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Identification of a *Cis*-Sex Chromosome Transition in Banded Geckos (*Coleonyx*, Eublepharidae, Gekkota)

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Abstract

Sex-determination systems are highly variable amongst vertebrate groups, and the prevalence of genomic data has greatly expanded our knowledge of how diverse some groups truly are. Gecko lizards are known to possess a variety of sex-determination systems, and each new study increases our knowledge of this diversity. Here, we used RADseq to identify male-specific markers in the banded gecko *Coleonyx brevis*, indicating this species has a XX/XY sex-determination system. Furthermore, we show that these sex-linked regions are not homologous to

the XX/XY sex chromosomes of two related *Coleonyx* species, *C. elegans* and *C. mitratus*, suggesting that a *cis*-sex chromosome turnover—a change in sex chromosomes without a concomitant change in heterogamety—has occurred within the genus. These findings demonstrate the utility of genome-scale data to uncover novel sex chromosomes and further highlight the diversity of gecko sex chromosomes.

1 INTRODUCTION

There are two forms of genetic sex determination (GSD) commonly found in sexually reproducing species, XX/XY sex chromosome systems, where heterogametic males inherit an X and Y chromosome, whereas females inherit two X chromosomes, and ZZ/ZW sex chromosome systems, where the female is the heterogametic sex with a Z and W chromosome, whereas males possess two Z chromosomes (Bull, **1983**). Sex chromosomes have evolved independently in many vertebrate lineages when an ancestral autosome acquired a sex-determining locus (Bull, **1983**; Bachtrog et al., **2014**). The evolutionary fate of these different sex chromosome systems can vary amongst lineages. Some degenerate over time from a morphologically identical (homogametic) chromosome pair to a visually distinct pair (heterogametic) through a process of recombination suppression precipitated to maintain linkage with a sexually antagonistic allele (Charlesworth, **1991**; Muller, **1914**; Ohno, **1967**), changes to the regulation of gene expression (Lenormand et al., **2020**; Lenormand & Roze, **2022**) or through neutral forces (Ironsides, **2010**; Jeffries et al., **2021**; Perrin, **2021**). These heteromorphic sex chromosomes can persist for millions of years, such as the XX/XY sex chromosomes of mammals and ZZ/ZW sex chromosomes of most birds (Cortez et al., **2014**; Veyrunes et al., **2008**). Other times, a sex chromosome system can remain stable for long periods of time, millions of years, with very little degeneration, including those of ratite birds (Ogawa et al., **1998**; Vicoso, Kaiser, & Bachtrog, **2013**; Zhou et al., **2014**), sturgeons (Kuhl et al., **2021**) and some skinks (Kostmann et al., **2021**). Finally, some groups experience frequent sex chromosome turnover so that related species may have non-homologous sex chromosome systems. Examples of this include the ZW and XY sex chromosome systems within medaka fish (*Oryzias*, Takehana et al., **2007**; Takehana et al., **2008**), the ZW and XY sex chromosomes of Japanese wrinkled frogs (*Glandirana rugosa*, Miura, **2007**; Ogata et al., **2008**) and the multiple XY sex chromosome systems of true frogs (*Rana*, Jeffries et al., **2018**).

Squamates (lizards and snakes) as a whole have more diverse sex-determination systems than other amniotes, but there is a high degree of sex chromosome conservation within most suborders or families (Pokorna & Kratochvíl, **2009**). These include the XX/XY sex chromosomes of skinks (Kostmann et al., **2021**), the ZZ/ZW sex chromosomes of lacertids (Rovatsos, Vukić, et al., **2016**), the XX/XY of most pleurodonts (Nielsen, Guzmán-Méndez, et al., **2019**; Rovatsos et al., **2014**) and the ZZ/ZW of caenophidian snakes (Matsubara et al., **2006**; Vicoso, Emerson, et al., **2013**). Gecko lizards, on the other hand, have evolved multiple sex chromosome systems (Gamble, Coryell, et al., **2015**), likely owing to a common ancestor that possessed temperature-dependent sex determination (TSD) (Pokorna & Kratochvíl, **2009**; Gamble, Coryell, et al., **2015**) with different daughter lineages subsequently evolving sex-determining loci on different syntenic blocks (Augstenová et al., **2021**). This has led to multiple XX/XY and ZZ/ZW sex chromosome systems in addition to several extant TSD species. Furthermore, there are four documented cases of intrageneric sex chromosome turnover, where different species within a genus have evolved different sex chromosome systems (*Sphaerodactylus*, Pinto et al. in press; *Cyrtodactylus*, Keating et al., **2021**; *Hemidactylus*, Gamble, Coryell, et al., **2015**; and *Gekko*, Yoshida & Itoh, **1974**; Solleder & Schmid, **1984**; Kawai et al., **2009**). Besides these geckos, intrageneric sex chromosome turnover in vertebrates has only been documented amongst some fish and amphibians, groups also known for dynamic and frequent sex-determination system transitions (Miura, **2007**; Ogata et al., **2008**; Myosho et al., **2015**; Jeffries et al., **2018**; Gammerdinger & Kocher, **2018**). Whether the frequent turnovers observed in geckos are a consequence of the ancestral TSD state during their early diversification, allowing separate lineages to independently evolve different sex chromosome systems, or some underlying biological mechanism promoting transitions is still unanswered (Augstenová et al., **2021**).

Here, we identify another intrageneric *cis*-sex chromosome transition—a change in sex chromosomes without a concomitant change in heterogamety—amongst members of the genus *Coleonyx*, commonly known as banded geckos, of the family Eublepharidae. Previous research has revealed a variety of sex-determination systems amongst eublepharid geckos. Incubation experiments demonstrated that *Eublepharis macularius* (Thorogood & Whimster, 1979; Viets et al., 1994; Wagner, 1980) and *Hemitheconyx caudicinctus* (Anderson, 1993; Viets et al., 1994) have TSD, wherein females develop at high and low incubation temperatures, whereas males develop at intermediate temperatures. Some species of *Goniurosaurus* displayed female-biased sex ratios when incubated at 25–28°C, whereas others had equal sex ratios at these temperatures (Seufer et al., 2005), suggesting the possibility that some species have TSD, whereas others have GSD, although a greater range of temperatures needs to be assessed to confirm this. TSD has also been suggested in *Aeluroscalabotes felinus*, but the available data are not sufficient to confirm this (Lui, 1996). Finally, cytogenetic data revealed the presence of a $X_1X_1X_2X_2/X_1X_2Y$ system in *Coleonyx elegans*, wherein heterogametic males have a neo-sex chromosome originating from a fusion between the ancestral sex chromosome and an autosome (Pokorná et al., 2010). This finding was later validated by identifying X-specific genes and using qPCR to identify the *C. elegans* sex chromosomes as homologous with chicken chromosomes 1, 6 and 11 (Pensabene et al., 2020). Homologous sex chromosomes were also identified in the closely related *C. mitratus*. However, two additional species of *Coleonyx* included in the study, *C. brevis* and *C. variegatus*, did not show the halved relative gene dose in males compared to females that would be indicative of a homologous sex chromosome system. Whilst incubation data indicate that *C. brevis* and *C. variegatus* do not have TSD (Viets et al., 1994), the specifics of the sex-determination system remain unknown. Here, we utilize RADseq data to demonstrate that *C. brevis* does indeed have a XX/XY sex chromosome system. However, synteny information reveals that the sex chromosomes of *C. brevis* are not homologous to those of *C. elegans* and *C. mitratus*, indicating a turnover has occurred amongst the banded geckos.

2 MATERIAL AND METHODS

Geckos were collected by hand near Hueco Tanks State Park in El Paso County, Texas, and via pitfall traps at the Indio Mountains Research Station in Hudspeth County, Texas, under the appropriate permits (Texas Parks and Wildlife Department SPR – 0290-019). All work was conducted under approved IACUC protocols (UTEP A-201004-1 and MU AR279). We extracted DNA from nine female and eight male *Coleonyx brevis* using the QIAGEN DNeasy Blood and Tissue Kit. We prepared RADseq libraries using a modified protocol from Etter et al. (2012) (Etter et al., 2012; Gamble, Coryell, et al., 2015). We digested genomic DNA using a high-fidelity *Sbf1* restriction enzyme (New England Biolabs, Ipswich, MA, USA) and ligated individually barcoded P1 adapters to each sample. We pooled samples into multiple libraries, sonicated and size selected for 200–500 bp fragments using magnetic beads in a PEG/NaCl buffer (Rohland & Reich, 2012). We then blunt-end repaired, dA-tailed and ligated pooled libraries with P2 adapters containing unique Illumina i7 (San Diego, CA, USA) indices. We amplified pooled libraries using NEBNext Ultra II Q5 polymerase (New England Biolabs) for 16 cycles and size selected a second time for 250–650 bp fragments that now contained full Illumina adapters. Libraries were sequenced using 150 bp reads on an Illumina NovaSeq 6000 at Psomagen, Inc., Rockland, MD.

We analysed the RADseq data using a previously described bioinformatics pipeline (Gamble, Coryell, et al., 2015). We demultiplexed, trimmed and filtered raw Illumina reads using the `process_radtags` function in STACKS (Catchen et al., 2011) (1.41). We used RADtools (Baxter et al., 2011) (1.2.4) to generate RADtags for each individual and identified candidate loci and alleles from the forward reads. We then used a custom Python script (Gamble, Coryell, et al., 2015) to identify putative sex-specific markers from the RADtools output, that is, markers found in one sex but not the other. The script also generates a list of ‘confirmed’ sex-specific RAD markers that exclude any sex-specific markers found in the original read files of the opposite sex, thus eliminating false positives. Finally, we used Geneious (Kearse et al., 2012) (R11) to assemble the forward and

reverse reads of ‘confirmed’ sex-specific RAD markers. These loci should correspond to genomic regions unique to a single sex, the Y or W chromosome, such that female-specific markers denote a ZZ/ZW system, whereas male-specific markers indicate an XX/XY system. We validated a subset of the ‘confirmed’ sex-specific markers with PCR. DNA primers (Table 1) were designed using Primer3 (Untergasser et al., 2012) in Geneious (R11). Additionally, we also tested the PCR primers on a related species with unknown sex chromosomes, *Coleonyx variegatus*. For *C. variegatus*, we used two males and two females to assess whether the *C. brevis* sex-specific primers might also amplify in a sex-specific pattern, which would indicate that the two species share a homologous sex chromosome system.

TABLE 1. Newly designed PCR primers used to validate sex-specific RADseq markers

Primer name	Sequence (5'-3')
Cb159-F1	ACCACAGGAAGCCTCTTAGGG
Cb159-R2	AGCTGCCCCATGTAAATCGTT
Cb145-F1	CCTGCAGGTAGATCCAGGATG
Cb145-R1	AGCTATGTTAAAGAGGGCAGGA
Cb141_157-F2	TCACCATAGCTCCATACCCCA
Cb141_157-R2	TGGGTTTCAGCTCAGTATTTGT
Cb302-F1	AGAGTAAATGCCCTTGCCCTT
Cb302-R1	AGACTCCTGAATGTGCAAGCA

We determined synteny of the *C. brevis* sex chromosomes using a step-wise strategy (Brelsford et al., 2017; Keating et al., 2020; Keating et al., 2021) by first BLASTing sex-specific RAD markers to a close relative with a sequenced genome and then subsequently BLASTin those larger fragments to the more distantly related syntenic reference, in this case chicken, *Gallus gallus*. We used an initial comparison with the leopard gecko (*Eublepharis macularius*) via BLAST (Altschul et al., 1990) to query male-specific *C. brevis* RAD markers to *Eublepharis* protein-coding genes and genome scaffolds (Xiong et al., 2016). *Eublepharis* BLAST hits were subsequently searched against chicken (*Gallus gallus*) protein-coding genes (International Chicken Genome Sequencing Consortium, 2004; Warren et al., 2017). Identification of syntenic regions allows us to assess the homology of sex-linked loci in *C. brevis* with chicken chromosomes. Using chicken chromosomes as a reference is standard in most studies of amniote synteny and we follow that convention here (Matsuda et al., 2005; Ezaz et al., 2009; Kawai et al., 2009; O'Meally et al., 2012; Rovatsos et al., 2014; Nielsen, Daza, et al., 2019; Augstenová et al., 2021; Kratochvíl et al., 2021).

3 RESULTS

We identified 161 511 RAD loci with two or fewer alleles. Of these, 625 markers were male specific and 0 were female specific. After checking the sex-specific markers against the reads from the opposite sex, we retained 576 ‘confirmed’ male-specific RAD markers. These results suggest that *C. brevis* has a XX/XY sex chromosome system, where the male-specific RAD markers are presumably found on the Y chromosome. We designed PCR primers for seven markers and validated four as sex specific (Figure 1). Two markers (Cb145 and Cb141_157) produced PCR bands for the male samples and no bands in females. One marker (Cb159) produced strong bands in males and fainter bands in the female samples. The fourth marker, Cb302, produced strong bands in males and two fainter bands in females, possibly due to off-target amplification or amplification of X-linked DNA if enough sequence similarity is retained with the Y-specific DNA from which the primers were designed. None of the markers produced a sex-specific pattern in *C. variegatus*.

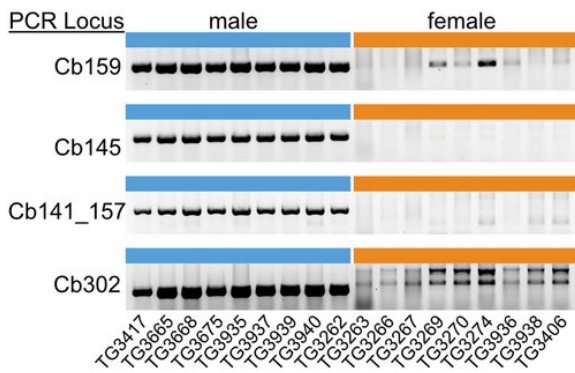


FIGURE 1. PCR validation of four male-specific markers in *Coleonyx brevis*. Marker names are listed to the right of the gels. The blue bars denote male samples and the orange bars denote female samples. Specimen identification numbers are listed below

After querying the male-specific RAD markers to the chicken genome, using *E. macularius* as an intermediate step, 35 markers successfully aligned to chicken and an additional 7 markers only aligned to the *E. macularius* genome (Table 2). When querying against protein-coding genes, again using *E. macularius* as an intermediate step, 7 markers aligned to the chicken genes and 13 markers aligned only to *E. macularius* genes. Nine markers had BLAST hits in both queries, of which three successfully aligned to chicken in both queries. These markers aligned to the same chicken chromosome in each query. Of the remaining six markers, one marker did not successfully align to chicken in either query, and only five aligned to the chicken genome when blasting against the genome itself and not protein-coding genes. Chicken chromosomes enriched with BLAST hits included chromosome 4q (14.3% of successful genome hits and 42.9% of successful gene hits), chromosome 28 (48.6% of genome hits and 57.1% of gene hits), chromosome 2 (14.3% of genome hits) and chromosome 24 (14.3% of genome hits). Three other chicken chromosomes (1, 3 and 7) had only a single BLAST hit (Figure 2, Table 2 and Table S1).

TABLE 2. BLAST queries of *Coleonyx brevis* male-specific markers against *Eublepharis macularius* and *Gallus gallus* genomes

<i>Coleonyx brevis</i> male-specific RAD marker	<i>Eublepharis macularius</i> scaffold	<i>Gallus gallus</i> chromosome
Coleonyx_brevis_Y_435	scaffold1701	1
Coleonyx_brevis_Y_26	scaffold58	3
Coleonyx_brevis_Y_7	scaffold3084	17
Coleonyx_brevis_Y_243	scaffold2272	24
Coleonyx_brevis_Y_261	scaffold2272	24
Coleonyx_brevis_Y_73	scaffold2272	24
Coleonyx_brevis_Y_195	scaffold2272	24
Coleonyx_brevis_Y_341	scaffold2272	24
Coleonyx_brevis_Y_104	scaffold999	28
Coleonyx_brevis_Y_193	scaffold999	28
Coleonyx_brevis_Y_242	scaffold999	28
Coleonyx_brevis_Y_379	scaffold999	28
Coleonyx_brevis_Y_438	scaffold999	28
Coleonyx_brevis_Y_532	scaffold999	28
Coleonyx_brevis_Y_49	scaffold999	28
Coleonyx_brevis_Y_39	scaffold1145	28
Coleonyx_brevis_Y_82	scaffold1145	28
Coleonyx_brevis_Y_166	scaffold1145	28
Coleonyx_brevis_Y_291	scaffold1145	28

Coleonyx brevis_Y_410	scaffold1145	28
Coleonyx brevis_Y_387	scaffold1602	28
Coleonyx brevis_Y_52	scaffold1682	28
Coleonyx brevis_Y_400	scaffold2351	28
Coleonyx brevis_Y_543	scaffold2351	28
Coleonyx brevis_Y_486	scaffold3818	28
Coleonyx brevis_Y_25	scaffold1334	2p
Coleonyx brevis_Y_332	scaffold1334	2p
Coleonyx brevis_Y_496	scaffold1334	2p
Coleonyx brevis_Y_516	scaffold1334	2p
Coleonyx brevis_Y_573	scaffold2388	2p
Coleonyx brevis_Y_172	scaffold414	4q
Coleonyx brevis_Y_145	scaffold843	4q
Coleonyx brevis_Y_240	scaffold843	4q
Coleonyx brevis_Y_547	scaffold843	4q
Coleonyx brevis_Y_284	scaffold1089	4q
Coleonyx brevis_Y_156	scaffold1150	No hit
Coleonyx brevis_Y_206	scaffold1150	No hit
Coleonyx brevis_Y_269	scaffold1150	No hit
Coleonyx brevis_Y_315	scaffold1150	No hit
Coleonyx brevis_Y_196	scaffold2597	No hit
Coleonyx brevis_Y_469	scaffold2597	No hit
Coleonyx brevis_Y_399	scaffold6395	No hit

Note: The *E. macularius* genome was used as an intermediate step to identify larger scaffolds before aligning to the *G. gallus* genome. All BLAST hits had an E-value of 0.

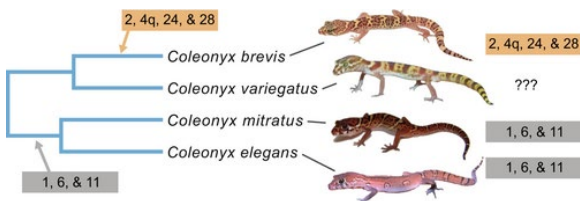


FIGURE 2. Phylogenetic tree of select *Coleonyx* species reproduced from Gamble, Greenbaum, et al. (2015). Boxes indicate the sex chromosome linkage group, represented by the homologous chromosome in chicken (*Gallus gallus*) for each species. The arrows indicate the likely origins of each sex-determining region based on common ancestry between extant species

4 DISCUSSION

Geckos display remarkable sex chromosome diversity when compared to other amniotes, and here, we increase this diversity as we describe a novel XX/XY sex chromosome in the banded gecko, *Coleonyx brevis*. This finding reveals the presence of an intrageneric *cis*-sex chromosome turnover as two other species of banded geckos, *C. elegans* and *C. mitratus*, have XX/XY sex chromosomes that are found on a different linkage group (Pensabene et al., 2020). These results suggest one of several possibilities: (1) that the ancestor of *C. elegans* and *C. mitratus* and the ancestor of *C. brevis* independently acquired XX/XY sex chromosomes, likely transitioning from a TSD ancestral state as two other species of eublepharid geckos, *Eublepharis macularius* and *Hemitheconyx caudicinctus*, have TSD, (2) either the XX/XY of the *C. elegans/mitratus* clade or *C. brevis* was ancestral in all three species and a *cis*-transition occurred in one of the lineages or (3) the ancestor to extant *Coleonyx* had some unknown/unsampled sex chromosome system and the sex chromosomes in *C. brevis* and the *C. mitratus/C. elegans* clade evolved independently. Given the data currently available, we

cannot distinguish amongst these alternative hypotheses. Identifying sex chromosomes in additional *Coleonyx* species could prove essential in this regard.

The above hypotheses (TSD to GSD or GSD to GSD) are also the two alternative explanations for the diversity of sex chromosome systems observed amongst extant geckos (Augstenová et al., 2021). For the first hypothesis, where TSD is the ancestral sex-determining mechanism in geckos, sex chromosomes evolved independently in different lineages. Following this acquisition, the sex chromosomes remained stable, leading to the distribution of sex chromosomes seen across the phylogeny today. Alternatively, in the GSD to GSD scenario, some ancestral sex chromosome system has led to repeated turnovers in different lineages and TSD observed in extant geckos is secondarily derived. An ancestral sex chromosome system might have remained in some extant species, whereas their relatives experienced the turnover, or the intermediate sex chromosome system could have been lost entirely. Such dynamic sex chromosome turnover is unusual amongst amniotes, but more prevalent amongst fish and amphibians (Jeffries et al., 2018; Lichilín et al., 2021; Takehana et al., 2007, Takehana et al., 2008; Miura, 2007, Ogata et al., 2008). As above, distinguishing between these scenarios is beyond the scope of the current data.

Greater taxonomic sampling is needed to distinguish between the above hypotheses. For *Coleonyx*, studies on related species could provide the relevant information. Unfortunately, the sex chromosome system of *C. variegatus* has yet to be revealed. *C. variegatus* samples were included in both Pensabene et al. (2020) and the current study but neither were able to validate *C. variegatus* as having sex chromosomes homologous to *C. elegans* / *C. mitratus* or *C. brevis*. There are two possible explanations for this. First, *C. variegatus* might have a different sex-determination system from the other three species. If so, this would highlight *Coleonyx* as a clade of extraordinarily diverse sex chromosome systems, even amongst geckos, and place it amongst genera with high rates of documented sex chromosome turnover such as *Rana* frogs (Jeffries et al., 2018) and *Oryzias* fish (Myosho et al., 2015). Alternatively, *C. variegatus* could have a homologous sex chromosome system to *C. brevis*, but there is not enough sequence similarity for the PCR primers to correctly bind to and amplify the sex-linked regions. Sex chromosomes often have higher rates of molecular evolution due to a lack of recombination and smaller effective population sizes that allow mutations to become fixed (Berlin & Ellegren, 2006; Ellegren, 2011). Whilst sex-specific primers designed for one species can sometimes work in a related species that shares the sex chromosome system (Fowler & Buonaccorsi, 2016; Hundt et al., 2019; Keating et al., 2020; Nielsen et al., 2020), this method is not always guaranteed to work. *C. variegatus* might likewise have sex chromosomes homologous with the *C. elegans-mitratus* clade but Pensabene et al. (2020) used qPCR to amplify X-linked genes, which are generally more conserved than the Y-linked genes we amplified here; this is a less likely scenario. Whichever the case, *C. variegatus* should be a priority for future studies in order to gain a greater understanding of the dynamics of sex chromosome evolution within banded geckos. Sampling other *Coleonyx* species, such as *C. fasciatus*, *C. gypsicolus*, *C. reticulatus* and *C. switaki*, will require significant effort, as all of these species have limited distributions and/or are microhabitat specialists and are rarely observed or collected (Dial & Grismer, 1992; Shedd & Murray, 2020). Studying other eublepharid species can likewise provide insight into the evolutionary history of sex determination within this family. Data suggest, but do not confirm (Lui, 1996), that the monotypic relative to *Coleonyx*, *Aeluroscalabotes felinus* (Agarwal et al., 2022; Gamble, et al., 2015), has TSD. Two species with confirmed TSD, *Eublepharis macularius* (Thorogood & Whimster, 1979; Viets et al., 1994; Wagner, 1980) and *Hemitheconyx caudicinctus* (Anderson, 1993; Viets et al., 1994) are in the sister clade to *Coleonyx* and *Aeluroscalabotes felinus* (Gamble, et al., 2015). This clade also contains species of *Goniurosaurus* with mixed support for GSD or TSD (Seufer et al., 2005) as well as the genus *Holodactylus* for which the sex-determination system remains unknown (Pokorná et al., 2010). Future research should focus on expanding sex determination information for these extant species in order to more accurately estimate the ancestral state of eublepharid geckos.

The sex chromosomes of *C. brevis* are composed of genomic fragments syntenic with chicken chromosomes 2, 4q, 24 and 28. Several of these chromosomes have been recruited into a sex-determining role in other amniotes. For example, chicken chromosome 2 is sex linked in *Python bivittatus* (Gamble et al., 2017), caenophidian snakes (Matsubara et al., 2006; Vicoso, et al., 2013) and the sphaerodactylid gecko genus *Aristelliger* (Keating et al., 2020). Chicken chromosome 4q is part of the sex chromosome system in geckos in the family Pygopodidae (Rovatsos et al., 2021). Chicken chromosome 24 is part of the sex chromosomes in the gecko genus *Saltuarius* (Augstenová et al., 2021). Chicken chromosome 28 is part of the sex chromosomes in monotremes and anguimorph lizards (Cortez et al., 2014; Kratochvíl et al., 2021; Rovatsos et al., 2019). Although all of these fragments have been observed as sex chromosomes in other amniotes before, this is a unique combination in *C. brevis*. It has been hypothesized that some autosomes may be predisposed to becoming sex chromosomes based on some genomic content that makes it more likely to serve a sex determination function (Graves & Peichel, 2010; O'Meally et al., 2012). For example, a chromosome may have a gene (or multiple genes) involved in the sex differentiation pathway that has been co-opted to act as the master sex determination gene controlling the entire cascade of regulatory networks (Herpin & Scharl, 2015). These so-called 'usual suspects', include genes like *Dmrt1*, *AMH* and *gsdf*, amongst others (Herpin & Scharl, 2015; Pan et al., 2021). Several of the 'usual suspects' show up on the *C. brevis* sex chromosomes, including *CTNNB1* and *SRD5A1* on chicken chromosome 2; *BMP1b* on chicken 4q and *AMH* on chicken 28. Although our current data offer no further insights into the identity of the *C. brevis* sex-determining gene, the occurrence of several likely candidates provides a jumping-off point for further investigation.

Here, we uncovered a sex chromosome turnover in the XX/XY sex chromosomes of *C. brevis*. This result was especially interesting as the sex chromosomes of *C. brevis* are not homologous to the XX/XY sex chromosomes of *C. elegans* and *C. mitratus*. Such novel sex chromosome diversity is becoming commonplace amongst geckos, as every recent study seems to identify a new sex-determination system (Keating et al., 2020, 2021; Pinto et al., *In press*; Pokorná et al., 2014; Augstenová et al., 2021; Gamble, et al., 2015; Rovatsos, et al., 2016; Rovatsos et al., 2021; Pensabene et al., 2020; Nielsen, et al., 2019). These frequent turnovers should make geckos a priority for future studies in order to uncover additional diversity and utilize this fascinating group of lizards as a model for studying sex chromosome evolution.

AUTHOR CONTRIBUTIONS

SEK, EG and TG conceptualized the study. EG, JDJ and TG collected the sample material. SEK conducted the laboratory work. SEK and TG conducted the bioinformatic analysis. SEK and TG co-wrote the manuscript. All authors contributed to the editing process and approved the final manuscript.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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