The invasive round goby *Neogobius melanostomus* and tubenose goby *Proterorhinus semilunaris*: two introduction routes into Belgium.

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Introduction

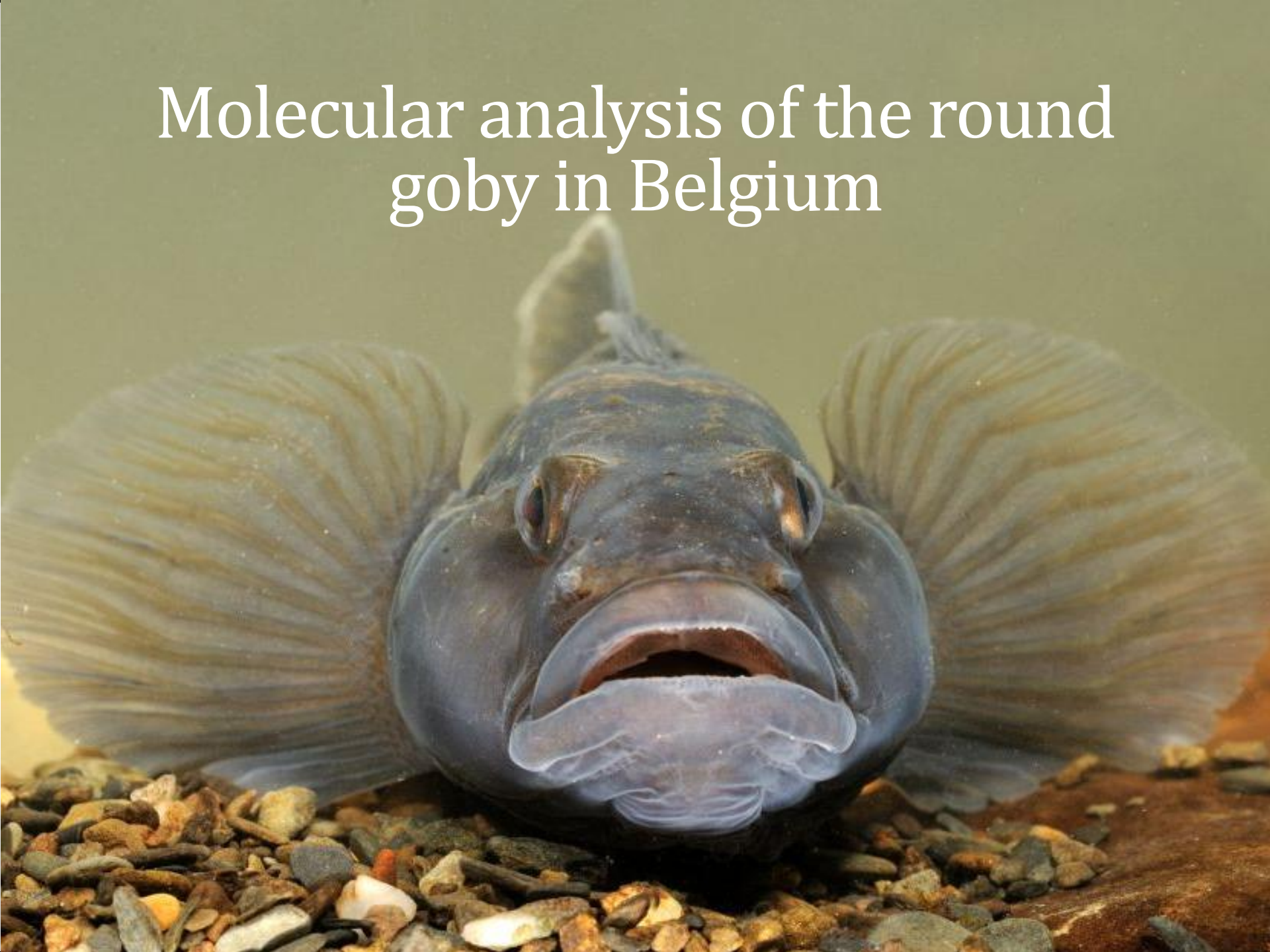
- Both species Ponto-Caspian origin
- Widely distributed across Europe and North America
  - Main transport mechanisms:
    - ballast water
    - opening Rhine - Main – Danube canal in 1992
- Proved to be invasive in introduced regions

- Reconstruction pathways
  - Molecular phylogeographic study
  - Parasitological investigation

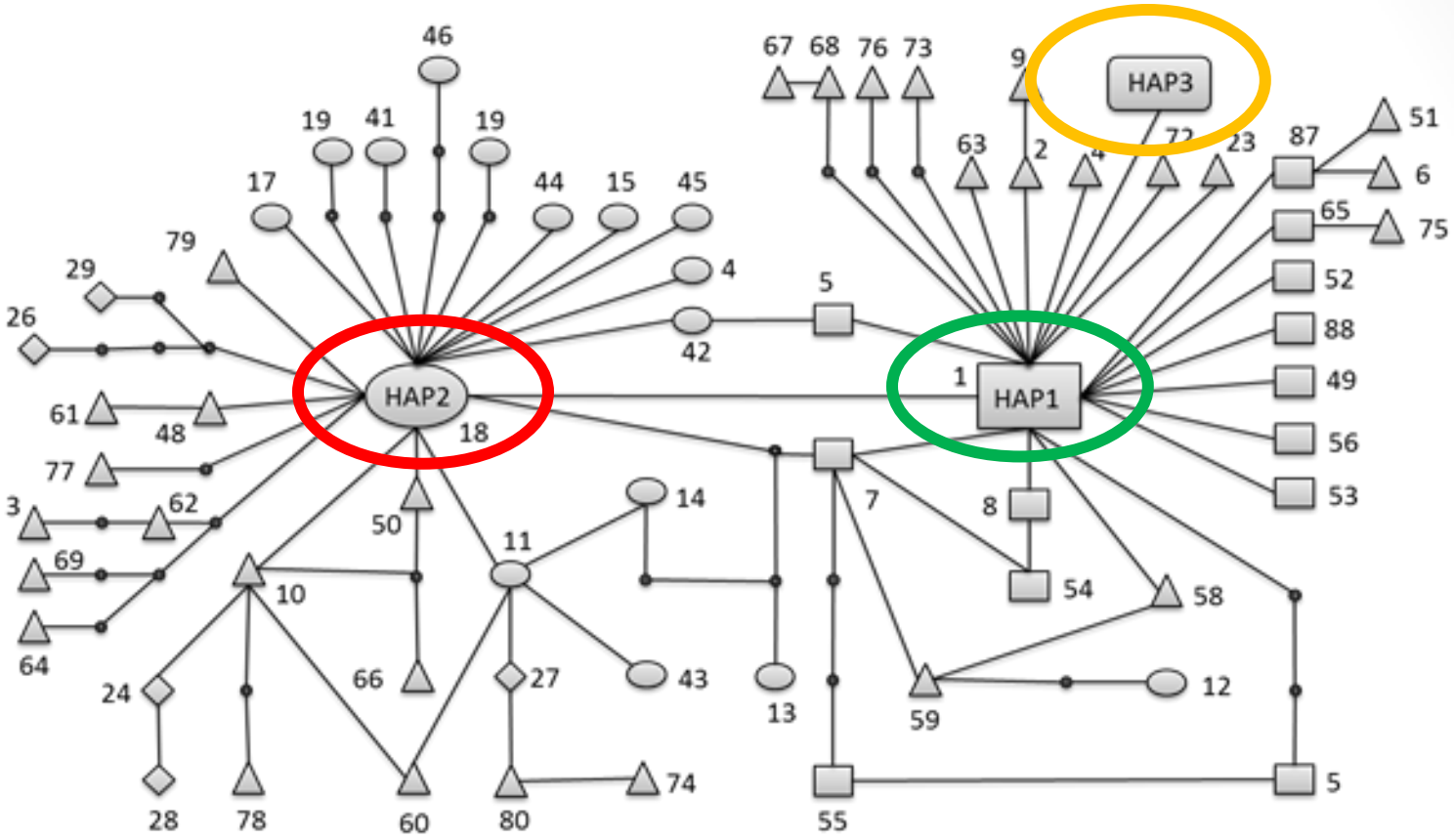
Material & Methods

- Electrofishing, fyke nets and angling (autumn 2011 & spring 2012)
  - 44 round gobies, 77 tubenose gobies and 72 fin clips of round goby
- Molecular analysis
  - The mitochondrial cytochrome b gene (cyt b)
  - Haplotype and nucleotide diversity
  - Sequence comparison on Genbank
  - Statistical parsimony analysis including Genbank sequences
- Parasitological analysis
  - Body, fins and gills
  - Parasite prevalence, abundance and infection intensity
  - Sequencing ITS rDNA fragment & morphological analysis

Molecular analysis of the round goby in Belgium



Statistical parsimony haplotype network



(▬) the Great Lakes and Black Sea drainages, (▲) Black Sea drainage, (⬬) Sea of Azov drainage, (♦) Volga

Statistical parsimony haplotype network

- Hap1 (88 %): identical to ame1 (Genbank), found ; Most dominant haplotype in Western Europe, North America and Ponto-Caspian Region.

- Hap2 (8 %): identical to ame18; groups together with sequencs from Sea of Azov drainages

- Hap3 (2 %): only one sample, no identical sequence known on Genbank

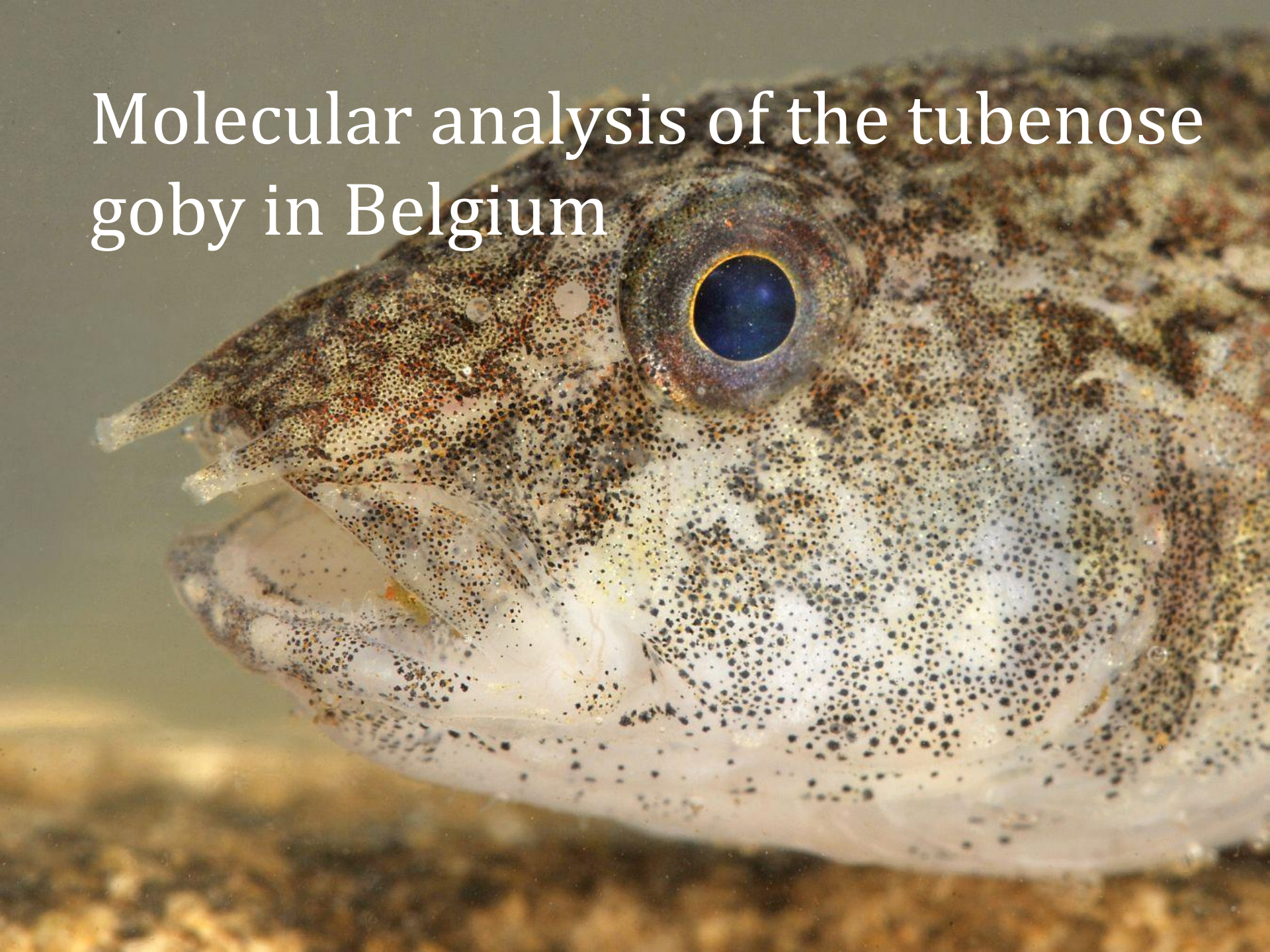
Haplotype (H_d) & nucleotide diversity (P_i)

		N_s	N_h	H_d	P_i ($x10^{-3}$)	P_i' ($x10^{3}$)
Belgium	Albert Canal	22	2	0.173	0.27	0.89
	Canal Gent-Terneuzen	10	3	0.378	0.38	1.18
	Zeeschelde	2	1	0	/	/
	Total	34	3	0.308	0.37	1.18
The Netherlands		11	1	0	/	/

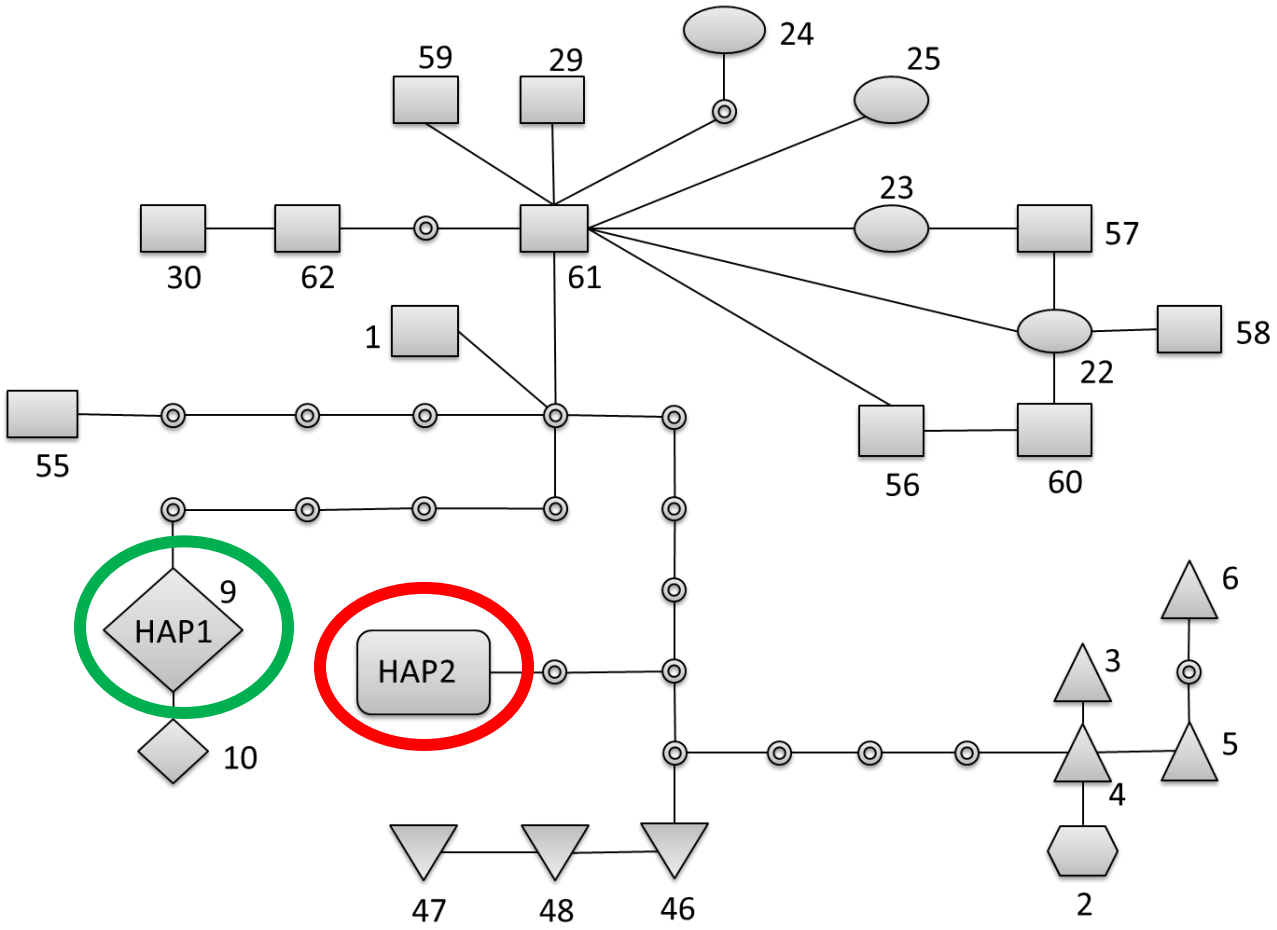
- Canal G-T: all 3 haplotypes; H_d and P_i highest
- the Netherlands: only Hap1
- H_d and P_i moderate in comparison with introduced North-American but lower towards endemic populations

=> Possibly multiple introductions

Molecular analysis of the tubenose goby in Belgium



Statistical parsimony haplotype network



(▬) Dniester and tributaries, Ukraine, (⬬) Odessa bay, Ukraine, (▲) Lake St. Clair and Superior, U.S.A., (▼) Simferopol, Ukraine, (●) Dnieper, Ukraine, (♦) Danube, Serbia, (▬) unknown outside Belgium.

Statistical parsimony haplotype network

- Hap1 (40/41): identical to Pro9 (Genbank); Together with Pro10 found in Danube river in Serbia. Differs at least 6bp from other haplotypes on Genbank.

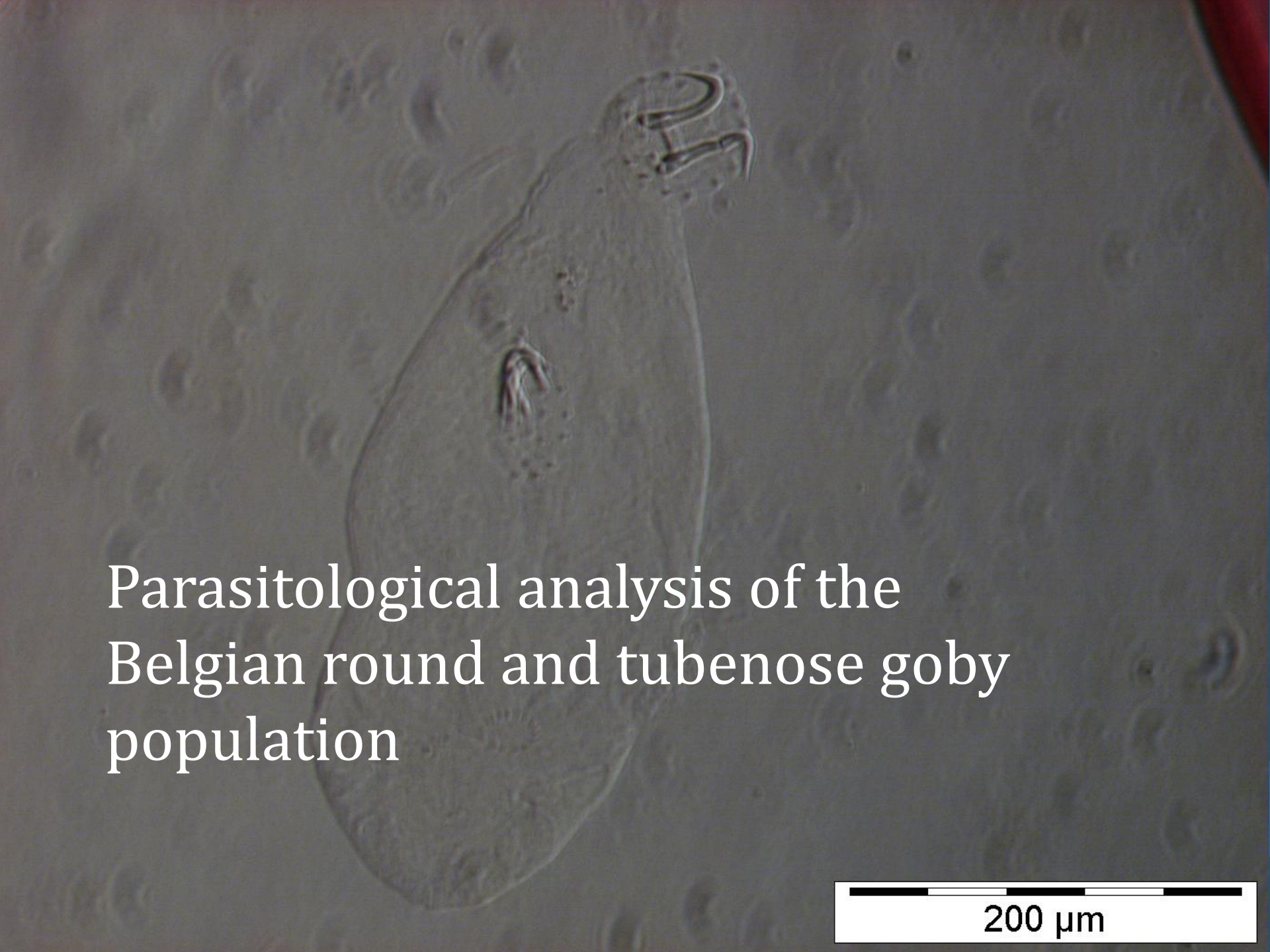
- Hap2 (1/41): only one sample, no identical sequence known on Genbank

Haplotype (H_d) & nucleotide diversity (P_i)

		N_s	N_h	H_d	P_i ($.10^{-3}$)	P_i' ($x10^3$)
Belgium	Canal of Zuid-Willems	20	1	0	/	/
	Mouth Kik -en Ziepbeek	20	2	0.10	2.39	10.20
	Canal of Beverlo	1	1	0	/	/
	Total	41	2	0.049	1.17	10.20

- H_d low in comparison with introduced North-American and endemic populations
- P_i high due to very divergent haplotypes

- H_d 6x lower than Belgian round goby population ($0,308.10^3$)

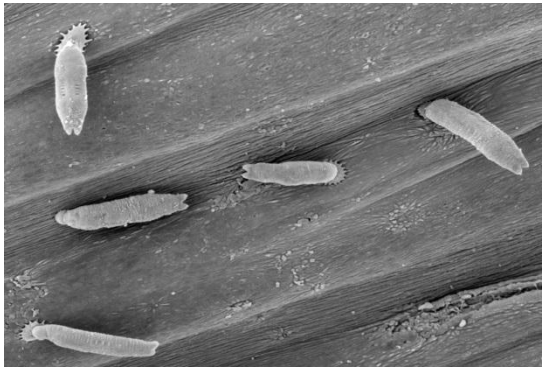

Parasitological analysis of the Belgian round and tubenose goby population
200 µm

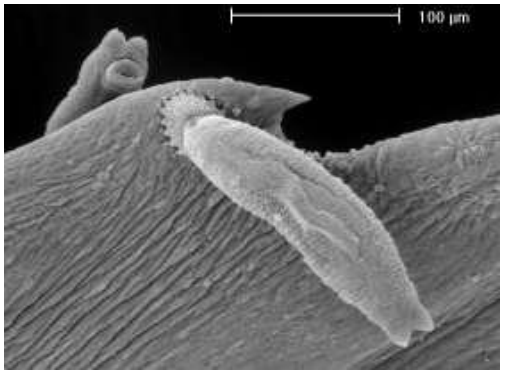
Prevalence (P%), Abundance (A) and Infection intensity (I) of *Gyrodactylus* sp.

		N_f	N_p	P (%)	A	I
Round goby	**Total**	**44**	**3**	**4.50**	**0.07**	**1.5**
	Albert Canal	31	0	0	0	/
	Zeeschelde	2	0	0	0	/
	Canal Gent-Terneuzen	11	3	18.18	0.27	1.5
Tubenose goby	**Total**	**73**	**163**	**82.19**	**2.23**	**2.72**
	Mouth Kikbeek	37	92	83,78	2.49	2.97
	Mouth Ziepbeek	7	13	85.71	1.86	2.17
	Canal of Beverlo	1	0	0	0	/
	Canal of Zuid-Willem	28	58	82.14	2.07	2.52

- Prevalence and infection intensity much higher for tubenose gobies

Gyrodactylus proterorhini





- Described on tubenose goby Southern Slovakia
- Introduced in Belgium with goby host
- Direct life cycle (no intermediate host) facilitates invasion of new areas
- Wide host range, spreading to other goby species is possible

Parasite results support our hypothesis of different pathways:

- Round goby:
  - *Gyrodactylus* only infects adults
  - Exchange of ballast water occurs just below water level (pelagic)
- Tubenose goby
  - Natural migration of adult infected fish
  - Less abrupt environmental changes during dispersal

Conclusion

- Round goby:
  - Possible introduction pathway: ballast water transport, possibly from the southern Dnieper River near the port of Kherson (Brown & Stepien, 2009)
- Tubenose goby:
  - Possible introduction pathway: migration through Danube-Main-Rhine Canal, opened in 1992 (Von Landwust, 2006)
- Parasite characteristics can help to evaluate possible introduction routes
- Co-introduction of parasites should be carefully monitored