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Addis Ababa University

School of Graduate Studies

College of Natural and Computational Sciences

Department of Zoological Sciences

(Ecological and Systematic Zoology)

Amphibian Diversity, Ecology and Threats in Kaffa Area, Southwest Ethiopia

By

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A Thesis Submitted to the Department of Zoological Sciences of Addis Ababa University in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in Zoological Sciences (Ecological and Systematic Zoology)

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This is to certify that the dissertation prepared by Abeje Kassie Teme, entitled: Amphibian diversity, ecology and threats in Kaffa area, southwest Ethiopia, and submitted in fulfillment of the requirements for the Degree of Doctor of Philosophy in Zoological Sciences (Ecological and Systematic Zoology) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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ABSTRACT

The current study was conducted on amphibian diversity, ecology and threats in the Kaffa Ethiopia from 2018 to 2022. This study aimed to provide a comprehensive description of the species composition and habitat association of amphibians in Kaffa. Data were collected during both the rainy and dry seasons using various methods, including opportunistic observations, audio recordings and visual encounters. Standardized transect sampling methods were used to gather data on the diversity and distribution of frog species. In order to capture a diverse range of frog species, drift fence and pitfall trap techniques were employed. To assess the knowledge, attitudes and perceptions of local communities regarding the conservation status of frog, 347 individuals from participatory forest management association were selected for the survey. Frog skin and toe swab were conducted to test the presence of amphibian Chytrid fungus in the area. The study focused on three distinct habitat types: wetlands, agricultural land, and riverine forests. These habitats were thoroughly explored to document the presence of frog species. A significant amount of effort was dedicated to the fieldwork, with a total of 3,672 person-hours spent in the study area. A total of 5,678 individual frogs belonging to 26 different species were recorded. These species represented eleven genera and eight families of frogs. Among the recorded species, six were not previously recorded in the Kaffa area, highlighting the importance of this study. The identification of the species was primarily based on their physical characteristics (morphology) and subsequently validated using genetic barcoding techniques. Molecular systematic analysis using mitochondrial 16S rRNA and COI gene sequencing was conducted to further confirm the species' identities. The earlier study using morphological identification mis-identified the species as *Leptopelis ragazzii*, but further analysis using DNA sequencing revealed that it is actually *Leptopelis vannutellii*. Similarly, the *Hyperolius* individuals were initially classified as *H. nasutus*, but DNA barcoding demonstrated that they belong to the species *H. howelli*. Additionally, three potential new species were discovered, namely *Leptopelis* sp. 1, *Leptopelis* sp. 2, and *Phrynobatrachus* sp. 1. In terms of abundance, the family Hyperoliidae was the most prevalent among the recorded frogs followed by Phrynobatrachidae, while Conrauidae was the least abundant. Wetland habitat supported the highest number of species, followed by the riverine forest, whereas agricultural land supported the lowest number of species. Shannon diversity index showed that wetlands had the highest values (2.131) followed by agricultural land (1.58). Among the environmental variables, temperature had a significant negative impact whereas precipitation had a non-significantly positive relationship with the abundance of frogs. Kaffa area is known for its rich variety of frog species, comprising about 35% of the country's amphibians. Unfortunately, these habitats have experienced fragmentation and degradation due to the expansion of agriculture, drainage of water from the wetlands, and the conversion of horticulture areas into field crops. To address these issues, we strongly recommend the active involvement of non-governmental organizations, district, and regional officials, as well as local communities in and around Kaffa, in the conservation and protection of wetlands and forests to maintain the diverse assemblage of frogs.

Keywords: Amphibia; Conservation; Diversity; DNA sequence; Habitat; Kaffa; New species

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DEDICATION

To my father Kassie Teme and my mother Mestewat Eskezia.

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Abbreviations and Acronyms

AES	Acoustic Encounter Survey
Bd	*Batrachochytrium dendrobatidis*
BLAST	Basic Local Alignment Search Tool
COI	Cytochrome C Oxidase Subunit I
DFPT	Drift Fence and Pitfall Trap
EBI	Ethiopian Biodiversity Institute
EWNHS	Ethiopian Wildlife and Natural History Society
GLM	Generalized Linear Model
IUCN	International Union for Conservation of Nature and Natural Resources
JCSI	Jaccard Coefficient Similarity Index
ML	Maximum Likelihood
NABU	The Nature and Biodiversity Conservation Union
NASA	National Aeronautics and Space Administration
NCBI	National Center for Biotechnology Information
NJ	Neighbor-Joining
NYU	New York University
PFM	Participatory Forest Management
SBL	Sum of Branch Length
SCSI	Sorenson's Coefficient Similarity Index
TAM	Technology Adoption Model
VES	Visual Encounter Survey
WBISPP	Woody Biomass Inventory and Strategic Planning Project
WCMC	World Conservation Monitoring Centre

List of morphometric character abbreviations

SVL	Snout-vent length
HW	Head width
HL	Head length
SL	Snout length
NS	Snout-nostril distance
IND	Inter-nares distance
EN	Eye-nostril distance
IOD	Inter-orbital distance
ED	Eye diameter
UEW	Upper eyelid width
FLL	Forearm length
HAL	Hand length
FinDW	Longest finger disc width
THL	Thigh length
TL	Tibia length
FL	Foot length
Toe4DW	Fourth toe disc width

1. INTRODUCTION

Amphibians are one of the most important groups of vertebrates that play a critical role in ecosystem functioning (Hocking and Babbitt, 2014). They are increasingly used as bioindicator organisms in pollution studies (Jongsma *et al*., 2014). Few studies show the discovery of new species each year. However, there is lack of comprehensive research on amphibians worldwide. There are over 8,674 described amphibian species globally where frogs and toads make up the majority, 88.1%, followed by newts and salamanders (9.4%) and caecilians (2.5%) (Amphibia Web, 2023). Amphibians are widely distributed except in the coldest, driest regions and the most isolated oceanic islands (Stuart *et al*., 2008; Foden and Stuart, 2009; Ceríaco *et al*., 2014).

Ethiopia's tropical position and generally humid temperature is ideal for the survival of a variety of amphibian species. Currently, the country is home to 78 known species of amphibians (Amphibia Web, 2023). As further study progresses, additional species are discovered (Smith *et al*., 2017; Goutte *et al*., 2019; Tiutenko and Zinenko, 2021; Goutte *et al*., 2021, 2022), particularly in the southwest of the country. A large number of these species are endemic to Ethiopia, and are local endemics in most cases.

Since the first half of the eighteenth century until 1986, very few studies have investigated the diversity of Ethiopian amphibians (Largen, 2001). There are still significant gaps in our knowledge of the country's amphibians although the last two decades have seen a renewed interest in the study of Ethiopian amphibians (Drewes and Roth, 1981; Evans *et al*., 2011; Abebe Ameha, 2012; Gower *et al*., 2012; Freilich *et al*., 2014; Freilich, 2015; Smith *et al*., 2017; Reyes-Velasco *et al*., 2018a and b; Goutte *et al*., 2019; Abeje Kassie and Afework Bekele, 2020; Tiutenko and Zinenko, 2021; Goutte *et al*., 2021, 2022). These recent studies have uncovered additional diversity of new species

(Smith *et al*., 2017; Goutte *et al*., 2019; Tiutenko and Zinenko, 2021; Goutte *et al*., 2021, 2022). This suggests the potential wealth of amphibian diversity in Ethiopia and in particular in the poorly explored southwest region of the country.

Understanding the potential habitats for wild animals is crucial for population assessment and conservation efforts. This is especially important in wild animal habitats outside protected areas that face high anthropogenic pressures. Unfortunately, like other wild animal species in Ethiopia, amphibians also face threats such as habitat loss, pollution and other anthropogenic activities. Amphibians are particularly vulnerable to environmental changes, leading to population decline. The global decline of amphibians has been well-documented over time (Archer *et al*., 2018; Thompson and Donelly, 2018; Zimkus *et al*., 2018), and the magnitude of the decline varies across geographical regions (Beebee and Griffith, 2005). For example, frog population decline in Africa is reported by Hirschfeld *et al*. (2016), habitat destruction (Wake and Vredenburg, 2008; Thompson and Donelly, 2018), disease (Cheng *et al*., 2011), climate change (Dukes and Mooney, 2004; Runting *et al*., 2017), invasive species, over-exploitation, and pollution (Stuart *et al*., 2004; Hof *et al*., 2011; Ficetola *et al*., 2014). Human-caused habitat destructions are considered significant contributors to the loss and extinction of amphibians globally (Stuart *et al*., 2004).

According to studies by Estrada *et al*. (2020) and Mengesha Asefa *et al*. (2020), habitat destruction has increased significantly in some parts of sub-Saharan Africa. This, undoubtedly, will have an impact on the distribution of associated flora and fauna that live in the forest and wetland habitats (Archer *et al*., 2018). To address these threats and conserve Ethiopia's amphibians, comprehensive studies are needed to document their diversity, distribution and ecology. Additionally, more effective conservation measures, such as establishment of protected areas and enforcement of regulations, are necessary to mitigate habitat destruction and pollution that in turn negatively affect amphibians.

This study provides a detailed analysis of amphibian species richness and distribution patterns in relation to habitat types in Kaffa of Ethiopia. The research emphasizes on the importance of Kaffa Forest and associated wetlands for amphibian conservation and provides insights into their diversity, distribution and habitat association.

1.1. Justification of the study

Amphibians in Ethiopia are not studied well. Despite some studies on the diversification of African tree frogs (Genus *Leptopelis*) in the highlands of Ethiopia (Abebe Ameha *et al*., 2013; Reyes-Velasco *et al*., 2018b; Goutte *et al*., 2022), the phylogeography, demography and taxonomy of grassland frogs (Genus *Ptychadena*) in the Ethiopian highlands (Reyes-Velasco *et al*., 2018a), evolution of frog species complex of the genus *Ptychadena* in the Ethiopian highlands (Abebe Ameha *et al*., 2013; Freilich *et al*., 2014; Smith *et al*., 2017; Goutte *et al*., 2021), diversity and conservation status of amphibians (Abebe Ameha, 2012) and amphibians and reptiles of Ethiopia and Eritrea (Spawls and Largen, 2010), little is known about amphibian diversity, distribution and their habitat association and threats in the country considering the diverse ecosystem types and altitudinal differences. The previous studies were also limited to some genera (for example; *Ptychadena* and *Leptopelis*) in the highland habitats of Ethiopia.

Despite importance of amphibian in the development of modern medicine, as indicator of environmental change and model animal for different research, they are neglected compared to other species and there is no detailed study on the distribution and ecological roles in Ethiopia.

Some of the remnant forests of Ethiopia that occur along the west and southwestern part of Ethiopia are the route of diversification to the highlands. Specifically, the Kaffa Natural Forest represents one of the last remnants of Afromontane Forest in Ethiopia with potential wetlands that can support amphibians. So far, except for some assessment of herpetofauna (reptiles and amphibians) in Kaffa Biosphere Reserve, there is no detail investigation on the distribution, population status, habitat association and threats of amphibians in Kaffa wetland and riverine forests. Such studies are important to establish priorities for long term conservation and management. Hence, this study provides valuable information on the diversity, distribution, habitat association and threats of amphibians in this area.

1.2. Objectives

1.2.1. General objective

- To investigate species diversity, abundance, distribution and threats of amphibians in the different habitat types of Kaffa area.

1.2.2. Specific objectives

- To assess the diversity and distribution of frogs
- To assess the impact of environmental factors on the diversity and abundance of frogs
- To describe the phylogenetic relationship of *Afrixalus* and *Hyperolius* frog species
- To assess the knowledge, attitude and perception of local community on frogs
- To assess the status of amphibian Chytrid fungus as a conservation threat.

1.3. Research hypotheses

In order to carry out the study on species composition, distribution, habitat association and threats of frogs in the study area, the following hypotheses were used as a guide:

- The Kaffa area possesses a significant diversity of amphibians distributed differently based on habitat type.
- There is a significant relationship between environmental factors and the abundance of frogs in the Kaffa area.
- The existing taxonomy of *Afrixalus* and *Hyperolius* species, which is based on morphological characters, aligns with the phylogenetic relationships inferred from 16S mtDNA markers
- The level of local communities' awareness and interest in the conservation of amphibians in the Kaffa area is significantly higher compared to people in other regions of Ethiopia or abroad.
- The amphibian Chytrid fungus poses a conservation threat to frog species in Kaffa area

2. LITERATURE REVIEW

2.1. Amphibian origin and taxonomy

According to Carroll (2001), it is believed that amphibians originated from Osteolepiform fish during the Devonian Period around 400 million years ago. Amphibians, which include frogs and toads (Anura), salamanders (Caudata), and caecilians (Gymnophiona), are a diverse group of animals with evolutionary lineages that can be traced back to the Early Carboniferous Period. However, the exact origin of these modern amphibians, collectively known as Lissamphibia, remains a topic of controversy in vertebrate evolution due to significant gaps in the fossil record and substantial morphological differences (Milner,1993)

The vicariant-origin hypothesis suggests that the ancient origins of amphibians can be understood by considering their distribution across different regions. Studies estimate that the crown group of amphibians originated during the Carboniferous to Permian Period, around 359 to 252 million years ago (Roelants *et al*., 2007; Pyron, 2011). This timeframe aligns with the existence of the Supercontinent Pangaea (Lomolino, 2010). However, there is no consensus among researchers regarding the exact evolutionary origins of extant amphibian groups (Marjanovic and Laurin, 2009).

For several years, the origin of lissamphibians has been a subject of intense debate, with a multitude of hypotheses proposed to explain their evolutionary roots, despite several comprehensive phylogenetic analyses. The current competing hypotheses regarding the origin of extant amphibians can be grouped into three categories, each proposing different evolutionary relationships and ancestral groups for Lissamphibia. Firstly, some research suggests that Lissamphibia is monophyletic and evolved from the Temnospondyls (depicted in Figs. 1a and b).

In this scenario, the proposed sister group of Lissamphibia could be Doleserpeton (Bolt, 1969), or a combination of Doleserpeton and Amphibamus (Ruta *et al*., 2003), or *Branchiosauridae* (Milner, 1990; Milner,1993), or a specific subgroup within *Branchiosauridae* (Trueb and Cloutier, 1991). Secondly, alternative hypotheses (shown in Fig. 1c) propose that Lissamphibia is monophyletic but originated from a group called "lepospondyls" (Vallin and Laurin, 2004). Thirdly, several studies have put forward a hypothesis of diphyly or triphyly of extant amphibians (illustrated in Fig. 1d). These hypotheses suggest that frogs and sometimes urodeles have their origins within the temnospondyls (sometimes different temnospondyl groups), while apodans and sometimes urodeles have their origins within the "lepospondyls" (Carroll, 2001; Schoch and Carroll, 2003; Lee and Anderson, 2006). These three hypotheses represent different perspectives on the evolutionary origins of modern amphibians and highlight the ongoing scientific discourse and investigation in this field.

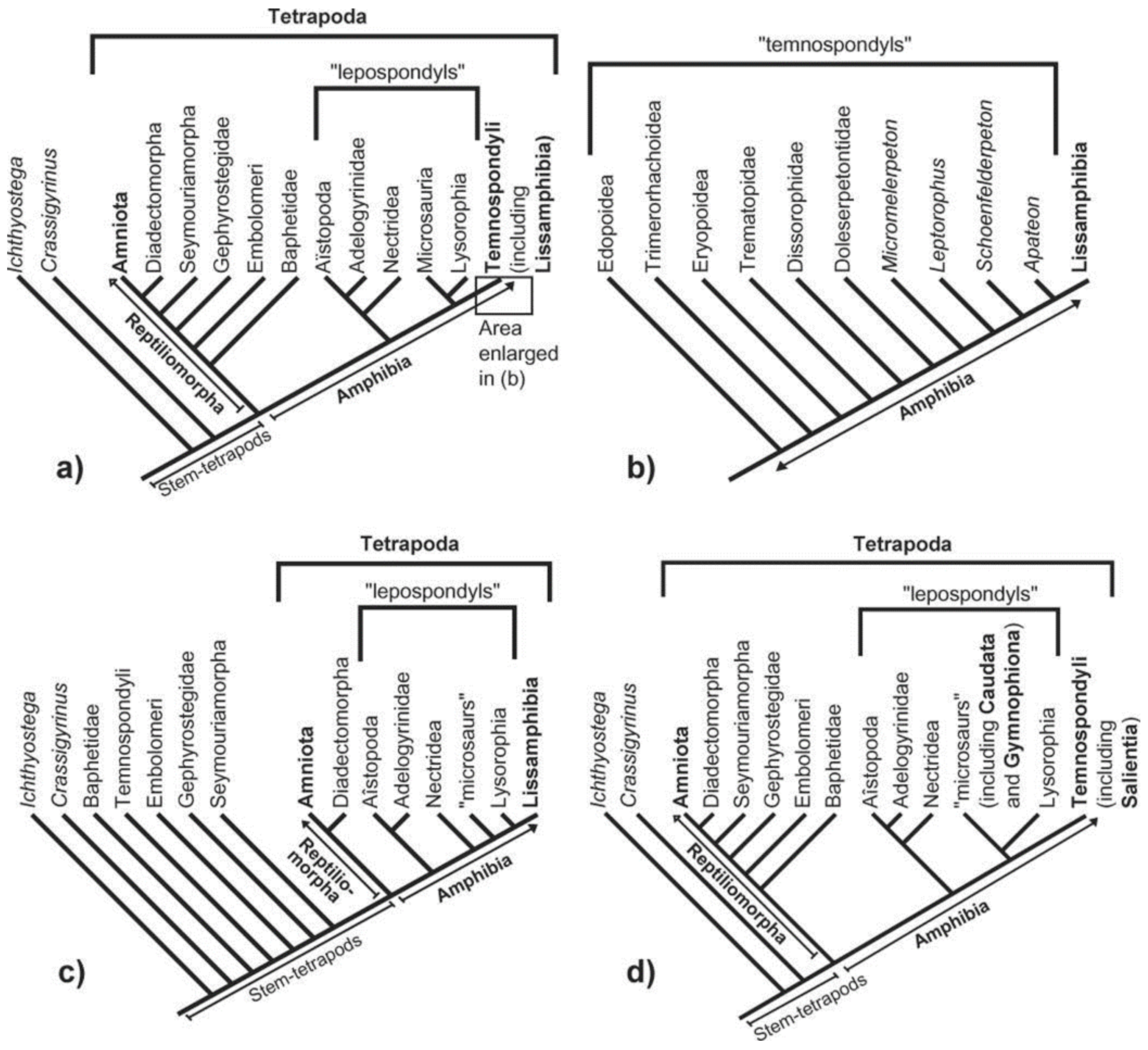


Figure 1. Phylogenetic hypotheses for the origin of extant amphibians: a) and b) Lissamphibia originated from within the group of extinct amphibians known as "temnospondyls." c) Lissamphibia traces its origin back to the group of extinct amphibians referred to as "lepospondyls." d) extant amphibians have a polyphyletic origin, indicating that they have multiple evolutionary origins (Adopted from Marjanovic and Laurin, 2007).

Traditionally, extant amphibians have been classified into three groups: Frog (frogs and toads), Urodela or Caudata (newts, salamanders) and Gymnophiona (caecilians) (AmphibiaWeb, 2023). These groups have been grouped together under the classification of Lissamphibia. However, this traditional view has been challenged in recent times (Anderson, 2008). There is a possibility that frogs, salamanders and caecilians may have evolved independently from different ancestral groups of Paleozoic amphibians. In fact, establishing the main lines of amphibian evolution faces challenges due to the relatively limited fossil record. Certain key groups like caecilians have very few fossils available and as a result the early history of modern amphibian groups remains poorly understood. Another difficulty arises from the fact that living frogs, toads and caecilians exhibit significant anatomical modifications. Frogs and toads are specialized for jumping, while caecilians have completely lost their limbs and are adapted for a burrowing lifestyle. These anatomical specializations make it challenging to find clear evidence of their ancestry (Marjanović and Lauri, 2007).

The classification and relationships of Paleozoic amphibians are also subject to controversy. In fact, if the likely ancestors of Amniota (reptiles, birds and mammals) are included in the Amphibia, then Amphibia becomes a paraphyletic group—a group that does not include all descendants of a common ancestor (Carrol, 2001).

2.2. Amphibian diversity

Amphibians exhibit a wide distribution across various regions and habitats, showing their remarkable adaptability and diversity as terrestrial vertebrates. With over 8,674 known species, amphibians can be found on every continent except Antarctica (Amphibia Web, 2023). The distribution of amphibians is influenced by both regional factors and specific habitat requirements.

Extensive studies have been conducted to explore the historical patterns of diversification within this group, as well as the distribution of species richness across different regions (Roelants *et al.*, 2007; Pyron and Wiens, 2013). Furthermore, researchers have investigated the early biogeographic history of amphibians, revealing evidence for Gondwanan vicariance, which suggests that major lineages diverged due to the separation of Gondwana (Van Bocxlaer *et al.*, 2006). Additionally, a Pangaean origin of early amphibians has been proposed, indicating that their ancestors originated from the Supercontinent Pangaea (Roelants and Bossuyt, 2005). These studies shed light on the evolutionary and biogeographic aspects of amphibians, providing valuable insights into their rich diversity and historical origins.

Amphibians have demonstrated remarkable stability in occupying specific ecological niches, implying that their historical dispersal between different continents may have been limited to similar climatic zones. Research findings indicate that transitions between tropical and temperate regions have been constrained due to niche conservatism (Pyron and Wiens, 2013). Nonetheless, the wide diversity of areas, habitats and climate that modern amphibians inhabit suggests that dispersal must have occurred multiple times (Buckley and Jetz, 2007). Previous studies have often assumed that long-distance over-water dispersal was highly unlikely for amphibians due to their low tolerance for salt (e.g., Van Bocxlaer *et al.*, 2006). However, emerging evidence increasingly supports the role of oceanic dispersal in explaining distributional patterns (Vences *et al.*, 2003).

The biogeographic history of amphibians from the Paleozoic to the Mesozoic Era was primarily shaped by the breakup and subsequent drifting of the supercontinents Pangaea, Laurasia and Gondwana (Roelants and Bossuyt, 2005; San Mauro *et al.*, 2005; Van Bocxlaer *et al.*, 2006). This vicariance process played a significant role in the distribution of most major extant amphibian lineages and its influence is still evident today. However, during the Cenozoic Era, a secondary wave of dispersal events occurred in many amphibian clades, involving overland range expansions, colonization through land bridges and even short-distance oceanic movements. These events have had a substantial impact on the global distribution of numerous clades (Pyron, 2014).

While long-distance oceanic dispersal of major lineages has been supported by evidence in at least three instances, the process of founder-event speciation does not appear to be the predominant force driving amphibian diversification. The ability of certain lineages to disperse and undergo diversification seems to be influenced by the specialization of ecological niches, where a restricted niche can limit distribution and increase extinction risks. Phenotypic adaptations also play a role, as certain eco-morphologies may be more or less suited to dispersal and colonization (Bewick *et al.*, 2012).

Both ecological and evolutionary processes, including speciation, extinction and dispersal, have exerted a significant and lasting impact on the diversity and distribution of amphibians over extended periods of time. These factors highlight the complex interplay between ecological niche specialization and phenotypic adaptations in shaping the evolutionary history of amphibians (Pyron, 2014).

Amphibians occupy diverse ecological niches, ranging from rainforests to rivers, streams, deserts and alpine environments. However, most amphibian species rely on moist environments for their survival, necessitating access to freshwater habitats. Tropical forests exhibit the highest levels of amphibian diversity, reflecting the favourable conditions and ample resources available in these regions. In contrast, temperate and arid regions generally have fewer amphibian species due to the challenges posed by drier conditions. Amphibians do not occur in marine environments, as they are adapted for life on land and freshwater ecosystems. The classification and relationships among zoogeographic regions vary depending on the specific taxonomic group and according to Holt *et al.* (2013), there are 20 regions identified for amphibians (Fig. 2)

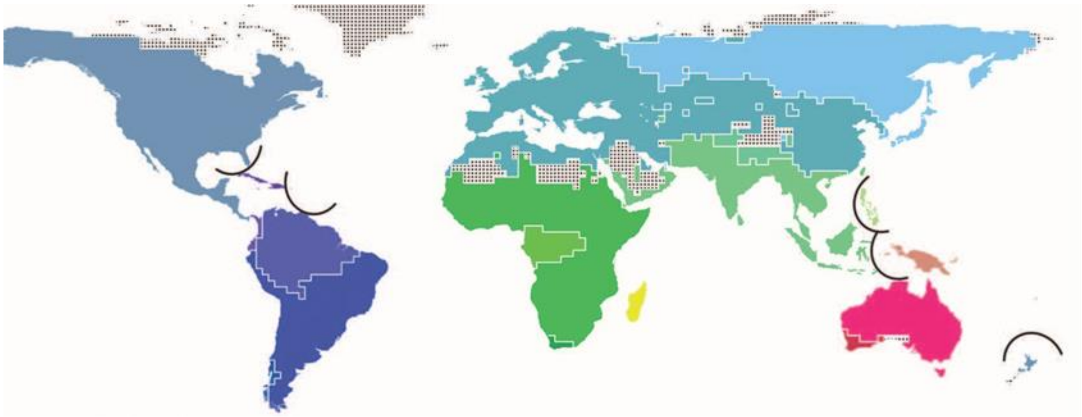


Figure 2. Map to illustrate the terrestrial zoogeographic regions of the world, focusing on species of amphibians. The variation in colours on the maps represents the extent of phylogenetic turnover among regions within each taxonomic clade. Dotted regions indicate areas where no species records are available, and Antarctica was not considered in the analyses (Source: Holt *et al*., 2013).

2.2.1. Diversity of amphibians in Africa

Africa is a continent renowned for its rich biodiversity and amphibians are no exception (McCarthy *et al*., 2005). The continent boasts a diverse array of amphibian species, ranging from frogs and toads to caecilians. The current diversity of amphibians in Africa is shaped by a combination of factors, including the continent's diverse habitats, climatic variations and historical events (Livingstone, 1993).

One of the remarkable aspects of amphibian diversity in Africa is the sheer number of species that inhabit various regions. According to Poynton (1999), Africa exhibits lower amphibian species richness compared to other continents, indicating a relatively lower abundance and diversity. There are about 815 known species of amphibians of which 788 are anurans, 23 caecilians and 4 salamanders (Channing and Rodel, 2019). This number is expected to increase as new species continue to be discovered and described (Amphibia web, 2023). The presence of a vast array of habitats, from lush rainforests to arid savannas and mountain ranges to coastal regions provides a multitude of niches that amphibians have adapted to, resulting in a remarkable variety of species (Channing and Rodel, 2019). For example, the Congo Basin, located in Central Africa, is known for its high amphibian diversity, with numerous species of frogs, toads and caecilians inhabiting the dense forests and wetlands (Nneji *et al*., 2023).

Climate also plays a significant role in shaping amphibian diversity in Africa (Channing and Rodel, 2019). The continent experiences a range of climatic conditions, including tropical, subtropical and arid regions. These variations in temperature, precipitation and seasonality contribute to the creation of distinct habitats and microhabitats, each supporting unique amphibian communities (Rohr *et al*., 2018; Huntley *et al*., 2019). For instance, the coastal regions of West Africa, with their humid and tropical climate, are known for hosting a rich assemblage of amphibian species adapted to the region's specific environmental conditions (Malhi *et al*., 2013).

Historical events have also influenced the current diversity of amphibians in Africa (Loader *et al.,* 2014). The continent has a complex geological and evolutionary history, including the formation of mountain ranges, the shifting of tectonic plates and the influence of ancient climatic changes (Penner *et al*., 2011). These factors have facilitated the isolation of populations, leading to the emergence of new species through processes such as allopatric speciation. The result is a mosaic of amphibian diversity across different regions of Africa (Portik *et al*., 2017).

It is important to note that the current understanding of amphibian diversity in Africa is still evolving. Many remote and understudied regions, such as Eastern Afromontane Biodiversity hotspot, the Congo Basin and the biodiversity hotspots of Madagascar (Nneji *et al*., 2023), hold the potential for the discovery of new species. However, ongoing habitat loss, climate change and other environmental pressures pose challenges to the conservation of amphibians in Africa. Mountainous regions in Africa, such as the Ethiopian highlands, the Eastern Arc Mountains and the Rwenzori Mountains, are biodiversity hotspots for amphibians (Barratt *et al*., 2017). Ethiopia, as a part of Africa, hosts its own unique amphibian fauna, with species adapted to its specific habitats and environmental conditions (Smith *et al*., 2017). Further exploration of amphibians in Ethiopia would provide valuable insights into their distribution patterns, ecological adaptations and conservation needs within the region.

2.2.2. Diversity of amphibians in Ethiopia

Ethiopia offers a unique chance for experts from different fields to explore the ecological and evolutionary dynamics that shape its ecological communities. An intriguing area of study lies in understanding how the region's geological past influences the biogeographical distribution of plants and animals. Despite Ethiopia's captivating characteristics, such as the Great Rift Valley, diverse topography and climate, there is lack of research examining the individual and combined effects of these factors on species colonization, speciation and extinction (Freilich *et al*., 2016; Smith *et al*., 2017).

Ethiopia's amphibian fauna is shaped by its diverse landscapes, which range from highland plateaus to lowland plains and tropical rainforests. The country's varied topography and climatic conditions create different habitats that support various amphibian species (Largen and Spawls, 2010). Some of the key regions and habitats known to host amphibians in Ethiopia include:

Ethiopian Highlands: The Ethiopian highlands, including the Bale Mountains, offer favourable environments for a variety of endemic species such as *Altiphrynoides osgoodi*, *Altiphrynoides* *malcolmi*, *Ericabatrachus baleensis*, *Ptychadena harenna*, *Balebreviceps hillmani*, as well as various *Leptopelis* species. In the Shoa highlands, endemic *Leptopelis* species occur, while *Leptopelis yaldeni* is found in the Gojam highlands.

Rift Valley Lakes: The Ethiopian section of the East African Rift Valley is distinguished by a chain of lakes that play a crucial role as habitats for amphibians. Shores of lakes such as Lake Abijatta, Lake Shala and Lake Ziway are recognized for supporting numerous amphibian species, including a variety of frogs and toads such as *H. viridiflavus*, *Sclerophrys langanoensis* and various *Ptychadena* species (Largen, 2001; Largen and Spawls, 2010).

Wetlands and Marshes: The wetlands and marshes of Ethiopia, specifically in the Gambella and Kaffa regions, host a wide array of amphibian populations. These habitats serve as important breeding grounds for various frog species, including *Afrixalus*, *Hyperolius* and *Ptychadena* species (Largen and Spawls, 2010; Smith *et al*., 2017)

Riverine Forests: Within the riverine forests of Ethiopia, including Kaffa Biosphere Reserve, a diverse range of amphibian species occur (NABU, 2020). Among them is the endemic *Leptopelis* species, specifically *Leptopelis vannutellii*.

2.3. Threats on amphibian

The diversity of extant amphibians is facing severe threats due to various pressures, including Anthropogenic activities like habitat loss, introduction of non-native species, pollution of their environments and urbanization (Cushman, 2006; Olson and Burnett, 2009), global climate change (Stuart *et al*., 2004; Blaustein *et al*., 2010), infectious diseases (Lips, 2016), endocrine-disrupting pollutants, ozone layer depletion (which exposes amphibians to harmful ultraviolet radiation affecting their skin, eyes and eggs). These pressures have led to the extinction of numerous amphibian species over the past few decades (Blaustein and Wake, 1990; Stuart *et al*., 2004). Aamphibians are declining even in habitats that seem healthy and suitable for them (Stuart *et al*., 2004). The most severe impacts usually occur when these threats interact with each other (Thomas *et al*., 2004; Hof *et al*., 2011). Amphibians are particularly vulnerable to environmental changes because they have delicate skin, eggs without shells and they rely on both land and water habitats (Vallan, 2000). When faced with high temperatures and dry climates, amphibians undergo various changes, including alterations in their growth rate, habitat preferences, activity levels, habitat utilization, reproductive capabilities and duration of their reproductive period (Crump, 2003). Some of the major amphibian threats recorded are anthropogenic factors, habitat loss, urbanization, predation, competition, disease and climate change.

2.4. Amphibian conservation action

Since the late 1980s, there have been alarming declines in amphibian populations, with reports of population crashes and mass localized extinctions occurring worldwide. These declines have positioned amphibian species as one of the most significant threats to global biodiversity (McCallum, 2007). In 2004, the International Union for Conservation of Nature (IUCN) reported that extinction rates for birds, mammals and amphibians were at least 48 times higher than natural rates and potentially as high as 1,024 times higher. It was estimated that in 2006, there were 4,035 amphibian species dependent on water at some stage of their life cycle. Out of these, 1,356 species (33.6%) were classified as threatened. However, this figure is likely to be an underestimation as it excludes 1,427 species with insufficient data for assessing their status (Hoekstra *et al*., 2010)

To address this crisis, a global strategy called the Amphibian Conservation Action Plan was released in 2005. This was developed by a collaboration of over eighty leading experts in the field. This plan outlines the necessary actions to mitigate amphibian decline and extinction over the subsequent five years, along with the associated costs. The Amphibian Specialist Group of the IUCN is at the forefront of efforts to implement a comprehensive worldwide strategy for amphibian conservation. Amphibian Ark, an organization established to carry out the ex-situ conservation recommendations of the plan, has been actively working with zoos and aquaria worldwide. They advocate for the creation of assurance colonies of threatened amphibians, encouraging these institutions to play a role in safeguarding the species (McCallum, 2007).

3. MATERIALS AND METHODS

3.1. Description of the study area

3.1.1. Location

Kaffa is located in the southwestern part of Ethiopia. Bonga, the main town of the zone, is 460 km further along the southwest of Addis Ababa. Kaffa is bordered on the south by Debub Omo, on the southwest by Bench Maji, on the west by Sheka, on the north by the Oromia Region, and on the east by Konta. Administratively, the zone is part of the Southwest Region of Ethiopia, and is divided into twelve Woredas (Fig. 3). The total land area of Kaffa zone is 10, 602.7 km^2.

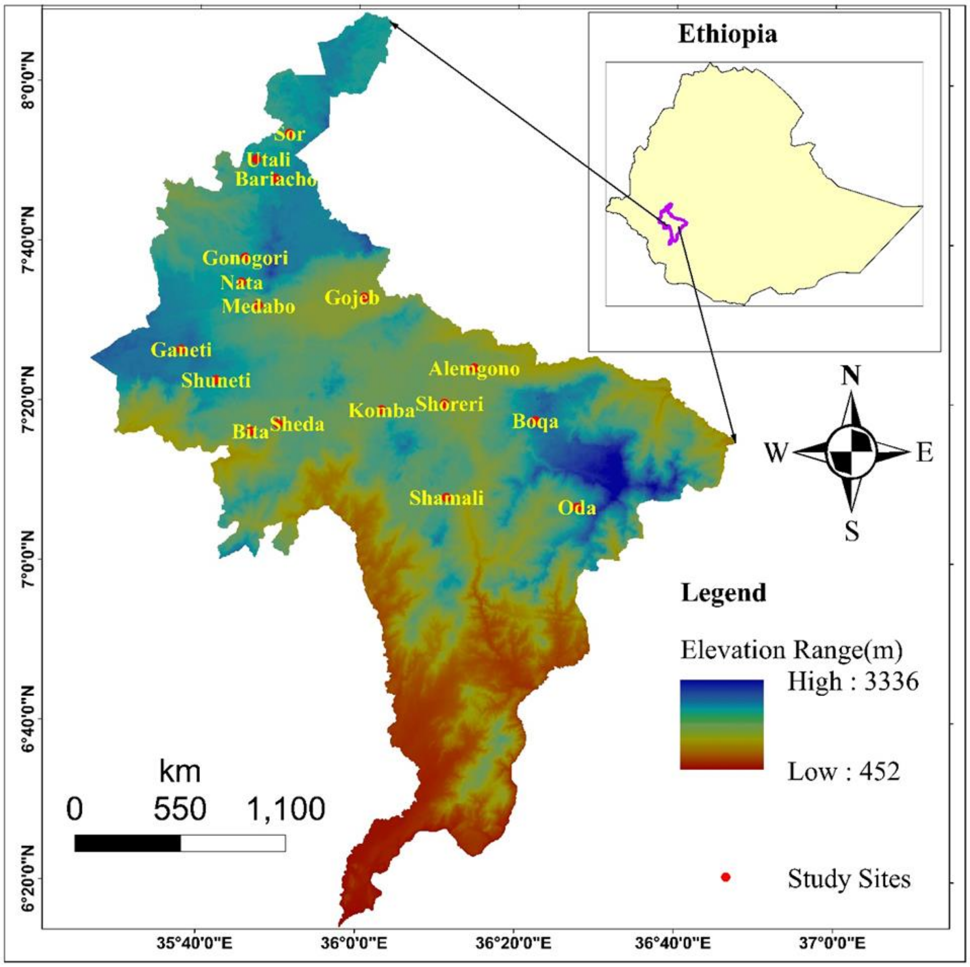


Figure 3. Map of Kaffa zone with seventeen study sites along elevation gradient

3.1.2. Geology

Ethiopia's magma dome and the formation of the East African Rift system both had a significant impact on the country's geology and tectonics. The Ethiopian highlands are built upon the Ethiopian magma dome, which was created by several volcanic eruptions and geological formations throughout several geological Eras (NABU, 2020). The present study area, Kaffa, is found on the western plateau of these highlands.

The various terrain features of Kaffa region are the outcomes of the Tertiary geological periods and the succeeding geomorphic processes. Precambrian rocks from the basement rock are heavily folded and faulted and they are covered with Mesozoic marine layers and Tertiary basalt traps. Rhyolites, trachytes, tuffs, ignimbrites, agglomerates and basalts are some of the Tertiary volcanic rocks. The majority of the substrates of Afromontane forests are established on these rocks (Tadesse Woldemariam, 2003).

3.1.3. Topography

The topography of Kaffa is characterized by severely fractured, rough highlands in the majority of the north and centre and undulating lowlands in the southern section. The natural topography of Kaffa area is extremely sloppy, ranging from 10% to over 60% (TAM Agribusiness, 2004). The altitude of Kaffa area typically ranges from less than 500 m asl in the southern region extending from undulating plains like in Decha Woreda (the lowest 500 m asl near the Omo River) to around 3000 m asl in the highlands of Menjiwo and Tello Woredas (peak 3348 m asl at Mount Shetra).

3.1.4. Drainage

Kaffa zone possesses several rivers. Gojeb River is the main one travelling from the north to the northeastern region of the zone, where it merges with Gibe River to form the Omo River.

Additional rivers include those that run toward the Omo and Gilo Rivers, including Woshi, Baro and Beko.

3.1.5. Soil

Soil map created by WBISPP (2004) reveals that nitosols are the dominant type of soil in Ethiopia's southwest highlands, including the Kaffa region. Nitosols and acrisols can both be found in Ethiopia's southwest highlands, mostly in areas with a significant rainfall. High rainfall causes significant soil leaching, which results in nitosols that are rich in non-soluble elements like iron and aluminum but lacking in soluble minerals like potassium, calcium and others. Due to leaching, these soils have a high concentration of iron (ferric) oxides, which give them their reddish-brown appearance. However, at present, these are frequently observed in both farmed areas and mountain grasslands. Additionally, according to Tadesse Woldemariam (2003), the soils (red or brownish ferrisols) originate from the volcanic parent material. The study region also contains limited eutricfluvisols, chromatic luvisols, chromatic vertisols and pellicvertisols (Tadesse Woldemariam, 2003; EWNHS, 2008)

Schmitt (2006) states that the soils of Kaffa region are ideal for cultivating coffee because they are rich in humus, well-drained and have a high water-holding capacity. The soils are described as deep red to brown red, lateritic loams or clay loams of volcanic origin, with moderate or medium fertility and with pH values ranging from 5.3 to 6.6. The pH levels of the soils in the Kaffa area are at the lower end of this range.

3.1.6. Climate

Kaffa area is located along the southwest of the country, where it experiences ample and frequent rainfall. This is a result of the existence of an evergreen forest cover on top of the ‘windward’ site of the moist monsoon winds. The climate is described by a bimodal rainfall pattern. There is a short-rainy season from February to April in addition to the main rainy season, which lasts from June to September. The lowest elevations receive 1,500 mm of annual rainfall on average, while the highest elevations receive 2,000 mm. Kaffa area has a wide range of topography and altitude, which results in several microclimatic variations from the typical rainfall patterns. The area experiences an average annual rainfall 2103 mm and maintains an average annual temperature 18.2 ^{o}C (Fig. 4).

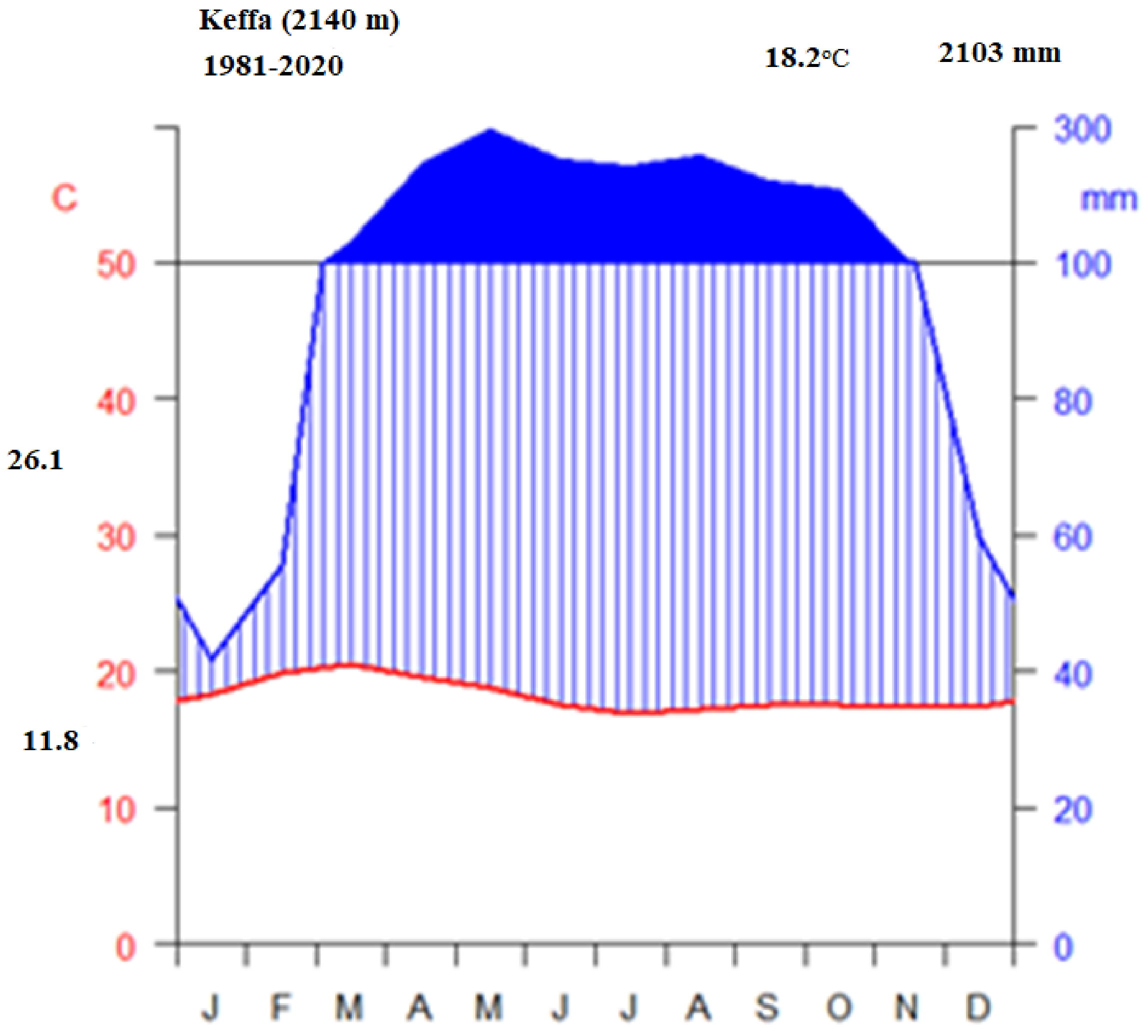


Figure 4. Climate diagram of the Kaffa zone (The climate point data was taken from NASA, 2021)

3.1.7. Wildlife and tourism

Kaffa zone harbours several described plant and animal species, and is home to a wide variety of plant species that are distinguished by dense forests, numerous endemic species and medicinal plants (Tekleab Bulo and Sanjeev, 2020).

Kaffa zone is home to more than 25% of Ethiopia's plant species, of which 30% are endemic. Bamboo forests, forest coffee, Afromontane woodlands, grasslands and enset are the most abundant plant types (Tekleab Bulo and Sanjeev, 2020). Kaffa is renowned for its abundance of animal diversity, encompassing a wide variety of species. For instance, Kaffa is home to an impressive assemblage of 300 insect species, including 63 dragonfly species. Furthermore, Kaffa provides habitat for 18 species of amphibians, 232 species of birds, six species of fish, ten species of reptiles, and an astonishing 55 species of mammals (NABU, 2020).

3.2. Materials

Plastic bags and jars for temporary containment, a dissecting kit for specimen examination, a Garmin GPS 72 device for location tracking, Sony Stereo Digital Voice Recorder, Nikon D7500digital camera for visual documentation, aquarium nets for capturing frogs and 150mm Dial caliper for precise measurements were used during the study period. In the laboratory, QIAGEN DNA extraction kits and chemicals such as QIAGEN Protease (Proteinase K), Buffer AL, ethanol (96–100%), Buffer AW1, Buffer AW2, and Buffer AE were employed to isolate DNA from frog samples. A PCR machine was utilized to amplify the extracted DNA. The Qubit system was used to accurately quantify the concentration of DNA. Additionally, an electrophoresis apparatus or electrophoresis system was employed to separate and analyze DNA based on its size.

3.3. Methods

3.3.1. Preliminary study

Before the actual data collection, basic information on the location, climate conditions, topography, habitat types and surrounding villages of the study area was carried out as the preliminary survey. In addition, forest and wetland types of possible presence of frogs, landscape, representative villages and representative habitat sites/blocks and their sizes were identified.

3.3.2. Sampling design

The study was carried out both during the wet and dry seasons from July 2018 to February 2022. The study area was stratified into three habitats: riverine forest, wetland and agricultural land (enset plantation) as shown in Fig. 5

We selected seventeen study sites (Fig. 3) located within existing macrohabitats, specifically enset plantations, riverine forests, and grassy wetlands as shown in Fig. 5. In total, we established fifty-one rectangular transects, with seventeen transects in each habitat type. Each transect had dimensions of 200 m in the north-south direction and 100 m in the east-west direction. For the purpose of data collection, we subdivided the entire length of each transect, which was 600 m long, into smaller units measuring 25 m. This division of the transect followed the methodology established by Rödel and Ernst (2004). We employed a method to distinguish and identify each subunit by using colored flag-tape.

The transect paths were intentionally kept open to allow for consistent walking speed throughout the surveys. We minimized extensive cutting and disturbance to important habitat features. The starting coordinate for each transect was consistently set at the southeast corner to ensure uniform geographic orientation across sampling units (Rödel and Ernst, 2004).

During the surveys, we recorded all amphibian specimens that were visible within a distance of 1 m on both sides of the transect, including both terrestrial and arboreal species. We employed a combination of Standardized Visual Encounter Survey and Acoustic Encounter Survey methods. These survey methods were not separated, and both were included in the analysis of the transect data within the study area (Appendix 1).

Figure 5. The three habitat types of the study area: Enset plantation (a/Gonogori and d/Oda), Ganeti wetland (b) and Ganeti riverine forest (c) (Photo: Abeje Kassie and Tariku, January, 2019)

3.3.3. Data collection

3.3.3.1. Frog diversity, abundance and distribution

Direct observation

Standardized visual and acoustic transect sampling methods were used to collect data on frog species diversity and distribution (Rödel and Ernst, 2004; Veith *et al*., 2004). Each transect was searched for four hours, each six days during the wet and dry seasons. A team of three persons sampled three transects per day at a constant speed from 6:00 to 9:00 a.m., in the morning and 6:00 to 9:00 p.m., in the evening using a head torch in order to enhance species detection and capture. Although many frog species call much later than 9:00 p.m, we were not allowed to stay in the field after 9:00 p.m. due to security concerns. Visual encounter survey, opportunistic observations, acoustic encounter surveys, and drift fence and pitfall trap methods developed by Heyer *et al*. (1994) and Rödel and Ernst (2004) were used to conduct the search. Drift fence and pitfall trap with a bucket (10 litres capacity) were used to collect frog species from the riverine forest habitat (Fig. 6). The drift fence was made of plastic sheet measuring 50 cm in height and 55 m in length. This structure was built to intercept and redirect frogs on the ground into pitfall traps. In all riverine forest habitats, transect lines with drift and pitfall traps were built. Ten (Eleven) buckets were placed deep into the ground at 3 m intervals along the drift fence at each site where the method was used. The drift fence and pitfall traps were checked twice a day, early morning and before sunset. An active search for frogs was conducted randomly in locations away from the transect lines, which included turning over logs, leaf litter, tree holes, rocks and other potential hiding places. Suitable habitats for sylvacaecilia grandisonae were identified and selected for sampling. These habitats typically included riverine forest, coffee leaf litter, or underground burrows. Systematic field surveys were conducted to locate and capture sylvacaecilia grandisonae. We carefully looked for them in their preferred habitats by digging in the soil, sifting through the layer of fallen leaves, and searching near bodies of water.

All captured specimens were placed in a separate plastic bag with moist leaves or grass for further identification. When possible, species identification was done on the field using published papers, keys and field guidebooks (Largen, 2001; Largen and spawls, 2010; Abebe Ameha, 2012; Channing *et al*., 2012; Channing and Rodel, 2019; Tiutenko and Zinenko, 2020; Goutte *et al*., 2021; Tiutenko and Zinenko, 2021). Individuals that were not identified on sites were collected as voucher specimens where they were euthanized by topical application of 20% benzocaine gel and fixed in a 10% formalin solution for 24-48 hours. Then, they were subsequently transferred to 70% ethanol for preservation and finally deposited at the Ethiopian Biodiversity Institute Museum, Addis Ababa. Tissue (from muscle/liver) was collected for further DNA analysis and preserved in 97% ethanol. Habitat type and geographic coordinates were recorded for all individual frogs sighted where geographic coordinates were determined using a Garmin GPS 72.

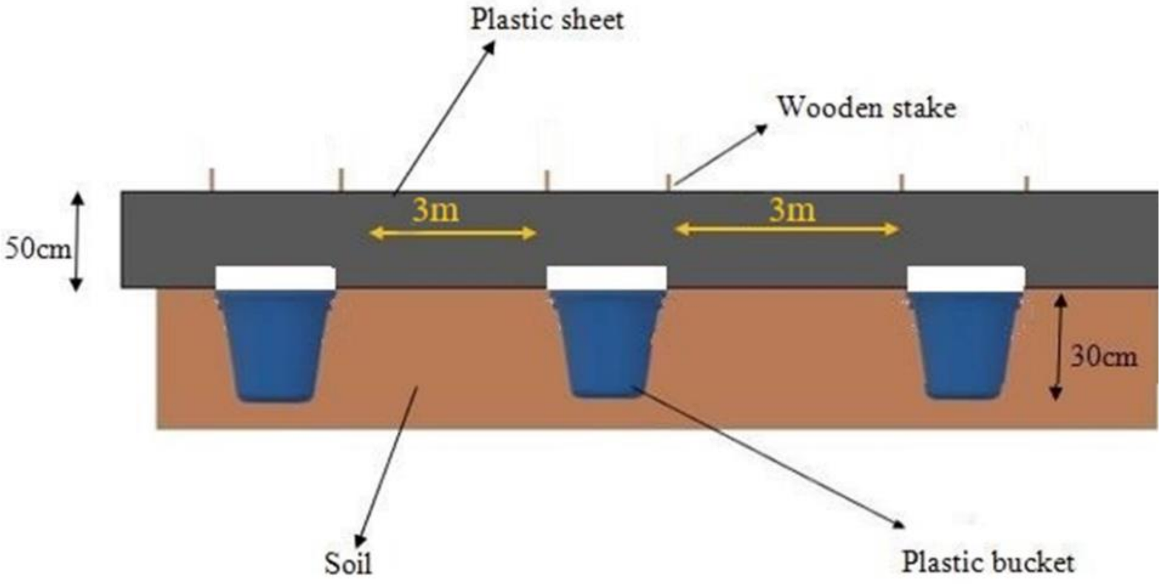


Figure 6. Sketch of drift fence and pitfall trap layout.

Advertisement call records

We recorded advertisement calls from different frog species *in - situ* (in the wetland, riverine forest and enset plantation of Kaffa area) at a distance of 0.5-2 m in order to avoid near-field effects or substantial attenuation of the sound. The taxonomic identity of populations and thus individuals was established using morphological traits and genetic barcoding. The sound recordings were made in June and July (2020 and 2021). Advertisement calls were recorded in the afternoon (between 6:00 and 9 p.m.), with air temperatures ranging from 17 to 25^{o}C with limited wind.

DNA barcoding

Species were tentatively identified in the field whenever possible, and DNA from liver/muscle tissue was also barcoded to confirm field identification and to determine whether any cryptic and/or candidate species were present. A sample of either liver or muscle tissue weighing 25 mg was collected.

All DNA extractions were carried out using the Qiagen Tissue and Blood kit in accordance with the manufacturer's instructions. DNA extraction was conducted at the Evolutionary Genomics Lab, New York University Abu Dhabi, Abu Dhabi, United Arab Emirates. Given the universal success of 16S primers and the widespread use of this mitochondrial region for frog barcoding, we chose to amplify this region, sequencing at least two individual representatives from each morphologically identified species (Fig. 7). The following were the PCR thermo-cycling conditions: 2 minutes at 94°C, followed by 35 cycles of 94°C for 30 seconds, 48°C for 30 seconds and 72°C for 1 minute, finishing with an elongation step of 72°C for 1 minute.

A 48 µl master mix PCR reaction volume was used, using the primer pair 16S_F, 5’-CGCCTGTTTAYCAAAAACAT-3’ and 16S_R, 5’- CCGGTYTGAACTCAGATCAYGT-3’. For *Leptopelis* species, Cytochrome c oxidase I (COI) was also sequenced to improve taxonomic identification using the primer pair COILEP-F1, 5’-GCATAGTCGGAACCGCTTTA-3’ and COILEP-R1, 5’-GGGTCGAAGAATGTGGTGTT-3’.

The PCR thermocycling conditions for COI were; 2 minutes at 94°C, followed by 30 cycles of 94°C for 30 seconds, 58°C for 30 seconds and 72°C for 1 minute, finishing with an extension step of 72°C for 1 minute. The BGISEQ-500 sequencing service was used to sequence unpurified PCR products in both forward and reverse directions. All amplicons were sequenced by BGI using Sanger sequencing in BGI Tech Solutions, Hongkong Co., Limited, P.R. China.

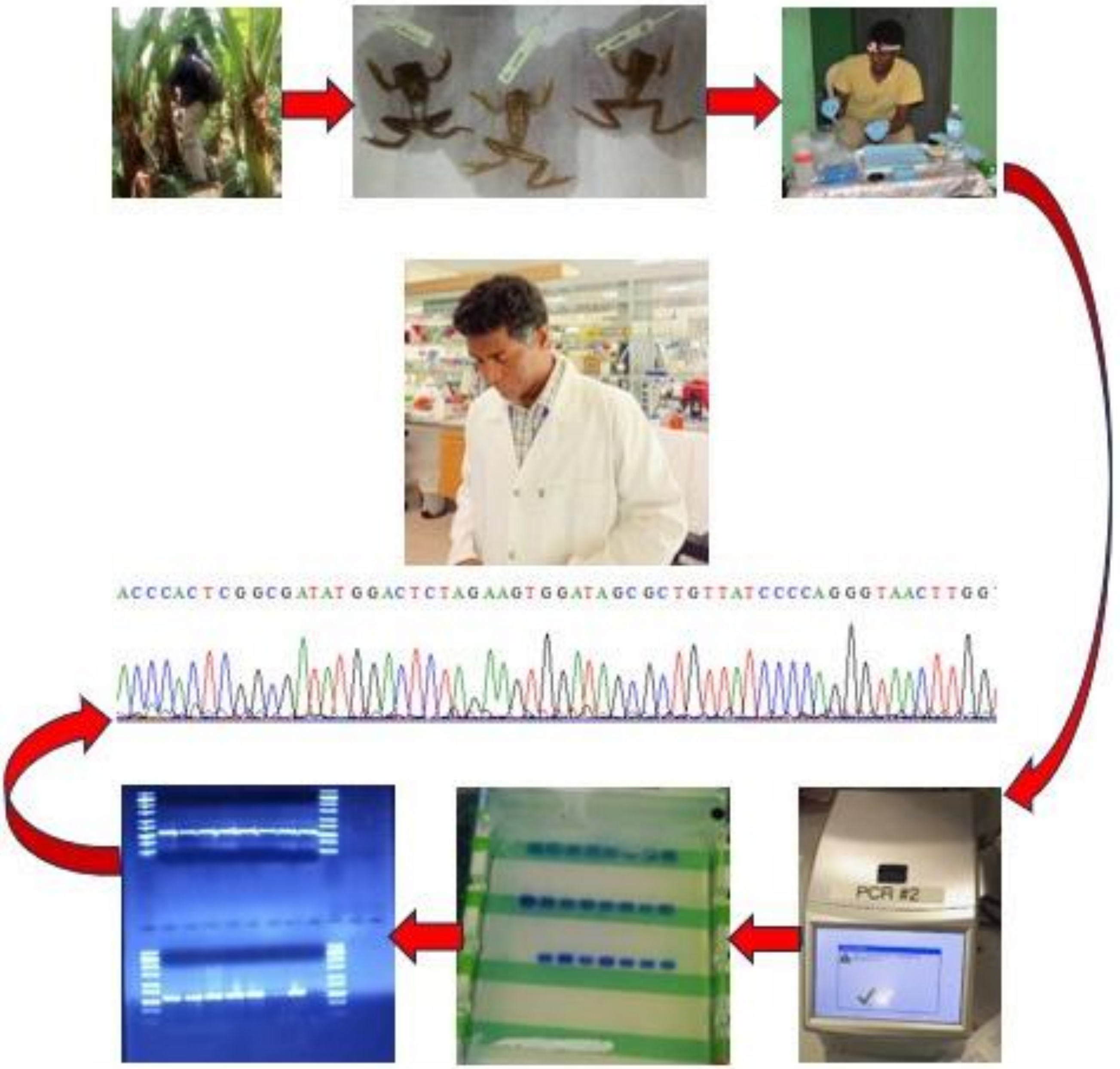


Figure 7. This figure illustrates the general procedures followed in the current study, from data collection to sequencing.

3.3.3.2. Knowledge, Attitude and Perception surveys on Frogs

A questionnaire survey was conducted to assess the knowledge, attitudes, and practices (KAP) of local communities regarding frogs, as well as their perceptions and attitudes towards frog and their habitat conservation. Direct observations were also carried out. Data were collected from selected Participatory Forest Management (PFM) associations in the Kaffa area. The total population of PFM members residing in the study area's buffer zone was 2625, distributed across 12 PFM associations. Using the purposivesampling technique developed by Yemane (1967), a sample size of 347 respondents, comprising 207 males and 140 females, were determined using the formula:

$$\boldsymbol{n} = \frac{\boldsymbol{N}}{\mathbf{1} + \mathbf{N}(\boldsymbol{e}^{\mathbf{2}})}$$

Where **N** represents the population size, **n** represents sample size and **e** represents the desired level of precision at a 95% confidence interval.

The main purpose of the questionnaire was to investigate attitudes, perceptions and major threats that affect frogs. The selection of respondents from each PFM association member was based on the proportional representation of members in each PFM site, as indicated in Table 1. The number of male and female respondents chosen also followed the proportional representation of male and female members in each PFM association (sites).

The questionnaire was initially prepared in English (Appendix 2) and Amharic and subsequently translated into the Kaffa (Kafi noono) language, as the majority of the respondents were Kaffa (Kafi noono) speakers. Additionally, Amharic language was utilized whenever necessary to provide further clarification of the questions and terms. All interviews were conducted by a skilled researcher who is a native speaker of Kaffa language and hired from the local area. Prior to each interview, participants were assured that their participation was entirely voluntary and that their responses and suggestions would be treated with confidentiality solely for the purposes of the study.

Table 1. Members of the PFM association selected for interview from different areas of Kaffa.

Woreda	PFM (Participatory Forest Management) Site	Male		Female		Total sample size
		Total	Selected	Total	Selected	
Gimbo	Agama	110	14	104	14	28
Gimbo	Kilira	100	13	34	6	19
Gewata	Addis Brihan	220	29	167	22	51
Bita	Bekiti	158	21	86	11	32
Bita	Kaweta	75	10	87	11	21
Bita	Eyo daneti	186	25	34	4	29
Adiyo	Chefe-hana	143	19	3	0	19
Tello	Sharesha	93	13	100	13	26
Gesha	Daditi	129	17	17	2	19
Bita	Kemo watigoti	90	12	110	15	27
Saylem	Odori goti	59	8	78	10	18
Decha	Bebenoch	202	27	240	31	58
	Total	**1565**	**207**	**1060**	**140**	**347**

3.3.3.3. Frog threat assessment

Swabbing of frogs for Amphibian Chytrid fungus (Batrachochytrium dendrobatidis (Bd))

Fieldwork was carried out in 12 locations throughout Kaffa area from 2018 to 2022 (Fig. 8) and sampling took place during the wet and dry seasons in the agricultural land, wetlands and riverine forests.

To assess Bd infection in frogs, the skin over the pelvic patch, ventral skin and plantar surface of each hind foot were swabbed using a sterile fine-tipped rayon swab (MW100-100; Medical Wire and Equipment Co.). After sampling, the tip of each swab was carefully placed in a 1.5 ml vial containing 95% ethanol to preserve DNA and prevent degradation caused by the tropical heat (Van Sluys *et al*., 2008). Subsequently, the vials with the swabs were shipped to Evolutionary Genomics Lab, New York University Abu Dhabi, Abu Dhabi, United Arab Emirates for further analysis.

To reduce cross-contamination when sampling, rigorous precautions were used. Each specimen was handled using surgical gloves, which significantly decreased the chance of cross-contamination. The animals were also held in fresh specimen containers (plastic bags) before being swabbed, removing the potential for residual contamination from earlier samples.

To prevent the transmission of Bd zoospores between sample sites, thorough cleaning and disinfection protocols were implemented for all equipment used during the fieldwork. These included cleaning and disinfecting specimen bags and other equipment using bleach. These measures were aimed to eliminate any potential contamination and reduce the risk of spreading Bd zoospores from one sampling location to another.

For the purification of DNA from the swabs, a PCR Purification Kit from Qiagen was utilized, following the instructions provided by the manufacturer. These standardized procedures ensured the efficient extraction and purification of DNA from the samples, facilitating subsequent genetic analyses and accurate assessment of Bd infection in the studied frog species.

DNA extraction and PCR amplifications were conducted following the protocols described by Annis *et al*. (2004) and Boyle *et al*. (2004). A total master mix of 23 ml was prepared, comprising 2.5 mM of the forward primer Bd1a (5'-CAGTGTGCCATATGTCACG-3') and reverse primer Bd2a (5'-CATGGTTCATATCTGTCCAG-3'), 0.45 mM of MgCl2, 0.8 NED Taq, and 2 mM of dNTP. As an alternative, we also utilized a different primer set consisting of the forward primer ITS1-3 Chytr (5'-CCTTGATATAATACAGTGTGCCATATGTC-3') and the reverse primer 5.8S Chytr (5'-AGCCAAGAGATCCGTTGTCAAA-3'). These PCR amplifications were crucial for detecting the presence of Bd and generating DNA fragments for subsequent analysis. The amplification conditions included an initial denaturation at 94°C for 3 minutes, followed by 35 cycles of 45 seconds at 94°C, 45 seconds at 60°C (the annealing temperature for the Bd1a and Bd2a primers), and 1 minute at 72°C. A final extension at 72°C for 3 minutes completed the amplification process.

The amplified DNA fragments were subsequently separated using gel electrophoresis. For this procedure, 1 g of agarose gel was prepared along with 100 ml of TAE buffer. To visualize the DNA fragments, 2µl of ethidium bromide was added to the gel. The gel was then subjected to electrophoresis, allowing the DNA fragments to migrate based on their size. Finally, the gel was examined under UV light and the presence or absence of Chytrid was determined by comparing the observed bands with the molecular weight ladder (Appendix 3).

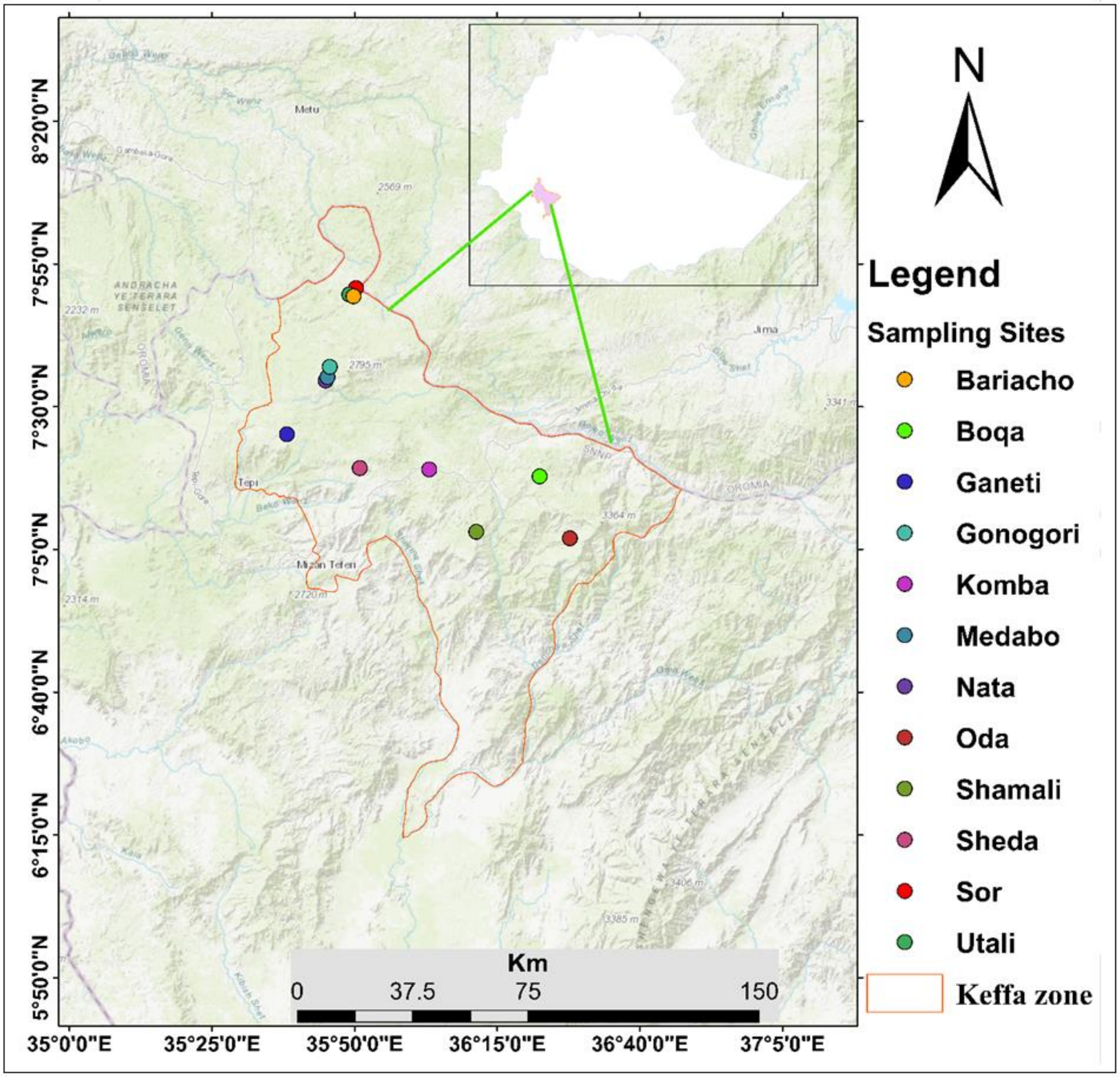


Figure 8. Geographical representation of surveyed sampling sites for Chytrid fungus in Kaffa area.

3.3.4. Data analyses

Frog species diversity of each habitat across the different sites was analyzed by using the formula of the Shannon-Wiener diversity index (H') (Shannon and Weiner, 1949; Magurran, 1988):

$$\boldsymbol{H' = -\sum_{i=1}^{s}(P_i \ln P_i)}$$

Where s is the number of frog species, P_i is the proportion of the total sample belonging to the **i** th frog species and **ln** is a natural logarithm. Species richness was the total number of frog species recorded in one community.

Simpson diversity index was calculated to determine the dominance of frog species using the formula:

Simpson diversity index: $\boldsymbol{D = 1 - \sum_{i=1}^{s}(P_i)^2}$

Evenness index of frog species in the study area was calculated using the following equation (Shannon and Weiner, 1949):

E =H'/ ln (s)

Where **E** = Shannon evenness index

H' = Shannon-Wiener diversity index,

ln = the natural logarithm.

s = the number of frog species recorded in one community.

Evenness index has a range of values from 0 to 1 and as the value approaches to 1 it means the species are evenly distributed and vice versa. Diversity indices between habitats were compared in Past software (Hammer *et al*., 2001) with Multiple Hutcheson t-tests using Package ecolTest version 0.0.1 (Hutcheson, 1970). Frog species composition and turnover were compared between habitats and study sites using Sorenson's Coefficient Similarity Index (SCSI) (Sørensen, 1948) and Jaccard Coefficient Similarity Index (JCSI) (Jaccard, 1912), calculated as:

$$\boldsymbol{JCSI} = \frac{\mathbf{a}}{(\mathbf{a} + \mathbf{b} + \mathbf{c})}$$

$$\boldsymbol{SCSI} = \frac{\boldsymbol{2a}}{(\mathbf{2a} + \mathbf{b} + \mathbf{c})}$$

where **a** is the number of species that are present in both habitats (site) 1 and habitat (site) 2, **b** is the number of species that are present only in one habitat (site) 1, and **c** is the number of species that are present only in habitat (site) 2. A low degree of similarity indicates a high rate of turn over as the index providing a range from 0 (there are no species shared by the two habitats) to 1 (two habitats have a completely identical set of species).

Sampling effort was assessed using a species accumulation curve generated by the Vegan package's *specaccum* function (Oksanen *et al*., 2019). Frog species diversity was estimated by the habitat type using Hill numbers developed by (Chao *et al*., 2014). This is the second approach to measure species diversity. We considered q = 0 (frog species richness), q = 1(exponential of Shannon's entropy index) and q=2 (inverse of Simpson's concentration index) and q representing the effective number of species. These analyses were performed using iNEXT online (Chao *et al*., 2016). We chose this approach in addition to traditional estimators because it allows us to compare species richness despite differences in sampling effort and/ most importantly, because it allows us to extrapolate and compare species richness at equal sample coverage even with the smallest samples (Chao *et al*., 2014). The iNEXT package (Hsieh and Chao, 2016) was used to generate rarefaction curves with 200 bootstraps to estimate 95% confidence limits (CL) and only records from transects (26 frog species). General search records were excluded from these analyses.

To analyze the relative importance of some environmental parameters such as temperature, precipitation, altitude and slope, we fitted generalized linear models (McCullagh and Nelder, 1989) to the data using the GLM function implemented in the statistical package nlme (Pinheiro *et al*., 2012). Since our data were not over dispersed, analyses were carried out with Poisson distribution and log link function. To demonstrate the significance of frog diversity indices at the p 0.05 level, Diversity t-test and Multiple Hutcheson t-test were used and all analytical data procedures were carried out using the R software package version 4.2.1 (R Core Team, 2022) and Paleontological Statistics (PAST) version 4.11 educational software package (Hammer *et al*., 2001).

Phylogenetic and molecular analyses

Mitochondrial sequences were visualized, trimmed and aligned in MEGA11 Software (Tamura *et al*., 2021) and were run through nucleotide BLAST (NCBI) totentatively identify species of frogs. Sequences retrieved from GenBank as well as the sequences we generated were used in a phylogenetic analysis using the Maximum Likelihood (ML) method (Saitou and Nei, 1987; Tamura and Nei, 1993), based on the Tamura-Nei distances. The final dataset that contained PCR products from all samples was 419 base pairs for *Phrynobatrachus* species and 505 base pairs for *Leptopelis* species. The GeneBank accession numbers were listed in Appendix 4. The robustness of the nodes was assessed by 1,000 bootstrap replicas of the data (Felsenstein, 1985). Evolutionary distances between sequences were computed using the p-distance method (Nei and Kumar, 2000).

Morphometric analysis

Species identification of amphibians was based on external morphological features (mainly colour pattern, shape of pupil and head, condition of tympanum, texture of skin, degree of webbing on hand and foot, and size). Measurements were performed for *Afrixalus* and *Hyperolius* genera using Standard Excel to determine the mean and standard deviation (SD) statistics. We employed ratios between various morphological parameters, such as Head Length to Snout-Vent Length (HL/SVL), Head Width to Snout - Vent Length (HW/SVL), Inter-Nares Distance to Head Width (IND/HW), Tibia Length to Snout-Vent Length (TL/SVL) and Thigh Length to Snout-Vent Length (THL/SVL), to adjust for body size when taking measurements. The significance difference among the measured species was tested using analysis of variance (ANOVA).

Frog sound analysis

Audacity software version 3.3.3 was used to analyze audio recordings of three frog species collected from the study area. The aim was to extract high-quality audio segments conveying the most important information. The waveform window was used to choose calls. For the spectrogram and oscillogram pictures, we used the Hann window, which has a size of 512 samples and used to analyze frog sound data using Raven Pro 1.6.5 software. In order to balance temporal and frequency resolution, the analysis was run with a 50% overlap and 256-sample hop size. The frequency grid size was set at 93.8 Hz, and the Discrete Fourier Transform (DFT) size was 512 samples (Lisa, 2023). We employed the note-centered approach (identifying unbroken units of sound as notes and their entirety as a call) and the notes of pulses, notes, and calls as described by Köhler *et al*. (2017). The advertisement call characteristics were compared using the Wilcoxon rank-sum test.

4. RESULTS

4.1. Amphibian diversity

The primary objective of the research was to evaluate amphibian diversity in Kaffa area, specifically focusing on caecilians, frogs, and toads. The only caecilian species known in Ethiopia is the Aleku caecilian (*Sylvacaecilia grandisonae)*. This species has been recorded from Jimma and Kaffa areas, particularly within the coffee plantation under leaf litter. However, our research findings solely encompass frog species. Consequently, our discussion will be limited to frogs.

4.2. Frog diversity, distribution and abundance in Kaffa area

4.2.1. Frog diversity

A total of 3,672 man-hours were spent for searching frogs in the study sites. 5,678 frog specimens were captured and identified. Although the majority of individuals could be identified unambiguously using morphology alone, it was not always the case in particular for individuals belonging to the *Ptychadena*, *Leptopelis* and *Phrynobatrachus* genera, and hence barcoding was computed. Barcoding of *Ptychadena* confirmed the validity of our morphological assignment and indicated the presence in the study area of a number of endemic grass frogs such as *P. beka*, *P. erlangeri*, *P. neumanni* and *P. doro* (Table 2).

Table 2. Number of specimens and relative abundance of frog species recorded in three habitat types of Kaffa (WL: Wetland; EP: Enset Plantation; RF: Riverine Forest)

Family	Species	Number of specimens				Relative abundance (Rank)
		WL	EP	RF	Total	
Hyperoliidae Laurent, 1943	*Afrixalus clarkei* Largen, 1974	784	115	25	924	16.27 (3)
	Afrixalus enseticola Largen, 1974	99	148	0	247	4.35 (8)
	Hyperolius howelli du Preez and Channing, 2013	478	0	0	478	8.41 (4)
	Hyperolius kivuensis Ahl, 1931	260	0	0	260	4.58 (7)
	Hyperolius viridiflavus Duméril and Bibron, 1841	247	0	13	260	4.58 (7)
	Kassina senegalensis Duméril and Bibron, 1841	23	0	0	23	0.41 (15)
	Paracassina obscura Boulenger, 1895	0	12	0	12	0.21 (20)
Arthroleptidae Mivart, 1869	*Leptopelis* sp. 1	22	0	0	22	0.39 (16)

	Leptopelis sp. 2	0	28	0	28	0.49 (14)
	Leptopelis cf. *susanae* Largen, 1977	2	0	0	2	0.04 (25)
	Leptopelis vannutellii Boulenger, 1898	0	167	289	456	8.03 (5)
Pyxicephalidae Bonaparte, 1850	*Amietia nutti* Boulenger, 1896	0	0	46	46	0.81 (12)
Conrauidae Dubois, 1992	*Conraua beccarii* Boulenger, 1911	0	0	7	7	0.12 (23)
Hemisotidae Cope, 1867	*Hemisus marmoratus* Peters, 1854	0	0	3	3	0.05 (24)
	Hemisus microscaphus Laurent, 1972	0	8	41	49	0.86 (11)
Phrynobatrachidae Laurent, 1941	*Phrynobatrachus* sp. 1	883	0	274	1157	20.38 (1)
	Phrynobatrachus natalensis Smith, 1849	1044	0	0	1044	18.38 (2)

Ptychadenidae Dubois, 1987	*Ptychadena anchietae* Bocage, 1868	48	7	0	55	0.97 (10)
	Ptychadena beka Goutte, Reyes-Velasco, Freilich, Kassie, and Boissinot, 2021	12	10	0	22	0.39 (17)
	Ptychadena doro Goutte, Reyes-Velasco, Freilich, Kassie, and Boissinot, 2021	15	0	0	15	0.26 (19)
	Ptychadena erlangeri Ahl, 1924	29	0	0	29	0.51 (13)
	Ptychadena neumanni Ahl, 1924	16	0	0	16	0.28 (18)
	Ptychadena nilotica Seetzen, 1855	382	6	5	393	6.92 (6)
	Ptychadena schillukorum Werner, 1908	11	0	0	11	0.19 (21)
Pipidae Gray, 1825	*Xenopus clivii* Peracca, 1898	93	0	17	110	1.93 (9)
	Xenopus largeni Tinsley, 1995	9	0	0	9	0.16 (22)
	Total	4457	501	720	5678	

In this study, we discovered that the small *Phrynobatrachus* in the Kaffa area probably belonged to an undescribed species (Table 2 and Fig. 9). The ML phylogram distinguished this new species, provisionally called *Phrynobatrachus* sp. 1, as a taxon distinct from other small *Phrynobatrachus* species previously recorded from Ethiopia. Based on the genetic distance matrix, there is a distance of 5.7% between *Phrynobatrachus* sp. 1 and *P. minutus*, a distance of more than 4.8% between *Phrynobatrachus* sp. 1 and *P. inexpectatus* and a distance of more than 9.5 % between *Phrynobatrachus* sp. 1 and the undescribed *Phrynobatrachus* sp. “Oromia” (Appendix 5).

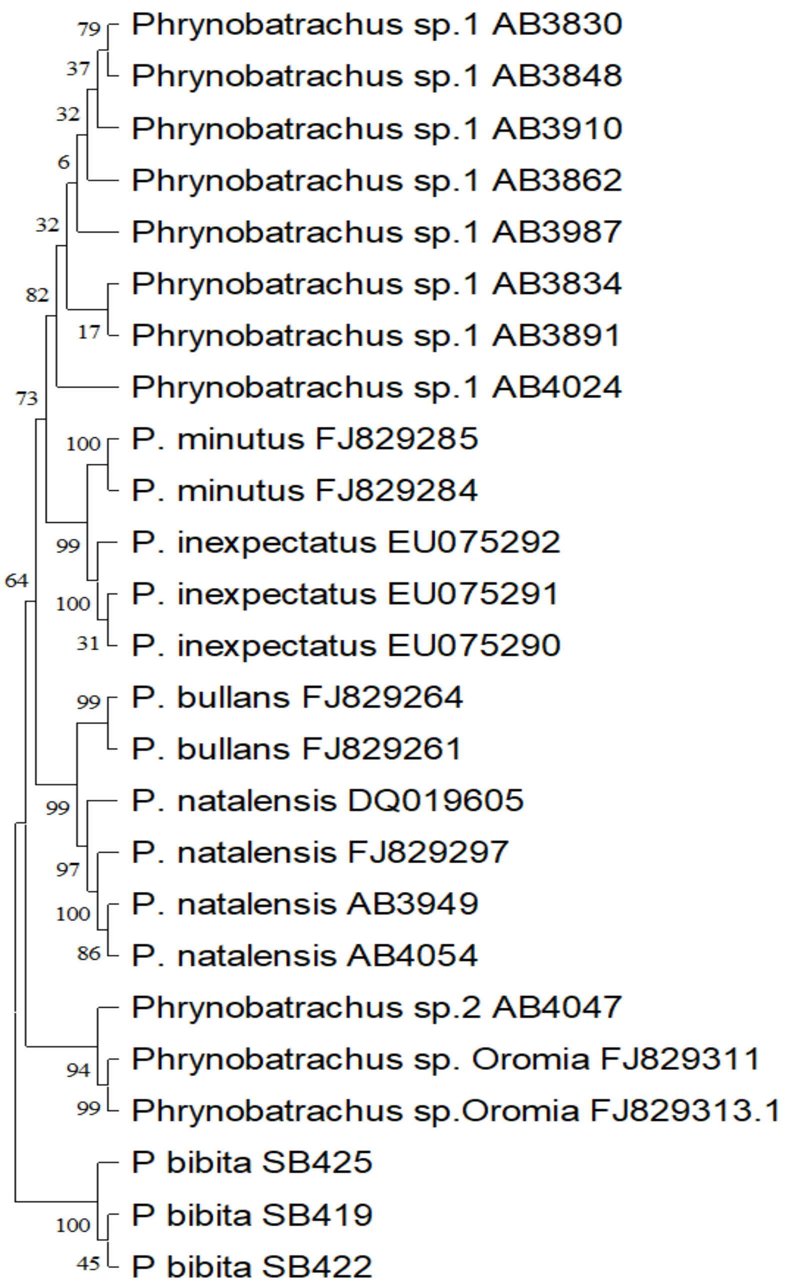


Figure 9. Phylogenetic tree was inferred by using the Maximum Likelihood (ML) method and Tamura-Nei model based on 16S gene. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Tamura-Nei model.

In addition, 11 specimens represented two undescribed *Leptopelis* species, *Leptopelis* sp. 1 and *Leptopelis* sp. 2 (Table 2 and Fig.10). The DNA barcodes of *Leptopelis* sp. 1 and *Leptopelis*sp. 2 were distinguished from previously recorded *Leptopelis* species found in NCBI (National Center for Biotechnology Information) using nucleotide BLAST (Basic Local Alignment Search Tool). Moreover, the genetic distance between the undescribed *Leptopelis* species and others is high as shown in Appendix 6 and Fig.10. Results of the ML tree (Fig.10) clearly grouped the *Leptopelis* sp. 1 and *Leptopelis* sp. 2 sequences separately and with high bootstrap support, from all other Ethiopian taxa. *Leptopelis* sp. 1 is 4.0% genetically distinct from *Leptopelis* sp. 2, 9.1% distinct from *Leptopelis susanae* and 12.9% distinct from *L. vannutellii*. Individuals of *Leptopelis* sp. 1 were collected from a grassy wetland at an altitude of 2,390 m, which is surrounded by pristine forest that is well preserved. *Leptopelis* sp. 1 has also been found in the Boqa wetland in Kaffa's Adiyo district at an elevation of 2,428 m. *Leptopelis* sp. 2 was collected from an enset plantation at an altitude of 2,427 m in Ganeti village of Bita district, about 90 km northwest of Bonga town.

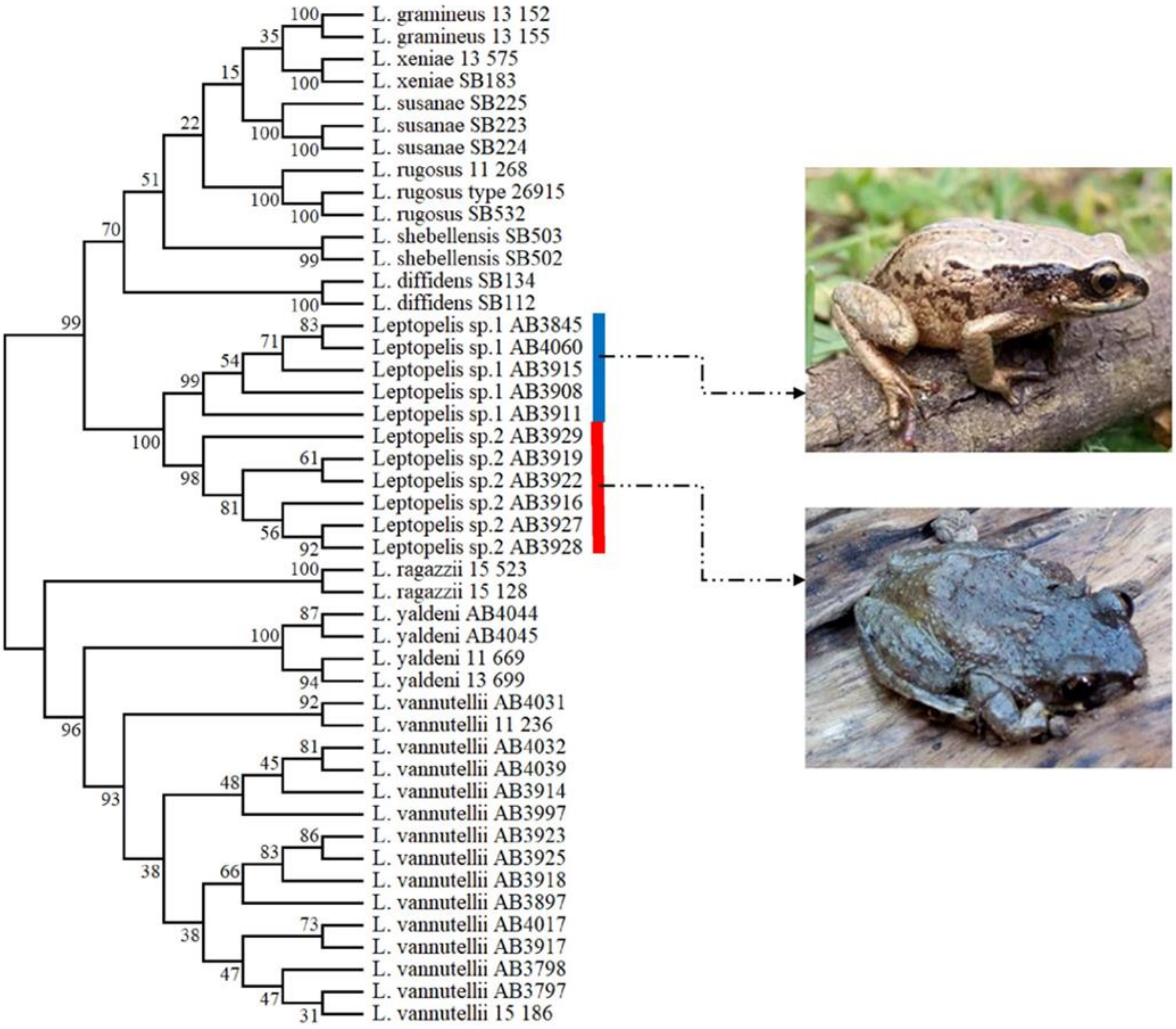


Figure 10. Phylogenetic tree created using the Maximum likelihood (ML) method and Tamura-Nei model, based on the COI gene, indicating the relationships of the two undescribed *Leptopelis* species to other *Leptopelis* species of Ethiopia. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Each species name is followed by the sequences' respective NCBI GenBank accession numbers. This analysis involved 46 nucleotide sequences.

Based on morphological characteristics such as the nature of the fingers and toe pad shape, the green dorsum, the black marking of a vertebral stripe, bifurcating anteriorly and confluent with the black interorbital bar that connects the upper eyelids (Fig. 11), we identified as *L. susanae*. However, it needs a DNA barcode to confirm *L. susanae* since this is relatively far from the only known locality for the species. As a result, we have given it the temporary name *L.cf. susanae*.

Figure 11. *Leptopelis cf. susanae* from Godofo/Ganeti wetland, Kaffa, southwest Ethiopia (Photo: Abeje Kassie, April, 2019).

Hyperolius nasutus was reported from Kaffa area identified using morphological characteristics using different identification keys. This study compared the sequenced 16S gene with recognized *Hyperolius* species from NCBI using BLAST and it showed that it is *H. howelli*, not *H. nasutus*.

Altogether, this study identified 26 different species of frog from eight different families in the area.

4.2.2. Frog distribution and abundance

Most species were found in the wetland habitat (19 species and 4,457 individuals), followed by riverine forest (10 species and 720 individuals) while agricultural land (enset plantation) supported the least (9 species and 501 individuals). The relative abundance of species varied with habitat types, with a few common species coexisting and with a significant number of rare species. *Phrynobatrachus* sp. 1 had the highest relative abundance (20.38) occurring in riverine forest and wetland habitats, followed by *Phrynobatrachus natalensis* (18.38%) which is only recorded in the wetland habitat. *Afrixalus clarkei* (16.27%) occurred in all the three habitat types. *L. cf. susanae* had the least relative abundance (0.04) and were found only in wetland habitats (Table 2).

Hyperoliidae was the most abundant family (2,204 individuals from seven species), followed by the Phrynobatrachidae (2,201 individuals from 2 species) and Conrauidae was least (seven individuals from single species) (Table 3). Hyperoliidae (seven species), Ptychadenidae (seven species) and Arthroleptidae (four species) were the families with the highest species richness, while Conrauidae and Pyxicephalidae had the lowest (only one species each) (Table 3).

Table 3. Relative abundance and species richness of frog family from Kaffa.

Family	Genus	Species	Abundance	Relative abundance (%)
Arthroleptidae	1	4	508	8.95
Hyperoliidae	4	7	2204	38.82
Conrauidae	1	1	7	0.12

Hemisotidae	1	2	52	0.92
Phrynobatrachidae	1	2	2201	38.76
Pipidae	1	2	119	2.10
Ptychadenidae	1	7	541	9.53
Pyxicephalidae	1	1	46	0.81
Total	11	26	5678	100.00

A total of 71 specimens (9.86%) representing six frog species were captured by drift fence and pitfall trap methods riverine forests (Table 4). Despite observing 10 species in the riverine forest habitat, only six of them were trapped using the drift fence and pitfall trap methods.

Among these species, *H. marmoratus* exhibited the highest relative abundance (66.67%) compared to others. *H. microscaphus* followed closely behind with a relative abundance of 60.97%, while P*tychadena nilotica* had a relative abundance of 40%. On the other hand, tree frogs such as *L. vannutellii* and *A. clarkei* and also *X. clevii* were not captured using drift fence and pitfall traps.

Table 4. Frog species trapped by drift fence and pitfall traps.

Species	DFPT (n)	VES/AES	Total number of specimens in RF (N)	Relative abundance (%) (n/N) * 100
Afrixalus clarkei	-	25	25	0

Hyperolius viridiflavus	2	11	13	15.38
Amietia nutti	7	39	46	15.22
Hemisus marmoratus	2	1	3	66.67
Hemisus microscaphus	25	16	41	60.97
Phrynobatrachus sp. *1*	33	241	274	12.04
Ptychadena nilotica	2	3	5	40
Conraua beccarii	-	7	7	0
Leptopelis vannutellii	-	289	289	0
Xenopus clivii		17	17	0
Total	71	649	720	9.86

NB: RF – Riverine Forest; drift fence and pitfall traps were constructed only in the riverine forest. DFPT- Drift fence and pitfall trap, VES- Visual encounter survey, AES- Acoustic encounter survey conducted in the riverine forest. N- number of individuals recorded in the riverine forest, n- number of individuals trapped in drift fence and pitfall traps.

4.2.3. Diversity indices of distribution of frogs

Shannon-Wiener indices were determined to represent the diversity of frogs in the agricultural (enset plantation), riverine and wetland habitats (Table 5). The diversity of frogs found in the agricultural land (enset plantation) was 1.58 (1.50, 1.65 at 95% CI), riverine forest 1.46 (1.38, 1.53 at 95% CI) and wetland 2.13 (2.10, 2.16 at 95% CI). Multiple Hutcheson t-tests showed that there was a significant difference between wetland and other habitats ($p < 0.05$), but no significant difference between agricultural land and riverine forest ($p > 0.05$).

Table 5. Frog diversity indices among habitats at the Kaffa area.

	WL	95% Lower	95% Upper	EP	95% Lower	95% Upper	RF	95% Lower	95% Upper
Taxa_S	19	19	19	9	9	9	10	10	10
Individuals	4457	4457	4457	501	501	501	720	720	720
Dominance_D	0.15	0.15	0.16	0.25	0.24	0.27	0.31	0.29	0.34
Simpson_1-D	0.85	0.84	0.85	0.75	0.73	0.76	0.69	0.66	0.71
Shannon_H	2.13	2.10	2.16	1.58	1.50	1.65	1.46	1.38	1.53
Evenness	0.72	0.71	0.73	0.72	0.68	0.75	0.63	0.60	0.66
Chao-1	19	19	19	9	9	9	10	10	10

Diversity t-test also indicated a significant difference between the wetland and agricultural land ($t = -14.275$, $df = 806.66$, $p < 0.05$) and wetland and riverine forest ($t = -13.799$, $df = 912.98$, $p < 0.05$) but differed between agricultural land and riverine forest ($t = 1.62$, $df = 1274.7$, $p > 0.05$). Shannon-Wiener (H) and Simpson (D) diversity indices indicated that wetland had the highest species diversity compared to riverine forest and agricultural land. As shown in Fig. 12D, in contrast to riverine forest, the species were more uniformly dispersed in agricultural land and wetland habitats.

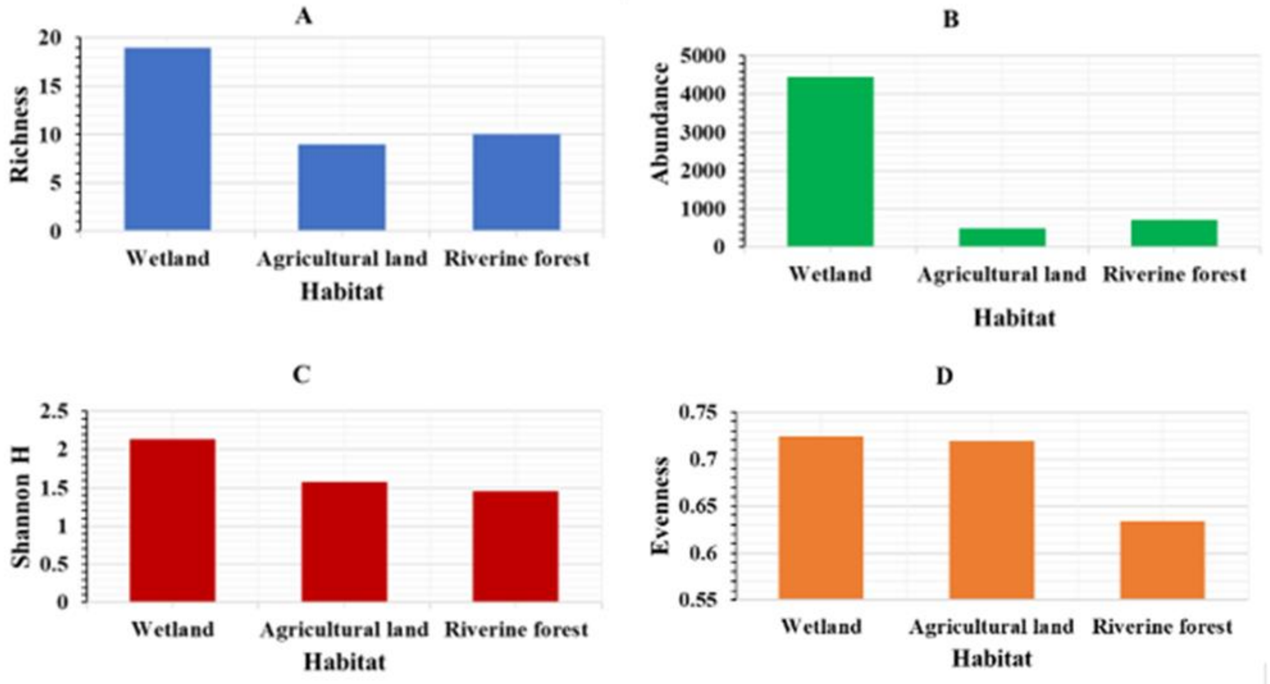


Figure 12. Diversity variation of frog species among habitats in Kaffa area. A) Richness B) Abundance C) Shannon _H diversity and D) Evenness

Wetland habitat displayed a greater abundance of individuals across various diversity measures such as evenness, Shannon, and Simpson diversity indices, as illustrated in the Fig. 12 and Table 5. However, we did not conduct a Renyi diversity profile to compare the diversity between the riverine and agricultural land habitats. This decision was made because the curve representing the riverine forest habitat intersected with both of the agricultural land curves, making it difficult to draw meaningful comparisons. In terms of overall diversity, regardless of the diversity index used, the riverine forest habitat was found to be the least diverse compared to the agricultural land habitat, as depicted in Fig. 13 and the accompanying Table 5.

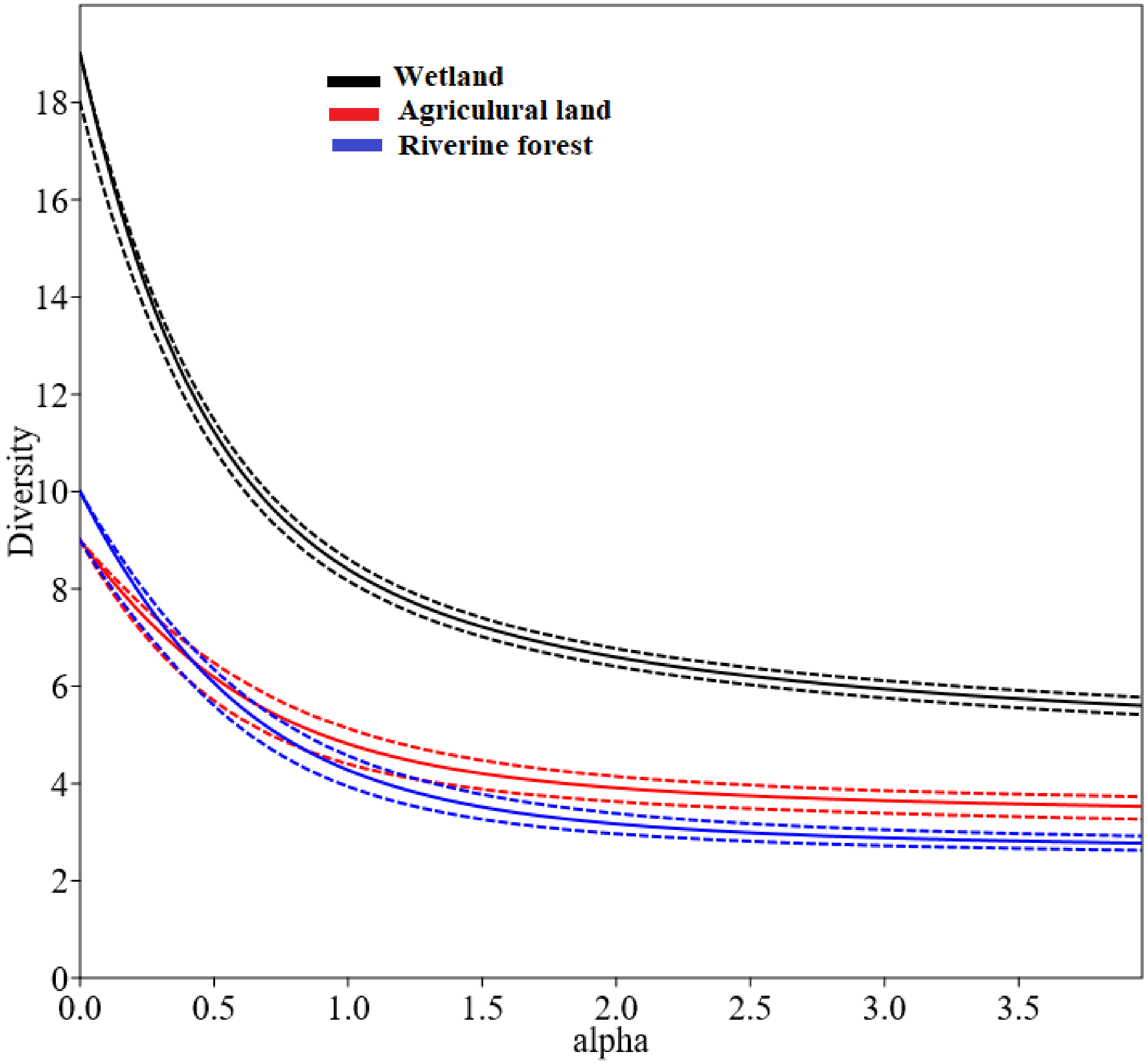


Figure 13. Renyi diversity profiles for the community diversity of frog species in the three habitat types with 95% confidence intervals after 9999 bootstraps. Alpha values: a = 0 richness; a = 1 Shannon index; a = 2 inverse Simpson index (1/D); and high a value approximates to Berger–Parker index.

4.2.4. Similarity Indices of frog species between habitats

Species similarity analysis between paired agricultural land and riverine forest habitats, agricultural land and wetland as well as riverine forest vs wetland habitats showed SCSI and JCSI values of less than 0.50 representing 40%, 34% and 39% of species similarity by SCSI and 25%, 21% and 24% by JCSI, respectively (Table 6).

Table 6. Sorensen's Coefficient Similarity Index (SCSI) and Jaccard Coefficient Similarity Index (JCSI) of frog species among the three habitat types.

Paired Habitats	Number of species				Similarity Indices	
	Unique to EP	Unique to RF	Unique to WL	Shared	SCSI	JCSI
AL Vs RF	5	6	-	3	0.40	0.25
AL Vs WL	4	-	14	5	0.34	0.21
RF Vs WL	-	5	14	5	0.39	0.24

EP = Enset plantation, RF=Riverine forest, WL=Wetland. Sorensen's and Jaccard similarity indices for each site are shown in Appendices 7 and 8.

Utali vs Shuneti had the highest Sorensen's similarity indices of 92% followed by Bariacho vs Nata, and Bariacho vs Medabo which had both 91%. Komba vs Ganeti, Gojeb vs Ganeti, and Shoreri vs Gojeb sites had the least similarity index of 11%, 13%, and 14%, respectively. In terms of frog species composition, Utali and Shuneti, which had the highest percentage of Sorensen's similarity indices (92 percent), were almost identical. All of the frog species found in Shuneti were also encountered in Utali, sharing six frog species. However, one species was found in Utali that was not noticed in the Shuneti site. In general, the results showed that Sorensen's similarity indices of frog species between all sites were low, falling below 50% in more than half of the paired sites.

4.2.5. Seasonal variation in frog species abundance

During the wet season, 25 frog species were observed, while 26 species were recorded during the dry season (Table 7). The wet season had relatively higher number of frog abundance than dry season (Table 7). However, the difference is not statistically significant. Frogs are more abundant in all three habitats during the wet season. Wetland had the highest frog abundance in both seasons compared to agricultural land and riverine forest and there is a significant difference among the habitat types ($p < 0.05$).

Table 7. Seasonal variation in abundance of frog species from three habitat types and percentage abundance per each season.

Species	Wet season				Dry season				Total
	WL	EP	RF	Total (%)	WL	EP	RF	Total (%)	
Afrixalus clarkei	497	65	17	579 (17.07)	287	50	8	345 (15.09)	924
Afrixalus enseticola	59	81	0	140 (4.13)	40	67	0	107 (4.68)	247
Amietia nutti	0	0	26	26 (0.77)	0	0	20	20 (0.87)	46
C. beccarii	0	0	4	4 (0.12)	0	0	3	3 (0.13)	7
Hemisus marmoratus	0	0	2	2 (0.06)	0	0	1	1 (0.04)	3
Hemisus microscaphus	0	3	24	27 (0.80)	0	5	17	22 (0.96)	49
Hyperolius kivuensis	173	0	0	173 (5.10)	87	0	0	87 (3.80)	260
Hyperolius howelli	250	0	0	250 (7.37)	228	0	0	228 (9.97)	478

Hyperolius viridiflavus	139	0	10	149 (4.39)	108	0	3	111 (4.85)	260
K. senegalensis	18	0	0	18 (0.53)	5	0	0	5 (0.22)	23
L. cf. susanae	0	0	0	0 (0.00)	2	0	0	2 (0.09)	2
Leptopelis sp.*1*	15	0	0	15 (0.44)	7	0	0	7 (0.31)	22
Leptopelis sp.*2*	0	15	0	15 (0.44)	0	13	0	13 (0.57)	28
L. vannutelli	0	104	179	283 (8.35)	0	63	110	173 (7.56)	456
Phrynobatrachus natalensis	602	0	0	602 (17.75)	442	0	0	442 (19.33)	1044
Phrynobatrachus sp.*1*	470	0	191	661 (19.49)	413	0	83	496 (21.69)	1157
Ptychadena anchietae	30	7	0	37 (1.09)	18	0	0	18 (0.79)	55
Ptychadena beka	7	7	0	14 (0.41)	5	3	0	8 (0.35)	22
Ptychadena doro	10	0	0	10 (0.29)	5	0	0	5 (0.22)	15
Ptychadena erlangeri	14	0	0	14 (0.41)	15	0	0	15 (0.66)	29
Ptychadena neumanni	10	0	0	10 (0.29)	6	0	0	6 (0.26)	16
Ptychadena nilotica	250	4	3	257 (7.58)	132	2	2	136 (5.95)	393
Ptychadena obscura	0	7	0	7 (0.21)	0	5	0	5 (0.22)	12
Ptychadena schillukorum	7	0	0	7 (0.21)	4	0	0	4 (0.17)	11
X. clivii	71	0	15	86 (2.54)	22	0	2	24 (1.05)	110
X. largeni	5	0	0	5 (0.15)	4	0	0	4 (0.17)	9
Total	2627	293	471	3391	1830	208	249	2287	5678

In both wet and dry seasons, the wetland habitat had the highest relative abundance i.e 80.0% during the dry season and 77.5 % during the wet season compared to other habitat types (Table 7).

Wetland habitats had a significant difference from other habitat types ($p < 0.05$). Wetland and agricultural land habitats had higher relative abundance during the dry season.

During the wet and dry seasons, there was a significant difference in Simpson 1-D diversity ($t = 2.1738$, $df = 4636.7$, $p = 0.02$) and Shannon H diversity ($t = -2.17$, $df = 4791.2$, $p = 0.03$) of frog species in the study sites (Table 8). Hutcheson t-test used to compare Shannon diversity between wet and dry seasons revealed a significant difference (Hutcheson t-statistic = 2.17, $df = 4794$, $p = 0.023$).

Table 8. Diversity variation and diversity T-test of frog species between wet and dry seasons (WS: Wet Season; DS: Dry Season).

	WS	DS	Diversity t-tests		
			t	Df	p (same)
Frog species	25	26			
Abundance	3391	2287			
Evenness	0.74	0.71			
Chao-1	25	26.5			
iChao-1	25	26.59			
ACE	25	26.23			
Simpson_D	0.1238	0.1324	2.32	4636.7	0.02

Shannon_H	2.47	2.41	-2.17	4791.2	0.03

As shown in Fig. 14, at q = 0, the blue line is above the red line, while at q=1 and 2, it is below the red line. This indicated that during the dry season, frog species richness is higher and Shannon H and Simpson 1-D is lower.

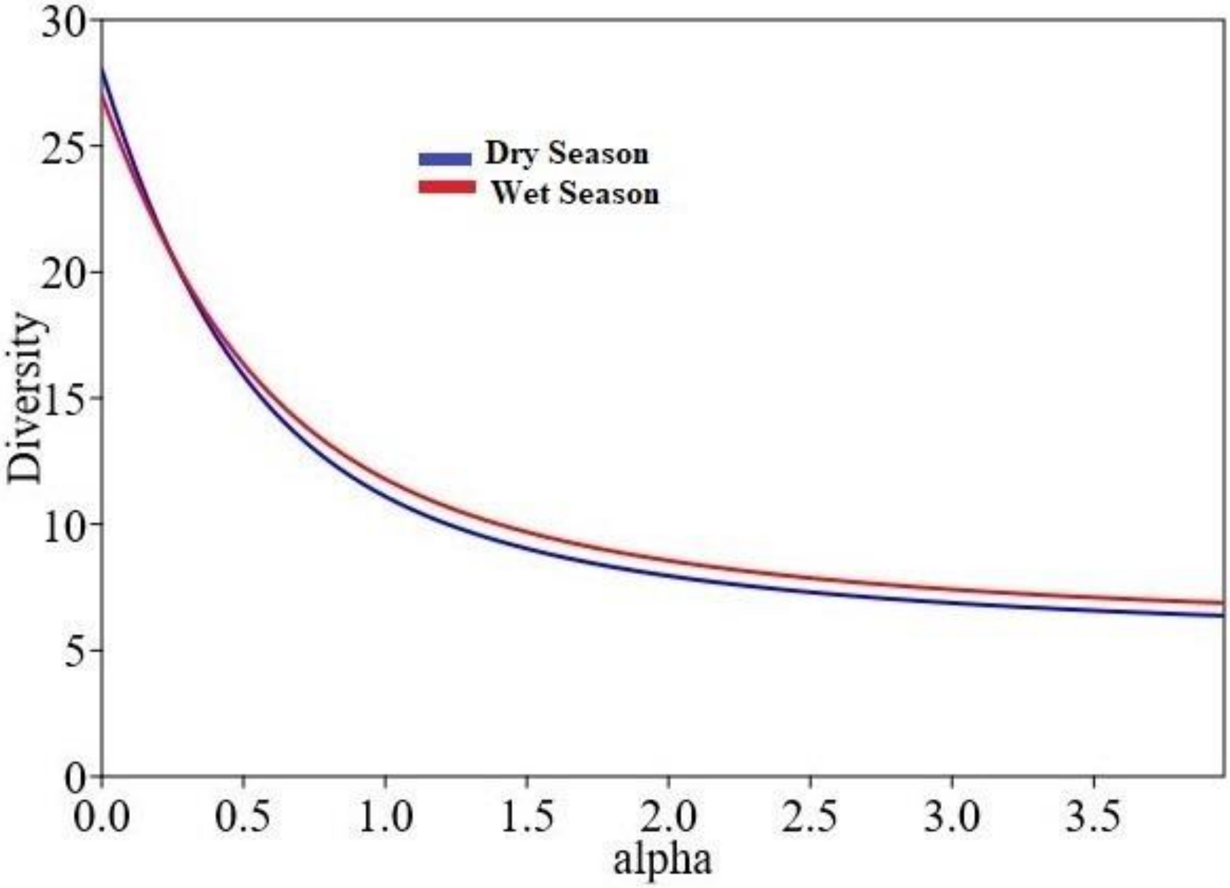


Figure 14. Variation of frog alpha diversity between seasons.

4.2.6. Effects of environmental variables on frog species abundance

The Poisson GLM analysis of frog species abundance across three habitat types for 51 transects yielded the best-supported model, which includes explanatory variables such as precipitation, temperature, slope and altitude (Table 9 and Fig. 15).

Table 9. Summary statistics for General Linear Model (GLM) for abundance relationships with selected environmental variables for frog species

		Abundance		
Predictors	Estimate	SE	z value	P
Intercept	1.132e+01	1.319e+00	8.582	< 2e-16 ***
Habitat (Riverine Forest)	1.089e-01	5.917e-02	1.840	0.0657
Habitat (Wetland)	2.093e+00	4.486e-02	46.649	< 2e-16 ***
Temperature	-5.106e-01	4.449e-02	-11.477	< 2e-16 ***
Precipitation	4.507e-04	2.775e-04	1.624	0.1044
Altitude	-9.363e-05	5.672e-05	-1.651	0.0988
Slope	-1.889e-02	3.387e-03	-5.579	2.43e-08 ***
Aspect	1.843e-03	1.491e-04	12.359	< 2e-16 ***

The model fitted to these data, supported the prediction for a positive relationship between precipitation and abundance although it is not a significant association (Fig. 15A). On the other hand, slope and altitude had a negative nonlinear relationship with the abundance of frog species. There was a significant relationship between abundance and slope, but not between abundance and altitude. Temperature had also a significant negative and linear relationship with frog abundance (Table 9 and Fig. 15B).

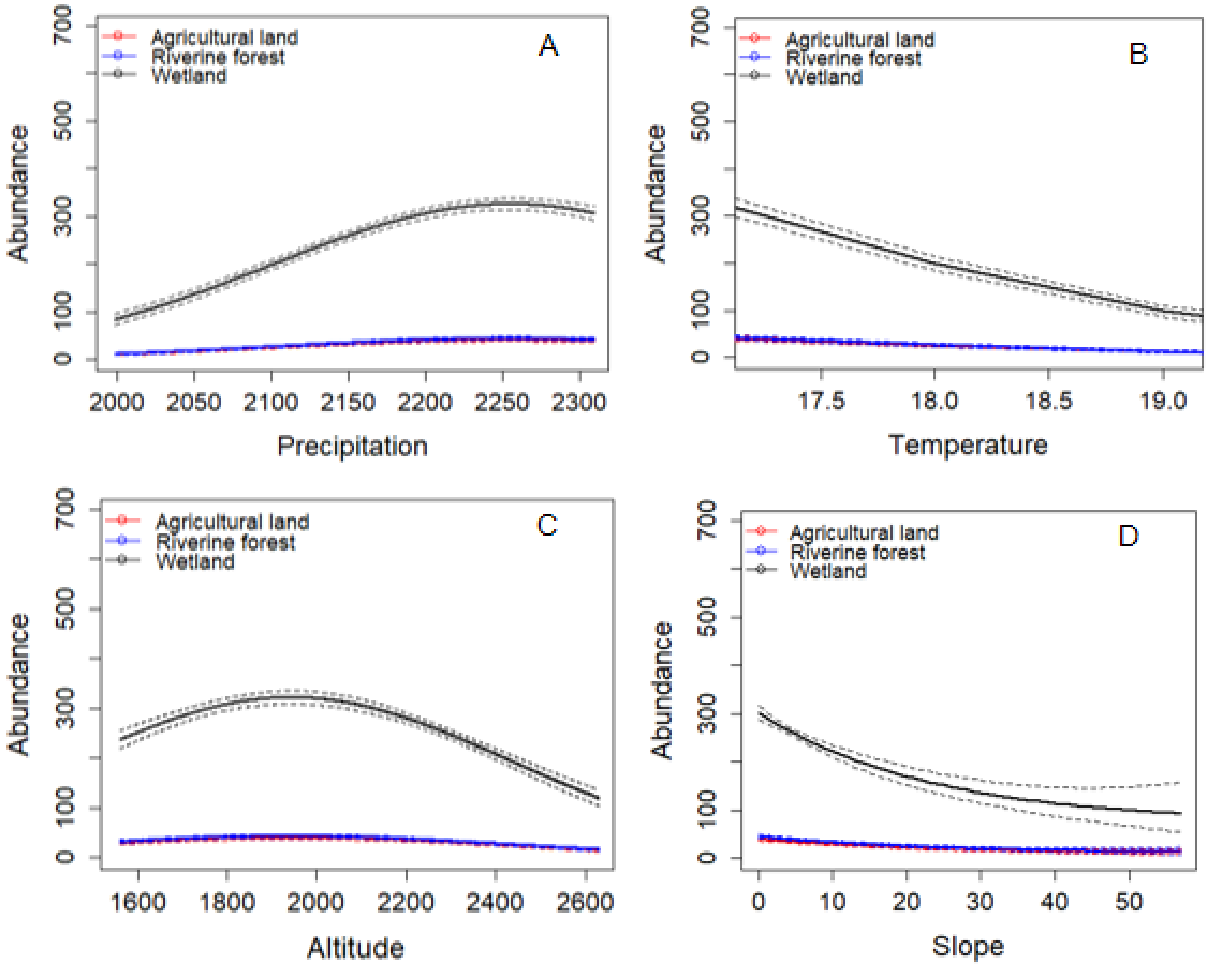


Figure 15. Effects of A) precipitation, B) temperature, C) altitude, and D) slope on the pattern of frog abundance across habitat types. Solid lines represent trends, while dashed lines represent the respective 95% credible intervals calculated using a Poisson GLM.

4.2.7. Frog species accumulation curve

The species accumulation curve depicts the rate at which new species are added as the sampling effort continues. Fig.16 shows that new species kept on being discovered as the study progressed in the area, albeit at a slower rate. At this sampling effort, the rarefied number of species based on 51 transects resulted in 26 species.

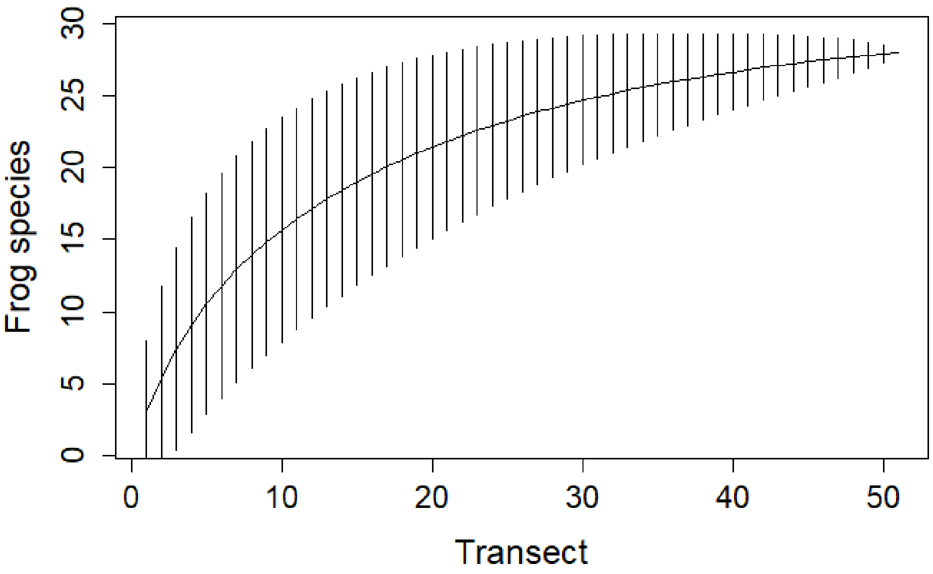


Figure 16. Species accumulation (rarefaction) curve (solid line) plotted against survey effort (number of transects surveyed) and vertical lines represent 95% confidence intervals.

4.2.8. Frog species richness estimation

Chao1 index was used to calculate the estimated total number of species per habitat type. Interpolation (rarefaction) and extrapolation curves showed that species richness ranged from 9 to 19 among habitats (Fig. 17). Measures of diversity show that wetland habitats had the highest diversity (Chao1 = 19 ± 0.76, H = 8.79 ± 0.12), followed by the riverine forest in species richness (Chao1 = 10 ± 0.59, H = 4.55 ± 0.17) and agricultural land (Chao1 = 9 ± 0.00, H = 4.85 ± 0.20).

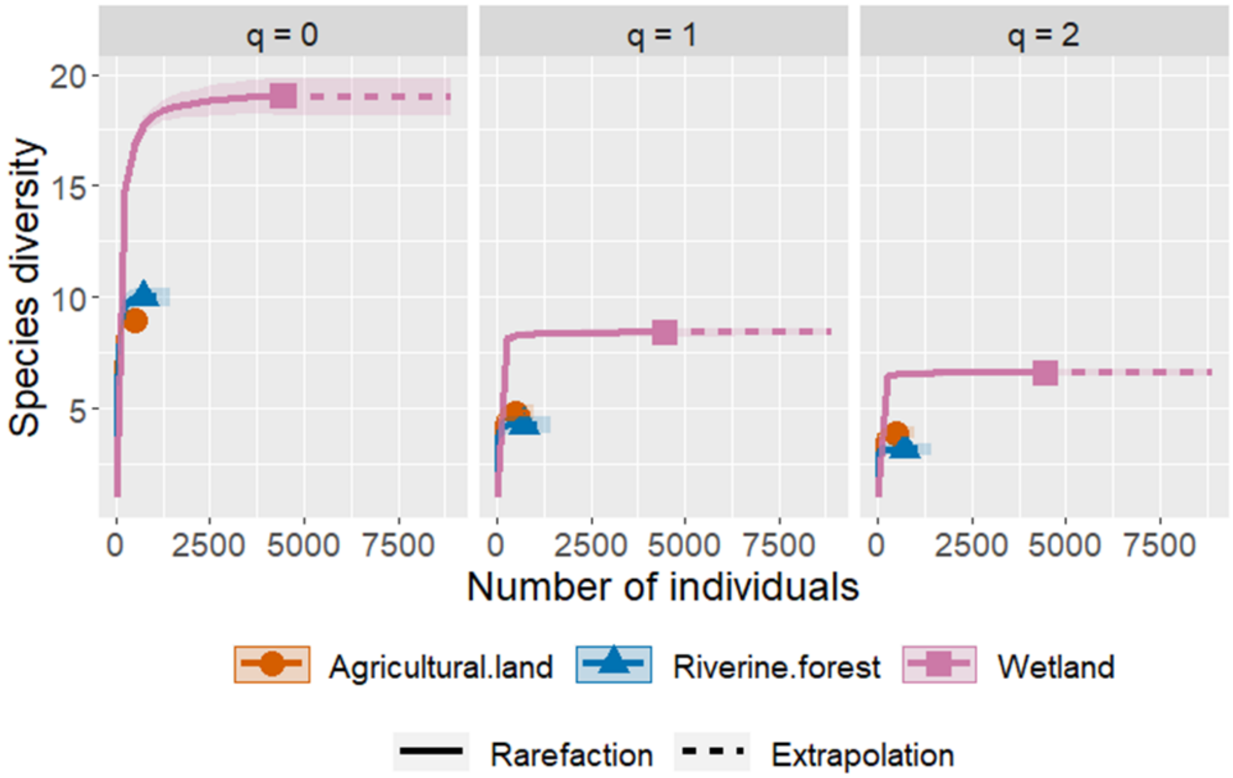


Figure 17. Comparison of the diversity of frog species in three habitat types through rarefaction solid lines and extrapolation dotted lines based on the number of individuals of the frog species. Species diversity was estimated using Hill numbers: q = 0 (frog species richness), q = 1 (exponential of Shannon's entropy index) and q = 2 (inverse of Simpson's concentration index).

4.3. Diversity, abundance and distribution of endemic frog species

4.3.1. Endemic frog diversity and abundance

A total of 3672 person-hours of sampling effort was exerted at sites representing all three habitats where 2988 specimens were recorded, 1208 during the dry and 1780 during the wet seasons. Fourteen endemic frog species belonging to the families Arthroleptidae (*Leptopelis cf. susanae*, *Leptopelis* sp.1, *Leptopelis* sp.2, and *Leptopelis vannutellii*), Hemisotidae (*Hemisus microscaphus*), Hyperoliidae (*Afrixalus clarkei*, *Afrixalus enseticola*, and *Paracassina obscura*), Phrynobatrachidae (*Phrynobatrachus* sp.1), Pipidae (*Xenopus largeni*), and Ptychadenidae (*Ptychadena beka*, *Ptychadena doro*, *Ptychadena erlangeri*, and *Ptychadena neumanni*) were recorded (see Table 10 and Fig. 18).

Figure 18. Photos of some of the frogs recorded from Kaffa area. a/*Amietia nutti* b/*Afrixalus enseticola* c/ *Xenopus clivii* d/*Ptychadena erlangeri* e/Leptopelis sp. 2 f/*Leptopelis vannutellii* g/*Leptopelis* sp. 1 h/*Kassina senegalensis* i/*Hyperolius viridiflavus* j/*Hyperolius howelli* k/*Conraua beccarii* l/ *Phrynobatrachus natalensis* m/*Afrixalus clarkei* n/*Paracassina obscura* o/ *Leptopelis cf. susanae* p/*Ptychadena nilotica* q/*Hyperolius kivuensis* (Photo: Abeje Kassie, 2019 - 2021)

Families Arthroleptidae and Ptychadenidae had the highest number of species (four species each), followed by Hyperoliidae (three species), and Hemisotidae and Pipidae that had just one species. *Leptopelis* sp.1, *Leptopelis* sp.2, *Leptopelis cf. susanae* (in Godofo /Ganeti wetland) and *Xenopus largeni* were endemic frog species recorded for the first time in the Kaffa area.

The highest beta diversity is found between wetland and agricultural land (0.63), followed by wetland vs riverine forest (0.60), and the lowest occurred between agricultural land and riverine forest (0.38).

In the current study, wetland habitats harboured more endemic species than the other habitat types. Agricultural land had the highest species diversity (H = 1.401) compared to riverine forest (H = 1.182) and wetland (H = 1.14) habitats. Evenness was also highest in agricultural land and lowest in wetland habitats (Appendix 9). However, dominance value was highest in the wetland habitat and lowest in the agricultural land.

Despite the fact that there was no significant difference in frog species richness between the wet and dry seasons, wetland habitat species richness varied across habitat types. However, there is no significant variation in diversity of endemic species across habitat types, seasons, or sites (Appendix 10).

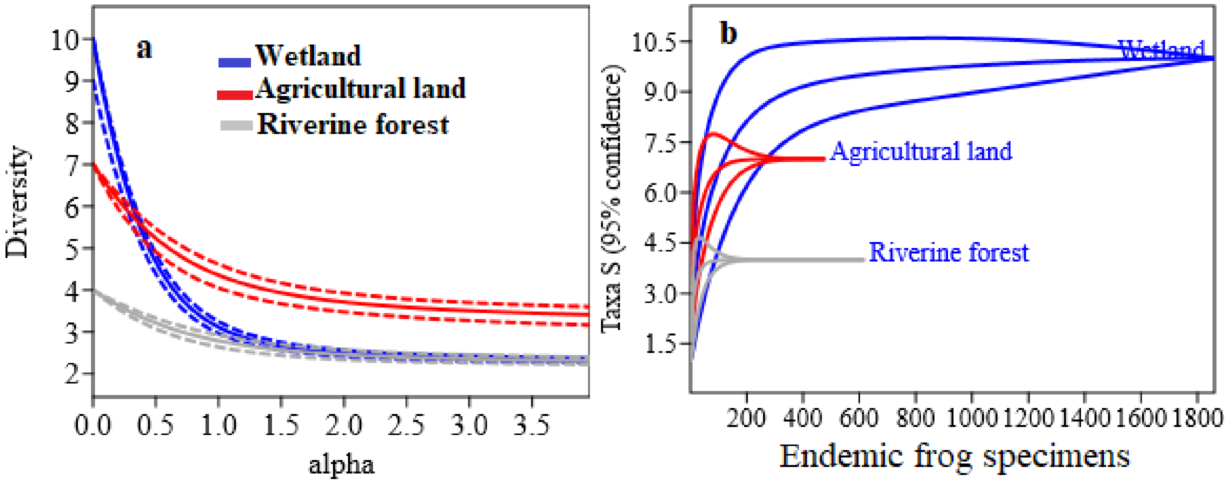
The abundance of endemic frog species recorded in the study sites showed that there was higher abundance during the wet season than the dry season (Table 10). However, the Kruskal-Wallis test showed that there is no significant difference among sites (H = 3.714, Hc = 3.714, $p > 0.05$). There was also no significant difference among the three habitat types in species richness (H = 1.041, Hc = 1.134, $p > 0.05$) although the number of individuals was highest in the wetland habitat compared to riverine forest and agricultural land (Table 10).

Table 10. Abundance of endemic frog species across the three habitat types during the wet and dry seasons (EP: Enset Plantation; RF: Riverine Forest; WL: Wetland)

Species	Wet season				Dry season				Total
	WL	EP	RF	Total (%)	WL	EP	RF	Total (%)	
Afrixalus clarkei	497	65	17	579 (17.07)	287	50	8	345 (15.09)	924
Afrixalus enseticola	59	81	0	140 (4.13)	40	67	0	107 (4.68)	247
Hemisus microscaphus	0	3	24	27 (0.80)	0	5	17	22 (0.96)	49
L. cf. susanae	0	0	0	0 (0.00)	2	0	0	2 (0.09)	2
*Leptopelis sp.*1	15	0	0	15 (0.44)	7	0	0	7 (0.31)	22
*Leptopelis sp.*2	0	15	0	15 (0.44)	0	13	0	13 (0.57)	28
L. vannutelli	0	104	179	283 (8.35)	0	63	110	173 (7.56)	456
*Phrynobatrachus sp.*1	470	0	191	661 (19.49)	413	0	83	496 (21.69)	1157
Ptychadena beka	7	7	0	14 (0.41)	5	3	0	8 (0.35)	22
Ptychadena doro	10	0	0	10 (0.29)	5	0	0	5 (0.22)	15
Ptychadena erlangeri	14	0	0	14 (0.41)	15	0	0	15 (0.66)	29
Ptychadena neumanni	10	0	0	10 (0.29)	6	0	0	6 (0.26)	16
Paracassina obscura	0	7	0	7 (0.21)	0	5	0	5 (0.22)	12
Xenopus largeni	5	0	0	5 (0.15)	4	0	0	4 (0.17)	9

Endemic frog diversity profiles

Wetland habitat showed a higher number of species in the diversity profile (higher values when a =0) (Fig.19a). However, although richness was high, evenness, Shannon, and Simpson diversity indices were low in the wetland. Renyi diversity profile was not performed to compare the diversity of the three habitats due to the reason that the wetland curve crosses both the agricultural and riverine forest curves. The riverine forest was the least diverse independently of the diversity index when evaluated with agricultural land (enset plantation) (Fig. 19a). Diversity profiles showed heterogeneity in the composition of several species among the three habitat types although statistically not significant ($p > 0.05$). The species accumulation curve for endemic frog data (Fig. 19b) indicates that most species are likely to occur in all habitat types, though wetland abundance was highest.



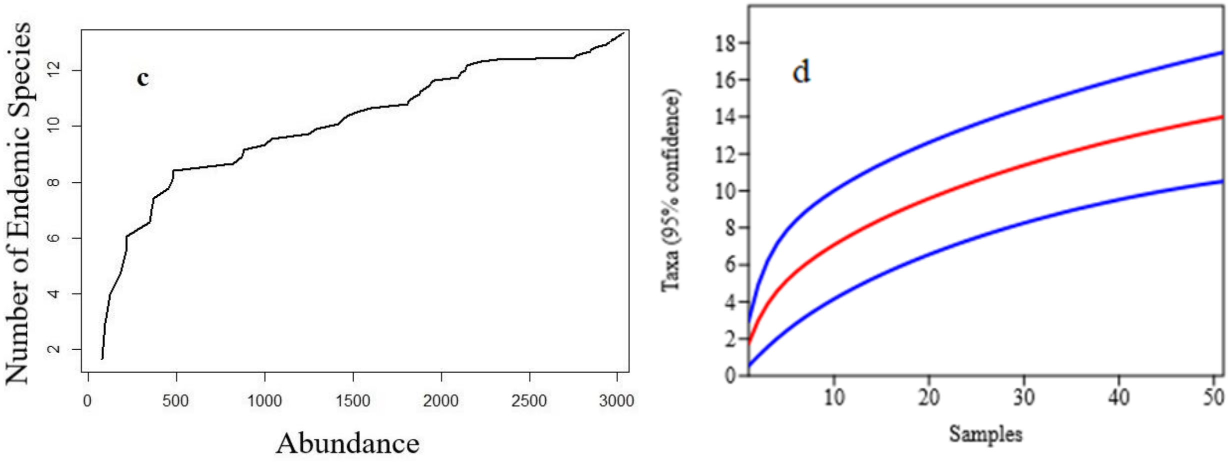


Figure 19. Accumulation curve, portraying the accumulative number of endemic frog species detected within Kaffa area. a) Renyi diversity profiles for the community diversity of endemic frog species in the three habitat types with 95% confidence intervals after 9999 bootstraps. Alpha values: a = 0 richness; a = 1 Shannon index; a = 2 inverse Simpson index (1/D); and high value approximating to Berger–Parker index. b) individual rarefaction curve. c) the accumulation curve of endemic frog species abundance with species richness d) sample rarefaction curve (the blue colors indicate 95% confidence intervals based on standard deviation).

The data set of endemic frog species accumulation curves at the research area revealed great variety in shape but did not follow the asymptotic pattern (Fig.19c). Despite a thorough transect search that included 3672 observation hours in the study area, our species accumulation curve suggests that many species were missed during the surveys (Fig. 19d). Our species richness estimates based on transect data indicate that 14 endemic species are feasible and our curve indicates that additional species are expected with increased sampling (Fig.19d).

Estimators to calculate the likelihood of finding unusual species occurrences showed that the sensitivity varied across estimators. For example, the estimators Chao and Chao.se predicted that at least one endemic species would be overlooked, whereas jack1 and jack2 predicted that more than three species would be missed. Except for Chao, which closely matches the observed species, none of the estimators performed well with these data. The data simply were not enough to give the estimators a better fit. Based on total pooled individuals, the best-fitting estimator is Chao predicted 14.19, which is 1.19 greater than the observed species.

Endemic frog community structure

The species composition cluster analysis yielded four primary groupings (Fig. 20). Each group was formed based on the transects' frog species composition. The habitats of group 1, Group 2, and Group 4 are dominated by wetland, agricultural land and riverine forest, respectively. However, Group 3 is dominated by wetland and Agricultural land.

Species composition characteristics in the agricultural land, riverine forest, and wetland habitats provided a dual revelation that perhaps predicts and explains the ecological position of the frog species at the different habitats. The results from the pairing of transects in the clustering showed similarities in species composition as seen in transects 39 and 51 and transects 31 and 32 among others. Most transects, however, appear to have a rather big ecological distance while being in the same location, for example, transects 2 and 3, transects 10 and 13, although for others the ecological distance is minimal.

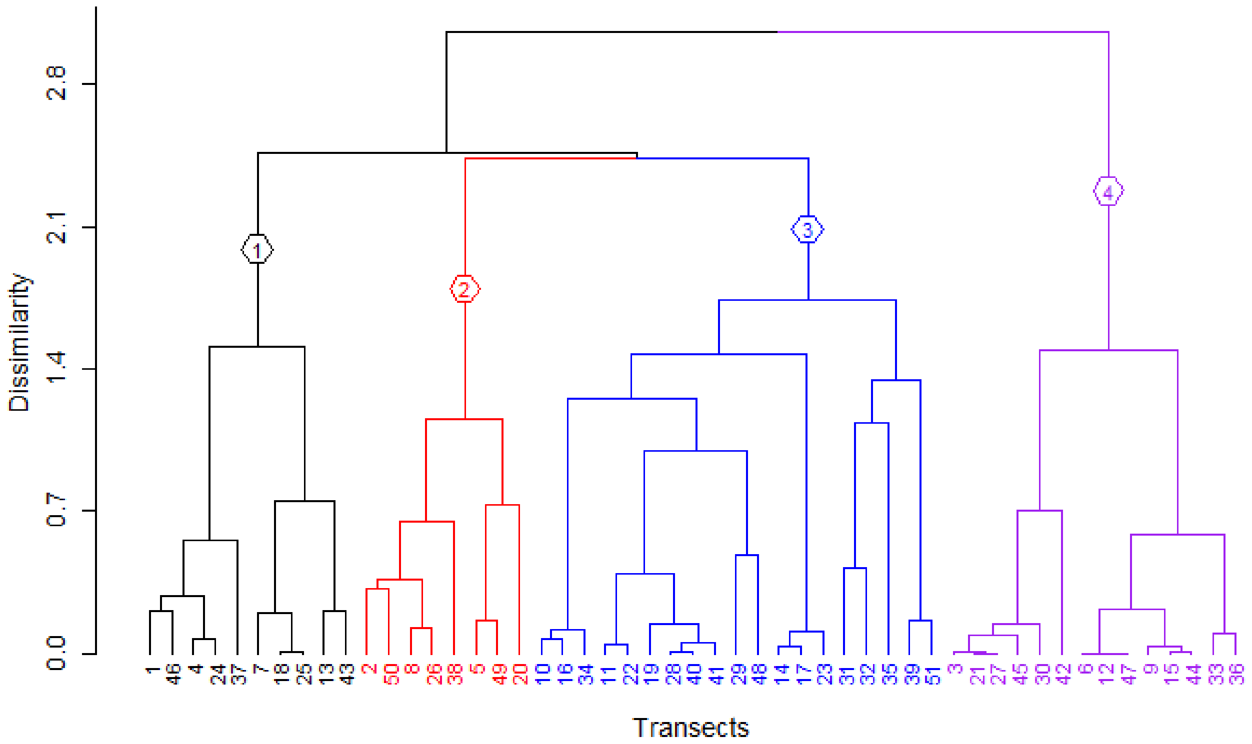


Figure 20. Cluster dendrogram for all 51 transects showing analysis of ecological distance to examine species composition for the entire area of the study sites; each cluster starts by combining the two most similar sites to each other and then proceeds by combining groups to groups; fusion of sites was carried out using average linkage method.

4.3.2. Endemic frog species distribution

Species similarity analysis between habitats revealed that agricultural land (enset plantation) and riverine forest are both home to 62% of the species recorded in the study area, with no difference during the wet and dry seasons. Species similarity is the lowest between agricultural land and wetland habitat during both dry (0.38) and the wet season (0.4) (Table 11).

Table 11. Sorensen similarity indices of endemic frog species.

Habitats	Dry season	Wet season
Agricultural land Vs Riverine Forest	0.62	0.62
Agricultural land Vs Wetland	0.38	0.40
Riverine Forest Vs Wetland	0.4	0.43

4.4. Phylogeny, morphometry and acoustic characteristics of selected frog species

The phylogeny, morphology and acoustic features of *Hyperolius* and *Afrixalus* species recorded in Kaffa area were characterized.

4.4.1. *Hyperolius* species

4.4.1.1. Phylogenetic analysis

Phylogenetics for *Hyperolius* species recorded in the study area are shown in Fig. 21. The optimal tree had sum of branch length (SBL) of 0.657. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying the Neighbor Joining method to a matrix of pairwise distances estimated using the Tamura Nei model. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. This analysis involved 31 nucleotide sequences. Codon positions included were 1st + 2nd + 3rd + Noncoding. There were 430 sites in the final dataset. The number of base differences per site from averaging over all sequence pairs is 0.11 and the standard error estimate(s) is 0.01 (Appendix 11).

The minimum genetic distance within *Hyperolius kivuensis* species is 0.0 and the maximum is 0.01 while the genetic distance within *Hyperolius howelli* species is 0.00. The minimum genetic distance within *H. viridiflavus* is 0.0 and the maximum is 0.01 (Table 12). The *H. kivuensis* revealed greater genetic variation from *H. howelli* with a genetic distance of 0.16 and less genetic variation from *H. balfouri* with a genetic distance of 0.075.

Table 12. Genetic variability of 14 *H. kivuensis* with 6 other *Hyperolius* species. Given are minimum (Min), maximum (Max) and mean values of uncorrected pairwise sequence divergence of the 16S rRNA gene; sd= standard deviation; N= number of individual interspecific comparisons; the bold text in the table is for species that was recorded in Kaffa area.

Species	Min	Max	Mean	SD	N
Hyperolius acuticeps	0.15	0.16	0.1575	0.00433	2
Hyperolius balfouri	0.07	0.08	0.075	0.005	1
Hyperolius microps	0.17	0.17	0.17	0	2
Hyperolius howelli	0.17	0.17	0.17	0	3
Hyperolius nasutus	0.14	0.15	0.145	0.005	2
Hyperolius viridiflavus	0.14	0.15	0.143	0.0047	6

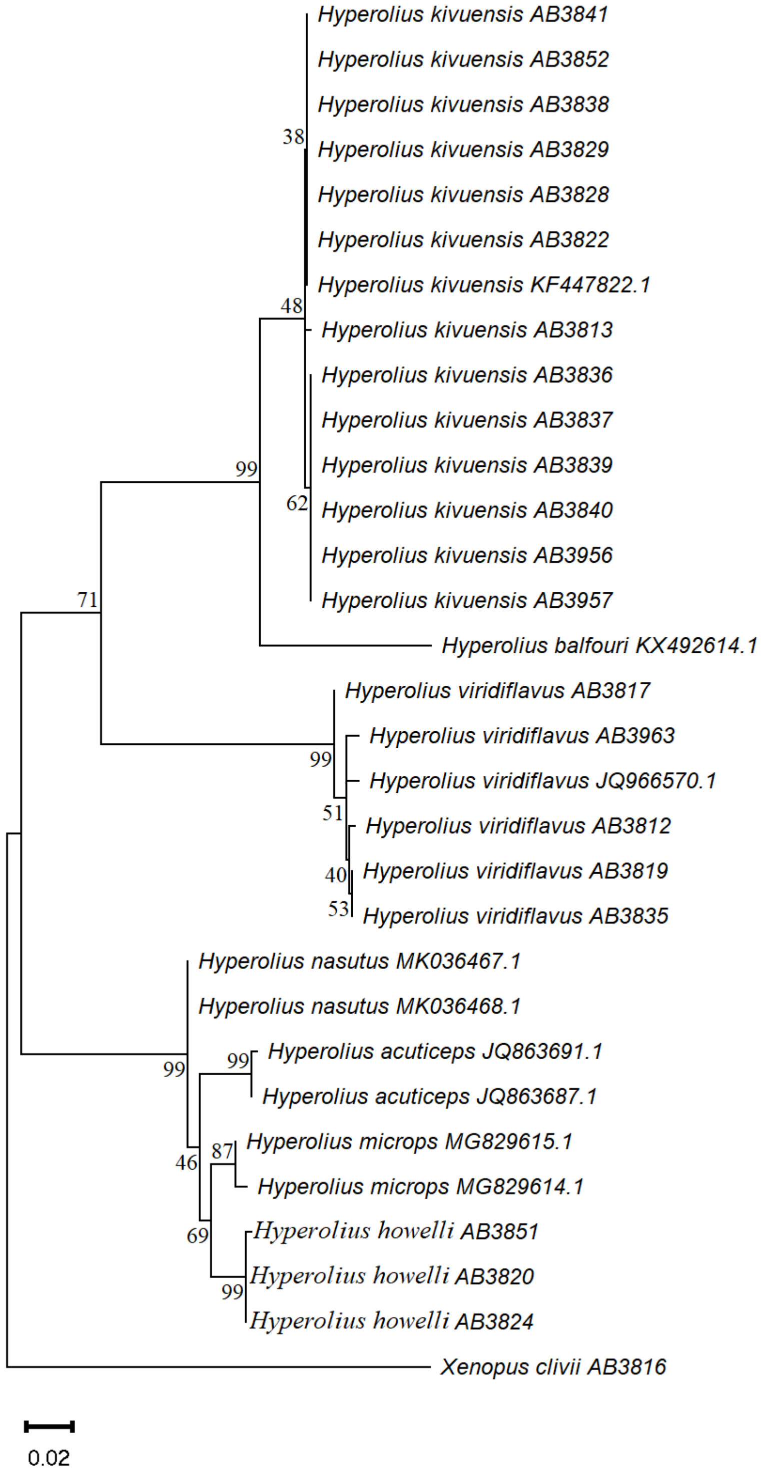


Figure 21. Maximum likelihood phylogram of species in the genus *Hyperolius* and *Xenopus clivi* as an outgroup, based on a comparison of 430 base pairs of the mitochondrial 16S rRNA gene.

Included are specimens from Kaffa area and samples taken from NCBI GenBank (with accession numbers). The numbers above nodes are percentage support values from maximum likelihood and maximum parsimony analyses.

4.4.1.2. Morphometry

Frog physical characteristics are quantified by morphometric measurements. In this study, we measured the 17 distinctive characteristics (Table 13) of frogs described in the methodology section for the *Hyperolius* species found in Kaffa area of southwest Ethiopia. These parameters are used to identify and characterize the three *Hyperolius* species. The mean snout vent length of male and female *H. viridiflavus* were 29.45 mm and 33.55 mm, respectively while it was 27.2 mm and 27.73 mm, respectively in *H. kivuensis*. A male *H. howelli* measured 15.78 mm and female 16.97 mm (Table 13). Analysis of variance (ANOVA) indicated a significant difference among the three female *Hyperolius* species (F=43.68, df=56.92, p=4.745E-26; p<0.05).

Table 13. Measurements (mean value ± sd in mm) of adult *H. viridiflavus*, *H. kivuensis* and *H. howelli*. "N" represents number of individuals. Ratios of parameters were computed from the mean of measurements.

Variables	*H. viridiflavus*		*H. kivuensis*		*H. howelli*	
	Male (N = 5)	Female (N =4)	Male (N = 8)	Female (N = 6)	Male (N=4)	Female (N = 6)
SVL	29.45 ± 0.65	33.55 ± 0.72	27.2 ±1.3	27.73 ± 3.3	15.78±0.3	16.97 ± 0.2
HW	9.85 ± 0.15	11.5 ± 0.36	9.0±0.4	9.23 ± 1.5	5.0±0.5	5.77 ± 0.3

HL	9.7 ± 1.3	9.0 ± 0.24	10.9 ± 1.5	9.5 ± 2	4.9 ± 0.4	5.17 ± 0.2
SL	4.4 ± 0.1	4.6 ± 0.24	4.5 ± 0.5	4.5 ± 0.4	2.70 ± 0.2	2.73 ± 0.2
NS	1.65 ± 0.15	1.6 ± 0.10	1.7 ± 0.3	1.77 ± 0.2	1.05 ± 0.15	1.13 ± 0.2
IND	3 ± 0	2.2 ± 0.23	3.5 ± 0.2	2.6 ± 0.3	2.0 ± 0.2	2.2 ± 0.1
EN	2.75 ± 0.05	3.1 ± 0.13	2.8 ± 0.3	2.73 ± 0.3	1.36 ± 0.4	1.6 ± 0.4
IOD	3.5 ± 0.5	5.1 ± 0.08	3.6 ± 0.4	3.57 ± 0.5	2.4 ± 0.3	2.4 ± 0.1
ED	3.55 ± 0.35	4.4 ± 0.15	3.2 ± 0.3	3.3 ± 0.2	2.0±0.3	2 ± 0.1
UEW	1.7 ± 0.2	2.3 ± 0.13	1.8 ± 0.3	1.67 ± 0.2	1.23 ± 0.25	1.3 ± 0.1
FLL	6.5 ± 0.1	7.4 ± 0.16	5.9 ± 1.0	6.07 ± 0.9	3.45 ± 0.12	3.5 ± 0.1
HAL	8.35 ± 0.35	9.6 ± 0.18	7.7 ± 0.6	7.27 ± 0.7	4.5 ± 0.23	4.53 ± 0.1
FinDW	1.6 ± 0.1	1.6 ± 0.05	1.4 ± 0.3	1.33 ± 0.2	0.90 ± 0.0	0.97 ± 0
THL	14.55 ± 0.25	15.6 ± 0.25	12.7 ± 1.5	11.8 ± 2	8.12 ± 0.34	8.3 ± 0.2
TL	14.05 ± 0.15	16.5 ± 0.25	12.1 ± 0.7	12.4 ± 1.2	8.50 ± 0.2	8.53 ± 0.4
FL	13.3 ± 0.3	15.5 ± 0.43	10.33 ± 0.8	11.03 ± 1.1	7.20 ± 0.3	7.3 ± 0.2
Toe4DW	1.6 ± 0.1	1.55 ± 0.05	1.32 ± 0.2	1.33 ± 0.2	0.65 ± 0.1	0.67 ± 0.2
HW/SVL	0.33	0.34	0.33	0.33	0.32	0.34
HL/SVL	0.33	0.27	0.40	0.34	0.31	0.30
IND/SVL	0.10	0.06	0.13	0.09	0.13	0.13

THL/SVL	0.49	0.47	0.47	0.42	0.51	0.49
TL/SVL	0.48	0.50	0.44	0.45	0.54	0.50

4.4.1.3. Acoustic characteristics

The auditory characteristics of the three identified species of *Hyperolius* frogs in the research area are as follows: The male advertisement call of *H. kivuensis* has a duration (Delta time) range of 0.21 to 0.88 seconds, with a mean duration of 0.56 seconds (SD ± 0.25 seconds). The mean peak frequency of the call was 2.5 kHz, (SD ± 0.06 kHz; range of 2.4 to 2.6 kHz). Additionally, the mean bandwidth of the call is 2.8 kHz, (SD ± 0.13 kHz; range of 2.5 to 2.9 kHz). These characteristics distinguish the call of *H. kivuensis* from those of *H. howelli* and *H. viridiflavus* (Table 14).

Table 14. Call duration, peak frequency and bandwidth of *H. kivuensis, H. howelli* and *H. viridiflavus*

Species	Delta time (s) (Mean ± SD, Min-Max)	No. pulses	Peak frequency (kHz) (Mean ± SD, Min-Max)	Bandwidth (kHz) (Mean ± SD, Min-Max)
H. kivuensis	0.56 ± 0.25, 0.21- 0.88	9	2.5 ± 0.06, 2.4 – 2.6	2.8 ± 0.13, 2.5 – 2.9
H. howelli	0.13 ± 0.07, 0.01 - 0.32	Repetitive pulses	4.4 ± 0.15, 3.8 – 4.7	1.0 ± 0.42, 0.52 – 3.7
H. viridiflavus	0.07 ± 0.02, 0.04 - 0.12	39	2.8 ± 0.03, 2.8- 2.9	2.6 ± 0.84, 0.17 – 3.0

Wilcoxon test revealed significant differences in the peak frequency (Hz) and delta time (s) between *H. kivuensis* vs *H. howelli*, *H. kivuensis* vs. *H. viridiflavus*, and *H. howelli* vs *H. viridiflavus* (Table 15). In addition, Wilcoxon test revealed that only *H. kivuensis* vs *H. howelli* and *H. howelli* vs *H. viridiflavus* showed significant results in the call bandwidth (Hz) (Table 15).

Table 15. Wilcoxon rank-sum test of *H. kivuensis, H. howelli* and *H. viridiflavus* for advertisement call characteristics

	Wilcoxon rank-sum test		
Species	Peak frequency (kHz)	Delta time (s)	Bandwidth (kHz)
H. kivuensis - *H. howelli*	W = 0; p = 0.00	W=525; p = 0.00	W=18; p = 0.00
H. kivuensis - *H. viridiflavus*	W = 0; p = 0.00	W=333; p = 0.00	W=101; p= 0.06
H. howelli - *H. viridiflavus*	W = 2183; p = 0.00	W=1830; p = 0.00	W=2111; p = 0.00

The oscillogram and spectrum of call of the three *Hyperolius* species are shown in Fig. 22. The male *H. kivuensis* is recognized for having a powerful call. The call consists of nine pulses in 45 seconds (Fig. 22a). Mostly, all *Hyperolius* species calls have been recorded while sitting on long reed grass stems and leaves, along the river's banks and in grassy wetlands. The male *H. kivuensis* was found calling at night, sitting and calling on reed grass (*Cyperus rotundus, Schoenoplectus confuses*) as depicted in Fig. 23a. However, *H. howelli* were observed both during the day and at night although the males were observed calling only during night time. The calls produced were continuous, repetitive with high pitch note in *H. howelli* (Fig. 22b). The vocalization of *H. viridiflavus* is commonly characterized as a sequence of brief, bell-like, high-pitched trills or chirps. These calls typically consist of an average of 39 pulses, as illustrated in Fig. 22c

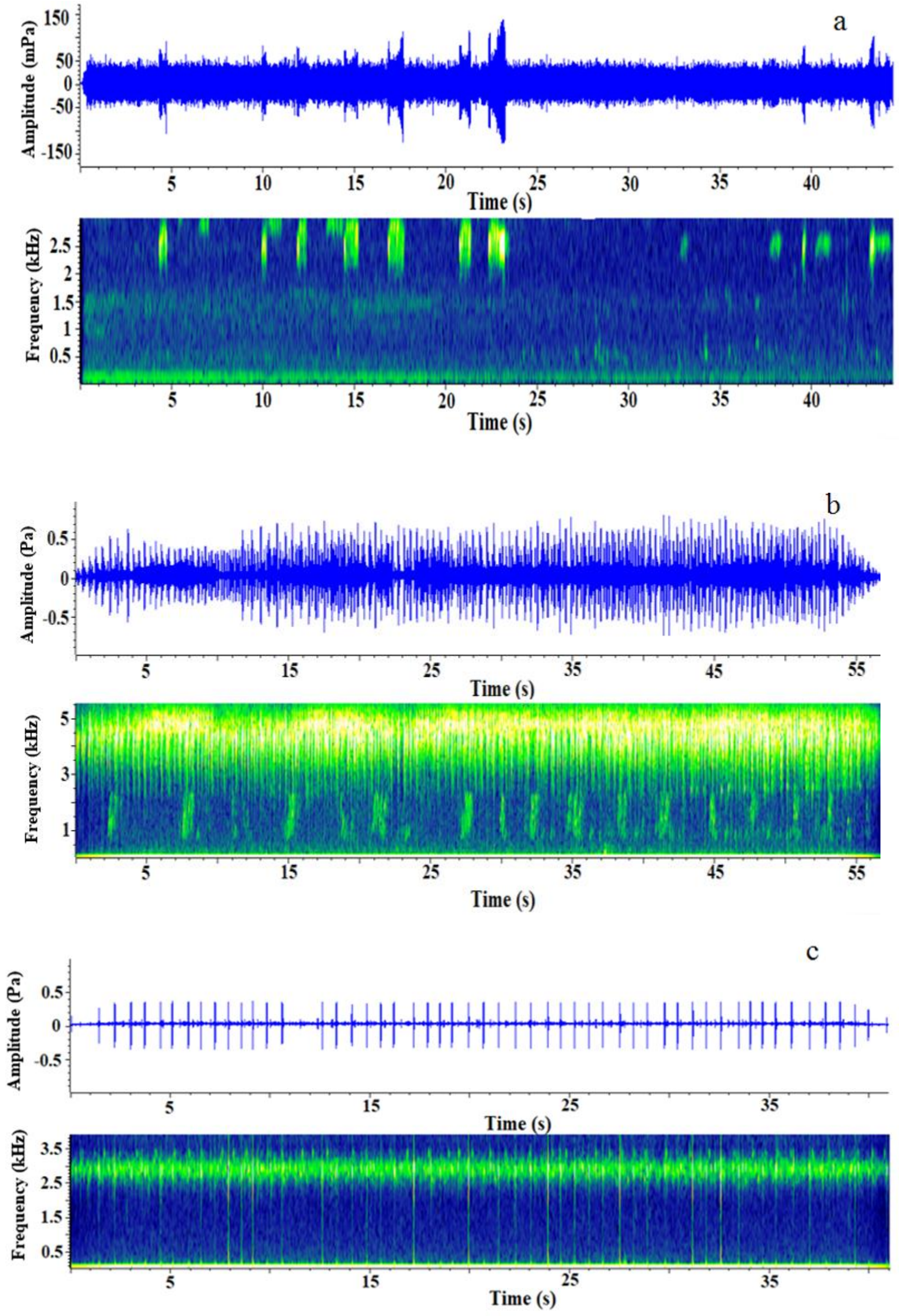


Figure 22. The oscillogram (top) and spectrogram view (bottom): (a) *H. kivuensis* (b) *H. howelli* and (c) *H. viridiflavus* from Kaffa

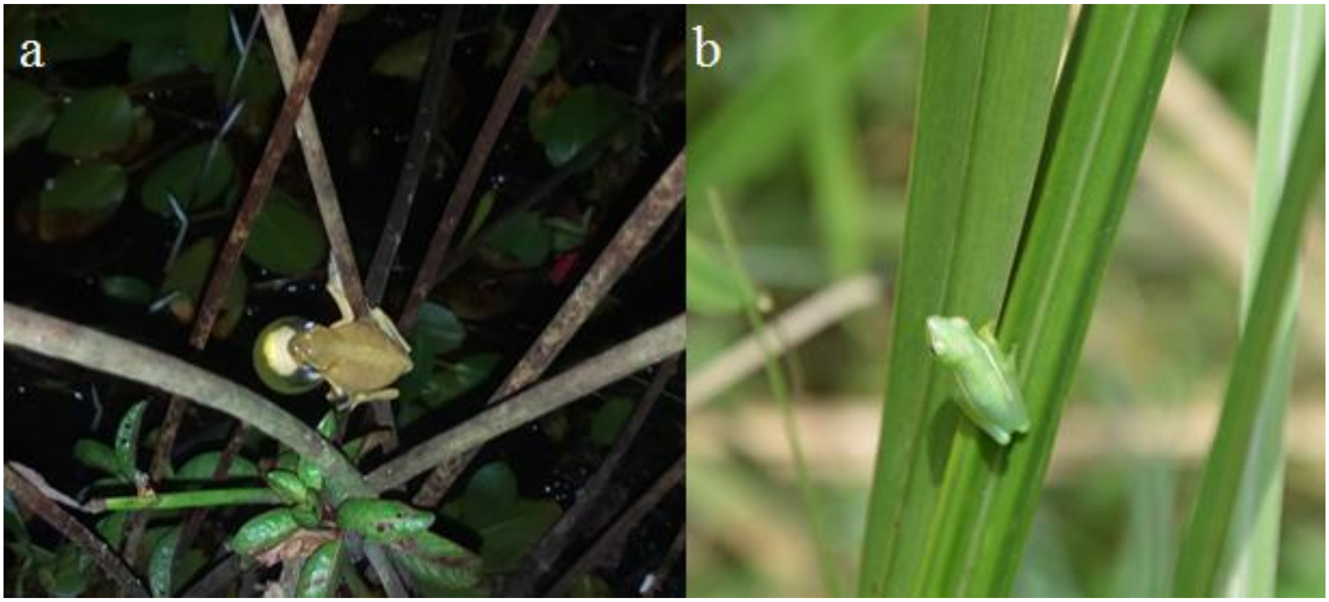


Figure 23. Habitat of *Hyperolius*. a) *H. kivuensis* at the reed long grass from Komba wetland sitted on *Schoenoplectus confuses* (‘Ketema’), b) *H. howelli* sitting on the reed grass in the Gojeb wetland (Photo: Abeje Kassie, July, 2021)

4.4.1.4. Distribution of Hyperolius species

We found three different *Hyperolius* species (*Hyperolius kivuensis*, *Hyperolius viridiflavus* and *Hyperolius howelli*) in Kaffa area (Fig. 24). All three *Hyperolius* species recorded from Kaffa are found on the reed grass (grassy wetlands) and at the shore of riverine forest. *Hyperolius viridiflavus* and *Hyperolius kivuensis* were found in Komba, Sheda, Alemgono and around Bita genet town wetlands. *Hyperolius viridiflavus* was recorded from riverine forest and ponds. An individual *Hyperolius kivuensis* was observed from Shoreri wetland. *Hyperolius howelli* were recorded only from Gojeb, Alemgono and Shida wetlands (Fig. 25). No species of *Hyperolius* is encountered above 2000 m asl but between 1561 and 1857 m asl. Depending on their specific habitat, individual *Hyperolius* species can exhibit varying coloration.

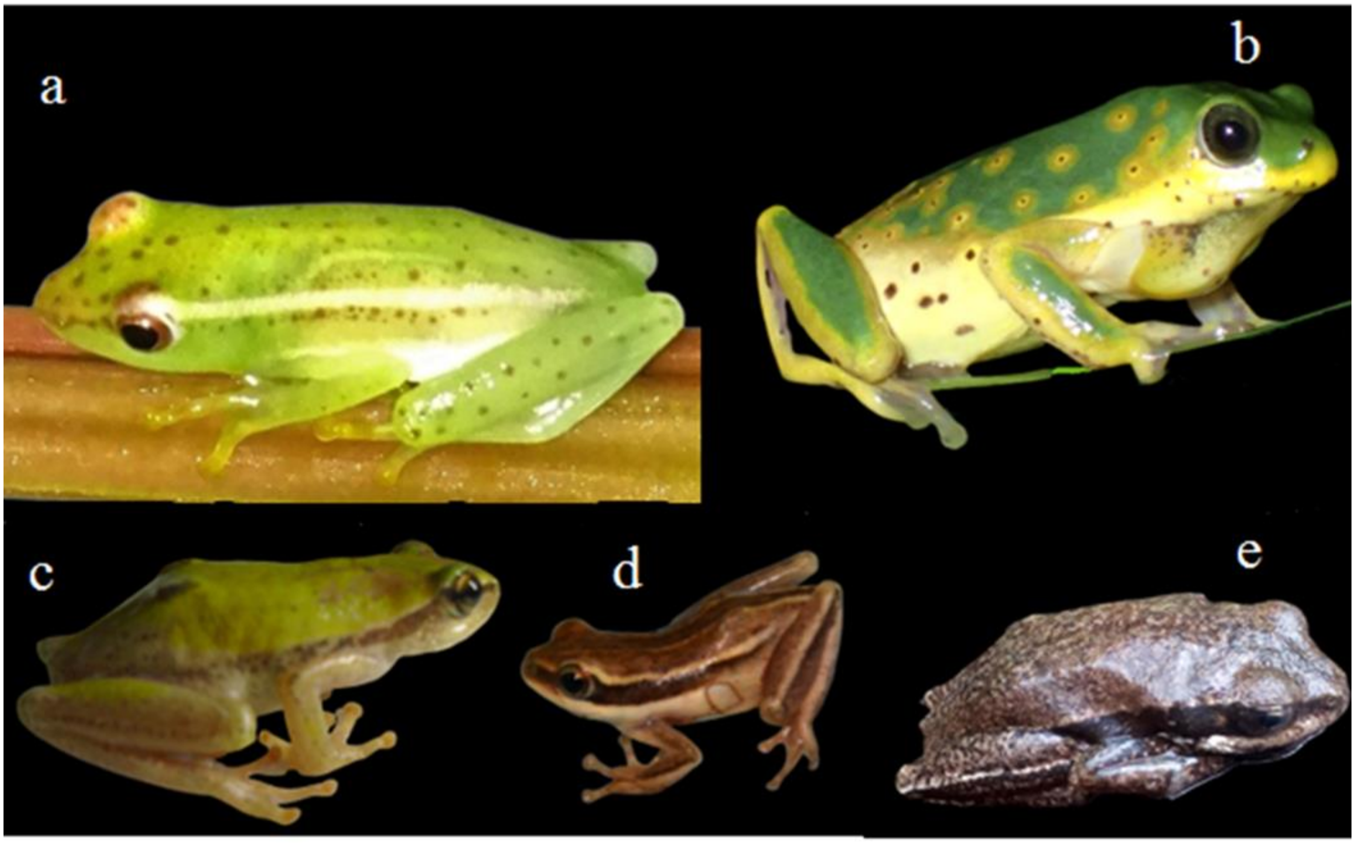


Figure 24. The three *Hyperolius* species recorded in Kaffa area. a) *H. howelli* b) *H. viridiflavus* and c, d and e are polychromatic features of *H. kivuensis* (Photo: Abeje Kassie, July, 2021).

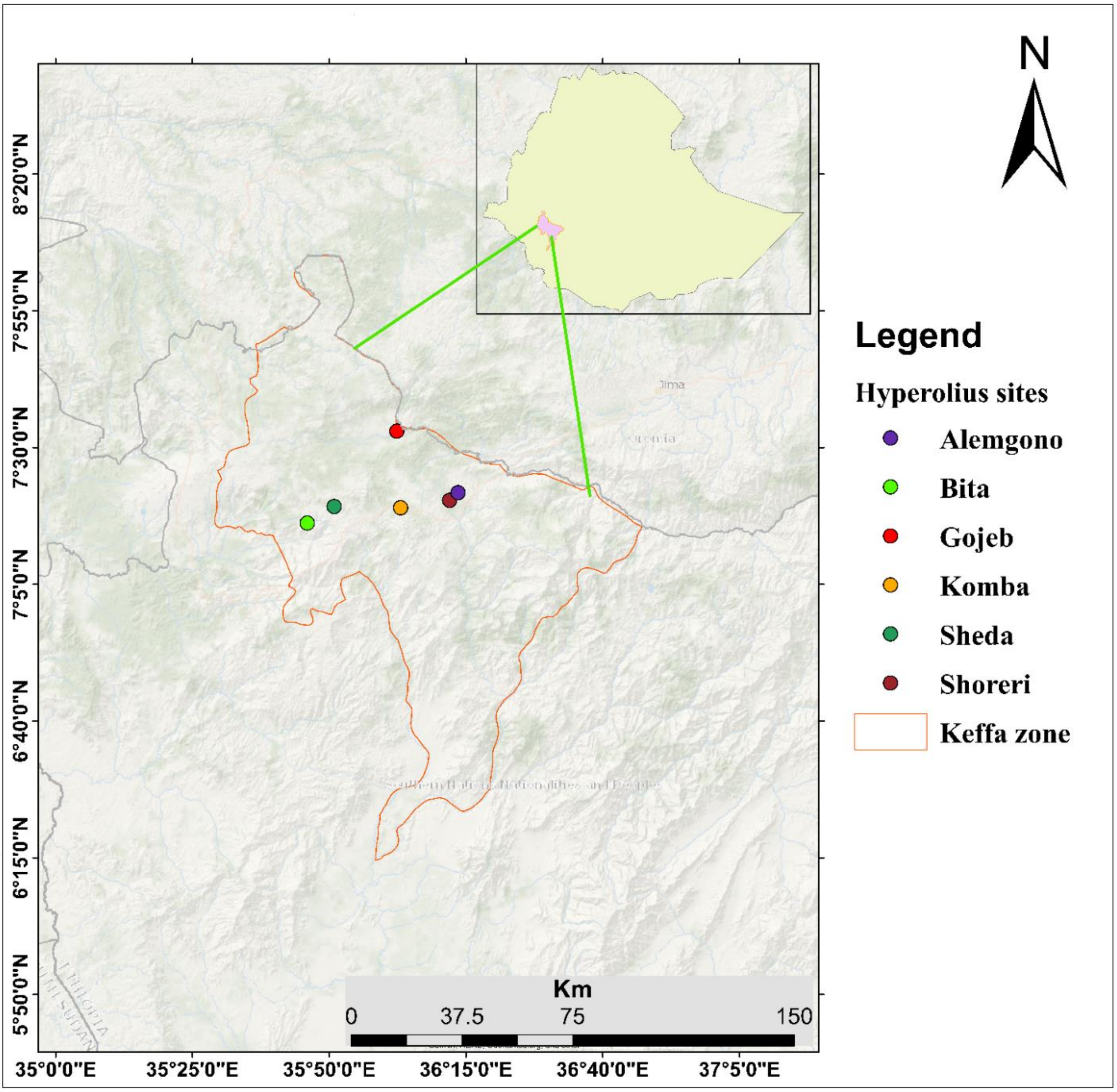


Figure 25. The distribution of *Hyperolius* species recorded in Kaffa area in the current study.

4.4.2. *Afrixalus* species

4.4.2.1. Phylogenetics

Phylogenetics for *Afrixalus* species recorded in the study area are shown on Fig. 26. The optimal tree had sum of branch length (SBL) of 0.734. The percentage of trees in which the associated taxa clustered together is shown next to the branches. The number of base differences per site from between sequences are shown. Standard error estimate(s) are shown above the diagonal Appendix 12). The rate variation among sites was modelled with a gamma distribution (shape parameter = 1). This analysis involved 30 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions with less than 95% site coverage were eliminated, i.e., fewer than 5% alignment gaps, missing data, and ambiguous bases were allowed at any position (partial deletion option).

There were a total of 456 positions in the final dataset The number of base differences per site from averaging over all sequence pairs is 0.11 and standard error estimate(s) is 0.01. The phylogeography and the evolutionary relationship of *A. clarkei* and *A. enseticola* recorded from Kaffa area were supported by bootstrap index of 60%. However, there was a clear distinct clade separation between *A. clarkei* and *A. enseticola* (Fig. 26). The minimum genetic distance within *A. clarkei* species was 0.0 and maximum was 0.02 while the genetic distance within *A. enseticola* species was 0.00. The minimum genetic distance between *A. clarkei* and *A. enseticola* was 0.13 and the maximum was 0.14 (Table 16).

Afrixalus enseticola reveals greater genetic variation from *A. fulvovittatus* with a genetic distance of 0.17 and less genetic variation from *A. delicatus* and *A. laevis* with a genetic distance of 0.118.

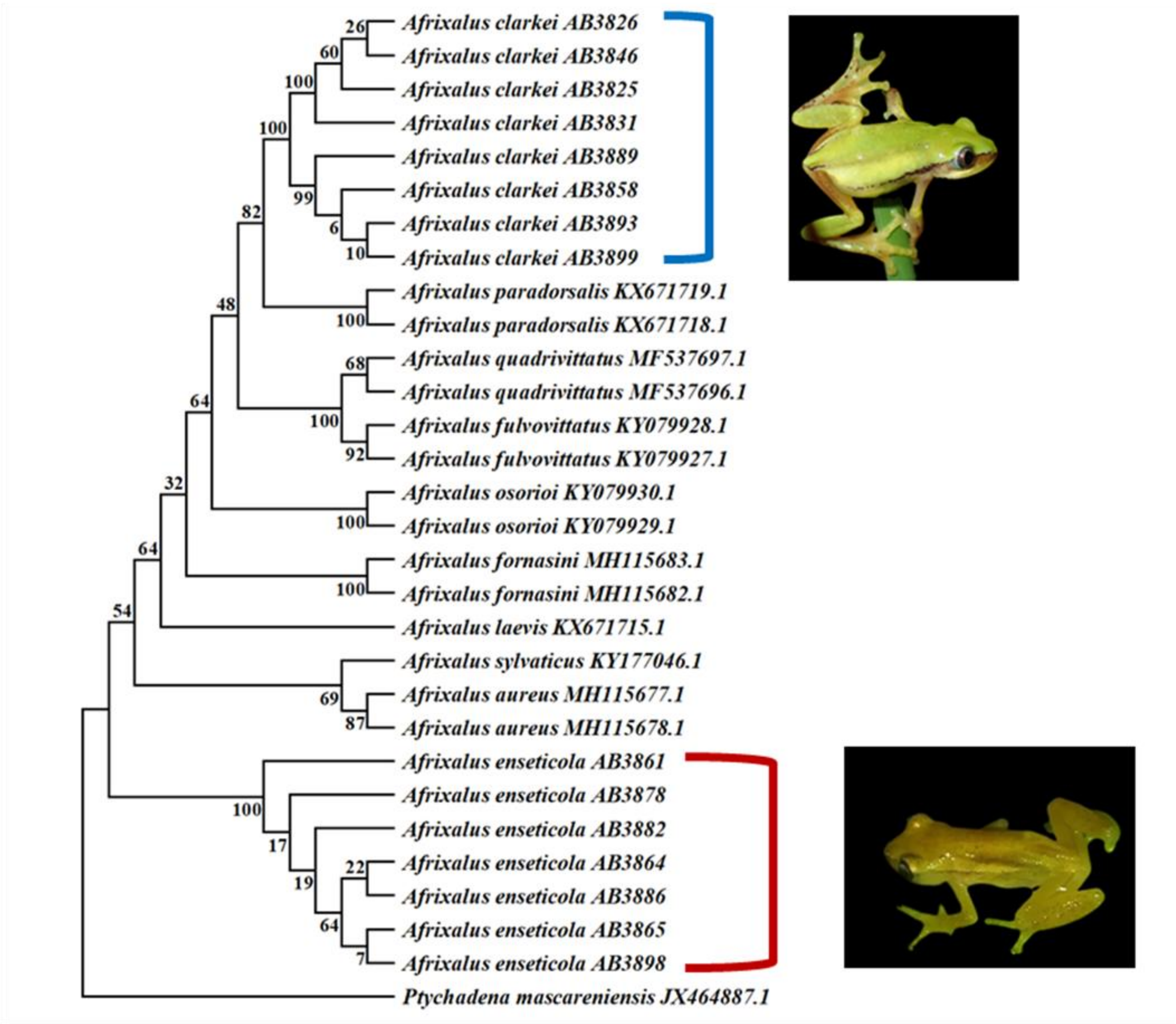


Figure 26. The maximum-likelihood (ML) phylogeny of the genus *Afrixalus* based on 16S mt RNA genes.

Table 16. Genetic variability of seven *Afrixalus enseticola* with 10 other species of African *Afrixalus*. Given are minimum (min), maximum (max) and mean values of uncorrected pairwise sequence divergence, presented in % of 348–515 bp of the 16S rRNA gene; SD = standard deviation; N= number of individual interspecific comparisons; the bold text in the table is for species recorded in Kaffa area.

	Min	Max	Mean	SD	N	Remark
A. aureus	0.11	0.11	0.11	0.00	2	
A. clarkei	0.13	0.14	0.131	0.01	8	
A. enseticola	0.0	0.0	0.0	0.0	6	Within ***A. enseticola***
A. fornasini	0.12	0.13	0.13	0.00	2	
A. fulvovittatus	0.16	0.17	0.16	0.00	2	
A. laevis	0.11	0.11	0.11	0.00	1	
A. osorioi	0.12	0.13	0.13	0.00	2	
A. paradorsalis	0.13	0.14	0.133	0.005	2	
A. quadrivittatus	0.16	0.16	0.16	0.00	2	
A. sylvaticus	0.12	0.12	0.12	0.00	1	
P. mascareniensis	0.20	0.20	0.20	0.20	1	

A. clarkei shows greater genetic variation from *A. enseticola* with a genetic distance of 0.131 and less genetic variation from *A. paradorsalis* with a genetic distance of 0.084 (Table 17). All of the new sequences generated from this study are deposited in GenBank, along with additional sequences obtained from the NCBI GenBank. Appendix 4 contains information about the vouchers' Id, locations, and GenBank accession. Based on the single mitochondrion gene study, eleven *Afrixalus* clades were identified. Each clade was considered a species, with minimum uncorrected p distances for 16S between them ranging from 0.08 to 0.17 (Table 17).

Table 17. Genetic variability of eight *Afrixalus clarkei* with ten other African *Afrixalus*. Given are minimum (Min), maximum (Max) and mean values of uncorrected pairwise sequence divergence of the 16S rRNA gene; sd= standard deviation; N= number of individual interspecific comparisons; the bold text in the table is for species that recorded in Kaffa area.

	Min	Max	Mean	SD	N	Remark
A. aureus	0.12	0.13	0.123	0.004	2	
A. clarkei	0.00	0.02	0.01	0.01	8	Within ***A. clarkei***
A. enseticola	0.13	0.14	0.131	0.003	7	
A. fornasini	0.10	0.11	0.103	0.004	2	
A. fulvovittatus	0.12	0.13	0.126	0.005	2	
A. laevis	0.11	0.11	0.11	0.00	1	
A. osorioi	0.09	0.11	0.10	0.006	2	
A. paradorsalis	0.07	0.08	0.08	0.00	2	
A. quadrivittatus	0.12	0.13	0.12	0.00	2	
A. sylvaticus	0.11	0.12	0.116	0.005	1	
P. mascareniensis	0.22	0.22	0.22	0.00	1	

4.4.2.2. Morphometry

In this study, we conducted morphometric measurements to quantify the physical characteristics of frogs, specifically focusing on the *Afrixalus* species. We measured 17 distinct characteristics as described in the methodology section. These parameters were utilized to identify and characterize the two *Afrixalus* species occurring in Kaffa.

The mean snout-vent length of male *A. clarkei* species was 19.15 mm, while for females it was 21.45 mm. For male *A. enseticola*, the mean snout-vent length was 20.4 mm, and for females, it was 26.6 mm. Male *A. clarkei* had a mean ratio of head length (HL) to snout-vent length (SVL) of 0.38 ± 0.02, whereas for the females, it was, 0.30. The male *A. clarkei* also exhibited a mean ratio of head width (HW) to snout-vent length (SVL) of 0.34 ± 0.01, while for the females, it was, 0.32 ± 0.02.

In *A. enseticola*, the male frogs had a ratio of HL/SVL of 0.33, while for females had 0.30. Male *A. enseticola* also showed a ratio of HW/SVL of 0.30, while for the females, it was, 0.28 (Table 18).

Table 18. Measurements (mean value ± sd in mm) of adult *A. clarkei* and *A. enseticola*. “N” represents number of *Afrixalus* species. Ratios of parameters were computed from the mean of measurements.

Variables	*A. clarkei*		*A. enseticola*	
	Male (N=10)	Female (N=8)	Female (N=4)	Male (N=7)
SVL	19.15 ± 1.55, 17.6 - 20.7	21.45 ± 1.05, 20.4 - 22.5	26.6 ± 2.1	20.4 ± 1.6
HW	6.55 ± 0.35, 6.2 - 6.9	6.8 ± 0.2, 6.6 - 7	7.5 ±0.4	6.2 ±0.35

HL	7.35 ± 0.95, 6.4 - 8.3	6.45 ± 0.35, 6.1 - 6.8	7.9 ±0.23	6.8 ±0.22
SL	2.55 ± 0.05, 2.5 - 2.6	3.2 ± 0.1, 3.1 - 3.3	3.3± 0.1	2.9 ±0.1
NS	0.95 ± 0.05, 0.9 - 1	0.85 ± 0.05, 0.8 - 0.9	1 ±0.06	1.0 ±0.0
IND	1.5 ± 0, 1.5 - 1.5	1.95 ± 0.05, 1.9 - 2	1.9 ± 0.0	2 ± 0.03
EN	1.6 ± 0.1, 1.5 - 1.7	2.35 ± 0.15, 2.2 - 2.5	2.3 ± 0.2	1.9 ± 0.14
IOD	2.25 ± 0.05, 2.2 - 2.3	2.3 ± 0, 2.3 - 2.3	2.6 ± 0.0	2.5 ± 0.03
ED	2.5 ± 0.1, 2.4 - 2.6	2.8 ± 0.3, 2.5 - 3.1	2.6 ± 0.2	2.8 ± 0.25
UEW	1.2 ± 0.2, 1 - 1.4	1.25 ± 0.25, 1 - 1.5	0.9 ±0.1	1.3 ±0.05
FLL	4.15 ± 0.25, 3.9 - 4.4	4.6 ± 0, 4.6 - 4.6	5.9 ± 0.34	4.5 ± 0.0
HAL	5.6 ± 0.2, 5.4 - 5.8	6.2 ± 0.2, 6 - 6.4	8.4 ± 0.1	6.1 ± 0.3
FinDW	1.05 ± 0.05, 1 - 1.1	1.15 ± 0.15, 1-1.3	1.5 ± 0.0	1.2 ± 0.03
THL	6.85 ± 0.45, 6.4 - 7.3	8.35 ± 0.45,7.9 - 8.8	10.8 ± 0.5	8.6 ± 0.26
TL	7.35 ± 0.35, 7 - 7.7	8.85 ± 0.35, 8.5 - 9.2	13 ± 0.25	9.4 ± 0.08
FL	7.65 ± 0.45, 7.2 - 8.1	9.15 ± 0.25,8.9 - 9.4	12.8 ± 0.30	9.2 ± 0.36
Toe4DW	0.75 ± 0.15, 0.6 - 0.9	1.1 ± 0.1, 1 - 1.2	1.6 ± 0.12	1.0 ± 0.15
HL/SVL	0.38 ± 0.02, 0.36 - 0.4	0.30 ± 0.00, 0.30 - 0.30	0.30 ± 0.0	0.33 ± 0.0
HW/SVL	0.34 ± 0.01, 0.33 - 0.35	0.32 ± 0.02,0.29 - 0.34	0.28 ± 0.01	0.30 ± 0.01
IND/SVL	0.08 ± 0.01, 0.07 - 0.09	0.09 ± 0.01, 0.08 - 0.10	0.07 ± 0.1	0.10 ± 0.03
THL/SVL	0.36 ± 0.01, 0.35 - 0.36	0.39 ± 0.04, 0.35 - 0.43	0.41 ± 0.03	0.42 ±0.0
TL/SVL	0.38 ± 0.01, 0.37 - 0.40	0.41 ± 0.00, 0.41 - 0.42	0.49 ± 0.02	0.46 ±0.03

4.4.2.3. Acoustic characteristics

Frogs belonging to the genus *Afrixalus* are well-known for their intricate and diverse vocalizations, which play a critical role in communication, mate selection, and territorial defense. In the study area, auditory characteristics were identified specifically for the *A. clarkei* species (Fig. 27).

The male advertisement call of *A. clarkei* has a duration (delta time) ranging from 0.2 to 0.5 seconds, with an average duration of 0.2 seconds (SD ± 0.1 seconds). The single call typically consists of nine distinct pulses (Fig. 27). In terms of peak frequency, the mean peak frequency of *A. clarkei* calls is measured to be 3.76 kHz, (SD ± 0.07 kHz). The range of peak frequencies falls between 3.66 kHz and 3.84 kHz. Additionally, the mean bandwidth of the calls was 0.66 kHz, (SD ± 0.05 kHz). The bandwidth ranges from 0.56 kHz to 0.75 kHz.

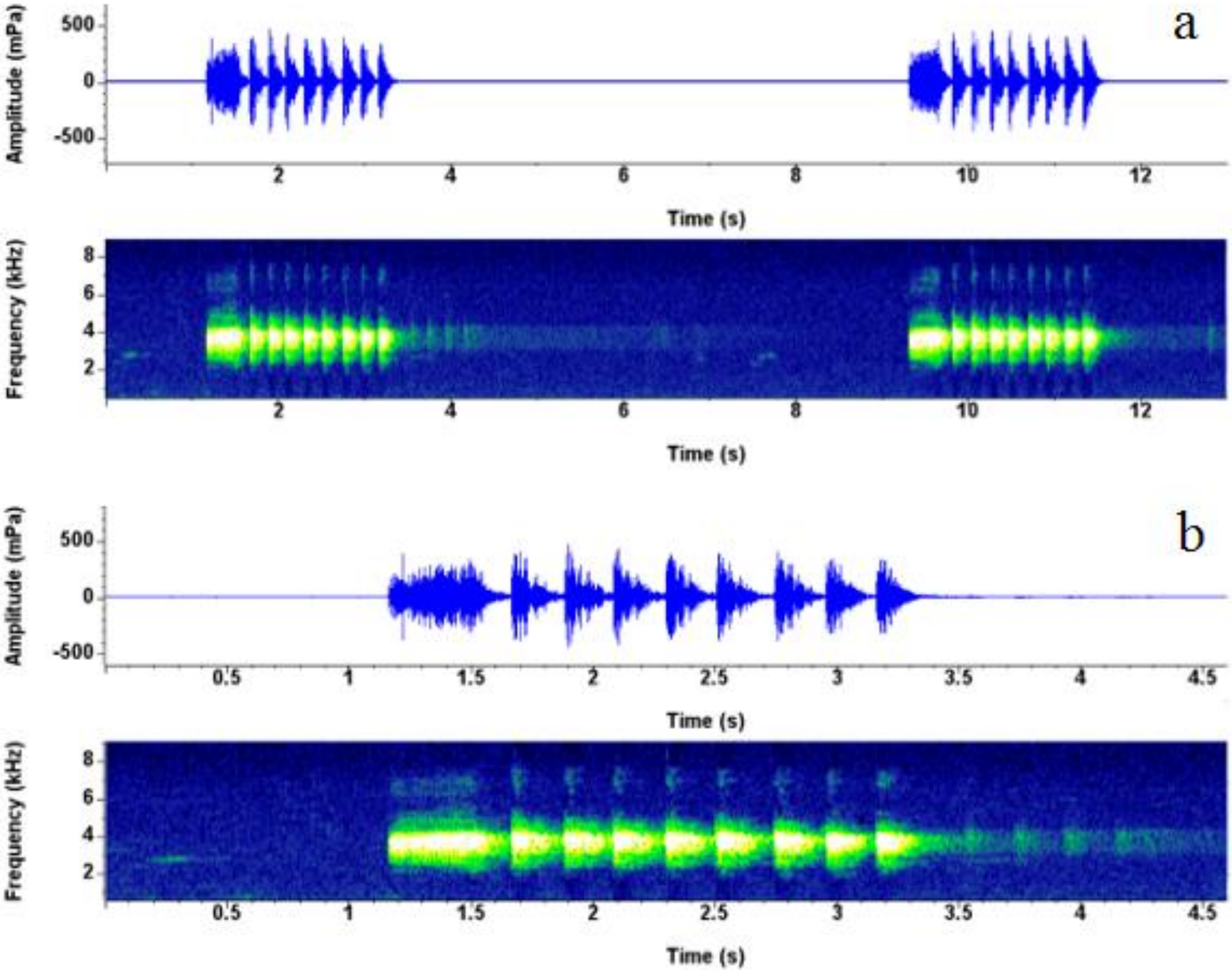


Figure 27. The oscillogram(top) and spectrogram view (bottom) of the call of *A. clarkei*. a) illustrating two calls composed of a series of 18 pulses (the smallest unit of a call) and b) a single call composed of 9 pulses.

4.4.2.4. Distribution of Afrixalus species

These species inhabit the same geographical region, indicating a sympatric occurrence. The study recorded their presence in diverse habitats, including wetlands, enset plantations and riverine forests (Fig. 28)

Figure 28. *Afrixalus* species. a) *A. clarkei* at the reed long grass from Boqa wetland, Adiyo Woreda, Kaffa b) *A. enseticola* at the edge of enset plantation in Medabo, Gesha Woreda, Kaffa (Photo: Abeje Kassie, February2020).

Afrixalus clarkei was specifically found in various locations within Kaffa area. These sites included Boqa wetland in Adiyo district, Medabo riverine forest in Gesha district, Komba enset plantation in Gimbo district, Shamali wetland in Decha district, and Sor riverine forest in Saylem district (Fig. 29). The elevation range at which *Afrixalus clarkei* was found in this area varied from 1561 to 2482 m asl. On the other hand, *Afrixalus enseticola* was recorded at elevations ranging between 1848 and 2351 m asl in the same area.

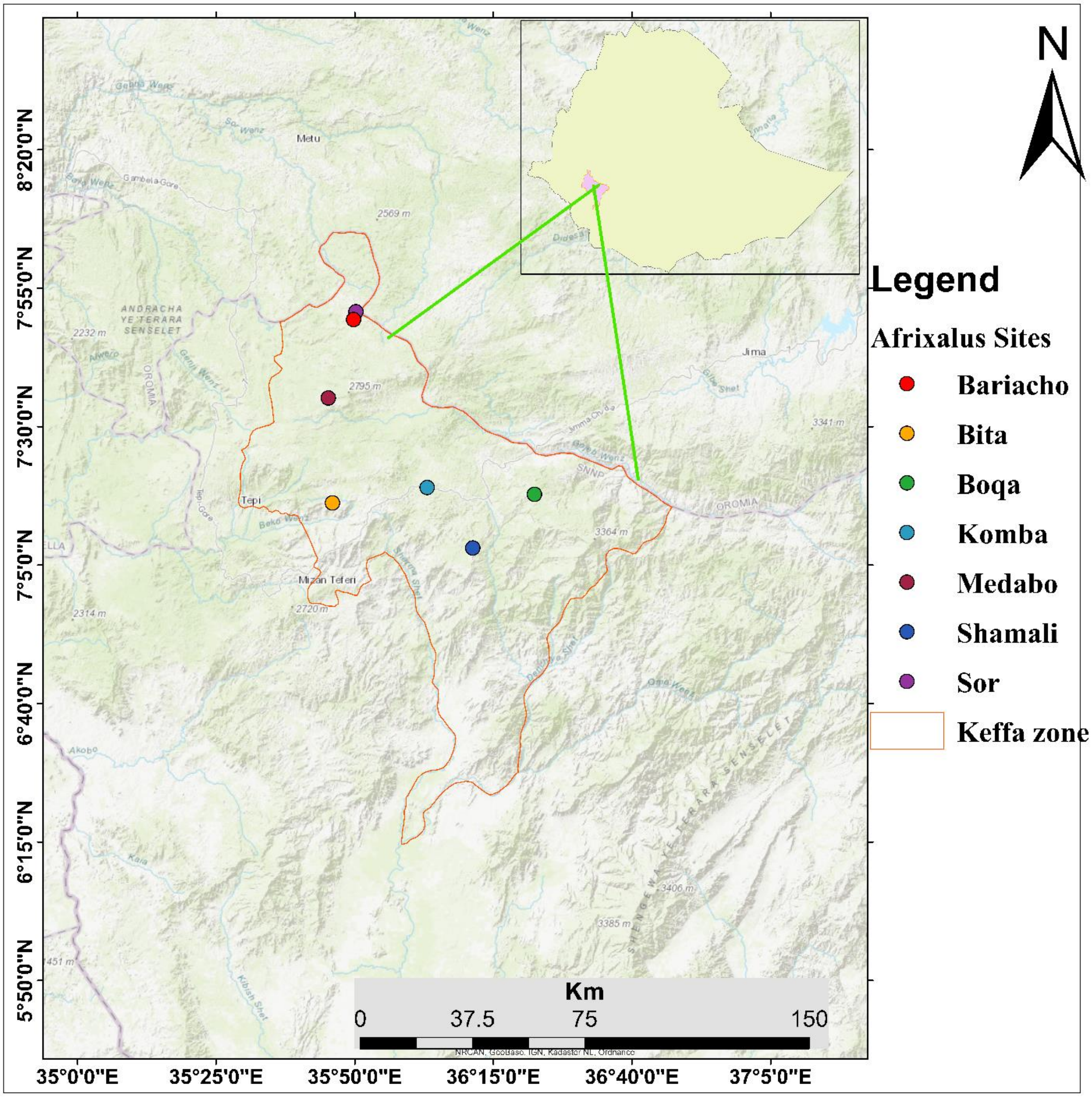


Figure 29. Distribution map of *Afrixalus* species around Kaffa area.

4.5. Knowledge, Attitudes and perceptions of local communities

Feedback from respondents exhibited substantial awareness and understanding of frogs, particularly when it comes to encountering frogs in diverse habitats. Of the respondents, 85.6% reported observing frogs in diverse habitats, including wetlands, ponds, and enset areas.

Specifically, 5.2% exclusively saw frogs in enset habitats, while 4.0% exclusively encountered them in wetlands. Moreover, 1.7% of the respondents mentioned observing frogs in both enset habitats and wetlands. Concerning the decline in frog populations, 80.1% of the participants indicated that the number of frogs had decreased in recent years (Table 19). This decrease was attributed to various factors, including reduced rainfall, climate change, population growth and agricultural expansion. Interestingly, the respondents noted that the number of frogs tends to increase significantly during the rainy season. Most respondents (75.5%) express willingness to actively participate in conservation efforts aimed at preserving frog habitats.

Among the participants, 53.6% responded that they do not experience fear when encountering frogs, while 46.4% admited to having fear of frogs. It was found that there is no statistically significant difference in the fear of frogs between genders ($p < 0.05$). The primary reason cited by individuals who are afraid of frogs is the feeling and thought of their smooth skin. These individuals reported feeling discomfort and fear when touching the smooth and cold skin of frogs (Table 19).

In the study area, various beliefs and perceptions are observed regarding frogs. There is a belief that when a woman holds a green frog (*Leptopelis vannutelli*) from the enset plantation in her hand, it prevents food, particularly boiled cabbage, from being spoiled for an extended period. Another belief suggests that a pregnant woman will give birth once a frog enters her house. In a different area within the Kaffa region, respondents mentioned that touching frogs can cause the entire body to swell and the presence of a frog in the house hinders the fermentation of teff, maize or wheat flour. Additionally, there is a belief that if someone kills a frog, their hand will tremble for many years. It is important to note these beliefs and perceptions, as they contribute to the local cultural understanding about frogs.

Table 19. Respondent feedback from Kaffa area regarding attitudes about the conservation of frogs.

		Preference of habitats				
		All habitat	Enset	Enset and wetland	Pond	Wetland
Sex	F	117	4	6	11	1
	M	180	14	0	1	13
Total		297 (85.6%)	18 (5.2%)	6 (1.7%)	12(3.5%)	14 (4.0 %)

		Is there any change in the number of frogs observed from the previous years?		
		I don't know	No	Yes
Sex	F	40	0	99
	M	27	2	179
Total		67 (19.3 %)	2 (0.6%)	278 (80.1%)

		Do you think that the habitat of frogs is being reduced?		
		I don't know	No	Yes
Sex	F	10	13	116
	M	2	28	178
Total		12 (3.5%)	41 (11.8%)	294 (84.7%)

		Seasonal preference		
		Dry season	I don't know	Rainy season
Sex	F	0	7	132
	M	2	4	202
Total		2 (0.6%)	11 (3.2%)	334 (96.2%)

		Do you think that frogs in this area should be conserved?		
		I do not know	No	Yes
Sex	F	26	13	100

	M	33	13	162
Total		59 (17.0%)	26 (7.5%)	262 (75.5%)

		Are you afraid of frogs?	
		No	Yes
Sex	F	85	54
	M	101	107
Total		186 (53.6%)	161 (46.4%)

4.6. Threats and conservation of frogs

4.6.1. The Amphibian chytrid fungus

A comprehensive examination was conducted in Kaffa area, where a total of 116 frog samples belonging to 12 different species were screened for the presence of Bd (amphibian chytrid fungus) (Table 20). Surprisingly, the findings revealed that all 116 frog samples tested negative for Bd, indicating the absence of the Chytrid fungus within the surveyed populations.

Table 20. Distribution and sample sites of swabbed frog species.

Frog species	Sites where frogs were swabbed and collected											
	Boqa	Dedifa	Deka	Ganeti	Haiti	Komba	Oda	Shamali	Sheda	Sor	Utali	Yadota
A. clarkei	1	1	1	-	-	3	-	2	-	-	4	2
A. enseticola	-	2	2	-	-	-	-	-	-	-	-	2
H. kivuensis	-	-	-	-	-	7	-	-	1	-	-	-
H. howelli	-	-	-	-	-	-	-	-	1	-	-	-
H. viridiflavus	-	-	-	-	-	2	-	-	-	-	-	-
Leptopelis sp.1	-	-	-	2	-	-	-	-	-	-	-	-
Leptopelis sp.2	-	-	-	3	-	-	-	-	-	-	-	-
L. vannutellii	-	-	-	-	5	-	15	2	-	3	-	7
Phrynobatrachus sp.*1*	4	2	1	1	3	-	1	5	-	-	11	5
P. erlangeri	-	-	-	-	-	-	-	3	-	-	-	1
P. neumanni	2	2	-	1	-	-	-	-	-	-	-	-
P. nilotica	-	-	-	-	-	-	-	-	2	-	2	2

4.6.2. Conservation status of frogs

According to the 2013 IUCN Red List status, our study area has revealed the presence of three Endangered species: *L. susanae*, *A. clarkei*, and *X. largeni*. Additionally, we have identified one species categorized as Vulnerable (*A. enseticola*) and another as Near Threatened (*P. erlangeri*) (Table 21). Despite extensive effort using different frog surveying techniques, including drift fences and pitfall traps, *Sylvacaecilia grandisonae*, the endemic caecilian species, was not recorded in the designated study area.

During our fieldwork, we directly observed the adverse effects of water drainage from the wetland and the establishment of *Eucalyptus* tree plantations on the wetland's edge, both of which have led to a decline in frog habitats. Furthermore, deforestation in the riverine forest was also observed. Additionally, we noted the conversion of enset plantations into field crops such as teff, maize and wheat.

Table 21. IUCN Red List Conservation status of frog species recorded in Kaffa area (LC: Least Concern; NT: Near Threatened; EN: Endangered; VU: Vulnerable; NE: Not Evaluated; * Not Assessed)

Family	Species	Endemism	IUCN Red list status
Arthroleptidae	*Leptopelis cf. susanae*	E	EN
	Leptopelis vannutellii	E	LC
	Leptopelis sp.1*		
	Leptopelis sp.2*		
Conrauidae	*Conraua beccarii*	E	LC

Dicroglossidae	*Hoplobatrachus occipitalis*		LC
Hemisotidae	*Hemisus marmoratus*		LC
	Hemisus microscaphus	E	LC
Hyperoliidae	*Afrixalus clarkei*	E	EN
	Afrixalus enseticola	E	VU
	Hyperolius kivuensis		LC
	Hyperolius howelli		LC
	Hyperolius viridiflavus		LC
	Kassina senegalensis		LC
	Paracassina obscura	E	LC
Phrynobatrachidae	*Phrynobatrachus sp.1*	E	LC
	Phrynobatrachus natalensis		LC
Pipidae	*Xenopus clivii*		LC
	Xenopus largeni	E	EN
Ptychadenidae	*Ptychadena anchietae*		LC
	Ptychadena doro	E	NE
Ptychadenidae	*Ptychadena erlangeri*	E	NT
	Ptychadena neumanni	E	LC
	Ptychadena nilotica		LC
	Ptychadena schillukorum		LC
Grandisoniidae	*Sylvacaecilia grandisonae*	E	LC

5. DISCUSSION

5.1. Diversity and abundance of frogs in Kaffa area

The findings highlight Kaffa area possesses a significant biodiversity of frog species. The highest number of frog species with a unique species composition is recorded. Previous studies by Abebe Ameha (2012), NABU (2020) and Goutte *et al*. (2021) have mentioned the existence of many of these species in Kaffa and its surrounding areas. However, this study revealed the presence of a few more species that were previously unrecorded in the Kaffa area. Some of these species are also distributed across various parts of Ethiopia, at elevations ranging from 1000 to 3000 m asl, but our genetic analyses have revealed that the specimens from the southwest region exhibit distinct characteristics compared to other Ethiopian populations (Manthey *et al*., 2017). In our study, we have documented the presence of *Amietia nutti* in the Sor Riverine Forest, marking it as a new record for the Kaffa area.

Our observations regarding the endemic frog species found in Kaffa revealed that the initially identified species of *L. ragazzii*, through DNA sequencing analyses, was found to be a different species, temporarily designated as *Leptopelis* sp. 2. Similarly, our BLAST search, genetic distance assessments and ML tree analysis have demonstrated that the small *Phrynobatrachus* species found in Kaffa are distinctly separate from the previously described *P. minutus* and *P. inexpectatus*. Consequently, we have provisionally assigned this species as *Phrynobatrachus* sp. 1.

Drayer and Richter (2016) and Nneji *et al*. (2019) have highlighted that wetlands serve as significant habitats for a diverse array of frog species. Consistent with this, our study has revealed that wetland habitat exhibited the highest abundance and species richness among the three habitats surveyed. We recorded a total of 4457 individuals belonging to 19 frog species in the wetland. This can be attributed to the fact that wetland ecosystems offer a wide range of food sources for these organisms, including invertebrates and zooplankton, as highlighted by Muro-Torres *et al*. (2020).

Species abundance varies across different habitats. While a few species were found to be common across all habitats, the majority of species were observed to be rare, with a large number of infrequent occurrences. This indicates a complex distribution pattern and highlights the diverse ecological preferences and niche requirements of the frog species in the study area. Inline with this study, Mindje *et al*. (2020) showed that the limited availability of physical niches in natural habitats for anurans results in intense competition among local species, leading to a decline in diversity due to competitive exclusion.

Habitat and environmental heterogeneity have been identified as contributing factors to the variation in species diversity (Silva *et al*., 2011; Tomé, 2011; Gouveia and Faria, 2015). Wetland habitats exhibit significantly higher frog diversities, measured by Shannon-Wiener (H) and Simpson (D) indices, compared to agricultural land and riverine forests. Similar findings have been reported in Brazil's Amazonian savannas and surrounding forests (Neckel-Oliveira *et al*., 2000), as well as in Trinidad's Aripo Savanna Scientific Reserve (Auguste and Hailey, 2018). The increased diversity observed in the wetland habitats during our study is possibly attributed to the greater availability of resources for foraging, reproduction, and predator cover, all of which influence frog habitat selection (Dodd, 2010).

The local communities in the study area predominantly depend on enset as a primary food source (Dagmawit Chombe and Endashaw Bekele, 2011; Borrell *et al*., 2019; Selamawit Araya *et al*., 2021). In Kaffa area, enset plantations exhibit a diverse structure, characterized by a mix of under-storey vegetation and scattered canopy trees. This particular habitat serves as a home for various species of frog, including *Leptopelis* sp.2, *L. vanuttellii*, *Afrixalus clarkei* and *Afrixalus enseticola*.

Consequently, enset plantation shows higher frog diversity and evenness compared to the riverine forests in the study area. However, due to low enset production resulting from diseases, local communities are dissatisfied and compelled to convert enset plantations into field crops such as teff, wheat, and maize. This shift in agriculture poses a significant threat to existing habitats. Supporting this finding, a separate study by Ndriantsoa *et al*. (2017) observed that banana vegetation plays a crucial role in maintaining frog diversity in Madagascar's fragmented landscape.

Drift fences and pitfall traps proved to be successful in capturing elusive and burrowing frog species like *Hemisus marmoratus* and *Hemisus microscaphus,* as well as active river frogs such as *Amietia nutti*, both during the day and at night. However, these particular methods were ineffective in capturing arboreal (tree) frog species like *L. vannutellii* and *Afrixalus clarkei*. The previous research by Weddeling *et al*. (2004) supports the notion that drift fences and pitfall traps are effective in capturing species that are surface-active, borrowing or cryptic.

These methods may have constraints when it comes to capturing specific frog species, especially those that reside under tree canopies or exhibit distinct movement patterns. This aligns with previous research, Delis *et al*. (1996), who found that drift fences are relatively inefficient at capturing tree frogs and other arboreal species. Additionally, Weddeling *et al*. (2004) discovered that while drift fences have been successfully employed for frog sampling in various locations, they are ineffective specifically in forest environments.

It is important to highlight that the study area contained a rich diversity of ten frog species within the riverine forest habitat. Nonetheless, the drift fence and pitfall trap methods only managed to capture six of these species. This indicates the necessity for alternative or supplementary sampling techniques to ensure a more comprehensive depiction of the frog community in the study area. Moreover, the effectiveness of this technique is significantly influenced by factors such as climatic conditions, seasons and the activity patterns of frogs, as supported by previous studies conducted by Enge (2005) and Todd *et al*. (2007).

5.2. Seasonal variation in species compostition of frogs

According to Giaretta and Menin (2004), the start and duration of reproductive seasons in frogs are directly influenced by weather conditions. The majority of frog species in tropical areas with seasonal climate reproduce during the rainy season (Nneji *et al*., 2019). Studies in Brazil (Giaretta *et al*., 1999) and Asia (Watanabe *et al*., 2005) have also found seasonal variation in frog communities, though there is still little information on this phenomenon in tropical forests. In tropical forests with clearly defined wet and dry seasons, Vonesh (2001) found that rainfall has a significant impact on the diversity and abundance of frog species. This is also true for the current study in the Kaffa area in terms of the diversity and abundance of frog species. Frog species diversity and the number of specimens in Kaffa area increased during the main rainy season, which can be attributed to a higher rate of adult movement around breeding sites (Giarettaand Menin, 2004). Abiotic factors that influence recruitment may be in charge of seasonal fluctuations in frog abundance, yet, predictions need to be verified with more research on Kaffa frogs. However, there is no significant difference between seasons in species richness. This fact might be partially associated with the lack of significant seasonal variation in frog species richness in the Kaffa area. High rainfall rates and an absence of a distinct dry season were observed in the study area.

5.3. Similarity of frog assemblages in Kaffa area

The species similarity tests in the current study revealed a clear distinction of species composition between all paired habitats and study sites. The dissimilarities of unique species composition could probably be associated with the contrasting ecological settings from different intensities of forest disturbance events in the studied forest areas (Hammond and Pokornỳ, 2020). According to Akoto *et al*. (2015), if the SCSI value is lower than 0.5; then the paired communities share different species composition; but if the index is greater than 0.5; then the paired communities share similar species composition. A study conducted in Nigeria by Nneji *et al*. (2019) found that forest and agricultural land have the highest similarity in species composition. In line with this; in the current study; the result showed that agricultural land and riverine forest habitats had the highest SCSI and JCSI.

5.4. Effects of environmental variables on frog species abundance

Although frogs are affected by both biotic and abiotic factors, this study focused solely on abiotic factors such as precipitation, temperature, aspect, altitude and slope. These variables can have an impact on the species richness, abundance and assemblages of frogs across a landscape. These impacts can be positive or negative and their effects have been thoroughly investigated (Blaustein *et al*., 2010). As a result, species react differently to different levels of factors (Wanger, 2009; Sirami *et al*., 2010; Cortés-Gómez, 2013). For example, altitudinal gradient and weather play a role in this because as altitude increases, species assemblages are negatively affected due to changes in temperature and habitat, resulting in fewer species being able to successfully establish populations outside their altitudinal range (Navas, 2002).

According to a recent meta-analysis using different taxonomic groups and geographic areas, species richness and abundance patterns that occur across the cliffs most frequently may be a hump-shaped pattern (Matavelli *et al*., 2022). In line with this, our observation of a hump-shaped pattern for abundance along the altitudinal gradient (Fig. 15C) is reliable with a number of empirical studies (Sanders and Rahbek, 2012).

5.5. Accumulation curve and species richness estimation

While the species cumulative curve usually levels off over time, it is still possible for local species diversity to increase. However, as the study continues, it becomes clear that putting in more effort to increase species richness results in only small improvements, as shown by richness estimators. This highlights the importance of using different sampling methods when conducting inventories to get a more comprehensive understanding of anurans (frogs and toads) (Maritz *et al*., 2007; Ribeiro-Jr *et al*., 2008, Costa-Campos and Freire, 2019), as species richness is closely related to the sampling effort (Costa-Campos and Freire, 2019).

Accumulation curves are great tools for measuring how efficient we are at recording all the different species in a particular place or environment. When we look at the accumulation curves for different habitats, we can see that they start to level off, which means we are capturing most of the species in those habitats. However, when it comes to agricultural land and riverine forests, we see that the curves level off at a relatively low number of species. This tells us that we need to do more sampling in those habitats to find more species that live there.

5.6. Endemic frog species of Kaffa area

The current study compiled the presence of 14 endemic frog species in Kaffa area, which constitutes more than 34% of Ethiopia's endemic frog species. This finding emphasizes the significance of this area as a a very suitable environment for the survival of these unique frog species. It is worth noting that all of the species observed in this study were previously reported to inhabit Kaffa area and its neighbouring regions (Abebe Ameha, 2012; NABU, 2020; Goutte *et al*., 2021). However, the discovery of Pipidae species, *Xenopus largeni*, in the Kaffa area is noteworthy. This species was previously only known to exist at high elevations in the vicinity of the Ethiopian Rift, specifically around the Bale mountains and the Tana Lake basin, where it thrived at altitudes ranging from 2300 to 3100 m (Largen and Spawls, 2010; Evans *et al*., 2011). Therefore, the identification of *X. largeni* in the Kaffa area represents a new and significant addition to the local frog fauna, expanding the known distribution range of this species.

In our recent study, we found *L. cf. susanae* in the Kaffa area, specifically in the Ganeti (Godofo) wetland. During our observations, we observed *L. cf. susanae* climbing on the grass, approximately 50 m away from the riverine forest. This finding is significant because it expands the known distribution range of this species. The first characterization of *L. susanae* was conducted by Largen (1977). At that time, it was believed to solely inhabit the Gughe Mountains of Ethiopia, thriving at elevations between 2600 and 3000 m (Largen and Spawls, 2010). However, further research conducted by Reyes-Velasco *et al*. (2018b) revealed that *L. susanae* is localized to the Gughe region. It is interesting to note that despite being an arboreal species, *L. susanae* was categorized within fossorial species populations. This suggests that the species has adapted to diverse habitats and exhibits unique behaviours.

Another noteworthy record was made by Abebe Ameha (2012), who genetically and morphologically confirmed occurrence of *L. susanae* in the Saja Forest, located approximately 60 km north of Bonga, the capital city of Kaffa. The Ganeti wetland, where we observed *L.cf. susanae*, is situated around 100 km west of Saja. This demonstrates that *L. cf. susanae* occupies various habitats within the Kaffa region, including an unfragmented environment consisting of a grassy wetland surrounded by remnants of pristine forest. Our findings highlight the habitat diversity of *L. cf. susanae* and its ability to thrive in different ecosystems. The presence of *L. cf. susanae* in the Ganeti wetland contributes valuable insights to our understanding of the species distribution and underscores the importance of preserving unfragmented habitats for the conservation of frog biodiversity.

During our research, we made an interesting observation of a single individual of *L. vannutellii* crawling along the road near the Enset plantation of the Daneti Kebele (Gonogori) following a heavy rainfall. This behaviour is atypical for species within the arboreal *Leptopelis* genus. Previous studies conducted by Portik and Blackburn (2016) have suggested that an arboreal habitat represents the ancestral state for *Leptopelis* species, with only a few species, such as *L. gramineus*, displaying adaptability to a fossorial habitat. Further investigation is necessary to gain a comprehensive understanding of the habitat preferences and behaviours exhibited by *L. vannutellii*. Additional research can shed light on whether this particular observation of *L. vannutellii* strolling on the road after heavy rain is an isolated incident or a recurring behaviour. By studying the habitat utilization patterns of this species, we can gain valuable insights into its ecological adaptations and better comprehend the factors influencing its distribution and behaviour.

The study found that among the three habitats surveyed, wetlands supported a greater number of endemic frog species. This suggests that wetland ecosystems offer a diverse range of frog habitats. Previous research by Drayer and Richter (2016) and Nneji *et al*. (2019) has also highlighted the significance of wetlands in supporting a wide diversity of frog species. Wetlands provide abundant food sources such as invertebrates and zooplankton, which contribute to the high frog diversity observed (Muro-Torres *et al*., 2020).

On the other hand, the Enset plantation exhibited the highest diversity index and evenness. This emphasizes the importance of agricultural areas, specifically Enset plantations, in future conservation efforts. Ndriantsoa *et al*. (2017) have noted that Enset plantation plays a crucial role in maintaining frog diversity in fragmented landscapes. In line with this, our study identified three Ethiopian endemic frog species, namely *L. vannutellii*, *A. clarkei*, and *A. enseticola*, within the

Enset plantation. Notably, *L. vannutellii* and *A. clarkei* are exclusively found in southwest Ethiopia. The local communities in the study area heavily rely on Enset as a food source (Dagmawit Chombe and Endashaw Bekele, 2011; Borrell *et al.*, 2019; Selamawit Araya *et al.*, 2021). However, due to the low production caused by diseases, the communities are dissatisfied and often convert Enset plantations into fields for other crops. This conversion poses threat to existing endemic frog habitats.

Furthermore, the protection and conservation of wetland habitats are of utmost importance due to the high taxonomic turnover observed in endemic frog species. Unfortunately, these habitats face degradation and pressures from activities such as overgrazing, agricultural expansion, water drainage, and eucalyptus plantations. These factors greatly impact the survival of the endemic frog species in the wetlands.

The study conducted by Rödel and Ernst (2004) revealed that different survey methods yielded varying results in terms of the richness of endemic frog species in riverine forests. This suggests that employing different methods is necessary for accurately estimating frog populations. In this research, the majority of frog species were documented using visual encounter surveys conducted along transects. However, only *Hemisus microscaphus* and *Phrynobatrachus* sp.1 species were captured using drift fences and pitfall traps out of the fourteen endemic frog species identified in the Kaffa area.

Drayer and Richter (2016) proposed that setting up pitfalls alongside drift barriers that encircle wetlands completely proves to be an effective approach for capturing most frog species and assessing their abundance. Unfortunately, in the current study, due to unsuitable conditions in the study area and the risk of buckets being displaced by water, it was not feasible to install drift fences and pitfall traps in the wetland habitat. Consequently, alternative methods such as visual encounter surveys, acoustic encounter surveys and dip netting were employed to document nine endemic frog species of Ethiopia

5.7. Phylogeny and acoustic characteristics of *Hyperolius* and *Afrixalus* frog species from Kaffa

Kaffa area in Ethiopia is renowned for its abundant wetlands, riverine forests and pristine forests, making it an ideal environment for studying both *Hyperolius* and *Afrixalus* frog species. However, previous comprehensive studies of Ethiopia's frog biogeography have primarily focused on the genera *Leptopelis, Ptychadena, and Xenopus* (Evans *et al*., 2011; Abebe Ameha *et al*., 2013; Reyes-Velasco *et al*., 2018a; Tiutenko and Zinenko, 2021; Goutte *et al*., 2022). Therefore, conducting research in this area allows for the characterization of *Afrixalus* and *Hyperolius* species, filling a gap in our understanding of these frog groups.

5.7.1. *Hyperolius* species

Kaffa area is a region of high biodiversity, with different habitats including riverine forests and grassy wetlands (NABU, 2020). In this research, three different types of *Hyperolius* frogs (*H. kivuensis*, *H. viridiflavus* and *H. howelli*) have been recorded in the reed grass wetlands, suggesting that the area supports an intricate ecosystem for frogs. These results support earlier findings where grassy wetlands are considered a significant habitat for *Hyperolius* frogs (Largen, 1998; Channing *et al*., 2002; Largen and Spawls, 2010; Mindje *et al*., 2020). The widespread distribution of *H. viridiflavus* and *H. kivuensis* in several wetlands around Kaffa region shows that these species may be relatively abundant and distributed in the area. The fact that *H. howelli* was found only found in few wetlands, on the other hand, may suggest the species-specific restricted range. The absence of *Hyperolius* species beyond 2000 m asl indicates that these frogs may have a possible elevation range limit due to physiological limitations and absence of suitable habitat. The elevation range of these species identified in this study (between 1561 and 1857 m asl) is in line with earlier findings of *Hyperolius* frogs (Largen and Spawls, 2010).

The findings of phylogenetic analysis for the *Hyperolius* species found in the study area offer significant new insights into the phylogeography history of these frogs. According to the analysis, each of the three studied species, *H. kivuensis*, *H. viridiflavus* and *H. howelli*, belong to a separate phylogenetic lineage, containing only a small amount of genetic variation within each species. The analysis's optimal tree, with a sum of branch length (SBL) of 0.657, showed that these species had undergone relatively little evolutionary divergence. Although Channing *et al*. (2002) designated *H. nasutus,* which have been found from Ethiopia (Largen, 1998), as *H. acuticeps*, it is at present recognized as *H. howelli* based on genetic distance, call type and morphometrics. The minimum and maximum genetic distances within each species indicate that there is relatively little genetic divergence within these lineages, which is consistent with earlier research on *Hyperolius* frogs in other parts of Africa. However, the genetic distance between *H. kivuensis* and *H. howelli* (0.17) indicates that these species are more distantly related than the other species in this study. This underlines the need for more investigation into the genetic and ecological diversity of *Hyperolius* frogs in Kaffa area and may reflect variations in their ecology, behaviour, or evolutionary history.

In studies of amphibians, such as frogs, where there is significant morphological variation both within and between species, morphometric data are particularly crucial to identify and distinguish between several species, as well as to look at patterns of morphological variation within and between populations used (Mohaymeni *et al*., 2022). The morphometric measurements in this study showed that the three *Hyperolius* species have a variety of morphological characteristics where female *H. viridiflavus* showed the longest mean SVL (32.9) while *H. howelli* recorded the smallest (16.97 ± 0.2). In terms of colour, Largen (1998) provided a thorough description of the colour of the *Hyperolius* species in Ethiopia where *H. viridiflavus* is considered polychromatic. We found that *H. viridiflavus* individuals display two different colorations, ranging from cream or pale gold to dark golden-brown or pinkish-brown on their dorsal surfaces in the study area. The dorsum itself can be pale to dark green and is occasionally unmarked, although it typically has irregular, spherical, yellow to orange dots. Each spot contains a tiny wart that is frequently rich red-brown in colour. *H. kivuensis* also exhibited different colorations from light green to dark brown dorsum and a black strip from the tip of the snout to its groin. In line with this, Dehling and Sinsch (2023) documented variations in color among *H. kivuensis* populations in Rwanda.

The findings reveal clear distinctions in the auditory characteristics of male advertisement calls among the three *Hyperolius* frog species: *H. kivuensis*, *H. viridiflavus* and *H. howelli*. These differences encompass various parameters such as the number of pulses, call duration, peak frequency, and bandwidth. Specifically, the *H. kivuensis* advertisement call is characterized by nine distinct pulses, whereas *H. viridiflavus* exhibits 39 pulses. In contrast, *H. howelli* produces a repetitive pulse pattern. Additionally, the call duration, peak frequency, and bandwidth of *H. kivuensis* significantly differ from those of *H. viridiflavus* and *H. howelli*. Furthermore, *H. viridiflavus* possesses a unique call that can be described as a metallic (small bell) click, whistle, or sound. The call of *H. viridiflavus* is both short and loud, making it audible over long distances. As a result, its unique acoustic properties make it easily distinguishable from the calls of the other two *Hyperolius* species. In a study carried out by Mindje *et al*. (2020) in the Mugesera wetland of Eastern Rwanda, researchers noted that the sound produced by *H. viridiflavus* could be readily differentiated from that of other *Hyperolius* species. However, the study also revealed significant variations in the sound within the same species across different habitats and between the wet and dry seasons.

According to AmphibiaWeb (2023), Kenya is home to 20 species of *Hyperolius*, Tanzania to 39 species and Uganda to 11 species. In contrast, only 4 species, of which three in Kaffa area have been documented in Ethiopia. This small number of reported species indicates that our knowledge of the diversity of *Hyperolius* frogs in Ethiopia is limited given the country's size and ecological diversity with potentially suitable habitats. It is expected that there are still a lot of new species waiting to be discovered in this area. Specifically, frogs are at risk of extinction as their habitats including wetlands and forests are disappearing at an alarming rate. Hence, prioritizing the conservation of suitable frog habitat and exhaustive research on the diversity of frog species is required in particular in southwest Ethiopia.

5.7.2. *Afrixalus* species

According to research conducted by Largen (1974), *Afrixalus clarkei*, was previously recorded only in specific areas on the edge of tropical deciduous forest in southwestern Ethiopia, specifically between 820 and 1800 m asl, in a moist tropical forest near Bonga (Largen, 1974; Largen and Spawls, 2010). However, recent studies have revealed new information about the species' distribution and habitat range (Mertens *et al*., 2016). The current research indicates that the home range of *A. clarkei* extends substantially. The species has been observed at altitudes ranging from 1561 to 2482 m asl, a substantial increase in the known altitudinal range. The known distribution of *A. clarkei* has also increased by approximately 100 km towards the northwest, reaching Sor village in the Saylem district and 40 km to the south, reaching Shamali in Decha. Additionally, the species was found in new habitats outside forested areas, including marshes, agricultural lands (such as enset plantations) and open disturbed landscapes suggesting its higher degree of tolerance against forest degradation. The discovery of *A. clarkei* species in Sor and Bariacho villages in Saylem, Shamali wetland in Decha, Boqa wetland in Adiyo and the Medabo enset plantation in

Gesha indicate that the species has a larger distribution than what was previously documented. These observations highlight the limited knowledge regarding frog distribution and conservation in this particular region of Ethiopia.

Afrixalus enseticola, another species of frog, has previously been documented in Kofole, which is situated in the West Arsi zone of the Oromia region in Ethiopia. This particular species occupies an altitude of 2600 m asl and shares its habitat with *Afrixalus clarkei* in the Bonga area, as reported by Largen and Spawls (2010) and Largen (1974). However, the current study has revealed that the habitat range of *Afrixalus enseticola* extends beyond its previous known range. Specifically, the species has been found in Saylem, a location located approximately 100 km away from the previously documented area. *Afrixalus enseticola* has been recorded in Gesha, a place situated about 80 km distant from Bonga. This expansion of habitat significantly broadens our understanding of the geographical distribution of *Afrixalus enseticola*.

5.8. Knowledge, Attitudes and Perceptions of people towards frog conservation

The survey respondents displayed an impressive level of awareness and knowledge regarding amphibians, particularly concerning their encounters with them in various habitats. They reported frequent interactions with frogs across diverse environments. However, a worrying trend emerged as participants observed a decline in frog populations over time, attributing it to factors such as reduced rainfall leading to habitat loss, climate change, human population growth, and agricultural expansion. Similarly, studies conducted in different parts of Ethiopia have also indicated that frogs are facing a decline due to habitat degradation, deforestation (Abebe Ameha, 2012), climate change, pollution from agrochemicals and disease (Gower *et al*., 2012), as well as the ongoing issue of deforestation (Gessesse Dessie and Kleman, 2007). Interestingly, respondents observed a significant increase in the number of frogs during the rainy season, indicating the species' dependence on favourable environmental conditions.

According to the study conducted by Tarrant *et al*. (2016), it was found that the attitudes, beliefs, and perceptions held by individuals can have harmful effects on animals and conservation efforts. In more recent research (Abeje Kassie, 2020), focused on the Oromo zone of the Amhara region, it was revealed that the local population showed a lack of interest in participating in the conservation of frogs in the area. The study revealed that negative superstitions and deep-rooted fears related to frogs and toads were prevalent, particularly within specific cultural contexts found in the study area. In line with this, Abebe Ameha *et al*. (2013) stated that some cultures in Ethiopia hold negative as well as positive attitudes and beliefs about frogs and toads. However, there is a positive aspect highlighted by the current research. The majority of the respondents demonstrated a willingness to actively engage in conservation activities that aim to protect frog habitats. This optimistic outlook suggests that local communities have the potential to contribute significantly to conservation initiatives. It also underscores the importance of raising awareness about the significance of frog conservation.

The survey findings indicated that 46.4% of the respondents acknowledged having a fear of frogs, while 53.6% did not experience fear when confronted with them. Interestingly, there was no significant difference in the fear of frogs observed between genders. Those individuals who expressed fear of frogs primarily attributed it to the perception of their smooth skin. The sensation of touching the smooth and cold skin of frogs caused discomfort and fear among these individuals. This aligns with a study conducted by Schlegel and Rupf (2010), which suggested that people tend to dislike frogs due to the slimy bodies of some frogs resembling disgust-inducing stimuli such as mucus or faeces (Davey, 1994). It is worthmentioning that some participants reported fear without providing specific reasons.

5.9. Threats and conservation status

5.9.1. Chytrid fungus

One of the focuses of the present study was to specifically investigate the presence of a disease threat from the chytrid fungus *Batrachochytrium dendrobatidis* (Bd) in the Kaffa area. Surprisingly, none of the sampled frogs showed any evidence of Bd infection. In a related study by Bielby *et al*. (2008), based on ecological and environmental variables, Ethiopian frog species were considered vulnerable to a decline associated with Bd infections as they shared notable similarities with species from other regions that had experienced declines linked to Bd. Moreover, Gower *et al*. (2012) conducted a comprehensive investigation in Kaffa and Bale regions during 2008 and 2009 and examined 120 frogs from 14 distinct species, where 51 individuals were infected with Bd. Interestingly, Bd was present in all surveyed locations except for Dinsho and Magano in the Bale Mountains. Furthermore, at least one specimen from each of the sampled genera exhibited positive results for Bd (Gower *et al*., 2012).

The absence of Bd in Kaffa in our findings suggests a potential absence or low prevalence of the fungus in this particular region during the time of the study. It may indicate that Kaffa area has not yet been significantly impacted by Bd or that the local frog species have developed some level of resistance against the fungus. It is important to note that the absence of Bd in the current study is limited to the specific time and area examined. Long-term monitoring and further research are necessary to assess the stability of these findings and to track potential changes in Bd prevalence over time. Nonetheless, the absence of Bd in Kaffa area represents a valuable baseline for future comparative studies and adds to our understanding of the spatial distribution and dynamics of Bd in frog populations.

5.9.2. Conservation status

Based on the 2013 IUCN Red List, among the species of frogs in Kaffa area, three species that are classified as Endangered: *L. susanae*, *A. clarkei* and *X. largeni*. Additionally, we have identified one species categorized as Vulnerable (*A. enseticola*) and another as Near Threatened (*P. erlangeri*). To document the presence of *Sylvacaecilia grandisonae*, the only caecilian species in Ethiopia, extensive efforts were made using various frog surveying techniques such as drift fences and pitfall traps, digging the soil at the suitable habitat and turning the leaf litter. However, this species was not recorded in the designated study area.

According to studies conducted by Assemian *et al*. (2015) and Nneji *et al*. (2019), wetland and swamp ecosystems provide a favourable environment for a higher diversity of frog species. This is likely due to the presence of various microhabitats, such as ponds, marshes and swamps, which offer different suitable habitats. In line with this, our study area, Kaffa, is known to contain abundant wetlands and pristine riverine forests, which play a crucial role as habitats for several frog species. Therefore, the research findings indicate that these microhabitats, along with the specific ecological requirements of each species, support the presence of species with in *Hyperolius*, *Ptychadena* and *Xenopus*.

However, the survival of these frog species is being threatened by overgrazing, agricultural expansion, water draining and the establishment of eucalyptus plantations, thus degrading and destroying their wetland habitats. Takele Taye et al. (2023) also showed that the *Eucalyptus* tree poses a threat to water bodies and wetlands as it depletes water sources through its high-water consumption. Given the high taxonomic turnover among frog species, the protection and conservation of wetland habitats are of utmost importance. Araújo *et al*. (2006) also support this notion, as their study demonstrated that the reduction in water availability resulted in a decrease in suitable habitats for frogs in Nigeria.

Another concern is the conversion of horticultural crops, particularly enset, which provides an important habitat for specific frog species like *Leptopelis* and *Afrixalus*, into field crops such as maize, wheat, and teff. This trend, observed among local communities, poses threat to the survival of frogs in the area. Nneji *et al*. (2021) highlighted this issue in their study, emphasizing that the transformation of agricultural fields degrades the natural habitats suitable for frogs, leading to a decrease in both species' richness and abundance.

6. CONCLUSION AND RECOMMENDATIONS

6.1. Conclusion

The study conducted in Kaffa area of southwestern Ethiopia has revealed the region's significance as a hotspot for biodiversity, particularly for frogs. The study recorded an impressive diversity of frog species, documenting a total of 26 species belonging to 11 genera across 8 families. Most of these have been recorded before while Eight species are new records in this area. The research documented a remarkable number of frog species, accounting for over 33% of Ethiopia's total frog species and 35% of endemics. This emphasizes the critical role of Kaffa area as an important habitat for frogs and underscores the significance of frog conservation. Furthermore, the research confirmed the presence of previously unreported species in Kaffa and its surrounding areas, that include *Amietia nutti*, *Hemisus marmoratus*, *Kassina senegalensis*, *Leptopelis* sp.1, *Leptopelis* sp.2 and *P. anchietae*. These findings shed light on the rich frog diversity in the region and highlight the importance of preserving and conserving the unique habitats found in Kaffa area.

Among the different habitats within Kaffa area, wetlands were the highest interms abundance and species richness of frogs. This can be attributed to the diverse array of food sources available in wetland ecosystems, including invertebrates and zooplankton. Riverine forests ranked second in terms of species richness and abundance, while enset plantation ranked last, suggesting that human-modified landscapes may have a detrimental impact on frog populations. The distribution of species varied across habitats, with a few common species coexisting alongside numerous rare species. Notably, *Phrynobatrachus* sp. 1 displayed the highest relative abundance, while *L.cf. susanae* had the lowest relative abundance and both species were exclusively found in wetland habitats. These findings emphasize the importance of preserving wetland ecosystems and highlight the need for conservation efforts to protect the diverse range of habitats present in Kaffa area in order to safeguard frog diversity.

The current study is important in that DNA sequencing was used for the first time to confirm species composition of the country's frog community in Kaffa area. This study is also the first to provide information on seasonal fluctuation of frog community and also high species richness and diversity of frog species across sites and habitats in Kaffa area. The frog species require a variety of habitat types, including grassy wetlands, enset vegetation, and riverine forest. Our results imply that all habitat types can be equally crucial for maintaining populations of different frog species and hence should be considered when developing management interventions.

This is specifically important in that the study area accounts for harbouring 35% of the endemic frog species of Ethiopia. In the current study, four endemic species that were new to the study area were discovered, implying that more species may still await to be discovered. According to the IUCN Red List, three of the endemic frog species recorded in this study were endangered (EN) (*A. clarkei*, *L. cf. susanae*, *X. largeni*), while one of them was vulnerable (VU) (*A. enseticola*) and *P. erlangeri* species was near threatened (NT). The endemic frog species, recorded in the study area, would make good flagship species for raising awareness of Ethiopia's conservation goals and promoting conservation efforts.

Our findings suggest that all habitat types may be equally important in supporting populations of various endemic frog species. This suggests that when designing management zones, these and possibly other habitat types should be taken into account. The destruction of wetlands, deforestation, and the conversion of enset plantations into cultivated fields pose significant threats to frog species. We hope that this study will inspire wildlife researchers, herpetologists, and conservationists to investigate the ecological patterns of frog communities across regions in order to provide valuable and relevant information to guide conservation and management programmes.

6.2. Recommendations

Kaffa area is home to a diverse range of fauna and flora, with frogs playing a crucial ecological role. Unfortunately, the forest cover in the region has largely been cleared for agricultural purposes and human settlement. Additionally, wetlands are being drained for irrigation, and various factors are leading to the decline of Enset plantations. The negative impacts of human activities on frog species in Kaffa area are evident.

To address this issue, it is recommended that local communities, district and regional officials, and non-governmental organizations working in Kaffa area focus their efforts on the conservation and protection of wetlands. These include preventing the illegal or unplanned drainage of wetlands and avoiding the plantation of eucalyptus trees near frog habitats. By adopting these measures, we can achieve the highest level of conservation possible for the frog fauna in the study area.

Furthermore, to effectively conserve the frog fauna in Kaffa, it is essential to implement conservation interventions to improve the management of horticultural crops, particularly enset plantations. These interventions will not only contribute to the conservation of frogs but also ensure food security for the local community, as well as providing suitable habitats for endemic frog species found in the country.

It is imperative to prioritize the creation of community awareness regarding the significance of conservation of frogs in Kaffa area. Raising awareness among local communities, stakeholders and policymakers about the importance of frogs and the need for their conservation is crucial.

The majority of the ecology of frogs in Kaffa area remains unstudied, highlighting the necessity for scientific research on frog species found in this region. Conducting research on the biology of Kaffa's frogs will provide valuable insights into their ecology and behaviour, aiding conservation efforts.

The study area boasts an appealing ecosystem with substantial potential to attract tourists and researchers. However, there is a lack of infrastructure, including roads (particularly in Gesha and Saylem districts), telecommunication networks, lodges, and other facilities. Therefore, it is vital for the government, the private sector and the local community to collaborate and invest in the necessary infrastructure to capitalize on the benefits of tourism.

Although the study area falls within Kaffa Biosphere Reserve, there is a significant human disturbance within and around the reserve. To address this issue, it is crucial to restrict the activities of local communities that contribute to disturbance within the biosphere reserve. By implementing regulations and promoting responsible behaviour, we can minimize the negative impacts on the ecosystem.

By taking proactive steps to conserve wetlands, protect riverine forests, and enhance the management of enset plantations, we can work towards the conservation of frog species in Kaffa area. These actions are vital for maintaining the ecological importance of frogs and promoting overall biodiversity in the region.

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8. APPENDICES

Appendix 1. Beliefs, Attitudes, and Perceptions of Local Communities towards amphibians in selected Kaffa Areas.

Data Collection Sheet

Dear respondents,

This questionnaire aims to collect information regarding the beliefs, attitudes, and perceptions of the people in the Kaffa area towards amphibians. Your genuine responses will play a crucial role in helping us understand the local community's viewpoint on these vertebrate animals. We assure you that all answers given will remain confidential and will be used solely for this study. We sincerely appreciate your cooperation and thank you in advance.

Socio-demographic data format:

1. Name ___________________________ Sex_________________ Age ____________
2. Educational status____
3. Occupational status____
4. How long did you live in this area? ____

Attitudes and beliefs to amphibian’s view and conservation

1. Do you know amphibians (እንቁራሪት አስተኔ (Amharic); ገፐቾ (Kafi noono))? A/ Yes B/ No

2. If “Yes”, where do you frequently observe these amphibians? A/ Enset plantation B/ riverine forest C/ wetlands d/ ponds e/ all habitats
3. Do you believe that frog habitats are being lost? a/ Yes b/ No c/ I don’t know
4. If your answer is “Yes”, explain how does the habitat of amphibians become lost?
5. In which season the number of amphibians will be high? A/wet season B/ dry season C/ no difference in season
6. Have there been noticeable changes in amphibian numbers over the past years? A/Yes B/No C/ I do not know
7. If your answer is “Yes” to question no.4.in what ways? A/declined B/increased
8. If there has been a decline in numbers, what do you think are the causes?

   ____

9. Do you experience fear when encountering amphibians? (a) Yes (b) No
10. If your answer is "Yes," what is the reason behind your fear of amphibians?
11. Are there traditional beliefs regarding amphibians in your area? A/Yes B/No C/ I do not know
12. If “Yes”, explain what belief exists.

   ____

13. Do you think amphibians should be conserved in this area? A/Yes B/ No C/ I do not know
14. If “Yes”, explain why?

   ____

15. Kaffa is an important area that harbors different fauna and flora species. Are you willing to actively contribute to the conservation of biological diversity, including amphibians, in this area? A/ Yes B/ No
16. If your response is "Yes," please explain the reasons behind your willingness to participate.
17. If your answer is "No," please provide an explanation for your decision.

Appendix 2. Sample collection information of 17 study sites and 51 transects.

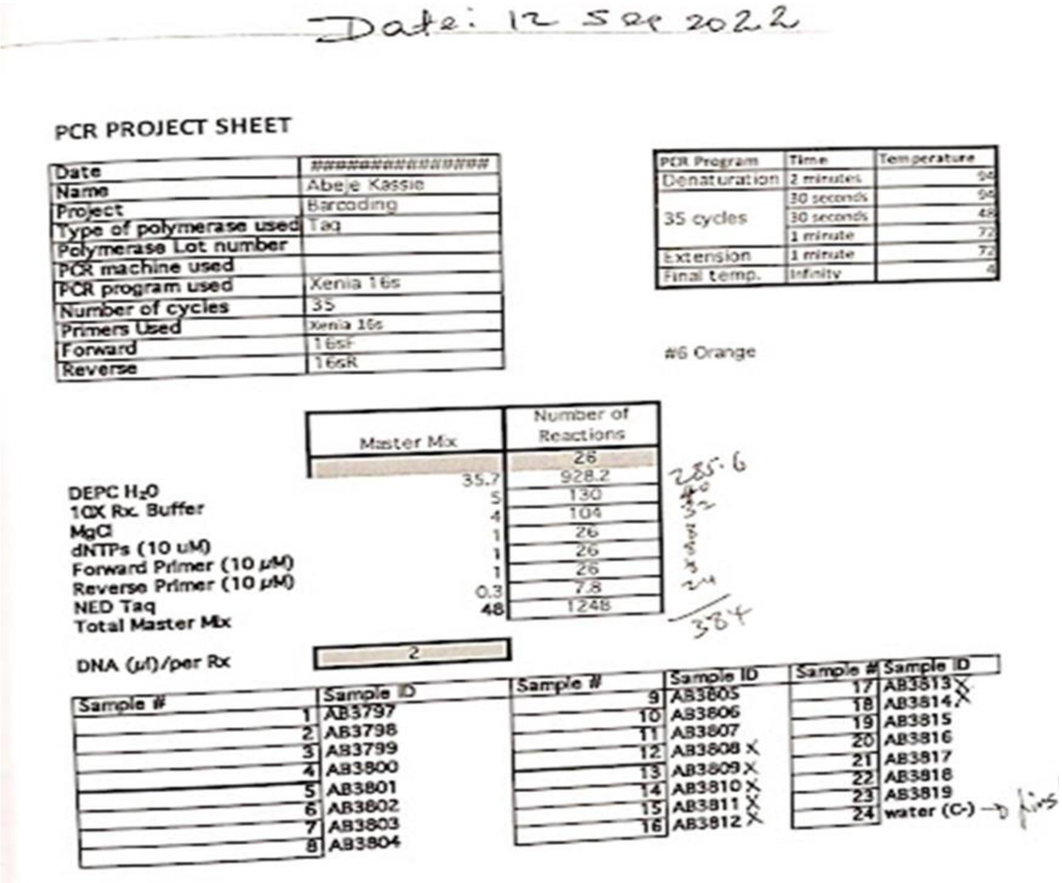
Transect	Site	Habitat	Annual Mean Temperature (°C)	Annual mean Precipitation (mm)	Latitude	Longitude	Altitude (m)
1	Bita	Wetland	17.2	2181.1	7.2702	35.7767	1853
2	Bita	Agricultural land	17.2	2181.1	7.2702	35.7665	1928
3	Bita	Riverine forest	17.2	2181.1	7.2810	35.7914	1847
4	Ganeti	Wetland	17.2	2181.1	7.4193	35.6356	2390
5	Ganeti	Agricultural land	17.2	2181.1	7.4270	35.6460	2427
6	Ganeti	Riverine forest	17.2	2181.1	7.4266	35.6443	2389
7	Shuneti	Wetland	17.2	2181.1	7.3837	35.6845	2298
8	Shuneti	Agricultural land	17.2	2181.1	7.3802	35.7297	2291
9	Shuneti	Riverine forest	17.2	2181.1	7.4185	35.7297	2353
10	Sheda	Wetland	17.2	2181.1	7.3204	35.8483	1857
11	Sheda	Agricultural land	17.2	2181.1	7.2867	35.8339	1879

Transect	Site	Habitat	Annual Mean Temperature (°C)	Annual mean Precipitation (mm)	Latitude	Longitude	Altitude (m)
12	Sheda	Riverine forest	17.2	2181.1	7.2795	35.8409	1797
13	Nata	Wetland	17.2	2181.1	7.5760	35.7485	2183
14	Nata	Agricultural land	17.2	2181.1	7.5869	35.7438	2231
15	Nata	Riverine forest	17.2	2181.1	7.5622	35.7607	2124
16	Medabo	Wetland	17.2	2181.1	7.5859	35.7542	2185
17	Medabo	Agricultural land	17.2	2181.1	7.5905	35.7635	2131
18	Medabo	Riverine forest	17.2	2181.1	7.5884	35.7639	2208
19	Gonogori	Wetland	17.2	2181.1	7.6158	35.7608	2135
20	Gonogori	Agricultural land	17.2	2181.1	7.6064	35.7608	2265
21	Gonogori	Riverine forest	17.2	2181.1	7.6152	35.7622	2157
22	Bariacho	Wetland	17.9	2141.2	7.8220	35.8299	2304
23	Bariacho	Agricultural land	17.9	2141.2	7.8210	35.8374	2351
24	Bariacho	Riverine forest	17.9	2141.2	7.8264	35.8306	2282
25	Utali	Wetland	17.9	2141.2	7.8270	35.8177	2283
26	Utali	Agricultural land	17.9	2141.2	7.8253	35.8162	2306
27	Utali	Riverine forest	17.9	2141.2	7.8306	35.8160	2270
28	Sor	Wetland	17.9	2141.2	7.8458	35.8370	2227
29	Sor	Agricultural land	17.9	2141.2	7.8366	35.8351	2270
30	Sor	Riverine forest	17.9	2141.2	7.8412	35.8352	2227

Transect	Site	Habitat	Annual Mean Temperature (°C)	Annual mean Precipitation (mm)	Latitude	Longitude	Altitude (m)
31	Gojeb	Wetland	17.2	2306.7	7.5503	36.0393	1561
32	Gojeb	Agricultural land	17.2	2306.7	7.5640	36.0440	1583
33	Gojeb	Riverine forest	17.2	2306.7	7.5491	36.0525	1563
34	Alemgono	Wetland	17.2	2306.7	7.3625	36.2259	1711
35	Alemgono	Agricultural land	17.2	2306.7	7.3548	36.2204	1739
36	Alemgono	Riverine forest	17.2	2306.7	7.3934	36.1663	1780
37	Shoreri	Wetland	17.2	2306.7	7.3391	36.2001	1603
38	Shoreri	Agricultural land	17.2	2306.7	7.3588	36.1874	1703
39	Shoreri	Riverine forest	17.2	2306.7	7.3626	36.2107	1671
40	Komba	Wetland	17.2	2306.7	7.3165	36.0510	1824
41	Komba	Agricultural land	17.2	2306.7	7.3138	36.0550	1868
42	Komba	Riverine forest	17.2	2306.7	7.3102	36.0677	1848
43	Boqa	Wetland	17.2	2306.7	7.2963	36.3736	2428
44	Boqa	Agricultural land	17.2	2306.7	7.2867	36.3667	2482
45	Boqa	Riverine forest	17.2	2306.7	7.2412	36.4520	2620
46	Shamali	Wetland	19.1	1998.7	7.1347	36.1884	1966
47	Shamali	Agricultural land	19.1	1998.7	7.1366	36.1924	1979
48	Shamali	Riverine forest	19.1	1998.7	7.2024	36.2832	1574
49	Oda	Agricultural land	19.1	1998.7	7.1157	36.4620	2204

Transect	Site	Habitat	Annual Mean Temperature (°C)	Annual mean Precipitation (mm)	Latitude	Longitude	Altitude (m)
50	Oda	Riverine forest	19.1	1998.7	7.1165	36.4624	2209
51	Oda	Wetland	19.1	1998.7	7.1031	36.4852	2527

Appendix 3. PCR standard format and Gel electrophoresis result for Frog DNA and Chytrid fungus (Bd)



Date: 12 Sep 2022

PCR PROJECT SHEET

Date	################
Name	Abeje Kassie
Project	Barcoding
Type of polymerase used	Taq
Polymerase Lot number	
PCR machine used	
PCR program used	Xenia 16s
Number of cycles	35
Primers Used	Xenia 16s
Forward	16sF
Reverse	16sR

PCR Program	Time	Temperature
Denaturation	2 minutes	94
35 cycles	30 seconds	94
	30 seconds	48
	1 minute	72
Extension	1 minute	72
Final temp.	Infinity	4

#6 Orange

	Master Mix	Number of Reactions
		26
DEPC H_2O	35.7	928.2
10X Rx. Buffer	5	130
MgCl	4	104
dNTPs (10 uM)	1	26
Forward Primer (10 µM)	1	26
Reverse Primer (10 µM)	1	26
NED Taq	0.3	7.8
Total Master Mix	48	1248

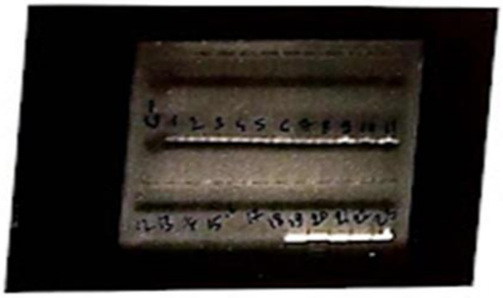
285.6
40
32
8
8
8
2.4
384

DNA (µl)/per Rx 2

Sample #	Sample ID
1	AB3797
2	AB3798
3	AB3799
4	AB3800
5	AB3801
6	AB3802
7	AB3803
8	AB3804

Sample #	Sample ID
9	AB3805
10	AB3806
11	AB3807
12	AB3808 X
13	AB3809 X
14	AB3810 X
15	AB3811 X
16	AB3812 X

Sample #	Sample ID
17	AB3813 X
18	AB3814 X
19	AB3815
20	AB3816
21	AB3817
22	AB3818
23	AB3819
24	water (C-) → fins



PCR PROJECT SHEET ✓

Date	14 September 2022
Name	Abeje Kassie
Project	Barcoding
Type of polymerase used	Taq
Polymerase Lot number	
PCR machine used	
PCR program used	Leptopelis
Number of cycles	30
Primers Used	COILEP
Forward	COILEP-F1
Reverse	COILEP-R1

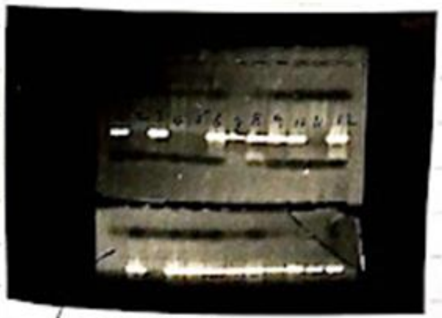
PCR Program	Time	Temperature
Denaturation	2 minutes	94
30cycles	30 seconds	94
	30 seconds	58
	1 minute	72
Extension	1 minute	72
Final temp.	Infinity	4

#6 Orange

	Master Mix	Number of Reactions
		26
DEPC H₂0	35.7	928.2
10X Rx. Buffer	5	130
MgCl	4	104
dNTPs (10 uM)	1	26
Forward Primer (10 µM)	1	26
Reverse Primer (10 µM)	1	26
NED Taq	0.3	7.8
Total Master Mix	48	1248

DNA (µl)/per Rx: 2

Sample #	Sample ID	Sample #	Sample ID	Sample #	Sample ID
1	AB3997	9	AB4031	17	AB4039
2	AB3998 ✗	10	AB4032	18	AB4041
3	AB4007	11	AB4033 ✗	19	AB4042
4	AB4014 ✗	12	AB4034	20	AB4044
5	AB4016 ✗	13	AB4035	21	AB4045
6	AB4017	14	AB4036	22	AB4059 ✗
7	AB4029	15	AB4037	23	AB4060
8	AB4030	16	AB4038	24	water (C-)



↳ flipped when scanning

it done again

PCR PROJECT SHEET

Date	15 September 2022
Name	Abeje Kassie
Project	Barcoding
Type of polymerase used	Taq
Polymerase Lot number	
PCR machine used	
PCR program used	Leptopelis
Number of cycles	30
Primers Used	COILEP
Forward	COILEP-F1
Reverse	COILEP-R1

PCR Program	Time	Temperature
Denaturation	2 minutes	94
30cycles	30 seconds	94
	30 seconds	52
	1 minute	72
Extension	1 minute	72
Final temp.	Infinity	4

#6 Orange

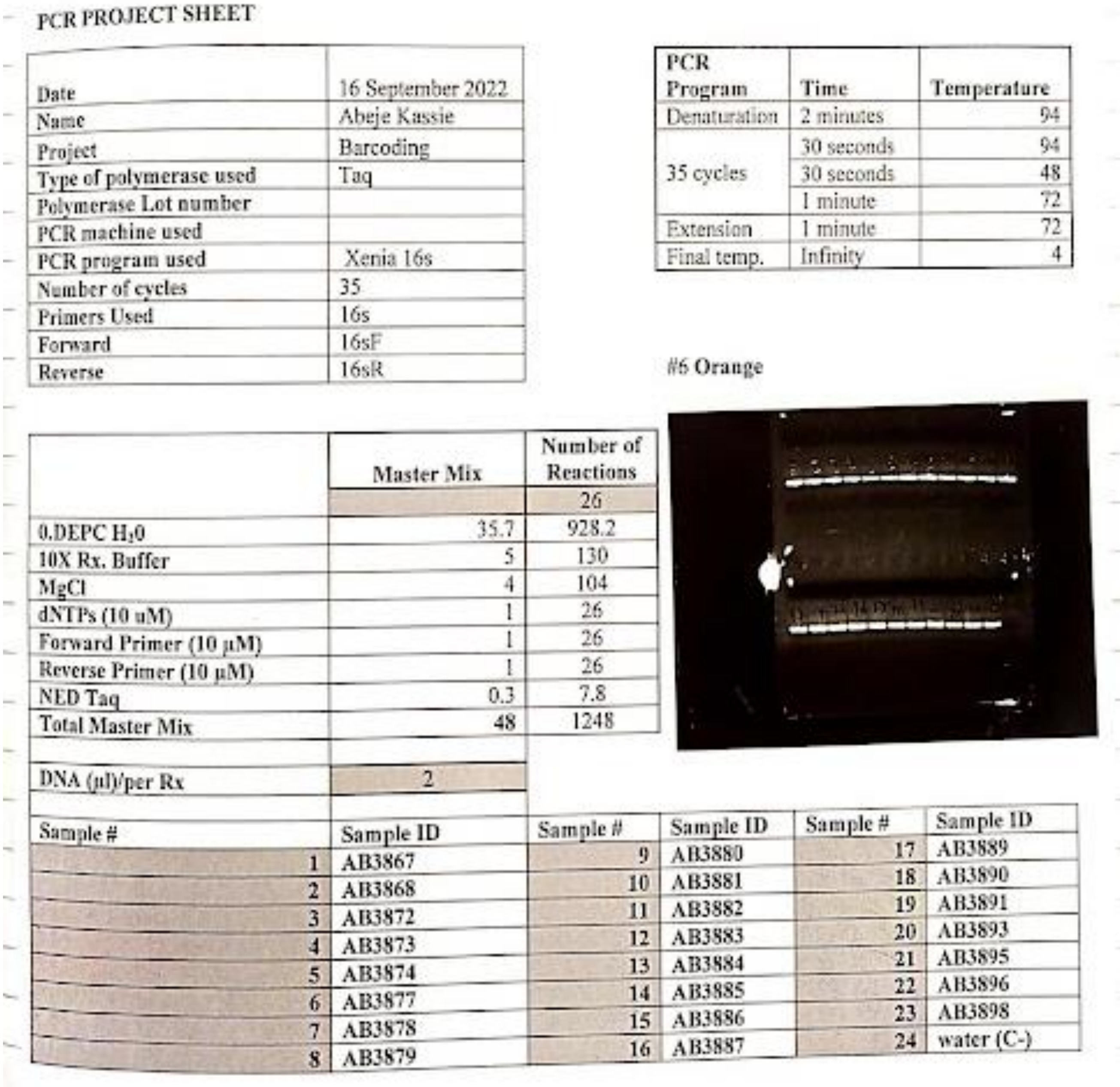
	Master Mix	Number of Reactions
		26
DEPC H_20	35.7	928.2
10X Rx. Buffer	5	130
MgCl	4	104
dNTPs (10 uM)	1	26
Forward Primer (10 µM)	1	26
Reverse Primer (10 µM)	1	26
NED Taq	0.3	7.8
Total Master Mix	48	1248

DNA (µl)/per Rx 2

Sample #	Sample ID	Sample #	Sample ID	Sample #	Sample ID
1	AB3797	9	AB3870	17	AB3900
2	AB3798	10	AB3871	18	AB3902 X
3	AB3815	11	AB3875	19	AB3905
4	AB3818	12	AB3876	20	AB3908 •
5	AB3845	13	AB3888	21	AB3911 •
6	AB3859	14	AB3892	22	AB3912 •
7	AB3860	15	AB3894	23	AB3913 •
8	AB3869	16	AB3897	24	water (C-)

AB3845 } are also worked on 16S primer
AB 3859
AB 3860

3797✓
3798✓
3815✓
3818✓



PCR PROJECT SHEET

Date	16 September 2022
Name	Abeje Kassie
Project	Barcoding
Type of polymerase used	Taq
Polymerase Lot number	
PCR machine used	
PCR program used	Xenia 16s
Number of cycles	35
Primers Used	16s
Forward	16sF
Reverse	16sR

PCR Program	Time	Temperature
Denaturation	2 minutes	94
35 cycles	30 seconds	94
	30 seconds	48
	1 minute	72
Extension	1 minute	72
Final temp.	Infinity	4

#6 Orange

	Master Mix	Number of Reactions
		26
0.DEPC H_20	35.7	928.2
10X Rx. Buffer	5	130
MgCl	4	104
dNTPs (10 uM)	1	26
Forward Primer (10 µM)	1	26
Reverse Primer (10 µM)	1	26
NED Taq	0.3	7.8
Total Master Mix	48	1248
DNA (µl)/per Rx	2	

Sample #	Sample ID	Sample #	Sample ID	Sample #	Sample ID
1	AB3867	9	AB3880	17	AB3889
2	AB3868	10	AB3881	18	AB3890
3	AB3872	11	AB3882	19	AB3891
4	AB3873	12	AB3883	20	AB3893
5	AB3874	13	AB3884	21	AB3895
6	AB3877	14	AB3885	22	AB3896
7	AB3878	15	AB3886	23	AB3898
8	AB3879	16	AB3887	24	water (C-)

PCR PROJECT SHEET

Date	19 September 2022
Name	Abeje Kassie
Project	Barcoding
Type of polymerase used	Taq
Polymerase Lot number	
PCR machine used	
PCR program used	Xenia 16s
Number of cycles	35
Primers Used	16s
Forward	16sF
Reverse	16sR

PCR Program	Time	Temperature
Denaturation	2 minutes	94
35 cycles	30 seconds	94
	30 seconds	48
	1 minute	72
Extension	1 minute	72
Final temp.	Infinity	4

#6 Orange

	Master Mix	Number of Reactions
		26
0.DEPC H_2O	35.7	928.2
10X Rx. Buffer	5	130
MgCl	4	104
dNTPs (10 uM)	1	26
Forward Primer (10 µM)	1	26
Reverse Primer (10 µM)	1	26
NED Taq	0.3	7.8
Total Master Mix	48	1248
DNA (µl)/per Rx	2	

Sample #	Sample ID	Sample #	Sample ID	Sample #	Sample ID
1	AB3926	9	AB3937	17	AB3945
2	AB3930	10	AB3938	18	AB3946
3	AB3931	11	AB3939	19	AB3947
4	AB3932	12	AB3940	20	AB3948
5	AB3933	13	AB3941	21	AB3949
6	AB3934	14	AB3942	22	AB3950
7	AB3935	15	AB3943	23	AB3951
8	AB3936	16	AB3944	24	water (C-)

PCR PROJECT SHEET

Date	20 September 2022
Name	Abeje Kassie
Project	Barcoding
Type of polymerase used	Taq
Polymerase Lot number	
PCR machine used	
PCR program used	Xenia 16s
Number of cycles	35
Primers Used	16s
Forward	16sF
Reverse	16sR

PCR Program	Time	Temperature
Denaturation	2 minutes	94
35 cycles	30 seconds	94
	30 seconds	50
	1 minute	72
Extension	1 minute	72
Final temp.	Infinity	4

#6 Orange

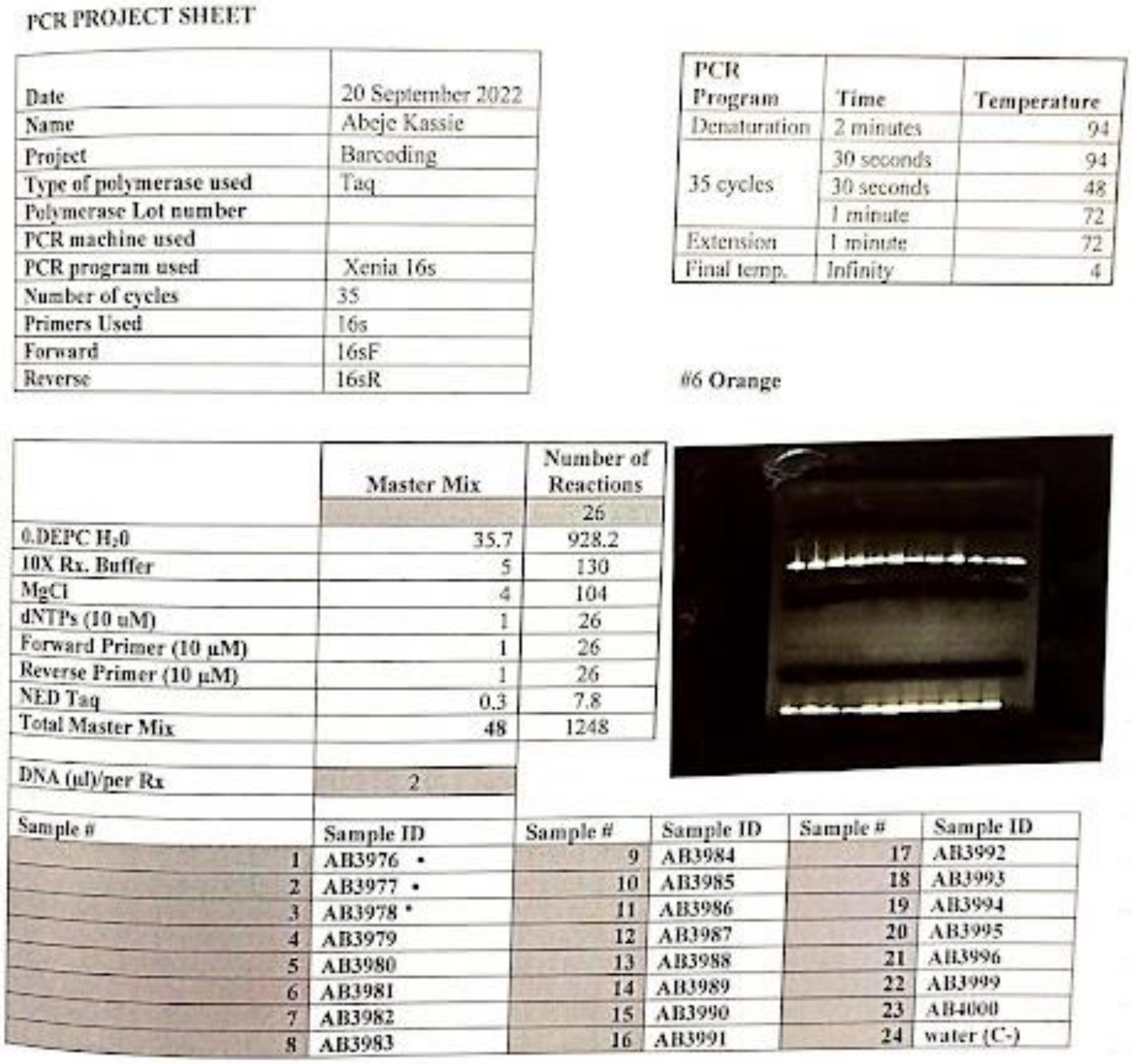
	Master Mix	Number of Reactions
		21
0.DEPC H_2O	35.7	749.7
10X Rx. Buffer	5	105
MgCl	4	84
dNTPs (10 uM)	1	21
Forward Primer (10 µM)	1	21
Reverse Primer (10 µM)	1	21
NED Taq	0.3	6.3
Total Master Mix	48	1,008
DNA (µl)/per Rx	2	

Sample #	Sample ID	Sample #	Sample ID	Sample #	Sample ID
1	AB4001	9	AB4010	17	AB4021
2	AB4002	10	AB4011	18	AB4022
3	AB4003	11	AB4012	19	water (C-)
4	AB4004	12	AB4013		
5	AB4005	13	AB4015		
6	AB4006	14	AB4018		
7	AB4008	15	AB4019		
8	AB4009	16	AB4020		

⇒ This is reworked at 50°c instead of 48°c but the result shows that the same

⇒ #8 is missing due pipeting problem

⇒ The lower DNA ladder bold due to 10 µl inst of 5µl by mistakem



PCR PROJECT SHEET

Date	20 September 2022
Name	Abeje Kassie
Project	Barcoding
Type of polymerase used	Taq
Polymerase Lot number	
PCR machine used	
PCR program used	Xenia 16s
Number of cycles	35
Primers Used	16s
Forward	16sF
Reverse	16sR

PCR Program	Time	Temperature
Denaturation	2 minutes	94
35 cycles	30 seconds	94
	30 seconds	48
	1 minute	72
Extension	1 minute	72
Final temp.	Infinity	4

#6 Orange

	Master Mix	Number of Reactions
		26
0.DEPC H_2O	35.7	928.2
10X Rx. Buffer	5	130
MgCl	4	104
dNTPs (10 uM)	1	26
Forward Primer (10 µM)	1	26
Reverse Primer (10 µM)	1	26
NED Taq	0.3	7.8
Total Master Mix	48	1248
DNA (µl)/per Rx	2	

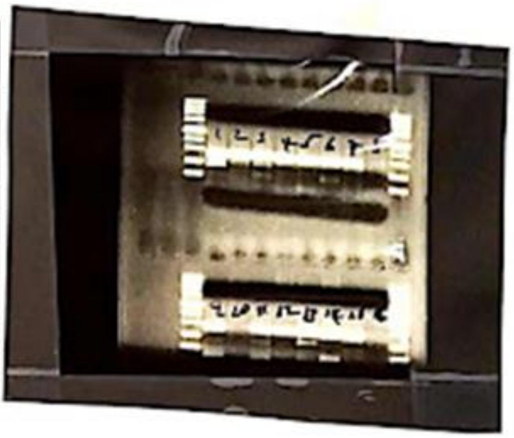
Sample #	Sample ID	Sample #	Sample ID	Sample #	Sample ID
1	AB3976 •	9	AB3984	17	AB3992
2	AB3977 •	10	AB3985	18	AB3993
3	AB3978 •	11	AB3986	19	AB3994
4	AB3979	12	AB3987	20	AB3995
5	AB3980	13	AB3988	21	AB3996
6	AB3981	14	AB3989	22	AB3999
7	AB3982	15	AB3990	23	AB4000
8	AB3983	16	AB3991	24	water (C-)

PCR PROJECT SHEET

Date	26 September 2022
Name	Abeje Kassie
Project	Barcoding
Type of polymerase used	Taq
Polymerase Lot number	
PCR machine used	
PCR program used	Xenia 16s
Number of cycles	35
Primers Used	16s
Forward	16sF
Reverse	16sR

PCR Program	Time	Temperature
Denaturation	2 minutes	94
35 cycles	30 seconds	94
	30 seconds	48
	1 minute	72
Extension	1 minute	72
Final temp.	Infinity	4

#6 Orange



	Master Mix	Number of Reactions
		18
0.DEPC H₂0	35.7	642.6
10X Rx. Buffer	5	90
MgCl	4	72
dNTPs (10 uM)	1	18
Forward Primer (10 µM)	1	18
Reverse Primer (10 µM)	1	18
NED Taq	0.3	5.4
Total Master Mix	48	864
DNA (µl)/per Rx	2	

Sample #	Sample ID	Sample #	Sample ID
1	AB3808	9	AB3835
2	AB3809	10	AB3998 ✗
3	AB3810	11	AB3899
4	AB3811	12	AB3903
5	AB3812	13	AB3904
6	AB3813	14	AB3907
7	AB3814	15	AB3909
8	AB3832 ✗	16	Water(C-)

PCR PROJECT SHEET

Date	3 October 2022
Name,	Abeje Kassie
Project	Barcoding
Type of polymerase used	Taq
Polymerase Lot number	
PCR machine used	
PCR program used	Chytrid
Number of cycles	35
Primers Used	
Forward	Bd1a
Reverse	Bd2a

PCR Program	Time	Temperature
Denaturation	3 minutes	94
35 cycles	45 seconds	94
	45 seconds	60
	1 minute	72
Extension	3 minutes	72
Final temp.	Infinity	4

	Master Mix	Number of Reactions
		26
DEPC $H_2$0	12.25	318.5
10X Rx. Buffer	2.5	65
MgCl (50 mM)	0.45	11.7
dNTPs (10 mM)	2	52
Forward Primer (10 µM)	2.5	65
Reverse Primer (10 µM)	2.5	65
NED Taq	0.8	20.8
Total Master Mix	**23**	598
DNA (µl)/per Rx	2	

Concentrations in Annis et al. 2004
1X
0.9mM
0.8mM
1 µM
1 µM
0.8U

Sample #	Sample ID	Sample #	Sample ID	Sample #	Sample ID
1	AB0047	9	AB3805	17	AB3814
2	AB3797	10	AB3807	18	AB3815
3	AB3798	11	AB3808	19	AB3816
4	AB3799	12	AB3809	20	AB3817
5	AB3800	13	AB3810	21	AB3818
6	AB3801	14	AB3811	22	AB3819
7	AB3802	15	AB3812	23	AB3820
8	AB3803	16	AB3813	24	water (C-)

PCR PROJECT SHEET

Date	3 October 2022
Name	Abeje Kassie
Project	Barcoding
Type of polymerase used	Taq
Polymerase Lot number	
PCR machine used	
PCR program used	Chytrid
Number of cycles	35
Primers Used	
Forward	Bd1a
Reverse	Bd2a

PCR Program	Time	Temperature
Denaturation	3 minutes	94
35 cycles	45 seconds	94
	45 seconds	60
	1 minute	72
Extension	3 minutes	72
Final temp.	Infinity	4

	Master Mix	Number of Reactions
		26
DEPC $H_2$0	12.25	318.5
10X Rx. Buffer	2.5	65
MgCl (50 mM)	0.45	11.7
dNTPs (10 mM)	2	52
Forward Primer (10 µM)	2.5	65
Reverse Primer (10 µM)	2.5	65
NED Taq	0.8	20.8
Total Master Mix	23	598
DNA (µl)/per Rx	2	

Concentrations in Annis et al. 20
1X
0.9mM
0.8mM
1 µM
1 µM
0.8U

Sample #	Sample ID	Sample #	Sample ID	Sample #	Sample ID
1	AB0024	9	AB0032	17	AB0040
2	AB0025	10	AB0033	18	AB0041
3	AB0026	11	AB0034	19	AB0042
4	AB0027	12	AB0035	20	AB0043
5	AB0028	13	AB0036	21	AB0044
6	AB0029	14	AB0037	22	AB0045
7	AB0030	15	AB0038	23	AB0046
8	AB0031	16	AB0039	24	water (C-) ?

PCR PROJECT SHEET

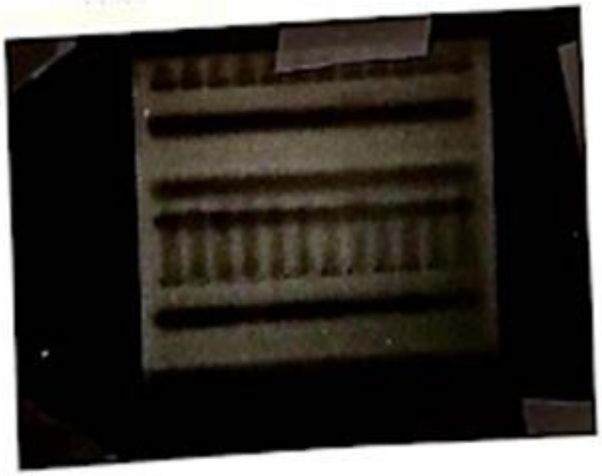
Date	4 October 2022
Name	Abeje Kassie
Project	Barcoding
Type of polymerase used	Taq
Polymerase Lot number	
PCR machine used	
PCR program used	Chytrid
Number of cycles	35
Primers Used	
Forward	~~Bd1a~~ Chytri ITS3
Reverse	~~Bd2a~~ Chytr-5.8S

PCR Program	Time	Temperature
Denaturation	3 minutes	94
35 cycles	45 seconds	94
	45 seconds	60
	1 minute	72
Extension	3 minutes	72
Final temp.	Infinity	4

	Master Mix	Number of Reactions
		26
DEPC H₂0	12.25	318.5
10X Rx. Buffer	2.5	65
MgCl (50 mM)	0.45	11.7
dNTPs (10 mM)	2	52
Forward Primer (10 µM)	2.5	65
Reverse Primer (10 µM)	2.5	65
NED Taq	0.8	20.8
Total Master Mix	23	598
DNA (µl)/per Rx	2	

Concentrations in Annis et al. 2
1X
0.9mM
0.8mM
1 µM
1 µM
0.8U

Sample #	Sample ID	Sample #	Sample ID	Sample #	Sample ID
1	AB3821	9	AB3829	17	AB3837
2	AB3822	10	AB3830	18	AB3838
3	AB3823	11	AB3831	19	AB3839
4	AB3824	12	AB3832	20	AB3840
5	AB3825	13	AB3833	21	AB3841
6	AB3826	14	AB3834	22	AB3842
7	AB3827	15	AB3835	23	AB3843
8	AB3828	16	AB3836	24	water (C-)



PCR PROJECT SHEET

Date	5 October 2022
Name	Abeje Kassie
Project	Barcoding
Type of polymerase used	Taq
Polymerase Lot number	
PCR machine used	
PCR program used	Chytrid
Number of cycles	35
Primers Used	
Forward	Chytr TS3
Reverse	Chytr-5.8s

PCR Program	Time	Temperature
Denaturation	3 minutes	94
35 cycles	45 seconds	94
	45 seconds	60
	1 minute	72
Extension	3 minutes	72
Final temp.	Infinity	4

	Master Mix	Number of Reactions
		26
DEPC H_2O	12.25	318.5
10X Rx. Buffer	2.5	65
MgCl (50 mM)	0.45	11.7
dNTPs (10 mM)	2	52
Forward Primer (10 µM)	2.5	65
Reverse Primer (10 µM)	2.5	65
NED Taq	0.8	20.8
Total Master Mix	23	598
DNA (µl)/per Rx	2	

Concentrations in Annis et al. 20
1X
0.9mM
0.8mM
1 µM
1 µM
0.8U

Sample #	Sample ID	Sample #	Sample ID	Sample #	Sample ID
1	AB3868	9	AB3876	17	AB3884
2	AB3869	10	AB3877	18	AB3885
3	AB3870	11	AB3878	19	AB3887
4	AB3871	12	AB3879	20	AB3888
5	AB3872	13	AB3880	21	AB3889
6	AB3873	14	AB3881	22	AB3890
7	AB3874	15	AB3882	23	AB3891
8	AB3875	16	AB3883	24	water (C-)

Appendix 4. Localities, collection and GeneBank accession numbers of taxa sequenced and aligned in this study.

Species	**Collection No.**	**Gene Bank Accession No.**	**Locality**	**Source**
Phrynobatrachus sp.1	AB3830	OQ398465	Kaffa, Ethiopia	This study
Phrynobatrachus sp.1	AB3834	OQ398466	Kaffa, Ethiopia	This study
Phrynobatrachus sp.1	AB3848	OQ398467	Kaffa, Ethiopia	This study
Phrynobatrachus sp.1	AB3862	OQ398468	Kaffa, Ethiopia	This study
Phrynobatrachus sp.1	AB3891	OQ398469	Kaffa, Ethiopia	This study
Phrynobatrachus sp.1	AB3910	OQ398470	Kaffa, Ethiopia	This study
P. natalensis	AB3949	OQ398471	Kaffa, Ethiopia	This study
Phrynobatrachus sp.1	AB3987	OQ398472	Kaffa, Ethiopia	This study
Phrynobatrachus sp.1	AB4024	OQ398473	Kaffa, Ethiopia	This study
Phrynobatrachus sp.2	AB4047	OQ398474	Chagni, Ethiopia	This study
P. natalensis	AB4054	OQ398475	Chagni, Ethiopia	This study
Phrynobatrachus sp.1	AB3830	OQ398465	Kaffa, Ethiopia	This study
Leptopelis sp.1	AB3845	OQ413091	Kaffa, Ethiopia	This study
Leptopelis sp.1	AB3908	OQ407700	Kaffa, Ethiopia	This study

Species	**Collection No.**	**Gene Bank Accession No.**	**Locality**	**Source**
Leptopelis sp.1	AB3911	OQ413101	Kaffa, Ethiopia	This study
Leptopelis sp.1	AB3915	OQ413102	Kaffa, Ethiopia	This study
Leptopelis sp.1	AB4060	OQ413100	Kaffa, Ethiopia	This study
Leptopelis sp.2	AB3916	OQ413180	Kaffa, Ethiopia	This study
Leptopelis sp.2	AB3919	OQ413183	Kaffa, Ethiopia	This study
Leptopelis sp.2	AB3922	OQ413184	Kaffa, Ethiopia	This study
Leptopelis sp.2	AB3927	OQ413187	Kaffa, Ethiopia	This study
Leptopelis sp.2	AB3928	OQ413188	Kaffa, Ethiopia	This study
Leptopelis sp.2	AB3929	OQ413189	Kaffa, Ethiopia	This study
L. yaldeni	AB4044	OQ413098	Chagni, Ethiopia	This study
L. yaldeni	AB4045	OQ413099	Chagni, Ethiopia	This study
L. vannutellii	AB4031	OQ413095	Kaffa, Ethiopia	This study
L. vannutellii	AB4032	OQ413096	Kaffa, Ethiopia	This study
L. vannutellii	AB4039	OQ413097	Kaffa, Ethiopia	This study
L. vannutellii	AB3914	OQ413103	Kaffa, Ethiopia	This study

Species	Collection No.	Gene Bank Accession No.	Locality	Source
L. vannutellii	AB3997	OQ413093	Kaffa, Ethiopia	This study
L. vannutellii	AB3923	OQ413185	Kaffa, Ethiopia	This study
L. vannutellii	AB3925	OQ413186	Kaffa, Ethiopia	This study
L. vannutellii	AB3918	OQ413182	Kaffa, Ethiopia	This study
L. vannutellii	AB3897	OQ413092	Kaffa, Ethiopia	This study
L. vannutellii	AB4017	OQ413094	Kaffa, Ethiopia	This study
L. vannutellii	AB3917	OQ413181	Kaffa, Ethiopia	This study
L. vannutellii	AB3798	OQ413090	Kaffa, Ethiopia	This study
L. vannutellii	AB3797	OQ413089	Kaffa, Ethiopia	This study
Hyperolius howelli	AB3820	OQ770355	Kaffa, Ethiopia	This study
Hyperolius viridiflavus	AB3817	OQ770356	Kaffa, Ethiopia	This study
Hyperolius viridiflavus	AB3819	OQ770357	Kaffa, Ethiopia	This study
Hyperolius kivuensis	AB3822	OQ770358	Kaffa, Ethiopia	This study
Hyperolius howelli	AB3824	OQ770359	Kaffa, Ethiopia	This study
Hyperolius kivuensis	AB3828	OQ770360	Kaffa, Ethiopia	This study

Species	Collection No.	Gene Bank Accession No.	Locality	Source
Hyperolius kivuensis	AB3829	OQ770361	Kaffa, Ethiopia	This study
Hyperolius kivuensis	AB3836	OQ770362	Kaffa, Ethiopia	This study
Hyperolius kivuensis	AB3837	OQ770363	Kaffa, Ethiopia	This study
Hyperolius kivuensis	AB3838	OQ770364	Kaffa, Ethiopia	This study
Hyperolius kivuensis	AB3839	OQ770365	Kaffa, Ethiopia	This study
Hyperolius kivuensis	AB3840	OQ770366	Kaffa, Ethiopia	This study
Hyperolius kivuensis	AB3841	OQ770367	Kaffa, Ethiopia	This study
Hyperolius howelli	AB3851	OQ770368	Kaffa, Ethiopia	This study
Hyperolius kivuensis	AB3852	OQ770369	Kaffa, Ethiopia	This study
Hyperolius viridiflavus	AB3835	OQ770370	Kaffa, Ethiopia	This study
Hyperolius viridiflavus	AB3963	OQ770371	Kaffa, Ethiopia	This study
Hyperolius kivuensis	AB3956	OQ770372	Kaffa, Ethiopia	This study
Hyperolius kivuensis	AB3957	OQ770373	Kaffa, Ethiopia	This study
Hyperolius viridiflavus	AB3812	OQ770374	Kaffa, Ethiopia	This study
Hyperolius kivuensis	AB3813	OQ770375	Kaffa, Ethiopia	This study

Species	Collection No.	Gene Bank Accession No.	Locality	Source
Hyperolius acuticeps	-	JQ863691	Malawi	NCBI Genebank
Hyperolius acuticeps	-	JQ863691	Malawi	NCBI Genebank
Hyperolius balfouri	-	KX492614	-	NCBI Genebank
Hyperolius kivuensis	-	KF447822	Uganda	NCBI Genebank
Hyperolius nasutus	-	MK036467	Angola	NCBI Genebank
Hyperolius nasutus	-	MK036468	Angola	NCBI Genebank
Hyperolius microps	-	MG829614	Mozambique	NCBI Genebank
Hyperolius microps	-	MG829615	Mozambique	NCBI Genebank
Hyperolius viridiflavus	-	JQ966570	Rwanda	NCBI Genebank
Xenopus clivii	AB3816	OQ975263	Kaffa, Ethiopia	This study
Afrixalus enseticola	AB3864	OQ779001	Kaffa, Ethiopia	This study
Afrixalus enseticola	AB3865	OQ779002	Kaffa, Ethiopia	This study
Afrixalus clarkei	AB3825	OQ779003	Kaffa, Ethiopia	This study
Afrixalus clarkei	AB3826	OQ779004	Kaffa, Ethiopia	This study
Afrixalus clarkei	AB3831	OQ779005	Kaffa, Ethiopia	This study

Species	**Collection No.**	**Gene Bank Accession No.**	**Locality**	**Source**
Afrixalus clarkei	AB3889	OQ779006	Kaffa, Ethiopia	This study
Afrixalus clarkei	AB3893	OQ779007	Kaffa, Ethiopia	This study
Afrixalus clarkei	AB3899	OQ779008	Kaffa, Ethiopia	This study
Afrixalus clarkei	AB3846	OQ779009	Kaffa, Ethiopia	This study
Afrixalus clarkei	AB3858	OQ779010	Kaffa, Ethiopia	This study
Afrixalus enseticola	AB3861	OQ779011	Kaffa, Ethiopia	This study
Afrixalus enseticola	AB3886	OQ779012	Kaffa, Ethiopia	This study
Afrixalus enseticola	AB3878	OQ779013	Kaffa, Ethiopia	This study
Afrixalus enseticola	AB3882	OQ779014	Kaffa, Ethiopia	This study
Afrixalus enseticola	AB3898	OQ779015	Kaffa, Ethiopia	This study
Afrixalus fornasini	-	MH115683	South Africa	NCBI Genebank
Afrixalus fornasini	-	MH115682	South Africa	NCBI Genebank
Afrixalus aureus	-	MH115678	South Africa	NCBI Genebank
Afrixalus aureus	-	MH115679	South Africa	NCBI Genebank
Afrixalus quadrivittatus	-	MF537697	Southeastern Gabon	NCBI Genebank

Species	**Collection No.**	**Gene Bank Accession No.**	**Locality**	**Source**
Afrixalus quadrivittatus	-	MF537696	Southeastern Gabon	NCBI Genebank
Afrixalus osorioi	-	KY079930	Republic of the Congo	NCBI Genebank
Afrixalus osorioi	-	KY079929	Republic of the Congo	NCBI Genebank
Afrixalus fulvovittatus	-	KY079928	Republic of the Congo	NCBI Genebank
Afrixalus fulvovittatus	-	KY079927	Republic of the Congo	NCBI Genebank
Afrixalus paradorsalis	-	KX671718	Cameroon	NCBI Genebank
Afrixalus paradorsalis	-	KX671719	Cameroon	NCBI Genebank
A. sylvaticus	-	KY177046	Kenya	NCBI Genebank
Afrixalus laevis	-	KX671715	Cameroon	NCBI Genebank
P. mascareniensis		JX464887		NCBI Genebank

Appendix 5. Uncorrected p-distances between the Phrynobatrchus species found in Ethiopia based on the 16S gene sequences.

Phrynobatrachus species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
P. bibita SB419																									
P. bibita SB422	0.00																								
P. bibita SB425	0.00	0.00																							
P. bibita SB440	0.00	0.00	0.00																						
Phrynobatrachus sp.1 AB3830	0.12	0.12	0.12	0.12																					
Phrynobatrachus sp.1 AB3834	0.12	0.12	0.12	0.12	0.00																				
Phrynobatrachus sp.1 AB3848	0.12	0.12	0.12	0.12	0.00	0.00																			
Phrynobatrachus sp.1 AB3862	0.12	0.12	0.12	0.12	0.00	0.00	0.00																		
Phrynobatrachus sp.1 AB3891	0.12	0.12	0.12	0.12	0.00	0.00	0.00	0.00																	
Phrynobatrachus sp.1 AB3910	0.12	0.12	0.12	0.12	0.00	0.00	0.00	0.00	0.00																
P. natalensis AB3949	0.18	0.17	0.17	0.17	0.16	0.16	0.16	0.16	0.15	0.16															
Phrynobatrachus sp.1 AB3987	0.12	0.12	0.12	0.12	0.00	0.00	0.00	0.00	0.00	0.00	0.15														
Phrynobatrachus sp.1 AB4024	0.12	0.12	0.12	0.12	0.00	0.00	0.00	0.00	0.00	0.00	0.16	0.00													
Phrynobatrachus sp.2 AB4047	0.13	0.13	0.13	0.13	0.09	0.09	0.09	0.09	0.09	0.09	0.16	0.09	0.09												
P. natalensis AB4054	0.18	0.17	0.17	0.17	0.16	0.16	0.16	0.16	0.15	0.16	0.00	0.15	0.16	0.16											
P. inexpectatus EU075292	0.11	0.11	0.11	0.11	0.05	0.05	0.05	0.05	0.05	0.05	0.16	0.05	0.05	0.09	0.16										
P. inexpectatus EU075291	0.11	0.11	0.11	0.11	0.05	0.05	0.05	0.05	0.05	0.05	0.16	0.05	0.05	0.09	0.16	0.00									
P. inexpectatus EU075290	0.11	0.11	0.11	0.11	0.05	0.05	0.05	0.05	0.05	0.05	0.16	0.05	0.05	0.09	0.16	0.00	0.00								
Phrynobatrachus sp. Oromia FJ829311	0.14	0.14	0.14	0.14	0.09	0.10	0.10	0.10	0.09	0.10	0.16	0.09	0.10	0.01	0.16	0.09	0.09	0.09							
Phrynobatrachus sp.Oromia FJ829313	0.14	0.14	0.14	0.14	0.09	0.10	0.10	0.10	0.09	0.10	0.16	0.09	0.10	0.01	0.16	0.09	0.09	0.09	0.00						
P. minutus FJ829285	0.10	0.10	0.10	0.10	0.05	0.06	0.06	0.06	0.05	0.06	0.16	0.05	0.06	0.10	0.16	0.02	0.02	0.02	0.11	0.11					
P. minutus FJ829284	0.10	0.10	0.10	0.10	0.05	0.06	0.06	0.06	0.05	0.06	0.16	0.05	0.06	0.10	0.16	0.02	0.02	0.02	0.11	0.11	0.00				
P. natalensis FJ829297	0.18	0.17	0.17	0.17	0.16	0.16	0.16	0.16	0.15	0.16	0.00	0.15	0.16	0.16	0.00	0.16	0.16	0.16	0.16	0.16	0.16	0.16			
P. natalensis DQ019605	0.15	0.15	0.15	0.15	0.14	0.14	0.14	0.14	0.14	0.14	0.08	0.14	0.14	0.14	0.08	0.14	0.14	0.14	0.15	0.15	0.13	0.13	0.08		
P. bullans FJ829264	0.15	0.15	0.15	0.15	0.14	0.14	0.14	0.14	0.13	0.14	0.12	0.13	0.14	0.14	0.12	0.14	0.14	0.14	0.15	0.15	0.14	0.14	0.12	0.11	
P. bullans FJ829261	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.14	0.16	0.16	0.17	0.14	0.16	0.16	0.16	0.17	0.17	0.16	0.16	0.15	0.13	0.04

Appendix 6. Uncorrected p-distances between the Leptopelis species of Ethiopia based on the COI gene sequences.

Leptopelis species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45
L. vannutellii AB3797																																													
L. vannutellii AB3798	0.00																																												
Leptopelis sp.1 AB3845	0.12	0.12																																											
L. vannutellii AB3897	0.01	0.01	0.12																																										
L. vannutellii AB3997	0.01	0.02	0.12	0.01																																									
L. vannutellii AB4017	0.01	0.01	0.13	0.01	0.02																																								
L. vannutellii AB4031	0.02	0.02	0.13	0.01	0.02	0.02																																							
L. vannutellii AB4032	0.02	0.02	0.13	0.02	0.02	0.03	0.03																																						
L. vannutellii AB4039	0.02	0.02	0.13	0.01	0.01	0.02	0.02	0.01																																					
L. yaldeni AB4044	0.04	0.04	0.12	0.04	0.04	0.04	0.03	0.05	0.04																																				
L. yaldeni AB4045	0.04	0.04	0.12	0.04	0.04	0.04	0.03	0.05	0.04	0.00																																			
Leptopelis sp.1 AB4060	0.12	0.12	0.00	0.12	0.12	0.13	0.13	0.13	0.13	0.12	0.12																																		
Leptopelis sp.1 AB3908	0.12	0.12	0.01	0.12	0.12	0.12	0.13	0.12	0.12	0.12	0.12	0.01																																	
Leptopelis sp.1 AB3911	0.11	0.12	0.01	0.12	0.12	0.12	0.13	0.12	0.12	0.12	0.12	0.01	0.01																																
L. vannutellii AB3914	0.01	0.01	0.13	0.01	0.01	0.02	0.01	0.02	0.01	0.04	0.04	0.13	0.12	0.12																															
Leptopelis sp.1 AB3915	0.12	0.12	0.00	0.12	0.12	0.12	0.13	0.12	0.12	0.12	0.12	0.00	0.00	0.01	0.12																														
Leptopelis sp.2 AB3916	0.12	0.12	0.04	0.12	0.12	0.12	0.12	0.12	0.12	0.11	0.11	0.04	0.04	0.04	0.12	0.04																													
L. vannutellii AB3917	0.01	0.01	0.13	0.01	0.02	0.01	0.02	0.03	0.02	0.04	0.04	0.13	0.13	0.12	0.02	0.13	0.13																												
L. vannutellii AB3918	0.01	0.01	0.12	0.00	0.01	0.01	0.01	0.02	0.01	0.03	0.03	0.12	0.12	0.12	0.01	0.12	0.12	0.01																											
Leptopelis sp.2 AB3919	0.12	0.12	0.04	0.12	0.12	0.12	0.12	0.12	0.12	0.11	0.11	0.04	0.04	0.05	0.13	0.04	0.00	0.13	0.12																										
Leptopelis sp.2 AB3922	0.12	0.12	0.04	0.12	0.12	0.12	0.12	0.12	0.12	0.11	0.11	0.04	0.04	0.05	0.13	0.04	0.00	0.13	0.12	0.00																									
L. vannutellii AB3923	0.01	0.01	0.12	0.00	0.01	0.01	0.01	0.02	0.01	0.03	0.03	0.12	0.12	0.12	0.01	0.12	0.12	0.01	0.00	0.12	0.12																								
L. vannutellii AB3925	0.01	0.01	0.12	0.00	0.01	0.01	0.01	0.02	0.01	0.03	0.03	0.12	0.12	0.12	0.01	0.12	0.12	0.01	0.00	0.12	0.12	0.00																							
Leptopelis sp.2 AB3927	0.12	0.12	0.04	0.12	0.12	0.12	0.12	0.12	0.12	0.11	0.11	0.04	0.04	0.04	0.12	0.04	0.00	0.13	0.12	0.00	0.00	0.12	0.12																						
Leptopelis sp.2 AB3928	0.12	0.12	0.04	0.12	0.12	0.12	0.12	0.12	0.12	0.11	0.11	0.04	0.04	0.04	0.12	0.04	0.00	0.13	0.12	0.00	0.00	0.12	0.12	0.00																					
Leptopelis sp.2 AB3929	0.12	0.12	0.03	0.12	0.12	0.12	0.13	0.12	0.12	0.12	0.12	0.03	0.04	0.04	0.13	0.03	0.01	0.13	0.12	0.01	0.01	0.12	0.12	0.01	0.01																				
L. ragazzii 15 523	0.06	0.06	0.12	0.06	0.06	0.07	0.06	0.07	0.06	0.07	0.07	0.12	0.12	0.12	0.06	0.12	0.11	0.07	0.06	0.11	0.11	0.06	0.06	0.11	0.11	0.11																			
L. ragazzii 15 128	0.06	0.06	0.12	0.06	0.06	0.06	0.06	0.06	0.05	0.07	0.07	0.12	0.12	0.12	0.06	0.12	0.11	0.07	0.05	0.11	0.11	0.05	0.05	0.11	0.11	0.11	0.00																		
L. vannutellii 11 236	0.02	0.02	0.13	0.01	0.02	0.02	0.00	0.03	0.02	0.04	0.04	0.13	0.13	0.13	0.01	0.13	0.13	0.02	0.01	0.13	0.13	0.01	0.01	0.13	0.13	0.13	0.07	0.06																	
L. vannutellii 15 186	0.00	0.00	0.12	0.01	0.01	0.01	0.01	0.02	0.01	0.04	0.04	0.12	0.12	0.12	0.01	0.12	0.12	0.01	0.01	0.12	0.12	0.01	0.01	0.12	0.12	0.12	0.06	0.06	0.01																
L. yaldeni 11 669	0.04	0.04	0.12	0.03	0.04	0.04	0.03	0.05	0.04	0.01	0.01	0.12	0.12	0.12	0.04	0.12	0.11	0.04	0.03	0.12	0.12	0.03	0.03	0.11	0.11	0.12	0.07	0.07	0.04	0.03															
L. yaldeni 13 699	0.04	0.04	0.12	0.03	0.04	0.04	0.03	0.05	0.04	0.01	0.01	0.12	0.12	0.12	0.04	0.12	0.11	0.04	0.03	0.12	0.12	0.03	0.03	0.11	0.11	0.12	0.07	0.07	0.04	0.03	0.00														
L. diffidens SB134	0.10	0.10	0.11	0.11	0.11	0.11	0.11	0.12	0.11	0.10	0.10	0.11	0.10	0.11	0.11	0.11	0.10	0.11	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.11	0.08	0.09	0.11	0.10	0.10	0.10													
L. diffidens SB112	0.10	0.10	0.11	0.11	0.11	0.11	0.11	0.12	0.11	0.10	0.10	0.11	0.10	0.11	0.11	0.11	0.10	0.11	0.11	0.10	0.10	0.11	0.11	0.10	0.10	0.10	0.09	0.09	0.11	0.10	0.10	0.10	0.01												
L. rugosus 11 268	0.09	0.09	0.10	0.10	0.10	0.10	0.10	0.11	0.10	0.10	0.10	0.10	0.09	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.09	0.09	0.10	0.09	0.10	0.10	0.07	0.07											
L. rugosus 26915	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.11	0.10	0.10	0.10	0.10	0.09	0.09	0.10	0.09	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.09	0.09	0.10	0.09	0.10	0.10	0.07	0.07	0.00										
L. rugosus SB532	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.11	0.10	0.10	0.10	0.10	0.09	0.09	0.10	0.09	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.09	0.09	0.10	0.09	0.10	0.10	0.07	0.07	0.00	0.00									
L. susanae SB225	0.10	0.10	0.10	0.10	0.10	0.10	0.11	0.11	0.10	0.10	0.10	0.10	0.09	0.10	0.10	0.10	0.09	0.11	0.10	0.10	0.10	0.10	0.10	0.09	0.09	0.09	0.09	0.09	0.11	0.10	0.10	0.10	0.06	0.06	0.05	0.05	0.05								
L. susanae SB223	0.10	0.10	0.10	0.10	0.10	0.10	0.11	0.10	0.10	0.10	0.10	0.10	0.09	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.11	0.10	0.10	0.10	0.06	0.06	0.05	0.05	0.05	0.00							
L. susanae SB224	0.10	0.10	0.10	0.10	0.10	0.10	0.11	0.10	0.10	0.10	0.10	0.10	0.09	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.11	0.10	0.10	0.10	0.06	0.06	0.05	0.05	0.05	0.00	0.00						
L. gramineus 13 152	0.11	0.11	0.11	0.12	0.12	0.12	0.12	0.12	0.11	0.11	0.11	0.11	0.10	0.11	0.12	0.11	0.10	0.12	0.11	0.11	0.11	0.11	0.11	0.10	0.10	0.10	0.10	0.10	0.12	0.11	0.11	0.11	0.08	0.08	0.06	0.06	0.06	0.06	0.06	0.06					
L. gramineus 13 155	0.11	0.11	0.11	0.12	0.12	0.12	0.12	0.12	0.11	0.11	0.11	0.11	0.10	0.11	0.12	0.11	0.10	0.12	0.11	0.11	0.11	0.11	0.11	0.10	0.10	0.10	0.10	0.10	0.12	0.11	0.11	0.11	0.08	0.08	0.06	0.06	0.06	0.06	0.06	0.06	0.00				
L. xeniae 13 575	0.10	0.10	0.11	0.11	0.11	0.10	0.11	0.10	0.10	0.10	0.10	0.11	0.11	0.11	0.10	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.10	0.11	0.11	0.10	0.10	0.10	0.08	0.08	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06			
L. xeniae SB183	0.10	0.10	0.11	0.11	0.11	0.10	0.11	0.10	0.10	0.10	0.10	0.11	0.11	0.11	0.10	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.10	0.11	0.11	0.10	0.10	0.10	0.08	0.08	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.00		
L. shebellensis SB503	0.10	0.10	0.11	0.10	0.11	0.10	0.11	0.10	0.10	0.10	0.10	0.11	0.11	0.11	0.10	0.11	0.11	0.11	0.10	0.11	0.11	0.10	0.10	0.11	0.11	0.10	0.10	0.10	0.11	0.10	0.10	0.10	0.08	0.08	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	
L. shebellensis SB502	0.09	0.09	0.11	0.10	0.10	0.10	0.10	0.10	0.10	0.09	0.09	0.11	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.11	0.11	0.10	0.10	0.10	0.10	0.10	0.10	0.09	0.10	0.09	0.10	0.10	0.07	0.07	0.05	0.06	0.06	0.05	0.05	0.05	0.06	0.06	0.06	0.06	0.01

Appendix 7. Sorenson similarity index between sites

	Bita	Ganeti	Shuneti	Sheda	Nata	Medabo	Gonogori	Bariacho	Utali	Sor	Gojeb	Alemgono	Shoreri	Komba	Boqa	Shamali	Oda
Bita	1																
Ganeti	0.33	1															
Shuneti	0.54	0.57	1														
Sheda	0.64	0.24	0.53	1													
Nata	0.48	0.46	0.55	0.43	1												
Medabo	0.48	0.31	0.55	0.43	0.80	1											
Gonogori	0.48	0.46	0.55	0.43	0.80	0.60	1										
Bariacho	0.54	0.43	0.67	0.53	0.91	0.91	0.73	1									
Utali	0.61	0.53	0.92	0.50	0.50	0.50	0.50	0.62	1								
Sor	0.29	0.31	0.36	0.43	0.40	0.40	0.40	0.55	0.33	1							
Gojeb	0.25	0.13	0.29	0.47	0.31	0.31	0.31	0.43	0.27	0.62	1						
Alemgono	0.50	0.30	0.33	0.67	0.35	0.35	0.35	0.44	0.32	0.47	0.60	1					
Shoreri	0.45	0.43	0.67	0.27	0.36	0.55	0.36	0.50	0.77	0.18	0.14	0.33	1				
Komba	0.62	0.11	0.25	0.63	0.27	0.27	0.27	0.38	0.24	0.53	0.44	0.64	0.25	1			
Boqa	0.48	0.59	0.53	0.44	0.57	0.57	0.43	0.67	0.63	0.43	0.35	0.48	0.53	0.32	1		
Shamali	0.52	0.53	0.77	0.63	0.67	0.67	0.50	0.77	0.71	0.67	0.40	0.42	0.46	0.35	0.63	1	
Oda	0.45	0.43	0.67	0.40	0.36	0.36	0.55	0.50	0.77	0.36	0.29	0.33	0.67	0.25	0.53	0.46	1

Appendix 8. Jaccard similarity between sites

	Bita	Ganeti	Shuneti	Sheda	Nata	Medabo	Gonogori	Bariacho	Utali	Sor	Gojeb	Alemgono	Shoreri	Komba	Boqa	Shamali	Oda
Bita	1.00																
Ganeti	0.20	1.00															
Shuneti	0.38	0.40	1.00														
Sheda	0.47	0.13	0.36	1.00													
Nata	0.31	0.30	0.38	0.27	1.00												
Medabo	0.31	0.18	0.38	0.27	0.67	1.00											
Gonogori	0.31	0.30	0.38	0.27	0.67	0.43	1.00										
Bariacho	0.38	0.27	0.50	0.36	0.83	0.83	0.57	1.00									
Utali	0.44	0.36	0.86	0.33	0.33	0.33	0.33	0.44	1.00								
Sor	0.17	0.18	0.22	0.27	0.25	0.25	0.25	0.38	0.20	1.00							
Gojeb	0.14	0.07	0.17	0.31	0.18	0.18	0.18	0.27	0.15	0.44	1.00						
Alemgono	0.33	0.18	0.20	0.50	0.21	0.21	0.21	0.29	0.19	0.31	0.43	1.00					
Shoreri	0.29	0.27	0.50	0.15	0.22	0.38	0.22	0.33	0.63	0.10	0.08	0.20	1.00				
Komba	0.44	0.06	0.14	0.46	0.15	0.15	0.15	0.23	0.13	0.36	0.29	0.47	0.14	1.00			
Boqa	0.32	0.42	0.36	0.29	0.40	0.40	0.27	0.50	0.45	0.27	0.21	0.31	0.36	0.19	1.00		
Shamali	0.35	0.36	0.63	0.45	0.50	0.50	0.33	0.63	0.56	0.50	0.25	0.27	0.30	0.21	0.45	1.00	
Oda	0.29	0.27	0.50	0.25	0.22	0.22	0.38	0.33	0.63	0.22	0.17	0.20	0.50	0.14	0.36	0.30	1.00

Appendix 9. Endemic frog diversity, richness and abundance among the transects and sites, in Kaffa area.

Transect	Season	Site	Habitat	Diversity	Richness	Abundance	Evenness
1	Dry Season	Bita	Wetland	1	4	29	0.68
2	Dry Season	Bita	Agricultural land	0.69	2	9	1.00
3	Dry Season	Bita	Riverine Forest	0.63	2	14	0.94
4	Dry Season	Ganeti	Wetland	0.81	4	32	0.56
5	Dry Season	Ganeti	Agricultural land	0	1	13	1
6	Dry Season	Ganeti	Riverine Forest	0	1	2	1
7	Dry Season	Shuneti	Wetland	0	1	20	1
8	Dry Season	Shuneti	Agricultural land	0	1	6	1
9	Dry Season	Shuneti	Riverine Forest	0.63	2	11	0.94
10	Dry Season	Sheda	Wetland	0	1	35	1
11	Dry Season	Sheda	Agricultural land	0	1	10	1
12	Dry Season	Sheda	Riverine Forest	0	1	3	1
13	Dry Season	Nata	Wetland	1.07	3	90	0.97

Transect	Season	Site	Habitat	Diversity	Richness	Abundance	Evenness
14	Dry Season	Nata	Agricultural land	0.62	2	21	0.93
15	Dry Season	Nata	Riverine Forest	0.63	2	14	0.94
16	Dry Season	Medabo	Wetland	0.78	3	152	0.73
17	Dry Season	Medabo	Agricultural land	0.58	2	17	0.89
18	Dry Season	Medabo	Riverine Forest	0	1	2	1
19	Dry Season	Gonogori	Wetland	0	1	20	1
20	Dry Season	Gonogori	Agricultural land	0.70	2	51	1.01
21	Dry Season	Gonogori	Riverine Forest	0	1	15	1
22	Dry Season	Bariacho	Wetland	0	1	12	1
23	Dry Season	Bariacho	Agricultural land	0.99	3	26	0.90
24	Dry Season	Bariacho	Riverine Forest	0.64	3	37	0.63
25	Dry Season	Utali	Wetland	0	1	92	1
26	Dry Season	Utali	Agricultural land	0	1	4	1
27	Dry Season	Utali	Riverine Forest	0.72	2	19	1.025
28	Dry Season	Sor	Wetland	0.59	2	20	0.90

Transect	Season	Site	Habitat	Diversity	Richness	Abundance	Evenness
29	Dry Season	Sor	Agricultural land	0	1	2	1
30	Dry Season	Sor	Riverine Forest	0.66	2	10	0.97
31	Dry Season	Gojeb	Wetland	0.72	2	8	1.03
32	Dry Season	Gojeb	Agricultural land	0	1	3	1
33	Dry Season	Gojeb	Riverine Forest	0.72	2	8	1.03
34	Dry Season	Alemgono	Wetland	0.61	2	25	0.92
35	Dry Season	Alemgono	Agricultural land	0	1	3	1
36	Dry Season	Alemgono	Riverine Forest	0.67	2	7	0.98
37	Dry Season	Shoreri	Wetland	0	1	7	1
38	Dry Season	Shoreri	Agricultural land	0	1	3	1
39	Dry Season	Shoreri	Riverine Forest	0	1	13	1
40	Dry Season	Komba	Wetland	0.63	3	23	0.62
41	Dry Season	Komba	Agricultural land	0.59	2	34	0.90
42	Dry Season	Komba	Riverine Forest	1.01	5	206	0.55
43	Dry Season	Boqa	Wetland	0	1	3	1

Transect	Season	Site	Habitat	Diversity	Richness	Abundance	Evenness
44	Dry Season	Boqa	Agricultural land	0.68	2	29	0.99
45	Dry Season	Boqa	Riverine Forest	0.54	2	19	0.86
46	Dry Season	Shamali	Wetland	0	1	3	1
47	Dry Season	Shamali	Agricultural land	0.77	2	5	1.08
48	Dry Season	Shamali	Riverine Forest	0	1	12	1
49	Dry Season	Oda	Agricultural land	0	1	5	1
50	Dry Season	Oda	Riverine Forest	0	1	4	1
51	Dry Season	Oda	Wetland	0	1	4	1
1	Rainy Season	Bita	Wetland	1.00	4	30	0.68
2	Rainy Season	Bita	Agricultural land	0.68	2	12	0.99
3	Rainy Season	Bita	Riverine Forest	0.39	2	17	0.74
4	Rainy Season	Ganeti	Wetland	0.68	3	38	0.66
5	Rainy Season	Ganeti	Agricultural land	0	1	15	1
6	Rainy Season	Ganeti	Riverine Forest	0	1	5	1
7	Rainy Season	Shuneti	Wetland	0	1	90	1

Transect	Season	Site	Habitat	Diversity	Richness	Abundance	Evenness
8	Rainy Season	Shuneti	Agricultural land	0	1	7	1
9	Rainy Season	Shuneti	Riverine Forest	0.69	2	16	1
10	Rainy Season	Sheda	Wetland	0	1	50	1
11	Rainy Season	Sheda	Agricultural land	0	1	11	1
12	Rainy Season	Sheda	Riverine Forest	0	1	4	1
13	Rainy Season	Nata	Wetland	1.0	3	230	0.94
14	Rainy Season	Nata	Agricultural land	0.6	2	27	0.90
15	Rainy Season	Nata	Riverine Forest	0.7	2	16	1.00
16	Rainy Season	Medabo	Wetland	0.5	2	65	0.86
17	Rainy Season	Medabo	Agricultural land	0.7	2	26	0.99
18	Rainy Season	Medabo	Riverine Forest	0.1	2	103	0.57
19	Rainy Season	Gonogori	Wetland	0	1	30	1
20	Rainy Season	Gonogori	Agricultural land	0.70	2	62	1.01
21	Rainy Season	Gonogori	Riverine Forest	0	1	16	1
22	Rainy Season	Bariacho	Wetland	0	1	14	1

Transect	Season	Site	Habitat	Diversity	Richness	Abundance	Evenness
23	Rainy Season	Bariacho	Agricultural land	1.02	3	31	0.92
24	Rainy Season	Bariacho	Riverine Forest	0.67	3	44	0.65
25	Rainy Season	Utali	Wetland	0	1	100	1
26	Rainy Season	Utali	Agricultural land	0	1	5	1
27	Rainy Season	Utali	Riverine Forest	0.54	2	19	0.86
28	Rainy Season	Sor	Wetland	0.65	2	30	0.96
29	Rainy Season	Sor	Agricultural land	0	1	5	1
30	Rainy Season	Sor	Riverine Forest	0.54	2	19	0.86
31	Rainy Season	Gojeb	Wetland	0.73	2	13	1.04
32	Rainy Season	Gojeb	Agricultural land	0	1	7	1
33	Rainy Season	Gojeb	Riverine Forest	0.72	2	10	1.03
34	Rainy Season	Alemgono	Wetland	0.27	2	108	0.65
35	Rainy Season	Alemgono	Agricultural land	0	1	3	1
36	Rainy Season	Alemgono	Riverine Forest	0.78	2	6	1.09
37	Rainy Season	Shoreri	Wetland	0	1	10	1

Transect	Season	Site	Habitat	Diversity	Richness	Abundance	Evenness
38	Rainy Season	Shoreri	Agricultural land	0	1	5	1
39	Rainy Season	Shoreri	Riverine Forest	0	1	11	1
40	Rainy Season	Komba	Wetland	0	1	20	1
41	Rainy Season	Komba	Agricultural land	0.41	2	23	0.75
42	Rainy Season	Komba	Riverine Forest	0.57	2	53	0.88
43	Rainy Season	Boqa	Wetland	1.07	5	223	0.58
44	Rainy Season	Boqa	Agricultural land	0	1	8	1
45	Rainy Season	Boqa	Riverine Forest	0.70	2	40	1.01
46	Rainy Season	Shamali	Wetland	0.56	2	26	0.87
47	Rainy Season	Shamali	Agricultural land	0	1	4	1
48	Rainy Season	Shamali	Riverine Forest	0.67	2	15	0.98
49	Rainy Season	Oda	Agricultural land	0	1	31	1
50	Rainy Season	Oda	Riverine Forest	0	1	17	1
51	Rainy Season	Oda	Wetland	0	1	10	1

Appendix 10. Output of the Linear Mixed-effects Model that depicts the variation of diversity and richness due to fixed and random factors.

Diversity					**Richness**			
Predictors	*Estimates*	*CI*	*p*	*df*	*Estimates*	*CI*	*p*	*df*
(Intercept)	0.25	0.10 – 0.41	**0.002**	65	1.42	1.04 – 1.80	**<0.001**	66
Habitat [Riverine Forest]	0.04	-0.13 – 0.20	0.663	82	0.09	-0.32 – 0.50	0.671	82
Habitat [Wetland]	0.13	-0.04 – 0.30	0.132	82	0.59	0.18 – 1.00	**0.006**	82
Season [Rainy Season]	-0.02	-0.16 – 0.12	0.771	82	-0.02	-0.36 – 0.32	0.908	82
Random Effects								
σ^2	0.12				0.73			
τ_{00}	0.02 $_{\textbf{Site}}$				0.14$_{\textbf{Site}}$			
ICC	0.17				0.16			
N	17$_{\textbf{Site}}$				17 $_{\textbf{Site}}$			
Observations	102				102			
Marginal R^2 / Conditional R^2	0.020 / 0.186				0.073/ 0.222			

Appendix 11. Uncorrected p-distances between the *Hyperolius* species of 16S gene sequences.

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
1	*H. microps* MG829614		0.00	0.02	0.02	0.01	0.01	0.01	0.01	0.02	0.01	0.02	0.02	0.02	0.01	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.01	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
2	*H. microps* MG829615	0.00		0.02	0.02	0.01	0.01	0.01	0.01	0.02	0.01	0.02	0.02	0.02	0.01	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.01	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
3	*H. kivuensis* KF447834	0.17	0.17		0.00	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.02	0.02	0.00	0.00	0.02	0.00	0.02
4	*H. kivuensis* KF447822	0.17	0.17	0.00		0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.02	0.02	0.00	0.00	0.02	0.00	0.02
5	*H. nasutus* MK036467	0.02	0.02	0.16	0.16		0.00	0.01	0.01	0.02	0.01	0.02	0.02	0.02	0.01	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.01	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
6	*H. nasutus* MK036468	0.02	0.02	0.16	0.16	0.00		0.01	0.01	0.02	0.01	0.02	0.02	0.02	0.01	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.01	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
7	*H. acuticeps* JQ863687	0.04	0.03	0.16	0.16	0.03	0.03		0.00	0.02	0.01	0.02	0.02	0.02	0.01	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.01	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
8	*H. acuticeps* JQ86369	0.04	0.04	0.17	0.17	0.03	0.03	0.00		0.02	0.01	0.02	0.02	0.02	0.01	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.01	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
9	*H. viridiflavus* JQ966570	0.18	0.18	0.16	0.16	0.17	0.17	0.17	0.17		0.02	0.01	0.00	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.00	0.01	0.02	0.02	0.01	0.02	0.02
10	*H. howelli* AB3820	0.02	0.03	0.17	0.17	0.03	0.03	0.03	0.04	0.18		0.02	0.02	0.02	0.00	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.00	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
11	*H. viridiflavus* AB3817	0.17	0.17	0.16	0.16	0.16	0.16	0.16	0.16	0.01	0.17		0.00	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.00	0.00	0.02	0.02	0.00	0.02	0.02
12	*H. viridiflavus* AB3819	0.17	0.17	0.16	0.16	0.16	0.16	0.16	0.16	0.01	0.17	0.00		0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.00	0.00	0.02	0.02	0.00	0.02	0.02
13	*H. kivuensis* AB3822	0.17	0.17	0.00	0.00	0.16	0.16	0.17	0.17	0.16	0.17	0.16	0.16		0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.02	0.02	0.00	0.00	0.02	0.00	0.02
14	*H. howelli* AB3824	0.02	0.02	0.17	0.17	0.02	0.02	0.03	0.03	0.18	0.00	0.17	0.17	0.17		0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.00	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
15	*H. kivuensis* AB3828	0.17	0.17	0.00	0.00	0.16	0.16	0.17	0.17	0.16	0.17	0.16	0.16	0.00	0.17		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.02	0.02	0.00	0.00	0.02	0.00	0.02
16	*H. kivuensis* AB3829	0.17	0.17	0.00	0.00	0.16	0.16	0.17	0.17	0.16	0.17	0.16	0.16	0.00	0.17	0.00		0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.02	0.02	0.00	0.00	0.02	0.00	0.02
17	*H. kivuensis* AB3836	0.17	0.17	0.00	0.00	0.16	0.16	0.16	0.17	0.16	0.17	0.16	0.16	0.00	0.17	0.00	0.00		0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.02	0.02	0.00	0.00	0.02	0.00	0.02
18	*H. kivuensis* AB3837	0.17	0.17	0.00	0.00	0.16	0.16	0.16	0.17	0.16	0.17	0.16	0.16	0.00	0.17	0.00	0.00	0.00		0.00	0.00	0.00	0.00	0.02	0.00	0.02	0.02	0.00	0.00	0.02	0.00	0.02
19	*H. kivuensis* AB3838	0.17	0.17	0.00	0.00	0.16	0.16	0.17	0.17	0.16	0.17	0.16	0.16	0.00	0.17	0.00	0.00	0.00	0.00		0.00	0.00	0.00	0.02	0.00	0.02	0.02	0.00	0.00	0.02	0.00	0.02
20	*H. kivuensis* AB3839	0.17	0.17	0.00	0.00	0.16	0.16	0.16	0.17	0.16	0.17	0.16	0.16	0.00	0.17	0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.02	0.00	0.02	0.02	0.00	0.00	0.02	0.00	0.02
21	*H. kivuensis* AB3840	0.17	0.17	0.00	0.00	0.16	0.16	0.16	0.17	0.16	0.17	0.16	0.16	0.00	0.17	0.00	0.00	0.00	0.00	0.00	0.00		0.00	0.02	0.00	0.02	0.02	0.00	0.00	0.02	0.00	0.02
22	*H. kivuensis* AB3841	0.17	0.17	0.00	0.00	0.16	0.16	0.17	0.17	0.16	0.17	0.16	0.16	0.00	0.17	0.00	0.00	0.00	0.00	0.00	0.00	0.00		0.02	0.00	0.02	0.02	0.00	0.00	0.02	0.00	0.02
23	*H. howelli* AB3851	0.02	0.03	0.17	0.17	0.03	0.03	0.03	0.04	0.18	0.00	0.17	0.17	0.17	0.00	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17		0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
24	*H. kivuensis AB3852*	0.17	0.17	0.00	0.00	0.16	0.16	0.17	0.17	0.16	0.17	0.16	0.16	0.00	0.17	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.17		0.02	0.02	0.00	0.00	0.02	0.00	0.02
25	*H. viridiflavus* AB3835	0.17	0.17	0.16	0.16	0.16	0.16	0.16	0.16	0.01	0.17	0.00	0.00	0.16	0.17	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.17	0.16		0.00	0.02	0.02	0.00	0.02	0.02
26	*H. viridiflavus* AB3963	0.17	0.17	0.16	0.16	0.16	0.16	0.16	0.16	0.01	0.17	0.00	0.00	0.16	0.17	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.17	0.16	0.00		0.02	0.02	0.00	0.02	0.02
27	*H. kivuensis* AB3956	0.17	0.17	0.00	0.00	0.16	0.16	0.16	0.17	0.16	0.17	0.16	0.16	0.00	0.17	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.17	0.00	0.16	0.16		0.00	0.02	0.00	0.02
28	*H. kivuensis* AB3957	0.17	0.17	0.00	0.00	0.16	0.16	0.16	0.17	0.16	0.17	0.16	0.16	0.00	0.17	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.17	0.00	0.16	0.16	0.00		0.02	0.00	0.02
29	*H. viridiflavus* AB3812	0.17	0.17	0.16	0.16	0.17	0.17	0.16	0.16	0.01	0.17	0.01	0.00	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.00	0.01	0.17	0.17		0.02	0.02
30	*H. kivuensis* AB3813	0.17	0.17	0.00	0.00	0.16	0.16	0.16	0.17	0.16	0.17	0.16	0.16	0.00	0.17	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.17	0.00	0.16	0.16	0.00	0.00	0.16		0.02
31	*H. balfouri* KX492614	0.20	0.20	0.10	0.10	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.11	0.19	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.20	0.11	0.19	0.19	0.11	0.11	0.19	0.11	

Appendix 12.Uncorrected p-distances between the *Afrixalus* species of 16S gene sequences.

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
1	*A. aureus MH115677*		0.00	0.02	0.02	0.02	0.02	0.01	0.02	0.01	0.01	0.01	0.02	0.02	0.01	0.01	0.02	0.02	0.01	0.01	0.02	0.02	0.01	0.02	0.02	0.02	0.02	0.02	0.02	0.01	0.02
2	*A. aureus MH115678*	0.00		0.01	0.01	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.02	0.01	0.02	0.02	0.02	0.02	0.02	0.02	0.01	0.02
3	*A. clarkei AB3825*	0.13	0.12		0.00	0.00	0.00	0.01	0.01	0.01	0.01	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.02
4	*A. clarkei AB3826*	0.13	0.12	0.00		0.00	0.00	0.01	0.01	0.01	0.01	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.02
5	*A. clarkei AB3831*	0.13	0.12	0.00	0.00		0.00	0.01	0.01	0.01	0.01	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.02
6	*A. clarkei AB3846*	0.13	0.12	0.00	0.00	0.00		0.01	0.01	0.01	0.01	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.02
7	*A. clarkei AB3858*	0.12	0.12	0.02	0.02	0.02	0.02		0.00	0.00	0.00	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.02
8	*A. clarkei AB3889*	0.12	0.12	0.02	0.02	0.02	0.02	0.00		0.00	0.00	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.02
9	*A. clarkei AB3893*	0.12	0.12	0.02	0.02	0.02	0.02	0.00	0.00		0.00	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.02
10	*A. clarkei AB3899*	0.12	0.12	0.02	0.02	0.02	0.02	0.00	0.00	0.00		0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.02
11	*A. enseticola AB3861*	0.11	0.11	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13		0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.02	0.02	0.02	0.01	0.01	0.01	0.02	0.02	0.02	0.02	0.02	0.02
12	*A. enseticola AB3864*	0.11	0.11	0.13	0.13	0.14	0.13	0.13	0.13	0.13	0.13	0.00		0.00	0.00	0.00	0.00	0.00	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
13	*A. enseticola AB3865*	0.11	0.11	0.13	0.13	0.14	0.13	0.13	0.13	0.13	0.13	0.00	0.00		0.00	0.00	0.00	0.00	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
14	*A. enseticola AB3878*	0.11	0.11	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.00	0.00	0.00		0.00	0.00	0.00	0.02	0.02	0.02	0.02	0.01	0.01	0.01	0.02	0.02	0.02	0.02	0.02	0.02
15	*A. enseticola AB3882*	0.11	0.11	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.00	0.00	0.00	0.00		0.00	0.00	0.02	0.02	0.02	0.02	0.01	0.01	0.01	0.02	0.02	0.02	0.02	0.02	0.02
16	*A. enseticola AB3886*	0.11	0.11	0.13	0.13	0.14	0.13	0.13	0.13	0.13	0.13	0.00	0.00	0.00	0.00	0.00		0.00	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
17	*A. enseticola AB3898*	0.11	0.11	0.13	0.13	0.14	0.13	0.13	0.13	0.13	0.13	0.00	0.00	0.00	0.00	0.00	0.00		0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
18	*A. fornasini MH115682*	0.11	0.11	0.10	0.10	0.11	0.10	0.10	0.11	0.10	0.10	0.12	0.13	0.13	0.12	0.12	0.13	0.13		0.00	0.02	0.02	0.01	0.01	0.01	0.01	0.01	0.02	0.02	0.02	0.02
19	*A. fornasini MH115683*	0.11	0.11	0.10	0.10	0.11	0.10	0.10	0.11	0.10	0.10	0.12	0.13	0.13	0.12	0.12	0.13	0.13	0.00		0.02	0.02	0.01	0.01	0.01	0.01	0.01	0.02	0.02	0.02	0.02
20	*A. fulvovittatus KY079927*	0.14	0.13	0.12	0.12	0.13	0.12	0.13	0.13	0.13	0.13	0.16	0.17	0.17	0.16	0.16	0.17	0.17	0.14	0.14		0.00	0.02	0.02	0.02	0.02	0.02	0.00	0.00	0.02	0.02
21	*A. fulvovittatus KY079928*	0.14	0.13	0.12	0.12	0.13	0.12	0.13	0.13	0.13	0.13	0.16	0.17	0.17	0.16	0.16	0.17	0.17	0.14	0.14	0.00		0.02	0.02	0.02	0.02	0.02	0.00	0.00	0.02	0.02
22	*A. laevis KX671715*	0.10	0.10	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.10	0.10	0.13	0.13		0.01	0.01	0.01	0.01	0.02	0.02	0.01	0.02
23	*A. osorioi KY079929*	0.13	0.13	0.10	0.10	0.11	0.10	0.10	0.11	0.10	0.10	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.12	0.12	0.14	0.14	0.10		0.01	0.01	0.01	0.02	0.02	0.02	0.02
24	*A. osorioi KY079930*	0.12	0.12	0.09	0.09	0.09	0.09	0.10	0.10	0.10	0.10	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.11	0.11	0.13	0.13	0.09	0.04		0.01	0.01	0.02	0.02	0.02	0.02
25	*A. paradorsalis KX671718*	0.12	0.11	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.12	0.12	0.12	0.12	0.10	0.10	0.09		0.00	0.02	0.02	0.02	0.02
26	*A. paradorsalis KX671719*	0.12	0.12	0.07	0.07	0.07	0.07	0.08	0.08	0.08	0.08	0.13	0.14	0.14	0.13	0.13	0.14	0.14	0.12	0.12	0.12	0.12	0.10	0.10	0.09	0.00		0.02	0.02	0.02	0.02
27	*A. quadrivittatus MF537696*	0.13	0.13	0.12	0.12	0.12	0.12	0.13	0.13	0.13	0.13	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.14	0.14	0.00	0.00	0.13	0.14	0.13	0.12	0.12		0.00	0.02	0.02
28	*A. quadrivittatus MF537697*	0.13	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.13	0.13	0.00	0.00	0.12	0.13	0.13	0.12	0.12	0.00		0.02	0.02
29	*A. sylvaticus KY177046*	0.06	0.06	0.11	0.11	0.12	0.11	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.13	0.13	0.09	0.12	0.11	0.10	0.11	0.13	0.13		0.02
30	*P. mascareniensis JX464887*	0.17	0.18	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.21	0.21	0.22	0.22	0.19	0.22	0.22	0.21	0.21	0.22	0.22	0.20	