



Article

The Cent Fonts Aquifer: An Overlooked Subterranean Biodiversity Hotspot in a Stygobiont-Rich Region

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Abstract: The South of France is a biodiversity hotspot within Europe. Here, we present a comprehensive review of surveys conducted in the Cent Fonts aquifer, an overlooked subterranean biodiversity hotspot embedded in a region rich in stygobiotic species and threatened by climate change and water abstraction projects. Key studies, spanning from 1950 to 2006, show a progression in survey methods and results, although troglobiotic species remain poorly documented. With 43 stygobiotic species recorded, the Cent Fonts is the richest stygobiont hotspot in France. Most species are regional endemics, a quarter of which are considered vulnerable by the IUCN. The Cent Fonts also hosts several relict species and is the type locality of four species. Such a high biological value clearly deserves to be preserved. Our analysis warns of a possible decline in biodiversity, as eight of the species recorded in the 20th century were absent from the 2006 survey, suggesting potential threats of unknown origin. The capture of the Cent Font springs for water abstraction is discussed as a potential threat to this ecosystem and its unique biodiversity. Three new species of stygobiotic molluscs are described, one of which was collected in the Cent Fonts.

Keywords: stygobiont; troglobiont; conservation; karst; subterranean diversity; conservation; water abstraction



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1. Introduction

1.1. Karst and Caves of the North-Montpellier Region

The Cent Fonts aquifer is located in the southern region of France, in the Mediterranean basin, and is part of a larger karstic system comprising the Hérault (2600 km²), Vidourle (800 km²), and Lez (200 km²) river basins. These regions are already acknowledged for their abundant and remarkable subterranean biodiversity [1,2].

1.2. Description of the Cent Fonts System

The karst system supplying the Cent Fonts is located in the western part of the northern Montpellier garrigues, formed by the limestone and dolomitic massifs located between Montpellier and the Cévennes. This karstic system develops within massive dolomites and

oolitic limestones of Bathonian age (Middle Jurassic). The Cent Fonts aquifer is a binary karstic system, receiving its water supply from both the rainfall on the Causse-de-la-Selle plateau and a sinkhole from the Buèges River, a tributary of the Hérault, situated more than 8 km upstream (Figure 1). The average altitude of the plateau that forms the Cent Fonts catchment area is about 300 m; the Cent Fonts springs are located at an altitude of 81 m on the right bank of the Hérault River. These springs emerge in the Bathonian dolomite, close to a fault. The system consists of about ten resurgences spread over a 300 m front, two observation points located a few meters higher, and the Cent Fonts cave, the entrance of which is situated a few meters above the observation points.

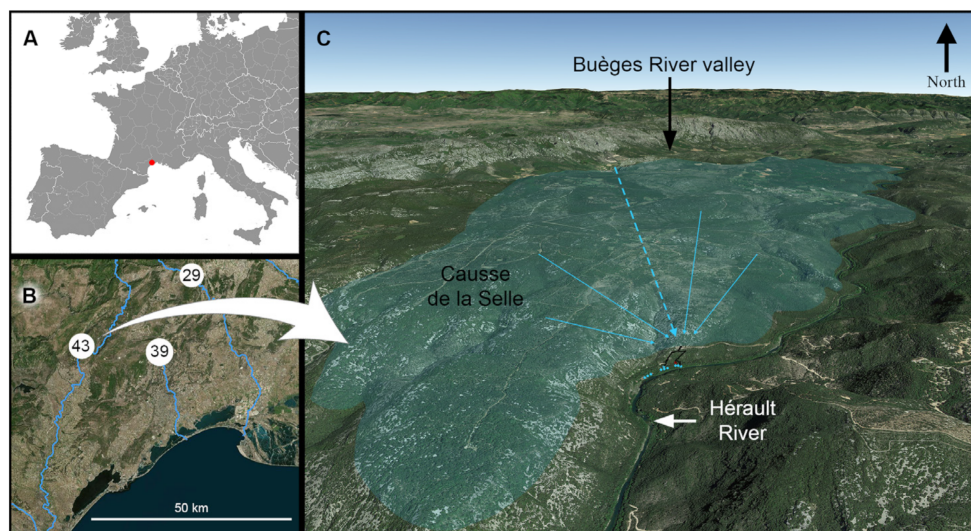


Figure 1. Location of the Cent Fonts springs. (A) In Western Europe. (B) Biodiversity hotspots of the Montpellier region, numbers refer to the number of stygobiotic species, 43 in the Cent Fonts, 39 in the Lez aquifer, and 29 in the Sauve spring. (C) The Causse de la Selle, aquifer of the Cent Fonts. Blue dots: springs; black line: cave topography. In bluish is the Causse-de-la-Selle plateau, which is the impluvium of the Cent Fonts system. To the north is the Buèges River, part of whose waters flow into the underground water system of the Cent Fonts (dotted blue line). Image © Google Earth.

The spring has been explored by cave divers, one of whom died in 1984. The divers reached a depth of -95 m, about 150 m from the cave entrance, and were blocked by a narrow passage.

The Cent Fonts system is the most important emergence of the Causse-de-la-Selle plateau. The land use in its catchment area consists mainly of evergreen oak forests *Quercus ilex* L., 1753 and extensive pastures. Human settlement in this area is very limited, and the presumed anthropogenic impacts are low. The Cent Fonts site falls within several protected areas (Natura 2000 site FR9101388—Gorges de l’Hérault; classified site; Grand Site de France; ZNIEFF (Natural Areas of Floristic and Faunistic Interest)). This site stands out for its remarkable vegetation associations (Salzman Pine forest); rare bird species such as the Bonelli eagle *Aquila fasciata* Vieillot, 1822 and the Cinereous vulture *Aegypius monachus* (Linnaeus, 1766); and some rare insect species, including an endemic beetle, *Cryptocephalus mayeti* Marseul, 1878. Rarely mentioned, however, is its exceptional richness in stygobiotic invertebrates.

1.3. History of Biological Studies

Following the description of the subterranean crustacean *Gallocaris inermis* (Fage, 1837) in the Gard department and its subsequent discovery in other aquifers bordering the Hérault, the aquatic fauna of the Cent Fonts massif has undergone a more extensive exploration. Initially, it was the subject of sporadic investigations utilizing rudimentary tools such as nets and baited traps [3–5], which revealed the presence of four large-sized

crustacean species, *Gallocaris inermis*, *Faucheria faucheri* (Dollfus & Viré, 1900), *Sphaeromides raymondi* Dollfus, 1897, and *Niphargus virei* Chevreux, 1896, and an ostracod species, *Sphaeromicola cebennica* Rémy, 1948, a parasite of *Sphaeromides* (Table 1).

Table 1. List of the stygobiotic species recorded in the Cent Fonts system from 1950 to 2006.

Classe	Sous-Classe	Ordre	[3–5]	Rouch et al. [6]	Olivier et al. [7]	This Paper	
Clitellata	Hirudinea	Arhynchobdellida	-	-	-	<i>Trocheta taunensis</i> Grosser, 2015	
			-	-	<i>Bythinella n. sp.</i>	<i>Bythinella sp.</i>	
			-	-	<i>Heraultiella exilis</i>	<i>Heraultiella exilis</i> (Paladilhe, 1867)	
			-	-	<i>Islamia moquiniana</i>	<i>Islamia cf. moquiniana</i>	
			-	-	<i>Paladilhia pleurotoma</i>	<i>Paladilhia pleurotoma</i> Bourguignat, 1865	
			-	-	<i>Bythiospeum bourguignati</i>	<i>Bythiospeum bourguignati</i> (Paladilhe, 1866)	
			-	-	<i>Moitessieria rolandiana</i>	<i>Moitessieria vidourlensis n. sp.</i>	
Gastropoda	Caenogastropoda	Littorinimorpha	-	-	<i>Moitessieria n. sp. 1</i>	<i>Moitessieria guilhemensis</i> Girardi & Boeters, 2017	
			-	-	<i>Moitessieria n. sp. 2?</i>	<i>Moitessieria sp.</i>	
			Decapoda	<i>Troglocaris inermis</i>	<i>Troglocaris inermis</i>	<i>Troglocaris inermis</i>	<i>Gallocaris (Troglocaris) inermis</i> (Fage, 1937)
			-	-	<i>Stenasellus buili</i>	<i>Stenasellus buili</i> Rémy, 1949	
			-	-	<i>Proasellus cavaticus cavaticus</i>	<i>Proasellus cavaticus</i> (Leydig, 1871)	
			-	-	<i>Microcharon doueti n. sp.</i>	<i>Microcharon doueti</i> Coineau, 1968	
			Isopoda	<i>Faucheria faucheri</i>	<i>Faucheria faucheri</i>	<i>Faucheria faucheri</i>	<i>Faucheria faucheri</i> (Dollfus & Viré, 1900)
			-	-	<i>Sphaeromides raymondi</i>	<i>Sphaeromides raymondi</i> Dollfus, 1897	
			-	-	-	<i>Niphargus laisi</i> Schellenberg, 1936	
			-	-	-	<i>Niphargus gallicus</i> Schellenberg, 1935	
Malacostraca	Eumalacostraca	Amphipoda	-	-	<i>Niphargus kochianus</i>	<i>Niphargus kochianus</i> Bate, 1859	
			-	-	<i>Niphargus pachypus</i>	<i>Niphargus pachypus</i> Schellenberg, 1933	
			<i>Niphargus orcinus virei</i>	<i>Niphargus orcinus virei</i>	<i>Niphargus virei</i>	<i>Niphargus cf. virei</i> Chevreux, 1896 clade A	
			-	-	<i>Salentinella angelieri</i>	<i>Salentinella angelieri</i> Delamare-Deboutteville & Ruffo, 1952	
			-	-	<i>Salentinella sp.</i>	<i>Salentinella delamarei</i> Coineau, 1962	
			-	-	<i>Salentinella delamarei</i>	<i>Salentinella delamarei</i> Coineau, 1962	
			Ingolfiellida	-	<i>Ingolfiella sp.</i>	<i>Ingolfiella thibaudi</i> Coineau, 1968	
			Bathynellacea	-	-	<i>Clamousella cf. delayi</i>	<i>Gallobathynella delayi</i> Serban, Coineau & Delamare-Deboutteville

Table 1. Cont.

Classe	Sous-Classe	Ordre	[3–5]	Rouch et al. [6]	Olivier et al. [7]	This Paper
			-	-	<i>Fabaeformiscandona</i> cf. <i>breuili</i>	<i>Fabaeformiscandona</i> cf. <i>breuili</i> (Paris, 1920)
			-	-	<i>Pseudocandona</i> <i>zschokkei</i>	<i>Marmocandona</i> cf. <i>zschokkei</i> (Wolf, 1920)
			-	-	<i>Schellencandona</i> cf. <i>simililampadis</i>	<i>Schellencandona</i> cf. <i>simililampadis</i> (Danielpol, 1978)
Ostracoda	Podocopa	Podocopida	<i>Sphaeromicola cebennica</i>	<i>Sphaeromicola cebennica</i>	-	<i>Sphaeromicola cebennica juberthiei</i> Danielpol, 1977
			-	-	<i>Candoninae</i> sp. 1, long, related to <i>Cryptocandona</i>	<i>Candoninae</i> sp. 1, cf. <i>Cryptocandona</i>
			-	-	<i>Candoninae</i> sp. 2, bean-shaped, related to <i>Pseudocandona</i>	<i>Candoninae</i> sp. 2, cf. <i>Pseudocandona</i>
			-	-	<i>Candoninae</i> sp. 3, triangular, related to <i>Pseudocandona</i> group <i>eremita</i>	<i>Candoninae</i> sp. 3, cf. <i>Pseudocandona</i> group <i>eremita</i>
			-	<i>Acanthocyclops rhenanus</i>	-	<i>Acanthocyclops rhenanus</i> Kiefer, 1936
			-	<i>Acanthocyclops stammeri westfalicus</i>	<i>Acanthocyclops venustus westfalicus</i>	<i>Acanthocyclops venustus westfalicus</i> (Kiefer, 1931)
			-	-	<i>Graeteriella</i> (<i>Graeteriella</i>) cf. <i>boui</i>	<i>Graeteriella</i> (<i>Graeteriella</i>) <i>boui</i> Lescher-Moutoué, 1969
		Cyclopoida	-	<i>Graeteriella unisetiger</i>	-	<i>Graeteriella</i> (<i>Graeteriella</i>) <i>unisetigera</i> Graeter, 1910
			-	<i>Paragraeteriella</i> n. sp.	-	<i>Graeteriella</i> (<i>Paragraeteriella</i>) <i>vandeli</i> Lescher-Moutoué, 1969
Copepoda	Neocopepoda		-	-	<i>Kieferiella delamarei</i>	<i>Kieferiella delamarei</i> (Lescher-Moutoué, 1971)
			-	<i>Speocyclops</i> sp.	-	<i>Speocyclops racovitzai</i> Chappuis, 1923
			-	<i>Ceuthonectes gallicus</i>	<i>Ceuthonectes gallicus</i>	<i>Ceuthonectes gallicus</i> Chappuis, 1928
			-	<i>Elaphoidella leruthi meridionalis</i>	<i>Elaphoidella leruthi meridionalis</i>	<i>Elaphoidella leruthi meridionalis</i> Chappuis, 1953
		Harpacticoida	-	-	<i>Nitocrella omega</i>	<i>Nitocrella omega</i> Hertzog, 1936
			-	<i>Nitocrella</i> cf. <i>hirta</i>	<i>Nitocrella hirta hirta</i>	<i>Nitocrella hirta</i> Chappuis, 1924
			-	<i>Ectinosomidae</i> sp.	<i>Pseudectinosoma vandeli</i>	<i>Pseudectinosoma vandeli</i> (Rouch, 1969)
Arachnida	Acari	Trombidiformes	-	<i>Soldanellonyx chappuisi</i>	-	<i>Soldanellonyx chappuisi</i> Walter, 1917
Insecta	Pterygota	Coleoptera				<i>Laemostenus</i> (<i>Actenipus</i>) <i>oblongus balmae</i> (Delarouzée, 1860) ¹

¹ This is the only trogllobiotic species collected so far in the Cent Fonts.

A second inventory dedicated to the Cent Fonts aquifer dates from 1967 to 1968 [6]. The system was studied under natural conditions, including all the springs and the cave. This study primarily aimed to characterize the stygobiotic fauna within the submerged zone

of the karst. More methods were used and the inventory of the stygobiotic fauna was more comprehensive. Thirty-nine crustacean species were collected, including 20 stygobiotic species belonging to the orders Decapoda, Amphipoda, Isopoda, and Copepoda (Table 1). The authors concluded that the Cent Fonts aquifer was “exceptionally rich”. The molluscs were not mentioned in this paper [6]. Their diversity in the northern Montpellier region was only studied later by Prié [8,9] and Girardi [10,11], but without focusing on the Cent Fonts aquifer.

In 2004, a water resource exploitation project prompted additional studies. A more thorough inventory of the stygobiotic fauna was conducted between 2005 and 2006 [7], employing extended filtrations of effluents during low-flow and flood periods, along with experimental pumping.

1.4. Threats

As mentioned above, the landscape surrounding the Cent Fonts system is relatively unaffected by human activities. However, this system is seen by the authorities as a major water resource for the entire department [12]. This human pressure on the water supply is believed to increase in the future, especially as the local climate is already dry and drought is expected to increase in the context of global warming [13].

1.5. Objectives

The aims of this paper are (i) to summarize the biospeleological studies carried out at Cent Fonts and in the surrounding caves, in particular the work of Olivier et al. [7], which has never been scientifically published; (ii) to update the taxonomy of the species present, with the description of new gastropod species; (iii) to highlight the interest in the site as a biodiversity hotspot; and (iv) to discuss the impact that the aquifer exploitation project could have on this hotspot.

2. Materials and Methods

We define the “Cent Fonts system” as the area drained by the Cent Fonts springs, extending from the Buèges River in the north to the Hérault River in the east and south, and an inactive valley to the east that, together with the rivers, outlines the boundaries of the “Causse de la Selle” (Figure 1). The stygobiotic fauna surveys were all carried out in the Cent Fonts springs, which line the right bank of the Hérault for about two hundred meters (Figure 1). The hyporheic zone of the Hérault was sampled just downstream of the springs, several meters away from the bank, using the Bou–Rouch Pump [14]. The Bou–Rouch pump allows large quantities of water to be pumped from the interstitial zone, at a depth of about 60 cm in the sandy clay alluvium. Troglobionts have not been inventoried in the Cent Fonts system. We list here species which most likely occur in the Cent Fonts, since they occupy many caves in the surroundings, outside of Figure 1.

The first surveys (1950–1951) used very simple methods such as dip nets and baited traps. Rouch et al. [6] in 1968 used a more comprehensive range of methods, including dip nets and baited traps; sight-hunting in each siphon (method only valid for large crustaceans); fine-netting (carried out in all siphons using a Bluter silk net); pumping with the Bou–Rouch pump in the Hérault River downstream of the springs; Karaman-Chappuis boreholes drilled in the siphon banks; and filtering of all the exurgences with Bluter silk nets of various mesh sizes, left in place and lifted every week. Some water outlets were filtered almost continuously from 15 November 1967 to 23 February 1968.

The same methods were used in the years 2005 and 2006: Bou–Rouch pumping (Figure 2), surbers, and spring water filtration, and baited traps, sight hunting, plus sediment sampling for mollusc shells in the springs and in the subterranean environment (Cent Fonts cave), respectively. The latter method consists of sampling sediment and leaving it in a bucket of spring water for a few days, in a cool and dark environment (e.g., a house cellar). As oxygen becomes scarce, the animals will try to return to the surface and can be caught on the sides of the bucket with flexible forceps. After a few days, when no

live snails are found, the sediment is dried out and poured into water again. The grains of sand will sink, and the empty shells will float to the surface and can be collected with a sieve.



Figure 2. Sampling the hyporheic zone of the Hérault River with the Bou-Rouch pump (© V. Prié).

All the data presented here have been deposited in the Inventaire National du Patrimoine Naturel (<https://inpn.mnhn.fr> (accessed on 6 November 2023)) database. The site number of the Cent Fonts is INPN 2047774. The sequences produced for the description of the new species (Appendix A) are deposited in GenBank, accession numbers PP050554 to PP050558 for COI; PP051254 to PP051258 for 16S; and PP057731 to PP057738 for 28S.

3. Results

The Lez system was considered to be the richest biodiversity hotspot for stygobiotic species in France [2]. We update here the checklist of stygobiotic species of the Lez basin given by Jourde et al. [15]: *Paladilhia umbilicata* (Locard, 1902) and *Bythiospeum articense* R. Bernasconi, 1985 are misidentifications, these species live far from the Lez system [16]; *Paladilhia subconica* Girardi, 2009 and *Moitessieria magnanae* Girardi, 2009 are considered endemic to their type locality in the Hérault basin; *Phagocata vitta* (Dugès, 1830) and *Proasellus coxalis* (Dollfus, 1892) are not stygobiotic species. Corrected in this way, the number of known stygobiotic species in the Lez system is 39. Following this study, the Cent Fonts aquifer appears as the richest system for stygobiotic taxa in France, with 43 species (Table 1). The terrestrial taxa, which are presumably not as rich as the aquatic ones, have not been studied in the Cent Fonts system itself. We present here the results of surveys carried out in neighboring caves located on the right bank of the Hérault valley, in the same geological context.

Where available, the IUCN Red List category is given for each species at global and national levels. Mollusc species were assessed at the global level in 2010 and at the regional level in 2021 (French Red List [17]). Although most species are regionally endemic, the 2010 (global) and 2021 (French) assessments sometimes differ. This is mainly due to an increased awareness of the threats to aquatic ecosystems, as human and climate change threats are increasingly documented. Most crustacean species have not been assessed at the global level, but a regional-level assessment is available [18].

3.1. Stygobionts

3.1.1. Clitellata Michaelsen, 1919; Arhynchobdellida Blanchard, 1894

- *Trocheta taunensis* Grosser, 2015 (= *T. bykowskii*)

Several populations of leeches named *T. bykowskii* have been discovered in Central and Western Europe [19]. Sket [20] was the first to suggest that *T. bykowskii* actually represents a species complex. Following Grosser [21], Lecaplain [19] considers the French populations to belong to *T. taunensis*. However, the records of *T. taunensis* in France are only from eastern France. The taxonomic status of the Cent Fonts population remains to be confirmed. The species was found in the Cent Fonts cave by F. Malard in 2002 (unpublished data).

3.1.2. Gastropoda Cuvier, 1795; Littorinimorpha Golikov & Starobogatov, 1975 Amnicolidae Tryon, 1863

- *Bythinella* sp.

A species of *Bythinella* was found in abundance in the springs of Cent Fonts (Figure 3a). It was considered a new species by Olivier et al. [7], based on the fact that it lives in a different aquifer from the regional stygobiotic *Bythinella* species described so far, i.e., *Bythinella navacellensis* Prié & Bichain 2009 endemic to the Larzac plateau (north-west of Cent Fonts) and *B. eutrepha* (Paladilhe, 1867) endemic to the Lez karst (south-west). Its identity remains unclear as no genetic data have been collected.

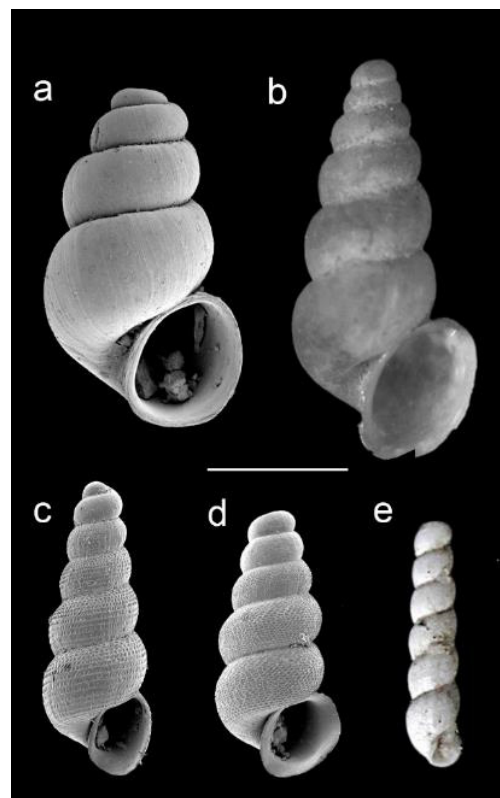


Figure 3. (a) *Bythinella* sp.; (b) *Paladilhia pleurotoma*; (c) *Moitessieria vidourlensis* n. sp.; (d) *Moitessieria guilhemensis*; (e) *Moitessieria* sp. nov.? or an anomalously shaped shell of *Moitessieria* sp. All these specimens were collected in the Cent Fonts sources. Scale: 1 mm.

Hydrobiidae Stimpson, 1865

- *Heraultiella exilis* (Paladilhe, 1867)

Heraultiella exilis lives in the hyporheic zone [22]. It sometimes occurs in springs but has never been found deep inside the caves. Here, it has been found in the hyporheic zone

of the Hérault River, and marginally in the springs. This species is protected in France and considered Vulnerable on both the international [23] and French Red List [17].

- *Islamia* cf. *moquiniana* (Dupuy, 1851)

The genus *Islamia* is awaiting molecular revision. *Islamia moquiniana* is described from the department of Lozère (type locality “. . . alluvions du Lot à Mende”), far from the Cent Fonts, and the specimens collected in the Hérault basin are morphologically different from those from the department of Lozère. It is therefore likely that the population found in the Cent Fonts is part of an undescribed species.

Moitessieriidae Bourguignat, 1864

- *Paladilhia pleurotoma* Bourguignat, 1865

P. pleurotoma is restricted to a few karst areas east of the Hérault River and west of the Rhône River. It is a cave specialist and has never been collected alive in the hyporheic zone. It is not certain whether the hyporheic zone can be used by this species as a corridor, as is the case for *Bythiospeum* species. Only one shell was found in the Cent Fonts cave (Figure 3b). This shell could be allogenic (transported there by flood). This species is protected in France. It was listed as Least Concerned in the IUCN international red list in 2010 [24] but re-evaluated as Vulnerable on the French Red List in 2021 [17].

- *Bythiospeum bourguignati* (Paladilhe, 1866)

This species lives mainly in the karst on the left bank of the Hérault [16]. It is found in the hyporheic zone of the Hérault and has been marginally collected in the sediments of the springs of the Cent Fonts. It is thought to reach its westernmost distribution limit in the Cent Font, which is also the westernmost limit of the genus. This species is protected in France. It was listed as Least Concerned in the IUCN international red list in 2010 [25] but re-evaluated as Near Threatened on the French Red List in 2021 [17].

- *Moitessieria vidourlensis* n. sp. (= *Moitessieria rolandiana* Bourguignat, 1864)

Most authors consider *M. rolandiana* as a widespread species in southern France, west of the Rhône River. However, Prié [9] showed that there is a strong genetic structure within the area of occurrence of *M. rolandiana*, which reflects the structure of the hydrographic network. A description based on morphometric and molecular data is provided hereafter (Appendix A). *M. vidourlensis* n. sp. (Figure 3c) is morphologically close to *M. rolandiana* but can be distinguished by morphometric analysis. This species is protected in France under the name *Moitessieria rolandiana*.

- *Moitessieria guilhemensis* Girardi & Boeters, 2017

This species was first recognized by Prié [8] based on morphological data (shells larger and smaller than that of *M. rolandiana*, Figure 3d), but was not described as a new species, because no genetic data were available, and the morphology has proven to be misleading for stygobiont species. However, Girardi and Boeters [26] could not wait and described the species as *M. guilhemensis*. This species is protected in France under the name *Moitessieria rolandiana*.

- *Moitessieria* n. sp.?

A spectacular shell was collected at the Cent Fonts (Figure 3e), perhaps an anomalously shaped shell, perhaps something new. As this is a single shell, we prefer not to consider it as a new species, pending further data, but we do report this remarkable form.

3.1.3. Malacostraca Latreille, 1802

Decapoda Latreille, 1802

- *Gallocaris (Troglocaris) inermis* (Fage, 1937)

This is a spectacular species (Figure 4), measuring up to 2 cm long, and is one of only two species of stygobiont decapod in France, endemic to a few aquifers in the Gard and

Hérault valleys where it is known from fewer than 10 localities. Interestingly, Rouch et al. [6] noted that this species only occurs in streamless waters inside the cave. Its supposed rheophobia may explain why it has never been collected in the springs, even during floods. It is listed as Near Threatened on the IUCN global Red List [27] and Vulnerable on the French Red List [18].



Figure 4. *Gallocaris inermis*, source of Sauve (Vidourle), ≈12 mm. © C. Alonso.

Isopoda Latreille, 1816

- *Stenasellus buili* Rémy, 1949

This species was described from the department of Aude, with isolated populations in the Corbières mountains and here in the Hérault valley. It is not evaluated at the international level, but it is listed as Near Threatened on the French Red List [18].

- *Proasellus cavaticus* (Leydig, 1871)

P. cavaticus is widespread in Western Europe, occurring in France along the Rhône–Rhine axis and in the Haut-Languedoc (it is also marginally present in the Atlantic basin). According to Henry [28], the population of Cent Fonts belongs to *P. cavaticus cavaticus* and is remarkable because it is the most western and the only place where *Stenasellus* and *Proasellus cavaticus* occur in syntopy. The species is considered Least Concern on the French Red List [18].

- *Microcharon doueti* Coineau, 1968

This species was discovered by Rouch by filtering the exurgences of the Cents Fonts (type locality) and then collected in the water table of the Orb River, west of the Hérault River. It is listed as Vulnerable on the French Red List [18].

- *Faucheria faucheri* (Dollfus & Viré, 1900)

This species was originally described by Adrien Dollfus and Armand Viré in 1900 as *Cæcosphaeroma faucheri* (family Sphaeromatidae), and it was reclassified by the authors in 1905 in the family Cirolanidae. Bertrand [29] lists a total of 21 localities, 10 in the upper Vidourle valley and the Hérault gorges and 11 in the eastern Corbières (Agly basin and its tributary the Verdoube). We (C.A.) add here another locality, the outlet of the Avencas cave, near Issensac, which extends the distribution of the species south to the coast. *F. faucheri* is listed as Least Concern on the French Red List [18].

- *Sphaeromides raymondi* Dollfus, 1897

S. raymondi (Figure 5) is a large species, up to 3 cm, known from a few caves in the Hérault department and the right bank drainage of the Rhône River, up to the Ardèche River. This species is mentioned in the literature from the 1950s and by Rouch et al. [6]

but was not found during the 2006 sampling. It is listed as Near Threatened on the French Red List [18].



Figure 5. *Sphaeromides raymondi*, Grotte exurgence de l’Avenas, Brissac, ≈18 mm. © J.C Queneau.

Amphipoda Latreille, 1816

- *Niphargus laisi* Schellenberg, 1936

The species is widespread in France and Germany. In France, its distribution is sporadic, from the Alsace in the north to the Rhône River aquifer near Lyon, and in the south in the Hérault basin. Its habitat is mainly represented by the hyporheic and phreatic zones. It is considered Data Deficient on the French Red List [18].

- *Niphargus gallicus* Schellenberg, 1935

This species is scarce in the southern half of France, where it lives in the porous aquifers of large alluvial floodplains (Rhône) and small streams (e.g., Triouzoune, St Angel), both in the phreatic and hyporheic zones. It has also been collected in karst areas (e.g., Prades-Le-Lez). *N. gallicus* is listed as Least Concern on the French Red List [18].

- *Niphargus kochianus* Bate, 1859

Niphargus kochianus had several subspecies, of which some are now considered as separate species. *Niphargus k. kochianus*, although frequently reported in France, is considered doubtful as it would not have a transcontinental distribution [30]. McInerney et al. [31] defined four distinct clades (A, B, C, D) based on molecular analysis. *N. kochianus* “D” would be the lineage present in France. The French form is sparsely distributed from the extreme north to the south (Pyrenees region), but is more common in the Rhône basin, Jura, and Ardèche regions. Given the large number of sites and specimens reported from France, it is difficult to provide a clear taxonomic status for the *N. kochianus* collected in the Cent Fonts system. Moreover, for this French “lineage D”, the number of sites and specimens in the 2014 study [31] appears to be very low.

From an ecological point of view, it is a small species, typically interstitial, living in cool waters and stable flow conditions. In French aquifers, *N. kochianus* is often found in the upwelling zones of rivers (e.g., Rhône), or in deep alluvial and phreatic zones (e.g., wells in the Albarine valley, Jura). It is also reported in karst, where it may find conditions for an interstitial lifestyle. *N. kochianus* is listed as Least Concern on the French Red List [18].

- *Niphargus pachypus* Schellenberg, 1933

Previously described as a subspecies of *N. kochianus*, it has been raised to species level and is now recognized as a highly divergent lineage [32]. It was collected from only few sites in the Netherlands and is also reported from Belgium and Luxembourg. In France, it is widespread, with more than a hundred localities. As a small-sized species, it is typically interstitial and particularly prefers cool and hydrologically stable areas, which explains its abundance in the deep alluvia of streams and in the phreatic zone. However, it has also

been collected in the karst areas, where it is probably associated with alluvial deposits. *N. pachypus* is listed as Least Concern on the French Red List [18].

- *Niphargus* cf. *virei* Chevreux, 1896 Clade A

This species is found mainly in France, but also in a few places in the Netherlands, Belgium, and Switzerland. In France, it is typically a karstic species. *Niphargus virei* has never been found in the porous aquifer, except for one specimen collected in the alluvia of the Rhône-Ardèche confluence, probably drifted from the surrounding karst. It is particularly common and abundant in the Jura and Ardèche massifs. Genetic studies have revealed the presence of three cryptic species in the French *virei* group [33]. The population of the Cent Fonts karst system belongs to the cryptic species “A”, located at the extreme south of the group’s geographical distribution. *N. virei* has been described from specimens collected in caves of the Jura mountains (grottes d’Arbois, Baumes-les-Messieurs, and Baumes-les-Dames). The nominal species should then belong to clade “B” of Lefebure et al. [33]. The most widespread of these cryptic species, Clade A (Figure 6), which is found from the Hérault to the Rhône and Moselle Rivers, is still awaiting formal description. This species is listed as Least Concern on the French Red List [18].

- *Salentinella angelieri* Delamare-Deboutteville & Ruffo, 1952

S. angelieri has a wide geographical distribution in Greece, Italy, and Spain. It is less common in France, where it is mainly recorded from the Rhône basin and the Hérault region. A population is also reported from Corsica. Two subspecies have been described from Croatia and Spain. In the Rhône floodplain, it is always collected in upwelling, i.e., in cool and stable interstitial water. It is listed as Near Threatened on the French Red List [18].

- *Salentinella delamarei* Coineau, 1962

This species is described from the phreatic waters of the Tech River in the department of Pyrénées-Orientales. It is reported only from France along the Rhône River, the Ardèche, and Hérault areas. Two subspecies have been described: *S. delamarei delamarei* and *S. delamarei macrocheles*. This species is listed as Least Concern on the French Red List [18].



Figure 6. *Niphargus* cf. *virei*, Grotte exsurgence de l’Avenca, Brissac, ≈15 mm. © J.C Queneau.

Ingolfiellida Hansen, 1903

- *Ingolfiella thibaudi* Coineau, 1968

This species has been reported from fewer than fifteen sites, from Ruoms in the Ardèche to Tarbes and the Saint Girons area in the Pyrenees region. It has been collected in both karst and porous aquifers (hyporheic and phreatic zones). In the Cent Fonts system, several specimens have been found in the spring sediments and in the hyporheic zone of the Hérault river (Figure 7). *I. thibaudi* is listed as Least Concern on the French Red List [18].



Figure 7. *Ingolfiella thibaudi*, Cent Fonts, ≈ 2 mm. © M.-J. Dole-Olivier.

Bathynellacea Chappuis, 1915

- *Gallobathynella* (*Clamousella*) *delayi* Serban, Coineau & Delamare Deboutteville 1971

This species was previously considered strictly endemic from the Clamouse Cave, a few kilometers downstream of the Hérault Valley, also on the right bank. The species is listed as Vulnerable on the French Red List [18].

3.1.4. Ostracoda Latreille, 1802; Podocopida Sars, 1866

- *Fabaeformiscandona* cf. *breuili* (Paris, 1920)

This species is widespread in Europe, from Poland to Spain, and certainly represents a number of subspecies or cryptic species. The taxonomic status of the Cent Fonts population needs to be clarified. On a European scale, *F. breuili* has been sampled in different habitats: wells, springs, the hyporheic zone of rivers, and, more rarely, in caves. In the Cent Fonts, *F. breuili* has only been sampled with exsurgence filtering, but not in the hyporheic zone. *F. breuili* is listed as Least Concern in the UICN French Red List [18].

- *Marmocandona* cf. *zschokkei* (Wolf, 1920)

Originally described in the genus *Candona*, it was then included in the genus *Pseudocandona*. Danielpol et al. [34] proposed the genus *Marmocandona* (whose type species is *Candona zschokkei* Wolf, 1920) for four stygobiotic species. This species is widespread in Western Europe: in Switzerland, Germany, Belgium, and France. The taxonomic status of this southern population needs to be clarified. *M. zschokkei* was often sampled in the hyporheic zone of large rivers, but also occurred in springs and wells. In the Cent Fonts, *M. zschokkei* was sampled with exsurgence filtering, but not in the hyporheic zone of the river. The species is listed as Least Concern on the UICN French Red List [18].

- *Schellencandona* cf. *simililampadis* (Danielpol, 1978)

This species was previously restricted to an artificial cave associated with the Vidourle spring at Sauve (Gard department). The taxonomic status of the population sampled in the Cent Fonts needs to be clarified. This species was sampled with exsurgence filtering. It is listed as Vulnerable on the French Red List [18].

- *Sphaeromicola cebennica juberthiei* Danielpol, 1977

This species is currently known from only two sites in the Hérault valley: the Cent Fonts and another cave a few kilometers upstream, also on the right bank of the Hérault River. It is mentioned in the literature from the 1950s and by Rouch et al. [6] but was not found during the 2006 sampling. *Sphaeromicola cebennica* is listed as Vulnerable on the French Red List [18].

- Candoninae sp. 1, 2, 3

Three other species of the subfamily Candoninae were sampled during the 2006 Cent Fonts study, but only with juveniles: a “long” form, related to the genus *Cryptocandona*; a “bean-shaped” form, related to *Pseudocandona*; and a triangular form, related to the *Pseudocandona* group *eremita*. Their taxonomic status still needs to be established by examination of adult specimens. However, although they could not be formally identified to the species level, they represent other species than those listed above.

3.1.5. Copepoda Milne Edwards, 1840

Cyclopoida Burmeister, 1834

- *Acanthocyclops rhenanus* Kiefer, 1936

This obligate groundwater cyclopoid shows a wide distribution in many groundwater habitat types of Europe. Its distribution covers several countries in central-eastern Europe, from France to Poland. The species shows no apparent habitat specialization, being recorded from almost all the groundwater habitat types. This species is mentioned by Rouch et al. [6] but was not found during the 2006 sampling. *A. rhenanus* is listed as Least Concern in France [18].

- *Acanthocyclops venustus (stammeri) cf. westfalicus* (Kiefer, 1931)

This species has an alternate representation in the current literature, and according to present knowledge, the accepted name for the subspecies *westfalicus* is *A. venustus venustus* [35]. The *venustus* group of the genus *Acanthocyclops* is in need of revision, and pending a clearer taxonomic assessment, the subspecies name *westfalicus* is provisionally maintained here. This subspecies has been recorded from Germany, Belgium, and France, and collected from phreatic habitats, the hyporheic zone of rivers, and aquifers in unconsolidated sediments. *A. venustus* is listed as Vulnerable on the French Red List [18].

- *Graeteriella boui* Lescher-Moutoué, 1974

This species is known only from France, with 11 records from both alluvial and karst aquifers, with a higher incidence in the saturated karst. It was originally described on the basis of specimens collected in the Gard department, but in the description, the author mentions the Ardèche and Hérault populations as belonging to the same species (“[The description of *Graeteriella boui* is based on individuals caught in the Gard department. Other forms collected in neighbouring departments reproduce the same characteristics; some differences, not sufficient to introduce new systematic subdivisions, are noted below]”). The population studied in the Hérault basin is that of the Cent Fonts, and Lescher-Moutoué [36] concludes the following: “The presence of *G. boui* in the Cent Fonts karstic system is all the more remarkable because two species of this genus have also been recorded in the same system: *G. unisetigera* and *G. (Paragraeteriella) vandeli* Lescher-Moutoué, 1969”. The species has also been collected from the karst aquifer of the Lez River. It is listed as Vulnerable on the French Red List [18].

- *Graeteriella unisetigera* (Graeter, 1908)

This species is considered by Fiers and Ghenne [37] to be a member of the cryptozoic fauna, as it has also been found in leaf litter and in other surface habitats (e.g., mosses) in Belgium, usually with some connection to groundwater. In spite of this situation, the species has several morphological characteristics that make it a good candidate for a widespread stygobiotic species in Europe, able to live in true groundwater habitats as well as in surface ecosystems dependent on groundwater. It is mentioned by Rouch et al. [6] but was not collected again during the 2006 sampling. *G. unisetigera* is listed as Least Concern on the French Red List [18].

- *Graeteriella (Paragraeteriella) vandeli* Lescher-Moutoué, 1969

Rouch et al. [6] mention “*Paragraeteriella n. sp.*”, without any further details. It was later described as *Paragraeteriella vandeli* by Lescher-Moutoué [38]. The type locality is the

Cent Fonts. It is known only from a single record from the Cent Fonts karstic system, which makes it spot endemic to this restricted area and rare in terms of abundance. At present, it has only been collected from the saturated karst. It was not found during the 2006 survey. *G. vandeli* is listed as Vulnerable on the French Red List [18].

- *Kieferiella delamarei* (Lescher-Moutoué, 1971)

This cyclopid species has exceptional stygomorphic features, such as a slender body, completely depigmented, long antennules, and long setae on the swimming legs, which make it a typical planktonic species swimming in underground karst lakes. This species is known from the Lez karst system and has also been collected from the Cent Fonts karst springs. The genus *Kieferiella* is monotypic and the only known species is from this restricted area in the south of France, making it a priority for conservation. It is listed as Vulnerable on the French Red List [18].

- *Speocyclops racovitzai* (Chappuis, 1923)

This species is mentioned by Rouch et al. 1968 as “*Speocyclops* sp. (en cours de détermination)”. It was not found during the 2006 sampling. *S. racovitzai* is present throughout southern France [39]. It shows a high degree of diversification in morphological micro-characteristics and is therefore divided into several subspecies with subtle morphological differences. No less important, some subspecies show overlapping distributions, raising doubts about their subspecific identity. The currently recognized subspecies need a taxonomic redefinition, but all are considered stygobionts. The nominotypical species also shows a wide distribution in the Pyrenees. It is listed as Least Concern on the French Red List [18].

Harpacticoida Sars G.O., 1903

- *Ceuthonectes gallicus* Chappuis, 1928

This species is widespread in France and always associated with groundwater habitats, both in alluvial and karst aquifers, with some preference for the latter [40]. It is endemic from France and is of Least Concern on the French Red List [18].

- *Elaphoidella leruthi meridionalis* Chappuis, 1953

The genus *Elaphoidella* is one of the most diverse harpacticoid genera in groundwater environments. In the study area, *E. leruthi meridionalis* is the only species recorded. It is known from several sites, mainly in southern France, with a clear preference for karstic groundwater, both in the saturated and unsaturated zones. *E. leruthi* is considered Data Deficient on the French Red List [18].

- *Nitocrella omega* Hertzog, 1936

The ameirid genus *Nitocrella* is considered to be of ancient direct marine origin and almost all species of this genus are known only from groundwater habitats [41]. This species collected from the Cent Fonts is rare in terms of occurrence and abundance, being known from only a few localities in France, Germany, and Hungary. It is listed as Vulnerable on the French Red List [18].

- *Nitocrella hirta* Chappuis, 1924

This species is widespread throughout Europe, with more than forty localities and collected from many groundwater habitat types. Four subspecies have been described. *N. hirta* is not evaluated on the French IUCN Red list.

- *Pseudectinosoma vandeli* (Rouch, 1969)

This minute harpacticoid was the first *Pseudectinosoma* species discovered in groundwater worldwide. The species was first mentioned by Rouch et al. in 1968 as “*Ectinosomidae* sp.”. A year later, Rouch described it and placed it in the marine genus *Sigmatidium*. It was only later that Galassi et al. [42] re-analyzed the type material of the type species of the marine genus *Sigmatidium* on the occasion of the discovery of the second representative

of the genus *Pseudectinosoma* in France, and they definitively placed this species in the genus *Pseudectinosoma*. The genus *Pseudectinosoma* is considered to be an ancient Tethyan relict found in the groundwater of Europe and Australia, probably the only remnant of an ancient fauna of direct marine origin. *P. vandeli* is known only from this area and has been collected in large numbers from the Cent Fonts karst system. The Cent Fonts is the type locality of the species, listed as Vulnerable on the French Red List [18].

3.1.6. Arachnida, Acari

- *Soldanellonyx chappuisi* Walter, 1917

This species is mentioned by Rouch et al. [6] but was not found (but not sought for) during the 2006 sampling.

3.2. Troglobionts

Unlike stygobionts, troglobionts have not been inventoried in the Cent Fonts system. The only troglobiont species collected in the Cent Fonts cave is the carabidae beetle *Laemostenus (Actenipus) oblongus balmae* (Delarouzée, 1860). There is currently no report of other troglobitic taxa in the Cent Fonts system itself. On the assumption that troglobionts are less drainage-dependent than stygobionts, we list below species which most likely occur in the Cent Fonts, since they occupy many caves in the surroundings.

3.2.1. Araneae

- *Palliduphantes sanctivincenti* (Simon, 1873)

This species is endemic from southern France, and it is widespread between the Pyrenees and the Alpes.

3.2.2. Opiliones

- *Peltonychia clavigera* (Simon, 1872)

The genus *Peltonychia* contains the first described travunioid species. This polyphyletic genus is known from central European caves (Pyrenees, central France, and the Alps). *Peltonychia clavigera* is distributed on both slopes of the Pyrenees and in the Cevennes where it is sporadic (Figure 8A).

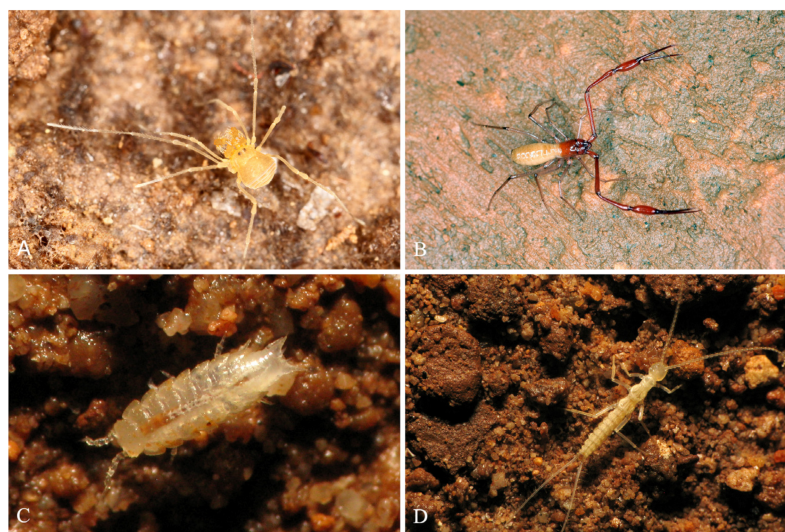


Figure 8. Some troglobite taxa from neighbouring karst systems. (A) Opiliones *Peltonychia clavigera*; (B) Pseudoscorpiones *Neobisium tuzetae*; (C) Isopoda *Trichoniscoides bonneti*; and (D) Diplura *Plusiocampa balsani*. © C. Alonso.

3.2.3. Pseudoscorpiones

- *Neobisium tuzetae* Vachon, 1947

N. tuzetae was described from the *Signal de la Montete* cave towards Quissac in the Gard department. This species is found in a large number of caves from the Hérault valley to the Larzac plateau (Figure 8B).

3.2.4. Isopoda

- *Trichoniscoides bonneti* Vandel, 1946

This endemic species is quite common in the caves of the limestone edge of the Cevennes, between the Hérault and Vidourle rivers (Figure 8C).

3.2.5. Diplura

- *Plusiocampa balsani* Conde, 1947

P. balsani is an endemic species found in many caves in the Massif Central. It is very common in all the caves of the Hérault valley (Figure 8D).

3.2.6. Collembola

- *Pseudosinella denisi* Gisin, 1954

This collembola is endemic from the sub-region (Gard, Ardèche, and Hérault Departments). It is widespread in caves around the Cent Fonts. It has clearly troglomorphic characteristics: eyeless, unpigmented, with elongated appendages and elongated claw.

- *Onychiurus ortus* Denis, 1935

O. ortus is endemic from the sub-region, in the departments of Hérault, Gard, and Aveyron. It is widespread in caves around the Cent Fonts. It has clearly troglomorphic characteristics: eyeless, unpigmented, and elongated claw.

3.2.7. Coleoptera

- *Laemostenus (Actenipus) oblongus balmae* (Delarouzée, 1860)

This is a widely distributed species, known from the Pyrenees to the southern and eastern edge of the Massif Central. The subspecies *balmae* is known from a few caves in the Gard and Hérault Departments, with one location in the Ardèche Department (Paiolive).

3.3. Stygophilic taxa

Five stygophilic species of Cyclopids have been collected in the Cent Fonts according to Lescher-Moutoué [39]:

- *Eucyclops serrulatus* (Fisher, 1851);
- *Paracyclops fimbriatus* (Fisher, 1853);
- *Acanthocyclops vernalis* (Fisher, 1853);
- *Megacyclops viridis* (Jurine, 1820), in the hyporheic zone of the Hérault River near the Cent Fonts exurgences;
- *Diacyclops languidoides* Lilljeborg, 1901.

3.4. Troglomorphic taxa and Parasites

Several other troglomorphic taxa are expected to be found in the Cent Fonts system, of which the most important are listed below.

Ixodida

- *Eschatocephalus vespertilionis* (Koch, 1844) is a common bat parasite.

Araneae

- *Lessertia denticelis* (Simon, 1884), a troglophile species, very common in the caves throughout the Hérault valley.

- *Meta bourneti* Simon, 1922, troglophile, very common in all caves in the area.
- *Meta menardi* (Latreille, 1804), troglophile, very common in all caves in the area.

Opiliones

- *Sabacon paradoxus* Simon, 1879, troglophile, found in cave entrances in France and Spain. It is very common in most caves in the Cévennes and in the Hérault karsts.

Julida

- *Blaniulus guttulatus* (Fabricius, 1798), troglophile, common in all caves of the region.

Isopoda

- *Oritoniscus delmasi delmasi* Vandel, 1933, endogeous and troglophile species, endemic to the southern Cévennes between the Vidourle and Lergue Rivers.
- *Phymatoniscus propinquus* (Brian, 1908), troglophile. The ocular area of this species is generally provided with a large single eyespot but in specimens of the Cents Fonts cave, eyes are completely invisible to external examination [43]. The species is common throughout the Cévennes in the Ardèche, Gard, and Hérault departments.

Coleoptera

- *Leptinus testaceus* P.W. Müller, 1817, is a troglophile, ectoparasite, and commensal of many species of micromammals. It lives mainly in subterranean mammal nests as well as in caves, on bat guano. This species is sporadic but known from many caves around the Cent Fonts system.

4. Discussion

4.1. A Biodiversity Hotspot Embedded in a Stygobiont Species-Rich Area

Only five stygobiont species were recorded from the Cent Fonts in the 1950s. Then, Rouch et al. [6] carried out a more extensive survey and found 20 stygobiont species. About 50 years later, another survey was triggered by an impact study of an important water extraction project, resulting in a total of 36 stygobionts [7]. Combining all this data, a total of 43 stygobiotic species have been identified, making the Cent Fonts system a hotspot of subterranean biodiversity in Europe (Table 1). Its stygobiont richness is higher than that of the better known Lez system (39 stygobionts), considered one of the most important biodiversity hotspots in the world [1,2]. The third-richest area of the southern Massif Central in France is the Sauve karstic system (29 stygobionts [44]), close to the Cent Fonts (Figure 1B). For the terrestrial fauna, it is expected that additional species will be found in the Cent Fonts, especially among the troglobionts known to occur in the surrounding caves (see Section 3.2,) as the Cent Fonts cave has been quickly sampled for troglobionts.

As pointed out by Rouch et al. [6], the rich fauna observed in the Cent Fonts includes groundwater genera of undoubtedly freshwater origin, such as *Elaphoidella*, *Ceuthonectes*, *Speocyclops*, and *Graeteriella* for copepods and *Gallocaris* for the decapods, and genera of no less certain marine origin such as *Microcharon* and *Sphaeromides* for isopods, *Ingolffiella* for ingolffiellids, and *Salentinella* for amphipods. Once again, the cave environment proves to be “the place of arrival of lineages of very different origins” [45]. In the stygobiotic molluscs, the origin of the family Moitessieriidae is still unclear, as all the published phylogenies have failed to anchor it in the global phylogenies of freshwater molluscs: the node linking it to the other taxa was not supported (e.g., Ref. [46]). This raises the question of the origin of this family, which could also be of marine origin.

4.2. Conservation Issues and Threats

The Cent Fonts system is the second-richest biodiversity hotspot in Europe for stygobiotic species and deserves conservation measures for this reason alone. A quarter of these 43 species are considered Vulnerable by the IUCN Red List. The Cent Fonts also hosts several relict species. Furthermore, it is the type locality of four taxa: *Sphaeromicola cebennica juberthiei*, *Graeteriella vandeli*, *Microcharon doueti*, and *Pseudectinosoma vandeli*. Type

localities should be preserved for future taxonomic work. Such a high biological value clearly deserves special attention.

Interestingly, eight species collected by Rouch et al. [6] (*Sphaeromides raymondi*, *Sphaeromicola cebennica juberthiei*, *Acanthocyclops rhenanus*, *Graeteriella vandeli*, *Graeteriella unisetigera*, *Speocyclops racovitzai*, *Diacyclops languidoides*, and *Soldanellonyx chappuisi*) were not collected in the 2006 inventory. Although the hydrological conditions are not documented by Rouch et al. [6], it is unlikely that the sampling conditions between November 1967 and February 1968 (4 months) were more favorable than during the whole period of the extensive survey carried out by Olivier et al. [7] between July 2005 and January 2006 (7 months), which included a major flood event. This difference in the results could be due to a lower probability of detection in 2006, but the sampling was more intensive, with water filtered for two years, a large team of experienced people both in the field and for the identification of the taxa, the use of improved collection methods, etc. So, if not the probability of detection, the absence of these species in 2006 could be due to local extirpation. It cannot be ruled out that these species are indeed in decline, but the reason for this is unknown. Although relatively well preserved in terms of land use, the Cent Fonts hydrosystem may be under unknown threats.

Water pollution from the surface is likely to be low, as this karst area has a very low human density. Climate change, which began in the early 1900s, could be a significant threat, but its effects on subterranean ecosystems are still poorly documented. However, severe droughts combined with increasing human pressure on the water resource especially in summer, with extreme fluctuations in water levels, are likely to affect subterranean ecosystems. Indeed, a short-term threat is the prospect of using this aquifer for drinking water. The Cent Fonts massif is recognized for the importance of its water supply and the quality of its water. An assessment of the volume of this resource and the possibilities for its exploitation was carried out in 2005 [12]. This study concluded that the drinking water reserve of the aquifer could not be mobilized for exploitation. However, this study is already disputed [47] and future needs may require greater resources.

4.3. Future Prospects

Troglobionts have been under-sampled, and it is likely that many more species will be found in future surveys, as described above. Intensive surveys by Rouch et al. [6] and Olivier et al. [7] have allowed the collection of many stygobiotic species, and only a few are expected to be added. However, some of the species collected in 1968 were not collected again in 2006. This may be due to local extinction and/or bias in the probability of detection. Intensive and regular surveys would give us a clearer picture of the biodiversity of the Cent Fonts and allow us to document its evolution and threats. However, these surveys require significant investment and are unlikely to be undertaken in the near-future to monitor the stygobiotic fauna.

Environmental DNA is the topical, cost-effective answer to unsatisfactory detection probabilities and the lack of taxonomic expertise. Several studies [48,49] have demonstrated its ability to detect up to 95% of aquatic organisms in surface streams, provided that optimized methods are implemented. Preliminary tests carried out in this karst with optimized methods (up to 250 L filtered, 12 PCR replicates, coverage of 300,000. . .) were promising, allowing the detection of most, but not all, of the gastropod and crustacean species known to occur in the area. Extensive work on sampling methods is needed to improve the detection probability. This approach deserves to be explored further and is probably the future for surveying and monitoring the fascinating stygobiotic ecosystems.

Author Contributions: Conceptualization, V.P. and M.-J.D.-O.; methodology, C.B., V.P. and M.-J.D.-O.; investigation, C.B., V.P. and M.-J.D.-O.; data curation, C.B., V.P., C.A., P.M., D.M.P.G. and M.-J.D.-O.; writing—original draft preparation, V.P.; writing—review and editing, V.P.; visualization, V.P. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: The data are available in the online open access database of the Inventaire National du Patrimoine Naturel (<https://inpn.mnhn.fr/accueil/index> (accessed on 6 November 2023)); INPN locality # 2047774.

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Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

We present here a brief description of three new species of the genus *Moitessieria*. These new species were described by Prié [9] in a Ph.D. thesis that is not considered an official publication according to the International Code of Zoological Nomenclature (ICZN). The names used in this earlier work are therefore *nomina nuda*. The descriptions are reproduced here with the proposed new names, in line with the current trend to avoid eponyms when describing new species.

Moitessieria species are very rarely collected alive and when they are, they are difficult to preserve, because ethanol does not penetrate into the shells, hence the paucity of available sequences on Genbank. The Niku-nuku method proposed by Fukuda et al. [50] was used systematically but failed in most cases. A *Moitessieria* shell can have 7 to 8 whorls, but the animal will retract to the first three whorls when stressed. As the opening at the shell mouth is less than 1/4 mm wide, it is unlikely that the ethanol ever comes into contact with the flesh. This probably explains why, in most cases, DNA amplification fails from *Moitessieria* specimens, or only one or two genes amplify. The genetic data presented here are therefore incomplete.

The gastropod family Moitessieriidae is the only family composed entirely of stygobionts. *Moitessieria rolandiana* was considered to be a widespread species in southern France [16]. This wide distribution contrasts with that of other species in the family, which are often restricted to a small karstic area, due to the fragmentation of subterranean habitats. Prié [9] showed that *M. rolandiana* is actually composed of three cryptic species, with each occupying a distinct karstic area, which supports their reproductive isolation by geographic barriers. They can be distinguished morphometrically, and molecular data corroborate their reproductive isolation. The species delimitation is based on morphometry, molecular, and distribution data.

Appendix A.1. Material and Methods

Appendix A.1.1. Biogeography

We used drainage basins as a hypothesis for where species limits are likely to occur. Drainage basin delimitations are based on the SANDRE database [51], which describes the subsurface hydrogeological units.

Appendix A.1.2. Morphometrics

Shells unambiguously attributable to the *Moitessieria* genus were collected in the four localities: 25 shells from the Folatière spring (close to the Gourneyras cave, locality 1 in Figure A2); 33 shells from the Cabrier spring (locality 2 in Figure A2); 18 shells from the Sauve Spring (locality 3 in Figure A2); and 12 shells from the Lirou River hyporheic zone (close to the Gour Noir spring, locality 4 in Figure A2). Shells were placed on an adhesive support in a standard position, i.e., with the columellar axis standing vertically, and then digitalized with a graduated scale using a stereomicroscope connected to a digital camera. Six parameters were recorded on each picture using ImageTool 3.00 [52]: height and width

of the shell, width of the last suture, width of the last whorl, and height and width of the aperture (Figure A1). These measures were log-transformed to minimize the size effect. Four ratios (H/W, H/LWW, AH/H, AH/AW) commonly used in the alpha-taxonomy of hydrobioid spring-snails, e.g., Refs. [53,54], were also calculated. Multivariate analyses were performed (Principal Component Analysis, PCA, and Linear Discriminant Analysis, LDA) to explore the distribution of these ten shell parameters using R [55].

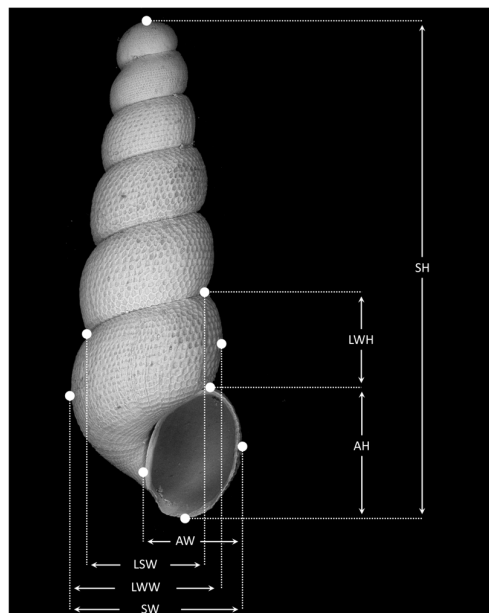


Figure A1. Measurements of a *Moitessieria* shell. SH: height of the shell; LWH: height of the last whorl; AH: height of the aperture; AW: width of the aperture; LSW: width of the suture of the last whorl; LWW: width of the last whorl; SW: width of the shell.

Appendix A.1.3. DNA Analyses

The whole specimens were used for the extractions, as their small size did not allow the animal to be taken out of the shell. DNA was extracted using the Nucleospin Tissue Kit (marketed by Macherey–Nagel), following the manufacturer’s protocol. Three partial gene sequences were amplified: a fragment of the cytochrome oxidase subunit I (COI) gene (barcode fragment of Folmer et al., [56]); a fragment of the rRNA 16S gene (universal primers of Palumbi [57]); and a fragment of the rDNA 28S gene (primers C1 and D3 [58]). Extractions, amplifications, and sequencing were performed by Genoscreen (France) and Eurofins (Germany) using standard protocols. Primer pairs were newly designed when the universal primer failed to amplify the 16S gene, 16sF2: AGTCGAGCCTGCCAGTGA and 16sR2: CAACCCTTAAAGACTTCTGCATCCTT; 16sF3: AGTCGRRCTGCCAGTGA and 16sR3: CAACCYTTAAAGACTTCTGCATCNTT. Sequences were automatically aligned using ClustalW multiple alignments implemented in BioEdit 7.0.5.3 [59]. The accuracy of automatic alignments was confirmed by eye. Only a few gaps, unambiguously aligned, were inferred for the 28S and 16S genes: they were conserved for the analyses. A topotype of *Paladilhia pleurotoma* Bourguignat, 1965 was included as an outgroup for both phylogenies. *Spiralix puteana* (accession numbers AF367635 and EU573992 [46]) was included as an outgroup for the mitochondrial analysis only.

Appendix A.2. Results

Appendix A.2.1. Biogeography

The region north of Montpellier in southern France is composed of distinct karst units, which have given rise to distinct faunal assemblages [9]. Not surprisingly, these distinct hydrosystems also support distinct species. The *Moitessieria* populations studied here

belong to four adjacent basins. One, the Tarn basin in the west, flows into the Atlantic. The Atlantic and Mediterranean drainages are the most isolated, especially because no stygobiont gastropods were found in the upstream hydrosystems. On the Mediterranean side, the Hérault (west), the Lez (centre), and the Vidourle (east) flow. Within these main basins, different hydrogeological units can be distinguished (Figure A2). While the surface relief creates ridge lines that distinguish these catchment areas, there may or may not be underground connections between the hydrogeological units. For example, the Larzac plateau flows north to the Vis River and south to the Lergue river, but the fauna is the same on both sides, reflecting the known subterranean connections between the two drainages. The same seems to occur between the upper Hérault and Vidourle drainages.

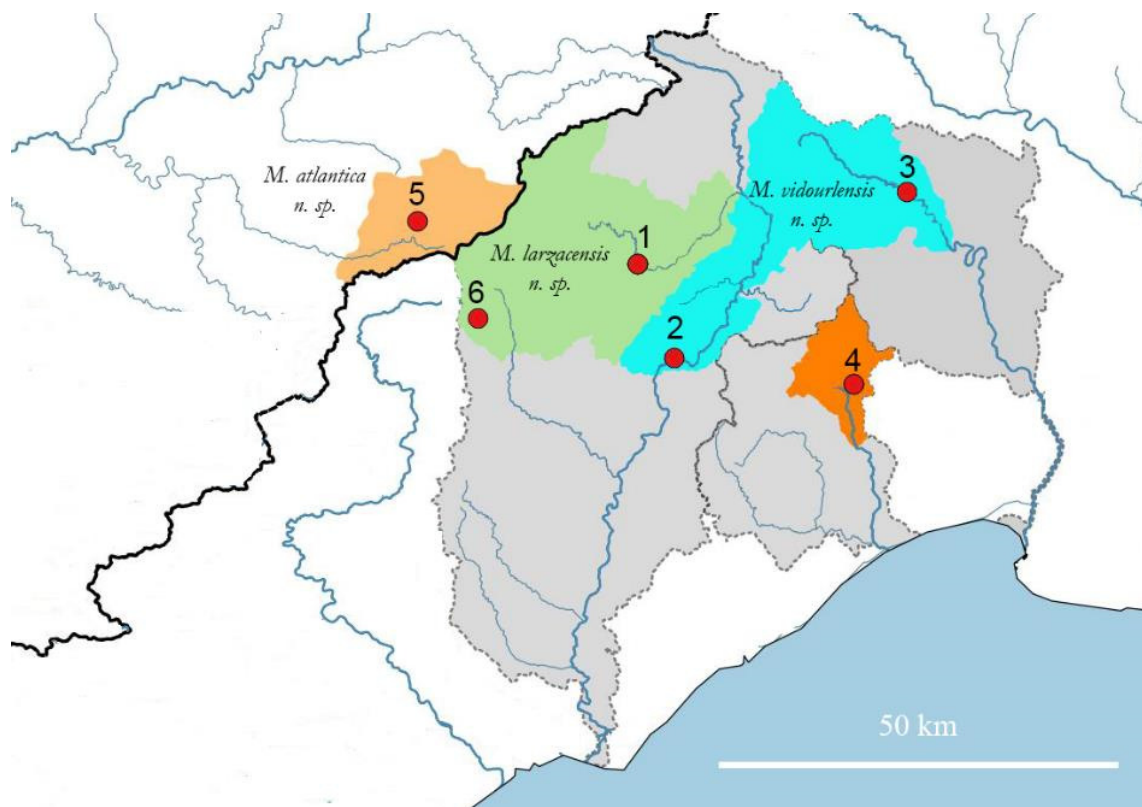


Figure A2. Biogeography of the subregion. Bold black line: separation between the Atlantic and Mediterranean drainages; thin dotted lines: separation between the major river drainages; blue lines: rivers; red dots: sampled populations, with numbers referring to the populations for which morphometric analysis was performed. The Mediterranean rivers' basins are highlighted in grey. The hydrogeological units are based on the SANDRE database. The numbers refer to the locations of the populations for which morphometric and/or molecular analyses were carried out (Figures A3 and A4).

Appendix A.2.2. Morphometry

Multivariate analyses allowed the populations from the Lez source (type locality of *Moitessieria rolandiana*), the Larzac plateau, and the upper Hérault/Vidourle to be distinguished. The populations from the upper Hérault and the upper Vidourle had the same morphology and could not be distinguished by morphometric analysis (Figure A3). Too few specimens were collected from populations 5 and 6 to be included in the morphometric analyses.

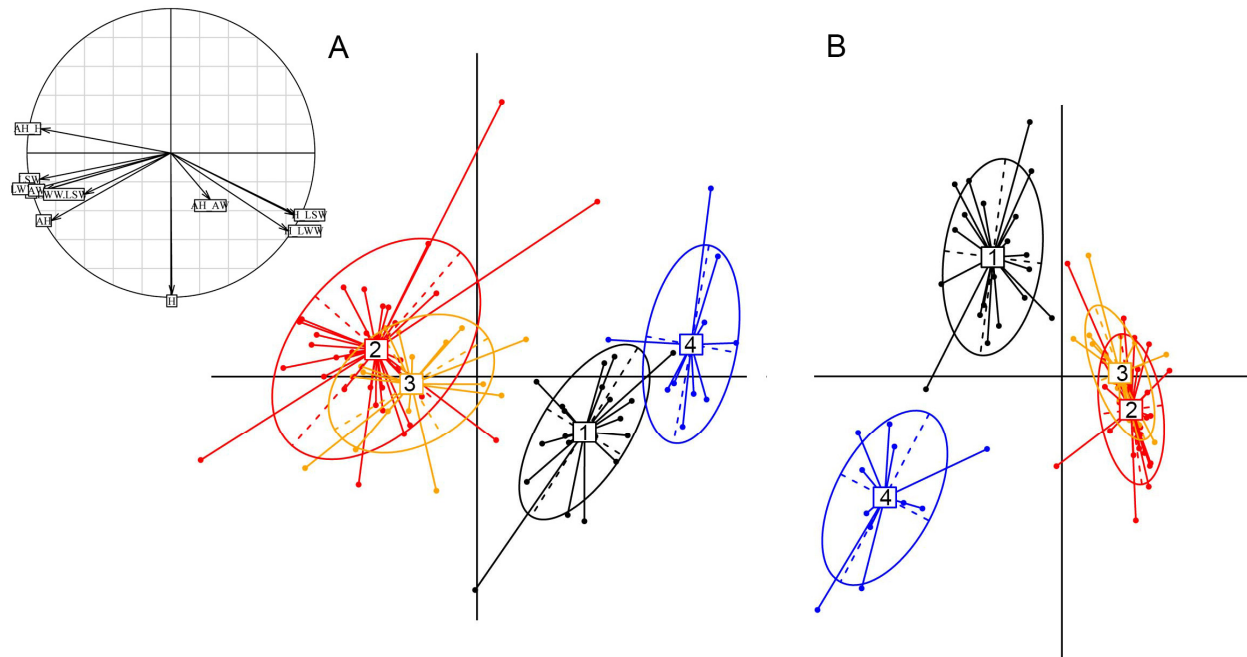


Figure A3. Multivariate analyses of the morphometrics of *Moitessieria* populations: (A) Principal Component Analysis; (B) Linear Discriminant Analysis. 1: Vis River, Larzac plateau, Hérault basin, *Moitessieria larzacensis* n. sp.; 2: Cabrier source, same system as the Cent Fonts, Hérault basin, *Moitessieria vidourlensis* n. sp.; 3: Sauve source, upper Vidourle, *Moitessieria vidourlensis* n. sp.; 4: Lez source, type locality of *Moitessieria rolandiana* (see Figure A1).

Appendix A.2.3. Genetics

Amplification was unsuccessful for several specimens or some of the genes studied, probably because *Moitessieria* species retract very deep into their shells, preventing contact between the tissues and the ethanol. The successful sequences of COI, 16S, and 28S obtained are given in Table A1. They support the biogeographic and morphometric analysis.

Table A1. Results of the tentative amplification of the three genes for the live specimens of *Moitessieria* collected. Location according to Figure A1.

Specimen	Location	COI	16S	28S
<i>Moitessieria rolandiana</i>	4 ¹	PP050555		
<i>Moitessieria rolandiana</i>	4 ¹	PP050556	PP051255	
<i>Moitessieria larzacensis</i> n. sp.	1	PP050554	PP051254	
<i>Moitessieria larzacensis</i> n. sp.	6	PP050557	PP051256	
<i>Moitessieria atlantica</i> n. sp. 1320	5			PP057732
<i>Moitessieria atlantica</i> n. sp. 1321	5		PP051257	PP057733
<i>Moitessieria atlantica</i> n. sp.	5			PP057734
<i>Moitessieria atlantica</i> n. sp.	5			PP057735
<i>Moitessieria vidourlensis</i> n. sp.	3			PP057731
<i>Moitessieria vidourlensis</i> n. sp.	2			PP057736
<i>Moitessieria vidourlensis</i> n. sp.	2			PP057737

¹ Type locality of *Moitessieria rolandiana*.

The mitochondrial genes (COI and 16S concatenated, Figure A4a) suggest that the population from the Larzac plateau is a separate species from *Moitessieria rolandiana* from the Lez drainage (type locality), as they diverge over 10% in COI, a threshold largely over interspecific divergences in related taxa [60–62]. This corresponds to a divergence of 3.8 to 4.7% in the 16S gene. No COI was available to compare the Atlantic population to *M. rolandiana*, but the 16S results can be transposed, as all mitochondrial genes share the same history.

The Atlantic population's divergence to the *M. rolandiana* type population is even higher, 6%. These results are congruent with the organization of the hydrogeological networks.

The nuclear gene (28S, Figure A4b), although it should be less variable, distinguishes the Atlantic population from the Mediterranean ones (only the upper Hérault and Vidourle basins analyzed here), but also, with a smaller divergence, the upper Hérault and upper Vidourle populations. Molecular data from more specimens are needed to determine whether the upper Vidourle and the upper Hérault populations are different species or not. A conservative attitude is adopted here and the upper Vidourle and the Cent Fonts populations are considered as belonging to the same species.

In summary, genetic data demonstrate that:

- (i) *M. larzacensis* n. sp. differs from *M. rolandiana* based on both COI and 16S.
- (ii) *M. atlantica* n. sp. differs from *M. rolandiana* and *M. larzacensis* n. sp. based on 16S.
- (iii) *M. vidourlensis* n. sp. differs from *M. atlantica* n. sp. based on 28S, but cannot be compared to *M. rolandiana* nor *M. larzacensis* n. sp. from the available molecular data. Only morphological differences, that are supported by geographical isolation, allow this species to be separated from *M. rolandiana* and *M. larzacensis* n. sp.

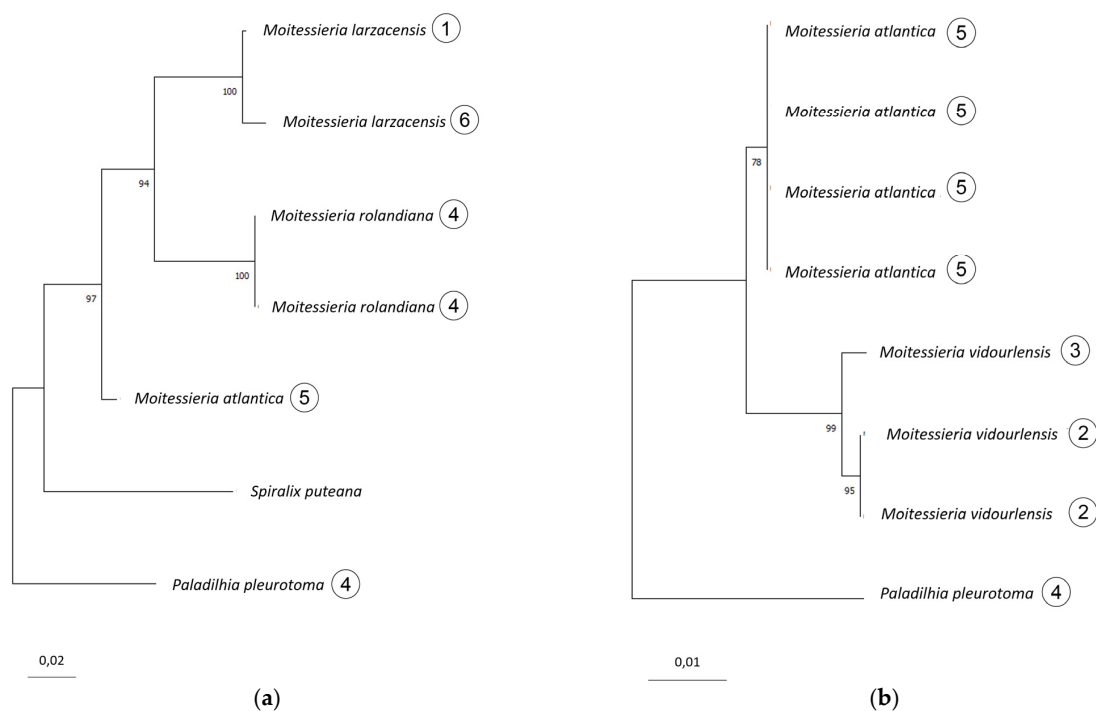


Figure A4. Phylogenetic tree of studied genes (Bayesian inference). (a) Concatenated COI + 16S; (b) 28S. Numbers correspond to localities in Figure A2.

Appendix A.2.4. Species Delimitation

Moitessieria rolandiana was considered to be a widespread species, distributed from the western tributaries of the Rhône River to the Garonne drainage [16]. Our results show that the name *Moitessieria rolandiana* should be restricted to the populations from the Lez drainage system. To the east, the adjacent Vidourle system hosts a distinct species, *M. vidourlensis* n. sp. The population sampled from the karst systems on the right bank of the Hérault River was morphologically and genetically similar to the Vidourle population and was therefore considered to belong to the same species. This distribution pattern involving two coastal river basins was unexpected. However, it is reminiscent of the distribution of some stygobiotic shrimps, *Gallocaris inermis*, *Proasellus cavaticus*, *Faucheria faucheri*, etc., a total of 12 crustacean species also known from both the Cent Fonts and Sauve (Vidourle) springs. It is therefore likely that hydrological connections exist, at

least sporadically, in the complex karstic network of the upstream Hérault and Vidourle drainages. *M. larzacensis* n. sp. is likely to live in the subterranean basin of the whole Vis River, probably on both sides, as *Moitessieria* species are known to live in the hyporheic zone and can therefore easily colonize the hydrosystems of both sides of the river. The hydrosystems of the Vis drainage are isolated from the Atlantic drainages to the west. It is therefore not surprising that the population of the Atlantic drainage belongs to a distinct species, described here as *Moitessieria atlantica* n. sp., due to geographic barriers.

Appendix A.2.5. Species Turbo-Taxonomy

Moitessieria vidourlensis n. sp. Prié 2024

Nomina nuda: *Moitessieria vasseuri* (Prié 2013)

Type material: holotype IM-2000-30145; paratypes: 28 shells (IM-2000-30146), deposited at the Museum national d'Histoire naturelle in Paris (Figure A5).

Type locality: the Sauve cave, in the Sauve (Gard department) municipality; 43°56'27.2394" N; 3°56'58.1568" E. The live specimen was collected while scuba diving, on the ground, with forceps, a few tens of meters from the entrance of the cave.

ZooBank record: urn:lsid:zoobank.org:act:B9A3A652-DDC5-4C18-A035-CA4574F8FE8E

Etymology: This species was originally dedicated to Frank Vasseur, a highly skilled subterranean scuba diver, who collected material from deep inside caves (including *M. larzacensis* n. sp.), and ensured VP's safety while diving in the Sauve cave in search of stygobiotic snails. We prefer to avoid eponyms here and give a name that reflects the distribution of the species.

Distribution: Pending further studies, the name *Moitessieria vidourlensis* should apply to the populations of *Moitessieria* from the Vidourle drainage, and the populations from the Causse-de-la-Selle. The species' distribution probably includes part of the Hortus karstic plateau between the Hérault and Vidourle drainages. Its eastward distribution limit is unknown.

Morphological characteristics: site 3—Vidourle: shell height: 1.67 (1.47–1.98) mm; shell width: 0.75 (0.65–0.98) mm; last whorl width: 0.65 (0.60–0.73) mm; N = 31; site 2—Cent Fonts: shell height: 1.74 (1.57–2.08) mm; shell width: 0.73 (0.65–0.82) mm; last whorl width: 0.62 (0.57–0.69) mm; N = 19.

Sequences GenBank accession numbers: PP057731, PP057736, PP057737 (28S).

Moitessieria larzacensis n. sp. Prié 2024

Nomina nuda: *Moitessieria tillierae* (Prié 2013)

Type material: holotype IM-2000-30143 (Figure A6); paratypes: 19 shells (IM-2000-30144), deposited at the Museum national d'Histoire naturelle in Paris.

Type locality: The Folatière spring, exurgence of the Folatière Cave, 43°51.871' N, 3°31.578' E. The live specimen used for DNA analyses was collected while scuba diving in the Gourneyras cave, 43°51'36.48 N; 3°31'38.64 E.

ZooBank record: urn:lsid:zoobank.org:act:D215581E-C50E-4707-9DA3-59B39B3FF3D1

Etymology: This species was initially dedicated to Annie Tillier, who successfully amplified the specimen collected from Gourneyras, at a time when *Moitessieria* specimen amplifications were systematically failing. We prefer to avoid eponyms here and give a name that reflects the distribution of the species.

Distribution: This species is known from molecular data only from the basins of the Vis River (north of the Larzac plateau) and the Lergue River (south of the Larzac plateau). It should therefore live in the entire hydrogeological network of the Larzac plateau. As *Moitessieria* species also inhabit the hyporheic zone, the Vis River does not represent a biogeographic barrier and the populations from the Blandas plateau are expected to belong to the same species. The distribution of *M. larzacensis* n. sp. is probably the same as that of *Bythinella navacellensis* [63], i.e., the subterranean watersheds of the Larzac and Blandas plateaus, drained to the north by the Arre, to the south by the Lergue, and in between by the Vis.

Morphological characteristics: shell height: 1.87 (1.65–2.33) mm; width: 0.63 (0.55–0.74) mm; last whorl width: 0.56 (0.50–0.67) mm; N = 20.

Sequences GenBank accession numbers: PP050554, PP050557 (COI), PP051254, PP051256 (16S).

Moitessieria atlantica n. sp. Prié 2024

Nomina nuda: *Moitessieria girardii* (Prié 2013)

Type material: holotype IM-2000-30147; paratypes: 11 shells (IM-2000-30148), deposited at the Museum national d’Histoire naturelle in Paris (Figure A7).

Type locality: The Gloriette spring, in the Sorgue drainage, municipality of Cornus (Aveyron department), 43°54′28.5114″ N; 3°10′38.0634″ E.

ZooBank record: urn:lsid:zoobank.org:act:BC712B05-70CF-4107-97B5-CBC94FE91325

Etymology: This species was initially dedicated to Henri Girardi, a famous French malacologist, author of many subterranean snails’ descriptions. We prefer to avoid eponyms here and give a name that reflects the distribution of the species.

Distribution: It is known only from the type locality, but presumably present elsewhere in the Sorgue River karstic drainages. Shells from a population sampled downstream (Saint-Paul-des-Fonts) fall within the morphological range of *M. atlantica* n. sp. and could belong to the same species.

Interestingly, no subterranean snail has been collected despite important sampling in the vicinity of the limit between the Atlantic and Mediterranean watershed. *M. atlantica* n. sp. is therefore geographically isolated from the other *Moitessieria* species described here.

Morphological characteristics: shell height: 1.72 (1.58–1.93) mm; shell width: 0.68 (0.66–0.73) mm; last whorl width: 0.62 (0.60–0.67) mm; N = 9. There are no morphometric analyses for this population, as the number of adult specimens collected was too low.

Sequences GenBank accession numbers: PP051257 (16S), PP057732, PP057733, PP057734, PP057735 (28S).

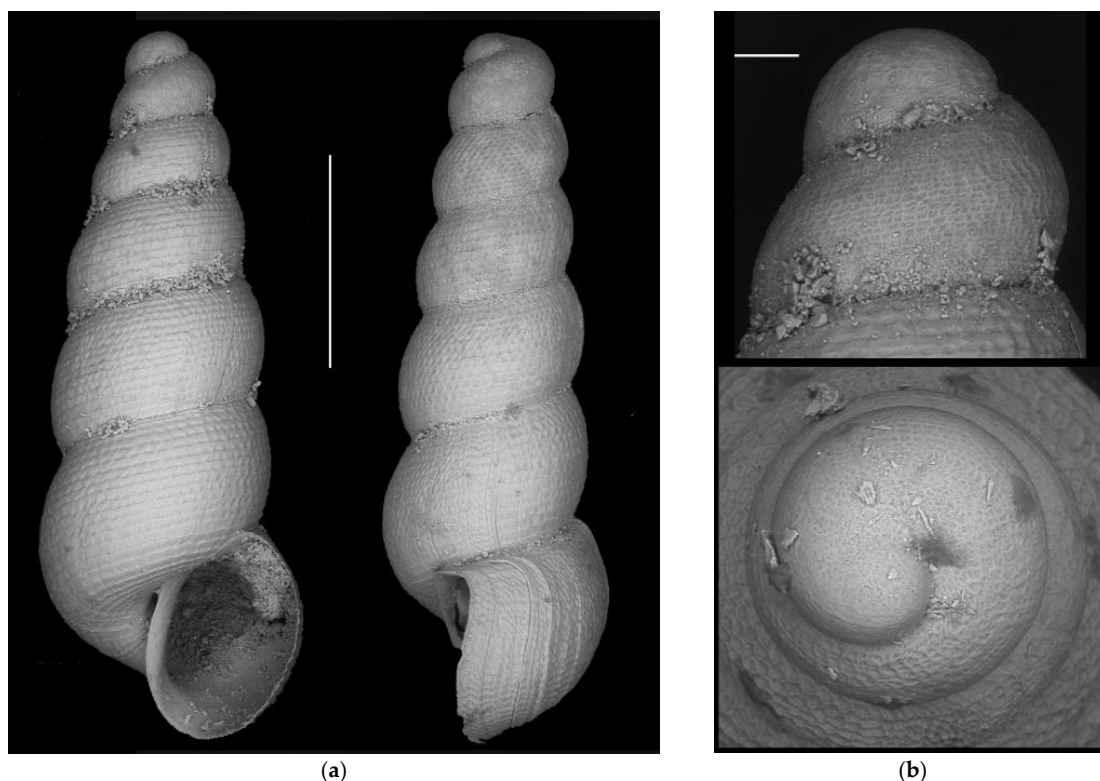


Figure A5. *Moitessieria vidourlensis*: (a) general view of the holotype (left) and of a paratype (right), scale = 500 μ m; (b) details of the protoconch of the holotype (above) and of a paratype (below), scale = 50 μ m.

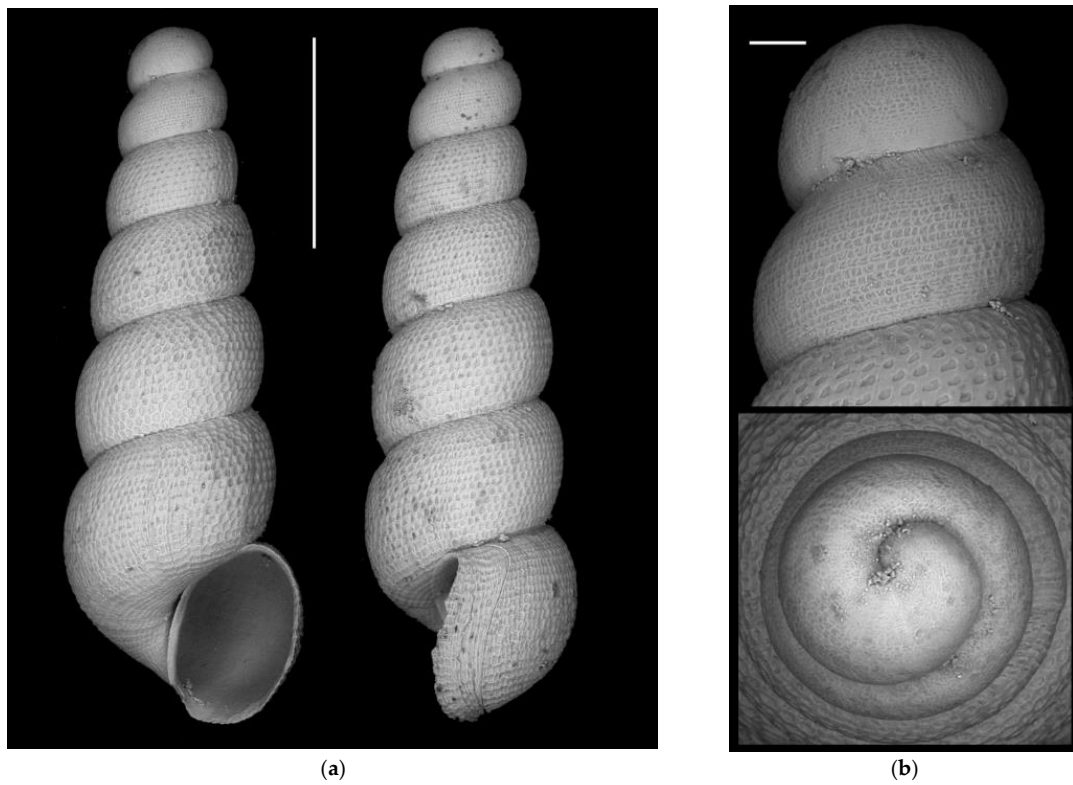


Figure A6. *Moitessieria larzacensis* n. sp.: (a) general view of the holotype (left) and of a paratype (right), scale = 500 μm ; (b) details of the protoconch of the holotype (above) and of a paratype (below), scale = 50 μm .

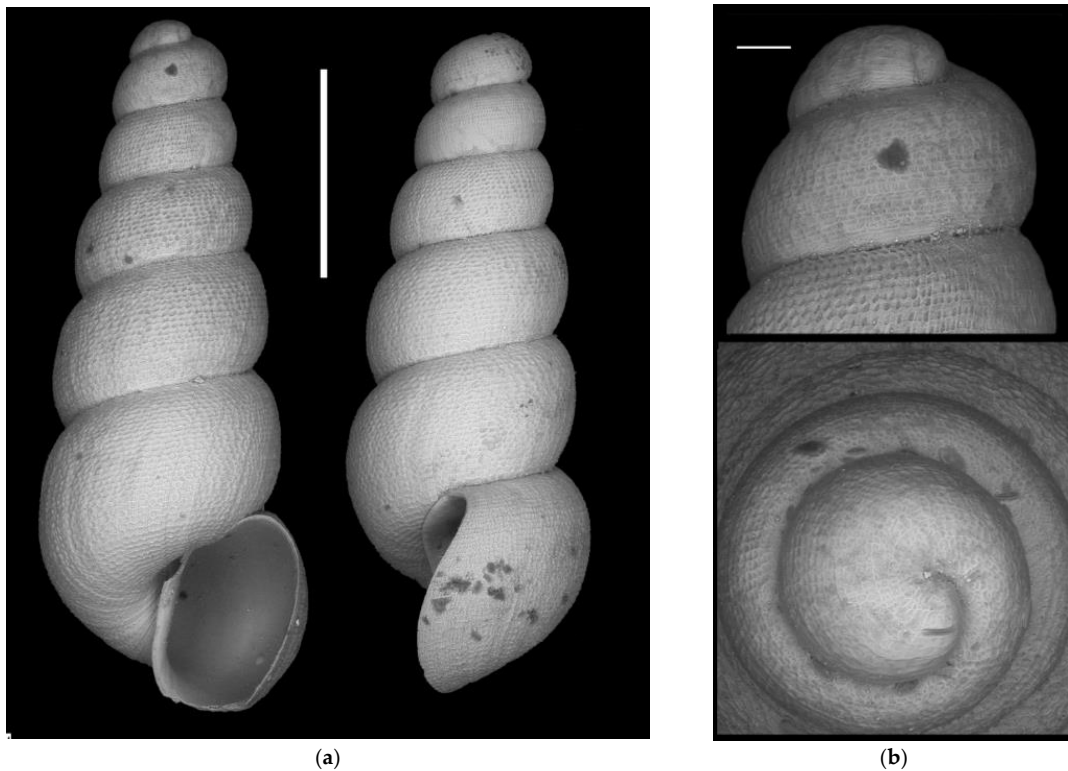


Figure A7. *Moitessieria atlantica* n. sp.: (a) general view of the holotype (left) and of a paratype (right), scale = 500 μm ; (b) details of the protoconch of the holotype (above) and of a paratype (below), scale = 50 μm .

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