SCIENTIFIC CONFERENCE

Le Bourget-du-Lac – July 2022

PRE-PRINT 2021

SYMPOSIUM 07
Biology, Subterranean life

Distribution, ecology, and diversity of the genus *Gallasellus* (Isopoda, Asellidae)

François LEFEBVRE[(1)], Michel CAILLON[(2)], Claude BOU[(3)], Florian MALARD[(4)] & Christophe J. DOUADY[(4)]

(1) 4 rue de Polverel, 19100 Brive-la-Gaillarde / SEPANSO, 1 rue de Tauzia, 33800 Bordeaux, France, francois.lefebvre@eco-analyses.com
(2) 19 rue des Villas, 86000 Poitiers, France, michel.sylvia.caillon@wanadoo.fr
(3) 52 chemin de la Fourestole, 81990 Cambon, France, bou.claude81@sfr.fr
(4) Université Claude Bernard Lyon 1, CNRS, ENTPE, UMR 5023 LEHNA, F-69622, Villeurbanne, France florian.malard@univ-lyon1.fr, christophe.douady@univ-lyon1.fr

Abstract

Until the nineties, *Gallasellus* (Isopoda, Asellidae) was known from a single species, *G. heilyi*, which was known only from the type-locality, the subterranean River of Bataillé (Gournay-Loizé, Deux-Sèvres, France). In 1992, *Gallasellus* was collected in a well in Oléron Island (Saint-Denis-d'Oléron, Charente-Maritime) at a distance of 100 km from the type-locality, indicating that the genus was more widely distributed. Here, we synthesize research conducted on *Gallasellus* since the 2010s and provide new findings on the systematics, distribution and ecology of that genus. *Gallasellus* is phylogenetically closely related to the North American genus *Caecidotea* and may contain at least 6 putative species. Its range spans over a maximum linear extent of nearly 200 km in limestone aquifers of the Poitou-Charentes Region. *Gallasellus* is a primary consumer feeding on organic detritus and microbial biofilm; it is preyed upon by *Niphargus ladmiraulti* (Amphipoda). The finding that *Gallasellus* is a potentially species-rich genus containing a mix of wide- and narrow-range species requires adapting conservation strategies.

Résumé

Distribution, ecologie et diversité de genus *Gallasellus* (Isopoda, Asellidae). Jusque dans les années 90, le genre *Gallasellus* (Isopoda, Asellidae) contenait une seule espèce, *G. heilyi*, connue d'une seule localité, la rivière souterraine de Bataillé (Gournay-Loizé, Deux-Sèvres, France). En 1992, *Gallasellus* fut collecté dans un puits de l'île d'Oléron (Saint-Denis-d'Oléron, Charente-Maritime) à une distance de 100 km de la localité type, indiquant que le genre était plus largement distribué. Dans les années 2010, un large programme d'échantillonnage a été mené à l'échelle de la région Poitou-Charentes et nous résumons ici les nouvelles connaissances acquises sur la systématique, la distribution, et l'écologie du genre.

1. Introduction

The term *Gallasellus* means literally Asellid of Gaul (i.e., "of France"). However, from the description of its type species, as *Asellus heilyi*, by LEGRAND (1956), to the description of the genus by HENRY & MAGNIEZ (1977), the genus *Gallasellus* was found to share many morphological similarities with the North American genus *Caecidotea*. Particularly, the endopod tip of male reproductive pleopod II has three processes (cannula, mesial and caudal processes) that are typically observed in the genus *Caecidotea* (WILLIAMS, 1970).

Until the nineties, *Gallasellus* was known from a single species, *G. heilyi*, which was known only from the type-locality, the subterranean River of Bataillé (Gournay-Loizé, Deux-Sèvres) (Fig. 1). In 1992, *Gallasellus* was collected in a well in Oléron Island (Saint-Denis-d'Oléron, Charente-Maritime) at a distance of 100 km from the type-locality, indicating that the genus was more widely distributed (MAGNIEZ & HENRY, 2001). Since then, some more specimens were collected in 2008-2009, and the first phylogenetic tree of Aselloidea showed a sister relationship between *Gallasellus* and *Caecidotea* (MORVAN *et al.*, 2013).

Date	Event	References
1955	Description of *Asellus heilyi* as a single-site species	Legrand (1956)
1970	Re-description as *Gallasellus heilyi*, morphological affinity with North American asellids, ecology and biology	Henry & Magniez (1970, 1977)
1992	Discovery of a new distant locality, distribution range extended to 100 km	Magniez & Henry (2001)
2010	Molecular systematics of Aselloidea	Morvan *et al.* (2013)
2013	Extensive sampling: distribution, ecology and biology	Lefebvre *et al.* (2016), Ercoli *et al.* (2019)
2013	Molecular diversity within *Gallasellus*	Morvan *et al.* (2013), Eme *et al.* (2018), this study

Figure 1: History of the genus Gallasellus.

From 2013 to 2015, an extensive collaborative sampling survey was carried out in the Poitou-Charentes Region. Here, we present the main findings of that survey and summarize knowledge on the biology, distribution, ecology and diversity of the genus *Gallasellus.*

2. Materials and methods

Biology and ecology

Data on sex ratio and body size sexual dimorphism were obtained from morphometric measurements of specimens collected during extensive sampling by LEFEBVRE *et al*. (2016). Knowledge on the biology and behavior of *Gallasellus* stems mainly from observations made by HENRY & MAGNIEZ (1977) during rearing of specimens from the type-locality. Knowledge on its trophic ecology is from stable isotope analyses conducted by ERCOLI *et al.* (2019). These authors used mixing models to estimate the proportions of available food resources exploited by *G. heilyi.*

To characterize habitat preferences of *Gallasellus*, we calculated its frequency of occurrence (number of positive sites / number of sampled sites) in consolidated rocks (mostly limestone, n=118 sampled sites) and unconsolidated rocks (mostly alluvial deposits, n=21). For occurrence in consolidated rocks, we distinguished between three different types of sites: subterranean river caves (n=20), springs (n=47) and wells (n=51).

Distribution

Mapping of the distribution of *Gallasellus* is based on sampling of 139 sites in the Poitou-Charentes Region. The protocol was described in LEFEBVRE *et al*. (2016). Samples were taken from caves, springs, wells and the hyporheic zone of streams. Samples were sorted under a stereomicroscope and specimens of *Gallasellus* were identified morphologically. Specimens were preserved in 96 % alcohol and molecular analyses were performed to delimit putative species (see below).

Diversity within Gallasellus

We used the COI gene-based threshold method defined by LEFÉBURE *et al*. (2006) to delimit putative species within *Gallasellus*. This species molecular delimitation method is based on the observation made from 1500 COI sequences belonging to 276 species of crustaceans that two clades diverging by more than 0.16 substitution per site, as measured by patristic distances, have a strong probability (ca 0.99 %) of belonging to different species. Putative species delimited with that molecular method are called molecular operational taxonomic units (MOTU).

Methods for extracting DNA, amplifying the mitochondrial cytochrome oxidase subunit I (COI) gene, building the COI phylogeny and delimiting MOTUs were provided by MORVAN *et al*. (2013). In the present study, the delimitation of MOTUs was performed on a data set containing a total of 2094 COI sequences of Aselloidea, including 53 sequences (specimens) of *Gallasellus* from a total of 28 sites.

3. Results

Biology and ecology

Mean body size in *Gallasellus* is 3.73 ± 0.68 mm (range: 2.16-5.65 mm, n=37 specimens) (Fig. 2). Growth is relatively fast: specimens reach a body size of 2.3 mm two months after hatching. Moult lasts 24 hours. Females are significantly more numerous (sex ratio 1M:2F, χ^2 test, p=0.02) and larger than males (body size: females: 3.91 ± 0.62 mm, n=27; males: 3.26 ± 0.61 mm; n=8, t-test, p=0.01) (Fig. 3). Life span is not known but exceeds 2 years. Females may produce 2 broods per year; the number of eggs of a 4.8-mm long female was 19 and egg diameter was 0.2 mm.

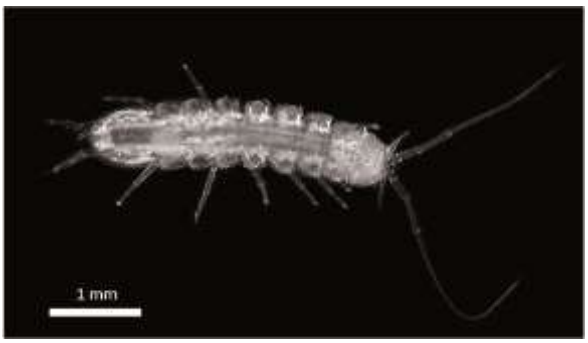

Figure 2: Photo of Gallasellus (courtesy of M. Delangle).

Type	Knowledge	References
Sex ratio	Female biased: number of F>M	Lefebvre *et al.*, 2016
Sexual dimorphism	Female biased: F larger than M	Lefebvre *et al.*, 2016
Reproduction	First brood at 7 months, 2 broods / year, 6-19 eggs / brood, egg incubation time: 35 days	Henry & Magniez, 1977; Lefebvre *et al.*, 2016
Chromosomes	26 pairs	Henry & Magniez, 1977
Diet	Primary consumer, organic detritus (50 %), microbial biofilm (38 %)	Ercoli *et al.*, 2019
Behaviour	Burrower when reared on clay	Henry & Magniez, 1977
Habitat preference	Mostly found in karstic water	Lefebvre *et al.*, 2016; this study

Figure 3: Biological and ecological knowledge of Gallasellus.

Gallasellus is a primary consumer feeding on organic detritus (50 %) and microbial biofilm (38 %). It is preyed upon by *Niphargus ladmiraulti* (Amphipoda). Observations

made during rearing showed that *Gallasellus* dug long galleries in clay. Mean water temperature at 22 occurrence sites was 13.1 °C (from 9.6 to 14.2 °C).

Distribution

Gallasellus was collected from a total of 32 sites belonging to four river catchments: Charente, Loire, Sèvre Niortaise and Dordogne (Fig. 4). Its presence in the Dordogne River is from a single collection which needs to be verified. Its range spans over a maximum linear extent of nearly 200 km. *Gallasellus* occurred at 23 % of sampled sites, but it has not yet been collected in unconsolidated rocks. In consolidated rocks, it occurred in 40, 30 and 20 % of sampled caves, springs and wells, respectively.

Diversity within Gallasellus

The delimitation of MOTUs using the COI gene-based threshold method as defined by LEFÉBURE *et al*. (2006) shows that the genus *Gallasellus* may contain 6 putative species (Fig. 4). Two of them are single-site species, whereas the other four have wider distributions spanning over a maximum linear extent of about 80-100 km. MOTUs were found to co-occur at three sites (Fig. 4).

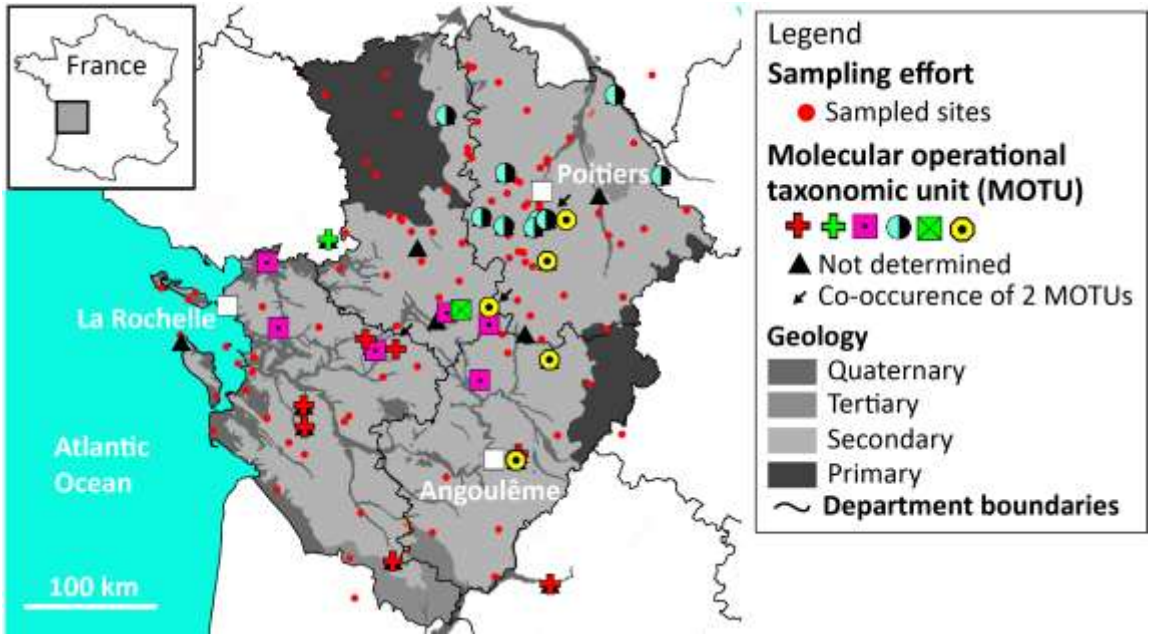

Figure 4: Distribution and diversity of Gallasellus in the Poitou-Charentes Region (France).

4. Discussions

Distribution

Increase in sampling effort resulted in a much wider known range of *Gallasellus*. Extensive sampling has indicated the genus is restricted to limestone aquifers. Unless *Gallasellus* has colonized hyporheic corridors of the Loire River catchment, its distribution should be constrained by the Armorican Massif to the North West and by the Central Massif to the South East. In the South, the Pyrenean region has been intensively sampled including by researchers of the CNRS laboratory of Moulis, but *Gallasellus* has never been found. A planned project dedicated to the study of the groundwater fauna of the Nouvelle-Aquitaine Region is intended to sample about 230 sites in south western France on a territory expanding over 58,000 km^2 (ALEZINE & LEFEBVRE, 2020). This project should help circumscribe the distribution and diversity of *Gallasellus*.

Biology and ecology

Knowledge of the biology and autecology of species is essential for conservation purposes. Contrary to many epigean asellids, *Gallasellus* is iteroparous, but its life span and life fecundity are not yet known. Obtaining such data would require long-term rearing. Rearing obligate-groundwater organisms is difficult, but optimal conditions for collecting *Gallasellus* specimens alive, transporting and rearing them were described in HENRY & MAGNIEZ (1977) and LEFEBVRE *et al.* (2016). Also, estimates of effective population size are not yet available, even though DELANGLE (2014) estimated the *census population size at the Fountain du Clos des Roches (*Roches-Prémarie-Andillé, Vienne*) to* 79 ± 7 individuals. Subterranean dispersal ways of *Gallasellus* remain to be elucidated. HENRY & MAGNIEZ (1977) suggested that the burrowing behaviour of *Gallasellus* might facilitate its dispersal along interstitial corridors. They further emphasized the importance of former alluvial corridors during Quaternary phases of low sea level for explaining the presence of *Gallasellus* in the Oléron Island (MAGNIEZ & HENRY, 2001). However, the genus has not yet been collected in unconsolidated rocks, suggesting limestone aquifers at greater depths might have contributed to its dispersal.

Organic matter detritus is an important component of the diet of *Gallasellus*. ERCOLI *et al*. (2019) proposed that the decrease in abundance of *Gallasellus* in the subterranean River of Bataillé might be due to wood removal operations. They suggested restoration efforts should be directed

towards reconnecting surface and subterranean ecosystems, including re-opening of sinkholes and traditional wells to promote inputs of decaying organic matter.

Diversity within Gallasellus

Increasing knowledge on the genus *Gallasellus* shows that it contains several species. The COI gene-based threshold method as defined by LEFÉBURE *et al.* (2006) is highly conservative in that it identifies highly divergent evolutionary units. However, the number of MOTUs is sensitive to sampling effort. Hence, MORVAN *et al*. (2013), EME *et al*. (2018) and the present study successively revealed 3, 4 and 6 MOTUs. A next important step consists in diagnosing and describing these MOTUs based on molecular and morphological criteria.

5. Conclusion

Increasing knowledge on the genus *Gallasellus* has important conservation implications. What has long been considered as a single-site monotypic genus is a potentially species-rich genus containing a mix of wide- and narrow-range species. Future sampling studies (ALEZINE & LEFEBVRE, 2020) will not only enable to direct conservation efforts to protect the diversity of *Gallasellus*, but they will also continue to reveal the unexpected diversity of groundwater fauna in south western France.

Acknowledgments

We gratefully thank all cavers for their help in collecting animals (especially members of the Spéléo-Club Poitevin), and members of France Nature Environnement in Nouvelle-Aquitaine (especially Poitou-Charentes Nature and Vienne Nature associations). We also thank Lara KONECNY-DUPRÉ for making the molecular work, Nathanaëlle SACLIER for delimiting MOTUs, Julian LEWIS for correcting the English and two anonymous reviewers for their comments.

References

ALEZINE T., LEFEBVRE F. (2020) Inventaire de la faune aquatique souterraine & Qualité des milieux en Nouvelle-Aquitaine (rapport de faisabilité) Ed. SEPANSO, Bordeaux, 43 p. (hal-02974506)

DELANGLE M. (2014) Écologie et biologie des populations de Gallaselle(s) en Poitou-Charentes. Ms Thesis, université de Poitiers, 72 p.

EME D., ZAGMAJSTER M., DELIĆ T., FIŠER C., FLOT J.-F., KONECNY-DUPRÉ L., PÁLSSON S., STOCH F., ZAKŠEK V., DOUADY C. J., MALARD F. (2018) Do cryptic species matter in macroecology? Sequencing European groundwater crustaceans yields smaller ranges but does not challenge biodiversity determinants. Ecography, 41, 424-436.

ERCOLI F., LEFEBVRE F., DELANGLE M., GODÉ N., CAILLON M., RAIMOND R., SOUTY-GROSSET C. (2019) Differing trophic niches of three French stygobionts and their implications for conservation of endemic stygofauna. Aquatic Conservation, 29, 2193-2203.

HENRY J.-P., MAGNIEZ G. (1970) Contribution à la systématique des Asellides (Crustacea Isopoda). Annales de Spéléologie, 25(2), 335-367.

HENRY J.-P., MAGNIEZ G. (1977) Observations sur *Gallasellus heilyi* (Legrand, 1956), représentant d'un nouveau genre d'asellide souterrain. Bulletin de la Société Zoologique de France, 102(2), 215-222.

LEFÉBURE T., DOUADY C. J., GOUY M., GIBERT J. (2006) Relationship between morphological taxonomy and molecular divergence within Crustacea: proposal of a molecular threshold to help species delimitation. Molecular Phylogenetics and Evolution, 40, 435-447.

LEFEBVRE F., FILLON B., GAILLEDRAT M. (2016) Étude et protection des gallaselles et de leurs habitats aquatiques souterrains en Poitou-Charentes. Ed. Poitou-Charentes Nature, Fontaine-le-Comte, 90 p. (hal-01350900)

LEGRAND J.-J. (1956) Contribution à l'étude de la faune cavernicole de l'ouest de la France. II. *Asellus Heilyi*, n. sp. Notes Biospéologiques, 11, 43-51.

MAGNIEZ G., HENRY J.-P. (2001) Présence d'un Asellide stygobie dans une île : causes et conséquences. Mémoires de Biospéologie, 28, 143-147.

MORVAN C., MALARD F., PARADIS E., LEFÉBURE T., KONECNY-DUPRE L., DOUADY C. J. (2013) Timetree of Aselloidea reveals species diversification dynamics in groundwater. Systematic Biology, 62(4), 512-522.

WILLIAMS W. D. (1970) A revision of North American epigean species of *Asellus* (Crustacea, Isopoda). Smithsonian Contributions to Zoology, 49, 1-80.