



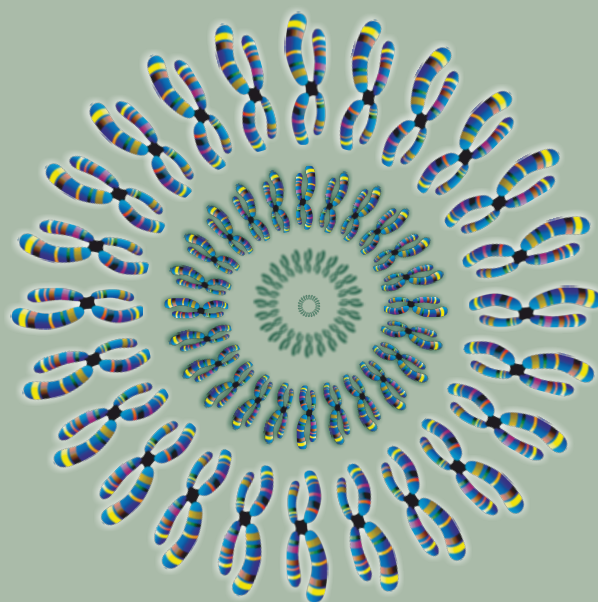
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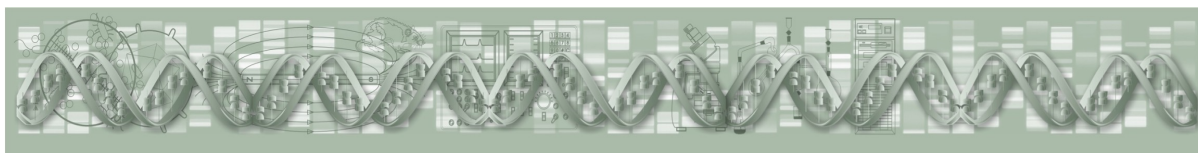
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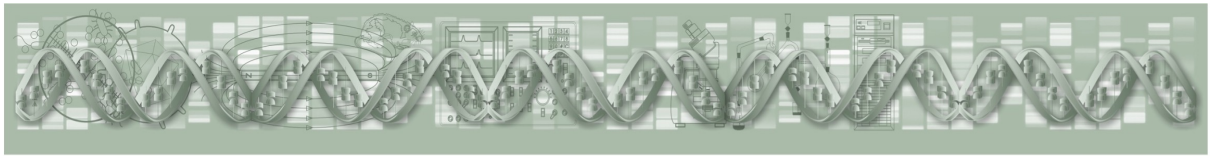
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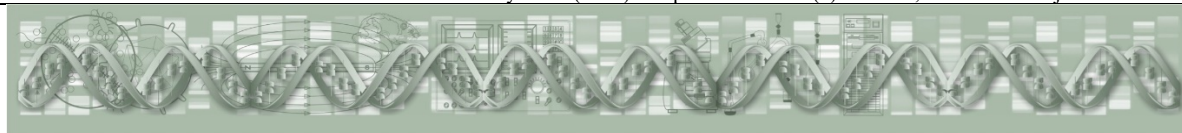
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UNSWEETENED MILK AND VITAMIN C SUPPLEMENTS AMELIORATE LEAD ACETATE INDUCED HEPATIC INJURIES THROUGH IMPROVEMENT IN LIVER FUNCTIONS AND HEMATOLOGY

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Abstract

Lead poisoning poses one of the major health challenges affecting all organ systems but mostly the nervous, renal, haematopoietic, liver and cardiovascular systems. This study investigated the effects of unsweetened milk and vitamin C supplements on markers of hepatic function and hematological parameters of lead acetate exposed albino rats. Twenty male albino rats were randomized into four groups of five rats each. Normal control received feed and water only. Lead group (Pb) received 80mg/kg body weight lead acetate. Standard control (Pb+Vit C) was given 80mg/kg lead acetate daily plus 100mg/kg of vitamin C, while treatment group (Pb+Milk) was given 80mg/kg lead acetate plus 400mg/kg milk. Animals were allowed access to feed and water. All administration was done once daily by oral gavage for 42 days. Biochemical analyses were done using standard procedures. Rats exposed to lead acetate showed a significant ($p < 0.05$) increase in the activities of alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase and total bilirubin level, indicating liver dysfunction with a significant ($p < 0.05$) decrease in plasma albumin when compared with the control groups. The results also showed significant ($p < 0.05$) decrease in red blood cell count, packed cell volume, hemoglobin, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration with significant elevations in the mean corpuscular volume and platelet concentrations when compared with the control. Unsweetened milk and vitamin C supplements ameliorated the negative effect of lead on the liver and improved hematological parameters.

Keywords: lead acetate, unsweetened milk, liver function and hematological parameters

Introduction

Lead exposure is a prevalent public health issue facing the populace as a result of its hazard effects on various physiological systems. Lead toxicity has posed a health threatening situation shown by nature of their environmental decadence (Alisha et al. 2017), especially since its

manufacturing transformations evolved. Almost all organ systems are associated with toxicity of heavy metals. However, the most susceptible systems include the cardiovascular, renal, haematopoietic, and nervous system (Abhay et al. 2024). Lead (Pb), one of the venomous heavy metals, has been implicated in various aspects of environmental and biological systems, especially in industrialized localities (Srivastava et al. 2024). Lead is one of the oldest deleterious agents known to mankind as its toxicity in both human and experimental animals can be traced back to the ancient times of the Romans, Arabs, Egyptians and Greeks (Jasbir et al. 2023). Lead exists in three isoforms: metallic lead, inorganic lead and organic lead, each with distinct properties and implications for health and its environment (Shafia 2022). Commonly encountered form of lead can enter the body through multiple routes including; ingestion, inhalation and dermal contact of their presence in food, air and tobacco leaves (Ashkan 2023). On absorption into systemic circulation, some of its components complexes with erythrocytes, and the remaining stays in plasma to be transported to other tissues (Elayat and Bakheelf 2010).

Lead-induced liver toxicity has been associated with oxidative stress, inflammation, and impairment of liver function parameters, which can have profound health implications (Ambreen 2024). The liver performs a critical role in detoxification, metabolism, and maintaining overall homeostasis in the body (Wu et al. 2023). As researchers seek innovative strategies to mitigate the adverse effects of lead exposure, the potential of unsweetened milk as a dietary intervention in alleviating liver and hematological damage requires thorough investigation.

Milk is an integral part of human and animal diets, recognized for its nutritional constituents. It's rich in essential nutrients, proteins, vitamins, minerals, and bioactive compounds with potential health-promoting properties (Felicito 2024). These components suggest that milk consumption could influence liver health by modulating oxidative stress, inflammation, and enzymatic activities that impact liver function and hematological parameters (Giovanna et al. 2023). Some studies have explored the potential hepatoprotective effects of milk and the specific impact of milk consumption on liver function parameters in the context of lead exposure remains under-investigated (Yasmin et al. 2023). Elucidating the potential benefits of unsweetened milk in mitigating lead-induced liver and hematological damage could offer a novel and practical dietary approach to reducing the adverse effects of lead toxicity. Therefore, this study investigated the effects of unsweetened milk and vitamin C supplements on markers of hepatic function and hematological parameters of lead acetate exposed albino rats.

Materials and Methods

Equipment, Chemicals and Reagents. All equipment used were optimally functional and chemicals/reagents were of high analytical quality. Lead acetate (Merck, Germany) was procured from Cjay Enterprise Ogoja Road, Abakaliki, Ebonyi State Nigeria while Peak[®] instant full cream unsweetened milk was purchased from St. Margret Umahi International Market, Lot 1, Abakaliki, Ebonyi State Nigeria.

Animal Collection. Adult albino Wistar rats with body weights of >100 g were used for this study. These rats were purchased from Pharmacy Department, University of Nigeria Nsukka, Enugu State, Nigeria. The rats were kept in steel cages in a conventional laboratory setting for a week of acclimatization, with free access to commercial basic diet (Top Feed Growers Mash) and water *ad libitum*. They were kept in the Animal House at the Presco Campus of Ebonyi State University, Nigeria.

Experimental Design. Twenty male albino rats were randomized into four groups of five rats each. Normal control (NC) received feed and water only. Lead group (Pb) received 80mg/kg body weight lead acetate (Okediran et al. 2017, Newairy and Abdou 2009). Standard control

(Pb+Vit C) was given 80mg/kg lead acetate daily plus 100mg/kg of vitamin C (Ait Hamadouche et al. 2012), while treatment group (Pb+Milk) was given 80mg/kg lead acetate plus 400mg/kg milk (Chuang et al. 2004). They were fed with commercial top feed growers mash and water. All administration was done once daily by oral gavage for 42days. Biochemical analyses was done using standard procedures.

Blood Sample collection. The experiment was carried out for 42days and blood samples collected via ocular puncture with the animals anesthetized with chloroform after 24hrs fasting. Blood samples were collected using lithium heparin bottles for other tests and EDTA bottles for hematology after which samples were centrifuged at 2000 x g for 5 minutes and the plasma was isolated into plain bottles and stored in refrigerator with temperature of -4°C for analysis.

Liver Function Test. The following parameters were measured: total bilirubin (TB) (Doumas et al. 1973), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) (Reitman and Frankel 1957), alkaline phosphatase (ALP) (Babson et al. 1966) and plasma albumin (ALB) (Doumas and Peters 1997).

Hematology Analysis. Hematological parameters were measured using the CELL-DYN Ruby Auto analyzer (Abbott, Abbott Park, IL, USA). The following hematological parameters were examined: red blood cell count (RBC), packed cell volume (PCV), hemoglobin concentration (HbC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and platelet count (PLT).

Statistical Analysis. Data generated were analyzed using the statistical package for social sciences software (Version 25) and thereafter subjected to one way analysis of variance (ANOVA).

Results and discussions

The results of this study showed a significant elevation ($P < 0.05$) in the activities of liver markers ALT, AST and ALP of lead acetate exposed rats relative to the control (Figure 1) with similar observation in Total Bilirubin level (Figure 2). The result in Figure 3 indicated a significant decrease ($P < 0.05$) in plasma albumin when compared with the control groups.

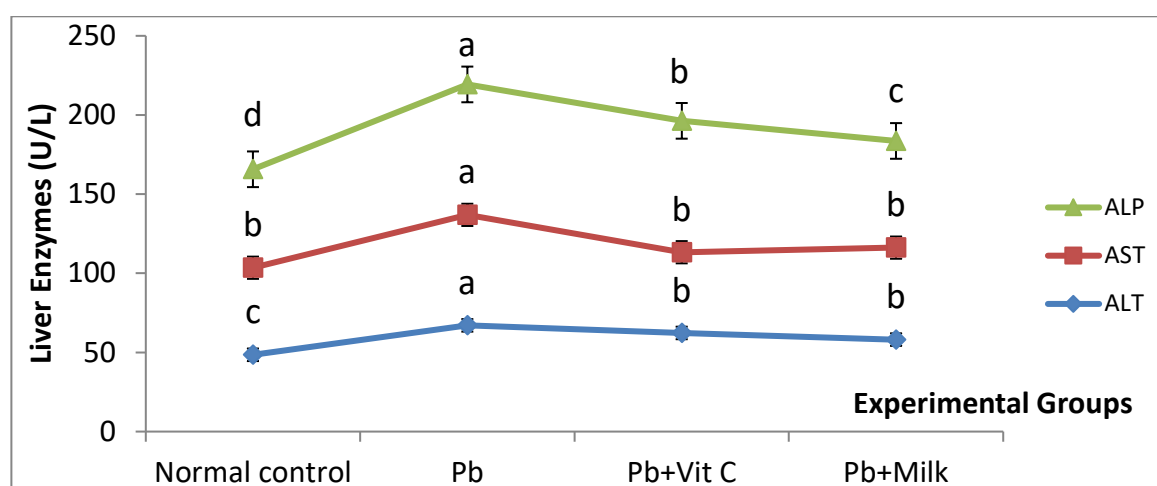


Figure 1. Effect of unsweetened milk and vitamin c supplements on liver enzymes in lead acetate exposed rats

Data are shown as mean $\pm$ SEM (n=5). **SEM:** Standard error of means. Coordinates with different alphabets are significantly different ($p < 0.05$).

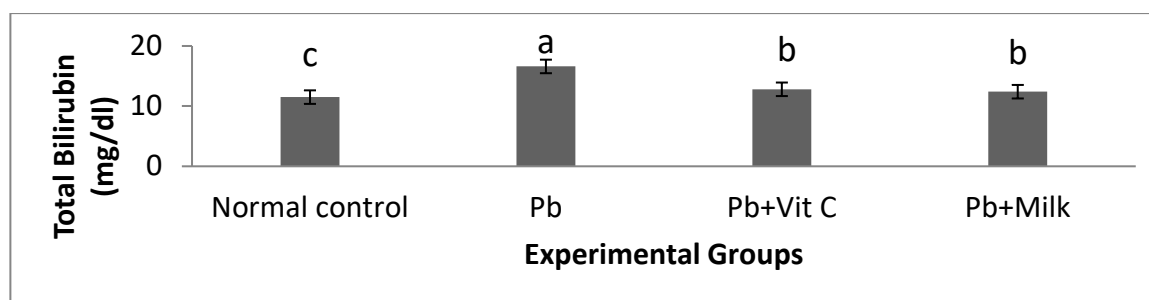


Figure 2. Effect of unsweetened milk and vitamin c supplements on plasma bilirubin level in lead acetate exposed rats

Data are shown as mean $\pm$ SEM (n=5). **SEM:** Standard error of means. Bars with different alphabets are significantly different ($p < 0.05$)

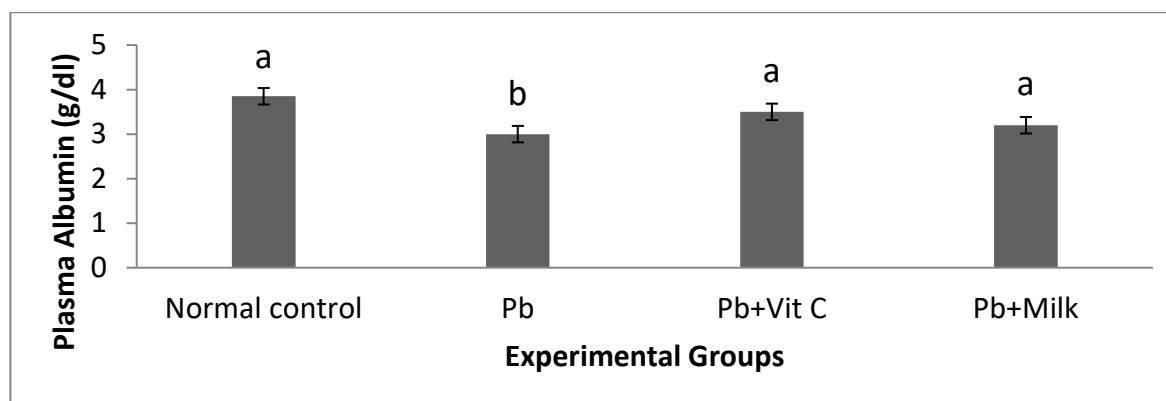


Figure 3. Effect of unsweetened milk and vitamin c supplements on plasma albumin level in lead acetate exposed rats

Data are shown as mean $\pm$ SEM (n=5). **SEM:** Standard error of means. Bars with different alphabets are significantly different ($p < 0.05$)

The administration of lead acetate to rats led to a significant ($P < 0.05$) elevation in plasma AST, ALT and ALP activities in the group treated without intervention when compared with the normal control, indicating that exposure to lead acetate may induce a detectable damage to the liver. The elevation in plasma ALT, AST and ALP could be as a result of an increase in cell membrane permeability due to damage exerted on the plasma membrane of hepatocytes associated with lead acetate hepatotoxicity. The result is in accordance with the works of Mehta et al. (2002) and Patil et al. (2007) who reported a significant increase in AST and ALT concentrations after treatment with lead acetate. The hepatotoxicity induced by lead acetate tends to damage the liver cells with a concomitant increase in serum concentrations of AST and ALT (Abdou et al. 2007). The increased level of total bilirubin observed in the results after induction of lead acetate could be initiation of hemeoxygenases that play an important role in heme catabolism or damage to the hepatocytes as discussed above or both (Alya et al. 2007). The groups that received 400mg/kg of milk and 100mg/kg of vitamin C revealed a significant ($P < 0.05$) decrease in Total bilirubin concentration, ALT, AST and ALP activities when compared to the group that received lead acetate only. This finding is in accordance with the works of Abdel-Mobdy et al. (2023), who reported that liver function parameters and bilirubin level in serum significantly decreased in all administered groups with the inclusion of the milk-induced groups relative to normal control. Our findings is also in agreement with the works of Magjeed (2005), Khan and Zohair (2011), and Abdel-Mobdy et al. (2021); who reported the hepatoprotective influence of camel milk on poisons; similarly, camel milk ameliorate the liver functions of lead acetate induced rats; they equally reported that camel milk's beneficial

impacts could be due to the incorporation of mineral compositions in it, which plays vital roles in decontamination and as nutraceuticals in the liver.

There was a significant ($P < 0.05$) reduction in the plasma albumin level of the rats exposed to lead acetate when compared with the normal control. The observed variations in plasma albumin is an indication of liver malfunction since it's responsible for proteins synthesis, mostly albumin (Mariam et al. 2023).

The results in Figure 4 and 5 indicated significant reduction ($P < 0.05$) in packed cell volume (PCV), hemoglobin (HB), red blood cell count (RBC), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) with a significant ($P < 0.05$) elevation in the mean corpuscular volume (MCV) and platelets count (Figure 6) when compared with normal control groups of lead acetate exposed rats.

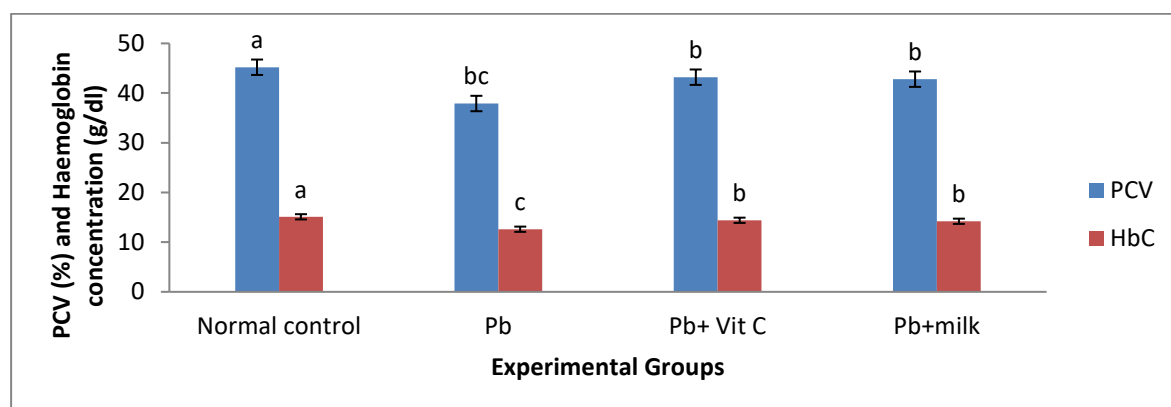


Figure 4. Effect of unsweetened milk and vitamin c supplements on packed cell volume (PCV) and haemoglobin concentration (HbC) of lead acetate exposed rats

Data are shown as mean $\pm$ SEM (n=5). **SEM:** Standard error of means. Bars with different alphabets are significantly ($p < 0.05$) different.

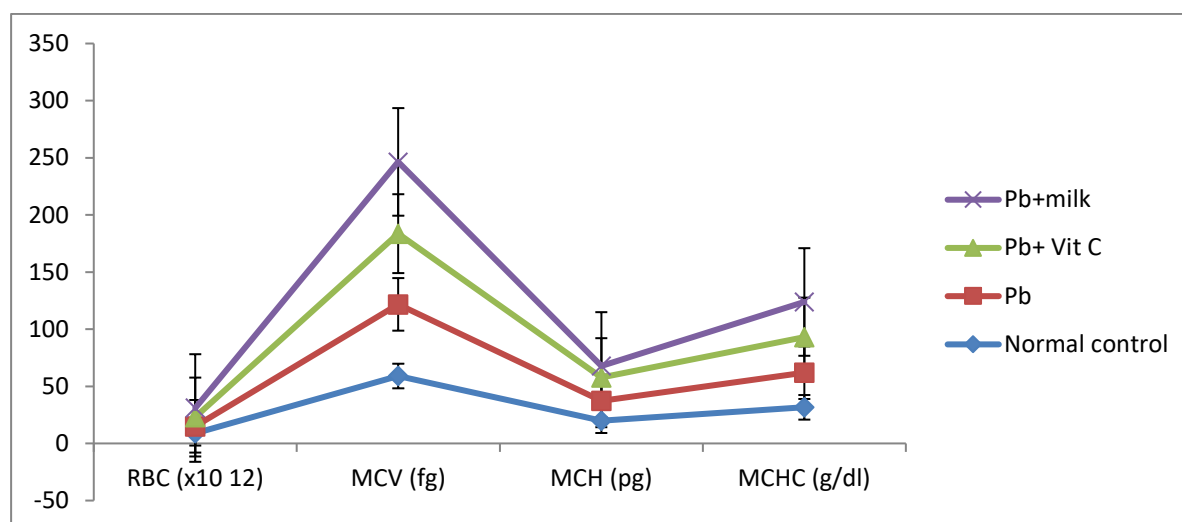


Figure 5. Effect of unsweetened milk and vitamin c supplements on red blood cell count (RBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and indices of lead acetate exposed rats

Data are shown as mean $\pm$ SEM (n=5). **SEM:** Standard error of means.

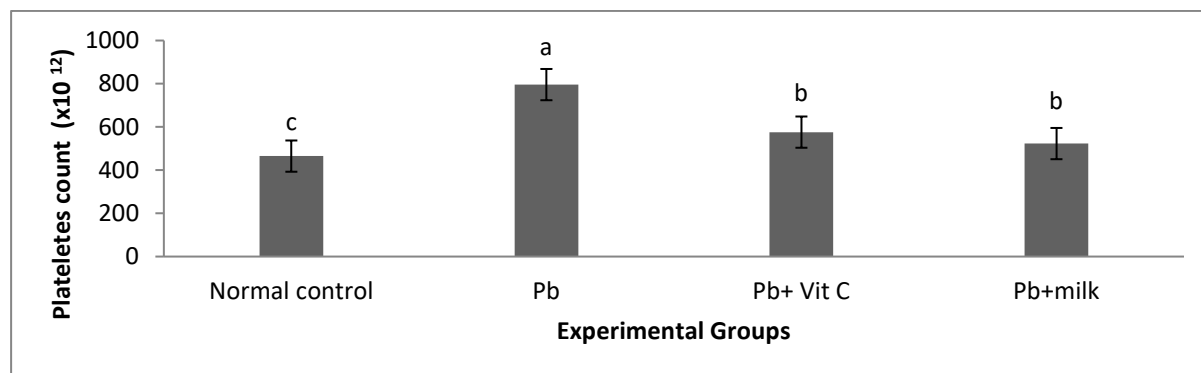


Figure 6. Effect of unsweetened milk and vitamin c supplements on platelets count of lead acetate exposed rats

Data are shown as mean $\pm$ SEM (n=5). **SEM:** Standard error of means. Bars with different alphabets are significantly ($p<0.05$) different.

The findings from this study suggest that unsweetened milk and vitamin c supplements may have a positive influence on haematological parameters in lead acetate exposed rats as shown in figure 4. There was a significant ($P<0.05$) reduction in PCV level in lead acetate group when compared to the normal control. Groups that received unsweetened milk and vitamin c supplements showed significant ($P<0.05$) elevation in PCV level in relation to lead acetate group. This indicates that milk consumption may help maintain healthy red blood cell volume in the presence of lead exposure. The decrease in PCV and HbC in the lead acetate exposed group aligns with the known hematotoxin effects of lead, which can impair erythropoiesis and haemoglobin synthesis (Katarina et al. 2024, Wahab et al. 2010). The improvement observed in the lead acetate + vitamin C group implies a potential ameliorative outcomes of vitamin C on lead-induced haematological changes. Notably, the lead acetate + milk group displayed results approaching those of the normal control, indicating that milk consumption might have a positive influence on blood parameters of animals exposed to lead acetate (Doris and Jorge, 2020). The protective effect of milk could be attributed to its nutritional composition, including proteins, vitamins, and minerals. Calcium and other components in milk might counteract the hematotoxin effects of lead, contributing to the observed improvements (Christian et al. 2021). These findings suggest that incorporating milk into the diet may have haematological benefits for individuals exposed to lead. However, it is pertinent to conduct further studies, including human trials, to validate these results and understand the underlying mechanisms.

The results in figure 5, indicates a significant reduction in the red blood cell (RBC) count in the group that received lead acetate. This aligns with the general understanding of adverse effects of lead on haematopoiesis. The increase in MCV as shown in the present study indicates changes in the size of red blood cells, potentially suggestive of alterations in their maturation. The decrease in MCH and MCHC in the same group further supports the notion of lead-induced anaemia, an indication of a decrease in haemoglobin content per red blood cell and its concentration.

Conversely, the incorporation of 100mg/kg body weight of vitamin C and 400mg/kg body weight of milk seemed to have a positive effect on red blood cell count and related indices, suggesting a potential protective role. The effects of vitamin C were more pronounced in comparison to milk, whereas milk appeared to play a role in enhancing red blood cell count, the sharp decrease in MCH and MCHC suggests that it might have a unique influence on the haemoglobin content and concentration within each red blood cell (Scholz-Ahrens et al. 2003, Torres et al. 1995).

Understanding the mechanisms behind these observed effects, especially the unexpected changes in MCH and MCHC in the milk-supplemented group, calls for further investigation. It's essential to explore how milk components interact with lead and influence erythropoiesis, haemoglobin synthesis, and red blood cell characteristics

In figure 6, the platelet count of animals given lead acetate increased significantly when compared to the normal control. This elevation in platelet count with lead acetate exposure aligns with the known association between lead exposure and inflammatory processes. Meanwhile, vitamin C and milk intervention tends to mitigate this effect. This suggests potential protective effects of these substances against lead acetate induced platelet alterations. An elevated platelet count (thrombocytosis) can have clinical implications, including an increased risk of thrombosis. On the other hand, excessively low platelet counts (thrombocytopenia) can lead to bleeding disorders. Therefore, understanding the factors that influence platelet count, including dietary components like milk, is essential for assessing their potential impact on overall health and homeostasis, especially in the context of lead exposure (Wati et al. 2023).

Conclusions

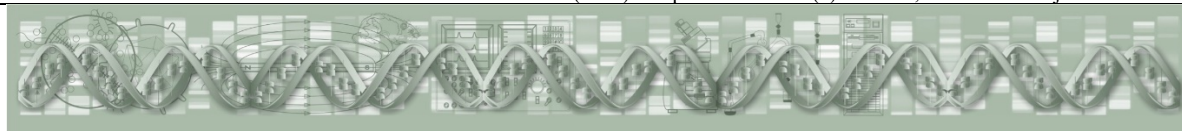
Our findings suggest protective effects of unsweetened milk and vitamin C supplements on hepatotoxicity and hematological damages associated with exposure to lead acetate in rats. Hence, unsweetened milk and vitamin C possess hepatoprotective potentials that deserve to be further evaluated towards understanding the underlying mechanisms of their hepatoprotective potentials in lead toxicity.

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INVESTIGATING THE *IN VIVO* EFFECTS OF COPPER COORDINATION COMPOUNDS WITH THIOSEMICARBAZONES ON ERYTHROCYTE REDOX BALANCE

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Abstract

Thiol-disulfide homeostasis plays a vital role in cellular and systemic functions, regulating biosynthetic reactions, growth, transport, repair, and redox signaling through the dynamic interplay between thiol (-SH) and disulfide (-S-S-) states. This study evaluated the effects of copper coordination compounds with thiosemicarbazones (CCTs) on thiol-disulfide metabolism in 120 rats (*Rattus norvegicus Albicans*). The animals were divided into 10 groups by sex, with the control group receiving saline and experimental groups (Groups 2–10) administered specific CCTs (10 µg/kg, subcutaneously) for 30 days. CCTs, known for their medicinal potential, particularly as anticancer agents, enhanced antioxidant defenses by increasing total and reduced glutathione (tGSH, rGSH) and decreasing oxidized glutathione (GSSG). These findings underscore the potential of CCTs in modulating redox balance and their promise in therapeutic applications, including cancer treatment.

Keywords: thiol-disulfide metabolism, erythrocytes, copper coordination compounds with thiosemicarbazones

Introduction

The tripeptide glutathione is a central component of an integrated antioxidant system that safeguards cells and tissues from oxidative stress (OS). Redox (oxidation-reduction) reactions are essential to cellular metabolism and homeostasis, producing electrophilic byproducts that are unavoidable. Cells expend significant energy synthesizing numerous proteins and processing both endogenous and dietary antioxidants to maintain redox equilibrium and protect critical cellular macromolecules from oxidative damage (Ribeiro et al. 2023).

Among these antioxidants, reduced glutathione (GSH) is particularly versatile. Composed of glutamate, cysteine, and glycine, GSH plays a key role in reduction and conjugation reactions, primarily through the sulfhydryl (-SH) group of cysteine. These reactions are crucial for neutralizing peroxides and detoxifying various xenobiotic compounds. Additionally, GSH is involved in regulating the cell cycle (Ribeiro et al. 2023). It is the most abundant cellular thiol,



with concentrations reaching 1 to 10 mM in many cell types (Wu 2004, Lapenna et al. 2023, Vázquez-Meza et al. 2023).

Glutathione is recognized as a critical cellular redox regulator, influencing key cell fate decisions, such as proliferation and apoptosis (Jones 2002, Watson et al. 2003, Lu 2020). Its abundance allows GSH to play a vital role in shielding cells from toxicity caused by excessive endogenous and exogenous substances (Ballatori 2009). Notably, GSH is the primary defense against various toxic heavy metal ions (Arthur, 2000). Additionally, GSH acts as a cofactor for the GSH peroxidase (GPX) enzyme family, which neutralizes hydrogen peroxide (H_2O_2) and lipid peroxides (Rinaldi et al. 2002). Through the activity of the glutathione S-transferase (GST) enzyme family, GSH also conjugates with diverse endogenous electrophiles and xenobiotics, ensuring their safe and efficient elimination (Rinaldi et al. 2002).

Human life relies on oxygen and aerobic processes, yet a consequence of these processes is the production of reactive oxygen species (ROS), which can be harmful to cellular components (Yi et al. 2016). ROS, including superoxide anion radicals ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), and hydroxyl radicals ($\cdot OH$), are typically generated during essential metabolic activities, such as protein synthesis and mitochondrial respiration (Valgimigli et al. 2023).

The oxidative effects of ROS are neutralized by the antioxidant capacity of cells, and this battle against OS maintains homeostasis (Krylatov et al. 2007). Within the cell, redox couples are specifically controlled, particularly in the mitochondria, endoplasmic reticulum, and nucleus (Jones et al. 2004). Additionally, extracellular compartments provide defensive barriers against external oxidants. Cysteine (Cys) and its disulfide form, cystine (CySS), constitute the principal low-molecular-weight thiol/disulfide couple in human plasma. The Cys/CySS pool serves as a central redox control point in biological signaling (Jones et al. 2002).

Organic compounds containing the sulfhydryl group are called thiols ($-SH$), composed of sulfur and hydrogen atoms. Thiol groups are highly susceptible to oxidation due to their $-SH$ nature. Disulfides ($-S-S-$) represent the most important class of dynamic, redox-sensitive covalent bonds formed between two thiol groups. Dynamic thiol-disulfide homeostasis (TDH) involves the reversible oxidation of thiols in proteins and reflects the levels of thiols and disulfides. This parameter is crucially associated with various biochemical processes, including the regulation of protein function, stabilization of protein structures, protection of proteins against irreversible oxidation of cysteine residues, chaperone function, and the regulation of enzymatic activities and transcription (Brülsauer et al. 2014, Ellgaard et al. 2018, Schmidt et al. 2023).

GSH acts as an essential cellular antioxidant with diverse protective functions. Maintaining normal GSH levels is therefore important for shielding cells against endogenous oxidants and low oxidative exposure.

ROS and nitrogen species (RNS) serve as significant signaling molecules, and changes in GSH levels can shift the threshold for this signaling. Research from various authors suggests that decreased GSH levels modify endothelial nitric oxide (eNO) signaling, affecting cellular responses (Sánchez-Rodríguez et al. 2019).

Thiosemicarbazones (TSCs) are a class of strong metal ion ligands currently being researched for various activities, including but not limited to anticancer treatment. In addition to these ligands, which exert their activity through interaction with metal ions, preformed metal-TSC complexes, particularly with essential metal ions such as iron, copper, and zinc, are also widely studied. Currently, it is unclear which are the active species, which complexes are present, and what their biological targets are. In this context, we have studied copper thiosemicarbazone complexes in preclinical studies regarding their activity in peripheral blood *in vivo*, focusing on thiol-disulfide metabolism.

The aim of the study was to investigate the influence of copper coordination compounds with thiosemicarbazones on thiol-disulfide metabolism in erythrocytes following subcutaneous administration *in vivo*.

Materials and Methods

Study Design

Novel local copper coordination compounds with thiosemicarbazones (CCTs), have been used in the study, including benzothiazole thiosemicarbazones (CMA-18, CMD-8, MG-22), phenyl thiosemicarbazones (CMC-34, CMJ-33, CMT-67), and allyl thiosemicarbazones (CMG-41, TIA-123, TIA-160). They were synthesized at the State University of Moldova, Republic of Moldova, in the "Advanced Materials in Biopharmaceutics and Technology" Laboratory (Gulea et al. 2008).

Experimental Design

This study utilized **120 laboratory white rats (*Rattus norvegicus Albicans*)**, comprising **60 males** (weight: 180–230 g) and **60 females** (weight: 210–228 g).

All experiments were conducted in accordance with ethical standards and were approved by the **Research Ethics Committee** of the "Nicolae Testemițanu" State University of Medicine and Pharmacy, Chisinau, Republic of Moldova (**Approval No. 73, dated 26.04.2017**).

Animal Maintenance

The animals were housed under **standard vivarium conditions** (temperature: $22 \pm 2^\circ\text{C}$, humidity: 55–60%, 12-hour light/dark cycle) with *ad libitum* access to standard laboratory chow and water.

The rats were randomly divided into the following groups:

Control Group (12 animals): 6 males and 6 females

Experimental Groups (108 animals):

- **54 males:** Subdivided into 9 groups of 6 animals each.
- **54 females:** Subdivided into 9 groups of 6 animals each.

Each experimental group received one of **9 biologically active copper coordination compounds with thiosemicarbazones**.

Screening and Preparations

The compounds tested were classified into three distinct groups based on their chemical structure:

1. **Benzothiazole derivatives of thiosemicarbazone:** CMA-18; CMD-8; MG-22;
2. **Phenyl thiosemicarbazone derivatives:** CMC-34; CMJ-33; CMT-67;
3. **Allyl thiosemicarbazone derivatives:** CMG-41; TIA-123; TIA-160;

Each compound was administered to the respective experimental group to assess its biological activity.

Table 1. Newly Studied Native Copper Coordination Compounds with Thiosemicarbazones

CMA-18	Chloro- {1-(1,2-benzothiazol-3-yl)-2-[1-(pyridin-2-yl)ethylidene]diazanido} copper
CMD-8	Chloro- {4-ethyl-2-[phenyl(pyridin-2-yl)methylidene]hydrazine-1-carbothioamido} copper
MG-22	Di-Chloro- {N'-(4-methoxyphenyl)-N,N-dimethylcarbamidothioato} copper
CMC-34	Chloro- {N'-[phenyl(pyridin-2-yl)methylidene]-N-pyridin-2-ylcarbamo-hydrazonothioato} copper

CMJ-33	Chloro- $\{4-(3\text{-methoxyphenyl})-2-[1-(\text{pyridin-2-yl})\text{ethylidene}]\text{hydrazine-1-carbothioamido}\}$ copper
CMT-67	Nitrato- $\{N\text{-phenyl-}N'-(\text{pyridin-2-ylmethylidene})\text{carbamohydrazonothioato}\}$ copper
CMG-41	Nitrato- $\{N'-[\text{phenyl}(\text{pyridin-2-yl})\text{methylidene}]-N\text{-prop-2-en-1-ylcarbamohydrazonothioato}\}$ copper
TIA-123	Di-Chloro- $\{N'-[\text{phenyl}(\text{pyridin-2-yl})\text{methylidene}]-N\text{-prop-2-en-1-ylcarbamohydrazonothioato}\}$ copper
TIA-160	Acetato- $\{2-([(\text{methylsulfanyl})(\text{prop-2-en-1-ylamino})\text{methylidene}]\text{hydrazinylidene})\text{methyl} \}$ enolato} copper

The test substances were dissolved in saline solution to the required volume and administered subcutaneously to the animals daily for 30 days at a dosage of 10 μg per kg of body weight. Twenty-four hours after the final administration of the local CCTs, the animals were euthanized in compliance with ethical standards and guidelines for laboratory animal care. Peripheral blood, the material for study, was collected in tubes containing a 6% K4-EDTA solution with a pH of 7.4 as an anticoagulant. The peripheral blood was centrifuged at 3000 rpm for 10 minutes. After centrifugation, the plasma was transferred to clean disposable Eppendorf tubes and stored in a refrigerator at -40°C until analysis. Simultaneously, the erythrocytes were washed three times with two volumes of 0.9% saline solution, followed by centrifugation. Subsequently, H_2O was added to lyse the erythrocytes according to their volume. The lysed erythrocytes were then transferred to tubes and stored at -40°C until use.

Assessment of the Action of CCT on Thiol-Disulfide Metabolism in Erythrocytes

Thiol-disulfide metabolism indices were measured using techniques adapted for application on the Synergy H1 microplate spectrophotometer (Hybrid Reader) (BioTek Instruments, USA). The activity of thiol-disulfide metabolism indices was evaluated by determining the following laboratory parameters: total glutathione (tGSH), reduced glutathione (rGSH), oxidized glutathione (GSSG), protein thiol (SH) groups, total thiol groups and free thiol groups.

Statistical Analysis

The statistical evaluation of the obtained data was performed using the Statistical Package for the Social Sciences (SPSS) software, version 23 (SPSS Inc., Chicago, IL, USA). After verifying the obtained data, the post-hoc Games-Howell multiple comparison test following One-Way ANOVA was used to highlight significant differences in thiol-disulfide metabolism parameters between the compared groups, with a significance threshold of $p < 0.05$. The median, interquartile range, and median percentage compared to the control group were calculated.

Results and discussions

Description of the action of CCT on glutathione metabolism

The study evaluated the impact of various biologically active copper coordination compounds with thiosemicarbazones (CCTs) on the glutathione enzyme activity in erythrocytes of healthy rats, revealing notable sex-based differences. Figure 1 presents graphically the statistical data reflecting these biochemical shifts.

Analysis of the effects of benzothiazole derivatives, particularly CMA-18, on glutathione enzyme activity in male rats indicated a significant increase in total glutathione (tGSH) levels by 33% ($p < 0.05$) and reduced glutathione (rGSH) by 73% ($p < 0.01$) compared to control values. Both CMA-18 and CMD-8 led to a marked decrease in oxidized glutathione (GSSG) levels by 52% and 54%, respectively (CMA-18, $p < 0.05$; CMD-8, $p < 0.001$).

In the phenyl derivative group, all administered compounds showed a non-significant increase in tGSH and rGSH levels across both sexes. However, GSSG levels in male rats were significantly reduced with CMC-34 and CMJ-33 by 31% and 40%, respectively ($p < 0.01$).

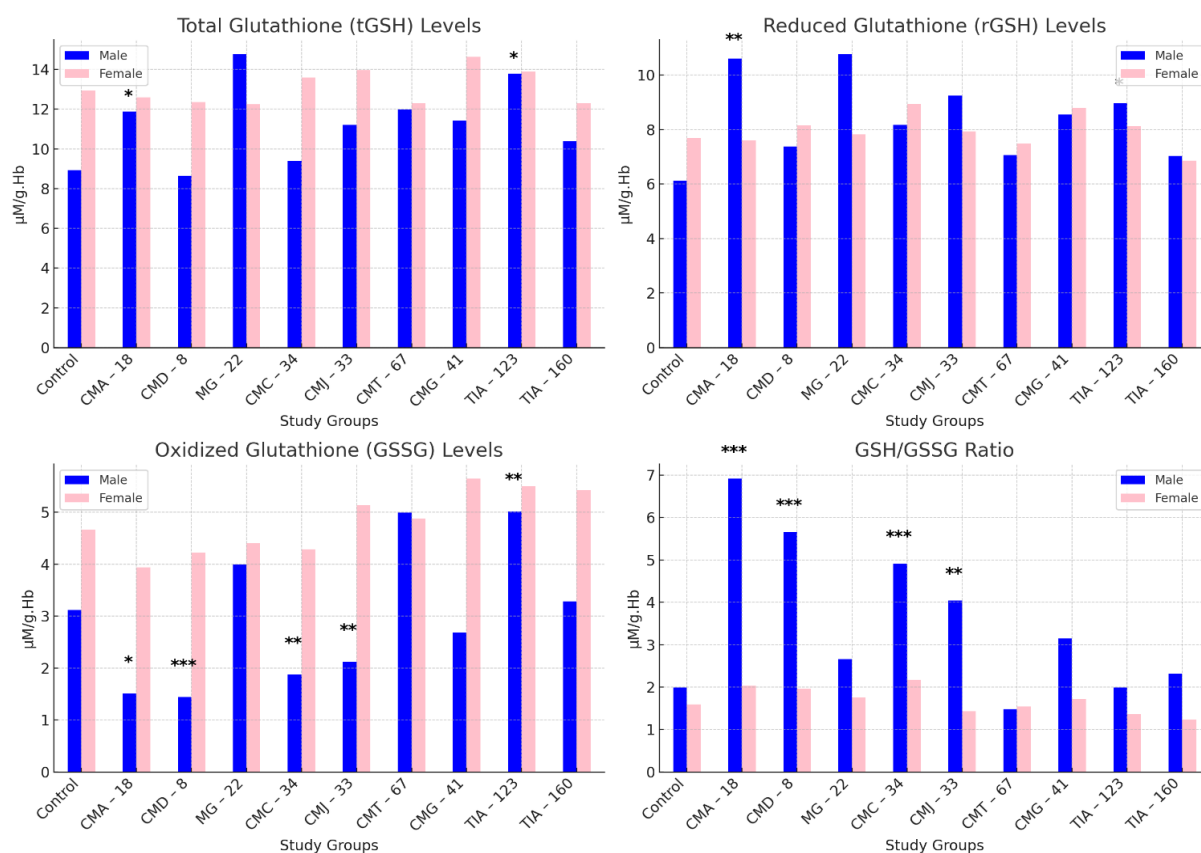


Figure 1. Changes in the activity of glutathione metabolism enzymes under the action of copper coordination compounds with thiosemicarbazones in erythrocytes *in vivo*

Note: Statistical significance compared to the control group: * – $p < 0.05$; ** – $p < 0.01$; *** – $p < 0.001$; **tGSH** – Total Glutathione, **rGSH** – Reduced Glutathione, **GSSG** – Oxidized Glutathione

Within the allyl group, significant enhancements were noted only with TIA-123 in male rats, showing a 54% increase in tGSH ($p < 0.05$) and a 47% rise in rGSH ($p < 0.05$), alongside a 61% elevation in GSSG levels ($p < 0.01$) compared to controls.

Effects of CCT on thiol-disulfide metabolism

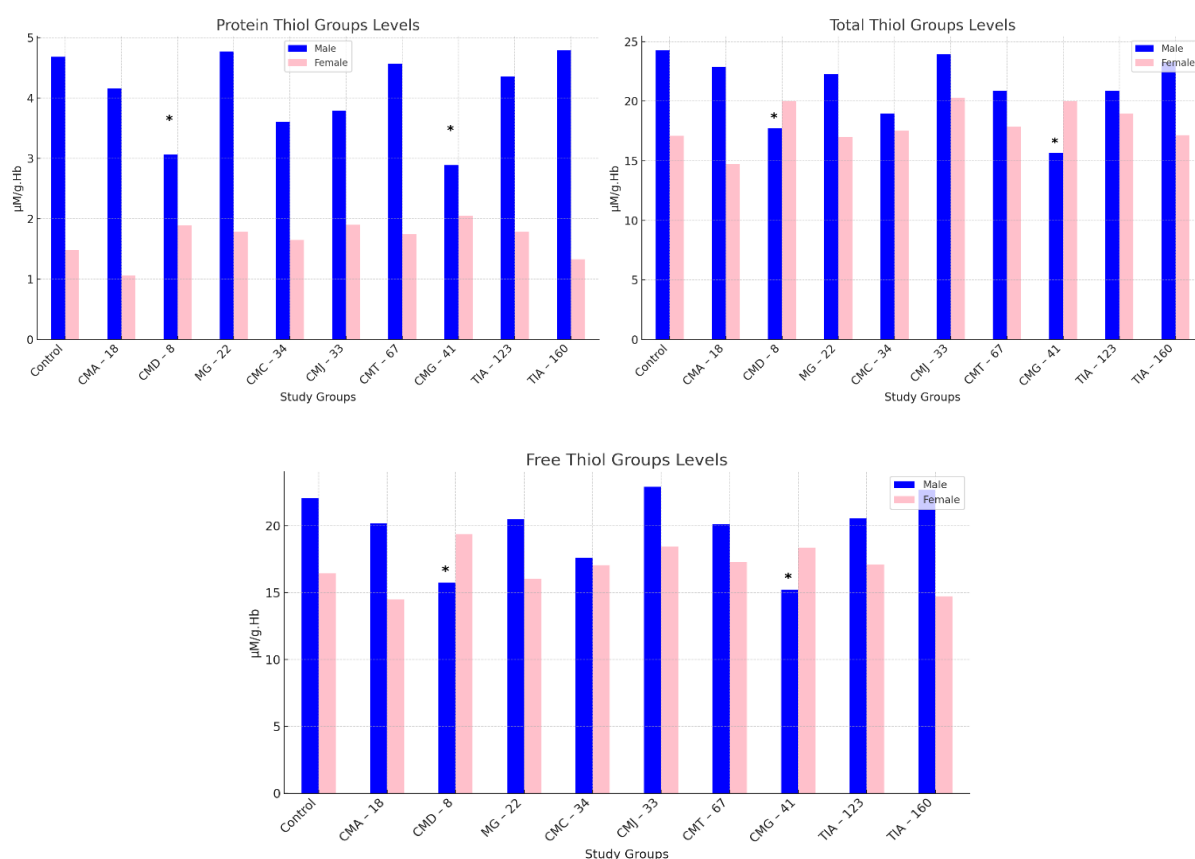


Figure 2. Evaluation of thiol groups level in rat erythrocytes following administration of copper coordination compounds with thiosemicarbazones

Note: Statistical significance compared to the control group: * – $p < 0.05$

The study also measured changes in thiol group levels in erythrocytes following administration of various CCTs (Figure 2), which highlighted both reductions and increases in thiol levels across different compounds and sexes. Significant reductions in male rats were observed with the benzothiazole derivative CMD-8, showing a 27-29% decrease in protein thiol, total thiol, and free thiol content ($p < 0.05$), and the allyl derivative CMG-41, which led to a 31-36% decrease ($p < 0.05$). In contrast, the remaining compounds demonstrated non-significant decreases ranging from 6-23% in thiol content relative to the control group.

In female rats, most compounds caused a non-significant increase in thiol levels, with enhancements ranging from 2-28% ($p > 0.05$). Notably, CMA-18 and TIA-160 led to slight reductions in thiol content in females, decreasing by 10-28% ($p > 0.05$).

These results underscore that specific CCTs modulate glutathione metabolism distinctly in male and female rats, likely due to underlying physiological differences between the sexes. GSH primarily exerts its antioxidant role through the action of GPx, which reduces hydrogen peroxide and lipid peroxides to maintain a balanced redox state by converting GSH into GSSG. This oxidized GSSG can be recycled back to GSH by glutathione reductase, a process essential for cellular redox homeostasis (Lu 2009). GPx and GST also facilitate organic peroxide reduction.

While catalase also breaks down hydrogen peroxide, it is confined to peroxisomes, rendering GSH crucial for protecting mitochondria from both physiological and pathological oxidative stress (Garcia-Ruiz et al. 2006, Chen et al. 2024). As the GSH/GSSG ratio dictates cellular redox potential, high OS prompts GSSG export from the cell or conjugation with protein

sulfhydryl groups, resulting in mixed disulfides. This mechanism prevents significant redox imbalances, as elevated OS can otherwise deplete cellular GSH (Lu 2009).

The modulation observed in this study supports the importance of the GSH/GSSG ratio in cellular redox signaling, with a higher GSH/GSSG ratio indicating a reduced state conducive to antioxidative processes. The ability of certain CCTs to enhance both tGSH and rGSH levels suggests that these compounds aid in maintaining redox homeostasis under oxidative conditions. Given that GSH is integral to the cell's antioxidant network, its upregulation by CCTs could enhance redox resilience during OS or inflammation. Conversely, elevated GSSG, particularly in male rats treated with TIA-123, may imply a compensatory response to high oxidative turnover, aligning with prior studies where increased GSH/GSSG ratios promoted cellular proliferation, while diminished ratios were associated with apoptotic pathways. This pro-oxidative redox environment could, therefore, influence cellular pathways that govern stress regulation (Anashkina et al. 2020, Ribeiro 2023).

The findings on thiol levels in Figure 2 also reveal CCT-induced changes in thiol-disulfide homeostasis, with notable thiol reductions in male rats for compounds CMD-8 and CMG-41, implying heightened oxidative activity. Such reductions might increase disulfide bond formation, suggesting a more oxidative cellular environment. Conversely, females displayed trends of boosted thiol levels, indicating an enhanced antioxidative response. This highlights a sex-specific ability to preserve thiol concentrations and underscores the relevance of thiol-disulfide balance in erythrocyte functionality and oxidative defense. These observations also suggest that males and females may differ in their response to CCT-induced oxidative stimuli, with implications for conditions relying on precise redox regulation.

A similar redox regulatory function of GSH has been previously documented, as it modulates cellular signaling via cysteine oxidation (Anashkina et al. 2020, Ribeiro 2023). Glutathionylation, where GSH binds reversibly to protein cysteine residues, creates glutathionylated proteins (Prot-SSG), which can alter protein activity, thereby protecting protein thiols from irreversible oxidation and helping conserve GSH under OS. Deglutathionylation, mediated by enzymes like glutaredoxin and sulfiredoxin, restores protein function using GSH as a reducing agent (Liu et al. 2010). The capacity for ROS and RNS to regulate proteins and signaling molecules through cysteine oxidation is well-documented, with GSH as a critical modulator in this process (Garcia-Ruiz et al. 2006, Liu et al. 2010, Anashkina et al. 2020, Lu 2020, Ribeiro 2023, Chen et al. 2024).

In the γ -glutamyl cycle, GSH is a sustained source of cysteine, essential given cysteine's instability and tendency to oxidize into cystine, which could produce harmful radicals. This cycle's involvement in GSH synthesis underscores its role in redox balance (Wu et al. 2004). Dysregulated GSH synthesis has been associated with aging, metabolic, and liver disorders (Wu et al. 2004).

The data affirm glutathione's effectiveness in maintaining oxidative balance. Thiols and disulfides participate in key biological functions, such as protein structure, redox homeostasis, and polymer secondary structure formation (Leichner et al. 2019). As an adaptive response to ROS, disulfide bonds formed within proteins contribute to self-repairing materials due to their reversible nature (Jin et al. 2013). Various studies confirm that altering the thiol-disulfide ratio modulates cellular processes: increased GSH/GSSG ratios promote proliferation, while lower ratios signal apoptosis (Nkabyo et al. 2002, Berndt et al. 2014).

Given ROS's multifaceted role in cellular regulation and cytotoxicity, targeted therapies against ROS must balance tumoricidal effects with normal cell protection. Future research should consider this complex regulatory network to develop redox-targeting treatments with precision (Yang et al. 2013).

The results herein reflect the selective biological effects of CCTs. Copper coordination compounds can directly affect the redox balance in erythrocytes by cycling between Cu(I) and

Cu(II) states, thereby influencing the formation and detoxification of reactive oxygen species (ROS). Specifically, these complexes may:

- Catalyze redox reactions: The copper ion can participate in Fenton-like or Haber-Weiss reactions, leading to ROS formation. Under controlled conditions, such activity may help regulate redox signaling (Malarz et al. 2018).
- Scavenge superoxide: Certain Cu-thiosemicarbazone complexes can mimic superoxide dismutase (SOD) activity, thereby reducing superoxide to hydrogen peroxide and mitigating oxidative stress (Menezes et al. 2024).
- Interact with GSH: Copper coordination compounds bind to glutathione, influencing its oxidation state. While this can transiently enhance OS, it also upregulates antioxidant defense pathways and maintains GSH/GSSG homeostasis (Hancock et al. 2011).
- Modulate protein sulfhydryls: By altering cysteine residues on hemoglobin and other proteins, these compounds can foster reversible glutathionylation, thus protecting protein thiols under oxidative conditions and stabilizing the redox environment in erythrocytes (Ramek 2021).

Through these mechanisms, copper coordination compounds with thiosemicarbazones display both prooxidant (facilitating ROS formation for signaling or cytotoxic effects) and antioxidant (SOD-like activity, GSH regeneration) properties, ultimately fine-tuning erythrocyte redox balance.

These results support the potential of CCTs as agents in redox modulation and warrant further exploration into their applications in oxidative stress-related pathologies as seen in other publications as described previously (Pantea et al. 2022, Pantea et al. 2023, Pantea 2023).

Conclusions

CCTs serve as metal ion ligands with promising therapeutic applications, particularly in anticancer treatments. The biological effects of copper coordination compounds with thiosemicarbazones are highly selective and varied, attributed to their complex actions across multiple cellular targets. Specifically, TSC metal complexes can induce toxicity by generating reactive oxygen species through the activation of molecular oxygen *via* metal ions, leading to the formation of radicals that potentially reduce cellular thiol content. These points to the necessity of understanding the complex interplay of physiological and pathological pathways involved in cellular redox balance.

In vivo studies demonstrate that CCTs selectively preserve thiol-disulfide homeostasis in erythrocytes by enhancing total and reduced glutathione levels and reducing oxidized glutathione, underscoring their antioxidant properties.

These findings suggest that CCTs hold therapeutic promise in cancer treatment by modulating cellular redox status through both antioxidant and prooxidant effects. Consequently, this research has the potential to contribute significantly to the development of novel therapeutic insights. The redox activity and biocompatibility of copper ions, the stability of copper coordination compounds in the bloodstream, and promising therapeutic outcomes *in vivo* collectively support the potential for clinical application of copper coordination compounds in cancer and redox-related pathologies.

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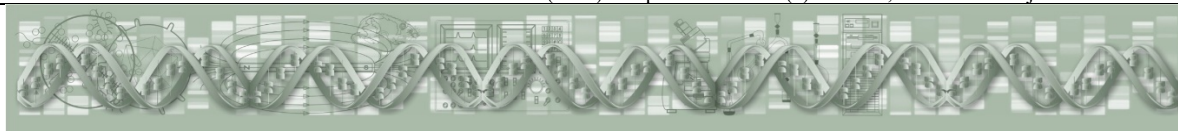
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CONTRIBUTION TO THE KNOWLEDGE OF ODONATA (INSECTA) IN MOLDOVA: IAȘI COUNTY

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Abstract

From 21-vi to 29-vi-2024 I made an odonatological study trip to Iași County, northeastern Romania. During this trip I examined 39 sites of both standing and flowing water and noticed 23 species of Odonata. Many sites are threatened by littering and drying out. The results of the trip demonstrate the summer aspect of the Southeast European dragonfly and damselfly fauna; neither spring nor autumn species were observed. Interesting from a faunistic point of view are the very early observation of immature *Sympecma fusca* at two sites, the northernmost record of *Somatochlora meridionalis* east of the Carpathians, and the almost complete absence of species of the genera *Lestes*, *Aeshna*, *Gomphus* s.l., *Libellula* and *Sympetrum*. The mentions of some species in the literature are critically examined.

Keywords: Romania, Moldova, Odonata, check-list, faunistics

Introduction

Moldova (Figure 1B) as one of the three major historical regions of the modern Romanian state is located in the northeastern part of the country (Figure 1A) and borders (in general) by the Carpathians to the west, the Ukraine to the north, the Prut River to the east and the Milcov, Siret and Danube rivers to the south.

Iași County (Figure 1C) with an area of *ca.* 5,476 km² is located in eastern Moldova. Its climate is temperate-continental (*sensu* Beck et al. 2023: Dfb, Prut valley: Dfa). The relief is hilly with heights between 22 m a.s.l. (Prut floodplain near Gorban in the south) and 593 m a.s.l. (in the Pădurea Tudora near Deleni in the northwest); it is characterized by valleys and floodplains of large and small rivers. The county is heavily influenced by agriculture (*ca.* 70 % of the area is agricultural land), forest covers *ca.* 18% of the county, and water bodies only take up *ca.* 0.75%. Rivers and streams of various sizes (Prut, Siret, Jijia, Bahlui, etc.) determine the hydrographic network, natural standing waters are almost completely absent (only a few oxbows of the rivers), whereas countless reservoirs of various sizes exist on many streams and rivers (all data: INS / DJS IS 2024).

The dragonfly fauna of Iași County is only moderately studied, with modern data almost completely lacking. The first mention dates from Cîrdei and Borcea (1949), who recorded a total of 22 species for nine locations in the county. Further investigations followed (Cîrdei 1956b, 1956c, Cîrdei and Bulimar 1961) and increased the total number of species mentioned for the county to 37. Cîrdei and Bulimar (1965) attempted to summarize all previous and numerous newer mentions. They listed a total of 32 species for Iași County, increasing the total number of species recorded here to 40. The summary by Lehrer and Bulimar (1979) showed mentions of 41 species and a total number of 43 species. Mancî (2011) examined the dragonfly



collection of the Iași Museum of Natural History and found 41 species collected in the county; this increased the total number of species reported for the county to 49. As part of his dissertation, Mancî (2012) created distribution maps of Odonata occurring in Romania and shows the occurrence of 48 species in the county. The total number rose to 51 species. Some publications based on limnological studies, some of which contained “unexpected” species (Nicoară et al. 2009, Ghețu 2012), were apparently not included in Mancî (2012). They increase the total number of records to 53 odonate species for Iași County. Recently, several chance observations of Odonata by laypeople have been published in open natural history databases on the Internet (e.g. iNaturalist.org; observation.org). However, a modern overview of the county's odonate fauna is missing.

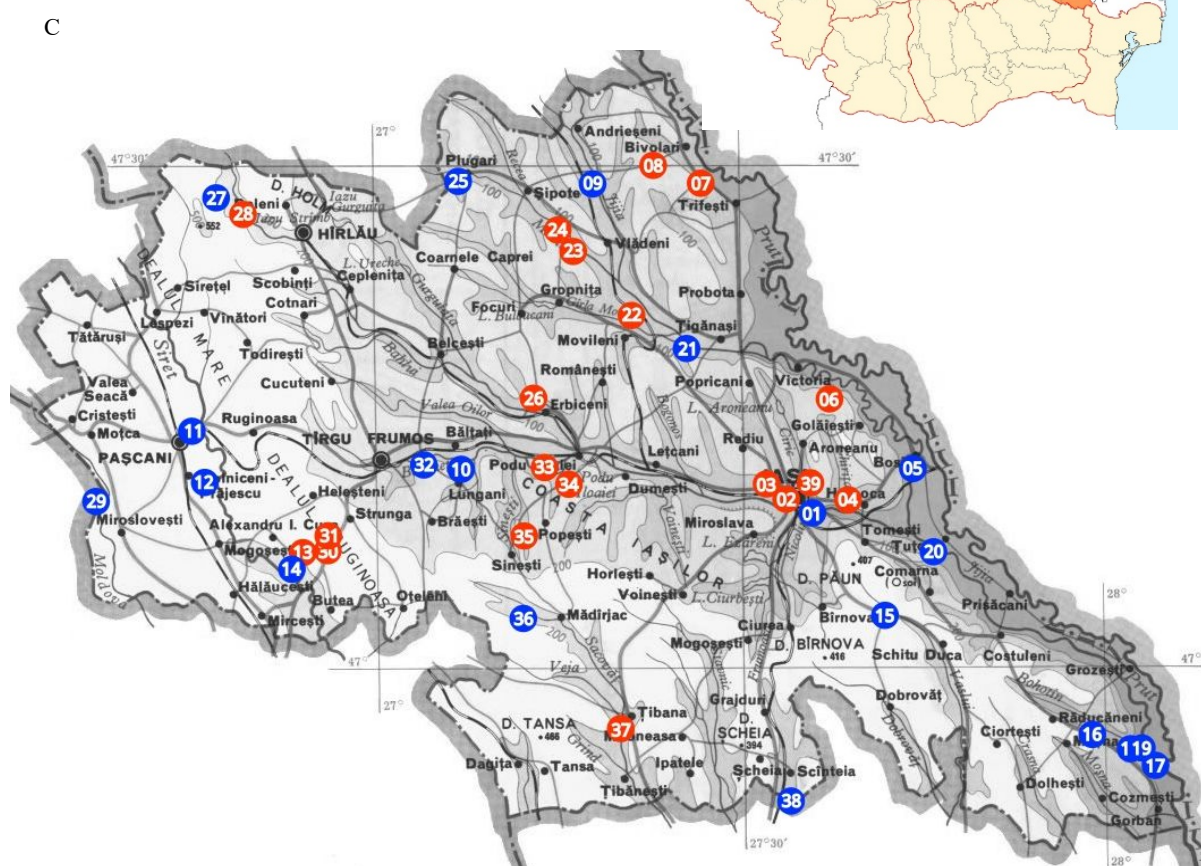


Figure 1. Situation of Romania (brown) within Europe (A) and Moldova (orange) with Iași County (green) within Romania (B), Iași County with the visited locations (C; red: standing waters, blue: flowing waters; see Table1)

A: © Blank political map from Alexrk2, CC BY-SA 3.0; slightly edited;

B: © Romanian Moldavia location map from Andrein, CC BY-SA 4.0; slightly edited;

C: background map: © Harta judetului Iasi (Harta Romaniei 2007-2024), edited.

Material and Methods

In order to get a modern overview of the dragonfly fauna of the county, I undertook a study trip from 21-iv to 29-vi-2024. To prepare for the trip, I searched maps and aerial photographs available on the Internet (Mapy.cz 1996-2024; ANCPI 2019-2024) for water bodies that were relatively easy to reach and freely accessible. Based on the location information in the existing literature (e.g. Cîrdei and Bulimar 1965, Mancu 2011, iNaturalist.org, observation.org), I included individual water bodies with evidence of dragonflies and damselflies in this pre-selection.

During my trip, I visited the preselected waters for varying lengths of time (between 15 minutes and 1 hour). The banks were walked for different lengths and all observed odonate species and their behaviour were recorded. I tried to document photographically each species that occurred, which was not always successful, especially with species of the Aeshnidae. Only once, I caught a specimen with an entomological net (diameter 40 cm) for reliable determination, but released it again afterwards. To search for larvae, I used a standard kitchen sieve (diameter 15 cm). I did not explicitly search for exuviae, but collected exuviae discovered by chance.

Imagines were identified using Lehmann and Nüß (2015) and Dijkstra et al (2020), for the identification of larvae and exuviae I used a digital microscope “DM-300” (Magainon, supra Foto-Elektronik-Vertriebs-GmbH, Kaiserslautern, Germany) and Brochard et al (2012), Gerken and Sternberg (1999), and Heidemann and Seidenbusch (2002). Coordinates of the locations were taken using the smartphone app “GPS Data” (examobile S.A, Bielsko-Biała, Poland) and verified by using the map services Mapy.cz (1996-2024) and ANCPI (2019-2024). The nomenclature follows Boudot and Kalkman (2015).



Figure 2. Examples of sites visited, top row: standing waters, bottom row: flowing waters
A) temporary pond (pond between Lacul Bogdănești and Lacul Hăbășești [loc. 31], 27-iv-2024); B) reservoir (Acumularea Podu Iloaiei [loc. 33], 28-iv-2024); C) stream (Bohotin [loc. 16], 25-iv-2024); D) channeled river (Jijia [loc. 09], 23-iv-2024)

Results

In total, I visited 39 water bodies (Table1; Figure 1C) throughout the county within nine days, including 19 standing (lentic; Figure 2 A,B) and 20 flowing water bodies (lotic; Figure 2 C,D). I visited location 02 twice. I observed dragonflies and damselflies at 37 locations. Location 36 (a stream) was dried up, at location 29 (Moldova River) only a very exhausted female *Calopteryx splendens* (Harris, 1782) was floating past on the water.

Table 1. Locations visited during this study

loc.	name ¹⁾ type	north ²⁾	east ²⁾	height ³⁾	nearest settlement date	species
01	Bahlui channeled river	47.1536	27.6066	61	Iași 21-iv	5
02	concrete pond in Parcul Copou garden pond	47.1781	27.5689	155	Iași 22-, 29-iv	2
03	reservoir in Grădina Botanică “Anastasiu Fătu” reservoir	47.1853	27.5494	65	Iași 22-iv	7
04	Acumularea Chirița reservoir	47.1678	27.6547	89	Dancu 23-iv	9
05	Jijia channeled river	47.2094	27.7378	35	Bosia 23-iv	6
06	oxbow of Jijia river (oxbow)	47.2822	27.6181	38	Luceni 23-iv	3
07	small reservoir in Soloneț River reservoir	47.4939	27.4503	56	Soloneț 23-iv	3
08	Acumularea Traian III reservoir	47.5094	27.3986	74	Traian 23-iv	2
09	Jijia channeled river	47.4869	27.3053	48	Spineni 23-iv	7
10	Bahluieț river	47.2108	27.1222	71	Bălțați 24-iv	4
11	Siret river	47.2469	26.7511	204	Pașcani 24-iv	2
12	Siret river	47.1842	26.7803	199	Cozmești 24-iv	2
13	Acumularea Mihail reservoir	47.1250	26.8994	214	Scheia 24-iv	2
14	Siret river	47.0994	26.8922	189	Scheia 24-iv	4
15	Vasluiș stream	47.0631	27.7036	230	Poeni 25-iv	2
16	Bohotin stream	46.9439	27.9936	63	Bohotin 25-iv	4
17	Jijia Veche river	46.9058	28.0814	26	Gura Bohotin 25-iv	4
18	drainage ditch ditch	46.9258	28.0475	26	Zberoaia 25-iv	3

Table 1. continued

19	Jijia Veche river	46.9269	28.0592	26	Zberoaia 25-iv	5
20	Jijia channeled river	47.1200	27.7664	32	Chiperești 25-iv	4
21	Jijia river	47.3214	27.4319	40	Țigănași 26-iv	7
22	Delta Moldovei reservoir	47.3536	27.3569	44	Movileni 26-iv	4
23a	Lacul Hălteni , southwest bay reservoir	47.4247	27.2678	52	Hălteni 26-iv	3
23b	Lacul Hălteni , middle north shore reservoir	47.4286	27.2831	52	Hălteni 26-iv	5
24	pond north of Miletin River temporary pond	47.4331	27.2555	54	Hălteni 26-iv	4
25	Miletin river	47.4839	27.1192	63	Plugari 26-iv	5
26	reservoir in Durușca (Buhușoia) River reservoir	47.2792	27.2319	76	Erbiceni 26-iv	5
27	Bahluieț stream	47.4747	26.7950	198	Pârcovaci 27-iv	5
28	Acumularea Pârcovaci reservoir	47.4542	26.8194	169	Pârcovaci 27-iv	2
29	Moldova river	47.1800	26.6200	254	Soci 27-iv	0
30	Lacul Copilași reservoir	47.1167	26.9444	218	Miclăușeni 27-iv	5
31	pond between Lacul Bogdănești and Lacul Hăbășești temporary pond	47.1339	26.9478	229	Hăbășești 27-iv	5
32	Bahluieț river	47.2127	27.0708	77	Prigoreni 27-iv	2
33	Acumularea Podu Iloaiei reservoir	47.2008	27.2439	62	Podu Iloaiei 28-iv	4
34	reservoir in Hărpășești River reservoir	47.1900	27.2703	68	Scobâlteni 28-iv	5
35	reservoir in Gâmboasa River reservoir	47.1440	27.2050	78	Obrijeni 28-iv	4
36	Balta Neagră stream	47.0597	27.2075	198	Mădârjac 28-iv	0
37a	Acumularea Tungujel , northeastern shore reservoir	46.9606	27.3428	157	Moara Ciornei 28-iv	5
37b	Acumularea Tungujel , southeastern shore reservoir	46.9422	27.3458	157	Moara Ciornei 28-iv	4
38	Rebricea river	46.8769	27.5689	131	Sasova 28-iv	2
39	dam between Lacul Ciric I and Lacul Ciric II reservoir	47.1825	27.6036	51	Iași 29-iv	5

Remarks: ¹⁾ bold = name taken from the geoportal (ANCPI 2019-2024); ²⁾ in, Datum: WGS84;
³⁾ in m above sea level

I observed 23 Odonata species (Table 2). All species except *Aeshna affinis* Vander Linden, 1820 and *Anax imperator* Leach, 1815 were documented photographically. Depending on the location, the number of species varied between one and nine (Table 1). The most common species were *Ischnura elegans* (Charpentier, 1825) (Figure 3A; observed at 25 locations), *Platynemesis pennipes* (Pallas, 1771) (23 locations), and *Orthetrum albistylum* (Selys, 1848) (Figure 3B; 21 locations). Only once I observed *Lestes barbarus* (Fabricius, 1798), *Calopteryx virgo* (Linnaeus, 1758), *Coenagrion ornatum* (Selys, 1850) (Figure 5), *Aeshna affinis*, and *Onychogomphus forcipatus* (Linnaeus, 1758) (Figure 6).

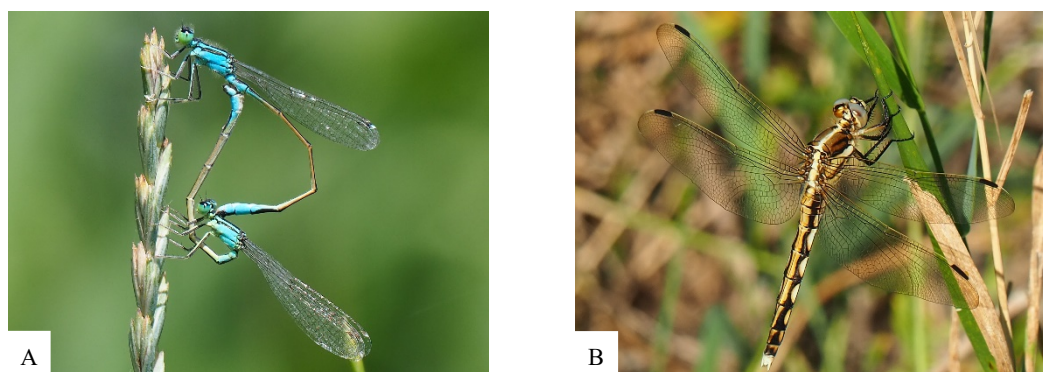


Figure 3. The most common species during this study: the most common damselfly was *Ischnura elegans* (A, pair in copula, Delta Moldovei [loc. 22], 26-iv-2024), the most common dragonfly was *Orthetrum albistylum* (B, female, Miletin river [loc. 25], 26-iv-2024)

At lentic habitats, I observed 16 species, only at lentic habitats four species: *Lestes barbarus*, *Coenagrion puella* (Linnaeus, 1758), *Enallagma cyathigerum* (Charpentier, 1840), and *Sympetrum fonscolombii* (Selys, 1840). At lotic habitats, I observed 19 species, only at lotic habitats seven species: *Calopteryx splendens*, *C. virgo*, *Coenagrion ornatum*, *Aeshna affinis*, *Onychogomphus forcipatus*, *Somatochlora meridionalis* Nielsen, 1935 (Figure 7), and *Orthetrum coerulescens* (Fabricius, 1798).

I searched for larvae at three locations (loc. 12, 15, and 27), but was only able to catch one single, very small (very young) larva of *Somatochlora cf. meridionalis* at loc. 15. This larva is in my personal collection.

At three locations, I discovered exuviae, all *Orthetrum cancellatum* (Linnaeus, 1758): loc. 08 one female, loc. 22 one male, and loc. 37b eight males and 15 females. All exuviae are in my personal collection.

The 23 species I observed correspond to *ca.* 32% of the 71 species mentioned for Romania (Manci 2012, Boudot and Kalkman 2015, Wildermuth and Martens 2019), *ca.* 43% of the 53 species mentioned for Iași County (Cîrdei and Bulimar 1965, Lehrer and Bulimar 1979, Nicoară et al 2009, Ghețeu 2012, Manci 2012) or 50% of the 46 species which I consider reliable to be part of the Iași County Odonata fauna (see Discussions and Appendix 1).

Discussions

Species spectrum. I deliberately chose the time of my trip, the end of June. My own experience (unpubl.) shows that summer species as well as the last spring species and also the first autumn species can be found. Unfortunately, I was not able to observe any spring species during my trip (too late?), and I also only observe very few autumn species (too early?). So, the results of my trip show only a picture of the summer species for Iași County and Moldova.

Table 2. Species, their behaviour and numbers at the respective locations.

no.	species	loc. ¹⁾ (behaviour ²⁾ : abundance class ³⁾
1	<i>Lestes barbarus</i> (Fabricius, 1798)	31 (F: II) ⁴⁾
2	<i>Sympecma fusca</i> (Vander Linden, 1820)	09 (F: I) ⁴⁾ ; 31 (F: I) ⁴⁾
3	<i>Calopteryx splendens</i> (Harris, 1782)	10 (F: IV); 11 (F: III); 12 (F: IV); 14 (F: I); 16 (F: V); 29 ⁵⁾ ; 32 (F, E: V)
4	<i>Calopteryx virgo</i> (Linnaeus, 1758)	27 (F: II)
5	<i>Platycnemis pennipes</i> (Pallas, 1771)	01 (F, M: III); 03 (F: II); 04 (F: II); 05 (F: II); 07 (F, E: II); 09 (F: II); 10 (F: II); 11 (F: III); 12 (F, C: V); 14 (F: IV); 15 (F: II); 16 (F, E: III); 18 (F: II); 19 (F: II); 21 (F: II); 22 (F: II); 25 (F, E: IV); 27 (F: IV); 30 (F: III); 31 (F: II); 32 (F: III); 38 (F, E: III); 39 (F: I)
6	<i>Coenagrion ornatum</i> (Selys, 1850)	16 (F: II)
7	<i>Coenagrion puella</i> (Linnaeus, 1758)	02 (F, C, E: II); 31 (F: II)
8	<i>Enallagma cyathigerum</i> (Charpentier, 1840)	26 (F: III); 34 (F: I); 35 (F: II)
9	<i>Erythromma viridulum</i> (Charpentier, 1840)	01 (F, M: II); 03 (F, C, E: IV); 04 (F: III); 05 (F, C: II); 06 (F, C, Em: V); 09 (F: III); 17 (F, C: V); 18 (F: I); 19 (F: II); 20 (F, E: III); 21 (F, C, E: III); 23b ⁶⁾ ; 25 (F: III); 26 (F: III); 37a (F: IV); 39 (F, E: III)
10	<i>Ischnura elegans</i> (Vander Linden, 1820)	01 (F, C: IV); 03 (F: II); 04 (F, C: II); 07 (F, C: II); 08 (F, C: V); 09 (F, C: IV); 10 (F: II); 13 (F: II); 14 (F: III); 17 (F, C: III); 18 (F: III); 19 (F: III); 20 (F: III); 21 (F, C: V); 22 (F, C: V); 23a (F, C: II); 23b (F, C: V) ⁷⁾ ; 24 (F: III); 25 (F, C: V); 26 (F, C: V); 30 (F, C: III); 33 (F: II); 34 (F, C: V) ⁸⁾ ; 35 (F, C: III); 37a (F, C: V) ⁹⁾ ; 37b (F, C: V) ⁷⁾ ; 39 (F: II)
11	<i>Ischnura pumilio</i> (Charpentier, 1825)	07 (F: II); 09 (M: II); 10 (F: II); 16 (F: I); 17 (F: I); 23b (F, C: IV); 24 (F, C: V); 26 (F: II); 33 (F, C: II); 35 (F, C: IV); 37b (F, M: V) ¹⁰⁾
12	<i>Aeshna affinis</i> Vander Linden, 1820	05 (F: I)
13	<i>Anax imperator</i> Leach, 1815	04 (F: I); 21 (F: I); 34 (F: I); 37a (F: II)
14	<i>Anax parthenope</i> (Selys, 1839)	01 (F: IV); 03 (F: II); 04 (F: III); 05 (F: I); 09 (F: I); 21 (F: I); 23a (F: I); 25 (F: I); 33 (F: I); 39 (F: I)
15	<i>Onychogomphus forcipatus</i> (Linnaeus, 1758)	27 (F: V)
16	<i>Somatochlora meridionalis</i> Nielsen, 1935	15 (F, [L]: III) ¹¹⁾ ; 27 (F: II)
17	<i>Crocothemis erythraea</i> (Brullé, 1832)	03 (F, C: II); 04 (F: III); 06 (F: II); 19 (F: II); 23b (F: I)

Table 2. continued

18	<i>Orthetrum albistylum</i> (Selys, 1848)	03 (F, C, E: IV); 04 (F: II); 05 (F: IV); 06 (F: II); 09 (F, E: II); 13 (F: I); 17 (F: II); 19 (F: I); 20 (F, E: III); 21 (F, C, E: V); 22 (F: III); 23b (F, C: V); 24 (F: II); 25 (F: III); 28 (F: II); 30 (F: II); 33 (F: I); 34 (F: III); 35 (F: IV); 37a (F: II); 39 (F, C, E: V)
19	<i>Orthetrum cancellatum</i> (Linnaeus, 1758)	01 (F, M, C: V); 03 (F, C, E: III); 04 (F, M: V); 08 (Ex: I); 20 (F: II); 22 (F, Ex: IV); 30 (F: III); 34 (F: II); 37b (Ex: IV); 39 (F: III)
20	<i>Orthetrum coerulescens</i> (Fabricius, 1798)	05 (F: II); 27 (F: II)
21	<i>Sympetrum fonscolombii</i> (Selys, 1840)	23a (F: II); 24 (F: I); 26 (F, C: II); 37a (F: IV); 37b (F: II)
22	<i>Sympetrum meridionale</i> (Selys, 1841)	04 (M: I); 14 (M: II); 21 (F: I); 23b (F: I); 28 (F: I)
23	<i>Sympetrum sanguineum</i> (O.F. Müller, 1764)	02 (F: I); 31 (F: III); 38 (F: II)

Remarks: ¹⁾ see Table 1. ²⁾ C: copula, E: egg laying, Em: emergence, Ex: exuvia(e), F: flight, L: larva, M: maiden flight. ³⁾ abundance class *sensu* Chovanec et al (2012) (see Table 3). ⁴⁾ immature. ⁵⁾ only one exhausted female floating on the water. ⁶⁾ no observation despite lush submerged vegetation. ⁷⁾ F: more than 1,000 specimens, C: more than 100 pairs. ⁸⁾ F: V, C: IV. ⁹⁾ F: V, C: V. ¹⁰⁾ F, M: more than 1,000 specimens. ¹¹⁾ one single, very small (very young) larva *Somatochlora cf. meridionalis*.

Previous records. My original goal was to revisit as many previously mentioned dragonfly locations as possible. In preparation for my trip, I therefore evaluated the literature available to me on the occurrence of dragonflies and damselflies in Iași County (see Introduction). However, most publications only listed the nearest settlements. Mancî (2011) lists highly accurate coordinates for the sites, but these refer to the center of the nearest settlement. It was therefore difficult to precisely locate many of the finds made so far. In order to stick to my schedule, previous sites could not be too far off my planned route. Therefore, I was finally only able to visit seven sites (four undoubted and three doubtful) from which observations had already been published (Table 4). Some species in these older publications appear very dubious from today's perspective. I discuss their records in the section "Remark to some families" below.

Threats. The waters in Iași County are exposed to various threats. One of the main problems is littering. Except for the streams (loc. 15, 27, and 36) and the pond in Parcul Copou (loc. 02),

Table 3. Abundance classes: imagines/100 m (Chovanec et al. 2012)

	I	II	III	IV	V
	(single)	(rare)	(frequent)	(abundant)	(extremely abundant)
Zygoptera without Calopterygidae	1	2-10	11-25	26-50	> 50
Calopterygidae and Libellulidae	1	2-5	6-10	11-25	> 25
Anisoptera without Libellulidae	1	2	3-5	6-10	> 11

Table 4. Visited locations with previously published records of Odonata in Iasi County with mentioned / observed species.

loc. ¹⁾	name of the location ²⁾ 4) species	source(s) ³⁾
01	Bahlui (undoubted) a) <i>E. viridulum</i> , <i>I. elegans</i> , <i>A. parthenope</i> b) <i>P. pennipes</i> , <i>O. cancellatum</i> c) –	i121855351, i170489327, i170488438
03	Grădina Botanică Iași (undoubted) a) <i>E. viridulum</i> , <i>C. erythraea</i> b) <i>I. elegans</i> , <i>P. pennipes</i> , <i>A. parthenope</i> , <i>O. albistylum</i> , <i>O. cancellatum</i> c) <i>L. barbarus</i> , <i>L. dryas</i> , <i>L. virens</i> , <i>S. fusca</i> , <i>I. pumilio</i> , <i>C. puella</i> , <i>C. pulchellum</i> , <i>A. imperator</i> , <i>I. isoceles</i> , <i>L. depressa</i> , <i>S. depressiusculum</i> , <i>S. meridionale</i> , <i>S. sanguineum</i> , <i>S. striolatum</i> , <i>S. vulgatum</i>	G, i18116208, i27717355
15	Bîrnova, Pd. Bîrnova, Bârnova, Pd. Bârnova (doubtful) a) – b) <i>P. pennipes</i> , <i>S. meridionalis</i> c) <i>S. fusca</i> , <i>C. splendens</i> , <i>C. virgo</i> , <i>C. ornatum</i> , <i>C. puella</i> , <i>E. cyathigerum</i> , <i>I. elegans</i> , <i>A. affinis</i> , <i>A. cyanea</i> , <i>A. mixta</i> , <i>C. heros</i> , <i>S. flavomaculata</i> , <i>L. depressa</i> , <i>O. coerulescens</i> , <i>S. flaveolum</i> , <i>S. sanguineum</i> , <i>S. striolatum</i> , <i>S. vulgatum</i>	A, C, D, E, F, G
23	Halceni Dam Lake (undoubted) a) <i>I. elegans</i> , <i>I. pumilio</i> , <i>A. parthenope</i> , <i>C. erythraea</i> , <i>O. albistylum</i> , <i>S. fonscolombii</i> , <i>S. meridionale</i> b) – c) –	H
33	Podu Iloaiei (doubtful) a) <i>I. pumilio</i> , <i>A. parthenope</i> b) <i>I. elegans</i> , <i>O. albistylum</i> c) –	G
37	Acumularea Tungujel (undoubted) / Țibana (doubtful) a) <i>E. viridulum</i> , <i>I. elegans</i> , <i>I. pumilio</i> , <i>A. imperator</i> , <i>O. albistylum</i> , <i>S. fonscolombii</i> b) – c) <i>O. cancellatum</i> , <i>S. flaveolum</i> , <i>S. meridionale</i> , <i>S. sanguineum</i> , <i>S. striolatum</i> , <i>S. vulgatum</i>	i168469464, i168469465 / G
39	Cîric (undoubted) a) <i>P. pennipes</i> , <i>E. viridulum</i> , <i>A. parthenope</i> , <i>O. albistylum</i> , <i>O. cancellatum</i> b) <i>I. elegans</i> c) <i>L. sponsa</i> , <i>L. dryas</i> , <i>S. fusca</i> , <i>C. splendens</i> , <i>C. lunulatum</i> , <i>C. puella</i> , <i>C. pulchellum</i> , <i>E. cyathigerum</i> , <i>I. pumilio</i> , <i>A. imperator</i> , <i>B. pratense</i> , <i>L. depressa</i> , <i>L. fulva</i> , <i>L. quadrimaculata</i> , <i>O. coerulescens</i> , <i>S. depressiusculum</i> , <i>S. flaveolum</i> , <i>S. sanguineum</i> , <i>S. striolatum</i> , <i>S. vulgatum</i>	A, B, C, D, E, F, G

Remarks: ¹⁾ (see Table 1). ²⁾ name of the location in previous publications (same site as my location: undoubtedly resp. doubtfully). ³⁾ A = Cîrdei and Borcea (1949), B = Cîrdei (1956a), C = Cîrdei (1956b), D = Cîrdei (1956c), E = Cîrdei and Bulimar (1961), F = Cîrdei and Bulimar (1965), G = Mancu (2011), H = Ghețu (2012), i12345678 = <https://www.inaturalist.org/observations/12345678/> ⁴⁾ species observed by me: a) only, b) also, c) not (crossed out: see corresponding family in Discussion)

I found garbage at all locations, mainly empty beverage containers, but also construction rubble, car tires, etc. It is hoped that the deposit system introduced in November 2023 (RetuRO 2024) will at least reduce the flood of empty beverage containers. However, the occasional discovery of containers with a deposit logo raises doubts about this.

Rivers and the reservoirs created along their course are in danger of drying up. Thus, Balta Neagră (a stream, loc. 36) was completely dried up, and Lacul Hălțeni (loc. 23) was largely without water. Between 1961 and 2012, the average air temperature in Iași County was 10.3°C, the average precipitation was 601 mm (Slave et al 2013). Compared to the period 1961-1990, the average air temperature in the period 2021-2050 will rise by up to 3.5°C, and the average precipitation will fall by up to 60 mm (Romanian Ministry of Environment, Waters and Forests 2022). For the same period, heat waves are forecast to increase in number (up to 50 %) and duration (up to 40%) (Antonescu et al. 2023). So, the climate will change in Iași County. This will also affect the Odonata fauna.

Ott (2010) summarizes the effects of climate change on Odonata. These include, for example, more prominent tendency for expansion in Mediterranean species (e.g. *Crocothemis erythraea* (Brullé, 1832): since the 1990s in Germany and Poland (Bernard et al. 2009, Ott et al. 2015), 2014 in Lithuania (Račkauskaitė and Gliwa 2015), 2015 in Denmark (Billquist et al. 2019)), more northerly breeding, also breeding in higher altitudes (e.g. *Chalcolestes viridis* (Vander Linden, 1825): Lemke 2021), or eclosion earlier in the season and overall alteration in the phenology (e.g. very early records of immature *Sympecma fusca* (Vander Linden, 1820) during my trip: see below).

Cerini et al. (2020) were able to demonstrate that climate change causes more species to become locally extinct than to be replaced by new species. The replacement occurs more in lentic than lotic habitats. Specialized species are more likely to be threatened with local extinction, whereas generalist species are more common among the new colonists. So, the current dragonfly fauna of Iași County is only a snapshot that will (continue to) change in the coming years and decades. A reduction in species diversity is expected.

Parasites. In addition to the dragonfly biting midge *Forcipomyia* (*Pterobosca*) *paludis* (Macfie, 1936) (Insecta: Diptera: Ceratopogonidae) (Martens et al. 2012, Wildermuth and Martens 2019: 869 ff., Lemke and Hryniuk 2022), dragonflies and damselflies are mainly parasitized by larvae of water and terrestrial mites (Wildermuth and Martens 2019: 859 ff.). A very severe infestation of these parasites can weaken the dragonfly to such an extent that it dies (Petzold 2006).

The Rubber-dinghy Water-mite *Limnochares aquatica* (Linnaeus, 1758) (Arachnida:

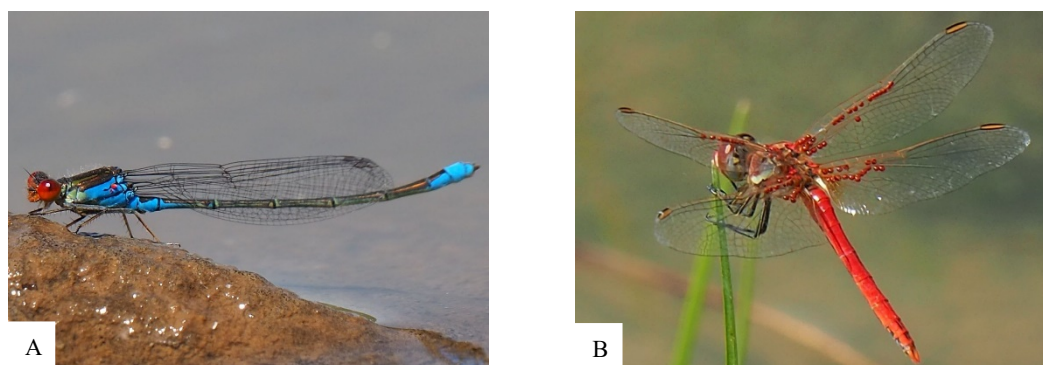


Figure 4. Examples of parasitic infestation in observed dragonflies and damselflies: A) male *Erythromma viridulum* infested with a larval *Limnochares aquatica* (red “dot” on the lateral thorax) (Acumularea Chirița [loc. 04], 23-iv-2024), B) male *Sympetrum fonscolombii* infested with more than 70 larval *Arrenurus papillator* (“pearls” on the wings) (Acumularea Tungujel [loc. 37b], 28-iv-2024)

Trombidiformes: Limnocharidae) is common and widespread in Europe. Its larva has a distinct red colouration and drop-shaped form. So far, only nine damselfly species and one dragonfly species have been identified as hosts (Wildermuth and Martens 2019: 864 ff.). I was able to observe an infestation of *L. aquatica* on two odonate species: one larva on a male *Erythromma viridulum* (Charpentier, 1840) (Figure 4A) and one larva on a female *Ischnura elegans*. Both records were on 23-iv at Acumularea Chirița near Dancu (loc. 04). Both *Erythromma viridulum* and *Ischnura elegans* are known as hosts of *L. aquatica* (Wildermuth and Martens 2019: 864 ff.).

Larvae of *Arrenurus papillator* (O.F. Müller, 1776) (Arachnida: Trombidiformes: Arrenuridae) are conspicuous as bright red balls, which are particularly recognizable as pearl-like structures on the wings of *Sympetrum* species (Wildermuth and Martens 2019: 862 ff.). On 28-iv at the southeast shore of Acumularea Tungujel near Moara Ciornei (loc. 37b) I observed a male *Sympetrum fonscolombii*, on whose four wings there were altogether more than 70 “pearls” (Figure 4B).

In addition to these two red larvae, other species of the genus *Arrenurus* parasitize Odonata (Zawal 2008). They appear as small grey-green pearls and adhere particularly to the thorax and the underside of the abdomen. Identification at species level is impossible based on photographic evidence (Wildermuth and Martens 2019: 859 ff.). The female *Ischnura elegans* at loc. 04, which was infested with at least one larva of *L. aquatica* (see above), was also parasitized by at least seven larvae of *Arrenurus* sp. They were positioned on the underside of the abdomen. At a temporary pond north of Miletin River near Hălçeni (loc. 24) I photographed on 26-iv a female *Ischnura pumilio* in copula, the underside of the thorax heavily infested with larvae of *Arrenurus* sp.

Remarks to some families

Lestidae. *Chalcolestes parvidens* / *viridis*: There is only one old record of “*Lesies* [sic!] *viridis* Vanderl.” from Iași County. Cîrdei and Borcea (1949) list it in their overview: “Lives around stagnant water, picked up from the branches of shrubs, Nicolina, August [1949].” (“Trăiește în jurul apelor stătătoare, recoltat depe [sic!] ramurile arbuștilor, Nicolina, August [1949].”) This record is repeated in Cîrdei and Bulimar (1965), Lehrer and Bulimar (1979) and Mancî (2012). *Chalcolestes parvidens* was described by Artobolevskij in 1929 as a new subspecies of *Chalcolestes viridis*. However, there is a large genetic distance between the two taxa, differences in diurnal activity and phenology, so that *C. parvidens* is considered a *bona species* (Wildermuth and Martens 2019: 44). Romania is situated in the broad transition zone between both species (Boudot and Dyatlova 2015a, Boudot and Willigalla 2015, Wildermuth and Martens 2019: 47, 53). Both *C. parvidens* and *C. viridis* occur in the neighboring Republic of Moldova (Bușmachi and Munjiu 2024). So it remains unclear whether Cîrdei and Borcea (1949) collected *C. viridis* or *C. parvidens*. Therefore, I only list the genus in the check-list (App. 1).

Lestes: The emerging of species of the genus *Lestes* observed so far in the Iași County (*L. barbarus*, *L. dryas* Kirby, 1890, *L. sponsa* (Hansemann, 1823), *L. virens* (Charpentier, 1825): Mancî 2011, 2012) begins in Central Europe from mid-May, in the Mediterranean countries even earlier (Wildermuth and Martens 2019: 57 ff.). By the time I travelled (end of June) imagines should already have emerged. Nevertheless, I was able to observe only one single specimen of a *Lestes* species: On 27-vi I saw an immature male *Lestes barbarus* at a temporary pond (loc. 31). Precisely because I visited different types of water bodies (stream, river, oxbow, channel, pond, lake), I also expected to observe other *Lestes* species. I can't explain why I saw only this one specimen and no others.

Lestes macrostigma (Eversmann, 1836): Mancî (2012) symbolizes with a blue dot a finding of this species after 1979 near Iași. The basis of this dot remains unclear. No specimen was found

in the Iași Museum of Natural History (Manci 2011). The corresponding distribution map on Manci's homepage (Manci *n.d.* [no date] e) also shows no record of this species in Moldova. So, the status of this species in Iași County remains unclear. However, an occurrence of *L. macrostigma* in the county seems possible. Bușmachi and Munjiu (2024) observed it in 2022 and 2023 in the village Măcărești, Republic of Moldova, only *ca.* 30 km southeast of Iași.

Sympecma fusca: With the observation of one male each on 23-vi (loc. 09: channeled Jijia River) and 27-vi (loc. 31: temporary pond), I was able to make very early observations of the summer generation of *S. fusca*. Their shiny wings identified them as very young specimens that had obviously emerged in the respective water body.

Sympecma is the only odonate genus in Europe that hibernate as adults. They return to the waters from February onwards and mate there. Egg laying was recorded up to June. In Central and Western Europe, the larvae develop rapidly within two to three months, and the imagines emerge from the beginning of July to September. The immatures initially stay near the water and then slowly retreat to their winter quarters, which can be up to several kilometers away from their home waters (Schweighofer 2011, Wildermuth and Martens 2019: 89 ff.). In southern France, emerging of *S. fusca* begins in early June (Boudot et al. 2017).

Of 145 evaluated data sets for Romania (Cîrdei and Bulimar 1965 with included references; Manci *n.d.* c with included references; iNaturalist.org; observation.org), 12 are from June and 26 from July (for Iași County there is only one data in these months: 1-vii-1990; Manci 2011). But only for one observation each from 29-vi (2023: iNaturalist.org/observations/170173201/) and 6-vii (2014: iNaturalist.org/observations/69051574/) it can be determined that these are specimens of the newly emerged summer generation. For all other data sets, it remains unclear whether these are specimens from the hibernating generation or the summer generation. When observing imagines of *Sympecma fusca* in June and/or July, special attention should be paid to indicating to which generation the observed specimens belong.

Platycnemididae. During my trip *Platycnemis pennipes* was the second most common species with observations at 23 locations (59 % of the locations visited). I observed this only species of the family in southeastern Europe in both lentic and lotic habitats. It was more common in lotic than in lentic habitats (observations in 84 % of lotic and 34 % of lentic habitats), and it was also more numerous in lotic (max. abundance class V; see Table 3) than in lentic habitats (max. abundance class III). Nevertheless, it also showed reproductive behaviour in lentic habitats (oviposition at loc. 07). I have not encountered *P. pennipes* in small ponds or in fish-rich or very murky waters. Remarkable because of the frequency I found: only 12 specimens of this species from five locations (all in Iași County) are deposited in the collection of the Iași Museum of Natural History (Manci 2011). It remains unclear whether the number of populations and/or the abundance of *P. pennipes* has increased or not. However, this museum has deposited 51 specimens from ten localities in Iași County of the most common species in my study, *Ischnura elegans*, and 54 specimens from seven localities in Iași County of my third most common species, *Orthetrum albistylum* (Manci 2011).

Coenagrionidae. The only representatives of the genus *Coenagrion* I observed were *C. ornatum* (loc. 16; Figure 5) and *C. puella* (loc. 02, 31). I was also expecting observations of *C. pulchellum* (Vander Linden, 1825) and *C. scitulum* (Rambur, 1842), which has already been recorded in Iași County (Manci 2011, 2012). These expectations have not been fulfilled. As with the genus *Lestes* (see above), I cannot explain why I did not see any other species.

Coenagrion lunulatum (Charpentier, 1840): There are two (or four) older reports for this species from Iași County. Cîrdei and Borcea (1949) found this species in summer 1949 at “Grasses around the swamps” (“Ierburile din jurul bălților”) at the locations Ciric and Zagavia in June and July respectively. Cîrdei (1956b) refers to Cîrdei and Borcea (1949), but mentions other sites: Cristești and Larga Jijiei, both with the date “VIII/49”. In Cîrdei and Bulimar (1965) these (false) locations are again given with reference to Cîrdei and Borcea (1949). Lehrer and Bulimar

(1979) take the original and the incorrectly reproduced locations and show in their compilation four locations for *C. lunulatum*. Mancî (2012) corrects the incorrect information from Cîrdei (1956b), Cîrdei and Bulimar (1965) and Lehrer and Bulimar (1979) and shows the two older mentions in his map: Cîric and Zagavia.

C. lunulatum is a Euro-Siberian taxon (Boudot et al. 2017) with a distribution range from Western Europe across Asia to Kamchatka. It is missing south of the Alps. It flies very early in the year, its flight period is very short (Wildermuth and Martens 2019: 195 ff.). Outside its core range in northern Central Europe, it has been recorded in Polish and Ukrainian Galicia and the high altitudes of the Romanian and Ukrainian Carpathians. There it is rare and lives in small, scattered populations (Boudot and Nelson 2015).

Because of the confusion regarding the locations of the species in Cîrdei and Borcea (1949), Cîrdei (1956b), Cîrdei and Bulimar (1965) and Lehrer and Bulimar (1979), its (current) rarity, its preference for higher altitudes in the south-eastern European countries (Boudot and Nelson 2015), and its recent Romanian records only in the heights of the Carpathians and the Apuseni Mountains (Flenker 2011, Mancî 2012), *C. lunulatum* should be removed from the check-list of Moldovan Odonata (Appendix 1). In any case, a (former) occurrence of this species in Iași County remains very doubtful.



Figure 5. *Coenagrion ornatum*, a species listed in Annex II of the EU Habitats Directive (European Community 1992), was observed only once (male, Bohotin stream [loc. 16], 25-iv-2024)

Erythromma najas (Hansemann, 1823): Lehrer and Bulimar (1979) show in their compilation five quadrants in Iași County where this species was observed. The basis of their entries remains unknown. In any case, Cîrdei and Bulimar (1965) did not mention the species for Iași County and Mancî (2011) does not find any specimens in the Iași Museum of Natural History. Mancî (2012) shows an old record (before 1979) of the species near Iași, but Mancî (*n.d.* d) does not

show any records for Iași County in its map. Except for a report by Kipping (1998), who observed *E. najas* on the same day at the same location (syntopic?) with *E. viridulum* as a similarly “common” species with similar reproductive behaviour, there are no modern records of *E. najas* in Moldova. I consider the mention in Kipping (1998) as a misidentification. *Erythromma najas* should be removed from the check-list of Moldovan Odonata (App. 1).

Ischnura elegans (Figure 3A) was by far the most common species in June 2024, both in terms of number of localities and number of specimens. I observed it at 25 locations (ca. 67 % of the locations with observations), at two locations it was abundant (class IV *sensu* Chovanec et al. 2012, see Table 3), at nine locations even extremely abundant (class V).

Female *I. elegans* occur in a number of colour variation, resulting from the combination of three mature colour morphs with ontogenetic colour changes (Sternberg 1999, Svensson et al. 2007, Wildermuth and Martens 2019: 257 f.; Dijkstra et al. 2020; Cordero-Rivera and Sánchez-Guillén 2024): the male-like morph typica (“androchrome” in Svensson et al. 2007 and Cordero-Rivera and Sánchez-Guillén 2024; “A-type” in Dijkstra et al. 2020), the morph infuscans (“B-type” in Dijkstra et al. 2020) and the morph infuscans-obsoleta (“aurantiaca” in Cordero-Rivera and Sánchez-Guillén 2024; “C-Type” in Dijkstra et al. 2020). Gorb (1999) notes that morph infuscans-obsoleta is missing in central Ukraine. Likewise, Dyatlova (2004) and Gosden et al. (2011) were unable to detect such females in southwestern Ukraine. Despite the frequency of the species and my special attention, I could not observe any females of this morph in Iași County. As in the studies of Gorb (1999), Dyatlova (2004) and Gosden et al. (2011), the morph typica was in the majority in my study: of 65 photographed females, 46 (ca. 71%) belonged to this morph, 19 (ca. 29%) were infuscans.

Nehalennia speciosa (Charpentier, 1840): Even with this species, there are contradictions in the information on the locations in the older literature. The species is not mentioned in Cîrdei and Borcea (1949), which presents results of odonatological surveys carried out in the summer 1949 in the (former) counties of Iași and Cîmpulung Moldovenesc. *Nehalennia speciosa* is mentioned for the first time in Cîrdei (1956b) as “new species for the R.P.R.” for six locations from two years (year 1949: three locations, two of them in Iași County; year 1953: three locations). An attached drawing shows the species-typical narrow transverse stripe on the rear edge of the head. Cîrdei and Bulimar (1965) only show the three locations from 1953 in their map, none of them in Iași County. Without further additions, Lehrer and Bulimar (1979) includes these three locations in their map. Mancî (2012) shows on his map the original mentions of Cîrdei (1956b), but moves the location Cucuteni (Cucuteni, commune Cucuteni; representation in Cîrdei and Bulimar 1965: Cucuteni, commune Lețcani).

According to Bernard and Wildermuth (2005), the records of 1949 should be recognized as a misidentification. Therefore, this species should be canceled from the check-list for both Iași County and Moldova (Appendix 1).

Aeshnidae. Of the seven species of this family recorded so far in Iași County, I was able to observe only three species: *Aeshna affinis*, *Anax imperator* and *A. parthenope* (Selys, 1839).

Aeshna affinis: I have observed this species only once: one male flew at loc. 05. It seems to be a rather rare species in Moldova, its main distribution areas in Romania are the Banat and the Danube Delta (Mancî 2012). Of 312 specimens deposited in the Iași Museum of Natural History, only 21 are from Iași County; they are also the only specimens from Moldova. In the literature (Cîrdei and Bulimar 1961, 1965; Lehrer and Bulimar 1979; Mancî 2011) it is mentioned for up to seven locations.

Aeshna cyanea (Müller, 1764) is a typical species of late summer and early autumn. Regarding them too, my trip was too early. It is questionable whether *A. cyanea* currently occurs in Iași County (and Moldova). It is only mentioned in older publications. Cîrdei and Borcea (1949), Cîrdei (1956c) and Cîrdei and Bulimar (1965) report an occurrence for two localities in Iași County. Lehrer and Bulimar (1979) show five quadrants with records of this species in the

county. Mancî (2012) discards these records and shows only two dots with older records (the locations of the other literature should appear as one dot), but no dot with younger records after 1979. There is no specimen deposited in the Iași Museum of Natural History (Mancî 2011). There are also no entries for Iași County and Moldova in the online databases (iNaturalist.org; observation.org). In July 2024, Bușmachi (in lit.) was able to observe two male *A. cyanea* for the first time for the Republic of Moldova in the Scientific Reserve “Plaiul Fagului”. This location is only *ca.* 25 km east of the Romanian-Moldovan border.

Aeshna mixta (Latreille, 1805) is another typical species of late summer and early autumn. My trip was too early to observe them. The specimens from Moldova deposited in the Iași Museum of Natural History were collected mainly in August, only exceptionally in July (17, 18 and 29-vii; Mancî 2011). Recent finds from Mancî (*n.d.* a, <https://observation.org/observation/288398697/>) date from September.

Anax parthenope was a common species, with observations at ten water bodies. It flew on both lentic and lotic water. The occurrence of this holo-Mediterranean (Boudot et al 2017) and northward expanding species (Kalkman and Proess 2015) also seems to have increased in Moldova and Iași County. Cîrdei and Bulimar (1961) reported the find of “several specimens” (“cîteva exemplare”) as larvae from a location near Iași, but did not mention this find in their compilation four years later (Cîrdei and Bulimar 1965). Lehrer and Bulimar (1979) show an additional find, which Mancî (2012) rejects. He shows only the old record of Cîrdei and Bulimar (1961) and the two newer locations of the specimens deposited in the Iași Museum of Natural History (Mancî 2011).

I could not observe either *Brachytron pratense* (Müller, 1764) or *Isoaeschna isoceles* (O.F. Müller, 1767); both already fly in late spring. The specimens from *B. pratense* deposited in the Iași Museum of Natural History were collected in May and early June, those from *I. isoceles* mainly in June, only two of them in the third decade (Mancî 2011). So my trip was too late to observe these species.

Gomphidae. Before my trip, I hoped to observe at least three species from this family: *Gomphus vulgatissimus* (Linnaeus, 1758), *Onychogomphus forcipatus* and *Stylurus flavipes* (Charpentier, 1825).

Gomphus vulgatissimus: There is only one mention of this species in Iași County: in the map in Lehrer and Bulimar (1979) is a dot near Iași. It remains unknown what this dot is based on. Neither Cîrdei (1956c) nor Cîrdei and Bulimar (1965) report a corresponding find. The map in Mancî (2012) lacks a corresponding dot.

Because this species has been mentioned only once in the literature, without any precise information on location, habitat and year, *G. vulgatissimus* should be excluded from the checklist of dragonflies and damselflies of Iași County (Appendix 1).

But it seems possible that this species occurs in wide and calmly flowing parts of the Jijia and Miletin rivers, possibly also in large reservoirs along their course (e.g. Acumularea Hălăeni, Lacul Vlădeni, Lacul Câmpeni). I also consider populations in the Prut River to be possible. During my visit, the current of the Moldova and Siret rivers proved to be too strong for the occurrence of *G. vulgatissimus*. This species, which is widespread in Central and Eastern Europe, inhabits mainly quiet streams and rivers in lowland areas, occasionally also the sandy banks of well-oxygenated lentic waters (Boudot et al. 2015a, Dijkstra et al. 2020: 190; Wildermuth and Martens 2019: 460). In the Republic of Moldova this species has been found several times in the Prut River (Bușmachi and Munjiu 2024). During my trip, the Romanian border police prohibited me from entering the Prut River, which marks the Romanian-Moldovan border. So I was not able to observe the species at the locations I visited. Thus, reliable evidence of this species is still lacking in Moldova.

Onychogomphus forcipatus (Figure 6): There is only one report of this species in Iași County: Ghețu (2012) reported the collection of one larva each in October 2010 and October 2011 in

Jijia River. On aerial photographs (Mapy.cz 1996-2024; ANCPI 2019-2024) the location shows strong similarities with the locations 05, 09 and 20 I visited. At these locations, the river was straightened as a channel, had a width of several meters and was accompanied by dikes. The water flowed leisurely. At Ghețeu's location, there are partly loose groups of bushes on the west bank, while the east bank has only a low herb layer.

However, the location where I found this species (loc. 27, Bahluieț stream) is completely different. I was able to observe 13 males over a length of about 80 m. Individual males sat on boulders lying in the stream and waited there for females, other males sunned themselves somewhat away from the stream on sunlit leaves of low vegetation. Only occasionally did small fights occur between the males over the stream, males outside the stream showed no aggression among each other. The Bahluieț stream was not a slowly flowing lowland river like the location at Ghețeu (2012), but a narrow, barely 1 m wide, clearly flowing stream in the middle of a large forest area. This probably corresponds to the vast majority of locations where this species has been found in Romania so far (Manci 2012, Manci *n.d.* b). So, I consider the mention in Ghețeu (2012) as a misidentification.



Figure 6. *Onychogomphus forcipatus* was observed only once. The habitat differs greatly from the habitat in Ghețeu (2012, see discussion; male, Bahluieț stream [loc. 27], 27-iv-2024)

Stylurus flavipes: This species has not yet been recorded in Iași County. Along with *Gomphus vulgatissimus*, *S. flavipes* is a species that inhabits quiet flowing streams and rivers in lowlands. However, the waters it inhabits are wider and flow more calmly than those inhabited by *G. vulgatissimus* (Boudot and Dyatlova 2015b, Wildermuth and Martens 2019: 500). I expected to observe imagines or to catch larvae on the right side of the Prut River. The species has been recorded in the Republic of Moldova on the left side of the Prut River (Munjiu et al 2014, Bușmachi and Munjiu 2024). Visits to pre-selected locations on the Prut River were thwarted

by the Romanian border police, who forbade me from staying near the river. Therefore, I could not find *S. flavipes*. So there are still no records of this species in Iași County.

***Cordulegastridae*.** At two streams that seemed suitable to me (loc. 15: Vasluiet stream, and loc. 27 Bahluiet stream) I used a sieve to search for buried larvae of *Cordulegaster* sp. I held the sieve under water and shoveled by hand fine soil (sand, fine gravel, detritus) into it. I repeated this process several times at several points, each over a stream length of 150 to 200 m. Unfortunately, I was not able to catch any larvae of *Cordulegaster* sp.

Manci (2011) lists several specimens of *Cordulegaster heros* Theischinger, 1979 deposited in the Iași Museum of Natural History, which were collected between 1974 and 1990 in the “Pd. Bârnova”. My loc. 15 is situated within the Bârnova forest, but slightly further northeast than the coordinates given by Manci (2011; but see my notes on the coordinates in Manci (2011) in the section “Previous records”). Unfortunately, I was unable to visit other streams in this forest and search for *Cordulegaster* larvae.

The drying up of smaller streams described in the section “Threats” has a particularly strong impact on *Cordulegaster heros*. Its larvae require between three and five years for their full development (Boda et al. 2015). Although *Cordulegaster* larvae can survive short dry periods hidden between rubble and in rock crevices (for *C. bidentata* Selys, 1843: Tamm 2018), they are unlikely to survive weeks or even months of streams drying up. Especially because their food is no longer available.

***Corduliidae*.** *Epitheca bimaculata* (Charpentier, 1825): In a large study conducted between March and December 2006, Nicoară et al. (2009) investigated the macroinvertebrate fauna from the river Ciric and the connected five dam lakes Lake Dorobanț, Lake Aroneanu, lakes Ciric I, Ciric II and Ciric III (Veneția). There they found, among others, a single larva, which they identified as “*Epitheca* sp.” They provide no information about the month of collection, the exact location, or the literature used for its determination.

The only European representative of the genus *Epitheca* is *Epitheca bimaculata*. It inhabits larger lentic waters with broad belts of reed, bulrush and sedges (Boudot et al. 2015b, Wildermuth and Martens 2019: 558 f.). In Romania, this species has been recorded twice so far: in the Carpathians and in the Crișana (Manci 2012). The larva of *E. bimaculata* is unmistakable. The abdomen is heavily spined dorsally and laterally, and there are two “humps” between the eyes (Gerken and Sternberg 1999, Brochard and van der Ploeg 2014). Due to the distinctive nature of the larvae and the mere mention of the genus instead of the name of the only European species in Nicoară et al. (2009), I consider the mention here to be a misidentification. So, *Epitheca bimaculata* should be removed from the check-list for Iași County (Appendix 1).

Somatochlora flavomaculata (Vander Linden, 1825): This species is first mentioned in Cîrdei and Borcea (1949) with two locations in Iași County. Later, these records are neither mentioned in Cîrdei (1956c) nor in Cîrdei and Bulimar (1961) or Cîrdei and Bulimar (1965). Only Lehrer and Bulimar (1979) again shows an occurrence of the species at the two locations in Iași County mentioned in Cîrdei and Borcea (1949). After that, this species was never mentioned again. Manci (2012) therefore does not show any locations of this species in Iași County or in Moldova. I consider the mention in Cîrdei and Borcea (1949) as a misidentification. So, *S. flavomaculata* should be removed from the check-list for both Iași County and Moldova (Appendix 1).

Somatochlora meridionalis / *metallica*: The taxon *Somatochlora meridionalis* was first described in 1935 by Nielsen as a subspecies of *Somatochlora metallica* (Vander Linden, 1825) (Wildermuth and Martens 2019: 589). Schmidt (1957) elevated the taxon to species rank without further justification. The species rank was controversial for a long time (Holuša 2009). Recently it has been generally accepted that *S. meridionalis* is a *bona species* (Wildermuth 2008).

As with other species (see above), the data on the taxon *S. metallica* in Cîrdei and Bulimar (1965) are confusing. They show two locations for this species in Iași County and refer to Cîrdei and Borcea (1949) and Cîrdei (1956a). However, these two sources do not mention any finds in Iași County. Lehrer and Bulimar (1979) includes the two wrong locations in their map for *S. metallica*. Mancu (2011) found four male specimens of *S. meridionalis* from three different locations in Iași County in the collection of the Iași Museum of Natural History. Mancu (2012) shows these three locations in his map for *S. meridionalis*. His map for *S. metallica* shows no dots either in Iași County or in Moldova. For Iași County, there are so far only records of *S. meridionalis* and no records of *S. metallica*.

Mancu (2016) recognizes a clear separation of the occurrence of both species: “In Romania, it appears to be a clear separation between the two also on ecological demands: *Somatochlora meridionalis* being found only near small and shaded running waters (mostly at low altitude); and *Somatochlora metallica* being found only at an altitude in habitats with standing waters.” So, *S. metallica* should be removed from the check-list for both Iași County and Moldova.



Figure 7. Yellow spot on the mesepimeron (red arrow) as identification feature of *Somatochlora meridionalis* (Wildermuth 2008, Dijkstra et al. 2020). (male, Vasluiț stream [loc. 15], 25-iv-2024)

I observed *Somatochlora meridionalis* twice: on 25-iv-2024 at loc. 15 (Figure 7) and on 27-iv-2024 at loc. 27. My loc. 15 corresponds to the locations “Pd. Bârnova” and “Pd. Bârnova - Pr. Nastea” in Mancu (2011). One male *S. meridionalis* was collected at each of these locations in 1985 and 1990, respectively. My loc. 27 is located ca. 80 km northwest of Bârnova forest. It represents the northernmost location of *S. meridionalis* in Romania and is also the northernmost location of this species east of the Carpathians (Mancu 2012, Bernard and Daraž 2015, Wildermuth and Martens 2019: 590). Although Bernard and Daraž (2015) could not find any specimens of this species in the streams they investigated in the Ukrainian Khotyn and

Chernivtsi Uplands, “an eastern ‘passage’ [for the northward spread of this species] must have also existed, i.e., the northward colonisation route proceeding along the eastern Subcarpathians and to the east of them.” (Bernard and Daraž 2015: 271). My loc. 27 also points to this. Further targeted searches for the species at streams in forests of the Moldovan Plateau in Romania, Republic of Moldova (where the species has not yet been recorded: Buşmachi and Munjiu 2024) and Ukraine are expected to yield new finds of *S. meridionalis* east of the Carpathians.

Libellulidae. Libellula: All three species of the genus *Libellula* (*L. depressa* (Linnaeus, 1758), *L. fulva* (O.F. Müller, 1764) and *L. quadrimaculata* (Linnaeus, 1758)) have so far been recorded in Iaşi County (Cîrdei 1956c, Cîrdei and Bulimar 1961, 1965, Lehrer and Bulimar 1979, Manciu 2011, 2012). Surprisingly, however, I was unable to observe any of these species. All three are late spring/early summer species and are on the wings in Central and Southern Europe from mid-May to late June (Kalkman and Chelmick 2015a, b, Kalkman et al. 2015a, Wildermuth and Martens 2019: 663, 670, 678). It is possible that the 2024 flight period in Iaşi County had already ended when I travelled.

Sympetrum: For most species of the genus *Sympetrum* my travel time was probably too early. Of the nine species recorded in Iaşi County (and Moldova) (Manciu 2011, 2012), I was only able to observe *S. fonscolombii*, *S. meridionale* (Selys, 1841) and *S. sanguineum* (O.F. Müller, 1764). The emergence period of *S. danae* (Sulzer, 1776), *S. depressiusculum* (Selys, 1841), *S. flaveolum* (Linnaeus, 1758), *S. pedemontanum* (O.F. Müller in Allioni, 1766), *S. striolatum* (Charpentier, 1840) and *S. vulgatum* (Linnaeus, 1758) starts in Central Europe from the end of May, the main flight period begins from the end of June (Wildermuth and Martens 2019: 740, 746, 752, 773, 790, 799). Similar times have also been observed in southern Europe (Kalkman et al. 2015b, Kalkman and Conze 2015, Kalkman and Kulijer 2015, Kalkman 2015), but there the main flight period of *S. striolatum* begins already in early May (Kalkman et al. 2015c) and of *S. vulgatum* already in mid-June (Kalkman et al. 2015d). So the species I observed show the typical summer aspect of my travel time. For most species of the genus *Sympetrum* my trip was too early.

Preliminary check-list of the Odonata in Iaşi County. The discussion resulted in the following preliminary check-list of the Odonata in Iaşi County (Appendix 1). Of the 53 odonate species ever mentioned for Iaşi County, seven are to be deleted.

Conclusions

During an odonatological study trip in Iaşi County from 21-vi to 29-vi-2024, I observed 23 species (11 Zygoptera, 12 Anisoptera). These species present the normal summer aspect of the dragonfly fauna of southeastern European countries. The dragonfly fauna in Iaşi County is exposed to various threats. As a result of climate change with rising temperatures and decreasing rainfall, more streams and small reservoirs will dry up. The banks of almost all of the bodies of water visited were littered, mainly with empty beverage and food packaging, but also with old tires and construction rubble. The literature consulted in preparation for the trip reveals many contradictions and obvious misidentifications. Through critical evaluation of the existing literature, the illustrated publications in open natural history online databases and my observations, a preliminary check-list of dragonfly species in Iaşi county is prepared. It contains 46 species; seven previously mentioned species are to be deleted. Intensive surveys in spring and autumn could reveal the presence of additional species.

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Appendix 1. Preliminary check-list of the Odonata in Iași County

no.	species ¹⁾ previous mention(s) ²⁾
LESTIDAE	
01	<i>Chalcolestes</i> sp. ³⁾ CBo49, CBu65, LBU79, Man12
02	<i>Lestes barbarus</i> (Fabricius, 1798) CBo49, Cir56b, CBu61, CBu65, LBU79, Man11, Man12
03	<i>Lestes dryas</i> (Kirby, 1890) LBU79, Man11, Man12
04	<i>Lestes macrostigma</i> (Eversmann, 1836) ³⁾ Man12
05	<i>Lestes sponsa</i> (Hansemann, 1823) CBo49, LBU79, Man11, Man12
06	<i>Lestes virens</i> (Charpentier, 1825) CBo49, Cir56b, CBu65, LBU79, Man11, Man12
07	<i>Sympecma fusca</i> (Vander Linden, 1820) CBo49, Cir56b, CBu65, LBU79, Man11, Man12, i208489913
CALOPTERYGIDAE	
08	<i>Calopteryx splendens</i> (Harris, 1782) CBo49, CBu65, LBU79, Man11, Ghe12, Man12, i126699249, i236711638
09	<i>Calopteryx virgo</i> (Linnaeus, 1758) CBu65, LBU79, Man11, Man12, i69685096, i69685228, i69685833, i69685954, i69686104
PLATYCNEMIDIDAE	
10	<i>Platycnemis pennipes</i> (Pallas, 1771) Cir56b, CBu61, CBu65, LBU79, Nea09, Man11, Ghe12, Man12, i170488438, i170489327
COENAGRIONIDAE	
	<i>Coenagrion lunulatum</i> (Charpentier, 1840) ³⁾ CBo49, Cir56b, CBu65, LBU79, Man12
11	<i>Coenagrion ornatum</i> (Selys, 1850) Man11, Man12
12	<i>Coenagrion puella</i> (Linnaeus, 1758) CBo49, Cir56b, CBu61, CBu65, LBU79, Nea09, Man11, Man12, i234572610
13	<i>Coenagrion pulchellum</i> (Vander Linden, 1825) LBU79, Man11, Man12
14	<i>Coenagrion scitulum</i> (Rambur, 1842) Man11, Man12
15	<i>Enallagma cyathigerum</i> (Charpentier, 1840) CBo49, CBu65, LBU79, Nea09, Man11, Ghe12, Man12
	<i>Erythromma najas</i> (Hansemann, 1823) CBu61, LBU79, Man12
16	<i>Erythromma viridulum</i> (Charpentier, 1840) CBo49, Cir56bm CBu65, LBU79, Man12
17	<i>Ischnura elegans</i> (Vander Linden, 1820) CBo49, CBu65, LBU79, Nea09, Man11, Ghe12, Man12, i27717355, i131475620, i171815334

Appendix 1. continued

18	<i>Ischnura pumilio</i> (Charpentier, 1825) CBo49, Cir56b, CBu65, LBU79, Man11, Man12
	<i>Nehalennia speciosa</i> (Charpentier, 1840)³⁾ Cir56b, Man12
AESHNIDAE	
19	<i>Aeshna affinis</i> Vander Linden, 1820 CBo49, Cir56c, CBu61, CBu65, LBU79, Man11, Man12
20	<i>Aeshna cyanea</i> (O.F. Müller, 1764) CBo49, Cir56c, CBu65, LBU79, Man11, Man12
21	<i>Aeshna mixta</i> Latreille, 1805 Cir56c, CBu65, LBU79, Man11, Man12
22	<i>Anax imperator</i> Leach, 1815 Cir56c, CBu61, CBu65, LBU79, Man11, Ghe12, Man12
23	<i>Anax parthenope</i> (Selys, 1839) CBu61, LBU79, Man11, Man12
24	<i>Brachytron pratense</i> (O.F. Müller, 1764) Cir56c, CBu65, LBU79, Man11, Man12
25	<i>Isoaeschna isoteles</i> (O.F. Müller, 1767) ⁴⁾ CBu61, LBU79, Man11, Man12, i166298019
GOMPHIDAE	
	<i>Gomphus vulgatissimus</i> (Linnaeus, 1758)³⁾ LBU79
26	<i>Onychogomphus forcipatus</i> (Linnaeus, 1758) Ghe12
CORDULEGASTRIDAE	
27	<i>Cordulegaster heros</i> Theischinger, 1979 Man11, Man12
CORDULIIDAE	
28	<i>Cordulia aenea</i> (Linnaeus, 1758) Nea09, Man12
	<i>Epitheca bimaculata</i> (Charpentier, 1825)³⁾ Nea09
	<i>Somatochlora flavomaculata</i> (Vander Linden, 1825)³⁾ CBo49, LBU79
29	<i>Somatochlora meridionalis</i> Nielsen, 1935 Man11, Man12
	<i>Somatochlora metallica</i> (Vander Linden, 1825)³⁾ CBu65, LBU79
LIBELLULIDAE	
30	<i>Crocothemis erythraea</i> (Brullé, 1832) CBu61, Man11, Man12
31	<i>Libellula depressa</i> (Linnaeus, 1758) Cir56c, CBu61, CBu65, LBU79, Man11, Man12, i126699820, i167396138
32	<i>Libellula fulva</i> (O.F. Müller, 1764) Cir56c, CBu61, CBu65, LBU79, Man11, Man12
33	<i>Libellula quadrimaculata</i> (Linnaeus, 1758) CBo49, Cir56c, CBu61, CBu65, LBU79, Man11, Man12

Appendix 1. continued

35	<i>Orthetrum brunneum</i> (Fonscolombe, 1837) LBo79, Man11, Man12
36	<i>Orthetrum cancellatum</i> (Linnaeus, 1758) CBo49, CBo65, LBo79, Nea09, Man11, Ghe12, Man12, i121855351, i167395349, i168469465, i203361582
37	<i>Orthetrum coerulescens</i> (Fabricius, 1798) Cir56c, CBo65, LBo79, Man11, Man12
38	<i>Sympetrum danae</i> (Sulzer, 1776) Cir56c, CBo65, LBo79, Man11, Man12
39	<i>Sympetrum depressiusculum</i> (Selys, 1841) CBo49, Cir56c, CBo65, LBo79, Man11, Man12
40	<i>Sympetrum flaveolum</i> (Linnaeus, 1758) CBo49, Cir56c, CBo65, LBo79, Man11, Man12
41	<i>Sympetrum fonscolombii</i> (Selys, 1840) Man11, Man12, i226180407
42	<i>Sympetrum meridionale</i> (Selys, 1841) CBo49, Cir56c, CBo61, CBo65, LBo79, Man11, Man12, i18116208, i192045593
43	<i>Sympetrum pedemontanum</i> (O.F. Müller in Allioni, 1766) Man11, Man12
44	<i>Sympetrum sanguineum</i> (O.F. Müller, 1764) Cir56c, LBo79, Man11, Man12, i94918325, i168469464, i189514528, i232968316, o286056972
45	<i>Sympetrum striolatum</i> (Charpentier, 1840) CBo49, CBo65, LBo79, Man11, Man12
46	<i>Sympetrum vulgatum</i> (Linnaeus, 1758) CBo49, CBo65, LBo79, Man11, Man12

Remarks: ¹⁾ bold = species observed during this study, crossed out = species mentioned in previous publications but their occurrence is unlikely (see corresponding family in section “Remarks to some families” of chapter “Discussion”). ²⁾ CBo49 = Cîrdei and Borcea (1949), CBo61 = Cîrdei and Bulimar (1961), CBo65 = Cîrdei and Bulimar (1965), Cir56b = Cîrdei (1956b), Cir56c = Cîrdei (1956c), Ghe12 = Ghețu (2012), LBo79 = Lehrer and Bulimar (1979), Man11 = Mancu (2011), Man12 = Mancu (2012), Nea09 = Nicoară et al (2009), i12345678 = <https://www.inaturalist.org/observations/12345678/>, o98765432 = <https://observation.org/observation/12345678/>. ³⁾ see corresponding family in section “Remarks to some families” of chapter “Discussion”. ⁴⁾ formerly known as *Aeshna* (*Anaciaeschna*) *isocles*, but with some unique combinations of morphological and colour characters that justify a separate genus (Schneider et al 2023).