Pranikee- Journal of Zoological Society of Orissa, ISSN 0970-4450
Volume XXVI, 2014, pp 1-12

ISSN 0970-4450

PRANIKEE

Journal of Zoological Society of Orissa

Volume- XXVI
2014

ISSN 0970-4450

PRANIKEE

Journal of Zoological Society of Orissa

Post Graduate Department of Zoology
Utkal University, Vani Vihar
Bhubaneswar - 751 004, Odisha, India

Volume XXVI, 2014

The emblem of Pranikee

The emblem "***NABAGUNJARA***" is a chimeric animal and a common motif of Odishan art and literature. It literally means "Nine form". This form has been described by poet Sarala Das in the Odia version of the epic Mahabharata. Apparently, Lord Krishna appeared in Nabagunjara form consisting of the body of an elephant, a leg each of a horse, a deer and a tiger respectively; throat of a peacock, tail in the form of a serpent, waist of a lion, hump of a bull and head of a cock, to fool his friend Arjuna. The Chimera was holding a lotus flower in a human hand. Arjuna had never seen such a creature in his life and guessed that this could not be a real animal but a form assumed by Lord Krishna and immediately bowed down at his feet. It is said that the human hand with the lotus provided the clue. In the paintings and sculptures however, the lotus is often replaced by a "Chakra" or the "stylized discus" of Lord Krishna. Chimeric forms are encountered in literature and art all over the world. However, a chimera of nine animals is uniquely Odishan. Therefore, it was considered to be an appropriate emblem for the Journal of Zoological Society of Odisha.

Padma Shri Prof. PriyambadaMohanty-Hejmadi
Former Editor

Zoological Society of Orissa

PRANIKEE

Journal of Zoological Society of Orissa

Abbreviation: Pranikee

Volume 26, Year-2014

CONTENTS **Page**

Research Articles

MORPHOLOGICAL EVOLUTION IN FROGS OF THE GENERA *FEJERVARYA*, *MINERVARYA*, *SPHAEROTHECA* AND *ZAKERANA* (DICROGLOSSIDAE)

Annemarie Ohler[1*], Sushil K Dutta[2] and Alain Dubois[1]

[1]Institut de Systématique, Évolution, Biodiversité, ISYEB –UMR 7205 – CNRS, MNHN, UPMC, EPHE, Muséum national d'Histoire naturelle, Sorbonne Universités, 57 rue Cuvier, F-75005, Paris, France.

[2] Nature Environment and Wildlife Society, Angul, Odisha, India.

[*]Corresponding author: Email: annemarie.ohler@mnhn.fr

ABSTRACT

Morphological differentiation measured by multivariate analysis of size corrected distance measurements was used to evaluate morphological evolution within the frogs referred to the "genera" *Fejervaya*, *Minervarya*, *Sphaerotheca* and *Zakerana*. Various phylogenetic relationships have been proposed between these four groups which either a monophyly or a paraphyly of *Fejervarya* and *Zakerana*, and either recognition of two genera or a single genus. Considering an adaptive approach to define genera, morphological differentiation is used as a measure for ecological adaptation. The results show absence of morphological differentiation between frogs referred to *Fejervarya* and *Zakerana*, but differentiation of *Minervarya* and *Sphaerotheca*. As no strong support for phylogenetic relationships between these groups is available, *Zakerana* should be considered a subgenus of *Fejervarya*, but *Minervarya* and *Sphaerotheca* maintained as genera.

Key words: Morphometry, genus concept, classification, Dicroglossidae.

INTRODUCTION

Studies in systematics made important progresses when methods for reconstructing phylogeny were developed (Hennig, 1950). In the recent decades, this conceptual advance combined with computer programmes allowed to analyse large datasets obtained by gene sequencing. This generated more and more robust hypotheses about phylogenetic relationships of a great number of taxa (Frost et al., 2006; Pyron and Wiens, 2009).

To transpose these new phylogenetic hypotheses into classifications, multi-evidence approaches named “integrative taxonomy” and “economy of change” were proposed (Dayrat, 2005; Vences et al., 2013). It is generally accepted that classifications should represent phylogenetic relationships and that these phylogenetic relationships are of hierarchical nature (Hennig, 1975). Beside these scientific aspects, taxonomy, the science that gives names to living kinds, has a much broader application as it allows retrieval of information. Thus, taxonomies are used in all fields of biology and also by national and international institutions and social fields (like trade, customs or conservation biology) that need such names.

One of the criticisms of this system is the usage of hierarchical units such as class, order, family, genus and species and various subunits which are not equivalent between groups. There is a large discussion in scientific literature about the species level with more than 20 species concepts recognized. Nevertheless, this basic unit of classification is generally accepted. But concerning the other hierarchical levels few theoretical approaches exist (Dubois, 1988b, 2008).

Inger (1958) proposed using an “adaptive approach” to define genera and provided concrete examples in amphibians. This approach proposes to correlate generic limits with adaptive zones and to consider various data on the morphology, genetics and also ecology and adaptation of the species to define genera. This proposal has rarely been applied to a classification because data on ecology and adaptations on most groups of animals, including amphibians, are too scarce and imprecise to allow robust definitions of groups.

Morphometric analysis of body measurement data allows to obtain a representation of the morphospace (Wills and Fortney, 2000) occupied by a group and to be compared to other such morphospaces. This method allows obtaining quantitative data on numerous characters on different body parts and can, thus, be considered a representation of the morphological adaptation (Dubois, 1988a). The method does not require a priori interpretation of the character states as adaptations to

particularecological niches. It measures similarity within a group and difference with other such groups. Morphometrical studies on species of several ranid genera showed a discontinuity in variation between groups that were considered genera (Ohler and Dubois, 1999).

We here propose to combine morphometrical analysis and phylogenetic hypothesis to make genus level proposals. The adaptive approach of the genus concept is used as a test, with the hypothesis that the species grouped in a genus occupy a morphospace. The groups resulting from phylogenetic analysis are used as units in a morphological analysis to test their morphological differentiation. This methodology is here applied to the classification of a group of dicroglossid genera that show various degree of morphological differentiation (Fig. 1): *Fejervarya* Bolkay, 1915 (16 species), *Minervarya* Dubois, Ohler and Biju, 2001 (2 species), *Sphaerotheca* Günther, 1859 (5 species) and *Zakerana* Howlader, 2011 (20 species).

Various relationships have been proposed for these genera. The tree published by Frost et al. (2006) proposed *Fejervarya* to be polyphyletic. Djong et al. (2007) obtained two monophyletic groups within *Fejervarya* that they informally named “large” and “small” groups. These groups show large geographical overlap. Kotaki et al. (2008) obtained conflicting relationship between south Asian and south-east Asian *Fejervarya* and *Spaerotheca* with mitochondrial and nuclear gene sequences. Mitochondrial genes support a monophyletic *Fejervarya* with *Sphaerotheca* as outgroup, whereas *Sphaerotheca* is within *Fejervarya* when using nuclear genes. Other studies show a sister group relationship of south Asian *Fejervarya* with *Sphaerotheca* (Kotaki et al., 2008) but this is poorly supported (Hasan et al., 2014). However, the monophyly of south Asian and south-east Asian *Fejervarya* is also statistically supported (Kotaki et al., 2010). Pyron and Wiens (2011) obtained a well supported monophyletic *Fejervarya* grouping south Asian and south-east Asian clade with *Sphaerotheca* as sister group.

When Howlander (2011) described the genus *Zakerana* (type-species *Rana syhadrensis*) for the south Asian frogs, the evidence was based on the results of Frost et al. (2006) without considering the conflicting results of the other authors. Additionally, including species into this genus was based on poor evidence, i.e., a single morphological character, body size, and geographical origin.

Molecular data on *Minervarya* are scarce. Kuramoto et al. (2007) proposed a phylogram of *Fejervarya* based on mitochondrial DNA from the Western Ghats positioning *Minervaryasahyadris* (previousely mentioned as *Fejervaryasyhadrensis*) within the group and transferred the species to *Fejervarya*.

Based on the hypothesis that the south-Asian and the south-east Asian *Fejervarya* have an independent phylogenetic history that could have led to different morphological adaptation, samples of specimens allocated to species representing the genera *Fejervarya*, *Minervarya*, *Sphaerotheca* and *Zakerana* were measured to obtain a multivariate representation of the morphological variation within and between these groups. Taxonomic consequences will be discussed.

MATERIAL AND METHODS

Specimens studied

Fejervarya limnocharis (Gravenhorst, 1829). Indonesia. Sumatra: FMNH 256752-53, males; FMNH 256769, male; FMNH 256768, female; Kalimantan: FMNH 256739, male; FMNH 256740, female; Java: RMNH 4287, female, neotype of *Rana limnocharis* Gravenhorst, 1829; FMNH 256727, male; FMNH 256729, male; FMNH 256790, male, FMNH 256721, female.

Minervarya chilapata Ohler, Deuti, Grosjean, Paul, Ayyaswamy, Ahmed and Dutta, 2009. India. West Bengal: ZSIC A10784, ZSIC A10787-88, ZSIC A10794, males, ZSIC A10791-92, females.

Minervarya sahyadris Dubois, Ohler and Biju, 2001. India Karnataka: MNHN 2000.3027-2029, females; MNHN 2000.3033, male; MNHN 2000.3034, female; SDB 708, male.

Sphaerotheca rolandae (Dubois, 1983). Sri Lanka: BMNH 1877.3.9.8, male; BMNH 1877.3.9.2, female and BMNH 1877.3.9.7, female, paratypes of *Rana (Tomopterna) breviceps rolandae* Dubois, 1983; BMNH 1973.3024, female, holotype of *Rana (Tomopterna) breviceps rolandae* Dubois, 1983.

Sphaerotheca breviceps (Schneider, 1799). India. Tamil Nadu: ZMB 3351-1, syntype of *Rana breviceps* Schneider, 1799; BMNH 1947.2.28.55, syntype of *Sphaerotheca strigata* Günther, 1859; BMNH 1947.2.28.58, syntype of *Sphaerotheca strigata* Günther, 1859; Uttaranchal: MNHN 2003.0791 Kerala MNHN 2003.1103 Karnataka: BMNH 1947.2.28.45, holotype of *Rana dobsoni* Boulenger, 1882. Nepal: MNHN 2003 and 0794MNHN 2003.3096

Zakerana nepalensis (Dubois, 1975). Nepal: MNHN 1975.1678, male.

Zakerana nilagirica (Jerdon, 1854). India. Tamil Nadu: MNHN 1984.2340, neotype of *Rana nilagirica* Jerdon, 1854, male; MNHN 1984.2334, 1984.2336, females.

Zakerana pierrei (Dubois, 1975). Nepal: MNHN 1975.1680, male, holotype of *Rana pierrei*; MNHN 1975.1688, female; MNHN 1975.1704, male.

Zakerana rufescens (Jerdon, 1853). MNHN 1984.2348, neotype of *Rana rufescens* Jerdon, 1853, male; MNHN 1984.2345, 1984.2347, females.

Zakerana syhadrensis (Annandale, 1919). India. Karnataka: MNHN 1989.2150, male; MNHN 2989.2151, female.

Fig. 1 Specimens belonging to the proposed genera *Fejervarya*, *Minervarya*, *Sphaerotheca* and *Zakerana*. From left to right, up to down: *Fejervarya limnocharis*, Vietnam; *Minervaryachilapata*, West Bengal, India; *Sphaerotheca dobsonii*, Odisha, India; *Zakerana syhadrensis*, Nepal.

Measurements

A total of 12 measurements were taken from 48 specimens either with the slide calliper of with an ocular micrometer. These measurements were chosen in order to estimate variation in size and shape of overall morphology. FOL – foot length (from the base of the inner metatarsal tubercle to the tip of the toe); HAL – hand length (from the base of the outer metacarpal tubercle to the tip of the toe); HL – head length (from the back of the mandible to the tip of snout); HW – head width; IBE – distance between the back of the eyes; IMT – length of inner metatarsal tubercle; ITL – inner toe length; MN – distance from the back of the mandible to the nostril; SVL –snout-vent length; TL – tibia length; TYD – greatest tympanum diameter; TYE – distance from tympanum to the back of the eye.

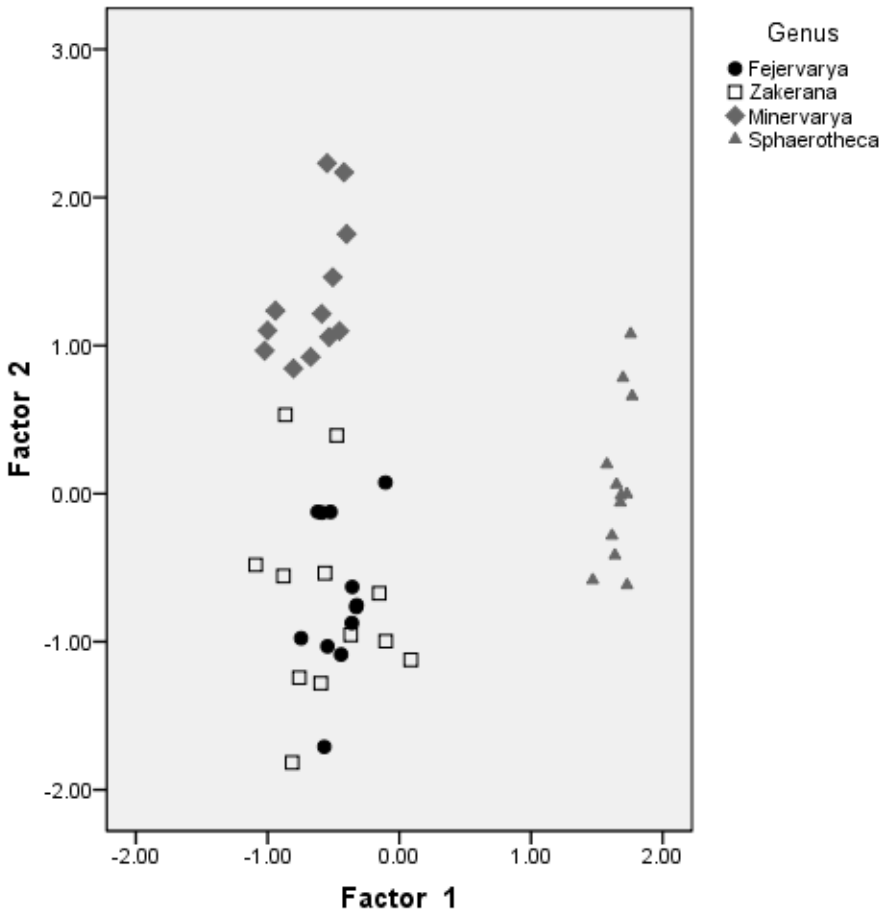

Fig. 2 Principal Component Analysis of 48 specimens belonging to the genera *Fejervarya*, *Minervarya*, *Sphaerotheca* and *Zakerana* obtained by analysis of 12 sizes corrected measurements. Factor 1 and Factor 2 are represented.

These measurements were corrected for size by dividing each measurement by the mean value of all measurements of a specimen and then transformed into the logarithm (Mosimann, 1970). Principal factor analysis was performed with the transformed variables and factors larger than 1 were retained for further statistical analysis and presentation as scatterplot. These factors were tested with Kruskal-Wallis for showing homogenous distribution. The factors rejected by Kruskal-Wallis were tested by Oneway Anova using ranked variables to obtain pairwise comparison. Tukey b was performed to propose statistically significant subgroups.

RESULTS

Three factors obtained by Principal Component Analysis have values higher than 1. They present a cumulative variation of 81.1 % (Table 1). The following variables have the highest loadings in Factor 1: HW, TL, TYD, IMT and ITL, whereas in Factor 2 the variables HL and MN, have the highest loadings. Kruskal-Wallis test shows significant differences between genera for first and second factor (Factor 1: Chi^2 = 28.4; df = 3; p = 0.000; Factor 2: Chi^2 = 32.2; df = 3; p = 0.000). Post hoc analysis using Tukey B proposes 2 groups for Factor 1, thus, pooling *Fejervarya*, *Minervarya* and *Zakerana*, and 3 groups for Factor 2 (Table 2), thus, pooling *Fejervarya* and *Zakerana* but separating *Minervarya*. *Sphaerotheca* is morphologically different from the other generaconcerning both factors.

Table 1 Results of Principal Component Analysis including 12 size corrected measurements of 48 specimens allocated to the genera *Fejervarya*, *Minervarya*, *Sphaerotheca* and *Zakerana*

Total Variance Explained

Component	Total	% of Variance	Cumulative %
1	5.799	48.323	48.323
2	2.580	21.497	69.820
3	1.356	11.299	81.120

Component Matrix

Component	1	2	3
LSVL	0.448	0.642	0.166
LHW	0.894	0.051	-0.146
LHL	-0.080	0.808	-0.394
LMN	-0.159	0.858	-0.294
LIBE	0.751	-0.454	-0.349
LHAL	0.454	0.640	0.436
LTL	-0.930	-0.196	-0.003
LFOL	-0.739	0.110	0.420
LTYD	-0.923	-0.010	-0.237
LTYE	0.297	-0.044	0.719
LIMT	0.905	-0.309	-0.061
LITL	-0.910	-0.111	0.029

Table 2 Proposed grouping obtained by post-hoc analysis (Tukey B) of Factor 1 and 2. Means for groups in homogenous subsets are displayed. N = 12 for all samples

	Genus	Subset for alpha = 0.05		
		1	2	3
Factor 1	*Minervarya*	-0.6578198		
	Zakerana	-0.5481503		
	Fejervarya	-0.4591751		
	Sphaerotheca		1.6651452	
Factor 2	*Zakerana*	-0.7277565		
	Fejervarya	-0.6769703		
	Sphaerotheca		0.0667739	
	Minervarya			1.3379529

DISCUSSION

The statistical analysis does not allow separation of specimens allocated to *Fejervarya* and *Zakerana*, whereas the specimens allocated to *Minervarya* and *Sphaerotheca* have distinct morphologies. If the rationale presented in the introduction is followed, then *Fejervarya* and *Zakerana* should be grouped in a single genus. Nevertheless this can be done only if monophyly of this group can be shown. Until now the relationship of the three groups, *Fejervarya*, *Sphaerotheca* and *Zakerana*, is not resolved, as there is no significant support for any of the three pairwise combinations.

The large morphological similarity of *Fejervarya* and *Zakerana* is of interest as it shows that although these two groups had a long independent evolutionary history, they kept a similar overall adaptation as represented by morphology, whereas the sister lineage *Sphaerotheca* shows a high morphological differentiation. *Minervarya* specimens are also morphologically very distinct. As these specimens are very small, it would be expected that this difference is due to size, but our data have been corrected for size with a very effective statistical method (Mossiman, 1970). The genus was erected on the basis of morphology and its phylogenetic position based on

genetic data is not resolved as conflicting hypothesis exist (Hasan et al., 2014; unpublished).

The phenomenon of morphological stasis and morphological anagenesis in sister groups is an interesting problem in evolutionary studies. Evolution of life is usually explained by accumulation of mutations and selection, but in the case of these frogs, despite genetic evolution as expressed by genetic divergence, morphology has remained grossly unchanged. Studies based on crossing of *Fejervarya* of specimens of Sri Lanka with specimens from Japan and Bangladesh show high lethality in early development and no tadpoles attained feeding stage (Sumida et al., 2007). The authors conclude of interrupted gene flow between frog lineages from south-east Asia and south Asia which confirms the independent evolutionary history of *Fejervarya* and *Zakerana*.

The results here are consistent with the results of Pyron and Wiens (2011) and interpretation of *Minervarya* and *Fejervarya* as a single genus, *Fejervarya*. If two genera should be recognized and the position of *Minervarya* within *Zakerana* was confirmed, then this genus should bear the first published name, *Minervarya*, but this would be conflicting with the morphological evidence presented here. As a matter of fact, the morphological differentiation of the two *Minervarya* species is very large compared to the conservative morphology within the other species. Following a conservative approach as defended by Vences et al. (2013), it is proposed to recognized a genus *Fejervarya* with two subgenera *Fejervarya* and *Zakerana*, and the genera *Minervarya* and *Sphaerotheca*.

The study on the morphological differentiation of the *Fejervarya* lineage gives interesting insights on the evolution of the group. To go further, strongly supported phylogenetic hypothesis should be available on taxonomically studied specimens from type localities. Morphometry could be used to measure morphological evolution and differentiation in frogs where patterns of morphological evolution are still improperly known.

REFERENCES

Dayrat B (2005) Toward integrative taxonomy. *Biological Journal of the Linnean Society*, 85: 407-415.

Djong TH, Islam MM, Nishioka M, Matsui M, Ota H, Kuramoto M, Khan MMR, Alam MS, Anslem DS, Khonsue W and Sumida M (2007) Genetic relationships and reproductive-isolation mechanisms among the *Fejervarya limnocharis* complex from Indonesia (Java) and other Asian countries. *Zoological Science*, 24: 360-375.

Dubois A (1988a) Some comments on the genus concept in zoology. *Monitore zoologico italiano*, 22: 27-44.

Dubois A (1988b) The genus in zoology: a contribution to the theory of evolutionary systematics. *Mémoires du Muséum National d'Histoire Naturelle Série A Zoologie*, 139: 1-123.

Dubois A (2008) Phylogenetic hypotheses, taxa and nomina in zoology. In: Minelli A, Bonato L and Fusco G (ed.), *Updating the Linnaean heritage: names as tools for thinking about animals and plants*. *Zootaxa* 1950: 51-86.

Faivovich N, Frost DR, Grant T, Haddad LIOFB, Bain RH, Haas A, Channing A, Wilkinson M, Sa RODE, Donnellan SC, Raxworthy CJ, Campbell JA, Blotto BL, Moler P, Drewes RC, Nussbaum RA and Lynch JD (2006) The amphibian tree of life. *Bulletin of the American Museum of Natural History*, 1-370.

Hasan M, Islam MM, Khan MMR, Igawa T, Alam MS, Djong HT, Kurniawan N, Joshy H, Sen YH, Belabut DM, Kurabayashi A, Kuramoto M and Sumida M (2014) Genetic divergences of South and Southeast Asian frogs: a case study of several taxa based on 16S ribosomal RNA gene data with notes on the generic name *Fejervarya*. *Turkish Journal of Zoology*, 38: 389-411.

Hennig W (1950) Grundzüge einer Theorie der phylogenetischen Systematik. *Deutscher Zentralverlag*, Berlin, pp 370.

Hennig W (1975) Cladistic analysis or cladistic classification - Reply. *Systematic Zoology*, 24: 244-256.

Howlader MSA (2011) Cricket frog (Amphibia: Anura: Dicroglossidae): two regions of Asia are corresponding two groups. *Bonnoprani - Bangladesh Wildlife Bulltin*, 5: 1-7.

Inger RF (1958) Comments on the definition of genera. *Evolution*, 12: 370-384, doi:10.2307/2405859.

Kotaki M, Kurabayashi A, Matsui M, Khonsue W, Hon Djong T, Tandon M and Sumida M (2008) Genetic divergences and phylogenetic relationships among the *Fejervaryalimnocharis* complex in Thailand and neighboring countries revealed by mitochondrial and nuclear genes. *Zoological Science*, 25: 381-390.

Kotaki M, Kurabayashi A, Matsui M, Kuramoto M, Djong TH and Sumida M (2010) Molecular phylogeny of the diversified frogs of genus *Fejervarya* (Anura: Dicroglossidae). *Zoological Science*, 27: 386-395.

Kuramoto M and Joshy SH (2007) New frogs from the Western Ghats. *Current Science* 93: 752-754.

Mosimann JE (1970) Size Allometry - Size and shape variables with characterizations of lognormal and generalized gamma distributions. *Journal of the American Statistical Association*, 65: 930-945.

Ohler A and Dubois A (1999) The identity of *Elachyglossa gyldenstolpei* Andersson, 1916 (Amphibia, Ranidae), with comments on some aspects of statistical support to taxonomy. *Zoologica Scripta*, 28: 269-279.

Padial JM and De la Riva I (2006) Taxonomic inflation and the stability of species lists: the perils of ostrich's behavior. *Systematic Biology,* 55: 859-867.

Pyron RA and Wiens JJ (2011) A large-scale phylogeny of Amphibia including over 2800 species, and a revised classification of extant frogs, salamanders, and caecilians. *Molecular Phylogenetics and Evolution*, 61: 543-583.

Sumida M, Kotaki M, Islam MM, Djong TH, Igawa T, Kondo Y, Matsui M, Anslem DS, Khonsue W and Nishioka M (2007) Evolutionary relationships and reproductive isolating mechanisms in the rice frog (*Fejervarya limnocharis*) species complex from Sri Lanka, Thailand, Taiwan and Japan, inferred from mtDNA gene sequences, allozymes, and crossing experiments. *Zoological Science*, 24: 547-562.

Vences M, Guayasamin JM, Miralles A and Riva I de la (2013) To name or not to name: criteria to promote economy of change in Linnaean classification schemes. *Zootaxa*, 3636: 201-244.

Wills MA and Fortey RA (2000) The shape of life: how much is written in stone? *Bioessays*, 22: 1142-1152

Pranikee- Journal of Zoological Society of Orissa, ISSN 0970-4450
Volume XXVI, 2014, pp 13-21

HUMAN-SLOTH BEAR INTERACTIONS: PRELIMINARY SURVEY FROM SEMILIGUDA RANGE OF KORAPUT FOREST DIVISION, SOUTHERN ODISHA, INDIA

Sharat Kumar Palita*, Tuhinansu Kar, Subrat Debata

Centre for Biodiversity and Conservation of Natural Resources, Central University of Orissa, Landiguda, Koraput, Odisha- 764 020, India.

*Corresponding author: skpalita@gmail.com

ABSTRACT

Human-Sloth bear interaction has become a common incident throughout the distributional range, including Koraput district of southern Odisha. A study was carried out to understand the nature and intensity of interaction in Semiliguda forest range of Koraput Forest Division, Odisha during February to April, 2014. Local villagers were interviewed regarding the presence of sloth bear in the area, followed by a sign survey method. All the victims were interviewed using a pre-designed questionnaire to understand the nature of conflict. A total of 25 sloth bear signs have been reported from the outskirt of villages, majority being feeding signs (64%) followed by scats (24%) and scratch mark on trees (12%). Most of the survey areas were found with termite mounds. Out of 33 bear mauling cases along with eight fatal incidents between the years 2007 to 2013, majority of the interactions (30.30%) occurred during early morning hours while victims were mostly engaged in cattle grazing (60%) and majority of the victims were males (87.87%). Agricultural crops of the local communities like Bengal gram, maize, sugarcane, groundnuts and tubers of *Dioscorea sps*were most frequently raided by sloth bear prior to harvest, making a heavy loss to the farmers.

Key words: Sloth bear, Mauling, Crop raiding, Sign survey.

INTRODUCTION

Human-wildlife interaction is fast becoming a critical threat to the survival of many globally endangered species, in particular to large and rare mammals. The numerous cases from countries all over the world demonstrate the severity of human-wildlife conflict and suggest an indepth analysis essential to understand the problem and support the conservation prospects of threatened and potentially endangered species. Globally, protected areas, though regarded as cornerstones of biodiversity conservation are under threat. Traditional approaches to wildlife conservation prove to

be largely ineffective in meeting the current challenges and conflicts, and several protected areas (PAs) are now in deep crisis. Human-wildlife interaction has been a well known problem in vicinities of protected and non-protected areas. Incidence of human casualties, livestock depredation and crop damage caused by wild animals e.g. Elephants, Tiger, Lion, Sloth bear, Leopard and many herbivores have been widely reported from various parts of India (Schultz, 1986; Chauhan and Singh, 1990; Indrukar et al., 1994; Saberwal et al., 1994; Sinha and Jha, 1994; Rajpurohit, 1996; Chandra, 1997; Mishra, 1997; Mankadan and Rahmani 1998; Bargali, 2003; Bargali et al., 2005; Chauhan, 2005a, 2005b; Palita and Purohit 2008). Of all these, currently human-sloth bear interactions in different parts of India assumes significance.

Of the four species of Ursidae found in India (Menon, 2014), sloth bear *Melursus ursinus* (Shaw, 1791) is wide spread. It is endemic to Indian subcontinent and has been reported from the Western Ghat hill ranges to the foot hill of the Himalayas (Bargali et al*.,* 2005). Once ranged throughout India, its historic range has been fragmented and descended because of large scale deforestation for various developmental activities. Apart from that, diminished food resources, poaching for gall bladder and cub lifting for bear dancing resulted in severe decline in population and local extinct in some areas. The current population of sloth bear in India has been confined to some protected areas as well as outside protected areas in close proximity to human habitations. Owing to its declining population and continuing anthropogenic threats to its habitat, sloth bear has been included in Schedule-I of the Indian Wildlife (Protection) Act, 1972 and Appendix-I in CITES. It has been also been designated as vulnerable in IUCN red data book.

Interaction of sloth bear with human has become a common incident throughout its distributional range, mostly at the fringe regions of protected areas or outside protected areas. Reduced forest cover and food resources outside protected areas (Akhtar et al*.,* 2004) have led to increased human- sloth bear conflicts, including frequent mauling (Bargali et al*.,* 2005). Simultaneously, the human interference in sloth bear habitat also leads towards the interaction(Sircar et al*.,* 2012). Because of its

frequent interaction with humans resulting major injury or fatal situations, sloth bear has been treated to be one of the most unpredictable and aggressive animals throughout its distributional range, thus losing local support for the conservation of the species throughout.

Sloth bear has been reported throughout Odisha and its interaction with human is very frequently reported in both print and electronic media. However, scanty attempt has been made to understand this situation in Odisha except Debata et al. (2012), whose study was mainly focused in Northern Odisha region. In this study, preliminary data on status and distribution of sloth bear in southern Odisha are developed with a note on its interaction with human.

MATERIALS AND METHODS

Study area

The study was carried out in Semiliguda Forest Range of Koraput Forest Division, southern Odisha, India (Fig. 1) between February to April, 2014. The area is located between 17.4° to 20.7°N latitude and 81.24°to 84.2°E longitude spreading over an area of 8379 sq. Km. The entire region comes under the Eastern Ghat regions in Odisha and characterized with a number of hill ranges interspersed with forests, agricultural land and human habitations. The historic forest cover has been drastically lost because of shifting cultivation. The remaining forest constitutes about 21.89% of the total geographical area of the district (Anonymous, 2013). The topography of Koraput varies from plain lands to high hills, with elevation gradients of 300 meter to 1,000 meter above mean sea level. The district is characterized by tropical to sub-tropical climate with an average annual rainfall of 1521.8 mm. The average temperature hovers around 30°C; however, the average relative humidity has been reported to be 70%.

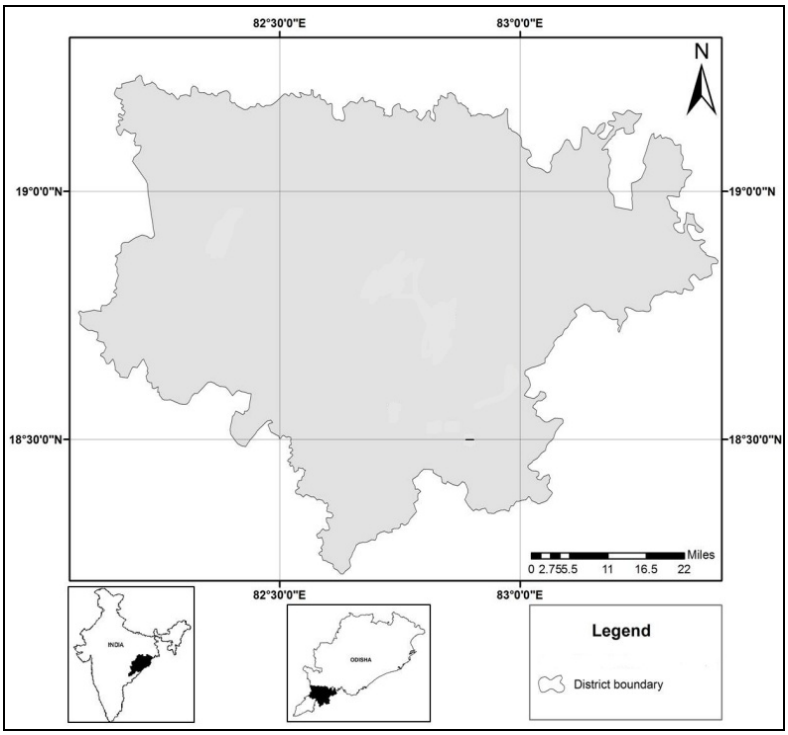

Fig. 1 Location of Koraput district.

Methods of data collection

To ascertain the presence of sloth bear in different forest covers of Semiliguda forest range of Koraput Forest Division, formal and informal interviews were conducted with the local villagers to obtain preliminary information on the presence of sloth bear in the area followed by a sign survey. The trails and foot paths along different forests were intensively searched for bear sign which included, foot mark, scratch mark on trees, feeding signs and scats. Whenever any sign was encountered, the location was recorded using a hand held Garmin etrex 10 GPS. Information on human mauling and compensation between the years of 2007 to 2013 were collected from the records of Forest department. All the victims were directly interviewed using a pre-designed survey format following Dhariya (2009) to understand the nature and intensity of conflict.

RESULTS

From the preliminary survey, sloth bears have been reported to be present in 15 reserve forests of Semiliguda forest range based on the views of local people and sign survey. In toto, 25 sloth bear signs have been reported throughout the study area, of which all of the signs were recorded at the outskirt of villages in the hilly terrains. Majority of the signs were found to be feeding signs (64%, n=16; Fig. 2) followed by scats (24%, n=6) and scratch mark on trees (12%, n=3). Most of the survey areas were found with termite mounds. Of all the feeding signs, 43.75% (n=7) were reported within agricultural lands in the vicinity of termite mounds.

A total 33 bear mauling cases resulting in eight fatal incidents have been reported from Semiliguda forest range (Table 1) between 2007 to 2013 of which majority of the incidents have been reported during 2011 (24.24%, n=8; Fig. 3). Majority of the attacks (30.30%, n=10) were reported to occur during early morning hour between 0400 to 0800 hour while victims were mostly engaged in cattle grazing (60%, n=6). Of all the victims, majority were males (87.87%, n=29). Apart from human injury, sloth bears have been reported to raid on agricultural fields of the local communities residing near sloth bear habitats. Discussion with local farmers indicated that, crop varieties like Bengal gram, maize, sugarcane, groundnuts and tubers of *Dioscorea* sps are the most frequently raided crops by Sloth bear prior to harvest, thus, making a heavy loss to the farmers. During the period of 2007 to 2013, a total amount of 11. 92 lakhs with an average of ≈1.70 lakh per year (Table 1) has been distributed among the victims in Semiliguda forest range of Koraput.

Fig. 2 Occurrence of sign and evidence of sloth bear.

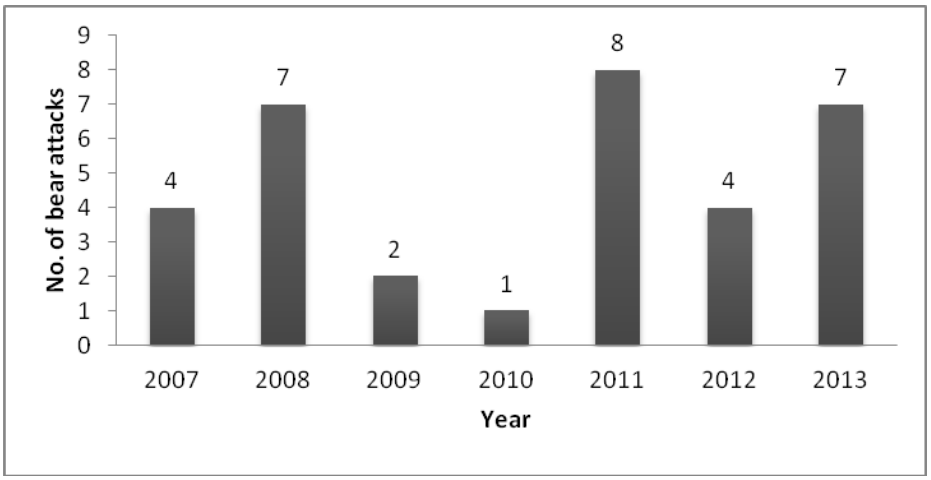

Fig. 3 Yearwise distribution of victims in Semiliguda forest range, Koraput, Odisha.

Table 1 Intensity of conflict and compensation payment in Semiliguda forest range, Koraput, Odisha

Year	No of victims injured	No of victims died	Total no of incidents	Total amount of compensation paid (Rs)
2007	3	1	4	106000
2008	3	4	7	406000
2009	2	0	2	4000
2010	1	0	1	2000
2011	7	1	8	229000
2012	4	0	4	20000
2013	5	2	7	425000
Total	**25**	**8**	**33**	**1192000**

DISCUSSION

Sloth bears are treated to be one of the most unpredictable animals throughout their distributional range because of their frequent interaction with human and behave very aggressively while accompanied by cubs (Prater, 1980; Debata et al., 2012; Pillarisett, 2012). The Koraput district, once famous for its forest cover, has been drastically changed. Majority of the native forest cover has been altered for human settlements, agricultural fields and road network, thus, making the population of sloth bear fragmented and isolated. Major part of the population in Semiliguda forest range belongs to tribal communities such as Kandh, Paraja, Gadaba etc. These people are highly dependent on forest resources for food, fuel, fodder and other livelihood requirements as well as mass hunting is an age old culture of these communities during celebration of Chaiti Parab where they venture in to the nearby forest for hunting of wild animals. Due to their direct attachment with forest, the people frequently come in interaction with sloth bears. Many times sloth bears have been reported to venture into villages, agricultural lands and other human dominated areas in search of food which increase the chances of bear attack on humans. The rising concern of human-sloth bear interaction leading to severe socio-economic loss has lost local support for the conservation of this endangered species throughout its distributional range (Yoganand et al., 2006).

The present study was a preliminary observation on this situation in southern Odisha. Thus, a long term study on the nature and intensity of conflict along with ecology of sloth bear is an utmost necessity for long term conservation of this species in southern Odisha.

ACKNOWLEDGEMENTS

Authors are very much thankful to the Divisional Forest Officer (DFO), Koraput Forest Division for sharing the conflict data with us and the Forest Range Officer, Semiliguda for providing field support to carry out the study. Thanks are due to the local communities for their support in the field.

REFERENCES

Akhtar N, Bargali, HS and Chauhan NPS (2004) Sloth bear habitat use in disturbed and unprotected areas of Madhya Pradesh, India. *Ursus*, 15: 203-211.

Anonymous (2013) India State of Forest Report 2013. Forest Survey of India, Ministry of Environment and Forests, Government of India, Dehradun.

Bargali HS (2003) Ecology of the problematic sloth bear (*Melursus ursinus*) and mitigation of human-bear conflicts in North Bilaspur Forest Division, Madhya Pradesh. Thesis, Saurashtra University, Rajkot, India.

Bargali, HS, Akhtar N and Chauhan NPS (2005) Characteristics of sloth bear attacks and human casualties in North Bilaspur forest division, Chhattisgarh, India. *Ursus*, 16: 263-267.

Chandra J (1997) Crop damage caused by blackbucks (*Antilope cervicapra*) at Karera Great Indian Bustard Sanctuary, and possible remedial solution. *Tigerpaper*, 4 (3): 26-32.

Chauhan NPS (2005a) Livestock depredation by tiger in and around Dudhwa National Park, Uttar Pradesh and mitigation strategies. *Indian Forester*, 131(10): 1319-1328.

Chauhan NPS (2005b) Human causalities by tiger in Katerniaghat Wildlife Sanctuary, Uttar Pradesh and mitigation strategies. *Indian Forester*, 131(10): 1347-1352.

Chuhan NPS and Singh R (1990) Crop damage by over abundant populations of Nilgai and Blackbuck in Haryana (India) and its management. In: *Proceedings of 14th Vertebrate Pest Conference, University of California, Davis*, pp 218-220.

Debata S, Sahu HK and Swain KK (2012) Human-Sloth bear conflict in Balasore Wildlife Division, Odisha, India. In: *Book of Abstracts of 21st International Conference on Bear Research and Management 26-30 November 2012*, *New Delhi, India,* pp164.

Dharaiya N (2009) Evaluating Habitat & Human-Bear Conflicts in North Gujarat, India, to Seek Solutions for Human-Bear Coexistence. *Final Report submitted to Rufford Small Grant Foundation*, pp i-v + 1-43.

Indrukar RN, Tyagi DK, Gujar SM and Patil VB (1994) The blackbuck farmer interface in Ahamadnagar and Sholapur districts of Maharashtra. *Proc. Workshop on wildlife damage problems and control 2-7 February 1994, Wildlife Institute of India, Dehradun.*

Manakadan R and Rahamani AR (1998) Crop damage by blackbuck *Antilope cervicapra* at Rollapadu Wildlife Sanctuary, Andhra Pradesh. *J. Bombay Natural History Society*, 95 (3): 408-417.

Menon V (2014) Indian mammals: a field guide. 1st edition. Hachette Book Publishing India Pvt. Ltd. Gurgaon, India.

Mishra C (1997) Livestock depredation by large carnivore in the Indian trans-Himalaya: conflict perception and conservation prospects. *Environmental Conservation*, 4 (4): 338-343.

Palita SK and Purohit KL (2008) Human-Elephant Conflict: Case Studies from Orissa and Suggested Measures for Mitigation. In: *Proceeding of HADP sponsored National Seminar on "Endemic and Endangered Species of Nilgiris" organised by the Dept. of AZBT & WLB, GAC College, Ooty, Tamil Nadu*, pp 86-99.

Pillariseth AM (2012) Sloth bear- Human Interactions. In : *Book of Abstracts of 21st International Conference on Bear Research and Management 26-30 November 2012*, *New Delhi, India,* pp178.

Prater HS (1980) The Book of Indian Animals. 3rd Edition. Bombay Natural History Society, Bombay, India.

Rajpurohit KS (1996) Study of wild animal damage problems in and around protected areas and managed forests in India. Phase-1, Madhya Pradesh, Bihar and Orissa. Report, Wildlife Institute of India, Dehradun, India.

Saberwal VK, Gibbs JP, Chellam R and Johnsingh AJT (1994) Lion-human conflict in the Gir Forest, India. *Conservation Biology*, 8(2): 501-507.

Schultz BO (1986) The management of crop damage by wild animals. *Indian Forester*, 112 (10): 891-899.

Sinha V.K. and M. Jha (1994) Effectiveness of change in cropping pattern in resolving human-wildlife conflict in context of wildlife degradadtion on cultivated crops in Amarvati. In : *Proceedings of the Workshop on wildlife damage problems and control 2-7 February 1994, Wildlife Institute of India, Dehradun.*

Sircar D, Basu S, Bodhankar S, Singh AK and Kaul R (2012) Factors governing the temporal and spatial distribution of human-sloth bear conflict in Madhya Pradesh, India. In : *Book of Abstracts of 21st International Conference on Bear Research and Management 26-30 November 2012*, *New Delhi, India,* pp 67.

Yoganand K, Rice CG, Johnsing AJT, and Seidensticker J (2006) Is the sloth bear in India secure? A preliminary repport on distribution, threats and conservation requirements. *J. Bombay Nat. Hist. Soc.*, 103(2-3): 172-181.

A CASE STUDY OF MALE-MALE TERRITORY COMBAT IN LEPTOBRACHIUM SMITHI MATSUI, NABHITABHATA AND PANHA, 1999 (ANURA: MEGOPHRYIDAE)

Dipankar Dutta[1], Jayanta Gogoi[1], Amalesh Dutta[2], Saibal Sengupta[1*]

[1]Department of Zoology, Arya Vidyapeeth College, Guwahati- 781 016, Assam, India.
[2]Department of Zoology, Gauhati University, Guwahati- 781 014, Assam, India.
*Corresponding author: E-mail address: senguptasaibal@yahoo.co.in

ABSTRACT

Many anurans show male male combat during breeding season where they mark and form territories. Male-male combat was evident in *Leptobrachium smithi* at Garbhanga Reserve Forest, Assam, India. Courtship and combat behaviour with a satellite male has been studied. Both advertisement call and distress call were recorded in field and analysed with soundruler. The advertisement call show higher pitch as compared to distress call.

Key words:*Leptobrachium smithi,* male male combat, advertisement call, distress call.

INTRODUCTION

*In many anurans a*ggressive behaviour is expressed amongst males of the same size for mates, specially where adult males hold territories and potential breeding opportunities. *During breeding season, males mark and form territories.* Territoriality is best defined as the extended defense of an area (through advertisement or aggression) that contains some limiting resource required for survival or reproduction. *In cases where there is occurrence of floating males (satellite male), the males try to show dominance to prove his superiority over the female. In anurans* difference in body size and weaponry are important sources of asymmetry in Fighting ability or resource holding potential during animal contests (Reichert, 1998). In anurans, the body size influences the male-male combat, verdict mostly goes in favour of larger ones (Wagner, 1989). In Male frog dominant frequency is negatively correlated with body size (Gerhardt, 1994) and male produces advertisement call with lower dominant frequency to publicize exaggerated size (Bee et al., 2000). The present study reports the sequence of events of mala-

male combat along with comparison of advertising call and distress call in *Leptobrachium smithi*.

MATERIALS AND METHODS

During field survey on 28th April 2012, while studying the reproductive behaviour in Leptobrachium smithi a male-male combat was observed at Garbhanga Reserve Forest (26°04.755' N, 91°46.122' E; area 114.65 sq. Km). A Garmin™ GPSMAP76 GPS was used to obtain geographic coordinates. The calls were recorded in a digital encoder (Make: Sony, Model ICD-UX533F/B) in the field at 28° C air-temperature and 87%-88% humidity (recorded with a Thermo-hygrometer, VICTOR-VC230A). To avoid disturbance that might arise due to air interference, the encoder mounted on a long stick was placed as close to the source as possible (< 30 cm). The interferences were cleared using audacity and analyzed in sound ruler (version 0.9.6.0) at FFT 256 length, 50% overlap and hamming window. Photographic documentation was carried out by using Cannon EOS 550D (Body) and EF-S 18 – 55 IS II kit (lens).

RESULTS

Garbhanga RF is a moist deciduous forest drained by numerous streams criss-crossing the whole area. During the month of April, dusk begins around 18.00-18.30 hour. A male started calling with the onset of dusk in the vicinity of the narrow peripheral part of the stream on 28th April 2012 was noticed. At around 19.45 hour, a female approached the male. As the female moved near, the male intensified the advertising call (Fig. 2) and within a span of 20 minutes mounted the female. The mounting pair then slowly started moving towards the stream. The pair could move only around 1.7 meters from its mounting point when it was witnessed a second male that was calling in the nearby area attacked the pair (Fig. 1). The second or satellite male floored the male which was on mount. Both the males indulged in wrestling with each other with emission of distress call (Fig. 3). In the mean time the female ran away from the site of fight to a safer place which is around 1.5 meters away. As the fight intensified, both the males became more aggressive and put each other upside down repeatedly. During fight, the males in bout reached a sloppy area of the hilly ground from

where they fell at a depth of 3 meters from the earlier spot down the hill and hold onto each other and the fight continued. After 12 minutes of fight, the male which was on mount (first male) surrendered and finally withdrawn. But the aggressive satellite male kept on charging the first male without releasing its hold around the leg of the later. Suddenly, with the advantage of a loose grip of the satellite male, the first male succeeded to get rid of from the clasp and hopped away. The satellite male however, did not get the chance to mate with the female.

The advertising call of the first male before mounting the female in the same evening comprised of 4 – 6 notes lasting 0.8-1.2 sec. A single note consists of 22 pulses and with dominant frequency 1366.39 Hz.

A comparison (Fig. 3) between the advertising call and distress call was made based on 9 different parameters (Heyer, 1971; Roy, 1993; Matsui, 1994). The dominant harmony was similar in both the calls; the total pulse number of the advertising call (21.71 Hz) was higher than the distress call (9.85 Hz) and the relative pulse peak was lower (0.66 Hz vs. 0.78 Hz). A higher frequency was shown in the advertising call than the distress call in terms of dominant frequency (1366.39 Hz vs. 1031.54 Hz); fundamental frequency (683.20 Hz vs. 515.77 Hz); pulse peak (0.48 Hz vs. 0.05 Hz). The pulse interval of the advertising call (17.93 m sec) was also higher than the distress call (4.95 m sec). The advertising call indicated a greater sound pressure level (2.66 db vs. 2.49 db) and also a larger bandwidth (591.54 Hz vs. 495.48 Hz).

DISCUSSION

The parabola of the curve (Fig. 3) drawn with the parameters discussed above indicates a higher pitch in the advertising call as compared to the distress call. However, pulses of lower frequencies seem to merge while those at the peak clearly differentiate.

Under the postulates of game theory, size matched individuals are more likely to fight, especially where adult males hold territories and potential breeding opportunities (Maynard Smith and Price, 1973). Territoriality has been classically defined as ‘‘any defended area’’ and is typically manifested by aggression toward intruders (Noble, 1939). For anurans, Mathis et al. (1995) proposed three necessary components of territoriality such as (1) resource limitation, (2) site tenacity and (3) resource defense. *Leptobrachium smithi* in the present study area has very limited resources especially in terms of availability of mate and breeding ground. Further, the body size of both males appears to be similar and thus, when the pair intrudes into the territory of the second male, the later defends the territory by fighting with vigour. The males at breeding site often show aggression for territoriality and resource defense (Duellman, 1966; Duellman and Savitzky, 1976; Wells, 2007; Costa et al., 2010). Aggression and territoriality also seem to be more common among species whose breeding strategies involve a limited number of breeding sites or who deposit eggs at calling sites (Duellman and Savitzky, 1976).

ACKNOWLEDGEMENTS

The authors are grateful to Indraneil Das and Kanto for their comments on a draft MS. Field assistance by Mridul Bora and Shah Nawaz Jelil is also acknowledged.

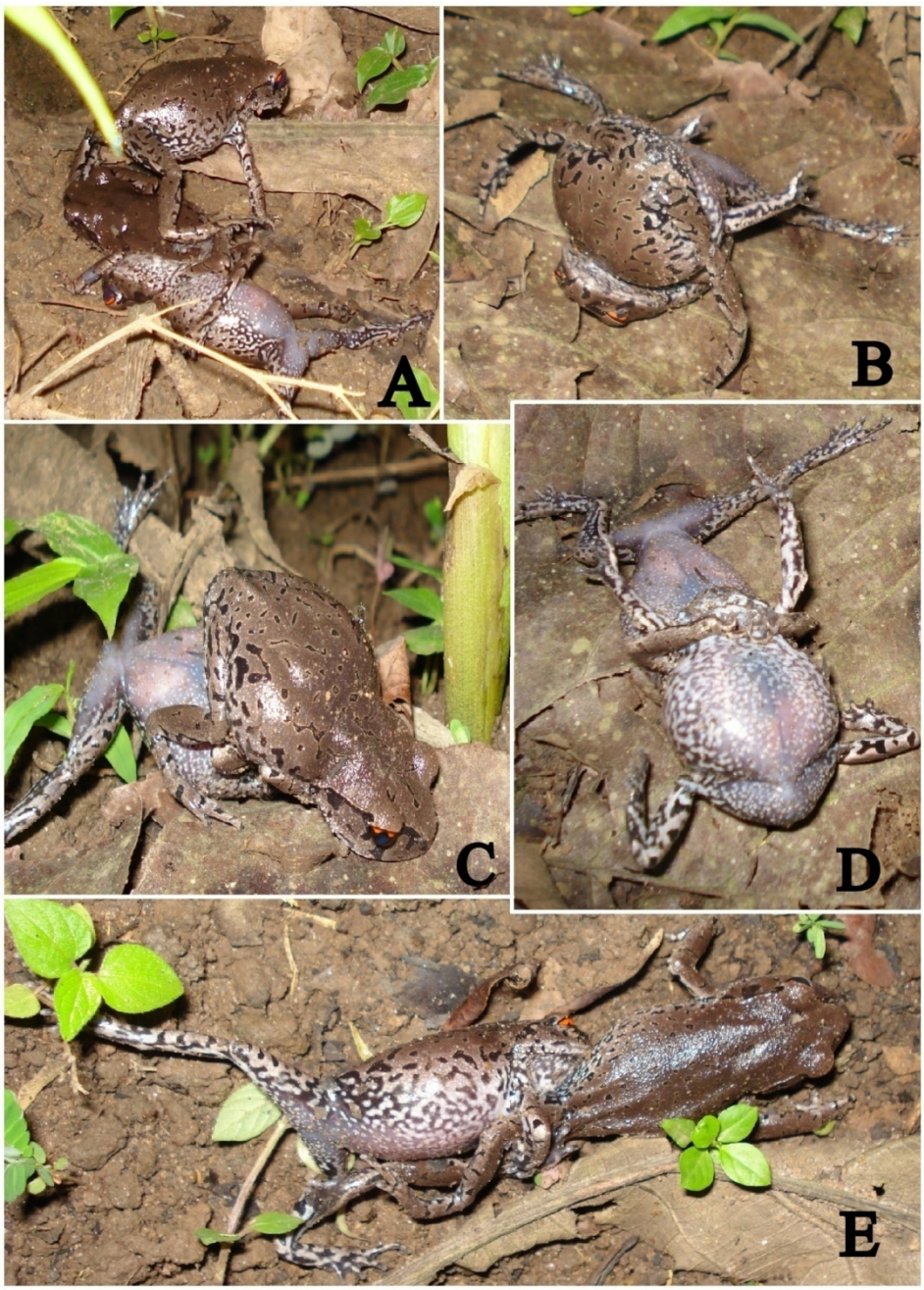

Fig. 1 Sequence of images showing male-male territory combat in *Leptobrachium smithi.*

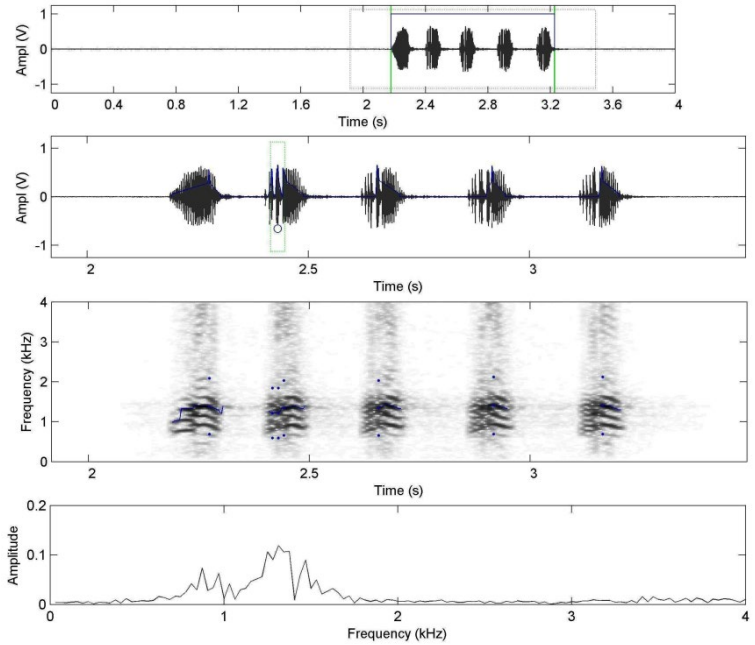

Fig. 2 Advertising call spectra of *Leptobrachium smithi.*

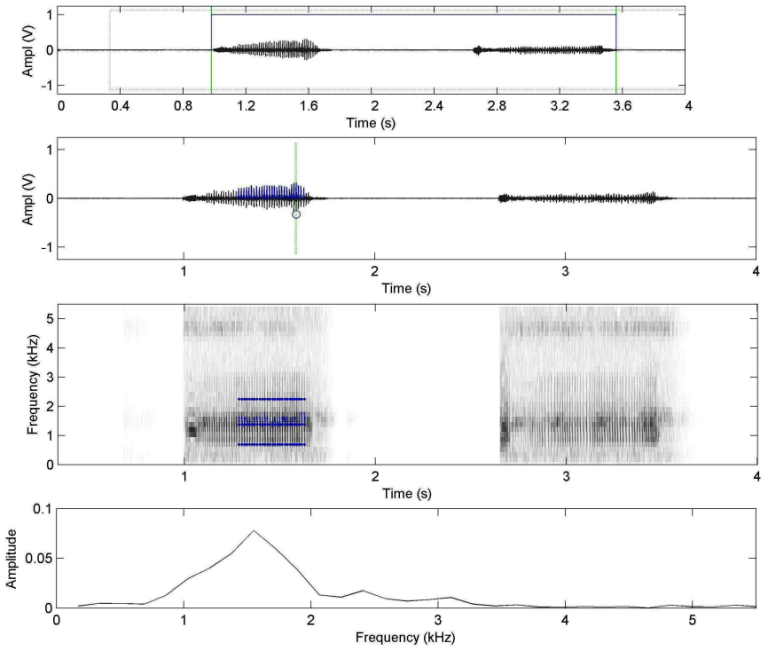

Fig. 3 Distress call spectra during male–male combat in *Leptobrachium smithi.*

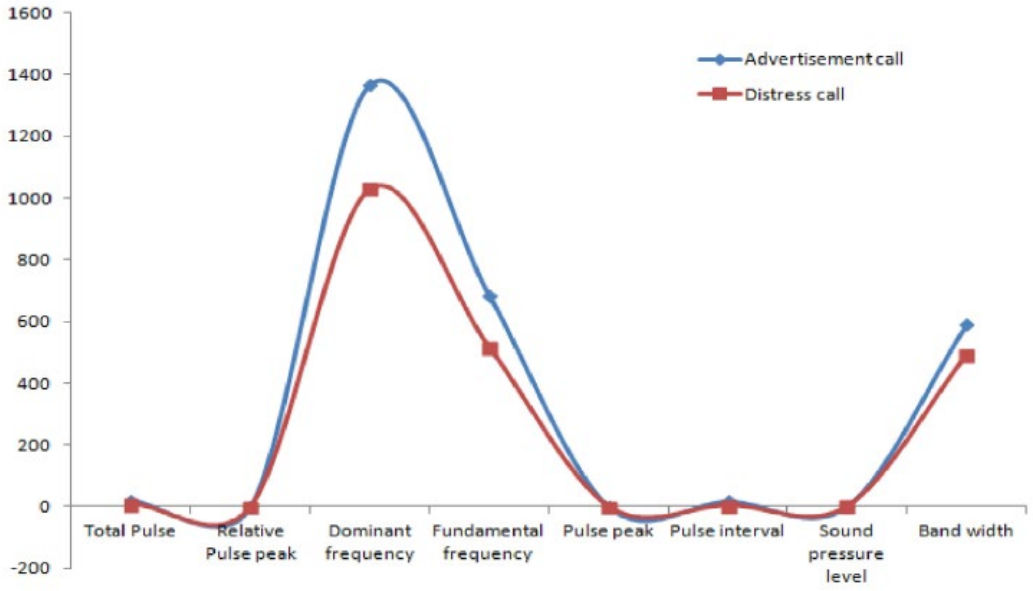

Fig. 4 Comparison between advertisement call and distress call.

REFERENCES

Bee MA, Perrill SA and Owen PC (2000) Male green frogs lower the pitch of acoustic signals in defense of terreitories: a possible dishonest signal of size? *Behavioral Ecology*, 11:169–177.

Costa TB, Guimaraes LD and Bastos RP (2010) Territorial and mating behaviour in *Phyllomedusa azurea* (Anura: Hylidae) at a temporary pond in west-central Brazil. *Phyllomedusa*, 9:99–108.

Duellman WE (1966) Aggressive behaviour in a neotropical frog, *Dendrobates granuliferus* (Anuran: Dendrobatidae). *Herpetologica*, 28:195–198.

Duellman WE and Savitzky AH (1976) Aggressive behaviour in a centrolenid frog with comments on territoriality in anurans. *Herpetologica*, 32:401–404.

Gerhardt HC (1994) The evolution of vocalization in frogs and toads. *Annual Review of Ecology and Systematics*, 25:293–324.

Heyer WR (1971) Mating calls of some frogs from Thailand. *Fieldiana*, 58:61–82.

Mathis A, Jaeger RG, Keen WH, Ducey PK, Walls SC and Buchanan BW (1995) Aggression and territoriality by salamanders and a comparison with the territorial behavior of frogs. In:Heatwole H and Sullivan BK (eds.), *Amphibian biology. Vol. 2. Social behavior.* Surrey Beatty and Sons, Chipping Norton, New South Wales, pp 633–676.

Matsui M and Wu GF (1994) Acoustic characteristics of treefrogs from Sichuan, China, with comments on systematic relationship of *Polypedates* and *Rhacophorus* (Anura: Rhacophoridae). *Zoological Science,* 11:485–490.

Maynard Smith J and Price GR (1973) The logic of animal conflict. *Nature,* 246:15–17.

Noble GK(1939) The role of dominance in the social life of birds. *Auk,* 56:263–273.

Reichert SE (1998) Game theory and animal contests. In: Dugatkin IA and Reeve HK (eds.), *Game theory and animal behavior.* Oxford University Press, New York, pp 64–93.

Roy D and Elephandt A (1993) Bioacoustic analysis of frog calls from Northeast India. *Journal of Bioscience,* 18:381–394.

Wagner WE Jr (1989) Fighting, assessment, and frequency alteration in Blanchard's cricket frog. *Behavioural Ecology and Sociobiology,* 25:429–436.

Wells KD (2007) The Ecology and Behaviour of Amphibians. University of Chicago Press, Chicago, Illinois.

ARCHITECTURE OF REPTILIAN EGGSHELL: AN ANALYSIS

Gunanidhi Sahoo

Department of Zoology, North Orissa University, Baripada – 757 003, Odisha, India.

e-mail: gunanidhi.nou@gmail.com

ABSTRACT

Reptiles are the first true terrestrial animals. The invasion of land could become possible due to the development of a shelled egg. The eggshell has several important functions ranging from protection to a source of essential minerals for the developing embryo. Structure of eggshells is quite diverse, i.e., from the small, flexible, parchment shelled eggs of many squamates to the large, rigid-shelled eggs of crocodilians. Shells of reptilian eggs are composed of an outer, inorganic calcareous layer underlain by an organic (shell) membrane comprised of multiple layers of fibres. The calcareous layer may be absent, very thin and patchy or thick in different groups of reptiles. Calcium carbonate of the calcareous layer may be present either as calcite or as aragonite. Fibres within a layer of the membrane may form a random felt-work or may be parallel, and may be closely apposed to one another or widely spaced. The arrangement and interaction of different shell layers in lepidosaurians, testudines and crocodilians is quite diverse.

Key words: Reptile, eggshell, calcium carbonate, shell units, membrane fibres.

INTRODUCTION

Females of all vertebrates produce eggs. Reproductive success in oviparous organisms depends upon the egg-laying ability and stability of the nest environment (Packard and Packard, 1988; Webb and Cooper-Preston, 1989). For example, it is suggested that crocodiles and turtles survived the mass extinction at the end of Cretaceous Period in part because they laid eggs in substrates that provided relatively stable temperature and humidity throughout incubation (Robertson et al., 2004). With the exception of the platypus and echidna, mammals provide the developing embryo with a suitable environment within the mother's womb. Reptiles could become the first completely land dwelling animals largely due to a shelled egg. Reptiles "invented" the eggshell a device that could keep the egg from drying out and allow reproduction away from water (or, at least, from extremely moist environments). For reptiles to survive on land, they had to be able to lay eggs on land, rather than, like amphibians in water. The problems were having a sufficient surface as to be able to exchange

gases, i.e., oxygen and carbon dioxide, and also to hold as much water without excessive loss as would be needed during the gestation of the embryo.

Parental care is not known among most reptiles. Therefore, other than the cost of nesting, the eggs of reptiles represent nearly the total reproductive investment in offspring by the females. The material and energy in a reptilian egg is primarily utilised for embryonic development within the eggs and secondarily for hatchling maintenance and growth. Because there exists wide differences in emergence time of hatchlings of different reptiles, the amount of stored material and energy required for hatchling survival may be reflected in different egg components and the amount of different fractions of the egg utilised for embryonic development.

The vast majority of contemporary reptiles are oviparous and deposit amniotic eggs with some sort of enveloping shell (Packard et al., 1988). A typical reptilian egg is composed of three physical parts namely the yolk, the albumen and the eggshell (Fig. 1). The eggshell of oviparous organisms (reptiles and birds) plays a complex, but only partially understood, role in development, particularly as a source of inorganic ions (Packard et al., 1992). Several researchers have reported on the calcium level of yolk, albumen and embryo of reptiles (Simkiss, 1962, 1967; Jenkins and Simkiss, 1968; Jenkins, 1975; Cox et al., 1984, Sahoo et al., 1998, 2009, 2010). The eggshell plays the most important mediating role between the living content and the ambient. Some major demands of the eggshell are permeability (to gas and water), mechanical stability, a potential calcium reserve, gluing (of sand grains) and so on (Schleich and Kastle, 1988; Sahoo et al., 2009, 2010). Modifications of shell structure probably have evolved for functional smoothness and evolutionary adaptations. Thus, in terms of survival of the oviparous species, the structure of the eggshell is of primary importance to the embryo, not only as a source of essential minerals, but also as a protective covering.

STRUCTURE OF EGGSHELL

The terms needed for a detailed description of various eggshell types were proposed by Schmidt (1943a, 1943b; Erben, 1970; Sauer, 1972; Packard, 1980; Hirsch 1983) (Fig. 2). The scanning electron microscopic (SEM) study reveals, in general, that the eggshell is composed of an outer variously thick calcium carbonate layer followed by an inner shell membrane (*membrane testacea*) and finally limited by a smooth boundary layer, facing the egg contents. The eggshell is covered by a cuticle of varying thickness in some cases. Structure of eggshells is quite diverse, ranging from the small, flexible, parchment shelled eggs of many squamates to the large, rigid-shelled eggs of crocodilians. None the less, certain common themes unite structure of eggs of all oviparous reptiles.

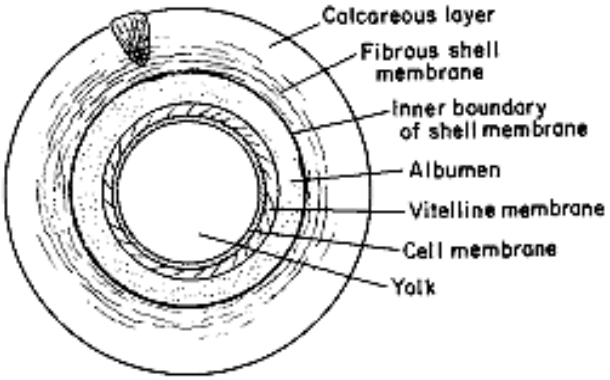

Fig. 1 Cross-section through a generalized egg of an oviparous, amniotic vertebrate showing the yolk and the *egg* membranes. Drawing is not to scale and size relationship has been exaggerated for clarity (after Packard and DeMacro, 2004).

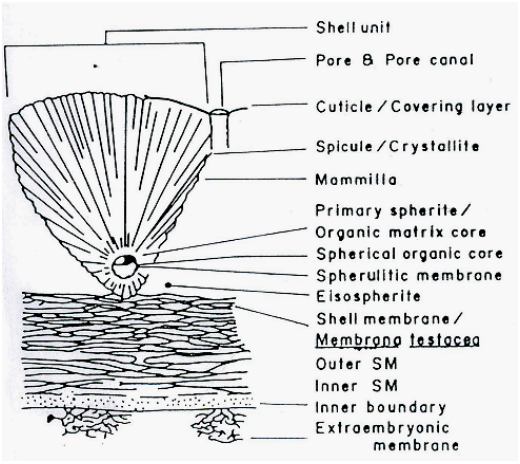

Fig. 2 Terminology of a typical eggshell (after Erben, 1970; Sauer, 1972 and Hirsch, 1983).

Calcareous layer

Shells of reptilian eggs (Fig. 3) are composed of an outer, inorganic layer underlain by an organic (shell) membrane comprised of multiple layers of fibres (Packard and Hirsch, 1986). Fibres within a layer of the membrane may form a random felt-work or may be parallel, and may be closely apposed to one another or widely spaced.

The inorganic layer of the eggshell consists of calcium carbonate in the form of calcite in crocodilians and oviparous squamates, but as aragonite in eggshells of testudinians (Erben, 1970; Solomon and Baird, 1976; Ferguson, 1982, 1985; Hirsch, 1983; Packard and Hirsch, 1986; Deeming, 1988, Sahoo et al., 1996, 1998, 2009). Diet or other environmental factors may affect the morph of calcium carbonate. For example, captive sea turtles (*Chelonia mydas*) may lay eggs containing both calcite and aragonite (Baird and Solomon, 1979), and eggshells of some captive pythons (*Python molurus bioittatus*) may be aragonitic rather than calcitic. Factors associated with captivity may also affect the chemical composition of the inorganic layer, in those shells of captive breeded Dragons (*Amphibolurus barbatus*) have been reported to consist of calcium phosphate (Schlëich and Kastle, 1988). Presence of calcium carbonate as calcite blocks sometimes make the eggs prone to fungal and other

microbial infections thereby affecting the hatchability and survival of the species (Solomon and Tippett, 1987). It is interesting to note that calcite and aragonite are found in invertebrate exoskeleton (Berman et al., 1993). The egg, the primary stage of development of an organism, has retained these crystal forms as the protective covering.

Eggs of contemporary reptiles exhibit a broad spectrum in the degree of development of the shell layer (Agassiz, 1857; Erben, 1970; Ewert, 1979; Sexton et al., 1979; Packard et al., 1982a, 1982b, 1982c). Comparing the shells of recent reptilian orders, three main types can be distinguished for the squamates, crocodiles and testudines (Fig. 2). Most squamates lay eggs with extremely flexible shells (Packard et al., 1977). In some species, the calcareous layer is absent and the eggshell consists only of a fibrous shell membrane; while in others, the outer surface of the shell membrane is dotted with isolated rosettes of calcareous material. In some others, a relatively thin calcareous layer forms the outer surface of the eggshell. The rigid-shelled crocodilian eggs have a well developed calcareous layer comprising bulk of the eggshell, and the double-layered shell membrane makes up a small fraction of the total shell thickness (Young, 1950, Ewert, 1979; Ferguson, 1982). The calcareous layer is organised into wedge-shaped shell units and the crystalline layer is penetrated by few pores. The testudines are intermediate between these two extreme forms. In rigid-shelled turtles (Trionichelidae, Chelidae, Kinosternidae and Testudinidae), the calcareous layer constitutes most of the thickness of the eggshell that organised into discrete shell units. The shell units fit together tightly and adjacent shell units inter-lock to yield a non-compliant layer of calcareous material (Erben etal., 1979; Packard, 1980; Packard et al., 1982a, 1982b, 1982c). Flexible-shelled turtle eggs (chelonids, chelydrids, most emydids, and sea turtles) are characterised by a well defined calcareous layer that usually of the same thickness as the shell membrane to which it is attached (Solomon and Baird, 1976; Ewert, 1979;; Packard, 1980;

Sahoo et al., 1996, 2009, 2010). The calcareous layer forms a rather loosely organised open matrix in which the individual shell units and pores are poorly defined.

The calcareous layer of eggshells of crocodilians, turtles, and some geckos consist of individual columns or shell units attached to the underlying shell membrane (Figs. 4a-e). Shell units in eggs of crocodilians and turtles have a knob-like or conical tip at one end (Figs. 4a-d) that provides for the attachment between the calcareous layer and the membrane (Packard, 1980; Ferguson, 1982, 1985; Ewert et al., 1984; Packard et al., 1984). The mode of attachment between shell units of rigid-shelled gecko eggs and fibres of the shell membrane has not been determined (Packard and Hirsch, 1986). When shell units abut closely and form a dense, highly organized, compact layer, the eggshell is rigid. By contrast, if shell units are not closely apposed or the calcareous layer is not organized into discrete shell units, the eggshell is flexible (Fig. 4e). In general, the calcareous layer is thick relative to the shell membrane in rigid-shelled eggs but equals in thickness to the shell membrane or is thin relative to the shell membrane in flexible-shelled eggs (Figs. 4a-f).

Flexible-shelled eggs, laid by most squamates (Figs. 4a-d), present a somewhat different situation from crocodilians and turtles in that the calcareous layer often occurs as a thin crust or as isolated deposits overlaying a shell membrane that itself is organized into a series of crests and troughs (Packard and Hirsch, 1986). The crystalline layer in squamate eggshells may be nodular or bulbous; may consist of plaques or rosettes or both; may have a bubbly, frothy, or fenestrated appearance; or may be relatively amorphous (Packard et al., 1982a, 1982b; Packard and Hirsch, 1986; Deeming, 1988; Schlëich and Kastle, 1988, Sahoo et. al., 1996, 1998, 2009).

Some authors have reported that squamate eggshell lack a calcareous layer, unfortunately, the surface of squamate eggshells can appear to lack a calcareous layer simply because this layer

does not always appear in the SEM to be obviously crystalline. Flexible shelled eggs laid by the tuatara (*Sphenodon punctatus*) represent an exception to the general pattern manifested by flexible-shelled eggs of other squamates (Packard, 1982c; Packard and Hirsch, 1986; Packard et al., 1988). Tuatara eggshells consist of loosely organized shell units or columns as do the flexible-shelled eggs laid by some turtles. However, the columns extent deep into the shell membrane and exhibit a more extensive association with fibres of the membrane that occurs in flexible-shelled eggs of turtles (Fig. 2f). However, all the layers may not be present in all the cases and the thickness is variable (Fig. 4).

The eggshells of most oviparous lepidosourians are fibrous and poorly calcified (Packard et al., 1982; Packard and Packard, 1991). The inorganic (mineral) content of the shell is usually restricted to the outermost portion and is comprised mainly of calcium carbonate in the form of calcite (Packard et al., 1982; Deeming, 1988; Packard and Packard, 1991). When present, calcite may form a thin layer, as found in eggs of the lizards (Packard et al., 1982) or occur as isolated aggregates.

Studies on the chemical composition of the eggshell of most of the lizards have not been carried out. The amount of calcium deposited during eggshell formation affects the thickness of the shell. Calcium deposition is important consideration in determining eggshell quality. Most good quality eggshell of this type of lizard contain approximately 2.2 gm of calcium in the form of calcium carbonate. The ionic content of the various shell layers, specifically looking at the distribution of magnesium, sodium, potassium, calcium within the eggshell can be identified by electron microscopy and x- ray analysis (Deeming, 1988).

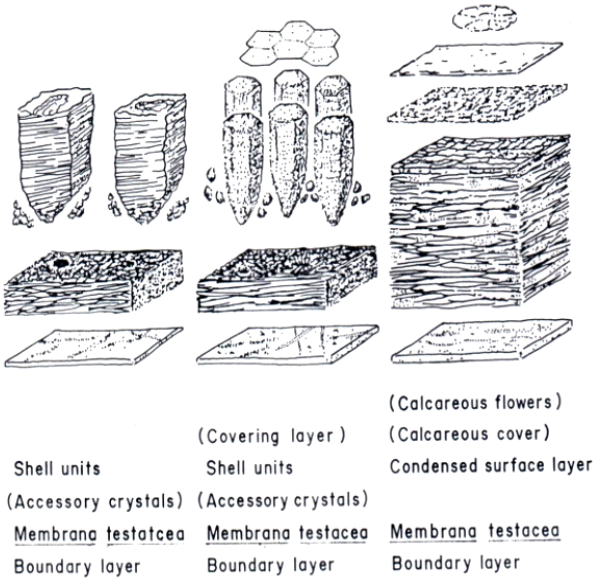

Fig. 3 Eggshells of three reptile groups in exploded view. Terms in brackets refer to Constituents lacking in some of the species. The relative thickness of the superimposed layers may greatly differ between taxa and even within eggs of one clutch (after Scleich and Kastle, 1988).

Shell Membrane

The calcareous layer is followed by a single or double-layered shell membrane. According to Packard (1980), when two shell membranes are present, the inner one generally exhibits a more fibrous structure. The membrane consists of a felt work of bifurcated, uneven, bound fibres arranged in multiple layers. The fibres of a given layer generally form a feltwork and are randomly oriented with respect to one another. Most studies of the ultrastructure of eggs of lepidosaurians have revealed that the fibres are hollow or contain cavities which are thought to aid the elasticity of the shell membrane and allow for swelling with water uptake (Sexton et al., 1979; Packard et al., 1982a). It is uncertain if the inter-fibrilar space changes through incubation.

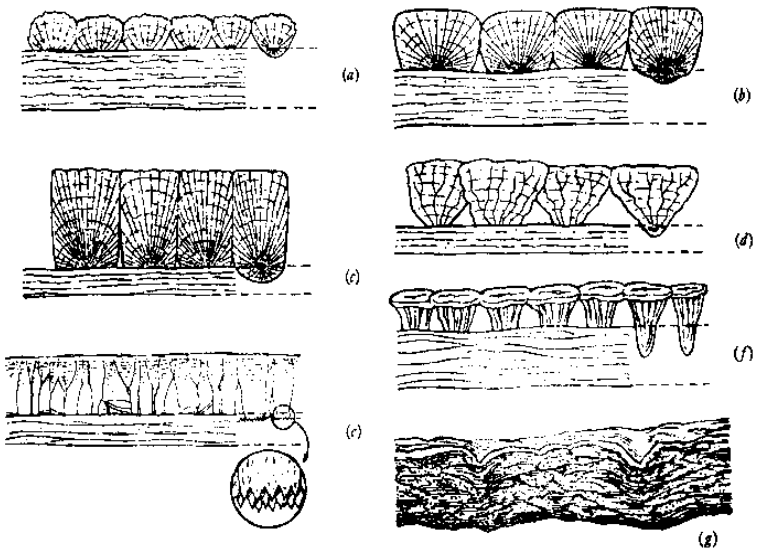

Fig. 4 Highly schematic diagrams of cross-sections of reptilian eggshells. The area to the right in (a)-(f) has been cut away to show the inner aspect of the shell units. (a) Flexible- shelled egg of a sea turtle (e. g., *Lepidochelys olivacea*). Shells units are small and irregular in shape, and the shell membrane is roughly the same thickness as the calcareous layer. (b) Flexible-shelled egg of turtles other than sea turtles (e. g., *Chelydra serpentina*). Shell units are more nodular, more regularly shaped, and the calcareous layer and shell membrane are about equal in thickness. (c) Rigid-shelled turtle egg (e. g., *Trionyx spiniferus*). Shell units are tall, columnar and abut tightly. The shell membrane is thin relative to the thickness of the calcareous layer. In all cases, shell units extend a short distance into the shell membrane and enclose fibres of the membrane, thereby providing for attachment between the calcareous layer and the membrane. (d) Rigid-shelled eggs of a crocodilian (e. g., *Alligator mississippiensis*). Shell units are roughly wedge-shaped and the shell membrane is thin relative to the calcareous layer. The conical tip of a shell unit encloses fibres of the shell membrane and thereby anchors the calcareous layer to the membrane. (e) Schematic cross-section of a rigid-shelled gecko egg (e. g., *Gekko gecko*). Shell units are less well defined and more difficult to trace through the thickness of the shell. The shell membrane is thin relative to the calcareous layer. The enlarged area in the circle shows the tip or inner aspect of shell units. The tip is comprised of fine, needle-like crystallites. The mode of attachment between the calcareous layer and the shell membrane has not been determined. (f) Schematic cross-section of a tuatara (*Sphenodon punctatus*) eggshell. The calcareous layer consists of irregularly- shaped shell units that extend deep into the fibrous shell membrane. (g) Schematic cross-section of the flexible eggshell laid by most oviparous squamates (e. g*., Callisaurus draconoides*). The shell consists of a fibrous membrane organized into a series of crests and troughs with the calcareous layer filling in the troughs and obscuring the crests to varying degrees. The calcareous layer seems simply to rest on the outer surface of the underlying shell membrane and does not enclose fibres of the membrane.

Fibres are the most important shell elements in reptiles. Each membrane fibre at ultrastructural level consists of an electron dense homogenous core surrounded by a less dense mantle (Solomon and Baird, 1977). Specific positions of these fibres act as nucleation sites for initiation of crystal growth. In many species, globules of varying size are associated with the fibres. In turtles, globules occur sporadically in different shell layers.

The number of fibrous layers in the shell membrane varies among species, up to eight layers in some turtles (Packard, 1980; Ewert et al., 1984), and the different layers comprising the shell membrane vary morphologically. This variability, coupled with the occasional occurrence of avian-like air cells in reptilian eggs has led to the interpretation that some reptilian eggs exhibit both an inner and outer shell membrane (Packard and Packard, 1979). However, air cells may also be formed between the shell membrane and the calcareous layer or between the shell membrane and the chorio-allantois (Manolis et al., 1987). Moreover, many reptilian eggs do not form air cells.

Interaction of the calcareous and membrane layers

In general, the shell membrane is tightly bound to the crystalline matrix. It is the tip of the shell unit that seems to provide for the attachment between a shell unit and a shell membrane. Invariably fibre tracks or grooves are present in that part of the tip that normally would be attached to the shell membrane in common snapping turtles (Packard, 1980), crocodiles and birds (Erben, 1970). Bond et al., (1980) observed that many eggshells are tough with a degree of flexibility. This implies that the shell membrane may function not only as a frame work upon which crystals grow but also as an important component of the mechanical properties of the shell. The rigidity or flexibility of reptile eggshell is possible due to the interaction of two basic features, the relative thickness of the membrane layer and morphology of the shell units. Both parallel and random arrangement of fibrils contributes to the mechanical

properties of the eggshell preventing any excess swelling of the oviposited eggs in the nest environment.

CONCLUSION

Future investigation in this field is important to understand the complex role the eggshell in development of the embryo. The diversities in the structure of eggshells of reptiles suggest the success of different groups (squamates, turtles and crocodiles) over the evolutionary period. It is generally believed that some of the squamates, with a thin calcareous layer and hence a thin eggshell, have led to the evolution of viviparity form oviparity.

REFERENCES

Agassiz L (1857) Contributions to the natural history of the United States of America. First Monograph, Vol.I, Little Brown & Company, Boston.

Berman A, Hanson J, Leiserowitz I, Koetzle TF, Weiner S and Addadi L (1993) Biological control of crystal structure: a widespread strategy for adapting crystal properties to function. *Science*, 259:776-779.

Baird T and Solomon SE (1979) Calium and aragonite in the egg shell of Chelonia mydas L. *J Exp Marine Biol Ecol*, 36: 295-303.

Bond GM, Scott VD, Cooke RH and Board RG (1980) Coorelation of hatching techniques with mechanical properties of their shells. In:Curry J, Vincent J, editors. The Mechanical Properties of Biological Materials. Cambridge University Press, Cambridge.

Cox DL, Thomas TJ, Meham P and Sexton OJ (1984) External incubation alters the composition of squamate eggshells. *Comp Biochem Physiol B* 79(3): 481-487.

Deeming DC (1988) Eggshell structure of lizards of two sub-families of the Gekkonidae. *Herpetological Journal* 1:230-234.

Erben HK (1970) Ultrastrukturen and Mineralisation rezenter and fossiler Eischalen bei Vogeln and Reptilien, *Biomineralisation*, 1:1-66.

Erben HK, Hoefs J and Wedepohi KH (1979) Paleobiological and isotopic studies of eggshells from a declining dinosaur species. *Paleobiology*, 5:380-414.

Ewert MA (1979) The embryo and its egg: Development and Natural History. In:Harless M, Morlock H, editors. Turtles: Perspectives and research. John Wiley & Sons, New York, pp 334-413.

Ewert MA, Firth SJ and Nelson CF (1984) Normal and multiple eggshells in batagurine turtles and their implications for dinosaurs and other reptiles. *Canadian J Zool*, 62:1834-1841.

Ferguson MWJ (1982). Increasing porosity of the alligator eggshell caused by extrinsic microbial degradation. *Experiantia*, 37:252-255.

Ferguson MWJ (1985) Reproductive Biology and Embryology of the Crocodilians. In: Gans C, Billett F, Maderson PFA, editors. Biology of the Reptiles, New York, John Willey & Sons, Vol. 14, 329-491.

Hirsch KF (1983) Contemporary and fossil chelonian eggshells. *Copeia,* 1983;382-97.

Jenkins NK and Smkiss K (1968) The calcium and phosphate metabolism of reproducing reptiles with particular reference to the adder (*Vipera berus*). *Comp Biochem Physiol,* 26:865-876.

Jenkins NK (1975) Chemical composition of the eggs of the crocodile (*Crocodylus novaeguineae*). *Comp Biochem Physiol,* 51A:891-895.

Manolis SC, Webb GJW and Dempsey KE (1987) Crocodile egg chemistry. In: Webb GJW, Manolis SC, Whitehead PJ, editors. Wildlife Management: Crocodiles and Alligators, Chipping Norton, New South Wales: Surrey Beatty and Sons Pvt Ltd, pp 445-72.

Packard MJ (1980) Ultrastructural morphology of the shell and shell membrane of eggs of common snapping turtles (*Chelydra serpentine*). *J Morphol,* 165:187-204.

Packard MJ and Hirsch KF (1986) Scanning electron microscopy of eggshells of contemporary reptiles. *Scanning Electron Microscopy*, 4:1581–1590.

Packard GC and Packard MJ (1988) The physiological ecology of reptilian eggs and embryos. In:Gans C, Huey RB, editors. Biology of the Reptilia, Vol. 16, New York: Alan R. Liss. pp 523-605.

Packard MJ and Packard GC (1984) Comparative aspects of calcium metabolism in embryonic reptiles and birds. In: Seymour RS, editors. Respiration and Metabolism in Embryonic Vertebrates, The Hague.

Packard MJ and Packard GC (1979) Structure of the shell and tertiary membranes of eggs of softshell turtles (*Trionyx spiniferus*). *JMorphol*, 159:131-43.

Packard MJ, Burns IK and Packard GC (1982a) Structure of shells from eggs of zebra-tailed lizards (Iguanidae: *Callisaurus adraconoides*). *Zool J Linn Soc,* 75(4):297-311.

Packard MJ and Demarco VG (1991) Eggshell structure and formation in eggs of oviparous reptiles. In:Deemings DC, Ferguson MWJ, editors. Egg Incubation: Its Effect on Embryonic Development in Birds and Reptiles, Cambridge University Press, pp 53–69.

Packard GC, Tracy CR and Roth JJ (1977) The physiological ecology of reptilian eggs and embryos, and the evolution of viviparity within the class reptilia. *Biol Rev* 52, 71-105.

Packard MJ, Hirsch KF and Meyer-Rochow VB (1982b) Structure of the shell from the eggs of the tuatara, *Sphenodon punctatus*. *J Morphol,* 174:197-205.

Packard MJ, Packard GC and Boardman TJ (1982c) Structure of eggshells and water relations of reptilian eggs. *Herpetologica,* 38:136-155.

Packard MJ and Packard GC (1991) Sources of calcium, magnesium and phosphorus for embryonic soft-shell turtles (*Trionyx spiniferus*). *J Exp Zool,* 258:151-157.

Packard MJ, Thompson MB, Goldie NK and Vos M (1988) Aspects of shell formation in eggs of the tuatara *(Sphenodon punctatus). J Morphol,*197:147-57.

Packard MJ, Phillips JA and Packard GC (1992) Sources of minerals for green iguanas (*Iguana iguana*) developing in eggs exposed to different hydric environments. *Copeia*, 1992:851–858.

Robertson DS, McKenna MC, Toon OB, Hope S and Laillegraven JA (2004) Survival in the first hours of the Coenozoic. *Geol Soc Am Bull,* 116:760-768.

Sahoo G, Sahoo RK and Mohanty-Hejmadi P (1998) Calcium metabolism in olive ridley turtle eggs during embryonic development. *Comp Biochem Physiol* A, 121:91-97**.**

Sahoo G, Mohapatra BK, Sahoo RK and Mohanty-Hejmadi P (1996) Ultrastructure and characteristics of eggshell of olive ridley turtles, *Lepidochelys olivacea*, from Gahirmatha. *Acta Anatomica,* 156:261–267.

Sahoo G, Sahoo RK and Mohanty-Hejmadi P (1998) Calcium metabolism in olive ridley turtle eggs during embryonic development. *Comp Biochem Physiol A,* 121:91–97.

Sahoo G, Mohapatra BK and Dutta SK (2009) Structural changes in olive ridley turtle eggshells during embryonic development. *Herpetological Journal,* 19:143-149.

Sahoo G, Mohapatra BK and Dutta SK (2010) Chemical composition and ultrastructure of shells of unfertilized eggs of olive ridley turtles, *Lepidochelys olivacea*. *Current Herpetology,* 29(1): 37–43.

Sauer EGF (1972) Ratitae eggshell and phylogenetic questions. *Bonn Zool Beitr,* 23:3-48.

Schleich HH and Kastle W (1988) Reptile Eggshells SEM Atlas. Stuttgart:Gustav Fischer.

Schmidt WJ (1943a) Uber den Afubu der Kalkschale bei den schildknteneiern. *Z MorpholOkoi Tiere*, 40:1-16.

Schmidt WJ (1943b) Uber den Bau der Kalkigen eischale vol *Lepidodactulos lugubris* OVB (Geckonidae). *Zool Anz,* 142:58-61.

Sexton OJ, Veith GM and Phillips DM (1979) Ultrastructure of the eggshells of two anoline lizards. *J Exp Zool,* 207:227-236.

Solomon SE and Tippett R (1987) The intra-clutch localisation of fungal hyphae in the eggshells of the leatherback turtle (*Dermochelys coriacea*). *Animal Technol*, 38(2):73-79.

Solomon SE and Baird T (1976) Studies on the eggshell (oviducal and oviposited) of *Cheloniamydas*. *J Exp Marine Biol Ecol,* 22:145–160.

Solomon SE and Baird T (1977) Studies on the soft shell membranes of *Chelonia mydas*. *J. Exp Marine Bio Ecol,* 27:83–92.

Simkiss K (1962) The sources of calcium for the ossification of the embryos of the giant leathery turtle. *Comp Biochem Physiol,* 7:71-79.

Simkiss K (1967) Calcium in Reproductive Physiology. Chapmann and Hall, London, pp 264.

Webb GJ and Cooper-Preston H (1989) Effects of incubation temperature of crocodiles and the evolution of reptilian viviparity. *Am Zoologist*, 29:953-971.

Young JD (1950). The structure and some physical properties of the testudine eggshell. *Proc Zool Soc London*,120:455-469.

AETIO-PATHOLOGY OF ORAL MUCOSAL CELLS DURING CARCINOGENESIS

Abhimanyu Mohanta[1], Prafulla K. Mohanty[2], Gadadhar Parida[3]

1*. UGC Research Fellow, P.G. Department of Zoology, Utkal University, Vani Vihar, Bhubaneswar, Odisha, India-751 004. E-mail: amohanta01@gmail.com

2. Professor, P.G. Department of Zoology, Utkal University, Vani Vihar, Bhubaneswar, Odisha, India-751 004. E-mail: prafulla.mohanty3@gmail.com

3. Former Professor and Head, Department of Oncopathology, Acharya Harihar Regional Cancer Centre (AHRCC), Cuttack, Odisha, India-753 007. E-mail: nituparida@yahoo.com.

*Address for correspondence-E-mail: amohanta01@gmail.com

ABSTRACT

Oral cancers are malignant neoplasms that affect the tissues of the mouth. Eventually, the multi-stage transformation of oral mucosal cell to a malignant one has been attributed to the consumption of tobacco and alcohol, some of the biological agents (oral lichen planus, Epstein-Barr virus, human papilloma virus), poor oral hygiene, age, gender, familial pre-disposition, changing food pattern, diet and modern life style. A progressive gross alteration in the form of leukoplakia, erythroplakia, benign and malignant tumors is characterized by pleomorphic cytological alteration. Being nucleus is the genetic store-house of the cell, any alteration at nuclear level leads to nuclear anomalies in general and the genetic components in particular. The frequency of micronucleation, multinucleation, anisonucleosis and nuclear-cytoplasmic ratio of the mucosal cells also increases from premalignant to malignant individuals. The most formidable drastic genotoxic effects in the form of pyknosis (ink-dot nucleus), karyorrhexis and karyolysis have also been observed in the exfoliated cytosmears of oral cancer patients. Presence of these nuclear anomalies indicates malignancy. The least number of cytological atypias and nuclear anomalies were found in the alcoholic group as compared with the other groups. The frequency of atypias and nuclear anomalies were observed to be in increased state from lower (30-49 years) to higher age groups (60-79 years). In a nut-shell, the multi-factorial effects are proved to be time-dose-dependent. More and longer the exposure, greater is the risk of malignant transformation and shorter is the survival expectancy.

Key words: Aetio-pathology, atypia, carcinogenesis, nuclear anomalies, pleomorphism

INTRODUCTION

Oral cancer remains a highly lethal disease and is one of the most debilitating and disfiguring of all malignancies. Multi-stage transformation of oral mucosal cell to a malignant one has been attributed to the multi-factorial effect. Aetiologically, all forms of tobacco (Anonymous, 1985; Nair et al., 1996; Lee et al. 2003; Anonymous, 2007; Madani et al., 2010), alcohol (Baan et al., 2007; Anonymous, 2010), oral lichen planus (OLP), Epstein-Barr virus (Jalouli et al., 2010), human papilloma virus (HPV) (Turner et al., 2011), poor oral hygiene and

modern life style have been reported to be strongly linked to the development of oral cancer. Various mutagens as well as carcinogens trigger the genetic component of the buccal epithelial cells to make mutant, aberrant and turn them into neoplastic cells. In due course of carcinogenesis, a progressive alteration in the form leukoplakia, erythroplakia, benign and malignant tumors are, no doubt, grossly noticed but microscopically a number of cytological atypias are observed. Not only cytological but also nuclear pleomorphism is well exhibited in the cytosmears of oral cancer patients. In addition to these, nuclear anomalies are frequently observed which may be attributed to the genotoxic effect of tobacco and alcohol (Mohanta et al., 2013).

In this context, early detection of oral cancer is a challege for dentists and pathologists. McKinley (2004) stated that the cytopathologists very often encounter problems in detection and diagnosis of the oral cancer patients as most of the oral squamous cells appear to be either well differentiated or moderately differentiated and mimic to be benign and non-neoplastic. Contrary to that, many benign and non-neoplastic lesions appear to be malignant neoplasms. Parida (2001) has also reported that a practical difficulty arises with well-differentiated squamous cell carcinoma in cytologic examinations as in many of these cases nuclear characteristic of malignancy, such as hyperchromatism and an increased nuclear-cytoplasmic ratio are not marked. Therefore, in the present study, the aetio-pathology of exfoliated oral mucosal cells during carcinogenesis was undertaken with respect to age, sex, oral sites and nature of addiction such as tobacco and alcohol for an early detection and diagnosis of the oral cancer cases.

MATERIALS AND METHODS

1. The Patients and collection of samples

Exfoliated scrape smears were collected from the clinically diagnosed 136 patients suffering from precancerous lesions and oral squamous cell carcinoma (OSCC) at the Out Patient Department (OPD) of Acharya Harihar Regional Cancer Centre (AHRCC), Cuttack, Odisha, during May 2007-May 2009. Smearing was undertaken on the pre-cleaned-coded microslides and the slides were fixed in aceto-alcohol (1:3) fixative, immediately. On the basis of International Classification of Diseases (ICD-10) by World Health Organization (WHO), two slides were smeared and prepared from each affected sites of the patient. Prior to the collection of samples, case-history of the patients related to their age, sex, food habits, oral hygiene and occupation were taken into account for detailed analysis. A parallel set of 136

samples were also collected from the non-addicted and non-cancerous individuals from different regions of Odisha which is called as control group.

This study was approved by Subject Research Committee (SRC) of Utkal University, Bhubaneshwar, Odisha, India and necessary permission was also obtained from the Director, AHRCC, Cuttack, Odisha, India for the same purpose.

2. Staining protocol and scoring of cytological atypias

Wet fixed cytosmears were stained by adopting Papanicolaou's staining protocol and counterstained with the Giemsa's solution. Different types of cytological atypias as well as nuclear anomalies were noted and photomicrgraphed by using computer-assisted Cat Cam 1.30 (1.3 Mega Pixel) microscope camera (Catalyst Biotech®, Maharashtra, India) which was fitted with hund®-Wetzler light microscope.

3. Statistical analyses

The findings were statistically analyzed and interpreted with respect to oral sites, sexes and nature of addiction.

RESULTS

Out of 136 oral cancer patients, 5(6.1 %) males and 6(11.1%) females were reported to have lesion at lip, 11(13.4%) males and 7(12.9%) females and 6(11.1 %) females were affected at alveolus and gingiva; 7(8.5%) males and 6(11.1%) females were suffering from lesion at floor of the mouth, 6(7.3%) males and 3(5.6 %) females were of palatal lesion, and 37(45.2%) males and 26(48.2%) were suffering from lesion at buccal mucosa. Based on the degree of pathogenicity 32(39.2%) males and 23(42.5%) females were recorded to be in premalignant, out of which 15(18.30%) males and 14(25.92%) females were of leukoplakia, 17(20.73%) males and 9(16.7%) females were of erythroplakia. Rest 50(60.8%) males and 31(57.5%) females were of malignant or cancerous ones (Table 1).

The recorded age of the patients varied from 30 to 87 years (Table 2). Therefore, the collected samples were grouped into three broad age groups, such as 30-49 years, 50-69 years and 70-89 years. Out of 82 males, 33 (40.28%) patients belong to 30-49 years, 34(41.5%) patients were aged between 50-69 years and 15(18.3%) patients come under 70-89 years. Out of 54 females, 11(20.4%) were aged between 30-49 years, 36(66.6 %) were grouped under 50-59 years and 7(13.7%) were of 70-89 years of age group. The

relative proportion of the patients was found to be more (51.5%) in the age group of 50-69 years than the other two.

On the basis of addiction (Table 3), out of 136 patients, 126(92.65%) were addicted to different forms of tobacco and alcohol for more than 15 years and the rest 10(7.35%) were absolutely non-addicted.Among males, the highest number with 24 cases were recorded in chewer-smokers group followed by 23 caces in purely chewers group. On the other hand, 20 females were recorded to be the highest in chewers group followed by 18 females in alcoholics group. Eight males and 6 females were used to chewing, smoking and drinking alcohol in their daily life and, thus, belong to chewers-smoker-alcoholic group.

On account of Broder's classification (Broder, 1926), three types of cells such as, well differentiated squamous cells (WDSC), moderately differentiated squamous cells (MDSC) and poorly differentiated squamous cells (PDSC), were observed during diagnosis in all types of squamous cell carcinoma (SCC). However, referring to the normal oral squamous cells, a number of atypical pleomorphic cells were observed in the exfoliated cytosmears from oral neoplasm. On the basis of their morphological peculiarities and nature of keratinisation, the atypical cells are named as Keratinized spindle cells (KSC), Keratinized tadpole cells (KTC), Keratinized strap (Anitschkow) cells (KSC-A), keratinized fibre cells (KFC), keratinized round cells (KRC), Micronucleated cells (MNC), Plump keratinized squamous cells (PKSC) and Non-keratinized malignant squamous cells (NMSC). Due to acidic (eosinophilic) cytoplasm, except the NMSCs, all these cells get keratinized and appear to be red, orange or yellow with Papanicolaou's stain (Fig.1).

Since, nucleus was the store-house of genetic information, any such alteration at nuclear level indicates the genetic alteration of the cells. In addition to different forms of tobacco and alcohol, many other carcinogens are involved at gene level to bring about such alteration. As a result, the cells behave aberrantly and become the cause of carcinogenesis.

The cytoplasm appears to be dense, and keratinized where as tremendous reactive changes are observed during different stages of oral carcinogenesis. In this case-control study, least number of cytological atypias and nuclear anomalies were found in the alcoholic group as compared with the other groups. The frequency of atypias and nuclear anomalies were observed to be in increased state from lower (30-49 years) to higher age (60-79 years) groups (Fig.2).

Nuclear anomalies (Table 3) like perinuclear halo, intranuclear halo, huggings (nuclear apposition), blebbing, crenation or folding of nuclear membrane are commonly observed during premalignant cases. Although hypochromatic nuclei are frequently observed in leukoplakia and erythroplakia, the nuclei get hyperchromatic in due course of carcinogenesis. Frequency of micronucleation, multinucleation, and anisonucleosis also increases from premalignant to malignant individuals. Micronucleation is considered to be a biomarker or onco-indicator duing oral carcinogenesis (Mohanta et al., 2013).

The most drastic genotoxic effect in the form of pyknosis (ink-dot nucleus), karyorrhexis and karyolysis has also been observed in the exfoliated cytosmears of oral cancer patients (Fig.3). Pyknosis (also called karyopyknosis) is the irreversible condensation of chromatin in the nucleus of a cell undergoing necrosis or apoptosis. It is followed by karyorrhexis, or fragmentation of the nucleus. The nuclear envelope becomes discontinuous and the DNA gets fragmented. The nucleus breaks into several discrete chromatin bodies or nnucleosomal units due to the degradation of DNA. Karyolysis is the complete dissolution of the chromatin of a dying cell due to the enzymatic degradation by endonucleases. It is usually preceded by karyorrhexis and occurs mainly as a result of necrosis, which is a form of traumatic cell death that results from acute cellular injury. In contrast, apoptosis is a naturally occurring programmed and targeted cause of cellular death. While apoptosis often provides beneficial effects to the organism, necrosis is almost always detrimental and can be fatal as in carcinogenesis.

DISCUSSION

Carcinogenesis is the progression from a normal healthy cell to a pre-malignant or a potentially malignant cell which is characterized by an ability to proliferate autonomously. The transformation process in which an apparently normal cell is transformed into a malignant cell consists of three stages namely initiation, promotion and progression. It involves a series of genetic steps and also epigenetic (outside the gene) changes. These changes include the aberrant expression and function of molecules regulating cell signaling, growth, survival, motility, angiogenesis (blood vessel proliferation), and cell cycle control (Scully, 2011). During this multi-stage process of transformation, the normal oral mucosal cells also get transformed into various atypical forms with or without any resemblance to the parent cell called cytological pleomorphism.

Use of the term premalignant lesion is now discouraged in favour of the phrase "potentially malignant disorders" (Warnakulasuriya, 2007). The lesions of most relevance are leukoplakia, erythroplakia, submucosal fibrosis and oral lichen planus in connection with the oral carcinoma.

The WHO working group defines leukoplakia as "a keratotic white patch or plaque that cannot be scraped off and cannot be characterized clinically or pathologically as any other disease." Therefore, a process of exclusion establishes the diagnosis of the disease. The prevalence of leukoplakia has been estimated at 2% (Forastiere et al., 2001), and the annual malignant transformation rate is estimated to be from 0.3% to 1% (Koch et al., 1999). On the other hand, erythroplakia is defined as a "fiery red patch or bright red velvety plaques that cannot be characterized clinically or pathologically as any other definable disease". These lesions are often symptomatic, have a degree of increased vascularity, and carry a high risk of harbouring dysplasia. In erythroplakia, the risk of malignant transformation is 10.3%, with high-grade dysplasia and carcinoma in situ carrying 24.1% risk (Mehanna et al., 2009).

Oral submucous fibrosis (OSMF) causes progressive trismus due to fibrosis of the connective tissues of the cheeks. It is strongly associated with betel nut or tobacco chewing and it is likely that the use of these carcinogens give it an association with oral cancer. The rate of malignant transformation is estimated at 0.5% per year (Walker et al., 2003). Chronic inflammation has long been reported to increase the risk of oral cancer. This may be the way in which candidiasis, syphilis, and chronic trauma from poor dentition contribute to a slight increase in risk of oral cancer.

Poor oral hygiene has been linked to increased carcinogenesis, the exact mechanism is unclear but it may be related to carcinogenic effects from the high burden of polymicrobial oral flora (Bloching et al., 2007). Lichen planus is an oral autoimmune condition with a number of morphological variants including reticular (fine white lacy lines), erosive (shallow ulcers), atrophic (thinned erythematous mucosa) and bullous (fluid filled vesicles). It can be symptomatic typically with a burning feeling or hypersensitivity of the affected mucosa. In fact, its status as a potentially malignant condition is debatable. Its risk of malignant transformation is below 1% per year (Lee et al., 2003).

The disproportionately higher incidence of carcinoma of the head-neck in relation to other malignancies in India, may be due to use of tobacco in various forms, consumption of alcohol, low socioeconomic condition related to poor hygiene, poor diet and rampant viral infections (Franceschi et al., 2000). The age adjusted incidence is reported from

approximately 3.4 to 13.8 per 1, 00, 000. Males are more often affected than females by a ratio of 1.5:1 (Warnakulasuriya, 2009).

A rising incidence has been noted in patients under 45years of age (Warnakulasuriya et al.,2007), with approximately 6% of oral cancers now being observed in this age group compared with 3% in 1973 (Llewellyn et al., 2001). It is a common cause of disability and death in the elderly. Over 50% of malignant neoplasm occurs in persons over fifth decade of life (Dix and Cohen, 1999; Parkin et al., 2001). Anisimov (2003) predicts that aging may predispose to cancer by several mechanisms, such as tissue accumulation of cells in late stages of carcinogenesis; alterations in homeostasis, in particular, alterations in immune and endocrine system and telomere instability linking aging and increased cancer risk. Increased susceptibility to the effects of tumor promoters is found both in aged animals and aged humans, as predicted by the multistage model of carcinogenesis.

Oral cancers account for the highest cancer related mortality among men aged 30-69 in India (Dikshit et al., 2012).Approximately, 96% of oral cancers are detected above the age of 40, and more than 50% of all cancers occur in persons older than 65 (Edwards et al., 2002). However, recent evidence indicates oral cancers are more prevalent in people younger than 40 years (Myers et al., 2000; Ries et al., 2003). In the present study, the relative proportion of the patients was found to be more (51.5%) in the age group of 50-69 years than the other two.

Larond et al., (2008) claim that oral cancer has no single factor but results from a variety of factors including the person's lifestyle, especially regarding tobacco and alcohol consumption. Aetiologically, all forms of tobacco use have strongly been linked to the development of oral cancer. Cigar and pipe smoking are associated with a greater risk of the development of oral cancer when compared with cigarette smoking, probably because most cigarettes have filters that reduce the load of carcinogen directly coming in contact with the oral mucosa (Rudolph and Atvis, 2003). However, there is a distinct geographical variation among the risk factors contributing to oral cancer. In the western population exposure to sunlight (lip cancer), cigarette-smoking, and alcohol consumption are the frontline etiologic culprits compared to the use of smokeless and combustible tobacco which are more prevalent in the South East Asian countries (Warnakulasuriya et al., 2007).

Carcinogens in tobacco (mainly polycyclic aromatic hydrocarbons-PAH) can cause an accumulation of genetic mutations in oral epithelial cells including *p53* mutation, mutation and loss of heterozygosity (H-RAS) and amplification (K-RAS and N-RAS) of the RAS

oncogenes leading to abnormal and uncontrollable cell division and growth (Scully and Bedi, 2000).

Oral neoplasia has been associated with chewing of tobacco with betel quid (BQ) in India and other Asian countries, whereas in western countries, cigarette smoking and heavy alcohol consumption are the main risk factors (Warnakulasuriya et al., 2007). The International Agency for Research on Cancer (IARC) confirms that smoking of various forms of tobacco (e.g., bidis, pipes, cigars and cigarettes) is carcinogenic in humans (Llewellyn et al., 2001).

Betel nut is the fourth most commonly used psychoactive substance in the world after caffeine, alcohol, and nicotine. Betel nut is present in a number of chewing products like mawa, paan, gutkha, khaini, and paan masala. Reasons for using betel nut include achieving euphoria, combating fatigue, increasing salivation, attaining satiation, and even seeking relief of toothaches (Pindborg and Sirsat, 1966; Aziz, 2010).

Chewing betel nut on a habitual basis is known to be deleterious to human health (Trivedy et al., 1999). The WHO and the IARC classified betel nut as a group 1 human carcinogen (Pankaj, 2010). The IARC considers paan and gutkha as substances containing known human carcinogens, of which the betel nut is believed to be the main culprit (Verma, 2011). Betel-quid induced lichenoid lesions mainly on buccal mucosa have been reported at quid retained sites. In chronic chewers, a condition called betel chewers mucosa is often found where the quid is placed. Betel nut chewing is implicated in oral submucous fibrosis (OSF) and its use along with tobacco can cause leukoplakia, both of which are potentially malignant in the oral cavity (Anand et al., 2014). Although it is widely accepted that the presence of tobacco in BQ plays an important role in the pathogenesis of oral squamous cell carcinoma, the carcinogenic potential of betel in the absence of other ingredients is less clear (Anonymous, 1985). Epidemiological data from several studies confirm that the habit of BQ chewing increases the relative risk of developing oral squamous cell carcinoma (Merchant et al., 2000; Jeng et al.,2001; Sharan et al., 2012; Gupta et al., 2013).

Chewing of the betel quid (often referred to as “paan”) is fairly common. The quid is usually made up of areca nut, catechu, slaked lime, and often tobacco, which are placed in a betel leaf and folded into the characteristic triangular shape. Chewing paan releases carcinogenic nitrosamines from the areca nut that can cause pre-neoplastic changes (Anonymous, 2012). Guha et al. (2013) have reported that eliminating consumption of betel quids in India alone could have prevent country’s burden of more than 37, 000 oral cancer cases in a year.

Alcohol, acting both independently as well as synergistically with smoking, has been implicated in oral carcinogenesis (Pindborg et al., 1968). More importantly, alcohol may act as a solvent and enhance the penetration of carcinogens into target tissues. Acetaldehyde, which is the alcohol metabolite, has been identified as a tumor promoter and is also known to be cytotoxic and causes production of free radicals (Einhorn and Wersall, 1967; Harty et al., 1997; Petti, 2003).Also, acetaldehyde, a metabolite of alcohol, is known to be a direct carcinogen by causing alteration of the *p53* gene and *Ras* oncogenes (Rudolph and Atvis, 2003). Acetaldehyde is responsible for the oral carcinogenic effect of ethanol, owing to its multiple mutagenic effects on DNA (Seitz and Stickel, 2007).

Lifestyle factors such as smoking, poor oral hygiene, and certain dietary deficiencies (folate, vitamin B6) or an excess of others (vitamin A/β-carotene), owing to unevenly composed diets or self-medication, also increase the risk for alcohol-associated tumors (McLaughlin and Gridley, 1988).Optimal levels of daily allowance of micronutrients like vitamin C, E, antioxidants, zinc, β-carotene, and folate are effective in prevention of oral cancer. (Taghavi and Yazdi, 2007). It has been reported that rapid urbanization leading to an unhealthy lifestyle, such as increased access to and utilization of tobacco in its various forms as well as abuse of alcohol, leads to an increased incidence of oral precancer and cancer (Anonymous, 2001). When tobacco use is combined with frequent alcohol consumption, the risk increases substantially as these two risk factors act synergistically exacerbating each other's harmful effects (Anonymous, 1985; Koch et al., 1999; Lee et al., 2003; Perera-Milla et al., 2003; Walker et al., 2003).

Oji and Chukwuneke (2007) in Enugu found that patients with oral cancer in their tertiary health centre gave no history of tobacco or alcohol misuse. They postulated that poverty, malnutrition, lack of education, poor oral hygiene and chronic malaria may be more important in the aetiology and severity of oral cancer in their series.

In this case-control study, the pattern of keratinization was observed from normal to malignant oral squamous cells. In normal cells, the cytoplasm is either hypokeratinized or non-keratinized and appear to be sky blue in color. On the other hand, except NMSC, almost all other detected cytological atypias are observed to be hyperkeratinized and appear to be deep orange in color. The atypical pleomorphic cells are observed to be thicker, denser and hyperkeratinized in patients habituated with any form of tobacco and alcohol than the non-addicted cancerous patients. Similarly, the exfoliated mucosal cells collected from occasionally habituated normal individuals are observed to be keratinized so far as non-habituated normal individuals are concerned in our study (Mohanta et al., 2014).

In the present study, out of 136 patients, 126(92.65%) were addicted to different forms of tobacco and alcohol for more than 15 years and the rest 10(7.35%) were absolutely non-addicted. Numerically more number of cytological atypias and nuclear anomalies were observed in chewer-smoker-alcoholic group than single (chewer, smoker, alcoholic) or double users (chewer-smoker) groups. Interestingly, the least number of cytological atypias and nuclear anomalies were found in the alcoholic group as compared to the other groups. The frequency of atypias and nuclear anomalies were observed to be in increased state from lower (30-49 years) to higher age groups (60-79 years) which corroborates with the earlier findings of time-dose response.

Human papilloma virus (HPV), Epstein-Barr virus (EBV) and *Candida albicans* are also found to be closely associated with benign and malignant oral lesions. HPV positivity is higher in tumors from the oral cavity (59%), pharynx (43%) and larynx (33%) (Gillison et al., 2000). *Candida albicans* can induce epithelial proliferation and can produce carcinogens from pro-carcinogens in vitro. Chronic hyperplastic candidosis presents as nodular or speckled-white mucosal plaques and are considered as potentially malignant oral epithelial lesions (Cawson, 1969).

Furthermore, people in low socio-economic group were less likely to have access to proper health services and health education that would empower them to make informed decisions and to improve their health and standard of living (Poul, 2008). A study in Canada shows that people in lower socio economic class were less likely to visit their dentist regularly and suggested that increased incidence of oral cancer in this group may also be related to their poor oral hygiene (Johnson et al., 2010).

CONCLUSION

The majority of oral squamous cell carcinomas are related to tobacco in various forms, areca nut or betel quid chewing, heavy alcohol drinking and dietary micronutrient deficiency. Being a part of modern life style, use of tobacco and areca nut, alone or in combination, khaini and gutkha, smoking and drinking account for the vast majority of oral cancers and oral potentially malignant disorders among the younger generation. Mostly, the genotoxicity of tobacco and alcohol induces cytological pleomorphism along with nuclear anomalies in premalignant and malignant oral neoplasm during carcinogenesis. Nuclear anomalies like hypochromasia, hyperchromasia, micronucleation, multinucleation, anisonucleosis, pyknosis (ink-dot nucleus), karyorrhexis and karyolysis are some of the sign and symptoms of aetiopathology of oral mucosal cells during carcinogenesis. Presences of these nuclear

anomalies in the exfoliated cytosmears indicate malignancy. The frequency of atypias and nuclear anomalies were observed to be in increased state from lower (30-49 years) to higher age groups (60-79 years). In a nut-shell, the multi-factorial effects are proved to be time-dose-dependent. Thus, the present study has a practical utility in the field of oral cytopathology in general and in early detection and diagnosis of the oral carcinoma in particular in the long run of carcinogenesis. However, in order to live a healthy life with a smiling face, unwanted and avoidable carcinogenic substances like tobacco and alcohol are suggested to be avoided forever.

ACKNOWLEDGEMENTS

Authors are thankful to the Head, P.G. Dept. of Zoology, Utkal University, Vani Vihar, Bhubaneshwar, Odisha for providing laboratory and library facilities; to the Director and Head, Department of Oncopathology, Acharya Haihar Regional Cancer Center (AHRCC), Cuttack, Odisha for permitting us to collect samples from the oral cancer patients and also for providing library and laboratory facilities. One of us (AM) is grateful to the University Grants Commission (UGC), New Delhi, India for awarding UGC Research Fellowship to undertake this project.

Table 1 Oral site and sex-wise collected samples

No	Oral sites (ICD-10)	Control Group		Premalignant				Malignant		Total
				Leukoplakia		Erythroplakia				
		Male	Female	Male	Female	Male	Female	Male	Female	
1	Lip	5 (6.1)	6 (11.1)	2 (13.3)	2 (14.3)	1 (5.9)	2 (22.2)	2 (4.0)	2 (6.45)	22 (8.8)
2	Tongue	11 (13.4)	7 (12.97)	4 (26.7)	2 (14.3)	3 (17.6)	1 (11.1)	4 (8.0)	4 (12.9)	36 (13.2)
3	Alveolus and gingival	16 (19.5)	6 (11.1)	3 (20.0)	2 (14.3)	2 (11.8)	2 (22.2)	11 (22.0)	2 (6.45)	44 (17.6)
4	Floor of the mouth	7 (8.5)	6 (11.1)	2 (13.3)	3 (21.4)	1 (5.9)	1 (11.1)	4 (8.0)	2 (6.45)	26 (9.5)
5	Palate	6 (7.3)	3 (5.6)	Nil (Nil)	Nil (Nil)	3 (17.6)	1 (11.1)	3 (6.0)	2 (6.45)	18 (6.5)
6	Buccal mucosa	37 (45.2)	26 (48.2)	4 (26.7)	5 (35.7)	7 (41.2)	2 (22.2)	26 (52.0)	19 (61.3)	126 (46.4)
7	Total	82	54	15	14	17	09	50	31	272

Source: Primary data. Figures in parentheses indicate the relative percentage.

Table 2 Age group and sex-wise oral cancer patients

No	Age groups in years	Male	Female	Total
1	30-49	33 (40.28)	11 (20.4)	44 (32.4)
2	50-69	34 (41.5)	36 (66.6)	70 (71.5)
3	70-89	15 (18.3)	07 (13.7)	22 (16.1)
Total	30-89	82(60.3)	54(39.7)	136 (100)

Source: Primary data. Figures in parentheses indicate the relative percentage.

Table 3 Degree of pathogenicity, age group, sex and addiction-wise individuals.

No	Group	Age group (in years)	Sex		Chewers		Smokers		Alcoholics		Chewers- smokers		Chewers-smokers-alcoholics		Non-addicted		Total
			M	F	M	F	M	F	M	F	M	F	M	F		F	
1	Pre-malignant	30-49	08 (9.7)	05 (9.3)	0.2 (8.6)	03 (15.0)	Nil (Nil)	Nil (Nil)	01 (8.4)	01 5.5	03 (12.5)	Nil (Nil)	02 (25.0)	Nil (Nil)	Nil (Nil)	01 (25.0)	13 (9.5)
		50-69	18 (22.0)	14 (25.9)	03 (13.0)	07 (35.0)	03 (33.04)	01 (50.0)	02 (16.6)	04 (22.3)	09 (37.5)	Nil (Nil)	01 (12.5)	02 (33.3)	Nil (Nil)	Nil (Nil)	32 (23.5)
		70-89	06 (7.3)	04 (7.4)	04 (17.4)	02 (10.0)	01 (11.1)	Nil (Nil)	Nil (Nil)	01 (5.5)	Nil (Nil)	01 (25.0)	01 (12.5)	Nil (Nil)	Nil (Nil)	01 (25.0)	10 (7.4)
2	Malignant	30-49	25 (30.5)	06 (11.1)	09 (39.1)	03 (15.0)	02 (22.2)	Nil (Nil)	03 (25.0)	03 (16.8)	06 (25.0)	Nil (Nil)	02 (25.0)	Nil (Nil)	03 (50.0)	Nil (Nil)	31 (22.8)
		50-69	16 (19.5)	22 (40.7)	04 (17.4)	04 (20.0)	02 (22.2)	01 (50.0)	03 (25.0)	08 (44.4)	03 (12.5)	03 (75.0)	02 (25.0)	04 (66.7)	02 (33.3)	02 (50.0)	38 (28.0)
		70-89	09 (11.0)	03 (5.6)	01 (4.4)	01 (5.0)	01 (11.1)	Nil (Nil)	03 (25.0)	01 (5.5)	03 (12.5)	Nil (Nil)	Nil (Nil)	Nil (Nil)	01 (16.7)	Nil (Nil)	12 (8.8)
All sites		30-89	82	54	23	20	09	02	12	18	24	04	08	06	82	54	136

Figures in parentheses indicate relative percentage. M-Male, F-Female

Table 4 Nuclear anomalies in normal, precancerous and cancerous groups in both sexes

No	Group	Nuclear membrane			Nuclear staining			Nuclear division		Nuclear condition		Nuclear texture				
		Regular	Irregular crenation	Folding	Normal	Hypo-chromasia	Hyper chromasia	Iso-nucleosis	Aniso nucleosis	Intact nucleus	Naked nucleus	Multi nucle-ation	Micro-nucleation	Piknosis	Karyo rrhexis	Karyo lysis
1	Control	+	-	-	+	-	-	+	-	+	±	-	±	-	-	-
2	Pre-malignant	±	+	±	-	±	±	±	+	±	±	±	+	+	+	+
3	Malignant	-	+	±	-	±	+	-	+	+	+	±	+	+	+	+

+, -, ± indicates the distinguishing features**:** (i) + Signifies usually present.**;** (ii) - Signifies usually absent.**;** (iii) ± Signifies sometimes present

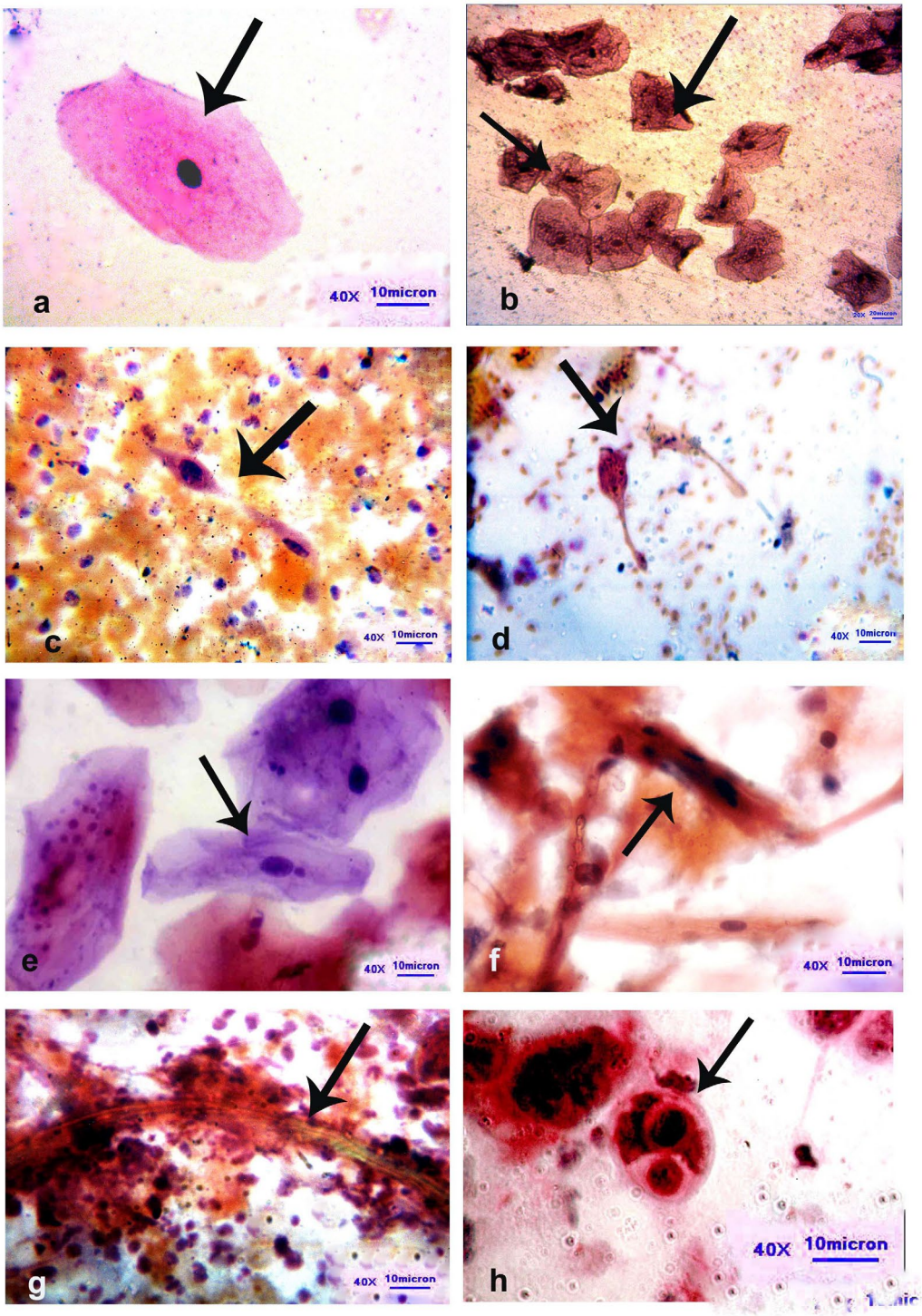

Fig. 1 Normal oral squamous cells and other cytological atypias: **a**.NOSC, **b**. PKSC, **c**.KSC, **d**.KTC, **e**.MNC, **f**. KSCA, **g**. KFC, **h**. KRC.

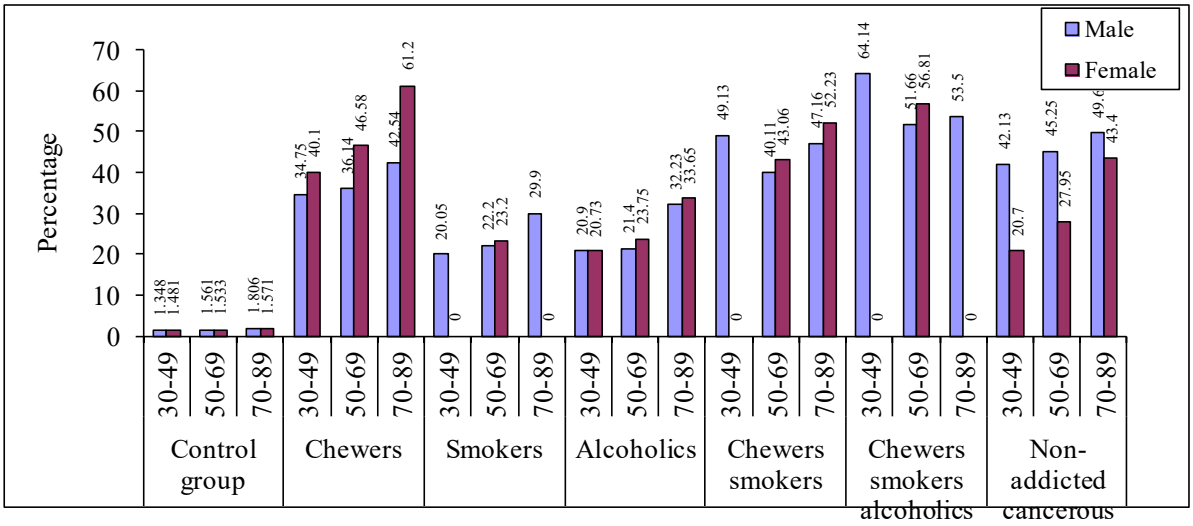

Fig. 2 Age group and sex-wise mean percentage of atypical cells in normal, addicted and non-addicted cancerous groups.

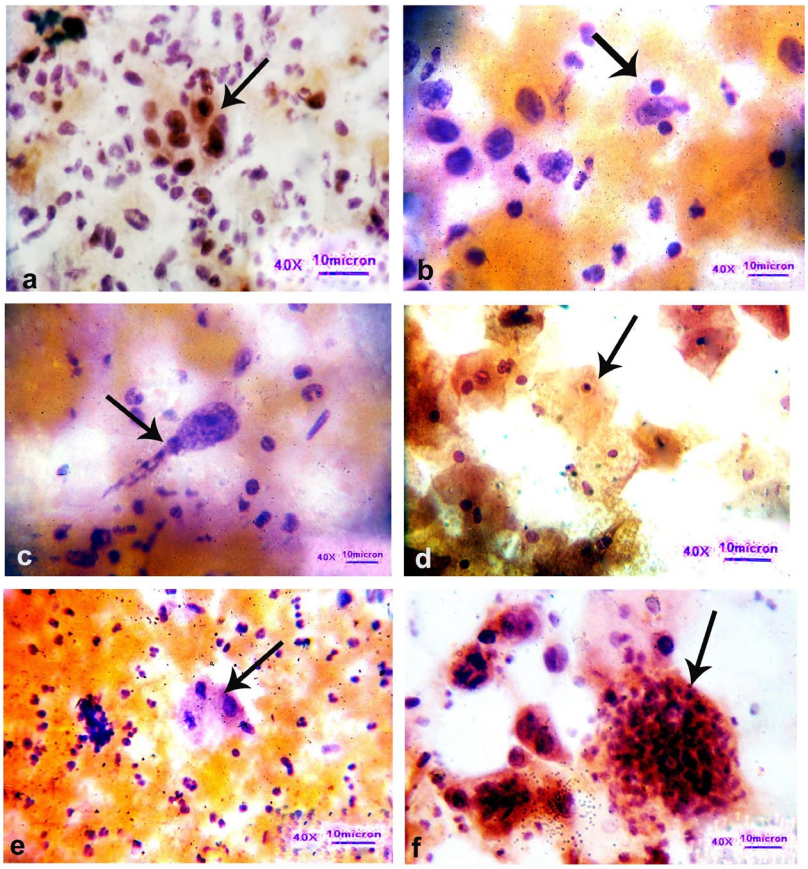

Fig. 3 Nuclear anomalies in oral squamous cells of cancer affected individuals: a. Anisonucleosis, b. Crenation, c. A tadpole-shaped nucleus with nuclear haloes, d.Karyopyknosis, e. Karyorrhexis, f. Karyolysis.

REFERENCES

Ahmed HG, Idris AM and Ibrahim SO (2003) Study of oral epithelial atypia among Sudanese tobacco users by exfoliative cytology. *Anticancer Res* 23(2C): 1943-1949.

Anand R, Dhingra C, Prasad S and Menon I (2014) Betel-nut chewing and its deleterious effects on oral cavity. *J Can Res Ther* 10 (3): 499-505.

Anisimov VN (2003) The relationship between aging and carcinogenesis: a critical appraisal. *Critical Reviews in Oncology/Hematology* 45 (3): 277-304.

Anonymous(1985) Tobacco habits other than smoking; betel-quid and areca-nut chewing; and some related nitrosamines. IARC Working Group. Lyon, 23-30 October 1984. *IARC Monogr Eval Carcinog Risk Chem Hum* 37:1-268.

Anonymous (2007) Smokeless tobacco and some tobacco-specific N-nitrosamines. Lyon, France: World Health Organization.*J Am Dent Assoc* 141:423-428.

Anonymous (2010) IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Alcohol consumption and ethyl carbamate. *IARC Monogr Eval Carcinog Risks Hum* 96: 1-1428.

Anonymous (2001) Oral Health. World Health Organization. Available from: http://www.afro.who.int/ oralhealth/epidemiology.html

Anonymous (2012) Betel quid and areca nut. In: Personal habits and indoor combustions :A review of human carcinogens. *IARC MonogrEval Carcinog Risks Hum* 100 (part E):1-538.

Aziz SR (2010) Coming to America. Betel nut and oral submucous fibrosis. *J Am Dent Assoc* 141 (4): 423-428.

Baan R, Straif K, Grosse Y, Secretan B, El Ghissassi F, Bouvard V, Altieri A and Cogliano V (2007) WHO International Agency for Research on Cancer Monograph Working Group. Carcinogenicity of alcoholic beverages. *Lancet Oncol* 8(4): 292-293.

Bloching M, Reich W, Schubert J, Grummt T and Sandner A (2007) The influence of oral hygiene on salivary quality in the Ames Test, as a marker for genotoxic effects. *Oral Oncol* 43 (9): 933-939.

Broders AC (1926) Carcinoma: grading and practical application. *Arch Pathol* 2: 376-381.

Cawson RA (1969) Leukoplakia and oral cancer. *Proc R Soc Med* 62 (6): 610-614.

Dikshit R, Gupta PC, Ramasundarahettige C, Gajalakshmi V, Aleksandrowicz L, Badwe R, Kumar R, Roy S, Suraweera W, Bray F,Mallath M, Singh PK, Sinha DN, Shet

AS, Gelband H and Jha P (2012) Cancer mortality in India: a nationally representative survey. *Lancet* 379 (9828):1807-1816.

Dix D and Cohen P (1999) On the role of aging in carcinogenesis. *Anticancer Res* 19 (1B):723-726.

Edwards BK. Howe HL, Ries L AG. Thun M J , Rosenberg HM, Yancik R , Wingo P A, Ahmedin JDVM and Feigal EG (2002) Annual report to the nation on the status of cancer, 1973–1999, featuring implications of age and aging on U.S. cancer burden. *Cancer* 94 (10):2766-2792.

Einhorn J and Wersall J (1967) Incidence of oral carcinoma in patients with leukoplakia of the oral mucosa. *Cancer* 20 (12): 2189-2193.

Forastiere A, Koch W, Trotti A and Sidransky D (2001) Head and neck cancer. *N Eng J Med* 345(26):1890-1900.

Franceschi S, Bidoli P, Herrero R and Munoz N (2000) Comparison of cancers of the oral cavity and pharynx worldwide: etiological clues. *Oral Oncol* 36 (1):106-115.

Gillison ML, Koch WM, Capone RB, Spafford M, Westra WH, Wu L, Zahurak ML, Daniel RW, Viglione M, Symer DE, Shah KV and Sidransky D (2000) Evidence for a causal association between human papillomavirus and a subset of head and neck cancers. *J Natl Cancer Inst* 92 (9):709-720.

Guha N, Warnakulasuriya S, Vlaanderen J and Straif K. (2014) Betel quid chewing and the risk of oral and oropharyngeal cancers: a meta-analysis with implications for cancer control. *IntJ Cancer* 135 (6):1433-1443.

Gupta B, Ariyawardana A and Johnson NW (2013) Oral cancer in India continues in epidemic proportions: Evidence base and policy initiatives. *Int Dent J* 63 (1):12-25.

Harty LC, Capraso NE, Hayes RB, Winn DM, Bravo-Otero E, Blot WJ, Kleinman DV, Brown LM, Armenian HK, Fraumeni JF Jr and Shields PG (1997) Alcohol dehydrogenase 3 genotype and the risk of oral cavity and pharyngeal cancer. *J Natl Cancer Inst* 89 (22):1698-1705.

Jalouli J, Ibrahim SO, Sapkota D, Jalouli MM, Vasstrand EN, Hirsch JM and Larsson PA (2010) Presence of human papilloma virus, herpes simplex virus and Epstein-Barr virus DNA in oral biopsies from Sudanese patients with regard to toombak use. *J Oral Pathol Med* 39(8):599-604.

Jeng JH, Chang MC and Hahn LJ (2001) Jeng JH, Chang MC, Hahn LJ. Role of areca nut in betel quid associated chemical carcinogenesis: current awareness and future perspectives. *Oral Oncol* 37:477-92.

Johnson S, McDonald JT, Corsten M and Rourke R (2010) Socio-economic status and head and neck cancer incidence in Canada: a case-control study. *OralOncol* 46:200-203.

Koch WM, Lango M, Sewell D, Zahurak M and Sidransky D (1999) Head and neck cancer in non-smokers: a distinct clinical and molecular entity. *Laryngoscope* 109(10):1544-1551.

Lee CH, Ko YC, Huang HL, Chao YY, Tsai CC, Shieh TY and Lin ML (2003) The precancer risk of betel quid chewing, tobacco use and alcohol consumption in oral leukoplakia and oral submucous fibrosis in southern Taiwan. *Br J Cancer* 88(3): 366-372.

Llewellyn CD, Johnson NW and Warnakulasuriya KAAS (2001) Risk factors for squamous cell carcinoma of the oral cavity in young people--a comprehensive literature review. *Oral Oncol* 37(5): 401-418.

Madani AH, Jahromi AS, Dikshit M and Bhaduri D (2010) Risk assessment of tobacco types and oral cancer**.** *Am J Pharm & Toxicol* 5 (1): 9-13.

McKinley ET (2004) General cytologic principles. (In B.F. Atkinsons (Ed) Atlas of Diagnostic Cytopathology, 2nd Edition. Saunder, Elsevier Inc., Philadelphia, pp 4-25.

McLaughlin JK and Gridley G (1988) Dietary factors in oral and pharyngeal cancer. *J Natl Cancer Inst* 80(15): 1237 – 1243.

Mehanna HM, Rattay T, Smith J and McConkey CC (2009) Treatment and follow up of oral dysplasia - a systematic review and meta-analysis. *Head and Neck* 31(12): 1600-1609.

Merchant A, Husain SS, Hosain M, Fikree FF, Pitiphat W, Siddiqui AR, Hayder SJ, Haider SM, Ikram M, Chuang SK and Saeed SA. (2000) Paan without tobacco: An independent risk factor for oral cancer. *Int J Cancer* 86(1):128-131.

Mohanta A, Mohanty PK and Parida G (2013) Genotoxicity of tobacco and alcohol on human oral mucosal cells. *Euro J Exp Biol* 3(2): 503-514.

Mohanta A, Mohanty PK and Parida G (2014) Pattern of keratinization in oral squamous cells during carcinogenesis. *IOSR J Dent and Med Sci* 13(7.IV):83-91.

Myers JN, Elkins T, Roberts D and Byers RM. (2000) Squamous cell carcinoma of the tongue in young adults: increasing incidence and factors that predict treatment outcomes. *Otolaryngol Head Neck Surg* 122(1): 44-51.

Nair J, Ohshima H, Nair UJ and Bartsch H (1996) Endogenous formation of nitrosamines and oxidative DNA-damaging agents in tobacco users. *Crit Rev Toxicol* 26 (2): 149–161.

Oji C and Chukwuneke F (2007) Oral cancer in Enugu, Nigeria, 1998-2003. *Br J Oral Maxillofac Surg* 45(4): 298-301.

Pankaj C (2010) Areca nut or betel nut control is mandatory if India wants to reduce the burden of Cancer especially cancer of the oral cavity. *.Int J Head Neck Surg* 1(1):17-20.

Parida G (2001) Diagnostic cells in scrape cytology of squamous cell carcinoma. *Acta Cytol* 45(6): 1085-1086.

Parkin DM, Bray FI and Devesa SS (2001) Cancer burden in the year 2000. The global picture. *Eur J Cancer* 37(Suppl 8): S4-S66.

Perea-Milla López E, Miñarro-Del Moral RM, Martínez-García C, Zanetti R, Rosso S, Serrano S, Aneiros JF, Jimenez-Puente A andRedondo M(2003) Lifestyles, environmental and phenotypic factors associated with lip cancer: a case–control study in southern Spain. *Br J Cancer* 88(11):1702–1707.

Petti S (2003) Pooled estimate of world leukoplakia prevalence: A systematic review. *Oral Oncol* 39(8):770-780.

Pindborg JJ, Jolst O, Renstrup G and Roed-Petersen B (1968) Studies in oral leukoplakia: a preliminary report on the period prevalence of malignant transformation in leukoplakia based on a follow-up study of 248 patients. *J Am Dent Assoc* 76: 767-71

Pindborg JJ and Sirsat SM (1966) Oral submucous fibrosis. *Oral Surg Oral Med Oral Pathol* 22(6):764-779.

Poul EP (2008) Oral cancer prevention and control- The approach of the World Health Organization. *Oral Oncol* 45(4-5):454-460.

Ries LAG, Eisner MP, Kosary CL, Hankey BF, Miller BA, Clegg L, Mariotto A, Fay MP, Feuer EJ and Edwards BK (2003) *SEER Cancer Statistics Review, 1975-2000*, National Cancer Institute. Bethesda, MD, http://seer.cancer.gov/csr/1975_2000/, 2003

Rudolph P and Atvis K (2003) Ulcerative conditions. In: Regezi J, Sciubba J, Jordan R (Eds.) Oral Pathology Clinical Pathologic Correlations. Saunders, Missouri, pp 52-55.

Scully C (2011) Oral cancer aetiopathogenesis; past, present and future aspects. *Med Oral Patol Oral Cir Bucal*, 16 (3): 306-311.

Scully C and Bedi R (2000) Ethnicity and oral cancer. *Lancet Oncol* 1(1): 37-42.

Seitz HK and Stickel F (2007) Molecular mechanisms of alcohol-mediated carcinogenesis. *Nat Rev Cancer* 7(8): 599-612.

Sharan R, Mehrotra R, Choudhury Y and Asotra K (2012) Association of betel nut with carcinogenesis: Revisit with a clinical perspective. *PLoS One* 7:e42759.

Taghavi N and Yazdi I (2007) Type of food and risk of oral cancer. *Archives of Iranian Medicine* 10 (2): 227 – 232.

Trivedy C, Warnakulasuriya S and Peters TJ (1999) Areca nuts can have deleterious effects. *Br Med J* 318:1287.

Turner DO, Williams-Cocks SJ, Bullen R, Catmull J, Falk J, Martin D, Mauer J, Barber AE, Wang RC, Gerstenberger SL and Kingsley K (2011) High-risk human

papillomavirus (HPV) screening and detection in healthy patient saliva samples: a pilot study. *BMC Oral Health* 11:28.

Verma S (2011) Areca nut (betel nut) chewing: A popular Indian cultural practice and its mucosal implications. *Int J Dermatol* 50(2): 229‑232.

Walker DM, Boey G and McDonald LA (2003) The pathology of oral cancer. *Pathology* 35(5): 376-383.

Warnakulasuriya S (2009) Global epidemiology of oral and oropharyngeal cancer. *Oral Oncol* 45(4-5):309-316.

Warnakulasuriya S, Johnson NW and van der Waal I (2007) Nomenclature and classification of potentially malignant disorders of the oral mucosa. *J Oral Pathol Med* 36(10): 575-580.

Warnakulasuriya S, Mak V and Möller H (2007) Oral cancer survival in young people in South East England. *Oral Oncol* 43(10): 982-986.

HAEMATOLOGY OF THE SNAKEHEAD FISH *CHANNA STRIATUS* (BLOCH, 1793)

Madhusmita Das[1]*, Bana Bihari Mahapatra[2] and Pravati Kumari Mahapatra[2]

[1]Department of Zoology, Government (Auto) College, Angul-759 106

[2] PG Department of Zoology, Utkal University, Vani Vihar, Bhubaneswar

Odisha- 751 004

*Corresponding Author: rinkoodas@gmail.com

ABSTRACT

In the present study the haematological parameter of male and female snakehead fish *Channa striatus* were evaluated. Sixty fishes were collected from the local fish market to evaluate the heath status of the fishes. The different parameters studied included Haemoglobin (Hb) content, packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), red blood cell (RBC) or erythrocytes count, White blood cell (WBC) count, Differential leukocyte count i.e. percentage of lymphocytes, monocytes, neutrophils, basophils and eosinophils, and morphometry of different blood cells. The Hb concentration, RBC, WBC and MCHC of male *Channa striatus* was found to be higher than the female *Channa striatus*. PCV and MCV value of the male *Channa striatus* were comparable to the female. MCV value of the male *Channa striatus* was found to be lower than the female.

Key words: *Channa striatus,* Haematology,

INTRODUCTION

Murrels, commonly called snakeheads belonging to the family Channidae (Ophiocephalidae), constitute the most common and dominant group of air breathing freshwater fishes and are highly regarded as food fish in the South and Southeast Asian countries (Wee and Tacon, 1982). Besides the high quality of their flesh in terms of taste and texture, they also have good market value due to the low fat, fewer intramuscular spines, and medicinal qualities (Haniffa et al*.,* 2011).

The blood composition of a fish reflects to some extent to metabolic and other physiological process (Clauss et al., 2008). Accordingly, haematology can be used as clinical tool for the investigations of physiological and metabolic alteration in fish (Obiniezue et al., 2014). So, regular monitoring of the fish blood is a diagnostic tool in establishing the health status of the fish in farm (Ebanasar and Jayaprakash, 1994). It helps in evaluating the response of difference of blood cell and its components in the conditions of physiological stress, as it quickly reflect the poor conditions of fish than other commonly measured parameters. The blood parameters like Red Blood Corpuscle (RBC), White Blood Corpuscle (WBC), Haemoglobin (Hb), Packed Cell Volume (PCV), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC) are commonly studied in fishes to assess the impact of pesticides in aquatic biota (Mullah et al., 2009). The importance of Packed Cell Volume (PCV) or haematocrit as an index of anaemia is well known in clinical medicine (Joshi, 1980). White Blood Corpuscles (WBC) plays an important role in defence mechanism and mainly comprises granulocytes, monocytes and lymphocytes (Joshi, 1980). Thrombocytes are involved in coagulation of blood (Tilak et al., 2007). Haematology has been widely used as potent bio indicator in aquatic toxicology (Samuel and George, 2000).

The aim of the present study was to find the physiological status of the fish *Channa striatus* which are commercially available in the local market of Bhubaneswar, Odisha for the public consumption. This paper also describes the hematology of both males and females of various size and weight groups.

MATERIALS AND METHODS

Collection of fish

Adult *Channa striatus* were collected from the local fish market, Bhubaneswar (20.2700° N, 85.8400° E), Odisha, India. They were transported to the laboratory and kept in the aquarium for acclimatization. Thirty males and thirty females of *Channa striatus* were used for the study. The males ranged from 15 cm to 18 cm with an average of 16.2 cm and the females ranged from 15 cm to 18 cm with on average of 16 cm. The male fish weighted 27 g to 30 g with an average of 28.8 g and the female fish weighted 22.7 g to 30.0 g with an average of 27.4 g.

Collection of Blood

Fishes were brought to the laboratory and anesthetized with MS222 (0.03%). Prior to the blood collection, specimens were weighted and total length was measured. The collected blood was kept in ethylene diamine tetra acetic acid (EDTA) vial. The blood was collected during morning hour to avoid the diurnal variation. This sample was used for haematological investigation. Fresh blood samples were used to prepare blood smear.

Methods used

The RBC and WBC count was done by haemocytometer method. Haemoglobin (Hb) concentration was determined by Sahli's haemometer. The packed cell volume (PCV) or haematocrit of blood was determined using heparinised capillary and microhaematocrit centrifuge. Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated from RBC, PCV and Hemoglobin (Hb) values using standard formulae (Thompson, 1969). Sizes of RBC, WBC and their nuclei were measured by an ocular micrometer using a Hund (H500) microscope. Photos of the blood cells were taken with the help of a Catcam 130 1.3 Megapixel Microscope Eyepiece Digital Camera connected to Hund H500 WETZLAR microscope.

Statistical analysis

Parametric descriptive statistics included measures of central tendency (arithmetic mean X), standard deviation (SD) and ranges.

RESULTS AND DISCUSSION

Hematological parameters investigated in the present study are represented in Table 1.

Morphology of blood cells

Erythrocytes or Red blood cells (RBCs)

In *channa striatus* erythrocytes were observed to occur in various forms. They were either oval, elliptical (Fig. 1A) or rounded (Fig. 1B) in shape with centrally placed nucleus. Some irregular forms such as tear drop were also observed (Figs. 1C and D).

Leucocytes or White blood cells (WBCs)

The leucocytes observed in the present study were of five types, i.e., lymphocytes, monocytes, eosinophils, basophils and neutrophils. The lymphocytes were rounded in shape.

The nuclei were rounded in shape in lymphocytes (Fig. 2A). Monocytes were rounded in shape and had eccentrically placed rounded nuclei (Fig. 2B). Eosinophils were also rounded in shape with segmented nuclei. Eosinophils had bilobed nuclei (Fig. 2C). Neutrophils were large and rounded in shape. They were easily distinguished from other cells due to their lobular and segmented nuclei. Bilobed nuclei (Fig. 2D) or U shaped or band neutrophils (Fig. 2E) were common. Basophils investigated were round cells having large dark violate stained granules over the irregular nuclei as well as entire cell (Fig.2F).

Platelets

Platelets were smaller in size and rounded in shape. The nuclei were large and rounded. The platelets were distributed widely. They were either found as single cell (Fig. 3A) or formed clusters on the blood smear (Fig. 3B).

Morphometry of blood cells

RBC and WBC

The length and breadth of erythrocytes remain 8.84±1.22 µm and 7.75± 0.76 µm in male and 8.43± 0.49 µm and 6.99 ± 0.47 µm in female. The surface areas occupied by the erythrocytes were more in male than female. The mean value of long axis and short axis of nuclei remained 4.35± 0.64 µm and 2.40 ± 0.51 µm in male and 4.15 ± 0.53 µm and 2.44 ± 0.39 µm in female. The surface areas of nuclei were more in male than female. The diameter of lymphocytes and basophils remains more in male than female. The diameter of the neutrophils, eosinophils and monocytes of female were higher than the male. The area occupied by lymphocytes and bosophils was mare in male than female. The area occupied by neutrophils, eosinophils and monocytes was more in female than male.

Absolute corpuscular values

The mean corpuscular volume (MVC) of *Channa striatus* male ranged from 26.29 to 26.83 μ^3 with a mean of 26.83 ± 0.296 μ^3. The mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) of *Channa striatus* male showed a mean value of 101.22 pg and 26.05 % respectively. The MCV, MCH and MCHC value of *Channa striatus* female showed a mean value of 26.924 μ^3, 101.172 pg and 25.88 % respectively. The value of MCV, MCH and MCHC in males was more than females. However, in the present study the values observed remained higher than previous reports for this species (Sasikala, 2011; Haniffa et al., 2011).

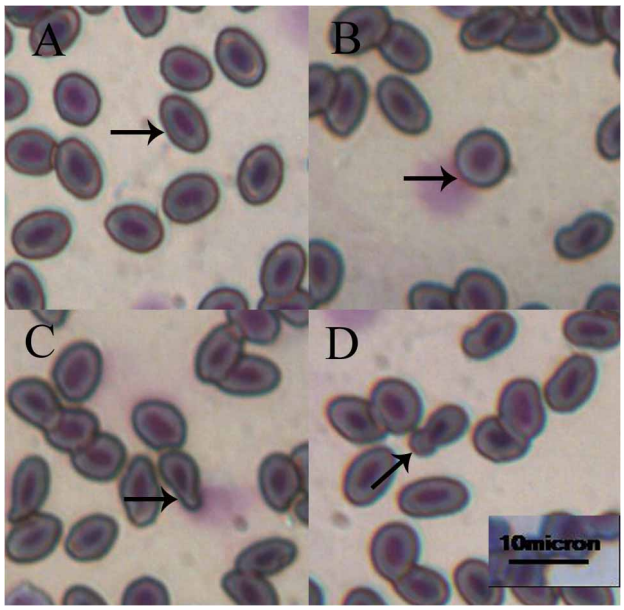

Fig. 1 Different shapes of erythrocytes found in *Channa striatus* (Arrow indicates the cells) (scale= 10µm) A) Elliptical, B) Round, C) Tear drop and D) Irregular shape.

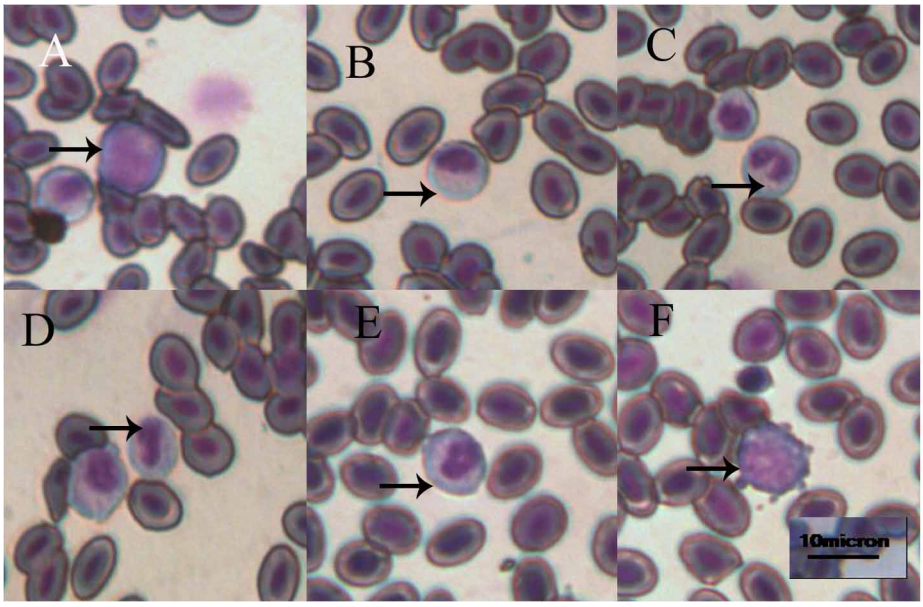

Fig. 2 Different type leucocytes found in *Channa striatus* (Arrow indicates the cell) (Scale=10µm) A) Lymphocytes, B) Monocytes, C) Eosinophil, D) Neutrophil, E) Band neutrophil and F) Basophil.

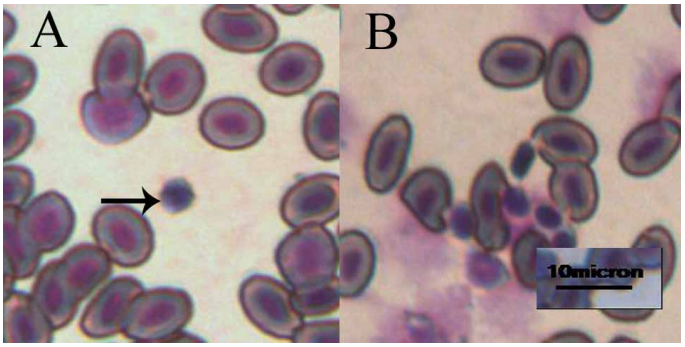

Fig. 3 Platelets found in *Channa striatus* A) Single platelet, B) Cluster platelets.

RBC count, WBC Count, Haemoglobin concentration and Packed cell volume

The mean number of RBC in males was 3.635 ±0.313/ mm³ of blood and in females 3.592 ± 0.300/ mm³ of blood. The number of RBC remained comparatively less in females than the males. The males showed higher number of WBC than females. The WBC count ranged from 3.87 to 4.6/ mm³ of blood in males and 3.89 to 4.66/ mm³ of blood in females. The mean WBC count was 4.27 × 10^3 ± 0.28/ mm³ in males and 4.16 × 10^3 ± 0.32/ mm³ in females. The gram percentage of haemoglobin per 100 ml of blood varied between 9.85 to 10.82/ 100 ml with mean of 10.518 ± 0.390/ 100 ml among males. In females it ranged from 9.95 to 10.88/ 100 ml with mean of 10.6 ± 0.391 ml. The mean value of PCV was more in females in comparison to males. RBC count in both male and female fishes was found to be higher in comparison to earlier findings for the same species (Sasikala, 2011) as well as *Channa punctatus* (Dube and Magar, 2013). But, WBC count of the present study was lower than the earlier reports for the same species (Sasikala, 2011). Hemoglobin percentage was also lower (Table1) than the normal healthy fishes of the same species studied by Haniffa et al. (2011).

Table 1 Hematological value of male and female *Channa striatus.*

Hematological Parameters	**Sex**	
	Male (X ± SD)	**Female (X ± SD)**
RBC count (millions /mm3)	3.635 ± 0.313	3.592 ± 0.300
Haemoglobin conc. (%)	10.518 ± 0.390	10.6 ± 0.391
Packed cell volume (%)	33.062± 0.394	33.092 ± 0.243
Mean corpuscular volume (μ^3)	26.86 ± 0.296	26.924 ± 0.406
Mean corpuscular haemoglobin (Pg)	101.22± 0.490	101.172± 0.750
Mean corpuscular haemoglobin conc. (%)	26.05 ± 0.45	25.888 ± 0.306
RBC, Length (µm)	8.834± 1.220	8.436 ± 0.499
RBC, Breadth (µm)	7.75 ± 1.220	6.99 ± 0.471
Surface area of erythrocyte (μm^2)	54.174 ± 12.475	45.604 ± 5.113
Length of RBC's nucleus (µm)	4.356 ± 0.694	4.158 ± 0.534
Breadth of RBC's nucleus (µm)	2.408 ± 0.514	2.444 ± 0.395
Surface area of RBC's nucleus (μm^2)	8.336 ± 3.021	7.66 ± 1.780
WBC count (No. of WBC/ mm^3)	$4.274 \times 10^3 \pm 0.285$	$4.168 \times 10^3 \pm 0.324$
Neutrophil (%)	18.8 ± 0.836	18.4 ± 1.140
Diameter of Neutrophil(µm)	9.12± 0.32	9.26 ± 0.45
Area occupied by neutrophil (μm^2)	65.35 ± 9.98	73.52 ±9.98
Lymphocyte (%)	58.4 ± 0.547	58.4 ± 0.547
Diameter of large lymphocyte (µm)	7.90 ± 0.21	7.87 ± 0.54
Area occupied by large lymphocyte (μm^2)	49.03 ±5.36	36.11 ± 5.68
Basophil (%)	3.00 ± 0.707	2.8 ± 0.836
Diameter of Basophil(µm)	11.90 ± 0.07	11.87 ± 0.55
Area occupied by Basophil (μm^2)	111.27 ± 11.20	110.52 ± 9.97
Eosinophil (%)	3.80 ± 0.836	4.2 ± 0.836
Diameter of eosinophil(µm)	10.77 ± 0.39	10.79 ± 0.65
Area occupied by eosinophil (μm^2)	90.97 ±20.13	91.31 ±10.25
Monocyte (%)	10.4 ± 1.949	9.8 ± 1.303
Diameter of monocyte(µm)	10.28 ± 0.50	10.38 ± 0.51
Area occupied by monocyte (μm^2)	83.03 ± 12.35	84.66 ± 12.34

X = Mean, SD = Standard deviation, Pg = picogram,

Differential leucocyte count

In males the percentage of lymphocytes ranged from 58 to 59 with a mean of 58.4 ± 0.547. Neutrophils ranged from 18 to 20, eosinophil from 3 to 5, basophils from 2 to 4 and monocytes from 8 to 13. The mean neutrophils, eosinophils, basophils and monocytes percentage were respectively, 18.8 ± 0.836, 3.8 ± 0.836, 3 ± 0.707 and 10.4 ± 1.949. In females percentage of lymphocytes range from 58 to 59 , neutrophils 17 to 20, eosinophils 3 to 5, basophil 2 to 4 and monocytes 9 to 11. The mean lymphocytes, neutrophils, eosinophils, basophils and monocytes percentage were found to be 58.4 ± 0.547, 18.4 ± 1.140, 4.2 ± 0.836, 2.8 ± 0.836 and 9.8 ±1.303, respectively. The percentage of lymphocytes remained same for both male and female fishes. The eosinophil percentage was higher in females than males. This may be due to the presence of the parasites. But, percentage of neutrophils, basophils and monocytes were higher in males than females.

Neutrophil to lymphocyte ratio

The mean value of neutrophil to lymphocyte (N/L) ratio of *Channa striatus* male (0.321 ±0.011) was more than the female (0.314 ± 0.020).

It is important to know the health status of fishes that are consumed by the human population. As hematological parameters indicate the health condition of the fishes, the present study provides information about the hematological parameters of the locally available *Channa striatus* for consumptions.

REFERENCES

Clauss TM, Dove ADM and Arnold JE (2008) Haematologic disorder of fish. *Veterinary Clinics Exotic Animal practice*. 11: 445-462.

Dube KV and Magar RS (2013) Effect of Lead Nitrate on the Haematological Parameters of *Channa punctatus. International Multidisciplinary Research Journal* 3(1):01-03

Ebanasar J and Jayaprakash V (1994) Evaluation of different diet for the cage culture of *Channa striatus. Journal of Inland fish, Sec, India.* 26(1): 59-66.

Haniffa MA, Abdul Kader and Mydeen KP (2011) Hematological Changes in *Channa striatus* experimentally Infected by *Aeromonas hydrophila. Bioresearch Bulletin* 4: 250-257.

Joshi BD (1980) Sex related variation in some haematologic value of certain fresh water Teleost. *Japanese Journal of Ichthyology.* 72(2): 235- 239.

Mullah MFA, Mamun MSA, Sarowar MN and Roy A (2009) Effect of stocking density on the growth and breeding performance of brood fish and larval growth and survival of school, *Channa striatus*. *J Bangladesh Agril Univ.* 7(4): 427-432.

Obiniezue, Nduka R, Bright C and Chirs I (2014) Histology and Activities of proteases, aminases and glutamate dehydrogenase in *clarias gariepinus* finger lings exposed to linear alkyl benzene sulphonat. *Advances in Life science and Technology*. 16: 147-156.

Samuel D and George DV (2000) Mineral counstituents of fresh water fish *Channa striatus* infected with acanthocephalan parasite. *Indian J. Fish*. 47(3):215-224.

Sasikala G (2011) Metasystox induced haematological modulation in the south Indian snakehead *Channa striatus. International Journal of Pharmaceutical and Biological Archives.*2(2): 775-777.

Thompson RB (1969) A short text book of Haematology. London Pitman Medical and Scientific Publ. Co. pp 153-158.

Tilak KS, Veeraich K and Batchirom MS (2007) Effect of phenol on haematological component of Indian Major Carp *Catla catla, Labeo rohita and Cirrhinus mrigala. Journal of Environmental Biology.* 28(2): 177-179.

Wee KL and Tacon AGJ (1982) A preliminary study on the dietary protein requirement of juvenile snakehead. *Bulletin of the Japanese Society of Scientific Fisheries,* 48: 1463-1468.

Zoological Society of Orissa

Brief History

Pranikee, the annual journal of Zoological Society of Orissa, publishes original research articles on Zoology.

The Society was founded in 1958 in order to promote effective communication between Zoologists through its publications, seminars and annual meetings.