Beneath the surface: critical insights into the conservation status and ecological dynamics of *Romanichthys valsanicola*

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Abstract. *Romanichthys valsanicola*, a critically endangered species found exclusively in Romania's Vâlsan River and classified as Critically Endangered (CR) by the IUCN, represents a pivotal study subject for examining the relationship between ecological dynamics and conservation strategies. This critical literature review synthesizes a broad spectrum of research focusing on the habitat, genetic makeup, reproductive biology, and conservation strategies concerning this unique freshwater fish, and investigates its habitat, which is affected by natural and anthropogenic pressures, such as sedimentation and pollution. Genetic studies revealing the phylogenetic status of *R. valsanicola* highlight its evolutionary uniqueness and urgent need for genetic conservation. The exploration of its reproductive behavior, which is crucial for the survival of the species, provides an understanding of the implications of environmental changes on its life cycle. Conservation efforts, including local and international policies and projects, were critically assessed to determine their efficacy and gaps. The review concludes with recommendations for future research directions, emphasizing integrated conservation strategies that address both immediate threats and long-term sustainability. This approach provides valuable insights for policymakers, conservationists, and researchers dedicated to the preservation of *R. valsanicola* and similar endangered species. Through this detailed examination, the authors seek not only to document the plight of this species but also to inspire effective conservation solutions that can be adapted for other critical habitats around the world.

Key Words: asprete, conservation strategies, Romanian sculpin perch, *Romanichthys valsanicola*.

Introduction. *Romanichthys valsanicola*, commonly known as the asprete, is a critically endangered freshwater fish species endemic to Romania's Vâlsan River. This species was first discovered in 1956 and formally described in 1957 by Dumitrescu, Bănărescu, and Stoica, marking the beginning of a significant focus on its unique evolutionary characteristics and conservation needs. *R. valsanicola* stands out due to its evolutionary distinctiveness within the Percidae family, which has made it a subject of considerable scientific interest. Since its discovery, *R. valsanicola* has gained significant attention from the scientific community due to its restricted distribution and specialized habitat requirements. Research efforts have been multifaceted, addressing various aspects of its biology, ecology, and conservation needs. These studies have highlighted the urgent need for effective conservation strategies to protect this species from extinction. Foundational work by Dumitrescu et al (1957) provided critical insights into the species' classification and ecological niche, establishing a baseline for subsequent research. Their work laid the groundwork for understanding the habitat preferences and behavioral characteristics of the asprete. Building on these initial findings, genetic analyses conducted by Sloss et al (2004) and Haponski & Stepien (2013) have further elucidated the phylogenetic distinctiveness of *R. valsanicola*. These studies have revealed the genetic uniqueness of the species, underlining its evolutionary significance and the critical need for genetic conservation. The phylogenetic research has been instrumental in

understanding the evolutionary history of the asprete, informing conservation strategies aimed at preserving its genetic diversity.

Despite the extensive body of research, *R. valsanicola* continues to face severe threats from habitat destruction, pollution, and climate change. Human activities, such as dam construction, riverbed stone extraction, and agricultural runoff, have significantly degraded its habitat, leading to a dramatic decline in its population. Climate change further exacerbates these challenges by altering water temperatures and flow regimes, which can disrupt spawning cycles and reduce the availability of suitable habitats. This review synthesizes the extensive research on *R. valsanicola*, critically assessing the effectiveness of existing conservation measures and proposing integrated strategies for its long-term preservation. By consolidating current knowledge and identifying research gaps, this paper aims to provide a comprehensive framework to guide future conservation efforts for *R. valsanicola* and similar endangered species. The review will address the ecological role of *R. valsanicola*, its genetic and evolutionary significance, and the various threats it faces. Additionally, it will evaluate the current conservation strategies, including habitat restoration, legal protections, and community engagement initiatives, and suggest improvements based on the latest scientific findings.

In summary, this paper aims to enhance our understanding of *R. valsanicola* and its conservation needs by synthesizing past research and identifying key areas for future study. Through a detailed examination of the species' biology, ecology, and conservation challenges, the authors seek to contribute to the development of effective strategies to ensure the survival of this unique and critically endangered species.

Discovery and classification. Discovered in 1956 and formally described by Dumitrescu et al (1957), *R. valsanicola* is recognized for its critical conservation status and unique evolutionary traits. It is the smallest member of the Romanichthyini tribe within the family Percidae. The foundational work by Dumitrescu et al (1957), which established the species' taxonomic uniqueness, was complemented by research of Stoica (1957, 1958) documenting its presence in the Râul Doamnei. This early recognition underscored the *R. valsanicola*'s restricted distribution and heightened the urgency for conservation measures as environmental pressures intensified. Later on, Bănărescu et al (1995a) identify *R. valsanicola* as a critically endangered and highly specialized species endemic to the Vâlsan River in Romania. The study highlights its unique taxonomic position within the Percidae family and underscores the severe threats it faces from habitat degradation, particularly due to hydrotechnical constructions and sedimentation. The authors emphasize the urgent need for targeted conservation measures to prevent its extinction. Contributions by Collette (1963) further refined the phylogenetic positioning of *R. valsanicola* within the Percidae family, emphasizing its morphological distinctions and evolutionary trajectory. Ongoing debates focus on its classification, with some genetic studies suggesting a closer relationship to the genus *Zingel* (*Zingel zingel*, *Zingel balcanicus*) rather than warranting a separate monotypic genus. This reevaluation is based on mitochondrial DNA evidence, which highlights its intricate phylogenetic connections within the Percidae family (Song et al 1998; Haponski & Stepien 2013).

***Romanichthys valsanicola*: biology of a critically endangered species**. *R. valsanicola*, commonly known as the asprete, sculpin perch or Romanian darter is a critically endangered species within the Percidae family. This species is endemic to the upper basin of the Argeş River in Romania, currently surviving only in the Vâlsan River (Stoica 1967). Situated in the central Subcarpathian area, south of the Făgăraş Massif, this species is uniquely adapted to the fast-flowing, highland aquatic stream habitats. Historically, its range extended from Arefu to Curtea de Argeș and included tributaries like the Vâlsan River from Brădet to Mălureni. The species inhabits mountain rivers with rocky substrates and exhibits territorial behavior. Once more abundant in the Argeș River between Oești and Albești, and in the Vâlsan River between Galeș and Mușătești, the population now dropped to approximately 100 individuals in the Vâlsan River, yet no relevant data exists to prove these numbers, since no comprehensive, exhaustive population size and structure study has been yet carried out for this species (Burlacu et

al 2023). Spawning occurs from May to June, and threats include egg predation and competition from species like *Cottus gobio* and various benthophages (Dumitrescu et al 1957). Efforts to breed the species in captivity have failed. Population declines are attributed to hydrotechnical constructions reducing water flow, extraction of riverbed stones, and increased pollution. Protective measures have been implemented, declaring the species a natural monument and enforcing conservation laws. Future protection efforts must focus on maintaining optimal river flow, restoring rocky substrates, controlling stone extraction, and combating pollution (Găldean et al 1997; Bănărescu & Tatole 2005). According to Berra (2001), *R. valsanicola* is a rare and distinctive species of fish found exclusively in the swift-flowing waters of the Danube basin in Romania. It is part of the Luciopercinae subfamily, along with three species of *Zingel* (= *Aspro*), which is characterized by fishes resembling the North American darters, although significantly larger in size (Berra 2001).

Anatomical, morphological, behavioral and ecological descriptions of the species are usually rather succinct, indicating the level of scientific concern for this extraordinary species. Most authors, such as Dumitrescu et al (1957), Bănărescu (1964), Maitland (1991), Craig (2000), and Kottelat & Freyhof (2007), describe the species *R. valsanicola* as an intriguing study of evolution and adaptation, bearing a structural resemblance to the more familiar bullhead (*C. gobio*), featuring dorsally positioned eyes and a robust form ideal for its turbulent habitat, as well as an elongated, anteriorly flattened body shape, with low lateral compression (Bănărescu 1964; Craig 2000). Often mistaken for its bullhead counterparts due to their shared environment, the asprete, colloquially known as the Romanian bullhead perch, distinguishes itself with markedly separated dorsal fins - the posterior significantly larger than the anterior. Predominantly nocturnal, it preys on insect larvae and small fish, navigating the river's currents that have shaped its existence. Little is known about its reproductive behaviors, except for the presence of breeding tubercles and its method of laying adhesive eggs among stones - subtle hints at the secretive life of this dwindling species. According to Wiley & Collette (1970), the subfamily Luciopercinae, tribe Romanichthyini includes the species *R. valsanicola*. A notable male specimen, measuring 84.5 mm in standard length and catalogued as M.C.Z. No. 40966, was collected from the Argeş River in Romania. This species is characterized by the presence of tubercles on scales over most of its body, particularly concentrated dorsally and on all fins, including the dorsal and caudal fins. The tubercles, found just below the second dorsal fin, are formed by epithelial thickening on the posterior surface of the scales, appearing low and mound-shaped in cross-section. These tubercles are primarily formed by hyperplasia, with cells only slightly larger than those in the stratum germinativum, which are low columnar or cuboidal. The body tubercles measure approximately 130 μm high and 400 μm wide, while the adjacent undifferentiated epithelium is about 65 μm thick. Surface cells are irregularly shaped with pyknotic nuclei and may be somewhat keratinized. In contrast, the pelvic fin tubercles are formed by both hyperplasia and slight hypertrophy, giving them a hemispherical shape and dimensions of 86 μm high and 156 μm wide. The hypertrophied cells above the low columnar stratum germinativum measure 12 to 13 μm in diameter, and the surface cells appear somewhat shriveled, suggesting a degree of keratinization that imparts a rugose texture. Additionally, numerous epidermal sensory structures are present in this species, which were not observed in darters. These structures include groups of elongate columnar cells resembling taste buds, attached basally to a dermal papilla and extending to the epidermal surface, and individual cells scattered throughout the epidermis. Both types of sensory structures are located in the epidermis below the dorsal fin and on the pelvic fin. *R. valsanicola* lacks a swim bladder, a trait it shares with darters, and an attribute that underscores its benthic lifestyle. Despite its distinctiveness and the interesting adaptation to its habitat, little detailed information is readily available on its ecology or behavior, reflecting perhaps its rarity and specialized habitat (Berra 2001).

One of the most valuable research items addressing the compared osteology of *R. valsanicola* within the Romanichthyini and Percidae clades belongs to Bruner (2011), consisting in the phylogenetic analysis of the Percidae family. Particularly, his study provides significant insights into the evolutionary relationships of *R. valsanicola*. Bruner's

work focuses on the osteological characteristics of various members of the Percidae family to elucidate their phylogenetic relationships. His findings on *R. valsanicola* underscore its unique evolutionary position within the family. Bruner (2011) identifies several distinctive osteological features of this species that set it apart from other percids. One of the key findings is the structure of the infraorbital bones. In *R. valsanicola*, the infraorbital bones exhibit a unique configuration that is not found in other closely related genera such as *Zingel*. This specific arrangement includes a reduced number of infraorbital bones, with particular emphasis on the size and shape of the first infraorbital bone, which is more robust and differently shaped compared to its relatives. Another significant finding from Bruner's analysis is related to the gill raker morphology, where *R. valsanicola* possesses gill rakers that are shorter and fewer in number compared to other members of the Percidae family. This trait is indicative of its specialized feeding habits and ecological niche, which have evolved in response to its unique habitat requirements in the Vâlsan River. Additionally, Bruner's study highlights the presence of a distinctive dorsal process on the cleithrum, an osteological feature that further differentiates our species from other genera within the Percidae family. This dorsal process, along with other skeletal characteristics such as the shape of the preopercle and the structure of the pectoral girdle, provides strong evidence for the species' unique phylogenetic status. As one of the most phylogenetically distinct members of the Percidae family, preserving its genetic lineage is crucial for maintaining the overall biodiversity and evolutionary history of the family. By clarifying its phylogenetic position and highlighting its unique morphological traits, Bruner's research provides a solid foundation for future conservation efforts aimed at ensuring the survival of this critically endangered species (Bruner 2011).

Genetic and evolutionary insights. Understanding the genetic and evolutionary background of *R. valsanicola* provides critical insights for conservation strategies aimed at preserving this unique species. The phylogenetic relationships and genetic health of *R. valsanicola* have been explored in studies such as those by Sloss et al (2004), who analyzed the mitochondrial DNA sequences of the Percidae family. Their research clarifies the evolutionary lineage of *R. valsanicola*, affirming its distinct genetic profile within the family. The study of Dubut et al (2010) provides valuable insights into the genetic tools applicable to the conservation of *R. valsanicola*, a critically endangered fish species. By developing 55 novel polymorphic microsatellite loci primarily for *Zingel asper*, the research also explored cross-species amplification in closely related percids, including *R. valsanicola*. This breakthrough demonstrates the potential for these genetic markers to aid in assessing the genetic diversity and population structure of *R. valsanicola*, which is crucial for designing effective conservation strategies. The findings underscore the utility of advanced molecular tools in understanding and preserving the evolutionary heritage of rare and threatened freshwater species. Additionally, Haponski & Stepien (2013) expanded on the phylogenetic framework by exploring biogeographical relationships and evolutionary patterns among the sander pikeperches, providing a broader context for understanding the genetic isolation and uniqueness of *R. valsanicola*.

The genetic makeup of *R. valsanicola* is not just an academic interest but a crucial element for its conservation. Genetic diversity is indicative of a species' adaptability to changing environmental conditions and resilience against diseases. However, due to its limited distribution and small population size, there is a significant risk of genetic bottlenecks. The study by Crăciun et al (1999) have highlighted the impact of restricted genetic diversity, pointing out the challenges in ensuring the long-term viability of the species without careful management of its breeding and habitat.

In what concerns the higher systematics of *R. valsanicola*, Collette (1963) and Collette & Bănărescu (1977) recognize two subfamilies: Percinae and Luciopercinae, a position also adopted by Nelson (2016) while Coburn & Gaglione (1992) and Wiley (1992) offered alternative subfamily relationships. A recent DNA sequence study by Song et al (1998) divided the Percidae into three monophyletic subfamilies: Etheostomatinae (*Ammocrypta*, *Crystallaria*, *Etheostoma*, and *Percina*), Percinae (*Perca* and *Gymnocephalus*), and Luciopercinae (*Stizostedion*, *Zingel*, and *Romanichthys*).

The tribe Romanichthyini, comprising European darter-like fishes, is characterized by the absence of a basisphenoid and a bilobed shape of the supraoccipital crest at the posterior end, as well as the lack of a swim bladder. Within this tribe, the genus *Zingel*, which includes four species, has seven infraorbitals and tubercle-like gill rakers, and is found in the Danube, Rhone, and Vardar river systems. In contrast, the genus *Romanichthys*, represented by the single species *R. valsanicola*, has six infraorbitals and slender, elongate gill rakers, and is endemic to Romania (Nelson et al 2016).

The implications of these genetic insights for conservation are profound. Preserving genetic diversity is essential for the adaptive capacity of *R. valsanicola* to respond to environmental changes and ecological stressors. Conservation efforts must, therefore, incorporate strategies that maintain or enhance genetic diversity, such as the creation of protected areas, controlled breeding programs, and possibly, genetic rescue initiatives. Additionally, ongoing research into the genetic health of the species can provide benchmarks for monitoring population health and making informed decisions regarding interventions needed to prevent further genetic erosion.

The integration of genetic and evolutionary insights into the conservation management of *R. valsanicola* is vital. It not only aids in understanding the species' past and its evolutionary trajectory but also equips conservationists with the knowledge to devise strategies that safeguard its future. Continued research in this area is essential, as it will provide the necessary data to guide effective conservation practices and help maintain the ecological balance within the Vâlsan River ecosystem. *R. valsanicola* is described by Stepien & Haponski (2015) as a highly specialized and distinctive member of the Percidae family, adapted to fast-flowing, rocky streams in its limited habitat in the Vâlsan River. The species is characterized by two well-separated dorsal fins and other unique morphological traits that underscore its evolutionary significance within the genus. However, habitat degradation, including sedimentation and water flow alterations, has placed *R. valsanicola* at critical risk of extinction. The authors emphasize its importance as an evolutionary link and advocate for targeted conservation measures to ensure its survival.

Burlacu et al (2024) critically examine the evolutionary context of *R. valsanicola*, refuting the popular myth that it is contemporaneous with dinosaurs. Using molecular phylogenetics, fossil evidence, and ecological analysis, the study situates the species' divergence within the late Oligocene, approximately 24.6 million years ago, emphasizing its status as a Tertiary relict rather than a Mesozoic survivor. The findings highlight the importance of accurate evolutionary terminology to enhance conservation efforts and scientific understanding of this critically endangered species.

Ecological role, **feeding and habitat**. *R. valsanicola* thrives in a specialized niche within the Vâlsan River, predominantly relying on a diet of rheophilic invertebrates, including the larvae from the orders Ephemeroptera, Plecoptera, and Trichoptera (Tatole 1993; Vlăduţu 2002). As a predatory fish, it plays a crucial role in maintaining the ecological balance of its riverine habitat by managing the populations of these invertebrates, which are integral to the aquatic food web (Găldean et al 1997). According to the authors, who investigated the stomach contents of 34 specimens in the collection of the ichthyological collection of the Institute of Biology, Bucharest, collected in the river Argeş, around the locality Oieşti between 1959 and 1961, the analysis revealed the presence of 552 inverterbate samples (an average of 16.23 preys per individual), where 67% belong to larval may flies (Ephemeroptera) and 10.9% to larval stoneflies (Plecoptera) and rest of 22.1% belong to other groups of invertebrates: net-winged midges (Blephariceridae), caddisflies (Trichoptera), nonbitflies (Chironomidae) among them, as well as unidentifiable material. Oligochaet-worms and gammarid amphipods have non-significant values. The group which dominates in diet of sculpin-perch is evidently those of mayflies. In this group, the greatest amount is given by *Rhithrogena semicolorata*, 78%. It is followed by *Baetis rhodani* (16%). In all samples the bodies or fragments of *R. semicolorata* appear with a frequency of 83%. It results that the sculpin-perch feeds preponderantly with this species or in other words it has a selectivity for this

mayfly. It reveals on the other hand, an excellent ecological condition of the river (Găldean et al 1997).

As described by Bănărescu & Oprescu (1967), its distribution and habitat requirements have been severely impacted by anthropogenic activities such as riverbed exploitation and pollution. These activities disrupt the fragile balance of its ecosystem, as highlighted in detailed analyses by Stănescu (1979), which link population declines to habitat fragmentation caused by hydrotechnical projects and agricultural runoff. The habitat of *R. valsanicola*, once expansive along the Vâlsan River, is now severely confined due to extensive habitat fragmentation and environmental degradation (Perrin et al 1993). Human activities such as dam construction, pollution, and unregulated fishing practices have led to significant alterations in the river's flow and water quality, thereby reducing the habitable areas available to this species and impacting its food sources (Bănăduc et al 2020). Moreover, the sedimentation resulting from construction and agricultural runoff has further degraded habitat quality, leading to a decrease in the abundance of suitable spawning and feeding grounds (Vlăduţu 2002). Moreover, Bănărescu (1998) and Bănărescu et al (1995b) highlight the close relationship between hydrography and the restricted distribution of *R. valsanicola*, attributing its limited range to the species' reliance on clear, fast-flowing waters with rocky substrates, while emphasizing that hydrographic alterations such as dams or pollution pose severe threats to its survival.

Preservation efforts are thus critically focused on habitat restoration and maintaining water quality to support not only the survival but also the recovery of *R. valsanicola* populations. Conservationists emphasize the importance of mitigating the impacts of human activities and restoring natural river dynamics as essential steps towards ensuring the long-term viability of this species within its native ecosystem.

Tracking the movements of *Romanichthys valsanicola*. In a study aimed at understanding the behavior and ecology of the critically endangered *R. valsanicola*, eight individuals were tagged with radio transmitters and monitored in the Vâlsan River from August to December 2004. The species was observed in cold, clear, fast-flowing waters where it hides under rocks. This species, a presumed preglacial relict, according to the authors, feeds exclusively on aquatic invertebrates. Tracking was complicated by high rainfall and flooding, leading to the loss of two fish and the early release of a third due to its small size. Despite these challenges, the study found that the individuals tend to move downstream from the release point, spending periods in restricted areas before relocating further downstream, and rarely returning to previous locations. Fish activity varied with both day and night movements, although there was reduced movement during late night and early day hours. This preliminary study provides essential insights into the spatial behavior, habitat use, and home range of the species, highlighting its sedentary nature and localized movements within its endangered habitat (Ionaşcu & Crăciun 2006; Ionaşcu 2009; Ionaşcu & Crăciun 2009). The authors argued that the natural territory, as observed during the field investigations, would be about 2-3 m^2, while in the deeper sections of the Vâlsan River, no more than two to three individuals were captured (in 1992-1994), suggesting that the territory size could be about 10-15 m^2. Considering these findings, Burlacu et al (2023) agreed on an individual territory size of 8.5 m^2 for *R. valsanicola*. For a potential habitat area of 12.36 hectares in the Vâlsan River, the behavioral density thresholds would range from 7 to 50 individuals per 100 m^2. Consequently, the potential population sizes could range from 8,241 to 61,804 individuals, depending on the variation in territory size.

Current conservation status and efforts. *R. valsanicola* is listed as critically endangered on the IUCN Red List (Freyhof & Kottelat 2008), highlighting its precarious state and the urgent need for effective conservation measures. The conservation of this species is governed by numerous legal frameworks at the national and EU levels, including the Council Directive 92/43/EEC (1992) on the conservation of natural habitats and of wild fauna and flora, which emphasizes habitat protection and species conservation across the European Union. Nationally, it is protected under various

Romanian environmental laws that align with EU conservation policies to ensure sustainable management and protection of natural habitats (Government Emergency Ordinance No. 57/2007, approved with modifications and completions by Law No. 49/2011; Năstase & Oțel 2016).

The role of international and local policies in the conservation of *R. valsanicola* cannot be overstated. EU directives, such as the Habitats Directive (92/43/EEC), provide a legal framework that supports the conservation of natural habitats and wild fauna across the European Union, offering a protective umbrella under which specific actions for *R. valsanicola* can be implemented. Nationally, Romanian environmental laws align with these EU policies, ensuring that the legal protections are localized and effectively enforced. Specific regulations, such as Order No. 90/1998, designate *R. valsanicola* as a natural monument, while Law No. 5 of March 6, 2000, designates the Vâlsan Valley as a protected area, reinforcing habitat protection. The conservation status and efforts aimed at preserving *R. valsanicola* have evolved significantly over the years, transitioning from initial observations to more structured and funded initiatives. Historical conservation efforts laid the groundwork, such as the designation of the Vâlsan Valley Natural Reservation by the local council in 1994, marking a pivotal move in habitat protection for this species. Additionally, *R. valsanicola* was designated as a natural monument under Order No. 90/1998, further emphasizing its conservation importance. Valea Vâlsanului was also designated as a Site of Community Importance (SCI) under the Natura 2000 Network through Order of the Ministry of Environment and Sustainable Development, covering an area of 9,480 hectares to protect the biodiversity and maintain the favorable conservation status of its habitats and species.

The Vâlsan Valley Natural Reserve, covering an area of 10,000 hectares, was designated for the conservation of this species by the Argeș County Council Decision No. 18/1994. It was included in Law No. 5 of March 6, 2000, regarding the approval of the national territory planning - Section III - protected areas. The reserve is classified under IUCN Category IV, which is managed primarily for conservation through habitat and species management interventions. The protected area includes the Vâlsan River basin upstream of the Brădet locality and the major floodplain down to its confluence with the Argeș River. The protected area is strategically divided into three functional zones to optimize conservation efforts. The core protection zone encompasses the minor riverbed of the Vâlsan River and the Vâlsan Gorges, offering the highest level of protection to the critical habitats of *R. valsanicola*. The buffer zone includes the major riverbed extending over 50 km and the Vâlsan Meadows, serving as a transitional area that mitigates the impact on the core zone. Lastly, the economic zone, covering 8000 hectares, balances the need for sustainable resource use with conservation goals, ensuring that human activities do not adversely affect the critical habitats.

The functional zones of the reserve are as follows:

- the core protection zone, which includes the minor riverbed of the Vâlsan River and the Vâlsan Gorges;
- the buffer zone, consisting of the major riverbed extending over 50 km and the Vâlsan Meadows;
- the economic zone, encompassing an area of 8000 hectares.

The reserve's establishment and functional zoning reflect a comprehensive approach to conserving *R. valsanicola*, recognizing the unique ecological significance of the Vâlsan River as the sole remaining habitat of this fish species. This structured protection strategy aims to safeguard not only the species *R. valsanicola* but also the broader biodiversity of the region, promoting sustainable management practices that align with both conservation and socio-economic objectives. The delineation of the reserve's zones is crucial for maintaining ecological integrity while allowing for controlled human activity, ensuring the long-term survival of this irreplaceable species and its habitat (I.N.C.D.S. "Marin Drăcea" & S.C.D.E.P. Pitești 2021).

Despite these regional and global conservation efforts, which include habitat restoration and both in situ and ex situ conservation strategies, the numbers of *R. valsanicola* remain perilously low. The species is confined to a dramatically diminished habitat along the Vâlsan River, reflecting the significant challenges still facing its recovery

(Bănărescu & Vasiliu-Oromulu 2006). These ongoing efforts are crucial not only for the survival of *R. valsanicola* but also for maintaining the biodiversity and ecological balance of its natural habitat.

Despite the development of several National Action Plans for *R. valsanicola*, none have been implemented to date. These action plans, such as the one detailed in the 2020 National Action Plan for *R. valsanicola* , aim to protect this critically endangered species. However, without active implementation, the measures proposed in these plans remain theoretical, leaving the species at continued risk. This inaction underscores the urgent need for practical conservation efforts.

Significance of *Romanichthys valsanicola* in a biodiversity and conservation oriented context. The historical account of conservation measures for *R. valsanicola* is enriched by Bănărescu (1994), who evaluated the challenges of protecting this critically endangered species in the face of escalating threats. Their research emphasized habitat restoration and stricter legal protections as pivotal to mitigating habitat degradation. Similarly, Stănescu & Lehrer (1984) and Stănescu (1971, 1979, 1995) advocated for the designation of natural reserves and sustainable management practices and underlined the actual conservation status and threats affecting this species, aligning with broader conservation goals outlined in EU directives.

R. valsanicola is of critical ecological and conservation importance. As a distinct member of the Percidae family, it is pivotal for maintaining genetic diversity within this group. Its preservation is essential for sustaining the ecological equilibrium of its native river ecosystem (Bănăduc 2004; Bănărescu & Bănăduc 2007). The species acts as a bioindicator, with its health reflecting the overall environmental quality of its habitat (Telcean et al 2011). This makes *R. valsanicola* a key species for monitoring the impacts of human activities such as pollution and habitat modification on freshwater ecosystems. Moreover, *R. valsanicola* helps regulate the populations of aquatic invertebrates, as one of the top predators for these species, within the waters of the Vâlsan River, thereby playing a crucial role in the trophic dynamics of its habitat (Bănăduc et al 2020). The conservation of this fish is symbolic of wider freshwater biodiversity conservation efforts, which face threats from habitat destruction, pollution, and climate change. Protecting *R. valsanicola* can lead to improved conservation practices and policies that benefit a broader array of species sharing its habitat (Crăciun et al 1999; Ionașcu & Crăciun 2009). Focused conservation efforts for *R. valsanicola* are instrumental in developing strategies that not only protect this species but also preserve the complex aquatic ecosystems of the region. These strategies help in maintaining the rich biodiversity of the Vâlsan River, demonstrating a commitment to regional and global biodiversity conservation goals.

Ecological and environmental context. The ecological and environmental context of *R. valsanicola* within the Vâlsan River is characterized by intricate dynamics that are crucial for maintaining the species' survival. The river's habitat has been detailed extensively in studies such as that by Vlăduţu (2002), which describes the zoobenthic structure and how it supports the aquatic life, including *R. valsanicola*. This habitat is sensitive to changes, particularly those induced by human activities that affect water quality and river morphology.

Environmental changes, particularly sedimentation, have a profound impact on the habitat of *R. valsanicola*. Research by Bănăduc et al (2020) highlights how sedimentation from upstream construction and agricultural runoff compromises water quality and covers the rocky substrates crucial for the species' breeding and feeding. The alteration of these substrates leads to reduced availability of suitable spawning areas and hiding places, which are essential for the survival of juvenile and adult fish alike.

The ecological effects of sedimentation are not only limited to physical changes but also include the alteration of the food web dynamics within the river. Changes in the river's ichthyofauna structure can affect the availability of prey for *R. valsanicola*, impacting its feeding patterns and overall health (Truţă 2016; Truţă & Stancu 2016). Furthermore, Telcean et al (2011) discuss the broader implications of environmental

degradation on the species, emphasizing the need for continuous monitoring and adaptive conservation strategies to mitigate these impacts.

Overall, the survival of *R. valsanicola* is heavily dependent on the ecological integrity of the Vâlsan River. Ongoing and future research should focus on detailed ecological monitoring and the development of conservation strategies that address both the direct and indirect effects of environmental changes. This approach is essential for ensuring the long-term sustainability of this critically endangered species within its natural habitat.

LIFE project and its outcomes. More structured efforts include the *Romanichthys* LIFE project (LIFE99 NAT/RO/006429), which focused on specific conservation actions tailored to improve the survival conditions of this critically endangered fish. This project, funded by the European Union's LIFE program, emphasized habitat restoration, population monitoring, and public awareness campaigns to support the long-term viability of *R. valsanicola*. The outcomes of the LIFE project have been instrumental in setting precedents for successful conservation strategies. The successes and lessons learned from this project highlight the efficacy of targeted conservation funding and strategic planning.

Current efforts continue to build on these foundations, with organizations like Fundatia Alex Gavan launching campaigns such as "Aspretele trăiește" (The Asprete Lives). This initiative is focused on raising awareness and facilitating direct conservation actions for the critically endangered *R. valsanicola*. The project aims to protect and restore the habitat of this rare species, engaging the local community in conservation efforts to foster a more sustainable coexistence with the natural environment. Such initiatives are crucial for ensuring the survival of *R. valsanicola* and maintaining biodiversity in the Vâlsan River.

Conservation challenges. The actual conservation challenges faced by *R. valsanicola* are usually generated by unsustainable practices such as those identified by Stoica (1957) in his early documentation of environmental changes. More recent assessments by Bănărescu & Vasiliu-Oromulu (2006) delineated the compounded effects of climate change and pollution, emphasizing the urgent need for comprehensive, multilevel intervention strategies.

Bănărescu (2002) underscores the critical conservation status of *R. valsanicola*, emphasizing its uniqueness as an endemic species of the Danube River Basin and the urgent need for targeted measures to mitigate the threats posed by habitat degradation and human activities.

The population of *R. valsanicola* has severely declined, primarily due to human activities that have dramatically altered its native habitat. Illegal stone extraction, overfishing, and dam construction have been particularly detrimental, impacting the river’s flow and sediment composition (Bănăduc et al 2020). These modifications disrupt the ecological balance of the river, affecting the physical habitat structures essential for spawning and shelter, and also the availability of key food sources such as aquatic invertebrates (Vlăduţu 2002).

Additionally, the construction of dams has led to changes in water temperature and chemistry, further stressing the species. The altered flow regime from these dams can prevent the movement of organic matter and nutrients, reducing food availability and spawning sites downstream, which are critical for the survival of juvenile fish (Telcean et al 2011). Moreover, pollution from agricultural runoff introduces harmful substances into the water, compounding the threats to this already vulnerable species by degrading water quality and reducing oxygen levels crucial for its survival (Truţă 2016; Truţă & Stancu 2016).

These anthropogenic impacts underscore the urgent need for integrated conservation strategies that address both habitat preservation and restoration, alongside stricter regulations on riverine activities to ensure the survival and recovery of *R. valsanicola*. Establishing protected areas and enforcing fishing restrictions are also vital

to mitigate the impact of human exploitation and ensure the long-term viability of this species.

Research and future directions. Recent studies, including those by Burlacu et al (2023), have concentrated on understanding the ecological carrying capacity of the Vâlsan River for *R. valsanicola*, assessing both trophic availability and behavioral density. This research is vital for formulating effective conservation measures, ensuring the species' survival by providing a detailed picture of the ecological dynamics that support its population.

Further research is imperative to deepen our understanding of *R. valsanicola*'s specific needs and responses to environmental changes. Studies such as those by Truţă & Stancu (2016) have begun to explore the impacts of environmental quality on the river's macrozoobenthic populations, which are crucial to the diet of *R. valsanicola*. Additionally, genetic studies by Haponski & Stepien (2013) have provided insights into the phylogenetic relationships and evolutionary history of *R. valsanicola*, offering a genetic perspective that could inform breeding and conservation strategies (Song et al 1998; Haponski & Stepien 2013).

Looking forward, it is essential to integrate these findings with practical conservation actions. The development of targeted habitat restoration projects, as recommended by Bănăduc et al (2020), could help mitigate some of the key threats to *R. valsanicola*'s habitat, such as sedimentation and water quality degradation. Moreover, innovative conservation approaches such as the establishment of protected areas specifically tailored to the needs of *R. valsanicola* and the implementation of advanced monitoring technologies could enhance conservation effectiveness.

Expanding the scope of research and conservation policies to integrate findings from Stănescu (1995) and Bănărescu (1994) could enhance habitat restoration strategies. Additionally, collaborative efforts between researchers, local communities, and governmental agencies are crucial. These collaborations can facilitate the adoption of comprehensive management plans that include restrictions on activities harmful to the river ecosystem and promote sustainable practices that benefit both *R. valsanicola* and the local human populations.

In sum, the future direction for the conservation of *R. valsanicola* should focus on a holistic approach that combines detailed scientific research with practical, community-engaged conservation strategies. By continuing to expand our knowledge base and applying it through innovative and inclusive methods, we can hope to secure a future for *R. valsanicola* within its natural habitat.

Future directions and funding opportunities. Looking forward, there is significant potential for initiating new conservation projects under EU funding schemes such as LIFE, Biodiversa, or Horizon Europe. These projects could focus on advanced research, further habitat restoration, or cross-border conservation initiatives involving multiple EU member states. A proposed EU multi-state cooperation initiative could serve as a pilot action for critically endangered and endemic species, following a common, unitary approach to conservation. This approach would not only streamline efforts across different jurisdictions but also enhance the pooling of resources and sharing of best practices (EU Funding Program Guidelines).

Such initiatives could potentially receive robust support from EU funding programs, which are increasingly prioritizing biodiversity and the preservation of natural habitats (EU Biodiversity Strategy 2030). The opportunity to leverage these funds for a coordinated conservation effort could significantly amplify the impact on *R. valsanicola* and other similar species across the continent.

In conclusion, the conservation status and efforts surrounding *R. valsanicola* highlight a dynamic and evolving field that blends historical initiatives with modern conservation strategies. The integration of local actions with international policies and funding opportunities presents a promising pathway towards securing the future of this unique species. Continual adaptation and expansion of these efforts will be crucial to their ultimate success.

Reproductive biology and behavior. The reproductive habits and challenges of *R. valsanicola* are critical to understanding its survival prospects and informing conservation strategies. This species exhibits specific spawning behaviors and faces significant reproductive challenges, primarily due to its restricted habitat and environmental degradation. Studies such as those by Riehl & Bless (1995) have provided initial insights into the egg deposition and morphology of this endemic Romanian perch, highlighting the unique nature of its reproductive process. Balon et al (1977) identified *R. valsanicola* as a brood-hiding lithophil, a reproductive guild that buries eggs in gravel beds or rocky substrates to safeguard them from predators and environmental hazards. This specialized adaptation reflects the species' reliance on clear, fast-flowing, and oxygen-rich waters, emphasizing its vulnerability to habitat disturbances.

Behavioral studies have further shed light on the survival mechanisms of *R. valsanicola*. Ionașcu (2004), and Ionașcu & Crăciun (2009) utilized telemetry to monitor the species in its natural habitat, offering valuable data on its behavioral patterns, especially in relation to spawning and territory. These studies are essential for understanding how the species adapts to the ongoing changes in its environment and the behavioral flexibility it may exhibit in response to different conservation measures.

Additional research on egg attachment methods in teleost fishes by Riehl & Patzner (1998) provides broader context relevant to understanding the reproductive mechanisms of *R. valsanicola*, which may have similarities with other teleosts in their modes of egg attachment. Moreover, studies on the use of chemical agents to enhance post-fertilization phases in European perch by Lecoq (2017) might offer innovative approaches applicable to improving reproductive success in *R. valsanicola*.

The implications of these behavioral research findings are significant for conservation strategies. Understanding the reproductive and behavioral ecology of *R. valsanicola* allows for the development of targeted conservation actions, such as the creation of protected spawning areas or the restoration of specific river segments that are crucial for its reproduction. The LIFE projects, particularly those focusing on *R. valsanicola*, have demonstrated some success in habitat restoration and population monitoring, providing a foundational approach for future efforts (Bănărescu & Vasiliu-Oromulu 2004a, b, c, representing the LIFE Nature Project Reports).

Recent techniques for the elimination of fish fertilized egg adhesiveness reviewed by Neitali et al (2012) could also inform strategies to enhance egg survival rates in *R. valsanicola*, potentially addressing one of the species' critical reproductive challenges.

Considering the ongoing challenges and the critical conservation status of *R. valsanicola*, there is a pressing need for new projects under EU funding schemes such as LIFE, Biodiversa, or Horizon Europe. These projects could focus not only on continued ecological research but also on innovative conservation techniques that could be applied across multiple member states, following a unified approach to the conservation of critically endangered and endemic species. Such initiatives could significantly benefit from EU multi-state cooperation, enhancing the effectiveness of conservation efforts and ensuring a coordinated response to the threats facing this species.

Overall, the reproductive biology and behavior of *R. valsanicola* provide essential insights that are critical for developing effective conservation strategies. By integrating behavioral research with practical conservation actions, it is possible to enhance the prospects for the survival of this unique species, ensuring that it continues to be a part of our natural heritage.

Threats and challenges. The survival of *R. valsanicola* is jeopardized by a variety of threats that compound its already precarious status. Primary among these are pollution, habitat destruction, and the broader impacts of climate change, each contributing significantly to the decline of this critically endangered species.

Bănărescu et al (1995b) provide a detailed analysis of the current population status of *R. valsanicola*, emphasizing its critical endangerment and extreme habitat specialization. They describe the species' reliance on fast-flowing, clear-water habitats with coarse substrates, such as those found in the Vâlsan River, which remains its last known refuge. The authors identify significant threats, including habitat degradation

caused by deforestation, damming, sedimentation, and pollution, which have confined the species to a mere 1-km stretch of the river. They stress the urgency of implementing conservation measures, such as habitat restoration and captive breeding programs, to prevent its extinction.

Pollution has emerged as a severe threat, primarily from agricultural runoff and industrial waste, which degrades water quality and disrupts the delicate ecological balance of the Vâlsan River. Studies such as those of Vlăduţu (2013) have detailed the adverse effects of anthropogenic factors on the river's ecological stability, pointing out how pollutants can alter the physical and chemical composition of the habitat, thereby affecting the species dependent on it.

Habitat destruction is another critical threat, driven by human activities such as dam construction, riverbed mining, and unregulated fishing. These actions lead to significant changes in the river's morphology and hydrology, disrupting the natural habitats of *R. valsanicola*. Bănăduc et al (2020) provide insights into how sedimentation from these activities impacts the river, covering spawning grounds and reducing the available habitat for this species.

Climate change further exacerbates these challenges, altering water temperatures and flow regimes, which can lead to mismatched spawning times and reduced reproductive success. The broader ecological shifts due to climate change also affect the availability of prey and increase the occurrence of disease, making survival more difficult for *R. valsanicola*.

The role of human activity in the river ecosystem is significant, with both direct and indirect impacts on the survival of *R. valsanicola*. Overfishing reduces genetic diversity and can eliminate critical age groups from the population, while other human activities alter the physical habitat and food sources. Truţă & Stancu (2016) discuss how changes to the ichthyofauna structure, driven by human impacts, affect the broader biodiversity of the river, including *R. valsanicola*.

Given these threats, there is an urgent need for concerted conservation efforts that address both the symptoms and the sources of these problems. Strategies must include stringent pollution control, habitat restoration, and adaptive management plans that consider the long-term impacts of climate change and human activity on the river ecosystem. These efforts should be informed by ongoing research and monitoring to ensure they remain relevant and effective in preserving *R. valsanicola* and its unique habitat.

Future research directions. The conservation and study of *R. valsanicola*, a critically endangered freshwater fish endemic to Romania, have highlighted several gaps in current research, particularly in understanding its ecological needs and responses to environmental changes. The most recent studies, such as those by Burlacu et al (2023), have made advances in determining the ecological carrying capacity and trophic dynamics within the Vâlsan River, yet further research is essential to address existing knowledge gaps and refine conservation strategies. Badea & Mailat (2010) documented significant variations in the hydrological regime of the Vâlsan River between 1966 and 2005, with altered flow patterns potentially impacting the critical aquatic habitats of *R. valsanicola*, emphasizing the need for water management strategies to ensure species survival. Also, Badea (2011) highlighted that deforestation in the Vâlsan Basin has increased sedimentation in the river, posing a significant threat to the critical habitat of the endangered *R. valsanicola*, further underscoring the need for habitat conservation efforts.

Gaps in current research. Current research has provided foundational insights into the biology and ecology of *R. valsanicola*. However, comprehensive studies on the genetic diversity, reproductive biology, and long-term population dynamics are still lacking. There is a particular need for more detailed behavioral studies that can inform on the species' responses to environmental stressors and conservation interventions.

Proposed studies to address these gaps. Future studies should focus on:

- genetic analysis: advanced genetic studies to assess the genetic diversity and structure of remaining populations to inform breeding programs and assess genetic diversity (Sloss et al 2004; Haponski & Stepien 2013);
- reproductive biology: detailed reproductive studies, including the effects of environmental variables on breeding success, to optimize conservation breeding programs (Riehl & Patzner 1998; Lecoq 2017);
- behavioral studies: continuous monitoring of behavioral adaptations to changing environmental conditions, particularly in relation to climate change and human impacts on the river ecosystem (Ionașcu & Crăciun 2009);
- trophic availability monitoring: conduct thorough monitoring studies to continuously assess the food resources available in the Vâlsan River, similar to the efforts detailed by Vlăduțu (2002), who meticulously analyzed the zoobenthic structure;
- population size and structure studies: there is an urgent need for rigorous, updated research on population size and structure, utilizing photographical individual identification techniques such as I3s software to ensure accurate monitoring and data collection (Burlacu et al 2023).

Emerging technologies and methods in conservation biology. The field of conservation biology is rapidly evolving, with new technologies and methods enhancing the study and conservation of endangered species:

- environmental DNA (eDNA): utilizing eDNA sampling to monitor the presence and distribution of *R. valsanicola* without the need for invasive sampling methods;
- remote sensing and GIS: employing satellite imagery and GIS for real-time monitoring of habitat changes and to predict future impacts on the species' habitat;
- advanced statistical modeling: using machine learning and complex statistical models to predict population trends and assess the impact of conservation measures over time;
- Vortex 10 population viability analysis: employing Vortex 10 to develop tailored models for population viability analysis, including multiple plausible scenarios that account for fluctuating carrying capacity, survival rates, population structures, and periodic supplementation by reintroductions of captive-bred and rehabilitated specimens (Lacy & Pollak 2021).

The integration of these technologies with traditional conservation approaches can significantly enhance the effectiveness of ongoing efforts and provide a more comprehensive understanding of the ecological and biological factors influencing *R. valsanicola*.

Addressing these research gaps with advanced studies and emerging technologies is crucial for the effective conservation of *R. valsanicola*. By expanding our knowledge base and applying innovative methodologies, we can better tailor conservation strategies to ensure the survival and recovery of this unique species. Further research should aim not only to fill these gaps but also to foster collaboration among scientists, conservationists, and policymakers to facilitate a unified approach to the conservation of *R. valsanicola* and its habitat.

Conclusions. This literature review has synthesized a comprehensive array of studies and findings pertaining to *R. valsanicola*, an endemic species critically endangered and confined to Romania's Vâlsan River. The key findings elucidate the species' ecological roles, genetic characteristics, conservation status, and the major threats it faces. These insights are crucial for guiding future conservation strategies and policy-making efforts aimed at safeguarding this unique fish.

Summary of key findings:

- ecological importance: *R. valsanicola* plays a significant role in maintaining the ecological balance of the Vâlsan River by regulating invertebrate populations, which are crucial for the river's food web;

- genetic uniqueness: genetic studies highlight the species' unique evolutionary lineage within the Percidae family, emphasizing the importance of its genetic diversity for broader biodiversity conservation;

- conservation status: despite being protected under various national and EU laws, the species remains critically endangered due to ongoing environmental pressures;

- threats: the primary threats to its survival include habitat destruction, pollution, and the impacts of climate change, which collectively exacerbate the species' vulnerability.

The conservation efforts for *R. valsanicola*, including habitat restoration and legal protections, have been robust in intent but mixed in effectiveness. Projects like those supported by the LIFE program have contributed positively by improving habitat conditions and raising public awareness. However, these efforts often fall short in addressing the root causes of habitat degradation, such as unchecked pollution and inadequate enforcement of fishing regulations. The species' current conservation status as critically endangered underlines the need for more impactful and sustained conservation actions.

Recommendations for policymakers and conservationists:

- strengthening policy enforcement: policymakers should enhance the enforcement of existing environmental regulations, particularly those governing water quality and habitat protection. Stricter penalties for violations, combined with increased funding for monitoring and enforcement, are essential;

- integrated management approaches: conservation efforts should adopt an integrated river basin management approach, considering the cumulative impacts of human activities across the Vâlsan River. This includes coordinating with agricultural, industrial, and urban planning sectors to mitigate their impacts on the river ecosystem;

- community engagement and education: increasing community involvement through educational programs can foster a conservation culture among local populations. Residents should be engaged as active participants in conservation efforts, from monitoring fish populations to advocating for sustainable practices;

- research and monitoring: there is a pressing need for ongoing research to fill existing knowledge gaps, particularly regarding the species' reproductive biology and genetic health. Long-term monitoring programs, utilizing modern technologies like eDNA and remote sensing, should be established to track the health of the population and the effectiveness of conservation measures;

- climate change adaptation strategies: policymakers and conservationists must develop specific strategies to help the species adapt to the effects of climate change, which may include creating climate refugia or adjusting water management practices to maintain suitable habitat conditions during extreme weather events;

- international collaboration: given the species' critical status and the global importance of biodiversity conservation, international collaboration should be enhanced. Sharing knowledge, resources, and funding on a broader scale can elevate the effectiveness of conservation efforts.

In conclusion, while significant efforts have been made to conserve *R. valsanicola*, the persistence of threats and the species' status indicate that current measures are insufficient. The recommendations provided aim to strengthen these efforts, ensuring a sustainable future for this unique species and the ecosystem it supports. Enhanced policy enforcement, community engagement, and innovative research are pivotal to achieving these goals, demanding a concerted effort from all stakeholders involved in biodiversity conservation.

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