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CARDIOPHORINE CLICK BEETLES (ELATERIDAE) OF BUXA TIGER RESERVE, WEST BENGAL, INDIA

SUTIRTHA SARKAR*, SUMANA SAHA**, DINENDRA RAYCHAUDHURI*

Cardiophorinae of Buxa Tiger Reserve, West Bengal, India is monotypic, being represented by the only genus *Cardiophorus* Eschscholtz that includes a new species *C. ferruginosus* and an endemic species *C. bucculatus* Candeze. Both the species are described and illustrated. Beside key to species, the relationship of the proposed new species is discussed.

Key words: click beetles, Cardiophorinae, *Cardiophorus ferruginosus* sp. nov., *Cardiophorus bucculatus*, Buxa Tiger Reserve, West Bengal, India.

INTRODUCTION

Cardiophorinae, a cosmopolitan subfamily (Chakraborty & Chakrabarti, 2006; Johnson & Cate, 2010) of elaterid beetles is known by 1.100 species belonging to 38 genera (Akhter *et al.*, 2011).

In contrast, even though a megadiverse country, Indian members of the subfamily in question are poorly explored. Only two genera, namely *Dicronychus* Burlle and *Cardiophorus* Eschscholtz, are known. The former is known by 2 species while the latter by 27 species (Vats, 1984; Vats & Chauhan, 1991; Punam *et al.*, 1997; Chakraborty & Chakrabarti, 2006).

From our faunistic survey (1994-2011) for the click beetles of Buxa Tiger Reserve, West Bengal, we conclude that cardiophorine elaterids is monotypic, being represented by *Cardiophorus* Eschscholtz. The genus is comprised of 2 species, a new species *C. ferruginosus* and an endemic species *C. bucculatus* Candeze. Beside providing diagnosis of the genus and a key to species, both species are suitably described and illustrated. The relationship of the proposed new species is also discussed.

The biotope. Buxa Tiger Reserve (26º30’ to 26º55' N and 89º20' to 89º55' E; MSL: 100 m to 1750 m; total area: 760.94 sq. km, core area: 385.02 sq. km; temperature: 6ºC to 30ºC; annual rainfall: 5000 mm) lies in the Alipurduar sub division of Jalpaiguri district of West Bengal. The reserve is the easternmost extension of the extremely bio diverse north Bengal. It is situated within 2 forest divisions, entire Buxa forest division of Alipurduar (Jalpaiguri) and part of Coochbehar forest division. The reserve includes 14 forest ranges. Many rivers and streams viz. Rydak, Sankos, Jainti, Bala and Dima traverse through the reserve.

The fragile "Terai Ecosystem" constitutes a part of this reserve. It is mainly moist tropical forest and subdivided into 8 subtypes: Sal Forest, Moist Mixed/Dry Mixed Forest, Wet Mixed Forest, Semi-evergreen Forest, Evergreen Forest, Hill Forest, Savannah Forest and Riverine Forest. Major plants include Sal, Simul, Siris, Khair, Champ, Sidha, Lali, Toon, Lampate, Mandane, Katus, Teak and Orchids. The fauna is represented by Elephant, Leopard, Gaur, Spotted Deer, Jungle Cat, Fishing Cat, varieties of birds, reptiles, amphibians and fishes. Recently, Raychaudhuri & Saha (2014) published an account on the insects and spiders of the reserve.

MATERIAL AND METHODS

The elaterids were collected and preserved following Chakraborty & Chakrabarti (2006). The collected samples were studied under Stereo Zoom Binocular Microscopes Zeiss SV11. All measurements are in millimeters, made with an eye piece graticule.

Abbreviations: LT = Light Trap; BTR = Buxa Tiger Reserve.

TAXONOMY

Family Elateridae Leach

Subfamily Cardiophorinae Candèze

Genus *Cardiophorus* Eschscholtz

Diagnosis. Head convex or flat, always much wider than long, frons usually arcuate, raised, with anterior margin projected, clypeus prominent. Mandibles short, bicuspid. Last joint of maxillary palp triangular. Antennae variable, narrow, usually extending beyond the posterior angle of prothorax, 1st segment large and ovoid, 2nd smallest, 3rd shorter to as long as 4th, last elongate and round. Prothorax often bulging, with sides round, posterior angles short, usually with a fine longitudinal carina, posterior margin with lateral short furrows, submarginal line present. Scutellum cordiform or heart shaped. Elytra round at apex. Prosternal spine truncate. Metasternum truncate or emarginate between mesocoxae. Metacoxal plates dilated, outwardly narrowed. Claw simple, dentate or bifid.

Distribution. Africa, Australia, China, India, Japan, Malayan Archipelago, Mexico, New Guinea, North America and Siberia (Chakraborty & Chakrabarti, 2006; Johnson & Cate, 2010).

Key to species

Body red brown; pronotum broader than long, with punctures simple, hind angles carinate; elytral interstriae not costiform posteriorly, with apex pointed; claws bifid

--- *ferruginosus* sp. nov.

Body yellow brown; pronotum longer than broad, with punctures double, hind angles acarinate; 7th and 8th elytral interstriae costiform posteriorly, apex truncate; claws dentate

---*bucculatus* Candeze

Cardiophorus ferruginosus sp. nov.

(Figs. 1-6; 13-14)

Type Material

Holotype. ♂, Newland, BTR, Jalpaiguri, West Bengal, India, 19.V.1997, Coll. D. Raychaudhuri.

Allotype. 1♀, Rajabhatkhawa/LT, BTR, Jalpaiguri, West Bengal, India, 25.V.2009, Coll. S. Sarkar.

Paratypes. 2♀♀, Newland, 19.V.1997, Coll. S. Saha/D. Raychaudhuri; 1♂, Panbari, 20.V.1997, Coll. S. Saha; 15♀♀, 3♂♂, Nimati/LT, Coll. S. Saha/ S. Sarkar/ D. Raychaudhuri, 28.V.2009; 1♀,1♂, Poro, 29.V.2009, Coll. S. Sarkar/ D. Raychaudhuri; 1♀, Rajabhatkhawa/LT, 03.IV.2010, Coll. S. Sarkar.

Description

Male (Holotype): Body length: 5.63, width: 1.69.

Body red brown. Pronotum slightly darker. Pubescence pale yellow, evenly distributed.

Head brown, pubescence pale yellow, moderately dense. Punctation simple. Frons broader than long, depressed, anterior margin strongly arcuate. Frontal carina complete across frons. Vertex flat to convex. Eyes brown, rounded, moderately large with distinct facets. Labrum brown, convex, broader than long, anterior margin convex. Mandibles moderate, brown with a tooth. Antennae brown, long, extended beyond the hind angle of pronotum. 1st segment robust, 2nd segment shorter than the 3rd. Vestiture starts on the 3rd antennomere.

Pronotum brown, broader than long, convex, punctation simple, dense. Sub marginal line absent. Anterior margin weakly convex, with anterior angle toothed. Carina present. Lateral margins round. Hind angle of pronotum subpointed. Posterior margin with lateral furrows.

Scutellum cordate, marginally black, medially red, anterior margin emarginate, pubescence dense.

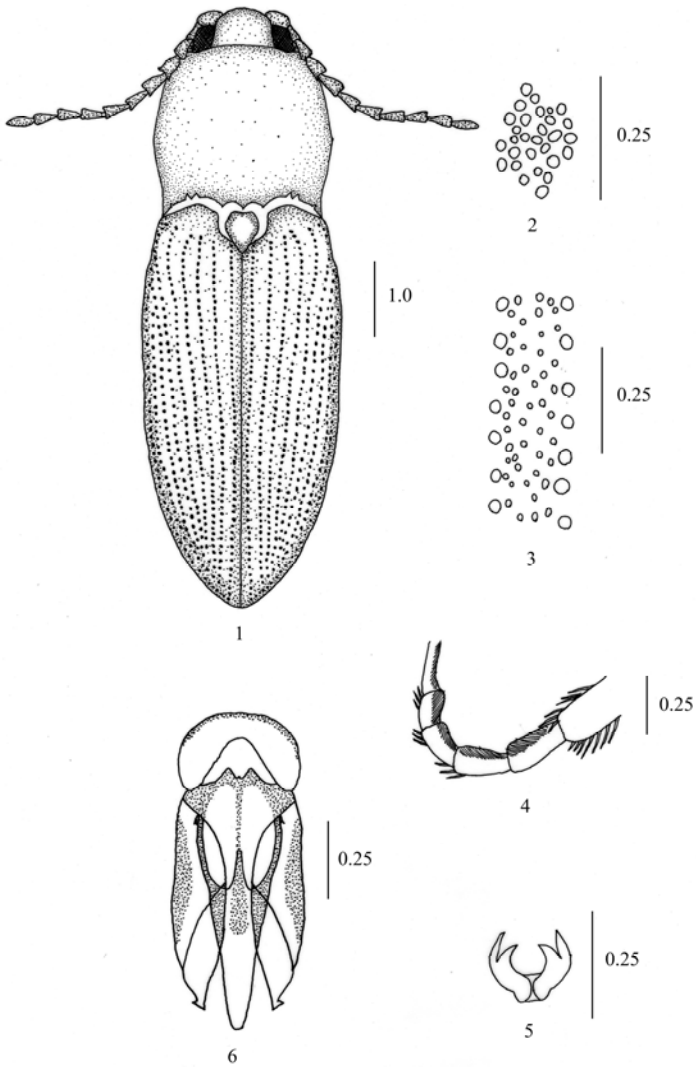
Elytra about 2.16 times longer than pronotum, reddish brown, striae punctuate. Interstriae slightly convex, moderately punctate with pale yellow pubescence, anteriorly marginate, apex pointed.

Prosternum convex, anteriorly with distinct chin piece, puncture simple, prosternopleural suture convex. Prosternal spine marginate, apically rounded, medially depressed.

Mesepimeron and mesepisternum not forming part of margin of mesocoxal cavity. Metacoxal plate round at middle.

Abdomen dark brown, punctation simple and moderately dense, pubescence dense.

Legs brown, moderately long. Tibia with 2 brown tibial spurs, claws bifid.



Figs. 1-6. *Cardiophorus ferruginosus* sp. nov. – Male (Holotype). 1. Dorsal habitus; 2. Pronotal puncture; 3. Elytral puncture; 4. Leg; 5. Claw; 6. Genitalia.

Figs. 13-14. *Cardiophorus ferruginosus* sp. nov. – Male.
13. Dorsal habitus; 14. Genitalia.

Genitalia. Basal piece wide, arms round at apex, anterior margin "V" shape, posterior margin weakly concave, laterial margin arcuate, posterior margin strongly sclerotized, thin, line like, rest feebly sclerotized; median lobe longer than parameres, arms strongly sclerotized compared to rest, long, nearly straight, pointed at base, not exceeding ventral posterior margin of parameres, median lobe little-finger like, furcae not reaching the anterior margin of parameres; parameres jointed, outer lateral margin of parameres basal to distal (upto joint) strongly sclerotized, medially weakly concave, distally notched, gradually narrowing from distal to apical, distal teeth small, pointed, apex acute.

Type deposition. Entomology Laboratory, Department of Zoology, University of Calcutta, registration no. EZC/elat./01/2014

Distribution. India: West Bengal.

Etymology. The species name is derived from the red brown nature of the body.

Remarks. The present species shows a close affinity to *Cardiophorus flexus* Vats and Chauhan, but can be separated by:

1. Red brown nature of the body (body black in *C. flexus*).
2. Pronotum subquadrate (pronotum distinctly broader than long in *C. flexus*).
3. Hind angle of pronotum pointed, acute (hind angle of pronotum truncate in *C. flexus*).
4. Prosternal spine marginate (prosternal spine without any margin in *C. flexus*).
5. Elytra pointed apically (elytra rounded apically in *C. flexus*).
6. Basal piece round without any conical anterolateral projection (basal piece straight with conical anterolateral projection in *C. flexus*).

The species is therefore recognized as new to science.

Cardiophorus bucculatus Candeze

Cardiophorus bucculatus Candeze, 1860. Mem. Soc. R. Sci. Liege, 15: 211.

(Figs. 7-12; 15-16)

Description

Male: Body length: 5.56, width: 1.27.

Body yellow brown. Head darker. Pubescence yellow brown, evenly distributed.

Head black, pubescence yellow brown, moderately dense. Punctation simple. Frons broader than long, concave, anterior margin strongly arcuate. Frontal carina complete across frons. Vertex more or less flat with median depression. Eyes brown, rounded, moderately large with distinct facets. Labrum brown, convex, broader than long, anterior margin convex. Mandibles moderate, brown, with a tooth above the middle on the incisor margin. Antennae yellow brown, long, extended beyond the hind angle of pronotum. 1st segment robust, 2nd and 3rd segments shorter than their following segments, rest subequal. Vestiture starts on the 2nd antennomere, last segment constricted near apex.

Pronotum yellow brown, longer than broad, convex, punctation double, moderately dense, sub marginal line short, anterior margin weakly convex, with anterior angle toothed, lateral margins round, sinuate before hind angle. Hind angle long, divergent, nearly acute. Posterior margin with lateral furrows.

Scutellum cordate, black, yellow brown distally, medially depressed, anterior margin emarginate, pubescence moderately dense.

Elytra about 2.44 times longer than pronotum, yellow brown, striae punctate, rest smooth, interstriae slightly convex, 7th and 8th interstriae of elytra costiform posteriorly, anterior margin marginate, apex truncate.

Prosternum convex, anteriorly with a laterally marked median contiguous chin piece, puncture simple, prosternopleural suture straight. Prosternal spine marginate, anterior part rounded, medially depressed.

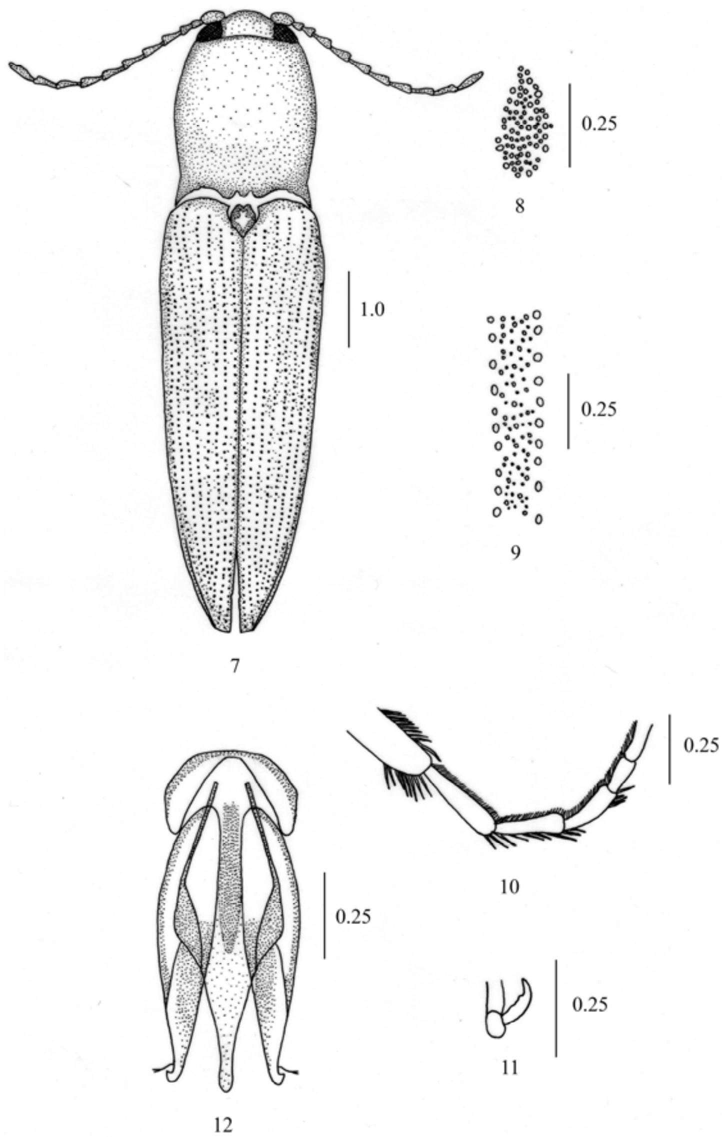
Mesepimeron and mesepisternum not forming part of margin of mesocoxal cavity.

Metacoxal plate rounded at middle.

Abdomen brown, punctation simple and moderately dense, pubescence moderately dense.

Legs brown, moderately long. Tibia with 2 brown tibial spurs, claws dentate.

Genitalia. Basal piece wide, arms round at apex, anterior margin broadly "V" shape, posterior margin medially convex, lateral margin arcuate, outer margin strongly sclerotized, thin and line like, rest feebly sclerotized; median lobe little longer than parameres, arms strongly sclerotized compared to rest, long, straight, clearly exceeding ventral posterior margin of parameres, median lobe gradually narrowing from proximal to medial, medial to apical parallel sided, apex rounded, furcae not reaching the anterior margin of parameres; parameres jointed, outer lateral margin of parameres basal to distal (up to joint) strongly sclerotized, distally deeply concave and forming posteriorly directed distal teeth, distal teeth small, pointed, apex rounded.



Figs. 7-12. *Cardiophorus bucculatus* Candeze – Male. 7. Dorsal habitus; 8. Pronotal puncture; 9. Elytral puncture; 10. Leg; 11. Claw; 12. Genitalia.

Figs. 15-16. *Cardiophorus bucculatus* Candeze – Male.
15. Dorsal habitus; 16. Genitalia.

Material examined. 2♀♀/1♀, Damanpur/LT, 27.III.2003/15.IV.2009, Coll. D. Raychaudhuri/ S. Sarkar; 1♂, Rajabhatkhawa/LT, 05.IV.2010, Coll. S. Sarkar.

Distribution. India: Haryana, Uttar Pradesh, West Bengal (Vats & Chauhan, 1991; Johnson & Cate, 2010).

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REFERENCES

AKHTER M.A., DRUMONT A., RIZVI S.A., AHMED Z., 2011, *Notes on Species of Cardiophorinae (Candeze, 1860) from Pakistan with Description of a New Species (Coleoptera: Elateridae) and New Records.* Pakistan Journal of Zoology, **43** (3): 477-481.

CHAKRABORTY P., CHAKRABARTI S., 2006, *A contribution to the fauna of click-beetles (Coleoptera: Elateridae) of West-Bengal.* Records of the Zoological Survey of India, Occ. Paper no. **254**: 1-220.

JOHNSON J., CATE C., 2010, http://insects.tamu.edu/ research/collection/ hallan/test/Arthropoda/ Insects/Coleoptera/Family/Elateridae.txt. (Accessed on 12.12.2014).

PUNAM G., SAINI M.S., VASU V., 1997, *New additions to genus Cardiophorus Eschscholtz (Coleoptera: Elateridae: Cardiophorinae) from India.* Journal of Entomological Research, **21** (1): 65-70.

RAYCHAUDHURI D., SAHA S., 2014, *Atlas of Insects & Spiders of Buxa Tiger Reserve*, Publ. West Bengal Biodiversity Board, Government of West Bengal & Nature Books India, 357 pp.

VATS L.K., 1984, *Two new species of Dicronychus Brullé (Cardiophorinae: Elateridae).* Annl. Ent., **2**: 7-9.

VATS L.K., CHAUHAN R.L., 1991, *The species of Cardiophorus from North India (Coleoptera: Elateridae)*. Research Bulletin (Science) of the Panjab University Science, **42** (1-4): 11-29.

VATS L.K., VASU V., 1993, *Internal organs of reproduction in Cardiophorus Esch. (Coleoptera: Elateridae: Cardiophorinae)*. Journal of Entomological Research, **17** (3): 159-162.

VATS L.K., VASU V., 1993, *A comparative account to evaluate the taxonomic importance of the male reproductive organs of click beetles (Coleoptera: Elateridae)*. Journal of Entomological Research, **17** (4): 233-238.

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SPIDER FAUNAL DIVERSITY OF TEA ECOSYSTEM OF ASSAM, INDIA

SUMANA SAHA*, TAPAN KUMAR ROY**, DHRUBA CHANDRA DHALI**, DINENDRA RAYCHAUDHURI**

Appreciating the necessity of spiders as bioresource against tea pests, the present work unfolds their spectrum in the tea ecosystem of Assam. The study area included 4 tea estates, Bukhial T. E., Jamguri T. E., Hunwal T. E. and Kotalgoorie T. E. Altogether 85 species under 53 genera distributed over 16 families could be recorded. These can broadly be categorized into 7 trophic groups. The decreasing order of the groups are Orb weavers (45.88%) > Stalkers (31.76%) > Ground dwellers (18.82%) > Foliage hunters (7.05%)> Space weavers (4.70%)>Ambushers (3.52%)≥ Sheet weavers (3.52%). Two species are considered new to science, 4 genera and 4 species from the country. Based on the species richness, the decreasing order of the tea estates are BTE (61.17%) ≥ JTE (61.17%) >KTE (38.82%) > HTE (35.29%). The present study leads to infer "tea system when closer to forests or experiences less pesticides, exhibits higher spider heterogeneity". Araneids and salticids are the dominant groups. Other than the Oriental representatives, Australian and Palaearctic elements are the next major groups. Nearly 38% of the species are found to be native.

Key words: araneofauna, diversity, tea ecosystem, Assam, India.

INTRODUCTION

Spiders are amongst the most omnipresent, numerous, generalist predators in both agricultural and natural ecosystems. They are nature's master spinners of silken webs. They exert considerable top down effect and have the potential to both lower and stabilize pest population making themselves excellent biological pest management candidates. In their absence, the world of insect pests would run amok creating havoc of our health and food resources. India is the world's 4th largest exporter of tea, a major, monoculture, permanently planted crop providing habitat continuity for many arthropod and nematode pests. Over the last few decades, India's share in world tea export declined consistently for several reasons. One of the most important reasons is residual effect in made tea. On the contrary, recent agricultural practices towards reduced pesticide use and ecological sustainability have lead to increased interests in spiders as potential tools (Hazarika *et al.*, 1994). As a consequence, the araneofauna of several cultivated crop systems, such as cotton, soybean, alfalfa, maize, rice, orchards like citrus, etc. are now well documented in some parts of the world (Barrion & Litsinger, 1995; Satpathi, 2004). But in India except the work of Roy (2014), documentation of the spider fauna of tea ecosystem is still wanting.

Above prompted to study the spider species assemblage in tea ecosystem of Assam, one of the seven northeastern sister states of India (longitude 89°42′ to 96° E, latitude 28°8′ to 28°2′ N) located south of the eastern Himalayas, a land of high rainfall, endowed with lush greenery and "unique tea". The tropical climate contributes to Assam's unique malty taste, a feature for which this tea is famous. Though "Assam" generally denotes the distinctive black teas, the region produces smaller quantities of green and white teas as well with their own distinctive characteristics. A large area (78.438 km^2) accordingly has been dedicated for tea cultivation.

The study area (Fig. 1) included 4 Tea Estates, namely Bukhial TE., Jamguri T.E., Hunwal T.E. and Kotalgoorie T.E. All the tea estates are of conventional type and within the jurisdiction of eastern Assam. Among them Kotalgoorie TE. lies close to Dessai Reserve Forest, more popularly known as Gibbon Wildlife Sanctuary. Again there is a judicious use of pesticides in both Bukhial and Jamguri Tea Estates as compared to the rest.

MATERIAL AND METHODS

Survey was conducted during the period of June, 2012 to May, 2014 in different sections of the referred tea estates in almost every month of any calendar year. Sampling was done by visual search, handpicking, inverted umbrella, bush beating, foliage, trunk and branch scanning, pitfall and leaf litter extraction. Collected samples were preserved following Tikader (1987) and Barrion & Litsinger (1995). The samples were studied using Stereo Zoom Binocular Microscopes (Olympus MSZ-BI, SZX-7 and Zeiss SV-11). Status of the taxa were determined with the help of Tikader (1970, 1980, 1982, 1987), Tikader & Malhotra (1980), Majumder & Tikader (1991), Barrion & Litsinger (1995), Sebastian & Peter (2009), Keswani *et al.* (2012), Metzner (2014) and Platnick (2014).

All materials are in the deposition of Entomology Laboratory, Dept. of Zoology, University of Calcutta, Kolkata.

RESULTS AND DISCUSSION

The survey yielded a total of 85 species and 53 genera, distributed over 16 families (Tables 1-2; Fig. 2). Araneids and salticids are the dominant groups (Fig. 3). The number of genera and species representing each family are given in Table 1. Of these 2 species are recognized as new to science, and 4 genera and 4 species are new to India. We further report 64 species as new from the state of Assam. *Oxyopes naliniae* Gajbe, 1999 is treated as the junior synonym of *O. matiensis* Barrion & Litsinger, 1995 (taxonomic detail will be dealt elsewhere) (Fig. 4). Of the 85 species, 32 are recognized as endemic to India (Fig. 4), thus exhibiting high endemicity (37.64%). The generated data represents 5.04%, 12.10% and 26.66% of the Indian species, genera and family respectively. Even though species richness is little higher during Premonsoon, always there remains a state of equilibrium throughout seasons. Analysis of zoogeographical distribution reveals that the fauna is largely Oriental (100%), followed by Australian (13.0%), Palaearctic (11.76%), Ethiopian (9.41%), Nearctic (2.35%) and Neotropical (2.35%). Species diversity is maximum in Bukhial and Jamguri T.E. (each with 52 species) and minimum in Hunwal T.E. (no. of species 30). Based on species diversity, the decreasing order of the tea estates are BTE (61.17%) ≥ JTE (61.17%) >KTE (38.82%)>HTE (35.29%). This leads to infer 'tea system when closer to forests or experiences less pesticides, exhibits higher spider heterogeneity'. Again on an average, any of the tea estates harbours at least 30 spider species at any point of time.

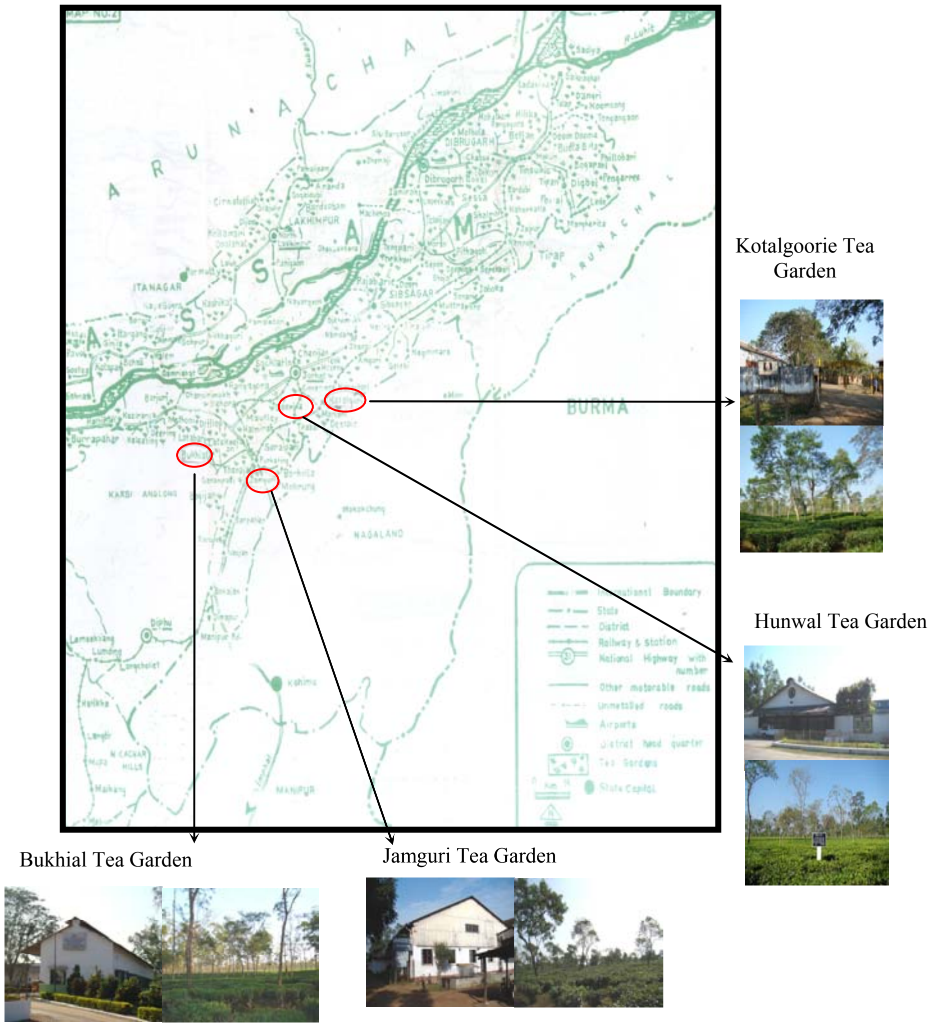

Fig. 1. Study Area.

Table 1
Species recorded from the tea estates of Assam

Taxa	Distribution		
	Tea Estates	**India**	**Elsewhere in the World**
I. Family Araneidae ▲1. *Araneus ellipticus* (Tikader & Bal)	BTE	Assam, West Bengal	Bangladesh, China, Laos
▲2. *Araneus mitificus* (Simon)	BTE, JTE, HTE, KTE	Assam, Andhra Pradesh, Chhattisgarh, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Manipur, West Bengal	Bangladesh, Hongkong, Japan, Malaysia, Myanmar, Pakistan, Philippines, New Guinea, Singapore, Thailand, Vietnam
▲3. *Argiope aemula* (Walckenaer)	BTE, JTE, HTE, KTE	Assam, Andaman & Nicobar Island, Andhra Pradesh, Chhattisgarh, Gujarat, Kerala, Madhya Pradesh, Maharashtra, Tamil Nadu, West Bengal	China, Indonesia, Malaysia, Myanmar, New Hebrides, Philippines, Sri Lanka, Taiwan, Thailand, Vanuatu
4. *Argiope pulchella* Thorell	BTE, JTE, HTE	Andaman Island, Arunachal Pradesh, Assam, Kerala, Madhya Pradesh, Maharashtra, Manipur, Orissa, Tamil Nadu, West Bengal	China, Indonesia; Malaysia, Myanmar
5. *Chorizopes* sp.	BTE, HTE	Assam, West Bengal.	-
▲6. *Cyclosa bifida* (Doleschall)	BTE, HTE, KTE	Assam, Arunachal Pradesh, Kerala, Meghalaya, Sikkim, West Bengal	Malaysia, New Guinea, Philippines, Sri Lanka
7. *Cyclosa confraga* (Thorell)	JTE	Assam, Gujarat, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Sikkim, West Bengal	Bangladesh, Malaysia
▲8. *Cyclosa hexatuberculata* Tikader	BTE, JTE, HTE, KTE	Assam, Kerala, West Bengal	Pakistan
▲9. *Cyclosa mulmeinensis* (Thorell)	BTE, JTE, HTE, KTE	Assam, Maharashtra, West Bengal	Africa, Japan, Malaysia, Myanmar, Philippines, Singapore, Taiwan
10. *Cyclosa quinqueguttata* (Thorell)	BTE, JTE, HTE	Assam, Sikkim, West Bengal	Bhutan, China, Myanmar, Taiwan
▲11. *Cyclosa simoni* Tikader	BTE, KTE	Assam, Sikkim, West Bengal	-
▲12. *Cyclosa spirifera* Simon	BTE, HTE, KTE	Assam, Arunachal Pradesh, Chhattisgarh, Madhya Pradesh, West Bengal	Pakistan
▲13. *Cyrtarachne avimerdaria* Tikader	JTE	Assam, Meghalaya, West Bengal	-
14. *Cyrtarachne inequalis* Thorell	BTE, JTE	Assam, Madhya Pradesh, West Bengal	Myanmar

▲15. *Cyrtophora cicatrosa* (Stoliczka)	JTE, HTE, KTE	Assam, Andaman & Nicobar Island, Gujarat, Kerala, Madhya Pradesh, Maharashtra, Punjab, Tamil Nadu, Uttar Pradesh, West Bengal	Pakistan, Bangladesh to Northern Territory, New Guinea
16. *Cyrtophora citricola* (Forskal)	KTE	Assam, Gujarat, Karnataka, Madhya Pradesh, Maharashtra, Punjab, Rajasthan, Tamil Nadu, West Bengal	Africa, Australia, Egypt, Europe, Madagascar, Malaysia, Myanmar, SriLanka
▲17. *Eriovixia excelsa* (Simon)	BTE, HTE	Assam, Bihar, Gujarat, Kerala, Maharashtra, West Bengal	Pakistan, Philippines, Indonesia, Taiwan
▲18. *Gasteracantha diadesmia* Thorell	JTE, HTE, KTE	Assam, Andaman & Nicobar Islands, Sikkim, West Bengal	Myanmar, Philippines, Thailand
19. *Gasteracantha kuhlii* C. L. Koch	BTE, JTE, HTE, KTE	Andaman & Nicobar Island, Assam, Bihar, Kerala, Sikkim, West Bengal	Bhutan, Hongkong, Indonesia, Japan, Malaysia, Myanmar, Philippines
▲20. *Gea subarmata* Thorell	BTE, JTE, HTE	Assam, Kerala, Uttar Pradesh, West Bengal	Bangladesh, Indonesia, Malaysia, Myanmar, New Guinea, Philippines, Singapore
▲21. *Neoscona bengalensis* Tikader & Bal	BTE, JTE, HTE, KTE	Assam, Andhra Pradesh, Kerala, Manipur, West Bengal	-
▲22. *Neoscona mukerjei* Tikader	HTE, KTE	Assam, Andhra Pradesh, Arunachal Pradesh, Kerala, Madhya Pradesh, Maharashtra, Manipur, West Bengal	-
23. *Neoscona nautica* (L. Koch)	HTE	Assam, Gujarat, Kerala, Madhya Pradesh, Maharashtra, Manipur, West Bengal	Cosmo tropical
▲24. *Neoscona punctigera* (Doleschall)	BTE	Assam, Maharashtra, West Bengal	Reunion to Japan
▲25.*Neoscona vigilans* (Blackwall)	JTE	Assam, West Bengal	Africa to Philippines, New Guinea
■26. *Neoscona yptinika* Barrion & Litsinger	JTE	Assam, West Bengal	Philippines
▲27. *Ordgarius sexspinosus* (Thorell)	BTE	Assam, Maharashtra, West Bengal	Japan, Malaysia, Myanmar, Singapore
▲28. *Parawixia dehaani* (Doleschall)	BTE	Assam, Karnataka, Kerala, Sikkim, West Bengal	Indonesia, Japan, Malaysia, Myanmar, New Guinea, Philippines, Polynesia
▲29. *Pasilobus kotigeharus* Tikader	JTE	Assam, Karnataka, Kerala, West Bengal	-

II. Family Corinnidae 30. *Castianeira* sp.	BTE	Assam	-
III. Family Gnaphosidae 31. *Scotophaeus* sp.	KTE	Assam	-
IV. Family Hersiliidae 32. *Hersilia savignyi* Lucas	BTE, JTE, HTE, KTE	Assam, Maharashtra, Tamil Nadu, Uttar Pradesh, West Bengal	Myanmar, Philippines, Sri Lanka
V. Family Linyphiidae ▲33. *Lephthyphantes bhudbari* Tikader	JTE	Assam, West Bengal	-
▲34. *Lephthyphantes lingsoka* Tikader	BTE, JTE	Assam, West Bengal	-
▲35. *Linyphia nicobarensis* Tikader	BTE, JTE, HTE, KTE	Assam, Nicobar Islands, West Bengal	-
VI. Family Lycosidae▲36. *Hippasa himalayensis* Gravely	BTE	Assam, Himachal Pradesh; Karnataka West Bengal	-
▲37. *Lycosa goliathus* Pocock	JTE, HTE	Assam, Maharashtra, West Bengal	-
▲38. *Lycosa phipsoni* Pocock	BTE	Assam, Maharashtra, West Bengal	China, Myanmar, Taiwan
▲40. *Pardosa birmanica* Simon	BTE, JTE, KTE	Assam, West Bengal; Andhra Pradesh, Bihar, Himachal Pradesh, Gujarat, Karnataka, Madhya Pradesh, Maharashtra, Meghalaya, Orissa, Punjab, Rajasthan, Tamil Nadu, Uttar Pradesh	Afghanistan, Bangladesh, China, Indonesia, Myanmar, Pakistan, Philippines
▲41. *Pardosa songosa* Tikader & Malhotra	HTE	Assam, Uttar Pradesh, West Bengal	Bangladesh, China
▲42. *Pardosa sumatrana* (Thorell)	HTE	Assam, Andhra Pradesh, Arunachal Pradesh, Bihar, Gujarat, Himachal Pradesh, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Manipur, Meghalaya, Rajasthan, Tamil Nadu, West Bengal	Bangladesh, China, Indonesia, Japan, Nepal, Philippines, Sri Lanka
VII. Family Miturgidae ▲43.*Cheiracanthium melanostomum* (Thorell)	JTE	Assam, Andaman & Nicobar Island, Bihar, Goa, Gujarat, Karnataka, Madhya Pradesh, Maharashtra, Mysore, Rajasthan, Tamil Nadu, West Bengal	Bangladesh, Bhutan, Myanmar, Sri Lanka
VIII. Family Nephilidae ▲44. *Herennia multipuncta* (Doleschall)	BTE	Assam, Arunachal Pradesh, Kerala, Maharashtra, Meghalaya, Tamil Nadu, West Bengal	China, Indonesia, Malaysia, Myanmar, Nepal, New Guinea; Philippines, Taiwan, Thailand

▲45. *Nephila kuhlii* Doleschall	BTE	Assam, West Bengal	Indonesia; Myanmar
IX. Family Oxyopidae ■46.*Oxyopes hotingchiehi* Sckenkel	BTE, JTE	Assam, West Bengal	China
▲47. *Oxyopes javanus* Thorell	BTE, JTE	Assam, Nicobar, West Bengal	Bangladesh, Cambodia, China, Indonesia, Malaysia, Myanmar, Philippines, Singapore, Thailand, Vietnam
▲48. *Oxyopes naliniae* Gajbe	BTE, JTE, KTE	Assam, Madhya Pradesh, West Bengal	-
▲49. *Oxyopes sakuntalae* Tikader	BTE, JTE	Assam, West Bengal	-
▲50. *Oxyopes shweta* Tikader	BTE, JTE, KTE	Assam, Arunachal Pradesh, Kerala, Manipur, Meghalaya, Sikkim, Tripura, West Bengal	China
▲51. *Oxyopes sunandae* Tikader	HTE	Assam, West Bengal	Meghalaya, Sikkim, Tripura
X. Family Pisauridae ▲52. *Pisaura gitae* Tikader	BTE, JTE, HTE	Assam, Andaman Islands, Kerala, Sikkim, West Bengal	-
XI. Family Salticidae ▲53. *Brettus albolimbatus* Simon	KTE	Assam, Kerala, West Bengal	Celebes, China, Indonesia, Sri Lanka
▲54. *Carrhotus viduus* (C. L. Koch)	JTE	Assam	Bintan Island, China, Indonesia, Malacca, Malaysia, Myanmar, Nepal, Penang Island, Singapore, Sri Lanka, Sumbawa
■55. *Chalcotropis* sp.	JTE, HTE	Assam	-
▲56. *Epeus indicus* Proszynski	JTE, HTE	Assam, West Bengal	Nepal
▲57. *Epocilla aurantiaca* (Simon)	BTE, HTE	Assam, Kerala, West Bengal	Malacca, Malaysia, Myanmar, Sri Lanka, Vietnam
▲58. *Hyllus semicupreus* (Simon)	BTE, JTE, KTE	Assam, West Bengal	Sri Lanka
▲59. *Marpissa decorata* Tikader	BTE, JTE, KTE	Assam, West Bengal	-
▲60. *Menemerus bivittatus* (Dufour)	KTE	Assam, West Bengal	Africa, South America
▲61. *Myrmarachne maratha* Tikader	KTE	Assam, Maharashtra, West Bengal	-
▲62. *Myrmarachne plataleoides*	JTE	Assam, Bihar, Maharashtra, Tamil Nadu, West Bengal	China, Malaysia, Singapore, Sri Lanka, Thailand
▲63. *Phidippus bengalensis* Tikader	BTE, JTE, HTE, KTE	Assam, West Bengal	-

▲64. *Phintella vittata* (C.L.Koch)	BTE, HTE	Assam, West Bengal	China, Indonesia, Malayasia, Myanmar, Philippines, Thailand, Vietnam
●65. *Plexippus levii* sp. nov.	BTE, HTE	Assam	-
▲66. *Plexippus paykulli* (Audouin)	BTE, JTE, HTE, KTE	Assam, Arunachal Pradesh, Kerala, Manipur, West Bengal	Africa, Europe, Myanmar, Philippines, Sri Lanka, and all warmer regions of the World
▲67. *Rhene decorata* Tikader	HTE	Assam, Maharashtra, West Bengal	-
●68. *Rhene wesolowskai* sp. nov.	HTE	Assam	-
▲69. *Telamonia dimidiata* (Simon)	BTE, JTE, HTE, KTE	Assam, Gujarat, Kerala, Maharashtra, West Bengal	Bhutan, Indonesia, Singapore
▲70. *Thiania bhamoensis* Thorell	BTE, HTE	Assam, Andaman Island, Kerala, West Bengal	China, Indonesia, Myanmar, Singapore
XII. Family Sparassidae 71. *Heteropoda kandiana* Pocock	JTE	Assam, Orissa, West Bengal	Sri Lanka
▲72. *Olios kiranae* Sethi & Tikader	JTE	Assam, Gujarat, West Bengal	-
XIII. Family Tetragnathidae 72. *Leucauge decorata* (Blackwall)	BTE, JTE, HTE, KTE	Assam, Bihar, Gujarat, Karnataka, Kerala, Maharashtra, Meghalaya, Orissa, Sikkim, Tamil Nadu, Uttar Pradesh, West Bengal	Africa, America, Bangladesh, Myanmar, Pakistan, Philippines, Sri Lanka, Thailand
▲73. *Leucauge pondae* Tikader	BTE, JTE, HTE, KTE	Assam Sikkim, West Bengal	-
▲74. *Tetragnatha ceylonica* O.P. Cambridge	BTE, JTE	Assam Kerala, West Bengal	Philippines, New Britain, New Guinea, South Africa, Seychelles, Taiwan, Thailand
■75. *Tetragnatha hasselti* Thorell	JTE, KTE	Assam, West Bengal	Bangladesh, Celebes, China, Indonesia, Myanmar, Thailand
▲76. *Tetragnatha javana* (Thorell)	JTE	Assam, Bihar, Karnataka, Orissa, Tamil Nadu, West Bengal	Africa to Japan, Bangladesh, Indonesia, Nepal, New Guinea, Philippines, South and South east Asia
▲77. *Tetragnatha maxillosa* Thorell	BTE, JTE, HTE	Assam, West Bengal	South Africa, Bangladesh, Cambodia, China, Indonesia, Japan, South Korea, Laos, Malaysia, Myanmar, New Hebrides, Philippines, Sri Lanka, Taiwan, Thailand, Vietnam

XIV. Family Theridiidae ▲78. *Achaeranea budana* Tikader	JTE	Assam, Sikkim, West Bengal	-
▲79. *Argyrodes gazedes* Tikader ▲80. *Chrysso angula* (Tikader)	JTE KTE	Assam, Kerala, Sikkim, West Bengal Assam, Andhra Pradesh, Arunachal Pradesh, Kerala, Sikkim	-
▲81. *Theridion indicum* Tikader	BTE	Assam, Andaman & Nicobar Island, West Bengal	-
XV. Family Thomisidae 82. *Misumena* sp.	BTE, JTE, KTE	Assam	-
▲83.*Ozyptila manii* Tikader	BTE	Assam, West Bengal	-
XVI. Family Uloboridae ▲84. *Uloborus danolisus* Tikader	JTE	Assam, Madhya Pradesh, Maharashtra, Nicobar Island, West Bengal	-
85. *Uloborus khasiensis* Tikader	BTE, JTE, HTE	Assam, Meghalaya, West Bengal	-

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Table 2
Summary of the recorded spider taxa of the tea gardens of Assam

	Bukhial Tea garden	**Jamguri Tea garden**	**Hunwal Tea garden**	**Kotalgoorie Tea garden**	**Total**
No. of family	13	13	9	10	16
No. of genera	37	33	27	23	53
No. of species	52	52	30	33	85
No. of individuals	308	292	194	231	1025
Sex ratio (♂/♀)	42 : 266	25 : 267	24 : 170	32 : 199	123 : 902

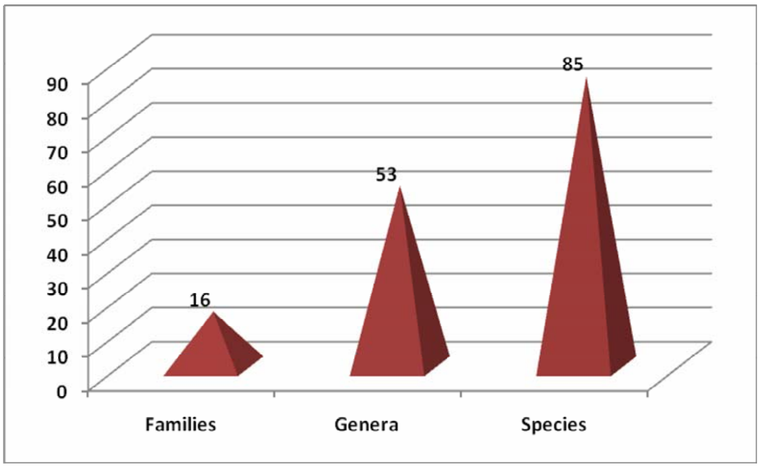

Fig. 2. Total no. of spider taxa trapped from Tea Estates of Assam.

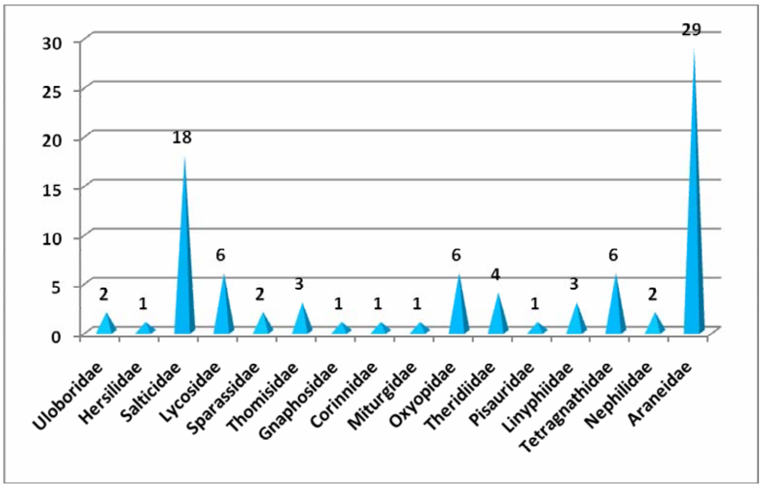

Fig. 3. Spider species trapped under different families
from the Tea Estates of Assam.

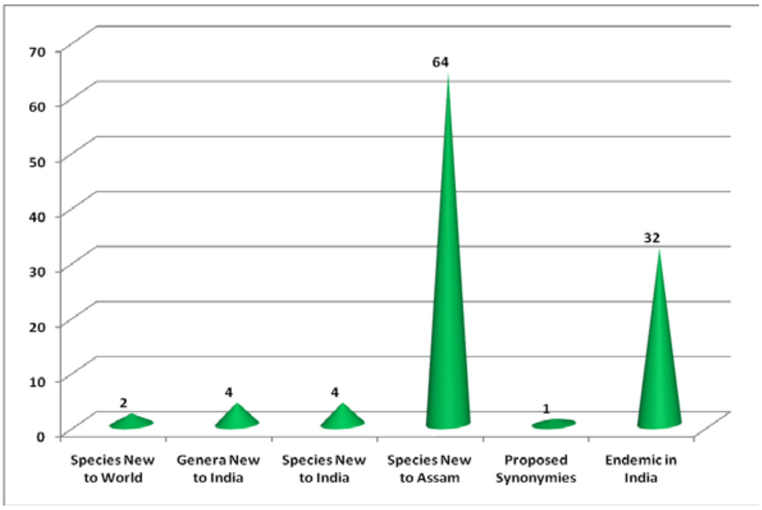

Fig. 4. Spider fauna of Assam-highlights.

Like anywhere else, spiders occupy several tropic niches in the tea system too. Based on their habit and habitats, they are broadly classified into Web builders and Wanderers or Non-web builders. The web forming spiders may be Orb weavers, Foliage hunters, Sheet weavers and Space weavers. Non-web forming spiders include Stalkers, Ground dwellers, Trunk dwellers (Foliage hunters) and Ambushers. Thus there are 7 trophic groups.

Orb weavers include members of the family Araneidae, Nephilidae, Tetragnathidae and Uloboridae. Their preys are trapped in the viscid and elastic silk on the periphery of the web. Most araneids are typical Orb weavers and nocturnal. However, they are frequently found in the webs during the day time and adopt the spin wrap attack method to subdue their prey. Among them, *Argiope pulchella* Thorell is very much common in tea bush, rehabilitation crop and fencing trees. *Cyclosa* spp. remain in the web with food particles and feces. *Neoscona* spp. enjoy the foliages, weeds or sometimes the dry tea leaves, perfectly matching with the back ground. Nephilids are the largest and golden Orb weavers. *Nephila kuhlii* C.L. Koch builds a large web to the extent of 30ft. between two shade trees or between shade trees and tea bushes and thus maximizing trapping area. Unfortunately they are rare probably due to their intolerance to pesticides. Tetragnathids are long jawed Orb weavers and therefore are good candidates of biological control. *Cyclosa spirifera* Simon, *Cyclosa mulmeinensis* (Thorell) and *Neoscona mukerjei* Tikader are the dominant species in Bukhial and Hunwal, Jamguri and Kotalgoorie Tea Estates respectively. *Tetragnatha* spp. are prevalent in the moist places near drainage and shaded areas. Uloborids spin modified version of Orb webs, ranging from triangular to a single thread, consisting of viscid cribellate silk in low bushes or between objects near the ground. They prefer cool and shady places. *Uloborus khasiensis* Tikader is the most frequent and typical in the tea ecosystem. None of the agronomic practices does affect the species, as they build their webs underside the tea leaves and tea bushes.

Foliage hunters accommodate, hersilids, miturgids, pisaurids and sparassids. Among them miturgids construct white, flattened, tubular sac or retreat with silk usually in rolled up leaves, foliages, under loose bark of shade trees and sometimes under stone. Types of sacs vary depending upon their biological need eg, resting, mating and breeding. Their usual habit of capturing prey is by sudden jumping and seizing with stout chelicerae. They use their front legs to detect and grab the prey. *Pisaura gitae* Tikader, the only pisaurid, is predominant in the tea ecosystem. Sparassids are usually found under the bark, in dry leaf litter and within folded leaves of tea bush and fencing trees. They do not spin webs, however make silken retreats.

Linyphids spin flat, dome or hammock shaped **sheet webs** with isolated threads forming scaffolding. The spider hangs upside down under the sheet without any retreat. Prey is bitten from below through the sheet and pulled through it, before being consumed. *Linyphia nicobarensis* Tikader is the most widely encountered species.

Most theridiids construct differently shaped 3D **space webs,** consisting of criss-cross viscid threads to catch flying insects. The threads break easily and glued prey becomes more entangled while trying to escape. Some species build special retreats within or out of the frame and use plant or soil particles to camouflage the web.

Stalkers consist of the members of the family Salticidae and Oxyopidae. Salticids, visually based stalking attackers, are frequent in tea trunk, both sides of leaves, fencing trees and shade trees. They are diurnal, typical non-weavers but make silken retreats in the form of a tube or sac fastened to various substrata to moult, sometimes to mate, egg laying or night shelter.

Dominant species are *Plexippus paykullii* (Audouin)<*Phidippus bengalensis* Tikader<*Marpissa decorata* Tikader<*Phintella vittata* (C.L. Koch). Most oxyopids chase their prey with great rapidity over bush canopy, jumping in habit and usually hide from a flying insect until within stalking distance. Others lie in wait near flowers and spring upon insects that visit flowers. These diurnal hunting spiders make no web but quickly detect the prey with good vision and catch it with legs. They are often found in grass, weeds and also fencing trees. *Oxyopes shweta* Tikader is the dominant species.

Ground dwellers comprise the members of the families Corinnidae, Gnaphosidae, Lycosidae, and some members of Salticidae. Lycosids are usually diurnal while some are nocturnal. They are typically non-weavers but make tunnels on cracks and crevices by weaving, usually found under stones or debris, at the plant base. Some prefer moist habitats. *Pardosa birmanica* Simon and *P. sumatrana* (Thorell) are most common in the tea ecosystem.

Thomisids are **ambushers.** They are primarily diurnal, non-weaving; usually live on plants and foliages and in winter hide in the cracks under stones and bark. On the bark, the body surface achieves a roughened texture to improve camouflage. They hunt by stealth and **ambush** with their powerful spinose legs and concealing colours.

The decreasing order of the groups are Orb weavers (45.88%)> Stalkers (31.76%)>Ground dwellers (18.82%)>Foliage hunters (7.05%)>Space weavers (4.70%)>Ambushers (3.52%)≥Sheet weavers (3.52%). Their food spectrum includes several major tea pests such as Tea mosquito bug *Helopeltis theivora* Waterh., Red spider mite *Oligonychus coffeae* (Nietner), *Aphis gossypii* Glover, eggs and adults of *Hyposidra talaca* (Walker) and *H. infixaria* Walker, shade tree pests *Diacrissia obliqua* Walker, *Oxyrachis tarandus* (Fabricius), *Eurema hecabe* (L.) and many stray species.

Besides predation, spiders have specific adaptation to match with the background such as leaves, flowers, grass, twigs, bark and the ground. In some cases the crypsis is extraordinarily precise. For example, most thomisids mimic the colours of flower heads wonderfully and prey on pollinators that approach. Such highly cryptic behaviour keeps them concealed in nature. Similar type of adaptation occurs in *Tetragnatha* spp. Some species of theridiids like *Theridion indicum* Costa use plant or soil particles to camouflage the web. Nocturnal, grey brown and black sparassid spiders are often with enough mottling to provide camouflage. *Hersilia savignyi* Lucas usually inhabit the shade tree trunk where they mimic the dry bark and spread the silk over the nearby area. *Herennia multipuncta* (Doleschall) also inhabits the shade tree trunk and camouflages with the bark. Thus selection for crypsis in similar types of habitat has lead to the repeated evolution of similar colouration in unrelated species.

Spiders may gain some protection from predators through their resemblance with aggressive or unpalatable ants, a range of other organisms, alive or dead and inanimate objects. For example, *Myrmarachne maratha Tikader* and *M. plataleoides* O. P.-Cambridge mimic with ants. *Cyclosa* spp. construct vertical “sticks” of prey remains, within the web and leave a gap in the centre where it stays. In *Argiope pulchella* Thorell the visibility of both the contrastingly coloured ventral and the UV reflecting dorsal side of the opisthosoma may increase the prey traps in the web.

Spiders are always dioecious. Apart from a few exceptions, the females are always larger than males. Sexual dimorphism is especially obvious in many tropical Orb weavers such as *Nephila*, *Gasteracantha* etc., in which the males appear to be dwarf. Females of *Nephila kuhlii* C.L. Koch are colourful, with size up to 5 cm, legs span up to 15 cm and build the largest orb web known, while males are many times smaller.

Cannibalism within spiders being a natural event, sociality is hardly expected. It is noteworthy to mention that actual number of social spiders is still unknown. Some theridiids of the gus *Argyrodes* Simon lead a kleptoparasitic life on the large webs of *Nephila* Leach and *Cyrtophora* Simon.

CONCLUSION

The tea ecosystem is stable and typical due to its regular management practices and continuous influx of energy and nutrients. But being a monoculture crop, it cannot favour diverse insect groups. On the contrary, it welcomes only few insect groups that are either pests or stray species. These species serve as a good food source to the inhabiting spiders. Study in the adjoining forests leads to guess that they opt ballooning as the means of movement, i.e. adjoining forests ↔ tea gardens.

Therefore they may be identified as a potential regulatory factor to address the issue of pest problems in tea.

The management plan that can be suggested is simply to augment the diversity of spiders in tea field. This can be achieved by:

i) Creation of forest fringe;

ii) Deposition of litters;

iii) Addition of organic mulch;

iv) Manipulation of spacing distance.

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REFERENCES

BARRION A.T., LITSINGER J.A., 1995, *Riceland Spiders of South and South East Asia*. CAB International, UK & IRRI, Philippines, 716 pp.

HAZARIKA L.K., BORTHAKUR M., SINGH K., SANNIGRAHI S., 1994, *Present status and future prospects of biological control of tea pests in North East India*. Proceedings of the 32nd Tocklai Conference, TRA, Tocklai Experimental Station, Jorhat, Assam, 169-177.

KESWANI S., HADOLE P., RAJORIA A., 2012, *Checklist of Spiders (Arachnida: Araneae) from India-2012*. Indian Journal of Arachnology, **1** (1), 129 pp.

MAJUMDER S.C., TIKADER B.K., 1991, *Studies on some spiders of the family Clubionidae from India*. Records of the Zoological Survey of India, Occasional Paper, **102**: 1-174.

METZNER H., 2014, *Worldwide database of jumping spiders (Arachnida, Araneae, Salticidae)*. (Available at: www.jumping-spiders.com; accessed on 30th November, 2014).

PLATNICK N.I., 2014, *The world spider catalog version, 14.5*. American Museum of Natural History (Available at: http://research.amnh.org./entomology/spiders/catalog 87-81/indexhtml; accessed on 30th November, 2014).

ROY T.K., 2014, *Spiders (Araneae: Arachnida) of Tea Ecosystem of Dooars, West Bengal*. Ph. D. Thesis, University of Calcutta, 488 pp.

SATPATHI C.R., 2004, *Predacious spiders of crop pests*. Capital Publishing Company, 188 pp.

SEBASTIAN P. A., PETER K.V., 2009, *Spiders of India*. Universities Press (India) Pvt. Ltd., 614 pp.

TIKADER B.K., 1970, *The spider fauna of Sikkim Himalaya India*. Records of the Zoological Survey of India, **64** (14): 1-84.

TIKADER B.K., 1980, *The Fauna of India, Araneae: Thomisidae (crab spiders)*. Zoological Survey of India, Calcutta, **1**: 1-247.

TIKADER B.K., 1982, *The Fauna of India, Spider: Araneae (Araneidae & Gnaphosidae)*. Zoological Survey of India, Calcutta, **II** (1): 533 pp.

TIKADER B.K., 1987, *Hand book of Indian spiders*. Ed. Director Zool. Surv. India, Calcutta, 251 pp.

TIKADER B.K., MALHOTRA M.S, 1980, *Fauna of India, spiders (Lycosidae)*. Zoological Survey of India, Calcutta, **I** (2): 248-447.

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PELECUS CULTRATUS (LINNAEUS, 1758) ON SITE MANAGEMENT DECISIONS SUPPORT SYSTEM – A CARPATHIAN NATURA 2000 SITE STUDY CASE

ANGELA CURTEAN-BĂNĂDUC*, IOANA-CRISTINA CISMAŞ*, DORU BĂNĂDUC**

The ADONIS: CE was utilised here in biology/ecology science field to create a broad framework management model for *Pelecus cultratus* fish species based on this species inventoried needs regarding the local habitats, the indicators which reveal the favourable conservation status and the proper measures, the identified threats and pressures on this fish species. If the suggested management directions will not be used in ROSCI0132, the *Pelecus cultratus* species is on the edge of its local extinction in the next 10-20 years. Such on species, on habitats and on site based management support systems for other fish species of Natura 2000 conservative interest should be done in the case of ROSCI0132.

Key words: *Pelecus cultratus*, Natura 2000 site, community interest fish species habitat necessities, threats, pressures, management, Transylvania, Romania.

INTRODUCTION

In order to guarantee the outlast of the most threatened species of the European continent, the EU states government representatives assumed the Habitats Directive in 1992, based on which their countries should make the necessary conditions for the conservation of all these species and habitats included in Directive (Annex 2), to conserve and to increase their ecological status.

The Natura 2000 sites in Romania, including for community interest fish species protection, were chosen for their relevance in relation with this species conservative importance. The designation of these European Natura 2000 net sites was realized based on specific selected criteria like: good geographical location, well conserved, healthy and stable fish populations, representative habitats, and low human impact. There are significant elements, based on which the Natura 2000 initiative can ameliorate the EU countries nature conservation: the increasing of the protected natural areas' surface; institution capacity strengthening; improving the citizens consciousness, realistic and functional management elements implementation for the protected areas (Papp & Toth, 2007).

Pelecus cultratus (Linnaeus, 1758) is a Community interest fish species. It is a freshwater, brackish, pelagic, good swimmer which lives in relative low altitude land areas. It is relatively frequent in littoral lakes. It reaches its sexual maturity at three – four years old and the reproduction happened in April – June period, starting at a temperature of around 12°C. They spawn in the midstream. It can reach a total length of 35-37 cm. Its food consists of plankton, terrestrial and aquatic benthic invertebrates, insects and also fish juveniles (Bănărescu, 1964; Bănărescu & Bănăduc, 2007).

In Romanian national territory the range of *Pelecus cultratus* was much restrained and fragmented in the last century due to the negative quantitative and qualitative impact of anthropogenic activities, which is different from one basin/ basin sector to another, and even from one protected area to another (Bănăduc, unpublished data).

The fish fauna structure, where *Pelecus cultratus* species was sampled, in Natura 2000 site Oltul Mijlociu-Cibin-Hârtibaciu (ROSCI0132) highlight extremely few individuals number as a straight effect of significant human activities impact. The range of distribution of these fish species populations and their very low abundance in this Natura 2000 site reveal the sinergical results of the Olt River basin lotic habitats quality diminishing (Curtean *et al*., 1999; Curtean-Bănăduc *et al*., 2007). Actually only in the upstream and in the downstream of this heavy impacted middle Olt River basin sector this species was still found in a very low conservative state.

In the present global trend in which the lotic systems change in more obvious valuable nature resources, the human impacts will diminish the quantitative and qualitative access to them (Gomoiu *et al*., 2009).

If this global trend will continue in the next decades, the so-called general approach management elements will be no more enough to be applied in various protected valuable areas, due to the fact that diverse microhabitat and habitat components should be evaluated, only after that specific management tools should be adapted in a flexible manner and proposed for specific local habitats/species conditions.

Lately, the elements of process modelling are more applied to obtain a "broad picture" of specific systems and/or actions of different domains. The elements of modelling processes are useful to understand the process stages for a realistic and functional management. Fundamentally the modelling tools are represented by software products, which are used to create and/or research models of business organizations, and to reveal information about the models. There are three main functions: validate the present situation, analyse the results of possible changings and suggest plans to change the present situation in a desired direction. In the end are suggested different ways to make diagrams which offer specific management elements (Hall & Harmon, 2005).

The principal aims of this study are: to show the present state of *P. cultratus* populations in the ROSCI0132 area; to show the present anthropogenic negative impact in terms of threats and pressures; to counsel through management elements proposal for the increasing of this fish species conservation status based on a specific designated management model, which includes habitat requirements of these species and specific habitat indicators, as a back up decisional system for management.

MATERIAL AND METHODS

The Natura 2000 site area ROSCI0132 (45.682778 latitude, 24.324444 longitude, 2826.10 ha surface, located between 314 and 568 a.s.l. m) is situated in Sibiu, Braşov and Vâlcea, Romanian administrative units. From the biogeographical point of view it is located in the Continental and Alpine European biogeographic regions. This protected area was designated also for ten fish species of conservative interest, included in the Annex 2 of the Habitats Directive (92/43/ EEC) *Pelecus cultratus* – Natura 2000 code 2522, *Zingel zingel*, *Zingel streber*, *Romanogobio kesslerii*, *Barbus meridionalis*, *Cobitis taenia*, *Sabanejewia aurata*, *Rhodeus amarus*, *Aspius aspius* and *Romanogobio uranoscopus*.

Pelecus cultratus individuals were sampled in 2010-2013 period, with fishing nets, identified in situ and released immediately in their habitats. The river sectors of the researched zone where *Pelecus cultratus* were identified are presented in Fig. 1.

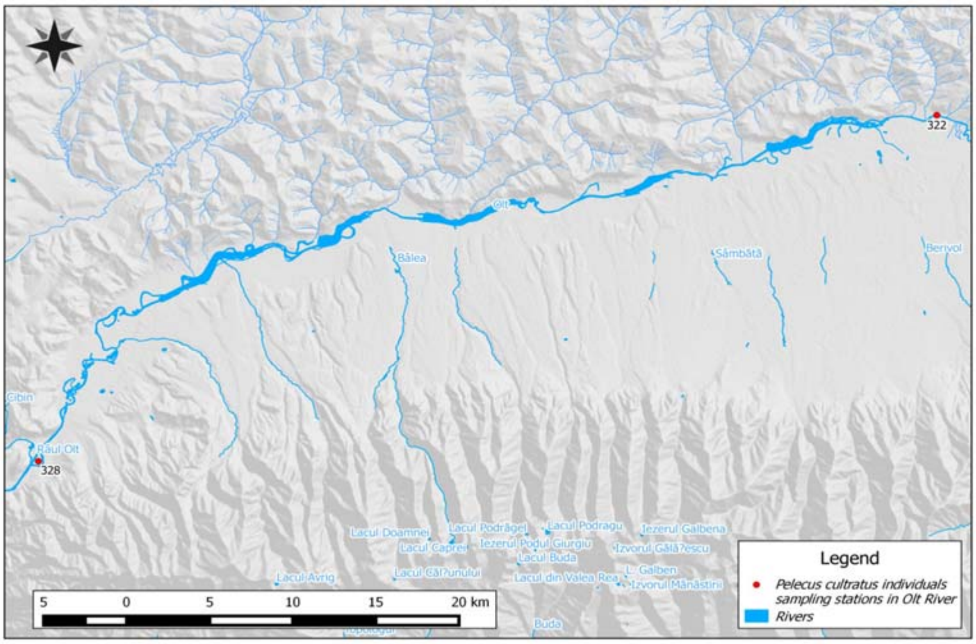

Fig. 1. *Pelecus cultratus* individuals sampling stations (322 and 328) in the Olt River.

Pelecus cultratus populations were under assessment in the study period and their ecological status was evaluated in the context of the local anthropogenic impact threats and pressures on these fish populations and habitats.

These fish populations conservation status were evaluated based on some specific designated criteria like: balanced distribution of fish on age classes, distribution surface, populations size and the low number of individuals of this species in the fish associations.The *Pelecus cultratus* species habitat needs, threats and pressures were ascertained based on their presence or absence and this fish species populations conservative status, in the studied area.

To determine the management elements, required to be taken to guarantee this species outlive in the studied area and unveil the necessary process, the authors utilise an adapted management model. Hence, the authors used ADONIS:CE, realised by Business Object Consulting. ADONIS: Community Edition, which is a free tool offered by the BOC Group which can be used as an entry point to Business Process Management and as a fitting way to become familiar with ADONIS. ADONIS: CE represents a feature rich stand-alone version of ADONIS with some limitations in comparison with the commercial edition. BPMN (Business Process Model and Notation) is a standardized specific modeling language which is right to be used for the visualisation of processes. The processes can be intelligibly and quickly modeled based on uniform notation.

RESULTS AND DISCUSSION

Ecological state evaluation of *Pelecus cultratus* populations

The conservation status of the species *Pelecus cultratus* populations in the Olt River sampling sectors 322 and 328 (Fig. 1) is exceptionally low in the context of: unbalanced distribution of individuals on age classes, populations size, and an exceptionally low percentage of fish individuals of this species in the structure of the local ichthyofauna. In the Olt River, the habitats of the studied species populations in the studied sectors are in exceptionally low conservation state, in respect of *P. cultratus* ecological needs.

Human pressures and threats

Along this research, the next threats and pressures on *Pelecus cultratus* were found: pressures – destruction/significant changes of natural habitats of these species, water pollution; threats – destruction of the habitats of this species, water pollution, lotic systems fragmentation, river regularization and mineral substratum overexploitation. All these human impact related pressures and threats, and their direct and indirect effects on the fish fauna were obvious in the last two decades in the middle Olt River sectors (Bănăduc, 1999), but efficient enough actions were not taken to diminish these negative effects.

Specific requirements

P. cultratus adults need relative large lotic ecosystems sectors with relatively deep (over four – five m depth) and fast flowing unpolluted water. They spawn in midstream and feed on plankton, terrestrial and aquatic invertebrates and alevines (Bănărescu,1964, Bănărescu & Bănăduc, 2007).

Specific habitat indicators

Based on *P. cultratus* species presence and relative abundance in the studied lotic sectors, some habitat indicators were proposed: surfaces in the minor riverbed with a depth of the water over four-five meters (66%) and flowing water surface percentage (66%).

Management measures

Conservation/ecological reconstruction of the natural morphodynamic of riverbeds; forbid the hydrotechnical constructions which change the lotic habitats in lenitic habitats and the nature of substratum. Construction of a fish leaders system which should diminish the important negative impact of the actual series of big dams, the last one finished in the last few years, the most downstream of them all in the middle of the Olt River course.

Riverbed mineral overexploitation should not be allowed and the exploitation should be forbidden in the few relatively fast or medium flowing sectors with relatively significant depth and rocky substratum, which remained unharmed by human impact. No less than five km between two successive riverbed mineral exploitations or/and stagnant/semi-stagnant water sectors should be approved. In the reproduction period (April-June) the fishing should be forbidden. The wastes abandonment in the riverbed and near wetlands should be banned. An integrated monitoring system with an objective on fish fauna should be implemented.

Site adjusted management model

The process for the on site management model is relying on activities (squares – see below), decisions (triangles – see below) and variables (circles – see below) (Figs. 3 and 4). The principal objects used to construct the *Pelecus cultratus* management model for ROSCI0132 with ADONIS: CE are presented below (Hall & Harmon, 2005).

A process  in this management model is a series of steps in which data is processed or transformed for different models. A process can be modelled relying on activities, decisions and subprocesses, documents attached to different activities and notes.

The activity in this management model is a part of a process and includes the tasks of the process. In the modeling process, there are activities that are based on decisions.

The decisions in the management model are main parts of this process because for every decision it can be selected a probability to accomplish the activities (used in the analysis and the simulation). To each decision it can be assigned a probability condition using variables and random generators.

Variables (steps which can be followed in accordance to the assignment of variables – defined in the transition conditions) and generators are defined (induce values to variables to which they are connected). The generator is associated with connectors by the decisions and the variables.

Model structured in subprocesses is supporting for a better process organization and understanding. The subprocess can work as a process. It is proposed to apply, especially when the model is very elaborate and on their base the user "enters" in the process from the higher to the lower level.

This paper modeled habitat ecological needs of *Pelecus cultratus* and the specific conditions that allow its good ecologic status. As it can bee seen in the figure below (Fig. 2 – Explorer Model groups), the targeted process is *P. cultratus*. The presented subprocesses revealed in the figure are part of the basic model. They can be used by a click, using Explorer Window or else from the starting model.

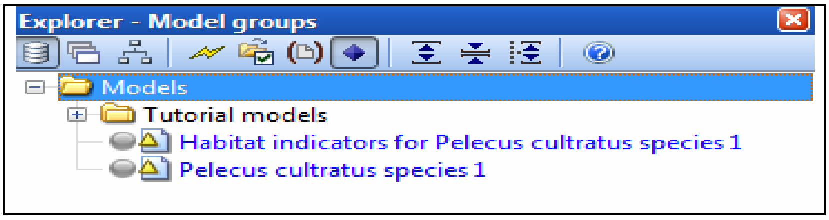

Fig 2. Table of content for modeled processes.

The basic process (Fig. 3) is called *Pelecus cultratus* species and is structured using the following modeling objects: two activities, a subprocess and a decision. Activities show the habitat specific requirements and current pressures and existing threats of *P. cultratus*. The description of each activity can be edited in the activity notebook. Next is subprocess Habitat indicators for *Pelecus cultratus* species (Fig. 4) and it is presented in detail in the following paragraph. A decision follows on whether indicators modeled in subprocess Habitat indicators for *Pelecus cultratus* species are or not in favorable conservation status. If they fulfill the conditions for favorable conservation status ("Conservation_state = Yes" probability = 30%), the process ends. If they do not fulfill the favorable conservation status ("Conservation_state = No" probability = 70%), it follows an activity where species current pressures and threats are described, then the process ends.

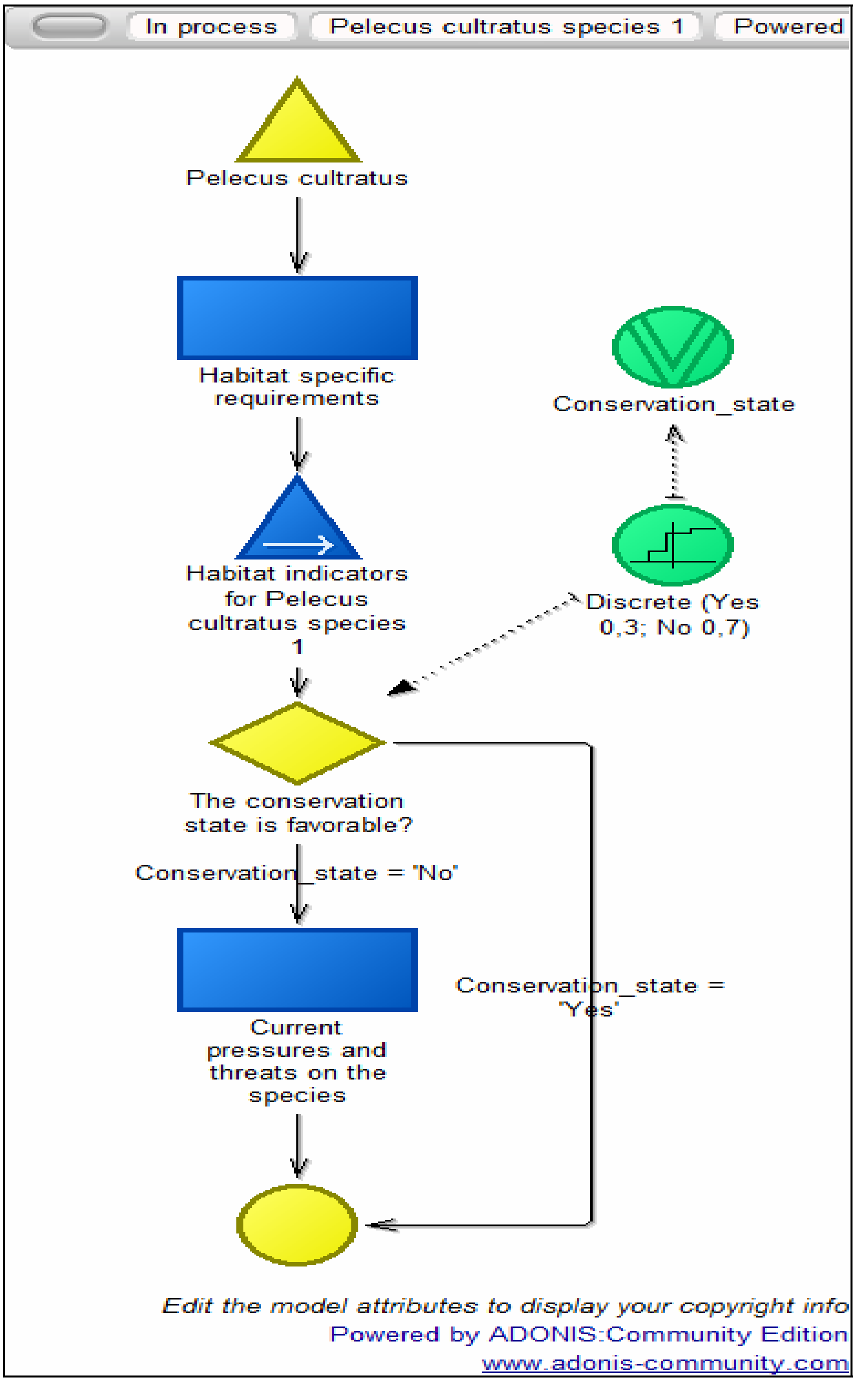

Fig. 3. *Pelecus cultratus* species – basic process.

Subprocess Habitat indicators for *Pelecus cultratus* species (Fig. 4) is structured in two decisions and seven activities. The decisions contain details about the status of the indicators and the percentage of favorable species conservation state. Because there are only two indicators, management measures of each indicator were not structured into subprocesses, but in activities that can take place in parallel – at the same time (activities 1-5).

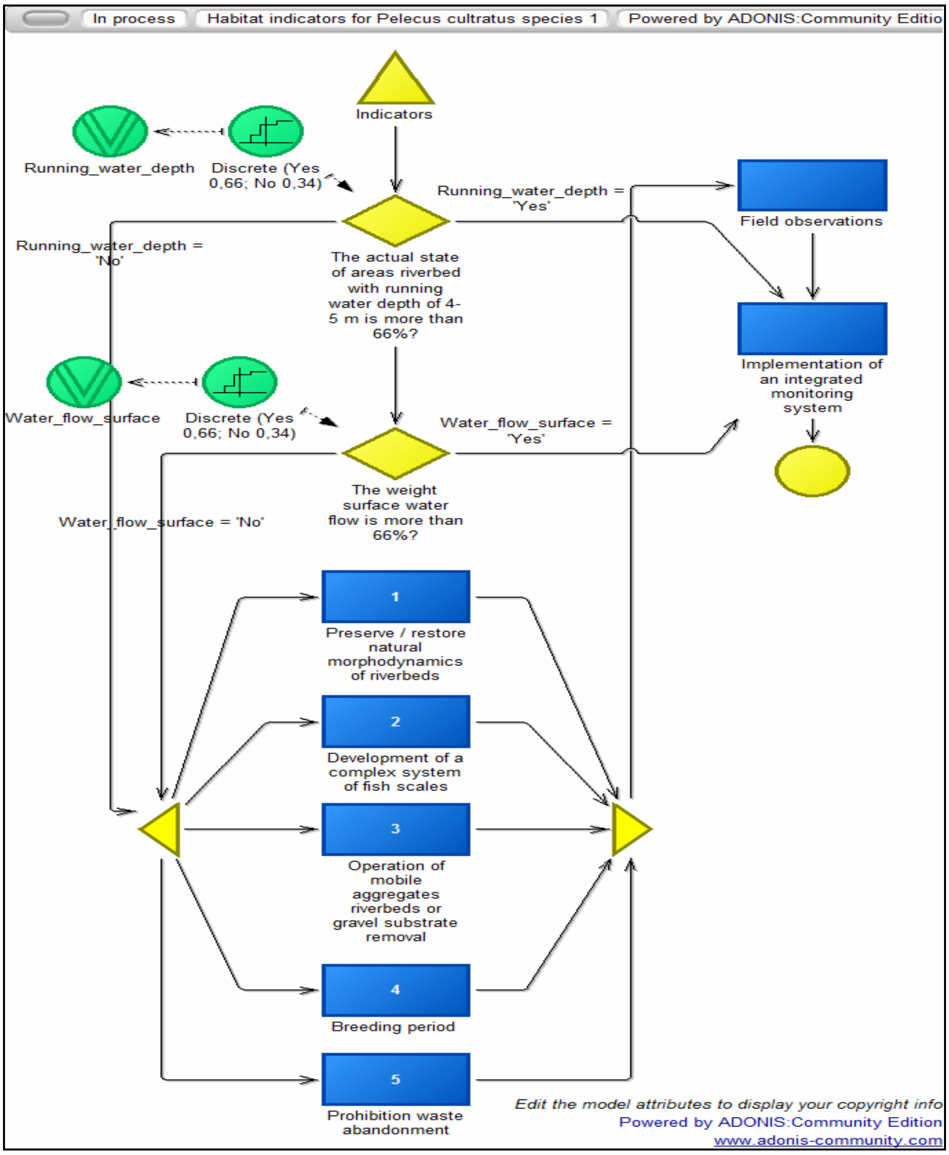

Fig. 4. Habitat indicators and management measures for *Pelecus cultratus*.

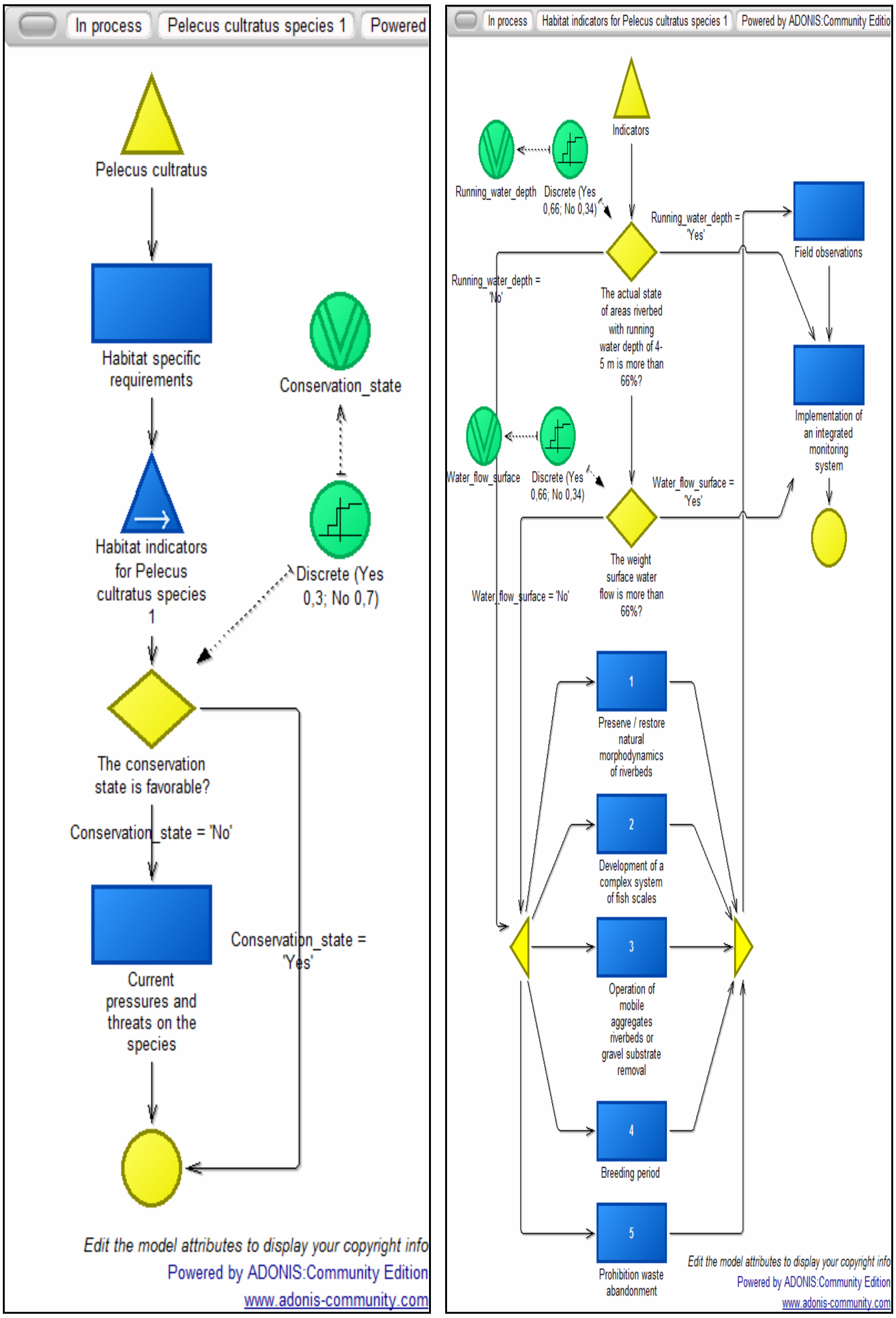

Fig. 5. The connection between processes (model framework).

Object modeling for parallelism consists of the object  which represents the beginning of parallelism and the object modeling  that shows its merging. If, for each indicator, the conservation state (Running_water_depth and Water_ flow_surface) is in the parameters, then it comes down to final activity – Implementation of an integrated monitoring system and the process ends. If indicators do not fulfill the desired percentage for the conservation status, follow the activities included in parallelism, and finally, the activities Field observations, Implementation of an integrated monitoring system and the process ends. The activities included in parallelism are exactly the management measures that should be taken to maintain favorable conservation status, and so, to ensure the livelihood of the species: Preserve/restore natural morphodynamics of riverbeds, Development of a complex system of fish scales, Control of mobile aggregates riverbeds or gravel substrate removal, taking into account the Breeding period and Prohibition waste abandonment.

Finally in this study there was created a framework model for management support (Fig. 5) for *Pelecus cultratus*.

CONCLUSIONS

The principal identified threats to the species *Pelecus cultratus* conservation status in ROSCI0132 Natura 2000 site are: destruction of the habitats of this species, water pollution, lotic systems fragmentation, river regularization and mineral substratum overexploitation; and the pressures: destruction/significant changes of natural habitats of this species and water pollution.

Very important for *Pelecus cultratus* species conservation are: conservation and ecological reconstruction of the natural morphodynamics of riverbeds; construction of a fish leaders system; riverbed mineral overexploitation control, fishing banning in April-June period; wastes abandonment control and an integrated monitoring system implementation.

The ADONIS: CE was used here in biology/ecology domain, creating a management model of *Pelecus cultratus* species that include its main necessities for habitat, the indicators that reveal a favourable ecological status – the proper measures, and the threats and pressures which influence this fish species.

If the proposed management elements are not implemented, this species will disappear in the studied area in the next 10-20 years.

This on site, on habitats and on species framework management support model for *Pelecus cultratus*, should be integrated in a management model for the local fish fauna. For this purpose such management systems for all fish species of community conservative interest should be realized for ROSCI0132 area.

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REFERENCES

BĂNĂDUC D., 1999, *Data concerning the human impact on the ichthyofauna of the upper and middle sectors of the Olt River*. Transylvanian Review of Systematical and Ecological Research, **1**: 157-164.

BĂNĂRESCU P., BĂNĂDUC D., 2007, *Habitats Directive (92/43/EEC) fish species (Osteichthyes) on the Romanian Territory*. Acta Ichtiologica Romanica, **II**: 43-78.

BĂNĂRESCU P., 1964, *Pisces-Osteichthyes, Fauna R.P.R.,* vol. XIII, Edit. Academiei R.P.R., București, pp. 962.

CURTEAN A., SÎRBU I., DRĂGULESCU C., BĂNĂDUC D., 1999, *Impactul antropic asupra biodiversității zonelor umede din bazinul superior și mijlociu al Oltului*. Edit. Univ. „Lucian Blaga" Sibiu, 102 pp.

CURTEAN-BĂNĂDUC A., BĂNĂDUC D., BUCȘA C., 2007, *Watershed Management (Transylvania, Romania) – implications, risks, solutions, Strategies to Enhance Environmental Security in Transition Countries, NATO Security through Science Series – C: Environmental Security*, Springer, pp. 225-238.

GOMOIU M.-T., ARDELEAN A., ARDELEAN G., ARDELEAN D.I., ONCIU T. M., SKOLKA M., KARÁCSONYI K., 2009, *Zonele umede - abordare ecologică*, Casa Cărții de Știință, Cluj-Napoca, 443 pp.

HALL C., HARMON P., 2005, *The Enterprise Architecture, Process Modeling & Simulation Tools Report*, Version 1.1 (2005) November, 2005 (Available at: http://mhc-net.com/whitepapers_ presentations/2005 Process Trends (040306).pdf)

PAPP D., TOTH C., 2007, *Natura 2000 Site Designation Process with a special focus on the Biogeographic seminars*, Second edition, Nagy D., Tripolszky S. and Schnell A.A. (Eds), pp. 1-36.

***Council Directive 92/43/EEC of 21 May 1992 (Available at: http://ec.europa.eu/ environment/ nature/conservation/species/habitats_dir_en.htm).

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CORVIDAE TOLERANCE TO HUMAN DISTURBANCE IN SETTLEMENT LANDSCAPES OF ZHYTOMIR (UKRAINE)

ALEX MATSYURA*, KAZIMIERZ JANKOWSKI**, ANASTASIA ZIMAROYEVA***

We determined the degree of anthropogenic tolerance of Corvidae species by analysis of flight initiation distance (FID) in rural and urban settlement landscapes. The FID significantly decreases in transition from rural to urban landscapes. We confirmed that Corvidae can successfully adapt to human presence and therefore can be used as the model species for modeling of synanthropization patterns. The results indicate that bird species with a high propensity to disperse and with large population sizes tend to decrease their FID more strongly along the urban-rural habitat gradient. This pattern was most apparent in the Rook. Based on FID, Corvidae species were classified as being tolerant to urban environmental conditions, with FID values showing decreasing trends along the urban-rural gradient, as in the Rook. Our results suggest that FID may be the relevant measure for analyzing birds' tolerance to urbanization and for assessing the speed by which species or populations can adjust or adapt to novel environmental conditions.

Key words: birds, adaptation, synanthropization, human tolerance, Corvidae, Ukraine.

INTRODUCTION

Urban environment is the place for the existence of those animal species which have wide norm of reaction and their responses on human factors correspond to adaptation potentials (Rezanov, 2006; Møller, 2008; Rahimov, 2011). Urbanization affects birds' survival, population structure, reproduction, and behavior. As urbanization increases, an understanding of how birds respond to the conversion of rural areas to urban ones is necessary to successfully conserve biodiversity. These modifications in land cover also provide a natural experiment for exploring how animals respond to modified environments.

An important behavioural adaptation of birds to urban environment expresses their high level of tolerance to human presence. One of the most accessible methods of estimation of bird tolerance to human presence is an evaluation of flight initiation distance, FID (Rezanov, 2002; Kelin & Spiridonov, 2009).

Flight initiation distance is the distance at which an animal begins to flee from an approaching predator (Møller, 2010). Because it is relatively easy to systematically approach animals until they flee, flight initiation distance is an excellent metric with which to quantify an individual's fearfulness in a particular circumstance.

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Urban habitats are characterized by environmental conditions that differ from those of nearby rural habitats (Jokimäki *et al*., 2005). Consequently, animals living in cities suffer selective pressures different from those suffered by animals inhabiting rural areas (Fernandez-Juricic & Schroeder, 2003; Møller *et al.,* 2012).

A striking difference is the higher density of humans that increases the frequency of animal–human interactions in urban areas. Because the density of human populations in urban areas is extremely high compared with nearby rural areas, urban habitats constitute suitable habitat only after adjustment to human proximity. Therefore, short flight initiation distances allow for coexistence with humans without disruption of foraging, low energy expenditure due to rare flights, and no release of stress responses.

Urbanization in birds was initially associated with migratory birds wintering in urban areas without any such wintering birds breeding there. Individuals can overcome cold winters with snow cover either through migration to areas with less severe conditions (Møller *et al*., 2013) or through adaptation to proximity of humans. Individuals with short flight initiation distances should do particularly well in urban compared with rural environments during cold winters, causing net growth in urban populations. In contrast, individuals with long flight initiation distances should do better in rural environments where there is less intense selection for ability to sustain proximity of humans and where predators continuously select for vigilance (Møller, 2008; Diaz *et al*., 2013). There is a hypothesis, that the greater the fluctuations of FID the higher the degree of species adaptation in urban landscape (Møller & Ibáñez-Álamo, 2012). Thus, urbanized populations should reduce flight initiation distance following cold winters, whereas rural populations should not.

Variation of bird FIDs is typically species-specific and depended upon character and orientation of intruder motion, the intensity of urban infrastructure, type of biotopes (Rezanov, 2006; Møller, 2008; Rahimov, 2011). Some researchers consider that degree of bird tolerance toward humans correlate with the starting distance of motion (Cooper, 1999; Møller, 2008; Cooke, 2003).

In this research we address the following questions: are Corvidae FID values generally lower in strongly urbanized areas than in more rural areas and do variations of Corvidae FID indicate their tolerance to urbanization?

MATERIAL AND METHODS

We collected behavioural responses of corvidae birds to the approach of human intruders at four points along the urban gradient (Zhytomir city, urban (small towns), suburban, and rural) from September, 2009 to August, 2012. We tested Rook (*Corvus frugilegus*), Eurasian Jackdaw (*Corvus monedula*), Hooded Crow (*Corvus cornix*), Eurasian Magpie (*Pica pica*), Eurasian Jay (*Garrulus glandarius*), and Common Raven (*Corvus corax*) in all the points.

All urban study sites included areas with multi-storey buildings, single family houses, roads and parks, while nearby rural areas had open farmland and woodland and did not contain continuous urban elements like multi-storey buildings, one family houses, roads and parks.

We conducted fieldwork around Zhytomyr (50°150'N, 28°40'E; Ukraine). The study sites were a mixture of urban and rural habitats, where we defined urban areas as those containing built-up areas with continuous houses or multistorey buildings, with the only interspersed areas being roads and city parks. Rural areas had open farmland, forests, moors, lakes, and other habitats with scattered houses and farm buildings that were never continuous.

Thus we selected two study sites at each of four levels of the urbanization gradient (defined by land cover, housing type, and average human density): urban (city center, apartments, 252 ± 143 [average residents per hectare^{-1} ± SD]); dense suburban (detached family housing, 46 ± 23); light suburban (detached family housing, 24 ± 17); and rural (villages, farms, detached family housing, 8.5 ± 5); thus, we had a total of four sites at each level and eight sites per city in order to use the urbanization gradient in our research (Marzluff *et al*., 2001). The distance between urban and rural study sites was 19-32 km and birds will not be prevented from moving between neighboring urban and rural habitats for distance reasons.

Under FID we understand the distance from the intruder to bird, from which a bird takes-off or perform short side fly (Møller, 2008). We determined the FID for six Corvidae species in different types of settlements and biotopes, according to Blumstein (2005, 2006). When determined the FID we choose a single bird, then began to move in its direction. Distance to the bird in the moment of its flight reaction was fixed as flight initiation distance.

Data were collected between 0700 and 1700 hours. We measured FID using the following methods: once a bird was spotted on the ground, the starting distance (distance between the bird and the observer) was recorded by the observer with a laser rangefinder (Stanley TLM 160i), and then it began walking directly at the bird at a steady pace, looking directly at the bird. Once the bird initiated fleeing, the observer stopped and the distance walked was subtracted from the starting distance to give the FID. Finally we recorded the escape behavior (flew, hopped or ran, or walked away). During the study period we performed 690 FID observations of Rook, 363 – of Eurasian jackdaw, 340 – of Hooded crow, 246 – of Eurasian magpie, 140 – of Eurasian jay, and 104 observations of the Common raven.

The statistical processing of data was made by MS Excel and Statsoft Statistica 11.0. Exploratory data analysis was performed before final analyses and any data lacking normality were logarithmically transformed. A one-factor ANOVA was carried out on continuous variables by use of GLM method. The Tukey test was used to test the differences between the means. For the data relating to the FID a Chi-square test was implemented. Summary statistics is presented as means ± one standard deviation, P-values < 0.05 were considered significant.

RESULTS

Distribution of FID of all the species was non-normal (Kholmogorov-Smirnov test, $p \leq 0.01$). The values of FID for Corvidae had high species-specific variation (Table 1) and depend on the type of habitat where the intruder was ($p < 0.001$, Generalized Linear Model Analyses of Variance).

Table 1

Mean values of Corvidae FID in settlements of Zhytomyr area, m

Species	N	M ± SE	Coeff. of variation	Range
Corvus frugilegus	690	8.1±0.2	77.8	0.7-42
Corvus monedula	363	7.2±0.2	59.7	0.5-30
Corvus cornix	340	7.4±0.3	71.6	1.2-37.8
Pica pica	246	11.5±0.5	63.4	3.1-60
Garrulus glandarius	140	14.4±0.8	66.5	4.5-60
Corvus corax	100	43.7±2.5	56.6	12.4-150

The lowest FID was registered for Eurasian jackdaw and the highest – for Common raven. In the conditions of central Ukraine the Eurasian jackdaw is an obligated synanthropic species (Rezanov, 2002; Rahimov, 2011) that is why the moderate values of its FID can testify Eurasian jackdaw adaptation to the factor of human disturbance. The high values of Common raven FID could be explained by its biotope preferences when this species normally exist out of settlements or on their fence surrounding villages, where the degree of human disturbance is insignificant. Therefore, the Common raven did not get used to human presence and consider people as potential danger more than other species.

We ranged the species according to their FID registered in the industrial city (in descending order): Common raven → Eurasian jay → Eurasian magpie → Rook → Hooded crow → Eurasian jackdaw. We found that FID of Corvidae is a variable index, which fluctuates almost by five times for different species. The coefficients of variation of FID of all the species exceed 50 percent that testifies considerable behavioral polymorphism of Corvidae. The highest values of CV were pre-determined for Rook (77.8%) that contributes to the situation when this species substantially prevails by quantity among all other Corvidae species in Zhytomyr area.

We performed a preliminary distribution of Corvidae species due to their human tolerance by hierarchical cluster analysis: high tolerance was typical for a jackdaw, rook, and hooded crow, middle – for Eurasian magpie and Eurasian jay, sub-zero man-impact tolerance was typical for raven, the FID of which are the highest and formed separate clusters on a tree-diagram (Fig. 1).

This suggested that the lower is the distance to human population and the higher is the infrastructure level of settlement development the more tolerant raven birds are to human presence (Table 2). Thus, the degree of birds' tolerance to human presence decreases along with the gradient of anthropogenic transformation of an area or rural-urban ratio.

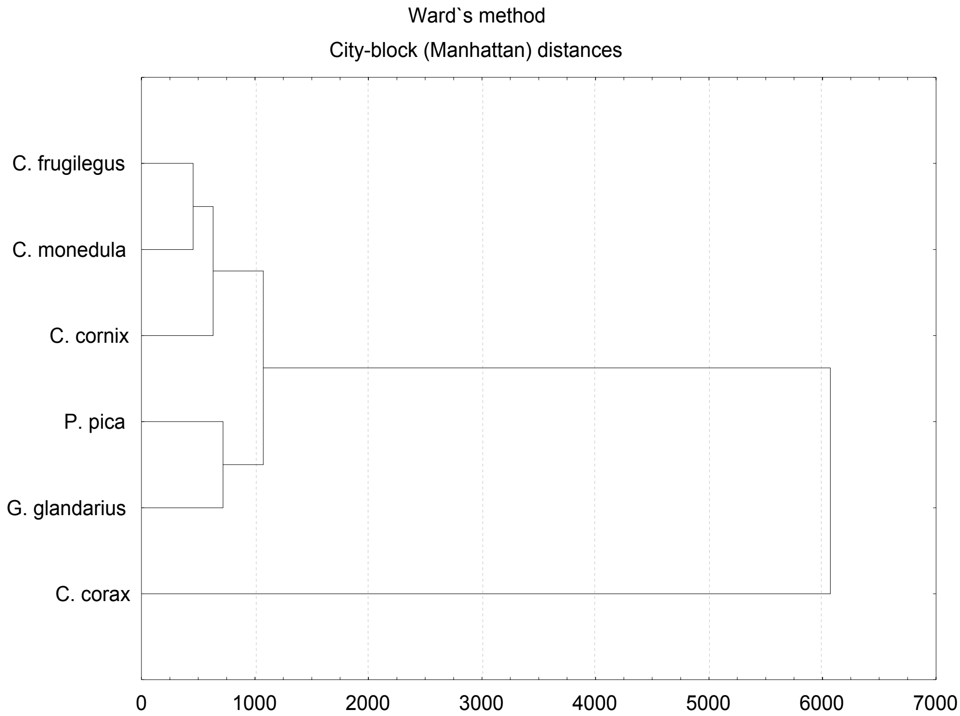

Fig. 1. Tree diagram of similarity of Corvidae FID (results of cluster analysis).

The FID of Corvidae differed greatly between four sites and, highly diminishes between the rural sites and light suburban sites, and light suburban sites and dense subsurban sites (Tukey, P < 0.05 in all cases). The difference in FID between urban and dense suburban areas was less pronounced (Tukey, P < 0.05).

Table 2

Mean, standard deviation and results of one-factor Generalised Linear Model Analysis of Variance for the FID of Corvidae in various types of settlements

Species	**Site**				**P-value**
	Rural	Light suburban	Dense suburban	Urban (Zhytomyr)	
Corvus frugilegus	12.2±0.5	7.3±0.6	6.1±0.5	5.2±0.2	0.001
Corvus monedula	10.5±0.4	6.7±0.4	6.4±0.4	4.9±0.3	0.001
Corvus cornix	10.6±0.7	6.8±0.5	6.2±0.5	5.5±0.3	0.001
Pica pica	14.0±0.8	10.6±0.6	8.9±0.7	8.3±0.4	0.001
Garrulus glandarius	16.3±1.1	11.6±2.2	10.7±1.6	10.3±0.6	0.001
Corvus corax	54.3±4.3	45.2±7.9	–	40.1±3.7	0.001
Sites mean*	12.72±2.5	8.6±3.5	7.66±2.4	6.84±2.3	0.001

* Results for *Corvux corax* were not applied for sites mean value.

DISCUSSION

The results indicate that bird species with a high propensity to disperse tend to decrease their FID more strongly along the urban-rural habitat gradient. This pattern was most apparent in the Rook. Based on FID, Corvidae species were classified as being tolerant to urban environmental conditions, with FID values showing decreasing trends along the urban-rural gradient, as in the Rook. Our results suggest that FID may be the relevant measure for analyzing birds' tolerance to urbanization and for assessing the speed by which species or populations can adjust or adapt to novel environmental conditions.

In general, the differences we found in flight initiation distances between urban and rural areas were similar to those reported in studies in other cities in Europe (Diaz *et al.*, 2013 a, b; Diaz *et al*., 2014; Legagneux & Ducatez, 2013; Leveau, 2013; McDonnell & Hahs, 2013; Møller & Tryjanowski, 2014; Møller, 2015).

European Magpies in Poland also have longer flight initiation distances in rural (36.3 m) than in urban areas (6.4 m; Ochla and Zielona Gora, respectively; Jerzak, 1995), but the difference is not as large as in our case. The exaggerated flight initiation distance in rural Colorado areas compared with those in Poland is likely due to the higher frequency of persecution by humans in rural Colorado. Furthermore, it was reported (Jerzak, 2001; Conole, 2014) that Black-billed Magpies had shorter flight distances in rural areas where they were not persecuted than in areas where they were persecuted (28.9 vs. 64.7 m). These data and our own results suggest that in certain rural areas it is a combination of lower human density (and, thus, less habituation to humans) and human discouragement behavior that increases birds' wariness of humans in comparison with urban areas. Our results suggest that crows adjusted their response depending on the general actions of the human population in the area. Corvidae in rural areas where persecution occurs may thus use a general rule of avoiding any human. The differences between Zhitomir and other European cities in humans' discouraging behavior toward birds across the urbanization gradient were reflected in how wary certain species were of humans. Ths pattern is likely to be replicated across similar gradients of directed discouraging behavior throughout other urban areas and should be considered when dealing with conservation or management of urban species. Tolerance among urban species appears to be related to their degree of habituation to people (McDonnell & Hahs, 2013; McGiffin *et al*., 2013; Shanahan *et al.*, 2014; Sol *et al*., 2014).

Behavioural, physiological and ecological flexibility may contribute to an urban bird's ability to tolerate a broad array of environmental conditions, including disturbed habitat. This flexibility may include traits such as a bird's ability to adjust behaviour in response to novel conditions, to resist detrimental physiological effects of breeding in urban habitat or to use novel resources, such as food types or nest sites. The characteristics of the behaviour, physiology and ecology of urban birds may be keys to their tolerance of a wide array of environments, predisposing them to succeed in human-disturbed habitat (Gendall *et al.*, 2015; McGiffin *et al.*, 2013; Møller & Tryjanowski, 2014; Møller, 2015).

Due to the urban birds had lower stress hormone levels than rural conspecifics. Sol *et al*. (2014) found that relative brain size and frequency of foraging innovations in these birds were positively correlated with a measure of potential for successful invasion into novel habitat.

CONCLUSIONS

We registered that FID of rural birds was almost twice as much compared to the urban populations; moreover, the latter had higher variation. Urban populations had consistently shorter flight distances than nearby rural populations, with the mean estimate being 6.8 m for urban birds and 12.7 m for rural birds, or an almost two-fold difference.

Thus, the FID of all studied Corvidae species appropriately reduces in transition from the less urbanized to more urbanized landscape. It confirms that birds are able to adapt successfully to human presence of man and could be used as model objects for the study of processes of synanthropization.

The results indicate that bird species with a high propensity to disperse tend to decrease their FID more strongly along the urban-rural habitat gradient. This pattern was most apparent in the Rook. Based on FID, Corvidae species were classified as being tolerant to urban environmental conditions, with FID values showing decreasing trends along the urban-rural gradient, as in the Rook. Our results suggest that FID may be the relevant measure for analyzing birds' tolerance to urbanization and for assessing the speed by which species or populations can adjust or adapt to novel environmental conditions.

REFERENCES

BLUMSTEIN D.T., 2005, *Flush early and avoid the rush: a general rule of antipredator behavior?* Behavioral Ecology, **21**: 440-442.

BLUMSTEIN D.T., 2006, *Developing an evolutionary ecology of fear: how life history and natural history traits affect disturbance tolerance in birds.* Animal Behavior, **71**: 389-399.

CONOLE L.E., 2014, *Degree of adaptive response in urban tolerant birds shows influence of habitat-of-origin.* Peer Journal, **2**: e306.

COOKE A.S., 2003, *Observation on how close certain passerine species will tolerate an approaching human in rural and suburban areas.* Biological Conservation, **18** (2): 85-88.

COOPER W.E., 1999, *Escape behavior by prey blocked from entering the nearest refuge.* Canadian Journal of Zoology, **77**: 671–674.

DIAZ M., CUERVO J.J., FLENSTED-JENSEN E., GRIM T., IBANEZ-ALAMO J.D., JOKIMAKI J., MARKY G., TRYJANOWSKI P., MOLLER A.P., 2013 a, *The geography of fear: a latitudinal gradient in anti-predator escape distances of birds across Europe.* Plos One, **8**: e64634.

DIAZ M., CUERVO J.J., FLENSTED-JENSEN E., GRIM T., IBANEZ-ALAMO J.D., JOKIMAKI J., MARKУ G., TRYJANOWSKI P., MOLLER A.P., 2013 b, *Population trends and flight initiation distance in different European countries.* Plos One, **8**: e64634.

DIAZ M., CUERVO J.J., GRIM T., FLENSTED-JENSEN E., IBANEZ-ALAMO J.D., JOKIMAKI J., MARKO G., TRYJANOWSKI P., MOLLER A.P., 2014, *Interactive effects of fearfulness and geographical location on bird population trends.* Behavioral Ecology, **26** (3): 716-721.

FERNANDEZ-JURICIC E., SCHROEDER N., 2003, *Do variations in scanning behavior affect tolerance to human disturbance?* Animal Behavior Science, **3**: 219-234.

GENDALL J., LILL A., BECKMAN J., 2015, *Tolerance of disturbance by humans in long-time resident and recent colonist urban doves.* Avian Research, **6** (7): 1-8.

JERZAK L., 1995, *Breeding ecology of an urban Magpie Pica pica in Zielona Gora (SW Poland).* Acta Ornitologica, **29** (3): 123-133.

JERZAK L., 2001, *Synurbanization of the magpie in the Palearctic.* Avian ecology and conservation in an urbanizing world, 403-425.

JOKIMÄKI J., KAISANLAHTI-JOKIMÄKI M.L., SORACE A., FERNÁNDEZ-JURICIC E., RODRÍGUEZ-PRIETO I., 2005, *Evaluation of the "safe nesting zone" hypothesis across an urban gradient: a multi-scale study.* Echography, **28**: 59-70.

KELIN E.A., SPIRIDONOV S.N., 2009, *Human tolerance of Eurasian Jackdaw in urban conditions*. In: *Ecology, evolution, and systematic of animals.* Riazan: 219-220

LEGAGNEUX P., DUCATEZ S., 2013, *European birds adjust their flight initiation distance to road speed limits.* Biological Letters, **9**: 20130417.

LEVEAU L.M., 2013, *Bird traits in urban–rural gradients: how many functional groups are there?* Journal of Ornithology, **154**: 655-662.

MARZLUFF J.M., BOWMAN R., DONNELLY R., 2001, *A historical perspective on urban bird research: trend, terms, and approaches.* In: Marzluff J.M., Bowman R., Donnelly R. (Eds.), *Avian Ecology and Conservation in an Urbanizing World.* New York, Kluwer: 20-47.

McDONNELL M.J., HAHS A.K., 2013, *The future of urban biodiversity research: moving beyond the low-hanging fruit.* Urban Ecosystems, **16**: 397-409.

MCGIFFIN A., LILL A., BECKMAN J., JOHNSTONE C.P., 2013, *Tolerance of human approaches by common Mynas along an urban–rural gradient.* Emu, **113**: 154-160.

MØLLER A.P., 2008, *Flight distance of urban birds, predation and selection for urban life.* Behavioral Ecology Sociobiology, **63** (1): 63-75.

MØLLER A.P., 2010, *Interspecific variation in fear responses predicts urbanization in birds.* Behavioral Ecology, **21**: 365-371.

MØLLER A.P., 2015, *Birds.* In: Cooper W.E. Jr., Blumstein D.T. (Eds.), *Escaping from predators: an integrative view of escape decisions and refuge use.* Cambridge, Cambridge University Press.

MØLLER A.P., IBÁÑEZ ÁLAMO J.D., 2012, *Escape behaviour of birds provides evidence of predation being involved in urbanization.* Animal Behavior, **84**: 341-348.

MØLLER A.P., TRYJANOWSKI P., 2014, *Direction of approach by predators and flight initiation distance of urban and rural populations of birds.* Behavioral Ecology, **25** (4): 960-966.

MØLLER A.P. *et al*., 2013, *Change in flight initiation distance between urban and rural habitats following a cold winter.* Behavioral Ecology, **24**: 1211-1217.

MØLLER A.P., DÍAZ M., FLENSTED-JENSEN, E., GRIM T., IBÁÑEZ-ÁLAMO J.D., 2012, *High urban population density of birds reflects their timing of urbanization.* Oecologia, **170**: 867-875.

RAHIMOV I.I., 2011, *Pred-adaptive bird features towards settlement in urban environment.* Bulletin of Baltic Federal University, **7**: 79-84.

REZANOV A.A., 2002, *Towards estimation of flight initiation distance*. In: *Ecology of Corvidae and urban landscapes.* Saransk: 112-123.

REZANOV A.A., 2006, *Human tolerance as a criterion for bird synanthropization estimation.* In: *Ornithological Research in Northern Eurasia.* Stavropol: 431-433.

SHANAHAN D.F., STROHBACH M.W., WARREN P.S., FULLER R.A., 2014, *The challenges of urban living.* In*:* Gil D., Brumm H. (Eds). *Avian urban ecology: behavioural and physiological adaptations*. Oxford, Oxford University Press: 3-20.

SOL D., GONZÁLEZ-LAGOS C., MOREIRA D., MASPONS J., LAPIEDRA O., 2014, *Urbanisation tolerance and the loss of avian diversity*. Ecology Letters, **17**: 942-950.

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EFFECT OF MODIFIED ATMOSPHERE STORAGE ON ADULTS CONTROL OF *SITOPHILUS GRANARIUS* (L.) AND *SITOPHILUS ZEAMAIS* (L.) INSIDE PACKED FOODSTUFFS

MOHAMMAD NATEQ GOLESTAN*, ALI ASGHR POURMIRZA**

Food packaging plays an important role in protecting foodstuffs from contaminations such as insects. Packaging wrappers are an essential factor in preventing the penetration and spread of stored product pests within packed foodstuffs. Wrappers used in this study were resistant to insect penetration and permeable for gases and, therefore, were appropriate to fumigation of packed foodstuffs. Experiments were performed with two weevils species, including *Sitophilus granarius* (L.) and *Sitophilus zeamais* Motschulsky, two foodstuffs, including wheat and rolled oats, four wrappers, including biaxially oriented polypropylene films (BOPP) with 40 and 80µm width, woven polypropylene perforated (WPP), non-woven polypropylene (NWPP) and 4 gas mixture of carbon dioxide and ozone, including 40% CO_2, 40% CO_2 + 50 ppm O_3, 40% CO_2 + 100 ppm O_3, 40% CO_2 + 150 ppm O_3. The result showed that mean mortality of maize weevil in gas mixture and wrapper treatments was more than wheat weevil at 0.05 level. More mortality was found in insects placed in wheat compared with rolled oats. In the wheat foodstuff, the packages made of BOPP film with 40 and 80µm width established most mortality. But in rolled oat's foodstuff, mortality in these packages was the least. In the rolled oat's foodstuff, most mortality performed in package made of non-woven polypropylene (Spunbond). Another result showed that adding 100 and 150 ppm O_3 to 40% CO_2, increased mean mortality of weevils compared with just 40% CO_2. Furthermore, it was found an interaction effect Gas mixture × Wrapper in the wheat foodstuff while this effect was not observed in rolled oats.

Key words: fumigation, ozone, *Sitophilus*, packaging, wrapper.

INTRODUCTION

Three species of *Sitophilus, S. granarius* (L.), *S. oryzae* (L.), and *S. zeamais* Motschulsky (Coleoptera: Curculionidae) are seed parasites of cereal crops such as wheat, corn, rice and sorghum. Furthermore, they are important economic pest species in stored grain (Campbell, 2002). Among these species, the maize and the rice weevils are particularly important in warmer climates (Correa *et al*., 2013) and maize weevil is one of the most serious cosmopolitan pests of stored cereal grain, especially of maize (*Zea mays* L.) in tropical and sub-tropical regions (Danho *et al*., 2002).

Food packaging as one of the most important parts of food industry is related with food security. Food packaging provides not only a method for transporting food safely, but it also extends the product's self-life via preventing from harmful bacteria, contamination and degradation (Chin, 2010). Although packaging can be security for food product, insect can enter into the goods during transportation, storage in the warehouse, or in retail stores, and also it is possible that the initial contaminants develop and destroy foodstuffs (Hou *et al*., 2004). Therefore, choice of wrapper that has a high resistance to insect penetration is inevitable. Furthermore, because it may be contaminated by stored product pests, the wrappers that have good permeability to gases can be suitable for fumigation during storage and transport.

Highland & Wilson (1981) believe that polypropylene has a higher resistance than polyethylene to insect penetration (with equal thickness). Bowditch (1997) found that the polypropylene film tested was resistant to penetration by 1st-instar larvae of *Ephestia cautella* (Walker). From 4 kinds of used polymers including polyethylene, polypropylene, polyvinylchloride and cellophane, polypropylene had the least permeability to insect and the pests were unable to penetrate this polymer and if penetration occurred, it was less (Allahvaisi *et al*., 2010). On the other hand, in recent years, especially biaxially oriented polypropylene films (BOPP) have become one of the most popular high-growth films in the world market (Lazic *et al*., 2010). Also in the study on BOPP 80 μm films it was expressed that BOPP films without holes and with the maximum number of micro-holes were the most suitable for controlling of *Tribolium confusum* Jacquelin du Val. in alive and non-alive foodstuffs respectively (Nateq Golestan *et al.*, 2015).

Sacks made from woven polypropylene are replacing jute sacks for commodity storage in developing countries. Woven polypropylene (WPP) sack manufacture was developed in Japan in the late 1960s and was quickly adopted in Europe, South Africa, Australia and North America. These sacks are lighter and relatively stronger than jute (Kennedy & Devereau, 1994). Non-woven polypropylene (Spunbonded) bags made of synthetic polymers were commercialized by the technology of Freudenberg (Germany) and Du Pont (USA) in the 1950s and 1960s. Many polymers, including polypropylene, polyester, polyethylene, polyamide, polyurethane, etc. are used in the spunbond process. Among various polymers, isotactic polypropylene (PP) is the most widely used polymer for spunbond non-woven production. Non-woven products made by using the spunbond process are used in different industries such as packaging (Lim, 2010). Food packaging with non-woven wrappers is developing and now is used for packaging products such as rice. This wrapper has a high permeability to gases and vapor.

Between used gases in modified atmospheres (MAs), CO_2 gas, is relatively safe to use, leaves no known residues, and readily penetrates packages of tightly compressed commodities (Keever, 1988). Bera *et al*. (2004) reported that carbon dioxide based MAs may be considered as one of the best alternatives to toxic and residue-building chemical fumigants for treatment of storage pests (Ahmdani, 2009). Ozone, a powerful oxidant, has numerous beneficial applications and is very familiar to the food processing industry. This gas has regulatory acceptance by the Food and Drug Administration (USA) (FDA 2001), and the Environmental Protection Agency's (USA) MSDS defines it as "pure air" (Mason *et al*., 2006).

When a product is packaged, contamination or initial contamination may be developed. Percentage of insect's penetration and contamination development also can depend on the type of packaging material. Therefore, finding the best wrapper for packaging is inevitable. This study offers a new model according to MAs called Gas-permeable Packaging (GPP) that can prevent insect's penetration into or out of the packaging a lot, and also has a good permeability to gases for eliminating probable initial and secondary contaminations during the supply process. It is notable that the current, the permeable packaging is used for fresh produce (Winotapun *et al*., 2010) and cold and hot bakery products (Yam, 2009).

MATERIAL AND METHODS

This study was carried out at Department of Plant Pest and Disease, Razavi Khorasan Research Center for Agriculture and Natural Resources during the years 2012-2013. Different concentrations of O_3 and CO_2 gases were tested on packages made of 4 wrappers, including 2 BOPP films with 40 and 80µm width, woven polypropylene wrapper laminated/perforated (WPP-L/P) and non-woven poly-propylene fabric (NWPP) filled with wheat and rolled oats. The gas mixture included 40% CO_2, 40% CO_2 + 50 ppm O_3, 40% CO_2 + 100 ppm O_3, 40% CO_2 + 150 ppm O_3.

Insects

The maize and the wheat weevils were collected from 2 silos in Mashhad (36°20'N 59°35'E), a city in Iran. Cultures were established and maintained on healthy uncontaminated food at 25 ± 2ºC and 65 ± 10% r.h. in plastic bottles and were closed with pieces of muslin cloth fixed by rubber bands. Rearing medium used was composed of corn and wheat soaked. All insects were cultured under moderately crowded conditions to ensure proper development and equal size of the resultant adults.

Supply of gases

Ozone gas was generated by ozone Generator, Ozonica series, Oz 100 models (www.ozoneab.com), that generate 100 gram/hour ozone from purified oxygen with 4 reactors. Purified oxygen produced by oxygen generator, LFY-I-5F-W model, provided by Longfei Group Co. Ltd., which produce purified oxygen 93% ± 3% with flow rate 0-5 L/min. Specified O_3 concentration was measured based on the volume of the chamber and the generator default. A local factory supplied CO_2 gas needed inside cylinders of 40 kg with 99.9% purity.

Wrappers

Woven polypropylene wrapper laminated/perforated was taken from Kabir Industrial Group located in Tehran, Iran and made of 95% PP + 2% PE + 2% $CaCO_3$ + 1% Color material and perforated by needle rollers with a distance 5 mm from each other. Non-woven polypropylene fabric was taken from Baftineh Ltd. located in Tehran, Iran and made from 100% PP with 90 gram/m^2 and white color. BOPP film rolls with 40 µm width were taken from Poushineh Industrial Group located in Tehran, Iran. We laminated 2 BOPP film rolls with 40µm width together and produced film 80µm.

At first, the packages 20×30 cm were filled with 1 kilogram of wheat and rolled oats separately. Then a cage (10 × 10 cm) containing 40 insects and 3 g food was entered into each package and sealed with a plastic press machine. Subsequently, packages were transferred into chamber 70×120×180 cm and placed horizontally at the bottom of it and the chamber was closed tightly. Afterwards, CO_2 gas (CO_2 cylinder with purity 99.9%) was injected into the upper left, and air exited from the bottom right until concentration of CO_2 was 40% and in the final step, we injected O_3 gas daily at a specified time and on reaching the specified concentration, ozone injection was stopped. A total of 7 injections with equal doses during 7 d were performed. During CO_2 injection and until 1 hour after O_3 injection, the system was circulated. During experiments, upper surface of packages was exposed to chamber atmosphere. Exposure period was considered 7 d at 25 ± 2°C, 35 ± 5% r.h. After exposure period, the specimens were transferred to a clean jar containing 3 g of food with the same condition. Mortality rates of the insects were recorded 6 h after termination of the treatment. Each test was replicatd 3 times on different days, and results were pooled.

Bioassay

In this experiment, we used adults of *Sitophilus granarius* and *S. zeamais* 7 ± 2 days old. Preliminary dose-mortality tests were carried out prior last experiment to determine a range of doses that produce 25 to 75% mortality at the lowest and the highest doses, respectively (Robertson *et al*., 2007). In ultimate experiment we compared average mortality in two foodstuffs and two *Sitophilus* species separately by independent sample's t-test and also analyzed mean mortality in gas mixture and wrapper treatments together by factorial experiment (4×4) in the completely randomized design. Comparison of the average mortality rates was performed by Tukey's test separately. All of data were analyzed with the Statistical Package for the Social Science (SPSS) software (SPSS Inc., 2007). First, mortality rates of various treatments were adjusted with Abbott's formula and then, for variance normalization of data; Arcsin square root of data was used in estimations.

RESULTS

In this study, mortality of *S. granarius* (0.9446 ± 0.03521) was lesser as compared to *S. zeamais* (1.0905 ± 0.03061) at 0.05 level, because, presumably, activity of *S. granarius* is less as compared to *S. zeamais*. Lesser active possesses less metabolic rate, lower respiration and less intake of fumigant (Ahmdani, 2009). Furthermore, mortality of weevils in the wheat foodstuff was (1.2580 ± 0.02860) more than rolled oat's foodstuff (0.7771 ± 0.01594) at 0.05 levels that seems respiration of wheat seeds and increasing CO_2 concentration during the period of fumigation to be the reason. The factorial experiment showed that both the gas mixture and wrapper factors have a significant effect on mortality at 0.05 level. In the wheat foodstuff, the interaction of the 2 factors (gas mixture × wrapper) was found while this effect was not found in rolled oats. The reason of this event should be followed in the foodstuff respiration rate and permeability value of wrapper. Totally, ANOVA test related to *S. granarius* in wheat explained 79% of the variance of mortality based on the independent variables. In this test, the gas mixture factor attained the largest effect size (ω^2= 0.39) and the interaction could explain 8% of the variance. About *S. zeamais* in wheat, ANOVA test explained 93% of the variance of mortality that the effect size of interaction was 22%. Conversely, statistics of squared Omega square (ω^2) obtained in the ANOVA test *S. granaries* and *S. zeamais* in rolled oats were nearly zero in interaction factors (Table 1).

Table 1

Factorial experiments for Gas mixture and Wrapper factors at insect and foodstuff treatments separately

S. V	*S. granarius* **in** wheat				*S. zeamais* **in** wheat				*S. granarius* **in** rolled oats				*S. zeamais* **in** rolled oats			
	df	MS	F	ω^2	df	MS	F	ω^2	df	MS	F	ω^2	df	MS	F	ω^2
Gas mixture (a)	3	0.431	37.32**	0.39	3	0.454	83.78**	0.32	3	0.071	28.88**	0.24	3	0.068	27.02**	0.47
Wrapper (b)	3	0.359	31.06**	0.32	3	0.555	102.42**	0.39	3	0.182	73.86**	0.63	3	0.038	15.26**	0.26
Gas × Wrapper	9	0.039	3.39**	0.08	9	0.110	20.22**	0.22	9	0.001	0.49 n.s.	–	9	0.001	0.53 n.s.	–
Error	32	0.012			32	0.005			32	0.002			32	0.003		
Total	47				47				47				47			
Ωa/Ωb				1.21				0.82				0.38				1.80

n.s ρ is not significant; ** ρ is significant at 0.01 level

Ωa/Ωb is equal to the ratio of gas mixture superiority to wrapper in mortality

Grouping mean mortality was performed for gas mixture and wrapper treatments separately by Tukey's test. The result showed that adding 50 ppm O_3 to the 40% CO_2 did not have a significant effect on the mortality rate in any of the 2 weevil species located in 2 types of foodstuffs (Table 2). It would be due to the different behavior of BOPP films with WPP and spunbond wrappers filled with wheat that adding 50 ppm ozone to carbon dioxide increased mortality in packages made of BOPP films but decreased mortality in packages made of WPP and NWPP wrappers (Fig. 1). In packages filled with rolled oats, similar behavior in all of wrappers was observed (Fig. 2). Mortality of weevils located in both of foodstuffs in 40% CO_2 + 100 ppm O_3 was more than 40% CO_2 + 50 ppm O_3. As a result, all of wrapper showed similar behavior increasing (Table 2). The most mortality rate occurred in the 40% CO_2 + 150 ppm O_3, and the least mortality was obtained in the 40% CO_2 and 40% CO_2 + 50 ppm O_3.

Table 2

Grouping arcsin $\sqrt{x}$ mean of mortality for gas mixture treatment

Gas mixture	N	*S. granarius* in **wheat**	*S. granarius* in **rolled oats**	*S. zeamais* in **wheat**	*S. zeamais* in **rolled oats**
40% Co_2	12	1.0189a	.6055a	1.1311a	.8206a
40% Co_2 + 50 ppm O_3	12	1.1256a	.6104a	1.1286a	.8300a
40% Co_2 + 100 ppm O_3	12	1.3256b	.7106b	1.3928b	.8949b
40% Co_2 + 150 ppm O_3	12	1.4378b	.7617b	1.5173c	.9834c
Std. Error		.03103	.01432	.02125	.01445
ρ is significant at 0.05 level					

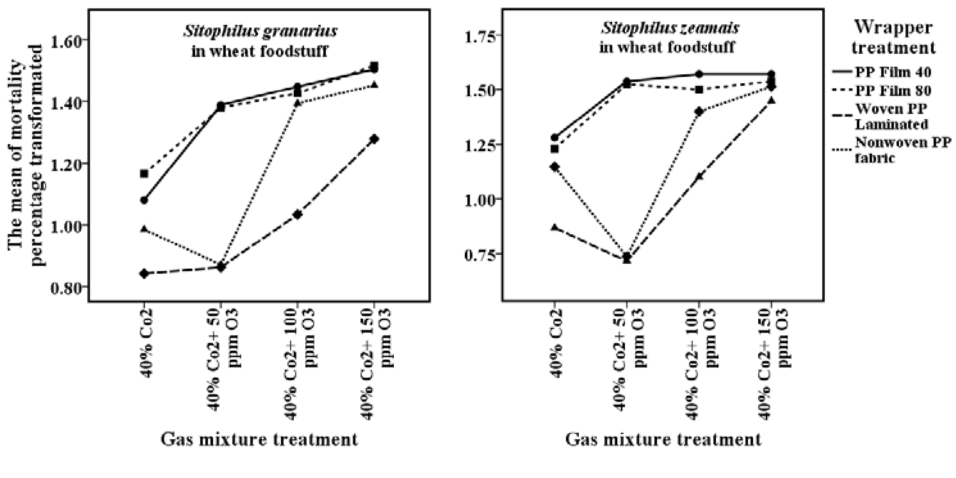

Fig. 1. Comparison of mean mortality in the wheat foodstuff in different gas mixtures under wrappers.

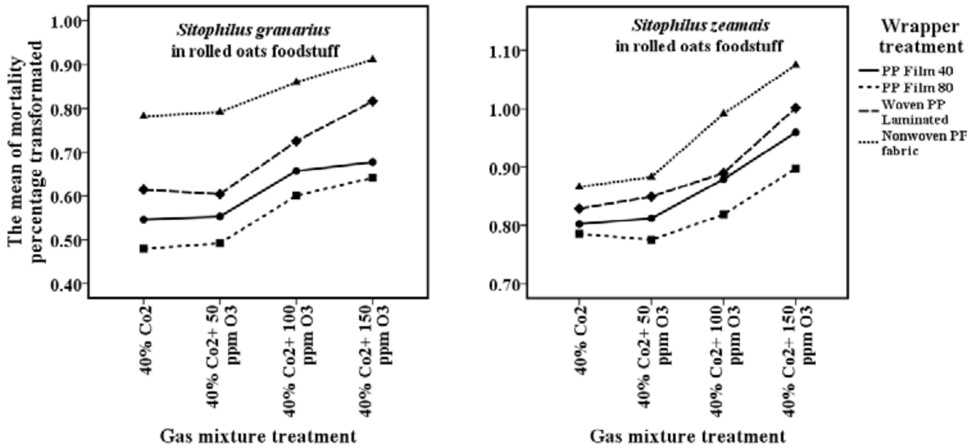

Fig. 2. Comparison of mean mortality in the rolled oats foodstuff
in different gas mixtures under wrappers.

Observations proved that the mean mortality in the wheat foodstuffs in packages made of BOPP films with 40 and 80 µm widths were equal and highest (Table 3) and this mortality reduced in WPP packages strongly and then, increased in spunbond bags considerably (Fig. 3). Accordingly because of the economic value and more flexibility, film 40 µm can be preferred. Although it should be considered that the film 80 µm resistance to insect penetration was more than film 40 µm. Arrangement of morality mean in the rolled oat foodstuff was in packages made of film 80 µm< film 40 µm< WPP wrapper< non woven PP fabric (Fig. 4). In wheat foodstuff, arrangement was WPP wrapper< Nonwoven PP fabric< films 40 and 80 µm (Table 3).

Table 3

Grouping arcsin $\sqrt{x}$ mean of mortality for wrapper treatment

Wrapper	N	***S. granarius*** in **wheat**	***S. granarius*** in **rolled oats**	***S. zeamais*** in **wheat**	***S. zeamais*** in **rolled oats**
PP Film 80	12	1.372c	.553a	1.447c	.819a
PP Film 40	12	1.355c	.608b	1.490c	.863ab
Woven PP-L/P	12	1.004a	.690c	1.033a	.892b
Nonwoven PP fabric	12	1.176b	.836d	1.199b	.954c
Std. Error		.03103	.01432	.02125	.01445

ρ is significant at 0.05 level

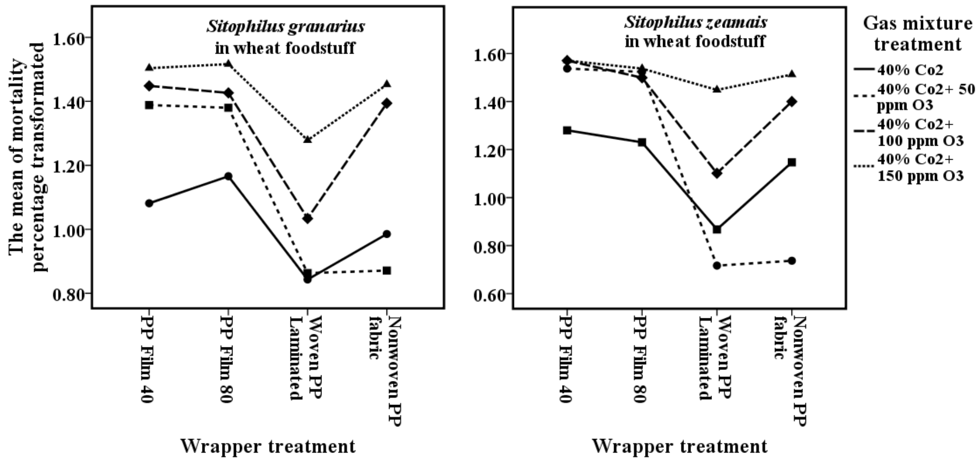

Fig. 3. Comparison of mean mortality in the wheat foodstuff in different wrappers under gas mixtures.

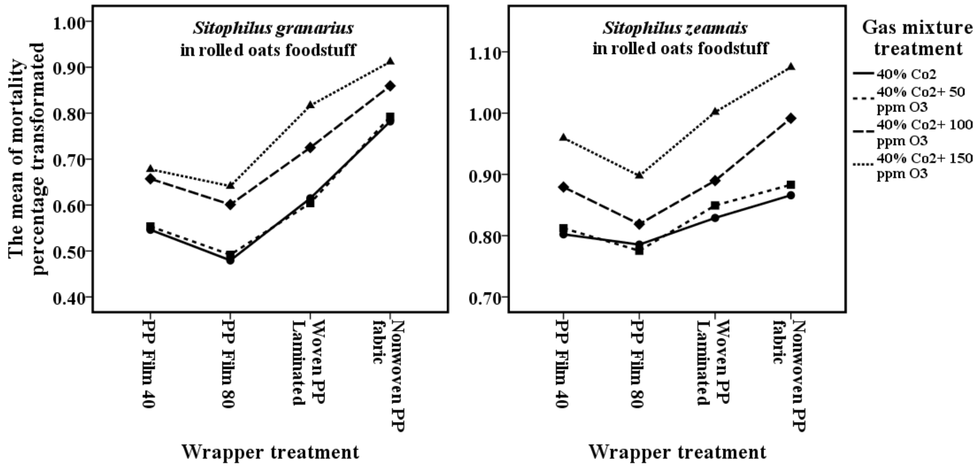

Fig. 4. Comparison of mean mortality in the rolled oats foodstuff in different wrappers under gas mixtures.

CONCLUSIONS

Two types of foodstuffs used are from the main stored products. Wheat grains are alive and breathing and other foodstuffs are not alive. Research shows that the seed respiration led to an increase in the CO_2 concentration within sealed packages, and this is an important factor influencing mortality (Moreno *et al*., 1991). And so, wheat respiration increased CO_2 concentration in the packages, and this condition led to elevation of pest mortality. Accordingly, it can be concluded that in alive products, packaging films with low permeability are proper for fumigation. Our results confirmed this assumption and BOPP film with thickness of 80 µm with the lowest permeability to gas was the most appropriate in wheat. On the other hand, in non-alive products such as rolled oats, because there is no respiration and no bio-increasing in the amount of gases within the packages, pest mortality was almost exclusively influenced by chamber atmosphere and with elevating CO_2 and O_3 concentrations, increased mortality was followed. Therefore, non-woven polypropylene fabric was suitable. BOPP film (80 µm) and non-woven polypropylene fabric showed the highest and lowest penetration resistance to insects, respectively.

About ozone gas, the results indicate that its decomposition on the grain surface occurs in two phases. In the first phase, due to the high interaction with the grains surface, the penetration rate is low and in the second phase, movement through the grain is rapid with very little impedance (Kells *et al.*, 2001; Dos Santos *et al*., 2007). For this reason, we used low doses of O_3 intermittently to achieve minimum damage to the product and maximum performance on the pest control. In this study, for control of weevils within foodstuffs packages in the chamber, in the case of rolled oats were applied only O_3 and CO_2 gases injected into chamber and in the case of wheat apart from the injected gases, an additional CO_2 gas produced by the respiration of wheat grains within the packages influenced on the pest control.

The results showed that in the wheat foodstuff (alive), the mortality of the adult weevils in the packaging with low permeability was more compared with packaging with high permeability. Conversely, in the rolled oat foodstuff (non-alive), this mortality in the packaging with low permeability was less compared with packaging with more permeability. Accordingly, we can hypothesize that a suitable model for packaging of alive foodstuffs is Low Gas-permeable Packaging (LGPP) and for packaging of non-alive foodstuffs, the High Gas-permeable Packaging model (HGPP) is an appropriate option. The results of Nateq Golestan *et al*. (2015) is also extremely consistent with this hypothesis.

Overall, it can be concluded that a mixture of O_3 gas was an appropriate treatment for control of weevils and use of O_3 gas with safe concentrations intermittently, and with specified intervals, can reduce the CO_2 concentrations used in modified atmospheres. Furthermore, the obtained mixture due to the use of two controlling agents can reduce the development of pest resistance compared to use of them separately. In addition, respiration of foodstuffs and permeability of wrapper can be factors influencing mortality.

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REFERENCES

AHMDANI M.S., 2009, *Phytosanitary Management of Trogoderma granarium Everts with Methyl Bromide Alternatives to Ensure Food Security and Safety*. Ph.D. thesis, Arid Agriculture University Rawalpindi, Pakistan, 427 pp.

ALLAHVAISI S., POURMIRZA A.A., SAFARALIZADEH M.H., 2010, *Control of Callosobruchs maculatus (Coleoptera: Bruchidae) in industry of packaging foodstuffs*. Romanian Journal of Biology and Zoology, **55** (2): 167-176.

BOWDITCH T.G., 1997, *Penetration of Polyvinylchloride and Polypropylene Packaging Films by Ephestia cautella (Lepidoptera: Pyralidae) and Plodia interpunctella (Lepidoptera: Pyralidae) larvae and Tribolium confusom (Coleoptera: Tenebrionidae)*. Journal of Economic Entomology, **90** (4): 1028-1031.

CAMPBELL J.F., 2002, *Influence of Seed Size on Exploitation by Rice Weevil*, *Sitophilus oryzae*. Journal of Insect Behavior, **15** (3): 429-445.

CHIN A., 2010, *Polymers for Innovative Food Packaging.* Worcester Polytechnic Institute, Massachusetts Press, 55 pp.

CORREA A.S., DE OLIVEIRA L.O., Braga L.S., Guedes R.N.C., 2013, *Distribution of the related weevil species S. oryzae and S. zeamais in Brazil*. Journal of Insect Science, **20** (6): 763-770.

DANHO M., GASPER C., HAUBRUGE E., 2002, *The impact of grain quantity on the biology of Sitophilus zeamais Motschulsky (Coleoptera: Curculionidae): oviposition, distribution of eggs, adult emergence, body weight and sex ratio.* Journal of Stored Product Research, **38**: 259-266.

DOS SANTOSE J.E., MARTINS M.A., FARONI L.A., DE ANDRADE M.P., CARVALHO M.C.S., 2007, *Ozonization Process: Saturation time, Decomposition Kinetics and quality of Maize Grains (Zea mays L.)*. Proceedings of IOA Conference and Exhibition, Valencia, Spain, 5.5: 1-6.

HIGHLAND H.A., WILSON R., 1981, *Resistance of polymer films to penetration by lesser grain borer and description of a device for measuring resistance*. Journal of Economic Entomology, **74**: 67-70.

HOU X., FIELDS P., TAYLOR W., 2004, *The effect of repellents on penetration into packaging by stored-product insects*. Journal of Stored Product Research, **40**: 47-54.

KEEVER D.W., 1988, *Use of carbon dioxide to disinfest a tobacco warehouse of the cigarette beetle*. Journal of Agricultural Entomology, **6** (1): 43-51.

KELLS S.A., MASON L.J., MAIER D.E., WOLOSHUK C.P., 2001, *Efficacy and fumigation characteristics of ozone in stored maize*. Journal of Stored Product Research, **37**: 371-382.

KENNEDY L., DEVEREAU A.D., 1994, *Observations on large-scale outdoor maize storage in jute and woven polypropylene sacks in Zimbabwe.* Proceedings of 6th International Working Conference on Stored Product Protection, Canberra, Australia, 290-295.

LAZIC V.L., BUDINSKI-SIMENDIC J., GVOZDENOVIC J.J., SIMENDIC B., 2010, *Barrier Properties of Coated and Laminated Polyolefin Films for Food Packaging*. Acta Physica Polonica A, **117** (5): 855-858.

LIM H., 2010, *A Review of Spun bond Process*. Journal of Textile and Apparel Technology and Management, **6** (3): 1-13.

MASON L.J., WOLOSHUK C.P., MENDOZA F., MAIER D.E., KELLS S.A., 2006, *Ozone: A new control strategy for stored grain*. Proceedings of 9th International Working Conference on Stored Product Protection, São Paulo, Brazil, 904-907.

MORENO E., REYES M.M., NIETO Z., RAMIREZ J., 1991, *Effect of hermetic storage on the quality of maize for tortillas*. Turrialba, **41**: 528-533.

NATEQ GOLESTAN M., GHOSTA Y., POURMIRZA A.A., VALIZADEGAN O., 2015, Study *on laser perforated films as gas permeable packaging for confused flour beetle (Tribolium confusum Jacquelin du Val.) control inside food packaging*. Journal of Stored Products Research, **60**: 54-59.

ROBERTSON J.L., RUSSELL R.M., PREISLER H.K., SAVIN E., 2007, *Pesticide bioassays with arthropods*. CRC Press, Boca Raton, Florida, 201 pp.

SPSS Inc., 2007, *SPSS for windows user's guide release 16*. SPSS Inc. Chicago, IL.

WINOTAPUN C., KERDDONFAG N., CHINSIRIKUL W., 2010, *Nd: YAG Laser perforation of Plastic Films for Enhanced Breathability*. Advanced Materials Research, 93-94: 79-82.

YAM K.L., 2009, *The Wiley Encyclopedia of packaging technology*, John Wiley and Sons, Lnc, Publication, 1366 pp.

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HISTOLOGICAL STRUCTURE OF VISUAL SYSTEM IN CASPIAN KUTUM (*RUTILUS FRISII KUTUM*) LARVAE AND FINGERLING

ZAHRA KHOSHNOOD

Visual system is one of the most important sense organs in freshwater and marginal seawater fishes. This system plays a role in nutrition, predation, reproduction etc., and it is one of the first organs which develops in fish embryos. Caspian kutum, *Rutilus frisii kutum*, is an economically and biologically important fish species of the Caspian Sea which millions of its fingerlings are reproduced and released into the Caspian Sea by Iran's government for stock recruitment annually. To investigate the normal structure of visual system in early life stages of *R. frisii kutum*, the newly hatched larvae and fingerlings of the releasing age were studied using histology. Results showed that the visual system in larvae and fingerling consists of slightly big round eyes, with flat cornea without eyelids. The general structure of eye was similar to the general structure of higher vertebrate eyes with the striking feature of the presence of a rete capillary system in the posterior parts of the eyes.

Key words: *Rutilus frisii kutum*, eye, histology.

INTRODUCTION

Vision has an important role in early life stages of the fish. Vision accuracy and specification develops during the ontogeny (Otten, 1981; Blaxter, 1986; Fernald, 1990; Miller *et al*., 1993). These changes in vision could lead to better avoidance from predators, due to better vision of the predator (Batty, 1989; Blaxter & Fuiman, 1990; Gamble & Fuiman, 1987) and increases finding of food and finally ended up in increasing the survival rate in early life stages. Caspian kutum, *Rutilus frisii kutum*, is one of the economically and ecologically important fish species of the Caspian Sea. Millions of its fingerlings were released into the Caspian Sea by Iran's government for stock recruitment, annually.

Knowing the normal structure of the body organs and physiology of the fish in the early life history could be beneficial for better handling in reproduction and culture facilities and also for every stock recruitment activities. Due to the lack of information on Caspian kutum body and tissue structure, the present study was conducted to investigate the normal histological structure of the vision system of the Caspian kutum larvae and fingerling.

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MATERIAL AND METHODS

The larvae and fingerlings of Caspian kutum (*Rutilus frisii kutum*) were obtained from Shahid Ansari Fish Proliferation and Culture Center, Rasht, Iran, in July 2011. Mean total length and mean body weight of fingerlings were 3.5 cm and 2.6 g respectively. For histological studies, fish were euthanized in 100 mg/L of MS222 and 100 mg/L of sodium bicarbonate and immediately immersed into Bouin's fixative for 24 hours, washed and dehydrated in an ascending series of ethanol for embedding in Paraffin (Merck). Following embedment in Paraffin (Merck), transversal and longitudinal sections of 6 μm were cut on a Leica RM2255 microtome and collected on glass slides and stained with Haematoxylin and Eosin (Khoshnood *et al*., 2010) and finally observed by light microscopy.

RESULTS

General structure of the eyes in Caspian kutum larvae was made up of slightly big round eyes with flat cornea without eyelids and there was a rete choroid at the posterior side of each eye (Fig. 1).

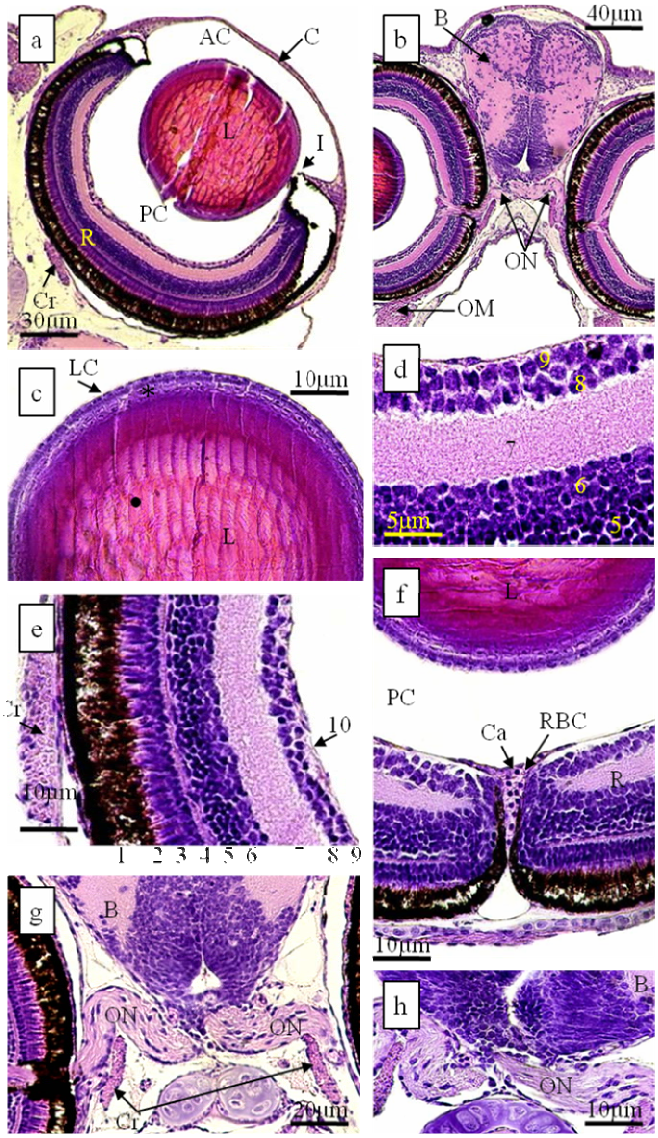
Each eye consists of three layers: 1) external layer or fibrous corneoscleral coat which consists itself of two parts, sclera which consists of connective tissue and made the cornea transparent in the anterior part; 2) the middle layer of uvea or vascular pigment coat; this layer was responsible for nutrition of the eye tissue and accommodation of the eye with light condition; 3) interior layer or photosensitive retina. The other parts of the eye were vitreous humor, aqueous humor and lens, the last being completely round and attached to the interior side of the cornea in touch with aqueous humor (Fig. 1). The position of the lens provides a wide vision sight for the fish. The lens was made up of non-capillary tissue with long prism cells orderly aligned to each other. The lens was encapsulated at the outer side by a layer of carbohydrate and glycoprotein (Fig. 1).

In corneoscleral coat, cornea was made up of a non pigmented squamous epithelium, a simple base membrane and a tin endothelium. The epithelium of cornea was multilayer and continued to the cranial skin.

The posterior wall of the eye ball made up of thick fibroblastic sclera continued. Unlike the transparent and non-pigmented cornea, sclera was non-reflective and covered by an outer layer of hyaline cartilage (Fig. 1).

The middle layer of the eye consists of the choroid and iris. Capillary network of the choroid surrounded the visual nerve and became highly developed in the posterior part of the eye and made a rete choroid.

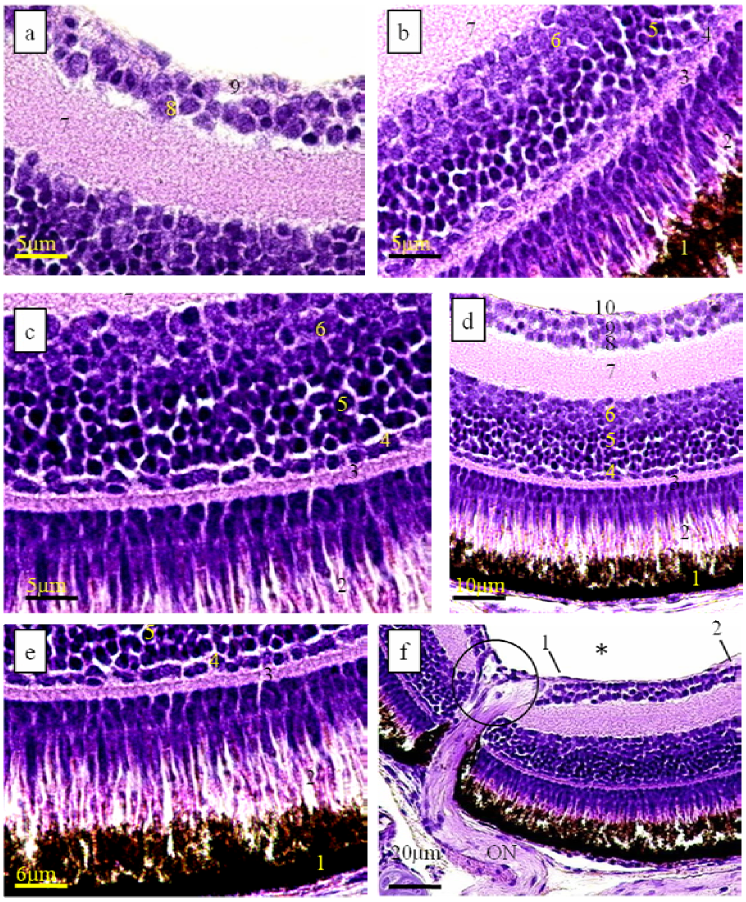
Iris developed from the choroid layer and becomes a thin layer in front of the lens (Fig. 2).



Different parts of the eye (a); optic nerves and optic lobes of the brain (b); lens with two groups of cells (*) and (•) (c); Different layers of retina (e); A magnified figure of 5-9 layers (d); Blind spot (f); rete choroid (g); Optic nerve covered by the myelin coat and gets to the brain right after leaving the eye ball (h).

Legend: C: Cornea; AC: Anterior Chamber; PC: Posterior Chamber; I: Iris; Cr: Choroid rete; B: Brain; ON: Optic Nerve; OM: Optic Muscle; LC: Lens Capsule; L: Lens; Ca: Capillary; RBC: Red Blood Cell.

Fig. 1. Histological structure of the eye in *R. frisii kutum* larvae (H & E staining).



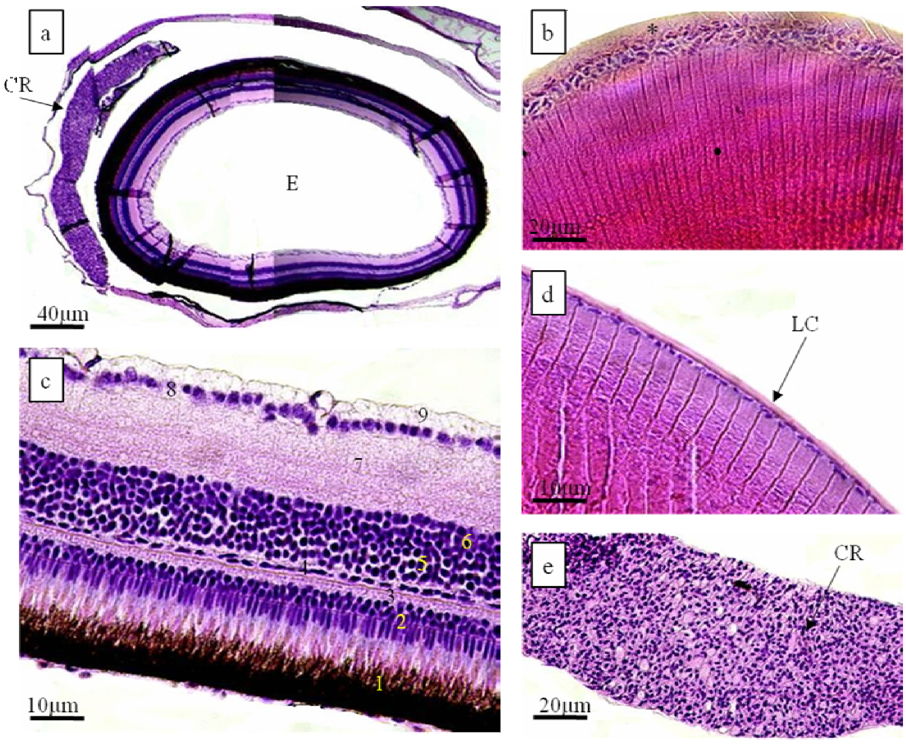
Magnified structure of the retina (a-e) of layers: 1-10. Posterior side of the eye (f) including afferent fibers (number 1) of ganglion cells (number 2) leaves the eye and made optic nerve. The (*) shows posterior chamber contains vitreous humor. Circle shows blind spot.
Legend: ON: Optic Nerve.

Fig. 2. Histological structure of the retina in *R. frisii kutum* larvae (H & E staining).

Retina was made up of 10 distinct layers: 1) The pigment epithelium, closely opposed to the choroid; 2) the elongated tips of cones and rods; 3) the external limiting membrane; 4) the outer nuclear zone containing the cell bodies and nuclei of the photoreceptors; 5) the outer plexiform layer; 6) the inner nuclear layer made up of the bipolar cells and associative glial cells; 7) the inner plexiform layer; 8) the nuclei of the ganglion cell layer; 9) the nerve fiber layer along the optic nerve to the brain; 10) the inner limiting membrane composed of the expanded processes of glial cells (Fig. 2).

Visual axis went through a pouch of concentrated cells of retina called the fovea. This area is called macula lutea due to the presence of yellow pigments. Fovea is the most sensitive and active part of the light sensitive retina. Close to this area afferent nerves come from retina which is called optic papilla and go together to make the optic nerve. At the site of the optic nerve leaving the eye ball, retina lacks any photoreceptors and this spot is called the blind spot or optic papilla (Fig. 2). At the central part of the optic nerve there was the central artery and central vein. The other parts of the optic nerve were strips of pial septa, myelin nerve fibers and the nucleus of neuroglial cells (Fig. 2).

Eye structure in Caspian kutum fingerlings was similar to the structure of larvae eye and the most important difference was the size of the eye ball which was bigger in fingerlings (Fig. 3).



Rete choroid (a). Round lens covered by a capsule of connective tissue (b and d). Anterior condensed cells (*) and posterior elongated cells (•) of lens (b). layers of retina (c). Enormous capillaries of rete choroid (e).
Legend: E: Eye; CR: Choroid rete; LC: Lens Capsule.

Fig. 3. Histological structure of the eye in *R. firisii kutum* fingerling (H & E staining).

DISCUSSION

At the time of hatching, eye in most species of teleosts is not differentiated. In this stage, eye is made up of lens and undifferentiated retina. At the beginning of external feeding the only functional cells of the eye are cone photoreceptors (Blaxter, 1975; Kawamura *et al*., 1984). Double cone photoreceptors developed lately during development and rod cells developed during and/or after juvenile or adult stages, as observed in *Clupea harengus* (Blaxter & Jones, 1967; Sandy and Blaxter, 1980), *Solea solea* (Sandy & Blaxter, 1980), *Pagrus major* (Kawamura *et al*., 1984) and *Hippoglossus hippoglossus* (Kvenseth *et al*., 1996). Results of the previous studies showed that in some species, some of the cone cells developed during embryonic stages and on the other hand rod cells were developed only after embryonic stage (Johns, 1982) and continued their growth and proliferation until maturation of the fish (Johns & Fernald, 1981; Szamier & Ripps, 1983). Compared to previous studies, results of the present study showed that in *R. frisii kutum* larvae the development of the retina begins during the embryonic stage and at the time of hatching the eyes were completely functional.

Although there are considerable differences in shape and structure of the eye in different fish species, the general structure is similar. The general parts of the eye in fish, like in higher vertebrates are: anterior chamber, iris, lens and posterior chamber. At the anterior part, the eye becomes flattened and the spherical lens almost gets in contact with the cornea. Cornea is a transparent layer of the sclera. Choroid with the high density of capillaries lined up between sclera and retina (Kvenseth *et al*., 1996). Retina with the main role of vision consists of a pigmented epithelium of photoreceptors (cones and rods), bipolar cells, ganglion cells and nerve fibers lead to the optic nerve (Kvenseth *et al*., 1996; Sandy & Blaxter, 1980).

On the first day of hatching, Caspian kutum newly hatched larvae have a completely developed eye structure which showed a direct development of the visual system in this species (Evans & Fernald, 1990) and it was different with the ones with metamorphic developments (Pankhurst *et al*., 1993; Pankhurst & Butler, 1996; Pankhurst & Hilder, 1998; Shand *et al*., 1999). In *R. frisii kutum* fingerlings, eye structure and tissue were similar to those of larvae except for the eye ball size which was significantly bigger in the fingerlings. This could be explained due to the previous findings which showed that the growth of the eye in teleosts is coupled with the total growth of the body and continued to the end of the growth period (Easter *et al*., 1977; Fernald, 1989). In almost all vertebrates, production of neural cells in retina completes during embryonic stages or right after hatching/ birth, however in teleosts, production of new cells in retina continued in juvenile to adult besides the embryonic and larval stages (Johns, 1977; Johns & Easter, 1977; Müller, 1952; Wagner, 1974), this long time of production of the cells in retina coupled with the significant growth of the eye surface in most fish which in some, in just few years the retina could become 100 times bigger (Fernald, 1989), it is worth mentioning that part of this enlargement is due to the development of the previously existing cells.

CONCLUSIONS

Results of the present study showed that the eye structure in *R. frisii kutum* at the hatching is fully functional and it could be assumed as an important role of the vision in survival abilities of the larvae and fingerlings of this species in their living environment.

REFERENCES

BATTY R.S., 1989, *Responses of marine fish larvae to visual stimulate*. Journal of the Marine Biological Association of the United Kingdom, **69**: 647-654.

BLAXTER J.H.S., 1975, *The eye of larval fish*, pp.: 427-443, *In*: Ali M.A. (Ed.), *Vision in Fishes*. New Approaches in Research, Plenum Press, New York.

BLAXTER J.H.S., 1986, *Development of sense organs and behavior of teleost larvae with special reference to feeding and predator avoidance*. Transactions of the American Fisheries Society, **115**: 980-1114.

BLAXTER J.H.S., FUIMAN L.A., 1990, *The role of the sensory systems of herring larvae in predator avoidance*. Journal of Marine Biology, **70**: 413-427.

BLAXTER J.H.S., JONES M.P., 1967, *The development of the retina and retinomotor responses in the herring*. Journal of the Marine Biological Association of the United Kingdom, **47**: 677-697.

EASTER S.S. JR., JOHNS P.R., BAUMANN L.R., 1977, *Growth of the adult goldfish eye 1. Optics.* Vision Research, **17** (3): 469–477.

EVANS B.I., FERNALD R.D., 1990, *Metamorphosis and fish vision.* Journal of Neurobiology, **21** (7): 1037-1052.

FERNALD R.D., 1989, *Fish vision*, pp.: 247-265, *In*: Finlay B.A. & Sengelaub D.R. (Eds.), *Development of the Vertebrate Retina*, Plenum Press, New York.

FERNALD R.D., 1990, *Teleost vision: seeing while growing*. Journal of Experimental Zoology, **256**, Supplement S5: 167-180.

GAMBLE J.C., FUIMAN L.A., 1987, *Evaluation of in situ enclosures during a study of the importance of starvation to the vulnerability of herring larvae to a piscine predator*. Journal of Experimental Marine Biology and Ecology, **113**: 91-103.

JOHNS P.R., 1977, *Growth of the adult goldfish eye*. III. Source of the new retinal cells. Journal of Comparative Neurology, **176**: 343-358.

JOHNS P.R., 1982, *The formation of photoreceptors in the growing retinas of larval and adult goldfish*. Journal of Neuroscience, **2**: 179-198.

JOHNS P.R., EASTER S.S., 1977, *Growth of the adult goldfish eye. II. Increase in retinal cell number*. Journal of Comparative Neurology, **176**: 331-342.

JOHNS P.R., FERNALD R.D., 1981, *Genesis of rods in teleost fish retina*. Nature, **293**: 141-142.

KAWAMURA G., TSUDA R., KUMAI H., OHASHI S., 1984, *The visual cell morphology of Pagrus major and its adaptive changes with shift from pelagic to benthic habitats*. Bulletin of the Japanese Society of Scientific Fisheries, **50**: 1975-1980.

KHOSHNOOD Z., MOKHLESI A., KHOSHNOOD R., 2010, *Bioaccumulation of some heavy metals and histopathological alterations in liver of Euryglossa orientalis and Psettodes erumei along North Coast of the Persian Gulf*. African Journal of Biotechnology, **9** (41): 6966-6972.

KVENSETH A.M., PITTMAN K., HELVIK J.V., 1996, *Eye development in the Atlantic halibut (Hippoglossus hippoglossus): differentiation and development of the retina from early yolk-sac stages through metamorphosis*. Canadian Journal of Fisheries and Aquatic Sciences, **53**: 2524-2532.

MARTOJA R., MARTOJA-PIERSON M., 1967, *Initiation aux techniques de l'histologie animale*. Masson et Cie, Paris, 345 pp.

MILLER T.J., CROWDER L.B., RICE J.A., 1993, *Ontogenetic changes in behavioral and histological measures of visual acuity in three fish species*. Environmental Biology of Fishes, **37**: 1-8.

MÜLLER H., 1952, *Bau and Wachstum der Netzhaut des Guppy (Lebistes reticulates)*. Zoologische Jahrbücher. Abteilung für Allgemeine Zoologie und Physiologie der Tiere, **63**: 275-324.

OTTEN E., 1981, *Vision during growth of a generalized Haplochromis species: H. elegans Trewavas 1933 (Pisces: Cichlidae)*. Netherlands Journal of Zoology, **31**: 650-700.

PANKHURST P.M., BUTLER P.E., 1996, *Development of the sensory organs in the greenback flounder, Rhombosolea tapirina*. Marine and Freshwater Behaviour and Physiology, **28**: 55-73.

PANKHURST P.M., HILDER P.E., 1998, *Effect of light intensity on feeding of striped trumpeter Latris lineata larvae*. Marine and Freshwater Research, **49**: 363-368.

PANKHURST P.M., PANKHURST N.W., MONTGOMERY J.C., 1993, *Comparison of behavioural and morphological measures of visual acuity during ontogeny in a teleost fish, Forsterygion varium, Tripterygiidae (Forster, 1801)*. Brain, Behavior and Evolution, **42**:178-188.

SANDY J.M., BLAXTER J.H.S., 1980, *A study of retinal development in larval herring and sole*. Journal of the Marine Biological Association of the United Kingdom, **60**: 59-71.

SHAND J., ARCHER M.A., COLLIN S.P., 1999, *Ontogenetic changes in the retinal photoreceptor mosaic in a fish, the black bream, Acanthopagrus butcheri*. Journal of Comparative Neurology, **412**: 203-217.

SZAMIER R.B., RIPPS H., 1983, *The visual cells of the skate retina: Structure, histochemistry and disc-shedding properties*. Journal of Comparative Neurology, **215**: 51-62.

WAGNER H.J., 1974, *Development of the retina of Nannacara anomala, with special reference to regional variations of differentiation*. Zeitschrift für Morphologie und Ökologie der Tiere, **79**: 113-131.

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LIVER FIBROLAMELLAR CARCINOMA LIKE TUMOR IN AN EXPERIMENTALLY IRON LOADED MOUSE

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Liver fibrolamellar carcinoma like tumor was found in the last survivor from an experimental group of iron loaded mice. This tumor was studied by histologic and electron-microscopic methods, in order to define the common features and the differences of this tumor type towards the liver fibrolamellar carcinoma in man. It was surprisingly to note the similarities of the aspect and structure of the oncocytes and fibrolamellar fibrosis in these two dfferent and distant tumors. A possible relation of the liver iron loading with the development of this tumor was a subject of interest.

Key words: iron loading, fibrolamellar stroma, oncocyte, mouse tumor.

INTRODUCTION

A special type of hepatic carcinoma, very different in comparison with the liver carcinomas known in mice (Turusov & Takayama, 1979), was observed in the liver of a surviving iron loading mouse, after 17 months from the beginning of the experiment. This type of liver carcinoma was morphologically characterized by "tumor cells with deep eosinophilic cytoplasm and a fibrous stroma formed of collagen fibers arranged in a lamellar fashion".

After a detailed documentation, we knew about the existence of a unique type of primary liver carcinoma in man, known under the name of liver fibrolamellar carcinoma (LFC). Its main morphological features were: tumor cells with deep eosinophilic cytoplasm and rich fibrous stroma arranged in a lamellar pattern (Edmondson, 1956; Peters, 1975). This tumor occurred in adolescents and young adults (Craig *et al*., 1980; Chun & Zimmitti, 2013).

The LFC features have been well documented about the morphological and biochemical characters (Craig *et al*., 1980; Berman *et al*., 1980; Fahri *et al*., 1982; Vecchio *et al*., 1984; Van Eicken *et al*., 1988; 1990; Torbenson, 2007; 2012) and also, about the genetic and molecular biology analyses (Ward & Waxman, 2011; Ward *et al*., 2010; Patonai *et al*., 2013; Malouf *et al*., 2014). This tumor was specific for man. Till now, its etiology remained unknown.

It became interesting to know the cellular mechanisms bound of the fibro-lamellar stroma occurrence in these two different and distant tumor entities. The results of the study of the histologic and ultrastructure traits of the fibrolamellar carcinoma like tumor observed in the iron loaded mouse were presented and compared with the data about this type of tumor in man.

MATERIAL AND METHODS

12 albino Swiss non imbred, young adult male mice of 22±2 g were treated with Ferrum Hausmann, as iron III-hydroxide polymaltose complex, by repeated intra-peritoneally (ip) inoculations, to obtain a loading of 2 mg iron/g body weight. After 17 months from the beginning of the treatment, the last surviving mouse was sacrificed. The liver presented diminished dimensions and tumor aspect.

Histology. Fragments of the tumor and surrounding liver tissue were fixed in 4% formaldehyde in saline and processed according to standard techniques for the paraffin embedding. The histological sections of 5 μm thickness were stained with hemalum-eosin (HE) for general tumor structure, Perls method to make evident ferric ions in hemosiderin deposits and PicroSirius red-Hemalum (PSR-H) the specific stain (Junquiera *et al*., 1979) for the collagen fibers.

Electron microscopy. Small pieces of about 1/1/1 mm, from the liver and the tumor tissue, were fixed in cold 2.5% glutaraldehyde in 0.1 M sodium cacodylic salt buffer pH 7.2, for 24 h at 4ºC. After careful washings in the same buffer, the samples were postfixed with 1.3%OsO_4 for 2 h, in the darkness and at the room temperature. Then, the material was dehydrated in ethyl alcohols of growing concentration degrees and processed for the embedding in Epon 812, according to standard techniques. The ultrathin sections were contrasted with uranyl acetate and lead citrate. The ultrastructural observations were performed with TEM-Jeol 100.

RESULTS

The histologic study showed that the lobular liver architecture was not preserved in the tumor territory. The typical structure of the hepatic lobules with portal triades, central veins, radially oriented sinusoids and hepatic cords, has never been observed.

The tumor tissue was formed of nodules (Fig. 1) of fibrolamellar carcinoma like tumor. They were often separated by PSR+ fascicles. The growing of the tumor nodules was aggressive, so the non invaded liver in vicinity appeared compressed.

The fibrolamellar nodules contained tumor cells arranged in short cords and in pseudoglandular acini (Figs. 1-3). Tumor cells were larger than the normal hepatocytes, polygonal or spindle in shape. Their cytoplasm was abundant, deep eosinophilic (oxyphilic), with a unique, large vesicular nucleus. Among these cells were noted small cells with pyknotic nuclei. Mitosis figures were rare. The pseudoglandular acini were formed by tumor cells arranged as cellular rosettes, sometimes with a central lumen. In the centre of such rosettes, fibrillar, amphophilic material was occasionally observed (Figs. 1-2). The short tumor cords and the pseudoglandular acini were surrounded by coiled fibrotic PSR+ fascicles, which tended to wrap up vast tumor areas (Figs. 3-4). The collagen fascicles presented a contracted rippled aspect. The tumor acini were arrested into the netting of these fibers (Fig. 5). Sometimes, in the tumor might be observed pseudoglandular acini isolated by large spaces filled with blood or other fluids (Fig. 3). Inflammatory cells: polymorphs, lymphocytes, monocytes were noted in some zones of the tumor.

In zones of non involved liver or with incipient tumorigenesis, the Perls + material might be observed in the hepatic cells, and in their nuclei (Fig. 6), but generally the iron was eliminated from the tumor. Detachments were produced between the atrophic cells which lined the inner face of the Glisson capsule and the tumor tissue. These areas were invaded by fluids or blood.

The electron microscopic observations presented the aspect of acini (Fig. 7) composed of three or more tumor cells. These bulky cells had the cytoplasm crowded with mitochondria. These organelles were swollen, with diffuse unclear matrix and poor defined cristae. The cellular nucleus was round with sinuous lining. Blocks of heterochromatin were located peripherally along the inner face of the nuclear membrane. The nucleoli were often observed. The cytoplasm presented some free ribosomes, a rough and smooth endoplasmic reticulum. Sometimes a Golgi complex was observed (Fig. 8). The glycogen was absent. In the cytoplasm electron dense inclusions were noted which might be accumulations of bile arrested in the tumor cells. Also secondary lysosomes, peroxisomes, sometimes siderosomes and lipid vesicles were noted. The most frequent and characteristic structures observed were bundles of microfilaments (microfibrils) (Figs. 9-10). These bundles formed of many fine parallel microfibrils were localized just near the cellular nucleus (Fig. 9). Such bundles run through the cellular cytoplasm towards the extracellular spaces, to meet other similar bundles of thin microfibrils originated from other tumor cells, and to form thicker fibrous structures of fine fibrils (Fig. 10).

The fibroblasts were observed (Fig. 11) with a characteristic long nucleus, little cytoplasm and the fascicle of collagen fibers with characteristic periodicity, located outside the cell. Another interesting image was represented by the nuclear ferritin aggregates with a peculiar aspect, eliminated in the cytoplasm of the tumor cells, in the vicinity of the normal hepatic tissue (Fig. 12).

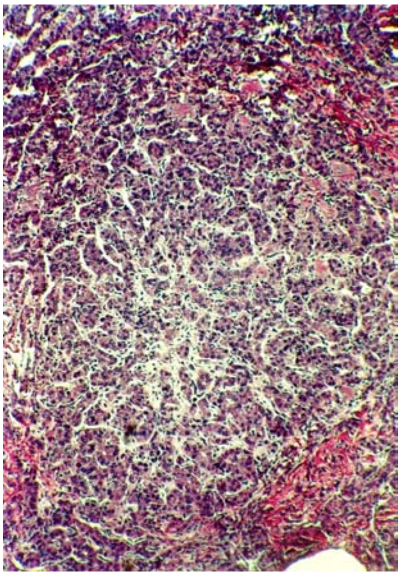

Fig. 1. Liver fibrolamellar carcinoma like tumor nodule with tumor cells forming small cords and pseudoglandular acini. PSR- Perls. Original magnification: × 90.

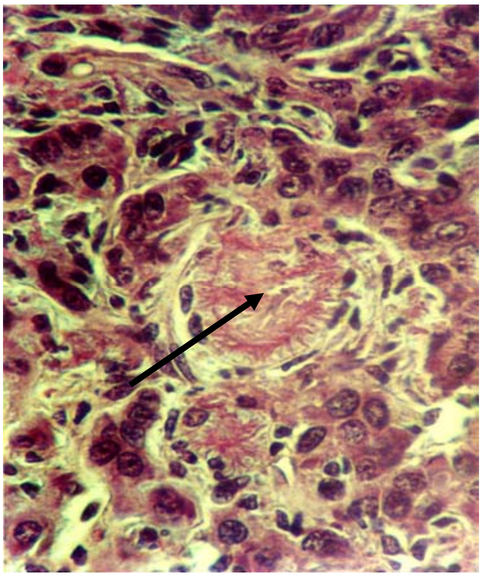

Fig. 2. Fibrillar amphophilic material (arrow) observed in the center of the cellular rosettes formed of the tumor cells. H-E. Perls. Original magnification: × 560.

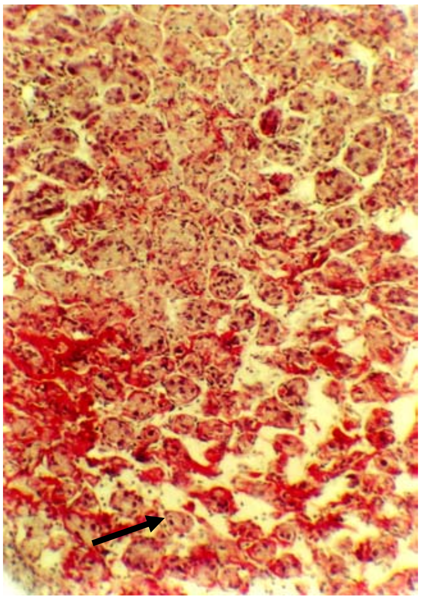

Fig. 3. Oncocytic tumor cells organized in pseudoglandular acini surrounded by fibrous PSR+ fascicles. Note the large interacinar spaces (arrow). PSR-H. Original magnification: ×140.

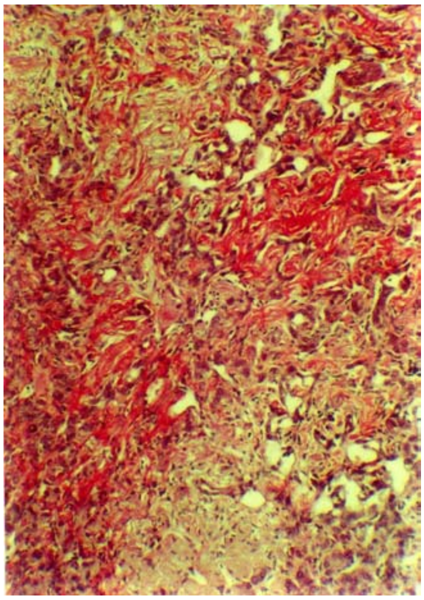

Fig. 4. PSR+ fibrolamellar fascicles invading the tumor cells. PSR-H. Original magnification: × 140.

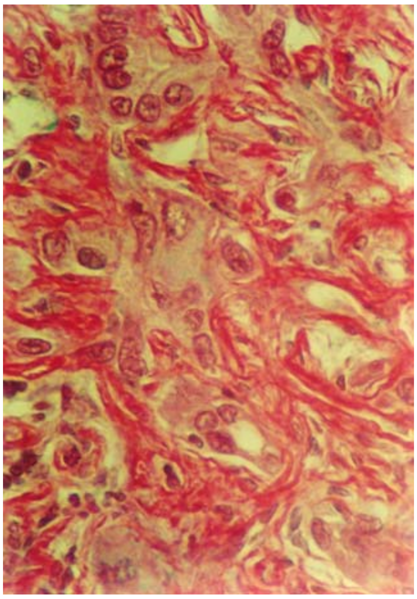

Fig. 5. Tumor cells in fibrotic area. PSR-H. Original magnification: ×560.

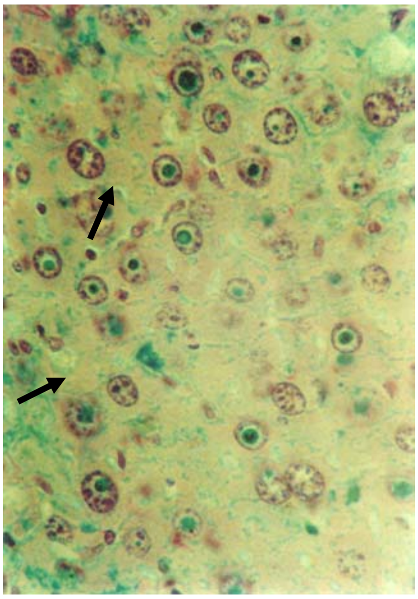

Fig. 6. Untransformed hepatic tissue. The Perls+ material was concentrated in siderosomes (arrow) and nuclear inclusions of ferritin (arrowhead). H-E Perls. Original magnification: × 560.

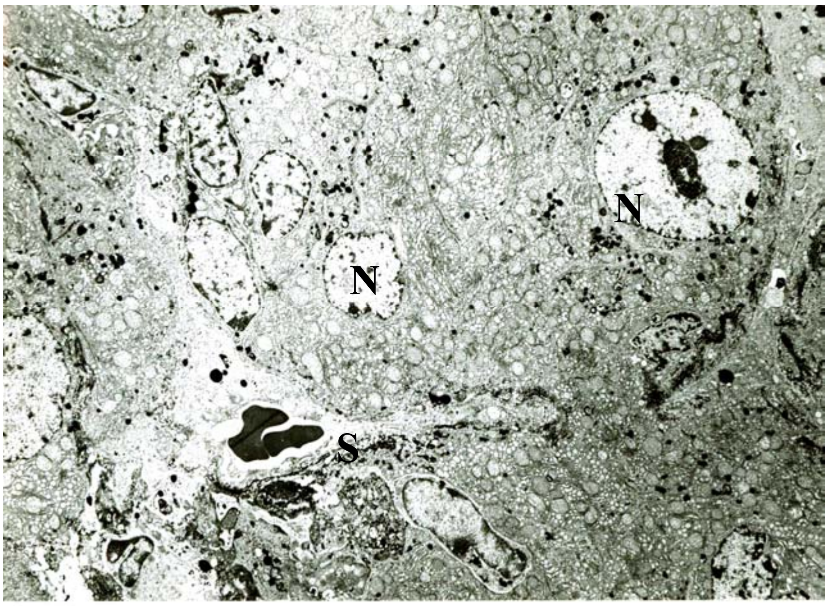

Fig. 7. Electron-microscopic aspect of a pseudoglandular acinus. Note the tumor cells nuclei (N), numerous mitochondria (m), rough endoplasmic reticulum, electron dense siderosomes. A sinusoid (S) seemed to be obstructed. Original magnification: × 3,000.

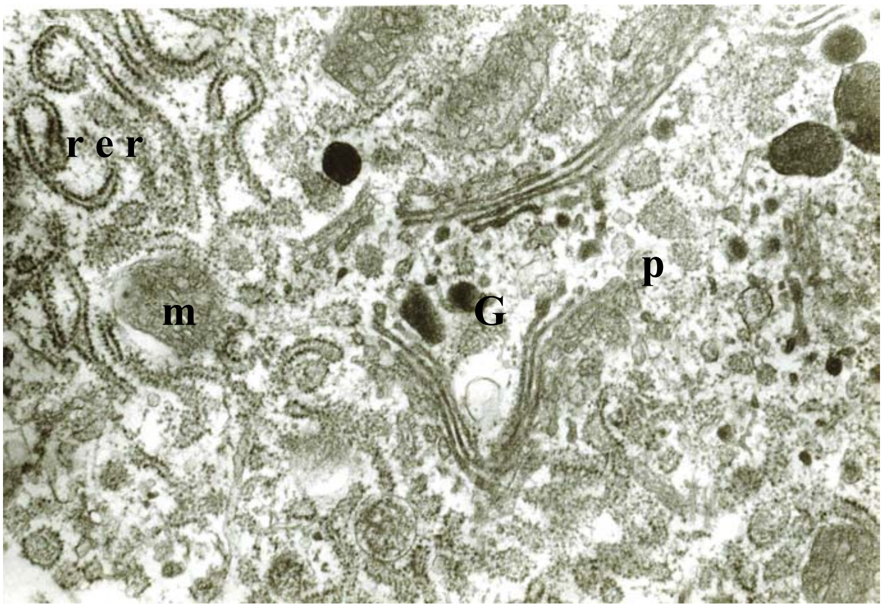

Fig. 8. Detail of the Golgi complex (G) in the tumor cell. Note: mitochondria (m) with diffuse matrix and few cristae, rough endoplasmic reticulum (rer), peroxisomes (p), siderosomes. Original magnification: × 30,000.

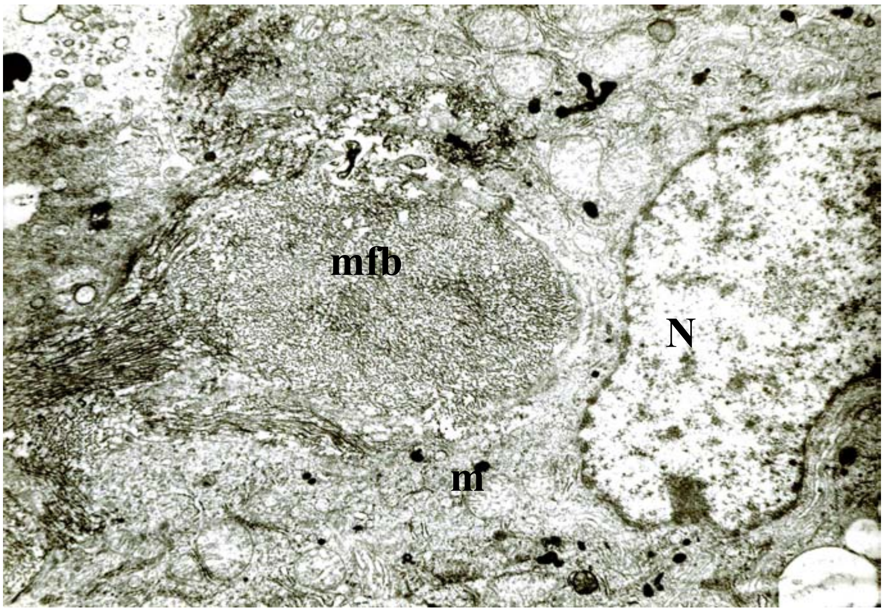

Fig. 9. Bundle of fine microfibrils (mfb) in transverse section, near the nucleus (N) of a liver fibrolamellar carcinoma like tumor cell. Original magnification: × 14,000.

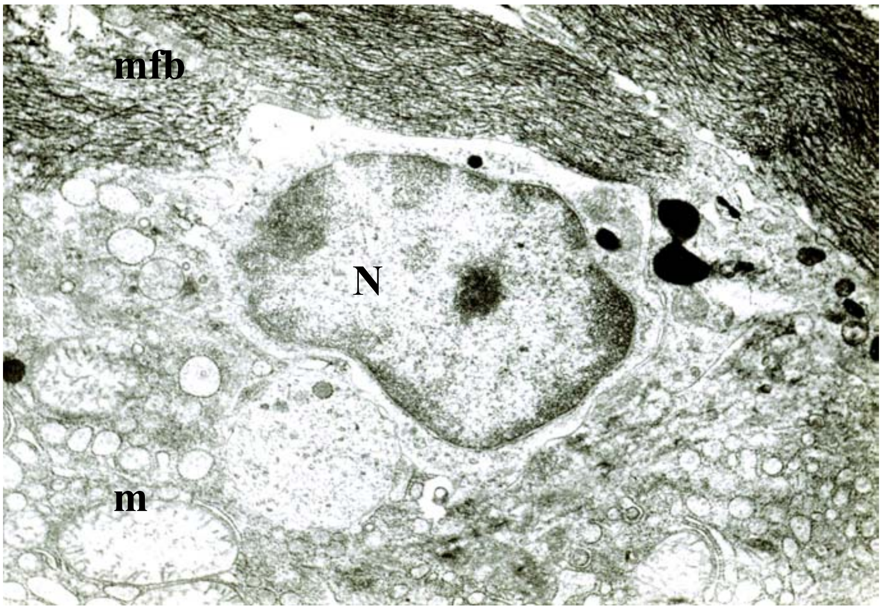

Fig. 10. Tumor cell surrounded by bundles of fine parallel microfibrils (mfb) in longitudinal section. Original magnification: × 14,000.

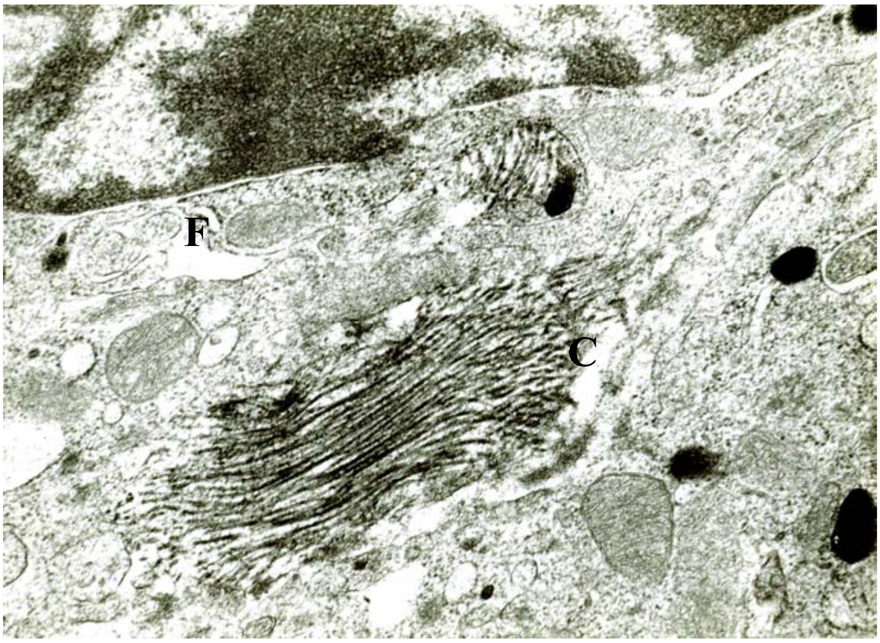

Fig. 11. Fibroblast (F) synthesizing collagen fascicle (C) with characteristic structure. Original magnification: × 28,000.

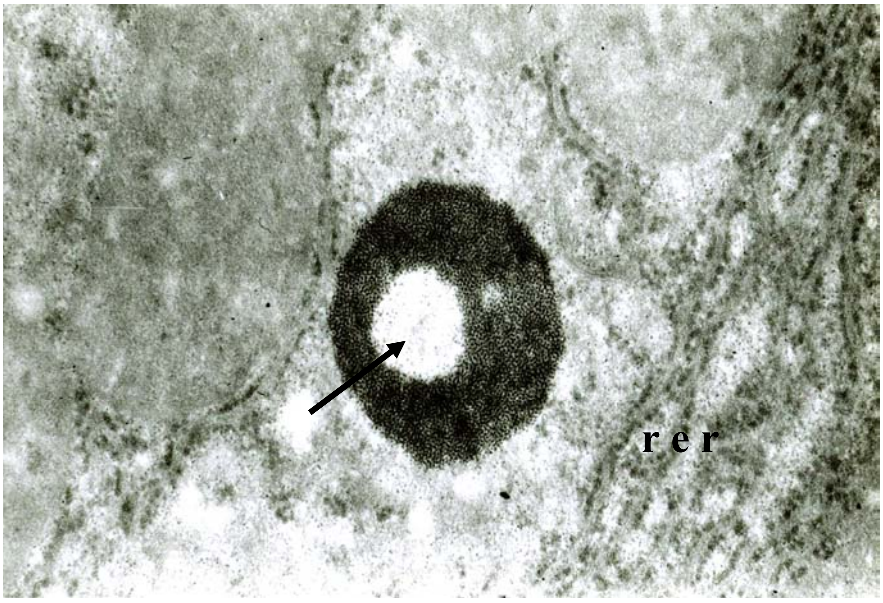

Fig. 12. Nuclear ferritin inclusion (arrow) with annular form and typical ferritin design which was eliminated in the cytoplasm. Note rough endoplasmic reticulum (rer). Original magnification: × 75,000.

DISCUSSION

From the literature dedicated to the liver fibrolamellar carcinoma, it became evident that this tumor type was unknown for the species *Mus musculus*.

In the volume II: “Mouse Tumors” (1979) from the volumes series entitled: “Pathology of Tumors in Laboratory Animals” edited by V.S. Turusov (1976-1982), there were presented spontaneous primary hepatic tumors and liver tumors induced by treatments with different carcinogenic substances. But, it was not presented any case of liver fibrolamellar carcinoma in mouse.

In fact, the scientists’ interest on the LFC in man and the publication of volumes about the tumors in laboratory animals happened nearly at the same time.

The first description of the LFC in man was made by Edmondson (1956) who established a differential diagnostic of this tumor and tumor like lesions in childhood, without giving a name for this tumor entity. The name “liver fibrolamellar carcinoma” was given by Peters (1975).

It seems that the liver fibrolamellar carcinoma was not a hepatic tumor presented exclusively in man. This tumor was described (Bettini & Marcato, 1992), in adult females of cattle. Unfortunately the authors presented only statistical data.

It was recorded the little proportion of 2% cases of liver fibrolamellar carcinoma of a total of 66 hepatic tumors, using Edmondson's definition and without morphological details.

Baithun & Pollock (1983) explained the name "oncocyte" in the case of fibrolamellar carcinoma cells. The oncocyte presented abundant, deep eosinophilic granular cytoplasm. The electron microscopic observations showed that the cytoplasm was loaded with the most numerous mitochondria, with few cristae and diffuse matrix. The authors reviewed this interesting eosinophilic modification in a large category of epithelial cells of different exocrine or endocrine glands: salivary, parathyroids, pituitary, adrenals, pancreas, liver and bronchial epithelia. When the tumors consisted only of oncocytes, the term oncocytoma was applied. The common feature observed in such oncocytoma was the increased number of mitochondria which replaced other organelles. In this situation the ability of the oncocytes to carry the normal cellular functions was restricted. Other authors (Vechio *et al*., 1984; Torbenson, 2007; 2012) agreed with the opinion that the oncocytes were deep eosinophilic due to the high number of mitochondria. The explanation of the high number of mitochondria in LFC was considered to result from the hypoxia established following the development of the fibrous stroma around the tumor cells.

The fibrolamellar stroma, the second LFC main character, was the most debated trait. Craig *et al*. (1980) observed on the EM photos multiple collagen fibrils with parallel orientation and characteristic banding, at intervals of 600-700 Å in the extra-cellular space. Also non-broad banded fibrils with a periodicity of 1100 Å were observed. Fahri *et al*. (1982) noted the presence of the collagen fibers and established without any argumentation that "the presence of dense eosinophilic lamellar bands of fibrous tissue is one of the hallmarks of this recently recognized variant of hepatocellular carcinoma". Vecchio *et al*. (1984) observed that among collagen bundles lined by fibroblasts "there were some elongated cells exhibiting the features of myofibroblasts with indented nucleus, nuclear bodies, cytoplasmic microfilaments and subplasmalemmal densities". Microfilaments were arranged in longitudinal bundles parallel with the long axis of the cells. Phillips *et al*. (1987) presented a fibrolamellar carcinoma cell with the aspect of a myoepithelial cell, with a band of parallel microfilaments placed on the cytoplasmic face of the cellular membrane. In his extensive review, Torbenson (2012) considered that the lamellar bands of fibrosis were composed of type I, III, and V collagen; occasionally, the tumor cells produced mRNA collagen.

The observation of the Mallory bodies in the LFC cells cytoplasm (Craig *et al.*, 1980) led to the great interest for the intermediate filaments of cytokeratine from the tumor cells cytoskeleton. Van Eiken *et al*. (1988) who already knew that the normal human hepatocytes expressed cytokeratins no. 8 and 18, and the bile duct cells presented the same cytokeratins and in addition cytokeratins no. 7 and 19, made the observation that the presence of Mallory bodies in the hepatocellular carcinoma indicated the changes in the organization of the cytoskeletal filaments during the neoplastic transformation. During the study of the pattern of cytokeratins in two cases of LFC, Van Eiken *et al*. (1990) found that the fibrolamellar carcinoma cells expressed cytokeratin polypeptids no. 8 and 18 and also the cytokeratin no. 7 and, in a little number of tumor cells, cytokeratin 19. More recent papers (Ward *et al*., 2010; Ward & Waxman, 2011) confirmed, by immunohistochemical evidences, the presence of the cytokeratins pattern of the hepatocytes and bile ducts cells in LFC. Also, the presence of the neuroendocrine markers was used for the differentiation between the fibrolamellar and hepatocellular carcinoma (Patonai *et al*., 2013; Malouf *et al*., 2014).

The great difference between LFC in man and the liver fibrolamellar carcinoma like tumor in mouse was that the first one was a primary tumor with unknown etiology, while the mouse tumor developed during a very long lapse of time (17 months) of irritable action upon the liver following the heavy iron overloading of 2 mg iron/g body. Iancu (1989) considered that the iron is essential for all cells, especially for those that divided rapidly such as microorganisms and tumor cells; the large body iron stores increased the risk of cancer.

At the beginning of the iron overloading process, the mouse hepatocytes cytoplasm presented many siderosomes and intranuclear inclusions of ferritin aggregates (Smith *et al.*, 1990; Prunescu & Prunescu, 1991). But, after a long lapse of time, the great avidity for the iron of the tumor tissue became evident. The tumor areas were lacked of the stain reactivity for iron (Perls stain negative). In the tumor cells cytoplasm, there were observed the peculiar structures of ferritin from the iron nuclear inclusions. These inclusions were excluded from the karyoplasm in course of mitosis developments. The delivered ferritin molecules might be probably used for tumor metabolic needs.

CONCLUSIONS

1. The main features of the hepatic tumor occurred in a mouse after 17 months of iron loading were similar with the morphological traits observed in human liver fibrolamellar carcinoma.

2. The deep eosinophilic cytoplasm of the tumor cells was a cytoplasm rich in mitochondria presenting important lesions, like in human LFC cases.

3. The fibrolamellar stroma was formed of PSR+ collagen fibers combined with microfibrils bundles originated from the tumor cells cytoskeleton.

4. The capacity of the mouse hepatocytes nuclei to concentrate ferritin molecules as nuclear inclusions represented an irritable condition but, at the same time, a source of available iron for the initiation and possibly genesis of the tumor.

REFERENCES

BAITHUN S.I., POLLOCK D. I., 1983, *Oncocytic hepatocellular tumor.* Histopathology, **7**: 107-112.

BERMAN M.M., LIBBEY P.N., FOSTER J.H., 1980, *Hepatocellular carcinoma. Polygonal cell type with fibous stroma. An atypical variant with favorable prognosis.* Cancer, **46**: 1448-1455.

BETTINI G., MARCATO P.S., 1992, *Primary hepatic tumor in cattle; a classification of 66 cases.* Journal of Comparative Pathology, **107** (1): 19-34.

CHUN S., ZIMMITI G., 2013, *Fibrolamellar carcinoma. Results.* Cancer Research., **190**: 101-110.

CRAIG J.R., PETERS R.L., EDMONDSON H.A., MASAO O., 1980, *Fibrolamellar carcinoma of the liver: a tumor of adolescents and young adults with distinctive clinico-pathologic features.* Cancer, **46**: 372-380.

EDMONDSON H.A., 1956, *Differential diagnostic of tumors and tumor-like lesions of the liver in infancy and childhood.* American Journal of Diseases of Children, **91** (2): 168-186.

FAHRI C.D., SHIKES R.H., SILVERBERG S.G., 1982, *Ultrastructure of fibrolamellar oncocytic hepatoma.* Cancer, **50**: 702-709.

IANCU TC., 1989, *Iron and neoplasia: ferritin and hemosiderin in tumor cells.* Ultrastructural Pathology, **13** (5-6): 573-584.

JUNQUIERA L.C.A., BIGNOLAS G., BRENTANI R.R., 1979, *A simple and sensitive method for the quantitative estimation of collagen.* Annalytical Biochemistry, **94** (1): 96-99.

MALOUF G.G., JOB S., PARADIS V., FABRE M., BRUGIÈRES L., SAUTIGNY P., VESCOVO L., BELGHITI J., BRANCHESREAU S., FAIVRES S., de REYNIÈS A., RAYMONDE E., 2014, *Transcriptional profiling of pure fibrolamellar hepatocellular carcinoma reveals an endocrine signature.* Hepatology, **59** (6): 2228-2237.

PATONAI A., ERDÉLYI-BELLE B., KOROMPAY A., SOMORÁCZ A., TÖRZÖK P., KOVALSZKY I., BARBAI T., RÁSÓ E., LOTZ G., SCHAFF Z., KISS H., 2013, *Molecular characteristics of fibrolamellar carcinoma.* Pathology Oncology Research, **19** (1): 63-70.

PETERS R.L., 1975, *Pathology of hepatocellular carcinoma,* pp.: 107-168. In: OKUDA K., PETERS R.L. (Eds.) *Hepatocellular Carcinoma,* Wiley, New York.

PHILLIPS J.M., POUCELL S., PATTESON J., VALENCIA P., 1987, *The Liver: An Atlas and Text of Ultrastructural Pathology, Chapter 7.* Raven Press, New York, 585 pp.

PRUNESCU C.C., PRUNESCU P., 1991, *Ferritin accumulation in nuclei of hepatocytes in polymaltosated iron injected mice.* Revue Roumaine de Biochimie, **28** (3-4): 169-172.

SMITH A.G., CARTHEW PH., FRANCIS J.E., EDWARDS R.E., DINSDALE D., 1990, *Characterization and accumulation of ferritin in hepatocyte nuclei of mice with iron overload.* Hepatology, **12** (6): 1399-1405.

TORBENSON M., 2007, *Review of the clinicopathologic features of fibrolamellar carcinoma.* Advances in Anatomic Pathology, **14** (3): 217-223.

TORBENSON M., 2012, *Fibrolamellar carcinoma: 2012 Update*. Scientifica, Vol. 2012, Art.ID 743790, Hindawi Publishing Corporation, 15 pp.

TURUSOV V.S., TAKAYAMA S., 1979, *Tumors of the Liver*, pp.: 193-235. *In*: TURUSOV V.S (Ed.), *Pathology of Tumors in Laboratory Animals*, *Vol. II, Tumors of the Mouse*. IARC Scientific Publications No. 23, International Agency for Research on Cancer, Lyon.

VAN EIKEN P., SCIOT R., PATERSON A., CALLEA F., KEW M., DESMET V.J., 1988, *Cytokeratin expression in hepatocellular carcinoma. An immunohistochemical study.* Human Pathology, **19**: 562-568.

VAN EIKEN P., SCIOT R., BROCK P., CASTEELS-VAN DAELE M., RAMAEKERS F.C.S., DESMET V. J., 1990, *Abundant expression of cytokeratin 7 in fibrolamellar carcinoma of the liver.* Hepatology, **17**: 101-107.

VECCHIO F.M., FABIANO A., GHIRLANDA G., MANNA R., MASSI G., 1984, *Fibrolamellar carcinoma of the liver. The malignant counterpart of focal nodular hyperplasia with oncocytic change.* American Journal of Clinical Pathology, **81**: 521-526.

WARD S.C., HUANG J., TICKOO S.K., THUNG S.N., LADANYI M., KLIMSTRA D.S., 2010, *Fibrolamellar carcinoma of the liver exhibits immunohistochemical evidence of both hepatocytes and duct bile differentiation.* Modern Pathology, **23** (9): 1180-1190.

WARD S .C., WAXMAN S., 2011, *Fibrolamellar carcinoma: a review with focus on genetics and comparison to the other malignant primary liver tumors.* Seminars in Liver Disease, **31** (1): 61-70.

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