

9th International Symposium on Terrestrial Isopod Biology

ISTIB 2014



26-30 June 2014 - Poitiers - France

University of Poitiers
Laboratory Ecology, Biology of Interactions - UMR CNRS 7267

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PROGRAMME

Thursday, June 26th

14:00-18:00 Registration

18:00-21:00 Welcome reception and cocktail party

Friday, June 27th

8:00-8:30 Registration

8:30-8:45 Opening ceremony

INTRODUCTORY TALK

8:45-9:15 HORNUNG E. - Tribute to Prof. M.R. Warburg and to his contribution to terrestrial isopod biology

Session SYSTEMATICS AND BIOGEOGRAPHY

Chairperson : S. TAITI

9:15-9:35 SFENTHOURAKIS, S. - Patterns of taxonomic diversity among terrestrial isopods

9:35-9:55 KAMILARI, M. - Congruence and conflicts in Greek *Trachelipus* species; implications for taxonomy, phylogeny and biogeography

9:55-10:15 KASHANI, G. - Recent findings on terrestrial isopods from Iran

10:15-10:45 : Coffee break

Chairperson : E. HORNUNG

10:45-11:05 SFENTHOURAKIS, S. - The diversification of isopods in Cyprus

11:05-11:25 ARAUJO, P. B. - Taxonomic revision of *Benthana Budde-Lund*, 1908 (Philosciidae)

11:25-11:45 KHEMAISSIA, H. - Biogeography of terrestrial isopods from Tunisian wetlands

11:45-12:05 TAITI, S. - Cave-dwelling terrestrial isopods from karst areas of Portugal

12:05-14:00 : Lunch

Session MORPHOLOGY AND PHYSIOLOGY

Chairperson : P. ARAUJO

- 14:00-14:20 SUSTR, V. - Autofluorescence of the body surface of cave isopod *Mesoniscus graniger*
- 14:20-14:40 ZIEGLER, A. - Ultrastructure and mineral phase distribution in the transparent cornea cuticle of the complex eye of *Ligia oceanica*
- 14:40-15:00 HORVATHOVA, T. - Effects of thermal and oxygen conditions on early development and growth in the terrestrial isopod *Porcellio scaber*
- 15:00-15:20 CSONKA, D. - Eco-morphological comparison on the marsupium of terrestrial isopods (Crustacea, Isopoda)

15:20-15:50 : Coffee break

Chairperson : M. ZIMMER

- 15:50-16:10 GRÈVE, G. - *Wolbachia*-induced feminization of the terrestrial isopod *Armadillidium vulgare* versus the masculinizing role of the androgenic hormone.
- 16:10-16:30 BADAWI, M. - Effect of the feminizing *Wolbachia* endosymbiont after a cross-species transfer among terrestrial isopods
- 16:30-16:50 GENTY, L. M. - A bug may hide another: cryptic *Wolbachia* in unfeminized lineages of *Armadillidium vulgare*

16:50-19:00 : Poster session and social hour (local foods and wines)

20:00-23:00 : Gala Dinner

Saturday, June 28th

Session EVOLUTIONARY BIOLOGY SESSION

Chairperson : R. KOSTANJSEK

- 8:30-8:50 DITTMER J. - The microbiota as a bacterial passport? Metagenomic insights from *Armadillidium vulgare*
- 8:50-9:10 BOUCHON, D. - *Rickettsiella* bacteria in terrestrial isopods: killers or helpers?
- 9:10-9:30 GILBERT, C. - Remarkable diversity of endogenous viruses in a crustacean genome

9:30-10:20 : Poster session (Coffee break)

Chairperson : A. ZIEGLER

- 10:20-10:40 DURAND, S. - Females don't choose the paternity of their offspring based on male genetic heterozygosity or dissimilarity to them in *Armadillidium vulgare*
- 10:40-11:00 VALETTE, V. - Influence of *Wolbachia* infections on multi paternity in *Armadillidium vulgare*
- 11:00-11:20 ACHOURI, M. S. - Genetic analysis of intraspecific variability in *Porcellionides pruinosus* (Brandt, 1833) from Tunisia
- 11:20-11:40 HARDY, C. - Evolutionary history of terrestrial isopods: Phylogenetic, Morphometric and Ecological approaches

11:40-13:00 : Lunch

13:00-19:30 : Excursion to Marais Poitevin

19:30-21:00 : Dinner

21:00-22:30 : Session grand public: Biodiversité et Agriculture

Sunday, June 29th

Session ECOLOGY AND ECOTOXICOLOGY Chairperson : B. LOMBARDO

- 8:30-8:50 CHARFI, F. - Terrestrial isopods in Tunisia after 20 years
- 8:50-9:10 NASRI-AMMAR, K . - Diversity of terrestrial Isopods from North Tunisian dorsal wetlands
- 9:10-9:30 TAJOVSKÝ, K. - Terrestrial isopods in alder fens of montane zone of the Outer Flysh Carpathians
- 9:30-9:50 DIXIE, B. - Effects of microclimate on behavioural and life history traits of terrestrial isopods : Implications for responses to climate change
- 9:50-10:10 GHEMARI, C. - Metal bioaccumulation in two species of Oniscideans: *Porcellio laevis* and *Porcellionides pruinosus* from Tunisian contaminated sites

10:10-10:40 : Coffee break

Chairperson : S. SFENTHOURAKIS

- 10:40-11:00 ZIMMER, M. - Relationship between genetic distance and stability of soil processes in microcosm communities of isopods and diplopods
- 11:00-11:20 HORNING, E. - Reproductive strategies of sympatric isopod species (Crustacea, Isopoda, Oniscidea)
- 11:20-11:40 MEDINI-BOUAZIZ, L. - Reproductive traits of *Porcellio variabilis* Lucas, 1946 (Isopoda, Oniscidea) from Tunisia
- 11:40-12:00 RICHARD, F.J. - Sexual affinity in terrestrial isopods: what does matter?

12:00-14:00 : Lunch

Chairperson : K. NASRI

- 14:00-14:20 ZAARAOUI, H. - Effects of disturbance on the diversity and abundance of terrestrial isopods in arid regions in Tunisia
- 14:20-14:40 BROLY, P. - Spatial heterogeneities, self-organization process, and aggregation behaviour in *Porcellio scaber*

- 14:40-15:00 HAMAIED, S. - Diversity and spatial distribution of Oniscidea in the National Park of Feija (N-W of Tunisia)
- 15:00-15:20 TUF, I.H. - Is defensive behaviour of woodlouse affected by its personality?
- 15:20-15:40 HASSALL, M. - What is the optimal group size for resting isopods ?

15:40-17:10 : Poster session (Coffee break)

17:10 - 18:00 : Closing ceremony
Discussions
Communication and poster awards
Biodiversity photography award

19:00 - 21:00 : Dinner

Monday, June 30th

Departure

INTRODUCTORY TALK
HORNUNG E.

Tribute to Prof. M.R. Warburg and to his contribution to
terrestrial isopod biology



<http://biology.technion.ac.il/>

Michael R. Warburg
professor emeritus

(May 31, 1931 Berlin, Germany – February 9, 2014 Haifa, Israel)

We have got the very shocking and sad news that Prof. Michael Warburg passed away on the 9th of February in Haifa.

He was one of the charismatic personalities and ‘spiritual sponsor’ of Isopodology for a long time. His first papers on Isopods were the results of his PhD work while he stayed as a student of the famous Prof. G.E. Hutchinson at Yale University (USA).

During his scientific career he was interested in different animal groups (ticks, scorpions, amphibians, reptiles besides terrestrial isopods) but there are common keywords that summarize his area of interest. The most important ones might be diversity, distribution, ecophysiology, reproductive systems and strategies. He wrote altogether more than 180 papers, over 75 abstracts, 2 books and was the co-editor of a Symposium Volume (from 1997, Haifa, Israel).

He was scientifically active till the last moments. Since his retirement, as a professor emeritus, he tried to summarize his results and share it with the scientific community. During the last 13 years he managed to write a series of accepted papers, among them 14 (partial) reviews on his favorite taxa. The idea of organizing the first symposium devoted to Isopods (London, 1984) originates from Prof. Warburg. The idea developed into a series of symposia, the newest one is expected to attract the isopodologists’ crowd to Poitiers this summer. It could be a worthy tribute to dedicate this meeting to Professor Michel Warburg’s memory.

Dr. Elisabeth Hornung, *professor of Ecology*
Budapest/Hungary, February 15. 2014.

Systematics and Biogeography

Chairpersons : S. TAITI, E. HORNING

Patterns of taxonomic diversity among terrestrial isopods

Sfenthourakis S.¹ and Taiti S.²

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²*Istituto per lo Studio degli Ecosistemi, Consiglio Nazionale delle Ricerche, Via Madonna del Piano 10, I-50019 Sesto Fiorentino, Florence, Italy, e-mail: stefano.taiti@ise.cnr.it*

The publication of the world catalog of terrestrial isopods some ten years ago by Schmalzfuss has facilitated research on isopod diversity patterns at a global scale. Furthermore, even though we still lack a comprehensive and robust phylogeny of Oniscidea, we do have some useful approaches to phylogenetic relationships among major clades which can offer additional insights into isopod evolutionary dynamics. Taxonomic diversity is one of many approaches to ‘biodiversity’ and, despite its sensitiveness to biases in taxonomic practice, has proved useful in exploring diversification dynamics of various taxa. In the present work, we attempt an analysis of taxonomic diversity patterns among Oniscidea based on an updated world list of species containing 3,710 species belonging to 527 genera and 37 families (data till April 2014). The analysis explores species diversity at the genus and family level, as well as the relationships between species per genera, species per families, and genera per families. In addition, we consider the structure of isopod taxonomic system under the ‘fractal’ perspective that has been proposed as a measure of a taxon’s diversification. Finally, we check whether there is any phylogenetic signal behind taxonomic diversity patterns. The results can be useful in a more detailed elaboration of Oniscidea systematics.

Keywords: global diversity, taxonomic diversity, systematics, fractal, phylogeny, species richness

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Congruence and conflicts in Greek *Trachelipus* species; implications for taxonomy, phylogeny and biogeography

Kamilari M.¹, Klossa-Kilia E.¹, Kiliass G.² and Sfenthourakis S.³

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The genus *Trachelipus* is comprised of stenoecious animals living in humid habitats, such as dense forests and around inland waters, which are generally threatened by human activities and climatic change. Species-level taxonomy within the genus has been based mainly on a few secondary sexual characters of males plus a restricted number of ambiguous somatic characters, which do not provide adequate taxonomic resolution.

Herein, we provide a comprehensive approach to the phylogenetic relationships and the genetic structure of more than 100 populations of endemic and European *Trachelipus* populations from both mainland and insular Greece (i.e. Greek endemics: *T. palustris*, *T. aegaeus*, *T. cavaticus*, *T. nsp*; European species: *T. camerani*, *T. squamuliger*, *T. rathkii*, *T. ratzeburgii*, *T. arcuatus*). We employ one nuclear (sodium-potassium ATPase α -subunit) and two mtDNA markers (COI and 16S rRNA), aiming to identify possible geographic correlation to their divergence and to evaluate the consequences of habitat fragmentation on the genetic structure of these hygrophilous animals.

Our results reveal incongruence with current taxonomy. Furthermore, the geographic patterns exhibited for some species (e.g. *T. aegaeus*) corroborate the established palaeoevents in the Hellenic region, whereas in other cases (e.g. *T. palustris*) are incongruent with known palaeogeography. The occurrence of more than one clade in mainland Greece raises the possibility of repeated episodes of clade expansion/contraction during Pleistocene glaciations. In any case, it is evident that in these relatively sedentary animals, habitat fragmentation promotes divergence at a very high rate.

Keywords: *phylogeography, genetic divergence, habitat fragmentation, evolution*

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Recent findings on terrestrial isopods from Iran

Kashani G. M.

Department of Biology, Faculty of Science, University of Zanjan, Zanjan, Iran

Iran is a big country containing many diverse habitats that can be potentially occupied by a huge number of terrestrial isopod species adapting in different conditions. The occurrence of both *Ligia* sp. (a supralittoral inhabitant) and *Hemilepistus* spp. (living in the arid areas) demonstrates the high capacity of this country for providing appropriate conditions for a wide range of terrestrial isopods. However, knowledge on terrestrial isopod fauna of this country is relatively poor. Most of the previous studies are only reporting the presence of certain species in Iran. The first species were reported by Budde-Lund in 1885, but by 2010, only 19 species had been reported from Iran, belonging to seven families and 11 genera. During the few recent years, the published and unpublished studies performed mainly by the author have added a dozen species to this number, so that the presence of more than 40 species in 28 genera and 13 families has been documented. Many parts of Iran are still uninvestigated and several new species are waiting to be described. Obviously, even these primary findings have dramatically increased our knowledge on the biodiversity and biogeography of these creatures in the region. It is anticipated that the terrestrial isopod fauna of Iran will reveal many interesting discoveries that will trigger studies also on other biological aspects of this taxon.

Keywords: Oniscidea, terrestrial isopods, taxonomy, Iran.

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The diversification of isopods in Cyprus

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Cyprus is an isolated island that is considered as ‘oceanic’ since, according to many authors, it was never connected to any mainland. Other authors assume a possible mainland connection during the Messinian Salinity Crisis, from ca. 5.6 to 5.3Ma. This topic being under debate, the fact is that the fauna of Cyprus is today a mixture of very old clades, with an age of several million years, and younger ones that managed to disperse to the island at more recent times. The isopod fauna of Cyprus is insufficiently known, with 24 species previously reported from the island. Intensive collecting during the past two years by the authors revealed the actual occurrence of at least 40 species, several of them new to science. In this work, we present results on a phylogeographic analysis of some isopod clades from Cyprus, including populations of the same or closely related taxa from adjacent mainland areas. We have applied cladochronology methods to estimate possible dates of sub-clade divergence, so as to identify the period when species and populations of Cyprus started to diverge from their mainland ancestors. We shall present a comparative account of several different species groups, discussing also similar published estimates from other taxa.

Keywords: phylogeography, molecular clock, genetic distance, geographic isolation, phylogeny

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Taxonomic revision of *Benthana* Budde-Lund, 1908 (Philosciidae)

Campos-Filho I.S.¹, Taiti S.² and Araujo, P.B.¹

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The family Philosciidae includes about 110 genera widely distributed in Australia, Southern Asia, Africa, Europe and Americas. The genus *Benthana* occurs in South America and includes 26 species plus two dubious species from Chile. The species in the genus are characterized by the presence of a lateral process on the male pleopod 1 exopod, the so-called dentiform protrusion. The aim of this study is to revisit the taxonomy of *Benthana* species, providing redescriptions or additional characters for the already known species and to describe new ones. Species used in this work were collected during expeditions carried out in the Brazilian states of Bahia, Minas Gerais, Espírito Santo, Rio de Janeiro, São Paulo, Paraná, Santa Catarina and Rio Grande do Sul. Additional material from collections of MZUSP, MNRJ was also examined. Fourteen species were redescribed: *Benthana albomaginata*, *B. bocainensis*, *B. dimorpha*, *B. bocainensis*, *B. iporangensis*, *B. longicaudata*, *B. longicornis*, *B. longipenis*, *B. moreirai*, *B. olfersii*, *B. picta*, *B. santosi*, *B. schubarti*, *B. sulcata* and *B. wernerii*; additional characters were described for six species: *B. araucariana*, *B. cairensis*, *B. convexa*, *B. taeniata*, *B. trinodulata* and *B. serrana*. In the material examined four species were recognized as new: two from the state of Espírito Santo, one from Bahia and one from Minas Gerais. Additionally, a pictorial key and distribution maps for the species are provided.

Keywords: Taxonomy, Neotropics, Atlantic Forest, Philosciidae, Benthana

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Biogeography of terrestrial Isopods from Tunisian wetlands

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In Tunisia, while wetlands are considered as remarkable habitats for their faunal and floral diversity, few studies on the biogeography and the diversity of terrestrial isopods were performed. To fill this gap, we carried out a field study in the supralittoral zone of 146 Tunisian wetlands belonging to eight types (rivers, lagoons, lakes, hill reservoirs, dams, chotts, beaches and sebkhas) and to five bioclimatic zones (humid, sub-humid, semi-arid, arid and saharian). Field work was done in April 2009-2010. Terrestrial isopods were collected in the morning by hand search with the same sampling effort each time.

During the study, 22 species of terrestrial isopods were collected belonging to nine families. The identified species belonged to different biogeographical categories: Mediterranean, Mediterranean-Atlantic, North Africa and circum-Sicilian islands, semi-arid and Saharan species. Some of these species are endemic to Tunisia or to North Africa.

Higher isopod abundance is observed both in the sub-humid and the semi-arid bioclimatic zones. From the northern to the southern regions, a latitudinal gradient in species richness had been shown: a gradual decrease of species richness towards the north is consistent both in total species number and in species number within species-rich families.

Porcellio variabilis (Ip = 41 %), *Porcellio laevis* (Ip = 32.8 %), *Chaetophiloscia elongata* (Ip = 18.4 %) and *Armadillidium pelagicum* (Ip = 17.8 %) are the most common species identified.

Keywords: Isopod fauna, Biogeography, Biodiversity, Wetlands, species richness

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Cave-dwelling terrestrial isopods from karst areas of Portugal

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The study of subterranean Oniscidea in Portugal has been neglected for almost seventy years. Only six troglobiotic species were previously known from karst caves. Recent investigations have been carried out in caves located in all the karst areas of mainland Portugal, i.e. Vimioso, Outil-Cantanhede, Serra da Boa Viagem, Sicó, Estremenho, Montejunto, Cesaredas, Cascais, Arrábida, Estremoz-Cano, Adiça-Ficalho and Algarve. This research has revealed new patterns of distribution and diversity for this group. The total number of oniscidean species known from Portuguese caves is now 29, of which 17 can be considered as troglobionts. One new genus and eight new species of Trichoniscidae have been discovered. The family Trichoniscidae with 15 species and the genus Trichoniscoides with eight species are the most diverse taxa. The family Porcellionidae has one troglobiotic species, *Porcellio cavernicolus*, previously known only from Sicó massif, but now found also in caves of the karst areas of Outil-Cantanhede and Mealhada, north of Mondego river, and in the Tomar region, largely increasing its distribution. *Trogeluma* with type-species *T. machadoi*, previously considered to be a subgenus of *Troglarmadillidium*, is erected to genus level. The entire oniscidean cavernicolous fauna from mainland Portugal is listed and mapped, including new localities. Biodiversity patterns for subterranean oniscidean isopods in Portugal are discussed. The richness of terrestrial isopods in the subterranean ecosystems of Portugal, and by extension of the Iberian Peninsula, requires a consistent sampling effort to increase our knowledge on this group of arthropods which, at the moment, includes the largest number of troglobiotic species.

Keywords: Oniscidea, troglobionts, distribution, mainland Portugal

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Morphology and Physiology

Chairpersons : P. ARAUJO, M. ZIMMER

Autofluorescence of the body surface of cave isopod *Mesoniscus graniger*

Šustr V.² and Giurginca A.¹

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The auto-fluorescence of the body surface of the cave isopod *Mesoniscus graniger* was found during analysis of the content of digestive tract of this animal. *Mesoniscus graniger* body surface showed blue auto-fluorescence under UV light (330-385 nm) using epi-fluorescence microscopy. Some morphological structures shine much more brightly than the remaining parts of the body.

This auto-fluorescence was confirmed in the living individuals using UV excitation light at 365 nm in the laboratory as well as in the field conditions using portable UV lamp. Field video-documentation was recorded in caves of Slovak Karst.

Preliminary spectroscopic measurements showed that the auto-fluorescence observed from living individuals of *M. graniger* may be due to some β -carboline or coumarine derivatives, by some reticulated structures, dityrosine or due to another compounds showing similar excitation - emission characteristics.

The presence of UV autofluorescence in other isopods, the possible adaptive significance of this phenomenon and using of the UV light in biospeleology were discussed.

Keywords: Mesoniscus graniger, fluorescence, UV, biospeleology

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Ultrastructure and mineral phase distribution in the transparent cornea cuticle of the complex eye of *Ligia oceanica*

Alagboso F. I.¹, Reisecker Ch.², Ruangchai S.¹, Hild S.² and Ziegler A.¹

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The cornea cuticle within the crustacean complex eyes is an interesting example of an exoskeleton with a bimodal function compromising between mechanical strength and transparency. The cornea forms arrays of microlenses that are part of the dioptric apparatus of each ommatidia. Using transmission and field-emission scanning electron-microscopy, EDX-analysis and Raman spectroscopic imaging we studied the ultrastructure and mineral phase distribution within the cornea of *Ligia oceanica*. Gradual thinning of the cuticle towards the interommatidial region rather than thickening of the central region brings about the shape of the microlenses, however, also a reduction of the mechanical strength. In species with a thin cuticle this limits the lens curvature and thus refractive power. In *L. oceanica* this is partly compensated by an unusual differential mineral distribution within the endocuticle. The ultrastructure of the cornea cuticle has significant differences from the cuticle of the surrounding head capsule or tergites. The epicuticle is much thicker and the distal layer of calcite much thinner than in the surrounding head capsule. Pore canals that play an important role for the mechanical properties of the cuticle, are restricted to regions between the ommatidia. Preliminary results on *Tylos europaeus* and *Helleria brevicornis* show that polyhedral structures of Mg-calcite are limited to the inter-ommatidial region as well. These adaptations likely minimise light scattering and/or improve light transmission. In the proximal region of the exocuticle, fibres form a peculiar reticulate structure around mineral filled canals probably improving the mechanical properties.

(Supported by the DFG within the priority programme SPP1420).

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Effects of thermal and oxygen conditions on early development and growth in the terrestrial isopod *Porcellio scaber*

Horváthová T., Antol, A., Kramarz P., Labecka A. M., Bauchinger U., Czarnoleski M. and Kozłowski J.

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Ectotherms that follow the so-called temperature-size rule (TSR) grow slowly in low temperatures, but they reach maturation later and consequently at a bigger size. This phenotypically plastic response is considered one of the most taxonomically widespread rules among ectotherms. Factors that govern this type of response to temperature are not fully understood because thermal conditions in the environment correlate with many other factors such as humidity and oxygen availability. We investigate the effect of temperature and oxygen concentration on life-history traits of terrestrial isopod species *Porcellio scaber*. Adult males and females were placed for mating to either 15°C or 22°C, at two oxygen levels (normoxia 22% and hypoxia 10% O₂). We recorded the rate at which gravid females appeared in each treatment and then we followed the growth of their progeny. We found that the length of marsupial development was significantly shorter in cold treatments, and over the first nine weeks of non-marsupial development, juveniles grew faster in warm than cold treatments. Oxygen conditions had no effect on development and growth. Our results suggest that the development and growth of early stages of *Porcellio scaber* is primarily affected by temperature and not by oxygen conditions.

Keywords: temperature-size rule, oxygen, life-history traits, juvenile Porcellio scaber

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Eco-morphological comparison on the marsupium of terrestrial isopods (Crustacea, Isopoda, Oniscidea)

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The brood pouch (marsupium) appears in the superorder Pericarida within Malacostraca (Crustacea). While in aquatic forms the marsupium serves only for mechanical protection of eggs and developing embryos, under terrestrial conditions it has evolved for providing - besides protection -, nutrition and optimal conditions for embryogenesis. Within the marsupium covered by oostegites, we can recognize fingerlike cotyledons which supply eggs and embryos with nutritive fluids. We have studied the morphology of marsupium in different isopod species. We compared species of different eco-morphological types (Schmalfuss, 1984). We used four different species: the cosmopolitan *Armadillidium vulgare* and the common *Cylisticus convexus* are conglobating ('roller') species; the habitat generalist *Trachelipus rathkii* and *Porcellium collicola* are non-conglobating ('clinger') species. Light microscopic studies have shown some similarities among the main structure of the species' brood pouch: in conglobating species (*A. vulgare*, *C. convexus*) the oostegites bend only slightly and the sternites arch into the body cavity to provide more space for the embryos. This leads to a displacement of the female's internal organs. In the non-conglobating forms the oostegites bend outwards when the growing embryos require more space. Our findings reconfirmed the results of Appel et al. (2011). The ultrastructure of cotyledons (TEM) showed the presence of secretory tissue. We plan to compare other species' marsupium related to different eco-morphological types and phylogenetic positions.

Keywords: marsupium, light microscopy, eco-morphology

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***Wolbachia*-induced feminization of the terrestrial isopod *Armadillidium vulgare* versus the masculinizing role of the androgenic hormone**

Cerveau N.¹, Saint-Jean A.¹, Bertaux J.¹, Geniez S.^{1,2}, Moumen B.¹, Kumar S.², Bouchon D.¹, Foster J.², Slatko B.E.² and Grève P.¹

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The sequence of the androgenic hormone (AH) was deciphered in the isopod *Armadillidium vulgare* more than 40 years after the discovery of the androgenic gland (AG) by Charniaux-Coton in 1954. This hormone is responsible for the differentiation of primary and secondary male sexual characters and might be the target of the feminizing α -proteobacteria *Wolbachia* which infect numerous isopod species. We examined AH gene expression during post embryonic *A. vulgare* development and showed that this gene is expressed before AG differentiation. We then amplified 12 new AH cDNAs in species belonging to five different families of the infra-order Ligiamorpha of terrestrial isopods. The phylogenetic relationships of AH sequences allowed to distinguish two main clades corresponding to members of the *Armadillidiidae* and the *Porcellionidae* families which are congruent with the narrow specificity of AG heterospecific grafting. We also sequenced the genome of the wVulC *Wolbachia* strain infecting *A. vulgare* and identified multiple secretion/export systems and putative effectors that might be involved in host/*Wolbachia* interactions. We then sequenced six new strains of *Wolbachia* with Next Generation Sequencing technologies that induce either host feminization or cytoplasmic incompatibility in terrestrial isopods. Comparative genomics were then performed to establish a pan-genome of *Wolbachia* and phylogenomics studies. We finally identified eukaryote-like proteins such as ankyrin repeats containing proteins that are known to be involved in host-symbiont interactions, including in bacterial virulence.

Keywords: Crustacean isopod, androgenic hormone, sex determination, symbiosis, *Wolbachia*

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Effect of the feminizing *Wolbachia* endosymbiont after a cross-species transfer among terrestrial isopods

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Sex determination in terrestrial isopods can be altered by the endosymbiont *Wolbachia*. Indeed, this intracellular alpha-proteobacterium is able to induce feminization of genetic males into functional females. Currently, no molecular genetic basis of feminization has been described. The isopod *Armadillidium vulgare* that harbours the feminizing strain wVulC offers an ideal ground to study feminization. Indeed, as it is undifferentiated at birth, we can study the feminization timing during development. Moreover, the effect of *Wolbachia* can be maintained during cross-transfection studies in a heterologous host. Thus, a cross-species transfection in a host with a different sexual differentiation timing will allow us to distinguish the effect of *Wolbachia* due to sexual differentiation from that due to development. This study aims to determine whether the feminizing strain wVulC of *A. vulgare* can also feminize the isopod host *Cylisticus convexus* following artificial transfection. First, as sexual differentiation timing is unknown in *C. convexus*, we described gonad morphological differentiation and reported an earlier differentiation timing compared to that of *A. vulgare*. Then, we injected wVulC in adult *C. convexus* males and observed the development of female sexual characters. Finally, we reported numerous intersexes and a sex-ratio bias toward females in the progenies of transfected females. These results collectively demonstrate the feminizing effect of wVulC in *C. convexus*. The stable establishment of the feminizing wVulC *Wolbachia* in a heterologous host now opens the avenue to comparative approaches to decipher the molecular processes of feminization in isopods. This work is funded by an ERC grant (EndoSexDet) to RC, which aims to identify the genetic factors implicated in the sex determination of *A. vulgare*.

Keywords: sex-ratio bias, intersexes, sexual differentiation, gonad morphology, transfection

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A bug may hide another: cryptic *Wolbachia* in unfeminized lineages of *Armadillidium vulgare*

Genty L.-M., Raimond M., Bouchon D. and Bertaux J.

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Wolbachia are widespread endosymbionts of arthropods and nematods. Among the *Wolbachia*-host symbioses a growing number of unexpected low titer infections has been described in bark beetles, *Drosophila paulistorum* complex of species, aphids, *D. simulans*, tse-tse fly and cherry fly. For 30 years our laboratory has bred distinct lineages of the terrestrial isopod *Armadillidium vulgare*, steadily infected or uninfected with *Wolbachia*. However, using Fluorescence *In Situ* Hybridization (FISH) and nested PCR, we showed that females from laboratory lineages regarded as uninfected were actually infected with cryptic *Wolbachia*. While *Wolbachia* have a feminizing effect in *A. vulgare*, the crypto-infected lineages have an equilibrated sex ratio. Moreover, *Wolbachia* remain undetected with PCR and TEM, the former standard methods to discriminate between infected and uninfected lineages.

Even more surprisingly, we also discovered a serendipitous crypto-*Wolbachia* in males of infected lineages, whereas they are considered as the individuals who escaped from the infection. In laboratory lineages both infected with *Wolbachia* and crypto-infected males, presented an opposite pattern of infection compared to crypto-infected females, with a huge infection in gonads and a low infection in the nerve chord. Moreover, we observed that *Wolbachia* infection pattern in the male gonads depends on the physiological cycle of the testis. Finally, we put in relation *Wolbachia* and crypto-*Wolbachia* localization patterns with the host phenotype, under the hypothesis that *Wolbachia* presence in some specific cells of the nerve chord is required to feminize woodlice.

Keywords: FISH, feminization, gonads, cryptic infection, *Wolbachia*

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Evolutionary Biology Session

Chairpersons : R. KOSTANJSEK, A. ZIEGLER

The microbiota as a bacterial passport? Metagenomic insights from *Armadillidium vulgare*

Dittmer J., Lesobre J. and Bouchon D.

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Symbiosis research has recently experienced a shift of focus, leading to the perception of a host as a ‘holobiont’, i.e. an individual harbouring and interacting with a diverse bacterial community, the microbiota. Terrestrial isopods represent an excellent new model system for multipartite animal-bacteria symbioses, particularly due to their well-characterized association with the endosymbiotic reproductive parasite *Wolbachia*. To date, three different feminizing *Wolbachia* strains have been identified in *Armadillidium vulgare*, presumably representing different co-evolutionary histories. However, the terrestrial isopod microbiome has never been analysed on a large scale, despite the fact that several other bacteria have been detected in several species. In order to fill this gap, 16S rRNA gene amplicon pyrosequencing was used to characterize the microbiota of *A. vulgare* on multiple levels: (i) In field vs. lab populations, (ii) in different host tissues, and (iii) depending on *Wolbachia* infection status. We demonstrate that individuals from different populations harboured distinct bacterial communities, which might be related to terrestrial isopod nutritional ecology. Moreover, *Wolbachia* represented the predominant member of the bacterial community in infected individuals, indicating that *Wolbachia* plays an important role within the terrestrial isopod microbiome. However, *Wolbachia* was not the only major player: *Candidatus Hepatoplasma crinochetorum*, a facultative symbiont previously reported from the midgut caeca, was for the first time observed to be highly abundant in all tested host tissues. The potential interactions of *Wolbachia* and *Ca. H. crinochetorum* constitute an interesting example for symbiont-symbiont relationships between two highly abundant members of a diverse bacterial community.

Keywords: Symbiosis, Bacterial Community, Wolbachia, Hepatoplasma

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***Rickettsiella* bacteria in isopods: killers or helpers?**

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Gammaproteobacteria of the genus *Rickettsiella* are well known as pathogenic bacteria in many arthropods (Bouchon et al. 2011). In aphids, they were recently expected to influence prey-predator interactions (Tsuchida et al. 2010) or to protect against a fungal pathogen (Lukasik et al. 2012), conferring benefits to their hosts. The wide distribution of *Rickettsiella* species and their various host taxa suggest a high infectivity and adaptability, which may be facilitated by their environmental transmission. They have been identified in several woodlice belonging to different genera such as *Armadillidium* sp., *Porcellio* sp., *Oniscus* sp., *Phyloscia* sp., *Asellus* sp., *Armadillo* sp. *Rickettsiella* from terrestrial isopods have been previously identified as *R. armadillidii*. According to the currently accepted taxonomy, three main *Rickettsiella* species are recognized, however species delineation and relationships are controversial (Cordaux et al. 2007; Bouchon et al. 2011).

Using the recently proposed Multilocus Sequence Typing (MLST) approach (Leclerque & Kleespies 2012), we investigated the phylogenetic relationships of *Rickettsiella* bacteria isolated from woodlice of different origins: *A. vulgare* and *A. nasatum* were found in sympatry in a grassland of Poitou-Charentes (France), the neotropical Brazilian woodlice species, *Circoniscus gagei* from Belém (PA) and *Atlantoscia floridana* as well as the introduced species *Porcellionides pruinosus*, collected in Porto Alegre (RS) (Brazil). Phylogenetic reconstructions were congruent in placing all *Rickettsiella* bacteria in a single clade. Within this clade, a subclade comprising *Rickettsiella* strains isolated from insects was found in all trees, while strains from terrestrial isopods formed deeper-branching clusters. Interestingly, results suggested that different *Rickettsiella* strains were present in asymptomatic *A. vulgare* compared to animals presenting clear symptoms of infection. Moreover, *Rickettsiella* found in the invasive species *P. pruinosus* were identical to bacteria found in the Palearctic woodlice whereas bacteria found in the Neotropical species were genetically distant. Our results suggest a high degree of *Rickettsiella* diversity in terrestrial isopods and that some genotypes present no signs of pathogenicity as shown in ticks (Kurtti et al. 2002, Tsuchida et al. 2013).

Keywords: *Rickettsiella*, bacterial pathogens, symbiotic interactions

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Remarkable diversity of endogenous viruses in a crustacean genome

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Recent studies in paleovirology have uncovered myriads of endogenous viral elements (EVEs) integrated in the genome of their eukaryotic hosts. These fragments result from endogenization, i.e., integration of the viral genome into the host germline genome followed by vertical inheritance. So far, most studies have used a virus-centered approach, whereby endogenous copies of a particular group of viruses were searched in all available sequenced genomes. Here we follow a host-centered approach whereby the genome of a given species is comprehensively screened for the presence of EVEs using all available viral sequences as queries. Our analyses revealed that 56 EVEs corresponding to 11 different viral lineages belonging to five viral families and one viral order became endogenized in the genome of the isopod crustacean *Armadillidium vulgare*. This is the largest diversity of EVEs ever reported in any species to date. We show that viral endogenization occurred recurrently during the evolution of isopods, and that *A. vulgare* viral lineages were involved in multiple host-switches that took place between widely divergent taxa. Furthermore, 32 *A. vulgare* EVEs have uninterrupted open reading frames, suggesting they result from recent endogenization of viruses likely to be currently infecting isopod populations. Overall, our work shows that isopods have been and are still infected by a large variety of viruses. It also extends the host range of several families of viruses and brings new insights into their evolution. More generally, our results underline the power of paleovirology in characterizing the viral diversity currently infecting eukaryotic taxa.

Keywords: paleovirology, viral diversity, viral host range, isopod crustacean, Armadillidium vulgare

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Male heterozygosity or dissimilarity does not influence the choice of paternity of offspring in *Armadillidium vulgare* females

Durand S. and Beltran-Bech S.

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Genetic diversity is a key factor in evolution conferring to natural populations and species the potential to face environmental changes and adapt through natural selection. At the individual level, genetic diversity has been shown to be positively correlated with many fitness parameters in several taxa. Producing more heterozygous offspring seems thus a promising investment, especially in species with risk of inbreeding. Mate choice mechanisms are expected to increase the genetic diversity in offspring, by mating with several partners, and/or choosing very heterozygous or genetically dissimilar mates. We tested this hypothesis in the species *Armadillidium vulgare*, a gregarious terrestrial isopod in which mechanisms of inbreeding avoidance could be expected. To do so, two males were proposed to one female and we waited until the brood was born. Microsatellite loci were used to determine the paternity of the brood. We also calculated the level of heterozygosity of females and males, and the genetic dissimilarity between each male and the female. Twelve broods out of 21 presented only one father, but there was no effect of male genetic heterozygosity or dissimilarity to the female at the paternity level, even when taking in consideration female heterozygosity and genetic dissimilarity of the two males. At the same time, the remaining nine broods had multiple fathers without specific choice for the male heterozygosity or intersexual dissimilarity at paternity level. Female mating choice based on neutral genetic characteristics seems thus nonexistent. Two hypotheses can explain our results: (1) a potential choice of female for traits other than male neutral genetic characteristics or (2) no choice of female and just a strategy of multiple mating resulting in multiple paternity which could be a way to increase genetic diversity in offspring at a lower cost than the one induced by choosing. This work improves our understanding of mate choice in terrestrial isopods and provides insights on the mechanisms at the basis of diversity maintenance.

Keywords: heterozygosity, genetic dissimilarity, mate choice, multiple paternity

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Influence of *Wolbachia* infections on multi paternity in *Armadillidium vulgare*

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Wolbachia are endosymbiotic alpha-Proteobacteria, which infect a large range of arthropods. These bacteria are transmitted vertically by the host female. In the terrestrial isopod *Armadillidium vulgare*, three different strains of *Wolbachia* were described. To favor their propagation, these strains convert the infected males into females able to reproduce. This conversion skews the sex-ratio in favor of females and could decrease the genetic diversity and the survival capacity of infected populations. However, Verne et al. (2012) showed that *Wolbachia* did not decrease the nuclear DNA genetic diversity in *A. vulgare*. One way to preserve such genetic diversity could be particular mating systems. Laboratory experimentations showed that multi paternity was possible in *A. vulgare*. However, such a result has never been shown in natural populations. Moreover, Moreau et al. (2001) showed that presence of *Wolbachia* in *A. vulgare* altered the reproduction behaviours: males preferred to reproduce with uninfected females. In this study, we sampled and isolated gravid females from natural *A. vulgare* populations. After the birth of pulli, we used microsatellite markers to genotype each offspring and their mother. Then, we inferred the number of fathers involved in each brood. We showed for the first time multi paternity in natural populations. Results showed that the mean number of male genitors involved in uninfected broods was higher than in infected broods. The mean numbers of male genitors in infected broods were different depending on the strains of *Wolbachia* which infect the broods. Coupled to mating choice, multi paternity could favor genetic diversity within populations.

Keywords: Multi paternity, Wolbachia, Armadillidium vulgare, Microsatellites

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Genetic analysis of intraspecific variability in *Porcellionides pruinosus* (Brandt, 1833) from Tunisia

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The cosmopolitan species, *Porcellionides pruinosus* (Brandt, 1833), characterized by a controversial taxonomic state and high ecological plasticity, exhibited a geographical variation of its morphological features and its reproduction pattern. In fact, some Tunisian populations had a seasonal reproductive period and other ones showed a reproductive activity.

Analysis with the scanning electron microscope showed important phenotypic variation between the populations studied in the form of the pleotelson and its lateral borders that seem to exhibit reliable differences between the specimens examined. Phenetic analysis of Tunisian populations exhibited an important heterogeneity, which is worth noting.

To complete and deepen this study by genetic analysis, 17 allozyme loci were studied, using horizontal starch gel electrophoresis, in 230 adult specimens belonging to these populations. Allozymic analysis showed that the Tabarka population was the most divergent from the other observed populations with the lowest gene flow (0.58) and the highest genetic distance (0.182).

The analysis of DNA 16S sequences reveals the absence of genetic variation between populations of Elfeija, Chebba, Monastir, Sbiba and Sned and a low one for the populations of Bella Regia and Bousalem. However, the population of Tunis has considerable variations in the primary structure of the sequences; also it has a substantial genetic diversity within intrapopulation. In addition, the genetic distance of this population compared to other populations allows us to say that it can be considered as a “new species”.

Keywords: Porcellionides pruinosus, variability, intraspecific, DNA 16S, population

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Evolutionary history of terrestrial isopods: Phylogenetic, Morphometric and Ecological approaches

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Oniscidea are the only group of Crustacea almost entirely composed of species completely independent from the aquatic environment for life and reproduction. They are a key taxon to study the transition from aquatic life to terrestrial life because of their gradual terrestriality adaptation. However, how ecological characteristics of this group do evolve remains unknown. The anatomy and the body plan are conserved among Oniscidea species with a typical division in three parts (cephalon, pereion and pleon). This conserved anatomy is opposed to a certain diversity of body shape by both an antero-posterior elongation and a dorso-ventral flattening. The geometric morphometrics analyses represent an interesting approach to measure these morphological gradual variations among species. Here we propose an original study relying on a new phylogenetic hypothesis based on molecular data of 21 Oniscidea species to measure the degree of phylogenetic signal in the evolution of Oniscidea morphology. We investigate the differences among species morphologies using 33 2D landmark data measured on Oniscidea exoskeletons by means of a generalized Procrustes analysis. First we estimate the degree of phylogenetic signal by estimating the sum of changes in shape along all branches of the phylogeny. In the second part we examine the relation between morphologic, ecologic and phylogenetic features and, consequently, their degree of dependence due to their evolutionary history. Such a study may help to identify the covariation between morphological and ecological features and permits to evaluate the results of the complex impact of past natural selection and adaptive evolution.

Keywords: Oniscidea, Phylogeny, Geometric morphometrics, Generalized Procrustes analysis, Phylogenetically independent contrasts

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Ecology and Ecotoxicology

Chairpersons : B. LOMBARDO, K. NASRI

Terrestrial isopods in Tunisia after 20 years

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Research on terrestrial isopods from Tunisia started at the end of the 19th century as a result of the explorations of foreign scientists and included descriptions of only few species.

In the last twenty years, my team at the UR “Systematics and Evolutionary Bioecology”, Faculty of Sciences of Tunis, has investigated many topics regarding this zoological group such as biosystematics, biodiversity, biogeography, genetics and population dynamics, reproductive strategies, behaviour, rhythm, etc. Two new species of *Porcellio* and two of *Armadillidium* were described and many species were newly recorded from Tunisia, e.g. *Ctenosia dorsalis*, *Platyarthrus caudatus*, *P. aiasensis* and *P. schoblii*, etc. Some other species previously recorded need confirmation using morphological and genetical analyses. In addition to the new species, other endemic species should have a special status in the IUCN Red List. Collection at more than 200 sampling localities in the whole country has clarified the geographic distribution of each species. Our studies focused also on forest areas, both on oasis and Majerda agroecosystems related to irrigation and agricultural practice, and on wetlands. Several works carried out on the biology and reproductive behaviour showed that the most studied species *Porcellionides pruinosus*, *P. sexfasciatus*, *Armadillidium pelgicum*, *Porcellio variabilis* and *P. lamellatus* exhibited a seasonal breeding pattern starting in late winter-early spring with a sexual rest in fall-winter. These species are semi-annual, since females produce up to three broods per year, iteroparous, as they were able to breed more than one time in a breeding period, and with a bivoltine life cycle, since each population produces two generations per year. Often female-biased sex ratio was a consequence of the effect of *Wolbachia* which induced the feminization of their hosts. The behavior of two burrowing semi-desert species, *Hemilepistus reaumuri* and *Porcellio albinus*, has also been studied

Keywords: woodlice, systematics, biodiversity, reproductive strategies, Tunisia

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Diversity of terrestrial Isopods from North Tunisian wetlands

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In order to assess the influence of wetland habitat types on the distribution of terrestrial isopods, we estimated species richness, relative abundance and diversity indices in the supralittoral zone of 95 wetlands in the northwestern part of Tunisia. The wetlands can be classified into six types: rivers, lagoons, lakes, hill reservoirs, dams and sebkhas. Sampling was conducted using eight quadrats of 50 * 50 cm, randomly placed and each observed for 20 minutes.

A total of 3255 Isopods belonging to 20 species were caught. The highest species richness (13 species) was found around the dams, followed by 12 species around the hill reservoirs and rivers, and 11 species around lagoons. Only five and four species were respectively found in the supralittoral zone of sebkhas and lakes. ANOVA test showed statistically significant differences in species diversity related to wetland habitat types. Moreover, a highly significant difference in mean abundance and mean densities was observed, as well as a positive relationship between species richness and both humidity and altitude. Cluster analysis showed a divergence of the lagoons compared to all other habitats. Among the edaphic and topographic factors analyzed, four were statistically different between wetland habitat types (soil sodium content, soil conductivity, soil pH and altitude) explaining partly the dissimilarities observed.

Keywords: Isopod fauna, Species richness, Distribution, Wetlands, Tunisian dorsal

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Terrestrial isopods in alder fens of the montane zone at the Outer Carpathian Flysch

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Most of the wetland habitats have been strongly affected by long-lasting human influence due to different management practices aimed at intensive use of forest and non-forest landscape for various purposes. Alder fens represent forested montane wetlands frequently threatened due to forestry management practices, drainage, clearing and subsequent changes in hydrology and degradation. In sub-montane and montane zone they represent specific sites of relatively small area, but with nutrient-rich conditions comparing to surrounding beech forests.

Terrestrial isopods, as characteristic inhabitants of wetlands, were investigated in nine selected alder fens (*Caltho-Alnetum*) differing in altitude, hydrology and degree of degradation; all were located at the Babiogórski National Park, Outer Flysch, Carpathians, Poland. Repeated sampling in plots during 2010-2012 provided data on terrestrial isopod assemblages. Additionally, three surrounding beech forest stands (*Dentario glandulosae-Fagetum*) at similar altitudes were sampled in 2010 to compare assemblages in both alder and beech stands. The results showed, that the Carpathian alder fens represent spots with high population densities of terrestrial isopods (ranging between 84-1115 ind.m⁻²) comparing to surrounding beech forests (3-32 ind.m⁻²). Alder patches play the role of key landscape structures for isopods in the mountain regions. Two of six species present are endemic for the Carpathians. Whereas the occurrence of *Trichoniscus carpaticus* was clearly correlated with higher altitude, that of *Hyloniscus mariae* was negatively correlated with this factor. Impacts of mountain fens degradation, hydrology as well as other environmental parameters on isopod assemblages will be discussed.

Keywords: alder fens, Carpathians, terrestrial isopods, altitude gradient, hydrology

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Effects of microclimate on behavioural and life history traits of terrestrial isopods : Implications for responses to climate change

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The sensitivity of terrestrial isopods to changes in both temperature and moisture make them suitable models for examining possible responses of arthropod macro-decomposers to predicted climate change. In South East England global climate change models predict that by 2060 air temperatures will have risen by 3.5°C, summer rainfall decreased by 50% and be restricted to fewer more intense episodes with more and longer periods of drought.

Effects of changes in both temperature and relative humidity on aggregation, consumption, growth and survivorship on species of isopods contrasting in their morphological and physiological adaptations to moisture stress have been investigated in laboratory microcosms. Responses to both temperature and relative humidity varied consistently between species, illustrating the importance of phylogenetic constraints in predicting how responses to climate change may differ intra-specifically. It is therefore difficult to predict overall effects of climate change on the contribution made by soil macro-arthropods as regulators of microbially mediated carbon dynamics in the soil.

For one of our study species the extent to which it stimulates microbially mediated emissions of carbon dioxide was monitored in the laboratory at different temperatures. We found that stimulation of microbial metabolism by isopods varied between temperatures, being significantly greater at low temperatures. We speculate that differences in the exponents of thermal reaction norms for micro-organisms and isopods might account for the apparent decrease in stimulation at higher temperatures.

The implications of these results for predicted regulation of fluxes in the soil carbon by soil macro-arthropods under future climates will be discussed.

Keywords: Temperature, moisture, stimulation of micro-organisms, behaviour, populations

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Metal bioaccumulation in two species of Oniscideans: *Porcellio laevis* and *Porcellionides pruinosus* from Tunisian contaminated sites

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Terrestrial Isopods that are important decomposers, are known for their resistance to high soil contamination by metal trace, making their use very helpful in biomonotoring. The aim of the present study was to compare metal bioaccumulation of two common species of *Porcellio laevis* and *Porcellionides pruinosus* exposed to the same contaminated soil belonging to industrial area polluted by metal trace elements in the North (1), Central (3) and South (1) of Tunisia. We hypothesized that these two species would accumulate metals according to their bioavailability in soil and in vegetation. These results will be discussed in relation to the soil and climatic of the different sites explored.

Keywords: Porcellio laevis, Porcellionides pruinosus, soil metal bioavailability, metal accumulation

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Relationship between genetic distance and stability of soil processes in microcosm communities of isopods and diplopods

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There is evidence for differences in environmental sensitivity (response traits) among functionally similar species providing stability to ecosystem processes. Under a scenario of climate changes, this relationship may help to predict which and how processes will be affected based on the differences between its community members. It has been proposed that the phylogenetic diversity of a community can be used as a proxy for differences in response traits, considering that closely related species tend to occupy similar niches. Here we test this relationship by manipulating the genetic distance between pairs of soil detritivores in a microcosm experiment, using eight species of isopods and three species of diplopods. The genetic distance was calculated using fragments of 18S rRNA and COI gene sequences. Each pair was assembled twice and distributed into two treatments, a “control” where soil moisture was maintained around 50% and a “low” treatment where moisture decreased over time. After four months, we evaluated fungal biomass and NO₃, NH₄ and PO₄ accumulation in the substrate. Preliminary results indicate higher NO₃ and PO₄ accumulation in the high-distance species pairs, but no differences between treatments, and stress species-specific effects on soil processes.

Keywords: Functional diversity, detritivores, ecosystem stability, soil processes

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Reproductive strategies of sympatric isopod species (Crustacea, Isopoda, Oniscidea)

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Terrestrial isopods as members of the decomposing ecosystem occur in a wide range of moist habitats, generally coexisting with other species of the macro-decomposer guild. Their overlapping food range and habitat preferences suggest that their coexistence is permitted by their different spatiotemporal resource utilization patterns, which is in turn determined by life-history strategies. In an empirical study, the reproductive strategies of three coexisting surface active isopod species (*Armadillidium vulgare*, *Protracheoniscus politus*, *Porcellium collicola*) were investigated in three meso-habitats within a characteristic central european forest habitat type. We found major differences in the reproductive strategies of the three sympatric isopod species, which differences were consistent across the different meso-habitats. *Armadillidium vulgare*, in contrast with the other two species, did not show large variances in seasonal population densities and in the ratio of gravid females throughout the season. On the contrary, *P. politus* and *P. collicola* exhibited large temporal variances in abundance. While both of the latter species had a distinctive peak in the ratio of reproducing females, the modality of their seasonal abundances was different. These results suggest that reproductive strategy differences, as major determinants of niche segregation, contribute to the stable coexistence of terrestrial isopod species.

Keywords: coexistence, terrestrial isopods, reproductive strategy

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Reproductive traits of *Porcellio variabilis* Lucas, 1946 (Isopoda, Oniscidea) from Tunisia

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Oniscid isopods inhabiting arid, temperate and tropical regions exhibited several reproductive traits like breeding period, female size at breeding, fecundity, reproductive allocation and life span. Here the breeding phenology and reproductive strategies of *Porcellio variabilis*, a woodlice endemic to North Africa, were investigated in the population of Béja (North of Tunisia). Monthly samples were taken during 18 months and 20 gravid females, collected in the field, were reared in the laboratory and followed till manca hatching. *Porcellio variabilis* is characterized by a seasonal reproduction period followed by a sexual rest phase during November, December and January. The highest percentage of ovigerous females was observed in May and June. Mean fecundity showed a great seasonal variation ranging between 29.612 ± 10.821 eggs for females caught in late summer and 125.920 ± 33.538 eggs for those collected in spring. *Porcellio variabilis* is an iteroparous species. In this case, females were able to produce one (95% of females), two (40% of females) or three broods (20% of females) during their reproduction period. The duration of the gestation period ranged between 34 days in the first brood and 14 days in the third one. Energy allocated to reproduction decreased from the first brood (26.29%) to the last one (4.62%). During the sampling period, eight cohorts were identified exhibiting a variable life span estimated to be 6 months for cohorts born in early spring and 12 for those born in fall. According to these characteristics, *Porcellio variabilis* tends towards an r-selected strategy. Finally, intra-species reproductive traits were discussed.

Keywords: Cohorts, life span, fecundity, reproductive investment, Porcellio variabilis

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Sexual affinity in terrestrial isopods: what does matter?

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Sexual selection predicts mate choice and mating preferences depending on *Wolbachia* infection status were studied in few models. In our model, *Armadillidium vulgare*, the endosymbiotic bacteria *Wolbachia* changes genetic males into functional females. *Wolbachia* impacts also host behavior and fitness. Moreover the hosts are gregarious and aggregation depends on abiotic and biotic factors such as individual affinity and infection status. Gregariousness may also increase the risks of inbreeding, so we could expect mechanism to prevent it.

Here we exposed an overview of different results to better understand the reproductive strategies in terrestrial isopods *Armadillidium vulgare*. We included the results of several published studies and new findings which address the ultimate and the proximate impact of sexual selection including individual life history and also sexual preferences and pairing behavior.

Keywords: Behaviour, Chemical profiles, Woodlice, Mate choice, Sexual selection

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Effects of disturbance on the diversity and abundance of terrestrial isopods in arid regions in Tunisia

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We investigated the diversity and abundance of terrestrial isopods according to habitat and the degree of disturbance of the natural environment. Three sites in arid areas were selected for this study in two years: 1) Site of Siliana an uncrowded area, located in the north-est, 2) site of Bouhedma a protected area, located in the center, and 3) site of Essateh, an overgrazed area, situated in the south-west of Tunisia. Seasonal samples of terrestrial isopods were collected by hand in each site using replicates in an area of 10m x10m.

During this study, 3058 specimens belong to 15 terrestrial isopods species from five families were collected. Terrestrial Isopod community structure differs among the three sampling sites. The highest species richness was recorded at Siliana and the lowest characterized the overgrazed area (Essateh).

Regression analysis between the abiotic factors and species abundance showed a positive relationship with the soil texture, vegetal associations and temperature for *Hemilepistus reaumuri* and *Porcellio albinus*, while *Porcellio laevis* and *Porcellionides pruinosus* were related to moisture. Canonical correspondence analysis (CCA) showed that penetrability, moisture and temperature had the greatest influence on the species distribution. *Hemilepistus reaumuri*, *Porcellio albinus* could be considered specialists and enfeoffed to specific environments, while *Porcellio variabilis* is a generalist species that is harvested in different environments.

Keywords: Diversity, specialist, generalist, arid area, disturbance

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Spatial heterogeneities, self-organization process, and aggregation behaviour in *Porcellio scaber*

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Porcellio scaber is one of the most common crustaceans on land. Essentially to limit its desiccation rate, this woodlouse aggregates in shelters. The relation between the behavioural responses to conspecifics and to environmental heterogeneities (shelters) is imperfectly known in woodlice as in many species. In previous studies with binary choice tests, we have shown that the aggregation behaviour in *Porcellio scaber* includes a strong social component (i.e. interattraction between individuals). Here, we report the effects of environmental heterogeneities (i.e. shelters) and some of their characteristics (size, fragmentation) on the aggregation process. One hundred *Porcellio scaber* were introduced in four set-ups (circular arena without shelter or including two small, one small or one large shelter(s)). Whatever the set-up used, a large aggregate (around 70 individuals) systematically takes place under one shelter or randomly in the arena without shelter. The rest of the population forms smaller aggregates diametrically opposed to the biggest one whatever the set-up. In view of these results, we conclude that the environmental heterogeneities only affect the location of the main aggregates and have a low impact on the global aggregation levels. We show that some underlying mechanisms inherent to the aggregation process (local amplification/ long-range inhibition) lead to a fragmentation of population and a regular spatial distribution of aggregates. A theoretical model involving the probability of leaving and joining an aggregate reproduces experimental results and highlights that simple individual rules lead to the amplification of collective patterns. These results therefore support a self-organized process in woodlice.

Keywords: woodlice, habitat selection, sheltering, interattraction, collective decision making

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Diversity and spatial distribution of Oniscidea in the National Park of Feija (N-W of Tunisia)

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Studies on woodlice have been often realized with the aim to assess ecosystem health. In this first woodlice investigation in a Tunisian protected area, we assessed the Oniscidean diversity in the National Park of Feija located in the Kroumirie area (NW Tunisia) hosting the most beautiful zeen oak forest in the world. Our main purpose was to record the species of terrestrial isopods, to analyse their distribution in relation to vegetational structure and several other biotic and abiotic factors (altitude, soil pH, soil humidity and temperature, air humidity and temperature, soil texture, litter thickness, stone density, shading, vegetation density) and to compare the terrestrial isopod diversity between the nine sampled habitats in the spring of three years, 2010, 2012 and 2013. Overall, ten species, belonging to four families (*Platyarthridae*, *Armadillidiidae*, *Porcellionidae*, *Philosciidae*) were reported and ascribed to four biogeographical categories. Several diversity indices were calculated showing variation between the nine habitats. Moreover, two multivariate analyses (ACP and CCA) were performed to assess the impact of environmental factors in each habitat and to quantify the distribution of the isopod species according to vegetational assemblage and other factors. The dissimilarities between the studied habitats were calculated using the Euclidean distance.

Keywords: woodlice, diversity indices, vegetational structure, Feija park, Tunisia

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Is defensive behaviour of woodlouse affected by its personality?

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During their evolution, terrestrial isopods were faced with new types of predators. Beside chemical defensive secretions (against spiders predominantly), they have developed behavioural protection known as tonic immobility or death feigning.

We tested 150 individuals of Common Rough Woodlouse. Woodlice were kept separately in plastic boxes. We evaluated promptness of evoke and duration of tonic immobility according three types of stimuli (touch, squeeze and drop). The experimental set included all three stimuli. Each stimulus was repeated several times (if necessary) to evoke tonic immobility. If evoked, endurance of immobility was measured. Sequence of stimuli during one experimental day was changed; all individuals were tested repeatedly for five days.

Altogether, 23% of tested woodlice reacted by tonic immobility. The touch was the weakest stimulus, it was necessary to repeat it a number of time to evoke response; contrary to touch, squeeze and drop were much stronger stimuli. Nevertheless, if woodlice postured immobility as response to touch, this response persisted for longer time. During set of three stimuli, the strongest stimulus was the third one. The length of reaction was affected by size of woodlouse, the smallest individuals feigned death for shortest time.

Beside mentioned factors, we evaluated personality of woodlice, i.e. effect of individual (its "ID") to its response. We found significant effect of individual to probability of posture as well as duration of this reaction. *Porcellio scaber* is the first species of terrestrial isopod with documented personality.

This research was supported by internal grant of Palacky University No. PrF_2013_016.

Keywords: Porcellio scaber, death feigning, thanatosis

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What is the optimal group size for resting isopods ?

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In this paper we develop a conceptual model of the trade-off between fitness costs due to reduced survivorship for under dispersed resting groups and fitness costs due to increased interference during foraging for highly aggregated resting groups. Predictions of the model are tested by analysing temporal and spatial variations in intraspecific aggregation and inter-specific associations of three species of terrestrial isopod from three different families over 25 years in four grassland sites.

Predictions from the model and laboratory studies, suggest that significant differences in aggregation would occur between isopod species as a consequence of differences in their morphological and physiological adaptations to the terrestrial environment. The predictions of the direction of such interspecific differences in aggregation were only partly supported by field data. We discuss how species specific differences in the importance of aggregation as an adaptive tactic in desiccation avoidance strategies may account for the apparent anomalies observed.

Predictions that terrestrial isopods would aggregate more in short open swards compared with tall thick ones, more when summer rainfall was lower, when litter temperatures were higher and at lower densities, were all strongly supported.

The possible implications for the interactions between microorganisms and aggregated populations of soil animals of predicted changes in temperature and rainfall patterns for future carbon dioxide emissions from soils are discussed.

Keywords : Central place foraging, fitness costs, soil carbon dynamics, grasslands, CO₂ emissions from soils

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Abstracts for poster presentations

Systematics and biogeography

Fossil record in Isopoda specifies the emergence of Oniscidea

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Within Crustacea, Oniscidea is the most diversified group fully living on land. However, despite the large size of the group (about 3600 extant species), fossil record of Oniscidea is quite scarce. Indeed, unlike insects, woodlice are difficult to fossilize both because of their strictly terrestrial mode of life and biochemical properties of their exoskeleton. Thus, origin and evolutionary history of Oniscidea are still poorly known. Some fossils of woodlice were reported in recent deposits, as in Dominican (15-20 Ma, Neogene) and Baltic ambers (35-40 Ma, Paleogene). Recently, the earliest attested woodlice fossils were reported in Aptian-Albian ambers (100-110 Ma, Cretaceous) from Spain, France and Burma. Considering Early Cretaceous palaeogeographical reconstructions, these fossils indicate that Oniscidea were already widely distributed at this time, suggesting an ancient origin of the group. Our review of the fossil record of both oniscidean and other isopodan sub-orders also supports an old origin of woodlice. Indeed, Oniscidea are phylogenetically bracketed by basal Carboniferous groups (as Phreatoicidea) and Permian-Triassic modern forms (as Sphaeromatidae). These observations consequently involve a pre-Pangaeian origin of the Oniscidea. In addition, a review of palaeobiogeographic, molecular and biological considerations supports our results. Therefore, we argue for a Late Paleozoic origin for Oniscidea (Permo-Carboniferous), followed by a global and rapid dispersal of the group on the Pangaea (Permo-Triassic). Taxonomic descriptions of Cretaceous specimens should considerably improve our knowledge of the evolutionary history of Oniscidea.

Keywords: phylogeny, fossil record, Cretaceous amber, palaeobiogeography, Carboniferous

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Preliminary data on the phylogeny of species included in *Hemilepistus*

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The genus *Hemilepistus* Budde-Lund, 1879 (Isopoda, Agnaridae), as currently perceived, consists of 20 species distributed in southern-southwestern Asia and the northern margins of the Sahara desert east of Algeria. It has attracted the interest of researchers especially due to the advanced social behavior of at least one of its species and their ability to cope with extreme dry environmental conditions. In the present work we present preliminary results of a phylogenetic analysis of species included in the genus, based on molecular markers and various phylogenetic methods. Up to now we mainly used species distributed in Iran plus populations of *H. reaumurii* from several parts of its distribution, as well as available sequences from other genera to serve as outgroups. Results provide evidence against the monophyly of the genus due to the possible misplacement of *H. elongatus* that seems to belong to a very distant clade (probably a different family altogether!). More species and characters will be evaluated in the near future so as to further resolve the phylogenetic history of these isopods.

Keywords: phylogeny, monophyly, Hemilepistus, Desertellio, molecular markers, genetic distance

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Terrestrial isopods fauna of the province of Qazvin, Iran

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The terrestrial isopod fauna of the province of Qazvin, central Iran, was investigated. While the northern parts of the province are mountainous, southern parts consist of broad planes. Formerly, only three species were reported from the province. In this study, totally 12 species belonging to five families were found: *Lygidium hypnorum* (Lygiidae), *Trachelipus* sp. (Trachelipodidae), *Armadillidium vulgare* (Armadillidiidae), *Porcellionides pruinosus*, *Proporcellio* sp. (Porcellionidae), *Armadillo cf. alievi* (Armadillidiidae), *Agnara persica*, *Hemilepistus klugii*, *H. elongatus*, *Desertonicus* sp., *Protracheoniscus major* and *Protracheoniscus* sp. (Agnaridae). The most abundant family was Agnaridae with six species in four genera. The most common species were *Protracheoniscus major* and *Armadillidium vulgare*, whereas *Lygidium hypnorum* and *Proporcellio* sp. were found only from one locality.

Keywords: Oniscidea, terrestrial isopods, Qazvin, Iran

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Phylogeny of the littoral family Halophilosciidae (Crustacea, Isopoda, Oniscidea)

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While several studies on the molecular phylogeny of different families or groups of species within a genus of terrestrial isopods have been produced in the recent past, to date no studies exist regarding the monophyly of single families or genera based on molecular analyses. In this study we aim to assess the phylogeny of both the family Halophilosciidae and the genera included within, using molecular and morphological traits. At present, the family Halophilosciidae consists of three genera: *Halophiloscia*, *Stenophiloscia* and *Littorophiloscia*. The genus *Halophiloscia* includes nine species mainly distributed on the coasts of the Mediterranean Sea, with three species endemic to the Canary Islands, and one species, *H. couchii* which is widely distributed in the Mediterranean, the Atlantic coast of Europe and northern Africa and also introduced to North and South America and Australia. The genus *Stenophiloscia* includes only three species from the Mediterranean coasts, Atlantic coasts of Europe and the Canary Islands. The genus *Littorophiloscia* has a wide distribution along the tropical and subtropical coasts with 21 species described so far. The monophyly of the family Halophilosciidae has been questioned several times in the past. Herein we provide the first results after the analyses of 33 individuals from the three genera of Halophilosciidae using the mitochondrial protein-encoding gene cytochrome c oxidase subunit I (COI), the mitochondrial 16S rRNA, and a fragment of the nuclear ribosomal 28S rRNA. We applied Maximum Parsimony (MP), Bayesian Inference (BI) and Maximum Likelihood (ML) analyses and calculated the genetic divergence within and among the taxa studied.

Keywords: Oniscidea, Halophilosciidae, morphology, molecular phylogeny

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Morphological variation related to life history and sexual immaturity of terrestrial isopods, implications for taxonomy

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Some morphological characters used for the identification of species of terrestrial isopod are secondary sex characters that develop with sexual maturity. Thus, although morphological features can change during the life history of a species, many descriptions do not refer to, or consider, these variations. This may cause taxonomic confusion. Moreover, specimens with short genital papillae are often found, even when body size is large. These specimens may be sexually immature, and thus their secondary sex characters may not be developed sufficiently. Thus, for accurate taxonomic identification of terrestrial isopods it is important to understand morphological variation related to life history and sexual immaturity.

The genus *Mongoloniscus* is distributed in East Asia (Japan, Korea and China) and includes 10 species (Schmalfuss, 2004). However, the taxonomic status of the genus is still confused. The aim of this study is to describe the morphological variations of *M. koreanus* Verhoeff, 1930 from Japan and Korea, in relation to life history and sexual immaturity.

We examined the life history of *M. koreanus* by collecting specimens every month. Four life history stages, manca, small, middle, and large, were identified. The shapes of morphological characters used for species recognition, e.g. pereopod 7 and pleopod 1, were compared among the life stages using an Elliptic Fourier Descriptor and scanning electron microscopy. The results showed that the morphological characters used by some authors to identify *M. koreanus* were found on large stage specimens only, while the morphological characters of the small and middle stages were similar to those of *M. nipponicus*.

Keywords: Elliptic Fourier Descriptor, Mongoloniscus, scanning electron microscopy, secondary sex characters

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Molecular data confirm species assignment of the genus *Ligia* (Crustacea Isopoda: Ligiidae) along the coastal zone of the Persian Gulf areas

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Coastal isopods of the genus *Ligia* exemplify an organism with very limited vagility and high restriction to a patchy habitat; therefore they can potentially show a high hidden or cryptic biodiversity. As an example, Khalaji-Pirbalouty and Wägele (2010) described two new species *L. persica* and *L. yemenica* from the Persian Gulf and Gulf of Aden, respectively. The current study uses molecular markers to identify and differentiate between three species (including *L. exotica*) of the genus *Ligia* from the coastal zone of the Persian Gulf and adjacent areas. A partial fragment of approximately 600 base pair of the mitochondrial gene; ribosomal RNA gene (16S), were earned from some collected material from the Persian Gulf, Yemen, India, Japan, and Taiwan. A phylogenetic tree was conducted based on Maximum Likelihood analysis. Our data clearly support the existence of three distinct lineages, with marked genetic differentiation between groups. However, the tree has not resolution among the three taxa, but clearly shows that they are different species. The samples from Japan, India and Taiwan are belonging to the same clade, which we assume is *L. exotica*. In addition, this study shows and confirms the reliability of the current morphological-based classification and identification of the isopods genus *Ligia*. It also suggests using molecular analysis to assess the real diversity of the genus *Ligia* in an era of vast human-induced biodiversity decrease.

Keywords: Ligia, Persian Gulf, Adan Gulf, 16S rDNA

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A GNU way to rapidly draw digital figures and plates: accurate scientific illustrations for biosystematics

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Nowadays only digital figures are accepted by the most important journals of biosystematics. These may be produced by scanning conventional drawings, made with high precision technical ink-pens, that normally use capillary cartridge and various pens for different line widths. Digital drawings techniques that use vector graphics, have already been described in literature to support scientists in drawing figures and plates for scientific illustrations; these techniques use several softwares and various hardware devices (such as digitiser boards or digital graphic pens).

The present work gives step-by-step instructions on how to make accurate line drawings with a new procedure that uses a raster graphic working on GIMP. Some of this method features are noteworthy:

- it is very accurate, producing detailed lines at the highest resolution;
- the raster lines appears as realistic ink-made drawings, whereas vector graphic gives an unnatural result;
- it is much faster either than the traditional way of making illustrations that the other procedures already described in literature;
- everyone can learn to use this simple technique; it can also be used by technical staff or even unexperienced volunteers;
- this method is completely free as it does not use expensive and licensed softwares.

Keywords: methodology, scientific illustration, GNU, digital drawings, GIMP

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Morphology and Physiology

The morphology of tergal cuticle in two terrestrial isopod species (Crustacea, Isopoda, Oniscidea)

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The main barrier between the body surface of terrestrial isopods and their environment is the protective sclerotized and mineralized tegumental cover, the cuticle. We have studied the cuticle of two conglobating but phylogenetically not related isopod species: the cosmopolitan *Armadillidium vulgare* and the synanthropic *Cylisticus convexus*. Both species occur frequently in the same habitat type in the studied area, in Central Europe. Structure and distribution of elements were compared using light (LM) and scanning electron microscopy (SEM), field emission scanning electron microscopy (FE-SEM), and electron microprobe analysis (EMPA).

The cuticle's main structure as revealed by light microscopic analysis (LM; cross section) is the same in the two species. The outer layer is the epicuticle and the procuticle. The procuticle is provided into two regions: the exocuticle and the multilayered endocuticle. Differences between the species cuticle occur in the epicuticle's surface morphology (tricornes, plaques). As in other Crustaceans, the terrestrial isopod cuticle consists of a composite material comprising an organic and an inorganic phase. The organic phase consists mostly of chitin-protein fibrils rather than fibres. Differences between the two species in the structure of organic phase comprise the form of the fibrils. Background subtracted spectra retrieved from spectral maps indicate a higher amount of calcium in the tergal cuticle of *A. vulgare* in comparison to that of *C. convexus*.

Keywords: tergal cuticle, light microscopy, scanning electron microscopy

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The integument morphology of the *Haplophthalmus movilae* from Movile Cave (Southern Dobrogea, Romania)

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Haplophthalmus movilae is one of the three species of terrestrial isopods described until now from the Movile Cave, one of the most important and unique hygrothermal cave systems. Detailed investigation of the cuticle structures has been undertaken using scanning electron microscopy in order to assess the influence of particular cave environment on the integument morphology of this species.

The body surface of *H. movilae* is characterized by two prominent pairs of short and tubercle-like tergal ribs on each pereionite, and a single dorsomedial widened tubercle formed by two rounded fused protuberances on the third pleonite. Each of the pereional tubercles is covered almost entirely by foliate jagged scales and especially the medial tubercles possess abraded tops apparently due to the contact of the body surfaces with cave sediments. The same foliate jagged scales were found on the labrum and on the last three pleonites where the broader scales are at their edges and more narrow and elongated in the medial part. Similarly, the uropods are covered by foliate scales gradually elongated from their base to the apex. A network of honeycomb-like polygonal scales covers the surface of the pereionites and the first two pleonites. Some of these polygonal scales present the same foliate structure. Tricorns, known as characteristic feature of the body surfaces of some isopod species, were not found but the other observed integument structures have a clear protection role. The water conservation function may be reduced or absent as the species permanently inhabits the highly humid cave environment.

Keywords: *Haplophthalmus movilae*, integument structures, SEM

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Pathogenesis, tissue distribution and host response to *Rhabdochlamydia porcellionis* infection in terrestrial isopod crustacean *Porcellio Scaber*

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Rhabdochlamydia porcellionis is known as intracellular pathogen in digestive glands of its primary host isopod *Porcellio scaber*. To analyze the influence of *R. porcellionis* infection, its pathogenesis and tissue distribution, and host response, we conducted light microscopic observations and localization of *R. porcellionis* by Fluorescent *In Situ* Hybridization (FISH). Digestive glands are confirmed as the main site of infection. From there *R. porcellionis* may spread to the lumen of digestive tract or into hemocoel, by release through apical membrane and basal membrane of digestive gland epithelial cells, respectively. Once in hemocoel, *R. porcellionis* is able to infect hindgut cells, hemocytes and hemopoetic tissues. Ventral nerve cord and gonads seem to be devoid of infection, despite the presence of *R. porcellionis* on their surface. Host response to *R. porcellionis* is mediated by aggregation of hemocytes, forming multilayered melanized nodules and exhibiting strong endogenous fluorescence. Asymmetric nodules are found on infected gut and occasionally on infected digestive glands. Nodules on ventral nerve cord and gonads appear symmetric and most likely serve to entrap bacteria released from digestive gland and gut cells. Our study also revealed high incidence of infection in isopod populations (up to 27%) and its detrimental effect during symptomatic phase, leading to death of infected animals, while the feeding experiment demonstrated significant decrease in feeding of symptomatic animals, without influence to agility, behavior or molting cycle.

Keywords: Rhabdochlamydia porcellionis, rough woodlouse, intracellular infection, nodulation, melanization, tissue distribution

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Comparative ultrastructure of the integument in troglobitic and epigeal woodlice (Isopoda: Oniscidea)

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We compared the ultrastructure and the relative thickness of the tergal cuticle in several species of troglobitic and non-troglobitic woodlice, focusing predominantly on representatives of the family Trichoniscidae, which includes the majority of troglobitic woodlice species.

The tergal integument was analyzed with light microscopy, transmission electron microscopy and scanning electron microscopy. By measuring the tergal cuticle thickness and relating it to the body-size of studied animals, we established that the tergal cuticle is thinner in troglobites than in non-troglobitic woodlice of similar size. As revealed by scanning electron microscopy, the endocuticles in troglobites generally consist of more numerous and thinner lamellae compared to cuticles of similar thickness in non-troglobites, whereas there are no evident differences in the structure of the exocuticle.

As demonstrated by transmission electron microscopy, the epicuticles in troglobites as well as non-troglobites are structurally diverse and display varying degrees of complexity. The inner epicuticles are simple and electron dense in all examined species, and are generally thinner relative to the entire thickness of the epicuticle in troglobites. The outer epicuticles, on the other hand, may be simple or consist of several interchanging electron lucent and electron dense sublayers. Surprisingly, outer epicuticles of both types can be found in troglobites as well as non-troglobites.

We can conclude that the thickness and structure of the integumental cuticle contribute to the evolutionary success in the cave environment. Nevertheless, some features of the cuticle in troglobites are diverse, likely reflecting their different life-styles.

Keywords: Trichoniscidae, cuticle ultrastructure, cave adaptations, crustaceans

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Impact of infection by feminizing bacteria *Wolbachia* on the metabolism of its host, the terrestrial isopod *Armadillidium vulgare*

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Bacteria of the genus *Wolbachia* are the most widespread in arthropods. Several studies have described that *Wolbachia* negatively influences some physiological performances of their host, such as survival, size, immune performance, locomotion, fecundity, fertility, sperm production, or the mating choice.

The realization of these different physiological performances depends on the amount of energy, also called aerobic metabolic capacity, available to the host to perform them. Then, the reduction of the host's physiological performances by *Wolbachia* could be the consequence of a reduction of the host's aerobic metabolic capacity by *Wolbachia*. Although the effect of the bacteria on its host's physiological functions has often been measured, the direct metabolic cost of the infection has never been measured directly. The host could possibly compensate a decreasing aerobic capacity by increasing its anaerobic capacity.

The objective of this study is to evaluate the metabolic cost of *Wolbachia* infection on aerobic metabolic capacity, anaerobic metabolism and oxidative stress in the woodlouse *Armadillidium vulgare*, considered as the major model for studying the impact of *Wolbachia*. Aerobic capacity will be measured across the mitochondrial capacity, of which it depends directly, at the cellular and molecular levels. Anaerobic metabolism and oxidative stress will be quantified at the cellular and molecular levels.

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Evolutionary Biology

***Wolbachia* mediated protection against pathogenic bacteria in two isopod models**

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Symbionts largely affect their hosts' life history traits and fitness. Previous studies showed that vertically transmitted symbionts can protect the host against pathogens. Among this symbionts, the *Wolbachia*, have recently been shown to confer protection to their hosts against a wide range of pathogens, especially viruses. The present study aims to test whether resident *Wolbachia* could confer protection to terrestrial isopods against invasive intracellular bacteria (*Salmonella typhimurium*, *Listeria ivanovii* but also the pathogenic *Wolbachia* strain wVulC). To do so, we assessed the survival during bacterial infections of *Armadillidium vulgare* and *Porcellio d. dilatatus* isopods when they are symbiotically associated or not with resident *Wolbachia*. We showed that when feminizing and CI *Wolbachia* have an effect on the survival of their hosts during the bacterial infection, this effect is always beneficial to the host. However, the intensity of the «anti-pathogenic» effect is clearly dependent on the *Wolbachia* strain and the genetic background of the host.

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Bidirectional cytoplasmic incompatibility caused by *Wolbachia* in the terrestrial isopod *Porcellio dilatatus*

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In the terrestrial isopod species *Porcellio dilatatus*, unidirectional Cytoplasmic Incompatibility (CI) between two morphs (*P. d. dilatatus* and *P. d. petiti*) caused by a *Wolbachia* strain (wPet) infecting the morph *P. d. petiti* has been previously described by experiments initiated four decades ago. Here, we studied another *Wolbachia* that has been recently detected in a population of the morph *P. d. dilatatus*. The MLST markers reveal that this *Wolbachia* is a new strain called wDil distinct from wPet also belonging to the isopod clade of *Wolbachia*. Quantifications of both *Wolbachia* strains in the gonads of the two *P. dilatatus* morphs revealed that all males exhibit similar *Wolbachia* titers while the titers in females depend on the *Wolbachia* strain they host. Crossing experiments showed that both wDil and wPet induced partial unidirectional CI with different intensities. Moreover, these two strains induced bidirectional CI when individuals were both infected with one of the two different *Wolbachia* strains. This way, we demonstrated that *P. dilatatus* can be infected by two closely related *Wolbachia* strains (wDil and wPet), that seem to have different modification-rescue systems. This does not constitute yet a proof of the influence of these CI-*Wolbachia* strains on the on-going speciation between *P. d. dilatatus* and *P. d. petiti*. However, this could constitute a good model where host of the same species harbours different *Wolbachia* strains that can participate to their divergence.

Keywords: cytoplasmic incompatibility, *Wolbachia*, *Porcellio dilatatus*, crossings, molecular characterisation, reproductive isolation

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The myth of symbionts in terrestrial isopod *Porcellio scaber*

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The evolutionary milestone of land invasion required several fundamental adaptations. Acquisition and maintenance of digestive tract symbionts may represent a key prerequisite for successful occupation of land allowing isopods to dwell on low-quality food sources. Digestion of cellulose and lignin in terrestrial diet might be facilitated by gut bacteria, the known example from the ruminant-symbionts association. Symbionts may additionally act as source of essential fatty acids and vitamins. Terrestrial isopods like *Porcellio scaber* harbour symbiotic bacteria in hindgut and hepatopancreas, however the mechanism of transmission and the nutritional role of symbionts remain elusive. We investigated the mode of symbiont acquisition and the role of bacterial symbionts as important food source through feeding manipulations by quantification of growth. In experiment I we provided different sources for symbiont inoculation through nutritional supplements to an artificial diet to test for the vertical (mother-offspring), horizontal (through faeces or contact with conspecifics) and environmental (through leaves) mode of transfer. Our results show that symbiont inoculation is mediated through horizontal and environmental transfer and question the existence of vertical transfer. In experiment II, we provided adults with nutritionally enriched (colonized with fungi and bacteria) and nutritionally not enriched diet for eight weeks. Isopods feeding on enriched diet grew significantly larger promoting an important role of microbes and fungi as direct source of nutrients. The here supported mode of symbiont acquisition and the effect of microbes and fungi on growth rates suggest that terrestrial life-style may have been facilitated through novel modes of symbiont uptake.

Keywords: symbiont transmission, nutrients, terrestrial diet

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Intra and interspecific aggregation affinity in terrestrial isopods

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The aggregation in terrestrial isopods is known to be dependent on humidity, brightness and temperature. Here we would like to compare the social component of intra- and inter-specific attractiveness for both male and female. We used seven species of terrestrial isopods: *Armadillidium vulgare*, *Armadillidium maculeatum*, *Oniscus asellus*, *Porcellio dilatatus*, *Porcellio scaber*, *Cylisticus convexus*, *Heleria brevicornis*.

In experimental arenas without shelters we measured the evolution of individual distances during one hour using eight individuals per species and all combinations of two species were done.

Next we collected individuals of each species to compare aggregation affinity and species chemical profiles.

Our results showed clear and different aggregation profiles at the intra-specific level. Moreover, we also found in mixed groups (inter-specific) different aggregation patterns linked to biological, chemical and phylogenetic component of the species. Each species present a specific chemical profile which could explain different level of intra-specific attractiveness.

Keywords: Behaviour, chemical profiles, woodlice

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Ecology and Ecotoxicology

***Hemilepistus reaumurii*: cuticle electron microscopy and chemical profiles**

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Cuticle from the desert Isopod *Hemilepistus reaumurii* (Tunisia, Bchechma, 35°49'77.5"N 010°10'04.0"E) was studied using Transmission Electron Microscopy (TEM) and gas chromatography (GC) in order to understand the role of *H. reaumurii* cuticle in the chemical communication and for this species survival.

The osmolarity was measured in order to determine the appropriate fixation solution: according to the value found (918±86.4 nmol/kg), the fixation solution is the one used for marine Isopods. After fixation and washing, samples were placed in the EM AMW (Automatic Microwave Tissue Processor for Electron Microscopy). Moreover, results obtained highlighted a difference concerning the thickness of the layer of chitin between reared animals since 6 months on the laboratory and those collected from natural habitat. Cuticular chemical analyses of *Hemilepistus reaumurii* were done by gas-chromatography.

We compared cuticular chemical profiles between different family of males and females as well as the faeces. We are currently processing statistical analysis in order to know if *H. reaumurii* present family and/or sex specific chemical profiles which could explain family recognition.

Keywords: *Hemilepistus reaumurii*, desert, Tunisia, social, cuticle, ultrastructure, Faeces, Chemical profile

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***Wolbachia* enhance tonic immobility in *Armadillidium vulgare* (Crustacea : Isopoda)**

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Key-words: stress, interindividual variability, symbiosis, behaviour

In many vertebrates, behavioural responses to stressors may be influenced by many factors, particularly sex or infection status. However, in arthropods, such effects remain little investigated. Our study focuses on the behavioural responses of the terrestrial isopod *Armadillidium vulgare* when it is subjected to repeated stressors. Moreover, in this species, infection with *Wolbachia* bacteria leads to the feminization of genetic males, turning them into functional females. Consequences of this infection on many hosts behaviour remain unknown. In order to address these questions, we exposed to repeated stressors: uninfected males and females, naturally and artificially infected females with *Wolbachia*. Our results revealed no differences concerning the duration of tonic immobility between males and females uninfected neither artificially infected by *Wolbachia*. Concerning naturally *Wolbachia* infected females, they stayed a significant longer time in tonic immobility after repeated stressors, than uninfected and artificially infected individuals. A strong interindividual variability was also observed within each group. To conclude, our study reveals that in addition of influencing the reproductive physiology of his hosts, *Wolbachia* infection affects their reactivity after repeated stressors. It could be interesting to test the evolutionary consequences of such a strategy.

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Preliminary studies on the interaction *Porcellio laevis* - Nematode parasite

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It is now established that several species of Arthropods are used as intermediate hosts for many nematode parasites. However, little is known about the prevalence and mean intensity of these parasites in Oniscoidae. The aim of this study is to report the presence of nematode parasite belonging to Spiruridae Order which is exclusively a parasite of the proventriculus of domestic and wild birds in natural population of *Porcellio laevis*. Seventy-five adult specimens of *Porcellio laevis* collected from wadi Joumin (Bizerte, Tunisia Northest) in spring 2014, were dissected. We noted that only females (N = 55) were parasitized with a prevalence of 44%. The mean intensity of parasitism is equal to 1.41. We also measured the body length of the parasite which is equal to 14mm. Preliminary results showed the existence of a positive correlation between on the one hand the size and weight of infected females, on the other hand the number of parasite encountered by the female. Such interaction host-parasite could to reduce the density of the natural population of terrestrial Isopods, particularly abundant in this region to tolerable values in their natural environment.

Keywords: Porcellio laevis, Nematode parasite, prevalence, mean intensity

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Reproductive patterns of three *Armadillidium* species (Oniscidea, Isopoda) in Tunisia

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The breeding patterns and reproductive strategies of three conglobating species of terrestrial isopods belonging to three populations: *Armadillidium* sp. from Ichlkeul Parc, *A. sulcatum* from El Feija Parc and *A. tunisiense* from El Jerissa were studied through rearing in the laboratory under natural conditions of temperature and photoperiod during 12 months from December 2010 to November 2011. These three species exhibited a seasonal reproduction followed by a resting period. Moreover, an intraspecific variation of the lag time (the number of days from the beginning of the experiment to the first parturial moult) was observed in the three species and was negatively correlated with the females weight. With the exception of *A. tunisiense*, virgin and isolated females of *A. sulcatum* and *A. sp.* realized spontaneously a normal vitellogenesis, followed by a parturial moult. Moreover, the onset of the female's reproduction of *A. sp.* and *A. sulcatum* was speeded up by the male presence. In fact, the mated females of these two species exhibited a shorter mean lag-time, a shorter period of preparturial intermoult and a smaller number of normal moults preceding the first parturial moult than virgin and isolated females. The presence of a male also extended the reproductive duration. Moreover, *A. sp.* and *A. tunisiense* exhibited a reproductive activity, whereas *A. sulcatum* showed a reproductive period. The fertility (number of released manca) was highly correlated with the weight of ovigerous females. These three species are iteroparous. The species-specific weight seemed to be the most determinant factor controlling the sexual differentiation and the sexual maturity, the gestation period, the mean fertility, the reproductive allocation and the parental investment.

Keywords: seasonal reproduction, North Africa, reproductive allocation, fertility, parental investment

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Biology, population structure, and field-growth rates of *Porcellio lamellatus* (Budde-Lund, 1885) (Isopoda, Oniscidea) at Bizerte lagoon (Tunisia)

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Porcellio lamellatus, a supralittoral terrestrial Isopod, is frequent along Atlantic river side's and Mediterranean coasts. Biology, population dynamics and life cycle were studied by monthly surveys performed from June 2006 to May 2008 on the supralittoral zone of Bizerte lagoon. Ovigerous females were present from February/March to October/November, suggesting that *Porcellio lamellatus* has a seasonal reproduction pattern. Two peaks of ovigerous females were observed, in September and in Mai. Spring peaks appear to be stronger than those observed in autumn (77 % and 64 % of females were carrying eggs in Mai 2007 and 2008 , respectively, compared to 49 % and 42 % in September 2006 and 2007, respectively). Female size was positively correlated with fecundity (number of eggs per brood) and fertility (numbers of embryos hatched per brood). Sex ratio was globally in favour of females (0.86). Furthermore, analysis of the size frequency polymodal distribution showed that five cohorts were identified in the first sampling date and nine new cohorts were detected during the next months. The minimal length of newly born cohorts was 1.39 mm. Lifespan was estimated at 4 to 8 months. *Porcellio lamellatus* behaved as a semi-annual species, with iteroparous females, and a bivoltine life cycle.

Keywords: Porcellio lamellatus, Breeding, Growth, Cohorts, Supralittoral

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Comparative study of the reproductive effort of four terrestrial isopods in the National Park Chaambi (Kasserine - west-central of Tunisia)

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The reproduction of terrestrial isopods has been the subject of many studies, in this one we aimed to study the reproductive effort of four species, which belong to two families, among eight species and three families, which were found in the National park of Chaambi, with the highest peak of Tunisia, mountain Chaambi, with an altitude of 1544 m.

In our study, we compare the reproductive effort of *Porcellio laevis*, *Porcellio variabilis*, *Porcellio djahizi*, *Armadillidium tunisiens*. The last two species are newly discovered and have not been studied before except for their morphological description. The data from this study were monitored weekly for 10 months from June 2013 to January 2014; the sampling of these species was done all over the seven floors in the National Park Chaambi, from 750m to 1544m. *Porcellio djahizi* and *Armadillidium tunisiens* were found from the second floor (850 m) to the sixth one (1400 m). However, *Porcellio variabilis* was found in the first and the second floors, and *Porcellio laevis* was collected only from the first one.

We have analyzed several traits concerning the population dynamics like: reproductive effort, fertility, parental investment, fecundity.

Keywords: *terrestrial isopods, reproductive effort, fertility, fecundity-Chaambi*

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Is the evaluation of isopods' diversity in an area dependent on the duration of sampling? (Crustacea, Isopoda, Oniscidea)

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The methodology of data collection, and more specifically the sampling period, is highly important in order to identify reliable diversity indicators among soil invertebrates. In general, samples collection takes place over a long period of time in order to assure an appropriate sample size for analysis. As a consequence, the procedure could be time consuming and costly in terms of materials and personnel involved. In this study, we investigated whether the abundance and species richness may be affected by seasonality (spring and/or autumn sampling versus throughout the year). Data were obtained following a standardized sampling method, continuously for twenty-four months in two protected areas of Sicily. Student's t-test, Mann-Whitney's test and Kolmogorov-Smirnov test were used to compare mean values of the number of species and individuals collected during the two years with mean values of the observations during even months, during odd months, and during the period between March and October excluding July and August. Comparison between results obtained in the two protected areas separately were also performed. For each period, no statistically significant differences were observed in the mean number of individuals and species. Notably, in all cases, the sex ratio remained constant. Thus, the abundance and species richness would have been unaffected even if the sampling would have been conducted only in even or odd months or just in the above mentioned period only one year.

Keywords: Oniscidea, biodiversity, sampling, comparison, optimization

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Fatty acid composition of *Porcellio albinus* and *Hemilipestus reaumuri* from Gafsa (SW of Tunisia)

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Despite their important biomass in the trophic chain, the biochemical composition of oniscideans has never been studied in Tunisia. In this topic, we analyzed and compared the fatty acid (FA) composition of these two xeric species, *Porcellio albinus* and *Hemilipestus reaumuri* collected in spring at Gafsa (SW of Tunisia). These species showed high similarities in their fatty acid composition made up of twenty-two fatty acids. Saturated fatty acid (SFAs), corresponding to the potential food, dominated in both species with 56% and 61% in *P. albinus* and *H. reaumuri*, respectively. Among them the myristic acid C14:0 showed higher ratio. They were followed by monounsaturated fatty acid (MUFAs) which displayed different proportion in both species (26% in *P. albinus* versus 19% in *H. reaumuri*) and high ratio of C18:1n-7 and C18:1n-9. In the polyunsaturated fatty acids (PUFAs), the omega-3 family exhibited the high value with stearidonic acid C18: 4n-3 in *P. albinus* and linolenic acid (LNA) C18: 3n-4 in *H. reaumuri*. Since that these two species are detritivorous, we hypothesize their capability to synthesize omega-3 and omega-6 fatty acid.

Keywords: fatty acids, PUFAs, *Porcellio albinus*, *Hemilipestus reaumuri*, Tunisia

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Terrestrial isopods, litter and soil: how their chemical signatures impact each other?

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As detritivores terrestrial isopods are a dominant component of the functional groups of decomposers with a remarkable effect on microflora. They fulfil a key role in organic matter turnover by determining the carbon cycle, the rates of carbon sequestration and gaseous emissions and soil organic matter transformation; they are well recognised as potential bioindicators in semi-natural grasslands. We compared the impact of *Armadillidium vulgare*, litter and soil on each other by investigating the chemical profile changes following experiments performed in microcosms and controlled conditions. Our results revealed that (1) the chemical signature of a soil without isopods is different from a soil harbouring isopods (2) the type of soil has an impact on *A. vulgare* chemical signatures. (3) moreover *A. vulgare* has also an impact on soil chemical signatures in both the artificial or cultivated soils (4) finally the results showed that the chemical signature of *A. vulgare* is depending upon their feeding. To conclude, terrestrial isopods, litter and soil chemical signatures can change over time following dynamic interactions between each other.

Keywords: cultivated plots, soil macrofauna, Armadillidium vulgare, microcosms, chemical signatures

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Oniscidea diversity in Vienne (central-western France)

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Terrestrial isopods or woodlice (Crustacea: Oniscidea) are well known in France (Vandel, 1960-1962), and particularly in Vienne (Poitou-Charentes) where local surveys were performed by Legrand (1954-1956). Owing to recent research works from the team Ecology Evolution Symbiosis (Laboratory of Ecological and Biological Interactions) and recent fieldworks from naturalists, the species diversity in Vienne can be updated and comparisons made over time; since 60 years, 37 species were encountered, belonging to ten families. Whatever the survey period 21 species were always listed, at least 31 species were found after the years 2000 (ten new species, but five species without recent sighting). These data give a good idea of the local effort for recording woodlice in Vienne as it was recently done for example in north-western France and in Auvergne. These surveys will greatly contribute to the national atlas of terrestrial isopods (Inventaire INPN n°I232). However, in Vienne, efforts should be continued towards tiny endogean/subterranean species (i.e., Trichoniscidae).

Keywords: soil macrofauna, woodlice, surveys, local diversity

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Woodlice and recolonization of disturbed habitats in the Région Nord-Pas-de-Calais (France)

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The région Nord - Pas-de-Calais is one of the most impacted French territories by artificialisation of the soil following not only its economical and industrial history but also the urbanization and the intensive agriculture. Woodlice - recognized as bioindicators of the soil quality and providing important ecosystem services, such as the decomposition of leaf litter- are studied in order to evaluate how different types of land use could foster the restoration of soil health. Three study sites were investigated by pitfalls and handsearch: (1) « Haute-Borne/Jardin de Cocagne » coming back to an environmentally- friendly agriculture with limitation of both pesticides and physical disturbance of the soil (2) « Union » former industrial site in urban zone and under conversion process (eco-district) (3) « Metaleurop Nord » located in the former mine and affected by the emissions from the past lead foundry; Cd, Pb, and Zn concentrations in these contaminated sites are assessed in soils before sampling terrestrial isopods. Moreover the concentration of copper is evaluated because of its effect on soil biological activities. Today these sites are a mosaic of managed landscapes for restoring the degraded habitats. So far we found seven woodlice species in the sites. Diversity indices are calculated and results discussed according to the level of contamination of the soils and the land-use practices.

Keywords: Land use, soil macrofauna, bioindicators, diversity, restoration

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Up and down: Comparison of two isopod populations from different altitudes

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Over the last century, ecosystems have undergone dramatic changes, accompanied by declines and changes in biodiversity at all levels, from biotopes to species and genetic variation within species. Considerable effort has been invested in ecological research to understand the functional importance of biodiversity as a regulator of ecosystem processes. However, intraspecific phenotypic variation has been considered rarely in this context. Consequently, during this study we want to examine how intraspecific variation affects ecosystem processes, more specifically, how intraspecific variation of an isopod species affects the decomposition process in forests. In addition, due to a postulated increase in extreme weather events in the future, we are also interested in how the intraspecific variation of such a species is affected by environmental extremes and how a population from a strongly fluctuating environment is different regarding leaf litter consumption and breakdown compared to a population from an environment with moderate environmental fluctuation.

Keywords: biodiversity, detritivores, environmental fluctuation, soil processes

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