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CONTENT

Geology-Paleontology

Biology

NYMPHAEA Folia naturae Bihariae	LII	5-32	Oradea, 2025

The Pleistocene vertebrates identified in Roșiori (Bihor County, northwestern Romania)

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Abstract. Roșiori is a locality in the Western Plain of Romania, between Oradea and Carei. Geologically, this area is part of the eastern margin of the Pannonian Basin. The sedimentary history of this basin concludes with Quaternary terrestrial accumulations, as the entire area had already emerged during that period. Large Pleistocene vertebrate

fossils are rare in these deposits, with reports coming from few localities. This rarity can be attributed to a continuous layer of Holocene rocks that overlays the Pleistocene ones, hindering extensive and detailed observations. In this context, the discovery of large herbivore bones and teeth at Roșiori is notable. This discovery resulted from gravel extraction for construction purposes in a gravel pit. The fossils indicate the presence of a cold-climate fauna in the Pleistocene, which includes the woolly mammoth, woolly rhinoceros, Irish elk, steppe bison and horse. The collection of these fossils has occurred sporadically, and the collectors were not paleontologists or geologists. Therefore, it is possible that the list of fossil vertebrates from this locality could be expanded and diversified in the future if extraction works continue. We can infer the existence of a cold steppe landscape inhabited by these mammals. Given the geological context of the area, it is reasonable to classify these fossils as belonging to Late Pleistocene, Weichsel/Würm. Continued collection of these fossils is important due to the data they provide about the evolution of Quaternary environments in western Romania. Furthermore, these fossils add educational and scientific value to the “Țării Crișurilor” Museum's paleontological collection in Oradea. Roșiori represents a newly identified Pleistocene locality featuring large vertebrates in Romania.

Key words: large mammals, late Pleistocene, cold climate, Pannonian Basin, Romania.

Vertebratele pleistocene identificate la Roșiori (Județul Bihor, nord-vestul României).

Rezumat. Roșiori este o localitate situată în Câmpia de Vest a României, între Oradea și Carei. Sub aspect geologic, această arie revine unei porțiuni a marginii estice a Bazinului Pannonic. Istoria sedimentară a acestui bazin este finalizată prin acumulări continentale cuaternare, întrega zonă fiind emersă în acea etapă. În astfel de depozite fosilele de vertebrate mari pleistocene sunt rare, fiind semnalate din relativ puține localități. Explicația unei astfel de rarități rezidă din pătura continuă a depozitelor holocene care le acoperă pe cele pleistocene, și care împiedică observațiile extinse și detaliate. În acest context, descoperirea unor oase și dinți de ierbivore mari la Roșiori este o excepție. Este datorată unor lucrări de extracție a balastului necesar construcțiilor din zonă, într-o balastieră. Fosilele dovedesc prezența în Pleistocen a unei faune de climat rece, ce include mamutul lânos, rinocerul lânos, cerbul megacer, bizonul de stepă și calul. Desigur, colectarea acestor fosile s-a realizat strict ocazional, iar colectorii nu au fost paleontologi sau geologi.

Este posibil ca lista vertebratelor fosile din această localitate să poată fi extinsă și diversificată sub aspect taxonomic în perspectivă, dacă lucrările vor continua. Putem să presupunem existența unui peisaj de stepă rece populat de aceste mamifere. În contextul geologiei zonei în discuție, suntem îndreptățiți să considerăm aceste fosile ca revenind ultimului glaciar, Weichsel/Würm. Colectarea acestor fosile se cuvine continuată, datorită mesajelor referitoare la evoluția mediilor cuaternare din vestul României. Totodată, astfel de fosile au și o valoare educativă și științifică, îmbogățind patrimoniul colecției de paleontologie a Muzeului Țării Crișurilor din Oradea, unde se află depuse. Roșiori este o nouă localitate pleistocenă cu vertebrate mari din România.

Cuvinte cheie: mamifere mari, Pleistocen superior, climat rece, Bazinul Pannonic, România.

Introduction

The plains that constitute the large Western Plain of Romania are geologically linked to the Pannonian Basin (Haas et al., 2012). In Romania, only the eastern edge of this extensive sedimentary basin is present, where it interfaces with the Carpathian orogenic structure (Paraschiv, 1979; Săndulescu, 1984; Mațenco & Radivojević, 2012).

Between the Middle Miocene and the Pliocene, the regions once submerged by Lake Pannon significantly shrank, and the salinity of the water gradually decreased, ultimately leading to entirely freshwater environments (ter Borgh et al., 2013). During the Pleistocene, the entire basin area within the Romanian sector emerged. Pools and marshes formed only in specific regions that were on the verge of subsidence (Paraschiv, 1979), likely with limited extent. Researchers have documented such occurrences in Jimbolia (Codrea et al., 1996) and Lovrin (Simionescu et al., 1989), both situated in southwestern Romania in Banat. These surfaces provided suitable habitats for large Pleistocene mammals, including the Irish elk, horse, woolly rhinoceros, and woolly mammoth. The presence of these mammals is supported by sporadic discoveries, particularly in terrace deposits of rivers flowing east to west and north-east to south-west, such as Barcău, Crișul

Repede, Crișul Negru, Crișul Alb, Mureș, Timiș, and Bega. Additional finds have emerged from quarrying activities, where Pleistocene and Holocene gravel is extracted for various construction projects, including roads and buildings. One such gravel pit operates in the northern part of Bihor County, in Roșiori (Fig. 1).

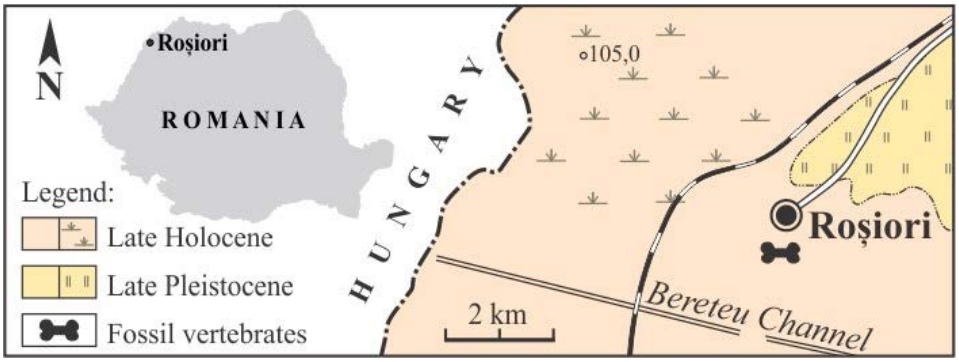

Fig. 1. Location of Roșiori locality and the geology of surrounding area (after Giușcă et al., 1965).

This locality is situated on the right bank of the Barcău River, about 25 km from Oradea, near the Romanian - Hungarian border. The gravel pit in Roșiori was utilized to extract gravel for local needs. During these operations, quarry workers uncovered teeth and postcranial bones of large Pleistocene mammals from the gravel. The bones were unearthed by Ionuț Cioară, who was operating an excavator for ballast extraction in the open pit. He noticed fragments of mammoth tusk in the sediment and retrieved them. On May 17, 2023, and then Ioan Mudura, the manager of the ballast site, notified the "Țării Crișurilor" Museum of Oradea (herein after, abbreviated as TCMNS) about the discovery. The following day, two of the authors (E.P. and L.V.) visited the site and brought the paleontological material to the museum, which included some large herbivore bones. All fossils were added to the museum's collection of fossil vertebrates.

This study identifies the fossils in question and emphasizes their presence in the specified locality. In the future, this report may be valuable for developing distribution maps of large Pleistocene herbivores within our country.

Geological setting

The Pannonian Basin is the largest intra-alpine sedimentary basin in Europe, bordered by the orogenic systems of the Alps, Carpathians, and Dinaric Mountains. Herein, we do not consider the Transylvanian Basin as part of the Pannonian Basin. We define the eastern boundary of the Pannonian Basin as the Poiana Ruscă Mountains and the Apuseni Mountains. To the northeast, we refer to the so-called "hidden mountains of Transylvania" (Szádeczky-Kardoss, 1925), which are simply an extension of the Inner Dacides of the Northern Apuseni Mountains (Săndulescu, 1984). Several synthesis papers have covered the genesis and structure of the Pannonian Basin (e.g., Haas et al., 2012; Balázs et al., 2016 and related references), making a detailed review of this topic. We will focus on a few key elements that relate specifically to the sector of interest for this paper.

The basement of the Pannonian Basin in Romanian territory comprises Alpine nappe structures that extend from the Carpathian regions into the basin basement (Bleahu et al., 1984; Visarion & Săndulescu, 1979). Within the Inner Dacides, which reach into the Roșiori area, significant tectogenesis occurred during the pre-Gosau, or intra-Turonian time span (Săndulescu, 1984). It was during this time that the thrust nappes covering the Bihor Unit, specifically the Codru Thrust Nappes and the Biharia Thrust Nappes systems, were formed. The latest Cretaceous ("Senonian") sedimentation, recorded within quasi-north-south oriented graben-type structures, is therefore considered to be post-tectogenetic (Ștefănescu et al., 1985). This episode was succeeded by a relatively extended period of emergence, which was associated with the erosion of metamorphic and Permo-Mesozoic formations, accounting for the absence of Paleogene and Lower Miocene deposits. Such deposits are lacking in both outcrops and well data (Giușcă et al., 1965, 1967; Țenu, 1984; Bordeianu et al., 2023).

Determining the extent to which sedimentary successions of these ages were initially absent or removed by erosional episodes is extremely challenging. Clarifying these details is essential, particularly through deep drilling in the Romanian sector. It is likely that this situation results from a non-depositional

interval. This conclusion is also informed by experiences from the southern part of the Pannonian Basin in Romania, specifically in Banat, from wells at Voiteg and Folea (Mutihac & Ionesi, 1974). The reports related to these wells indicated the presence of Paleogene deposits; however, subsequent drilling data from the 1980s contradicted these paleogeographic models. The arguments supporting the existence of Paleogene deposits in Banat relied on the presence of “nummulites”. Nevertheless, the new boreholes have yielded large Badenian foraminifera (V.A.C., personal observation) from the depths previously classified as Paleogene. The presence of Middle Miocene (Badenian) deposits is logical. If Paleogene rocks were indeed present, outlining a credible paleogeographic model to explain their occurrence in that area would have been exceedingly difficult. Furthermore, even Mutihac et al. (2004) no longer reference the Paleogene deposits in question, but without providing any explanation. For the “Eocene” deposits mentioned by Paraschiv (1979) "south of the Mureș," which are characterized by "calcareous breccia, conglomerates, sandstones, and black shales, reaching 300 m in thickness", relevant paleontological evidence is still necessary.

However, north of Roșiori, Campanian to the Oligocene rocks is known to exist within the Szolnok Flysch Unit (Nagymarosy & Báldi-Beke, 1993), associated with the Maramureș-Szolnok Trough. The direct influence of this unit by a south-southeast extension indicated by marine deposits in the Jibou Formation (northwestern Transylvanian Basin, Maastrichtian-Early Eocene; Mészáros, 1995), remains unconfirmed (Gheerbrant et al., 1999).

The expansion of the Pannonian sedimentary basin to its outlines is widely accepted to have occurred during the Middle Miocene as a result of the large Badenian marine transgression. A series of sub-basins linked to the Pannonian Basin are currently beginning to evolve, extending deeply into the western edge of the Carpathians. The nearest sub-basins to the locality of Roșiori are the Șimleu and Borod sub-basins (Paucă, 1964; Istocescu et al., 1970; Istocescu & Istocescu, 1974; Popa, 2000). In both these sub-basins and the adjacent sector of the Pannonian Basin, the Neogene sedimentary succession commences in the Badenian and continues up to the Upper Miocene level (Pannonian s. str.,

Pontian; Codrea et al., 2002; Codrea & Margin, 2009) extending even into the Pliocene. The discovery of some large vertebrates could argue for such geological age, such as *Anancus arvernensis* (CROIZET & JOBERT, 1828) at Firiteaz (Arad County; Codrea & Iuga, 2006) and Huta (Bihor County; Jurcsák & Popa, 1977; Codrea, 2008) or *Mammut praetypicum* (SCHLESINGER, 1919) at Păgaia (Codrea et al., 2005, 2021; Codrea, 2008).

The geophysical logs of geothermal water and hydrocarbon wells effectively illustrate the sediment infilling process in the Pannonian Basin. The electric well logs reveal that after extensive sequences dominated by marls, with very few inter-beddings of sand or sandstone, there is a transition starting from the Pontian to repeated alternations of clays and sandstones. The deepest Pontian arenitic beds exhibit thicknesses on the order of meters, with a limited presence of clay, which grants them excellent fluid-collecting properties, particularly for geothermal waters. Over time, as sedimentation progressed, the sand layers became thinner and their clay content increased, thereby reducing their collector qualities, often referred to as “dirty collectors.” The alternating layers of marl and clay increasingly dominate, illustrating the sedimentary basin's filling and the development of river networks that supplanted the former lake areas. Subsequently, in the northwestern part of the country, the area became emerged, resulting in a recorded thick pile (over hundreds of meters) of clastic rocks, evident on the geophysical logs (V.A.C., personal observation).

The Quaternary deposits, which are particularly relevant to this study primarily consist of clastic rocks such as boulders, gravels, and sands associated with river terrace deposits. As previously demonstrated, the thickness of these Quaternary deposits is influenced by the evolution of the river network. For instance, the terraces of the Crișul Repede River between Aleșd and Oradea show significant variation in extent and thickness exposing asymmetric distribution, with the left bank exhibiting much greater coverage than the right bank. Additionally, the thickness is affected by the underlying deep geological structures of the graben type. This influence is evidenced by the diving zones associated with several tectonic blocks within the basin, including those at Variaș, Jimbolia-

Tomnatec-Lovrin-Sânicolau Mare, Zarand-Socodor, Biharea-Girișu de Criș, Sânicolau de Munte, Moftinu-Căuaș, and Satu Mare-Carei-Marghita (Paraschiv, 1979; Țenu, 1981). The tectonic block structure resembles a "piano keyboard", characterized by alternating raised and sunken blocks, which contributes to these geological effects (e.g., Bordeianu et al., 2023). The overall direction of tectonic block subsidence in the basin trends from east to west. In areas of subsidence, true depo-centers for sediments developed during the Quaternary.

According to geological maps and drilling data, the area surrounding Roșiori exhibits a significant presence of Upper Pleistocene (Weichsel/Würm) deposits reaching close to the surface, which are predominantly overlain by clay-rich Holocene deposits. The right bank of the Barcău Canal hosts this geological formation. The open pit where the fossil vertebrates were extracted from is mining clastic sedimentary rocks belonging to the Barcău River Pleistocene terrace, buried by the Holocene rocks and the actual alluvia (Fig. 2). The sedimentary material processed by the river likely originated from an upper Pleistocene alluvial fan situated east of Roșiori, which was nourished by erosion from the Plopiș Mountains (part of the Inner Dacides).

Fig. 2. Ballast mining in the Roșiori open pit (photo: V.L.).

The Pannonian Basin exhibits a positive thermal anomaly (Paraschiv & Cristian, 1973; Veliciu, 1987; Airinei, 1987). This phenomenon results from an extension phase characterized by the thinning of the lithosphere and crust, which occurred during the Middle Miocene, beginning in the Badenian and continuing through the Sarmatian *s. str*. This process later slowed down during the post-Sarmatian time (Veliciu, 1987 and related references), coinciding with an increase in sedimentary infilling.

The Quaternary environments, shaped by a favorable geological setting, created optimal conditions for the development of large mammals during the Pleistocene. These Ice Age fossils represent a geological heritage that deserves to be valued.

Materials and methods

The specificity of collecting these fossils deprives them of several details that would have been extremely valuable. We are thinking particularly about taphonomy, but also about a possible vertical distribution of taxa, in the sequence of the geological deposit of origin. They were extracted by dredging the ballast, and on the edge of the quarry they were separated from classes such as boulders, gravel, and sand.

E.P. prepared the skeletal remains by mechanically removing silt from the surface of the bones. The most challenging aspect of preparing the rhinoceros's mandible was cleaning the spaces once nesting the tooth rows, which were filled with pebbles, using a vibrating cutter. Once the bones were cleaned and prepared, E.P. soaked them in a solution of Paraloid B72 dissolved in 3-5% acetone.

The fossils are housed in the vertebrate paleontology collection at the "Țării Crișurilor" Museum of Oradea, within the Natural Sciences Department (abbreviated as TCMNS). Each fossil is mentioned in the text with its associated inventory number specified.

The measurements were taken with a caliper with a tolerance of 0.1 mm. and for the Irish elk deer measurements followed Heintz (1970) and Vislobokova (2012);

for the horse bones, Eisenmann (1979), Eisenmann & Beckouche (1986) and Samson (1975); and for the rhinoceros, Guérin (1980, 2010) and Mazza (1988). The photos were taken using a Sony DSC-RX100M5 camera.

The lower premolars were marked as *p* and the molars as *m*. The measurements for the long bones and their abbreviations are specified in text, for each taxon.

Systematic paleontology

Class Mammalia LINNAEUS, 1758

Order Proboscidea ILLIGER, 1811

Genus *Mammuthus* BROOKES, 1828

cf. *Mammuthus primigenius* BLUMENBACH, 1799

Material: tusk fragment (TCMNS 7809/2; Fig. 3).

Description. The fragment of ivory indicates the presence of a proboscidean within the vertebrate association at Roșiori. Several fragments of ivory were collected, but after being collected from the extracted gravel, the ivory fragments underwent a harsh drying process that significantly harmed the fossil's preservation. As a result, the fragments were disintegrated into multiple pieces that currently cannot be reconnected. Visible fragments of dentin and cement exist. Consequently, we lack details regarding the internal structure of the ivory, particularly the Schreger patterns. The remaining fragments do not allow for credible estimates of the ivory's circumference. However, we can infer that it belonged to a relatively young specimen, as the fragments likely originated from the middle and apical segments of the tusks. Thus, we lack clear and convincing evidence for a more precise systematic assignation of this fossil. Nevertheless, it is clear that the ivory is from a proboscidean, and considering the other fossils in the vertebrate association, we have reason to believe it belonged to a woolly mammoth (*Mammuthus primigenius*). For such an assignation, though, a well-preserved fragment of ivory would be necessary to indicate morphological features related to curvature and circumference, or ideally, cheek teeth that could provide information

about lamellar frequency, enamel thickness, and similar characteristics, all essential for taxonomy.

Fig. 3. cf. *Mammuthus primigenius*: tusk fragment TCMNS 7809/2.

Order Perissodactyla

Genus *Equus* LINNAEUS, 1758

Equus cf. *germanicus transilvanicus* TEODOREANU, 1926

Material: tibia (TCMNS 7809/9; Fig. 4 A-F), two right metatarsals (TCMNS 7809/7; Fig. 5 A-F and TCMNS 7809/8; Fig. 5 G-L).

Description. The tibia is fully preserved. It features an extensive proximal articular surface, while the diaphysis narrows towards the middle portion, continuing as such up to the distal articulation.

Measurements (mm):

TCMNS 7809/9 - Length (L) = 372; Proximal breadth (PB) = 114.5; Proximal articular breadth (PAB) = 109.5; Proximal depth (PD) = 97; Proximal articular breadth (PAD) = 92; Breadth of the mid-shaft diaphysis (BD) = 51; Depth of the mid-shaft diaphysis (DD) = 41; Distal supra-articular width (DSW) = 90.3; Distal articular breadth (DAB) = 70; Distal supra-articular depth (DSD) = 54; PB/L x 100 = 30.78; BD/L x 100 = 13.71; DSW/L = 24.27; DSD/DSW = 59.80

For the tibia, we have relatively limited metric data for comparisons. Generally, the materials collected from Romania yield few data due to the scarcity of samples (e.g. Samson, 1975). Under these circumstances, where the sampling

is statistically insignificant, a comparative discussion is not useful. Nevertheless, we noted the similarity in length between the tibia from Roșiori and that of *E.* cf. *transilvanicus* TEODOREANU, 1926 as indicated by the aforementioned author (Table 13; MBSN 32.565). The other dimensions and indices also show values close to those reported for the horse from Transylvania by Samson (1975).

Both metatarsals are complete, but like the giant deer, they show signs of likely long-distance pre-burial transport.

Measurements (mm):

TCMNS 7809/7 – greatest length (L) = 279; greatest proximal breadth (PB) = 58; proximal depth (PD) = 49.5; breadth of the articular facet for *os tarsale* III (large cuneiform; BAT) = 49; breadth in the middle of the shaft (BD) = 39.5; mid-shaft depth (antero-posterior diameter; DD) = 32.5; distal supra-articular breadth (DSW) = 57.4; distal supra-articular depth (DSD) = 32; depth of the sagittal crest (DSC) = 42; least depth of the medial condyle (DMC) = 31.5; greatest depth of the medial condyle (DMCg) = 35.4; PB/L x 100 = 20.78; BD/L x 100 = 14.16; DSW/L = 20.57; DSD/DSW = 55.75
TCMNS 7809/8 - greatest length (L) = 272; greatest proximal breadth (PB) = 53; proximal depth (PD) = 46.2; breadth of the articular facet for *os tarsale* III (large cuneiform; BAT) = 48; breadth in the middle of the shaft (BD) = 35; mid-shaft depth (antero-posterior diameter; DD) = 30; distal supra-articular breadth (DSW) = 48; distal supra-articular depth (DSD) = 30; depth of the sagittal crest (DSC) = 35; least depth of the medial condyle (DMC) = 27.5; greatest depth of the medial condyle (DMCg) = 30; PB/L x 100 = 19.48; BD/L x 100 = 12.86; DSW/L = 17.65; DSD/DSW = 62.5

The measurements for the horse metatarsals from Roșiori align closely with those specified by Samson (1975) for *Equus* cf. *transilvanicus* and *E. spelaeus* ssp. The discussions surrounding the systematics of European Pleistocene horses have a long-standing history that we do not wish to elaborate on here. However, we concur with the perspective expressed by Spassov & Iliev (1998), who classifies this type of East European broad-hoofed horse as *E. g. transilvanicus*, underlying Samson's mixture of bones between the two above mentioned taxa.

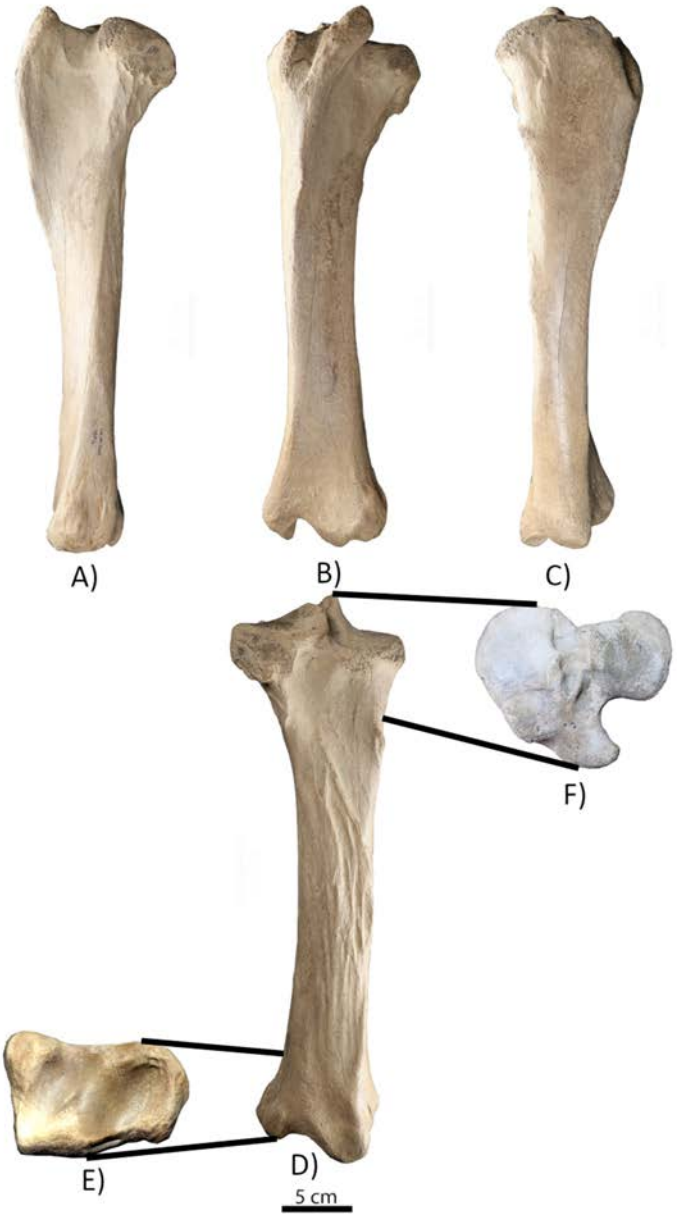

Fig. 4. *Equus* cf. *germanicus transilvanicus*, tibia: A TCMNS 7809/9 – anterior view; B, C – lateral views; D – posterior view; E – distal articulation, ventral view; F – proximal articulation, dorsal view.

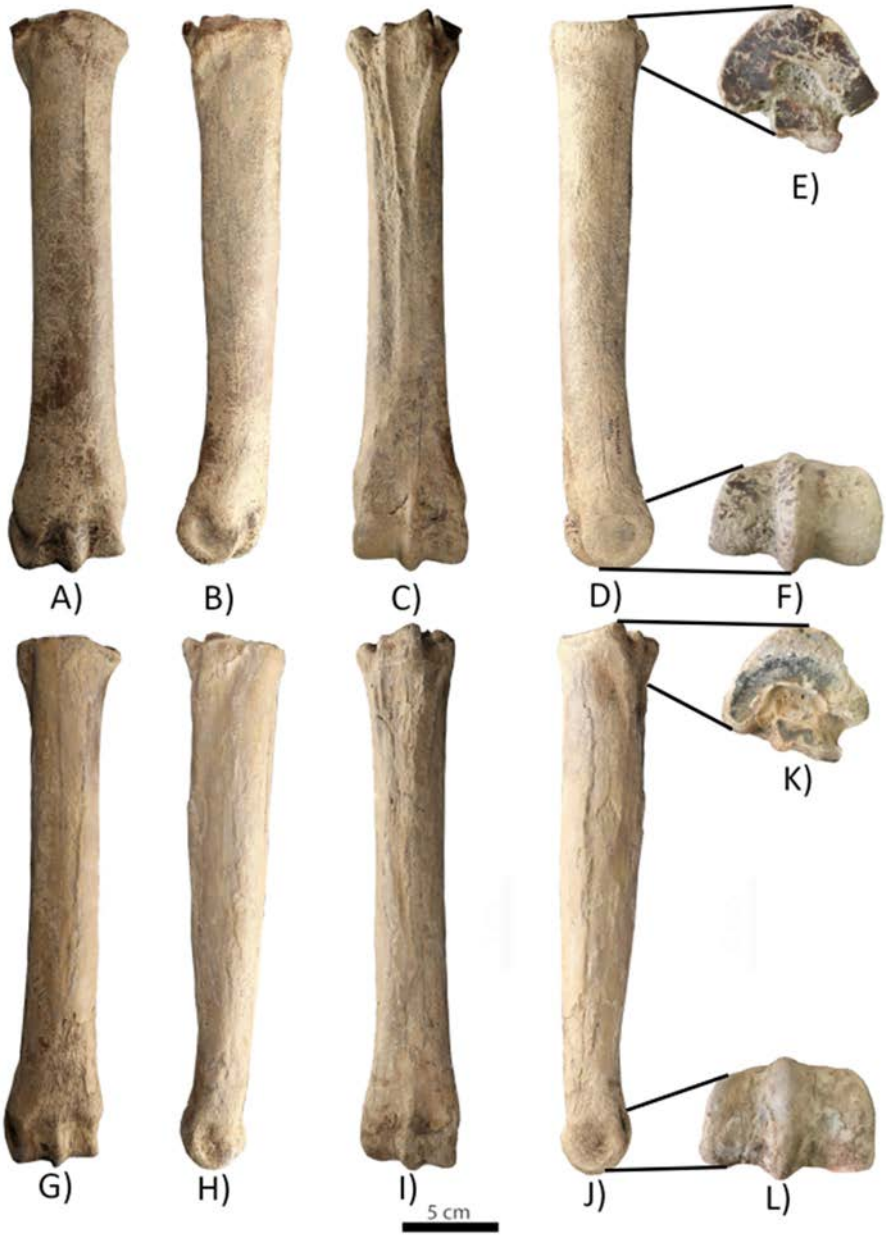

Fig. 5. *Equus* cf. *germanicus transilvanicus*, metatarsals: TCMNS 7809/7. A - anterior view; B, D – lateral views; C – posterior view; E – proximal articulation, dorsal view; F – distal articulation, ventral view; TCMNS 7809/8. G - anterior view; H, J – lateral views; I – posterior view; K – proximal articulation, dorsal view; L – distal articulation, ventral view.

Genus *Coelodonta* BRONN, 1831

Coelodonta antiquitatis (BLUMENBACH, 1799)

Material: mandible, preserving partial right and left horizontal rami and partial symphysis (TCMNS 7809/1; Fig. 6 A-B); fragmentary left humerus (TCMNS 7809/10).

Description. The mandible exhibits several damaged areas, including the anterior part of the symphysis, the inner and outer alveolar borders on both sides, the cheek teeth sockets and posterior portions of the horizontal rami. None of the cheek teeth have been preserved; it is evident that they left their sockets during the pre-burial transport of the mandibular fragment by river streams as indicated by the sandy sediment filling these sockets. As the sockets are missing, determining the precise posterior limit of the symphysis in relation to the premolars' positions in the dental rows it is not possible in this state of preservation. However, we can estimate that this limit was situated somewhere within the segment of the first premolars (p1-p3). The mandibular foramens are visible on both lateral sides, but their relationships with the premolars above cannot be clearly established. Despite these damages, the size and the convex shape of the ventral margins of the horizontal rami in lateral views are sufficient arguments for classifying this fossil as belonging to the woolly rhinoceros.

Measurements (mm):

TCMNS 7809/1 - Length of the fragmentary mandible = + 433; Length of the symphysis (LS) = + 82.5; Breadth of the incisive corpus (BI) = ca. 60; Maximum breadth of the horizontal rami (Bhr) = 68.

The diaphysis fragment of the left humerus retains very few diagnostic features, although it does exhibit a robustness characteristic of this rhinoceros species.

Measurements (mm):

TCMNS 7809/10 - transverse diameter of diaphysis (TDD) = 81; antero-posterior diameter of diaphysis (APD) = 84

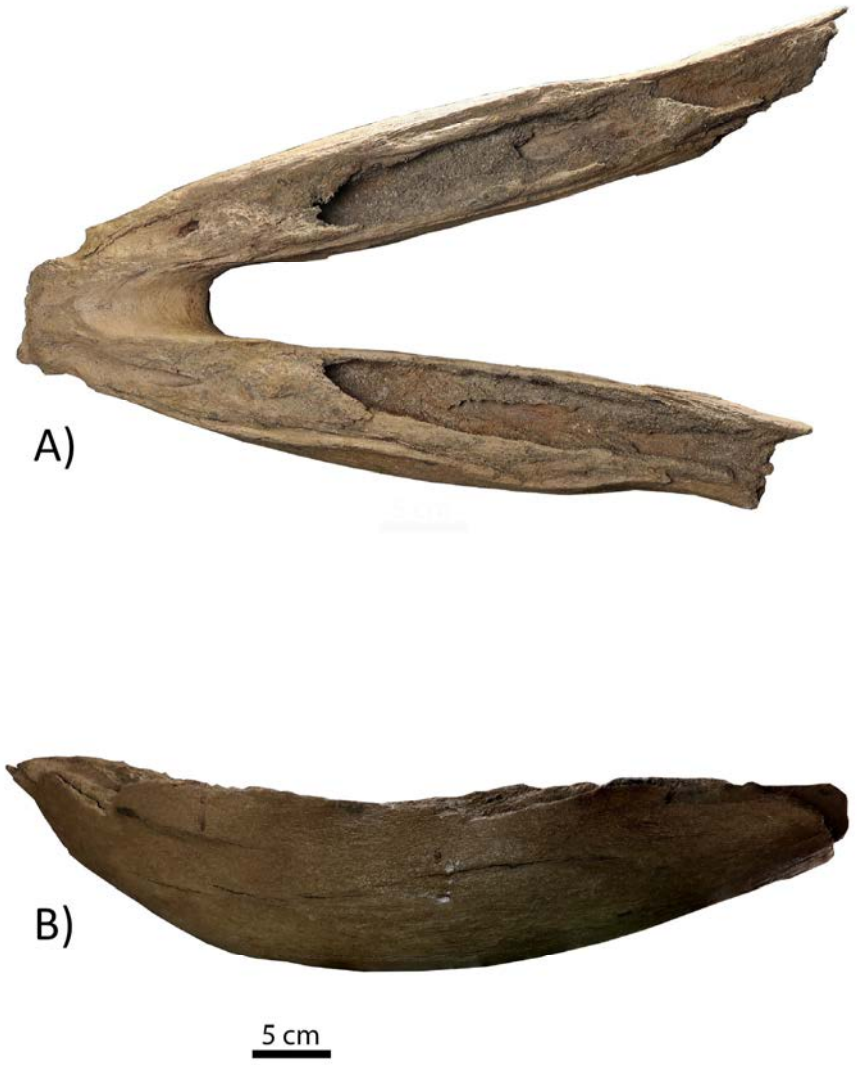

Fig. 6. *Coelodonta antiquitatis*, mandible: TCMNS 7809/1
A – dorsal view; B – lateral view.

Order Artiodactyla OWEN, 1848
Family Cervidae GOLDFUSS, 1820
Subfamily Cervinae GOLDFUSS, 1820
Genus *Megaloceros* BROOKES, 1828
Megaloceros giganteus (BLUMENBACH, 1803)

Material: right metacarpal III+IV (TCMNS 7809/6; Fig. 7 A-F).

Description. The metacarpal was heavily rolled prior to burial, resulting in a series of longitudinal ridges that are significantly worn down by erosion, as are the articular surfaces too. On the proximal articular surface of the metacarpal, the synovial hollow is completely closed and enclosed in porous area (Breda, 2005). Regarding this bone, the distinctive features that differentiate cervids from bovids, as already noted by Heintz (1970; Fig. 26), are observable. In general, the morphologic details accord with the ones outlined by Breda (2005). The metacarpal is long and robust, with the width of the distal joint being significantly large (see comparative data in Vislobokova, Fig. 34).

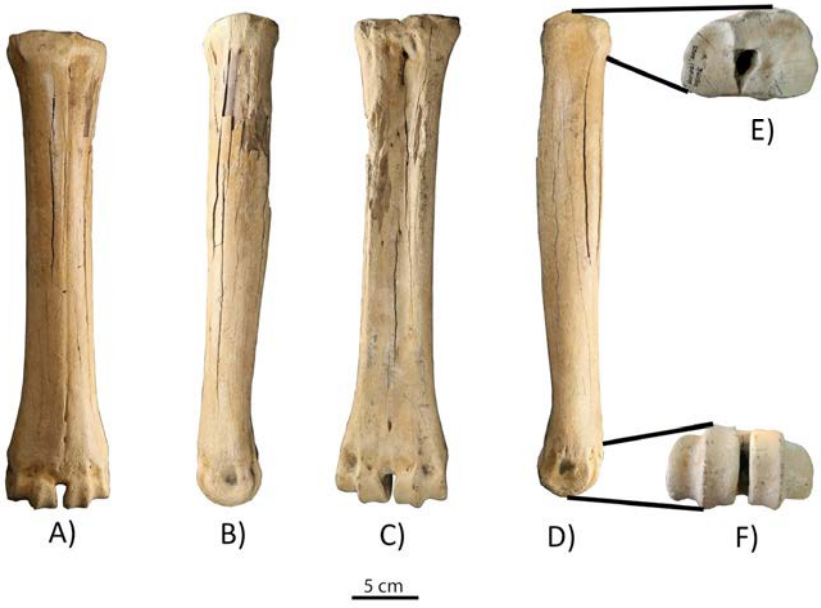

Fig. 7. *Megaloceros giganteus*, TCMNS 7809/6 metacarpal III + IV: A – anterior view; B, D – lateral views; C – posterior view; E- proximal articulation in dorsal view; F – distal articulation in ventral view.

Measurements (mm):

TCMNS 7809/6 - Length (L) = 332.5; Breadth of the proximal surface (Dtp) = 71; Depth of the proximal surface (DAPp) = 47.2; Breadth in the middle of the shaft (DTm) = 37.4; Mid-shaft depth (DAPm) = 37.4; Breadth of the distal articulation (DTd) = 73.5; Depth of the distal articulation (APDd) = 45; Dtm/L x 100 = 11.25; DTd/L x 100 = 22.11; DTm/L = 11.25

Family Bovidae GRAY, 1821

Genus *Bison* HAMILTON SMITH, 1827

Bison priscus BOJANUS, 1827

Material: left metatarsal (TCMNS 7809/5; Fig. 8 / A-F).

Description. Regarding this bone, the distinctive features that differentiate cervids from bovids, as already noted by Heintz (1970; Figs. 36-38) can be noticed. On the anterior face there is a groove whose termination is reaching the distal supra-articular termination, different from those of Cervids. In the distal articulation there are prominent crest-guides, although this feature has been largely obscured by the rolling erosion the bone was subjected to. The posterior face is not very concave and the crests that outline this concavity were also largely eroded by the pre-burial transport.

Measurements (mm):

TCMNS 7809/5 - Length (L) = 305; Breadth of the proximal surface (Dtp) = 71; Depth of the proximal surface (DAPp) = 66; Breadth in the middle of the shaft (DTm) = 46.4; Mid-shaft depth (DAPm) = 39; Breadth of the distal articulation (DTd) = 77.07; Depth of the distal articulation = 45; DTm/L x 100 = 15.2; DTd/L x 100 = 25.02

The length exceeds the ones of the actual bison's and wisent's (Skinner & Kaisen, 1947) and is bigger compared with the specimens from Habarra Grotte (Prat et al., 2003; Tab. 34).

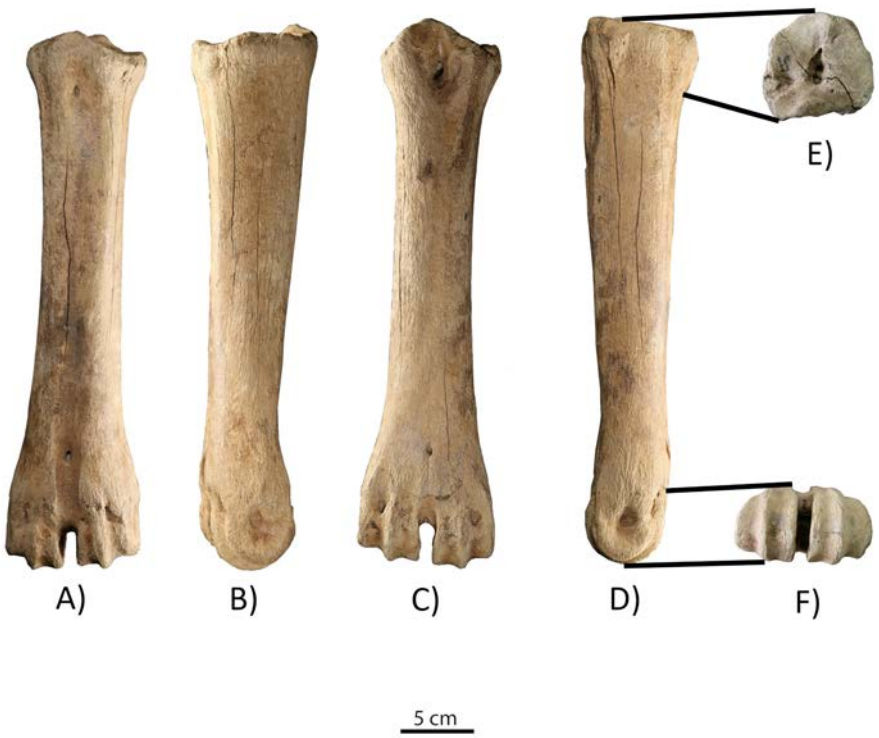

Fig. 8. *Bison priscus*, metatarsal: TCMNS 7809/5 A – anterior view; B, D – lateral views; C – posterior view; E- proximal articulation in dorsal view; F – distal articulation in ventral view.

Discussions

The large Pleistocene herbivores from Roșiori illustrate instances of poor fossilization, primarily due to the relatively long transport before their final burial. Additionally, the sampling is limited regarding the number of specimens available for each taxon, which restricts comparison opportunities. The diagnostic elements necessary for systematic classification are rather few, and the limited number of specimens hinders the ability to outline clearly defined osteometric trends.

The study of the morphology and biometry of the cheek teeth would have been beneficial for understanding the evolutionary stage of the last Pleistocene

mammoth, known as the woolly mammoth. However, as of now, no mammoth premolars or molars have been found at Roșiori.

In the case of the *Megaloceros*, the antlers would have been valuable for systematic detailing, particularly for determining the subspecies (Croitor, 2008, 2021, and related references). Unfortunately, such specimens were not recovered, and given the mechanized ballast extraction methodology, we do not have an optimistic outlook for retrieving large, intact bones from the studied locality either. Croitor (2021) noted that this deer entered Western Europe in three distinct stages, leading to the separation of three subspecies. While we can tentatively assume that the materials from Roșiori belong to the nominate subspecies *M. m. megaceros* (BLUMENBACH, 1799), we lack sufficient convincing evidence to substantiate this classification. Nevertheless, the size of the metacarpal could suggest an evolved representative from the late Upper Pleistocene for this locality. The systematics of *Megaloceros* is a controversial and inadequately understood topic. The establishment of a clear ancestor and the differentiation of evolutionary lineages are still subjects that require clarification (e.g. Lister, 1994; Lister et al., 2005; van der Made, 2006, 2019; van der Made & Tong, 2008; Vislobokova, 2011, 2012, 2013; Croitor, 2018, 2021). Apart from *Megaloceros*, another Cervid could be present in this locality.

The presence of the steppe bison in this association, although based on a single hind leg bone, is valuable for the interpretation of the paleoenvironment. This bison species is rarely reported in western Romania. A locality where it is reported from is Oradea (xxx, 1974).

The metatarsals are significant, as they confirm the presence of horses within the overall fauna. However, regarding the Upper Pleistocene horses from Romania, several details remain insufficiently clarified, particularly concerning *Equus transilvanicus*. This species is based on cranial material, but the P2-M2 series was reportedly lost by Samson (1975). The stratigraphic position of these findings is still debated, particularly concerning the site at Sf. Gheorghe "La Moară", where railroad construction took place at the beginning of the 20th century. It is plausible, as suggested by Samson, that the material originates from the base

of a loess layer, which he dated to the "Lower Würm". The additional material from Ghidfalau I, attributed to the same species by the Romanian paleontologist, is crucial for understanding this Pleistocene horse amidst the aforementioned uncertainties. Nonetheless, we align with the viewpoint of Spassov & Iliev (1998) and classify it as a subspecies of *E. germanicus*.

In western Romania, *E. germanicus* was noticed in localities as Oradea or even in a larger sample at Râpa, near Tinca, in a *Crocuta spelaea* den (xxx 1974, Csák, 1978). This material would still need a closer study.

Concerning the taphonomy, we have no reason to believe that the vertebrates originate from different stratigraphic levels, as the ballast extraction method involved successive strips of the same gravel layer, which was mixed with sandstones and silt, all located at approximately the same depth. Consequently, we are not concerned about the origin of the bones found within faunal associations of varying geological ages. We believe they are from the Upper Pleistocene, but we cannot determine which Weichsel/Würm stage they belong to. However, some precautions must be taken, as a mixture of Pleistocene vertebrate fossils with bones that fell during the extraction of ballast from more recent Holocene deposits cannot be ruled out.

For the geographical area where Roșiori is situated, we propose that a cold steppe landscape characterized the late Upper Pleistocene, featuring sparse patches of shrubs and trees in limited areas. The presence of the woolly rhinoceros, known for its grazing capabilities, serves as an indicator of this environment. The presence of horses is another argument for such environment, and the presence of the steppe bison is iconic.

The area has yielded a series of large herbivore fossils, particularly those of the ultimate Pleistocene mammoth, reported from localities such as Sălard and Ciubulcut (Apostol, 1968; Jurcsák, 1983; Vörös, 1983). The nearest locality from which remains of mammoth, woolly rhinoceros, giant deer, and horse (“*E. scythicus*”) have been reported, described as having "the appearance of household waste" is Galoșpetreu (Dealul Legii; xxx, 1974). However, it is questionable whether household waste is actually discussed in this context,

stronger evidence is needed. At Valea lui Mihai, according to the same bibliographic source, remains of *E. germanicus* were discovered in 1935, six meters deep at a location referred to as "Dienes-tag" (p. 397). This concise list highlights the rarity of such discoveries in northwestern Romania. We already mentioned the horse finds at Oradea and Râpa.

Conclusions

The association of large herbivores from the Upper Pleistocene of Roșiori includes marker taxa for this time span: wooly mammoth (*Mammuthus primigenius*), giant deer (*Megaloceros giganteus*), steppe bison (*Bison priscus*), wild horse (*Equus* cf. *germanicus transilvanicus*), and wooly rhinoceros (*Coelodonta antiquitatis*). Another Cervid is present, but for instance is unclear if it is a Pleistocene representative or a Holocene one. In this context, this association bears similarities to the one of large herbivores documented from Galoșpetreu, Valea lui Mihai, and Oradea, particularly from the Salca river terrace (Jurcsák, 1983; Jurcsák & Moisi, 1983; Codrea, 2008).

It is clear that Roșiori is a locality where systematic monitoring is needed in the ballast quarry from which the fossils described here originate. Such monitoring could, on one hand, enhance the understanding of Upper Pleistocene mammal associations, and on the other hand, enrich the collections at the Țării Crișurilor Museum in Oradea.

A series of analyzes would be necessary to get a clearer picture of these fossils, such as absolute age dating or pollen analyzes that would help reconstruct the Pleistocene environments in northwestern Romania.

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References

Airinei, Șt. 1987 - Geotermia cu aplicații la teritoriul României. Editura Științifică și Enciclopedică, 412 pp., București.

Apostol, L. 1968 - Particularités morphologiques des molaires de proboscidiens fossiles quaternaires de Roumanie, conservées dans la collection du Musée d'Histoire Naturelle « Grigore Antipa ». *Travaux du Musée d'Histoire Naturelle „Grigore Antipa"* **9**: 581-616.

Balázs, A., Matenco L., Magyar I., Horváth F., Cloetingh S., 2016 - The link between tectonics and sedimentation in back-arc basins: New genetic constraints from the analysis of the Pannonian Basin. *Tectonics*, 35 pp., DOI:10.1002/2015TC004109.

Bleahu, M., Mantea, G., Bordea, S., Panin, Ș., Ștefănescu, M., Sikić, K., Haas, J., Kovács, S., Péró, C., Bérczi-Makk, A., Konrád, G., Nagy, E., Rálisch-Felgenhauer, E., Török, A. 1994 - Triassic facies types, evolution and paleogeographic relations of the Tisza megaunit. *Acta Geologica Hungarica* **37** (3-4): 187-234.

Bordeianu, M., Novac, M., Codrea, V., 2023 - New data on the Biharia Horst documented by deep drilling. *Nymphaea* **50**: 5-21.

Breda, M., 2005. The morphological distinction between the postcranial skeleton of *Cervalces*/*Alces* and *Megaloceros giganteus* and comparison between the two Alceini genera from the Upper Pliocene–Holocene of Western Europe. *Geobios* **38**:151–170.

Codrea, V. A. 2008 - Fossil proboscideans in Inner Carpathian area (Romania). *Sargetia, Acta Musei Devensis, Series Scientiae Naturae* **21**: 31-38.

Codrea, V., Tanţău I., Fărcaş S., Wanek F., Chira C., 1996 - Etudes préliminaires concernant le Quaternaire de Jimbolia (Banat, Roumanie). *Studia Universitatis Babeş-Bolyai, Geologia* **41**(2): 103-115.

Codrea A.V., Venczel M., Popa E., 2005 - New finding of *Mammut praetypicum* (Proboscidea, Mammalia), a zygodont mastodon from Păgaia (NW Romania). *Acta Palaeontologica Romaniae* **5**: 67–71.

Codrea V., Iuga G., 2006 - A mastodon (Proboscidea, Mammalia) finding at Firiteaz (Arad District). *Studii şi cercetări, Geology-Geography* **11**: 15–24.

Codrea V., Fărcaş C., Săsăran E., Dica P., 2002 - A Late Miocene mammal fauna from Derşida (Sălaj district) and its related paleoenvironment. *Studia Universitatis Babeş-Bolyai, Special issue* **1**: 119-132.

Codrea V., Margin C., 2009 - The environments of the Uppermost Miocene vertebrates from Derșida (northwestern Rumania, Salaj district). *Oltenia, Studii și comunicări, Științele Naturii* **25**: 385-390.

Codrea A.V., Venczel M., Solomon A. Al., Sabău I., Bordeianu M., Fărcaș C., 2021- How many Pliocene mastodon species lived in Romania? *Marisia, Natural Sciences* **1**: 65-84.

Croitor, R. 2008 - Giant deer *Megaloceros giganteus* (Cervidae: Mammalia) from Late Pleistocene of Moldova. *Oltenia Studii și Comunicări Șiințele Naturii* **24**: 262–266.

Croitor R., 2018 - Plio-Pleistocene Deer of Western Palearctic: Taxonomy, Systematics, Phylogeny. *Institute of Zoology of the Academy of Sciences of Moldova*, Chișinău, 139 pp.

Croitor, R., 2021 - Taxonomy, systematics and evolution of giant deer *Megaloceros giganteus* (Blumenbach, 1799) (Cervidae, Mammalia) from the Pleistocene of Eurasia. *Quaternary 2021* **4:** 36 pp. https://doi.org/10.3390/quat4040036

Csák C., 1978 - *Crocuta spelaea* (Goldfuss) în Pleistocenul Superior de la Râpa (Bazinul Beiuș). *Nymphaea*, **6**: 183-204.

Eisenmann V., 1979 - Les métapodes d'*Equus sensu lato* (Mammalia, Perissodacyla). *Géobios* **12**: 863-886.

Eisenmann V., Beckouche S., 1986 - Identification and discrimination of metapodials from Pleistocene and Modern *Equus*, Wild and Domestic. In: Equids in the Ancient World (Eds.: Meadow H.R., Uerpmann H.-P.), Tubinger Atlas des Vorderen Orients: Reiche A., *Naturwissenschaften*, **19**(1): 117-163.

Gheerbrant E., Codrea V., Hosu Al., Sen S., Guernet C., de Lapparent de Broin F., Riveline J., 1999 - Découverte de vertébrés dans les Calcaires de Rona (Thanétien ou Sparnacien), Transylvanie, Roumanie: les plus anciens mammiferes cénozoiques d'Europe Orientale. *Eclogae geologicae Helvetiae* **92**: 517-535, Basel.

Giușcă, D., Bleahu, M., Ghenea, C., Ghenea, A. 1965 - Harta geologică scara 1:200000, folio Oradea L-34-X; L-34-IV. Institutul Geologic, București.

Giușcă, D., Bleahu, M., Lupu, M., Borcoș, M., Lupu, D., Bițoianu, C. 1967 - Harta geologică scara 1:200000, folio Șimleul Silvaniei L-34-XI. Institutul Geologic, București.

Guérin C., 1980 - Les rhinocéros (Mammalia, Perissodactyla) du Miocène términal au Pléistocène supérieur en Europe Occidentale. Comparaison avec les espèces actuelles. *Documents du Laboratoire de Géologie de Lyon* **79**(1-3): .

Guérin, C., 2010 - *Coelodonta antiquitatis praecursor* (Rhinocerotidae) du Pléistocène moyen final de l'Aven de Romain-la-Roche (Doubs, France). In: Guérin, C., Malvesy, T. (eds.), L'Aven Pléistocène Moyen Final de Romain-la-Roche (Doubs, France). *Revue de Paléobiologie* **29**(2): 697-746.

Haas, J., Nagymarosy, A., Hámor, G. 2012 - Genesis and evolution of the Pannonian Basin. pp. 149-200. In: Haas, J. (eds.) - *Geology of Hungary. Regional Geology Reviews*. Springer, Berlin, Heidelberg.

Heintz, E., 1970 - Les cervidés villafranchiens de France et d'Espagne. *Mémoires du Muséum National d'Histoire Naturelle, Série C : Sciences de la Terre* 1970, 303 p., Paris.

Istocescu, D., Mihai, A., Diaconu, M., Istocescu, F., 1970 - Studiul geologic al regiunii cuprinse între Crişul Repede şi Crişul Negru. *Dări de seamă ale ședințelor Institutului de Geologie, LV/5 Tect. Geol. Reg., (1967-1968)*: 89-106, Bucureşti.

Istocescu, D., Istocescu, F., 1974 - Consideraţii geologice asupra depozitelor neogene ale bazinului Crişurilor. *Studii și Cercetări de Geologie, Geografie, Geofizică (Geol.), Acad. RSR*, **19**: 115-127.

Jurcsák T., 1983 - Răspîndirea proboscidienilor în nord-vestul României. *Nymphaea* **10**: 65-85.

Jurcsák T., Popa E., 1977 - *Anancus arvernensis* Croizet et Jobert din Pliocenul de la Huta (Bihor, Romania), *Nymphaea* **5**: 71–93.

Jurcsák T., Moisi N., 1983 - Conservarea şi restaurarea scheletului de mamut descoperit la Oradea. *Nymphaea* **10**: 143-150.

Lister, A.M.T., 1994 - The evolution of the giant deer, *Megaloceros giganteus* (Blumenbach). *Zoological Journal of the Linnaean Society* **112**: 65–100.

Lister A.M., Edwards C.J., Nock D.A.W., Bunce M., van Pijlen I.A., Bradley, D.G., Thomas M.G., Barnes I., 2005 - The phylogenetic position of the 'giant deer' *Megaloceros giganteus*. *Nature* **438**: 850–853.

Made van der, J. 2006 - The evolution and biogeography of the Pleistocene giant deer *Megaloceros giganteus* (Cervidae, Mammalia). *Courrier Forschunginstitut Senckenberg*, 256: 117–129.

Made van der, J. 2019 - The dwarfed “giant deer” *Megaloceros matritensis* n. sp. from the Middle Pleistocene of Madrid – a descendant of *M. savini* and contemporary to *M. giganteus*. *Quaternary International* **520**: 110–139.

Made van der, J., Tong, H.W., 2008 - Phylogeny of the giant deer with palmate brow tines *Megaloceros* from west and *Sinomegaceros* from east Eurasia. *Quaternary International* **179**: 135–162.

Matenco, L., Radivojević D. 2012 - On the formation and evolution of the Pannonian Basin: Constraints derived from the structure of the junction area between the Carpathians and Dinarides. *Tectonics* **31**(6), TC6007, doi:10.1029/2012TC003206.

Mazza P., 1988 - The Tuscan Early Pleistocene rhinoceros *Dicerorhinus etruscus*. *Palaeontographica Italica* **75**: 1-87.

Mészáros, N., 1995 - Marine deposits in the continental deposits of the Jibou Formation. *Muzeul Bistrița-Năsăud, Studii și cercetări* **1**: 59-61.

Mutihac V., Ionesi L., 1974 - *Geologia României*. Editura Tehnică, 646 pp., București.

Mutihac V., Stratulat M. I., Fechet R. M., 2004 - *Geologia României*. Editura Didactică și Pedagogică, 249 pp., București.

Nagymarosy A., Báldi-Beke M., 1993 - The Szolnok unit and its probable paleogeographic position. *Tectonophysics* **226**: 457-470.

Nicorici, E., 1972 - *Stratigrafia Neogenului din Sudul Bazinului Şimleu*. Editura Academiei R.S.R., 160 pp., București.

Paraschiv D., 1979 - Romanian Oil and Gas Fields. Institute of Geology and Geophysics. *Studii tehnice şi economice, Prospecțiuni și explorări geologice* **13**: 382 p., București.

Paraschiv D., Cristian M., 1976 - Asupra particularităților regimului geotermic în NE Depresiunii Pannonice. *Revista Petrol și Gaze* **24**(11): 655-660.

Paucă M., 1964 - Bazinul neogen al Silvaniei. *Anuarul Comitetului Geologic* **34**(1): 39-101.

Popa M., 2000 - Lithostratigraphy of the Miocene deposits in the eastern part of Borod Basin (North-Western of Romania). *Studia Universitatis Babeș-Bolyai, Geologia* **45**(2): 93-103.

Prat F., Delpech F., Cancel N., Guadelli J.-L., Slott-Moller R., 2003 - Le bison des steppes, *Bison priscus* Bojanus, 1827, de la grotte d’Habarra à Arudy (Pyrénées-

Atlantiques). *PALEO, Revue d'Archéologies Préhistorique* **15**, Cahier spécial: 1-102.

Samson P., 1975 - Les Equidés fossiles de Roumanie. *Geologica Romana* **14**: 165-352.

Săndulescu M., 1984 - *Geotectonica României*. Editura Tehnică, 336 pp., București.

Simionescu T., Codrea V., Trelea N., 1989 - La faune quaternaire de Lovrin (dép. Timiș), Banat. *Analele Ştiinţifice ale Universităţii "Al. I. Cuza" Iasi (serie nouă), Geologie-Geografie* **35**: 41-53.

Skinner M. F., Kaisen O. C., 1947 - The fossil bison of Alaska and preliminary revision of the genus. *Bulletin of American Museum of Natural History* **89**: 126-256.

Spassov N., Iliev N., 1998 - The late Pleistocene and Holocene horses of East Europe and the polyphylethic origin of the domestic horse. In: M. Stefanovich, H. Todorova, H. Hauptmann (eds.). *James H. Gaul in memoriam*. The James Harvey Gaul Fondation, Sofia, pp. 371 - 390.

Szádeczky-Kardoss, G. 1925 - *Munții ascunși din Nordvestul Transilvaniei*. Dări de Seamă ale Ședințelor Institutului Geologic **13**: 136-142.

Ștefănescu M., Ionescu Fl., Niculin M., Popescu-Brădet L., Teodorescu V., Spinoche E., Popescu I., Polonic P., 1985 - Secțiune geologică Șilindru-Suplacu de Barcău-Buciumi-Sălișstea Veche-Cojocna-Tăul Zaului, Sc. 1: 200000, 3-A. *Institutul de Geologie și Geofizică,* București.

ter Borgh M., Vasiliev I., Stoica M., Knežević S., Matenco L., Krijgsman W., Rundić L., Cloetingh S., 2013 - The isolation of the Pannonian basin (Central Paratethys): New constraints from magnetostratigraphy and biostratigraphy. *Global and Planetary Change* **103**: 99–118.

Țenu A., 1981 - *Zăcămintele de ape hipertermale din Nord-Vestul României*. Editura Academiei R.S.R., 208 pp., București.

Veliciu Ș., 1987 - Geothermics of the Carpathian area. *Anuarul Institutlui de Geologie și Geofizică* **67**: 81-116.

Visarion M., Săndulescu M., 1979 - Structura subasmentului Depresiunii Pannonice în România. *Studii și Cercetări de Geologie, Geofizică, Geografie, seria Geofizică* **17**(2): 191-201.

Vislobokova I.A., 2011 - Historical development and geographical distribution of giant deer (Cervidae, Megacerini). *Paleontological Journal* **45**(6): 674–688.

Vislobokova I.A., 2012 - Giant deer: origin, evolution, role in the biosphere. *Paléontological Journal* **46**: 643–775.

Vislobokova, I.A., 2013 - Morphology, taxonomy, and phylogeny of megacerines (Megacerini, Cervidae, Artiodactyla). *Paléontological Journal* **47**: 833–950.

Vörös I. 1983 - Elephantiden-Reste aus dem Karpathenbecken. *Fragmenta Mineralogica et Palaeontologica* **2**: 61-84.

xxx (Jurcsák T., Béczy T., Marossy A., Paina I., Polis R.), 1974 - Monumente ale Naturii. In: Repertoriul monumentelor din Judetul Bihor (ed.: Sever Dumitrașcu), *Muzeul Țării Crișurilor*: 320-399.

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Sirenians between myth and science

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Abstract: Sirenians (also known as sea-cows), as we know them today, represent an order of fully aquatic herbivorous mammals, with a rich fossil record that spans from the Paleogene to the Holocene. In fact, sirenians are the only marine full herbivorous mammals, the others having different diets. Although they are peaceful herbivores, sirenians have fueled an entire legend about their supposed, often imaginary, deeds. Since ancient times, their name has been associated with creatures from the myths and legends

of numerous nations, linked to hybrid beings, adopting the appearance of humanoid birds and later, semi-human (especially women), semi-aquatic creatures with scales and a fish tail. Sometimes, they were interpreted as being beneficial for sailors or, on other occasions, malevolent. In this article, we present how mermaids have influenced mythology throughout human history, as well as the scientific aspect of these magnificent creatures. This article does not aim to be a comprehensive approach to the subject, but rather to highlight the most illustrative elements related to the topic at hand. From a scientific perspective, these mammals are challenging subjects regarding their evolution, especially concerning their adaptations to the aquatic environment. From the quadrupedal forms of the Early Eocene, there was a gradual transition to forms that are completely dependent on water and do not return to terrestrial life. Geological history records forms of very large sizes (which became extinct in historical times), as well as representatives of more modest sizes. In Romania, these mammals are recorded from the Eocene and Oligocene. A single report from the Middle Miocene (Badenian) remains highly debatable.

Key words: sirenians, sea-cows, sirens, mermaid, mythology, science

Rezumat: Sirenienii între mit și știință. Sirenienii (cunoscuți și sub numele de vaci de mare), așa cum îi cunoaștem astăzi, reprezintă un ordin de mamifere erbivore complet acvatice, cu un bogat registru fosil care se întinde din Paleogen în Holocen. De fapt, sirenienii sunt singurele mamifere erbivore marine, celelalte având diete diferite. Deși sunt erbivore pașnice, sirenienii au alimentat o întreagă pleiadă de legende despre faptele lor presupuse, adeseori imaginare. Din cele mai vechi timpuri, numele lor a fost asociat unor creaturi din miturile și legendele numeroaselor seminții, legate de ființe hibride adoptând înfățișarea unor păsări umanoide și mai târziu, a unor creaturi semi-umane (în special femei), acvatice, cu solzi și coadă de pește. Uneori, ele erau interpretate ca fiind benefice pentru marinari, iar în alte ocazii, malefice. În acest articol, vom prezenta modul în care sirenele au influențat mitologia de-a lungul istoriei umane, precum și câteva aspect științifice ale acestor mamifere magnifice. Contribuția de față nu își propune a fi o abordare cuprinzătoare, cu pretenții ehaustive asupra subiectului, ci mai degrabă să puncteze cele mai ilustrative elemente referitoare la tema în discuție. Din perspectivă științifică, aceste mamifere sunt subiecte provocatoare prin prisma evoluției lor, în special în privința

adaptărilor lor la mediul acvatic. Pornind de la forme cvadrupede eocen inferioare, pe parcursul timpului geologic s-a petrecut o tranziție treptată către forme care sunt complet dependente de apă și nu mai revin la viața terestră. Istoria geologică consemnează forme de talii foarte mari (care au dispărut în timpurile istorice), precum și reprezentanți de dimensiuni mai modeste. În România, aceste mamifere sunt consemnate din Eocen și Oligocen. O unică semnalare din Miocenul Mediu (Badenian), din Banat, rămâne extrem de discutabilă.

Cuvinte cheie: sirenieni, vaci de mare, sirene, mitologie, știință

Introduction

Sirenians, in popular terms known as ‘sea-cows’, represent an order of actual fully aquatic mammals. By their very nature, sirenians are unique, as they are the only aquatic mammals that are obligate herbivores, with all others being either filter-feeders or macro-carnivores. Additionally, sirenians also possess distinct morphological features, such as streamlined bodies, cetacean or paddle-like tail flukes, lack of hind limbs and the osteological phenomenon known as pachyosteosclerosis, where the bones become denser and the internal marrow is either reduced, or vanished.

While there are only five living species of sirenians living today, in the geological past, beginning from the Paleogene, they were vastly more numerous and widespread, with many fossil remains being discovered almost all around the world and providing a clear evolutionary history of this order.

However, despite the wealth of scientific data we have about sirenians, the knowledge pertaining to them began in ancient times, when such mammals represented the stuff of myth and legend. The situation is not surprising: in ancient times and in the first human communities, the tendencies of deification were extremely widespread. In the beginning, nymphs, fauns, gods, or goddesses could dwell in every stone, in every tree or animal.

Herein, we will underline the mythological aspects of sirenians, where their name originated from and how they transitioned from fantastic, mythical beings to living, scientifically documented organisms. We will review the perceptions that these marine mammals have generated, many of them stemming from ignorance, from a limited understanding of these animals. It is a fascinating group of mammals, but they are under the threat of extinction. In this regard, it is enough to mention the recent extinction, in historical times, just a few centuries ago, of a large-sized form that lived in the cold waters of the northern Pacific Ocean, called Steller's sea cow, *Hydrodamalis gigas* (ZIMMERMANN, 1780).

Mythological aspects of sirenians

The earliest facts regarding sirenians originated mostly from the stories told by sailors from Antiquity, who were able to get a glimpse of these marine mammals. From these stories sirenians began to be associated with the mermaids of myths and legends. According to Mircea Eliade, a myth is ”a complex cultural reality which tells a sacred history from the primordial times. After the intervention of supernatural beings, man becomes a mortal, cultural being” (Eliade, 1978).

The myth of the siren is certainly to be sought in the cultures of peoples that were connected to the sea and navigation. Consequently, those who had early concerns related to navigation were undoubtedly the Phoenicians. In their mythology, the goddess Atargatis (or Ataratheh, or Derceto) appears with the appearance of a mermaid (Fig. 1). The first story depicting mermaids appeared in Assyria, which told of how the goddess Atargatis (Astarte in Phoenician and Aphrodite in Greek) (Figs. 1, 6) (Dell, 2010; Dorofeeva, 2014) fell in love with a mortal and mothered the Assyrian queen Semiramis. Feeling ashamed for what she had done, the goddess jumped into a lake near her temple in Ascalon (Ashkelon) and turned into a fish. However, because she would be allowed to keep her divine beauty, the upper part of her body remained unchanged.

Fig. 1. Derceto, from Athanasius Kircher, ”Oedipus Aegyptiacus”, 1652
(Source: public domain https://en.wikipedia.org/wiki/Atargatis)

But undoubtedly, the myth of the sirens was widely spread by the ancient Greeks, a category of sailors dedicated to the sea. The representatives of the order Sirenia are sometimes referred to as ‘sirens’, the name itself originating from the infamous evil creatures of ancient Greek mythology. In the confines of myth and legend, sirens were described as bizarre creatures that used their enchanting songs to hypnotize and lure traveling sailors to the rocky coasts of their islands (Orchard, 1995; Mittman & Dendle, 2013), where their ships would crash and sink, although it is unknown if this was the sirens’ true intention. Originally the poets Vergil and Ovid wrote that the sirens lived on a group of small islands, at the time known as *Sirenum scopuli*, however others have since referred to other islands as the home of the creatures. Pliny the Elder mentions Greek historian Dinon of Colophon (ca. 360–340 BC), who stated that sirens are aggressive predatorial creatures which originate from India (Rackham, 1967), but even Pliny himself doubted this theory (Dorofeeva, 2014).

Originally, according to Greek scholars and philosophers, sirens were chimeric creatures, bearing the head of a woman attached to an avian body, sometimes being confused with harpies, which are another mythical avian creature (Dixon-Kennedy, 1998). The most well-known appearance of sirens in literature is in Homer's "Odyssey", where the main hero Odysseus (Ulysses), who ordered his men to tie him to the mast of their ship, fill their ears with wax and continue sailing, following the sorceress Circe's advice (Fig. 2) (Pope, 1880; Kun, 1964). In this manner, he was the only one who was able to listen to their songs. After passing the sirens as they sang, the crew survived and some authors and Greek scholars believed that if one were to hear the sirens' song and survive, the sirens would die (Fowler, 2013).

Figure 2: Painting by John William Waterhouse depicting Ulysses tied to the mast, while the sirens attack (Source: public domain https://en.wikipedia.org/wiki/Ulysses_and_the_Sirens_ %28Waterhouse%29)

Interestingly enough, this humanoid avian image also appears in Egyptian mythology as well, called '*ba*', as a way of explaining the soul's departure from the body (Fig. 3).

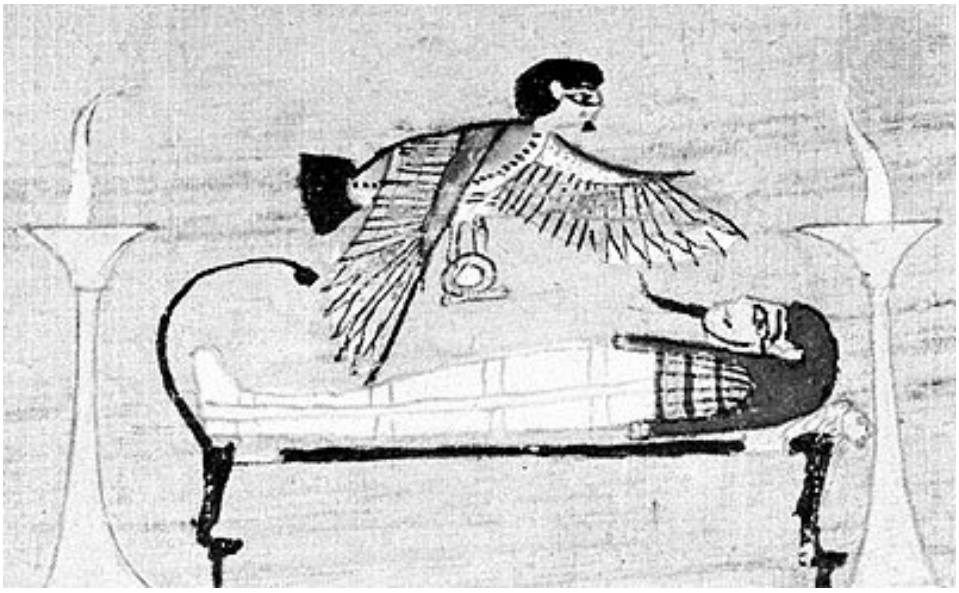

Figure 3: *Ba* hovering over a dead man, from a papyrus of the ”Book of the Dead”; in the British Museum (Source: public domain https://www.britannica.com/topic/ba-Egyptian-religion)

Later, sirens were described as having fully human bodies with the addition of a pair of avian wings on their backs (Austern & Naroditskaya, 2006), which leans closer to the myth of how the goddess Demeter gave the sirens wings to find Persephone in the Underworld (Kun, 1964). This form could be the origin of the modern popular image of angels in Christian myth. Additionally, the image of humans with avian wings on their backs also appeared in mythologies of other cultures, a prominent example being the *Tengu* (Fig. 3), a mischievous flying human or goblin from Japanese mythology.

In short, not to stray away from Greek mythology, it should be noted that sirens could also be associated with rivers and their deities. Such mythical characters were Pisinoe (Peisithoe), Aglaope, and Thelxiepiathe, daughters of the river god Achelous and Melpomene (Appolodorus, ”Epitome 7”).

Figure 4: *Tengu* statue (Source: public domain https://en.wikipedia.org/wiki/Tengu)

One thing that is for certain is that the image of the sirens of antiquity has nothing in common with the marine mammals called sirenians or sea-cows. However, by the time of the Early Modern Period the image of the sirens changed completely. While the partial human features and enchanting songs did remain, their physical appearances were drastically altered by medieval Christian scholars. Many, such as St. Jerome in his Commentary on Isaiah (ca. 404 – 410 A.D.) used the word ‘siren’ as a Latin translation for words such as jackals, demons or dragons (Dorofeeva, 2014; Scheck, 2015). For example, the Romanian translations of the orthodox Bible and the Hungarian translations of the protestant Bible use in Isaiah 13:22 the word ‘jackal’, while the 1660 translation by Károli Gáspár of the protestant Bible indicates the word ‘dragon’ (‘*sárkány*’). On the other hand, Isidore of Seville (ca. 560 – 636 A.D.) mentions in his ”Etymologiae” (”The Etymologies of Isidore of Seville”) that sirens, apart from being an allegory for worldly temptation and lust, ”live in the waves” because a ”wave created Venus”, the name Venus being how the Romans referred to the goddess Aphrodite (Barney et al., 2006). It is possible that the association of the name ”siren” and half fish women might have begun from here.

While the image of half human - half fish creatures was prevalent during the ancient times, such creatures were mostly shown as ”mermen”. A well-known example being *Dagon* (Fig. 5), an ancient Mesopotamian and Babylonian water god with the upper body of a man and the lower body of a fish (Bible, 1805).

Figure 5: Stone carving of *Dagon* (source: public domain https://theancientbridge.com/2015/06/confronting-pseudo-archaeological-memes-pt-3-was-dagon-of-the-philistines-a-fish-god/?print=print)

Another example from ancient Babylonia is of a merman by the name of Oannes, who has the head of a man, as well as a head and tail of a fish. This was a good character, in the sense that he taught humans about agriculture and scientific writing during the day, while at night he would return to the waters of the Persian Gulf (Kernbach, 1989; Dorofeeva, 2014). However, the first recorded merman in ancient writings was *Ea*, a Sumerian god of water (Waugh, 1960). With the passage of time however, mermen were described more and more as ugly and bestial. A notable example would be in Howard Philips Lovecraft’s literary work ”Shadow over Innsmouth”, where the author describes a race of grotesque humanoid fish creatures, named the Deep Ones.

Female versions of mermen, known as ”mermaids”, also occasionally appeared as protective figures. The name of these mythological creatures might have derived from the word *kuliltu*, which in Mesopotamian means ”fish-woman” (Black & Green, 1992). The word *kuliltu* first appeared as an association to a being known as *Kulullû*, meaning ”fish-man”, which was a Mesopotamian, mythological water dwelling monster (Black & Green, 1992).

Figure 6: Nabatean statue of Atargatis, in the Jordan Archaeological Museum (source: public domain https://en.wikipedia.org/wiki/Atargatis)

Although this story depicts a mermaid, the first visual depictions of mermaids simply showed creatures with full fish bodies and only the head was that of a human. This ‘fish with human heads’ image persisted all the way in Japan with the water *yokai*, known as *Ningyo* (Yoda & Alt, 2008).

There is even a legend about the sister of Alexander the Great, Thessalonike, who changed into a mermaid after her death. Said to now live in

the Aegean Sea, she would ask passing sailors if her brother is still alive. If the answer is 'yes', then she would leave and calm the waters, however if the answer is 'no', then she would become enraged and would cause a sudden storm that could drowns the unfortunate sailors (Mitakidou et al., 2002; Russell, 2013). However, this is a more modern legend, which can be traced back to an 'Alexander Romance' entitled *Phylláda tou Megaléxandrou,* first printed in 1680, during the Ottoman Greek period.

One of the first artistic English depiction of a mermaid can be found in the Norman Chapel, within Durham Castle (Wood, 2010). Like the banshee of Irish folklore, mermaids are represented in English folklore as symbols of bad women, foretelling disasters to come (Briggs, 1976). These 'disasters' were often a reference for approaching harsh weather. Sometimes mermaids were even described as having monstrously sized bodies, reaching up to 160 ft in length (49 m) (Briggs, 1976). This size aspect of mermaids disappeared as time went on, and with the exception of certain pop-culture media, mermaids are no longer described as gigantic leviathans. Mermaids have been featured in many English and Irish works of literature, and while most are described as malevolent creatures, some are also good natured, such an example being a legend from the village of Zennor from Cornwall, England, where a mermaid fell in love with a human after hearing him sing (Watkins, 1962).

As we have already shown, sometimes mermaids were said to inhabit freshwater environments as well (Watson, 1908; Ivanits, 1992), which might have spawned the various myths and legends of ravenous female water spirits, nymphs or water fairies (e.g. the Naiads of Greek mythology, the Rusalkas of Slavic folklore and the *Lostrița* from Romanian folklore), as well as myths of other mermaid-like creatures (e.g. the seal-like Selkie from Scottish and Norse mythology) (Fig. 7). In Romanian mythology, the *Știma apei* or *Vâlva apei* is an evil spirit that drowns people and turns them into 'water people'. The *Știma* comes out of the water during the night and lures people in. Her daughters are said to be *'the daughters of the sea with the bodies of women, the tail of a fish and fins as white as the seafoam.'* They are said to drown handsome men to

take them to their palaces, while capsizing the boats of ugly men (Antonescu, 2009).

In Poland, there are several legends pertaining to a mermaid by the name of Syrenka. The most prominent of these is when Syrenka, after parting ways with her sister, swam up the Vistula River and settled in the part of the river's bank where the city of Warsaw is today. While the legends of her origin are many, what they have in common is that eventually Syrenka becomes a protector of the city, even being represented in the Warsaw coat of arms (Fig. 8) (Wasilewski & Kostrzewa, 2018). A similar coat of arms can be found in the Reformed Church in Cluj-Napoca. It belonged to countess Bethlen, née Sarolta, baroness Wesselényi of Hadad. The coat of arms depicts a mermaid with a crown on her head and holding three flowers, a clear resemblance to the coat of arms of Warsaw (Nagy, 1865). While the Warsaw mermaid is armed with a sword in her right hand and a shield in her left, the Transylvanian one is much more peaceful, holding a bouquet of three flowers in her right hand (Fig. 8).

Figure 7: Painting of a Naiad approaching a sleeping Hylas, by John William Waterhouse (source: public domain https://en.wikipedia.org/wiki/Naiad)

Similar symbolism can be seen in the present day, specifically in the case of the Starbucks logo, which is presented as a mermaid with two tails and a crown

upon her head (Fig. 9). According to their website, the mermaid was chosen as a symbolism for how their coffee attracts consumers, similarly to how the mythological beings attract sailors with their voices. An inspired example of marketing with roots in ancient legends, this is valid if we abstract from other meanings that the siren in question presents.

In his treatise *A Book on Nymphs, Sylphs, Pygmies, and Salamanders, and on the Other Spirits*, Swiss alchemist Paracelsus von Hohenheim describes mermaids as the offspring of a human and a water elemental, as an attempt of the latter to acquire an immortal soul (Temkin et al., 1996). Paracelsus' book led to the writing of de La Motte Fouqué's novella 'Undine', and eventually to Hans Christian Andersen's famous mermaid fairy tale 'The Little Mermaid'.

(left) and the coat of arms of the Wesselényi family (right) (Photo by Veress László). Right: Warsaw coat of arms, depicting Syrenka (source: public domain https://en.wikipedia.org/wiki/Coat_of_arms_of_Warsaw)

Figure 9: Starbucks logo (source: public domain https://en.wikipedia.org/wiki/Starbucks)

In China, mermaids are referred to as *jiaoren*, meaning ‘shark-people’. Mermaids have also made appearances in other Asiatic cultures, examples being the princess Hwang-ok of an underwater kingdom named Naranda from Korean mythology (Keith & Sung Ae, 2018) and the legend of Yaobikuni from Japan, who consumed the flesh of a *Ningyo* and lived far longer than humans naturally do (Fig. 10) (Yoda & Alt, 2008).

Figure 10: Woodblock-printed flier of Ningyo
(source: public domain https://en.wikipedia.org/wiki/Ningyo)

In western, central and southern Africa mermaids appear as unpredictable water spirits, known as '*Mami Wata*' (or sometimes *sirene* or *madame poisson*), which are said to grant favors of beauty and wealth to people they are interested in. However, a *Mami Wata*'s mind can easily shift, and she can become interested in someone else quickly, taking her previously given favors away and bestowing them to another. This characteristic reflects the dual nature of such spirits in African folklore (Bastian, 2005).

In Hinduism an example of mermaids can be found in the tail of Suvannamaccha, a golden mermaid princess and daughter of Ravana, who falls in love with the Hindu god Hanuman (Desai, 1980).

Since the 7th and 8th century many medieval bestiaries, such as the *Liber monstrorum*, have popularized the idea of women with fish lower bodies, referred to as 'mermaids', with a desire to tempt men into their aquatic domains and devour them (Dorofeeva, 2014). However sometimes these creatures are described as having additional avian feet.

While the word 'mermaid' might have originated during the 1200s, some authors, such as Guillaume le Clerc and Bartholomaeus Anglicus used both 'siren' and 'mermaid' interchangeably, which resulted in both names referring to just one being (Guillaume, 1970; Steele, 1905). Even most European countries use the word 'siren' in one form or another when speaking about mermaids.

Nowadays naturalists and cryptozoologists confirm that the legends and sightings of mermaids have in fact been sightings of Sirenians, although some of these sightings were also confirmed to be hoaxes.

According to historians, Christopher Columbus, during his exploration of the Caribbean in 1492, reported sightings of three 'mermaids', or 'sirens' as they were referred to by him (Haase & Reinhold, 1993). The following quote from the logbook of his first voyage, which was transcribed by father Bartolomé de Las Casas, briefly describes what he saw: *'On the preceding day, as the Admiral was proceeding toward the Rio del Oro, he said that he had quite distinctly seen three Sirens emerging from the sea, but that they were not as beautiful as they are said to be, for their faces had some masculine traits'* (Haase & Reinhold, 1993). The

mentioning of these creatures not being beautiful and having ‘masculine traits’, stems from earlier beliefs that the male counterparts of sirens (mermen) have much more bestial features, as opposed to their female counterparts. This was one of the reasons why it was deduced later that what Columbus saw that day weren’t actual mermaids, but manatees. This would later be confirmed as the first sighting of manatees in North America.

From a morphological standpoint, it is easy to see why Sirenians have such a strong connection to the mythological side of natural history. Like mermaids, sirenians have streamlined bodies with only a pair of front limbs, are devoid of hind limbs and have a fish-like horizontal tail fin, like cetaceans (with manatees being the exception since they have round, paddle-like fins).

However, during the 13th and 14th centuries, explorers and scientists would often mix folklore, myths and legends of old with natural observations to presumably better understand the world around them and add an air of mystery around the theories they put forward. Columbus is once again an example of this type of thinking, as the North American ‘mermaid’ sighting wasn’t the first he made. Several years earlier he had already seen manatees while journeying near the coast of Guinea (Haase & Reinhold, 1993), which are now identified as West African manatees (*Trichechus senegalensis*, LINK 1795*).* However, even then, even though what he saw were animals and not mythical sea monsters, he firmly believed that what he saw were indeed sirens.

Another example is Spanish captain Gonzalo Fernández de Oviedo Valdés, who in 1526 gave some of the first accurate descriptions and sketches of living sirenians, in this case the West Indian manatees. However, when he presented his findings, the scientists at the time changed the name of the creatures he described, so that instead of manatees there would be mermaids. Until the 19th century, such misinterpretations continued to exist in regions of the world populated by living sirenians. However, when scientists became wiser and began interpreting these sightings in a more logical and realistic manner, the scientific Order ‘Sirenia’ was created, and mermaids were rejected from science as simply being creatures of myth and fantasy (Fattori, 2009).

Scientific approach

Since the beginning of civilization, humanity always held the need to organize everything to better understand the world. With the passage of time the process of organization has come to be known as taxonomy. In the field of biology, taxonomy involves the identification and study of new or old specimens, referred to in this sense as ‘taxa’. Over time those in the field of biology have further organized these taxa, based on their morphological and biological characteristics, known today as ‘Classification’ (Bellows et al., 1999).

In his book *‘The Origin of Species’*, naturalist Charles Darwin (1809-1882) briefly described sirenians as thus: *‘The sirenian form a very distinct group of mammals, and one of the most remarkable peculiarities in the existing dugong and lamantin is the entire absence of hind limbs without even a rudiment being left; but the extinct Halitherium had, according to Professor Flower, an ossified thigh-bone ‘articulated to a well-defined acetabulum in the pelvis’, and thus makes some approach to ordinary hoofed quadrupeds, to which sirenian are in other respects allied’* (Darwin, edition 2003). From this description, Darwin theorized that the sirenians belong to the *Ungulate* (hoofed) clade, and further research has shown that sirenians belong to a separate clade, known as *Paenungulates* (almost hoofed).

The Order Sirenia is divided into four families, from which two families are known only from the Eocene and are extinct, and two have survived into historic times (Bajpai et al., 2009). The two extant families are: Trichechidae, which includes the West Indian manatee (*Trichechus manatus* LINNAEUS, 1758), the African manatee (*T. senegalensis* LINK, 1795) and the Amazonian manatee (*T. inunguis* NATTERER, 1883), with the second family being Dugongidae, which includes the dugong (*Dugong dugon* MÜLLER, 1776). The West Indian manatee is further classified into two subfamilies, consisting of the Florida manatee (*Trichechus manatus latirostris* HARLAN, 1824) and the Caribbean or Antillean manatee (*Trichechus manatus manatus* LINNAEUS, 1758). The Dugongidae also includes the largest known sirenian, Steller’s sea

cow (*Hydrodamalis gigas*), however due to human predation it is now extinct (Berta, 2020; Walker, 2015). There are also some accounts that suggest that Steller's sea cow might still exist in isolated areas in the north Pacific and related seas, however, similarly to the Tasmanian tiger, currently there is no concrete evidence to support this theory.

The two extinct families of Sirenia from the Eocene are Prorastomidae and Protosirenidae. While morphologically they resemble Condylarths (Tabuce et al., 2008), they possess many characteristics (such as pachyostotic bones and enlarged rostrums) that indicate their position in the Sirenia evolutionary line (Domning et al., 2010). Besides the two previously mentioned sirenian families, several sirenian genera from the Eocene exist which are classified as '*incertae sedis*' (uncertain taxonomic placement), such examples being *Indosiren, Paralitherium, Sirenavus, Miodugong* etc. (Kordos, 2002). The fossil record of the Eocene sirenians is reasonably well documented, with specimens found on every continent, except for Australia and Antarctica (Zalmout et al., 2003).

The first adequately formulated cladogram of the Order Sirenia was put together by Robert J. G. Savage, which was soon followed by detailed cladistic analysis done on other members of the order (e.g. *Trichechidae, Rytiodontinae* and *Metaxytherium*) (Domning, 1994). Specifically, Domning (1994) revised Savage's work and extended it to encompass every known sirenian species, both extant and extinct. This in turn was followed up by Manja Voss (2014) with slight alterations being made to show her work on the taxonomic revision of *Halitheriinae* (Voss, 2014).

The stratigraphic range of sirenians within Romania is highly extensive, although discoveries of fossils pertaining to these mammals are thus far restricted to the interior of the Paleogene Transylvanian sedimentary basin. The first sirenian fossils in Romania were discovered by Johann Michael Ackner in the Eocene limestone deposits from Turnu Roșu (formerly known as Porcești; Anonymous, 1850a, b), and later were analyzed and interpreted by Hermann von Meyer (*fide* Koch, 1894, in Șuraru & Codrea, 1988). This discovery was later

followed by subsequent finds, many of which were mentioned by Koch (1891, 1894, 1900) in his works, beginning with occurrences in the upper Lutetian strata from Iara and Valea Chioarului, the lower Priabonian limestones from Leghia, and upper Priabonian Cluj Limestone deposits from Cluj-Napoca (Mănăștur, Hoia Hill, Cheile Baciului etc.) and the surrounding areas (Mera, Jebuc, Stana, Baba, Bucium, Bizușa-Băi etc.) (Fuchs, 1959, 1970, 1973, 1988, 1990; Șuraru & Codrea, 1988). The frequency of sirenian fossils in Oligocene strata lowers significantly, as a possible side effect of the '*Grande Coupure*' turnover event, although fossils of Early Oligocene sirenians were found in localities such as Cluj-Napoca, Mera, Bizușa-Băi, Stoiceni etc. (Fuchs, 1971; Veress et al., 2025). Late Oligocene sirenian fossils are yet to be discovered, as of the writing of this document. The same can be said regarding Miocene deposits. Florei (1962) mentioned having discovered a possible sirenian fossil rib fragment in the Miocene deposits of Zorlențu Mare, within the Banat region, but the fossil is now lost. Sirenian teeth are also noteworthy fossils, particularly as they are extremely rare.

Of note is a sirenian fossil discovered outside the Transylvanian Basin. The fossil in question refers to a rib from which several fragments have been recovered, some of them lacking direct connections. It was unearthed in the Albești Nummulitic Limestone, near the Albești village, at the Albești Muscel commune, in the Argeș district (Grigorescu, 1967; Veress & Codrea, 2020). Only a single fragment of this fossil bone can be retrieved in the paleontological collections of the University of Bucharest, the other being apparently lost (information received from Dr. Ștefan Vasile by V.A.C.).

Conclusions

Since the beginning of human civilization sirenians have been a part of our history and culture. Whether simply by name or by stories, from evil humanoid avians, to beautiful aquatic maidens, to fascinating evolutionary landmarks, sirenians always were of a special kind of interest for humanity.

At first, the word siren was used to name enchanting avian creatures that would lure unsuspecting sailors to their demise, with the most infamous being the story of Odysseus's confrontation with the beasts. This imagery of humanoid avians persisted throughout history, in different cultures (Egyptian, Japanese, and Christianity), while the name siren was later associated with an aquatic creature: the mermaid. Even before this association, legends of human–fish hybrids have existed since the time of Babylon, with the image of half man and half fish creatures being dominant. Overtime, depictions of female aquatic humanoids became more and more prevalent, along with the name siren becoming associated with these beings. Sirens or mermaids became mythological icons, being represented even in certain heraldries and being depicted as protectors.

Until the 19th century mermaids retained a position in science as living creatures; however, once researchers began to properly examine the evidence, they separated the mythical from what is real, thereby leaving sirens as fictitious creatures, while Sirenians became some of the most fascinating vertebrates. Their fossil record already shows an extensive and detailed evolutionary journey; however, the more fossils of these animals we discover, the more we can learn about them and their ecology, as well as unearth potentially new species.

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References

Anonymous 1850a, b - Siebenbürgische Petrefacten in der Sammlung des Herrn Michael Ackner, Pfarrer in Hammersdorf. *Verhandlungen und mitteilungen des Siebenbürgischen vereins für naturwissen schaften zu Hermannstadt* **11**: 171-175, Hermannstadt.

Antonescu R., 2009 - *Dicționar de simboluri și credințe tradiționale românești*, Editura TIPOMOLDOVA, 724 pp., Iași.

Appolodorus - *Odyssey & Telegonia, Epitome 1*, Chapter 18, source: https://www.theoi.com/Text/ApollodorusE.html#7

Austern, L., Naroditskaya, I. 2006 - *Music of the Sirens*. Indiana University Press, 440 pp., Bloomington and Indianapolis.

Bajpai, S., Domning, D. P., Mishra V. P. 2009 - A new middle Eocene sirenian (Mammalia, Protosirenidae) from India. *Neues Jahrbuch für Geologie und Paläontologie.* **252** (3): 257–267, Stuttgart.

Barney, A.S., Lewis, J.W., Beach, A.J., Berghof, O., Hall, M. 2006 - *The Etymologies of Isidore of Seville*. Cambridge University Press, 245 pp., Cambridge, New York, Melbourne, Madrid, Cape Town, Singapore, São Paulo.

Bastian, L.M. 2005 - *Nwaanyi Mara Mma: Mami Wata, the More Than Beautiful Woman*. Department of Anthropology, Franklin & Marshall College, https://web.archive.org/web/20050417071004/http://server1.fandm.edu/departments/anthropology/mami.html

Bellows, T.S., Fisher, T.W., Caltagirone, I.E., Dahlsten, D.L., Gordh, G., Huffaker, C.B. 1999 - *Handbook of Biological Control Principals and Applications of Biological Control*. ACADEMIC PRESS, 1046 pp., San Diego, San Francisco, New York, Boston, London, Sydney, Tokyo.

Berta, A. 2020 - *Return to the Sea, The Life and Evolutionary Times of Marine Mammals*. University of California Press, 224 pp., Berkeley, Los Angeles, London.

Bible (*Szent Biblia vagy Az Istennek Ó és Új Testamentomában foglaltatott egész Szent Írás*), translated by Károli Gáspár, Új Kiadás Trattner Mátyás Kiadó, Pest, 1805, Bír. 16:23, 1. Sám. 5:2, 243 pp.

Bible (*Biblia cu ilustrații, Versiune diortosită după Septuaginta*, redactată și adnotată de Bartolomeu Valeriu Anania Arhiepiscopul și Mitropolitul Clujului sprijinit pe numeroase alte osteneli), Editura Jurnalul Național, vol. 1-8, 2,826 pp., Editura Litera, Tipărit la Monitorul Oficial.

Black, J., Green, A. 1992 - *Gods, Demons and Symbols of Ancient Mesopotamia, an Illustrated Dictionary*. The British Museum Press, 1,990 pp., Great Britain.

Briggs, K. 1976 - *An Encyclopedia of Fairies, Hobgoblins, Brownies, Bogies, and Other Supernatural Creatures*. First American Edition, 272 pp., New York.

Darwin, C. 2003 - *The Origin of Species by Means of Natural Selection of the Preservation of Favored Races in the Struggle for Life*. Signet Classics, 359 pp., New York.

Dell, C. 2010 - *Monștrii, un bestiar al bizarului*. Editura ART, 192 pp., Rochester, Vermont, Toronto, Canada.

Desai, N.S. 1980 - *Hinduism in Thai Life*. Popular Prakashan, 135 pp., Bombay.

Dixon-Kennedy, M. 1998 - *Encyclopedia of Greco-Roman Mythology*. Library of Congress Cataloging-in-Publication Data, 370 pp., Santa Barbara, California, Denver, Colorado, Oxford, England.

Domning, D.P. 1994 - A phylogenetic analysis of the Sirenia. In: Berta, A., Deméré, T. (eds.) - *Contributions in marine mammal paleontology honoring Frank C. Whitmore Jr., Proceedings of the San Diego Society of Natural History* **29**: 177–189, San Diego, California.

Domning, D.P., Gingerich, P.D., Zalmout, I.S. 2010 - Sirenia, In: Werdelin, L., Sanders, W.J. (eds.), *Cenozoic Mammals of Africa*. 2nd edition, University of California Press: 147-160, Berkeley, Los Angeles, London.

Dorofeeva, A. 2014 - The Siren: A Medieval Identity Crisis. *Mittelalter. Interdisziplinäre Forschung und Rezeptionsgeschichte* 1-5. https://doi.org/10.58079/rgtt

Eliade, M. 1978 - *Aspecte ale mitului*. Editura Univers, 212 pp., București.

Fattori, D. 2009 - The Mermaid Song. The Taulanne sirenian site in France, one of the oldest in the world. https://www.paleonature.org/paleotraveling/166-the-mermaid-song-the-taulanne-sirenian-site-in-france

Florei, N. 1962 - Noi forme de Gastropode tortoniene de la Zorlențul-Mare (Banat). *Studia Universitatis Babeș-Bolyai, Geologia-Geographia* **7**(1): 63–73, Cluj-Napoca.

Fowler, L. R. 2013 - *Early Greek Mythography, Volume 2: Commentary*. Oxford University Press, 848 pp., New York.

Fuchs, H. 1959 - Sziréna-lelet Erdélyből (Ein Sirenenfund aus Siebenbürgen). *Földtani Közlöny* **89**(3): 326–328, Budapest.

Fuchs, H. 1970 - Schädelfragment einer Sirene aus dem Eozän von Cluj, SR Rumänien. *Geologie* **19**(10): 1185–1190, Berlin.

Fuchs, H. 1971 - Contribuții la cunoaşterea raspîndirii stratigrafice şi geografice a sirenidelor în Bazinul Transilvaniei. *Buletinul Societății de Științe Geologice din Republica Socialistă România* **13**: 195–200, București.

Fuchs, H. 1973 - Contribuţiuni la cunoaşterea sirenidelor fosile din Bazinul Transilvaniei (IV): Asupra unui fragment de humerus din Cheia Baciului (Cluj). *Studia Universitatis Babeş-Bolyai, Seria Geologie-Mineralogie* **18**(2): 71–77, Cluj.

Fuchs Brassói, H. 1988 - Adatok az Erdélyi-medence ásatag szirénjeinek ismeretéhez (VI.): Alkarcsontok a kolozsvári (Cluj-Napocai) felsőeocénből. *Földtani Közlöny* **118**(1): 49–60, Budapest.

Fuchs Brassói, H. 1990 - Einige Angaben zur Kenntnis von fossilen Sirenen (VII). Sirenenzähne aus der Umgebung von Cluj (Klausenburg, Rumänien). *Földtani Közlöny* **120**(1–2): 89–92, Budapest.

Grigorescu, D. 1967 - Asupra prezenfei unor fragmente scheletice de sirenide din Paleogenul de la Albești-Muscel. *Analele Universității Bucureşti, Seria Ştiințele Naturii (Geologie-Geografie)* **16**(1): 73–78, București.

Guillaume le Clerc de Normandie 1970 - *Le bestiaire divin*. Slatkine Reprints, 323 pp., Genéve.

Haase, W., Reinhold, M. 1993 - *The Classical Tradition and the Americas: European images of the Americas and the classical tradition. Volume I, European Images of the Americas and the Classical Tradition, Part I*. Walter de Gruyter & Co., D-10785, 203 pp., Berlin.

Ivanits, J.L. 1992 - *Russian Folk Belief*. M. E. Sharpe Inc., 272 pp., Armonk, New York, and London England.

Keith, S., Sung Ae, L. 2018 - Legend of the Blue Sea: Mermaids in South Korean folklore and popular culture, In: Hansen, J., Sato, M., Russel, G., Khercha, P. (eds.) - *Scaled for Success: The Internationalisation of the Mermaid*. John Libbey Publishing Ltd.: 69-89. United States of America.

Kernbach, V. 1989 - *Dicționar de mitologie generală*. Editura Științifică și Enciclopedică, 183 pp., București.

Koch, A. 1891 - A review of the Transylvanian fossil mammals. *A Magyar orvosok és természetvizsgálók 1890 Augusztus 16-20-ig, Nagyváradon tartott XXV vándorgyűlésének történeti vázlata és munkálatai*: 456-466, Budapest (in Hungarian).

Koch, A. 1894 - Die Tertiarbildungen des Beckens der Siebenbürgischen Landestheile. I. Paleogene. auswirkungen auf die Oligozäne, und in der Nachbargebieten. *Acta Geologica Hungarica*, I Z: 263 – 290, Budapest.

Koch, A. 1900 - A systematic review of the fossil vertebrate remains from the lands of the Hungarian crown. *Magyar orvosok és természetvizsgálók vándorgyűlései* **30**: 526-560, Budapest (in Hungarian).

Kordos, L. 2002 - Eocene Sea Cows (Sirenia, Mammalia) from Hungary. *Fragmenta Palaeontologica Hungarica* **20**: 43-48, Budapest.

Kun, N.A. 1964 - Legendele și miturile Greciei Antice. Editura Științifică, 542 pp., București.

Mitakidou S., Manna L.A., Kanatsouli M. 2002 - *Folktales from Greece: A Treasury of Delights*. Libraries Unlimited, 95 pp., Colorado.

Mittman, S.A., Dendle, J.P. 2013 - *The Ashgate Research Companion to Monsters and the Monstrous*. Ashgate, 558 pp., England.

Nagy, I. 1865 - *Magyarország családai czímerekkel és nemzékrendi táblákkal, tizenkettedik kötet*. Kiadja Ráth Mór, Pest, 158 pp., Budapest.

Orchard, A. 1995 - *Pride and Prodigies: Studies in the Monsters of the Beowulf Manuscript*. University of Toronto Press, 263 pp., Toronto, London.

Pope, A. 1880 - *The Odyssey of Homer*. New York: John Wurtele Lovell, Publisher, 14 & 16 Astor Place, 384 pp., New York.

Rackham, H. 1967 - *Pliny's Natural History, with an English Translation, in Ten Volumes*. Volume III, Libri VII-XI. Harvard University Press, Cambridge Massachusetts, William Heinemann Ltd., book 10, 140 pp., London.

Russell, E. 2013 - *Literature and Culture in Late Byzantine Thessalonica*. Bloomsbury, 22 pp., London, New Delhi, New York, Sydney.

Scheck, P.T. 2015 - *St. Jerome: Commentary on Isaiah: Including St. Jerome's Translation of Origen's Homilies 1-9 on Isaiah*. Newmann Press, 1106 pp., New York.

Steele, R. 1905 - *Medieval Lore from Bartholomew Anglicus*. Alexander Moring Ltd., the de la More Press, 222 pp., London.

Șuraru, N., Codrea, V.A. 1988 - Un dinte premolar (p4 dext) de sirenid (Mammalia, Sirenia) în orizontul Calcarului de Cluj, de la Cluj-Napoca: Cheile Baciului. *Nymphaea* **18**: 689-695, Oradea.

Tabuce, R., Asher, R.J., Lehmann, T. 2008 - Afrotherian Mammals: a review of current data. *Mammalia* **72**: 2–14, New York, Berlin.

Temkin, C.L., Rosen, G., Zilboorg, G., Sigerist, H.E. 1996 - *Four Treatises of Theophrastus Von Hohenheim Called Paracelsus*. The Johns Hopkins University Press, 280 pp., Baltimore, London.

Veress, L., Codrea, V.A. 2020 - A freshwatersoft-shelled turtle in the Albești Limestone. *Muzeul Olteniei Craiova. Oltenia. Studii şi comunicări. Ştiinţele Naturii* **36**(1): 7–20, Craiova.

Veress, L., Codrea, V.A., Venczel, M. 2025 - Overview of the Transylvanian Paleogene sea cows' distribution records, with a focus on a new Oligocene locality. *North Western Journal of Zoology* **21**(6): 61-72, Oradea.

Voss, M. 2014 - On the invalidity of *Halitherium schinzii* Kaup, 1838 (Mammalia, Sirenia), with comments on systematic consequences. *Zoosystematics and Evolution* **90**: 87-93, Berlin.

Walker M. 2015 - The giant cow that swam the ocean, http://www.bbc.com/earth/story/20150613-the-giant-cow-that-swam-the-ocean?referer=https%3A%2F%2Fen.wikipedia.org%2F

Wasilewski, J., Kostrzewa, A. 2018 - Syrenka Tattoos: Personal Interpretations of Warsaw Symbol. *Shima* **12**(2): 144 – 162, Sydney.

Watkins, V. 1962 – Affinities: The Ballad of the Mermaid of Zennor. *Poetry* **95**(1): 11-14, London.

Watson, E.C. 1908 - Highland Mythology. *The Celtic Review* **5** (17): 67, Edinburgh.

Waugh, A. 1960 - The Folklore of the Merfolk. *Folklore* **71** (2): 73.

Wood, R. 2010 - The Norman Chapel in Durham Castle. *Northern History* **47**(1): 9-48, England.

Yoda, H., Alt, M. 2008 - Yokai Attack!: The Japanese Monster Survival Guide. Kodansha USA, 265 pp., Tokyo, New York, London.

Zalmout, I. S., Ul-Haq M., Gingerich P.D. 2003 - New Species of Protosiren (Mammalia, Sirenia) from the Middle Eocene of Balochistan (Pakistan). *Museum of Paleontology, The University of Michigan*, Ann Arbor, **31**(3): 79-87, Michigan.

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Phytopathogens associated with the sweet potato crops cultivated in Romania

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Abstract. The sweet potato, *Ipomoea batatas* (L.) Lam., gained attention in Romania for both agronomists and consumers, due to its productivity, and nutritional importance, respectively. However, the crop is now challenged by the emergence of common and targeted fungal phytopathogens, some severely reducing the yield and reducing storability, due to an increased risk of post-harvest spoilage. The aim of the paper is to document the relevant fungal pathogens affecting the sweet potato grown in the sandy soils of Dăbuleni

area, with particular interest on field-associated infections. Besides symptomatology, plant pathogens were identified based on their morphology, and through laboratory analysis. The microbiologic studies consisted in fungal isolation in pure cultures, and their identification using the Biolog technique and the side cultures method. These two techniques allowed species or genus identification based on pathogen physiological profile and microscopic traits. Among diseases, *Fusarium* wilt is highlighted to be the most common and damaging. To mitigate the disease impact and support the sustainable development of sweet potato cultivation, there is a need for dedicated disease management strategies focused on this soil-borne pathogen.

Keywords: Biolog FF, *Fusarium* wilt*, Ipomoea batatas*, leaf spots, myxomycetes, slide cultures.

Introduction

Sweet potato (*Ipomoea batatas* [L.] Lam.) is a globally significant tuber crop, that has gained increasing attention in Romania. Consumers are mostly focused on its' nutritional value and organic production, while agronomists are pleased with the crops' high productivity and tubers market value. Moreover, sweet potato ensures diversification of local agriculture, while expanding the crop rotation possibilities. Although traditionally underutilized in Eastern Europe, recent agronomic trials in various regions of Romania, including South-West Oltenia, have demonstrated promising yields and biochemical profiles under temperate continental conditions (Dinu et al., 2021 a, b). The crop's expansion aligns with broader European goals of sustainable agriculture and functional food production.

However, expanding this crop in enlarged areas and new agroecosystems raises concerns regarding phytosanitary risks, particularly the emergence or adaptation of pests and phytopathogens. In Romania, the lack of historical cultivation data complicates the prediction and management of biotic stressors. Studies have highlighted the susceptibility of sweet potato to a wide range of threats (Mounika et al., 2025).

Given the crop's emerging role in Romanian agriculture, this study aims to document the relevant fungal pathogens affecting the sweet potato grown in the sandy soils of Dăbuleni area, with particular interest on field-associated infections. The findings will contribute to the following development of integrated pest management strategies tailored to Romanian conditions and support the safe expansion of sweet potato, as a resilient and nutritionally valuable crop.

Materials and methods

Sweet potato production was carried out at the Research-Development Station for Field Crops on Sandy Soils (SCDCPN) Dăbuleni, in Dolj County, Romania. Their entire production cycle from seed-tubers selection, bedding the tubers to induce sprouting in greenhouse conditions, shoots propagation, field planting and cultivation, harvesting and storing were performed at SCDCPN Dăbuleni.

Sweet potato diseases, encountered in the greenhouse and field conditions were first identified based on their symptomatology. To identify the pathogens involved in the infection, the symptomatic plant material was microbiologically analyzed at the Research-Development Institute for Plant Protection (RDIPP) Bucharest, Romania. Laboratory analysis consisted in pathogen isolation on Dichloran Rose Bengal Chloramphenicol Agar or Potato Dextrose Agar supplemented with antibiotics. In most cases, symptomatic plant samples were maintained for some days in humid chambers, at room temperature, to promote fungal growth and sporulation, as well as to improve pathogens' isolation.

Purified isolated pathogens were analyzed by Biolog FF technique (Singh, 2009) and slide cultures (Tunç et al., 2025) on Malt Extract Agar (MEA), to identify the filamentous fungi.

For identification with the Biolog system (Biolog, Hayward, CA, USA), the purified fungi were grown on MEA for 10 to 14 days, then conidia were harvested with a cotton-ended sterile swab and resuspended in FF inoculation fluid provided

by the manufacturer. The spore suspension of 75% turbidity was inoculated in 96 wells Biolog FF MicroPlates using 100µL/well. Plates were then incubated at 26ºC for 3 to 5 days, repeatedly analyzed spectrophotometrically at 490nm and 750nm using a Biolog Microstation Reader. Collected optical density (OD) values were used to calculate color and turbidity data saved in ASCII files (American Standard Code for Information Interchange). Two formulas were used to process the OD collected values, one for color data (Equation 1), and the other for turbidity data (Equation 2), as follows:

$$\text{OD data on FF printouts for color data} = \\ = [(\text{OD490nm}_x - \text{OD490nm}_{A1}) \\ - (\text{OD750nm}_x - \text{OD750nm}_{A1})] \times 1000 \quad \text{(Equation 1)}$$

$$\text{OD data on FF printouts for turbidity data} \\ = (\text{OD750nm}_x - \text{OD750nm}_{A1}) \times 1600 \quad \text{(Equation 2)}$$

where x is any well from A2 to H12, while A1 is the control well

Results were processed with the MicroLog 3 software, version 5.2., which automatically revealed the fungal identification at genus or species level, based on their phenotypic similarity and distance from other fungal taxons found in the Biolog FF database.

Slide cultures were prepared on MEA growth medium. The agar blocks were placed on sterile microscopic slides, inoculated and axenic covered with sterile coverslips, then incubated in moist chambers to allow the undisturbed development of conidiophores and spores. The coverslips were afterwards mounted in lactophenol cotton blue, for microscopic observation. For this, the coverslips were detached with a tweezer from the slide cultures, after their incubation and fungal development, and then placed on a clean microscopic slide bearing a droplet of lactophenol cotton blue. The stain pigmented with blue the hyaline mycelia and spores, allowing an improved visualization of the microscopic features of the specimen under the microscope. Nikon Eclipse E200 optic microscope, having 10X and 40X objectives, was used for microscopic

examination. Dedicated guidelines and manuals for fungal identification were consulted to recognize the plant pathogens (Leslie and Summerell, 2006; Taralova et al., 2011).

Results and discussion

Greenhouse contaminants during sweet potato sprout induction

During sprout induction, no important plant pathogens were found. Most probably, the high quality and proper selection of the seeding tubers led to reduced phytosanitary issues. However, some slime molds were identified within greenhouse sprout induction causing disturbances. Symptoms revealed slime molds such as *Fuligo* species, most probably *F. septica* (Figure 1a) and *F. gyrosa* (Figure 1b), as well as *Stemonitis axifera*. (Figure 1c).

These Myxogastria slime molds, also known as Myxomycetes, are saprotrophs that usually develop on decaying wood and soil organic matter. Although they are not plant pathogens, these slime molds were able to superficially colonize sweet potato shoots and basal leaves, only on their surface.

a. b. c.

Figure 1. Myxomycetes slime molds
a. *Fuligo septica*, b. *F. gyrosa*, c. *Stemonitis axifera*.

Similar myxomycetes were previously mentioned on sweet potato grown at SCDCPN Dăbuleni (Coteț et al., 2021). Most probably, the inoculum was brought into the greenhouse with the soil mixture added in the raised beds. This mixture included forest topsoil, along with sand, peat and NPK fertilizer. Additionally, the frames used for the raised beds were built from old wooden planks that also favored slime molds' survival and opportunistic development during subsequent growing seasons.

Field phytopathogens of sweet potato

Field-grown sweet potato was mainly challenged by two diseases, plant wilt (Figure 2a) and leaf spots (Figure 2b).

a. b.

Figure 2. Sweet potato plant diseases
a. *Fusarium* wilt, b. leaf spot

Visual analysis of the wilted plants revealed basal stem rot. Various samples, such as stems (Figure 3a), plant crown (Figure 3b) and tuberizing roots (Figure 3c), were therefore collected from symptomatic wilted plants and submitted to laboratory analysis for pathogen identification.

a. b. c.

Figure 3. Late symptoms of *Fusarium* spp. infections on stem (a), plant crown (b) and tuberizing roots (c) of sweet potato

The infected plant parts were detached, cleaned, chopped and placed into humid chamber to ensure pathogen proliferation on the host plant tissue. After days of incubation at room temperature, fungal fruiting bodies have developed. Sporodochia bearing *Fusarium* type conidia were identified after direct microscopic evaluation (Figure 4).

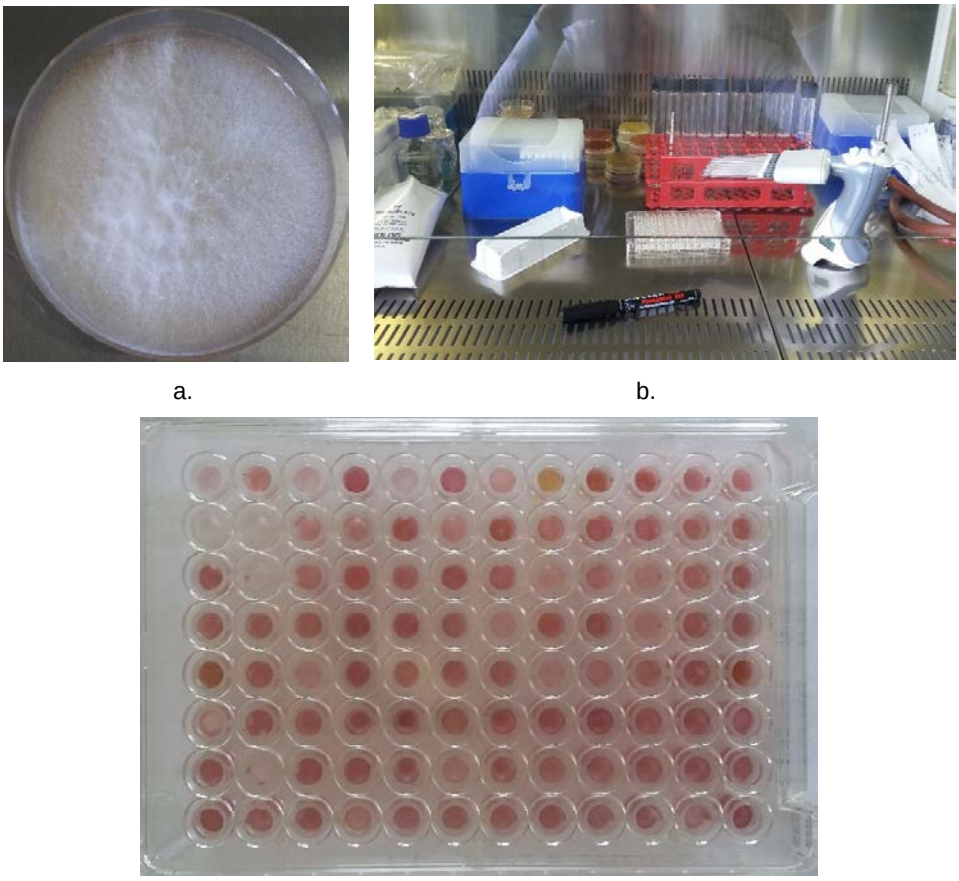
a. b.

Figure 4. *Fusarium* sp. macroscopic and microscopic view (40X magnification)
a. sporodochia on plant crown, b. hyaline fusiform macroconidia with 3 to 5 transversal septa

Macroconidia with 3 to 5 septa were mainly seen in sporodochia developed on diseased plant crown. While on stems the infecting *Fusarium* spp. produces abundant microconidia, and less abundant macroconidia of 3 septa. In the case of symptomatic tuberizing roots, microconidia formed on phialides were most predominant.

After transferring the fungal inoculum on agar plates, the isolates were purified and grown on MEA. Spore suspension prepared in FF inoculation fluid and grown on various single carbon sources in Biolog FF microplates revealed reliable phenotypic identification after 3 to 5 days of incubation (figure 5). The Biolog FF technique provided specific phenotypic patterns. For each strain, the fungal profile of various nutrients utilization was compared to a broad database of 710 taxa. Spectrophotometric analysis at two wavelengths was processed before scoring the results. The optical density at 490 nm collected data on tetrazolium redox dye reduction of 95 single carbon sources compared to a negative control well. Increased respiration revealed metabolic activity and fungal nutritional preferences, which induced the redox dye color change that was measured afterwards.

Alongside, optical density at 750 nm was also recorded to estimate fungal biomass (Pinzari et al., 2016). The row data was processed and the semiautomated Biolog system provided the fungal identification results. Four fungal species were detected, based on carbon source utilization profile and tetrazolium redox dye reduction (Table 1).



a. b.

c.

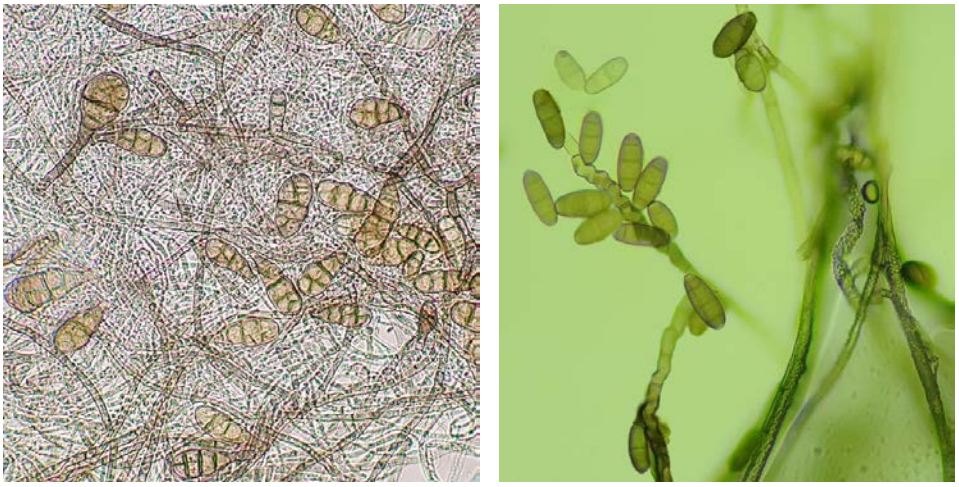
Figure 5. *Fusarium oxysporum* identification through Biolog FF technique
a. pure culture on MEA, b. aspects from Biolog FF microplate inoculation,
c. phenotypic pattern of *F. oxysporum* on the 96 well Biolog FF microplate.

Table 1. *Fusarium* species identification using the Biolog FF technique

No.	*Fusarium* species	Similarity with database FF	Distance between identified strain and other FF proposal
1	*F. oxysporum*	0.684 to 0.753	5.360 to 5.795
2	*F. equiseti*	0.654	5.761
3	*F. proliferatum*	0.618	5.616
4	*F. verticillioides*	0.703	5.873

Most studies on sweet potato wilt mention *F. oxysporum* as the responsible pathogen for this disease (Paul et al., 2020; Wang et al., 2024; Lee et al., 2025). Moreover, Mousa et al. (2018) mention the special form *F. oxysporum* f.sp. *batatas* being adapted to wilt sweet potato, without causing infections in non-*Convolvulaceae* plants. *F. equiseti* was also found to be associated with sweet potato plant wilt, alongside *F. oxysporum* (Bibi et al., 2024). However, some *F. verticillioides* isolated are mentioned to be much more virulent than other *F. oxysporum* (Gunua, 2010). Among the sweet potato pathogens, *F. proliferatum* and *F. solani* (Scruggs and Quesada-Ocampo, 2016; Yang et al., 2018) are also mentioned, although they are involved in root rot.

Among the leaf spots of sweet potato, two pathogens were found, one belonging to *Alternaria*, and the other to *Curvularia* genus. These pathogens were identified based on conidia morphology. *Alternaria* sp. has clavate-shape, pluricellular conidia, with transversal and longitudinal septa (Figure 7a), while *Curvularia* sp. has slight curve-shape conidia with 3 transversal septa. Both fungi are dematiaceous, with dark pigmented spores and mycelia. By slide cultures *Alternaria* conidia were shown to arise in small chains, while *Curvularia* showed geniculate conidiophores (figure 7b).



a. b.

Figure 7. Phytopathogenic fungi associated with sweet potato leaf spot diseases (40X magnification)
a. *Alternaria* sp. mycelia and spores, b. *Curvularia* sp. conidiophore bearing conidia

Complex studies, based on morphologic traits and genetic analysis, performed on *Alternaria* leaf pathogens, revealed various species infecting sweet potato, among which *A. bataticola*, *A. ipomoeae* and *A. neoipomoeae* are listed (Woudenberg et al., 2014). *Curvularia* sp. was also mentioned to produce leaf infections on sweet potato (Baharon et al., 2019). Among the pathogenic *Curvularia* species associated with sweet potato, *C. plantarum* was identified to cause leaf spots (Lai et al., 2022), while *C. spicifera* was related to black rot (Wu et al., 2025). However, within this study, the identification of leaf spot pathogens was limited to genus level.

Conclusion

The main phytosanitary problems of sweet potato plants cultivated in Romania remain *Fusarium* wilt and leaf spots. However, some *Fusarium* spp. infections can contaminate plant crown and tuberizing roots, increasing the incidence of root rot after

harvest. Accurate identification of sweet potato field pathogens under Romanian growing conditions is essential for understanding the major disease constraints of this crop. This allows targeted disease management strategies to reduce infection incidence and minimize tuber infection risks, and future yield losses.

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References

Baharon, M. N., Zulkifli, N. S. A., Baharom, N. A., Yunus, S. M. 2019 - Antifungal activity of selected plant extracts against *Curvularia* sp. infecting local purple sweet potato (*Ipomoea batatas*). *Malaysian Journal of Microbiology* **15** (4): 342–345. https://doi.org/10.21161/mjm.1915411

Bibi, A., Mubeen, F., Rizwan, A., Ullah, I., Hammad, M., Waqas, M. A. B., Ikram, A., Abbas, Z., Halterman, D., Saeed, N. A. 2024 - Morpho-molecular identification of *Fusarium equiseti* and *Fusarium oxysporum* associated with symptomatic wilting of potato from Pakistan. *Journal of fungi (Basel, Switzerland)* **10** (10): 701. https://doi.org/10.3390/jof10100701

Coteț, G., Coșoveanu, A., Radu, I., Diaconu, A., Mitrea, R., 2021 - Slime mold - sweet potato association on sandy soils of Romania. *Scientific Papers. Series A. Agronomy* **64** (1): 60–67.

Dinu, M., Soare, R., Hoza, G., Becherescu, A. D., Babeanu, C. 2021 - Bioactive compounds content and antioxidant activity in the leaves of some sweet potato cultivars (*Ipomoea batatas* L.). *Scientific Papers. Series B, Horticulture* **65** (1): 415–422.

Dinu, M., Soare, R., Babeanu, C., Hoza, G., Sima, R., 2021 - Nutraceutical value and production of the sweet potato (*Ipomoea batatas* L.) cultivated in South-West of Romania. *Journal of Central European Agriculture* **22** (2): 285–294. https://doi.org/10.5513/JCEA01/22.2.2982

Gunua, T. G. 2010 - A review of sweet potato scab and studies on *Fusarium* wilt of sweet potato (*Ipomoea batatas* (L.) Lam.) and approaches to its management. MPhil Thesis, School of Agriculture and Food Sciences, The University of Queensland.

Lai, J., Liu, T., Liu, B., Kuang, W., Song S. 2022. First report of *Curvularia plantarum* causing leaf spot on sweet potato (*Ipomoea batatas*) in China. *Plant Disease* **106** (6): 1753. https://doi.org/10.1094/PDIS-08-21-1665-PDN

Lee, J. G., Ji, J. H., Ryu, S., Park, J. W., Paul, N. C., Park, S., Im, S., Han, G.H., Sang, H. 2025 - Control of sweet potato *Fusarium* wilt and black rot by endophytic *Bacillus velezensis* with improved lipopeptide production. *BMC Microbiology* **25**: 603. https://doi.org/10.1186/s12866-025-04334-4

Leslie J. F., Summerell, B. A. 2006 - *The Fusarium laboratory manual*. First Edition. Bloackwell Publishing Ltd., Oxford, UK. ISBN-13: 978-0-8138-1919-8

Mounika, V., Gowd, T. Y. M., Lakshminarayana, D., Krishna, G.V., Yogesh, M., Reddy, S. I. V., Soumya, B. K., Chamuah, S., Singh, Y. D., Reddy P.M. 2025 - Sweet potato (*Ipomoea batatas* (L.) Lam.): a comprehensive review of its botany, nutritional composition, phytochemical profile, health benefits, and future prospects. *European Food Research and Technology* **251**: 3225–3239. https://doi.org/10.1007/s00217-025-04828-5

Mousa, T. A. A., Farag, M. F., Armanious, H. A., Salem, A. A., Galal, A. A. 2018 - Fusarium wilt of sweet potato caused by *Fusarium oxysporum* f. sp. *batatas* in Egypt. *Egyptian Journal of Phytopathology* **46** (1): 21–35.

Paul, N. C., Park, W., Lee, S., Chung, M. N., Lee, H. U., Yang, J. W. 2020 - Occurrence of sweet potato (*Ipomoea batatas*) wilt and surface rot disease and determining resistance of selected varieties to the pathogen in Korea. *Plants (Basel, Switzerland)* **9** (4): 497. https://doi.org/10.3390/plants9040497

Pinzari, F., Ceci, A., Abu-Samra, N., Canfora, L., Maggi, O., Persiani, A. 2016 - Phenotype MicroArray™ system in the study of fungal functional diversity and catabolic

versatility. *Research in Microbiology* **167** (9–10): 710–722. https://doi.org/10.1016/j.resmic.2016.05.008

Scruggs, A. C., Quesada-Ocampo, L. M. 2016 - Etiology and epidemiological conditions promoting *Fusarium* root rot in sweet potato. *Phytopathology* **106** (8): 909–919. https://doi.org/10.1094/PHYTO-01-16-0009-R

Singh, M. P. 2009 - Application of Biolog FF MicroPlate for substrate utilization and metabolite profiling of closely related fungi. *Journal of Microbiological Methods* **77** (1): 102–108. https://doi.org/10.1016/j.mimet.2009.01.014

Taralova, E.H., Schlecht, J., Barnard, K., Pryor, B. M. 2011 - Modelling and visualizing morphology in the fungus *Alternaria. Fungal Biology* **115** (11): 1163–1173. https://doi.org/10.1016/j.funbio.2011.08.002.

Tunç, G., Uçar, F., Telli, O., Lima, N., Boiu-Sicuia, O.A. 2025 - Fungal contaminants in Turkish bottled water and their mycotoxin-producing potential. *GMS Hygiene and Infection Control* **20**: 41. https://dx.doi.org/10.3205/dgkh000570

Wang, J., Chen, P., Zhao, T., Huang, X., Zong, J., Luo, Q., Peng, C., Wu, X., Qiu, F., Zhao, D., Xiang, L., Zhang, Y., Yang, C., Zhang, F., Liao, Z., Fu, Y., Zeng, J. 2024 - Biosynthesis of scopoletin in sweet potato confers resistance against *Fusarium oxysporum*. *Journal of Agricultural and Food Chemistry* **72** (14): 7749–7764. https://doi.org/10.1021/acs.jafc.3c09389

Woudenberg, J. H. C., Truter, M., Groenewald, J. Z., Crous, P. W. 2014 - Large-spored *Alternaria* pathogens in section Porri disentangled. *Studies in Mycology* **79**: 1–47. https://doi.org/10.1016/j.simyco.2014.07.003

Wu, Y., Zhang, F., Zhang, S., Zhang, J., Zhao, S., Qiu, Z., Zhu, M. 2025. *Curvularia spicifera* causing black rot on *Ipomoea batatas* in China. *Plant Disease* **109** (10): 2207. https://doi.org/10.1094/PDIS-03-25-0477-PDN

Yang, J.-W., Nam, S. S., Lee, H.-U., Choi, K.-H., Hwang, S.-G., Paul, N. C. 2018 - *Fusarium* root rot caused by *Fusarium solani* on sweet potato (*Ipomoea batatas*) in South Korea. *Canadian Journal of Plant Pathology* **40**: 90–95.

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The catalogue of the „Natura2000" coleoptera species from the entomological collection of the „Țării Crişurilor" Museal Complex of Oradea

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Abstract. The entomological collection housed in the Natural Sciences Section of the "Țării Crișurilor" Museal Complex in Oradea plays a vital role in documenting regional biodiversity and supporting conservation efforts. Among these holdings are 135 specimens out of which 132 adults and three larvae, belonging to six Coleoptera species

listed under the Natura 2000 conservation framework. This study provides the first integrated inventory and taxonomic reassessment of these protected taxa within the museum collection. Through an inventory and taxonomic assessment of the collection, the following species were identified: *Carabus rothi hampei*, *Carabus variolosus*, *Cerambyx cerdo*, *Morimus asper funereus*, *Rosalia alpina* and *Lucanus cervus*. Together, these species represent 30% of all Coleoptera taxa protected at the national level in Romania. Most specimens were collected from the vicinity of Oradea, offering valuable insights into the historical distribution of these taxa. The findings highlight not only the zoogeographical significance of the collection but also its value as a reference for monitoring biodiversity changes over time. Ultimately, the presence of these species within the museum's holdings underscores the essential role of scientific collections in biodiversity documentation, conservation research, and the implementation of effective ecological strategies under the Natura 2000 network.

Key words: Coleoptera, Natura2000, "Țării Crişurilor" Museum, museum collection, protected species.

Introduction

The Natural Sciences Section of the "Țării Crișurilor" Museal Complex in Oradea preserves a scientifically significant entomological collection that encompasses six Coleoptera species of high conservation interest, all formally listed under the Natura 2000 framework. Although numerically limited, this assemblage is taxonomically and conservationally noteworthy: taken together, the recorded species represent nearly one-third (30%) of the beetle taxa afforded legal protection at the national level in Romania, as specified in Government Emergency Ordinance No. 57/2007 (O.U.G. 57/2007). The majority of specimens originate from Oradea and its immediate surroundings, a circumstance that confers particular value to the collection by documenting the historical presence, local distribution patterns, and habitat associations of threatened Coleoptera within an intensively transformed lowland landscape. As such, the collection constitutes an important

archival resource for understanding long-term faunal dynamics, informing regional biodiversity assessments, and evaluating the extent to which contemporary conservation areas encompass historically suitable habitats for these taxa.

These data highlight the dual importance of historical entomological collections as they function not only as repositories of past biodiversity but also as indispensable instruments for contemporary conservation science. By documenting the species' former distributions, habitat affinities, and potential shifts in occurrence over time, such collections provide an essential baseline against which long-term ecological change can be assessed. Their value extends beyond faunistic reconstruction since, when integrated into broader biodiversity evaluations, museum specimens contribute to more accurate assessments of species' conservation status, refine habitat suitability analyses, and enhance the evidence base underlying Natura 2000 management decisions. Consequently, they play a critical role in guiding both regional and national conservation policies and in strengthening the scientific foundation of ongoing monitoring and habitat-protection efforts.

Material and methods

The identification of specimens was conducted through the visual inspection of associated labels and individuals, and the extracted data were systematically compiled into a curated digital database. Despite the limitations inherent to historical collections, such as incomplete or imprecise locality information, a significant portion of the specimens retains high scientific value due to their relevance for biogeographical and conservation-oriented analyses. The analysed material was collected from 22 distinct localities, of which five fall within the boundaries of designated Natura 2000 sites, and twelve are situated in their vicinity. This spatial pattern highlights the collections' relevance for documenting both protected and adjacent habitats.

In the case of *Carabus rothi hampei*, species confirmation was performed following the diagnostic criteria established by Turin, Penev, & Casale (2003). For the remaining species, visual examination of morphological features was

conducted, and all available label data were systematically recorded and organized into a dedicated database.

Collection sites were classified according to their spatial relation to Romanian Natura 2000 protected areas, as either located within ("Inside" = I) or in the immediate vicinity ("Vicinity" = V), based on the delineations provided by Brînzan (2013), and are presented in detail in Table 1.

For spatial representation, a distribution map at a scale of 1:1,475,053 was generated using QGIS v. 3.40 “Bratislava,” applying the EPSG:3844 coordinate reference system (Pulkovo 1942(58) / Stereo 70) to ensure geospatial accuracy and regional compatibility.

The nomenclature was revised in accordance with the updates proposed by Bartolozzi & Sprecher-Uebersax (2006), Bousquet et al. (2003), and Sama & Löbl (2010). Notably, the species formerly identified as *Morimus funereus* is now recognized as *Morimus asper* ssp. *funereus*, following the reclassification by Sama & Löbl (2010). Where applicable, inventory numbers of the analysed specimens are provided in brackets to ensure full traceability and facilitate future reference.

All analyses and graphical outputs were generated in R (R Core Team 2024) using ggplot2 for plot construction (Wickham 2016). Basic data processing was performed with dplyr (Wickham et al. 2023), while axis scaling and formatting were handled using the scales package (Wickham & Seidel 2020). Perceptually uniform colour palettes were provided by viridisLite (Garnier 2024).

Table 1. The correspondence between the collecting places and the Natura2000 protected areas

Nr.	Collecting place	Nearest locality	Natura2000 site (I/V)
1	Bacea (Ilia)	Ilia	Defileul Mureşului (V), Râul Mureș between Brănișca and Ilia (V)
2	Băile Felix	Sânmartin	Betfia (V)
3	Băile 1 Mai	Rontău (Sânmartin)	Lacul Peţea (I)
4	Gilău	-	-
5	Gligoreşti	-	Munţii Apuseni – Vlădeasa (V)
6	Lăzăreni	-	Codru Moma (V)
7	Meseş Mts.	Meseşenii de Jos	-
8	Micula	-	Râul Tur (V)

9	Negreşti (as Negreşti-Oaş)	-	-
10	Nojorid	-	-
11	Oradea	-	Valea Roşie (V)
12	Paleu forest	Paleu	Valea Roşie (V)
13	Râtu Florilor	-	Platoul carstic Padiş (I)
14	Salonta	-	Pescăria Cefa – Pădurea Rădvani (V)
15	Săldăbagiu forest	Săldăbagiu de Munte	Defileul Crişului Negru / Valea Roşie (V)
16	Sâmbăta (BH)	-	-
17	Scărişoara Tomasca	Scărişoara	Peştera, Platoul carstic Padiş / Trascău(I)
18	Scăriţa-Belioara	Poşaga de Sus	Muntele Mare (Botanic reserve) (I)
19	Slănic? Maybe Slănic-Prahova or Slănic-Moldova	Slănic-Prahova or Târgu Ocna	-
20	Ştei	Nucet (ex Petru Groza)	Munţii Apuseni – Vlădeasa (V)
21	Glighii valley	Rontău (Sânmartin)	Lacul Peţea (I)
22	Vadu Crişului	-	Muntele Şes (V)

Abbreviations used:

A.T.U. = Administrative Territorial Units (counties)

leg. = legit (“collected by”, Lat.)

s.d. = sine data („without data”, Lat.)

spec(s) = specimen(s)

sub nom. = sub nomen (“under the name” for “[previously] identified as”, lat.)

Counties: AB – Alba; BC – Bacău; BH – Bihor; CJ – Cluj; PH – Prahova; SM - Satu Mare; SJ – Sălaj

Results

The examined material consists of 135 Coleoptera specimens, 132 adults and three larvae, belonging to six species and was assigned to the following taxa: *Carabus (Morphocarabus) rothi hampei* (27 specimens), *Carabus (Hygrocarabus) variolosus* (5 specimens), *Cerambyx cerdo* (3 specimens), *Morimus asper funereus* (6 specimens), *Rosalia alpina* (7 specimens), and *Lucanus cervus* (87 specimens, including 84 adults and three larvae).

Class: Insecta

Subclass: Pterygota

Order: Coleoptera Linnaeus, 1758

Superfamily: Caraboidea Latreille, 1802

Family: Carabidae Latreille, 1802

Subfamily: Carabinae Latreille, 1802

Tribe: Carabini Latreille, 1802

Genus: *Carabus* Linnaeus, 1758

1. *Carabus (Morphocarabus) rothi hampei* Küster, 1846

1 spec., sub nom. *Carabus comptus hampei,* Oradea (BH), V.1973 [22812]; 1 spec. sub nom. *Carabus comptus hampei*, Negreşti (AB), VIII.1985; 1 spec., sub nom. *Carabus comptus hampei* ab. *eximius*, Băile Felix (BH), VI.1986 [22811]; 1 spec., sub nom. *Carabus comptus hampei* Gligoreşti (AB), .VII.1987; 1 spec, sub nom. *Carabus comptus hampei,* Bacea (Ilia) (HD), VII.1989; 3 specs, sub nom. *Carabus comptus hampei,* Micula (SM), VI.1992; 2 specs, sub nom. *Carabus comptus hampei* ab. *dacicus*, s.d. [22829, 22830]; 4 specs, sub nom. *Carabus comptus hampei gutiiensis*, s.d. [22831, 22832, 22384, 22835]; 1 spec., sub nom. *Carabus comptus hampei* ab. *hunyadensis*, s.d. [22833]; 2 specs, sub nom. *Carabus comptus hampei* ab. *telekii*, s.d. [22836, 22837]; 10 specs sub nom. *Carabus comptus hampei* ab. *zilahiensis*, s.d. [22813, 22814, 22815, 22816, 22817, 2218, 22819, 22820, 22821, 22822].

2. *Carabus (Hygrocarabus) variolosus* Fabricius 1787

3 specs, Meseş Mts., VI.1990 [22714, 22715, 22716]; 2 specs, Gilău, VI.2001 [22717, 22718].

Superfamily: Chrysomeloidea

Family: Cerambycidae Latreille, 1802

Subfamily: Cerambycinae Latreille, 1802

Tribe: Cerambycini Latreille, 1802

Genus: *Cerambyx* Linnaeus, 1758

3. *Cerambyx cerdo* Linnaeus, 1758

1 spec., leg. M. Bălan; 2 specs, s.d.

Tribe: Rosaliini Fairmaire, 1864

Genus: *Rosalia* Audinet-Serville, 1835

4. *Rosalia (Rosalia) alpina* (Linnaeus, 1758)

1 spec. ♀, Oradea (BH), 30.VI.1963, leg. T. Jurcsák, det. M. Paina [23161]; 1 spec. ♂, Râtu Florilor (M. Bihor) (sic!) (BH), 11.VIII.1966, leg. et det. M. Paina [23160]; 1 spec. ♂, Scăriţa-Belioara (AB), 11.VIII.2006, leg. E. Posmoşanu, det. A. Gagiu [24488]; 1 spec. ♂, Salonta (BH), leg. et det. M. Paina [23176]; 1 spec. ♀, Salonta (BH), leg. et det. M. Paina [23175]; 2 specs, s.d.

Subfamily: Lamiinae Latreille, 1825

Tribe: Lamiini Latreille, 1825

Genus: *Morimus* Brullé, 1832

5. *Morimus asper funereus* Mulsant, 1863

6 specs, s.d. [3579].

Superfamily: Scarabaeoidea Latreille, 1802

Family: Lucanidae Latreille, 1804

Subfamily: Lucaninae Latreille, 1804

Genus: *Lucanus* Scopoli, 1763

6. *Lucanus* (*Lucanus*) *cervus* (Linnaeus, 1758)

3 specs ♂, Slănic (PH/BC), 15.VIII.1953, leg. L. Galamb [23108-23110]; 3 specs ♀, Slănic (PH/BC), 15.VIII.1953, leg. L. Galamb [23106, 23107, 23146]; 2 specs ♀, Lăzăreni (BH), 10.X.1955, leg. T. Jurcsák [23111, 23112]; 1 spec. ♂, Băile 1 Mai (BH), 3.VI.1956, leg. P. Perényi [5531]; 4 specs ♂, Băile 1 Mai (BH),

7.VI.1957 [23120, 23124, 23133, 23142]; 1 spec. ♂, Scărişoara Tomasca (sic!) (AB), leg. T. Jurcsák 29.VI.1957 [23114]; 1 spec. ♀, Scărişoara Tomasca (sic!) (AB), leg. T. Jurcsák 29.VI.1957 [23115]; 2 specs ♀, Băile 1 Mai (BH), 15.VII.1957 [5531]; 1 spec. ♀, Oradea, Săldăbagiu forest (BH), 25.VII.1957 [5531]; 1 spec. ♂, Băile 1 Mai (BH), 25.VII.1957 [23132]; 3 specs ♂, Sâmbăta (BH), 4.VI.1958 [23127, 23136, 23150]; 1 spec. ♀, Sâmbăta (BH), 4.VI.1958 [23154]; 11 specs ♂, Băile 1 Mai (BH), 7.VI.1958 [23122, 23125, 23126, 23128, 23129, 23134, 23135, 23140, 23143, 23148, 23155]; 8 specs ♀, Băile 1 Mai (BH), 7.VI.1958 [5530, 23117, 23137, 23138, 23139, 23145, 23151, 23152, 23153]; 1 spec. ♂, Băile 1 Mai (BH), 17.VI.1958 [23121]; 2 specs ♂, Băile 1 Mai (BH), 19.VI.1958 [23118, 23119]; 1 spec. ♀, Băile 1 Mai (BH), 19.VI.1958 [23116]; 1 spec. ♂, Ştei (BH), 25.VI.1958 [23123]; 1 spec. ♀, Ştei (BH), 25.VI.1958 [23147]; 1 spec. ♀, Băile 1 Mai (BH), 4.VII.1958 [23131]; 1 spec. ♂, Băile 1 Mai (BH), 18.VII.1958 [23130]; 1 spec. ♀, Băile 1 Mai (BH), 21.VII.1958 [23141]; 2 specs ♂, Oradea, Paleu forest (BH), 9.VI.1963, leg. P. Perényi [5531]; 1 spec. ♀, Oradea, Paleu forest (BH), 9.VI.1963, leg. P. Perényi [5531]; 1 spec. ♀, Oradea, Paleu forest (BH), 16.VI.1963, leg. P. Perényi [5531]; 1 spec. ♀, Oradea, Paleu forest (BH), 23.VI.1963, leg. P. Perényi [5531]; 1 spec. ♂, Oradea, Paleu forest (BH), 9.VI.1963 [5531]; 3 specs ♂, Oradea, Paleu forest (BH), 16.VI.1963 [5531]; 1 spec. ♀, Săldăbagiu (BH), 17.V.1964; 1 spec. ♀, Oradea (BH), 31.V.1978, det. A. Gagiu, coll. Bălan [1661]; 2 specs ♂, Băile 1 Mai (BH), 17.VI.1978, det. A. Gagiu, coll. Bălan [1764, 1765]; 4 specs ♀, Băile 1 Mai (BH), 17.VI.1978, det. A. Gagiu, coll. Bălan [1766, 1767, 1768, 1769]; 2 specs ♂, Paleu (BH), 23.VIII.1989, det. A. Gagiu, coll. Bălan [2140, 2141]; 2 specs ♂, Nojorid (BH), 30.VIII.1994, det. A. Gagiu, coll. Bălan [2142, 2143]; 1 spec. ♀, Vadu Crişului (BH), 24.VI.2006, leg. E. Posmoşanu, det. A. Gagiu [24474]; 1 spec. ♀, Oradea (BH), X.195 (sic!), leg. T. Jurcsák [23113]; 1 spec. ♂ s.d. [5531]; 1 spec. ♂, s.d., coll. Bălan; 1 spec. ♀, s.d., coll. Bălan; 5 specs s.d. (coll. M. Bălan); 5 specs s.d. 1 spec. (larva), Băile 1 Mai, Glighii valley (BH), 12.IV.1958 [5531]; 1 spec. (larva), Băile 1 Mai (BH), 26.VII.1958 [5531]; 1 spec. (larva), Săldăbagiu forest (BH), 10.IV.1966 [5531].

Following the identification of toponyms or, where applicable, the nearest localities, all specimen records were systematically compared against the spatial extent of Natura 2000 protected areas. Of the 22 identified collection localities, five were found inside the boundaries of designated Natura 2000 sites, while twelve were located in their immediate vicinity. The spatial distribution of these records is illustrated in a georeferenced map generated using QGIS software (Figure 1), providing a visual representation of their relationship to protected areas.

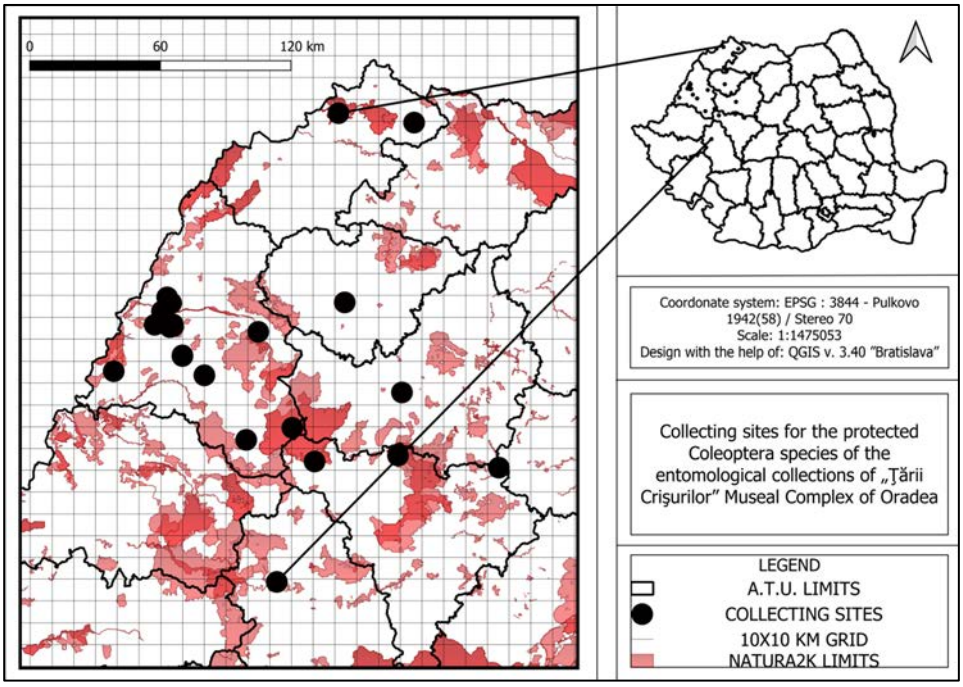

Fig. 1. Collecting sites of the investigated protected Coleoptera specimens from the entomological collection of the “Ţării Crișurilor” Museal Complex (Oradea), plotted within the regional administrative units (A.T.U.) of north-western Romania. The black circles represent individual collecting localities, overlaid on a 10 x 10 km reference grid and the boundaries of the Natura 2000 protected areas (shaded in red). The inset map shows the position of the study region within Romania. Coordinate system: EPSG 3844 – Pulkovo 1942(58) / Stereo 70.

The conservation status of the analysed entomological material is generally assessed as favourable. The specimens are systematically curated in multiple entomological boxes, arranged primarily according to the period of collection. In several cases, the material forms part of larger assemblages donated by various entomologists, thereby enhancing its historical and scientific value. Each specimen is mounted on standardised black-enameled entomological pins, predominantly size no. 2. The labelling reflects a combination of historical and contemporary practices: while a substantial number of specimens bear handwritten labels, more recent additions employ printed labels to improve legibility and ensure standardisation. Although some labels are incomplete, the majority of the specimens are adequately documented, preserving their research and conservation utility.

A noteworthy aspect of the collection pertains to the *Lucanus cervus* specimens, several of which were collected from the same locations and, in at least one instance, within the same year. At the time of collection, the potential ecological impacts of over-sampling were not yet fully recognised. However, the recurrence of records from the same sites may also indicate the historical presence of stable and abundant populations, as suggested by the frequency and success of collection events.

The temporal coverage of the dataset spans 53 years, from 1953 to 2006, during which a total of 132 adult specimens and three larvae were recorded. Annual collection rates ranged from 1 to 30 individuals, with a notable peak in 1958, when 33 specimens were collected (Figure 2). This long-term dataset provides valuable insights into species population dynamics, historical sampling intensity, and ecological conditions over time, thereby reinforcing the significance of the collection for both retrospective and contemporary entomological research.

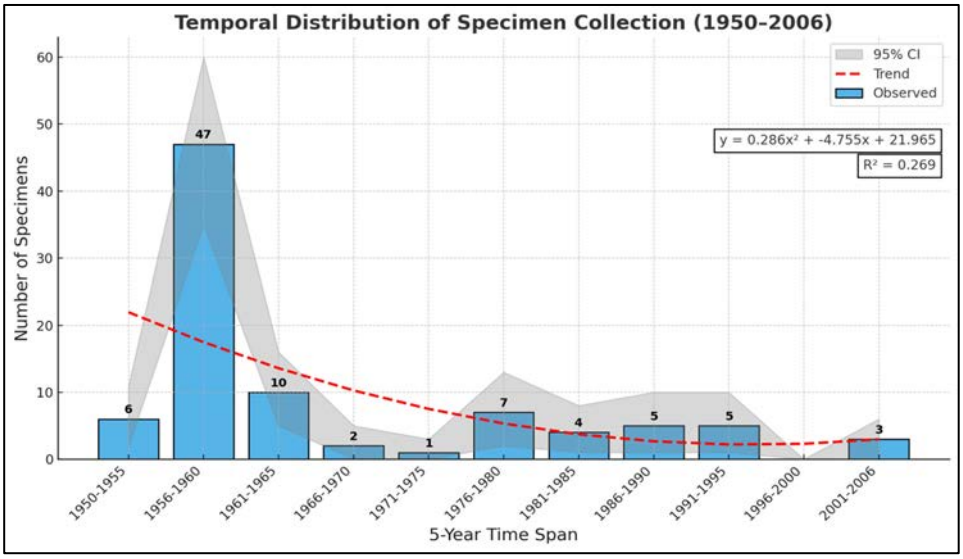

Fig. 2. Temporal distribution of the investigated specimens (n = 132) collected between 1950 and 2006, aggregated into 5-year intervals. Bars represent the observed number of specimens per interval, with values annotated above each bar. The grey polygon denotes the 95% confidence interval around the fitted quadratic trend (red dashed line), indicating a pronounced collecting peak during 1956–1960 followed by a long-term decline in specimen acquisition. The inset equation and R^2 value summarize the fitted model describing the temporal trend.

As can be seen in Figure 2, the collection activity peaked markedly between 1956 and 1960, with 47 specimens recorded, representing the highest intensity of collection effort. This peak is substantially higher than in all other periods, suggesting either a targeted collecting campaign or favourable ecological conditions during that interval. This intense activity is followed by a sharp decline: only 10 specimens were collected during 1961–1965, and this downward trend continues into the 1970s, reaching a minimum of 1 specimen in the 1971–1975 interval. A secondary, modest rise is observed between 1976 and 1985, with the number of collected specimens fluctuating between 4 and 7, possibly reflecting a partial revival of collecting activity or sporadic fieldwork. From the late 1980s onward, the number of specimens collected remains relatively low and stable, ranging between 3 and 5 per interval, except for a slight increase in 2001–2006.

The fitted quadratic trend ($y = 0.286x^2 – 4.755x + 21.965$; $R^2 = 0.269$) suggests a general decline in collection intensity over time. However, the moderate coefficient of determination ($R^2 = 0.269$) indicates that the model explains only about 27% of the variance in specimen counts, implying that other factors, possibly institutional, environmental, or personal, contributed significantly to collection dynamics.

The phenograms generated from the collected specimens (Figure 3) demonstrate limited relevance, primarily due to the relatively small number of specimens available for most taxa. However, the data for *Lucanus cervus* stands out as both significant and representative, providing meaningful insights into the species' distribution and ecological patterns within the Bihor region.

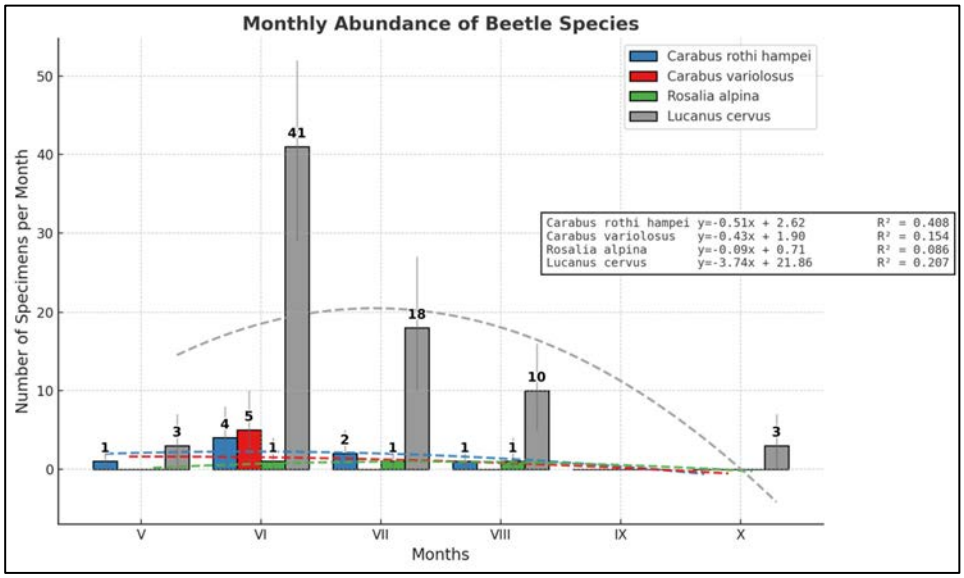

Fig. 3. Monthly phenogram of the investigated beetle species (n = 132). Bars represent the number of specimens collected per month. Species-specific linear trend lines (dashed) illustrate seasonal activity patterns. Error bars denote standard errors. Regression equations and R^2 values for each species are shown in the inset.

Figure 3 illustrates the monthly distribution of collected specimens for four protected Coleoptera species: *Carabus rothi hampei*, *Carabus variolosus*, *Rosalia alpina*, and *Lucanus cervus*. Polynomial or linear trend lines are fitted to each species, with their respective regression equations and coefficients of determination

(R^2) shown in the legend. The data indicate a clear phenological peak in *Lucanus cervus*, with a sharp increase in abundance in June (41 specimens), followed by a gradual decline through July (18 specimens) and August (10 specimens), and a minor reappearance in October (3 specimens). This pronounced unimodal distribution suggests that *L. cervus* adults are most active and detectable in early to mid-summer, which is consistent with known emergence patterns of this saproxylic species. The regression model ($y = -3.74x + 21.86$, $R^2 = 0.207$) captures the overall trend moderately, reflecting the species' temporal clustering in June.

In contrast, *Carabus rothi hampei* displays a more even distribution with relatively low abundance, peaking at 4 individuals in June. Its linear regression ($y = -0.51x + 2.62$) achieves the highest R^2 among all species (0.408), indicating a moderately strong declining trend over time. *Carabus variolosus* and *Rosalia alpina* exhibit low and sporadic presence across the months, with peak counts of 5 and 1 respectively, both occurring in June. Their regression models show weak fit ($R^2 = 0.154$ for *C. variolosus*, $R^2 = 0.086$ for *R. alpina*), suggesting either low detectability or insufficient sample size to model temporal trends robustly.

Overall, the graph underscores June as the peak activity period for most of the studied beetle species, with *Lucanus cervus* contributing most significantly to the monthly totals. This pattern may reflect both biological phenology and collector effort. The inclusion of standard error bars further indicates some degree of uncertainty around monthly values, especially in *L. cervus* due to its high variability.

The observed spatial correlation offers valuable insights into the persistence of the original populations from which the examined specimens were collected, despite the fact that sampling occurred several decades ago. The apparent continuity of these populations suggests that designated protected areas have been effective in maintaining suitable habitat conditions and ecological integrity, thereby facilitating the long-term survival of these species. This underscores the critical role of conservation measures implemented within Natura 2000 and similar frameworks, affirming the efficacy of protected areas in sustaining biodiversity across extended temporal scales. Moreover, the findings reflect the ecological resilience of the species concerned and their capacity to

withstand environmental fluctuations, provided that essential habitat requirements are met and preserved.

Discussions

The examined material represents a comprehensive regional archive of protected Natura 2000 Coleoptera from north-western Romania, offering valuable historical insights into population status, habitat continuity, and long-term faunal stability. A notable feature of the collection is the strong correspondence between historical collecting sites and areas that today form part of the Natura 2000 network. Many specimens originate precisely from localities that are now protected, suggesting long-standing ecological suitability for these species and highlighting the value of historical datasets for assessing whether current protected areas effectively encompass the core habitats of conservation-relevant taxa.

The temporal structure of the collection is also informative. The pronounced collecting peak of the late 1950s corresponds to a period of intensified entomological activity within Romanian research institutions, while later declines likely reflect shifting research priorities, reduced institutional capacity, or increased legal restrictions related to the capture of protected species. The specimens are generally well preserved, mounted in a standardized manner, and labelled with clear and legible data. The presence of larval material of *Lucanus cervus* adds particular value, as historical documentation of larval stages is rare and provides opportunities for ecological and developmental comparisons. Additionally, the preservation of older determinations (including historical nomenclature) and several aberrant or local morphotypes offers future potential for taxonomic revision, morphometric analyses, and studies on intraspecific variation.

Several limitations must be acknowledged. A portion of specimens lacks complete locality information, and the uneven temporal distribution of collecting effort restricts the strength of long-term quantitative analyses. Similarly, observed phenological patterns reflect a combination of biological signals and sampling bias. Nevertheless, despite these constraints, the dataset constitutes a reliable

and scientifically robust baseline for reconstructing historical distributions of protected Coleoptera in the region and provides essential context for contemporary biodiversity assessments and conservation planning.

Conclusions

Of the 20 Coleoptera species listed under the Natura 2000 Directive in Romania, six were identified within the entomological collection of the “Țării Crișurilor” Museal Complex in Oradea. This collection includes a total of 135 Natura 2000 specimens, originating from 22 distinct localities in 6 counties. Of these, five localities are situated within officially designated Natura 2000 sites, while twelve are located in their immediate vicinity. The temporal span of specimen acquisition extends from 1953 to 2006, with a marked peak in collecting activity occurring between 1956 and 1960.

The examined specimens possess considerable scientific and cultural significance. As biodiversity vouchers, they serve as critical reference material for species identification, distribution mapping, and ecological assessments. Furthermore, they contribute to the zoogeographical understanding of protected taxa, documenting historical distribution patterns that are essential for long-term monitoring. Beyond their scientific value, these specimens also hold museological importance, particularly in the context of current conservation legislation that prohibits the collection of such protected species from the wild. As such, the collection represents not only a record of past biodiversity but also a preserved heritage resource of enduring relevance to both science and conservation.

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this work. Special thanks are also extended to the manager of the “Ţării Crişurilor” Museal Complex of Oradea for granting access to the entomological collection, which was instrumental to this study.

References

Bartolozzi, L., Sprecher-Uebersax, E. 2006 – ”Family Lucanidae, Latreille, 1804”. In: Löbl, I. & Smetana, A. (eds.) 2006 – *Catalogue of Palaearctic Coleoptera*. Volume 3. Scarabaeoidea-Scirtoidea-Dascilloidea-Buprestoidea-Byrrhoidea. Apollo Books, Stenstrup, 690 pp.

Bousqet, Y., Březina, B., Davies, A., Farkač, J., Smetana, A. 2003 – ”Tribe Carabini Latreille, 1802”. In: Löbl, I. & Smetana, A. (eds.) 2003 – *Catalogue of Palaearctic Coleoptera*. Volume 1. Archostemata-Myxophaga-Adephaga. Apollo Books, Stenstrup, 819 pp.

Brînzan, T. (ed.) 2013 - *Catalogul habitatelor, speciilor și siturilor Natura 2000 în România*. Fundația Centrul Național pentru Dezvoltare Durabilă, 784 pp.

Garnier, S. 2024 - *viridisLite: Default color maps from ‘matplotlib’*. https://CRAN.R-project.org/package=viridisLite

O.U.G. nr. 57/20.06.2007 privind regimul ariilor naturale protejate, conservarea habitatelor naturale, a florei și faunei sălbatice (Monitorul Oficial nr. 442 din 29 iunie 2007).

R Core Team, 2024 - *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/

Sama, G. & Löbl, I. 2010 – ”Remaining Cerambycidae”. In: Löbl, I. & Smetana, A. (eds.) 2010 – *Catalogue of Palaearctic Coleoptera*. Volume 6. Chrysomeloidea. Apollo Books, Stenstrup, 924 pp.

Turin, H., Penev L. & Casale, A. (eds.) 2003 - *The Genus Carabus in Europe. A Synthesis*. Co-published by Pensoft Publishers, Sofia-Moscow & European Invertebrate Survey, Leiden, 512 pp.

Wickham, H. 2016 - *ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag New York. https://ggplot2.tidyverse.org

Wickham, H. & Seidel, D. 2020 - *scales: Scale Functions for Visualization*. https://CRAN.R-project.org/package=scales

Wickham, H., François, R., Henry, L. & Müller, K. 2023 - *dplyr: A Grammar of Data Manipulation*. R package version. https://CRAN.R-project.org/package=dplyr

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Ex situ conservation works of the thermal rudd (*Scardinius racovitzai*): captive breeding and sperm cryopreservation

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Abstract. The present study details the recent results of the ex situ conservation program for the thermal rudd (*Scardinius racovitzai*), a cyprinid species currently classified as Extinct in the Wild (EW). Several propagation attempts were conducted using broodstock acquired from various institutions: the Muzeul Țării Crișurilor (Oradea), Schönbrunn Zoo (Vienna), Tropicarium and Oceanarium (Budapest) and Hungarian University of Agriculture and Life Sciences (Gödöllő). During the course of the program, sperm samples were successfully cryopreserved, the existing sperm bank of the species was expanded. The offsprings from the captive breeding trials were restocked in the above listed institutes.

Keywords. thermal rudd, *Scardinius racovitzai*, ex situ conservation, captive breeding

Introduction

This work is part of an international cooperation aiminig the rescue of an Extinct in the Wild (EW) fish species, the thermal or Racoviţa rudd *Scardinius racovitzai* G. J. Müller, 1958 (Ford, 2024; Iftime and Oţel, 2025). The thermal rudd is a pannonian-biogeographical warm-age relict species, which was endemic only in Peţea Lake (Kottelat and Freyhof, 2007) that also provided the habitat for the thermal snail *Microcolpia parreyssii* (R. A. Philippi, 1847) and the Egyptian white water-lily *Nymphea lotus var. thermalis* (DC) Tuzson. The lake and the surrounding territories are located near Oradea, Romania, and under

nature conservation protection (Peţea Spring Natural Reserve) (Müller, 2014; Șerban & Grigoraș, 2018). The present taxonomic status of the species is disputed (Horváth et al., 2025). First, it was described as a separate taxon of Miocene origin by G. J. Müller (1958). Later, these species-specific characteristics were investigated by Berinkey (1960). According to his work, these are not permanent, and not such that differentiation on a species level can be applied to them. The thermal rudd was considered as subspecies of the common rudd (*Scardinius erythrophthalmus*) by Bănărescu (1964). Later, a hypothetical recent isolation from the common rudd was supposed based on morphological and behavioural studies (Bănărescu, 2002; Crăciun 1997, 1998). Popescul et al. (2017) demonstrated in their molecular study that the species ''does not form its separate clade". It is currently suggested that this fish represents a warm-water adapted ecotype or subspecies of the common rudd (Müller et al., 2018).

The body structure of the fish can be described mainly with a compact body and head (Figure 1). Unlike the common rudd, the body length of the adult fish is only 10-12 cm, the head is wider and the number of vertebrae 36-37. The most important external distinguishing characteristics are the rounded pectoral fins, while those of the common rudd are pointed (Telcean and Cupșa, 2006). The thermal rudd has been the subject of several morphological investigations. Based on traditional morphometric traits, the species exhibits a slender body shape, a relatively large head-to-body ratio, and elongated fins, reflecting its adaptation to the stable environmental conditions of its thermal habitat (Grigoraş et al., 2014; 2015). Structural analysis of the scales revealed well-developed radial and concentric line systems, while annual growth rings were less distinct, a feature associated with minimal temperature fluctuations (Șerban and Grigoraş, 2018). The current morphometric status of the population shows reduced size variability and homogenization of certain characters compared to earlier observations, indicating a decline in genetic divergence due to small population size and long-term isolation (Grigoraş et al., 2015).

Figure 1. The thermal rudd (*S. racovitzai*).

The Peţea Lake has dried up (probably permanently) in recent years due to the exploitation of geothermal springs that provided its water supply. In 2011 and 2012, the increasing thermal water abstraction for the surrounding buildings resulted in decreasing water levels in the pond until such an extent that it almost shrank to a puddle (Grigoraș et al., 2014; Müller et al., 2014). The intervention into the life of this thermal ecosystem was urgent. Fish (N = 60) and snails (N = 40) were saved by the scientists of Muzeul Ţării Crişurilor and transported into a 700 L aquarium. Despite the efforts of recreating normal water levels by pumping water back into the pond, the pond dried up in 2014. In 2013, a Romanian-Hungarian cooperation started with the leadership of the Muzeul Ţării Crişurilor financed by the Mohamed bin Zayed Species Conservation Fund. The aim of the work was the protection of the two endangered species (the thermal rudd and Parreys' snail) by different tools of ex situ conservation such as captive breeding, reintroduction of the juveniles and sperm cryopreservation (Müller, 2014; Müller et al., 2018).

In 2014, 20 fish from the saved broodstock were transported to Gödöllő where they were also kept in a 700 L tank. Two different artificial propagation methods were applied. In the induced spawning trial, the selected fish (N = 9) were treated with 200 IU hCG/fish with intraperitoneal hormone injection. After successful spawning, the eggs were incubated in another 110 L aquarium. From this technique, approximately 5000 juveniles were reared. In another

experiement, induced propagation technique was used. 7 fish (2 males, 5 females) were injected 10 mg/kg body weight acetone-dried carp pituitary extract. Semen was collected from the males which was later cryopreserved in the way demonstrated by Horváth et al. (2012). After 18 h latency time, the stripped eggs were in vitro fertilised. The sperm quality of the fresh and the cryopreserved sperm samples was investigated. The use of fresh sperm resulted significantly higher fertilisation and hatching rates (Müller et al., 2018).

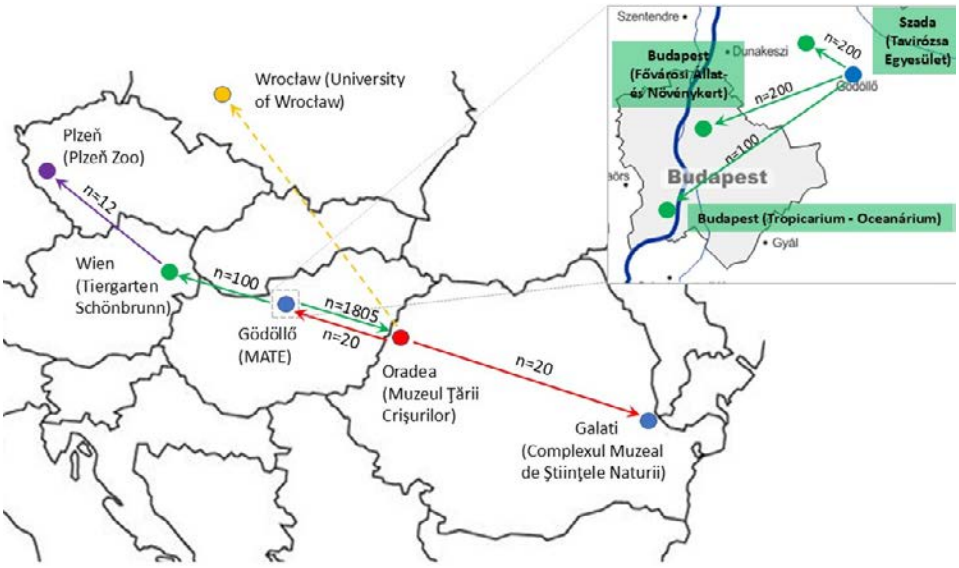

Figure 2. The different trasportation routes and the number of the transported specimens. The different colours of the arrows indicate different populations (red – orginal broodstock from Peţea Lake, green – Gödöllő propagation in 2014, purple – Vienna spawning, yellow – planned transport from Oradea).

After the successful captive breeding procedures, the offsprings were transported to several institutes and locations to provide the biggest probability of survival (from March 2014 to December 2014; Figure 2). These target places werre the following: Peţea Lake (Oradea - ROM), Tavirózsa Association (Szada – HUN), Tiergarten Schönbrunn (Wien – AUS), Tropicarium and Oceanarium (Budapest – HUN), Budapest Zoo (Budapest – HUN). The vast majority of the fish were re-introduced to their original habitat, Peţea Lake (*N* = 1805, 20 March

2014). 400 fish were relocated to Szada (*N* = 400, 13 June 2014.), 100 fish to Vienna, 100 to the Tropicarium and Oceanarium and the rest to the Budapest Zoo (*N* = 200, Müller et al., 2018). In 2024, an international collaboration was relaunched, involving the Hungarian University of Agriculture and Life Sciences, the Tiergarten Schönbrunn (Austria), Muzeul Țării Crișurilor (Romania), the Tropicarium and Oceanarium (Hungary), and the Galați Aquarium (”Răsvan Angheluță” Museum Complex of Natural Sciences, Aquarium Department, Romania). The primary goal of this cooperation was to promote ex situ conservation efforts related to the species, focusing primarily on establishing a viable offspring population based on the existing broodstock. The present study summarize the most recent findings of the conservation program concerning this fish species.

Material and methods

Artificial propagation trials in Gödöllő

The preparation of the broodstock in Gödöllő was carried out using the initial stock of thermal rudd introduced to Hungary in 2014 (*N* = 6; mean body weight ± SD: 66.06 ± 2.77 g). The first stage of the work involved a general assessment of the reproductive status of the broodstock, which was conducted through a preliminary spawning trial using a suspension of common carp (*Cyprinus carpio*) pituitary extract as an inducer.

In order to improve the reproductive readiness of the fish, the broodfish were transferred to a small pond (diameter: 8 m; depth: 0.3–0.8 m) located in the Botanical Garden of the Hungarian University of Agriculture and Life Sciences (MATE), Gödöllő. This pond, locally referred to as the “Körmedence = Circular Basin”, was designated for semi-natural conditioning, and continuous monitoring and preparatory management were undertaken there. Physical water parameters were measured on a daily basis, whereas chemical water quality indicators were assessed weekly. To enhance dissolved oxygen concentrations, a solar-powered aeration device was installed, and decomposing organic

material residues (mainly leaves and other macrophytes) were gradually removed from the bottom.

Following several months of pond-based conditioning, the broodfish were recaptured, and subsequently transported to the Fish Biology Laboratory of MATE. The fish were stocked into a 500 L aquarium equipped with an external filtration system, where they underwent a short acclimation period. Thereafter, a second preliminary spawning trial was conducted using pituitary suspension to re-evaluate their reproductive condition. Following this trial, the broodfish were subjected to intensive conditioning under controlled laboratory conditions in the same 500 L aquarium system to further enhance their readiness for induced propagation.

After further intensive conditioning, a third artificial reproduction experiment was conducted on 29 April 2025. All the fish in this experiment (N = 5; 54.06 ± 18,30 g) were treated with 10 mg carp pituitary extract/kg body weight in the evening. During the experiment, water temperature was raised from 26 to 27 ºC. In the morning hours, the broodstock and the occurence of spawning were checked in every hour. After spawning, the female showing partial ovulation was stripped two times and the obtained eggs were fertilised using the dry method (in vitro fertilisation). The fertilised egg batches – those from the induced spawning and also from the in vitro fertilisation – were incubated in another aquarium. The hatched larvae were also reared in the same aquarium. From the start of the feeding stage, the fry were fed on selected plankton (<0.15 mm) collected exclusively from the ponds of the Botanical Garden in the first five days, which was later gradually changed to newly hatched *Artemia salina* nauplii and then to Aller Infa (0.1-0.4 mm).

Spawning and sperm cryopreservation experiments
at the Schönbrunn Zoo

First spawning trial (11–12 November, 2024)

During the first spawning experiment, three females and two males (41.38 ± 9.99 g) were hormonally induced by intraperitoneal administration of carp

pituitary suspension at a dose of 10 mg/kg body weight. The broodfish were maintained during the latency period in a mobile 500 L aquarium supplied with aeration, where artificial plants were provided to serve as potential spawning substrates. Fish and the occurrence of ovulation were inspected hourly during the morning hours.

For sperm cryopreservation, six additional males (23.69 ± 14.12 g) were selected and treated with the same pituitary suspension. In the morning hours, milt was collected by gentle stripping and aspiration with an automatic pipette. Sperm quality was assessed using a Computer-Assisted Sperm Analysis system (CASA, Sperm Vision™ v. 3.7.4). Spermatozoa were activated with a solution previously developed for common carp (45 mM NaCl, 5 mM KCl, 30 mM Tris, pH 8.0; Saad et al., 1988). Sperm concentration was also determined using the CASA system. Samples were diluted in an immobilising solution (150 mM glucose, 75 mM NaCl, 30 mM KCl, 1 mM $Na_2HPO_4 \times 12H_2O$, 1 mM $MgCl_2 \times 6H_2O$, 1 mM $CaCl_2 \times 2H_2O$, 20 mM Tris, 0.5% BSA; pH 8.0 ± 0.2; osmolality: 385 mmol/kg). For sample 2, 0.5 µl of sperm was diluted in 20 µl of immobilising solution, while for sample 5, 0.5 µl of sperm was diluted in 100 µl of immobilising solution for concentration determination. Sperm cryopreservation was feasible for only two samples, as the remaining ones did not meet the minimum quality or volume requirements. For cryopreservation, an extender successfully applied in cyprinids was used, composed of: 150 mM glucose, 75 mM NaCl, 30 mM KCl, 1 mM Na2HPO4 * 12H2O, 1 mM MgCl2 * 6H2O, 1 mM CaCl2 * 2H2O, 20 mM Tris, and 0.5% BSA (pH: 8.0 ± 0.2; osmolality: 385 mmol/kg). A 1:9 dilution ratio (sperm extender+cryoprotectant) was utilized for freezing, adding 10% methanol as cryoprotectant. The diluted samples were loaded into 0.5 mL plastic straws. Freezing was conducted for 3 minutes in liquid nitrogen vapour, positioning the straws 3 cm above the surface of the liquid nitrogen. The different steps of sperm analysis and cryopreservation are presented in Figure 3.

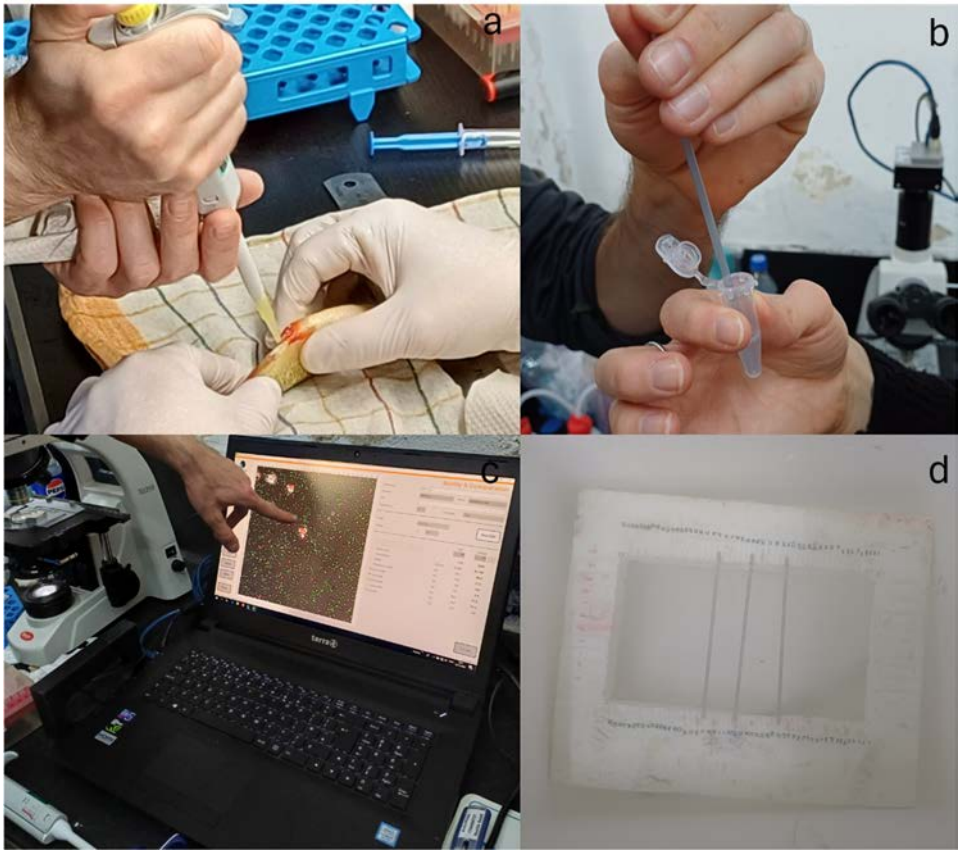

Figure 3. The different steps of sperm analysis and cryopreservation. a, sperm collection by automatic pipette; b, filling the cryostraw; c, computer-assisted sperm analysis; d, cryopreservation of the samples in liquid nitrogen vapour.

Second spawning trial (31 March – 2 April, 2025, Vienna)

The second spawning trial followed a similar protocol to the first experiment. The experiment was divided into two sub-experiments. In the first trial, the older generation was used, while in the second one, younger fish were utilised. A total of 15 individuals (13.96 ± 11.76 g) were hormonally induced with intraperitoneal injection of carp pituitary suspension (10 mg/kg body weight). Following treatment, the broodstock, consisting of 12 females and 3 males, was maintained during the latency period in a mobile 500 L, aerated aquarium. Spawning was monitored hourly during the morning hours.

Induced spawning experiments in Muzeul Ţării Crişurilor (Oradea)

For the experiment conducted on 8-9 April, 2024, a total of 10 females and 5 males (14.46 ± 3.11 g) were selected on the basis of externally visible secondary sexual characteristics. All broodfish, irrespective of sex, were hormonally induced with an intraperitoneal injection of carp pituitary suspension at a dose of 10 mg/kg body weight (Figure 4, top left). After this treatment, the fish were transferred to a 150 L aquarium equipped with aeration and heating, which was also supplied with artificial plants serving as potential spawning substrates (Figure 4, top right).

Figure 4. a, inraperitoneal hormone injection; b, spawning aquarium; c, fertilised egg batches on plastic plant.

Egg batches deposited on the artificial plants during the morning inspections were carefully removed from the aquarium (Figure 4, bottom picture) and incubated separately in a designated hatching tank. The resulting larvae were initially reared on selected plankton (<0.15 mm) collected exclusively from the ponds of the Botanical Garden, in order to minimise the risk of pathogen introduction. From day 5 onwards, the larvae were fed ad libitum with newly hatched *Artemia nauplii*, later supplemented with Aller Infa (0.1-0.4 mm).

Results and discussion

Besides the last experiment, the spawning trials conducted in Gödöllő have not resulted in viable offsprings. Egg batches obtained following hormonal induction were, in most cases, of poor quality, irrespective of the conditioning environment (natural or artificial). The most probable factors contributing to this outcome are the old age of the female broodstock (>11 years) and potential underlying health issues. One female showed the most typical signs of dropsy (ascites), which after detection was separated from the other fish. The conditioning period under artificial circumstances (the time between two spawning events) was maintained for 119 days at an average water temperature of 25.5 ºC (3,035 day-degrees). This thermal sum proved sufficient for the broodstock involved in the experiment to develop a new set of mature oocytes. This result is similar to observations made by Horváth (1985) regarding common carp (*Cyprinus carpio*) kept under intensive conditions. The hormonal induction resulted in 110-120 fertilised eggs, from which 90-100 larvae hatched.

During the first spawning attempt at Schönbrunn Zoo, ovulation was induced in one of the females following carp pituitary treatment. The stripped eggs were successfully fertilised. However, embryonic development was irregular during embryogenesis, and a high incidence of deformity was observed in the non-feeding larvae after hatching. Sperm sample analysis allowed the

cryopreservation of two of the examined samples. Currently, 10 straws (0.5 ml each) - 3 from the 2 individuals kept at Schönbrunn Zoo, and 7 more from 2014 - are cryopreserved and stored at the Institute of Aquaculture and Environmental Safety, Hungarian University of Agriculture and Life Sciences. In the second spawning event, carp pituitary treatment successfully induced ovulation in 2 females. The stripped egg batches fertilised in vitro showed initial development. However, all embryos gradually perished before hatching, which is presumably also attributable to poor egg quality.

During the artificial propagation trial performed at Muzeul Țării Crișurilor, spawning was successfully induced via hormonal treatment. The number of fertilised eggs obtained from the spawning was estimated to be 150–160. The larvae (approximately 130 individuals) hatched on day 3 post-fertilisation and commenced feeding on day 2 post-hatching.

The juveniles from the Oradea stock were restocked to Muzeul Țării Crișurilor (N = 50) and to Schönbrunn Tiergarten (N = 80), respectively. The 85 offsprings from Gödöllő stock were transported to the Tropicarium and Oceanarium in Budapest.

Genetic diversity is a key factor in term of ex situ conservation of different fish species (Meffe, 1986). In the case of fish species classified as Extinct in the Wild (EW), negative genetical effects as the genetic bottleneck can play a crucial role. During our works, the deformities observed in some cases of the adults and the bad quality of eggs may be the precursors of these processes. Therefore, in subsequent work, the various breeding strategies for *S. racovitzai* must be based upon different genetic markers (Schwart et al., 2007). Furthermore, it should be noted that the aim of this study did not include the clarification of the taxonomic status of *S. racovitzai*. The objective of this work was the conservation of a Cyprinid species - or subspecies, local, or relict ecotype population of common rudd (*S. erythrophthalmus*). The procedures and the whole international cooperation detailed previously are to be considered adaptable and applicable to other severely endangered cyprinids.

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References

Bănărescu, P. 1964 – Pisces – Osteichthyes (peşti ganoizi şi osoşi). *Fauna R. P. R., Vol. XIII*. Editura Academiei, București (in Romanian).

Bănărescu, P. 2002 – Species and subspecies of fish fauna and lampreys endemic or almost endemic to the drainage area of the river. *TISCIA Monograph Series* **6**: 167–172.

Berinkey, L. 1960 – On the systematical position of *Scardinius racovitzai* Müller, 1958. *Vertebrata Hungarica* **2**: 143–152.

Crăciun, N. 1997 – Ethological researches on *Scardinius racovitzai* from the Thermal Lake 1 Mai, Oradea. *Studii şi Cercetări* **2**: 79–88.

Crăciun, N. 1998 – *Cercetări de etologie comparată a unor peşti marini şi dulcicoli din fauna României*. PhD thesis, Institutul de Biologie al Academiei Române, Bucureşti (in Romanian).

Ford, M. 2024 – *Scardinius racovitzai*. The IUCN Red List of Threatened Species 2024: e.T19948A137328770.

Kottelat, M., Freyhof, J. 2007 – Handbook of European freshwater fishes. Cornol, Berlin, 646 pp.

Grigoraș, G., Müller, T., Gagiu, A., Bontaș, I., Șerban, C., Roșca, M. 2014 – Ex situ conservation of the thermal rudd (*Scardinius racovitzai*): a general review of work and results. *Nymphaea* **41**: 155–160.

Grigoraș, G., Müller, T., Gagiu, A., Șerban, C., Bontaș, I. 2015 – Preliminary results regarding the present morphometric characters of thermal rudd *Scardinius racovitzai* Müller 1958 from Peștea Spring Natural Reserve. *Transylvanian Review of Systematical and Ecological Research* **17**(2): 79–85.

Horváth, Á., Jesenšek, D., Csorbai, B., Bokor, Z., Raboczki, É., Kaczkó, D., Bernáth, G., Hoitsy, Gy., Urbányi, B., Bajec, S. S., Snoj, A. 2012 – Application of sperm cryopreservation to hatchery practice and species conservation: a case of the Adriatic grayling (*Thymallus thymallus*). *Aquaculture* **358**: 213–215.

Horváth, J., Tóth, A., Weissenbacher, A., Gagiu, A., Bernáth, G., Nagy, B., Varga, Á., Nagy, A., Grigoraș, G., Pándi, I., Ivánovics, B., Müller, T. 2025 – Ex situ conservation of the thermal rudd *Scardinius racovitzai*. *Oryx* 1–1.

Horváth, L. 1985 – Egg development (oogenesis) in the common carp (*Cyprinus carpio* L.). in: Muir J. F., Roberts R. J. (eds.): Recent Advances in Aquaculture. *Springer*, 31–77, Boston.

Iftime, A., Oțel, V. 2025 – An annotated systematic checklist of the Romanian ichthyofauna. *Zootaxa* **5654**(1): 1–64.

Meffe, G. K. 1986 – Conservation genetics and the management of endangered fishes. *Fisheries* **11**(1): 14–23.

Müller, T. 2014 – Kísérlet a váradi maradványcsiga (*Melanopsis parreysi*) és a Racovitza-kele (*Scardinius racovitzai*) megmentésére. *Halászat* **107**(3): 13–14.

Müller, T., Bernáth, G., Horváth, Á., Várkonyi, L., Grigoraș, G., Gagiu, A., Urbányi, B., Zarski, D., Freyhof, J., Cameron, T. 2018 – Artificial propagation of the endangered Romanian endemic warm-water rudd (*Scardinius racovitzai* Müller 1958, Cyprinidae, Cypriniformes) for conservation needs. *Egyptian Journal of Aquatic Research* **44**(3): 245–249.

Popescul, O., Grigoraș, G., Șerban, C., Ciorpac, M., Gorgan, L. D. 2017 – Phylogeny of *Scardinius* genus inferred from nuclear (Rag1) and mitochondrial (Co1) sequences. *Deltas and Wetlands* **4**: 21–28.

Saad, A., Billard, R., Theron, M. C., Hollebecq, M. G. 1988 – Short-term preservation of carp (*Cyprinus carpio*) semen. *Aquaculture* **71**(1-2): 133-150.

Șerban, C., Grigoraș, G. 2018 – Structural and morphometric study of scales in Pețea rudd (*Scardinius racovitzai* Müller 1958). *Applied Ecology and Environmental Research* **16**(5): 6063–6076.

Schwartz, M. K., Luikart, G., Waples, R. S. 2007 – Genetic monitoring as a promising tool for conservation and management. *Trends in Ecology & Evolution* **22**(1): 25–33.

Telcean, I., Cupșa, D. 2006 – Püspökfürdő endemikus hala: a Racovitza-kele (*Scardinius racovitzai*). *Halászat* **99**(3): 135.

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Rehydration of dehydrated wet specimens from the collection of the Țării Crișurilor Museum

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Abstract

This study presents a curative conservation protocol for the rehydration of dehydrated wet specimens from the collection of the Țării Crișurilor Museum, Oradea. The methodology involves a stepwise process including photographic documentation, washing, glycerin pre-rinsing, buffered rehydration (pH 7.4), and reintegration into preservation fluids. Results indicate that moderately dehydrated specimens responded positively, with improved tissue volume, coloration, and structural integrity. The study contributes to regional conservation literature by offering a replicable model for fluid-preserved specimen care and underscores the role of documentation and interdisciplinary collaboration in heritage science.

Keywords: rehydration, wet specimens, zoological collections, conservation, museology

Introduction

Wet specimen collections represent a vital segment of museum heritage, preserving anatomical, zoological, and botanical materials in fluid media for scientific, educational, and cultural purposes. These specimens, often fixed in formalin and stored in ethanol or glycerin, offer irreplaceable insights into regional biodiversity, historical research practices, and morphological variation. The Țării Crișurilor Museum, located in Oradea, Romania, holds a diverse array of such specimens, including vertebrates, invertebrates, and botanical samples, many of which are integral to its natural history and educational programs. However, like many institutions with legacy collections, the museum faces the challenge of specimen dehydration—a gradual process resulting from evaporation, seal failure, or prolonged exposure to suboptimal environmental conditions (Simmons, 2014; Singer, 2014; Cersoy & Herbin, 2022; Clothworkers Foundation, 2022).

Dehydration compromises both the visual integrity and scientific utility of fluid-preserved specimens. Shrinkage, discoloration, and tissue collapse can obscure diagnostic features and render specimens unsuitable for research or display. In response, rehydration has emerged as a conservation strategy aimed at restoring anatomical form and stabilizing compromised samples. While historically under-documented, recent literature has begun to consolidate best practices for rehydration, emphasizing gradual moisture reintroduction, compatibility with original fixatives, and close monitoring to prevent microbial growth or structural damage (Neisskenwirth, 2020; Cersoy & Herbin 2022; Clothworkers Foundation, 2022).

Protocols typically begin with vapor-phase humidification, allowing tissues to soften without direct immersion. This is followed by staged immersion in aqueous or low-concentration ethanol solutions, progressing toward the original preservation medium. For specimens originally fixed in formalin, buffered rehydration solutions may be required to mitigate chemical interactions and prevent further tissue degradation (Neisskenwirth, 2020; Singer, 2014; Simmons, 2014).

The Țării Crișurilor Museum's initiative to rehydrate its dehydrated wet specimens aligns with broader conservation trends in Europe, where museums are increasingly investing in the restoration and digitization of fluid collections. This effort not only preserves the scientific and educational value of the specimens but also enhances public engagement by enabling renewed display and interpretation. Moreover, rehydration offers an opportunity to reassess collection management practices, including container integrity, fluid composition, and environmental controls. By documenting its methodology and outcomes, the museum can contribute to regional and international discourse on wet specimen conservation, potentially informing future guidelines and collaborative research (Clothworkers Foundation, 2022).

This paper proposes a tailored rehydration protocol for the Țării Crișurilor Museum, drawing on recent conservation literature and adapting it to the museum's specific collection profile and institutional infrastructure. It outlines the scientific rationale, procedural steps, and monitoring strategies necessary for successful rehydration, while also considering ethical and curatorial implications. Through this initiative, the museum reaffirms its commitment to preserving biological heritage and fostering interdisciplinary dialogue across conservation science, museology, and regional biodiversity studies.

Contextual overview

The wet specimen collection of the Țării Crișurilor Museum in Oradea represents a scientifically and educationally valuable segment of the institution's heritage. It includes zoological samples preserved in fluids such as formalin and ethanol, assembled over decades to reflect both regional biodiversity and historical conservation practices. Beginning in 2006, the collection underwent a complex relocation process to the museum's new headquarters, a transition that spanned more than ten years. This extended period of movement and storage introduced significant conservation challenges, including partial or complete evaporation of preservation fluids,

deterioration of glass containers, and compromised sealing systems (Golban & Lobonț, 2023).

According to Golban and Lobonț (2023), the museum's wet collection underwent a comprehensive evaluation to assess its preservation status following this transfer (Figures 1-3). The assessment revealed that approximately 40% of the specimens' showed signs of fluid evaporation, with varying degrees of degradation in both the preservation medium and container integrity. Most of the affected specimens were originally fixed in formalin and later transferred to ethanol, a transition that, without proper buffering, contributed to microbial growth and tissue discoloration. Around 25% of the jars had compromised seals or structural damage, needing full replacement. In cases of partial fluid loss, conservators replenished the jars with the same type of preservation fluid — either formalin or ethanol — to maintain chemical consistency.

The team also recorded the types of containers used, noting that glass jars with metal lids were more prone to corrosion and leakage, while newer polymer-sealed containers offered improved durability. In terms of specimen categorization, the collection includes vertebrate samples (65%), invertebrates (20%), and miscellaneous anatomical preparations (15%), with the vertebrate group showing the highest rate of fluid degradation. This statistical breakdown helped prioritize conservation efforts, focusing first on specimens most vulnerable to microbial contamination and structural collapse.

During condition assessments, museum specialists identified multiple forms of degradation: tissue collapse, discoloration, microbial contamination, and structural damage to containers. Depending on the severity, curative measures were applied — such as replenishing evaporated fluid with identical substances, replacing damaged jars, and re-labeling specimens. Rehabilitation of the specimens has already begun, with rehydration protocols and fluid replacement underway to restore tissue integrity and prevent further deterioration.

The curative strategy also involved creating a registry that logs each specimen's condition, treatment history, and photographic documentation, enabling longitudinal tracking and comparative analysis. This data-driven

approach not only supports internal conservation planning but also aligns with internationally recognized conservation protocols, facilitating future benchmarking and collaborative research.

Beyond its scientific value, the collection holds strong educational and exhibition potential. Wet specimens offer the public a direct view of organismal morphology, enriching the museum's narrative on biodiversity and conservation. In this context, curative conservation and rehydration efforts are essential for maintaining the collection's functionality and ensuring future generations can access and learn from this fragile heritage.

Causes and mechanisms of dehydration in wet specimens

Dehydration in wet specimens is a multifactorial process driven by physical, chemical, and biological mechanisms that compromise both the preservation medium and the specimen itself. One of the primary causes is the failure of container seals, which allows evaporation of the preservation fluid — typically ethanol, formalin, or glycerin — and exposes specimens to air. Cato and Williams (1993) first emphasized the vulnerability of fluid collections to seal degradation, noting that even minor leaks can lead to significant fluid loss over time. This remains a central concern in contemporary conservation, particularly for legacy collections stored in glass jars with rubber or cork closures (Singer, 2014; Dangeon et. al, 2021; Sablier et al, 2021).

Environmental conditions such as temperature and relative humidity fluctuations exacerbate dehydration. Elevated temperatures accelerate evaporation, while low humidity can draw moisture from specimens even through intact seals. Stauderman (2012) and Clothworkers Foundation (2022) highlight the importance of climate-controlled storage environments, warning that uncontrolled microclimates can lead to both desiccation and microbial contamination. Inadequate ventilation and exposure to light further contribute to chemical degradation of the preservation fluid, altering its pH and reducing its antimicrobial efficacy.

Chemical instability within the preservation medium is another key mechanism. Formalin, for instance, can polymerize over time, forming paraformaldehyde precipitates that reduce fluid clarity and preservation quality. Ethanol may absorb atmospheric moisture or leach compounds from specimen tissues, leading to dilution and acidification. Observations have been made that such chemical shifts can accelerate tissue collapse and discoloration, particularly in specimens that were not properly buffered during initial fixation. These changes are often subtle and cumulative, requiring regular monitoring to detect early signs of degradation (Simmons, 2014; Schiller et al, 2014; Mayfield-Mewer, 2016; Domański et al, 2023; SPNHC, 2025).

Biological factors also play a role. When fluid levels drop below the specimen or preservatives lose their potency, microbial growth can occur. This is especially problematic in specimens originally fixed in formalin but later transferred to ethanol without adequate rinsing or buffering. Documented cases of fungal colonization in anatomical collections, linking contamination to both fluid degradation and poor container hygiene exist, so preventive measures such as antimicrobial additives and periodic fluid replacement are recommended to mitigate these risks (Simmons, 2014; Cersoy & Herbin, 2022; Domański et al, 2023; SPNHC, 2025).

In summary, dehydration of wet specimens arises from a complex interplay of seal failure, environmental stress, chemical degradation, and biological contamination. Addressing these mechanisms requires an integrated conservation strategy that includes airtight containment, climate control, fluid monitoring, and routine maintenance. Understanding these processes is essential for designing effective rehydration protocols and ensuring the long-term stability of fluid-preserved collections.

Rehydration methodology

The rehydration protocol applied to dehydrated wet specimens in the Țării Crișurilor Museum follows a structured, stepwise approach designed to restore tissue integrity while minimizing chemical and mechanical stress. Initially, each

specimen is carefully photographed and labeled prior to any intervention, ensuring that its pre-treatment condition is documented for comparative analysis. This visual record supports both curatorial transparency and future research.

Before applying the protocol to inventoried specimens, a preliminary trial was conducted on non-inventoried fish samples (Figure 4). This test phase allowed conservators to evaluate the efficacy of the rehydration steps, monitor tissue response, and refine the timing and chemical composition of each stage. Based on the successful outcome of this trial, the first inventoried specimens selected for rehydration were *Natrix natrix* (inv. 1203) (Figure 5), *Coronella austriaca* (inv. 8547) (Figure 6), and *Canis familiaris* (inv. 8498) (Figure 7).

The first active step involves washing the specimen with distilled water, a process that removes surface residues and prepares the tissue for gradual rehydration. Following this, the specimen is immersed in a 5% glycerin solution diluted in water for a period of 6 to 12 hours (Figures 8). This pre-rinsing phase serves to gently reintroduce moisture into the tissue matrix while stabilizing cellular structures through glycerin's humectant properties.

Throughout the process, specimens are periodically checked to monitor changes in texture, coloration, and structural response. These observations guide the timing and progression of the next phase, which involves immersion in a buffered rehydration solution composed of water and either sodium bicarbonate or trisodium phosphate, adjusted to a neutral pH of 7.4 (Figures 11-12). This solution facilitates deeper tissue rehydration while maintaining chemical compatibility. Specimens remain in this solution for a minimum of six hours, with continued monitoring to assess rehydration efficacy and detect any adverse reactions. This protocol aligns with the methodology outlined by Neisskenwirth (2020), who emphasizes the importance of gradual chemical rehydration and buffer stabilization in restoring dried biological specimens.

Once rehydration is deemed complete, specimens are transferred into clean containers filled with the appropriate preservation fluid—typically ethanol, depending on their original fixative (Figures 13-16). This final step ensures long-term stabilization and reintegration into the museum's collection. Throughout the

entire protocol, detailed notes are taken at each stage, documenting procedural steps, specimen responses, and any deviations from standard practice. This meticulous record-keeping supports reproducibility and contributes to the development of best practices for the conservation of wet specimens.

This procedure will be systematically applied to the remaining specimens in the collection that exhibit signs of dehydration or fluid degradation, ensuring consistent restoration and preservation across the entire inventory.

Results and discussions

Following the implementation of the rehydration methodology, several key observations were recorded. Specimens that had suffered partial dehydration — characterized by tissue shrinkage, discoloration, and surface hardening— responded positively to the protocol. The pre-rinsing phase with 5% glycerin solution facilitated gentle moisture reintroduction, while the buffered rehydration bath (pH 7.4) helped restore pliability and reduce brittleness. In most cases, tissue expansion was gradual and uniform, with minimal signs of rupture or microbial bloom. Periodic monitoring confirmed that specimens retained their anatomical contours and regained translucency, particularly in small vertebrates.

These results are consistent with findings from the Muséum National d'Histoire Naturelle in Paris, where similar rehydration efforts have been documented. Cersoy and Herbin (2022) reported that buffered solutions and controlled immersion times were critical in restoring anatomical specimens without compromising historical fixatives. Likewise, the anatomical collections at the Jagiellonian University Medical Museum in Kraków have benefited from glycerin-based rehydration protocols, particularly for specimens stored in formalin for extended periods, as noted in recent case studies (Domański et al, 2023).

In comparison, the Natural History Museum in London has emphasized the importance of container integrity and environmental control as complementary factors in successful rehydration. Their conservation team observed that specimens rehydrated in sealed, climate-stable environments showed better long-term

retention of restored features (Sablier et al, 2021). The Țării Crișurilor Museum's results support this observation: specimens rehydrated and transferred to new, airtight containers maintained their improved condition over several weeks, with no signs of fluid discoloration or tissue regression (Golban and Lobonț, 2023).

Notably, the museum's documentation practices — photographing specimens before and after treatment, recording fluid composition, and noting procedural deviations — mirror best the practices outlined by the Society for the Preservation of Natural History Collections (SPNHC, 2022). This level of detail not only supports internal quality control but also contributes to broader conservation literature, especially for institutions operating with limited resources.

Challenges remain, particularly with severely dehydrated specimens where tissue collapse was irreversible. In such cases, rehydration improved external appearance but did not fully restore internal anatomical features. These limitations are echoed in reports from the Centre de Recherche sur la Conservation (CRC 2021) in France, where researchers caution that rehydration is not a universal remedy but a targeted intervention best suited for moderately compromised specimens.

Conclusions

The rehydration of dehydrated wet specimens from the Țării Crișurilor Museum has demonstrated that targeted, stepwise intervention can effectively restore anatomical integrity, stabilize preservation conditions, and extend the functional lifespan of fluid-preserved collections. Through a protocol involving glycerin pre-rinsing, buffered immersion, and meticulous monitoring, specimens previously compromised by evaporation and tissue collapse showed measurable improvements in volume, coloration, and structural resilience.

These outcomes affirm the relevance of curative conservation in regional museums, especially those managing legacy collections affected by prolonged relocation or environmental instability. The success of the protocol aligns with documented practices in European institutions such as the Muséum National d'Histoire Naturelle (Cersoy & Herbin, 2022), the Jagiellonian University Medical

Museum (Santamarina & Ruiz, 2016), and the Natural History Museum in London (Russell & Kane, 2003), where rehydration has been used not only to recover specimen morphology but also to reintegrate compromised samples into educational and research contexts.

The Țării Crișurilor Museum's approach—emphasizing documentation, chemical compatibility, and container renewal—reflects a growing consensus in conservation science: that fluid-preserved specimens require dynamic care strategies tailored to their material history and institutional context. The practice of photographing specimens before and after treatment, recording procedural variables, and monitoring long-term outcomes aligns with internationally recognized conservation protocols. These measures not only contribute to a valuable body of regional and global conservation data and supports future benchmarking efforts and the development of best practices in specimen preservation.

While the protocol proved effective for moderately dehydrated specimens, limitations were noted in cases of severe tissue collapse or microbial contamination. These challenges underscore the need for preventive conservation measures, including climate control, seal integrity checks, and regular fluid replacement. As highlighted by Clothworkers Foundation (2022) and SPNHC (2022), rehydration should be viewed not as a universal remedy but as a targeted tool within a broader preservation framework.

In conclusion, the rehydration initiative at the Țării Crișurilor Museum offers a replicable model for similar institutions seeking to restore and preserve fluid collections. It demonstrates that with careful planning, interdisciplinary collaboration, and adherence to international standards, regional museums can successfully rehabilitate vulnerable specimens and reaffirm their role as custodians of biological heritage**.**

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References

Cato, P. S., Williams, S. L. 1993 - Guidelines for developing policies for managing collections. *Collection Forum* **9**(1): 1–8.

Centre de Recherche sur la Conservation (CRC). 2021 - Research on the Conservation of Fluid Collections. Paris: CRC, Muséum National d'Histoire Naturelle. Accesed October 2025 https://hal.science/search/index/?q=CRC%20fluid%20collections.

Cersoy, S., Herbin, M. 2022 - Wet collections: preserving their values for the future. *Heritage for the Future, Science for Heritage* (Paris: Muséum National d'Histoire Naturelle, 2022): 453–457.

Clothworkers Foundation. 2022. - *Standards in the Care of Wet Collections*. Conservation and Collections Care. Accessed October, 2025. https://conservation.myspecies.info/node/33.

Dangeon, M., Cornet E., Brambilla L. 2021. - Conservation-Restoration of a Botanical Museum Fluid Collection: Practice and Research. Neuchâtel: Haute École Arc Conservation-Restauration, HES-SO University of Applied Sciences and Arts Western Switzerland.

Domański, J., Janczura, A., Wanat, M., Grajzer, M., Simmons, J. E., Domagała, Z., Szepietowski, J. C. 2023. "Preservation Fluids of Heritage Anatomical Specimens—A Challenge for Modern Science." *Journal of Anatomy* 243 (1): e13854. https://doi.org/10.1111/joa.13854.

Golban, D., Lobonț, G. 2023 - *Conservarea curativă a preparatelor umede din colecția Muzeului Țării Crișurilor Oradea – Complex Muzeal*. *Crisia* **53**(1): 579–584.

Mayfield-Meyer, T. 2016. "Distinguishing Between Ethanol and Isopropanol in Natural History Collection Fluid Storage." Field Museum, Division of Amphibians and Reptiles.

Neisskenwirth, F. 2020 - Rehydration of dried-out specimens: a new approach. *Collection Forum* **34**(1): 73–86.

Sablier, M., Davron, C., Herbin, M. 2021. - Characterization of the Jar Sealants in the Fluid Collections of the Muséum National d'Histoire Naturelle. HAL Open Archive, October.

Schiller, E., Haring, E., Däubl, B., Gaub, L., Szeiler, S. 2014. "Ethanol Concentration and Sample Preservation Considering Diverse Storage Parameters: A Survey of Invertebrate Wet Collections of the Natural History Museum Vienna." *Annalen des Naturhistorischen Museums Wien, Serie B* 116: 41–68.

Simmons, J. E. 2014 - *Fluid preservation: A comprehensive reference*. Rowman & Littlefield.

Singer, R. A. 2014 - Are dehydrated specimens a lost cause? A case study to reclaim fluid-preserved specimens. *Collection Forum* **28**(1-2): 16–20.

SPNHC (Society for the Preservation of Natural History Collections). 2025. *Fluid Collection Monitoring*. Accesed October 2025. https://spnhc.org/fluid-collection-monitoring/.

SPNHC 2022 - *Preservation and Management of Fluid-Preserved Biological Collections*. Society for the Preservation of Natural History Collections, 45-47.

Stauderman, S. D. 2012 - *Environmental management for collections*. Smithsonian Institution.

Figure 1 – Containers used for the fluid preserved specimens

Figure 2 – Damaged containers found at assessment

Figure 3 – Damaged and dried out specimens in the collection

Figure 4 - Assessing the rehydration protocol on non-inventoried fishes

Figure 5 – *Natrix natrix* - condition before rehydration treatment

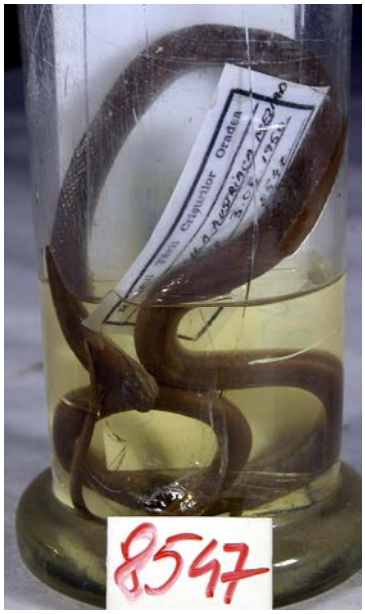

Figure 6 - *Coronella austriaca* - condition before rehydration treatment

Figure 7 – *Canis familiaris* – condition before rehydration treatment

Figure 8 - *Coronella austriaca* in 5% glycerin solution in water

Figure 9 – *Canis familiaris* in 5% glycerin solution in water

Figure 10 – *Natrix natrix* transferred in 5% glycerin solution in water

Figure 11- *Coronella austriaca* in water with sodium bicarbonate (pH 7.4)

Figure 12 – *Natrix natrix* in water with sodium bicarbonate (pH 7.4)

Figures 13 - *Canis familiaris* transferred into clean container filled with the appropriate preservation fluid and labeling

NYMPHAEA Folia naturae Bihariae	LII	123-127	Oradea, 2025

In Memoriam Elisabeta Popa
(1944-2025)

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I met Elisabeta Popa in July 1978, when I accompanied my aunt, Emilia Tallodi, a geologist at the time at the Dobrești Mining Company, at the museum. The purpose of our visit was to hand over to paleontologist Tiberiu Jurcsák the bones discovered in the Lower Cretaceous bauxite deposits at Cornet-Lens 204, which became famous for its dinosaur, pterosaur and bird remains. It was late in the evening, but Elisabeta Popa, known as "Vetuța" by her close friends, still prepared Triassic marine reptile bones with Tiberiu Jurcsák. They both became my mentors and friends.

Born in 1944 in Rogoz, Bihor County, Elisabeta Popa graduated from the “Emanoil Gojdu” High School in Oradea in 1962. Seven years later, she joined the Bihor County Museum, formerly known as the Crișana Country Museum, through a transfer from the Crișana Regional Council.

Initially, she was employed in an administrative position. Due to the nature of her job, she had the opportunity to become familiar with each department and thus became very involved in the preparation and preservation of the paleontological material held by the Natural Sciences Department, where she would occasionally visit the paleontology laboratory.

Elisabeta Popa began preparing certain materials as early as 1970, working on several restorations of fossil vertebrate specimens, under the direct supervision of T. Jurcsák, head of the Natural Sciences Department, who guided her steps in the field of bone restoration and fossil preparation. In 1974 he suggested that she specialize in paleontological preparation and restoration. From that moment on, she participated in all the museum's paleontological excavations and in the recovery of skeletal remains that occasionally appeared on construction sites.

She was involved in recovery excavations at the site of the former Beer Factory in Oradea in 1972, which brought to light a woolly mammoth skeleton. This excavation led to the reconstruction of a large part of the mammoth skeleton, which, since its first exhibition and to this day, has been one of the museum's main attractions.

Also, in 1974 she began specialized training organized by the Council of Culture for certification as a bone preparator-restorer, courses which she completed successfully. She graduated from a series of different specialization courses and reached all hierarchical levels in the field of paleontological restoration, obtaining the title of expert restorer in paleontological material in 2004.

Other sites she worked on, which took several years to complete, were the paleontological excavations in Peștiș and Lugașu de Sus. From there, she prepared important marine reptiles from the Middle Triassic (Anisian), such as the partial skeleton of *Nothosaurus transsylvanicus* Jurcsák, 1973, a new species of marine reptile.

Another site to which Elisabeta Popa contributed as a restorer was the Bears' Cave in Chișcău, discovered in 1975, where, together with Tiberiu Jurcsák, she has contributed to the preservation of the skeletal remains of *Ursus spelaeus*, *Panthera leo spelaea*, as well as a partial skeleton of *Capra ibex carpathorum*.

Starting with 1978, she took part in the excavations of vertebrate fossil remains dating from the Early Cretaceous discovered in the bauxite deposits at Lens 204-Cornet, unique in the world in terms of their fossilization. Elisabeta Popa managed to prepare thousands of skeletal elements of dinosaurs, pterosaurs, and birds from this site.

She also was involved in the Romanian - French research project together with fellow museographers Radu Huza and Erika Posmoșanu, and paleontologists Jean-Michel Mazin and Franck Metayer from the University of Poitiers, France, which aimed to collect new paleontological material from Lugașu de Sus, carried out in 1995.

Elisabeta Popa collaborated with Professor Dr. Dan Grigorescu from the Department of Paleontology at the University of Bucharest, participating in excavations that led to the discovery of dinosaur skeletal remains from the Late Cretaceous of the Hațeg Basin.

Between 1994 and 1998, she was part of the British-Romanian research project coordinated by Prof. Dr. Michael Benton from the University of Bristol, Great Britain, which led to the reopening of the bauxite mine at Lens 204-Cornet and the collection of new dinosaur skeletal remains. She also participated in preparing this paleontological site for filming by the *Learning Channel* from London for the film titled *Dwarf Dinosaurs* from Romania, broadcasted by the *Discovery Channel*.

Elisabeta Popa, together with Dr. Márton Venczel, Radu Huza, and Dorina Golban, brought to light fossil remains of mastodons from Păgaia and Pleistocene vertebrates from Subpiatră.

Another important moment in the restorer's career was the discovery in Tăşad of some skeletal remains of a whale from the Middle Miocene, which she prepared.

During her five decades at the Țării Crișurilor Museum, Elisabeta Popa has professionally and passionately prepared and restored over 25,000 fossils, increasing the value of the institution's paleontological collections.

Elisabeta Popa published with T. Jurcsák and other authors the results of the paleontological research they performed or the preparation, restauration and conservation of the paleontological material.

List of E. Popa's publications in chronological order:

1. Jurcsák, T., **Popa, Elisabeta,** 1977 – *Anancus arvernensis* Croiz et Jo0b din Pliocenul de la Huta (Bihor, Romania), *Nymphaea*, V, Oradea, pp. 71-93.

2. Jurcsák, T., **Popa, Elisabeta**,, 1977 – Săpătura de salvare de la Huta (jud. Bihor), *Muzeul și educația socialistă*, Ed. Muzeul de Istorie al RSR, 1.78–5, București.

3. Jurcsák, T., **Popa, Elisabeta** 1978 – Resturi de dinozaurieni în bauxitele de la Cornet (Bihor). *Nymphaea*, VI, 61-64.

4. Jurcsák, T., **Popa Elisabeta,** 1979 – Dinozaurieni ornitopozi din bauxitele de la Cornet (Munții Pădurea Craiului). *Nymphaea*, VII, 37-75.

5. Jurcsák,T., Poliş, Rozalia, Ignat, Doina, Şerban, M., **Popa, Elisabeta,** 1980 – 1981 Date privind fauna fosilă a Peşterii Urşilor (Munţii Bihor), *Nymphaea*, VIII-IX, 161-257.

6. Jurcsák, T., **Popa Elisabeta,** 1981 – Conservarea vestigiilor paleontologice în Peştera Urşilor de la Chişcău, *Cercetări de conservare şi restaurare a patrimoniului muzeal,* Naţionale de Istorie, Bucureşti, I, pp 201-204.

7. Jurcsák, T., **Popa Elisabeta,** 1983 – Reptile zburătoare în bauxitele de la Cornet, notă preliminară. *Nymphaea*, X, 7-15.

8. Jurcsák, T., **Popa, Elisabeta,** 1983 – La faune de dinosauriens du Bihor (Roumanie). 325-388. *In* Buffetaut, E, Mazin, J.-M. and Salmon, E. (eds.), *Actes du Symposium paléontologique G. Cuvier, Montbéliard*. Ville de Montbéliard, Paris.

9. Jurcsák, T., **Popa Elisabeta**, 1984 – Pterosaurians from the Cretaceous of Cornet, Roumania. *In* Reif, W.-E. and Westphal, F. (eds.), *Third symposium on Mesozoic terrestrial ecosystems, short papers*. Attempto, Tübingen, 117-118.

10. Tallodi, Erika, **Popa, Elisabeta,** Venczel, M., Huza, R.R., 1995. – Cercetări paleoherpetologice din Bihor, *Naturalia, Studii şi Cercetări*, Piteşti, 157-162.

11. **Popa, Elisabeta,** Tallodi, Erika, Huza, R.R., Mazin, J-M., 1996 – Les sites triasiques de Peştiş et de Lugaş - Bihor, Roumanie, historique et. Perspectives, *Nymphaea Folia Naturae Bihariae, 22, 43-51*, Oradea.

12. Tallodi-Posmoșanu, Erika, **Popa, Elisabeta,** 1997. – Notes on a Camptosaurid dinosaur from the Lower Cretaceous bauxite, Cornet – Romania*, Nymphaea, Folia Naturae Bihariae, 23-25: 35-44.*

13. **Popa Elisabeta,** Golban, Dorina, 2000 – Conservarea şi restaurarea pieselor scheletice de proboscidieni în colecţia Muzeului Ţării Crişurilor, *Acta Musei Porolissensis* Zalău, XXIII vol.II, pg. 745-759

14. **Popa Elisabeta**, Golban Dorina, 2003 – Restaurarea și conservarea scheletului de *Ursus spelaeus* de la Peştera Urşilor – Chişcău, *Nymphaea, Folia naturae Bihariae*, XXX, p.191-197

15. Codrea, V., Venczel, M., **Popa, Elisabeta**, 2005 — New finding of *Mammut praetypicum* (Proboscidea, Mammalia), a zygodont mastodon from Păgaia (NW Romania). *Acta Palaeontologica Romaniae,* 5, 67-71.

16. Venczel, M., Hír, J., Huza, R., **Popa, Elisabeta**, Golban, Dorina, 2005 – A New Middle Miocene Vertebrate Fauna from Subpiatră, *Nymphaea, Folia naturae Bihariae*, XXXII, p. 23-38.

17. Golban, Dorina, **Popa, Elisabeta,** 2006 – Simonkai's herbarium / alphabetical index of specimens from the Ţării Crişurilor Museum's collection, *Nymphaea, Folia naturae Bihariae* XXXIII, p. 103-135.

Elisabeta Popa near the restored woolly mammoth skeleton, together with Tiberiu Jurcsák and Noemi Moisi (1976)

E. Popa washing the dinosaur bone-bearing bauxite during the 1978 fieldwork at Cornet – Lens 204

E. Popa preparing the dinosaur bones from the bauxite (1993)

The dinosaur collection of the Țării Crișurilor Museum, prepared by E. Popa (1995)

The British – Romanian research team (1995)

E. Popa had prepared fossils for over five decades (2014)

Note to authors

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Landing, E. (ed.): *Studies in Stratigraphy and paleontology*. New York State Museum, Bulletin 481: 149-160, New York.

Taylor, T. N. 1981 - *Palaeobotany: An introduction to fossil plant biology.* McGraw-Hill Book Co., New York, 589 pp.

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