), toothed/fork-like fore and middle femora widened (upper and lower edge) (Figure 4B), protruding eyes (Figure 4C) ....*Morphopoides folipes* (Hancock, 1908).

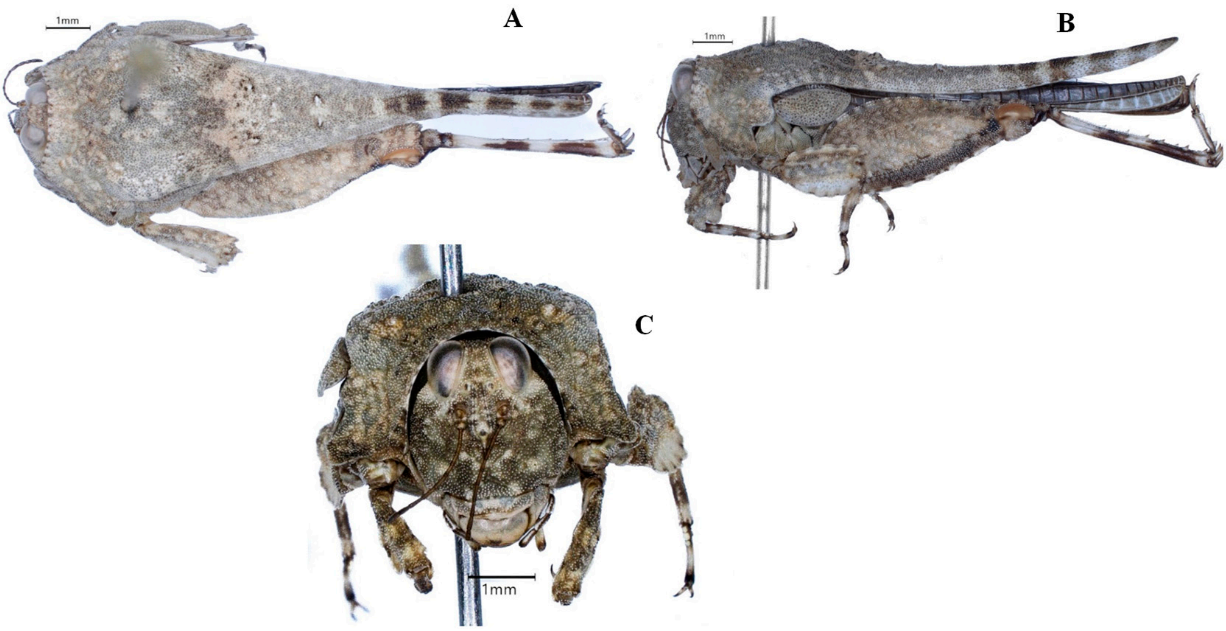

Figure 3. *Morphopoides tessmanni*, female; (**A**) body, dorsal view; (**B**) body, lateral view; (**C**) head parts, anterior view.

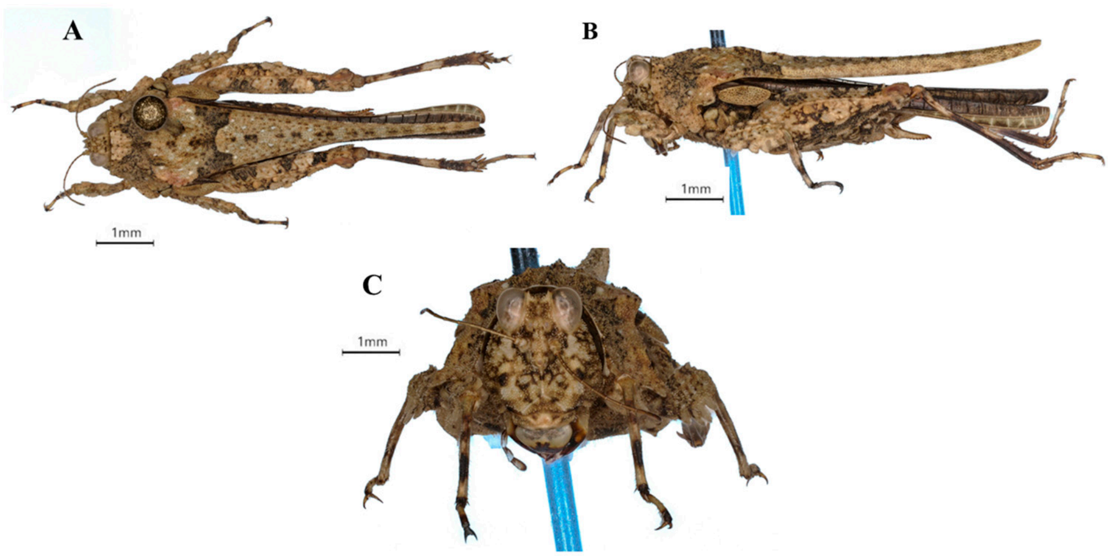

Figure 4. *Morphopoides folipes*, female; (**A**) body, dorsal view; (**B**) body, lateral view; (**C**) head parts, anterior view.

Distribution records:

Both species are rarely found in Ethiopia. *Morphopoides tessmanni* was recorded from two sampling areas, one in the northern parts (Amahara region; Addis Zemen district; Addis Zemen River shore) and the other in the western (Oromiya region; West Shawa; Tokke Kutaye district; Boji River shore) parts of Ethiopia (Figure 1).

In the present collection campaign, *Morphopoides folipes* was found only in the eastern parts of Ethiopia, Eastern Oromiya, and Harari region (Dherer Walda River shore near Babile), which is at a lower altitude (1394 m.a.s.l.) (Figure 1).

Continental distribution records:

In addition to being distributed in Ethiopia, *M. tessmanni* is distributed in Niger, Ghana, and Togo, while *M. folipes* is found in the D.R. of Congo and Mozambique [37].

Habitat: Riverbeds and shores at altitudes of 1394–1988 m.a.s.l. in Ethiopia.

Subfamily Tetriginae Serville 1838

Tribe Tetrigini Serville 1838

The African genera of this tribe were revised by [9], except for *Tettiella* Hancock, 1908, and the doubtful *Pseudosystolederus* Günther, 1939. Two genera are present in Ethiopia: *Paratettix* Bolivar, 1887, and *Leptacrydium* Chopard, 1945. We refer to [9] for a key to these genera.

Genus *Paratettix* Bolívar 1887

Two new species of *Paratettix* were recorded in Ethiopia, but the previous records of *Paratettix meridionalis* could not be confirmed. It is possible that *Parattetix subpustulatus* will be found in Ethiopia. A more detailed search in the lowlands would be necessary to detect this species, as it generally prefers lower altitudes.

Key to species of *Paratettix* Bolívar, 1887, in northeastern Africa and the Mediterranean:

3. Hind tibia uniform brown; middle femora with straight borders.. *Paratettix subpustulatus* (Walker, 1871).
4. Hind tibia with one or two white rings; middle femora with undulated borders 2
5. Hind tibia with one basal white ring, sometimes a faint second one in the middle; frontal costa not incurved at the base; middle femora slightly undulated...*Paratettix tanai* sp. nov.
6. Hind tibia with two white rings; frontal costa incurved at the base; middle femora strongly undulated 3
7. Ratio of fastigium/eyes + fastigium 24–28%; hind tibia gray with white rings .... *Paratetix geminus* sp. nov.
8. Ratio fastigium/eyes + fastigium 29–33%; hind tibia brown with white rings . . ..*Paratettix meridionalis* (Rambur, 1838).

***Paratettix tanai* sp. nov. Devriese, Fite, and Husemann**

Zoobank ID: 61CB2F76-D32A-4C4B-BA36-B083F8E432DF

Figure 5A–L

Type material:

Holotype: ♀, Ethiopia, Lake Tana, Zege Peninsula, 11°69′32″ N, 37°31′97″ E, 23 February 2023, M. Husemann leg.

Paratypes:

28 ♂, 6 ♀, Ethiopia, Lake Tana, Zege Peninsula, 11°69′32″ N, 37°31′97″ E, 23 February 2023, collector; M. Husemann leg.; 6 ♂, 11 ♀, Ethiopia, Addis Zemen, shore and bed of river (altitude 1988 m.a.s.l.), 12°11′36″ N, 37°77′36″ E, 1 March 2023, collector; M. Husemann leg.; 4 ♂, 1 ♀, Ethiopia, Tara Gedam Church Forest (altitude 2291 m.a.s.l.), 12°12′59″ N, 37°74′73″ E, 28 February 2023, collector; M. Husemann leg; 1 ♀, Ethiopia, Oromiya, West Shawa, Caliya, Sokondo, Gura forest wetlands (altitude 2278 m.a.s.l.), 9° 22′40″ N, 37°25′12″ E, 19 July 2023, collectors; Tarekegn F. and Hunde B. leg.; 4 ♀, 9 ♂, Ethiopia; Amhara, Gonder, Addis Zemen River shores and beds (altitude 2278 m.a.s.l.), 12°07′19″ N, 37°46′39″ E, 25 February 2023, collector; Tarekegn F. leg.; 9 ♀, 14 ♂, Ethiopia, Oromiya, West Shawa, Toke Kutaye, Boji River (altitude 1935 m.a.s.l.), 8°59′18″ N, 37°47′53″ E, 10 March 2023, collector; Tarekegn F. leg.; 2 ♀, 2 ♂, Ethiopia, Oromiya, East Hararge, Haramaya district, Dhangago, near a water riverbed (altitude 1746 m.a.s.l.), 9°22′00″ N, 41°57′00″ E, 23 February 2023, collector; Tarekegn F. leg.; 6 ♀, 11 ♂, Ethiopia, Oromiya, East Wallaga, Guto Gida, wetland (altitudes 2278 m.a.s.l.), 9°0′60″ N, 36°34′55″ E, 7 February 2023, collector; Tarekegn F. leg.; 26 ♀, 52 ♂, Ethiopia, Oromiya, West Shawa, Ambo district, farmland (altitude 2169 m.a.s.l.), 8°59′26″ N, 37°47′56″ E, 18 April 2023, collectors; Tarekegn F. and Baritu T. leg; 3 ♀, 10 ♂, Ethiopia, Oromiya, West Shawa, Mexi River shores (altitude 2358 m.a.s.l.), 8°58′53″ N, 37°57′38″ E, 22 April 2023, collector; Tarekegn F. leg.; 2 ♀, 8 ♂, Ethiopia, Oromiya, Shagar city, Galan, Sidam Awash, (altitude 2106 m.a.s.l.), 8°49′55″ V, 38°49′19″ E, collector; Tarekegn F. leg.; 3 ♀, 10 ♂, Ethiopia, Benishangul Gumuz, Assosa, riverside (altitude 1355 m.a.s.l.), 9°57′20″ N, 34°39′14″ E, 3 July 2023, collector; Tarekegn F. leg.; 4 ♀, 8 ♂, Ethiopia, Oromiya, West Shawa, Caliya, Sokondo Gura forest, wetlands (altitude 2278 m.a.s.l.), 9°22′40″ N, 37°25′12″ E, 19 July 2023, collectors; Tarekegn F. and Hunde B. leg.; 5 ♀, 14 ♂, Ethiopia, Oromiya, Shagar city, Managasha, near Suba Forest (altitude 2522 m.a.s.l.), 9°23′81″ N, 38°34′45″ E, collectors; Tarekegn F. and Gamachu F. leg.; 12 ♀, 7 ♂, Ethiopia, Oromiya, West Shawa, Ejere, Tullu Korma Forest, wetland (altitude 2131 m.a.s.l.), 9°01′23″ N, 38°21′52″, 3 August 2023, collector; Tarekegn F. leg.; 7 ♀, 16 ♂, Ethiopia, Oromiya, West Hararge, Ciro, Laga Bulte River shores (altitude 1732 m.a.s.l.),

9°02′53″ N, 38′′53′45″ E, 18 August 2023, collector; Tarekegn F. leg.; depository, SMNK, Karlsruhe, Germany.

Diagnosis:

Medium-sized species of brown creamy spottled color (Figure 5A,B) with dark gray posttibia with a basal white ring and sometimes another less pronounced ring at the middle of the tibia (Figure 5G). The color is highly variable. The upper part of the costa in the lateral view is slightly extended beyond the eyes without incurring (Figure 5C). Middle femora with undulated borders. Median carina of pronotum with one or two elevations. Pronotum with short pronotal disk (Figure 5A).

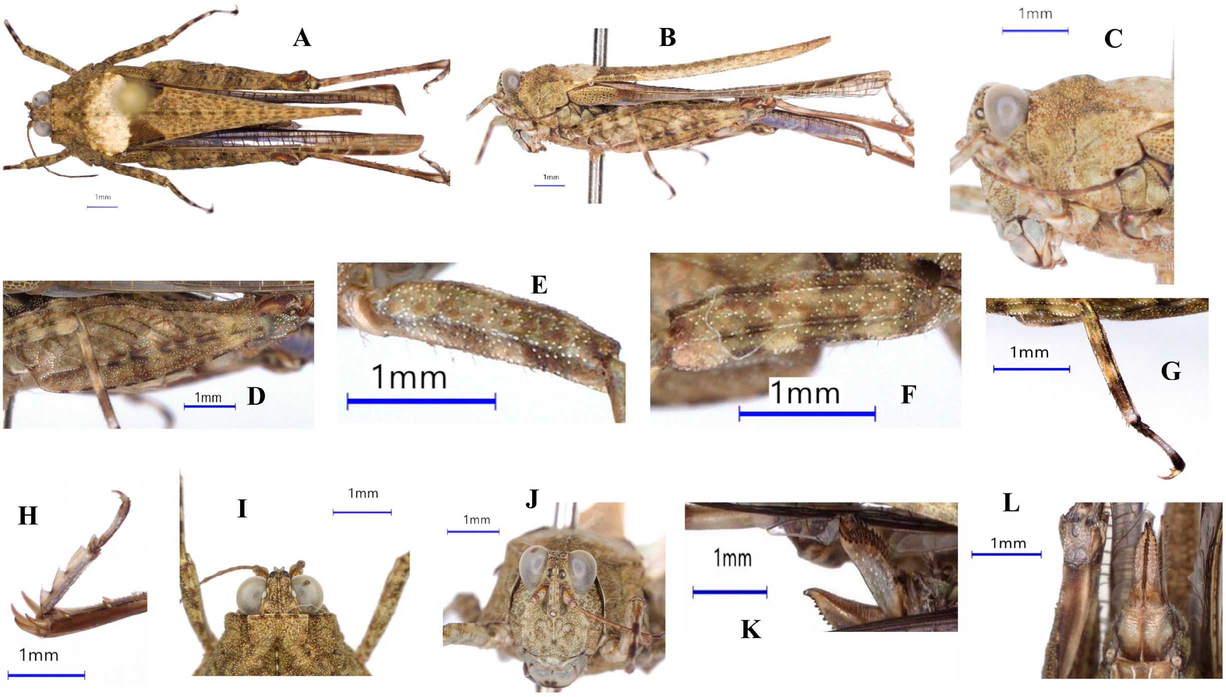

Figure 5. *Paratettix tanai* sp. nov., holotype female; (**A**) body, dorsal view; (**B**) body, lateral view; (**C**) head parts, lateral view; (**D**) hind femora, lateral view; (**E**) femur of the foreleg, lateral view; (**F**) midfemur, lateral view; (**G**) hind tibia, lateral view; (**H**) tarsus, lateral view; (**I**) head and anterior pronotum, dorsal view; (**J**) head, anterior view; (**K**) Ovipositor, lateral view; and (**L**) subgenital plate, ventral view.

Etymology:

The species name is derived from the name of Lake Tana, where the holotype and many paratypes were found. The Zege Peninsula is a peninsula located on the southern shore of Lake Tana, one of the oldest great lakes in Ethiopia, and is the source of the Blue Nile River. It is 600 km northwest of Addis Ababa, the capital city of Ethiopia. It is considered an area sacred to the Ethiopian Orthodox Tewahedo Church, boasting multiple historical monasteries. In contrast to the surrounding arable farmland region, the Zege Peninsula is notable for its dense coffee forest and is covered by a tropical forest. This originates from a religious prohibition in the area on cutting trees, plowing land, and raising large animals.

Description:

Fastigium narrow, narrower than width of an eye (Figure 5I). Transverse carinae are L-shaped; frontal costa in lateral view projects slightly before the eyes and is slightly incurved. The median carina is distinct. Superior ocelli in the middle of the eyes (Figure 5J). Antennae with 13 segments (including the scapus and pedicle), the 11th last segment is slender. Pauropronotal disk nearly flat, median carina in lateral view with one or two elevations. Lateral carinae long, extending before the anterior end of the interscapular area. The pronotum is much longer than the body and 1–2 mm longer than the hind femora (Figure 5A,B). Disk rugose, covered with small warts (Figure 5A). The humeral angles are relatively broad and angular. The lateral lobes of the paranota are contiguous to the body.

Anterior and median femora are slender, and the border of the median femora is undulated. Hind femora are relatively broad, and the superior carina is angled. Genicular teeth are medium-sized (Figure 5K,L). Posttibia dark gray with one conspicuous white band, sometimes a second, less conspicuous band in the middle of the tibia. Pulvilli with acute ends (Figure 5H). The third segment of tarsi is nearly as long as the first segment. Coloration is variable, mostly gray or brown with spots, often with two dark spots, and sometimes with whitish patches or white or cream-colored superior parts.

Measurements (n = 22):

Pronotum length: ♂, 7.9–10.8 mm; ♀, 8.4–11.6 mm. Pronotum width: ♂, 2.1–2.4 mm; ♀, 2.5–3.1 mm. Width of the vertex/width of the eyes: ♂24–28%, ♀27–29%. Length of the middle femur: ♂, 1.6–1.9 mm; ♀, 1.8–2.4 mm. Length of the hind femur: ♂, 4.7–5.5 mm; ♀, 5.1–6.5 mm. Width of the hind femur: ♂, 1.5–1.8 mm; ♀, 1.8–2.2 mm (Table 2).

Table 2. Comparison of some *Paratettix* species with the newly described species from Ethiopia.

Key Characters	Sex	*P. subpustulatus*	*P. macrostenus*	*P. tanai* sp. nov.	*P. geminus* sp. nov.	*P. meridionalis*
Frontal costa		not incurved	not incurved	not incurved	incurved	incurved
Eyes D/H		<1	<1	1	>1	>1
Fastigium/total width of eyes + fastigium		26–30%		24–29%	24–28%	29–33%
Carinae of the lateral lobes of the pronotum		not pronounced	not pronounced	not pronounced	very pronounced and curved	very pronounced and curved
Middle carina of the pronotum		nearly straight	nearly straight	with one or two conspicuous elevations	with two conspicuous elevations	with one or two conspicuous elevations
Pronotal disk L/B		>1	>1	<1	<1	<1
Pronotal length	female	10–14 mm	10–12 mm	8.5–11.5 mm	7.5–11 mm	8–10 mm
	male	8.5–12.5 mm	8.5–12.5 mm	8–11 mm	7–9 mm	7.5–9 mm
Pronotal breadth	female	2.5–3.2 mm	2.2–2.6 mm	2.5–3.2 mm	2.3–3.2 mm	2.7–3.0 mm
	male	2.0–2.5 mm	1.8–2.5 mm	2.1–2.4 mm	1.9–2.9 mm	2.0–2.5 mm
Borders of the midfemur		nearly straight	nearly straight	undulating	strongly undulating	strongly undulating
posttibia		brown	brown	dark gray with a basal ring, sometimes with an unclear ring at the middle	dark gray with 2 clear rings	dark brown with 2 clear rings
Coloration		mostly dark brown, sometimes with white patches	dark brown	spottled with darker and white patches	spottled with darker and white patches	spottled with dark and white patches

Differential diagnosis:

The species is somewhat intermediate between *Paratettix subpustulatus* and *P. meridionalis*. These species have been put in different "groups" by [9]: the *Paratettix scaber* species group with uniform brown hind tibia and the *Paratettix* (*meridionalis*) species group with ringed hind tibia.

Initially, it appeared difficult to determine in which group to place this species and whether this could be *Parattetix macrostenus*, a taxon described from Kenya. We had the opportunity to study the specimens in the Karlsruhe museum on which [47] based his description of *P. macrostenus. Paratettix macrostenus* is clearly identical to *P. subpustulatus*. It differs only in its very narrow pronotum and narrow fastigium. *Paratettix tanai* sp. nov. from Ethiopia is quite different and belongs to the *Paratettix* (*meridionalis*) species group. It differs from the above-mentioned Kenyan form of *P. subpustulatus* in (1) the middle carina of the pronotum, which has one or two elevations; (2) the ratio of the broadest part of the pronotum to the anterior part; (3) the undulating borders of the mid-femur; (4) the coloration of the posttibia; and (5) the general coloration.

Paratettix tanai differs from *P. scaber* in (1) the coloration of the posttibia, (2) the presence of two elevations on the middle carina of the pronotum, and (3) the general coloration. *Paratettix scaber* is known only in southern Africa, which is approximately 5000 km away from Ethiopia. Finally, *Paratettix ruwenzoricus* (Rehn, 1914) clearly differs in that (1) it is nearly always brachypronotal, (2) it has a uniform brown posttibia, and (3) it has a frontal costa that clearly projects before the eyes in lateral view.

Within the *Paratettix (meridionalis)* species group, *P. tanai* is characterized by (1) the frontal costa only very narrowly protruding before the eyes (differentiating it from *P. africanus* (Bolivar, 1908)), (2) the lateral lobes contiguous to the body (differentiating it from *P. overlaeti* (Günther, 1979) and *P. lamellitettigodes* (Günther, 1979), common species in central Africa, (3) the head that is not elongated (differentiating it from *P. spretus* (Günther, 1979), (4) the fastigium that is not narrowing toward the anterior part (differentiating it from the much smaller *P. pallipes* (Walker, 1871)), (5) the pronotum with one or two elevations (differentiating it from *P. gibbosulus* (Günther, 1968) and (6) the frontal costa that has no incurvation at the bottom, the hind tibia with only one faint ring and the only slightly undulating middle femora (differentiating it from *P. meridionalis* and *P. geminus* sp. nov.).

Distribution records:

This new species is widely distributed across many zones of Ethiopia, including the Oromiya, Benishangul Gumuz, and Amahara regional states (Figure 1).

Habitat: Common species of river shores and beds and wetlands at altitudes of 1100–2300 m.a.s.l in Ethiopia.

Paratettix geminus **sp. nov. Devriese, Fite, and Husemann**
Zoobank ID: 4453989D-CF38-4945-9DFF-A5B08CDD6F7C
Figure 6A–J

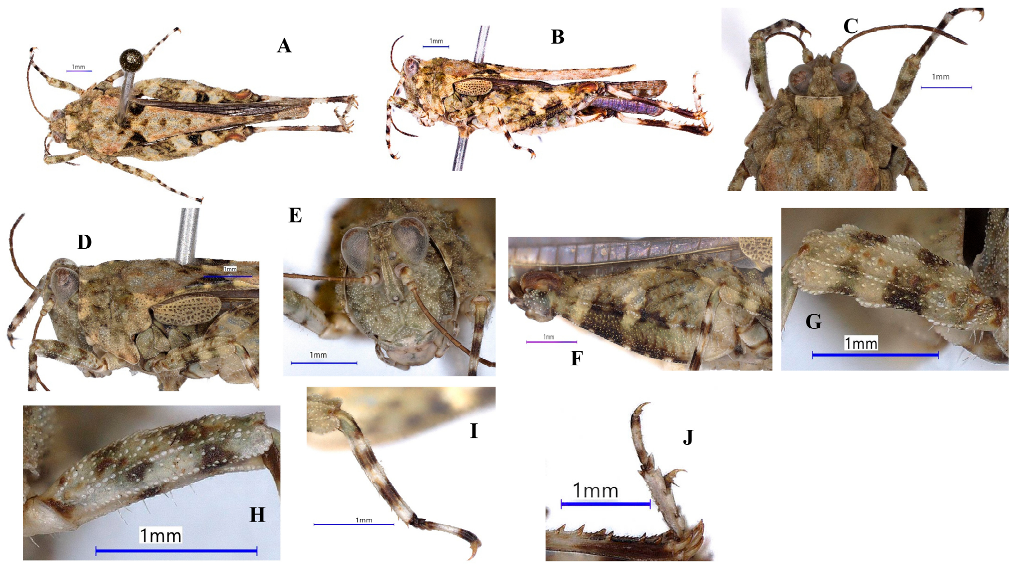

Figure 6. *Paratettix geminus* sp. nov., holotype female; (**A**) body, dorsal view; (**B**) body, lateral view; (**C**) head and anterior pronotum, dorsal view; (**D**) head and anterior pronotum, lateral view; (**E**) head,

anterior view; (**F**) hind femora, lateral view; (**G**) midfemur, lateral view; (**H**) femur of the foreleg, lateral view; (**I**) hind tibia, lateral view; (**J**) tarsus, lateral view.

Type material:

Holotype: ♀, Qaarsaa Lake, Jimma Raree district, Horro Guduru Wallaga, Oromiya, Ethiopia, 9°22′40″ N, 37°25′12″ E, 19 July 2023, Tarekegn F. leg.

Paratypes: 20 ♀, 4 ♂; Ethiopia; Oromiya; Horo Guduru Wallaga; Jimma Raree; Qaarsaa water pond (altitude 2279 m.a.s.l.), 9°22′40″ N, 37°25′12″ E, collectors; Tarekegn F. and Basha G. leg.; 11 ♂, Ethiopia, Amhara region, Gonder, Addis Zemen, Tara Gedam, 12°08′44″ N, 36°44′33″, 1 February 2023, collector; Tarekegn F. leg.; 15 ♀, 7 ♂, Ethiopia, Amhara, Addis Zemen, Tara Gedam, Addis Zemen riverbed (altitude 2291 m.a.s.l.), 12°07′19″ N, 37°46′39″ E, 25 February 2023, collector; Tarekegn F. leg.; 14 ♂, 13 ♀, Ethiopia, Oromiya, West Shawa, Ambo district, farmland (altitude 2169 m.a.s.l.), 8°59′26″ N, 37°47′56″ E, 18 June 2023, collectors; Tarekegn F. and Baritu T. leg.; 7 ♀, 7 ♂, Ethiopia, Oromiya, West Shawa, Mexi River shores (altitude 2358 m.a.s.l.), 8°58′53″ N, 37°57′38″ E, 22 June 2023, collector; Tarekegn F. leg.; 1 ♀, 1 ♂, Ethiopia, Oromiya, Shagar city, Galan, grassland near a dry river (altitude 2103 m.a.s.l.), 8°49′55″ N, 38°49′19″ E, 28 June 2023, collector; Tarekegn F. leg; 1 ♂, Ethiopia, Benishangul Gumuz, Assosa, riverside (altitude 1355 m.a.s.l.), 9°57′20″ N, 34°39′14″ E, 3 July 2023, collector; Tarekegn F. leg.; 1 ♀, Ethiopia, Oromiya, West Shawa, Caliya, Sokondo Gura forest, wetlands (altitude 2278 m.a.s.l.), 9°22′40″ N, 37°25′12″ E, 19 July. 2023, collectors; Tarekegn F. and Hunde B. leg.; 1 ♀, Ethiopia, Oromiya, West Shawa, Ejere, Tullu Korma Forest, wetland (altitude 2131 m.a.s.l.), 9°01′23″ N, 38°21′52″ E, 3 August 2023, collector; Tarekgn F. leg.; 6 ♀, 10 ♂, Ethiopia, Oromiya, West Shawa, Bako Tibe, Gibe River (altitude 1663 m.a.s.l.), 9°07′32″ N, 37°02′51″ E, 7 October 2023, collector; Tarekegn F. leg.; 2 ♀, 1 ♂, Ethiopia, East Arsi, Harero, river shores (altitude 2355 m.a.s.l.), 7°00′30″ N, 39°21′97″ E, 19 October 2023, collector; Tarekegn F. leg.; 3 ♀, Ethiopia, Southwest Ethiopian People, Bonga forest (altitude 1581 m.a.s.l.), 7°17′13″ N, 36°14′10″ E, 14 September 2023, collector; Tarekegn F. leg; 4 ♀, 11 ♂, Ethiopia, Oromiya, Jimma, Amiyo Gojeb River shore (altitude 1298 m.a.s.l.), 7°24′39″, 36°23′22″ E, 15 September 2023, collector; Tarekegn F. leg.; 3 ♀, 1 ♂, Ethiopia, Oromiya, Shagar city, Galan, Sidam Awash, (altitude 2106 m.a.s.l.), 8°50′38″ N, 38°20′48″ E, collectors; Tarekegn F. and Basha G. leg.; 2 ♀, Ethiopia, Oromiya, West Shawa, Asgori riverbed, (altitude 2455 m.a.s.l.), 8°58′17″ N, 38°05′26″ E, 24 October 2023, collector; Tarekegn F. leg.; 2 ♀, 1 ♂, Ethiopia, Oromiya, Shagar city, Managasha, near Suba forest (altitude 2522 m.a.s.l.), 9°23′81″ N, 38°34′45″ E, 16 October 2023, collectors; Tarekegn F. Gamachu F. leg., depository, SMNK, Karlsruhe, Germany.

Diagnosis: Small to medium-sized species of brown creamy spottled color with dark gray posttibia with two white rings (Figure 6A,B,I). In the lateral view, the upper part of the costa extends beyond the eyes, with an incurvation (Figure 6B,D). The median carina of the pronotum with two elevations (Figure 6B,D). Pronotum with a short pronotal disc (Figure 6C). Middle femora with undulated borders (Figure 6G).

Etymology: The species name reflects the morphological similarity to *Paratettix meridionalis* (Rambur 1838) and is derived from the Latin *geminus* for siblings.

Description: Fastigium narrow, less than the width of an eye (fastigium/total breadth of eyes + fastigium 25–28%) (Figure 2). Transverse L-shaped carinae; frontal costa in lateral view projecting before the eyes, curved with an incurvation. The median carina is distinct. Scutellum in anterior view is narrow, nearly reaching the fastigium. Superior ocelli at the middle of the eyes. Antennae with 13 segments (including scapus and pedicel), the last 11 segments slender.

Macropronotal. Pronotum with a nearly flat disk and median carina in lateral view at two elevations. Lateral carinae long, extending before the anterior end of the interscapular area. Pronotum is much longer than the body and 1–2 mm longer than the hind femora.

Disk rugous, covered with small warts. Humeral angles are relatively broad and angled. The lateral lobes of the paranota are contiguous to the body.

Anterior and median femora are broad, and the border of the median femora is clearly undulated. Hind femora are relatively broad, and the superior carina is angled. Genicular teeth are medium-sized. Posttibia dark gray with two white bands. Pulvilli with an acute end. The third segment of the tarsi is nearly as long as the first segment. Coloration is variable, most of the time gray with dark spots, often with two dark spots on the disk, sometimes with a whitish patch or a white or cream-colored superior part.

Measurements (*n* = 20): Pronotum length: ♂6.8–9.2 mm, ♀7.7–11.1 mm. Pronotum width: ♂1.9–2.9 mm, ♀2.3–3.2 mm. Width of the fastigium/total width of the eyes + fastigium: ♂24–28%, ♀24–28%. Length of the middle femur: ♂1.7–2.0 mm, ♀1.8–2.3 mm. Length of the hind femur: ♂4.0–4.7 mm, ♀4.8–5.2 mm. Width of the hind femur: ♂1.4–1.6 mm, ♀1.6–1.9 mm.

The variability in size is important. A population of Addis Zemen (Amhara region) has very small specimens, while most other populations are larger.

Differential diagnosis: This species closely resembles *P. meridionalis*, and without being able to compare specimens of both, they could be easily confused. The fastigium of *P. geminus* is narrower, and the eyes are larger and especially higher. The clearest distinction is the ratio of fastigium/eyes + fastigium (Figure 2). Another difference is the coloration of the posttibia, which is dark gray in *P. geminus* and brown in *P. meridionalis*. The separate identity was determined by genetic analysis.

Distribution records: *Paratettix geminus* is widely distributed across various geographical regions in Ethiopia, especially the Oromiya, Benishangul Gumuz region, and Southern Ethiopian People's Regional States (Figure 1).

Habitat: Common species of river shores and wetlands at an altitude of 1298–2300 m.a.s.l. in Ethiopia.

Genus *Leptacrydium* (Chopard, 1945)

General characteristics

We recorded this genus in Ethiopia for the first time with one new species, *Leptacrydium naqamteensis* sp. nov. The main identification characters for the genus *Leptacrydium* (Chopard, 1945) in Ethiopia are as follows: usually dark spots on the pronotum; brachypronotal, elytra, and alae very short; strong median carina in the whole length of the pronotum and not interrupted before the anterior margin; transverse carinae of the fastigium parabolic or semicircular; relatively broad fastigium that extends before the eyes and L-shaped fastigium; fastigium almost as broad as an eye; median femora without broad projections; and brachypronotal with very short hind wings/alea.

***Leptacrydium naqamteensis* sp. nov. Fite, Devriese, and Husemann n.sp.**

Zoobank ID: 5D13EAFC-1857-4B9D-A7BC-06ADE1236764

Figure 7A–E

Type material:

Holotype: ♀, Ethiopia, Naqamtee, Guto Gida, East Wallaga, Oromiya, 9°06′00″ N, 36°34′55″ E, 7 February 2023, collector: Tarekegn F. leg.

Paratypes: 2 ♂, 10 ♀, Ethiopia, Oromiya, East Wallaga, Guto Gida (altitude 2097 m.a.s.l.), 9°06′00″ N, 36°34′55″ E, 7 February 2023, collector: Tarekegn F. leg.; 2 ♂, 2 ♀, Ethiopia; West Shawa, Oromiya, Caliya, Gedo, and Laga Abba Galata River shores (altitude 2502 m.a.s.l.), 9°00′47″ N, 37°27′38″ E, 8 November 2023, collectors; Tarekegn F. and Obsi B. leg.; 1 ♀, Ethiopia, Harari, Dherer Walda, river shores (altitude 1460 m.a.s.l.), 9°17′11″ N, 42°14′03″ E, 10 October 2023, collector; Tarekegn F. leg.; 3 ♀, Ethiopia, West Shawa, Oromiya, Asgori, riverbed (altitude 2455 m.a.s.l.), 8°58′17″ N, 38°05′26″ E, 29 October 2023, collectors; Tarekegn F. and Amanuel S. leg.; depository, SMNK, Karlsruhe, Germany.

Diagnosis: Small species (Figure 7A,B). Fastigium is wide, extending above and before the small eyes (Figure 7C–E). Brachypronotal, middle carina of the pronotum without interruption (Figure 7B), anteriorly arched. Elytra and alae are short, the former is one-fifth of the pronotal length, and the latter is approximately three times the length of the elytra, much shorter than the pronotum.

Etymology: The species name is derived from the name of the city of Leqa Naqamtee, the area where the holotype and paratypes were found.

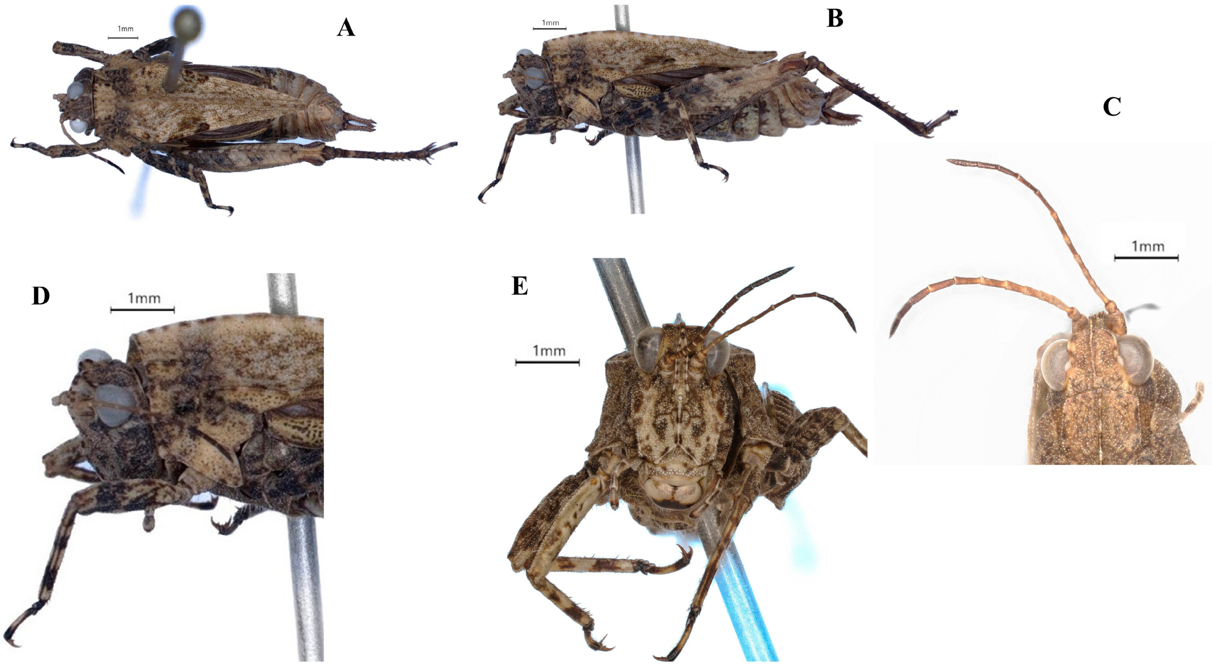

Figure 7. *Leptacrydium naqamteensis* sp. nov., holotype female; (**A**) body, dorsal view; (**B**) body, lateral view; (**C**) head and anterior pronotum, dorsal view; (**D**) head and anterior pronotum, lateral view; (**E**) head parts, anterior view.

Description: Fastigium wide (Figure 7C), approximately 45% of the width of the eyes and fastigium combined, transverse carina of parabolic to semicircular shape. rontal costa in lateral view projects before and above the eyes (Figure 7D) and is curved without incurvation at the bottom (Figure 7E). The median carina is distinct (Figure 7B). Scutellum in anterior view is narrow, nearly reaching the fastigium. Superior ocelli are slightly above the middle of the eyes. Antennae with 13 segments (including scapus and pedicel), the last 11 segments slender.

Brachypronotal. Pronotal disk is slightly rooflike, the median carina is not interrupted, and in lateral view, it is anteriorly arched. Lateral carinae long, extending before the anterior end of the interscapular area. Pronotum is longer than the body and 1.5 mm shorter than the hind femora. Anterior border of the pronotum is very slightly triangular. Disk is relatively smooth, covered with small warts. The humeral angles narrow, with a rounded angle. The lateral lobes of the paranota are contiguous to the body.

Anterior and median femora are slender, and the border of the median femora is slightly undulated. Hind femora are relatively wide, and the superior carina is straight. Genicular teeth are medium-sized. Posttibia dark gray with two white bands. Pulvilli with an acute end. The third segment of the tarsi is only half the length of the first segment.

Coloration is variable; most of the time it is middle brown with gray spots, sometimes with two dark spots on the pronotal disk. The final three segments of the antennae were blackish.

Measurements (*n* = 7): Pronotum length: ♂6.0 mm, ♀6.3–6.8 mm. Pronotum width: ♂2.0 mm, ♀2.1–2.4 mm. Width of the vertex/width of the eyes: ♂43–45%, ♀42–46%. Length of the middle femur: ♂2.0 mm, ♀2.0–2.1 mm. Length of the hind femur: ♂5.2 mm, ♀5.2–5.7 mm. Width of the hind femur: ♂1.6 mm, ♀1.7–1.8 mm.

Differential diagnosis: The new species closely resembles *Leptacrydium gratiosum.* It differs from smaller eyes in that the fastigium extends more before and above the eyes, and the transverse carinae are more rounded. The pronotum, elytra, and alae are much shorter than the brachypronotal form of *L. gratiosum*. The middle femora are slightly undulating in *L. naqamteensis*. The third segment of the tarsi is shorter than the first, whereas they are nearly equal in *L. gratiosum*. Only brachypronotal specimens are known in *L. naqamteensis* (Figure 7A–E).

Distribution records: New species widely distributed across varying geographical regions in Ethiopia, especially Oromiya (West Shawa; Asgori and Caliya near Gedo town; Laga Abbaa Galata River shore), East Wallaga (Guto Gida district near the city of Naqamtee, wetlands), and the Harari Region, Dherer Walda, and Dherer River Shores (Figure 1).

Habitat: Common species of river shores and wetlands at an altitude of 1298–2300 m.a.s.l. in Ethiopia.

Tribe Criotettigini (Kevan, 1966)

Very recently, the tribe Criotettigini is placed in a newly established subfamily Criotettiginae [49]. Previously, this tribe was placed in Tetriginae and before which most genera of the tribe were placed in Scelimeninae, but ever after Günther divided the subfamily into "Scelimeninae verae" and "Scelimenae spuriae" [45], it became clear that this subfamily was not monophyletic. The tribe Criotettigini is characterized by genera with (1) U-shaped transverse carinae of the fastigium, (2) macropronotal forms, i.e., with alae as long as the pronotum, and (3) a flat pronotal disc with many warts and tubercules. The pronotal lobes, on which the subfamilies of Bolivar (1887) were based, are very variable in this tribe: sometimes contiguous to the body (*Coptotettix*), sometimes expanded and quadrangular, but in most genera with spines (*Criotettix*, *Eucriotettix*, *Afrocriotettix*, etc.), and even within a single species, they can be variable (e.g., *Loxilobus bantu*).

Genus *Dasyleurotettix* (Rehn, 1904)

Dasyleurotettix infaustus (Walker, 1871)

A single specimen of this widespread African species has been found in Ethiopia at Dherer Walda (Harar region) (Figure 1). We report the species here in Ethiopia for the first time. The main identification characteristics for the species are as follows: a rugulose vertex, a vertex width of an eye about two-times, antenna slender (15 segments including scapus and pedicle), anterior margin of the pronotum straight and articulated diamond shaped, pronotum and alea reaching the knee of hind femur and rough pronotum, median carina of the pronotum interrupted before the anterior margin, depressed pronotal form, truncate cephalic margin, facial scutellum with an inverted V-shaped from anterior view, brachypronotal form with equal length of hind wings/alea form, usually with dark spots on the hind, mid and fore femora legs, undulated middle femora, hind tibia dark brown with one clear white ring, fore and middle tibia with two unclear white rings, fore and middle tarsi with one clear white ring at the middle, lower margins of anterior and middle femora straight with thick hind femora (Figure 8A–C).

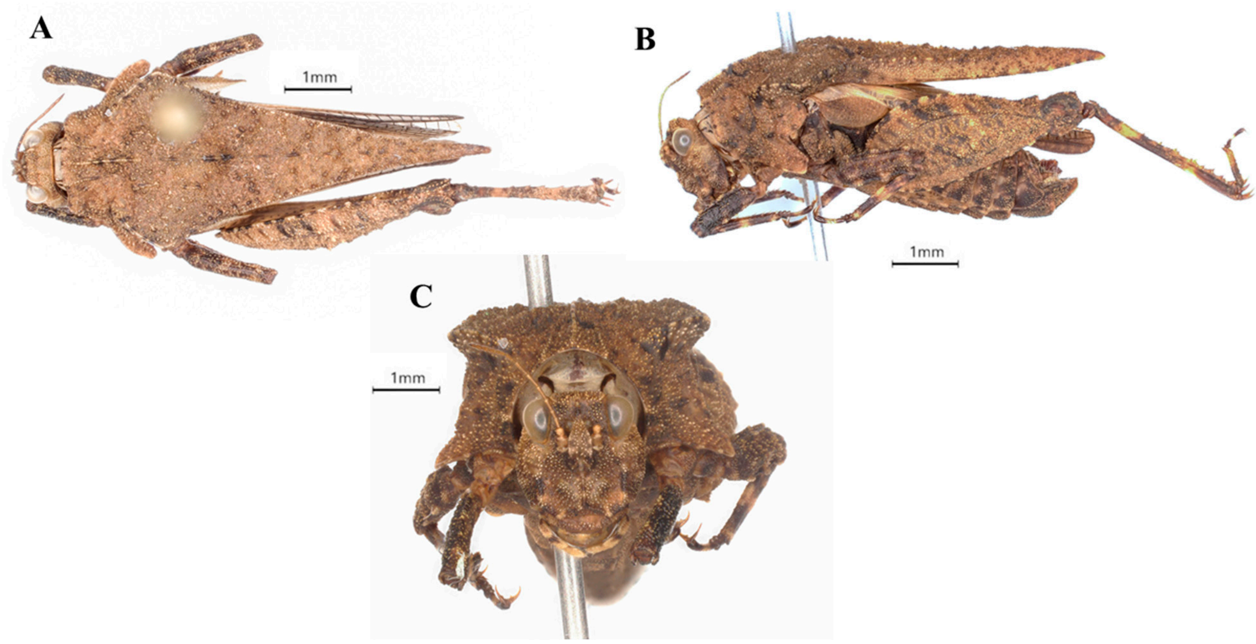

Figure 8. *Dasyleurotettix infaustus*, female; (**A**) body, dorsal view; (**B**) body, lateral view; (**C**) head parts, anterior view.

Distribution: Widespread species restricted to Africa, known from South Africa, Rwanda, Congo, Cameroon, Ghana, Togo, Sierra Leone, Botswana, Malawi, Eritrea, Liberia, Ivory Coast, Kenya, Mozambique, Tanzania, Uganda, Guinea, Zambia, Angola, and Zimbabwe. However, it is currently recorded only from one location in Ethiopia, and further surveys are needed to determine its distribution status.

DNA barcoding results

From the total Tetrigidae samples we collected for this study, we generated 35 new sequences (NCBI GenBank accession numbers: PV383318, PV441804-PV441835, and PV444646-PV444647) for Ethiopian Tetrigidae, which represent all six species reported here. We also used three sequences from Portugal for *P. meridionalis* and from Germany for *Tetrix subulata* from GenBank for comparison and as a reference. The ML and BI phylogenetic trees of Ethiopian Tetrigidae clearly separate the specimens into five clades representing the genera *Paratettix* (Bootstrap (BS): 99), *Leptacrydium* (BS: 99), *Morphopoides* (BS: 97–100), *Tetrix* (BS: 99), and *Dasyleurotettix* (Figure 9). The three tribes and five genera are monophyletic (Figure 9). Additionally, each morphologically identified species represents a monophyletic clade, which is supported by high bootstrap values and posterior probabilities (Figure 9): *P. tanai* sp. nov., *P. geminus* sp. nov., *L. naqamteensis* sp. nov., *D. infaustus*, *M. tessmanni*, and *M. folipes*. Interestingly, both of the new *Paratettix* species (*P. tanai* sp. nov. and *P. geminus* sp. nov.) are distinct from each other and *P. meridionalis* from Portugal and *Tetrix subulata* from Germany. Moreover, the phylogenetic tree clearly revealed three monophyletic tribes (*Tetrigini* (blue-colored clade), *Criotettigini* (green-colored clade), and *Xerophyllini* (red-colored clade)) (Figure 9).

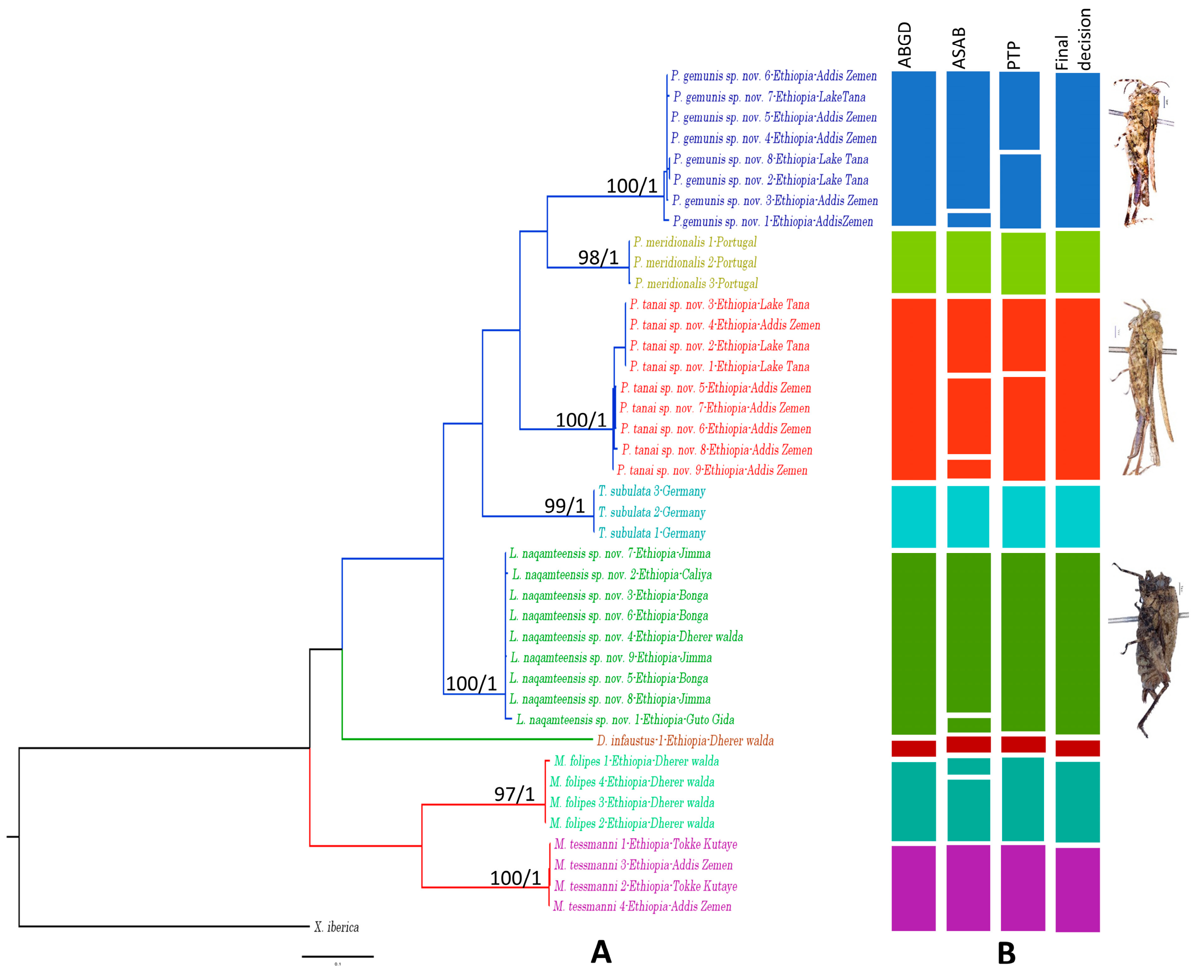

Figure 9. Phylogenetic tree and species delimitation of Tetrigidae. (**A**) Phylogenetic inference of Ethiopia and other European Tetrigidae species inferred from COI gene data, including one outgroup (*Xya iberica*, OR974754), based on maximum likelihood (PhyML) and Bayesian inference (BI). Bootstrap support (BS) (%) and the Bayesian posterior probability (PP) values are indicated on the branches. (**B**) The result of species delimitation analyses; the columns are the results of three delimitation approaches (ABGD, ASAP, and PTP) based on COI gene data and the final decision (**far right**). The putative species (MOTUs) inferred by the COI gene data are shown in different colored bars.

Pairwise distances were estimated by using the P-distance model with the option for pairwise deletion. Interspecific distance ranged from 0.0016 to 0.285 (Supplementary Material S1).

- **Species delimitation results**

Species delimitation was conducted with three models: ABGD, ASAP, and PTP algorithms, which grouped the sequences into 8, 13, and 10 molecular operational taxonomic units (mOTUs) for the Tetrigidae species, respectively (Figure 9). The species delimitation analysis largely supported the phylogenetic tree created with the COI gene, with the exception of the *Paratettix tanai* sp. nov., *Paratettix geminus* sp. nov., and *Leptacrydium naqamteensis* sp. nov. clade when ASAP and PTP models are used.

The ABGD analysis identified eight mOTUs, separating *P. tanai* sp. nov., *P. geminus* sp. nov., *L. naqamteensis* sp. nov., *M. folipes*, *M. tessmanni*, *D. infaustus*, *T. subulata*, and *P. meridionalis* into distinct mOTUs, supporting them as distinct species using the phylogenetic tree created with the COI gene. Whereas, the ASAP models generated 13 mOTUs divided *P. geminus* sp. nov., *L. naqamteensis* sp. nov., and *M. folipes* each into two and *P. tanai* sp. nov. into three theoretical species. Additionally, PTP methods generated 10 mOTUs, which separated both *P. tanai* sp. nov. and *P. geminus* sp. nov., each into two distinct hypothetical species. However, morphological description and phylogenetic analyses indicated that *P. geminus* sp. nov. is a single species, and similarly, *L. naqamteensis* sp. nov. and *P. tanai* sp. nov., also should be classified as one species each, showing a strong bootstrap value of 97–100%.

The combined findings from the morphological description, phylogenetic analysis, and species delimitation validated the identification of five genera (*Paratettix*, *Tetrix*, *Leptacrydium*, *Morphopoides*, and *Dasyleurotettix*), which include eight distinct species, i.e., *P. tanai* sp. nov., *P. geminus* sp. nov., *P. meridionalis*, *T. subulata*, *L. naqamteensis* sp. nov., *M. tessmanni*, *M. folpes*, and *D. infaustus*.

4. Discussion

We here report six species of Tetrigidae in Ethiopia, three of which are new to science and are described herein: *P. tanai* sp. nov., *P. geminus* sp. nov., *L. naqamteensis* sp. nov., *M. tessmanni*, *M. folipes*, and *D. infaustus*. We provide the first DNA barcodes and species delimitation for Tetrigidae in Ethiopia and show their value for species identification and discovery. All six newly identified species are clearly monophyletic and represent distinct units. In the following, we discuss the results in more detail. The present study demonstrates that sampling poorly studied regions can result in spectacular results.

Distribution and faunistics

Paratettix

Paratettix is among the most common, but also the most cryptic and difficult to identify Tetrigidae. To date, 22 species of *Paratettix* are known from Africa [50]. In Ethiopia, only a single species, *P. meridionalis*, had been registered thus far. Interestingly, we could not confirm previous records of *Paratettix meridionalis*. The species was not present in our samples. This was only clarified after a genetic study of Ethiopian specimens, which are now described as *Paratettix geminus* sp. nov., whose morphology is very similar to that of *P. meridionalis*. Another species, *P. tanai* sp. nov., is also new to science and could likewise be mistaken for *P. meridionalis*.

Paratettix meridionalis is reported from wide parts of West Africa, Niger, Liberia, and Togo [9], and northern African countries, such as Algeria, Morocco, and Libya [5]. In Ethiopia, the species had been recorded from a single location in the past, the Oromiya regional state (Harari) [9]. Here, we sampled across a wider geographical range and identified the specimens on the basis of both morphological and DNA barcoding data. We did not find *P. meridionalis* in the current collection campaign in Ethiopia, but instead discovered two new *Paratettix* species, *P. tanai* sp. nov. and *P. geminus* sp. nov., which are predominantly found in Ethiopia, and both species coexist across varying geographical ranges from east to west and from north to south in Ethiopia. Both are common species of middle and lower altitudes in Ethiopia, ranging from 1298 to 2502 m.a.s.l. To date, we know nothing about their distribution in other neighboring countries, but considering that they have long been overlooked in Ethiopia and that they are rather cryptic, it may suggest that they have a wider distribution across the continent and perhaps beyond. Specimens from Algeria, northeastern Africa, and the Middle East must be studied to determine where the boundary is between the two new species and *P. meridionalis*. First results, suggest that specimens from the Jordan Valley in Israel also belong to *P. geminus* sp. nov., and hence, the species may be much further distributed than is currently known.

Some other species of the genus may be expected in Ethiopia, namely, *P. subpustulatus*, *P. ruwenzoricus*, and *P. gibbosulus*, which have been recorded from diverse regions of some East African countries [9]. Hence, more research across Ethiopia is needed to fully understand the *Paratettix* community of the country.

Both *P. tanai* sp. nov. and *P. geminus* sp. nov. are found together near and on the shores of water bodies and feed on growing algae of polluted water in Ethiopia (Figure 10). This may indicate that *Paratettix* could play a role in the recycling of nutrients and hence, may be considered an ecosystem service provider.

Figure 10. *Paratettix tanai* sp. nov. and *P. geminus* sp. nov. feed in situ on growing algae in Shagar city (Galan, Sidam Awash, near a drying riverbed and shore), Oromiya, Ethiopia, 2023.

Leptacrydium

The genus *Leptacrydium* is known from Africa with five species, two of which were discovered only recently from Angola [9]. The only relatively widespread species thus far is *L. gratiosum*. We record *Leptacrydium* in Ethiopia for the first time, with one new species, *L. naqamteensis* sp. nov., currently the only known species of this genus for the country. It is distributed across varying geographical regions in Ethiopia, but not as widely as *P. tanai* sp. nov. and *P. geminus* sp. nov. *Leptacryidium naqamteensis* sp. nov. was also found coexisting with the two species of *Paratettix* described herein. Globally, the genus is restricted to the African continent; *L. gratiosum* is found in Togo, Congo, Cameroon, Uganda, Tanzania, and Kenya [5]. Hence, it may be expected to find this species in Ethiopia in the future as well, or to assign some of the other Eastern African populations to the newly described taxon.

Morphopoides

Morphopoides is found in Africa with four species (including one from Madagascar). *Morphopoides tessmanni* and *M. folipes* are rather widespread from West to East Africa, but with very scattered records thus far [50]. Here, we record these two species for Ethiopia for the first time. *Morphopoides tessmanni* has been reported mainly from tropical environments in countries such as Togo and the Central African Republic, while *M. folipes* has been reported only from Congo and Mozambique [50]. *Morphopoides folipes* appears to be a rare species, and only four specimens were collected alongside a sandy riverbed and the shores of the Dherer River in Eastern Oromiya, Ethiopia.

Dasyleurotettix

Dasyleurotettix is found in Africa with two species widely distributed across various geographical regions of Africa, including South Africa, from West Africa to East Africa. We report this genus here for Ethiopia for the first time. Although we assessed numerous localities, we recorded only one specimen of *D. infaustus* from one area of eastern Oromiya, alongside the Dherer River shores characterized by sand and stones mixed with vegetation. Whether the species is truly so rare or if any seasonal aspects may play a role will have to be solved in the future.

The species is widespread in Africa and is known from South Africa, Rwanda, Congo, Cameroon, Ghana, Togo, Sierra Leone, Botswana, Malawi, Eritrea, Liberia, Ivory Coast, Kenya, Mozambique, Tanzania, Uganda, Guinea, Zambia, Angola, and Zimbabwe.

DNA Barcoding and Species Delimitation

In this study, we provide the first DNA barcodes for African Tetrigidae, clearly indicating that the new species *P. tanai* sp. nov., *P. geminus* sp. nov., and *L. naqamteensis* sp. nov. are genetically distinct from each other and the reference species we used. All of the Ethiopian species are barcoded here for the first time; the data represent important resources for future molecular identification and phylogenetic studies of these groups, especially since these species can be considered cryptic and are difficult to identify morphologically. Furthermore, we demonstrated that COI sequences provide sufficient information for the species delimitation of Ethiopian Tetrigidae. A comparable approach to identify Tetrigidae species on the basis of COI gene sequences was used by [22], who successfully sequenced some species of Tetrigidae, *Tetrix kraussi*, *Paratettix meridionalis*, *Tetrix depressa*, and *Tetrix bipunctata*, from Europe. More recently, [21] also barcoded five species of Tetrigidae—*Paratettix meridionalis*, *Tetrix depressa*, *Tetrix ceperoi*, *Tetrix nodulosa*, and *Tetrix undulata*—from Portugal. On the basis of these reference sequences and our data, we clearly demonstrate that our new species is distinct from *P. meridionalis*. Supported by morphological differences, we demonstrate the high value of integrative taxonomy for understudied cryptic species groups.

In the present study, three molecular species delimitation approaches—two distance-based methods and one tree-based approach—were used to better assess the consistency and repeatability of the inferred species delineations, and ABGD was found to be the best species delimitation model for these Tetrigidae groups compared with ASAP and PTP. The ABGD approaches clearly identified eight species consistent with the phylogenetic tree. Supporting this result, various research reports also observed the strong performance of ABGD in analyzing the *Polypedilum* [51] and non-biting midges [52] based on COI barcode datasets. Nevertheless, considering the morphological consistency and the distinctly singular clustering results of the phylogenetic tree for both *P. tanai* sp. nov., *P. geminus* sp. nov., and *L. naqamteensis* sp. nov., these samples should be considered as one species each. Tree-based methods have demonstrated overfitting in several previous studies, frequently resulting in an increased number of mOTUs [53,54]. The bPTP does not require an ultrametric tree as an input [31]. Rather, it adds Bayesian support values into the input tree to delimit species. The high Bayesian support values at nodes indicate that all descendants of that node are probably species [51,55]. This model identified several lineages of *P. tanai* sp. nov., *L. naqamteensis* sp. nov., and *P. geminus* sp. nov. This division may indicate significant intraspecific genetic variation and possibly phylogeographic patterns within the species.

5. Conclusions

Here, we performed the first systematic analyses of Tetrigidae in Ethiopia. We report six new species for the country, three even new for science, which are described here. Furthermore, we demonstrate the high value of barcodes and show that our new species represents independent genetic units, which is further confirmed by species delimitation. However, further research should include more material from diverse regions and more comprehensive taxon sampling using multiple genes, including nuclear genes, to understand the diversity of Tetrigidae across Africa. Moreover, their distribution and ecological significance should be explored.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/taxonomy5030049/s1, Supplementary Material S1 [44,56].

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Data Availability Statement: All the newly generated COI sequences in this study have been deposited in the NCBI GenBank with the accession numbers PV444646-PV444647, PV441804-PV441835, and PV383318.

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