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Chromosomal Evolution in Psittaciformes. Revisited

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Abstract

With colourful plumage, charismatic character and vocal learning abilities, parrots are one of the most striking and recognizable bird groups. Their attractiveness has drawn human attention for centuries, and members of the Psittaciformes order were, also, among the first avian species to be subject to cytogenetic studies which have contributed to understand their taxonomic and evolutionary relationships.

We present here the karyological results collected by the study of thirteen parrot species new to karyology. These results are additionally supported by G banded preparations obtained in five species.

The order Psittaciformes is an interesting example of a, typically, non migratory avian lineage with Gondwanaland origin, whose evolutionary radiation has been shaped by the Cenozoic geographic and climatic events that affected the land masses derived from the Gondwanaland continental split.

We discuss the results of our studies, in conjunction with the previously compiled Psittaciformes cytogenetic data to delineate a picture of the chromosomal evolution of the order, concurrently with the biogeographic history of the lands in the southern Hemisphere.

Considering the available data on parrot cytogenetics, a "standard parrot karyotype pattern" is proposed for evolutionary comparisons.

Several biogeographic, and phylogenetically related "karyogram patterns" are also identified, and mechanisms of chromosome rearrangement that associate this patterns among them, and with the standard parrot karyotype pattern are proposed. These schemes on parrot chromosomal variation are discussed in relation to the general avian chromosome evolutionary theses proposed by cytogenetic and molecular genomic researchers.

Keywords: chromosome, cytotaxonomy, karyotype, evolution, parrots, Psittaciformes

1. Introduction.

Around 94 to 108 parrot taxa are listed as critically endangered, endangered or vulnerable (BirdLife International, 2000; IUCN, 2015). This current situation has been motivated mainly by hunting, illegal trade, and habitat destruction (Collar et al., 1992; IUCN, 2015), and places Psittaciformes among the most threatened avian groups.

In the case of some species (Spix macaw being, probably, one of the most emblematic) their immediate survival relies on “Ex-Situ” conservation efforts, and sex determination of the monomorphic species has become mandatory for the adequate management of specimens involved in captive propagation efforts.

Before polymerase chain reaction (PCR) techniques were widely available and used for sexing purposes (Morinha, Cabral, & Bastos, 2012), conservation veterinarians considered that cytogenetic analysis could become a helpful non-invasive tool to determine sex in avian sexually uniform species. (Bercovitz, 1989; Delhanty, 1989). However, the presence of nucleated red blood cells made the lymphocyte culture, routinely used in mammal karyology, difficult to apply in avian species. Alternative approaches were developed for avian cytology using feather pulp

culture technique and short term bone marrow cell culture. Anyhow, the samples required for these studies were short lived and difficult to obtain. Consequently avian karyotype studies were never widely available for sexing purposes, despite some company efforts (Avian Research Associates Lts, UK).

Nevertheless, the cytogenetic results obtained with Psittaciformes have also yielded useful data for taxonomical comparisons, and they may be used to study the genetic relationships and evolutionary divergence among species (Caparroz & Duarte, 2004; Christidis, Shaw, & Schodde, 1991; De Boer & Belterman, 1980; De Lucca & De Marco, 1983; De Lucca, Shirley, & Lanier, 1991; De Lucca, 1974, 1984, 1985; Duarte & Caparroz, 1995; Duarte & Giannoni, 1990; Francisco & Galetti J., 2001; Goldschmidt, Nogueira, Monsores, & Souza, 1997; Joshua, Unpublished, 1994; Joshua & Parker, 1993; Lunardi, Francisco, Rocha, Goldschmidt, & Galetti JR, 2003; Nogueira, De Souza, Goldschmidt, Da Silva, & Monsores, 2006; Ray Chaudhuri, Sharma, & Ray Chaudhuri, 1968; Rothfels, Aspden, & Mollison, 1963; Schmutz & Prus, 1987; Valentine, 1987, 1990; Van Dongen & De Boer, 1984). They also may contribute to the comprehension of avian chromosome evolutionary mechanisms (Burt, 2002; de Oliveira Furo, Kretschmer, O'Brien, Ferguson-Smith, & de Oliveira, 2015; Shoffner, 1974; Skinner & Griffin, 2012; Stock, Arrighi, & Stefos, 1974; Takagi & Sasaki, 1974; Tegelström, Ebenhard, & Ryttman, 1983; Tegelström & Ryttman, 1981).

Cytogenetic studies reveal the organization of a species genome in discrete assemblages called chromosomes which are recognizable under the optical microscope. The complete set of chromosomes in a species is the karyotype, and karyotypes may evolve as the species diverge. The variations in karyotype morphology among closely related species are consequently of one, or more changes in chromosome morphology, number or both.

Unfortunately, the rate of karyotype evolution has not been accurately correlated with the rate of speciation (Tegelström et al., 1983), neither the direction of the changes can always be exactly determined, unless we identify unequivocally the ancestral karyogram into a group of related species.

We present here, the cytogenetic study of thirteen parrot species new to karyology. We used feather pulp culture technique and short term bone marrow cell culture in order to get these results. Additionally, G banded preparations were obtained in five species.

At least 69 Psittaciformes taxa karyotypes have been studied and published by previous authors, while some results remain unpublished at the present time (Joshua, Unpublished, 1994).

This report aims to define the main events in the Psittaciformes chromosomal evolution, and to establish the correlations between karyological changes with historical biogeographical events, with phylogenetic relationships and, finally, with the current hypotheses on chromosome avian evolution.

1.1 Taxonomical and Biogeographic Considerations.

The order Psittaciformes has been, customarily, considered a well differentiated avian lineage (Collar, 1997; Forshaw & Cooper, 1989). Molecular genomics, also considers parrots a monophyletic group, sister to Passeriformes (Hackett et al., 2008; Suh et al., 2011). However, relationships within the order were never in complete agreement, and several systematic arrangements, based in different criteria have been proposed (Collar, 1997; Forshaw, 2010; Glenny, 1957; Joseph, Toon, Schirtzinger, Wright, & Schodde, 2012; Morony, Bock, & Farrand, 1975; Mudge, 1902; Peters, 1937; Smith, 1975; Thompson, 1899; Verheyen, 1956; Winkler, Billerman, & Lovette, 2015).

The most widely used taxonomical classification of the avian order Psittaciformes considers 353 species and two families; Cacatuidae and Psittacidae (Collar, 1997; Rowley, 1997).

Cacatuidae is divided in three subfamilies; Cacatuinae, Calyptorhynchinae and Nymphicinae.

Psittacidae has two subfamilies; Loriinae and Psittacinae.

Psittacinae is additionally divided in nine tribes; Psittrichadini, Nestorini, Strigopini, Micropsittini, Cyclopsittacini, Platycercini, Psittaculini, Psittacini and Arini.

This classification has been recently revised, and New Zealand endemic Nestorini and Strigopini tribes are now considered as members of a third family of Psittaciformes; Strigopidae (Winkler et al., 2015).

According to their current native areas (Collar, 1997) the extant parrot taxa are assigned to the Afrotropical, Australian, Indo-Malayan, Malagasy, or Neotropical biogeographic regions (Schweizer, Seehausen, & Hertwig, 2011). Present distribution of parrots, only exceptionally span over more than a single biogeographic region (Collar, 1997; Forshaw & Cooper, 1989; Forshaw, 2010).

2. Material and Methods

Chromosome studies were performed on nine species belonging to the subfamily Psittacinae (fam. Psittacidae), three species to the subfamily Loriinae (fam. Psittacidae), and one species from the family Cacatuidae.

Three parakeets included in the tribe Platycercini; *Psephotus chrysopterygius*, (Golden-shouldered Parrot), *Purpureicephalus spurius* (Red-capped Parrot), *Barnardius barnardi macgillivrayi* (Mallee Ringneck) and the Cacatuidae *Callocephalon fimbriatum* (Gang gang Cockatoo) have an Australian distribution. The three Loriinae species *Trichoglossus haematodus massena* (Rainbow Lorikeet ssp. massena), *Trichoglossus flavoviridis* (Yellow and green Lorikeet) and *Charmosyna josefinae* (Josephine's Lorikeet), also inhabit the Australian region.

Three species, appertaining to the tribe Psittaculini; *Tanygnathus megalorynchos* (Great-billed Parrot), *Tanygnathus lucionensis talautensis.* (Blue-napped Parrot) and *Prioniturus discurus* (Blue crowned Racquet tail), naturally occur in the Indo Malayan region.

The Psittacini *Poicephalus robustus* (Cape Parrot) has an Afrotropical distribution.

Finally, two species inhabit the Neotropical region and belong to the tribe Arini; *Aratinga finschi* (Crimson-fronted Parakeet) and *Triclaria malachitacea* (Blue-bellied Parrot). We also present the karyotype of the also Neotropical *Graydidascalus brachyurus* (Short-tailed Parrot) (Previously described by Caparroz & Duarte, 2004) for support and for comparisons.

Chromosome studies were carried out with captive female specimens endoscopically sexed or sexually dimorphic. In every case the study was limited to a single female.

The material used for obtaining the chromosome spreads was feather pulp from growing feathers (Van Tuinen & Valentine, 1982). The only exception was the case of *Triclaria malachitacea,* where we used tibiotarsal bone marrow (Christidis, 1985) from a female specimen which recently died as consequence of an accident. The bone marrow sample was, in this case, directly placed in culture medium.

The feather pulp was collected, and immediately was mechanically separated with a scalpel blade, after that, it was incubated at 37ºC for 20-40 min. in a 10% trypsin solution for enzymatic disassociation. The cellular suspension was then cultured with Ham's F 10 medium supplemented with foetal bovine serum, Hepes and antibiotics in standard conditions for cell culture without CO_2 supplementation. The incubation temperature was 39ºC. After 3-4 days, cellular growth was optimal for harvesting cells with colchicine at a final concentration of 0'06 mg/ml for two hours. Metaphase spreads were stained with 10% Giemsa for 10 min. (Belterman & De Boer, 1984; Bercovitz, 1989; Biederman & Lin, 1982; Delhanty, 1989; Zartman, 1973).

In five species (*Psephotus chrysopterygius*, *Trichoglossus haematodus massena, Charmosyna josefinae, Tanygnathus megalorynchos* and *Prioniturus discurus*), G bands were produced by the Trypsin-Giemsa method. The slides, kept 2 days in the fridge, were aged in 15% hydrogen peroxide for 2.5 min, washed in methanol, and dried for at least 30 min. Slides were trypsinized for 10 seconds, and stored in buffer pH 6.8 before staining in 10% Giemsa for 12 min. (Bulatova & Radjabli, 1974).

The nomenclature criteria of the chromosomes follow the recommendations suggested by Levan, Fredga, & Sandberg (1964).

3. Results.

Purpureicephalus spurius, Barnardius barnardi macgillivrayi and *Psephotus chrysopterygius.*

The karyotypes of the Australian Parakeets *Purpureicephalus spurius, Barnardius barnardi macgillivrayi* and *Psephotus chrysopterygius* are very similar. G banding stain slides were also produced on *Psephotus chrysopterygius* (Figures 1, 2 and 3).

The diploid chromosome number is about 62, with 6 pairs of autosome macrochromosomes.

The first and second pairs are composed of large metacentric chromosomes.

Pairs 3, 4, 5 and 6 are acrocentric.

The remaining autosomes are microchromosomes.

The Z chromosome is submetacentric (acrocentric in *Psephotus chrysopterygius*), and the W is a small acrocentric chromosome.

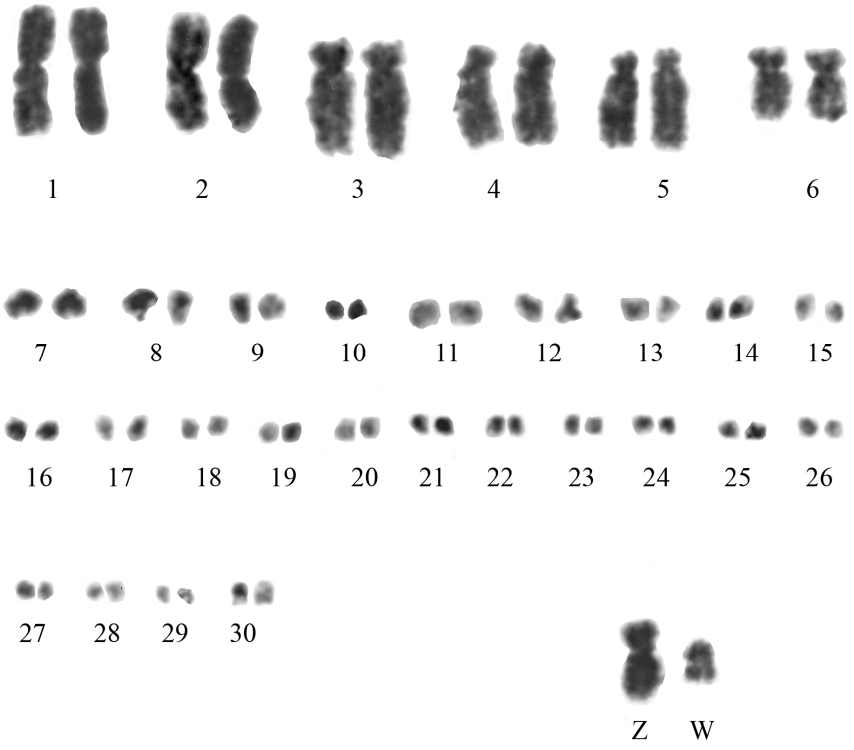

Figure 1. Proposed karyotype of *Purpureicephalus spurius*

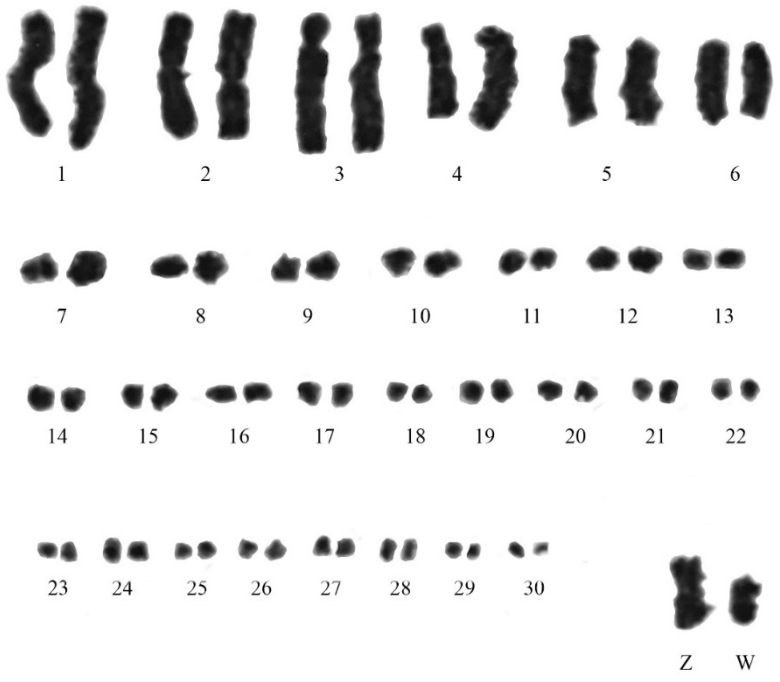

Figure 2. Proposed karyotype of *Barnardius barnardi macgillivrayi*

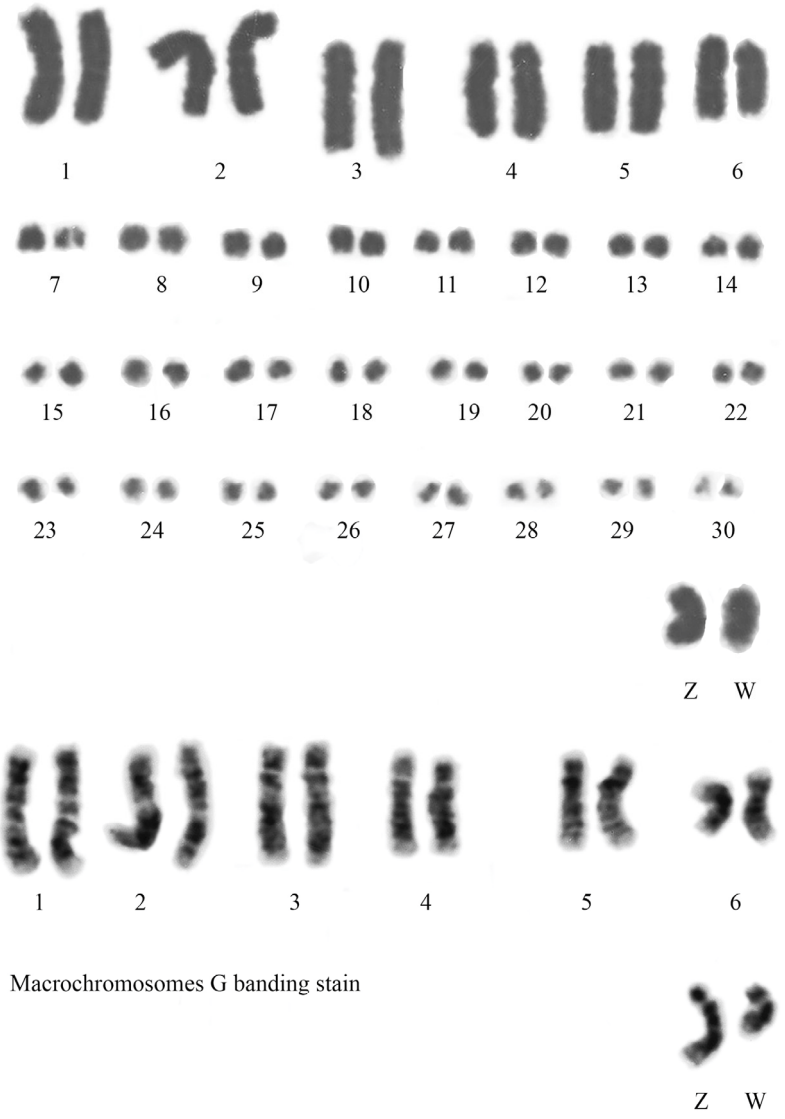

Figure 3. Proposed karyotype and G band stain of *Psephotus chrysopterygius*

Callocephalon fimbriatum.

The total chromosome number is about 92. We recognized eight pairs of autosome macrochromosomes (Figure 4).

Chromosome pairs 1 and 2 are acrocentric.

The chromosome pair 3 of this particular female shows a chromosomal heterozygosity. One of the chromosome complements being telocentric and smaller than the acrocentric complement. This difference could be the result of a heterozygous robertsonian translocation.

The remaining macrochromosome pairs 4, 5, 6, 7 and 8 are telocentric. Microchromosomes are also telocentric, as far as the centromere position can be discerned.

The sex chromosome pair is interpreted as follows: the Z chromosome is metacentric, while the W chromosome is submetacentric.

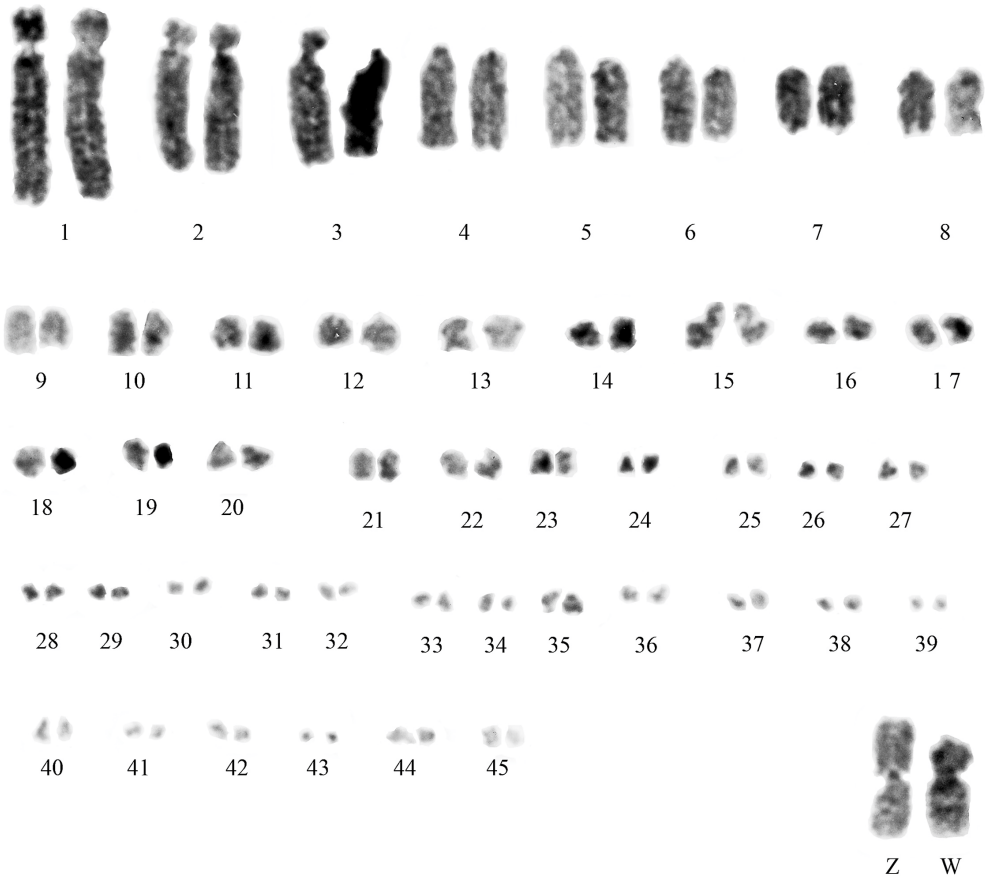

Figure 4. Proposed karyotype of *Callocephalon fimbriatum*

Trichoglossus haematodus massena.

The diploid chromosome number is about 48, with seven pairs of autosome macrochromosomes. G banding slides were also produced (Figure 5).

The first pair is metacentric.

Pairs 2, 3 and 4 are acrocentric.

Pairs 5 and 6 are submetacentric to metacentric.

Pair 7 is telocentric.

The remaining autosomes are considered microchromosomes.

The Z chromosome is submetacentric, and the W chromosome is metacentric.

Trichoglossus flavoviridis.

The diploid chromosome number is about 46, with seven pairs of autosome macrochromosomes (Figure 6).

The first pair is metacentric.

Pairs 2, 3 and 4 are acrocentric.

Pair 5 are 6 are submetacentric.

Pair 7 is telocentric.

The remaining autosomes are considered microchromosomes. Pairs 8 and 9 are large microchromosomes, and are metacentric.

The Z chromosome is a submetacentric, and the W chromosome is slightly smaller, and submetacentric too.

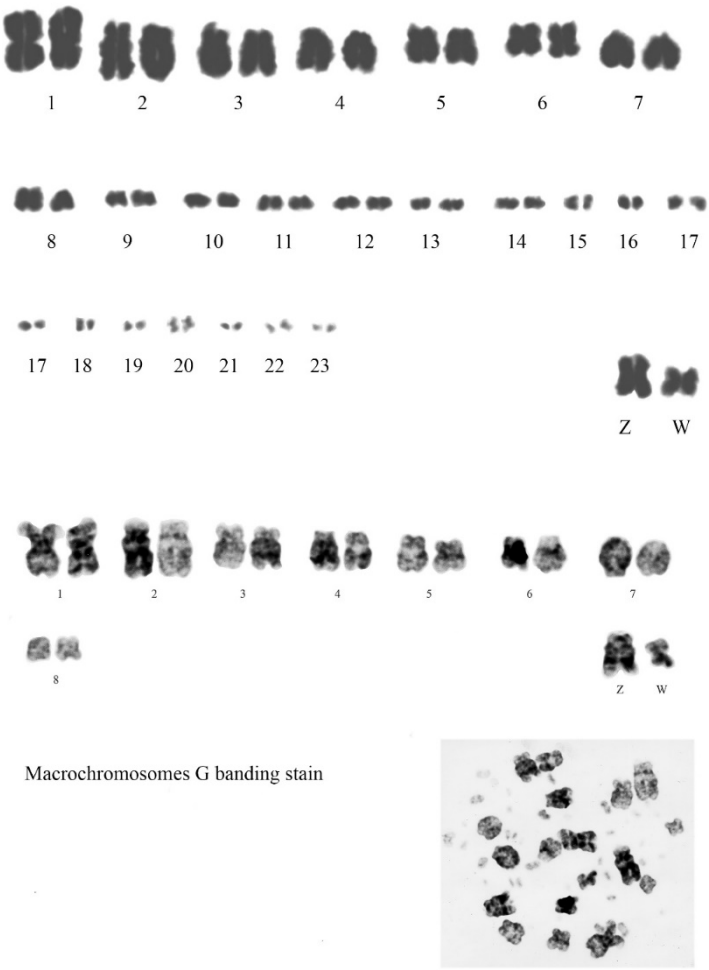

Figure 5. Proposed karyotype and G band stain of *Trichoglossus haematodus massena*

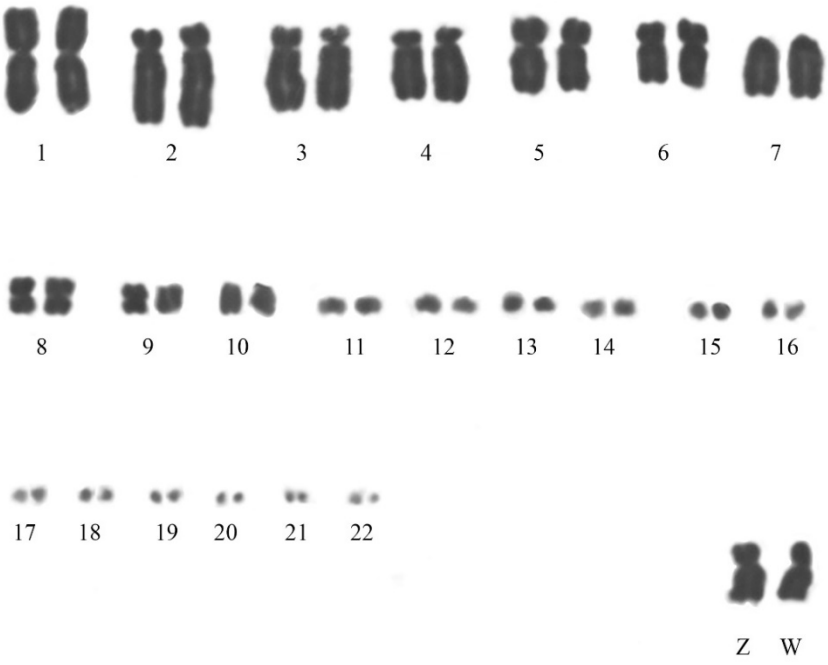

Figure 6. Proposed karyotype of *Trichoglossus flavoviridis*

Charmosyna josefinae.

The diploid chromosome number is about 64, with eight pairs of autosome macrochromosomes (Figure 7). G banding stain slides were also produced (Figure 8).

Pairs 1, 2 and 3 are acrocentric.

Pairs 4, 5 and 6 are metacentric to submetacentric.

Pairs 7 and 8 are telocentric.

The remaining autosomes are considered microchromosomes.

The Z and W chromosomes are metacentric and similar in size.

Tanygnathus megalorynchos and *Tanygnathus lucionensis talautensis*.

The karyotypes of *Tanygnathus megalorynchos* and *T. lucionensis talautensis* are very similar. We describe them together (Figures 9 and 10). G banding was performed on slides obtained from *Tanygnathus megalorynchos*.

The diploid chromosome number is about 56 to 62, with seven pairs of autosome macrochromosomes.

The first pair is metacentric.

Pairs 2, 3, 4, and 5 are acrocentric.

Pairs 6 and 7 are telocentric.

The remaining autosomes are microchromosomes.

The Z chromosome is submetacentric, and the W chromosome is a smaller submetacentric chromosome.

Prioniturus discurus.

The diploid chromosome number is about 60, with seven pairs of autosome macrochromosomes. G banding stain slides were also produced (Figure 11).

The first pair is metacentric.

Pairs 2, 3, 4, and 5 are acrocentric.

Pairs 6 and 7 are metacentric.

The remaining autosomes are considered microchromosomes.

The Z chromosome is submetacentric, and the W chromosome is a smaller submetacentric chromosome.

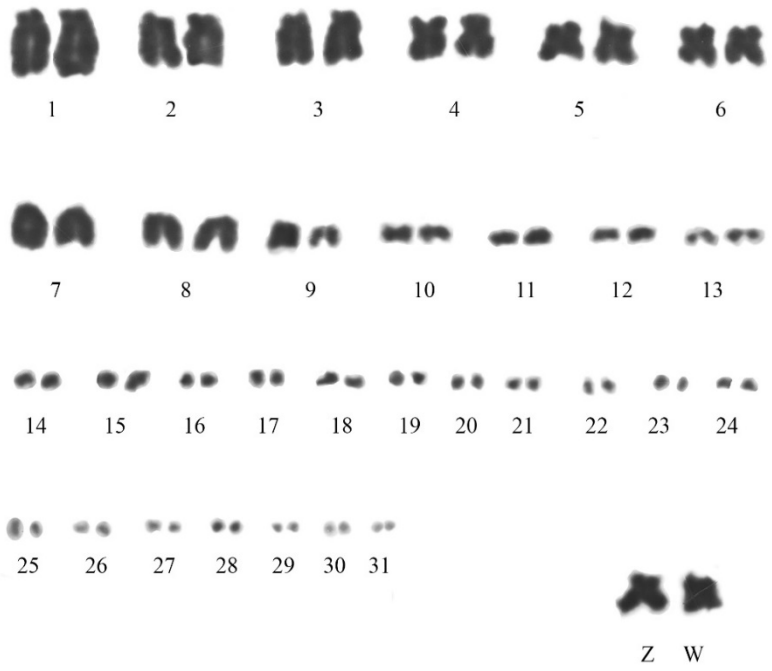

Figure 7. Proposed karyotype of *Charmosyna josefinae*

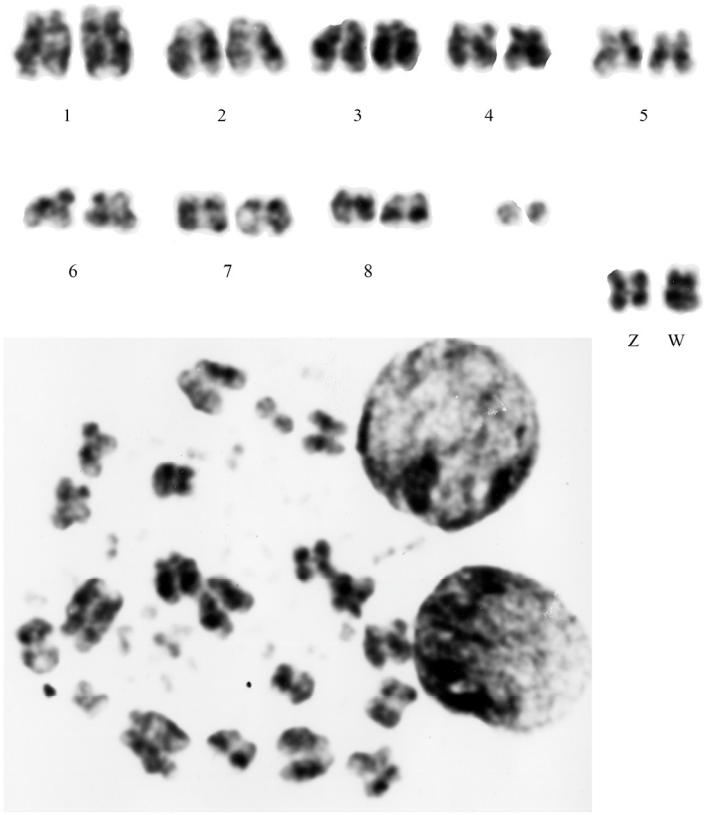

Figure 8. G banding stain karyotype of *Charmosyna josefinae*

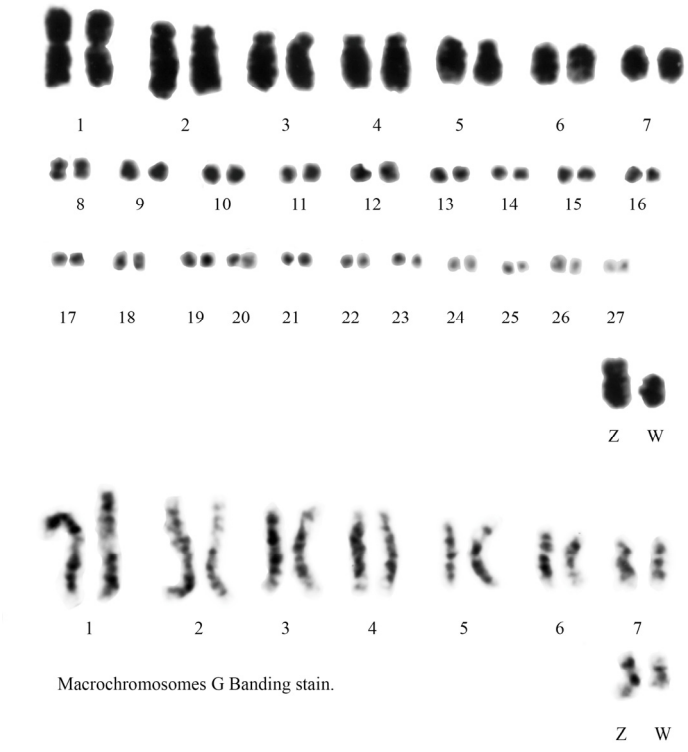

Figure 9. Proposed karyotype and G band stain of *Tanygnathus megalorynchos*

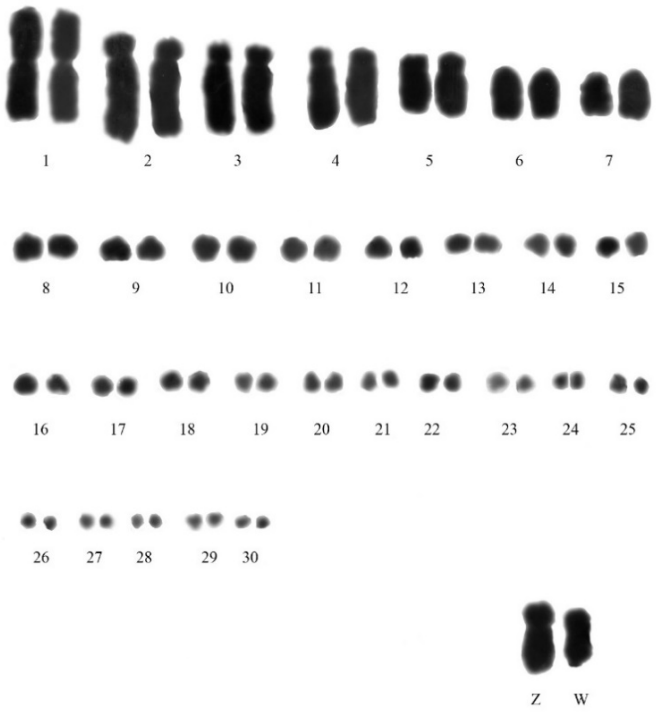

Figure 10. Proposed karyotype of Tanygnathus lucionensis talatuensis

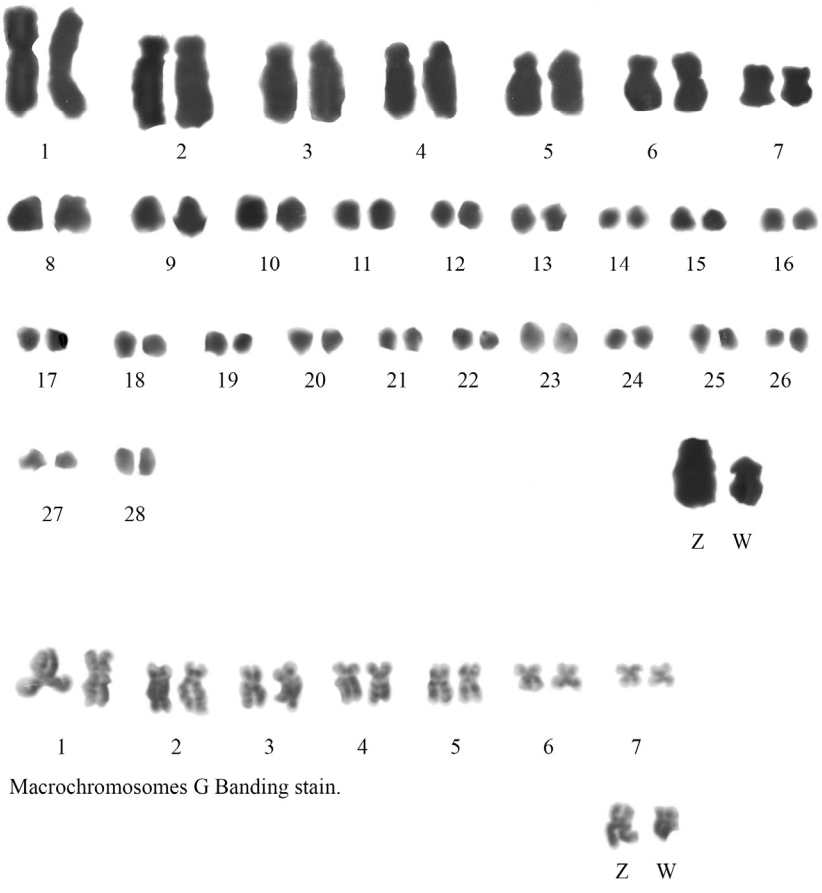

Figure 11. Proposed karyotype and G band stain of *Prioniturus discurus*

Poicephalus robustus.

Poicephalus robustus shows a diploid chromosome number of 60, with seven pairs of autosome macrochromosomes (Figure 12).

The first pair is composed of large metacentric chromosomes.

Pairs 2, 3, 4, 5, and 6 are considered acrocentric.

The seventh pair is telocentric.

The remaining autosomes are microchromosomes.

The sex pair is composed of two metacentric chromosomes, Z being larger than W.

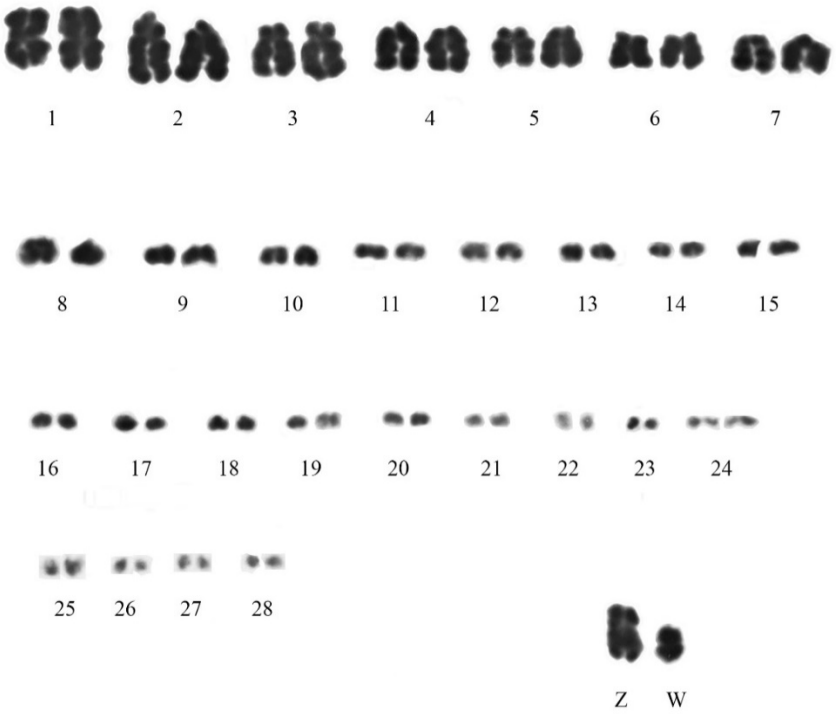

Figure 12. Proposed karyotype of *Poicephalus robustus*

Aratinga finschi.

The diploid chromosome number of *Aratinga finschi* is estimated to be 50. Seven pairs of autosome macrochromosomes are recognized (Figure 13).

The first pair consists of large metacentric chromosomes.

Pairs 2, 3, 4, 5, 6 and 7 are acrocentric.

The remaining autosomes are microchromosomes. Pair 8 is a large metacentric microchromosome.

The Z chromosome is submetacentric. The W chromosome is an acrocentric chromosome.

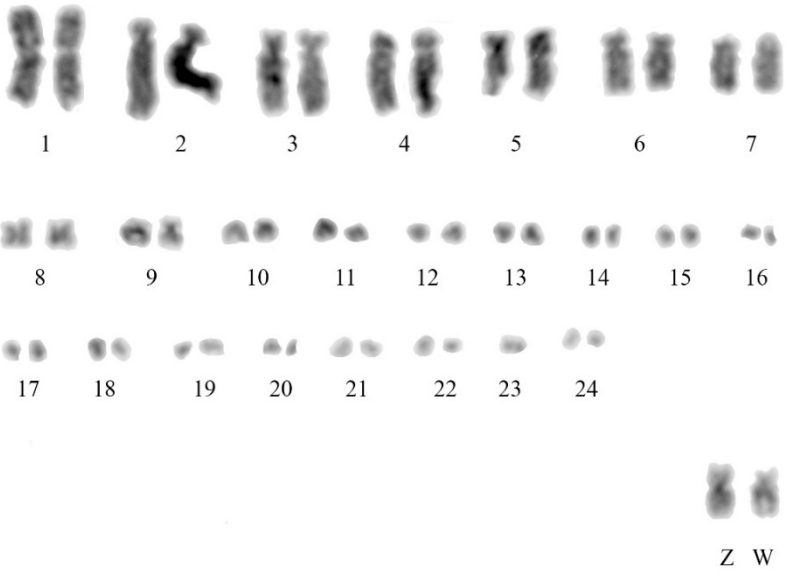

Figure 13. Proposed karyotype of *Aratinga finschi*

Triclaria malachitacea.

The diploid chromosome number of *Triclaria malachitacea* is estimated to be 54, of which seven pairs of autosome macrochromosomes are recognized (Figure 14).

The first pair is composed of large metacentric chromosomes.

Pairs 2, 3, 4, 5, and 6 are acrocentric.

The seventh pair is metacentric.

The remaining identifiable autosomes are microchromosomes, the larger are metacentric chromosomes.

The Z chromosome is metacentric. The W chromosome is acrocentric.

Graydidascalus brachyurus.

This karyotype has been previously described by Caparroz and Duarte, (2005). Our findings are in agreement with its description. However, we will interpret as eight, the number of macrochromosomes for comparisons (Figure 15).

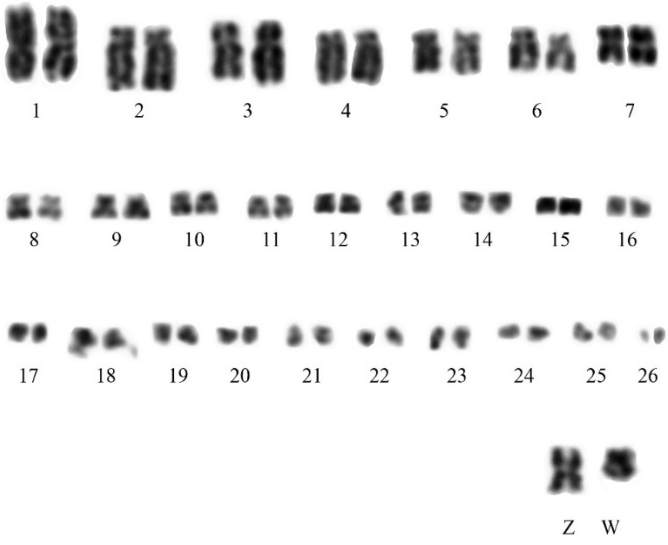

Figure 14. Proposed karyotype of *Triclaria malachitacea*

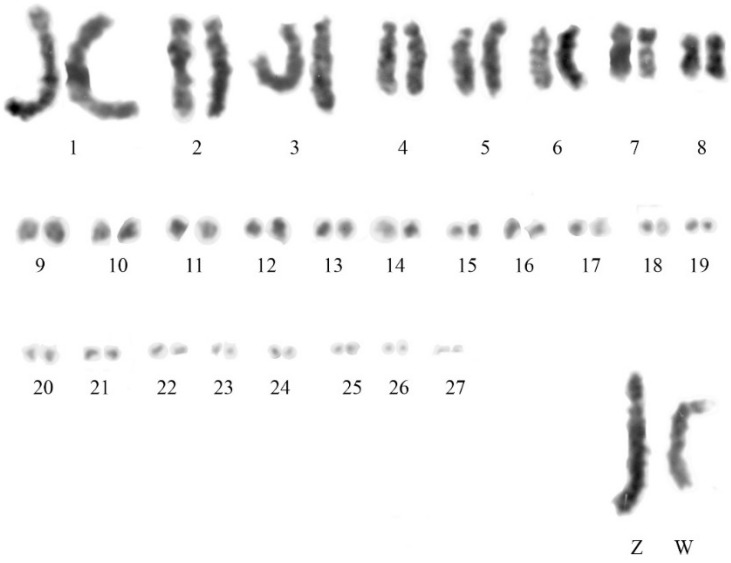

Figure 15. Proposed karyotype of *Graydidascalus brachyurus*

4. Discussion.

4.1 Avian Cytogenetic Considerations

The chromosomes of birds, some primitive amphibians and most reptiles are characterized by great variation in size. It is common to speak about macro- and microchromosomes, although there is not always a sharp borderline between the two groups, and intermediate size chromosomes or large microchromosomes may also be considered (Bloom, Delany, & Muscarella, 1993).

Cytogenetic data suggest that the organization of avian genomes remains highly conserved in evolution, as karyotype patterns within the majority of bird species are broadly similar to one another (Christidis, 1990; Skinner & Griffin, 2012; Tegelström et al., 1983; Tegelström & Ryttman, 1981). Moreover an avian standard karyotype has been defined (Christidis, 1990; Tegelström & Ryttman, 1981). This standard bird karyotype has a diploid number of 80, with 16 macrochromosomes and 64 microchromosomes.

Present day availability of some avian genome sequences, or techniques based on chromosome painting with DNA probes have improved our ability to reconstruct chromosome evolutionary changes (de Oliveira Furo et al., 2015; Nanda, Karl, Griffin, Schartl, & Schmid, 2007; Seibold-Torres et al., 2016). Interchromosome evolutionary changes have been considered to occur rarely during avian diversification suggesting that mechanisms exist to preserve a static overall karyotype structure (Romanov et al., 2014). Despite interchromosome conservation, intrachromosome changes are considered common (Nanda et al., 2007; Skinner & Griffin, 2012). A correlation between rates of speciation, and the observed number of intrachromosome rearrangements has been suggested, as a faster rate of change has been observed in the most diverging orders Passeriformes and Psittaciformes (de Oliveira Furo et al., 2015; Nanda et al., 2007; Romanov et al., 2014; Seibold-Torres et al., 2016; Skinner & Griffin, 2012). It has also been suggested that the presence of syntenic blocks of genome, associated with evolutionary breakpoint hotspot regions, may explain most events in the avian chromosomal evolution (Larkin et al., 2009; Skinner & Griffin, 2012).

Whole chromosomes are, in fact, large syntenic blocks, and comparative cytogenetic descriptions are yet crucial for understanding the role of chromosome rearrangements in evolution. Technical resolution of cytogenetics is smaller than genome sequence analysis, or chromosome painting techniques. However recurrent karyological observations of chromosome rearrangements on preferential sites lead to conclusions which may be considered analogous to the above mentioned hypotheses regarding conservation of large syntenic blocks of genome, and the existence of preferential evolutionary breakpoint regions (Christidis, 1990; Skinner & Griffin, 2012; Takagi & Sasaki, 1974; Tegelström & Ryttman, 1981).

Regarding to chromosomal evolution among Psittaciformes, the cross examination of the available cytological data indicates a considerable karyotype variability within the order. The diploid number of parrot karyotypes ranges from 2n = 46, found in *Agapornis roseicollis* (Christidis, Shaw, et al., 1991) and *Trichoglossus flavoviridis*

(This report), to a number close to ninety; 2n = 86 in *Forpus xanthopterygius* (De Lucca & De Marco, 1983), 2n = 92 in *Callocephalon fimbriatum* (This report). As previously considered in avian karyotypes (Tegelström & Ryttman, 1981), parrot karyotypes with a high diploid number have a large number of telocentric chromosomes, whereas parrot karyotypes characterized by a smaller diploid number tend to show more biarmed macrochromosomes. In this second case, , a tendency also seems to prevail that there is a presence of metacentric large microchromosomes, which are difficult to classify consistently as macro- or microchromosomes. These observations apparently suggest that repeated events of fission may have been prevalent in the chromosomal evolution of "telocentric" parrot karyotypes, whereas fusions may have been dominant in the case of the "biarmed" karyotypes (Burt, 2002; Christidis, Shaw, et al., 1991; Tegelström & Ryttman, 1981). However, we also suggest in this report, that some chromosomal changes in parrots, can also be explained, as a consequence of pericentric inversions or arm deletion-addition events (See below).

Between these extreme karyograms, where chromosome changes seem to precipitate in one direction or another, there is a dominant assembly of parrot karyotypes defined by intermediate karyological traits, which are apparently conserved in the chromosomal evolution of Psittaciformes. Moreover we may be able to discriminate in this assembly of intermediate parrot karyotypes some karyotype patterns which are, usually, correlated to biogeographic and phylogenetic congruent assemblies.

In the same way a standard avian karyotype has been statistically determined (Tegelström & Ryttman, 1981), the existence of some common karyological features in the dominant assembly of intermediate parrot karyotypes may lead us to the proposal of a standard parrot karyotype pattern to be used as reference in the discussion on Psittaciformes chromosomal evolution.

Ideally, this standard parrot karyotype pattern, should be a close relative to the ancestral karyotype, from which, present day parrot karyotypes have been derived. Sometimes it may be suggested (Joshua, 1994), that the most frequent karyogram pattern shown by the species of a taxonomic group, can be the ancestral form, where most chromosomal features are conserved. However the accuracy of this assumption is not always granted, as a frequent karyotype could be the reflection of a successful rearrangement, experiencing subsequent and generous diversification, and furthermore, the direction of the chromosome changes cannot be always accurately determined.

Some specific karyotypes have been previously considered as an ancestral parrot karyotype by, at least, two independent researchers, and in different biogeographic contexts; *Psittacula krameri* and *P. alexandri* karyotypes in Old World parrots (Christidis, Shaw, et al., 1991), and similarly the macaw-conure *typical* karyotype in Neotropical parrots (Valentine, 1990). Both karyotypes share common features, and can be considered as derived from each other (Christidis, Shaw, et al., 1991).

Our proposal of a standard parrot karyotype pattern is a flexible one which integrates the two previous approaches to an ancestral parrot karyotype (Christidis, Shaw, et al., 1991; Valentine, 1990), but considers a certain degree of variation. In spite of this flexibility, the standard parrot karyotype pattern proposal assumes that most chromosome features in parrot karyotypes seem to be conservative.

This proposed standard parrot karyotype pattern is characterized by the following features:

- A diploid chromosome number of 60 (± 10).
- Seven pairs of autosome macrochromosomes.
- There is a big metacentric pair, which is usually the largest autosome pair.
- Second, third and fourth pairs are acrocentric.
- Pairs 5, 6 and 7 are more variable (metacentric, acrocentric to telocentric). This variation can be explained by microchromosome centric fusion, centric fission, or pericentric inversion events affecting these chromosomes.
- A metacentric to submetacentric Z chromosome, well conserved in Psittaciformes, with some noticeable exceptions.

4.2 Biogeographic and Evolutionary Considerations

Parrots, as several other biological groups, e.g., the also forest dweller and no migratory avian order Trogoniformes (Moyle, 2005), show a tropical and geographically discontinuous, range of distribution.

This biogeographic distribution pattern is often attributed to Gondwanaland split vicariance, a process through which the geographic range of species, or biological communities was split in discontinuous parts during the fragmentation of the Gondwana continent, subsequently leading to allopatric speciation events (Cracraft, 1973, 2001).

The question of when modern birds (Neornithes) first diversified has generated much debate among avian systematists. Fossil evidence generally supports a Cenozoic diversification; the Tertiary radiation hypothesis (Feduccia & Martin, 1976; Feduccia, 1995, 2003; Olson, 1985), whereas the Gondwanaland origin hypothesis, or pre K-T radiation hypothesis, mainly supported by molecular data, favors an earlier diversification in the Cretaceous period (Brown & Toft, 1999; Cracraft, 1973, 2001; Pacheco et al., 2011; Sibley & Ahlquist, 1990; Slack et al., 2006).

Today, even if the fossil record has not yet supported this hypothesis (54 MY old, early Eocene *Mopsitta tanta* represents the oldest parrot fossil found to date (Waterhouse, Lindow, Zelenkov, & Dyke, 2008)), phylogenetic analysis and molecular clock estimates, confronted to the paleogeographical events, indicate that parrots have originated in Gondwanaland during the Cretaceous, and it is generally agreed that most of the diversification of the group took place in Cenozoic Palaeogene times (Ericson, 2012; Schweizer, Seehausen, Güntert, & Hertwig, 2010; Wright et al., 2008). This conclusion is in general agreement with a hypothesis on the vicariant origin of parrots mediated by the breakup of Gondwanaland (Cracraft, 1973, 2001; Schweizer et al., 2010; Wright et al., 2008).

However, there are some difficulties in establishing the temporal and causal relationships between geological and biological events (Wright et al., 2008), and vicariance alone cannot explain the distribution patterns of several parrot groups, neither the divergence time estimates among taxa (Kundu, Jones, Prys-Jones, & Groombridge, 2012; Schweizer et al., 2010).

Molecular genomic results seem to indicate an Eocene divergence of Psittaciformes in three basal lineages (Pacheco et al., 2011; Rheindt et al., 2014; Schweizer et al., 2010, 2011; Tavares, Baker, Pereira, & Miyaki, 2006; Wright et al., 2008), which correspond to the most recent taxonomical classification of the order in three families; Strigopidae, Cacatuidae and Psittacidae (Winkler et al., 2015).

Early divergence of New Zealand Strigopidae parrots: kea (*Nestor notabilis*), kaka (*Nestor meridionalis*) (Tribe Nestorini) and kakapo (*Strigops habroptilus*) (Tribe Strigopini), has been (not unanimously) correlated to the Cretaceous separation of New Zealand from Gondwana 82-85 MYA (Pacheco et al., 2011; Rheindt et al., 2014; Schweizer et al., 2010, 2011; Tavares et al., 2006; Wright et al., 2008), and it was cautiously assumed to be caused by vicariance (de Kloet & de Kloet, 2005; Wright et al., 2008), although alternative scenarios are contemplated (Pacheco et al., 2011; Rheindt et al., 2014; Schweizer et al., 2011; White et al., 2011).

Australian family Cacatuidae is the next group to diverge from the main group of Psittaciformes, apparently in sympatric coexistence with other parrot lineages (Astuti, Azuma, Suzuki, & Higashi, 2006; Mayr, 2010; Schweizer et al., 2011; White et al., 2011; Wright et al., 2008). Their basal diversification during the Oligocene, and ulterior radiation in early to middle Miocene has been well documented (Pacheco et al., 2011; Rheindt et al., 2014; Schweizer et al., 2011; White et al., 2011).

Diversification of the large family Psittacidae has been affected by climatic and geological events experienced by the land masses that resulted from the Gondwanaland split, as well as by key innovations in some groups like Lories (Kundu et al., 2012; Schweizer et al., 2010). Vicariance has been considered the original diversification force in this family of, currently, mostly non migratory birds, but a complementary and ulterior mechanism of transoceanic dispersion (helped or mediated by island bridges), followed by local radiations (Kundu et al., 2012; Schweizer et al., 2010), has been consistently proposed, accordingly to the tectonic, geographic, climatic, and biological events in the Indo Pacific oceanic region during Palaeogene (Ali & Aitchison, 2008; F. H. Glenny, 1954; Hall, 2002; Kundu et al., 2012; Li & Powell, 2001; Moss & Wilson, 1998; Schweizer et al., 2010).

We discuss these complex events affecting the diversification and the cytogenetic evolution of the Psittacidae, in a regional analysis.

4.2.1 Australian Species

Australia conformed an important portion of the Gondwanaland continent where parrots originated and diversified initially (Collar, 1997; Rowley, 1997). Today Australia harbours a large diversity of parrots, and the tectonic, climatic and biological events of East Gondwana, comprising India, East Antarctica, Australia, Madagascar, the Seychelles, and other microcontinental blocks, during Palaeogene, have been influential in the early diversification of Psittaciformes, as some of the oldest phylogenetically recognized lineages (Nestorini-Strigopini, Cacatuidae) arose in this biogeographic region. (Gibbons, Whittaker, & Müller, 2013; Kundu et al., 2012; Li & Powell, 2001; Rheindt et al., 2014; Ribas, Moyle, Miyaki, & Cracraft, 2007; Schweizer et al., 2011). Moreover, the ulterior northward migration of the Australian plate, together with climatic changes, seems to have been influential in the Miocene onwards diversification of parrots, and has contributed to shape the present day parrot diversity and distribution in the Australasian Pacific area (Schweizer et al., 2010; White et al., 2011; Wright et al., 2008).

Regarding chromosomal diversity, we consider in Australian parrots several karyogram patterns corresponding to Platycercini parakeets, Cacatuidae, Loriinae and Australian Psittaculini (See Indo-Malayan species).

4.2.1.1 Platycercini Tribe (Fam.: Psittacidae).

Members of the Platycercini tribe are familiarly known as *rosellas*. They are a diversified parrot group occupying many Australian habitats, from temperate forest to semiarid woodlands (Collar, 1997).

Molecular phylogenetic studies on Platycercini suggest the existence of a well-defined assembly of genera integrating a "Platycercini core" (*Platycercus, Northiella, Barnardius, Eunymphicus, Psephotus, Purpureicephalus, Cyanoramphus, Prosopeia, Lathamus*) with close relatives (*Pezoporus, Neophema, Neopsephotus*) (Joseph et al., 2012), well differentiated from Australian Psittaculini (*Alisterus. Aprosmictus, Polytelys*), where *Melopsittacus undulatus* is excluded, and considered phylogenetically relative to Loriinae species (Joseph, Toon, Schirtzinger, & Wright, 2011; Schweizer et al., 2011). The same studies suggest that diversification of this group took place at relatively recent times, influenced by the aridification of the Australian continent beginning in the middle Miocene.

Regarding the karyological data available, a great similarity has been observed within the karyotypes of the Platycercini Australian parakeet species studied to date (Christidis, Shaw, et al., 1991; Christidis, 1990; Joshua, Unpublished, 1994; This Report). It seems that this Australian parakeet karyotype pattern is shared with other Australian (*Platycercus, Purpureicephalus, Barnardius, Eunymphicus, Psephotus*) and New Zealand (*Cyanoramphus, Prosopeia, Lathamus*) Platycercini genus (Joshua, 1994), which consequently can be considered as a chromosomally defined and uniform group. These results suggest that genic mechanisms of evolution, or intrachromosome rearrangements, rather than interchromosome changes have played a major role in Platycercini diversification.

The Platycercini karyotype pattern does not belong to the standard parrot karyotype pattern we have proposed: this Platycercini karyotype has two pairs of large metacentric autosomes conforming pairs 1 and 2, and the number of autosome macrochromosomes is six. It has been suggested (Christidis, Shaw, et al., 1991) that this karyotype pattern is derived from an ancestral Old World karyotype similar to the Asiatic *Tanygnathus, Eclectus, Psittacula krameri* and *P. alexandri* karyotypes through an event of robertsonian fusion between telocentric autosome pairs 6 and 7. A similar explanation has been suggested to interpret the karyotype of *Psittacula cyanocephala* (Ray Chaudhuri et al., 1968) which is rather similar to the Australian Platycercini karyotypes.

Despite this karyotype resemblance between Platycercini and *Psittacula cyanocephala* (shared also with the Neotropical species *Forpus xanthopterygius* (De Lucca & De Marco, 1983; De Lucca et al., 1991)), an hypothetical chromosome evolutionary relationship between Platycercini and Psittaculini would not agree with the current molecular phylogenetic results: A major clade of Old World parrots which includes Platycercini, Loriinae, *Agapornis ssp.* and Psittaculini has been defined (Winkler et al., 2015), however similar studies, also indicate a closer relationship of Platycercini to a monophyletic assemblage of Melopsittacus, Loriinae, and Cyclopsittacini, rather than with Psittaculini (Joseph et al., 2011; Schweizer, Güntert, & Hertwig, 2013).

Assuming that a chromosome mutational change has occurred between Psittaculini and Platycercini, and that this change has occurred as proposed here (fusion event), the Platycercini karyotype should have been derived from a standard parrot karyotype ancestral to this monophyletic group, or alternatively from a more ancient ancestor shared also with Psittaculini.

4.2.1.2 *Callocephalon fimbriatum* and the Cockatoo Family

Cockatoos are native to the Australian region, and identified by the presence of movable head crest, among other characteristics.

This family diverged from other parrots during the Eocene, and diversified initially in three lineages over the Oligocene (White et al., 2011). This lineage divergence corresponds with the actual taxonomic arrangement of the family in three subfamilies; Nymphicinae, Calyptorhynchinae and Cacatuinae (Collar, 1997). Ulterior diversification of the *Cacatua* genus occurred throughout the early Miocene to Pliocene, a period of parallel evolution of modern Australian environments, habitats and biotas (Pacheco et al., 2011; Rheindt et al., 2014; Schweizer et al., 2011; White et al., 2011), which also affected Platycercini radiation.

Several cockatoo karyotypes have been previously studied (Christidis, Shaw, et al., 1991; Joshua & Parker, 1993; Schmutz & Prus, 1987; Van Dongen & De Boer, 1984). This work, extends the chromosome data available on 6 genera and 11 species, (Joshua & Parker, 1993; Joshua, Unpublished, 1994) with a description of the karyotype of the gang gang cockatoo (*Callocephalon fimbriatum*).

Chromosomal evolution in cockatoos has been explained in terms of a succession of centromere fusions between micro- and macrochromosomes starting off from an hypothesized ancestral cockatoo karyotype, where telocentric chromosomes predominate (Christidis, Shaw, et al., 1991). This telocentric karyotype pattern is recognizable in karyotypes of many living cockatoo species (e.g. *Cacatua* and *Calyptorhynchus* genera) (Christidis, Shaw, et al., 1991; Schmutz & Prus, 1987; Van Dongen & De Boer, 1984).

We suggest a different explanation. *Nymphicus hollandicus*, which has been previously considered a Polyteline or Platycercini parakeet (Adams, Baverstock, Saunders, Schodde, & Smith, 1984; Condon, 1975) is the sole member of a monotypic lineage at the base of the cockatoo radiation (White et al., 2011). We consider the karyotype of *Nymphicus hollandicus* (Christidis, Shaw, et al., 1991; Joshua & Parker, 1993) as a variation of the standard parrot karyotype pattern that we have previously defined (the largest chromosome pairs are a metacentric and three acrocentric ones, whereas the telocentric chromosome pair 5 differs from Indo Malayan Psittaculini typical karyotypes). Moreover, its karyotype can be considered akin to the karyotype of *Nestor notabilis* (De Boer & Belterman, 1980), from the lineage Strigopidae, as they share a very similar chromosome complement.

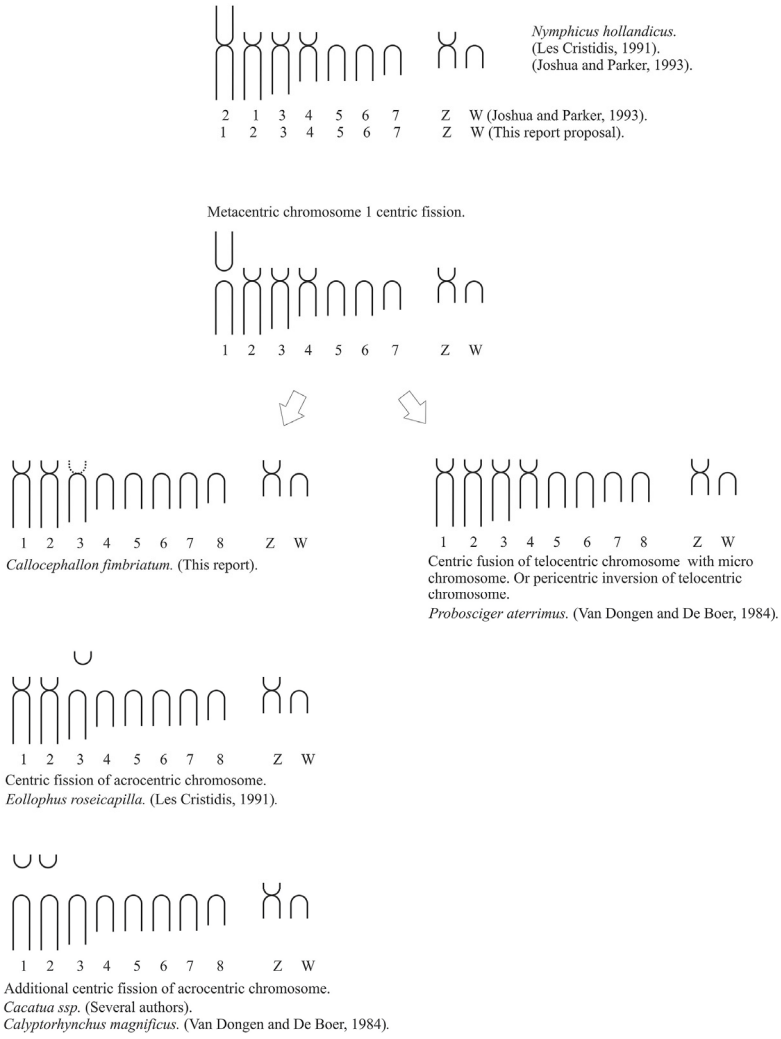

Figure 16. Scheme on suggested chromosome evolutionary events in Cacatuidae Successive events of centric fissions in an ancestral cockatoo karyotype similar to *Nymphicus hollandicus* karyotype lead to the typical telocentric cockatoo karyotypes, and also, to intermediate Cacatuidae karyotypes.

We consider that the original event in chromosome cockatoo evolution was a centric fission of the metacentric large autosome pair 1, in a founder cockatoo species with an ancestral karyotype similar to the present day *Nymphicus hollandicus* karyotype, resulting in a typical cockatoo karyotype composed of eight pairs of macrochromosome autosomes. This hypothetical basal member of the cockatoo family could also have been very close to the base of the Psittaciformes order radiation too.

The karyotypes of *Probosciger aterrimus* (Van Dongen & De Boer, 1984), *Callocephalon fimbriatum* (This report) and *Eolophus roseicapillus* (Christidis, Shaw, et al., 1991), have eight large autosome pairs, without any large metacentric. They, still have some acrocentric autosomes. Successive events of biarmed chromosome fission affecting these acrocentric may account for the existence of the typical telocentric chromosome arrangement of *Cacatua sp*. (Christidis, Shaw, et al., 1991; Joshua & Parker, 1993; Schmutz & Prus, 1987) and *Calyptorhynchus magnificus (C. banksii)* (Van Dongen & De Boer, 1984) karyotypes.

Phylogenetic studies on *Callocephalon fimbriatum* and *Eolophus roseicapillus* have identified this two species as sister taxa within the Cacatuinae (White et al., 2011), in an intermediate position between *Nymphicus hollandicus* and the remainder cockatoos. The cytogenetic results presented here seem to support this close relationship and transitional evolutionary position.

The chromosome heterozygosity shown by this particular female also can be interpreted as if the intermediate karyotype arrangements in cockatoos were a transitory chromosome organizations (De Lucca & De Marco, 1983). Unfortunately a single female Gang gang cockatoo was available for the present cytogenetic studies and further work on this species is necessary.

4.2.1.3 Subfamily Loriinae.

Lories, with 53 species (Collar, 1997), are a very diverse group of parrots. It has been suggested that they arose in Papua New Guinea in the middle Miocene (at relatively recent times) (Joshua, 1994; Schweizer et al., 2011; Schweizer, Wright, Peñalba, Schirtzinger, & Joseph, 2015) and ulteriorly dispersed colonizing many land territories of the Indo Australasian Pacific Ocean.

The position of the continents has experienced little variation, during the diversification period of the subfamily (Moss & Wilson, 1998). In this case, the suggested trigger event leading to the group radiation, are not tectonic episodes, but the morphological innovations that drive them to the nectarivorous diet (brush-tip tongue, modified gizzard musculature, etc.) (Collar, 1997; Forshaw & Cooper, 1989). "Nectar may have been, in this part of the world, a spatially widespread, and underutilized niche which would have allowed Lories to disperse, and successfully establish populations on oceanic islands, which was then followed by allopatric speciation, and eventually secondary sympatry through range expansion" (Schweizer et al., 2011, 2015).

Some Loriinae karyotypes have been studied: *Trichoglossus haematodus* and, *Lorius hypoinochrous* (Christidis, Shaw, et al., 1991); *Lorius hypoinochrous, L. lory, Phighys solitarius* (De Lucca et al., 1991). This report studies *T. h. massena*, *T. flavoviridis* and *Charmosyna josefinae*. Loriinae has been, also studied, quite exhaustively by Joshua (1994, and Unpublished data).

Accordingly to the lectures of this author, most of the genera share a similar karyotype. *Eos, Chalcopsitta, Lorius, Trichoglossus, Neopsittacus* and *Glossopsitta* have karyotypes which can be considered derived among them, whereas *Pseudeos, Vini* and *Charmosyna* have different karyotype patterns (Joshua, 1994).

Karyotypes of *Trichoglossus haematodus* (no subspecies specified) and *Lorius hypoinochrous* (Christidis, Shaw, et al., 1991); *Lorius hypoinochrous, L. lory* and *Phighys solitarius* (De Lucca et al., 1991) are very similar. Christidis (1991) considered the karyotype of *T. haematodus* and *Lorius hypoinochrous* as closely related to the ancestral Old World karyotype represented by *Psittacula krameri* and *P. alexandri* karyotypes, and explains the karyological differences as consequence of robertsonian translocations or pericentric inversions.

The karyotypes of the *Trichoglossus* taxa described in this report differ from the previously studied *Trichoglossus sp.* karyotypes on the shape of chromosome pair 7, which is telocentric in the taxa we studied.

Charmosyna josefinae karyotype shows some original features when compared to the previously described Loriinae karyotypes. It has been suggested that *Charmosyna, Vin*i, and *Pseudeos* genera integrate a genuine, and possibly heterogeneous, karyological group among Loriinae (Joshua, 1994). Recent molecular phylogenetic studies suggest that *Charmosyna* genus is not monophyletic (Schweizer et al., 2015).

Charmosyna josefinae karyotype could be considered as derived from the *Trichoglossus- Lorius* type karyotype through the fission of metacentric chromosome 1, resulting in two smaller telocentric chromosome pairs 7 and 8.

As there are species of *Charmosyna* yet to be studied, and some unpublished data, this hypothesis has to be additionally addressed.

Molecular genetic studies consider Loriinae species relative to *Melopsittacus undulatus* (Joseph et al., 2012; Schweizer et al., 2013; Wright et al., 2008). Relationships among *Melopsittacus* (Platycercini), Loriinae, Cyclopsittacini, Micropsittini, *Agapornis* and *Loriculus* (Psittaculini), have also been proposed based on morphological (Mayr, 2008), and biochemical (Christidis, Schodde, Shaw, & Maynes, 1991) evidences. However,

Melopsittacus genus has been assigned to Platycercini by most authors (Collar, 1997; Forshaw & Cooper, 1989; Smith, 1975).

The karyotype of *Melopsittacus undulatus* (Rothfels et al., 1963; Van Brink, 1959; Van Dongen & De Boer, 1984) shares common features with the karyotype of Loriinae *Trichoglossus haematodus massena*, and *Trichoglossus flavoviridis* presented in this report. In both cases, the seven pairs of autosome macrochromosomes, are very much alike in shape and size and, they are followed by large biarmed microchromosomes.

Agapornis roseicollis karyotype (Christidis, Shaw, et al., 1991) has the lowest diploid number found in Psittaciformes, and an unusual high number of macrochromosomes (ten autosome pairs), suggesting that several fusion events have conformed this karyogram (Nanda et al., 2007). It is difficult to propose karyological relationships between this species and other parrots. On the other hand, *Loriculus vernalis* karyotype (Ray Chaudhuri et al., 1968) has the usual number of seven macro- autosomes, but in this case, telocentric chromosomes predominate. Both karyotypes are apparently unrelated. *Psittaculirostris* species (Cyclopsittacini) have been studied by Joshua (Unpublished). No *Micropsitta* species karyotypes were available, to our knowledge, for comparison. *Micropsitta* genus has been recently related to Psittaculini (Winkler et al., 2015).

4.2.2 Psittaculini Tribe. Indo-Malayan and Australian Species

Most parrots from the Indo Malayan biogeographic region are integrated into the tribe Psittaculini. Psittaculini species are also found in the Australian region. Biogeographic data and molecular clock estimates (Schweizer et al., 2013, 2011), indicate that the ancestors of Indo-Malayan Psittaculini parrots (*Psittacula, Tanygnathus, Prioniturus, Psittinus, Loriculus*), colonized Indo Malaysia from Australia during Oligocene Miocene times, after the Australian continent approached Asia from the south (Hall, 2002).

The Australian species (*Aprosmictus, Alisterus* and *Polytelis* genus), recently assembled into his own taxonomical tribe Polytelini (Winkler et al., 2015) are now considered a monophyletic group (Joseph et al., 2011; Schweizer, Güntert, & Hertwig, 2012). A sister relationship between them and a monophyletic assemblage of Indo Malayan Psittaculini species (*Psittacula, Tanygnathus, Eclectus, Prioniturus, Psittinus, Geoffroyus*) has been suggested (Joseph et al., 2011).

The cytogenetic studies performed, so far in Psittaculini parrots show certain heterogeneity, and the evolutionary relationships within their karyotypes are, sometimes, difficult to determine.

A first Psittaculini karyogram pattern may be recognized in several genera of Indo Malayan Psittaculini species. The karyotypes of the *Tanygnathus* species described in this report are very similar to *Psittacula krameri, Psittacula alexandri* (Ray Chaudhuri et al., 1968) and *Eclectus roratus* (De Lucca et al., 1991) karyotypes. This Indo Malayan Psittaculini karyotype pattern has been previously considered as the ancestral Old World parrot karyotype (Christidis, Shaw, et al., 1991).

As previously exposed in the Australian regional analysis, *Psittacula cyanocephala* (Ray Chaudhuri et al., 1968) has a different karyotype which is very similar to the Australian Platycercini karyotype pattern. This is also the case in the Neotropical species *Forpus xhantopterygius*. These taxa are not usually considered related among them (Joseph et al., 2011; Schweizer et al., 2011). This karyological resemblance seems to point to a similar, and independent chromosomal event of fusion between macrochromosomes (Christidis, Shaw, et al., 1991), affecting a presumptively chromosome mutation prone region.

An additional karyotype is recognizable in *Prioniturus discurus*. *Prioniturus* is a genus characterized by an elongated shaft of central tail feathers with spatulate tips (racket tail). *Prioniturus* genus has been recently undergoing events of insular dispersion and allopatric diversification in the Wallacean, Sundaland and Phillipine biogeographic regions (Schweizer et al., 2012).

Prioniturus discurus shows a standard parrot karyogram consisting on seven macrochromosomes, but differs from the Indo Malayan Psittaculini karyotype pattern in the shape of the chromosome pairs 6 and 7 (metacentric instead of telocentric). This karyogram is very similar to *Psittrichas fulgidus* karyotype, and also, to the typical long tailed Neotropical karyotype pattern (See below).

Australian Psittaculini (Collar, 1997) or Polytelini parakeets (Winkler et al., 2015) have different karyotypes.

Alisterus scapularis karyotype (Christidis, Shaw, et al., 1991) has a large diploid number (76), and eight pairs of macrochromosomes. A first pair of metacentric and a second pair of acrocentric chromosomes are conserved with the standard parrot karyotype; metacentric pair 3 has an enigmatic origin; pairs 4, 5, 6 and 7 are metacentric to acrocentric and pair 8 is telocentric.

The karyotype of *Polytelis alexandrae* (De Lucca et al., 1991) is similar to the *A. scapularis* karyotype.

These karyotypes appear to represent a karyogram pattern characteristic of Polytelini (Joshua, Unpublished), but the karyological relations between Polytelini and Indo Malayan Psittaculini karyotypes, or other parrot karyotypes, are difficult to establish. The previously mentioned phylogenetic relation between Polytelini and Indo Malayan Psittaculini parakeets (Joseph et al., 2011) does not seem to be immediately supported by the cytogenetic results available today.

4.2.3 African Species. Tribe Psittacini

Genetic molecular analysis results indicate that African Psittacini, and Neotropical Arini are sister taxa (Kundu et al., 2012; Schweizer et al., 2010, 2011). The common ancestor of these biogeographically separated lineages lived in the Antarctica, and accordingly to the molecular clock estimates was separated from the Australian parrot lineages at about 40 MY, which corresponds to the time the two continents finally separated. (Ali & Aitchison, 2008; Kundu et al., 2012; Li & Powell, 2001; Schweizer et al., 2013, 2010, 2011). Ulterior phylogenetic separation and independent colonization of the Neotropics and Africa by parrots living in the Antarctica, began in the early Oligocene (35 MYA) (Schweizer et al., 2010, 2011; Tavares et al., 2006), as a result of climate change towards cooler conditions.

Poicephalus robustus, the African species studied here, has a karyotype very similar to other Psittacini African species such as *Psittacus erithacus* (De Boer & Belterman, 1980) and other *Poicephalus* species (De Lucca et al., 1991; Joshua, Unpublished), suggesting a close relationship.

It was proposed (Christidis, Schodde, et al., 1991) that the karyotype of *Psittacus erithacus,* was derived from the ancestral Old World karyotype (Indo Malayan Psittaculini karyotype pattern) by robertsonian fusion of a telocentric chromosome pair (6 or 7) with a microchromosome (or a pericentric inversion). An additional macrochromosome rearrangement of the remaining telocentric pair 7 separates this Psittacini karyotype type from the typical long tailed Neotropical parrot karyotype type (See below). All three karyotype patterns may be integrated in the standard parrot karyotype pattern, we have defined in this work.

A sister relationship between Arini and Psittacini gathered together with the New Guinea monospecific tribe Psittrichadini has been suggested (Winkler et al., 2015). Cytogenetic studies also show that, in spite of the geographic and taxonomic distance the *Psittrichas fulgidus* karyotype (Van Dongen & De Boer, 1984) is very similar to long tailed Arini species karyotypes (The also Indo Malayan parrot *Prioniturus discurus* (This report) shows a similar karyotype too).

It has been hypothesized that other African parrots have a different origin, and that *Agapornis* (Psittaculini), and the Psittacini genus *Coracopsis* living in Malagasy have independently colonized Madagascar and Africa through long distance dispersal across the Indian Ocean from Australasia (Kundu et al., 2012; Schweizer et al., 2010). *Agapornis roseicollis* karyotype (Christidis, Shaw, et al., 1991) seems unrelated to Psittacini typical karyotype. *Coracopsis* karyotype has been studied by Joshua (Joshua, 1994).

4.2.4 Neotropical Species

Mitochondrial DNA sequence analysis, (Miyaki et al., 1998), supported by other data, among them, morphology (Schodde, Remsen, Schirtzinger, Joseph, & Wright, 2013; Sick, 1993) indicates that there are two phylogenetic groups of Neotropical parrots: long tailed Neotropical parrots, which roughly includes macaws, conures and others, and short tailed Neotropical parrots which includes, at least, the genera *Amazona*, *Pionus* and *Graydidascalus*. The separation among this groups of Neotropical parrots occurred at the Oligocene-Miocene boundary (de Kloet & de Kloet, 2005; Miyaki et al., 1998; Ribas et al., 2007; Tavares, Yamashita, & Miyaki, 2004), a time of climate amelioration where forest habitats expanded in present day South America, leading to biotic changes, and the radiation and diversification of several forest dweller animals groups, like the Platyrrhini monkeys. (Flynn, Wyss, Charrier, & Swisher, 1995).

The cytological studies performed so far, have revealed several karyotype patterns in the New World parrots.

One karyotype pattern is prevalent in the so considered group of long tailed Neotropical parrots. This typical long tailed Neotropical karyogram, or *macaw conure karyotype,* is characterized by the presence of seven pairs of macrochromosomes; pair 1 is composed of large metacentric chromosomes; pairs 2, 3, 4, 5, and 6 are acrocentric, and pair 7 is metacentric. The metacentric Z and smaller acrocentric W are also similar in all genera.

The monotypic genus *Triclaria malachitacea* and *Aratinga finschi,* described in this report, are integrated in this karyological group, where we may include the genera; *Anodorhynchus* (Lunardi et al., 2003), *Ara* (Francisco & Galetti J., 2001; Van Dongen & De Boer, 1984), *Cyanopsitta* (Duarte & Giannoni, 1990), *Aratinga* (De Lucca, 1974, 1984; Goldschmidt et al., 1997; This report), *Guarouba* (Goldschmidt et al., 1997), *Nandayus*,(Francisco & Galetti J., 2001), *Propyrrhura* (Francisco & Galetti J., 2001), *Pionites* (Francisco, Lunardi, & Galetti Jr., 2001),

Pionopsitta (Francisco et al., 2001), *Deroptyus* (Lunardi et al., 2003), *Brotogeris* (De Lucca et al., 1991; De Lucca, 1985) and *Pyrrhura* (De Lucca et al., 1991; Valentine, 1987).

However some internal variation is observed in this otherwise karyological uniform group. The centromere position of macrochromosome pairs 6, 7, and (depending on the author ordination criteria) 5, may be variable, at least, in species of *Aratinga, Pyrrhura,* and *Brotogeris* (Christidis et al., 1991; De Lucca et al., 1991; De Lucca, 1984, 1985; Joshua, Unpublished, 1994; Valentine, 1987).

Another karyotype pattern occurs in the species of the genus *Amazona*. This pattern is characterized by the presence of 8 autosome macrochromosome pairs with telocentric pairs 1, 5, 6 and 7. (Aquino & Ferrari, 1990; De Boer & Belterman, 1980; Duarte & Caparroz, 1995; Schmutz & Prus, 1987; Valentine, 1990; Van Dongen & De Boer, 1984).

According to recreations of the possible chromosome mutational events, performed with image software (Photoshop CS®, Adobe) (See Figure 17), *Amazona* karyotypes derivate from the typical macaw-conure karyotype. Our suggestion is that the metacentric pair 1 of an ancestral parrot karyotype similar to the present day macaw-conures, experienced a pericentric inversion, at the times the *Amazona* lineage separated from other Neotropical parrots, leading to the apparition of a characteristic telocentric pair 1 in *Amazona* karyotypes. We consider that the chromosome pairs 2, 3 and 4 are conserved in both karyogram types, whereas chromosome pairs 5, 6 and 7 have evolved to telocentric chromosomes in *Amazona* genus typical karyotypes, through chromosome mutational events of centric fissions, or alternatively, pericentric inversions (No depicted). We also consider that there is not an increase in the number of macrochromosome autosomes in *Amazona* genus, but that the large metacentric microchromosome pair 8 found in the typical long tailed Neotropical karyogram has been considered as a macrochromosome by *Amazona* cytologists, but a microchromosome by *macaw-conure* cytologists.

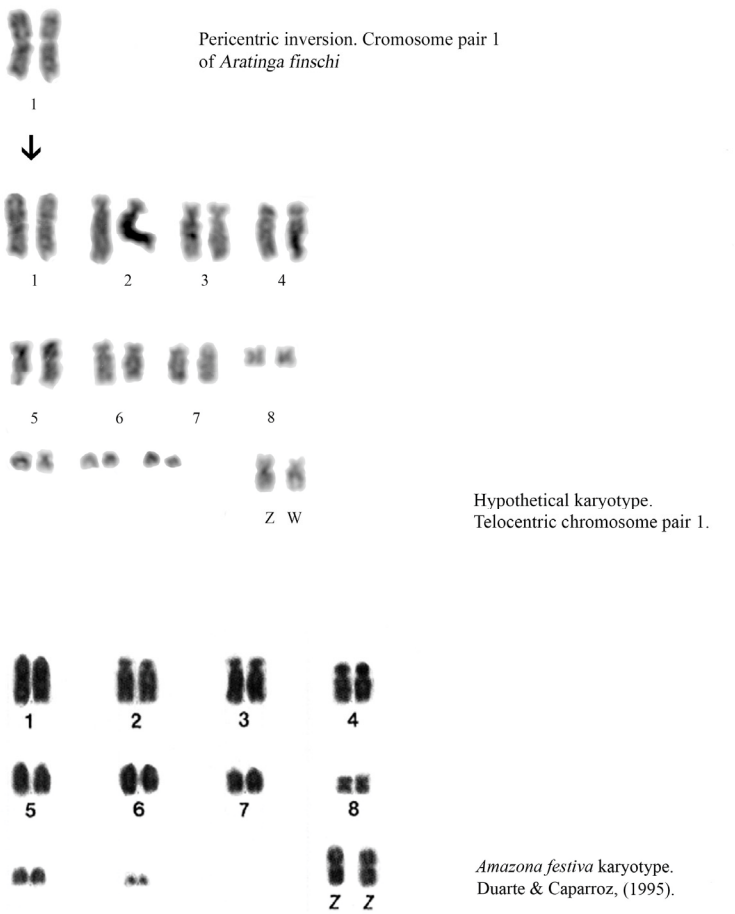

Figure 17. Proposed chromosome evolution in *Amazona* genus. Recreation of possible chromosome mutational events, performed with image software (Photoshop CS®, Adobe), suggest that a pericentric inversion of metacentric chromosome pair 1 of a macaw-conure karyotype may lead to the typical *Amazona* genus karyotypes

A further karyotype pattern is observed in the species of the genus *Pionus* (Caparroz & Duarte, 2004; De Lucca et al., 1991), and also in *Amazona* (*Alipiopsitta*) *xanthops*, (Duarte & Caparroz, 1995), and *Graydidascalus brachyurus* (Caparroz & Duarte, 2004).

Pionus karyotype consists of 6 pairs of macrochromosomes (Caparroz & Duarte, 2004). Pairs 1, 2, 3, 4 and 5 are acrocentric, and pair 6 is telocentric. The acrocentric Z-chromosome of *Pionus* appears to be the largest chromosome of the series. This is a striking feature, considering the conservative pattern of the Z chromosome in parrot karyotypes, quite constantly composed of a middle size metacentric chromosome.

Graydidascalus brachyurus (Caparroz & Duarte, 2004), and *Amazona xanthops*, (Duarte & Caparroz, 1995; Valentine, 1990) have eight pairs of macrochromosomes. Their karyotypes are very similar to each other and to *Pionus* karyotypes, but they differ from *Pionus* in pairs 7 and 8, which are larger, and metacentric. As *Pionus* karyotypes do; *Graydidascalus brachyurus* and *Amazona xanthops* karyotypes also show a large acrocentric Z chromosome (larger than any autosome).

The taxonomic position of *Graydidascalus brachyurus* and *Amazona xanthops* has been discussed for some time. Cytogenetic data (Caparroz & Duarte, 2004), molecular genomic analysis results (Russello & Amato, 2004), morphological and behavioural observations (Silva, 1991), suggest a close relationship between this two species. Recently, the taxonomic position of *Amazona xanthops* has been revised, and it has been classified in the monotypic genus *Alipiopsitta* (Winkler et al., 2015).

Our suggestion is that the mutational chromosome events leading to the *Pionus, Graydidascalus brachyurus* and *Amazona xanthops* group karyotypes have to be considered independent from the evolutionary changes observed in the *Amazona* genus.

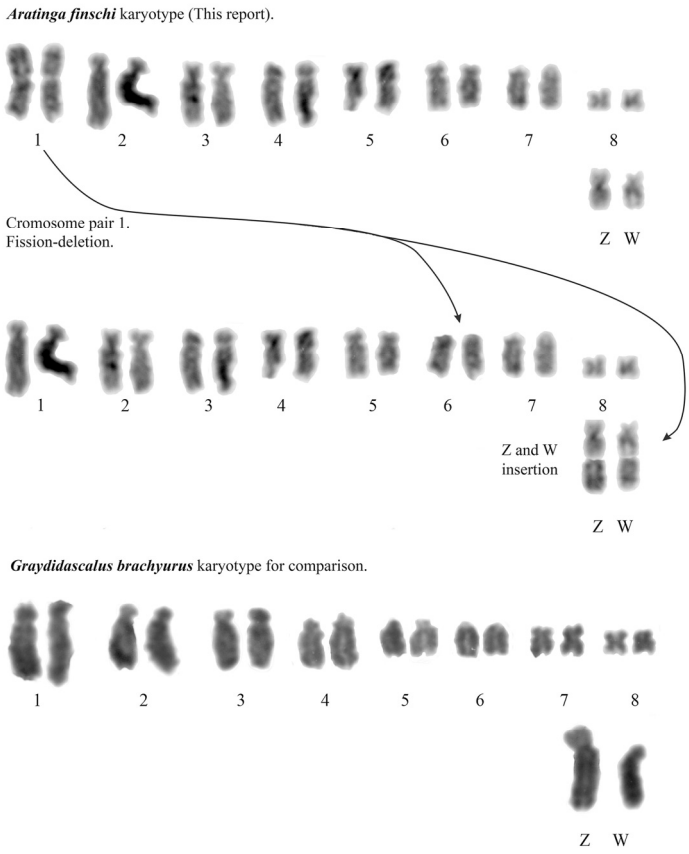

Figure 18. Proposed chromosome evolution in *Graydidascalus brachyurus Alipiopsitta*. Recreation of the possible chromosome mutational events, performed with image software (Photoshop CS®, Adobe), In this case we suggest that a fission-deletion of metacentric chromosome pair 1 of a macaw-conure karyotype may have played a role in the chromosomal evolution of *Pionus, A. xanthops-Graydidascalus* karyotypes

Accordingly to graphical recreations performed on possible chromosome rearrangement events in Neotropical parrots (See Figure 18), we hypothesize that the acrocentric chromosome 1 of *Pionus, A. xanthops-Graydidascalus* karyotypes is derived from the acrocentric chromosome 2 of a macaw-conure karyotype type. Metacentric chromosome 1 of this macaw-conure karyotype type may have experienced a fission-deletion to conform telocentric chromosome 6 of *Pionus, A. xanthops-Graydidascalus* karyotypes, while the remaining chromosome material derived from this fission-deletion has been inserted to the Z and W chromosome increasing their size.

The karyotype of *Pionus* genus can be related to the *A. xanthops-Graydidascalus* karyotypes, through centric fissions or arm deletion of the chromosomes 7 and 8 to shape a complement with 6 recognizable macrochromosomes.

A fourth Neotropical karyotype pattern is observed in *Forpus xhantopterygius* (De Lucca & De Marco, 1983; De Lucca et al., 1991). This species shows a karyogram with morphological resemblances to the Australian Platycercini parakeets. The presence of: six large autosomes and two large metacentric first pairs, suggest a centric fusion among small telocentric chromosomes, or, eventually, a relationship with Australian Platycercini (Brereton, 1963; Cracraft, 1973).

Phylogenetic relations of congeneric *Forpus passerinus* were studied (Wright et al., 2008). A independent clade, was defined for this single species (Schodde et al., 2013).

A scheme on suggested chromosome evolutionary events in Neotropical parrots is presented in Figure 19.

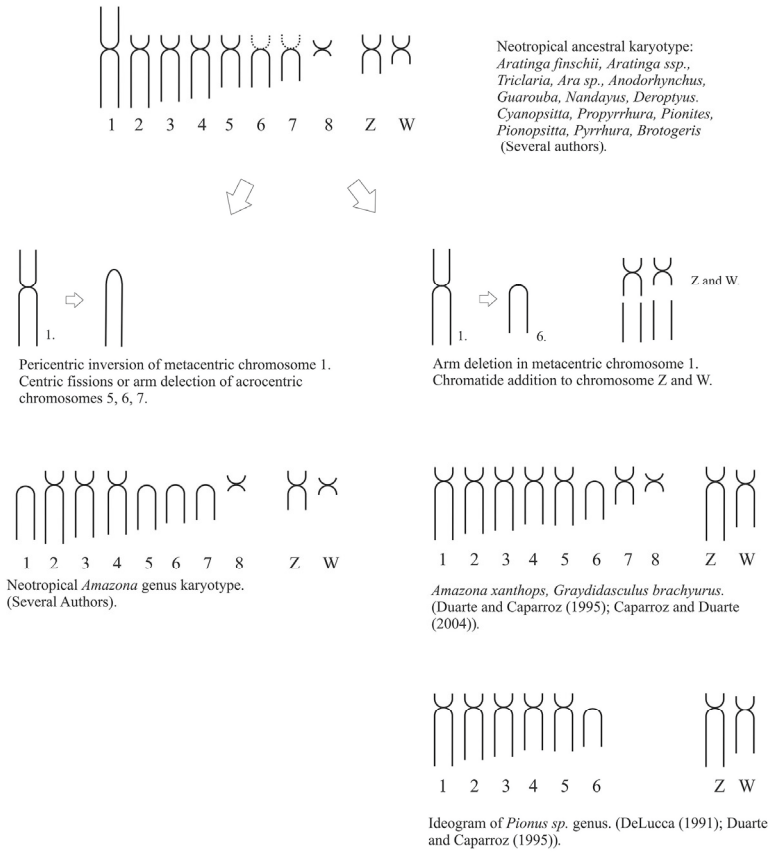

Figure 19. Scheme on suggested chromosome evolutionary events in Neotropical parrots. Mutations in autosome 1 account for the evolution of the short tailed karyotype patterns in Neotropical parrots, starting in an ancestral Neotropical karyotype pattern well conserved in most "long tailed Neotropical parrots"

5. Conclusion

The main events on parrot chromosome evolution are consistent with the biogeographic and phylogenetic evolution of the order, but the rate of chromosome change, when compared to the results of molecular phylogenetic analysis, is rather unpredictable. It is possible to infer correlations between molecular phylogenetic

results, and the cytogenetically observed chromosome variations. The karyotype changes provide strong support to the divergence nodes detected by molecular techniques. However, the sensitivity of cytogenetics to predict divergence topology is not as good.

The nature of the observed events that configure the chromosomal evolutionary history of parrots is compatible with the previous observations on the mechanisms of avian chromosome evolution: there are very stable regions in the complement, and preferential rearrangement sites which recurrently experience interchromosome rearrangements. These rearrangements consist mainly in centric fusions, or fissions, affecting primarily to microchromosomes (as far as it is possible their detection), and secondly to the smaller autosome macrochromosomes.

The presence of conserved chromosomal features in the diverse parrot karyotypes studied to date, has led us to define a standard parrot karyotype for reference and for comparisons.

A scheme on suggested chromosome evolutionary events in Psittaciformes is presented in Figure 20.

Figure 20. Scheme on suggested chromosome evolutionary events in Psittaciformes Order

High variability has been observed on chromosomes six and seven in the proposed reference parrot karyotype pattern. But rearrangements of these pairs do not show a precise orientation. Biogeographic distribution, or

phylogenetic relationships may apparently define a karyogram pattern (Christidis, Shaw, et al., 1991), which can be unexpectedly broken away by the presence of regional variations, which sometimes may be reminiscent of other karyological patterns.

Apparently, three independent cases of fusion between these two pairs have occurred in the Platycercini tribe, *Psittacula cyanocephala*, and *Forpus xanthopterygius*, resulting in karyotypes composed of six autosome macrochromosomes, and two first pairs of metacentric.

Metacentric pair one, in the proposed reference parrot karyotype pattern behaves more conservatively. However fission of this pair has been proposed to explain the chromosomal evolution of three independent groups; Cacatuidae family, *Charmosyna josefinae* (Loriinae), and also *Loriculus vernalis.* We suggest that mutations involving this chromosome pair are also responsible for the chromosomal divergence observed in genera *Amazona* (pericentric inversion), and also in the case of genus *Pionus, Graydidascalus*, *Alipiopsitta*, where an arm deletion is hypothesized.

Very conservatives are acrocentric chromosome pairs 2, 3 and 4 (and usually 5).

Metacentric chromosome Z is conservative among Psittaciformes. We suggest that a mutational episode of chromatin addition is responsible for changes observed in Z chromosome of *Pionus, Graydidascalus*, and *Alipiopsitta* karyograms.

Chromosome heterozygosity was observed in chromosome pair 3 of *Callocephalon fimbriatum*. As previously suggested, this situation can be interpreted as the occurrence of heterogozyous robertsonian translocation.

Polytypy due to robertsonian differences occurs in a number of animals, notably small mammals, as shrews (Castagné, Mehmeti, & Hausser, 1994; Wyttenbach, Narain, & Fredga, 1999; Zima, Fedyk, Fredga, & Hausser, 1996) and Rodents (Bauchau, Smets, Viroux, Nootens, & Caritat, 1990; Hauffe & Searle, 1998; Lyapunova & Vorontsov, 1984; Matthey & Petter, 1970; Nachman & Searle, 1995; Tettenborn & Gropp, 1970; Wahrman, 1972). This situation is often termed “robertsonian fan” (Lyapunova & Vorontsov, 1984; Matthey & Petter, 1970).

Classic chromosomal speciation models assume that heterozygous individuals have a reduced fitness or low fertility (Dobzhansky, 1933), although recent observations indicate otherwise (Castagné et al., 1994; J. a Coyne & Orr, 1998; J. A. Coyne, Meyers, Crittenden, & Sniegowski, 1993; Hauffe & Searle, 1998; Navarro & Ruiz, 1999), and chromosomal speciation models are under review (Faria & Navarro, 2010; Rieseberg, 2001).

Some cases of retained chromosome polymorphism have been described in Passeriformes populations (Capanna, Rantucci, & Civitelli, 1987; Thorneycroft, 1966), and also in some Psittaciformes individual cases (De Lucca & De Marco, 1983; Joshua, Unbublished; Valentine, 1990).

De Lucca and De Marco (1983) have considered the evolutionary significance of the chromosomal polymorphism accordingly to: “1) As the response to frequent genetic-environmental interaction. 2) As an isolating mechanism instrumental in speciation. 3) As a process conferring heterozygote advantage”.

The interpretation of the chromosome heterozygosity of chromosome pair 3 in *Callocephalon fimbriatum* is limited by the fact that only one individual has been examined to date.

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