



Data Article

Karyological and bioinformatic data on the common chameleon *Chamaeleo chamaeleon*



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ABSTRACT

The data presented in this paper stand as supplementary information of the associated article “Karyological characterization of the common chameleon (*Chamaeleo chamaeleon*) provides insights on the evolution and diversification of sex chromosomes in Chamaeleonidae” [1]. This work provides (i) raw experimental data on the karyology of the common chameleon *Chamaeleo chamaeleon* and (ii) the results of bioinformatic analysis on sex-specific and repeated DNA sequences found in the same species. The karyological information here presented includes traditional staining method (Giemsa staining) and sequential C-banding + fluorochromes performed on Tunisian samples of the species. The sequence data include the alignments of the isolated DNA sequences with homologous sequences found in squamate Short Read Archives (SRAs) and the results of searches in public nucleic acid databases.

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Specifications Table

Subject	Agricultural and Biological Sciences
Specific subject area	Animal Science and Zoology
Type of data	Ecology, Evolution, Behaviour and Systematics Image Figure
How data were acquired	Karyological data were acquired using different staining/banding techniques (Giemsa staining, sequential C-banding + fluorochromes) followed by observations with optical (Giemsa staining) and epifluorescence (C-banding + fluorochromes) microscope. Molecular data were acquired by sequencing the amplicons obtained using the primers reported in [2] from DNA of a male of <i>C. chameleo</i> . The obtained sequences were manually edited with Chromas Lite 2.1.1. (Technelysium Pty Ltd, South Brisbane, AU) and assembled with BioEdit 7.2.5. [3]
Data format	Raw Analyzed .tif images
Parameters for data collection	Metaphase plates were searched at 10x and 20x magnification and recorded at 100x magnification either with optical or epifluorescence microscopy. Observations in epifluorescence were carried out using filter cubes for Chromomycin A ³ (CMA) and DAPI (excitation/emission wavelength, 445 nm /575 nm and 358 nm/ 461 nm, respectively).
Description of data collection	Data were collected through optical (Giemsa staining) and epifluorescence (C-banding + fluorochromes) microscope. Collection of molecular data was performed by search for identity of the obtained sequences [1] using BLAST Short Read Sequences (SRA) Archive of male 4 of <i>C. calyptratus</i> (Accession number SRX3547644) [2]. Alignments among C.Cham-RADseq of <i>C. chameleo</i> and SRA sequences of <i>C. calyptratus</i> were performed using BioEdit [3]
Data source location	Latitude and longitude (and GPS coordinates) for collected samples: Agareb (Tunisia) 34°25'60" N, 10°10'60" E (32 S 608722.99 m E, 3810839.52 m N); Tourief (Tunisia) 36°20'18 N, 8°35'9" E (32 S 462833.91 m E, 4021866.03 m N); Zéramdine (Tunisia) 35°34'16" N, 10°44'6" E (32 S 631429.31 m E, 3911883.93 m N)
Data accessibility	Raw karyological data can be found with this article. Sequence data can be found as reported in [1].
Related research article	Sidhom M., Khaled S., Chatti N., Guarino F.M., Odierna G., Petraccioli A., Picariello O., Mezzasalma M. Karyological characterization of the common chameleon (<i>Chamaeleo chamaeleon</i>) provides insights on the evolution and diversification of sex chromosomes in Chamaeleonidae. Zoology. In press https://doi/10.1016/j.zool.2019.125738

Value of the Data

The karyological data presented here show undifferentiated karyotypes, in terms of chromosome number, morphology and distribution of heterochromatin, between different sexes of *C. chamaeleon*, indicating that sex chromosomes are at an early evolutionary stage.

Cytogeneticists as well as evolutionary and molecular biologists can benefit from these data. Further comparative karyological analyses on other species and genera of Chamaeleonidae can highlight differences in chromosome number/morphology, heterochromatin content and distribution, and distinct sex determination systems.

The alignments with the newly generated DNA sequences, previously isolated in *C. calyptratus*, show their conservation in *C. chamaeleon*.

Comparative molecular and bioinformatic analyses on the isolated DNA sequences will add information about their conservation and distribution in other chameleon, squamate and vertebrate taxa.

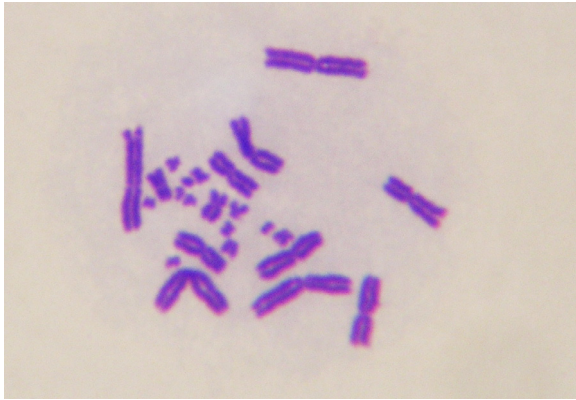


Fig. 1. Metaphase plate of a male specimen of *C. chamaeleon* from Agareb (Tunisia) stained with 5% Giemsa solution.

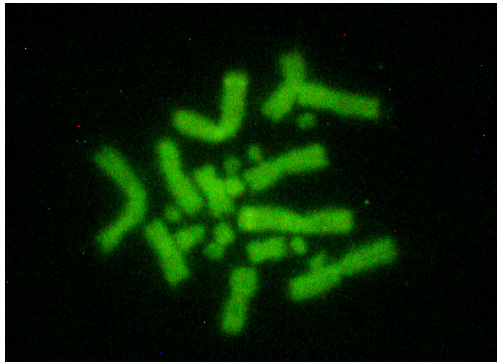


Fig. 2. Metaphase plate of a female specimen of *C. chamaeleon* from Tourief (Tunisia) with C-banding + CMA3.

1. Data Description

The data presented in this paper include the supplementary information of a thorough karyological characterization, molecular and bioinformatic analyses on Tunisian samples of the common chameleon *C. chamaeleon* described in the associated paper [1]. Figure 1 shows a metaphase plate with $2n=24$ chromosomes stained with Giemsa solution of a male common chameleon specimen. Figure 2 and 3 represent a sequential C-banding + CMA3 (Fig. 2) and + DAPI (Fig. 3), performed on a metaphase plate from a female specimen of the common chameleon. Figure 4 shows the distribution of best hits from query of the Cham 57 sequence isolated in [1] on SRA (Short Read sequence Archive) sequences of males 1 - 8 of *C. calyptratus* reported in [2]. Figure 5 shows the alignment with C.cham-RADseq (Restriction site-Associated sequence)_M2 sequence isolated in [1] and homologous sequences found in squamate SRAs [2]. Figure 6 shows the alignment with C.cham-RADseq_M3 sequence isolated in [1] and homologous sequences found in squamate SRAs [2]. Figure 7 shows the alignment with C.cham-RADseq_M12 sequence isolated in [1] and homologous sequences found in squamate SRAs [2]. Figure 8 shows the alignment with C.cham-RADseq_M13 sequence isolated in [1] and homologous sequences found in squamate SRAs [2].

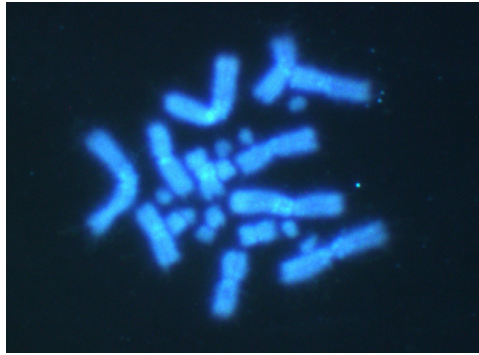


Fig. 3. Metaphase plate of a female specimen of *C. chamaeleon* from Zéramdine (Tunisia) with C-banding + DAPI.



Fig. 4. Distribution of best blast hits of Cham 57 sequence isolated in [1] on SRA sequences of males 1 - 8 of *C. calypttratus* (Accession numbers SRX3547644 - SRX3547651) [3].

2. Experimental Design, Materials, and Methods

We performed different chromosome staining techniques and banding methods to determine the number and morphology of chromosomes and to characterize heterochromatin distribution of the study specimens of the common chameleon. Chromosomes were obtained from cell suspensions using the air-drying method [4]. Specimens were injected with 0.01ml/ g body weight of a 0.1% vinblastine solution (Sigma), and after 2 h they were deeply anaesthetized by exposure to profound exposition to ethyl ether vapours. Chromosomes were obtained from bone marrow using the air-drying method, namely: 5 ml of a hypotonic solution of KCl 0.075 M were injected through medullar canal of femurs and cells collected in a test tube. After centrifugation at 1000 rpm, cells were fixed in methanol-acetic solution (3:1) and dropped (20 microliter) on a slide. Chromosomes were stained with conventional technique (5% Giemsa solution at pH 7 for 10 min.) and with sequential C-banding + CMA+DAPI following [5-7]. In brief, chromosomes were incubated in $\text{BA}(\text{OH})_2$ at 40°C (2 min.), washed first in HCl 0.2N and then in distilled water, then incubated in 2xSSC (Sodium Saline Citrate) for 15 min. After washing in distilled water, chromosomes were stained for 20 min in CMA (50 $\mu\text{m}/\text{ml}$ in McIlvaine buffer pH7), then washed

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          10      20      30      40      50      60      70      80      90      100
C. cham-RADseq_M2 ctgaaagacaaccaccaagcgCTTGTAGTCTGACTGCTGACAACCATTCCTCCCTTTAAAGCTTTCGAGGCATGCAGCAATTTTGTGATTTTGATGTGC
|||||
SRA1 CTGAAAGACAACCACCAAGCGCTTGTAGTCTGACTGCTGACAACCATTCCTCCCTTTAA-GCTTTCGAGGCATGCAGCAATTTTGTGATTTTGATGTGC
|||||
SRA2 TCCCCCTTTAAAGCTTTCGAGGCATGCAGCAATTTTGTGATTTTGATGTGC

          110     120     130     140     150     160     170     180     190     200
C. cham-RADseq_M2 ACAATGTCCTTGTGACTCCAGAGCTGTGCAAAATTATTCCTTTCTGCTCCTGGTGCAAAGCCAGCACTTCAAAGGATGAAGCTCTGGCTTGGTTAGTA
|||||
SRA1 ACAATGTC      SRA3 CCAGAGCTGTGCAAAATTATTCCTTTCTGCTCCTGGTGCAAAGCCAGCACTTCAAAGGATGAAGCTCTGGCTTGGTTAGTA
|||||
SRA2 ACAATGTCCTTGTGACTCCAGAGCTGTGCAAAATTATTCCTTTCTGCTCCTGGTGCA

          210     220     230     240     250     260     270     280     290     300
C. cham-RADseq_M2 CTCTCCAATCTTACCAGGCACATGAATTTGGAGCCAGATTATAGTTTCTGAGTCTCACAACCCTGTCTCAACTAAATGAGAATCCTGAGAGGATATTAT
|||||
SRA3 CTCTCCAATCTTACCAGGCACAT      SRA5 GTCTCAACTAAATGAGAATCCTGAGAGGATATTAT
|||||
SRA4 CTCTCCAATCTTACCAGGCACATGAATTTGGAGCCAGATTATAGTTTCTGAGTCTCACAACCCTGTCTCAACTAAATGAGAATCCTGAGAGGATATTAT

          310     320     330     340     350     360     370     380     390     400
C. cham-RADseq_M2 TTTTATGTTACCTCTCTTTTAAAGAGATTTTAAAAGTGGTCTTTTCTTAATCTACTTCACAATGTTGGCAAATTGACAGCAGGCCAACTAGGGTTGCT
|||||
SRA4 TTTTAT      SRA6 GGTTCCTTTTCGTAATCTACTTCACAGTGTGGCAAATTGACAGCAGGCCAACTAGGGTTGCT
|||||
SRA5 TTTTATGTTACCTCTCTTTTAAAGAGATTTTAAAAGTGGTCTTTTCTTAATCTACTTCACAATGTTGGCAAAT

          410     420     430     440     450
C. cham-RADseq_M2 TGACGTCTGTATGTGGTTTGAATGTGAGGatcacaccaatggcagtccta
|||||
SRA6 TGAAGTCTGTATGTGGTTTGAATGTGAGGatcacaccaatggcagtccta

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Fig. 5. Alignment of Short Read Archive (SRA 1-6) of RAD seq of *C. calypratus*: male 4 (Accession number SRX3547644) [3] versus the sequence C.cham-RADseq_M2 of male (Cham57) of *C. chamaeleon* (99% identity) isolated in [1]. Primer pairs are indicated in lower cases.

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          10      20      30      40      50      60      70      80      90     100
C. cham-RADseq_M3  aggaactgtgtgagctctcaatcaATGTTTCCTCATGTTATGCATCAGAGAATATTTTTTCAGAAATCCCTATACTTTTCAGAGCAAACGATTTTCATTG
|||||
SRA1  AGGAACTGTGTGAGTCTCAATCAATGTTTCCTCATGTTATGCATCAGAGAATATTTTTTCAGAAATCCCTATACTTTTCAGAGCAAACGATTTTCATTG
|||||
|||||
SRA2  TTCATTG

          110     120     130     140     150     160     170     180     190     200
C. cham-RADseq_M3  CTTGCCCTAGGTTTAAGAACCCTCAGCACTTGGTTTGTCTTCCAGAACATTAGACTATGTGTTTTGAATTAAGAAAAGGGAGTTTTCAATGGCCAGCCACAT
|||||
SRA1  CTTGCCCTAGGTTTAAGAACCCTCAGCACTTGGTTTGTCTTCCAGAACATTAGACTATGTGTTTTGAATTAAGAAAAGGGAGTTTTCAATGGCCAGCCACAT
|||||
SRA2  CTTGCCCTAGGTTTAAGAACCCTCAGCACTTGGTTTGTCTTCCAGAACATTAGACTATGTGTTTTGAATTAAGAAAAGGGAGTTTTCAATGGCCAGCCACAT
|||||

          210     220     230     240     250     260     270     280     290     300
C. cham-RADseq_M3  GAGTTTCTCCTGCCAGCCACATCAGAATGAATGGGTCTAAAACAAAACCAGAGCATTACGGACCTGGGGTCAATTATTGTCACATAATAATATATTAGGT
|||||
SRA3  GAGTTTCTCCTGCCAGCCACATCAGAATGAATGGGTCTAAAACAAAACCAGAGCATTACGGACCTGGGGTCAATTATTGTCACATAATAATATATTAGGT
|||||
|||||
SRA4  TAATAATATATTAGGT

          310     320     330     340     350     360     370     380     390     400
C. cham-RADseq_M3  ACCCTACTGTTGTGTTTGCAGATAAGAAGGCATCTCACTCGTAGGTACGATCAAACAACAGAGCTATTTATPAAAAATCTCGAGAAAAACATGGGCTGCAG
|||||
SRA4  ACCCTACTGTTGTGTTTGCAGATAAGAAGGCATCTCACTCGTAGGTACGATCAAACAACAGAGCTATTTATPAAAAATCTCGAGAAAAACATGGG
|||||
|||||
SRA5  TTTATTAGCAGCTCGAGAGAA-CATGGGCTGCAG

          410     420     430     440     450     460     470     480     490     500
C. cham-RADseq_M3  AGTCAGCAAAACCAACAACCTTATATATGGGTAGAATGTCAGTTAACATTGGTTACATTTGCATGGGCTTATAGAGAAGCAGGAATAACATTCTGATAAAAT
|||||
SRA5  AGTCAGCAAAACCAACAACCTTATATATGGGTAGAATGTCAGTTAACATTGGTTACATTTGCATGGGCTTATAGAGAAGCAGGAATAACATTCTGATAAAAT
|||||
|||||
SRA6  ATGGGCTTATAGA-AAGCAGGAATAACATTCTGATAAAAT

          510     520     530     540     550     560     570     580
C. cham-RADseq_M3  CAGCAGCTATCTGGGGTGTGTTTCAACTGGCACCTTTGCTCTTATTATATAACAAGATGGgctctgatctttttgtggaa
|||||
SRA6  CAGCAGCTATCTGGGGTGTGTTTCAACTGGCACCTTTGCTCTTATTATATAACAAGAT-GGCTCTGAGCTTTTTtggtggaa

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Fig. 6. Alignment of Short Read Archive (SRA 1-6) of RAD seq of *C. calypttratus*: male 4 (Accession number SRX3547644) [3] versus the sequence C.cham- RADseq_M3 of the male (Cham57) of *C. chamaeleon* (99.9% identity) isolated in [1]. Primer pairs are indicated in lower cases.

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      10      20      30      40      50      60      70      80      90      100
C. cham-RADseq_M12 caacctcctgcccagggttctccatgaaagctggcgcccttctctgaggaatgagaaccagccttacttccagatgtgtcaggctcagcccaccgagtaagta
SRA1 CAACCTCTTGCAGGGATTCTCCATCAAGCTGGTGCCTTCTCTGAGGAATGAGAACCAGCCTTACTTCCAGATGTGTCAAGCTCAGGCCACCAGGTAAGTA
SRA2 GCCCACCAGTAAGTA

      110      120      130      140      150      160      170      180      190      200
C. cham-RADseq_M12 CAATGGGTGAGAAGCTTTCCTCTCCCTGCGGATTACGCCATAGAGTTAGAATATCTGGTAGCAAGAAATGGTCACAGGAATGAGGGATGGAACATCCG
SRA1 C SRA3 GCAAGAAATGGTCACAGGAATGAGGGATGGAACATCAG
RA2 CAATGGGTGAGAAGCTTTCCTCTCCCTGCGGATTACGCCATAGAGTTAGAATATCTGGTAGCAAGAAATGGTCACAGGAATGAGGGATGGA

      210      220      230      240      250      260      270      280      290      300
C. cham-RADseq_M12 TGCCCTTCTTGGGACAGTTAAGGACCTTTCCTTGGTATCCAGACTGAGGTATCTTCACTTGGCTGTTGGAAAAGTATAGGAAAGCAACTGAAGAATG
SRA3 TGCCCTTCTTGGGACAGTTAAGGACCTTTCCTTGGTATCCAGACTGAGGTATCTTCACTTGGCTGTTGG
SRA4 TGGCTGTTGGAAAAGTATAGGAAAGCAACTGAAGAATG

      310      320      330      340      350      360      370      380      390      400
C. cham-RADseq_M12 AGCCCCATGTACCCATGGAGATGCTCCTCACATGGCAACTCTGCCACGAGGTTGTGCAATGGATGCACGCTATGTATGAGGCCCTTAAGAACAATTTTTC
SRA4 AGCCCCATGTACCCATGGAGATGCTCCTCACATGGCAACTCTGCCACGAGGTTGTGCAATGGATGCACGCT
SRA5 CGAGGTTGTGCAATGGATGCACGCTATGTATGAGGCCCTTAAGAACAATTTTTC

      410      420      430      440      450      460      470      480      490      500
C. cham-RADseq_M12 AGAGCAGGAGTGTACGACGCTGATCCCACAGCCCTTGCAAAGAGCAGCCACAGGGCAAACCTGGGCTGAGGATTctgggtaaatcctccacctc
SRA5 AGAGCAGGAGTGTACGACGCTGATCCCACAGCCCTTGCAAAGAGCAGCCACAGG
SRA6 GAGCAGGAGTGTACGACGCTGATCCCACAGCCCTTGCAAAGAGCAGCCACAGGGCAAACCTGGTGCCTGAGGATTCGCGCTAATCCTCCACCTC

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Fig. 7. Alignment of Short Read Archive (SRA 1-6) of RAD seq of *C. calypttratus*: male 4 (Accession number SRX3547644) [3] versus the sequence C.cham-RADseq_M12 of male (Cham57) of *C. chamaeleon* (identity 99.9%) isolated in [1]. Primer pairs are indicated in lower cases.

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                10      20      30      40      50      60      70      80      90     100
C. cham-RADseq_M13 atttggcattctcaggggaagGGAAGCCACACATATCCATTCTGTGCTGCCAACGCT-----GGTAGAGGGCTCCCTGCACAACCCCTTTTGT
|||||
SRA1  ATTTGGGCATCCTCAGGGAAGGGAAGCCACACATATCCATTCTGTGCTGCCAACGCTGGACTTCCGAGCAGGTAGAGGGCTCCCTGCACAACCCCTTTTGT
|||||
SRA2  TTG

                110     120     130     140     150     160     170     180     190     200
C. cham-RADseq_M13 GTGTGAAGGACCATGGGGAGCCACCAGTACCCCTAAGAACACAGCCATGCACCCAGGGTAAGTGGGGCCCTGGTTTGTGGGGCGGGAGTGG-CAAGGT
|||||
SRA1  GTGTGAAGGACCATGG                               SRA3  CCAAGG-TAAGTGGGGGGCTGGTTTGTGGGGCGGGTGGGGTCAAGGT
|||||
SRA2  GTGTGAAGGACCATGGGGAGCCACCAGTACCCCTAAGAACACAGCCATGCACCCAGGGTAAGTGGGGCCCTGGTTTGTGGGGCGGGAGTGG-CAAGGT

                210     220     230     240     250     260     270     280     290     300
C. cham-RADseq_M13 GTCATGAGTTTAGCCTAACAAACCTCACGACACTTTGATTTTAGTAATATAGAAAAGTCCCATGCACACTACTGACAAAGTCTCTTAAGCAATCTGGCAacc
|||||
SRA2  GTCATGAG                               SRA4  AAAAGTCCCATGCACACTACTGACAAAGTCTCTTAAGCAATCTGGCAACC
|||||
SRA3  GTCATGAGATTAGCCTAACAAACCTCACGACACTTTGATTTTAGTAATATAGAAAAGTCCCAT

                310
C. cham-RADseq_M13 agctcaagaagacagca
|||||
SRA4  AGCTCAAGAAGACAGCA

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Fig. 8. Alignment of Short Read Archive (SRA 1-4) of RAD seq of *C. calypttratus*: male 4 (Accession number SRX3547644) [3] versus the sequence RADseq Cham_M13of male (Cham57) of *C. chamaeleon* isolated in [1] (identity 95.3%). Primer pairs are indicated in lower cases.

in McIlvaine buffer and stained with DAPI (0.5 $\mu\text{m}/\text{ml}$ in McIlvaine buffer) for 10 minutes. After washing in McIlvaine buffer, slides were mounted with antifade solution (80% glycerine + 0,2% of DABCO, DiAzoBiCicloOctane). Determination of the chromosome number, karyotype reconstruction and chromosome classification were performed according to Levan et al. [8].

Metaphase plates were searched using the objectives 10 or 20x with optical (for Giemsa staining) and fluorescence microscope (for sequential C-banding) and recorded with objective 100x.

The DNA sequences isolated in [1] were blasted in BLAST Short Read Sequences (SRA) Archive of male 4 of *C. calyptratus* (Accession number SRX3547644) [2]. Alignment among the DNA sequences isolated in [1] and SRA sequences of *C. calyptratus* were performed using BioEdit [3].

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships which have, or could be perceived to have, influenced the work reported in this article.

Acknowledgments

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