Molecular taxonomy of *Spirochona gemmipara* (Ciliophora, Chonotrichia), an epibiont living on the gills of *Gammarus fossarum*. First results.

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In memory of Denis Lynn (1947-2018)

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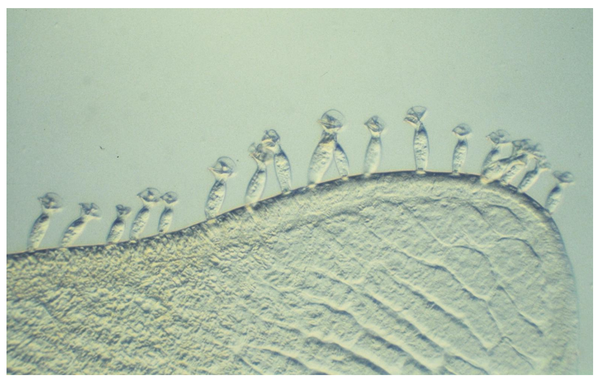
ABSTRACT. The chonotrichs (Chonotrichia, Ciliophora) live settled on diverses appendices (antennas, pereopods, gills, etc) of several kinds of marine and freshwater crustaceans (Decapods, Nebaliaceas, Isopods, Amphipods, …) [1]. The chonotrichs reproduce principally by budding, and exhibit complex life cycles driven by the moulting of their hosts [2]. The chonotrich taxonomy was established in the milestone book of Jankowski (1973) [1] which described more than 40 genera and 100 species living all around the world. Ultrastructural datas are known for some species [3, 4] and molecular datas are actually restricted to 2 species only [5, 6]. Here, we present the SSU seq of *Spirochona gemmipara* sampled in several localities between Geneva and Lausanne.

pulex versus *fossarum* ?

In the '80, the species status of the gammarid basibiont was not clear.
Actually, the morphological distinction of the 2 species (based on the length of the 3rd uropod) is well established, and DNA sequences give unambigous responses.

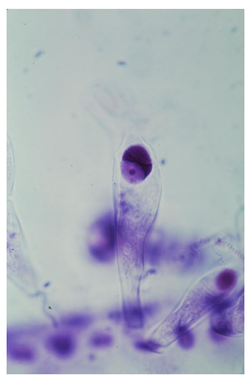
According to COX sequences, the individuals sampled in station B and H all belong to *G. fossarum*.



Gill of *G. fossarum*, live (phase contrast)

S. gemmipara, live (Nomarski)

S. gemmipara, live



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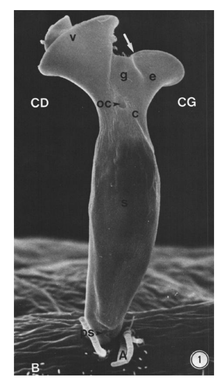
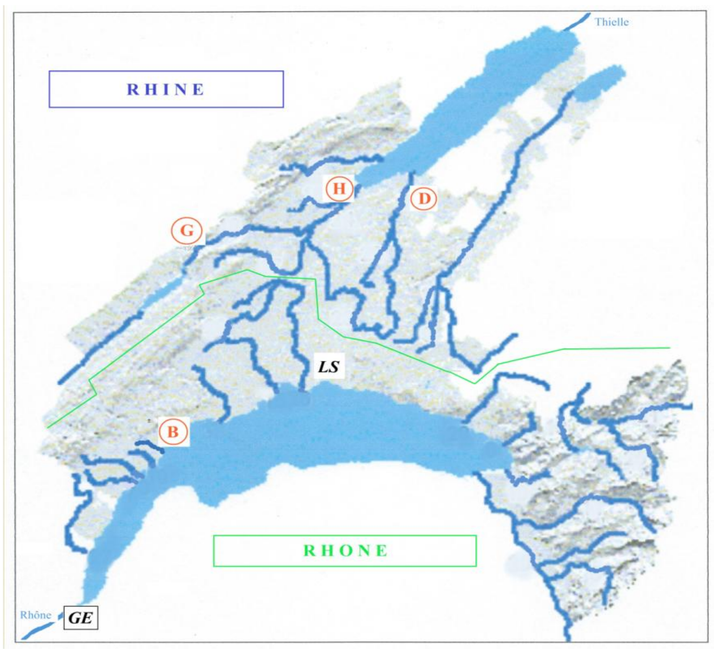


SEM

MATERIAL AND METHODS

The material was sampled in different watercourses between Geneva and Lausanne (Switzerland) in autumn 2018.
Water flow was roughly estimated, air and water temperatures were measured.
Stains and wood branches were displaced with a metallic hook ; moving gammarids were collected in a metallic strainer (Ø 20 cm) and immediately reserved in a small glass jar.
In laboratory, gammarids were kept living in an air-conditionned cabinet, at 10 °C, with a 12/24 h illumination period.
Gammarids were examined under a Wild M5 binocular and gills were resected with a pair of fine tweezers ; the edge of colonized gills were cut off with iridectomy cissors.
Gills fragments were examined and framed under an Olympus BH-2 microscope, then processed for DNA extration.
DNA extractions were done with a Qiagen «Blood and Tissue Extraction kit», and/or in guanidin, after Chomzynsky and Sacchi (1987).
SSU gene fragment were ampified with primers CIL_F (TGG TAG TGT ATT GGA CWA CCA) and CIL_R (TCT RAT YGT CTT TGA TCC CYT) (Stoeck, 2014); products were purified with a Roche «High Pure PCR Cleanup Micro Kit» and then directly sequenced (with the amplification primers) .
Sequences were edited in CodonCode Aligne and BioEdit.
Phylogenetic tree was constructed wih Seaview.



CODES, STATIONS, RIVERS, SEQUENCES.

A	Gollion-Aclens	*La Senoge*	KY349104.1
B	Gland	*La Dullive*	B-3-3a, B57_37
D	Yvonnand	*La Mentue*	D1_71, D2_72
G	Vallorbe	*L'Orbe*	G1_76
H	Orbe	*L'Orbe*	H8_77

The green line separates «Rhone» watershed from «Rhine» watershed .

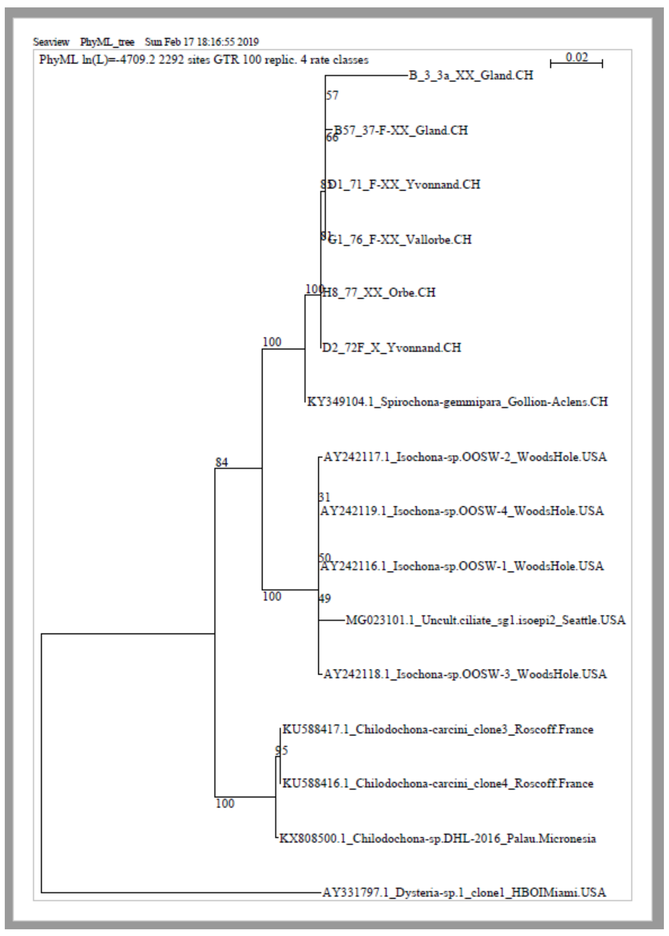
GE = Geneva *LS* = Lausanne

KY349104.1 *S. gemmipara* 18s SSU 1119 bp. (82F – 1055R) DH. Lynn (12-DEC-2016)

RESULTS AND DISCUSSION

The six new swiss spirochones sequences form a solid group, facing 2 other solid groups, the isochonines and the childochonines.
The "uncultured" sequence, coming from organisms not cytologically identified, could be an isochonine or a lobochonine (two close species) or one other chonotrich.
The dysterids are considerd to be chonotrich ancestors. The sequence clearly "roots" the three chontrich groups.

Gammarids were collected in 3 rivers, and 6 stations.
Rivers from station A (Gollion) and B (Gland) belong to the Rhone watershed, while rivers from station D, G and H belong to the Rhine watershed.
The resolution between the new sequences is poor. This is probabely due to the short SSU fragment (ca 600 bp) analyzed. So, it is actually not possible to know if the spirochones of the Rhone and Rhine watershed are different.



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