Acta Parasitologica
https://doi.org/10.1007/s11686-023-00675-0

SHORT COMMUNICATION

First Molecular Identification of a *Goussia* Parasite from a New World Invasive Blenny

Michael J. Jowers[1,2,3] · Raquel Xavier[1,2] · Oscar M. Lasso-Alcalá[4] · Elena Quintero-T[4] · Jorge L. S. Nunes[5] · Tommaso Giarrizzo[6,7] · Fabiola S. Machado[7] · Jesús Gómez[8] · M. Pilar Cabezas[9]

Received: 19 September 2022 / Accepted: 10 March 2023

Abstract

Purpose Introduced or invasive fish are susceptible to new parasites but can simultaneously carry infectious parasites from their native range towards new hosts. Screening these parasites is key to address the health of fish populations and spread of diseases.

Methods In this study, we sequenced a Coccidia parasite, for the first time from the blenny *Omobranchus sewalli*, introduced in the northern coast of Brazil with an Indo-Pacific origin.

Results Only one individual was infected, its genetic sequence matched (over 99%) with two lineages of undetermined species, belonging to the genus Goussia, sequenced from three marine fish species (*Mulloidichthys flavolineatus, Lutjanus kasmira*, and *Selar crumenophthalmus*) in Hawaii.

Conclusions Phylogenetic analysis suggests considerable differentiation between the Goussia detected and other Goussia spp. sequenced from North Atlantic marine fish, thus we cannot exclude the possibly that this parasite was carried by *O. sewalli* from its native Indo-Pacific range.

Keywords Infection · Brazil · *Omobranchus* · *Goussia* · Fish

Introduction

Fish host a variety of Apicomplexa microparasites from two major groups, the Coccidia Leuckart 1897, that infect several organs, and the Hematozoa Vivier 1982, which occur in host blood cells [e.g., [1, 2]]. Among the piscine Coccidia Leuckart, 1879, those from the genus *Goussia* Labbé 1896, are the most commonly reported in fish globally, especially in freshwater environments. These parasites have important negative effects on host fitness such as focal necrosis, malabsorption, and starvation [3–6]. Therefore, monitoring and screening for their presence is important, as they are a threat to wild fish and to aquaculture species [7–9]. While most *Goussia* spp. were described parasitizing freshwater fish, some members of the group parasitize marine and brackish fish and some species infect amphibians [2, 9–12]. Evidence

Michael J. Jowers and Raquel Xavier have contributed equally to this work.

✉ Michael J. Jowers
michaeljowers@hotmail.com

1 CIBIO/InBIO (Centro de Investigação em Biodiversidade e Recursos Genéticos), Universidade do Porto, Campus Agrario De Vairão, 4485-661 Vairão, Portugal

2 BIOPOLIS Program in Genomics, Biodiversity and Land Planning, CIBIO, Campus de Vairão, 4485-661 Vairão, Portugal

3 Departamento de Zoología, Facultad de Ciencias, Universidad de Granada, 18071 Granada, Spain

4 Museo de Historia Natural La Salle, Fundación La Salle de Ciencias Naturales, Caracas, Venezuela

5 Laboratório de Organismos Aquáticos, Departamento de Oceanografia e Limnologia, Universidade Federal do Maranhão, São Luís, MA, Brazil

6 Instituto de Ciências do Mar (LABOMAR), Universidade Federal do Ceará (UFC), Avenida da Abolição, 3207 Fortaleza, Brazil

7 Núcleo de Ecologia Aquática e Pesca da Amazônia (NEAP), Universidade Federal do Pará (UFPA), Belém, PA, Brazil

8 Universidad Metropolitana, Caracas 1073, Venezuela

9 Department of Biogeographical Ecology and Evolution, Centro de Ciências do Mar (CCMAR), Universidade do Algarve, Faro, Portugal

Published online: 27 April 2023

suggests that the genus has a worldwide distribution, thus screening these parasites in recently established non-native fish populations, combined with phylogenetic analysis, can provide valuable insights into the possible generalist biology of *Goussia*.

The muzzled blenny *Omobranchus punctatus* (Valenciennes, 1836) poses a fascinating case of a species formally thought to be one of the most widely distributed blenny species, having been introduced outside its native range through transport associated with ship's ballast water and biofouling, which would favour long-distance dispersal [13–15]. Since it was considered a native fish of the Indo-Pacific region, its distribution was thought to range from the Persian Gulf, in the Western Indian Ocean and the Arabian Sea, South-East Asia, Japan, Australia and the Fiji Islands, in the Western Pacific Ocean, with the species being recently recorded worldwide [e.g., [13, 14]]. Recently, Cabezas et al. [15] presented morphological and molecular data showing the diversity within *O. punctatus*. They uncovered three mitochondrial lineages dating to the Pleistocene, corresponding to a species complex with three species, which were morphologically supported. One of these species, *O. sewalli* (Fowler 1931), was recognized as the invasive species recently established in the Western Atlantic [15].

This study aimed to screen coccidian parasites from an introduced population of *Omobranchus sewalli* in the North of Brazil ($n = 28$) by Cabezas et al. [15], using genetic identification by sequencing a fragment of the 18S rRNA gene. This is the only gene commonly used to characterize genetically fish Apicomplexa, with 18S rRNA 'barcode' sequences representing the most comprehensive database available to date, encompassing lineages distributed throughout the globe [e.g., [16, 17]].

Materials and Methods

DNA was extracted from fins and caudal tissue previously stored in 96% ethanol from 28 specimens of *Omobranchus sewalli* captured from intertidal reefs at 6 localities along the Brazilian coast (Curuçá and Salinópolis in Pará State; Araçagy and São Marcos in Maranhão State; Barra Grande locality in Piauí State; and Jericoacora in Ceará State) [15]. We used the PureLink Genomic DNA Mini Kit (Invitrogen, Paisley, UK), and screened for the presence of fish coccidia using a PCR approach which has been previously shown to be highly sensitive to detect infections in fish [5]. Thus, a portion of the parasites' 18S rRNA gene was targeted using the primer pairs HEP300 and HEP900 (~600 bp) [18]. PCR amplifications were performed in a 25 µl reaction volume consisting of 5 µl of template DNA, 10 × buffer $MgCl_2$ free (Invitrogen), 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 1 µM of each primer, 0.4 U Platinum Taq DNA polymerase (Invitrogen), and double-distilled water to volume. PCR conditions were the following: initial denaturation of 4 min at 94 °C, followed by 40 cycles of 45 s at 94 °C, 50 s at 60 °C and 1 min at 72 °C. A final extension step was performed at 72 °C for 10 min. The resulting PCR products were purified and bidirectionally sequenced at GENEWIZ (Leipzig, Germany).

The obtained sequences were edited with Sequencher v5.4.6 (Gene Codes Corporation, Ann Arbor, MI, USA), and checked for potential contaminations using GenBank's BLASTn search [19]. The final dataset, consisting on one parasite sequence obtained in this study (accession number OM021887) and 16 18S rRNA sequences from their close relatives (selected through using the BLAST algorithm in GenBank), was aligned using MUSCLE [20] in MEGA X [21], and checked for the presence of pseudogenes by translating sequences into amino acids.

Phylogenetic relations of parasite sequences were analysed for Bayesian Inference analysis using MrBayes v3.2.6 [22]. The best model of sequence evolution (TIM + I + G) was selected following the AIC using jModelTest v2.1.6 [23] and a sequence of *Cystoisospora* sp. (GenBank accession number AB519675) was included as an outgroup. Four independent runs (each with four Markov chains for 3×10^7 generations) were performed. Trees and parameters were sampled every 1,000 generations, with the heating parameter set to 0.25. The convergence of the analyses was validated by the standard deviation of split frequencies being lower than 0.01 and by graphical monitoring of the likelihood values over time using Tracer v1.7.1 [24]. The majority-rule consensus tree was estimated by combining results from duplicated analyses, after discarding 25% of the total samples as burn-in. The consensus tree was visualized and rooted using FigTree v1.4.4 [25], and later prepared as a graphic with the software Inkscape v1.0.1 (http://www.inkscape.org).

Results and Discussion

Of the 28 Brazilian fish specimens screened for the presence of parasites, only one individual from São Marcos, Maranhão, was found infected. The results from the phylogenetic analysis confirmed the close relation between the parasitic lineage sequenced herein and species of epicelular *Goussia* sequenced from various fish, suggesting it belongs to this genus (Fig. 1).

Results from the BLAST search showed that the 18S sequence of the *Goussia* from *O. sewalli* shared 99.83% similarity with the sequences of *Goussia* sequenced from three fishes, *Mulloidichthys flavolineatus* (Lacepède, 1801), *Lutjanus kasmira* (Fabricius, 1775) and *Selar crumenophthalmus* (Bloch, 1793) captured in Hawaii (accession number HM117907-8) [2, 26, Whipps, pers. comm.] (Fig. 1). Our findings suggest that the matched *Goussia* sp. from

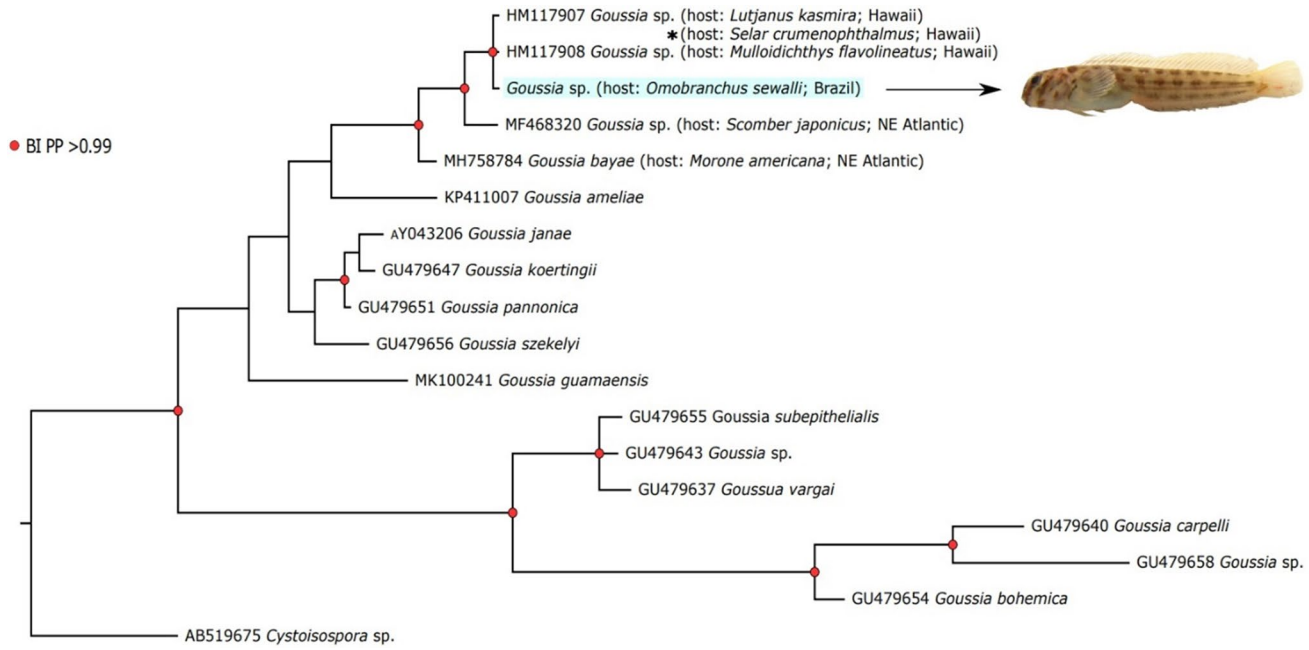

Fig. 1 Phylogenetic relationships between *Goussia* sp. from Brazil and other species of the genus, as inferred from sequences of partial 18S rRNA gene (578 bp) analysed by BI method. The sequence generated in the present study (highlighted in blue). Bayesian posterior probabilities (BPP) over 0.99 are represented by red circles at nodes. Asterisk: although there is no sequence in Genbank for *Selar crumenophthalmus*, the name is included in the tree to show all three fish species with close confirmed genetic identification of *Goussia* sp. to *Omobranchus sewalli*. Photo credit O. M. Lasso-Alcalá. The sequence of *Cystoisospora* sp. (GenBank accession number AB519675) was included as an outgroup

the Pacific Ocean (i.e., Hawaii) is widespread [2] and that it quite likely corresponds to a generalist species, having multiple unrelated hosts. According to historical and morphological analyses, it seems that *O. sewalli* was first introduced to the Western Atlantic Ocean on slave boats from the Bay of Bengal (Madras or, more probably, Calcutta, Indian Ocean) to Trinidad and Tobago, (South America), secondarily spreading to Venezuela, Panama and Colombia, and then to different Brazilian localities [see [13, 15]]. From a geographical proximity, our finding could suggest that arrival to South America occurred through the Pacific, from the Panama Canal, as this is a heavily navigated maritime route. Nevertheless, as mentioned in Lasso-Alcala et al. [13], the Panama Canal did not open until 1914, and the first ichthyofaunal surveys in the Pacific coast between 1935 and 1937 [26] followed by McCosker and Dawson [27] never found *O. sewalli* in the Pacific versant of the canal and this remains the case to date [28, 29].

Although the sequence of *Goussia* from *O. sewalli* from Brazil is not closely related with any sequence of *Goussia* species sequenced from the Atlantic Ocean, we cannot fully discard the hypothesis that this is in fact a widely distributed *Goussia* species, being already present in the South Atlantic Ocean before *O. sewalli*'s arrival. There are in fact piscine *Goussia* species that presumably occur both in the Pacific and Atlantic Ocean (e.g., *Goussia cruciata* (Thélohan, 1894) [30, 31]; *Goussia clupearum* (Thélohan, 1894), reviewed in Xavier and Saraiva [32]). Doubts remain as to whether *Goussia* species with worldwide distributions could be a species complex [e.g., [30]], since genetic analysis of many coccidian species has revealed the existence of multiple cryptic species, especially among those with wide distributions [e.g. [33]]. Although host specificity of parasite lineages may be common, the lack of significant divergence between *Goussia* sp. genetic lineages found in different hosts would suggest that these likely represent host generalists [2].

A recent study on *Goussia guamaensis* [4], that parasitizes the ornamental freshwater fish *Thoracocharax stellatus* (Kner, 1858), captured in the basin of the Guamá River, municipality of Belém, northern Brazil, reported a prevalence of 26% and has a 92% similarity to our species, an endemic host species to South America [4]. Interestingly, phylogenetic analyses recovered its position basal to the sequences used in our analyses and ordination plot of the Principal Components Analysis (PCA) of morphometric data found a clear distinction between environments (marine vs. freshwater). The low prevalence of *Goussia* (2.8%) found in our study contrasts that of other studies [4, 5, 34], but likely relies on the type of tissue examined and sampling techniques (only muscle tissue was available to our study, vs. liver and spleen homogenates for example in other studies). In addition, out of the ten samples from Maranhão (five

from São Marcos and five from Araçagy) only one specimen was infected (from São Marcos). The population study of Cabezas et al. [15] showed that *O. sewalli* reported three haplotypes from each of these two localities, suggesting that they are all part of the metapopulation and gene flow in ongoing, which could suggest much higher expected prevalence in the region.

Due to the harmful effect that some *Goussia* species have on their fish hosts as well as their potential wide host range and distribution, we suggest further studies should be performed to ascertain the pathogenicity of this parasite and determine whether it is widespread among native fish fauna or weather *O. sewalli* maybe acting as a vector for the spread of Indo-Pacific parasitic lineages.

Acknowledgements Thanks to J.D. Carrillo Briceño (University of Zurich, Switzerland) for his collaboration in the shipping of some of the samples studied. We are grateful to Christopher Whipps for comments regarding other fish hosts in Hawaii.

Author Contributions MJJ, RX and MPC conceived the study. MJJ and RX wrote the paper and run the analyses. OML-A, EQ-T, JLSN, TG, MPC conducted the laboratory analyses. FSM and JG provided samples, material preparation and reviewed the manuscript. All authors read and approved the final manuscript.

Funding Michael J. Jowers was funded by the Fundação para a Ciência e a Tecnologia, FCT, SFRH/BPD/109148/2015 and Next Generation Europe (Maria Zambrano grant). Raquel Xavier was funded by FCT under the Programa Operacional Potencial Humano—Quadro de Referência Estratégico Nacional funds from the European Social Fund and the Portuguese Ministério da Educação e Ciência, 2020.00854. CEECIND/CP1601/CT0001. Work supported by the European Union's Horizon 2020 Research and Innovation Programme under the Grant Agreement Number 857251.

Data Availability Genbank.

Declarations

Conflict of Interest The authors declare no competing interests.

Ethical Approval NA.

Consent to Participate Informed consent was obtained from all individual participants included in the study.

Consent for Publication Informed consent was obtained from all individual participants included in the study.

References

1. Cook CA, Sikkel PC, Renoux LP, Smit NJ (2015) Blood parasite biodiversity of reef-associated fishes of the eastern Caribbean. Mar Ecol Prog Ser 533:1–13. https://doi.org/10.3354/meps11430
2. Whipps CM, Fournie JW, Morrison DA, Azevedo C, Matos E, Thebo P, Kent M (2012) Phylogeny of fish-infecting *Calyptospora* species (Apicomplexa: Eimeriorina). Parasitol Res 111:1331–1342. https://doi.org/10.1007/s00436-012-2969-8
3. Lovy J, Friend SE (2015) Intestinal coccidiosis of anadromous and land locked alewives, *Alosa pseudoharengus*, caused by *Goussia ameliae* n. sp. and *G. alosii* n. sp. (Apicomplexa: Eimeriidae). Int J Parasitol Parasites Wildl 4:159–170. https://doi.org/10.1016/j.ijppaw.2015.02.003
4. da Silva MF, de Araújo RS, da Silva DT, Sanches O, Hamoy I, Matos E (2019) Gastric coccidiosis in *Thoracocharax stellatus* caused by *Goussia guamaensis* n. sp. (Apicomplexa: Eimeriidae) from the Amazon region. J Fish Dis 42:905–912. https://doi.org/10.1111/jfd.12992
5. Xavier R, Cardoso J, Barroso R, Nogueira S, Cruz C, Pereira A, Saraiva A (2020) Effects of *Goussia* infecting the blue whiting and phylogenetic placement of *Goussia* infecting marine fish off Northern Portugal. Parasitol Res 119:2139–2147. https://doi.org/10.1007/s00436-020-06727-y
6. Xavier R, Barroso R, Cardoso J, Cruz C, Pereira A, Saraiva A (2021) Phylogenetic affinities and infection patterns of *Goussia* infecting *Sardina pilchardus* from the NE Atlantic. Acta Parasit 66:693–698. https://doi.org/10.1007/s11686-020-00319-7
7. Bricknell IR, Bron JE, Bowden TJ (2006) Diseases of gadoid fish in cultivation: a review. ICES J Mar Sci 63:253–266. https://doi.org/10.1016/j.icesjms.2005.10.009
8. Gjurčević E, Kužir S, Baždarić B, Matanović K, Debelić I, Marino F, Drašner K, Rosenthal BM (2017) New data on *Eimeria dicentrarchi* (Apicomplexa: Eimeriidae), a common parasite of farmed European sea bass (*Dicentrarchus labrax*) from the mid-eastern Adriatic. Vet Arhiv 87:77–86
9. Xavier R, Severino R, Pérez-Losada M, Gestal C, Freitas R, Harris DJ, Veríssimo A, Rosado D, Cable J (2018) Phylogenetic analysis of apicomplexan parasites infecting commercially valuable species from the Northeast Atlantic reveals high levels of diversity and insights into the evolution of the group. Parasit Vectors 11:63. https://doi.org/10.1186/s13071-018-2645-7
10. Molnár K, Ostoros G, Dunams-Morel D, Rosenthal B (2012) *Eimeria* that infect fish are diverse and are related to, but distinct from, those that infect terrestrial vertebrates. Infect Genet Evol 12:1810–1815. https://doi.org/10.1016/j.meegid.2012.06.017
11. Rosenthal BM, Dunams-Morela D, Ostoros G, Molnár K (2016) Coccidian parasites of fish encompass profound phylogenetic diversity and gave rise to each of the major parasitic groups in terrestrial vertebrates. Infect Genet Evol 40:219–227. https://doi.org/10.1016/j.meegid.2016.02.018
12. Matsche MA, Adams CR, Blazer VS (2019) Newly described Coccidia *Goussia bayae* from white perch *Morone americana*: morphology and phylogenetics support emerging taxonomy of *Goussia* within piscine hosts. J Parasitol 105:1–10. https://doi.org/10.1645/18-67
13. Lasso-Alcalá O, Nunes JLS, Lasso C, Posada J, Robertson R, Piorski NM, Van Tassell J, Giarrizzo T, Gondolo G (2011) Invasion of the Indo-Pacific blenny *Omobranchus punctatus* (Perciformes: Blenniidae) on the Atlantic Coast of Central and South America. Neotrop Ichthyol 9:571–578. https://doi.org/10.1590/S1679-62252011000300010
14. Cabezas MP, Lasso-Alcalá OM, Xavier R, Jowers MJ (2020) First genetic record of the non-native muzzled blenny *Omobranchus punctatus* (Teleostei: Blenniidae) in the Atlantic Coast of Central and South America. J Fish Biol 96:841–846. https://doi.org/10.1111/jfb.14268
15. Cabezas PM, Lasso-Alcalá OM, Quintero-T E, Xavier R, Giarrizzo T, Nunes JLS, Machado FS, Gómez J, Pedroza WD, Jowers MJ (2022) Clarifying the taxonomy of some cryptic blennies (Blenniidae) in their native and introduced range. Sci Rep 12:9514. https://doi.org/10.1038/s41598-022-12580-z
16. Hayes PM, Smit NJ (2019) Molecular insights into the identification and phylogenetics of the cosmopolitan marine fish blood parasite, *Haemogregarina bigemina* (Adeleorina:

Haemogregarinidae). IJP-PAW 8:216–220. https://doi.org/10.1016/j.ijppaw.2019.01.006
17. Renoux LP, Dolan MC, Cook CA, Smit NJ, Sikkel PC (2017) Developing an apicomplexan DNA barcoding system to detect blood parasites of small coral reef fishes. J Parasit 103:366–376. https://doi.org/10.1645/16-93
18. Ujvari B, Madsen T, Olsson M (2004) High prevalence of *Hepatozoon* spp. (Apicomplexa, Hepatozoidae) infection in water Pythons (*Liasis fuscus*) from tropical Australia. J Parasitol 90:670–672. https://doi.org/10.1645/GE-204R
19. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. J Mol Biol 215:403–410. https://doi.org/10.1016/S0022-2836(05)80360-2
20. Edgar RC (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res 32:1792–1797
21. Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: molecular evolutionary genetics analysis across computing platforms. Mol Biol Evol 35:1547–1549. https://doi.org/10.1093/molbev/msy096
22. Ronquist F, Huelsenbeck JP (2003) MRBAYES 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19:1572–1574. https://doi.org/10.1093/bioinformatics/btg180
23. Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: more models, new heuristics and parallel computing. Nat Methods 9:772–772. https://doi.org/10.1038/nmeth.2109
24. Rambaut A, Drummond AJ, Xie D, Baele G, Suchard MA (2018) Posterior summarization in Bayesian phylogenetics using Tracer 1.7. Syst Biol 67:901–904. https://doi.org/10.1093/sysbio/syy032
25. Rambaut A (2018) FigTree-version 1.4.4, a graphical viewer of phylogenetic trees. http://www.tree.bio.ed.ac.uk/software/figtree/
26. Hildebrand SF (1939) The Panama Canal as a passageway for fishes, with lists and remarks on the fishes and invertebrates observed. Zoologica 24:15–45
27. McCosker J, Dawson C (1975) Biotic passage through the Panama Canal, with particular reference to fishes. Mar Biol 30:343–351. https://doi.org/10.1007/BF00390639
28. Cohen AN (2006) Chapter III: species introductions and the Panama Canal. In: Gollasch S, Galil BS, Cohen AN (eds) Bridging Divides, vol 83. Springer, Dordrecht. https://doi.org/10.1007/978-1-4020-5047-3_5
29. Robertson DR, Allen GR (2015) Shorefishes of the Tropical Eastern Pacific: online information system. Version 2.0 Smithsonian Tropical Research Institute, Balboa, Panamá. https://www.biogeodb.stri.si.edu/sftep/en/pages
30. González-Kother P, González MT (2014) The first report of liver coccidian *Goussia cruciata* in jack mackerel, *Trachurus murphyi*, from the South Pacific and its relationship with host variables. Parasitol Res 113:3903–3907. https://doi.org/10.1007/s00436-014-4134-z
31. Gestal C, Azevedo C (2005) Ultrastructure of *Goussia cruciata* (Apicomplexa: Coccidia) infecting the liver of horse mackerel, *Trachurus trachurus* (L.), from Ibero-Atlantic waters. J Fish Dis 28:125–132. https://doi.org/10.1111/j.1365-2761.2005.00611.x
32. Xavier R, Saraiva A (2021) Coccidiosis of the liver of the blue whiting. ICES Identification Leaflets for Diseases and Parasites in Fish and Shellfish No. 72, Copenhagen, Denmark, 12 pp. https://doi.org/10.17895/ices.pub.5437
33. Cantacessi S, Riddell S, Morris GM, Doran T, Woods WG, Otranto D, Gasser RB (2008) Genetic characterization of three unique operational taxonomic units of *Eimeria* from chickens in Australia based on nuclear spacer ribosomal DNA. Vet Parasitol 152(3–4):226–234. https://doi.org/10.1016/j.vetpar.2007.12.028
34. Work T, Rameyer R, Takata G, Kent M (2003) Protozoal and epitheliocystis-like infections in the introduced bluestripe snapper *Lutjanus kasmira* in Hawaii. Dis Aquat Organ 57:59–66. https://doi.org/10.3354/dao057059