DNA barcoding of midges (Diptera: Chironomidae) First episode: building the base

Alexander Klink

Presentation on the DNA barcoding conference of Naturalis June 3 2015

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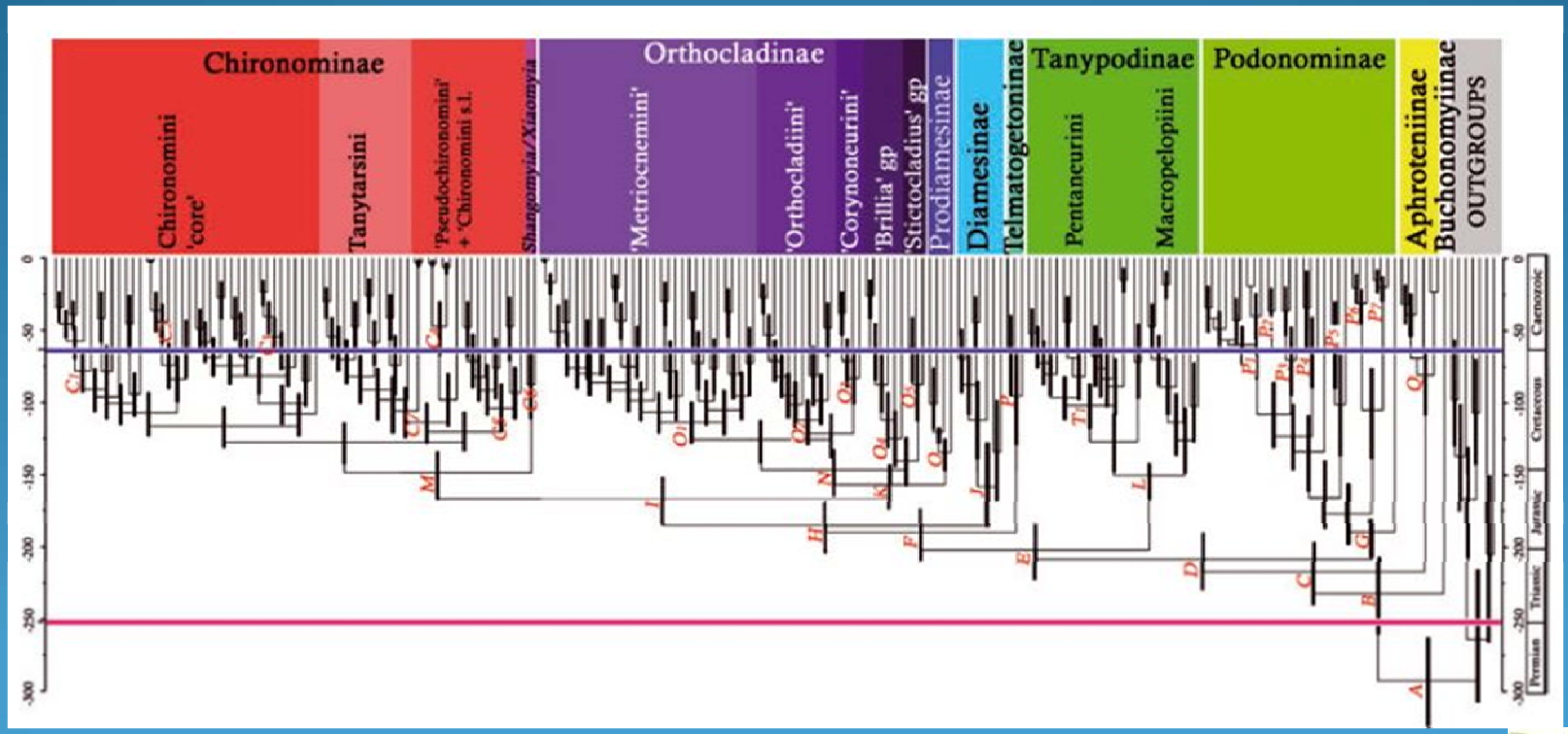
Chironomidae date back to Trias and all main groups are present since 60 My BP



After Cranston et al. (2011)

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Life stages for identification of Chironomidae: Larva

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Life stages for identification of Chironomidae: Pupa and pupal exuviae

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Life stages for identification of Chironomidae: Adult ♂

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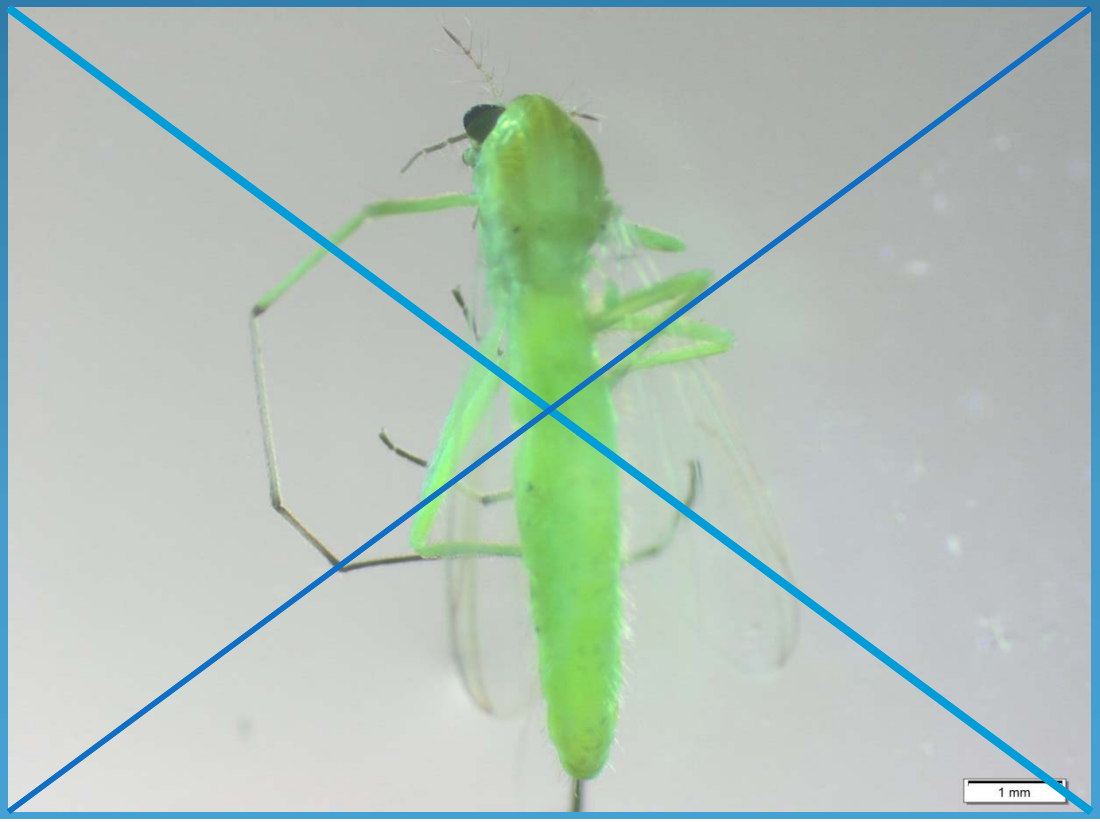
Life stages for identification of Chironomidae: Adult ♀



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Role of preservation on DNA recovery in routine aquatic samples

Stage	Larva			Pupa			Pupal exuviae		
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

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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

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Products:

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

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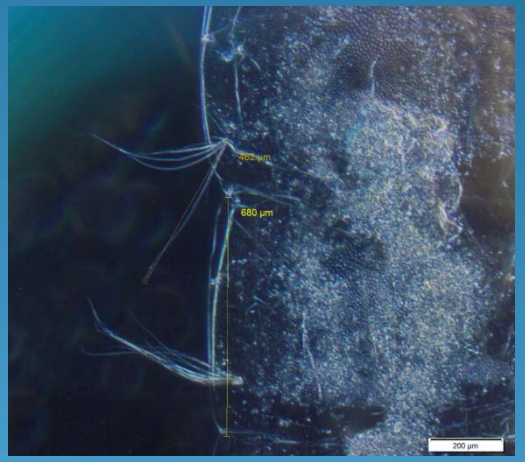
Products:

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

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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 pilitarsis (Zett. 1850)	LP1	99,85 early release ES	Hirvenoja, 1973
Location	Ter Apel Bosbeek		AACATTATATTTTATTTTCGGGGCTTGATCAGGAATAGTAGGTACTTCCCTAAGAATCTT			
Sample	HA1106-13		AATTCGAGCTGAATTAGGTCATGCCGGATCATTAATTGGTGATGATCAAATTTATAATGT			
Date	27-4-2008		TATTGTTACAGCTCATGCTTTTGTAATAATTTTTTTCATAGTTATACCTATTTTAATTGG			
X coord.	268912		AGGATTCGGAAACTGATTAGTTCCTTTAATGTTAGGAGCTCCTGATATAGCTTTCCCTCG			
Y coord	544795		GATAAATAATATAAGTTTTTGATTATTACCACCTTCTCTCACCTTACTTCTTTCAAGTTC			
Leg	WHA		AATTGTTGAAAACGGGGCTGGGACAGGGTGAACTGTTTATCCTCCTCTTTCTTCAGGAAT			
Det	A. Klink		TGCCCACGCCGGAGCTTCTGTTGATTTAGCTATCTTTTCACTTCATTTAGCGGGTATTTC			
Idendifation date	26-1-2015		TAGTATTTTAGGAGCAGTAAATTTTATTACTACTGTAATTAATATACGGTCAGAAGGAAT			
			CACTTTAGATCGAATACCTTTATTTGTTTGATCAGTTATTATTACTGCAATTCTATTGTT			
			ATTATCTTTACCTGTTTTAGCAGGAGCTATTACTATATTATTAACTGATCGAAATTTAAA			
			TACTTCATTCTTTGATCCTGCTGGAGGAGGAGACCCAATTTTATACCAACATTTATTT			

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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. pilitarsis. 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

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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 in stead of just having Micropsectra sp. until slide mounting was done, the specimens 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

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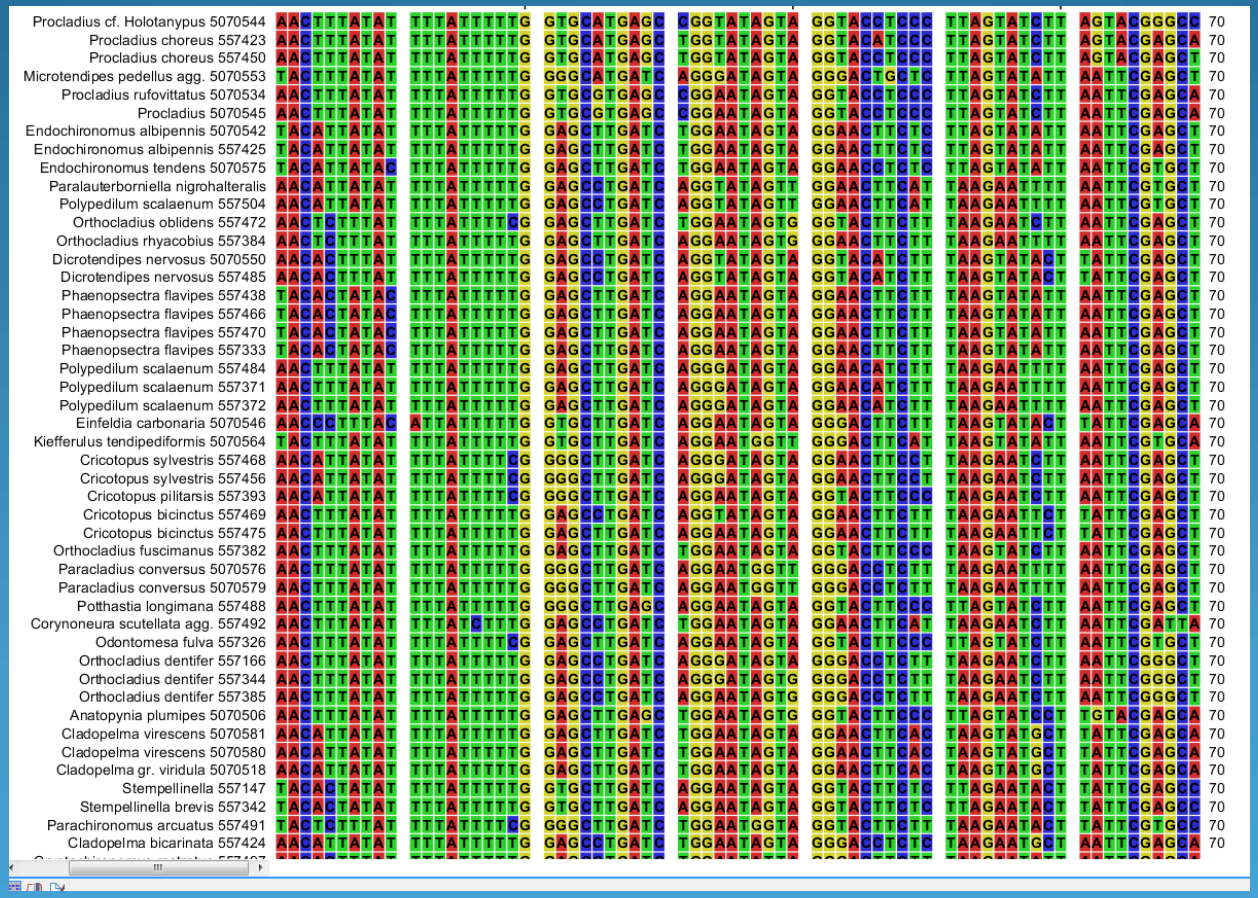
Products:

- Taxonomical backup 1: Aligning sequences



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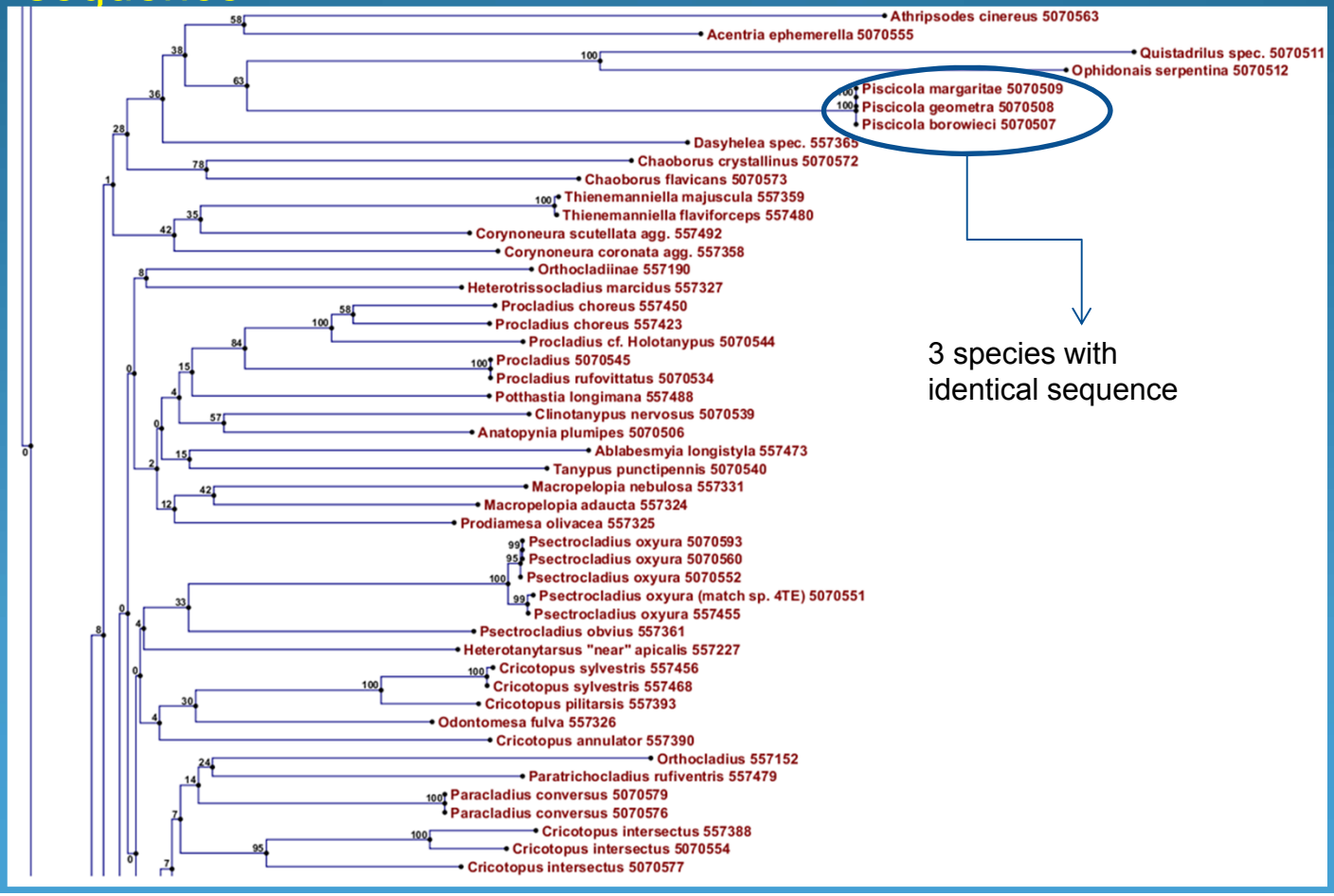
Products:

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



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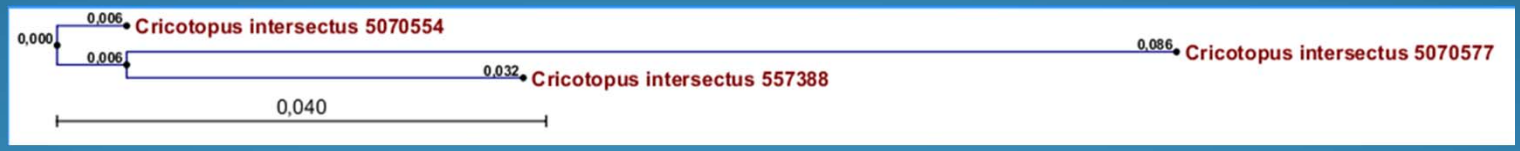
Products:

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



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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

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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

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Thank you for your attention!