



A current view of classification and sampling of terrestrial snails: A review

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Abstract

The snails are second large and most successful invertebrates on the face of the earth yet remained ignored and unidentified due to the lesser interest of scientist. Due to this these have not been successfully documented in the Red Data Book. Out of 693 documented extinctions 42% are from Phylum Mollusca. The species delimitation is still an issue. The shells have phenotypic plasticity and variation due to which relying completely on the morphology of the shell becomes strongly confusing to systematists. The biochemical and molecular markers are important in understanding freshwater gastropod diversity along with morphological characters. However species specific markers have not been much explored in the field of malacology due to which most of the work in the last two decades reveals species characterization on the basis of RAPD markers. Still there are gaps need to be filled as there are many traits that are plastic and have been used in description of many species in taxonomic reviews and therefore are not very useful in determination of systematic relationships. The shell size is directly proportional with some ecological factors as well as geographical features. Species concept and sampling strategies needs to be reconsidered and redefined when studying land snails.

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Introduction

Land snail biodiversity, is defined as the number of native species per unit of land area, (Holland and Cowie, 2009). The snails are second large and most successful invertebrates after members of the Class Insecta (Abbot, 1989 and Hapman, 2009). Although the molluscs are one of the most diverse group, yet there are only a few scientists who have tried to explore the field of malacology. The most effective approach to the biodiversity conservation is the compilation and publication of the Red Data Book (Bouchet, 1997).

The total documented species of freshwater and terrestrial molluscs are 708 and 1222 respectively, out of which 42% of the 693 extinctions were from Phylum Mollusca, comprising 260 gastropods and 31 bivalves (Baillie *et al.* 2004). Invertebrates are not generally noticed by the biologists for conservation studies however most of the work is being done on terrestrial vertebrate and their extinctions have been well documented (Lydeard *et al.*, 2004).

The members of the class Gastropoda are extreme importance for the ecosystem due to their various ecological services *i.e.* bioindicators, intermediate hosts for various parasites, source of protein and calcium in the food chain, especially during breeding season of the birds, due to which their taxonomy is of extreme importance.

However, their study of ecological habits, development of sampling strategies and exploration of the species specific markers are important to be considered to reduce confusion for the correct identification of the species. The biological species concept cannot be applied to many species in gastropods due to the fact that they are uniparental in nature guiding us to the use of phylogenetic species concept. Various ecological factors lead to the development of ecophenotypes of the species leading to the synonymies.

This review covers the discussion on the taxonomy, ecology and sampling strategies need to be devised to study the status of this ignored part of the ecosystem.

Fresh Water Gastropods Species Delimitation

The identification and classification of the freshwater gastropods is generally done on the basis of morphological as well as shell characteristics for the past two centuries. The qualitative and quantitative data is rarely provided by authors at comparable levels and the original descriptions that are provided with descriptive terms mostly have different interpretations, which depends on the prior experience of the taxonomist (*i.e.* “broadly conic” vs “tapering”).

Usually, description of specimens on the basis of juvenile shells, single shells, or sometimes even partial shells and the level of natural variation at the level of philosophy of taxonomy is not considered. The identification keys for the classification of many groups are available (Thompson, 1999; Wu *et al.*, 1997; Burch, 1982) however; they are difficult to understand for amateur taxonomist as there is an inconsistency in differential diagnostic and descriptive approach among them. The shells have phenotypic plasticity and variation due to which relying completely on the morphology of the shell becomes strongly confusing to systematics. In fresh water the phenotypic plasticity is well documented (Adams, 1900; Holomuzki and Biggs, 2006; Heller *et al.*, 2005) and has been generally observed due to environmental pressures (Prezant *et al.*, 2006; DeWitt, 1998; Krist, 2002).

According to Schilthuzen *et al.* (2006), the biotic factors play major role in the allopatric speciation. Due to genetic divergence and geographical variation the shell morphology also varies strikingly, as the geographical differentiation evolves the shell shape and behavior. The level of the shell variation that is related to the local adaptation or adaptation due to environmental pressures as compared to the characters valid species is not completely understood. As noted by Burch (1982) there are many generic groups that have been classified on the basis of shell characteristics and the characters, which have been used historically, have many intervening forms at one or the other point.

The areas which are considered to be extremely important for the identification of species or subspecies, are the intervening areas as they are the regions where there is intergradation, especially in case of the allopatric populations (Mayr, 1991). The hypothesis that the mouth of the Gulf of California is a barrier for the migration of fauna into and out of the Gulf has been rejected with the help of sliding window technique, and it was confirmed that the fauna in the Gulf of California has not been structured by this isolated water body (Fredensborg, 2006). In the unrelated taxa there may be the presence of similar shapes and sculptures of shell (Minton and Lydeard, 2003; Minton *et al.*, 2003; Chambers, 1980). There are many phenotypes within a same species or population of species of fresh water molluscs which can be distinguished and their presence pose severe problems in the process of identification (Dillon, 1984). This also gives convincing evidence that shell structure and sculptures cannot be used only for the exact identification and accurate species delineation.

There are other morphological characters which add more information about the gastropods that help in the confirmation of the correct taxonomic unit. The characters like radula morphology and soft-part anatomical characters have been proved as very important, along with the shell characters, in classification of the freshwater gastropods as they are strongly non plastic (Schander and Sundberg, 2001; Falniowski and Szarwoska, 1995 Brown and Berthold, 1990) however, it varies in others (Minton, 2002; Dazo, 1965). The radula taxonomically is a very useful character in many groups of gastropod molluscs (Kohn *et al.*, 1999).

It is extremely important phenotypic character as it has a basic relationship with diet and environment (Andrade and Solferini, 2006). The combination of an array of morphological features, as well as its microanatomy in *Acochlidian* gastropod *Hedylopsis ballantinei*, plays a key role in the construction of *Acochlidian* phylogeny (Sommerfeldt, 2005).

The characters, which can be strongly informative but have not been completely explored for their utility during identification of freshwater gastropods are mucous composition, body color, gamete recognition, and genetic loci code associated with reproduction or for traits under selection. According to Malaquis (2006) the major taxonomic features in the snails are external morphology, shell, animal coloration, jaws, radula, gizzard plates, seminal duct, prostrate gland, egg masses and penis. In almost all species with European origin except *Haminoea orbignyana*, the anatomical data is considered to be the most important for identification, considering male reproductive system as the most important. The genital-anatomical characters can be equally mistrusted like conchological characters, when used at the tribe level, especially when they are in conflict with the distributional and conchological patterns (Weerd, 2004).

The snail species, which have opposite chirality have genitalia on the wrong side due to which they are not able to mate and are reproductively isolated. The chirality in the snails originates during the early stages of the development and can be observed in the adults later. Single genetic locus generally determines the coiling of the shell which is on the basis of delayed inheritance. 90% of the snails have dextral coiling, while the sinistral coiling is due to the mutation in the genera and families, which are entirely dextral. It is generally considered there should be the strong frequency dependent selection against the establishment of the specific chirality as dextral species are in minority and find it difficult to have a mating partner (Schilthuizen and Davison, 2005). *Xeropicta derbentina*, shows plasticity and can switch from annual cycle to biennial cycle depending on the population density or climatic conditions (Kiss *et al.*, 2005).

The biochemical and molecular features are potential characters that help in understanding of freshwater gastropod diversity, in addition to the morphological characters (Mangenelli *et al.*, 2001; Raahauge and Kristensen, 2000; Hershler and Liu, 2004; Minton and Lydeard, 2003).

Still there are gaps need to be filled as there are many traits that are plastic and have been used in description of many species in taxonomic reviews (Cuezzo, 2003; Hovingh, 2004; Haase, 2003) and therefore are not very useful in determination of systematic relationships (Wullschleger and Jokela, 2002; Dillon *et al.*, 2002; Backeljau *et al.*, 2001; Parmakelis *et al.*, 2003).

Ecology

The lands molluscs generally live on a varied range dead and decayed material or living herbaceous plants, fungus, algae, rotting wood and bark. They feed on empty snail shells, live snails, nematodes, animal wastes and carcasses, and even rasp limestone rock or cement (Nekola, 2012). The gastropods are found under large stones, old logs, lying in wood, under the decaying bark of trees on the wet lichens-clad barks, on the damp moss near waterfalls, on walls, on the leaves of the shrubs, the plantain and bamboos, under decaying leaves, beneath the surface of ground in worm burrows, in the roots of plants and in the exuviae left by floods on river banks and many shells were found on land washed by the flood (Blanford and Godwin (1908).

The faunistic composition of the land snails varies with vegetation types and habitat (Burch, 1956; Nekola, 2002; Ports, 1996; Stamol, 1993; Stamol, 1991; Theler, 1997; Van Es and Boag, 1981; Wärebörn, 1970; Young and Evans, 1991). The contrasting difference in the distribution pattern between forest faunas and open-ground is not confined to terrestrial snails; other soil invertebrate members also show the same including fungus-feeding microarthropods (Branquart *et al.*, 1995).

According to Nekola (2003) conservation of snails require protection of soil surface architecture. The structure of the community in molluscs is best expressed by soil moisture content (Machintosh 2002) and the protection of the architecture of soil is required for their conservation (Nekola, 2003). Nearly 90% of snails live in top soil surface, upto 5 cm (Hawkins *et al.*, 1997). The architecture of organic litter (Alvarez and Willig, 1993), and the soil which forms the underlying layer (Hermida *et al.*, 2000), can also put a strong influence on the structure of the terrestrial gastropod community.

They have possibly some kind of competition, for the inorganic nutrients, with the plant roots (Lavelle *et al.*, 1995). The other important feature of terrestrial gastropod communities is the thickness of organic litter as the abundance of snails (Berry, 1973), community composition (Barker and Mayhill, 1999) diversity (Locasciulli and Boag, 1987) of terrestrial gastropods often has a positive correlation with litter depth. Machintosh *et al.*, (2002) studied the macro fauna in the Ranong mangrove in southern Thailand and found low molluscan diversity in the area of tin mining (pollutants), whereas in the areas of *Rhizophora* plantation (higher organic matter) there was the highest diversity.

The anthropogenic disturbance may impact the duff soil more severely as compared to the turf soil; however, the grassland snails face a negative impact due to heavy grazing (Cameron and Morgan-Huws, 1975). The differences in the faunal composition may be due to disturbance resulting in changes in the architecture soil surface. The organic litter layers are less complex, structurally, and are thinner in undisturbed turf soils which goes less to compaction when compared to the duff sites (Nekola, 2003), whereas sheep grazing helps the rare gastropod species to survive by providing them habitats that are suitable for them. The heterogeneity of the vegetation and a complex cover of top soil help co-existence of more species, whereas there is a significant reduction of the gastropod abundance and diversity due to the homogenous grazing (Labaune and Magnin 2002) and fire-management (Nekola, 2002b).

The *Monadenia fidelis* and *Deroceras reticulatum* were identified as indicator species, in unburned wetland prairie. On the other hand, *Vertigo modesta* and *Catinellar hederi* were found to be the indicator species for burned habitat. The terrestrial gastropod species richness was found to be low in the burned area, however, there is an increase in the abundance of molluscs after the first post burn year, in which it was low. The source population of the molluscs and other related animals should be provided an opportunity to colonize them by conducting the burns as prescribed according to the refuges,

without prior information about their response to fire, since it appears that fire helps in the decrease of molluscs' abundance and diversity in wetland prairies (Severens, 2005). The species survival is closely correlated with the ground moisture and the presence of bryophyte cover that remains intact due to which snail remains unaffected after clear cutting. In the buffer strips, species number remains unaffected while there is a decrease in the individuals' number. This indicates that in the riparian forests buffer-strip retention is a successful practice for protection of land snails. The moist or wet floor of the forest generally serve as refuge even when studied at very small scales i.e. hollow and shallow crevices, which help in the survival of the gastropod fauna for long term. Clear cutting decreased the number of individuals from each site along with decrease in the mean number of species (Hylander *et al.*, 2004).

Initial declines of snail fauna in temperate and boreal forests after fire have been reported Karlin (1961), which was reconfirmed by Waldén (1998) and Strayler *et al.* (1986) with similar effect of clearcutting. Shrubs or trees which are broad leaved generally help in species density and richness after disturbance (Hawkins *et al.*, 1997). Poor snail communities are found in the coniferous plantations that are when compared to forests that are old grown (Shikov, 1984).

A very short term disturbance effects are very long lasting for the snail survival as the land snails are generally poor dispersers (Baur and Baur, 1988), which may lead to local extinction for a long period of time (Hylander, 2004). Two things are extremely important, for species that cannot survive any disturbance, firstly the dispersal capacity of species for the recolonization in those areas where they are no longer observed (Green and Johnson, 2000); and secondly, the spatial structure of patches in which its source populations are generally found (Kafka *et al.*, 2001).

The juveniles member of most of the species are very sensitive to dessication (Asami, 1993) and a positive correlation exist between the moisture of the soil and gastropod distribution (Prior, 1985).

The number and abundance depend on the presence of bryophyte layers, and is more intact in the wet areas as compared to the drier areas. This protects the litter layers underlying these bryophytes, on which the snails survive, and the moisture content of the soil. Generally, during sunny days there are dry and hot conditions, which modify the characteristics of the top soil litter of the clearcuts (Chen *et al.*, 1993) and as a result many snails are killed.

Hawkins *et al.* (1997) reported that some of the snails have developed their survival strategies from severe conditions. They quickly move to the deep soil layers where the site is unaffected. The wet or moist soil served as a useful refuge as and even this moist ground predicts the survival of the snails (Prior, 1985). The areas are generally cleared and prepared by sweeping up the well-drained, steep lime stone slopes. This results in the vegetation cover loss, greater impact of sun radiations, loss of topsoil, resulting in desiccation, greater risk of fire, and in the reduction of the number of drought-intolerant snail species (Vermeulen and Whitten, 1999). The lime stone hills may take years to recover (Kiew, 2001) once damaged by fire and even if recovered after a long period becomes overgrown by secondary vegetation of grasses, invasive climbers, and pioneer tree species (Vermeulen and Whitten, 1999).

The limestone outcrops in the Southeast Asia are rich in terrestrial gastropods with 74 species in 16000 sampled individuals. Prosobranchia species are extremely endemic to very short ranges and are restricted to the limestone regions and are less abundant in the disturbed sites as compared to Pulmonata snails. The gastropod diversity are not found to be different between undisturbed and disturbed sites in most sites. This leads to the result that Prosobranchia may lead to extinctions if continuously exposed to disturbances.

The organisms which have high calcium carbonate requirement and high pH along with low tolerance for acidity need high limestone content regions for their growth (Schiltuizen, 2005a).

Snails generally have high calcium requirement for their reproduction and shells (Graveland *et al.*, 1994) and their abundance has a positive correlation with both pH and Calcium carbonate (CaCO₃) (Schilthuizen *et al.*, 2003; Waldén, 1995). There are a few groups, which are extremely endemic to these limestone hills, showing the presence of 106 species which are obligately calcicolous snails on 28 karst hills. Out of these, 70 species were restricted to a single hill, as they have extremely high requirement of calcium due to which they are found exclusively on limestone and show great diversity (Tweedie, 1961).

Vermeulen and Whitten (1999) found that in the state of Sarawak there are nearly 50 species of the terrestrial gastropods which are endemic to only one large outcrop of karst. In Pulmonata, there is less pronounced impact of inter relationship of calcicoly and endemism as compared to the Prosobranchia. Not only this, while comparing Pulmonata with Prosobranchia, the desiccating effects of terrestrial life are more pronounced on Prosobranchia due to its open mantle cavity (Solem, 1984; 1974). Nearly 50 percent of the species belonging to terrestrial gastropod in Malaysia belong to the order Prosobranchia, but their distribution is more patchy when compared to the order Pulmonata (Schilthuizen *et al.*, 2002), which shows their tolerance ranges are narrow for humidity and various abiotic factors.

Due to human intervention, in Southeast Asia the limestone is exposed to deterioration and degradation as excavation for obtaining marble, cement, for construction of roads is leading to the extinction of various site endemic species (Vermeulen, 1994). In Turkey, limestone meadows are in danger to be affected by human activities and are important site for snail conservation. They have 24 species out of which, 21 are native and 3 are located in Istanbul (Orstan, 2005).

There are other ecological factors which are important to assess the diversity and distribution of the snail species such as bottom sediments, flow of water, diversity of macrophyte. The number of species depends on the number of bottom sediments,

an increase in the flow velocity decreased the number of the species density and snail number while snail species richness is not affected by the vegetation density except *Elodea canadensis* and a few other species (Strzelec and Królczyk, 2004). The adverse climatic conditions do greatly effect pulmonate species i.e., *Albinaria caerulea*, which survives in adverse conditions as they can prevent themselves from desiccation by showing a series of morphological and behavioral characteristics that can support their survival during adverse climatic conditions as there is no correlation between the climatic conditions and biochemical variables (Giokas, 2005).

The shell size is directly proportional with some ecological factors as temperature and algal abundance while the callus thickness and shell size decreases with an increase in density of individuals (Takahiro, 2006). Elkarmi and Ismail (2007) studied the effect of the temperature of the water on the morphometrics, age and growth of two morphologically different species *Melanoides tuberculata* and found that the size and life of the snails in the hot water springs is almost twice greater than the snails of the pools having ambient temperature. While according to the Bergmann's rule there is a positive relationship between latitude and body size of the species which could not be confirmed for Northwest European snails (Hausdorf, 2003).

Chokor and Oke (2009) reported that the snail diversity is decreasing in Nigeria due to deforestation as they found that the number of species, rather than species composition, was greatly reduced in the sunny places as there was a highly significant difference in species number between shady places and in the sun plot. Sunlight and moisture content of the soil seems to a major impact on the composition of terrestrial gastropod community in dry and sunny habitats. However, the other important possible driving factors are relative humidity and temperature but not sunlight (Suominen, 1999). It was concluded that fire, plant species, plant number, herb cover, slope aspect, and habitat humidity are the important ecological factors that predict the snail abundance at the sites and also be used as biological indicator of ecological changes (Anna, 2005).

Sampling and the Quadrat Size

Manual searching is done while sampling within a quadrat during night and/or day of suitable microhabitats and is generally standardized with the help of searching effort and is written in the unit of person hours or per meter square (m²). For these studies the bulk method is generally followed i.e. beating of the specific number of branches over an inverted umbrella. The sampling may be carried out by random collection sieving and sorting of a specific amount of leaf litter or soil (Emberton *et al.*, 1996) or following the method of Cameron and Pokryszko (2005). The sampling may be carried out with a variety of ways. The living slugs and snails can be sampled at night for two person hour in circle with a radius of 3-m without disturbing the litter and returning the animals after identification (Bloch *et al.*, 2007).

The time for sampling and sampling site can vary as De Winter and Gittenberger (1998) sampled by spending two hours by two people in quadrats measuring 20 × 20 m quadrats in Cameroon. This can also be done by 0.5 h tree trunks searching, beating the vegetation and collection of the well defined amount leaf litter, which can be later dried, sieved and manual searching in the laboratory. A quadrat sampling measuring 4 × 2 m at the base of limestone rocks in west Malaysian karst forests were sampled with the help of collection of litter and topsoil which was later enriched with the help of coarse sieving, floatation, drying and further sieving (Clements *et al.* 2008).

Most of the scientists have adopted nearly same sampling methods for comparable results (De Chavez and De Lara, 2011; Cameron *et al.*, 2003; Schilthuizen and Rutjes, 2001; Winter and Gittenberger, 1998) while others are of the view that sampling strategies should be optimized to have a complete picture (Cameron and Pokryszko, 2005). Incidence- based completeness estimators (ICE) (Chao and Lee, 1992; Chazdon *et al.*, 1998) were computed by Liew *et al.* (2008) for hexaplets of quadrates samples in non-limestone and limestone

forests throughout Malaysia and it was found that there was a 90% completeness irrespective of the intensity of quadrat sampling, whereas the one which were far from limestone area were quite incomplete. Optimization of the sampling methods is of extreme importance for the analysis of the local species diversity studies for community-ecological purposes rather than comparing species diversity with other studies. An inventory of a quadrat is shows nearly a true status if all species actually present are found (Cameron and Pokryszko 2005). Species “presence” becomes an elusive concept when studying poor dispersers as land snails. The demes are extremely narrow and is less than tens of metres to less even in large-bodied tropical and subtropical land snails, whereas active dispersal rates range from 1-5 m (Schilthuizen *et al.*, 2005b; Giokas and Mylonas, 2004; Parmakelis and Mylonas, 2004; Schilthuizen and Lombaerts, 1994). The tropical snails communities are often dominated by complex thereby reducing heterogeneity much below the scale of standard quadrates.

In other words, a 20 × 20 m quadrat may contain 35 species, but only subsets of these may actually be coexisting in the sense that they share the same resources and potentially engage in ecological interactions with one another (MacArthur, 1965). It is important to keep in mind that such clumping in land snails is fundamentally different in character from similar patterns of micro-scale clumping in organisms with mobile individuals, gametes, or seeds; in the latter cases, micro-scale clumping of adults affects demographic and genetic population structure much less than in land snails, and some of the unevenness seen in adjacent quadrates along transects (Schilthuizen *et al.*, 2002) may thus be real rather than sampling artefacts. It also means that, in order to decide on the quadrat size adequate for capturing true alpha diversity in a certain habitat (Rosenzweig, 1995). The largest quadrat size that has an S not significantly different from q could be taken as the preferred quadrat size (Schilthuizen 2011) when fitted in Arrhenius equation $S = q + cAz$ (Rosenzweig, 1995).

Sampling Previous Generations

A very important issue which comes under consideration is the inclusion of the empty shells, when appear in a sample, at least in the studies on the non-acidic soils. Most of the scientists include empty shells while sampling necessarily as in the tropical forest it becomes really difficult to get live snails and slugs while some authors have limited themselves to the contemporary populations by sampling living snails only (Alvarez and Willig, 1993; Bloch *et al.*, 2007). Clements *et al.* (2008) and Schilthuizen *et al.* (2002), explained of sampling empty shells only while in other studies living snails along with empty shells were sampled. According to Schilthuizen *et al.* (2005a), the majority of the specimens in the Pristine and Bornean karst forest were the empty shells containing less than 1% living animals.

There are advantages and disadvantages of sampling empty shells, depending on the study objectives. Considering the advantages of the above mentioned sampling strategy is that almost the entire malacofauna can be obtained in an unbiased fashion because otherwise there will be a biasness towards the larger and thicker shelled species to survive weathering for much longer. These sampling methods have greatly supported to answer questions related to species- area relationships (Clements *et al.*, 2008). In the forest strata, generally all shelled snails and semi-slugs, end up as empty shells on the forest floor unless they live in enclosed spaces like phytotelmata (Kitching, 2000), nest ferns (Ellwood and Foster, 2004), or social insect nests (Eguchi *et al.*, 2005). Sampling shells from the forest floor is thus comparable in efficiency to insecticide fogging for canopy insects (Coddington *et al.*, 1991).

The changes in the species composition can be revealed, making comparisons between the present day species with the previous diversities and can understand the level of changes in the species composition. Empty shells are considered to be the representatives of the contemporary malacofauna, which is only possible if the shells are younger than an average snail generation (Cernohorsky *et al.*, 2010).

Basically the rate of degradation of shells on the forest floor is still unknown. According to Cameron and Pokryszko (2005), the empty shells which are present in the calcareous areas may be actually a part of ancient or subfossils, while Solem (1984) states that the rate of shell decay is less than ten days when the forest floor litter is wet and occur in a period of three weeks during dryer periods.

The places where land use has been majorly changed, the presence of old specimens in the sample is particularly problematic (Schilthuizen *et al.*, 2005a), which is quite important for biogeographical related problems specific to species- area relationships (Clements *et al.*, 2008). The effect of logging, quarrying and forest to plantation on the snail community has been studied with the help of the inclusion of empty shells in the samples (Oke *et al.*, 2008; Schilthuizen *et al.*, 2005a; Tattersfield *et al.*, 2001b). The impacts of these disturbances can be underestimated if the samples include the remains of the fauna from predisturbed time. The same is in the case of vertebrates as their bones are left as remanants of predisturbed times (Terry, 2010a; 2010b). The empty shell species can be compared with contemporary species to calculate diversity in an area and if there is no difference and other procedural problems then the samples can be pooled (Rundell and Cowie, 2004; Cameron and Pokryszko, 2005) and can be considered the present-day populations.

In the field of conservation biology the roles of systematics are well understood and accepted for many organisms. The role of systematics and taxonomy for the species protection and management of freshwater gastropods has not been reviewed. A thorough review of literature in systematics and taxonomy of freshwater gastropods, with recent examples in the International scenario, to identify the key roles of these fields in species delineation, taxonomy and final designation of conservation management units is discussed (Perez and Minton, 2008).

Defining the Theoretical Conservation Management Unit

The major pillar for the increase in the success of conservation measures is the identification of the appropriate conservation management unit. The populations and species which need to be protected, as they have unique life history and are genetically diversified that helps them to adapt according to their environment and other historical events, are called conservation management unit (Perez and Minton, 2008). The taxonomist does not have to undertake routine identifications of materials collected during archeological or ecological collections.

However it is the duty of an ecologist to carry out such expeditions, who wants the material to be identified. Primarily the taxonomists have to draw clear boundaries (taxonomic entities) across vague outlines. The major responsibility of the taxonomists is to provide scientific names and data so that there can be the development of conservation guidelines by the lay men, agency workers, decision makers and legislators. Another responsibility of the taxonomists is to convey these scientific changes to the end users as these change are for good reasons, these changes in name is a sign of better understanding of a group of organisms or single organisms.

These changes resulting in instability brought about due to additional data, must be understood and willingly accommodated by the managers. To grant protection status and proper recognition of managements units, different organization working at the national level frequently conduct reviews of species, group taxonomy and natural history of these species (Nicholopoulos, 1999, COSEWIC, 2006). It is a major responsibility of a taxonomist to identify synonym species and summarize the already available accumulated knowledge. Unfortunately, a several scientific species give umbrella to single species which makes the inventory difficult due to which scientific names change for several reasons. There are few reasons due to which name changes become a necessity. Firstly a species may have been described by different scientists and resultantly even named more than once as they belong to different sexes, geographical regions or due to lack of knowledge of earlier descriptions.

The conservation plans are implemented prior to the appropriate definition of management units, which can give very negative results for the conservation of species (Awise and Nelson, 1989; Greig, 1979). The conservation plan was established prior to the research on freshwater snail *Leptoxis crassa anthonyi* (Minton and Savarese, 2005), which clearly indicates that the units of the conservation management (USFWS, 1997), which were inconsistent with the evolutionary history of population while they had been established on the basis of the assumption that the genetic and geographical distribution were correlated (Minton and Savarese, 2005). Field survey resulted in establishment of a number of new records during Pennsylvania Land Snail Atlas Project. These new records (104 specimens) were not introduced species and were likely having been simply overlooked. The conservation is mainly focussed on the forest species and species like *Gastrocopta procera* which are open field species are not included or are undersampled. These new records indicate the dire need for surveys so that the gaps in knowledge about land snail distributions can be filled (Pearce, 2010).

The freshwater snail literature includes examples working plans for conservation of fresh water snails with detailed information about the taxonomic work. The recent studies on *Hydrobiidae* (Ponder *et al.*, 1989; 1993; 1994; 1995; 2000; Hershler, 1994; Hershler *et al.*, 2007 a,b; ; Hershler and Liu, 2004; Hershler and Ponder, 1998) has helped understanding of the taxonomy and systematics of these species. For example, the two separate management units were distinguished i.e. *Assimineia cienegensis* from *Assimineia pecos*, for conservation with the help of new molecular data as well as the morphological and conchological studies, when studied with the help of electron microscope (Hershler *et al.*, 2007a) a rare as well as disjunct populations in the Cuatro Cie'negas basin, Coahuila (Mexico).

The most of the taxonomic studies on the snails are done on the basis of shell measurements characters, while further work on gastropods has revealed two problems about species delineation with this approach.

Plasticity in the shell character in the gastropods makes the demarcation of the taxonomic unit difficult. Secondly, there is a great chance of the exclusion of morphologically cryptic, valid species from the list. Therefore for the accurate identification of the species there is the need of methods which reveal the taxonomic status in more detail, in addition to shell morphology (Mayden, 1997; Adams, 2001).

The precise description and demarcation of species on the basis of its distinguishing characters, helps to understand its systematic relationship of the group under study. The taxonomists are relying on the biological species concept, abbreviated as (BSC), for many years, which supports identification of species on the basis of reproductive isolation (Dillon *et al.*, 2002, de Queiroz, 2005a). Later faults and failure in the biological species concept to meet the standards were suggested (Wheeler and Meier, 2000) and species concept based on lineage such as unified (de Queiroz, 1999; 2005b) and the phylogenetic (Cracraft, 1983) species concepts became popular.

The freshwater gastropods literature also includes examples of thorough systematic work that has extremely supported and has showed fruitful results in conservation plans of freshwater gastropods (Holznagel and Lydeard, 2000; Mulvey *et al.*, 1997; Lydeard *et al.*, 2000; Minton and Lydeard, 2003; Roe *et al.*, 2001; Roe and Lydeard, 1998). The species cannot be identified on the basis of morphological characters only while species identification on the basis of lineage-based concepts is extremely valueable (Wilke and Falniowski, 2000).

The identification on the basis of the lineage based method considers species as independent evolving units without considering the criteria on the basis of which the species has been described and delineated. The description of species on the basis of lineage gives more understanding and recognition about the management units corresponding with the subspecies level approach (IUCN, 2001). According to the United States Endangered Species Act (USFWS, 2003) protection should be provided at the subspecies level rather than the distinct populations.

In case of the countries like, Canada (COSEWIC, 2006) and Australia (Australian Government Attorney-General's Department 1999) the protection must be extended to smaller, groups at the subspecific level. When addressing taxa at the subspecific levels groups that are of evolutionarily significance (Ryder, 1986) are of prime attraction; to be considered as it relies more on the populations that are genetically and morphologically distinct populations rather than on a specific concept. There are a few questions, which arise regarding level of distinctness, in the context of evolution (Pennock and Dimmick, 1997).

There is the development of very low level of agreement as the protection at and below species level has been recognized by most of the conservationists. The best approach applicable to delineate the units for conservation management allows the flexibility with reference to the organism; however, specific exceptions are there and existence of few changes is to do systematic studies in combination with the population genetic data regardless of the taxonomic level. This helps to recognize more valid species (Pfenninger and Magnin, 2001; Ponder *et al.*, 1994) and as well in policy making for conservation management units. The major benefit of modern systematics is the increased objectivity and recognition of biodiversity (Isaac *et al.*, 2004; Wheeler and Cracraft, 1996). The goal of defining and identifying species can be accomplished scientifically with the help of lineage-based approach to species delineation which helps to reduce the negative effects of statistical errors (Mc Garvey, 2007).

Species Abundance Distributions

The researchers of the tropical land snail expressed and demonstrated the patterns of intraspecific rarity and commonness that have been found (Oke and Alohan, 2006; De Winter and Gittenberger, 1998; Schilthuizen *et al.*, 2002; 2005a; Schilthuizen and Rutjes, 2001). Species abundance distribution has been a point of interest for the scientists and researchers due to many reasons. This is because in this way the community can be understood in a more better way rather than by just counting the species,

as in this way heterogeneity and abundance can be incorporated, which is the basis for the calculations (Magurran, 2004). Secondly in understanding species abundance distribution, the rare-species tail can also provide information regarding the estimation species number missed giving true scenario of species richness.

Chao (1984) estimated the number of singletons and doubletons in the sample with the help of a simple non parametric estimator. Various scientists found high percentages of singletons i.e., (23%, 12%, and 11%) (Fontaine *et al.*, 2007b; De Winter and Gittenberger, 1998; Liew *et al.*, 2010) with high estimations of richness. Thirdly, the species abundance distribution helps us to understand changes in species dominance induced due to the season (De Winter and Gittenberger, 1998) disturbance on the basis of changes in physical factors (Schilthuizen *et al.*, 2005a) and to deduce the ecological processes developing the community structure. The log normal distribution seen in the natural communities including gastropods can be approached by a model (Sugihara *et al.*, 2003; Sugihara, 1980) for the subdivision of niche space in sequence.

A sigmoid shaped curve is formed in a Whittaker plot when a log normal distribution was calculated. Whenever there is the over representation of the rare species a distinct right-skew is often observed (Tokeshi, 1999). This niche free model can predict the skew in a better way (Hubbell, 2001) as it fills the slots randomly in a community. The predictions of particular models was tested by the goodness of fit to understand the potential ecological processes that structures the communities for some of the non-tropical and tropical land snail (Cameron and Pokryszko, 2005). However species abundance distribution studies available in the literature largely suffer from biased sampling methodology. There are many patterns which are quite similar in properties with that of SAD (species abundance distribution) which are present in nature which can lead to complex dynamical properties. This limits to understand the ecological structuring processes from species abundance distribution alone (Nekola and Brown, 2007).

These models are mostly applicable to only one guild in a same community, which in case of the gastropods needs a little more elaboration. If the spatial scale for the determination of species abundance diversity is quite large then the chances of obtaining patterns due to ecological reasons rather than statistical reasons declines rapidly (Sizling *et al.*, 2009). A community of species which are performing ecological function that are similar (Rosenzweig, 1995); and they compete for almost the similar resource (Hubbell, 2001) is called a guild. It would be probably wrong to consider tropical terrestrial gastropods communities as guilds. Although meager knowledge about tropical terrestrial gastropod' autecology is available and there are many predators in the African faunas (Wronski and Hausdorf, 2008; De Winter and Gittenberger, 1998), which are in high numbers including fungivores, foliovores and detrivores, even considering the non-molluscivores, yet there is another factor to be considered and measured, is its body size.

The snail's body mass varies in several magnitude throughout its life, and there are stronger ecological interactions in the individuals of the similar size so animals of different sizes should be considered as separate communities as individuals of the same size have stronger ecological interactions so different size classes should be really considered as different communities. This gives us an idea that there is a difference in the community of the juveniles and adults. This is not necessary that this community is composed of land snails only, rather this community may include other invertebrates with low mobility e.g. isopoda, diplopoda and certain coleopteran, and these snails will have a stronger competition than it would have with other snails, as occurs in the rocky intertidal community in marine invertebrates (Connell, 1961; Wootton, 1994).

The shells of the molluscs can be easily collected and studied due to which it has been a source of extreme attraction by professional and amateurs for the molluscan alpha taxonomy. However, phenotypic plasticity mostly results in differences in shell morphology due to which past descriptions were often unjustified.

The plasticity in the phenotype due to ecosystem variation poses a difficulty in morphospecies identification (van Damme and Pickford, 2003) due to which adult shell shape and ornamentation become unreliable characters. The same difficulty arises when identifying on the basis of radula morphology (Michel, 1994), due to this methods are needed which give evidence of the separate species besides morphological methods.

A system based on shell morphology and colour was devised by Samadi *et al.* (1999), which were later supported molecular investigations and this system of morphological identification system was used by Facon *et al.* (2003) and Genner *et al.* (2004). The understanding of importance of molecular methods taxonomy was done by Facon *et al.* (2003). They studied the invasion of *Melanoides* of the New World using molecular methods and suggested that these morphs were paraphyletic and the two morphs camouflaged in Lake Malawi (Genner *et al.* 2004) and according to the authors they originated from Southeast Asia.

This has been supported by development of molecular DNA markers to find the genetic relationships among taxa at a higher and more advanced level to get information about their phylogeny and biodiversity and they can be further used to solve general biological problems (Kupriyanova, 2000; Antonov, 2000). The cases of synonymization of species in molluscs are probably higher as compared to other marine invertebrate phyla as a result of number of genetic studies. There are examples of the *Nautilus* (Wray *et al.*, 1995) and *Donax* as studied by Adamkewicz and Harasewych, 1996 that clearly show the fact that there may be cases where genetic data does not support drastic differences between taxa that are sympatric on the basis of shell morphology. While on the other hand, very less morphological differences in shell or other various characters have been confirmed by the genetic data to be important taxonomically (Izuka *et al.*, 1996; Borsa and Benzie, 1993; Johnson and Cumming, 1995; Oliverio, 1995; Mokady *et al.*, 1994; Parsons and Ward, 1994; Thollessen, 1998; Sanjuan *et al.*, 1997).

In molluscs, there are very less chances of the discovery of morphological similar groups which are extremely divergent, however it may occur; for example, in cephalopods (Yeatman and Benzie, 1994) in the deep sea (Craddock *et al.*, 1995; Peek *et al.*, 1997). In allopatric studies, the genetic and environmental basis for the differences are mostly confused and the uncertainty in the taxonomy on the basis of shell variability is worsened. In some taxa there is a very less or no genetic differentiation when comparing in same geographic entity while on the other hand a large amount of genetic differentiation is found taxa, at generic level (Adamkewicz and Harasewych, 1996; Côte-Real *et al.*, 1996a, b).

There are zones of overlap along continuous coastlines, which are narrow may have a little evidence of hybridization providing definite evidence for species boundaries (Liu *et al.*, 1991; Ridgway *et al.*, 1998), while sometimes quite complex patterns of hybridization are observed. Using a variety of approaches in studying several molluscan genera have helped to reduce the complexity of the results making them more understandable. Depending on the taxa and geographical locale, a variety of patterns of hybridization have been revealed in studies of *Mytilus* (Beynon and Skibinksi, 1996; Quesada *et al.*, 1998; Gardner, 1996; Comesaña *et al.*, 1999).

The importance and actuality of the molecular markers in the field of taxonomy can be understood from its application. On the other hand using different molecular markers may lead to a conflict, due to different reasons, within the views of the zoologists about the morphological taxonomy. The complexity of the molecular data analysis is explained by modeling of the evolutionary processes, on the basis of the various assumptions, which proceed at unequal and nonuniform rates in different taxa. However some scientists think that with the advent of the sequencing of human genome the significance of molecular marker studies has been diminished, while others consider it to be a huge task when comparing with the identification with the help of molecular markers reducing the comparison of genomes to that of analysis of genome parts (Lavrentieva, 1999).

The molecular biologists working on the evolutionary perspectives of the species need to learn about the cladistic approach in systematics (Pesenko, 1989). The traditional taxonomists accomplishing the task with the help of cladistics develop phylogenetic relationships among elementary evolutionary units, and are unable to suggest a better method. The satisfaction is achieved when the conclusions drawn from both approaches lead to a common end. However, if the results do not coincide then the preference is given to those more reasonably integrating the molecular and morphological results.

Population and Evolutionary Genetics

In case of the cryptic and ambiguous characteristics the taxonomic identification becomes extremely complex for which the use of molecular markers becomes the need and have been proved to be a useful (Park *et al.* 2005, Miura *et al.* 2005, Westheide *et al.* 2003, Douek *et al.* 2002). The RAPD-PCR, Randomly Amplified Polymorphic DNA-Polymerase Chain Reaction, technique is a comparatively cheap and simple method capable of differentiating taxa without the the information about their genomes (Welsh and McClelland 1990, Williams *et al.* 1990).

RAPDs are dominant markers that result from the use of short (10 bases long) primers (synthetic oligonucleotides) of random sequence that can amplify multiple segments of genomic DNA by PCR. The number of segments depends on the number of sites of the genome recognized by a particular primer. The main reason for the success of RAPD analysis is the gain of a large number of genetic markers that require small amounts of DNA without the need of a molecular characterization of the genome of the taxa under study. Species in which such markers were used include oyster genera *Crassostrea*, *Saccostrea*, and *Striostrea* (Klinbunga *et al.*, 2000), amphipods (Gammarus: Costa *et al.*, 2004) and the tropical abalones *Haliotis asinina*, *Haliotis ovina* and *Haliotis varia* (Klinbunga *et al.*, 2004).

The RAPD technique has received a great deal of attention from population geneticists (Hedrick, 1992) because of its simplicity and rapidity in revealing DNA-level genetic variation. The utility of RAPD markers in the phylogeny of cichlid fishes (Sultmann *et al.*, 1995) and the genus *Xiphophorus* (Borowsky, *et al.*, 1995) gave support to classical hypotheses of their phylogenetic relationships. RAPD markers have been successfully used to detect genetic variability in *Gliricidia* (Chalmers, *et al.*, 1992) mosquito species and populations (Kambhampati *et al.*, 1992) closely related species of black Aspergilli (Megnegneau, *et al.*, 1993), cocoa (Russel *et al.*, 1993.), medfly (Baruffi, *et al.*, 1995) and parasitic protozoa (Tibayrenc *et al.*, 1993). Species-specific markers were developed in species and strains of microorganism (Welsh and McClelland, 1990; Skibinski, 1994; Fani *et al.*, 1993). Clone-specific markers have been identified in hydroids (Hadrys *et al.*, 1992) and in fungal mycelia (Smith *et al.*, 1992).

The technique has also been used to study genetic variation in several fish species. Bardacki and Skibinski, (1994) and Naish *et al.*, (1995) used RAPD markers to discriminate between commercially important tilapia species, subspecies and strains of tilapia. RAPD markers were also generated for several tropical fish species representing 7 families (Dinesh *et al.*, 1993). Furthermore, RAPD analysis revealed high levels of genetic variation among individuals from the same broodstock of sea bass (*Dicentrarchus labrax*) (Allegrucci *et al.*, 1995). Finally, 721 strain-specific RAPD markers were identified in 2 laboratory strains of zebrafish (Johnson *et al.*, 1994). RAPD markers unique to individuals from one species within a genus will be species-specific (inter-specific). Similarly, genus-specific markers can be generated if the fragment is a unique polymorphism to individuals belonging to a certain genus. Species-specific markers can be used in inter-specific gene flow and hybrid identification. Similarly, population-specific markers will be useful in identification of hybrid populations (Hadrys *et al.*, 1992).

Inter-specific gene flow was shown between two *Iris* species, *Iris hexagona* and *I. vulva* using species-specific RAPD markers (Arnold *et al.*, 1991). F1 hybrids from different inbred lines of maize were identified using AP-PCR (Welsh, *et al.*, 1991). RAPD polymorphism detected among individuals within a given species has been used to determine paternity and kinship relationships in large progenies of dragonfly (Hadrys *et al.*, 1992).

In this study, *Anax parthenope* males guard ovipositing females. It was suggested that the male might guard a female in order to assure a subsequent mating rather than immediate fertilisation. RAPD analysis of several unrelated males, the guarding male, the guarded female and offspring clutches demonstrated that the guarding male was the father. A specific RAPD marker was found in the guarding male and in the offspring but was rare in the population as a whole. Parentage analyses with RAPD markers are based on the presence of diagnostic markers (present in only 1 of the putative parents) in the offspring.

A high frequency of non-parental RAPD bands has been reported in primate pedigrees (Riedy *et al.*, 1992). However, Scott *et al.*, (1992) found much lower frequencies of non-parental RAPD bands in beetles (*Nicrophorus tomentosus*) and strawberries (*Fragaria vesca*). The presence of species-specific RAPD characters makes it possible to use these markers in studying hybridization in the context of speciation. The data on individual variation of parental populations are required for experiments on interspecific hybridization upon investigating, e.g., reticulate evolution. The probability of speciation through cross evolution is particularly high for plants (Grechko, 2002).

The genital differences in *Zonitoides nitidus* and *Zonitoides excavatus*, are not clearly found but there are clear differences in the shell morphology. A great variation has been revealed by using (RAPD) markers in *Zonitoides excavatus*. Sperm transfer has not been observed to be completely absent.

The species status of both taxa is supported by the strong genetic differentiation suggested by allozyme and RAPD data (Jordaens *et al.*, 2003). Due to this it has become extremely important to use molecular techniques so that they can be identified with more accuracy. Histone gene primers have been developed for land snails and it has been observed that land snails and bivalves seem to differ in their histone gene organization. Nucleotide polymorphism is of significant importance to understand phylogenetics and systematics of closely related species and genera. The coding regions exhibit no amino acid substitution among land snail species (Armbruster, 2005). Apparently, one can expect to find any locus among RAPD fragments depending largely on the primer specificity. Thus, if adequately used and interpreted, the RAPD method is an informative modern tool for studying genetic diversity of species in nature.

Most of the studies on the land snails have been carried out approximately a century before in the Indian subcontinent. Hutton (1842) collected and studied the land snails of neighborhood of Bolan pass, from Suliman range and the hills of south laying west to Indus. According to the Blanford and Godwin (1908), in the areas of Sawat, Dir or Chitral no terrestrial snails have been observed except *Petraeus* snails, while in the Kuram valley only very few species were found.

They worked chiefly on the conchological side of the families Testacellidae and Zonitidae of Indian subcontinent. Pokryszko *et al.*, (2009) collected 3500 dry shells and described 22 species out of which 12 were new species from 77 localities. The specimens were preserved in alcohol. The shell variations in most species were described and nine species were illustrated with figures of detailed reproductive system. In the Northern area of Pakistan there is highest diversity of Pupilliods due to wetter climate and wider altitudinal range. Ten out of twenty two species were considered to be endemic to Pakistan, while the distribution of the rest of species was extended to other regions i.e. Asia, Europe and Holarctic. Pupilliod fauna showed great diversity of Palaearctic/Holarctic influence on general.

Recently 329 specimens of snails were collected by Ali (2006) from sugarcane fields of village Gatti Faisalabad pertaining to 6 families and 9 genera belonging to order Pulmonata. Recently in a study done by Altaf (2016) the snails in the agroecosystem of Faisalabad were found to belong to two suborders, seven families, nine genera, fifteen species out of which six species have been reported first time in this region. Molecular characterization of snails was done with the help of randomly amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) technique for understanding the biodiversity in this region. On the basis of Analysis of Molecular Variance there was found 11% variation among populations of the five habitats and 89% variation within populations in species population found in all the habitats. Genetic similarity among snail species was estimated by Nei's genetic similarity indices showing a range of 0.5 to 0.74.

Maximum genetic similarity was found between *Ariophanta belangeri bombayana* and *Ariophanta bistrialis taprobanensis* as well as *Ariophanta belangeri bombayana* and *Ariophanta solata*. Minimum genetic similarity based on Nei's genetic indices was observed among *Cerņuella virgata* and *Ariophanta bistrialis cyix*. Considering the *Zoectecus insularis* and *Juvenile Zoectecus insularis* they are quite distant from each other in the cluster due to which it is expected that might belong to some new species, which need further investigations. An overall trend of distribution was that we progress towards south, the diversity of the snail species in Faisalabad gets highly reduced. The diversity indices in all the habitats of the agroecosystem were highly significant while in ditches the results were non-significant.

In the light of above meagre scientific studies we can say that the studies related to the faunal distribution of snails have been ignored and there is a scanty and scattered knowledge about the snails in this area of the world.

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