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The Wetlands Diversity

Editors

Angela Curtean-Bănăduc & Doru Bănăduc

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IN MEMORIAM

Ernst Heinrich Philipp August Haeckel
(1834 – 1919)

Ernst Heinrich Philipp August Haeckel, the eminent German zoologist and evolutionist who was one of the period's most enthusiastic advocates of Darwinism, was born on 16 February 1834 in Potsdam, and died on 9 August 1919 in Jenna. He projected and proposed original ideas on the evolutionary descent of humans and he asserted that phylogeny is briefly and partially repeated in the process of ontogeny ("ontogeny recapitulates phylogeny").

Haeckel grew up in Merseburg. He studied in Würzburg and in Berlin University, where one of his professors, Johannes Müller, began to take him on expeditions to the North Sea coasts and to kindle his interest in sea organisms.

This contact with marine biology directed Haeckel's interests towards biology, but initially he took a medical degree, to satisfy his family's plans for him, at Berlin in 1857. For a while he practiced medicine; and travelled in Italy, where he painted and even considered art as a path. At Messina he researched Radiolaria one-celled protozoans.

The direction of Haeckel's interest was induced by reading Charles Darwin's On the Origin of Species by Means of Natural Selection. Meanwhile, in 1861 he obtained a dissertation in zoology at Jena University. In 1862 he was appointed associate professor of zoology, and that year, when he published his Radiolaria monograph, he asserted his understanding and acceptance of Darwin's theory of evolution. Since then he began to be a strong supporter of Darwinism, and he started lecturing to wide lay audiences on the theory of descent. For Haeckel, this was only the starting point, with effects and results to be sought further. In 1865 he was appointed full professor in Jena University, where he remained in charge until his retirement in 1909.

Haeckel's best-known published works were: Generelle Morphologie der Organismen (General Morphology of Organisms) and Die Perigenesis der Plastidule (The Generation of Waves in Small Vital Particles).

Haeckel brought debate to substantial and valuable biological questions. His gastraea theory, tracing multicellular animals to a theoretical two-layered ancestor, aroused both analysis and deliberations. His attraction to systematization along evolutionary lines drive to his very important improvements in the knowledge related to some invertebrate taxa such as radiolaria, medusa, siphonophores, and sponges.

Gathering and building collections, Haeckel founded the Phyletic Museum in Jena and also the Ernst Haeckel Haus; the latter contains his books and archives, and it cares for many other memorabilia of his extraordinary life and prestigious professional work.

The centenary of Haeckel reminds us of his lifelong devotion to natural sciences in a heroic stage of the history of the theory of evolution, a beautiful and remarkable life under the signs of art-like science.

The Editors

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Preface

In a global environment in which the climate changes are observed from few decades no more only through scientific studies but also through day by day life experiences of average people which feel and understand allready the presence of the medium and long-term significant change in the "average weather" all over the world, the most comon key words which reflect the general concern are: heating, desertification, rationalisation and surviwing.

The causes, effects, trends and possibilities of human society to positively intervene to slow down this process or to adapt to it involve a huge variety of aproacess and efforts.

With the fact in mind that these aproaces and efforts shuld be based on genuine scientific understanding, the editors of the *Transylvanian Review of Systematical and Ecological Research* series launch three annual volumes dedicated to the wetlands, volumes resulted mainly as a results of the *Aquatic Biodiversity International Conference*, Sibiu/Romania, 2007-2017.

The therm wetland is used here in the acceptance of the Convention on Wetlands, signed in Ramsar, in 1971, for the conservation and wise use of wetlands and their resources. **Marine/Coastal Wetlands** - Permanent shallow marine waters in most cases less than six metres deep at low tide, includes sea bays and straits; Marine subtidal aquatic beds, includes kelp beds, sea-grass beds, tropical marine meadows; Coral reefs; Rocky marine shores, includes rocky offshore islands, sea cliffs; Sand, shingle or pebble shores, includes sand bars, spits and sandy islets, includes dune systems and humid dune slacks; Estuarine waters, permanent water of estuaries and estuarine systems of deltas; Intertidal mud, sand or salt flats; Intertidal marshes, includes salt marshes, salt meadows, saltings, raised salt marshes, includes tidal brackish and freshwater marshes; Intertidal forested wetlands, includes mangrove swamps, nipah swamps and tidal freshwater swamp forests; Coastal brackish/saline lagoons, brackish to saline lagoons with at least one relatively narrow connection to the sea; Coastal freshwater lagoons, includes freshwater delta lagoons; Karst and other subterranean hydrological systems, marine/coastal. **Inland Wetlands** - Permanent inland deltas; Permanent rivers/streams/creeks, includes waterfalls; Seasonal/intermittent/irregular rivers/streams/creeks; Permanent freshwater lakes (over eight ha), includes large oxbow lakes; Seasonal/intermittent freshwater lakes (over eight ha), includes floodplain lakes; Permanent saline/brackish/alkaline lakes; Seasonal/intermittent saline/brackish/alkaline lakes and flats; Permanent saline/brackish/alkaline marshes/pools; Seasonal/intermittent saline/brackish/alkaline marshes/pools; Permanent freshwater marshes/pools, ponds (below eight ha), marshes and swamps on inorganic soils, with emergent vegetation water-logged for at least most of the growing season; Seasonal/intermittent freshwater marshes/pools on inorganic soils, includes sloughs, potholes, seasonally flooded meadows, sedge marshes; Non-forested peatlands, includes shrub or open bogs, swamps, fens; Alpine wetlands, includes alpine meadows, temporary waters from snowmelt; Tundra wetlands, includes tundra pools, temporary waters from snowmelt; Shrub-dominated wetlands, shrub swamps, shrub-dominated freshwater marshes, shrub carr, alder thicket on inorganic soils; Freshwater, tree-dominated wetlands; includes freshwater swamp forests, seasonally flooded forests, wooded swamps on inorganic soils; Forested peatlands; peatswamp forests; Freshwater springs, oases; Geothermal wetlands; Karst and other subterranean hydrological systems, inland. **Human-made wetlands** - Aquaculture (e. g., fish/shrimp) ponds; Ponds; includes farm ponds, stock ponds, small tanks; (generally below eight ha); Irrigated land, includes irrigation channels and rice fields; Seasonally flooded agricultural land (including intensively managed or grazed wet meadow or pasture); Salt exploitation sites, salt pans, salines, etc.; Water storage areas, reservoirs/barrages/dams/impoundments (generally over eight ha); Excavations; gravel/brick/clay pits; borrow pits, mining pools; Wastewater treatment areas, sewage farms, settling ponds, oxidation basins, etc.; Canals and drainage channels, ditches; Karst and other subterranean hydrological systems, human-made.

The editors of the *Transylvanian Review of Systematical and Ecological Research* started and continue the annual sub-series (*Wetlands Diversity*) as an international scientific debate platform for the wetlands conservation, and not to take in the last moment, some last heavenly "images" of a perishing world …

This 20.2 volume included variated researches from diverse wetlands around the world.
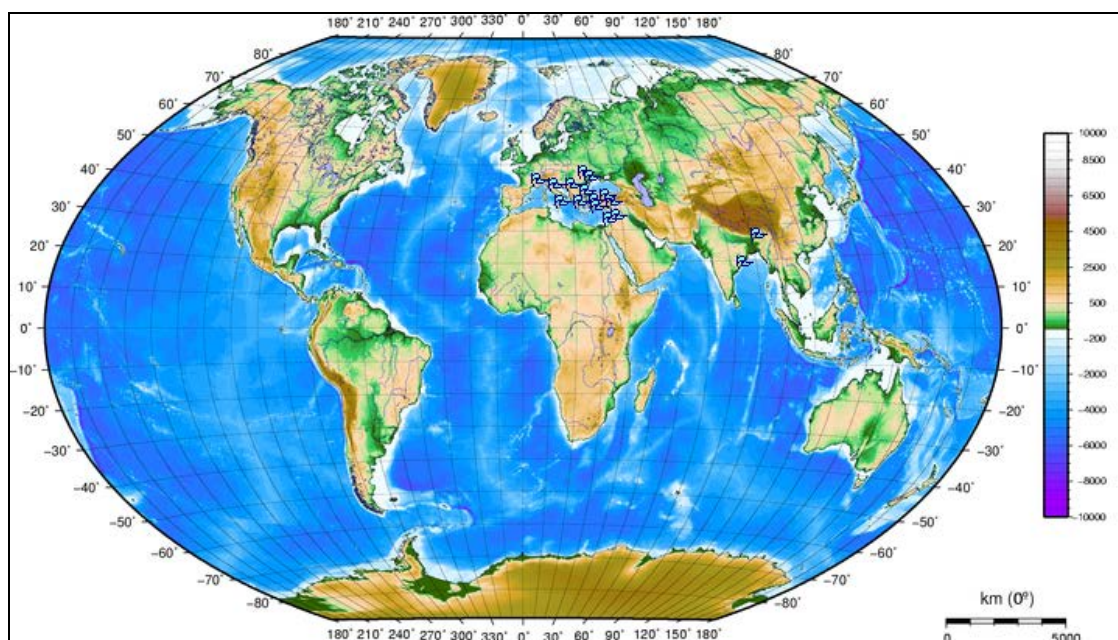
The subject areas (⮂) for the published studies in this volume.

No doubt that this new data will develop knowledge and understanding of the ecological status of the wetlands and will continue to evolve.

Acknowledgements

The Editors

CHARACTERISTICS OF DISSOLVED ORGANIC NITROGEN IN MUNICIPAL AND BIOLOGICAL NITROGEN REMOVAL WASTEWATER TREATMENT PLANTS IN JORDAN

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ABSTRACT

The investigation is conducted on the biochemical form and characteristics of wastewater-derived DON in three different WWTPs in Jordan. The main eliminations of DON and biodegradable dissolved organic nitrogen (BDON) noticed along the treatment course are in the Irbid (ITP). Dissolved combined amino acids (DCAA) and dissolved free amino acids (DFAA) in the outlet accounted for less than 4% of the outlet DON of all plants. The DON from the outlet was composed of 90% hydrophilic compounds which stimulate algal growth. The study provided information for future improvement of WWTPs of Jordan and for adjusting the assortment of DON elimination systems to comply with stricter limits.

RÉSUMÉ: Caractéristiques de l'azote organique dissous dans les usines municipales de traitement des eaux usées par enlèvement biologique de l'azote en Jordanie.

La forme biochimique et les caractéristiques du DON dérivé des eaux usées dans trois WWTP différentes en Jordanie ont été étudiées. Les principal éliminations de DON et d'azote organique dissous (BDON) biodégradable tout au long du traitement ont été observées à Irbid (ITP). Les acides aminés combinés dissous (DCAA) et les acides aminés libres dissous (DFAA) dans l'exutoire représentaient moins de 4% du DON de toutes les usines. Plus de 90% du DON émis était sous forme de composés hydrophiles qui stimulent la croissance des algues. L'étude a fourni des informations pour l'amélioration future des stations d'épuration de la Jordanie et pour l'assortiment de systèmes d'élimination de DON pour respecter des limites de décharge d'azote plus basses.

REZUMAT: Caracteristicile azotului organic dizolvat în stațiile municipale de tratare a apei uzate cu treaptă biologică pentru eliminarea nitraților din Iordania.

Au fost analizate forma biochimică și caracteristicile DON provenit din apa uzată în trei WWTP din Iordania. Cele mai importante eliminări ale DON și azotului organic dizolvat biodegradabil (BDON) în timpul procesului de tratare au fost constatate la Irbid (ITP). Aminoacizii combinați dizolvați (DCAA) și aminoacizii liberi dizolvați (DFAA) din emisar au însumat aproximativ sub 4% din DON deversat pentru toate stațiile. Peste 90% din DON deversat a fost format din compuși hidrofili care stimulează dezvoltarea algală. Studiul oferă informații pentru viitoare îmbunătățiri ale WWTPs iordaniene și pentru ajustarea sistemelor de eliminare a DON pentru a respecta limite mai stricte de azot în emisar.

INTRODUCTION

Pollution is one of the main human negative impact on water qality (Sandu et al., 2008; Yildiz et al., 2010; Akkoz, 2016; Al-Rufaie, 2016; Khoshnood, 2017). The most significant source of anthropogenic nitrogen to surface waters is the domestic wastewater runoff that also has distinct effects on the water quality, especially in effluent-dominated waters (Pagilla et al., 2008; Bronk et al., 2010; Liu et al., 2012; Huo et al., 2013). Biological enhanced nitrogen removal (BENR) processes that eliminate most of the dissolved inorganic nitrogen are widely used in the domestic wastewater treatment to decrease cultural eutrophication of the reception waters (Czerwionka et al., 2012). The dissolved organic nitrogen (DON) is one of the remaining fractions of nitrogen in the outlet of the effective BENR process that influence the surface water (Pagilla et al., 2006; Liu et al., 2012) Previous studies showed that algae and plankton are sensitive to the bio available DON of the outlet (Pehlivanoglu-Mantas and Sedlak, 2006, 2008; Sattayatewa et al., 2009; Simsek et al., 2013). Nowadays, DON is currently important in wastewater treatment plants (WWTPs) because of highly concerns such as increase and stimulate algal growth (Pehlivanoglu-Mantas and Sedlak, 2004) and forming nitrogenous compounds by-product *N*-nitrosodimethylamine (Pehlivanoglu-Mantas and Sedlak, 2006, 2008; Lee et al., 2007). The emerging worries for wastewater-derived DON have augmented the requirement to define their concentrations, structure and properties.

DON is commonly determined by subtracting dissolved inorganic nitrogen (DIN, the sum of ammonium, nitrate and nitrite) concentrations from the total dissolved nitrogen (TDN) concentrations. Low DON concentration quantification in waters with high DIN/TDN ratio using existing methods tends to be inaccurate and DON measurements often have high standard deviations. (Lee et al., 2007) Some pre-treatment methods were used to increase the accuracy and precision of DON quantities, (Lee el al., 2007) and nanofiltration (NF) pre-treatment (Xu et al., 2010). The limitations of available measurement methods dissatisfied investigators considering the nature and behaviour of DON in wastewater treatment plants. As a substitute to measuring DON as a bulk parameter in wastewater, investigators have measured specific organic nitrogen containing compounds such as dissolved free and combined amino acids (DFAA and DCAA). (Pehlivanoglu-Mantas and Sedlak, 2008) To characterize the unidentifiable wastewater-derived DON, the molecular weight (MW) distributions of DON are also measured. Most studies presented that about 70% of wastewater-derived DON still cannot be considered with currently available methods. (Pehlivanoglu-Mantas and Sedlak, 2006, 2008; Simsek et al., 2012)

Previous studies on wastewater-derived DON focused on two important research enquiries: where DON is removed or produced in BERN processes and what is the effect of BERN process on DON (Czerwionka et al., 2012; Simsek et al., 2013). Sattayatewa et al. (2009) informed that about 28-57% of the effluent DON was bio available or biodegradable by using diverse kinds of test species.

The present study aimed to get more information on wastewater-derived DON in wastewater treatment plants and to assess the concentrations of amino acids in wastewaters without sample pre-concentrating. We measured the concentration and removal percentage of both forms of amino acids (DFAA and DCAA) at dissolved organic nitrogen and bio available dissolved organic nitrogen in triggered sludge and trickling filter wastewater from treatment processes.

MATERIAL AND METHODS

Sample sources

Samples were obtained from three different treatment plants effluent, which are Al-Zarqa (ZTP), Irbid (ITP), and al Mafraq (MTP) (Fig. 1). The selected plants treat about 90% of the domestic wastewater in Jordan and serve more than two million inhabitants (Bataineh et al., 2002); the treatment process is in table 1.
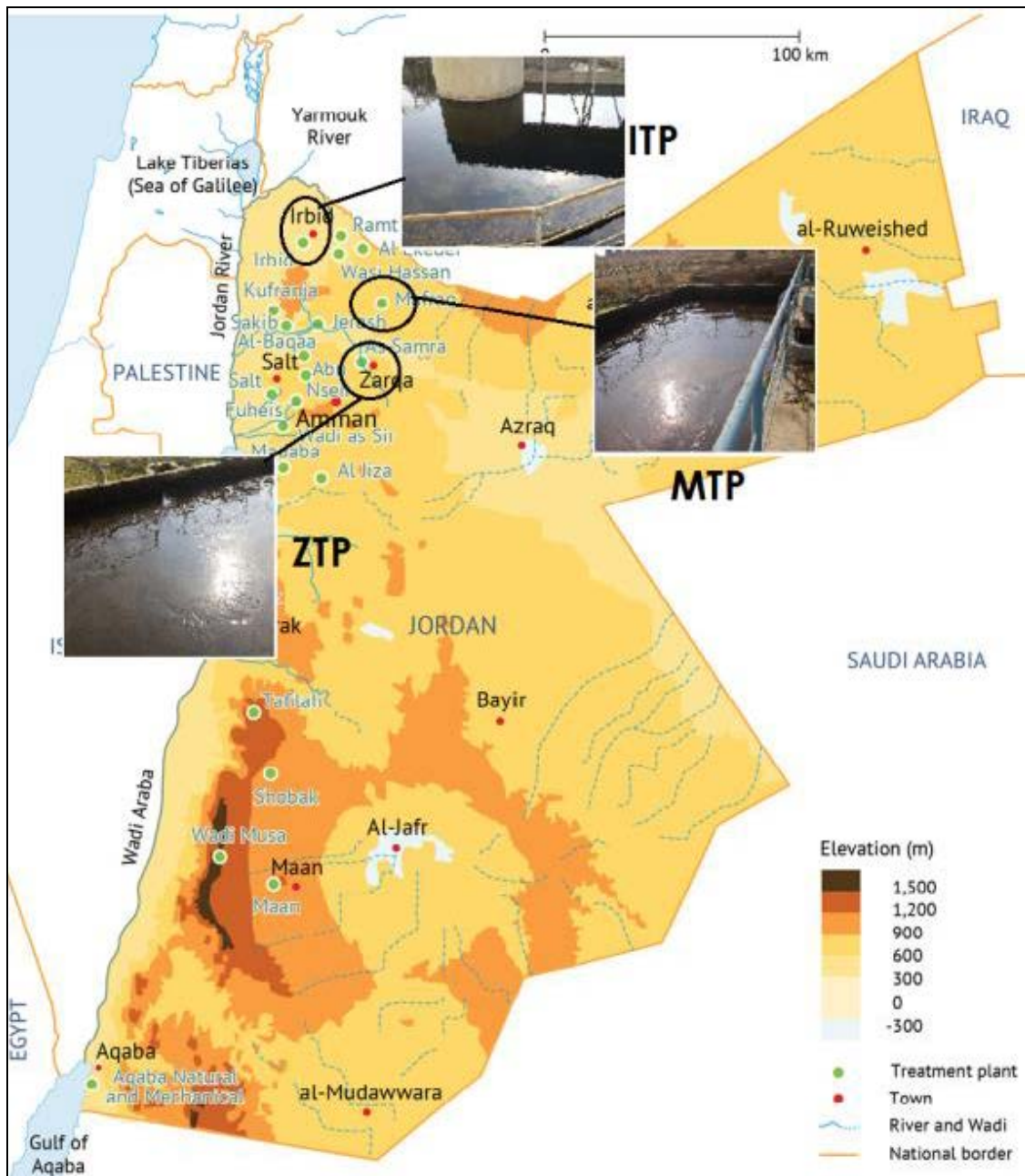
Figure 1: Jordan map indicating the sampling sites.

Table 1: The treatment plants concerned in the study (Bataineh et al., 2002).

Plant	Treatment process	Remarks
Al-Zarqa	Stabilization ponds (natural anaerobic lagoons) aeration, facultative	
Irbid	Screen, grit removal, primary sedimentation, biological process, secondary sedimentation, disinfections	Trickling filter and activated sludge
Mafraq	Screen, grit removal, biological process, secondary sedimentation, polishing pond, infiltration, disinfections	Activated sludge with nitrogen removal technique

All plants must adhere to discharge limits for biochemical oxygen demand (BOD) and ammonia (based on the receiving river flow rate) but are not subject to any total nitrogen or total phosphorus limits.

Collection and preparation

The samples are from the wastewater treatment plants collected on three different dates, specifically February, April and June of 2015. All samples were collected in polyethylene containers (acid-washed and rinsed with ultrapure water) (Mill-Q, Millipore Corp. USA), then delivered to the laboratory on ice, filtered through 0.45μm cellulose acetate membranes upon arrival, and stored at 4°C in the dark according to Huo et al. (2013).

DFAA and DCAA determination procedures

To prevent contamination by laboratory controls, pre-cleaned glassware, which include filters, were used in all laboratory work. The glassware was prepared by washing in alkaline detergent, placing in 1N HCl overnight followed by rinsing with organic free water obtained with distilled water and then heating in an oven at 110 ℃ for 10 to 12 h according to Wedyan and his colleagues (Wedyan et al., 2008). To identify the amino acids in rainwater, samples filter through 0.45 μm cellulose acetate filters then are kept at 5°C until analysis. All samples are run in triplicates.

The amino acids in the samples are separated into the following fractions (i) the dissolved free amino acids (DFAA) (i.e. those uncombined amino acids directly extractable into water), (ii) the dissolved hydrolysable amino acids (DHAA) (i.e. those combined amino acids directly extractable into water and released by hydrolysis), (iii) total dissolved amino acids (DTAA = DFAA – DHAA).

The recovery of 14 individual amino acids spiked into water prior to the hydrolysis step ranged between 92.1 and 99.3%. All analyses were carried out in triplicate for each sample according to Huo et al. (2013) modified the high amplitude.

RESULTS AND DISCUSSION

The variations of DON in each plant were studied in previous work and presented in table 2 (Wedyan et al., 2016).

Table 2: Concentration (mg-N/L) of Dissolved Nitrogen in Different locations, (average ± SD) (Wedyan et al., 2016).

Dissolved Nitrogen	ZTP	ITP	MTP
DNO_3-N	6.81 ± 2.40	6.33 ± 3.80	7.83 ± 6.49
DNO_2-N	0.48 ± 0.040	0.70 ± 0.048	0.013 ± 0.002
DNH_4-N	0.039 ± 0.0030	0.70 ± 0.48	0.06 ± 0.019
DTN	19.30 ± 6.60	41.8 ± 3.39	33.21 ± 5.01
DNO_3-N	6.81 ± 2.40	6.33 ± 3.80	7.83 ± 6.49

As shown in table 2, average DON concentrations in ZTP, ITP and MTP influent ranged from 0.039 to 33.21 mg l^{-1} as N. The results of this study are consistent with the concentration range previously reported in BENR plant effluents (Pagilla et al., 2006; Pehlivanoglu-Mantas and Sedlak, 2008; Sattayatewa et al., 2009; Czerwionka et al., 2012; Huo et al., 2013).

Pehlivanoglu-Mantas and Sedlak, (2008) propose that DON was challenging to eliminate during biological treatment. The information regarding the biodegradable portion of the DON (BDON) profile along the treatment train will help recognize the roles of the wastewater treatment process in the removal of this fraction of DON (Simsek et al., 2013 Huo et al., 2013).

Distribution of dissolved free (DFAA) and combined amino acids (DCAA).

Measurements on the distribution of DFAA and DCAA concentrations are from the treatment of ZTP, ITP and MTP samples to obtain information of the composition of inlet and outlet dissolved amino acids (DAA) and the effect of treatment processes on DFAA and DCAA (Figs. 2a-d).
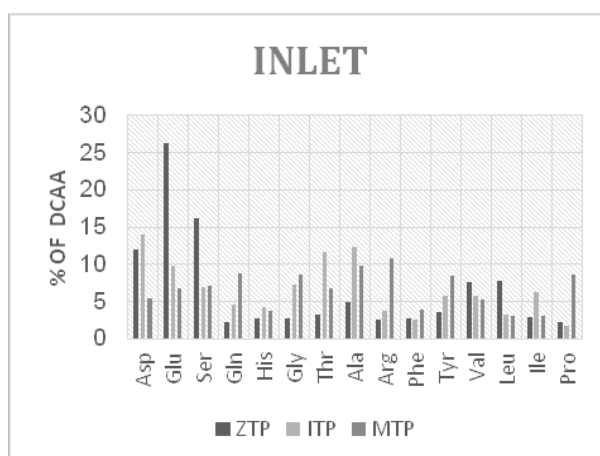
Figure 2a: DFAA and DCAA concentrations percentages of ZTP, ITP and MTP samples (inlet and outlet).
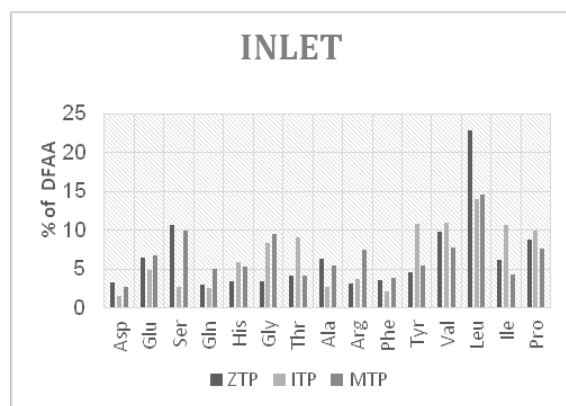
Figure 2b: DFAA and DCAA concentrations of ZTP, ITP and MTP samples (inlet and outlet).
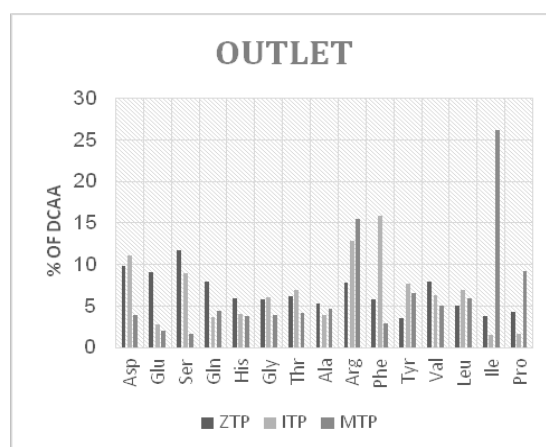
Figure 2c: DFAA and DCAA concentrations of ZTP, ITP and MTP samples (inlet and outlet).
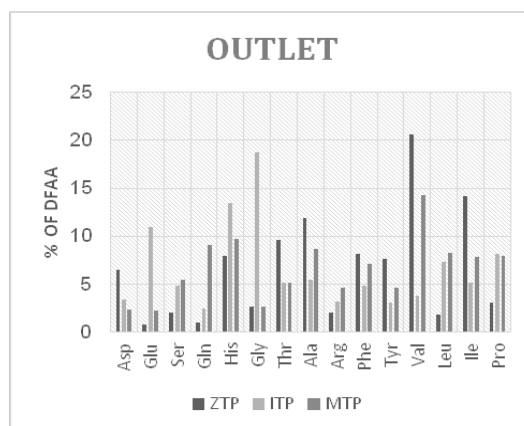
Figure 2d: DFAA and DCAA concentrations of ZTP, ITP and MTP samples (inlet and outlet).

Figures 2a-d, show both concentrations of inlets and outlet in the wastewater treatment plants of DFAA and DCAA values. The reduction of the proportion levels of the DFAA and DCAA along the treatment by 56% and 63%, respectively. While the DCAA concentrations ranged from 7.35 to 16.6 µM in the inlet and ranged from 0.79 to 5.88 µM in the outlet that covering for 2.27-4.42% of the inlet DON and 1.72-6.40% of the outlet DON, respectively. And also the DFAA contributed about 0.1-0.62% of DON in the inlet and about 0.61-0.97% of outlet. The results show that the DCAA proportions in the different plants in Jordan showed a significantly higher than DFAA proportions.

The DFAA and DCAA concentration measurements are from different locations in the studied WWTPs (Fig. 3). The DFAA concentration ranged from 0.011 to 0.071 µl/ml in the inlet, and from 0.0018 to 0.06 µl/ml in the outlet, whereas the concentration of DCAA ranged from 0.038 to 4.85 µl/ml in the inlet, and from 0.038 to 0.77 µl/ml in the outlet. It was also found that the concentration of DFAA in ZTP, ITP and MTP inlets were 0.73 ± 0.015, 0.71 ± 0.014 and 0.72 ± 0.015 µl/ml respectively. In contrast, the concentration of DCAA in ZTP, ITP and MTP inlets were 4.67 ± 0.97, 6.67 ± 1.06 and 5.08 ± 0.58 µl/ml respectively. To compare this with the outlets treated wastewater it was found that the concentration of DFAA in ZTP, ITP and MTP were 0.33 ± 0.15, 0.31 ± 0.14 and 0.39 ± 0.21 µl/ml respectively, and the concentration of DCAA in ZTP, ITP and MTP were 2.28 ± 0.1, 2.45 ± 0.14 and 2.95 ± 0.18 µl/ml respectively. The DCAA concentration differs by an average of 18% over the course of treatment between the different plants, whereas the DFAA differs by an average of 1.4% only. The removal ratios of DFAA varied between plants with values of around 56% at ZTP, 52% at ITP and 45% at MTP, and that seems to depend on the treatment used. The removal ratios of DCAA were varied between plants as well with values of around 51% at ZTP, 63% at ITP and 42% at MTP.

The concentrations of DFAA and DCAA in the inlets of all WWTPs were within ranges reported in previous studies (Parkin and McCarty, 1981; Confer et al., 1995; Grohmann et al., 1998; Dignac et al., 2000). Supposing a characteristic DON concentration of 143 µM (~ 2.0 mg N l^{-1}) in the wastewater inlets, the DFAA concentration accounted for between 0.3 and 3% of the DON, while the DCAA accounted for 1.5-13% of the DON (Parkin and McCarty, 1981; Confer et al., 1995; Dignac et al., 2000). The comparatively low concentrations of DCAA and DFAA in the inlet samples most likely formed during biological treatment since amino acids and proteins are readily removed by microorganisms (Confer et al., 1995; Pehlivanoglu-Mantas and Sedlak, 2008).
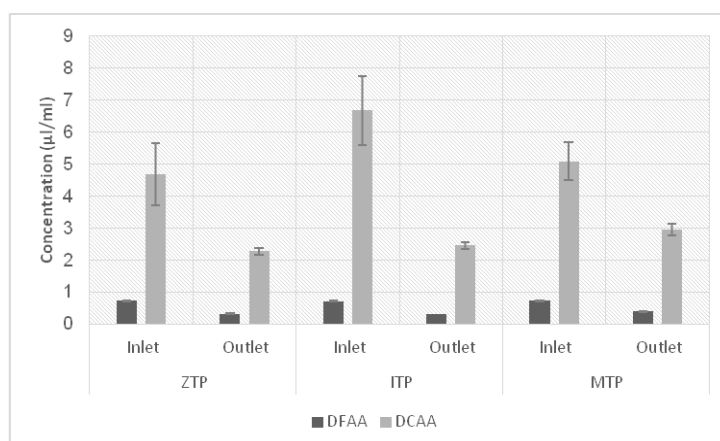
Figure 3: Concentration of DFAA and DCAA at the wastewater treatment plants (µl/ml).

While most of the wastewater-derived DON cannot be recognized with accessible methods (~ 70%) (Pehlivanoglu-Mantas and Sedlak, 2008), DON can be categorized by its molecular weight (MW). Pagilla and his colleagues (2008) suggested that the low molecular weight DON fractions with less than one kDa are urea, amino acids, DNA, peptides and various artificial compounds and that the DON group with more than one kDa are composed of fulvic acids and humic substances. Biological nitrogen removal processes, for example, the activated sludge process, have been viewed as successful in expelling low sub-atomic weight DON, while high MW DON is viewed as recalcitrant to this sort of treatment (Dignac et al., 2000; Pagilla et al., 2008). However, the results of DON molecular weight distributions at the three studied WWTPs and in this study indicated that about half of the DON can go through a one kDa ultra filter, and low MW DON cannot successfully be detached by the biological nitrogen removal processes or the membrane biological removing process (MBR). Shon et al. (2005) found that microfiltration or nanofiltration may not be very good in eliminating wastewater-derived DON due to the polluting problems often met in micro and/or nanofiltration. This research highlight that the low MW DON fractions probable appeared concurrently with the biological treatment. This research also found that the DON concentrations raised in the oxic zone as explained above. Consequently, the conditions and processes responsible for low MW DON production need additional studies. Suitable operating conditions or treatment processes should be employed to decrease effluent low molecular weight DON to inferior levels.

Hydrophobicity of outlet

The estimated outlet percentage of DON was separated into hydrophobic and hydrophilic fractions. The overall recovery of DON in the two fractions ranged from 91.3% to 97%, with an average of $94.7 \pm 0.9\%$. The hydrophobic DON accounted for an average of $1.11 \pm 0.01\%$ of the total DON in the outlet samples of ZTP (Fig. 4), and an average of $1.07 \pm 0.02\%$ of the total DON in the outlet samples of ITP (Fig. 4) and an average of $0.88 \pm 0.02\%$ of the total DON in the outlet samples of MTP. The results of this study found that most DON fractions were hydrophobic compounds, which was similar to the previous studies. (Pehlivanoglu-Mantas and Sedlak, 2008; Liu et al., 2012) All the studies proposed that the hydrophobic fractions would be much more easily removed by adsorption of activated sludge in the biological treatment systems, while the hydrophilic compounds have a low attraction for the surfaces of organic particles (Pehlivanoglu-Mantas and Sedlak, 2008). Furthermore, the C:N ratios of the hydrophobic fraction and hydrophilic fraction were 11.4 ± 0.2, 15.7 ± 2.3 and 11.8 ± 0.9 in this study. The hydrophilic fractions have low C:N ratios representative the presence of amino acids and proteins, which result in N-rich hydrophilic fractions. (Leenheer et al., 2007) Some studies suggested that bioavailability of hydrophobic and hydrophilic DON by the algal bioassay tests, the results showed that hydrophilic DON, which accounted for around 80% of the waste DON, encouraged algal growth, whereas the remaining DON as hydrophobic DON had no effect on algal growth during a 14-day incubation period Liu et al. (2012). Thus, the hydrophobic DON may be measured to eliminate from the outlet total nitrogen principles, while hydrophilic DON in wastewater treatment plants with different biological nutrient removal (BNR) processes might be compact by using alternative biological treatment systems such as reverse osmosis, activated carbon adsorption (Krasner et al., 2009).
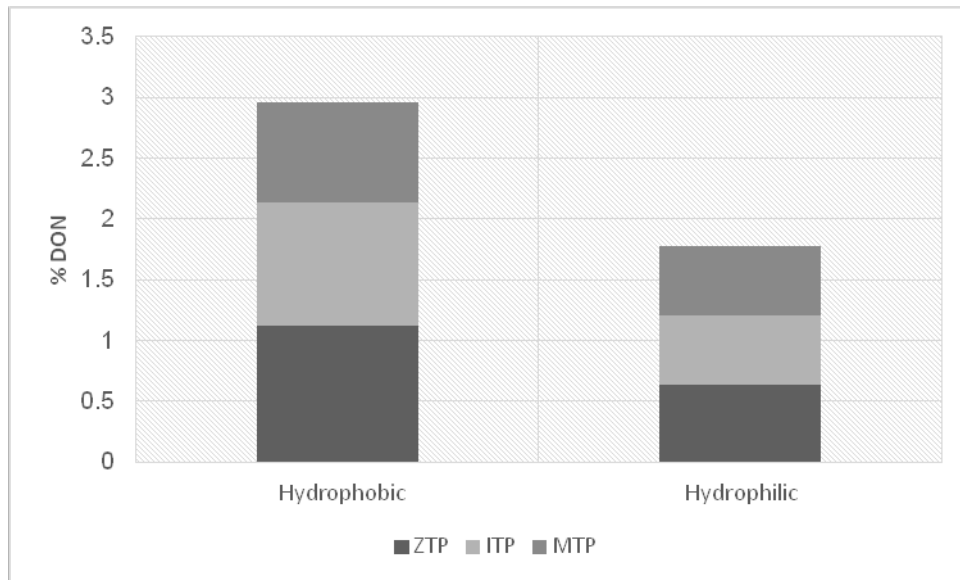
Figure 4: Hydrophobic and hydrophilic fractions of outlet DON.

CONCLUSIONS

This research supply a valuable image of the occurrence and treatment of DON in WWTPs. The dominant removal fractions of DON and biodegradable dissolved organic nitrogen (BDON) along the treatment progress were noticed in the Irbid (ITP). Dissolved combined amino acids (DCAA) and dissolved free amino acids (DFAA) in the outlet accounted approximately for less than 4% of the outlet DON of all plants. Over 90% of outlet DON was composed of hydrophilic compounds which provoke algal growth. The research offer important data for planned improving of WWTPs of the studied area and for the preference of DON removal systems to meet greater demanding nitrogen discharge limits.

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POLLINATION ECOLOGY OF THE SPECIES
MOLLUGO CERVIANA (L.) SER. (MOLLUGINACEAE)

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KEYWORDS: *Mollugo cerviana*, soil binder, facultative autogamy, anemochory, ombrohydrochory, hydrochory.

ABSTRACT

Mollugo cerviana is an annual herb which usually grows throughout the year in open dry sandy and sandy-loamy soils, but also occurs in moist habitats, especially in cultivated lands. *Haplothrips* uses the flowers for breeding and feeding; the feeding activity affects pollination. The flowers have specialized floral structural and functional behaviours for self-induced and spontaneous autogamy while keeping the options open for insect pollination after anthesis; it is facultative autogamous which is reflected in pollen-ovule ratio and natural fruit and seed set rates. Seed dispersal modes include anemochory, ombrohydrochory and hydrochory.

ZUSAMMENFASSUNG: Bestäubungsökologie von *Mollugo cerviana* (L.) Ser. (Molluginaceae).

Mollugo cerviana ist ein einjähriges Kraut, das in der Regel das ganze Jahr über auf offenen trockenen, sandigen und sandig-lehmigen Böden wächst, aber auch in feuchten Lebensräumen, vor allem in Kulturlandschaften, vorkommt. *Haplothrips* verwendet die Blüten zur Aufzucht und Fütterung; die Nahrungsaufnahme beeinflusst die Bestäubung. Die Blüten haben sich auf ein floral strukturelles und funktionelles Verhalten selbstinduzierter und spontaner Autogamie spezialisiert, während die Möglichkeiten für Insektenbestäubung nach der Anthese offen bleiben; die Pflanze ist fakultativ autogam, was sich im Pollen-Samen-Verhältnis und den natürlichen Frucht- und Samensatzraten widerspiegelt. Ihre Samenausbreitungsmodi umfassen Anemochorie, Ombrohydrochorie und Hydrochorie.

REZUMAT: Ecologia polenizării la *Mollugo cerviana* (L.) Ser. (Molluginaceae).

Mollugo cerviana este o plantă anuală care crește în mod normal pe soluri uscate, nisipoase și nisipo-argiloase, pe tot parcursul anului, dar se dezvoltă și în habitate umede, în special în locuri cultivate. *Haplothrips* folosește florile pentru reproducere și hrănire; Aportul alimentar afectează polenizarea. Florile se specializează în comportamentul structural și funcțional floral pentru autogamia auto-indusă și spontană, în timp ce posibilitățile de polenizare a insectelor rămân deschise după anteză; este autogamă facultativă, ceea ce se reflectă în raportul dintre semințele de polen și rata naturală a ratelor de fructe și semințe. Modurile de propagare a semințelor include anemochoria, ombrohidrochoria și hidrochoria.

INTRODUCTION

Pollination is an important part of plants' life (Solomon Raju, 1998), a key element for mangrove flora ecology and conservation (Aluri, 2013). It is a successful tool for maximizing the gene flux (Almeida-Soares et al. 2010). *Mollugo* genus is native to tropical and a warm temperate part of North and South America, but it is also distributed in Europe, Africa and Asia. The name derives from the Latin word "mollis" meaning soft (Short, 2002). *M. cerviana* is native to India, Sri Lanka, Pakistan and Bangladesh (Parvathamma and Shanthamma, 2000). It is a C_4 species distributed in hot arid regions from pantropics to temperate regions (Christin et al., 2010). It is valuable in medicine for treating different diseases and ailments (Parvathamma and Shanthamma, 2000; Rajamanikandan et al., 2011; Sahu et al., 2012).

It is know little about the pollination ecology of Molluginaceae, in which nectar-secreting tissue is present in almost all species. In few genera, showy sepals or petals have evolved, both of which suggest entomophily (Watson and Dallwitz, 1992; Kubitzki et al., 1993). Syrphid fly, *Mesogramma marginata* pollinates *Mollugo verticillata* (Robertson, 1928). The widely spread, weedy species of *Mollugo verticillata, M. nudicaulis*, and *M. cerviana* are self- and insect-pollinated (Pax and Hoffmann, 1934; Bogle, 1970). In Taiwan, *M. pentaphylla* is a minor pollen source for *Apis mellifera* (Lin et al., 1993). In South India, honey bees use *Mollugo* species as a pollen source and reciprocate the plants with pollination (Ponnuchamy et al., 2014). This study investigate how *M. cerviana* reproduce in semi-dry and dry habitats with scarce pollinators. The objective of the present study is to know how various aspects pollination ecology contributes for the reproductive success through seed mode in dry habitats.

MATERIAL AND METHODS

Mollugo cerviana wild patches grow in open dry and semi-dry areas of Visakhapatnam and its surroundings (17°42'N latitude and 82°18'E longitude) were selected for study during March 2015-May 2017. Field trips were conducted to record phenological aspects. Ten inflorescences which have not initiated flowering on five plants were tagged and followed to record anthesis schedule and the timing of anther dehiscence. Twenty five fresh flowers were used to record the floral morphological details. Nectar could not be measured and analysed due to its secretion in minute quantity which was further depleted by thrips during mature bud and flower life. Twenty mature, but un-dehisced anthers, two anthers each per flower/plant from ten plants were collected and examined for pollen output as per the protocol described in Dafni et al. (2005). The calculation of pollen output per flower and pollen-ovule ratio was done as per the formulas described in Cruden (1977). Ten flowers each from five individuals were used to test stigma receptivity. It was tested with hydrogen peroxide from mature bud stage to flower closure/drop as per Dafni et al. (2005). Seventy inflorescences were tagged prior to the initiation of their flowering and followed for three weeks to record fruit and seed set rate in open-pollinations. The fruit and seed morphological characteristics were observed in detail to evaluate their adaptations for dispersal by different means. Fields visits were made during rainy season to note the aspects of seed germination and production of new plants. Based on the timings of maturation of anthers and receptivity of stigmas, the sexual system was defined and also elaborately explained its functionality to achieve self-induced autogamy, spontaneous autogamy, geitonogamy, and xenogamy. The positions of stamens and stigmas during and after anthesis were observed to evaluate how they facilitate spontaneous autogamy during anthesis and flower closure. Further, observations were also made to evaluate as to how these positions preclude self-pollination when flowers stay open. The flower buds were used by thrips for breeding and feeding and in this context their role in pollination was observed.

RESULTS

Phenology. The species is a small, glabrous, slender annual herb common in open dry sandy and semi-dry soils along roadsides, waste places, bare ground and dry river beds (Fig. 1a). Due to its very low ground habit, wiry reddish orange stems and thin linear leaves its presence is usually overlooked. The stems are numerous, upright, thin and stiff. Leaves are sessile, grey-green and linear with acute apex; they arise in whorls on the stem, but some are in a rosette at the base. The plant appears simultaneously in vegetative, flowering and fruiting phases in different populations growing in different habitats throughout the year (Fig. 1b). An individual plant, however, has a short life cycle of three months from seed germination to seed dispersal. Although it appears throughout the year, it shows robust vegetative growth and profuse flowering and fruiting during July-October when the soil is damp due to the occurrence of rains. The flowers are borne on seven-eight mm long pedicels in dichotomous and trichotomous umbellate cymes produced terminally or in leaf axils.
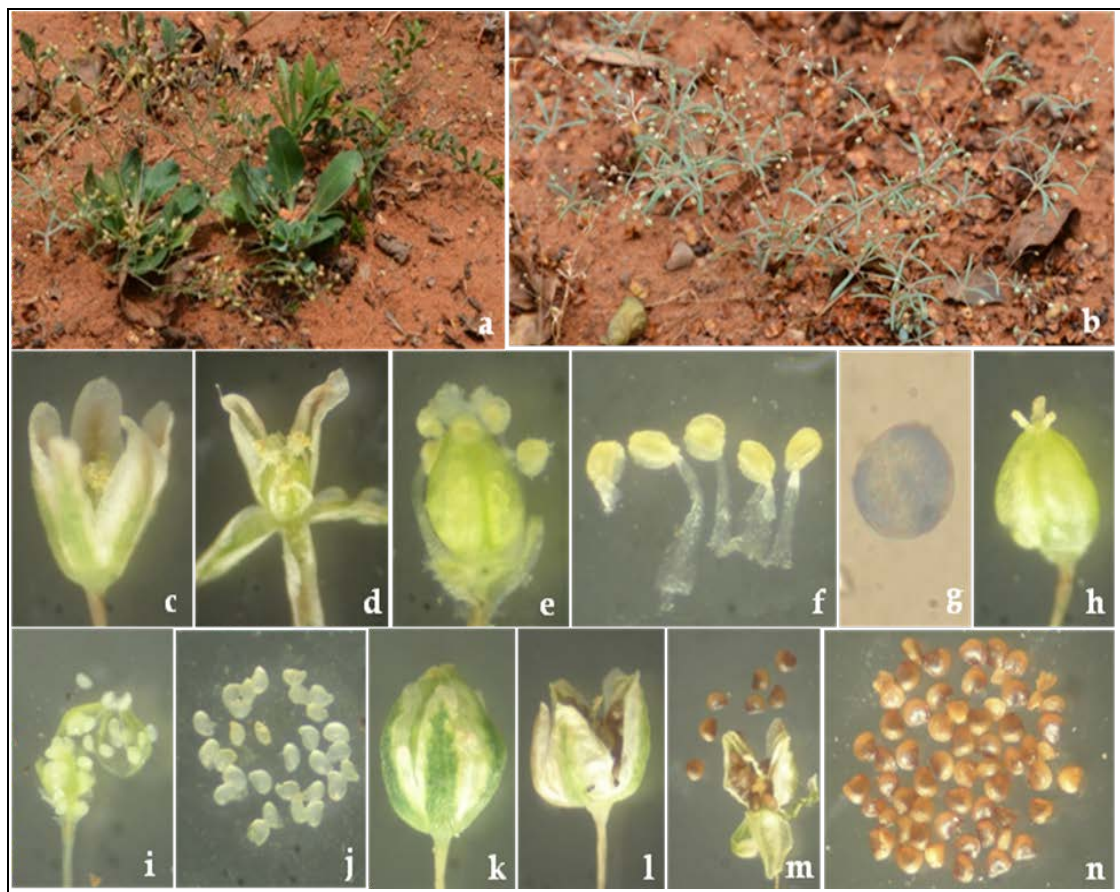
Figure 1a-n: *Mollugo cerviana*: a. Habitat with *Mollugo cerviana* and *M. nudicaulis*,
b. *Mollugo cerviana* in flowering phase, c. and d. Flowering-opening phase,
e. Position of stigmatic lobes and anthers at the same height contacting each other at anthesis,
f. Dehisced anthers, g. Pollen grain, h. Ovary with three stigmas, i. and j. Multi-ovuled ovary,
k. Maturing fruit, l. and m. Dehisced fruit capsule, n. Seeds.

Flower morphology. The flowers are small (2.52 ± 0.4 mm long, 1.51 ± 0.5 mm wide), whitish green on adaxial side and green on abaxial side, odourless, actinomorphic and bisexual. A monochlamydeous perianth of five herbaceous scarious, elliptic to oblong, 2.45 ± 0.4 mm long, 1.13 ± 0.2 mm wide long free tepals with white margins represent sepals and petals. The stamens are five, anti-tepalous, free but connate at base, white, 1.22 ± 0.3 mm long with dorsifixed, golden yellow, less than one mm long and dithecous anthers. The ovary is light green, tri-carpellary, tri-locular syncarpous with 58.2 ± 8.16 D-shaped ovules arranged on axile placentation (Figs. 1i-j). The style is absent but the ovary is terminated with three free stigmas (Fig. 1h). The stigmas are minutely denticulate with membranous flaps.

Floral biology. Mature buds open during 07,00-08,00 h. Individual buds take five to 10 minutes from partial to a full opening (Figs. 1c-d). The flowers are homogamous as the anthers and stigmas attain maturity at the same time during anthesis; the former dehisce by longitudinal slits (Fig. 1f), and the latter continue receptivity until the noon of the 2nd day. The pollen output is 159.7 ± 14.5 per anther and 798.5 ± 69.5 per flower. The pollen-ovule ratio is 14:1. The pollen grains are pale yellow, spheroidal, tri-colpate, tri-zonoaperturate, granulated, tectum scabrate, and 21.9 ± 4.12 µM (Fig. 1g). The nectar secretes in traces during mature bud stage. The tepals with the stamens and stigmas close back by 10,00-11,00 h.

Pollination mechanism and pollinators. 20-35% of pollen grains found in dehisced anthers collected during anthesis possess pollen tubes indicating *in situ* germination. Further, the pollen tubes are also present on the stigma. The pollen germination and formation of tubes both within the dehisced anthers and on the stigma indicate the presence of self-induced autogamy. During and after anthesis, the dehisced anthers and receptive stigmas contact with each other due to their close proximity and their position at the same height due to which autogamy occurs (Fig. 1e). Further, the stamens and stigmas contact each other very closely during the closing of the flower assuring autogamy if it did not occur during the open state of the flower. Any insects never visited the flowers. *Haplothrips* sp. (Thysanoptera: Thripidae) used flower buds for breeding and flowers for feeding. The larvae emerged from the eggs in synchrony with anthesis and nectar production in flowers. The larvae and adults foraged for pollen and nectar. Pollen dusts individual thrips during their movements within the flowers. They carried 87 to 176 pollen grains on their body setae, wings and legs. The thrips dispersed the pollen on free denticulate and membranous stigmas due to their active movement, rubbing of abdomen against the stigmatic surface, cleansing of their body parts with their hind legs and also by their wing combing mechanism. The homogamous flowers were found to facilitate self-pollination in the same or different flowers of the same plant. As the plant occurs as small or large populations, thrips could fly to migrate to the flowers of other closely spaced plants and effect cross-pollination by feeding on the foliage.

Fruiting ecology and seed dispersal. Fruits mature within 8-10 days. The stamens and stigmas are persistent and remain inside due to the closure of the flower. The tepals bulge gradually and protect the bulging ovary in which the seeds form and mature (Fig. 1k). Natural fruit set is 91.27% and seed set is 61.94%. Fruit is a loculicidal three-valved broadly-ellipsoid capsule, stalked, membranous, and densely pubescent, 2.35 ± 0.36 mm and 1.85 ± 0.23 mm wide. The seeds are arranged in two rows in each locule. They are tiny, brown, shiny, D-shaped and faintly striate dorsally (Fig. 1n). The seed coat is studded with minute granular excrescences with reticulate ornamentation. Dry capsules break open when fruit pericarp and tepals are dry and expose the seeds (Figs. 1l-m). But the seeds remain and gradually separate and fall to the ground on their own on clear sunny days. On rainy days, the water droplets falling on the dehisced capsules washout seeds to the ground. Further, water acts as an efficient

dispersal agent for the dispersal of seeds fallen on the soil during rainy season. Seeds do not have adaptations for wind dispersal. But, wind disperses the dry cymes together with dry dehisced capsules to short distances and subsequently the seeds fall to the ground from capsules. Therefore, seed dispersal modes include ombrohydrochory, hydrochory and anemochory. The seeds produced from plants growing in cultivated lands have the potential to be dispersed as a cereal grain contaminant and in effect agricultural produce movement contributes to seed dispersal and expansion of its distribution.

DISCUSSION

This study finds that *Mollugo cerviana* with its low ground habit populates the soil and for this reason is often called carpetweed. The plant grows throughout the year displaying vegetative, flowering and fruiting phases in different populations. However, the wet season confines its robust growth, profuse flowering and fruiting individual plants complete their life cycle within three months from seed germination to seed dispersal. Similarly, Owens and Lund (2009) reported that *M. cerviana* is a herbaceous ephemeral species and completes its life cycle in a very short time. This study finds that the inflorescence is a dichotomous or trichotomous umbellate cyme in *M. cerviana* suggesting that the soil moisture and nutrient environment regulate the branching of inflorescences and the production rate of flowers.

The floral descriptions of *Mollugo* species provided by different authors (Goncalves, 1978; Matthew, 1995; Pullaiah, 2000; Pullaiah and Mohammed, 2000; Bora and Kumar, 2003) are not accurate and/or complete. The present study provides details of the floral descriptions, especially of perianth, androecium and gynoecium in *M. cerviana* as these are important from the pollination of point view. In this species, perianth typically consists of five tepals which serve the function of calyx (sepals) and corolla (petals). The abaxial surface of the perianth serves the role of calyx while the adaxial surface of the perianth serves the role of corolla due to the display of two different colors on each surface. However, the perianth acting as both calyx and corolla is unable to attract any insect pollinators in pollinator-deprived or pollinator-available habitat. Such a situation explains that *M. cerviana* is not dependent on insect foragers for pollination. Ronse De Craene (2010) reported that in *Mollugo*, the androecium generally consists of five stamens alternating with the sepals. *M. cerviana* flowers produce a fixed number of five stamens, and all are opposite to tepals suggesting that there is no process evolving to produce flowers with three or four stamens. Further, the plant produces trimerous ovary with three stigmas; each carpel with a variation in ovule number. Despite the absence of vector-mediated pollination, the plant produces high fruit and seed set rates indicating that this plant is facultative autogamous.

Peter et al. (2004) reported that the temperature and relative humidity are probably important cues determining flower opening in the afternoon. In the present study, the anthesis during morning time in *M. cerviana* is attributable to its predominance in open, dry habitats where herbaceous flora usually does not grow. The absence of insect foraging activity on *M. cerviana* could be attributable to its common occurrence in pollinator-excluded or deprived habitats and production of tiny flowers which can be overlooked or unnoticed by foragers.

Bittrich (1990) reported that in Molluginaceae, *Adenogramma* is the only genus which produces one-seeded nutlets. All other genera produce capsules with many seeds which become exposed by loculicidal dehiscence. Soerjani et al. (1987) reported that *Mollugo pentaphylla* is hydrochorous. In the present study, *M. cerviana* produces fruits within or slightly more than a week time. The fruit is an ellipsoid 3-valved capsule and it breaks open to disperse seeds during sunlight days. But, on rainy days, the fruits when filled with water expel seeds and water violently. Wind also disperses dry cymes along with dry dehisced capsules to short distance and then seeds find their way into the ground. The seeds disseminated through these modes further dispersed by rain water during rainfall. Therefore, *M. cerviana* species is anemochorous, ombrohydrochorus and hydrochorous.

Narayana (1962) and Hofmann (1973) noted that *Mollugo* species produce seeds with a primordium-like swelling on the funiculus and this structure is considered to be a vestigial aril. *M. cerviana* produces tiny, brown, shiny, D-shaped seeds with a faintly striate dorsal surface. Minute granular excrescences with reticulate ornamentation stud the seed coat. Since the seeds of these plant species lack any aril or strophiole-like structure that usually serves as food for ants, the possibility for myrmecochory is ruled out. Wagner et al. (1999) noted that *Mollugo* species produce fruit capsules and inside seeds that lack means of external attachment for dispersal by animals. This study agrees with this report because *M. cerviana* lacks external structures to aid seed dispersal by animals and hence there is no possibility of seed dispersal by animals.

Bittrich and Ihlendfeldt (1984) reported that *Mollugo* seeds germinate by means of an operculum. *M. cerviana* propagates by seeds and reseeds itself, often forming colonies. It produces several batches of populations in a year, and the seeds germinate as soon as they disperse, but their germination is related to soil moisture which plays an important role in breaking the seed coat.

As a therophyte, this species it is best adapted to survive in open dry habitats as it takes advantage of any sign of temporary humidity that allows it to complete its life cycle quickly. Jurado et al. (1991) reported that *M. cerviana* does not form a dense cover that inhibits other vegetation and compete well in crowded conditions. The present study also indicates that *M. cerviana* does not grow in shaded habitats or form dense populations that inhibit other vegetation.

Brockington et al. (2009) reported that *Mollugo cerviana* is the only known C_4 species in Molluginaceae. Christin et al. (2010) reported that *M. cerviana* being a C_4 species is distributed in hot, arid regions of tropical and temperate latitudes. The present study also shows that *M. cerviana* with C_4 photosynthesis grows only in dry habitats which are almost devoid of other vegetation and also devoid of pollinator fauna. This finding is in line with the statement by Lundgren et al. (2015) that C_4 species are usually abundant in warm but not cool environments and this photosynthetic pathway is physiologically advantageous for their niche broadening in warm environments. In *M. cerviana*, genetic variation achieved through thrips pollination is essential to expand and invade dry habitats.

CONCLUSIONS

Mollugo cerviana as an annual facultative autogamous therophyte grows throughout the year in open dry sandy and sandy-loamy soils, and also in moist habitats. The flowers have specialized floral structural and functional behaviors for self-induced and spontaneous autogamy while keeping the options open for insect pollination after anthesis but the insects never visited the flowers. Seed dispersal is polychorous involving anemochory, ombrohydrochory and hydrochory. The seeds germinate immediately after dispersal, but soil moisture is required to rupture the seed coat. The plant is best adapted to survive in open dry habitats as it takes advantage of any sign of temporary humidity to complete its life cycle quickly and acts as a soil binder and also moisture accumulator in the root zone.

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PARTICULARITIES OF GREY ALDER (*ALNUS INCANA*) STANDS IN RIPARIAN GALLERIES ALONG STREAMS OF THE SOUTHERN TRANSYLVANIAN TABLELAND (ROMANIA)

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ABSTRACT
Grey Alder (*Alnus incana* (L.) Moench.) stands are distributed in the Carpathian area on alluvial deposits with sediments of different grain size as galleries along rivers and streams of the sub-montane to the middle montane level. The geological structure (Tertiary sedimentary deposits) in relation to the hydromorphological processes is the base for the structure of the stream valleys, which are in part very deep. Under the special microclimate of these deeper stream stretches with lower temperatures there are favourable conditions for the development of grey alder stands.

ZUSAMMENFASSUNG: Merkmale der Grauerlen- (*Alnus incana*) Bestände in den Galeriewäldern entlang der Bäche des Süd-Siebenbürgischen Hochlandes.
Bestände der Grauerle (*Alnus incana* (L.) Moench.) sind in den Karpaten, einschließlich das Hochland von Siebenbürgen auf alluvialen Ablagerungen unterschiedlicher Korngröße von der submontanen bis zur mittleren montanen Stufe als Galerien entlang der Flüsse und Bäche verbreitet. Die geologische Struktur (Tertiäre Ablagerungen) bildet in enger Verbindung mit den hydromorphologischen Prozessen die Voraussetzung für die Form der teils tief eingeschnittenen Bachtäler. Unter den mikroklimatischen Bedingungen dieser Täler finden sich im Hügelland günstige Bedingungen für die Entwicklung von Grauerlenbeständen.

REZUMAT: Caracteristicile galeriilor ripariene cu arin alb (*Alnus incana*) din sudul Podișului Transilvaniei.
Grupări de arin alb (*Alnus incana* (L.) Moench.) sunt răspândite în aria Carpaților pe depozite aluviale de diferite fracțiuni granulometrice sub formă de galerii de-a lungul râurilor și pârâurilor din etajul submontan până în cel montan mijlociu. Structura geologică (depozite de sedimente terțiare) constituie în relație cu procesele hidro-morfologice baza pentru forma văilor pârâurilor, pe porțiuni adânc săpate în straturile de sedimente. In condițiile particulare microclimatice cu temperaturi mai scăzute ale acestor segmente de râu există condiții favorabile pentru dezvoltarea grupărilor de arin alb în perimetrul podișului.

INTRODUCTION

Grey alder (*Alnus incana* (L.) Moench.) stands are distributed in the Carpathian area on alluvial deposits of different sediment grain size as galleries along rivers and streams of the sub-montane to the middle montane level, their optimum being at the level of beech and beech-fir forests (Georgescu, 1952; Ciocârlan, 2009). But they can also be observed at lower altitude in the Tertiary Tableland or Highland of Transylvania. In some valleys such as those of the rivers Mureș and the Someș, they descend as far as the plains. Sites with Grey alder in the plain are mentioned from Criş, Argeş and Ialomița valleys (Georgescu, 1952). Generally Grey alder occurs on seeping wet or temporary flooded areas, alkaline and nutrient-rich, mostly calcareous, loose and aerated, raw, gravelly-sandy clay soils and slumping marls (Oberdorfer, 2001). Such sites are characteristic also for Carpathian Basin i.e. the Transylvanian Highlands.

The Southern Transylvanian Tableland is characterised by a large network of small streams, tributaries to the larger rivers crossing the tableland, all of them under a higher or lower human impact. The small streams have in common that their springs are situated in the Tertiary Highlands, the riverbeds have a natural or nearly natural structure; the streams are crossing various geological layers of sandstone, marl, clay and loam. Due to this fact the size of sediments in the stream bed are highly variable, from fine clay, loamy and sandy sediments to those with gravel or larger stony materials. The discharge is generally very low, but during high floods, raised by heavy rain falls, they can have a high discharge, accompanied by pronounced hydro-morphological dynamics with erosion and deposition processes. Depending on the crossed layers, the erosion can be different from stretch to stretch in the course of the river. (Roşu, 1980; Posea et al., 1982; Badea et al., 1983; Ielenicz, 1999; Dobros, 2005; Drăgulescu, 2005; Mountford and Akeroyd, 2005; Curtean-Bănăduc, 2005)

On the streams and streamlets exists in the whole Southern Transylvanian Tableland mostly well-structured gallery-like softwood forests with characteristic species such as White willow (*Salix alba*) and Crack willow (*Salix fragilis*) adapted to the high humidity of the river banks and the changing water levels. Black alder (*Alnus glutinosa*) occurs infrequently and generally with low abundance dominance values. The gallery-like softwood stands of the tableland are all included in the priority habitat type 91E0 *Alluvial forests with *Alnus glutinosa* and *Fraxinus excelsior* (Alno-Padion, Alnion incanae, Salicion albae) (EUR 28, Gafta and Mountford, 2008) (Fig. 1). From the phytocoenolocical point of view the grey alder stands are included in the association Alnetum incanae Lüdi 1921 taking part of the Class Querco-Fagetea Br.-Bl. Et Vlieg. 37 em., order Fagetalia sylvaticae Pawlowski in Pawlowski, Sokolowski et Wallisch 1928, Alliance Alno-Ulmion Br.-Bl et Tx. 1943 (Syn. Alno-Padion Knapp 1948), sub-alliance Alnenion glutinoso-incanae (Oberdorfer, 1992). The grey alder stands of lower altitude in the submontane area and descending to the colline level, as mentioned for large parts of Europe (Schwabe, 1985; Oberdorfer, 1992), are similar to those of the Transylvanian Tableland.

The present study deals with the occurrences of grey alder (*Alnus incana*) stands in the Southern part of the Transylvanian Tableland, i.e. from a part of the tableland, situated on the left river bank of the Târnava Mare River, in particular from the Natura 2000 site "Sighişoara-Târnava Mare". On stretches of some tributary streams and streamlets such as the Şapartoc-Albeşti, V. Dăii, V. Robului upstream of Sighişoara, Şaeş at Sighişoara and Criş, Laslea/Roandola, as well as Valchid downstream of the municipality of Sighişoara, particular sites with grey alder (*Alnus incana*) can be found. The site conditions and ecological factors for their occurrence were scarcely studied and are remarkable for the tableland conditions.

From montane, submontane and colline wet river and stream valleys of Romania is described the association Telekio speciosae-Alnetum incanae Coldea (1986) 1990 with the characteristic species *Telekia speciosa* and *Alnus incana* and as accompanying species, among others, *Impatiens noli-tangere*, *Circaea lutetiana*, *Festuca gigantea, Rubus caesius, Stachys sylvatica, Euphorbia amygdaloides, Salvia glutinosa, Scrophularia nodosa, Brachypodium sylvaticum* and *Cornus sanguineus*, most existing as well on the sites with *Alnus incana* of our study area. The special conditions for the occurrence of grey alder at low altitude arises in general due to the structure of valleys, the content of calcareous substrate (calcareous marl) and the cold air accumulation in the deeper valleys (Oberdorfer, 1992).

Figure 1: Riparian gallery forest of white and brittle willow (*Salix alba* and *Salix fragilis*) on the Șaeș Stream (Schaaserbach) between Daia (Denndorf) and Apold (Trappold) July 2013.

MATERIAL AND METHODS

During the vegetation periods of the years 2011-2017 field studies concerning the riparian habitats, in particular of the priority habitat type alluvial forests with *Alnus glutinosa* and *Fraxinus excelsior* (Alno-Padion, Alnion incanae, Salicion albae) were carried out on different streams of the Southern Transylvanian Tableland. Samples were taken according to the method of Braun-Blanquet with the seven degree abundance-dominance scale (Braun-Blanquet, 1964; Borza and Boşcaiu, 1965). The samples were used for detailed studies concerning the ecology, species composition and structure of the phytocoenoses. Special attention was given to the subtype 44.2 Alnioin incanae in strong relation with the subtype 44.13 Salicion albae, and the accompanying tall herbaceous vegetation on the fringe of the riparian galleries. Considered too were aspects concerning the structure of the habitats in

strong relation with the water dynamics, the grain size of sediments and the succession of the vegetation along ecological gradients from the river banks to the higher elevations of the river valleys. From upstream to downstream along the Târnava Mare River the following tributary valleys have been studied: Șapartoc, Daia, Criș, Laslea/Roandola, and Valchid Valley.

The samples taken are included in phyto-coenological tables and grouped according to characteristic species of the phytocoenological units or according to ecological gradients and presented as well in the context of the European Union habitats (Gafta and Mountford, 2008; EUR28, 2013). The indicator values for light requirements of the species, wetness (W/F) and nitrogen (N) are included as well in the table according to Ellenberg et al. (2001). The nomenclature of species is given according to Sârbu et al. (2013) and Ciocârlan (2009).

RESULTS AND DISCUSSION

The occurrence of grey alder (*Alnus incana*) stands is in strong relation to the geomorphological structure of the Tertiary tableland and the hydro-morphological processes. The different layers are build by lime-rich marl, clay, sandstone and conglomerates. Over long time erosion and deposition processes there result different structured valleys with various depth and grain sized sediment materials transported during high floods. In deeper valley stretches an accumulation of cold air creates special microclimate conditions which allow the development of small grey alder galleries at low altitude, similar to those of the Carpathian montane level. Such grey alder galleries similar to those of the study area are mentioned also from other parts of Europe as well under conditions of cold air accumulation (Oberdorfer, 1992; Schwabe, 1995). These galleries with transition character or typical characteristics of montane grey alder galleries are interlocked with tall herbaceous fringe communities (habitat type 6430 Hydrophilous tall herb fringe communities of plains and of montane to alpine levels, EUR 28). The fringe communities occurring in the area are build by species with a transition character from hilly to submontane and montane, or they have typical montane character. The montane species are represented mostly by *Petasites hybridus*, *Telekia speciosa*, and *Salvia glutinosa* and other characteristic species of wet sites. Generally, the hills between 350 and 500 m in height present in the study area a vegetation including many montane elements, which give the vegetation of the area a transition character from lower tableland hills to those of the submontane and montane level.

On some stretches of the tributaries of the Târnava Mare River in the area of the Natura 2000 site Sighișoara-Târnava Mare, where the valley due to the changing morphological structure of the underground is deeper, as on the stretches dominated by willows, typical sites of grey alder have been registered and monitored. These riparian sites are strongly interlocked with the galleries dominated by white and crack willow and are located in the valleys of Șapartoc-Albești, Saeș, Criș, Laslea, Roandola, and Valchid (Fig. 2; Tab. 1).

The grey alder (*Alnus incana*) as a characteristic stand-building species is accompanied by white willow (*Salix alba*) and crack willow (*Salix fragilis*), both present with high constancy (IV). Their presence is characteristic for these small grey alder galleries at the submontane and hills level of the Southern Transylvanian Tableland. The most characteristic species of the herbaceous layer of these alder stands are the tall herbaceous species *Telekia speciosa*, *Equisetum telmateia, Petasites hybridus* and *Salvia glutinosa*, which generally are present mostly as belts along streams at the level of beech forests, but by lesser coverage degree of the tree crowns they are part of the inner of the riparian gallery. Together with the above-mentioned species, *Cirsium oleraceum* characteristic for Calthion and Molinietelia communities is the most constant accompanying species in the herbaceous layer.

On the bottom level of the herbaceous layer the presence of *Chrysosplenium alternifolium*, characteristic for riparian forests, with temporary flooding, or seepage areas is remarkable (Ellenberg et al., 2001; Oberdorfer, 2001). The occurrence of *Rubus caesius*, locally of high abundance-dominance values, indicates that grey alder stands occurs at low altitude only on lime-rich sites as was noted also in grey alder phytocoenoses of Central Europe (Oberdorfer, 1992). Also the presence of elm (*Ulmus minor*) and oak (*Quercus robur*) is characteristic for the submontane and hills level of the grey alder stands. The bloodtwig dogwood (*Cornus sanguinea*) is characteristic as well for the low level grey alder stands and described as the typical *Cornus sanguinea*-form of the Alnetum incanae in Central Europe (Schwabe, 1985; Oberdorfer, 1992).

The high number of wetness indicator species (values seven and eight) as well as species characteristic for temporary flooded areas (=), underlines the character of the wet habitat along the streams. The number of half shade (values five and six) and half light indicator species (seven), demonstrate that the riparian gallery is more or less small and open with a middle crown degree. The indicator species for nutrient (nitrogen) rich soils (values seven and eight) are corresponding to the conditions of temporarily flooded areas. Only the presence of species such as *Arctium lappa, Urtica dioica* and *Sambucus nigra* (value nine) indicate high nutrient content, due to human influence by rubbish deposition on the stream banks.

The constancy of *Telekia speciosa* and the species combination of the samples was the reason to include the grey alder phytoconoses of the left tributaries of Târnava Mare in the association Telekio speciosae-Alnetum incanae Coldea 1990 (Sanda et al., 2008; Coldea, 2015).
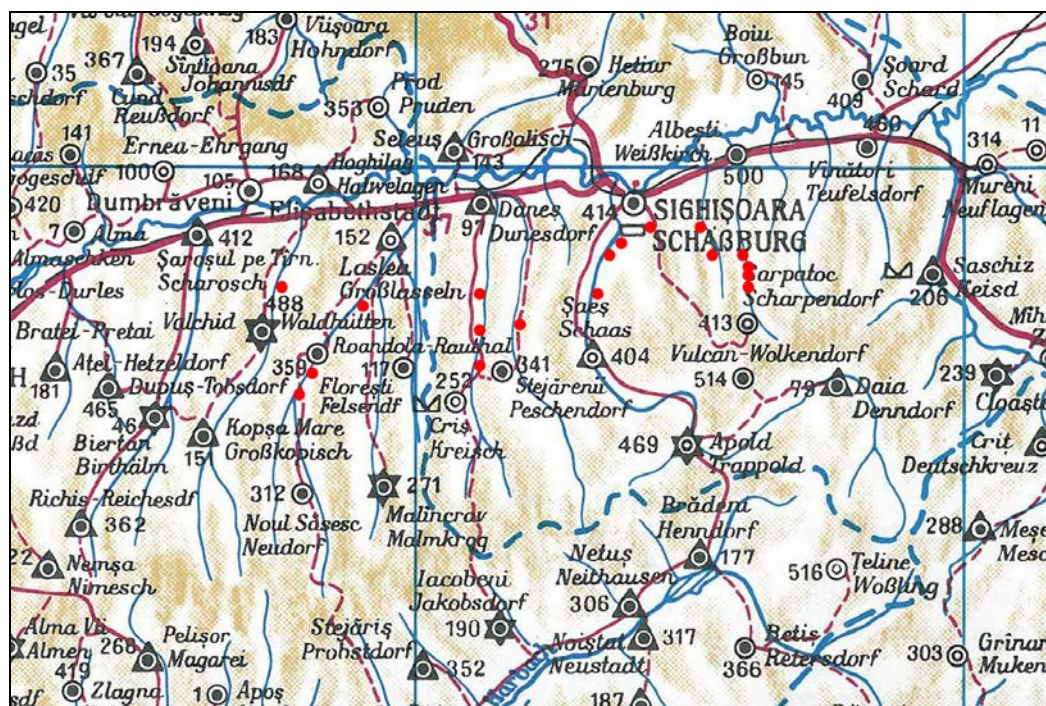
Figure 2: Distribution of grey alder sites on the left tributaries of the Târnava Mare River,
Natura 2000 site Sighişoara/Târnava Mare
(map basis for the distribution points: detail of the map from Fabini, 1999).

Table 1: Ass. Telekio speciosae-Alnetum incanae Coldea 1990.

L	U/W	N	Serial number	1	2	3	4	5	6	7	8	9	10	
			Nr. sample monitoring progr.	42	43	44	23	17	9	8	12	E	7	
			Locality	Sa	Sa	Sa	Sae	Cr	Cr	Cr	L/R	Roa	Va	
L	U/W	N												C
			Tree layer											
6	7 =	x	*Alnus incana*	3	4	3	2	2	1	3	2	3	3	V
5	8 =	7	*Salix alba*	.	+	2	3	2	3	2	2	1	2	V
5	8 =	6	*Salix fragilis*	+	+	+	.	3	3	1	1	2	.	IV
5	9 =	x	*Alnus glutinosa*	.	.	3		.	.	.	1	.	.	I
5	x	x	*Ulmus minor*	.	.	.	.	.	.	1	.	.	2	I
4	6	7	*Tilia platyphyllos*	.	.	.	1	.	.	.	.	.	.	I
5	5	6	*Acer campestre*	.	.	.	.	1	.	.	.	.	.	I
5	4	8	*Robinia pseudaccacia*	.	.	.	.	+	+	.	.	.	1	II
5	6	7	*Juglans regia*	.	.	.	.	.	.	.	1	.	.	I
7	x	x	*Quercus robur*	.	.	.	.	.	.	.	1	.	.	I
4	x	7	*Fraxinus excelsior*	.	.	.	.	.	.	.	1	.	.	I
5	7	6	*Populus alba*	.	.	.	.	.	.	.	.	.	1	I
			Shrubs layer											
7	5	9	*Sambucus nigra*	+	+	.	+	.	+	1	.	+	+	IV
7	5	x	*Cornus sanguinea*	3	.	.	.	1	.	2	.	1	2	III
6	5	5	*Evonymus europaea*	.	.	.	.	+	1	1	.	1	+	III
6	x	7	*Rubus caesius*	1	.	+	.	2	+		+	3	.	III
6	7 =	x	*Alnus incana*	.	+	.	.	.	.	.	+	.	+	II
5	4	3	*Evonymus verrucosa*	.	.	.	.	3	.	.	.	1	.	I
5	x	x	*Ulmus minor*	.	.	.	.	.	.	.	.	.	1	I
8	x =	x	*Salix purpurea*	.	.	2	.	.	.	.	.	1	.	I
5	6	7	*Acer negundo*	.	.	.	+	2	.	.	.	.	.	I

Table 1 (continued): Ass. Telekio speciosae-Alnetum incanae Coldea 1990.

L	U/W	N	Serial number	1	2	3	4	5	6	7	8	9	10	C
			Nr. sample monitoring progr.	42	43	44	23	17	9	8	12	E	7	
			Locality	Sa	Sa	Sa	Sae	Cr	Cr	Cr	L/R	Roa	Va	
			Climbing plants/liana											
8	6	9	*Calystegia sepium*	+	.	+	.	+	+	.	+	+	.	III
6	8 =	8	*Humulus lupulus*		.	.	.	.	2	+	+	+	.	II
7	9 =	8	*Echinocystis lobata*	.	.	+	.	.	.	+	+	+	.	II
7	5	7	*Clematis vitalba*	+	.	.	.	.	1	.	.	.	.	I
6	6	6	*Vitis sylvestris*	.	.	.	.	.	1	.	.	.	1	I
6	9 =	7	*Cucubalus baccifer*	.	.	.	.	.	.	.	+	+	.	I
			Tall herbaceous layer	.	.	.	.	.	.	.	.	.	.	.
5	8	5	*Equisetum telmateia*	+	.	+	2	.	.	.	+	+	+	III
7	8 =	8	*Telekia speciosa*	1	+	.	1	3	.	.	+	+	.	III
7	8 =	8	*Petasites hybridus*	+	3	.	+	.	.	.	2	1	.	III
4	6	7	*Salvia glutinosa*	2	2	+	.	+	.	.	.	.	.	II
6	7	5	*Cirsium oleraceum*	+	.	+	+	.	+	+	+	+	.	IV
7	8	4	*Angelica sylvestris*	+	.	.	+	.	.	+	.	.	.	II
6	8	4	*Scirpus sylvaticus*	2	.	+	+	.	.	.	3	.	.	II
7	7	8	*Eupatorium cannabinum*	+	.	+	+	.	.	.	.	+	.	II
8	x	6	*Solidago canadensis*	.	.	.	+	.	.	+	.	+	+	II
6	8	x	*Lysimachia vulgaris*	+	.	.	.	.	.	.	.	+	.	I
8	7	6	*Rudbeckia laciniata*	+	.	+	.	.	.	.	.	.	.	I

Table 1 (continued): Ass. Telekio speciosae-Alnetum incanae Coldea 1990.

L	U/W	N	Serial number	1	2	3	4	5	6	7	8	9	10	
			Nr. sample monitoring progr.	42	43	44	23	17	9	8	12	E	7	
			Locality	Sa	Sa	Sa	Sae	Cr	Cr	Cr	L/R	Ro a	Va	
L	U/W	N												C
			Herbaceous layer											
x	6	9	*Urtica dioica*	2	.	.	.	+	3	+	3	+	2	IV
5	6	8	*Aegopodium podagraria*	+	+	.	+	3	.	2	.	3	3	IV
7	8 =	8	*Myosoton aquaticum*	+	.	.	+	+	.	+	.	1	.	III
7	8 =	8	*Polygonum hydropiper*	2	+		.	+	.	+	.	.	.	II
4	8 =	5	*Chrysospleniu m alternif.*	.	.	.	.	2	.	2	2	.	3	II
7	9 =	7	*Lycopus europaeus*	+	.	+	+	.	.	.	.	.	.	II
3	5	6	*Brachypodium sylvaticum*	2	.	+	.	+	.	.	.	.	.	II
7	x	7	*Elymus repens*	.	.	2	.	+	.	.	+	+	.	II
7	8 =	7	*Mentha longifolia*	.	.	+	+	.	.	+	.	.	.	II
7	8	5	*Myosotis scorpioides*	.	.	+	.	.	.	+	.	.	+	II
7	9 =	7	*Poa palustris*	.	.	.	+	.	.	.	+	+	.	II
7	7	8	*Symphytum officinale*	.	.	.	+	.	.	+	.	+	.	II
6	7	7	*Ranunculus repens*	.	.	+	+	+	.	.	.	+	.	II
6	6	7	*Glecoma hederacea*	.	.	.	+	2	.	+	+	+	+	III
4	6	7	*Stachys sylvatica*	.	.	.	.	+	+	.	2		.	II
4	6	x	*Lysimachia nummularia*	.	.	.	+	+	.	.	.	+	.	II
7	6	8	*Erigeron annuus*	+	.	.	+	+	.	.			+	II
7	x	8	*Galium aparine*	+	+	.	.	+	.	.	.	+	.	II
7	5	6	*Dactylis glomerata*	.	.	.	+	+	.	.	+	+	.	II

Table 1 (continued): Ass. Telekio speciosae-Alnetum incanae Coldea 1990.

			Serial number	1	2	3	4	5	6	7	8	9	10	
			No. sample monitoring progr.	42	43	44	23	17	9	8	12	E	7	
			Locality	Sa	Sa	Sa	Sae	Cr	Cr	Cr	L/R	Roa	Va	
L	U/W	N												C
8	4	4	Melandrium album	.	.	.	+	.	+	.	+	+	.	II
7	5	6	Galeopsis tetrahit	.	.	+	.	+	.	.	.	+	.	II
7	5	8	Galeopsis speciosa	+	.	.	+	+	+	+	.	.	.	III
9	5	9	Arctium lappa	+	+	+	−	+	.	.	.	.	.	II
6	5	8	Chelidonium majus	+	+	+	.	.	.	.	.	.	.	II
7	5	x	Prunella vulgaris	+	+	.	+	.	.	.	.	.	.	II
4	5	7	Geum urbanum	.	.	.	.	+	.	.	+	+	.	II

Names of the localities /streams: Şa = Şapartoc 81, 2, 3, Sae = Şaeș (4), Cr = Criș, L/Roa = between Laslea and Roandola, Roa = Roandola, Va = Valchid;

Data and point of sampling Şa = Şapartoc, 4.07.2013: 46 12 40,4 N, 24 50 48.9 and 2 sampling point more in distance of hundred meters in the neighbouring area; Şae = Şaeș 46 08 435 N, 24 47 678 E; Cr = Criș (5): between Daneș and Criș, 13.09.2012: 46 2009 198 35379 N, 24 704 803985213 (E); Criș (6) downstream the bridge, 13.09.2012; Criș (7): near to the place of number fife on the upstream lying meander; 8. L/Roa = 46 10 789 N, 024 37 661 E, alt 371; 9. Roa, two km upstream the village, 46 09 479 N, 024 36 075 E, 375 m altitude; 10. Va = downstream the village, on the right river bank: 14.07.012, 46 12 786 N, 24 36 081 E.

Species present with + in one sample (constancy I): column 1: *Rumex obtusifolius, Arctium minus, Oxalis stricta*; column three: *Chenopodium album, Juglans regia, Pyrus pyraster, Robinia pseudacacia, Rorippa amphibia, Rosa canina, Salix triandra*; column four: *Agrimonia eupatoria, Carpinus betulus, Cichorium intybus, Crataegus monogyna, Pulicaria dysenterica, Silene vulgaris*; column five: *Acer negundo, Asarum europaeum, Corylus avellana, Deschampsia caespitosa, Dipsacus laciniatus, Heracleum sphondylium, Lamium maculatum, Stellaria media*; column six: *Senecio paludosus*; column nine: *Ajuga reptans, Carex hirta, Festuca gigantea, Lactuca serriola, Phragmites australis, Stellaria holostea, Thalictrum flavum, Viola odorata*.

Species occurring in two samples (constancy I): columns one and three: *Bidens tripartita, Geranium phaeum, Holcus lanatus, Lapsana communis, Trifolium repens, Tussilago farfara*; column one and six: *Agrostis stolonifera*; columns two and seven: *Xanthium strumarium*; columns three and four: *Achillea millefolium, Sium latifolium*; columns three and six: *Juncus effusus*; columns four and five: *Taraxacum officinale*; Columns five and nine: *Stachys palustris, Pulmonaria officinalis*; columns six and nine: *Artemisia vulgaris*; columns seven and nine: *Lythrum salicaria*; columns eight and nine: *Vicia cracca*.

Comparing the extent of riparian gallery stretches build by grey alder stands with those dominated by white and brittle willow, the firsts are shorter and more localized in the area with deep valleys and special microclimate conditions. In all studied valleys they have a similar structure and species combination, but there are also some differences from upstream to downstream concerning the structure of the vegetation layers, and the species composition (Tab. 1). These differences of the riparian gallery forest stretches are caused almost by human intervention in longer time through cutting trees, burning, and deposition of garbage (near to the villages). But in comparison with the stretches dominated by willows the stretches with grey alder are in a better and more natural state.

The most extended and representative grey alder galleries in the study area are those of the Șapartoc Valley east of Sighișoara, the most eastern of the monitored valleys. In that area from its source in the surrounding hills of the highland to the mouth of the Șapartoc Stream into the Târnava Mare River can be followed a succession of interlocking phytocoenoses as they are known from canon like deep valleys, constituting forests of ravines ("Schluchtwälder") with steep stream sides.

The Șapartoc Stream collects its waters from a small spring and seepage waters of the slopes near to the village of Șapartoc. The vegetation of the area is characterized by grasslands developed after forest clearance around the village in the upper part of a beech forest (Fig. 3). In that area many occurred and continue to do so. In the beginning the small water course of the valley crossing the forest have no special riparian wet vegetation, the beech forest with here and there sycamore (*Acer pseudoplatanus*), ash (*Fraxinus excelsior*) and largleaved lime (*Tilia platyphyllos*) reaching to the river bank. Such forests of slopes, screes and ravines are – according to the list of habitat types of the FFH-Directive – included in the habitat type 9180 (EUR 28, Gafta and Mountford, 2008).

The typical wet riparian species accompanying the stream through the beech forest are mainly the tall herbaceous *Telekia speciosa* (Fig. 5), *Petasites hybridus* and *Equisetum telmateia*. These species occur together or in separate groups, constituting the first riparian vegetation belt. This is followed by the development of a small grey alder belt, which is broadened to the downstream part (Fig. 3). The tall herbaceous vegetation remains further as a well contoured belt (Figs. 4 and 5), but the species takes part together with other herbaceous species also of the layers of the riparian forest, where the light conditions are appropriate. The most compact herbaceous belt accompanying the stream is that of *Petasites hybridus* (Fig. 6). Near to the mouth into the Târnava Mare River the riparian gallery of grey alder is changing through a transition area including occurrences of black alder (*Alnus glutinosa*) disappearing completely on the lowest level of the valley. At the mouth it is replaced by stands of white and crack willows, due to changes in site conditions (Tab. 2). The ravine-like character on the upper and middle stretch is lost due to slopes with smaller inclination on the opening to the Târnava Mare River valley. The succession and the changes in species composition are clearly visible from upstream to downstream.
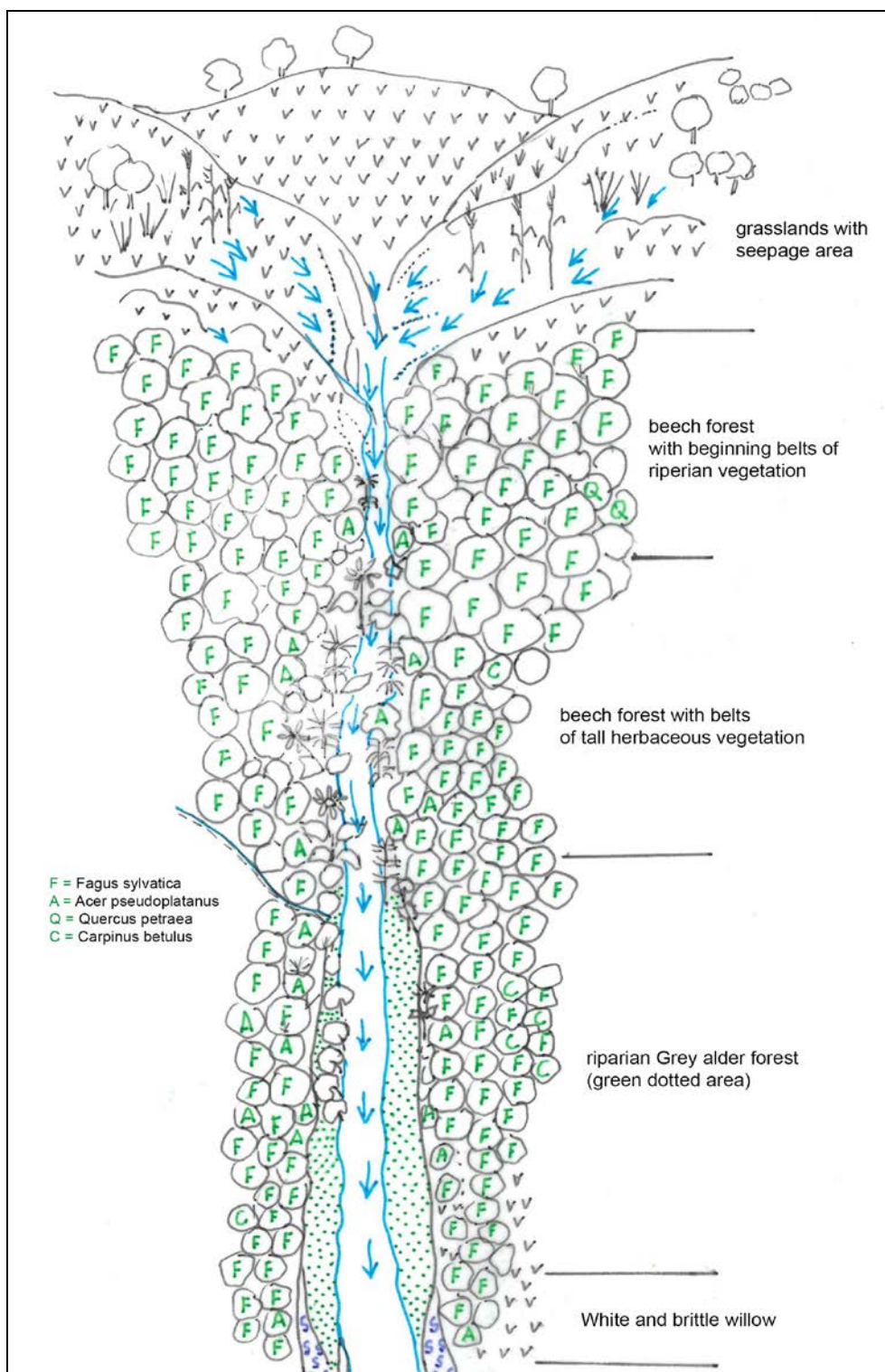
Figure 3: Scheme of the structure and distribution of the vegetation of the Şapartoc Valley with grey alder stands (Natura 2000 site Sighișoara/Târnava Mare).

Table 2: Succession of the riparian vegetation from upstream to downstream in the Şapartoc Valley (Fig. 3); all the samples included in the table were realized along the Şapartoc Valley from upstream to downstream on 4th July 2013.

Serial number	1	2	3	4	5	6	7	8	9	10
Sample no. monitoring	39	40	40a	41	42	42b	43	44	45	46
Tree layer and shrubs shrubs layer										
Fagus sylvatica	2	2	.	2	.	.	.	.	.	.
Acer pseudoplatanus	2	2	2	2	.	.	.	.	.	.
Acer pseudoplatanus	.	.	.	1	.	.	.	.	.	.
Popus tremula	1	1	2	.	.	.	.	.	.	.
Tilia platyphyllos	1	.	.	1	.	.	.	.	.	.
Robinia pseudoacaccia	1	.	.	1	.	.	.	.	.	.
Carpinus betulus	.	.	1	.	.	.	.	.	.	.
Fraxinus excelsior	.	.	.	1	.	.	.	1	.	.
Acer campestre	.	.	.	.	1	+	.	.	.	.
Alnus incana	.	.	.	.	2	4	4	3	.	.
Alnus incana	.	.	.	.	2	2	+	.	.	.
Alnus glutinosa	.	.	.	.	.	.	.	3	.	.
Salix alba	.	.	.	.	.	.	.	2	2	+
Salix fragilis	.	.	.	+	+	+	.	+	4	3
Salix alba	.	.	.	.	.	.	.	.	+	2
Salix purpurea	.	.	.	.	.	.	.	2	.	+
Salix triandra	1	.	.	.	.	+	.	+	.	.
Corylus avellana	1	2	1	+	.	.	.	.	+	.
Cornus sanguinea	.	1	.	+	3	.	.	.	+	.
Sambucus nigra	.	.	+	+	1	2	+	+	2	+
Evonymus europaeus	.	.		+				+	.	+
Robinia pseudaccacia	+	.	+	+	+	.	.	.	+	.
Rubus caesius	+	.	1	+	+	2	+	.	+	.
Climbing plants/lianas										
Clematis vitalba	.	+	.	+	+	.	.	+	.	.
Calystegia sepium	.	.	.	.	+	.	.	+	+	.
Echinocystis lobata	.	.	.	.	.	+	.	+	+	.
Humulus lupulus	.	.	.	.	.	.	.	+	2	1
Tall herbaceous layer										
Telekia speciosa	1	3	3	2	1	1	3	+	.	.
Petasites hybridus	3	+	+	4	4	3	+	.	.	.
Eqisetum telmateia	3	+	2	.	+	4	3	.	.	+
Salvia glutinosa	1	3	+	2	+	2	+	+	.	+
Cirsium oleraceum	2	+	3	.	2	+	2	+	.	.
Scirpus sylvaticus	.	.	+	+	+	.	.	+	+	.
Eupatorium cannabinum	+	.	.	+	+	.	+	1	+	+

Table 2 (continued): Succession of the riparian vegetation from upstream to downstream in the Şapartoc Valley (Fig. 3); all the samples included in the table were realized along the Şapartoc Valley from upstream to downstream on 4th July 2013.

Herbaceous layer										
Aegopodium podagraria	.	+	+	+	1	2	2	+	+	.
Myosoton aquaticum	.	+	+	+	+	3	+	.	.	.
Urtica dioica	.	.	+	3	+	+	+	.	.	.
Brachypodium sylvaticum	.	.	2	.	2	+	+	.	.	.
Trifolim repens	.	.	+	.	.	+	.	+	.	.
Erigeron annuus	.	.	+	.	.	+	.	+	.	.
Arctium lappa	.	.	.	.	+	+	.	.	+	.
Chelidonium majus	.	.	.	.	+	+	+	+	.	.
Galium aparine	.	.	.	.	+	+	.	+	.	.
Rudbeckia laciniata	.	.	.	.	+	+	+	.	.	.
Galeopsis speciosa	.	.	.	.	+	+	.	+	.	.
Tussilago farfara	.	.	.	.	+	2	.	.	.	.
Polygonum hydropiper	.	.	.	.	.	+	.	+	.	+
Elymus repens	.	.	.	.	.	2	+	2	+	2
Artemisia vulgaris	.	.	.	.	.	.	.	.	.	2

Figure 4: Grey alder phytocoenose with *Equisetum telmateia* on the upper Şapartoc Stream.

Figure 5: *Telekia speciosa* on the edge of the grey alder gallery middle Șapartoc Valley.

Figure 6: Grey alder gallery in the middle Șapartoc Valley with tall herbaceous belt dominated by *Petasites hybridus*.

CONCLUSIONS

The occurrence of grey alder stands in the southern part of the Transylvanian Tableland is strongly related to the special site conditions given by the geomorphological structure of the deepened streambeds and the accumulation of cold air, which create a special microclimate and special ecological conditions for their existence in the area. These conditions exist generally only on small stretches along the streams. The grey alder stands are strongly interlocked with the larger spread white and crack brittele willow stands, these lasts building characteristic riparian galleries all over the Southern Transylvanian Tableland. Between the characteristic species exists with high constancy tall herbaceous species with a montane distribution area such are *Telekia speciosa, Petasites hybridus, Equisetum telmateia*, and *Salvia glutinosa*. These species have their main distribution area on streams of the level of beech forests, descending sometimes on lower altitudes of the submontane and hills level. Due to their rare occurrences under the above-mentioned special site conditions, such grey alder stands – included in the habitat type 91E0 merit more attention in the frame of management plans for Natura 2000 sites with existing grey alder stands.

ACKNOWLEDGEMENTS

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THE DISTRIBUTION OF THE ALIEN SPECIES
PENAEUS AZTECUS IVES, 1891 (DECAPODA, PENAEIDAE)
IN THE MEDITERRANEAN SEA

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ABSTRACT

The present paper is based on the literature review and the recent information about the distribution range of the alien brown shrimp *Penaeus aztecus* Ives, 1891 in the Mediterranean Sea. This alien species has spread throughout the Mediterranean Sea (especially the eastern part) mainly through ship/ballast water introductions and has presently been reported by eight countries (27 localities). Introduction pathways and chronological distribution patterns of the alien brown shrimp species are discussed. *Penaeus aztecus* forms dense populations along the coast of the Turkish Mediterranean Sea, and it has a potential for colonising the native habitats of autochthonous species.

ZUSAMMENFASSUNG: Die Verbreitung der fremdländischen Art *Penaeus aztecus* Ives, 1891 (Decapoda, Penaidae) im Mittelmeer.

Vorliegende Arbeit stützt sich auf eine Erfassung der Fachliteratur und auf rezente Informationen betreffend die im Mittelmeer eingebürgerte Art *Penaeus aztecus* Ives, 1891. Diese adventive Art hat sich im gesamten Mittelmeer, vor allem in dessen östlichem Teil vorwiegend durch Balastwasser der Schiffe ausgebreitet und wurde bereits aus acht Ländern insgesamt an 27 Orten gemeldet. Ebenso wurden die Verbreitungswege und das chronologische Verbreitungsmodell der Art dargestellt. *Penaeus aztecus* bildet dichte Populationen entlang der türkischen Mittelmeerküste und hat unter den invasiven Garnelenarten das Potential die Lebensräume der einheimischen Arten zu besiedeln.

REZUMAT: Distribuţia speciei străine *Penaeus aztecus* Ives, 1891 (Decapoda, Penaidae) în Marea Mediterană.

Prezenta lucrare se bazează pe o trecere în revistă a literaturii şi pe informaţii recente referitoare la distribuţia speciei adventive *Penaeus aztecus* Ives, 1891 din Marea Mediterană. Această specie adventivă s-a răspândit în întreaga Mediterană (în special în partea estică) în principal introdusă prin apa de balast a navelor şi a fost semnalată în opt ţări (27 de localităţi). Am prezentat de asemenea, căile de introducere şi modelul cronologic de distribuţie al acestei specii. *Penaeus aztecus* formează populaţii dense de-a lungul coastei Turce a Mării Mediterane. Între speciile invazive de creveţi, *Penaeus aztecus* are potenţial de colonizare a habitatelor native ale speciilor autohtone.

INTRODUCTION

The alien species invasions are one of the most important conservation issues around the world (Welcomme, 1988; Lodge et al., 1998; Strahm and Rietbergen, 2001; Lansdown et al., 2016; Anastasiu et al., 2017; *, GISD). Biological invasions of alien species constitute a significant environmental problem and one aspect of global change in the marine environment (Özcan et al., 2010; Katsanevakis et al., 2014; Galil et al., 2018). According to Galil et al. (2018) 726 alien marine species were listed in the Mediterranean Sea. An average of 10 alien species per year has penetrated in this sea (Galil, 2009).

The Mediterranean Sea is affected by invasive marine species by means of the Suez Canal. Most of pathways for the introduction of exotic species are via hull fouling and/or ballast water from ships (Özcan et al., 2010).

The brown shrimp *Penaeus aztecus* Ives, 1891 is commonly distributed throughout the north-western Atlantic Ocean (from Massachusetts to the Gulf of Mexico and the north-western Yucatán) (Perez Farfante, 1969; Tavares, 2002).

The species was first recorded from the Antalya Bay, Levantine Sea coast of Turkey and it was hypothesized its introduction to the eastern Mediterranean Sea was due to ballast water (Deval et al., 2010). Then, it has been reported from the coast of Egypt (Sadek et al., 2018), Greece (Nikolopoulou et al., 2013), Montenegro (Marković et al., 2014), Italy (Cruscanti et al., 2015; Zava et al., 2018), Israel and France (Galil et al., 2017). *Penaeus aztecus* has also been reported from different localities of the Mediterranean Sea (Gökoğlu and Özvarol, 2013; Kapiris and Apostolidis, 2014; Zenetos and Giavasi, 2015; Minos et al., 2015; Bakır and Aydın, 2016; Kapiris and Minos, 2017; Kampouris et al., 2018; Zava et al., 2018). The economic importance of the zoogeographical distribution of this species in the Mediterranean Sea and its distribution in the İskenderun and Mersin Bays where areas of important shrimp fisheries are present.

MATERIAL AND METHODS

According to the existing literature (Web of Sciences, Google Scholar, Researchgate etc.) the zoogeographic distribution and expansion of *Penaeus aztecus* was attempting to be presented. In Turkey, the samplings were carried out in İskenderun and Mersin Bays between December 2010 and March 2017. The specimens of *Penaeus aztecus* were captured by means of trawl hauls on the sandy muddy seabed at depths of 20-35 m (Fig. 1). The specimens were identified using the keys in Perez Farfante (1969, 1988) and Tavares (2002). The specimens were preserved in 4% formalin and deposited at the Faculty of Marine Sciences and Technology of the Iskenderun Technical University, Turkey (collection of Dr. T. Özcan).
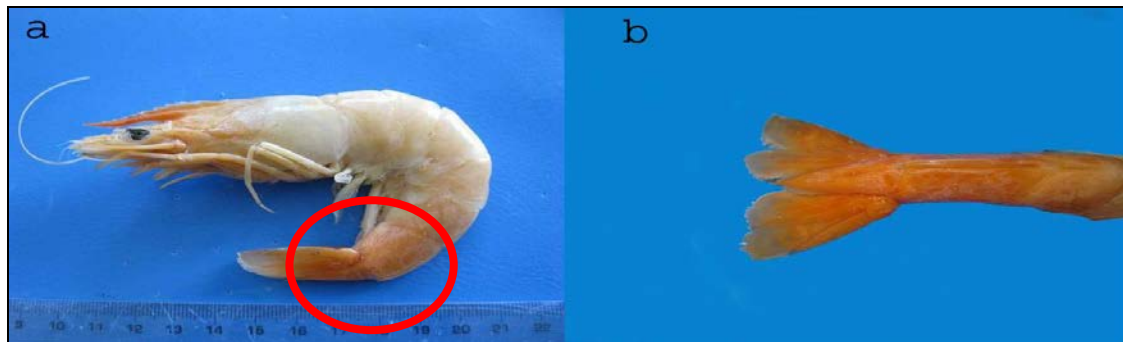

Figure 1: *Penaeus aztecus* a: Lateral view, ♀ mm;
b: Dorsal view of sixth abdominal somite, telson and uropods.

RESULTS AND DISCUSSION

Recently, *Penaeus aztecus* has been one of the species that has rapidly entered in the Mediterranean Sea (Zava et al., 2018). The distribution area of this species in this sea has expanded. Previous local records of *Penaeus aztecus* on the Turkish Mediterranean coast were given from the Bays of Antalya, Mersin, and İskenderun (west side: Yumurtalık Bight) (Gökoğlu and Ozvarol, 2013). *Penaeus aztecus* is denser in the Yumurtalık-Karataş region within the Iskenderun Bay and it is predicted to be evaluated economically in terms of its population size. After one year, the species first reported in Turkey has expanded to the Finike coast and the Iskenderun Bay increasing its expansion area by approximately 500 km (Gökoğlu and Özvarol, 2013). *Penaeus aztecus* was reported on the shores of Damietta Egypt (Sadek et al., 2018) and Thermaikos Gulf, Greece (Nikolopoulou et al., 2013) after one year due to introduction by ship ballast waters. The expansion between 2013 and 2018 are as follows; Boka Kotorska Bay, Adriatic Sea, Montenegro (Marković et al., 2014), Corfu Island, Ionian Sea, Greece (Kapiris and Apostolidis, 2014), Thermaikos Gulf (Kevrekidis, 2014) and Nestos estuaries, Aegean Sea, Yunanistan (Minos et al., 2015), Castiglione della Pescaia, Tyrrhenian Italy (Cruscanti et al., 2015), Kyllini (Zenetos and Giavasi, 2015) and Chalki Island, Greece (Kondylatos and Corsini-Foka, 2015), Gulf of Lion (France), Israeli coast (Galil et al., 2017), Sicily between Porto Empedocle and Mazara del Vallo, Italy (Scannella et al., 2017); Vivari Lagoon, Argolikos Gulf-Greece, Aegean Sea (Kapiris and Minos, 2017); Çandarlı and Ildır Bay, Aegean Sea, Turkey (Bakır and Aydın, 2016); Chieti, Ortona, Mola di Bari and Termoli, Adriatic Sea (Zava et al., 2018) and Vlora Bay-Albania, Adriatic Sea and Marzamemi, Ionian coast of Sicily (Kampouris et al., 2018) (Fig. 2 and Tab. 1).
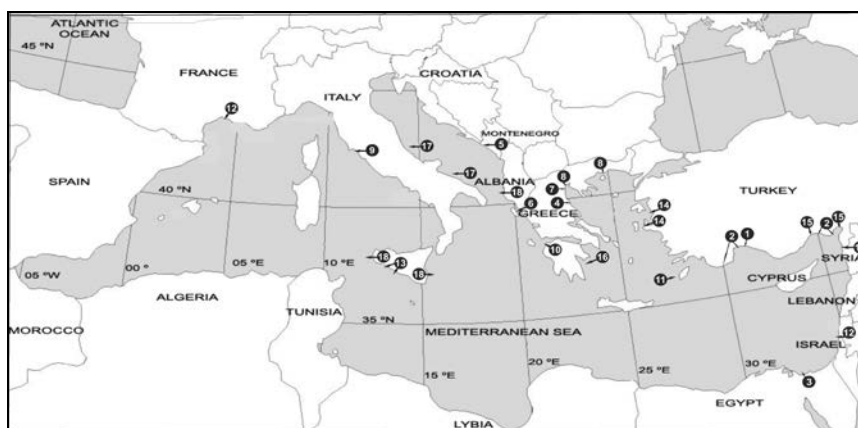
Figure 2: Current map showing distribution locations of records of *Penaeus aztecus* in the Mediterranean Sea, in chronological order: 1: (Deval et al. 2010); 2: (Gökoğlu and Özvarol 2013); 3: (Sadek et al. 2017); 4: (Nikolopoulou et al. 2013); 5: (Marković et al. 2013); 6: (Kapiris and Apostolidis, 2014); 7: (Kevrekidis 2014); 8: (Minos et al. 2015); 9: (Cruscanti et al. 2015); 10: (Zenetos and Giavasi, 2015); 11: (Kondylatos and Corsini-Foka, 2015); 12: (Galil et al., 2016); 13: (Scannella et al., 2017); 14: (Bakır and Aydın, 2016); 15: (Present study); 16: (Kapiris and Minos, 2017); 17: (Zava et al., 2018); 18: (Kampouris et al., 2018). For details see table 1.

According to Deval et al. (2010), *Penaeus aztecus* was captured with native and lessepsian species in the İskenderun and Mersin Bay. After *Penaeus aztecus* was reported for the first time in Antalya Bay, in eight years, its distribution in the Mediterranean was expanded.

Table 1: Chronological distribution of *Penaeus aztecus* found in the Mediterranean Sea.

No.	Authors	Region	Date reported
1.	Deval et al., 2010	Antalya, Turkey	24 December 2009
2.	Gökoğlu and Ozvarol, 2013	Finike, İskenderun Bay, Turkey	June 2011
3.	Sadek et al., 2018	Damietta, Egypt	2012
4.	Nıkolopoulou et al., 2013	Thermaikos Gulf, Greece	2012
5.	Marković et al., 2014	Boka Kotorska Bay, Montenegro	19 September 2013
6.	Kapiris and Apostolidis, 2014	Corfu Island, Greece	November 2013
7.	Kevrekidis, 2014	Thermaikos Gulf, Greece	22 November 2013
8.	Minos et al., 2015	Thermaikos Gulf and Nestos estuaries, Greece	November 2013 to March 2014
9.	Cruscanti et al., 2015	Castiglione della Pescaia, Italy	6th August 2014
10.	Zenetos and Giavasi, 2015	Kyllini, Greece	October 2014
11.	Kondylatos and Corsini-Foka, 2015	Chalki Island, Greece	1st November 2014
12.	Galil et al., 2017	Le Grau du Roi, Gulf of Lion, France and Israel	30 April 2015
13.	Scannella et al., 2017	Sicily between Porto Empedocle and Mazara del Vallo, Italy	3rd November 2015
14.	Bakır and Aydın, 2016	Çandarlı Bay and Ildır Bay, Turkey	December 2015 to February 2016
15.	Present study	Mersin Bay, İskenderun Bay, Turkey	December 2010 to March 2017
16.	Kapiris and Minos, 2017	Vivari Lagoon, Argolikos Gulf-Greece, Aegean Sea	–
17.	Zava et al., 2018	Chieti, Ortona, Mola di Bari, Adriatic Sea Termoli, Adriatic Sea	2015, December 2016, November 2017
18.	Kampouris et al., 2018	Vlora Bay-Albania, Adriatic Sea; Marzamemi, Ionian coast of Sicily; Mazara del Vallo to Pozzallo	21 May and 2nd June 2018 24 March 2018

CONCLUSIONS

Recent studies reported that *Penaeus aztecus* which extends the distribution area in the Mediterranean, may have a potential negative effect on the native species of the Mediterranean such as *Penaeus kerathurus* (Kevrekidis, 2014). Consequently, it is likely that the existing shrimp species will start to compete with other shrimp species in the Mediterranean Sea. It is known, *Penaeus aztecus* prefers the coastal areas so many bays and estuaries in the Mediterranean ecosystem are suitable habitats for this species. *Penaeus aztecus* competes with many native and exotic species in the eastern Mediterranean (Kevrekidis, 2014).

The eastern Mediterranean Sea (particularly Iskenderun Bay) is affected by invasion of exotic shrimps via Suez Canal and hull fouling and/or ballast water from ships. Due to that, most of scientific surveys should be performed in İskenderun Bay to have a detailed understanding about the impact exotics have on native species or competition between native and exotic species. Yet, we need to carry out the surveys in areas economically affecting shrimp fisheries in the Iskenderun Bay and Mediterranean Sea.

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MORPHOLOGY OF THE GENUS *GYMNOCEPHALUS* (PISCES) FROM THE LOWER DANUBE RIVER

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ABSTRACT

The current study completed the information regarding the phenotypic variability in the Danube ruffe (*Gymnocephalus baloni*). We also assessed the phylogenetic relationship of *G. baloni* with the other two species of the genus *Gymnocephalus* from the Lower Danube River.

Ten morphological characters were the most useful together for discriminating between *G. baloni* and *G. cernua* from the Lower Danube River. In addition, we found a more streamlined body shape in *G. baloni* compared with the described holotype, which could be in connection with fish phenotypic response to ecological characteristics of the Lower Danube River.

RESUMÉ: Morphologie du genre Gymnocephalus (Pisces) du cours inférieur du Danube.

L'étude actuelle a complété l'information concernant la variabilité phénotypique de la ruffe du Danube (*Gymnocephalus baloni*). On a également évalué la relation phylogénétique de *G. baloni* avec les deux autres espèces du genre *Gymnocephalus* du cours inférieur du Danube.

Dix caractères morphologiques ont été les plus utiles pour différencier *G. baloni* et *G. cernua* du cours inférieur du Danube. De même, on a trouvé une forme de corps plus hydrodynamique chez *G. baloni* par rapport à l'holotype décrit, ce qui pourrait être en rapport avec la réponse phénotypique des poissons aux caractéristiques écologiques du cours inférieur du Danube.

REZUMAT: Morfologia genului Gymnocephalus (Pisces) din cursul inferior al Dunării.

Studiul a completat informațiile privind variabilitatea fenotipică a ghiborțului de Dunăre (*Gymnocephalus baloni*). S-a evaluat, de asemenea, relația filogenetică dintre *G. baloni* și celelalte două specii din genul *Gymnocephalus* din cursul inferior al Dunării.

Zece caractere morfologice au fost semnificative pentru discriminarea speciilor *G. baloni* și *G. cernua* din cursul inferior al Dunării. De asemenea, s-a evidențiat forma hidrodinamică mai accentuată a speciei *G. baloni* comparativ cu holotipul, aspect care ar putea fi în legătură cu răspunsul fenotipic al peștilor la caracteristicile ecologice ale fluviului Dunărea în cursul inferior.

INTRODUCTION

The highly diversified and complex Lower Danube River area geoecosystems, generated in time and space by a unique mixture of biotopes and biocoenosis, has formed a highly dynamic and extremely rich fish fauna (e.g. there are 137 fish species only in the Danube Delta) (Antipa, 1909; Bănărescu, 1964; Bănărescu and Bănăduc, 2007; Bănăduc et al., 2016).

In the last century, compared to Antipa (1905, 1909, 1915, 1933, 1934), the Lower Danube-Danube Delta-Black Sea's area habitats heterogeneity and native economic and conservation important fish species diversity and stock abundance have a significantly decreasing trend, and currently, there is no scientific indication that this trend will stop in the near future (Bănăduc et al., 2016). In this present context, it is evident that the regional fish species of conservative and/or economic interest should be studied from a high number of points of view.

Three species of the genus *Gymnocephalus* are widespread in the Lower Danube River basin: the widespread ruffe, *Gymnocephalus cernua* (Linnaeus, 1758), the Danube ruffe, *Gymnocephalus baloni* (Holčík and Hensel, 1974) and the yellow pope, *Gymnocephalus schraetser* (Linnaeus 1758), the last two of these Eurasian fish species being indexed in the Romanian Vertebrate Red List as vulnerable (Bănărescu, 2005) and are protected by Habitats Directive – 92/43/EEC (Bănărescu and Bănăduc, 2007). There is another species of ruffe reported in scientific studies from the Black Sea basin, the Don ruffe, *Gymnocephalus acerina* (Gmelin, 1789) but the information about its presence in the Prut River, a tributary of the Danube River has not been confirmed by some recent ichthyological studies (Davideanu et al., 2008).

The species from the Eurasian genus *Gymnocephalus* date to about 13.4 Mya (Stepien and Haponski, 2015). They show different patterns in body shape, colours and meristic characters as lateral line scale and fin rays counts (Bănărescu, 1964; Holčík and Hensel, 1974; Kottelat and Freyhof, 2007; Stepien and Haponski, 2015).

Gymnocephalus baloni has long been treated as *G. cernua* and just recently described as a specific rank by Holčík and Hensel (1974), the species being poorly known (Bănărescu, 2005; Ratschan, 2012). After its first description, several ichthyological studies reported the first presence of populations in different parts of the Danube River drainage such as the Drava River, Slovenia and Croatia (Povž et al., 1997), and the Mur River, Austria (Ratschan, 2012). A recent study of Tsyba and Kogodiy (2017) notices the presence of the species in another river from Black Sea basin (middle Dniepr/Nistru drainage, Ukraina).

There are several studies from recent years that report *G. baloni* species from different rivers in the Romanian Danube River drainage area: the Danube River – downstream to the town of Brăila, including the Danube Delta arms, the Danube River between Turnu-Severin and Moldova Nouă, as well as the rivers Criş, Someş, Mureş, Ialomiţa, Argeş, Olt, Vedea, Timiş, Tisoviţa, and Berzasca (Oţel et al., 1993; Kováč, 1994; Bănărescu and Bănăduc, 2007; Oţel, 2007; Bănăduc, 2009; Telcean and Cupşa, 2009, 2012; Oţel and Năstase, 2010; Györe et al., 2013; Bănăduc et al., 2014, 2016; Nuţă et al., 2016; Năstase and Oţel, 2016; Bulat, 2017; Năstase et al., 2017).

However, no publications are known to us to describe the phenotypic variability of populations from the Romanian Danube River basin.

The main aim of our research was to analyse the morphometric features of *G. baloni* from the targeted area and compare them with data published by other authors in recent years, regarding the fish populations from the Danube River basin (Bănărescu, 1964; Holčík and Hensel, 1974; Specziár and Vida, 1995; Kottelat and Freyhof, 2007; Geiger and Schliewen, 2010; Stepien and Haponski, 2015; Tsyba and Kokodiy, 2017).

The intra- and inter-population morphological variations in fish are put in evidence by several authors (Karakousis et al., 1991; Wimberger, 1992; Turan, 1999; Shao et al., 2007). Various references sustain the habitat-associated morphological divergence as a common pattern in fish (Foster et al., 2015).

The morphological characters for reliable discrimination between two of these sympatric species are also analysed in our study. An analysis of phenotypic variation in morphometric or meristic characters is the method most commonly used for fish population delineation (Karakousis et al., 1991; Shao et al., 2007; Samaradivakara et al., 2012). This approach is still commonly used in fish biology, taxonomic studies and assessment of the relationship between various taxa (Turan, 1999), despite the development of molecular techniques (Shao et al., 2007).

Another goal of our research was to analyse the phylogenetic relationship between the sister taxa *G. cernua* and *G. baloni* from the targeted area. Although there is a strong similarity between *G. baloni* and *G. cernua* and a clear discrimination of these two species from *G. schraetser* (with more hydrodynamic shape of the body and also specific colour features), there are still different opinions expressed in the studies of last decades, regarding their phylogenetic relationship (Specziár and Vida, 1995; Geiger and Schliewen, 2010).

Thus, the first taxonomical description of *G. baloni* by Holčík and Hensel (1974) allowed the authors to identify a closer relationship between *G. baloni* and *G. cernua*, placing them in the same subgenus, *Acerina*, while the remaining two elongated species (*G. schraetser* and *G. acerina*) are placed in the subgenus *Gymnocephalus*. Later, Geiger and Schliewen (2010), using genetic techniques, have questioned the phylogenetic position of *G. schraetser* within the genus *Gymnocephalus*, while the new results of Stepien and Haponski (2015), based on molecular data, have suggested a sister – taxa relation between *G. baloni* and *G. schraetser*.

MATERIAL AND METHODS

The morphometric data are from a total of 49 fish specimens in the Lower Danube River area, of them: 12 specimens of the Danube ruffe, *G. baloni*, and 16 specimens of the striped ruffe, *G. schraetser* were sampled near the confluences of the Danube River with the rivers Prut and Siret at Galaţi City (sea mile 80-76 from Black Sea) while 21 specimens of the widespread ruffe, *G. cernua* were sampled at Brateş fish farm pond (Galaţi County), which has a Danube River water supply. The random sampling of studied fish specimens occurred in 2015, between June and October. Specimen collection was performed using hook – and – line method.

All collected specimens were fixed in 10% formalin at the site of collection and transported to the laboratory.

A CANON A590 IS camera is used to take digital pictures of the left side of each fish individual.

Bănărescu (1964) and Holčík and Hensel (1974) describe a total of 18 morphometric measurements (Fig. 1) taken. They are as follows:

- SL – standard length (mm);
- BODYDEPTH – maximum body height;
- CAUDPEDDEPTH – minimum height of caudal peduncle;
- PREDORSDIST – predorsal distance;
- CAUDPDLENGTH – length of caudal peduncle;
- PREVENTDIST – pre-ventral distance;
- PREANALDIST – pre-anal distance;
- PVDIST – distance between pectoral and ventral fin insertions;
- VADIST – distance between ventral and anal fin insertions;
- DLENGTH – length of dorsal fin base;
- ALENGTH – length of anal fin base;
- ADEPTH – the distance from the insertion of the anal fin to the end point of the highest ray of anal fin;
- PLENGTH – pectoral fin length;
- VLENTGTH – ventral fin length;
- HEADLENGTH – the distance from the tip of the snout to the ventral base of the largest opercula spine;
- HEADDEPTH – head depth;
- MOUTHLENGTH – preorbital length;
- EYEDIAMETER – eye diameter.

The values express a percentage of the standard length, with the exception of the head depth, mouth length and eye diameter, which count as a percentage of the head length. The mean values of each morphological character, as well as the confidence intervals ($P < 0.05$), were computed using descriptive statistics function with Systat 10.2.
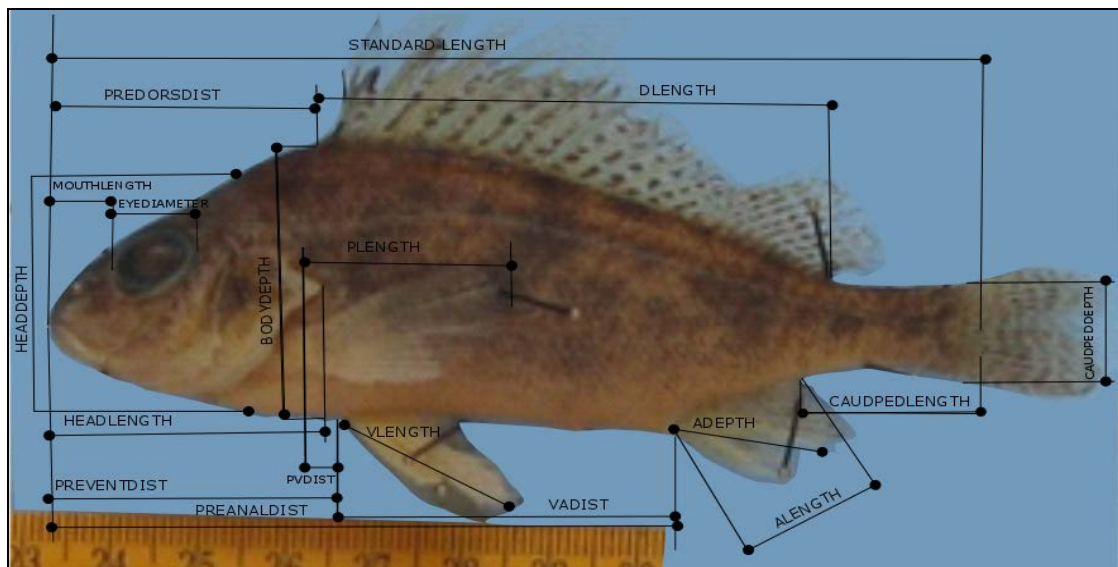

Figure 1: Morphometric characters taken on the ruffe species
(picture of *Gymnocephalus baloni*) studied in the Lower Danube River basin.

A multivariate approach, including factor analysis (FA) and discriminant analysis (DA), was applied for the morphometric assessment, using Systat 10.2. For DA, a linear discriminant analysis, using a backward stepwise method, based on Mahalanobis distances (developed by Fisher, 1936, in Engelman, 2005), was performed to establish the most relevant morphometric features that together discriminate the samples of *G. baloni* and *G. cernua* (Engelman, 2005).

MorfoJ 1.05f applies the geometric methods in this study (Klingenberg, 2011) and 16 landmarks are defined and also recorded as two-dimensional coordinates, using TPS Digitize (Rohlf, 2016a), TPS Relative Warps (Rohlf, 2016b), and TPS Utility (Rohlf, 2016c) software. The landmarks were selected to provide a homogeneous frame of the entire shape. The shape information was extracted by Procrustes superimposition, which removes variation in size, position and orientation from data on landmark coordinates (Klingenberg, 2011).

The multivariate statistical analysis of body shape uses coordinates of the superimposed landmarks, and includes principal components analysis (PCA), discriminant analysis (DA), and canonical variate analysis (CVA) in order to address the main question: the current variability of the ruffe species populations from the Lower Danube River basin, around Mm80 area.

The geometric, statistical analysis provides necessary information regarding the fish body shape variation between groups. The interspecific study used the 49 ruffe individuals.

The statistical test is made using the MorphoJ software. After landmark data importing and shape information extracting with Procrustes superimposition (Klingenberg, 2011), several techniques of multivariate tests data were used to analyse the substantiating patterns in the groups and among the variables. The PCA and CVA statistic tools allow the examination of the relationship among variables and cases in a single block of data (Heyman and Noble, 1989).

RESULTS AND DISCUSSION

The morphological characters (Fig. 1) obtained through body measurements of the three ruffe species, as well as the confidence intervals with 95% confidence level, are listed in table 1.

The standard body length of *G. baloni* individuals ranged between 61.72-107.71 mm (minimum – maximum values), the standard body length of *G. cernua* was 87.74-110 mm, and this of *G. schraetser* was 161.99-186.46 mm.

Discrimination between *G. baloni* and its more widespread congener *G. cernua* assessed by the univariate analysis indicated an overlapping of the ranges in some morphometric characters, except the body depth, length of the caudal peduncle, ventral-anal fin distance, length of dorsal base and eye diameter, which is consistent with references (Holčík and Hensel, 1974).

The received results were compared to those published by other authors (Tabs. 2-4), with several studies made in recent years (Bănărescu, 1964; Holčík and Hensel, 1974; Specziár and Vida, 1995; Kottelat and Freyhof, 2007; Geiger and Schliewen, 2010; Stepien and Haponski, 2015; Tsyba and Kokodiy, 2017).

Table 1: Main morphological characters of the three ruffe species *Gymnocephalus baloni*, (n = 12 specimens) *Gymnocephalus cernua* (n = 21 specimens), and *Gymnocephalus schraetser* (n = 16 specimens) studied in the Lower Danube River basin (confidence intervals, $P < 0.05$; % of the standard length except the head depth, mouth length and eye diameter that are expressed as % of head length). Explanations of morphological characters are in the material and methods.

	Morphological character *	Value of morphological character (Confidence interval, in % of standard length, L, which is in mm unit)		
		Gymnocephalus baloni	*Gymnocephalus cernua*	*Gymnocephalus schraetser*
1.	SL	(82.4 – 101.8)	(94.3 – 100.5)	161.99 – 186.46
2.	BODYDEPTH	27.6 – 29.1	25.7 – 26.4	22.12 – 23.17
3.	CAUDPEDDEPTH	8.5 – 9.3	8.6 – 9.1	6.78 – 7.16
4.	PREDORSDIST	29.5 – 31.9	29.7 – 30.9	26.81 – 28.66
5.	CAUDPDLENGTH	19.8 – 21.8	21.1 – 22.4	18.94 – 20.82
6.	PREVENTDIST	32.1 – 34.1	33.6 – 34.8	29.08 – 30.79
7.	PREANALDIST	63.8 – 66.7	64.3 – 65.8	66.51 – 68.84
8.	PVDIST	3.5 – 4.5	2.8 – 3.6	3.50 – 4.73
9.	VADIST	31.1 – 34.9	30.6 – 31.8	36.48 – 39.21
10.	DLENGTH	54.1 – 56.8	53.0 – 54.7	55.83 – 57.97
11.	ALENGTH	14.4 – 17.0	13.9 – 15.2	12.06 – 13.60
12.	ADEPTH	19.2 – 23.2	19.5 – 20.8	17.53 – 19.77
13.	PLENGTH	21.4 – 24.0	22.2 – 23.6	17.76 – 18.66
14.	VLENTGTH	21.1 – 22.6	20.6 – 22.2	18.50 – 19.50
15.	HEADLENGTH	30.4 – 32.7	31.5 – 32.6	25.39 – 28.43
16.	HEADDEPTH	75.7 – 79.9	72.3 – 74.8	68.88 – 79.50
17.	MOUTHLENGTH	26.4 – 29.8	27.5 – 29.6	37.51 – 41.49
18.	EYEDIAMETER	29.2 – 31.9	27.3 – 29.2	24.46 – 28.73

Our results were consistent with some authors (Tab. 2), however showed differences in some of the morphological features when compared with other scientifical researches (the body depth, predorsal distance, caudal peduncle length, preventral distance, and preanal distance).

Table 2: Main morphological characters of *Gymnocephalus baloni* specified by various references (% of the standard length except for the head depth, mouth length and eye diameter). Explanations of morphological characters are in the material and methods.

Morphometric character	Hungarian (Budapest) area of the Danube River (Specziár and Vida, 1995)	Hungarian (Szigetköz) area of the Danube River (Specziár and Vida, 1995)	The Upper Diepr River, Ukraine (Tsyba and Kokodiy, 2017)	The Middle Diepr River, Ukraine (Tsyba and Kokodiy, 2017)	Holotype (Holčík and Hensel, 1974)	Paratypes (Holčík and Hensel, 1974)	Others (noted as ZICU by Holčík and Hensel, 1974)	Other material (Holčík and Hensel, 1974)	Holčík and Hensel, 1974	Lake Ammersee, Germany (Geiger and Schliewen, 2010)
STANDLENGTH	78.8	97.5	96.4	93.2	107.3	96.5	112.6	93.7	no data	101.7
BODYDEPTH	29.9	31.2	31.6	31.4	31.6	31.2	35.2	31.0	30.8	32.1
CAUDPEDDEPTH	8.1	8.2	8.4	8.3	8.4	8.7	10.4	8.7	8.6	8.9
PREDORSSDIST	32.4	31.4	37.2	37.1	35.3	33.8	40.0	34.4	34.1	35.4
CAUDPDLENGTH	21.2	20.2	21.0	16.8	18.6	19.7	22.4	19.9	19.8	17.9
PREVENTDIST	36.1	36.7	35.3	36.4	39.4	39.1	41.7	38.8	38.7	37.0
PREANALDIST	66.8	69.1	68.0	69.7	70.3	70.3	77.8	70.2	no data	no data
PVDIST	3.5	4.5	6.0	4.9	12.6	12.3	12.9	12.1	12.0	no data
VADIST	30.7	32.5	32.3	34.7	32.7	34.7	38.6	34.3	no data	no data
DLENGTH	50.6	52.4	56.75	56.9	58.2	56.4	64.8	56.7	56.3	no data
ALENGTH	11.9	10.7	14.8	14.8	13.9	14.4	18.0	14.5	14.4	14.6
ADEPTH	16.7	14.7	17.2	16.6	no data	no data	no data	no data	no data	no data
PLENGTH	21.0	20.4	22.1	21.1	20.1	19.4	23.8	19.8	no data	19.7
VLENGTH	22.4	21.4	21.8	22.9	22.3	21.4	24.8	21.9	no data	20.3
HEADLENGTH	33.0	31.5	31.8	32.2	25.6	30.1	35.1	30.5	no data	29.2
HEADDEPTH	64.2	73.0	80.2	76.1	105.1	85.7	82.1	84.9	no data	84.3
MOUTHLENGTH	no data	0.0	32.4	35.2	39.8	33.5	32.2	33.1	no data	35.5
EYEDIAMETER	28.8	28.3	31.4	29.9	33.6	28.6	28.8	29.2	no data	31.8

The body depth of *G. baloni* sampled from the Lower Danube River is smaller than the parameter measured on the holotype (Tab. 2) and smaller than other samples from the Danube River basin showing the specific phenotypic response to lotic habitat and unsteady swimming behaviour of fish. We can mention here the results of Foster et al. (2015), which highlight the general influence of the body shape differences by different habitat types and adaptive phenotypic features in the sympatric species to relative environmental gradients.

Table 3: Main morphological characters of *Gymnocephalus cernua* specified by various references (% of standard length except for the head depth, mouth length and eye diameter). Explanations of morphological characters are in the material and methods.

Morphometric character	G. cernua x G. baloni (Holčík and Hensel, 1974)	Holčík and Hensel, 1974	Syntype (Geiger and Schliewen, 2010)	Brackish Elbe River, Germany (Geiger and Schliewen, 2010)	The Elbe River, Germany (Geiger and Schliewen, 2010)	Lake Ammersee, Germany (Geiger and Schliewen, 2010)	Hungarian (Budapest) area of the Danube River (Specziár and Vida, 1995)	Baltic Sea (Geiger and Schliewen, 2010)	Hungarian (Szigetköz) area of the Danube River (Specziár and Vida, 1995)	Romanian Danube River basin (Bănărescu, 1964)	The Middle Diepr River, Ukraine (Tsyba, and Kokodiy, 2017)	Tge Upper Diepr River, Ukraine (Tsyba, and Kokodiy, 2017)
STANDLENGTH	no data	no data	101.1	100.3	80.5	98.9	91.9	101.3	84.9	145.0	103.8	93.3
BODYDEPTH	30.2	25.8	35.3	27.0	26.6	27.0	25.8	23.4	27.2	29.5	25.3	27.0
CAUDPEDDEPTH	8.9	8.2	10.1	9.2	9.4	8.8	8.0	7.5	8.2	8.4	7.7	7.8
PREDORSSDIST	34.8	32.7	36.6	38.3	36.2	35.1	30.8	34.2	31.8	34.0	35.2	35.2
CAUDPDLENGTH	20.9	21.6	17.9	20.1	21.2	20.2	22.9	23.3	23.1	21.0	20.1	24.6
PREVENTDIST	36.6	36.4	39.1	36.8	35.0	35.2	35.0	32.2	34.4	no data	33.9	33.3
PREANALDIST	no data	no data	no data	no data	no data	no data	67.2	no data	67.5	no data	67.2	65.3
PVDIST	11.5	9.7	no data	no data	no data	no data	3.2	no data	2.6	no data	4.3	5.9
VADIST	no data	no data	no data	no data	no data	no data	32.3	no data	33.2	no data	34.6	32.1
DLENGTH	5.7	53.8	no data	no data	no data	no data	50.8	no data	50.3	no data	53.5	53.2
ALENGTH	13.4	12.5	15.0	14.7	13.0	12.4	9.9	11.4	9.3	no data	12.3	11.9
ADEPTH	no data	no data	no data	no data	no data	no data	16.7	no data	15.7	no data	14.7	14.8
PLENGTH	no data	no data	21.1	23.6	20.6	20.1	20.5	18.5	22.2	22.0	20.4	20.8
VLENGTH	no data	no data	22.0	22.5	20.4	19.8	21.6	18.6	22.6	21.8	21.3	19.7
HEADLENGTH	no data	no data	30.6	33.2	31.3	29.5	31.8	28.7	31.7	31.3	30.9	30.5
HEADDEPTH	no data	no data	86.3	71.4	72.2	75.9	59.7	69.3	64.0	no data	66.6	71.0
MOUTHLENGTH	no data	no data	33.9	36.9	35.6	37.1	0.0	36.6	0.0	32.8	34.0	30.2
EYEDIAMETER	no data	no data	29.9	28.1	31.1	29.6	26.6	31.6	29.6	27.3	27.6	29.7

Table 4: Main morphological characters of *Gymnocephalus schraetser* from the Lower Danube River basin (Bănărescu, 1964). Explanations of morphological characters are in the material and methods.

Morphometric character	Value (% of standard length except the head depth, mouth length and eye diameter)
STANDLENGTH	170.0
BODYDEPTH	21.8
CAUDPEDDEPTH	6.8
PREDORSSDIST	32.8
CAUDPDLENGTH	20.5
PREVENTDIST	no data
PREANALDIST	no data
PVDIST	no data
VADIST	no data
DLENGTH	no data
ALENGTH	no data
ADEPTH	no data
PLENGTH	18.6
VLENGTH	19.8
HEADLENGTH	31.5
HEADDEPTH	no data
MOUTHLENGTH	46.0
EYEDIAMETER	22.7

In the same way, as an adaptive answer of fish morphology to water flow regime and other ecological parameters, we recorded smaller values in Danube ruffe body characters than the holotype description for some biometric characters such as predorsal distance, preventral distance, preanal distance. These differences were not recorded by comparing measurements of the fish population sampled from Hungarian Danube River section (Tab. 2) and also to populations in the upper and middle basin of the Dnieper/Nistru River (Tab. 2).

A suggestive image of the morphological variation and relationship among species from the genus *Gymnocephalus* sampled from the Lower Danube River (Mm80), compared with results from the measurements of fish from the various Eurasian rivers obtained by different authors (Tabs. 2-4) was assessed by a hierarchical clustering single linkage method. The dendrogram of figure 2 displays the output of the hierarchical clustering, which represent the trees that put in evidence, the similarity, and dissimilarity between the results.

The cluster based on our results reveales a more advanced degree of morphological similarity of fish with data referring to fish sampled from the upper and middle Dniepr/Nistru Basin (Tabs. 2-4) and the Hungarian stretch of Danube River (Tabs. 2-4). The distances between our results and the clusters of Danube ruffe holotype/paratype (Tab. 2) and widespread ruffe paratype (Tab. 3) are bigger (Fig. 2).

The dendrogram sustains the identified dissimilarity between *G. schraetser* (according to data described by Bănărescu, 1964 on 26 specimens from Danube, Timiş, Bega, Mureş rivers, Romania, Tab. 4) and the other two ruffe species (2.20 Euclidean distance).
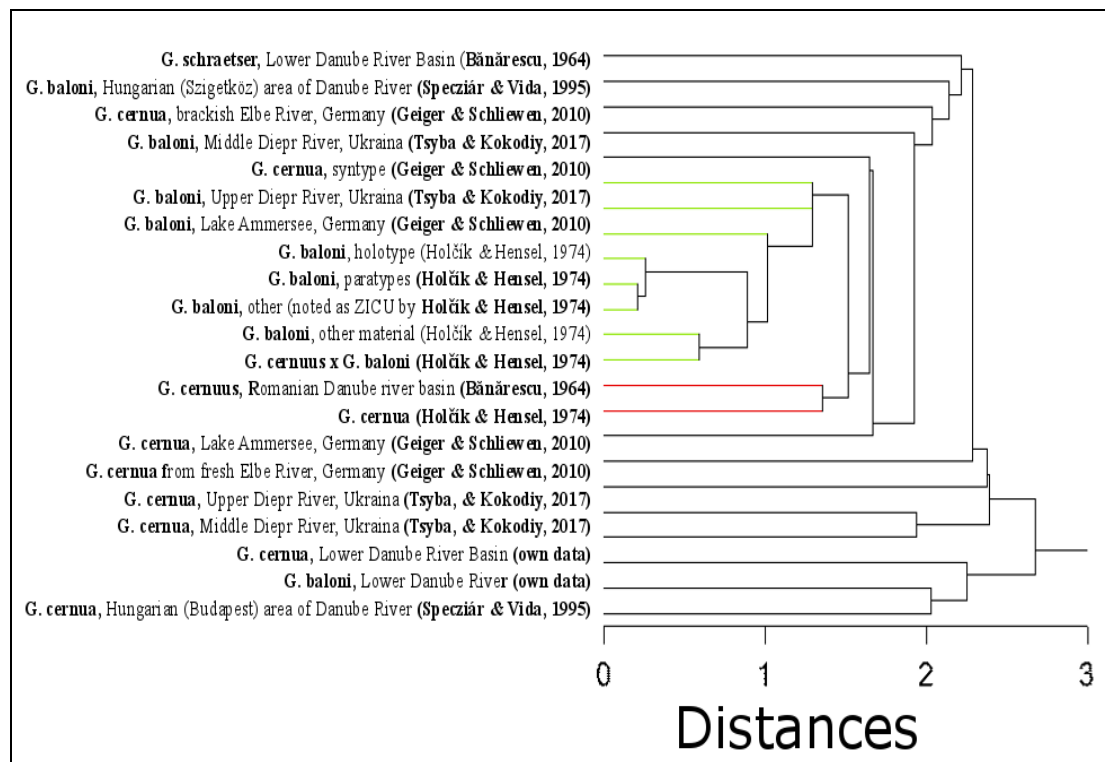
Figure 2: Dendrogram of the hierarchical clustering analysis based on morphometric data.
The algorithm used was the single linkage clustering (nearest neighbour)
with the Euclidean distance as a similarity measure.

Aco-variance matrix of the Procrustes coordinates of each sample is used to perform the PCA, and the first two components summarised 67.166%, which represent more than the half of total variance.

The scatter plot of PC scores from figure 3 displays the patterns in the relations between observations. PC1 separated *G. schraetser* (blue circle) from the other two studied species of interest while PC2 separated *G. baloni* (red circle) from *G. cernua* (green circle) (Fig. 3).
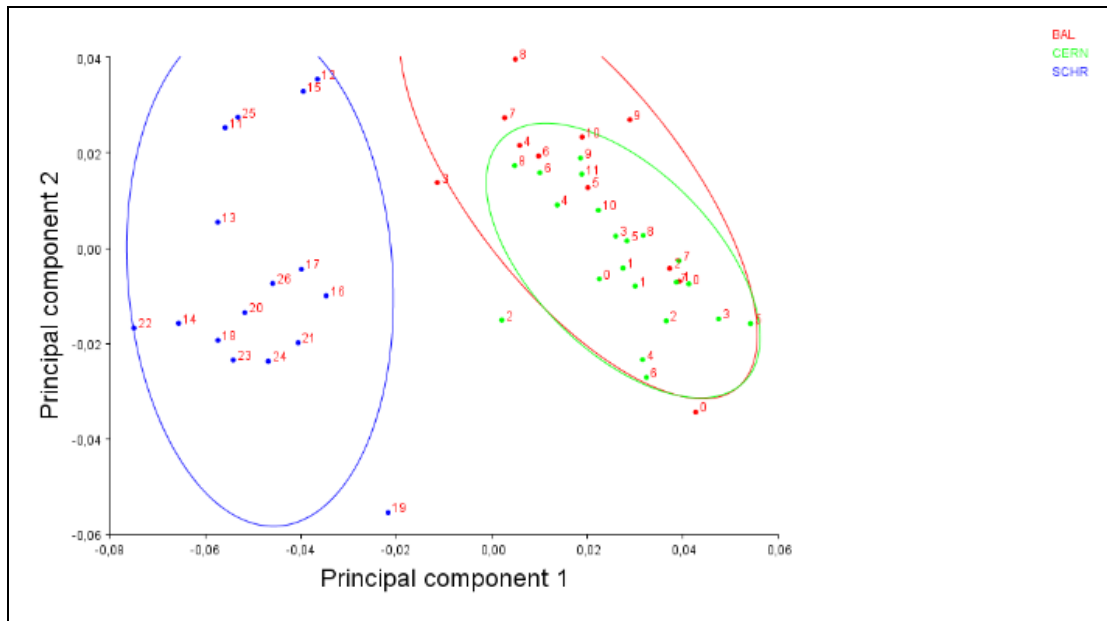
Figure 3: PC scores and the disposition of specimens in the morphospace of the multivariate analysis. The coordinates of PCA plot are grouped by equal frequency confidence ellipses (P = 0.9), which separate *G. schraetser* (blue circle) *G. baloni* (red circle), and *G. cernua* (green circle).

In order to have an optimised representation of the differences among groups, we performed a canonical variate analysis (CVA) that allows a different type of coordination analysis, which improves the separation of the specified groups (species/ecotypes; Klingenberg, 2011).

Figure 4 represents the shape changes associated with the canonical variate in the species from the genus *Gymnocephalus* in the Lower Danube. The scale factor for canonical variate (CV) shape changes is a unit of Mahalanobis distance of within-group shape changes (Klingenberg, 2011). We observed the variation in the pairs of fin insertions, eye diameter, head length and body depth, which is in connection with the elongation degree of body shape.
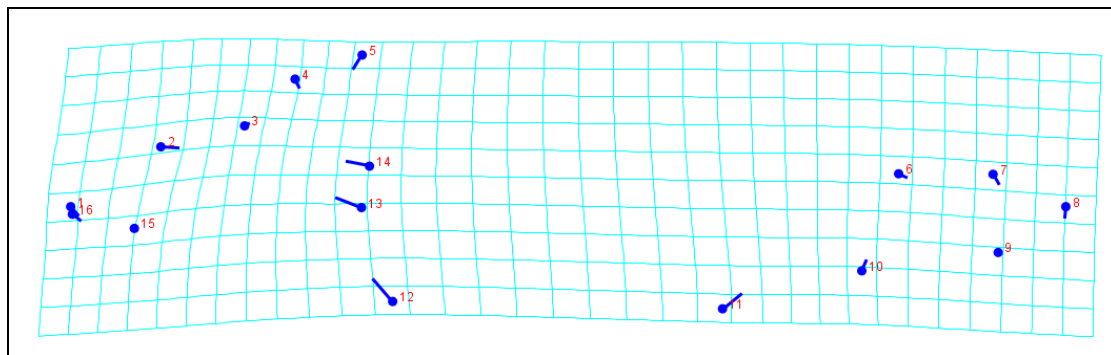

Figure 4: Transformation grid illustrating the shape variation associated with canonical variates (CV) in the ruffe species from the Lower Danube River. The scale for CV shape changes is in units of Mahalanobis distance.

The CVA generated the matrices of farthest Mahalanobis distances between the groups of *G. baloni* and *G. schraetser* (18.3364) and the smallest distances between *G. baloni* and *G. cernua* (8.7875). The P-values from permutation tests (10.000 permutation rounds) for Mahalanobis distances among the groups were statistically significant ($P < 0.05$).

The CVA chart represents the scatter plot of the canonical variate scores, displaying the species differentiation. The figure 5 allows the description of the relationship among the specimens of the groups by plotting the sample centroids of 90% confidence ellipses of the first two canonical variates CV1 and CV2.
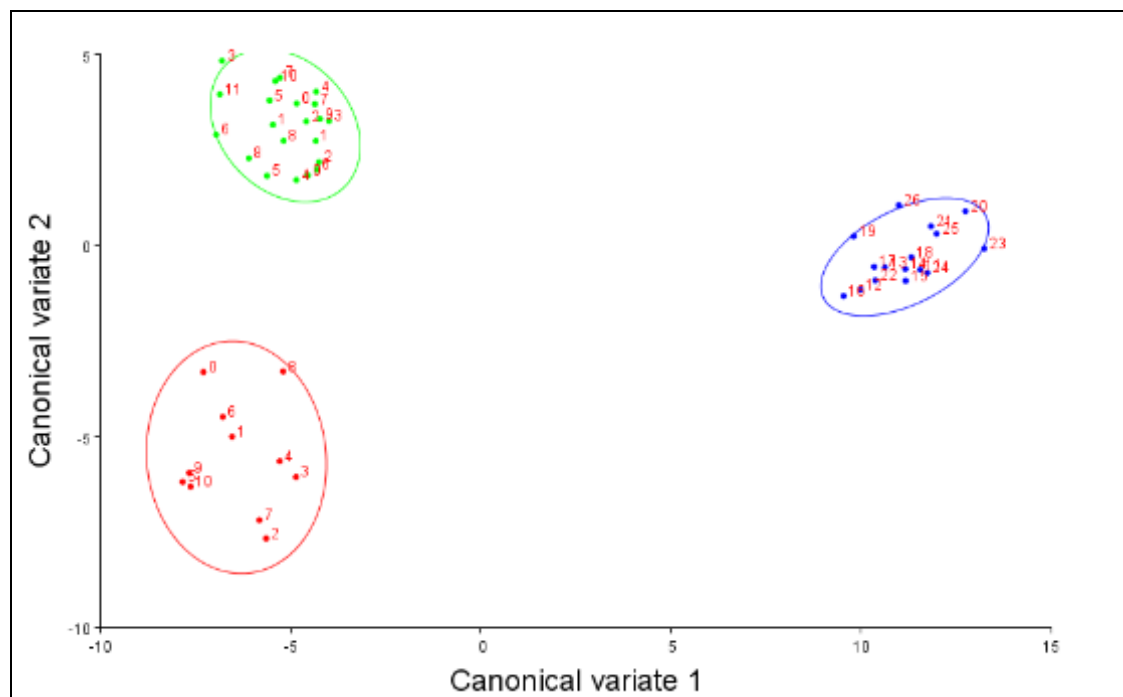

Figure 5: Transformation grid illustrating the shape variation
associated with canonical variates (CV) in the ruffe species from the Lower Danube River.
The scale for CV shape changes is in units of Mahalanobis distance.

The statistical analysis of morphometric data is for *G. baloni* and *G. cernua.* The objective is in order to point out the phenotypic characters for reliable discrimination between these two species.

The performed factor analysis reveals the first three principal components sum up 59.267%, which means that the first three factors explain more than a half of the variance of all body variables (Fig. 6). Based on the variables with high loadings, it may be seen that PC1 includes the significant, positive contribution of the dorsal fin length, eye diameter, head depth and the negative contribution of the head length, mouth length, preanal, predorsal, preventral and preorbital distances (Fig. 6a). In the same way, PC2 indicates the positive contribution of characters as the pectoral-ventral fin distance, ventral-anal fin distance, and preanal distance, and the negative contribution of the caudal peduncle length (Fig. 6b). PC3 only reveals the positive contribution of the body depth, ventral length, anal fin length, and anal fin depth.
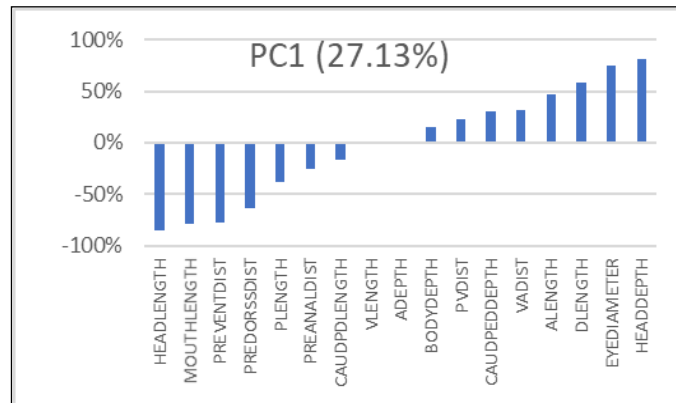
a)
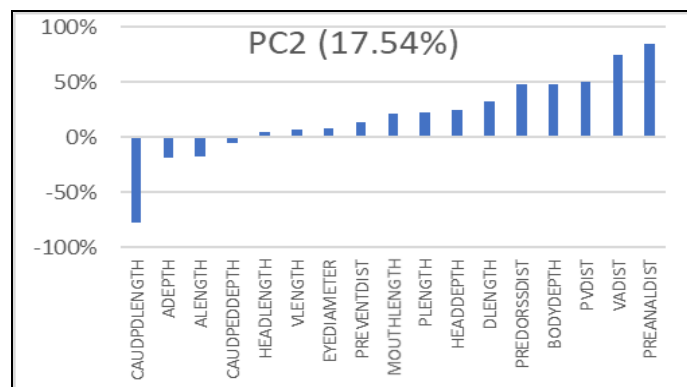
b)
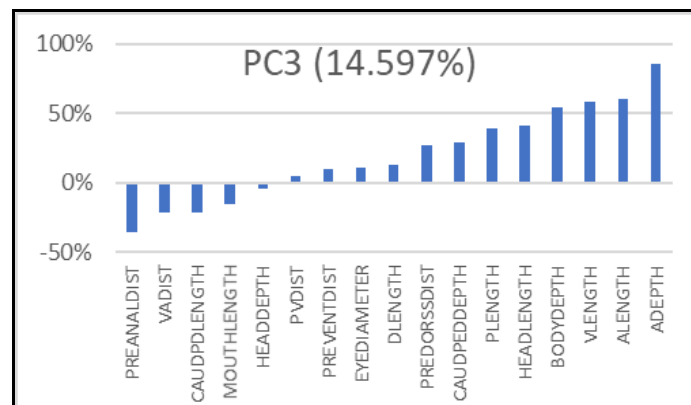
c)

Figure 6a-c: Variables loaded in the factor analysis of the morphometric measurement values
of *Gymnocephalus baloni* and *Gymnocephalus cernua* (n = 32);
the full names of variables are in material and methods.

We tested the differences between samples with T test. Table 5 notes the significant differences in the main characters of fish from the two groups (P < 0.05) as a character "S".

Table 5: Mean values of variables (%) in *Gymnocephalus baloni* and *Gymnocephalus cernua*. Explanations of morphological characters are in the material and methods. *S* – significant differences.

No.	Morphological character	*G. baloni* mean (%)	*G. cernua* mean (%)
1.	BODYDEPTH[S]	28.327	26.043
2.	CAUDPEDDEPTH	8.945	8.819
3.	PREDORSSDIST	30.691	30.305
4.	CAUDPDLENGTH	20.827	21.767
5.	PREVENTDIST	33.118	34.181
6.	PREANALDIST	65.282	65.076
7.	*PVDIST*[S]	4.000	3.205
8.	*VADIST*[S]	32.982	31.210
9.	*DLENGTH*[S]	55.473	53.833
10.	ALENGTH	15.664	14.538
11.	ADEPTH	21.191	20.171
12.	PLENGTH	22.673	22.914
13.	VLENGTH	21.845	21.362
14.	HEADLENGTH	31.555	32.048
15.	*HEADDEPTH*[S]	77.800	73.581
16.	MOUTHLENGTH	28.118	28.552
17.	*EYEDIAMETER*[S]	30.518	28.248

Having in view the similarity between *G. baloni* and *G. cernua*, a multivariate test for a morphometric comparison between the collected samples from the Danube River population (sea mile 80 from Black Sea) is used to complete the data regarding the morphological variation in the ruffe species and assess its plasticity.

A linear discriminant analysis using a backward stepwise method allows establishment of the most relevant morphometric features of *G. baloni* and *G. cernua* that together discriminate between the two samples of the fish species.

The between groups F-matrix and analysis of the disparities between the groups and all the variables (Engelman, 2005) reveals the correct classification of the ruffe samples with the farthest distance between *G. baloni* and *G. cernua* (6.754).

The reliability assessment of the discriminant analysis of morphometric characters of fish from the two species, computed by Wilks' lambda statistical index (Engelman, 2005) proved a significant discrimination with an advanced degree of confidence (Wilks' lambda = 0.1514, prob = 0.00008, P < 0.05).

The F-to-remove statistics (Engelman, 2005) from table 6 allows us to conclude that 10 of the morphological characters are the most useful together for discriminating among the species (the head features, body depth, the distances between pectoral, ventral and respectively anal fins, and the lengths of fin bases).

Table 6: F-to-remove statistics of the main morphometric characters assessed by the backward stepwise method for discrimination among the ruffe species: *Gymnocephalus baloni* and *Gymnocephalus cernua*. Explanations of morphological characters are in the material and methods.

Variable	F-to-remove	Tolerance
BODYDEPTH	22.69	0.200
PREDORSDIST	6.11	0.219
PREANALDIST	14.09	0.115
PVDIST	18.43	0.255
VADIST	8.27	0.332
ALENGTH	4.15	0.334
PLENGTH	5.56	0.356
VLENGTH	3.65	0.560
HEADLENGTH	6.09	0.057
HEADDEPTH	4.16	0.09

The classification matrix showed a high percentage of classification validation in all cases (100% correct classification).

Jackknife a form of cross-validation using a method of leaving out one case confirms the correct classification of the two sympatric ruffe species. The relative lower percent in the Jackknife panel (73% correct) could be attributed to the high number of predictors and small size of the ruffe samples.

The canonical scores plot generate the graphic with the confidence ellipse ($P < 0.05$) of each group, which is centred on the centroid of each group (Fig. 7). Based on the body measurement values, the groups are distinct.
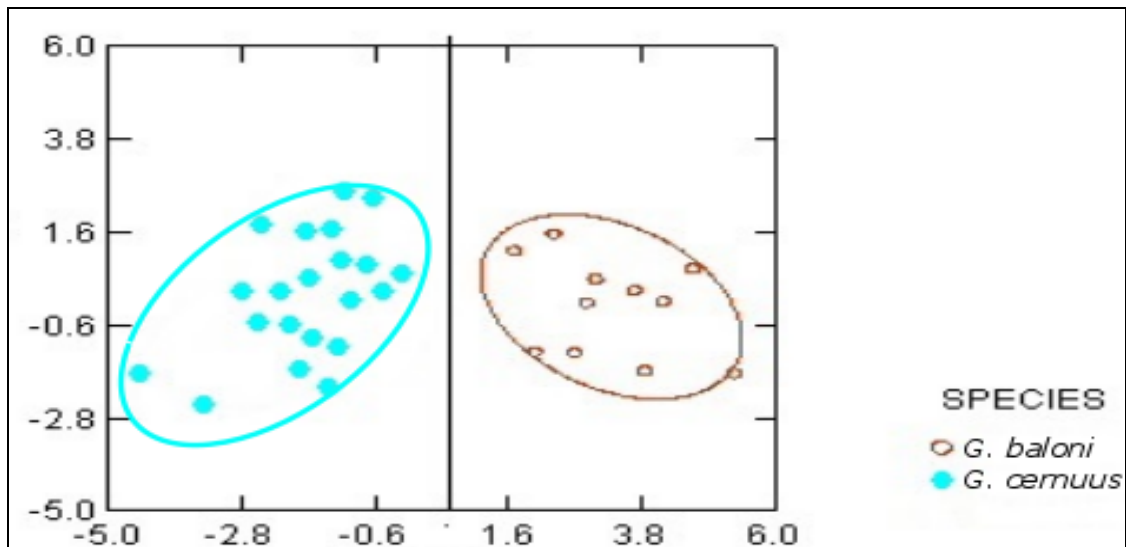

Figure 7: Diagram of canonical scores (95% confidence level) based on 18 morphological characters (Tab. 1) for *Gymnocephalus cernua* (blue circle) and *Gymnocephalus baloni* (brown circle) of the samples on which a discriminant analysis was applied (95% confidence ellipses are drawn around group centroids, and the variance proportions represent each canonical function as indicated).

CONCLUSIONS

The current paper completes the information regarding the body plasticity of the Danube ruffe (*Gymnocephalus baloni*) and its relationship with the other two species of the genus *Gymnocephalus* from the Lower Danube River.

We revealed a closer relationship between the population of *Gymnocephalus baloni* from the Lower Danube River and those from the middle stretch of the Danube River and the populations identified in rivers from the eastern part of Europe.

We found a closer phylogenetic relationship between the sister taxa *G. cernua* and *Gymnocephalus baloni* and a distance from *Gymnocephalus schraetser*, results that are consistent with some of previous studies.

Ten morphological characters are the most useful together for discriminating among the ruffe species from the Lower Danube River.

We also found a slightly less hydrodynamic body of *Gymnocephalus baloni* from the Lower Danube River comparing with the description of the holotype, which could be in connection with the phenotypic response of fish to environment.

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DISTRIBUTION REVISED- FIFTEEN YEARS OF CHANGES IN THE INVASION OF A FRESHWATER FISH, *PSEUDORASBORA PARVA* (TEMMINCK AND SCHLEGEL, 1846) IN TURKEY

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KEYWORDS: Non-native, topmouth gudgeon, introduction, distribution, Anatolia.

ABSTRACT

The aim of the present paper is to revise the distribution range of the invasive freshwater fish species, topmouth gudgeon *Pseudorasbora parva* (Temminck and Schlegel, 1846) in Turkey, based on the literature review. After the first report of the species from the Meriç River in Thrace (European part of Turkey) in 1982, it has spread across the country mainly through human-mediated accidental introductions. The species has now been reported from 66 water bodies in total. Besides the introduction pathways, we also present chronological distribution pattern of the species. This information is useful especially for conservation endemic ichthyofauna due to the negative impact of *Pseudorasbora parva*, particularly through introducing an emerging infectious eukaryotic intracellular pathogen on the fungal-animal boundary, *Sphaerothecum destruens* Arkush et al., 2003.

ZUSAMMENFASSUNG: Eine überprüfte Verbreitung- fünfzehn Jahre von Veränderungen betreffend die Invasion des Süßwasserfischs *Pseudorasbora parva* (Temminck und Schlegel, 1846) in der Türkei.

Ziel der vorliegenden Arbeit ist es, auf Grund der Durchsicht neuerer Fachliteratur das Verbreitungsgebiet des Süßwasserfischs *Pseudorasbora parva* (Temminck und Schlegel, 1846) in der Türkei zu revidieren. Nach einer ersten Meldung der Art im Meriç-Fluss in Thrakien (europäischer Teil der Türkei) im Jahr 1982 hat sich die Art im Land vorwiegend über zufälliges Einschleppen durch den Menschen verbreitet und wurde gegenwärtig insgesamt aus 66 Gewässern gemeldet. Es werden auch die Einschleppungswege und die chronologischen Verbreitungsmodelle der Art dargestellt. Diese Information wird als nützlich erachtet, vor allem im Hinblick auf den Erhalt der endemischen Fischfauna, wegen des negativen Einflusses von *Pseudorasbora parva* durch das Einschleppen eines eukaryotischen, intrazellularen ansteckenden Pathogens an der Pilz-Tier Grenze *Sphaerothecum destruens* Arkush et al., 2003.

REZUMAT: Distribuţie revizuită- cinsprezece ani de schimbări în invazia unui peşte de apă dulce, *Pseudorasbora parva* (Temminck şi Schlegel, 1846) în Turcia.

Scopul prezentei lucrări este revizuirea ariei de distribuţie a speciei invazive de apă dulce, *Pseudorasbora parva* (Temminck şi Schlegel, 1846) în Turcia, pe baza revizuirii literaturii. După o primă raportare a speciei în râul Meriç din Tracia (partea europeană a Turciei) în 1982, sa răspândit în ţară în principal datorită introducerilor antropice accidentale şi a fost raportată dintr-un număr total de 66 de bazine de apă. Sunt de asemenea prezentate căi de introducere şi modele cronologice de distribuţie a specie. Această informaţie se crede că este utilă în special pentru conservarea ihtiofaunei endemice. Efectul negativ a murgoiului bălţat, este reprezentat în particular prin introducerea unui patogen eucariot intracelular infecţios emergent la graniţa fungi-animale, *Sphaerothecum destruens* Arkush et al., 2003.

INTRODUCTION

In general, freshwater ecosystems and its endemic specific fauna are adversely affected by introduction of non-native species, pollution and drought (Marr et al., 2013). Non-native fish species are usually introduced into freshwater ecosystems through transportation, aquaculture and biological control along with fish stocking, fisheries and pet trade (Allan and Flecker, 1993; Maitland, 1995; Ruesink, 2005; Özcan, 2008; Tarkan et al., 2015). In the case of being invasive, non-native fish species have great potential to impact native biota both economically and ecologically (Cucherousset and Olden, 2011).

Turkey has a rich freshwater fish biodiversity with high number of endemic species (Tarkan et al., 2015). In total, 377 species have been reported so far; the majority belongs to the Cyprinidae family (Çiçek et al., 2015). In Turkey, there are approximately 157 endemics (Çiçek et al., 2015) and 30 introduced freshwater fish species being overall establishment success over 60% (Tarkan et al., 2015). One successfully non-indigenous fish species is *Pseudorasbora parva* (Temminck and Schlegel, 1846), which is an invasive species of the European fish fauna that influence native and endemic fishes by generating damages (Curtean-Bănăduc and Bănăduc, 2007-2008; DIAS, 2017; GISD, 2017; NISD, 2017) including in pond fish farming as a strong competitor (Csorbai et al., 2014) and healthy carrier of a deadly pathogen *Sphaerothecum destruens* Arkush et al., 2003 (Gozlan et al., 2005).

Pseudorasbora parva was originally discovered in Nagasaki, Japan and they are also naturally distributed in China and Siberia (Berg, 1949). This species was recorded in Europe in the 1960s; they were possibly introduced through the movement of Chinese carps for fish farming (Gozlan et al., 2010) and the species was reported in Turkey for the first time in 1982 from Meriç River (Thrace – European part of Turkey) (Erk'akan, 1984), then from Aksu River (Anatolia – Asian part of Turkey) in 1994 (Wildekamp et al., 1997). Ekmekçi and Kırankaya (2006) reviewed the distribution of the species in Turkey and reported seven new locations for the species. Since then, several new locations have been recorded for the species except for Eastern part of the country (e.g. Uğurlu and Polat, 2007; İlhan and Balık, 2008; Şaşı and Berber, 2013; Çınar et al., 2013; Bakaç et al., 2017; Gül et al., 2017; Karakuş et al., 2017; Küçük et al., 2018). Since distribution pattern of *Pseudorasbora parva* has not properly been reviewed despite its presence in Turkish inlands for almost four decades and available information on the distribution range is obsolete, we aimed to present current distribution and introduction pathways of the species in Turkey.

MATERIAL AND METHODS

A literature review was performed in Web of Science, and supplemented by Google Scholar, starting with the species name ("*Pseudorasbora parva*" and "topmouth gudgeon") and country of interest ("Turkey") as well as complimentary words ("new", "record", "locality", "occurrence", "distribution", "spread", "range" and their combinations). Also, available journal archives, reports and grey literature were reviewed. All location reports were tabulated and visualized with a table and figure, respectively by enumerating them chronologically. The introduction rate of *Pseudorasbora parva* was calculated as the average number of new locations introduced per decade after Ribeiro et al. (2009).

RESULTS AND DISCUSSION

For Turkish freshwater bodies, *Pseudorasbora parva* is considered one of the most ecologically damaging species that is rapidly spreading and become established (Ercan et al., 2015; Tarkan et al., 2015). Our near-comprehensive literature screening presented that *Pseudorasbora parva* is widely distributed in Turkey, particularly in the regions located in western part of the studied country; Aegean, Mediterranean and Marmara regions (Fig. 1).

The average rate of introduction to Turkey of *Pseudorasbora parva* since the first introduction of the species in early 1980s up to the date is 1.7 per decade. For the period 1990-2020 and 2000-2020 it is 2.2 and 2.9 species per decade (Fig. 2). Although this rate is now almost the same (2.9 vs. 2.8) for the last two decades (i.e. 2000-2010 and 2010-2020), it is likely that the latter would be higher with potential new location reports in next two years until 2020 (Fig. 2). This considerable increase could apparently be attributable to lack of ichthyofaunal surveys and the species' misidentification especially before 2000s where the former is still true for the eastern part of the country (Fig. 1). Indeed, there is a relatively long time (i.e. 12 years) and distance (approximately 900 km) between first (Erk'akan, 1984) and second record (Küçük and İkiz, 2004) of the species but after 2000s, new reports of the species has remarkably increased, mostly from the regions located between first two reported locations (Tab. 1; Fig. 1). The fourth report of the species was in late 1990s from a basin (Sakarya Basin), which is located in between those locations (Ekmekçi, 2000).

Based on the pattern of *Pseudorasbora parva* introductions since the first report in Turkey, it is likely that the actual distribution of the species in its introduced range reflects a "stepping-stone" type of invasion model, which is characterized as "further introduction from initial one" (Gozlan et al., 2010). This is because the major vectors for the introduction of *Pseudorasbora parva* to Turkey have been indicated as government authorized aquaculture and stocking programmes to establish and support cage aquaculture, and commercial fisheries (Tarkan et al., 2015). Hence, the accidental transfer and release of *Pseudorasbora parva* within the translocations of native cyprinids (mainly common carp *Cyprinus carpio*) for aquaculture characterize the primary pathway of *Pseudorasbora parva* introduction into its expanded range in Turkey. Our review of location reports of the species supports this suggestion as it has been reported from distant locations in different times, i.e. does not follow a regular distribution pattern (Tab. 1; Fig. 1). Indeed, second record of *Pseudorasbora parva* in Anatolia after its first report of in Thrace strongly suggests that the expansion of the species by natural ways is not likely, as this freshwater gudgeon species cannot pass two salt water barriers (i.e. İstanbul and Çanakkale straits), which separates European and Asian parts of Turkey. Recent findings of the species in a remote location (i.e. Gökçeada Island) have strengthened this contention (Bakaç et al., 2017).
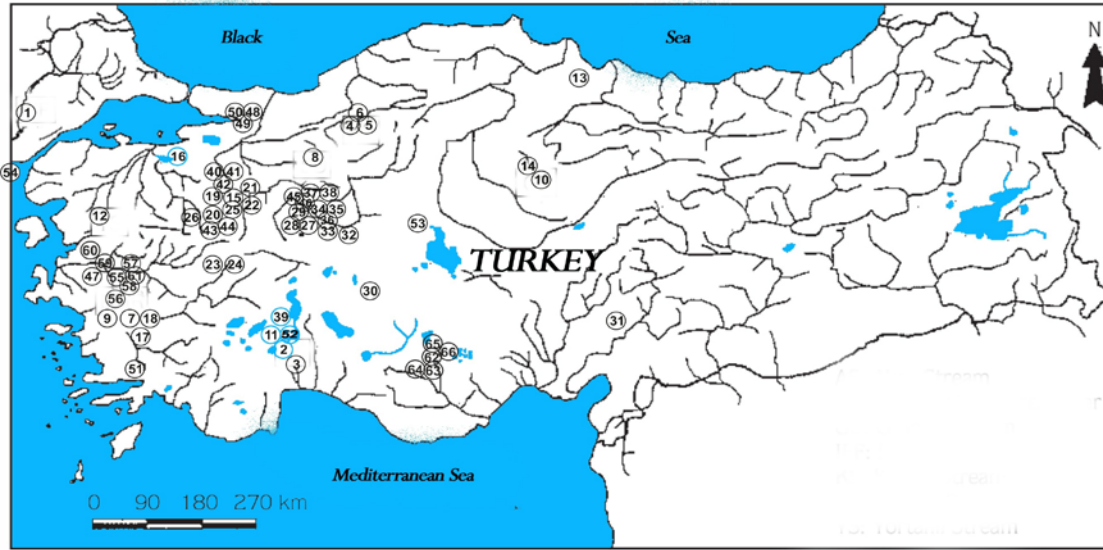
Figure 1: Updated distribution map of *Pseudorasbora parva* in Turkish inlands by location:
1. Meriç River; 2. Karacaören I-II reservoirs; 3. Aksu River; 4. Kirmir Stream; 5. Kirmir Stream; 6. Kirmir Stream; 7. Topçam Reservoir; 8. Sarıyar Reservoir; 9. Dipsiz-Çine Stream; 10. Gelingülü Reservoir 11. Gölcük Lake 12. Yortanlı Stream 13. Bekdiğin Pond
14. Gelingülü Reservoir; 15. Ağaçköy Stream, Felek Stream and Enne Reservoir; 16. Uluabat Lake; 17. Kemer Reservoir; 18. Topçam Reservoir; 19. Sekiören Pond; 20. Pazarlar Pond; 21. Doğluşah Pond; 22. Söğüt Reservoir; 23. Altınbaş Pond; 24. Göğem Pond; 25. Enne Reservoir; 26. Kayaboğazı Reservoir; 27. Çatören Reservoir; 28. Kunduzlar Reservoir; 29. Erenköy Pond; 30. Meyil Lake; 31. Evri Stream; 32. Günyüzü II Pond; 33. Koçaş Pond; 34. Mercan Pond; 35. Bahçeçik Pond; 36. Kaymaz Reservoir; 37. Yenice Reservoir; 38. Gökçekaya Reservoir; 39. Eğirdir Lake; 40. Küçükelmalı Pond; 41. Kızıldamlar Reservoir; 42. Dodurga Reservoir; 43. Dumlupınar Pond; 44. Çavdarhisar Pond; 45. Porsuk River; 46. Akın Creek; 47. Marmara Lake; 48. Bayraktar Reservoir; 49. Kirazoğlu Reservoir; 50. Davuldere Reservoir; 51. Ula Reservoir; 52. Onaç Reservoir; 53. Mogan Lake; 54. Gökçeada Reservoir; 55. Demirköprü Reservoir; 56. Afşar Reservoir; 57. Gördes Stream; 58. Marmara Lake; 59. Gediz River; 60. Demirci Stream; 61. Kemerdamları Drainage Channel; 62. Gödet Creek; 63. Gödet Reservoir; 64. Deliçay Reservoir; 65. İbrala Reservoir; 66. Ayrancı Reservoir; for details see table 1.

According to Hanel et al. (2011), *Pseudorasbora parva* can establish abundant populations in stagnant bodies of water compared to running waters. This is in line with our review showing that out of 66 water bodies, 50 were stagnant water (lake, reservoir and pond) and only 16 were running water (creek, stream and river) (Tab. 1). Also, reviewed resources confirmed that it become established more abundantly in still waters. High phenotypic plasticity in fitness related traits such as growth, early maturity, fecundity, reproductive behaviour (paternal care) and the ability to cope with novel pathogens has predisposed the *Pseudorasbora parva* to be a strong invader (Gozlan et al., 2010). Recently, *Pseudorasbora parva* in Turkey has been shown to grow better than both native and non-native populations (Akbaş et al., 2015). Further, *Pseudorasbora parva* was considered as a serious threat to native and endemic fish species in several water bodies of Turkey that have high biodiversity richness (Ekmekçi and Kırankaya, 2006; Özcan, 2008; Polat et al., 2011).

Notably, the most compelling issue regarding the invasion of *Pseudorasbora parva* is that it is a healthy carrier of a rosettte agent *Sphareothecum destruens*, a generalist pathogen on the animal-fungal boundary (Gozlan et al., 2005). After the experimental evidence of the pathogen in *Pseudorasbora parva* (Gozlan et al., 2005), first field evidence was provided from Turkey (Sarıçay River, Milas, SE Turkey) (Ercan et al., 2015), which also proved its introduction via *Pseudorasbora parva* to sea bass farms and linking the pathogen to severe declines in threatened European endemic freshwater fishes (i.e. 80% to 90% mortalities).

Table 1: Chronological list of reported locations of *Pseudorasbora parva* in Turkish inlands.

No.	References	Location	Report Date
1.	Erk'akan, 1984	Meric River-Üpsala/ Edirne	16 September 1982
2.	Küçük and İkiz, 2004	Karacaören I-II Reservoirs/Aksu River/Isparta-Burdur	November 1994-October 1996/September 2002-August 2003
3.	Wildekamp et al., 1997	Aksu River/ Antalya	29-30 July 1996
4.	Ekmekçi, 2000	Sakarya Kirmir Stream	1999
5.	Ekmekçi, 2000	Kirmir Stream	1998
6.	Ekmekçi and Kırankaya, 2006	Kirmir Stream	January 1998
7.	Şaşı and Balık, 2003	Topçam Reservoir/ Aydın	June 1999-June 2000
8.	Ekmekçi and Kırankaya, 2006	Sarıyar Reservoir	November 1999
9.	Barlas and Dirican, 2004	Dipsiz-Çine Stream/ Aydın	November 1999 and February 2001
10.	Ekmekçi and Kırankaya, 2006	Gelingülü Reservior, Kızılırmak/Yozgat	June/2002-December 2004
11.	Yeğen et al., 2015	Gölçük Lake/ Isparta	17 May 2003
12.	Ekmekçi and Kırankaya, 2006	Yortanlı Stream/Bakırçay/ İzmir	–
13.	Uğurlu and Polat, 2007	Bekdiğin Pond/ Samsun	June 2003-September 2005
14.	Yalçın-Özdilek et al., 2013	Gelingüllü Reservoir/ Yozgat	Summer 2003 and Summer 2005
15.	İlhan and Balık, 2008	Ağaçköy Stream – Felek Stream, Enne Reservoir/Kütahya	Summer 2004 and 2005
16.	Çınar et al., 2013	Uluabat Lake (Apolyont)/Bursa	January 2006 and December 2006

Table 1 (continued): Chronological list of reported locations of *Pseudorasbora parva* in Turkish inlands.

17.	Şaşı and Berber, 2013	Kemer Reservoir/ Aydın	April 2007 to February 2008
18.	Şaşı and Berber, 2013	Topçam Reservoir/ Aydın	April 2007 to February 2008
19.	Yeğen et al., 2015	Sekiören Pond/ Kütahya	03 May 2007
20.	Yeğen et al., 2015	Pazarlar Pond/ Kütahya	22 May 2007
21.	Yeğen et al., 2015	Doğluşah Pond/ Kütahya	24 May 2007
22.	Yeğen et al., 2015	Söğüt Reservoir/ Kütahya	24 May 2007
23.	Yeğen et al., 2015	Altınbaş Pond/ Uşak	14 June 2007
24.	Yeğen et al., 2015	Göğem Pond/ Uşak	14 June 2007
25.	Yeğen et al., 2015	Enne Reservoir/ Kütahya	28 August 2007
26.	Yeğen et al., 2015	Kayaboğazı Reservoir/ Kütahya	30 August 2007
27.	Yeğen et al., 2015	Çatören Reservoir/ Eskişehir	24 October 2008
28.	Yeğen et al., 2015	Kunduzlar Reservoir/ Eskişehir	24 October 2008
29.	Yeğen et al., 2015	Erenköy Pond/ Eskişehir	01 October 2008
30.	Özuluğ et al., 2013	Meyil Lake (Konya)	2008
31.	Özuluğ et al., 2013	Evri Stream (Kahramanmaraş)	2008
32.	Yeğen et al., 2015	Günyüzü II Pond/ Eskişehir	28 April 2009
33.	Yeğen et al., 2015	Koçaş II Pond/ Eskişehir	28 April 2009
34.	Yeğen et al., 2015	Mercan Pond/ Eskişehir	29 April 2009
35.	Yeğen et al., 2015	Bahçeçik Pond/ Eskişehir	29 April 2009
36.	Yeğen et al., 2015	Kaymaz Reservoir/ Eskişehir	30 April 2009
37.	Yeğen et al., 2015	Yenice Reservoir/ Eskişehir	26 May 2009

Table 1 (continued): Chronological list of reported locations of *Pseudorasbora parva* in Turkish inlands.

38.	Yeğen et al., 2015	Gökçekaya Reservoir/ Eskişehir	27 May 2009
39.	Yağcı et al., 2014	Eğirdir Lake/ Isparta	March 2010 and June 2011
40.	Yeğen et al., 2015	Küçükelmalı Pond/ Bilecik	06 May 2010
41.	Yeğen et al., 2015	Kızıldamlar Reservoir/ Bilecik	06 July 2010
42.	Yeğen et al., 2015	Dodurga Reservoir/ Bilecik	18 August 2010
43.	Yeğen et al., 2015	Dumlupınar Pond/ Kütahya	19 Auguıst 2010
44.	Yeğen et al., 2015	Çavdarhisar Reservoir/ Kütahya	19 August 2010
45.	Yeğen et al., 2015	Porsuk River/ Eskişehir	16 August 2011
46.	Yeğen et al., 2015	Akın Creek/ Eskişehir	13 September 2011
47.	İlhan and Sarı, 2013	Marmara Lake/ Manisa	March 2012-February 2013
48.	Keskin et al., 2013	Bayraktar Reservoir/ -İzmit	2012
49.	Keskin et al., 2013	Kirazoglu Reservoir/ -İzmit	2012
50.	Keskin et al., 2013	Davuldere Reservoir/ -İzmit	2012
51.	Keskin et al., 2013	Ula Reservoir/ -Muğla	2012
52.	Yeğen et al., 2015	Onaç Reservoir/ Burdur	02 October 2013
53.	Gül et al., 2017	Mogan Lake/ Ankara	January-December 2014
54.	Bakaç et al., 2017	Gökçeada Reservoir/ Çanakkale	08 June 2016
55.	Karakuş et al., 2017	Demirköprü Reservoir/Manisa	March-August 2017
56.	Karakuş et al., 2017	Afşar Reservoir/ Manisa	March-August 2017
57.	Karakuş et al., 2017	Gördes Stream/ Manisa	March-August 2017

Table 1 (continued): Chronological list of reported locations of *Pseudorasbora parva* in Turkish inlands.

58.	Karakuş et al., 2017	Marmara Lake/ Manisa	March-August 2017
59.	Karakuş et al., 2017	Gediz River/ Manisa	March-August 2017
60.	Karakuş et al., 2017	Demirci Stream/ Manisa	March-August 2017
61.	Karakuş et al., 2017	Kemerdamları Drainage Channel/Manisa	March-August 2017
62.	Küçük et al., 2018	Gödet Creek/ Karaman	2015-2017
63.	Küçük et al., 2018	Gödet Reservoir/ Karaman	2015-2017
64.	Küçük et al., 2018	Deliçay Reservoir/ Karaman	2015-2017
65.	Küçük et al., 2018	İbrala Reservoir/ Karaman	2015-2017
66.	Küçük et al., 2018	Ayrancı Reservoir/ Karaman	2015-2017
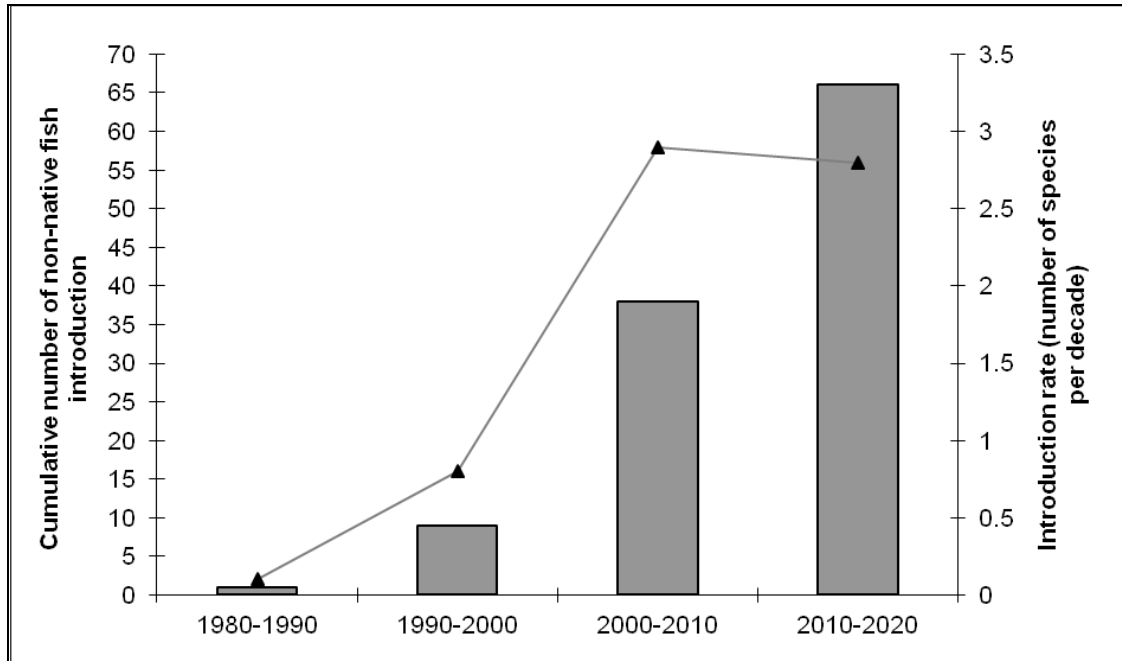
Figure 2: Temporal pattern of introduction of *Pseudorasbora parva* into Turkish watersheds. Cumulative number of the species introduction (left axis) and the rate of the species introduction per decade (right axis) is presented.

CONCLUSIONS

Overall, our near comprehensive review points out rapid and wide spread distribution of *Pseudorasbora parva* in Turkey and suggests that it would increasingly continue to spread in the future. It is most probable that the number of new records of the species in Turkey will increase with more frequent ichthyofaunal researches especially in the eastern part of Turkey.

To this end, *Pseudorasbora parva* introductions and related infectious diseases should constantly be monitored and necessary management actions with robust risk assessments should follow given rich native and endemic freshwater biodiversity in Turkey.

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MANAGEMENT ELEMENTS FOR TWO ALBURNINAE SPECIES, *ALBURNUS ALBURNUS* (LINNAEUS, 1758) AND *ALBURNOIDES BIPUNCTATUS* (BLOCH, 1782) BASED ON A DECISION-SUPPORT SYSTEM STUDY CASE

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KEYWORDS: Bleak, Schneider, fish habitat needs, human activities negative effects, conservation management elements.

ABSTRACT

ADONIS:CE has been used as a base to create a support-system management decision-making model for *Alburnus alburnus* (Linnaeus, 1758) and *Alburnoides bipunctatus* (Bloch, 1782) species. Investigation of the habitat necessities and the identification of the necessary elements for a good status of conservation of these two fish species populations has revealed the pressures and threats to these congener species, for which specific management activities have been finally recommended.

ZUSAMMENFASSUNG: Management Elemente für zwei Alburninae Arten, *Alburnus alburnus* (Linnaeus, 1758) und *Alburnoides bipunctatus* (Bloch, 1782) anhand einer Fallstudie als Grundlage für ein Management-Entscheidungssystem.

ADONIS:CE wurde für die Entwicklung eines Modells verwendet, das als Grundlage für ein Entscheidungssystems in Managementfragen für die Arten *Alburnus alburnus* (Linnaeus, 1758) und *Alburnoides bipunctatus* (Bloch, 1782) dienen soll. Untersuchungen betreffend Habitatansprüche und Feststellung der notwendigen Elemente für einen guten Erhaltungszustand der Populationen dieser Fischarten ließen deutlich die Gefährdungen der beiden verwandten Arten erkennen, für die ein entsprechendes Management vorgeschlagen wird.

REZUMAT: Elemente de management pentru două specii de Alburninae, *Alburnus alburnus* (Linnaeus, 1758) şi *Alburnoides bipunctatus* (Bloch, 1782) bazate pe un studiu de caz al unui sistem de suport decizional.

ADONIS:CE a fost utilizat pentru a crea un model de sistem-suport pentru luarea deciziilor de management pentru speciile *Alburnus alburnus* (Linnaeus, 1758) şi *Alburnoides bipunctatus* (Bloch, 1782). Investigarea necesităţilor de habitat şi a elementelor necesare pentru un statut bun de conservare al populaţiilor acestor două specii, au relevat presiunile şi ameninţările asupra acestor specii congenere pentru care au fost recomandate în final măsuri specifice de management.

INTRODUCTION

Regardless of variation in need and reserve induced by the dynamic of fishing activities results, fish remain a significant source of food in many regions (*, 2002). Fish population management systems, to assure the protein and also the game needs of the humans, need to be complex, innovative, and highly addaptative to the local/regional habitat, biotic and human-related conditions (Cochrane, 1999). The increasing desire for more fish protein is evident everywhere, and this threat demands a focused, creative struggle in identifying practical answers for conservative and economic issues (Agnew et al., 2009; Monte-Luna et al., 2016).

Usually only the conservation and high-direct, economic valuable fish species benefit from adapted management plans, and very rarely the indirect economic valuable fish species, which consist the trophic base for upper trophic level fish species (Bănăduc et al., 2011).

Too often, many different fish species, belonging to a certain overspecific taxonomic group with different economic and/or conservation value, are confused by the local fisherman (Oţel, 2007), situations in which a proper conservation is hard to implement and sustain. In some such cases, specific on site and on species adapted management systems can offer integrated management elements, which is the goal of this Târnava Mare River study case.

The Alburninae subfamily (Actinopterygii, Cypriniformes, Cyprinidae) include over eighteen species (Fish Base, 2018) including *Alburnus alburnus* (Linnaeus, 1758) and *Alburnoides bipunctatus* (Bloch, 1782). A relatively common fish species in the Târnava Mare River, with relative, similar morphological and colour aspects, these two fish species, especially in young age classes (Bănărescu, 1964, 2005), can be difficult to identify, creating problems in their populations' assessment, monitoring, and management.

The Târnava Mare Basin is a well known area under constant and variable human impact and effects on local biota (Cupşa, 2005; Sîrbu, 2005; Momeu and Péterfi, 2005; Robert and Curtean-Bănăduc, 2005), including species of fish fauna containing *Alburnus alburnus* and *Alburnoides bipunctatus* (Bănăduc, 2005; Curtean-Bănăduc, 2005; Păpuc et al., 2017).

There is no general accepted "golden rule" in Carpathian streams and rivers fish populations' optimum management, but it is obvious that one feature is usually correlated with beneficial consequences, namely science-based adequate management, the goal of this study relaying on this specific approach.

In nature conservation, modeling is frequently used to obtain the "large picture" of various systems and/or actions of peculiar domains. The pieces of the modeling process are practical in discriminating the specific phases of adaptive species and their environment management. Using ADONIS:CE, we can construct models that support management objectives. This type of model targets three operational sectors important for environment conservation: 1) to determine the present state, 2) to assess the effects of modifications and 3) to suggest actions to improve the actual state in a desired way. Convincingly, diversified diagrams can be developed to highlight management elements (Hall and Harmon, 2005).

MATERIAL AND METHODS

The researched area, the Târnava River watershed (Fig. 1) is located in the central area of the Romanian Carpathians arch, running off the Transylvania Depression, in precisely its southern sector of the Târnavelor Plateau. With a watershed of 6,157 km^2, a length of 249 km and a falling elevation of about 1,250 m, the Târnava River is one of the main tributaries of the Mureş River, delineating 21% of its watershed. It is composed of the confluence of Târnava Mare River (3,606 km^2 watershed surface; 221 km length) and Târnava Mică River (2,049 km^2 watershed surface and 191 km length) near Blaj locality. (Tufescu, 1966; Posea et al., 1983)
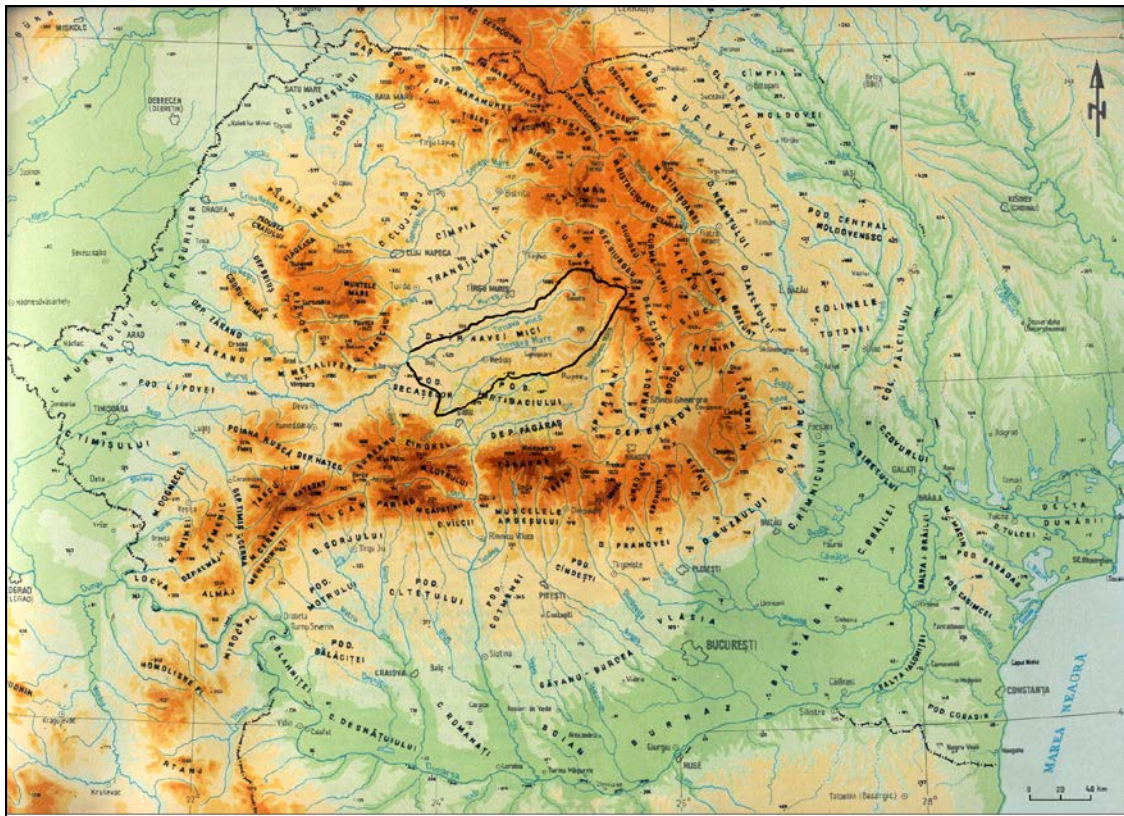
Figure 1: The Târnava River basin location (Bănăduc, 2005).

Alburnus alburnus and *Alburnoides bipunctatus* are a well known species in Romania (Bănărescu, 1964) with a relatively large distribution in Transylvanian medium and large rivers (Bacalu, 1997; Bănăduc, 1999; Bănărescu et al., 1999; Bănărescu, 2005; Curtean-Bănăduc and Bănăduc, 2007; Telcean and Cupşa, 2009; Bănăduc et al., 2013, 2014; Bănăduc and Curtean-Bănăduc, 2014; Telcean et al., 2014; Cocan et al., 2015; Păpuc et al., 2017; Stavrescu-Bedivan et al., 2017; Voicu et al., 2016, 2017) where the studied area is located.

These fish species individuals were found for this research and analyzed in Târnava River in 2016; all of them were immediately released alive after an in situ identification, in their natural habitat.

Supplementary reference data for these fish species' presence and ecological status were based on a similar approach study of Bănăduc (2005) and on the local fisherman's captures.

The researched habitat characteristics of the fish populations were evaluated based on specific selected criteria including: population size, size of range, the balanced allocation of fish in age classes, and high/low number of individual fish species individuals in fish communities.

The local lotic habitat necessities, pressures, and threats on the two fish species were studied in connection with their populations' ecological status, the correlations between them and the conservation situation of these species.

An in situ-on species adaptable management model was projected to build up a suitable management plan that would protect the researched fish species that are living in the studied lotic sectors, with a priority on required processes.

The ADONIS:Community Edition (ADONIS:CE), made-up by the Business Object Consulting (BOC) Group, was applied here. This software is a freely accessible form of ADONIS with few restraints (in comparison with the commercial version). It uses a Business Process Model and Notation (BPMN), a standardized modeling language that supports detectable processes. ADONIS:CE is typically used as an access point to Business Process Management. These processes can be modeled using compatible notation.

RESULTS AND DISCUSSION

The study results reveal that the main common threats on *Alburnus alburnus* and *Alburnoides bipunctatus* fish species are: modifying and fragmentation of specific habitats, water pollution, and overfishing and poaching.

Identified specific requirements

Both the juveniles and adults need a significant/close to natural water flow and relatively high depths of the water (minimum 0.5 m), with rocky-sandy substrata, variable speed of the water flow, and not abundant aquatic vegetation.

Proposed specific habitat indicators

In the studied lotic sectors, principal habitat indicators as causes for the presence/absence and abundance of *Alburnus alburnus* and *Alburnoides bipunctatus* are: average water flowing surface speed (proportion 50% of the river), relatively slow water flowing surface speed (proportion 50% of the river), mixed sandy with rocky substrata (50% of the river), and water surface with relatively high depth of the water (50% of the river).

Management measures

Management characteristics have been a suggestion for analytical research and, a request for managers which face many pressures and threats on lotic systems. As a result, there are many viewpoints and models which fluctuate based on source, system, and design intricacy. The management indicators can be match based on a process which includes six levels (Krause and Mertins, 1999): designing a process value chain model, determining the key success factors, giving the description of the performance indicators, data acquiring and checking, assessment of the performance indicators, and putting into action process.

This pathway based on a model is sustained by the learning process which appears while realising the process maps; and set up the need for management elements grouped around the record sheets of management measures. It is meaningful to highlight that constructing the essentiality to identify an indicator set for assessment of an entity's overall achievement, the proposed model find the preeminent value delivery process, to which an indicator set for process assessment can be designated, which are generated by diagnosing the success factors for the process and for the entity's performance (Miricescu, 2011, 2014).

Appropriately with this model, we propose that the main management measures include the conservation of: the natural morphology of the lotic systems – natural dynamic of banks and water flow regime; rocky-sandy substratum and relatively deep water depth; the forbiddance of the disposing of wastes in water and on the banks; keeping a medium level of the water including in drought periods based on avoiding of important water removals and use; decreasing water pollution; and implementation of a long term monitoring system for fish.

Adjusted model for the site management

The proposed model of the two species of fish *Alburnus alburnus* and *Alburnoides bipunctatus* uses common objects of the ADONIS:CE for modeling business processes (BPM), namely: the beginning of the process ( ), activities ( ), decisions ( ), parallelism ( ) and merging ( ) – in the case of parallel activities, notes ( ), subprocesses ( ) – processes that are used within the basic process, variables ( ) and generators ( ) – are used to highlight the percentage of achievement of the habitat indicators (the percentage indicators that ensure conservation status) and the end of the process ( ).

To better visualize the model structure proposed for the two fish species, figure 2 highlights the inter-model references between processes modeled.
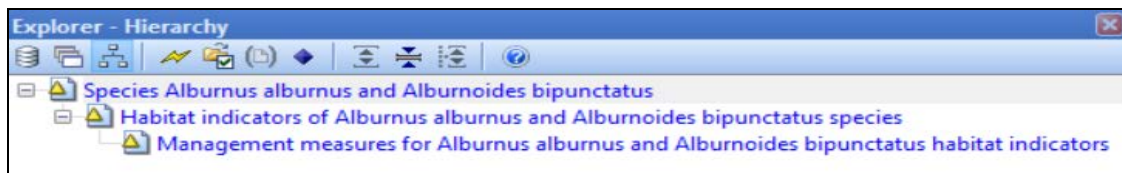
Figure 2: Hierarchy of modeled process and inter-model references.

Model description

The basic process of these two fish species modeled (Fig. 3) is conceived as a description of them, being presented with the help of the following activities: the habitat type, the specific identified requirements (these were modeled with the help of parallelism and merging – independent activities), continues with the "Habitat indicators of *Alburnus alburnus* and *Alburnoides bipunctatus* species" subprocess call (Fig. 4), then follows another two activities field observations, pressures and threats on the habitat, and implicitly, on the species *Alburnus alburnus* and *Alburnoides bipunctatus* and the process ends. With the help of notes, it was possible to graphically exemplify the characteristics of certain process activities.

The "Habitat indicators of *Alburnus alburnus* and *Alburnoides bipunctatus* species" subprocess (Fig. 4) have the same characteristics as a process and contain the specific habitat indicators – proposed for this research, the decisions for verifying them – whether or not they ensure the favourable conservation status of the studied two species *Alburnus alburnus* and *Alburnoides bipunctatus*, the management measures to be taken (subprocess – Fig. 5) and the final activity, the implementation of a long-term fish species monitoring system.

For example, the model begins with the first indicator ("Average water flowing surface speed" – proportion 50% of the river), and the first decision verifies whether it does or does not meet fair conservation status (basically, this was compared to the current state of the indicator – resulting from field measurements – with favourable conservation status).

If for this indicator, the favourable conservation status is fulfilled (the "YES" branch of the decision, variable: Water_flowing_surface_speed = "Yes", probability: 99%), then the model continues with the second indicator. If the first indicator does not fulfil the favourable conservation status (the "NO" branch of the decision, variable: Water_flowing_surface_speed = "No", probability: 1%) then, the model continues with the management measure subprocess – goes through every measure – after which, it returns to the first indicator, and once again checks (after the management measures applied) whether or not the fair conservation status is fulfilled. A loop is formed and the process does not go through the other indicators unless the current indicator meets that condition.
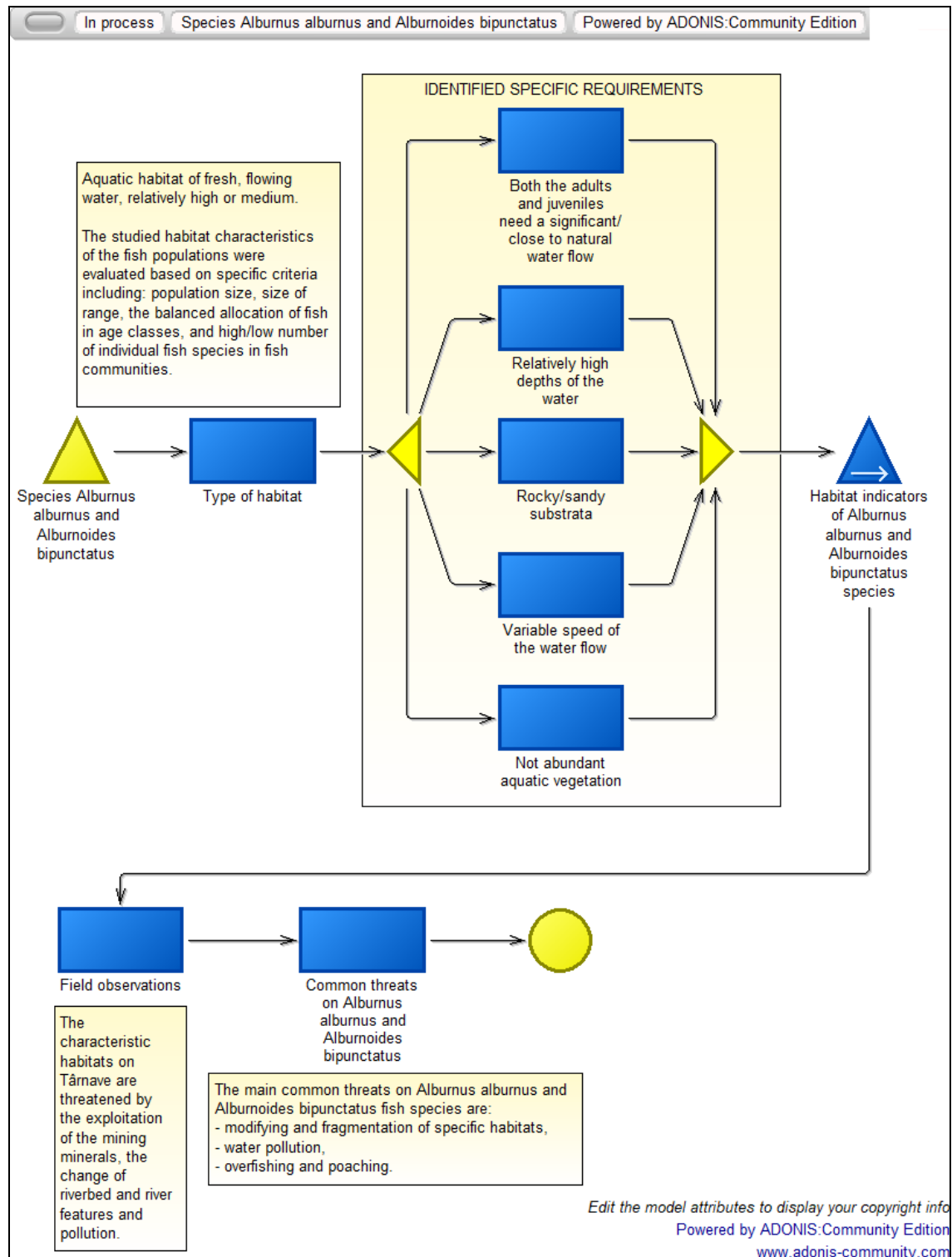
Figure 3: Species *Alburnus alburnus* and *Alburnoides bipunctatus*
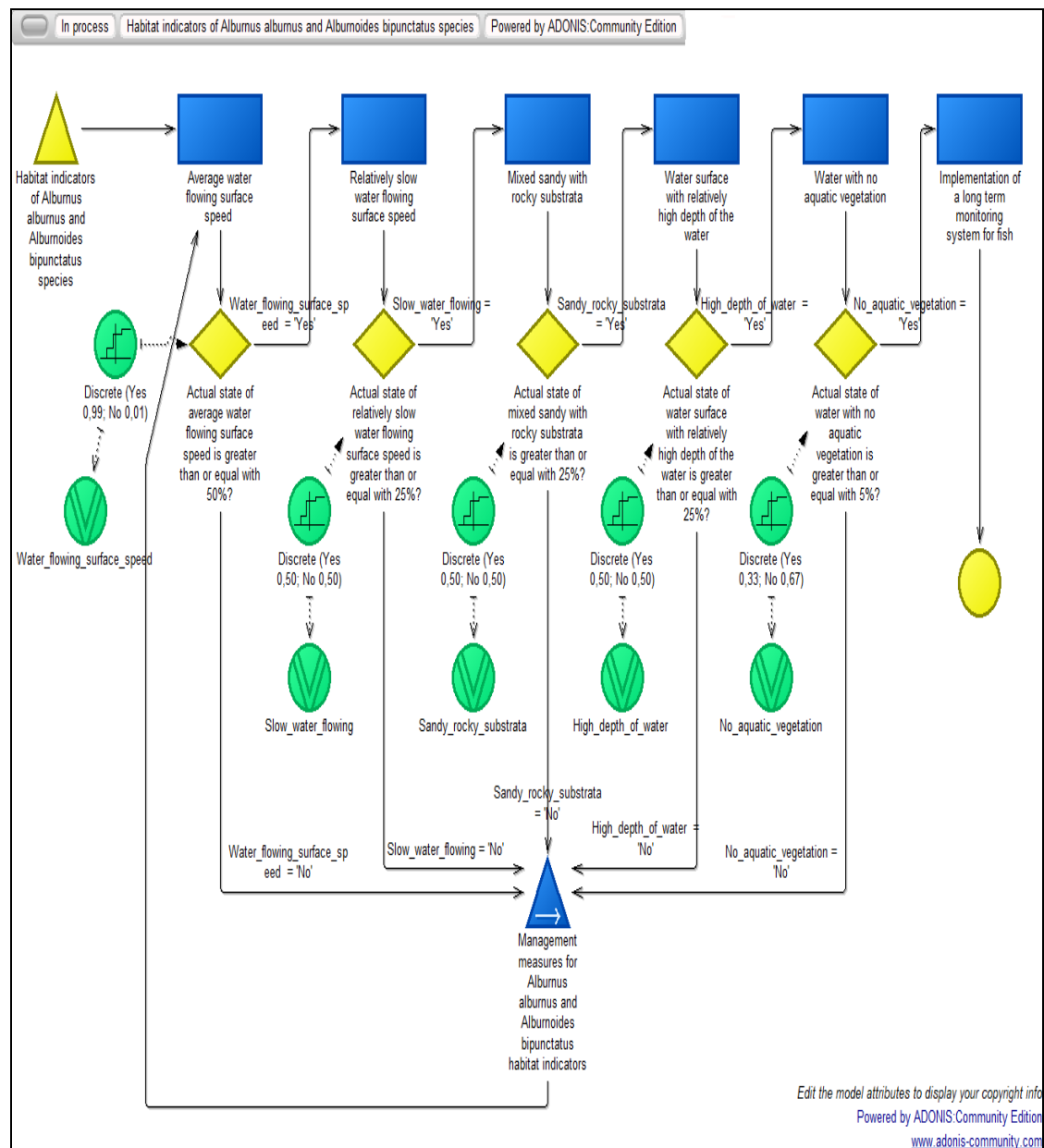– critical requirements of habitat.

Figure 4: Habitat indicators of *Alburnus alburnus* and *Alburnoides bipunctatus* species.

The last subprocess shows the management measures (Fig. 5) model made only with activities. Here are the management measures that should be taken to ensure that the *Alburnus alburnus* and *Alburnoides bipunctatus* species preserve their favourable conservation status. Among these, we mention the conservation of the natural morphology of the lotic systems, the preservation of the rocky and sandy substrate, the prohibition of the discharge into the rivers of any type of waste, the maintenance of a medium level of water during periods of drought.
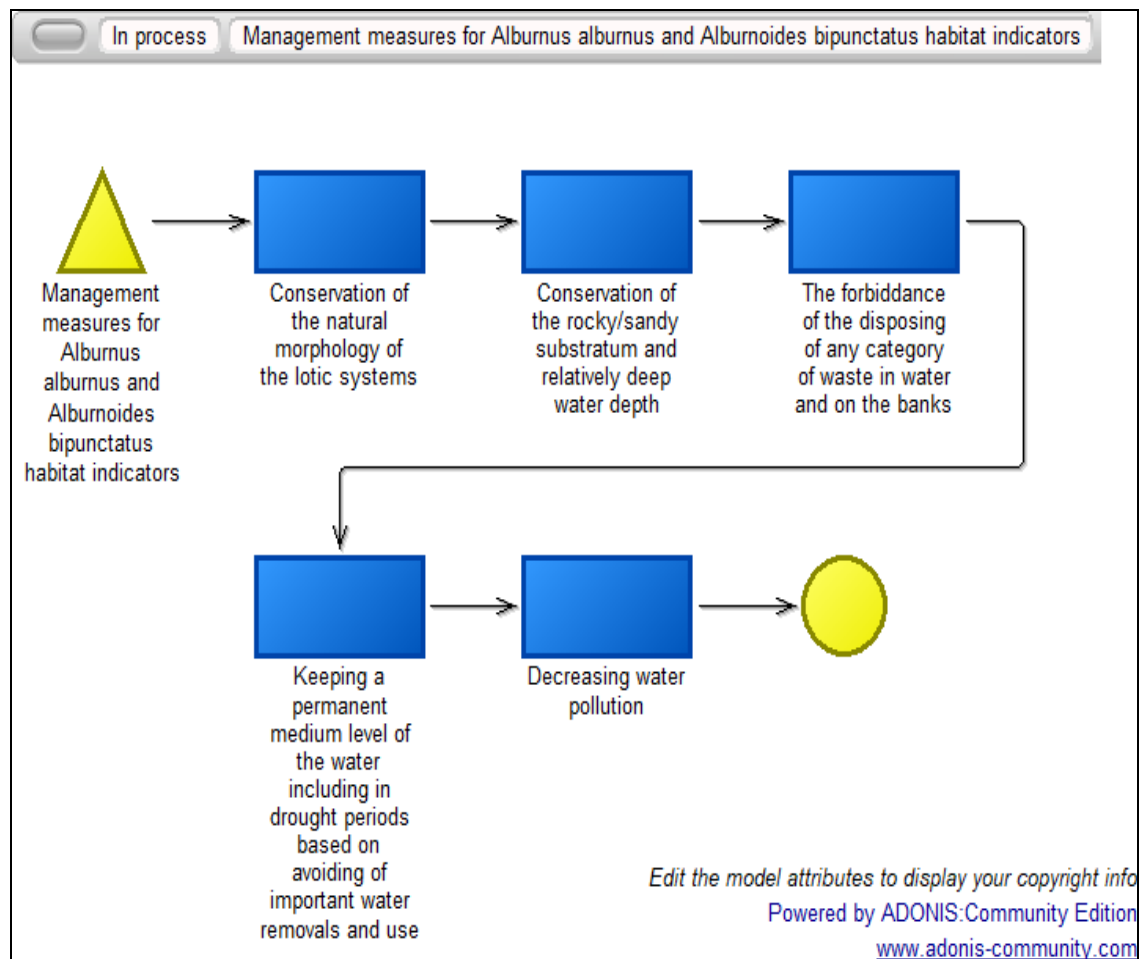
Figure 5: Management measures for *Alburnus alburnus* and *Alburnoides bipunctatus* habitat indicators.

CONCLUSIONS

The principal recorded pressures and threats to the fish species *Alburnus alburnus* and *Alburnoides bipunctatus* in the studied lotic sectors of the Târnava Watershed were the following: modifying and fragmentation of characteristic lotic habitats, water pollution, and overfishing and poaching.

Critical for *Alburnus alburnus* and *Alburnoides bipunctatus* fish species conservation are the following: the characteristic/natural riverbed morphodynamics guardianship, the diminishment of the existing flowing water habitats fragmentation, the prohibition of riverbed heavy exploitation, the riverine vegetation preservation, the ecological restoration of the riverbeds' characteristic morphodynamic, complex waste management, reducing water pollution, effective poaching restriction, and the creation of an integrated monitoring system where the fish fauna is a core element.

In this particular research, a necessary model for decisions in management in order to back the two *Alburninae* species was produced, ready to be implemented in the researched area.

The ADONIS:CE was used here for fish conservation in an area of concern, coming up with a specific management model for *Alburnus alburnus* and *Alburnoides bipunctatus* fish species that contain their main necessities regarding the habitat, and the elements that reveal a good ecological status. The suggested management elements help to prevent and/or diminish the identified pressures and negative effects on these species' populations.

This particular on-site, on habitats and on species management decisions supporting model scheme for *Alburnus alburnus* and *Alburnoides alburnoides*, will be more effective if integrated in a management model for the Târnava Watershed fish associations.

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