Molecular taxonomy of the Spirochonidae (Chonotrichia, Ciliophora) from the Baïkal Lake. First results.

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ABSTRACT. The Baïkal Lake appeared some twenty-five million years ago, and is actually the biggest freshwater lake in the World.
It harbours more than 600 plant and 1500 animal species, including more than 250 freshwater amphipod species. These crustaceans host a number of epibionts, among which the ciliates are represented by various kinds of Peritrichia, Suctoria and Chonotrichia.
According to the Jankowski book, («Chonotricha», 1973), at least 10 species (in 3 genera) of the Spirochonidae family live on diverse appendages of Baïkal amphipods.
In order to complete the cytological datas, we planed to obtain molecular data.
In july 2018, during a 9-days scientific cruise on the Baïkal Lake, we have collected amphipods from sediments dredgings.
More than 100 specimen were examined and dissected under binocular, 15 of them carrying spirochonids.
The gills were micro-photographed and stored in ethanol.
In 2019, DNA was extracted, SSU-amplified and sequenced. The first molecular datas are presented here.

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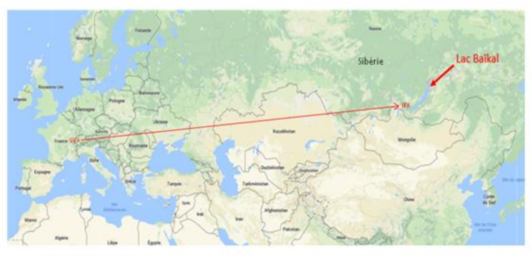
In memory of Denis Lynn (1947-2018)

GENEVA – IRKUTSK - BAIKAL



GVA : Geneva IRK : Irkutsk

Geneva - Irkutsk: ca. 7'000 km
6 time zones : GVA + 6 h
Flight : Geneva - Moscow / Moscow – Irkutsk

IRKUTSK – BAÏKAL LAKE

The Baïkal Lake	The lake of superlatives :
	- the oldest freshwater lake (25 million years) ; - in volume, the world's biggest lake (23'600 km³) ; Tanganika Lake : 18'900 km³. - the world's deepest lake, with a maximum depth of 1,642 m ; - more than 300 affluents (north : sup. Angara ; south : inf. Angara ; ...) - 600 plant species ; - 1'550 animal species, with 350 amphipods species (1/3 of the known species) ; - the water temperature ranges from 4 °C to 16 °C in summer ; - the lake is covered by ice 5 months a year ; Notes : - The **Baikal seal** or nerpa (*Pusa sibirica*) is found throughout Lake Baïkal. It is one of only three entirely freshwater seal populations in the world, the other two being subspecies of ringed seals. - The **sponge** *Lubomirskia baicalensis*, looking like mexican cactus, can reach 1m high ; it is colored in green by symbiotic *Zoochlorellas*. - The **copepod** *Epischura baikalensis* forms 90% of the planctonic mass.

THE BAÏKAL SPIROCHONIDAE PROJECT

The "Baïkal Spirochonidae Project" was initiated at the ICOP_2017_Prague, by Lynn – Fahrni – (Sherbakov).

Background :
Baïkal Lake is a paradise for amphipods (250 species) and for the chonotrich Family Spirochonidae .
According to Jankowski, the family counts 2-3 genera (*Cavichona*, *Spirochona*, *Serpentichona*?), with about 25 different species, leaving on some 30 different host species.

Goals : **Exploring the Jankowski's work on the Spirochonidae Family.**
The main goals of the study are:
- Obtaining a clear phylogenic tree of the family;
- Clarification of some synonymies (*Serpentichona* / *Spirochona* - *anthus*),
- (*Cavichona* / *Spirochona* –*elegans*), (*Cavichona* / *Spirochona* - *elegantula*);
- Clarification of the "genus" status of some (several) "similar" species;
- Possible new genera or species;
- Relationships between hosts and epibionts;
- Possible co-evolution between hosts and epibionts (Sherbakov).

Tasks : The tasks were roughly distributed as follow:
- Fahrni: - collection of Spirochonidae living on amphipods from Baïkal Lake (July 2018);
- Lynn: - DNA extraction, sequencing, phyllogenomy of the collected Spirochonidae ;
- Sherbakov: - organisation of the sampling cruise on the Baïkal Lake;
  - phylogenomic of the collected amphipods hosts.

Funding: Lynn, Fahrni (?), Sherbakov.

Actual state:
- I have participated to the 2018 sampling cruise on Baïkal Lake (12-21.07.2018).
- I have recolted gills bearing chonotrichs (photos) from some 18 amphipods hosts; gills are preserved in EtOH (for DNA extraction), in osmium (for protargol staining), and in glutaraldehyde (for SEM examination). Cytological studies in progress.
- I keep the EtOH preserved gills samples.
- Dr. Sherbakov keeps the 18 amphipods specimen (in EtOH) for molecular identification and phylogenomic study.

The tragedy : Denis Lynn died accidentally on 26.06.2018.

29.10.2018 : J. Fahrni restart the molecular work, hosted in the laboratory of Ass. Prof. J. Pawlowski *

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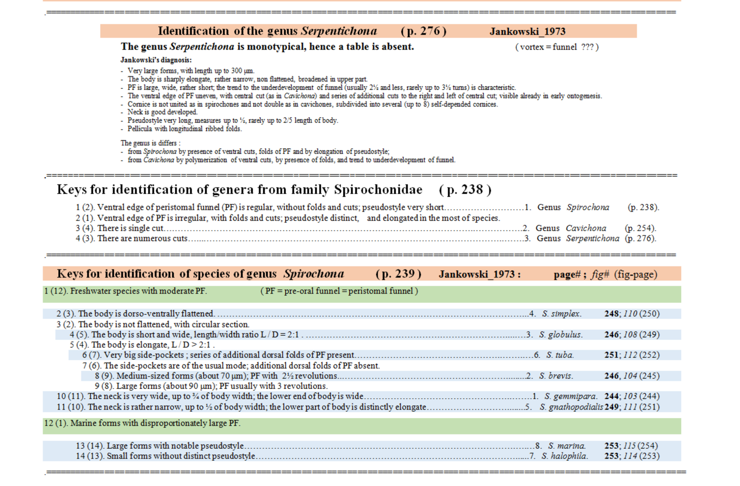
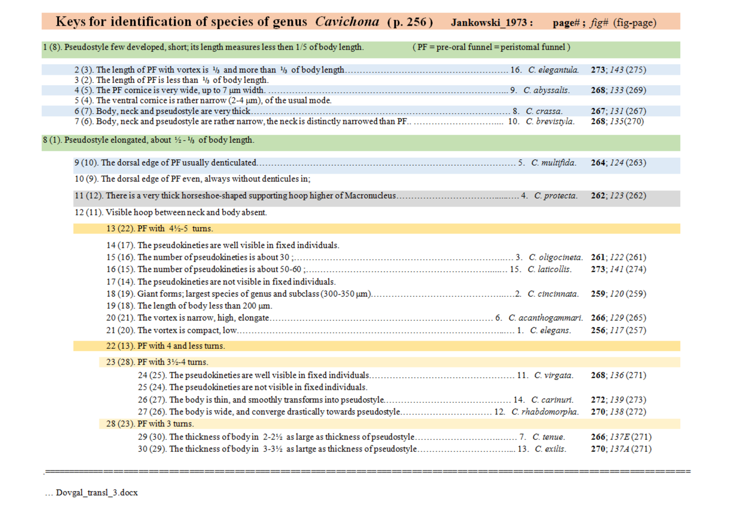
THE JOB : to get a phylogenomic tree corresponding to the huge morphological diversity described by JANKOWSKI (1973).

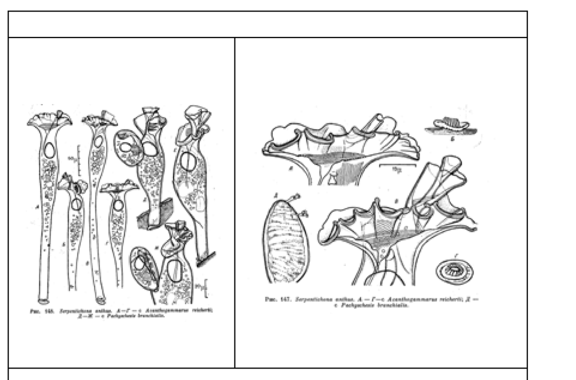
The Spirochonidae (Jankowski, 1973)
- 3 genera : *Cavichona, Spirochona, Serpentichona ;*
- 25 species :
  - *C. abyssalis ; acanthogammari ; brevistyla ; carinuri ; cincinnata ; crassa ; elegans ; elegantula ; exilis ; laticollis ; multifida ; oligocineta ; protecta ; rhabdomorpha ; tenue ; virgata .*
  - *Spirochona brevis ; gemmipara ; globulus ; gnatopodialis ; halophila ; marina ; simplex ; tuba .*
  - *Serpentichona anthus .*

JANKOWSKI's DETERMINATION TABLES





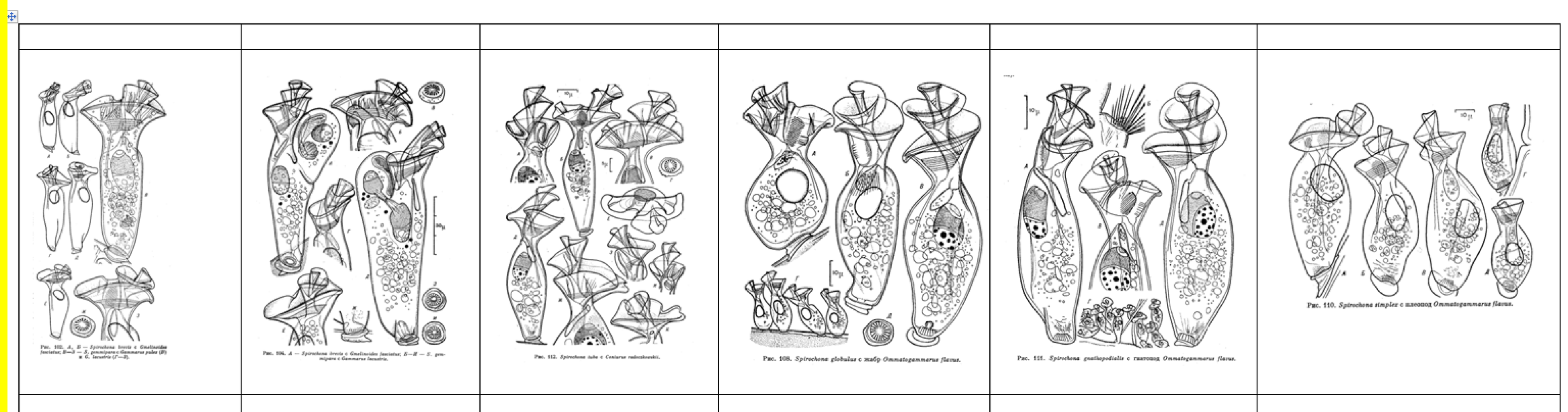
Genus *Serpentichona* morphology



Serpentichona anthus Swarczewsky, 1928
syn : *Spirochona anthus* Swarczewsky, 1928 (Cf. J. 1973).
Acanthogammarus reicherti

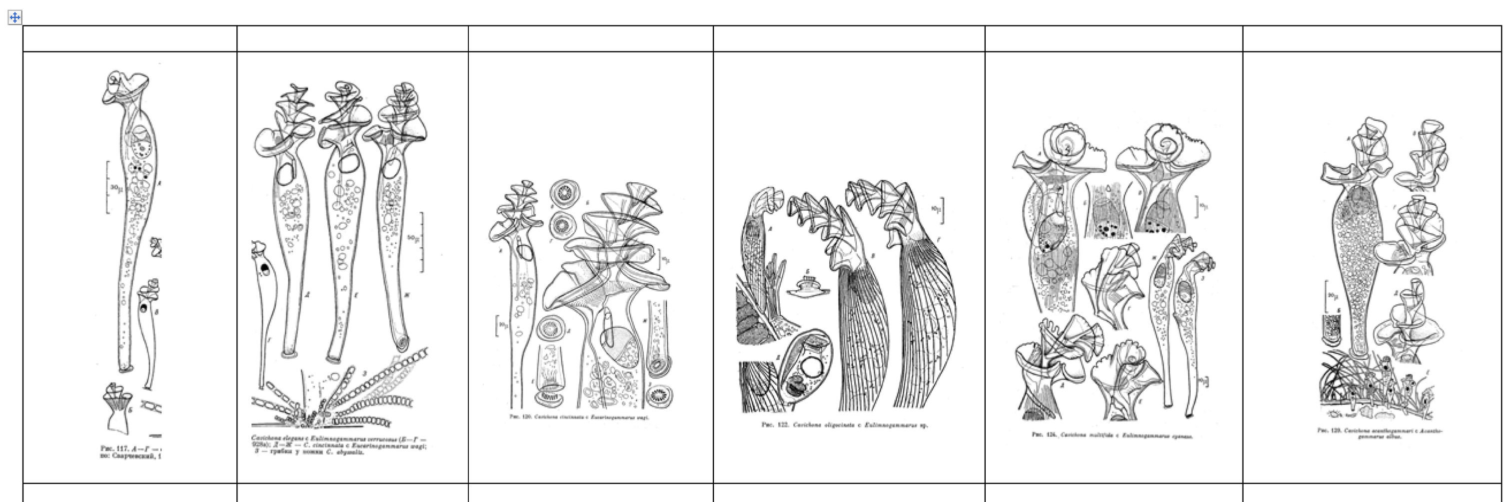
Serpentichona_morph-2.docx

Genus *Spirochona* morphology (JANKOWSKI, 1973) Spirochona_morph-2.docx



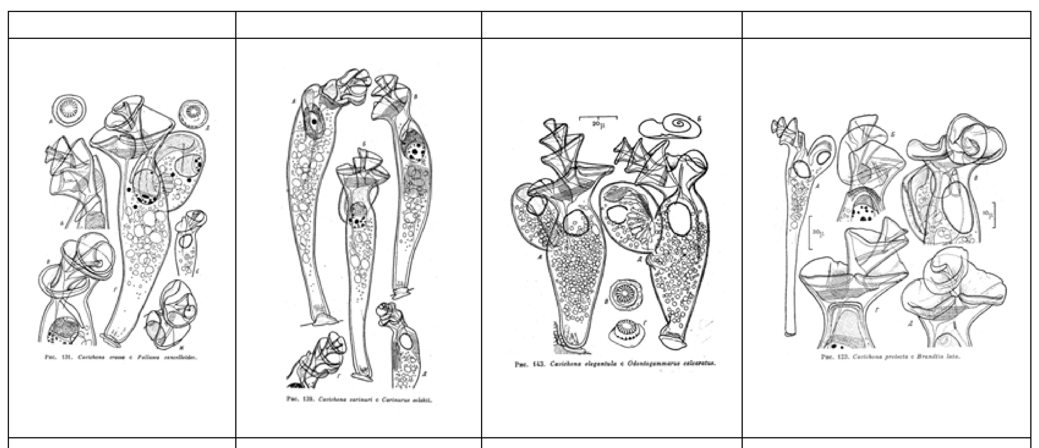
Spirochona - *gemmipara*. *G. pulex*, *G. lacustris*	*Spirochona* - *brevis*, J. 1973. *Gmelinoides fasciatus*	*Spirochona* - *tuba*, Sw. 1928. *Acanthogam*., *Odontogam*., *Coniurus*	*Spirochona* - *globulus*. *Ommatogammarus flavus* *Pallasea kessleri*	*Spirochona* - *gnathopodialis*. *Ommatogammarus flavus*	*Spirochona* - *simplex*. *Ommatogammarus flavus*

Genus *Cavichona* morphology (JANKOWSKI, 1973) Cavichona_morph-2a.docx



Cavichona elegans. *Eulimnogam. verrucosus*	*Cavichona cincinnata*. *Eucarinogammarus wagi*	*Cavichona cincinnata*. *Eucarinogammarus wagi*	*Cavichona oligocineta*. *Eulimnogammarus sp.*	*Cavichona multifida*. *Eulimnogammarus cyaneus*	*Cavichona acanthogammari*. *Acanthogammarus albus*

Genus *Cavichona* morphology (JANKOWSKI, 1973)



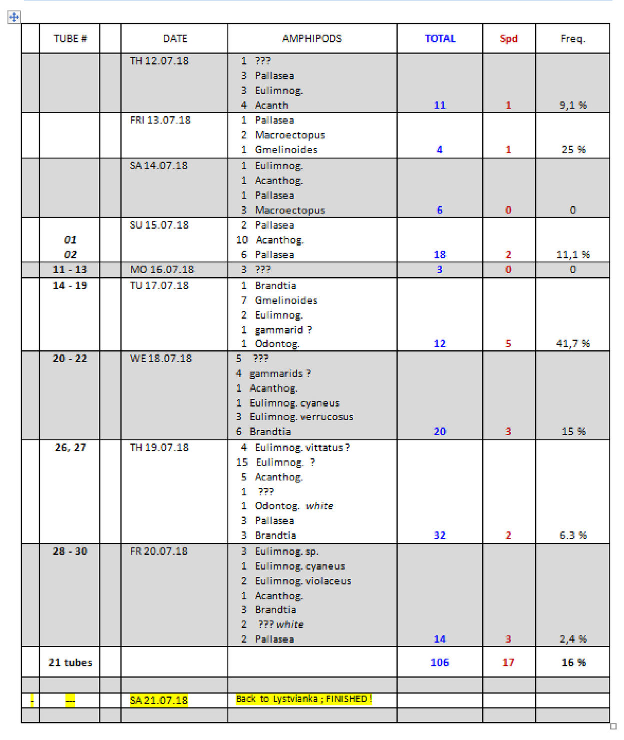
Cavichona crassa. *Pallasea cancelloides*	*Cavichona carinurii*. *Carinurus solskii*	*Cavichona elegantula* *Odontogam. calcaratus*	*Cavichona protecta* *Brandtia lata*

Spirochona gemmipara – Presentation

① *Gammarus fossarum*, living. ② Position of the gills. ③ Gills ( ), ventral (SEM). ④ Gill of *G. foss.* with several *S. gemmipara*. ⑤ Living *S. gemmipara* (DIC) ⑥ *Spg*, live (DIC) ⑦ *Spg*, fixed (SEM) ⑧ *Spg*, fixed (Protargol) ⑨ *Spg*, fixed (Feulgen)

Preliminary molecular work

BAÏKAL 2018 – Amphipods samplings



"Species" and number of amphipods collected during the cruise (12-21.07.2018).
- in blue : total number (by day) of amphipods collected and examined.
- in red : total number (by day) of amphipods bearing Spirochonidae.
- in black : frequency (in percent) of amphipods bearing Spirochonidae.

cruise-sampling_3.docx

BAÏKAL – MOLECULAR 1

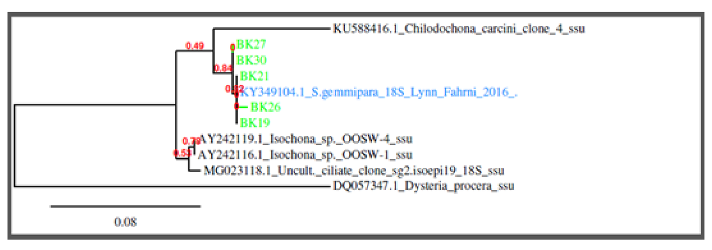
BLAST IDENTIFICATION OF THE CILIATES AND THEIR HOSTS (BAÏKAL_July 2018)

Amplif_date	Sample	PCR/no	Minigel	Seq.	BLAST	PCR/no	Minigel	BLAST	HOST
		CIL				CoxV			
18.03.2019	BK 19		+	+	*Spg*_Lynn 99%		+	FJ756323	*Eul-cyaneus* 87%
	BK 21		+	+	*Spg*_Lynn 99%		+	FJ756323	*Eul-cyaneus* 87%
	BK 26		+	+	*Spg*_Lynn 98%		+	FJ756344	*Pall_kessleri* 95%
	BK 27		+	+	*Spg*_Lynn 99%		+	AY926654	*Brandtia lata* 94%
	BK 30		(-)	(-)	*Spg*_Lynn 99%		+	AY926654	*Brandtia lata* 96%
		CIL				CoxV			
13.06.2019	BK 15.1	63	-	-	-	83	(+)		*Eul-vittatus/verrucosus*
	BK 19.1	64	-	-	-	84	(+)		---
	BK 20.1	65	(+)	(+)	Suct. *Acineta*	85	+		*Pallasea/Babr/Nyphargus*
	BK 20.2	66	-	-	-	86	+		*Pallasea/Babr/Nyphargus*
	BK 28.1	67	-	-	Suct. *Acineta*	87	+		*Eul-virridis/verrucosus*
	BK 28.2	68	-	-	Suct. *Acineta*	88	+		*Eul-verruc/Garajewia/G-balc.*
	BK 29.1	69	+	+	Suct. *Acineta*	89	++		---
	BK 29.2	70	+	+	Suct. *Acineta*	90	++		*Acanthogam-victorii*
	BK 29.3	71	+	+	Peritr. *Vorticella*	91	++		*Acanthogam-victorii*
	BK 32.1	72	+	-	-	92	+		*Brandtia_lata/latissima*
	BK 33.1	73	(+)	-	-	93	(+)		*Brandtia_lata/latissima*
		CIL							
24.06.2019	BK 15.1	102	-		-				
	BK 19.1	103	-		-				
	BK 20.2	104	-		-				
	BK 29.1	105	+		*Acineta flava*				
	BK 29.2	106	++		*Acineta flava*				
	BK 32.1	107	(+)		*Spg_Lynn*				

baïkal – molecular_2.docx

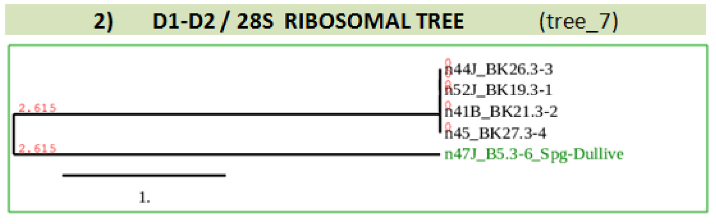
BAIKAL – MOLECULAR 2 Baikal_trees_2.docx

1) CIL / 18S RIBOSOMAL TREE



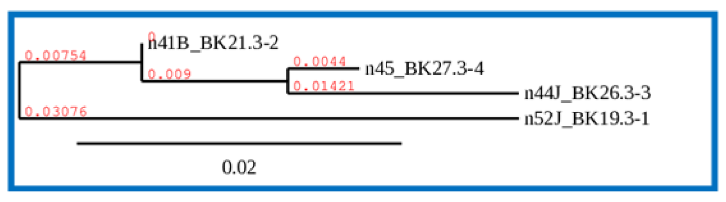
- in black : Available Chonotrichia sequences (with *Dysteria* as root) ;
- in green : Baikal sequences (this study) ;
- in blue : *S. gemmipara*_Lynn_2016 .

2) D1-D2 / 28S RIBOSOMAL TREE (tree_7)



- in black : Baikal sequences (this study) ;
- in blue : *S. gemmipara*_Dullive_2019.

2) D1-D2 / 28S RIBOSOMAL TREE (tree_9) Baikal only !



- in black : Baikal sequences (this study) ;

CONCL.: The D1-D2 fragments contain very SLIGHT differences !

BAIKAL – MOLECULAR 3

RESULTS

1) The number of Baikal spirochonids sequences obtained is low : (**5 sequences** / 106 amphipods analysed) !
2) With 18S (CIL), the 5 sequences are 99% identical between them ;
3) With 18S (CIL), the 5 sequences are 99% identical to the *S.gemmipara*_Lynn_2016 seq. ;
4) With 18S (CIL), the 5 sequences are 99% identical to the *S.gemmipara*_Fahrni_2019 seq. (unpubl.) ;
5) The 18S (CIL) sequences of several other ciliates (Suctoria, Peritrichia) were obtained ;
   The 18S (CIL) gene fragment is not appropriate to discriminate different species (if any ?) ;
6) Other gene fragments are tested (V9 ; ITS; 28s) ; works in progress ;
7) **Five D1-D2 (28S) sequences were obtained; they contain very SLIGHT differences between them ! (see " BAIKAL – MOLECULAR 2 ")**
8) All gene fragments tested gave results with positive control species (*Paramecium*, *Vorticella*, ...) ;
9) **The amphipods molecular identication with the CoxV fragment is satisfying .**

CONCLUSIONS

1) General contamination with *S.gemmipara*_Fahrni_2019 material ? unlikely (see tree_7) ?
2) The D1-D2 sequences must be checked; then sequenced again;
3) The D1-D2 amplifications and sequencing must be done again;
4) New DNA extractions are needed;
5) Getting new amphipods (and spirochonids !) from an other Baïkal_2019 cruize ? (Dr. D. Sherbakov's help needed.)
6) Looking for others gene fragments needed .

LITERATURE

1. JANKOWSKI, AW. **1973**. Infusoria subclass Chonotricha. Faune URSS, 2 (1): 1-355. (Akad. Nauka SSSR, St.-Petersburg).
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