Karyotypic divergence reveals that diversity in the *Oecomys paricola* complex (Rodentia, Sigmodontinae) from eastern Amazonia is higher than previously thought

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**Abstract**

The genus *Oecomys* (Rodentia, Sigmodontinae) is distributed from southern Central America to southeastern Brazil in South America. It currently comprises 18 species, but multidisciplinary approaches such as karyotypic, morphological and molecular studies have shown that there is a greater diversity within some lineages than others. In particular, it has been proposed that *O. paricola* constitutes a species complex with three evolutionary units, which have been called the northern, eastern and western clades. Aiming to clarify the taxonomic status of *O. paricola* and determine the relevant chromosomal rearrangements, we investigated the karyotypes of samples from eastern Amazonia by chromosomal banding and FISH with *Hylaeamys megacephalus* (HME) whole-chromosome probes. We detected three cytotypes for *O. paricola*: A (OPA-A; 2n = 72, FN = 75), B (OPA-B; 2n = 70, FN = 75) and C (OPA-C; 2n = 70, FN = 72). Comparative chromosome painting showed that fusions/fissions, translocations and pericentric inversions or centromeric repositioning were responsible for the karyotypic divergence. We also detected exclusive chromosomal signatures that can be used as phylogenetic markers. Our analysis of karyotypic and distribution information indicates that OPA-A, OPA-B and OPA-C are three distinct species that belong to the eastern clade, with sympatry occurring between two of them, and that the “*paricola* group” is more diverse than was previously thought.

**Introduction**

The arboreal genus *Oecomys* (Rodentia, Sigmodontinae) currently comprises 18 recognized species distributed from southern Central America to southeastern Brazil in South America, and is the most speciose genus of the Oryzomyini tribe. However, the actual number is
uncertain, considering that morphological, phylogenetic (mtDNA and nuDNA), classic cytogenetics and chromosome painting analyses have shown that there is wide-ranging diversity within some lineages [1–6]. Although these multidisciplinary approaches have helped researchers to comprehend better the distribution range and taxonomy of Oecomys, the resolution of O. bicolor, O. catherinae, O. cleberi, O. mamorae, O. paricola and O. roberti remains controversial. These taxa have been proposed to constitute species complexes, but additional sampling with more accurate analysis are required to distinguish the actual evolutionary units [1,4].

In particular, O. paricola exhibits a wide and uncertain distribution in central Brazil, from south of the Amazonas River to northeastern Peru [1] (Fig 1). This group was recovered as monophyletic by Suárez-Villota et al. [4], with high support values in maximum likelihood (ML) and Bayesian inference (BI) analyses performed with the concatenated genes, Cytb, IRBP and iBF7. The authors also recovered three distinct evolutionary lineages named the northern, eastern and western clades with high support within O. paricola, suggesting that the group represents a complex of different species. Beyond molecular identification, these evolutionary units can also be discriminated based on diploid number (2n), autosomal fundamental number (FN) and morphological traits [4]. The northern clade contains samples from the Marajó Island, north of Brazil in the Amazon biome, with 2n = 70, FN = 72 (locality no. 1 in the Fig 1); the eastern clade includes samples from Belém, north of Brazil in the Amazon biome, with

Fig 1. Map showing the distribution area and sampling points for Oecomys paricola with available cytogenetic data from the literature. 2n (diploid number), FN (autosomal fundamental number) and the clades identified for O. paricola (northern, eastern and western; sensu [4]) are also shown. The localities mentioned are: Tauari farm, Chaves, Marajó Island—PA (locality 1); Utinga Reserve, Belém—PA (locality 2); Uruçu-Una—PI (locality 3); Cláudia—MT (locality 4). All localities are from Brazil, and from the states of Mato Grosso (MT), Pará (PA), and Piauí (PI). The map was made using QGIS v. 3.10.7. Geographic distribution of Oecomys paricola is based on sample points provided by Patton et al. [1] and Suárez-Villota et al. [4]. The database was obtained from DIVA-GIS [7]. The shapefiles data containing countries limits, hydrography and elevation were obtained in the link https://www.diva-gis.org/gdata. In this link we selected the shapefiles from every country that is showed in both Figs 1 and 5. An O. paricola specimen is shown below. Scale bar: 5 cm. Photo by WOS.

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2n = 70, FN = 76 and 2n = 68, FN = 72 (locality no. 2 in the Fig 1), and specimens from the Cerrado biome in the states of Piauí, Maranhão and Tocantins, with 2n = 70, FN = 76 (locality no. 3 in the Fig 1); and the western clade includes samples from the state of Mato Grosso, with 2n = 70, FN = 74 (locality no. 4 in the Fig 1), south of the Amazon biome in central Brazil.

The level of information obtained from classic cytogenetics alone by using conventional staining, C-banding, and G-banding is lower than that achieved by FISH (fluorescence in situ hybridization) with whole-chromosome painting probes. The latter approach provides a better resolution of chromosomal evolution at many taxonomic levels, ranging from differences among populations of the same species and detection of complex rearrangements such as translocations and pericentric inversions [8–12], to the identification of conserved chromosomal segments among distinct mammalian groups that can be used as phylogenetic markers [10,13–19]. Furthermore, comparative chromosome painting analysis has been used as a valuable method to delineate species limits. In rodents, the information obtained by this approach has helped to elucidate the diversity within some groups and to establish geographic boundaries [12,20,21].

In the genus Oecomys, Malcher et al. [2] and Suárez-Villota et al. [4] used a multi-pronged approach to analyze samples of O. catherinae. By employing karyotypic, morphological and molecular methods, the groups reached similar conclusions, both proposing that O. catherinae populations from the Amazon (2n = 62, FN = 62) and Atlantic Forest biomes (2n = 60, FN = 62) are two distinct species. Although the two taxa from Amazonia and Atlantic Forest exhibited a low intraspecific molecular divergence of 1.6–3.1% [4] and 1.7% [2], they exhibited some distinct morphological characteristics. Suárez-Villota et al. [4] used C-banding analysis and proposed that the karyotypic divergence was due to one fission event, while Malcher et al. [2] used C-banding, G-banding and chromosome painting with HME (Hylaeamys megacephalus) whole-chromosome probes [22] and showed that the divergences were caused by one translocation and one fusion/fission event.

Taking into account the diversity observed in the “paricola group”, we set out to investigate the karyotypes of O. paricola from four localities of eastern Amazonia, aiming to improve their taxonomic delineation and determine the chromosomal rearrangements in this group. Toward this end, we performed a comparative analysis through classical cytogenetics and chromosome painting using HME whole-chromosome probes [22]. We also compared the taxa from the present study with other species from previously published studies using HME whole-chromosome probes [2,12,19,20,22–24].

Here, we reveal the chromosomal rearrangements that distinguish samples of O. paricola and describe exclusive chromosomal signatures for this taxon. We also propose that the samples in the present study correspond to three distinct species and discuss how chromosomal rearrangements may have played a part in the speciation process.

Materials and methods

Samples

The specimens were collected using live animal traps (Sherman) and pitfall traps [25], and the captures were authorized by the Brazilian Environment Department under license (IBAMA 02047.000384/2007-34). JCP has a permanent field permit (number 13248) from the Instituto Chico Mendes de Conservação da Biodiversidade. The specimens were deposited at the zoological collections of Museu Paraense Emílio Goeldi (MPEG) and the Museu de Zoológica da Universidade Federal do Pará (MUFPA). Both institutions are in Belém, Pará state, Brazil (Table 1). The Comitê de Ética em Pesquisa Animal da Universidade Federal do Pará approved this research (Permit 68/2015).
The cells used to prepare metaphase chromosomal preparations were obtained from bone marrow extraction performed according to Ford & Hamerton [27] and by cell culture of skin biopsy performed as described by Morielle-Versute [28]. The C-Banding [29] and G-Banding [30] techniques used slides bearing chromosomal preparations. C-banding was performed on G-banded metaphases to enable correct chromosomal assignment. FISH experiments followed Yang et al. [31] and were performed with 24 whole-chromosome probes from *Hylaeamys megacephalus* (HME) [22] made by degenerate oligonucleotide primed PCR (DOP-PCR) of flow-sorted chromosomes [31,32], of which three corresponded to two pairs of HME chromosomes each (HME (9,10), (13,22) and (16,17)). The labeling was made either with biotin-16-dUTP (Boehringer Mannheim), fluorescein isothiocyanate (FITC)-12-dUTP (Amersham) or Cy3-dUTP; the detection of the biotin probes was made with avidin-Cy3 or avidin-FITC.

The slides with chromosomal preparations were denatured for 2 minutes at 70% formamide, 2×SSC at 65˚C for 1 minute; the HME probes were denatured for 15 minutes at 60˚C before adding to the slides. After 72 hours hybridization at 37˚C, the slides were washed (2x formamide 50%, 2x (2xSSC), 1x (4xSSC/Tween) at 40˚C) [31,32]. The detection of the biotin probes was made with avidin-Cy3 (red) or avidin-FITC (green); for identification of the chromosomes pairs the counterstaining was made with DAPI (4',6-diamidino-2-phenylindole; blue).

**Image capture and analysis**

Digital images of banded karyotypes were gathered using an Olympus BX41 microscope and a CCD 1300QDS digital camera, and analyzed using the GenASIs software v. 7.2.7.34276. FISH images were obtained using a Nikon H550S microscope and a DS-Qi1Mc digital camera, and analyzed using the Nis-Elements software. The karyotypes were organized according to Levan et al. [33], with modifications. The final images were edited using Adobe Photoshop CS6.

### Table 1. Cytogenetic data available in the literature and obtained in the present study for *Oecomys paricola*. Species analyzed with *Hylaeamys megacephalus* probes in the present study are highlighted in bold in the leftmost column. The clades for *O. paricola* (northern, eastern and western; sensu [4]) are also shown within parentheses. Localities 1–4 refer to those mentioned in Fig 1; localities 2 and 5–7 are from the present study. Brazilian (BR) states are Mato Grosso (MT), Pará (PA) and Piauí (PI). The museum numbers of specimens analyzed in the present study are provided. Abbreviations: diploid number (2n); autosomal fundamental number (FN); Museu Paraense Emílio Goeldii (MPEG); Museu de Zoologia da Universidade Federal do Pará (MUFPA); male (♂); female (♀).

| Species Karyotype | Locality | Museum Number |
|-------------------|----------|---------------|
| *O. paricola* (Northern clade) | 2n = 70/ FN = 72 | 1 BR, PA: Tauparí farm, Chaves, Marajó Island (00˚ 39’S 50˚11’W) |
| *O. paricola* (Eastern clade) | 2n = 70/ FN = 76 | 2 BR, PA: Utinga Reserve, Belém (01˚25’4.39’S 48˚25’41.74’W) |
| *O. paricola* (Eastern clade) | 2n = 70/ FN = 76 | 3 BR, PI: Urucuí-Unia (8˚55’38.54’S 45˚11’35.07’W) |
| *O. paricola* (Western clade) | 2n = 70/ FN = 74 | 4 BR, MT: Cláudia (11˚41’4.49’S 54˚52’21.22’W) |
| *O. paricola* cytotype A (Eastern clade) | 2n = 72/ FN = 75* | 5 BR, PA: Utinga Reserve, Belém (01˚25’4.39’S 48˚25’41.74’W) |
| *O. paricola* cytotype B (Eastern clade)** | 2n = 70/ FN = 75 | 6 BR, PA: Expedido Ribeiro Community, Santa Bárbara (01˚13’02.31’S 48˚16’33.63’W) |
| *O. paricola* cytotype C (Eastern clade)** | 2n = 70/ FN = 72 | 7 BR, PA: Barcarena (01˚31’13.12’S 48˚41’25.09’W) |
| *O. paricola* cytotype A (Eastern clade) | 2n = 72/ FN = 75 | MPEG 39699♂, MPEG 39703♀ Present study |
| *O. paricola* cytotype B (Eastern clade)** | 2n = 70/ FN = 75 | MUFPA 377♂, MUFPA 372♀ Present study |
| *O. paricola* cytotype C (Eastern clade)** | 2n = 70/ FN = 72 | MPEG 39892♂, MPEG 39894♂, MPEG 39906♂, MPEG 39907♂, MPEG 39908♀ Present study |

*Previously assigned with 2n = 68/FN = 72 [26] but corrected to 2n = 72/FN = 75.

**The designation of the phylogenetic clade (sensu [4]) was based on distribution and karyotypic data from the present study.

**Cytogenetics**

The cells used to prepare metaphase chromosomal preparations were obtained from bone marrow extraction performed according to Ford & Hamerton [27] and by cell culture of skin biopsy performed as described by Morielle-Versute [28]. The C-Banding [29] and G-Banding [30] techniques used slides bearing chromosomal preparations. C-bandung was performed on G-banded metaphases to enable correct chromosomal assignment. FISH experiments followed Yang et al. [31] and were performed with 24 whole-chromosome probes from *Hylaeamys megacephalus* (HME) [22] made by degenerate oligonucleotide primed PCR (DOP-PCR) of flow-sorted chromosomes [31,32], of which three corresponded to two pairs of HME chromosomes each (HME (9,10), (13,22) and (16,17)). The labeling was made either with biotin-16-dUTP (Boehringer Mannheim), fluorescein isothiocyanate (FITC)-12-dUTP (Amersham) or Cy3-dUTP; the detection of the biotin probes was made with avidin-Cy3 or avidin-FITC.

The slides with chromosomal preparations were denatured for 2 minutes at 70% formamide, 2xSSC at 65˚C for 1 minute; the HME probes were denatured for 15 minutes at 60˚C before adding to the slides. After 72 hours hybridization at 37˚C, the slides were washed (2x formamide 50%, 2x (2xSSC), 1x (4xSSC/Tween) at 40˚C) [31,32]. The detection of the biotin probes was made with avidin-Cy3 (red) or avidin-FITC (green); for identification of the chromosomes pairs the counterstaining was made with DAPI (4’,6-diamidino-2-phenylindole; blue).
Results

Classic cytogenetics

The karyotype of O. paricola cytotype A (OPA-A) has 2n = 72 and FN = 75, with autosomes comprising 32 acrocentric pairs (pairs 1 to 32), two meta/submetacentric pairs (pairs 33 and 34) and one heteromorphic pair (pair 35; submetacentric and acrocentric homologue). The X chromosome is a large submetacentric and the Y chromosome is a medium submetacentric (Fig 2A). The karyotype of O. paricola cytotype B (OPA-B) has 2n = 70 and FN = 75 with autosomes comprising 30 acrocentric pairs (pairs 1 to 30), three meta/submetacentric pairs (pairs 31 to 33) and one heteromorphic pair (pair 34; submetacentric and acrocentric homolog); the X chromosome is a large submetacentric, and the Y chromosome is a medium submetacentric (Fig 2B). The karyotype of O. paricola cytotype C (OPA-C) has 2n = 70 and FN = 72 with autosomes comprising 32 acrocentric pairs (pairs 1 to 32) and two meta/submetacentric pairs (pairs 33 and 34); the X chromosome is a large submetacentric, and the Y chromosome is a medium submetacentric (Fig 2C).

The constitutive heterochromatin is distributed at the centromeric regions of almost all autosomes, in all three cytotypes; two autosomal pairs carry large heterochromatic blocks on the short arms of: OPA-A 33 and 34, OPA-B 31 and 32, OPA-C 33 and 34; the X chromosome carries a large heterochromatic block on the short arm, and the Y chromosome is almost entirely heterochromatic (Fig 3).

Molecular cytogenetics

Chromosome painting with all 24 Hylaeamys megacephalus (HME) whole-chromosome probes were performed on Oecomys paricola cytotypes A (OPA-A; 2n = 72/FN = 75), B (OPA-B; 2n = 70/FN = 75) and C (OPA-C; 2n = 70/FN = 72), and yielded 41, 39 and 40 hybridization signals, respectively. No hybridization signals were obtained on heterochromatic regions, as well on the Y chromosome and the short arm of the X chromosome. Table 2 summarizes these results.

From the 24 HME whole chromosome probes, seven (HME 6, 7, 8, 12, 18, 24 and 26) hybridized to whole chromosomes and one (HME 21) hybridized to part of one chromosome on the three OPA cytotypes; eleven probes showed multiple signals, from them nine (HME 2, 3, 4, (9,10), (13,22), 14, (16,17), 19 and 23) hybridized to two chromosomes each, and two (HME 1 and 5) showed signals in three chromosomes each on the three OPA cytotypes; the HME X chromosome hybridized to Xq due to the presence of a large heterochromatic block at Xp (Fig 2, Table 2).

The remaining four HME probes showed varied hybridization patterns among the three OPA cytotypes: HME 15 and 25 hybridized to whole chromosomes of OPA-A and OPA-C, while in OPA-B these probes hybridized on two chromosomes each; HME 20 showed multiple signals and hybridized to parts of two chromosomes each on OPA-A and OPA-C, while in OPA-B hybridized to part of one chromosome; HME 11 hybridized to a whole chromosome on OPA-B, and it is fragmented into three blocks on OPA-C and four blocks on OPA-A (Fig 2, Table 2).

All three OPA cytotypes exhibited four chromosomal pairs that corresponded to more than one HME homeolog: HME 20/(13,22), (13,22)/21, 4/19 and 19/14. Another chromosomal pair that exhibited the HME 20/11 is shared only by OPA-A and OPA-C (Fig 4).

Discussion

Chromosomal variability in Oecomys paricola

Rosa et al. [26] used chromosomal banding and Ag-NOR techniques to describe two karyotypes with 2n = 68, FN = 72 and 2n = 70, FN = 76 for O. paricola samples collected at the
Fig 2. G-banded karyotypes with chromosome painting revealed by Hylaeamys megacephalus (HME) whole-chromosome probes [22] of (a) O. paricola cytotype A (OPA-A; 2n = 72/FN = 75), (b) O. paricola cytotype B (OPA-B; 2n = 70/FN = 75) and (c) O. paricola cytotype C (OPA-C; 2n = 70/FN = 72). An asterisk indicates a centromere; “H” indicates a large block of constitutive heterochromatin.

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Utinga Reserve (Table 1). Here, we used C-banding, G-banding and FISH with HME probes to reanalyze the same samples previously assigned with 2n = 68, FN = 72, and found that the correct karyotype is 2n = 72, FN = 75. We did not have access to the chromosomal preparation.
of samples with $2n = 70$, $FN = 76$, and thus were unable to confirm this karyotype. Thus, we will only consider the karyotype from our study with $2n = 72$, $FN = 75$ as representative of samples from Utinga Reserve. We designate this karyotype (Table 1, locality 2) as *O. paricola* cytotype A (OPA-A). We also describe two other cytotypes: *O. paricola* cytotype B (OPA-B; Figure 3) and *O. paricola* cytotype C (OPA-C; $2n = 70$, $FN = 72$).

![Karyotypes](https://doi.org/10.1371/journal.pone.0241495.g003)

Fig 3. C-banded karyotypes of (a) *O. paricola* cytotype A (OPA-A; $2n = 72$, $FN = 75$), (b) *O. paricola* cytotype B (OPA-B; $2n = 70$, $FN = 75$) and (c) *O. paricola* cytotype C (OPA-C; $2n = 70$, $FN = 72$).
2n = 70, FN = 75) from Santa Bárbara and Barcarena (Table 1, localities 5 and 6, respectively); and *O. paricola* cytotype C (OPA-C; 2n = 70/FN = 72) from Marabá (Table 1, locality 7).

The comparative analysis of chromosomal number (2n) and morphology (FN) among samples of *O. paricola* from distinct localities of the Amazon and Cerrado biomes (Table 1) showed that these taxa exhibit only two diploid numbers (70 and 72), with variability seen in the FN (72, 74, 75 and 76). The differences in 2n could be caused by fusion/fission events, while those in the FN could be caused either by pericentric inversions or centromeric repositioning [34]. We detected one heterozygous pericentric inversion by the presence of a heteromorphic pair with submetacentric and acrocentric homolog in OPA-A and OPA-B; this is responsible for FN = 75, and is absent from its counterpart in the acrocentric pair of OPA-C and in other *O. paricola* karyotypes.

Differences in the sizes of the X and Y chromosomes were observed among *O. paricola* samples. Suarez-Villota et al. [4] described the X and Y chromosomes of karyotypes with 2n = 70, FN = 74, 76 as “slightly heterochromatic”. However, our C-banding results showed large heterochromatic blocks at the short arms of the X chromosomes, whereas the Y chromosomes were almost entirely heterochromatic. The three OPA cytotypes also exhibited two bi-armed pairs with heterochromatic blocks (Fig 3). These variations in the sex and autosomal chromosomes were most likely caused by amplification/deletion of constitutive heterochromatin; this
is a frequent event in rodents [18,35] but is unlikely to be involved in the speciation process, since heterochromatin usually does not contain functional genes and would not have a reproductive impact or generate deleterious meiotic products [36].

**Speciation hypothesis in the “paricola group”**

Observing the distribution, karyotypic, molecular and morphological data of *O. paricola* from the literature and the present study can suggest insights into how the speciation process may have acted in the “paricola group”. The literature shows that this taxon occurs in six out of eight Amazon areas of endemism recognized for terrestrial vertebrates: the taxon is present in the Belém, Xingu, Tapajós, Rondônia, Inambari, and Napo areas of endemism, as well as in the Marajo Island [1,37]. The role of Amazonian rivers as a barrier to species distribution was proposed by Wallace [38]. Since then, many studies in terrestrial vertebrates as rodents, primates and birds have shown that the more significant rivers of the Amazon basin can act as allopatric barriers to gene flow and contribute to species diversification in Amazonia [6,20,37,39,40]. This could explain the morphological differences found between the eastern and western clades of the “paricola group” mentioned by Suárez-Villota et al. [4], as differences in craniodental measurements, pelage coloration and morphology of the incisive foramen and subsquamosal fenestra, since the former clade occurs in the Belem and Xingu areas of endemism, while the latter occurs in the Tapajós area of endemism [4,37] (Fig 5).

Although no significant morphological or molecular difference was found within the eastern clade [4], our comparative chromosome painting analysis showed that OPA-A (2n = 72, FN = 75) and OPA-C (2n = 70, FN = 72) diverged due to one fusion/fission event and one heterozygous pericentric inversion. This was identified based on the presence of a heteromorphic pair in OPA-A (pair 35) that is also present in OPA-B (pair 34). OPA-B (2n = 70, FN = 75) differs from OPA-A and OPA-C by five and four fusion/fission events, respectively, and one translocation (Fig 6).

Although OPA-A and OPA-C are differentiated by a few chromosomal rearrangements, they occur in distinct areas of endemism: The former is found in the Belém area of endemism and the latter in the Xingu area of endemism; they are separated by the Tocantins River, which could act as a barrier to the distribution and gene flow between these two taxa. The sample collection points of OPA-A and OPA-B were located at isolated points in the metropolitan region of Belém, where only 30% of the original forest cover remains [41] (Fig 5). The karyotypic differences discussed above between these two apparently sympatric species, the lack of a strong geographic barrier and the absence of heterozygous karyotypic forms indicate that there is no gene flow between them.
Similar results were found by Rocha et al. [5], who analyzed the Cytb genetic structure of *Oecomys aff. roberti* (= *O. tapajinus*) populations from the mid-Araguia River in central Brazil, with the aim of testing how the river influenced their locomotion habits, habitat preferences and gene flow. The authors found a correlation between genetic and geographic distances, as this taxon exhibits stable and isolated populations, but did not observe any genetic difference related to the opposite riverbanks. This indicates that the isolation of *Oecomys* taxa can occur in the absence of a strong geographic barrier.

Da Silva et al. [11] discussed some biological features of rodents such as a high reproductive rate, the birth of several individuals per gestation in a short period, and a low vagility that favors endogamy that may allow the formation of an assemblage of taxonomically closely related individuals, denominated “demes” [42]. In a scenario that the same rearranged chromosomal form arises in different individuals within a population, these features could increase the probability of interbreeding between these heterozygotes. Thus, in a few generations a homozygous subpopulation for this rearranged form could arise [11].

The leading role of chromosomal rearrangements in the speciation of rodents [43] has been discussed in various studies [11,12,44,45]. Fusion/fission events and pericentric inversions are cited as the main events in the chromosomal reorganization of rodents [43], and translocations are also thought to be a strong barrier for hybridization in nature leading to diversification and speciation, as reported for rodents of genus *Ellobius* [21]. The occurrence of chromosomal
rearrangements in allopatric subpopulations could act as a barrier to gene flow in a secondary contact caused by the geographic expansion of the new chromosomal forms [46]. Thus, chromosomal rearrangements could act as post-zygotic blockage of gene flow and play a leading role in the speciation process, potentially explaining the sympatry occurring between OPA-A and OPA-B, but in OPA-C the allopatric effect caused by the Tocantins River would relegate chromosomal rearrangements to a secondary role in the speciation process.

The pattern we found for OPA-A, OPA-B, and OPA-C three distinct cytotypes was not reflected in the Cytb cladogram presented by Suárez-Villota et al. [4], who recovered the *O. paricola* eastern clade as a single entity. We note that the authors of the prior study did not include any specimen from the Tapirapé-Aquiri region (Fig 5, locality 7), and thus their topology may not include OPA-C. However, the authors did include Cytb sequences that correspond to one of the three cytotypes recognized here from the Utinga Reserve (OPA-A,
2n = 72, FN = 75; Fig 5, locality 2) and also from Barcarena (Fig 5, locality 6), which were obtained from GenBank and originally described by Rosa et al. [26]. Although Suárez-Villota et al. [4] did not provide the specimen karyotype from Barcarena, we karyotyped samples from this locality and from Santa Bárbara (Fig 5, locality 5) that exhibit 2n = 70, FN = 75 (OPA-B). We conclude that the OPA-A and OPA-B karyotypes were represented in the Cytb phylogeny from Suárez-Villota et al. [4] and that the chromosomal divergences described herein were not reflected in the Cytb sequence data.

In summary, our results indicate that OPA-A, OPA-B and OPA-C are three distinct species that belong to the O. paricola eastern clade (sensu [4]), with sympatry occurring between OPA-A and OPA-B. In this sense, the “paricola group” is more diverse than was reported previously, and a review of taxonomy of this group is needed to fully address the geographical limits and taxonomic delimitations. Lastly, detailed phylogeographic studies are necessary to improve our understanding of the speciation process in the genus *Oecomys*.

### Chromosomnal signatures for *Oecomys*

Previously published studies using HME whole-chromosome probes allowed the proposition of chromosomal signatures for the Sigmodontinae subfamily (HME 7/(9,10), 8, 1/12, 6/21, 11/(16,17), 5/(16,17), 20/(13,22), 15, 19/14/19, 24, and 26) and the Oryzomyini tribe (HME 8a, 8b, 18, and 25) [2,12,19,20,22–24]. We herein performed a comparative chromosome painting analysis with those data (S1 Table) and our findings are consistent with the above proposals.

Regarding genus *Oecomys*, only *O. catherinae* from Pará (OCA-PA; 2n = 62, FN = 62) and *O. catherinae* from Rio de Janeiro (OCA-RJ; 2n = 60, FN = 62) had previously been analyzed by chromosome painting with HME probes [2]. Our comparative analysis among OPA-A, OPA-B, OPA-C, OCA-PA and OCA-RJ karyotypes revealed the following specific signatures for this group: the syntenic block HME (13,22)/21 and the fragmentation of HME 1 into three blocks are exclusive traits for the *Oecomys* genus; HME (9,10)/14/5, 23/19/11 and 26/11 are exclusive traits for OCA-PA and OCA-RJ; and HME 4/19 and the fragmentation of HME 3 into two blocks are exclusive traits of OPA-A, OPA-B and OPA-C.

Although the syntenic block HME 20/(13,22) is considered to be an ancestral trait of the Sigmodontinae, in both OPA-A and OPA-C it is present as a derived form; this is due to a translocation that generates the syntenic block HME 20/(13,22) and 20/11, which are exclusive traits for OPA-A and OPA-C.

In the future, the use of comparative chromosome painting in other *Oecomys* species could help improve the taxonomic delineation, particularly in those taxa that are proposed to constitute species complexes (e.g., *O. bicolor*, *O. catherinae*, *O. cleberi*, *O. mamorae*, *O. paricola* and *O. roberti*) and in which morphological and/or molecular methods could not fully establish species boundaries.

### Conclusions

Our comparative chromosome painting analysis show that OPA-A, OPA-B and OPA-C differ by fusion/fission events, translocations and pericentric inversions (or centromeric repositionings), and allow the detection of chromosomal signatures that can be used as phylogenetic markers for genus *Oecomys* and species *O. paricola* and *O. catherinae*. Our results also indicate that OPA-A, OPA-B and OPA-C are three distinct species that belong to the eastern clade, with sympatry occurring between OPA-A and OPA-B. Moreover, we suggest that chromosomal rearrangements have played a leading role in the speciation process of OPA-A and OPA-B, but in OPA-C the allopatric effect caused by the Tocantins River would relegate
chromosomal rearrangements to a secondary role, and that the *Oecomys paricola* complex is more diverse than was previously alleged.

**Supporting information**

S1 Table. FISH signals detected for Sigmodontinae species, as assessed based on hybridization with *Hylaemys megacephalus* (HME) whole-chromosome probes [22].

(DOCX)

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**References**

1. Patton JL, Pardiñas UFJ, D’Elía G. Mammals of South America. Volume 2, Rodents. The University of Chicago Press: Chicago, MI, USA, 2015; pp. 140–277.

2. Malcher SM, Pieczarka JC, Geise L, Rossi RV, Pereira AL, O’Brien PCM, et al. *Oecomys catherinae* (Sigmodontinae, Cricetidae): Evidence for chromosomal speciation? PLoS ONE. 2017; 12(7): e0181434. https://doi.org/10.1371/journal.pone.0181434 PMID: 28727788

3. Pardiñas UFJ, Teta P, Salazar-Bravo J, Myers P, Galliari CA. A new species of arboreal rat, genus *Oecomys* (Rodentia, Cricetidae) from Chaco. J. Mammal. 2016; 97: 1177–1196.

4. Suárez-Villota EY, Carmignotto AP, Brandão MV, Percequillo AR, Silva MJJ. Systematics of the genus *Oecomys* (Sigmodontinae: Oryzomyini): molecular phylogenetic, cytogenetic and morphological approaches reveal cryptic species. J. Mammal. 2016; 97(4): 1177–1196. https://doi.org/10.1093/jmammal/gyw070
5. Rocha RG, Ferreira E, Fonseca C, Justino J, Leite YL, Costa LP. Seasonal flooding regime and ecological traits influence genetic structure of two small rodents. Ecol. Evol. 2014; 4: 4598–4608. https://doi.org/10.1002/ece3.1336 PMID: 25558355

6. Saldanha J, Ferreira DC, da Silva VF, Santos-Filho M, Mendes-Oliveira AC, Rossi RV. Genetic diversity of **Oecomys** (Rodentia, Sigmodontinae) from the Tapajós River basin and the role of rivers as barriers for the genus in the region. Mamm. Biol. 2019; 97: 41–49. https://doi.org/10.1016/j.mambio.2019.04.009

7. Hijmans RJ, Guarino L, Bussink C, Mathur P, Cruz M, Barrentes I et al. DIVA-GIS. Vsn. 5.0. A geographic information system for the analysis of species distribution data. 2004. Manual available at http://www.diva-gis.org.

8. Engelbrecht A, Taylor PJ, Daniels SR, Rambau RV. Chromosomal Polymorphisms in African Vlei Rats, **Otomys irratus** (Muridae: Otomyini), Detected by Banding Techniques and Chromosome Painting: Inversions, Centromeric Shifts and Diploid Number Variation. Cytogenet. Genome Res. 2011; 133: 8–15. https://doi.org/10.1159/000323416 PMID: 21228562

9. Graphodatsky A, Ferguson-Smith MA, Stanyon R. A short introduction to cytogenetic studies in mammals with reference to the present volume. Cytogenet. Genome Res. 2012; 137: 83–96. https://doi.org/10.1159/000341502 PMID: 22846392

10. Tian Y, Nie W, Wang J, Ferguson-Smith MA, Yang F. Chromosome evolution in bears: reconstructing phylogenetic relationships by cross-species chromosome painting. Chromosome Res. 2004; 12: 55–63. https://doi.org/10.1023/b:chro.0000092399.59998.f0 PMID: 14984102

11. Da Silva WO, Rodrigues da Costa MJ, Pieczarka JC, Rissino J, Pereira JC, Ferguson-Smith MA et al. Identification of two independent X-autosome translocations in closely related mammalian (**Proechimys**) species. Sci. Rep. 2019; 9: 4047. https://doi.org/10.1038/s41598-019-40593-8 PMID: 30858413

12. Oliveira da Silva W, Pieczarka JC, Rodrigues da Costa MJ, Ferguson-Smith MA, O’Brien PC, Mendes-Oliveira AC, et al. Chromosomal phylogeny and comparative chromosome painting among **Neacomys** species (Rodentia, Sigmodontinae) from eastern Amazonia. BMC Evol. Biol. 2019; 19: 184. https://doi.org/10.1186/s12862-019-1515-z PMID: 31601183

13. Jauch A, Wienberg J, Stanyon R, Arnold N, Tofanelli S, Ishida T, et al. Reconstruction of genomic rearrangements in great apes and gibbons by chromosome painting. PNAS. 1992; 89: 8611–8615. https://doi.org/10.1073/pnas.89.18.8611 PMID: 15288669

14. Scherthan H, Cremer T, Arnason U, Weier H, Lima-de-Faria A, Frönicke L. Comparative chromosome painting discloses homologous segments in distantly related mammals. Nat. Genet. 1994; 6: 342–347. https://doi.org/10.1038/ng0494-342 PMID: 8054973

15. Mao X, Nie W, Wang J, Su W, Feng Q, Wang Y, et al. Comparative cytogenetics of bats (Chiroptera): The prevalence of Robertsonian translocations limits the power of chromosomal characters in resolving interfamily phylogenetic relationships. Chromosome Res. 2008; 16: 155–170. https://doi.org/10.1007/s10577-007-1206-2 PMID: 18293110

16. Azevedo NF, Svartman M, Manchester A, Moraes-Barros N, Stanyon R, Vianna-Morgante AM. Chromosome painting in three-toed sloths: A cytogenetic signature and ancestral karyotype for Xenarthra. BMC Evol. Biol. 2012; 12: 36. https://doi.org/10.1186/1471-2148-12-36 PMID: 22429690

17. Perelman PL, Beklemisheva VR, Yudkin DV, Petrina TN, RozhnovVV, Nie W, et al. Comparative chromosome painting in Carnivora and Pholidota. Cytogenet. Genome Res. 2012; 137: 174–193. https://doi.org/10.1159/000334138 PMID: 22889959

18. Romanenko SA, Perelman PL, Trifonov VA, Graphodatsky AS. Chromosomal evolution in Rodentia. Heredity. 2012; 108: 4–16. https://doi.org/10.1038/hdy.2011.110 PMID: 22086076

19. Oliveira da Silva W, Malcher SM, Pereira AL, Pieczarka JC, Ferguson-Smith MA, O’Brien PCM, et al. Chromosomal Signatures Corroborate the Phylogenetic Relationships within Akodontini (Rodentia, Sigmodontinae), Int. J. Mol. Sci. 2020; 21: 2415. https://doi.org/10.3390/ijms21072415 PMID: 32244440

20. Oliveira Da Silva W, Pieczarka JC, Ferguson-Smith MA, O’Brien PCM, Mendes-Oliveira AC, Sampaio I, et al. Chromosomal diversity and molecular divergence among three undescribed species of **Neacomys** (Rodentia, Sigmodontinae) separated by Amazonian rivers. PLoS ONE. 2017; 12(8): e0182218. https://doi.org/10.1371/journal.pone.0182218 PMID: 28763510

21. Romanenko SA, Lyapunova EA, Saïdov AS, O’Brien PCM, Serdyukova NA, Ferguson-Smith MA, et al. Chromosome Translocations as a Driver of Diversification in Mole Voles **Elllobius** (Rodentia, Mammalia), Int. J. Mol. Sci. 2019; 20: 4466; https://doi.org/10.3390/ijms20184466 PMID: 31510061

22. Nagamachi CY, Pieczarka JC, O’Brien PCM, Pinto JA, Malcher SM, Pereira AL, et al. FISH with whole chromosome and telomeric probes demonstrates huge karyotypic reorganization with ITS between two species of Otomys (Rodentia, Otomyini) - **Hylaemys megacephalus**, **Cerradomys langiuthli**, Chromosome Res. 2013; 21: 107–119. https://doi.org/10.1007/s10577-013-9341-4 PMID: 23494775
23. Suárez P, Nagamachi CY, Lancerone C, Malloré MT, O’Brien PCM, Ferguson-Smith MA et al. Clues on syntenic relationship among some species of Oryzomyini and Akodontini Tribes (Rodentia: Sigmodontinae). PLoS ONE. 2015; 10(12): e0143482. https://doi.org/10.1371/journal.pone.0143482 PMID: 26642204

24. Pereira AL, Malcher SM, Nagamachi CY, O’Brien PCM, Ferguson-Smith MA, Mendes-Oliveira AC, et al. Extensive chromosomal reorganization among species of New World murid rodents (Cricetidae, Sigmodontinae): Searching for phylogenetic ancestral traits. PLoS ONE. 2016; 11(1): e0146179. https://doi.org/10.1371/journal.pone.0146179 PMID: 26800516

25. Santos-Filho M, De Lázari PR, Sousa CPF, Canale GR. Trap efficiency evaluation for small mammals in the Southern Amazon. Acta Amaz. 2015; 45, 187–194. https://doi.org/10.1590/1809-4392201401953

26. Rosa CC, Flores T, Pieczarka JC, Rossi RV, Sampaio I, Rissino JD, et al. Genetic and morphologic variability in South American rodent Oecomys (Sigmodontinae, Rodentia): evidence for a complex of species. J. Genet. 2012; 91: 265–277. https://doi.org/10.1007/s12041-012-0182-2 PMID: 23271012

27. Ford CE, Hamerton JL. A colchicine, hypotonic-citrate, squash sequence for mammalian chromosomes. Stain. Technol. 1956; 31: 247–251. https://doi.org/10.3109/10520295609113814 PMID: 13380616

28. Morille-Versute E, Varella-Garcia M. A simple and fast procedure to grow bat fibroblasts from lung biopsies for cytogenetic studies. Rev. Bras. Genet. 1995; 18: 503–505.

29. Sumner AT. A simple technique for demonstrating centromeric heterochromatin. Exp. Cell. Res. 1972; 75: 304–306. https://doi.org/10.1016/0014-4827(72)90558-7 PMID: 1639399

30. Sumner AT, Evans HJ, Buckland RA. New technique for distinguishing between human chromosomes. Nature (Lond) New Biol. 1971; 31: 282. https://doi.org/10.1038/newbio232031a0 PMID: 4105244

31. Yang F, Carter NP, Shi L, Ferguson-Smith MA. A comparative study of karyotypes of muntjacs by chromosome painting. Chromosoma. 1995; 103:642–652. https://doi.org/10.1007/BF00357691 PMID: 7587587

32. Telenius H, Carter NP, Bebb CE, Nordenskjo M, Ponder BAJ et al. Degenerate oligonucleotide-primed PCR: general amplification of target DNA by a single degenerate primer. Genomics. 1992; 13(3):718–725. https://doi.org/10.1016/0888-7543(92)90147-k PMID: 1639399

33. Levan A, Fredga K, Sandberg AA. Nomenclature for centromeric position on chromosomes. Hereditas. 1964; 52: 201–220.

34. Rocchi M, Archidiacono N, Schempp W, Capozzi O, Stanyon R. Centromere repositioning in mammals. Heredity. 2012; 108: 59–67. https://doi.org/10.1038/hdy.2011.101 PMID: 22045381

35. Kasahara S. Introdução à Pesquisa em Citogenética de Vertebrados. 1st ed: Ribeirão Preto, São Paulo, Brazil. Sociedade Brasileira de Genética; 2009.

36. King M. Chromosomal rearrangements, speciation and the theoretical approach. Heredity. 1987; 59: 1–6. https://doi.org/10.1038/hdy.1987.90 PMID: 3610656

37. Silva JMC, Rylands AB, Fonseca GAB. O destino das Áreas de endemismo. Megadiversidade. 2005; 1 (1): 124–131.

38. Wallace A. On the monkeys of the Amazon. Proceedings of the Zoological Society of London. 1852; 20: 107–110.

39. George TK, Marques SA, de Vivo M, Branch LC, Gomes N, Rodrigues R. Levantamento de mamíferos do Pará-Tapajós. Brasil Florestal. 1988; 63.

40. Ribas CC, Alexo A, Nogueira ACR, Miyaki CY, Cracraft J. A paleobiogeographic model for biotic diversification within Amazonia over the past three million years. Proc. R. Soc. B. 2012; 279: 681–689. https://doi.org/10.1098/rspb.2011.1120 PMID: 21795268

41. Amaral DD, Vieira ICG, Almeida SS, Salomão RP, Silva ASL, Jardim MAG. Checklist of remnant forest fragments of the metropolitan area of Belém and historical value of the fragments, State of Pará, Brazil. Bol. Mus. Para. Emílio Goeldi. Cienc. Nat., Belém. 2009; 4(3): 231–289.

42. Gilmour J, Gregor J. Demes: A Suggested New Terminology. Nature. 1939; 144, 333. https://doi.org/10.1038/144333a0

43. Romanenko SA, Volobouev V. Non-Sciuromorph rodent karyotypes in evolution. Cytogenet. Genome. Res. 2012; 137: 233–245. https://doi.org/10.1159/000339294 PMID: 22699115

44. Patton JL, Sherwood SW. Chromosome Evolution and Speciation in Rodents. Ann. Rev. Ecol. Syst. 1983; 14: 139–158.

45. Taylor PJ. Patterns of Chromosomal Variation in Southern African Rodents. J. Mamm. 2000; 81(2): 317–331. https://doi.org/10.1644/1545-1542(2000)081<0317:PCVSI2.0.CO;2

46. Rieseberg LH. Chromosomal rearrangements and speciation. Trends Ecol. Evol. London. 2001; 16(7): 351–357. https://doi.org/10.1016/s0169-5347(01)02187-5 PMID: 11403867