

DNA barcoding of midges (Diptera: Chironomidae)

First episode: building the base

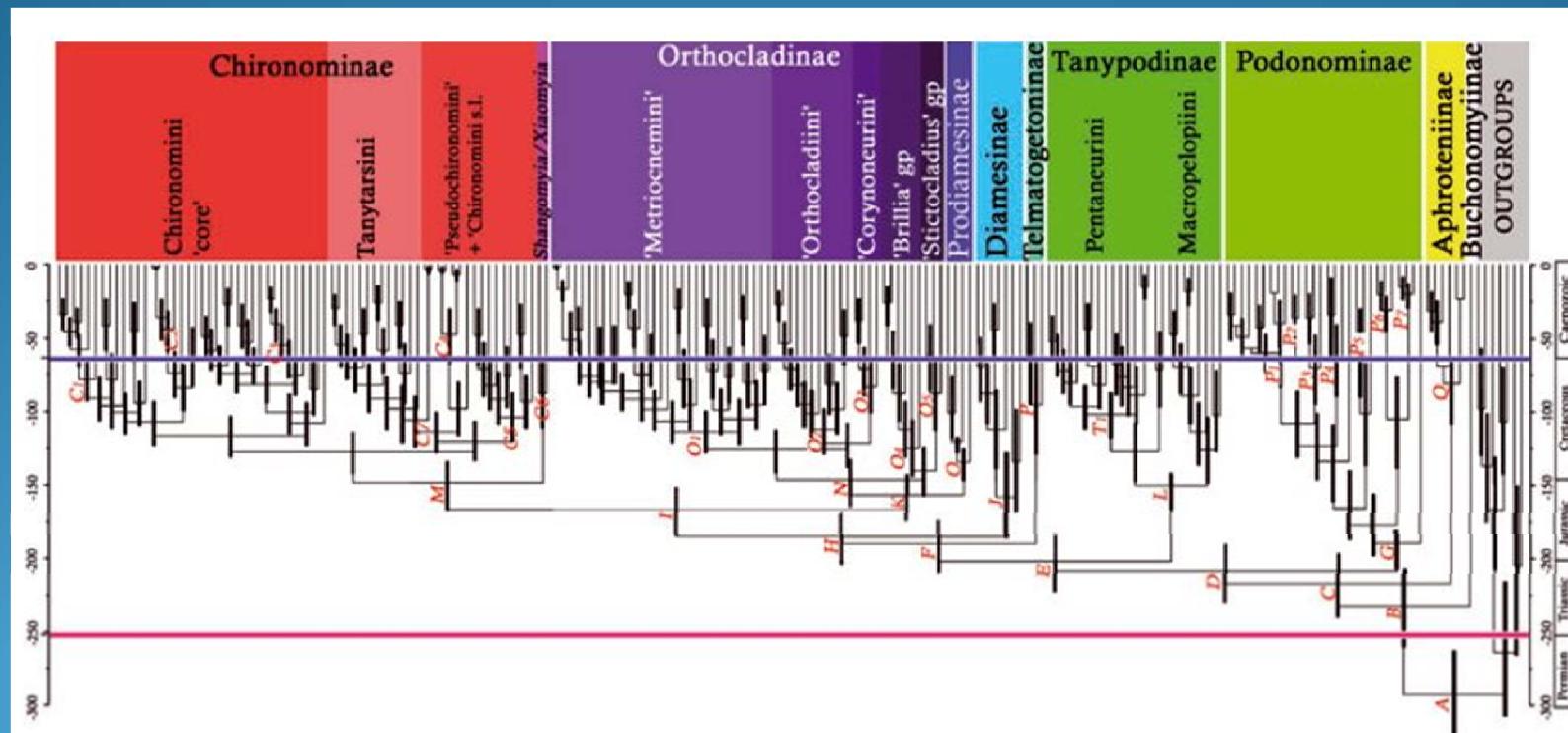
Alexander Klink



Presentation on the DNA barcoding conference of
Naturalis June 3 2015



Chironomidae date back to Trias and all main groups are present since 60 My BP



After Cranston et al. (2011)

Life stages for identification of Chironomidae: Larva



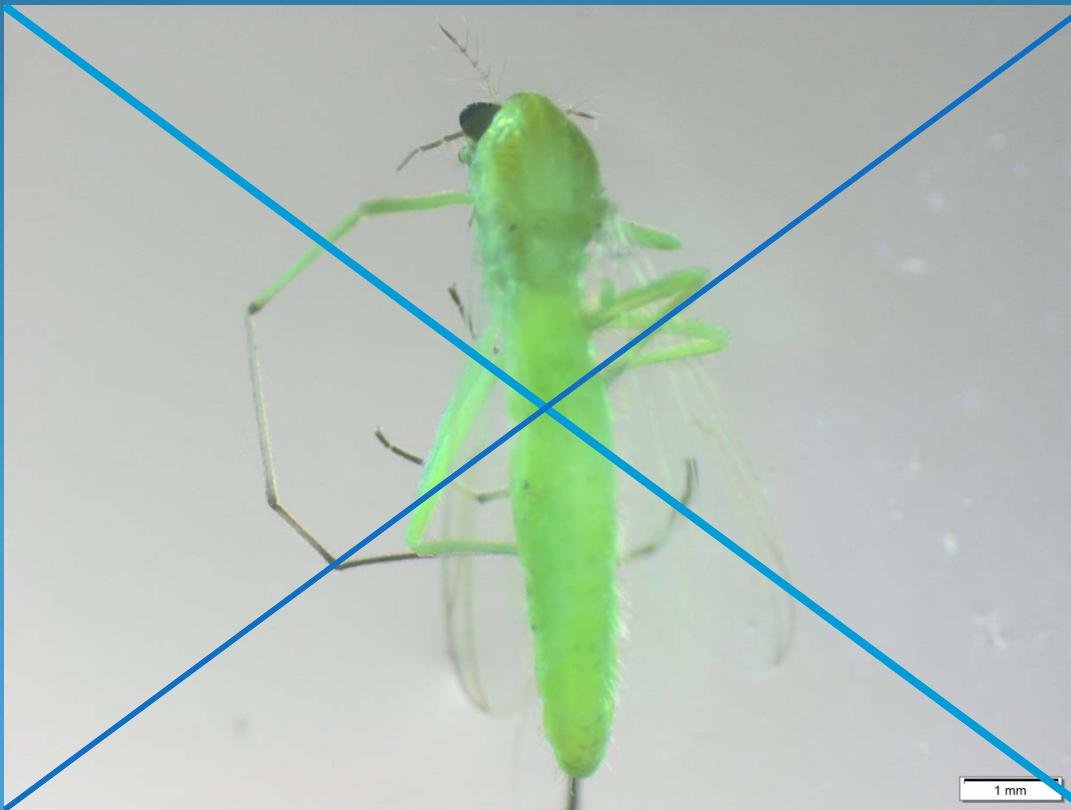
Life stages for identification of Chironomidae: Pupa and pupal exuviae



Life stages for identification of Chironomidae: Adult ♂



Life stages for identification of Chironomidae: Adult ♀



Role of preservation on DNA recovery in routine aquatic samples

Stage	Larva			Pupa			Pupal exuviae		
	< 1	> 2	≥ 5	< 1	> 2	≥ 5	< 1	> 2	≥ 5
Years in storage before recovering DNA	< 1	> 2	≥ 5	< 1	> 2	≥ 5	< 1	> 2	≥ 5
Conservation in the field of bottom sample and sorting within a month	< 50	10-50	0	< 50	10-50	0	0-10	0	0
Conservation in the field of wood sample and sorting within a month	> 75	25-75	0	> 75	25-75	0	0-10	0	0
Cooling any sample soon after collection and sorting within 48 hours	> 75	25-75	0	> 75	25-75	0	0-10	0	0

Identification of 1300 species in the west Palearctic

Identification literature	Larva	Pupa	Pupal exuviae	Adult ♂ only
Klink & Moller Pillot (2003)	480 sp. & gr.			
Langton & Visser (2003)		appr. 1000 sp.	appr. 1000 sp.	
Langton & Pinder (2007)				590 sp. UK
Hundreds of references				1262 sp. EU

Products:

- Vouchers: here in microscopic slides (preferably sealed in DMHF which is water soluble)



Products:

Pictures of vouchers on the internet and the literature reference of the species identification. Here *Cricotopus pilitarsis* (Zett. 1850) identified with Hirvenoja (1973): a stage new to science



Pupal thoracic horn



Larval mentum and premandible



Pupal L4 brushes on abdominal segments

Products:

- Sequence, match in BOLD, identification reference and geo-data

RMNH Voucher	Plate	Well	Taxon	Stage	BOLD match	Ident. Ref.
RMNH.INS.557393	BCP0061-30	F-10	Cricotopus pilatensis (Zett. 1850)	LP1	99,85 early release ES	Hirvenoja, 1973
Location	Ter Apel Bosbeek		AACATTATATTTATTTGGGGCTTGATCAGGAATAGTAGGTACTCCCTAAGAACCTT			
Sample	HA1106-13		AATTGAGCTGAATTAGGTATGCCGGATCATTAATTGGTGATGATCAAATTATAATGT			
Date	27-4-2008		TATTGTTACAGCTCATGCTTGTAAATAATTTCATAGTTACCTATTAAATTGG			
X coord.	268912		AGGATTGGAAAATGATTAGTTCCCTTAATGTTAGGAGCTCTGATATAGCTTCCCTCG			
Y coord	544795		GATAAATAATATAAGTTTGATTATTACCAACCTCTCACCTTACTCTTCAGTT			
Leg	WHA		AATTGTTAAAACGGGGCTGGGACAGGGTGAATCTTATCCTCCTTTCTCAGGAAT			
Det	A. Klink		TGCCACGCCGGAGCTCTGTTGATTAGCTATCTTCACTCATTAGCGGGTATTTC			
Idendification date	26-1-2015		TAGTATTAGGAGCAGTAAATTACTACTGTAATTAAATACGGTCAGAAGGAAT			
			CACTTAGATCGAACCTTATTGTTGATCAGTTATTACTGCAATTCTATTGTT			
			ATTATCTTACCTGTTAGCAGGAGCTATTACTATATTAACTGATCGAAATTAAA			
			TACTCATTCTTGCCTGGAGGAGACCAATTACCAACATTATTT			



Products:

- Sequence shared with Chironomidae group with reaction the same day.

Hei Alexander,

Thanks for sharing this with us. It is an interesting find and nice to see another example of life stage association in Chironomidae using DNA barcodes!

Your sequence match with a Cricotopus male from Central Norway which I identify as cf. pilatarsis. It is a male, but as you know the separation of males in the sylvestris group can be quite challenging. The identification of your specimen can help us be more confident on the identification of ours.

The specimen is part of an ongoing DNA barcode and faunistic survey in Central Norway that continues until the end of 2016. In addition will this material be included in a Cricotopus project where we compare material from several geographical regions. We have quite broad taxonomic sampling from several species groups.

Do you intend to submit your sequence to BOLD and make it available to the research community this way? Since you already have a registered voucher it would be a nice addition to the reference library

All the best,
Elisabeth



Products:

- Sequentie Micropsectra klinki match with *M. recurvata* in BOLD: an example of self-purification of the BOLD database

Dear Alexander,

Elisabeth and I checked the specimen and it looks that we mixed two numbers when assigning the ID in BOLD. Thus instead of just having *Micropsectra* sp. until slide mounting was done, the specimen was named *M. recurvata*. The slide mount has now been made and we can confirm that the adult male from the area around Oslofjord indeed is *Micropsectra klinki*! The name in BOLD is now updated.

Thank you very much for your help and for sharing your sequence!

Best wishes,
Torbjørn



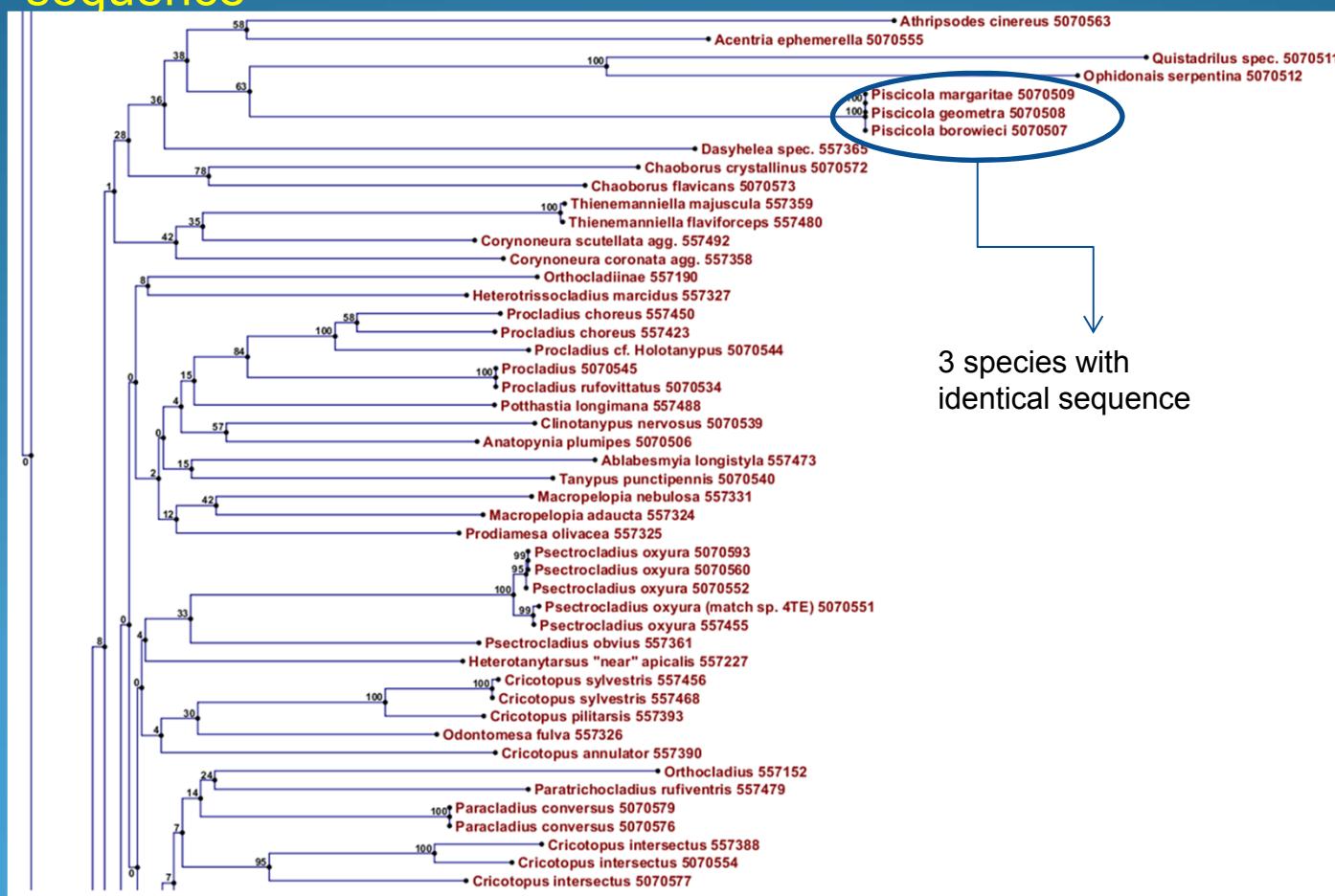
Products:

- Taxonomical backup 1: Aligning sequences

Procladius cf. Holotanypus 5070544	AAC TTT TAT	TTT A TTTTG	G TG CAT GAGC	GGG AT AGTA	GGT ACCT CCC	TTAGT AT CTT	ACT AC GGG	70
Procladius choreus 557423	AAC TTT TAT	TTT A TTTTG	G TG CAT GAGC	TGG AT AGTA	GGT ACAT CCC	TTAGT AT CTT	ACT AC GAG	70
Procladius choreus 557450	AAC TTT TAT	TTT A TTTTG	G TG CAT GAGC	TGG AT AGTA	GGT ACCT CCC	TTAGT AT CTT	ACT AC GAG	70
Microtendipes pedellus agg. 5070553	TAC TTT TAT	TTT A TTTTG	GGG CAT GATC	AGGG AT AGTA	GGG ACT GGT	TTAGT AT ATT	AAT TC GAG	70
Procladius rufovittatus 5070534	AAC TTT TAT	TTT A TTTTG	G TG CGT GAGC	GGG AT AGTA	GGT ACCT CCC	TTAGT AT CTT	AA TT CGA G	70
Procladius 5070545	AAC TTT TAT	TTT A TTTTG	G TG CGT GAGC	GGG AT AGTA	GGT ACCT CCC	TTAGT AT CTT	AA TT CGA G	70
Endochironomus albipennis 5070542	TAC ATT TAT	TTT A TTTTG	G AG CTT GATC	TGG AT AGTA	GG AAC T TTC	TTAGT AT ATT	AA TT CGA G	70
Endochironomus albipennis 557425	TAC ATT TAT	TTT A TTTTG	G AG CTT GATC	TGG AT AGTA	GG AAC T TTC	TTAGT AT ATT	AA TT CGA G	70
Endochironomus tendens 5070575	TAC ATT TAT	TTT A TTTTG	G AG CTT GATC	TGG AT AGTA	GG AAC CCT CCC	TTAGT AT ATT	AA TT CGT G	70
Paralauterborniella nigrohalteralis	AAC ATT TAT	TTT A TTTTG	G AG CCT GATC	AGG AT AGTT	GG AAC TT CAT	TAAGA ATT TT	AA TT CGT G	70
Polydipidum scalaenum 557504	AAC ATT TAT	TTT A TTTTG	G AG CCT GATC	AGG AT AGTT	GG AAC TT CAT	TAAGA ATT TT	AA TT CGT G	70
Orthocladius oblidens 557472	AAC TCT TAT	TTT A TTTTG	G AG CCT GATC	TGG AT AGTG	GG TACT T CT	TAAGA ATT TT	AA TT CGA G	70
Orthocladius rhyacobius 557384	AAC TCT TAT	TTT A TTTTG	G AG CCT GATC	AGG AT AGTG	GG AAC TT CCT	TAAGA ATT TT	AA TT CGA G	70
Dicrotendipes nervosus 5070550	AAC AC T TAT	TTT A TTTTG	G AG CCT GATC	AGG AT AGTA	GG TAC AT CCT	TAAGT AT ACT	TA TT CGA G	70
Dicrotendipes nervosus 557485	AAC AC T TAT	TTT A TTTTG	G AG CCT GATC	AGG AT AGTA	GG TAC AT CCT	TAAGT AT ACT	TA TT CGA G	70
Phaenopsectra flavipes 557438	TAC ACT TAC	TTT A TTTTG	G AG CCT GATC	AGG AT AGTA	GG AAC CCT CCC	TAAGT AT ATT	AA TT CGA G	70
Phaenopsectra flavipes 557466	TAC ACT TAC	TTT A TTTTG	G AG CCT GATC	AGG AT AGTA	GG AAC CCT CCC	TAAGT AT ATT	AA TT CGA G	70
Phaenopsectra flavipes 557470	TAC ACT TAC	TTT A TTTTG	G AG CCT GATC	AGG AT AGTA	GG AAC CCT CCC	TAAGT AT ATT	AA TT CGA G	70
Phaenopsectra flavipes 557333	TAC ACT TAC	TTT A TTTTG	G AG CCT GATC	AGG AT AGTA	GG AAC CCT CCC	TAAGT AT ATT	AA TT CGA G	70
Polydipidum scalaeum 557484	AAC TTT TAT	TTT A TTTTG	G AG CCT GATC	AGG GA TAGTA	GG AAC AT CCT	TAAGA ATT TT	AA TT CGA G	70
Polydipidum scalaeum 557371	AAC TTT TAT	TTT A TTTTG	G AG CCT GATC	AGG GA TAGTA	GG AAC AT CCT	TAAGA ATT TT	AA TT CGA G	70
Polydipidum scalaeum 557372	AAC TTT TAT	TTT A TTTTG	G AG CCT GATC	AGG GA TAGTA	GG AAC AT CCT	TAAGA ATT TT	AA TT CGA G	70
Einfeldia carbonaria 5070546	AAC CCT TAC	ATT A TTTTG	G TG CCT GATC	AGG AT AGTA	GG AAC TT CCT	TAAGT AT ACT	TA TT CGA G	70
Kiefferulus tendipediformis 5070564	TAC TTT TAT	TTT A TTTTG	G TG CCT GATC	AGG AT AGTT	GG AAC TT CCT	TAAGT AT ATT	AA TT CGT G	70
Cricotopus sylvestris 557468	AAC ATT TAT	TTT A TTTTG	G GG CCT GATC	AGGG AT TAGTA	GG AAC CCT CCT	TAAGA ATT TT	AA TT CGA G	70
Cricotopus sylvestris 557456	AAC ATT TAT	TTT A TTTTG	G GG CCT GATC	AGGG AT TAGTA	GG AAC CCT CCT	TAAGA ATT TT	AA TT CGA G	70
Cricotopus pilularis 557393	AAC ATT TAT	TTT A TTTTG	G GG CCT GATC	AGGG AT TAGTA	GG TACT CCT	TAAGA ATT TT	AA TT CGA G	70
Cricotopus bicinctus 557469	AAC ATT TAT	TTT A TTTTG	G GG CCT GATC	AGGT AT AGTA	GG AAC CCT CCT	TAAGA ATT TT	AA TT CGA G	70
Cricotopus bicinctus 557475	AAC ATT TAT	TTT A TTTTG	G GG CCT GATC	AGGT AT AGTA	GG AAC CCT CCT	TAAGA ATT TT	AA TT CGA G	70
Orthocladius fuscimanus 557382	AAC ATT TAT	TTT A TTTTG	G AG CCT GATC	TGG AT AGTA	GG TACT CCT	TAAGT AT CCT	AA TT CGA G	70
Paracladius conversus 5070576	AAC ATT TAT	TTT A TTTTG	G GG CCT GATC	AGGA AT TGGTT	GG AAC CCT CCT	TAAGA ATT TT	AA TT CGA G	70
Paracladius conversus 5070579	AAC ATT TAT	TTT A TTTTG	G GG CCT GATC	AGGA AT TGGTT	GG AAC CCT CCT	TAAGA ATT TT	AA TT CGA G	70
Pothastia longimana 557488	AAC ATT TAT	TTT A TTTTG	G GG CCT GATC	AGGA AT TAGTA	GG TACT CCT	TTAGT AT CCT	AA TT CGA G	70
Corynoneura scutellata agg. 557492	AAC ATT TAT	TTT A TTTTG	G AG CCT GATC	TGG AT AGTA	GG AAC CCT CCT	TAAGA ATT TT	AA TT CGA G	70
Odontomesa fulva 557326	AAC ATT TAT	TTT A TTTTG	G AG CCT GATC	TGG AT AGTA	GG AAC CCT CCT	TAAGA ATT TT	AA TT CGG G	70
Orthocladius dentifer 557166	AAC ATT TAT	TTT A TTTTG	G AG CCT GATC	AGGG AT AGTA	GG AAC CCT CCT	TAAGA ATT TT	AA TT CGG G	70
Orthocladius dentifer 557344	AAC ATT TAT	TTT A TTTTG	G AG CCT GATC	AGGG AT AGTG	GG AAC CCT CCT	TAAGA ATT TT	AA TT CGG G	70
Orthocladius dentifer 557385	AAC ATT TAT	TTT A TTTTG	G AG CCT GATC	AGGG AT AGTG	GG AAC CCT CCT	TAAGA ATT TT	AA TT CGG G	70
Anatopynia plumipes 5070506	AAC ATT TAT	TTT A TTTTG	G AG CCT GATC	TGG AT AGTG	GG TACT CCT	TTAGT AT CCT	TGT AC GAG	70
Cladopelma virescens 5070581	AAC ATT TAT	TTT A TTTTG	G AG CCT GATC	TGG AT AGTG	GG AAC CCT CCT	TAAGT AT CCT	TA TT CGA G	70
Cladopelma virescens 5070580	AAC ATT TAT	TTT A TTTTG	G AG CCT GATC	TGG AT AGTG	GG AAC CCT CCT	TAAGT AT CCT	TA TT CGA G	70
Cladopelma gr. viridula 5070518	AAC ATT TAT	TTT A TTTTG	G AG CCT GATC	TGG AT AGTG	GG AAC CCT CCT	TAAGT AT CCT	TA TT CGA G	70
Stempellinella 557147	TAC ACT TAT	TTT A TTTTG	G TG CCT GATC	AGG AT AGTA	GG TACT CCT	TTAGT AT CCT	AA TT CGA G	70
Stempellinella brevis 557342	TAC ACT TAT	TTT A TTTTG	G TG CCT GATC	AGG AT AGTA	GG TACT CCT	TTAGT AT CCT	AA TT CGA G	70
Parachironomus arcuatus 557491	TAC TCT TAT	TTT A TTTTG	G GG CCT GATC	TGG AT TGGTT	GG TACT CCT	TTAGT AT CCT	AA TT CGT G	70
Cladopelma bicarinata 557424	AAC ATT TAT	TTT A TTTTG	G AG CCT GATC	TGG AT AGTA	GG AAC CCT CCT	TAAGA ATT ACT	AA TT CGA G	70

Products:

- Taxonomical backup 2: 3 described species with an identical sequence



Products:

- Taxonomical backup 3: Larvae identified as *Cricotopus intersectus* comprise at least 2 distinct species



Conclusions and strategy:

- Larvae are only partly suitable in this stage of the project since less than half of them can be identified on species level
- Pupae are an ideal stage and they should be set aside for this project in routine sampling of the water boards
- Adult ♂♂ should be sampled on light in areas with much aquatic nature throughout the Netherlands
- BOLD lacks any kind of information that can back up the identification of its vouchers
- For the first time ever, we can make a quantum leap on the larval taxonomy by comparing their sequence with that of their pupal and adult stages



Future episodes:

- It will take a few years before we have a database sound enough for WFD purposes. But when the point is there we can process huge amounts of routine samples.
- The human resource that is released from the visual identification will prove to be crucial for:
 - Evaluating all these data
 - Upgrading the WFD assessment tools
 - Developing sound knowledge for the ecological rehabilitation of the Dutch surface waters



Thank you for your attention!

