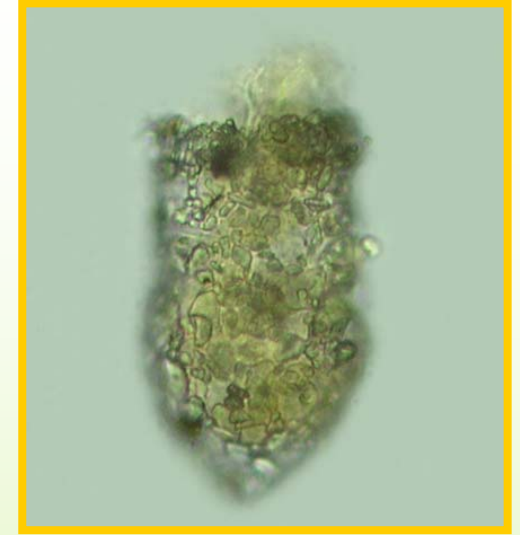


Barcoding Ciliates - A Phylum With Highly Divergent CO1 Sequences



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Phylum Ciliophora

Characterized by

- Infraciliature
- Nuclear dimorphism
- Conjugation



The Cytochrome Oxidase Subunit 1 Gene in Ciliates

First successfully amplified in *Tetrahymena thermophila*, *T. pyriformis*, and *Paramecium aurelia*

CO1 is 2000-2200 nucleotides long and very AT-rich

Barcoding region is 980 nucleotides long, due to an 108 aa insert

Origin of the sequenced species

ATCC (*Tetrahymena* spp.)

Spain (scuticociliates endosymbiotic in fish)

Italy (*Colpidium* sp., *Dexiostoma* sp., *Drepanomonas revoluta*, *Dexiotricha* sp.)

Russia (*Paramecium* spp.)

Guelph (mostly undetermined species)

Grand River Basin (*Carchesium polypinum* and other peritrich species)

Sampling

- cultures received from colleagues or culture collections
- water samples from various lakes and rivers
- petri dishes submersed for 1-2 weeks in rivers of the Grand River Basin

Cultures

- Cells are isolated and - if possible - clonal cultures are established
- various media, e.g., Cerophyl, PPYE, spring water and grains

Lab Procedures

DNA extraction

- Epicentre Masterpure Kit or Chelex[®]

PCR amplification

- Diamond Taq
- various amplification protocols necessary for different groups
- several primer pairs developed

Purification

- MinElute Kit
- sometimes direct sequencing possible

Sequencing

- both directions with 3730 DNA Analyzer

Post-sequencing analyses

- Contigs constructed with Sequencher
- BLAST Search
- Alignment with Mega 3.1
- Phylogenetic analyses with MrBayes, PAUP, PHYLIP

Problems

- Culture Collections have only few genera available
- Axenic cultures only sometimes possible
- Maintenance of cultures is time consuming
- Determination of species requires in many cases Protargol staining and microscopical analysis
- Primer design progresses very slowly
- Standardisation of molecular procedures not (yet?) possible

Where do we stand?

Most CO1 gene sequence data available are restricted to members of the Class Oligohymenophorea

We have...

77 isolates of 28-35 species in 16 genera, representing the four subclasses Hymenostomatia, Scuticociliatia, Peritrichia, and Peniculia

We also have...

Sequences of 2 or 3 species from other classes

A) Insert Region

thermo	FGGFANFLIP	YHVGSKDVAY	PRLNSIGFWI	QPCGYILLAK	IGFLRPQFWR	YYDKTSFSFFLEKMKYNQY	KEYK--NDYL	FYLDPLKKEI	T-DDHSFF--	WKARKVIKLP	QYSVFSFVPL	KLMMW----	----K	
bergerL.....D.....F.....	S.....T.....F.....	
colpoL.....I.....S.....F.....	
ostomaI.....S.....	
chattY.....L.....L.....T.....S.....F.....D.....R.....G.....E.....F.....I.....L.....S.....Q.....I.....--I.....T.....V.....E.....S.....Q.....S.....I.....L.....I.....	
multiF.....A.....N.....L.....L.....F.....Y.....K.....P.....F.....P.....N.....M.....L.....V.....N.....Y.....--D.....T.....I.....N.....N.....V.....I.....--V.....N.....L.....S.....D.....L.....S.....D.....N.....M.....T.....I.....F.....F.....	
catenF.....A.....N.....L.....L.....F.....Y.....K.....P.....F.....P.....N.....M.....L.....V.....N.....Y.....--D.....T.....I.....N.....N.....V.....I.....--V.....N.....L.....S.....D.....L.....S.....D.....N.....M.....T.....I.....F.....F.....	
tylis	L.....L.....F.....L.....L.....I.....M.....N.....V.....Y.....T.....N.....N.....E.....V.....E.....N.....Y.....K.....M.....K.....N.....S.....T.....H.....T.....N.....	A.....F.....F.....L.....--	D.....E.....T.....I.....N.....F.....F.....I.....T.....K.....T.....G.....S.....	F.....F.....K.....E.....G.....N.....R.....D.....P.....K.....	Y.....K.....Y.....D.....A.....T.....E.....N.....H.....T.....E.....N.....	L.....I.....L.....	T.....E.....N.....H.....T.....E.....N.....	T.....I.....E.....N.....H.....T.....E.....N.....	T.....I.....E.....N.....H.....T.....E.....N.....	T.....I.....E.....N.....H.....T.....E.....N.....	
polCR3	LE.....Y.....F.....L.....N.....L.....F.....L.....I.....M.....S.....M.....L.....E.....Y.....T.....T.....E.....V.....F.....S.....G.....L.....T.....V.....D.....N.....F.....L.....Q.....Q.....Y.....K.....Y.....--	D.....E.....I.....F.....H.....N.....I.....K.....K.....S.....I.....S.....N.....Y.....	D.....Y.....S.....R.....L.....L.....V.....L.....K.....	D.....A.....T.....K.....D.....I.....D.....K.....L.....	H.....D.....F.....T.....Y.....R.....K.....L.....R.....	D.....Q.....A.....I.....L.....D.....A.....E.....L.....	H.....K.....G.....M.....	
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thecodG.....Y.....F.....V.....I.....F.....Q.....P.....E.....V.....V.....N.....F.....S.....I.....L.....L.....S.....L.....L.....I.....L.....S.....I.....S.....I.....E.....	



thermo	TMINY-PESF	WYAASRVVQ-	-----SRR	KKVFPVTKCSA	RTLTTAGWTF	ITPSSNKY	TGVGSQDILI	LSVVFAGIST	TISFTNLLIT	RRTLAMPGLR	HRRVLMFPVT	ISIFLTLRML	AT
berger
colpo
ostoma
chatt
multi
caten
tylis
polCR3
olCR23
icella
otrich
hryoid
nophry
ienavi
gl_SD
i SR10
tetGB
cauda
multi
bursa
woodr
ne WS
anomom
coKMP2
ambi
thecod



B) CO1 as marker for species identification

Genetic distances:

T. thermophila d = 0 - 0.01

P. caudatum d = 0 - 0.08

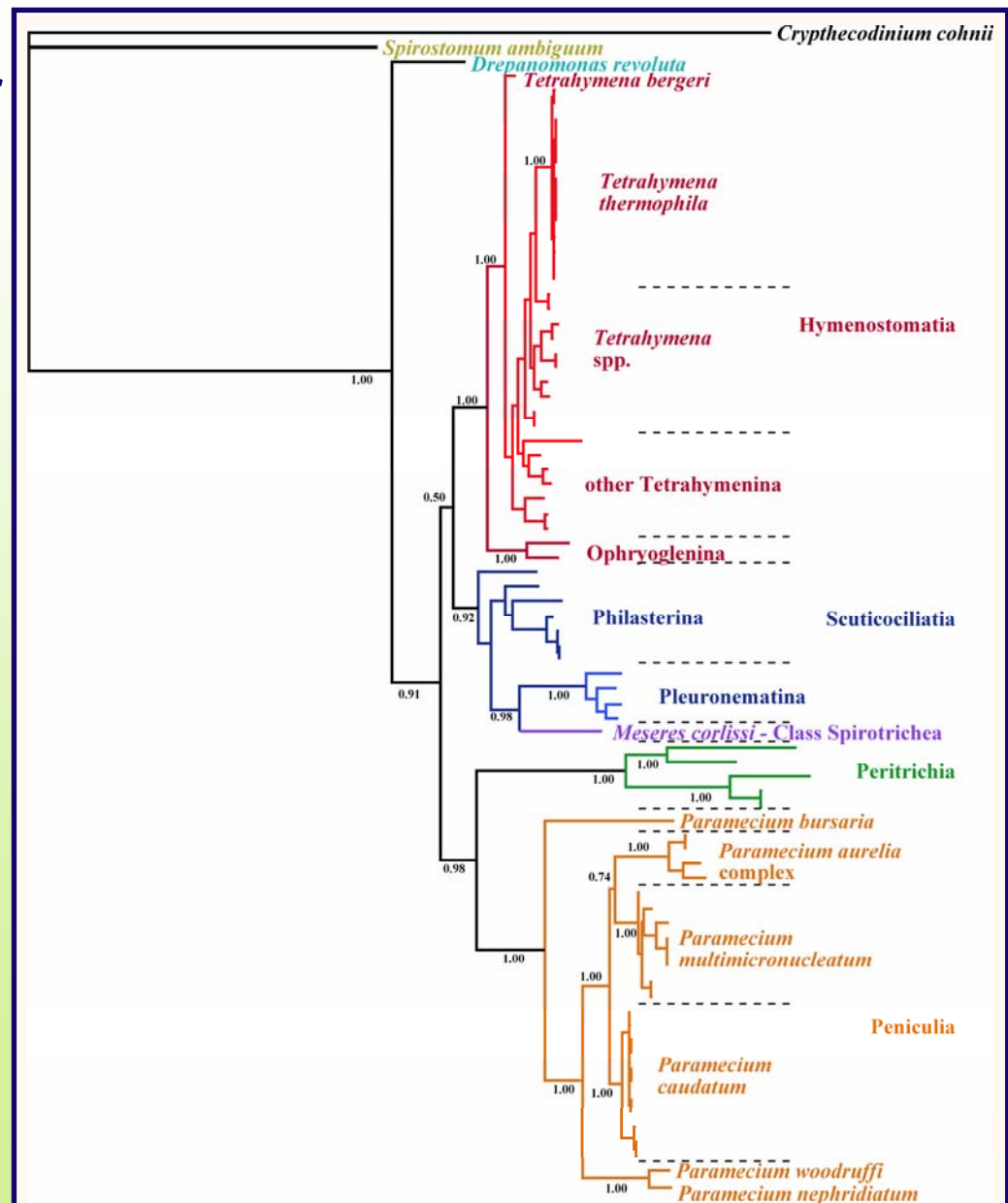
Tetrahymena spp. d = 0.03 - 0.13

Paramecium spp. d = 0.11 - 0.39

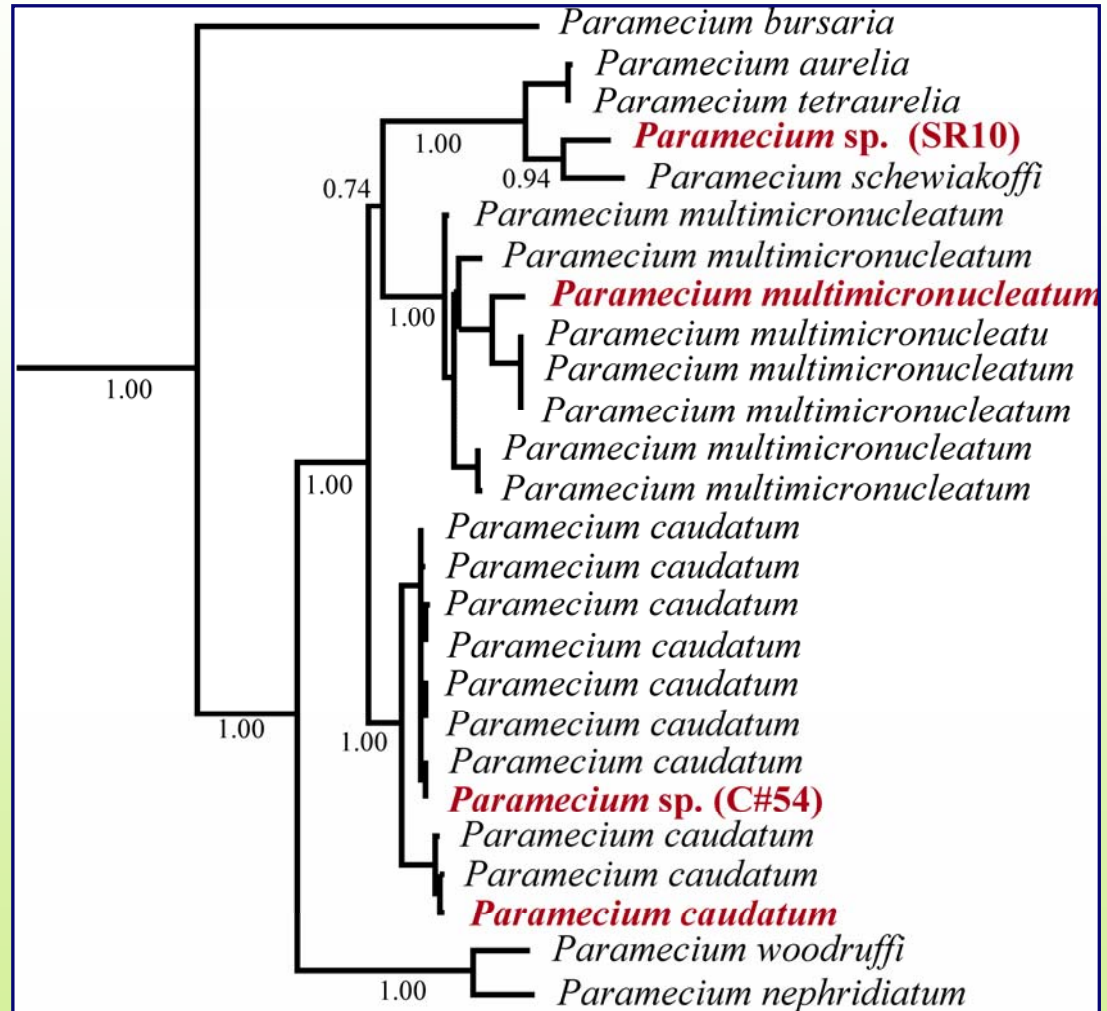
Peritrichia d = 0.14 - 0.21

Hymenostomatia d = 0.03 - 0.20

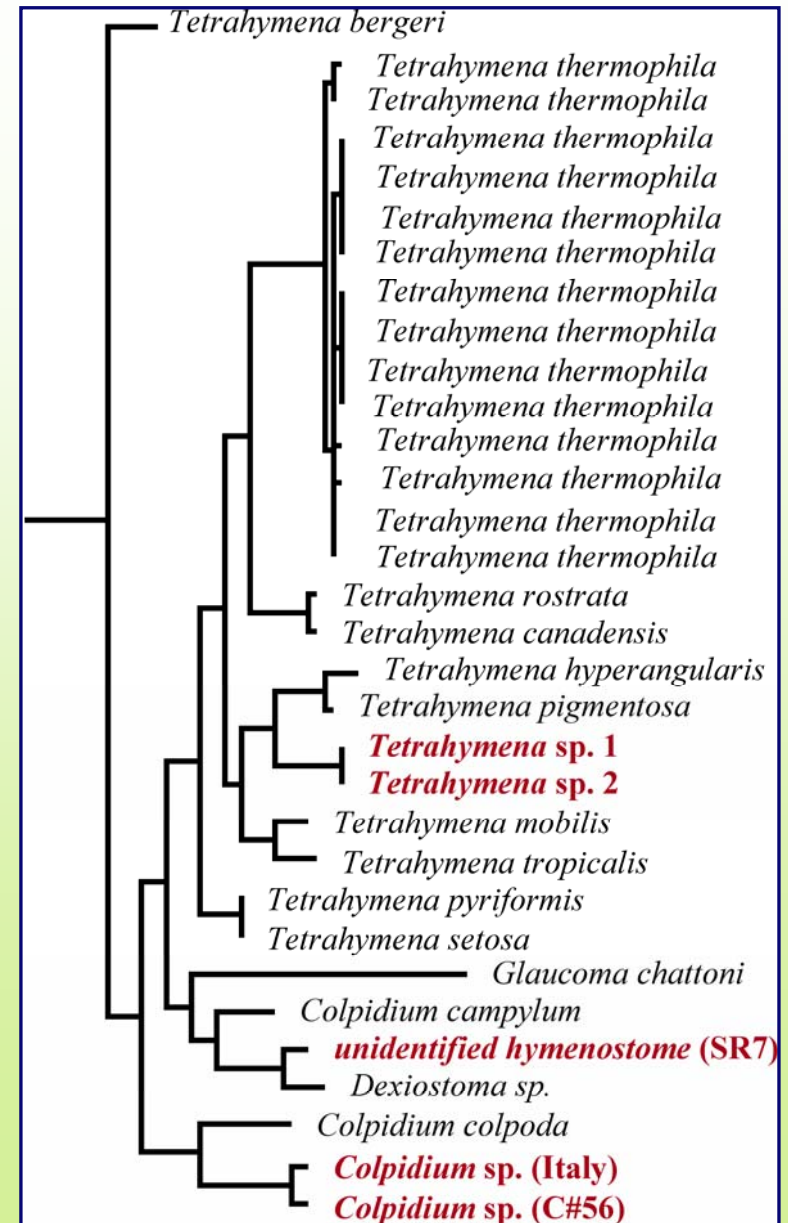
Scuticociliatia d = 0.05 - 0.28



C) Identifying Species



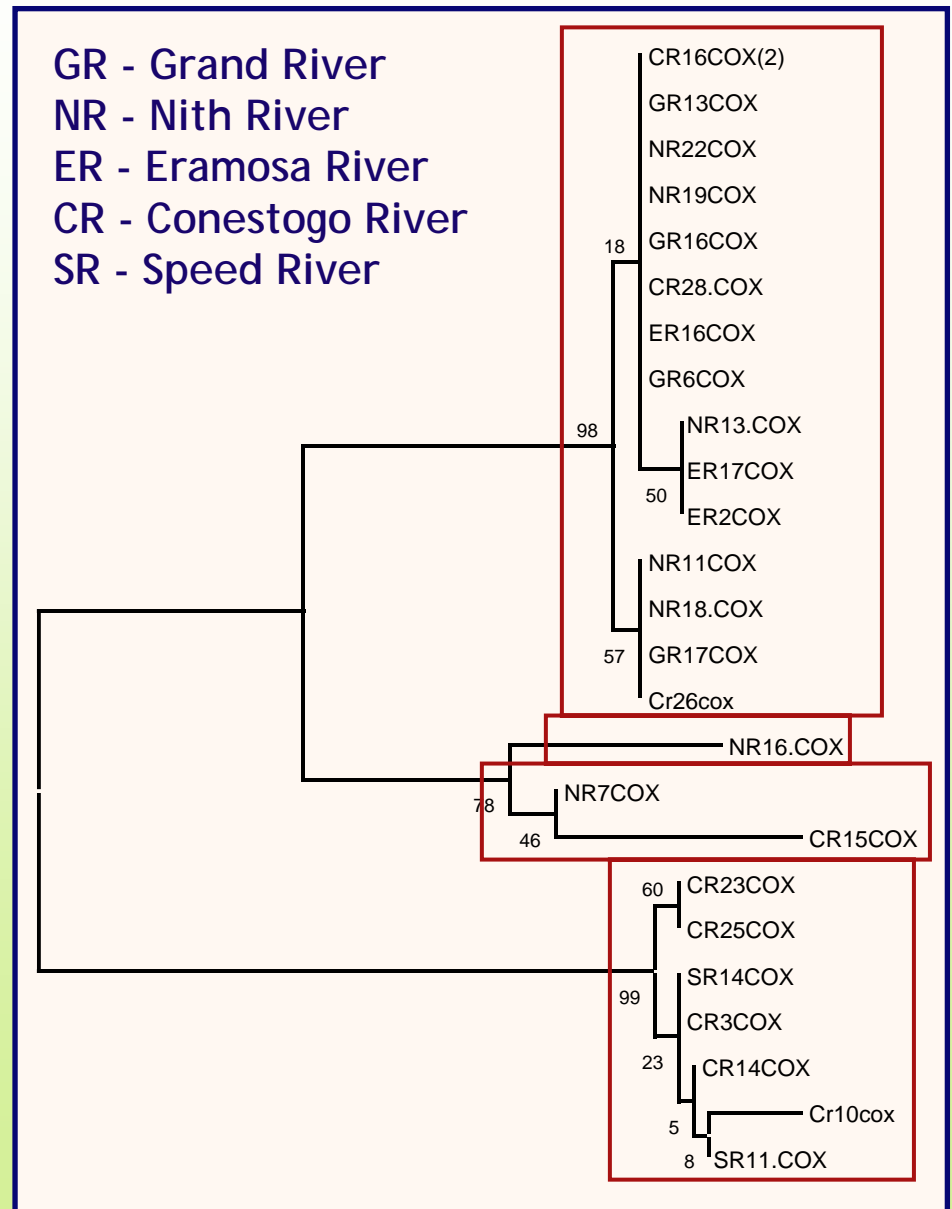
C) Identifying Species



D) Phylogeography



Populations of *Carchesium polypinum*



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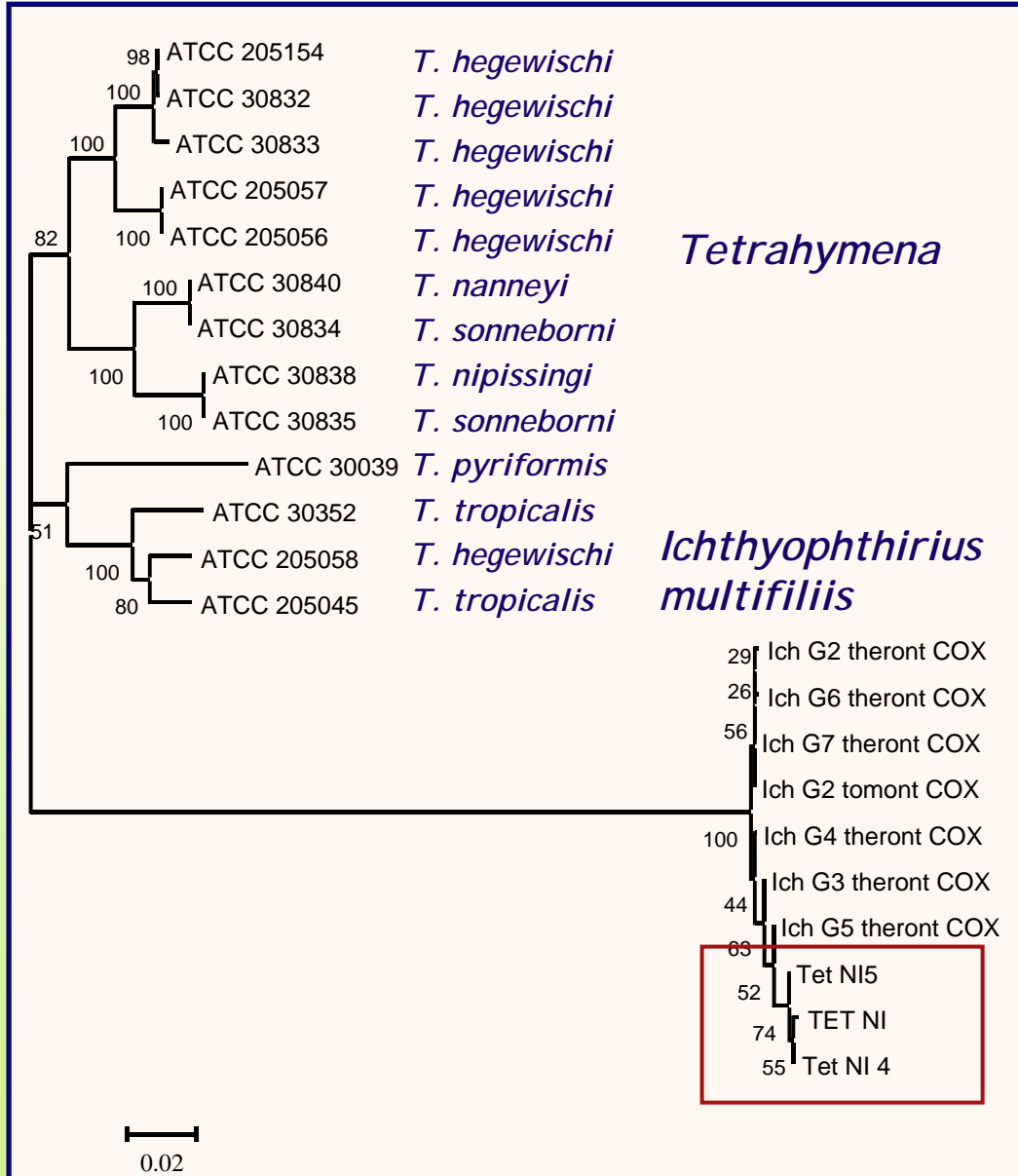
- Genome Canada, Ontario Genomics Institute, Natural Sciences and
Engineering Research Council of Canada

- Micro*scope Website (<http://starcentral.mbl.edu/microscope>)





C) Identifying and Validating Species



B) CO1 as marker for species identification

Genetic distances:

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