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BOOK REVIEW

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OVERVIEW ON THE *ROMANIAN FAUNA* ACADEMIC COLLECTION

"*People and years pass and are getting off in the mists of time, rare being those destined to remain alive in the memory of their descendants, through their work and life*".
Dragomir Hurmuzescu

SANDA MAICAN*, DUMITRU MURARIU, MARIAN-TRAIAN GOMOIU†

The project of publishing the monographic *Romanian Fauna* Academic Collection - 16 volumes and 307 fascicles expected to be published in the volume Guide published by the Romanian Academy Publishing House (1951), was carried out at a steady pace, starting with the publication of the first volume of Protozoa in the same year. 71 years after the beginning of the activities within the Romanian Fauna Study Group within the Romanian Academy, only 97 fascicles were published (about 26,570 pages), which represent about 30% of the total number of issues expected to be published. With rare exceptions, the completion of the *Romanian Fauna* was interrupted. Therefore, it is necessary to pay more attention or reconsider the importance of research on systematics and animal taxonomy to research on systematics and animal taxonomy, with results that can be published in the *Romanian Fauna*, especially for groups that are not yet represented in this Collection. It is also necessary to update the information published in the *Romanian Fauna* – including by taking in account the latest data obtained in related fields (ecology, physiology, molecular biology, etc.). The updating and completion of the *Romanian Fauna* is necessary both in terms of the importance of fundamental research (taxonomy, systematics, biology, ecology) and the practical significance of species distribution, identification and establishment of strategies for monitoring and protecting endangered species and those with declining populations, the application of optimal control measures in case of invasions of pests, etc.

Keywords: Romanian Fauna Academic Collection, taxonomy, systematics, published volumes and fascicles.

THE FUNDAMENTAL AND APPLIED IMPORTANCE OF ANIMAL TAXONOMY AND SYSTEMATICS RESEARCH

Currently, research in many areas of biology, on biodiversity knowledge, the identification of threatened and endangered species, those considered to be important indicators of environmental quality and ecosystem status, the estimation of the population trend for certain scientifically and economically important species, setting of long-term protection measures for species and habitats, biological pest control, rational use of biological resources for sustainable development, ecological land management, etc. is based on accurate taxonomic, systematic, ecological and zoogeographical knowledge of flora and fauna and the correct identification of taxa.

Knowledge of biodiversity has become a priority for the whole world. Why? Because, more than ever, natural capital is under unprecedented anthropogenic pressure, more and more species endangered with extinction.

The Convention on Biological Diversity and all the events related to this document have highlighted the importance of worldwide taxonomic and systematic research. One of the important objectives mentioned in the international biodiversity study programs is the identification, description, revision and naming of taxa. The correct identification and "problem" of the biological species term definition are the basis for establishing and implementing strategies for biodiversity protection. But, before they can protect and save the species, they need to be known. And the identification of plant and animal species, knowledge of the origin and relationships between them, decoding the diversity of the living world in general are characteristics of taxonomy and systematics, among the oldest sciences in the vast field of biology (Gomoiu & Maican, 2021).

Since the second half of 19th century, the Romanian scientist Grigore Antipa considered that "*Sine systematica, chaos!*", systematic research, along with taxonomy, contributing to the substantiation of many fields of biological sciences.

Starting with the second half of the 20th century, the Romanian taxonomist researchers, although well experts in flora and fauna, hardly succeeded in obtaining funds for projects on knowledge of biodiversity from a taxonomic, systematic, ecological and zoogeographical point of view.

FROM THE HISTORY OF THE *ROMANIAN FAUNA COLLECTION* – "THE TREASURE COLLECTION" OF THE ROMANIAN ACADEMY

In the interwar period, between 1919 and 1948, so suggestively called by Academician Dan Berindei "the time of fulfillment" (History of the Romanian Academy, 2006), Romanian biology had a favourable evolution, with the establishment of new universities, institutes and research stations, due to the economic development and valuable human resource, formed mainly in the countries of western Europe, especially in Germany and France. An important part in the field of descriptive zoology was played by the school created by Andrei Popovici-Bâznoşanu (1876–1969), a real nursery of systematic zoologists, zoogeographers and ecologists, who enjoyed international recognition (Negrea, 2003).

The reorganization in 1948 of the old Academy, into the Academy of the Romanian People's Republic (R.P.R.), significantly influenced the development of botany and zoology research in our country. Knowing the flora and fauna of Romania has become an objective assumed by the new leadership of the Academy. Within this context, in 1949, the Flora and Fauna teams were established, the inventory of plant and animal species, along with other aspects (geographical, geological research, etc.) representing a major concern, being included in the State Plan, within the Academy activity (Gomoiu & Maican, 2021).

The knowledge of the fauna – "*one of the natural richness of the country*" – was required both theoretically (taxonomically, systematically, ecologically, zoogeographically) and economically, the rational use of this natural resource and pest control requiring accurate knowledge of the composition of the fauna, biology and species distribution (Botnariuc, 1966).

In the "R.P.R. Fauna study group" the most valuable zoologists in the country were included. The activity of this group began by making a general classification of the Animal Kingdom up to the Class level, and to the studied groups up to the Order level. From the beginning, the main purpose of Fauna study group was the elaboration of the fascicles from the *R.P.R. Fauna Series*. This Series, considered by Academician Nicolae Botnariuc "*a national work of success*", was to be a synthesis of zoological research in our country, containing 16 volumes, each volume including several fascicles.

Fauna of Romania is a fundamental work of national importance that appears under the auspices of the Romanian Academy, initially with the title *Fauna of the People's Republic of Romania* (1951–1965), later *Fauna of the Socialist Republic of Romania* (1965–1989) and *Fauna of Romania* (after 1989).

Among the Romanian biologists who contributed to the initiation of activities within the *Romanian Fauna* we mention: Mihai Băcescu and Theodor Buşniţă (for Hydrobiology), Eugen Pora and Nicolae Şanta (Animal Physiology), Radu Codreanu (Animal Morphology), Mihai A. Ionescu (Systematics and Animal Ecology), Mihai I. Constantineanu (parasitoid ichneumonological fauna), Gheorghe Nichita (Limnology), Wilhelm Knechtel (Agricultural Entomology), Constantin C. Georgescu, Grigore Eliescu and Ion Popescu-Zeletin (Forest Entomology and Forestry), and others.

In 1951, at Academician Traian Săvulescu's initiative, president of the Romanian Academy, the volume *Guide* was published (part I), which describes the characteristics of the phyla and classes, the keys to identify classes and orders, the terminology used (Codreanu & Băcescu, 1951).

The research of the *Fauna* team initially targeted a small number of taxa. The criteria underlying the selection of the groups of animals to be processed in the *Romanian Fauna* were determined by the economic importance of the group and the number of existing specialists. Later, as zoological research developed in Romania, the number of those involved and the taxa studied increased considerably.

Since 1949, the *Fauna Collection* has been designed to be published in 16 volumes and 307 fascicles (Niţu, 2006). These works are indispensable for Romanian zoologists, whether beginners or seniors, but also for foreign specialists involved in the creation of monographs of various taxonomic groups or synthesis works (e.g., *Fauna Europaea*). Fauna fascicles are useful tools for specialists interested in the taxonomy of the group, as well as a wider audience, including professors, students, amateurs, researchers in museums, faculties of natural sciences and environmental protection institutes, etc.

The Fauna fascicles are also useful guides of taxa identification and include both reported species on the Romanian territory and species whose presence in the country should be possible. These "*…are designed according to a common plan, with a general part, which deals with the chapters: history, morphology, phylogeny, economic importance and research methods, and a systematic part, including a detailed description of orders, families, genera, species etc., based on the identification keys, as well as the complete data regarding their biometrics, ecology and distribution*" (Niţu, 2006).

Over time, the contents of Fauna fascicles have evolved. If at the beginning the works were elaborated on the basis of existing biological materials in older collections, over time the collecting new materials in many areas of the country intensified, and the study of groups became more thorough, which allowed a more complex presentation of the analyzed taxa (Botnariuc, 1966).

The scientific publications in the *Fauna* Collection varied from year to year (Fig. 1 and Fig. 2). In the first 25 years after the first volume, the publications were regular, with at least one issue published annually. The most prolific years, in terms of the number of pages published, were 1960 and 1959, and in terms of the number of issues or fascicles were 1955 and 1951. After 1975, the pace of publication decreased, with no volumes appearing year after year (1976, 1986–1988, 1990–1994, 1996–1999, 2007–2013, 2017–2020).

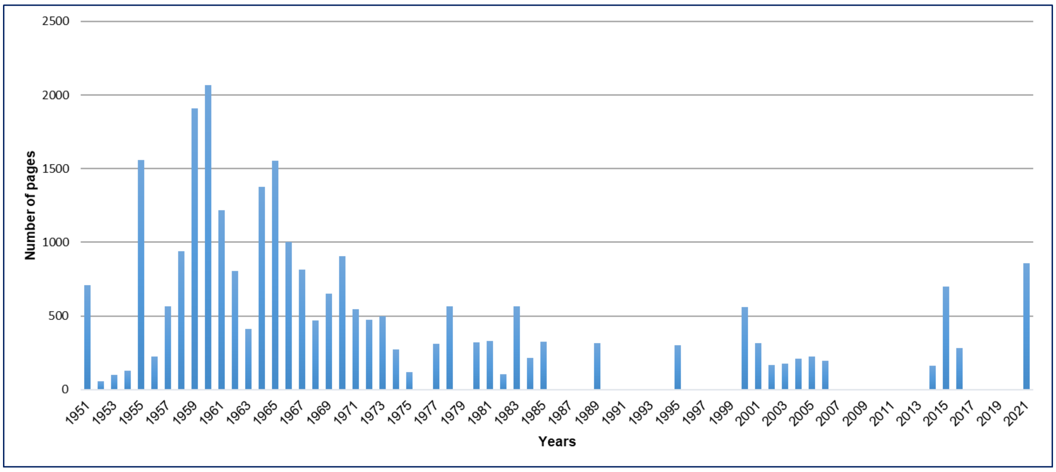

Fig. 1. Annual number of published pages in the *Romanian Fauna.*

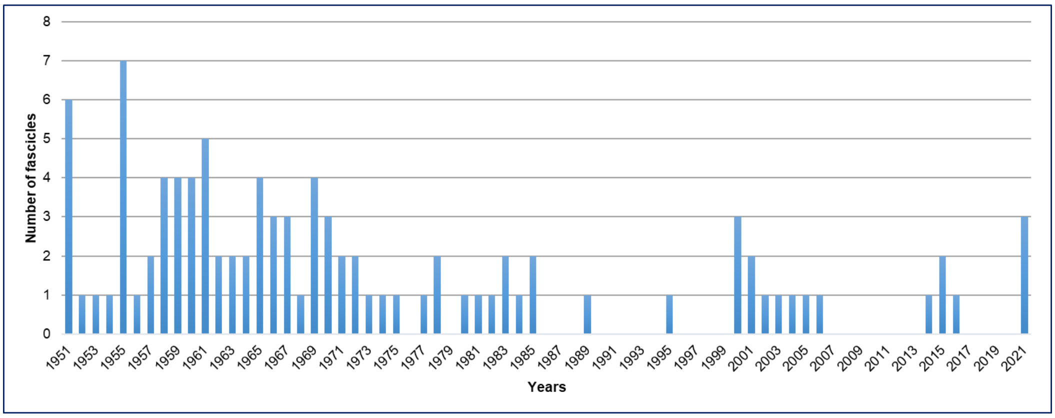

Fig. 2. Annual publication frequency of the *Romanian Fauna* fascicles.

At least one fascicle of each volume appeared. A number of 97 fascicles (including *Guide* volume) were published in the period 1951–2021. They were signed by 72 authors (46 as first author), totaling about 26,570 pages (Table 1). This represents approximately 30% of the total number of issues expected to be published, 38 issues being developed within the former Team of Taxonomy and Animal Systematics, from the Institute of Biology of the Romanian Academy.

Of the 97 published fascicles, 82 (22,593 pages) refer to invertebrates and 14 (3,977 pages) to vertebrates.

From the Insecta Class – the dominant group in terms of the number of animal species, five volumes were expected to be published. In volumes VII, VIII, IX and X were published 33 fascicles, and in volume XI (Lepidoptera and Diptera Orders) appeared 15 fascicles. In volume X, dealing with the Coleoptera Order, with approximately 6,800 known species in the Romania (Nitzu, 2004), only seven fascicles were published, signed by some of the few coleopterologists in the country.

In the periods 2000–2006 and 2014–2021 were published several fascicles of volumes VIII, IX, X, XI (Insecta), XV (Aves) and XVI (Mammalia).

Beginning with 2000, among the publications from volume Mammalia, Dumitru Murariu (Editor-in-Chief of the *Romanian Fauna* since 2021) had a sustained activity, which has substantial contributions to the knowledge of mammals in our country. His experience and professional results are presented in over 25 books about mammals in Romania and around the world, of which seven issues (three in English) are published in the *Romanian Fauna*.

Currently, Academician Dumitru Murariu almost finished Fascicle 5 (Carnivora, in English version) as well as Fascicle 2 (Rodentia, in English too). Also, the issue that deals with the general part of the Hymenoptera Order (of which 12 fascicles were already published) is being finalized by Professor Irina Teodorescu.

Table 1

The list of volumes and fascicles published in the *Romanian Fauna*

Nr. crt.	Volume	Fascicle	Taxonomic group	Authors	Year	Number of pages
1.	**GUIDE (first part)**			Radu Codreanu, Mihai Băcescu (Coord.)	1951	252
2.	**Vol. I PROTOZOA**	*1*	Hypermastigina	Adriana Murgoci	1951	36
3.		*2*	Rhizopoda, Euamoebidea	Iosif Lepşi	1960	435
4.		*3*	Tubulinea, Cls. Corycida and Cls. Elardia (Ord. Arcellinida)	Stoica Godeanu	2021	417
5.		*4*	Stramenopiles, Cls. Labirinthulomycetes (Ord. Amphytremida), Cercozoa, Cls. Thecofilosea and Cls. Silicofilosea (Ord. Euglyphida)	Stoica Godeanu	2021	221
6.	**Vol. II**	*1*	Plathelminthes, Cls. Monogenoidea	Elena Roman-Chiriac	1960	150
7.		*2*	Trochelminthes, Rotatoria	Ludovic Rudescu	1960	1192
8.		*3*	Nematoda, Mermithidae	Dan Coman	1961	62
9.		*3*	Trochelminthes, Gastrotricha	Ludovic Rudescu	1967	295
10.		*4*	Trematoda	Elena Chiriac and Maria Udrescu	1973	497
11.		*5*	Porifera, Potamospongiae	Ludovic Rudescu	1975	117
12.	**Vol. III MOLLUSCA**	*1*	Gastropoda, Pulmonata	Alexandru V. Grossu	1955	519
13.		*2*	Gastropoda, Prosobranchia and Opistobranchia	Alexandru V. Grossu	1956	222
14.		*3*	Bivalvia	Alexandru V. Grossu	1962	427
15.	**Vol. IV CRUSTACEA, TARTIGRADA**	*1*	Cumacea	Mihai Băcescu	1951	95
16.		*2*	Phyllopoda	Nicolae Botnariuc and Traian Orghidan	1953	100
17.		*3*	Mysidacea	Mihai Băcescu	1954	126
18.		*4*	Amphipoda (brackish forms and freshwater forms)	Sergiu Cărăuşu, Ecaterina Dobreanu and Constantin Manolache	1955	410
19.		*5*	Bathynellacea	Lazăr Botoşăneanu	1959	37
20.		*6*	Copepoda, Cyclopidae (freshwater forms)	Andriana Damian-Georgescu	1963	207
21.		*7*	Tardigrada	Ludovic Rudescu	1964	403
22.		*8*	Copepoda, Calanoida (freshwater forms)	Andriana Damian-Georgescu	1966	131
23.		*9*	Decapoda	Mihai Băcescu	1967	351
24.		*10*	Ostracoda, Cytheridae	Francisca-Elena Caraion	1967	165

Table 1 (continued)

Nr crt	Volume	Fascicle	Taxonomic group	Authors	Year	Number of pages
25.	**Vol. IV CRUSTACEA, TARTIGRADA**	*11*	Copepoda, Harpacticoida (freshwater forms)	Andriana Damian-Georgescu	1970	254
26.		*12*	Cladocera	Ştefan Negrea	1983	399
27.		*13*	Isopoda, Oniscoidea	Vasile Gh. Radu	1983	168
28.		*14*	Isopoda, Oniscoidea, Crinochaeta	Vasile Gh. Radu	1985	159
29.	**Vol. V ARACHNIDA**	*1*	Acarina, Trombidoidea	Zicman Feider	1955	190
30.		*2*	Acaromorpha, Ixodoidea	Zicman Feider	1965	407
31.		*3*	Araneae, Fam. Lycosidae	Ion E. Fuhn and Floriana Niculescu-Burlacu	1971	256
32.		*4*	Araneae, Fam. Clubionidae	Cleopatra Sterghiu	1985	168
33.		*5*	Araneae, Fam. Salticidae	Ion E. Fuhn and Viorel F. Gherasim	1995	302
34.	**Vol. VI**	*1*	Chilopoda, Anamorpha	Zachiu Matic	1966	269
35.		*2*	Chilopoda, Epimorpha	Zachiu Matic	1972	224
36.	**Vol. VII INSECTA (I)**	*1*	Protura	Mihai A. Ionescu	1951	48
37.		*2*	Diplura	Mihai A. Ionescu	1955	51
38.		*3*	Ephemeroptera	Constantin Bogoescu	1958	190
39.		*4*	Orthoptera (Saltatoria, Dermaptera, Blattodea, Mantodea Orders)	Wilhelm K. Knechtel and Andrei Popovici-Bâznoşanu	1959	337
40.		*5*	Odonata	Filimon Cîrdei and Felicia Bulimar	1965	275
41.	**Vol. VIII INSECTA (II)**	*1*	Thysanoptera	Wilhelm K. Knechtel	1951	263
42.		*2*	Isoptera	Mihai A. Ionescu	1951	24
43.		*3*	Homoptera, Psylloidea	Ecaterina Dobreanu and Constantin Manolache	1962	379
44.		*4*	Homoptera - general part	Ecaterina Dobreanu and Constantin Manolache	1969	102
45.		*5*	Homoptera, Aleyrodoidea, Aleyrodinae	Ecaterina Dobreanu and Constantin Manolache	1969	154
46.		*6*	Neuroptera (Planipennia)	Béla Kis, Carol Nagler and Constantin Mîndru	1970	346
47.		*7*	Plecoptera	Béla Kis	1974	273
48.		*8*	Heteroptera - general part and Superfamily Pentatomoidea	Béla Kis	1984	214
49.		*9*	Heteroptera, Coreoidea and Pyrrhocorioidea Superfamilies	Béla Kis	2001	99
50.	**Vol. IX INSECTA (III)**	*1*	Hymenoptera, Apinae	Wilhelm K. Knechtel	1955	114
51.		*2*	Hymenoptera, Cynipinae	Mihai A. Ionescu	1957	248
52.		*3*	Hymenoptera, Apoidea (Fam. Apidae, Subfam. Anthophorinae)	Victoria G. Iuga	1958	271
53.		*4*	Hymenoptera, Fam. Ichneumonidae, Subfam. Ichneumoninae, Tribe Ichneumoninae-Stenopneusticae	Mihai I. Constantineanu	1959	1249

Table 1 (continued)

Nr crt	Volume	Fascicle	Taxonomic group	Authors	Year	Number of pages
54.	**Vol. IX INSECTA (III)**	*5*	Hymenoptera, Fam. Ichneumonidae, Phaeogeninae and Alomyinae Subfamilies	Mihai I. Constantineanu	1965	510
55.		*6*	Hymenoptera, Cynipoidea, Fam. Ibaliidae, Subfam. Ibaliinae, Fam. Figitidae, Subfam. Aspicerinae, Anachartinae, Figitinae, Fam. Cynipidae, Eucoilinae and Charipinae Subfamilies	Mihai A. Ionescu	1969	290
56.		*7*	Hymenoptera, Fam. Ichneumonidae, Ephialtinae, Lycorininae, Xoridinae and Acaenitinae Subfamilies	Mihai I. Constantineanu and Constantin Pisică	1977	305
57.		*8*	Hymenoptera, Symphyta, Tenthredinoidea, Fam. Tenthredinidae, Selandriinae, Tenthredininae and Heterarthrinae Subfamilies	Xenia G. Scobiola-Palade	1978	248
58.		*9*	Hymenoptera, Symphyta, Tenthredinoidea, Fam. Tenthredinidae, Blennocampinae and Nematinae Subfamilies	Xenia G. Scobiola-Palade	1981	328
59.		*10*	Hymenoptera, Fam. Ichneumonidae, Subfam. Mesochorinae	Mihai I. Constantineanu and Gheorghe I. Mustaţă	1982	105
60.		*11*	Hymenoptera, Fam. Braconidae: general part, Cardiochilinae, Microgastrinae, Acaeliinae and Miracinae Subfamilies	Matilda Lăcătuşu and Constantin Filipescu	1989	315
61.		*12*	Hymenoptera, Fam. Ichyneumonidae, Cteniscinae, Tryphoninae, Thymaridinae and Sphinctinae Subfamilies	Mihai I. Constantineanu, Raoul Constantineanu, Irinel Gh. Constantineanu	2000	268
62.	**Vol. X INSECTA (IV)**	*1*	Coleoptera, Fam. Cicindelidae	Sergiu Panin	1952	56
63.		*2*	Coleoptera, Fam. Carabidae (*Cychrus* Fabricius and *Carabus* Linnaeus genera)	Sergiu Panin	1955	148
64.		*3*	Coleoptera, Fam. Scarabaeidae, Melolonthinae and Ruthelinae Subfamilies	Sergiu Panin	1955	124

Table 1 (continued)

Nr crt	Volume	Fascicle	Taxonomic group	Authors	Year	Number of pages
65.	**Vol. X INSECTA (IV)**	*4*	Coleoptera, Fam. Scarabaeidae, Coprinae, Geotrupinae, Aphodiinae, Aegialinae, Hybosorinae, Ochodaeinae, Orphninae, Troginae, Glaphyrinae, Sericinae, Hoplinae, Dynastinae, Valginae, Trichiinae and Cetoniinae Subfamilies	Sergiu Panin	1957	319
66.		*5*	Coleoptera, Fam. Cerambycidae	Sergiu Panin and Nicolae Săvulescu	1961	523
67.		*6*	Coleoptera, Fam. Carabidae (Tribe Bembidiini)	Eugen Niţu	2006	196
68.		*7*	Coleoptera, Fam. Buprestidae	Sergiu Panin, Nicolae Săvulescu and Adrian Ruicănescu	2015	390
69.	**Vol. XI INSECTA (V)**	*1*	Lepidoptera, Fam. Aegeriidae	Aurelian Popescu-Gorj, Eugen Niculescu and Al. Alexinschi	1958	199
70.		*2*	Diptera, Fam. Tabanidae	Gheorghe Dinulescu	1958	279
71.		*3*	Diptera, Fam. Syrphidae	Petru Şuster	1959	287
72.		*4*	Diptera, Fam. Oestridae	Gheorghe Dinulescu	1961	168
73.		*5*	Lepidoptera, Fam. Papilionidae	Eugen Niculescu	1961	107
74.		*6*	Lepidoptera, Fam. Pieridae	Eugen Niculescu	1963	203
75.		*7*	Lepidoptera, Fam. Nymphalidae	Eugen Niculescu	1965	364
76.		*8*	Diptera, Fam. Simuliidae	Gheorghe Dinulescu	1966	603
77.		*9*	Lepidoptera, Fam. Tineidae	Iosif Căpuşe	1968	467
78.		*10*	Lepidoptera, the general part	Eugen Niculescu and Frederic König	1970	306
79.		*11*	Diptera, Fam. Asilidae	Mihai A. Ionescu and Medeea Weinberg	1971	288
80.		*12*	Diptera, Fam. Calliphoridae	Andy Lehrer	1972	251
81.		*13*	Diptera, Fam. Chironomidae	Paula Albu	1980	318
82.		*14*	Diptera, Fam. Ceratopogonidae (genus *Culicoides*)	Adriana Damian-Georgescu	2000	142
83.		*15*	Diptera, Fam. Chironomidae, Subfam. Orthocladiinae	Victoria Tatole	2003	175

Table 1 (continued)

Nr crt	Volume	Fascicle	Taxonomic group	Authors	Year	Number of pages
84.	**Vol. XII CHAETOGNATHA; ECHINODERMA; STOMOCHORDATA, TUNICATA, CEPHALOCORDATA, CYCLOSTOMATA, CONDRICHTHYES**	*1*	Cyclostomata and Chondrichthyes	Petru-Mihai Bănărescu	1969	107
85.	**Vol. XIII PISCES (II)**		Osteichthyes	Petru-Mihai Bănărescu	1964	972
86.	**Vol. XIV AMPHIBIA and REPTILIA**	*1*	Amphibia	Ion Fuhn	1960	289
87.		*2*	Reptilia	Ion Fuhn and Ştefan Vancea	1961	353
88.	**Vol. XV AVES**	*1*	Gaviiformes, Podicipediformes, Procellariiformes, Pelecaniformes	Ion Cătuneanu, János Korodi-Gál, Dan Munteanu, Sergiu Paşcovschi and Emil Vespremeanu	1978	317
89.		*2*	Galliformes, Ciconiiformes	Dan Munteanu (Coord.); autori: Gabriel Chişamera, Alin David, Simon Dieter, Nicolae Onea, Angela Petrescu, Eliana Sevianu, Alexandru N. Stermin	2015	308
90.	**Vol. XVI MAMMALIA**	*1*	Insectivora	Dumitru Murariu	2000	152
91.		*1*	Insectivora (the english version)	Dumitru Murariu	2014	163
92.		*2*	Rodentia	Alexandrina Popescu and Dumitru Murariu	2001	214
93.		*3*	Chiroptera	Nicolae Valenciuc	2002	166
94.		*3*	Chiroptera (the english version)	Dumitru Murariu, Gabriel Chişamera, D. Ştefan Măntoiu, Irina Pocora	2016	283
95.		*4*	Lagomorpha, Cetacea, Artiodactyla, Ord. Perissodactyla (without recent species)	Dumitru Murariu	2004	209
96.		*4*	Lagomorpha, Cetacea, Artiodactyla, Ord. Perissodactyla (without recent species) (the english version)	Dumitru Murariu	2021	221
97.		*5*	Carnivora	Dumitru Murariu and Dan Munteanu	2005	223

The activity of the *Romanian Fauna* is coordinated by an Editorial Committee, which, over time, has included a number of personalities who, although constrained perhaps by the time and place in which they lived, through the results of research have managed to contributes significantly to the development of zoology in our country. Among them, we mention: Wilhelm K. Knechtel, Mihai A. Ionescu, Mihai I. Constantineanu, Constantin Manolache, Vasile Gh. Radu, Nicolae Botnariuc, Radu Codreanu, Mihai Băcescu, Constantin Motaş, Petru-Mihai Bănărescu and others.

The contributions of the Romanian specialists, as first authors of the *Romanian Fauna* issues, summarized in the total number of pages and published fascicles, are presented in Fig. 3 and Fig. 4.

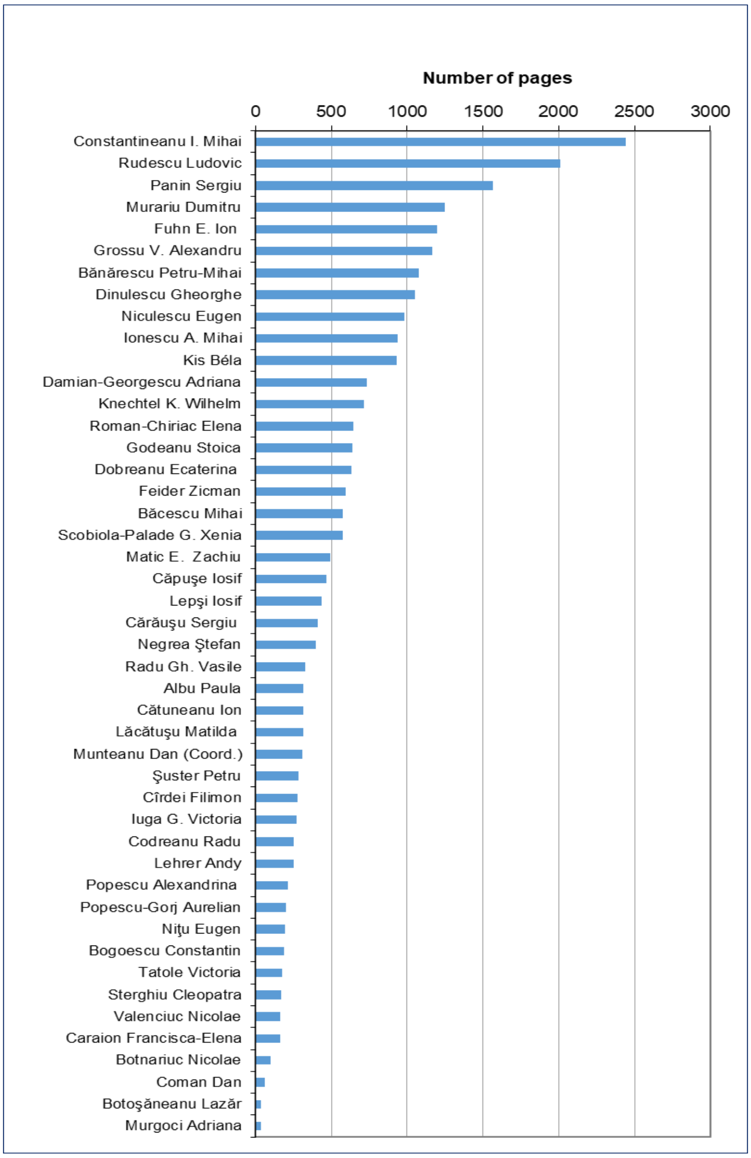

Fig. 3. *Romanian Fauna* – First author contribution
by the number of published pages between 1951–2021.

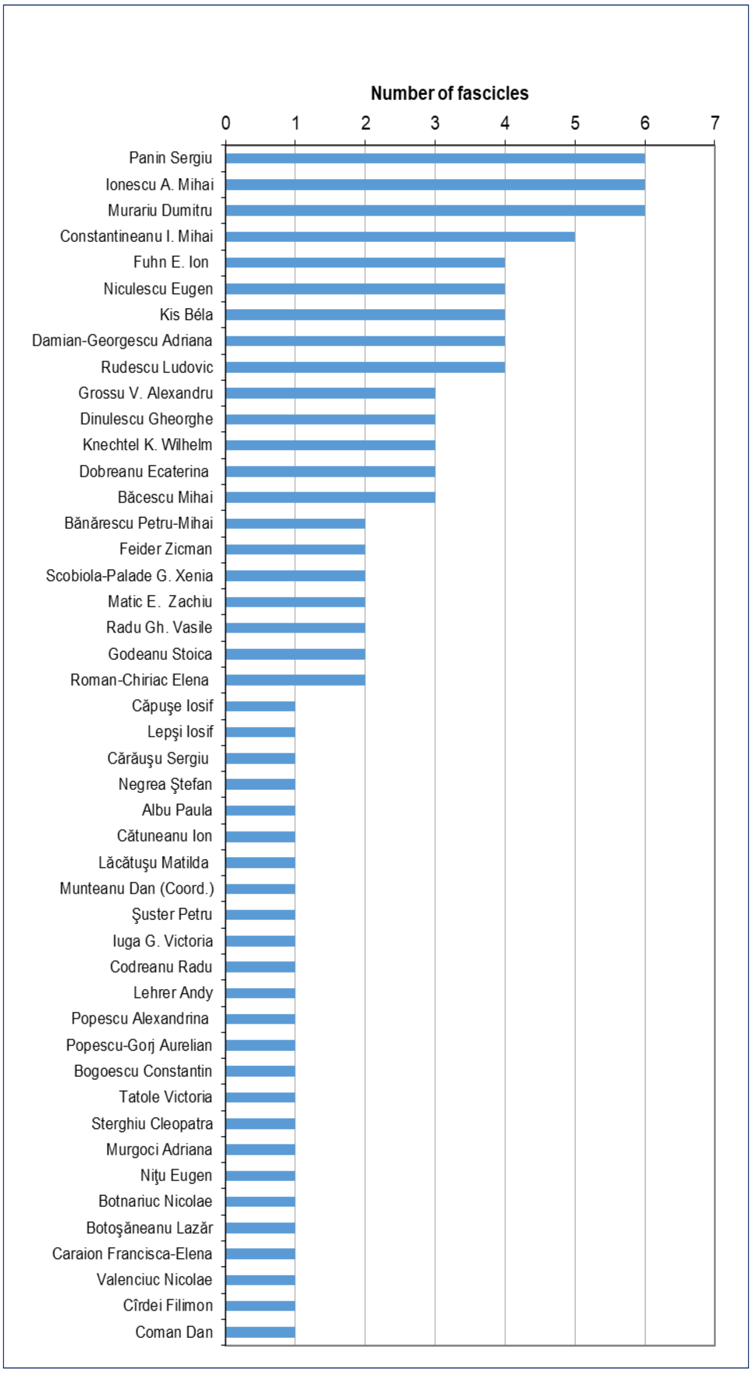

Fig. 4. *Romanian Fauna* - First author contribution
by the number of published fascicles between 1951 – 2021.

The information taken from the biographies of some of the first contributors to the *Romanian Fauna*, exemplified below, is eloquent and demonstrates that they were already well-known personalities in the field of zoology, who had published important papers on the systematic group studied, impacting the national and international scientific community. At that time, the international scientific circles were surprised by the "impetuous" appearance of this work, for foreign specialists being a real revelation the existence in Romania of a nucleus of trained zoologists, who were able to accomplish a high work of scientific value in a short time (Radu, 1966).

Mihai I. Constantineanu (1894–1993) – exceptional entomologist, Emeritus university Professor and researcher of international prestige, school creator, specialized in the study of entomophagous insects of Ichneumonidae family, wrote in the *Romanian Fauna* in 1959 a monumental volume of 1,249 pages and, in 1965, a volume of 510 pages. But, Mihai I. Constantineanu had thoroughly mastered the "books of biology", especially zoology, since the time when he was a student at the high school of zoology – "Alexandru Ioan Cuza" University in Iași, under the guidance of the great naturalists Ion Borcea, Ion Simionescu, Paul Bujor, Ion Scriban, etc. He began studying ichneumonids as early as 1924, when he took specialization courses in Vienna, the Museum of Natural History, and the Plant Protection Station in Austria, and then completed his studies at the Institute of Zoology in Berlin (1927–1929). In addition to studying the Ichneumonidae family, Mihai I. Constantineanu was also interested in certain aspects of pest insects and biological control. He studied the structure of insect eyes, graduating from the "Friedrich Wilhelm" University in Berlin. He continued his specialization in France (1929–1930) at the National Agronomic Institute, the Marine Biological Station at Banyuls-sur-Mer, the Roskoff Marine Station, the National Museum of Natural History in Paris and in Italy – at the Higher Institute of Agronomy at Porches. Together with his students, he also published a series of fascicles from the *Romanian Fauna*: with Constantin Pisică in 1977, with Gheorghe I. Mustaţă in 1982, with Raoul Constantineanu and Irinel Gh. Constantineanu in 2000. In the impressive work of Professor Mihai I. Constantineanu (including over 5,500 pages), 2,419 species of ichneumonids from Romania are reported, of which 70 species, 120 subspecies and 176 varieties are new to science (Constantineanu & Constantineanu, 2021).

Knechtel Wilhelm Karl (1884–1967) – Romanian entomologist of German origin, attended Herăstrău High School and studied in Germany, in Stuttgart-Hohenheim (1904–1907). He specialized in plant protection in Naples – Italy, then at the Institute of Biology in Berlin (1912–1914). He has published a series of papers on pest insects, especially on tisanopters (*Thysanoptera in Romania. Morphological studies; Pest insects in Romania and the means to control them*). He became a Professor at the School of Viticulture in Chișinău – Moldova (1918–1920), then a Professor at the Faculty of Agronomy in Bucharest (1940–1944).

Academician Wilhelm K. Knechtel is considered the founder of the Romanian School of Agricultural Entomology. Within the Entomology Section of the Descriptive Zoology Laboratory of the Faculty of Natural Sciences in Bucharest, coordinated by W. K. Knechtel, five fascicles were published in the *R.P.R Fauna* Collection.

Iosif Lepși (1895–1966) belongs to the gallery of some notable personalities from the interwar period. With higher education in Bucovina, in Chernivtsi, he became a doctor of biological sciences, professor, zoologist, nature researcher, geographer and director of the National Museum of Natural History in Chișinău. He studied, developed and implemented the development programs of the Museum, the various methods of establishing collections and popularizing cultural values in Bessarabia. The *History of Romanian Sciences* (Pop & Codreanu, 1975) mentions that Iosif Lepși published taxonomic papers on ciliates and other free protozoans (Berlin, 1926; Romanian Academy, 1927, 1929), as well as general research in hydrobiology, fauna and geography, especially in Dobrogea. He also wrote two important monographs: *Euamoebidea* (1960) and *Protozoologia* (1965) and carried out the first limnological studies on the Tăbăcărie, Siutghiol and St. Ana lakes.

Victoria Iuga-Raica (n. 1900) was a brilliant representative of Romanian biology. She graduated from private secondary school and high school in Bucharest, but due to the WWI, she had her baccalaureate in Roman city – Moldova, her refuge place. She then attended the Faculty of Natural Sciences in Bucharest, with renowned professors: A. Popovici-Bâznoşanu, Ion Atanasiu, Ludovic Mrazec, Traian Săvulescu. She was enrolling in a Doctorate School at the Department of Animal Morphology, led by the world-renowned scholar and cytologist Dimitrie Voinov, who was also her first husband. From 1920 to 1940 she had a teaching career, going through the ranks of assistant, lecturer and professor at the Faculty of Natural Sciences in Bucharest.

As Head of Department at the "Grigore Antipa" National Museum of Natural History, she founded the Entomology Collection. Between 1949–1959 she also cumulated the position of senior researcher in the Fauna Collective of the Romanian Academy. She has done research internships in various institutions abroad: Aquariums in Trieste and Naples in Italy as well as well as in Monaco and Roscoff Marine Research Station in France, Museums of natural History in Budapest, Vienna, Paris, London in various laboratories and libraries of Universities in France and England, Institute of Zoology of the Russia (former Soviet Union) Academy from Leningrad (today Sankt Petersburg). The results of her research can be found in over 100 published scientific papers, in which she dealt with topics of cytology, histology, systematics, ecology.

Mihai C. Băcescu (1908–1999) – Romanian zoologist, oceanologist and museologist, full member of the Romanian Academy, attended the Faculty of Natural Sciences in Iași, where he attended lectures given by professors Ion Borcea and Paul Bujor. From the second year of his studies, he was appointed preparator at the Department of Animal Morphology, specializing in aquatic and marine fauna, especially in crustaceans and fish. In 1933 he began his doctorate training and spent five years at the Agigea Marine Zoological Station and at the Cape Caliacra Resort, managed by the Bio-Oceanographic Institute of Constanța, founded by Dr. Grigore Antipa. In 1938 he defended his doctoral thesis on Mysidaceae in Romanian waters. At the recommendation of Paul Bujor and Emil Racoviță, in 1939 he obtained a scholarship in France, where the young scientist made an excellent impression at the National Museum of Natural History in Paris, the Oceanographic Museum in Monaco and in the various Marine Biology Stations where he worked. Later, he went through the steps of international scientific affirmation, leading the National Museum of Natural History "Grigore Antipa" and in the Romanian Academy, becoming the founder of the Romanian Schools of Biological Oceanography, Carcinology and Museography. Here, Mihai Băcescu created an important school of taxonomists with woorld-class carcinologists: Modest Guţu, Zaruhi Muradian, Iorgu Petrescu, Ileana Negoescu, etc.

Ludovic Iosif Urban Rudescu (1908–1999) was a hydrobiologist and naturalist, with college and university studies in Chernivtsi – Bucovina. After graduation, he followed various specializations in Berlin, Stockholm, Lund, Uppsala, Gothenburg, etc. In 1948, he became a researcher at the Institute of Cellulose and Paper in Bucharest, then at the Institute of Biology. Doctor in natural sciences, specialized in biology, hydrobiology and reed farming, since 1967 he has been Head of Department at the "Traian Săvulescu" Institute of Biology in Bucharest. He became a member of the American Society of Limnology and Oceanography, the American Society of Microscopy, and the International Society of Limnology. A lover of hiking and hunting, he devoted much of his time to microscopic studies of rotifers, gastrotrichs and tardigrades in the benthos and plankton microfauna, about which he published over 2,000 pages in the *Romanian Fauna* Collection.

Mihail Andrei Ionescu (1900–1988) was a famous entomologist, professor at the University of Bucharest. He graduated from the Faculty of Science in Bucharest and specialized at the Museum of Natural History in Vienna and the Institute of Biooceanography in Split. He became a professor of entomology at the Faculty of Biology - University of Bucharest (1949–1968). He formed a Romanian entomology school by developing entomological education, with eminent disciples (Matilda Lăcătușu, Constanța Tudor, Nicolae Toniuc, Irina Teodorescu, Medeea Weinberg), with whom he had a close collaboration. He made significant contributions in the fields of insect systematics, ecology and zoogeography (Protura, Diplura, Collembola, Termitina, Cynipoidea, Asilidae, etc.).

Alexandru Grossu (1910–2004) is considered one of the most valuable malacologists of the 20th century, who distinguished himself both in his teaching and in his scientific research. From 1951 he was Professor and Cancellor of the

Pedagogical Institute of Bucharest, and from 1955 Professor at the Faculty of Biology - University of Bucharest, for a period of 20 years. The results of his research and his passion for the study of mollusks have been materialized in the description of dozens of new gastropod species for science, in the pages of over 160 scientific articles, including three fascicles (Mollusca) published in the "*Romanian Fauna*" (1955, 1956, 1962).

Petru-Mihai Bănărescu (1921–2009), valuable Romanian zoologist and biogeographer, nationally and internationally recognized for his significant contributions in the fields of zoological taxonomy, systematics and biology of marine and freshwater fish species. The results of his research have been included in over 350 scientific papers, including two issues published in the *Romanian Fauna* (*Pisces-Osteichthyes* and *Cyclostomata and Chondrichthyes*), as well as the volumes: *Principles and problems of zoogeography*. *Zoogeography, Biogeography* (in collaboration), *Principles and methods of systematic zoology*, *The Fresh Water Fishes of Europe*. His research has focused on the systematics of the Cyprinidae and Cobitidae families in Europe and Asia, the zoogeography of freshwater fauna, the principles of taxonomy, the problems of species and speciation, and, equally, the problems of nature conservation. The volume *Pisces-Osteichthyes*, published in the *Romanian Fauna* Collection, remains today the most valuable and comprehensive monograph on freshwater and marine fish in our country. At the Institute of Biology of the Romanian Academy, Petru-Mihai Bănarescu created a valuable collection of freshwater fish from Eurasia, North America, partly Africa, this collection being later donated to "Grigore Antipa" National Museum of Natural History, Bucharest.

REASONS FOR UPDATING AND COMPLETION OF THE *ROMANIAN FAUNA* ACADEMIC COLLECTION

Although the publication of the 97 issues in the *Romanian Fauna* is an extremely necessary and valuable achievement, over time, the Romanian zoological community has moved away from the project of publishing and finalizing this national work – a fundamental "document" of nature in our country. At present, 71 years after the publication of the volume *Guide*, with rare exceptions, the completion of the *Fauna* has been interrupted. In comparison, the world of plants in our country is much better known, through the complete publication of the *Romanian Flora*, materialized in 13 volumes, published between 1952–1964, under the coordination of Academician Nicolae Sălăgeanu.

The interest of Romanian zoologists, especially of young people, for the so-called classical sciences (taxonomy, systematics) has decreased, which has contributed to a shortage of specialists in the study of certain groups, especially invertebrates. In the past, in our country, research on invertebrates has been less numerous, compared to those on vertebrates. Due to their specific diversity and extremely high abundance, invertebrates have a significant ecological impact on the living world, so the training of specialists in the study of invertebrates is urgently needed.

Although traditional methods of working in taxonomy are recognized and give correct and objective results, they also have obvious disadvantages, the training of a specialist in a certain taxonomic group requiring a lot of time, experience and sustained work. According to Popescu (2008) "…*it is necessary to maintain at an acceptable level the scientific research that refers to the classical biological disciplines (botany, zoology). In Romania we have the chance to still have very good specialists in classical taxonomy both in botany and in zoology [...]. Molecular taxonomy cannot be done without classical taxonomy. The use of modern methods in taxonomy complements the research undertaken by classical methods*".

CONCLUSIONS AND PROPOSALS

1. For the scientific attestation of all animal species from the natural capital of the country in the *Romanian Fauna* Collection – a work of a large scientific scale, a collective effort is required to complete it, by publishing new fascicles related to taxonomic groups that were not processed, mentioned in the volume *Guide*.

2. Due to the recent progress in the fields of animal systematics and taxonomy, by including new information obtained in related fields (ecology, physiology, molecular biology), it is necessary to update the first edition of the *Romanian Fauna* fascicles.

3. This update is necessary because the systematic classification and nomenclature of species have changed over time, some species have been synonymized both by deepening classical morphology research and by clarifications brought by molecular biology studies. It is also necessary to bring "up to date" the knowledge about the spread of species worldwide and in Romania, to confirm the presence in the country of some species mentioned in the older works, requiring new data collection.

4. In order to continue and update the *Romanian Fauna* first of all it is necessary the identification of qualified human resources, but also the training of new researchers, especially for groups of animals less or not at all represented in *Fauna* – young people with a vocation for zoological research, in close collaboration and exchange of information with specialists in our country and with those abroad.

5. School teachers and university professors, scientific researchers from the Research Institutes of the Romanian Academy and other biological research institutes, museographers of "Grigore Antipa" National Museum of Natural History and other museums of natural sciences, etc. can meet in a *Society of Zoologists in Romania*, following the model of other countries.

6. Zoologists-taxonomists must be more involved in joint projects on the taxonomic study of national fauna, by funding with priority complex research programs on knowledge of specific biodiversity and the distribution and abundance of animal organisms in our country. Young people interested in a career in the field of zoological research may be encouraged to choose for their studies in doctoral theses a group of organisms that have not been treated in the *Romanian Fauna*.

7. Greater attention should be paid to research into the study of invertebrates, the largest group in the Animal Kingdom. Despite their impressive diversity and importance in the living world, interest in the study of “these little animals” has waned due to difficulties in identification and, implicitly, the small number of specialists. Invertebrate species are insufficiently known from a taxonomic, systematic, biological point of view and are less present in the Lists of protected species by national and international regulations, compared to larger animals.

8. For the information contained in the *Romanian Fauna* issues to be accessible to the international scientific community, it is necessary to translate them in English.

9. Romania has a remarkable faunal diversity (about 33,696 animal species are known, of which about 33,085 species of invertebrates and 611 species of vertebrates), of which about a third have been studied. In this context, the continuation and deepening of taxonomy, systematics and zoogeography research in Romania, the results of which will be published in the *Romanian Fauna*, will have to become a priority assumed by the entire zoological community in our country, so that the over 70 years old project of the Romanian Academy to be completed.

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APETHYMUS FILIFORMIS (HYMENOPTERA: TENTHREDINIDAE), NEW HOST FOR FIVE PARASITOID SPECIES (HYMENOPTERA: BRACONIDAE, ICHNEUMONIDAE)

RAOUL CONSTANTINEANU*, CAMIL ȘTEFAN LUNGU-CONSTANTINEANU

The paper presents some aspects about the attack and biology of *Apethymus filiformis* (Klug.), a non-indigenous defoliator pest of the sessile oak forest stands, *Quercus petraea* (Matt.) Liebl., 1784. It was recorded for the first time in Romania in 1999, but misidentified as *Apethymus abdominalis* Lep., a synonim of *A. cereus* Klug., an accepted species, but not present in the Romanian fauna. We established five new host – parasitoid relationships. In the laboratory we obtained from *Apethymus filiformis* larvae, by rearings, five new ichneumonoid parasitoid species: *Charmon extensor* (Linnaeus) (Hymenoptera: Braconidae: Charmontinae), *Temelucha ophtalmica* (Holmgren) (Hymenoptera: Ichneumonidae: Cremastinae), *Gelis cinctus* (Linnaeus) (Hymenoptera: Ichneumonidae: Cryptinae), *Diplazon laetatorius* (Fabricius) (Hymenoptera: Ichneumonidae: Diplazontinae), and *Phytodietus ornatus* (Desvignes) (Hymenoptera: Ichneumonidae: Tryphoninae).

Keywords: *Apethymus filiformis*, Ichneumonoidea, parasitoids, new host, *Quercus petraea*.

INTRODUCTION

Apethymus filiformis (Klug.) was recorded in Romania for the first time by Ciornei & Mihalache (1999), but misidentified as *Apethymus abdominalis* Lep., a synonym of *A. cereus* Klug., a species which is not present in the Romanian fauna, according to Fauna Europaea. During the period 1999 – till now, in some papers, *Apethymus filiformis* was misidentified as *Apethymus abdominalis* Lep. (Ciornei *et al*., 2001, 2003, 2005). Later, it was recorded correctly by Popa (2006), being identified by Constantineanu Raoul.

Apethymus filiformis (Fig. 1) is a widespread species in Europe, being previously reported from 21 countries (acccording to Fauna Europaea).

It is not known how it was introduced in Romania, but in the absence of its natural enemies, it produced outbreaks in some forests in Moldova, Romania. Later, in Romania, some biological limiting factors for *Apethymus filiformis* were recorded: the oophagous parasitoid *Trichogramma* sp. (in the Heltiu, Păltinata and Cornățel forests, Silvicultural District Căiuți, Bacău county) (Ciornei *et al*., 2005). In the same forests, Ciornei *et al*. (2005) recorded also the following predator birds: *Dendrocopos major* (L., 1758), *Picus canus* Gmel., 1788 (Picidae), *Lanius collurio* L., 1758 (Laniidae), *Ficedula albicollis* (Temminck, 1815) (Muscicapidae), *Phylloscopus collybita* (Vieillot, 1817) (Phylloscopidae), *Sturnus vulgaris* L., 1758 (Sturnidae), *Sylvia atricapilla* (L., 1758), *Sylvia communis* (Latham, 1787) (Sylviidae), *Coccothraustes coccotraustes* (L., 1758) (Fringillidae) and *Emberiza citrinella* L., 1758 (Emberizidae). Later it was recorded correctly by Popa (2006), being identified by Raoul Constantineanu.

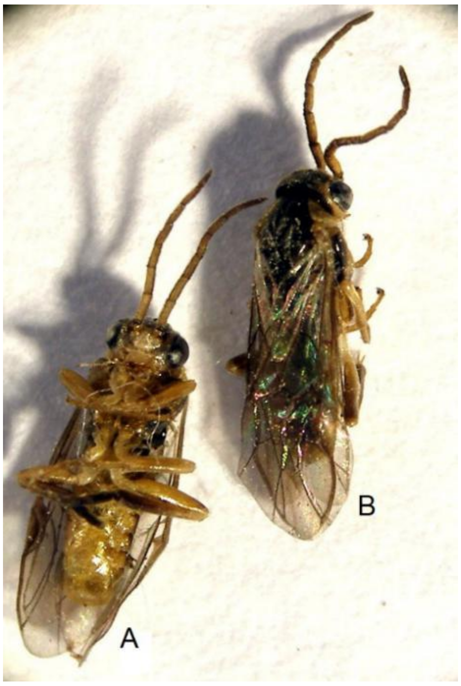

Fig. 1. *Apethymus filiformis* (Klug.), adults: A – ventral view; B – dorsal view (orig).

Ciornei *et al*. (2005) catched in the Barber traps many species of Carabidae (Coleoptera) and they supposed that especially the following species: *Molops piceus* (Panz.), *Pterostichus oblongopunctatus* (F.), *Carabus arvensis* Herbst, *Calosoma inquisitor* (L.) and *Abax ovalis* (Duftschmid) can limit the populations of *Apethymus filiformis*.

MATERIAL AND METHODS

The field observations of the attack of *Apethymus filiformis* and the collectings of its larvae, were made during the period of May 2005 – June 2007 in Heltiu, Păltinata and Cornățel forests, Silvicultural District Căiuți, situated in the Trotuș valley, between the localities Onești (Bacău county) and Adjud (Vrancea county), Eastern Romania. Păltinata and Cornățel are sessile oak and beech mix forest stands and Heltiu is a sessile oak stand. The last stage larvae of *Apethymus filiformis* (Fig. 2), were collected especially from the stalks of the sessile oak trees. At each scientific trip we collected 100 larvae of *Apethymus filiformis* at the following forests: Heltiu (10.05.2005 and 12.06.2006), Păltinata (12.05.2006) and Cornățel (02.05.2007). It were studied four different samples of 100 larvae each.

Laboratory rearings were performed as follows: each larva of *Apethymus filiformis* collected from infested oak forests, was introduced into an individual new vials of 30 ml capacity, labeled and checked every day. Each vial was covered with a cotton plug, to provide the air needed for the larva to survive. Each larva was fed with clean oak leaves, uninfested with other pests. There were provided conditions close to those in nature, regarding temperature (20–21°C), humidity, feeding. After a few days, the last stage larvae turned into eonymph (Fig. 3), a stage from which the parasitoids emerged.

Fig. 2. *Apethymus filiformis* – last instar larva (orig.).

Fig. 3. *Apethymus filiformis* – eonymph (orig.).

RESULTS

From the *Apethymus filiformis* larvae reared in the laboratory, we obtained the adults of the following five ichneumonoid parasitoids: *Charmon extensor* (L.) (Hymenoptera: Braconidae: Charmontinae) (Fig. 4, Fig. 5), *Temelucha ophtalmica* (Holmgr.) (Hymenoptera: Ichneumonidae: Cremastinae) (Fig. 6), *Gelis cinctus* (L.) (Hymenoptera: Ichneumonidae: Cryptinae) (Fig. 7), *Diplazon laetatorius* (F.) (Hymenoptera: Ichneumonidae: Diplazontinae) (Fig. 8) and *Phytodietus ornatus* (Desv.) (Hymenoptera: Ichneumonidae: Tryphoninae) (Fig. 9, Fig. 10).

Fig. 4. *Charmon extensor*, ♀ – lateral view (orig.).

Fig. 5. *Charmon extensor*, ♂ – dorsal view (orig.).

Fig. 6. *Temelucha ophtalmica*, ♀ – dorsal view (orig.).

Fig. 7. *Gelis cinctus*, ♀ – dorsal view (orig.).

Fig. 8. *Diplazon laetatorius*, ♀ – dorsal view (orig.).

Fig. 9. *Phytodietus ornatus*, ♀ – dorsal view (orig.).

Fig. 10. *Phytodietus ornatus*, ♂ – dorsal view (orig.).

From sample no 1 emerged one individual of *Gelis cinctus*, from sample no 2 emerged one individual of *Charmon extensor*, from sample no 3 emerged three individuals of parasitoid species: *Charmon extensor*, *Temelucha ophtalmica* and *Phytodietus ornatus*, from sample no 4 emerged three individuals of parasitoid species: *Temelucha ophtalmica*, *Phytodietus ornatus* and *Diplazon laetatorius* (Table 1).

The parasitization degree varied between 1 to 3%, which was very low, but the presence of these ichneumonoid parasitoid species in the population of *Apethimus filiformis* is very important because it were obtained for the first time from this host.

Table 1

Parasitization degree of each sample

Sample	**Forest**	**Collecting date**	**Parasitoid**	**Parasitization degree**
1	Heltiu	10.05.2005	*Gelis cinctus*	1%
2	Heltiu	12.06.2006	*Charmon extensor*	1%
3	Păltinata	12.05.2006	*Charmon extensor* *Temelucha ophtalmica* *Phytodietus ornatus*	3%
4	Cornățel	02.05.2007	*Temelucha ophtalmica* *Phytodietus ornatus* *Diplazon laetatorius*	3%

The laboratory rearings lasted 8 to 16 days (Table 2).

Table 2

The parasitoids of *Apethymus filiformis* larvae

Parasitoid species	**Forest**	**Collecting date of *Apethymus filiformis* larvae**	**Emerging date of parasitoid species**	
Charmon extensor	Păltinata	12.05.2006	25.05.2006	
	Heltiu	12.06.2006	20.05.2006	
Temelucha ophtalmica	Păltinata	12.05.2006	25.05.2006	
	Cornăţel	02.05.2007	18.05.2007	
Gelis cinctus	Heltiu	10.05.2005	21.05.2005	
Diplazon laetatorius	Cornăţel	02.05.2007	17.05.2007	
Phytodietus ornatus	Păltinata	12.05.2006	22.05.2005	
	Cornăţel	02.05.2007	16.05.2007	

CONCLUSIONS

Apethymus filiformis (Klug.) is a new host for the following five ichneumonoid parasitoids: *Charmon extensor* (L.) (Hymenoptera: Braconidae: Charmontinae), *Temelucha ophtalmica* (Holmgr.) (Hymenoptera: Ichneumonidae: Cremastinae), *Gelis cinctus* (L.) (Hymenoptera: Ichneumonidae: Cryptinae), *Diplazon laetatorius* (F.) (Hymenoptera: Ichneumonidae: Diplazontinae) and *Phytodietus ornatus* (Desv.) (Hymenoptera: Ichneumonidae: Tryphoninae).

These parasitoid species belonging to the Ichneumonoidea Superfamily, have adapted to the pest invasive species from Romania, representing the parasitoid complex of *Apethymus filiformis*.

Apethymus filiformis is the first non-Lepidoptera host of the parasitoid *Charmon extensor* (L.).

Apethymus filiformis was previously misidentified, by different authors in Romania, as *Apethymus abdominalis* Lep., a synonym of *A. cereus*, species which is not present in the Romanian fauna.

The *Apethymus filiformis* status in Romania is updated.

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IMPACT OF INDUCED STRESS ON PARASITIC AND FREE LIVING NEMATODES FROM SELECTED AREAS OF GUJARAT: A PRELIMINARY STEP AIMED AT NEMATODE CONTROL

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Nematodes are a diverse group of soil fauna, the group of roundworms belonging to the phylum Nematoda. More than 14,000 nematode species have been described, distributed in almost every habitat, from free-living forms to parasitic types. In the present study, we identified five nematode species from Fenugreek leaves (*Trigonella foenum-graecum* L.), Green onion (*Allium fistulosum* L.) and Ginger (*Zingiber officinale* Roscoe), from freshwater bodies, as well as from neighbouring privately owned farmlands in the Village Boriavi, District Anand, Gujarat. After identification and classification nematodes were cultivated on Nematode Growth Medium (NGM) plates and assays for various stress parameters such as salinity, heat and pH were carried out to evaluate the influence of altered physical conditions on growth and survival of these organisms. The data obtained would facilitate measures for the control of agricultural pest nematodes. Identification studies revealed the presence of *Prodontorhabditis* spp. from Green onion (*Allium fistulosum*), *Eudorylaimus* spp. from Ginger (*Zingiber officinale*), *Doryllium minor* and *Radopholus* spp. from root soil of *Trigonella foenum-graecum* L. Anand district and *Aporcelaimus* spp. from freshwater bodies, Ahmedabad. Salinity stress was observed to increase the mortality rate in *Prodontorhabiditis* spp., whereas, specified increased salt concentration appeared to have growth promoting effects in juveniles of *Doryllium minor* and *Radopholus* spp. The results of the heat stress assay indicated that temperatures above 40ºC prove lethal for all test nematode species, with fatal effects in all developing stages. The results of the pH stress assay suggested that plant parasitic nematode species showed optimum growth at pH of 8 for *Radopholus* spp. Hence, maintaining pH conditions of pH 9 would prove effective in controlling the plant parasitic nematodes i.e. *Radopholus* spp. and this pH level would not adversely affect free living nematode species i.e. *Prodontorhabditis* spp., since they showed favourable growth conditions at a pH range from 8.5 to 9.0. Thus, the study helped to identify crucial stress conditions, which could help curtail growth of parasitic nematodes by altering the ambient soil environment.

Keywords: Salinity Stress, pH Stress, *Radopholus* spp., *Prodontorhabditis* spp., Heat stress.

INTRODUCTION

Nematodes have successfully adapted to nearly every ecosystem from marine to freshwater, in varied soils, from polar to tropic regions, as well as parasites of animals and plants. Plant parasitic and free living nematodes are known to tolerate a wide range of environmental disturbances and are recognized to adapt quickly, a phenomenon that aids in the evaluation of environmental health. Nematodes are useful indicators of environmental quality and have been reported to show sensitivity to a wide range of stress factors such as heat, salinity, temperature and pH (Dong *et al*., 2018). Stress assays are generally carried out using the free living, marine nematode *Caenorhabditis elegans*, as it is a research model species and a representative of phylum Nematoda. Consequently, *C. elegans* survival assays have proven to be key tools for studying stress response and physiological processes including aging, stress resistance and immunity. A remarkable advantage of using *C. elegans* for such assays is that synchronized isogenic populations are simple to obtain since the worm usually exists as a self-fertilizing, hermaphrodite that produces hundreds of isogenic progeny (Park *et al*., 2017). However, stress assays on plant parasitic nematodes are rarely done although such assays are inevitable, as these parasites have a major impact on agricultural yield, quality of food, as well as on the economy of agricultural outcome.

Stress assays mainly disrupt protein homeostasis (e.g., Heat stress assay) and in addition, adversely affect physiological activity, certain morphological features and behavioural patterns. Hence, evaluation of stress induced alterations and a study of the outcome of stress assays would provide valuable information for controlling plant parasitic nematodes in agriculture. It is thus, an environmentally friendly mode for sustainable agricultural development.

Many nematodes are well adapted to abiotic stress and are capable of cryptobiosis (hidden life): the ability to enter a state of suspended metabolic activity during unfavourable environmental conditions (drying, heat and cold). While not all nematodes are capable of cryptobiosis, the ones that are can often survive for years in a cryptobiotic state awaiting favourable conditions that will trigger their revival. The ability of nematodes to undergo cryptobiosis and overcome stress is one reason why some nematode species are very difficult to control or eradicate from a field (Kumar & Yadav, 2020). Some nematode species like *C. elegans* activate a specific and unique stress response when subjected to a combination of multiple abiotic and biotic stresses. Plant parasitic nematodes have been found to reduce stress tolerance levels in plants which lead to quality loss and yield loss of crop in agricultural fields (Atkinson & Urwin, 2012).

The influence of stressors on soil-borne pathogens has been a neglected area in stress research as most studies have been directed towards understanding stress response in plant parasitic nematodes (PPN) since they contribute to vast agricultural losses (Eastburn *et al*., 2010). Infestation of crops with plant-parasitic nematodes can exacerbate or counteract the effects of abiotic stress on plants, as their parasitic attack on roots severely disrupts plant water absorption (Bird, 1974; Smit & Vamerali, 1998). Very few sporadic research reports are available regarding the physiological stress responses of soil or plant parasitic nematodes in the State of Gujarat and hence there is now an urgent need to investigate altered stress conditions in nematodes of this region to provide insights for their control and suppression. Monitoring, curtailing and eradication of nematode attack are imperative to create avenues for developing healthy plants that maintain high yields.

The aim of this study was to evaluate the impact of induced stress on certain selected plant parasitic and free living nematodes collected from agricultural fields, using standard stress assays. The results obtained after carrying out the selected stress assays are beneficial for standardization of specific plant parasitic nematode cultivation methods under laboratory conditions. In addition, the data obtained following stress assays would help to reveal sensitivity of nematode species to altered environmental conditions and this in turn would prove useful in designing control measures to curb growth and survival of nematodes. Research to evaluate stress resistance provides valuable information regarding the interaction between internal or external stresses and biological processes, such as cellular homeostasis (Park *et al.*, 2017).

MATERIAL AND METHODS

Sample collection

Since most Plant Parasitic Nematodes (PPNs) are found around the plant roots, collection of rhizosphere samples was carried out in January 2020 from a private farmland in the Village Boriavi, District Anand, Gujarat State (22.61ºN, 72.93ºE). Soil samples were collected from 15–20 cm depth. After collection, the samples were kept moist and were contained in pre-cleaned polythene bags. Samples were stored in a cool, dry place between 16–20ºC. Following sample collection, nematode extraction and purification were carried out using the standard sieving method for free living terrestrial nematodes (Cobb, 1918) and for freshwater nematode identification and purification done by following differential floatation method (Abebe *et al.*, 2006).

Identification and classification

After extraction and purification nematodes were visualized by using Stereo Zoom (Nikon) Research microscope (LM 1080). Images were captured using Motic camera with dedicated Motic Image Plus software. Identification of the nematodes was carried out using two primary dichotomous keys (Tarjan *et al.*, 1977; Mekete *et al.*, 2012). Nematodes were identified up to genus and species level by succeeding dichotomous key for a particular genus. The following species were identified and used in the stress experiments: *Radopholus* spp., *Aporcelaimus* spp., *Prodontorhabditis* spp., *Eudorylaimus* spp. and *Doryllium minor*.

Salinity stress assay

Salinity stress assay was performed on cultivated *Radopholus* spp., *Prodontorhabditis* spp. and *Doryllium minor* species following the protocol given by Hill *et al*. (2014). Nematodes were exposed to different saline solutions having different concentrations of NaCl i.e. 0.25%, 0.50%, 1%, 2%, 4%, 8% and 16% in triplicates, for a duration of 30 minutes at 22ºC and then the washed nematodes were seeded on freshly prepared NGM plates. Nematodes were observed for motility and readings were taken after 1, 24 and 48 hrs. (Hill *et al*., 2014).

Heat stress assay

Heat stress assay on agar plates seeded with *Radopholus* spp., *Prodontorhabditis* spp. and *Doryllium minor* species was done according to the method stated by Zevian & Yanowitz (2014). The heat stress was induced at 35ºC and 40ºC temperature in a laboratory incubator in triplicates. At each temperature adult worms were exposed for durations of 1 hour, 1½ hour and 2 hours. The exposed nematodes were then maintained at normal cultivation temperature i.e. 30ºC in a separate laboratory incubator for further evaluation of heat stress effect (Zevian & Yanowitz, 2014).

pH stress assay

The procedure given by Park *et al*. (2017) was followed for the pH stress assay carried out on *Radopholus* spp., *Prodontorhabditis* spp. and *Doryllium minor* species. The nematodes were seeded on nutrient media having different pH range i.e. pH 2, 3, 4, 5, 6, 8, 9 and 10. Nematodes were observed after 1, 24, 48 and 72 hours for evaluation of pH stress effect on test nematodes and results were recorded in triplicate.

As stipulated by Park *et al.* (2017) for each stress assay, active and healthy adult worms were taken for the tests from culture.

Statistical analysis of the data

Each parameter was expressed as Mean±S.E. The Student's *t*-test was calculated using Microsoft Excel 2010. EC_{50} Values for effective salt concentration for all test species were calculated using HN-NonLin V 1.1 Software (Sharma *et al*., 2016).

RESULTS

Identification

The identified five nematode species belonged to five different genera i.e. *Radopholus* spp., *Aporcelaimus* spp., *Prodontorhabditis* spp., *Eudorylaimus* spp. and *Doryllium minor*. Observations carried out indicated that in the selected areas under study from Gujarat state, infestation of *Prodontorhabditis* spp. was prevalent on green onion (*Allium fistulosum* L.), while in Ginger rhizomes (*Zingiber officinale* Roscoe) *Eudorylaimus* infection was recorded. The presence of *Doryllium minor* and *Radopholus* spp. was observed from root soil of *Trigonella foenum-graecum* L. in Anand district.

Salinity stress assay

Nematodes selected for evaluation of salinity stress were exposed to different concentration of NaCl and it was observed that *Prodontorhabditis* spp. had transitioned into a state of dormancy due to unfavourable conditions for a period of 1 to 2 hours. However, after 24 hours when the nematodes were transferred to normal growth media, recovery and restoration of normal growth was observed in 0.25% and 0.50% salt concentrations. Hence from the salt stress assay it was evident that the most favourable range of NaCl concentration was found between 0.25–0.50% (Fig. 1).

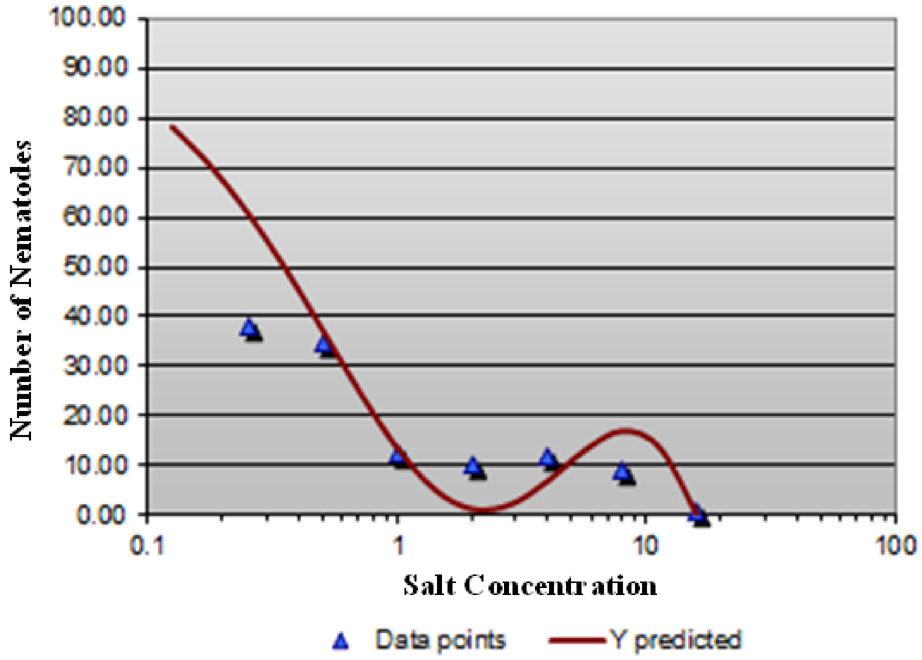

Fig. 1. Salinity Stress Assay on *Prodontorhabditis* spp.

Concentration dependent cessation in motility was also observed for *Prodontorhabditis* spp. after 30 minutes of exposure. A lower recovery rate was noticed. Effective concentration which leads to 50% mortality was observed at

0.35% NaCl concentration. Hence, it was observed that *Prodontorhabditis* spp. was highly sensitive to salinity variations and concentrations of NaCl at and above 0.5% prove unfavourable for this species.

In *Doryllium minor* species although there was a decline in motility observed during the first hour and after 48 hours more than 80% of the nematodes had shown recovery and become motile up to 2% salt concentration. Recovery rate was found to be initiated and increases after 24 hours. *Doryllium minor* showed tolerance to a comparatively broader range of salt concentration i.e. from 0.25% to 0.50% (Fig. 2). LC_{50} for *D. minor* found to be 0.5%.

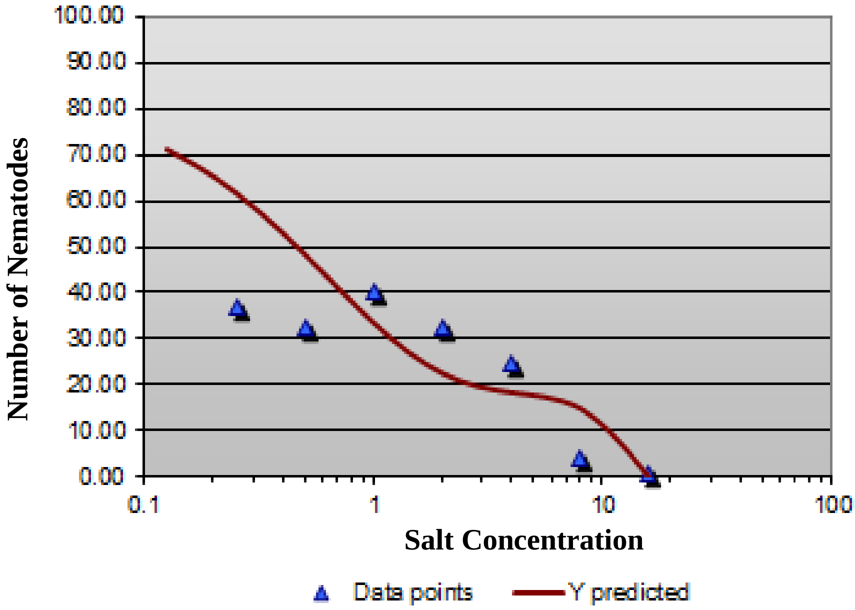

Fig. 2. Salinity Stress Assay on *Doryllium minor*.

Radopholus spp. showed best growth and survival at 1% NaCl concentration after 48 hours of recovery. However, the favourable range of salt concentration was observed between 0.25%–2%. Most dormant adults and juveniles were observed up to 24 hours; however after 24 hours recovery rate increases in case of *Radopholus* spp. (Fig. 3). LC_{50} for *Radopholus* spp. found to be 3.73%.

After the Salinity Stress Assay, results obtained from the viability test (Fig. 4) indicated that the favourable range of salt concentration for 3 different treated nematode species. The most favourable salt concentration for *Prodontorhabditis* spp. ranges from 0.25 to 0.50%. Suitable NaCl concentration for *Doryllium minor* ranges from 0.25 to 2% however most growth was observed at 0.25% NaCl concentration. When cultured in concentrations of 1% NaCl, *Radopholus* spp. retained maximum viability. Hence, all tested nematode species have their unique favourable range of salt environment in which they show maximum growth rate.

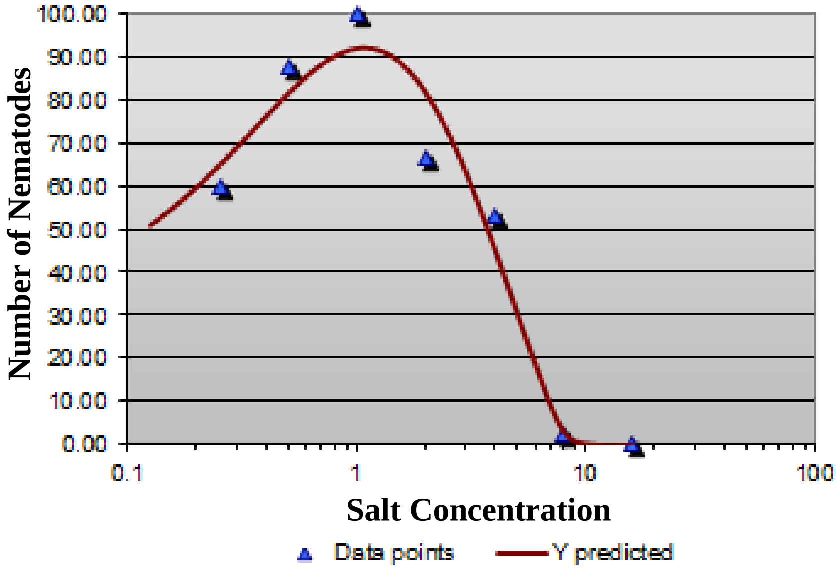

Fig. 3. Salinity Stress Assay on *Radopholus* spp.

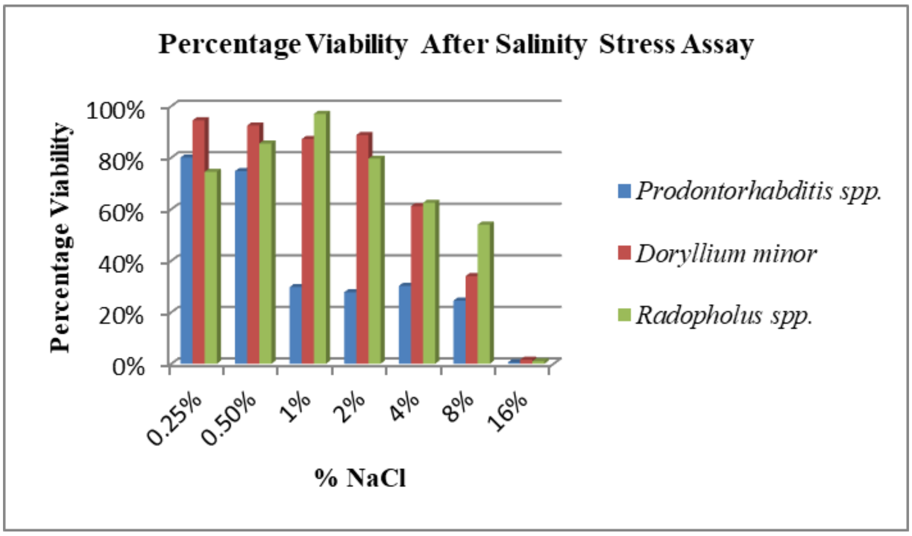

Fig. 4. % Viability after Salinity Stress Assay.

Heat stress assay

The soil free living nematodes as well as plant parasitic nematodes can tolerate higher temperature fluctuations as compared with freshwater and saline water nematodes. Therefore the temperatures selected for this study were 35ºC and 40ºC, temperatures which are higher than the normal optimum temperature range for soil and plant parasitic nematodes. After subjecting the cultured nematodes to heat shock of 35ºC (Fig. 5) and 40ºC (Fig. 6) for 30 minutes, one hour and two hours, all test species showed dauer formation, retreating to a condition of stasis, as a basic response to stressful conditions. Following heat stress, the feeding rate was found to be increased in case of *Prodontorhabditis* spp. *Doryllium minor*, the ginger parasitic nematode species was able to tolerate higher temperatures. As the time of exposure to heat stress increases the rate of dormancy and rate of dauer formation also increases. After duration of two hours of incubation at 40ºC (Fig. 6), a significant decline in motility was observed. On the other hand, at 40ºC for all the three incubation time durations, almost all nematodes turned dormant. Therefore, temperatures of 40ºC induce heat stress conditions in the nematodes, which appeared to be highly unfavourable temperature for all the test nematodes to withstand.

At 35ºC formation of the dauer larvae was found to be most prominent as an adaptive behaviour found in almost all free living as well as PPNs. However, after stress recovery of dauer larvae to normal motility and viability was observed. The 40ºC heat stress was proven to be most lethal for all tested species within the span of two hours of exposure (Fig. 6). By comparing tested PPNs with free living nematode species, the green onion free living nematode, *Prodontorhabditis* spp. showed better growth, tolerance and survival during and after heat stress for 30 minutes and one hour. Species identified from farmland soil samples, *Radopholus* spp. manifested the least survival and tolerance.

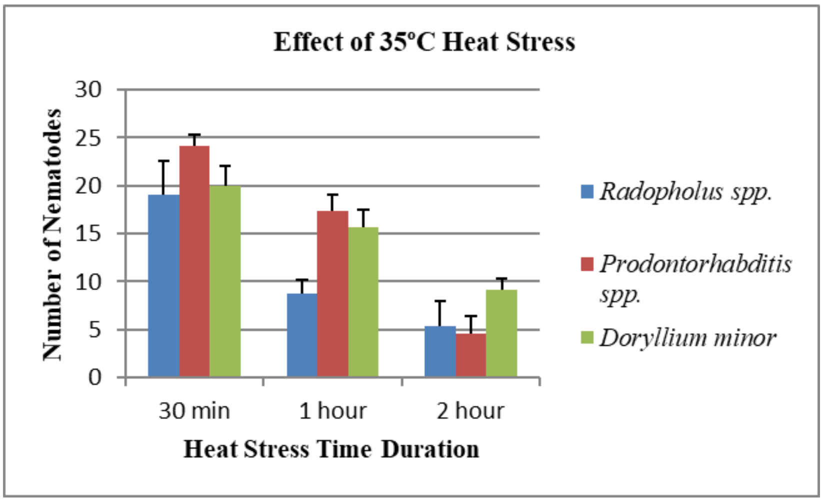

Fig. 5. Heat Stress Assay at 35ºC; Bars represent mean± S.E. (n=6).

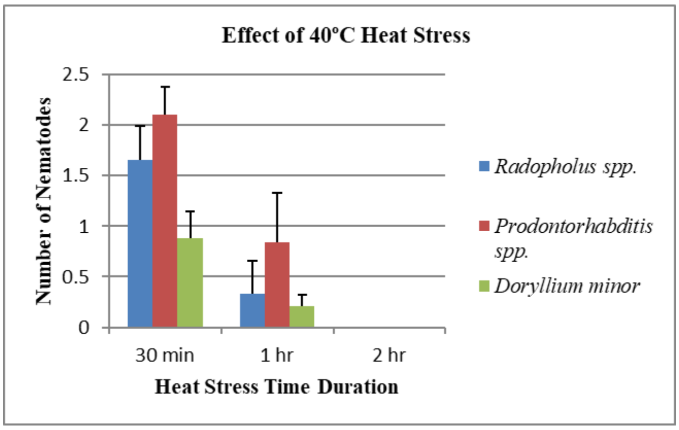

Fig. 6. Heat Stress Assay at 40° C. Bars represent mean± S.E. (n=6).

pH stress assay

Evaluation of the response of the Nematodes under study to varied pH levels was done employing the pH stress assay on *Radopholus* spp. The stress induces hibernation in all tested nematode species due to change in pH. It was observed that soil nematode *Radopholus* spp. was unable to survive under acidic pH range and showed best growth rate in a basic environment that reflects the adaptation of these species to agricultural soil environment having higher pH range. *Radopholus* spp. showed best growth rate at an alkaline pH between 8 and 9. As shown in Table 1, after 72 hours of incubation in media of varied pH, nematodes showed best recovery in terms of survival and growth at pH 8. As the pH range increases above neutral nematode growth was also found to be increased. While in acidic pH nematodes were unable to survive. At a pH range of 4 to 6, the nematodes showed minimum growth which was less than growth rate found at pH 8.

Table 1

pH stress effect and recovery of *Radopholus* spp. at 25° C; Values are mean± S.E. (n=6)

pH	1 hour	24 hour	48 hour	72 hour
2	29.6±0.92	12±3.25	4.7±1.07	3.7±1.0
3	29.1±0.11	8.2±0.08	7.5±0.96	6.2±1.60
4	25.5±0.90	34±5.43	38.9±3.67	205.1±2.30
5	29.9±1.07	75.5±4.90	210.1±2.30	254.39±1.90
6	26±0.60	46.6±1.48	397.7±1.89	589.1±1.56
8	32.1±2.56	50.5±1.23	527.4±5.23	1052.7±4.17
9	22.5±1.60	58.3±1.40	422.1±2.13	746.9±6.32
10	27.3±0.04	41.2±1.45	375.2±1.17	446±8.60

As *Prodontorhabditis* spp. which was identified from samples of green onion, is a slow growing species; the induced pH stress for 48 hours led to a significant decline in the number of nematodes as compared with the *Radopholus* spp. after a similar duration in the pH stress assay. The favourable pH for better growth and multiplication of this species occurred between pH 8 to 9, as shown in the Table 2. Hence soil, free living as well as plant parasitic nematodes have basic pH range favours their growth and multiplication. As shown in the Table 3, the favourable pH range for *Doryllium minor* ranges from 8 to 9. Furthermore acidic pH increases mortality rate in all test nematode species.

Table 2

pH stress effect and recovery of *Prodontorhabditis* spp. at 25º C;
Values are mean ± S.E. (n=6)

pH	1 hour	24 hours	48 hours	72 hours
2	30.23±0.45	6.21±0.84	2.09±0.37	1.08±1.34
3	29.77±0.81	8.49±0.54	5.18±0.62	2.46±0.24
4	31.19±1.26	8.73±0.89	6.08±0.47	2.07±0.92
5	31.04±0.67	17.87±1.54	24.16±1.04	62.15±1.14
6	29.28±0.81	23.14±1.29	32.89±1.20	78.70±0.47
8	30.47±0.91	31.84±0.46	59.47±0.26	98.24±0.57
9	32.54±0.57	33.56±0.74	60.48±0.82	104.08±0.69
10	29.45±1.13	30.93±1.25	29.15±1.45	30.14±1.02

Table 3

pH stress effect and recovery of *D. minor* at 25º C; Values are mean ± S.E. (n=6)

pH	1 hour	24 hours	48 hours	72 hours
2	28.23±0.89	2.18±1.84	1.09±0.41	0.71±0.49
3	30.77±0.57	4.09±0.58	3.18±0.27	1.24±1.57
4	29.19±1.26	5.63±1.45	2.08±0.87	1.09±0.49
5	31.45±0.81	8.81±0.54	4.16±1.17	2.15±1.72
6	28.92±0.81	24.18±1.67	36.79±1.26	67.80±0.78
8	30.24±0.57	32.48±0.85	89.51±0.68	127.53±1.67
9	29.73±0.61	31.96±0.34	57.74±1.81	98.08±0.95
10	32.14±1.47	27.37±1.46	39.41±0.38	75.24±1.72

DISCUSSIONS

As suggested earlier, based on research in other organisms by Haverkort *et al.* (1991), our results also indicated that exposure to different stressors could result in multiple biological defects affecting survival, life span, development, reproduction, locomotion and behaviour. The data obtained in the present study also revealed that there was a correlation between the favourable pH range for PPNs with that of the soil and root pH. In addition, results from heat stress assays indicated that the range of temperature required for optimum growth of the nematodes was similar to that found in and around the cultivation field. These observations therefore, provide vital information for establishing ambient conditions requisite for the maintenance of PPN under laboratory conditions, for studying stress pathways in these organisms and for studying the response of the nematodes during altered stress conditions (Duangjan *et al*., 2019). The effect of stress on slow growing (*Prodontorhabditis* spp.) as well as fast growing (*Radopholus* spp.) parasitic nematodes was also observed. This investigation also provides new insights for developing strategies for the natural, chemical-free control of plant parasitic nematodes which attack agricultural fields in large numbers. By altering any one of these parameters and thus inducing stress in the nematodes their survival and growth could effectively be curtailed.

According to the research carried out by Munoz (2003) longevity of *C. elegans* is strongly correlated with heat tolerance and oxidative stress, as after stress assays nematodes were unable to recover damage caused by stresses. In the current investigation, the results obtained for selected nematodes also showed similar correlation between life span and temperature tolerance. However, according to Zhang *et al*. (2021) in some circumstances the enhancement of heat and oxidative stress resistance can be developed which is reflected by prolonged lifespan in *C. elegans* under stress condition with some morphological, physiological changes and genetic mutations. The results obtained following the stress assays reveal that different plant parasitic nematodes manifest different responses towards stress conditions as in case of *D. minor* formation of dauer and the feeding rate was found to be increased after heat stress; a similar behaviour was also performed by *C. elegans* during stress assays.

The stress assays also helped in standardization of species specific cultivation methods. The relationship between longevity and thermal stress was also observed for PPNs and free living *Prodontorhabditis* spp. as well as development of stress resistant progeny after stress was produced same as in case of *C. elegans*. However, this occurred only up to certain temperature limit; at or above 40ºC the nematodes were unable to survive. Induction of heat stress was observed to lead to dauer formation and thermo-tolerant progeny formation which are similar to the study carried out by Munoz (2003). The heat stress assay data also revealed that a temperature of 40ºC proved to be significantly lethal for all soil free living as well as PPN adults and juveniles. Hence this study provides evidence of thermo-tolerance, a protective behaviour of juveniles and adults, in the species identified from the samples collected from different vicinities in Gujarat.

The mechanisms that activate stress resistance have been elucidated by Munoz (2003). Also thermo tolerance leads to dauer formation after stress assays which lead to morphological changes along with changes in feeding behaviour in nematodes hence producing unhealthy cultures in case of *Prodontorhabditis* spp. Therefore, the range of pH, stress reveals that all tested species have varied favourable range of salt concentration. Favourable pH range for *D. minor* and *Prodontorhabditis* spp. ranges from 8 to 9. *Radopholus* spp. showed best survival and growth at pH 8. Heat and Salt concentration highly need to be focused. From our investigation the highly and precisely favourable range of pH was found to be 8 for *Radopholus* spp. as well as for *Doryllium minor*. However, the precise favourable pH for *Prodontorhabditis* spp. was found to be 9.

CONCLUSIONS

In summary, all long lived adults are sensitive to different stresses provided to them and also showed favourable range of pH, Temperature and Salt concentration to assist their multiplication and growth. Also, heat stress induced thermo-tolerant progeny was produced in *Prodontorhabditis* spp. In addition, exposure of the animals to thermal stress for a short duration marks an increased resistance to heat and increased longevity. Investigating the effect of salt, heat and pH stress prove significant in supressing the growth of nematodes at certain ranges which can be efficient in PPN control.

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OBSERVATIONS ON PRESENCE, ABUNDANCE, DYNAMIC AND DIVERSITY OF BIRD SPECIES (AVES) FROM THE DANUBE EVERGLADE (DOLJ COUNTY, ROMANIA)

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ALMA LIOARA NICOLIN[***], DUMITRU MURARIU[****]

A valuable Data base getting along 25 years on 34 bird species from a protected area (Site Ramsar Bistreț, Dolj County, Romania) illustrates permanent presence of 24 water fowl with significant yearly differences between reported taxa. Ecological indexes and species abundance revealed an avian richness in 1998 and the lowest number of species in 2021. These differences could be on the one side because of natural conditions (temperature, relative humidity, drought), and on the other side – because of anthropic pressure, mainly hunting. Therefore the statute of the surveyed protected area should be a real refuge for the protected bird species in a part of the Danube Everglade.

Keywords: Bird conservation, ROSPA0010, water fowl, bird census.

INTRODUCTION

Over time, climate change has been shown to be a key factor in changes in bird distribution and abundance (Trautmann, 2018). Thus, birds are considered indicators of ecosystem condition (Gregory & Strien, 2010; Li *et al*., 2021). Among bird species, the most sensitive to climatic conditions are waterbirds (Jordán, 2017). They are used as important bio-indicators of wetland habitat change (Rahman & Ismail, 2018). In this context, it is essential to monitor the presence and activity of waterbirds to provide an early warning of the danger to our environment (Burger, 2006; Rahman & Ismail, 2018). Waterbirds have also been used as sentinel species in various environmental toxicology issues (Zhang & Ma, 2011; Rahman & Ismail, 2018).

According to the Ramsar Convention (1994), waterbirds are “ecologically dependent on wetlands”. In this context, waterbirds use wetlands both for wintering – longer periods, and for 'stopovers' – for shorter periods (Warnock, 2010; Maneas *et al*., 2020).

One of Romania's important wetlands on the Danube Plain is the Bistreț Lake Complex (Dolj County). It has been declared a Special Protection Area since Romania joined to the European Union and since 2012 it has also received Ramsar site status, in order to conserve 24 waterbird species listed in Annex I of the Birds Directive (Ridiche *et al*., 2021). These include the Red-breasted Goose, the Lesser white-fronted goose, the Dalmatian Pelican or the Ferruginous duck, species that use the site for feeding, resting or nesting.

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The aim of our study was to analyse the diversity and abundance of waterbirds and shorebirds species wintering in ROSPA0010 Bistreț, by analysing data collected in the International Waterbird Census, coordinated by Wetlands International (IWC) between 1988 and 2022.

MATERIAL AND METHODS

Study area

The study area is Lake Bistreț, which is one of the largest natural lakes in the Danube Plain, located in Dolj County (Ridiche & Vișan, 2008) and covers an area of 2.030 ha (Fig. 1).

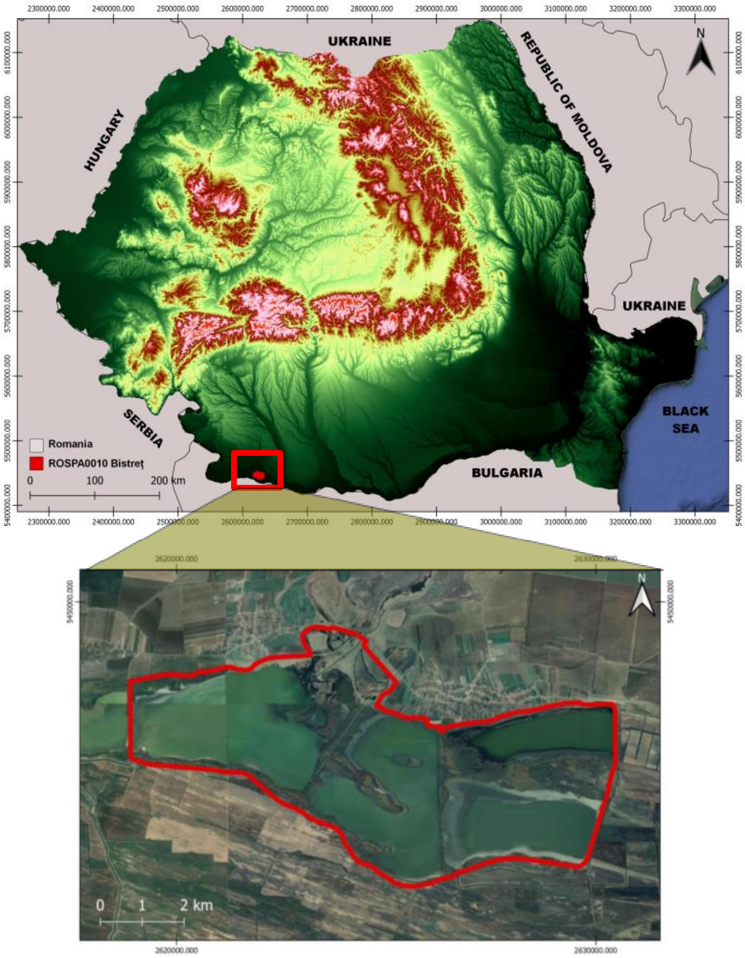

Fig. 1. Study area (© Simona Dumitrița Chirilă).

Data collection

The study analysed a dataset of observations collected over a 13-year period, with an output at the same time of year: mid-January, from 1988 to 2022. Between 1988 and 2019 data were collected from participants of the International Waterbird Census, that dataset was accessed from the online database of the Romanian Ornithological Society (Ornitodata) and the data collected between 2020–2022 are personal observations.

Field equipment consisted of Leica 42x10HD binoculars, Leica Apo-Televid 82 W telescope with Leica Vario 25-50x WW ASPH eyepiece and Canon 70D camera with Sigma 150–600 mm Contemporary telephoto lens.

Statistical analysis

Five statistical indices (abundance, Shannon index, Evenness index, Margalef index and Simpson index) were calculated in PAST version 4.03 (Hammer *et al*., 2001). Differences in the number of species and the number of individuals between years were analysed using Kruskal-Wallis and Mann-Whitney non-parametric post-hoc tests. The map of the study area was produced in QGIS version 3.24.1.

RESULTS

During the study period, 34 bird species from four orders were recorded. In turn, 24 species are aquatic and ten species are shorebirds, classified in the following seven families: Anatidae (19), Scolopacidae (5), Phalacrocoracidae (3), Laridae (3), Rallidae (3), Recurvirostridae (1) and Charadriidae (1). Most observations (19) were made in 2022, when the highest number of individuals (11,169) was also recorded.

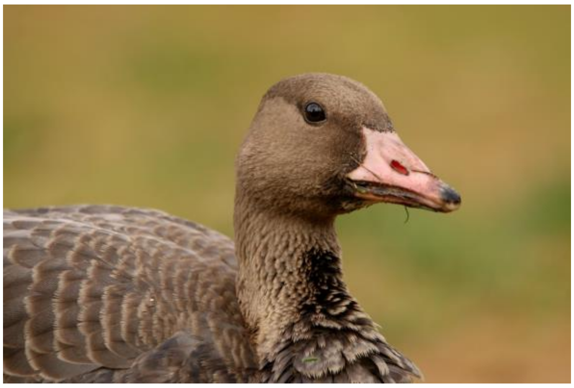

Fig. 2. Greater white-fronted goose (*Anser albifrons)* (photo: Alexandru Cătălin Birău).

Of the 34 species identified, if we consider only the aquatic ones, we found that 92% of them belong to the Order Anseriformes, in which the species *Anser albifrons* was dominant in number of individuals. The other three orders were represented as follows: Gruiformes – 3.4% species – through *Fulica atra*; Pelicaniformes – 1.9%, with most individuals of *Phalacrocorax carbo*; Charadriiformes with waders species, among which *Larus ridibundus* was the most frequent.

Of the 34 species of waterbirds and shorebirds, *Anser albifrons* (42.57%), *Anas crecca* (19.06%) and *Anas platyrhynchos* (17.35%) had the highest percentage frequency, and the species with the lowest frequency were *Vanellus vanellus* (0. 002%), *Gallinula chloropus* (0.002%), *Tachybaptus ruficollis* (0.002%), *Tringa stagnatilis* (0.004%), *Rallus aquaticus* (0.004%) and *Recurvirostra avosetta* (0.012%).

Fig. 3. Little grebe (*Tachybaptus ruficollis*) (photo: Alexandru Cătălin Birău).

According to IUCN (International Union for Conservation of Nature) criteria, 77% of the bird species surveyed are classified as non-Endangered, 12% are Near Threatened with extinction, and 12% are Vulnerable (i.e. at high risk of extinction). The species classified as Vulnerable are represented by: *Anas acuta*, *Aythya ferina*, *Cygnus columbianus* and *Vanellus vanellus*.

Some of the bird species analysed are also protected by Annex 1 of the Bern Convention: *Mergellus albellus*, *Cynus cygnus*, *Microcarbo pygmaeus*, *Recurviristra avosetta* and *Aythya nyroca* and by the Bonn Convention: *Calidris alpina*, *Larus cachinnans*, *L. canus*, *L. ridibundus*, *Limosa limosa*, *Numenius arquata*, *Recurvirostra avosetta*, *Tringa erythropus*, *T. stagnatilis* and *Vanellus vanellus*.

Fig. 4. Pygmy cormorant (*Microcarbo pygmaeus*) (photo: Alexandru Cătălin Birău).

Table 1

Characteristics of birds from the ROSPA0010 site

Taxa	Common name	No. observations	No. individuals	IUCN		Bern Convention	Bonn Convention
				Europe	Global		
Anseriformes							
Anatidae							
Anas acuta	Pintail	3	47	VU	LC		
Anas crecca	Eurasian teal	12	7849	LC	LC		
Anas platyrhynchos	Mallard	28	7146	LC	LC		
Anser albifrons	Greater white-fronted goose	15	17533	LC	LC		
Anser anser	Greylag goose	9	2481	LC	LC		
Mareca penelope	Eurasian wigeon	5	169	LC	LC		
Mareca strepera	Gadwall	8	356	LC	LC		
Tadorna tadorna	Common shelduck	8	366	LC	LC		
Netta rufina	Red-crested pochard	1	19	LC	LC		

Table 1 (continued)

Mergellus albellus	Smew	5	51	LC	LC	x	
Mergus merganser	Common merganser	1	60	LC	LC		
Spatula clypeata	Northern shoveler	5	91	LC	LC		
Aythya ferina	Common pochard	2	185	VU	VU		
Aythya fuligula	Tufted duck	1	8	NT	LC		
Aythya nyroca	Ferruginous duck	1	75	LC	NT	x	
Bucephala clangula	Common goldeneye	7	248	LC	LC		
Cygnus columbianus	Tundra swan	9	485	VU	LC		
Cygnus cygnus	Whooper swan	10	117	LC	LC	x	
Cygnus olor	Mute swan	18	629	LC	LC		
Charadriiformes							
Charadriidae							
Vanellus vanellus	Northern lapwing	1	1	VU	NT		x
Gruiformes							
Rallidae							
Fulica atra	Eurasian coot	8	1405	NT	LC		
Gallinula chloropus	Common moorhen	1	1	LC	LC		
Rallus aquaticus	Water rail	1	2	LC	LC		
Recurvirostridae							
Recurvirostra avosetta	Pied avocet	1	5	LC	LC	x	x
Laridae							
Larus cachinnans	Caspian gull	6	58	LC	LC		x
Larus canus	Common gull	4	52	LC	LC		x
Larus ridibundus	Black-headed gull	10	646	LC	LC		x
Scolopacidae							
Calidris alpina	Dunlin	2	82	LC	LC		x
Limosa limosa	Black-tailed godwit	2	117	NT	NT		x
Numenius arquata	Eurasian curlew	4	78	NT	NT		x

Table 1 (continued)

Tringa erythropus	Spotted redshank	1	11	LC	LC		x
Tringa stagnatilis	Marsh sandpiper	1	2	LC	LC		x
Pelecaniformes							
Phalacrocoracidae							
Microcarbo pygmaeus	Pygmy cormorant	8	69	LC	LC	x	
Phalacrocorax carbo	Great cormorant	9	735	LC	LC		
Tachybaptus ruficollis	Little grebe	1	1	LC	LC		

Table 2 shows the summary statistics on waterbird species from the ROSPA0010 site: number of observations, number of individuals, Simpson index, Evenness index, Margalef index, Shannon index and abundance.

Table 2

Summary statistics regarding the species of water birds from the ROSPA0010 site

Year	**Taxa_S**	**Individuals**	**Simpson**	**Evenness**	**Margalef**	**Shannon**	**Abundance**
1988	4	6130	0.31	0.44	0.34	0.56	2.43
2000	8	1129	0.74	0.56	1.00	1.50	0.55
2001	7	964	0.64	0.50	0.87	1.26	0.01
2005	12	507	0.76	0.48	1.77	1.74	0.01
2006	6	49	0.81	0.92	1.29	1.70	0.03
2007	6	1075	0.53	0.49	0.72	1.08	0.12
2010	15	421	0.85	0.59	2.32	2.18	0.00
2011	9	340	0.52	0.38	1.37	1.24	0.07
2013	3	807	0.02	0.35	0.30	0.06	0.01
2018	2	40	0.50	1.00	0.27	0.69	0.05
2020	15	10203	0.54	0.22	1.52	1.18	0.00
2021	12	7843	0.69	0.36	1.23	1.46	0.01
2022	19	11169	0.54	0.16	1.93	1.14	0.01

H specific diversity (Shannon index) of waterbird and shorebird species ranged from 0.06 to 2.18. The lowest diversity (0.06) was reported in 2013 and the highest diversity (2.18) was reported in 2010 (Table 1). The F-value was 1.051, showing no significant differences between years.

Waterbirds abundance ranged from 0.00 to 2.43 and was not significantly different (p>0.05). The highest value (2.43) was found in 1988, when 6130 individuals and four species were recorded. These species showed the following characteristics in the period 1988–2022: *Bucephala clangula*, with seven observations and 248 individuals; *Anas crecca*, with 12 observations and 7849 individuals; *Anas platyrhynchos*, with 28 observations and 7146 individuals and *Mareca penelope*, with five observations and 169 individuals.

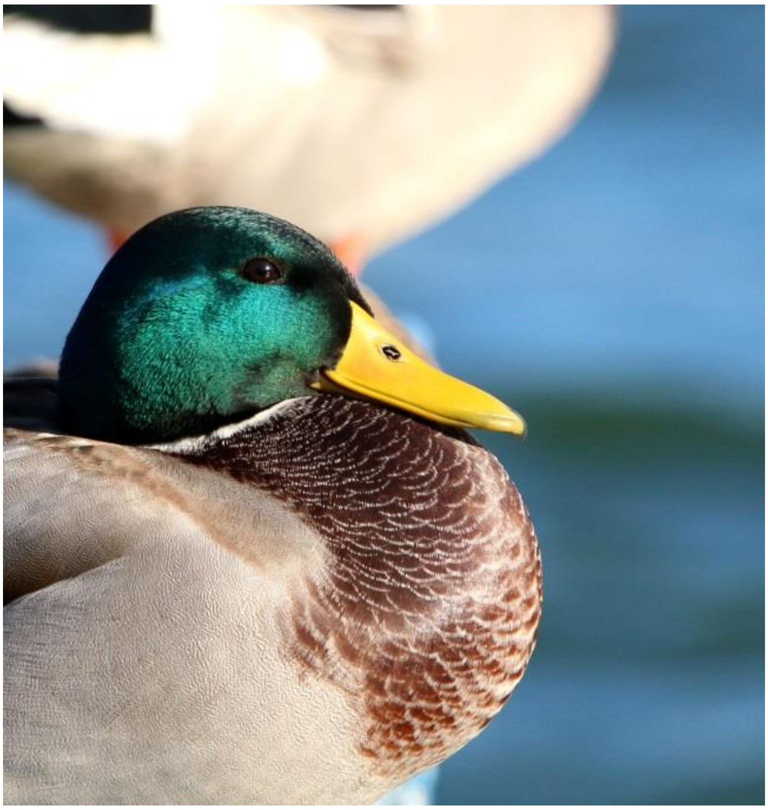

Fig. 5. Mallard (*Anas platyrhynchos)* (photo: Alexandru Cătălin Birău).

Uniformity (Evenness index) of waterbird and shorebird species ranged from 0.16 to 1.00. The highest value was reported in 2018 – the year in which *Cynus olor* and *Larus cachinnas* recorded the most individuals. In the case of *C. olor*, 18 observations and 629 individuals were recorded, and in the case of *L. cachinnans*, six observations and 58 individuals were recorded. The lowest Evenness index value was recorded in 2022, when the highest richness was reported, with 11,169 individuals and 19 species recorded. In this year, besides *Anser albirons*, *A. crecca* and *Anas platyrhynchos*, which recorded the most individuals, another species was

Anser anser. For the latter, nine observations were recorded, 2481 individuals, of which 1778 individuals were reported in 2022.

The species richness (Margalef index) of the bird community ranged from 0.30 in 2013 to 2.32 in 2010. The Simpson index averaged 0.570, indicating a moderately high degree of diversity/heterogeneity.

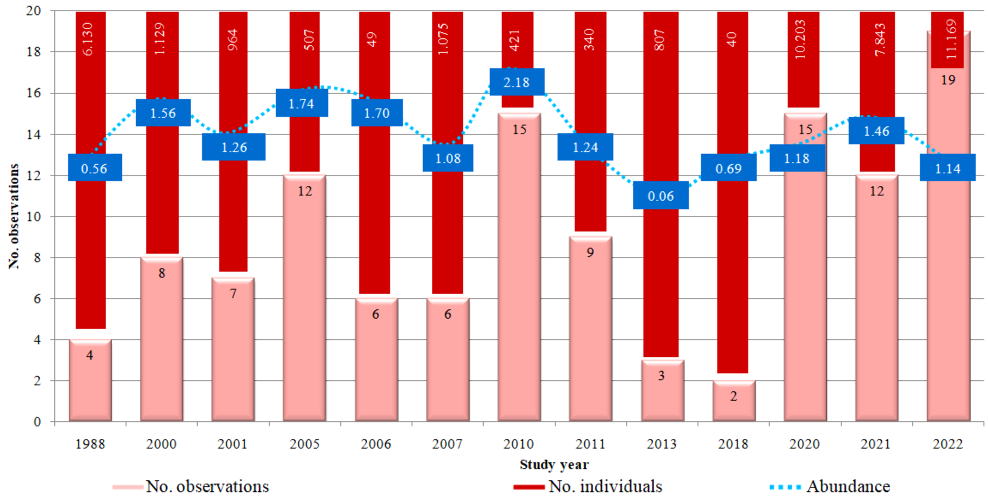

Fig. 6. The number of observations, the number of individuals and abundance in the period 1988–2022.

DISCUSSIONS

The low abundance and lower number of species in some years can be attributed both to natural factors: low temperatures leading to the freezing of water bodies, and to anthropogenic causes: hunting activities, in this case the difference between the first years of the study (1988–2020) and the last two years (2021–2022) – when hunting was stopped in Romania during the winter season – is evident, this factor being reflected in the higher number of birds present during our assessment.

CONCLUSIONS

1. The Bistreț Ramsar site is home to important wintering populations of Romania's wintering waterbird species. During the study, 24 aquatic species were identified, among which in recent years, the Greater white-fronted goose – *Anser albifrons* (17,533 individuals), the Common Teal – *Anas crecca* (7,849 individuals), the Mallard – *Anas platyrhynchos* (7,146 individuals) and the Greylag goose –

*Anser anser (*2,481 individuals) have stood out with large numbers. The highest number of observations was for the *Anas platyrhynchos* (28 observations).

2. There are differences in the species identified from year to year, with the most significant differences occurring between the early years of the survey – when data were collected by other participants in the International Waterbird Census, so we cannot be sure whether the differences were due to observer or natural causes – and the last 3 years – when we started the International Waterbird Census.

3. As far as shorebirds are concerned, their numbers are low and have not been consistently present throughout the study period. This is normal, given the tendency of many species to migrate to warmer places in winter. Of the ten shorebird species identified, species of the genus *Larus* recorded high numbers: the Common gull – *Larus canus* (52 individuals), the Caspian gull – *Larus cachinnans* (58 individuals) and the Black-headed gull – *Larus ridibundus* (646 individuals).

4. The highest diversity (2.42) of waterbird and shorebird was recorded in 2021, while the lowest bird diversity (0.08) was observed in 2013. Between years, there was a relatively significant difference in diversity. In this context, the variation in bird diversity between years is due to weather conditions and disturbance caused by hunters.

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HERONRIES ON A POPULOUS URBAN AGGLOMERATION AND SUBURBS ON THE SOUTHWEST COAST OF INDIA: NESTING SPECIES AND NESTING TREES

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Despite the invaluable ecosystem services provided by the wetlands and the wetland birds, the habitat that is grappling with the reverberations of climate change is increasingly subjected to anthropogenic disturbances worldwide and is deteriorating and decimated incessantly. The present study was conducted in the Indian state of Kerala on the South-western coast of India, during breeding season coinciding with the South-West Monsoon (June–August 2021) to document the existing and previously unknown heronries of the landscape. 22 heronries comprising of five species were documented. The Indian Pond Heron (PH) and Little Egrets (LE) with presence in almost all the sites, dominated the survey neck and neck with 782 (43.5%) and 763 (42.4%) nests respectively. Cormorants were confined to very few sites (5) and built 252 (14.0%) nests and 216 (12.0%) by Little Cormorant (LC) and 36 (2.0%) by Indian Cormorants (IC). Only a single nest (0.06%) of Purple heron was found during the survey, inconspicuously placed in a Reed bed. A total of 28 species of trees and one Reed Bed were utilized by the birds for nesting during the survey period. The results indicate how the colonial nesting water birds are faring in the district and provide a concrete baseline for future conservation and management work.

Keywords: Heronry, nest, nesting species, nesting tree, heron, egret, cormorant.

INTRODUCTION

Ecosystem services provided by wetlands have been relatively well studied and their value has been estimated in an increasing number of cases (MWO, 2012). Water birds too provide invaluable ecosystem services by playing key functional roles in many aquatic ecosystems, including as predators, herbivores and vectors of seeds, invertebrates and nutrients. Waterbirds can maintain the diversity of other organisms, control pests, be effective bio indicators of ecological conditions, and act as sentinels of potential disease outbreaks (Green & Elmberg, 2014).

Waterbirds themselves can be considered as “ecosystems” in that they act as hosts for a wide variety of parasites and commensalists, often specific to a small number of bird species, including an unknown number of parasite species yet to be described. In some cases, the presence of these parasites makes a major contribution to the total biodiversity in aquatic ecosystems (Green & Elmberg, 2014). These roles have often been overlooked and the attention shifts to the negative ones. “Ecological disservices” (Dunn, 2010), such as transmission of diseases that can potentially affect humans (Hubalek, 2004), or conflicts with fisheries (Carss & Marzano, 2005; Harris *et al*., 2008), are well-known and often wielded as pretext for hostility to wetland birds. However even when all kinds of disservices are taken into account to evaluate the “net contribution” of water birds, the positive ecological consequences of waterbirds outweigh the disservices.

India, home to a myriad of biodiversity rich wetlands spread across all the biogeographic zones is experiencing significant biodiversity depletion and habitat loss. The ruthless plundering of resources of the wetlands exerts immense pressure on the dependent flora and fauna, sparing none including the tree nesting colonial waterbirds, who breed in single or mixed-species colonies (heronries) typically, located in wetlands and associated areas. Having a monitoring programme for heronry birds, which include species of storks, ibises, spoonbills, herons and cormorants, is the need of the hour in India because of its relevance for conservation (Rahmani, 2012). Many of the fish eating heronry birds are apex predators in the aquatic food chains. Therefore, any fluctuation in their principal food source fish (for instance, due to climate change) is likely to be picked up in monitoring exercises, alongside changes in their foraging habitat due to urbanization (Urfi, 2010) or pollution.

In the last century, many heronries across the Indian landscape have been lost (Subramanya, 1996). Disturbance at nesting sites affect nesting behaviour which results in abandonment of the site (Carney & Sydeman, 1999; Roshnath & Sinu, 2017). In Kerala too, some of these changes have impacted colonial nesting water birds, resulting in the loss of heronries (Roshnath & Sashikumar, 2019).

Climate variation may influence bird populations both in their breeding and non-breeding areas, affects breeding success and survival (reviewed in Newton, 1998; Lande *et al*., 2003). Previous studies of herons (e.g., cattle and little egrets, grey heron) (Hafner *et al*., 1992, 2002; Marchant *et al*., 2004; North & Morgan, 1979; Bennetts *et al*., 2000) have emphasized the same. Birds may physiologically respond to changes in temperature and precipitation caused by climate change (Steen & Powell, 2012; Pavón-Jordán *et al*., 2019). Climate change causes major shifts in the features of water bird habitats (Wormworth & Mallon, 2006) and, in conjunction with irregular monsoons, has altered the distribution of their nesting habitats (Urfi, 2011; Jabaraj & Gopi, 2020). Colonial nesting waterbirds breed in a select few locations. Even a small disturbance to those sites may have profound consequences on the waterbird populations. Continued management interventions are required to sustain these dynamic sites for long term conservation (Frank *et al*., 2021). Thus, information related to nesting locations and breeding periods is vital in long term monitoring of waterbirds in relation to the current climate change (Urfi, 2011).

Kerala has about 15 species of resident and breeding waterbirds nesting in various heronries across the state (Sashikumar *et al*., 2011). Heronry Nest Count, an annual statewide survey of water bird breeding colonies of Kerala coincides with the monsoon (June – August). A survey of nests in water bird breeding colonies in Kollam District was conducted to gain an insight on the current distribution and abundance information on colonial nesting water birds for conserving populations, resolving management conflicts stemming from increasing and expanding populations, and providing the data necessary to manage water bird populations at the local and regional scale. These concerns resulted in a comprehensive survey throughout the district.

The objectives of the survey were to conduct a comprehensive inventory of water bird breeding sites and populations in the district. The specific objectives of the present study were (i) to document and map the existing heronries, (ii) to document the relative abundance of the breeding colonial nesting water bird species and nests across the district, (iii) to understand the nesting tree preferences, nesting heights and document the threats faced by the nesting birds.

Study area

Kollam is a southern district of Kerala bearing Latitude and Longitude 8.99° 00' N 76.87° E respectively (District plan 2018, Kerala State Planning Board), on the South-western coast of India, flanked by the Arabian Sea on the West, Tamil Nadu on the East, Alapuzha and Pathanamthitta districts on the North and Thiruvananthapuram district on the South. The climate of the district is tropical humid with the hot season spreading during the months of March to May, followed by the South-West Monsoon from June to September. After a short spell of dry weather, the North-West Monsoon starts by November and continues through the months of December and January. The average annual rainfall is about 2700 mm and the temperature fluctuates between 22.4°C and 36°C (Nair & George Mathew, 2020). Kollam is home to two of the three Ramsar sites in Kerala-Sasthamkotta Lake, the largest fresh water lake in Kerala and Ashtamudi, a massive, multi-branched water body. Total length of sea coast in the District is about 37 km. Sandy loams are found along the coastal belt, and the forest soil is found in the eastern forest belt. The rest of the district has laterite soil. Major rivers like Pamba, Achancovil, Kallada and Ithikara River endows the district with perennial supply of water. About 70 percent of the work force is engaged in agriculture. The total area of land under cultivation is around 2,18,267 hectares. The major crops grown here are paddy, tapioca, coconut, rubber, pepper, banana, mango and cashew.

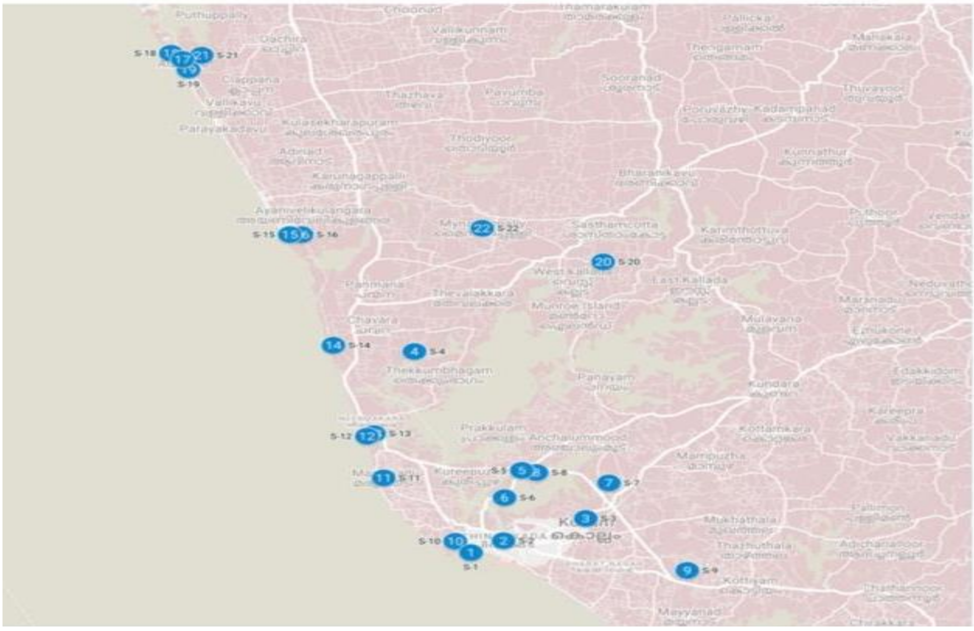

Fig. 1. Map showing location of Heronry of Kollam.

(S1-Vady Harbour; S2-Chinnakada; S3-MG Colony; S4-KOchuveettil Colony; S5-Pallivettuchira; S6-Pernazhikam; S7-Mangad; S8-Thrikadavoor; S9-Saarkarakulam; S10-Kothalavayal; S11-Maruthady; S12-Neendakara Harbour; S13-Neendakara Residential; S14-IRE; S15-Kodi; S16-Kochupalam; S17-Edachira; S18-Kazhukanthuruth; S19-Chanthakadavu; S20-Kakkathoppu; S21-Ayiramthengu; S22-Mynagappally).

MATERIAL AND METHODS

The information about the congregation of water birds was obtained from e-Bird, previous surveys (Roshnath *et al*., 2019) and the citizen responses to our newspaper advertisements for heronry bird sites. Local tree climbers too were approached to gain information about nesting sites, prompted with pictures and photographs from field guides to gather evidence of occurrence. Water bodies were located using Google Earth and its surroundings were surveyed for the presence of breeding colonies of colonial nesting water birds.

The Heronry Survey was conducted during July-August 2021 as part of the Annual Kerala State Heronry Survey that coincides with the South West Monsoon. Trained students (10) in groups of two, with at least three years of bird watching experience volunteered for the survey, as much as possible under the supervision of the District coordinator. All the sites were randomly checked by the District coordinator to verify the observations. A total of 720 hours spreading three months was spent on active observation by the teams, excluding periodic breaks and travels. The count was taken at the beginning and end of the survey period and arithmetic mean of the same was recorded. Heronries separated at least by 500 m was considered separate entity.

Cold searching (Sutherland *et al*., 2004), i.e., searching visually for nests in all potential nesting habitat in the study area was the method used for finding nests. Only the apparently active or occupied nests and the trees hosting them were counted. Water birds in the breeding colonies were identified, counted, and information pertaining to the GPS location of the heronries, number and details of nesting species and nesting trees were documented.

The proximity of the heronries to water bodies and human habitation was also noted. Though not originally part of the survey, roosting sites were also noted. Standard software was used for quantitative and qualitative analysis of the data. Survey limitations: the eastern forest area was excluded from the survey.

RESULTS AND DISCUSSION

Composition of heronry bird species in the Kollam District

The study was conducted in Kollam district of Kerala state, and covered 22 sites. The study found a total of 1,798 nests on 581 trees of 28 species and a Reef Bed in human-altered habitats. Although 28 tree species were hosting the nests of heronry birds, *Cocos nucifera*, *Mangifera indica*, *Azadirachta indica*, *Casuarina equisetifolia*, *Artocarpus hirsutus*, *Tectona grandis*, etc. become the crucial nesting trees of the heronry birds in the urban areas. The abundance of individuals of a tree species correlates strongly with the number of individual birds and species visitation.

22 active nesting sites from Kollam district (Fig. 1) were found during the survey period. Of the five species of colonial nesters observed and recorded during the heronry survey (Table 1), three belonged to Ardeidae family: Little Egret (*Egretta garzetta*), Indian Pond Heron (*Ardeola grayii*), and Purple Heron (*Ardea purpurea*); rest were Phalacrocoracids: Indian Cormorant *Phalacrocorax fuscicollis* and Little Cormorant (*Microcarbo niger*).

581 trees belonging to 28 species and a reed bed hosted 1798 nests of five heronry bird species (Table 1). Highest number of nests was recorded for IPH 782 (43.5%) followed by LE 763 (42.4%), LC 216 (12%), IC 36 (2.0%), and PH 1 (0.06%).

Table 1

Summary of number, height of nesting trees of different species and nest numbers per tree recorded during 2020–2021 in the study area

Nos.	Common Name	Scientific Name	Tree Count	Nest Count					Mean Nest Height (m)
				IPH	**LE**	**LC**	**GH**	**IC**	
1	Teak	*Tectona grandis*	16	28	132				10.61
2	Badam	*Terminalia catappa*	3	52	5				11.72
3	Coconut	*Cocos nucifera*	353	282	323	34		8	12.32
4	Mahagony	*Swietenia mahagoni*	11	27	6	6		8	10.9
5	Anjili (Wild Jack)	*Artocarpus hirsutus*	19	48	9	21			8.64
6	Acacia	*Acacia crassicarpa*	1	2					7.23
7	Acacia Manjium	*Acacia manjium*	6	2	49	5			6.85
8	Mango	*Mangifera indica*	48	129	16	30			6.63
9	Pride of India (Manimaruth)	*Lagerstroemia speciosa*	1		2				7.28
10	Black Jamun	*Syzygium cumini*	1	1	3				9.32
11	Kattadi /Kite tree	*Casuarina equisetifolia*	24	14	45	24			8.67
12	Malabar Tamarind	*Garcinia cambogia*	1	2					5.83
13	Jackfruit	*Artocarpus heterophyllus*	13	34	3	10			8.12
14	Tamarind	*Tamarindus indica*	7	22	12	35		8	8.41
15	Pulivaka/Ceylon Rose Wood	*Albizia odoratissima*	1	3					6.23
16	White cotton tree	*Ceiba pentandra*	1			2			5.87
17	Peral	Ficus bengalensis	2		4	22		12	6.54
18	NutMeg	*Myristica fragrans*	1	1					5.32
19	Portia Tree (sheelanthy)	*Thepesia populnea*	5	4	21	11			5.21
20	Gooseberry	*Phyllanthus acidus*	3	3					3.82
21	Neem	*Azadirachta indica*	32	56	69				3.52
22	Pala	*Alstomia scholaris*	3		7				7.43
23	Udi	*Lania coromandadica*	3	10	10				6.61
24	Copper pod	*Peltophorum terocarpum*	8	22	23				8.72

Table 1 (continued)

Nos.	Common Name	Scientific Name	Tree Count	Nest Count					Mean Nest Height (m)
				IPH	LE	LC	GH	IC	
25	Subabul	*Leucaena leucocephala*	13	18	9	16			7.91
26	Spanish Cherry	*Mimusops elenji*	1	3					6.23
27	Peepal (Arayal)	*Ficus religiosa*	2	15	4				7.81
28	Nochi	*Vitex negundo L.*	1	4	11				2.86
1	Reed		1				1		1
	Total = (28 +1)		**581**	**782**	**763**	**216**	**1**	**36**	
	Total No of Nests	***1798***							

Cormorants were confined to 5 sites- Kodi, Kochupalam, Kazhukanthuruth, Kakkathoppe and IRE and built 252 (14.0%) nests: 216 (12.0%) by Little cormorant and 36 (2.0%) by Indian Cormorants. These were the sites that had maximum species' nests (4/5). Cormorants nested only when there were nests of LE and IPH. They failed to show in similar large heronries of Vaddy and Neendakara. The water logged area near Muscat Service Station (9.1200, 76.4816) at Ayiramthengu was the only site where a Purple heron nest was found in the reed bed. Interestingly no other heronry bird nests could be located there, making it the smallest nest count and species count of the survey. Single species nests made by Pond herons were found in some sites. Egrets were not seen nesting as single species in any of the sites. Little egret's nests were seen along with the nesting colony of cormorants. All the 22 heronries were seen closely associated with wetland and human habitations (Fig. 2, Fig. 3, Fig. 4).

Composition of nesting tree species in the Kollam District

In Kerala, coconut palm is the most extensively cultivated crop (7,56,890 ha in 2018–2019. It grows virtually everywhere in the state (Kumar & Kunhamu, 2022). In the heronry areas, almost all 3–12m coconut trees were found to host heronry bird nests. LE, IPH, LC and IC preferred mostly coconut trees to other nesting trees. 353 coconut trees were chosen by the 4 species to build a total of 647 nests. Mixed nests of IPH and LE were seen in the same coconut tree on different fronds and inflorescences; cormorants nested separately. Mango trees, the inevitable components of homesteads of the state ranked second among the tree species the heronry birds chose for nesting; 48 mango trees hosted 175 nests. LE built only 16 nests, while IPH made 129 nests in mango trees. Neem, casuarina, jacktree, wildjack, teak were also chosen by the birds in good numbers. A teak tree at Neendakara had the highest nest count of 29, whereas Nutmeg was the tree with the least nest count (1) and tree count (1) (Fig. 5, Fig. 6).

Fig. 2. Left to right: Little Cormorant nest on *Mangifera indica*; Indian Cormorant nest on *Ficus bengalensis*; Little egret nest on *Ceiba pentandra*; Little Egret nest on *Cocos nucifera.*

Fig. 3. Left to right: Little Cormorant nest on *Acacia manjium*; Pond Heron nest on *Mangifera indica*; Pond Heron nest on *Artocarpus hirsutus*; Indian Cormorant nest on *Peltophorum terocarpum*.

Fig. 4. Left to right: Pond Herons nests on *Azadirachta indica*, *Cocos nucifera*, *Tectona grandis* and *Ficus religiosa.*

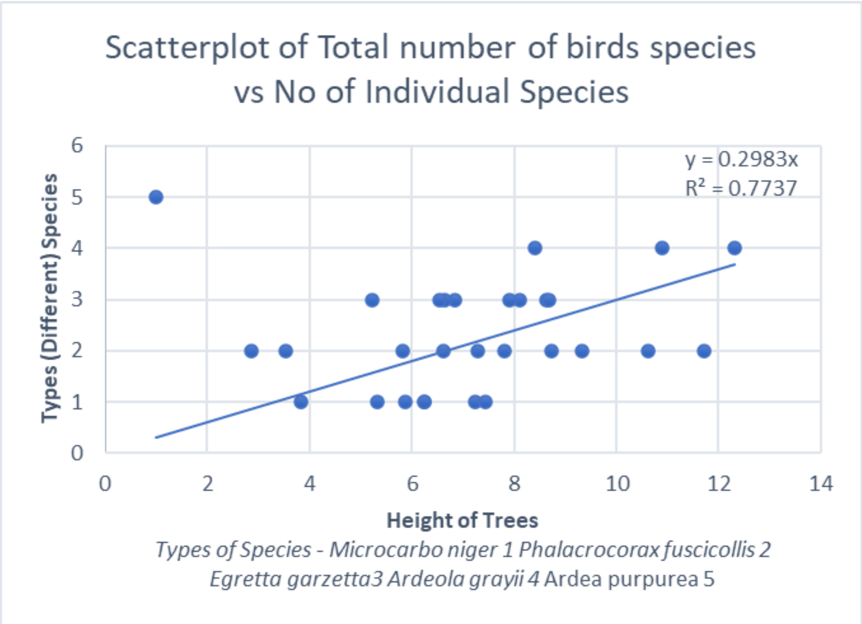

Fig. 5. Correlation analyses for the height of the tree versus the types of individual birds.

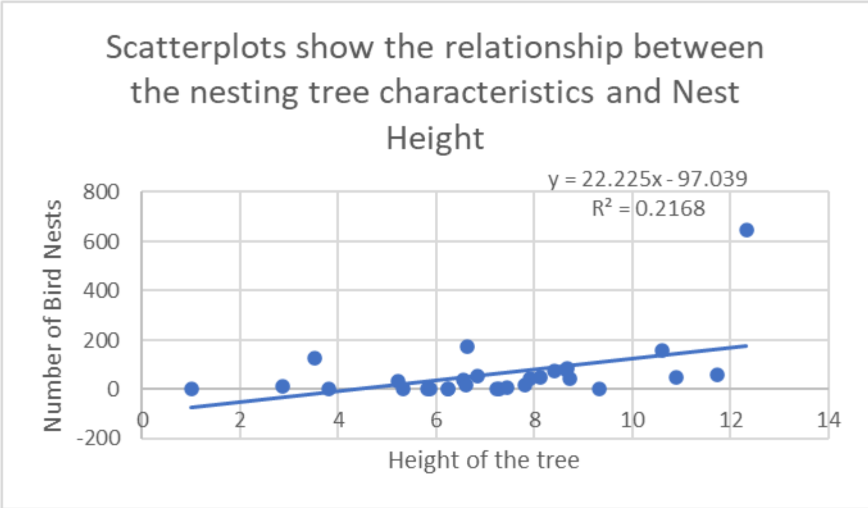

Fig. 6. Correlation analyses for the height of the individual tree versus the number of individual birds' nests.

Proximity of Heronry to Water bodies and human habitation

Three of the major heronries with maximum nesting were located in the premises of busy fishing harbours. All the heronries except the one at Mynagappally were in the midst of human habitations with many of the nests on trees overhanging the houses and busy roads. Majority of nesting trees recorded are coconut trees, which is a cultivated crop and a homestead tree. It was noted that the heronries both big and small were located very near to water bodies at a distance of 5–250 m from water bodies. Closest ones were ponds, paddy fields and lakes while the distant water body was sea.

Kollam, the sothern part of Kerala is inhabited by a wide range of wetland birds. Most of the 15 species that nest or breed in Kerala have been reported from the district too by eBird and avid birdwatchers. Wetland areas in and around the city provides suitable feeding and breeding grounds for these birds, which they use for perching, nesting, foraging and safe haven from predators. The study area being home to two of the three Ramsar sites of Kerala, by itself is offering conducive habitat to the wetland birds. The lakes, rivers, waterways, canals, swamps, mangroves and numerous paddy fields, all, support the heronry birds in one way or the other.

Three major heronries (Vaddy, Neendkara & Kazhukanthuruth) are directly depending on the adjoining fishing harbours for their foraging. Here we have noted that the LE are depredating on the catches of fishing vessels and the landings. In Vaddy and Neendakara harbours, the birds can be seen in large numbers on the truss and roof of the fish handling areas, waiting patiently to scoop up fish from the baskets of head load workers. These birds seem to have lost foraging skills as they are mostly depredators and scavengers. The heronries are found in crowded residential areas and busy public areas frequented by the general public. In the district that's ranked 48th most populous agglomerations of India (Census of India, 2001), urban and rural demarcation in terms of population density is just namesake.

Areas that are rich in the context of suitable large trees, feeding ground nearby and low predation pressure, probably set the city a preferred breeding ground for the waterbirds (Roshnath & Sinu, 2017). Along with nesting locations, year-round nutrition resources, less predatory pressure and stable climatic conditions offered by an urban ecosystem (Fischer *et al*., 2012; Seedikkoya & Azeez, 2012; Ajitha & Jose, 2015; Griffin *et al*., 2017; Roshnath *et al*., 2019). Many authors have suggested that a low predatory pressure favours water bird nesting activities in urban areas so, the nest site selection, nesting preference, and architecture of nests directly correlate with the predation rate (Garg, 2016). Results and findings of this survey conforms to the findings of the previous studies with regard to the nesting and proliferation of heronry birds on areas that have suitable large trees, nearby feeding grounds, less predatory pressure, favourable climate. The abundance of the LE nests in the three major heronries can be correlated to the "free meal" the LE effortlessly scoops from the landed fish and discards.

All tree characteristics individually and in interaction with each other predicted the occupancy and the abundance of nests in trees, which is in agreement with the findings of previous studies (Post, 1990; Ranglack *et al*., 1991; Minias & Kaczmarek, 2013). Previous studies suggest that the average height of the nesting trees of heronry birds is 6–11 m (Telfair, 1983; Hilaluddin *et al*., 2006; Sashikumar & Jayarajan, 2007), which might vary with the habitat (Telfair, 1983, 1994; Narayanan, 2014). In the present study the nesting heights recorded are in the range of 2.86-12.32 m. Thorough monitoring of the heronry sites showed that even though other trees with desired characters are present in the vicinity, birds choose to nest in trees that were selected previously (Kelsall & Simpson, 1980; Visser *et al*., 2005). In Nadal, pond-herons nest in small rain tree with less-extent canopy, even though large canopied rain trees were abundant. Street trees are important habitats for birds and other urban taxa (Nagendra & Gopal, 2010). Heronry birds' nests have differential predatory pressure from the birds of prey, snakes, and mouse in the urban and wild natural habitats (King, 1983; Walask, 1990; Gliwicz *et al*., 1994).

Greater abundance of suitable large trees, low predation pressure, and additional foraging places in the close neighborhood (e.g., fish markets, garbage pile near coast, e.g. Vady and Thangassery harbor) might have set the city a preferred breeding ground for the heronry birds. Though Kollam ranks 4th in the state with 0.530 km^2 mangrove cover constituting 2.71% of Kerala's mangrove forest, we couldn't locate a single nesting in the mangroves even after repeated boat based surveys. The observations indicate their affinity to the urban areas for nesting, over the conventional mangroves and the like. The study finds that the majority of nesting trees are located in wetland areas, nonresidential areas including industrial area, and residential plots (Subramanya, 1996; Sashikumar & Jayarajan, 2007). The affinity of heronry birds to towns and cities was also reported previously in other parts of India (Subramanya, 1996; Sashikumar & Jayarajan, 2007; Urfi, 2010), and elsewhere (Des Granges & Reed, 1981; Henny *et al*., 1989; Vennesland & Butler, 2004; Vergara *et al.*, 2006). Although heronry birds are highly acclimated to the disturbed environments such as urban areas (Urfi, 2006, 2010; Møller *et al*., 2008), vertical and horizontal expansion of cities, and irresponsible solid waste management are sources of concern for the continued conservation of this important functional group of birds in wetland and urban ecosystems. Notifying the nesting trees as protected sites and proper management of identified nesting trees could help in conservation of these breeding birds.

Heronry at IRE, the government owned processing company, maintained a good population of Little Cormorants and little egrets. The nesting started very recently though it remained a roosting site for several years. Compared to other heronries, human disturbances were very less because of the conservation awareness of company.

Kazhukanthuruth, Kakkathoppe, Edachira & Chanthakadvu regions of Vellanathuruth were found to be a suitable place for breeding as well as roosting. Most of the nesting trees were *Cocos nucifera, Mangifera indica* and *Tamarindus indica.* Indian Pond heron, Little Egret, Indian and Little cormorants breeds here making it a site with conservational priority. Several hundreds of cormorants can be seen on the lakes, especially on the estuary. But nesting birds have been very limited and restricted to faraway sites in the northern end of the district. Thorough search for cormorant nests in the estuarine area, the mangroves and other suitable habitats in rest of the district was futile. Though eBird records of several species of heronry birds are reported from the area, only five species could be found nesting during the survey. Cattle egrets seen at times in hundreds even in breeding plumage during the survey period didn't yield a nest. Storks, grey and reef herons, Oriental darters and Ibises seen in different parts of the district failed to be represented in the nesting survey. 50 or more Oriental darters were seen roosting on a rain tree along with several cormorants and egrets in the middle of the city even during the survey period. Though the site is very old with confirmed roosting of more than 15 years, no nest was found. Search for nests on several mangrove patches of the district was in vain.

CONCLUSIONS

The information compiled here serves as a baseline of recent and available historical distribution and abundance of heronries with details of both nesting species and nesting trees. Wildlife management agencies, conservationists and town planners can benefit from the baseline data as it gives insight into the current colony locations, status and trends of colonial nesting waterbirds and shall be of assistance to them in making decisions about protection through population and land use management. Finally, future surveys and monitoring can be planned using these data as a comprehensive baseline inventory and atlas of these colonies.

The survey results have been provided to Kollam Social Forestry Division, Forest & Wildlife Department of Kerala, the Indian Rare Earths Kollam, the District administration and the State Heronry Group, so that appropriate management strategies can be implemented. Neendkara, Vaddy and Edachira area heronries that are dense waterbird colonies shall be recommended for protection from disturbances through the application of appropriate protective notations or seasonal sanctuaries.

Human bird conflict and other threats

All the heronry sites with nests on trees were in the areas of dense human habitation. Even when the nesting trees were scattered in and around residential areas, the noisy bird, their droppings, falling chicks and the food remnants from the nests caused severe inconveniences to residents and passers-by alike. The obnoxious odour emanating from the accumulated decomposing excreta, dead chicks and leftovers of rotting fish makes life miserable. Vehicles and dresses are soiled by the droppings. Piling up of excreta on trees is blamed for yield and crop loss. Hapless laymen have resorted to retaliatory measures in some heronries by bursting fire crackers, pelting stones, dislodging nests, trimming branches or felling trees outright. Outbreaks of avian flu elsewhere and ecological disservices are used as a pretext to vindicate the hostilities. Thus, general awareness should be given to the local people living there focusing the importance of birds in the ecosystem. A heronry at Neendakara, reported in earlier studies, had very few nests this season as the teak trees were trimmed. Uncontrolled rapid urbanisation, land reclamation, tree felling, ghost nets and gears, poaching are all taking toll on the heronry birds. A concerted effort led by the Forest & Wildlife Department with the active participation of the residents and NGOs is the need of the hour to address the grievances and pave way for the conservation of the heronries. As water birds are considered to be the ecological indicators of wetland ecosystem health, care should be taken to maintain the population of them.

Posters regarding the nesting bird species could be posted under the trees to draw public attention and create awareness. If removing the nesting trees is inevitable for the expansion of the cities, we recommend selective removal of the non-nesting trees in the vicinity first. Local governments may consider erecting heronry guards above the bus waiting shelters if nesting trees are located in such places (Sashikumar C., pers. comm.).

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AREA DEPENDANT DISTRIBUTION OF AVIFAUNA IN THE SACRED GROVES OF NORTHERN KERALA, INDIA

PAZHEDATH VASUDEVAN KRISHNANAND, KARUMAMPOYIL SAKTHIDAS ANOOP DAS

The sacred groves otherwise called sacred forests are small patches of natural habitats that act as a refugee for many species which are being protected by religious reasons. Seven sacred forests, are selected that belongs to Kerala, India having the areas varies between 3 and 6.4 hectares. The bird diversity in these selected sacred forests were studied from December 2017 to May 2019. The point count method was used for the bird survey which recorded 88 species of birds. A higher bird species richness was observed in Nelair kottam (6.4 ha) with 49 species, which is the largest study sites among the study sites and the less number of bird species belongs to Poil kavu (4.4 ha) with 14 species of birds. Shannon-Weiner Index was employed to determine the bird species diversity. Species-Area relationship in these ecosystems is also estimated. This study highlights that the area of the sacred forest is one of the important factors in determining the species richness and abundance of birds.

Keywords: Avifauna, sacred groves, point count, Shannon-Wiener Index, species-area relationship.

INTRODUCTION

Sacred forests, otherwise called sacred groves, are fragments of landscapes containing vegetation, life forms and geographical features, delimited and protected by human societies in a relatively undisturbed state, which usually have a religious connotation (Hughes & Chandran, 1998). These forests have historical existence from the pre-agricultural era and most of these conserve pristine vegetation (Gadgil & Vartak 1975). Religious and cultural beliefs are the major driving force behind the conservation of these forest patches (Ormsby & Bhagwat, 2010). They vary in size depending on their location and management profile. These are considered to be a small conservative area that provides ground for maintaining biodiversity and thus helps in the conservation of local and endemic species (Bhagwat *et al.*, 2005; Kushalappa & Raghavendra, 2012). This is a very old tradition and still exists in various forms across the globe (Bhagwat & Rutte, 2006). Even though the estimation of the number of sacred groves found in India is difficult, recorded around 13,720 sacred groves from 19 states (Gokhale *et al.*, 2001).

The state of Kerala, is also rich in sacred groves, recorded around 2000 sacred groves contributing 0.15% of land. The total area of these sacred groves varies from 0.01 hectares to 24 hectares, with Kammadam kavu (24 hectares) of Kasargod district as the largest sacred grove. Sacred groves are called “kavu” in Malayalam (local language of Kerala), in which some are managed by private temple trustee, some are under devaswam boards, and some are owned by private family members (Chandrashekara & Sankar, 1998), thus signifies the close association of sacred groves with the people. These small conservatory patches support birds even in adverse conditions (Brandt *et al.*, 2013). This study examined the bird abundance, diversity, bird species richness in the sacred groves and also how the distribution of birds are related with the size or area of an ecosystem. This study also tests the species-area curve whether the relationship between bird species and area of sacred groves favors this curve.

Study area

The present study is conducted in seven sacred groves, three from Kasargod, two in Kannur and two sacred groves in Kozhikkode of Northern Kerala, India (Fig. 1). The detailed description of the selected study sites is given in the Table 1.

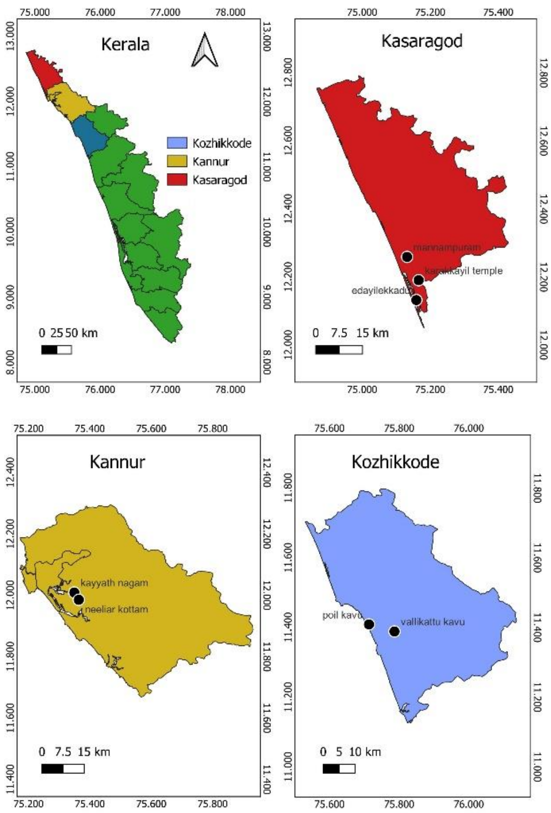

Fig 1. Selected study sites.

Table 1

Selected study sites description

Sl no	Sacred grove	Area (ha)	Coordinates	Ownership	Water source	Adjacent habitat
1.	Mannampuram kavu (Kasargod)	3	12.258608 75.132317	Malabar devaswam board	pond	Home steads
2.	Karakkayil kavu (Kasargod)	6	12.190213 75.166295	Private temple trust	Pond	field
3.	Neliar kottam (Kannur)	6.4	11.985404 75.363928	Private temple trust	Well	Home steads
4.	Kayyath nagam (Kannur)	6	12.009116 75.349348	Private temple trust	Pond	Hill top
5.	Edayilekadu kavu (Kannur)	5.2	12.131795 75.159621	Private trust	pond	Fields, home steads
6.	Poil kavu (kozhikkode)	4.4	11.408626 75.71358	Private temple trust	Nil	Home steads
7.	Vallikkatu kavu (kozhikkode)	6.3	11.38834 75.786571	Malabar devaswam board	stream	Fields

MATERIAL AND METHODS

Fixed point count method was used to determine the bird species abundance and diversity (Bibby *et al.*, 1992; Raman, 2003) with five minutes duration after three hours of sunrise with 25 metre apart from each point. All the birds heard or seen within 30 metre radius at each point are recorded using binoculars (10 × 50) and the birds were identified using field guides (Grimmet & Inskipp, 2005).

The details included the number of bird species, the number of individual species and the distance from point of observation. This bird count was repeated once every two months from December 2017 to May 2019.

The obtained data is analyzed to detect the species richness abundance and diversity of bird species. The association between the area of patch and bird species is also analyzed to obtain the area-dependent distribution of birds in the selected sacred groves. All the data were analyzed using Microsoft excel 2011 version and SPSS software 2011 version.

RESULTS AND DISCUSSION

Bird species richness and diversity analysis

A total of 88 bird species were recorded from the seven sacred groves which belong to 41 families and 13 orders. Passeriformes were the most dominant (67%), followed by Piciformes (4.8%) and Coraciiformes (4.8%) (Table 2).

Table 2

Family wise distribution of birds

Sl. no	Family	Order
1.	Corvidae	Passeriformes
2.	Leiothrichidae	
3.	Nectariniidae	
4.	Sturnidae	
5.	Pycnonotidae	
6.	Dicruridae	
7.	Dicaeidae	
8.	Muscicapidae	
9.	Pittidae	
10.	Timaliidae	
11.	Oriolidae	
12.	Monarchidae	
13.	Chloropseidae	
14.	Rhipiduridae	
15.	Phylloscopidae	
16.	Acrocephalidae	
17.	Cisticolidae	
18.	Paridae	
19.	Laniidae	Passeriformes
20.	Vangidae	
21.	Pellorneidae	
22.	Zosteropidae	
23.	Turdidae	
24.	Campephagidae	

Table 2 (continued)

Sl. no	Family	Order
25.	Aegithinidae	
26.	Alcippeidae	
27.	Sittidae	
28.	Megalaimidae	Piciformes
29.	Picidae	
30.	Cuculidae	Cuculiformes
31.	Alcedinidae	Coraciiformes
32.	Meropidae	
33.	Hemiprocnidae	Apodiformes
34.	Strigidae	Strigiformes
35.	Accipitridae	Accipitriformes
36.	Apodidae	Apodiformes
37.	Phasianidae	Galliformes
38.	Rallidae	Gruiformes
39.	Psittaculidae	Psittaciformes
40.	Columbidae	Columbiformes
41.	Ardeidae	Pelecaniformes

Species abundance was more in Vallikkatu kavu (6.3 ha) with 131 individuals. Neliar kottam (6.4 ha) has 110 individuals where as 86 individuals were recorded from karakkayil kavu (6 ha). Least number of individual bird species was from Poil kavu (4.4 ha) with 25 individuals (Table 3).

Table 3

Descriptive statistics

Camp	**Mannampuram (3 ha)**	**Karaka kavu (6 ha)**	**Neliar kottam (6.4 ha)**	**Edayilekadu kavu (5.2 ha)**	**Kayyath Nagam (6ha)**	**Poil kavu (4.4ha)**	**Vallikkatu kavu (6.3ha)**
No. of species	27	43	49	33	30	14	37
Species abundance*	52	86	110	79	79	25	131
Shannon index	1.303	1.574	1.635	1.534	1.41	0.801	1.473
Mean individuals	0.591	0.977	1.25	0.898	0.898	0.284	1.489
Standard deviation*	1.21	1.313	1.464	1.26	1.547	1.241	2.344

*Species abundance - It is the number of individual birds belongs to different species in respective study sites;
*Standard deviation - Deviation from the mean value. Most of the data points are adjacent to each other indicting close resemblance between study sites in species composition and sample 6 is deviated more indicates Vallikkatu kavu has difference in bird species composition and showing more diverse bird species than others.

Neliar kottam (6.4 ha) had the most number of bird species whereas Poil kavu (4.4 ha) recorded 14 species (Fig. 2). Bird species diversity is determined by Shannon- Weiner index (Table 3) and feeding guild structure is also determined (Fig. 3). Most of them were insectivorous (37/88).

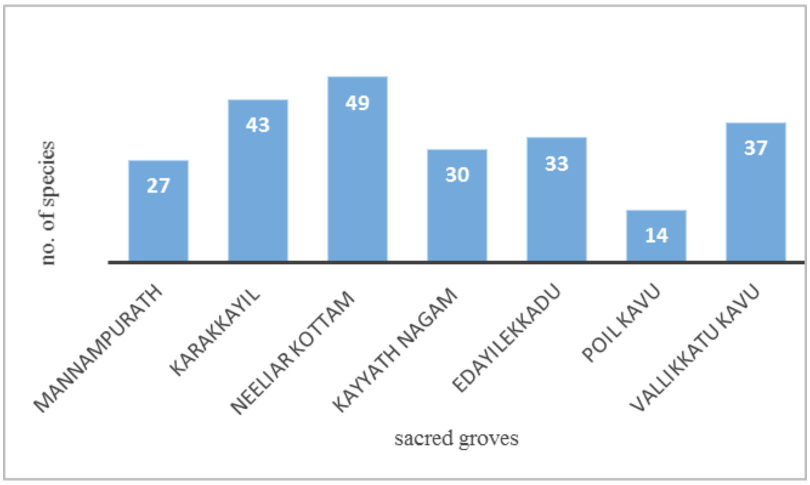

Fig. 2. Number of bird species in study sites recorded of Northern Kerala.

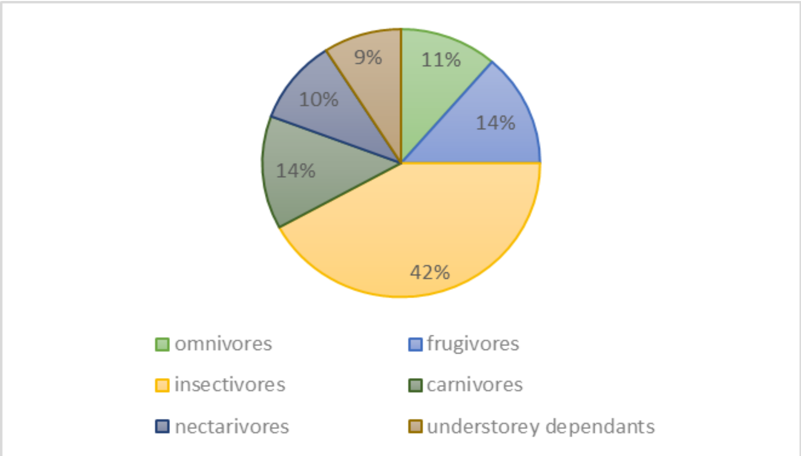

Fig 3. Feeding guild structure of birds.

Species richness or number of bird species belongs to each sacred grove is determined. The study sites, when arranging in descending order of species richness, will follow the pattern-Neliar kottam (49 bird species)>Karakkayil kavu (43)> Vallikkatu kavu (37)>Edayilekadu kavu (33)>Kayyath Nagam kavu (30)> Mannampurath kavu (27)> Poil kavu (14).

Neliar kottam contribute more percentage of bird species (55.68%), Karakkayil kavu has 48.86% of total identified bird species. Vallikkatu kavu recorded 42.04% bird species, Edayilekadu kavu has 37.5% of total bird species whereas Kayyath Nagam kavu has 34.09% bird species. Mannampurath kavu recorded 30.68% species of birds and the least percentage of bird species is recorded in Poil kavu with 15.90% of the total recorded bird species from all the selected study sites.

The sacred groves provide roosting and nesting sites for many birds. Poil kavu sacred grove is a roosting site for brown fish owl, brahminy kite. The threatened species white bellied sea eagle is nested in Edayile kadu sacred grove, its nesting and breeding were reported from here in 2000 (Palot, 2011), and even this time also, this species continues to choose Edayile kadu sacred grove as its nesting site, which is a good sign that these small patches act as refugee sites for endemic and endangered species.

To find out the area dependency of birds, a graph is plotted with bird species abundance and Area (ha) as coordinates, by using logarithmically transformed species numbers and area (Fig. 4). This study favors the species-area curve graph with R value equals 0.65 (R^2 = 0.4245) indicating that area of the ecosystem and the bird species richness has some correlation between themselves highlights that area of an ecosystem is a major factor that determine the bird species diversity and also indicates, apart from the area, some other factors influence the species diversity. The diversity of bird species is dependent on multiple factors, if the area is the primary factor, then a linear graph would be obtained in this study, thus highlighting the role of some other factors such as vegetation, climatic conditions, seasonal variations in determining the species abundance in an ecosystem.

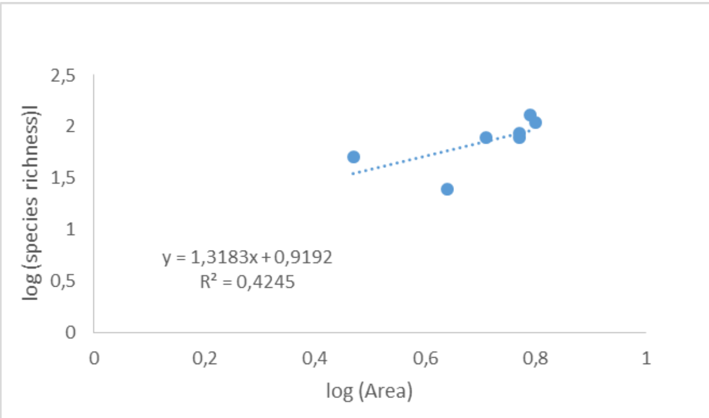

Fig. 4. Bird species-area relationship observed in the sacred groves of Northern Kerala.

The most widely used model to examine the species area relationship is $S=cA^Z$, where S is species richness, A is the area, and c and z are constants. The value of slope (z), as plotted from this study is 1.31 and the intercept (c) is 0.91 (Fig. 4). The species-area relationship of birds are similar with other studies (Brashares *et al.*, 2001; Olivier *et al.*, 2013).

Species-area models have become the primary tool to predict baseline extinction rate for species in isolated habitats and have influenced conservation and land use planning worldwide (Brashares *et al.*, 2001). Extinction is an ongoing process ad its rate is predicted to increase due to increase rate of habitat conversion and to the extension debt, which is the future ecological cost of current habitat destruction, where the loss of natural habitats causes time-delayed extinctions (Tilman *et al.*, 1994). Conversion of natural habitats by human would reduce the species number and may leads to extinctions. An extinction debt may present in the human modified landscapes (Báldi & Vörös, 2006; Szabo *et al*., 2011), where the conversion of habitats results in species loss. The extinction debt is predicted by using species area curve (Olivier *et al*., 2013). This may happen in the sacred groves too, where the human activities such as deforestation, encroachment in these small patches would result in the species loss in future. As seen from this study, in case of white bellied sea eagle, which is nested in the sacred grove, it may lose its habitat when any anthropological disturbance occur in that landscape in future.

Sacred groves can be considered as examples for in situ biodiversity conservation (Sharma & Devi, 2014) .These are responsible for conserving natural resources by religious informal rules and regulations (Narasimha, 2015; Singh *et al*., 2017). These are not categorized as formal protected areas but may have conservational importance as that of protected areas (Boraiah *et al.*, 2003). Many endemic species are being conserves in these small islets and the proper management of these patches would ensure the protection of native species.

The details of bird species recorded from all the studied sacred groves is given in Table 4.

Table 4

Bird species recorded in each sacred groves

Sl no	**Common name**	**Species**	**Family**	**Mannampuram**	**Karakayil kavu**	**Neliar kottam**	**Edayile kadu**	**Kayyath Nagam**	**Poil kavu**	**Vallikkatu kavu**
1	House crow	*Corvus splendens*	Corvidae	5	4	4	6	4	5	5
2	Rufous treepie	*Dendrocitta vagabunda*	Corvidae	3	5	5	5	3	1	4
3	White cheeked barbet	*Megalaima viridis*	Megalaimidae	2	4	4	2	4	1	3
4.	Asian koel	*Eudynamys scolopaceus*	Cuculidae	0	3	3	1	2	0	0

Table 4 (continued)

Sl no	Common name	Species	Family	Mannampuram	Karakayil kavu	Neliar kottam	Edayilekadu	Kayyath Nagam	Poil kavu	Vallikkatu kavu
5	White throated kingfisher	*Halcyon smyrnensis*	Alcedinidae	1	2	1	2	1	0	2
6	Crested tree swift	*Hemiprocne coronata*	Hemiprocnidae	0	1	3	2	2	0	1
7	Yellow billed babbler	*Turdoides affinis*	Leiothrichidae	4	3	5	4	7	0	6
8	Purple sunbird	*Cinnyris asiaticus*	Nectariniidae	1	1	2	1	2	0	6
9	Chestnut tailed starling	*Sturnia malabarica*	Sturnidae	3	0	0	0	0	0	0
10	Red vented bulbul	*Pycnonotus cafer*	Pycnonotidae	2	1	2	1	4	0	5
11	Indian golden oriole	*Oriolus kundoo*	Oriolidae	1	2	2	1	3	0	4
12	Jungle owlet	*Glaucidium radiatum*	Strigidae	1	1	1	0	1	0	2
13	Greater racket tailed drongo	*Dicrurus paradiseus*	Dicruridae	6	7	7	4	6	10	8
14	Purple rumped sunbird	*Leptocoma zeylonica*	Nectariniidae	2	1	3	2	2	0	4
15	Nilgiri flowerpecker	*Dicaeum concolor*	Dicaeidae	2	1	2	1	0	0	2
16	Black rumped flameback	*Dinopium benghalense*	Picidae	2	3	2	2	2	1	3
17	Black naped oriole	*Oriolus chinensis*	Oriolidae	0	1	2	2	1	0	1
18	Pale billed flowerpecker	*Dicaeum erythrorhynchos*	Dicaeidae	2	0	2	0	0	0	0
19	Black drongo	*Dicrurus macrocercus*	Dicruridae	1	2	1	1	0	1	2
20	Red whiskered bulbul	*Pycnonotus jocosus*	Pycnonotidae	2	0	4	3	6	0	12
21	Oriental magpie robin	*Copsychus saularis*	Muscicapidae	4	0	1	1	3	0	5
22	Greater coucal	*Centropus sinensis*	Cuculidae	1	2	2	1	3	1	4
23	Black kite	*Milvus migrans*	Accipitridae	1	0	0	0	0	0	0
24	Indian pitta	*Pitta brachyura*	Pittidae	1	1	1	0	0	0	2
25	Blue Capped rock thrush	*Monticola cinclorhynchus*	Muscicapidae	1	0	0	0	2	0	0
26	Dark fronted babbler	*Rhopocichla atriceps*	Timaliidae	3	0	0	2	0	0	0
27	blue tailed bee eater	*Merops philippinus*	Meropidae	1	0	2	0	0	0	0
28	Asian green bee eater	*Merops orientalis*	Meropidae	0	2	1	0	0	0	0

Table 4 (continued)

Sl no	**Common name**	**Species**	**Family**	**Mannampuram**	**Karakayil kavu**	**Neliar kottam**	**Edayilekadu**	**Kayyath Nagam**	**Poil kavu**	**Vallikkatu kavu**
29	Black naped oriole	*Oriolus chinensis*	Oriolidae	0	1	2	1	0	0	2
30	Indian paradise flycatcher	*Terpsiphone paradisi*	Monarchidae	0	1	4	2	2	0	0
31	Common myna	*Acridotheres tristis*	Sturnidae	0	3	4	0	3	0	5
32	yellow browed bulbul	*Acritillas indica*	Pycnonotidae	0	1	0	0	0	0	0
33	Ashy drongo	*Dicrurus leucophaeus*	Dicruridae	0	1	0	0	0	0	3
34	crested serpent eagle	*Spilornis cheela*	Accipitridae	0	1	1	0	1	0	1
35	Tikell's blue flycatcher	*Cyornis tickelliae*	Muscicapidae	0	1	0	0	0	0	0
36	Golden fronted leafbird	*Chloropsis aurifrons*	Chloropseidae	0	2	0	0	0	0	0
37	White browed fantail	*Rhipidura aureola*	Rhipiduridae	0	3	3	0	0	0	0
38	large billed leaf warbler	*Phylloscopus magnirostris*	Phylloscopidae	0	2	0	0	0	0	0
39	Blyth's reed warbler	*Acrocephalus dumetorum*	Phylloscopidae	0	1	2	0	0	0	0
40	common tailorbird	*Orthotomus sutorius*	Acrocephalidae	0	2	0	2	0	0	0
41	ashy prinia	*Prinia socialis*	Cisticolidae	0	1	0	0	0	0	0
42	cinereous tit	*Parus cinereus*	Paridae	0	2	0	0	0	0	0
43	brown shrike	*Lanius cristatus*	Laniidae	0	1	0	1	0	0	0
44	malabar woodshrike	*Tephrodornis sylvicola*	Vangidae	0	2	0	2	2	0	0
45	malabar barbet	*Megalaima malabarica*	Megalaimidae	0	2	0	2	0	0	0
46	common kingfisher	*Alcedo atthis*	Alcedinidae	0	1	0	0	2	0	0
47	brown cheeked fulvetta	*Alcippe poioicephala*	Alcippeidae	0	2	0	0	0	0	0
48	puff throated babbler	*Pellorneum ruficeps*	Pellorneidae	0	2	3	2	3	0	0
49	Indian white eye	*Zosterops palpebrosus*	Zosteropidae	0	2	1	0	0	0	0
50	orange headed thrush	*Geokichla citrina*	Turdidae	0	2	0	0	0	0	0
51	indian robin	*Saxicoloides fulicatus*	Muscicapidae	0	2	2	1	0	0	0

Table 4 (continued)

Sl no	Common name	Species	Family	Mannampuram	Karakayil kavu	Neliar kottam	Edayilekadu	Kayyath Nagam	Poil kavu	Vallikkatu kavu
52	pied bushchat	*Saxicola caprata*	Muscicapidae	0	1	1	0	0	0	0
53	Bronzed drongo	*Dicrurus aeneus*	Dicruridae	0	0	2	2	0	0	0
54	Orange minivet	*Pericrocotus flammeus*	Campephagidae	0	0	0	0	2	0	2
55	Crimson backed sunbird	*Leptocoma minima*	Nectariniidae	0	0	2	2	0	0	0
56	Common iora	*Aegithina tiphia*	Aegithinidae	0	0	1	0	0	0	1
57	Jerdon's leafbird	*Chloropsis jerdoni*	Chloropseidae	0	0	2	0	0	0	0
58	Verditer flycatcher	*Eumyias thalassinus*	Muscicapidae	0	0	2	0	0	0	0
59	Asian brown flycatcher	*Muscicapa latirostris*	Muscicapidae	0	0	1	0	0	0	0
60	coppersmith barbet	*Megalaima haemacephala*	Megalaimidae	0	0	2	2	0	0	0
61	Greenish warbler	*Phylloscopus trochiloides*	Phylloscopidae	0	0	1	0	0	0	0
62	Loten's sunbird	*Cinnyris lotenius*	Nectariniidae	0	0	2	0	0	0	4
63	Little spiderhunter	*Arachnothera longirostra*	Nectariniidae	0	0	1	0	0	0	0
64	Flame throated bulbul	*Rubigula gularis*	Pycnonotidae	0	0	1	0	0	0	0
65	Laughing dove	*Spilopelia senegalensis*	Columbidae	0	0	2	0	0	0	0
66	Indian jungle crow	*Corvus culminatus*	corvidae	0	0	2	0	0	0	0
67	Black naped Monarch	*Hypothymis azurea*	Monarchidae	0	0	1	0	0	0	0
68	large billed leaf warbler	*Phylloscopus magnirostris*	Phylloscopidae	0	0	2	0	0	0	0
69	common hawk cuckoo	*Hierococcyx varius*	Cuculidae	0	0	1	1	0	0	1
70	indian tit	*Machlolophus aplonotus*	Paridae	0	0	0	2	0	0	0
71	small minivet	*Pericrocotus cinnamomeus*	Campephagidae	0	0	0	3	0	0	0
72	brown hawk owl	*Ninox scutulata*	Strigidae	0	0	0	0	1	0	0
73	little swift	*Apus affinis*	Apodidae	0	0	0	2	0	0	0
74	Indian nuthatch	*Sitta castanea*	Sittidae	0	0	0	3	0	0	0
75	Jungle myna	*Acridotheres fuscus*	Sturnidae	0	0	0	2	3	0	0
76	spotted dove	*Spilopelia chinensis*	Columbidae	0	0	0	0	1	1	4

Table 4 (continued)

Sl no	Common name	Species	Family	Mannampuram	Karakayil kavu	Neliar kottam	Edayilekadu	Kayyath Nagam	Poil kavu	Vallikkatu kavu
77	black hooded oriole	*Oriolus xanthornus*	Oriolidae	0	0	0	1	0	0	0
78	mottled wood owl	*Strix ocellata*	Strigidae	0	0	0	0	1	0	0
79	white bellied sea eagle	*Haliaeetus leucogaster*	Accipitridae	0	0	0	2	0	0	0
80	Emerald dove	*Chalcophaps indica*	Columbidae	0	0	0	0	0	3	0
81	Brown fish owl	*Bubo zeylonensis*	Strigidae	0	0	0	0	0	1	0
82	Indian pond heron	*Ardeola grayii*	Ardeidae	0	0	0	0	0	0	3
83	Rose ringed parakeet	*Psittacula krameri*	Psittaculidae	0	0	0	0	0	0	5
84	Gray breasted prinia	*Prinia hodgsonii*	Cisticolidae	0	0	0	0	0	0	1
85	Grey junglefowl	*Gallus sonneratii*	Phasianidae	0	0	0	0	0	0	7
86	Cattle egret	*Bubulcus ibis*	Ardeidae	0	0	0	0	0	0	6
87	shikra	*Accipiter badius*	Accipitridae	0	0	0	0	0	0	1
88	White breasted waterhen	*Amaurornis phoenicurus*	Rallidae	0	0	0	0	0	0	4
				52	86	110	79	79	25	131

CONCLUSIONS

Sacred groves are small conservatory regions that are protected based on religious aspects. Apart from religious importance, these patches are responsible for the conservation of species and are also home to numerous bird species as seen from this study. Despite its closeness to human habituations and small area/size, these patches act as a refugee to many living species, as seen in the case of white bellied sea eagle. Poil kavu (4.4 ha), even though this has an ample area to support bird life, limited number of bird species is reported from here as compared to other sacred groves in this study. It highlights that, apart from area, there are some other factors responsible for the bird diversity. Level of disturbance, human activities inside the sacred grove may also affect the species abundance. Proper fencing, management policies would promote conservation of these small patches thereby conserving the endemic and endangered species. Conservation and proper management of these islands are necessary in the human dominated landscapes since their presence ensures the ecosystem balance.

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ELECTRON MICROSCOPIC INVESTIGATIONS OF THE TUMOR-STROMA INTERFACE DURING SKIN BASAL CELL CARCINOMA DEVELOPMENT

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The aim of this paper is to know subtle infrastructural alterations of cancer cells *per se* as well as associated tumor stroma of basal cell carcinoma (BCC). From the external part of the tumor mass towards the associated stroma a gradually alteration of histoarchitecture is remarkable. Basement membrane and hemidesmosomes are severely altered or totally abolished. Tumor cells affronted to the stromal microenvironment shed extracellular microvesicles. Because of high fragility of tumor associated microvasculature, extravasated inflammatory cells become intermingled with tumor cells. Inside of tumor mass, particular conduit systems delimited by tumor cells can be detected. A recently described cell phenotype termed telocyte is present inside of peritumoral stroma of all our investigated cases of BCC. These peculiar stromal cells establish both homo- and heterocellular junctions.

Keywords: Basal cell carcinoma, tumor-stroma interface, defective hemidesmosomes, shedding membrane vesicles, tumor microvasculature, conduit system, telocytes.

INTRODUCTION

Cancer is considered an irreversible disease mainly due to gradually accumulation of one or some mutations and/or deregulation of oncogenes and tumor suppressor genes and chromosomal abnormalities. This model corresponds to the so called somatic mutation theory (SMT) of cancer (Sonnenschein & Soto, 2000, 2014). SMT posits that cancer begin with a single mutation in a somatic cell followed by successive mutations (Baker % Kramer, 2007). Because many authors reported conflictual data *versus* the SMT, an alternative theory was elaborated termed tissue organization field theory (TOFT). TOFT states cancer arises and maintain as a result of defective cell-cell and cell-extracellular matrix communication (Soto & Sonnenschein, 2004; Baker & Kramer, 2007; Bizzari *et al*., 2014; Monti *et al*., 2022). Some infrastructural aspects observed in this study may contribute to sustain also the second theory. The large majority of human cancers have an epithelial origin accounting for circa 80% of all malignancies.

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Like any other type of cancer, skin tumours occur when damaged DNA of some epidermal cells is unrepaired and mutations are installed so that affected epidermal epithelial cells proliferate uncontrolled. Stratified epithelial epidermal cells mainly represented by keratinocytes become in direct contact with the hostile environment. Cancer (a malignant tumor) develops as a progressive multi-step process in which involved cells undergo consecutive genetic alterations and in cooperation with stromal cells gradually acquire phenotypic changes so that transformed cells will grow rapidly and uncontroled. Mention must be made that there is a body of evidence that alterations in the tumor cells themselves are not sufficient to generate a tumor so that an adequate stromal microenvironment is a necessary condition (Fusenig *et al*., 2002; Mueller & Fusenig, 2004; Mirancea & Mirancea, 2010a,b). Indeed, a malignant tumor is a complex ecosystem: (1) genetically altered neoplastic cells and (2) the associated tumor stroma represented by (a) connective tissue cells, imported cells plus (b) extracellular matrix and (3) the embedded microvasculature (Fusenig *et al*., 2002; Mirancea *et al*., 2013; Moroşanu *et al.*, 2013).

There are two main categories of skin cancer: (1) melanoma and (2) non melanoma. Non melanoma skin cancers are mainly comprised of (a) basal cell carcinoma (BCC) which accounts for about 80% of all non melanoma skin cancers and (b) squamous cell carcinoma (SCC) (Mirancea *et al*., 2013; Căruntu *et al.*, 2014). In terms of classification of BCCs, Reiter *et al.* (2021) showed that the most common dermoscopic features of BCC were arborizing vessels (59%), shiny white structures (49%), and large blue-gray ovoid nests (34%), which allow the systematic classification of BCC histopathologic subtypes. More than 26 different subtypes of BCC are known, but the more common types include: nodular, micronodular, superficial, morpheaform, infiltrative and fibroepithelial (Basset-Seguin & Herms, 2020; McDaniel *et al*., 2021). Because approximately 29% of patients with a primary BCC lesion will develop at least one such lesion in their lifetime, it is necessary to pay more attention to the profile of risk factors specific to each patient in order to have a better view of the differential diagnosis and long-term prognosis (Bartos, 2019). Taking into account the histopathological aspects and the molecular alterations, it can be stated that the probability of developing BCC is the result of a complex cumulation of factors that interact with each other: genetic, phenotypic and environmental factors (Dika *et al*., 2020).

A tumor is a complex ecosystem formed by neoplastic genetic altered cells and associated tumor stroma represented by different cell phenotypes and extracellular matrix represented by soluble mater and fibrils. There was a great interest to know morphologic aspects of tumor cells *per se*, so that a growing body of results was accumulated (Cheville, 2009; Mirancea & Mirancea, 2010a,b; Mirancea *et al*., 2010, 2014; Moroşanu *et al.*, 2013). Nevertheless, there are still unknown subtle infrastructural alterations of both tumor and extracellular microenvironment which compose the tumour ecosystem. The aim of this paper is to know such peculiar abnormalities which accompany tumor development and tumor cell behaviour.

Here we report about some original observations obtained by electron microscopic investigations of different tumoral specimens from human skin involved in BCC development. Our electron microscopic investigation of the basal cell carcinoma tumors from all investigated cases provide relevant aspects concerning ultrastructure of both tumor cell *per se* as well as associated tumor stroma, including tumor microvasculature and a recently identified cell phenotype termed telocyte.

MATERIAL AND METHODS

In order to perform transmission electron microscopic investigations, small tissue fragments about 2–3 mm^3 from the skin tumors resulted by surgery for diagnostic and curative therapy from the patients with clinic diagnostic for Basal Cell Carcinoma (surgeon got patients' consent) were processed following the routine TEM protocol (Mirancea *et al*., 2007; Mirancea & Mirancea, 2010a). Semithin sections of Epon-embedded tissue fragments were stained with 1% toluidine blue for light microscopy. Ultrathin sections were cut using a diamond knife and collected on 200 mesh grids and double counterstained with uranyl acetate and subsequently lead citrate. The grids were examined by a transmission electron microscope JEOL JEM-1400 operated at an acceleration voltage of 80kV. Several electron microscopic images were digitally colored.

RESULTS

Light microscopy examination

In spite of the fact that histopathological subtypes diagnosis is very important in view of planning patient management, so far, there is no unified and generally accepted classification of BCCs (Saldanha *et al*., 2015). In order to get an accurately diagnosis is very important the modality of biological material excision. It seems that the whole excision of the presumed malign cutaneous lesion is recommended comparing with shave and biopsy specimens because only the whole specimen offers the possibility to detect the biological transformation of BCC which tends to be much evident at the base and edges of the growing neoplasm (Crowson, 2006; Tanese, 2019). Indeed, in almost our investigated BCC cases, there is a clear difference in both light and electron microscopic aspects, depending on the level of the place we focus microscopic the section: from the external region of the tumor mass towards the associated stroma, a gradually alteration of histoarchitecture is detectable (Figs. 1–5) (Moroşanu, 2019, PhD thesis). This aspect is better visible by electron microscopic examination (Figs. 6–10) (Moroşanu, 2019, PhD thesis).

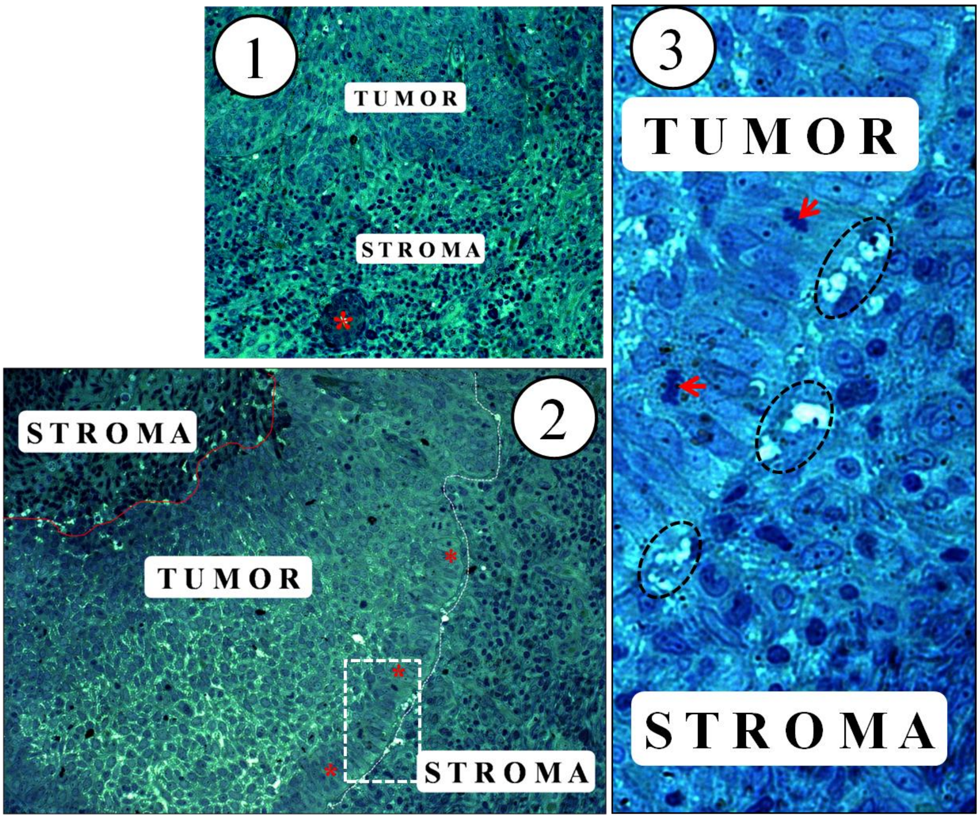


Fig. 1. An overview shows few tumor strands which penetrate inside of the rich epithelioid phenotype stroma (see details in Fig. 2 and Fig. 3). An islet of tumor cells can be detected deeply inside of the stroma (asterisk) (ob. 20x).

Fig. 2 and Fig. 3. A massive mass of tumor at the tumor-stroma interface shows at one side large columnar cells (asterisks), some of them being in mitosis (framed area detailed in Fig. 3, arrows; ob. 20x). Many lacunae can be seen between palisaded basal cells and adjacent stroma (elliptic areas) (ob. 40x).

At the tumor stroma interface, many lacunae can be detected (Fig. 3) (Moroşanu, 2019, PhD thesis). Such kind of lacunae fused and slit-like (cleft-like) spaces are formed (Fig. 4) (Moroşanu, 2019, PhD thesis). Crowson (2006) also reports about slit-like (cleft-like) spaces between palisaded basal cells and adjacent stroma in different morphological subtypes of BCC. Saldanha *et al.* (2015) consider that presence of lacunae or cleft-like between tumor cells and adjacent stroma contributes to differentiate BCC from other tumors.

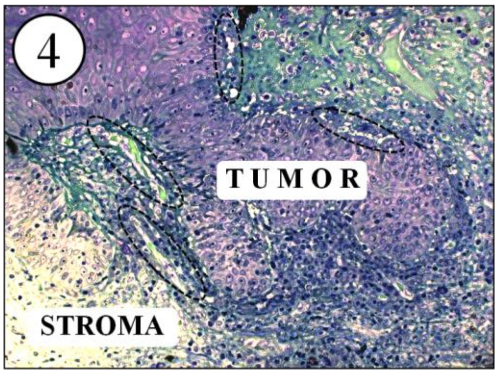


Fig. 4. A very extensive strand o tumor cells penetrates deeply inside of a fibrotic stroma. Clefts-like are formed by adjacent lacunae fusion at the tumor-stroma interface (elliptic areas) (ob. 20x).

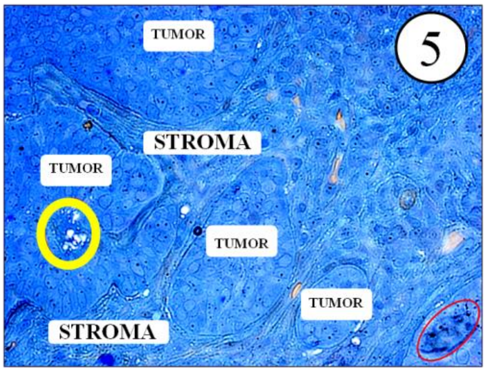


Fig. 5. Overview from a very large tumor showing many cords of tumor cells intermingled with restraint strands of stromal tissue. Yellow elliptic area indicates a primitive lumen formed by coalescence of small lacunae inside of a tumor with adenoid (pseudoglandular) while red elliptic area indicates necrotic cells inside of the tumor strand (ob. 40x).

Electron microscopic investigation

In all investigated skin basal cell carcinoma tumors, an overview from the external part of the tumor mass towards the associated stroma shows a gradually alteration of histoarchitecture. Large nuclei are almost euchromatic and nucleolated. Tumor cells still express some of characteristic but impaired infrastructures of the normal epidermis: limited as number and impaired as ultrastructure desmosomes, totally absence or defective hemidesmososmes, reduced amount of kerain intermedium filaments. Some intercellular spaces can be detected (Fig. 6) (Moroşanu, 2019, PhD thesis). In the middle of the tumor mass, the paucity of both keratin intermedium filaments and desmosomes are remarkable. Moreover, intercellular spaces are much numerous and larger (Fig. 7) (Moroşanu, 2019, PhD thesis).

Inside of the tumor mass much close to the tumor stroma, by hard the epidermal cell phenotype as origin of tumor cells can be identified. Very scanty keratin intermedium filaments and illusive desmosomes can be detected. In such circumstances, numerous and large wide intercellular spaces are formed (Fig. 8) (Moroşanu, 2019, PhD thesis).

At the tumor stroma interface, no basement membrane can be detected but an amorphous material is interposed. Hemidesmosomes are missing or defective hemidesmosomes can be detected (not shown). Ultrastructurally, two tumor cell phenotypes can be distinguished: cells with euchromatic nuclei and cells with heterochromatic nuclei. Some tumor cell exhibit cell projected towards the stroma suggesting an invasive behavior, but also exhibits some remnants of keratin intermedium filaments. Large apparently vide spaces or filled with amorphous material are visible at the tumor stroma border (Fig. 9) (Moroşanu, 2019, PhD thesis).

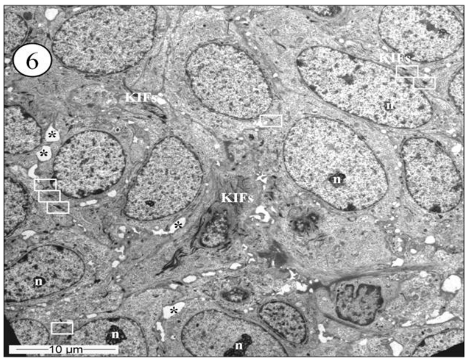


Fig. 6. An overview from the external part of the tumor mass shows an altered histoarchitecture. Large nuclei are almost euchromatic and some nucleols (n) can be seen. Tumor cells still express some of characteristic but impaired infrastructures of the normal epidermis: limited as number and impaired as ultrastructure desmosomes (framed areas), reduced amount of keratin intermedium filaments (IFs). Some intercellular spaces can be detected (asterisks).

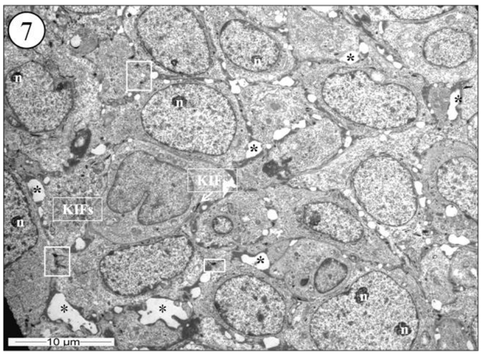


Fig. 7. A successive overview towards the middle of the tumor mass shows a similar aspect from the Fig. 6, but the paucity of both keratin intermedium filaments (KIFs) and desmosomes (framed areas) are remarkable. Intercellular spaces (asterisks) are much numerous and larger. n = nucleols.

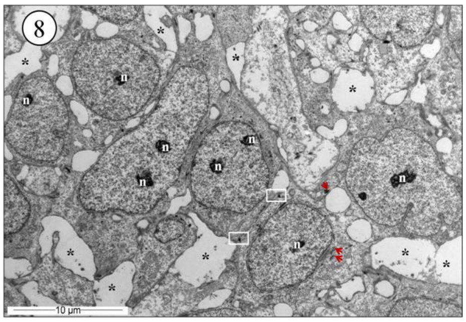


Fig. 8. A succesive image from the middle of the tumor mass towards of the tumor stroma. By hard the epidermal cell phenotype as origin of tumor cells can be identified. Very scanty keratin intermedium filaments (red arrows) and illusive desmosomes (framed areas) can be detected. Intercellular spaces are very numerous and some are very large (asterisks). n = nucleols.

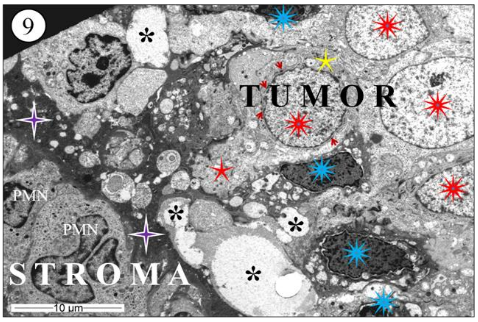


Fig. 9. At the tumor stroma interface, no basement membrane can be detected but an amorphous material is interposed (4-point stars). Ultrastructurally, two tumor cell phenotypes can be distinguished: cells with euchromatic nuclei (red ten point stars) and cells with heterochromatic nuclei (blue ten point stars). One tumor cell with euchromatic nucleus exhibits uropodial cell extension towards the stroma (five point red arrow) while the the rest of cytoplasm (five point yellow star) exhibits some remnants of keratin intermedium filaments (red arrows) around the nucleus. Large apparently vide spaces or filled with amorphous material (asterisks) are visible at the tumor stroma border. Inflammatory stromal cells (PMN) in close vicinity to tumor cells can be seen.

Inflammatory stromal cells in close vicinity to tumor cells can be seen. In some areas, extravasated blood cells become intermingled with invasive tumor cells (Fig. 9 and Fig. 10) (Moroşanu, 2019, PhD thesis).

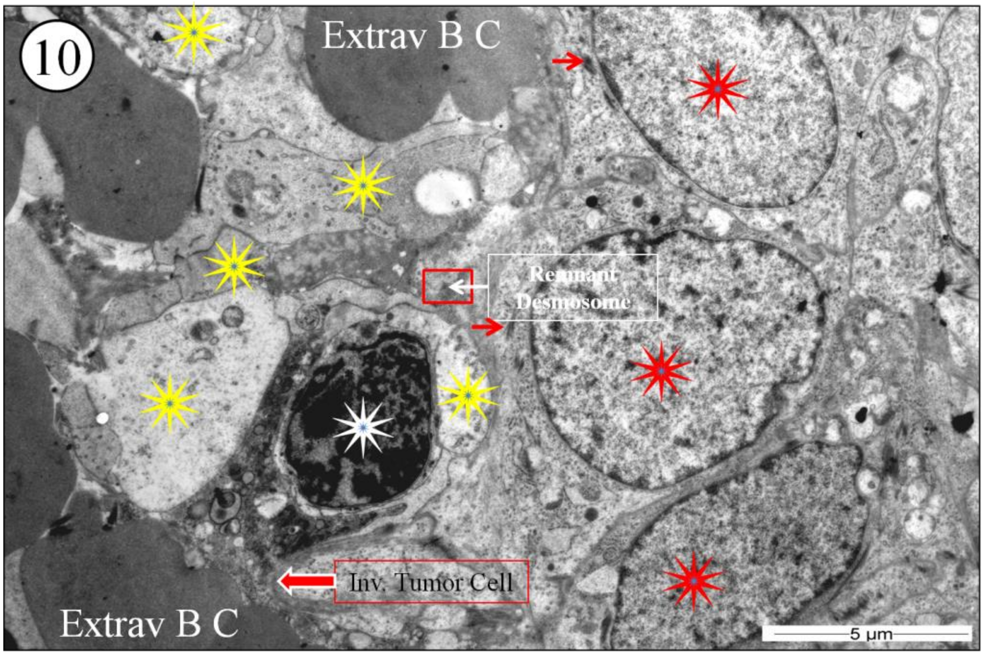
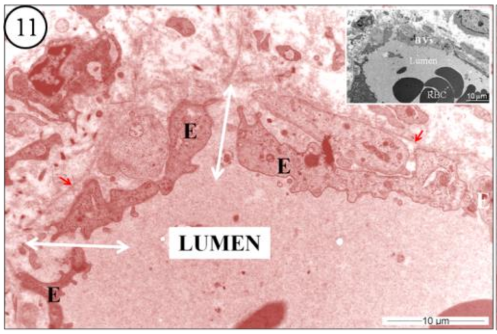


Fig. 10. An intermingled of tumor cells with extravasated blood cells (Extrav B C). Some tumor cells have euchromatic nuclei (ten point stars) with very scanty keratin intermedium filaments (red arrows) and remnant desmosome. One tumor cell has a nucleus with large blocks of condensed chromatin (white ten point star) while some others are in advanced stage of degradation (yellow ten point stars). A tumor cell extension deeply penetrates into adjacent stroma (large arrow).

Very often, small tumor blood vessels are very fragile with large gaps between adjacent endothelial cells so that leukocytes and red blood cells will extravasate. Moreover, some blood vessels are defective for basement membrane and pericytes (Fig. 11 and Fig. 12) (Moroşanu, 2019, PhD thesis).

Interestingly, inside of tumor mass, particular conduit systems delimited by tumor cells still preserving some keratin intermedium filaments and desmosomal junctions can be detected. The central part of the conduits is represented by collagen fibrils cross sctioned (Fig. 13) (Moroşanu, 2019, PhD thesis).



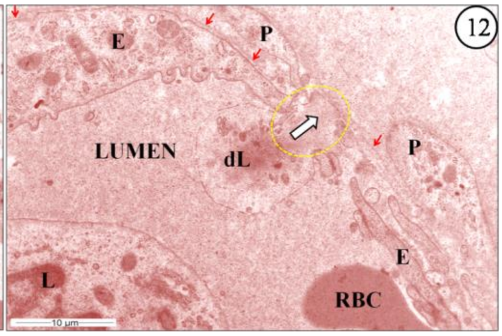


Fig. 11. Between the tumor cells facing tumor stroma, desmosomal junctions are missing (white elliptic area). A tumor cell exhibit microvesicles (red elliptic area). Delivered membrane vesicles (red asterisks) can be seen. Basement membrane is missing but dense amorphous material is deposited (yellow asterisks). No hemidesmosome can be detected. A gap in plasma membrane of the tumor cell is visible (large arrow). For overview see inset **a.** Tumor cell still express keratin intermedium filaments (inset **b**, KIFs).

Fig. 12. A very fragile small blood vessel (for larger view, see the insert) exhibits endothelial cells (E) missing interendothelial junctions so that large gaps can be seen (double arrows). Small red arrows indicate fragments of basement membrane. BVs = blood vessel. RBC = red blood cells.

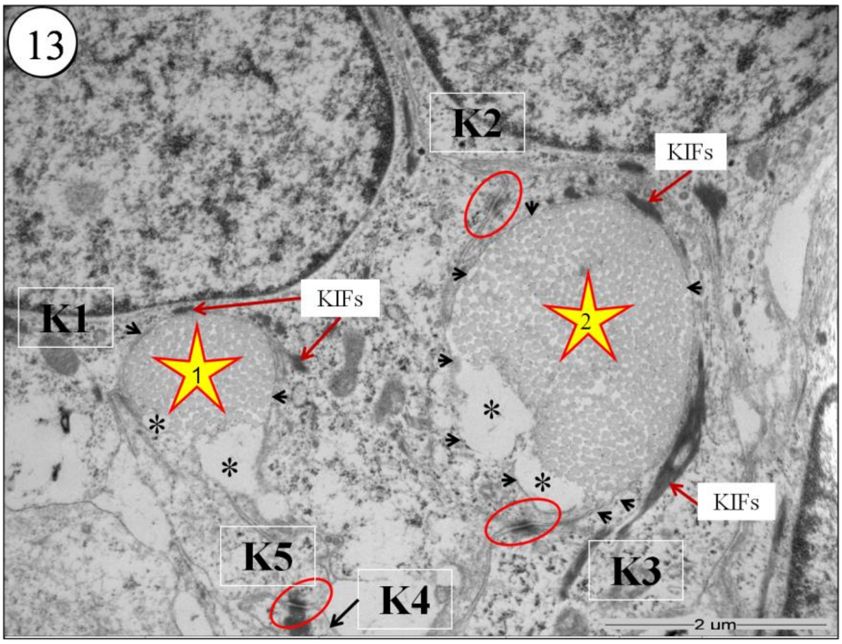


Fig. 13. A tumor blood vessel exhibits a large gap between two adjacent endothelial cells (E) so that a leukocyte (dL) is engaged in diapedesis. An interrupted basement membrane facing endothelial cells can be detected (red small arrows). Pericytes (P) are devoid of any basement membrane.

When present, nucleated telocytes with telopods can be detected inside of the tumor stroma but mention must be made, they are relative far from the tumor cells. Telocytes esthablish homo- and/or heterocellular junctions (Fig. 14) (Moroşanu, 2019, PhD thesis). Fig. 15 (Moroşanu, 2019, PhD thesis) depicts plasma cell membrane recombination between a telocyte and a tumor stromal cell. Moreover, at this level, microvesicles of different size can be seen but is difficult to know if these originate from the stromal cell or are delivered by the telocyte. Fig. 16 depicts a homotypic junction between two telopodes located inside of a fibrotic tumor stroma (detailed in Fig. 17) (Moroşanu, 2019, PhD thesis).

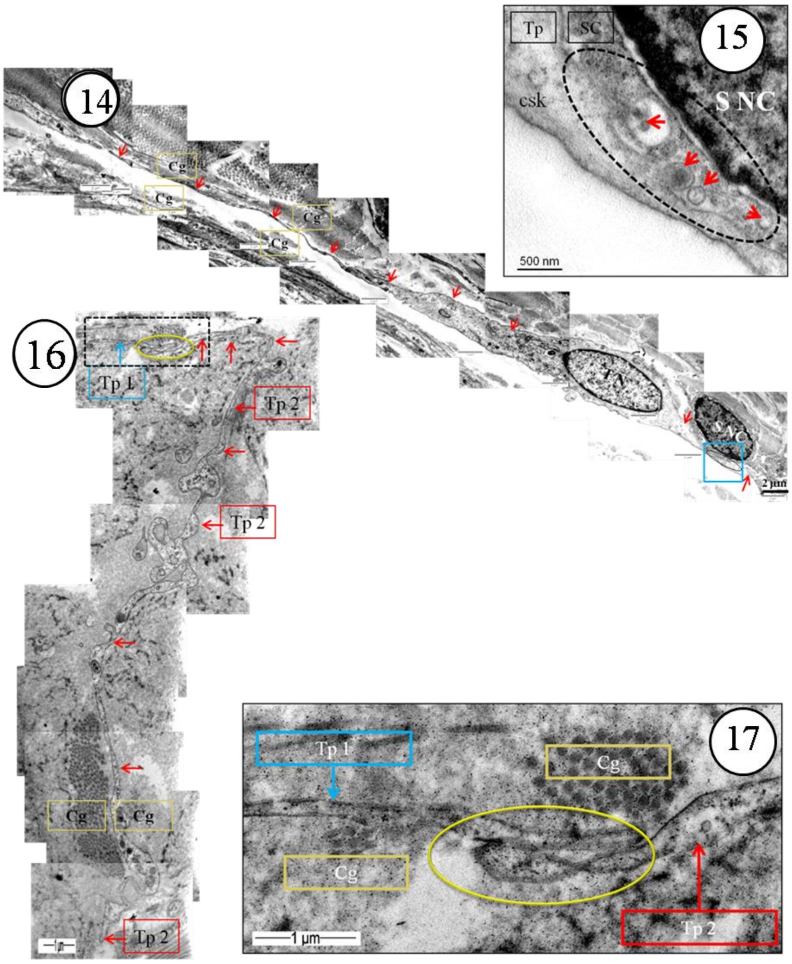


Fig. 14. Inside of tumor mass, there are particular conduit systems (big stars 1 and 2) delimited by tumor cells (K1-K5) still preserving some keratin intermedium filaments (KIFs) and desmosomal junctions (elliptic areas). The conduits with a core represented by collagen fibrils cross sectioned are delimited by plasma membranes of tumor cells (arrows). Empty spaces inside of each conduit system can be seen (asterisks).

Fig. 15. A nucleated telocyte exhibiting two telopods (red arrows) were detected inside of the tumor stroma but relative far from the tumor cells. A stromal nucleated cell (SNC) become in direct contact with one telopode.
Fig. 16. Detail of the framed area in Fig. 15 shows a plasma cell membrane recombination (elliptic area) established between telopode (Tp) and tumor stromal cell (SC). Microvesicles of different sizes can be seen (red head arrows) but is difficult to know if these originate from the stromal nucleated cell (SNC) or are delivered by the telocyte. Csk=cytoskeleton.
Fig. 17 and Fig. 18 depict a homotypic junction (elliptic areas) between two telopodes (TP 1 and TP 2) located inside of a fibrotic tumor stroma. Cg = collagen fibers.

DISCUSSIONS

Tumor-stroma interactions. The tumor microenvironment

Normal human skin (NHS) represented by (1) a pavimentous stratified epithelium termed epidermis of ectodermal origin attached to (2) the dermis of mesenchymal origin and, more profound, (3) the hypodermis is considered the second larger organ of the human body. Skin is a vital organ. The epidermis is the first defense line or barrier against environmental injuries for the skin, resting on the dermis underneath. In the NHS between epidermis and subjacent dermis there is a so called dermal-epidermal junctional zone (DEJZ). At the dermal-epidermal interface, a specialized extracellular matrix (ECM) highly organized at the molecular level composed of glycoproteins and proteoglycans appears as a continuous anhist infrastructure known as the basement membrane (BM) which separates and also connects epidermis to the dermis (Hashmi and Marinkovich, 2015). Depletion or functional deficiencies of any BM component may be lethal at some stage of development or around birth or may induce impairment in the skin function late in the life. Skin may be involved in inborn or acquired skin diseases.

Because ECM functions as a reservoir of various types of growth factors, cytokines and chemokines, the intense remodeling process it undergoes in cancer affects the processes of angiogenesis, proliferation or cell mobility, by specifically regulating signaling pathways (Jarvelainen *et al.*, 2009; Chen and Nunez, 2010; Kavasi *et al*., 2022).

Histoarchitecture and ultrastructure of the epithelial tumor. Cell-cell relationships inside of tumor

During normal skin morphogenesis as well as in *in vitro* reconstructed skin equivalents a permanent interplay exists between epithelial cells and adjacent fibroblast cells (Mackenzie, 1994; Stark *et al*., 2001; Marionnet *et al*., 2006). Now days appears more clearly that associated stroma to the tumor plays a major role in tumor initiation, tumor growth and invasive behaviour of malignant cells during metastasis (Mueller & Fusenig, 2004; Mirancea *et al*., 2010, 2014). That is the reason why we focus our electron microscopic investigations also on the tumor stroma. Interestingly, in this context electron microscopic examination showed that inside of tumor mass there is a gradually change of tumor cell phenotype so that, cells located at the tumor-stroma interface are ultrastructurally very different from the tumor cell located at the tumor external surface (opposite and far from the stromal tissue).

This study related to some peculiar ultrastructural alterations detected in some histiotypic human skin basal carcinoma both inside of tumoral mass as well as associated tumor stroma. In all investigated cases a severe histoarchitecture disarrangement of the epidermal tumor cells is remarkable (Figs. 6–7) (Moroşanu, 2019, PhD thesis). The paucity of desmosomal junctions and associated keratin filaments may partially explain this aspect (Fig. 8) (Moroşanu, 2019, PhD thesis). In fact, loss of adhesive regulation is a hallmark of almost invasive carcinoma types, a prerequisite to dissemination in ectopic places to form secondary tumors (Kimura *et al*., 2007; Mirancea *et al*., 2013, 2014; Moroșanu *et al*., 2013).

Basement membrane at the tumor stroma interface

In normal human skin the DEJZ is represented by specific type molecules arranged in particular infrastructures: hemidesmosomes ($\alpha 6\beta 4$ integrin, bullous pemphigoid 180 kDa and 230 kDa, HD 1/plectin, basement membrane (laminin and type IV collagen) and anchoring fibrils (type VII collagen) (Mirancea *et al*., 2001, 2010; Mirancea & Mirancea, 2010a; Neve *et al*., 2014; Hashmi & Marinkovich, 2015). Basement membrane is a potential source and bifunctional for pro- and antiangiogenic molecules (McDonald & Baluk, 2002; Neve *et al*., 2014).

DEJZ is severely altered during epidermal carcinogenesis so that, accordingly with the tumor progression BM becomes illusive or even totally disappearing (Figs. 9, 10) (Moroşanu, 2019, PhD thesis). When present, it appears as redundant patches of basement membrane. In fact, DEJZ alteration is a hallmark of a bad evolution of skin BCC.

Alteration of hemidesmosomal junctions

BM alteration is associated with another important abnormality, namely absence of hemidesmosomal junctions or presence of defective hemidesmosomes. Either absence or defective hemidesmosomes were also reported in BCC and SCC (Mirancea *et al*., 2010; Moroşanu *et al*., 2013).

Absence of hemidesmosomes, BM and anchoring fibrils may explain the lacunae ad slit-like stromal retraction characteristic in BCC (Crowson, 2006).

The tumor microenvironment and inflammatory cells inside of the tumor stroma

A solid tumor is a site of chronic inflammation. Inflammatory cells are coming from the blood flow in response to the pro-angiogenic factors delivered from the tumor cells and their accomplices from the tumor microenvironment (Mueller & Fusenig, 2004). In all our investigated cases of BCC, especially at the tumor-stroma interface we detected altered (fragile) microvasculature and, consequently extravasated free inflammatory bold cells become in close contact with tumor cells, as is depicted in Fig. 9 and Fig. 10 (Moroşanu, 2019, PhD thesis). Similar aspects were reported by Mirancea *et al*. (2014) and Constantin *et al*. (2015) in both neuroendocrine and exocrine pancreatic tumors. In an elegant experimentally study, Arwert *et al.* (2010) demonstrated that differentiated epidermal cells triggered by inflammatory infiltrate can initiate tumor formation without reacquiring the ability to divide, by recruiting undifferentiated cells to become incorporated into the tumor and form its proliferative compartment.

Shedding membrane vesicles

There are many players involved in cell-cell and cell-extracellular matrix communications. Very tightly mechanisms of control represented by autocrine and paracrine factors maintain normal local tissue homeostasia. Now is well stated that bioelectric signals play a major role in cell-cell communications (Lobikin *et al*., 2012). Moreover, small lipoprotein sacks termed extracellular vesicles (ECVs) produced by all body cells and a special cell phenotype called telocytes present and described in almost all organs (Popescu *et al*., 2005, 2010; Rusu *et al*., 2012a; Mirancea *et al*., 2013) are added to the list of mediators involved in cell-cell and cell-extracellular matrix. Almost eukaryotic cell phenotypes release into their microenvironment, including blood and body fluids, a heterogeneous mixture of vesicular (infra)structures organelle-like, often termed extracellular (micro)vesicles (ECMVs) or extracellular organelles (Mathivanan *et al*., 2010). ECMVs which include (1) exosomes, (2) shedding membrane vesicles, (3) apoptotic blebs are spherical bilayered proteolipids vesicles with a mean diameter of 20-1.000 nm. Exososmes are formed inside the cell in multivesicular bodies, whereas the other two types bud off from the plasma membrane (Smythies, 2015). These are generated *via* diverse biological mechanisms triggered by diverse pathways involved in different cellular activities: intercellular communications, pathogenesis including oncogenic transformation etc. Now is well documented that EMCVs contain bioactive molecules as growth factors and their receptors, adhesion molecules, signaling molecules, DNA, mRNA, microRNA sequences, proteases, lipids so that ECMVs appear as bioactive cargoes which play important roles in patho-physiology, including facilitation of tumor growth (Hendrix & Hume, 2011; Lee *et al*., 2011; Cismașiu & Popescu, 2015; Kok and Yu, 2020).

In almost all our investigated cases of BCCs as well as SCCs we detected shedded microvesicles by tumor cells (Mirancea *et al.*, 2010, 2013). Cancer cells release or deliver large amounts of ECMVs which can be transferred also to non-transformed cells (stromal cells as fibroblasts, endothelial cells and to the inflammatory infiltrated cells). Such events may contribute to tumor angiogenesis as well as to tumor migration, invasion and ectopic dissemination in order to develop secondary tumor (metastasis), drug resistance, and cancer stem cell hierarchy (Lee *et al*., 2011).

Microvasculature

Like in many other tumor types, in our investigated patients diagnosed with basal cell carcinoma in different degrees, we observed leakiness of tumor vessels (Fig. 11 and Fig. 12) (Moroşanu, 2019, PhD thesis). There is a general agreement that tumor blood vessels are abnormal, namely (1) defective and leaky endothelium, (2) impaired associated basement membrane or redundant layers of basement membrane as a continuous vascular remodeling and (3) dissociation of pericytes (McDonald & Baluk, 2002; Stratman *et al*., 2009; Stratman & Davis, 2012). Low-molecular-weight vascular-disrupting agents induce severe damage of the tumor associated microvasculature causing an extensive tumor necrosis while the blood vessels in normal tissues remain relatively intact (Tozer *et al.*, 2005). The evaluation of the microvasculature and its status is even a tool in establishing the differential diagnosis, the subtypes of BCC and their aggressiveness, namely by the dermoscopic evaluation of the vascular model (Lupu *et al*., 2019).

Tumor microvasculature is abnormal in their constitution as is depicted in Fig. 11 and Fig. 12 (Moroşanu, 2019, PhD thesis). That appears leaky with remarkable increased vascular permeability, so that, both red and white blood cells disseminate inside or in peritumoral spaces (Mirancea *et al*., 2010; Stratman & Davis, 2012; Mirancea *et al*., 2014; Constantin *et al*., 2015). Consequently, delivered inflammatory blood cells become rich sources of pro-inflammatory agents, a prerequisite for the well known tumor status as a disease that do not heal (Dvorak, 1986).

Conduit system

In one of the skin BCC case we investigated, a special structure termed conduit system was detectable. Such kind of conduits has been described for lymph nodes, spleen and thymus (Sixt *et al*., 2005; Drumea-Mirancea *et al*., 2005; Roozendaal *et al.*, 2008, 2009; Mirancea & Mirancea, 2010a). A conduit of lymph nodes and spleen is composed of a network of collagen fibers with periodicity enwrapped by fibroblastic reticular cells (Drumea-Mirancea *et al.*, 2005). Immune electron microscopic examination of a conduit system detected inside of the human thymus showed that such kind of 3-D tubes are represented by fibrillar collagens surrounded by a laminin-5-containing membrane (Drumea-Mirancea *et al*., 2005). Moreover, desmosomal junctions were detected (Drumea-Mirancea *et al*., 2005;

Mirancea & Mirancea, 2010a). Different from the above more complex conduit system, in our skin BCC case we investigated, a conduit system also exhibit a core of fibrillar collagens, but the basement membrane is missing, so that, only plasma membranes of tumor cells surround fibrillary collagen and, associated soluble extracellular micromedium. One the other hand, also epithelial cells namely tumor keratinocytes exhibit defective desmosomes and some intermedium filaments but no hemidesmosome or hemidesmosome-like junction is detectable. Inside of a conduit some apparently wide spaces can be seen (Fig. 13) (Moroşanu, 2019, PhD thesis). Mention must be made that, from our best knowledge, so far, this is for the first time when such particular structure is detected and described in a case of BCC. These unique structures seem to play an important role for the transport of fluids and some low weight soluble molecules as cytokines, chemokines (Sixt *et al.*, 2005; Drumea-Mirancea *et al*., 2005; Roozendaal *et al*., 2008; Roozendaal *et al*., 2009; Morgado *et al*., 2020; Novkovic *et al*., 2020).

Telocytes

Recently, a new interstitial/stromal cell phenotype termed telocyte presents inside of almost all tissue type gain a lot of interest. Telocytes (TCs) have been described in the interstitium of many normal tissues as heart (Popescu *et al*., 2005; Popescu & Faussone-Pellegrini, 2010; Rusu *et al*., 2012b), skin (Rusu *et al*., 2012 a), esophagus (Rusu *et al*., 2012c), trachea (Rusu *et al*., 2012d), uterus (Roatesi *et al*., 2015) or tissues involved in different diseases as heart failure (Richter & Kostin, 2015), oviduct disease (Yang *et al*., 2015a,b), skin diseases (Mirancea *et al*., 2013; Manole *et al.*, 2015), human brain tumor (Mirancea *et al.*, 2022). So far, ultrastructural characteristics remain the modality for precisely identification of TCs (Popescu *et al*., 2010; Mirancea *et al.*, 2013). A specific Immuno Histo Chemical (IHC) marker has not yet been found, but several IHC markers have been found that have variable expression in TCs from different tissues (Roatesi *et al*., 2015). The immunophenotype of TC includes double immunostaining with CD34/CD117/Kit (mainly for cell body) or CD34/vimentin (mainly for telopodes). TC may also express caveolin -1, CD44, NOS-2, desmin, cadherin-11 and PDGF-R beta (Popescu & Nicolescu, 2013). *In vitro*, in primary culture, cardiac TCs were positive for CD34/c-kit, CD34/vimentin, and CD34/PDGFR-□ as well as for mesenchymal maker CD29 (Bei *et al*., 2015 b).

We also detected this special cell phenotype, namely telocyte, inside of tumor stroma in our investigated cases of BCC (Figs. 14-17) (Moroşanu, 2019, PhD thesis). TCs were detected relative far from the tumor cells. There is a body of evidence that TCs might be involved in many physiological and pathological processes. By their homocellular junctions TCs connect each other (Fig. 16 detailed in Fig. 17 – Moroşanu, 2019, PhD thesis) while *via* hetero-cellular junctions TCs can be connected with other stromal cells (Fig. 14 detalied in Fig. 15 – Moroşanu, 2019, PhD thesis) as endothelial cells, nerve ending, putative stem cells, mast cells, macrophages, fibroblasts etc. By homo- and heterocellular junctions, TCs can form an interstitial 3D network able to modulate tissue homeostasis and development as well as pathogenesis of some disorders. Moreover, owing to their close relationship with stem cells and or/progenitor cells in almost all tissue types as was reported in heart (Popescu, 2011), skin (Ceafalan *et al*., 2012). TCs influence/support tissue regeneration (Mirancea *et al*., 2013; Manole *et al*., 2015; Smythies, 2015; Bei *et al.*, 2015a; Cismașiu & Popescu, 2015). Smythies & Edelstein (2014), consider that TCs network might be well regarded as a very primitive nervous system. TCs may be involved in morphogenetic bioelectrical signalling (Edelstein & Smythies, 2014). Mention must be made that TCs damages, reduced number of telocytes *per se* as well as the 3-D interstitial architectural alteration by reduced number of telocytes homo- and heterocellular junctions have been reported in different pathologies as cardiac diseases, skin cancers etc. (Mirancea *et al*., 2013; Moroșanu *et al*., 2013; Manetti *et al.*, 2014; Yang *et al*., 2015a,b; Richter & Kostin, 2015; Díaz-Flores *et al*., 2021).

TCs are able to release extracellular vesicles which may play a role in intercellular communications, cell signaling, maintaining tissue homeostasis (Mirancea *et al.*, 2013; Cretoiu & Popescu, 2014). Fig. 15 (Moroşanu, 2019, PhD thesis) depicted a heteocellular junction between a telocyte and a stromal cell where plasma cell membranes recombinate and microvesicles are present in way to be delivered. Such kind of extracellular organelles are considered to be involved in paracrine long distance signaling (Popescu, 2011; Cretoiu *et al.*, 2012; Mirancea *et al*., 2013).

There are many reports which emphasize that telocytes play a major role as integrators of many intercellular functions in normal tissue (Smythies, 2015), as well as in tissues involved in different pathologies, including tumor development (Mirancea *et al*., 2013; Moroşanu *et al.*, 2013; Manetti *et al.*, 2014, 2015). In a very recently published paper Mirancea *et al.*, (2022) emphasize that inside of the human severely altered brain tumor stroma many telocytes still keeping their homocellular junctions are visible, suggesting a kind of mechanically protection against aggressive growth of the tumor brain.

CONCLUSIONS

Our study pointed out some original relevant dynamics alterations of the tumor stroma interface. Basement membrane and hemidesmosomes are severely altered or totally abolished. Vesicular (infra)structures organelle-like termed extracellular (micro)vesicles are released by tumor cells at the tumor-stroma interface. Tumor associated capillary are often devoid of basement membrane and pericytes so that extravasated inflammatory cells become intermingled with tumor cells. Inside of tumor mass, particular conduit systems delimited by tumor cells can be detected. A recently described interstitial/stromal cell phenotype termed telocyte is present inside of peritumoral stroma of all our investigated cases of BCC. These peculiar stromal cells, mostly located far from the tumor cells, establish both homo- and heterocellular junctions.

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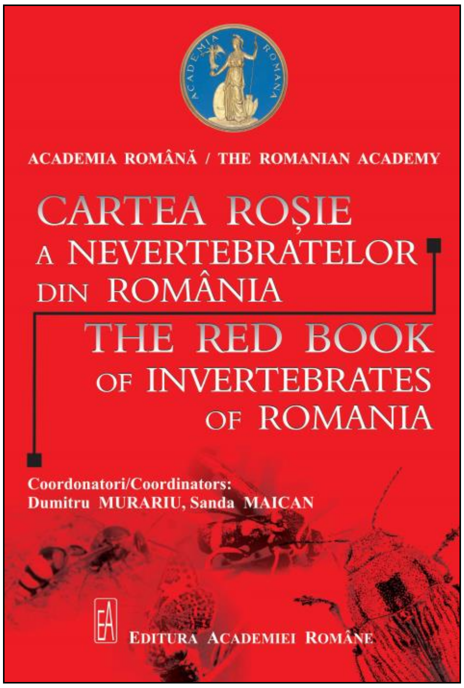
Dumitru MURARIU, Sanda MAICAN (Coord.), 2021. *Cartea Roșie a Nevertebratelor din România/The Red Book of Invertebrates of Romania.* Romanian Academy Publishing House, Bucharest. 49 authors, 451 pages, 364 pictures and habitus drawings, 81 habitats pictures, 364 distribution maps. ISBN 978-973-27-3357-8



Generally, the *Red Books* are public documents that present rare and threatened species of plants, fungi and animals in certain areas. The purpose of the publication of these Books is to help specialists to establish effective measures to protect endangered species and to provide scientific information for Habitat Monitoring Programs and rare or endangered species.

Thus, the Red Books provide scientific information on species and subspecies in the world, help establish measures to biodiversity conservation, clarify (at the time of printing) which species are threatened or even extinct, serve as a scientific basis for research and protection of all species from around the globe.

Among the main objectives of the *National Strategy for Biodiversity Conservation (*2010–2020) and the *EU's 2021–2027 Multiannual Program* is the one related to the elaboration and updating of the Red Lists and Books on the flora and fauna of the member countries.

The project to publish the *Red Book of Invertebrates of Romania (RBIR)*, started in 2016, took place under the coordination of the Department of Biological Sciences of the Romanian Academy and is the continuation of an older initiative that belonged to the late Dr. Dan Munteanu, corresponding member of the Romanian Academy, former president of the *Commission for the Nature Monuments Protection.*

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Romania was one of the few countries that did not yet have a *Red Book of Invertebrates*, in the neighboring states being published several years ago Red Books of the entire fauna. In this context, the publication of the *RBIR* is a necessary and commendable contribution, which supports the sustained efforts, globally and nationally, to ensure the survival of endangered species. This is now more important than ever when we are witnessing a sharp, often irreversible, loss of biodiversity on Earth. Globally, increasing species extinction and accelerating declining biodiversity, highlighted by the increasing number of taxa in the category of rare, threatened or endangered species, have become increasingly worrying.

Despite the apparent neglect and lack of interest in invertebrates wildlife protection (at all levels, from government to the most active organizations in biodiversity conservation), invertebrates play key roles in the structure and functioning of ecosystems, due to their specific diversity and extreme abundance. Large, this group represents over 90% of all animal species on Earth.

RBIR is a reference monograph, long awaited by the zoological community and by all those interested in nature protection, which covers an important gap in the Romanian literature. This volume joins two other previously published monographs: *The Red Book of Vertebrates of Romania* (2005) and the *Red Book of Vascular Plants in Romania* (2009). In addition to these volumes, this synthesis is bilingual (Romanian/English), thus becoming accessible to foreign specialists.

The work was carried out by a team of 49 zoologists from universities and research institutes in the academic network, including museums of natural sciences in Bucharest, Iași, Sibiu, Cluj-Napoca, Craiova, Constanța, Bacău, Timișoara, Oradea, Brașov under the coordination of Dr. Dumitru Murariu, member of the Romanian Academy, and Dr. Sanda Maican, senior scientist researcher at the Institute of Biology Bucharest of the Romanian Academy. In the absence of a specially funded project, the two coordinators managed to mobilize renowned zoologists, specialists in the study of invertebrates, around the idea of voluntarily developing a *Red Book of Invertebrates*, from the data collected each year, patiently and meticulously by each colleague, in the conditions of a permanent precarious financing.

The volume totals 451 pages and is structured in seven chapters, at the end of each chapter being inserted the *References* related to the species of interest presented in the book.

In the first chapter are analyzed taxa from the Porifera, Cnidaria, Xenacoelomorpha, Nemertea, Bryozoa, Annelida, and Mollusca Phyla and also from the Hexanauplia and Malacostraca (Arthropoda) Classes. Chapter 2 includes representatives of the Arachnida and Chilopoda Classes. Chapters 3, 4, 5 and 6 are dedicated to the insect species of the Odonata, Orthoptera, Mantodea, Coleoptera, Lepidoptera, Hymenoptera and Diptera Orders. The last chapter refers to the Class of Leptocardii in the Chordata Phylum.

Very good quality iconography and a significant number of bibliographic sources, many of them dated recently, come to complete the scientific value of the information presented.

The higher taxa with the rank of Phylum, Class, Order and Family are presented in systematic order, and the genera and species in alphabetical order. The *RBIR* contains information on 364 species of terrestrial, freshwater and marine invertebrates (classified in 16 Classes, 44 Orders and 115 Families) from the Romanian fauna, considered a priority for conservation. Of the total number of species evaluated and included in the monograph, most are represented by insects: 260 species, belonging to seven Orders and 56 Families.

The authors wrote in bilingual format (Romanian/English), for each species evaluated, a complex *Sheet* that includes photos of the habitus (sometimes of the preferred habitat), as well as information on:

- current taxonomic classification;
- general geographical distribution, in Romania as well;
- habitat and ecology of the species;
- protection status in Romania and in other countries;
- estimating the population and population trend of evolution (where there are information);
- the main threats to the species;
- conservation measures.

The distribution map in Romania is presented for each species, the localities/sites where the species was reported being marked with different colors (gray dots - for older mentions, up to 1990, and red dots - for reports after 1990). The presentation of the relevant information for each species with the help of the sheets gives a unitary structure to the paper, despite the large number of authors and the diversity of the taxonomic groups presented.

The conservation status of each species has been established on the basis of criteria developed by the *International Union for Conservation of Nature.* The assessment of the current ecological situation of the 364 invertebrate species has resulted in the following worrying percentage distribution:

- 1.4% - Extinct species (EX);
- 17.0% - Critically Endangered species (CR);
- 31.6% - Endangered species (EN);
- 38.2% - Vulnerable species (VU);
- 8.8% - almost endangered species/Near Threatened (NT);
- 2.7% - species for which there are not enough data to be included in one or another endangered category/Data Deficient (DD);
- 0.3% - species that are not currently endangered/Low Concern (LC).

At the end of the volume is a welcome alphabetical *Index* of the scientific names of the invertebrates evaluated, very necessary for the reader to quickly identify the taxon of interest.

This monograph is a useful working tool for establishing actions to improve the *National Strategy for Biodiversity Conservation* and environmental policy change, which are essential for the protection of natural resources. *RBIR* offers decision makers the opportunity to complete the list of species in need of protection, to rethink the national network of protected areas based on the contribution and experience of specialists in the country.

It should also be noted that invertebrates are underrepresented in European legislation, not including a number of taxonomic categories presented in the *RBIR*.

Due to the insufficient number of specialists in the taxonomy of some groups of invertebrates, but also the fact that not all specialists have expressed their willingness to participate "voluntarily" in this monograph, some groups of invertebrates (e.g. freshwater unions) do not are found in *RBIR*.

Given that the Red Lists and Red Books are "living" working tools that need to be updated from time to time, depending on the anthropogenic impact and species dynamics, we recommend that the next edition of *RBIR* include the contributions of more specialists and, implicitly, species from taxonomic groups that are not present in this first edition.

The *Red Book of Invertebrates of Romania* is a very important reference source, with rigorously documented information for both specialists in academic institutions (universities, research institutes) and other institutions involved in environmental protection such as: entomologists, other zoologists, agronomists, forestry engineers, etc., as well as for biology teachers in pre-university education.

This monograph will also be useful for various civil society organizations that are carrying out activities of inventory, monitoring and protection of the biodiversity in Romania, as well as for policy makers of protected areas management.

We appreciate the effort of all – authors and coordinators, who contributed to the publication of this first edition of *Red Book of Invertebrates of Romania*, a valuable and true "scientific document" whose importance will be validated by time.

Congratulations to the 49 invertebrate specialists and the two coordinators, who through an unconditional involvement, over a long period of time, managed to complete this important and complex project.

Bucharest, June 6, 2022 Acad. Octavian POPESCU

Sorin GEACU, 2021. *Teriofauna din sud-estul Moldovei. Condiții de mediu, populații, răspândire, impact antropic* (*The Teriofauna of South-eastern Moldavia. Environmental Conditions, Populations, Spread, Anthropic Impact*). Romanian Academy Publishing House, Bucharest. 239 pages, 101 figures, 53 tables, 319 bibliographical references, summary in English. Preface by Acad. Dumitru Murariu.

The paper is an extensive contribution to the study of mammal fauna in Romania, in a region understudied in this respect, but interesting from a zoogeographical point of view, located at the contact between steppe and forest-steppe areas and mesoxerophilous and mesophilous deciduous forests, which is reflected in the complex character of the faunal composition. The paper is based on extensive field research, in addition to information provided by forestry staff, hunters and locals, as well as information taken from Forestry Office archives and other sources of documentation. A special merit of the author is their openness to work with people with a lot of experience in forestry and hunting, which substantially fill in the perspective over the structure and dynamics of mammal fauna within the researched area, which is quite difficult to consistently follow in all its complexity. Additionally, the laborious research of the rich documentary material from the archives of forestry offices and other institutions with responsibilities in the field of wildlife, as well as the study of an extensive bibliography, led to obtaining valuable additional information on various aspects concerning the relationship between animal populations and environmental factors, or with anthropic actions, as they developed over time, since the first part of the 20th century and in some cases since even more distant times. The author's special ability stands out, that of correlating and integrating a multitude of informative data into an overarching perspective based on both solid zoological knowledge and a geographical, spatial-temporal interpretation.

The author highlights the role of natural factors in the current distribution of mammal species, as well as the strong anthropogenic influences, both indirect, caused by the anthropogenic change in landscape and the various agricultural practices, and direct, caused by actions on wildlife, hunting or combating species deemed to be harmful on the one hand and, on the other hand, through colonization, the provision of food for game animals and other protective measures. In the case of large herds (the hare, the red fox, the wild boar, the roe deer), a number of animals were also extracted, to be removed to other areas.

The most substantial part of the book is the presentation, in a systematic order, of each species. These presentations indicate the bio-ecological characteristics and the geographical dissemination or, for rare species, observational data (often the places where the species are reported are also shown on the map of the region). For the more abundant species, the living and hunted numbers are also added and sometimes, for the species better known by the locals, even the toponyms that attest to the presence of the species at various points in more distant times. For some species (e.g., the hare, the common hamster, the common mouse, the Asiatic jackal), the damage they inflict is also included. For the species that are of hunting interest, yearly tables are presented, comprising the number of hunted specimens. In particular, the situation of the fallow deer is under analysis, a species introduced to the region, which has fluctuated greatly in numbers and areas it occupies. The roe deer has also seen a sharp decline in numbers in the first half of the 20th century, followed by repopulation and expansion of its living area, and subsequently by a further decline of the area and large fluctuations in numbers, both due to anthropogenic pressure and to natural causes (droughts, heavy snowfall, very low temperatures, floods). Among the species that have recently appeared in the Romanian fauna through spontaneous expansion are the muskrat, the raccoon dog and the jackal. The author analysed in detail the way they entered the region and expanded their area, as well as the numerical fluctuations of the general population.

Another chapter contains detailed and well-documented information on various mammals that have disappeared from the region under investigation or that have randomly appeared. Ever since the 19th century, most reports refer to the large number of wolves, that caused damage and against which substantial control measures were taken. Subsequently, as a result of intense counter-measures, their numbers decreased to the point of becoming extinct from the constant fauna. Currently, only sporadic specimens from outside the region are to be found. The occasional intrusion of the elk, the red deer, and the European mink is also analysed.

Yet another chapter is dedicated to the mammals that inhabited the region during the Pliocene and Pleistocene, known due to the discovery of fossil bones in natural openings, loessoid deposits or in the terraces of rivers that flow through the region. The presentation of the Holocene paleofauna is also conveyed, largely based on the remains discovered following archaeological research, highlighting the high frequency of macromammals in the forest biotope, which indicates a greater expansion of the forest in past historical eras. The chapter on the protection of the teriofauna mentions the various normative acts by which a series of mammals are protected, given that they hold the status of vulnerable, endangered or even critically endangered species.

The conclusions are quite significant, as they highlight the causes that determine the dynamics of mammal populations, and can provide valuable guidance on the best possibilities for the protection of species of zoogeographic interest and management of species that may become a danger to the balance of natural biocenoses or may even impact economic activities. Thus, a number of species that are in decline are mentioned, alongside the "population explosions" of various rodent species, as well as the effects of the expansion of non-native species such as the jackal or the muskrat. The effects of restricting the physical range of some species are also highlighted, sometimes leading to a vulnerability to the point of extinction. The lack of connectivity between some areas (the case of the pine marten) can also have a negative impact on genetic diversity. So, in addition to the valuable theoretical and zoogeographical contribution, the work of Dr. Sorin Geacu is also of practical interest for all those who concern themselves with the implementation of effective actions to protect both the biocenoses, and nature as a whole.

Bucharest, April 4, 2022 Prof. univ. dr. Cristina MUICĂ