



REVIEW ARTICLE

A review on the conservation genetic studies of Indian amphibians and their implications on developing strategies for conservation[†]

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Abstract. Amphibians show a very high level of diversity and endemism and are facing global declines from the past few decades. Studies have shown that the molecular tools can be helpful in their conservation efforts. In India, more than 80% of amphibians are endemic and most show a narrow range of distribution. Most of the Indian amphibians lack information on their genetic diversity. In this study, we review the overall trend on amphibian studies in India with the specific focus on conservation genetics. Overall, of the 173 studies, only 14 dealt with the conservation of amphibians through genetic tools and five studies estimated the genetic diversity or gene structure. Here, we discuss the gaps and provide future directions on how genetic studies can be helpful in Indian amphibian conservation.

Keywords. population genetics; conservation; hot spots; phylogeography; genomics; frog.

Introduction

Amphibians show a very high level of diversity and endemism and are facing a global decline due to the ever-increasing anthropogenic activities from the past few decades (Wake 1998; Stuart *et al.* 2004). Among the vertebrates, amphibians are the most threatened species with extinction (IUCN 2019). Owing to their poor dispersal abilities and sensitive skin, amphibians are highly prone to habitat destruction along with the other factors like climate change, disease, pesticides and invasive species (Blaustein and Kiesecker 2002). Studies have shown that the habitat modification has led to the population declines in many species of amphibians (Collins and Storer 2003; Arntzen *et al.* 2017). Fragmentation of habitats could also narrow the genetic base of a species with severe implications for future generations (Cushman 2006). However, of all the ecological impacts of the population decline, genetic consequences have been the least realized aspect.

To accelerate the conservation efforts on amphibian populations, studies estimating genetic diversity and fitness are

informative, useful in assessing the status of amphibian populations and species (Storfer *et al.* 2009). At the global level, the population and landscape genetic studies of amphibians are increasingly investigated as studies show that spatial distribution of genetic diversity can boost conservation efforts (Emel and Storfer 2012).

At present, India has 437 amphibian species (Frost 2019), of these 80% are endemic and concentrated in three biodiversity hot spots, namely the Western Ghats, Himalayas and northeast India (part of Indo-Burma hot spot). About 4% of the amphibian species are critically endangered, 2% are near threatened, and others have not yet been assessed by IUCN (Dinesh *et al.* 2019). As more amphibian species are discovered in the country, with most of them being endemic and range restricted, it is essential to synthesize and understand the amphibian conservation in India. During the last decade alone, there has been a spurt in the discovery of amphibians in India and the use of modern genetic analysis have led to the higher species diversity than previously reported (Aravind *et al.* 2004). Specifically, the genetic studies have led to new insights into the amphibian diversity especially in the Western Ghats, which has seen many species being discovered. The increase in the number of species

[†]This is one of the articles of collections on 'Conservation Genetics'.

Table 1. List of conservation genetic studies from India (1990–2018).

	Journal	Taxon	Marker	Reference
1	<i>Journal of Zoology</i>	<i>Ichthyophis bombayensis</i>	Mitochondrial	Gower et al. (2007)
2	<i>ACM Digital Library</i>	<i>Nyctibatrachus major</i>	Microsatellites	Meenakshi et al. (2010)
3	<i>Conservation Genetic Resources</i>	<i>Indirana beddomii</i>	Microsatellites	Nair et al. (2011)
4	<i>Biotechnology, Bioinformatics and Bioengineering.</i>	<i>Fejervarya keratensis</i>	Microsatellites	Raveendran et al. (2011)
5	<i>Conservation Genetics Resources</i>	<i>Fejervarya sahyadrensis</i>	Microsatellite	Aggarwal et al. (2012)
6	<i>BMC Research Notes</i>	Five species of <i>Indirana</i>	Microsatellites	Nair et al. (2012a)
7	<i>Conservation Genetics</i>	<i>Indirana beddomii</i>	Microsatellites	Nair et al. (2012b)
8	<i>Animal Conservation</i>	<i>Indirana</i> spp.	Mitochondrial	Nair et al. (2012c)
9	<i>Mitochondrial DNA Part A</i>	<i>Pseudophilautus wynaadensis</i>	Mitochondrial	Gopalan et al. (2016)
10	<i>Conservation Genetic Resources</i>	<i>Euphyctis karaavali</i>	Mitochondrial (whole genome)	Anoop et al. (2017)
11	<i>Zootaxa</i>	<i>Fejervarya manoharani</i>	Mitochondrial (whole genome)	Kiran et al. (2017)
12	<i>Conservation Genetic Resources</i>	<i>Indirana semipalmata</i>	Mitochondrial (whole genome)	Kumar et al. (2017)
13	<i>Mitochondrial DNA Part B</i>	<i>Microhyla ornata</i>	Mitochondrial	Garg et al. (2018)
14	Ph.D. thesis	<i>Nyctibatrachus jog</i> <i>N. kempholeyensis</i>	Microsatellites	Hebbar (2018)

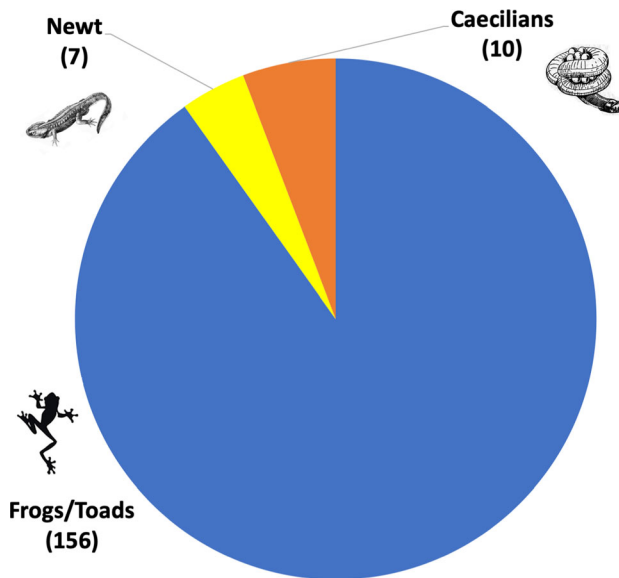


Figure 1. Number of studies undertaken on amphibians in India (1990–2018).

is remarkable, making it one of the highest percentages of endemic species in India; also, more than 100 species have been described during the last decade alone. However, despite a spurt of discoveries, recent work on amphibians have been mainly restricted to systematics and to some extent to ecology. Except for a few amphibians, very little information is available at the population and species levels. Considering that many amphibian species in India are rare, have patchy and possibly fragmented distribution or comprise of small and fewer populations, they have neither been adequately recorded nor monitored over time, especially in view of human-induced changes in the habitat. Similarly, studies on extinction risks and population vulnerability have not yet been carried out for most species.

Conservation genetic studies provide accurate information and understanding of the processes that led to the current endangered state or rarity of the species (DeSalle and George 2004). Quantification of genetic diversity, gene-flow, inbreeding depression in natural populations of the species provide additional information that affects endemic and endangered species. Such studies can provide immediate and genetically based responses to the detrimental effects of these processes (DeSalle and George 2004). With the advent of high-throughput DNA sequencing and genotyping technology, the scope of conservation genetics studies has expanded across the globe. Amphibian populations show high genetic structure at short distances and are poor dispersers, thus can be useful model systems for studying conservation genetics (Beebee 2005; Zeisset and Beebee 2008).

In this brief review on the amphibian genetic studies, we collated data on amphibian research in India beginning from 1990 till 2018. This review aims to understand the overall trend in amphibian studies in India and carry out a detailed

analysis of factors shaping conservation genetic studies of amphibians in India.

Methods

We conducted an extensive literature review of studies published in the last three decades (between 1990 and 2018) using Web of Science knowledge (<http://apps.webofknowledge.com>) database as well as Google Scholar search engine at two levels. In the first level, we included search for an overall amphibian studies using the keywords (amphibian or frogs or toads or newt or caecilians and India). In the next level, we focussed on the conservation genetics studies of Indian amphibians using the keywords (amphibian or frogs or toads or newt or caecilians) and (conservation or population genetics or conservation genetics or genetic diversity and India). The results are presented in the tabular and graphical format (table 1; figures 1–3).

Results

Overall, we found 173 studies on Indian amphibians. These studies were grouped into three orders: frogs or toads, Himalayan newt and caecilians into following categories like ecology, conservation, physiology and toxicology, taxonomy, phylogeography, behaviour and conservation genetics. We also recorded whether the studies were conducted on adults or tadpoles. There were a total of 156 studies on frogs and toads, seven on Himalayan newt and 10 on caecilians (figure 1). Most of the studies were conducted on adults and a very few on tadpoles, except physiology and toxicology categories which showed the maximum studies on tadpoles. The overall majority of amphibian studies focussed on taxonomy. There were only 14 studies related to conservation genetics of the Indian amphibians (table 1; figure 2), of which 13 were published on frogs, only one on the genetic diversity of a caecilian, and no studies are yet published on

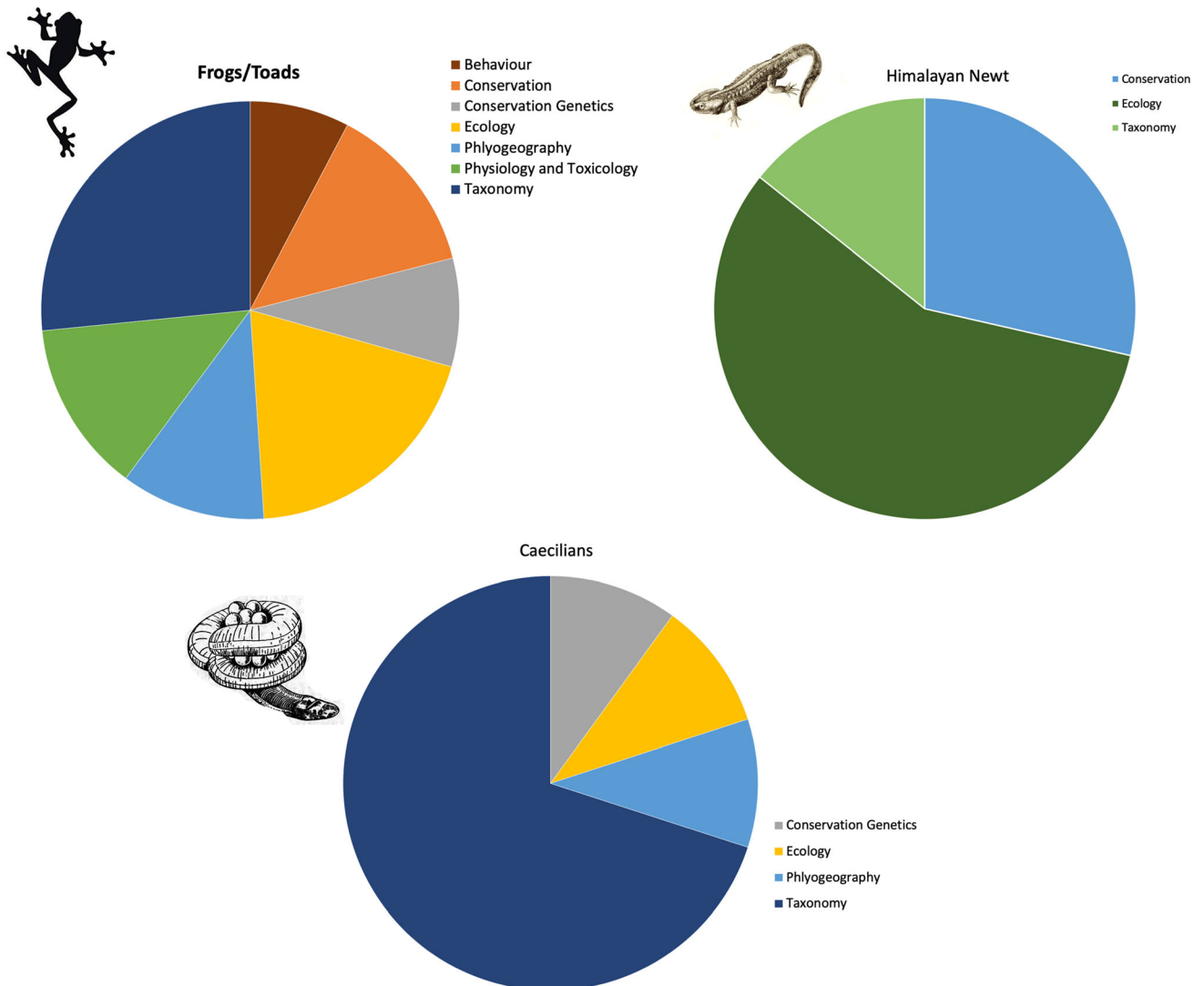


Figure 2. Studies undertaken on different categories of amphibians.

the Himalayan newt. The studies were published in the last decade and were conducted on endemic frogs and a caecilian of the Western Ghats. Among the molecular markers, both microsatellites and mitochondrial markers were used, but microsatellites were the preferred choice. Majority of the studies were technical papers and focussed on the development of the microsatellite markers or mitochondrial genomes. Based on the region wise, the markers were developed for species found in the Western Ghats of Kerala, and there was underrepresentation of species from the other regions of India. Only five of 14 studies assessed the genetic diversity.

Discussion

Although India is one of the major global biodiversity hot spots for amphibians, there is a lack of studies on conservation genetics. Globally, species that are rare, endemic, endangered, or have patchy geographic distribution have often been the target species for studying their genetic diversity so that the estimate can help in ensuring their survival either through translocation or captive breeding to avoid extinction. From this review, it is evident that the genetic studies on endemic and endangered Indian amphibians are far too few. Studies on Himalayan newt and caecilians are also underrepresented. It is imperative that more research should be focussed on the conservation genetics of Indian amphibians so that adequate strategies could be developed for their management.

The field of conservation genetics has focussed mainly on warm-blooded species and hence globally amphibian conservation genetic studies are minimally represented (Jehle 2010). A review on amphibian conservation genetic studies between 2000 and 2009 contributed just 7.6% to publications in journals like *Molecular Ecology* and *Conservation Genetics* (Jehle 2010). Even in India, amphibian conservation genetics research has not yet caught the attention as much as research on charismatic species like tigers or the elephants. A comparison between amphibians and tiger related conservation genetic studies from India shows that

studies on tiger have doubled (figure 3). Multidisciplinary approaches like landscape genetics and next-generation sequencing technologies have been utilized to prioritize their conservation needs (Natesh et al. 2017; Reddy et al. 2017). While maintaining the genetic reserves of the Indian tiger is crucial, it is equally important to study the genetic variation of diverse amphibians of India considering that the populations of several amphibian species are too small and could be under the verge of extinction.

Genetic analysis of species and their populations relies on the quantification of inbreeding depression, effective population size, population genetic structure, population viability and gene flow across populations. Considering that there is a high number of endemic and rare amphibians in India, it is important that future studies should focus on amphibian genetic studies. Molecular tools could help in addressing questions like determining management units, bottlenecks among the populations and identifying potential barriers or dispersal corridors. We discuss below the five broad study areas where molecular tools can be valuable in amphibian conservation in India.

Genetic markers and effective population size

Molecular markers have been useful in delineating amphibian species complexes and have helped in describing new species from India (Priti et al. 2016; Garg et al. 2018). This is an important area in which molecular tools had a significant influence on the amphibian systematics in India. The use of molecular tools has helped in resolving cryptic species, which has been a contentious issue in a number of groups (Biju et al. 2014; Mahony et al. 2018). However, they have still not been utilized to study the population genetics of Indian amphibians. For example, molecular markers can help in estimating the sufficient population size which has been considered as extremely valuable in amphibian species management (Shaffer et al. 2015). Estimating effective population size is useful, especially for those species that are difficult to study in the wild. It has also helped in understanding the population expansion, decline or those that are at the risk of extinction (Funk et al. 1999). For example, a comparative genetic study of the four ranid frogs from the USA showed that three of them had a very low effective population size (<50), attributing the cause to habitat fragmentation or population bottleneck (Phillipsen et al. 2011). Since small effective population size is linked to population viability, the authors suggested mitigating anthropogenic activities through habitat restoration and connectivity could help in amphibian conservation. Understanding the effective population size will be very critical from an Indian point of view, as India has a high number of endemic amphibian species and many of their habitats are under threat either due to deforestation or various developmental activities. It is imperative that Indian amphibian

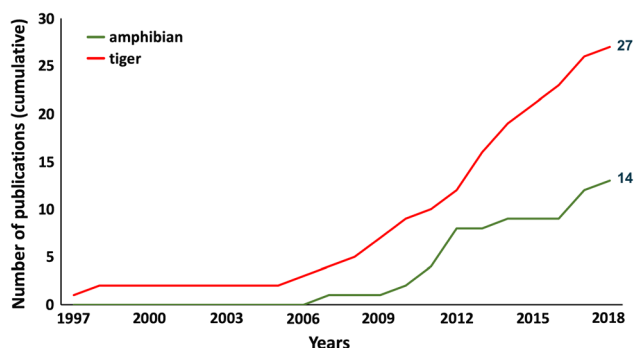


Figure 3. The number of cumulative publications of tiger and amphibians in the conservation genetics field in India.

research includes such genetic studies which can be beneficial to amphibian conservation and management.

Conservation units

Genetic markers have been used to designate conservation units to populations within a species (Waples 1991; Moritz 1994). These are based on high genetic distinction and establishing such units can maximize the evolutionary potential of the species (Funk *et al.* 2012). The commonly included conservation units are evolutionary significant units (ESUs) and management units (MUs). According to Waples (1991), ESUs are populations that are reproductively separate from other populations with unique adaptations. However, the most widely used criterion of ESUs is of Moritz (1994), where populations that are reciprocally monophyletic for their mitochondrial markers with strong divergence at nuclear loci are considered as an ESU. But a recent study suggests that populations that lack reciprocal monophyly but exhibit distinct mitochondrial DNA haplotypes can also be considered as ESU (Magalhães *et al.* 2017). Demarcating populations as ESUs has been applied for amphibian conservation elsewhere. For example, based on the evidence from mitochondrial markers, study on the Malagasy poison frog (*Mantella bernhardi*) showed that the species lacked habitat connectivity and showed high genetic differentiation between north and south populations distributed in southeast Madagascar (Vieites *et al.* 2006). The authors proposed both the north and south populations to be separate ESUs. Recently this approach has also been used to conserve highly threatened anurans belonging to sooglossid frogs of the Seychelles archipelago (Jim *et al.* 2019).

MUs are crucial for managing natural populations of species and monitoring anthropogenic threats (Palsbøll *et al.* 2007). They are much smaller than ESUs and are demographically independent lacking evolutionary depth (Funk *et al.* 2012; Shaffer *et al.* 2015). A study on the genetic variation on the alpine newt (*Triturus alpestris*), at a small geographical scale from Poland showed that the populations were genetically distinct and physically separated warranting separate MUs (Pabijan *et al.* 2005). In India, such studies are yet to be carried out on amphibians but have already been conducted for reptiles like the Olive ridley turtles (Shanker *et al.* 2004). Since ESUs and MUs help in preserving the intraspecies diversity, their application will be extremely useful to maintain species evolutionary potential (Shaffer *et al.* 2015). Such studies on Indian amphibians can prove instrumental in their conservation.

Landscape genetics

Landscape and its topology can influence the genetic variation of populations (Neville *et al.* 2012). This field of study

where the influence of geographical and environmental features on gene flow and genetic structure is studied is known as landscape genetics (Manel *et al.* 2003). Globally, landscape genetics approach has been extensively applied to amphibians. In amphibians, dispersal is influenced by landscape features such that the genetic variation can be a function of both natural landscape features like topography as well as anthropogenic effects like habitat destruction or modification (Spear *et al.* 2005). Recently a landscape genetic study on montane grassland Nimba toad (*Nimbaphrynoides occidentalis*) showed that elevation, land-use history and life history traits influenced the genetic variation of toad where elevation and life history traits had a positive influence on their population connectivity while adverse effects of mining (land-use history) was mitigated by ongoing gene flow (Sandberger-Loua *et al.* 2018). Another study on marbled newts (*Triturus marmoratus*) showed that the agricultural landscapes led to the genetic structuring of newt and might act as a barrier to their movement (Costanzi *et al.* 2018). Landscape genetics approach has also been used to understand the wildlife diseases. For example, both genetic and environmental factors were studied to predict amphibian pathogen *Batrachochytrium dendrobatidis* (Bd) dynamics in northern leopard frog (*Lithobates yavapaiensis*) populations. The study showed that environmental data predicted infection intensity and genetic diversity alone predicted mortality, but when both the factors were combined, they predicted infection prevalence (Savage *et al.* 2015). The authors suggest combining both genetics and environmental factors for better understanding of Bd which can help to prioritize conservation of frogs. Such a framework is yet to be applied on the Indian amphibians. Since landscape genetics has the potential to provide insights on how landscape affects the contemporary gene flow in amphibians, such studies can be valuable in designing appropriate management actions for the Indian amphibians.

Phylogeography and conservation

Phylogeographic studies which combine phylogenetics with biogeographic studies can be helpful in amphibian conservation. Such studies can be useful in identifying biodiversity regions, unravel the effect of the climate on species persistence (Wang *et al.* 2018) or help in understanding historical aspects of species distribution (Zeisset and Beebe 2008). It can also help in identifying species lineages or help in understanding the phylogenetic diversity which is a measure of the functional diversity and evolutionary potential (Shaffer *et al.* 2015). Globally, such studies have been extensively carried out to understand the evolutionary processes of amphibians. For example, a study on red spotted toad (*Bufo punctatus*) from North America showed three divergent clades probably separated between Miocene to early Pliocene by vicariance events (Jaeger *et al.* 2005), thus providing information about the past events influencing the

dispersal and distribution of the species. Similarly study on critically endangered Chinese giant salamander showed seven divergent clades due to low dispersal ability and upliftment of mountains (Liang *et al.* 2019). In India, phylogeographic studies have been helpful in discovering new species and new families (Biju and Bossuyt 2003). Such studies, especially on Western Ghats amphibians have been beneficial in understanding how historical events influence the species distribution. For example, the study on species from *Nyctibatrachus* genus indicated that although the Nyctibatrachidae family originated in the Indian subcontinent, extant nyctibatrachids diversified in Eocene. The species are poor dispersers and show mountain associated clade endemism (Van Bocxlaer *et al.* 2012). Similarly, a study on caecilians from the Western Ghats and the Eastern Ghats attempted to understand the role of wet zone fragmentation in shaping caecilian diversity (Gower *et al.* 2016). The study showed that during the mid Cenozoic might have led the fragmentation of the wet zone to the intervening dry zone which acted as a barrier for the low vagile caecilians. However, the application of phylogeographic studies to understand the genetic variation and population history of Indian amphibians is limited. Since the past events influences how species are distributed and phylogeography can provide insights about the spatial distribution of genetic diversity, more such studies should be undertaken which can provide exciting insights into the evolutionary processes of the Indian amphibians.

Genomics for conservation

Conservation genetics studies until a few years ago relied on a few neutral markers like amplified fragment length polymorphism (AFLP), mitochondrial markers and microsatellites. However, the focus has now turned into genomics which involves the use of thousands of molecular markers generated using next-generation techniques. In recent years, genomic technologies have assisted in increasing the efficiency of the field of conservation genetics and in helping conservation decision-making. Studies suggest that such massive data acquisition, storage and analysis can be extremely useful for the conservation of the species (McMahon *et al.* 2014). For amphibians, one of the critical areas where genomics has been valuable is in understanding Chytridiomycosis: a fungal disease that is one of the major causes of global amphibian decline. In one of the studies, Ellison *et al.* (2014), studied comparative transcriptomics to quantify gene expression responses to the pathogen *Batrachochytrium dendrobatidis* in different South American amphibian species. The results showed that species that were resistant to the pathogen showed higher skin integrity and low skin inflammation, whereas those that were highly susceptible showed suppression in splenic T-cell genes. Another area of interest where genomics can be used is in understanding the local adaptation of the species. Local

adaptation is a genetic change in a species that evolved to suit its environment in response to natural selection (Blanquart *et al.* 2013). Such studies have provided insights on how tadpoles inhabit geothermal streams (Pastenes *et al.* 2017), how environment affect evolutionary history and speciation in frogs (Wang *et al.* 2018), finding genes that determine the persistence of the populations (Bonin *et al.* 2006) as well as genes that are crucial for amphibian development (Richter-Boix *et al.* 2013). While there are numerous benefits of genomics approach, overall very few amphibian genomes have been sequenced but with affordable sequence costs, many non-model amphibian genomes are being sequenced (Shaffer *et al.* 2015). In India, not a single amphibian genome has been sequenced yet. As genomics can contribute immensely to the conservation of amphibians as well as provide evolutionary insights, future studies should try to utilize genomic approaches for amphibian management. Given the population density and the ever-increasing anthropogenic pressure on the natural ecosystems in India, it is imperative that along with taxonomic and ecological studies, genetic studies should be carried out to monitor and safeguard the viability of a number of threatened amphibians. Further studies should include conservation genetics, phylogenetics and landscape genetics-based approaches, which can predict threats and risk to the amphibians, thus helping in building strategies for their conservation.

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