The vitellogenin genes in Cynops orientalis: New insights on the evolution of the vtg gene family in amphibians

Federica Carducci | Maria A. Biscotti | Adriana Canapa | Marco Barucca

Dipartimento di Scienze della Vita e dell’Ambiente, Università Politecnica delle Marche, Ancona, Italy

Correspondence
Maria A. Biscotti, Dipartimento di Scienze della Vita e dell’Ambiente, Università Politecnica delle Marche, Via Brecce Bianche 60131 Ancona, Italy.
Email: m.a.biscotti@univpm.it

Funding information
Ministero Italiano dell’Università e della Ricerca, Grant/Award Number: 040017_R.SCIENT.A_2018

Abstract
The vitellogenins (Vtgs) are glycolipophosphoproteins that play a key role in constituting nutritional reserves for embryo development in nonmammalian vertebrates. However, additional functional roles have been evidenced. These vtg genes are present in multiple copies, different in number and sequences in various vertebrate lineages. The comprehension of the vtg gene family evolutionary history remains a matter of intense interrogation for this field of research. In tetrapods, information about vtg genes are limited to few taxa. Up to date concerning amphibians, detailed studies have been conducted only in Anura. Therefore, in this study, to further increase knowledge about vtg genes in Amphibia class, the urodele Cynops orientalis (Amphibia: Caudata) was analyzed and four complete vtg sequences were obtained. Moreover, genomic data available for the caecilians Microcaecilia unicolor and Rhinatrema bivittatum (Amphibia: Gymnophiona) were also included. In these amphibians, our findings evidenced the presence of a vtgI sequence ortholog to that of tetrapods, absent in Anura. Moreover, microsyntenic, phylogenetic, and gene conversion analyses allowed postulating two hypotheses to explain the complex evolutionary history of this gene family.

KEYWORDS
amphibians, gene evolution, vertebrates, vitellogenin, phylogeny, transcriptomics

1 | INTRODUCTION

The vitellogenins (Vtgs) are glycolipophosphoproteins constituted by a large multidomain apolipoprotein (Barucca et al., 2010; Canapa et al., 2007, 2012; Carducci et al., 2019; Verderame & Scudiero, 2017). The complete amino acid Vtg sequences have a signal polypeptide, a heavy chain lipovitellin including four subdomains (N-sheet, α-helix, C-sheet, and A-sheet), a phosvitin, a light chain lipovitellin, and a von Willebrand factor type D domain containing a β′-component (β′-c) and a C-terminal coding region (Ct) (Carducci et al., 2019). These proteins play a key role in constituting nutritional reserves for embryo development in nonmammalian vertebrates. They are mainly synthesized in the female liver but also in males, at lower levels (Barucca et al., 2010; Canapa et al., 2007, 2012; Verderame & Scudiero, 2017). Their involvement in egg buoyancy, toxin transport and antimicrobial and antioxidant activities was reported in different species (Reading et al., 2017).

There is an increasing number of evidence about the presence of multiple vtg genes in different vertebrate lineages that makes difficult the reconstruction of vtg gene family evolution (Finn et al., 2009; Finn & Kristoffersen, 2007). In brief, as reviewed in our recent works (Biscotti et al., 2018; Carducci et al., 2019), up to three vtg genes have been found...
in nonteleost fishes. In particular, in lampaerids (Ichthyomyzon unicuspis and Petromyzon marinus) a single gene has been reported while three vtg genes have been found in the elephant shark (Callorhinichus milii), in the spotted gar (Lepisosteus oculatus), and in the bichir (Acipenser schrenckii). In teleost fish, the vtg gene number is higher, with a maximum of eight genes in zebrasid (Danio rerio; Yilmaz et al., 2019). In basal sarcopterygians, three vtg genes have been identified in coelacanths (Latimeria menadoensis and L. chalumnae) (Canapa et al., 2012), and four genes in the lungfish (Protopterus annectens) (Biscotti et al., 2018). Three vtg genes have been described for birds and turtles, and a single gene has been retrieved in platypus (Ornithorhynchus anatinus) (Babin, 2008; Biscotti et al., 2018; Finn & Kristoffersen, 2007). Phylogenetic and microsyntenic analyses have revealed a clear orthology for vtg genes of birds and turtles and vtgC genes of fish (Babin, 2008; Biscotti et al., 2018). For the remaining vtg genes tandemly organized in cluster, the orthology has not yet been defined (Carducci et al., 2019). In amphibians, past studies have considered only species belonging to the Anura order. In particular, three vtg genes have been described and their orthology to other tetrapod vtg genes is not clear (Brawand et al., 2008). Analyses performed on the Xenopus genome have not evidenced the presence of a vtg gene orthologous to vtgC of other tetrapods (Biscotti et al., 2018). No information is available about the other two orders: Caudata, the sister clade of anurans, and Gymnophiona, the early-branching lineage of amphibians. In the present work, transcriptomic resources related to the urodele C. orientalis (common name: fire-bellied newt) and genomic data of Gymnophiona were analyzed to get new insights onto the vtg evolutionary scenario in amphibians, a basal clade of tetrapods. Our results evidenced for the first time in amphibian species the presence of a vtgC gene showing orthology to those of birds and reptiles. Moreover, microsyntenic, phylogenetic, and gene conversion analyses allowed us to postulate two hypotheses explaining the evolutionary history of this intriguing gene family.

2 | MATERIALS AND METHODS

Specimens of C. orientalis were obtained from a local dealer during the reproductive season. Three females and three males were anesthetized with MS222 at 2 g/l and sacrificed. All experimental procedures were approved by the Italian ethical committee Ministero della Salute (authorization n° 2E1BD.N.LYB) and all methods were performed in accordance with the relevant guidelines and regulations. Testes, ovaries, and female livers were dissected. Total RNA was extracted and used for sequencing using methodologies published by Biscotti et al. (2020).

2.1 | Cynops orientalis sequences identification and characterization

Transcripts corresponding to vtg genes were retrieved from the high-quality transcriptome assembly of C. orientalis published by Biscotti et al. (2020) using a tBLASTn sequence homology search. Two complete sequences and five partial transcripts were obtained. These latter were used to obtain two further vtg complete sequences. The missing portions were completed through a polymerase chain reaction (PCR)-based approach starting from the complementary DNA synthesized from the female liver RNA samples processed in Biscotti et al. (2020). Reverse transcription was done using Superscript III First-strand Reaction Mix (Thermo Fisher, Invitrogen) and random primers. PCRs were performed in a thermal cycler using Platinum Taq DNA Polymerase (Thermo Fisher, Invitrogen). The complete list of primers used in this study and the amplification profile are provided in Figure S1. PCR products were sequenced using Sanger sequencing technology. Pairwise distance between the obtained sequences was calculated using a tool in MEGA X (Kumar et al., 2018). The four nucleotide sequences were translated using EMBL/EBI Sequence Translation Tool (https://www.ebi.ac.uk/Tools/st/emboss_transeq/). Protein function was annotated through the Gene Ontology database using the web server PredictProtein (https://predictprotein.org/; Rost & Liu, 2003). These sequence data have been submitted to the GenBank databases under accession number MZ064525-MZ064528.

2.2 | Phylogenetic analyses

In the phylogenetic analysis, the four Vtg sequences obtained in this study for C. orientalis (Caudata order) and the five sequences collected from NCBI (https://www.ncbi.nlm.nih.gov/) for Microacaelia unicolar and Rhinatremia bivittatum, two species belonging to Gymnophiona order, were used. In this analysis, additional eight amphibian sequences (four sequences of Xenopus laevis and three of X. tropicalis for Anura order and one sequence of Andrias davidianus for Caudata order) and 60 Vtg amino acid sequences belonging to other vertebrates were included. The complete list of accession numbers of sequences was reported in Table S1. The alignment was performed with ClustalOmega (https://www.ebi.ac.uk/Tools/msa/clustalo/) using default parameters. The phylogenetic analysis was carried out with MrBayes-3.2 (Huelsenbeck et al., 2001). The Jones amino acid model (Jones et al., 1992) was identified by the MrBayes program (Ronquist et al., 2012) with a posterior probability of 1.00. 1,000,000 generations were run, and sampling conducted every 100 generations. Stationarity was defined as the condition where the standard deviation of split frequencies reached 0.003. The first 2500 trees were discarded as the burn-in. The sequence of silver lamprey L. unicuspis was used as an outgroup. The adequacy of posterior samples taken from the Monte Carlo Markov Chain analysis was assessed estimating the effective sample size (ESS) using Tracer (Rambaut et al., 2018). ESS value was more than 200.

2.3 | Gene conversion analysis

The program GENECONV (www.math.wustl.edu/sawyer/geneconv/gconvdoc.pdf; Sawyer, 1989) determines the extent of gene conversion in a set of sequences. It seeks protein segments for which a
pair of sequences are sufficiently similar to suggest that gene conversion occurred. Inner fragments are evidence of a possible gene conversion event between ancestors of two sequences in the alignment. The output results are ranked by p-values and presented in a spreadsheet manner. The alignment of Vtg amino acid sequences for each amphibian species was used as input and tested using GENECONV to look for gene conversion tracts. The analysis was conducted using default parameters exception made for p (protein sequences); w123 (internal random number generator); lp (list of pairwise) setting 2,000,000 permutations; finally, as gscale/g0/g2, and/g7 mismatch penalties were used (data not shown).

2.4 | Microsyntenic analysis in basal sarcopterygians

The microsyntenic arrangement of vtg genes were obtained for the caecilian M. unicolor and the lungfish Neoceratodus forsteri. The strategy adopted consists in a BLAST analysis on the genome of interest using the "Whole Genome Sequencing Shotgun" tool. The identification of vtg genes and their upstream and downstream flanking genes allowed the comparison with coelacanth (basal sarcopterygian) and tetrapods available in Biscotti et al. (2018).

2.5 | Gene expression analyses

C. orientalis transcriptomic trimmed reads were mapped against the four Vtg nucleotide sequences in CLC Genomics Workbench v.12 environment (Qiagen). The alignment between the vtg sequences and trimmed reads of the three female livers and the three female gonads was performed with the RNA-seq mapping tool, setting mapping parameters as highly stringent (length fraction 0.9 and similarity fraction 0.9).

Gene expression levels were computed as transcripts per million (TPM) (Falcon & Gentleman, 2008), as this metric allows to efficiently compare gene expression levels both within and between samples. We followed the same strategy described in Biscotti et al. (2020), using a subset of 1694 unequivocal single-copy othologs.

3 | RESULTS

Two complete vtg sequences and five partial transcripts were obtained from the high-quality transcriptome of C. orientalis. Using a PCR-based approach two additional vtg sequences were reconstructed from the partial fragments. The attribution to the vtg gene family was checked through a BLAST sequence similarity search. The four vtg transcripts were named vitellogenin C. orientalis I (vtgCol), vtgColl, vtgColII, and vtgColIV and were of 5331, 5849, 5490, and 5479 bp long, respectively.

The pairwise distance was of about 46% between vtgCol and the other three sequences and within these latter ranging from 27.6% to 29.4%. Moreover, vtg sequences were searched and identified in the sequenced genomes of two caecilian species available in public databases. A total of three vtg sequences for the M. unicolor and two for the R. bivittatum were retrieved and used for phylogenetic, gene conversion, and microsyntenic analyses.

The phylogenetic analysis was based on a total of 77 amino acid sequences (24 species) whose 17 belong to 6 species of amphibians (Table S1). In the phylogenetic tree, two main clades can be observed: One characterized by the sequences of tetrapods together with those of lungfish P. annectens (Clade A) and the other constituted by sequences of L. chalumnae and L. menadoensis, A. schrenckii, L. oculatus, and teleosts (Clade B). Finally, in external position, three groups were present: Vtgi sequences of tetrapods, Vtg sequences of C. mili, and VtgC sequences of teleosts. In particular, the Vtgi sequences of tetrapods included that one found in the caecilian M. unicolor and the VtgCol belonging to the salamander C. orientalis (Figure 1).

The relationships evidenced in the Clade A were of interest for this study. Indeed, one subgroup (A1) of this clade was composed of Vtg sequences of the turtle Pelodiscus sinensis, the birds Gallus gallus, Taeniopygia guttata, and Ficedula albicollis, and the mammal O. anatinus. The second one (A2) can be further divided into three subgroups corresponding to the Vtg sequences of Anura (X. tropicalis and X. laevis), Caudata (C. orientalis and A. davidianus) and Gymnophiona (R. bivittatum and M. unicolor) with these latter as sister group compared to the sequences of other amphibians. In particular, the Vtg sequences of Anura, Caudata, and Gymnophiona and those of birds did not group following the species phylogenetic relationships but the sequence orthology. Analyses using GENECONV were conducted to search possible gene conversion events that occurred in amphibians (C. orientalis, M. unicolor, R. bivittatum, X. tropicalis, X. laevis). Setting the gscale (mismatch penalty) parameter as 2, for C. orientalis results evidenced one region of 158 residues length between VtgColI and VtgColV and a region of 327 residues in VtgColl that might have undergone to gene conversion. For X. tropicalis, a fragment of 89 residues length was evidenced between Vtg1 and Vtg2. For X. laevis, M. unicolor, and R. bivittatum relevant tracts in which gene conversion events might have occurred were not evidenced. Setting the mismatch penalties as 0 and 7 no further tracts that underwent gene conversion were evidenced, exception made for X. laevis in which two regions of 40 and 48 residues were detected between Vtg2a and Vtg1. All results here reported had p-values less than 0.05. Results involving regions having small lengths were not taken into account.

Previous studies have evidenced that the vtg genes are located in two regions (Babin, 2008), named M (multiple vtg genes) and S (single vtg gene) (Biscotti et al., 2018). In this study, the arrangement of vtg genes was investigated in the genome assemblies of lungfish and caecilians (Figure 2) and compared with coelacanth and tetrapods in Biscotti et al. (2018). The microsyntenic analysis revealed that the three vtg genes identified in caecilians are located two in the M region and one in the S region. Regarding the lungfish N. forsteri the four vtg genes were located in the M region. The presence of a vtg gene in the S region in lungfish cannot be excluded since our search on available genome data did not allow to identify the flanking genes suggesting that this region is not correctly assembled. In Figure 2 the microsyntenic arrangement of
C. orientalis, whose genome is not available, was hypothesized on the basis of the results obtained in the phylogenetic analysis.

The transcriptional activity of four vtg genes identified in Cynops was evaluated through RNA-seq. The analysis performed on data obtained from three female livers showed a remarkable activity of vtgCoII, vtgCoIII, and vtgCoIV (Figure 3a). In particular, in all samples, the vtgCoIV TPM values were the highest, followed by those of vtgCoII and vtgCoIII. Overall, in liver tissues, the vtgCoI was characterized by negligible expression values compared to other vtgs. Different expression levels observable in the three female livers can be explained following histological observations made for these female gonads and published in Biscotti et al. (2020) in which clear asynchronous developmental stages among samples emerged.

Transcriptional activity of vtgs was found also at gonadal level, although with lower TPM values, both in the case of male and female individuals (Figure 3b). In gonads, the expression of vtgs is mainly referred to vtgCoIII. Overall, in liver tissues, the vtgCoI was characterized by negligible expression values compared to other vtgs. Different expression levels observable in the three female livers can be explained following histological observations made for these female gonads and published in Biscotti et al. (2020) in which clear asynchronous developmental stages among samples emerged.

Transcriptional activity of vtgs was found also at gonadal level, although with lower TPM values, both in the case of male and female individuals (Figure 3b). In gonads, the expression of vtgs is mainly referred to vtgCoIII. Overall, it can be affirmed that the vtgCoI was not expressed at gonadal level. The Gene Ontology functional annotation of isolated Vtg sequences evidenced a nutrient reservoir activity for the VtgCoII and VtgCoIV and a lipid transfer activity for the VtgCoIII. No functional annotation was obtained for VtgCoI (data not shown).

4 | DISCUSSION

The analysis of C. orientalis transcriptome coupled with sequence reconstruction through PCR approach allowed to identify four sequences attributable to the vtg gene family. The phylogenetic analysis clearly showed the orthology of one sequence to the VtgI of tetrapods together with one sequence identified in the caecilian M. unicolor. The presence of this gene in two orders of Amphibia demonstrated that this gene was already present in the common ancestor of this clade and its absence in anurans was due to secondary loss. These findings were confirmed at microsyntenic level in caecilians whose genome was available.

In previous works (Babin, 2008; Biscotti et al., 2018), the microsyntenic arrangement of vtg genes revealed that in sarcopterygians these genes are located into two regions, named M and S region. While a single gene (vtgI) is located in the S region, multiple genes are present in the M region. The microsyntenic analysis conducted in the present work on caecilian M. unicolor showed the same distribution of vtg genes located in this region as the result of independent lineage-specific duplications from a unique ancestral gene on the basis
also of phylogenetic analyses. Indeed, while in the phylogenetic tree the sequences of VtgII of birds and those of the turtle P. sinensis constituted a paraphyletic group to that of the VtgIII of the same species evidencing a clear orthology, the sequences of amphibians clusterized for order underlining the missing of orthology between the Vtgs of amphibians and other tetrapods.

However, it has been hypothesized that the nonallelic gene conversion (NAGC) phenomena could explain the failure of phylogenies to reconstruct the duplication of the vtg genes located in the locus M occurred in the amphibian ancestor (Braasch & Salzburger, 2009). NAGC is the copying of a genetic sequence from a "donor" region to an "acceptor" in which the donor and the acceptor are at distinct genetic loci. These recombination events can easily occur when the paralogous sequences are erroneously aligned because of their high similarity. This is common in recent tandem gene duplicates while, over the time, mutations accumulate and sequences diverge leading to the disappearance of this phenomenon (Harpak et al., 2017). The occurring of NAGC between genes tandemly arranged can determine their clusterization due to the homogenization of the sequences rather than clusterization in phylogenetic analysis with ortholog sequences present in other species (Braasch & Salzburger, 2009). Therefore, regarding the vtgs of amphibians, two hypotheses can be suggested (Figure 4). According to the first hypothesis, in the common ancestor of amphibians two vtg genes were present: vtgI and vtgII/III ancestral genes. The vtgI gene is currently present in the Gymnophiona and Caudata lineages, while this gene was lost in anurans. The vtgII/III was undergone to a tandem duplication independently in all three amphibian orders. Therefore, caecilians show three genes and the newt C. orientalis has four genes, the vtgI and three genes derived from additional duplications of the vtgII/III gene. Data available do not allow to know if this condition is shared in urodeles. In anurans, the presence of three genes is due to two duplication events from the vtgII/III gene in the ancestor of this clade. In X. laevis, a fourth gene is derived by a polyploidization event occurred in the genome of this species. This model is in agreement with the phylogenetic tree and excludes that NAGC events have influenced phylogenetic relationships between sequences (Figure 3a). In the second hypothesis, the amphibian ancestor had three genes: vtgI, vtgII, and vtgIII, the latter two derived from a duplication of an ancestral gene. Subsequently, when the vtgII was highly similar to the vtgIII, independent gene conversion events occurred in the ancestors of the three amphibian orders. Therefore, in amphibians, three genes are expected as currently observed in Gymnophiona. In C. orientalis and probably in all urodeles a further duplication led a fourth gene. A similar event occurred in anurans in which, however, the vtgI was lost. Past gene conversion events occurred in the ancestor are not efficiently traced due to the increase of sequence divergence. However, this second hypothesis is supported by the analysis performed with GENECONV that evidenced possible gene conversion events between the vtg genes. The presence of three genes also in reptiles and birds allowed us to hypothesize that this condition was already present in the ancestor of amniotes. Therefore, the duplication event that led to vtgII and vtgIII might be dated at 400 Mya (or even before) in the common ancestor of tetrapods.

Recently the presence of multiple vtg genes has been correlated with several functions, beside as source of yolk nutrients for early developmental stages (Carducci et al., 2019). For example, in acanthomorph fish,
the heavy chain of VtgAa lipovitellin is highly degraded during oocyte maturation, producing a pool of free amino acids that has an effect on oocyte hydration and egg buoyancy (Finn & Kristoffersen, 2007). In Takifugu pardalis, a Vtg subdomain is able to bind and transfer tetraodotoxin from liver to ovary where it is accumulated in eggs as a repellent against predators and as pheromone able to attract males (Yin et al., 2017). Furthermore, other papers evidenced also antimicrobial activity (Liu et al., 2009; Shi et al., 2006; Zhang et al., 2005) and antioxidant activity for Vtg (Li & Zhang, 2017; Sun & Zhang, 2015).

The different expression levels of vtg genes in Cynops suggested that they might be involved in different functions. Indeed, the vtgCoII and vtgCoIV showed a high expression in livers of sexual mature females suggesting a role in yolk formation as also confirmed by functional annotation. The vtgCoII showed a lower expression in the liver compared to vtgCoI and vtgCoIV and its activity was also detected in male and female gonads. Overall, the expression of vtgs in gonads was due to autosynthesis, a process that occurs in gonads contrarily from heterosynthesis that occurs in the liver. The not gender-related expression of vtgs has been proposed to not be correlated to nutritional functions (Carducci et al., 2019). The vtgCoII originated with vtgCoIV from a duplication event, as also evidenced in the phylogenetic tree in which they group together, might have acquired a new function in the gonads of both sexes. Indeed, the annotation with Gene Ontology showed a "lipid transfer activity" for this sequence. In the analyzed tissues, no transcriptional activity was evidenced for vtgCoI suggesting that its role might be played in a different developmental stage or in a different tissue.

5 CONCLUSIONS

Solving the puzzle of the evolutionary history of vtg gene family remains a challenge for this study field. Certainly, past studies conducted on basal sarcopterygians provided precious knowledge to comprehend the number and genomic organization of these genes in the common ancestor of tetrapods. However, in this taxon information about vtg genes are limited to few species with lineages not yet examined. Therefore, analyses here conducted, taking into account species belonging to urodeles and caecilians, increased our knowledge in the Amphibia class. Our results evidenced that the vtg genes number varies between evolutionary lineages of this clade. Moreover, the identification of homologous genes to vtgl of tetrapods in Gymnophiona and
Caudata led us to affirm that this gene was already present in the common ancestor of this class and that Anura experienced a secondary loss. Expanding the data set to other tetrapod species will certainly contribute to define dynamics underlying the evolution of this gene family and functional studies will be necessary to definitely comprehend the roles of the duplicated vtg genes.

ACKNOWLEDGMENTS

Maria A. Biscotti, Marco Barucca, and Federica Carducci were involved in the phylogenetic and microsyntenic analyses. Federica Carducci and Marco Barucca did the sequence reconstruction, the analysis of gene expression, and functional annotation. All authors contributed to the interpretation of the

FIGURE 4  Schematic representation of the two hypotheses proposed for the evolution of vitellogenin gene family in amphibians. In the upper scheme (a) the evolutionary hypothesis based on the results obtained from phylogenetic analysis is showed; in the lower scheme (b) evolutionary hypothesis, in which gene conversion events have affected the phylogenetic relationship between sequences, is illustrated. Their detailed explanation is provided in the text. Black-filled squared are vitellogenin genes located in the M region and gray-filled square represents the single gene located in the S region (vtgI). Black and gray striped squares are referred to hypothesized vtg genes whose presence cannot be excluded. Crossed square indicates the loss of the vtg gene in locus S. Black curved arrows above filled squares indicate duplication events. White curved arrow indicates polyploidization-related duplication event occurred in Xenopus laevis. Dashed rectangles indicate hypothesized gene conversion events.
data and wrote the paper. Marco Barucca, Maria A. Biscotti, and Adriana Canapa conceived the study. All authors have given final approval for the version to be published.

CONFLICT OF INTERESTS
The authors declare that there are no conflict of interests.

PEER REVIEW
The peer review history for this article is available at https://publons.com/publon/10.1002/jez.b.23067

DATA AVAILABILITY STATEMENT
All sequences included in the study are available in the public data bank and accession numbers are reported in Table S1.

REFERENCES
Babin, P. J. (2008). Conservation of a vitellogenin gene cluster in oviparous vertebrates and identification of its traces in the platypus genome. Gene, 413, 76–82.
Barucca, M., Biscotti, M. A., Forconi, M., Regoli, F., Olmo, E., & Canapa, A. (2010). Characterization and phylogenic analysis of vitellogenin coding sequences in the Antarctic fish Trematomus bernacchii. Journal of Experimental Zoology. Part B, Molecular and Developmental Evolution, 314, 645–652.
Biscotti, M. A., Barucca, M., Carducci, F., & Canapa, A. (2018). New perspectives on the evolutionary history of vitellogenin gene family in vertebrates. Genome Biology and Evolution, 10, 2709–2715.
Biscotti, M. A., Carducci, F., Barucca, M., Gerdol, M., Pallavicini, A., Scharlt, M., Canapa, A., & Adolfi, M. C. (2020). The transcriptome of the newt Cynops orientalis provides new insights into evolution and function of sexual gene networks in sarcopterygians. Scientific Reports, 10, 5445.
Braasch, I., & Salzburger, W. (2009). In ovo omnia: Diversification by duplication in fish and other vertebrates. Journal of Biologe, 8, 25.
Brawand, D., Wahl, W., & Kaessmann, H. (2008). Loss of egg yolk genes in mammals and the origin of lactation and placentation. PLoS Biology, 6, e63.
Canapa, A., Barucca, M., Gobri, S., Benedetti, M., Zucchi, S., Biscotti, M. A., Olmo, E., Nigro, M., & Regoli, F. (2007). Vitellogenin gene expression in males of the Antarctic fish Trematomus bernacchii from Terra Nova Bay (Ross Sea): A role for environmental cadmium? Chemosphere, 66, 1270–1277.
Canapa, A., Olmo, E., Forconi, M., Pallavicini, A., Makapedua, M. D., Biscotti, M. A., & Barucca, M. (2012). Composition and phylogenic analysis of vitellogenin coding sequences in the Indonesian coelacanth Latimeria menadoensis. Journal of Experimental Zoology. Part B, Molecular and Developmental Evolution, 318, 404–416.
Carducci, F., Biscotti, M. A., & Canapa, A. (2019). Vitellogenin gene family in vertebrates: Evolution and functions. The European Zoological Journal, 86, 233–240.
Falcon, S., & Gentleman, R. (2008). Hypergeometric testing used for gene set enrichment analysis. Bioconductor Case Studies (pp. 207–220). Springer.
Finn, R. N., Kolarevic, J., Kongsdahg, H., & Nilsen, F. (2009). Evolution and differential expression of a vertebrate vitellogenin gene cluster. BMC Evolutionary Biology, 9, 2.
Finn, R. N., & Kristoffersen, B. A. (2007). Vertebrate vitellogenin gene duplication in relation to the “3R hypothesis”: Correlation to the pelagic egg and the ocean radiation of teleosts. PLoS One, 2, e169.
Harpak, A., Lan, X., Gao, Z., & Pritchard, J. K. (2017). Frequent nonallelic gene conversion on the human lineage and its effect on the divergence of gene duplicates. Proceedings of the National Academy of Sciences of the United States of America, 114, 12779–12784.
Huelsenbeck, J. P., Ronquist, F., Nielsen, R., & Bollback, J. P. (2001). Bayesian inference of phylogeny and its impact on evolutionary biology. Science, 294, 2310–2314.
Jones, D. T., Taylor, W. R., & Thornton, J. M. (1992). The rapid generation of mutation data matrices from protein sequences. Computer Applications in the Biosciences: CABIOS, 8, 275–282.
Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K. (2018). MEGA X: Molecular evolutionary genetics analysis across computing platforms. Molecular Biology and Evolution, 35, 1547–1549.
Li, H., & Zhang, S. (2017). Functions of vitellogenin in eggs. Results and Problems in Cell Differentiation, 63, 389–401.
Liu, Q. H., Zhang, S. C., Li, Z. J., & Gao, C. R. (2009). Characterization of a pattern recognition molecule vitellogenin from carp (Cyprinus carpio). Immunobiology, 214, 257–267.
Rambaut, A., Drummond, A. J., Xie, D., Baele, G., & Suchard, M. A. (2018). Posterior summarisation in Bayesian phylogenetics using Tracer 1.7. Systematic Biology, 67, 32–904.
Reading, B. J., Sullivan, C. V., & Schilling, J. (2017). Vitellogenesis in fishes, Reference module in Life Sciences. Elsevier.
Ronquist, F., Teslenko, M., Van Der Mark, P., Ayres, D. L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M. A., & Huelsenbeck, J. P. (2012). MrBayes 3: Efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology, 61, 539–542.
Rost, B., & Liu, J. (2003). The PredictProtein server. Nucleic Acids Research, 31, 3300–3304.
Sawyer, S. A. (1989). Statistical tests for detecting gene conversion. Molecular Biology and Evolution, 6, 526–538.
Shi, X., Zhang, S., & Pang, Q. (2006). Vitellogenin is a novel player in defense reactions. Fish Shellfish Immunology, 20, 769–772.
Sun, C., & Zhang, S. (2015). Immune-relevant and antioxidant activities of vitellogenin and yolk proteins in fish. Nutrients, 7, 8818–8829.
Verderame, M., & Scudiero, R. (2017). Estrogen-dependent, extrahepatic synthesis of vitellogenin in male vertebrates: A mini-review. Comptes Rendus Biologies, 340, 139–144.
Yilmaz, O., Patinote, A., Nguyen, T., Corn, E., Pineau, C., & Bobe, J. (2019). Genome editing reveals reproductive and developmental dependencies on specific types of vitellogenin in zebrafish (Danio rerio). Molecular Reproduction and Development, 86, 1168–1188.
Yin, X., Kiriaki, A., Ohita, A., Kitani, Y., Ishizaki, S., & Nagashima, Y. (2017). A novel function of vitellogenin subdomain, WVF type D, as a toxin-binding protein in the pufferfish Takifugu pardalis ovary. Toxicon, 136, 56–66.
Zhang, S., Sun, Y., Pang, Q., & Shi, X. (2003). Hemagglutinating and antibacterial activities of vitellogenin. Fish and Shellfish Immunology, 19, 93–95.

SUPPORTING INFORMATION
Additional Supporting Information may be found online in the supporting information tab for this article.

How to cite this article: Carducci, F., Biscotti, M. A., Canapa, A., & Barucca, M. (2021). The vitellogenin genes in Cynops orientalis: New insights on the evolution of the vtg gene family in amphibians. Journal of Experimental Zoology Part B: Molecular and Developmental Evolution, 1–8. https://doi.org/10.1002/jez.b.23067