# Study on the Vector Role for *Calicophoron daubneyi* of Some Aquatic Snails form Western Romania

**Cătălin Bogdan Sîrbu[1], Ioan Peț[2], Florica Morariu[2], Claudia Alexandrina Goina[3], Beatrice Ana-Maria Sîrbu[1], Sorin Morariu[1]**

*[1]Banat's University of Agricultural Sciences and Veterinary Medicine "King Michael I of Romania" from Timisoara, Faculty of Veterinary Medicine, 300645-Timisoara, Calea Aradului 119, Timiș, Romania*
*[2]Banat's University of Agricultural Sciences and Veterinary Medicine "King Michael I of Romania" from Timisoara, Bioengineering Faculty of Animal Resources, 300645-Timisoara, Calea Aradului 119, Timiș, Romania*
*[3]"Victor Babes" University of Medicine and Pharmacy, 300041-Timisoara, Eftimie Murgu 2, Timiș, Romania*

**Abstract**
In the last decade, *Calicophoron daubneyi* parasites have been found in animals in many European countries. Its development and spread are predicated on the presence of numerous intermediate hosts, but mainly on the presence of the aquatic snail *Galba truncatula*. Natural infestations of three freshwater snail species with *Calicophoron daubneyi* were studied from April to June 2020 in western Romania. Were collected 235 snails belonging to the species *Galba truncatula* (115 snails (48.94%)), *Stagnicola palustris* (48 snails (20.43%)), *Planorbis corneus* (72 snails (30.64%)). Out of a total of 235 snails harvested, 165 were positive for the presence of cercariae, but of these only 93 (39.57%) had *Calicophoron daubneyi* cercariae. These results indicate that *Calicophoron daubneyi* may be able to infect and grow in aquatic snail populations in western Romania. *Calicophoron daubneyi* has demonstrated its ability to adapt to an intermediate host in a new environment. The aim of this study was to identify aquatic snail species in grassland areas on the basis of shell morphological characteristics and to identify juvenile life cycle forms of the trematode *Calicophoron daubneyi* from aquatic snails, intermediate hosts, by PCR method in order to determine areas where infestation with this parasite causes economic losses to livestock farmers.

**Keywords**: *Calicophoron daubneyi*, *Galba truncatula*, *Planorbis corneus*, *Stagnicola palustris*, ruminants, snails.

## 1. Introduction

Trematodes are among the most important animal pathogens worldwide. They are a concern in many temperate countries due to climate change [1-3], which is why the prevalence of trematodes and the occurrence of trematode diseases is increasing worldwide [4]. Due to the absence of a vaccine, control of trematodes is largely achieved by anthelmintic administration, but this practice is under threat due to the spread of trematode resistance to some anthelmintics [5].
In our country, the trematodes of economically important animals are produced by helminthes of the family *Plathelmintes*, class *Trematoda*, subclass *Monogenea* and *Digenea* [6].
Paramphistomes need an intermediate host for the life cycle to take place. This intermediate host is represented by aquatic snails of the genera *Galba*, *Stagnicola*, *Planorbis*. Larval forms of trematodes include sporocysts, redia and cercariae, which form in the body of the snail [7]. The many genera and species of parasites in the family Paramphistomidae have different intermediate host preferences, which is a major factor determining the potential geographical range of each parasite species.
The dynamics of snail-containing ecosystems should be monitored in several areas, so that knowledge of the distribution of both snail populations and parasitic diseases in these areas

* Corresponding author: Florica Morariu
Email: floricamorariu@usab-tm.ro

can help control snail populations, improving animal health. [3].
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## 2. Materials and methods

*Study area*
The study was carried out in three counties in the western part of Romania, Timiș, Caras-Severin and Hunedoara (between 45º47`N 21º21`E, 45°09′N 22°04′E and 45°46′ N 22°55′ E). The study area has a temperate-continental climate, with rich vegetation in floristic composition covering an area of approximately 46,266 km² with a total of 285,882 cattle and 2,808,138 sheep population. From April to June 2020, 235 aquatic snails were collected. From Timiș county 132 snails were collected, from Caras Severin county 79 snails were collected and from Hunedoara county 24 snails were collected.

*Snail collection*
The snails were collected in plastic containers with water from the sampling site and transported to the Parasitology and Parasitic Diseases Department of the Faculty of Veterinary Medicine in Timisoara, where they were kept at laboratory temperature for 7 to 10 days in order to release possible cercarias in the water in the glass (Figure 1).

**Figure 1.** Snails collected from the 3 counties surveyed

The presence of cercariae was revealed by examining the water in the glass in which the snails were kept under a stereomicroscope.

*DNA extraction and polymerase chain reaction*
The first step in the molecular analysis was the isolation of parasite genomic DNA from the sample to be analyzed. This extraction was performed using the Bioline Tissue Protocol Kit (BIOLINE®).
The PCR reaction was performed following the technique described by Lotfy and co-workers [8] in 2010 with some minor modifications.
Two primers were used: forward primer - GA1 (5'-AGA ACA TCG ACA TCT TGA AC-3') and reverse primer - BD2 (5'-TAT GCT TAA ATT CAG CGG GT3').
A MyTaqTM Red Mix Master Mix (BIOLINE®) was used for the reaction. The final volume of the PCR reaction was 25 µl, of which 12.5 µl MyTaqTM Red Mix (BIOLINE®), 1 µl GA1 primer, 1 µl BD2 primer (diluted to a concentration of 10 pmol/µl according to the protocol described by the manufacturer) DNA extracted from the test sample and ultrapure water. The amplification program was performed with the My Cycler thermocycler (BioRad®). This program included DNA denaturation steps at 95°C for 1 minute, 32 cycles of: denaturation at 95°C for 30 seconds, hybridization at 49°C for 30 seconds and extension at 72°C for 30 seconds followed by incubation at 4°C.
Amplicon analysis and control was performed by horizontal electrophoresis in a 1.5% agarose gel electrophoresis submersion system with the

addition of MidoriGreen fluorescent dye (Nippon Genetics® Europe) at 120 V and 90 mA for 60 minutes.

## 3. Results and discussion

Investigations of the morphology of snails collected from the external environment showed that they belong to the family *Lymnaeidae*, species *Galba truncatula* and *Stagnicola palustris*, and to the family *Planorbidae*, species *Planorbis corneus*, based on the morphological characteristics of the shell (Figure 2), presented by Hurtrez-Boussès in 2005 [9]. Measurements were made using a stereomicroscope MOTIC SMZ-140.

**Figure 2.** Morphological characteristics of the shells of the snails *Galba truncatula*, *Stagnicola palustris* and *Planorbis corneus* collected for study

*Galba truncatula* has a shell that is 7 to 8 mm high, about 12 mm long and 3 to 3.5 mm high. It also has an elliptical or oval opening of about 4 mm in size, which in most cases ends bluntly at the top. The snail's shell is made up of 5 to 6 spirals, its surface finely ridged. The second species identified is the aquatic snail *Stagnicola palustris*. This snail has a shell height of between 10 and 20 mm and a width of between 10 and 15 mm; the aperture is approximately 6 mm in size, it is pointed at the top and the outer edge falls curved. The shell of the snail is made up of 6 spirals that increase in size regularly, and on the surface of the shell there are straight or slightly convex striations and the last species identified is represented by *Planorbis corneus*, it has a height of 11 to 12 mm and a width of about 27 mm; the aperture has a size of about 13 mm, it has an oblique and reniform appearance. The shell of this snail has 5 spirals in one plane (Figure 3).

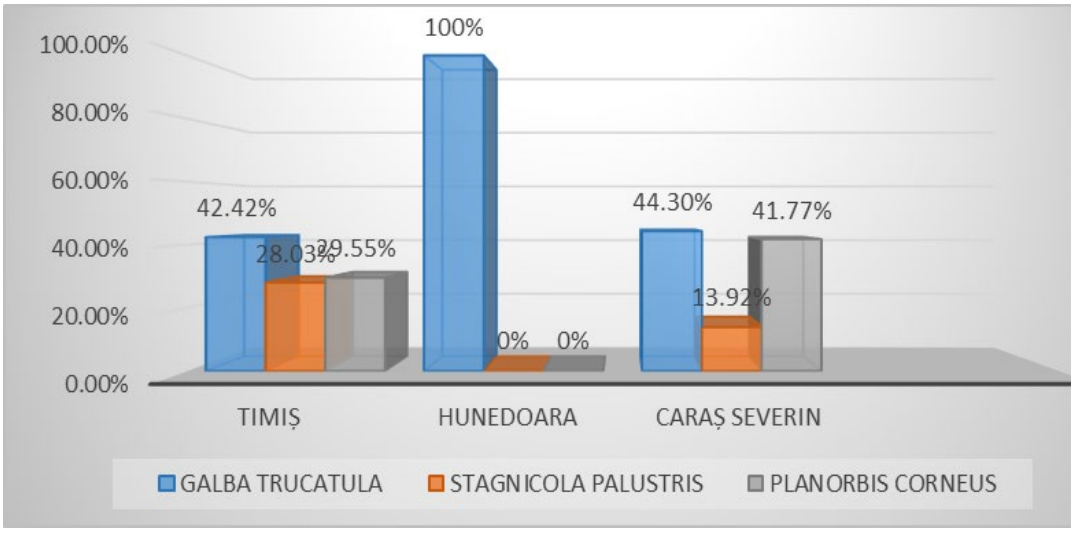


**Figure 3.** Snail species identified in the 3 counties surveyed

From Timiș county, 132 snails were collected, of which 56 (42.42%) were identified as *Galba truncatula*, 37 (28.03%) were represented by *Stagnicola palustris*, and 39 snails (29.55%) belonged to the family *Planorbidae*, species *Planorbis corneus*. A total of 24 snails (100%) belonging to the family *Lymnaeidae*, species *Galba truncatula*, were identified in Hunedoara county. From Caraș Severin county 79 snails were collected, of which 35 (44.30%) were represented by *Galba truncatula*, 11 (13.92%) were identified as *Stagnicola palustris*, and 33 (41.77%) belonged to the *Planorbidae* family, species *Planorbis corneus*. After identification, the snails were individually relocated to containers of water and kept at the light and temperature of the laboratory in order to collect possible cercariae leaving the snail, intermediate host (Figure 4).

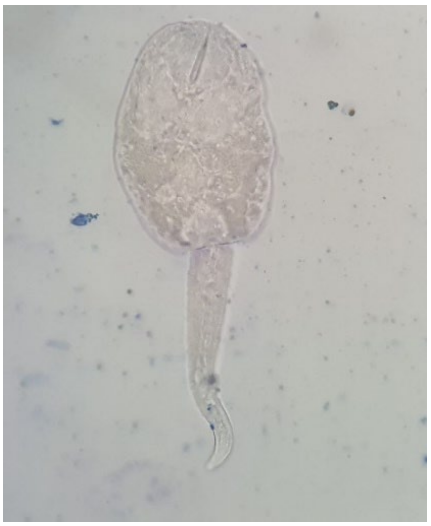

**Figure 4.** Cercaria of *Calicophoron daubneyi*

After amplification of DNA extracted from snails harvested from water, following the technique described by Lotfy et al. in 2010 [8], it was revealed that not all snails harvested were parasitized with juvenile forms of *Calicophoron daubneyi*.

Out of a total of 235 snails harvested, 165 were positive for the presence of cercariae, of which only 93 (39.57%) showed *Calicophoron daubneyi* cercariae.

From Timiș county 132 snails were collected, of which 87 showed cercariae, but only 51 (38.64%) were of *Calicophoron daubneyi*, from Caraș Severin county 79 snails were collected, of which 62 showed cercariae, but only 37 (46.84%) were of *Calicophoron daubneyi*, and from Hunedoara county 24 snails were collected, of which 16 had cercariae, but only 8 (33.33%) were of *Calicophoron daubneyi* (Figure 5).

This study is the first to record the prevalence of *Calicophoron daubneyi* in the snails *Galba truncatula*, *Stagnicola palustris* and *Planorbis corneus* in Romania. The prevalence obtained in this study was 39.57%. The recorded prevalence levels of *Calicophoron daubenyi* infesting snails, intermediate hosts, were within the prevalence's recorded in studies from France (20.6% [10], 44.7% [11]) and Spain (52.7% [12]). Increased prevalence of *Calicophoron daubneyi* in snail populations was associated with increased numbers of *C. daubneyi* eggs shed by ruminants on pastures containing intermediate hosts.

It is important to reduce the contamination of grassland with trematode eggs in order to reduce the future population of infesting snails, but this is usually ignored by livestock farmers [13].

Of the total number of snails harvested in this study, only 96 (40.85%) harboured *Calicophoron daubneyi* cercariae.

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With numerous snail species, *Galba trucatula*, *Stagnicola palustris*, *Planorbis corneus*, found in the western part of Romania, *Calicophoron daubneyi* is more likely to infest ruminants in

these snail populations. *Calicophoron daubneyi* has demonstrated its ability to adapt to different intermediate hosts in different habitats.
Similar results were observed in two other regions of France with regard to the prevalence of *C. daubneyi* and the intensity of infestation of the snails *Galba trucatula*, *Planorbis corneus* [14].

This study identified *Galba truncatula*, *Stagnicola palustris* and *Planorbis corneus*, aquatic snails, unlike France where *Lymnaea truncatula*, *Lymnaea glabra*, *Omphiscola glabra*, *Galba truncatula*, *Lymnaea neotropica*, *Lymnaea viatrix* var. *ventriosa* [6, 10, 11, 14-18], and *Galba truncatula* has been identified in Spain [12] and UK [19].

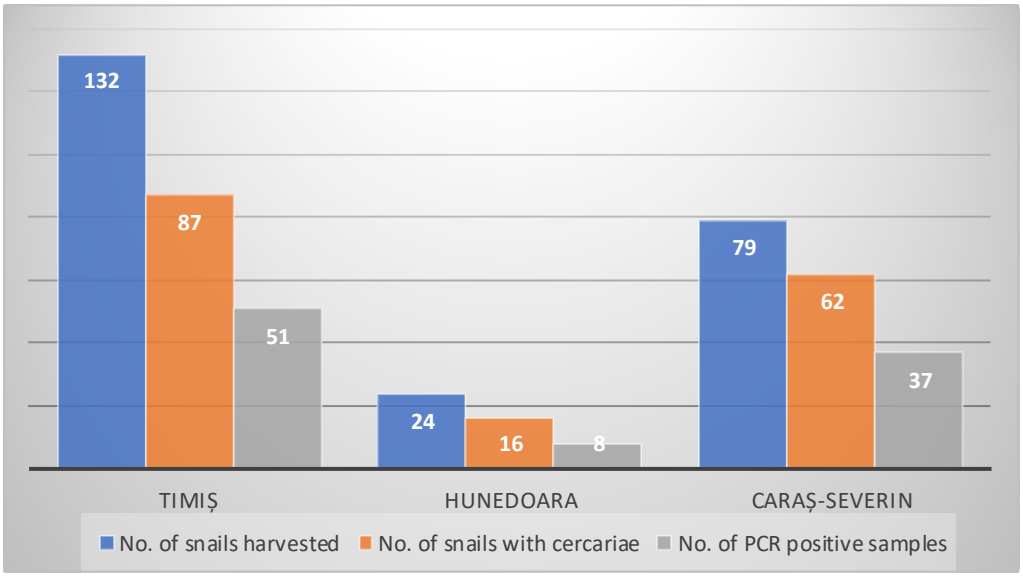


**Figure 5.** Graphical representation of PCR results from snails released from snails harvested from the 3 counties

In this study, a significantly higher prevalence could be observed in all examined habitats (100%) from which snails were harvested and in which paramphistoma DNA was identified, in contrast to the UK where only in 8 out of 19 (42.11%) examined habitats was paramphistoma DNA identified [20].

## 4. Conclusions

This study demonstrates that *Calicophoroon daubneyi* infects *Galba trucatula*, *Stagnicola palustris* and *Planorbis corneus* in western Romania.
Of the total number of snails harvested, 93 (39.57%) showed confirmed cercariae of *Calicophoron daubneyi* by PCR examination.
The prevalence of parasitism with juvenile forms of *Calicophoron daubneyi* varied according to snail collection site, from 25% to 61.54%.

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