

REVIEW ARTICLE

UPDATED REVIEW OF AMPHIBIAN DIVERSITY, DISTRIBUTION AND CONSERVATION IN ETHIOPIA

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ABSTRACT: Ethiopia has a diverse amphibian fauna occurring in various ecosystems, from savanna to alpine highlands. Except for a taxonomic study on amphibians by Largen in 2001, little is known about the molecular systematics, evolution, population biology and conservation status of the different species. The Ethiopian Highlands are particularly important habitats as several endemic amphibian genera and species are restricted to these highly fragmented areas. For Ethiopia, five of the 24 genera, and 26 of the 64 known species are endemic. As is the case for amphibians worldwide, the survival of these species faces threats from habitat degradation, climate change, and a pathogenic fungal disease. Several factors might explain our low level of knowledge on Ethiopian amphibians; these are associated to culture and belief, education and training, and economic value. Taxonomic and biogeographic re-assessment and field surveys in unexplored areas is needed to understand the natural history and population status of Ethiopian amphibians. This paper tries to summarize existing knowledge on the amphibians of Ethiopia pertaining mainly to their taxonomy and systematics, a brief outline of the amphibian families of Ethiopia, geographic distribution and conservation. It discusses past and ongoing studies, existing gaps of knowledge, future needs in the area, and the application of phylogeography to resolve taxonomic complexities and outline the distribution patterns of some problematic groups.

Key words/phrases: Amphibian diversity, Biodiversity conservation, Ethiopian Highlands, Great Rift Valley, Phylogeography, Systematics.

I) INTRODUCTION: THE STATE-OF-KNOWLEDGE

Amphibian biology has been one of the zoological disciplines to which little attention was given in Ethiopia until recently. This is evident from the fact that despite having diverse amphibian fauna in its varied ecosystems, little is known about the updated taxonomy, evolution, population biology and

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conservation status of the different species. The relative lack of research on amphibians can be attributed to several factors that are discussed below. The starting point to prepare this review paper is the catalogue compiled by Largen (2001a) and a similar book by Largen and Spawls (2010). There are few recent publications that appeared during the past decade with some updates on a few taxonomic groups. Therefore, in this article, we will try to briefly discuss some of the background information on earlier works, main updates from recent and ongoing research, existing gaps of knowledge, future needs, some methods of diversity assessment and prioritization for conservation.

The following abbreviations have been used throughout the text: AAU = Addis Ababa University (Ethiopia); AMNH = American Museum of Natural History (New York); BSE = The Biological Society of Ethiopia (Addis Ababa); CI = Conservation International; CNS = College of Natural Sciences (Addis Ababa University); DNA = Deoxyribonucleic acid; EFAP = Ethiopian Forestry Action Program; EPA = Environmental Protection Authority (Addis Ababa); EWCA = Ethiopian Wildlife Conservation Authority (Addis Ababa); EWNHS = Ethiopian Wildlife and Natural History Society (Addis Ababa); EWNRA = Ethio-Wetlands and Natural Resources Association (Addis Ababa); FAG = Freiwilligen Akademischen Gesellschaft (Basel) (Voluntary Academic Society); FfE = Forum for Environment (Addis Ababa); GPS = Global Positioning System; IBC = Institute of Biodiversity Conservation (Addis Ababa); IUCN = International Union for Conservation of Nature; MCZ = Museum of Comparative Zoology (Harvard University); MSNG = Museo Civico di Storia Naturale di Genova Giacomo Doria (Genoa); mtDNA = Mitochondrial DNA; Mya = Millions of years ago; NHM = Natural History Museum (London); NLU = Institut für Natur-, Landschafts und Umweltwissenschaften (University of Basel) (Institute for Nature-, Landscape- and Environmental Sciences); PCR = Polymerase chain reaction; SNE = Stipendiencommission für Nachwuchskräfte aus Entwicklungsländern (Basel) (Scholarship Commission for Trainees from Developing Countries); UB = University of Basel (Switzerland); UTA = University of Texas Arlington (Arlington); WWF = World Wildlife Fund; ZMB = Universität Humboldt Zoologisches Museum (Berlin); ZNHM = Zoological Natural History Museum (Addis Ababa University).

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A) Amphibian biology in Ethiopia

The comprehensive review by Largen (2001a) summarizes field collection of amphibians in Ethiopia by earlier European and North American visitors and researchers (including some Ethiopian academicians) from the first half of the nineteenth century until 1986, and ten years of analytical work referencing museum collections. This review provides a background for all the necessary taxonomic information, and is therefore an excellent starting point for future research, including the ongoing studies outlined here. Earlier and recent field collections of amphibians from Ethiopia are kept in natural history museums in Ethiopia and elsewhere, the major ones including the ZNHM (AAU), NHM (London), MCZ (Harvard), AMNH (New York), MSNG (Genoa), ZMB (Berlin), the Field Museum (Chicago), and UTA (Arlington).

The few previous studies conducted on Ethiopian amphibians mainly focused on taxonomy, geographic distribution, conservation (e.g., Poynton, 1999; Largen, 2001a), and a few other biological aspects such as feeding behaviour and natural history (Grandison, 1978; Wake, 1980; Drewes and Roth, 1981; Grandison, 1981). Previous taxonomic descriptions were based on morphology and the ecology of each species (Largen, 2001a). Larger phylogenetic analyses of mainly molecular data using DNA samples of amphibian species from around the world have resulted in various taxonomic changes (Frost *et al.*, 2006; Frost and AMNH, 2010). We have extracted and compiled a summary of the complete list of the currently recognized amphibian species of Ethiopia from the IUCN online database (IUCN, 2010) (Table 1); some major nomenclatural changes are applied from Frost *et al.* (2006) and Wilkinson *et al.* (2011). This review paper currently recognizes 64 amphibian species recorded as occurring in Ethiopia, belonging mostly to the Order Anura, with a single species representing Caecilians (Apoda), and no representative of salamanders (Caudata).

B) The amphibian families of Ethiopia

Following large-scale revision of the taxonomy of amphibians of the world (Frost *et al.*, 2006; Frost and AMNH, 2010) and other specific updates (e.g., Channing *et al.*, 2002; Zimkus, 2008; Wilkinson *et al.*, 2011), it would be appropriate to summarize the current taxonomic status of Ethiopian amphibians. After the publication of the Catalogue of Amphibians of Ethiopia in 2001 (Largen, 2001a), several changes have been made on the placement of families, genera, species, and on nomenclature. Five of the 24

genera, and 26 of the 64 species are endemic to Ethiopia. Seven of the genera (with their respective families) are monotypic (Table 2). Brief updates on the known families of Ethiopian amphibians are presented below.

1) Indotyphlidae Lescure, Renous & Gasc 1986

Indotyphlidae are diagnosed as caecilians with imperforate stapes, inner mandibular teeth, some teeth bicusped, eye at the border of the squamosal and maxillopalatines, and either viviparity with neither scales nor secondary annuli, or oviparity (Wilkinson *et al.*, 2011). Only a single species, *Sylvacaecilia grandisonae*, represents the legless amphibians (Apoda/Gymnophiona) in Ethiopia. Based on morphology, this species has previously been grouped under the Family Caeciliidae, until revised morphological study (similarity with Indo-Seychellean species) grouped it under Indotyphlidae (Wilkinson *et al.*, 2011). The molecular phylogeny of *Sylvacaecilia* remains unknown. Specimens were recorded only from a few localities in the southwestern part of the country. There are no encounters of this species during scientific explorations made in its known range in the past three decades. Consequently, there are no molecular data or detailed information on the biology and population status of the species.

2) Arthroleptidae Mivart 1869

Arthroleptids are small frogs exhibiting forked omosterna that, with the exception of *Arthroleptis* (a non-Ethiopian genus), have a typically biphasic life history. Many of the arthroleptids, including *Leptopelis*, have vertical pupils. Molecular data are very helpful to recognize Arthroleptids as a group (Frost *et al.*, 2006). This family of tree frogs is represented in Ethiopia by a single genus (*Leptopelis*) containing six known species. Previously *Leptopelis* was placed under Family Hyperolidae. Five of the Ethiopian species of *Leptopelis* are endemic to the Ethiopian Highlands. One of these highland treefrogs (*L. gramineus*) is a fossorial (ground-dwelling) species. The only non-endemic species (*L. bocagei*) has been recorded from lowland areas in the western and southern parts of the country. One species (*L. susanae*) is considered to be Endangered.

3) Brevicipitidae Bonaparte 1850

Brevicipitids lack ossified sphenethmoids, have extremely short head and exhibit direct development (Parker, 1934). This family is represented in Ethiopia by a single endemic species (*Balebreviceps hillmani*). This species is found in the Bale Mountains National Park at very high altitudes,

inhabiting moist surfaces under mosses growing on the stems and branches of ericaceous forest. The population size of this species is very low, and hence the species is recognized as Endangered.

4) Bufonidae Gray 1825

This family is one of the complex and problematic groups to diagnose based on adult morphology. Larval morphology (such as diastema in larval lower lip paillation, rudimentary or absent larval lungs; Haas, 2003) and molecular data (Frost *et al.*, 2006) were recently used to understand the systematics of Bufonidae. This family is represented in Ethiopia by four genera and thirteen species. Most species in this family were previously grouped under the paraphyletic Genus *Bufo* (Frost *et al.*, 2006). Currently, only *Bufo dodsoni* is kept in its previous placement; nine Ethiopian species of *Bufo* are placed under the Genus *Amietophrynus*, and one species under *Poyntonophrynus*. *Altiphrynoides osgoodi* was previously placed under the Genus *Spinophrynoides*. There is one Endangered species (*Altiphrynoides malcolmi*) in this family, restricted only to high altitudes in the Bale Mountains. *Amietophrynus langanoensis* and both species of *Altiphrynoides* are endemic to Ethiopia. Bufonidae in Ethiopia occupy various habitats and are widespread in many parts of the country.

5) Dicroglossidae Anderson 1871

No morphological synapomorphies optimize to this branch, but molecular similarity is decisive (Frost *et al.*, 2006). There is only one non-endemic species, *Hoplobatrachus occipitalis*, of Least Concern status recorded in Ethiopia. This species was previously placed under the Genus *Euphlyctis*. It is distributed in moist savannas in western Ethiopia and at the shore of Lake Abaya in the Rift Valley. Earlier, the Genus *Hoplobatrachus* was grouped under Family Ranidae until its recent placement under Dicroglossidae.

6) Hemisotidae Cope 1867

Besides synapomorphies in larval features, members of this family lack vomers, middle ear, and ductus lacrimosus, and exhibit fusion of vertebrae 8 and 9 (De Villiers, 1931). These frogs have pointed snout to burrow into the ground (Channing, 1995). There are two Ethiopian species representing the snout-burrower frogs, Genus *Hemisus*; and both species have a Least Concern status. The geographic distribution of *Hemisus* in Ethiopia is restricted to the western plateau.

Table 1. List of amphibian species of Ethiopia with their conservation status (extracted and compiled from online records of IUCN, 2010).

Family	Scientific name	Taxonomic authority	Common name	Endemicity	IUCN Red List status
Indotyphlidae Lescure, Renous & Gasc 1986	<i>Sylvacaecilia grandisonae</i>	(Taylor 1970)	Aleku Caecilian	Endemic	Data Deficient
Arthroleptidae Mivart 1869	<i>Leptopelis bocagii</i>	(Günther 1865)		Non-endemic	Least Concern
	<i>Leptopelis gramineus</i>	(Boulenger 1898)	Badditu Forest Treefrog	Endemic	Least Concern
	<i>Leptopelis ragazzii</i>	(Boulenger 1896)	Shoa Forest Tree Frog	Endemic	Vulnerable
	<i>Leptopelis susanae</i>	Largen 1977	Susana's Forest Tree Frog	Endemic	Endangered
	<i>Leptopelis vannutellii</i>	(Boulenger 1898)	Dimme Forest Tree Frog	Endemic	Vulnerable
	<i>Leptopelis yaldeni</i>	Largen 1977	Grassland Forest Tree Frog	Endemic	Near Threatened
Brevicipitidae Bonaparte 1850	<i>Balebreviceps hillmani</i>	Largen & Drewes 1989	Bale Mountains Tree Frog	Endemic	Endangered
Bufonidae Gray 1825	<i>Altiphrynoides malcolmi</i>	(Grandison 1978)	Malcolm's Ethiopian Toad	Endemic	Endangered
	<i>Altiphrynoides osgoodi</i>	(Loveridge 1932)	Osgood's Ethiopian Toad	Endemic	Vulnerable
	<i>Amietophrynus asmarae</i>	(Tandy <i>et al.</i> 1982)	Asmara Toad	Non-endemic	Least Concern
	<i>Amietophrynus blanfordii</i>	(Boulenger 1882)	Blanford's Toad	Non-endemic	Least Concern
	<i>Amietophrynus garmani</i>	(Meek 1897)	Garman's Toad	Non-endemic	Least Concern
	<i>Amietophrynus kerinyagae</i>	(Keith 1968)	Keith's Toad	Non-endemic	Least Concern
	<i>Amietophrynus langanoensis</i>	(Largen <i>et al.</i> 1978)	Lake Langano Toad	Endemic	Data Deficient
	<i>Amietophrynus maculatus</i>	(Hallowell 1855)	Lesser Square-marked Toad	Non-endemic	Least Concern
	<i>Amietophrynus regularis</i>	(Reuss 1833)	African Common Toad	Non-endemic	Least Concern
	<i>Amietophrynus steindachneri</i>	(Pfeffer 1893)	Steindachner's Toad	Non-endemic	Least Concern
	<i>Amietophrynus xeros</i>	(Tandy <i>et al.</i> 1976)	Subdesert Toad	Non-endemic	Least Concern
	<i>Bufo dodsoni</i>	Boulenger 1895	Dodson's Toad	Non-endemic	Least Concern

Family	Scientific name	Taxonomic authority	Common name	Endemicity	IUCN Red List status
	<i>Poyntonophrynus lughensis</i>	(Loveridge 1932)	Somali Toad	Non-endemic	Least Concern
Dicroglossidae Anderson 1871	<i>Hoplobatrachus occipitalis</i>	(Günther 1858)	Crowned Bullfrog	Non-endemic	Least Concern
Hemisotidae Cope 1867	<i>Hemisis marmoratus</i>	(Peters 1854)	Marbled Snout-burrower	Non-endemic	Least Concern
	<i>Hemisis microscaphus</i>	Laurent 1972	Ethiopian Snout-burrower	Endemic	Least Concern
Hyperoliidae Laurent 1943	<i>Afrixalus clarkei</i>	Largen 1974	Clark's Banana Frog	Endemic	Vulnerable
	<i>Afrixalus enseticola</i>	Largen 1974	Ethiopian Banana Frog	Endemic	Vulnerable
	<i>Afrixalus quadrivittatus</i>	(Werner 1907)		Non-endemic	Least Concern
	<i>Hyperolius acuticeps</i>	Ahl 1931		Non-endemic	Least Concern
	<i>Hyperolius balfouri</i>	(Werner 1908)		Non-endemic	Least Concern
	<i>Hyperolius kivuensis</i>	Ahl 1931	Kivu Reed Frog	Non-endemic	Least Concern
	<i>Hyperolius viridiflavus</i>	(Duméril & Bibron 1841)	Common Reed Frog	Non-endemic	Least Concern
	<i>Kassina maculifer</i>	(Ahl 1924)	Spotted Kassina	Non-endemic	Least Concern
	<i>Kassina senegalensis</i>	(Duméril & Bibron 1841)	Senegal Kassina	Non-endemic	Least Concern
	<i>Kassina somalica</i>	Scortecci 1932		Non-endemic	Least Concern
	<i>Paracassina kounhiensis</i>	(Mocquard 1905)	Kouni Valley Striped Frog	Endemic	Least Concern
	<i>Paracassina obscura</i>	(Boulenger 1894)	Ethiopian Striped Frog	Endemic	Least Concern
	Microhylidae Günther 1858	<i>Phrynomantis somalicus</i>	(Scortecci 1941)	Somali Rubber Frog	Non-endemic
Petropedetidae Noble 1931	<i>Conraua beccarii</i>	(Boulenger 1911)	Beccari's Giant Frog	Non-endemic	Least Concern
Phrynobatrachidae Laurent 1941	<i>Phrynobatrachus bullans</i>	Crutsinger <i>et al.</i> 2004		Non-endemic	Least Concern
	<i>Phrynobatrachus inexpectatus</i>	Largen 2001	Bore River Frog	Endemic	Data Deficient
	<i>Phrynobatrachus minutus</i>	(Boulenger 1895)	Ethiopian Dwarf Puddle Frogs	Endemic	Least Concern

Family	Scientific name	Taxonomic authority	Common name	Endemicity	IUCN Red List status
	<i>Phrynobatrachus natalensis</i>	(Smith 1849)	Natal Dwarf Puddle Frog	Non-endemic	Least Concern
Pipidae Gray 1825	<i>Xenopus clivii</i>	Peracca 1898	Peracca's Clawed Frog	Non-endemic	Least Concern
	<i>Xenopus largeni</i>	Tinsley 1995	Largen's Clawed Frog	Endemic	Data Deficient
	<i>Hildebrandtia macrotympanum</i>	(Boulenger 1912)	Plain Burrowing Frog	Non-endemic	Least Concern
	<i>Ptychadena anchietae</i>	(Bocage 1868)	Anchieta's Ridged Frog	Non-endemic	Least Concern
	<i>Ptychadena cooperi</i>	(Parker 1930)	Cooper's Grassland Frog	Endemic	Least Concern
	<i>Ptychadena erlangeri</i>	(Ahl 1924)	Erlanger's Grassland Frog	Endemic	Near Threatened
	<i>Ptychadena filwoha</i>	Largen 1997	Filwoha Grassland Frog	Endemic	Data Deficient
	<i>Ptychadena harensa</i>	Largen 1997	Bale Grassland Frog	Endemic	Data Deficient
Ptychadenidae Dubois 1987	<i>Ptychadena mascareniensis</i>	(Duméril & Bibron 1841)	Mascarene Grassland Frog	Non-endemic	Least Concern
	<i>Ptychadena nana</i>	(Perret 1980)	Arussi Grassland Frog	Endemic	Data Deficient
	<i>Ptychadena neumanni</i>	(Ahl 1924)	Neumann's Grassland Frog	Endemic	Least Concern
	<i>Ptychadena porosissima</i>	(Steindachner 1867)		Non-endemic	Least Concern
	<i>Ptychadena pumilio</i>	(Boulenger 1920)		Non-endemic	Least Concern
	<i>Ptychadena schillukorum</i>	(Werner 1907)	Sudan Grassland Frog	Non-endemic	Least Concern
	<i>Ptychadena tellinii</i>	(Peracca 1904)	Central Grassland Frog	Non-endemic	Least Concern
	<i>Ptychadena wadei</i>	Largen 2000	Tisisat Grassland Frog	Endemic	Data Deficient

Family	Scientific name	Taxonomic authority	Common name	Endemicity	IUCN Red List status
	<i>Amietia angolensis</i>	(Bocage 1866)	Angola River Frog	Non-endemic	Least Concern
Pyxicephalidae Bonaparte 1850	<i>Cacosternum boettgeri</i>	(Boulenger 1882)	Boettger's Dainty Frog	Non-endemic	Least Concern
	<i>Ericabatrachus baleensis</i>	Largen 1991	Bale Mountains Frog	Endemic	Endangered
	<i>Tomopterna cryptotis</i>	(Boulenger 1907)	Common Sand Frog	Non-endemic	Least Concern
Ranidae Rafinesque 1814	<i>Hylarana galamensis</i>	(Duméril & Bibron 1841)	Senegal Golden-backed Frog	Non-endemic	Least Concern
Rhacophoridae Hoffman 1932	<i>Chiromantis kelleri</i>	(Boettger 1893)	Keller's Foam-nest Frog	Non-endemic	Least Concern

Note on Table 1: The earlier *Hyperolius nasutus* is currently recognized as *Hyperolius acuticeps* (Channing *et al.*, 2002). *Phrynobatrachus bullans* Crutsinger *et al.* 2004 was not included in Largen (2001a), but has been recently recorded in Ethiopia by Zimkus (2008), but not yet included in the IUCN records. After Frost and AMNH (2010), the following changes have been made on the taxonomic placement of some Ethiopian genera: *Bufo* to *Amietophrynus* or to *Poyntonophrynus* (except for *Bufo dodsoni*); *Spinophrynoidea* to *Altiphrynoidea*; *Euphlyctis* to *Hoplobatrachus*; *Rana/Afrana* to *Amietia*; and *Afrixalus clarkeorum* to *Afrixalus clarkei*. There are eight species listed in Frost and AMNH (2010) but for which information on their distribution in Ethiopia is not given either by Frost and AMNH or by IUCN. These are: *Xenopus muelleri*, *Bufo pentoni*, *Hildebrandtia ornate*, *Ptychadena oxyrhynchus*, *Leptopelis kivuensis*, *Hyperolius poweri*, *Pyxicephalus edulis*, *Lanzarana largeni*. To avoid confusion, we have omitted these species from our revised list until further updates confirm their occurrence in Ethiopia.

Table 2. Summary of the number of families, genera and species of Ethiopian amphibians (number of endemics in parentheses).

Order	Family	Number of Genera	Number of Species	
Gymnophiona	Indotyphlidae Lescure, Renous & Gasc 1986	1(1)	1(1)	
	Arthroleptidae Mivart 1869	1	6(5)	
	Brevicipitidae Bonaparte 1850	1(1)	1(1)	
	Bufoidea Gray 1825	4(1)	13(3)	
	Dicroglossidae Anderson 1871	1	1	
	Hemisotidae Cope 1867	1	2(1)	
	Hyperoliidae Laurent 1943	4(1)	12(4)	
	Anura	Microhylidae Günther 1858	1	1
		Petropedetidae Noble 1931	1	1
		Phrynobatrachidae Laurent 1941	1	4(2)
		Pipidae Gray 1825	1	2(1)
		Ptychadenidae Dubois 1987	1	14(7)
		Pyxicephalidae Bonaparte 1850	4(1)	4(1)
		Ranidae Rafinesque 1814	1	1
Rhacophoridae Hoffman 1932		1	1	
Total number	15 families	24 (5)	64 (26)	

7) Hyperoliidae Laurent 1943

Hyperoliids are unique among frogs in having a distinctive gular gland (Drewes, 1984). Four genera containing twelve species represent the hyperoliids in Ethiopia. Hyperoliid frogs are distributed in many parts of the country, although each genus may have its own restricted range. Species belonging to *Afrivalus* and *Hyperolius* are predominantly arboreal, having extended discs on the tips of their toes. The genera *Kassina* and *Paracassina* are ground-dwelling. *Hyperolius nasutus* Günther 1865 has been included in the list of Ethiopian amphibians by Largen (2001a), but excluded from the list by Frost and AMNH (2010) and IUCN (2010); it is recently identified as *Hyperolius acuticeps* (Channing *et al.*, 2002). Two Ethiopian species belonging to the genus *Afrivalus* are considered to have Vulnerable status.

8) Microhylidae Günther 1858 (1843)

This family is represented in Ethiopia by a single, non-endemic and Least Concern species, *Phrynomantis somalicus*. Based on differences in the morphology and breeding behaviour between *Phrynomantis* and *Phrynomerus*, Largen and Spawls (2010) suggest the generic assignment of the Ethiopian species to be under *Phrynomerus*. However, *Phrynomantis* is the valid generic name in IUCN (2006) and Frost and AMNH (2010). *Phrynomantis* typically has aquatic, exotrophic microhylid larvae (Altig and

McDiarmid, 1999). The single juvenile Ethiopian specimen was collected from Negelle area close to Genale River.

9) Petropedetidae Noble 1931

No morphological synapomorphies are evident in this group, although the molecular data are decisive (Frost *et al.*, 2006). *Conraua beccarii* is the only species representing Petropedetidae in Ethiopia. This species has earlier been placed under Family Ranidae. It is a non-endemic, Least Concern species distributed in Ethiopia at lower and mid-altitudes to the northwest of the Great Rift Valley.

10) Phrynobatrachidae Laurent 1941

Phrynobatrachids are small terrestrial and semi-aquatic frogs with poorly understood species boundaries, a typically biphasic life history and eggs laid in water. They lack digital discs, usually retain an outer metatarsal tubercle (Laurent, 1986) and have a distinctive tarsal tubercle (Channing, 2001). Molecular characterization is decisive in recognizing members of this family. Although Frost *et al.* (2006) includes *Ericabatrachus* in this family (see note below), we maintained its placement under Pyxicephalidae as used in IUCN (2010). There are four species of *Phrynobatrachus* in Ethiopia, two being endemic, and none are categorized as threatened. The Genus *Phrynobatrachus* was formerly placed under Ranidae. One of the four species, *P. bullans*, has been included in the Ethiopian records list only recently (Zimkus, 2008). The Ethiopian distribution of *Phrynobatrachus* is widespread from lowlands to highlands excepting the eastern lowlands.

11) Pipidae Gray 1825

Pipids are highly aquatic frogs that have inguinal amplexus and that vocalize using the hyoid apparatus to make clicks (Rabb, 1960). Two species of clawed frogs, *Xenopus*, are found in Ethiopia, distributed on both sides of the Rift Valley. One of them is endemic, and none of them are listed as threatened. Molecular data indicate co-distribution of the two species in the highlands on both sides of the Great Rift Valley (Evans *et al.*, 2011).

12) Ptychadenidae Dubois 1987

The synapomorphies of the exemplar group, *Ptychadena*, include absence of or rudimentary otic plate, absence of (neo)palatines and other osteological (Clarke, 1981) and larval (Haas, 2003) morphological features. This is one of the complex group of anurans in terms of morphological taxonomy and systematics. Two Ethiopian genera of frogs, *Hildebrandtia* (Burrowing

Frog) and *Ptychadena* (Grassland Frog) are included in this family. These genera were previously placed under Family Ranidae. The recorded distribution of *H. macrotympanum* in Ethiopia is at the southern border of the country in arid lowland areas; this is a non-endemic and Least Concern species. The Genus *Ptychadena* is a diverse group of solely African frogs. In Ethiopia, there are thirteen known species of *Ptychadena*, widely distributed in the lowlands and/or highlands. None of the Ethiopian species of *Ptychadena* were classified as threatened (IUCN, 2010). According to Largen (2001a), five strictly highland species and two lowland species are endemic to Ethiopia.

13) Pyxicephalidae Bonaparte 1850

The grouping in this taxon is morphologically heterogenous, but geographically coherent and molecular evidence for monophyly is strong (Frost *et al.*, 2006). Four genera are included in the Ethiopian Pyxicephalidae: *Amietia*, *Cacosternum*, *Ericabatrachus* and *Tomopterna*. Each of these genera contains only one species in Ethiopia. *Ericabatrachus baleensis* is the only endemic species in this group, and is enlisted as Endangered. Frost *et al.* (2006) suggest inclusion of the Genus *Ericabatrachus* in the Family Phrynobatrachidae based on its “*Phrynobatrachus*-like” appearance. However, *Ericabatrachus* was not included in the molecular investigation in Frost *et al.* (2006); therefore, we maintained its placement under Pyxicephalidae as used in IUCN (2010). *Amietia angolensis* was previously placed under Genus *Rana/Afrana*, until its revision in Frost *et al.* (2006). Largen and Spawls (2010), however, retained the old nomenclature. With the exception of *E. baleensis* that is restricted to high altitudes in the Bale Mountains, the other three species of Ethiopian Pyxicephalidae are widespread from lowlands to higher altitudes.

14) Ranidae Rafinesque 1814

Molecular data are decisive in recognizing this branch, as morphological characterization has been historically complex (Frost *et al.*, 2006). Previously, Ranidae used to be the most diverse family of Ethiopian amphibians, containing 25 species grouped under eleven genera (Largen and Spawls, 2010). Most of these species are now taken out of Ranidae and placed into various other families. Currently, only one species, *Hylarana galamensis* (earlier known under the Genus *Amnirana*), represents Ranidae in Ethiopia. *Hylarana* has been a historically ambiguously diagnosed genus, and was difficult to characterize morphologically (Frost *et al.*, 2006). *H. galamensis* has been recorded in Ethiopia from the west and southwest

lowlands, and is a non-endemic, Least Concern species.

15) Rhacophoridae Hoffman 1932

Although a few groups are primarily terrestrial, rhacophorids are predominantly tree frogs, sharing with basal ranids expanded digital pads and with mantellids the characteristic of intercalary phalangeal elements. There are also some larval features that may be synapomorphies to this family. *Chiromantis kelleri* is the only representative of Rhacophoridae in Ethiopia. Its known geographic range in Ethiopia is in the lowland savannah and semi-desert habitats in the southern and eastern parts of the country. This arboreal frog is non-endemic and is not under threatened status.

C) The Ethiopian Highlands and their biogeographic relevance

The Ethiopian Highlands are clusters of highly fractured chains of mountains surrounded by vast areas of lowlands, and are part of the Eastern Afromontane Biodiversity Hotspot in Eastern Africa (Poynton, 1999; Myers *et al.*, 2000; CI and McGinley, 2009), and constitute 50% of the land above 2,000 m (Yilma Seleshi and Demarée, 1995) and about 80% of the land above 3,000 m (Yalden, 1983; WWF, 2010) in Africa. There are several peaks in excess of 4,000 m, the highest being Ras Dejen at 4,550 m. The Great East African Rift Valley splits these highlands into the northwestern and the southeastern parts, which are further split by several major drainage systems (Fig. 1). The highlands are characterized by vast plateaus, deep gorges, steep escarpments, highland lakes, Afroalpine moorlands, Afromontane forests, riverine forests, and highland grasslands. Many of these areas have been sites of ancient civilizations that were accompanied by age-old farming practices, which have resulted in severe erosion of the landscapes (Girma Tadesse, 2001). The fractures in the landscapes have through time created diverse micro- and macro-habitats for a variety of fauna and flora. Extremes such as isolated peaks of high mountains have become homes for many globally unique endemics, such as the Ethiopian Wolf and Mountain Nyala.

The Ethiopian Highlands are climatically important in trapping moist air that mainly comes from the Indian Ocean, and providing precipitation to the country. Average annual rainfall varies between 600 mm per year in Tigray (the north) and more than 2,000 mm per year in the southwestern highlands (Krauer, 1988). The rainfall in this area has, however, been subject to historical variability, facing declines during the second half of the 20th century (Yilma Seleshi and Demarée, 1995; Osman and Sauerborn, 2002).

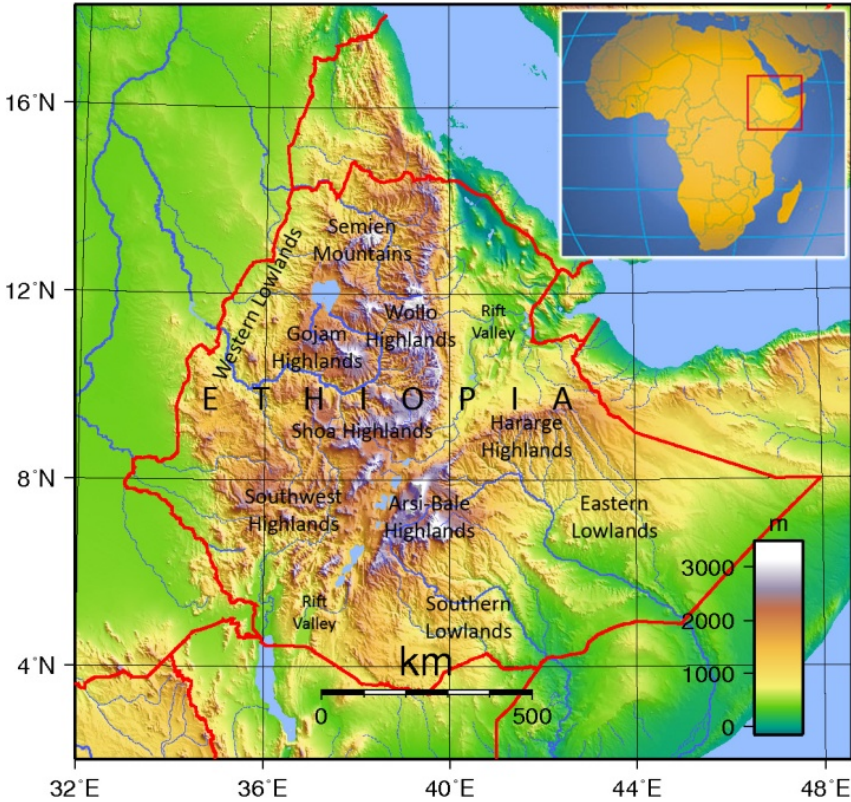


Fig. 1. Topographic map of the Ethiopian Highlands and major lowlands including the Great East African Rift Valley, and fractured mountain ranges (Modified from source: Sadalmelik, I. (2007); downloaded from http://en.wikipedia.org/wiki/File:Ethiopia_Topography.png).

Demographically, the highlands harbour over 80% of Ethiopia's human population, engaged in agriculture and urban activities. In general, the national population is increasing at a high rate – about ten fold in the past 60 years (CI and McGinley, 2009). Pressure from increased human population usually leads to expansion of settlements to uninhabited natural areas, thereby disturbing wildlife and natural habitats. For instance, a study made in the Belete-Gera Forest in the southwestern forests showed that coffee plantations and encroachment negatively influenced up to 49% of the accessible natural forest (Cheng *et al.*, 1998). Historically, the northern and central parts of the Ethiopian Highlands were subject to ancient civilization and agricultural practices for thousands of years, negatively impacting the vegetation cover and faunal diversity of the area (Reader, 2009); in recent times, despite varying figures, many reports agree on the very high level of deforestation that took place in the highlands in the past few decades

(EFAP, 1994; Reusing, 2000).

The highlands of Ethiopia are the main repositories of moist forests and wetlands in Ethiopia, which are known to be home for, among others, a diverse and unique amphibian fauna. In general, mountain ranges in tropical regions are seen to be important because they harbour much diversity at species, lineage and allelic levels (Hewitt, 2004), with the reasons for this being unclear. Because of the nature of their delicate skin that is used for respiration, most amphibians dwell in moist habitats, such as swamps/bogs, streams, lake shores, and moist forests (Pough *et al.*, 2003). Drying or decreased moisture of such habitats brings in fragmentation into micro-habitats and local disappearance of populations of amphibians. At its extreme, this could lead to extinction of species that have narrow geographic distribution and specialized resource requirements (IBC, 2005).

So far, about 32.5% of the roughly 6,000 known amphibian species in the world are reported to be globally threatened (Stuart *et al.*, 2004). Habitat destruction, together with climate change, is accounted as being the main reason for the decline. In addition, chytridiomycosis, a pathogenic disease caused by the fungus *Batrachochytrium dendrobatidis*, is another emerging cause for the global decline of amphibian populations and extinctions (Rödder *et al.*, 2009; Lötters *et al.*, 2010). A recent study on the prevalence of this disease in Kenya has confirmed its widespread occurrence in various habitats – but more pronounced in areas where the temperature is relatively lower (Kielgast *et al.*, 2010). This work concludes that detailed study of the nature and distribution of the fungus is necessary in different parts of Africa, especially in the high altitudes. As inferred from predictive models that combine ecological niches, climate envelope and known occurrences of the fungus, the Ethiopian Highlands are expected to be one of the potentially most suitable sites to harbour *B. dendrobatidis*, implying that amphibians in this area could be highly susceptible for the disease (Rödder *et al.*, 2009; Lötters *et al.*, 2010). A preliminary study on the prevalence of the fungus in amphibians of the Ethiopian Highlands indicated that about one-half of the studied specimens tested positive for the fungus (Gower *et al.*, 2012). The correspondence of high level of diversity in mountain areas and high chytrid presence makes concerns for Ethiopian fauna a high priority.

Mountain ranges remain ideal places for the survival of lineages through climatic changes, and hence for genome divergence (Hewitt, 2004). The Ethiopian Montane has the highest rank of percentage of endemic genera and species of amphibians within biogeographic provinces of the

Intertropical Montane region in Africa (Poynton, 1999). It is also understandable that many undiscovered species of amphibians still remain in species-rich tropical countries (Köhler *et al.*, 2005). Therefore, the Ethiopian Highlands require high priority research on amphibian systematics for focused conservation. Timely action is needed when one considers the value of amphibians as indicators of habitat change, and the current scale of human interference in these habitats.

D) The Ethiopian Lowlands

The Ethiopian lowlands are relatively warm regions having altitudes as low as -115 m (below sea level). Contrasting to the uplifted Ethiopian plateau, the underlying lowlands form part of the Horn of Africa Biodiversity Hotspot (CI and McGinley, 2007). The lowlands are contained in one of three broader types of areas: the Rift Valley, border lowlands, or major river gorges. The Rift Valley runs through the Ethiopian Highlands from northeast (wider and deeper part) to southwest (narrower and elevated part). The northeastern part in Ethiopia is a terrestrial dead end blocked by the Red Sea, whereas the southwestern end connects with lowland areas in western and southern Ethiopia, northwestern Kenya and South Sudan. The border lowlands surround Ethiopia in the west, south and east connecting it with Sudan, South Sudan, Kenya, Somalia and Djibouti. Within the major river basins and sub-basins of Ethiopia run several river gorges slicing the mountain ranges and connecting the highlands with the Rift Valley and the border lowlands. For instance, the Abay (Blue Nile) Gorge starts at Tisisat Falls some 30 km south of Lake Tana and runs close to the northwestern border with Sudan, having at some places a depth of about 1.6 km. Omo, Gibe, and Awash are some of the other major rivers that have deep gorges.

The lowlands of Ethiopia are generally characterized by warmer and drier climate that is continuous with other parts of eastern, southern, central and northern Africa. Many of the species of amphibians occurring in these areas therefore appear to have wide and continuous distribution. Although these habitats are suitable for some species of amphibians, they are not renowned as places for high endemism as seen in the highlands.

II) MAJOR GAPS IN AMPHIBIAN RESEARCH AND CONSERVATION IN ETHIOPIA

Several factors contribute to the low level of understanding and concern on Ethiopian amphibians. These can be broadly summarized as: 1) the bad reputation that many local people had towards amphibians and reptiles (based on our field observation in the past two decades), and 2) so far no

known commercial importance of these animals in Ethiopia (e.g., no report of export of amphibians from Ethiopia; Earth Trends, 2003). Accordingly, until recently, little coverage has been given to the study of amphibians by higher education and research institutions and conservation organizations in the country. The following discussion elaborates the abovementioned points based on our observation on the local practices of various communities and education/research institutions.

Culture, belief, and economic value: To our knowledge, amphibians are not used as sources of food for humans in Ethiopia, in particular in the highlands where most of the population lives. Age-old and conserved culture and beliefs as well as availability of easily-accessed alternative sources of protein (such as beef, lamb, fish, cereals and other crops) have made amphibians to be excluded from the dishes. Ornamental uses or aquaculture practices also are not known. There are, however, some uses of amphibians (e.g., toads) for traditional medicine by local practitioners. Otherwise, it seems there is no clearly reported economic benefit gained from amphibians in Ethiopia. In general, Ethiopian amphibians are the least focused of all other major groups of vertebrates in terms of economic and social contribution.

Education and research: The existing effort of zoologists who specialized on non-amphibian vertebrate groups or other biological fields (mammalogists, ornithologists, ichthyologists, geneticists, museum curators) to train students on amphibian biology is highly appreciated. Although there is basic knowledge on the biology of amphibians in general among students at different levels of education in Ethiopian schools and universities, there is very limited, or no understanding on the taxonomy and conservation status of the country's amphibians. The curricula seem to give less coverage to Ethiopian amphibians than the big-sized, more visible and reputed wildlife such as mammals, birds, and fish. Absence of local professional herpetologists specifically skilled as amphibian biologists appears to be a factor for this gap of knowledge. Recently, the Centre for Environmental Science of the School of Graduate Studies of AAU had one of the co-authors of this paper (Samy Saber) employed for a few years as the only herpetologist. The ZNHM of this university has some collections of amphibians that are taken care of by volunteer non-amphibian zoologists and a curator. The ZNHM and vertebrate laboratory of the Department of Zoological Sciences (AAU) appear to be the only educational and research places where Ethiopian amphibians are given some space and attention; however, courses on amphibian biology (or herpetology) are not given to

students as compulsory requirements. Therefore, the current little coverage of amphibian biology in Ethiopia mainly sees the field as small component of trainings given in broader fields such as Biology and Zoology.

As is the case in the formal education system of Ethiopia mentioned above, professional associations (such as the BSE and the EWNHS) give limited attention to amphibians. If there may be an issue to deal with research or publication on Ethiopian amphibians, professional support would be possible mainly from foreign experts. Existing local and foreign professional associations have not established links between professionals, the general public and several government organizations in the transfer and exchange of ideas and knowledge on amphibians and their relevance for development and conservation in Ethiopia. We do not see any public awareness programs on the values of amphibians through mass media, posters, publications in local journals and other means of communication that can serve as informal means of educating the broader non-scientific community.

Conservation of amphibians and their habitats: The main factors that might prevent effective conservation of threatened amphibians in Ethiopia are lack of knowledge on their biology (Largen, 2001a), and ever-increasing habitat degradation - mainly deforestation (EFAP, 1994; Yilma Seleshi and Demarée, 1995; Tadesse Woldemariam Gole *et al.*, 2002) (Fig. 2). The remaining refugia for some of the threatened highland species of amphibians are confined to small areas (some protected) and fragments of highland forests (example, Bale Mountains, Southwest forests) (Largen, 2001a; Weinsheimer *et al.*, 2010). EWCA, as a national overseer of wildlife protection, is playing a direct role in conservation of amphibians through recognizing the value of these fauna, promoting research, and indirectly through protecting areas that are mainly reputed for their mammal and bird species. Some other local organizations such as IBC, EWNRA, EPA, and FfE are providing direct or indirect contribution towards conserving amphibians and their habitats. These organizations try to protect, among others, suitable amphibian habitats such as forests, grasslands, wetlands and aquatic habitats; but this is not directly intended to protect the amphibians there. However, the effort of all governmental and non-governmental institutions is constrained by the absence of skilled amphibian biologists in the country and inadequate baseline data. Historical aspects of some of the problems in research and conservation have been pointed out in the preface of this article.



Fig. 2. Protected swampy grassland and forest amphibian habitat in the Bale Mountains (top), and habitat degradation in the southwest highlands (bottom) in Ethiopia.

III) ONGOING RESEARCH, FUTURE NEEDS AND METHODS

A) Recent activities

There are some recently communicated works on Ethiopian amphibians focused on systematics and distribution (Zimkus, 2008; Zimkus and Blackburn, 2008; Zimkus *et al.*, 2010; Zimkus and Schick, 2010; Evans *et al.*, 2011; Abebe Ameha Mengistu, 2012), distribution modelling (Weinsheimer *et al.*, 2010), as well as conservation of threatened endemics (Abebe Ameha Mengistu, 2012; Gower *et al.*, 2013) and prevalence of chytridiomycosis (Gower *et al.*, 2012). Graduate students from AAU

(Ethiopia) and the UB (Switzerland) have addressed research topics focused on preliminary studies on the genetics, occurrence, distribution and conservation of some species of Ethiopian amphibians (Andualem Tsige Missale, 2008; Roman Kassahun, 2009; Schwaller, 2009; Wendwesen Tito, 2009).

For a more complete knowledge on Ethiopian amphibians and to recommend more reliable conservation options, we need to understand not only the taxonomic status and distribution of each species, but also their life history, including among others, habitat, behaviour (feeding, breeding, etc) and interactions with parasites. This in turn requires a multi-disciplinary approach and concerted effort of all stakeholders. In this line, as a higher learning and research institution, the Section of Biogeography (UB), in collaboration with the College of Natural Sciences (AAU) and EWCA, has conducted biogeographic studies on Ethiopian amphibians. This has helped to improve the skills of local scientists, to build institutional capacity, and to recommend prioritized conservation options. One part of this collaborative project dealt with the diversity, geographic distribution and conservation of some amphibians in the Ethiopian Highlands.

B) Future needs

Future research, development and protection of amphibians in Ethiopia require a systematic approach where more urgent needs and appropriate methods are identified and prioritized, and research resources utilized efficiently. Some geographic areas and species are less surveyed than others; most research topics other than taxonomic issues have not been well addressed for most species. Even for taxonomic assessment, most of the earlier taxonomic methods relied on morphology only with some reference to ecology (Largen, 2001a). All of the existing professional herpetologists and laboratories working on Ethiopian amphibians are based in Europe and North America; and natural history museums in these two continents hold all of the holotypes of the known species of Ethiopian amphibians. These kinds of institutional and professional capacities need to be established in Ethiopia to enable more feasible research locally (Largen, 2001a), and for a better implementation of practical conservation actions.

Upcoming project activities should focus on prioritized research problems that could be based on taxa, geographic areas, or habitats. The methodologies to be used should incorporate a combination of classical and modern techniques. As the basis for all other studies on amphibian biology, we will discuss the suitable taxonomic research needs and modern

approaches required to achieve a better understanding of amphibians in this region.

Taxa and geographic areas: Taxonomic units with diverse and complex, endemic, threatened, or data deficient populations should take priority over others for taxonomic assessment. Taxonomic groups with diverse species and populations contain a lot of genetic information pertaining to the evolutionary history of the group; endemic, threatened or data deficient taxa also are the only representatives of their kind to preserve their respective evolutionary histories. Most of the 26 known endemic amphibians of Ethiopia are found in the highlands, which are surrounded by vast lowlands linked with other parts of eastern and central Africa. In an effort to re-assess the taxonomy of some of these taxa, there are ongoing studies on various families and genera. For instance, the taxonomic status of species within the Genus *Phrynobatrachus* in Ethiopia has been assessed by Largen (2001b) and is being revised at a continental level for Africa (Zimkus, 2008; Zimkus and Blackburn, 2008; Zimkus *et al.*, 2010; Zimkus and Schick, 2010). These taxonomic assessments were achieved using molecular techniques, in conjunction with traditional morphological approaches. The biogeography of *Phrynobatrachus* was also considered (e.g., Zimkus *et al.*, 2010). The most intensive investigation of the phylogeography and conservation status of *Leptopelis* and *Ptychadena* in the Ethiopian Highlands and the Rift Valley has been done very recently (Abebe Ameha Mengistu, 2012). Further assessment of other highland groups such as many members of the families Bufonidae (*Altiphrynoides*), Brevicipitidae (*Balebreviceps*), Pyxicephalidae (*Ericabatrachus*), and Hyperolidae (*Kassina*, *Paracassina*, *Afrixalus*, *Hyperolius*) is underway as part of a broader project for the Eastern Afromontane amphibian biodiversity (Simon Loader, pers. comm.). The mysterious fossorial life of *Sylvacaecilia grandisonae* (Taylor 1970) (Indotyphlidae) and its aquatic-breeding behaviour (accounted for having a larval stage), unlike other African caecilians, has attracted attention of researchers (David Gower, pers. comm.) (Fig. 3). This Ethiopian endemic has been encountered in the wild in the southwest forests of the country a few decades ago and only on a few occasions recently.



Fig. 3. Holotype of *Sylvacaecilia grandisonae* (Gymnophiona), BM 1969.1589. The Natural History Museum, London.

The genera that are diverse and have wide geographic distribution in Ethiopia include *Amietophrynus* (Bufonidae), *Leptopelis* (Arthroleptidae), *Ptychadena* (Ptychadenidae), and members of the family Hyperoliidae. These are groups to which priority can be given based on their diversity and endemism. For instance, *Leptopelis* and *Ptychadena* (Fig. 4) together comprise 30% of all known species of Ethiopian amphibians. Species belonging to these genera have been reported to be some of the most problematic groups for taxonomy due to their complexity and similarities in morphology and geographic distribution (Largen, 1977; 1997; 2001a; Richards and Moore, 1996; Vences *et al.*, 2004). Other less-diverse families and genera may need priority because of their endemism, monotypic representations, and threatened conservation status (e.g., *Ericabatrachus*, *Balebreviceps*; Gower *et al.*, 2013).



Fig. 4. Top-left: *Leptopelis yaldeni* from Debre Markos, type locality (AM-016); top-right: ventral view of *L. ragazzii*, from Ghedo (BM 1976.971 and BM 1976.974); bottom-left: *Ptychadena wadei* from Andassa, near Tis-Abay area (AM-021); bottom-right: ventral side of *P. cooperi*, near Bore (BM 1975.1817).

Geographically, the Ethiopian Highlands are home for most of the endemic and threatened species of amphibians as contrasted with the lowlands that have widespread populations of species shared with other parts of Africa. Deforestation, habitat degradation, agriculture and urbanization are increasing and replacing natural forested, grassland and aquatic habitats. The majority of the country's human population also dwells in the highlands. Within the highlands themselves, some areas have been relatively better surveyed than others, and some highland segments have more suitable amphibian habitats than other fragments. These scenarios mean that it is necessary and timely to conduct research on amphibian species in the highland parts of Ethiopia.

The Ethiopian Highlands are split apart by the Rift Valley into northwestern and southeastern mountain ranges; this geologic event has been happening for a long period of time (since around 30 Mya; Arndt and Menzies, 2005). Further separation of mountain ranges by deep gorges to form smaller segments could imply the possibility of having vicariant taxa across mountain ranges (Cox and Moore, 2005). The Gughe Mountain, Kaffa

Highlands, and central Shoa plateau in Ethiopia can be some of the good examples for this. That is, the patchy mountain ranges might possess their own separate assemblages of species belonging to separate taxa (at least for some species) instead of the wide distribution previously proposed (for *Leptopelis ragazzii*: Largen, 1977; 2001a; Weinsheimer *et al.*, 2010; for *Ptychadena neumannii*: Largen, 1997; 2001a). A similar (but not same) case has been reported for amphibian diversity in different segments of the Eastern Arc Mountains in Tanzania (Poynton *et al.*, 2007), that are part of the Eastern Afromontane Biodiversity Hotspot, to which the Ethiopian Highlands also belong to. Therefore, assessment of any association between phylogenetic, morphological, and/or geographic groupings of species will enable to outline clear biogeographic patterns at species or population level.

C) Highlighting the methods

Implementing modern techniques such as molecular systematics of Ethiopian amphibians in local institutions would require establishment of a broader-scope laboratory for animal systematics. Until availability of facilities and funding permits, it is possible to work on taxonomy using traditional morphological and ecological data, as well as through collaboration with other overseas institutions. In this regard, phylogenetic and biogeographic investigation would benefit from data from genetic, bioacoustics, behavioural, morphological, and/or geographic/ecological studies. As a modern tool to understand the evolutionary history, relationship and distribution of organisms, the following section discusses the potential and prospects of phylogeographic studies. The flowchart in Fig. 5 shows a summary of the steps to be followed in investigation of amphibian diversity, distribution and conservation in Ethiopia.

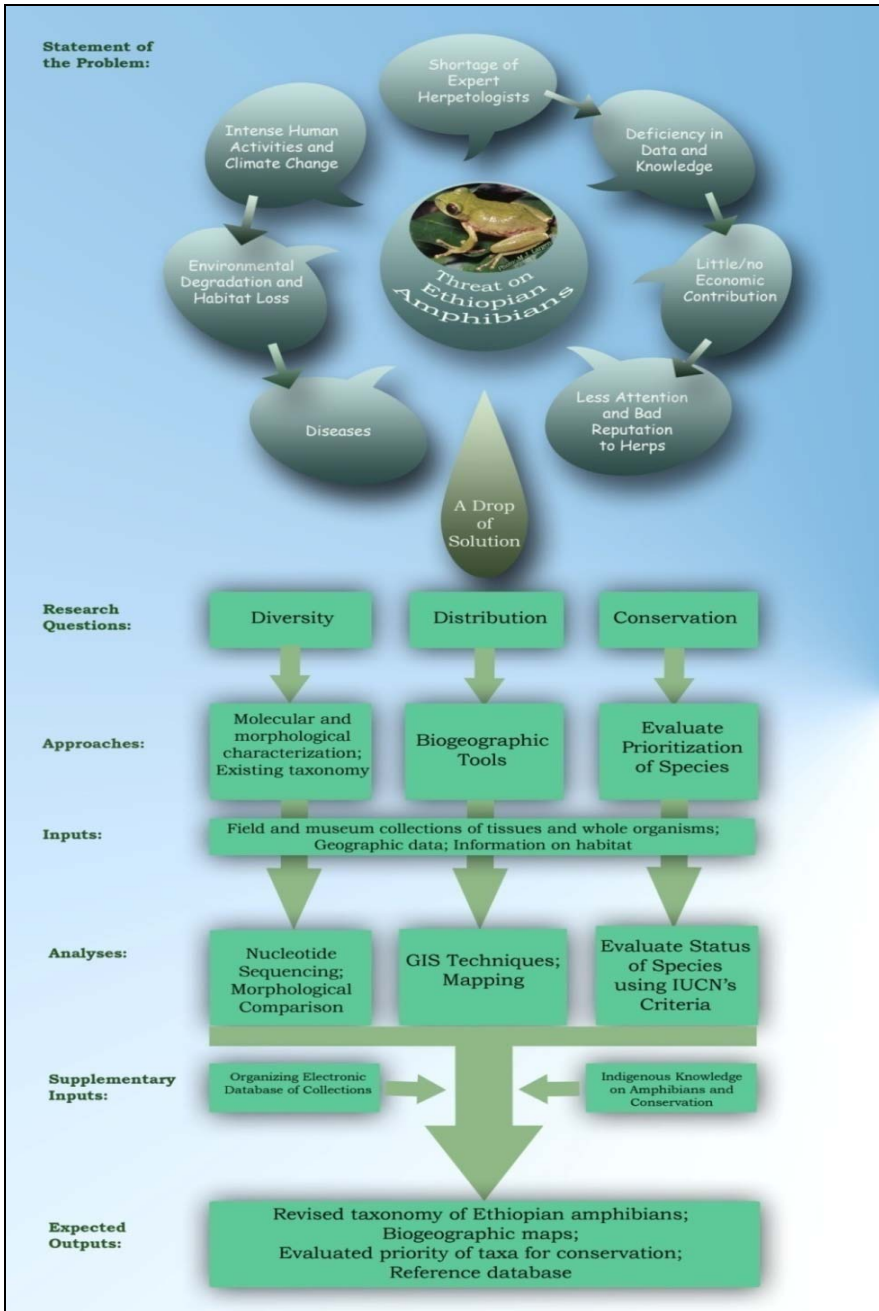


Fig. 5. Flowchart summarizing the current research questions and possible answers outlined in the project concept on amphibian diversity, distribution and conservation in Ethiopia (extracted from Abebe Ameha Mengistu *et al.*, 2009 and Abebe Ameha Mengistu, 2012).

1) Phylogeography as a tool

Phylogeography is a field that deals with the geographical distribution of genealogical lineages, their spatial relationships and evolutionary history as populations, sub-species and species (Avice, 2000). As a new and rapidly developing field, phylogeography is nowadays serving a great deal in the study of systematics, biogeography and conservation of many organisms. This has been enabled through an easier access to mtDNA sequences of most animal species (Avice, 1998). Because mtDNA has a relatively fast rate of nucleotide divergence, it is well suited to examine events over the last few million years (Hewitt, 2004). In particular, anuran amphibians have several advantages in phylogeographic studies; that is, they have generally low individual mobility – which consequently results in genetically highly structured populations (Beebee, 1996), are relatively easy to sample, and have a worldwide distribution and substantial species diversity (Zeisset and Beebee, 2008).

Evaluation of the recent status of genetic exploration of amphibians indicated that there are reported taxonomic and geographic disparities. In 2006, there were no gene sequences for 86% of species of Gymnophiona and 76% of Anura, and for 76% of African species (Vences and Köhler, 2006). In view of this, intensified sampling in remote tropical regions and specific research for key taxa and highly endangered species were necessary.

For the African amphibian species for which genetic exploration has been conducted, it has been found to be helpful to understand phylogenetic relationships at different taxonomic levels, and to correct previous misplacement of taxa outside of their close relatives (paraphyly). For instance, the molecular phylogeny of hyperoliid tree frogs of Africa has been assessed using the 12S and 16S genes of the mtDNA. Based on this data, the Family Hyperoliidae was recognized as being a paraphyletic assemblage having the genus *Leptopelis* as a basal group forming a group outside of hyperoliids (Richards and Moore, 1996; Vences *et al.*, 2003). Later, this has been further refined and *Leptopelis* was taken out of the hyperoliids and placed under the Family Arthroleptidae. Karyological data also supported that *Leptopelis* does not form a monophyletic group with the other hyperoliids (Odierna *et al.*, 2007).

Among Ethiopian amphibians, gene sequences exist only for very few of the known species. Extensive barcoding of as many species of amphibians as possible would enable to test the traditional taxonomy and to see clearer

relationships of the populations and species that potentially have been geographically isolated for millions of years. To achieve this, we can use the 16S gene, which is a highly conserved mitochondrial marker, but having common mutations in some variable regions, and ensuring a sufficient amount of mutations among species. This gene has been recommended as an important additional standard DNA barcoding marker for amphibians and other vertebrates (Vences *et al.*, 2005; Avise, 2009). Generating 16S data of many other African taxa would mean placement of Ethiopian taxa on a broad scale is also possible.

Progress is being made in identifying appropriate molecular markers – the 12S and 16S genes more commonly applied – and production of more reliable and taxon-specific primers. Furthermore, the development of software to analyze molecular (sequence) data and geographic information continues rapidly. The prospect of utilizing molecular systematics is a highly valuable tool to solve problems of understanding and conserving amphibian diversity. An example for the useful contribution of molecular data to better understand amphibian diversity has been presented by Vieites *et al.* (2009) in revealing the underestimated amphibian diversity of Madagascar, and the phylogeography of the cosmopolitan species *Ptychadena mascareniensis* (Vences *et al.*, 2004).

However, as is the case with the use of morphological characters and ecology to make species distinctions, the use of molecular techniques also has the problem of setting threshold values to delimit species boundaries. Therefore, the assignment of a minimum value of genetic distance to make species distinctions remains a subjective decision. Another weakness seen while using mitochondrial genes as genetic markers is that these genes contain phylogenetic information of only the maternal lineage, masking the complete evolutionary history of a species that comes from both parents. Despite the limitations, mitochondrial genes are currently widely used to understand genetic relationships and supplement taxonomic diagnosis of species. In this regard, the techniques that can be used to conduct phylogeographic assessment of Ethiopian amphibians are highlighted below.

2) Field sampling and specimen handling

To conduct studies on the diversity and distribution of amphibians in Ethiopia in general, it is preferable to organize field surveys and sampling of whole specimens and tissues during the main rainy season (Amharic: *kiremt*, from June to August) and/or the dry season (Amharic: *bega*, from December to February). Various localities and habitats, including tropical forests,

Afroalpine moorland, streams, swamps, highland grasslands, forest edges and inhabited/agricultural lands, are suitable places for amphibians. For molecular studies, cutting external body parts or taking tissue samples from the liver by dissecting the animal, would damage potentially useful diagnostic morphological characters. It is therefore preferable to take tissue samples from muscles of adults or tail/toe clips of metamorphs or tadpoles, and store them at -20°C in 96% ethanol. To preserve whole animals, the specimen is first fixed in 5% formaldehyde solution, rinsed with pure water, and then kept in 70% ethanol solution for further morphological study. If conservation-related survey on chytrid fungus infection (prevalence of chytridiomycosis) is needed, swabs can be taken from the skin of sampled specimens. Basic field sampling and museum preservation techniques of amphibians are presented in Abebe Getahun and Abebe Ameha Mengistu (2006). Advertisement calls and the behaviour of amphibians are species-specific and are some of the most important data that help in taxonomic studies. Recording geographic data such as GPS coordinates, elevation, habitat type, and locality name and other details, as well as taking high resolution photographs of specimens and habitats should accompany field sampling of specimens. The date and time of collection, name(s) of collector(s), and tag numbers are also important information to have during field collection. Proper documentation and organization of the raw data in natural history museums and/or printed or electronic databases helps to produce good quality results and makes future access to the data easier.

In addition, museum collections of Ethiopian amphibians can be used to deal with historical materials for morphological comparison and referencing with fresh/new collections. The majority of these collections are housed in the ZNHM-AAU (Addis Ababa), NHM (London), MCZ (Harvard), ZMB (Berlin), and MSNG (Genoa). Most of these museums have kept very old collections and type materials that are important to revise the taxonomy of the complex and less-studied Ethiopian amphibians. These specimens can be accessed through study visits and loans arranged between collaborating institutions and the museums.

3) Molecular work

Basic preparations for laboratory work should be followed using guidelines such as those outlined in Hillis *et al.* (1996) and DeSalle *et al.* (2002). Collection of genetic data in molecular laboratories is conducted first by extracting and purifying total DNA, and then by amplifying the DNA using PCR. DNA sequences of small parts of the mitochondrial DNA (mtDNA)

and nuclear genes provide phylogenetic information that can give estimates of evolutionary relationships, genetic distances and divergence times of populations and species. Analysis of molecular data requires several steps and analytical models incorporated in different computer programs. The commonly used software include MEGA (Tamura *et al.*, 2011), PAUP* (Swofford, 2002), and jModeltest (Guindon and Gascuel, 2003; Posada, in press). Analysis of biogeographic data (mapping distributions, estimating areas, predicting potential habitats) incorporates the use of software such as ArcGIS (Environmental Systems Research Institute, Inc., Redlands, California) and DIVA-GIS (Hijmans *et al.*, 2005).

The results of analysis of molecular, morphological and geographic /ecological data provide various results. That is, the results could verify/validate the taxonomic status of known taxa, reveal cryptic taxa (morphological conservatism), or conspecifics (morphological plasticity). Accordingly, assessment of geographic distribution data could provide with range retention, extension or shrinkage, thereby changing implications for conservation. As there could be taxonomic discrepancies between molecular and morphological assessments, the general definition for candidate species (Vieites *et al.*, 2009) can be applied to evaluate the taxonomic status of newly revealed groups among populations. In this definition, 1) **Unconfirmed Candidate Species (UCS)** are deep genealogical lineages of unknown status, data deficient for morphology, ecology, and distribution; 2) **Confirmed Candidate Species (CCS)** show genetic divergence combined with a distinct difference in either morphology or in a character that mediates premating isolation; and 3) **Deep Conspecific Lineage (DCL)** are deep genealogies above a threshold value typical for comparisons among closely related species in the group of animals under study. These definitions can be modified and applied for different taxa (e.g., genera) depending on the respective results of genetic, morphological and geographic data. Overall, these approaches serve to make estimates of species diversity or evolutionary significant units.

4) Evaluation of conservation status

The outputs of the phylogeographic analyses are important inputs to assess the conservation status of the species under consideration. If a previously single species becomes split into several phylo- or morpho-species, or if a presumed widely distributed species turns out to have very limited geographic range and/or if the habitat of a species has deteriorated through time, then these would imply that the conservation status of the species in

question may need revision and prioritization. Conversely, species or populations that genetically prove to be conspecifics, or those for which new records indicate extension of previously known geographic ranges, or when habitats have remained intact or become protected, then the conservation status of the species can be revised to have lower ranks. These evaluations are based on the standard criteria set for the IUCN Red List of species (IUCN, 2010). In addition to geographic ranges, it would be important to consider, if any, information on the trend and status of populations or habitats, prevalence of amphibian diseases, possible threats and opportunities pertinent in a specific range. Based on these assessments, the researcher can prioritize the studied taxa for further research and more practical conservation planning.

In summary, effective preservation of Ethiopian amphibians and proper utilization by humans as living natural resources can be achieved through more complete understanding of their taxonomy and natural history. Concerted and well-organized research and conservation programs on amphibians in unexplored areas, coupled with training of amateurs, semi- and highly-skilled professionals, could serve as means of building the capacity of local education institutions and wildlife conservation organizations. Proper handling of old museum specimens and curation of newly collected materials will enable future researchers to make more detailed studies, and for students to have suitable reference materials.

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