


RESEARCH NOTE

Open Access



A phylogeny of the genus *Limia* (Teleostei: Poeciliidae) suggests a single-lake radiation nested in a Caribbean-wide allopatric speciation scenario

Montrai Spikes^{1,2}, Rodet Rodríguez-Silva², Kerri-Ann Bennett³, Stefan Bräger⁴, James Josaphat⁵, Patricia Torres-Pineda⁶, Anja Ernst¹, Katja Havenstein¹, Ingo Schlupp^{1,2} and Ralph Tiedemann^{1*} 

Abstract

Objective: The Caribbean is an important global biodiversity hotspot. Adaptive radiations there lead to many speciation events within a limited period and hence are particularly prominent biodiversity generators. A prime example are freshwater fish of the genus *Limia*, endemic to the Greater Antilles. Within Hispaniola, nine species have been described from a single isolated site, Lake Miragoâne, pointing towards extraordinary sympatric speciation. This study examines the evolutionary history of the *Limia* species in Lake Miragoâne, relative to their congeners throughout the Caribbean.

Results: For 12 *Limia* species, we obtained almost complete sequences of the mitochondrial cytochrome *b* gene, a well-established marker for lower-level taxonomic relationships. We included sequences of six further *Limia* species from GenBank (total N = 18 species). Our phylogenies are in concordance with other published phylogenies of *Limia*. There is strong support that the species found in Lake Miragoâne in Haiti are monophyletic, confirming a recent local radiation. Within Lake Miragoâne, speciation is likely extremely recent, leading to incomplete lineage sorting in the mtDNA. Future studies using multiple unlinked genetic markers are needed to disentangle the relationships within the Lake Miragoâne clade.

Keywords: Cytochrome *b*, Island biogeography, Fresh water fish, Phylogeny

Introduction

The Caribbean is considered one of the most important global biodiversity hotspots [1]. The largest biodiversity is

found in the Greater Antilles (Cuba, Hispaniola, Jamaica and Puerto Rico) where a remarkable diversification is observed in freshwater fishes [2–5], amphibians [6, 7], reptiles [8, 9], invertebrates [10–12] and plants [13, 14], putatively driven by a complex geological history, environmental heterogeneity, and the tropical climate [15, 16].

Adaptive radiations typically occur when a set of open niches becomes available because of a key innovation or the arrival of a founder species, which subsequently differentiates to occupy these niches [17]. Many classical examples are linked to islands, as Darwin's Finches on the Galapagos islands, all of which go back to a

The original online version of this article was revised to provide additional funding information.

*Correspondence: tiedeman@uni-potsdam.de

¹ Unit of Evolutionary Biology/Systematic Zoology, Institute of Biochemistry and Biology, University of Potsdam, Karl-Liebknecht-Straße 24-25, Haus 26, 14476 Potsdam, Germany

Full list of author information is available at the end of the article



© The Author(s) 2021, corrected publication 2022. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>. The Creative Commons Public Domain Dedication waiver (<http://creativecommons.org/publicdomain/zero/1.0/>) applies to the data made available in this article, unless otherwise stated in a credit line to the data.

single ancestor [18–20]. Research on Darwin's Finches also highlighted the role of hybridization in speciation [21]. Other well-explored radiations include Hawaiian silverswords [22–24] and Hawaiian honeycreepers [25]. In all these examples, molecular evidence has played an important role in understanding the evolutionary processes of speciation. Probably the best-known examples from the Caribbean region are *Anolis* lizards [26] and *Eleutherodactylus* frogs [27].

Poeciliidae are freshwater livebearing fishes that have experienced an enormous radiation in aquatic environments of the West Indies with three endemic genera (*Girardinus*, *Quintana* and *Limia*) distributed in the Antilles [3, 4, 28, 29]. The Caribbean is also the site of two lesser known radiations in isolated inland lakes, both of which involve fishes of the genus *Cyprinodon* [30–33]. These Caribbean fishes share many characteristics with the most prominent example of radiation in freshwater fishes, the cichlids in lakes of the Rift Valley of East Africa, where each lake has produced a distinct cichlid fauna [34–36]. One of the important drivers for speciation in these fishes seems to be feeding specializations [33, 37, 38]. Furthermore, as generally predicted from the theory of island biogeography [39] and recently empirically confirmed for island birds [40, 41], the number and diversity of species in both the Rift Valley lakes and Greater Antilles correlates with the size of the habitat.

Among livebearing fishes of the Greater Antilles, the origin of the different lineages and species composition within each genus may show peculiar patterns [39, 42]. *Limia* is part of the unique freshwater fish fauna of the Greater Antilles. It is found in most freshwater habitats in Hispaniola, ranging from hypersaline lagoons to relatively cool mountain streams [43, 44]. *Limia* species are generally feeding generalists [2, 45, 46]. Their distribution indicates a radiation on Hispaniola [2, 47], with 19 of the 23 known species found on this island [46, 48] (Additional file 1). By contrast, on Cuba, Jamaica, and Grand Cayman, only one species each is found [28, 44, 49]. Within Hispaniola, nine *Limia* species (*L. fuscomaculata*, *L. garnieri*, *L. grossidens*, *L. immaculata*, *L. islai*, *L. mandibularis*, *L. miragoanensis*, *L. nigrofasciata*, *L. ornata*) have been described from a single site, Lake Miragoâne. This lake is one of the largest freshwater lakes in the Caribbean and is located in the southwestern part of Haiti. It comprises an isolated, endorheic drainage [50]. A *Limia* radiation there was hypothesized by Rivas [2] and has received renewed attention through the description of two new species from the lake [43, 45]. However, few studies have examined the evolutionary history of the fishes found in Lake Miragoâne.

Without specific attention to Lake Miragoâne, some studies of *Limia* have resolved the general phylogeny of

the genus. Current literature suggests *Limia* to form a monophyletic group with the genera *Pamphorichthys*, *Mollienisia*, *Micropoecilia*, and *Poecilia*, with *Limia* as sister taxon to *Poecilia* [51–53]. *Limia melanogaster* is the most basal species, branching off early and colonizing Jamaica [2]. *Limia melanogaster's* divergence was followed by the colonization of Hispaniola, where the species diverged into over 20 recognized species [44]. Nested within the species native to Hispaniola are *L. vittata* and *L. caymanensis* [2, 54] which are the only species native to their respective islands, Cuba and Grand Cayman [28, 44]. Most previous analyses target only a few species [52, 53, 55]. The most comprehensive phylogeny to date used nine species of *Limia*. Among them were only two native to Lake Miragoâne, *Limia nigrofasciata* and *Limia islai* [2, 44, 46], such that Riva's hypothesis of a local radiation within Lake Miragoâne [1] could so far not been tested.

Our study comprises 18 out of 23 currently recognized species of *Limia*. It is particularly novel regarding its more comprehensive sampling of Lake Miragoâne, including five of its native species. We expected that if a local radiation event did occur in Lake Miragoâne, those species native to the lake should form a monophyletic clade.

Main text

Materials and methods

Sampling

Ingroup sampling consisted of 67 individuals representing 18 species of *Limia* (Additional files 2, 3). Twelve *Limia* species were obtained from wild-caught populations. Sequences from six *Limia* species were obtained from GenBank: *L. garnieri*, *L. melanonotata*, *L. pauciradiata*, *L. rivasi*, *L. versicolor*, and *L. sulphurophila*. Outgroup sampling consisted of eight individuals representing three species of *Poecilia*, the sister taxon to *Limia* [28, 44, 55]: *P. dominicensis* [45], *P. hispaniolana* [56], both endemic to Hispaniola, and *P. mexicana* from the Atlantic side of Mexico. We used four to five individuals per species, except in cases where sampling was limited.

Molecular methods

We targeted the mitochondrial (mt) cytochrome *b* gene, a well-established marker for lower-level taxonomic relationships as in recent radiations (see [57] for a fish example) for which we obtained an almost complete sequence.

Genomic DNA was extracted from muscle tissue using a cetyl trimethylammonium bromide (CTAB) protocol [58]. DNA concentration was measured using a NanoDrop ND-1000 and ranged from 2.7 to 120 ng/μl. Via Polymerase Chain Reaction (PCR), we amplified 1127 bp of the mitochondrial cytochrome *b* gene. Primers and

reaction profiles were modified from Hrbek et al. ([51]; Additional file 4). Except for *L. vittata*, *P. dominicensis*, and *P. hispaniolana*, the primer combinations L14725 and H15981 were used. 1 µl DNA isolate was used during amplification (increased to 2 µl if DNA concentration was below 20 ng/µl). PCR reactions contained 0.12 µl of 5 U/µl MyTaq mtDNA polymerase (Bioline), 0.5 µl of each 10 µM primer, 5 µl of 5 × MyTaq reaction buffer and HPLC H₂O up to a final volume of 50 µl. PCR products were sequenced using Applied Biosystems™ BigDye™ Terminator v3.1 Cycle Sequencing Kits (ThermoFisher), purified with ExoSAP (Exonuclease I [59] and Antarctic Phosphatase [60]) according to manuals from New England Biolabs, and run on an Applied Biosystems™ 3500 sequencer.

Phylogenetic and haplotype network analyses

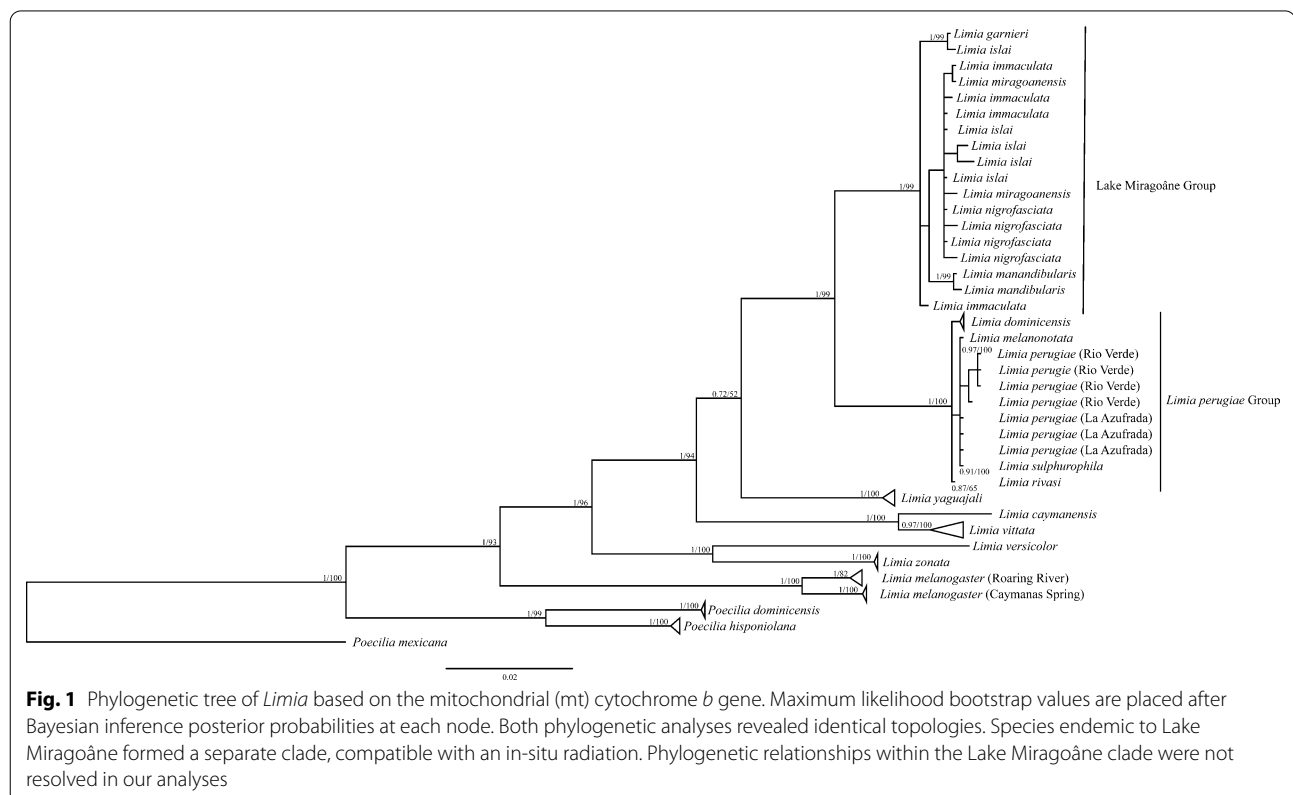
Sequences were manually edited and aligned with ClustalW in BioEdit v.7.2 [61, 62] and 1127 bp of cytochrome *b* were used in phylogenetic analyses. Potential mutation saturation was assessed with DAMBE [63]. We conducted Maximum Likelihood analyses using RAxML GUI v.2.0 [64, 65] and assessing clade support via 10,000 rapid bootstrap pseudoreplicates. Separately, we conducted Bayesian analyses in MrBayes v.3.2.7. [66], where we ran four Markov chains for 10,000,000 iterations,

sampling every 1000 iterations, with three heated chains and one cold chain and default parameters unlinked across partitions. Convergence was assessed using Tracer v.1.7. All parameter estimates were verified to have been sampled sufficiently (ESS >200). We removed the first 25% of our trees as burn-in, such that 3002 trees were retained. Nodes were considered with bootstrap support (BS) and Bayesian posterior probability (PP) greater than 70 and 0.95, respectively [67, 68]. A haplotype network was constructed within PopArt [69] using a median joining network [70]. Genetic distances between taxonomic groups were calculated in MEGA [71].

Results

There was no indication of mutation saturation in our data set (Additional file 5). Maximum Likelihood and Bayesian trees revealed nearly identical topologies for interspecific relationships (Fig. 1). In both trees, there is strong support that the species found in Lake Miragoâne are monophyletic (BS = 97; PP = 1.0). However, within Lake Miragoâne, *L. mandibularis* is the only species resolved as a monophyletic group, while the phenotypically described species *L. islai*, *L. immaculata*, *L. miragoanensis*, and *L. nigrofasciata* form a polytomy.

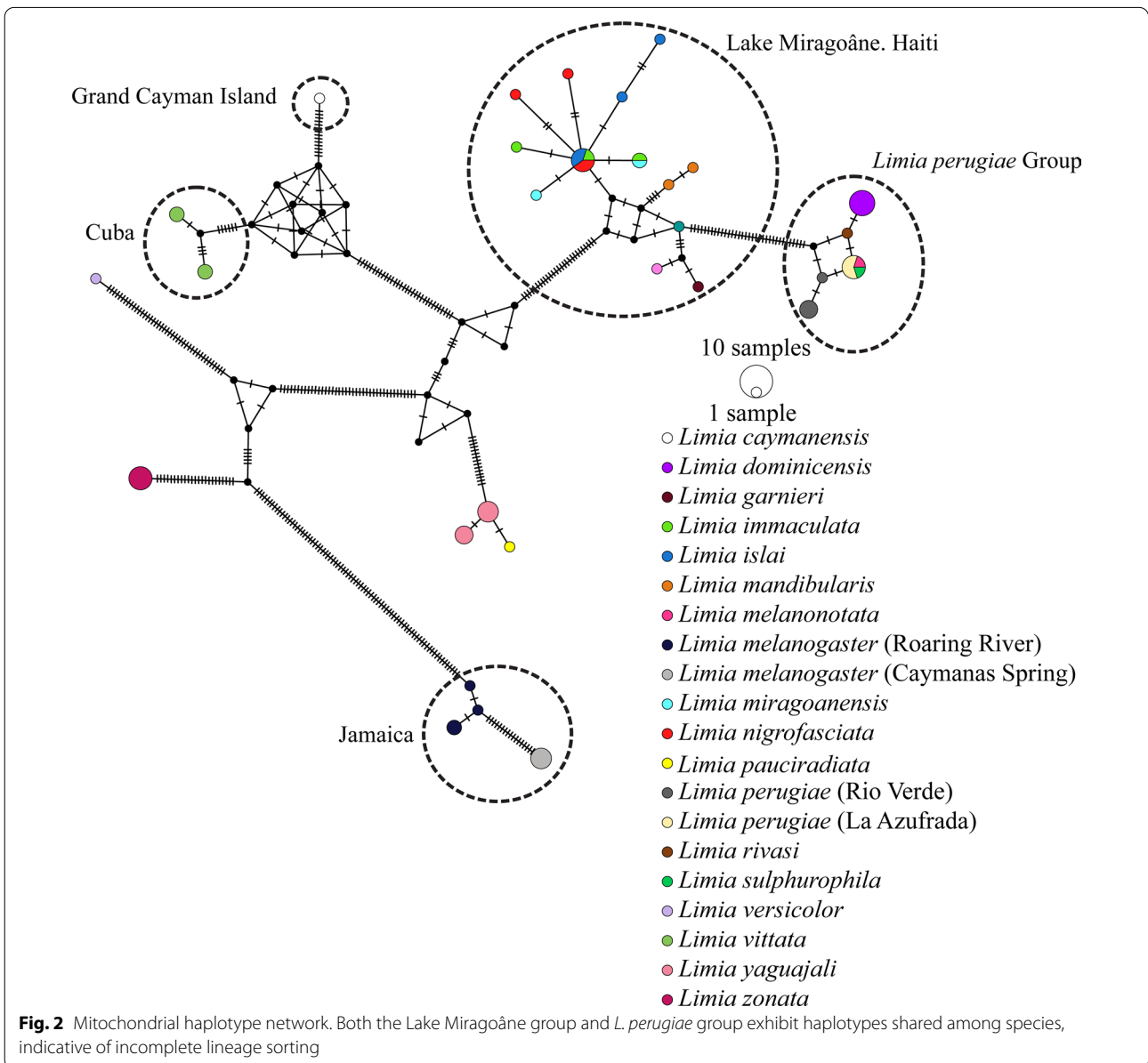
For the majority of taxa outside Lake Miragoâne, both species monophylies and respective taxonomic



relationships were well supported. *L. yaguajali* is sister to a clade consisting of *L. perugiae*, *L. dominicensis*, and the species in Lake Miragoâne, but this node has only moderate support (BS = 61; PP = 72). We found significant genetic divergence among two populations of *L. melanogaster* on Jamaica (BS = 100; PP = 1.0; Additional file 6). The haplotype network (Fig. 2) confirms divergent *Limia* evolution among the different Caribbean islands. Within Lake Miragoâne and in the *L. perugiae* group, some mitochondrial haplotypes were shared among morphological species.

Discussion

The five *Limia* species from Lake Miragoâne are recovered as a well-supported clade in all phylogenetic analyses, indicating an in situ radiation. However, taxonomic relationships within the clade were not resolved. These species have likely diverged too recently for complete lineage sorting and reciprocal monophyly to evolve at a single maternally inherited locus like cytochrome *b*. Alternatively, the observed pattern could be due to species hybridization, introgression, or phenotypic species misassignment [72]. However, the distinct morphological differences of the species found in Lake Miragoâne make



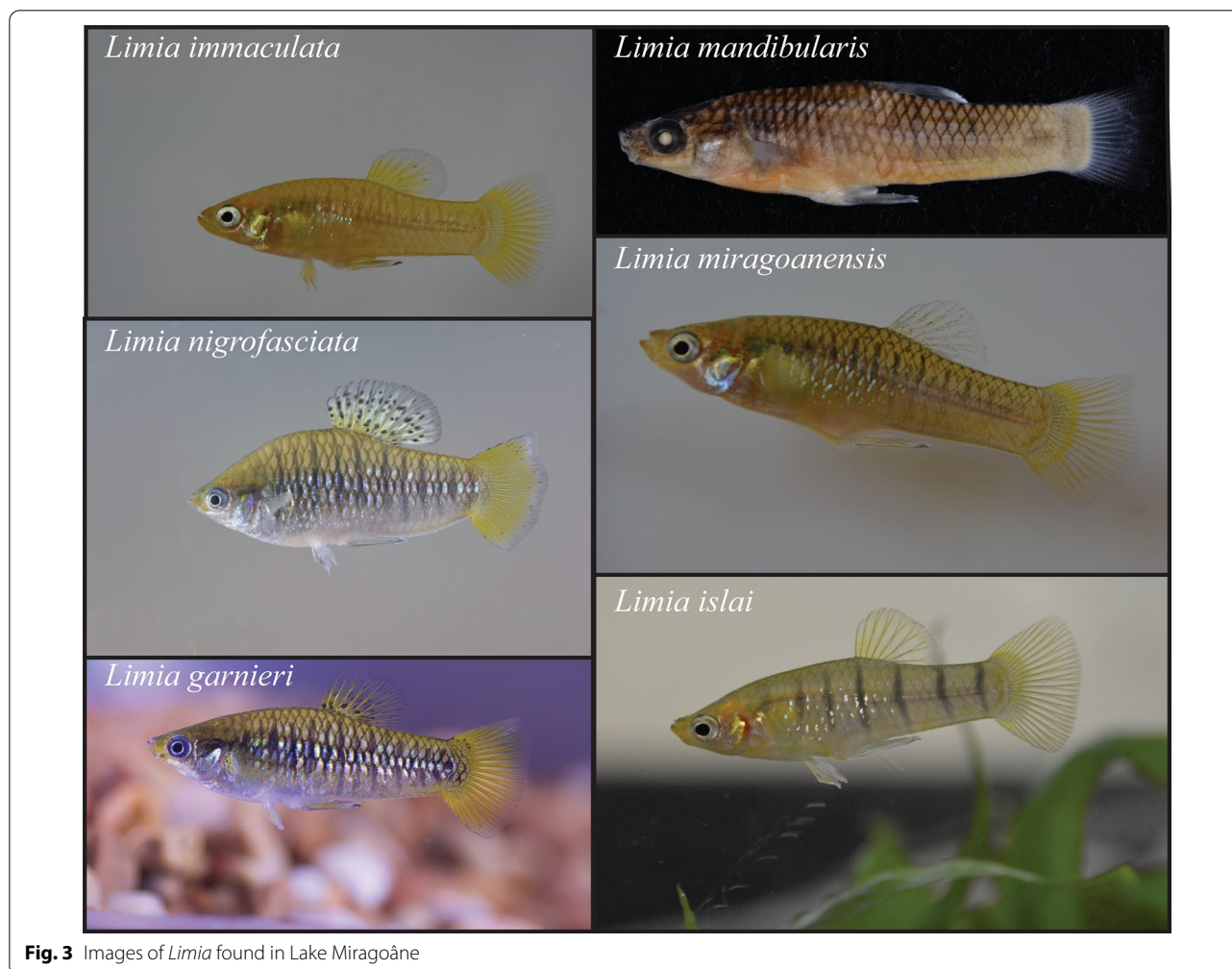


Fig. 3 Images of *Limia* found in Lake Miragoâne

us reluctant to attribute the polytomy to phenotypic species misassignment (Fig. 3).

Most other relationships supported in our phylogenetic analyses are consistent with the findings of previous phylogenetic studies [28, 44]. *Limia melanogaster*'s basal placement (Fig. 1) corroborates initially colonization of Jamaica before radiating across other islands in the Greater Antilles [41], lending additional support to the Greater Antilles and Aves Ridge (GAAR) landia hypothesis [44, 73]. The divergence within *L. melanogaster* may be the first evidence of a further cryptic speciation event in *Limia* (Additional file 5). *Limia vittata* from Cuba and *L. caymanensis* from Grand Cayman group within the Hispaniola clade. These two species are likely the sister taxa to *L. yaguajali* which is found in the north of Hispaniola. This coincides with geological evidence that eastern Cuba and north-central Hispaniola were likely connected as a single magmatic arc during the Paleocene-Eocene [74] until the Oligocene [75]. Together, the biogeographic and

geological evidence suggests that a *L. vittata* ancestor reached Cuba from the north of Hispaniola and subsequently *L. caymanensis* ancestor reached Grand Cayman from Cuba. Alternatively, they may have reached Cuba via open ocean migration, which has been found in freshwater fishes [5].

The *L. perugiae* group also exhibits a shallow phylogeny with short branches and one haplotype shared across species, again indicating a recent divergence or incomplete lineage sorting. *L. perugiae* is found from hypersaline lagoons to cool freshwater streams and dominates another large lake on Hispaniola, Lake Enriquillo. *Limia perugiae* is also widely distributed throughout Hispaniola with many isolated populations. The combination of *L. perugiae*'s diverse life history strategies and fragmented populations may promote cryptic speciation. However, given our inability to genetically resolve this group, phenotypic plasticity could be an alternative explanation.

It is known that *Limia* species from Lake Miragoâne all inhabit extremely similar niches [76] and they likely

possess similar life histories, perhaps with the exception of *L. mandibularis*. This species has well-developed and anteriorly projected lower jaw, deviating from other poeciliids and potentially reflecting specializations in diet [48]. Marked sexual dimorphism, with males such as in *L. nigrofasciata* being extremely ornamented, suggests that sexual selection is also present. Therefore, it is plausible that both natural and sexual selection might—independently or in concert—act as drivers in the potential radiation of *Limia* in Lake Miragoâne.

Limitations

We present initial evidence for a potential radiation in Lake Miragoâne, yet we recognize the limitations of a single-gene phylogeny. Our preliminary findings are supported by morphometric data that show distinct phenotypic differences between multiple *Limia* populations [1, 43, 45]. The use of multiple unlinked markers, such as microsatellites or SNPs, along with increased population sampling are imperative to understand the radiation event within this clade, as is true for the *L. perugiae* group as well. Such analyses may also resolve the relationship of *L. yaguajali* with *L. vittata* and *L. caymanensis*. We acknowledge that 18 species represent only a subset of the 23 known species of *Limia*, therefore future studies should continue to increase species sampling. Furthermore, our methodology cannot rule out ongoing hybridization between species in Lake Miragoâne, however, the species we keep in the International Stock Center for Livebearing Fishes, appear to breed like regular species.

Abbreviations

BS: Bootstrap support; CTAB: Cetyl trimethylammonium bromide; GAAR: Greater Antilles and Aves Ridge; *L.*: *Limia*; mt: Mitochondrial; *P.*: *Poecilia*; PCR: Polymerase chain reaction; PP: Bayesian posterior probability.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s13104-021-05843-x>.

Additional file 1: Figure S1. Biogeographical distribution of *Limia* species on the Greater Antilles. Note the high species number on Hispaniola, suggesting a radiation on that island.

Additional file 2: Table S1. Taxon list including collection localities, country of origin, coordinates, date of collection, and accession numbers. 'NA' denotes the information is either unavailable or inapplicable.

Additional file 3: Figure S2. Sampling sites on Hispaniola. LIM: *Limia immaculata*, LIS: *L. islai*, LMA: *L. mandibularis*, LMI: *L. miragoanensis*, LNI: *L. nigrofasciata*, LPEaz: *L. perugiae* (La Azufrada), LPErv: *L. perugiae* (Rio Verde), LDO: *L. dominicensis*, LVE: *L. versicolor*, LZO: *L. zonata*, LYA: *L. yaguajali*, PDO: *Poecilia dominicensis*, PHI: *P. hispaniolana*.

Additional file 4: Table S2. Cytochrome *b* PCR primer pairs and temperature profiles. Primer sequences are listed from 5' to 3' ends. For primers not developed during this study, references are provided.

Additional file 5: Figure S3. DAMBE saturation plot for our cytochrome *b* data set. There is no indication of saturation, as transitions (s) exceed transversions (v) and both are linearly correlated to genetic distance.

Additional file 6: Table S3. p-distance between genetically analysed species and populations of *Limia* and *Poecilia* based on mitochondrial cytochrome *b*.

Acknowledgements

We thank the members of the Tiedemann research group, in particular Marijke Autenrieth, for their assistance in the molecular and associated data analysis. We also thank Waldir Miron Berbel Filho for his feedback on previous versions of this manuscript. This work was made possible by contributions of the International Stock Center for Livebearing Fishes at the University of Oklahoma. We also thank the graduate advisory committee of Montra Spikes who reviewed previous versions of this manuscript. This work is in partial fulfillment of Montra Spikes' doctoral dissertation. This work was approved by the University of Oklahoma IACUC (R18-005).

Authors' contributions

Conception/design of the work: MS, IS, RT. Data collection, data analysis and interpretation: MS, IS, RT, RRS, K-AB, SB, JJ, P T-P, AE, KH. Drafting the article: MS, IS, RT. Critical revision of the article and final approval: MS, IS, RT, RRS, K-AB, SB, JJ, P T-P, AE, KH. All authors read and approved the final manuscript.

Funding

Financial support was provided by the University of Potsdam. Open Access funding enabled and organized by Projekt DEAL.

Availability of data and materials

Sequence data are available at Genbank under Accession Numbers MW355516–MW355575.

Declarations

Ethics approval and consent to participate

This work was approved by the University of Oklahoma IACUC (R18-005). Fishes were collected under corresponding permits of the authorities of The Dominican Republic and The Republic of Haiti. Fishes collected in Cuba were collected under permit number 44/18.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Author details

¹Unit of Evolutionary Biology/Systematic Zoology, Institute of Biochemistry and Biology, University of Potsdam, Karl-Liebknecht-Straße 24-25, Haus 26, 14476 Potsdam, Germany. ²Department of Biology, University of Oklahoma, 730 Van Vleet Oval, Norman, OK 73019, USA. ³Department of Life Sciences, The University of the West Indies (Mona Campus), Kingston, Jamaica. ⁴German Oceanographic Museum (DMM), Katharinenberg 14-20, 18439 Stralsund, Germany. ⁵Caribaea Initiative and Université Des Antilles, Guadeloupe, Kingston, Jamaica. ⁶Museo Nacional de Historia Natural Prof. "Eugenio de Jesús Marciano", Avenida Cesar Nicolás Penson, 10204 Santo Domingo, República Dominicana.

Received: 3 August 2021 Accepted: 10 November 2021

Published online: 25 November 2021

References

1. Helmer E, Ramos O, López TDM, Quinones M, Diaz W. Mapping the forest type and land cover of Puerto Rico, a component of the Caribbean biodiversity hotspot. *Caribb J Sci.* 2002;38(3–4):165–83.
2. Rivas L. Eight new species of Poeciliid fishes of the genus *Limia* from Hispaniola. *Gulf Mex Sci.* 1980. <https://doi.org/10.18785/negs.0401.03>.

3. Rosen DE, Bailey RM, Reeve M. The poeciliid fishes (Cyprinodontiformes): their structure, zoogeography, and systematics. *Bulletin of the AMNH*; v. 126, article 1. 1963. <http://digitallibrary.amnh.org/handle/2246/1123>. Accessed 24 Jun 2020.
4. Doadrio I, Perea S, Alcaraz L, Hernandez N. Molecular phylogeny and biogeography of the Cuban genus *Girardinus* Poey, 1854 and relationships within the tribe *Girardinini* (Actinopterygii, Poeciliidae). *Mol Phylogenet Evol.* 2009;50(1):16–30.
5. de León JLP, León G, Rodríguez R, Metcalfe CJ, Hernández D, Casane D, et al. Phylogeography of Cuban *Rivulus*: evidence for allopatric speciation and secondary dispersal across a marine barrier. *Mol Phylogenet Evol.* 2014;79:404–14.
6. Alonso R, Crawford AJ, Bermingham E. Molecular phylogeny of an endemic radiation of Cuban toads (Bufonidae: Peltophryne) based on mitochondrial and nuclear genes. *J Biogeogr.* 2012;39(3):434–51. <https://doi.org/10.1111/j.1365-2699.2011.02594.x>.
7. Gómez-Rodríguez C, Díaz-Paniagua C, Bustamante J, Portheault A, Florencio M. Inter-annual variability in amphibian assemblages: implications for diversity assessment and conservation. *Aquat Conserv Mar Freshw Ecosyst.* 2010;20(6):668–77. <https://doi.org/10.1002/aqc.1134>.
8. Gior RE, Gifford ME, Larson A, Losos JB, Schettino LR, Lara ARC, et al. Partial island submergence and speciation in an adaptive radiation: a multilocus analysis of the Cuban green anoles. *Proc R Soc Lond B Biol Sci.* 2004;271(1554):2257–65. <https://doi.org/10.1098/rspb.2004.2819>.
9. Gifford ME, Larson A. In situ genetic differentiation in a Hispaniolan lizard (*Ameiva chrysolaelma*): a multilocus perspective. *Mol Phylogenet Evol.* 2008;49(1):277–91.
10. Matos-Maraví P, Núñez Águila R, Peña C, Miller JY, Sourakov A, Wahlberg N. Causes of endemic radiation in the Caribbean: evidence from the historical biogeography and diversification of the butterfly genus *Calisto* (Nymphalidae: Satyrinae: *Satyrini*). *BMC Evol Biol.* 2014;14(1):199. <https://doi.org/10.1186/s12862-014-0199-7>.
11. de Weerd DRU, Robinson DG, Rosenberg G. Evolutionary and biogeographical history of the land snail family Urocoptidae (Gastropoda: Pulmonata) across the Caribbean region. *J Biogeogr.* 2016;43(4):763–77. <https://doi.org/10.1111/jbi.12692>.
12. Čandek K, Agnarsson I, Binford GJ, Kuntner M. Biogeography of the Caribbean *Cyrtognatha* spiders. *Sci Rep.* 2019;9(1):397.
13. Santiago-Valentin E, Olmstead RG. Historical biogeography of Caribbean plants: introduction to current knowledge and possibilities from a phylogenetic perspective. *Taxon.* 2004;53(2):299–319. <https://doi.org/10.2307/4135610>.
14. Ackerman JD, Trejo-Torres JC, Crespo-Chuy Y. Orchids of the West Indies: predictability of diversity and endemism. *J Biogeogr.* 2007;34(5):779–86. <https://doi.org/10.1111/j.1365-2699.2006.01676.x>.
15. Ricklefs R, Bermingham E. The West Indies as a laboratory of biogeography and evolution. *Philos Trans R Soc B Biol Sci.* 2008;363(1502):2393–413. <https://doi.org/10.1098/rstb.2007.2068>.
16. Rodríguez-Silva R, Schlupp I. Biogeography of the West Indies: a complex scenario for species radiations in terrestrial and aquatic habitats. *Ecol Evol.* 2021;11(6):2416–30. <https://doi.org/10.1002/ece3.7236>.
17. Schluter D. The ecology of adaptive radiation. Oxford: OUP; 2000. p. 302.
18. Lack D. Evolution of the Galapagos finches. *Nature.* 1940;146(3697):324–7.
19. Grant PR, Grant BR. Unpredictable evolution in a 30-year study of Darwin's finches. *Science.* 2002;296(5568):707–11.
20. Lack D. Darwin's finches: an essay on the general biological theory of evolution. New York: Harper; 1961. p. 236.
21. Lamichaney S, Han F, Webster MT, Andersson L, Grant BR, Grant PR. Rapid hybrid speciation in Darwin's finches. *Science.* 2018;359(6372):224–8.
22. Baldwin BG, Kyhos DW, Dvorak J, Carr GD. Chloroplast DNA evidence for a North American origin of the Hawaiian silversword alliance (Asteraceae). *Proc Natl Acad Sci.* 1991;88(5):1840–3.
23. Baldwin BG, Sanderson MJ. Age and rate of diversification of the Hawaiian silversword alliance (Compositae). *Proc Natl Acad Sci.* 1998;95(16):9402–6.
24. Barrier M, Baldwin BG, Robichaux RH, Purugganan MD. Interspecific hybrid ancestry of a plant adaptive radiation: allopolyploidy of the Hawaiian silversword alliance (Asteraceae) inferred from floral homeotic gene duplications. *Mol Biol Evol.* 1999;16(8):1105–13.
25. Lerner HRL, Meyer M, James HF, Hofreiter M, Fleischer RC. Multilocus resolution of phylogeny and timescale in the extant adaptive radiation of Hawaiian honeycreepers. *Curr Biol CB.* 2011;21(21):1838–44.
26. Losos JB, Jackman TR, Larson A, de Queiroz K, Rodríguez-Schettino L. Contingency and determinism in replicated adaptive radiations of island lizards. *Science.* 1998;279(5359):2115–8.
27. Hedges SB, Duellman WE, Heinicke MP. New World direct-developing frogs (Anura: Terrarana): molecular phylogeny, classification, biogeography, and conservation. *Zootaxa.* 2008;1737(1):1–182.
28. Hamilton A. Phylogeny of *Limia* (Teleostei: Poeciliidae) based on NADH dehydrogenase subunit 2 sequences. *Mol Phylogenet Evol.* 2001;19(2):277–89.
29. Reznick DN, Furness AI, Meredith RW, Springer MS. The origin and biogeographic diversification of fishes in the family Poeciliidae. *PLoS ONE.* 2017;12(3):e0172546. <https://doi.org/10.1371/journal.pone.0172546>.
30. Humphries JM, Miller RR. A remarkable species flock of pupfishes, genus *Cyprinodon*, from Yucatán, México. *Copeia.* 1981;1981(1):52–64.
31. Plath M, Strecker U. Behavioral diversification in a young species flock of pupfish (*Cyprinodon* spp.): shoaling and aggressive behavior. *Behav Ecol Sociobiol.* 2008;62(11):1727–37. <https://doi.org/10.1007/s00265-008-0601-9>.
32. Strecker U. Genetic differentiation and reproductive isolation in a *Cyprinodon* fish species flock from Laguna Chichancanab, Mexico. *Mol Phylogenet Evol.* 2006;39(3):865–72.
33. Martin CH, Wainwright PC. Trophic novelty is linked to exceptional rates of morphological diversification in two adaptive radiations of *Cyprinodon* pupfish. *Evolution.* 2011;65(8):2197–212. <https://doi.org/10.1111/j.1558-5646.2011.01294.x>.
34. Meyer A, Kocher TD, Basasibwaki P, Wilson AC. Monophyletic origin of Lake Victoria cichlid fishes suggested by mitochondrial DNA sequences. *Nature.* 1990;347(6293):550–3.
35. Turner G. Speciation mechanism in Lake Malawi cichlid: a critical review. *Arch Hydrobiol.* 1994;44:139–60.
36. Turner GF, Seehausen O, Knight ME, Allender CJ, Robinson RL. How many species of cichlid fishes are there in African lakes? *Mol Ecol.* 2001;10(3):793–806. <https://doi.org/10.1046/j.1365-294x.2001.01200.x>.
37. Albertson RC, Streebman JT, Kocher TD. Directional selection has shaped the oral jaws of Lake Malawi cichlid fishes. *Proc Natl Acad Sci.* 2003;100(9):5252–7.
38. Husemann M, Tobler M, McCauley C, Ding B, Danley PD. Evolution of body shape in differently coloured sympatric congeners and allopatric populations of Lake Malawi's rock-dwelling cichlids. *J Evol Biol.* 2014;27(5):826–39. <https://doi.org/10.1111/jeb.12353>.
39. MacArthur RH, Wilson EO. The theory of island biogeography. Monographs in population biology. Princeton: Princeton University Press; 1967.
40. Valente L, Phillimore AB, Melo M, Warren BH, Clegg SM, Havenstein K, et al. A simple dynamic model explains the diversity of island birds worldwide. *Nature.* 2020;579(7797):92–6.
41. Lack D. Island biology illustrated by the land birds of Jamaica. *Syst Bot.* 1976;1(2):180.
42. Furness AI, Reznick DN, Avise JC. Ecological, evolutionary and human-mediated determinants of poeciliid species richness on Caribbean islands. *J Biogeogr.* 2016;43(7):1349–59. <https://doi.org/10.1111/jbi.12736>.
43. Haney D, Walsh S. Influence of salinity and temperature on the physiology of *Limia melanonotata* (Cyprinodontiformes: Poeciliidae): a search for abiotic factors limiting insular distribution in hispaniola. *Caribb J Sci.* 2003;1:39.
44. Weaver PF, Cruz A, Johnson S, Dupin J, Weaver KF. Colonizing the Caribbean: biogeography and evolution of livebearing fishes of the genus *Limia* (Poeciliidae). *J Biogeogr.* 2016;43(9):1808–19. <https://doi.org/10.1111/jbi.12798>.
45. Rivas L. A new species of poeciliid fish of the genus *Poecilia* from Hispaniola, with reinstatement and redescription of *P. dominicensis* (Evermann and Clark). *Gulf Mex Sci.* 1978. <https://doi.org/10.18785/negs.0202.02>.
46. Rodríguez-Silva R, Weaver PF. A new livebearing fish of the genus *Limia* (Cyprinodontiformes: Poeciliidae) from Lake Miragoane, Haiti. *J Fish Biol.* 2020. <https://doi.org/10.1111/jfb.14301>.
47. Vergara R. Desarrollo evolutivo de la ictiofauna dulceacuicola cubana con especial referencia a los ciprinodontiformes. La Habana: Editorial Academia; 1992.

48. Rodriguez-Silva R, Torres-Pineda P, Josaphat J. *Limia mandibularis*, a new livebearing fish (Cyprinodontiformes: Poeciliidae) from Lake Miragoane, Haiti. *Zootaxa*. 2020;4768(3):395–404.
49. Burgess GH, Franz R. Zoogeography of the Antillean freshwater fish fauna. Biogeography of the West Indies: past, present, and future. Pemberley: Sandhill Crane Press; 1989.
50. Curtis JH, Hodell DA. An isotopic and trace element study of ostracods from Lake Miragoane, Haiti: a 10,500 year record of paleosalinity and paleotemperature changes in the Caribbean. In: Climate change in continental isotopic records. Washington: American Geophysical Union (AGU); 1993. p. 135–52. <https://doi.org/10.1029/GM078p0135>.
51. Hrbek T, Seckinger J, Meyer A. A phylogenetic and biogeographic perspective on the evolution of poeciliid fishes. *Mol Phylogenet Evol*. 2007;43(3):986–98.
52. Meredith RW, Pires MN, Reznick DN, Springer MS. Molecular phylogenetic relationships and the evolution of the placenta in *Poecilia* (*Micropoecilia*) (Poeciliidae: Cyprinodontiformes). *Mol Phylogenet Evol*. 2010;55(2):631–9.
53. Pollux BJA, Meredith RW, Springer MS, Garland T, Reznick DN. The evolution of the placenta drives a shift in sexual selection in livebearing fish. *Nature*. 2014;513(7517):233–6.
54. Rivas LR, Fink WL. A new species of Poeciliid Fish of the genus *Limia* from the island of Grand Cayman, B.W.I. *Copeia. Ecol Freshw Fish*. 1970;1970(2):270–4.
55. Goldberg DL, Landy JA, Travis J, Springer MS, Reznick DN. In love and war: The morphometric and phylogenetic basis of ornamentation, and the evolution of male display behavior, in the livebearer genus *Poecilia*. *Evolution*. 2019;73(2):360–77. <https://doi.org/10.1111/evo.13671>.
56. Evermann BW, Clark HW. New fishes from Santo Domingo. *Proc US Natl Mus*. 1906;30(1478):851–5.
57. Feulner PGD, Kirschbaum F, Mamonekene V, Ketmaier V, Tiedemann R. Adaptive radiation in African weakly electric fish (Teleostei: Mormyridae: Campylomormyrus): a combined molecular and morphological approach. *J Evol Biol*. 2007;20(1):403–14. <https://doi.org/10.1111/j.1420-9101.2006.01181.x>.
58. Toonen RJ. Microsatellites for ecologists: non-radioactive isolation and amplification protocols for microsatellite markers. *PLoS ONE*. 1997. <https://doi.org/10.1371/journal.pone.0055990>.
59. Enroth CH, Fehler AO, Poulsen LD, Vinther J. Excess primer degradation by Exo I improves the preparation of 3' cDNA ligation-based sequencing libraries. *BioTechniques*. 2019;67(3):110–6. <https://doi.org/10.2144/btn-2018-0178>.
60. Sambrook HC. Molecular cloning: a laboratory manual. Cold Spring Harbor: CSHL Press; 1989.
61. Hall TA. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp Ser*. 1999;41:95–8.
62. Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, et al. Clustal W and Clustal X version 2.0. *Bioinformatics*. 2007;23(21):2947–8.
63. Xia X, Xie Z. DAMBE: software package for data analysis in molecular biology and evolution. *J Hered*. 2001;92(4):371–3. <https://doi.org/10.1093/jhered/92.4.371>.
64. Stamatakis A. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics*. 2014;30(9):1312–3.
65. Silvestro D, Michalak I. raxmlGUI: a graphical front-end for RAxML. *Org Divers Evol*. 2012;12(4):335–7. <https://doi.org/10.1007/s13127-011-0056-0>.
66. Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, et al. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Syst Biol*. 2012;61(3):539–42.
67. Hillis DM, Bull JJ. An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. *Syst Biol*. 1993;42(2):182–92. <https://doi.org/10.1093/sysbio/42.2.182>.
68. Erixon P, Svennblad B, Britton T, Oxelman B. Reliability of Bayesian posterior probabilities and bootstrap frequencies in phylogenetics. *Syst Biol*. 2003;52(5):665–73.
69. Leigh JW, Bryant D. popart: full-feature software for haplotype network construction. *Methods Ecol Evol*. 2015;6(9):1110–6. <https://doi.org/10.1111/2041-210X.12410>.
70. Bandelt HJ, Forster P, Röhl A. Median-joining networks for inferring intraspecific phylogenies. *Mol Biol Evol*. 1999;16(1):37–48.
71. Kumar S, Stecher G, Li M, Niyaz C, Tamura K. MEGA X: molecular evolutionary genetics analysis across computing platforms. *Mol Biol Evol*. 2018;35(6):1547–9.
72. Choleva L, Musilova Z, Kohoutova-Sediva A, Paces J, Rab P, Janko K. Distinguishing between incomplete lineage sorting and genomic introgressions: complete fixation of allospecific mitochondrial DNA in a sexually reproducing fish (Cobitis; Teleostei), despite clonal reproduction of hybrids. *PLoS ONE*. 2014;9(6):e80641.
73. Iturralde-Vinent M, MacPhee RDE. Paleogeography of the Caribbean region: implications for Cenozoic biogeography. *Bulletin of the AMNH*: no. 238. Caribbean paleogeography. 1999. <http://digitallibrary.amnh.org/handle/2246/1642>. Accessed 14 Nov 2020.
74. Draper G, Barros JA. Cuba (chapter 4). In: Caribbean geology: an introduction. Berlin: Springer; 1994. p. 65–85.
75. Iturralde-Vinent MA. Cuban geology: a new plate-tectonic synthesis. *J Pet Geol*. 1994;17(1):39–69. <https://doi.org/10.1111/j.1747-5457.1994.tb00113.x>.
76. Farr JA. Premating behavior in the subgenus *Limia* (Pisces: Poeciliidae): sexual selection and the evolution of courtship. *Z Für Tierpsychol*. 1984;65(2):152–65. <https://doi.org/10.1111/j.1439-0310.1984.tb00096.x>.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Ready to submit your research? Choose BMC and benefit from:

- fast, convenient online submission
- thorough peer review by experienced researchers in your field
- rapid publication on acceptance
- support for research data, including large and complex data types
- gold Open Access which fosters wider collaboration and increased citations
- maximum visibility for your research: over 100M website views per year

At BMC, research is always in progress.

Learn more biomedcentral.com/submissions

