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Genetic Diversity and Population Structure of the European Eel (Anguilla anguilla) in Baltic Lakeland

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***Abstract. Anguilla anguilla* (Linnaeus, 1758) is the unique catadromous fish species in the Latvian fauna. The Baltic Lakeland area representing naturally recruited and introduced eels. In recent decades, its population has decreased not only in Latvia, but also in Europe. European eel critically endangered due to overfishing of glass eels, blocking of migratory paths, deaths in HPS turbines, water pollution and diseases and parasites. Only some bodies of water are freely accessible to natural migration of eels in Latvia.**

Currently, its position has been recognized as being critical, and a range of normative acts have been adopted for its restoration, such as the Regulation EC 1100/2007. The restocking programs of the European eel *Anguilla anguilla* have been conducted for nearly one century in Latvia.

This study provides the first data on population structure of freshwater eels in Baltic Lakeland, for use in eel conservation and management of aquaculture on a regional and/or global scale. By analysing the sequences of the Cyt *b* gene of mtDNA for individuals caught in locations throughout in Baltic Lakeland, we determined the population genetic structure of *A. anguilla* in the area. The diversity of haplotypes was studied in ten waterbodies from part of Baltic Lakeland, namely Lake Sīvers, Lake Usmas, Lake Ķišezers, Lake Liepājas, Lake Alūksnes, Lake Rāznas, Lake Vialikija Švakšty, Lake Svir, Lake Myadzyel, Myadzelka river. Additionally, this study investigated the affinity of the Latvian populations to other *A. anguilla* populations around the world.

This is the first report about eel's population genetic diversity in Baltic Lakeland. Haplotype variation was different in all investigated waterbodies. In current studies seven new unique haplotypes were detected. Eel population in Baltic Lakeland shows quite high genetic diversity and rapid population expansions, which possibly is results of intensive restocking program. Sequences characterized *Anguilla rostrata* in Lake Alūksnes were detected.

Keywords: Anguilla anguilla, cyt b, genetic diversity, haplotype.

I. Introduction

European eel (*Anguilla anguilla* (L.)) is a catadromous euryhaline fish characterized by a complex biological cycle involving marine, brackish, and freshwater habitats [1]. The Baltic Lakeland area representing naturally recruited and introduced eels. In recent decades, its population has decreased not only in Latvia, but also in Europe. To increase the local eel production in Latvia, the restocking plan has been conduct since 1927. During 1960 to 1988 by the government, almost 30 million of glass eels imported from France were regularly released in 51 Latvian lakes [2]. After 1990, eel was mentioned in the fishing haul statistics in 16 lakes, but only four of these water bodies are freely accessible to natural migration of eel, and in the others eels which were released in the 1960s-1990s were caught [3]. The restocking programs of the

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European eel have been conducted for nearly one century in Latvia [2]. European eel critically endangered due to overfishing of glass eels, blocking of migratory paths, deaths in HPS turbines, water pollution and diseases and parasites. In Latvia, *A. anguilla* was one of the most expensive fish that fishermen were most interested in. Currently, its position has been recognized as being critical, and a range of normative acts have been adopted for its restoration, such as the Regulation EC 1100/2007 [2]. Although the restocking plans were conducted for a long time in Latvia, the following restocking efficiency was seldom evaluated, nor was the contribution of restocked eels to the local population examined. In Latvia, information on the genetic diversity and population structure of this species is necessary for resource management.

The aim of present research is to evaluate the genetic diversity of *Anguilla anguilla* population in Baltic Lakeland.

In order to address this paucity of information, sequencing of the entire mitochondrial control region (mtDNA) was carried out for *Anguilla anguilla* from Baltic Lakeland. By analysing the sequences of the Cyt *b* gene region of mitochondrial DNA for individuals caught in locations throughout in Baltic Lakeland, was determined the population structure of *A. anguilla* in the area. Additionally, this study investigated the affinity of the Latvian population to other *A. anguilla* populations around the world.

II. Materials and Methods

A. Sample Collection

To evaluate the efficiency of the eel restocking program and reveal the migratory life histories of European eels in Baltic Lakeland waters, a total of 34 individuals were collected. Eel samples were collected in 2014 - 2020 from seven lakes, namely Lake (further L.) Usmas, L. Liepajas, L. Kisezers, L. Aluksnes, L. Vaidavas, L. Raznas, L. Sivers in Latvia and from four waterbodies, namely L. Myadzyel, L. Svir, Myadzyelka river, L.Vialikija Svaksty in Belarus (Fig. 1), in accordance with the monitoring plan of fish resources.

Fig. 1. Sampling sites of studied European eel.

B. DNA Extraction and Mitochondrial DNA Sequencing

Genomic DNA was extracted from muscle tissue or fins according to the salt-extraction method of Aljanabi and Martinez [4], which earlier was used in genetic researches of water animals [5], [6]. The quality and quantity of DNA samples were determined using spectrophotometer BioSpec-Nano. The extracted DNA was stored at -20ºC until analysis. For the analysis, the DNA was diluted to a concentration of 10 ng/µL. The primers for the Cyt *b* gene (forward primer: 5`CCTCCTTCTTCTTTATCTGCCT 3`; reverse primer: 5`GTTTTCTAGTCAACCTGCTAATGG 3`) were used [7], [8]. Polymerase chain reaction (PCR) was performed using ABI 9700 thermocycler with a total reaction volume of 10 µL, containing ddH2O, 10xPCR buffer, 25mM MgCl2, 2mM dNTP Mix, 3pmol of each primer, 0.1U Taq DNA polymerase and 100 ng template DNA. Amplification started with an initial denaturation step for 5 min at 95 °C, followed by 35 cycles (denaturation for 45 s at 94 °C, annealing for 45 s at 56 °C, elongation for 1 min at 72 °C), and ended with a final elongation step for 5 min at 72 °C. The length of the amplified fragment was approximately 550bp. The amplified product was evaluated electrophoretically for quality using agarose gels and was purified using EXS-500 ExS-Pure™ Enzymatic PCR purification kit (NimaGen). Samples were sequenced in both directions using Big-Dye Terminator v1.1 Cycle Sequencing Kit (Thermofisher) on ABI 310 automated sequencer.

C. Data Analysis

The sequences were assembled and aligned using the ClustalW algorithm [9] in MEGA 11 [10]. The haplotype number (*Nh*), haplotype diversity (*h*), nucleotide diversity (π), number of polymorphic sites (*S*), number of mutations (η), average number of nucleotides differences (*k*), Tajima's D and Fu's Fs were calculated using DnaSP v6.12 [11]. The haplotype network was constructed using the Median Joining (MJ) [12] algorithm, implemented in NETWORK 10.2.0.0 software [13].

III. Results and Discussion

A total of 34 individuals were sequenced, total number of aligned sites was 394, number of polymorphic sites 26 and conserved sites 368. A total of 18 different haplotypes were identified (TABLE 1). Haplotype diversity (*Hd*) for all the 394 sequences was calculated to be 0.914 +/- 0.032 SD and nucleotide diversity π was 0.0075+/- 0.0017 SD. Average number of nucleotide differences *k*= 2.95. Totally there were noted 27 point mutations and nine parsimony informative sites. In spite of high haplotype diversity, low nucleotide diversity value suggested, that there are small differences between haplotypes. Both Tajima's D and Fu's Fs statistics were negative (- 1.94 and -10.39) and statistically significant ($P < 0.05$). That indicates deviations from neutrality. The combination of high haplotype and low nucleotide diversity in present research can be a signature of a rapid population expansion from a small effective population size as a consequence of intensive restocking program. The rapid eel's population expansion, shown by mtDNA

markers, was also revealed in eel's population from waterbodies in Lithuania [8].

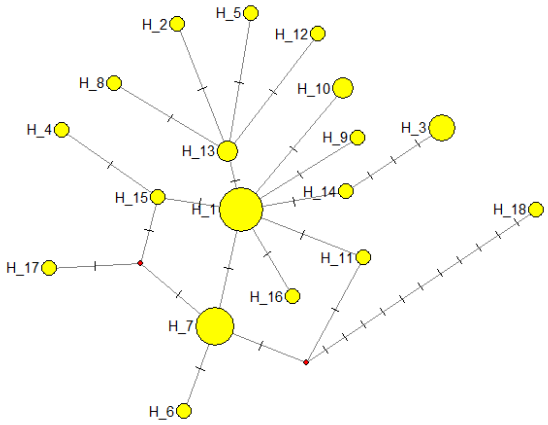

Fig. 2. Haplotype network of *Anguilla anguilla* based on *Cyt b* gene sequences (the dashes between the nodes indicate particular mutations, the size of the circles is proportional to the haplotype frequency).

Detected haplotypes were separated into two haplogroups based on haplotype frequency and by minimum mutational steps (Fig. 2). Network reflects haplotype diversity, variability and relationship between detected haplotypes.

Altogether 18 haplotypes were revealed (TABLE 1). Haplotype H_1 was revealed in eel's samples from five waterbodies, namely L.Usmas, L. Kisezers, L. Vaidavas, L. Liepajas and L. Myadzyel. Haplotype H_1 is widespread across *Anguilla anguilla* distributional range. This haplotype was reported for 26 isolates from eight localities (data from GenBank) [14], [15]. Another haplotype, which was detected in five waterbodies (L. Myadzyel, L. Raznas, L. Usmas, L. Sivers and Myadzyelka river) is haplotype H_7.

TABLE 1 HAPLOTYPE VARIATION DETECTED AMONG 34 *ANGUILLA ANGUILLA* SAMPLES FROM 11 WATERBODIES IN BALTIC LAKELAND (ONLY VARIABLE SITES WITH SEQUENCE POSITION GIVEN IN FIRST ROW ARE SHOWN. IDENTTITY WITH THE FIRST SEQUENCE IS MARKED BY THE DOT, SUBSTITUTION IS MARKED BY A DIFFERENT BASE LETTER (A, T, G OR C))

Position / **Haplotypes**	**38**	**47**	**89**	**122**	**140**	**153**	**176**	**179**	**203**	**209**	**224**	**242**	**250**	**254**	**272**	**281**	**284**	**308**	**311**	**317**	**362**	**377**	**380**	**386**	**389**	**392**	**Frequency**
H_1	A	T	A	T	C	C	T	G	A	C	G	C	T	A	T	C	A	A	A	G	C	C	C	T	G	G	8
H_2	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	1
H_3	.	.	.	C	.	.	.	.	G	.	.	.	.	.	.	T	.	.	.	A	.	.	.	.	.	.	3
H_4	.	.	.	.	.	.	C	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1
H_5	.	C	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1
H_6	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	1
H_7	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	6
H_8	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	1
H_9	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	1
H_10	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	2
H_11	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	1
H_12	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	1
H_13	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	2
H_14	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1
H_15	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1
H_16	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1
H_17	.	.	.	.	T	.	.	A	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1
H_18	.	.	.	.	.	T	.	.	.	T	A	T	A	.	C	.	.	G	G	A	.	.	T	C	A	A	1

H_7 haplotype is also widespread across *Anguilla anguilla* distributional range. Identical sequences were reported for ten *Anguilla anguilla* isolates and for one *Anguilla rostrata* isolate (data from GenBank) [15], [17]. In current studies three haplotypes were revealed, which occur each in two waterbodies. That were H_3 in L. Kisezers and L. Sviras, H_10 in L. Sivers and L. Vialikija Svaksty, H_13 in L. Sviras and L. Vialikija Svaksty. The sequence similar to H_3 haplotype has been reported in GenBank for only one isolate of *Anguilla anguilla* x *Anguilla rostrata* hybrid from Greenland [17]. The sequence of haplotype H_10 was quite rare. Identical sequences have been reported in GenBank only for two *Anguilla anguilla* isolates from Cantabrian Sea in Spain and from Ireland's waters [15], [17]. Sequences similar to haplotype H_13 in present study have been reported in GenBank for two *Anguilla anguilla* x *Anguilla rostrata* hybrid's isolates from Greenland [20] and for three *Anguilla anguilla* isolates from other waterbodies [2], [15].

The sequences of five haplotypes (namely, H_11, H_14, H_15, H_16 and H_17) are quite rare in present research. That were revealed each in one waterbody only.

But identical sequences in GenBank have been reported as widespread. For instance, sequences of haplotype H_11 has been reported for 27 *Anguilla anguilla* isolates from waterbodies in Spain, Ireland, France and Sweden [14], [16], [21]. Whereas sequence of haplotype H_14, which was detected in L. Sviras, has been reported for one *Anguilla anguilla* isolate only [22]. Sequences of haplotype H_15, which was revealed in L. Sviras also, has been reported for two *Anguilla anguilla* isolates from France and Ireland waterbodies and two *Anguilla anguilla* x *Anguilla rostrata* hybrid's isolates from Iceland waterbodies [18], [16]. Sequence of haplotype H_16, which was detected in L. Usmas only, in GenBank has been reported for *Anguilla anguilla* x *Anguilla rostrata* hybrid's isolates from Iceland [18]. Finally, very rare sequence of haplotype H_17, which was detected in Myadzyelka river, has been reported for only one *Anguilla anguilla* isolate in France waterbodies [23].

In current studies new unique haplotypes were detected, namely H_8 in L. Myadzyel, H_9 in L. Sivers, H_12 in L. Sviras, H_2 and H_4 in L. Kisezers, H_5 and H_6 in L. Liepajas. New revealed haplotypes have not been deposited to GenBank yet.

The last haplotype H_18 was detected in L. Aluksnes only, but that sequence has been reported in GenBank for 20 isolates as *Anguilla rostrata* (American eel) from different waterbodies in USA. Lake Aluksne is a waterbody which is not freely accessible to natural migrations.

So, definitely, we have *Anguilla rostrata* individuals or *Anguilla anguilla* x *Anguilla rostrata* hybrids in L. Aluksnes and can speak about anthropogenic invasion as a result of incorrect resource for restocking.

These two species (European eel and American eel) spawn in Sargasso Sea (Atlantic Ocean) and therefore American eel or its hybrids could not be as a potential threat to biodiversity of European eel. However, these two species have similar biology and, therefore, could enter trophic interactions and compete. In addition, translocated species, may promote pathogen pollution in the invaded area leading to the emergence of diseases.

IV. Conclusions

This is the first report about eel's population genetic diversity in Baltic Lakeland. Eel population in Baltic Lakeland shows quite high genetic diversity and rapid population expansions, which possibly is results of intensive restocking program. American eel's haplotype detected in sample from Lake Aluksnes, indicate possible anthropogenic invasion, which may be a result of incorrect resource for restocking.

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REFERENCES

[1] M. Y. Elgendy, A. M. Kenawy, and A. E. Noor El-Deen, "*Gyrodactylus anguillae* and *Vibrio vulnificus* infections affecting cultured eel, *Anguilla anguilla*", Com. Sci., vol. 7, no. 1, pp. 1–11, May 2016, https://doi.org/10.14295/cs.v7i1.1248

[2] Y.-J. Lin, J.-C. Shiao, M. Plikshs, A. Minde, Y. Iizuka, I. Rashal and W.-N. Tzeng, "Otolith Sr:Ca Ratios as Natural Mark to Discriminate the Restocked and Naturally Recruited European Eels in Latvia", American Fisheries Society Symposium, vol. 76, pp. 1-14, Jan. 2011, https://doi.org/10.1111/j.1095-8649.2009.02471.x

[3] Ē. Aleksejevs and J. Birzaks, "Long-term Changes in the Ichthyofauna of Latvia's Inland Waters", Scientific Journal of Riga Technical University, Environmental and Climate Technologies, vol. 7, pp. 9-18, Jan. 2011, https://doi.org/10.2478/v10145-011-0022-2

[4] S. M. Aljanabi and I. Martinez, "Universal and rapid salt-extraction of high quality genomic DNA for PCR based techniques", Nucleic Acids Research, vol. 25, pp. 4692-4693, Nov. 1997, http://dx.doi.org/10.1093/nar/25.22.4692

[5] A. Morozova and N. Shkute, "Genetic characteristics of the *Dreissena polymorpha* population in Latvia (Lake Rāzna) as part of the European population", Archives of Biological Sciences, 11 p., March 2023, https://doi.org/10.2298/ABS230202011M

[6] J. Oreha and N. Škute, "Current genetic structure of European vendace *Coregonus albula* (L.) populations in Latvia after multiple past translocations". Animal Biodiversity and Conservation, vol. 45, no. 2, pp. 161-173, May 2022, https://doi.org/10.32800/abc.2022.45.0161

[7] A. Ragauskas, D. Butkauskas, A. Sruoga, V. Kesminas, I. Rashal and W. -N. Tzeng, "Analysis of the genetic structure of the European eel *Anguilla Anguilla* using the mtDNA D-loop region molecular marker", Fisheries Science, vol. 80, pp. 463-474, May 2014, https://doi.org/10.1007/s12562-014-0714-1

[8] A. Ragauskas, D. Butkauskas and M. Bianchini, "Distinct matrilines in the panmictic population of the European eel *Anguilla anguilla*?", Aquat. Living Resour., vol. 30, no. 21, June 2017, https://doi.org/10.1051/ALR/2017021

[9] J. D. Thompson, D. G. Higgins and T. J. Gibson, "CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice", Nucleic Acids Res., vol. 22, no. 22, pp. 4673-80, Nov. 1994, https://doi.org/10.1093/nar/22.22.4673

[10] K. Tamura, G. Stecher and S. Kumar, "MEGA11: Molecular Evolutionary Genetics Analysis Version 11", Mol. Biol. Evol., vol. 38, no. 7, pp. 3022-3027, June 2021, https://doi.org/10.1093/molbev/msab120

[11] J. Rozas, A. Ferrer-Mata, J. C. Sánchez-DelBarrio, S. Guirao-Rico, P. Librado, S. E. Ramos-Onsins and A. Sánchez-Gracia, "DnaSP 6: DNA Sequence Polymorphism Analysis of Large Data Sets", Mol Biol Evol., vol. 34, no. 12, pp. 3299-3302, Dec. 2017, https://doi.org/10.1093/molbev/msx248

[12] H.-J. Bandelt, P. Forster and A. Röhl, "Median-joining networks for inferring intraspecific phylogenies", Mol. Biol. Evol., vol. 16, no. 1, pp. 37-48, Jan. 1999, https://doi.org/10.1093/oxfordjournals.molbev.a026036

[13] C. Solís-Lemus, P. Bastide, C. Ané, "PhyloNetworks: A Package for Phylogenetic Networks", Mol. Biol. Evol., vol. 34, no. 12, pp. 3292-3298, Dec. 2017, https://doi.org/10.1093/molbev/msx235

[14] Y. Minegishi, J. Aoyama, J. G. Inoue, M. Miya, M. Nishida and K. Tsukamoto, "Molecular phylogeny and evolution of the freshwater eel's genus Anguilla based on the whole mitochondrial

genome sequences", Mol. Phylogenet. Evol., vol. 34, no. 1, pp. 134-146, Jan. 2005, https://doi.org/10.1016/j.ympev.2004.09.003

[15] Y. S. Lin, Y. P. Poh and C. S. Tzeng, "A phylogeny of freshwater eels inferred from mitochondrial genes", Mol. Phylogenet. Evol., vol. 20, no. 2, pp. 252-261, Aug. 2001, https://doi.org/10.1006/mpev.2001.0969

[16] M. W. Jacobsen, J. M. Pujolar, M. T. Gilbert, J. V. Moreno-Mayar, L. Bernatchez, T. D. Als, J. Lobon-Cervia and M.M. Hansen, "Speciation and demographic history of Atlantic eels (*Anguilla anguilla* and *A. rostrata*) revealed by mitogenome sequencing", Heredity, vol. 113, no. 5, pp. 432-442, May 2014, https://doi.org/10.1038/hdy.2014.44

[17] J. Aoyama, M. Nishida and K. Tsukamoto, "Molecular Phylogeny and Evolution of the Freshwater Eel, Genus Anguilla", Molecular Phylogenetics and Evolution, vol. 20, no. 3, pp. 450-459, Sept. 2001, https://doi.org/10.1006/mpev.2001.0959

[18] J. Frankowski and R. Bastrop, "Identification of *Anguilla anguilla* (L.) and *Anguilla rostrata* (Le Sueur) and their hybrids based on a diagnostic single nucleotide polymorphism in nuclear 18S rDNA", Mol. Ecol. Resour., vol. 10, no. 1, pp. 173-176, Jan. 2010, https://doi.org/10.1111/j.1755-0998.2009.02698.x

[19] R. Gonzalez-Sevilla, S. Perez-Benevente, J.A. Gonzalez, S. Jimenez, A. Diez and J.M. Bautista, "FishTrace: Genetic Catalogue, Biological Reference Collections and Online Database of European Marine Fishes", [Online]. Available: GenBank, https://www.ncbi.nlm.nih.gov/nucleotide/EF427618.1?report=genbankandlog$=nucltopandblast_rank=2andRID=1YNGWD3901N . [Accessed March 27, 2023].

[20] J. Frankowski, R. Hedeholm, P. R. Moeller, I. Lindner, E. Magnussen, M. Sanchez and R. Bastrop, "Hybrid ancestry of Greenlandic Nimeriaq (*Anguilla anguilla* x *A. rostrata*)", [Online]. Available: GenBank, https://www.ncbi.nlm.nih.gov/nucleotide/HG794922.1?report=genbankandlog$=nucltopandblast_rank=4andRID=1YNT09DS01N . [Accessed March 27, 2023].

[21] M. Noren, S.O. Kullander and M. Kallersjo, "FishTrace: Genetic Catalogue, Biological Reference Collections and Online Database of European Marine Fishes", [Online]. Available: GenBank, https://www.ncbi.nlm.nih.gov/nucleotide/EU492326.1?report=genbankandlog$=nucltopandblast_rank=1andRID=1YP9MJ7A013 . [Accessed March 27, 2023].

[22] J. Frankowski, R. Hedeholm, P. R. Moeller, I. Lindner, E. Magnussen, M. Sanchez and R. Bastrop, "Hybrid ancestry of Greenlandic Nimeriaq (*Anguilla anguilla* x *A. rostrata*)", [Online]. Available: GenBank, https://www.ncbi.nlm.nih.gov/nucleotide/HG794873.1?report=genbankandlog$=nucltopandblast_rank=5andRID=1YNY8F1S01N . [Accessed March 27, 2023].

[23] V. S. Verrez-Bagnis, M. Jerome, O. Mouchel, S. Iglesias and P. Pruvost, "FishTrace: Genetic Catalogue, Biological Reference Collections and Online Database of European Marine Fishes", [Online]. Available: GenBank, https://www.ncbi.nlm.nih.gov/nucleotide/EU223997.1?report=genbankandlog$=nucltopandblast_rank=1andRID=1YPHHPGF013 . [Accessed March 27, 2023].