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Atlantic sturgeon *Acipenser oxyrinchus* and alien sturgeon species in Polish waters: can biometric analysis assist species discrimination and restoration?

Sławomir Keszka,[1] Remigiusz Panicz[2]

[1] Department of Aquaculture, Faculty of Food Sciences and Fisheries, West Pomeranian University of Technology, ul. K. Królewicza 4, 71-550 Szczecin, Poland, e-mail: skeszka@zut.edu.pl.
[2] Department of Meat Technology, Faculty of Food Sciences and Fisheries, West Pomeranian University of Technology, ul. K. Królewicza 4, 71-550 Szczecin, Poland, e-mail: rpanicz@zut.edu.pl.

Keywords bycatch, countable and measurable characters, multidimensional analysis, restocking, species identification

Abstract Biometric character analyses were conducted on Atlantic sturgeon *Acipenser oxyrinchus*, a species included in a re-establishment programme in the Baltic Sea basin. The study sought to identify measurable and countable characters most useful for distinguishing *A. oxyrinchus* from three alien species found in open waters of Poland: *Acipenser baerii*, *Acipenser gueldenstaedtii*, and *Acipenser stellatus*. Measurable characters that contributed most to discrimination included preorbital distance, eye diameter, ventral fin base to anal fin base, and postorbital distance. Among countable characters, the number of lateral scutes made the greatest contribution. The data from the present study may be used to supplement existing systematic keys and lay the foundations for creating a catalogue or atlas of popular species and interspecific hybrids of sturgeon, including their complete systematic determination.

Jesiotr ostronosy Acipenser oxyrinchus oraz obce gatunki jesiotrów w wodach Polski: czy analiza biometryczna uzupełni identyfikację i restytucję gatunków?

Słowa kluczowe analiza dyskryminacyjna, cechy biometryczne, identyfikacja gatunków, przyłów, restytucja gatunku

Streszczenie W niniejszej pracy przedstawiono wyniki analizy cech biometrycznych osobników jesiotra ostronosego *Acipenser oxyrhinchus oxyrhinchus*, gatunku objętego restytucją w basenie Morza Bałtyckiego. Przy wykorzystaniu technik aglomeracyjnych podjęto próbę wyjaśnienia, które z cech mierzalnych i policzalnych mają największą przydatność przy odróżnianiu *Acipenser oxyrinchus oxyrinchus* od trzech gatunków obcych, łowionych w wodach otwartych Polski, tj. jesiotra syberyjskiego (*Acipenser baerii*), rosyjskiego (*Acipenser gueldenstaedtii*) oraz siewrugi (*Acipenser stellatus*). Wyniki analiz wykazały, że największy udział w dyskryminacji na podstawie cech mierzalnych miały: długość rostrum (R), odległość między płetwami V i A (VA) oraz przestrzeń zaoczna (OP), natomiast wśród cech policzalnych była to liczba tarczek bocznych (SL). Wyniki uzyskane w niniejszej pracy wzbogacają aktualnie

stosowane klucze do identyfikacji gatunków jesiotrów oraz mogą stanowić podstawę do opracowania katalogu bądź atlasu powszechnie występujących jesiotrowatych jak i ich hybryd międzygatunkowych.

Introduction

During the interwar period, when Poland's borders differed from the present and included the river Dniester and tributaries of the Danube river, Polish ichthyofauna included four species of *Acipenser*: Atlantic sturgeon *Acipenser sturio* (L. 1758) also known as the common sturgeon, the sterlet *Acipenser ruthenus* (L. 1758), the starry sturgeon *Acipenser stellatus* (Pallas, 1771), and the rare, ship sturgeon *Acipenser nudiventris* (Lovetsky, 1828; Kulmatycki, 1919, 1922, 1932, 1933; Starkiewicz, 1922). Numbers of *A. sturio* in Polish waters were already in decline in 1908–1910 (Grabda, 1971) and earlier along some stretches of the Vistula River (Sasorski, 1922), while, in the Oder River, it was a rare species even at the beginning of the 20th century (Kulmatycki, 1921). The last specimens of *A. sturio* in Polish waters were collected from the Vistula in the mid-1960s (Żelechowska, 1964).

Many years later, DNA studies of museum specimens revealed that a species similar to *A. sturio*, the Atlantic sturgeon *Acipenser oxyrinchus* (Mitchill, 1815), existed for 800 years in the Baltic Sea catchment area, and which, being able to better adapt to changing climatic conditions, supplanted *A. sturio* (Ludwig et al., 2002). Thus *A. oxyrinchus*, generally regarded as extinct in the Baltic Sea (Kolman et al., 2011a, b), has been included in the re-establishment plan. Using fish from the St. John River in Canada, stocking has been conducted since 2004 in locations where the species was recorded in the past: the Drawa, a tributary of the Oder (70,000 individuals) and the Drwęca, a tributary of the Vistula (51,000 individuals). An agreement between the Inland Fisheries Institute in Olsztyn and the Leibnitz Institute of freshwater Ecology and Inland Fisheries in Berlin has allowed the introduction of juvenile *A. oxyrinchus* to the rivers (Kolman et al., 2008).

Alien sturgeon species are appearing with increasing frequency in European waters, especially the Siberian sturgeon *Acipenser baerii* (Brandt, 1869) and the Russian sturgeon *Acipenser gueldenstaedtii* (Brandt, Ratzeberg, 1833) that are escapees from fish farms (Nabiałek, 1974; Filipiak, 1996; Elvira, Almodovar, 1997; Keszka, Stepanowska, 1997; Keszka et al., 2011). Individuals are often observed after periods of high water, especially near rivers supplying fish farms (Maury-Brachet et al., 2008). The occurrence of alien sturgeon in the natural environment is a highly controversial issue, especially in connection with the programme being undertaken for restocking *A. oxyrinchus*. Interspecific competition from alien species, disease transfer, and potential risk of hybridization also hinder attempts at restoring the species, especially with the increasing abundance of the alien species (Kempter et al., 2009; Gessner et al., 1999, 2010).

A necessary prerequisite for the success of sturgeon restoration is identification of exotic species and their elimination according to national regulations, and, if required, adjusting international codes and guidelines (Gessner et al., 2010). The identification of *Acipenser* species is problematic because of similarities among species due to high plasticity of biometric characters (Ruban, Sokolov, 1986; Guénette et al., 1993; Ruban, Panaiotidi, 1994; Paaver, 1997). Correct identification is also complicated by individual plasticity and human interference through the creation of hybrids (Loy et al., 1997). The aim of the present study was to conduct biometric analysis of *A. oxyrinchus* and compare data with that reported for *A. gueldenstaedtii*, *A. baerii*, and *A. stellatus*, as well as to use multidimensional analysis to select diagnostic characters useful for rapid identification of sturgeon in bycatch.

Material and methods

Fifty dead juvenile specimens of *A. oxyrinchus* were collected from seine fishers in three locations (N 53°43′59″; E 014°28′11″; N 53°44′22″, E 014°27′51″, N 53°43′10″, E 014°26′42″) in the Szczecin Lagoon near the town of Stepnica, from 21 May to 9 June 2008. The fish, some of which were tagged, originated from German stocking (Gessner et al., 2008) undertaken as part of a species restoration plan (Figure 1). Specimens were frozen using the method proposed by Keszka and Krzykawski (2008). Measurements were taken with calipers of 0.01 mm accuracy according to figure published by Kempter et al. (2013). Masses of individuals were measured with electronic scales with 0.01 g accuracy. Twenty-one measurable and five countable biometric characters were compared with characters of

Figure 1. *Acipenser oxyrinchus* collected from the Szczecin Lagoon

Source: fhoto by S. Keszka.

A. baerii (n = 336), *A. gueldenstaedtii* (n = 99), and *A. stellatus* (n = 69) (Keszka, 2000; Keszka, Raczyński, 2006). Information on measurable characters for these 3 species were published by Keszka and Raczyński (2006), Keszka and Krzykawski (2008), Keszka et al. (2009). All individuals of alien species originated from warm water aquaculture systems in Nowe Czarnowo and Gryfino (NW Poland). Biometric characters of the studied species are given in Table 1.

Table 1. Biometric data for four sturgeon species

Species	Location/number of specimens (n)	L_T [mm]			Mass [g]		
		min	max	mean	min	max	mean
Acipenser oxyrinchus	Roztoka Odrzańska – Stepnica (50)	209.8	680.00	351.39	31.0	1,122.7	202.49
Acipenser baerii	Cage culture – Nowe Czarnowo (276)	300.5	690.00	334.90	50.0	1,245.0	274.20
Acipenser gueldenstadtii	Cage culture – Nowe Czarnowo (334)	230.0	29.09	27.07	34.0	207.0	112.89
Acipenser stellatus	Cage culture – Gryfino (69)	235.0	390.00	327.55	21.9	137.0	67.43

Statistical analysis was performed using Statistica 10.1. Measurable and countable characters were summarized calculating minimum and maximum values, arithmetic mean, and standard deviation.

Per cent of total length (L_T) of measurable characters and absolute values for countable characters were used for conducting stepwise discriminant analysis and canonical discriminant analysis. Results were analysed taking into consideration the classification matrix and values of standardized coefficients for canonical variables and visualized as scatter plots of canonical scores.

Results

Measurable Characters

Length and mass were comparable for all specimens (Table 1). Measurable characters of *A. oxyrinchus* presented as absolute values (mm) and as per cent calculated with respect to total length (L_T) are presented in Table 2. Among the five countable characters, standard deviation for the number of dorsal scutes and the number of ventral scutes were the lowest and therefore similar to the mean value (Table 3).

Table 2. Measurable characters of the *Acipenser oxyrinchus* given as absolute values and as per cent total length L_T

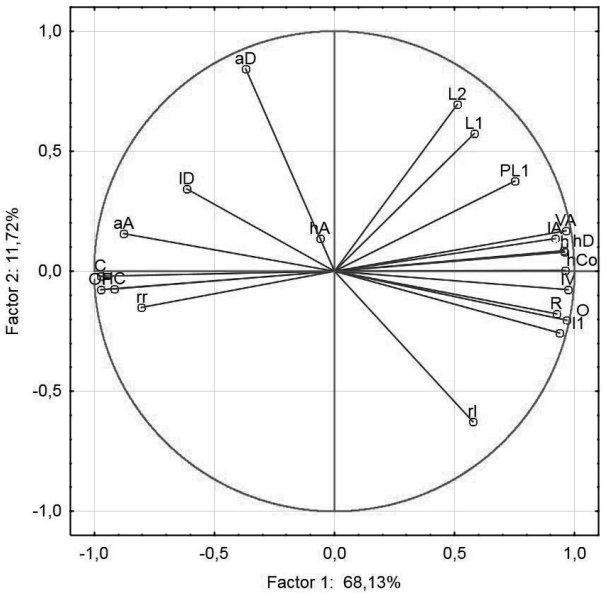
Symbol and character description	Value [mm]				Value [% Lt]			
	min	max	mean	sd	min	max	mean	sd
1	2	3	4	5	6	7	8	9
L_T – Total length	209.80	680.00	351.39	121.48				
L_F– Fork length (Smitt's length)	178.70	580.00	301.86	100.27	79.41	154.30	86.65	90.99
L_S – Standard length	166.30	545.00	284.41	96.74	75.30	147.66	81.45	90.67
C – Length of head	56.60	161.20	91.20	26.80	23.14	44.92	26.48	30.04
R – Preorbital distance (length of snout)	26.40	86.20	47.87	14.44	11.13	23.44	13.88	10.67
O – Horizontal eye diameter	50.20	11.70	70.22	10.69	10.58	30.32	20.14	00.31
OP – Postorbital distance	22.60	65.20	36.02	11.27	90.46	18.98	10.41	10.32
HCo – Head depth (at centre of eye)	13.40	37.50	20.72	50.86	50.21	10.25	60.05	00.77
HC – Head depth (at nape)	18.90	55.40	31.31	90.79	60.29	14.26	90.05	00.99
H – Maximum body depth	21.20	72.40	38.85	13.13	80.18	20.41	11.18	10.62
lD – Length of dorsal fin	12.70	43.10	22.92	70.97	50.43	12.79	60.56	00.99
Hd - Depth of dorsal fin	12.80	51.70	22.91	80.41	50.39	11.19	60.54	00.88
aD – Predorsal dostance	132.00	418.40	220.48	72.60	60.02	85.04	63.16	30.71
poD – postdorsal distance	11.30	51.70	23.97	90.13	50.11	14.75	60.81	10.28
h – Maximum body depth (last depth of caudal penducle)	50.30	17.00	90.35	30.20	20.23	40.34	20.68	00.31
lA – Length of pectoral fin	60.80	24.00	12.78	40.56	20.85	60.56	30.66	00.56
hA – Depth of anal fin	10.30	40.90	21.05	80.06	40.58	10.25	50.95	00.80
pl1 – Length of caudal penducle	20.90	80.70	39.67	14.55	90.84	21.60	11.29	10.62

1	2	3	4	5	6	7	8	9
pl2 – Distance from anal fin tip to tip of middle rays in caudal fin	33.60	117.30	58.79	20.58	14.98	29.92	16.80	10.98
lV – Length of ventral (pelvic) fin	10.20	39.10	20.88	70.75	40.42	11.23	50.94	00.96
VA – distance from ventral fin base to anal fin base	17.50	83.30	35.63	15.39	70.54	22.13	10.10	20.36
PV – Distance from pectoral fin base to ventral fin base	61.70	208.30	104.13	36.23	24.40	54.92	29.80	30.95
aV – Preventral distance	117.50	363.60	193.41	63.10	52.19	98.36	55.59	60.35
aA – Predeorsal distance	138.10	434.90	232.10	76.54	62.43	118.85	66.64	70.72
SRC – Width of snout at base of barbels	13.40	33.90	21.30	60.04	40.84	90.96	60.24	00.97
ll – Length of barbel	80.20	24.00	13.33	30.68	30.08	90.73	30.98	10.08
rc – Distance from tip of snout to cartilaginous arch of mouth	12.90	55.90	31.18	90.40	50.32	15.57	90.08	10.46
rl – Distance from tip of snout to middle barbel	12.00	36.80	19.85	60.64	40.93	10.12	50.70	00.72
rr – Distance from tip of barbel to cartilaginous arch of mouth	28.50	91.30	51.02	15.22	12.02	25.57	14.83	10.89
SO – Width of mouth	80.70	28.60	15.06	40.61	30.27	70.17	40.37	00.55
SRr – Width of snout at the base of mouth	15.30	46.70	27.09	80.17	50.45	13.36	70.90	10.21
iO – Interorbital distance (skull width)	13.50	43.50	22.52	70.27	50.68	10.98	60.49	00.71
BC – Maximum width of head	19.50	64.00	33.20	11.34	80.16	16.39	90.54	10.21
lP – Length of pectoral fin	24.10	46.80	30.52	60.89	90.95	12.88	11.39	00.70

Table 3. Countable morphological characters of *Acipenser oxyrinchus*

Character	Min	Max	Mean	sd
Number of dorsal scutes	9	12	10.28	0.67
Number of lateral scutes	21	30	25.22	1.76
Number of ventral scutes	7	10	8.56	0.67
Number of dorsal fin rays	28	41	36.46	2.46
Number of anal fin rays	22	29	24.30	1.45

The characters showed a low variation range. In order to reduce the signal and the number of characters for further comparison, principal component analysis (PCA) was conducted for the 21 measurable characters of the 4 sturgeon species. The value of χ^2 was high (cumulative inertia: 0.999, χ^2 = 6,304.226, P = 0.000), which indicated that the species differed substantially with respect to measurable characters. Figure 2 shows distribution and direction of vectors on the graph depicted by the first two principal components of 21 morphological variables.



R – Preorbital distance (length of snout), LF– Fork length, LS– Standard length, C – Length of head, O – Horizontal eye diameter, OP – Postorbital distance, HCo – Head depth (at centre of eye), HC – Head depth (at nape), aD – Predorsal distance, lD – Length of dorsal fin, Hd – Depth of dorsal fin, h – Maximum body depth (last depth of caudal penducle), lA – Length of pectoral fin, hA – Depth of anal fin, Pl1 – Length of caudal penducle, lV – Length of ventral (pelvic) fin, VA – distance from ventral fin base to anal fin base, aA – Preanal distance, l1 – Length of barbell, rl – Distance from tip of snout to middle barbell, rr – Distance from tip of barbel to cartilaginous arch of mouth.

Figure 2. Results of the principal component analysis for measurable characters with the highest contribution to species differentiation. Projection of the variables on the factor-plane (1 × 2)

The PCA revealed that the measurable characters with the highest contribution to differentiation of the 4 sturgeon species included head characters along with distance from ventral fin base to anal fin base (VA) and length of ventral (pelvic) fin (lV). Characters with the highest contribution to the first two principal components were selected for stepwise discriminant analysis. All compared measurable characters were taken into account in the discriminant function model (Wilks' lambda: 0.00014 approx. F (27.2065) = 1,512.9 $P < 0.0000$). A classification matrix for the stepwise discriminant analysis generated for nine measurable characters of the species was characterized by a high level of correctness (99.5828% Table 4).

The classification matrix displayed the lowest correctness for *A. oxyrinchus* and *A. stellatus*, and the highest for *A. baerii* and *A. gueldenstaedtii*. Only individual specimens of *A. oxyrinchus* might be incorrectly classified as *A. stellatus* or *A. gueldenstaedtii* (Table 4). On the basis of both discriminant functions, canonical discriminant analysis clearly discriminated the four species with respect to their biometric characters (Figure 3).

Table 4. Classification matrix of the stepwise discriminant analysis for measurable characters of four sturgeon species, rows: observed classification; columns: expected classification

	% correct	*Acipenser oxyrinchus* $P = 0.06815$	*Acipenser gueldenstadtii* $P = 0.37691$	*Acipenser baerii* $P = 0.46453$	*Acipenser stellatus* $P = 0.09040$
A. oxyrinchus	95.9184	47	1	0	1
A. gueldenstadtii	100.0000	0	271	0	0
A. baerii	100.0000	0	0	334	0
A. stellatus	98.4615	1	0	0	64
Total	99.5828	48	272	334	65

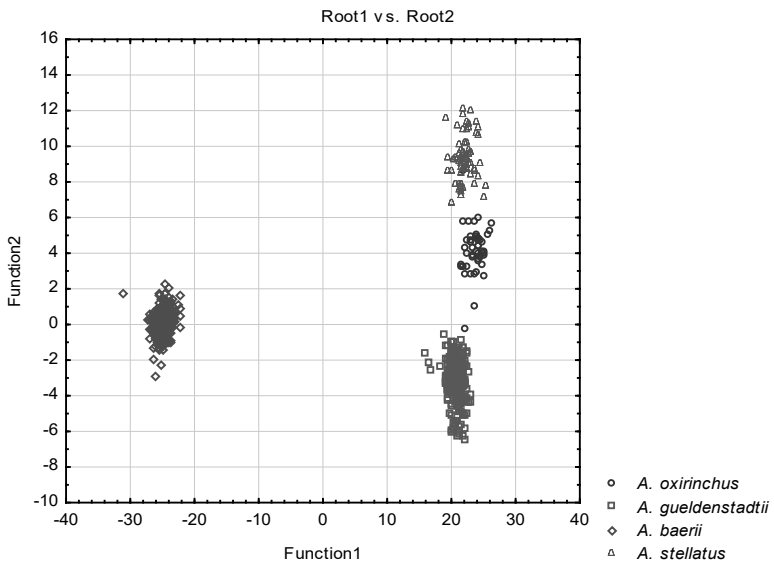


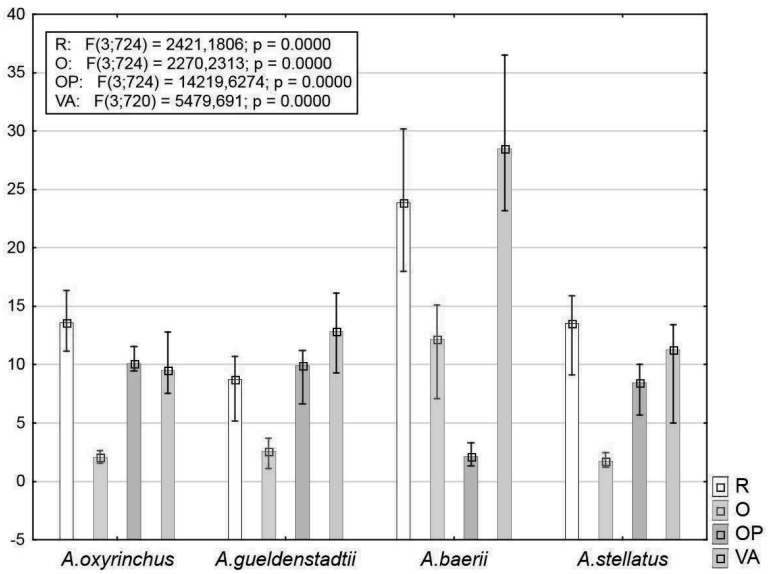
Figure 3. Scatter plot of canonical scores for the analysis of measurable characters of the 4 sturgeon species (root 1; root 2)

The first discriminant function separated *A. gueldenstaedtii*, *A. oxyrinchus*, *A.* stellatus, and *A. baerii*. The second function discriminated between *A. oxyrinchus* and *A. gueldenstaedtii* and also between *A. stellatus* and *A. gueldenstaedtii*. According to the position of *A. baerii* on the graph, the second function situate this species between *A. oxyrinchus* and *A. gueldenstaedtii*. In the first function (root 1), character VA made the most significant contribution to discrimination, while, in the second function (root 2), the significantly contributing characters included horizontal eye diameter and preorbital distance. Postorbital distance had a significant contribution only in the first function (Table 5, Figure 4).

Table 5. Standardized coefficients for canonical variables of measurable characters, Root1-3 – classification functions

	Root 1	Root 2	Root 3
OP	0.6763	–0.43705	0.10452
HCo	–0.5037	–0.70146	0.17790
R	–0.3309	2.31381	1.03342
O	–0.7311	–2.71539	1.55823
VA	–0.8352	–0.04078	0.21091
l1	0.1216	1.03025	–2.47056
HC	0.4408	0.37853	0.21382
C	0.3019	0.00752	0.31132
lV	–0.2852	–0.13161	–0.05984
Eigenvalue	336.0406	9.75159	0.94491
Cumulative per cent	0.9692	0.99727	1.00000

OP – Postorbital distance; HCo – Head depth; R – Preorbital distance; O – Horizontal eye diameter; VA – Distance from ventral fin base to anal fin base; l1 – Length of barbell; HC – Head depth; C – Length of head; lV – Length of ventral (pelvic) fin.



VA – distance from ventral fin base to anal fin base, R – Pre-orbital distance (length of snout), O – Horizontal eye diameter, OP –Postorbital distance.

Figure 4. Comparison of the median values of characters providing the greatest contribution to discriminant functions for the 4 sturgeon species

Countable Characters

The analysis of standardized coefficient values for canonical variables indicated number of lateral scutes as the character with the most significant contribution to discrimination (Table 6).

Table 6. Standardized coefficients for canonical variables of countable characters, Root 1 – first classification function; Root 2 – second classification function

	Root 1	Root 2	Root 3
SL	0.941708	–0.095766	–0.517779
SV	–0.092696	1.007434	–0.054934
SD	0.163372	0.049238	1.054514
Eigenvalue	6.258933	0.324818	0.000476
Cumulative per cent	0.950595	0.999928	1.000000

SL – Number of lateral scutes, SV – Number of ventral scutes, SD – Number of dorsal scutes.

Correctness of the classification matrix for countable characters was calculated as 79.92%, lower than for measurable characters (Table 7).

Table 7. Classification matrix of the stepwise discriminant analysis for countable characters of four sturgeon species, rows: observed classification; columns: expected classification

Species	% correct	*A. oxyrinchus* $P = 0.09042$	*A. baerii* $P = 0.60579$	*A. gueldenstaedtii* $P = 0.17902$	*A. stellatus* $P = 0.12477$
A. oxyrinchus	98.00	49	0	0	1
A. baerii	96.72	0	324	9	2
A. gueldenstaedtii	7.07	0	92	7	0
A. stellatus	89.86	7	0	0	62
Total	79.93	56	416	16	65

In spite of there being relatively fewer *A. oxyrinchus*, 98% of *A. oxyrinchus* individuals were correctly discriminated. The highest possibility of an error based on countable characters was observed for *A. gueldenstaedtii*, which showed similarity of meristic characters with *A. baerii*, as well as the distinctiveness of the *A. oxyrinchus* from other species on the basis of having significantly fewer lateral scutes (Figure 5).

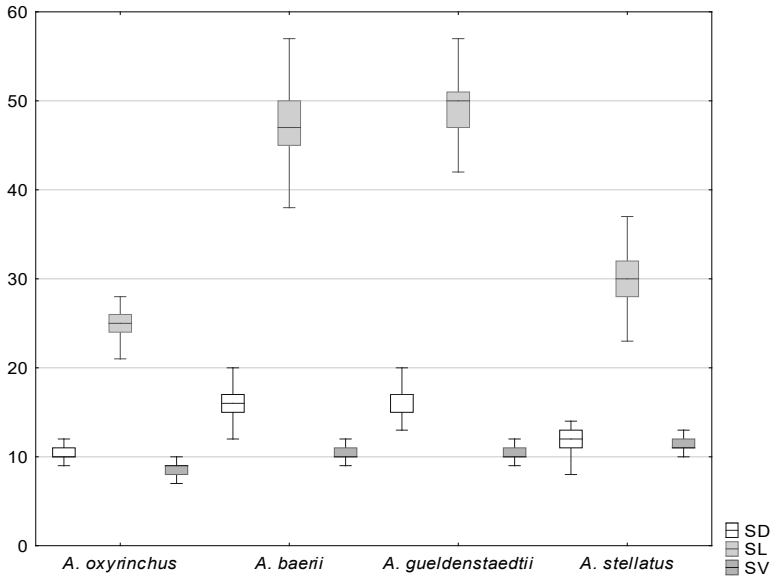


Figure 5. Median values of countable characters: number of dorsal scutes, number of lateral scutes and number of ventral scutes in four sturgeon species

Discussion

The data from the present study may be used to supplement existing systematic keys and lay the foundations for creating a catalogue or atlas of popular species and interspecific hybrids of sturgeon, including their complete systematic determination (Krylova, 1997). Correct identification is especially important in the context of species restoration. Stocking of juvenile European sea sturgeon and *A. oxyrinchus* as part of their re-establishment programme in Europe (Elvira, Gessner, 1996; Gessner et al., 2010) along with the co-occurrence of alien species, requires an informational campaign for users of the waters, focusing on distinguishing between native and non-native species. A reference book containing a simple and user-friendly key with sound taxonomic data is necessary. The data available for comparison with the results of the present biometric analyses were conducted on only a small number of living specimens (Artiukhin, Vecsei, 1999; Debus, 1999). When examining 27 museum specimens and a single live sturgeon, it was noted that variability of some characters of the sturgeon head, mainly due to scraping the substratum while feeding, precluded considering distances connected with snout structure as distinguishing characteristics. Comparison of dorsal and lateral scutes suggested that *A. oxyrinchus* differs from *A. sturio* from the Gironde (Atlantic Ocean) and the Rioni (Black Sea) rivers (Ninua, 1976). The analyses discussed in the present paper compared morphometric data of *A. oxyrinchus*, *A. stellatus*, *A. gueldenstaedtii*, and *A. baerii*. Among measurable characters expressed as per cent of L_T, preorbital distance, distance from ventral fin base to anal fin base (VA), horizontal eye diameter (O), and postorbital distance (OP) had the highest contribution to discrimination (Figure 4). Mainly on the basis of VA, the first function clearly discriminated

between *A. baerii* and those of *A. oxyrinchus, A. gueldenstaedtii*, and *A. stellatus* (Figure 3). A longer VA distance distinguished *A. baerii* from the other three sturgeon species (Figure 4). In the second function, preorbital distance contributed significantly to discrimination due to the positive value of the canonical variable coefficient; whereas, in the second function, horizontal eye diameter contributed significantly to discrimination (Table 5). On the basis of this analysis it can be concluded that the shorter the rostrum of an *A. oxyrinchus* individual, the higher the possibility of mistaking it for *A. gueldenstaedtii*. Distinguishing *A. oxyrinchus* from *A. baerii* may present difficulties due to considerable changes of the rostrum length during the lifetime of the latter, as well as the existence of short-rostrum and long-rostrum forms of *A. baerii* (Keszka et al., 2009). Thus, the risk of an erroneous classification is high in the case of juvenile *A. baerii* individuals, which have a longer rostrum than adults of the species. The risk of mistaking *A. oxyrinchus* for another species on the basis of measureable characters is the highest with respect to *A. stellatus*. The second classification function, with rostrum length having the major contribution to discrimination, clearly distinguished between the *A. gueldenstaedtii* and *A. baerii* grouping and the *A. stellatus* and *A. oxyrinchus* grouping while the first classification function separated the group of *A. baerii* from the groups of the remaining three sturgeon species.

Based on the five analysed countable characters, exotic species are clearly distinguishable from *A. oxyrinchus*. The number of lateral scutes had the highest contribution to discrimination (Table 6). In the sample, the number of scutes ranged from 21 to 30, with a mean of 25.22. The value was lower than that observed for *A. oxyrinchus* in the St Lawrence River in Canada (28.67) (Artiukhin, Vecsei, 1999) and considerably lower than found for exotic species occurring in Polish waters (Keszka, Heese, 2003; Keszka, Krzykawski, 2008; Keszka et al., 2009). Low variability of the countable characters in *A. oxyrinchus* specimens in the present study might be due to the limited number of broodstock used to produce stocking material as well as to an effect of artificial rearing conditions on juvenile fish, as had been noted in the case of other cultured sturgeon species (Ruban, Sokolov, 1986).

Exotic sturgeon introduction into European waters may occur from fish released by aquarium owners and hobbyists who want to dispose of large individuals, sturgeons intentionally released by fishing clubs and associations, and accidental escape from ponds and farms. Despite clear regulations applying to alien species in aquaculture under the European Commission Regulation (EC) no. 535/2008 of 13 June 2008 rules for implementation of the Council Regulation (EC) no. 708/2007 concerning use of alien and locally absent species in aquaculture, a fourth source of exotic sturgeon introduction involves artificial breeding and release (Britton, Davis, 2006). The occurrence of non-native sturgeon species in German and Polish coastal waters and river estuaries has been growing since the beginning of the 1990s (Spratte, Rosenthal, 1996; Keszka, Stepanowska, 1997; Arndt et al., 2000, 2002; Keszka, Heese, 2003; Keszka et al., 2011). The presence of exotic sturgeon species was also observed in the Gironde River in France in 1999 after a period of storms that caused water levels to rise and resulted in the escape of several thousand *A. baerii* from fish farms. This presented a serious problem for inland fishers and anglers in distinguishing between two sturgeon species, of which one (*A. sturio)* was strictly protected and the second (*A. baerii*) needs to be eliminated from open waters (Gessner et al., 2010). Alien species release, taking place in spite of the prohibitions connected with environmental protection, should be considered a potential threat, since alien species may have a negative effect on native species and their populations, especially in strongly altered ecosystems (Leppäkoski et al., 2002).

Apart from the above implications for alien species introduction, genetic implications cannot be dismissed, as sturgeons display a potential for hybridization (Kozhin, 1964). Current

information suggests that the hybrids are fertile, which means that mixed genetic material is passed on to the next generation, resulting in a dispersal of the original genetic information and reducing adaptation ability to habitats. The existence of the first hybrids created as a result of release of exotic *A. baerii* has been confirmed by genetic analyses conducted on specimens collected from the Danube River (Ludwig et al., 2009).

Avoidance of loss connected with fisheries is an important principle behind protecting the remaining native populations *in situ* (Kapusta et al., 2008), Gessner et al., 2010), Key factors in effective restoration of a population include decreasing fishing-associated mortality and obtaining a high level of acceptance and support from the fishery sector. Measures that need to be taken include instituting and monitoring programs to limit accidental catch based on the ability of fishers to identify species. The example of *A. sturio* in France and preliminary results obtained from Germany clearly show that this is possible (Gessner et al., 2010).

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Anatomy of the female reproductive system and sperm storage of the viviparous caecilian *Typhlonectes natans* (Gymnophiona: Typhlonectidae)

Jakub Dymek,[1] Anna Dymek,[2] Artur Osikowski[3]

[1] Department of Comparative Anatomy, Institute of Zoology and Biomedical Research, Jagiellonian University, Gronostajowa 9, 30-387 Kraków, Poland, e-mail address: kuba.dymek@doctoral.uj.edu.pl
[2] Department of Comparative Anatomy, Institute of Zoology and Biomedical Research, Jagiellonian University, Gronostajowa 9, 30-387 Kraków, Poland, e-mail address: anna.tyrkalska@doctoral.uj.edu.pl
[3] Department of Animal Anatomy, Institute of Veterinary Science, University of Agriculture in Krakow. Al. Mickiewicza 24/28, 30-059 Kraków, Poland, e-mail address: a.osikowski@ur.krakow.pl

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Abstract Here, we describe the anatomy of the female reproductive system of the aquatic, viviparous caecilian *Typhlonectes natans*. We conducted our research on two adult females using light microscopy and reflected our results with the available literature data on the anatomy of the reproductive systems of caecilian amphibians. The female reproductive system of *T. natans* consisted of longitudinal, paired ovaries incorporated with fat bodies, paired oviducts and the cloaca. The ovaries were arranged in segmental mannered germinal nests and the oocytes were in various stages of development. Ovulated, postvitellogenic oocytes were present in the oviducts of both females. The wall of the oviduct consisted of three layers: the mucous membrane, layers of muscles and serous membrane. The presence of wedge-like cells, not previously reported in the mucous membrane of the caecilian oviduct is postulated. Although the females possessed ovulated eggs, no sperm were detected in any part of the oviducts leaving the question of eventual female sperm storage in *Typhlonectes natans* open.

Anatomia żeńskiego układu rozrodczego oraz system przechowywania nasienia żyworodnego płaza beznogiego *Typhlonectes natans* (Gymnophiona: Typhlonectidae)

Słowa kluczowe Typhlonectidae, płazy beznogie, układ rozrodczy, żyworodność, przechowywanie plemników

Streszczenie W artykule opisano budowę anatomiczną żeńskiego układu rozrodczego żyworodnego płaza beznogiego *Typhlonectes natans*. Badania z wykorzystaniem mikroskopii świetlnej przeprowadzono na dwóch dojrzałych samicach. Otrzymane wyniki porównano z danymi literaturowymi dotyczącymi żeńskiego układu rozrodczego płazów beznogich. U *T. nantans* składa się on z wydłużonych, parzystych jajników oraz parzystych jajowodów uchodzących do kloaki. W pobliżu jajowodów znajdują się wydłużone ciała tłuszczowe. Segmentowane jajniki zawierają gniazda oogoniów oraz oocyty będące na różnych etapach oogenezy, natomiast w świetle jajowodów u obu samic stwierdzono owulujące oocyty. Ściana jajowodu składa się z trzech warstw: śluzowej, mięśniowej i surowiczej. W warstwie śluzowej stwierdzono

występowanie komórek przypominających komórki klinowe, co nie było dotąd obserwowane u płazów beznogich. Pomimo obecności owulujących oocytów w żadnym rejonie jajowodu niezaobserwowano plemników, co pozostawia pytanie o występowanie magazynowania plemników u samic tego gatunku wciąż otwartym.

Introduction

Various aspects of the biology of caecilians (Gymnophiona) still remain poorly understood. Members of this group live in tropical regions of the southern hemisphere under the ground or in the water (Jared et al., 1990). Caecilians exhibit internal fertilization: males possess an introminent organ termed phallodeum (e.g., Tonutti, 1931), which penetrates the female's cloaca during copulation (Kupfer et al., 2006). Fertilization occurs in the anterior part of the oviduct (Wake, Dickie, 1998). Approximately two thirds of the species are oviparous, and the rest are viviparous (e.g., Wake, 1977; San Mauro et al., 2014).

The first descriptions of the female reproductive tract in Gymnophiona were provided by Müller (1832) and Rathke (1852). There have been an increasing number of studies describing the anatomy of the reproductive systems of caecilians including both phylogenetically basal oviparous [*Ichthyophis supachaii* Taylor, 1960 (Pewhom et al., 2015); *I. beddomei* Peters, 1880 (Masood-Parwees, Nadkarni, 1993); *I. glutinosus* Linnaeus, 1758; *Siphonops annulatus* Mikan, 1820 (Sarasin, Sarasin, 1887); *Gegeneophis ramaswamii* Taylor, 1964 (Beyo et al., 2007)] and derived viviparous species [e.g., *Chthonerpeton indistinctum* Reinhardt, Lütken, 1862 (Berois, De Sa, 1988); *Typhlonectes compressicauda* Duméril and Bibron, 1841 (Exbrayat et al., 1983)], but an understanding of the morphology, anatomy and function of these reproductive systems still lags behind that of anurans and caudates.

Typhlonectes natans Fischer In Peters, 1880 is an aquatic, viviparous caecilian that lives in rivers, swamps and lakes in the northern areas of South America (e.g., Fuhrmann, 1914). This species' range covers the Cauca and Magdalena rivers in Columbia and Lake Maracaibo in Venezuela (e.g., Fuhrmann, 1914). Its body has a length ranging from 45–55 cm. Sexual dimorphism is manifested in body length (females are larger) and the shape of the cloaca (Kupfer, 2009).

The aim of this study was to describe for the first time the anatomy of the female reproductive tract of viviparous *T. natans* and to compare the results with the available literature of both oviparous and viviparous species. Special attention was paid to the histological structure of each part of the reproductive tract and the search for putative places of female sperm storage in the oviduct. We performed the research using light microscopy.

Materials and methods

We focused on two adult *T. natans* females from the Municipal Zoological Garden (Łódź, Poland). Individuals were assigned to a species on the basis of the number of lobes surrounding the vent and on the basis of genetic study. The two females were kept with two males in a 400-liter aquarium filled with 140 liters of water and a sandy bottom. The temperature of the water was electronically maintained at 26–27°C. The animals were fed raw beef, earthworms and vitamins *ad libitum*. The total lengths of the females at the moment of sacrifice were 503 and 527 mm.

The animals were killed via immersion in a 0.1% water solution of tricaine methanesulfonate, MS – 222 (Sigma®, St. Louis, MO, USA). After opening up the abdominal cavity, the reproductive tracts of the females were dissected (Figure 1). The materials were preserved in Bouin fixative

for one week. Later, the material was dehydrated in a graded series of ethanol (70%, 96%, 100%), cleared in toluene and embedded in Paraplast® Regular (Sigma®, St. Louis, MO, USA). The obtained material was sliced on a microtome to create transverse sections 7 micrometers thick that were then affixed to slides. Selected slides were treated with Hematoxilin – Eosin (HE), Passini method and Mallory's trichrom for general cytology and with Alcian Blue & Periodic Acid Schiff stain (AB+PAS) for histochemical cytology. After treatment, the slides were enclosed in a Thermo Scientific Shandon Consul Mount and observed using a light microscope. The slides were photographed using a Nikon COOLPIX P6000 camera. For each individual, approximately 150 slides were prepared representing the entire reproductive duct. Measurements of the structures were obtained using ImageJ software.

Results

The female reproductive tract of *T. natans* consisted of a pair of ovaries accompanied by twisted fat bodies, paired oviducts and the cloaca (Figure 1).

(c) cloaca; (dt) digestive track; (fb) fat bodies; (ova) ovary; (ovi) oviduct. Scale bar = 10 mm.

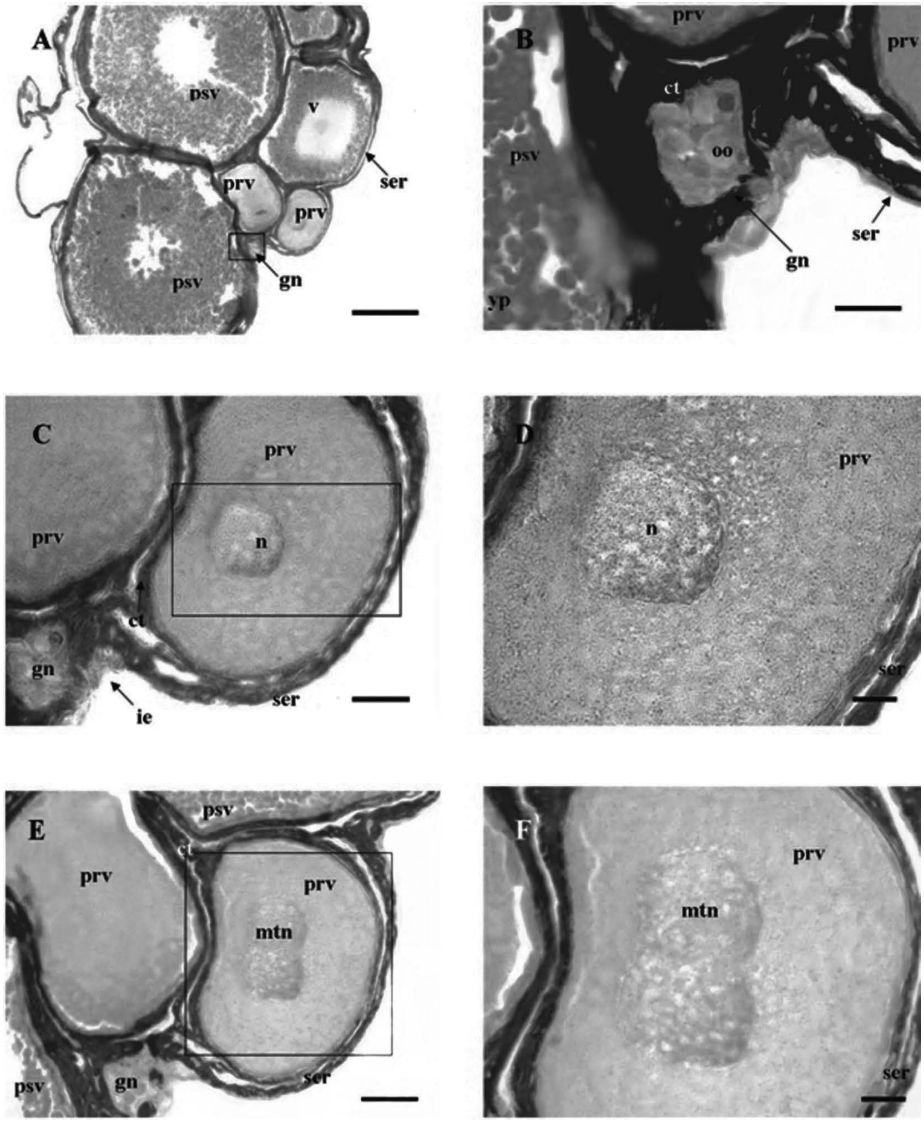
Figure 1. Gross morphology of the female reproductive system of *Typhlonectes natans*

Oocytes at various stages of development were observed in the ovaries and in the oviducts of both females.

Ovary and oocytes

The ovaries of both individuals were approximately 90 mm in length and fewer than 3 mm in diameter. They were connected to the respective kidneys and fat bodies by a mesovarium.

The ovaries were surrounded by connective tissue and enveloped by peritoneal epithelium. Several germinal nests and oocytes in different stages of development (including previtellogenic, vitellogenic and postvitellogenic oocytes) were present inside the ovaries (Figure 2A), and they varied in size from 240–1,300 µm. The oogonia were grouped into germinal nests, which were arranged in a segmental manner (Figure 2B). These structures were oocytes inside of the ovary were separated by connective tissue, not surrounded by follicular cells. The germinal nests were situated between oocytes.



(A) gross structure (scale bar = 300 µm). (B) germinal nests of oogonia (scale bar = 20 µm). (C, D) previtellogenic cells with the eccentric nucleus (scale bar C = 50 µm, D = 30 µm). (E, F) previtellogenic cells with the multiple nuclei (scale bar E = 50 µm, F = 30 µm). (ct) conective tissue; (gn) germinal nests; (ie) islets of epithelium; (mtn) multiple nuclei; (n) nucleus; (oo) oocyte; (prv) previtellogenic oocyte; (psv) postvitellogenic oocyte; (ser) serosus membrane; (v) vitellogenic oocyte; (yp) yolk platelets.

Figure 2. Histological structure of the ovary of *T. natans*. Passini staining

Previtellogenic oocytes (Figure 2C–F) were found at the periphery of ovary, which implied that progressive ovarian zonation was present. These cells were surrounded by membrana granulosa of the ovary and a thin layer of connective tissue. The cell nucleus of a previtellogenic oocyte

was spherical and relatively large compared with the entire cell (Figure 2C–D). The nucleus contained numerous nucleoi. In addition, multiplying of the number of nuclei in some oocytes was observed (Figure 2E–F). Previtellogenic oocytes did not contain supplementary material or pigment grains. In the late previtellogenic phase, the oocytes became larger and began to accumulate supplementary material such as platelets of yolk and lipid droplets. In this phase, the oocytes had clearly visible islets of glycoprotein materials.

Oocytes in the vitellogenic phase were easy to identify because they contained peripherally arranged platelets of heterogeneously sized yolks, which increased with later vitellogenic stages. Large and numerous yolk platelets were found in the cortical layer of the vitellogenic oocytes, as well as pigment grains, that had accumulated in the animal hemisphere. Interestingly, the theca cell layer was thicker in oocytes in the vitellogenic phase than in oocytes in the previtellogenic phase.

The postvitellogenic oocytes were the largest in size (approx. 1mm). The nuclei of the postvitellogenic cells were large and irregularly shaped. Furthermore, there were densely arranged platelets of yolk in the cytoplasm of postvitellogenic oocytes. The surface layer of the ooplasm contained cortical grains.

We observed the atresia of some developing follicles. Atresia was established on the basis of characteristic changes in the nucleus shape and degenerative changes of the oocytes. The nuclei of the artretic follicles were shrunken and had an irregular shape.

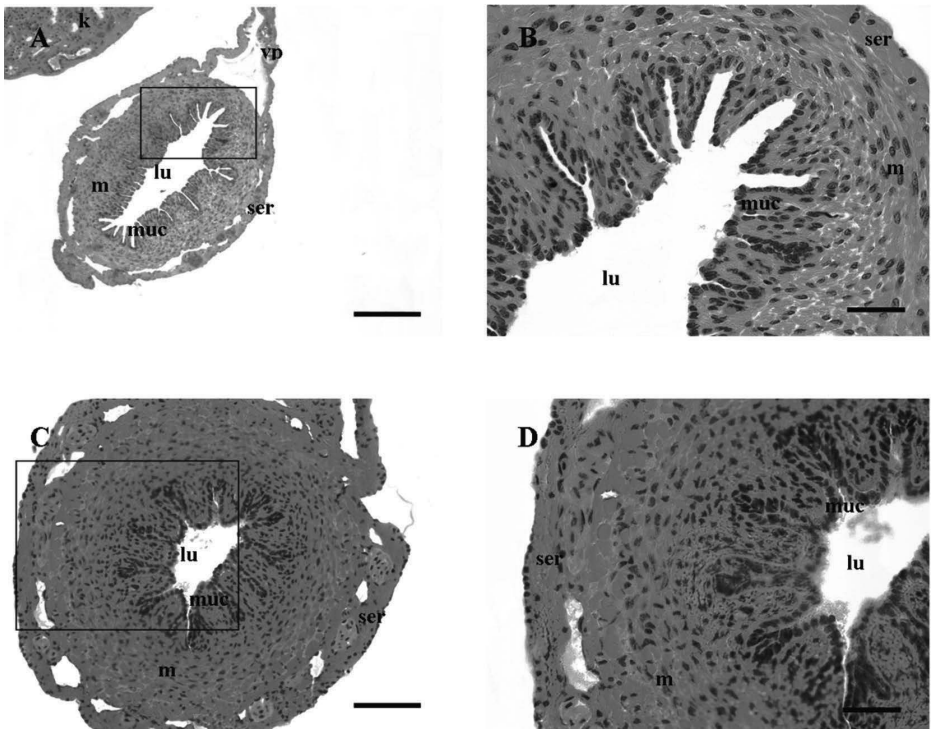
Fat bodies

The multi-lobed, leaf-like fat bodies were observed parallel to the reproductive tract of the studied females (Figure 1). They were extremely extensive (approximate width: 10 mm) and they stood out in their size compared with other internal organs.

Oviduct

The length of the oviducts in both of the tested animals was approximately 140 mm. The oviducts consisted of two morphological units: starting with the funnel, the anterior part with a larger lumen and a relatively thin layer of muscles (Figure 3A–B) and the rear part, the so-called pars uterine, which had a thick layer of muscle and lower lumen (Figure 3C–D). The oviduct wall had a layered structure with the mucous membrane lining the lumen, a layer of muscles and the external serous membrane (Figure 3). In the lumen of the oviduct, we found mucous aggregations as well as singular epithelial cells, especially in the pars uterina.

The mucous membrane consisted of a simple columnar epithelium (Figure 4A–D) composed of three types of epithelial cells: ciliated cells, unciliated secretion cells (Figure 4A–B) and a few wedge-like cells (Figure 4A). The epithelium formed numerous villus-like folds, which were directed into the oviduct lumen. On the top of these folds, a larger proportion of ciliated epithelial cells were visible (Figure 4B). The number and height of the folds of the mucous membrane depended on location within the oviduct; longer and more numerous folds occurred in its anterior part. Under the epithelium, a layer of connective tissue with numerous fibers was present (Figure 4A–B).



(A, B) front part of the oviduct (scale bar A = 300 µm, B = 50 µm). (C, D) rear part of the oviduct, so-called pars pars uterina (scale bar C = 100 µm, D = 50 µm). Hematoxylin & eosin staining. (k) kidney; (lu) lumen; (m) muscles; (muc) mucous membrane; (ser) serous membrane; (vp) visceral peritoneum.

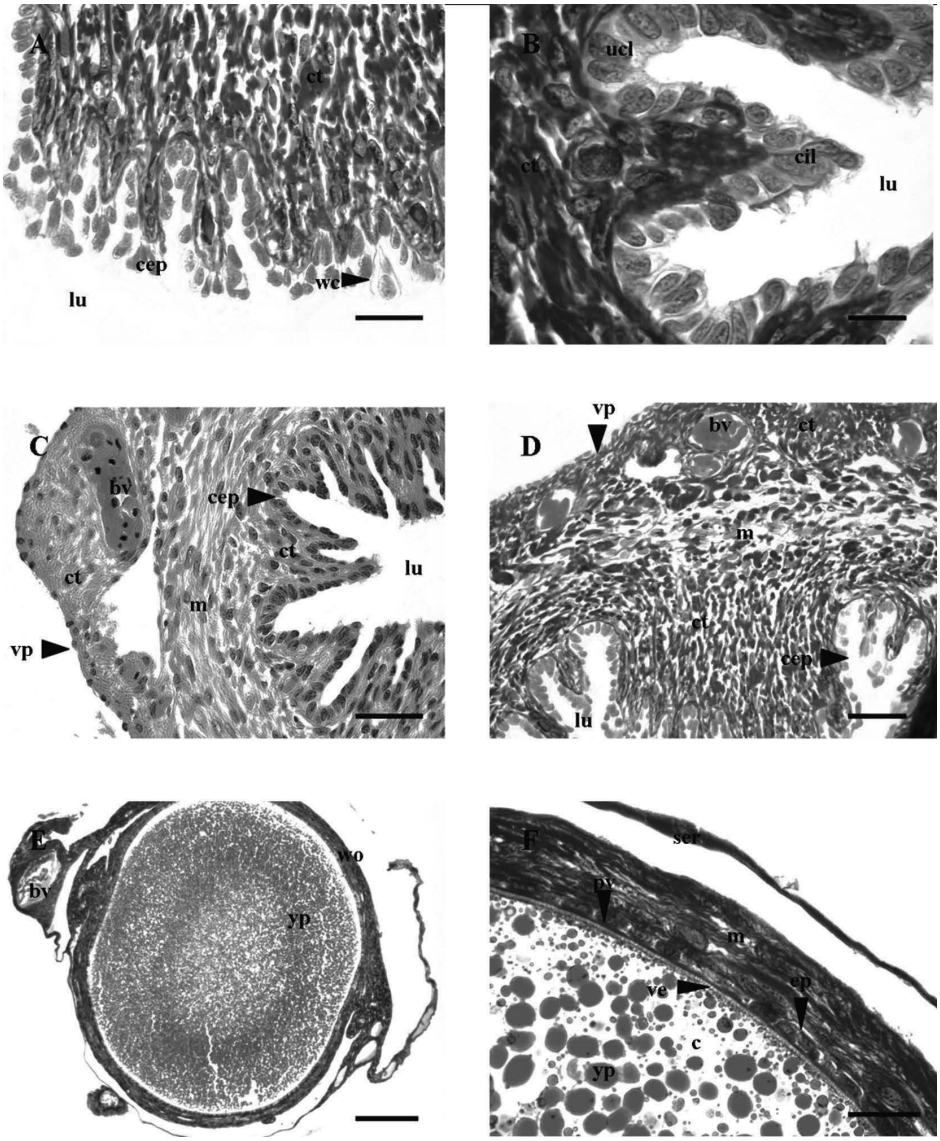
Figure 3. Anatomical structure of the oviduct of *T. natans*

There was a layer of smooth muscles just below the mucous membrane (Figure 4C–D). The thickness of the muscle layers depended on the part of oviduct and, as noted above, the posterior part of the oviduct – the so-called "uterine" – had the thickest layer of muscles.

The outermost layer of the oviduct wall was a serous membrane. It consisted of connective tissue covered with visceral peritoneum (Figure 4C–D). Numerous blood vessels and elastic fibers were visible. Visceral peritoneum surrounded the oviduct and nearby located kidney.

The oviducts were highly extensible, which was clearly seen in slides containing the passing oocytes (Figure 4E–F). In such places, the walls of the oviduct wall were stretched, which rendered the mucous folds and muscular layers very thin.

The oocytes that were passing through the oviduct were unfertilized. Since these oocytes were filled with yolk platelets, they were in the postvitellogenic phase.

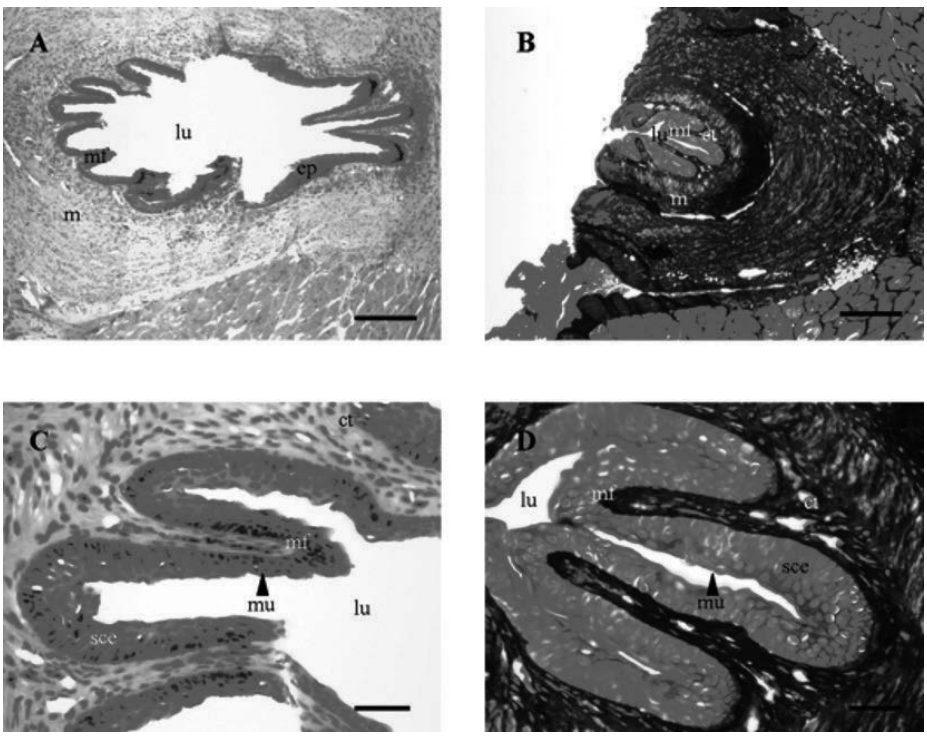


(A, B) mucous membrane of the wall of the oviduct (scale bar A = 30 µm, B = 20 µm). (C, D) structure of the wall of the oviduct (scale bars = 50 µm). (E, F) mature oocyte passing through the oviduct (scale bar E = 300 µm, F = 30 µm). (A, B, D, F) Mallory`s trichrome staining; (C) Hematoxilin – eosin staining; (E) Passini staining. (bv) blood vessel; (c) cytoplasm; (cep) cilindrincal epithelium; (cil) ciliated cell; (ct) connective tissue; (ep) epithelium; (lu) lumen; (m) muscles; (pv) previtelline space; (ser) serous membrane; (ucl) unciliated cell; (ve) vitelline envelope; (vp) visceral peritoneum; (wc) wedge like cell; (wo) wall of oviduct; (yp) yolk platelets.

Figure 4. Histological structure of the oviduct of *T. natans*

Cloaca

The cloacal walls consisted of an epithelial layer, connective tissue and a layer of circular and longitudinal muscles (Figure 5A). The lumen of the cloaca was star-like (Figure 5A). Epithelium with connective tissue created mucosal folds, which projected into the lumen of the cloaca (Figure 5A). The folds were more branched in the anterior part of the cloaca than in the posterior part (Figure 5A–B). Because of this irregular branching, the lumen of the cloaca was tortuous. Stratified columnar epithelium formed the lining of the cloaca (Figure 5C–D). The epithelial cells possessed large nuclei and low levels of cytoplasm. We noted that the anterior part of the cloaca was lined with a thinner layer of epithelium than the posterior part (Figure 5C–D). The epithelium contained round secretion cells with medium-sized nuclei and abundant cytoplasm. The secretions of these cells could be stained with alcian blue (AB), which suggests that the mucus consisted of carboxylated glycosaminoglycans (AB+) but lacked neutral carbohydrates (PAS–). A thin layer of mucus covered the walls of the cloaca (Figure 5C–D).



From the anterior part (A, C) to the posterior part (B, D) (scale bars A, B = 300 µm, C, D = 50 µm). (A, C) Hematoxilin – eosin staining; (B, D) Mallory`s trichrome staining. (ct) connective tissue; (lu) lumen; (m) muscle; (mf) mucosal fold; (mu) mucus; (ps) process of skin; (sce) stratified columnar epithelium.

Figure 5. Histological structure of the cloaca of *T. natans*

Discussion

Presented study revealed, that female reproductive system of *T. natans* possess a similar organization as other previously described members of Gymnophiona [e.g., *Hypogeophis rostratus* (Oyama, 1952); *Ch. indistinctum* (Welsch et al., 1977), *Dermophis mexicanus* (Wake, 1980); *T. compressicauda* (Exbrayat, 1983, 1984, 1986, 1988, 1992, 1996) and *I.* cf. *kohtaoensis* (Kuehnel, Kupfer, 2012)]. Their anatomy consists of paired ovaries with attached fat bodies, paired oviducts and a cloaca.

The ovaries of viviparous *T. natans* were paired, elongated structures. Their length (approximately 90 mm in both of the studied individuals) was similar to that of *T. compressicauda* (Exbrayat, 2009), in which the ovaries of adult specimens were 60–100 mm in length. Fat bodies attached by connective tissue alongside the ovaries were observed. The lumen in ovaries was not detected. Originally, the occurrence of the lumen in the ovaries of Gymnophiona was reported by Tonutti (1931), but this phenomenon has not been confirmed more recently and is accordingly considered to be an artifact (Exbrayat, 2009). The ovaries consisted of germinal nests and oocytes in various stages of development: previtellogenic, vitellogenic and postvitellogenic. In amphibians, oogonia are organized into various structures: in anurans they are called germ patches (Masood-Parwees, Nadkarni, 1993), and in Urodela and Gymnophiona they are termed germinal nests (Gomes et al., 2012). The germinal nests of *T. natans* are arranged in a segmental manner as previously observed in *T. compressicauda* and oviparous *I. beddomei* (Masood-Parwees, Nadkarni, 1993; Exbrayat, 2006). In *Ch. indistinctum* (Berois, De Sa, 1988) and *I. supachai* (Pewhom et al., 2015), germinal nests are randomly scattered throughout entire ovary.

Roughly a dozen oocytes in different stages of development we observed in the oviducts of both of the studied *T. natans* females. This finding agrees with observations of other Gymnophiona in which the number of oocytes in the oviducts varied from 3–30. However, the number of oocytes also depends on the current reproductive phase of the animal; more oocytes are found in pregnant females than in non-pregnant ones (Exbrayat, 2009).

One of the general differences between oviparous and viviparous caecilians is that the oviducts of oviparous species are situated some distance from the ovaries; in viviparous species the funnel is in some distance from the ovary, and the oocytes are ovulated directly into the oviduct (Exbrayat, 2009), which was also confirmed in this study. The oviducts of oviparous species are typically divided into three regions: the pars recta, the pars convolute and the pars uterina (Exbrayat, 2009; Kuehnel, Kupfer, 2012); the oviducts of viviparous species are divided into two regions: anterior and posterior (Exbrayat, 2009). Our results confirmed this general rule since the oviducts of *T. natans* are clearly composed of two parts: the anterior part, with a large lumen and a thin layer of muscle, and the posterior part, pars uterina, characterized by a small lumen and a thick layer of muscles. These observations are similar to the results reported for other viviparous genera: *Chthonerpeton* (Parker, Dunn, 1964), *Dermophis* (Wake, 1980) and closely related *T. compressicauda* (Exbrayat, 1984, 1986, 1988, 2009). It is accepted that fertilization occurs in the anterior part of the oviduct, which also contains the glands that produce the mucous envelope of eggs (Exbrayat, 2009; Kuehnel, Kupfer, 2012).

The inner walls of *T. natans* oviducts consisted of folds partially filling the oviductal lumen. Anteriorly the folds contained a larger fraction of ciliated cells than other regions of the oviductal mucous membrane. There were no differences in the structure of the epithelium in the dorsal and abdominal parts of the oviduct. This finding is inconsistent with observations of oviparous *I. supachaii* in which the anterior and the middle parts of the oviduct consisted of two distinct epithelia:

a simple columnar epithelium in the dorsal part and a ventral side lined with pseudostratified columnar epithelium (Pewhom et al., 2015). Interestingly, the epithelial folds in *T. natans* contain wedge-like cells, which not have been previously reported in caecilians (Oyama, 1952; Wake, 1970; Welsch et al., 1977; Gomes, 2012). Cells of this type are rare in the oviductal mucosa, which may explain the lack of information about their presence in other caecilians. The function of these wedge-like cells in the oviducts is unknown; they are likely immature secretion cells (Young et al., 2010). The second type of cells, which are found in the epithelium of the mucous membrane, are ciliated cells, and these cells enable active transport of secreted mucus alongside the oviduct via the undulating movements of cilia (Young et al., 2010). The third type of epithelial cells, the unciliated ones, are single-celled glands with an exocrine type of secretion.

Epithelial secretions of the oviduct can perform several functions. One of these functions may be to protect the oviduct walls against mechanical damage during the transportation of oocytes and fetuses. Epithelial secretions may also protect the oocytes from damage. Another important function of epithelium secretions in viviparous species is nutrition of fetuses. After the yolk stocks are depleted, fetuses scrape oviductal epithelium by fetal teeth and feeding on them (Wake, 1970). Wake (1970) described the enlargement of glandular areas that contained developing fetuses within the oviducts of *Gymnopis multiplicata* Peters, 1874. Welsch et al. (1977) noted that the oviductal epithelium of *Ch. indistinctum* produces secretions rich in free amino acids and carbohydrates during the early stage of pregnancy. In the late stages of pregnancy, the material was rich in lipids (Welsch et al., 1977). Oviductal epithelial secretions can also create a barrier preventing sperm from traveling too far into the oviduct prior to ovulation by creating physiological demarcation line consisting of glandular secretions (Kuehnel, Kupfer, 2012). Mucus can create a specific biochemical environment around sperm and enable them to maintain their vitality for a long time; this time duration corresponds to the time that these cells are stored in females.

Oviductal wall stretching, described in Gymnophiona by Wake (1970), was also observed herein when oocytes of a diameter of 1 mm passed through. When large oocytes passed through the oviducts, the walls of the ducts became extremely thin and the mucosal folds were not visible. The muscle layer was much thinner than in other parts of the oviduct. After the passage of the oocyte, the oviductal wall returned to its previous shape and diameter.

The anatomy of the cloaca of *T. natans* exhibits numerous similarities with previously described viviparous species, but there were histochemical differences in mucus composition. The mucus of *T. natans* consists of carboxylated glycosaminoglycans (AB+), but lack neutral carbohydrates (PAS–). In the cloaca of *I. supachaii*, the mucus is reactive to AB and PAS, suggesting that this secretion is a mixture of neutral and acid mucopolysaccharides (Pewhom et al., 2015).

No sperm were noted in the oviducts of either of the examined *T. natans* females. This result is surprising since both females possessed ovulated eggs in their oviducts. Kuehnel and Kupfer (2012) reported that oviparous females of *I.* cf. *kohtaoensis* (Family: Ichthyophidae) store sperm between mucous folds of their oviducts. This finding implies that at least *I. kohtaoensis* does not possess specialized sperm storage organs, spermatheca, that are common in tailed amphibians (Urodela). At this time, we were unable to compare the location of sperm storage between oviparous and viviparous caecilians, but we nonetheless propose the hypothesis that females of viviparous taxa may store sperm in a similar way as oviparous species i.e., in spaces between mucous folds in the anterior part of the pars uterina. A lack of spermathecae and overall similarity in oviductal anatomy of viviparous *T. natans* and oviparous *I.* cf. *kohtaoensis* (Kuehnel, Kupfer, 2012), in which sperm storage was detected between the mucosal folds of the oviducts, allows us to

formulate such a hypothesis. A possible explanation for the lack of sperm in the reproductive ducts of female *T. natans* is that long-term sperm storage might not occur, most likely as a consequence of viviparity or a more simply the females have been sacrificed prior copulations. However it cannot be excluded that the lack of sperm was a consequence of captivity conditions, which for unknown reasons may prevent copulations.

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A review on research studies of marine and brackish water meiofauna from Odisha, India

Tapas Chatterjee,[1] Chandrabhotla Annapurna,[2]
Bhikari Charan Guru,[3] Baban Ingole[4]

[1] Crescent International School, Bario, Govindpur, Dhanbad 828109, Jharkhand, India, e-mail: drtchatterjee@yahoo.co.in
[2] Dept of Zoology, Andhra University, Visakhapatnam, India 530003, e-mail: cannapurna@rediffmail.com
[3] Deartment of Zoology, Utkal University, Vani Vihar, Bhubaneswar 751004, Orissa, India, e-mail: bcguru50@rediffmail.com
[4] Biological Oceanography Division, National Institute of Oceanography, Dona Paula, Goa-403004, India, e-mail: baban.ingole@gmail.com

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Abstract In any marine system, meiofauna plays a key role in the functioning of the food web and sustain important ecological processes. Benthic research has long been carried out both in spatial and temporal scale on the distribution, species diversity, community structure and abundance. Very few works have been reported on meiofauna along Odisha coast during last few decades. The present work is aimed to review how much work has actually made along Odisha Coast, East Coast of India during last few decades.

Aktualny stan wiedzy na temat mejofauny wybrzeża Odisha (Indie)

Słowa kluczowe mejofauna, słonawa woda morska, przegląd, Odisha, Indie

Streszczenie W każdym systemie morskim mejofauna odgrywa kluczową rolę w funkcjonowaniu sieci pokarmowej, odpowiadając za wiele ważnych procesów ekologicznych. Mimo prowadzenia od dawna szeroko zakrojonych badań bentosu w zakresie zmian rozmieszczenia, różnorodności gatunkowej, struktury społeczności i liczebności, niewiele wiadomo na temat mejofauny wschodniego wybrzeża Indii. W artykule przedstawiono stan wiedzy na temat mejofauny wybrzeża Odisha z ostatnich kilku dekad.

Introduction

Term meiofauna derived from the Greek word ‘meio’ means the smaller and was introduced and defined by Mare (1942) and suggested 2 mm for the topmost size limit for meiofauna but Solwedel (2000) commented that ‘today 1mm is commonly accepted as the upper size limit for meiobenthic investigations.’ Wigley and McIntyre (1964) used 74 µm as lower size limit for the meiobenthos, while Thiel (1966) used 65 µm, while Dinet (1973), Thiel (1971) set the lower limit to 50 µm and 42 µ respectively. Solwedel (2000) commented that today a lower size limit of 32 µm

seems to be commonly accepted. Generally, organisms larger than 500 µm are called macrofauna; between 500µm and 63 µm are called meiofauna and smaller than 63 µm are called microfauna.

Generally, benthic communities are much more diverse in terms of species richness than those of the surface and mid water layers (the so called pelagic realm). Approximately 98% of all marine species are supposed to belong to the benthos (Peres, 1982). "Permanent meiofauna" are species of meiofaunal size throughout their lives, while "temporary meiofauna" are of this size only when they are immature. Meiofauna are also known to be sensitive indicators of environmental disturbances and have great potential as pollution indicators, by giving attention to their species diversity, abundance and biomass. The reason which makes them potential indicators is that they live in very high numbers in small areas, have high diversity, have limited mobility which makes them consistently and intimately exposed to their immediate environment, and have short life cycles. Meiofauna also plays a key ecological role in linking detritus (and prokaryotic) resources with higher trophic levels: in fact most of the meiofaunal taxa eat microalgae, prokaryotes and detritus; and it is a well known fact that meiofauna are a good food source for macrofauna and fishes. Meiofauna and nematodes, based on laboratory and in-situ experiments, are in fact able to influence microbial activities and to graze their production (Pusceddu et al., 2014). Meiofauna are ubiquitous in marine soft-sediment communities, form an important link in transferring carbon primary and secondary production to higher trophic levels (Baguley et al., 2008). Although their biomass is generally low, their high abundance and high metabolic and reproductive rates render them potentially important in benthic fluxes of carbon and nutrients (Kuipers et al., 1981; Coull 1999; Moens et al., 2005).

In terms of total marine biomass meiobenthos constitute ≈ 80% and is of great importance in the marine ecology and marine mineralogy (McIntyre, 1969), making meiobenthic investigation a very active research field (Kitazato et al., 2003; Gwyther, 2004). Meiofauna has been regarded as an important component in benthic ecosystems due to their small size, high abundance and fast turnover rates (Heip et al., 1985; Coull, 1999). This component of the benthos exhibits high abundance, diversity and productivity in most sedimentary habitats and plays an important role in marine benthic food chains (Gee, 1989).

Eventhough much work was done on the distribution, species diversity, community structure, abundance of meiobenthos and effect of pollution on meiobenthos with respect to environmental parameters worldwide, also in and around Indian waters. But only a limited works have been reported on benthos especially on meiobenthos from Odisha coast. Recently Sharma (2016) attempted a review on macrobenthos studies of Odhisa coast. In the present study we are undertaking a detailed review of meiobenthic research conducted in brackishwater and coastal Odhisha, East Coast of India during the last few decades (1969–2015).

Brief description of Orissa coast

Odisha (formerly Orissa) is one of the 29 states of India, located in eastern India. It is surrounded by the states of West Bengal to the north-east, Jharkhand to the north, Chhattisgarh to the west and north-west, and Andhra Pradesh to the south. Odisha has 485 kilometers (301 miles) of coastline along the Bay of Bengal from Balasore to Ganjam.

The Orissa coast has bulged out in the middle portion from Brahmagiri at Chilika lake in the southwest (SW) to Chandrabali in the north north-east (NNE) where rivers the Mahanadi, Brahmani and Baitarani form a delta. In this portion, the coast is convex while from Chandbali to the Subarnarekha river mouth it is concave. If a straight line is drawn from Chandipur off

Balashore coast to Gopalpur, the recent delta formation in the Post-tertiary period in the middle portion becomes quite obvious by the protruding nature of the coast.

The bay-sandbars in the mouth of the Chilka lake, Devi river mouth and on the left bank of the Mahanadi mouth; and at other rivers Kushabhadra near Konark and Rushikulya are the best examples. In the Mahanadi mouth, the complex-spit with a number of hooks is formed due to the offshore long current and the strong longshore drift during the rainy season when the load discharge in the Mahanadi is the maximum. The high tidal prism keeps the mouths of the Devi, the Mahanadi, the Brahmani, the Baitarani and the Rushikulya open to form estuaries. In the north, along Balasore, the coast is crescent shaped embayment with inter tidal flat development and the mesotidal regime is quite conspicuous .

The salinity variation along the Orissa coast is quite large (18–35 PSU). The thermal regime of coastal plain of Orissa is mostly mega thermal type and the climate is either moist, sub humid or dry sub humid type. The rainfall in the coastal districts is mostly contributed by the monsoon depression during the southwest monsoon season (June–September), and cyclonic storms during post-monsoon (October-November) and pre – monsoon (March–May) period. The rainfall pattern along coastal stretch is maximum in the north and gradually decreases towards south.

Some remarks on coastal habitats and beaches and ecologically importance systems are as follows:

***Gopalpur*:** Gopalpur is located at Ganjam district in the southern part of Odisha, India, around 160 km south of Paradip and 260 km north of Visakhapatnam. It is a natural, deep sea port on the east coast of India. Gopalpur lies on a 4 km stretch of barren coast line, with no mangrove or tropical forests. The coast line falls in the rain-shadow of the region.

***Talsari*:** *Talsari* is located at the border of Orissa and West Bengal where River Subarnarekha meets the Bay of Bengal. There is a large mud flat, which remains submerged during high tides. The village is criss-crossed by the canals connected to the tributaries of the river Subarnarekha at its confluence with the Bay of Bengal. A natural Mangrove cover consisting of *Sonneratia apatala*, *Exocaria agalocha* and *Acanthus* sp., is coming up on the south west side.

***Bhitarkanika*:** The Bhitarkanika Mangroves are a mangrove wetland in Kendrapara district of Odisha cover an area of 650 km^2. It is the 2nd largest mangrove ecosystem of India which consists mangrove forests, rivers, creeks, estuaries, backwater, accreted land and mud flats. The Bhitarkanika Mangrove ecosystem flourishes in the deltaic region, formed by rich alluvial deposits of Barahmani and Baitarani river. It receives inputes of untreated domestic and industrial wastes (including organic matter, oil and heavey metals). The Bhitarkanika Mangroves are home to 55 of India's 58 known mangrove species.

***Balaramgari*:** Balaramgari is a flat exposed sandy strip along the north east coast of India at Chandipur of Balasore district, Odisha. It consists estuarine flow from Burhabalganga river, and sand bar formation at the mouth of estuary. The sediment is composed of medium to fine sand, silt and clay.

***Puri and Konark*:** Puri and Konark beaches are sandy beach, flat, exposed beach. Due to Tourist, beaches are mostly affected by anthropogenic activities. Hotels and restaurants dotting along the Puri beach also are responsible for polluting the area by letting their effluents into the sea.

***Chilka lagoon (Lake)*:** is a brackish water lagoon, spread over the Puri, Khurda and Ganjam districts of Odisha state on the east coast of India, at the mouth of the Daya River, flowing into the Bay of Bengal, covering an area of over 1,100 km^2. It is the largest coastal lagoon in India. Chilika Lake is a shallow bar-built estuary with large areas of mudflats. The western and southern margins of the lake are fringed by the Eastern Ghats hill range. Several inland rivers, which bring silt into

the lake, control the northern end of the lake. A 60 km long barrier beach called Rejhansa, formed by northerly currents in the Bay of Bengal, resulted in the formation of this shallow lake and forms its eastern side. As an ephemeral lake, it's water surface area varies from 1,165 km^2 (449.8 sq mi) in the summer monsoon season to 906 km^2 (349.8 sq mi) in the winter dry season.

Results

We presented here some brief idea about the major research publications which dealt with the meiofauna of Orissa coast.

In one of the early publication Rao (1969) reported more than 100 interstitial species of diverse invertebrate groups collected in December 1966, during a preliminary faunistic survey of Odisha (Orissa) coast at Puri and Konarak. He commented that intertidal and vertical distribution of fauna showed that majority of species inhabits medium sands with moderate water saturation below surface near mid- tide level. In general, most of the foraminifera member occurred near the low water level; Archiannelida, Polychaeta, Ostracoda occurred between low- and mid-water levels at sediment depths of 10–30 cm below surface. The distribution of some gastrotrichs, kinoehynchs showed preference to deeper layer of sands 30–50 cm below surface towards the mid-tide level, associated with low oxygen content. Species of nematods, oligichaets, copepods, isopods, Acarina occurred sporadically at all levels and depths of the intertidal sand, indicating their adaptation to diverse environmental factors.

Nagabhusanam and Rao (1969) made preliminary observations on- a collection of shore fauna of Orissa coast. They reported more than 160 species of meiofauna. Nagabhusanam (1972) reported interstitial meiofauna (mentioned in the paper as microfauna) along the Puri and Konarak coast. He has reported different species of meiofauna belong to Foraminifera, Coelenterata, Turbellaria, Nematoda, Gastrotricha, Kinorhyncha, Nemartina, Rotifera, Annelida, Ostracoda, Tardigrada and Mollusca. He further reported that fine grades of sand yielded very poor interstitial fauna, possibly due to paucity of interstitial space and blocking of the interstices due to organic matters.

Pattanaik (1971) reported the seasonal abundance and bottom fauna of Chilka lagoon. He studied month-wise abundance during April, 1963 to March, 1964. Highest population density was during October to December and lowest during July to September (monsoon). The meiobenthos (author reported as microbenthos) was dominated by Foraminifera and nematodes.

Sarma and Satapathy (1978) reported phytal fauna in and around Balugaon in Chilka lake. They studied five algal species viz, *Cladophora glomerata*, *Enteromorpha compressa*, *Chaetomorpha linum*, *Polysophonia serialarioides* and *Potamogeton pectinatus*. They did not separate macro- and meiofauna was and thus provided group-wise density for all epifauna. They suggested that the algae and sediment accumulated on the thalli and of the environment affected the qualitative and quantitative association and distribution of the phytal fauna. *Potamogeton* followed by *Cladophora*, *Enteromorpha*, *Chaetomorpha*, *Polysiphonia* supported faunal abundance.

Subsequently, Pattnaik and Mohanti (1980) reported preliminary observations on foraminifera from the Mahanadi estuary and Ganti and Mohanti (1989) reported the recent foraminifera from the Hukitola Bay. In their subsequent study Ganti and Mohanti (1992) have reported 25 species of foraminifera from the inner shelf of Orissa.

While reporting four new species of Gastrotricha from Odisha coast Rao (1981a) reported a new species of macrodasyid Gastrotricha *Crasiell indica* from the intertidal zone of Gopalpur beach; three species of Gastrotricha viz. *Tubanella indica* from intertidal sand of Gopalpur beach

Tetranchyrodorma littoralis and *Cephalodasya caudatus* from intertidal zone of Puri beach (Rao, 1981b) were reported. Sarma et al. (1981) documented phytal macro- and meiofauna of *Gracilaria lichenoides* from Kalijai off Chilka lagoon, *Potamogeton pectinatus* and *Halophyla ovata* Parikud, Chilka lake (lagoons) In *Potamogeton pectinatus* meiofauna comprised 17 animal groups and dominated by nematodes. In *Halophyla ovalis* 16 animal taxa were observed and copepods were dominant group. They observed that the fauna of more silted *Potamogeton* was dominated by nematodes followed by tanaidaceans and copepods; relatively less sedimented thalli of *Halophila* inhabited by crustaceans (copepods, crustacean naupli and ostracods) and nematodes; least silted thalli of *Gracelaria* occupied by copepods and ostracods. Rao (1989) investigated meiofauna of eight localities on the coast near Chandipur, Bhadrakh, Paradwip, Konarak, Puri, Rambha Bay (Chilka Lake), Bahuda Estuary and Gopalpur. The material reported here comprises in all 130 species of the diverse groups of meiofauna: Hydrozoa (2 species), Turbellaria (4 species), Nematoda (27 species), Gastrotricha (22 species), Kinorhyncha (2 species), Archiannelida (12 species), Polychaeta (18 species), Ostracoda (1 species), Copepoda (35 species), Of the total 130 species dealt in the community 16 (12.4%) are cosmopolites, 45 (34.6%) eurytopics occurring on warm temperate and tropical beaches, 64 (49.2%) Indian Ocean forms and 5 (3.8%) endemics.

Pattanaik and Rao (1990) studied the composition and distribution of interstitial meiofauna of the sandy beach at Gopalpur, south Odhisa coast. In this paper a total of 15 meiofaunal group were recorded. Harpacticoid copepods are the most dominant group, forming nearly 70% of the total interstitial population followed by nematodes (14.68%). Other common groups are Turbellaria, Archeannelida, Polychaeta and Isopoda. They reported meiofauna population densities at low, mid and high tide level.

A new species of halacarid mite *Copidognathus sambhui* was described from Chilka lagoon (Chatterjee, 1991). Chatterjee and Sarma (1993) also reported new record of another halacarid mite *Copidognathus sideus* from Chilka lagoon. Rao and Sarma (1994) presented the seasonal abundance and breeding cycles of 15 species of meiobenthic harpacticoid copepods inclusive of ovigerous females inhabiting the littoral sediments of Parikud islands in Chilka lagoon.

Sarma and Wilsanand (1994) reported the littoral meiofauna of Bitrakanika mangroves sediments of Mahanadi river system, in the Orissa state, east coast of India. It included 11 major faunal taxa, of which nematodes were the dominant group. Chatterji et al. (1995a) reported seasonality in meiofaunal distribution from Balramgarhi coast. The meiofauna was comprised of eight faunal groups viz. Nematoda, Harpacticoida, Tubellaria, Ostracoda, Lamellibranchiata, Amphipoda, Isopoda and Cladocera encountered in different seasons. Maximum density of meiofauna was recorded during the southwest monsoon season. The major part of the faunal group consisted of Cladocera (47.70%), followed by nematodes (15. 71%) and harpacticoids (14.12%). They have reported Cladocera *Diaphanosoma excism* in Balaramguri beach in relatively higher densities particularly during the southwest monsoon season. This is a typical freshwater form recorded in several states in India (Chatterjee et al., 2013). Chatterji et al. (1995a,b) also reported that densities of Cladocera was maximum at low tide level and minimum at high tide level during fair and southwest monsoon season. They further commented that occurrence of these cladocerans in estuarine beach could be due to land runoff because the discharge of the river Burhabalanga also reported to be high during southwest monsoon season. Rao and Satapathy (1996) worked on demecology of Kinorhyncha of Chilka lagoon (Bay of Bengal). They reported for the first time *Echinoderes* sp and *Pycnophyes* sp in the Chilka lagoon. Both species were found in sediments as well as phytal (*Halophyla ovata*) biotopes. *Pycnophyes* sp constituted 80–90% of the total kinorhynch population. In this paper tri-monthly values of environmental parameters, sediment as

well as numbers and biomass of phytal Kinorhyncha was provided. It was reported that the density distribution of Kinorhyncha in different sampling sites through seasons reveals the maximum abundance of the organisms corroborates with the salinity distribution than with any other co-chemical parameters. It was observed that the abundance or distribution of kinorhynchs related with salinity, sediment nature and vegetation cover of the bottom. Fine grained sediments with a detrital surface layer were more favourable for the development of phytal fauna.

While working on the meiofauna of outer channel of Chilka lagoon Sarma and Wilsanand (1996) reported the presence of 12 major taxa, of which nematodes and copepods were the dominant. The average total meiofaunal densities were significantly correlated with the sediment temperature, pH and salinity. No significant correlation was found between total meiofaunal densities, mean grain size and the organic matter. Kameswar Rao et al. (2000) reported foraminifera from the Chilka lagoon. A total of 69 foraminiferal species belonging to 27 genera and 19 families have been identified from the sediment samples. *Miliammina fusca*, *Ammobaculites exiguus*, *Ammonia beccarii* and *A. tepida* were the most abundant species.

Jayalakshmy and Kameswar Rao (2001) reported 69 foraminiferal species from the sediment samples collected from the Chilka Lake. These species have been quantitatively studied in regard to their relative abundance and distribution. Frequency distribution study of Foraminifera shows that *Miliammina fusca, Ammobaculites exiguus, Trochammina hadai, Jadammina macrescens, Gaudryina exilis, Ammonia beccarii* (Linne), *A. tepida* and *Asterorotalia dentata* are the dominant species of the fauna. The sites dominated by M. *fusca* and *A. exiguus* are mostly inside the lagoon, while those dominated by *A. beccarii* (Linne) besides T. *hadai,* G. *exilis, Hanzawaia asterizans, H. nitidula,* A. *tepida,* A. *dentata, Elphidium crispum* and *E.galvestonense* are in the outer channel of the lake.

Jayalakshmy and Kameswar Rao (2003) reported Multivariate statistical study with a factor analysis of Foraminiferal fauna from the surface sediments of the Chilka Lake along the east coast of India has been studied as regards distribution of its assemblages. Among the species recorded, 16 species during post-monsoon season (November) and 34 species during pre-monsoon season (May) form the differential factor groups providing maximum information for the distribution of the fauna. It is obvious from the factor score distributions of stations against latitude (N) and species with respect to average absolute abundance that the species observed are from a continuously varying population least affected by insidious environmental changes in the study area.

Jayalakshmy and Kameswar Rao (2006) reported some aspects of foraminifera from Chilka lagoon. They concluded that ≈30% of the species always clustered together during early pre- and late-monsoon, indicating the possibility of species succession during November. Moreover the species replacementtakes place over a seasonal cycle. This finding could be an indication of the seasonality of foraminiferal community through species succession.

Paikaray et al. (2012) has undertaken a study to understand the species composition, population density and relative abundance of meiobenthos occurring in the intertidal beach sediment of South Orissa Coast, especially in mouth area of the Rushikulya estuary and on the beach sand of Gopalpur. During the study, salinity values in the Rushikulya estuary ranged from 21.8 to 26.8 PSU, whereas at Gopalpur it ranged from 27.3 to 30.8 PSU. From the granulometry analysis it was found that sediment particles remained predominantly sandy. Nematodes formed the dominant group in all the stations followed by crustaceans and polychetes. Among the crustaceans, Harpacticoid copepods were more abundant than the others. The population size of meiobenthos during the study period exihibited moderate variation between different stations as well as

different months. Annapurna et al. (2012) reported free living nematode *Sphaerolaimus balticus* Schneider from Paradweep.

Jaikumar and Annapurna (2012) made a comprehensive study of the species composition, abundance and biomass of the meiobenthic fauna in general, and the free-living marine nematode community structure of the Chilka lagoon in particular. The samples were obtained from 36 stations spread over four pre-determined sectors inside the Chilka lake namely, southern sector, central sector, northern sector and outer channel along the east coast of India. Observations on meiobenthos presented in the thesis are based on 154 samples collected inside the Chilka Lake during monsoon.1 (Oct'04), pre monsoon (Jan'05), post monsoon (May'05) and monsoon.2 (Oct'05). During this investigation a total of six diverse meiofaunal taxa represented by nematodes (58.76%), foraminiferans (11.79%), copepods (9.22%), amphipods (7.56%), kinorhynchs (6.69%) ostracods (4.63%) and others (1.35%) were encountered. Nematodes represented 58.76% of the total meiofauna with greater diversity when compared to the remaining groups. In the present study, in nematodes, altogether there were 84 species represented by 61 genera in 25 families were reported from different seasons. The dominant nematode species encountered during this study include *Metalinhomoeus longiseta*, *Metalinhomoeus filiformis, Metalinhomoeus* sp*., Sabatieria punctata*, *Sabatieria* sp., *Terschellingia gourbaultae*, *Sphaerolaimus balticus*, *Sphaerolaimus papillatus, Daptonema procerum*, *Microlaimus* sp*., Phanoderma* sp*., Paralinhomoeus* sp., and *Viscosia* sp. The foraminiferans represented by *Miliammina* sp., *Ammobaculites sp*., *Trochammina sp., Ammonia* sp. The harpecticoid copepods represented by *Stenhelia* sp., *Ectinosoma* sp., *Harpacticus* sp., *Enhydrosoma* sp., kinorhynchs represented by 2 genera namely *Echinoderes bengalensis, Pycnophyes* sp. The ostracods were represented by *Phlyctenophora* sp., *Tanella* sp., and *Cypridopsis* sp .Since nematodes constituted one of most important faunal group in view of their numerical abundance and species richness, they were examined and studied in detail in the study area and correlated with the environmental parameters. One of the objectives of this study was to locate the presence of specific nematode species assemblages for the Chilka lagoon, east coast of India. Three communities could be distinguished in the lagoon, named after the most important (determining) species: *Sabatieria* sp. (Group 1), *Metalinhomoeus longiseta* (Group 2) and *Metalinhomoeus* sp (Group 3) representing southern sector and outer channel; central sector and northern sector categories respectively. This is perhaps surprising since lagoon assemblages must be viable over a greater range of environmental conditions than those found subtidally in brackish water habitats, and suggests that despite their isolated nature, dispersal opportunities remain sufficient to maintain the input of estuarine/marine species. Bhattacherjee et al. (2013) reported benthic foraminiferal assemblages along coastal Orissa. Thirty-nine species (belonging to 6 order and 23 families) of Foraminifera were collected from three coastal areas viz. Rushikulya, Devi and Gahirmata.

Datta et al. (2014) made a new record of a nematode species Oncholaimellus brevicauda from sediments of Talsari. Baliarsingh et al. (2015) reported a first record of *Desmoscolex falcatus* (nematode: Adenphorea: Desmoscolecida: Desmoscolecidae) from Rushikulya estuary, Odisha, India.

Recently, Ansari et al. (2015) reported the nematode assemblage from the Chilka lagoon and provided a checklist of 64 free-living marine nematode species belonging to 32 genera and 13 families. Among these, *Oncholaimus oxyuris* has been reported as new distributional record from the Indian waters. The distribution of encountered nematode species was investigated in relation with environmental variables such as salinity and sediment texture throughout the lagoon. Accordingly the nature of sediments varied from sandy to silt/clay. Most of the nematode species were found in high salinity zone.

Discussion

It is interesting to note that meiofaunal of the Orissa coast although have been studies since 1970's very few studies deals with the overall biodiversity of meiofauna. In the early investigations G.C Rao and co-warker's have contributed significant to the meiofaunal ecology, taxonomy and biogeography. Sediment samples collected in late 1966 ZSI more than 480 interstitial species of diverse invertebrate fauna (1969). The early investigations of ZSI indicated the presence of very rich benthic meiofauna (microfauna). The intertidal and vertical distribution of fauna showed that majority of species inhabits in medium sands with moderate water saturation below the surface near mid- tide level. The distribution of some gastrotrichs, kinorhynchs showed preference in sediment deeper layer down to 30–50 cm below surface. Species of nematods, oligichaets, copepods, isopods, acarids occurred sporadically along the beach gradient indicating their adaptation to diverse environmental factors.

Nagabhusanam and Rao (1969) made preliminary observations on- a collection of shore fauna of Orissa coast. They reported more than 160 species of meiofauna. In early 1980's Sarma and Satpathy (1978) studies the phytal fauna of the Chilika lagoon and Sarma and Rao (1980) investigated the meiofauna of the Chilika lagoon. The fauna was comprised of 17 major taxa and was dominated by Foraminifera (>71%). This was followed by nematodes (19%) and copepods. Chattarji et al. (1990) studies the meiofaunal of northern Orissa. While our knowledge has been certainly improved with the recent findings of Ingole, Ansari et al. and Annapurna et al. This is particularly true for the meiofauna of the southern Orissa, especially on the nematode species distribution from the Chillika lagoon. However, we do not know much about the seasonality and species distribution with respect to rest of the Orissa Coast.

Based on this review we recommend that following studies may be initiated at the early stage which will help in understanding the seasonal species abundance, distribution and overall role of meiofauna benthic food web: 1) Meiofaunal community structure in different coastal marine habitats. 2) Meiofaunal community structure in different coastal marine habitats. 3) Response of meiofaunal to physical disturbance (dredging, sewage dumping, oil spill, harbour development, dumping of various pollutants. 4) Microfauna-Meiofauna-macrofaunal interactions. 5) Role of meiofauna in feeding preference of demersal feeding fish species. 6) Meifauna of the ecologically sensitive/specialized habitats such as – Turtle nesting grounds; Breeding beaches of Horseshoe Crab; Seagrass beds; Subtidal corals habitats; Phytal rocky habitats (though in very less area rocky algae found); Mangrove mudflats.

Conclusions

Scattered attempts have been made to understand the quantitative nature and community structure of benthos from different regions of the country. A number of benthic studies in Indian seas were published; most of them pertaining to studies on major estuaries or backwaters and shallow coastal regions. In most studies, much attention was given to macrobenthos part and very often the role played by meiobenthos is neglected. This may have been partly due to their smaller size and lack of taxonomic expertise. This lacuna in the information regarding meiobenthos led to embark upon the present study. The review of meiofaunal study with reference to Odisha coast suggested that even though in the different marine environments such as beaches, tidal flats, nearshores, continental shelf, deep sea, salt marshes, mangroves and other related environments, distribution and diversity have been documented; there is certainly a need for more research

efforts. Most of the available published studies have dealt with group taxon/level and were not undergone to species level identification. In India, meiofaunal study mainly concentrated on Nematoda and Harpacticoida (Copepoda), foraminifera and to certain extent to Polychaeta. Further, the biomass study, interrelationship with tropic level, pollution monitoring systems, culture aspects with reference to aquaculture and mariculture related activities, drugs from the sea, etc. in relation to meiofauna are also other research areas where the marine biologist should concentrate and work for the developmental activities for the humankind.

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Initial research on necrophagous true flies (Diptera) in Gryfino Commune

Tomasz Czernicki,[1] Grzegorz Michoński,[2] Agnieszka Szlauer-Łukaszewska,[3] Aleksandra Bańkowska,[4] Andrzej Zawal[5]

[1] Department of Invertebrate Zoology and Limnology, Institute for Research on Biodiversity, Faculty of Biology, University of Szczecin, Wąska 13, 71-415 Szczecin, Poland; e-mail: tczernicki@gmail.com
[2] Department of Invertebrate Zoology and Limnology, Institute for Research on Biodiversity, Faculty of Biology, University of Szczecin, Wąska 13, 71-415 Szczecin, Poland; e-mail: grzegorz.michonski@usz.edu.pl
[3] Department of Invertebrate Zoology and Limnology, Institute for Research on Biodiversity, Faculty of Biology, University of Szczecin, Wąska 13, 71-415 Szczecin, Poland; e-mail: agnieszka.szlauer-lukaszewka@usz.edu.pl
[4] Department of Invertebrate Zoology and Limnology, Institute for Research on Biodiversity, Faculty of Biology, University of Szczecin, Wąska 13, 71-415 Szczecin, Poland; e-mail: aleksandra.bankowska@usz.edu.pl
[5] Department of Invertebrate Zoology and Limnology, Institute for Research on Biodiversity, Faculty of Biology, University of Szczecin, Wąska 13, 71-415 Szczecin, Poland; e-mail: andrzej.zawal@usz.edu.pl

Keywords forensic entomology, north-western Poland, field experiment, Calliphoridae, forensics

Abstract As a result of research carried out in June and July 2017, at five sites in north-western Poland with different characteristics, 769 individuals belonging to three dipteran families of forensic significance were collected. The most abundant family was Calliphoridae, which was represented by 8 species (*Calliphora vicina*, *Calliphora loewi*, *Lucilia bufonivora*, *Lucilia caesar*, *Lucilia illustris*, *Lucilia sericata*, *Lucilia silvarum*, and *Lucilia richardsi*). The experiment examines differences in the dominance of individual species depending on the habitat type and the time of year, and also shows correlations between environmental parameters and individual taxa.

Wstępne badania nad fauna muchówek (Diptera) nekrofagicznych w gminie Gryfino

Słowa kluczowe entomologia sądowa, północno-zachodnia Polska, eksperyment terenowy, Calliphornidae, kryminalistyka

Streszczenie W wyniku badań przeprowadzonych w czerwcu i lipcu 2017 roku, na pięciu stanowiskach o zróżnicowanej charakterystyce w północno-zachodniej Polsce zebrano 769 osobników należących do 3 rodzin muchówek mających znaczenie w kryminalistyce. Najliczniej występującą rodziną była Calliphornidae, którą reprezentowało 8 gatunków (*Calliphora vicina, Calliphora loewi, Lucilia bufonivora, Lucilia caesar, Lucilia illustris, Lucilia sericata, Lucilia silvarum, Lucilia richardsi*). Doświadczenie sprawdza różnice w dominacji poszczególnych gatunków w zróżnicowaniu względem typu siedliska oraz w odstępstwie czasowym, a także pokazuje korelację między parametrami środowiskowymi, a poszczególnymi taksonami.

Introduction

The main goal of forensic entomology is to determine the time elapsed since death, i.e. the post mortem interval (PMI), by means of quantitative and qualitative analysis of necrophagous insects found on the body, especially when the use of other medico-legal methods is difficult (Włodarczyk, 2007). This science is also helpful in reconstructing the circumstances and place of death (when the body has been moved) and can provide information about the victim, such as health condition or medical negligence. The bodies of preimaginal stages of necrophages accumulate heavy metals, chemicals and toxic substances. They can also collect the spores of pathogenic microbes, thus indicating the cause of death.

Determination of the date of death is based on two main elements. First, insects colonize the body in a predictable and known order, and they appear on it in separate groups known as waves of succession. Secondly, the rate of development of the preimaginal stages of each insect species is also known, and is closely dependent on environmental conditions. In forensic entomology, all factors that may affect the decomposition rate of the body are taken into account, such as air temperature, humidity, insolation, light, and rain (Kaczorowska, 2014).

True flies (Diptera) are the first to arrive at the corpse, and among these, blow flies (Calliphoridae) play the most important role. Their larvae eat decaying organic matter and indicate the time elapsed from death to the discovery of the body (Merritt et al., 2000).

The classic method of species identification is assessment of the morphological features and anatomy of a preserved specimen using special identification keys. Preparation of the cephalopharyngeal apparatus can be used for this purpose. Unfortunately, this is a tedious and difficult task, even for an experienced entomologist. An additional difficulty is the lack of detailed morphological data for some insect families or the need to revise available data, especially in relation to eggs and preimaginal stages. Due to the high degree of similarity of many species, it is easy to make a mistake during identification, resulting in an erroneous estimate of the date of death (Skowronek, 2009).

An alternative method of identifying insects of forensic significance is currently being developed, using methods based on molecular biology and genetics. The material for analysis can be DNA located in the nucleus (nDNA) or in the mitochondria (mtDNA). Pioneer research is being carried out at the Museum and Institute of Zoology, Polish Academy of Sciences in Warsaw to implement the HRM-PCR method (high-resolution DNA melting analysis-polymerase chain reaction), based on analysis of high-resolution DNA denaturation curves in conjunction with PCR and preparation of reference DNA samples of blow flies (Calliphoridae) occurring in Poland (Skowronek, Chowaniec, 2010). Research is also being conducted on the barcoding method, which exploits the COI (encoding 1 subunit of cytochrome oxidase) specific for each species. This method enables rapid and efficient species identification (Bogdanowicz, Rogalla, 2009).

The numbers of taxa and specimens on the body, the rate of colonization, and the development time of species found on cadavers depends on multiple factors, both biotic and abiotic (Piotrowski, 1990). The most important factor of these include climatic conditions, i.e. air temperature and humidity. Other important factors for establishing the date of death include the geographic location and type of habitat where the body is located, its position relative to the sun, the cause of death, and where and how the body was concealed. Knowledge of the developmental biology and behaviour of animals found on cadavers is also very important (Kaczorowska, 2014).

In Poland, experimental field research is mainly conducted by teams of scientists from Poznań and Toruń, who have published many specialist articles and journals devoted to this field.

Research on the use of molecular biology and genetics to identify insects of forensic significance is carried out continually (Skowronek, 2012). This is a cause for optimism, although for a number of reasons it will be some time before specialized forensic entomological laboratories or 'body farms' appear in Poland (Mikołajczyk, 2009).

The aim of this study was to determine the composition of dipteran fauna feeding on pork liver, which mimicked a cadaver for the purposes of the experiment, taking into account different types of habitats and time intervals before the body was revealed.

Study area

The study area comprised fields and forests in the settlement of Szczawno, located in the Gryfino Commune in the West Pomeranian Voivodeship. The geographic location of the commune influences the local climatic conditions. The basic meteorological parameters of the area, such as the average level of precipitation, dominant wind directions and strength, and average temperatures, are representative of most of Western Pomerania. The growing season lasts on average about 210 days, the average yearly temperature ranges from 6.5 to 7°C, and precipitation varies between 600 and 650 mm. An important feature of the local climate is variable and irregular weather, associated with both the easy movement of large masses of air and the complex relief of the terrain (Korzeń et al., 2007).

Five stations were established in the field, differing in surrounding vegetation, insolation, and distance from households and water bodies. The sites are shown on the map and designated with symbols (Figure 1).

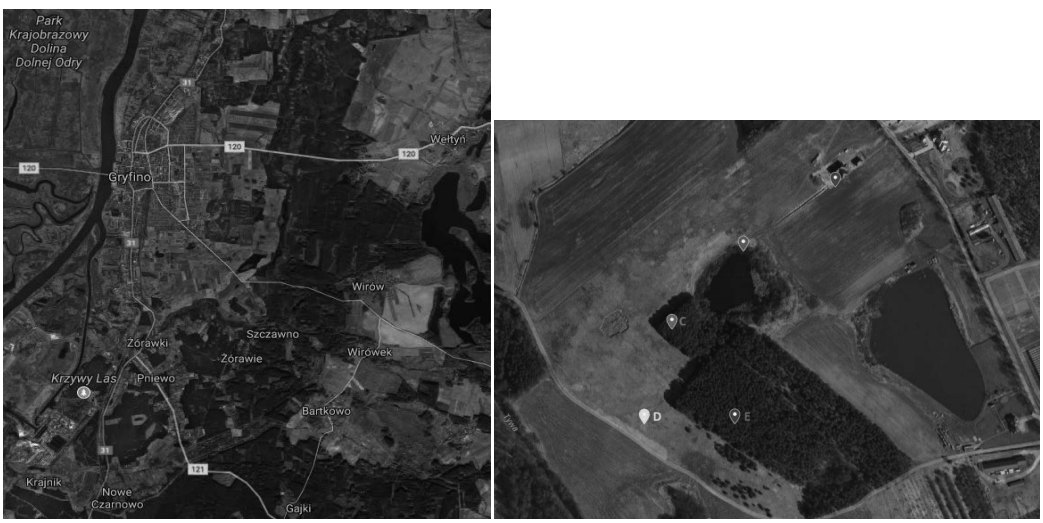

Figure 1. Study area with sites indicated

The first station (A) was located directly next to a household, between a garden and a wheat field. The perceived temperature was 22–25.9°C on June 6 and 29.6–29.9°C on July 19. The sun

exposure at this site was estimated to be high. During the day, swallow activity was observed over the field, which may have influenced further results. The distance from water was about 200 m.

The second site (B) was located directly by a fishing pond, about 200 m away from the nearest household. There was lush vegetation around the pond, composed mainly of grasses and herbaceous plants. The site has strong sun exposure and the perceived temperature was 25.7–28.2°C on June 6 and 27.3–29.3°C on July 19.

The third station (C) was set up at the edge of a coniferous forest. The tree density here was small, and the forest was directly adjacent to a field. Insolation was estimated as medium, and the perceived temperature was 25.3–25.7°C on June 6 and 22.2–25.5°C on July 19. The station was about 300 m from the nearest household and nearly 100 m from a nearby pond. During the July trial, all of the material had been plundered by scavengers. The remaining fragments of the bloodied, plastic bag in which the livers were kept served as a lure enabling insect collection.

The fourth station (D) was set up on fallow land, in the shade of a single small tree. Sunlight exposure and perceptible air temperature during the day were the highest of all sites: 28.1–31.4°C on June 6 and 26.5–30°C on July 19. Considerable ant activity was also observed during the day, in both the June and July trials. The vegetation was dominated by grass with an admixture of herbaceous plants, and there was a wheat field nearby. The site was located about 420 m from the nearest household and about 200 m from a body of water.

The fifth station (E) was located in a coniferous forest with broadleaf undergrowth. This site had the least sunshine, being protected from the sun by dense tree crowns and shrubs. The litter here was also densely overgrown with grasses. The temperature was 26.7–26.9°C on June 6 and 24.3–25.1°C on July 19. The site was about 380 m from the nearest household and about 200 m from the nearest water body.

Material and methods

The study was based on material collected on June 6 and July 19, 2017. In order to effectively carry out the experiment, the stations were secured against scavengers and the equipment needed to carry out the field experiment was assembled (nets and collecting bottles).

The material for the study comprised 800 g portions of pork liver that were frozen until needed for the experiment. Two days before the field experiment, the meat was thawed and exposed to outdoor weather conditions to enhance the properties making it attractive to necrophages. The material was then placed at each of the previously selected sites. Necrophages foraging on the livers were caught repeatedly throughout the day using a net, and then placed in previously prepared collecting bottles containing ethyl acetate. At each site the daytime temperature was measured and a GPS device was used to obtain the geographic coordinates. The sites were then marked on a map of the area and the distance from water bodies and houses was determined. The level of insolation at each site was estimated as well, using a three-point scale.

The collected material was transported to the laboratory for analysis. Flies were identified to family and species using a stereoscopic microscope and keys by Draber-Mońko (2014). The results were analysed by creating a database in Microsoft Excel 2010 and calculating the dominance index. Then the statistical significance of differences in the abundance of fauna collected in June and July was calculated using the Mann-Whitney U test. The Kruskal-Wallis test was used to verify the statistical significance of differences in the dominance of individual species. Spearman correlation and correspondence analysis were used to analyse the effect of environmental parameters on the abundance of necrophagous fauna.

Results

During the analysis of the collected material, we identified three families of necrophagous flies of forensic significance: Calliphoridae, Sarcophagidae and Muscidae. Seven species from the family Calliphoridae were found in the June trial (*Calliphora vicina* Robineau-Desvoidy, *Lucilia sericata* Meigen, *L. bufonivora* Moniez, *L. silvarum* Meigen, *L. illustris* Meigen, *L. caesar* Linnaeus, and *L. richardsi* Collin) and seven species in the July trial (*Calliphora vicina*, *C. loewi* Enderlein, *Lucilia sericata*, *L. silvarum*, *L. illustris*, *L. caesar*, and *L. richardsi*). The results are presented in Table 1 below.

Table 1. Total material collected in the study

Species	6 June 2017						19 July 2017						
	A	B	C	D	E	subtotal	A	B	C	D	E	subtotal	total
Calliphora loewi Enderlein, 1903									1			1	1
Calliphora vicina Robineau--Desvoidy, 1830	2		2			4			2			2	6
Lucilia bufonivora Moniez, 1876	1	1				2							2
Lucilia caesar Linnaeus, 1758	26	37	54	10	45	172	93	127	38	22	102	382	554
Lucilia illustris Meigen, 1826	1	1	7		4	13	10	13			2	25	38
Lucilia richardsi Collin, 1926				1		1				2		2	3
Lucilia sericata Meigen, 1826	4	1		3		8	14	9	2	15		40	48
Lucilia silvarum Meigen, 1826	12	11				23	11	16		2		29	52
Muscidae	1	2				3					1	1	4
Sarcophagidae	6	10	1	4	3	24	7	5	15	3	7	37	61
Total	53	63	64	18	52	250	135	170	58	44	112	519	769

The table presents the numbers of taxa identified in the June and July trials. The species *Lucilia bufonivora* was found in the June material, with one specimen each at sites A and B. This species did not appear at any site in the July trial. The material collected in July contained one individual of the species *Calliphora loewi* at station C, which did not appear anywhere else. The later material was also more abundant in terms of quantity. We caught and identified 250 dipterans in June and 519 in July, from the families Calliphoridae, Sarcophagidae and Muscidae, for a total of 769 insects. The statistical significance of differences in the abundance of fauna collected in June and July was calculated using the Mann-Whitney U test, with a result of $Z = -1.25336$; $p = 0.2$. Thus the test showed that the differences in numbers between the two samples were not statistically significant.

The dominance structure is presented in the form of pie charts, separately for each station and each trial (June and July) (Figures 2–6).

The highest dominance index at station A for both trials was obtained for *Lucilia caesar*, with values of 49.1% in the June and 68.9% in the July. The values for other species were lower, e.g. *L. silvarum* with 22.64% in the first trial and 8.2% in the second trial, and *L. sericata* with 7.6% in the June trial and 10.4% in the July trial. Flies of the Sarcophagidae family attained dominance indices of 11.3% and 5.2%, respectively. The last species in both samples was *L. illustris*, which accounted for 1.9% and 7.4% of the material at the first site respectively. In addition, the material

from the first trial included the species *Calliphora vicina* (3.8%) and *L. bufonivora* (1.9%), as well as flies of the family Muscidae (1.9%).

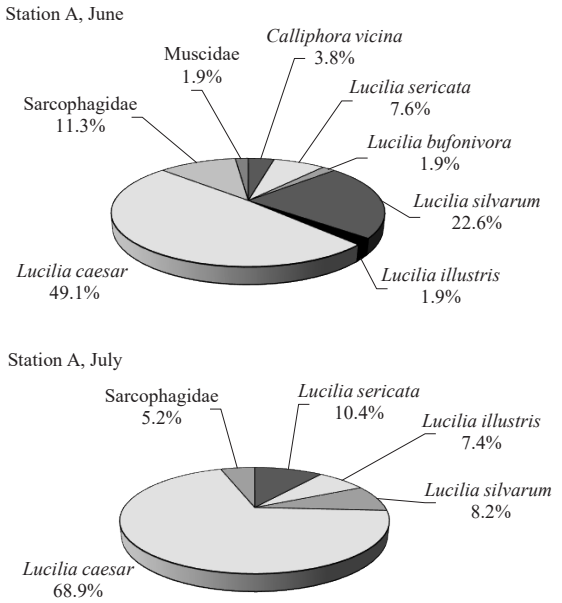

Figure 2. Dominance indices at station A in June and July

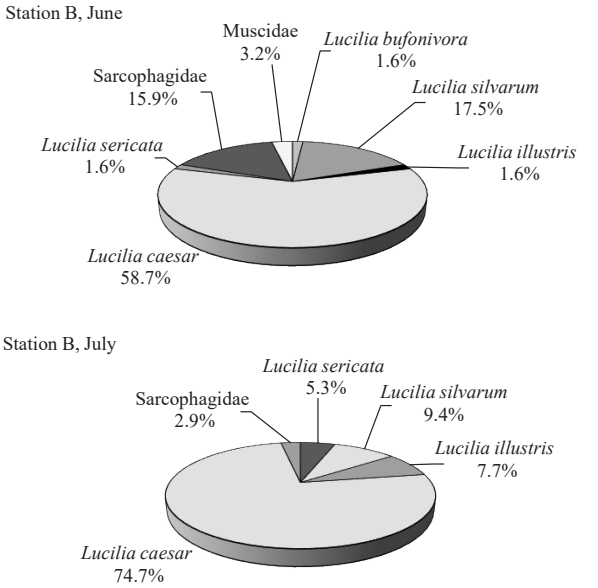

Figure 3. Dominance indices at station B in June and July

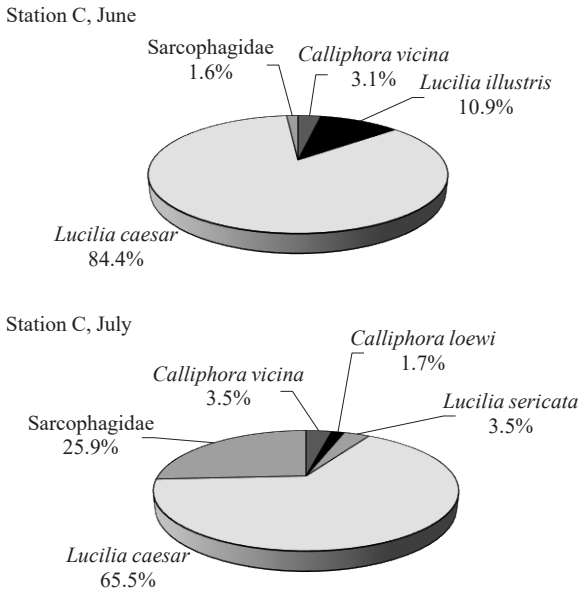

Figure 4. Dominance indices at station C in June and July

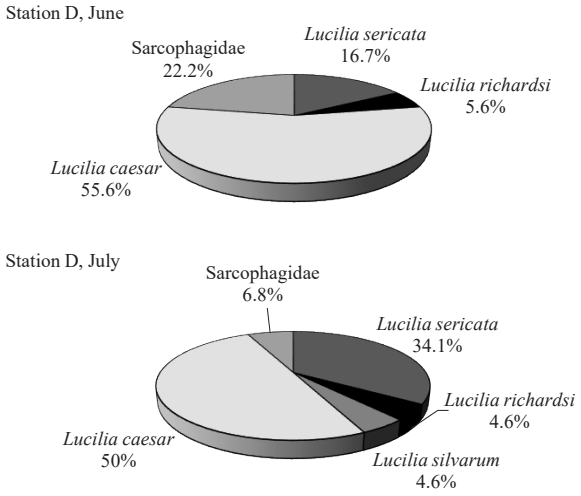

Figure 5. Dominance indices at station D in June and July

Station B was also the most attractive site for flies of the species *Lucilia caesar*, which accounted for as much as 58.7% of the June material and 74.7% of the July material among all specimens. The second highest value here was noted for *L. silvarum*, accounting for 17.5% and 9.4% of flies caught respectively. *L. illustris* (1.6% and 7.7%) and *L. sericata* (1.6% and 5.3%) were

more active in July. Activity of dipterans from the family Sarcophagidae was also noted at station B, with a dominance index of 15.9% for the June trial and 2.9% for the July trial. In addition, the first sample contained flies from the family Muscidae (3.2%) and the species *L. bufonivora* (1.6%).

Station C was also dominated by the species *Lucilia caesar*, which accounted for 84.4% of the material from the first trial and 65.5% of the material from the second trial. *Calliphora vicina* attained a similar dominance index in the two samples, with values of 3.1% for June and 3.5% for July. Flies from the family Sarcophagidae accounted for 1.6% of the material from the first trial but as much as 25.9% in the second. In addition, the first sample included the species *L. illustris* (10.9%), which was not detected in the second trial. On the other hand, the second sample had the species *L. sericata* (3.5%) and *C. loewi* (1.7%), which were not found in the first trial.

Station D had relatively similar fauna in the two trials. The largest share at this site fell to *Lucilia caesar*, reaching values of 55.6% and 50%. Ranking second were flies of the species *L. sericata*, with a dominance index of 16.7% in the June trial and as much as 34.1% in July. Flies of the family Sarcophagidae accounted for 22.2% of the flies in the June material for this site and 6.8% in the July trial. *L. richardsi* had a similar share in both trials, with a dominance index of 5.6% for the first and 4.6% for the second. In the July material, *L. silvarum* was detected as well, constituting 4.6% of the total number of individuals.

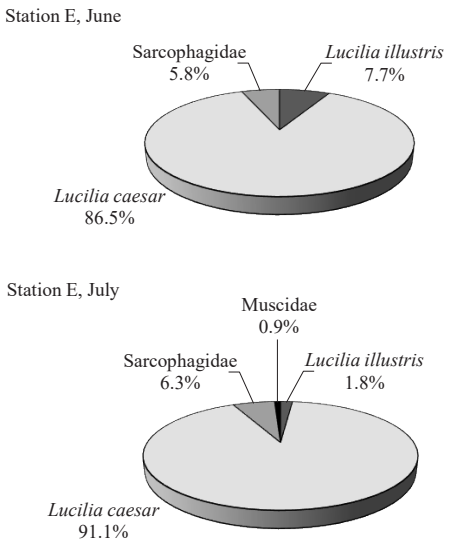

Figure 6. Dominance indices at station E in June and July

Station E had the highest proportion of flies of the species *Lucilia caesar*. The dominance index for this species reached 86.5% for the first trial and 91.1% for the second trial. Flies of the family Sarcophagidae had a similar share in both trials (5.8 and 6.3%). *L. illustris* was more active in June and accounted for 7.7% of flies at this site, as compared to July, when its dominance index was 1.8%. Flies of the Muscidae family were only detected at this site in July, in the amount of 0.9% of all specimens.

The results presented above show that the largest share at each station, in both June and July, fell to the species *Lucilia caesar*. The dominance index for this species ranged from 49.1% to 91.1%. Other important species in terms of quantity were *L. sericata* and *L. silvarum*, whose proportions at each station were strongly linked to the location and the month when the material was collected.

The Kruskal-Wallis test was used to test differences in the dominance of each species, resulting in statistically significant data. The result of the Kruskal-Wallis test was H (9,N = 100) = 57.78997; $p \leq 0.02$.

This was followed by Spearman correlation analysis, which showed strong correlations between individual species and environmental parameters, such as the distance from the site to water bodies and buildings, temperature, insolation and vegetation type (Table 2).

Table 2. Spearman's rank order correlation for individual species

	Distance from water	Distance from buildings	Temp.	Sunlight	Coniferous forest	Mixed forest	Fields	Fallows
C. loewi	−0.26352	0.00000	−0.52543	−0.26352	0.52705	−0.16667	0.00000	−0.37268
C. vicina	−0.17252	−0.30861	**−0.72616**	−0.17252	0.51755	−0.32733	0.34503	−0.48795
L. bufonivora	−0.19764	−0.53033	−0.26271	0.39528	−0.39528	−0.25000	0.39528	0.27951
L. caesar	−0.30277	−0.36927	−0.08537	−0.38534	0.30277	0.34816	−0.22019	−0.38925
L. illustris	−0.23758	−0.51250	0.18577	−0.11180	0.02795	0.17678	0.02795	−0.17788
L. richardsi	0.39284	**0.70273**	0.50477	0.39284	−0.39284	−0.24845	0.00000	0.55556
L. sericata	0.13932	−0.19938	0.51853	**0.80805**	**−0.72446**	−0.61679	**0.66873**	**0.66989**
L. silvarum	−0.27990	**−0.69834**	0.25130	**0.73657**	**−0.73657**	−0.46585	0.61872	0.60417
Muscidae	−0.10206	−0.31950	−0.36177	0.05103	−0.20412	0.16137	0.00000	0.07217
Sarcophagidae	−0.24923	−0.44584	−0.26994	0.08308	−0.05538	−0.08757	0.19385	−0.01958

Positive correlations were obtained for the following:
- the species *Lucilia richardsi* with distance from buildings,
- the species *Lucilia sericata* with insolation and open areas such as fields or fallows,
- the species *Lucilia silvarum* with insolation.

Negative correlations were obtained for the following:
- the species *Calliphora vicina* with high temperature,
- the species *Lucilia sericata* with coniferous forests,
- the species *Lucilia silvarum* with distance from buildings and coniferous forests.

DCA analysis for the abundance of individual taxa of necrophagous fauna showed that the length of the gradient represented by the first ordinate axis was below 3.0, so that direct RDA analysis could be performed to determine the relationship between the abundance of individual taxa and environmental parameters (ter Braak, 1986; ter Braak et al., 1988). The RDA analysis (Figure 7) showed a strong correlation for the species *Lucilia sericata* with insolation and open areas, such as fields and fallows. The species *L. silvarum* and *L. bufonivora* showed a correlation with decreasing distance from households, while the reverse was true of *Calliphora loewi* (correlation with increasing distance from buildings and coniferous forest). *L. richardsi* was the only species associated with an increase in perceived temperature. *L. illustris* was linked to the proximity of a water body. No clear results were shown for the remaining taxa.

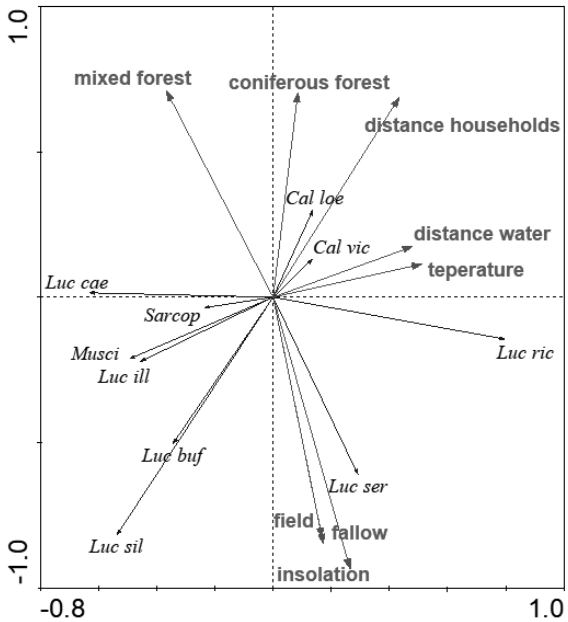

Figure 7. RDA analysis of relationships between abundance of individual taxa and environmental parameters

Discussion

The analysis of the material collected in the field revealed the presence of three families of forensic significance: Calliphoridae, Sarcophagidae and Muscidae. The most abundant was the Calliphoridae, in which the following species were identified: *Calliphora vicina*, *Calliphora loewi*, *Lucilia caesar*, *Lucilia illustris*, *Lucilia silvarum*, *Lucilia bufonivora*, *Lucilia sericata* and *Lucilia richardsi*. The most abundant species was *Lucilia caesar*, accounting for 49.06% to 91.07% of all the material, depending on the site and trial (June or July).

In temperate climate conditions, eight waves of succession of insects are observed on unburied carcasses. The aforementioned arthropod families and species represent the first and second waves of succession. The first wave of succession comprises flies of the family Calliphoridae, such as *Calliphora vicina*, *Calliphora vomitoria* and *Lucilla* spp., as well as *Musca domestica*, *Musca autumnalis* and *Muscina stabulans* of the family Muscidae. The appearance of these insects signifies the initiation of autolysis in the body. The second wave of succession of unburied carcasses consists of flies of the genera *Lucilia* and *Protophormia* and the species *Cynomya mortuorum* (Calliphoridae), as well as *Sarcophaga* spp. (Sarcophagidae), which arrive at the start of putrefaction. Both waves occur within the first three months after death (Kaczorowska et al., 2002).

Comparison of the first and second trials, bearing in mind the time interval separating them, indicates that June favours greater diversity of necrophages, which was particularly evident at sites A and B. This may be influenced by the lower perceptible temperature at these sites in June, as some species are negatively phototropic and avoid flying in full sunlight and high temperatures. Among dipterans, species of the genus *Calliphora* prefer shade, while *Lucilia* and *Sarcophaga* prefer light (Smith, 1986). Knowledge of the preferences of insects and their classification

according to preference for light or shade is useful in establishing whether a body has been moved or how long it has been exposed to sunlight.

There were over twice as many individuals in the second (July) samples, irrespective of the type of habitat, except for the site where scavengers had removed the food source, leaving only fragments of the bloodied bag. Inadequate protection of the sample contributed to the negative U Mann-Whitney test result for the statistical significance of differences in the abundance of collected fauna. Without the third site, the result would certainly have been statistically significant. This may have been influenced by the seasonal activity of necrophagous insects, observed mainly in temperate climate zones. This is obviously an individual characteristic of the species, but in the local weather conditions, fauna is richest in terms of quantity in late spring and summer. Insects found on the body represent multiple generations in the season and fly from early spring to late autumn, but are not able to reproduce during the entire period (Smith, 1986).

The most similar results between the two months in which the field experiment was conducted were obtained at sites D and E. The fourth site (D) was the furthest removed from any households, as well as from water bodies. The liver was exposed in the field, in the shade of a single small tree. The perceived temperature in both trials exceeded 30°C. The conditions prevailing at the fourth site were reflected in the number of insects arriving at the food source, limiting their number (Nuotreva, 1959). While *Lucilia caesar* was the most abundant species, a high dominance index was also noted here for *L. sericata*. Cragg (1956) describes this species as flying only in spring and summer, on hot days, laying eggs on surfaces heated to 30°C or with strong sunlight. The Spearman correlation analysis and RDA analysis revealed a clear, strong correlation of this species with insolation and open areas such as fields and fallows. Site D was therefore an ideal place for laying eggs, which contributed to the significant percentage share of this species.

Site E was a mixed forest. In theory, this site should have a large diversity of arthropod species, due to the abundant vegetation and favourable conditions for shade-loving insects. Research on the succession of insects on unburied carcasses in Polish forests has been conducted by Matuszewski et al. (2008 and 2010a), in the forests of Wielkopolska in 2005–2007. They recorded 14 species of flies and 16 species of beetles on the body of a domestic pig (Matuszewski, 2010a). Another example of research in this area is an experiment conducted by Bourel et al. (1999), who studied the fauna of unburied carcasses in the dune zone of the coast of northern France. The greatest diversity of taxa was observed on the bodies lying in the mixed forest: 42 arthropod species, of which 18 were of the order Diptera. The food source in their case consisted of fresh rabbit carcasses, which may have additionally contributed to differences in the results (Smith, 1986). In the experiment carried out for the present study, the station in the mixed forest attracted the lowest diversity of species and proved to be an ideal site for feeding by flies of the species *Lucilia caesar*. This species has been described by Matuszewski et al. (2010b) as the most common species in forests, appearing at every site during their experiment. The dominance index for this species was 86.54% in June and increased to 91.07% in July, marginalizing the activity of other species.

The greatest differences between the June and July trials were obtained at site C, located at the edge of a forest, which could have resulted in forest fauna mixing with open area species (Fiedler, 2008). In the June sample, 10.94% of the dipterans collected here were of the species *Lucilia illustris*, which was not found in the July sample. In July, we found flies of the species *L. sericata* and *Calliphora loewi*, described by Günther Enderlein as a Holarctic species, attracted by the smell of stinkhorns. Differences in the results could in this case again be due to the

disappearance of the intended food source in the July trial, so that dipterans were attracted only by the bloody fragments of the bag in which the liver was stored.

The Spearman correlation and RDA analyses showed several connections between individual taxa and environmental parameters. Particularly noteworthy is the correlation of the previously mentioned species *Lucilia sericata* with high insolation and open habitats, such as fields and fallows, and its negative correlation with mixed and coniferous forests. Another species with unambiguous results in both analyses is *L. silvarum*, which was linked to the proximity of households. The results of the Spearman correlation analysis also indicate several other links between taxa and environmental parameters, but these were not reflected in the RDA analysis. The second analysis, in turn, showed correlations that were not found in the results of the first analysis. Apart from the sensitivity and specificity of each of the analyses, the ambiguity of the results was additionally due to the small number of samples, i.e. replications, and the small number of specimens representing individual taxa collected during the field experiment, sometimes limited to a few individuals.

The field experiment was not without flaws, which should be avoided by those repeating the experiments carried out in this study or planning similar ones. In the future, the number of trials should be increased and spread out over time and the research area should be expanded. In addition, each individual sample should contain necrophages caught over several consecutive days. The means of constructing the trap itself should also be improved so that scavengers will not be able to disturb the food source. Adherence to these suggestions should improve the research in terms of both quantity and quality.

Conclusions

1. The date of exposure (June or July) of pork liver does not significantly affect the qualitative differences in the taxa present on the food source, but has a significant effect on quantitative differences.
2. Some taxa show a strong correlation with specific environmental parameters:
   – *Lucilia sericata* with strong insolation and open areas such as fields and fallows,
   – *Lucilia silvarum* with proximity to buildings.
3. The species described as *Lucilia caesar* was the most common species in the study area, irrespective of the date and the environment where the food source was exposed.

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Present status of water mite species (Acari, Hydrachnidia) described by Motaş, Tanasachi and Orghidan from Romania

Mirela Cîmpean,[1] Karina Paula Battes[2]

[1] Babeş-Bolyai University, Faculty of Biology and Geology, Department of Taxonomy and Ecology, 5-7 Clinicilor Street, 400006, Cluj-Napoca, Romania, e-mail: mirela.cimpean@ubbcluj.ro
[2] Babeş-Bolyai University, Faculty of Biology and Geology, Department of Taxonomy and Ecology, 5-7 Clinicilor Street, 400006, Cluj-Napoca, Romania, e-mail: karina.battes@ubbcluj.ro

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Abstract The present paper investigates the validity of the water mite species (Acari, Hydrachnidia) described from Romania by Motaş, Tanasachi and Orghidan. The Romanian researchers from the "Emil Racoviţă" Speleology Institute, Romanian Academy, Bucharest, had a prolific activity between 1939 and 1963: among the taxa new to science described by these authors, 34 species and 3 subspecies had their type localities in Romania. More than 50 years later, 23 species and one subspecie described by the team coordinated by Motaş are still valid. 17 of these taxa were found all over Europe, while 7 were not recorded outside Romania until present day.

Obecny status wodopójek (Acari, Hydrachnidia) z Rumunii opisanych przez Motaşa, Tanasachiego i Orghidana

Słowa kluczowe gatunki synonimiczne, gatunki obowiązujące, gatunki wątpliwe, gatunki niepewne

Streszczenie W niniejszym artykule zbadano aktualność gatunków wodopójek (Acari, Hydrachnidia) opisanych z terenu Rumunii przez Motaşa, Tanasachi i Orghidana. Główna aktywność rumuńskich badaczy z Instytutu Speleologii "Emil Racoviţă", Akademii Rumuńskiej w Bukareszcie, przypadała na lata 1939–1963. Wśród nowych gatunków opisywanych przez tych autorów locus typicus 34 gatunków i 3 podgatunków znajdowały się na terenie Rumunii. Ponad 50 lat później, 23 gatunki i jeden podgatunek opisane przez zespół prowadzony przez Motaşa są nadal aktualne. 17 z tych taksonów odnotowano na terenie całej Europy, a 7 innych do dzisiaj stwierdzonych jest jedynie na terenie Rumunii.

Introduction

Hydrachnidia (the water mites), also called Hydrachnellae or Hydracarina, represents the most important group of freshwater Arachnida, with more than 6,000 species described worldwide (Di Sabatino et al., 2008). The Palaearctic region is one of the best investigated areas, with the highest number of species recorded (1,642 species) (Di Sabatino et al., 2008).

The first Romanian study on water mites (Acari, Hydrachnidia) was conducted in 1923, when Motaş published „Contribution à l'étude des Acariens d'eau douce de Roumanie" (Motaş, 1923). More than 70 scientific papers were published between 1923 and 1972 by Motaş, Tanasachi and Orghidan, dealing with water mites from different environments: rivers, lakes and interstitial habitats.

In 1979, Konnerth-Ionescu published an inventory of all water mite species from Romania, with their exact locations. 267 water mite species and 18 subspecies were listed, from 367 sampling locations considered until then (Konnerth-Ionescu, 1979).

An updated and complemented list of water mite species from Romania was published in recent years. It included 251 species recognized at present in Romania (Cîmpean, 2011).

Discussions

Between 1939 and 1963, Constantin Motaş, Jeanne Tanasachi (Şoarec before marriage) and Traian Orghidan described 34 species and 3 subspecies new to science in Romania (Table 1) (Motaş, Şoarec, 1939; Şoarec, 1939; Motaş, 1940, 1959; Motaş, Tanasachi, 1944, 1946, 1948a, 1948b, 1960, 1963; Motaş et al., 1946, 1947a, 1947b, 1947c, 1957a, 1957b, 1958; Tanasachi, Orghidan, 1955).

23 water mite species and one subspecie from those described by the team coordinated by Motaş are still valid, according to the Hydrachnidia taxonomic revisions made at an European scale (Table 1).

Table 1 Water mite species new to science, described by Motaş and his team (the current status, the type localities, the European distribution and the preferred habitats)

No.	Taxa	Species status	Type localities	Distribution	Habitat
1	2	3	4	5	6
1.	*Stygothrombium racovitzai* (Motaş, Tanasachi, 1946)	*Species dubia* (Davids et al., 2007). Future material from the Romanian populations is necessary.	The Crişul Repede River in Şuncuiuş village (Motaş, Tanasachi, 1946)	–	–
2.	*Tadjikothyas fibulata* (Motaş, Tanasachi, 1957)	Valid species	Rheocren spring tributary of Lake Greaca (drained) (Motaş et al., 1957b)	Limnofauna: 12, Y*	Crenophylous (springs and small rivers) (Motaş et al., 1957b; Motaş, 1959)
3.	*Dacothyas savulescui* (Motaş, 1959)	Valid species	Spring in Comana village, 30 km south from Bucharest (Motaş, 1959)	Limnofauna: 12* Romania	Crenobiont (springs) (Motaş, 1959)
4.	*Vietsthyas fonticola* (Motaş, Tanasachi, 1957)	A junior synonym to *Tartarothyas micrommata* Viets, 1934	–	–	–

1	2	3	4	5	6
5.	*Lebertia holsatica nitida* (Motaş, Tanasachi, 1963)	Non valid subspecies This subspecies is insufficiently defined and agree with *L. semireticulata* Viets, 1925 (Gerecke, 2009; Di Sabatino et al., 2010)	–	–	–
6.	*Torrenticola jeanneli* (Motaş, Tanasachi, 1947)	Valid species	The Târlungul Stream (near Satul-Lung village, Braşov county); The Bîrsa Stream, tributary of the Olt River; The Cerna River (Motaş et al., 1947b)	Limnofauna: 4, 5, 7, 10* Central and Southeastern Europe. Only known from a few sites (Di Sabatino et al., 2010)	Low- and middle order streams, only known from hyporheic
7.	*Kawamuracarus chappuisi* (Motaş, Tanasachi, 1946)	Valid species *K. chappuisi* was proposed to be a synonym of *K. vardaricolus* Viets, 1942. A resolution is hampered by the loss of type material of *K. chappuisi* (Gerecke et al., 2016). Future material from the Romanian populations could solve this discussion.	The Valea Drăganului Stream, tributary of the Crişul Repede River (Bihor county) (Motaş, Tanasachi, 1946)	Limnofauna: 10* Southeastern and eastern Europe, one record from the eastern part of the area covered, Poland (Gerecke et al., 2016)	Interstitial waters of low- and middle order streams
8.	*Atractides latipalpis* (Motaş, Tanasachi, 1946)	Valid species	The Valea Drăganului Stream, tributary of the Crişul Repede River (Bihor county) (Motaş, Tanasachi, 1946)	Limnofauna: 2, 4, 5, 7, 8, 9, 10, 11, 13, 18* Central and southeastern Europe, United Kingdom (Di Sabatino et al., 2010)	Hyporheobiont

1	2	3	4	5	6
9.	*Atractides magnirostris* (Motaş, Tanasachi, 1948)	*Species dubia* Possibly synonym to *A. acutirostris* (Motas, Angelier, 1927) (Gerecke, 2003)	The Sadul Stream, right tributary of the Cibin River (the Olt River catchment area); the Bughea Stream (in Câmpul-Lung), tributary of the Rîul Târgului River (the Argeş River catchment area) (Motaş, Tanasachi, 1948b)	–	–
10.	*Atractides microphtalmus* (Motaş, Tanasachi, 1948)	Non valid species Species similar with *A. denticulatus* (Walter, 1947) and possible also with other species of the *cisternarum*-species group (Di Sabatino et al., 2010). Further investigations on the geographical variation in species of this group are necessary (Gerecke, 2003)	–	–	–
11.	*Atractides nodipalpis intermedius* (Şoarec, 1939)	*Subspecies dubia* (Gerecke, 2003)	The Secu Stream, tributary of the Ozana River (the Siret River catchment area) (Şoarec, 1939)	–	–
12.	*Atractides phreaticus* (Motaş, Tanasachi, 1948)	Valid species	The Rîul Mare Stream, left tributary of the Olt River (near Porumbacul de Sus village); the Sebeş Stream, left tributary of the Olt River; the Bogata Stream, left tributary of the Olt River (Motaş, Tanasachi, 1948b)	Limnofauna: 4, 7, 8, 9, 10* Central and southeastern Europe, southern France (Di Sabatino et al., 2010)	Hyporheobiont
13.	*Atractides prosiliens* (Motaş, Tanasachi, 1948)	Valid species	The Bughea Stream, tributary of the Rîul Târgului River (the Argeş catchment area) (the Southern Carpathians) (Motaş, Tanasachi, 1948b)	Limnofauna: 10* Romania, doubtful records from Austria and southern France (Di Sabatino et al., 2010)	Hyporheobiont

1	2	3	4	5	6
14.	*Atractides pygmaeus* (Motaş, Tanasachi, 1948)	Valid species	The Rîuşor Stream, tributary of the Dâmboviţa River (the Southern Carpathians) (Motaş, Tanasachi, 1948b)	Limnofauna: 3, 4, 5, 7, 10* the Alps, the Carpathians, Montenegro (Di Sabatino et al., 2010)	Hyporheobiont
15.	*Atractides sokolowi* (Motaş, Tanasachi, 1948)	Valid species	The Bughea Stream, tributary of the Rîul Târgului River (the Argeş catchment area) (the Southern Carpathians) (Motaş, Tanasachi, 1948b)	Limnofauna: 7, 10* Eastern and southeastern Europe; from area covered recorded in Poland (Di Sabatino et al., 2010)	Rhithrobiont
16.	*Atractides szalay* (Motaş, Tanasachi, 1948)	A junior synonym to *A. oblongus* (Walter, 1944)	–	–	–
17.	*Atractides elegans* (Motaş, Tanasachi, 1948)	*Species incertae* Species similar with *A. orghidani* Motaş & Tanasachi, 1960 (Di Sabatino et al., 2010).	The Sadul Stream, right tributary of the Cibin River (the Olt River catchment area) (Motaş, Tanasachi, 1948b)	–	–
18.	*Atractides orghidani* (Motaş, Tanasachi, 1960)	Valid species	The Vîrghiş Stream, tributary of the Olt River in Mereşti village (Harghita county) (Motaş, Tanasachi, 1960)	Limnofauna: 3, 5, 6, 9, 10, 13, Y* Romania, Greece, Montenegro, Italy, France, Turkey (Di Sabatino et al., 2010)	Rhithrobiont, hyporheophilous
19.	*Feltria mira* (Motaş, Tanasachi, 1948)	*Species incertae* Species similar with *F. motasi* (Schwoerbel, 1961; Gerecke, 2012; Gerecke et al., 2016)	The Prahova River (near Azuga city) (Motaş, Tanasachi, 1948a)	–	–
20.	*Feltria amplexa* (Motaş, Tanasachi, 1944)	Valid species (Further investigations are necessary)	Rheocren spring, right tributary of the Valea Rea Stream in Sinaia city (Motaş, Tanasachi, 1944)	Limnofauna: 10*	–
21.	*Feltria halberti* (Motaş, Tanasachi, 1957)	A junior synonym to *F. rouxi* (Walter, 1907)	–	–	–
22.	*Feltria simionescui* (Motaş, Soarec, 1939)	A junior synonym to *F. zschokkei* (Koenike, 1896)	–	–	–
23.	*Forelia aspidiophora* (Motaş, 1959)	Valid species (Further investigations are necessary)	The Cocora Stream, left tributary of the Ialomiţa River, near Peştera village (the Bucegi Mountains) (Motaş, 1959)	Limnofauna: 10*	–

1	2	3	4	5	6
24.	*Albaxona lundbladi* (Motaş, Tanasachi, 1947)	Valid species	The Bogata Stream, tributary of the Olt River (the Southern Carpathians) (Motaş et al., 1947b)	Limnofauna: 3, 4, 5, 7, 8, 9, 10, 18, Y* British Isles, central, southern and southeastern Europe (Gerecke et al., 2016)	Hyporheobiont
25.	*Paraxonopsis inferorum* (Motaş, Tanasachi, 1947)	Valid species	The Târlungul Stream (near Satul-Lung village, Brașov county) (Motaș et al., 1947b)	Limnofauna: 2, 3, 4, 5, 6, 9, 10, 11, 14, 15* Central, eastern and southern Europe (Gerecke et al., 2016)	Hyporheobiont
26.	*Paraxonopsis vietsi* (Motaş, Tanasachi, 1947)	Valid species	The Cerna River (Herculane) (Motaș et al., 1947b)	Limnofauna: 2, 3, 7, 10, 12* Central and southern Europe (Gerecke et al., 2016)	Hyporheobiont
27.	*Erebaxonopsis brevipes* (Motaş, Tanasachi, 1947)	Valid species	The Târlungul Stream (near Satul-Lung village, Brașov county); The Cerna River (Herculane) (Motaș et al., 1947b)	Limnofauna: 1, 3, 10* SW Palearctic (Gerecke et al., 2016)	Hyporheobiont
28.	*Aturus paucisetus* (Motaş, Tanasachi, 1946)	Valid species Future material from the Romanian population is necessary, because after the loss of the holotype, morfological data are mostly based on central European material (Gerecke et al., 2016)	The Crișul Repede River in Șuncuiuș village (Motaș, Tanasachi, 1946)	Limnofauna: 2, 4, 7, 10* Central and southeastern Europe (Gerecke et al., 2016)	Low and middle order streams, preferably in the hyporheic (Gerecke et al., 2016)
29.	*Kongsbergia dentate folioligera* (Motaş, Tanasachi, 1958)	Valid subspecies (Further investigations are necessary)	The Salătruc Stream, tributary of the Topolog River (Motaș et al., 1958)	Limnofauna: 10*	–
30.	*Kongsbergia d-motasi* (Motaş, Tanasachi, 1958)	Valid species (Further investigations are necessary)	The Salătruc Stream, tributary of the Topolog River; the Rîul Mare Stream, tributary of the Olt River (Motaș et al., 1958)	Limnofauna: 7, 10*	–
31.	*Kongsbergia pectinigera* (Motaş, Tanasachi, 1946)	Valid species	The Valea Drăganului Stream and the Crișul Repede River (Bihor county) (Motaș, Tanasachi, 1946)	Limnofauna: 1, 3, 4, 5, 7, 8, 9, 10* Southern Europe, south-central and eastern Europe (Gerecke et al., 2016)	Hyporheobiont

1	2	3	4	5	6
32.	*Mideopsis fonticola* (Tanasachi, Orghidan, 1955)	*Species incertae* Species similar with *Nudomideopsis latipalpis* E. Angelier, 1963 (Gerecke et al., 2016)	Olguța Gabor's fountain from Ponor village (Hunedoara county) (Tanasachi, Orghidan, 1955)	–	–
33.	*Bogatia maxillaris* (Motaş, Tanasachi, 1948)	Valid species	The Bogata Stream, left tributary of the Olt River (close to Bogata village) (Motaş, Tanasachi, 1948a)	Limnofauna: 3, 6* Romania, Macedonia and Sicily (Gerecke et al., 2016)	Hyporheobiont
34.	*Chappuisides thienemanni* (Motaş, 1959)	Valid species (Further investigations are necessary)	The Bogata Stream, tributary of the Olt River; the Rîușorul Stream, tributary of the Dîmbovița River, near Cîmpulung Muscel; the Dopca Stream, left tributary of the Olt River; the Valea Drăganului River (the Apuseni Mountains); the Valea Sighișteului Stream (the Apuseni Mountains) (Motaș, 1959)	Limnofauna: 10*	–
35.	*Phreatohydracarus mosticus* (Tanasachi, Orghidan, 1955)	Valid species	Wells from Ponor village (Hunedoara county) (Tanasachi, Orghidan, 1955)	Limnofauna: 10* Romania	Hyporheobiont
36.	*Arrenurus iassiensis* (Motaş, 1940)	Valid species (Further investigations are necessary)	Pond on the right bank of the Bahlui River, near Iași (Motaş, 1940)	Limnofauna: 16*	–
37.	*Arrenurus lundbladianus* (Motaş, Tanasachi, 1958)	A junior synonym to *A. corsicus* (E. Angelier, 1951)	–	–	–

* Information from the database based on data published by Viets (1978) in Limnofauna Europaea, updated in 1.09.2016 and published on watermite.org (2017) (1: Iberian Peninsula, 2: The Pyrenees, 3: Italy, 4: The Alps, 5: Dinaric Western Balkan, 6: Hellenic Western Balkan, 7: Eastern Balkan, 8: Western Highlands, 9: Central Highlands, 10: The Carpathians, 11: Hungarian Lowlands, 12: Pontic Province, 13: Western Lowlands, 14: Central Lowlands, 15: Baltic Province, 16: Eastern Lowlands, 18: Great Britan, Y: Israel, Turkey, Syria).

However, 6 out of the 24 valid taxa need further investigations, since holotype and paratype specimens are missing: *Feltria amplexa* (Motaş, Tanasachi, 1944), *Forelia aspidiophora* (Motaş, 1959), *Kongsbergia dentate folioligera* (Motaş, Tanasachi, 1958), *Kongsbergia d-motasi* (Motaş, Tanasachi, 1958), *Chappuisides thienemanni* (Motaş, 1959), *Arrenurus iassiensis* (Motaş,

1940). These species were not classified during the latest European revision (Davids et al., 2007; Di Sabatino et al., 2010; Gerecke et al., 2016), but they are still considered valid in the databases, according to information published by K.O. Viets (1978), updated in 1.09.2016 and posted on watermite.org (2017).

Table 1 depicts updated information on the European distribution of valid water mite species described by the team coordinated by Motaş, together with the habitats they inhabit and the type localities they were described from.

Recent studies reported the following species in Romania: *Kawamuracarus chappuisi* and *Aturus paucisetus* in the Crişul Repede River (Pavelescu, Cîmpean, 2002–2003); *Phreatohydracarus mosticus* and *Atractides* cf. *latipalpis* in the Arieş River (Moldovan et al., 2011); *Torrenticola jeanneli* in the Someşul Cald River (Cîmpean, 2011).

The number of new species described by the team coordinated by Motaş versus the number of valid species during the 24 years (1939–1963) is depicted in Figure 1. The highest number of new described species was in 1948 (10), with 5 valid at present.

Four water mite species were proposed as candidates for the European Red List: *Tadjikothyas fibulata*, *Kawamuracarus chappuisi*, *Chappuisides thienemanni* and *Phreatohydracarus mosticus* (Cîmpean, 2014). Two species might need protection and could join the list, due to their restricted distribution: *Dacothyas savulescui* and *Atractides prosiliens*.

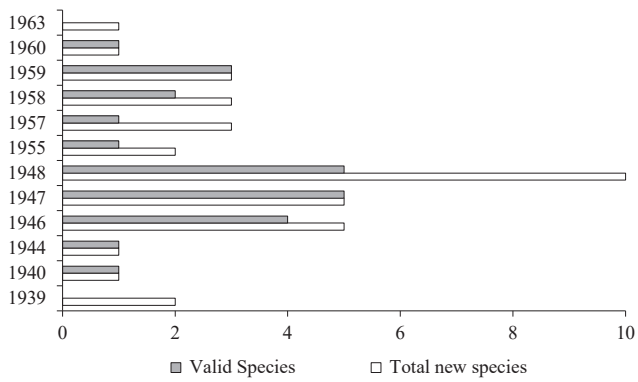

Figure 1. Total new versus valid water mite species (Acari, Hydrachnidia) described by Motaş, Tanasachi and Orghidan from Romania

Since several holotype and paratype specimens are missing, further investigations are mandatory for some water mite species, by revisiting the sampling regions.

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Prey abundance supporting unusual water mite (Acari: Hydrachnidia) community in a sublacustrine spring and tributary river

Ivana Pozojević,[1] Ivančica Ternjej,[2] Zlatko Mihaljević,[3] Sanja Gottstein,[4] Natalija Vučković,[5] Valentina Dorić,[6] Mario Rumišek[7]

[1] Division of Zoology, Faculty of Science, University of Zagreb, Rooseveltov trg 6, 10000 Zagreb, Croatia, e-mail: ivana.pozojevic@biol.pmf.hr
[2] Division of Zoology, Faculty of Science, University of Zagreb, Rooseveltov trg 6, 10000 Zagreb, Croatia, e-mail: ivancica@biol.pmf.hr
[3] Division of Zoology, Faculty of Science, University of Zagreb, Rooseveltov trg 6, 10000 Zagreb, Croatia, e-mail: zlatko.mihaljevic@biol.pmf.hr
[4] Division of Zoology, Faculty of Science, University of Zagreb, Rooseveltov trg 6, 10000 Zagreb, Croatia, e-mail: sanja.gottstein@biol.pmf.hr
[5] Division of Zoology, Faculty of Science, University of Zagreb, Rooseveltov trg 6, 10000 Zagreb, Croatia, e-mail: natalija.vuckovic@biol.pmf.hr
[6] Eko-monitoring d.o.o., Kučanska ul. 15, 42000 Varaždin, Croatia, e-mail: doric.valentina@gmail.com
[7] Independant researcher, Vinogradska 140c, 44320 Kutina, e-mail: rumisek.mario@windowslive.com

Keywords meiofaunal food web, predator-prey interactions, karst water community, sublacustrine spring, karst river

Abstract Water mites are predators on microcrustations (Copepoda, Cladocera, Ostracoda) and insect larvae (mostly Diptera). Their communities usually have great diversity with high species richness but relatively low abundance. This may have important implications for poorly investigated predator-prey interactions of water mites in natural habitats. A sublacustrine karst spring Torak and the mouth of its tributary river Čikola were examined seasonally from August 2016 to June 2017, with the aim of determining water mite distribution connected to prey availability. Triplicate samples were taken with an Ekman dredge on three distinct depth zones (littoral, sublittoral and profundal zone) at both study sites. Both, the karst spring and the tributary river, had typical lentic water mite communities with no rhithrobiont or crenobiont taxa present. Water mite species richness, abundance and alpha diversity were not governed by depth, but rather by prey availability. Greater copepod, ceratopogonid and chironomid abundances were found to positively correlate with higher abundances of water mites. The population of *Limnesia connata*, previously reported from lime-poor waters exclusively, was most probably supported by vast prey availability, thus unexpectedly surviving in this karst environment. *L. connata*, *Neumania vernalis* and *Piona longipalpis* were recorded for the first time in Croatia, while *L. connata* was recorded for the first time in the Dinaric karst.

Dostępność pokarmu w źródle krasowym i ujściu rzeki warunkuje występowanie nietypowych zgrupowań wodopójek (Acari: Hydrachnidia)

Słowa kluczowe sieć pokarmowa w mejofaunie, interakcje drapieżnik–ofiara, zgrupowania wód krasowych, źródło na dnie jeziora, rzeka krasowa

Streszczenie Wodopójki są drapieżnikami odżywiającymi się mikroskorupiakami (Copepoda, Cladocera, Ostracoda) i larwami owadów (głównie Diptera). Ich zgrupowania zazwyczaj charakteryzują się dużym zróżnicowaniem gatunkowym, ale stosunkowo niską liczebnością. Może to mieć duże znaczenie dla wciąż słabo zbadanych relacji drapieżnik–ofiara w naturalnych siedliskach. Celem badań przeprowadzanych w okresie od sierpnia 2016 do czerwca 2017 roku w znajdującym się na dnie jeziora, krasowym źródle Torak i ujściu rzeki Čikola było zbadanie rozmieszczenia wodopójek w zależności od dostępności pokarmu. Próby pobierano chwytaczem Ekmana w trzech strefach głębokości (litoral, sublitoral i profundal) w obu badanych miejscach, za każdym razem wykonując trzy powtórzenia. Zarówno źródło krasowe, jak i dopływająca rzeka charakteryzowały się typowo lenitycznym zgrupowaniem wodopójek, bez obecności rhithrobiontów i krenobiontów. Bogactwo gatunkowe wodopójek, liczebność i wskaźniki różnorodności nie były związane z głębokością, lecz z dostępnością pożywienia. Stwierdzono, że większe liczebności widłonogów, ceratopogonidów i chironomidów korelują dodatnio z większą liczebnością wodopójek. Populacja *Limnesia connata*, poprzednio opisywana wyłącznie z wód ubogich w wapń, była najprawdopodobniej wspierana przez ogromną dostępność pokarmu, co niespodziewanie przełożyło się na jej przetrwanie w tym krasowym środowisku. *L. connata*, *Neumania vernalis* i *Piona longipalpis* zostały odnotowane w Chorwacji po raz pierwszy, natomiast *L. connata* została po raz pierwszy odnotowana na Krasie Dynarskim.

Introduction

Predation is a biotic interaction that links water mites to different taxonomic groups and life stages of benthic invertebrate communities (Goldschmidt, 2016). Predatory aquatic adult and deutonymph water mites feed on insect larvae and other small invertebrates (Martin, Stur 2006; Stoch et al., 2011) such as microcrustations (Copepoda, Cladocera, Ostracoda) and insect larvae -mostly Diptera (Martin, 2005; Stoch et al., 2011). Their communities usually have great diversity with high species richness but relatively low abundance (Di Sabatino et al., 2008). This may have important implications for poorly investigated predator-prey interactions of water mites, mostly known from observations and ex-situ studies (Martin, 2005), while the distribution patterns and co-occurrence of water mites and their prey in natural habitats are poorly investigated.

Depth has been shown as an important abiotic driver in water mite composition and structure across lentic habitat profiles (Zawal et al., 2013). Concerning both depth and prey availability, the objectives of this study were: 1) to examine the effect of depth on water mite abundance, species richness and diversity and 2) to examine the dispersal of water mites in connection to prey abundance.

Materials and methods

Study area

Torak is a sublacustrine karst spring situated on the southeastern bank of the upstream end of a Lakeland formed by the Čikola river near the Skradinski buk waterfall. The altitude is

approximately 50 m above sea level. The submerged spring is circular, having a diameter of about 180 m. The eye of the spring is 47 m deep and is a tributary of the Čikola River (Figure 1).

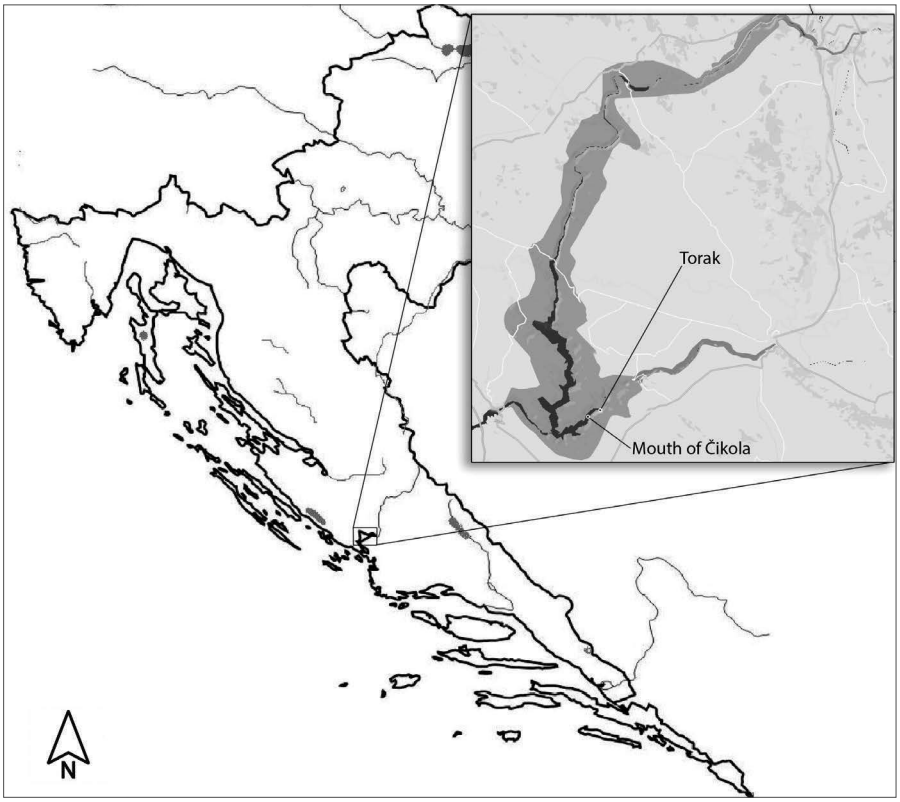

Figure 1. Map of the study area with the locations of the two sampling sites in Krka National Park

The three sampling depths (littoral, sublittoral and profundal zone) were sampled at approximately 2, 10 and 45 meters.

The second sampling site Čikola is a Dalmatian river with the length of 47 km and is Krka's largest tributary. The Čikola River enters the Krka above Skradinski Buk. The lower reach of the river and the Torak spring lie within the boundaries of Krka National Park (Terzić, Frangen, 2010). At our sampling site, Čikola is at its lowest reach and is very slow flowing, even showing seasonal stratification. The three sampling depths (littoral, sublittoral and profundal zone) were sampled at approximately 2, 10 and 25 meters.

Both sites can be referred to as semi-lentic habitats with some flow present, but with stratification occurring regularly.

Sampling and laboratory methods

Each site was sampled four times (once per season) from August 2016 to June 2017. Triplicate samples were taken with an Ekman dredge (15 × 15 cm surface) on three distinct depth zones (littoral, sublittoral and profundal zone) at both sites. In total, we collected 72 benthic macroinvertebrate subsamples (36 from each site). All benthic samples of macroinvertebrate fauna were preserved in 96% ethyl alcohol. Water mites from the samples were subsequently isolated and fixed in Koenike's solution. All water mite individuals were determined to either species or genus (some deutonymphs) level using general keys by Tuzovskij (1990), Davids et al. (2007), Di Sabatino et al. (2010) and Gerecke et al. (2016). All water mite specimens are deposited at the Department of Biology, Faculty of Science, Zagreb, Croatia. The number of specimens per water mite species were classified as Male/Female/Nymph.

Diversity indices

The Shannon's (H'), Simpson's (1-Λ), Fisher's and Margalef (d) diversity indices and the Pielou's evenness index (J') were calculated for water mite assemblages at each site using Primer Version 6 software (Clarke, Gorley, 2006).

Data analysis

We used Pearson's correlation coefficient to determine if a significant correlation could be detected between depth and water mite species richness, abundance and diversity. We used five different indices at each site, as they all have slightly different approaches when calculating alpha diversity.

We used Pearson's correlation coefficient to determine significant correlations between diversity indices and water mite abundance with prey availability.

All values were log(x + 1) transformed before testing. For all tests, $p < 0.05$ indicated statistical significance. All statistical analyses were done using Statistica, 13.0 (TIBCO Software Inc., 2017).

Results and discussion

In total, only thirteen water mite specimens from four taxa were collected during the study period, proving once again the diverse, but not abundant nature of water mite distribution. Both, the karst sublacustrine spring and tributary river, had typical lentic water mite communities with no rhithrobiont or crenobiont taxa present. The most abundant taxon was *Neumania vernalis*, recorded in both sites with eight specimens found (1/1/6). *Arrenurus* sp. (0/0/2) and *Piona longipalpis* (0/1/0) were found only in Čikola river, while *Limnesia connata* was found solely in Torak (1/1/0).

Water mite specimens were not found in the profundal zone. The thermocline, as found by Zawal et al. (2013) seemed to pose an obstacle for water mite colonisation of the deeper layers of our two study sites. However, water mite species richness, abundance and alpha diversity were found not to be governed by depth. Neither specific abundances nor indices based on water mite assemblages were found to correlate with depth significantly (Table 1).

Table 1. Relationships between water mite species richness, diversity and/or abundance variables and depth. The values of the rank correlations (r) and their probabilities (p) for depth variable

	Depth	
	r	p
Hydrachnidia	–0.101	>0.05
Arrenurus sp.	–0.417	>0.05
Limnesia connata	0.318	>0.05
Neumania vernalis	0.154	>0.05
Piona longipalpis	0.036	>0.05
Species richness	–0.062	>0.05
Margalef index	–0.025	>0.05
Pielou evenness	0.012	>0.05
Fisher index	–0.166	>0.05
Shannon index	–0.073	>0.05
Simpson index	0.011	>0.05

Greater copepod, total microcrustacean, ceratopogonid and chironomid abundances were found to significantly correlate with higher abundances of water mites, indicating that water mite spatial distribution was governed by prey availably, rather than depth (Table 2).

Table 2. Relationships between prey and water mite abundance. The values of the rank correlations (r) and their probabilities (p) (significant values in bold)

	Hydrachnidia abundance	
	r	p
Chironomidae	**0.4503**	**<0.050**
Ceratopogonidae	**0.6565**	**<0.001**
Microcrustacea (all)	**0.5060**	**<0.010**
Ostracoda	0.2250	>0.050
Cladocera	0.2230	>0.050
Copepoda	**0.5180**	**<0.010**

New records for the water mite fauna of Croatia

A checklist of water mites in Croatia was published by Pešić (2002), which was later on broadened within the research on water mites of the Balkan Peninsula (Pešić et al., 2010, 2018). These check lists are missing records from Matoničkin and Pavletić (1959) (*Woolastookia rotundifrons*) and a doctoral thesis from Romana Latiger (1988) in which groundwater water mites were determined by Prof. Schwoerbel (*Partnunia angusta*, *Panisus torrenticolus* and *Protzia squamosa*). These make up a total of 64 water mites recorded in Croatia, and with the current paper three new species are added, making the total number of water mite species found in Croatia 67.

Limnesia (*Limnesia*) *connata* Koenike, 1895

Only two specimens found in the sublacustrine spring confirms the records of van Haaren and Tempelman (2009) who stated that this species is widely distributed, but rather scarce in abundance and is not numerous anywhere. A discrepancy was found with other recordings of the habitat preferences of this species such as: lime-poor waters, quagfens and temporary waters (Schwoerbel, 1956; Smit, van der Hammen, 1996). Two adult specimens (1/1/0) were found in the littoral zone in winter. The population of *Limnesia connata*, previously reported from lime-poor waters exclusively, was most probably supported by vast prey availability, thus unexpectedly surviving in this karst environment.

Neumania (*Neumania*) *vernalis* (Müller, 1776)

In total eight specimens were found (1/1/6) at both sampling sites. The species was found in spring and autumn, in the littoral and sublittoral zone. The findings of Pešić et al. (2007) that stated: "the species is common in eutrophic to dystrophic standing waters" is in slight discrepancy with the findings of this study where the species was found in both the lower reaches of Čikola river and the sublacustrine karst spring Torak, which we consider to be semi-lentic habitats.

Piona longipalpis (Krendowskij, 1878)

This ubiquitous species was found in the sublittoral zone of the slow flowing, lower reaches of Čikola river. Only one specimen was recorded (0/1/0) in autumn. The species is not habitat specific and is widespread in Europe (Gerecke et al., 2016).

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A faunistic and ecological characterization of the water mites (Acari: Hydrachnidia) of the Bukowa River (central-eastern Poland)

Robert Stryjecki,[1] Aleksandra Bańkowska,[2] Magdalena Szenejko[3]

[1] Department of Zoology, Animal Ecology and Wildlife Management, University of Life Science in Lublin, Akademicka 13, 20-950 Lublin, Poland, e-mail: robstry@wp.pl
[2] Department of Invertebrate Zoology and Limnology, University of Szczecin, 71-415 Szczecin, Wąska 13, Poland. alekbankow@gmail.com
[3] Department of Ecology and Environmental Protection, Institute for Research on Biodiversity, Faculty of Biology, University of Szczecin, Poland, e-mail: magdalena.szenejko@usz.edu.pl

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Abstract The water mite communities of the Bukowa River were found to be similar to those of other lowland rivers in Poland. An element specific to the Bukowa River was a much higher abundance of *Lebertia inaequalis* than in other Polish rivers. Another distinctive element was the very high numbers of *Arrenurus crassicaudatus*, but this taxon should be considered allochthonous – its presence in the river was due to the periodic inflow of water from fish ponds. The largest synecological group was rheophiles and rheobionts, which together accounted for 80% of the fauna. The very large quantitative share of rheobionts and rheophiles is indicative of the natural character of the river, and the physicochemical parameters confirm its good water quality. More individuals (1,764) and species (47) were caught in the lentic zone of the river than in the lotic zone (1,027 individuals, 32 species). The species most associated with the lotic zone and fast water flow were *Sperchon clupeifer* and *Hygrobates calliger*. Data from the Bukowa River, as well as other rivers of the Janów Forests Landscape Park, indicate that the most characteristic habitat for *Lebertia inaequalis* is the lentic zone, with abundant water vegetation. The typical habitats of Hygrobates setosus are marginal pools and zones with slower water flow. *Mideopsis crassipes* and *Torrenticola amplexa* were associated with the upper course of the river. *Sperchon clupeifer* was associated with lotic habitats of the middle and lower course of the river, and *Hygrobates setosus* and *Forelia variegator* were associated with habitats with slower flow in the middle and lower stretches of the river.

Faunistyczna i ekologiczna charakterystyka wodopójek (Acari: Hydrachnidia) rzeki Bukowa (Polska środkowo-wschodnia)

Słowa kluczowe strefa lenityczna, strefa lotyczna, profil podłużny rzeki, grupy synekologiczne, różnorodność gatunkowa

Streszczenie Uznano, że zbiorowiska wodopójek z rzeki Bukowej są podobne do innych zbiorowisk z rzek

nizinnych w Polsce. Elementem charakterystycznym dla rzeki Bukowej była znacznie większa liczebność *Lebertia inaequalis* niż w innych polskich rzekach. Innym wyróżniającym ją elementem była bardzo wysoka liczba *Arrenurus crassicaudatus*, ale ten takson powinien być uważany za allochtoniczny – jego obecność w rzece była spowodowana okresowym napływem wody ze stawów rybnych. Największą grupą synekologiczną były reofile i reobionty, które łącznie stanowiły 80% fauny. Bardzo duży ilościowy udział reobionów i reofili świadczy o naturalnym charakterze rzeki, a parametry fizykochemiczne potwierdzają jej dobrą jakość wody. Więcej osobników (1764) i gatunków (47) zostało złowionych w strefie lenitycznej rzeki niż w strefie lotycznej (1027 osobników, 32 gatunki). Gatunkiem najbardziej związanym ze strefą lotyczną i szybkim przepływem wody były S*perchon clupeifer* i *Hygrobates calliger*. Dane z rzeki Bukowej, a także z innych rzek Parku Krajobrazowego Lasy Janowskie wskazują, że najbardziej charakterystycznym siedliskiem dla *Lebertia inaequalis* jest strefa lenityczna z obfitą roślinnością wodną. Typowe siedliska *Hygrobates setosus* to zastoiska i strefy o wolniejszym przepływie wody. *Mideopsis crassipes* i *Torrenticola amplexa* były związane z górnym biegiem rzeki. *Sperchon clupeifer* był związany z siedliskami lotycznymi środkowego i dolnego biegu rzeki, a *Hygrobates setosus* i *Forelia variegator* były związane z siedliskami o wolniejszym przepływie w środkowych i dolnych odcinkach rzeki.

Introduction

The water mite fauna of flowing water bodies in Poland is less well-known than the Hydrachnidia fauna of standing waters. Data on water mites of flowing waters in lowland areas of Poland can be found in works by Pieczyński (1960), Bazan-Strzelecka (1964, 1986), Biesiadka (1970, 1972), Biesiadka and Kasprzak (1977), Kowalik (1981), Cichocka (1996a, 1996b, 2006), Stryjecki (2009, 2010), Stryjecki et al. (2012), Zawal and Sadanowicz (2012), Stryjecki and Kowalczyk-Pecka (2013a), Zawal and Kowalik (2013), Bańkowska et al. (2015) and Zawal et al. (2017). Hydrachnidia communities of rivers in the highlands of Poland are described in studies by Kowalik (1981), Kowalik and Biesiadka (1981), Stryjecki and Kowalczyk-Pecka (2013b), Kowalik et al. (2014) and Biesiadka et al. (2015). The rivers of mountainous areas and foothills are by far the least well researched. The most important works on these areas include studies by Kupiszewska (1965), Biesiadka (1974, 1979), Biesiadka and Cichocka (1993). Among the studies cited above, particularly valuable are those which take into account longer stretches of rivers or their entire course (Bazan-Strzelecka, 1964; Biesiadka, 1970, 1979; Kowalik, 1981; Cichocka, 1996a, 2006; Zawal et al., 2017). Due to the lack of studies on the water mite fauna of the rivers of Poland, there is a need for more intensive research on flowing water bodies to provide more complete knowledge of the Hydrachnidia communities of these ecosystems.

Polish rivers are undergoing increasing anthropogenic transformations, most commonly water pollution, but also other forms of human impact, such as regulation of the river bed and modification of the structure of the banks and bottom of watercourses (Biesiadka, 1972; Biesiadka, Kasprzak, 1977; Kowalik, 1981; Kowalik, Biesiadka 1981; Cichocka, 1996a; Zawal, Kowalik, 2013; Zawal et al., 2015). The effect of human impact is increasing impoverishment of fauna. Therefore, there is an urgent need to document the state of Hydrachnidia fauna of rivers, especially natural and unpolluted watercourses (Cichocka, 1996a, 2006).

In addition to species that have established habitat preferences and are characteristic of watercourses (rheobionts and rheophiles), there are taxa that are found in both flowing and standing water bodies. An example of such a species is *Hygrobates longipalpis*. In the European Hydrachnidia identification key by Gerecke et al. (2016), standing and slow-flowing waters are specified as the habitat of *H. longipalpis*. In the catalogue of Polish fauna (Biesiadka, 2008), the

species is referred to as a lake species that is also found in flowing waters. This species is classified in different ways in the literature – both as stagnophile (Kowalik, 1981; Zawal, Kowalik, 2013) and as a rheophile (Cichocka, 1996; Zawal, Sadanowicz, 2012). Intensified research on rivers, apart from identification of the fauna of these ecosystems, may contribute to a fuller understanding of the habitat requirements and ecological character of certain species, especially taxa encountered in both flowing and standing water bodies. An additional issue is the provision of new information on taxa that have only recently been recognized as separate species. An example is *Hygrobates setosus*, which is a sister species of *H. nigromaculatus*. The river species (*H. setosus*) was finally separated from the lake species (*H. nigromaculatus*) relatively recently (Martin et al., 2010). Older publications on flowing waters include *H. nigromaculatus* in the list of species, although it is highly likely that the species in question was *H. setosus*. Documentation of the habitat preferences of *Hygrobates setosus* is important because it contributes to more complete knowledge of the habitat preferences of this species.

The aim of the study was a detailed faunistic and ecological analysis of the water mite fauna of the natural, unpolluted Bukowa River (central-eastern Poland). We analysed variation in environmental factors, species composition of fauna, dominance structure, species diversity, faunistic similarity, distribution of fauna in the transverse and longitudinal profiles of the river, and synecological structure, as well as the habitat preferences of selected species.

Study area and sites

The Bukowa River is 54.2 km long (Michalczyk, Wilgat, 1998). For its entire length it flows through the Biłgoraj Plain mesoregion, which is part of the Sandomierz Basin geographic macroregion (Kondracki, 2014). The Bukowa River is a right-bank tributary of the San River. The river begins in a swamp near the village of Korytków. The headwaters of the river, a stretch of a dozen or so metres flowing south-west, has the appearance of a ditch. The river valley dissects a sandy, mostly forested area with numerous sand dunes. The Bukowa River basin covers an area of 650.8 km^2. In the upper and middle course of the river, its basin is highly asymmetrical, with all major tributaries coming from the right side. In its final stretch, the river flows through the valley of the San River, which it falls into at an altitude of 147 m above sea level. The difference in altitude between the beginning of the river and its mouth is about 75 m, for an average drop of 1.4‰. The Bukowa discharges on average 4.9 m^3/s into the San (Michalczyk, Wilgat, 1998). The Bukowa River is the largest river in the Janów Forests Landscape Park. The park is located at an altitude of 150–220 m above sea level, and its relief is not very diverse. The forests are crossed by numerous parallel valleys of small rivers and streams flowing from the edges of upland areas. An important element of the hydrographic network of the park is its numerous swamps and peatlands. Numerous wetlands and fragments of river valleys are occupied by large fish pond complexes situated among forests (Rąkowski et al., 2004).

Five sites were designated on the Bukowa River.

Site 1 – in Korytków Duży (50°36′52.5″N; 22°37′43.5″E)

A site located about 5 km from the source. The river here had the character of a meadow stream. It was regulated, with a bed about 1 m wide and 0.2–0.5 m deep. The bottom was sandy; by the banks and in places with abundant vegetation it was composed of silt or sand and silt. *Veronica anagallis-aquatica* L., *Sparganium erectum* L. em. Rchb. s. s. and *Elodea canadensis* Michx. grew in the lotic zone. *Scirpus sylvaticus* L., *Juncus effusus* L., *Galium palustre* L., *Carex*

vesicaria L., *C. canescens* L. and flooded grasses grew by the banks. In places with a slow current, the surface of the water was covered with *Lemna minor* L. and *L. trisulca* L. There were large fluctuations in the water flow at this site, depending on precipitation (Table 1).

Site 2 – in Szewce (50°35′27.1″N; 22°30′34.7″E)

A site located about 15 km from the source. The river here had the character of a meadow stream. The river bed was winding, lay deep below the ground surface, and was about 1.5 m wide, in places (at the meanders) up to 3 m. The depth was varied, from 0.8 to 1.3 m. Lotic zone: bottom covered with a thin layer of sandy sediments with compact clay underneath, nearly devoid of vegetation. Sparse *Fontinalis* sp. was present on sunken trunks and branches. Lentic zone: sparse, flooded vegetation by the banks, including *Galium uliginosum* L., *G. palustre*, *Carex nigra* Reichard and *Iris pseudacorus* L.

Site 3 – in Momoty Górne (50°36′02.5″N; 22°25′12.7″E)

A site located about 23 km from the source, within a forest. The width of the riverbed varied from 8 to 10 m. This section of the river was hydro-morphologically varied. Over a dozen or so metres there were riffles, pools, and sandbanks. Water flow was laminar, in some places turbulent. Depth varied from 0.2 m to 1.5 m, on average 0.5 m. The bottom was sandy over nearly the entire cross-section of the river, but rocky over a stretch that was similar to a mountain river, and composed of sand and silt or silt in the marginal pools. Aquatic vegetation was sparse. In the lotic zone, a few isolated specimens of *Sparganium erectum* and *Fontinalis* sp. were present. *Veronica anagallis-aquatica*, *Mentha aquatica* L. and flooded *Poa palustris* L. grew by the banks. Water from fish ponds was periodically discharged into the river.

Site 4 – in Łążki Przymiarki (50°37′11.8″N; 22°17′51.5″E)

About 33 km of the course of the river, within a forest. The river meandered slightly. It lay quite deep below the ground surface, and was about 8 m wide and 1 m deep. The bottom was sandy over nearly the entire transverse profile of the river, with small amounts of silt in the marginal pools. Numerous embankments and shoals. Water current quite fast (Table 1), uniform water flow. Vegetation sparse. In the lotic zone there were isolated stems of *Sparganium erectum*. Closer to the banks there was more abundant *Elodea canadensis*, *Batrachium aquatile* (L.) Dumort. and *Potamogeton pusillus* L. Flooded *Scirpus sylvaticus*, *Sium latifolium* L., *Phalaris arundinacea* L. and *Poa palustris* grew by the banks.

Site 5 – in Szwedy (50°37′12.7″N; 22°14′12.2″E)

Site located about 40 km from the sources. Shaded, hydro-morphologically diverse stretch of the river. Width up to 13 m, depth varied, on average 0.6 m. Sandy sediments, not very thick, with clay shale underneath. In the vegetation zone by the banks there were sediments of clay and silt, but the bottom was rocky over a stretch that was similar to a mountain river. The current was quite fast (Table 1), the flow was turbulent in places, uniform over the course of the year. The lotic zone was nearly devoid of vegetation; there were only a few clumps of *Potamogeton crispus* L. and aquatic mosses forming mats. In the lentic zone, *Scirpus sylvaticus* and flooded grasses were dominant.

Methods and material

Field research

The field research was conducted mainly from April to November 1996 and from May to October 1997. Samples were taken once a month. At each site samples were taken from two zones of the river: the lentic zone (near the riverbanks) and the lotic zone (in the middle of the river). Sampling was done with a hand net. The net had a round frame with a 0.25 m diameter and 250 µm mesh size. A single sample was taken over a distance of about 10 m. A total of 160 samples were collected. The material comprised a total of 2,791 individuals. The material collected in the field was transported to the laboratory and segregated in white cuvettes. The following works were used for identification of water mites: Viets (1936), Sokolov (1940), Davids et al. (2007), Di Sabatino et al. (2010), and Gerecke et al. (2016). Species nomenclature and systematics were adopted according to Davids et al. (2007), Di Sabatino et al. (2010) and Gerecke et al. (2016). Allocation of species to synecological groups was based on literature data (Smit, van der Hammen, 2000; Davids et al., 2007; Di Sabatino et al., 2010; Gerecke et al., 2016), taking into account the specificity of Poland (Biesiadka, 2008) and the region under investigation (Kowalik, 1984).

During collection of hydrobiological samples, the basic physical and chemical indicators of the water were measured: temperature (°C), pH, electrolytic conductivity (µS/cm), dissolved oxygen (mg O_2/l), and water saturation with oxygen (%). The measurements were made using a Slandi kit (TM204 thermometer, PH204 pH meter and CM204 conductivity meter) and an Elmetron CX401 multifunction meter. The water current was determined by the floating object method, by measuring the object's flow time over a distance of 10 m.

Statistical analyses

Descriptive statistics (sums, means, range, and standard deviation) were calculated using PAST ver. 3.16/2017 software (Hammer et al., 2001). The same software was used to calculate the Shannon–Wiener index (H'). Analyses of quantitative faunistic similarities based on the Bray–Curtis formula were carried out using BioDiveristy Pro ver. 2 software (McAleece et al., 1997). Similarity dendrograms were generated using BioDiveristy Pro software. Group Average was used to create clusters. Correspondence analysis (CA) was also performed using this software.

The normality of the data distribution was checked by the Shapiro-Wilk test in Statistica 13.1 software. The Mann-Whitney U test (Z) was used to compare two independent samples. The Kruskal-Wallis test (H) or one-way ANOVA (depending on the data distribution) was used to compare multiple independent samples. The Spearman correlation coefficient (R_S) was used to determine the relationship between parameters. All tests were carried out in Statistica 13.1 software. The statistical significance level was set at $p < 0.05$.

Results

Physicochemical properties of the water and water current

The water temperature ranged from 2.6°C to 20.1°C. Both extreme values were found at Site 1 (Table 1), and the greatest temperature fluctuations during the study period were found at this site (±SD 4.81). In the longitudinal profile of the river, the average water temperature at the first two sites was higher than at the other three (Table 1), but the differences in water temperature

between sites were not statistically significant (ANOVA F(4, 68) = 0.33332, p = 0.85462). Water pH values ranged from 6.00 to 8.01 (Table 1). Differences in pH between the sites were statistically significant (ANOVA F(4, 68) = 3.3571, p = 0.01442). Post-hoc tests showed a statistically significant difference (Tukey test, p = 0.0256) in pH between Site 2 (lowest mean pH) and Site 5 (highest mean pH; Table 1). In the longitudinal profile of the river, the first two sites had lower average water pH than the other three. Electrolytic conductivity ranged from 123 to 684 µS/cm (Table 1). The lowest mean electrolytic conductivity was found at Site 2 (202 µS/cm) and the highest at Site 3 (273 µS/cm). Differences in electrolytic conductivity between the sites were not statistically significant (Kruskal-Wallis test: H (4, N = 73) = 5.613654, p = 0.2299). The oxygen content in the water ranged from 6.40 to 11.78 mg O_2/l (Table 1). There was substantial variation in oxygen conditions between the sites, but the differences were not statistically significant (ANOVA, F(4, 49) = 2.2947, p = 0.07248). The best oxygen conditions were found at Site 5 (average 8.69 mg O_2/l, ±SD 1.92), and the worst at Site 1 (average 6.61 mg O_2/l, ±SD 1.77). The oxygen saturation of the water ranged from 38.0% to 115.7% from 6.40 to 11.78 mg O_2/l (Table 1).

Table 1. Values of analyzed environmental parameters (range; mean; ±SD). a, b – the differences in the values between particular sites were statistically significant

Parameter	Site 1	Site 2	Site 3	Site 4	Site 5
Temperature (°C)	2.6–20.1; 12.5; ±4.81	4.7–19.7; 12.7; ±4.57	4.8–16.5; 11.7; ±3.72	4.1–16.9; 11.2; ±4.08	3.9–17.0; 11.4; ±4.36
pH	6.51–7.30; 6.88; ±0.21	6.00–7.61; 6.81[a]; ±0.49	6.10–7.72; 7.10; ±0.39	6.70–7.79; 7.16; ±0.33	6.45–8.01; 7.25[b]; ±0.43
Electrolytic conductivity (µS/cm)	138–468; 259; ±98.68	123–317; 202; ±63.68	132–684; 273; ±145.01	125–274; 210; ± 43.46	144–441; 253; ±82.27
Dissolved oxygen (mg O_2/l)	3.60–9.91; 6.61; ±1.77	5.60–10.80; 8.02; ±1.58	5.90–10.66; 7.82; ±1.56	5.40–11.78; 8.53; ±2.09	5.60–11.66; 8.69; ±1.92
Water saturation with oxygen (%)	38.0–82.4; 63.9[a]; ±13.65	55.0–93.4; 79.2; ±12.33	57.0–87.2; 75.0; ±9.75	56.0–109.9; 81.7[b]; ±17.13	56.0–115.7; 83.9[b]; ±16.2
Water current (m/s)	0.12–0.62; 0.33[a]; ±0.16	0.14–0.66; 0.37[a]; ±0.17	0.21–0.50; 0.39[a]; ±0.10	0.43–1.0; 0.62[b]; ±0.13	0.50–0.83; 0.68[b]; ± 0.12

Differences in the values of this parameter between sites were statistically significant (ANOVA F(4, 49) = 3.4750, p = 0.01413). Post-hoc tests showed statistically significant differences in oxygen saturation between Sites 1 and 4 (Tukey test, p = 0.040) and between Sites 1 and 5 (Tukey test, p = 0.013). In the longitudinal profile of the river, dissolved oxygen and water saturation with oxygen increased along the course of the river (Table 1). Water current values fluctuated between 0.12 and 1.00 m/s (Table 1). The differences observed in this parameter between sites were statistically significant (ANOVA, F(4, 65) = 18.361, p = 0.00000). These differences were found for the group of Sites 1, 2 and 3, where the current was slower, and Sites 4 and 5, where it was much faster (Table 1).

Water mite fauna

General characteristic, the structure of dominance and species diversity

A total of 2,791 individuals belonging to 51 species, 24 genera and 15 families were caught (Table 2).

Table 2. Qualitative and quantitative composition of water mite fauna found in the Bukowa River. SG – synecological group: Rb – rheobionts, Rp – rheophiles, St – stagnobionts and stagnophiles, S/R – species occurring both in running and stagnant waters; le – lentic zone of the river, lo – lotic zone of the river, To – total at the site

No.	Species	SG	Site 1			Site 2			Site 3			Site 4			Site 5			In the River		
			le	lo	To	le	lo	To	le	lo	To	le	lo	To	le	lo	To	le	lo	To
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1.	*Limnochares aquatica* (L.)	St				34	18	52										34	18	52
–	*Eylais* sp.	–		1	1	1	1	2										1	2	3
2.	*Hydrodroma torrenticola* (Walt.)	Rp				3		3				2		2				5		5
3.	*Thyopsis cancellata* (Protz)	Cr										1		1	3		3	4		4
4.	*Hydryphantes planus* Thon	St													3		3	3		3
–	*Hydryphantes* sp.	–	1		1							2		2				3		3
5.	*Nilotonia borneri* (Walt.)	S/R													1		1	1		1
6.	*Lebertia fimbriata* Thor	Rp				2	11	13	14	7	21	11	10	21	7	12	19	34	40	74
7.	*L. oblonga* Koen.	Rp				19	45	64	38	21	59	14	6	20	12	15	27	83	87	170
8.	*L. rivulorum* Viets	Rb				1	6	7	2	1	3	3	1	4	10	14	24	16	22	38
9.	*L. dubia* Thor	Cr		1	1							1		1				1	1	2
10.	*L. inaequalis* (Koch)	Rp	25	42	67	62	43	105	80	17	97	49	10	59	29	18	47	245	130	375
11.	*L. insignis* Neum.	Rp	2		2	10	7	17	3	2	5	11	3	14	5	2	7	31	14	45
12.	*L. pilosa* Maglio	Rp				2	5	7										2	5	7
13.	*L. porosa* Thor	Rp				5	25	30		3	3	2		2	1	4	5	8	32	40
–	*Lebertia* sp. (deutonymphs)	–	4	1	5	15	7	22	11	3	14	10	1	11	1	3	4	41	15	56
14.	*Sperchon clupeifer* Piers.	Rb				3	7	10	15	58	73	13	27	40	11	35	46	42	127	169
15.	*S. papillosus* Thor	Rb														1	1		1	1
16.	*S. setiger* Thor	Rb					3	3	4	6	10	38	20	58	5	10	15	47	39	86
–	*Sperchon* sp. (deutonymphs)	–							1	3	4							1	3	4
17.	*Sperchonopsis verrucosa* (Protz)	Rb											1	1		1	1		2	2
18.	*Teutonia cometes* (Koch)	S/R				10	1	11	3		3	10		10	5		5	28	1	29
19.	*Torrenticola amplexa* (Koen.)	Rb				90	94	184	9	1	10	3	1	4	3		3	105	96	201
20.	*Albia stationis* Thon	Rp										3		3				3		3
21.	*Axonopsis complanata* (Müll.)	St				1		1										1		1
22.	*Parabrachypoda modesta* (Koen.)	Rp				8	5	13	3		3				2		2	13	5	18
23.	*P. montii* (Maglio)	Rp				4	3	7										4	3	7
24.	*Ljania bipapillata* Thor	Rp										1		1				1		1
25.	*Atractides distans* (Viets)	Rb				1		1				3		3	1	1	2	5	1	6
26.	*A. nodipalpis* Thor	Rb				7	16	23	3	5	8	10	10	20	4	8	12	24	36	63
27.	*A. spinipes* Koch	Rb				1		1										1		1

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
–	*Atractides* sp. (deutonymphs)	–														1	1		1	1
28.	*Hygrobates calliger* Piers.	Rb				46	67	113	20	40	60	22	16	38	8	34	42	96	157	253
29.	*H. fluviatilis* (Ström)	Rb	4	1	5	10	23	33				5		5	1	4	5	20	28	48
30.	*H. longipalpis* (Herm.)	S/R	54	15	69		1	1				1		1	4	1	5	59	17	76
31.	*H. setosus* Bess.	Rp	2	1	3	2	2	4	33		33	35	3	38	27	2	29	99	8	107
–	*Hygrobates* sp. (deutonymphs)	–				4	1	5		1	1				1	1	2	5	3	8
32.	*Limnesia maculata* (Müll.)	St							9	7	16	1	1	2				10	8	18
33.	*L. undulatoides* Davids	St							5		5							5		5
–	*Limnesia* sp. (deutonymphs)	–							5	1	6							5	1	6
34.	*Forelia brevipes* (Neum.)	St							2		2							2		2
35.	*F. liliacea liliacea* (Müll.)	St							5		5							5		5
36.	*F. variegator* (Koch)	S/R	2	1	3	5	7	12	19		19	29	5	34	35	4	39	90	17	107
37.	*Nautarachna crassa* (Koen.)	Rp				1		1	6		6							7		7
38.	*Piona carnea* (Koch)	St										1		1				1		1
39.	*P. paucipora* (Thor)	St							9		9							9		9
40.	*P. pusilla* (Neum.)	St							7		7	1		1				8		8
–	*Piona* sp. (deutonymphs)	–							162	7	169	2		2		1	1	164	8	172
41.	*Neumania limosa* (Koch)	St							3	1	4							3	1	4
42.	*Unionicola crassipes* (Müll.)	St							1		1							1		1
43.	*Arrenurus crassicaudatus* Kram.	St							137	10	147	7	1	8	7	1	8	151	12	163
44.	*A. cylindratus* Piers.	Cr		1	1														1	1
45.	*A. globator* (Müll.)	St				1		1	1		1							2		2
46.	*A. mediorotundatus* Thor	St													2		2	2		2
47.	*A. tubulator* (Müll.)	St							2		2							2		2
48.	*A. sinuator* (Müll.)	St								1	1								1	1
–	*Arrenurus* sp. (deutonymphs)	–							1		1							1		1
49.	*Mideopsis crassipes* Soar	Rp	51	39	90	64	25	89	10	1	11	10		10	10	1	11	145	66	211
50.	*M. orbicularis* (Müll.)	St							8	5	13				1		1	9	5	14
51.	*M. roztoczensis* Bies. et Kow.	Rp				27	10	37	8		8	25		25	12		12	72	10	82
–	*Mideopsis* sp. (deutonymphs)	–				1		1	2		2				1		1	4		4
Total individuals			145	103	248	440	433	873	641	201	842	326	116	442	212	174	386	1,764	1,027	2,791
Total species			7	8	9	26	22	28	29	17	31	28	15	29	26	19	28	47	32	51

The dominant species (dominance > 5%) in the material were *Lebertia inaequalis* (13.4%), *Hygrobates calliger* (9.1%), *Mideopsis crassipes* (7.6%), *Torrenticola amplexa* (7.2%), *Lebertia oblonga* (6.1%), *Sperchon clupeifer* (6.1%) and *Arrenurus crassicaudatus* (5.8%). The number of specimens caught at individual sites ranged from 248 to 873 (Table 2). Differences in the

number of specimens caught at individual sites were not statistically significant (Kruskal-Wallis test: H (4, N = 150) = 3.408295 p = 0.4920). The number of species caught at individual sites ranged from 9 to 31 (Table 2). A high correlation was found between the number of specimens caught and the number of species found ($R_S = 0.84$, $p < 0.05$).

More individuals and species were found in the lentic zone of the river (1,764 individuals, 47 species), than in the lotic zone (1,027 individuals, 32 species), but the differences in the number of individuals caught in these two zones were not statistically significant (Z = 0.9645, p = 0.3347). The dominant species in the lentic zone were *Lebertia inaequalis* (13.9%), *Arrenurus crassicaudatus* (8.6%), *Mideopsis crassipes* (8.2%), *Torrenticola amplexa* (6.0%), *Hygrobates setosus* (5.6%), *H. calliger* (5.4%) and *Forelia variegator* (5.1%). The dominant species in the lotic zone were *Hygrobates calliger* (15.3%), *Lebertia inaequalis* (12.7%), *Sperchon clupeifer* (12.4%), *Torrenticola amplexa* (9.3%), *Lebertia oblonga* (8.5%) and *Mideopsis crassipes* (6.4%).

Greater species diversity was found in the lentic zone than in the lotic zone (H' = 2.99 and H' = 2.72, respectively). As a rule, species diversity was greater in the lentic zone at individual sites (Figure 1). The exception was Site 2, where slightly greater species diversity was found in the lotic zone (H' = 2.52 vs. H' = 2.43 in the lentic zone).

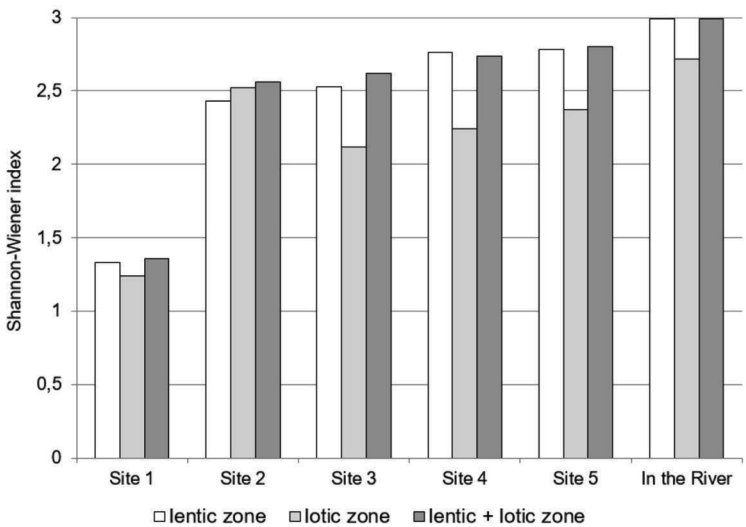

Figure 1. Shannon-Wiener index values at particular sites and lentic and lotic zones in the Bukowa River

Faunistic similarities

The faunistic similarities between sites ranged from 19.4% to 78.3%. The similarity dendrogram shows the distinctiveness of the fauna at Site 1, and a group of sites with similar fauna (Sites 3–5; Figure 2A). The similarities of the fauna of Sites 3–5 ranged from 54.8% to 74.3%. In the analysis at the habitat level, the distinctness of the fauna of the lentic and lotic zones at Site 1 from the zones at other sites also stands out (Figure 2B). Within the upper branch of the dendrogram, the highest similarity was found between the lentic and lotic zones at Site 2 (72.1%). In the group comprising Sites 3–5, the fauna of the lentic zones formed one cluster, and the fauna of the lotic zones formed another cluster (Figure 2B).

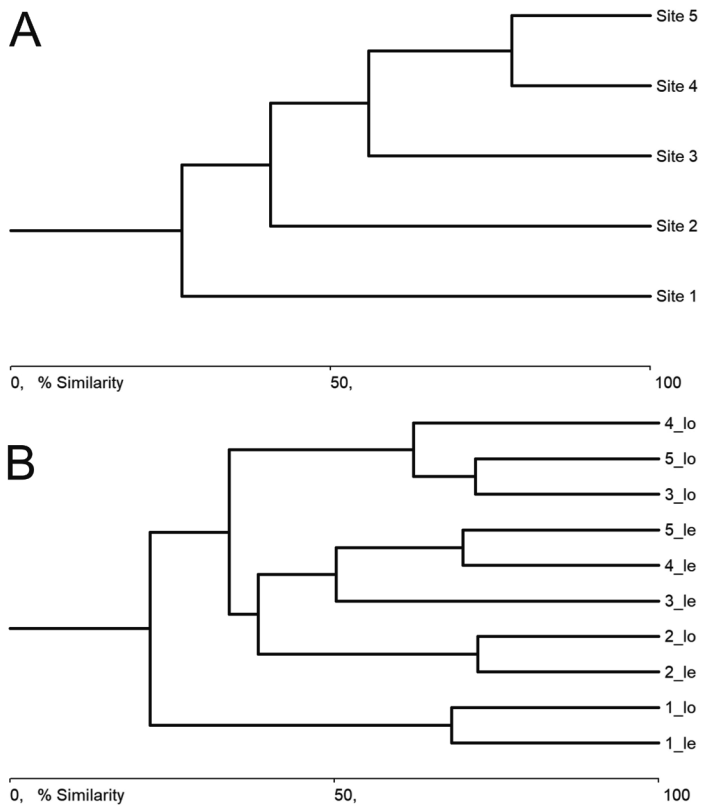

Figure 2. Faunistic similarities between sites (A) and zones (B) in the Bukowa River. 1–5 – sites, le – lentic zone, lo – lotic zone

Synecological structure of fauna

Rheophiles were the largest synecological group in the Bukowa River (45.6%, 15 species), followed by rheobionts (34.4%, 11 species). The group of stagnobionts and stagnophiles comprised many species, but had a much smaller percentage share (11.6%, 18 species). Species occurring in both running and standing waters (four species) accounted for 11.6%. Three crenophilous species were also distinguished in the structure of the fauna, but very few of these were caught (seven individuals in total; Table 2). Differences were found in the synecological structure of the fauna between the lentic and lotic zones of the river. In the lentic zone, there was a larger proportion of rheophiles, stagnobionts and stagnophiles, and species occurring in both running and standing waters (Figure 3). The last two groups of species, which can be considered less typical of rheocoenoses, together accounted for 27.8% of the fauna. In the lotic zone, rheobionts constituted 51.5% of the fauna. Stagnobionts and stagnophiles and the group of species occurring in both running and standing waters together accounted for only 8.1% in the lotic zone of the river (Figure 3).

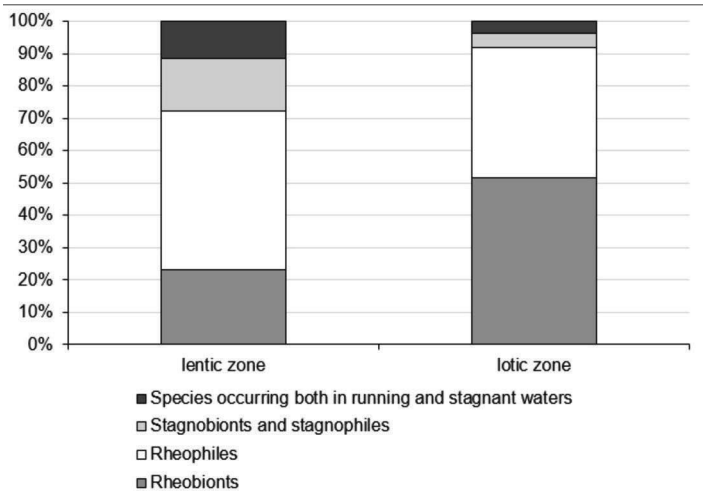

Figure 3. Quantitative synecological structure of water mite fauna in the Bukowa River

Occurrence and distribution of water mites in the transverse profile of the river

For the nine most abundant species (>100 individuals), distribution among the river zones was analysed. The species most associated with the lotic zone were *Sperchon clupeifer* (75.1% of individuals caught in this zone) and *Hygrobates calliger* (62.1% of individuals; Figure 4). Both species were found at all sites except Site 1 (Table 2). *Lebertia oblonga* and *Torrenticola amplexa* were found in equal numbers in both river zones (Figure 4). Another two species, *Lebertia inaequalis* and *Mideopsis crassipes*, were caught more frequently in the lentic zone, but they

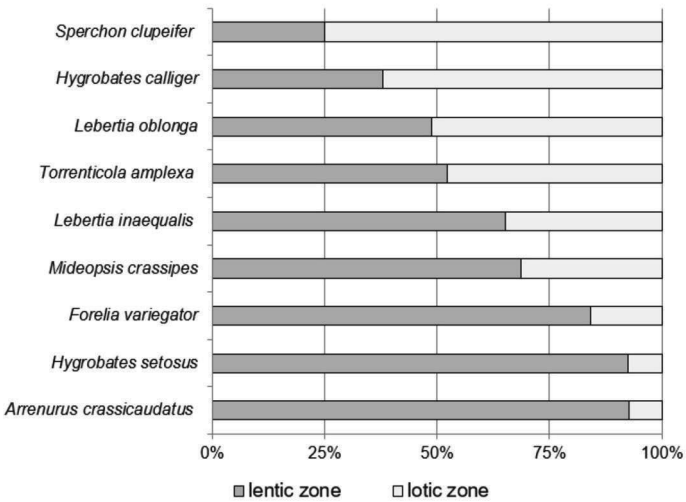

Figure 4. Percentage share of the most numerous species in lentic and lotic zone in the Bukowa River

were also quite numerous in the lotic zone (Table 2, Figure 4). Two species, *Forelia variegator* and *Hygrobates setosus*, can be considered to be associated with the lentic zone; the majority of individuals were caught in this zone (Figure 4). Both species were found in the lentic zone at all sites (Table 2). *Arrenurus crassicaudatus*, of which 92.6% of individuals were caught in the lentic zone, was only abundant at one site (Site 3), so it cannot be considered a typical element of the lentic zone of the Bukowa River.

The occurrence of water mites in the longitudinal profile of the river

The fewest individuals (248) were caught at Site 1 (Figure 5, Table 2). Between Sites 1 and 2 there was a sharp increase in the number of individuals, reaching the highest value of all sites (873 individuals). From Site 2, the number of individuals caught decreased along the course of the river; slightly fewer water mites were caught at Site 3 than at Site 2, and at Sites 4 and 5 a sharp decrease was noted in the number of specimens caught (Figure 5, Table 2). The curve showing the changes in the number of species along the course of the river was similar: after the minimum at Site 1 (9 sp.), there was a sharp increase in the number of individuals caught, reaching a maximum at Site 3 (31 sp.), and then the number of species decreased along the course of the river, but not as dramatically as the number of individuals (Figure 5).

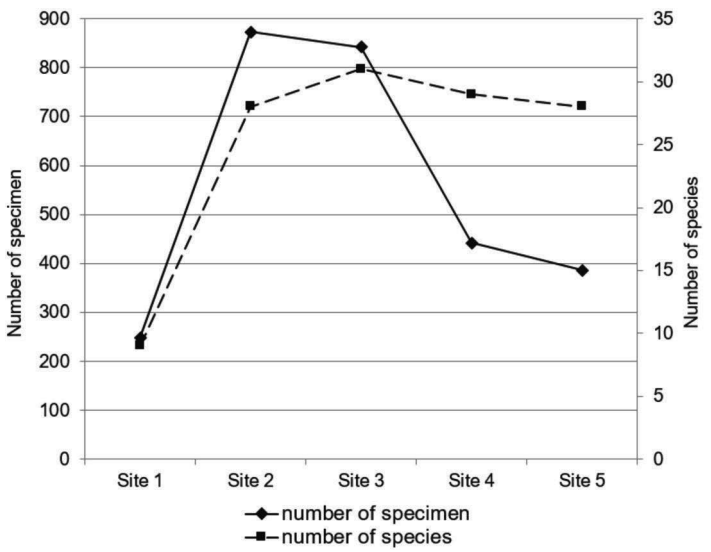

Figure 5. Changes in the number of specimen and species of water mite fauna along the longitudinal profile of the Bukowa River

More detailed analyses of distribution in the longitudinal profile of the river were conducted for the nine most abundant species (abundance > 100 individuals). For the upper course of the river, the presence of *Mideopsis crassipes* (Sites 1 and 2) and *Torrenticola amplexa* (Site 2) was characteristic (Figure 6, cloud 1). Both species were caught in high numbers in both the lotic and lentic zones (Figure 4, Table 2). The position of three species – *Lebertia oblonga*, *L. inaequalis* and *Hygrobates calliger* – near zero on the X axis (site gradient – from Site 1 to Site 5) indicates

relatively uniform distribution of these species along the course of the river. *Arrenurus crassicaudatus* was present in high numbers only in the lentic zone of Site 3 (Figure 6, Table 2). Cloud 2 in the CA diagram indicates zonal distribution of *Sperchon clupeifer*. This species was associated with Sites 3–5, and more specifically, with the lotic zones of these sites (Figure 6). Cloud 3 in the CA diagram groups two species, *Hygrobates setosus* and *Forelia variegator*, and two sites (4 and 5), and more specifically, the lentic zones of these sites (Figure 6). Therefore, both species can be regarded as characteristic of the middle course of the river and the beginning of the lower course, and at the same time, as taxa characteristic of the lentic zones of the river (Figure 4).

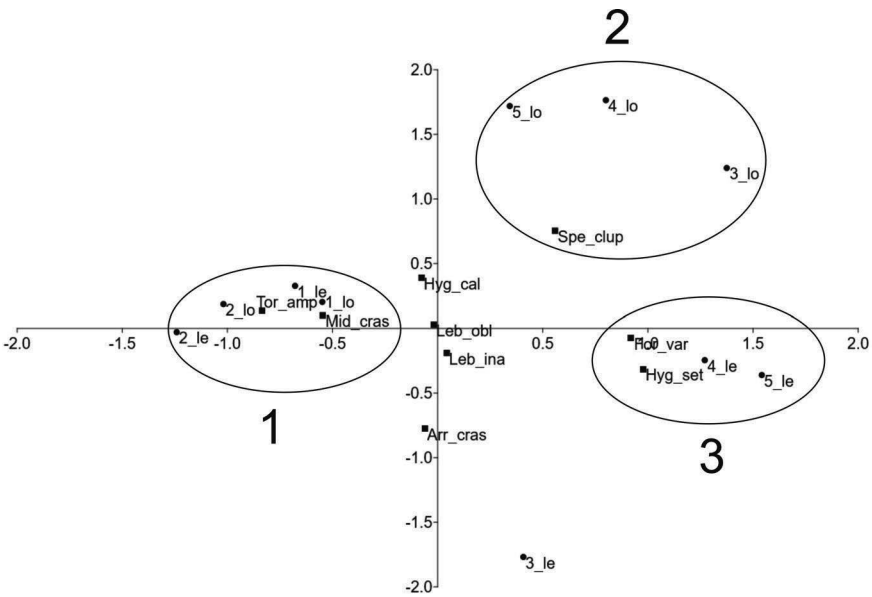

Figure 6. Correspondence analysis (CA) showing occurrence of the most numerous species along the longitudinal profile of the Bukowa River

Discussion

The water mite communities of the Bukowa River were to a certain degree similar to those of other lowland rivers in Poland, but we can point out certain distinctive elements that distinguish this river from other rivers of Poland. An element specific to the Bukowa River was a much higher abundance of *Lebertia inaequalis* than in other Polish rivers. This species was even more abundant in other rivers of the Janów Forests Landscape Park (Stryjecki, 2002). *L. inaequalis* has also been found to be the most abundant species of the genus *Lebertia* in other rivers of the Biłgoraj Plain (Zawal, Kowalik, 2013). In the neighbouring geographic macroregion of Roztocze, *L. inaequalis* has been a permanent component of the fauna of rivers, but not so abundant as in the rivers of the Sandomierz Basin (Kowalik, 1981; Stryjecki, Kowaliczyk-Pecka 2013b; Kowalik et al., 2014; Biesiadka et al., 2015). *L. inaequalis* has been caught in other lowland rivers of Poland (Biesiadka, 1972; Cichocka, 1996a, 2006; Zawal, Sadanowicz, 2012; Zawal et al., 2017), but in none of these has it reached such abundance and dominance as in the Bukowa River and other

rivers of the Biłgoraj Plain. *L. inaequalis* has not been found in some other rivers of Poland (Bazan-Strzelecka, 1964; Biesiadka, 1970). This analysis indicates that the abundance of *L. inaequalis* in the rivers of the Sandomierz Basin macroregion and the Biłgoraj Plain mesoregion is a regional characteristic.

The very high abundance of *Arrenurus crassicaudatus*, which had the status of dominant in the Bukowa River, requires separate explanation. Very high numbers of *A. crassicaudatus* were noted at only one site – just downstream of the mouth of a canal discharging water from fish ponds, in which it was one of the most numerous species (Stryjecki et al., 2015). *A. crassicaudatus* should be considered an allochthonous element of the Hydrachnidia communities of the Bukowa River, present in the river due to human impact.

A feature common to the Bukowa River and other Polish rivers was the high abundance of the rheobiontic species *Hygrobates calliger* and *Sperchon clupeifer*. *Hygrobates calliger* has attained the status of dominant in certain Polish rivers (Biesiadka, 1970; Cichocka, 1996a), and has been caught in very high numbers in others (Zawal et al., 2017). *Sperchon clupeifer* has also been found in high numbers in other Polish rivers (Kowalik, 1981; Zawal et al., 2017), sometimes attaining dominant status (Cichocka, 1996a). Other species that were fairly abundant in the Bukowa River and have been caught in other Polish rivers (Biesiadka, 1970, 1972; Kowalik, 1981; Cichocka, 1996a, 2006; Zawal, Sadanowicz, 2012; Zawal et al., 2017) include *Lebertia oblonga*, *Sperchon setiger*, *Hygrobates fluviatilis*, *H. setosus* and *Atractides nodipalpis*.

Lowland watercourses have highly diverse water mite fauna, which includes rheobiontic and rheophilic as well as stagnophilic elements, and its character varies with the size of the river and the velocity of the current (Zawal, Sadanowicz, 2012). In the Bukowa River the largest synecological group was rheophiles and rheobionts. These two synecological groups usually dominate in rivers, particularly natural rivers or those little affected by human impact (Biesiadka, 1970, Cichocka, 1996b; Zawal et al., 2017). The dominance of rheophiles and rheobionts has also been noted in other lowland rivers of Poland (Biesiadka, 1970; Cichocka, 1996a, 2006; Zawal, Sadanowicz, 2012; Zawal et al., 2017), as well as upland and lowland rivers of central-eastern Poland (Kowalik, 1981; Stryjecki, Kowaliczyk-Pecka 2013b; Zawal, Kowalik, 2013; Kowalik et al., 2014; Biesiadka et al., 2015). The very large quantitative share of rheophiles and rheobionts in the Bukowa River is indicative of the natural character of this river. According to many authors, human impact and transformation of aquatic ecosystems cause a change in water mite fauna: rheophilous species, with narrow tolerance for environmental factors (e.g. water current and temperature or nutrient level), are gradually replaced by species with a broader ecological valence, which are often also present in standing water bodies (van der Hammen, Smit, 1996). The values of the physicochemical parameters confirmed the good quality of the water of the Bukowa River.

Fewer species and individuals were caught in the lotic zone of the Bukowa River. Impoverishment of fauna was noted in particular on the sandy bottom with sparse aquatic vegetation. Impoverishment of fauna in such habitats has also been reported by other authors (Kowalik, 1981; Cichocka, 1996a). Fewer individuals and species were caught in the lotic zone of the Bukowa River because this zone was uniform in terms of habitat (sandy bottom and lack of plants). In the lowland Pasłęka River, with well-developed current habitats, over twice as many individuals were caught in the lotic zone of the river than in the marginal pools (Cichocka, 1996a). In the Bukowa River many more individuals and species were caught in the marginal pools and in the central part of the river, in places with abundant aquatic vegetation. The results confirm the relationship between abundance of water mites and the degree of abundance of vegetation in water courses (Kowalik, 1981; Cichocka, 1996a, 2006; Zawal, Sadanowicz, 2012; Zawal et al., 2017).

The species most associated with the lotic zone and fast water flow in the Bukowa River were *Sperchon clupeifer* and *Hygrobates calliger*. The results confirm literature data on the preferences of these species for habitats associated with fast water flow (Biesiadka, 1970; Kowalik, 1981; Cichocka, 1996a; Gerecke et al., 2016; Zawal et al., 2017). At the first two sites in the river, the fauna was similar in the lotic and lentic zones. The substantial similarity of the fauna of these two zones was due to the narrowness of the river, which was conducive to intensive migration of species between zones. In the case of small rivers, the presence of species preferring current habitats is usually noted over the entire cross-section of the river (Zawal, Sadanowicz, 2012).

The most abundant species in the Bukowa River, *Lebertia inaequalis*, was caught in higher numbers in the lentic zone (65.3% individuals). According to Kowalik (1981), *L. inaequalis* shows a considerable predisposition for environments with a moderate current and submerged vegetation. In the Pasłęka River, it has been found to be associated with lentic zones without vegetation (Cichocka, 1996a). In the Krutynia River, *L. inaequalis* was most abundant in the lotic zone (Cichocka, 2006). According to Smit and van der Hammen (2000), *L. inaequalis* is found in both running and standing waters. In the Netherlands it has been found in lowland streams (natural and channelized), man-made spring brooks, large lakes, and large canals. In standing water bodies it occupies zones with waves. In Poland it is a typical species of flowing water bodies (Biesiadka, 2008). Data from the Bukowa River, as well as other rivers of the Janów Forests Landscape Park (Stryjecki, 2002), indicate that the most characteristic habitat for this species is the lentic zone of rivers, with abundant aquatic vegetation.

It is worth noting the habitat preferences of *Hygrobates setosus*. *H. setosus* is a sister species of *H. nigromaculatus*. The river species (*H. setosus*) was finally separated from the lake species (*H. nigromaculatus*) relatively recently (Martin et al., 2010). In earlier studies on flowing water bodies, *H. setosus* was identified as *H. nigromaculatus* (Kowalik, 1981). In many other publications the species identified as *H. nigromaculatus* was probably *H. setosus*. This is indicated by its abundance in the flowing water bodies discussed by the authors and its classification in these studies as a rheophilous species (Biesiadka, 1979; Cichocka, 1996a, Kowalik et al., 2014), whereas *H. nigromaculatus* is a typical lake species (Martin et al., 2010). In the Bukowa River *H. setosus* showed a very clear preference for the lentic zone of the river (92.5% of individuals were caught here). In other rivers of Poland, *H. setosus* has also been caught mainly in marginal pools and habitats with a slow current (Kowalik, 1981; Zawal, Sadanowicz, 2012; Zawal et al., 2017). Documentation of the habitat preferences of *Hygrobates setosus* is important because it contributes to more complete knowledge of the habitat preferences of this species. The results from the Bukowa River confirm literature data indicating that it is a species typical of flowing water bodies, inhabiting marginal pools and zones with slower water flow.

Forelia variegator was also associated with the lentic zone of the Bukowa River. This is a species found in both flowing and standing water bodies (Biesiadka 2008; Gerecke et al., 2016), so its preference for the lentic zone of rivers is natural. The results obtained confirm the preference of this species for places with a slower water flow in running water bodies (Kowalik, 1981; Cichocka, 1996a, 2006; Zawal et al., 2017).

Site 1 in the river was markedly different from the other sites – the fewest species and individuals were caught here. Over this stretch, the river had the form of a straight ditch. A similar phenomenon of quantitative impoverishment in the upper, unregulated stretch of a river has been found in the Pasłęka River (Cichocka, 1996a). The results confirm literature data indicating that regulation of a river channel causes impoverishment of water mite fauna, mainly the disappearance of rheobionts and rheophiles (Biesiadka, 1972; Martin, 1996; van der Hammen, Smit,

1996). From Site 2 the number of individuals caught began to decline. This was due to habitat conditions: at Sites 3–5 the bottom was sandy with very sparse vegetation over nearly the entire cross-section of the river. In such habitats water mite fauna is usually less abundant than in places with more aquatic vegetation and a larger share of organic sediments (Kowalik, 1981; Cichocka, 1996a; Zawal, Sadanowicz, 2012). The fairly large number of individuals and maximum number of species at Site 3 were due to the periodic influx of pond water. Allochthonous stagnobiontic species which do not naturally occur in the river entered it together with this water. The influx of these species increased the species richness at the site and the total species richness in the Bukowa River, but disturbed the natural character of the Hydrachnidia communities of the river. Human impact consisting in the introduction of species alien to river fauna should be considered a negative phenomenon.

Zonal occurrence of certain species was noted along the course of the Bukowa River. *Mideopsis crassipes* and *Torrenticola amplexa* were associated with the upper course. This type of distribution of these species should be considered an individual characteristic of this river, as both of these species have been caught mainly in the middle course of the Pasłęka River (Cichocka, 1996a), and in the middle and lower course of the Krąpiel River (Zawal et al., 2017). *Sperchon clupeifer* was associated with the middle and lower course of the river, and more precisely, with the lotic zones of these stretches of the river. At Sites 3–5, a lower average water temperature was recorded than in the upper course of the river, as well as higher oxygen content and oxygen saturation. Furthermore, the water current in the middle and lower course of the river was much faster than in the upper course. For *Sperchon clupeifer*, as a hemistenothermal rheobiont (Kowalik, 1981), these environmental factors had a fundamental influence on its distribution in the longitudinal profile of the river. In the lowland Krąpiel River, this species was also caught in the largest numbers in the lower course of the river (Zawal et al., 2017). *Hygrobates setosus* and *Forelia variegator* were associated with the middle and final stretch of the river (Sites 3-5), and more specifically, with the lentic zones of these stretches. Both species are characteristic of lentic environments by the shore (Kowalik, 1981; Zawal, Sadanowicz, 2012; Zawal et al., 2017), which are poorly developed in the upper course of watercourses.

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A meta-analysis of genetic and environmental factors impacting on obesity

Agata Kopiniak,[1] Ewa Rębacz-Maron[2]

[1] Student of biology at the Wrocław University of Environmental and Life Sciences
[2] University of Szczecin, Department of Vertebrate Zoology and Anthropology, Institute for Research on Biodiversity, Faculty of Biology, ul. Wąska 13, 71-415 Szczecin, Poland, e-mail: rebae@univ.szczecin.pl

Keywords obesity, environmental factors, civilization diseases, genetic factors

Abstract *Background*: excess body weight in the populations of both developed and developing countries has been a health, social and economic issue for more than 30 years. The causes of overweight and obesity are complex and rooted in many different factors. The reasons why people gain weight need to be explored to prevent obesity-related diseases, to improve the quality of life and health status in the populations. *Aim*: to discuss genetic and environmental factors impacting on obesity. *Materials and methods*: a literature-based meta-analysis. *Results*: various factors contributing to excessive weight gain need to be explored; obesity prevention measures should include health-promoting education, changing eating habits in families as well as recipes and menu options in catering, and also by encouraging sports activity at every ontogenetic stage. Patients with genetic susceptibility to obesity should be supported in prophylaxis through education, elimination measures (diet) and the necessary pharmacological management so that they can remain active and independent for as long as possible. *Conclusions*: overweight and obesity are now a global problem. In only two generations, health complications due to excess adiposity have become hard to ignore. The process is parallel to the progress of civilisation, with the related stress, processed food, lack of exercise, insufficient sleep. Today, people have access to many modern conveniences in everyday life, and therefore more time for leisure, but still, maintaining normal (healthy) weight is becoming increasingly difficult.

Metaanaliza czynników genetycznych i środowiskowych wpływających na otyłość

Słowa kluczowe otyłość, czynniki środowiskowe, choroby cywilizacyjne, czynniki genetyczne

Streszczenie *Tło*: nadmierna masa ciała mieszkańców państw rozwiniętych i rozwijających się jest problemem zdrowotnym, społecznym i ekonomicznym od ponad 30 lat. Przyczyny nadwagi i otyłości są złożone i mają niejedno podłoże. Należy szukać powodów tycia w celu zapobiegania chorobom powiązanych z otyłością, aby podnieść jakość życia i stan zdrowia społeczeństw. *Cel*: omówienie czynników genetycznych i środowiskowych mających wpływ na otyłość. *Materiał i metoda*: metaanaliza na podstawie piśmiennictwa. *Wyniki*: należy szukać przyczyn nadmiernego tycia na różnych aspektach; zapobiegać otyłości poprzez informację prozdrowotną, zmianę nawyków żywieniowych w rodzinach, w żywieniu zbiorowym, a także zachęcać do działań prosportowych na każdym etapie ontogenezy. Pacjentów z otyłością uwarunkowaną

genetycznie należy wspierać w profilaktyce poprzez edukację, działania eliminacyjne (dieta) i konieczne leczenie farmakologiczne, tak aby chorzy jak najdłużej byli aktywni i samodzielni. *Podsumowanie*: nadwaga i otyłość to obecnie problem ogólnoświatowy. Zaledwie w ciągu dwóch pokoleń notuje się powikłania zdrowotne z powodu nadmiernego otłuszczenia. Proces ten jest równoległy z rozwojem cywilizacyjnym, związanym z nim stresem, przetworzoną żywnością, brakiem ruchu, zbyt małą ilością snu. Obecnie ludzie dysponują technicznymi udogodnieniami w życiu codziennym, a przez to mają więcej wolnego czasu, a mimo to utrzymanie prawidłowej (zdrowej) masy ciała jest coraz trudniejsze.

Introduction

According to the World Health Organisation (WHO) obesity is defined as excessive fat accumulation that may present a risk to health. Since 1975, there has been a threefold increase in the prevalence of overweight and obesity, which is now considered to be an epidemic of the 21st century. Obesity is a medical, social and economic issue, leading directly to approx. 13% of premature deaths regardless of sex or skin colour, and its management accounts for between 2% and 7% of healthcare spending in European countries (Wąsowski et al., 2013).

The roots of obesity are still an object of study. Research on the underlying causes of overweight and obesity has currently moved into the field of molecular genetics, as the impact of genetic factors on its development is increasingly recognised. Without the knowledge of various factors contributing to excess body weight, it cannot be effectively countered and treated.

One anthropometric measure of normal body weight is BMI (Body Mass Index). BMI = body weight [kg]/height [m]^2. BMI scores are interpreted as follows: BMI < 16.00 indicates grade 3 thinness; BMI 16.00–16.99 indicates grade 2 thinness; BMI 17.00–18.49 indicates grade 1 thinness; BMI 18.50–24.99 is the normal (healthy) range; BMI 25.00–29.99 indicates grade 1 overweight; BMI 30.00–39.99 indicates grade 2 overweight; BMI 40.00 ≥ indicates grade 3 overweight (Physical status: WHO, 1995).

According to WHO data, in 2016 as many as 39% of men and women aged 18 and older were overweight. Countries with the highest percentage of the population having BMI scores in excess of 30 kg/m^2 include: the USA (36.2%), Saudi Arabia (35.4%), Libya (32.5%), Turkey (32.1%), Egypt (32.0%), Iraq (30.4%).

In order to mitigate the adverse consequences of excessive adiposity on human life and health, it is necessary to undertake measures aimed at reducing the body weight in obese individuals.

The aim of this paper is to discuss the genetic and environmental factors impacting on obesity based on the available literature.

Classification of obesity in clinical practice according to its causes

A. Simple (primary, common) obesity, which is caused by energy surplus, that is caloric intake which exceeds the body's needs for metabolic processes and energy expenditure related to physical activity. The underlying causes of this type of obesity are primarily environmental; excessive consumption of highly processed and calorie-dense foods, with very high levels of animal fats and simple carbohydrates, plus the lack of physical activity.

B. Secondary obesity, presenting in the course of such endocrine disorders as: hypothyroidism, Cushing's disease and Cushing's syndrome, growth hormone deficiency, hypogonadism and some genetic conditions: Prader-Willi syndrome, Lawrence-Moon-Biedl syndrome, Turner syndrome, Klinefelter syndrome, as well as hypothalamic dysfunction in the course of degenerative diseases,

inflammatory conditions, developmental defects, tumours, injuries of the central nervous system and as a result of prolonged use of certain medication, such as: corticosteroids, antidepressants and antiepileptic drugs.

Based on anthropometric tests

The abdominal type (central adiposity, android fat distribution, pot belly, apple shape) – with fat accumulating around the abdominal area. It is associated with an increased risk of cardiovascular complications, metabolic syndrome and certain types of cancer.

Gynoid or peripheral obesity, pear shape – where excess fat accumulates in the buttocks, hips and thighs. This type develops in women going through puberty, signalling hormonal health and fertility (Szymocha et al., 2009).

Certain anthropometric measures are useful in determining the type of body fat distribution, namely WC and WHR:

- Waist circumference, WC – indicates normal body weight when WC < 80 cm (women), and WC < 94 cm (men); the overweight range is WC = 80 to 88 cm (women), and WC = 94 to 102 cm (men); and obesity when WC > 88 cm (women), and WC > 102 cm (men).
- The waist-to-hip ratio (WHR); in women WHR should not exceed 0.8, and should preferably amount to 0.7, while in men the optimal WHR is 0.95 and the ratio should not exceed 1.0.

Environmental factors responsible for the development of obesity

Highly processed food

The most common contributing factor is the consumption of too much highly processed, calorie-dense foods. Also, portion sizes tend to be larger now than they used to. People tend to eat more snacks, like cakes, sweets or chocolate bars, which are composed predominantly of fat and sugar and are therefore rich in calories. On a daily basis, no more than 50–55% of the calorie intake should come from carbohydrates. What is more, simple sugars, which are present in sweets and sugary beverages, should account for no more than 10% of the daily energy intake (Białkowska, 2011). Cakes, chocolate, sweets or wafers are rich in sucrose and have little nutritional value other than being calorie-dense. Consequently, excessive consumption of carbohydrate-rich products promotes obesity.

The consumption of easily digestible carbohydrates has also surged in recent years. The increase is mainly in the consumption of energy drinks and sweetened juices. To reduce production costs, the extra sugar often comes from glucose-fructose syrup, which is relatively about 40% sweeter than sucrose. Excessive consumption of fructose leads to adverse metabolic effects, potentially contributing to elevated blood levels of very low-density lipoprotein (VLDL) and triglycerides. This process results in fatty liver (hepatic steatosis).

Excessive fructose consumption was found to be associated with arterial hypertension in young people (Nguyen et al., 2009). Drinks containing fructose promote the development of type 2 diabetes. There have been cases of young people dying from an overdose of energy drinks, especially following intensive physical exercise.

Fats

Too much fat in the diet is another factor contributing to the development of obesity. There is a relationship between the amount of fat consumed and BMI scores (Białkowska, Szostak, 2010). Fat is a high-calorie food and requires much less energy to convert into the fat reserve compared to the conversion of carbohydrates. Digested fat is used by cells as a source of energy and as a building material of fatty compounds, cellular membranes, hormones, etc.

What matters is not only the quantity, but also the quality of fat in the diet. Usually, animal-source foods contain large quantities of saturated fatty acids and cholesterol. Animal fat stimulates appetite and leads to adverse metabolic effects, resulting in the premature development of atherosclerosis. In the development of this disease, the type of fat eaten is crucially important. Another essential factor is the balance of polyunsaturated omega-3 to omega-6 fatty acids. Excess amounts of dietary fat significantly increase the energy-density of one's diet. A fat-rich diet with insufficient amounts of fibre will lead to weight gain and increase appetite.

Calcium

Insufficient supply of calcium also contributes to the development of obesity. Studies show that calcium intake is associated with body mass index. A lower calcium content in the diet corresponds to an increased risk of overweight. It has also been demonstrated that higher calcium consumption (up to 1000 mg/d) coupled with a reduced calorie intake helps achieve faster weight loss (Zemel, 2005). Dietary calcium participates in the blood coagulation process, plays a role in the function of the nervous and muscular systems, as well as regulates energy metabolism by influencing the metabolism of adipocytes, thermogenesis and lipolysis. Insufficient amounts of calcium in the diet contribute to the accumulation of lipids within fat cells (adipocytes), as a result of inhibiting lipolysis in fatty tissue (Major et al., 2008). Increased consumption of calcium may help reduce weight gain and accumulation of fat in adipocytes by stimulating lipolysis (Zemel, 2003; Van Loan, 2009).

Physical activity

Little or no physical activity is another factor which has been confirmed as a contributor to obesity. Physical inactivity is implicated not only in the development of obesity, but also in other chronic conditions. It has been demonstrated that if a lifestyle marked by no physical activity is adopted in adolescence, it tends to persist in adulthood. According to a 2017 study by Wojciech Zgliczyński, one in eight children in Poland is obese or overweight (Zgliczyński, 2017). Factors contributing to this state of affairs include widespread avoidance of physical education classes in school and spending too much leisure time at the computer and/or television. According to a survey conducted by the National Food and Nutrition Institute in Warsaw, the time spent by young people between the ages of 11 and 15 in front of the computer and television on average exceeds 4 hours a day.

Regular physical activity effectively prevents the development of obesity and many other conditions such as: diabetes (type 2), arterial hypertension, osteoporosis, cancer, and even depression. Physical activity is also helpful in the treatment of obesity and prevents excessive accumulation of fatty tissue in the body. The benefits of increased physical activity include: fat loss, increased muscle and bone mass, reversal of the undesirable decrease in resting energy expenditure caused by a bad diet, reduction of elevated insulin levels, improved glucose tolerance and lipid profile,

improved fitness, reduction of resting and exercise blood pressure and heart rate, easier long-term maintenance of a dietary regime, improvement of general well-being and mental health; improvement of emotional state, including reduction of depressive moods and anxiety (Brownell, Wadden, 1999; van Baak, Saris, 1999).

Medication

Some medicines not only contribute to the development of obesity, but also make it harder to treat. Medication groups impacting on obesity include: progestogens, psychoactive and antidepressant drugs, serotonin antagonists, certain antihistamine, anti-allergy and antidiabetic medicines as well as steroids (Zahorska-Markiewicz, 2009). Obesity may result from changes in the anatomy of tissues and organs, e.g. abnormal hypothalamic function due to brain tumours, trauma, or inflammatory conditions.

Obesity may be caused by various endocrine disorders, related to hormone deficiency or excess. These include: Cushing's disease, hypothyroidism, pseudohypoparathyroidism, hyperprolactinemia, growth hormone deficiency, hypogonadism, polycystic ovary syndrome, insulinoma (insulin-secreting tumour, islet cell carcinoma).

Psycho-emotional factors

Psycho-emotional factors also play a role in the development of obesity. Currently, the lifestyles in many communities are characterised by high levels of stress and a fast pace. To cope with that, people consume large amounts of high-energy foods, alcoholic beverages, energy drinks, abuse nicotine and medication. An unbalanced diet is very often accompanied by little or no physical activity. Stress leads to an increased secretion of cortisol by affecting the hypothalamic-pituitary-adrenal axis (Bose et al., 2009). These factors contribute to the development of central adiposity, which may in consequence lead to the development of atherosclerosis later in life.

Interestingly, central adiposity did not seem to affect previous generations. This can be observed in old photographs and films. Even if the people in them were "heavier built", they were unlikely to carry a pot belly. Most likely, the reason for fat accumulation in the visceral cavity among the industrialised populations lies in stress, fast-paced lifestyles and even air pollution (Henneberg, Grantham, 2014).

With the progress of science, researchers from many different fields turned their attention to the psychological determinants of obesity. These include: failing mechanisms of self-regulation, individual beliefs and expectations, personality traits and problems coping with stress and emotions (Ogińska-Bulik, 2007). A person suffering from self-regulation disorders loses control over the amount of consumed food, which leads to "overfeeding".

Many scholars believe that stress and inability to cope with problems are the main causes of overeating. The so-called emotional eating is a form of a coping mechanism, distracting the person from painful experiences, and a way of avoiding threats or compensating for distress (Pietrzykowska, Wierusz-Wysocka, 2008). As food is now easily accessible, for many people it is the easiest and most effective way of dealing with tension. The increased appetite is a consequence of negative emotions, discomfort and depressed mood (Stice et al., 2005).

Consumption of carbohydrates soothes negative emotions by increasing serotonin levels, which in turn improves mood. Unfortunately, carbohydrates only have a temporary effect, the bad mood returns very quickly, and the person reaches for sweets again. A vicious circle is created, which may lead to the binge eating disorder or eating anarchy.

Insomnia

Insomnia contributes to the development of obesity. Eating disorders caused by sleep problems, e.g. the night eating disorder (NES) and sleep-related eating disorder (SRED), may cause weight gain. Symptoms of SRED are found in people who wake up in the night and eat large amounts of high-calorie food. The disorder differs from NES in that sufferers of the former have no memory of eating at night.

The night eating syndrome is diagnosed in people who are sleepless at least 3 nights a week, and who become "ravenously hungry" after 7 p.m. and eat more than 50% of their daily food ration at that time. Patients eat small portions throughout the night, they take no pleasure in eating, but feel compelled to do it nonetheless (Jakuszkowiak, Cubała, 2003). NES patients can be observed to have metabolic disorders related to the reduced secretion of leptin and melatonin, and increased release of ghrelin, galanin and cortisol (Stunkard, Allison, 2003).

There is a relationship between sleep duration and susceptibility to weight gain. Inadequate sleep disturbs the levels of ghrelin and leptin, and increases cravings for high-energy processed foods (Gangwisch et al., 2005).

Vitamin D

Another factor impacting on obesity is vitamin D deficiency. There are several reasons for vitamin D deficiency in obese individuals, and one of them is insufficient synthesis in the skin. Skin synthesis is the main source of endogenous vitamin D, accounting for approx. 80% of vitamin D supply in the body. Provitamin D3 is produced in the skin as a result of exposure to UVB radiation. Vitamin D3, otherwise known as cholecalciferol, undergoing enzymatic hydroxylation processes in the liver and kidneys, is converted to calcitriol – an active vitamin D metabolite. Calcitriol stabilises a number of processes and metabolic changes by interacting with the membrane and nuclear receptor. To date, there is no clear answer as to the causes of vitamin D deficiency – insufficient exposure to solar radiation or impairment of the skin synthesis process (Wąsowski et al., 2012).

According to Kull et al. inadequate skin synthesis is the likely cause for vitamin D deficiency in obese individuals. Their study involved 367 participants. The authors proved that sunbathing results in higher concentrations of calcifediol in the serum (Kull et al., 2009). In turn, Florez et al. (2007) demonstrated that obese individuals who exercise outdoors are 47% less likely to have hypovitaminosis D compared to those with obesity who exercise indoors (Florez et al., 2007). It is worth highlighting that people who do not get much exercise, spend a lot of time indoors and avoid going outside are more likely to suffer from depression.

Yvonne Foss claims that vitamin D deficiency in the obese causes the body to switch to the so-called "winter metabolism" (Foss, 2009). The intensity of sunlight decreases in the autumn, which in turn slows down metabolism and results in weight gain due to the accumulation of fat.

Intestinal flora (microbiota)

An unhealthy gut flora has been confirmed to be involved in the onset of obesity. The human gastrointestinal tract is inhabited by approx. 1,000 species of bacteria. Depending on the section of the intestine, the numbers vary greatly. The composition of the microbiota is likewise varied: the small intestine contains predominantly gram-negative aerobes, whereas the large intestine contains both gram-positive and gram-negative bacteria. 98% of the intestinal bacteria belong to

the genera Bacteroides and Firmicutes (Żak-Gołąb et al., 2014). The role of commensal bacteria in the gastrointestinal tract has not been fully explained to date. They are known to be involved in the fermentation of undigested foods and the absorption of electrolytes, to take part in the growth and maturation of erythrocytes and epithelial cells of the large intestine, to affect the immune function and gastrointestinal motility, to participate in the synthesis of vitamins and degradation of toxins and carcinogens.

Both human and animal studies have shown that the Bacteroides to Firmicutes ratio is altered in obese individuals – with increased numbers of Firmicutes. Weight loss contributes to increasing the ratio of Bacteroides in the gut flora, pro-rata to the number of kilograms lost (Ley et al., 2006). The findings of Furet et al. indicate that this is due to changes in the diet and reduced caloric intake, rather than the weight loss itself (Furet, 2010). Despite the studies, it is still not known why the obese have relatively more Firmicutes in the gut flora. It is believed that Firmicutes thrive in those eating a typical Western diet, rich in fats and sugars (Mosley, 2017). It was also observed that a low-carbohydrate, low-fat diet leads to an increased number of Bacteroides colonies in feces.

Genetic factors responsible for the development of obesity

Monogenic obesity

To date, more than 200 cases of single-gene mutation obesity have been described. Mutations affect at least 11 genes; they are inherited according to Mendelian rules and the majority involve the melanocortin receptor 4 (Rankinen et al., 2006). Experimental studies into the genetics of obesity had started already in the mid-20th century, mainly on mice with significant hereditary obesity. Thanks to those studies, it was later possible to identify analogous irregularities in human genes and discover the key hypothalamic mechanisms governing appetite and satiety. Knowledge of these mechanisms made it possible to understand how obesity develops in individuals with defects along the pathway regulating eating behaviour.

Mutations in the leptin gene and leptin receptor gene

Leptin is an adipokine, and is also referred to as the “satiety hormone”. Its gene was first cloned in mice and then in humans. It plays an important role in the hypothalamic appetite control, participates in regulating bone mass, is involved in the regulation of the immune system and the reproductive function. Up until recently, the mutation in the leptin receptor gene (LEPR) was considered rare, but innovative studies carried out in many different countries attracted additional attention to the phenomenon (Farooqi et al., 2007). It is an autosomal recessive inherited disease. A LEPR mutation accounted for 3% of the severely obese individuals examined as part of the study.

Leptin deficiency, which is caused not only by insufficient leptin, but also a defect in its receptor, presents with a similar clinical phenotype. It is characterised by high-grade obesity with the onset in the first months of life, and accumulation of excess fat in the trunk and limbs (Farooqi et al., 2002). Patients present with extreme hyperphagia (excessive hunger) which causes behavioural changes, with the affected individuals constantly seeking food and behaving aggressively when the food is restricted. Obesity is mainly due to the excessive caloric intake, as the basal metabolic rate is not dependent on leptin concentration. One important clinical finding for patients with LEPR mutation is that there is no specific difference in the severity

of hyperlectinemia compared to other obese individuals. Earlier, it had seemed to be typical for patients with a receptor defect, but recent studies have shown this characteristic not to be of key importance (Męczekalski et al., 2008).

Carriers of the LEP/LEPR mutation do not exhibit the phenotype typical for homozygotes, but they show increased levels of adiposity. In patients with a LEP mutation, adipokine supplementation provides satisfactory results. It remedies all of their symptoms, suppressing appetite, which makes it possible to shed the excess kilograms, restore normal immune function and regulate the function of the pituitary-gonadal axis (Heymsfield et al., 2006).

Mutations of the melanocortin-4 receptor (MC4R)

Melanocortin plays a key role in the regulation of appetite by the hypothalamus. It is a ligand binding mainly to type 4 MSH receptor. To date, more than 90 mutations have been described, of the frameshift or missense type (Yeo et al., 1998; Vaisse, 1998). The most common monogenic form of obesity is that related to an MC4R defect. According to studies, mutations in this gene are responsible for 2.4–4.0% of severe obesity cases, where BMI exceeds 35 or 40 [kg/m^2]. It is the most common autosomal dominant disorder (Alharbi et al., 2007). A 2007 study led to the discovery of a mutation involving constant activation of MC4R, causing severe obesity. Apart from obesity, patients presented a wide range of phenotypes. Where hyperphagia used to be regarded as ubiquitous, it was found not to affect all patients (Dubern et al., 2007; Lubrano-Berthelier et al., 2006). The mutation can only be diagnosed by molecular tests. No effective therapy has been developed to date (Xiang et al., 2007).

Syndromic obesity

Obesity can be concomitant with order disorders, i.e. intellectual disability or organ dysfunction within a single clinical phenotype. Such syndromes have a genetic background and may be due to defects in more than one gene, though this is not a rule.

Prader-Willi syndrome

Prader-Willi syndrome (PWS) is the most common and long-known genetically mediated disorder related to obesity. The first case was described back in the 19th century. Ledbetter et al., (1982) linked the syndrome to a chromosomal aberration, however no specific mechanisms have been discovered to date which are responsible for the symptoms presenting in the syndrome (Ledbetter et al., 1982).

Genetically, the syndrome results from a deletion in the paternal copy of chromosome 15 in the 15q11–q13 region or a uniparental 15 disomy, where both copies of the chromosome originate from the mother. Occasionally, the syndrome may be caused by an unbalanced translocation or an imprinting defect. In 2006, Varela et al. discovered that the characteristic phenotypic expression of Prader-Willi syndrome is also present in cases involving a deletion in the region that includes the SIM1 gene (Varela et al., 2006). The syndrome is characterised by a number of anomalies. Symptoms vary depending on the individual and calendar age. Mental development is often normal, without cognitive deficit. In turn, motor development is delayed. Patients struggle with intense cravings for food, which leads to behavioural changes such as: aggression if food is withheld or stealing food. Individuals with Prader-Willi syndrome present with numerous endocrinopathies, e.g. hyperinsulinemia, hypogonadism secondary to hypothalamic dysfunction

with underdeveloped reproductive organs, and growth hormone deficiency which results in short stature. The molecular pathogenesis of the syndrome has not been fully explained. On the other hand, the genes included in the defective region of chromosome 15 are known. Some products of those genes (NDN, MAGEL2) are strongly expressed in the central nervous system, specifically in the hypothalamus and this is the probable cause for many dysfunctions, including obesity (Muscatelli et al., 2000; Lee et al., 2000).

New whole-genome studies have led to the discovery of numerous genes involved in the development of obesity, whose expression is abnormal in Prader-Willi syndrome (ADI, POR2, MC2R, HCRT, OXTR) (Bittel et al., 2007). Complications secondary to obesity (type 2 diabetes, venous thrombosis, cardiovascular disease) are some of the more common causes for premature death in patients with the syndrome.

Polygenic obesity

Obesity with a polygenic basis is not very well understood. Many genes occurring in the population are polymorphic and lead to a tendency to accumulate excess fat in favourable circumstances (Męczekalski et al., 2008). It was observed that the genes involved in the pathogenesis of monogenic obesity also participate in the pathogenesis of polygenic obesity. Many genes are confirmed to be implicated in both types of obesity, albeit with a different extent of gene deficiency.

PPARγ – peroxisome proliferator-activated receptor gamma [γ]

PPARγ are transcriptional factors belonging to the nuclear receptor superfamily and, by regulating the expression of many genes involved in adipogenesis, metabolism of carbohydrates and lipids as well as adipokine synthesis, they are implicated in various metabolic disorders including obesity, insulin resistance and hypertension (Chmielewska-Kassassir et al., 2013). Studies focusing on PPARγ isoform 2 revealed that SNP at position 115 (Pro111Gln) of this receptor is associated with obesity risk, and at position 12 (Pro12Ala) with the risk of obesity and type 2 diabetes (Deeb et al., 1998; Franks et al., 2007). It was found that SNP in this location affects body weight depending on the type of diet: in the comparison of groups eating a low-fat vs. high-fat diet, the carriers of the alanine variant showed no differences in BMI increase, whereas homozygotes with prolin on a high-fat diet put on weight (Memisoglu et al., 2003). Looking back at that study, one may observe the importance of the interplay between genes and the environment in the development of polygenic obesity. The tendency to gain weight is determined by genes, but its phenotypic expression depends on environmental factors (physical activity, dietary composition in terms of quality and quantity).

Endocannabinoid system and CNR1 gene polymorphisms

Hemp (*Cannabis sativa*) is a herbaceous plant which has been used in folk medicine for many years. Numerous studies have been devoted to exploring its chemical composition and properties. Analyses aimed at understanding the mechanism of cannabinoid action led to the discovery of the first cannabinoid receptor (CB). A hypothesis was formulated that animal and human bodies contain substances which have similar properties to cannabinoids of plant origin and which are responsible for activating CB receptors. The hypothesis was confirmed when in 1992 the first of the endogenous ligands for the CB receptors was identified (Begg et al., 2005).

The endocannabinoid system (ECS) is composed of CB1 and CB2 receptors, belonging to the family of 7-transmembrane receptors, non-CB1 and non-CB2 receptors, ligands for those receptors and enzymes involved in the synthesis, uptake and degradation of those ligands (Komorowski, Stępień, 2007). The ECS plays an important role in modulating energy balance. It influences hunger regulation by the central nervous system. CB1 receptors are found in the hypothalamus and are responsible for appetite stimulation, related to the starvation response (Harrold, Williams, 2003; Kirkham, 2005). Activation of the CB receptor stimulates appetite and has an antiemetic, analgesic and sedative effect (Di Marzo, Matias, 2005).

By 2011, scholars managed to sequence 1973 polymorphisms of individual nucleotides in the CNR1 gene. Studies conducted in American, European, Chinese and Japanese populations demonstrated that CNR1 gene polymorphisms are associated with body weight, fat distribution, energy expenditure and metabolic disorders.

A study in a Swiss cohort of 865 obese men showed a relationship between polymorphism in rs806381 G-allele of the CNR1 gene and polymorphism in rs2023239 T-allele vs. BMI scores (Benzinou et al., 2008). Similarly, in a French cohort a relationship was found between polymorphism in rs806381 G-allele of the CNR1 gene and BMI scores (Benzinou et al., 2008).

Aberrant thermogenin (UCP1) expression

There two main types of human adipose tissue: white adipose tissue (WAT) and brown adipose tissue (BAT). WAT and BAT differ in terms of morphology, function and anatomical location. The key function of white adipose tissue is to store energy in the form of triacylglycerols, while brown adipose tissue is involved in the regulation of non-shivering thermogenesis (Saely et al., 2012). Non-shivering thermogenesis provides for temperature regulation in warm-blooded animals. WAT and BAT also importantly differ in the number of mitochondria and the structure of mitochondrial membranes (Saely et al., 2012). Mitochondria are much more abundant in BAT cells. The inner membrane of those mitochondria contains a protein – thermogenin (UCP1), which is directly responsible for generating energy in the form of heat (Nicholls, Locke 1984; Wijers et al., 2009). Thermogenin is regarded as a marker of brown adipose tissue (Wójcik, 2011).

Thermogenin – uncoupling protein (UCP1) belongs to the group of transport proteins. Genes encoding UCP1 and adrenergic receptors have been associated with an increased susceptibility to gain weight (Stosio et al., 2016). The first polymorphism of the UCP1 gene to be described involved an adenine to guanine change at position 3826 upstream of the TATA box, in the promoter region (A-3826G, rs1800592) (Cassard-Doulcier et al., 1996). This polymorphism does not play a key role in the pathogenesis of obesity, but it was found that replacing an A-allele with a G-allele favours weight gain. Other polymorphisms which may be associated with obesity include: A-1766G polymorphism (rs3811791) at the 5' flanking region (Kim et al., 2005) and nonsynonymous polymorphism Ala64Thr (rs45539933), where G is replaced with A at +1068 in exon 2 of the UCP1 gene (Jia et al., 2010).

Dysfunction of the β3-adrenergic receptor has an adverse effect on UCP1. The Trp64Arg polymorphism in β3-adrenergic receptor was studied and found to affect the protein-receptor interaction, and to demonstrate a synergic effect with A-3826G polymorphism (Clement et al., 1996).

Oppert et al., (1994) conducted the first study which confirmed the existence of A-3826G polymorphism. Their findings proved that the presence of the G-allele was related to increased body weight (Oppert et al., 1994). In turn, a study by Kogure et al. (1998) demonstrated that

individuals with a G-allele at the polymorphic site A-3826G burn even 200 kcal/day less than those without the mutation (Kogure et al., 1998). The study conducted in a group of obese Japanese confirmed that carriers of the G-allele found it harder to lose weight.

IgG-mediated food allergy

The mechanism of the IgG-mediated food hypersensitivity involves the human body recognising food particles as potentially harmful foreign bodies. In IgG-mediated food allergy, incompletely digested food particles continually enter the bloodstream through the intestinal lining, which means that the concentration of immune complexes is high. These complexes travel through the bloodstream, spreading the inflammation process to new tissues and organs. This can lead to numerous health conditions, e.g. obesity and type 2 diabetes (Gałęcka et al., 2013; Zawisza, 2010; Bartuzi, 2009). The likely immediate cause of the intolerance is a compromised intestinal barrier, with larger gaps between enterocytes. Increased intestinal permeability is most commonly related to a history of infections: viral, bacterial, parasitic; exposure to environmental toxins, physical and mental stress, influence of alcohol, medication and a diet rich in preservatives and dyes (Brodzicki, 2006).

Upon exposure to a food allergen which is an integral part of the diet the inflammation process becomes chronic and leads to organ and system dysfunctions (Gałęcka et al., 2013). Characteristically, IgG-mediated allergy causes a delayed response, occurring from 8 to 72 hours after ingesting the allergen. Some scholars hypothesised that there is a link between IgG-mediated food hypersensitivity, generating a free-radical state and cytokin production, and the accumulation of adipose tissue in the body (Frank et al., 2014). Clinical dieticians believe that the right course of action in order to lose excess weight is to test for IgG-mediated food hypersensitivities and then start appropriate dietary treatment.

A chronic inflammatory response is characterised by elevated levels of inflammatory mediators, primarily TNF-α, CRP and proinflammatory interleukins. This may lead to the development of insulin resistance and, consequently, type 2 diabetes. TNF-α is produced in the cells of the immune system as well as adipocytes and muscle cells. The level of TNF-α increases in proportion to the amount of body fat. It is responsible for adverse metabolic changes and inhibits insulin receptor function by reducing receptor phosphorylation (Moller, 2000). Seen as insulin cannot bind to its blocked receptor, its production increases, leading to the development of hyperglycaemia and hyperinsulinemia. Blood glucose levels remain high, despite the fact that the glucose is not used in metabolic processes but merely accumulated in the muscles in the form of fatty acids. This leads to changes in body composition, increasing the body fat percentage and leading to obesity (Lorenzo et al., 2008; Nieto-Vazquez et al., 2008). Enhanced secretion of TNF-α causes damage to leptin receptors, disrupting the hunger inhibition response.

Erlinger et al. (2003) conducted a study in which they proved that an elimination diet has a different effect on weight loss in those whose obesity is related to a persistent chronic inflammation. Inflammatory conditions reduce the effectiveness of the elimination diet and increase the risk of cardiovascular disease (Erlinger et al., 2003).

Wilders-Trusching et al. (2008) conducted a study in which they proved a link between the level of IgG antibodies and CRP in obese children. They demonstrated that obese children have significantly higher IgG antibody values (Wilders-Trusching et al., 2008).

Conclusions

Year by year the number of obese people in the world is growing. In highly developed countries, obesity is an increasingly problematic health, social and economic issue. Regarded as a civilisation threat, it may in the future reach pandemic proportions. Eating just a few extra calories on a regular basis may in the long run add up to weight gain.

The development of obesity may be attributed to a multitude of environmental factors, associated mainly with the contemporary lifestyle, with its unlimited access to processed and calorie-dense food. Today's eating habits provide for larger portions and too many portions, without maintaining adequate intervals between meals, coupled with the wrong quantity and quality of nutrients from the diet. The habit of snacking in between meals has become the norm, due to the easy accessibility of food (Jakuszkowiak, Cubała, 2004). It is also worrying that obesity affects more and more children who give up physical activity and spend their spare time in front of the computer. Such a lifestyle is characteristic of many communities experiencing high levels of stress and a fast-paced life. The inability to cope with problems leads to overeating.

Genetic factors, including single-gene and multifactorial mutations, also play a part in the development of obesity. Genetic research started in the mid-20th century and helped prove that obesity genes can be inherited according to Mendelian rules. The most extensively studied Prader-Willi syndrome is a classic example of a monogenic mutation. Polygenic obesity is not yet fully understood. In light of the prevalence of polygenic obesity, however, the diagnostics and treatment of such patients is of crucial importance. In favourable circumstances, polymorphism manifests itself in a tendency to accumulate excess fat.

The earlier the onset of obesity, the higher the prevalence of its related complications. It has been proven that obesity is implicated in increased mortality and is a risk factor for the development of diseases affecting the cardiovascular, nervous, immune and endocrine systems.

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Long-term changes in the numbers of waterbirds at an important European wintering site

Dominik Marchowski,[1] Łukasz Ławicki,[2] Sebastian Guentzel,[3] Jacek Kaliciuk,[4] Zbigniew Kajzer[5]

[1] Ornithological Station, Museum and Institute of Zoology, Polish Academy of Sciences. Nadwiślańska 108, 80-680 Gdańsk, Poland, e-mail: dominikm@miiz.waw.pl
[2] West Pomeranian Nature Society. Wąska 13, 71-415, Szczecin, Poland, e-mail: izuza@interia.pl
[3] West Pomeranian Nature Society. Wąska 13, 71-415, Szczecin, Poland, e-mail: limicola@interia.pl
[4] West Pomeranian Nature Society. Wąska 13, 71-415, Szczecin, Poland, e-mail: zboxpl@gmail.com
[5] West Pomeranian Nature Society. Wąska 13, 71-415, Szczecin, Poland, e-mail: atricilla@interia.pl

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Abstract Waterbirds were regularly monitored in north-western Poland from 2002 to 2018. Counts took place in the most important areas where wintering waterbirds concentrate in this region, the most important wintering ground for this group of birds in Poland and one of the most important in Europe. In addition to the wintering function, this area also has a very important function as a stopover site for significant numbers of African-Eurasian Flyway migrants. The average number of all waterbirds covered by the study from 2002 to 2018 was 117,000. Numbers in recent years (2011–2018) have increased significantly ($P < 0.001$) compared to the first half of the study (2002–2010). The most important species found here during the non-breeding period were: Greater Scaup *Aythya marila* (mean number for 2002–2018: 20,600), Tufted Duck *A. fuligula* (26,700), Common Pochard *A. ferina* (1,500), Smew *Mergellus albellus* (1,400), Goosander *Mergus merganser* (7,700), Great Crested Grebe *Podiceps cristatus* (840), Eurasian Coot *Fulica atra* (8,400), Mute Swan *Cygnus olor* (1,100), Whooper Swan *C. cygnus* (890), Taiga/Tundra Bean Goose *Anser fabalis sensu lato* (13,000) and White-fronted Goose *A. albifrons* (6,500). The importance of this region at the European scale for migratory and wintering waterbirds has increased, and this presents new challenges to the institutions responsible for protecting these areas.

Długoterminowe zmiany liczebności ptaków wodnych w ważnym w skali Europy miejscu zimowania

Słowa kluczowe Natura 2000, obszary chronione, kaczki, łąbędzie, gęsi, zmiany klimatyczne, estuarium Odry, północno-zachodnia Polska, tereny podmokłe

Streszczenie Od 2002 do 2018 roku prowadzono regularny monitoring ptaków wodnych w północno-zachodniej Polsce. Liczenie przeprowadzono w najważniejszych miejscach zimowania ptaków wodnych w tym regionie, będących jednocześnie ważnymi zimowiskami w Polsce i Europie.

Poza funkcją zimowiska, miejsca te jednocześnie są ważnymi miejscami przystankowymi dla migrantów przelatujących z Euroazji do Afryki. Średnia liczba wszystkich ptaków wodnych w latach 2002–2018 na badanym obszarze wynosiła 117 000 osobników. W porównaniu z latami 2002–2010 w ostatnich ośmiu sezonach zimowych (lata 2011–2018) znacząco wzrosła liczba zimujących ptaków na Pomorzu Zachodnim. Najważniejszymi gatunkami obserwowanymi na badanym obszarze w okresie pozalęgowym były: ogorzałka *Aythya marila* (średnio 20 600 osobników), czernica *A. fuligula* (26 700), głowienka *A. ferina* (1500), bielaczek *Mergellus albellus* (1400), nurogęś *Mergus merganser* (7700), perkoz dwuczuby *Podiceps cristatus* (840), łyska *Fulica atra* (8400), łabędź niemy *Cygnus olor* (1100), łabędź krzykliwy *C. cygnus* (890), gęś zbożowa/tundrowa *Anser fabalis sensu lato* (13 000) i gęś białoczelna *A. albifrons* (6500). Znaczenie tego regionu dla ptaków migrujących i zimujących w skali Europy rośnie, co tworzy nowe wyzwania dla instytucji odpowiedzialnych za ochronę tych obszarów.

Introduction

Bird monitoring has become an important tool in environmental monitoring. Such programmes have been implemented for many decades (Magurran et al., 2010). Waterbirds have been used as bioindicators in environmental monitoring for many years (Wetlands International, 2010). Many international institutions, such as HELCOM (Helsinki Convention on the Protection of the Marine Environment of the Baltic Sea Area), ICES (International Council for the Exploration of the Sea), BirdLife International or the European Union, use bird monitoring data to predict changes in the environment. The International Waterbirds Census (IWC) is a worldwide programme that has been monitoring waterbirds for many years (Mundkur et al., 2017). As part of this programme, wintering waterbirds are counted on one occasion in January, the count usually taking place at the same time each year using the same methodology (Wetlands International, 2010). The West Pomeranian Nature Society (Polish: Zachodniopomorskie Towarzystwo Przyrodnicze, hereafter ZTP) is a non-governmental organisation that carries out such monitoring on the one of the most important waterbird wintering areas in Europe. The results of the January counts are transferred to Wetlands International based in the Netherlands, the organization that coordinates the IWC. In addition to the January counts, ZTP carries out two extra counts using the same methodology in November and March. As a result, we have more precise information on the numbers and distribution of birds, not only during the winter but also during the autumn and spring migrations. The site monitored by ZTP is not accidental. The north-western part of Poland is one of the most important waterbird wintering areas in Europe (Skov et al., 2011), and the region is particularly important for species such as Greater Scaup *Aythya marila* (hereafter Scaup), Common Pochard *Aythya ferina* (hereafter Pochard), Goosander *Mergus merganser* and Smew *Mergellus albellus* (Marchowski et al., 2017, 2018). This article summarises the results of the monitoring of several key bird species and the changes in their numbers over a period of 17 years.

Materials and methods

Study area

The study area lies in north-western Poland and covers the most important areas where waterbirds congregate during the non-breeding period. These areas are located mainly along the Odra River valley and in the Odra estuary, which includes the Szczecin Lagoon, the Kamień Lagoon and Lake Dąbie. The monitoring also covers Lake Miedwie and the fishponds in

Dzwonowo – these two sites lie beyond the Odra valley and the estuary (Figure 1). The study site covers an area of around 1,200 km² and about 420 km of river banks and waterbody shores.

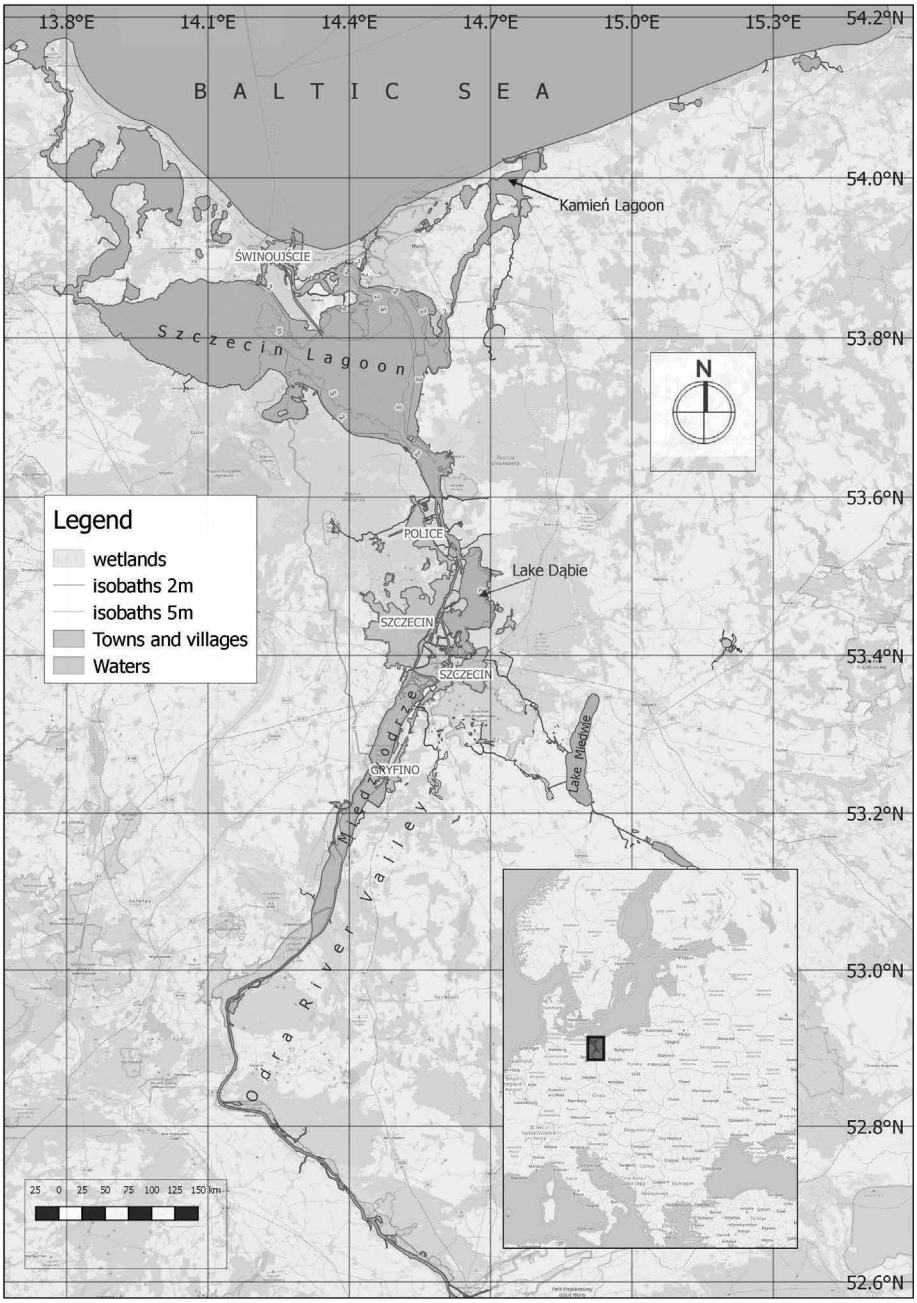

Figure 1. The study area

Odra Valley. The section between Kostrzyn and Szczecin (120 km) is characterised by quite diverse habitats. There are regulated sections with stone groynes and flood embankments; unregulated sections that are periodically flooded such as Kostrzyneckie Rozlewisko, around the village of Piasek, or the section from Czelin to Kłosów; oxbow lakes and mosaics of wetland habitats from flooded meadows, reed beds and compact willow scrub to waterlogged forests between Widuchowa and Szczecin. Also typical of the region are habitats strongly transformed by man, such as ports, harbours, shipyards, wharves, sewage treatment plants and artificially heated waters: such habitats occur mainly in the vicinity of the Szczecin–Police–Gryfino conurbation in the middle of the study area.

Odra River Estuary. The average and maximum depths of the estuary are 3.8 and 8.5 m, respectively (the dredged shipping lane cutting across the bottom of Lagoon from Baltic Sea to the port of Szczecin is 10.5 m deep) (Radziejewska, Schernewski, 2008). The waters of the Szczecin Lagoon, Kamień Lagoon and Lake Dąbie are brackish. The salinity in the central part varies from 0.3 psu (practical salinity unit) to 4.5 psu (mean = 1.4 psu) and declines with increasing distance from the sea (Radziejewska, Schernewski, 2008). Periodic inflows of water from the Pomeranian Bay (salinity ~7 psu) take place through the Świna Strait and, to a lesser extent, through the Dziwna and Peene Straits (the latter in the German part of the ORE). Large communities of benthic organisms such Zebra Mussels *Dreissena polymorpha*, which occur here on a very large scale (Wolnomiejski, Woźniczka 2008), provide a source of food for benthic feeding birds (Marchowski et al., 2015, 2016).

Lake Miedwie. In comparison to the Odra estuary, Lake Miedwie is deep, its maximum depth being 43.8 m. As it lies at a height of 14 m above sea level, this makes it the place with the largest cryptodepression in Poland (29.8 m below sea level). The lake has an area of 35 km² and a shoreline 39 km in length. The shores of the lake are mainly surrounded by a landscape of arable fields, meadows, pastures and wetlands.

Dzwonowo fish ponds. The ponds are used for fish farming, producing stocking material and carp for human consumption. The 36 ponds cover a total area of 365 hectares. The largest pond for commercial carp production is 138 hectares in area.

Methods

Our counts included Grebes (Podicipediformes), Ducks, Geese and Swans (Anseriformes) and Gruiformes. The species nomenclature and the systematic order used in the article are in accordance with the latest version of the HBW and BirdLife Taxonomic Checklist (HBW & BirdLife International, 2017). The following way of listing species in the text (e.g. Marchowski et al., 2015, 2017), standard in ornithological publications, has been adopted: when the species name appears for the first time, the full English name is given along with the full scientific name (genus and species – in italics); whenever a species is referred to again in the text, the English name only is used.

A total of 46 counts were conducted in 2002–2018. When calculating the mean number of birds for each season, a season was treated as the period from November (the first count) in one year to January and March (the second and third counts) in the following year. Only one count took place in the 2001/2002 season (in January), and no counts were done in the 2006/2007 season. Therefore, we have data for 15 full seasons. No observations were made during extreme weather conditions (heavy rain, high wind, strong wave action). Counts were carried out on the ground and from the air; the alternative terms ‘platform’ and ‘census method’ are used in the text

to refer to the 'count method'. All count results were raw data: numbers were not processed by any calculations, such as distance analysis. We used 'total count' methods with both platforms. This 'total count' method has also been used in other studies (Joasen, 1968, Savard, 1982, Kingsford, 1999, Voslamber, van Turnhout, 1999, Laursen et al., 2008). A team of about 20 trained and experienced observers was involved in all the counts.

A slow-flying, high-wing aeroplane was used for the aerial counts. The average flight speed was about 100 km/h and the average flying height was about 80 m above the water. This gave a roughly 1500 m wide band within which birds could be recorded. The flight route was designed to cover as much of the water surface as possible; we estimated that coverage was thus approximately 95% of the area surveyed. Only the birds in a very small part of the middle of the Szczecin Lagoon (the largest water body in the survey area – see Figure 1) were not counted.

Ground counts were usually done on foot, although cars were also involved. Each observer was equipped with 10 x 40 or 10 × 50 binoculars and tripod-mounted spotting scopes with variable magnification, usually 20–60×. During the counts, observers walked along the same routes, stopping every few hundred metres to scan the area with binoculars and/or spotting scope and then count the birds. Alternatively, counts were conducted from vantage points accessible by car. We used the best vantage points and routes, dividing the study area up into areas that were visible from such points or routes so that no counted areas overlapped and no parts of the study area were overlooked. The present analysis took only birds on the water into consideration. All the counts were carried out from the same routes and observation points.

The methodology of the ground and aerial counts is consistent with generally accepted standards in this field (Komdeur et al., 1992; Wetland International, 2010) and is described for our study area in more detail by Marchowski et al. (2018).

Statistical calculations

We analysed the data from 15 seasons in the period 2002–2018. All the statistics were performed using R software (R Development Core Team 2014). Because of the small sampling size (n = 15 seasons) we could not assume a normal distribution, so we used the simple bootstrap method to estimate means, standard error and confidence intervals. Bootstrap works well with samples that have less than 40 elements, because it involves resampling (Jain et al., 1987). To check the statistical significance of the differences between the first half of the study period (2002–2010) and the second half (2011–2018), we used a permutation test based on resampling without replacement. This test does not rely on assumptions about the distribution of the data. Permutation tests work by resampling the observed data many times in order to determine a p-value for the test (Hothorn et al., 2015). Results were considered statistically significant for $P < 0.05$ and close to statistical significance for $P < 0.1$.

Results

The average number of waterbirds in the non-breeding periods in the studied area in 2002-2018 was 116,968 (±5,498 SE). The mean autumn number was 128,933 (±14,244 SE), the mean spring number was 122,994 (±11,152 SE) birds, and the mean winter number was 98,997 (±10,774 SE). The numbers from each season are shown separately in Figure 2. The mean number from the first half of the survey (2002–2010) was 86,542 (±7,101 standard error – SE, confidence intervals

– 95% CI: 73,294; 101,032) ind., while that from the remaining seasons was 147,403 (±8,439 SE 95% CI = 131,191; 164,170) ind.; this indicates that the number of birds increased significantly over time (P < 0.001) (Figure 3). The lowest number of waterbirds was recorded in January 2010 – 38,320, and the highest in November 2017 – 225,335.

The most important bird species

Scaup *Aythya marila*. The mean for 2002–2018 was 20,613 (±1790 SE), the lowest number (2 birds) was recorded in January 2004, and the highest number (83,330) in November 2014 (an exceptionally high number (95,500) was recorded outside the counting period, in April 2011) (Ławicki, Guentzel, 2012). The mean number from the first half of the survey (2002–2010) was 11,320 (±2,590 SE; 95% CI: 6,883; 16,942); for the second half (2011–2018) the figure was 28,740 (±4,264 SE; 95% CI = 20,847; 37,522), indicating that the numbers of these ducks increased significantly over time (P = 0.002) The most important area for Scaup was the Szczecin Lagoon; high numbers were also regularly recorded on Lake Dąbie.

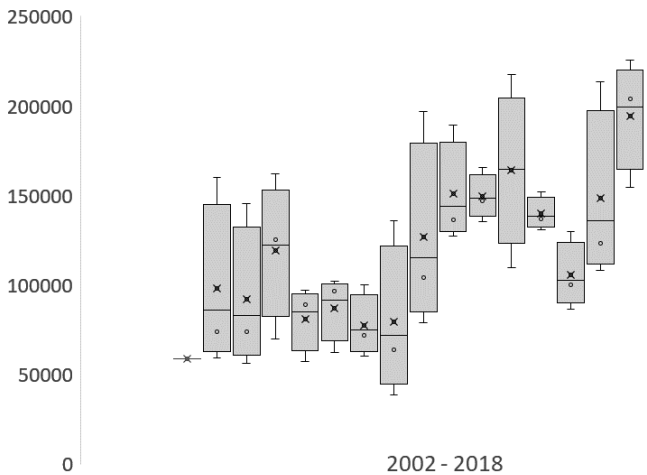

Figure 2. Changes in the abundance of all waterbird species counted in the whole study area in 2002–2018. Results of three counts during the non-breeding period (November, January and March). The years on the x-axis represent all three counts in one season, e.g. 2003 is the 2002/2003 season, etc. Only one count was carried out in the 2002 season (2001/2002) (in January). Each plot box gives the following information: minimum value, maximum value, intermediate value (small circle), mean (small ×), median (line), upper and lower quartile (box).

Tufted Duck *Aythya fuligula*. The mean for 2002–2018 was 26,680 (±1,635 SE) birds, the lowest number (516) was recorded in January 2004, and the highest one (57,276) in November 2014. The mean number for the first half of the survey (2002–2010) was 18,912 (±2,885 SE; 95% CI: 13,386; 24,676); for the second half (2011–2018) it was 33,483 (±3,093 SE; 95% CI: 27,341; 39,455), indicating that the number of birds increased significantly over time (P = 0.003). The most important area for Tufted Duck was the Szczecin Lagoon, but high numbers were also regularly

recorded on Lake Dąbie. Tufted Duck was much more dispersed than Scaup and was regularly observed in other areas such as the Odra Valley and Lake Miedwie.

Figure 3. All species of waterbirds in the study area, aggregate numbers from the first half of the study period (2002–2010) and from the second half (2011–2018). Each plot box gives the following information: minimum value, maximum value, intermediate value, mean (small ×), median (line), upper and lower quartile (box).

Pochard *Aythya ferina*. The mean for 2002–2018 was 1,483 (±156 SE) birds, the lowest number (0) was recorded in January 2003, and the highest number (4,935) in January 2005. The mean number for the first half of the survey (2002–2010) was 1,839 (±357 SE; 95% CI: 1,163; 2,557); for the second half (2011–2018) it was 1,171 (±160 SE; 95% CI: 871; 1,497). This indicates that the number of birds decreased over time, but the result is marginally insignificant (P = 0.1). The most important area for Pochard was the Szczecin Lagoon, while high numbers were also regularly recorded on Lake Dąbie. Like Tufted Duck, Pochard was more dispersed than Scaup and was regularly sighted in other areas such as the Odra Valley and Lake Miedwie.

Common Goldeneye *Bucephala clangula*. The mean for 2002–2018 was 3,493 (±170 SE) birds, the lowest number (198) was recorded in January 2010, and the highest number (10,971) in March 2011. The mean number for the first half of the survey (2002–2010) was 2,846 (±333 SE; 95% CI: 2,212; 3,515); for the second half (2011–2018) it was 4,055 (±435 SE; 95% CI: 3,265; 4,965). Numbers of these ducks thus increased significantly over time (P = 0.05). Goldeneye was dispersed throughout the study area, the highest numbers being found in the Szczecin Lagoon, on Lake Dąbie and in the Odra Valley.

Eurasian Coot *Fulica atra*. The mean for 2002–2018 was 8,356 (±561 SE) birds, the lowest number (563) was recorded in January 2003, and the highest number (24,836) in November 2004. The mean number for the first half of the survey (2002–2010) was 7,675 (±1,382 SE; 95% CI: 5,247; 10,619); for the second half (2011–2018) it was 8,952 (±846 SE; 95% CI: 7,361; 10,673). The increase in numbers was not significant over time (P = 0.45). Coot was dispersed all over the study area, but the most important subareas were Lake Dąbie, the Kamień Lagoon and the Odra Valley.

Smew *Mergellus albellus*. The mean for 2002–2018 was 1,388 (±97 SE) birds, the lowest number (116) was recorded in January 2006, and the highest one (3,770) in January 2009. The mean number for the first half of the survey (2002–2010) was 1,465 (±212 SE; 95% CI: 1,059; 1,889); for the second half (2011–2018) it was 1,311 (±229 SE; 95% CI: 887; 1,781), indicating insignificant changes over time (P = 0.63). Smew were found throughout the study area, but the largest concentrations were in the Szczecin Lagoon, on Lake Dąbie and the Kamień Lagoon.

Goosander *Mergus merganser*. The mean for 2002–2018 was 7,670 (±673 SE) birds, the lowest number (429) was recorded in November 2016, and the highest number (24,000) in January 2009. The mean number for the first half of the survey (2002–2010) was 8,751 (±1,643 SE; 95% CI: 5,646; 12,070); for the second half (2011–2018) it was 6,589 (±1,255 SE; 95% CI: 4,297; 9,205), indicating an insignificant decrease over time (P = 0.31). Goosander was found throughout the study area, but the largest flocks were in the Szczecin Lagoon and on Lake Dąbie.

Great Crested Grebe *Podiceps cristatus*. The mean for 2002–2018 was 839 (±78 SE) birds, the lowest number (70) was recorded in January 2003, and the highest one (8,100) in January 2014. The mean number for the first half of the survey (2002–2010) was 522 (±107 SE; 95% CI: 349; 761); for the second half (2011-2018) it was 1,155 (±161 SE; 95% CI: 854; 1,485), indicating a significant increase over time (P = 0.002). Great Crested Grebe was found on large water bodies, hence the largest concentrations were in the Szczecin Lagoon and on Lake Miedwie. It was rather rare in the Odra Valley.

Mallard *Anas platyrhynchos*. The mean for 2002–2018 was 13,418 (±588 SE) birds, the lowest number (1,300) was recorded in March 2005, and the highest number (26,500) in March 2018. The mean number for the first half of the survey (2002–2010) was 12,522 (±1,210 SE; 95% CI: 10,227; 14,970); for the second half (2011–2018) it was 14,315 (±1,406 SE; 95% CI: 11,606; 17,108); this indicated a non-significant increase over time (P = 0.349). Mallard was found everywhere, but there were sites where considerable numbers congregated, especially during poor weather. During below-zero temperatures, these ducks tended to remain in the Szczecin – Police – Gryfino conurbation, but in frost-free winters, they could be found in localities such as the Kostrzyneckie Rozlewisko in the Odra Valley and small bays on Lake Dąbie, Lake Miedwie and the Kamień Lagoon.

Mute Swan *Cygnus olor*. The mean for 2002–2018 was 1,115 (±41 SE) birds, the lowest number (157) was recorded in January 2010, and the highest one (2,500) in January 2016. The mean number for the first half of the survey (2002–2010) was 932 (±90 SE; 95% CI: 763; 1114); for the second half (2011–2018) it was 1,299 (±78 SE; 95% CI: 1,154; 1,460), a significant increase over time (P = 0.04). Important areas for Mute Swan were extensive water bodies such as the Szczecin Lagoon, the Kamień Lagoon, Lake Dąbie and Lake Miedwie.

Whooper Swan *Cygnus cygnus*. The mean for 2002–2018 was 892 (±66 SE) birds, the lowest number (18) was recorded in March 2014, and the highest number (2,700) in March 2004. The mean number for the first half of the survey (2002–2010) was 769 (±118 SE; 95% CI: 562; 1021); for the second half (2011–2018) it was 1,016 (±159 SE; 95% CI: 712; 1,333), a non-significant increase over time (P = 0.23). Whooper Swan was seen more often and in greater numbers in the Odra Valley, on Lake Miedwie and the eastern Szczecin Lagoon.

Tundra Swan *Cygnus columbianus*. The mean for 2002–2018 was 34 (±3 SE) birds, the lowest number (0) was recorded during several counts, while the highest number (142) was recorded in March 2004. The mean number for the first half of the survey (2002–2010) was 46 (±8 SE; 95% CI: 30; 63); for the second half (2011–2018) it was 23 (±6 SE; 95% CI: 13; 34), indicating a significant

decrease over time (P = 0.03). This species was found mainly on Lake Miedwie, the Dzwonowo fish ponds and in the Odra Valley.

Tundra/Taiga Bean Goose *Anser fabalis sensu lato* (Bean Goose complex *Anser fabalis/ serrirostris*). The mean for 2002–2018 was 13,330 (±1113 SE) birds, the lowest number (89) was recorded in March 2005, and the highest number (30,000) in January 2013. The mean number for the first half of the survey (2002–2010) was 14,545 (±2,036 SE; 95% CI: 10,767; 18,730); for the second half (2011–2018) it was 12,114 (±1,810 SE; 95% CI: 8,672; 15,744) – a non-significant decrease over time (P = 0.389). The main area for Bean Goose complex was the Odra valley, mainly the Kostrzyneckie Rozlewisko and Międzyodrze. Other important sites were Lake Miedwie and the Kamień Lagoon. It must be emphasized that the vast majority of Bean Goose complex wintering and migrating through our study area belongs to Tundra Bean Goose *Anser serrirostris*.

White-fronted Goose *Anser albifrons*. The mean for 2002–2018 was 6,454 (±845 SE) birds, the lowest number (0) was recorded in January 2010, and the highest number (40,400) in March 2003. The mean number for the first half of the survey (2002–2010) was 8,602 (±2,192 SE; 95% CI: 4,775; 13,307); for the second half (2011–2018) it was 4,304 (±717 SE; 95% CI: 2,993; 5,792), indicating a marginally non-significant decrease over time (P = 0.07). Geese gather in similar places, creating mixed flocks. Hence, the most important areas are the same for White-fronted Goose as for Bean Goose complex. The proportion of White-fronted Goose in mixed flocks was slightly higher at Międzyodrze than at other sites.

Discussion

In north-western Poland, numbers of water birds as a group have increased over the last two decades. This situation can be explained by climate warming, because this phenomenon has been already demonstrated for some waterbird species wintering at these latitudes (Lehikoinen et al., 2013, Pavon-Jordan et al., 2015, Marchowski et al., 2017). The shifting boundaries of birds' ranges as a result of climate change may be variously manifested in the study area: numbers of some species are on the increase, others are decreasing. Looking at the individual species, the situation is much more complex than the overall pattern would suggest: the causes of changes in numbers can be quite different. Depending on the species, increases or decreases in number may have the same cause, or the same direction of change may have different causes (Marchowski et al., 2017).

Goosander numbers have decreased in our study area, but during the period of study (2002–2018) the changes were not significant. Before the start of this research, however, Goosander numbers were higher. Some 30 years ago, mean winter numbers (1991–1993) were 27,900 with a maximum of 33,100 birds (Meissner et al., 1993, Meissner, 1994); more recently (2016–2018), mean winter numbers were 14,600 ind. with a maximum of 21,300. More advanced calculations, taking into account such predictors as the size of the entire flyway population and ice cover, showed a decreasing trend in 2002–2016 (Marchowski et al., 2017). The probable decrease in abundance can be explained by the wintering grounds shifting farther east and north, where numbers have increased (Lehikoinen et al., 2013). The same applies to Smew: in the early 1990s, mean winter numbers of this species were 7,300 (1991–1993) with a maximum of 19,700 birds (Meissner, Kozakiewicz, 1992), whereas in recent years (2016–2018) the mean winter number was down to 1,400 with a maximum of 1,900 birds. That Smew's wintering areas have shifted further to the north and east has been demonstrated by Pavon-Jordan et al. (2015).

The reverse situation applies to two other species that winter in our area – Scaup and Tufted Duck. The abundances of these species have increased significantly in the last two decades: the

reason behind these changes is the same as in the two previous species. But the difference is that the main wintering grounds of Scaup and Tufted Duck are situated farther west and south in Europe than our study area. Hence, as a result of the eastward and northward shift of their wintering grounds, the abundances of these two species have locally increased (Lehikoinen et al., 2013, Marchowski et al., 2017).

In some species, number reductions can be explained by global trends. A good example is Tundra Swan, in which both the flyway and local populations have decreased significantly (Wetlands International, 2017). In contrast, local growth trends of other species are in line with global ones (Wetlands International, 2017, 2018). Our local examples are Mute Swan, Common Goldeneye and Great Crested Grebe, the numbers of which have all significantly increased.

With regard to Pochard, the lack of significant changes in the study period and the steep decline in the global populations of this species (Fox et al., 2016) highlights the importance of the study area for this species.

The numbers of two species – Whooper Swan and Mallard – have remained at the same level during the last two decades. The flyway population of the first has increased strongly (Wetlands International, 2017), so our local situation could also be explained by the shift of these birds' wintering grounds further to the north and east, but there is no proof of this. In contrast, the lack of directional changes in Mallard numbers in the study area along with a moderate decrease in the entire flyway population (Wetlands International, 2017) is hard to explain, because the population trends in this species as reported by various sources have been variously assessed (e.g. BirdLife International, 2015, Wetlands International, 2018).

The situation regarding the final two species is likewise not very clear and hard to explain. The trends differ from the global ones, but they cannot be explained by a shift in the wintering range because there is no proof of this. For example, the flyway population of Tundra Bean Goose (subspecies *Anser serrirostris rossicus*, mainly wintering in the study area) is growing but in our area it is stable; in contrast, the flyway population of White-fronted Goose is stable (Wetlands International, 2018) but its numbers in our study area are decreasing (marginally non-significantly). This may be due to the slightly different methodology adopted at the beginning of the research period, and to the fact that more sites have been monitored (for Geese only) including the German side of the Odra valley.

In summary, we can say that north-western Poland (the province of Western Pomerania) remains an important wintering area for waterbirds in Europe. Natura 2000 sites were established here to provide effective protection for this group of birds. The effects of climate change and other causes have led to changes in the numbers of individual species and whole groups. On balance, however, the importance of this region of Europe as a wintering site for waterbirds has increased, the main reason for this being climate warming. This presents new challenges to the institutions responsible for environmental conservation in these areas. The individual fates of each species are different – some species are decreasing, others are increasing. A thorough knowledge of the causes of these phenomena at the level of particular species and their life cycle will enable them to be effectively protected in these important locations.

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New record of *Copidognathus andhraensis* (Acari: Halacaridae) from the west coast of India, Arabian Sea

Tapas Chatterjee

Crescent International School, Bario, Govindpur, Dhanbad 828109, Jharkhand, India, e-mail: drtchatterjee@yahoo.co.in

Keywords *Copidognathus andhraensis*, West coast India, Arabian Sea

Abstract *Copidognathus andhraensis* Chatterjee, Annapurna & Chang, 2004 is reported here from Anjuna beach, Goa, west coast of India (Arabian Sea) among algal sediment. This is the first report of this species from west coast of India and also from the Arabian Sea.

Nowe stwierdzenie *Copidognathus andhraensis* (Acari: Halacaridae) z Zachodniego wybrzeża Indii, Morze Arabskie

Słowa kluczowe *Copidognathus andhraensis*, zachodnie wybrzeż Indii, Morze Arabskie

Streszczenie *Copidognathus andhraensis* został stwierdzony na plaży Anjuna, (Goa, zachodnie wybrzeża Indii, Morze Arabskie) pośród osadów alg. Jest to pierwsze stwierdzenie tego gatunku z zachodniego wybrzeża Indii, a także z Morza Arabskiego.

Introduction

Copidognathus is most species rich genus in the family Halacaridae (see Bartsch, 2009). In India, species of the genus *Copidognathus* were recorded from Chilka lagoon, Odisha, east coast of India (Chatterjee, 1991a; Chatterjee, Sarma, 1993); Visakhapatnam, Andhra Pradesh, east coast of India (Chatterjee, 1991b, Chatterjee, Annapurna, 2002, 2003; Chatterjee et al., 2003, 2004); Kerala, west coast of India (Chatterjee, Sarma, 1993; Chatterjee, 2000); Maharastra, west coast of India (Chatterjee, Chang, 2004); Goa, west coast of India (Chatterjee, Guru, 2011; Chatterjee, 2015) and Andaman & Nicobar Islands (Chatterjee, 1991c, 1992, 1995, 1996, 1997, 1999a,b; Chatterjee, De Troch, 2003; Sarma, Chatterjee, 1991; Chatterjee, Guru, 2013).

Copidognathus andhraensis was first described from Palm beach, Visakhapatnam, Andhra Pradesh, east coast of India among phytal samples (macroalgae) (Chatterjee et al., 2004). This species was also recorded from Singapore among colonies of Bryozoa (Bartsch, 2013).

The specimen of *C. andhraensis* used in the present study was collected from Goa, India, among macro algae, makes it the first recording of the species from west coast of India and also first report from the Arabian Sea.

Material and methods

One male among mixed macro algae growing on rocky coast of Anjuna beach (Lat. 15° 34′ 58″ N; Long. 73° 44′ 28.54″ E), Goa, west coast of India, 20th May 2011, coll. T. Chatterjee.

After preliminary observation specimen was processed for SEM study.

Specimen for scanning electron microscopy (SEM) was prefixed overnight at 4°C in 2.5% glutaraldehyde, followed by post fixation in 2% cold osmium tetroxide. After dehydration through a graded series of ethanol (50–100% at 10% interval) for 30 minutes each, the material was critical point dried, and coated with a platinum-palladium mix in a high evaporator, and then examined with a scanning electron microscope.

The following abbreviations are used in the text and figure legends: AD, anterior dorsal plate; AE, anterior epimeral plate; ds, dorsal setae 1–2 on the idiosoma; GA, genitoanal plate; GO, genital opening; OC, ocular plate(s); PAS, parambulacral seta(e); PGS, Perigenital setae.

Results and Discussion

Copidognathus andhraensis Chatterjee, Annapurna & Chang 2004
Copidognathus andhraensis – Chatterjee et al. 2004, 75–78, Figs. 1A–G, 2A–D.
Copidognathus andhraensis – Bartsch 2013, 111–119, Figs. 1–20.

Brief Description: Small median elevated area (areola) on AD; setae ds_1 on posterior side of median areola and pair of gland pores on anterior side of this areola. Distance between two gland pores almost (or more than) twice than that of two setae of ds_1. A pair of middle costae restricted to posterior third of the posterior dorsal plate. Anterior cornea on OC large, posterior cornea subdivided into two small corneae; ds_2 on near anteromedian corner of OC. Pair of oblong punctate areolae present within area representing first pair of epimera on AE (Figure 1B). Magnified view of epimeral pore on AE shown in Figure 1C. In male paragenital areolae enlarged anteriorly and occupying almost entire length of GA, but not touching anterior end of GA. In present male, distance between anterior end of GO and that of GA equaling about 1.2 times of GO length (Figure 1A). In male about 40 PGS present, about 5 pairs close to and about 30setae in a wide ring around GO (Figures 1A, D). Legs slender (Figure 1A). Pectinate setae on leg I–IV: 0–2–1–0 (Figures 2A, B). Tarsus I with 2 doublets eupathid PAS. Tarsus II with 2 singlet eupathid PAS. Paired claws II to IV with pectines extending along inner flank of claws (Figures 2C–E).

Distribution: Indian Ocean - Andhra Pradesh, east coast of India, Bay of Bengal (Chatterjee et al 2004); Goa, west coast of India, Arabian Sea (Present report); Pacific Ocean – Singapore (Bartsch, 2013).

Remarks: Bartsch (2013) commented about pair of gland pores on the AD and pair of ds_1 shown in Fig 1B of Chatterjee (2004) 'distance between pores about the same as between the pair of setae', but in Fig 1B of Chatterjee (2004) and in present specimen showing that the distance between two gland pores more than twice than that between two setae of ds_1 as found in Singapore specimens. The position of ds_2 in the present study is found near anteromedian corner of OC similar with Bartsch (2013). Claws also found similar with Singapore specimens in present study.

There are many natural species groups in the genus *Copidognathus*. One of these groups is the '*tricorneatus'* group. At present, 14 species of this species group have been recorded. *Copidognathus andhraensis* Chatterjee, Annapurna & Chang has been reported among phytal algal samples from Andhra Pradesh, east coast of India (Chatterjee et al., 2004), among algal

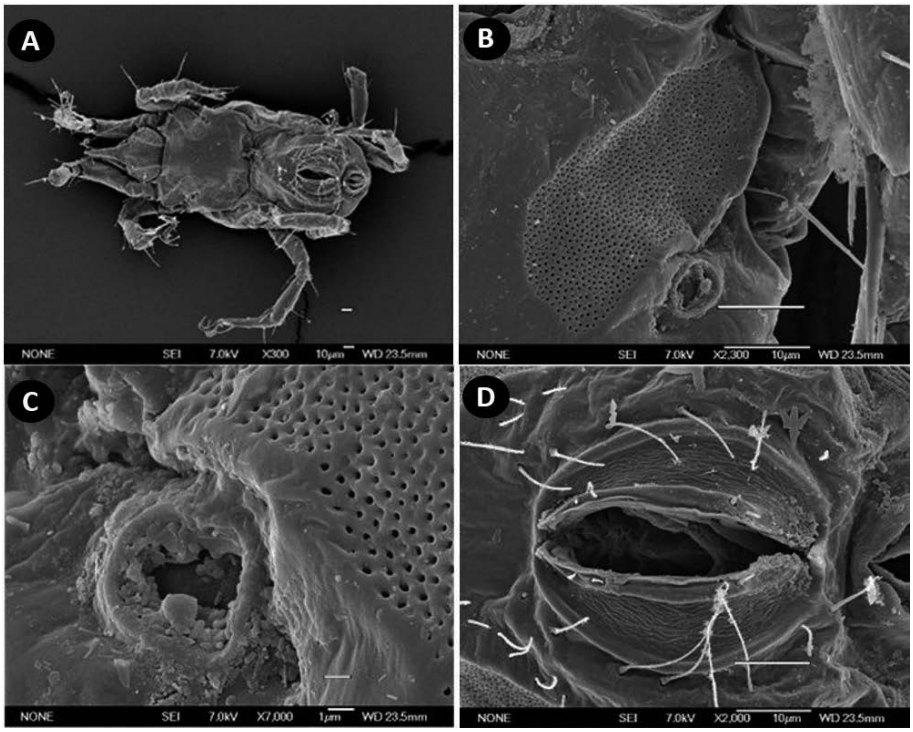

Figure 1. *Copidognathus andhraensis* Chatterjee, Annapurna and Chang, 2004 male. A. Idiosoma, ventral; B. Ventral areola on AE; C. Magnified view of epimeral pore on AE; D. GO. (Scale bars: A, B, D = 10 µm, C = 1 µm)

sediments from Anjuna beach Goa, west coast of India (present report) and among bryozoa from Singapore (Bartsch, 2013). *Copidognathus dictyotellus* Bartsch was reported among corals, algae and sponges in Rottnest Island, Western Australia (Bartsch, 1997, 1998). Bartsch (1993) reported *C. dictyotus* Bartsch from Babi Besar Island, Malaysia, South China Sea, among dead coral blocks and calcareous algae, in and below the tidal range. The species *C. hummelincki* (Viets) was reported from Bonaire and Aruba, Caribbean Sea (Viets, 1936) and also among algae *Halemida* from Florida, USA (Newell, 1947). *Copidognathus kagamili* Newell was reported in northeastern Pacific, Aleutian Islands from boulders covered with corals, sponges, and hydrozoans at about 75 m depth (Newell, 1950). Otto (2001) reported *C. leptoporus* Otto from Great Barrier Reef in northeastern Australia among coarse sand, coralline algae, and hydroids at 0.5m to 9m depth. Otto (2001) also reported C. *megaloporus* Otto, 2001 from Great Barrier Reef in northeastern Australia (Otto, 2001). *Copidognathus longipes* Bartsch was recorded from northwestern Atlantic (Josephine Bank), taken from a depth of 210-240 m (Bartsch, 1973). *Copidognathus mucronatus* Viets was reported from Black Sea (Viets, 1928; Bartsch, 1997, 2001) taken from Phyllophora, Rhodophyta (Viets, 1928). *Copidognathus quadricostatus* (Trouessart) and *C. trouessarti* (Voinov) were reported from northeastern Atlantic and Mediterranean (Trouessart, 1894; Voinov, 1896; Bartsch,1991, 1997).The species *C. quadricostatus* and *C. trouessarti* often found together amongst sublittoral bryozoans and barnacles (Bartsch, 1997). *Copidognathus spophaie* Pepato

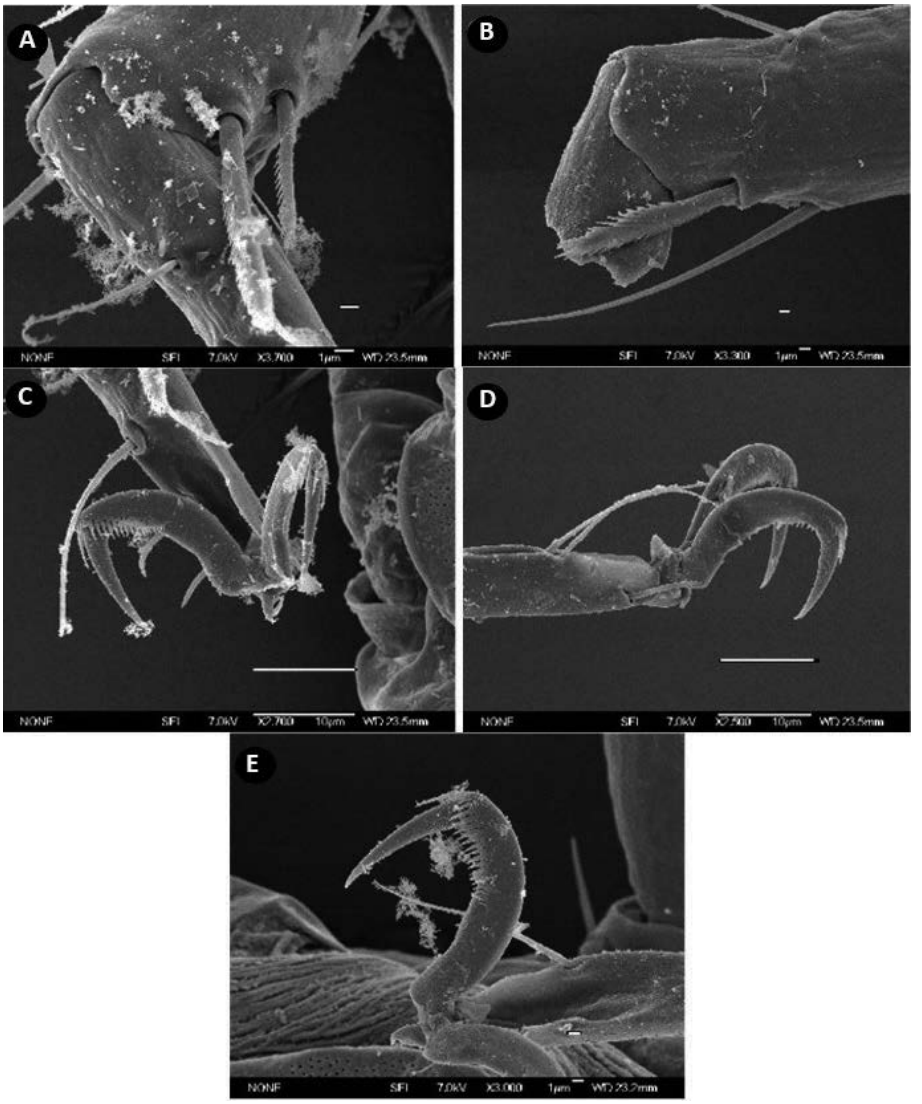

Figure 2. *Copidognathus andhraensis* Chatterjee, Annapurna and Chang, 2004 male. A. Part of tibia II showing pectinate setae; B. Part of tibia III showing pectinate seta; C. Part of tarsus II showing claws; D. part of tarsus III; E. Claw of tarsus IV. (Scale bars: A, B, E = 1 µm ; C, D = 10 µm)

& Tiago was reported among *Sargassum cymosum* from intertidal rocky shore at Lázaro Beach, Brazil (Pepato, Tiago, 2005). *Copidognathus tricorneatus* was reported from eastern Australia, Sydney, from a substratum with corals and sponges (Viets, 1938; Bartsch, 1997). *Copidognathus xaixaiensis* Proches was reported from the middle littoral rocks on the breakwater wall facing the resort in Xai-Xai, Mozambique (Proches, 2002). Three species: *C. andhraensis*, *C. dictyotellus*, and *C. xaixaiensis* are known from Indian Ocean region.

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Bioinformatics analysis of the promoter sequence of the *9f-2.8* gene encoding germin

Izabela Szućko, Urszula Kowalska, Lidia Skuza

University of Szczecin, Institute for Research on Biodiversity, Faculty of Biology, Department of Molecular Biology and Cytology, ul. Wąska 13, 71-415 Szczecin, Poland, e-mail: izabela.szucko@usz.edu.pl

Keywords *Triticum aestivum* L., bioinformatic tools, germin protein, *in silico* analyse

Abstract Bioinformatics is a field of study having an enormous potential, allowing to solve a number of problems arising as a result of dynamic development of natural sciences with the use of computer science methodologies. It is widely used and constitutes a basis for most scientific research conducted in the field of molecular biology. The aim of this study was *in silico* analysis of the promoter sequence of the *9f-2.8* gene encoding isoform of the germin protein considered as a germination marker in common wheat's (*Triticum aestivum* L.). The gene mentioned above has already been characterized, however, with the use of experimental methods instead of bioinformatics. Analysis with the use of TSSP and TSSPlant software identified the promoter region and classified it as the TATA-box containing promoter. For *9f-2.8* gene including 2.8 kbp, the TSSP software indicated that the TATA-box sequence was located in the position 1665 nt, while the TSSPlant tool showed that TSS [+1] was located in the position 1699 nt. At the second stage, transcription factors were analyzed. Four main families of transcription factors were detected within the analyzed region: MADS, AP2, bZIP and NAC. The most common were MADS-box and bZIP motifs. In the final step of analysis the presence of CpG islands have been checked using the PlantPAN software. The region which could be potentially considered as CpG island have been detected and localized. Software used in analysis above is free online tool.

Analiza bioinformatyczna sekwencji promotora genu *9f-2.8* kodującego germinę

Słowa kluczowe *Triticum aestivum* L., narzędzia bioinformatyczne, białko germina, analiza *in silico*

Streszczenie Bioinformatyka jest dyscypliną nauki, w której tkwi olbrzymi potencjał. Dyscyplina ta rozwiązuje wiele problemów powstałych w wyniku dynamicznego rozwoju nauk przyrodniczych przy użyciu metodologii nauk informatycznych. Ma szerokie zastosowanie i jest bazą dla prowadzenia większości badań naukowych z dziedziny biologii molekularnej. Celem artykułu jest analiza *in silico* promotora genu *9f-2.8* kodującego izoformę (9f-2.8) białka germiny uważaną za marker kiełkowania u pszenicy zwyczajnej (*Triticum aestivum* L.). Gen ten był już wcześniej scharakteryzowany, jednak do jego analizy korzystano z metod eksperymentalnych, nie obliczeniowych. Analiza bioinformatyczna za pomocą programów TSSP i TSSPlant pozwolila zidentyfikować promotor i potwierdziła jego klasyfikacje do grupy promotorów z motywem TATA-box. W genie *9f-2.8* liczącym 2.8 kpz program TSSP wykazał, że sekwencja TATA-box

znajduje się w pozycji 1665 nt, zaś narzędzie TSSPlant wskazało, że TSS [+1] znajduje się w pozycji 1699 nt. Drugim etapem była analiza czynników transkrypcyjnych. W analizowanym obszarze wyróżniono cztery główne rodziny czynników transkrypcyjnych: MADS, AP2, bZIP oraz NAC, z których najliczniejsze były motywy MADS-box oraz bZIP. Ostatnią częścią analiz była kontrola obecności wysp CpG. Zastosowano program PlantPAN, dzięki któremu zlokalizowano region spełniający warunki, pozwalające uznać go za wyspę CpG. Programy, których użyto do scharakteryzowania tych sekwencji są darmowe i ogólnodostępne online.

Introduction

Germins are group of proteins present in germinating grains of cereals, among others, in wheat (*Triticum aestivum*) and barley (*Hordeum vulgare*), as well as in some dicotyledonous species, such as *Arabidopsis thaliana* or mustard (*Sinapis arvensis*). However, they mainly occur in the cell wall of monocotyledons. They form an extensive and diverse set of proteins characteristic of plants (Nowakowska, 1998). Germin is characterized by a homopentameric structure and a mass of 125 kDa. Increased concentration of germins, and thus increased expression of genes encoding these proteins is a hallmark of the germination process, hence they are called the germination marker (Nowakowska, 2001). In addition, these proteins are involved in plant defense responses to stress caused by abiotic or biotic factors (Lane, 2000; Davidson et al., 2009). Considering the essence of the functions of this group of proteins, it seems necessary to complement knowledge in this field not only through experimental techniques, but also *in silico* analysis. The rapid development of technology over the past years, which was also associated with enormous progress in bioinformatics, significantly broaden and facilitated the possibilities of conducting analyses. Bioinformatics is based on a variety of mathematical methods, which in a simple and rapid way allow for a detailed analysis of a given sequence. There are many publicly available, free bioinformatic programs that are a great tool for gene analyses (Baxevanisa, Ouellette, 2004, Xiong, 2006, Higgs, Attwood, 2008). Thanks to the use of a number of bioinformatic tools, it is possible to supplement and correct data obtained through the application of experimental methods.

Gene expression is a highly complex and strictly regulated process, because its subsequent stages are closely related and dependent on each other (Szopa et al., 2003). Each stage on the way from the gene to the functional protein can be subject to regulation. In eukaryotes, many cellular processes are regulated at the level of transcription. This process is complicated and we distinguish two main parts in it: transcription initiation and RNA synthesis and processing. The key issue for initiating transcription is to build initiation complexes, whose primary element is the promoter (Molina, Grotewold, 2005). The promoter is a fragment of a sequence lying on a DNA strand, upstream from the transcription start site (TSS) of the gene that has regulatory functions. It is not directly involved in the transcription, but it defines its beginning, direction, time and place (Porto et al., 2014). The identification of promoters and their regulatory elements is one of the main challenges of modern bioinformatics as well as structural and functional genomics, and it allows to predict the expression profiles and location of genes in plants (Rombautus et al., 2003; Porto et al., 2014).

The structure of promoters varies depending on the type of polymerase that will transcribe the gene. However, common elements can be distinguished in all of them, *i.e.*, elements of the basic promoter, *i.e.*, TATA-box, Inr, BRE, DPE, MTE and regulatory elements in its vicinity. They are present upstream the basic sequences. Their presence does not have a decisive influence on the transcription, which is possible even in their absence, but then it occurs with lower

efficiency. There are many factors that determine transcription initiation depending on the type of polymerase involved in this process (Roy, Singer, 2015).

Over 3,000 genes are involved in transcription in plants, and more than half of them encode transcription factors (TFs). The transcription process relies on a number of transcription factors that, through binding to specific DNA sequences, form regulatory regions (Hernandez-Garcia, Finer, 2014). Transcription factors mainly regulate the transcription initiation phase, which is one of the most important points in gene expression regulation. TF regulating effects include interactions with *cis*-acting regulatory elements (CAREs). During this process, TFs function as regulatory *trans*-acting elements that bind to specific *cis*-regulatory elements in the promoters of target genes to activate or repress expression of the target genes. Transcription factors are subject to a complicated classification based on DNA-binding motifs, nevertheless, among plants the most characteristic families include: MADS, AP2, NAC, bZIP, MYB, DATF and WRKY.

In contrast to gene prediction, *in silico* prediction of plant promoters is still underdeveloped; one of the main problems is to define the promoter's location. Although the bioinformatic databases are constantly being updated, there is still a lack of clear and unambiguous descriptions of genomic segments that contain all the elements required for transcription activation. While there are studies on this subject for the model organisms like *Arabidopsis thaliana* or *Medicago truncatula*, it is significantly more difficult to obtain data for plants not belonging to this group.

The following work concerns promoter sequence analysis of the *9f-2.8* gene encoding one of the two germin protein isoforms (9f-2.8) using bioinformatics tools. The previous data on the structure of these genes date back to 1991. Lane and others relied then on tedious methods based on the creation of genomic libraries, restriction enzyme digestions and sequencing.

Materials and Methods

The *9f-2.8* gene sequence (accession number M63223) deposited in the NCBI database (National Center for Biotechnology Information) was the base material for the bionformatic analysis. Publicly available bioinformatic programs were used to determine and analyze promoters, *i.e.*, TSSP – in order to determine the promoter region, TSSPlant – TSS position, CisBP – to analyze transcription factors and their DNA binding motifs and PlantPan – to analyze the occurrence of CpG islands.

Results

Analysis in the TSSP program showed that the promoter belonged to the group of promoters containing the TATA box. The TAT-box of the *9f-2.8* gene (total sequence length – 2822 nt) is located at position 1695 nt. The TSS position (1699 nt) was determined using the TSSPlant program. Sequences characteristic for the binding of transcription factors belonging to four different families were distinguished in the analyzed gene: MADS, bZIP, NAC and AP2. It was observed that factor motifs belonging to the MADS box were the vast majority, mainly: TaMADS#11 and VRN-B1 (Tables 1 and 3).

The distribution of motifs in the promoter sequences is random, and none of the motif families are present only in one region of the analyzed sequence. There are many motifs belonging to the bZIP family. The motifs of the NAC and AP2 families are significantly less common. The sequences of individual motifs are presented in Table 2 and Figure 1.

Analysis of the gene sequence encoding the germin isoform using PlantPAN showed the presence of the CpG island. PlantPAN has found the CpG island in the *9f-2.8* gene, starting at position 1412, with a length of 1234 nucleotides (Table 2).

Table 1. Families of transcription factors in the promoter of the *9f-2.8* gene

Family of TFs	Name	Number of motifs
MADS box	TaMADS#11 VRN-B1	11 11
bZIP	HBP1A_WHEAT HBP1B_WHEAT EmBP-1	3 2 3
NAC/NAM	NAC69-1	3
AP2	A5JTT1_WHEAT	5

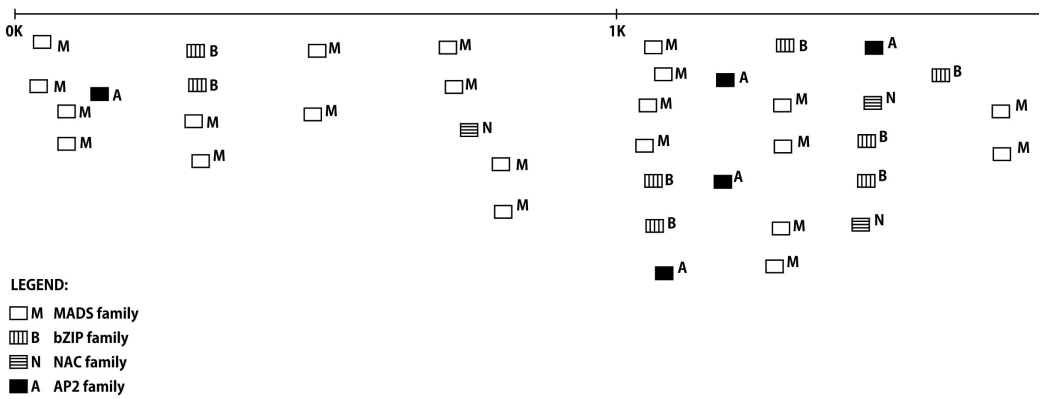

Figure 1. Distribution of motifs in the promoter of the *9f-2.8* gene

Table 2. Motif sequences in the promoter of the *9f*-2.8 gene

Name	Sequence
1	2
TaMADS#11	ATATATATGGAAGG CCAAAAAAAAT TGCATTTTTGC TATACCGAAAAATGTATT TCTTTTTTTGC ATAAATATCGTACA TTATATATTATTGGCAAC CAGAAACAAAACAAGAAGAGA TTCTCGGCTTTTTCATTTTGC TTTCTCTCTTTTGCT TCCCCTATATAAAGGACT

1	2
VRN-B1	ATATATATGGAAGG CCAAAAAAAAT TGCATTTTTGC TATACCGAAAAATGTATT TCTTTTTTTGC ATAAATATCGTACA TTATATATTATTGGCAAC CAGAAACAAAACAAGAAGAGA TTCTCGGCTTTTTCATTTTGC TTTCTCTCTTTTGCT TCCCCTATATAAAGGACT
HBP1A_WHEAT	TGACACGTTA CGACGTGT TGCCACGCAA
HBP1B_WHEAT	CATGTCGTCA AGTGACGACA
EmBP-1	TGACACGTTA CGACGTGT TGCCACGCAA
NAC69-1	ATTTGACTA CCTTGACTC CACGCAAG
A5JTT1_WHEAT	CGCAGCCC TGCGCCGACG TACGCCGCAG CGGCGGCG CAGCCGGCAG

Table 3. CpG islands in the *9f-2.8* gene, PlantPAN program

Start	Finish	Length	Frequency C+G
1412	2663	1234	0,53

Discussion

Regulation at the level of transcription plays the most important role in the activation or inhibition of expression and is largely controlled by gene promoters and *cis* elements present in their region. The structure of gene promoters transcribed by polymerase II has been most accurately characterized so far. The structure of its promoters has been described in many prokaryotic and eukaryotic organisms, *i.e.*, yeast, fruit fly or human (Morton et al., 2014). The majority of research conducted so far on promoters focused on understanding the structure and functioning of animal promoters, therefore, a clear advantage of information about them is observed when analyzing data available in the scientific literature. However, this information forms a foundation for understanding the structure of plant promoters. Analyses are constantly being conducted to find out their structure and properties depending on their sequence, including curved DNA

(Pandey, Krishnamachari, 2006). Model organisms, which during the studies became the starting point for describing plant promoters, included *Drosophila melanogaster, Mus musculus* as well as *Homo sapiens* (Butler, Kadonaga, 2002; Smale, Kadonaga, 2003; Riva, 2012). Research on gene promoters in plants is essential for understanding the global mechanism of gene regulation. Performing a series of bioinformatic analyses on the sequence of a gene encoding germin isoform was designed to analyze the promoter sequences as well as to check the effectiveness and reliability of the programs and to supplement the data published in the 1990s.

Comparing the results with those available in the literature, we could see both a number of similarities, but also differences that may have arisen from the application of different analytical tools (Lane et al., 1991, Nowakowska, 1998, 2001). Differences obtained during the analysis of the data obtained with bioinformatic tools are frequently observed. This is influenced by the quantity of data contained in individual databases as well as model organisms, on the basis of which the programs were designed. The PromPredict computing predicting program is often used to predict promoter regions. It has been used to find promoter regions, *e.g.*, in *A. thaliana* or *O. sativa*. The effectiveness and correctness of promoter region predictions using this program reaches 90%. It analyzes differences in DNA stability of neighboring upstream and downstream regions in relation to TSS (Shahmuradov et al., 2017).

The result for determining the promoter region of the *9f-2.8* gene was consistent with the data available in the literature. The TATA-box position was also similar (1 nt shift) (TSSPlant, 1665-; Lane and others 1991, 1664-). Lane et al. (1991) analyzed the promoter regions of genes coding for two germin isoforms in common wheat. They qualified the analyzed promoter structure to TATA-box promoters, which was confirmed in this work using the TSSPlant program. Promoters containing the TATA box are regulated by the action of biotic and abiotic stimuli. Germins, whose intensive synthesis is observed during germination, are a great example of genes containing the TATA-box motif. The germination process in which they participate is strongly dependent on abiotic factors. Lack of optimal environmental conditions, which include temperature, humidity, soil type, water access or adequate light exposure, may result in plant growth inhibition and development. What is more, pathogen attacks, exemplifying biotic factors, also stimulate the expression of germins, involved in plant defense reactions through the participation in cell wall cross-linking.

The analyses localized the TSS at the –1699 nt position. The obtained results meet the condition characteristic for promoters with the TATA box, according to which the start site of transcription is located at a distance of about 30–40 base pairs from the TATA-box. Conventional methods used to determine TSS are based on technologies of low and medium throughput, *i.e.*, EST/cDNA or MPSS modification. For *A. thaliana*, the PEAT analysis was applied to determine TSS sites, using transcript digestions with TAP; it allows detecting potential TSS sites and subsequently analyzing them based on the already known TFBS signals (Morton et al., 2014).

The analysis of the sites responsible for transcription factor binding is the key to understanding expression regulation, because they are mainly responsible for the transcription process to occur. In the following tests, the CIS-BP program was used to classify TFs, thanks to which it was possible to determine CAREs characteristic for plants in the analyzed sequence. Four families, characteristic of plant promoters, were localized in the analyzed region: MADS, AP2, bZIP and NAC. Bioinformatic analysis allowed to reveal their sequences, the distribution of individual families against each other and their location, which indicated their presence in both the distal and proximal region of the promoter. Thirty-eight sequence motifs were distinguished.

Complex interactions between protein and DNA lead to activation, enhancement or suppression of transcription. Germins, in addition to their key role during germination, participate in plant reactions related to stress response. The presence of the Dreb1 motif belonging to the AP2 family of genes encoding germin isoforms demonstrated during the analysis confirmed their involvement in stress reactions (Yaish et al., 2010). The Dreb1 motif was detected in *A. thaliana* in the *Rd29A* gene promoter and was associated with the dehydration-triggered plant response (Hernandez-Garcia, Finer, 2014). Motifs belonging to the NAC family have also been localized in *A. thaliana*, and similarly as AP2, they are involved in responses to drought (Tran et al., 2004). The remaining transcription factor motifs, *i.e.*, MADS-box and bZIP are also related to stress response induced by abiotic factors (Jakoby et al., 2002; Schütze et al., 2008). The MADS family additionally participates in processes related to gametophyte development (Heijmans et al., 2012). Literature data indicate that unidirectional AT repeats are present in the promoter region of the *9f-2.8* gene. Nine sequences known as RY, *i.e.*, purine pyrimidine AuxRE-type sequences (auxin-responsive element), conditioning the response to auxin, can also be distinguished. In addition, there were characteristic sequences, similar to the AuxRE element mentioned above, and PS was one of them. It is a homologous fragment to the *PS-IAA4/5* gene. There were also "TG" sequences in this region. The AS motif is another element present in the promoter of this gene, responsible for binding the ASF-1 protein (Nowakowska, 2001). It is possible to regulate transcription factors using compounds, such as auxins, gibberellins, salicylic acid and certain ions or chlorides (Lane et al., 1991; Nowakowska, 1998, 2001).

The use of the PlantPAN program allowed to localize the CpG island, not only in the promoter region, but in the entire sequence of the analyzed gene. Generally, it is assumed that CpG islands are motifs characteristic of animal genomes (Sakowicz, Frasiński, 2014). However, there are references in the literature and the results of analyses demonstrating the presence of these motifs also in plants. Sequence analysis of the *Arabidopsis thaliana* genome, showed the presence of CG-rich segments. Most segments rich in CpG were associated with genes, hence they can be used as landmarks when identifying genes in plants. CpG motifs present in plants meet the criteria applied to identify animal CpG islands (Ashikawa, 2001). PlantPAN indicated the region recognized as the CpG island in the *9f-2.8* gene at 1412-2663. It means that the CpG island is located partly in the promoter region. The presence of CpG islands in plants has been the object of numerous studies for a long time. Epigenetic modifications, *i.e.*, DNA methylation, chromatin remodeling and histone modifications are hereditary changes that affect the expression of genes, and thus the phenotype of organisms. Among them, DNA methylation has the greatest impact on gene expression in plants and animals. DNA methylation involves CpG dinucleotides and CpNpG sites (N = A, C or T). CpG-rich regions are known as CpG islands. They must meet three conditions to classify them as CpG islands: a) they must contain over 50% of CG dinucleotides, b) the length of the CpG/CpNpG region should be more than 200 bp and c) the ratio of observed to expected dinucleotides should be above 0.5. CpG islands present near TSS can regulate tissue-specific gene expression. The plant genome contains more CpG dinucleotides compared to human DNA. Cytosine methylation on CpG islands in the promoter region has been shown to limit the access of transcription factor binding, which inhibits expression. Cytosine methylation patterns are not static, they change with development or under the influence of environmental conditions in the whole plant genome. DNA methylation plays a significant role in plant embryogenesis, seed development, in regulating the immune response to pathogenic infections, environmental adaptations and resistance to stress. Methylation errors cause defects in embryogenesis, *i.e.*, abnormal cell divisions or partial sterility as well as development retardation and plant size reduction.

CpG/CpNpG analyses indicate the presence of CpG/CpNpG islands in the second half of the promoter region (3' end) in all *OsPRs* genes, except for *OsPR2*, whereas they are absent in *AtPR*, indicating that the CpG islands are present in *Oryza sativa* genome in the *PR* genes (parthogenesis related), whereas they are missing in the *PR* genes in *A. thaliana*. The CpG islands observed only in the *PR* genes of rice indicate that monocotyledonous genomes contain more GC motifs than dicotyledonous ones (Kaur et al., 2017).

Knowledge of the promoters' architecture is crucial for understanding the transcription regulation, which is fundamental to basic life processes. More detailed molecular analyses of these sequences are connected with the isolation of promoter sequences and associated elements, which is a critical point during the regulation of the introduced transgenes, including genes encoding proteins and non-coding sequences involved in gene silencing through the RNAi mechanism.

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