

A Decade of Gene Diversification by Viral Hemorrhagic Septicemia (VHS) since its first appearance in the Laurentian Great Lakes



By Carol Stepien

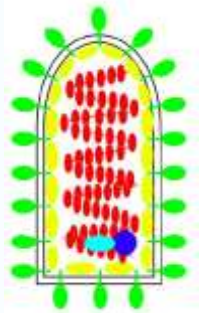
Great Lakes Genetics/Genomics Laboratory, University of Toledo

*With PhD Student Megan Niner & Graduated PhD student Lindsey Pierce
(now at Cleve. Clinic)*

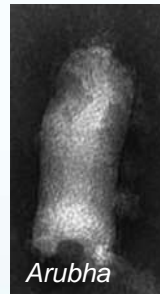


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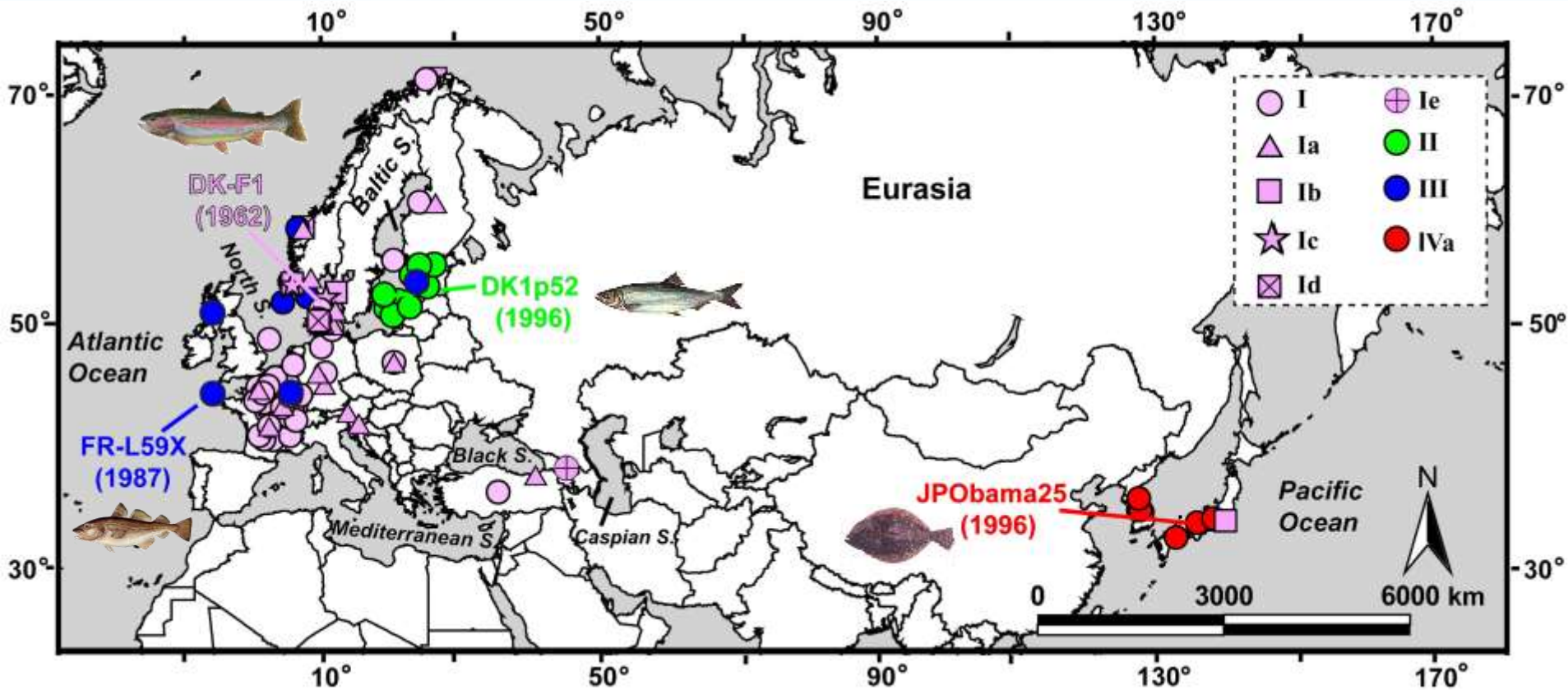
Viral Hemorrhagic Septicemia virus (VHSV)



- RNA bullet-shaped *Novirhabdovirus*
- 11,158 nt, 6 genes: 3'**N-P-M-G-Nv-L**'5
 - Unique **Nv (Nonvirion)** gene to this group
 - Related to IHNV, Snakehead rhabdovirus
- One of world's most important finfish diseases
- External & internal hemorrhages
- Infects >80 fish species across N. Hemisphere
- New substrain (IVb) emerged in the Great Lakes~ decade ago
 - Large outbreaks across GL
 - ~30 species killed



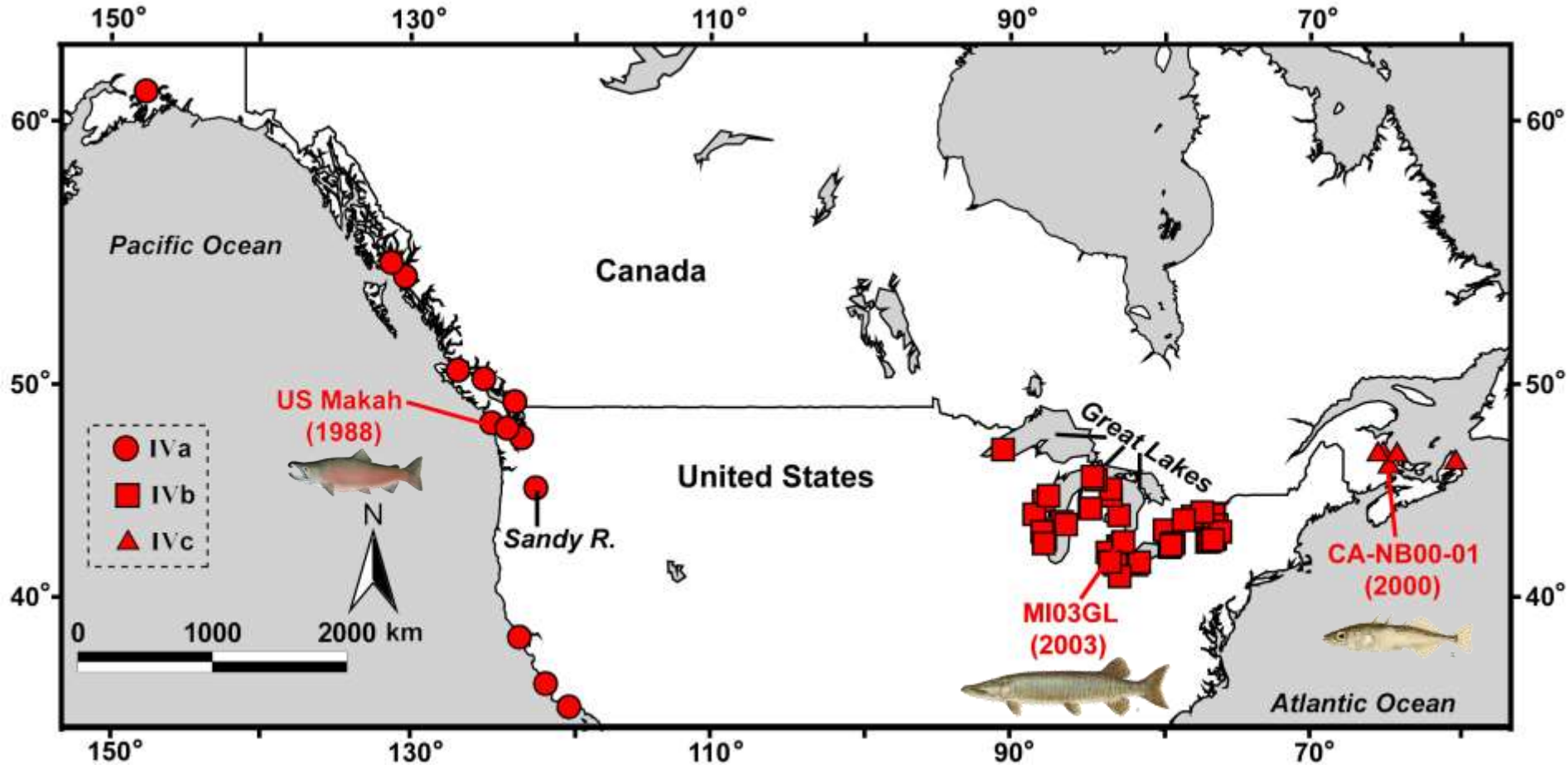
VHSV Distribution in Eurasia



(Dates=first known occurrences)

- First discovered in 1938 European rainbow trout aquaculture
- Now: Four strains (I, II, III, & IV) & several substrains
(adapted from Pierce & Stepien 2012, Mol Phyl Evol)

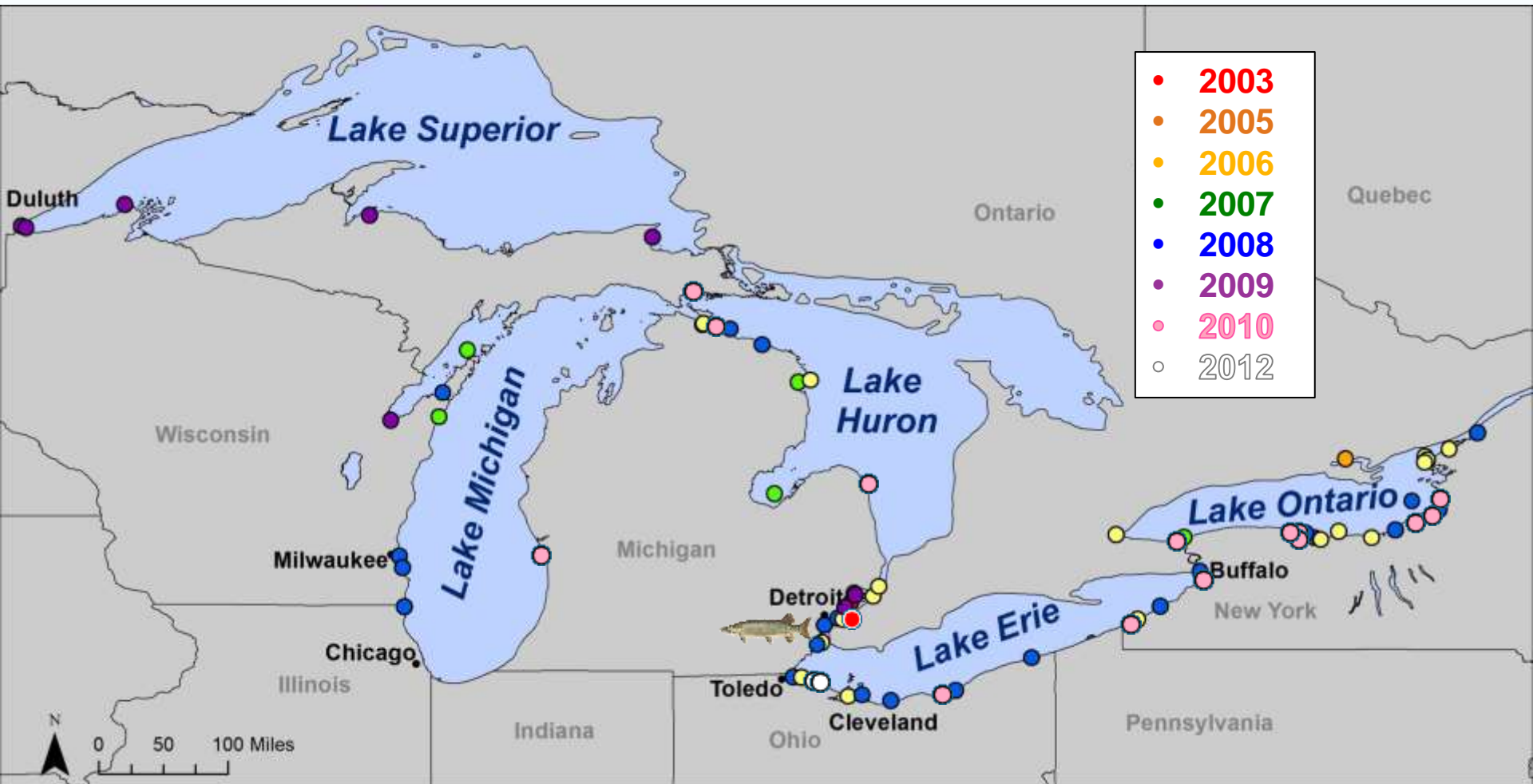
VHSv-IV in North America



(Dates=first known occurrences)

Three geographically separated substrains of IV (IVa, b, c)
(adapted from Pierce & Stepien 2012, *Mol Phyl Evol*)

VHSV-IVb Spread in the Great Lakes



*Data compiled from: J. Sieracki & J. Bossenbroek, UT (2003-2009);
Cornwell et al. 2015 (2010 samples); Stepien et al. 2015 (2012 samples)*

Major Species Infected by VHSV-IVb in the Great Lakes



Black crappie
Pomoxis nigromaculatus



Bluegill
Lepomis macrochirus



Brown bullhead
Ameiurus nebulosus



Pumpkinseed
Lepomis gibbosus



Burbot
Lota lota



Emerald shiner
Notropis atherinoides



Freshwater drum
Aplodinotus grunniens



Common carp
Cyprinus carpio



Channel catfish
Ictalurus punctatus



Gizzard shad
Dorosoma cepedianum



Lake herring
Coregonus artedii



Chinook salmon
Oncorhynchus tshawytscha



Rainbow trout
Oncorhynchus mykiss



Smallmouth bass
Micropterus dolomieu



Trout perch
Percopsis omiscomaycus



Walleye
Sander vitreus

VHSv Impacts on Great Lakes Region

- **Commercial & sport fishing**
- **Tourism & public perception**
- **Secondary pathogens & biological wastes**
- **Aquaculture, hatchery & baitfish industries**



Andy Noyes, NYSDEC



Lake Erie Center

VHSv Characteristics & Spread

Ecology:

- Stable 3-15°C in water
- Long-lived in water (~14 days)
- Fish shed virus up to 15 weeks post infection
- Continue to shed virus after recovery in times of of stress

Transport:

- Fish migration (spawning)
- Ballast water from fishing boats
- Bait fish may transport
- Birds' feet
- Benthic invertebrates (leeches & *Diporeia* spp.)
- Aquatic turtles



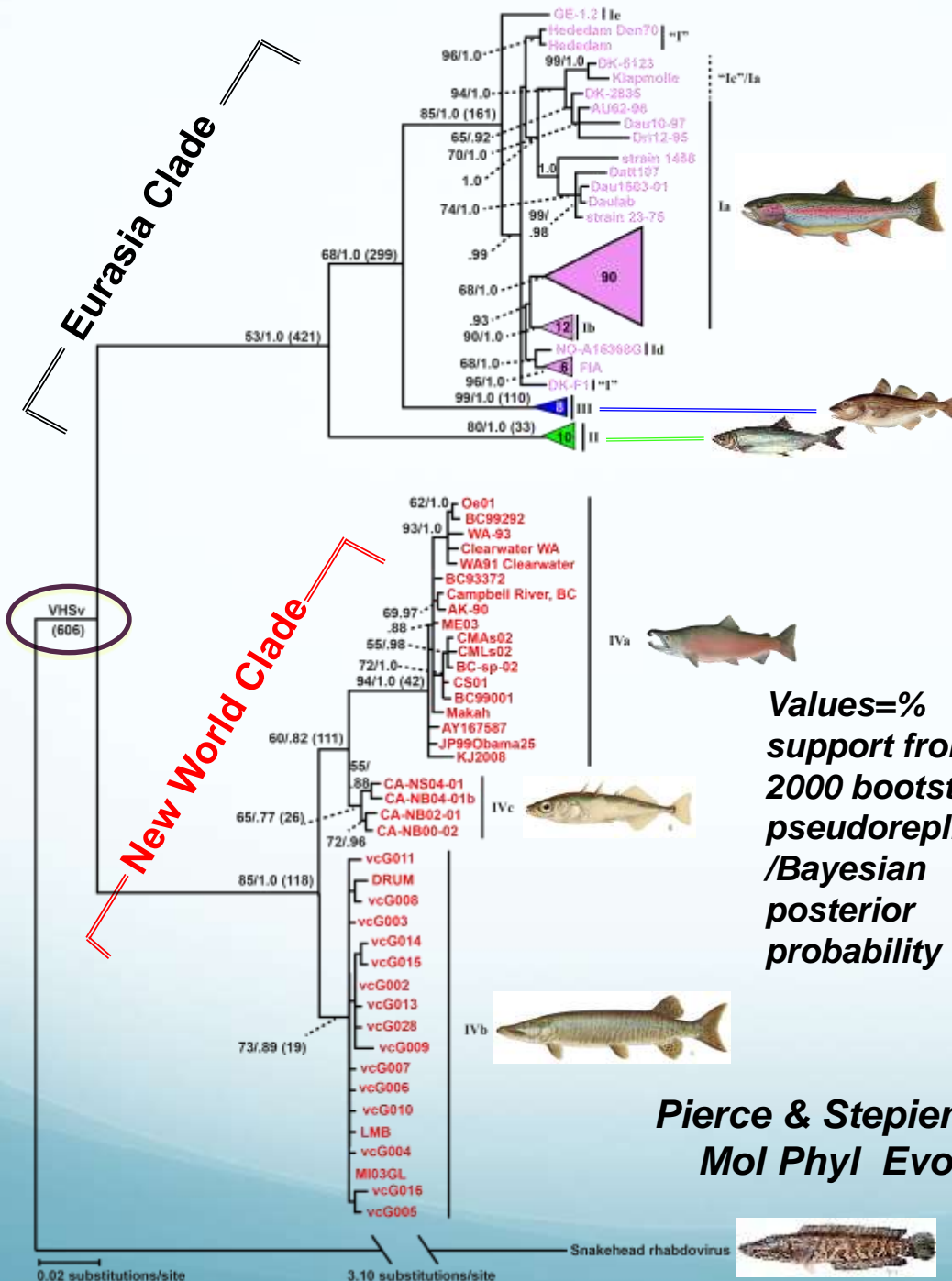
Study Objectives

1. Evaluate the overall phylogenetic & biogeographic history & relationships of VHSV
2. Survey/ Test for current infection across the GL with our PLOS-One (2013) assay
3. Discern the evolutionary diversification patterns & virus-host coevolution of IVb in the Great Lakes



Part 1. Biogeographic & Phylogenetic Results

(G, Nv, N, M, P- genes)



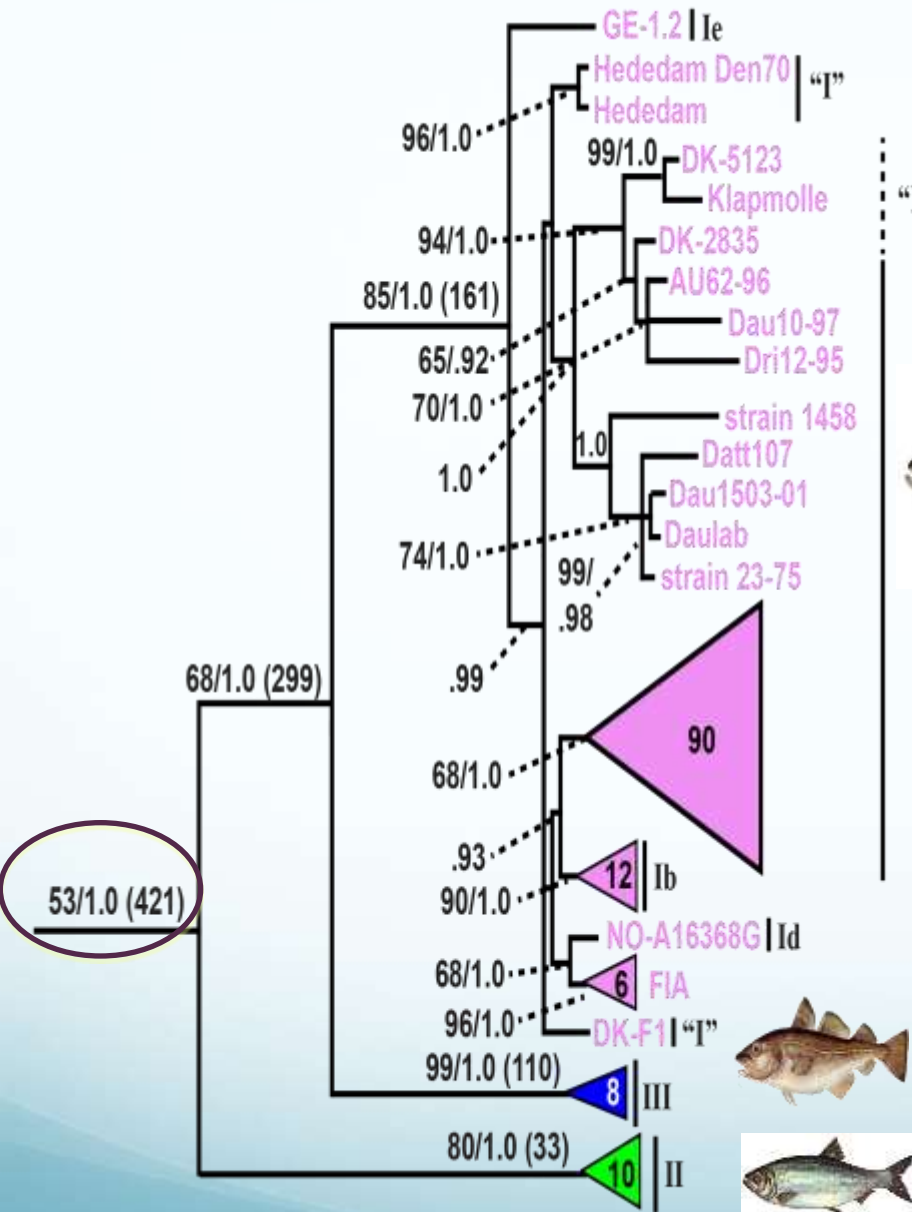
- Phylogenetic analyses: Maximum Likelihood & Mr. Bayes
- VHS likely originated from a marine ancestor in N Atlantic
- Diverged ~600ya into 2 primary clades:
 - I, II, III (Eurasia)
 - IV (New World)
 - IV has 3 substrains, which also are geographically distinct

Values=% support from 2000 bootstrap pseudoreplicates /Bayesian posterior probability

Pierce & Stepien 2012, Mol Phyl Evol



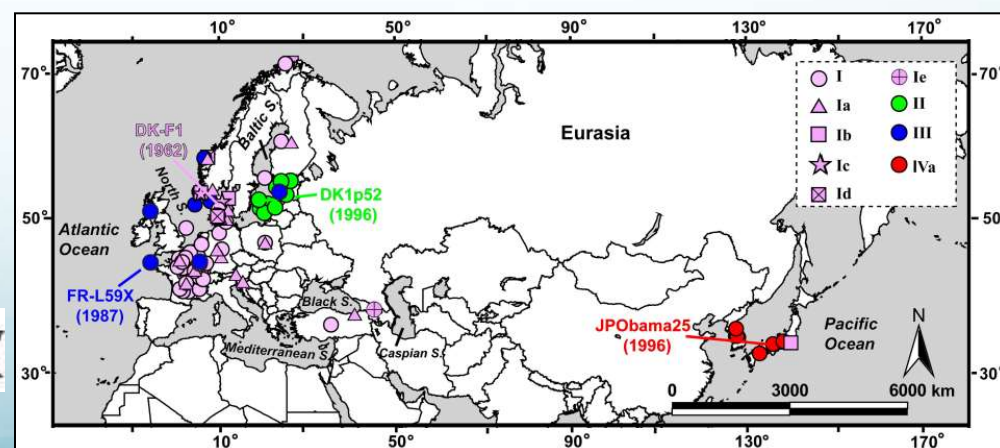
VHSv Eurasian Clade: Strains I, II & III



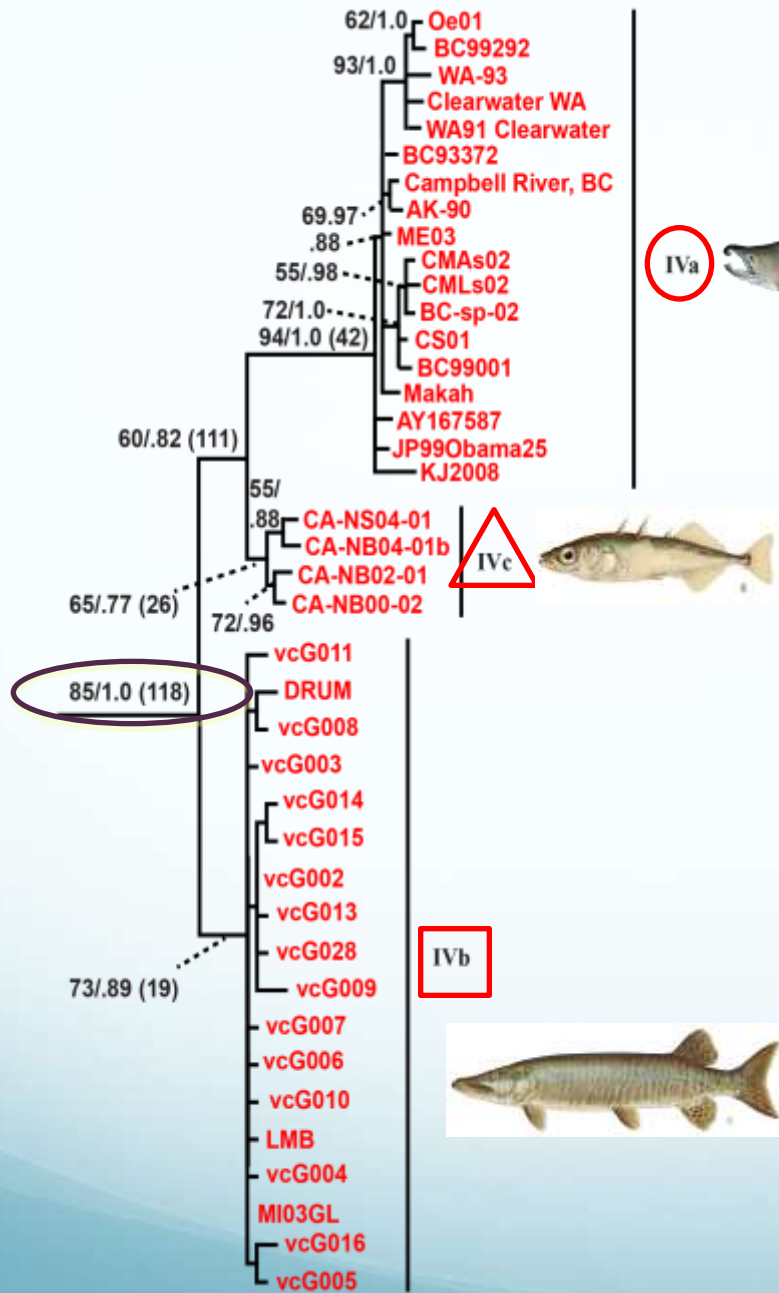
"Ic"/Ia



- The I-III clade then diverged ~420ya into 2 clades:
 - II (marine) – Baltic Sea region
 - I (marine/estuarine/freshwater) + III (marine/estuarine)
- Strains I & III diverged ~300ya
 - III – separated in the North Atlantic Ocean & North Sea
 - I – radiated in European salmonids



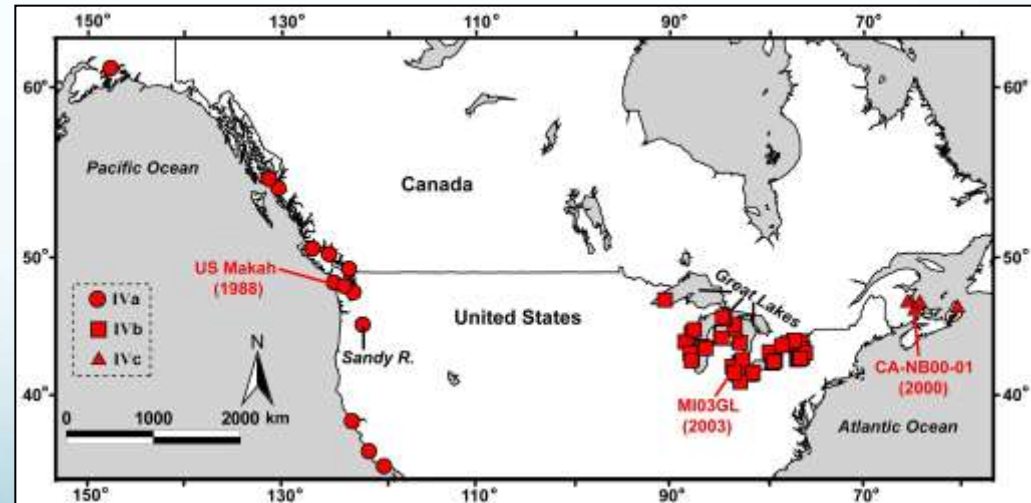
VHSv New World Clade: Strain IV



- Likely originated in marine fishes in N Atlantic

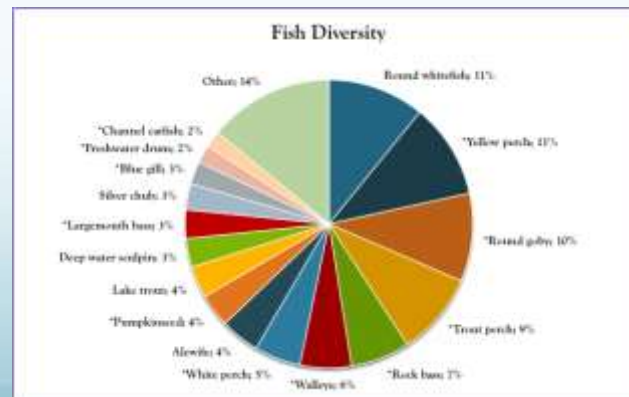
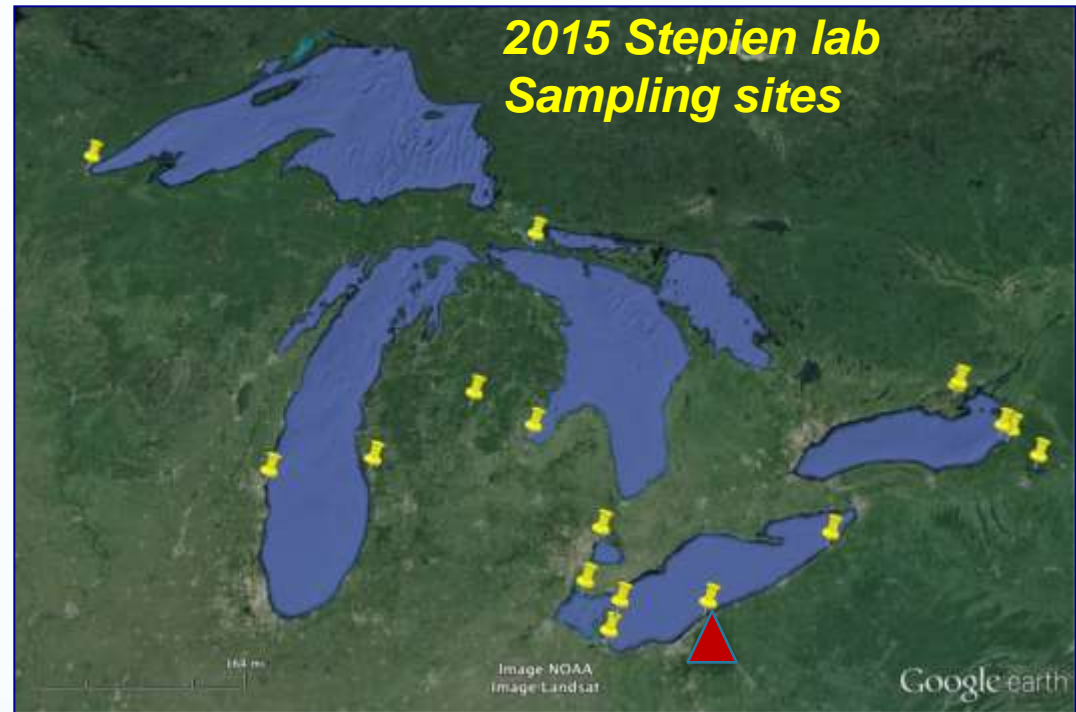
- **IV** diverged ~120ya into 3 distinct substrains:

- **IVa** = (~40ya) N Pacific Ocean
- **IVc** = (~25ya) NW Atlantic Ocean
- **IVb** = (~20ya) Great Lakes



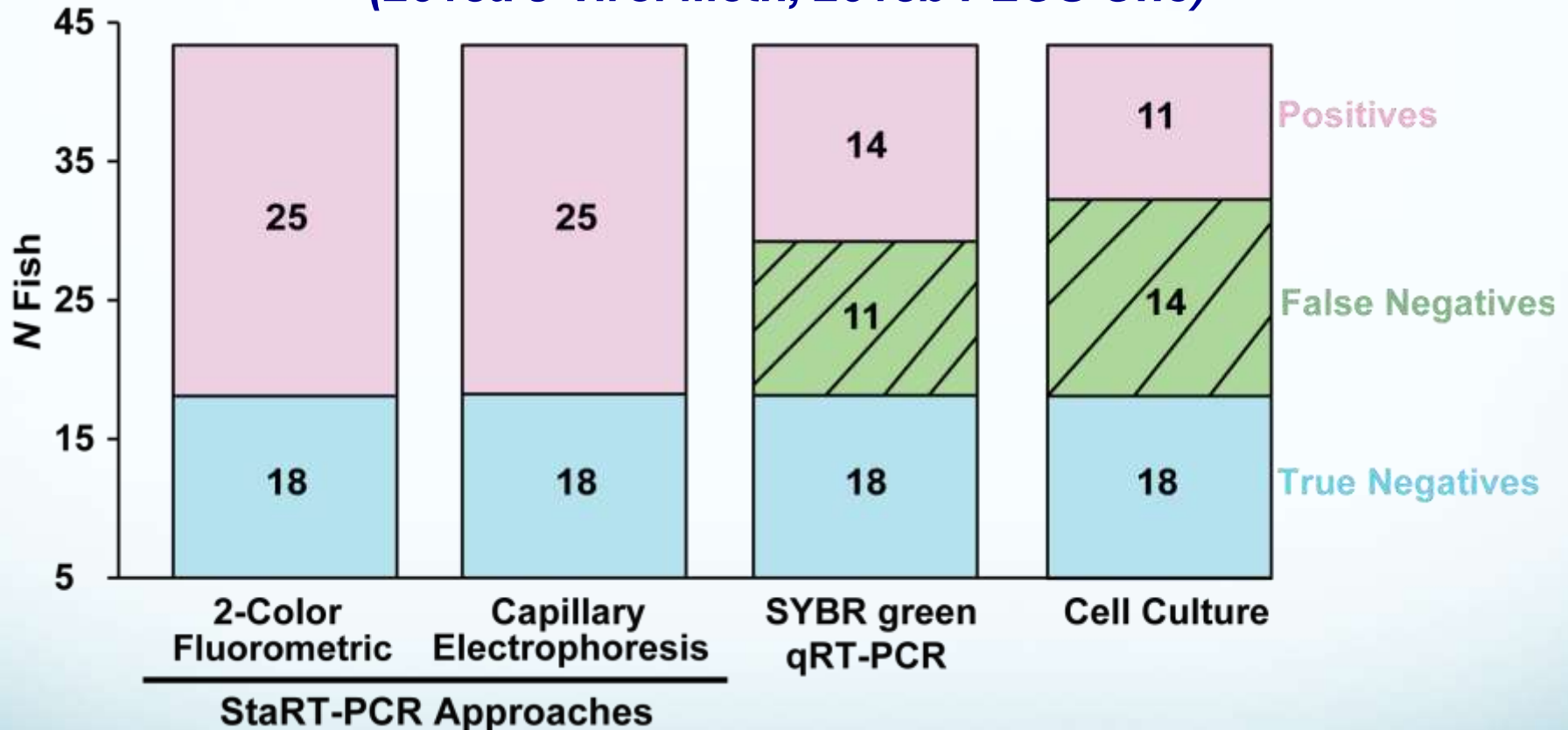
Part 2: 2015 VHSV-IVb Sampling Results

- Students (*PhD candidate Megan Niner & undergraduate Shelby Edwards*) sampled 1,193 fishes with federal & state agencies
 - April-July 2015
 - 40 fish species
 - Positives only at Fairport Harbor, central Lake Erie
- Screened pooled groups & individuals with our SYBR Green qPCR Assay (PLOS ONE 2013)
- Followed by our 2-color fluorescence quantitative assay
 - Uses synthetic internal standards
 - Pierce, Willey, Leaman, Shepherd, Stepien 2013a,b (*J Virological Methods, PLOS ONE 2013*)



Developed New Accurate Assays to Detect & Quantify VHSV

*Pierce, Willey... Leaman, Shepherd, Stepien
(2013a J Virol Meth, 2013b PLOS One)*

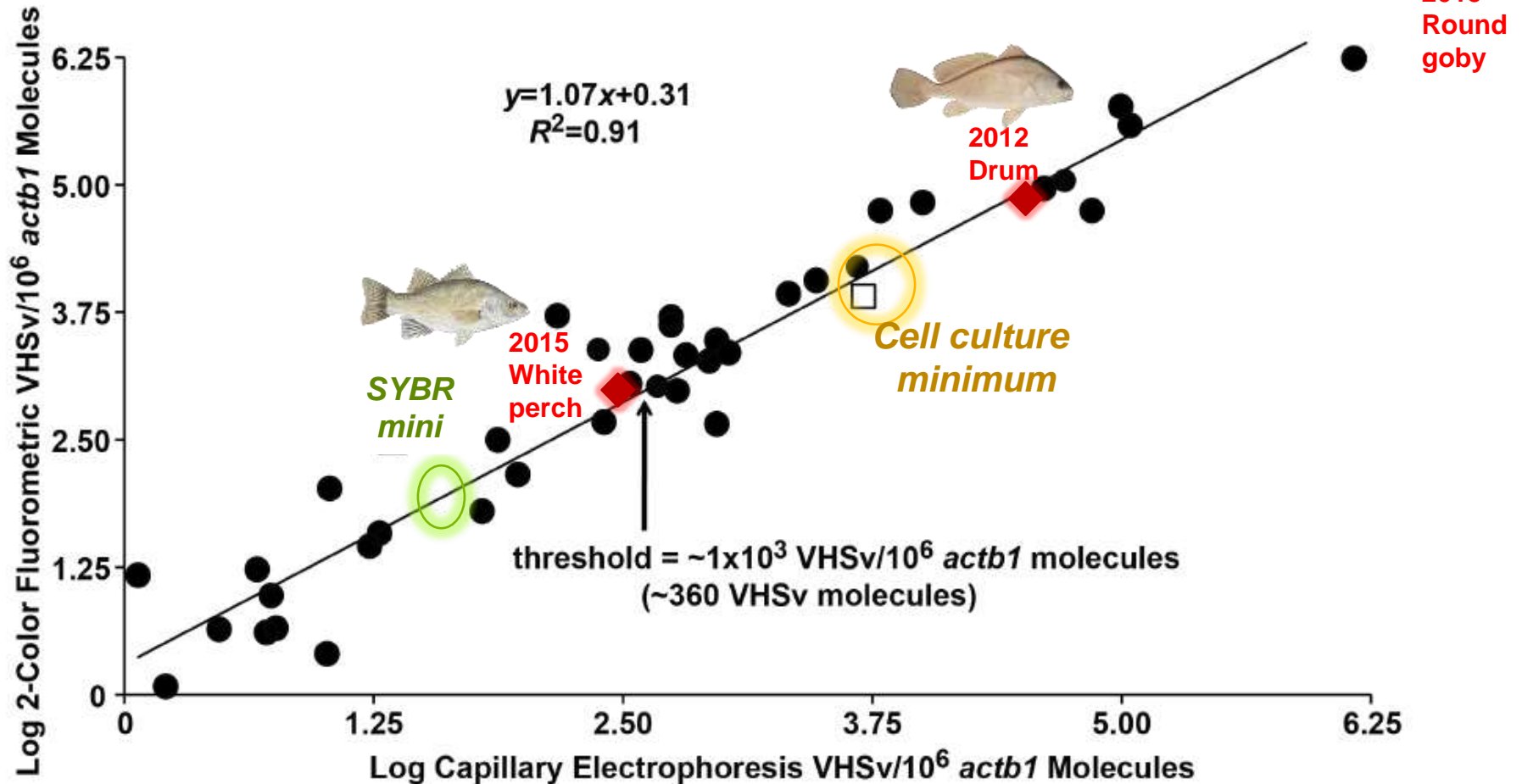


- 1) Both StaRT-PCR assays detected the same # of positives & negatives
- 2) Both were more sensitive than SYBR green qRT-PCR ($\chi^2=5.68$, $p=0.02$) & Cell Culture ($\chi^2=9.36$, $p=0.002$)
- 3) False negative rates=38% SYBR green & 44% Cell Culture

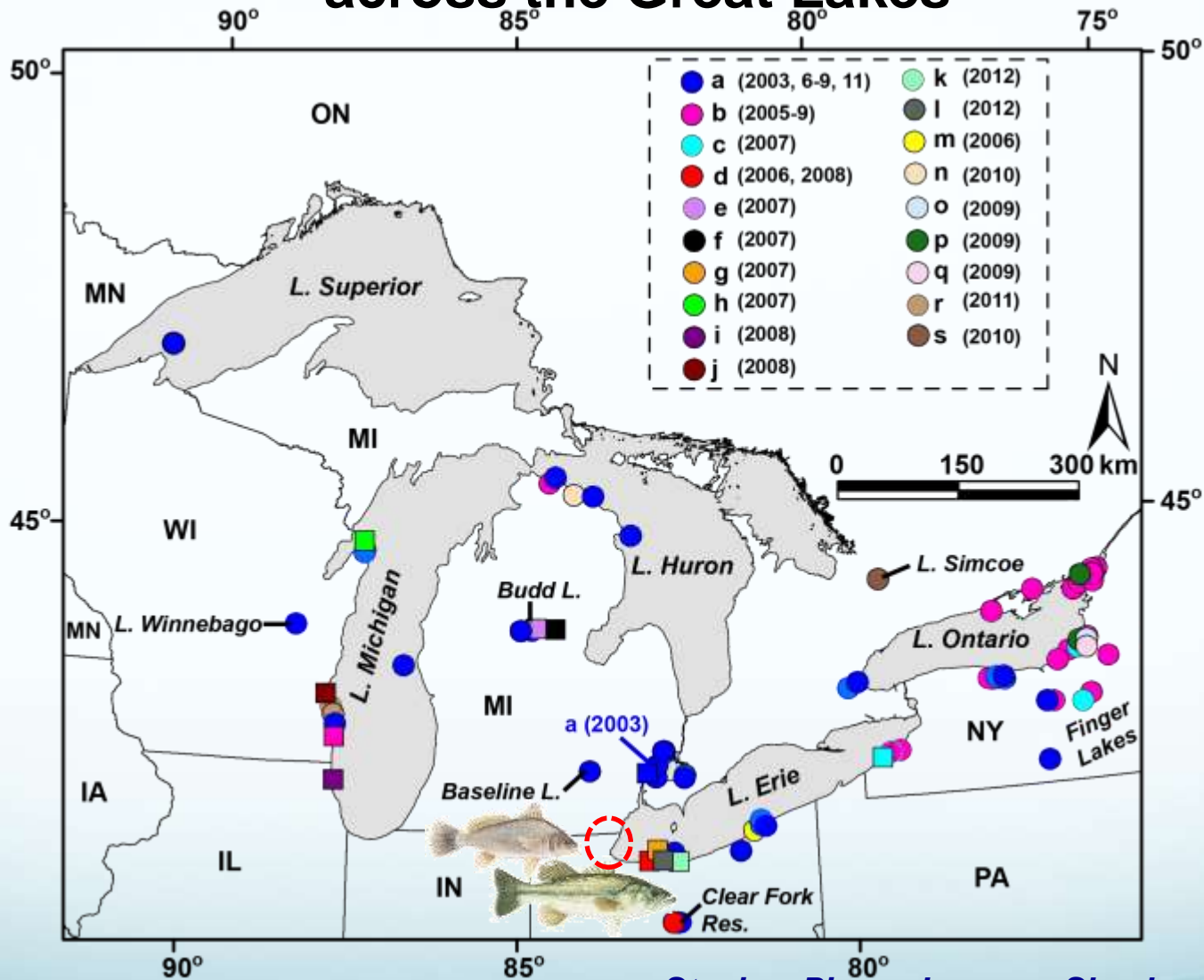
VHSv Concentration Analyses

Adapted from:

Pierce, Willey... Shepherd, Leaman, Stepien 2013 PLOS One with New Data



Part 3: Genetics/Genomics over Time & Space across the Great Lakes

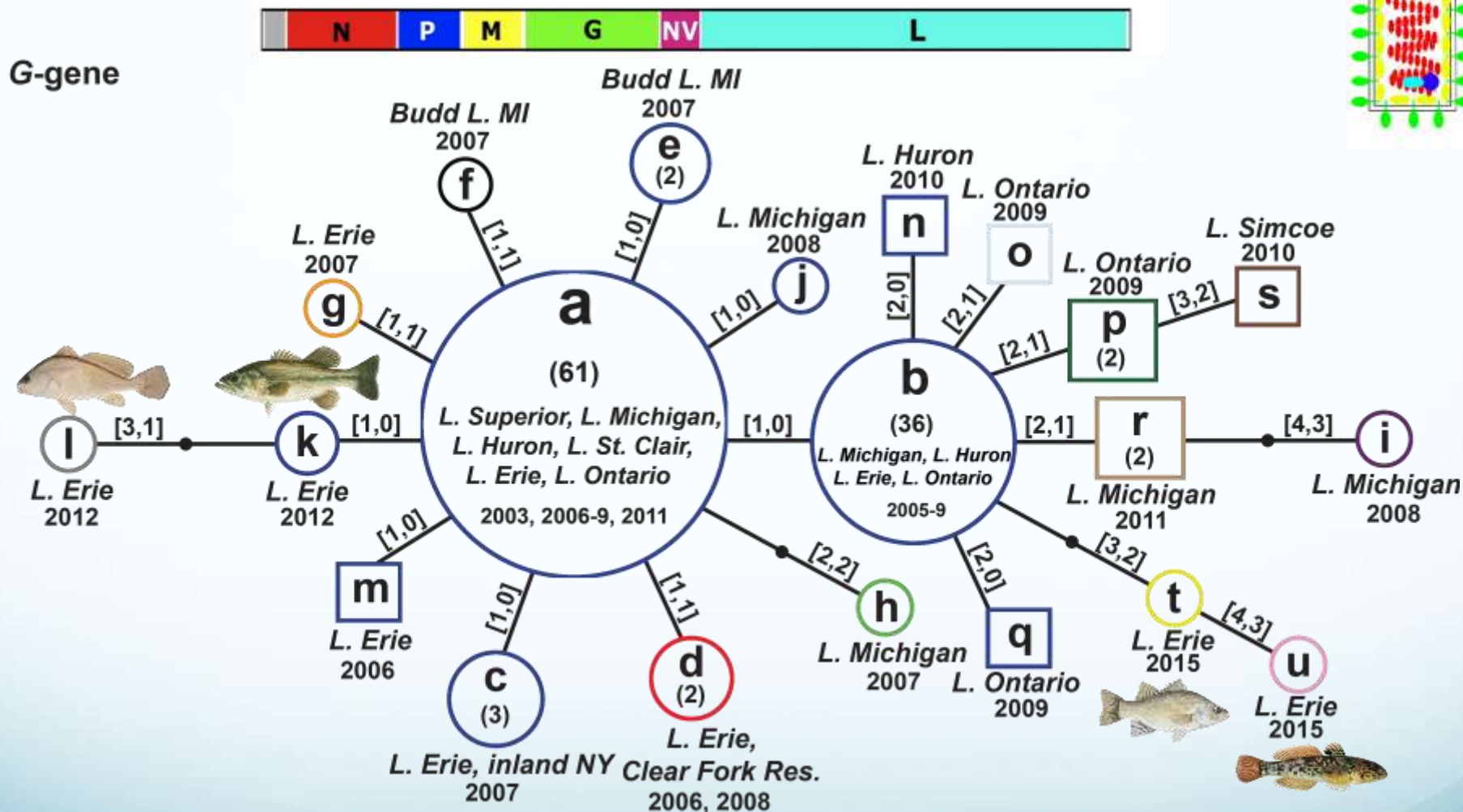
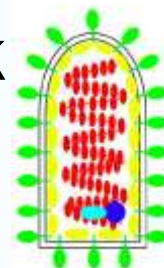


*Stepien, Pierce, Leaman, Shepherd, Niner 2015
PLOS One*

With info from Dr. Gael Kurath, USGS, Seattle

- 21 G-gene haplotypes identified to date
- We are sequencing these for other genes

3. Evolutionary Patterns of VHSV-IVb: *G*-(glycoprotein) gene Haplotype Network

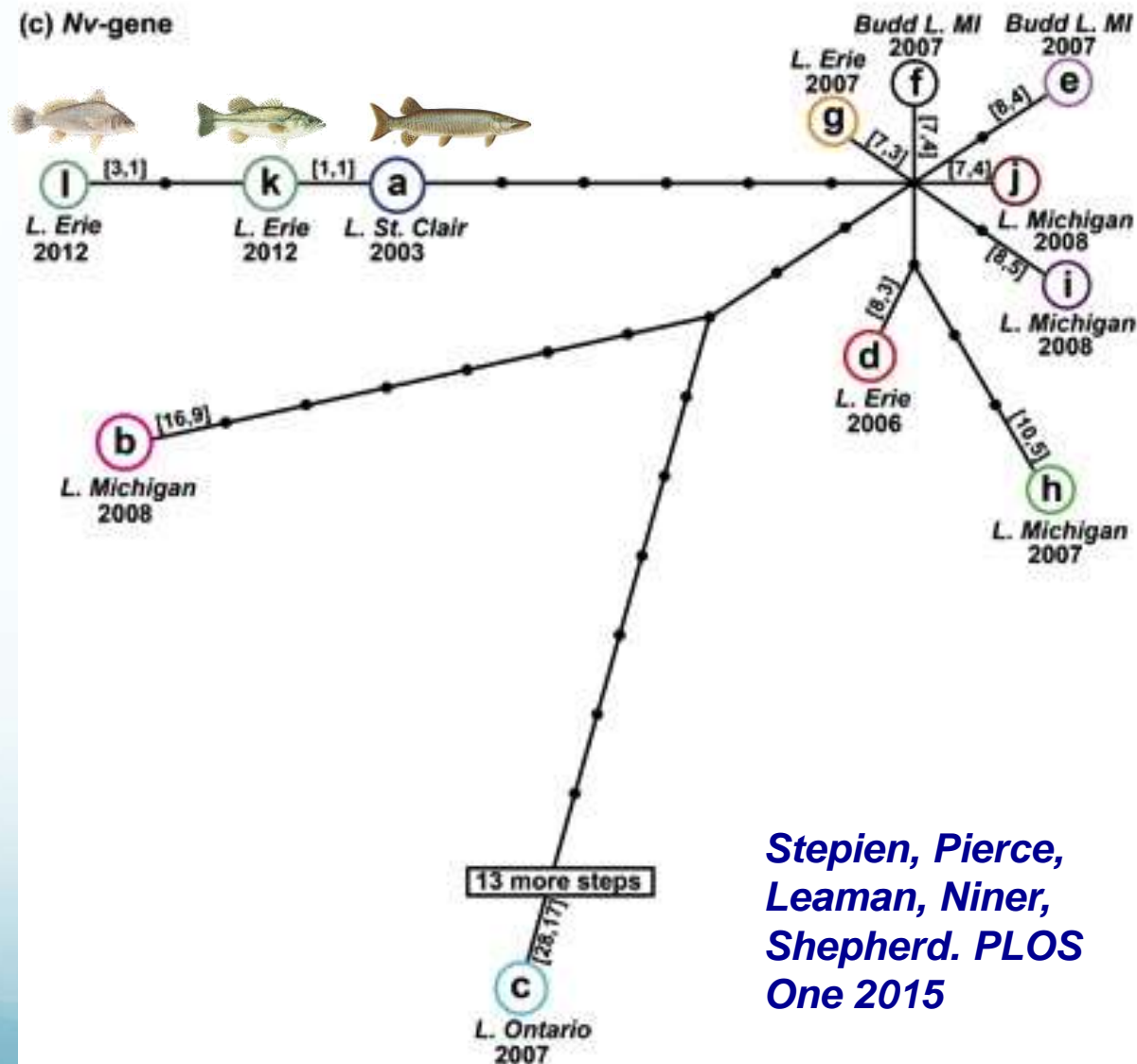


- Two central haplotypes -a & b- abundant & widespread; likely the oldest
- Several haplotypes radiated in a “star-like” pattern (i.e., quasispecies)
- G evolves at ~1/10 rate of NV
- G functions in attachment & entry into host cells

VHSv-IVb *Nv*-gene Haplotype Network



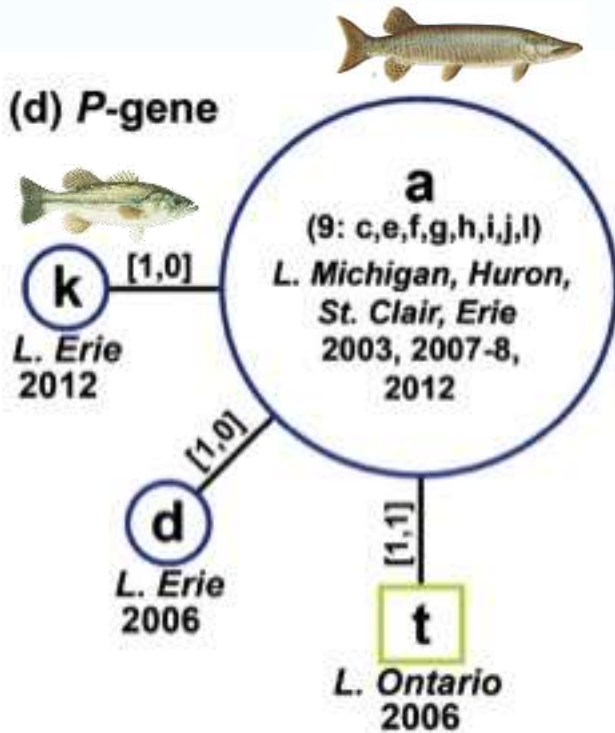
(c) *Nv*-gene



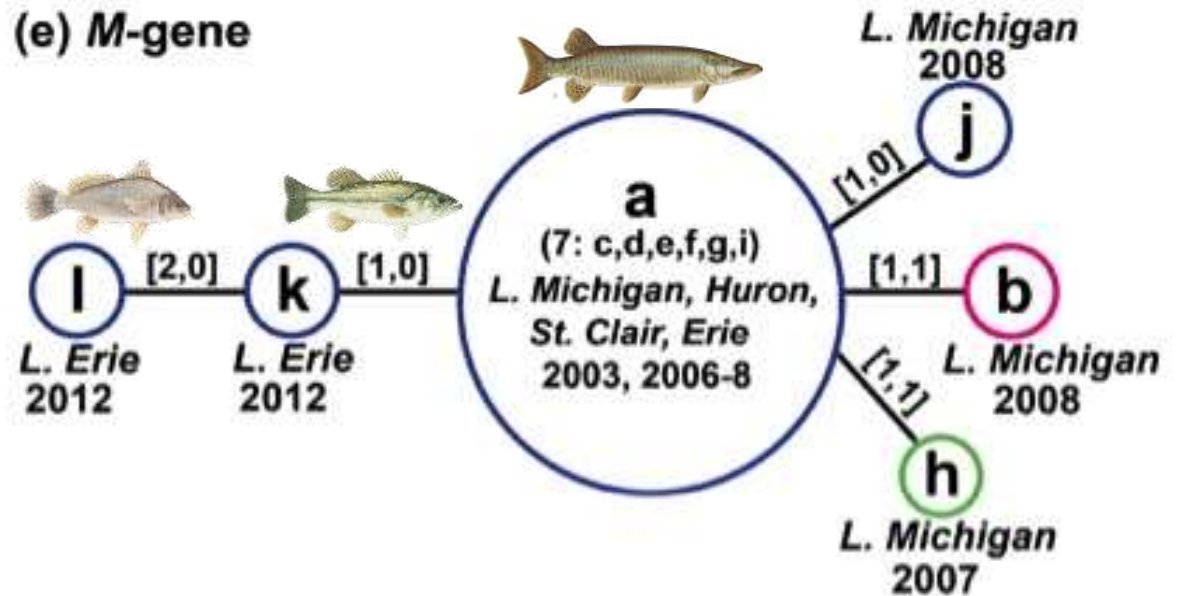
Stepien, Pierce,
Leaman, Niner,
Shepherd. PLOS
One 2015

- *Nv* evolves fastest
 $\mu=2.1 \times 10^{-3}$
- All G-gene variants also have unique *Nv* sequences
- All but one *Nv* variants differed in amino acids
- Functions in viral replication
- Anti-apoptotic role in early infection
- Involved in pathogenicity
- New paper: influences interferon pathway in fish host (Cano et al. 2016, Veterinary Microbiology)

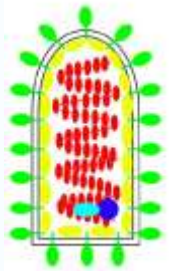
VHSV-IVb *P* & *M*-gene Haplotype Networks



(e) *M*-gene



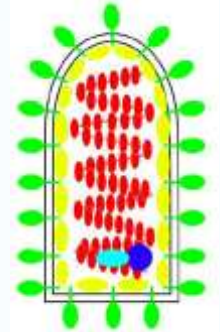
- *P*-functions in viral replication
- may help inhibit host response
- *M*-assists in viral budding
- may also inhibit host response



- More evolutionarily conserved
- Evolve at 1/15 NV's rate

VHS Summary to Date

- **VHSv likely originated in the N Atlantic**
 - Marine reservoir
 - Split into 2 primary clades: Eurasia & New World
 - Successful invader of freshwater systems
- **VHSv-IVb may have invaded the Great Lakes via St. Lawrence Seaway**
- **IVb has diversified rapidly into many unique variants**
- **Follows a quasispecies mode of evolution**
- ***Nv* gene evolves the fastest (>N>G>P>M)**
- **Current directions:**
 - Testing for new variants, sampling across GL
 - Whole genome sequencing
 - Analyzing virulence of variants in challenge experiments
 - Testing knock-out gene variants (w/ Dr. Vikram Vakharia, U Md)



Thank You:

Virus & Samples:

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Agencies & Universities:

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